Effect of food conditioning on gonadic activity in the oyster *Pinctada margaritifera*

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ABSTRACT

Preliminary experiments allowed defining basic requirements of *Pinctada margaritifera* for somatic and gonadic growth in controlled conditions. This study was continued by observation of the gametogenesis at a cellular level. In April/May 2008, 125 oysters were conditioned for 30 days in 25 tubular tanks (30 L). Water was renewed 4 times/hour. A mixed (v:v) diet of *Isochrysis galbana* (T-Iso) and *Chaetoceros gracilis* was supplied continuously. A control batch of 125 oysters was maintained in trays in the lagoon. An initial sample of 30 individuals was made. Gonadic changes were first characterized using a gonadal development index (GDI) based on the ratio of the gonad area to the total visceral mass area on a sagittal section of the body. Histological sections were made to analyse gametogenesis cellular lines. Initial and final GDI distribution of control oysters did not vary and followed the normal law. In conditioned oysters GDI distribution appeared flattened. Analysis of GDI data showed that reproductive effort was significantly lower (P<5%) in conditioned oysters. Histological analysis of female gonad revealed that oocyte size increased significantly in conditioned females but not in controls. Male cell lines observation showed that only spermatozoa remained in conditioned oyster. Gametogenesis process appears then to have been stopped or slowed down in conditioned animals. This experiment showed that the techniques allowed to finish the development of already present germinal cells but seemed to put an end to gametogenesis. Further studies will focus on the capacity of *P. margaritifera* to maintain its gonad activity in captivity.

Keywords

Pearl oyster - reproduction – gametogenesis- broodstock conditioning.

1. INTRODUCTION

The control of the reproduction is a major requirement for the domestication of any species. Therefore, the physiological mechanisms controlling the gonadic activity must be understood for a best conditioning of broodstock in the laboratory. This requires a comprehensive understanding of the influence of environmental factors such as temperature and trophic resources. In the wild, the pearl oyster, *Pinctada margaritifera*, undergoes continuous gametogenesis because the trophic conditions and temperature are very stable and favourable all year round [1]. As a consequence of the relative abundance of mature pearl oysters, hatcheries may rely on wild broodstock for production and basic knowledge on *P. margaritifera* broodstock conditioning is mostly lacking.

Chlorophyll *a* concentration is a bulk estimator of phytoplankton biomass but give no information concerning the nutritional state of the pearl oyster. Chlorophyll *a* range is 0.1 to 0.8 µg L^{-1} in Polynesian lagoons such as Takapoto (Northern Tuamotu) [2] [3]. In the laboratory, transposition of these data in microalgae concentrations gives an equivalent of 1000 to 8000 cell mL^{-1} of *Isochrysis aff. galbana* (T-*Iso*) [4]. The qualitative requirements estimated through the analysis of sterols and fatty acid during the oogenesis show that essential polyunsaturated fatty acid could be supplied by a diet composed of 2 algae species: T-*Iso* and *Chaetoceros calcitrans* [5]. This study describes a preliminary experiment aiming at defining basic requirements of *P. margaritifera* for gonadic growth in controlled conditions. The effort of reproduction was followed by measurement of gonad size. The intensity of gametogenesis was analysed numerically and an histological analysis of gonad was focused on the presence or absence of gonia in male gonad and oocytes size for female.

2. MATERIAL AND METHODS

In April 2008, 125 oysters were conditioned for 30 days in 25 30L tubular tanks (∅ 150mm, L 800mm .). Water was renewed 4 times/hour. A mixed (v:v) diet of *Isochrysis galbana* (T-Iso) and *Chaetoceros gracilis* was supplied continuously. Algae concentration was checked daily at inlet and outlet of each tank. A control batch of 125 oysters was maintained in trays in the lagoon. An initial sample of 30 individuals was made.

Sampled oysters were opened and flesh was removed. After flesh dissection, the visceral masses (VM) were placed in salted 10% formalin during 72h before being transferred into ethanol 70% for histology. VM were then cut according to the sagittal plan. An image of the section was obtained using a desktop scanner. The digital pictures were then analysed using the ImageJ software. Gonad size was characterized using a gonad development index (GDI) which is the ratio of the gonad surface (G) to the VM area of a sagittal section (GDI = G/VM).

For histological analysis, the gonads were dehydrated through a graded series of ethanol, embedded in paraffin, sectioned as 3–4µm slices on a rotary microtome, stained with Giemsa dye and finally mounted on microscope glass slides. Male gametogenesis was analysed for the presence or absence of the different germinal cells (GC). Normal gametogenesis was characterized by the presence of all types of germ cells from gonia to spermatozoa. Two situations were described as abnormal gametogenesis : the absence of any germ cell other than spermatozoa or the presence of clusters of gonia associated with spermatozoa.

Female gametogenesis was studied by measurement of oocyte diameters following the procedures described by [6].

Breeding capacity was checked at day 0 and day 30 by spawning induction. Cleaned oysters were placed in a flat tank where they were submitted to 20°C. After 20h, the temperature was raised to 30°C. Spawning generally occurs 1h30 after the thermal shock. 50 oysters were stimulated, for each condition (T0 lagoon, T30 lagoon and T30 conditioning)

One way Anova followed when possible by the Fisher *post hoc* test was used to analyse the effect of the conditioning on the effort of reproduction (GDI)

3. RESULTS

3.1 Environmental conditions

Average temperatures in lagoon and conditioning tanks were respectively of 26.4°C and 26.1 °C. Mean algae concentration at the inlet of the tanks was of 15000±4000 cells mL^{-1} while mean algae concentration at the outlet was of 6000±2000 cell mL^{-1}.

3.2 Spawning

After one month, 65 % of the conditioned animals responded to the stimulation instead of 7% for the lagoon population (among them 4 individuals released oocytes).

3.3 Effort of reproduction

Initial and final GDI distribution of control pearl oysters did not vary and followed approximately the normal law (figure 1a, b). In conditioned oysters GDI distribution appeared flattened due to the relatively higher proportion (around 20%) very low GDI (figure 1c).

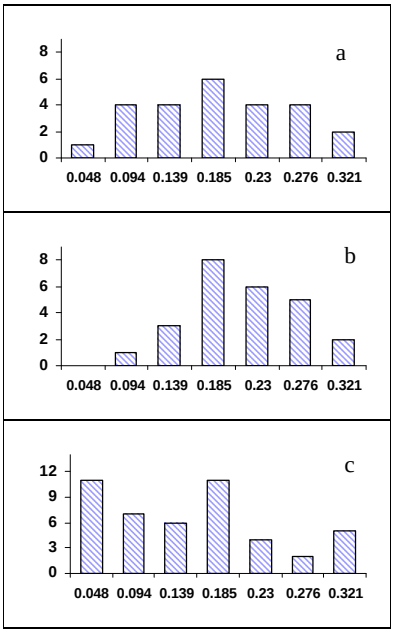

Figure 1. GDI distribution of T0 oyster reared in lagoon (a), T30 oysters in lagoon (b) and T30 conditioned pearl oysters (c).

Analysis of GDI data showed that reproductive effort was significantly lower (P<5%) in conditioned oyster (table 1).

Table 1. Gonadal development index (GDI) from oysters reared in lagoon and conditioned oysters

Origin	GDI±std	n
Lagoon T0	0.17±0.07[a]	25
Lagoon T30	0.19±0.06[a]	25
Conditioning T30	0.13±0.09[b]	46

3.4 Histology

Male cell lines observation showed that only spermatozoa remained in conditioned pearl oysters. The gametogenic process appeared then to have stopped or slowed down in conditioned animals (table 3) since gonia had disappeared along the tubular epithelium.

Table 3. Percentage of oysters exhibiting continuous GC lines, gonia clusters and absence of GC lines in oysters reared in lagoon and in conditioned oysters

Origin	Continuous	Clusters	Absence
Lagoon	100%	0%	0%
Conditioning	0%	44%	56%

Ten females reared in lagoon and 8 conditioned females were studied. Oocyte size increased significantly in conditioning but not in controls females. In conditioned female the smallest oocytes disappeared (figure 2).

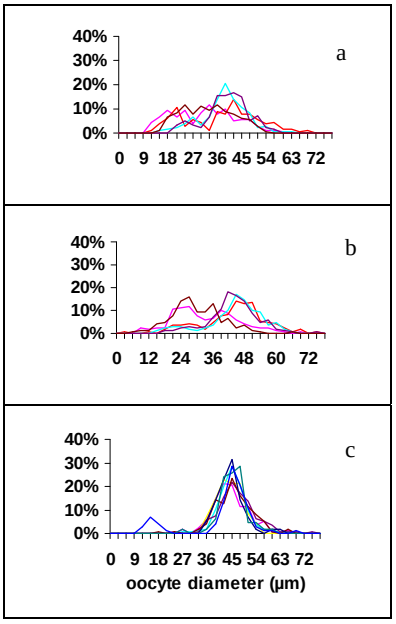

Figure 2. Oocyte size frequency of T0 oyster reared in lagoon (a), T30 oysters in lagoon (b) and T30 conditioned pearl oysters (c).

4. DISCUSSION-CONCLUSION

At the end of the 30 days experiment, most of conditioned pearl oysters responded to spawning stimulation. The response of control population reared in lagoon was significantly lower.

The distribution of GDI of control pearl oysters indicated that the gonads evolved slowly between the beginning and the end of the experiment in the natural environment. The flattened distribution of GDI of conditioned oysters could however indicate that some oysters spawned during the conditioning period. The GDI analysis did not show a positive effect of conditioning on gonad size.

Histological analysis revealed an interruption of gametogenesis in conditioned animals. The observations made on control pearl oyster showed the permanence of the production of gametes with the presence of all types of germinal cells between gonia and mature cells (spermatozoa and ripe oocytes). In conditioned males, no gonia could be observed in 56% individuals, only clusters of gonia remained in the others. Similarly, female gametogenesis appeared to be also interrupted : no previtellogenic oocytes remained in the gonads at the end of the conditioning period, all oocytes had been recruited as ripe oocytes. So far, this consequence of conditioning has not been reported in bivalves : even at low temperature and low food, the gametogenesis is not interrupted in conditioned *Crassostrea gigas*. The importance of energetic reserves has been pointed out in this species [7]. Pearl oysters have no specialized reserve tissue and very few information is available on their energy management. This species appears to be very dependant to food availability.

The conditioning of *P. margaritifera* allowed the maturation of the gonads by developing already present germinal cells to maturity. Despite the interruption of germinal cells production, the conditioned oysters spawned after 1 month, whereas the oysters reared in lagoon which had maintained a complete germinal cell line did not spawn. Around 20% of conditioned oysters with low GDI could have spawned during the conditioning and the absence of GC hampered the gonad recovery.

The control and the conditioned pearl oysters dealt with environmental conditions (mainly trophic resource considering that the temperatures were not really different). Food availability is known to be an important factor for bivalve development, affecting broodstock energy reserves and gametogenesis, duration of the maturation process, fecundity, quality and quantity of eggs, and larval development [7] [8] [9] [10].

Further studies will focus on the factors that could allow *P. margaritifera* to maintain its gonad activity in captivity. Works will be done on the effect on the trophic level and temperature because the functioning of bivalve for growth and reproduction is based on the relationship with the environmental conditions such as water temperature and food level [8]. Investigations about oyster reproduction need to be undertaken more deeply in order to enhance hatcheries performances and to get a better insight on the reproductive behaviour and its implication in natural populations.

5. ACKNOWLEDGMENTS

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