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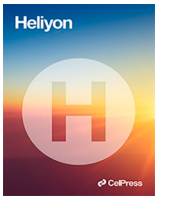

Review article

Freshwater pearl culture in Bangladesh: Current status and prospects

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ABSTRACT

Freshwater pearl farming is an emerging sector of aquaculture in Bangladesh which plays a growing role at major jewelry markets. With some improved techniques, high quality image or designer pearls are now produced from freshwater mussels *Lamellidens marginalis*. Yet it is difficult to reach in conclusion as the quantities produced, culture techniques used, and the upgrading of the existing culture technique are not well documented. Furthermore, many obstacles such as proper dissemination of culture technologies among the interested peoples, optimization of the culture environment and culture methods, standardization of breeding protocol and so on need to be addressed by the scientific community. This review article reports for the first time about the status of freshwater pearl culture in Bangladesh highlighting the fundamentals of pearl production, culture techniques used in farms, challenges, and prospects for upgradation of current culture principles in Bangladesh.

1. Introduction

The pearl is the only gemstone produced by a biological entity and may be reflected as an organic gemstone. Globally, marine and freshwater pearl culture has proven to be the most emerging aquaculture system with variant color, low production cost, shorter production period and high market value [1,2]. Following the commercialization of freshwater pearl cultivation in the late 1960s and early 1970s, advancements in technology resulted in enhanced production capabilities, yielding larger quantities of pearls with heightened lustre. Nowadays, this ancient activity can be compared to the land-based agriculture system, which could be beneficial for income generation of large arrays of people. Most importantly, rural artisan communities can easily adopt pearl culture system to sustain their livelihood [3,4].

Numerous Asian countries such as China, Japan, India, Vietnam, Philippines, Thailand, South Korea, Malaysia, and Myanmar have carried out the culture of freshwater pearl on a large scale and accomplished research to meet the global demand [3–5]. Currently China is the leading freshwater pearl producing country contributing over 95% of the world's freshwater pearl production [6] and achieved tremendous progress in culturing freshwater pearls in triangular mussels *Hyriopsis cumingii* [7]. However, global pearl production has declined and the current production accounts for 703 tons which is worth about 6 million US dollar [8]. Therefore,

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pearl culture is considered as a multi-billion-dollar sector of the aquaculture industry. Realizing the scope and importance of pearl culture, Bangladesh government had initiated the pearl culture project through the Bangladesh Fisheries Research Institute during the period 2014 to 2019 and introduced a base technology of growing pearls in freshwater mussels.

Pearl culture in Bangladesh is generally evolved with four major species *viz.*, *Lamellidens marginalis, L. corrianus*, *L. jenkensianus* and *L. phenchooganjensis*, whereas *L. marginalis* is reported as the most suitable species for producing high quality pearls [9]. Generally, two culture methods of freshwater mussels are used in Bangladesh *viz.*, grazing method, and hanging net bag method. Nuclei pearl production technique was first experimented in China and it is the most common process of nuclei pearl production. Furthermore, image or designer pearl production technique also gives better results. In Bangladesh, freshwater pearl culture is highly concentrated on image or designer pearl production. Despite the growing popularity of pearl culture, detail information on culture techniques, culturing areas, production figures and the characteristics of the pearls themselves are not well documented.

In this review, we summarize for the first time, the current context of freshwater pearl culture in Bangladesh in terms of selection of suitable mussels, various culture techniques, updates of culture information and prospects of this activity. We also analyze the fundamental issues regarding the freshwater pearl culture practiced in other countries of the world.

2. A brief history of freshwater pearl culture

Throughout history, there has been a persistent interest in artificially cultivating pearls through human intervention. In the 18th and 19th centuries, research on the formation of pearls predominantly took place in Europe. Nevertheless, the subsequent experiments on pearl culturing conducted globally during the early 20th century. The People's Republic of China possesses the earliest recorded documentation of historical endeavours in the field of pearl cultivation. In 1168, Wenchangzalu authored a publication detailing a technique for creating hemispherical cultured pearls, also known as cultured blisters. This method involved utilizing the cockscomb pearl mussel species, scientifically known as *Cristaria plicata*. Subsequently, in 1127, Hou-Tchen Fou examined the advancements made by Yang and achieved success in cultivating original shell-attached Buddha pearls. This was accomplished by inserting images of Buddha, crafted from thin lead plates [10].

During the period from the 19th century to the early 20th century, numerous efforts were undertaken across various regions worldwide, driven either by scientific interest or the pursuit of industrialization, with the objective of cultivating pearls. In the year 1825, J.E. Grey made a declaration regarding the possibility of producing pearls through artificial means, namely by introducing a fragment of nacre into the interstitial region between the shell and mantle. Following the technique proposed by Grey, J. Waltl conducted an experiment in 1838 to produce pearls using *M. margaritifera*. In 1896–1898 in Iowa, USA, Vane Simmonds conducted an experiment by inserting a small bead into the gap between mantle and shell and discovered the appropriateness of anesthetic chemicals to operate mussels [11]. In 1908–1909, Chmielewski tried to produce hemispherical pearls with shell using freshwater mussels [12]. During that period, in 1907, a Japanese scientist Tokichi Nishikawa unravelled the mystery behind the pearl production and proposed the 'Pearl Sac theory' [13]. Modern round pearl cultivation owes its founding and status to development of the Mise-Nishikawa-method in Japan in the early 1900s [2]. In addition, there was a vigorous promotion of techniques aimed at elevating the quality of pearls to a gem level. This was accompanied by advancements in the procedure of nucleus insertion, enhancements in surgical tools, and the development of culture management techniques. As a result, the fundamental framework of pearl culturing techniques was nearly perfected by approximately 1930 [14].

Subsequently, the market for pearls has experienced significant growth, expanding multiple times over and currently representing a multi-billion-dollar segment within the aquaculture business. Commercial pearl production is presently being conducted in various countries globally, such as China, Japan, Australia, Indonesia, French Polynesia, Cook Islands, Philippines, India, Sri Lanka, Bangladesh, Myanmar, Thailand, Malaysia, and Mexico [3,15]. China and Japan are recognised as the primary sources of freshwater and marine pearls, respectively. China holds the distinction of being the largest global producer of pearls, encompassing both marine and freshwater varieties. In fact, China's pearl production reached a remarkable 3540 tonnes, constituting over 98% of the total global pearl production [5].

3. Fundamentals of shell formation and nacre synthesis in molluscs

The only gemstone created by a living organism is the pearl. Molluscs can produce pearls as an immunological response to a foreign particle [16]. Anatomically, shells are superimposition of two to five calcified layers and one organic layer (framework of polysaccharides and proteins e.g., conchiolin). This process of shell formation is referred to as 'biomineralization' which is a common phenomenon for both marine and freshwater molluscs [17–19]. Biomineralization is a genetically and environmentally controlled process [20,21] and this basis of genesis is still unknown in freshwater molluscs [22–25]. Organic layer formed through biomineralization is generally thin and called periostracum which remains non-eroded throughout the life of an animal. Right below the periostracum, a mineralized layer is formed which is composed of elongated crystals defined as the prismatic layer. These prisms are made of calcite, one of the six polymorphs of calcium carbonate. Followed by the prismatic layer, 50% of the shell thickness is defined by the nacreous layer which is aragonite, also called mother-of-pearl. It is the internal lustrous layer and is always aragonitic. Cultivation of pearl is generally practiced in two different groups of mother-of-pearl shell, *viz.*, marine pearl oysters of the family Pteriidae and freshwater pearl mussels of the families Unionidae and Margaritiferidae [5]. In Japanese pearl oyster, the ventral part of the mantle (mantle edge) forms the prismatic layers, whereas the dorsal part of the mantle (pallium) forms the nacreous layers. The formation of these two layers is regulated by more than 70 novel candidate genes [26]. The nacreous layer plays a key role in the synthesis, storage and mobilization of all constituents needed to form the pearl [18].

In general, the beauty and luster of pearls are determined by the surface nacreous layer. The nacre exhibits exceptional mechanical properties due to its distinctive and well-structured design across various length scales [Fig. 1(a and b)]. These properties encompass a remarkable blend of stiffness, strength, impact resistance, and toughness [27–31]. Nacre secreted from the nacreous layer consists of polygonal to rounded tablets arranged in broad, regularly formed, parallel sheets [32]. These tablets are made of aragonite with a thickness varies between 0.5 and 1 μm and a lateral extension of few microns. Depending on the arrangement of the tablets, nacre are of three types; brick wall nacre or sheet nacre in which crystals are positioned in staggered in rows, just like bricks in a wall; row-stack nacre in which tablets are showed vertical stacking in vertical sections perpendicular of length axes and stair step stacking in vertical sections parallel to their length axes [32]; and columnar nacre in which flat tablets are forming the piles of crystals [33]. This accurate and orderly assembled crystal and organic matrix adds luster in pearls and provide resistance to the nacreous layer against fracture [34, 35]. Crystal size of the nacre in freshwater mussels is known to vary in thickness with the space, shell size and the period of rearing [36].

Shell matrix proteins control the crystal phase, shape, size, nucleation, and aggregation of $CaCO_3$ crystals [37]. The process of pearl formation involves two consecutive stages: (i) irregular calcium carbonate ($CaCO_3$) deposition on the nucleus and (ii) $CaCO_3$ deposition that becomes more and more regular until the mature nacreous layer has formed on the nucleus. Complete pearl sac is formed within the 5th day of implantation. An irregular phase of $CaCO_3$ continues up to 20 days of implantation. The deposition pattern started to change on the 25th day when large crystals with polycrystalline shaped aragonite are formed on the nacre-prism transition layer. On the 30th day, nucleus divides into irregular shapes and on 35th day, a nacreous layer formed around the nucleus which indicated smooth surface mature pearl luster [37].

Sometimes, abnormal biomineralization results in the production of vaterite which is an unstable phase of $CaCO_3$ [38]. Vaterite was recently discovered in freshwater bivalves and in pearl producing Asian mussels [38,39]. Vaterite is known to influence the final quality of pearls [40]. Most freshwater pearls are usually lustrous. Pearls with luster contain aragonite crystals as their inorganic component and are referred to as aragonite pearls, however certain pearls with vaterite crystals as their inorganic component are referred to as vaterite pearls and these pearls have no luster [40–42]. The lusterless pearls are relatively dirty and yellowish brown [38]. Therefore, the higher the content of vaterite in pearls the lower its quality is. Selection of the donor and host bivalves with colorful and lustrous nacre layer are also important to produce quality pearls as because final pearl color and overtone are resembling that of the nacre layer [24,25,]. Genetic parameters of donor and host determine the heritability for nacre weight and thickness, darkness and color of pigmentation, surface defects and overall grade [43]. As a result, selecting donors and hosts with excellent luster, golden color and higher growth traits would enable the cultivation of larger and more brilliant golden pearls [44]. Furthermore, age of the donor and host are also essential to speed up the biomineralization and nacre deposition, and the subsequent production of larger and higher quality cultured pearls. Immunological activities of hemocytes or plasma of freshwater mussels are also reported to regulate the quality of cultured pearls [45].

4. Pearl culture in freshwater mussels

4.1. Types of pearls

In nature, nacre is secreted by mussel epithelial cells as a body's reaction for strange agents such as bacteria [46]. While in pearl

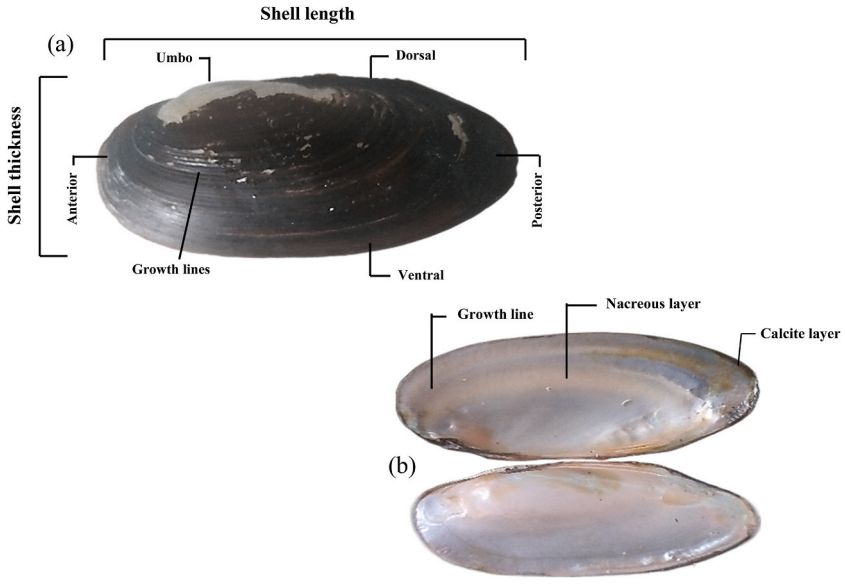

Fig. 1. Shell morphology of *L. marginalis*, (a) external, (b) internal.

mussel culture, grafts from recipient pallia are transplanted with or without nuclei into the mantle cavity or gonad of mother mussel, whereas the proliferation of epithelial cells forming pearl sac and various proteins are secreted within this sac to form the nacreous layers [47–50]. As a result, two types of cultured pearls are generally produced: non-nucleated (originated from only mantle tissue) and nucleated pearls (generated from nuclei and mantle tissue) [51].

4.1.1. Non-nucleated pearl

In non-nucleated pearl culture technique, a large piece of mantle tissue from a donor mussel of the same species (called saibo', or 'graft') or different species (called 'xenografts') is cut out, the marginal zone is removed, and several strips are cut with a specific dimension of 2 × 2 to 4 × 4 mm [52,53]. After that, the small strips are smoothed using specific tools to facilitate the grafting process [54,55]. Currently, young mussels are used to prepare the mantle grafts as because it is thin enough and enable its rolling into a rounder ball, which facilitate the nucleation with larger (4 × 4 mm) and thinner cuts of mantle grafts [56]. Pearls produced through non-nucleated pearl culture technique are generally called rice pearls and using this process, one freshwater mussel is reported to produce up to 50 pearls in one implantation period [57].

4.1.2. Nucleated pearl

In nucleated pearl culture technique, the non-nucleated technique is modified to produce greater value round pearls by re-operating the same recipient with nuclei [56,58]. Production of round pearls requires a round nucleus to be implanted with a piece of mantle tissue by grafting process [56,57]. Another type of nucleated pearls is image/design pearl whereas image nucleus is implanted into the mantle cavity of mussel [Fig. 2(a-c)].

4.2. Characteristics of nuclei

Nuclei (bead) used for grafting are generally manufactured from shells, plastics and paraffin [57]. The number and optimal nucleus diameter are known to influence the thickness of nacre. For instance, implantation of nuclei in the mussel *Anodonta woodiana* at 2 grains/individual with a diameter of 10 mm has been reported to produce 17 μm layers after 9 months of cultivation [40]. Image bead (size of nuclei up to 1 cm) is also used to produce designer pearl in freshwater mussels. The shapes of the nuclei in round pearl production are usually spherical or hemispherical, while operator's preference is the basis for the choice of image or designer nuclei selection. One recent development in the use of nuclei for pearl production is the use of organic nuclei to produce beaded baroque cultured pearls from oyster. These pearls are larger in sizes and characterized with high visually appealing luster [59]. Organic nuclei are also containing a bio-coating of fibronectins which facilitate the healing of mussels after the surgical operation.

4.3. Implantation method of nuclei

Nuclei in the freshwater mussels can be implanted in three main processes: mantle cavity, mantle tissue and gonad implantation [60] depending on the types of pearl desired to produce i.e., designer pearl, round pearl, or rice pearl etc.

4.3.1. Mantle cavity implantation

In mantle cavity implantation method, the nucleus is inserted within the cavity of outer mantle layer and the inner surface of the shell. In this method, mantle grafts are not necessary as the outer mantle layer secrets the necessary nacre to coat the nuclei. Mantle cavity implantation method is mostly used for image or designer and half round pearl (known as mabe) production. Mantle cavity implantation method also ensures higher survival and growth of mussels and can produce maximum thickness of nacreous layer [61].

4.3.2. Mantle tissue implantation

In mantle tissue implantation method, several incisions are made in the mantle to form small pockets. These pockets are used for the implantation of mantle grafts along with or without nuclei to produce nucleated small round pearls and non-nucleated irregular small round pearls or rice pearl.

Fig. 2. Different types of freshwater cultured pearl, (a) non-nucleated or blister pearl, (b) nucleated pearl, (c) image or design pearl.

4.3.3. Gonad implantation

Moreover, in gonad implantation method, small incision is made in the gonad of the host mussel and the nuclei together with the grafts are inserted to produce regular round pearls. Implantation of nuclei is generally carried out during summer season (May–June) as because high temperature favors the growth of bivalves. Once the surgery is successful and the wounds heal, graft tissue proliferates and produce pearl sac of which nacre layers are deposited over the nuclei bead [17]. Generally, each pearl sac is formed within 20–30 days of implantation. However, technical skills, species involved, and rearing environment of the inoculated bivalve may influence the pearl sac formation [52]. Using the mantle cavity and mantle tissue implantation approach, the success rate of pearl formation is 60–70%, compared to 25–30% with gonadal implantations [62].

4.4. Grafting process affecting pearl quality

Origin of graft tissue influences the quality of the produced pearls. For instance, pearl cultivators of China usually cut pieces of tissue grafts from the posterior mantle lobe of the mussel as because in that place the mother-of-pearl has the desired color and luster. They are also used to place the sacrificed tissue grafts into the pockets of the same posterior mantle lobes of the host mussel. Therefore, the produced pearls were improved in more color and luster [63]. However, in many cases, good quality pearls are not produced due to the death of the host mussel soon after the grafting process, rejection of graft and mantle, and the production of low-quality pearls with irregular shape and organic layer [59]. To avoid the aforementioned challenges, Chinese technicians introduced three stage seeding strategies depending on the pearl produced: (i) producing non-beaded keshi pearls after one year culture without sacrificing the host mussel (first generation), (ii) these first generation host mussels are re-seeded with a few coin-shaped beads to create larger, baroque pearls known as “fireballs”, (iii) re-seeding the first generation host mussels with one round, shell-made bead to produce larger and rounder pearls [53,58,65. Mussels used in the second and third strategies are considered as second-generation mussel and they possess a fully grown pearl sac which facilitates the nucleation of bead without the graft tissue [63]. These methods are beneficial because it reduces the rejection rates and make it easier for pearls to develop entirely [56].

4.5. In vitro pearl culture

In vitro pearl culture could be a promising option to overcome the limitations of existing in vivo pearl culture methods. Attempts have already been made to develop in vitro pearl production with volatile success [17,46,64–66]. Nacre crystals were formed when pearl producing bivalves’ mantle epithelial cell culture were performed [17,64,67]. Nacre crystals formed from epithelial cell culture are composed of Ca and S [64]. Although to date in vitro pearl production was not possible, this method is potentially used to establish the quality of pearl mussel species by screening their pearl formation efficiency. Nacre secretion from pallial mantle epithelial cells was evident in some selected media [46].

5. Grading of pearls

Prior to sale, the cultured pearls are graded according to six main value factors such as pearl nacre quality and thickness, size, shape, color, luster, and surface quality [24,68,69]. The thickness of nacre is a crucial factor in determining the value of pearls, as it serves as the fundamental basis for all other attributes. A pearl lacking a substantial and well-structured nacre coating will exhibit diminished aesthetic appeal compared to its counterparts and will also experience a reduced lifespan over time.

Assuming all other variables remain constant, larger pearls are considered superior. Like diamonds, the size of an object can have a significant impact on its price, resulting in exponential increases as each millimetre of size is added. This phenomenon can be attributed to the fact that the cultivation of larger pearls is considerably more challenging, requiring a significantly longer production period, resulting in their scarcity.

When considering the shape of a pearl, it is crucial to prioritise the presence of evenly symmetrical shapes. Such shapes are not only aesthetically pleasing but also highly versatile for various pearl jewellery designs. Nevertheless, pearls that possess a perfectly spherical shape are widely regarded as the epitome of excellence and are commonly referred to as the “gold standard.” Following perfectly spherical pearls, the subsequent shapes that garner significant popularity include smooth Drops, followed by Buttons and Baroques.

The phenomenon of pearl coloration is attributed to structural colours, arising from the intricate interplay of multiple reflections within the nanolayered structure of nacre, commonly referred to as mother of pearl. Color pigments inside the nacre layers and some trace element compounds are also considered as essential components of pearl coloration. The price of pearls is not significantly influenced by colour, as it primarily depends on personal preference. When selecting a colour, a general recommendation is to consider one’s skin tone. While the process of pearl grading encompasses various intricate elements, pink pearls are typically favored.

Among all the value factors, it can be argued that lustre holds the utmost significance. The concept of lustre pertains to the degree of brightness and shininess exhibited by a pearl. Luster can be characterized as the intrinsic radiance emanating from a pearl, resulting from the interaction of light entering the pearl and subsequently reflecting towards the observer via the layers of nacre. The lustre of a pearl is contingent upon the uniformity and smoothness of its nacreous layers.

The final and highly significant factor in assessing the value of a pearl is surface quality, which pertains to the cleanliness, smoothness, and absence of imperfections on its surface. Blemishes encompass a range of imperfections, such as abrasions, bumps, spots, and wrinkles. Nevertheless, significant concerns regarding surface quality typically manifest as chips and gaps, thereby diminishing the worth of even the most radiant pearls.

A multitude of diverse grading systems are employed worldwide for the evaluation of pearls. Regrettably, a universally accepted standard does not exist. However, some of the most common peal grading systems are, Japanese grading (Mikimoto grading) system, Tahitian grading system, Chinese grading system and Kyllonen grading system. In all the grading system, pearls produced from the industry are graded by the ruling pearling industry using some alphabets (e.g., AAA, AA, A, B; or A, B, C, D). The high-quality pearls having regular shape, excellent luster and almost no defects on their surface are graded as 'AAA' under one grading system and 'A' under different system. The high-quality pearls with regular shape, very good luster and few defects on their surface are graded as 'AA' or 'B'; good-quality pearls as for shape and luster and nearly 50% defects are categorized as 'A' or 'C'; and bad-quality pearls with average luster, considerable defects and no commercial value are graded as 'B' or 'D' under two different systems respectively [53]. However, literature regarding the grading system of image pearls yet not been reported.

6. Environmental impact on quality of pearls

Following implantation of graft tissue and nuclei, survived mussels are moved to the grow-out phase in pond or river. They are placed in plastic nets and suspends from vertical ropes attached to foam buoys, recycled plastic bottles, or bamboo branches. Depending on the types of pearls, two to three mussels are placed and cultured for 6–48 months in each plastic net [56,63,70]. Cultured pearls are generally deposited after approximately 18 months of implantation [71,72]. However, culture duration, internal structure and color of cultured pearl are affected by changes in the external environment and genetics of both donor and host mussel [4,73,74]. Water temperature is known to stimulate the nacre and pearl deposition rates. Temperature controls the metabolic rate of the mollusks and higher temperature leads to faster rate of nacre deposition [75]. Therefore, growing pearls in the summer produces heavier,

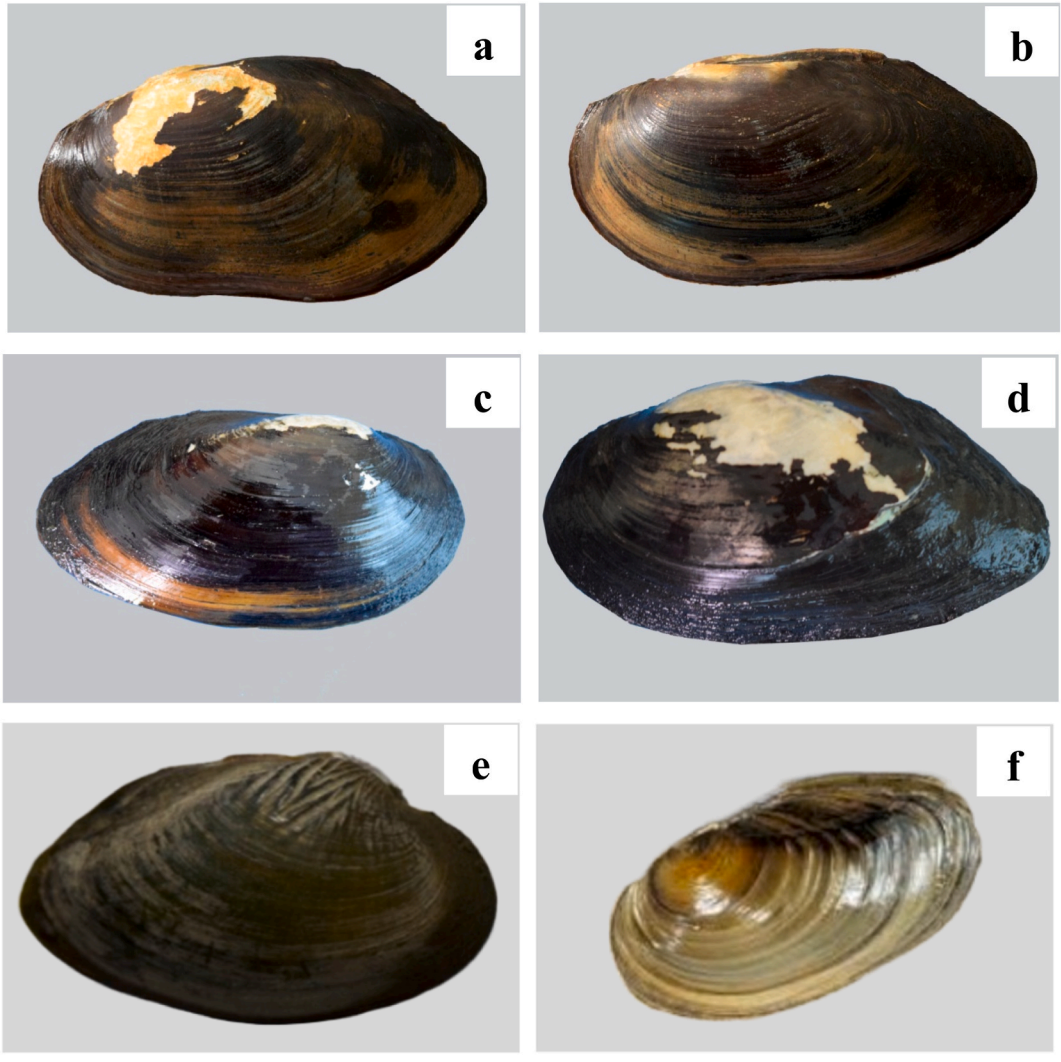

Fig. 3. Pearl producing mussel species in Bangladesh; (a) *L. marginalis*; (b) *L. corrianus*; (c) *L. jenkinsianus*; (d); *L. phenchooganjensis*; (e) *P. corrugata*; (f) *P. exilis*.

thicker pearls that are valued on the market. However, higher temperature may sometimes lead to lower quality pearls. As evident in oysters, rapid growth in higher temperature often results in beaten surface appearance in cultured pearls which is commonly known as "hammering effect" [2,76]. Furthermore, higher temperature might also cause higher mortality rate in the oysters [76]. Low water temperature, on the other hand, resulted in thinner nacre tablets, and improved pearl quality with better luster and fewer flaws [77, 78]. Consequently, the farmers chose to harvest the pearls during the cooler months of the year [79,80]. Furthermore, the thickness of the nacre tablets in the shell and pearl biomineralization is said to be influenced by the amount of food consumed [81]. As the iridescence and color of the shell depend on the last few layers of nacre and on the structure of nacre, it is necessary to provide sufficient food (microalgae) to facilitate the pearl mineralization during the last few months of the culture period [82]. pH influences the crucial physiological process of nacre deposition in mollusks [83]. Acidification of water could hinder biomineralization process of shells by making the less availability of $CaCO_3$ in water, the building blocks to make their shells. Shell fouling is reported to hasten by acidic or alkaline environments because of the increased rate of periostracum layer removal [84]. Water pH has also direct effects on survival and image pearl production rate in freshwater mussels, *L. marginalis* [74].

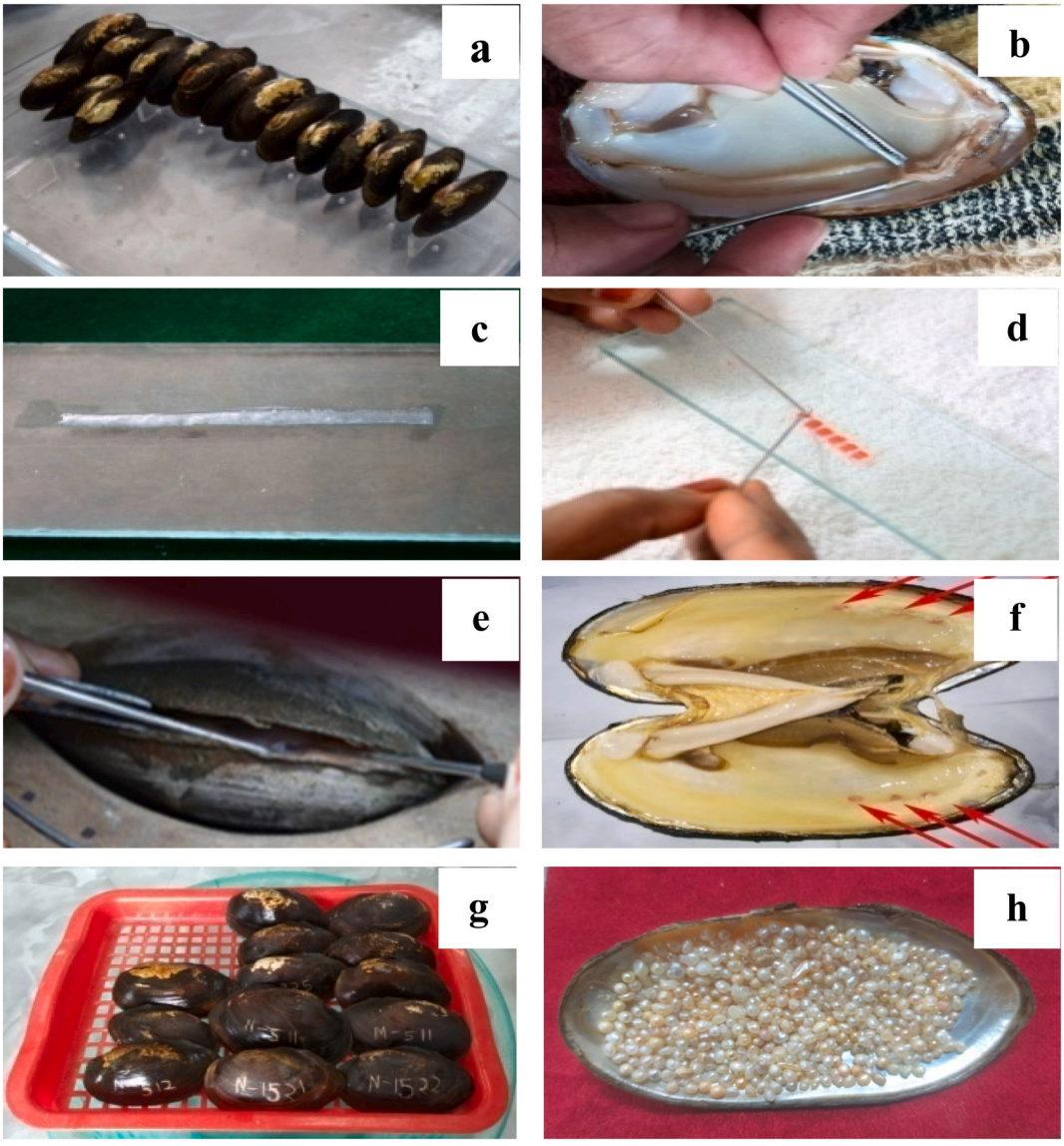

Fig. 4. The seeding technique adapted in Bangladesh to produce non-nucleated rice pearls from the freshwater mussel, *L. marginalis*: (a) prepared mussel for operation; (b) collection of mantle tissue from donor mussel; (c) separated mantle tissue strip; (d); preparation of mantle allograft; (e) transplantation of mantle allograft in host mussel; (f) incision of pockets for allograft transplantation; (g) tagging of transplanted mussel; (h) produced rice-pearl.

7. Present status of freshwater pearl culture in Bangladesh

In Bangladesh, research on pearl culture was first initiated at Bangladesh Fisheries Research Institute (BFRI) in 1999. However, the first comprehensive research on this technology was carry out under the project 'Development and dissemination of pearl culture technology' within the period of 2014–2019. The specific objectives of this project were development of pearl culture technology, propagation of pearly mussel, development of nuclei for round pearl production and provide training to the fishermen, rural women, entrepreneurs to disseminate pearl culture technology in Bangladesh. Firstly, the project had identified demographic features of the mussel stock in Bangladesh. In the survey, the project had identified seven potential pearl producing mussel species [85]. These species were *Lamellidens marginalis*, *Pilyroconcha exilis, L. corrianus, L. jenkinsianus, L. phenchooganjensis* and *Parreysia corrugata*. Among these species, *Parreysia corrugata* is vulnerable, *L. phenchooganjensis* is endangered in nature and *Pilyroconcha exilis* has the poorest distribution throughout Bangladesh. Among the other three species, *L. marginalis* is larger in size and has wider distribution throughout the country [Fig. 3(a-f)]. Another survey was conducted during 2016–2017 to determine the status of pearl producing mussels in the Meghna River of Bangladesh [86]. Available species of mussels reported in the river were *L. marginalis*, *L. corrianus*, *L. phenchooganjensis*, *L. jenkinsianus*, *Parreysia corrugata*, and *Meretrix meretrix*. From the identified species, *L. marginalis*, *L. jenkinsianus*, *L. corrianus* and *L. phenchooganjensis* were reported to have pearl forming potentials [87]. However, considering body size, survival rate and pearl production, *L. marginalis* has been identified as the most suitable species for pearl culture. Based on the above information, *L. marginalis* was selected as the target species for developing the next stages of the project (non-nucleated rice pearl, nuclei pearl and image pearl).

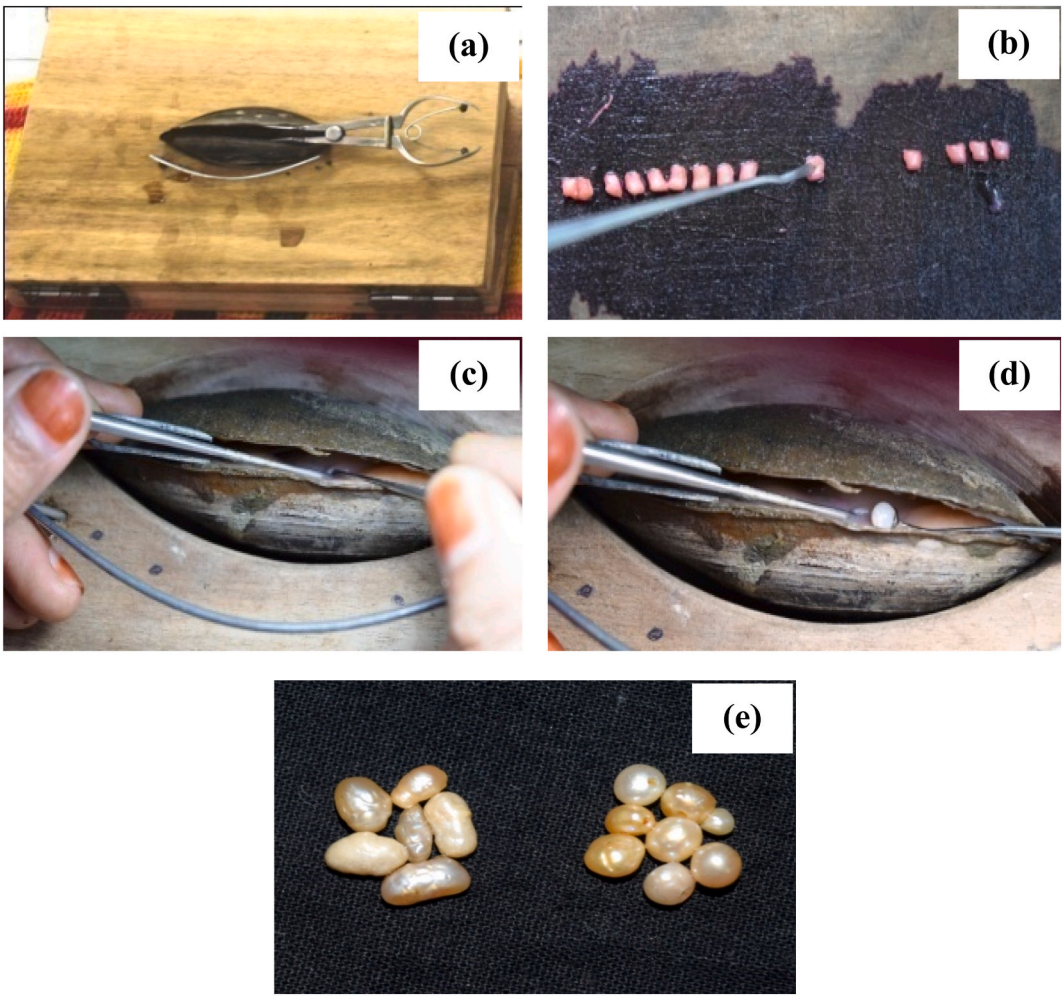

Fig. 5. The seeding technique adapted in Bangladesh to produce nuclei pearls from the freshwater mussel, *L. marginalis*: (a) prepared mussel for operation; (b) preparation of mantle allograft; (c) pocket making in mantle tissue (d) transplantation of mantle allograft in host mussel; (e) produced nuclei pearl.

7.1. Non-nucleated rice pearl production

At the first stage of non-nucleated rice pearl production, number of mantle allograft and culture methods was optimized. For this purpose, disease free, healthy and young mussels were collected from different aquatic habitats of Mymensingh region and stocked in a pond at Freshwater Station, BFRI, Mymensingh, Bangladesh. The average shell length, width and age of stocked mussels were 8.93 ± 0.30 cm, 4.91 ± 0.23 cm and 1–1.5 years, respectively. The culture ponds were fertilized with 5 kg organic manure, 125 g TSP and 100 g urea/decimal fortnightly to enhance the primary productivity. To maintain the optimum level of pH and calcium in pond, 0.5 kg lime/decimal was also applied to the ponds fortnightly. Collected mussels were cultured for 30 days before used in the operation. After collecting the mussels from the ponds for operation, they were kept in cistern for seven days in tube wells water for acclimatization and removing dirt from the intestine and internal body organs. In the laboratory, the acclimatized mussels were kept at ventral side downwards in perforated tray for 24 h to remove water. Selected donor mussels were sacrificed, and two layers of mantle tissue were separated along the pallial line. After that, separated mantle tissues were sliced to produce mantle allograft (2 × 2 mm) which was used for transplantation in host mussel. Host mussel was opened gently about 8–10 mm (depending on the size) and an incision pocket was made in the mantle tissue by curve-head needle. The small mantle allograft (2–10) was then inserted into the incision pocket and the operation was completed [Fig. 4(a-h)]. After the operation, host mussels were tagged and kept in nylon bags (diameter 20 cm, mesh size 1 cm) at the rate of 3 mussels/net bag and put up at 0.2 m depth in post-operative care units (ferro-cemented cistern of 5000 L capacity) at a stocking density of 150 mussels/cistern without food for 7 days. The mussels were fed with natural food for following 21 days in the cistern and observed daily to remove dead mussels. After one month of post-operative care, the mussels were transferred to the grow-out phase in ponds for three years until the harvest of rice pearls. In the grow-out phase, the mussels were cultured following the strategy used in China where two to three mussels were gathered in plastic net bags and hanged from vertical ropes (distance between two bags is 0.25–0.30 m and between two vertical ropes is 1.5 m) tied to plastic buoys and bamboo branches [56,63]. The net bags containing mussels were hanged at a constant depth of 0.30–0.35 m in the pond. In this method, survival rate of the mussels was influenced by the number of mantle allograft transplanted, whereas the highest survival rate was recorded for four allografts per mussel [88]. However, mussels containing six allografts performed better in terms of pearl production rate, thickness of the nacre

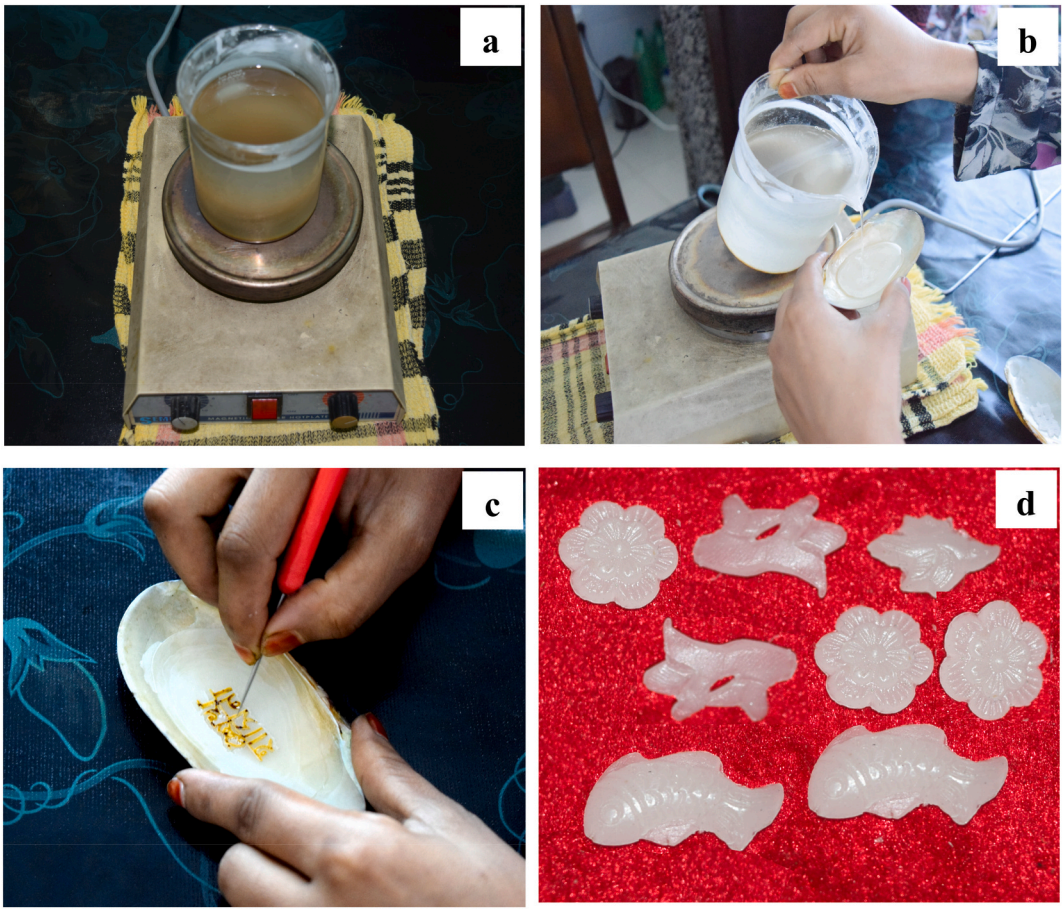

Fig. 6. Preparation of paraffin image; (a) melting of paraffin wax; (b) transfer of paraffin wax into the shell; (c) designing of paraffin layer; (d); prepared paraffin image.

layer, and luster and shape of the produced pearls [89]. A study was also conducted to compare the production rate and quality of the pearls between net bag hanging and grazing method, whereas more shiny pearls was produced in net bag handing method.

7.2. Nuclei pearl production

Rice pearls are usually smaller in size and low economic value in Bangladesh. Therefore, research was conducted to produce nuclei pearl. During the initial stage, potentiality of the nuclei materials to produce pearl and the optimal number of nuclei was unknown, so the study was conducted to select the appropriate nuclei materials and to optimize the suitable number of nuclei per host mussel. Two types of nuclei were used in the study such as, stelon nuclei produced from the shells of *Lamellidens marginalis* and *L. corrianus* and shell bead nuclei from shells of native cockle *Anadara granosa* with three different sizes (2, 2.5 and 3 mm). Following the similar procedure of selection and preparing of the donor and host mussels, implantation of one spherical nucleus in the incision pocket of the left valve was inserted [Fig. 5(a-e)]. Two mm sized shell bead nuclei from native marine mussel *A. granosa* recorded better quality pearl [90].

7.3. Image or design pearl production

Image or design pearls are produced in a picture or image format. The principle is, when an image (bird, fish, flower etc.) inserted into the mantle cavity, it covers by the nacre layer to produce image pearl [91]. Images can be produced from either paraffin or shell of donor mussel. To prepare paraffin image, liquid paraffin is poured on concave side of the dead shell which is previously socked with soybean oil. Thickness of the paraffin layer is maintained at approximately 1.5–3.5 mm [Fig. 6(a-d)]. After solidification of the paraffin layer, desired image or sculpture are designed with a needle. To produce shell image, desired designs are drawn on the concave side of the shell with a pencil. After that, a grinding machine was used to cut the shell according to the design [Fig. 7(a-d)].

To insert the paraffin or shell image, selected live mussels are opened about 8 mm, attached mantle tissue is detached to make pocket accordance to the size of prepared images. Following the insertion of images into the mantle cavity, trapped air is removed with gentle pressure. After the operation completed, the mussels are kept at an upward position on the tray to examine the rejection of the images by the host mussels [Fig. 8(a-h)]. Size and shape of the inserted image determines the quality of produced pearls. Several

Fig. 7. Preparation of shell image; (a) drawing on the dead mussel shell; (b) designing of the shell image; (c) polishing of shell image; (d); prepared shell image.

studies have conducted to optimize the size and shape of the images. Attempts has been made to optimize the size of paraffin image in net bag handing method [92]. Among five different sizes of paraffin image (3.5 × 1.5 cm^2, 3 × 1.5 cm^2, 2.5 × 1.5 cm^2, 2 × 1.5 cm^2, and 1.5 × 1.5 cm^2) it was concluded that paraffin image with a size of 2.5 × 1.5 cm^2 was suitable to produce shinny and lusters image pearl in *L. marginalis*. Study was also undertaken to optimize the size of shell image (3 × 1.5 cm^2, 2.5 × 1.5 cm^2, 2 × 1.5 cm^2, and 1.5 × 1.5 cm^2) by examining the quality of pearls in terms of the thickness of nacre layer and lusters of the pearl. The study revealed that shell image with a size of 2 × 1.5 cm^2 was most suitable for lusters shinny pearl production in *L. marginalis* [92].

During the project period, quality of the produced pearls with paraffin and shell images was compared. It was observed that, after eight months of culture period, pearl production rate in paraffin and shell image were 21 and 16.5%, respectively. Furthermore, image pearl produced with shell image had medium to high shiny luster compared to the pearls produced with paraffin image. Therefore, the market price of shell image pearl was higher compared to paraffin image pearl. The project findings also indicated that higher culture period (11 months) had positive impact on the quality of image pearls in terms of luster shininess compared to lower culture period (7 months) [93] (Tanu et al., 2022). However, apart from the above optimization, sometimes produced pearls are being less lustrous and gloomy which reduces its market value. To overcome this incidence, treatment of produced pearls with 10% C_2H_5OH was reported to enhance the luster and self-life of image pearls [94]. The main features of the different types of pearls produced in Bangladesh are

Fig. 8. The seeding technique adapted in Bangladesh to produce paraffin and shell image pearls from the freshwater mussels, *L. marginalis*: (a) preparation of mussels for operation; (b) opening of mussel with stopple (8–11 mm); (c) separation of mantle tissue with spatula; (d); soaking of paraffin image in water; (e) soaking of shell image in water; (f) insertion of image into the mantle cavity; (g) removing water and air from mantle cavity; (h) tagging of operated mussels.

shown in Table 1.

8. Challenges and prospects

Freshwater pearl culture has a great economic potential in Bangladesh. Despite its economic value, efforts are relatively diminutive for countrywide dissemination and adoption of this technology. After eight years of research, pearl culture in Bangladesh from L. mariginalis is still experimental. Although, six potential species have been identified as pearl producing, only one species is mostly cultured for pearl production. Therefore, a complete demographic study is essential to confirm its wider distribution in the country. Regardless of this, the potentiality of the other species for pearl production is needed to be thoroughly explored. Furthermore, despite the huge potentiality of marine oysters and mussels for pearl production, they are remained untouched. Collection of mussels in Bangladesh still depends on the natural sources and therefore, existing wild collection are continuously depleting the stocks. Another challenge for pearl culture in Bangladesh is the supply of the sufficient number of adequately sized mussels. Experience achieved from the pearl project identified the smaller size of host mussel as the main barrier to the nuclei pearl production. The project has reported mass mortality of the implanted mussels in that period. However, non-nucleated and designer or image pearls are also not up to the mark because of some quality issues. Therefore, efforts are essential to overcome the shortfalls concerning the high mortalities during round pearl production and the overall quality improvement of cultured pearls. In this consent, selective breeding can be considered vital for the production of quality pearls.

Pearl size is generally influenced by heredity, the environment, and how those things are interacted [96]. Therefore, improvement in size, shape, luster, and color in recent years are the results of the selection of potential mussel species, use of younger mussels for nucleation, reduction in the number of pieces inserted into the host mussel and improvement in tissue nucleation technique (rolling a thinner piece of mantle into a round shape), a substantially longer culture period and frequent changes in the culture environment [63]. Previous study has shown that selection of larger shell lengths given positive results in terms of pearl size and quality [97]. For instance, improvement in body length or weight of *Hyriopsis cumingii* in culture indirectly improved pearl size and weight [98]. Potentiality therefore exists to the use of shell phenotypic characters as indicator for selective breeding of mussels, as reported in case of *P. margaritifera* [99]. During the project period, some individuals of *H. cumingii* were introduced from China and their growth performances were compared with the native *L. marginalis* and *L. corrianus*. The findings of the experiment showed that despite the larger size of *H. cumingii*, it showed lower relative growth rate compared to other native species. Being an exotic species, perhaps *H. cumingii* was unable to adapt with the environmental conditions of Bangladesh, which might be responsible for lower growth rate.

Breeding protocols of *L. marginalis* are not discovered yet in Bangladesh. Recent studies on *L. marginalis* are focused on the annual gametogenic and ovarian cycle [100,101]. Similarly, it is required to find out the induced breeding protocol and hatchery-reared glochidia culture to improve the quality of the gametes, survival of larvae and recruitments of healthy juveniles for captive culture of mussels. Preliminary trials on breeding of *L. marginalis* under captivity had also been successful for the first time in Bangladesh, which is creating a hope to utilize one of the abundantly available mussel species for culture in the country.

The environmental conditions such as temperature and food are also reported to affect the shell growth, gene expression level of shell matrix protein in the mantle, the mineralizing tissue of the shell, mortality, and finally pearl quality of bivalves [15,102]. The temperature of the culture environment has a significant impact on the rate of nacre deposition, with higher temperatures accelerating

Table 1
Main features of the different types of pearls produced in Bangladesh.

Features	Types of pearls					References
	Non-nuclei rice pearl	Nucleated		Image or design pearl		
		Shell bead nuclei	Stelon nuclei	Paraffin image	Shell image	
Transplantation process	Mantle tissue	Mantle tissue	Mantle tissue	Mantle cavity	Mantle cavity	[65] Siddique et al. (2020b)
Nucleus (mm)/Image size (× 1.5 cm^2)	–	2–3	2–3	1.50–3.50	1.50–3.00	[65,90] Tanu et al. (2021), Siddique et al. (2020b)
Number of mantle tissue/nucleus/ image inserted	2–10	4–14	2–10	1	1	[85] Tanu et al. (2019a)
Survival rate (%)	62–77	27.67–50	25.33–39.67	13–49	10–18.60	[85,86,91,93,95] Miah et al. (2000), Hossain et al. (2004), Tanu et al. (2019a), Siddique et al. (2020b), Tanu et al. (2021)
Rate of pearl production (%)	10–34	2.67–4.67	0.67–3.33	18.50–20	10–18.60	[85,88,91,93] Tanu et al. (2019a), Siddique et al. (2020a; 2020b), Tanu et al. (2021)
Nacre thickness (mm)	2.12–5.19	0.10–0.30	0.10–0.30	0.23–0.71	0.21–0.41	[61,85,91,92] Tanu et al. (2019a; 2019b; 2021; 2022)
Pearl shape	Button, oval, round	–	–	–	–	
Pearl colour	Ash, orange, pinkish, silvery, white Shinny and moderate to good luster	–	–	Shiny luster	White, Ash, Pink, Orange	[85,91] Tanu et al. (2021), Tanu et al. (2019a)

nacre deposition and lower temperatures slowing nacre deposition rates. However, slower deposition of nacre at lower temperature is reported to increase nacre quality [103]. Stable phytoplankton production in adequate amounts is also needed to ensure commercial grade pearl production [95]. The present review work shows that optimizing the culture environment with suitable water quality, depth of the pond and food availability can uphold the commercial pearl culture in Bangladesh. Furthermore, intervention in the accessibility of knowledge, operation techniques and an organized supply chain and sufficient market demand are essential. Skilled manpower in operation technique is critical to avoid mortality after surgery through infection, disease, shell boring and biofouling [95].

Mussel farming in Bangladesh is not so popular when compared with fish and shrimp farming. The main reason is the limited number of pearl farmers, and the sector is still not well-organized in the country. Although some entrepreneurs have ventured into pearl farming, the number is rather insignificant, and how the market chain is structured and how much a farmer or collector at the beginning of the chain will make from harvested or produced pearls are uncertain. Therefore, insufficient knowledge on market opportunities is a hampering factor in the initiation of pearl culture activities, which needs to be investigated further. In this situation, strong extension network is needed to disseminate the existing culture technologies and advances throughout the country.

Concerning the challenges and prospects, the following recommendations are made in view of these review outcomes.

- Efforts should be made on induced breeding for mass seed production and selective breeding for the improvement of pearl quality.
- In depth study should be conducted on the effects of pH, temperature, and other environmental factors on growth, survival, and pearl formation rate in operated mussels. Such findings could ensure optimum environmental conditions required for better growth, survival, and pearl quality in the grafted mussels.
- Modernization of grafting techniques and development of factory-based pearl mussel culture technologies should be revised, which will lead to the production of improved quality pearls.
- Introduction and efficiency test of different pearl treatment methods for enhancing pearl luster and shelf life.
- Collaboration should be developed between the producers and jewelers, which will play a key role in the creation of improved markets of cultured pearls.
- In addition, specification of low-grade pearls should be formulated to enhance the value of these pearls in pharmaceuticals, healthcare products, nutritional foods and beauty products.

9. Conclusion

This study demonstrates that Bangladesh has the environmental and biological resources for pearl culture activities, including the existence of species of pearl-bearing mussels and the availability of freshwater, to become a promising aquaculture-based, environmentally friendly, and viable model for socioeconomic development. Recent advancement in the selection of pearl producing mussels, appropriate surgical implantation procedure, post-operative care of the implanted mussels and captive breeding protocols broaden the scope of pearl culture in Bangladesh. However, the present research thrusts are on optimization of culture environment, post-harvest value addition of cultured pearls, establishment of market chain and in vitro culture strategy on pearl production. Concern attention needs also to be paid on large scale captive production of pearl bearing mussels and the development of skilled manpower to expand the adoption of freshwater pearl culture technology in Bangladesh.

Ethical clearance statement

The design and utilization of animals in current research have been approved by the Ethical Standard of Research Committee of Bangladesh Agricultural University Research System (BAURES), Bangladesh Agricultural University, Mymensingh-2202, Bangladesh (Ref. 1126/BAURES//38).

CRediT authorship contribution statement

Mohammad Ferdous Siddique: Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Md Ayenuddin Haque:** Writing – review & editing, Resources, Methodology, Data curation. **Arun Chandra Barman:** Writing – review & editing, Formal analysis, Data curation. **Mohosena Begum Tanu:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis. **Md Shahjahan:** Writing – review & editing, Resources, Methodology, Conceptualization. **M. Jasim Uddin:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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