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Recent Trends in Freshwater Pearl Farming in India

Shailesh Saurabh, Sweta Pradhan and Sonal Suman

Abstract

Cultured pearls have an important place in international trade. The Vedas, the Bible, and the Koran all mentioned pearls, and they are regarded as one of the highest honours. Pearls are generated in nature when an irritant, such as a sand grain or a parasite, is swept into the pearl molluscs and lodged within it, where it is coated with micro-layers of nacre, a lustrous substance made up of 80–90 per cent aragonite crystals of CaCO_3 . The ICAR-Central Institute of Freshwater Aquaculture (CIFA), Kausalyaganga, Bhubaneswar, India, has created a base technology for cultivating pearls in freshwater habitats, recognising the scope and value of freshwater pearl production. Indian pond mussel, *Lamellidens marginalis* is the major species used in freshwater pearl aquaculture. In addition, ICAR-CIFA has pioneered a novel feature of freshwater pearl farming. The Institute has also taken the lead in disseminating freshwater pearl culture technology to the country's fish farming communities, entrepreneurs, researchers, and students to build a sustainable model for the country's socio-economic development. In this chapter, we will briefly cover pearls and their types, their historical significance, the spread of pearl mussels of freshwater origin in various countries, pearl biomineralisation, pearl farming techniques, and factors affecting pearl quality, among other things.

Keywords: cultured pearl, *Lamellidens marginalis*, CIFA, mussels, farming

1. Introduction

The “Queen of Gems,” pearl, has a long history of cultural significance and great commercial demand, making it one of the most profitable aquaculture ventures in countries with extensive bivalve resources. Pearl harvesting was once restricted to wild aquatic resources, but with the innovation and standardisation of pearl producing processes, it has become a profitable aquaculture practice. The stimulation of a defence mechanism, where some kind of irritant prompts the bivalves to exude the glossy nacre, which coats the irritant by progressively evolving into layers to create the pearl, is the essential biology responsible for the production of pearly gems [1]. The pearl production process and cultured pearls are driven by this defence event, which is replicated in captivity. However, the ability to make the gem is not found in all molluscan bivalves; rather, only those species with a nacreous layer beneath their shell may make pearls [2].

Commercial pearl production is currently taking place in a number of nations throughout the world, including China, Japan, Australia, Indonesia, French Polynesia, Cook Islands, Philippines, India, Sri Lanka, Bangladesh, Myanmar, Thailand, Malaysia, and Mexico [3, 4]. China and Japan are the main producers of

freshwater and marine pearls, respectively, whereas China is the world's biggest producer of pearls, including both marine and freshwater pearls, with 3540 tonnes produced, accounting for 98% of global pearl production [2]. According to the recent FAO (2020) data, freshwater pearl production is continuing to decline, both in terms of volume and value [5], as seen in **Figures 1** and **2**. It's difficult to answer the question of what can be causing the drop in freshwater pearl output year after year. However, a number of causes, including urbanisation, pollution, climate change, longer pearl production cycles, habitat degradation, sedimentation, a scarcity of qualified manpower, pesticide issues, and the lack of implantation kits, may be to blame for the fall in freshwater pearl output. It is imperative that actions be taken by fisheries departments, policymakers, planners, research organisations, financing

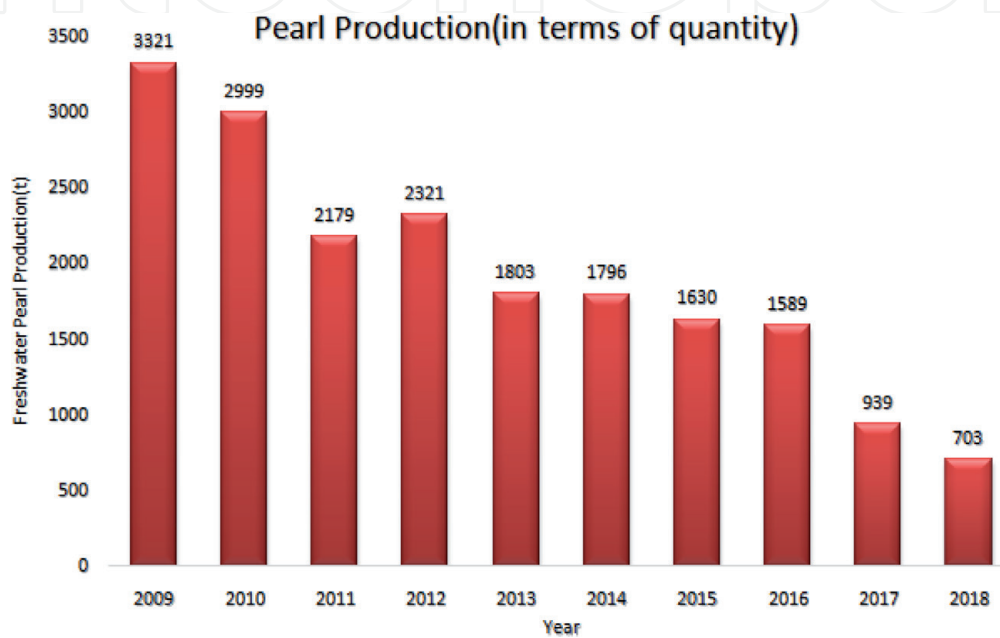


Figure 1. Global freshwater pearl production in terms of quantity (2009–2018; FAO 2020). X-axis: The annual year of pearl production; Y axis: Freshwater pearl production in tonnes.

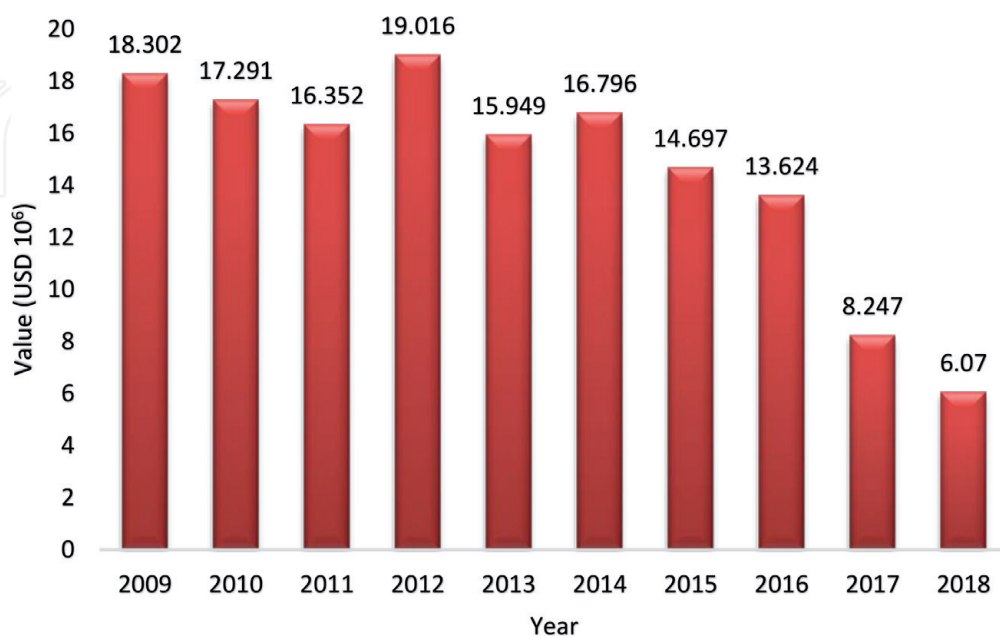


Figure 2. Total output value of freshwater pearl production (2009–2018; FAO 2020). X-axis: The annual year of pearl production; Y-axis: Value of pearl production.

agencies, export agencies, and others to increase freshwater production output, which will aid in the long-term development of the pearl business. In the marine sector, the pearls from the oyster species *Pinctada maxima* followed by *Pinctada margaritifera* dominate the pearl industry [2].

ICAR-CIFA is instrumental in recognising the importance of freshwater pearl culture in India and creating standardised culture technologies for pearl production in freshwater systems. Collection of mussels, pre-operative care, preparation and implantation of nucleus, post-operative care, culture, and harvest are the six essential phases in the creation of cultured pearls. Of these processes, the precise implantation of nucleus is the most crucial. In comparison to other aquaculture species, pearl cultivation necessitates patience and skill.

2. What is a pearl?

A pearl is a gemstone that is generated by living organisms and is the only one of its kind. Bivalves create pearls as a result of an immunological reaction caused by a foreign particle introduced into their body. The gem is made up of 82–86% of aragonite crystals of calcium carbonate and organic proteins, which form the pearl's matrix, where calcium crystallises [6, 7]. One of the most prevalent proteins identified in pearls is conchiolin. It resembles mother of pearl, the inner nacreous lustrous layer of the shell.

3. Historical development of pearl farming

The English word pearl is derived from the Latin word *pirula* which means pear. The name comes from the fact that pearls are frequently pear-shaped. Since the dawn of time, people have been fascinated by pearls. Pearls were given the term “mukta” in classical Sanskrit, which means “purity” or “escape,” alluding to the spirit of the mollusc's desire to escape and solidify as a pearl. Back in the days, procurement of the pearls was done by collecting them from the wild. The creation of these structures in the oyster has long been a source of speculation among ancient philosophers and naturalists. The Rig Veda, the oldest of the Indian Vedas, mentions pearls. Pearl presents are mentioned in Chinese literature dating back to 2200 B.C. Pearls are mentioned in the Indian epics Ramayana and Mahabharata. In Hindu literature, the fabled origin of pearls is linked to Krishna, the eighth avatar of Vishnu, the most significant Hindu divinity. On Pandai's wedding day, Krishna retrieved pearls from the depths of the sea to adorn her and Indian brides still wear pearls on their wedding days today [8]. According to another narrative, the pearl was a prize for Krishna's victory over the monster Pankagna, and he used it to ornament his bride. In ancient China, it was believed that pearls originated in the brain of the fabled dragon. They were regularly delivered as tributes to emperors by foreign princes and found sparkling in the centre of representations of Gods [8]. Throughout Hebrew literature, the Bible, and the Koran, pearls are mentioned in awe-inspiring terms. King Vijaya, who invaded Sri Lanka in the 6th century B.C., is reported to have lavished pearl offerings to his father-in-law, the Pandyan King of Madurai. Megasthenes, the Greek Ambassador at Chandragupta Maurya's court in the 3rd century B.C., and later travellers like Marco Polo (1260–1300 A.D.) and others have left illustrious records of India's and Sri Lanka's pearl fishery. The Persian Gulf, the Red Sea, and the Gulf of Mannar's pearl bank have all been recognised as producing Oriental pearls. For at least three thousand years, oyster pearl fishing has been documented in the Gulf of Mannar [9]. Pearls reached its zenith in the Roman Empire [10].

In the 12th century A.D., the Chinese found a feasible method for producing pearly Buddha images from the cockscomb pearl mussel, *Cristaria plicata*. There were attempts to make pearls by hand all over the world outside of China. In 1761, Carl von Linnaeus, a Swedish biologist, claimed to have developed a method for producing pearls artificially in freshwater mussels [10]. The Japanese solved the mystery by creating blister pearls from the pearl oyster in 1893 and free spherical pearls from the pearl oyster in 1907. Tokichi Nishikawa and Tatsuhei Mise brought this perliculture technology to Japan after it was created by British biologist William Saville-Kent in Australia. In 1916, Nishikawa acquired the patent and married Mikimoto's daughter. Later, in Japan, Kokichi Mikimoto, who is regarded as the pioneer of modern pearl cultivation techniques in the nineteenth century, began commercial pearl manufacturing [2], which paved a path towards the present day culture technologies. To distinguish the Japanese pearls from the "natural pearl" produced by wild oysters/mussels, the term "cultured pearl" was coined. Marine pearl farming began in India in the early 1970s, while freshwater pearl culture began in 1989. The marine pearl culture technique was introduced by the ICAR-Central Marine Fisheries Research Institute, whereas the freshwater pearl culture technology was developed by the ICAR-Central Institute of Freshwater Aquaculture. Farmers have expressed a strong desire to participate in the program offered by the ICAR research institute. From 2012 to 2020, more than 2000 entrepreneurs were trained on various elements of freshwater pearl farming through a total of 21 training programmes. A large number of stakeholders have adopted this technology at their backyard as a result of these. Furthermore, a user-friendly manual in various Indian languages has been created to meet the needs of various kinds of aspirants and to reach out to more farmers. Moreover, with the collaboration of ICAR-CIFA scientists, DD Kissan Delhi Doordharsan National Channel, New Delhi produced a documentary film on 'Moti Ke Kheti', which has been highly welcomed by many sections of the Indian population. Later on, DD Kissan released a documentary film on YouTube in order to make freshwater pearl technology accessible to a larger number of people throughout the world.

4. Distribution of freshwater pearl mussels

As per the studies conducted world-wide, the first appearance of pearl producing molluscs precedes to 530 million years [11] with 10,000 species of bivalve reported from across the planet [12]. China harbours more than 100 species of freshwater mussel out of which 10 species belonging to the Unionidae and Margaritiferidae family are being utilised for commercial production of pearls. Few of them include *Hyriopsis cumingii*, *C. plicata*, *Lamprotula leai*, *Lamprotula rochechouarti* and *Margaritiana dahurica*. The freshwater pearl market of China is occupied by the pearls obtained from *H. cumingii* followed by *C. plicata* as they offer the ease of operation along with better quality pearls [2]. The freshwater pearl industry of Japan is dominated by *Hyriopsis schlegelii* and *Margaritiana dahurica* [2]. The species *H. schlegelii* has strong ability to secrete nacre. Another freshwater mussel species native to North America, *Potamilus alatus*, has the ability to produce high quality black pearls [2]. Due to over-exploitation of European pearl mussel *Margaritifera margaritifera* in search of pearl the species now comes under endangered category and lots of projects are running to revive the natural population of this important species. The distribution of different freshwater pearl mussel is compiled and presented in **Table 1**.

India is a home to around 3270 molluscan species and 1100 out of them are bivalves [62] The marine bivalves count reaches up to 625 species, 88 of which are

Country/Place	Species	References
Bangladesh	<i>Lamellidens marginalis</i>	[13–15]
	<i>Lamellidens corrianus</i>	[14]
	<i>Lamellidens jenkinsianus</i>	
	<i>Lamellidens phenchooganjensis</i>	
	<i>Parreysia corrugata</i>	
	<i>Parreysia favidens</i>	
	<i>Parreysia daccaensis</i>	[16]
China	<i>Cristaria plicata</i>	[17]
	<i>Hyriopsis cumingii</i>	[18, 19]
	<i>Lamprotula tortuosa</i>	[20]
	<i>Lamprotula leai</i>	[21]
	<i>Lamprotula rochechouarti</i>	[21]
	<i>Lanceolaria glayana</i>	[22]
	<i>Hyriopsis schlegelii</i>	[23]
Czech Republic	<i>Margaritifera margaritifera</i>	[24]
Europe	<i>Margaritifera auricularia</i>	[25]
	<i>Margaritifera margaritifera</i>	[26]
Finland	<i>Margaritifera margaritifera</i>	[27]
France	<i>Margaritifera margaritifera</i>	[28]
Germany	<i>Margaritifera margaritifera</i>	[29]
India	<i>Lamellidens marginalis</i>	[30]
	<i>Lamellidens corrianus</i>	
	<i>Parreysia corrugata</i>	
Indonesia	<i>Anodonta woodiana</i>	[31]
Ireland	<i>Margaritifera margaritifera</i>	[32]
	<i>Margaritifera durrovensis</i>	
Japan	<i>Hyriopsis schlegelii</i>	[33]
	<i>Margaritifera laevis</i>	[34]
	<i>Margaritifera togakushiensis</i>	
	<i>Cristaria plicata</i>	[35]
	<i>Margaritiana dahurica</i>	[2]
Malaysia	<i>Hyriopsis bialata</i>	[36]
Mexico	<i>Psoronaias crocodilurum</i>	[37]
	<i>Potamilus alata</i>	
Morocco	<i>Margaritifera marocana</i>	[38]
Nepal	<i>Lamellidens marginalis</i>	[39]
North America	<i>Quadrula sp.</i>	[40]
Norway	<i>Margaritifera margaritifera</i>	[41]
Philippines	<i>Cristaria plicata</i>	[42]
Poland	<i>Margaritifera margaritifera</i>	[43]
Portugal	<i>Margaritifera margaritifera</i>	[44]

Country/Place	Species	References
Russia	<i>Margaritifera margaritifera</i>	[45]
	<i>Margaritifera dahurica</i>	[46]
	<i>Margaritifera middendorffii</i>	
	<i>Margaritifera laevis</i>	
	<i>Cristaria plicata</i>	[47]
Scotland	<i>Margaritifera margaritifera</i>	[48]
South Korea	<i>Cristaria plicata</i>	[49]
Spain	<i>Margaritifera auricularia</i>	[50]
Sweden	<i>Margaritifera margaritifera</i>	[51]
Taiwan	<i>Anodonta woodiana</i>	[31]
Thailand	<i>Hyriopsis (Limnoscapha) myersiana</i>	[52, 53]
	<i>Hyriopsis desowitzi</i>	[52]
	<i>Chamberlainia hainesiana</i>	[52]
	<i>Hyriopsis bialatus</i>	[54]
	<i>Cristaria plicata</i>	[55]
Turkey	<i>Unio terminalis</i>	[56]
	<i>Potamida litoralis</i>	
	<i>Leguminaia wheatleyi</i>	
	<i>Anodonta pseudodopsis</i>	
USA	<i>Quadrula ebena</i>	[57, 58]
	<i>Quadrula undulate</i>	
	<i>Unio sp.</i>	
	<i>Pleurobema oesopus</i>	
	<i>Tritogonia verrucosa</i>	
	<i>Margaritifera margaritifera</i>	
Vietnam	<i>Sinohyriopsis cumingii</i>	[59, 60]
	<i>Cristaria bialata</i>	[61]
	<i>Sinanodonta elliptica</i>	
	<i>Sinanodonta woodiana</i>	
	<i>Lamprotula leai</i>	

Table 1.
Distribution of freshwater pearl mussels in different countries.

endemic [63]. As far as the freshwater mussel species are concerned, around 52 species have been reported from Indian waters including stagnant to slow flowing water bodies [64]. Nevertheless, large scale production of pearls is being carried with three species categorised under the Unionidae family i.e. *L. marginalis*, *L. corrianus* and *Parreysia corrugata* [30, 65].

5. Biomineralisation of pearls

Biomineralisation is a common occurrence in a variety of species [66]. A pearl is a well-known organo-mineral composite product of biomineralisation that is

composed of more than 95% calcium carbonate (CaCO_3) and less than 5% organic molecules [6, 67, 68]. The process by virtue of which the pearl develops in mussel/oyster is otherwise termed as biomineralisation and the outer epithelium of the mantle plays an important part in pearl biomineralisation. It is formed when an external stimulus like a foreign body or a parasite is fortuitously trapped in the bivalve followed by biomineralisation around the non-native particle via the deposition of pearl nacre in micro-layers. Nevertheless, this phenomenon is restricted to those bivalve species whose interior surface of the shell carries the nacreous layer. The peculiarity of pearl formation being restricted to certain species of bivalves can also be supported by the fact that a prominent resemblance in the CaCO_3 and matrix protein component lies between the pearl and nacreous layer of the organism's shell.

Biomineralisation is attributed to a complex physiological process wherein various matrix proteins and calcium metabolism regulatory proteins secreted from the mantle epithelium act together to form the lustrous product that is preceded by the formation of the pearl sac around the foreign particle by the proliferation of the outer epithelium of the mantle [6, 7, 69–71]. The matrix proteins contribute to the pearl formation process in forming a biomineral framework and simultaneously regulating the nucleation as well as the growth of calcium carbonate crystals [6, 7]. Microstructure analysis of pearls reveals that the crystallising layers of the pearl include three polymorphs of CaCO_3 that include aragonite, calcite and vaterite, which also govern the quality of pearls [6]. The mantle shell of molluscan species consists of three layers, the outer periostracum, middle prismatic and the inner nacreous layer [7, 72, 73]. The periostracum, a layer of strongly cross-linked proteins that covers the shell's external surface, is produced by the mantle [74]. Secretions from the edge region of the mantle (mantle edge) form the prismatic layer while the inner part of the mantle (mantle core) makes up the nacreous layer or “mother-of-pearl”, which is the most widely studied structural motif. Studies on the crystal structures of molluscan shells reveal that the prismatic and nacreous layer consists of calcite and aragonite, respectively [67, 73] and this polymorph formation is regimented by the differences in the mantle epithelium's secreted protein [7, 67]. The third polymorph, vaterite is an unstable and rarely occurring form of CaCO_3 that has been exclusively reported in freshwater pearl mussel species [6, 75, 76].

6. Classification of pearls

Pearls can be broadly classified into 3 different types (**Figure 3**). They are as follows-

6.1 Natural pearls

These pearls form naturally in the environment when mussels ingest a foreign particle without the need for human involvement. Natural pearls have a small core or nucleus with thicker crystalline pearl nacre. It has an uneven shape and is quite small. Due to the margins of the overlaying aragonite crystals, the surface of natural pearl has a rough texture.

6.2 Cultured pearls

It's similar to the natural pearls formed in mussels, except instead of an organism accidentally absorbing a foreign particle, a nucleus and a mantle graft are surgically implanted into the mussel. It is possible to create pearls of desired size, shape,

colour, and lustre using the culture method of obtaining natural pearls. They come in a variety of shapes and sizes, including round, half-round, and designer pearls, (Figure 4) depending on the nucleus utilised.

6.3 Artificial or imitation pearls

Artificial pearls are made by covering a hard, spherical core or base with pearl-like materials to substitute natural or cultured pearls. From low-cost gleaming paints to synthetic pearl essences derived from fish scales, the coating can indicate a difference. Artificial pearls, unlike natural or cultured pearls, have a smooth surface texture and leave a scratch on the surface when rubbed against a sharp object figure depicts the 3 types of pearls and not artificial pearl, only.

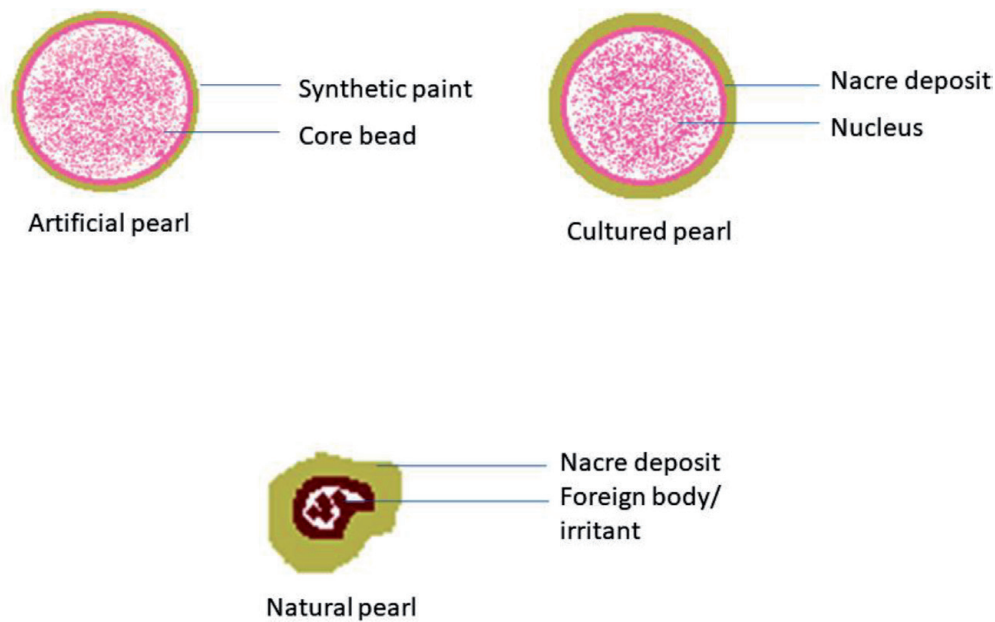


Figure 3.
Graphics depicting distinct types of freshwater pearls.



Figure 4.
*Production of designer pearl in *L. marginalis*.*

7. Freshwater pearl farming technology

Freshwater pearl farming procedures vary depending on the surgery performed on the pearl mussel's internal anatomy and the type of ideal pearl result. Cultured pearl production involves six basic steps: mussel collection, pre-operative conditioning, nucleus preparation and surgical insertion, post-operative care, pond culture of implanted mussels, and harvest. To avoid graft and mantle rejection and minimise the post-operative mortality rate, implantation can be done at any time of year, with the exception of May–June (summer season). The different steps involved in pearl farming are given below:

7.1 Collection of mussels

The study was conducted at ICAR-CIFA, Kausalyaganga farm, Bhubaneswar, Odisha, India (Lat. 20° 11' 06''-20° 11' 45''N; Long.85° 50'52''-85° 51'35''E). Mussels are hand-picked and harvested by competent employees from culture pond, ideally early in the morning. The mussels are harvested and stored in water for short distance travels to avoid stress during transportation. While collecting mussels, the mussel size requirement is the most significant consideration. It has been proven that pearl mussels with a shell length of 8–12 cm and a weight of 35 g or more can produce pearls.

7.2 Pre-operative conditioning

Pre-operative conditioning allows appropriate relaxation of the adductor muscles and keeps the animal's metabolic rate low to get the best possible result throughout the surgical implantation process. Prior to surgery, pearl mussels are subjected to pre-operative conditioning for 24 to 48 hours after being collected from cultured ponds. The mussel was stocked in 200 litre FRP tanks at 60 mussels per tank during acclimation. The experimental tank was filled with filtered pond water. Green water should be offered to them as a source of nutrients. During the study, the following physico-chemical water quality parameters were measured: dissolved oxygen 5.6 to 6.5 mg l⁻¹; pH 7.5 to 7.6; nitrites 0.020 to 0.030 mg l⁻¹; ammonia 0.05 to 0.08 mg l⁻¹ and temperature 27 to 29°C. Further, 5% mortality of the mussel was also observed during this period.

7.3 Preparation and surgical implantation of nuclei

It is the most crucial stage in the pearl-growing process. The cultivated pearl design that will be used as the end result will define the type of nucleus to be developed. Some of the nuclei that can be made are designer, half-round, and round nuclei. The nucleus can be prepared using one of two methods. The first step involves mixing acrylic powder with its solvent in a 1:1 ratio to make slurry that is utilised to prepare all of the nucleus forms. The second method, on the other hand, comprises a time-consuming technique that involves bleaching, powdering, and sieving dead mussel shells to get a finely crushed shell powder. The shell powder is then mixed with araldite glue to make dough, which is then cast on moulds to make designer and half-round nuclei, or rolled on the palm to make circular nuclei. Prior to either the acrylic powder-solvent slurry or the shell powder-araldite dough to the moulds, make sure they are lubricated to make it easier to remove the nuclei from the moulds.

The ultimate pearl to be generated determines the method of implantation to be used. There are three different kinds of implantation procedures. The list is as follows:

7.3.1 Mantle cavity implantation

This is the easiest way of the three, requiring the least amount of skill and expertise. The nucleus is implanted into the cavity between the outer mantle layer and the mussel shell's inner surface in this procedure. The outer mantle layer serves as the source of nacre secretion; hence mantle graft is not employed in this procedure.

7.3.2 Mantle tissue implantation

The nucleus, together with the mantle graft, is implanted into pockets formed in the recipient mussel's mantle tissue on the posterior side, in both the left and right lobes (**Figure 5**).

7.3.3 Gonadal implantation

A small incision is made in the recipient mussel's gonad and the nucleus together with a single mantle graft or sandwiched between two pieces of graft are then put into the incision. Along with the round nucleus, a live graft of 2 to 3 mm is introduced from the pallial mantle ribbon. Sometimes, infections can occur in the mussel after the graft and nucleus have been implanted; these infections can be treated with broad-spectrum antibiotics, which also assist to restore the immunological status of the operated mussel.

The success rate of pearl generation in the mantle cavity and mantle tissue implantation methods is 60–70 percent, but it is 25–30 percent in gonadal implantations.

7.4 Post-operative care

The implanted mussels' recuperation necessitates post-operative care, which is a key phase in the production of freshwater pearls. In this step, 7–10 days post the implantation the mussels are monitored closely. The shell valves are given enough care to guarantee that they can open and close freely for respiration. The use of a broad-spectrum antibiotic in the water in post-operative care units at a rate of 1–2 ppm is favourable to the survival and wound healing of the implanted mussels. Mussels in post-operative care are given green algae grown in the lab. During post-operative care, there is a risk of rejection of the implanted nucleus and graft, which can be reduced by lowering stress in the post-operative care tanks by maintaining water level, feeding, and aeration. It is vital to keep in mind that proper post-operative care reduces the likelihood of abnormal pearls by preventing nucleus extrusion soon after implantation.



Figure 5.
Implantation of live graft pieces into the mantle of mussel.

7.5 Culture in ponds

To prevent post-operative mussel mortality and nucleus bead rejection, freshwater mussel implantation is done all year in India, except during the extreme hot months (May to June). Pearl culture operations can be carried out in traditional carp culture ponds (2.5 m deep) with a clay-soil basis and somewhat alkaline waters. Ponds free from aquatic macrophytes and algal blooms, such as *Microcystis* and *Euglena*, are perfect for pearl culture. Bamboo poles are used as rafts in the ponds to hang the pearl mussels that have been implanted at a stocking density of 50,000/ha. The mussels are hanged in 1.5 mesh sized nylon bags of 30 cm x 13 cm @ 2 mussels per bag.

Pond management is crucial during the culture stage, especially in terms of natural food production and water quality control via liming or fertilisation, as it affects the quality and quantity of pearl production. The addition of green water (*Chlorella sp.*, *Chlorococcum sp.*, and *Scenedesmus sp.*) to the pearl culture ponds at regular intervals as direct mussel feed has been revealed to be the most effective technique for maintaining the pearl yielding mussel standing crop. The “open culture method” can be used to cultivate green algae in ferro-cement tanks (200 litres) positioned along pond dykes. Cow dung (10,000 kg/ha/yr), urea (100 kg/ha/yr), and single super phosphate (100 kg/ha/yr) are used to fertilise the water in the tanks in equal monthly instalments. When the fertilisers degrade in 10 to 15 days and green water appears, the improved water is placed into the pearl culture ponds. According to reports, freshwater mussels feed on algae belonging to the Chlorophyceae (green algae), Bacillariophyceae (diatoms), and Cyanophyceae (blue green algae) families. Diatoms, green algae (*Chlorella*, *Chlorococcum*, *Scenedesmus*, etc.) and blue-green algae (*Spirulina*) are the most usually favoured algal species by the freshwater mussel *L. marginalis*. Mussels may ingest a wide variety of particulate organic materials since they are mucoid filter feeders. A routine health check-up of the cultured mussels should be done at biweekly intervals because there is still a high risk of mortality of operated mussels due to internal incision, limited food availability, and parasitic infection. As a result, mussels in net bags should be removed, inspected, and cleaned before being returned. Because the mussels remain sedentary and static inside the enclosures, algal development can occur as a result of heavy nutrient loading, which should be avoided at all costs. The pond's physico-chemical parameters and water level are monitored during the culture period. Temperatures between 25 and 30 degrees Celsius are optimal.

7.6 Harvest of pearls

Depending on the implantation method utilised, the size and quantity of nuclei implanted, the health of the mussels, and the pond environment, pond culture of operated mussels can take anywhere from a year to 18 months. Harvesting occurs at the end of the culture period, where the mussels are checked and processed individually in order to obtain the ultimate product, pearls. A biological system produces pearls through a natural process. Because pearls are created through a natural process, they have a wide range of look and quality. After harvest, the pearls are subjected to value addition through surface cleaning, bleaching and dyeing, or both cleaning and bleaching, in order to maintain uniformity in colouring and quality, which may improve their marketability.

8. Pearl quality, factors affecting and quality enhancement

The value of a pearl, like any other valuable gem, is decided by its quality, which is decided by several qualities of the pearl such as shape, size, colour, lustre, and

surface complexion [69, 77]. Given that a pearl is the result of a complex biological phenomenon, diversity in pearls is unavoidable and is influenced by both the genetic makeup of the individual and the impact of many environmental factors. It can be deduced that genetics, environment, and genotype-by-environment interactions influence the overall quality of pearls [78, 79]. Pearls are classified into three varieties based on their appearance to the naked eye: nacreous pearls, prismatic pearls, and organic pearls, with nacreous pearls being the most valuable [80]. Although controlling and regulating the quality of cultured pearls at the genetic level is a time-consuming task that necessitates extensive research, changes in culture methods and environmental conditions can significantly improve pearl quality. Pearl quality is also affected by other factors like the host and donor oysters [70], or by exogenous physico-chemical and biological stressors [81]. Various factors that can be regulated to enhance the pearl quality are listed below.

8.1 Host

Individual mussels selected for surgical nucleus implantation must be healthy and of a suitable size.

8.2 Donor mussel and graft tissue preparation

The donor mussel used to get the graft tissue should have a well-developed and healthy mantle and be of the desired size. The mantle graft should be carefully selected, cleaned, cut, and trimmed, and the graft tissues should be kept in good quality water with the proper level of chemicals while the surgical procedures are being performed.

8.3 Implantation

The most important phase in the production of cultured pearls is nucleus implantation. A successful implantation technique is defined, in addition to the technician's skills, by the identification of an appropriate location for nucleus and graft insertion. More skill and patience are necessary to appropriately position and orient the graft tissue in contact with the nucleus, as well as for several nucleus implantations in a single individual.

8.4 Mussel convalescence

For the mussels to recover from the effects of narcotisation, they need regular water changes or a gentle flow through. Furthermore, the mussels should be given ample time to heal after the incision for nucleus implantation before being stocked in culture ponds.

8.5 Tool maintenance

Before usage, surgical instruments should be sharp, rust-free, and thoroughly sterilised.

8.6 Temperature

Temperature is known to influence the metabolic rate of every organism. Although higher temperatures encourage mussel growth and nacre deposition, the quality of the pearls produced suffers as a result.

8.7 Quantity and quality of natural feed

The type and amount of plankton supplied to the mussels during the culture stage is also a quality determining factor because quality and quantity of nacre secretion of the organism depends on plankton composition.

8.8 Culture period

The mussels must be nurtured for the requisite period of time to develop the proper thickness of nacre surrounding the nucleus, which contributes to the pearl's overall size, colour, and shine. The culture period might last anywhere from 12 to 18 months, depending on the type of nucleus used and implantation employed.

The raw pearl obtained from the implanted freshwater mussels are inapt to be directly used as a jewel. The pearls are value added after harvesting in order to improve quality and maintain consistency. They are subjected to a variety of procedures, including cleaning, bleaching, dyeing, or both. For cleaning or bleaching, hydrogen peroxide, ether solvents, water, and alcohol are used in varied amounts depending on the need. Ultra sonication, for example, is a physical treatment process that removes any clinging contaminants. Other chemicals, such as EDTA, sodium hypochlorite (NaOCl), and calcium hypochlorite (bleaching powder), can help remove adherent particles by chelating, oxidising, or bleaching them. Pearls have been demonstrated to shine when treated with EDTA, NaOCl, chlorine, and hydrogen peroxide [82].

Studies have shown that the colour of cultured pearl can be enhanced using radiation and chemical treatment. 48 h exposure to $5.423\% \times 10^{-2}$ M rad of gamma radiation, treatment in 1.2% eosin solution for 24 h, 20% iodine solution for 48 h and 0.2% silver nitrate solution for 24 h result in distinct changes in the colour of pearl from white to black, pink, yellow and metallic brown or black, respectively. It was also observed that the coloration caused by gamma radiation and silver nitrate was everlasting [82].

9. Identification of pearl

There are a few characteristics that can be used to establish the originality of a pearl, and they are as follows:

9.1 Irregularities

Minor irregularities in colour signify a pearl to be original in contrast to which artificial pearls are large, symmetrical and perfectly matched in all possible way.

9.2 Examination of drill hole

You can detect if a pearl is natural or not by poking a small hole in it and looking at it under a magnifying glass. The crystallisation layers of a real pearl can be seen using a magnifying lens.

9.3 Tooth test

To identify between genuine and imitation pearls, gently rubbing the pearl between the teeth is a frequent and fairly reliable test. A natural pearl's surface has a gritty and sandy feel due to the unique architecture of the nacreous surface, but an artificial pearl has a smooth texture and hence a smooth feel when rubbed on teeth.

9.4 Lustre test

An artificial pearl's lustre is limited to its surface, and scratching it against a rough surface removes the shiny coating. A genuine pearl, on the other hand, is formed by the subsequent deposition of nacre in layers, thus the lustrous nacre layers run deep from the surface up to the nucleus, and scratching the pearl surface is difficult due to the compactness of the calcium carbonate crystallisation.

9.5 Spectroscopy

Micro-infrared spectroscopy, Raman spectrometry, scanning electron microscopy and X-ray diffraction are all commonly employed to distinguish between freshwater and saltwater cultured pearls [83].

10. Grading of pearls

Natural cultured pearls that are commercially accessible are graded according to their quality [84], as shown below.

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

11. Challenges in freshwater pearl culture

Despite the fact that pearl farming is a profitable business, there are a number of challenges to overcome when cultivating the pearl. One of the most crucial aspects is the mussel's capacity to survive following implantation. Another challenge is determining the right quality of the pearl after it has been obtained. In freshwater mussels, standardisation of breeding technology needs an utmost priority, successful breeding occurs in mussels; however mussel larvae survival is a serious challenge. i.e., glochidia adhesion to the secondary host (fish) is problematic. The mussel larval cycle requires a secondary host, such as a fish, to complete the life cycle. The lack of competence in pearl culture technology is one of the primary challenges. The sector's development is further hampered by a lack of a proper extension network to propagate existing culture technology.

12. Prospects of freshwater pearl culture in India

Farmers in developing countries like India have a limited grasp of modern aqua farming procedures, including pearl farming, which should be followed in their respective fields. Through research, teaching, and training, many people are working hard to transfer this critical technology of pearl farming to the needy. Many farmers, entrepreneurs, and women who are interested in this subject have already

received training in recent years. Freshwater pearl farms have been developed in various states across the country, including Odisha, Maharashtra, Gujarat, West Bengal, Bihar, Uttar Pradesh, Chhattisgarh, Kerala, and a few more that are still in the early phases of development with the technical support of ICAR-CIFA. Larger round pearls and designer pearls in various designs such as goddess Laxmi, Holy Cross, Ganesh, and other beautiful shapes are currently in high demand in India. Time has come to educate more people about pearl farming, as this aquaculture technology is expected to generate a lot of employment and money. To produce excellent pearls in a shorter period, new developments in freshwater pearl farming should be introduced into farmer's fields in the future.

13. Conclusion

The ICAR-Central Institute of Freshwater Aquaculture has made significant progress in areas such as the identification of newer biocompatible nuclei, surgical implantation technique, mussel pre and post-operative care, graft and nucleus rejection minimization, pond culture of implanted mussels, and pearl value addition. Efforts are also being made to develop low-cost technologies for farmers through the use of basic, readily available equipment in pearl cultivation. Glochidial larvae culture *in vivo* and *in vitro* has also been the bailiwick of recent research. The technology of freshwater pearl culture is also being disseminated with a focus on farmers, investors, state government officials, researchers, and students across the country to enhance technical skills and popularise freshwater pearl culture technology to new heights. However, the limited availability of skilled personnel for precise implantation and a lack of effective marketing networks are the two most significant barriers to successful pearl farming adoption. Commercialization of this skilled technology necessitates a well-thought-out strategy at the regional and farm levels, as well as entrepreneurial development and participation. To summarise, freshwater pearl farming has a large economic, social, and environmental impact. In the future, this pearl-growing method is expected to become one among the most well-known components of India's freshwater aquaculture.

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Conflict of interest

The authors declare no conflict of interest.

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Author details

Shailesh Saurabh^{1*}, Sweta Pradhan¹ and Sonal Suman^{1,2}

1 ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Odisha, India

2 ICAR-Central Institute of Fisheries Education, Mumbai, India

*Address all correspondence to: ssaurabh02@rediffmail.com

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