Homogenic and xenogenic implantation in pearl mussel surgery

Pearl culture technology is a developed sector in countries like China and Japan. Realizing the potential, several other countries have taken up this practice in the aquacultural operations. Pearl culture technology in India is in its infancy. However, the base technology to produce cultured pearls has been standardized[1] and more attention is being paid to improve the implantation technique. The Indian pearl mussel *Lamellidens marginalis*, widely distributed throughout the country in majority of the freshwater bodies, is known to produce lustrous pearls. China has made tremendous progress in culturing freshwater pearls employing the triangular mussel *Hyriopsis cumingii*[2,3], through which pink-to-purplish coloured quality pearls are produced. It is understood that in bivalve molluscs, mantle is the key organ for secreting shell material and assists in repair and regeneration of shell[4] and the pallial mantle plays an important role in pearl production. Wada[5] studied mantle transplantation in freshwater mussels, viz. *H. schlegelli*, *Cristaria plicata* and *Anodonta calipygos* by both allografting and xenografting methods. Some reports are also available in which experimental works are done to study the quality and colouration of pearls through genetic[6]; irradiation procedures[7] and through xenogenic implantations[8]. Besides the standard implantation procedure employing the indigenous mussel sp. *L. marginalis*, *L. corrianus* and *Parreysia corrugata*, xenogenic implantations were also made earlier[9]. However, there is no data available involving Indian and exotic mussel species. Thus the present experimental work was undertaken to study the possible role of xenografts in rate of pearl sac production and to determine the probability of pearl production through xenogenic implantation between the Indian pearl mussel *L. marginalis* and exotic sp. *H. cumingii* (Figure 1).

The Indian pearl mussel sp. *L. marginalis* and the exotic sp. *H. cumingii* of average length 10.0 cm and 13.4 cm and wet weight 100 g and 176 g respectively were selected and conditioned in the laboratory prior to surgery. 50 numbers of healthy recipient mussels were segregated in each experimental set-up. In the control, homografting was done in the Indian and exotic species separately whereas in xenografting, one set consisted of the exotic mussel species *H. cumingii* as the donor and *L. marginalis* as the recipient and in the second set, *L. marginalis* as donor with *H. cumingii* as the recipient. Mantle tissue implantation was made in all the recipient mussels following the standard surgical procedures[10]. In the exotic species, six implantations were made in each recipient mussel (two nucleated and four non-nucleated) whereas in the Indian mussel only four (two nucleated and two non-nucleated) mantle tissue implantations were made. All the mussels after implantations were subjected to post-operation care for one week. The implanted mussels were then transferred to the natural pond environment for rearing for a period of twelve months. Regular monitoring of the experimental stock was done with periodical sampling. In order to study the pearl sac content, microscopic examination of smears of the pearl sac fluid was carried out at regular intervals.

In the post-operation care phase, one recipient Indian mussel with exotic graft had rejected two nuclei along with grafts after 24 h of surgery. However, no rejections or mortality were encountered in other sets of mussels. Pearl sac formations were recorded after 15 days of implantation in Indian mussels with the xenografts and in exotic mussels with

Figure 1. ***a***, Exotic pearl mussel *H. cumingii*; ***b***, Indian pearl mussel *L. marginalis*.

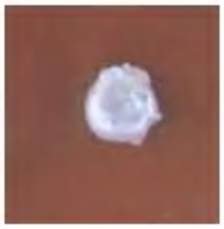

Figure 2. A non-nucleated pearl through xenogenic implantation with *H. cumingii* as recipient and *L. marginalis* as donor.

Table 1. Comparative data on the homogenic and xenogenic implantations between *L. marginalis* and *H. cumingii*

	Homogenic implantation		Xenogenic implantation	
	L. marginalis	*H. cumingii*	Recipient *L. marginalis* Donor *H. cumingii*	Recipient *H. cumingii* Donor *L. marginalis*
Pearl sac formed (%)	88	80	70	50
Dominance of pearl sac content	Aragonite	Aragonite	Calcite	Calcite
Pearl produced (%)	70	65	20	2
Calcite matter produced (%)	0	0	50	70

Figure 3. A few nucleated pearls through xenogenic implantation with *L. marginalis* as recipient and *H. cumingii* as donor.

Figure 4. A few non-nucleated pearls through xenogenic implanation with *L. marginalis* as recipient and *H. cumingii* as donor.

Indian pearl mussel grafts, pearl sac formation was observed in 20 days. Pearl sac formation through xenografting between *H. schlegelli*, *Cristaria plicata* and *Anodonta calipygos* takes 17 days. However, lower rate of pearl sac production has been reported in *Anodonta woodiana* as donor and *H. schlegelii* as recipient[11] which could be because of incompatibility between the donor and the recipient mussel. Homografting was made in Indian pearl mussels and exotic species separately, and within 15 days pearl sac was formed and sixty days of culture revealed 88% and 80% of functional pearl sacs respectively. In the pearl sac fluid, numerous minute crystalline structures of aragonite were observed, indicating the normal bio-mineralization process[12–14]. Through xenogenic implantation of *L. marginalis* (recipient) with *H. cumingii* (donor) grafts, a total of 70% pearl sacs were formed but *vice versa*, only 50% pearl sacs, a few of abnormally larger sizes were recorded (Table 1). The sampled pearl sac fluid with xenografts showed dominance of calcite over aragonite. The requirement of a total culture period of 12 months to complete nacre deposition over the implanted nucleus has been reported earlier[15]. However, three months of culture alone was required in the present experiment for nacre deposition. From the control sets, both nucleated and non-nucleated pearls could be recovered successfully after twelve months of culture, indicating compatibility between the implanted homograft and the host. From the recipient *H. cumingii* with indigenous grafts, only one lustrous silvery-white graft pearl was produced (Figure 2). Fifty percent of the retained nuclei had dull grey deposits and from the graft implantation site, chalky brittle masses were recovered. In the recipient *L. marginalis* with exotic grafts, a few nucleated pearls (Figure 3) and non-nucleated pearls (Figure 4) of pink-to-purple hue were retrieved. The retained implanted nuclei had calcite coating and separately numerous minute calcite graft pearls were recovered. Though the success rate of peals produced through xenogenic implantation was low (20% with *L. marginalis* as recipient and 2% with *H. cumingii* as recipient), the quality nacreous pearls produced supports the observation of Machii and Nakahara[16], indicating potentials. This study is the first attempt to indicate the possible role of xenografts in pearl mussel surgery and opens a potential avenue for further detailed investigation on graft–host relationships at the biochemical and cellular levels.

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Early diagnosis of white spot viral syndrome using rapid gill staining technique

White spot viral syndrome caused by the white spot syndrome virus (WSSV) is the major cause of mortality and morbidity in shrimps, resulting in huge losses to shrimp farmers in coastal areas. It has been reported that during 1994–95, this disease struck the booming aquaculture industry and caused a loss of around 20,000 tons of shrimp worth about Rs 250–350 billion[1]. This is a dreadful disease and is difficult to identify in the early stages. The diagnosis of viral infection is made possible with PCR and monoclonal antibody-based techniques[2,3], but these methods are expensive and the facility is not available everywhere. Early diagnosis will provide an opportunity to manage the disease in a comparatively better manner and will also help in preventing the rapid spreading of the disease. In general, the prevalence of WSSV can be observed by visual symptoms like appearance of white spots on the carapace and other external parts of the body. However, diagnosis at this stage of infection is of no use, as the shrimps start dying due to the disease. Thus, diagnosis of WSSV before it shows external symptoms is of practical significance. This will be useful at least to regulate the disease in the preliminary stage. In the present correspondence, a tool for rapid diagnosis of WSSV has been described as a reproducible protocol for the aqua industry. Diagnosis of the disease can be made by adopting wet squash preparation of the tissue and observing it through a microscope after fixing and staining the tissue for observation of hypertrophied nuclei (symptom for WSSV), as the WSSV is known to replicate in the nucleus of the host by forming inclusion bodies[4,5]. This is a novel method of identification of infection in the asymptomatic phase using a nuclear stain, developed in our laboratory based on the principles of existing stains. For developing the stain, the components were mixed in the proportions shown in Table 1.

For employing the rapid gill staining technique, live shrimps were collected from the culture ponds for experimental analysis. Staining experiments were conducted on the gill filaments obtained from shrimps showing external symptoms as well as those not showing any symptoms. However, the shrimps were collected from the same ponds. After plucking the gill filaments from the shrimps, they were placed on a glass slide for squash preparation and the smear was prepared using another glass slide. The gill filament was chosen because WSSV is a systemic ectodermal mesodermal baculovirus. The smear was fixed on the glass slide with gentle heating. After fixing the smear, the slide was flooded with the stain for 5 min. The glycerol component in the stain is useful to prevent the rapid drying of the stain and phenol will act as cytotoxicant for integration of stain. The slides were washed and observed under different objectives of the compound microscope for the occurrence of hypertrophied nuclei. Under the 40× objective, the early stages of infection with WSSV were observed; which revealed the occurrence of basophilic, blue-coloured, hypertrophied nuclei in the gill filament. This might be because of the onset of basophilic, central inclusions surrounded by marginated chromatin. In the later stages of infection, some of the eosinophilic nuclei have shown Cowdry type-A inclusions which are characterized by a central dark region followed by a clear zone of chromatin in circular shape (Figure 1). In the advanced stage of infection, large hypertrophied, basophilic and blue-coloured nuclei were distinctly seen (Figure 1). It was quite interesting to

Table 1. Composition of the developed stain

Component	Quantity
Trypan blue	0.60 g
Eosin Y	0.20 g
Phenol crystals	0.50 g
Sodium chloride	0.30 g
Glycerol	20.00 ml
Distilled water	80.00 ml
pH	7.0–8.0