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Influence of preoperative food and temperature conditions on pearl biogenesis in *Pinctada margaritifera*

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ABSTRACT

Trophic conditions and water temperature strongly influence bivalve physiological processes and metabolism. In black-lip pearl oyster *Pinctada margaritifera*, these parameters have been shown to affect shell biomineralization. The present study investigated the effect of preoperative food level (i.e., microalgal concentration) and temperature on pearl biomineralization. Donor and recipient oysters were conditioned at different levels of food and temperature during the preoperative phase to evaluate the influence of these factors on 1) pearl retention rate (grafting success), 2) expression of genes involved in biomineralization in the mantle and pearl sac and 3) pearl quality traits. Our study confirmed the influence of both microalgal concentration and temperature on shell growth. Food level of donor oysters was decisive for pearl biomineralization, with donors that had been fed at a high microalgal concentration producing pearl sacs with significantly higher biomineralization capabilities and faster nacre establishment during early stages of pearl formation. However, food level showed no effects on quality traits of the pearls harvested 12 months postgrafting, while preoperative temperature only influenced the relative expression of two genes in pearl sacs at 12 months postgrafting. No significant effects of the preoperative conditioning of recipient oysters were detected in either experiment considering gene expression measurements and pearl quality traits. However, mortality was significantly lower in grafted recipient oysters fed at an intermediate trophic level. Finally, pearl weight was shown to be positively correlated with recipient oyster growth.

1. Introduction

The black-lip pearl oyster *Pinctada margaritifera* (Linnaeus 1758) is farmed to produce black cultured pearls – unique gems generated by a living organism – in several countries in tropical and subtropical regions. In French Polynesia, pearl production is a major industry, with the exportation of pearl products reaching 63 million Euros in 2014 (Talvard, 2016). Production sites are located in the Society, Gambier, and Tuamotu archipelagos, whose pearl production accounts for > 95% of the world's black cultured pearls in terms of value (Cartier et al., 2012). As reported by Southgate et al. (2008a), pearl production involves four phases: (1) preoperative oyster conditioning, (2) the surgical grafting operation, (3) postoperative care, and (4) oyster culture and pearl harvest. Preoperative conditioning consists of reducing the metabolism and gametogenic activity of pearl oysters for 28–40 days prior to grafting (Aji, 2011; Gervis and Sims, 1992; Southgate et al., 2008a). Some pearl producers use preoperative conditioning, including lower water temperature, deliberate over stocking, reduction of food and oxygen levels, and placing of the pearl oysters deeper in the water column prior to the graft operation, as these actions are considered to decrease pearl rejection and improve pearl quality (Aji, 2011; Gervis and Sims, 1992; Southgate et al., 2008a). These rearing practices have not, however, been standardized nor tested under controlled conditions.

Surprisingly, the impact of the environment on cultured pearl biomineralization has been little documented, and previous studies have mainly focused on postoperative maintenance. For instance, the proportion of high-quality pearls harvested 4 months postgrafting was found to be significantly higher in recipient oysters that had undergone a low salinity treatment during the 14 days following the graft operation than in those reared conventionally (Atsumi et al., 2014). Temperature is considered an important factor for obtaining high-quality pearls, and winter is usually considered the best season to harvest pearls. Low temperatures are believed to reduce pearl oyster

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metabolism and lead to thinner mineral lamellae in the final layers of nacre laid down on the pearls, thereby enhancing their luster (Alagarswami, 1987; Menzel, 1991). However, to the best of our knowledge, no study has yet examined the effect of environmental factors experienced during the preoperative conditioning period on the subsequent pearl biomineralization process. The surgical procedure known as "grafting" is carried out by skilled technicians following preoperative conditioning. A small piece of the mantle, the tissue responsible for shell mineralization, is cut from a donor oyster and inserted along with a spherical nucleus (consisting of mollusk shell or synthetic material) into the gonad of another pearl oyster, the "recipient" (Kishore and Southgate, 2016; Southgate et al., 2008a). The external epithelial cells of the graft proliferate and cover the nucleus to form a pearl sac, a process that takes approximately 30 days following the grafting operation (Cochennec-Laureau et al., 2010). The first pearl layers are not homogeneous, as they show high variability in thickness and composition, as well as a remarkable association of organic and mineral materials (Cuif et al., 2008). The basal layer of the pearl, produced by the very first secretion of the pearl sac starting 21 days postgrafting, is usually composed of thin organic layers mostly consisting of proteins, with the mineral material present as dispersed microgranules of aragonite and calcite (Cuif et al., 2011). Two months after grafting, radial microstructures perpendicular to the surface of the nucleus appear due to the formation of organic envelopes. These microstructures form prisms composed of calcite or aragonite. This prismatic aragonite is specific to pearl microstructure and has never been observed in mollusk shells. Finally, a regular and parallel nacreous layer composed of aragonite tablets is established during pearl formation. Its production may occur directly onto the organic layer or may be delayed for a few months (Cuif et al., 2011). Therefore, the mineralization capabilities of the graft could be critical for the development of nacreous layers during the early stages of pearl formation and for obtaining high-quality pearls.

Pearl biomineralization results from complex molecular processes. The pearl sac epithelium synthesizes shell matrix proteins (SMPs), which play a major role in pearl biomineralization. Numerous SMPs have been characterized and some genes encoding these proteins have been identified in pearl oysters (Joubert et al., 2010; Marie et al., 2012; Montagnani et al., 2011; Suzuki et al., 2009). SMPs are thought to partly regulate the formation of the prismatic and nacreous shell layers (Marie et al., 2012). Notable examples of nacreous layer-related proteins include Pif177, known to specifically bind to aragonite crystals (Suzuki et al., 2009); MSI60, which is involved in the formation of aragonite crystal (Sudo et al., 1997); and Pearlin, which exhibits calcium- and chitin-binding properties (Montagnani et al., 2011). In the prismatic layer, Aspein is involved in the calcite precipitation process (Isowa et al., 2012), while Prismalin14 plays an important role in regulating calcification of the prismatic layer (Suzuki et al., 2004). Some proteins such as Nacrein are important for shell formation and are implicated in the mineralization processes of both the aragonitic nacreous and the calcitic prismatic layers (Miyamoto et al., 2005).

Pearl production is also a complex process that involves genetic contributions from two oysters (donor and recipient), which may be affected by the environment. Although the donor oyster is primarily responsible for the expression pattern of biomineralization genes in the pearl sac at both genomic (Arnaud-Haond et al., 2007) and transcriptomic levels (McGinty et al., 2012), the recipient oyster is strongly suspected to regulate pearl sac metabolism (Le Pabic et al., 2016) The grafter skills also influence pearl biomineralization and quality (Ky et al., 2014, 2015b). Recently, significant correlations have been demonstrated between pearl quality traits and some donor and recipient characteristics, such as a positive correlation between pearl nacre deposition and recipient shell growth or significant donor effects on pearl nacre deposition, luster, shape and defects (McDougall et al., 2016). To date, very little attention has been paid to the effects of environmental factors on pearl biomineralization. The purpose of our study was therefore to investigate under controlled conditions the effects of food level (microalgal concentration) and temperature during the preoperative phase to test their influence on: 1) the pearl retention rate, 2) the molecular mechanism involved in biomineralization in both the mantle and pearl sac and 3) pearl quality traits.

2. Material and methods

2.1. Biological material

Wild *P. margaritifera* pearl oysters aged 18 months were obtained by spat collection and transferred by air from Arutua lagoon to Vairao lagoon. These animals were then left in the lagoon for an acclimatization period of at least one month before the trophic and temperature conditioning experiments were conducted.

2.2. Shell labeling and deposition rate

The pearl oysters were immersed for 12 h in a 150 $mg{\cdot}L^{-1}$ calcein (Sigma-Aldrich) solution prepared using 0.1-mm filtered seawater 5–6 days before the conditioning experiments. Both the donor and the recipient shells were sawn along the dorsoventral axis at different sampling time using a SwapTop Trim Saw (Inland, Middlesex, UK). The ventral sides of the shell cross-sections were observed by epifluorescence microscopy using a Leica DM400B microscope (I3 filter block and LAS V.8.0 software for size measurements). The shell deposit rate (SDR, $\mu m{\cdot}day^{-1}$) was calculated by dividing the thickness of the new nacre deposits formed during the experimental time by the number of days that had elapsed since the marking (Linard et al., 2011). A mean of two measurements was calculated for each cross section.

2.3. Experimental design

2.3.1. Experiment 1: microalgal concentration conditioning experiment

A total of 392 pearl oysters with a mean height of 10.5 ± 0.4 cm and a mean weight of 157.1 ± 27.7 g were divided among eight 500-L tanks in which microalgal concentrations were gradually increased over a period of 5 days. The pearl oysters were then reared for 30 days in April 2014. During the 1-month conditioning experiment, the pearl oysters were divided into two groups fed a mixed diet composed of two microalgae: 2/3 *Tisochrysis lutea* (T-iso) and 1/3 *Chaetoceros gracilis*, at an overall concentration of 10,000 or 40,000 $cells{\cdot}mL^{-1}$ supplied continuously using Blackstone dosing pumps (Hanna). Tanks were sampled automatically every 3 min for fluorescence and temperature measurements. The intermediate concentration is considered as an optimal food concentration for *P. margaritifera* (Yukihira et al., 1998) and the high concentration is close to ingestion saturation (Le Moullac et al., 2013). During this experiment, the mean temperature was 28.1 ± 0.5 °C. Twelve pearl oysters (3.1%) died during the conditioning period, 10 (2.6%) were not grafted at the end of conditioning because of their apparently poor health status (weak resistance of the adductor muscle prior to shell opening), 10 were used as donor oysters, and 360 were grafted (Fig. 1, see Section 2.4 for a detailed description of this procedure).

2.3.2. Experiment 2: temperature-conditioning experiment

A total of 378 pearl oysters with a mean height of 10.8 ± 0.5 cm and a mean weight of 175.4 ± 35.5 g were divided among eight 500-L tanks in which temperatures were gradually increased or decreased over a period of 5 days. The pearl oysters were then reared for 30 days in June 2014. Then the pearl oysters were split into two groups, which were exposed to water temperatures of 22 and 30 °C, respectively. In French Polynesia, the monitoring of temperature data over 10 years (Ifremer sources) showed that water temperature is rarely lower than 22 °C and higher than 30 °C. The lower temperature is recorded in the Gambier and Australes archipelagoes whereas the higher is recorded in