International Journal of Natural and Social Sciences
Print: 2617-6637 < ISSN > Online: 2313-4461
IJNSS

Preparation of nuclei and optimization the size of Nuclei for pearl production in freshwater mussel, *Lamellidens marginalis* in Bangladesh

Mohammad Ferdous Siddique*, Mohosena Begum Tanu, Arun Chandra Barman, Md. Moniruzzaman, Sonia Sku, Nazmul Hossen, Yahia Mahmud

Bangladesh Fisheries Research Institute (BFRI), Mymensingh-2201, Bangladesh

ARTICLE INFO

Article history

Received: 25 January 2020
Accepted: 27 February 2020

Keyword

Stelon nuclei, shell bead nuclei, nuclei size, nuclei pearl, freshwater mussel

***Corresponding Author**

Mohammad Ferdous Siddique
siddique.bfri@gmail.com

ABSTRACT

No technique is yet developed in Bangladesh for the culture of nuclei pearl. The current research was conducted to prepare suitable sized nuclei and optimization the size of nuclei for nuclei pearl culture in Bangladesh at Bangladesh fisheries research institute (BFRI), Mymensingh from July 2017 to June 2018. During the experiment stelon nuclei produced from shells of *Lamellidens marginalis* and *L. corrianas* and shell bead nuclei produced from shells of native cockle *Anadara granosa* were inserted with three different sizes (2, 2.5 and 3 mm), tagged and cultured for 12 months in net bag hanging method. During 12 months of culture period maximum survival rate (45.33%) was found after insertion of 2 mm sized shell bead nuclei while 36.33% and 27.67% survival rate was found after insertion of 2.5 and 3mm sized shell bead nuclei. Besides Survival rate were 39.67%, 32.33%, 25.33% mussels with 2mm, 2.5mm and 3 mm in stelon nuclei insertion. Among the different type and sized nuclei 2mm shell bead nuclei produced from *A. granosa* was found suitable for native *L. marginalis* with highest nuclei pearl production rate (4.67%) and thick nacre layer (0.3 ± 0.05 mm). Nuclei pearl production rate with nacre layer was recorded 3.67%(0.1±0.2mm), 1.33%(0.1±0.03mm) for 2.5mm and 3mm shell bead nuclei while it was 3.33%(0.2±0.5mm), 2.67%(0.1±0.05mm) and 0.67%(0.1±0.01) for 2mm, 2.5mm and 3mm stelon nuclei respectively. Two mm sized shell bead nuclei from native marine mussel *Anadara granosa* recorded better quality pearl.

INTRODUCTION

Glittery, lustrous and colorful pearl is a precious gem, which is one of the most attractive objects of adoration and called the queen of the jewels all through the ages. Today there is a great demand for cultured freshwater pearls and China produces 95% of freshwater pearls sold in the world market (Prabu, et al. 2010). The initial Mise-Nishikawa method that was later developed by Kokichi Mikimoto and his company who brought round cultured pearls to the international market from 1919 onwards (Simkiss and Wada, 1980; Strack, 2006). Spherical shell beads are used as nuclei to produce round pearls. The nuclei used in the cultured pearl process are produced in Japan from shell belonging to the Family Unionidae, which includes some of the freshwater mussels and clams. The genera include Amblema, Quadrula, Pleurobema and Megalonais (Wada 1973). Nuclei should be made from the same crystalline morph of calcium carbonate as that coating them (aragonite) in order to be physically compatible (Roberts and Rose, 1989). The nucleus of a pearl, although it is not typically visible in a harvested pearl, is extremely important in the culturing process. Pearl nucleus manufactured from freshwater mussel shells has been made pearls into a perfect sphere and polished. Without a quality nucleus it is impossible to create a quality pearl. The method of culturing round pearls was exposed at the inauguration of the 20th century. Producing such cultured pearls required three things: a host oyster, a donor oyster's saibo (mantle tissue) and a nucleus (Taylor and Strack, 2008; Hänni, 2012). The grafted mantle cells slowly form a pearl sac around a spherical nucleus (pearl sac is complete after about 30 days, Cochennec-Laureau et al., 2010), which is responsible for secreting and depositing regular layers of nacre onto the nucleus and eventually leading to a cultured pearl. The basic method of forming such beaded cultured pearl didn't change much since its beginning. Bangladesh has large inland water bodies with area of 4760894 ha knowing the potentiality; BFRI has been started pearl culture from 1999 and successfully produced pearl in pearl producing native mussel such as *Lamellidens marginalis, L. corrianus, L. phenchooganjensis* and *L. jenkinsianus* (Tanu et al., 2019). In Bangladesh some studies has been documented the technique of rice pearl and image pearl

How to cite this article: Siddique M.F., Tanu M.B., Barman A.C., Moniruzzaman M., Sku S., Hossen M.N. and Mahmud Y. (2019). Preparation of nuclei and optimization the size of nuclei for pearl production in freshwater mussel, *Lamellidens marginalis* in Bangladesh. International Journal of Natural and Social Sciences, 7(1): 86-92. DOI: 10.5281/zenodo.5295651

culture. Unfortunately preparation of nuclei and optimization the size of produced nucli for the nuclei pearl culture in native mussel has yet not be developed. Considering above context the present study was undertaken for preparing nuclei and production of pearl inserting the nucle into the mantle of the freshwater mussel *L. marginalis*.

MATERIALS AND METHOD

Experiment was conducted at Bangladesh Fisheries Research Institute (BFRI), Mymensingh. Shells of Freshwater Uninoids species *L. marginalis* and *L. corrianas* were collected from different ponds, lakes and rivers of Bangladesh. Shell size of collected specimens was measured with vernier calipers. The first type of nucleus (stelon beads), was prepared by using commercially available araldite glue (hardener and resin). This nuclear material was made from the dead shell pieces of the freshwater mussel *Lamellidens marginalis* and *L. corrianas.* For the second type of nuclei (shell bead) shells of *Xancus pyrum, Anadara granosa, Mercenaria mercenaria, Melo melo, Strombus canarium, Tridacna squamosa, Lobatus raninus* collected from Sonadia and Moheshkhali, Cox's Bazar. The equipment for the process was designed from Velu et al., 1973 and Taburiaux, 1985.

Stelon Nuclei

For the preparation of the stelon nucleus, dead shells of *L. marginalis* and *L. corrianas* was collected and subjected to thorough washing in water to remove dirt and sand particles. The dry flesh materials were scraped out. The shells were then dipped in 5000 ppm chlorine solution (50g of bleaching powder containing 10% chlorine in 0.1 liter of water) for twenty four hours to forty eight hours. The completely lye- peeled shells were sorted out and then continuously washed in tap water. These were then kept in an oven maintained at 60°C, for more than two hours and then sun dried, for a longer duration to ensure the complete removal of chlorine from the treated shells. The dried shells were made into small pieces by using a mortar and then finely grinded by means of an electric grinder. The powdered shells were then processed through a sieve of 0.01 - 0.05 mm mesh size. The commercial glue Araldite hardener and resin (that acts as a binder) was mixed in a ratio of 1:1 to prepare a paste. To this paste the sieved shell powder was added gradually to prepare dough of thick consistency. The ratio of the shells powder to the paste was 5:1. Immediately, nuclear material of desired shape and size was prepared and allowed to air dry. Later, they were stored in dust free close containers. The nuclear material was boiled in water, air dried and cooled few hours prior to implantation. Following the above procedure three sizes (2mm, 2.5mm and 3mm of diameter) of nuclei were made.

Shell Bead Nuclei

Locally available Shells of *Xancus pyrum, Anadara granosa, Mercenaria mercenaria, Melo melo, Strombus canarium, Tridacna squamosa, Lobatus raninus* were processed into spherical beads of different diameters, generally 2–3mm, through cutting, grinding, shaping and polishing using appropriate machines and tools. The equipment (Figure 1) consisted of two grinding wheels, fixed inside suitable housings, and fitted on to an ordinary bench drill worked with a 0.75—1 H.P. electric motor. Two pairs of carborundum wheels with different layers of grooves were used. The housing of the top wheel provided with a taper shank which was inserted into the spindle of the drill. The bottom wheel was larger and fixed on to the platform of the drill which oscillates to and fro from the axis of the pillar of the drill with a sliding action. A metal guard provided in the housing of the upper wheel prevents the beads, kept in between the wheels during grinding, from being scattered due to centrifugal action.

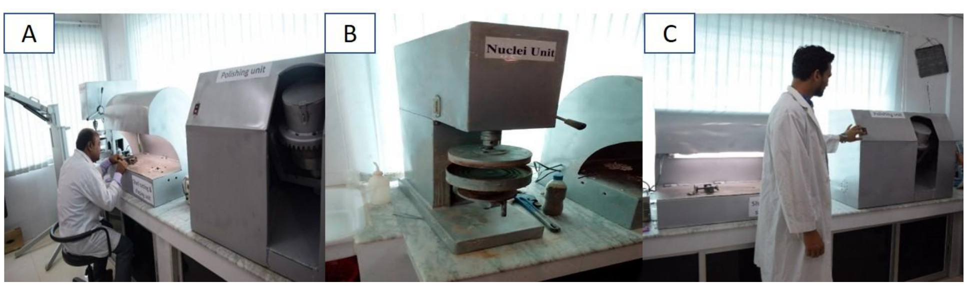


Figure 1: Nuclei making equipment A. Shell cutting unit B. Nuclei unit C. Polishing unit

Nuclei making procedure

The shells were cut into small pieces with a chisel and the sharp edges were ground off with an ordinary grinder. These were roughly sorted out into different sizes. Ground pieces of more or less similar sizes were then placed on the bottom wheel and the top wheel was lowered until it begins to touch the shell pieces. The rough-surfaced wheels were used first. When the motor is switched on, the top wheel rotates at 1500-2000 rpm, while the bottom wheel oscillates at a frequency of 50-60 oscillations per second. A gentle flow of water on the wheels is maintained so that the shell powder was turned into a paste which remains in between the wheels to facilitate smooth grinding. Due to simultaneous rotary and oscillatory movements of the upper and lower wheels, the pieces of shell were turned into spherical beads. The diameter of the beads was controlled by adjusting the gap in between the two wheels by means of a lever. The beads were sorted out and again fed into the machine after replacing the first set of wheels by smooth-faced wheels. The beads obtained was White and spherical and have a smooth surface. They were treated with a concentrated solution of hydrochloric acid/ nitric acid for 5 minutes, after which they were thoroughly washed. The shell beads were then polished with 30% HCl for 30 minutes for polished appearance in a wooden barrel placed at 45^0angle with a rotation of 500rpm. Following the above procedure three sizes (2mm, 2.5mm and 3mm of diameter) of shell bead nuclei were made.

Standardization of nuclei material

Different sized (2mm, 2.5mmand 3mm of diameter) of the both stelon and shell bead nuclei and collected standard nuclei was assessed to know the roundness, dimensional accuracy, thermal expansion characteristics, effect of drilling and specific gravities. Roundness was assessed by measuring the largest and smallest diameters of the nuclei with a dial micrometer. The thermal expansion characteristics (below 140°C) were determined for each nucleus type. The effect of drilling was assessed by drilling up to 3 holes in different directions in a number of nuclei. The specific gravities of the three nucleus types were compared by measuring the volume of water displaced by a known mass of each material.

Standardization of nuclei size

For standardization of nuclei size mussels were inoculated with produced different sized (2mm, 2.5mm and 3mm of diameter) of both stelon and shell bead nuclei (Table .2)

Mussel collection

Mussels (*L. marginalis*) were collected randomly from ponds of Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh. *L. marginalis* was selected as it was identified the best pearl producing mussel species for mantle transplantation in Bangladesh by Hossain et al., *(*2004). For nuclei transplantation young, healthy and strong mussel with broad and distinct growth line and without disease and injury were selected. The average length, width and age of sorted mussels were 9.0 cm, 5.0 cm and 1-1.5 years respectively.

Mussel rearing

Ponds with sandy soil, clean water and pollution free bottom were selected for the rearing of mussels. To stimulate the growth of plankton, organic and inorganic fertilizer was applied fortnightly. The doses of organic and inorganic fertilizer were 5 kg organic manure, 0.125kg T. S. P. and 0.1kg urea per decimal. Lime was applied at 0.5kg/decimal fortnightly to keep the pH in optimum range. These mussels were reared in pond until transplantation process.

Grafting tools and chemicals

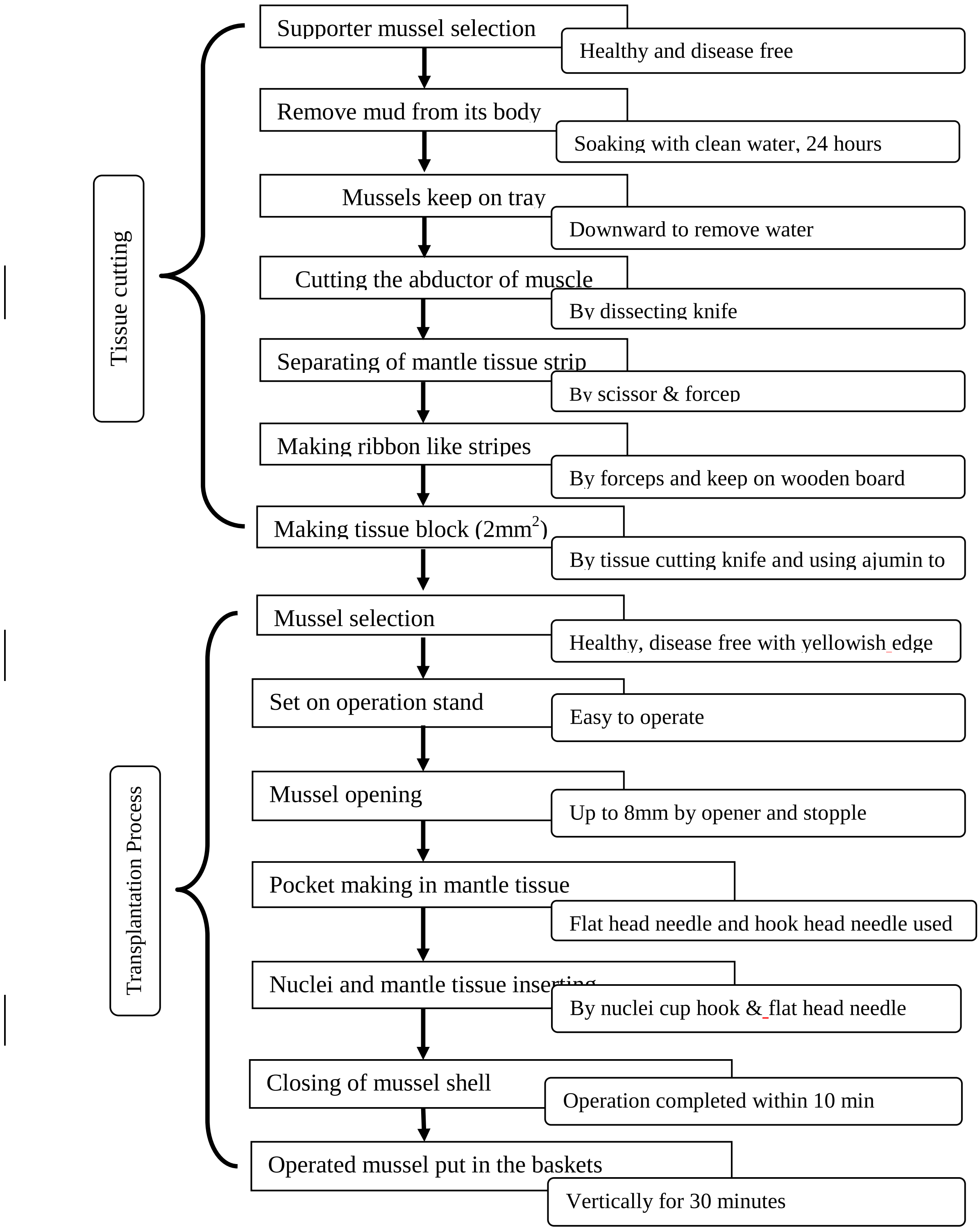
Different types of tools and chemical were used for grafting of mussels (Tanu et al., 2019). These are mussel dissecting knife, needle, forceps, sponge, glass board, tissue cutting knife, mussel opener, stopple, flat head needle, hook head needle, operating stand, tray, dropper ajumin, and 70% alcohol. The best uses for these tools and chemicals are stated in Figure 1.

Pre conditioning

Before transplantation, mussels were kept without food for 7 days to remove the inner mud and dirt from the mussel body. Then the mussels were brought to laboratory and kept them in downward position on a porous basket to remove the water.

Inoculation and culture of grafted mussels

The mussels were divided into two groups; the supporter and the recipient mussels. For inoculation and culture of grafted mussels three steps were followed: Tissue cutting, Transplantation Process and Post conditioning. The details of these processes are described in Figure 1.


Tissue cutting
Supporter mussel selection
Healthy and disease free
Remove mud from its body
Soaking with clean water, 24 hours
Mussels keep on tray
Downward to remove water
Cutting the abductor of muscle
By dissecting knife
Separating of mantle tissue strip
By scissor & forcep
Making ribbon like stripes
By forceps and keep on wooden board
Making tissue block (2mm2)
By tissue cutting knife and using ajumin to
Transplantation Process
Mussel selection
Healthy, disease free with yellowish edge
Set on operation stand
Easy to operate
Mussel opening
Up to 8mm by opener and stopple
Pocket making in mantle tissue
Flat head needle and hook head needle used
Nuclei and mantle tissue inserting
By nuclei cup hook & flat head needle
Closing of mussel shell
Operation completed within 10 min
Operated mussel put in the baskets
Vertically for 30 minutes

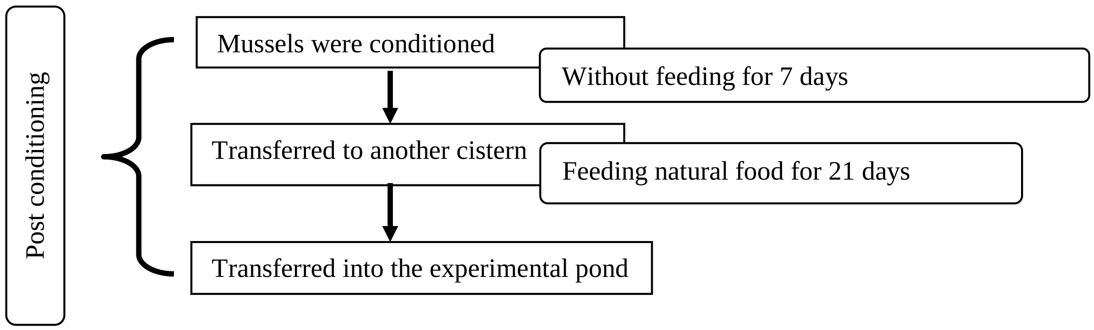


Figure 1: Procedure for inoculation and culture of grafted mussels

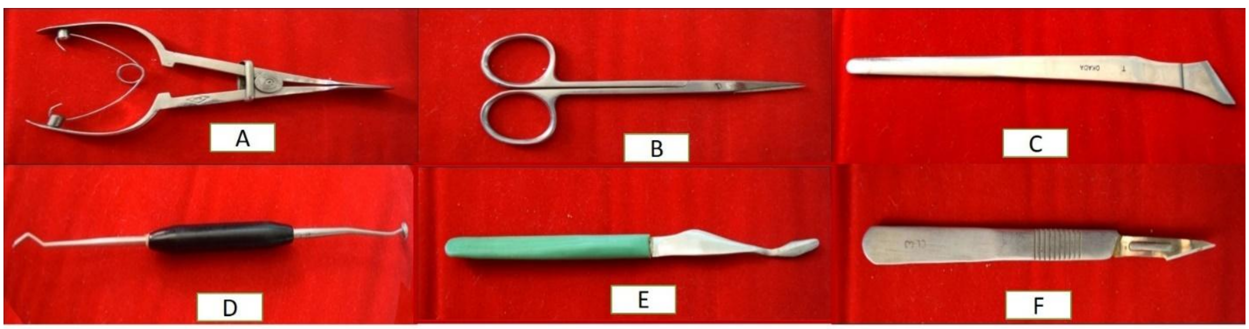


Figure 2: Instruments used for nuclei Nuclei inoculation in *L. marginalis*A. Mussel opener B. Scissor C. Mantle tissue cutter D. Nuclei cup hook E. Tissue adjusting oar F. Scalpel

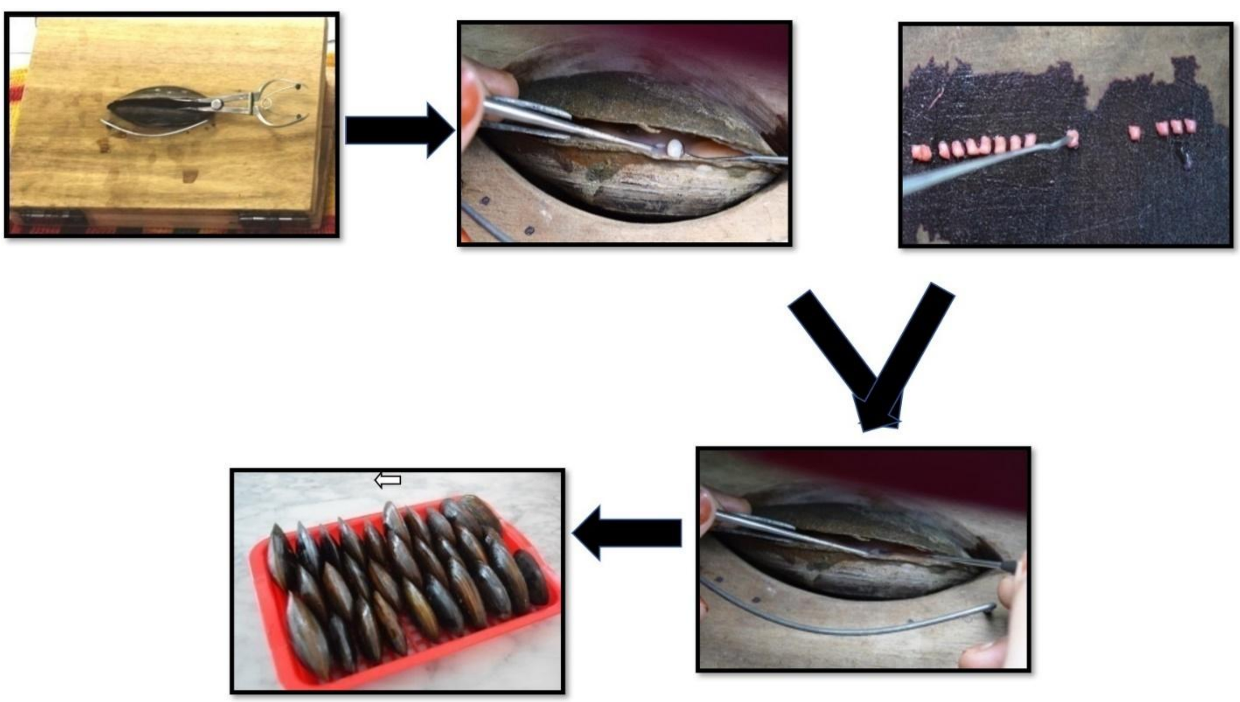


Figure 3: Steps of Nuclei inoculation in *L. marginalis*

Table 1: Design of the experiment for standardization of nuclei size

Culture method	Nuclei type	Nuclei size(mm)	Mussel used for pearl production
Net bag hanging	Stelon nuclei	2	*Lamellidens marginalis,*
		2.5	
		3	
	Shell bead nuclei	2	
		2.5	
		3	

Mussel stocking and management

After one month post-operative care, the operated mussels were kept in the culture ponds of Freshwater Station, Mymensingh. Research pond was splitted by bamboo fence (bana). These inoculated mussels were then kept in cistern for post-operative treatment and then transferred to the culture pond for 12 months. in net-bag hanging method. Stocking density of mussels and fish was 80 mussels/decimal and 30fish/decimal (*Catla catla* 6, *Labeo rohita* 10, *Cirrhinus cirrhosus* 10, *Labeo calbasu* 4) respectively for maximum use of the water body. Three transplanted mussels were stocked in a net bag and hanged from a rope at 30-35cm depth with float. The rope stretched across the pond in the surface of water. The distance between adjacent two bags were 25-30cm and two hanging rope were 1.5m.

Organic and inorganic fertilizer was given fortnightly to the pond at the rate of 5kg cow dung, 0.125kg T.S.P and 0.1kg urea per decimal. Liming was applied fortnightly at the rate of 0.5kg dolomite/decimal. Survival rate of the operated mussel will be monitored once in a month. Water temperature, pH, plankton growth, and organic matter, NH_4-N, DO and Ca^{2+} was monitored fortnightly. After 12 months culture, survival rate and pearl production rate of the operated mussel were observed.

RESULT AND DISCUSSION

Nuclei preparation

A total of 1,500 nos stelon nucleus with a size of 2, 2.5and 3 mm was prepared in the Pearl laboratory, BFRI, Mymensingh from dead shells of shells of *L. marginalis* and *L. corrianas.* Besides another 3,500 nos of shell bead nuclei were made from shells of *Xancus pyrum, Anadara granosa, Mercenaria mercenaria, Melo melo, Strombus canarium, Tridacna squamosa, Lobatus raninus.* The roundness, dimensional accuracy, thermal expansion characteristics and effect of drilling. Roundness was assessed by measuring the largest and smallest diameters of the nuclei with a dial micrometer. The thermal expansion characteristics (below 140°C) were determined for each nucleus type. The effect of drilling was assessed by drilling up to 3 holes in different directions in a number of nuclei. It was observed that shells of native marine cockle *Anadara granosa* was found preferable for its shell thickness, hardness and availability. In case of stelom nuclei dead shells of dead shells of shells of *L. marginalis* and *L. corrianas might* be used for nuclei production.

Optimizing nuclei size

Each 300 mussels were operated with different sizes (2mm, 2.5mm and 3mm) stelon and shell bead nuclei. Survival rate were 39.67%, 32.33%, 25.33% mussels with 2mm, 2.5mm and 3 mm stelon nuclei while 45.33%, 36.33% and 27.67% with 2mm, 2.5mm and 3mm shell bead nuclei respectively (Table 2). Among the different type and sized nuclei 2mm shell bead nuclei size was found suitable for native *L. marginalis* with highest nuclei pearl production rate (4.67%) with nacre layer of 0.3 ± 0.05 mm. Nuclei pearl production rate with nacre layer was recorded 3.67%(0.1±0.2mm), 1.33%(0.1±0.03mm) for 2.5mm and 3mm shell bead nuclei while it was 3.33%(0.2±0.5mm), 2.67%(0.1±0.05mm) and 0.67% (0.1±0.01) for 2mm, 2.5mm and 3mm stelon nuclei respectively.

Table 2: Nuclei pearl production

Nuclei type	Size of nuclei (mm)	No. of operated mussel	Survival rate (%)	Nuclei containing mussel	Nuclei pearl number	Nuclei pearl production rate (%)	Nacre layer (mm)
Stelon nuclei	2	300	39.67	10	10	3.33	0.2±0.5
	2.5	300	32.33	8	7	2.67	0.1±0.05
	3	300	25.33	2	2	0.67	0.1±0.01
Shell bead nuclei	2	300	45.33	14	14	4.67	0.3±0.05
	2.5	300	36.33	11	11	3.67	0.1±0.2
	3	300	27.67	4	4	1.33	0.1±0.03

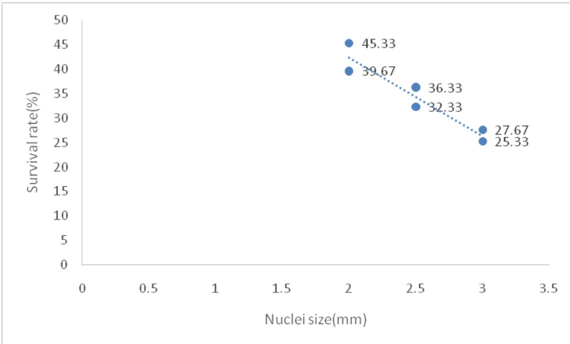


Figure 4: Survival rate in different sized shell nuclei inoculation

Miah et al., (2000) found 80% mussel survival rate by inserting stone, fish eye (without any mantle tissue) in *L. marginalis* for one month rearing and recorded highest pearl production in stone and lowest in the sand. In mantle cavity implantation nacreous layer was found 0.35 and 0.20 mm respectively (Pandey and Singh 2015). Rahayu et al., (2013) implanted shells nucleus of 10 mm diameter and observed the highest layer thickness of 17 µm after 9 months cultivation of freshwater mussel *Anodonta woodiana*.

CONCLUSION

The size of the pearl depends on the nucleus used to make the pearl. The nucleus is very important in the production of spherical pearls. Stelon nuclei can be produced from the dead shells of locally available *Lamellidens marginalis* and *L. corrianas*. Shell bead nuclei produced through the designed nuclei making machineries with native cockle *Anadara granosa* with a size of 2mm resulted best with highest survival rate, nuclei pearl production and thick nacre layer formation although the number of nuclei containing survived mussel was low and the rejection rate was high. More research work should be done to establish suitable production of both stelon and shell bead nuclei which is important for the future of sustainable pearl culture in native mussel.

REFERENCES

Cochennec-Laureau N, Montagnani C, Saulnier D, Fougerouse A, Levy P, Lo C (2010). A histological examination of grafting success in pearl oyster *Pinctada margaritifera* in French Polynesia. Aquatic Living Resources, 23(01), pp. 131-140.

Hanni HA (2012). Natural pearls and cultured pearls: A basic concept and its variations. *The Australian Gemmologist*, 24(11): 256-266.

Hossain MA, Sultana N, Azimudin K, Hussain MG and Mazid MA (2004). Selection of freshwater pearl mussel species for mantle transplantation in Bangladesh. Bangladesh Journal of Fisheries Research, 8(2): 113-116

Miah MI, Rahman ASMK, Rahmatullah SM, Saha JK and Islam MA (2000). Culture of pearl in freshwater mussels *(Lamellidens marginalis).* Bangladesh Journal of Fisheries Research, 4: 57-60.

Pandey A and Singh A (2015). Freshwater pearl culture: Scope and importance in North West States of India Department of Aquaculture. College of Fisheries, Guru Angad Dev Veterinary and Animal Science University, Ludhiana (Punjab) India. 10(2):43-45.

Prabu LD, Arun Sudhagar S and Ferosekhan S (2010). Fresh Water Pearl Production Integrated with Ornamental Fishes: For Profit Motive. Aquafind. URL: aquafind.com/articles/Freshwater-Pearls.

Rahayu SYS, Solihin DD, Manalu WN and Affandi R (2013). Nucleus pearl coating process of Freshwater mussel *Anodonta woodonia* (Unionidae). Journal of Bioscience, 20: 24-30

Simkiss K, Wada K (1980). Cultured pearls—commercialized biomineralisation. Endeavour, 4(1):32-37.

Strack E (2006). Pearls. Ruhle-Diebener-Verlag, Stuttgart, Germany.Strayer D.L., Downing J.A., Haag W.R., King T.L., Layzer J.B., Newton.

Strayer DL, Downing JA, Haag WR, King TL, Layzer JB,, Newton TJ and Nichols SJ (2004). Changing perspectives on pearly mussels, North America's most imperiled animals. BioScience, 54(5):429-439

Taburiaux J (1985). Pearls–Their Origin, Treatment and Identification. Chilton Book Company, PA, USA, 247.

Tanu MB, Barman AC, Siddique MF, Sku S and Mahmud Y. (2019). Production and quality of mantle tissue transplanted pearls in *Lamellidens marginalis* cultured in different locations of Mymensingh district, Bangladesh. Journal of the Bangladesh Agricultural University, 17(3), 424–429.

Velu M, Alagarswami K and Qasim SZ (1973). Technique of Producing Spherical Shell Beads as Nuclei for Cultured Pearls. Indian Journal of Fish, 20 (2): 672-676.

Wada K (1973). Modern and Traditional Methods of Pearl Culture. Underwater Journal, February, 28-33.