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Nucleus Pearl Coating Process of Freshwater Mussel *Anodonta woodiana* (Unionidae)

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The limiting factor which is a weakness of sea water pearl production are high costs, the risk of major business failures and a long coating time. From the issue of freshwater pearls appear to have prospects of alternative substitution for sea water pearl. This present study aimed to evaluate effect of loads (the number and diameter nucleus) on freshwater pearl coating process and the number and size of the appropriate nucleus diameter, to produce the optimum coating thickness of half-round pearls. The research consists of experimental implantation of 2, 4, and 6 nucleus number per individual mussel was maintained by the method stocked in hapa in bottom waters. Observation method and factorial randomized block design used in the study of the influence of the load to the successfulness of pearl coating and the pearl layer thickness. The results showed that *A. woodiana* can be utilized as a producer of freshwater pearls. In addition, the number of optimum nucleus that can be attached to the mussel *A. woodiana* was 2 grains/individuals with a diameter of 10 mm. Shells implanted with the optimum nucleus diameter and number of pearls produced the highest layer thickness of 17 µm after 9 months cultivation. This result was good enough compared with the layer thickness of sea water pearl production after the same cultivation time.

Key words: *Anodonta woodiana*, blister pearl, coating process

INTRODUCTION

Pearl business world initially dominated by sea water pearls. At the national and international markets, blister pearls have a specific market segment and are generally produced by the oyster species *Pteria penguin*. The limiting factor which is a weakness of sea water pearl production are high cost, the risk of major business failures and a long forming time, starting from 1.5 to 3 years. From the issue of freshwater pearls appear to have prospects of alternative substitution for sea water pearls because the color varies, production costs are relatively low, and the formation of a relatively short time, less than 1 year (Rachman *et al*. 2006). Currently China is the supplier of 95% of the world's freshwater pearl production (Mamangkey *et al*. 2009). Pearl is produced from shellfish (mussel) *Hyriopsis cumingii* with production area in almost all freshwater areas in the country (Dan & Ruobo 2002). In Japan, since 1949, freshwater pearl mussels produced by other species, namely *Hyriopsis schlegeli* (Rachman *et al.* 2006; Rahayu *et al*. 2009). Other type of shells that resemble the morphology of *Hyriopsis* sp. by Moorkens (1999) is *Anodonta* sp. (Family Unionidae).

Physiologically, *A. woodiana* have the ability to produce nacre and the prismatic crystal, pearl producer (Ram & Gayatri 2003). Attachment of nucleus will induce blister on mussel shell to form a blister pearls. In addition, the microstructure and composition of amino acids influence the formation of shell and pearl or nacre layer. According Suwignyo *et al*. (2005), *A. woodiana* was easily breed. This mussel has a high reproductive capacity because it can breed more than once a year. Anodonta woodiana in Taiwan only spawn in the summer, but in Indonesia

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this type spawn any time of the year (Rahayu *et al*. 2009).

Shellfish aquaculture activities of *Margaritifera* sp. was in pond nature, have been conducted by the Center for Freshwater Aquaculture Development (BBPBAT) of Sukabumi, West Java, since 2006. Best blister pearl layer thickness for 8 months of maintenance is at 6 µm with a survival rate of 83.3% and the highest percentage of the results of implantation of 93.3% at 30 cm depth of the pool. However, at the end of maintenance obtained only 40 individuals which was implanted or target was reached only 12.5% (Rachman *et al*. 2006). Thus, the type of shellfish is not recommended to be developed as a producer of freshwater pearls.

A. woodiana first discovered in Indonesia in 1971 in Cibalagung Inland Fisheries Research Institute, Bogor. Although the shells of this type is the introduction animal, *A. woodiana* has long been adapted and also has great economical and ecological potencies in Indonesia (Hamidah 2006). *A. woodiana* live in public pond areas as benthic population abundantly. The waters condition have a prospect for a culture development of this mussel. According to Aldridge (1999), *Anodonta* sp. have a faster growth when it compared with *Margaritifera* sp. because it requires a faster time for the formation of shell layers. The fastest growth rate was shell height and the slowest one found on the shell thickness.

If we see a more detailed anatomy and tissue biochemical processes, it turns out *Anodonta* sp. crystaline able to deposit calcium carbonate ($CaCO_3$) in the form of aragonite crystals, known as nacre, and the fundamental building blocks of crystalline hexagonal prismatic layer calsite conchiolin ($C_{32}H_{48}N_2O_{11}$) on the inner shell layer. This is a potential biological advantages that can be used to produce freshwater pearls (Aldridge 1999). Lately, these kinds of shells used for pearl production (Berni *et al*. 2004). This present study aimed to evaluate effect of loads (the number and diameter nucleus) on freshwater pearl coating process and the number and size of the appropriate nucleus diameter, to produce the optimum coating thickness of half-round pearls.

MATERIALS AND METHODS

Mussel Samples and Blister Nucleus. Mussel tests used in this study is mussel *A. woodiana* derived from Cisaat, Sukabumi. The amount was 480, which was 360 for treatment, and 120 for control. The average size of 12 cm shell length, shell width 8 cm and the average individual weight of 290 g. Nucleus half-round pearl formation as a starter made of beads made from acliric, with a nucleus diameter of 10 and 12 mm and high of 6 mm (Figure 1).

Implantation Procedure of Half Round Pearl Nucleus Activity Stages. Implantation of half-round pearl nucleus (Winanto 2004) begins with preparation of mussel parent, implantation equipment and nucleus half-round pearls. Before implantation, mussel length measured with digital calipers and weighed with a digital balance. Next nucleus is placed in the shell holder. Then the mussel shell was opened with a shell mounted opener and peg (wedge) between both of shells so it was not close again. Then the coat hook is revealed by a spatula with a hook.

Furthermore, the inner shell of mussel (nucleus location will be attached), dried and cleaned with cotton. Then the nucleus is taken with the carrier, then the bottom was given a drop of cyanoacrylate adhesive. Then the nucleus is placed and attached to the pallial line (the inner shell growth lines) on the position in the middle of pallial line with a nucleus number (Figure 2A); on the left and right pallial line with the nucleus number of two with distance of 3 cm (Figure 2B); and on the left, center and right pallial line with the nucleus number of three with distance of 2.5 cm (Figure 2C).

After the nucleus is attached properly, the coat is returned to its original position and the shell closed. Mussel which has been implanted subsequently immersed in 10 ppm solution of KMnO4 for 2 hours in the basin of fiber, as prevention of infection after implantation. After the treatment described above, then these mussel was conditioned inside the hapa for 2 weeks, to know that death and release nucleus. Mussel who filled the nucleus and is still alive then fed into the hapa with a density of 40 individuals/ hapa. While in the control pond, mussel that are not implanted into the hapa stocked with a density of 40 individuals/hapa.

Activity Goals. This activity aims to evaluate: (i) the influence of load (number and size of the nucleus diameter) of the physiological processes in freshwater pearl coating, (ii) biomineralization process on the precise number and size of nucleus, to produce optimum coating thickness of half round pearls.

Expected Activity Results. The implantation techniques are expected to be an implantation standard technique of freshwater pearls mass production in Indonesia.

Data Analysis. Data results of the survival, growth and pearl coating process study was analyzed by analysis of variance (ANOVA). To view the main effect of the differences among the treatments

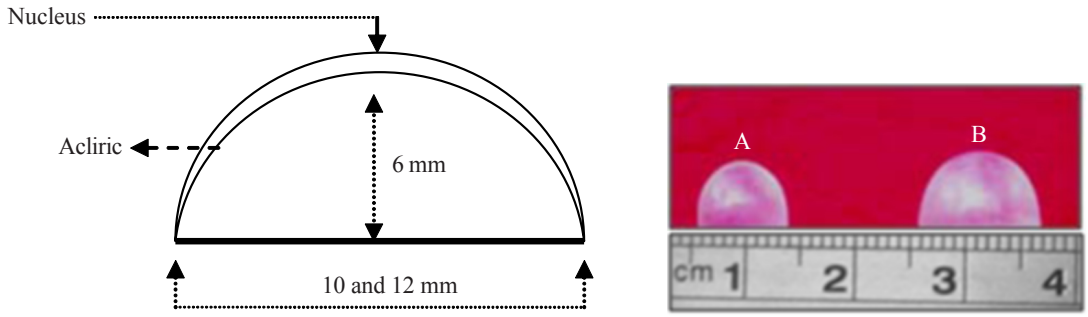

Figure 1. Nucleus half-round pearls were used in the experiment (side view) in diameter (A) 10 and (B) 12 mm.

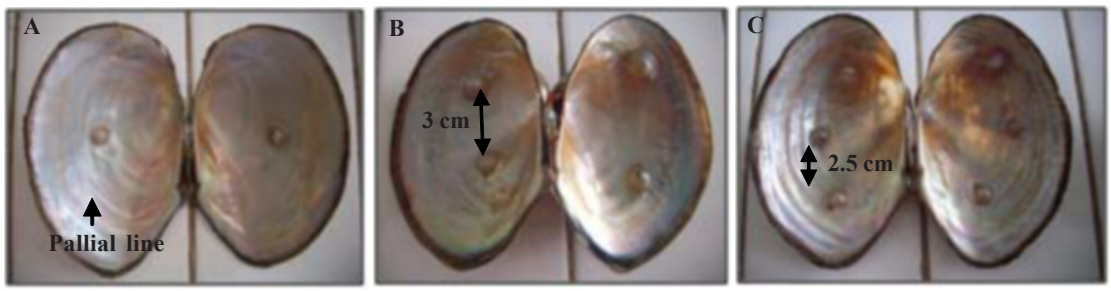

Figure 2. The position of the laying of the nucleus half-round in shell (A). *woodiana* position in the middle pallial line (B) on the left and right pallial line and (C) on the left, center and right pallial line.

Table 1. Pearl layer thickness (µm) of A. *woodiana* implanted by treatment with 0, 2, 4, and 6 nucleus per individual and size of 10 and 12 mm during maintenance

Nucleus diameter (mm)	Pearl layer thickness (i m) Total nucleus/individual			Mean
	2	4	6	
10	17.00 ± 1.00	9.33 ± 1.53	4.00 ± 1.00	10.11 ± 5.75[a]
12	12.67 ± 1.15	5.33 ± 1.53	1.33 ± 0.58	6.44 ± 1.63[b]
Mean	14.83 ± 2.56[a]	7.33 ± 2.58[b]	2.67 ± 1.63[c]	

Different letters in the same row and column, indicate significant difference between treatments ($P < 0.05$) at the 0.05 level.

continued with Tukey test, at 5% level of confidence interval. Analysis of interaction between the nucleus diameter is done by orthogonal polynomial contrasts further test, at 5% level of confidence interval. Data processing was performed using SPSS software version 17 for Windows. This analysis is used in order to predict or estimate the quantitative effects of a treatment of test animals.

RESULTS

The Thickness of the Pearl Layer. Results of mussel pearl coating thickness measurement with a different number and diameter of the nucleus during the maintenance period of 9 months appeared to be seen in Figure 3 and 5. Decrease in the thickness of layers of pearls occur along with the increasing number and diameter of the nucleus. The results of analysis of variance showed that the number and size of the nucleus significantly affect the thickness of the layer of pearl ($P < 0.05$) and there was no interaction effect ($P > 0.05$) between the number of nucleus with its size. Treatment I (nucleus number of 2 nucleus per individual) and size of 10 mm has the highest value of the pearl layer thickness compared with the other treatments (Table 1).

Diameter and Distance Among Stem Cell Room Mantel. Histologic analysis of the specific characteristics of mantle structure are analyzed in control (without giving the nucleus) and treatment (2, 4, and 6 nucleus per individual and 10 and 12 mm diameters). Histological analysis showed the existence of mantle cell network file Mussel-shaped rod (column), because the mantle is composed of a network file rod-shaped cells.

Based on the measurements under a microscope with a magnification of 50 x then seen that the comparison between control and treatment from the beginning (0 months) until the end (9 months plating), cell size became larger but the density becomes less and less (Figure 4). From the results shown a decrease in diameter stem cell coat along with the increase in the number and diameter of the nucleus.

Comparison of diameter and distance between the living mantle tissue stem cells between control and treatment showed that the high number and size of the nucleus resulted on the small both of stem cell diameter and growth distance of mantle tissues. Increasing the load resulted in increased levels of stress that interfere with the growth of mussel mantle, as shown by the decreasing diameter and increasing

Figure 3. Pearl layer thickness on (I) 3 months, (II) 6 months and (III) 9 months.

the distance between the space of mussel mantle tissue stem cells.

The Successfulness of Pearl Coating 3, 6, and 9 Months. Results of measurement of successfulness pearl coating Mussel with different diameters of the nucleus and during the maintenance period of 9 months appear in Figure 6. There is a tendency that the successfulness of pearl coating decreases with increasing number and size of the nucleus.

DISCUSSION

Histological analysis of control with a coat of mussel implanted in this study also showed impaired

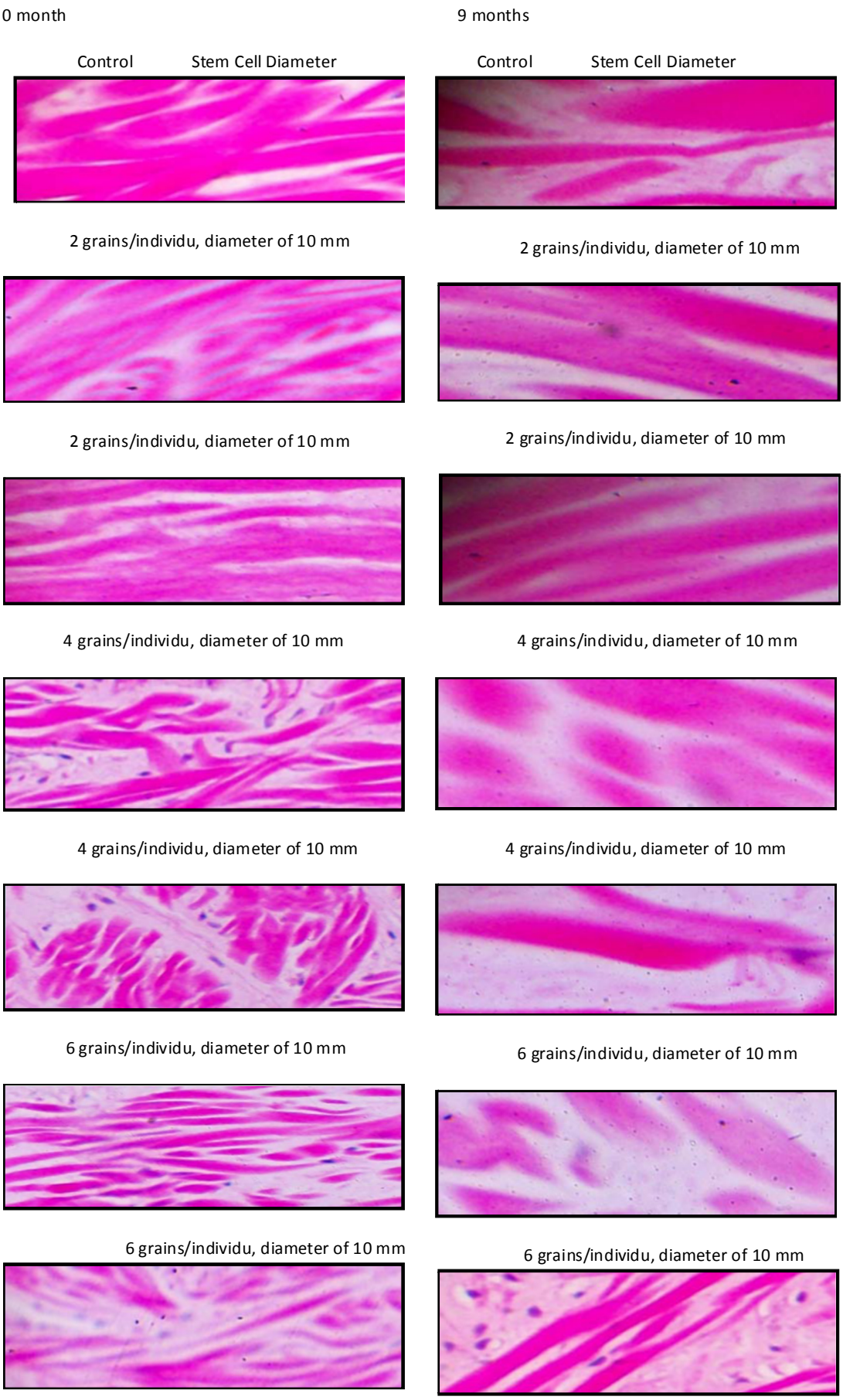

Figure 4. Histologic analysis of mantle from 0 month until the end (9 months) cultivation.

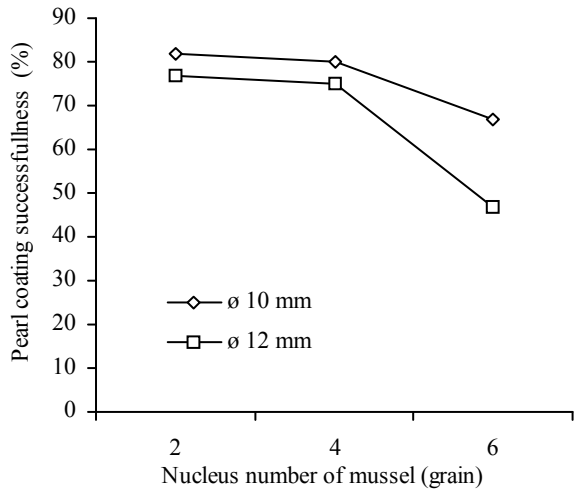

Figure 5. The successfulness of pearl coating (%) implanted with mussel treatment of 2, 4, and 6 nucleus per individual and diameter of 10 and 12 mm during cultivation.

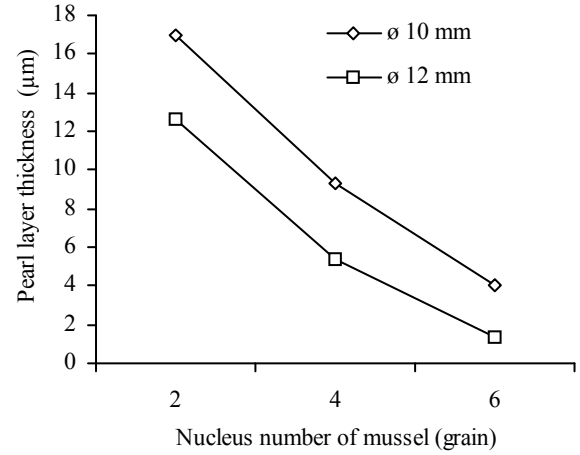

Figure 6. Pearl layer thickness (m) mussel implanted with treatment of 2, 4, and 6 nucleus per individual and diameter of 10 and 12 mm during cultivation.

growth due to mussel nucleus insertion. According to Salmon *et al*. (2005) *Pinctada fucata* which has been given 500 MGL-1 benzocaine and the edge of his coat was taken, showed 100% survival rate after 4 weeks of treatment. Oysters have regenerated from the missing mantle tissue. After three weeks, histologic analysis of the coat showed a complete regeneration of the mantle and its structure. This discovery proves that the oyster mantle tissue recovered from the shooting coat and can be used as backup host for the oyster farming operation. In the molecular phylogeny of giant clams research, at the same condition, a small piece of mantel tissues was cut off from seven species i.e. *T. crocea*, *T. maxima*, *T. squamosa*, *T. derasa*, *T. gigas*, *Hippopus hippopus*, and *H. porcellanus* were collected from several locations in the Indonesian Archipelago during the field trips in 2004 and 2005. They carried out under water in order to minimise the sampling impact (Nuryanto *et al*. 2007).

In the tropics like Indonesia, where this research located with high temperatures almost all year, the secretion of nacre faster than in the four seasons (Alagarswarni *et al*. 1991). According to Kripa *et al*. (2007) on oyster *Pinctada fucata* is possible to produce layers of pearls within 10 months of maintenance. Thickness deposition of pearl in the Southwest coast of India to 9 times higher than the deposition in Japanese waters and 2.2 to 2.3 times than in the Southeast Indian Coast. The quality of pearls produced are also higher when the nucleus size of 5 mm in diameter with a total percentage coating of 72.4% and only 13.7% are not coated with nacre. Nacre deposition is strongly influenced by water temperature, at high temperatures will accelerate the deposition of nacre, while low temperatures reduce the pearl coating, but increases the quality of pearls (Barik *et al*. 2004). Result of physical water quality measurement of this research, media temperatures ranging from 25.10 to 25.90 °C. It contributed to the speed of nacre secretion that produce a thick layer of pearl as stated by Winanto *et al*. (2009). According to Suwignyo *et al*. (2005), A. *woodiana* live well in waters with temperature of 24 to 29 °C.

According to Table 1, in this study the number and size of the nucleus is inserted in the mussel *A. woodiana* significantly affect the percentage of pearl coating. This is in line with the statement above, namely the amount and the optimum nucleus diameter (2 per individual nucleus, diameter 10 mm) produced the highest percentage of pearl coating. Despite having the speed advantage pearl coating process, implantation technology still needs to be improved (Kripa *et al*. 2007).

There is no case folds results of this study. This may be caused by the implanted nucleus form of non-nucleus round blister. According to Kripa *et al*. (2007), production of Akoya pearl oyster Pinctada fucata in the Southwest Coast of India, producing a percentage of nacre deposition on the implanted nucleus is 4 µm per day (in nucleus diameter of 5 mm) and 3 µm per day (diameter 6 mm). Among the crop that is not used (reject) are usually due to thin layers of nacre (without coating after 317 days of maintenance) is also available creases/bumps coat on one side or two opposite sides on the oyster shell. Some of these mirrors is a dry tissue, as part of a low calcification, but generally these folds is the uneven deposition of nacre. In *P. margaritifera*, calcified bulge is called a "tail" and found to contain inflammatory cells that die (Gervis & Sims 1992;

Friedman & Southgate 1999). Salmon *et al*. (2005) states that in the mantle tissue of bivalves that live in both of the sea and in freshwater role in issuing mother of pearl or nacre layer. For instance, *Pinctada* sp. and *A. woodiana* have the same protein and enzyme inside its body, although in a different composition according to its physiological metabolism and environment.

In this study, the maximum thickness of pearl found on the mussel of the inserted number 2 nucleus per individual with a diameter of 10 mm, and there is no "tail" (the bulge coat). Thick layer of two-fold compared to the implantation with 4 nucleus will however be greater 5.5 time when compared with the implantation of six nucleus. Based on this, thought that the proper method of insertion which produces a maximum thickness of the pearl.

In conclusion, burden (number and diameter nucleus) affect physiological processes in freshwater pearl coating: the greater the number and diameter of the nucleus increases stress levels wich produced nacre layer coating reduction. Further result, an increasing number and diameter of this nucleus will cause a decrease repons eating mussel, survival rate, growth and pearl coating. Biomineralisasi process is relatively good on the amount of nucleus 2 grains/ individuals with a diameter of 10 mm. On the mussel are implanted with a nucleus diameter and number of pearls produced the highest layer thickness of 17 µm, while the number of nucleus 4 grains/individuals is only able to produce coatings of 9 µm and 6 grains/ individuals at 5 µm. At the nucleus of the ideal (2 grains/individuals with a diameter of 10 mm) reached maximum growth mussel *A. woodiana*. This result was good enough compared with the layer thickness of sea water pearl production after the same cultivation time.

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REFERENCES

Alagarswarni K, Dhamaraj S, Velayudhan TS, Chelam A, Victor ACC, Gandhi AD. 1991. Larva rearing and production of spat of pearl oyster *Pinctada fucata* (Gould). *Aquaculture* 32:87-301.

Aldridge DC. 1999. The morphology, growth and reproduction of unionidae (Bivalvia) in fenland waterway. *J Moll Stud* 65:47-60. http://dx.doi.org/10.1093/mollus/65.1.47

Barik SK, Jena JK, Ram KJ. 2004. $CaCO_3$ crystallization in primary culture of mantle epithelial cells of freshwater pearl mussel. *Curr Sci* 8:16-25.

Berni P, Bitossi S, Salvato M, Orlandi M, Salviati J, Silvestri M, Megale P, Orlandi P, Billiard R. 2004. Enhancing the Local Production of Alternative Freshwater Pearls, High quality, Environmentally Sustainable Mixed Farming Techniques. Italy: Freshwater Pearl Culture. Ceresole d'Alba Pr. p 179-185.

Dan H, Gu Ruobo. 2002. Freshwater pearl culture and production in China. *J Aquac Asia* 7:1-10.

Friedman KJ, Southgate PC. 1999. Growth of black lip pearl oysters *Pinctada margaritifera* collected as wild spat in the Solomon Island. *J Shellfish Res* 18:159-167.

Gervis MH, Sims NA. 1992. The Biology and Culture of Pearl Oysters (Bivalvia: Pteridae). Manila: International Centre for Living Aquatic Marine Resources (ICLARM) Studies and Reviews. 21:1-49.

Hamidah A. 2006. Effect of using different types of fish as a host of survival glochidia mussel Taiwan (Anodonta woodiana Lea). *Biota* 11:185-189.

Kripa V, Mohamed KS, Appukuttan KK, Velayudhan TS. 2007. Production of akoya pearls from the Southwest coast of India. *Aquaculture* 262:347-354. http://dx.doi.org/10.1016/j.aquaculture.2006.09.047

Mamangkey NGF, Salmon HA, Southgate PC. 2009. Use of Anaesthetics with the Silver Lip Pearl Oyster, *Pinctada maxima* (Jameson). *Aquaculture* 288:280-284. http://dx.doi.org/10.1016/j.aquaculture.2008.12.008

Moorkens EA, Costello MJ, Speight MCD. 2005. Status of the freshwater pearl mussels Margaritifera margaritifera and Margaritifera durrovensis in the Nore Barrow, and Suir river tributaries. *Irish Naturalist J* 24:127-131.

Nuryanto A, Solihin DD, Soedharma D, Blohm D. 2007. Molecular phylogeny of giant clams based on mitochondrial DNA cytochrome C oxidase I gene. *Hayati J Biosci* 14:162-166.

Rachman B, Winanto T, Maskur. 2006. Effect of various depth against implantation process and nucleus coating pearls in oysters *Margaritifera* sp. on controlled pool. *Impasja* 2:86-95.

Rahayu SYS, Duryadi D, Affandi R, Manalu W. 2009. Ekobiologi kerang mutiara air tawar (*Anodonta woodiana*, Lea). *Omni Akuatika* 8:27-32.

Ram JK, Gayatri M. 2003. Homogenic and xenogenic implantation in pearl mussel surgery. *Curr Sci* 85:23-25.

Salmon AH, Fernandez EM, Southgate PC. 2005. Use of relaxants to obtain saibo tissue from the blacklip pearl oyster (*Pinctada margaritifera*) and the Akoya pearl oyster (*Pinctada fucata*). *Aquaculture* 246:167-172. http://dx.doi.org/10.1016/j.aquaculture.2004.12.010

Suwignyo S, Widigdo B, Wardiatno Y, Krisanti M. 2005. Avertebrata Air. Depok: Penebar Swadaya.

Winanto T. 2004. Status of pearl culture in Indonesia. *J Shellfish Research* 13:354.

Winanto T, Soedharma D, Affandi R, Sanusi H. 2009. Effect of temperature and salinity on physiological response pinctada maxima pearl oyster larvae (Jameson). *J Biol Indon* 6:51-69.