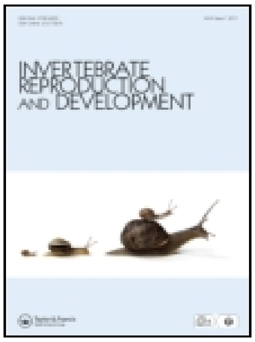

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Influence of environmental variables on the growth and reproductive cycle of *Crassostrea* (Mollusca, Bivalvia) in Guaratuba Bay, Brazil

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The production of oysters in Guaratuba Bay, in the state of Paraná, Brazil, is still a mixed activity of mariculture and extractivism. The sustainable development of this production requires the monitoring of environmental, genetic, reproductive, and zootechnical variables. This study evaluated the importance of these variables on *Crassostrea* oyster production. Data were obtained between September 2009 and February 2011 from six evaluations: water quality, oyster larvae in plankton, capture of oyster spat by artificial collectors, molecular identification of collected spat and larvae, continuous evaluation of the reproductive maturity stage of adult oysters, and zootechnical performance achieved by experimental production. Temperature, dissolved oxygen, and water transparency were the environmental factors that had the most influence on the reproductive cycle (e.g. gonadal development and spat capture) and on the growth of the oyster. The highest rates for spat uptake were recorded in the summer, coinciding with the peak of sexual maturity of adults. The growth of the oysters, in terms of the weight of the meat, was related to the gonadal stage.

Keywords: oyster production; seasonality; performance; molecular identification

Introduction

Crassostrea Sacco, 1897 oysters are bivalve mollusks that inhabit shallow coastal waters from the equatorial zone to the temperate zone between the latitudes of 64°N and 44°S (Rios 1994; Hedgecock 1995). The production of these mollusks ranks them as the second most important species for aquaculture worldwide (FAO 2009), especially because their cultivation involves low installation costs, uses easily obtained material and capturable spat, and generates satisfactory levels of profit (Bautista 1989).

The production of native oysters is an income-generating activity that contributes to the conservation of estuaries, potentially reduces pressure on natural stocks, and promotes sustainable use of the environment (Haws et al. 2010). In Brazil, two native species of oyster are of zootechnical interest: *Crassostrea brasiliana* (Lamarck, 1819) and *Crassostrea rhizophorae* (Guilding, 1828). The first species is generally found on rocky coasts and mangrove roots (*Rhizophora mangle L.*). It is present predominantly in the infralittoral zone, with a distribution ranging from the state of Santa Catarina, southern Brazil, to the state of Pará, in the northern region (Ignacio et al. 2000). The latter species has a distribution from Uruguay to the Caribbean and is generally attached to mangrove roots, rocks, and hard substrata in the mesolittoral zone (Rios 1994). *Crassostrea brasiliana* is considered to be the best species for cultivation in Brazilian estuarine regions and has greater economic and zootechnical yield (Pereira et al. 2001).

Another species of zootechnical interest is the Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), which was introduced into Brazil for commercial cultivation. This species spread to natural habitats in South America, increasing the concern about its possible environmental impact on native oyster species (Escapa et al. 2004; Pie et al. 2006; Christo et al. 2008; Melo et al. 2009).

Similar to other bays in southern Brazil, Guaratuba Bay supports fishing activity, almost exclusively on an artisanal scale, by communities living on the shores of the estuary (Chaves et al. 2002). The location of the natural oyster banks favors extractivism and leads to intense exploitation, which can cause irreversible damage to the population structure of local species (Erse and Bernandes 2008). Oyster cultivation is an alternate income source for small-scale fishing and has contributed to the conservation of the natural stocks of the bay (Andriguetto-Filho et al. 2009).

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Various parameters can influence the success of oyster culture, such as the systems and techniques of cultivation, the biology of the species and requirements for its cultivation (Lucas 2008). The quality of the water influences the physiology and adaptation of the animal to the environment (Pereira et al. 2003). Despite being euryhaline, eurythermic, and adapted to an estuarine environment, the best biological development of *Crassostrea* species for aquaculture occurs under specific salinity and temperature conditions (Siqueira et al. 2010). In addition, the dissolved oxygen concentration, marine currents, and suspended particulate material also influence *Crassostrea* aquaculture (Galvão et al. 2000).

With regard to the reproductive cycle, the commercial exploitation of these mollusks is both directly and indirectly influenced by the sexual characteristics of the species, and by environmental and seasonal stimuli that influence gonadal maturation and the release of gametes (Cledón et al. 2004).

Variations in zootechnical and reproductive results achieved in oyster cultivation in estuarine regions cannot be attributed to a single cause but rather to the interaction of factors such as seasons, biological factors, and environmental processes (Hofstetter 1990). However, few studies have investigated the cumulative effects of environmental and biological processes and how they contribute to changes in the structure of oyster populations (Ramos and Castro 2004). To provide a better understanding of the system where the *Crassostrea* species are inserted, and to enable effective management of stocks from natural farming and banks, it is necessary to identify and quantify the factors that affect the population development of *Crassostrea* species (Dekshenieks et al. 2000). Siqueira et al. (2010) state that knowing the influence of these factors can optimize the efforts of producers at every stage of the cultivation process.

Unsatisfactory results, in terms of zootechnical performance or development, reveal problems during the cultivation planning and/or a mismatch between cultivation and the environment, especially among small producers (Galvão et al. 2009). For the safe and effective development of cultivation methods, it is necessary to understand aspects of the reproductive biology of the species, such as the periods of greatest spawning and recruitment of larvae. In addition, it is also important to monitor the water quality in the cultivation environment (Erse and Bernardes 2008). In Brazil, few studies have been developed to assess the relationship between the biological development of *Crassostrea* species and environmental factors (Lemos et al. 1994; Miranda and Guzenski 1999; Ramos and Castro 2004; Tureck et al. 2004; Christo et al. 2008; Guimarães et al. 2008; Siqueira et al. 2010).

This study aimed to identify the main environmental sources of variation and the extent of their effects on the biological parameters of *Crassostrea* oysters.

Material and methods

Sampling

Biological material and data were collected in Guaratuba Bay, an estuarine system located in the state of Paraná, southern Brazil (25°51.8′S, 48°38.2′W). The oyster species collected were *C. brasiliana*, *C. rhizophorae*, and *C. gigas*, which can be found both in nature and in commercial farms located in the region.

Data came from six different and associated studies: (1) monitoring of water quality parameters; (2) collection of water for research on oyster larvae in plankton; (3) capture of spat using artificial collectors; (4) molecular identification of spat and larvae; (5) evaluation of the stage of reproductive maturity of the oysters; and (6) assessment of the zootechnical performance in experimental cultivation. The data were collected monthly between September 2009 and February 2011.

Sampling sites

Based on a previous survey on natural banks of oysters in Guaratuba Bay (Erse and Bernardes 2008), three sites were selected for water, plankton monitoring, spat collection, and for the collection of adult oysters. The sites were distinguished by the influence of continental, coastal, and marine waters on the physical and chemical variables of the water at each location. The first site (called "Vicente" – 25°51.154″S, 48°36.481″W) was located closer to the mouth of the bay and was greatly influenced by coastal and marine waters. The second site (called "Cabaraquara" – 25°49′59.8″S, 48°34′41.6″W) was situated inside the bay, with both salt- and freshwater from coastal and river water. The third site (called "Parati" – 25°47.866″S, 48°36.477″W), located farther into the bay, was heavily influenced by freshwater from the rivers that flowed into the bay (Figure 1).

Water sampling and plankton analysis

During the study, the temperature, salinity, dissolved oxygen, and transparency of the water were measured twice a day (at the flood and ebb tides, during the morning and afternoon, respectively, following the local tide tables), at a depth of 90 cm. Every 2 weeks, 1000 L of water was filtered, using a mechanical pump and a 65 μm net, for plankton collection at the three sites. The material retained in the net was fixed in a 4%

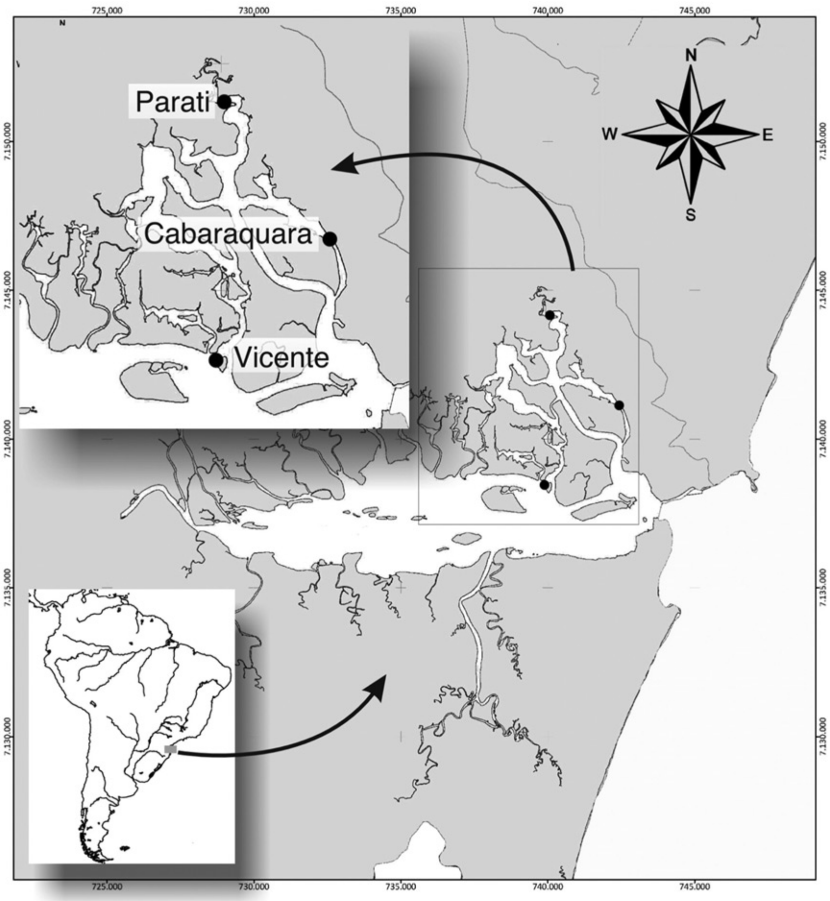

Figure 1. Location of Guaratuba Bay and identification of collection sites for plankton and spat samples of *Crassostrea* oysters (Ludwig et al. 2011).

buffered formaldehyde solution. In the laboratory, the identification and quantification of *Crassostrea* spp. larvae were made from the plankton set according to the protocol of Boltovskoy (1999). Throughout the whole study period, 102 samples were analyzed.

Spat recruitment

The natural recruitment and development of spat were evaluated using artificial collectors installed near the mangrove swamps. The collectors consisted of PVC plates with $50 \times 21\,cm^2$ rectangles, which were sanded to make their surfaces rougher to facilitate the settlement of oyster spat. Each collector consisted of three plates, horizontally positioned in parallel, that were tied together to be arranged in separate floors, 5 cm apart, 30 and 100 cm deep in the water column. The collectors ($n = 132$) remained in the water for short periods (30 days), intermediate periods (60 days), and long periods (90 days) to accomplish both maximum recruitment and monthly counting. After the exposure periods, the collectors were withdrawn from the water and replaced by new collectors. A numbered label was attached to each collector plate for later identification. In the laboratory, the attached spat were removed, sieved by size, and counted. It was not the objective of this study to test the different exposure periods or the depth of the artificial collector. Therefore, all the data were pooled for further analysis.

Genetic identification

All oyster larvae in the plankton and the spat settled on the artificial collectors from natural banks were fixed in 92% ethanol and subjected to posterior genetic identification. This material, which had been collected in the field, had its nuclear and mitochondrial DNA amplified through PCR (polymerase chain reaction) by using species-specific primers to identify the specimens as *C. brasiliana*, *C. rhizophorae*, or *C. gigas* (Ludwig et al. 2011). To estimate the monthly count of each species in the estuary, we multiplied the monthly frequency of genetically identified larvae and spat of each species by their monthly quantification.

Reproductive cycle

The study of the reproductive cycle of the oysters that were sampled from the three sites was based on the histological analysis of gonads. Oysters ($n = 36$/month) were sent alive to the laboratory, under refrigeration, for extracting gonadal tissue and the histological identification of their reproductive stage. As proposed by Galvão et al. (2000), these stages were defined as immature (or not possible to evaluate the gonad), maturing (gametogenesis), mature (gametes ready for reproduction), and spawned (or voided, in the case of males). In the same animals, we also measured the height (the distance between the umbo and posterior end of the shell), length (the measure perpendicular to the height), and thickness (dorsal-ventral axis) using a caliper accurate to 0.01 mm resolution (Galtsoff 1964).

Growth performance

The experimental cultivation of *Crassostrea* spp. was carried out to evaluate the zootechnical performance. The oysters were settled on previously numbered net cages and subjected to monthly evaluation throughout the study. We monitored the following biological and biometric parameters: height, length, and thickness of the whole animal with closed shells, growth and mortality rates. To determine the meat weight, we opened the shells by cutting the adductor muscle and removed the fresh meat. After kiln drying at 60°C for 72 h, the meat was weighted again to determine its dry weight and was used for further analyses (Forcelini et al. 2009).

Data analysis and statistical treatment

The results of these six subprojects were tabulated in a single database and grouped by month and year of collection. The original measures of central tendency and dispersion for each variable were maintained, making individual and joint analyses possible. The statistical methods were defined on a case-by-case basis, depending on the specifics, assumptions, and objectives for the evaluation of each variable.

To improve the statistical treatment and technical evaluation of the results, the raw data of the larvae count in the plankton and the number of spat that had settled down on the collector plates were transformed into a dimensionless rate. This value was related to the maximum monthly count for each one of the variables during the period of evaluation and was multiplied by 100 so that it was expressed as a percentage.

The influence of each environmental variable on the biological characteristics, the significance of the respective effects and the determination coefficient of the equations were defined by multiple regression analyses. The environmental variables that should be used in each equation to predict biological outcomes were determined by the significant coefficients. To evaluate the inter-relation among groups of variables, a canonical correlation and a principal component analysis was performed. For all of the tests, we considered 95% confidence intervals. We used SPSS Statistics v.18 and Statistica v.8 for the statistical evaluations.

Results and discussion

Data description

The biological variables were not significantly different among the three sampling sites, allowing the data to be grouped for further analyses. Furthermore, the results of reproductive maturity and the production performance of the assessed species were pooled because no PCR analysis was performed for identification.

Values for the biological and environmental variables were grouped by season and are listed in Table 1. Significant differences ($p < 0.05$) were detected among seasons for all studied variables. The differences in the variables analyzed over the seasons revealed the importance of seasonal factors on water quality parameters and the relationship between these quality parameters and the biological cycles of the *Crassostrea* spp. in the estuary.

Considering the entire monitoring period, data for the entire variables were fitted ($p > 0.05$) to the Gaussian normal curve (Shapiro–Wilk test, $p > 0.05$) with homoscedasticity (Bartlett test, $p > 0.05$) among the months and the seasons of the year, which enabled the evaluation of these variables using parametric statistical methods without mathematical transformations (Table 2). There was a significant difference between the coefficients of variation (Levene test) of the distribution of the two variables relative to dissolved oxygen (in mg/L and as percent of saturation). In spite of the fact that the two variables were obtained from the same sampling and at the same locations and times, the absolute measurement showed a lower coefficient of variability than the proportional value ($p < 0.05$), and this result was used in further analyses.

Water quality parameters

The temperature during the study ranged from 20.0°C to 27.2°C, which matched the optimal temperature for the studied species: from 22°C to 34°C, according to Barliza and Quintana (1992). However, Fabioux et al. (2005) suggested that the maximum temperature for the reproductive development of *C. gigas* is 30°C.

In this study, salinity varied between 15.3 and 19.4 PSU, with an average of 17.1 (±1.0) PSU throughout the period. Brown and Hartwick (1988)

Table 1. Seasonal values for the zootechnical, reproductive, and environmental variables of *Crassostrea* spp. in Guaratuba Bay from September 2009 to January 2011.

Variables	Summer	Autumn	Winter	Spring
Temperature (°C)	25.8 ± 1.4^{a}	23.8 ± 3.1^{b}	20.6 ± 1.6^{c}	24.0 ± 2.0^{b}
Salinity (PSU)	16.8 ± 0.9^{b}	17.0 ± 1.3^{b}	18.2 ± 0.6^{a}	17.2 ± 0.7^{ab}
Dissolved oxygen (mg/L)	5.9 ± 0.8^{b}	6.0 ± 0.8^{b}	6.5 ± 0.4^{a}	6.4 ± 0.4^{ab}
Dissolved oxygen (%)	73.5 ± 11.2^{b}	83.3 ± 5.1^{a}	74.5 ± 4.3^{b}	81.3 ± 8.2^{ab}
Transparency (cm)	83.2 ± 21.7^{b}	91.8 ± 31.0^{b}	132.8 ± 20.4^{a}	98.1 ± 27.9^{b}
Sexually mature animals (%)	66.7 ± 9.3^{ab}	51.6 ± 13.9^{b}	25.1 ± 17.2^{c}	77.6 ± 14.2^{a}
Larvae in plankton (%)*	61.7 ± 27.7^{a}	20.9 ± 16.4^{b}	6.4 ± 2.8^{c}	17.6 ± 16.3^{b}
Recruitment of spat (%)*	46.3 ± 33.0^{a}	14.0 ± 2.3^{b}	0.1 ± 0.1^{c}	5.6 ± 9.2^{bc}
Growth of meat weight (%)	8.9 ± 0.2^{b}	31.4 ± 0.8^{a}	11.6 ± 0.2^{ab}	-7.6 ± 0.1^{c}
Mortality (%)	4.5 ± 1.0^{b}	4.9 ± 1.1^{b}	7.0 ± 2.2^{a}	4.3 ± 0.7^{b}

Notes: Different superscript letters in the same row differ significantly ($p < 0.05$) by a Fisher test.
*Percent in relation to the maximum monthly values observed during the period of data collection.

Table 2. Descriptive statistics of water variables in Guaratuba Bay from September 2009 to January 2011.

	Temperature (°C)	Salinity (PSU)	Oxygen (mg/L)	Oxygen (%)	Transparency (cm)
Mean	23.9	17.4	6.2	78.2	98.7
Standard deviation	2.2	1.0	0.6	8.7	28.9
Coefficient of variation (%)	9.28	5.78	9.56	11.11	29.26
Minimum	20.0	15.3	5.0	62.3	52.5
Maximum	27.2	19.1	7.0	90.1	155.0
Normality (probability)	0.36	0.84	0.08	0.43	0.21

evaluated the effect of salinity on *C. gigas* and concluded that the highest rates of body growth were recorded between 20 and 25 PSU. According to Gomez et al. (1995), areas suitable for the cultivation of *Crassostrea* species should have a salinity between 25 and 35 PSU. Miranda and Guzenski (1999) concluded that the best salinity for the production of *C. rhizophorae* spat in the laboratory varied between 25 and 30 PSU. However, at salinities below 8 PSU, adult oysters close their shells and stop filtering, which impairs their growth and reproductive development (Taylor et al. 2004). Andriguetto-Filho et al. (2009) reported that the salinity in Guaratuba Bay varied inversely with the rainfall because, with high rainfall, the river level and the amount of water flowing from the river into the estuary increased, consequently causing a decrease in the estuarine salinity. This finding explained why the salinity was significantly lower in the summer (16.8 PSU) and autumn (17.0 PSU), as these seasons historically have higher rainfall in the region (Chaves and Bouchereau 1999).

In this study, the lowest mean temperature (20.6°C) and highest salinity (18.2 PSU) of the water were registered during the winter. Aiming at maximizing the survival rate, Guimarães et al. (2008) suggested that the cultivation of *C. rhizophorae* should be performed in estuaries with a salinity between 15 and 25 PSU. We observed the highest mortality rates during the winter months. Tureck et al. (2004) recorded increased mortality for *C. gigas* in the autumn in a study performed in Babitonga Bay (on the northern coast of Santa Catarina state, Brazil). The authors suggested that lower salinity, combined with a reduced water temperature and low values of dissolved oxygen in the autumn, contributed to the signs of stress displayed by the oysters. Miranda and Guzenski (1999) reported an increase in *C. rhizophorae* mortality at temperatures lower than 22°C.

The dissolved oxygen ranged between 5.0 and 7.0 mg/L. Throughout the study period, the monthly minimum values always remained above those recommended by Gomez et al. (1995). According to the authors, a proper area for *Crassostrea* cultivation in a mangrove region should have a concentration of dissolved oxygen between 2 and 5 mg/L. Nevertheless, the results observed for the growth, survival, and reproductive activity in our study revealed that the high level of dissolved oxygen in Guaratuba Bay was not a limiting factor for the normal physiological processes of the oysters.

There was a significant increase in water transparency in the winter. In estuaries, the suspended matter in water has usually been related to resuspended sediments from the bottom, wind, rainfall, tidal

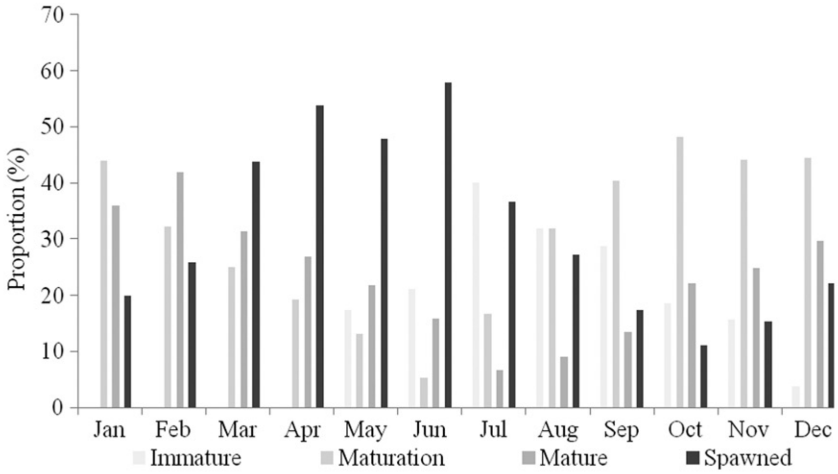

Figure 2. Proportion of *Crassostrea* spp. in Guaratuba Bay at different stages of gonadal development, presented as average monthly results for the years 2009 and 2010.

currents, and the concentration of phytoplankton (Gabrielson and Lukatelich 1985). There was lower rainfall in the winter, so the development of phytoplankton may have been impaired as a function of the lower water temperature and the scarcity of nutrients transported from the land (Chaves and Bouchereau 1999).

Reproductive cycle

Based on histological analysis of the gonads, immature animals were present from May to December, while reproductive or sexually mature animals (i.e. maturing and mature groups) were more concentrated in spring and summer (Table 1, Figure 2). Comparing the proportion of animals at sexual maturity with the total number of animals that had spawned, we determined that the peak for spawning occurred in the summer months; there was a greater proportion of maturing animals during these sampling periods. After the summer (between January and March), there was a drop in the frequency of mature animals and an increase in the proportion of those that had spawned.

The higher proportion of animals that had spawned in the summer and autumn corroborates the results obtained by Galvão et al. (2000), who studied the reproductive cycle of *C. brasiliana* in Cananéia (São Paulo state). Akaboshi and Pereira (1981) associated rainfall with a high frequency of individuals in the gamete release phase of *C. brasiliana* during this period of the year. In a study with *C. virginica* in Mexico, Zamora et al. (2003) found a significant negative correlation between salinity and the reproductive events of gametogenesis and spawning. Oocytes from *C. virginica* living under conditions of estuarine salinity frequently exhibit cytolysis of the gonad cells when subjected to conditions of rapid decreases in salinity, culminating in the release of gametes (Galtsoff 1964).

In addition to salinity, Lenz and Boehs (2011) suggested that other factors should be considered when examining the processes of the stimulus, intensity, and duration of reproductive events. Among these factors, the availability of food has an important influence, primarily because of the requirement to accumulate nutrient reserves for the later release of gametes (Hopper et al. 1998). The effect of salinity and temperature can be reflected in changes in nutritional quality and the abundance of food (Zamora et al. 2003; Nishida et al. 2006). The difference in the photoperiod in middle latitudes, as in the case of Guaratuba Bay, can also stimulate the accumulation of glycogen and trigger the process of gamete release (Grotta and Lunetta 1982). Fabioux et al. (2005) stated that the gametogenic cycle of *C. gigas* is modulated, accelerated or delayed by the water temperature, as well as by the direct influence of photoperiod. Regarding this information and compared to previous studies, it was possible to conclude that the significant decreases in water salinity and transparency that were associated with the temperature rise in the spring and summer months were decisive for the maturity and spawning of oysters in Guaratuba Bay.

Larvae in plankton and spat recruitment

After the peak of sexual maturity, the highest concentration of oyster larvae in the plankton was observed, this was succeeded by the highest concentration of spat that settled on the artificial collectors. Higher rates of capture of spat were observed from November to April. In December 2009, the highest number of larvae (154 organisms in 1000 L of filtered water) in the plankton throughout the

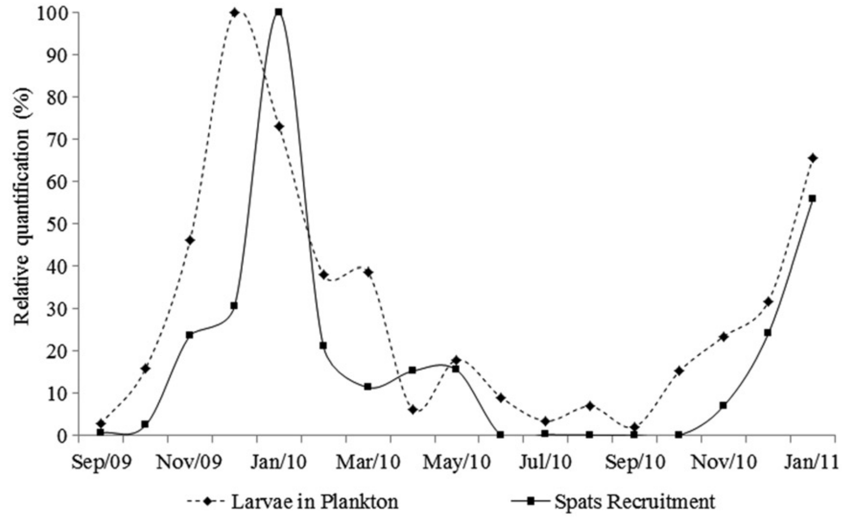

Figure 3. Quantification of larvae in plankton and of spat recruitment of *Crassostrea* spp. in Guaratuba Bay, presented as a percentage of the maximum monthly value of the period.

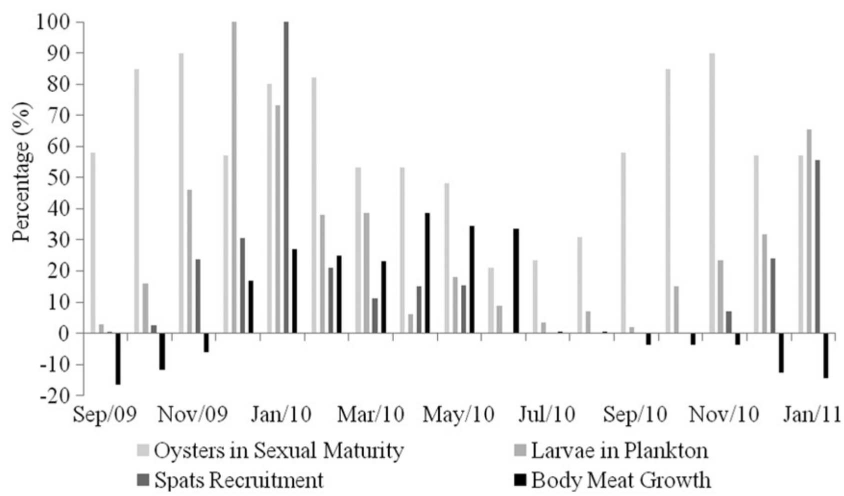

Figure 4. Temporal variation of the proportion of animals at sexual maturity, the presence of larvae in the plankton, the recruitment of spat on artificial collectors, and the growth of meat for *Crassostrea* spp. in Guaratuba Bay from September 2009 to January 2011.

experimental period was recorded. Subsequently, in January 2010, the highest spat recruitment was recorded (5780 spat on all collector plates). The rate of spat recruitment was insignificant during the other months (Figure 3).

Nalesso et al. (2008) observed that the recruitment of *Crassostrea* oyster spat in the Benevente River estuary (Espírito Santo state, Brazil) started in September and lasted until April and was higher from November to February, a trend that was associated with higher temperatures and salinity of the estuarine water. The collection of natural oyster spat can be a critical factor for the success of oyster cultivation, mainly because of a serious deficit in the supply/demand ratio for spat in the region and throughout Brazil, which is directly influenced by the costs and levels of management of commercial enterprises (Henriques et al. 2010; Tanaka et al. 2010). The low recruitment between winter and spring and the significant decrease in the autumn indicated that the use of artificial collectors for spat capture is viable only in the summer.

Animals exhibited significantly greater growth in the autumn and winter in the experimental cultivation, followed by loss weight in the spring, when there was a higher percent of maturing animals. We observed a significant reduction in the weight of the meat during the spawning season (Figure 4).

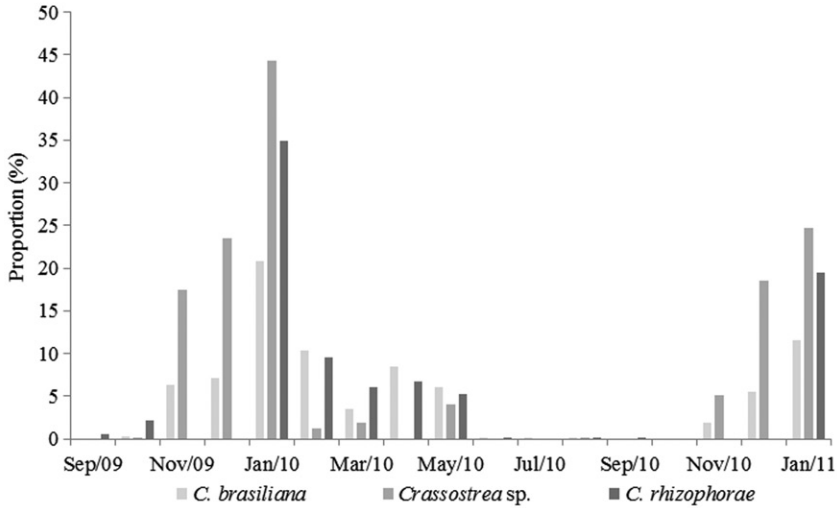

Figure 5. Monthly proportion of genetic identification of *Crassostrea* spp. for spat settled on artificial collectors in Guaratuba Bay from September 2009 to January 2011.

Genetic identification

Over the year, there was a similar proportion of the oyster species *C. rhizophorae*, *C. brasiliana*, and *Crassostrea* sp. as determined by genetic identification. These species were found in the larval stage in the plankton and as spat settled on artificial collectors, as described by Ludwig et al. (2011). For these authors, the third species was genetically unidentified, although it exhibited high similarity with *C. gigas*. However, our use of only PCR could not differentiate *C. gigas* from *Crassostrea* sp. We obtained proportions of 36%, 33%, and 31% of the total as *C. rhizophorae*, *C. brasiliana*, and *Crassostrea* sp., respectively. These percentages were similar when compared with each other using Fisher's exact test ($p > 0.05$).

The temporal distribution was distinct for the three species, with separate and evident peaks for each species. During the summer and autumn, higher quantities of larvae and spat were found in the estuary, with the prevalence being the unidentified *Crassostrea* (41%). However, in the winter, the percentage of larvae of this species decreased to 11%. For *C. rhizophorae*, the seasons with greatest absolute values were summer and autumn, but in terms of proportional values, there was a higher prevalence (62%) of larvae of this species in the spring; the same result was observed for *C. brasiliana* (Figure 5).

General environmental influence

According to the multiple regression analysis, the linear variation of environmental factors could explain the variation in gonadal development stages, sexual maturity, spat collection, and body growth ($p < 0.05$; Table 3). In contrast, no environmental factor was able to explain the variation in mortality over time, which was verified by the experimental cultivation of oysters. The environmental variable that most significantly interfered with the biological indices was the concentration of dissolved oxygen, while temperature had the least influence. The regression equations did not provide significant coefficients for the *y*-intercept. Predictive equations should not possess values for the *y*-intercept, considering the incompatibility of the occurrence of any biological process for these species wherein the results of all environmental variables are all zero (i.e. water at 0°C, 0 PSU and 0 mg/dL of dissolved oxygen).

Tureck et al. (2004) observed significant coefficients ($p < 0.05$) for the variables: water temperature, salinity, pH, and concentration of dissolved oxygen by applying multiple regression analysis to data of the influence of environmental variables on biometric indices for *C. gigas*. In the same study, the authors identified that cultivated oysters in locations with lower variation in environmental parameters had growth indices that were significantly higher than cultivated oysters in locations where the same parameters showed constant variation.

The joint variation of the environmental variables interfered with the joint variation of the biological variables using canonical correlation analysis. The redundancy of variance between these groups was 53.8%. The principal component analysis indicated that the environmental data explained 74.8% of the variation in the biological data, with a positive impact for temperature and dissolved oxygen, but a negative impact for salinity and transparency (Figure 6). Ramos and Castro (2004) verified that the environmental variables that had the most significant influence on the growth of *C. rhizophorae*, when employing principal component analysis, were suspended particulate material, current velocity, salinity, and dissolved oxygen.

Table 3. Coefficients obtained from multiple linear regression analysis of environmental and biological variables of *Crassostrea* spp. in Guaratuba Bay from September 2009 to January 2011, with the significance of each coefficient.

	Equation coefficients (probability)					
Variables	*Y*-intercept	Temperature (°C)	Salinity (PSU)	Oxygen (mg/L)	Transparency (cm)	R^2
Gonadal development[a]	–	−0.108	0.188	0.733	−0.398	0.705
	(0.990)	(0.804)	(0.044)	(0.011)	(0.390)	(0.043)
Sexual maturity of animals (%)	–	−0.017	0.052	0.439	−0.704	0.729
	(0.637)	(0.967)	(0.821)	(0.049)	(0.135)	(0.057)
Larvae in plankton (units/mL)	–	0.604	−0.419	−0.095	0.107	0.467
	(0.753)	(0.200)	(0.092)	(0.071)	(0.822)	(0.169)
Recruitment of spat (units/m^2)	–	1.268	−0.680	0.326	−0.995	0.878
	(0.284)	(0.002)	(0.002)	(0.049)	(0.009)	(0.004)
Body growth (%)	–	−0.564	−0.170	0.732	−0.702	0.859
	(0.510)	(0.092)	(0.320)	(0.002)	(0.052)	(0.007)
Mortality (%)	–	0.948	−0.740	0.676	0.226	0.515
	(0.916)	(0.442)	(0.339)	(0.271)	(0.863)	(0.579)

Note: [a]Score for gonadal development, ranging from 0 (for animals with immature gonads) to 4 (for spawned animals).

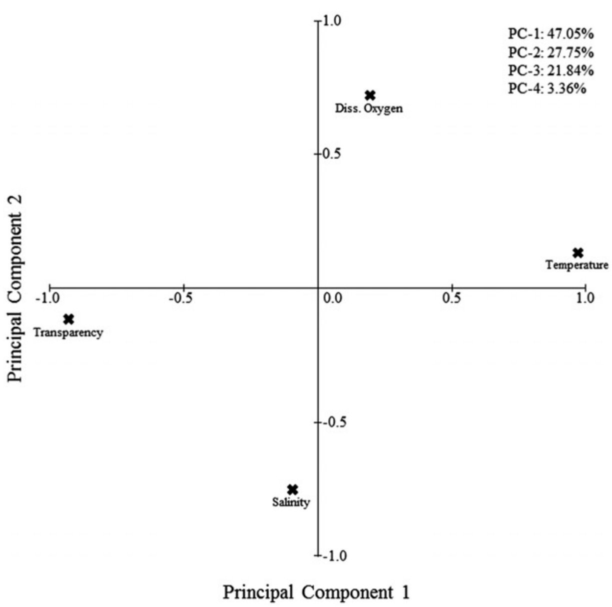

Figure 6. Graphical representation of the principal component analysis for the environmental variables in accordance with the biological parameters of *Crassostrea* spp. variation, in Guaratuba Bay from September 2009 to January 2011.

The authors suggested that high tidal levels, current velocity, and seasonality were dominant factors that acted directly and indirectly on the ecosystems and on cultivated oysters, as well as on the behavior of the variables studied.

Christo et al. (2008) observed that the higher values of the index of body condition and the index of meat production of *C. rhizophorae* have been associated, also by means of principal component analysis, with high water temperatures, greater availability of phytoplankton, and higher rainfall, combined with lower salinity and water transparency. According to the authors, these environmental factors were capable of explaining 87.67% of the total variability of the data.

In conclusion, temperature, dissolved oxygen, and water transparency were the environmental factors that had the most influence on the reproductive cycle (e.g. gonadal development and spat capture) and the growth of the *Crassostrea* species in Guaratuba Bay. Furthermore, the relationship among the biological parameters is also important for an overall evaluation. This project proved to be quite adequate for understanding the environmental and biological processes related to the natural life cycle of *Crassostrea* spp. in Guaratuba Bay and their influence on production management. New studies of the influence of other environmental variables on oysters, such as water pH, the quantification of chlorophyll, suspended particulate matter, tidal dynamics, photoperiod, and rainfall levels, can improve the biological knowledge of studied species in this estuary.

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