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Effects of pH on image pearl production in freshwater mussels (*Lamellidens marginalis*) under controlled temperature

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ABSTRACT

The environmental conditions such as pH are reported to affect the matrix protein, mineralizing tissue of the shell and finally the quality pearls in bivalves. However, limited information is available on the relationship between pH and the pearl characteristics of freshwater bivalves. Considering the fact, the present study was designed to optimize the pH in pearl culture of *Lamellidens marginalis*. Three different treatments were assigned considering three level of pH as T_1 (7.5), T_2 (8.0) and T_3 (8.5) at controlled temperature (28°C). All the mussels were inoculated with paraffin image and cultured for 180days in glass aquariums. After the culture period, the mussels were dissected and the image pearls were assessed for nacre thickness and pearl production rate. Results showed that pH had no significant effect on shell length and weight increment, while significant increase in survival and pearl production rate were observed at treatment T_1 (pH = 7.5). Furthermore, the studied three different levels of pH had no significant effect on nacre thickness and nacre weight of the produced image pearls. The present study concluded that, considering the higher survival and pearl production rate at treatment T_1, grafted mussels should be cultured preferably at pH 7.5. The findings of the current study might be helpful for strengthening culture environment of image pearl in *L. marginalis*.

INTRODUCTION

Along with many other abiotic parameters, the hydrogen ion concentration (pH) of a solution plays a role in determining whether aquatic organisms can live there, how large they can grow, how many offspring they can have, and where they may be found. It is known that the physiological process of growth, which serves as an integrator of many other physiological outcomes, is the result of three separate processes: cell division, assimilation, and cell expansion (Ndubuisi et al., 2015).

Molluscs secrete their shells by an extracellular matrix-mediated physiologically regulated biomineralization process (Mann, 2001). Calcium carbonate ($CaCO_3$) and an organic cell-free matrix released by the external mantle epithelium combine to form the natural biomaterial that is a mollusc shell. Despite its importance, this organic matrix outside the cells contributes just a small amount to the shell's overall composition (Weiner and Lowenstam, 1986). Several microstructural layers of the shell and pearl are laid down because of the calcifying shell matrix interacting with the crystal surface to orient its nucleation and govern $CaCO_3$ crystal polymorphisms, in the form of aragonite or calcite (Marie et al., 2012). Bivalves that produce pearls are sensitive to changes in water's pH (Strack, 2006). The nacreous layer, the prismatic layer, and the organic layer make up the three layers that make up a pearl oyster shell (Fougerouse et al., 2008). The outer layer, known as the periostracum, plays a significant role in protecting the shells of pearl-producing bivalves from fouling. Shell fouling is hastened by acidic or alkaline environments because of the increased rate of outer layer removal (Buschbaum et al., 2007). Above the prismatic layer, the bivalves that produce pearls secrete fresh nacre. Tablets of nacre are derived from aragonite crystals on the

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nanoscale. The nacreous layer is formed when aragonite crystals develop and fuse together (Saruwatari et al., 2009). The nacre produced by pearl bivalves is used in the cultivated pearl business, thus these animals are raised specifically for this purpose. The farmed pearl industry's output may be severely hampered if pH influences the crucial physiological process of nacre deposition (Gosling, 2003). In terms of water quality, the freshwater pearl mussel is quite delicate (Bauer et al., 1980; Klupp, 1983 and Heming et al., 1988).

Image pearls are produced in freshwater unionid mussels using images in a picture or image format either from paraffin or shell of the donor mussels (Tanu et al., 2021). However, the quality of the image pearls depends on thickness of the nacre layer and lusters of the pearl. Limited information is available on the relationship between pH and the shell increment and weight gain of freshwater bivalves in the world (Joubert et al., 2014). At neutral pH the mantle of mussels synthesizes and secrets more organic matrix than at acid or alkaline pH (Andong and Anjing, 1999) which resembles that pH effect the growth and pearl formation of inoculated mussels. According to Xu et al. (1988) pH is crucial to the growth of mollusc. Neutral pH allow faster growth, and nacre secretion in freshwater mussel (Xu et al., 1988; Andong and Anjing, 1999) whereas, low pH hinder shell growth (Heming et al., 1988; Ren et al., 2014). Therefore, it is important to evaluate the possible effects of pH on the growth, survival, and image pearl production in freshwater mussels. Current study aimed to examine the growth, survival, image pearl production and nacre accumulation of inoculated freshwater mussels (*L. marginalis*) at different pH under controlled temperature.

MATERIALS AND METHODS

Location and duration of the experiment

The experiment was conducted at the Pearl Research Laboratory (PRL) at Bangladesh Fisheries Research Institute (BFRI), Mymensingh, Bangladesh (24°43'49.6"N, 90°25'27.4"E) from December 2019 to July 2020.

Collection and pre-operative treatment of mussels

Freshwater mussels with a yellow margin on the shell were gathered from the different water bodies of Mymensingh region. The species was selected for the experiment due to its availability, easy image inoculation, high survival, and higher rate of pearl production (Hossain et al. 2004; Tanu et al. 2019). Collected mussels were sorted and similar sized mussels were stocked in the pond complex of BFRI. Before stocking, ponds were fertilized with inorganic (12g TSP and 10g Urea per decimal) and organic fertilizer (5kg mustard oil cake per decimal) to grow planktonic food materials. After rearing for three months in the ponds, the mussels were collected and cleaned to remove algae and other waste materials and transferred into the cemented cistern ($2.42\times1.88\times1.00$ m^3). In the cistern the mussels were conditioned with continuously exchanging aerated water. During the conditioning period, the mussels were also disinfected with $KMnO_4$ at 2 ppm/m^3. The conditioned mussels were reared for four weeks with planktonic foods. Water in the cistern was siphoned regularly to clean feces and other waste materials. Before harvesting, the mussels were starved for 7 days (Tanu et al., 2022) to hardening for image pearl operation. Before transferring to the laboratory, pre-operative mussels were measured for shell length and weight. In the laboratory, mussels were held in a downward posture (hinge upward) in a porous basket for 2 h to permit water to remove from the internal organs.

Paraffin image preparation

Molds for paraffin image were prepared from the shells of dead mussel. Liquid wax was poured into the concave side of the shell and agitated to form a thin layer of approximately 1.25 mm. A needle was then used to draw an image or sculpture on the solidified wax layer. Prepared image (1.75x1.5 cm^2 in size and 1.25 mm in thickness) with mold was then collected and preserved for further inoculation in the mussel. Soybean oil was applied in the dead shell's concave side before waxing to facilitate the easy separation of the paraffin image (Siddique et al., 2020).

Inoculation of paraffin image and post-conditioning

Prepared images were washed with distilled water for cleaning and making it slippery. A total of 270 healthy mussels were selected for the inoculation of image pearls. A mussel opener was used to open the valves up to 6-8 cm. About 2.5 cm area of the mantle tissue was gently lifted from the inner surface of the shell using a spatula and the image mold was inserted into the mantle cavity. Gentle scrapping with a spatula was made to remove the extra air from the cavity area. Finally, the operated mussels were kept in an upward direction (hinge down) into a purported tray until they were transferred into the cistern (Tanu et al., 2021). The inoculated mussels were then tagged on the shell surface through scrapping with a mini grinding machine (Dera, DKEG 3mm), and transferred into the cistern for the post-operative treatments. Image inoculation tools used in the operation were stated in Table 1. After grafting, mussels were conditioned with flowing water without feeding for 7 days. Post-operative mussels were fed with phytoplankton at a density of 60 × 10^3 cells/L for 21 days (Tanu et al. 2022).

Table 1: Image operation tools adopted from Tanu et al. (2022)

Tool	Function
Stainless steel grafting stand	To hold the mussel in a fixed position.
Gill adjusting oar/ spatula	To adjust the gill and visceral mass into an appropriate position during the operation
Stainless steel Mussel opener	A mussel opener was used to open the mussel.
Stainless steel stopple	Used to hold the two shell valves apart.
Stainless steel forceps	Forceps used for transferring the paraffin wax image mold into position within the mussel.
Porous plastic tray	A porous tray was used for placing operated mussels.
Mussel shell	Dried mussel shells were used as the substrate to facilitate preparing the paraffin image.
Paraffin image	Paraffin wax was liquidated, shaped, and sculpted to make the image
Plastic dropper bottle, paraffin wax, glass beaker, and heater	A dropper bottle was used for cleaning the dirt, and paraffin wax, beakers, and a heater for producing the image or design.

Experimental design

Operated mussels were transferred into nine glass aquariums each having a capacity of 300 L (100 x 60 x 60 cm^3). Nine aquariums were distributed into three treatments with three replicates. Before stocking, initial length and weight of the operated mussels were measured with a digital calipers (INGCO, HDCD 28200) and electronic balance (Radwang WLC/600/A_1), respectively.

Experiment was performed to estimate the effect of controlled temperature during the study period. Operated mussels were reared for three pH regimes (T_1, 7.5, T_2, 8.0 and T_3, 8.5) at constant temperature (28°C) by optimizing the ratio of H_2SO_4 or NaOH in each treatment. The mussels were cultured maintaining a light-dark cycle of 12:12h (Loayza-Muro and Elías-Letts, 2007). Feces and other waste materials were removed regularly by siphoning (Submersible pump, RS-21000). Electronic aerators (Resun, 9902) were used for oxygenation of the aquariums. Inoculated mussels were fed with cultured plankton with a density of 60 × 10^3 cells/L (Tanu et al., 2022).

Measurement of water quality parameters

A Celsius thermometer was set in each aquarium to observe the water temperature during the experiments. The pH was monitored regularly using a digital pH meter (HANNA, HI 98129).

Growth performance of inoculated mussels and pearl production rate

The culture system was monitored regularly for the counting and removal of dead mussels. After 180 days of culture period, the mussels were harvested. The final length and weight of the survived mussels were measured. Length increment, weight increment, survival rate of the mussels and image pearl production rate were calculated according to Suryawanshi and Kulkarni (2019) using the following equations:

$$\text{Percentage length Increment (LI\%)} = \frac{\text{Final length-Initial length}}{\text{Initial length}} \text{ x } 100$$

$$\text{Percentage weight increment (WI\%)} = \frac{\text{Final weight-Initial weight}}{\text{Initial weight}} \text{ x } 100$$

$$\text{Survival Rate (SR\%)} = \frac{\text{No. of stocked mussel - No. of dead mussel}}{\text{No. of stocked mussel}} \text{ x } 100$$

$$\text{Percentage of image pearl production (IPP\%)} = \frac{\text{No. of inoculated mussel - No. of (Dead mussel + mussel rejected image)}}{\text{No. of inoculated mussel}} \text{ x } 100$$

Measurement of nacre thickness and weight

The shell containing the image pearls was washed with tap water and dried. The image pearls were separated from the shells (by cutting with Optilab ANAND 388001), rinsed in distilled water, and dried. Finally, the attached image with the nacre layer was pulled out from the inner shell of dissected mussels. Nacre thickness and nacre weight of the harvested image pearl was measured with a digital micrometer (Mitutoyo, IP 65) and electric balance according to Ky et al. (2013). The following equations estimated nacre thickness and nacre weight:

Nacre thickness (NT) =
Final image pearl thickness - Initial paraffin image thickness
Nacre weight (NW) =
Image pearl weight– Paraffin image weight

Statistical analysis

Data incurred during the experiments were summarized, tabulated and expressed as Mean ± SD. One-way analysis of variance was used to examine the differences in length increase, weight gain, nacre thickness, and nacre weight gain over a range of pH and temperature conditions. The Tukey's HSD test was used to compare the means.The relationship between temperatures and pH, length increment, weight gain, nacre thickness, and nacre weight gain were explored using Pearson's correlation. Significance was assigned at 5% level (p<0.05). All the data analysis was performed using IBM SPSS™Statistics 26.0 software.

RESULTS

Initial shell length, final shell length, final length increment, initial weight, final weight, weight increment, survival rate, image pearl production, paraffin image thickness, image pearl thickness, nacre thickness, paraffin image weight, image pearl weight, nacre weight of the inoculated mussels was given in the Table 2.

Table 2: Growth, survival and image pearl production (Mean ±SD) in *L. marginalis* at different pH

Parameters	**Treatments**		
	T_1 (7.5)	**T_2 (8.0)**	**T_3 (8.5)**
Initial shell length (cm)	8.89±0.05[a]	8.90±0.06[a]	8.88±0.04[a]
Final shell length (cm)	9.51±0.05[a]	9.51±0.06[a]	9.48±0.05[a]
Shell length increment (%)	6.94±0.23[a]	6.85±0.23[a]	6.76±0.25[a]
Initial weight (g)	59.25±0.77[a]	59.18±0.78[a]	59.02±0.70[a]
Final weight (g)	66.46±0.88[a]	66.31±0.86[a]	66.06±0.72[a]
Weight increment (%)	12.17±0.34[a]	12.05±0.19[a]	11.92±0.26[a]
Survival rate (%)	62.22±5.09[a]	48.89±1.92[b]	36.67±3.34[c]
Image pearl production (%)	54.45±3.85[a]	43.33±3.34[b]	31.11±5.09[c]
Paraffin image thickness (mm)	1.27±0.04[a]	1.28±0.04[a]	1.25±0.05[a]
Image pearl thickness (mm)	1.54±0.04[a]	1.54±0.05[a]	1.51±0.06[a]

Nacre thickness (mm)	0.26±0.02[a]	0.26±0.01[a]	0.26±0.01[a]
Paraffin image weight (mg)	257.08±8.24[a]	257.1±7.52[a]	255.68±8.36[a]
Image pearl weight (mg)	342.49±9.23[a]	342.23±7.76[a]	340.46±8.79[a]
Nacre weight (mg)	85.41±3.34[a]	85.13±3.04[a]	84.79±2.57[a]

Different superscript letters in the same row indicate significant difference (p<0.05) while similar superscript letter indicate insignificant difference (p>0.05).

Length increment of inoculated mussels at different pH

The mean initial shell length of the inoculated mussels was 8.89±0.05, 8.90±0.06 and 8.88±0.04 cm, in T_1, T_2 and T_3, respectively (Table 2). After the culture period of 180 days, mean final shell length of mussel at different pH level was recorded 9.51±0.05, 9.51±0.06 and 9.48±0.05 cm in T_1, T_2 and T_3, respectively (Figure 1A). The shell length increment was 6.94±0.23% in T_1, 6.85±0.23% in T_2, and 6.76±0.25% in T_3. Shell length increment at the end of the experiment was not significantly different (p>0.05) in T_1, T_2 and T_3 (Figure 1B).

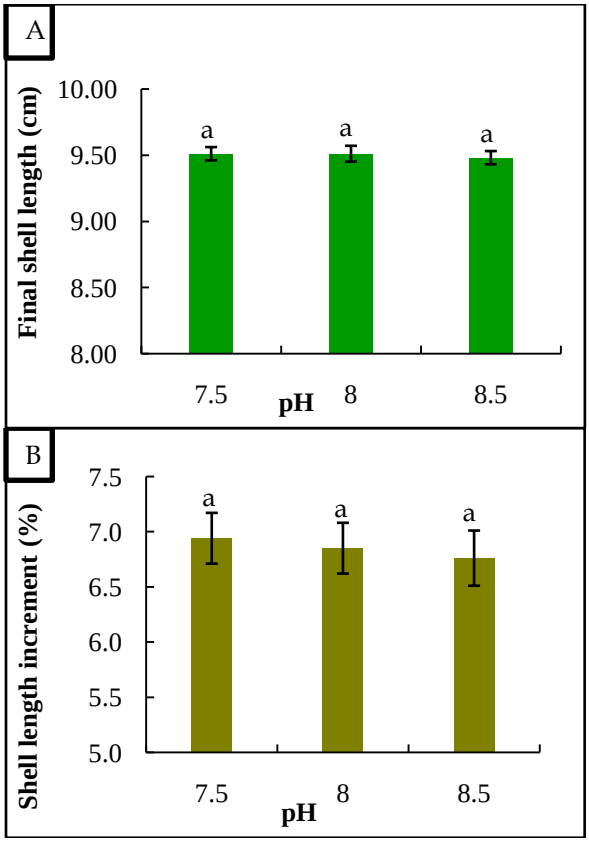

Figure 1: Final length (A) and percentage of length increment (B) of *L. marginalis* at different pH. Treatment bars with similar superscript letters indicate insignificant difference (p>0.05).

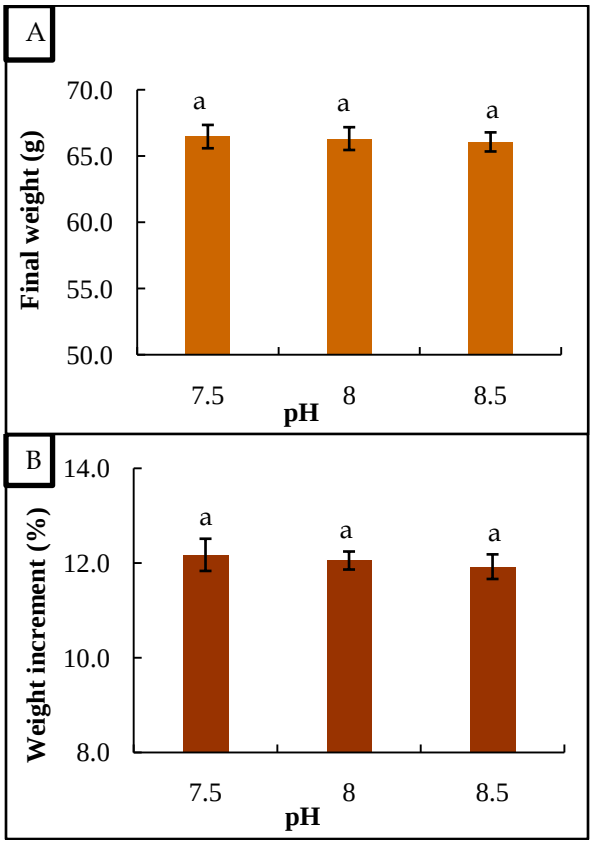

Figure 2: Final weight (A) and percentage of weight increment (B) of *L. marginalis* at different pH. Treatment bars with similar superscript letter indicate insignificant difference (p>0.05).

Weight gain of inoculated mussels at different pH

In the present experiment, the mean initial weight of the operated mussels was 59.25±0.77, 59.18±0.78 and 59.02±0.70 g in T_1, T_2 and T_3, respectively (Table 2). At the end of the culture period, the mean final weight was recorded 66.46±0.88, 66.31±0.86 and 66.06±0.72 g in T_1, T_2, and T_3, respectively (Figure 2A). After 180

days of culture period, the highest weight increment was recorded in T_1 (12.17±0.34 %) followed by T_2 (12.05±0.19%) and T_3 (11.92±0.26 %). Weight increment was consistent (p>0.05) in T_1, T_2 and T_3 during the experiment (Figure 2B).

Survival and pearl production rate of grafted mussels

Survival and pearl production rate of the experimental mussels are shown in Figure 3. Mussels reared at different pH showed significantly (p<0.05) higher survival rate in T_1 (62.22±5.09%), followed by T_2 (48.89±1.92%), and T_3 (36.67±3.34%) (Figure 3A). Similar to survival rate, significantly higher image pearl production rate (p<0.05) was observed in T_1 when compared with T_2 and T_3. Image pearl production rate was also significantly different between T_2 and T_3. Image pearl production was 54.45±3.85% in T_1, 43.33±3.34% in T_2, and 31.11±5.09% in T_3 (Figure 3B).

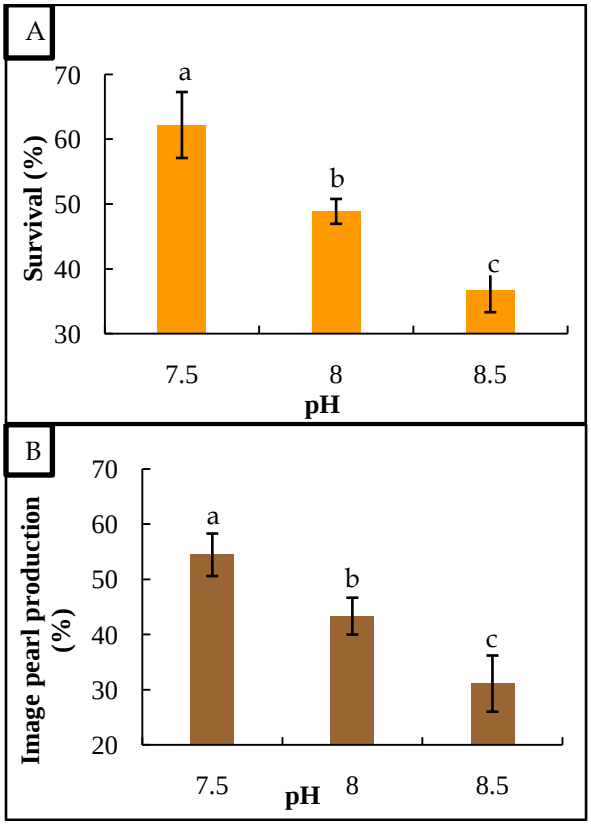

Figure 3: Survival (A) and image pearl production (B) of the inoculated *L. marginalis* at different pH. Treatment bars with different letters denote significant differences (p<0.05).

Nacre accumulation in grafted mussels

Mean thickness of the paraffin images were 1.27±0.04, 1.28±0.04, and 1.25±0.05 mm in T_1, T_2 and T_3, respectively during the inoculation period. After the culture period of 180 days at different pH level at constant temperature, thickness of the image pearls was measured 1.54±0.04, 1.54±0.05 and 1.51±0.06 mm in T_1, T_2 and T_3, respectively (Table 2). Mean thickness of the images were increased and nacre thickness was 0.26±0.02, 0.26±0.01, 0.26±0.01 mm in T_1, T_2 and T_3, respectively (Figure 4A). After the experimental period, harvested image pearl's nacre weight was measured 85.41±3.34 mg in T_1, 85.13±3.04 mg in T_2, and 84.79±2.57 mg in T_3 (Figure 4B).No statistically significant differences were noted among the treatments (p>0.05) in terms of either nacre thickness neither nacre weight (Table 2).Image pearls produced at different treatments are shown in Figure 5.

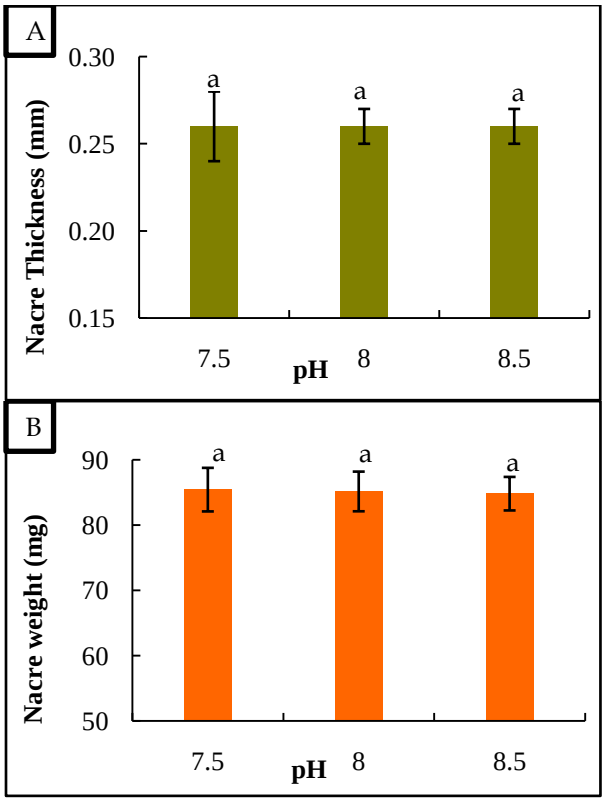

Figure 4: Nacre thickness (A) and nacre weight (B) of the harvested image pearls from the inoculated *L. marginalis* at different pH. Treatment bars with similar letters indicate insignificant difference (p>0.05).

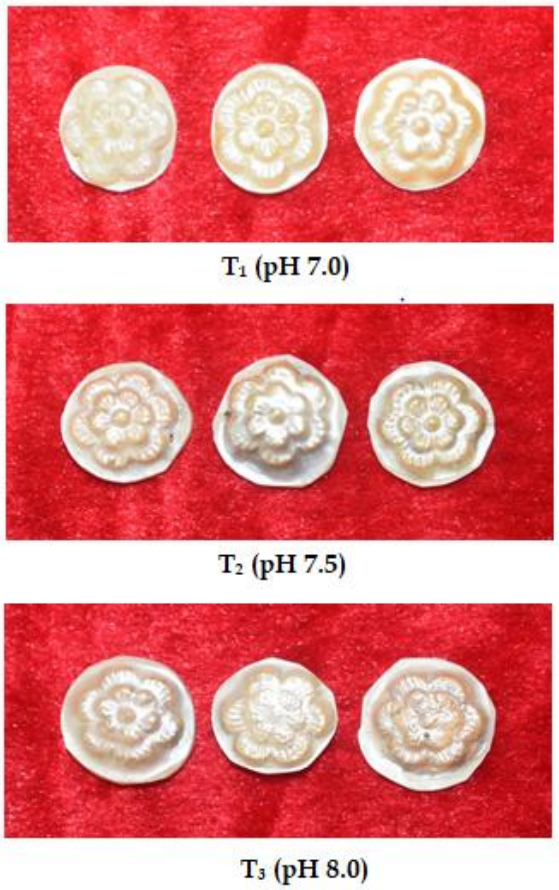

Figure 5: Image pearls produced from the inoculated *L. marginalis* at different pH.

DISCUSSION

The present experiment demonstrated the effect of different pH levels on the growth, survival rate, nacre thickness and nacre weight of *L. marginalis*. Growth rate, which was calculated in the simplest form from changes in tissue weight or shell length, is a common way to measure the fitness of bivalve organisms (Norkko et al., 2006). No Significant differences in length and weight of *L. marginalis* were recorded at pH 7.5, 8.0 and 8.5 in treatment T_1, T_2 and T_3, respectively. Previous studies have shown that pH plays a vital role in the growth and survival of freshwater mussels (Areekijseree et al., 2004; Uddin et al., 2013; Sangsawang et al., 2019; Niogee et al., 2019; Siddique et al., 2020). Water pH is known to influence the physiology of *L. marginalis* by changing extracellular acid-base balance, metabolic activities and feeding behavior (Heming et al., 1988; Islam et al., 2020). This variation in the shell increment and weight increment of the freshwater mussel might be due to the variability of species, habitat and ecological differences (Gosling, 2003).

During the study period, the survival rate along with the pearl production rate of grafted mussels was significantly higher at pH level 7.5 compared to pH 8.0 and 8.5, respectively, which might be due to the oxidative injury resulting from increased reactive oxygen species and malondialdehyde levels at higher pH (Liu et al., 2020). However, our result contradicts the findings of Heet al. (2017), who reported an insignificant effect of pH on the survival and growth of Chinese razor clam *Sinonovacula constricta*. Changes in environmental pH coincide with the internal shift in pH, which can affect the chemical composition of growth-layered mantle tissue and influence the health condition and even death of mussels (Bibby *et al.*, 2008; Cole et al., 2016). However, the effect of pH may be more profound with the combination of other factors such as hardness, alkalinity, disease and post-operative disinfection (McGladdery et al., 2007). Image pearl retained in all the surviving mussels in the present experiment indicated the grafter professionalism and the suitability of environmental conditions. Image pearl production rate in the current investigation was higher compared to the findings of Siddique et al. (2020), who reported lower survival (20%) and pearl production rate (20%) in the net bag handing method in a perennial pond. The higher survival and pearl production rate in the present study might be due to the controlled environment. Furthermore, the higher pearl production rate at lower pH (7.5) in the present study is presumably due to higher wound healing capacity than higher pH (8.0 and 8.5). However, freshwater mussels' reduced survival and pearl production rate is worth further study.

In the present study, pH level was found to show an insignificant effect on nacre thickness and nacre weight. Jin et al. (2012) reported that improving body length and weight indirectly improved pearl size and weight. The process of nacre biomineralization is closely connected with nacre thickness and weight. As already seen in *Pinctata maxima*, high biomineralization potential may have contributed to a more considerable nacre

deposition (Strack, 2006; Kono et al., 2000). A hundred to thousand aragonite crystals and protein matrices alternatively overlay on the nucleus to produce the nacre (Zhang and Xu, 2013). The epithelial cells' ability from the mantle tissue's outer surface to synthesize various calcium carbonate polymorphs determines the biomineralization phenomena in molluscs (Wilbur, 1964; Watabe, 1988; Addadi and Weiner, 1992). Although biological and genetic processes govern biomineralization, water chemistry also affects the elemental and mineral composition of the shell (Bourgoin, 1990; Pitts and Wallace, 1994; Belcher et al., 1996). Therefore, nacre thickness and nacre weight at pH 7.5 to 8.0 might be due to better biomineralization success.

CONCLUSION

In conclusion, the present study demonstrated that water chemistry significantly affects survival and image pearl production in freshwater mussels. An optimal pH level of 7.5 showed higher survival and image pearl production. Furthermore, length increment, weight gain, nacre thickness and nacre weight indicated less stress to the operated mussel at these pH levels. Although changes in structural properties and luster were evident at higher pH (8.0 and 8.5) and causes behind this phenomenon require further investigation.

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REFERENCES

Addadi L and Weiner S (1992). Control and design principles in biological mineralization. Angewandte Chemie International Edition, 31(2), 153-169.

Andong Q and Anjing S (1999). Effects of pH on nacre secretion of freshwater pearl mussel (*Hyriopsis cumingii*). Dongwuxuebao. [Acta Zoologica Sinica*]*, *45*(4), 361-370.

Areekijseree M, Engkagul A, Kovitvadhi U, Thongpan A, Mingmuang M, Pakkong P and Rungruangsak-Torrissen K (2004). Temperature and pH characteristics of amylase and proteinase of adult freshwater pearl mussel, Hyriopsis (*Hyriopsis) bialatus*) Simpson 1900. Aquaculture, 234(1-4), 575-587.

Bauer G, Schrimpff E, Thomas W and Herrmann, R. (1980). Zusammenh~ ingezwischendem Bestandsrfickgang der Flussperlmuschel (*Margaritifera margaritifera*) imFichteigebirge und der Gew~ isserbelastung. Arch. Hydrobiol, *88*, 505-13.

Belcher AM, Wu XH, Christensen RJ, Hansma, P. K., Stucky, G. D., & Morse, D. E. (1996). Control of crystal phase switching and orientation by soluble mollusc-shell proteins. Nature, 381(6577), 56-58.

Bibby R, Widdicombe S, Parry H, Spicer J andPipe R (2008). Effects of ocean acidification on the immune response of the blue mussel *Mytilus edulis*. Aquatic Biology, *2*(1), 67-74.

Bourgoin BP (1990). *Mytilus edulis* shell as a bioindicator of lead pollution: considerations on bioavailability and variability. Marine Ecology Progress Series, *61*(3), 253-262.

Buschbaum C, Buschbaum G, Schrey I and Thieltges, DW (2007). Shell-boring polychaetes affect gastropod shell strength and crab predation. Marine Ecology Progress Series, *329*, 123-130.

Cole VJ, Parker LM, O'Connor SJ, O'Connor WA, Scanes E, Byrne M and Ross PM (2016). Effects of multiple climate change stressors: ocean acidification interacts with warming, hyposalinity, and low food supply on the larvae of the brooding flat oyster *Ostrea angasi*. Marine Biology, *163*, 1-17.

Fougerouse A, Rousseau M and Lucas JS (2008). Soft tissue anatomy, shell structure and biomineralization. In: Southgate PC and Lucas J (Editors), The Pearl Oyster. Elsevier Press, Amsterdam, The Netherlands.pp 75–100.

Gosling E (2003). Bivalve molluscs: biology, ecology and culture. Fishing News Books, Oxford. pp. 443.

He M, Lai QF, Yao ZL, Sui YM, Lu JX, Gao PC, Zhou, K (2017). Effects of pH and temperature on carbon budget of *Sinonovacula constricta*. Chinese Journal of Ecology, *36*(4) 1014–1019.

Heming TA, Vinogradov GA, Klerman AK and Komov VT (1988). Acid-base regulation in the freshwater pearl mussel *Margaritifera margaritifera*: Effects

of emersion and low water pH. Journal of Experimental Biology, 137(1), 501-511.

Hossain MA, Sultana N, Azimuddin K, Hussain MG, and Mazid MA (2004). Selection of freshwater pearl mussel species for mantle transplantation in Bangladesh. Bangladesh Journal Fisheries Research,8, 113–116.

Islam S, Hasan T, Siddique MF, Moniruzzaman M, Hossain MS and Uddin MJ (2020). Effects of pH on filtration of freshwater pearl mussel Lamellidens marginalis, Lamarck (Bivalvia: Unionidae) under laboratory conditions. Bangladesh Journal of Fisheries, 32(2), 221–228.

Jin W, Bai Z, Fu L, Zhang G and Li J (2012). Genetic analysis of early growth traits of the triangle shell mussel, *Hyriopsis cumingii*, as an insight for potential genetic improvement to pearl quality and yield. Aquaculture International, 20, 927-933.

Joubert C, Linard C, Le Moullac, G, Soyez C, Saulnier D, Teaniniuraitemoana V, Ky CL and Gueguen Y (2014). Temperature and food influence shell growth and mantle gene expression of shell matrix proteins in the pearl oyster *Pinctada margaritifera*. PloS ONE, 9(8), 1–9.

Klupp R (1983). Das Perlmuschel problem. Fischwirt, 33, 34-36

Kono M, Hayashi N and Samata, T (2000). Molecular mechanism of the nacreous layer formation in *Pinctada maxima*. Biochemical and biophysical research communications, 269(1), 213-218.

Ky CL, Blay C, Sham-Koua M, Vanaa V, Lo C andCabral P (2013).Family effect on cultured pearl quality in black-lipped pearl oyster *Pinctada margaritifera* and insights for genetic improvement. Aquatic Living Resources, 26(2), 133-145.

Liu Y, Li L, Zheng L, Fu P, Wang Y, Nguyen H, Shen, X and Sui Y (2020).Antioxidant responses of triangle sail mussel *Hyriopsis cumingii* exposed to harmful algae *Microcystis aeruginosa* and high pH. Chemosphere, 243,125241.

Loayza-Muro R, Elías-Letts R (2007). Responses of the mussel *Anodontites trapesialis* (Unionidae) to environmental stressors: effect of pH, temperature and metals on filtration rate. Environmental Pollution,149(2), 209–215.

Mann S (2001). Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry, Oxford University Press, New York. pp. 222.

Marie B, Joubert C, Tayalé A, Zanella-Cléon I, Belliard C, Piquemal D, Cochennec-Laureau N, Marin F, Gueguen Y and Montagnani C (2012). Different secretory repertoires control the biomineralization processes of prism and nacre deposition of the pearl oyster shell. Proceedings of the National Academy of Sciences, 109(51), 20986-20991.

McGladdery SE, Bondad-Reantaso MG and Berthe FC (2007). Pearl oyster health management. FAO Fisheries Technical Paper, 503, 19

Ndubuisi UC, Chimezie AJ, Chinedu UC, Chikwem IC andAlexander U (2015). Effect of pH on the growth performance and survival rate of *Clarias gariepinus* fry. International Journal of Research in Biosciences, 4(3), 14-20.

Niogee SR, Tonni KF, Barman AC, Tanu MB, Sku SONIA and Uddin MJ (2019). Ovarian cycle of freshwater pearl mussel, *Lamellidens marginalis* (Lamarck, 1819) collected from a culture pond in Bangladesh. Asian Fisheries Science, 32(3), 117-123.

Norkko J, Thrush SF and Wells RMG (2006). Indicators of short-term growth in bivalves: Detecting environmental change across ecological scales. Journal of Experimental Marine Biology and Ecology, 337(1), 38-48.

Pitts LC and Wallace GT (1994). Lead deposition in the shell of the bivalve, *Mya arenaria*: an indicator of dissolved lead in seawater. Estuarine, Coastal and Shelf Science, 39(1), 93–104.

Ren G, Wang Y, Qin J, Tang J, Zheng X andLi Y (2014). Characterization of a novel carbonic anhydrase from freshwater pearl mussel *Hyriopsis cumingii* and the expression profile of its transcript in response to environmental conditions. Gene, 546(1), 56–62.

Sangsawang A, Kovitvadhi U and Kovitvadhi S (2019). The effect of water temperature on the early-life development, growth and survival of the freshwater mussel *Hyriopsis bialata.* Aquaculture, 510, 311-317.

Saruwatari K, Matsui T, Mukai H, Nagasawa H and Kogure T (2009). Nucleation and growth of aragonite crystals at the growth front of nacres in pearloyster, *Pinctada fucata*. Biomaterials, 30(16), 3028-3034.

Siddique MF, Barman AC, Tanu MB, Sku S and Mahmud Y (2020). Optimizing pearl production inoculating paraffin image in freshwater mussel (*Lamellidens marginalis*) cultured in perennial ponds. Journal of the Bangladesh Society for Agricultural Science and Technology,17,1–5.

Strack E (2006).Pearls. Ruhle-DiebenerVerlag GmbH and Co. KG, Stuttgart, Germany. pp. 707.

Suryawanshi AV and Kulkarni AN (2019). Mussel culture by using different culture methods and estimation of growth parameters of *Lamellidens Corrianus* from Nanded Region, Maharashtra. International Journal of Applied and Natural Sciences,8, 9–18.

Tanu MB, Barman AC, Siddque MF, Sku S, Hossen MN, Southgate PC and Mahmud Y (2022). Impact of culture period on quality of image

pearls produced by the freshwater mussel, *Lamellidens marginalis*, in Bangladesh. Journal of Shellfish Research,41(1), 75–83.

Tanu MB, Barman AC, Siddique MF, Sku S, Hossen MN, Rayhan A and Mahmud Y (2021). Determining of suitable size of paraffin image for image pearl production in freshwater mussel (*Lamellidens marginalis*) in Bangladesh. International Journal of Fisheries and Aquatic Studies, 9, 222-226.

Tanu MB, Barman AC, Siddique MF, Sonia S and Yahia M (2019). Production and quality of mantle tissue transplanted pearls in *Lamellidens marginalis* cultured in different locations of Mymensingh district, Bangladesh. Journal of the Bangladesh Agricultural University,17(3), 424–429.

Uddin MJ, Jeung HD, Yang HS, Kim BK, Ju SJ and Choi KS (2013). Quantitative assessment of reproductive effort of the Manila clam *Ruditapesphilip pinarum* in a lagoon on Jeju Island (Korea) using enzyme-linked immunosorbent assay. Invertebrate Reproduction and Development, 57(4), 316-324.

Watabe N (1988). Shell structure, form and function. The Mollusca,11, 69–104.

Weiner S and Lowenstam HA (1986). Organization of extracellularly mineralized tissues: A comparative study of biological crystal growth. Critical reviews in biochemistry, 20(4), 365-408.

Wilbur KM (1964).Shell formation and regeneration. In: Wilbur KM and Yonge CM (Editors), Physiology of Mollusca- Volume 1. Academic Press, New York. pp. 243–287.

Xu Z, Xu Z andWu Q (1988). Effects of temperature on the physiology of *Hyriopsis cumingii*. Fisheries Science,7**,**9–11.

Zhang G and Xu J (2013). From colloidal nanoparticles to a single crystal: New insights into the formation of nacre's aragonite tablets. Journal of Structural Biology, 182(1), 36-43.