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Variation in cultured pearl quality traits in relation to position of *saibo* cutting on the mantle of black-lipped pearl oyster *Pinctada margaritifera*

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ABSTRACT

Cultured pearl production from *Pinctada margaritifera* uses the biomineralization capacities of the mantle graft, the *saibo*, which is usually obtained from only the middle mantle section of the donor oyster. To evaluate the potential for using other parts of the mantle, this study explores and describes the cultured pearl quality traits, pearl size, shape, surface defects and colour parameters obtained with *saibo* from the entire length of the mantle, comprising the four following sections: 1) posterior, 2) connection with the gills, 3) middle, (the section usually used commercially), and 4) anterior. Rates of nucleus retention and oyster mortality were also recorded and compared between sections. For this, two experimental grafts were designed and conducted in two contrasting culture sites, using 10 selected wild donor oysters in each to perform a total of 1536 grafts. Mantle section comparison revealed that the anterior section was different from the three other sections, showing: 1) the lowest nacre deposition rate in terms of weight and thickness, 2) the palest pearls, with lowest rate of the attractive overtone colour and the 3) a lower rate of pearls with lustre. For pearl circles and shape, no difference was recorded among the different mantle sections. Posterior, connection and middle sections showed similar pearl quality traits, revealing how the number of high quality *saibo* obtainable from the same batch of donors can easily be increased, thus benefitting the *P. margaritifera* pearl industry. This finding could provide significant benefits to pearl farmers and the further development of current pearl grafting practices.

1. Introduction

Nucleated pearls are produced by species of the *Pinctada* genus (Southgate et al., 2008). The cultured pearl industry is one of the largest in the Asia-Pacific region. The cultured pearls produced from the black-lipped pearl oyster *P. margaritifera* (Linnaeus, 1758) (Bivalvia, Pteriidae) come mostly from French Polynesia (around 90% of world production), where pearls are the top export industry and the second largest source of income (after tourism). Other countries of the Pacific region, such as the Cook Islands, Fiji or Micronesia, have also developed pearl farming industries based on *P. margaritifera* (Cartier et al., 2013). Two main particularities characterize the aquaculture of *P. margaritifera* and its development in French Polynesia. First, *P. margaritifera* is particularly abundant in the lagoons of French Polynesia compared with other Pacific regions. Its aquaculture is based on natural spat collection from wild stock during the reproductive season, without need for any supply from hatcheries or divers/fisherman collecting adult individuals. This is not the case in other Pacific regions or, to an extent, for the exploitation of *P. maxima* (Indonesia, Myanmar) and *P. fucata* (Japan, China), for which the supply is hatchery-dependent. Second, the French Polynesian pearl industry is made up of hundreds of independent pearl producers (536 in 2014) spread geographically across 26 atolls and islands located in three archipelagos: 79.0% in Tuamotus, 14.5% in Gambier and 6.5% in Society (statistics from DRMM: *Direction des Ressources Marines et Minières*). Consequently, great variation in cultured pearl quality trait is observed at harvest, in relation to the multiplicity of cultural practices and disparate environmental regimes.

Cultured pearl quality is defined by five visual and measurable parameters (Matlins, 2002; Taylor and Strack, 2008; Ky et al., 2013). First, the "size", which includes the diameter (8–20 mm) and the weight of the sample, with the largest and heaviest pearls being the most valuable. Second, the shape, which can be round, near round, button-

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shaped, oval, pear to drop shape, or completely uneven/asymmetrical ("baroque"), with the perfectly round and symmetrical fetching the highest prices. *P. margaritifera* also produces a large number of circled pearls compared with *P. fucata* (Ito, 2009). Third, the colour, which has two components: the darkness level and the colour categories. The latter is a combination of bodycolor (mainly due to different pigments), and overtone (the secondary or physical colour caused by diffraction/diffusion of the light on the pearl's surface). The combination of body and secondary colours produces a large spectrum of visually perceived colours in *P. margaritifera*, from white to anthracite black, going through pink, green, blue, cream, aubergine, bronze, or a mix of nearly all of these colours that offers a rainbow effect known as peacock. Fourth, the lustre, or reflection of light from the edges of the cultured pearl, defines the shininess/glossiness of the surface. Finally, fifth, the surface quality, which is an assessment of the (often microscopic) imperfections that can mark a cultured pearl, such as signs of scratches, small holes, unevenness, abrasions, spots and roughness. It has been estimated that only 5 to 10% of pearls harvested can be qualified as being of top *gem* quality following the Tahitian regulatory control standards; these provide about 95% of farms' income (Ellis and Haws, 1999; Haws, 2002). This illustrates the great variation in pearl quality in relation to the entire production process. The key step of the graft operation is particularly important for subsequent pearl quality (Southgate and Lucas, 2008; Ky et al., 2015a).

At harvest, cultured pearl quality can be considered the result of a complex and multi-factorial equation where genetics, the environment and their interactions all play key deterministic roles. Controlling quality is complex as two animals are involved; a factor that makes pearl oyster an interesting animal model for phenotype transmission and determination studies. The origin of such variations is the pearl oyster mantle, which is a metabolically and transcriptionally active tissue, indispensable for mollusc shell formation and growth, in which the transcriptional activity of biomineralization genes has been shown to be particularly high (Clark et al., 2010). It is these biomineralization properties that are exploited for pearl production. As mentioned above, formation of a cultured pearl requires two animals: a small piece of mantle tissue (the *saibo*) is dissected from a donor oyster and inserted with a round bead of nacre (a nucleus) into the gonad of a recipient oyster (Gervis and Sims, 1992; Taylor and Strack, 2008). Approximately 18 months after implantation, a pearl is harvested and *P. margaritifera* recipients are often re-implanted to produce a second pearl (*surgreffe*) (Demmer et al., 2016; Kishore and Southgate, 2016a). *Saibo* donors are carefully selected based on the visible quality (colour, lustre and surface quality) of their inner shell nacre, as the mantle tissue used to make the graft will influence the quality traits of resulting pearls (Alagarswami, 1987; Taylor, 2002), including for example their colour (Wada, 1985; Wada and Komaru, 1996). Suitable donors are sacrificed and both the anterior (where the byssus are located) and posterior (position of the large adductor muscle region) sections of the mantle are removed from each valve and discarded (Taylor and Strack, 2008; Kishore and Southgate, 2015). The remaining middle section is known as the "commercial" part of the mantle. It lies between the anterior and posterior sections and does not include the junction with the gills. The inner epithelial layers of mantle are then removed from the outer section of mantle tissue responsible for secreting the periostracal shell layers and are cut into square pieces for *saibo*. This "commercial" section is routinely and empirically used, as it corresponds to the part of the mantle that both maximizes shell growth and deposits the broadest part of the inner shell colouration band (Fig. 1). Consequently, the number of useable *saibo* is commonly 30–40 pieces per donor oyster (both valves) in *P. margaritifera* of 2–3 years old (Ky et al., 2014).

The present study focused on cultured pearl quality trait variation at the within-donor level, i.e., the variation resulting from the relative position of origin of the excised *saibo* on the mantle. Four mantle tissue sections were compared: posterior, connection zone with the gills (gills were attached to the mantle lobe), middle (commercial section) and anterior (Fig. 1). For this, we performed a duplicated experimental graft (two rearing locations), using *saibo* cut from the four sections (both valves) of 20 wild donor oysters selected on the basis of having a common "green" phenotype basis, with a real *saibo*-pearl traceability. Analysis of the cultured pearl quality traits recorded should reveal any variations associated with the different mantle sections and thus the *saibo* positions. This study was the first designed to examine this question and will provide important information that could benefit the *P. margaritifera* pearl industry.

2. Materials and methods

2.1. Animals

Pinctada margaritifera var. *cumingi* pearl oysters, both donors and recipients, were obtained from the wild, i.e., by natural spat collection, from two distinct locations: 1) Mangareva island (Gambier archipelago, French Polynesia) and 2) Takume atolls (Tuamotu archipelago, French Polynesia). Passive techniques were employed for catching spat using commercial collectors, as described in Ky et al. (2014). After nearly one year of subsurface rearing (3–5 m below the surface), the young pearl oysters (4–5 cm in diameter) were then removed from the collectors on which they had developed. These juveniles were pierced and tied together onto a CTN (Cord Technical Nakasai) rearing system, where they were left until grafting. This procedure involves drilling a small hole through the base of the shell in the dorsal-posterior region, which does not affect living tissue.

Two different age groups of donor pearl oysters were used: 1) around 3 years old, with a mean (± SE) dorso-ventral measurement of 112.5 ± 9.3 mm in the GMR site ($N = 10$), and 2) 18 months old with 55.4 ± 9.2 mm in the RRR site (N = 10). Donors were selected visually by an expert grafter for their dominant green inner shell phenotype (Figure1; Table 1). To discern the inner shell colour for this set, the grafter used a speculum to gently pry open the oyster valves (Ky et al., 2017a, 2017b).

Recipient pearl oysters aged almost 20 months in the Mangareva site (GMR), with mean (± SE) dorso-ventral measurement of 76.30 ± 0.65 mm, were randomly selected from a set of healthy animals and taken from the CTN, detached, and stored ready to be used in the grafting procedure. Recipient pearl oysters in Raroia atoll (RRR) were also obtained from the same pool as the donors.

2.2. Experimental graft design

The grafting procedure and culture were carried out at two commercial pearl farms operated by: 1) Regahiga Pearl farm, at Atiaoa bay, on the island of Mangareva, GMR (23°07′ S, 133°58′ W; Gambier archipelago, French Polynesia) and 2) Heimoana Poe Pearl farm, in Raroia atoll, RRR (15°56′ S, 142°22′ W; Tuamotu archipelago, French Polynesia).

The grafting operation was conducted by one expert in each pearl farm, as described in Ky et al. (2015a). The nuclei used for this purpose were made from the shells of freshwater mussels: 1) 1.8 BU size (equivalent to 5.45 mm diameter, 0.26 g weight; Imai Seikaku Co. Ltd., Japan) in the GMR site, and 2) 2.4 BU size (6.054 mm diameter, 343 mg weight - Nucleus Bio, Hyakusyo Co. Japan) in RRR site. The thickness and hardness of the nacreous layers of these beads show specific gravity and thermal conductivity that make them particularly suitable for use as cultured pearl nuclei (Gervis and Sims, 1992).

The epithelial cells required for grafting were excised from the entire mantle of the selected donor pearl oysters, including all the following sections: posterior, connection, middle and anterior (Fig. 1; Table 1). A total of 801 and 735 grafts were performed in GMR and RRR, respectively. Table 1 shows the number of grafts made from each donor oyster and the number of grafts per mantle section for the two sites. All the grafted oysters were checked for nucleus retention/