Microstructures of the larval shell of a pearl oyster, *Pinctada fucata*, investigated by FIB-TEM technique

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**ABSTRACT**

The structure of the larval shell of a pearl oyster, *Pinctada fucata*, has been investigated at several growing stages mainly using the focused ion beam (FIB) sample preparation technique and transmission electron microscopy (TEM). Until 12 h from fertilization, the larva does not have any calcified shells. After 18 h from fertilization, the embryo is covered with the first shell made of aragonite with the *c*-axis normal to the shell. The cross-sectional view of the shell shows a columnar contrast, but plan-view observation revealed that the columnar contrast does not correspond to individual crystals but is related to dense polycyclic {110} twins in the aragonite crystal. After 48 h from fertilization, the larvae formed a new aragonite layer under the initial layer; a homogeneous layer with globular contrast. The *c*-axis of the globules is normal to the shell. The orientation of the other axes is aligned locally but random in general. Additionally, larvae consisting of monolithic calcite as the inner layer were found at this stage. One to three weeks from fertilization, a new aragonite layer with a prismatic contrast is formed under the homogeneous layer. This layer consists of prismatic grains of aragonite, with their *c*-axes parallel to the prisms. High-angle annular dark-field (HAADF) images suggest that a considerable amount of organic molecules may be contained in the homogeneous layer but not in the inner prismatic layer, implying that the texture of each layer is related to the amount of organic molecules incorporated.

**Keywords:** *Pinctada fucata*, larval shell, FIB-TEM, aragonite, {110} twin, HAADF

**INTRODUCTION**

Mollusk shells have been studied extensively to understand the biomineralization process there, but most of the research is for adult shells, and those for larval stages are limited. In the larvae, the shell formation process begins from a part of the ectoderm, called the shell field, which invaginates to form the shell gland (Kniprath 1980). The first shell is called prodissococonch-I, and the shell called prodissococonch-II is formed outside prodissococonch-I (Rees 1950; Waller 1981). Waller (1981) observed the larval shell structure of an oyster, *Ostrea edulis*, and suggested that the larval shell after 7 and 15 days from fertilization is composed of three layers: the outer prismatic layer, a granular homogeneous layer, and the inner prismatic layer. Weiss et al. (2002) also suggested that the larval shell of *Crassostrea gigas* and *Mercenaria mercenaria* is composed of three layers with similar structures. Mao et al. (2001) observed the larval shell and the juvenile shell structure of a pearl oyster, *Pinctada margaritifera* after 6, 17, and 27 days from fertilization (the larval shell) and 40 days, 2 to 4 months from fertilization. They separated the shell 17 and 27 days from fertilization into prodissococonch-I and prodissococonch-II.

Since the report by Stenzel (1964), the CaCO₃ polymorph comprising the larval shells has been reported to be aragonite, based on XRD analyses (Taylor et al. 1969; LaBarbera 1974; Carricker and Palmer 1979; Waller 1981; Kobayashi 1980, 1981; Watabe 1988; Castilho et al. 1989; Medaković et al. 1989, 1997; Mao Che et al. 2001). However, Hasse et al. (2000) and Miyazaki et al. (2009) suggested also from XRD analyses the existence of amorphous calcium carbonate (ACC) besides aragonite. Weiss et al. (2002) suggested that ACC is formed in the larval shell as the precursor phase of aragonite, by using Raman spectroscopy and other techniques.

In these previous works, observations of the larval shell have been conducted mainly using scanning electron microscopy (SEM), but few have been reported using transmission electron microscopy (TEM). Lee (1990) analyzed the larval shell (prodissococonch-II) of *Crassostrea virginica* by polarized microscopy and TEM. He suggested that it is crystallized as aragonite with its *c*-axis perpendicular to the shell surface. Kudo et al. (2010) investigated the larval shell of *Crassostrea nippona* after 14 and 72 h from fertilization using TEM with the focused ion beam (FIB) technique to prepare the TEM specimens. They reported that the shell consists of two layers; the outer layer...