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by Cyska Lumenta

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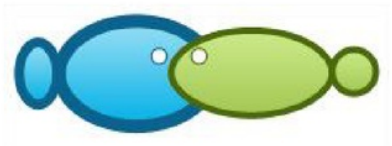

Micro tablet of nacre layer in the shell of freshwater bivalve *Anodonta woodiana*

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Abstract. The presence of shell nacre layer in bivalves is important information for pearl culture. Freshwater mussel, *Anodonta woodiana*, can be used in pearl culture maintained in a controlled manner to determine shell development, microstructure of nacre layer in particular. Using a Scanning Electron Microscope, observations were made on the structure of micro tablet nacre. Results concluded that the crystal size of the nacre of *A. woodiana* varied in thickness with space and shell size, rearing time, and rearing media. The optimum thickness of the tablet was averagely 0.720 µm at 110 mm shell length. The size of these shells could be a maximum size and should be considered in the cultivation of pearl oysters, *A. woodiana*.
Key Words: nacre microstructure, pearl, SEM, crystals, thickness.

Introduction. Pearl from freshwater oysters has recently been known, and become an interesting object in aquaculture development (Dan & Ruobo 2002; Misra et al 2009; Liu et al 2014; Bai et al 2016; Li et al 2017). Pearl formation in nature, in deed, occurs when an alien material enters the shell and cannot be removed so that irritation occurs (Hänni 2012). As a response to the irritation, the oyster activates calcium carbonate secretion as the 'nacre' on the shell is formed which then gradually covers the material.

Pearl formation is basically a product of biomineralization process indicated with nacre layer formation on the oyster or mussels shell. The information is just provided in either marine or freshwater oysters, such as *Hyriopsis cumingii*, *H. schlegeli*, *H. myersiana*, *Cristaria plicata*, *Unio pictorum*, *Lamellidens marginalis*, *Parreysia corrugata*, *Chamberlainia hainesiana*, *Elliptio complanata*, and *Villosa lienosa* (Dan & Ruobo 2002; Misra et al 2009; Feng 2011; Kovitvadhi & Kovitvadhi 2013; Marie et al 2017). *Anodonta woodiana* is known to be invasive in many worldwide locations (Watters 1997; Cichy et al 2016; Benson 2017), where the pearl growing experiments have been conducted in Indonesia (Lumenta 2012; Rahayu et al 2013), but the microstructure of the nacre as biomineralization product is not well studied.

In relation with the limited information, particularly the appropriate initial size selection of *A. woodiana* for pearl implantation on the shell, a study was carried out to determine the microstructural development of the nacre. The microstructure is a biomineral crystal naturally composed in the shell.

Material and Method. The microstructure development of the nacre was descriptively approached through *A. woodiana* cultivation. It was done for 8 months, from March to October 2014. This Chinese pond mussel was collected from the bank of Tondano Lake (North Sulawesi, Indonesia). The rearing was controlled in the restricted concrete tank and net placed in the pond. In these both 1x1x0.4 m tanks, 60 individuals of oysters at initial length of about 62 mm were held. Chlorophycea and Cyanophycea were available in the pond previously fertilized and taken by the oysters as natural food during the culture. For the concrete tank, water was changed one a week taken from pond water.

Both concrete pond waters had mean temperature of 27°C, pH of 6-7, dissolved oxygen of 5.5-6.7 mg L^{-1}, and CO_2 of 2.2-2.9 mg L^{-1}, respectively.

The oyster and shell sample handling used container tank, plastic tank, and knife or scissor/shell softener. Measurement and analysis were carried out in the working laboratories.

Growth observations during the culture were done through 4 sampling activities, i.e. after 2, 4, 6, 8 months of the rearing. As many as 7 individuals were randomly taken from both concrete tank and pond, the shells were measured, and selected to obtain the nacre layer for further analysis. They were then cleansed, dried, and sorted to obtain intact shells, especially internal surface.

To observe the shell layer, vertical dissection of the shell was done using a shell cutter. The shell preparat was then observed on the outer ('*periostracum*'), the middle ('*prismatic*'), and the inner layers ('*nacre*'). Moreover, to observe and document the microstructure of the formed crystal tablet, the prepared shell layer (Figure 1) was then faced on a Scanning Electron Microscope (JSM-6510LA, Japan Electron Optics Laboratory Company). This instrument gives the image of the shell layer that could be monitored and determined the size, and saved on the computer facility.

Data analysis was processed using descriptive statistics. In this case, data tabulation and graphic presentation are basis of descriptive interpretation on the microstructural development of the crystal tablet from biomineralization of the oyster, *A. woodiana,* shell.

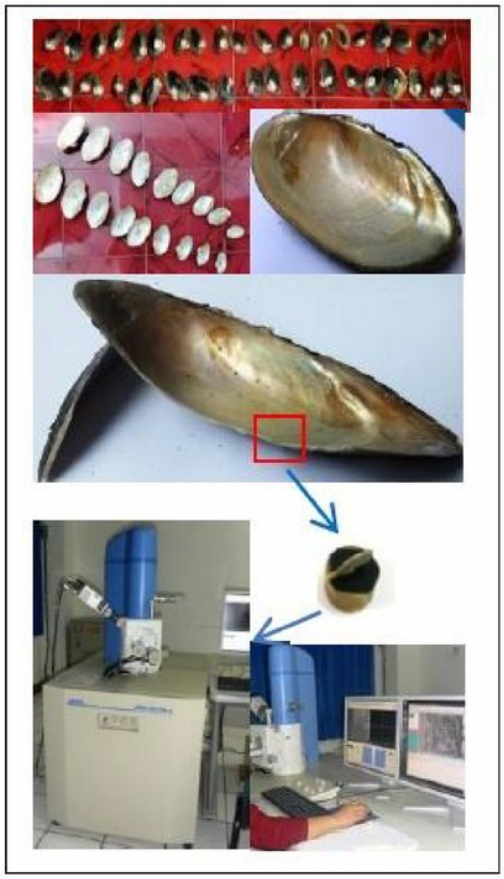

Figure 1. Measurement of nacre sample.

Results and Discussion. The oysters culture in 2 different tanks grew in shell length increment. From the average initial size of 62 mm, the oyster shell grew, but it was different with rearing time, either measured in the first 2 months or the next 2 months (Figure 2).

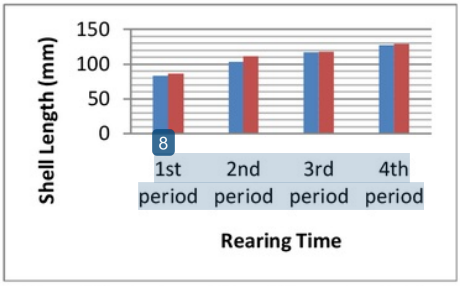

Figure 2. Shell length with rearing time: blue - concrete tank; red - pond.

Figure 2 shows that the shell length does not give significantly different response to culture media, particularly concrete tank and pond, even though there is slightly higher growth observed in the oyster shell length cultured in the pond than that in the concrete tank. It could result from space and food availability, in which pond could provide more sufficient space and food source. This growth trend of the oyster cultured in pond using net bags is in line with the previous finding (Lumenta 2012). However, they had slow growth when the rearing was done in more than 4 months.

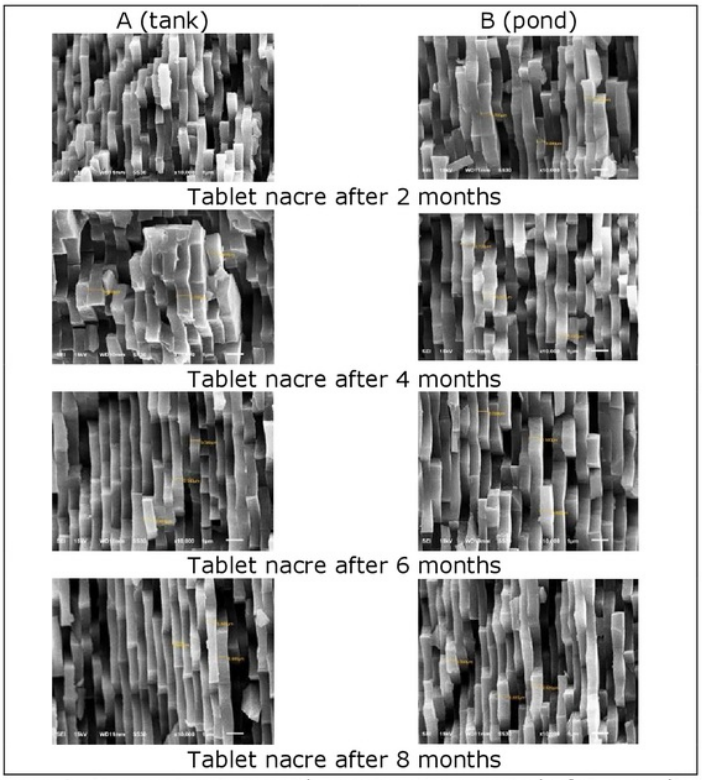

Figure 3. Nacre structure with rearing time period of *A. woodiana*.

As oyster shell, the crystal tablet structure develops differently as well. Figure 3 shows the nacre structure as crystal tablet layer indicated with different thickness. The tablet is composed of aragonite crystal and/or vaterite crystal (Wehrmeister et al 2007; Ma et al 2011; Debruyne 2008; Gerhard et al 2017) and separated by polysaccharide and protein fiber space (Marin et al 2012; Liu et al 2017) appears different in thickness, not only on the nacre part, but also from rearing time and tank.

Different tablet thickness in the micro-space of the nacre is related with nucleation and crystal growth and its orientation. According to Addadi & Weiner (1997) and then Marie et al (2017), the crystal growth is determined by matrix protein. As polymorph of calcium carbonate that easily changes to calcit, Ma et al (2011) stated the stability of aragonite is inferior to calcite thermodynamically, therefore, calcium carbonate crystals in nature are most in the form of calcite.

The nacre crystal tablet of the oyster cultivated in ponds is generally thicker than than that in the concrete tank. This phenomenon is in line with the shell length increment (Figure 2). Although information support is still few, it is apparent to agree with Furuhashi et al (2009) and Wojtas et al (2012) that environment affects the crystal formation.

The development of crystal tablet thickness appears to be optimum at the rearing of 110 mm shell length-sized oyster. It was recorded in the shell crystal tablet thickness of the oysters reared up to 4 months, with mean thickness of 0.720 µm. Furthermore, rearing period up to 8 months, in both concrete tank and pond, shows relatively similar crystal tablet thickness, even though the shell size is still growing. Thus, this result could become basic consideration in shell size selection to achieve optimal chance to grow pearl, i.e. less than 110 mm.

According to Soldati et al (2008), vaterite is only encountered in freshwater pearl formed of implanted mantle tissue core, but neither in the pearl of core implantation nor marine pearl. However, according to Blank et al (2003), considering a number of information, the crystal tablets of the pearl could reach adult or mature at 0.5 µm thick. Moreover, Addadi & Weiner (1997) stated that nacre was an internal brightness of the nacre layer in the shell, the pearl itself is composed of the entire nacre. In its formation, the epithelial cells responsible for early formation develop the organic matrix causing the crystal formed as available space grow far from mantle tissue providing raw materials for its growth. Marie (2008) found that in molecule and supramolecule, organic matrix plays active role in micro-environmental space organization where mineralization occurs, crystal growth. Thus, protein matrix does not only participate in the construction of nacre organic structure, but control the nucleation and the growth of aragonite crystal as well, and determine the specific polymorph of the nacre calcium carbonate (Xie et al 2011).

Conclusions. The crystal tablet composing the nacre of *A. woodiana* shell varied in thickness with space and shell size, rearing time, and rearing media. The optimum tablet thickness was measured at an average of 0.720 µm long in the oyster of 110 mm shell length. This size could become maximum size considerable for cultivation of freshwater mussel, *A. woodiana.*

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