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GROWTH PERFORMANCE COMPARISON OF *PINCTADA MARGARITIFERA* JUVENILES PRODUCED BY THERMAL SHOCK OR GONAD SCARIFICATION SPAWNING PROCEDURES

CHIN-LONG KY,[1]* CHRISTOPHE LAU,[2] MANAARII SHAM KOUA[1] AND CÉDRIK LO[2]

[1]*Ifremer, UMR 241, EIO, Labex Corail, Centre du Pacifique, BP 7004, 98719 Taravao, Tahiti, Polynésie Française;* [2]*Direction des Ressources Marines et Minières, BP 20, 98713 Papeete, Tahiti, Polynésie Française*

ABSTRACT The black-lipped pearl oyster *Pinctada margaritifera* is of high economic importance in French Polynesia. A recent decline in pearl value led to the initiation of several genetic breeding programs aiming to improve production traits, including oyster shell growth, which dictates the time of grafting, size of the implanted nuclei, and biomineralization capacities. We assessed shell diameters on hatchery-produced spat to analyze juvenile growth performance of four half-sib families derived from polyandry (one dam crossed with two sires) and polygyny (two dams crossed with one sire) using gametes obtained by thermal shock or gonad scarification. Spat growth was monitored over 3 mo, with shell diameter measured weekly. Results revealed that the spawning method had no significant effect on juvenile growth; however, the half-sib families produced with the polygyny mating design showed significant differences in average shell diameter between dams throughout the experiment, whereas none were observed between sires with the polyandry mating design. Precocious larval size selection within each family was performed by separation into batches of small, medium, and large size, and their maintenance through juvenile stages, providing the possibility for early growth selection. These findings are important for genetic breeding programs (1) as breeding of sires and dams exhibiting the most colorful inner shell phenotypes would be possible with the gonad scarification spawning procedure without affecting overall growth performance and (2) because *P. margaritifera* is a protandrous hermaphrodite species, genetic selection strategies in the pearl industry must take into account the differential influence of polygyny and polyandry mating designs.

KEY WORDS: pearl oysters, *Pinctada margaritifera*, juvenile, growth performance, spawning procedure

INTRODUCTION

The black-lipped ''pearl oyster'' *Pinctada margaritifera* (Linnaeus, 1758) (Bivalvia, Pteriidae) is a saltwater mollusk distributed throughout the Indo-Pacific region. This species is particularly abundant in the lagoons of the atolls and islands of French Polynesia, where it is reared for cultured pearl production. Other countries of the Pacific region, such as the Cook Islands, Fiji islands, or Micronesia, have also developed pearl farming industries based on *P. margaritifera* (Cartier et al. 2013). In French Polynesia, black cultured pearls are (1) the top exportation resource and (2) the second economic resource after tourism. In 2014, there were 536 pearl producers (statistics from DRMM, the *Direction des Resources Marines et Minières*) across 26 atolls and islands, concentrated in three archipelagos: Tuamotu (79.0% of the producers and 83.0% of commercially produced pearls), Gambier (14.5% of all producers and 15.5% of commercially produced pearls), and Society (6.5% of all producers and 2.0% of commercially produced pearls). This pearl industry has been in decline for the last decade due to a combination of two main factors: the world economic crisis and overproduction, whereby quantity has been favored over quality. This critical situation has led to a decrease in pearl value on the international market (Wane 2013). Although quality control measures have been promoted to improve the image of cultured pearls, the need to improve pearl quality over quantity remains a major concern for this industry in French Polynesia. For this, one of the solutions is genetic improvement of stocks through selection of both donor and recipient oysters. Indeed, production of a cultured pearl requires two animals: a small piece of mantle tissue (a graft) is dissected from a donor oyster and inserted with a round bead of nacre (a nucleus) into the gonad of a recipient oyster. Approximately 18 mo after implantation, a pearl is harvested and oysters are sometimes reimplanted to produce a second pearl (*surgreffe*).

Aquaculture of *Pinctada margaritifera* in French Polynesia is still based on natural spat collection from wild stocks, mainly from the atolls of Ahe, Takaroa, Takume in the Tuamotu Archipelago, and from the lagoons of the Gambier archipelago. Spat are collected in these lagoons during the reproductive season, where they settle onto artificial collectors and are left up to 6 mo for pregrowing. They are then transferred to different pearl farm locations, where they are grown in various culture systems. Although spat can be collected in the wild in French Polynesia, difficulties in collecting natural spat in areas where the resources for *P. margaritifera* are not abundant or are of low quality have led other countries (Fiji islands and Micronesia) to start breeding programs with hatchery-produced spat. In Japan, Australia, and south-east Asia, hatchery programs have also been developed for other pearl oyster species (*Pinctada fucata* and *Pinctada maxima*) to meet the needs of resource production. To date, the use of hatchery-produced spat in conjunction with selective breeding strategies in French Polynesia has only been developed at an experimental scale and not yet by any private company.

Producing high-quality pearls through family genetic selection remains one of the main challenges for future aquaculture development of *Pinctada margaritifera*. For pearl size, which is one of the most important pearl quality traits, with the largest pearls being the most valuable, genetic selection needs to be performed with both appropriate donor and recipient families.

*Corresponding author. E-mail: chinky@ifremer.fr
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Tayale et al. (2012) demonstrated the influence of graft source on seven pearl quality traits (donor effect) including nacre thickness and pearl weight using wild donors of *P. margaritifera*. The same observations were made by Ky et al. (2013) using farmed donors produced in the hatchery. These findings are of interest to the cultured pearl industry as selection for donors showing high potential of nacre deposition could increase pearl quality (Blay et al. 2014). For the recipient, the use of large oysters allows the grafter to introduce a larger nucleus into the gonad. A study made on *Pinctada fucata* by Wada and Komaru (1996) showed a positive correlation between the weight of the shell of a recipient oyster and the weight of the pearl produced. Selection for traits like growth performance on both donors and recipients for breeding programs offers great potential for increasing cultured pearl quality. Additionally, knowledge on *P. margaritifera* has substantially improved over the last few decades. The captive breeding and rearing of this species of over its entire life cycle has been possible for several years now and genetic programs for selected donor oysters have been initiated at Ifremer (French Research Institute for Exploitation of the Sea) in French Polynesia. One part of the Ifremer program is to establish selected lineages for quality traits of interest through progeny testing. Several first-generation donor families have already been bred and reared.

Growth performance has been examined in several pearl oyster species. Studies on *Pinctada margaritifera* growth were done on wild oysters (Pouvreau et al. 2000) and on hatchery juveniles, although the latter focused on the culture techniques (Alagarswami et al. 1989, Southgate & Beer 2000) or environmental effects (temperature, salinity, density, algal ration) on the juveniles (Doroudi et al. 1999, Doroudi & Southgate 2000). Several studies have also focused on the effect of culture technique and environmental factors (density, fouling effect, salinity) on the growth of *Pinctada maxima* (Taylor et al. 1997a, 1997b, 1998) and *Pinctada fucata* (Alagarswami et al. 1989). The main objective of the present study was to evaluate the juvenile growth performance of hatchery spat produced with two different spawning procedures and two different mating designs, so as to assess these approaches for potential usefulness in the development of selective breeding programs.

MATERIALS AND METHODS

Breeding Design and Spawning Procedure

Four half-sib families of *Pinctada margaritifera* were produced in the Arutua atoll (Tuamotu Archipelago, French Polynesia) by breeding three selected females (named B, C, and N) with three selected males (named 1, J, and R) from the wild broodstock pearl oysters. Selection of these genitors was made on the basis of (1) the outer shell variant phenotypes, with "black," "yellow," and "red" colors for genitors N, J, and R, respectively; and (2) the "green" inner shell color phenotype for genitors 1, B, and C (Fig. 1A). Crosses were made according to two types of breeding design and based on two different spawning methods (described below). The resulting half-sib families were called (1) families NJ and NR, obtained by mating dam N with the two sires J and R using natural spawning procedures, and (2) families C1 and B1, obtained by mating dams C and B with sire number 1 using the gonad scarification procedure (Fig. 1B).

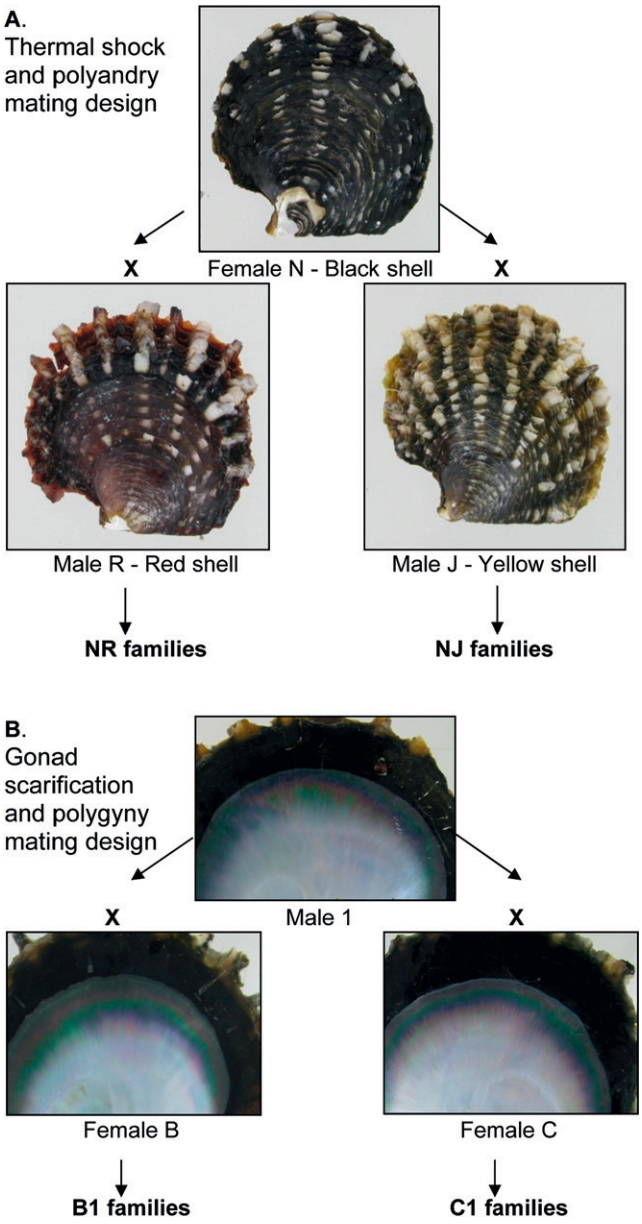

Figure 1. ***Pinctada margaritifera*** **mating designs used to produce the four half-sib families (NJ, NR, B1 and C1) from (A) genitors chosen according to outer shell color phenotypes, spawned by the thermal shock and crossed according to a polyandry mating design (NJ and NR families), and (B) genitors selected according to inner shell color phenotypes, with gametes obtained by the gonad scarification procedure and crosses made according to a polygyny mating design (B1 and C1 families).**

The spawning procedure used to produce the NJ and NR half-sib families was the same as described by Ky et al. (2015). Spawning was triggered by thermal shock: the pearl oysters were placed in cooled seawater at 20°C for one night before being plunged into seawater at 31–32°C. Soon after spawning started, male and female oysters were placed in separate containers for gamete collection.

The spawning procedure used to obtain the B1 and C1 half-sib families was a variant of the one described by Tanaka and Kumeta (1981). Gametes were obtained by gonad scarification.

Female oysters were opened, tissues cut away, and the visceral mass isolated. The gonad was then abundantly rinsed and placed in 1-µm-filtered seawater. The female gonad was striped by making several incisions allowing oocytes to be released. Once freed, the oocytes were filtered through an 85-µm mesh to remove tissue and other debris, and then rinsed and collected on a 25-µm mesh screen. At this point, the oocytes were not sufficiently mature for fecundation, having a specific "drop" shape (Fig. 2A). Artificial maturation was achieved by placing the oocytes in a 6-mM seawater ammonia solution and checking them for maturity every 5–10 min until they had attained a round shape (Fig. 2C) with the germinal vesicle no longer clearly visible (Fig. 2B). After 50–60 min, the eggs were ready for fertilization. Male oyster gonads were prepared in a similar manner as described for females. Male gonads were then stripped by making several incisions. Sperm were collected in a beaker with 25-µm mesh screen to remove tissues. For fertilization, sperm were introduced into the container with matured oocytes. *In vitro* fertilization occurred and after 15 min, the eggs were washed with filtered seawater (25-µm mesh screen for washing off excess sperm). Fertilized eggs were put in 150-l containers filled with 1-µm-filtered seawater held at 27°C for hatching.

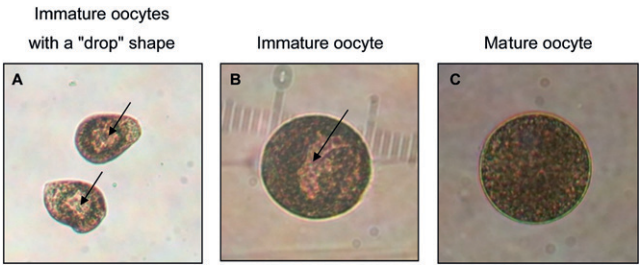

Figure 2. Artificial maturation of *Pinctada margaritifera* oocytes in ammonia solution. (A) Immediately after female gonad striping, immature oocytes appeared in "drop" shape. (B) After 30–50 min, immaturity of the oocytes was shown by the presence of a germinal vesicle. (C) After about 60 min, oocytes presented no germinal vesicle, were in round shape, and were ready for fertilization. Arrows indicate the germinal vesicles.

Experimental Design of the Larval, Seed, and Juvenile Rearing of Pinctada margaritifera

After 24 h, the D larvae were collected on 40-µm mesh screens, and each half-sib family was transferred (in bottles) by plane to the Ifremer hatchery facilities. On their arrival, each half-sib family was individually transferred to a 30-l rearing tank filled with 1-µm-filtered seawater at 27°C, where they were reared until settlement. Veliger larvae were initially reared at a concentration of 20 larvae/ml. D-shaped larvae were fed for 6 days on a tropical microalgae species: *Isochrysis galbana* (T-Iso). From the 7th day after fertilization, they were fed on a mix of two microalgae (*I. galbana* and *Chaetoceros gracilis*). During larval rearing, seawater was renewed every two and a half hours with 200 ml/min water flow and 40% renewal rate per hour. Tanks were cleaned every 2 days and larvae were sieved on 60-µm and 80-µm mesh at days 8 and 15, respectively, to remove remains of dead larvae. During tank cleaning, larvae were concentrated in a 2-l graduated cylinder and their number was assessed in a sample ranging from 200 µl to 1 ml in volume.

At day 18 postfertilization, each family was divided into three batches on the basis of their size using appropriate mesh screens: small-sized batch "P" (ranging from 100 to 120 µm), medium-sized batch "M" (ranging from 120 to 150 µm) and large-sized batch "G" (>150 µm). This allowed to obtain 12 batches: NJ P, NJ M, NJ G, NR P, NR M, NR G, B1 P, B1 M, B1 G, C1 P, C1 M, and C1 G. The larval phase lasted 21 days, when the corresponding pediveliger stage was reached.

The pediveliger larvae were transferred in micronursery batches of ~10,000 individuals for settlement into downwellers (Ø 40 cm, height 40 cm, mesh 130 µm) set out in flowthrough raceways (200 × 50 × 50 cm), with four downwellers per raceway. Correspondence between downwellers and families was randomly selected among the three raceways. Filtered seawater (5 µm) was continuously supplied, with a mixture of the microalgae *T-Iso* and *Chaetoceros gracilis* at an average concentration of 20,000 cell/ml. After 1 wk, the nonsettled larvae were eliminated. The meshes were cleaned every day by brushing their external side. After ~45 days, the settled spat (2–5 mm in size) were detached using a brush and transferred to the nursery.

In the nursery, the transferred spat were reared in 36 raceways of 90 × 20 × 20 cm (corresponding to a volume of 30 l), at a density of 1,000 individuals per raceway. Correspondence between raceways and families was randomly selected among the 36 units. This density prevents any mortalities and growth competition during the entire experiment till 141 days. In addition, these 36 raceways corresponded to triplicate of the 12 batches (provided from the micronursery). Unfiltered seawater was added with a suspension of microalgae produced in outdoor tanks at a renewal rate of 100 l/h. The 10-m^3 outdoor algal tank contained a bloomed mix of *T-Iso* and *Chaetoceros gracilis* in filtered seawater, cultivated with a fertilizer consisting of 78 g Ballance® Triple superphosphate and 75 g Ballance® urea.

Spat Biometry: Shell Diameter Measurements

Biometry measurements of the spat were conducted once in a week from day 65 to day 141 postfertilization. Fifty to one hundred individuals from each batch and each family were randomly sampled. Three samples were collected per family corresponding to each batch. The sampled spat were then placed in a 14-cm glass Petri dish and meticulously separated from one another. A 20-cm Petri dish was used later as the spat grew larger. The spat used for the measurements were not sacrificed and subsequently returned to their respective raceway.

Measurement of the shell diameter was performed by image analysis pictures of spat obtained using an Epson Perfection 4990 Photo scanner at 600-dpi resolution. The pictures were treated using Adobe Photoshop CS3 extended. Individual measurement of spat was obtained using ImageJ software following a modified procedure described in Moriceau (2010). Diameter was ultimately determined from the shell area corresponding to each individual.

Statistical Analysis

To assess differences in growth between juveniles issued from crosses made with the two spawning methods, data from each method were pooled, NJ/NR for the gonad scarification procedure and B1/C1 for the thermal shock, before comparison using Mann–Whitney tests. Additionally, data from each family were pooled by considering all size batches together to evaluate

the overall growth rate for each family. Half-sib comparisons, NJ versus NR and B1 versus C1, were performed. Differences in the spat mean shell diameter were evaluated using Mann–Whitney tests. Kruskal–Wallis tests were used to test differences among size batches (P, M and G) within each family. If the overall test was significant, a pairwise Wilcoxon test with a Bonferroni correction was performed.

In all tests, P values lower than 0.05 were considered significant. All analyses were performed using R software (version 2.14.1).

RESULTS

Effect of Spawning Procedure on Pinctada margaritifera *Juvenile Growth*

Growth performance showed no significant difference between juveniles produced from gametes obtained by thermal shock (NJ and NR half-sib families) and gonad scarification (C1 and B1 half-sib families) during the period day 78–141 postfertilization (Fig. 3), although, significant differences in size were observed from day 65 until day 78 postfertilization ($P < 0.00001$ at day 65, $P \leq 0.03$ at day 71, and $P < 0.00001$ at day 78). From the beginning to the end of the experiment, juvenile size increased up to four times, whatever the prior spawning procedure, from 2.4 to 10.4 mm diameter on average. Although the present study was focused on juvenile growth, no impact on growth and survival rate has been detected during larval stage period (data not shown).

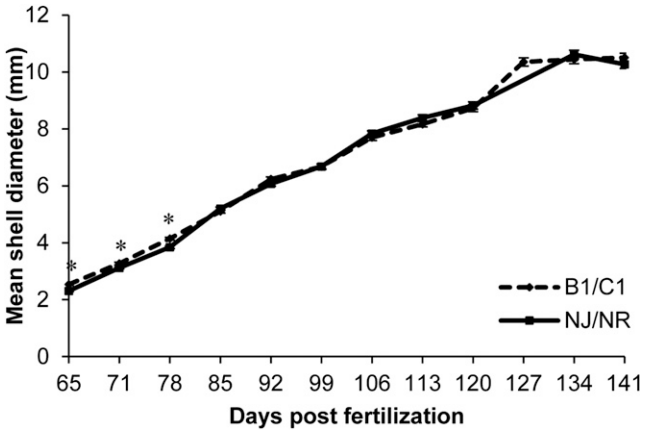

Figure 3. Growth monitoring through shell diameter biometry (mm) of *Pinctada margaritifera* juveniles from half-sib families produced from gametes obtained by thermal shock (NJ and NR) or gonad scarification procedures (B1 and C1). Growth evolution was recorded from 65 to 141 days postfertilization. Values and error bars represent average diameter ± SE, respectively. Data points significantly different at $P < 0.05$ are indicated by an asterisk (*).

Half-sib Family Effect

Average growth of NJ and NR half-sib families (produced by polyandry mating design) was not significantly different during the experiment except at days 65 ($P < 0.0001$), 92 ($P < 0.001$), and 99 ($P < 0.001$) postfertilization (Fig. 4A).

Comparison between B1 and C1 half-sib families (produced by a polygyny mating design) revealed a significant difference, with C1 showing the largest size at days 65–85, 106, and 120–141 postfertilization (Fig. 4B). At this last point, mean diameters for B1 and C1 were 9.7 and 11.5 mm, respectively. The C1 family was 19% larger at that point and was on average 14% larger throughout the study. No significant differences were observed at days 92, 99, or 113 postfertilization.

Intra Half-sib Family Growth Performance

For each of the four half-sib families, growth of the three size batches G, M, and P was significantly different (Fig. 5).

For the NJ half-sib family and from day 65 to day 78 postfertilization, mean shell diameter was not significantly different, with an average of 2.2 mm (Fig. 5A). From day 85 postfertilization until the end of the experiment, the G batch grew significantly faster than the P and M batches. These latter two batches showed no significant difference throughout the study except at 106 and 113 days postfertilization. At the end of the experiment, the G batch shell mean diameters had increased up to seven times compared with their initial size, whereas the P and M batches had only increased by a factor of 4. At day 141 postfertilization, G showed 57% and 73% higher shell diameter than the P and M, respectively.

For the NR half-sib family, the mean shell diameters of the three batches showed no significant differences on days 65, 71, and 85 postfertilization (Fig. 5B). From day 92 postfertilization until day 134, batch G grew significantly faster than batches P and M. During this period, mean diameters of batch G were on average 17% and 13% higher than those of batches P and M, respectively.

For the B1 half-sib family, mean diameter started at 1.7 mm for batch P, 2.2 mm for batch M, and 2.7 mm for batch G, respectively; and finally attained 8.9 mm (+417%), 9.4 mm (+333%) and 11.3 mm (+315%), respectively (Fig. 5C). Batch G remained significantly larger in size than the other two batches during the entire period studied. In addition, P and M batches were not significantly different in size from day 92 to day 141 postfertilization: 31% and 18% larger on average than batches P and M, respectively. At the end of the experiment (day 141), batch G was 27% and 21% larger than batches P and M, respectively.

For the C1 half-sib family, batch G was significantly larger in size than batches P and M over the entire experimental period (Fig. 5D). From day 71 until day 92 postfertilization, batch P appeared to be significantly smaller than batches M and G. At the end of the experiment, batch G had grown to five times its initial size, whereas batches P and M had only increased four times. In addition, the C1 batch G had grown 45% and 47% larger than the P and M batches, respectively, at day 141. On average, batch G was 31% and 26% larger than batches P and M, respectively.

DISCUSSION

Effect of Spawning Procedures on Growth Performance

The comparison of mean shell diameters between spat produced with the two spawning methods (thermal shock and gonad scarification) showed no clear difference at the juvenile growth stage. Although the mean shell diameters of the two groups of spat showed significant differences from day 65 to day 78 postfertilization, the values were still quite close. These differences could be explained by small variations in the spat size at these early stages that statistically segregate the two

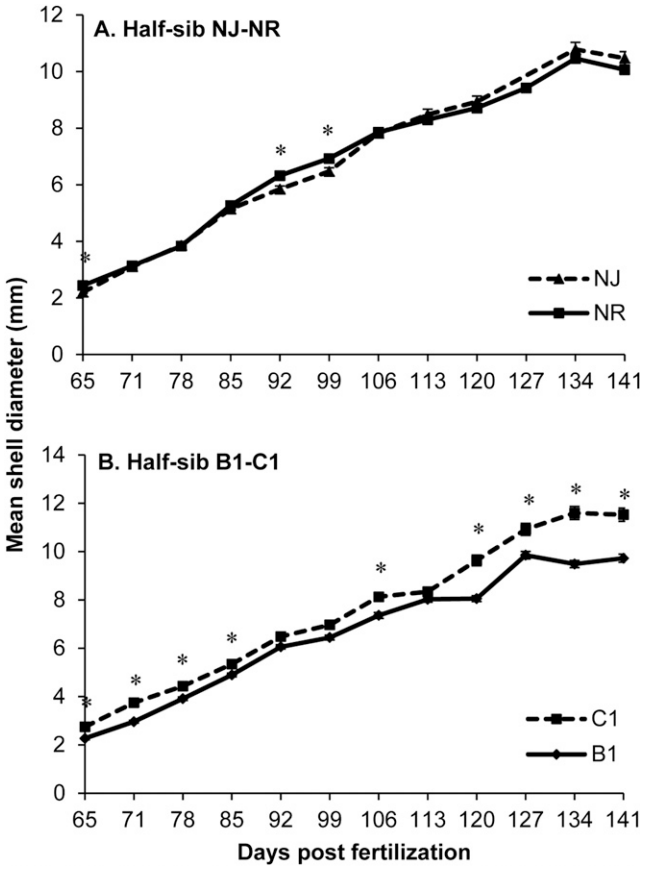

Figure 4. Growth monitoring through shell diameter biometry (mm) of *Pinctada margaritifera* juveniles between (A) half-sib families produced by crossing one dam and two sires (NJ and NR) or (B) by crossing two dams and one sire (B1 and C1). Growth evolution was recorded from 65 to 141 days postfertilization. Values and error bars represent average diameter ± SE, respectively. Data points significantly different at $P < 0.05$ are indicated by an asterisk (*).

groups. Juvenile growth for the two groups seems to have remained equal throughout the experiment, because the comparison of mean shell diameter showed almost the same growth pattern, especially at the end of the study where both groups showed the same mean increase in mean shell diameter, about 8.3 for the NJ/NR group and 8.5 mm for B1/C1. This suggests that using the gonad scarification procedure to collect gametes potentially has no impact on the growth of the offspring at the juvenile stage and it seems that it does not affect their shell biomineralization performance. Studies on the effect of scarification on the size and growth of offspring are rare. The thermal shock method used here does not require the animals to be sacrificed, whereas the gonad scarification procedure requires the oysters to be opened to collect the gametes and is therefore destructive. For selective breeding programs, the gonad scarification could show advantageous perspectives because color selection on the interior nacreous side of the shell can only be made once the shell has been opened. With the thermal shock procedure, such selection would be difficult because the shells of the animals are barely open and inner shell color would therefore be hard to judge. Quality trait selection using the gonad scarification procedure could produce

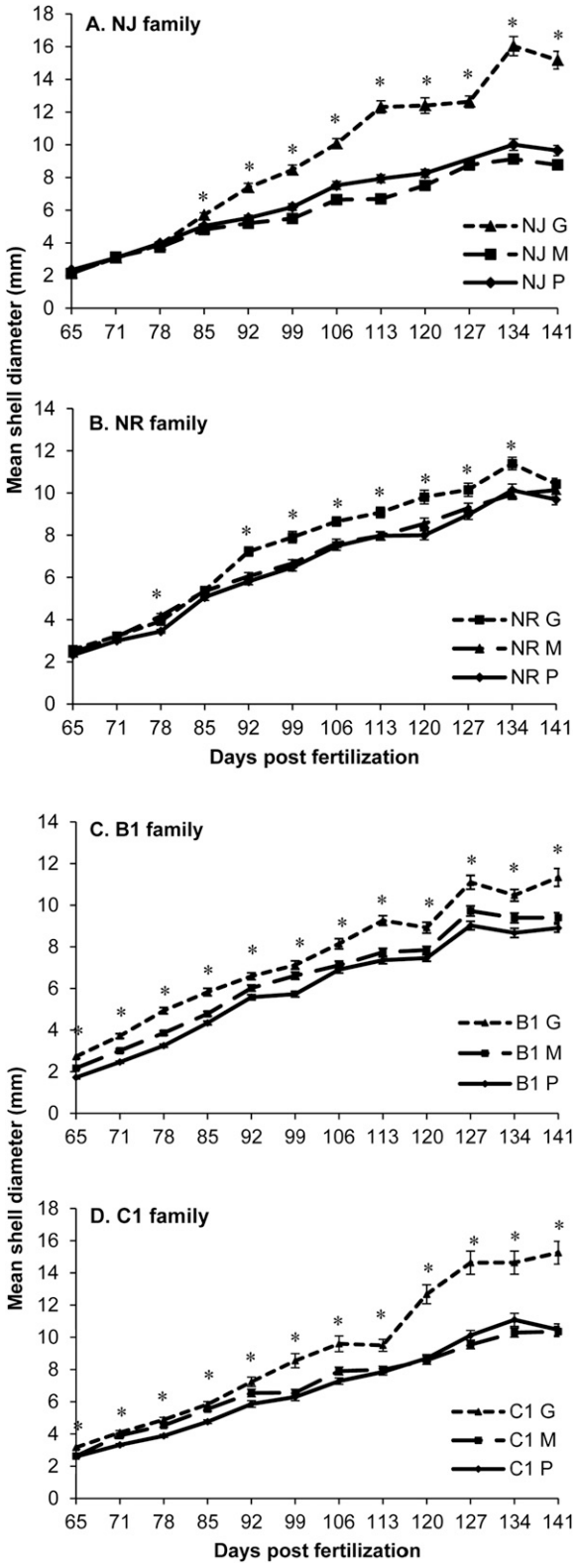

Figure 5. Growth monitoring through shell diameter biometry (mm) of *Pinctada margaritifera* juveniles among size batches (large: G; medium: M; and small: P) of each family: (A) NJ family, (B) NR family, (C) B1 family, and (D) C1 family. Growth evolution was recorded from 65 to 141 days postfertilization. Values and error bars represent average diameter ± SE, respectively. Data points significantly different at $P < 0.05$ using a Kruskal–Wallis test are indicated with an asterisk (*).

potential high-quality donors because it has been shown that there is a family effect of donors on cultured pearl quality (Tayale et al. 2012, Ky et al. 2013), with evidence of a relation between cultured pearl color and the biomineralization capabilities of the mantle cells of the graft correlated with the interior shell color of the donor oyster (Tayale et al. 2012). Using a donor oyster with a yellow inner shell color, for example, these authors observed a large proportion of yellow pearls in the harvest.

Half-sib Family Effect

Comparison of the NJ and NR half-sib families, which were produced by crossing one dam with two sires, showed no significant differences in spat mean shell diameters. With the opposite mating plan: two dams and one sire, families B1 and C1 showed clear significant differences in shell diameters throughout the experiment. As this result was found between the progenies of two females crossed to the same male suggests that there could be a maternal effect on the size of the offspring. In contrast, the lack of such an effect in the progenies of one female crossed with two males implies that the paternal contribution does not notably affect the size of the offspring. Maternal effect of different animal species on egg size and offspring has already been reported in the literature. Cruz and Ibarra (1997) reported a maternal effect on egg characteristics in another mollusk *Argopecten circularis*. Allen et al. (2008) showed that maternal environment of invertebrate *Bugula neritina* has an effect on the size of offspring. Heath et al. (1999) also reported a maternal effect on the early life offspring size in chinook salmon *Oncorhynchus tshawytscha*, although this influence decreased as the offspring aged. In contrast, the results of the B1 and C1 families in the present study suggest that there would be no benefit of the contribution of the males to their progenies growth performance. The implication of these results for *Pinctada margaritifera* genetic selection programs could be important. Selection of females for their quality traits and the potential of their progenies as donors may have interesting applications in genetic selection programs. The benefit of the selection of females for cultured pearl quality traits such as nacre weight and thickness has also been reported in another experiment using half-sib families produced by polyandry and polygyny mating designs (Ky et al. 2015). Although selection of females could offer such benefits, *P. margaritifera* is a protandric species and the low numbers of females in both natural and cultivated conditions could be a restraint to such selection. Selection of males, although it may not offer such clear benefits as with females, remains an interesting prospect for genetic programs.

Growth Performance of Size Groups within the Families

From day 65 to day 141, average size of the spat of the four families increased by about four times; however, growth showed large variations among different families and size batches, especially the G groups, which showed increase of shell diameter to be higher in three out of four families, especially in the C1 and NJ families. Shell growth is the result of the interaction of several factors. The process controlling the growth is directly related to the biomineralization process. Joubert et al. (2014) reported that the molecular processes and gene expression involved in biomineralization is influenced by environmental conditions. Studies have demonstrated that food and temperature play a key role in the growth of *Pinctada margaritifera* (Doroudi et al. 1999, Linard et al. 2011). In addition, the density can also affect growth rate (Sims 1994, Wada & Komaru 1994, Taylor et al. 1997). High densities can affect physiological processes relating to food or oxygen availability. But, in this study, inherent genetic differences could probably be responsible for the shell diameter difference between the batches observed. Indeed, this study (1) showed the same rearing conditions in the raceways (with a random attribution between the triplicate batches of families and raceways) and (2) used a low rearing density (1,000 individuals per raceway) that was far from a limiting growth condition. In the same way as for shell color, differences in growth rate caused by differences in the biomineralization process could result from gene expression differences, although the relevant genes are regulated by varying environmental conditions (Joubert et al. 2014). Further molecular investigations are needed to assess the regulation process of the parts of the genome responsible for these variations. Selection for high growth performance exhibited by spat like G group could have interesting implications for pearl quality, because growth through nacre deposition rate is related to the biomineralization process. Indeed, Blay et al. (2014) showed a correlation between high nacre deposition potential of the donor oyster and cultured pearl quality improvement.

CONCLUSION

This study found that the gonad scarification procedure neither impairs nor improves juvenile growth of hatchery spat produced by this method compared with spat produced by thermal shock, as no clear difference was seen in the growth performance of these groups. The results of this study are interesting for selective breeding programs and the cultured pearl industry as the gonad scarification procedure allows farmers to select genitors on the basis of characters such as inner shell color which is only observable on sacrificed animals and therefore not possible with thermal shock–induced spawning. These selections are likely to be performed on female oysters, as traits like juvenile growth seem to be inherited through a maternal contribution rather than a paternal one. Juvenile size indeed showed a clear difference between half-sib families when two dams were crossed with one sire. Genes controlling growth through biomineralization process are to be regarded as well. The analysis of gene expression involved in the biomineralization should help to identify markers and improve the understanding of these processes implicated in animal growth performance but also in the cultured pearl traits important for the industry.

Although only two pairs of half-sib families were used in this study, this experiment shows the potential of the gonad scarification procedure and polygyny mating design. Further studies exploring this potential should be considered to improve and assist selective breeding programs for cultured pearl quality in *Pinctada margaritifera*. In French Polynesia, private hatchery programs are developing to explore the possibility of supplying

farmers with hatchery-produced oysters to complement natural spat collection. Ongoing studies on genetic selection on pearl quality will provide support for these programs to help them to increase cultured pearl quality and reverse the decline that the industry has been struggling with for a decade.

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