# AQUABYTE SECTION

## **Aquabyte** is the Newsletter of the Network of Tropical Aquaculture Scientists

Freshwater pearl culture, the techniques for which originated in China during the 12<sup>th</sup> century, is becoming a booming industry. The Biwa pearls of Japan, with a production of 8-10 tons per year, are much in demand throughout the world. In recent years, Bangladesh, India, the Philippines, Thailand and Vietnam have initiated commercial production of freshwater pearls. Freshwater pearl culture methods developed in India are featured in this issue.

With an increasing demand for fish, farmers are looking at ways of integrating aquaculture with agriculture and utilizing unproductive agricultural lands for aquaculture. There are two papers dealing with the subject in this issue.

M.V. Gupta

### Freshwater Pearl Culture in India

K. Janaki Ram

#### Abstract

Freshwater pearl culture is growing as a source of employment and income in many Southeast Asian countries. Bangladesh, Thailand, the Philippines and Vietnam have initiated freshwater pearl culture projects in recent years. In India, the Central Institute of Freshwater Aquaculture is carrying out research on identification of suitable local pearl mussel species, defining appropriate surgical implantation procedures, developing post-operative care procedures and captive pond culture of mussels.

#### Introduction

The world trade of pearls, which is well over US\$2 billion per year, is controlled by a few countries (Fassler 1991). The culture of pearls in a freshwater environment is akin to the cash crops of land-based agriculture. Freshwater pearl culture probably has an edge over its marine counterpart in terms of wider area of farming, absence of fouling, boring and predatory organisms, and overall cost effectiveness of the operations. The majority of Southeast Asian countries have distinct advantages such as a conducive climate, strategic location on sea and air trade routes, traditional marketing environments and vast resources of fresh water with abundant distribution of qualitatively rich pearl mussel fauna. Most importantly, there are rural artisan communities suitable for the development and adoption of freshwater pearl culture, which is also beneficial in terms of import substitution and export earnings.

Realizing the scope and importance of freshwater pearl culture, the Central Institute of Freshwater Aquaculture (CIFA), Kausalyaganga, Bhubaneswar, India, initiated research in 1987 and evolved a base technology of growing pearls in the freshwater environment. This pa-

per gives a brief history of pearl culture and describes freshwater culture in India.

### Biomineralization of Pearls

The pearl is both mystic and beautiful with a soft color and cool luster. This is perhaps the only gem in nature which does not require lapidary treatment. The pearl is composed of about 82% to 86% aragonite crystals of calcium carbonate, 10% to 14% organic matrix, a sclero-protein termed as conchiolin and 2% to 4% water. A pearl has 3.5 to 4.5 hardness on Moh's scale with a specific gravity of 2.7.

Theoretically, any shelled mollusc can produce a pearl of some sort, but only those molluscs which have a pearl lining or pearl nacre on the interior of the shell surface can produce lustrous pearls. A pearl has structural and chemical properties similar to the pearl nacre of the shell interior. Its shape is determined by the irritant foreign body and its quality by the nature of secretions of the pearl sac. Thus the outer epithelium of the mantle tissue is the "keynote" in the "orchestra" of biomineralization of a pearl (Simkiss and Wada 1980).

Japanese scientists have recently demonstrated that pearl luster is basically caused by certain genes. The corresponding protein has been isolated and named *nacrein*. It is hoped that the isolation of the gene and its specific proteins can lead to commercial production of pearls outside the molluscan body in the near future.

#### Status of Freshwater Pearl Culture in the World

In Japan, freshwater pearls have been cultured commercially since 1935. The Japanese freshwater pearl mussel Hyriopsis schlegeli, which abounds in Lake Biwa in Shiga Province in Honshu of central Japan, was transplanted to the northeastern Lake Kasumiga-ura in 1963. It is reported that 47 culture farms with a water spread area of 110 ha in Lake Biwa and 16 farms with an area of 13 ha in Lake Kasumiga-ura are in operation, producing 8 to 10 tons of cultured pearls per year (Kafuku and Ikenoue 1983; Ward 1985; Simrad 1992). The Japanese freshwater cultured pearls are popularly called

"Biwa pearls" and are much in demand throughout the world.

The genesis of modern freshwater pearl culture can be traced from the twelfth century when the practice of producing pearlcoated Buddha images by inserting these images into the mussel Cristaria plicata in Lake Tahu, China was initiated. Following the success of Japan in freshwater pearl culture, China has made tremendous progress in the development of inland pearl culture employing the local mussel Hyriopsis cumingii. The first batch of Chinese freshwater culture pearls entered international markets in 1970 and the yields are reported to be over 100 tons per year (Ward 1985). Today, the Southeast Asian markets are flooded with Chinese rice pearls which form the mainstay of the pearl trade. Currently, research is aimed at developing pearls under in vitro conditions (Shi 1983). Taiwan recently initiated several commercial freshwater pearl culture projects (Liu-Fu-Guang 1993).

In Europe, the freshwater pearl mussels *Margaritifera* sp. and *Unio* sp. are well distributed (Bauer 1986). The Scottish pearls were traditionally fished from the Tay, Earn, Teith and other rivers in Scotland and north Wales. The mussel *M. margaritifera* in the River Conway has historical significance in the pearl fishing industry in Wales (Jones 1973).

The Mississippi River basin in the United States of America is the main source of raw material for manufacturing the shell bead nuclei used for pearl culture operations. Until recently, the empty shells of local larger freshwater mussels Quadrula ebena, Q. undulata, Unio sp., Pleurobema oesopus, Tritogonia verrucosa and

Megalanias sp. were exported to Japan for the shell bead industry. Efforts to develop indigenous freshwater pearl culture technology in the U.S. are underway and pearl farms have already appeared in California and Tennessee (Fassler 1992).

Among Southeast Asian countries, Bangladesh, Thailand, the Philippines and Vietnam have initiated commercial freshwater pearl culture projects in recent vears (Fassler 1994). Bangladesh, about 150 kg of natural pearls are harvested annually from the mussels Lamellidens marginalis and Parrevsia deccaensis (Anon. 1986). In the Philippines, the accidentally introduced freshwater mussels Cristaria plicata and Anodonta sp. are extensively used for production of cultured pearls. Vietnam commenced research in 1977 and semi-commercial freshwater pearl culture operations began in 1988. The Research Institute for Marine Products (RIMP) at Hai Phong has developed the base technology for culturing pearls from Hyriopsis cumingii, Cristaria bilata, Anodonta jourdyi and Lamprotula sp. mussels which are abundant in the northern Cau, Thuong, Chau Giang, Day and Lucnam rivers. The shell of the riverine mussel Lamprotula sp. is the main source of indigenous shell bead material for pearl culture operations in Vietnam (Ha Dac Thang, pers. comm.).

#### Freshwater Pear' Culture Research in dia

Pearl culture at CIFA involves three common species of mussels and the different implantation procedures. The biological and physico-chemical conditions required in the culture of the implanted mussels are discussed in this paper.

#### **Species Involved**

Two local genera, Lamellidens and Parreysia, are important for pearl culture. There are more than fifty species of these genera distributed throughout the country (Subba Rao 1989). Of these, the common species L. marginalis, L. corrianus and P. corrugata are being employed for pearl culture operations (Janaki Ram 1989). The basic information on the indigenous freshwater pearl culture technology has been detailed by Janaki Ram and Tripathi (1992). A summary of the steps involved is indicated in Fig. 1.

#### **Implantation Procedures**

Until now, shell beads imported from Japan constituted the essential input in pearl culture operations. It has been demonstrated that certain inexpensive and bio-compatible acrylic materials (which are locally available), can be employed as nuclei in freshwater pearl culture (Janaki Ram 1993). The pearls produced using the acrylic beads are fairly comparable to the traditional shell bead nucleated pearls. The implantation methods vary depending on the type of pearl products targeted, as shown in Fig. 2.

Mantle Cavity Insertion. In this method, appropriate nuclei of up to 1.0 cm diameter are placed in the mantle cavity of pearl mussels (L. marginalis and L. corrianus) of 8 to 10 cm in shell length. The implanted mussels are reared in ponds for 12 months. The products are generally shell-attached, half round or designer pearls depending upon the shape of the nucleus implanted.

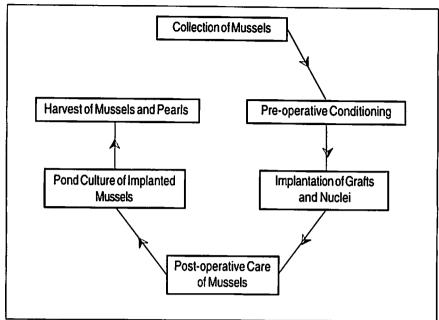


Fig. 1. Steps in freshwater pearl culture.

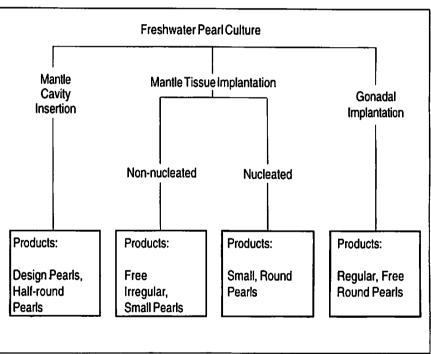


Fig. 2. Implantation methods and products.

Mantle Tissue Implantation. The procedure involves placement of a donor mantle graft (2 to 5 mm²) in the space between the outer and inner epithelial layers of the left and right mantle lobes of the recipient mussels. A small

nucleus (<3 mm dia) is also placed along with the graft, depending on the size and development of the mantle tissue of recipient mussels. The implanted mussels are transferred to a pond environment for a culture period

of 12 months. The pearls produced are either unattached, irregular to oval graft pearls or round, nucleated pearls.

Gonadal Implantation. In this method, the donor mantle graft (2 mm²) and a nucleus (3 to 6 mm dia), is implanted in the gonad of the recipient mussels. The gonad implanted mussels are maintained in post-operative care units with antibiotic support for 7 to 10 days to minimize the rejection of the implanted grafts and nuclei before being transferred to pond culture units. The pond culture period varies from 6 to 12 months depending on the size and number of nuclei implanted. Unattached, regular, round culture pearls are produced.

The success rate of pearl formation has been recorded at 60% to 70% in mantle cavity and mantle tissue implantation methods, and about 25% to 30% in gonadal implantation procedures. The color of the half-round and design pearls produced through mantle cavity insertion generally follows the color of the shell interior of the mussels employed. In the case of mantle tissue and gonadal implantation methods, the color of the pearls varies from silvery white, golden yellow to deep pink depending upon the nature of the donor mantle grafts employed (Janaki Ram et al. 1994).

It has been observed that the implanted mantle graft epithe-lium leads to the enveloping of the nucleus in the form of a pearl sac in about 15 days, and that the microvilli of the pearl sac epithelium constitutes the cellular basis for crystallizations of aragonite calcium carbonate, the first step in pearl formation (Janaki Ram and Misra 1997).



Half-round cultured pearls produced through mantle cavity insertion.



Designer cultured pearl produced through cavity insertion.

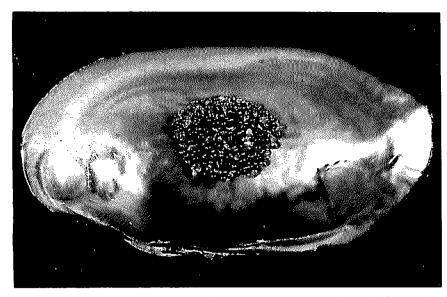


A collection of irregular non-nucleated culture pearls in a shell produced through mantle tissue implantation.

Currently, efforts are focused on culturing pearl nacre-secreting mantle epithelial cells under *in vitro* conditions. The pallial mantle epithelial cells can be grown under culture conditions in about 40 days and the viabil-

ity and functionality of the *in vitro* grown cells is being established. The *in vitro* cell culture studies have an application potential for ensuring a uniform quality of culture pearls produced.

<sup>&</sup>lt;sup>1</sup> The details on *in vitro* cell culture studies are being published separately.



A collection of nucleated culture pearls in a shell produced through gonadal implantation.

## Captive Culture of Implanted Mussels

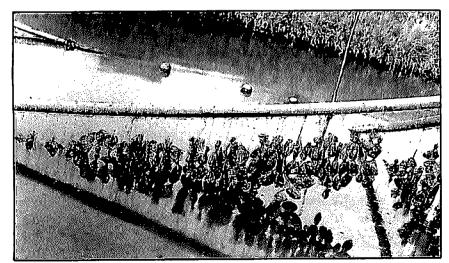
Mussel implantation is carried out throughout the year, except during the summer months (May to June) to minimize post-operative mussel mortality and rejection of implanted grafts and nuclei. Traditional carp culture ponds (2.5 m deep) with clay-soil base and slightly alkaline waters are suitable for pearl culture operations. Ponds without aquatic microphytes and algal blooms such as *Microcystis* sp. and *Euglena* sp. are ideal for

pearl culture. Implanted mussels at a density of 50 000 to 75 000 individuals per hectare are placed in nylon bags (1.0 cm mesh; 12 x 14 cm) at 2 mussels per bag and hung at a depth of 1.5 to 2.0 m in ponds over bamboo or PVC tubing platforms. Some of the desirable water and soil parameters for pearl culture are:

Water

pH: 7.5 to 8.5

Total alkalinity: 75 to 150 ppm Total hardness: 40 to 75 ppm Dissolved calcium: 25 to 50 ppm



Implanted mussels in pond culture units.

Soil

pH: 6.5 to 7.5

Organic carbon: 1.0% to 2.5% Available nitrogen: 25 to 75

mb/100 g of soil Hydrogen sulphide : Nil

Periodic addition of "green water" (Chlorococcum sp. and Scenedesmus sp.) to pearl culture ponds as direct mussel feed has been observed to be ideal for proper maintenance of the pearl bearing mussel standing crop. The green water can be developed by "open culture method" in a series of ferro-cement tanks arranged along the pond embankment. The water in the tanks is fertilized with cattle manure (10 000 kg/ha/yr), urea (100 kg/ha/yr) and single super phosphate (100 kg/ha/yr) in equal monthly installments. When the fertilizers degrade in 10 to 15 days and green water develops, the enriched water is channeled into the pearl culture ponds. By virtue of being mucoid filter feeders, the mussels can accept a variety of particulate organic materials as feed. The pearl mussels in captive culture conditions can be maintained on a diet of powdered rice bran and groundnut oil cake (1:1 ratio) at 1% of the body weight of the mussels provided on alternate days (Misra et al. 1995).

#### Conclusion

The development of freshwater pearl culture technology in India is of recent origin compared to Japan and China. However, considerable progress has been made in areas such as identification of suitable local pearl mussel species and definition of appropriate surgical implantation procedures, short-term post-operative care of the im-

planted mussels minimizing nucleus and graft rejection rates, and long-term captive pond culture of implanted mussels until harvest. Presently, the research thrust is on post-harvest value addition of the cultured pearls and on pearl mussel seed production. Concerted attention is also being paid to demonstration and training to develop technical expertise in the region to expand the adoption of the freshwater pearl technology.

#### Acknowledgments

The author is thankful to Dr. S. Ayyappan, Director, Central Institute of Freshwater Aquaculture (CIFA) for the facilities accorded. He also acknowledges the Department of Biotechnology, Ministry of Science and Technology, Government of India for the financial assistance provided from 1992 to 1996.

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