MATURATION OF NATIVE OYSTER *Crassostrea gasar* AT DIFFERENT DIETS IN THE LABORATORY*

Cassio de Oliveira RAMOS [1]; Jaime Fernando FERREIRA [2]; Cláudio Manoel Rodrigues de MELO [2]

ABSTRACT

This study evaluated the influence of different microalgae diets on gonadal tissue maturation in the native oyster *Crassostrea gasar* in the laboratory, between March and May 2010, totalizing 60 days. Ninety-six oysters, collected from an experimental farm located on Florianópolis/SC, were transferred to the laboratory and maintained in three different diet treatments: *Isochrysis galbana*, *Chaetoceros müelleri* and a mix diet of both species in a 1:1 ratio. The oysters were conditioned in 3 L experimental units at a water flow rate of 300 mL min^{-1} and constant aeration. Food was provided in a continuous flow system, at a density of 16 x 10^4 cells mL^{-1}. The mean water temperature and salinity during the experimental period were 24.36 ± 1.23 ºC and 29.4 ± 3.08, respectively. Fortnightly, six oysters randomly sampled from each treatment were examined for histological analysis. The condition index was analyzed at the beginning and the end of the experiment. The initial mean height and weight of the oysters were 68.25 ± 6.73 mm and 60.89 ± 14.19 g, respectively, and the final mean height and weight were 69.20 ± 5.97 mm and 70.04 ± 17.42 g, respectively. The sex ratio for the treatments was 1.2 males for each female and 19.5% of the oysters were considered indeterminate. The condition index was not affected by treatments and there was no improvement on the gonadal tissue maturation of the native oyster *C. gasar* subjected to different microalgae diets during the conditioning period.

Keywords: Microalgae; *Chaetoceros müelleri*; *Isochrysis galbana*; gonadal development

MATURAÇÃO DA OSTRA NATIVA *Crassostrea gasar* SUBMETIDA A DIFERENTES DIETAS EM LABORATÓRIO

RESUMO

Este estudo avaliou a influência de diferentes dietas microalgais sobre a maturação do tecido gonádico da ostra nativa *Crassostrea gasar* em laboratório, entre março e maio de 2010, totalizando 60 dias. Noventa e seis ostras, coletadas em cultivo experimental localizado em Florianópolis/SC, foram transferidas para o laboratório e submetidas a três tratamentos de alimentação: *Isochrysis galbana*, *Chaetoceros müelleri* e uma dieta mista das duas espécies na proporção de 1:1. As ostras foram acondicionadas em unidades experimentais de 3 L com vazão de 300 mL min^{-1} de água e aeração constante. A alimentação foi fornecida em sistema de fluxo contínuo, na densidade de 16 x 10^4 células mL^{-1}. A temperatura e salinidade média da água durante o período experimental foram 24,36 ± 1,23 ºC e 29,4 ± 3,08, respectivamente. Quinzenalmente, foram realizadas análises histológicas de seis indivíduos amostrados aleatoriamente de cada tratamento. O índice de condição foi calculado no início e no fim do experimento. A altura e peso inicial das ostras foram 68,25 ± 6,73 mm e 60,89 ± 14,19 g, respectivamente, e a altura e peso final foram 69,20 ± 5,97 mm e 70,04 ± 17,42 g, respectivamente. A proporção sexual dos tratamentos foi 1,2 machos para cada fêmea e 19,5% das ostras foram consideradas indeterminadas. O índice de condição não foi afetado pelos tratamentos e não houve melhora na maturação do tecido gonádico da ostra nativa *C. gasar* submetida a diferentes dietas microalgais durante o período de acondicionamento.

Palavras chave: Microalgas; *Chaetoceros müelleri*; *Isochrysis galbana*; desenvolvimento gonádico

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INTRODUCTION

The genus *Crassostrea* comprises several species that can be found growing on different regions of the coastal areas. In Brazil, there were three species of *Crassostrea*: an introduced species, *Crassostrea gigas* (THUNBERG, 1793), and two native species, *Crassostrea gasar* (ADANSON, 1757) (=*Crassostrea brasiliana* (LAMARCK, 1819)) and *Crassostrea rhizophorae* (GUILDING, 1828). Recent studies on molecular biology demonstrated that *C. brasiliana* and *C. gasar* are identical (MELO *et al*., 2010), and the nomenclature *C. gasar* should be maintained, for being the oldest one. There is also a record of a fourth species of the genus *Crassostrea* (*Crassostrea* sp. canela) to the Northern Brazil, Canela Island, in the state of Pará, considered exotic, and that has a strong relationship with the Indo-Pacific species (VARELA *et al*., 2007). This species was also reported in a study in Southern Brazil, in Babitonga Bay, on the Northern coast of the state of Santa Catarina (TURECK, 2010).

The mangrove oyster, *C. gasar*, constitutes an important source of income in many communities along the Brazilian coast. This species is geographically distributed along the coast of Central West Africa (LAPÈGUE *et al*., 2002), and South America, from French Guiana to Southern Brazil (LAPÈGUE *et al*., 2002; LAZOSKI *et al*., 2011).

The commercial feasibility of this species has been demonstrated (PEREIRA *et al*., 2003), however, it is unlikely that natural environment seeds will reach the commercial demand, thus, studies are needed to learn about the production of such species in the laboratory. Methods of conditioning have been well established for other species of the genus *Crassostrea*, for instance *Crassostrea virginica* (DUPUY and RIVKIN, 1972) and *C. gigas* (FUJITA, 1934; BREESE and MALOUF, 1975).

Among the most important stages in the production of bivalves in the laboratory, there is the maturation of breeding (HELM and BOURNE, 2004). Besides temperature, one of the key factors during this period is the diet. It is directly related to the energy reserves of breeding, length of maturation process, fertility, quality and quantity of eggs, and larval development (BERNTSSON *et al*., 1997; UTTING and MILLICAN, 1997; HENDRICKS *et al*., 2003).

In bivalves, the energy is stored as glycogen, lipids and proteins when there is plenty of food and it is subsequently used in the production of gametes, when metabolic demand is high (MATHIEU and LUBET, 1993). Glycogen is considered the most important reserve (WHYTE *et al*., 1990) and it is stored in several body tissues. For a proper maturation, oysters must have a large amount of stored glycogen (LOOSANOFF and DAVIS, 1952). Glycogen is mainly used in the synthesis of lipids during the vitellogenesis (UTTING and MILLICAN, 1997) and the whole process of final gamete maturation depend on that, because this will ensure good fertilization rate and formation of larvae (BREESE and MALOUF, 1975).

Mollusc gonad maturation depends on an appropriate microalgae diet (MURANAKA and LANNAN, 1984). The nutritional value of the diet depends on the type of microalgae used. Although there is a big difference in the composition of microalgae, related to the class and species, protein is always the main organic compound, followed by lipid and carbohydrate (COUTEAU, 1996). According to SPENCER (2002), microalgae with high nutritional value must have large amounts of fatty acids (especially for long chains, called polyunsaturated fatty acid – "PUFA'S").

Among the different species of microalgae used in aquaculture, *Chaetoceros müelleri* and *Isochrysis galbana* stand out due their high nutritional value (MOURA JR. *et al*., 2006). COUTEAU (1996) claims that species of diatoms, such as *C. müelleri*, have significant concentrations of eicosapentaenoic acid (20:5, EPA), while high concentrations of docosahexaenoic acid (22:6 ω 3, DHA) are found in *Isochrysis* sp. Therefore, the supply of mixed diets probably contains the biochemical diversity needed not only for growth, but also for gametogenesis (MADRONES-LADJA *et al*., 2002).

Bivalve production in the laboratory is directly related to the quality and quantity of microalgae provided (HELM and BOURNE, 2004). Cultivation of phytoplankton in the

laboratory of molluscs can represent 30-50% of the total costs of production (JEFFREY and GARLAND, 1987; HELM, 1990; COUTEAU and SORGELOOS, 1992; BOROWITZKA, 1999; PONIS *et al.*, 2003). Thus, the choice of the appropriate microalgae is a critical step in the maintenance of breeding (RICO-VILLA *et al.*, 2006). From this assumption, the present study assesses the influence of three microalgae diets on gonadal tissue maturation in the native oyster *C. gasar* in the laboratory.

MATERIALS AND METHODS

Mature adults of *C. gasar* from the third generation and the same batch of animals originated from spawning in the laboratory and reared at an experimental farm on Ponta do Sambaqui Beach (27º28'30"S, 48º33'40"W), Baía Norte, Florianópolis/SC, Brazil, were collected and maintained in three microalgae diet treatments in the laboratory from March to April 2010, totalizing 60 days. The first treatment was composed of the microalgae *Isochrysis galbana* (ISO); the second one was composed of *Chaetoceros müelleri* (CM); and the third, of a mixed diet of both species in 1:1 ratio (IC). The microalgae were produced in sealed system, using the culture medium semi-defined "f/2" (GUILLARD, 1975) modified by adding silica to the diatom.

For each treatment, thirty two oysters were maintained into 3 L experimental units in three replicates, totalizing nine experimental units, with a continuous water flow system of 300 mL min^{-1} and constant aeration for 60 days. Food was also provided in a continuous flow system at a density of 16 x 10^4 cells mL^{-1}, methodology routinely used in the laboratory. The mean temperature and salinity of the water (± standard deviation) in the treatment tanks were 24.36 ± 1.23 ºC and 29.4 ± 3.08, respectively. At the beginning of the experiment, five oysters of the same batch were examined for histological analysis and five for condition index in order to verify the influence of the diets on the gonad maturation.

Fortnightly, six oyster chosen randomly and without replacement from each treatment were examined for histological analysis, totalizing four samplings. Although the number of animals has decreased throughout the experiment, microalgae density was kept constant for each oyster and thus, did not affect the investigation. After sectioning the adductor muscle and removing the soft tissue, a section of the gonad was performed in the anteroposterior direction measuring approximately 0.7 cm and was fixed in Davidson's solution for 48 hours (HOWARD and SMITH, 1983). Histological slides were prepared with 5 μm thick sections, stained with Harris hematoxylin and eosin (HE) and examined using an optical microscope to determine sex and gonadal development stages (HOWARD and SMITH, 1983).

The condition index (CI = [dry weight of the meat/ (total weight - weight of the shell)] x 100) was calculated at the beginning and the end of the experiment according to the methodology described by CROSBY and GALE (1990). At the end of the experimental period, the eight remaining oysters of each treatment were examined for the condition index analysis. To prepare the samples, the heights of the oysters (maximum dimension from the hinge to the growth edge, mm) were calculated using a digital caliper with an accuracy of 0.01 mm. The oysters were weighed (total weight) using a digital balance with an accuracy of 0.001 g. Then, after sectioning the adductor muscle and removing the soft tissue, the meat and shell were weighed separately (wet weight) and incubated at 68 °C for 48 hours to obtain the dry weight, in accordance with the method described by LAWRENCE and SCOTT (1982).

The gender of the oyster was designed as follows: male, female, hermaphrodite or indeterminate. The gonad stage determination was made based on the qualitative classifications of SAUCEDO and SOUTHGATE (2008) (Table 1), and on quantitative classifications by means of stereological analysis, using the M-42 test system (Weibel # 2) (WEIBEL *et al.*, 1966).

Table 1. Description of the stages of native oyster *Crassostrea gasar* gonadal development according to SAUCEDO and SOUTHGATE (2008).

Stages	Female	Male
Gametogenesis	Not juxtaposed follicle walls with intra and interfollicular spaces. Oocytes in different stages of development, mostly pedunculated and attached to the wall. Free oocytes have more spherical shape. Presence of connective tissue between the follicles.	Within the follicles along the wall, it is possible to distinguish several strains of germ cells. The sperm accumulates in the lumen and eosinophilic tails are evident in this direction. The amount of connective tissue decreases between the follicles because of its expansion due to sperm accumulation.
Repletion / pre-spawning	Juxtaposed follicles, thickly populated with mature gametes without intra-and interfollicular spaces. The polygonal shaped oocytes are mostly detached from the wall. Little or no visible connective tissue between the follicles.	The germ cells almost disappear being restricted to a small margin in the cellular wall. The follicles are distended, filled with dense agglomerations of sperm with flagellum oriented to the lumen. Almost total absence of connective tissue.
Partial spawning	Many follicles contain oocytes which are usually free in the lumen. Little connective tissue with intra and interfollicular spaces. Follicle walls with the appearance of fragility.	The sperm is expelled from the follicle, that assumes an unsteady appearance partially empty and with broken appearance walls. There is little interfollicular connective tissue with the presence of intrafollicular spaces.
Complete spawning	Collapsed follicles totally or partially empty with remaining gametes. It starts resorption of oocytes that were not expelled. Connective tissue begins to develop between the follicles.	Collapsed follicles with remaining gametes, sperm are degenerating. It starts resorption of sperm that were not expelled. Connective tissue begins to develop between the follicles.
Rest / Indeterminate	Undifferentiated cells of the germinal epithelium so that it may not be possible to distinguish the sexes. There is rarely any evidence of gonadal tissue. The connective tissue occupies most of the part between the collapsed follicles.	

The stereological classification was performed using the Weibel graticule, and the image was superimposed onto the histology slides. The cells were counted at 42 points in five separate areas of the slides. After counting, the mean for the five areas was calculated for subsequent classification. The following seven cell types were determined for the cell count: mature gonads, gonads in development, intracellular space, extracellular space, follicle wall, connective tissue and collapsed gonad. The later stages were categorized as described in Table 1.

- Gametogenesis: greater than 10% of the cells on slide were in the development.
- Repletion: greater than 20% of the cells on the slide were mature cells.
- Partial spawning: greater than 10% of cells on the slide appeared to be collapsed.
- Complete spawning: greater than 75% of the cells on the slide appeared to be collapsed.
- At rest: greater than 40% of the cells on the slide were connective tissue cells.

Data were organized in a completely randomized factorial design (3x4) with three diets, four sampling periods and six replications (animals); except for the condition index, for which there was no factorial scheme, as the effect of period was not considered. The frequency of animals at each stage of maturation (data of histological and stereological analyses) was examined using generalized linear models method (NELDER and WENDDERBURN, 1972), with the cumulative logit link function. The same approach was employed for the condition index with the identity link function. Analyses of "deviance" (ANODEV - a generalization of ANOVA for Generalized Linear Models) were performed to determine the statistical significance of the effects on gonadal maturation or on the condition index, using the GENMOD procedure of SAS® (SAS, 2003).

RESULTS

The oysters used throughout the experimental period were sexually mature, with initial mean height and weight of 68.25 ± 6.73 mm and 60.89 ± 14.19 g, respectively. At the end of the experimental period the mean height and weight were 69.20 ± 5.97 mm and 70.04 ± 17.42 g, respectively. The mean sex ratio for the treatments in the sampling period was 1.2 male for each female, and 19.5% of the oysters were considered indeterminate. No simultaneous hermaphrodites were found.

There was significant correlation ($\rho = 0.71$) between the methodologies used to determine the stages of maturation (histology and stereology). The analysis of deviance (Table 2) indicated that there were no significant differences among the diet treatments during the experimental period and nor among the dates of sampling for the histological and stereological analyses. Interaction between the effects was not significant at 0.01 level.

The photomicrographs of the *C. gasar* cross sections at different stages of gonadal tissue development are shown in Figures 1, 2 and 3.

At the beginning of the experiment, 60% of the oysters were in resting stage, 20% were in gametogenesis, and 20% were in repletion. In the first sampling, partial spawning stage represented 50% in IC and CM treatments. In the ISO treatment, 65% of the oysters were in gametogenesis stage.

Table 2. Analysis of deviance (ANODEV) to assess the differences among microalgae diets, in different harvest dates, for histological and stereological analyses.

Source of Variation	HISTOLOGY				STEREOLOGY			
	DF*	Deviance	χ^2	Pr > χ^2	DF*	Deviance	χ^2	Pr > χ^2
Intercept		356.98				386.3889		
Treatments	2	354.44	1.27	0.53	2	378.0503	4.17	0.12
Dates of sampling	3	353.80	0.32	0.96	3	372.0268	3.01	0.39
Interaction Treatments x Dates	5	326.41	13.7	0.02	5	354.7770	8.62	0.13

**DF: Degrees of freedom*

The gametogenesis stage presented the highest prevalence among all treatments, reaching its peak in the fourth sampling in treatment ISO (100%), however, oysters in this stage were observed in all samplings and treatments. Animals in repletion stage were observed after 15 days in the treatments IC and CM, and after 60 days in the treatment CM, in low percentage (16%). The highest percentage of oysters in partial spawning occurred at 15 days (66% IC and CM, 33% in ISO), with reduction in the subsequent samplings, and in the fourth sampling (60 days) no oysters were observed at this stage. Oysters in complete spawning stage were observed in the second (30 days) (CM - 16%) and third sampling (45 days) (IC and ISO - 16%) (Figure 4).

The condition index was not affected by treatments (Table 3 and Figure 5).

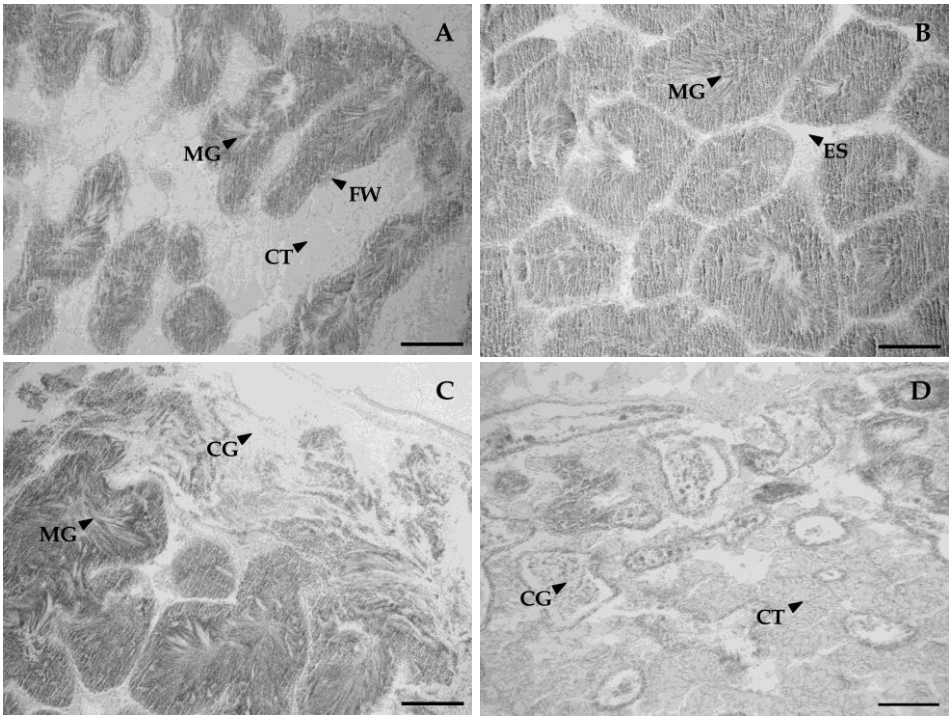

Figure 1. Photomicrographs of the gonadal tissue development stages of the native oyster *Crassostrea gasar* male specimens. A. Gametogenesis, B. Repletion / pre-spawning, C. Partial spawning and D. Complete spawning. Bar: 100 μm. 400X. MG: mature gonads; CT: connective tissue; FW: follicle wall; ES: extracellular space; CG: collapsed gonad.

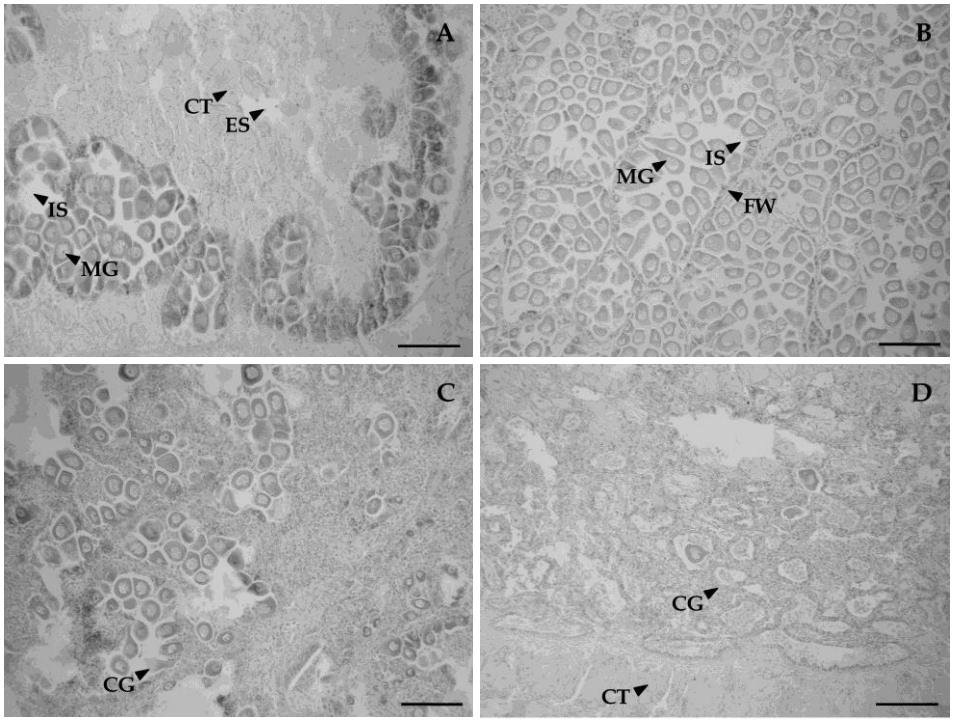

Figure 2. Photomicrographs of the gonadal tissue development stages of the native oyster *Crassostrea gasar* female specimens. A. Gametogenesis, B. Repletion / pre-spawning, C. Partial spawning, and D. Complete spawning. Bar: 100 μm. 400X. MG: mature gonads; IS: intracellular space; ES: extracellular space; CT: connective tissue; FW: follicle wall; CG: collapsed gonad.

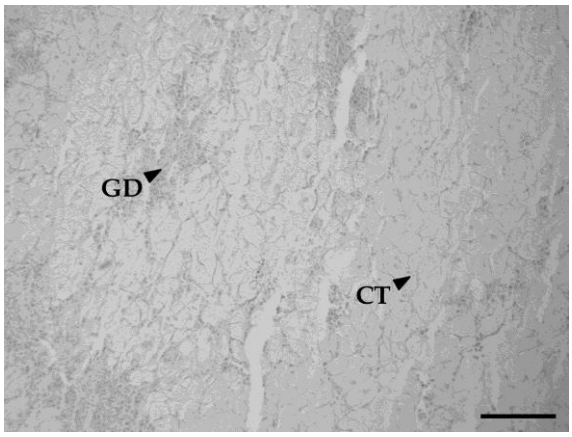

Figure 3. Photomicrographs of the gonadal tissue development stages of the native oyster *Crassostrea gasar*. Indeterminate. Bar: 100 μm. 400X. GD: gonad in development; CT: connective tissue.

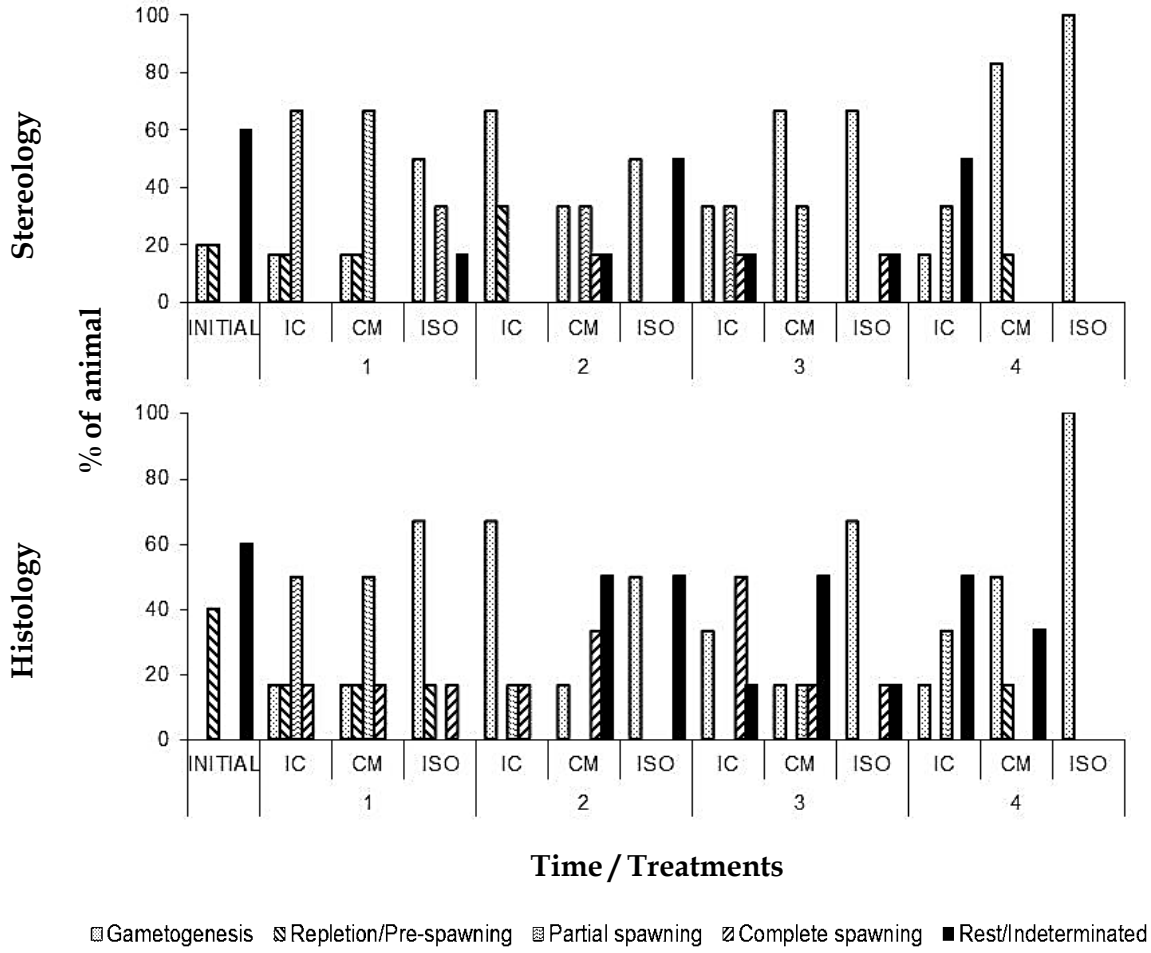

Figure 4. Percentage of the gonadal tissue development stages of the native oyster *Crassostrea gasar* subjected to different microalgae diets, acquired by means of histological and stereological analyses. ISO = *Isochrysis galbana*; CM = *Chaetoceros müelleri*; IC = *Isochrysis galbana* + *Chaetoceros müelleri*. Time: 1 = 15 days; 2 = 30 days; 3 = 45 days; 4 = 60 days.

Table 3. Analysis of deviance (ANODEV) to assess the differences among diet treatments for the condition index.

Source of Variation	DF*	2 Log Likelihood	Chi-Sq	Pr
Intercept		-75.89		
Treatments	2	-72.05	3.84	0.15

**DF: Degrees of freedom*

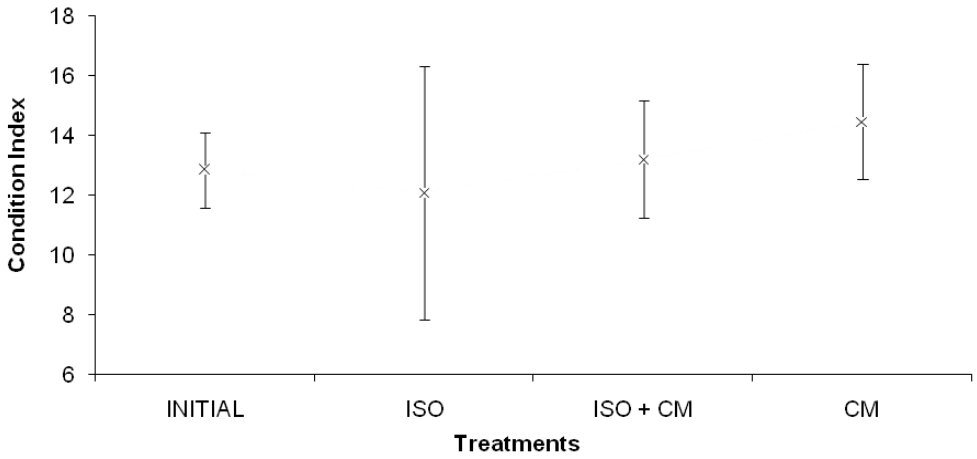

Figure 5. Mean condition index (± standard deviation) of native oyster *Crassostrea gasar* in the different diet treatments.

DISCUSSION

The availability of food can be the most significant factor in the maturation of the gonadal tissue in bivalves (GRIFFITHS, 1977). Oysters that lack organic reserves in their gonads fail to achieve full development (NASCIMENTO and PEREIRA, 1980). This fact agrees with the proposed by BAYNE (1976), in which gametogenesis, in some species, is hindered in unfavorable environmental conditions. However, in favorable environmental conditions, with abundance of phytoplankton, there is an increase of gametogenesis (NEWELL *et al.*, 1982).

Filter-feeding bivalves obtain most of their energy and nutritional requirements from microalgae (VOLKMAN and BROWN, 2005). Biochemical components such as proteins, lipids, fatty acids, and vitamins are essential to promote growth and good health, and these substances are abundant in microalgae (SOUDANT *et al.*, 1998). Several species of microalgae are used in aquaculture to feed bivalves at different stages (BROWN *et al.*, 1998; VOLKMAN and BROWN, 2005); however, the flagellates *Isochrysis* sp. and *Pavlova* sp. and the diatom *Chaetoceros* sp. are preferred because of their small size, rich biochemical and energy profiles, temperature tolerances, and ease of cultivation (MARTÍNEZ-FERNÁNDEZ *et al.*, 2006; MARTÍNEZ-FERNÁNDEZ and SOUTHGATE, 2007; RIVERO-RODRÍGUEZ *et al.*, 2007).

Among many microalgae identified for purposes of aquaculture, species of the genus *Chaetoceros* are extensively used as food (SIMON, 1978; SMITH *et al.*, 1993). The microalgae, *C. müelleri*, is one of the prominent species of microalgae used as a food source for the growth of some commercial species because of its profile of the fatty acids, its size suitable as larval food and its valve little silicified offering little resistance (BROWN *et al.*, 1997).

Several authors have described *I. galbana* and *C. müelleri* as the best microalgae monospecific diet species for the production of molluscs in the laboratory, such as *Ostrea edulis* (ENRIGHT *et al.*, 1986), *Pecten maximus* (HELM and LAING, 1987;

LAING *et al.*, 1987), *Pinctada maxima* (TAYLOR *et al.*, 1997), *Pecten margaritifera* (SOUTHGATE *et al.*, 1998; MARTÍNEZ-FERNÁNDEZ *et al.*, 2006) and *Crassostrea corteziensis* (RIVERO-RODRÍGUEZ *et al.*, 2007). However, MULLER-FEUGA *et al.* (2003) reported that mixed diets of microalgae increases the chances of a balanced diet, making it necessary to have a clear understanding of the nutritional component needs to supply such diets.

For the Japanese oyster *C. gigas*, temperature manipulation is routinely used to induce gamete maturation in research and production laboratories, and different quality and quantity of the algal diet are used to improved fecundity and condition broodstock (BUCHANAN *et al.*, 1998; MARTINEZ and PÉREZ, 2003; PRONKER *et al.*, 2008). URIARTE *et al.* (2004) feeding *C. gigas* with two mixed diets of *I. galbana* e *Chaetoceros gracilis* with different protein supplements, observed that oyster matured in four weeks, however, there was no significant difference in the condition index of the treatments.

DUNPHY *et al.* (2006) analyzed the feeding capabilities of the oyster *Ostrea chilensis* trough the selective removal and consumption of natural planktonic assemblages and artificial inert particles (polystyrene beads) and suggested that difficulties in the broodstock culture might be caused by inappropriate microalgae diets. In the scallop *Mimachlamys asperrima*, O'CONNOR *et al.* (2000) observed higher fecundity when the animals were fed with *C. gracilis*. LIU *et al.* (2008) demonstrated that there was no maturation in the clam *Clinocardium nuttallii* after a 13 weeks conditioning period at 16 °C and with different combinations of the microalgae *I. galbana*, *C. gracilis*, *Thallasiosira pseudonana* and *Tetraselmis suecica*. On the other hand, differences in gonadal tissue maturation were observed in *Mytilus edulis* fed with different mixed diets of *Pavlova lutherii*, *Chaetoceros calcitrans* and *Skeletonema costatum* in the laboratory (PRONKER *et al.*, 2008). Contrary, in the present study, there were no significant differences in the gonadal maturation of *C. gasar* fed with the different microalgae diets.

Seed production in laboratories is often not possible during the autumn / winter periods, when standard methods of conditioning are used (LE PENNEC, 1998). Bivalves that live in regions with temperate waters have a recovery phase or resting phase, which occurs during the autumn and winter, when food becomes scarce (RUIZ *et al.*, 1992). The conditioning of *O. edulis* in the autumn is not always efficient (WILSON, 1981). COCHARD and DEVAUCHELLE (1993) have reported that, occasionally, specimens of *P. maximus* that were conditioned in the autumn are not stimulated with increasing temperature.

Studies of CHÁVEZ-VILLABA *et al.* (2002) and FABIOUX *et al.* (2005) demonstrated that oysters *C. gigas* obtained after major summer spawning events and maintained at elevated temperature and with sufficient food, as used routinely in conditioning method, could not reconstitute their stock of germ cells to initiate gametogenesis. These findings could explain the failure in the maturation of the gonadal tissue of the native oysters in the present study. Our results demonstrated that *C. gasar* conditioned in the laboratory between the period from March to April / 2010, beginning of the autumn, showed no improvement in gonadal tissue maturation, observed by the large amount of oysters in resting and gametogenesis stages, even after the conditioning period. Hence, the oysters could have entered in a dormancy period in which they were not able to become reproductively active without an environmental trigger.

Bivalve artificial maturation is only successful for animals that have already developed their sexual cells, at least on the initial phase of their growth (BAYNE *et al.*, 1975). For *C. virginica,* the reabsorption of residual oocytes is important for a satisfactory conditioning (DUPUY *et al.*, 1977). SASTRY (1979) has shown that in some pectinids, the maturation of gametes can only occur once they have completed the complex tasks of post-spawning. In oyster species *C. gasar* collected from their natural environment in Guaratuba Bay, PR, Brazil, in March 2002, it was observed that 94% of animals had their gonads in empty stage, indicating a possible spawning in the previous month (CHRISTO, 2006). Similar results were observed in the present study. At the beginning of the experiment, 60% of the oysters were in rest stage, what could indicated a possible spawning in previous months. After 15 days, 65% of the animals in ISO treatment were observed in

gametogenesis, demonstrating the gonadal recovery with the beginning of the accumulation of the energy reserves.

GOMES (2009) reports that in March/2009, it began the period in which most of the oysters in the region of Sambaqui / Florianópolis were at spawning and reabsorption stages. In the present study, 60% of the oysters from the same region were in resting stage at the beginning of the study period, and this fact suggests that the oyster probably were in a post-spawning period, which may have hindered the maturation of gametes. Similar results were observed in the present study, with oysters in complete spawning stage after 30 and 45 days of conditioning, and oysters in gametogenesis stage in the whole experimental period. Studies regarding the gonadal maturation of oysters, performed in different periods of the year, are crucial to determine the ideal period for the conditioning of animals from natural environment, and also to determine the time required for the storage of energy reserves, appropriate for the beginning of gametogenesis in the laboratory.

Conditioned breeding in laboratories require 6-8 weeks to reach the stage of spawning in winter and early spring, and a progressively shorter as they approach natural breeding seasons (UTTING and SPENCER, 1991). Breeding of *Argopecten purpuratus* fed with *I. galbana*, *C. gracilis* and *C. calcitrans* for 48 days, did not show mature gametes (MARTINEZ *et al.*, 1992). The conditioning of *M. edulis* breeding for six weeks, fed with different microalgae diets, improved spawning and fecundity rates (PRONKER *et al.*, 2008). In *C. gigas* conditioned for eight weeks, there was a significant increase in the condition index of the oysters fed with *C. calcitrans*, while those fed with *T. suecica* had reduced values of CI compared to the other treatments, and those fed with *Isochrysis* sp. (T-Iso) maintained their physiological state (DELAPORTE *et al.*, 2003). In the present study, despite the conditioning period of eight weeks of the oysters, no significant differences were found for CI values among the different diets.

Further study involving a larger sample of oysters will be necessary to confirm if throughout the period of storage in laboratory, there may be specific times for the oysters maturation to be influenced by different microalgae diets.

CONCLUSION

In the present study, there was no improvement on the gonadal tissue maturation of native oyster *C. gasar* subjected to different microalgae diets during the conditioning period. Our results suggest that oysters conditioned after the natural spawning period showed no signs of maturation probably due to low stock of germ cells to initiate gametogenesis.

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