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Md. Akbal Husen
Senior Scientist (S4), Fishery Research Station (FRS), Pokhara, Kaski, Nepal

Tek Bahadur Gruung
Former Executive Director, Nepal Agricultural Research Council (NARC), Nepal

Corresponding Author:
Md. Akbal Husen
Senior Scientist (S4), Fishery Research Station (FRS), Pokhara, Kaski, Nepal

An update on freshwater pearl cultivation research and development in Nepal

Md. Akbal Husen and Tek Bahadur Gruung

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Abstract
This paper elucidates some recent updates on the status of pearl farming research, development, and its future perspectives in Nepal. For pearl farming, a locally available *Lamellidens marginalis*, freshwater mussel was identified to use. We collected the mussels from outlet canals of fisheries research facility, brought to laboratory and skillfully inserted beads (nucleus) in the mantle cavity of the mussels and transported and hung back in a productive fish pond for the response. We kept observing the mussels periodically, where the mussels responded to grow white coated glister as the initial stages of pearl. After the continued experiments for 18 months in the Rupa Lake and ponds of FRS, Begnas Lake, Kaski, Nepal, the final stage half round and image pearl was harvested. Our preliminary experiments and their findings demonstrated that pearl farming is possible in Nepal. Since this is one of the newest technologies of aquaculture in Nepal, therefore in present paper we have given more emphasis on handling mussels for procedural bead preparation, implantation into mantle cavity, and general growth trends of the pearl.

Keywords: Mussels, pearl culture, *Lamellidens marginalis*, aquaculture

1. Introduction
A pearl is a beautiful, shiny and hard natural gemstone which growth inside the shell of a mussel, used in jewelry since time ancient [1-4]. The pearl is also known to have some medicinal values [5-8]. Most of the pearls available in international trade are cultured pearls [3] produced using freshwater mussels in ponds, rivers, or lakes. China and Japan are the main producers of freshwater and marine pearls, whereas China is the world's biggest producer of pearls, including both marine and freshwater pearls contributing about 98% of global pearl production [9-11].
Recently India and Bangladesh have also shown promising advancement in pearl culture research and production [3, 4, 12-18]. Pearl culture is an ancient activity that gradually transformed into one of the leading and more profitable aquaculture industries worldwide [19]. Freshwater pearl culture in South Asia is also growing rapidly as a source of employment and income [14]. In Nepal, different species of mussels are known to occur [1-2]. As much as it is known there has been no study reported the pearl cultivation practices from Nepal. Since the present updates on cultivation potential of pearls might have implications, therefore our aim is to elucidate some of the recent updates on pearl cultivation technologies.

2. Materials and Methods
2.1 Study sites
All experiments described in this case study was perforated at Fishery Research Station (FRS), Begnas, Pokhara and Rupa Lake (Figure 1) of Pokhara Valley, Kaski in Gandaki Province of Nepal [2]. Begnas Lake is the second biggest lake (328 ha) situated at 28°10’26.2″N and 84°05’50.4″E, 650 m above mean sea level. Fishery research station is situated at the western part of Begnas Lake at Damside. Lake Rupa (100 ha) is the third biggest lake in Pokhara valley having its watershed located between 28°08’N to 28°10’N and 84°06’E to 84°07’E, at 600 m above mean sea level [20] lying close Lake Begnas at 600 m above sea level.

Fig 1: Map showing the location of Pokhara (A), FRS, Begnas Fish Farm (B), native mussels *Lamellidens marginalis* (C) and outer and inner surfaces of the mussels (D).

2.2 Preliminary experiments

In the preliminary experiments, native mussels *(Lamellidens marginalis*), were collected from drainage canals of FRS Begnas [2]. We performed simple trials to examine whether there will be any response on insertion of foreign particles inside the mantle of the mussel. For this we selected mussels having about 80-110 gm body weight almost identical mussels from natural stocks that were available in water drainage. Then, the mantle cavity of mussels was opened and representing the bead, small pebbles were gently pushed in the mantle cavity. After closing both shells carefully as much as possible without injuries, mussels were transferred in small net-bags and hung in ponds for time-to-time examination for pearl formation. After a series of preliminary experiments of experiments were conducted such as captive management of (*Lamellidens marginalis*), study on designing spherical pearl development, observation of the responses of *L. marginalis* after implantation of beads, and pearl development stages in experiments performed in various locations during fiscal year 2020-2023. The overall details procedures are presented briefly below.

Fig 2: Some of the tools for weight (A) and length (B, C) measuring and handle the mussels for insertion of nucleus inside the mantle cavity for pearl formation (D).

2.3 Initiation of pearl projects

Nepal Agricultural Research Council (NARC) has initiated preliminaries studies on pearl cultivation at its Fisheries Research Station, Pokhara started form year 2017 [2]. A project entitled "Developing freshwater pearl culture using local bivalves" was initiated which was headed by Dr. Md. Akbal Husen under NARC at FRS, Begnas. Under this project two workers were sent for exposure training at Indian Council for Agricultural Research (ICAR), Central Institute of Fisheries Education (CIFE), Mumbai in the year 2019. This project is running till date and exploring the package practices of freshwater pearl farming in Nepal.

2.4 Collection, identification and rearing of bivalves (*Lamellidens marginalis*) in captivity

On collection, identification and rearing of Lamellidens marginalis more relevant information has been illustrated in [1] and [2]. The mussel *Lamellidens marginalis* (Fig. 2) for the present study was collected from water drainage fishponds in Begnas. This mussel was identified based on the keys given by several authors [21-23].

Preliminary experimentation to develop freshwater pearl were carried out from 2017 in Begnas fish farm farming technology in Nepal. Later *Lamellidens marginalis* mussels were reared and maintained in the ponds of FRS, Pokhara. Mussels are known to subsist on phytoplankton for energy and nutrients. For the phytoplankton growth, pond was manured with cowdung@10000kg/ha/yr., Urea @ 100 kg/ha/yr. and DAP @ 100 kg/ha/yr. Fertilizers were applied in the ponds on the monthly basis as for getting optimum level of plankton.

2.5 Procedure for bead preparation

The shells of dead mussels were soaked into lime and water solution for 20-25 days and stirred periodically. After the desired soaking period when the shells become whitish, the shells were taken out, washed with water and sun-dried (Figure 3B). The sun-dried shells were grinded (Figure 3C) into mortar and pastel to make powder (Figure 3D). The powder was sieved with muslin cloth. To make the dough of fine shell powder, the araldite adhesives (Figure 3E) were taken equal part, mixed equally and then shells powder was added as required amount and mixed again for dough preparation (Figure 3F). The small quantity of dough is taken out and put on molds to make the images (Figure 3G-H). The spherical bead is made by using finger and palm. The flow diagram of bead making procedure is presented in figure 3 (A-H). The measurements of beads were taken with digital Vernier clippers. The measurements of beads were taken with

digital Vernier clippers. The circular beads diameter was 10.32±0.55mm and weight was 0.13±0.03gm, and the image bead length was 25.43±2.20mm, width was 23.55±2.17mm and weight was 1.47±0.58 gm was used for mantle cavity implantation.

Fig 3: Procedural steps for preparation of bead or nucleus using the outer shell of mussels. Dead outer shells (A), cleaned shell (B), grinded shell (C), grounded powder (D), adhesive (E), dough prepared by outer shell (F), mold used for image bead preparation(G), and images for insertion in the mantle cavity (H and I)

2.6 Mantle cavity implantation

The tools of mussel's implantation and rearing materials such as spatula, knife, mortar and pistel and instrument box, net, mussel's shell opener, forceps, wooden pegs were procured from market. For the mantle cavity implantation**,** the healthy mussels were collected from the outlet canals of fishery Research Station, Begnas facilities manually and kept in 100-liter plastic bucket with water. The length, width, height and weight of *Lamellidens marginalis* was taken before implantation by use of digital Vernier caliper (Mituotyo, Japan) and electronic balance (Libror ED 3200D, SHIMADZU corporation Ltd.). For the mantle cavity implantation, mean weight of mussels (Mean ±SD, 65.9±14.0 gm) and length (Mean ±SD, 91.33±7.10 mm) was used. These mussels were of 3-5 years old group.

In the pre-operative condition**,** the collected mussels were kept in a bucket at the rate 1 mussel per liter with continuous supply of tap water for three days in laboratory condition to loosen the adductor of mussels for easy mantle cavity implantation. On the day of surgery, the mussels were arranged in a tray. The mussels two valves were carefully opened with help of mussel's opener for about 0.8 cm then wooden peg inserted (Figure 4A). During the implantation processes, special attention was given to make sure to avoid disturbances to mussels because small sounds was found to closing of valve which delayed the process of implantation. For implantation, the mantle was detached from mantle cavity with the help spatula and small opening was made. The round or designed (images of Ganesh, Buddha, etc.) beads were inserted into the mantle cavity region of mussel with the help of forceps and push deeper with scapula (Figure 4C). After bead insertion, the grafted mussels were placed in the 100-liter bucket and treated with turmeric powder. The mussels were kept for 7 days in the laboratory conditions in the bucket with continuous supply of tap water. Mantle cavity implantations were done in 300 mussels.

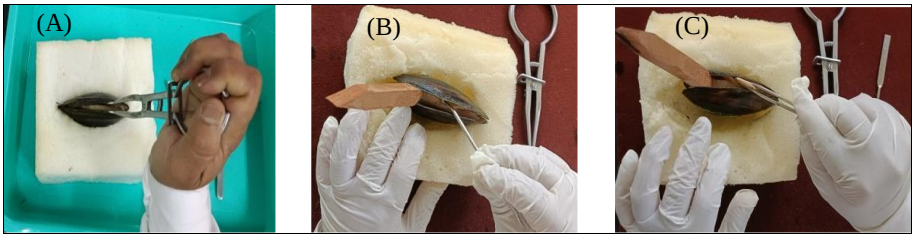

Fig 4: Nucleus or bead implantation process, mussels kept in tray before surgery and opening the bivalve cavity applying gentle pressure with a fork (A),B), shells opening and detaching with wooden piece (B), finally nucleus or bead insertion in mantle cavity (C)

2.7 Culture of mussels in ponds and lake

Three hundred implanted mussels were placed in the nylon bags @ 2 pieces /bag. The mussels were hanged in the three batch(each batch containing 50 mussels) with the help of rope in the FRS, Begnas ponds and also three batch(each batch containing 50 mussels) hang in the cage frame of Rupa lake. The mussels were cultured for 18 months. The experiments were repeated twice for accuracy. On the basis of weekly monitoring of water quality, the mussels cultured ponds were frequently fertilized with organic and inorganic fertilizer to maintain phytoplankton population. In the Rupa Lake, the mussels were cultured on natural planktons of lake. Periodical checking of mussels in 15 days with removal of dead ones and cleaning of bags was carried out throughout the culture period of 18 months.

2.8 Observations of pearl development

In the preliminary experiments the mussels were harvested and checked for the pearl development stage in every three months and the observations were noted. On the basis of observations of earlier experiments, in the next experiments, the mussels were harvested after twelve and eighteen months

from FRS ponds and Rupa Lake. These mussels were brought in laboratory of FRS, Begnas. The mussels were sacrificed and all inner part of mussels was removed and thoroughly cleaned with tap water. Each mussel was checked for the pearl development. The pearls were screened as nacre coating and luster. The survived mussels containing partial nacre coating and full nacre coating were segregated and counted. Pearl grading was done as descriptions given by [24].

2.9 Statistical analysis
The data of mussel's weight and length, bead length and weight, thickens of nacre, survival of mussels, grading of pearl were entered in MS excel and were analyzed statistically and expressed as mean (±SD) and standard deviation and graphs using IBM SPSS statistics version 25.0 software.

3. Results and Discussion
3.1 Mussel species diversity of Nepal
Nepal is rich in water resources and freshwater mussel which produces pearl are available in easily accessible natural water bodies like ponds, ditches, canals and river, etc. in Nepal [23, 25-31]. There are 40 species of class Bivalvia belongs to two order and four families are recorded from different water bodies of Nepal [1]. Among these two families Unionidae and Amblemidae mussels are important for freshwater pearl culture. More than 18 species of freshwater mussels have been used for freshwater Pearl farming around the world [11, 12, 14, 32-34]. The species which are important for the pearl culture found in Nepal are: *Lamillidens corrianus*, *Lamillidens jenkinsianus*, *Lamillidens marginalis*, *Parreysia favidens pinax*, *Parreysia corrugata laevirostris* Some of the above species are abundant in the drainage canal of the Fisheries Research Station, Pokhara, Kaski, Nepal.

3.2 Initial pearl development
In the preliminary experiments, acceptance of inserted bead or nucleus (stone, coarse sand) in mussels, *Lamillidens marganalis* within 3-6 months was the positive indication for the pearl development process. After 9 to 12 months culture periods, 50 to 90% white coated glister as the initial stages of pearl were noted. The indication of acceptance of foreign materials and some coating on the inserted bead seems to be a success towards the pearl culture innovation in Nepal. This acceptance and attachment of beads and development luster on the attached beads is the clear indication that this species could be used as the pearl culture in Nepal also.

3.3 Pearl harvest
After the continuous experiments, during the years 2022 and 2023, final stage designer and half round pearls were harvested from Rupa Lake and ponds of FRS, Begnas, Nepal which were harvested after 18 months of culture of implanted mussel's and it's were hanging in the nylon bags. In the present study, progressive nacre developments on implanted beads were observed in the *Lamillidens marginalis* and it has good luster (Figure 5). The methods of mental cavity implantation and rearing of implanted mussels in nylon bag is the most suitable combination for producing freshwater pearls in *L. marginalis* [35].The present study showed that in the 18 months culture periods, the survival percentage of mental cavity implanted mussels were 63-65% varied in lake and ponds(Figure 6). Sakpal & Singh (2000) [13] reported 70% survival of *L. marginalis and* Pandey & Singh, (2015) [35] was recorded 85% survival of implanted mussels with mantle cavity implantation method. The survivability of mussels (65%-71%) in *L. marginalis* in the entire experiments [36].

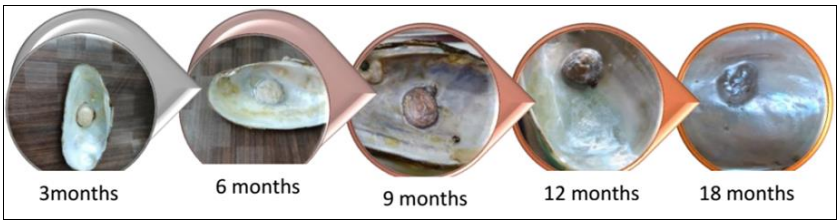

Fig 5: Temporal growth pattern of the pearl in mussel's cavity.

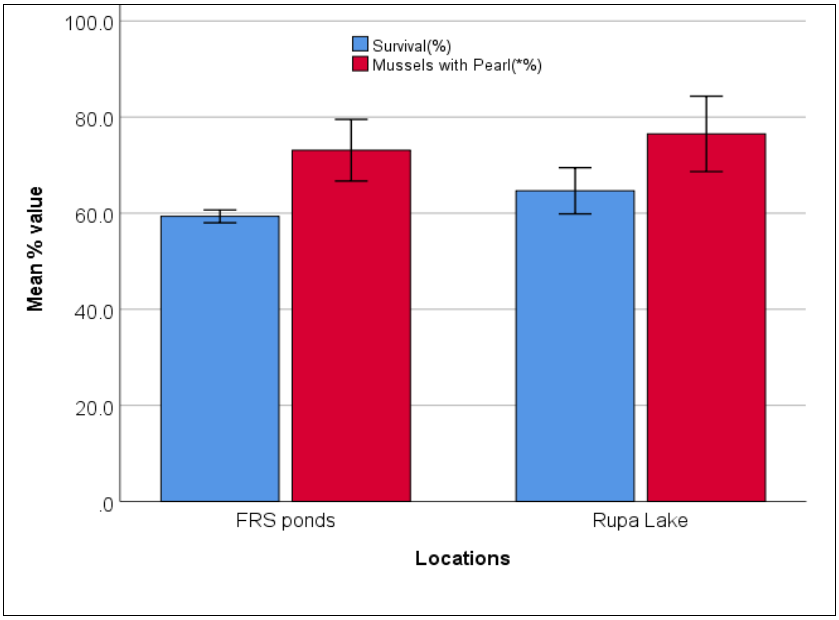

Fig 6: The survival (%) and pearl production (%) in the mussels 18 months reared in FRS ponds and Rupa Lake

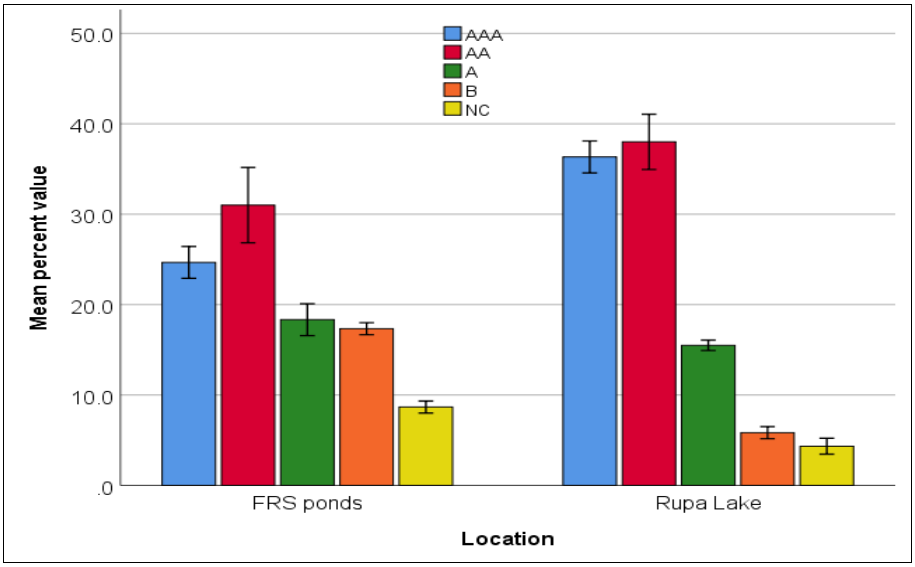

Fig 7: Showing pearl grade percentage of designer pearl and half round pearl from harvest mussels after 18 months. culture periods in FRS ponds and Rupa Lake (AAA- Excellent lusters and high quality; AA- Good lusters and good quality; A- good lusters but medium quality; B- Good luster with irregular surface; NC- shine is low; the nacre layer is weak culture periods in FRS ponds and Rupa Lake

In the present study, the thick nacre was observed as maximum 1.35 mm in the half round pearl and minimum 0.45 mm in deginer pearl (Table 1). Sakpal & Singh (2000) [14] reported 0.071 to 0.106 mm thick nacreous layer around pearl nuclei in mantle cavity implantation method. The maximum and minimum thickness of nacreous layer was 0.35 and 0.20 mm respectively in mantle cavity implantation method [35]. The highest quality pearls with a mean nacre thickness of 0.71 mm were produced after 11 months [36].Previous studies also reported that half round pearl could be obtained from the *Lamillidens marginalis* and image pearl could be obtained easily in 12-18 months [12, 14]. On the basis of present study, it can be concluded that the mussels used for pearl farming should be above 80 gm size and implanted mussels should be reared for 12-18 months in the ponds with high density of plankton for the good results. The thicknesses of the nacre making up the image pearl and pearl luster were both improved with longer culture periods [36].

Table 1: Pearl colour, shape and nacre layer in mussels reared in FRS Begnas ponds and Rupa Lake

Locations	Pearl colour	Shape / size	Nacre layer (mm)
FRS Begnas ponds	Silvery white, creamy, pinkish	Half round	0.85-1.11
	Silvery white, creamy, pinkish	designer pearl	0.45-0.75
Rupa Lake	Silvery white, creamy, pinkish	Half round	0.85-1.35
	Silvery white, creamy, pinkish	designer pearl	0.45-0.87

The survival and pearl having mussel's percentage was found higher in the Rupa Lake in comparison to FRS, Begnas pond (Figure 6). The high-grade pearl was harvested from Rupa Lake than the FRS, Begnas pond (Figure 7). These differences are due to water quality and culture environment. The Rupa Lake is deeper (4 m) and rich in plankton while FRS, Begnas pond is very shallow (0.8 m) and lower phytoplankton availability mussel for growth of pearl in cultured. 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 [18].

3.4 Adoptions possibilities of fresh water pearl culture technology in Nepal

Nepal is rich in water resources and freshwater mussel which produces pearl are available in easily accessible natural water bodies like ponds, ditches, canals and river, etc. in Nepal. The pearl culture is favorable in Nepal due to expansion of area of pond fish farming in various regions of the country in which pearl culture could be integrated and thus, it is easy to extend in rural area of Nepal. In addition, the favorable warm weather with nine months culture period in the Terai region could make it feasible in Nepal.

Pearl culture in Nepal could provide a source of income and increased the livelihood of farmers. The designers pearl (Lord Budha, Bishnu etc), are related to belief of people of Nepal and India for betterment in their life and the tourist of Nepal, India as well as other country will easily pay for designer's pearl, which will increase the farmers income and develop entrepreneurship in the country providing employment opportunities. Pearl has also medicinal value and its uses are increasing. In addition, strong demand for pearls across Asia-Pacific and it is an emerging market. provide. These freshwater mussels are edible and consumed as a source of protein delicacy since tradition among many ethnic communities in many parts of Nepal. Mainly in the Tharu community, it is consumed largely. This could be an alternative food to be used to alleviate malnutrition in the rural area of Nepal. As the freshwater pearl farming in local mussels will be expanded, the importance of mussels will be realized by people and its culture as well as conservation awareness will be initiated. Likewise, the people will know its nutritional value. The interest of many farmers towards the pearl farming are increasing day by day. Some of the Rural municipalities have already organized three days intensive training of freshwater pearl farming for their farmers.

4. Conclusion

These initial findings demonstrated that pearl farming could

be an attractive approach research and development in Nepal. With the advent of pearl farming technology, it is likely that the wider areas used for warm water fish farming could also be integrated with pearl culture for additional income, livelihood and human nutrition as well in Nepal. These freshwater mussels are edible and consumed as a source of protein delicacy since tradition among many ethnic communities in many parts of Nepal. The pearl is also used for medicine. The farmers are interested to adapt this technology in Nepal. For the wider adaptation of this technology in Nepal, well-planned strategy at national, regional, institutional, farm levels and entrepreneurial involvement needed to implement. More species of mussels should be tested and its further research should be carried for implantation methods, propagation and nursing's of mussels to develop sustainable the freshwater pearl culture technology in Nepal.

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6. Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

7. References

1. Husen MA, Gurung TB, Nepal AP. Freshwater pearl culture: an initiative in Nepal. In: Proceedings of the 2nd NEFIS International Convention. Kathmandu, Nepal: Nepal Fisheries Society (NEFIS); 2018. p. 137-144. Available from: www.nefis.org.np
2. Husen MA, Prasad S, Gurung TB. Prospect of pearl culture in Nepal. In: Proceedings of the 10th National Workshops on Livestock and Fisheries Research. Kathmandu, Nepal: Nepal Agricultural Research Council; 2017. p. 58-63.
3. Saurabh S, Pradhan S, Suman S. Recent trends in freshwater pearl farming in India. In: Ray S, Mukherjee S, editors. Update on malacology. 2022. DOI: 10.5772/intechopen.99281.
4. Siddique MF, Haque MA, Barman AC, Tanu MB, Shahjahan M, Uddin MJ, *et al*. Freshwater pearl culture in Bangladesh: current status and prospects. Heliyon. 2024;10(3). DOI: 10.1016/j.heliyon.2024.e29023.
5. Suryawanshi AV, Kulkarni AN. Comparative study of length-weight relationship of *Parreysia corrugata* (Muller) and *Lamellidens marginalis* (Lamark) from Nanded region, Maharashtra (India). Indian J Life Sci. 2014;3(2):77-80.
6. Chiu HF, Hsiao SC, Lu YY, Han YC, Shen YC, Venkata KK, *et al*. Efficacy of protein-rich pearl powder on antioxidant status in a randomized placebo-controlled trial. J Food Drug Anal. 2018;26(1):309-317. DOI: 10.1016/j.jfda.2017.05.010.
7. Chen X, Peng LH, Chee SS, Shan YH, Liang WQ, Gao JQ, *et al*. Nanoscaled pearl powder accelerates wound repair and regeneration *in vitro* and *in vivo*. Drug Dev Ind Pharm. 2019;45(6):1009-1016. DOI: 10.1080/03639045.2019.1593436.
8. Loh XJ, Young DJ, Guo H, Tang L, Wu Y, Zhang G, *et al*. Pearl powder - An emerging material for biomedical applications: A review. Materials. 2021;14(11):2797. DOI: 10.3390/ma14112797.
9. FAO. Global aquaculture production online dataset. Food and Agriculture Organization of the United Nations, Rome; 2016. Available from: http://www.fao.org/fishery/statistics/global-aquaculture-production/en
10. Zhu C, Southgate PC, Li T. Production of pearls. In: Goods and services of marine bivalves. 2019. p. 73-93. DOI: 10.1007/978-3-319-96776-9.
11. Ali S, Rawat RS. A review on global status of freshwater mussel: pearl culture. Int. J Creative Res Thoughts. 2023;11(8):2320-2882.
12. Miah MI, Rahman ASMK, Rahmatullah SM, Saha JK, Islam MA. Culture of pearl in freshwater mussels (*Lamellidens marginalis* Lamarck). Bangladesh Fish Res. 2000;4(1):57-61. Available from: http://hdl.handle.net/1834/32272
13. Sakpal RR, Singh H. Effect of different methods on implantation of nucleus in freshwater mussel *Lamellidens marginalis*. In: Singh UP, Chauhan RS, Sharma AP, editors. Proceedings of the National Symposium on Fish Health Management and Sustainable Aquaculture. Pantnagar, India; 2000.
14. Misra G, Jena J, Kumar K. Freshwater pearl crop: an emerging enterprise in the Indian subcontinent. Aquaculture Asia, 2009, 14(4).
15. Dwivedi BK, Tripathi A, Dwivedi H, Solanki RN, Singh A, Saiful M. Standardization of freshwater pearl culture technology in Uttar Pradesh. Bioved. 2017;28(1):163-180. Available from: http://biovedjournal.org/bv28(1)/27.pdf
16. Rathor VS. Induced designer pearl production in freshwater mussel Lamellidens corrianus. J Sci Technol Res. 2017;1(4):16-19.
17. Tanu MB, Barman AC, Siddique MF, Sku S, Mahmud Y. Production and quality of mantle tissue transplanted pearls in *Lamellidens marginalis* cultured in different locations of Mymensingh district, Bangladesh: comparison of production and quality of cultured pearls. J Bangladesh Agric Univ. 2019a;17(3):424-429.
18. Tanu MB, Barman AC, Siddique MF, Sku S, Hossen MN, Mahmud Y, *et al*. Assessing the number of mantle tissue in non-nuclei pearl production in freshwater mussels *Lamellidens marginalis*. Bangladesh J Shellfish Res. 2019b;18:55-64.
19. Taylor J, Strack E. Pearl production. In: Southgate PC, Lucas JS, editors. The pearl oyster: Biology and culture. Amsterdam: Elsevier Science; 2011. p. 273-302.
20. Gurung TB. Restoration of small lakes through cooperative management: A suitable strategy for poverty-laden areas in developing countries? Lakes Reservoirs Res Manag. 2007;12(4):237-246.
21. Subba Rao NV. Handbook of freshwater molluscs of India. Kolkata: Zoological Survey of India; 1989.
22. Ramakrishna Dey A. Handbook on Indian freshwater molluscs. Kolkata: Zoological Survey of India; 2007. p.

288-289.
23. Nesemann H, Sharma S, Sharma G, Khanal SN, Pradhan B, Shah D, *et al*. Aquatic invertebrates of the Ganga river system (Mollusca, Annelida, Crustacea). 2007;1:11-56.
24. Ruiz-Rubio H, Acosta-Salmón H, Olivera A, Southgate PC, Rangel-Dávalos C. The influence of culture method and culture period on quality of half-pearls ('mabé') from the winged pearl oyster Pteria sterna, Gould, 1851. Aquaculture. 2006;254(1-4):269-274. Available from: http://www.elsevier.com/locate/aqua-online
25. Takayasu K, Gurung DD, Matsuoka K. Some new species of freshwater bivalves from the Mio-Pliocene Churia Group, west-central Nepal. In: Transactions and proceedings of the Paleontological Society of Japan. 1995;179:157-168.
26. Subba BR, Ghosh TK. Land molluscs from eastern and central Nepal. J Bombay Nat Hist Soc. 2001;97(4):58-61.
27. Subba BR. Molluscan checklist of Ghodaghodi Tal area, Kailali district. Our Nature. 2003;1(1):1-2.
28. Subba BR, Pandey MR. Molluscan diversity of Jhapa district, eastern Nepal. J Nat Hist Mus. 2005;22:22-27.
29. Yadav CNR, Subba BR. Freshwater molluscs from Mudbalwa Village Development Committee, Rautahat, Nepal. Our Nature. 2008;6(1):82-83.
30. Subba BR, Ghosh TK. Report on some terrestrial molluscs from different regions of Nepal. J Nat Hist Mus. 2008;23:78-81.
31. Wagle SK, Jha A, Gautam A. Diversity of edible aquatic mollusks and their nutritional contribution in selected Terai districts of Nepal. In: Sustainable fisheries & aquaculture diversification: Proceedings of the 2nd NEFIS International Convention. Kathmandu, Nepal: Nepal Fisheries Society (NEFIS); 2021. p. 31-50.
32. Akamatsu S, Zansheng LT, Moses TM, Scarratt K. The current status of Chinese freshwater cultured pearls. Gems Gemology. 2001;37(2):96-113.
33. Janakiram K. Freshwater pearl culture technology development in India. J Appl. Aquaculture. 2003;13(3-4):341-349.
34. AMNH. American Museum of Natural History (AMNH), Central Park West at 79th Street, New York, NY 10024-5192. 2017. Available from: http://www.amnh.org/exhibitions/pearls/freshwater-pearls (Accessed on 23 February 2017).
35. Pandey A, Singh A. Effect of different pearl nuclei implantation and rearing methods on survival, growth and pearl formation in freshwater mussel, *Lamellidens marginalis* in Punjab. Ecol Environ Conserv. 2015;21.
36. Tanu MB, Barman AC, Siddique MF, Sku S, Hossen MN, Southgate PC, *et al*. Impact of culture period on quality of image pearls produced by the freshwater mussel, *Lamellidens marginalis*, in Bangladesh. J Shellfish Res. 2022;41(1):75-83. DOI: 10.2983/035.041.0104.