

Nacre characterization of pearl oysters *Pinctada margaritifera* from Arakan waters, North Sulawesi, Indonesia

by Cyska Lumenta 3

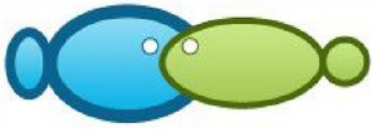
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Nacre characterization of pearl oysters *Pinctada margaritifera* from Arakan waters, North Sulawesi, Indonesia

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Abstract. Mother of pearl inside the pearl oyster *Pinctada margaritifera* was studied to provide information on the diversity of the aragonite microtablets and their biomineral compositions. This information can be used to reveal the specific characters of the nacre of the oyster from North Sulawesi coastal waters and as a basis for the oyster size selection in pearl culture. Thirty samples of oysters from Arakan waters had been prepared for their shells and then the nacre layer was analyzed using Scanning Electron Microscope and Energy Dispersive X-Ray Spectroscopy. Results showed that the nacre-composing microtablets varied with shell size, with thickness of 0.36-0.90 μm . The maximum thickness was found in *P. margaritifera* of 80-90 mm shell length. The biomineral composition contained in the tablet is $\text{Ca} > \text{Mo} > \text{Na} > \text{Al} > \text{Mg} > \text{Se} > \text{Fe} > \text{Mn} > \text{Cr}$.

Key Words: nacre, biomineral, microtablet, aragonite, oyster.

Introduction. Mother of pearl or nacre is internal layer naturally formed in bivalve shell living in marine or freshwater environments. This biomineralization yield has been used since about 4500 BC as a decorative material as marked from the ruins of Bismaya in Mesopotamia (Strack 2006). It is generally understood that natural pearls and domestic pearls from pearl culture are formed by the nacre layer.

Pearls from oyster cultivation are produced through grafting of shell-based core and mantle tissue sheet of the donor oysters. This tissue grows and secretes nacre that encloses the nucleus inserted into the host oyster. The thickness of the nacre layer and the pearl quality of *Pinctada margaritifera* and *P. maxima* are determined by the oyster origin, gene, culture location, and its biomineralization (Addadi et al 2006; Taylor & Strack 2008; Jerry et al 2012; Blay et al 2013; Chang et al 2016). The layer is composed by a series of aragonite microtablets that possess various sizes with species, oyster size and living environment (Ky et al 2016; Le Pabic et al 2016). These microtablets have mean size of 44 ± 23 nm (Rousseau et al 2005), but according to Watanabe in Debruyne (2014) it ranges from 0.2 to 2 μm . For the nacre and pearl in freshwater oyster *Anodonta woodiana*, the aragonite microtablet reached 0.720 μm (Lumenta 2012; Lumenta et al 2017). According to Le Pabic et al (2016), the thickness of pearl nacre from cultivated pearl shows significant correlation with that of *P. margaritifera* shell reported by Ky et al (2017) as pearl oysters have specific ability to yield pearl of the widest color range among all species of pearl oysters.

P. margaritifera recently found in a number of coastal waters of Minahasa Peninsula, North Sulawesi, are cultured to produce pearls. Since the development of the shell nacre has not been well studied, this study was initiated from nacre characterization of *P. margaritifera* collected from Arakan coastal waters, Minahasa Peninsula. The objective is to specifically determine the thickness of the aragonite microtablet of the nacre and its biomineral content.

Material and Method. Characterization of nacre was carried out on oyster samples obtained from dives in a coral reef area on the coast of Arakan, Minahasa Peninsula. In this case, the oysters *P. margaritifera* were collected proportionally according to its size using a hook knife which was then accommodated in a sample container. Species determination and shell height and hinge length measurements were carried out in the field followed by the separation of organs and shells.

As many as 30 shells of various sizes were studied in the laboratory, starting with cleaning using sodium hypochlorite (NaOCl, 5%), rinsing, and drying. Furthermore, the nacre image on the shell was digitized to facilitate measurement of its area using ImageJ. To examine the microstructure, a piece of nacre layer was prepared measuring 1.0 cm².

A scanning electron microscope (SEM) facilitated with energy dispersive x-ray spectroscopy (EDS) was used to observe the nacre. This equipment (JSM-6510 LA) describes the specimen morphology and can plot the major element contained. To characterize the sample from the chemical point of view, EDS was utilized to determine the microelement composition of the nacre. The analysis resulted in image data and graphs that were then used to describe the objectives of the study.

Result and Discussion. The nacre studied were taken from 60-115 mm shell long-sized oyster. The nacre area ranged from 187 to 684 mm² with a mean of 385±176 mm². In a horizontal view of the inner shell shape (Figure 1), the shape of the shells appears to be round in the lateral line. The inside of the shell shows a silvery nacreous layer surrounded by a dark black shell, therefore the common name is the "black-lip" oyster. In a 1000x magnification scale, the nacreous layer displays a plain surface which in certain parts is grouped with rows of cell grains that mark the growth line of the layer. According to Farre et al (2011), nacre is a layer that grows over the aragonite layer that wraps the prismatic layer, which is the last stage of shell formation.

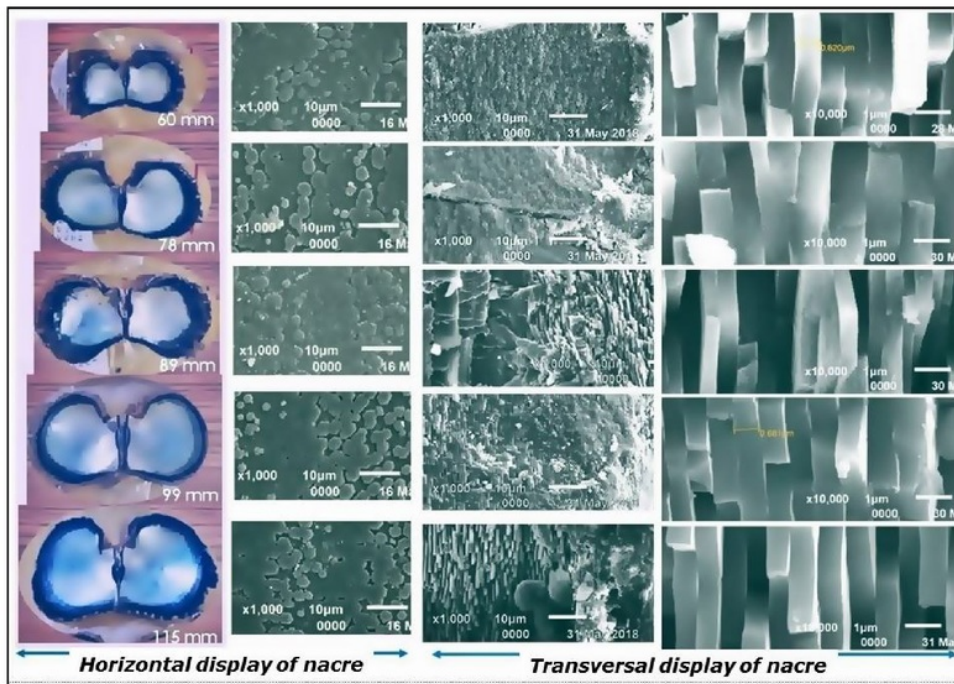


Figure 1. Nacre of *P. margaritifera* from Arakan waters.

Observation of the nacre layer transversely clearly shows a set of polygonal aragonite tablets arranged horizontally separated by an interlamellar membrane (Figure 1). As previously reported, the structure looks like brick wall and its organic matrix-like

adhesive (Addadi et al 2006; Nudelman et al 2008; Rousseau et al 2009; Marie et al 2012). On a 1000x magnification scale, aragonite tablets appear to be related to the prismatic layer which in the growth of shells subsequently has the opportunity to form aragonite tablets. While according to the scale of 10000x enlargement, nacre tablets are characterized by a variety of thicknesses.

The thickness of aragonite microcrystal tablet of the nacre layer showed different sizes with shell size. All observations and measurements of the microtablet thickness are presented in Figure 2. The thickness of microtablet of the nacre ranges from 0.357 to 0.904 μm , and for the shell, it ranges from 60 to 115 mm wide. This picture also informs that microtablets are generally relatively small in small oyster shells. It reflects that the development of the aragonite crystal formation follows the individual growth of the oyster indicated with the shell size change. The aragonite microtablet thicker than 0.586 μm appears to be maximum in *P. margaritifera* with size range of 80-90 mm. Blank et al (2003), based on a number of previous information, stated that the aragonite microcrystal tablets of the nacre could reach maturity at 0.5 μm thick.

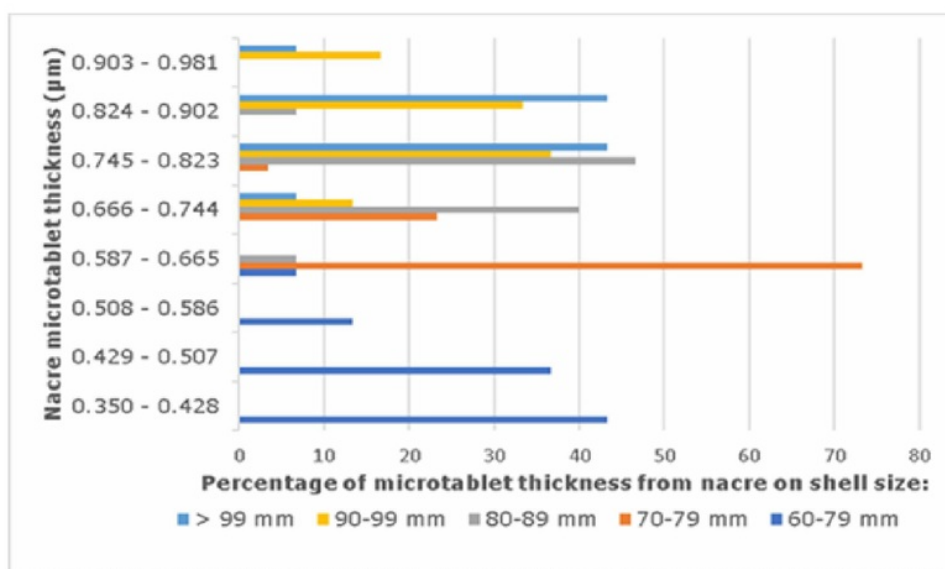


Figure 2. Microtablet thickness composition of *P. margaritifera* nacre.

As an organized inorganic-organic composite material secreted by the epithelial cells of the mantle tissue of the mollusk, Addadi et al (2006) stated that nacre formation was initiated by mantle epithelial cells that secrete crystalline precursors as well as amorphous calcium carbonate, together with organic matrix molecules which consist mostly of proteins and polysaccharides. Its formation, according to Kroger (2009), is a longitudinal calcite layer formation tightly structured in perpendicular orientation to the shell growth direction, followed by calcium carbonate crystallization that alters the amorphous calcite to crystal aragonite layer. The organic matrix plays active roles in organizing the mineralization microenvironment, in crystal nucleation, crystal growth toward certain crystallographic axis, and in terminating the crystal growth (Marie et al 2012). Thus, protein matrix participates not only in constructing the organic skeleton of the nacre, but in controlling the nucleation and the growth of the aragonite crystal and determining the particular polymorph of the nacre as well (Xie et al 2011). Besides, previous studies on *Anodonta woodiana* found that the microtablet development of the nacre was influenced by the modifications of the culture media (Lumenta 2012; Lumenta et al 2017). Despite requiring gradual supporting information, it seems to be similar to Furuhashi et al (2009) and Wojtas et al (2012) that microtablet formation is also affected by the environment condition where the oyster live.

Nacre as the output of calcium carbonate crystallization contains biomineral elements dominated by calcium (Ca) content, > 90% (Figure 3). Beside calcium, nacre oysters is indicated with the presence of microtablets containing molybdenum (Mo), selenium (SE), ferrum (Fe), mangan (Mn), chrome (Cr), aluminium (Al), magnesium (Mg), and sodium (Na). Similar elements are also found in the nacre of *A. woodiana* (Chen et al 2017) some of which contribute to the color appearance of the nacre. While the Al elements in alumina (Al₂O₃) crystallized are generally blue in ruby and sapphire (Baron & Torrent 2013), their presence is markedly different in the nacre shells of *A. woodiana* from a number of locations in the Minahasa area (Lumenta & Kalesaran 2017).

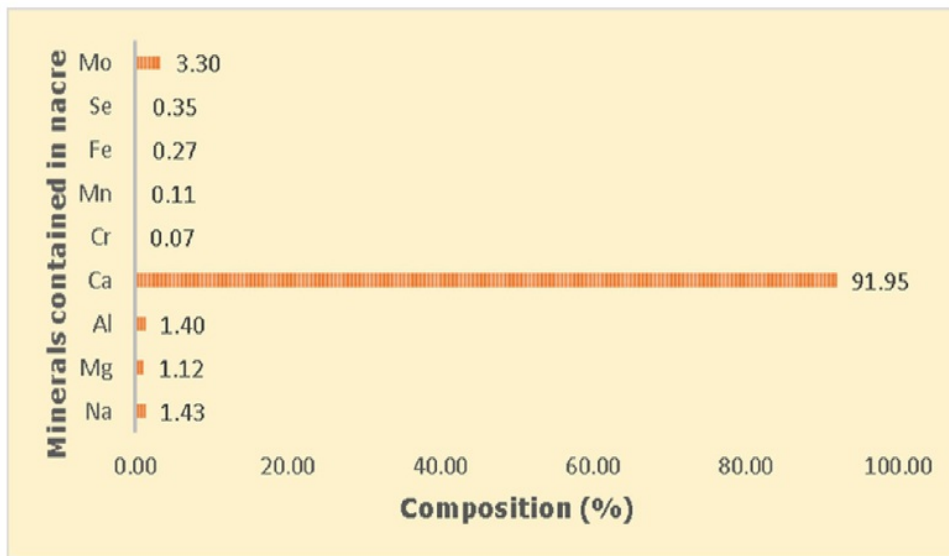


Figure 3. Biomineral composition of *P. margaritifera* nacre.

Conclusions. The oyster shells of *P. margaritifera* from Arakan waters were characterized by the nacre composed of various aragonite microtablets as thick as 0.357-0.904 μm . These variations tended to be maximum in the oysters with 80-90 mm shell length. The minerals contributing to the nacre colors were dominated by Ca, and other microelements were Mo, Na, Al, Mg, Se, Fe, Mn, and Cr.

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