PANAMJAS
Pan-American Journal of Aquatic Sciences

Recruitment of oysters of *Crassostrea* genus (Bivalvia, Ostreidae) in Guaratuba Bay, PR, Brazil

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Abstract. Larval development of *Crassostrea* is characterized by three larval phases: larva D, Umbo and Pediveliger. In Pediveliger the larvae are near settlement and metamorphosis in hard substratum. In this work, recruitment of oysters' larvae of genus *Crassostrea* (*C. rhizophorae* and *C. brasiliana*) was studied in Guaratuba Bay (Paraná – Brazil) through analysis of artificial collectors placed in three points: Point I (bay´s entrance), Point II (cultivation park) and Point III (medium sector of the bay) and changed biweekly from June/2003 to June/2004. Due to the impossibility to differentiate the recruits of the two oysters' species by the shell morphology, the oyster's spats of the two species were counted jointly on both sides of the plates, to determine the number of settled individuals. The results showed an increase in the number of settled individuals between October and March with successive peaks during this period. The medium sector of the bay showed the larger density of recruits along the studied period. An important application of the present research would be in the improvement of oyster cultivation based in seed collection from natural environment.

Key-words: larvae, collectors, spats, Guaratuba Bay, South Atlantic.

Resumo. Recrutamento de ostras do gênero *Crassostrea* (Bivalvia, Ostreidae) na baía de Guaratuba, PR, Brasil. O desenvolvimento das larvas de ostras do gênero *Crassostrea* é caracterizado em fases larvais: larva D, umbo e Pediveliger. No estágio Pediveliger as larvas estão próxima da metamorfose e assentamento no substrato consolidado. Neste trabalho o recrutamento das larvas de ostras do gênero *Crassostrea* (*C. rhizophorae* e *C. brasiliana*) foram estudados na baía de Guaratuba (Paraná, Brasil) através da análise de coletores artificiais em três locais: ponto I (entrada da baía), ponto II (parque de cultivo) e ponto III (setor médio da baía) e a variação quinzenal entre junho/2003 a junho/2004. Devido à impossibilidade de diferir os recrutas das duas espécies nativas de ostras a partir da morfologia da concha, as sementes das duas espécies foram contadas em conjunto de ambos os lados das placas, para determinar o número de indivíduos fixados. Os resultados mostraram um aumento no número de indivíduos fixados entre os meses de outubro e março, com sucessivos picos durante este período. O setor médio da baía mostrou a maior densidade de recrutas ao longo do período estudado. Uma aplicação importante da presente pesquisa seria no aperfeiçoamento do cultivo de ostras com base na coleta de sementes do ambiente natural.

Palavras chaves: larvas, coletores, sementes, baía de Guaratuba, Atlântico Sul.

Introduction

During early developmental stages (D-larvae and Umbo-larvae), *Crassostrea* spp. larvae are abundant in surface plankton, sinking to deeper

waters during late (pediveliger) stages (Andrews 1983, Boehs & Absher 1997, Baker 2003, Finelli & Wethey 2003). At settlement, substrate selection is a key factor controlling species distribution at local scales (Southworth & Mann 2004, Christo & Cruz 2009). The recruitment process in space and time scales can also supply information about the behavior of the adult oyster in the environment through the observation of the patterns of reproduction, spawns, estimates of the larval period, dispersion and growth associated to environmental factors (Southworth & Mann 2004, Wilson *et al.* 2005). Among these factors temperature, salinity, primary productivity and local hydrodynamic are very important (Boehs & Absher, 1997, Nalesso *et al.* 2008, Kim *et al.*2010, Haase *et al.* 2012).

Researches with artificial substrate are quite spread in oyster culture, and have been used especially in areas where the artificial production of larvae in laboratory is unviable due to the high cost or when the objective is to increase settlement rate in the environment. In the recruitment process the interaction among genetic and environmental factors can also contribute to the substratum selection and settlement rate (Baker 2003, Finelli & Wethey 2003, Silveira *et al.* 2011). In the present study, the selection of the polypropylene substratum was based on previous works accomplished in Paranaguá Bay, on the influence of the substratum=in the settlement of oysters' larvae (Absher & Christo 1991, Christo & Absher 1993). The selection of the type of artificial substratum for the collectors is important in the optimization of spat recruitment in the nature (Sorabella & Luckenbach 2003, Jordan & Coakley 2004, Padilla & Klinger 2005, Su *et al.* 2007). The emphasis of the studies has been in the selection of the size of the collectors and materials of low cost and handling with the optimization purpose in the recruitment of larvae in nature (Mann 2001, Sorabella & Luckenbach 2003, Jordan & Coakley 2004, Padilla & Klinger 2005, Christo & Cruz 2009, Zanette *et al.* 2009). Therefore, this work had as objective to verify the space and time variation of larval recruitment of *Crassostrea* oysters (*C. rhizophorae* and *C. brasiliana/C. gasar*) through artificial collectors and identify their relationship to the environmental variables that act on the process of settlement of the seeds in the natural environment.

Materials and Methods

Guaratuba Bay situated on the coastal plain of southern Brazil (Fig. 1), has an area of 50.19 km^2 and four cross-estuary sections (bay mouth; middle; São João river mouth; Cubatão river mouth) were established (Marone *et al*. 2006). The climate of the region is humid mesothermal. Marone *et al.* (2006) describes very dynamic situation, with strong currents (more than 2.0 $m.s^{-1}$ at the mouth), semi-diurnal tides of up to 1.5 m range (0.65 m at neap tide), moving up to $75x10^6m^3$ of water at spring tide and $32x10^6km^2$ at neap tides in a complex tide propagation pattern (progressive, mixed and stationary).

Oyster's recruitment was analyzed, in the period from June 2003 to June of 2004, through artificial collectors placed at three locals of Guaratuba Bay, as follows: Point I (25°51'20''S; 48°34'39''W – bay mouth) - close to Sepultura's Island, in the entrance of the bay, where natural banks of oysters occur in rocky coasts. Point II (25°49'56"S; 48 °34'48"W - middle) - is located in a cultivation park of *C. brasiliana* in Pinheros' River, formed by a tide channel in the northeast portion of the bay between Veiga Island and the continent. This channel has an approximate width of 150m, length of 2000m. Point III (25°52'12''S; 48°38'21''W - middle) - near Garças' Island, in the medium sector of the bay has a mean depth of (Fig. 1). In each point 2 collectors with 5 plates of polypropylene each were placed, to 1 m depth from the sea surface. This depth was selected in order to prevent the establishment of native oyster according with preference of larvae settlement. *C. rhizophorae* is predominant intertidal and *C. brasiliana*/*C. gasar* is predominant infralittoral. A total of 10 plates were disposed in strings of galvanized wire and each plate with an area of 10 cm^2 was placed 4 cm apart. Every 15 days the collectors were substituted and taken to the laboratory. Jointly to spat sampling environmental factors were measured: temperature with a mercury thermometer - precision of 0.1 °C; salinity with manual refractometer - precision of 1 unit; seawater transparency with Secchi disk. Pluviosity data was obtained from the meteorological station of Guaratuba of the Technological Institute SIMEPAR (Meteorological System of Paraná).

Due to the impossibility to differentiate the recruits of the two oysters' species by the shell morphology, the oyster's spats of the two species were counted jointly on both sides of the plates, to determine the number of settled individuals. The number of spats settled in each plate was transformed in densities, expressed in individuals/100 cm^2.

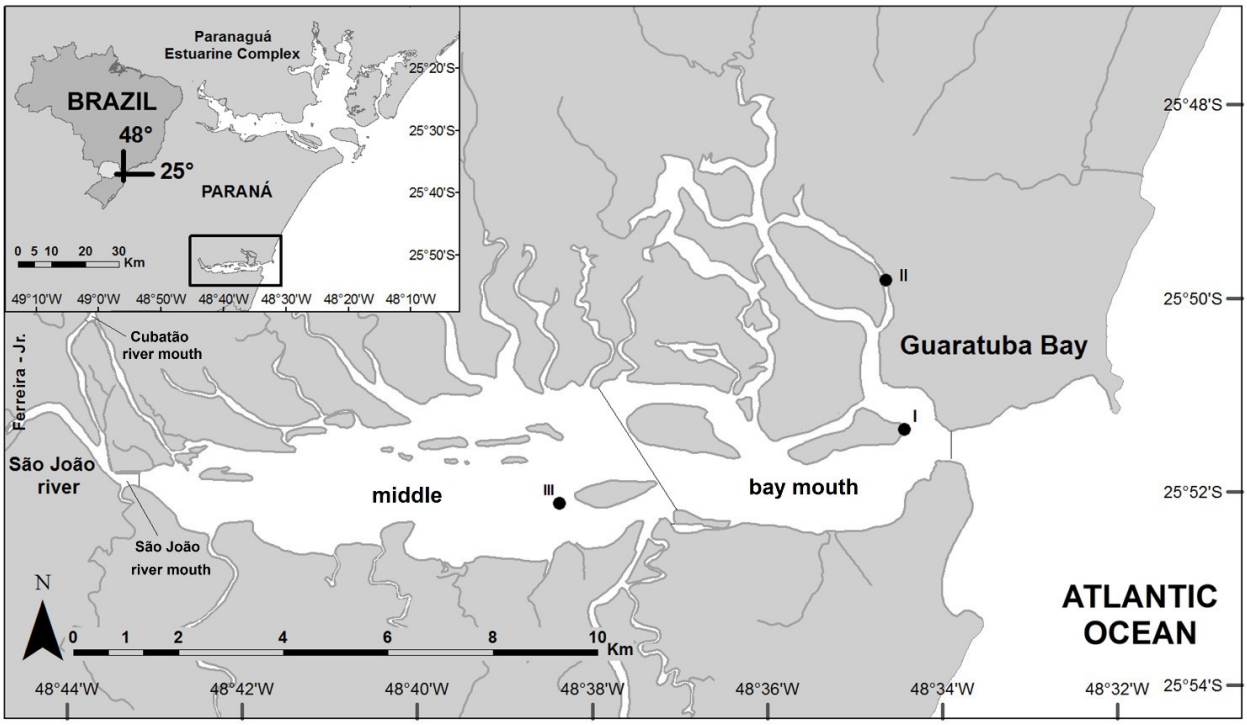

Figure 1 - Location of points in Guaratuba Bay, Paraná – Brazil. Points: I (near Sepultura Island), II (cultivation park) and III (medium sector of the bay). Sections: bay mouth; middle; São João river mouth; Cubatão river mouth (Marone *et al*. 2006).

Spats data were transformed (square root). After evaluated the normality and homogeneity of the data, one-way ANOVA was used to test for significant differences (a=0.05) in larvae densities (dependent variable) between plates (1 to 5) and side of the plate area (upper and lower). A two-way ANOVA was used to test for significant differences (a=0.05) in larvae densities (dependent variable) between points and stations sampled (independent variable). The correlation between the biological parameters and the environmental parameters was put in evidence through a multivariate analysis of ordination as a PCA (Principal Component Analysis) (Legendre & Legendre 1983, Valentin 2000), with the seasonal averages of the variables.

Results

Recruitment of oysters: The analysis of the results of the recruitment of oysters showed an increase in the number of individuals settled between the months of October/2003 and March/2004 with successive picks during this period. An annual average of 112 ±77.61 spats.100cm^{-2} was observed at Point I; Ponto II, 81 ±59.59 spats.100cm^{-2} and Ponto III an average of 193 ±22.71 spats.100cm^{-2}. The largest densities observed were in spring (October/2003) and autmn (March/2004), where the Ponto III (medium section of the bay) showed a larger density of settled individuals (679.5 spats.100cm^{-2}) in this period (Fig. 2).

Comparing the sampled points, the variance analysis showed that the differences in the recruits' densities were highly significant (F=49.35; p<0.001) (Fig. 3). There was significant difference between the number of recruits and the period sampled (F=68.78; p=0.001) (Fig. 4).

In the three studied points a more expressive recruitment is observed at springtime and a lower amount in the winter period (F=14.20; p<0.01) probably justifying the high significant interaction between the season and recruits (Fig. 2-4). Comparisons of density values of spats among plates (p=0.9183) and the upper and lower inferior side of the plate (p=0.2559).

Environmental parameters: Seawater temperature had an annual variation of 7.4°C with values that oscillated between 19°C and 27°C and annual average of 23°C in Points I, II and III. The highest temperatures were registered in the period from spring (October/2003) to autumn (March/2004). The annual average of the salinity was 26 in the Ponto I; 24 in Point II and 19 in Point III, with annual amplitude of 10, 6 and 13 in the Points I, II and III, respectively. The smallest values were registered in Point III, at the medium area of the bay.

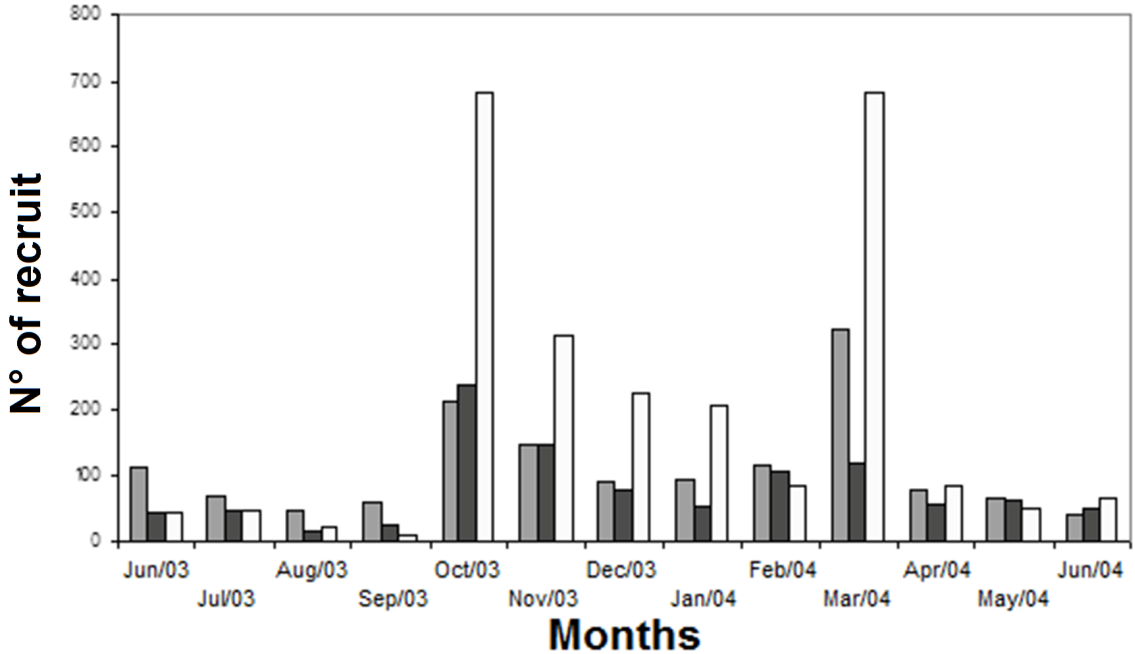

Figure 2 - Monthly average of the number of recruits in Points I (light grey), II (dark grey) and III (white) during the studied period in Guaratuba Bay, Paraná – Brazil.

The annual average of the depth of extinction of the Secchi Disk was 1.60 m in the Ponto I; 1.15 m in Point II and 1.25 m in Point III, with annual amplitude of 1.10, 0.80 and 1.90 m in the Points I, II and III, respectively. The largest value of transparency of the water was verified in the Point I (2.20 m) that corresponds to the area at the entrance of the bay and the smallest registered value (0.50 m) was registered in summer (February/2004) in Point III.

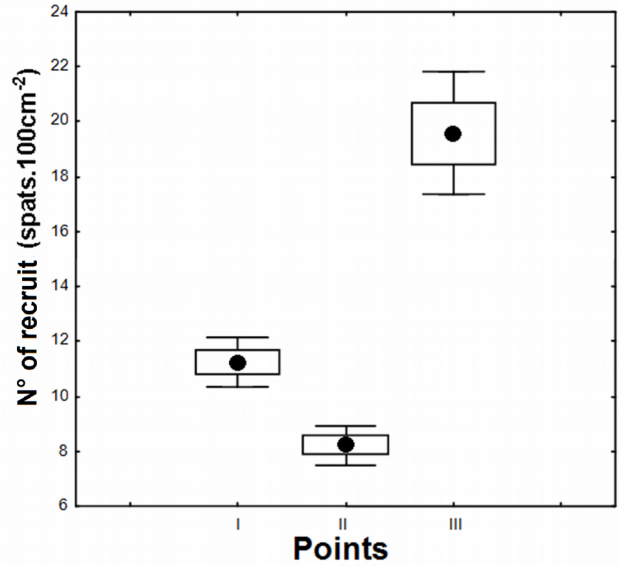

Figure 3 - Distribution of the number of recruits (spats.100cm $^{-2}$) in Points I, II and III, during the studied period in Guaratuba Bay, Paraná – Brazil. ●: mean; C: stander error; ⌶: stander deviation.

Environmental and biological interactions: Result of PCA analysis evidenced that in the points I, II and III the recruits' density was accentuated in the spring/2003 and summer/2004 in conditions of high temperature and less saline waters. Pluviosity had little influence on the recruits' density, while water transparency didn't interfere in the recruitment. In Points I and III an inverse relationship of recruitment to the transparency of the water was observed, however, the salinity didn't have influence on the recruits' density (Fig. 5).

Discussion

In this study, the settlement of oysters' spat, does not present a preference close to the bottom, as verified by Wakamatsu (1973), Akaboshi & Pereira (1981), Christo & Cruz (2009) for these organisms; because significant differences were not observed in the number of recruits between the upper and lower sides of the plates. This behavior can be related to the little depth of the bay and/or temperature, since Andrews (1983) suggests that oysters' larvae tend to stay in layers of water masses with more favorable temperature. Nalesso *et al.* (2008) observed spat settlement was significantly higher on oyster shell, tile and tire collectors. Hossain *et al.* (2013) in three sites in the Bangladesh coast observed higher performance of windowpane shell for spat settlement when comparing four different substrates: dead oyster shell, live oysters, windowpane shell and stone/rubble. Silveira *et al.* (2011) in laboratory

experiments of larval settlement and spat recovery rates of *Crassostrea brasiliana* selected plastic collectors due to its pliability, favoring spat detachment.

A continuous reproductive pattern is observed in the oyster from Guaratuba Bay what favors the presence of larvae in the plankton along the whole year with temperature playing an important role in this pattern, high density of larvae and gonadal maturation coincides with periods of temperature elevation (Christo 2006). This fact indicates that the spawning activity influence the pattern of recruitment of the oysters (Southworth & Mann 2004, Wilson *et al.* 2005). Nalesso *et al.* (2008) in five sites in the Benevente river estuary, Espírito Santo State – Brazil observed that recruitment of oysters´ spat occurred throughout the year, being higher from November to February. Similar increase was observed in recruitment of oysters´ spat of Guaratuba bay, where there was an increase from October to March.

Related with water transparency the highest values correspond to periods where the rate of rainfall was low, especially verified in the winter months; while in the hottest and rainy periods there was an accentuated increase in turbidity. This fact might have happened due to the development of phytoplankton and/or suspension of the sediment due to little depth of the area, especially verified in Point II that corresponds to the medium sector of the bay. The river basins that contribute for the total runoff into the Guaratuba bay represents a surface area of 1,724 km, with a drainage density of 1.87 rivers.km^{-1} , presenting a dendritic drainage pattern and a mean river runoff of at least 80m^{3}.s^{-1} (Marone *et al.* 2006). It is important to note, also, the role of the relative importance between tides and river runoff, which seasonal changes, moving the bay's dynamic from a highly stratified to a well mixed estuary in different time scales (Marone *et al.* 2006).

These patterns of surface sediment being dragged in the direction of the inside of the bodies of water, mainly during the periods of intense rains, are according with the results of the transparency of the water obtained in the present study.

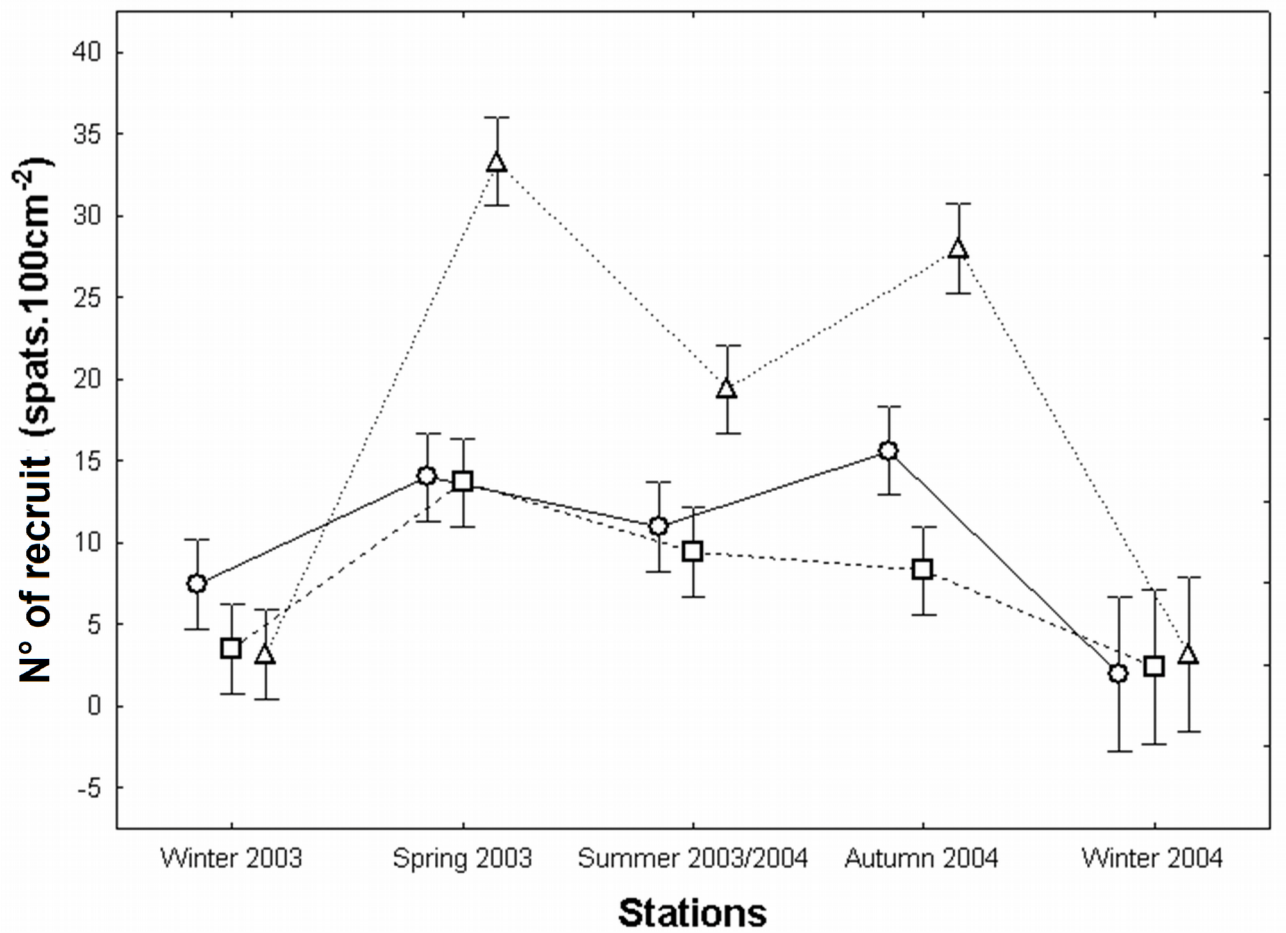

Figure 4 - Seasonal averages of the number of recruits (spats.100cm $^{-2}$) in Points I (○), II (□), and III (Δ) during the studied period in Guaratuba Bay, Paraná – Brazil. ⊥: standar deviation.

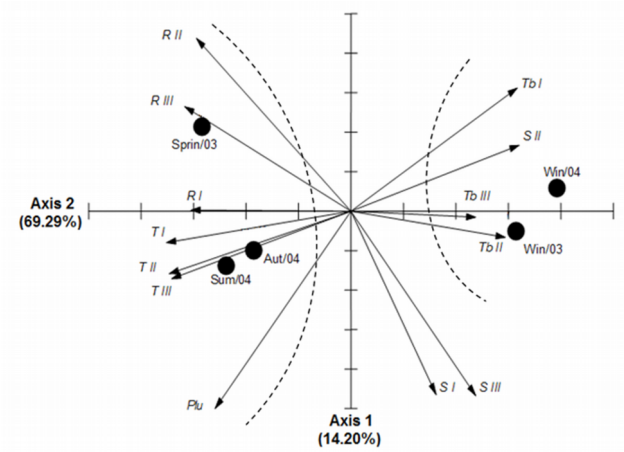

Figure 5 - Graphic representation of Principal Components Analysis (PCA) in Points I, II and III, with the projection of the vector-variables, being: R = recruits; T = temperature (C°); S = salinity; Tb = water transparency (m) and Plu = pluviosity (mm); Sum = summer; Aut = autmn; Win = winter; Sprin = spring, with the Seasonal observations in the factorial plan I and II. Mean seasonal values in the sampled period from June/03 to June/04.

There is a conspicuous stratification attributed to the geomorphologic characteristics of the Guaratuba bay with an entrance channel strait and deep, added by the time of the water residence calculated by the referred authors as being of approximately 9.3 days (Marone *et al.* 2006), this characteristic may contribute to the retention of the larvae in the internal sections of the bay. Study to investigate the larval transport of eastern oyster, *Crassostrea virginica*, in two American estuarine systems indicated the influence of hydrodynamics, seasonality and the population dynamics of oysters increased larval retention near the spawning area, thus providing a favorable condition for local recruitment of oysters (Kim *et al.* 2010, Haase *et al.*2012)

However, the analysis of the environmental parameters, suggests that the hottest waters favor the recruitment and the permanence of larvae in the plankton, while no direct relationship was observed in relation to the water transparency in the studied points. Finally, those results may have an important application in commercial exploitation of oyster farms in the area that depends on seed collection from natural environment. Nalesso *et al.* (2008) recommend that collectors be placed in the water from October to December (to collect spat during the rainy months) in high salinity areas, and that they should be transferred to areas of low salinity from April, to avoid the rainy season at estuarine sites. An accentuated reduction in larvae and recruits density was observed in February/2004 in Point III, where the smallest value of water transparency (0.50 m) was registered. This fact can be justified by the period of the collectors' substitution. Point III, close to Garças' Island, is a place often visited by tourists' boats of small and medium size especially in this time of the year. The great circulation of high-speed boats in this area can cause the sediment suspension and displacement of water, what probably interfered with the distribution of larvae and recruits due to disturbances caused in the environment in this period. In this estuarine system 8 marina can be identified located in the cities of Matinhos and Guaratuba (SETPT 2012), as well as continuous flow of ferry boats in their outlet, responsible for the crossing Matinhos - Guaratuba that happens daily along the year (DER 2015). According with DER (2015), in the summer period 3 ferry-boats and 3 tug boats are used for 24 hours to assist the demand of the tourist season, besides the presence of a shipyard of maintenance of the six embarkations (ferry-boats and tug boats).

Analyzing evidencing the recruitment seasonally, being the seasons the independent variables, Point I showed a high number of individuals in autumn, in the Point II in spring and Ponto III in autumn and spring, this fact could be associated to the elevation of the seawater temperature, salinity and water transparency. Perbiche-Neves *et al.* (2010) in five sites in Guaratuba Bay (sections: bay mouth and middle) observed spatial and seasonal variations for the micro-zooplankton in the bay, with peaks of abundance (due to meroplankton) in the summer, associated to the highest water temperatures. The number of oyster spat was positively correlated with the salinity and water temperature, revealing that areas with higher salinities and summer months were better for spat collection in the Benevente river estuary, Espírito Santo State – Brazil (Nalesso *et al.* 2008). Studies observed the number of oyster settlements in three locations of Bangladesh coast indicated that main factors were an increase in suspended solids, low salinity, due to heavy rains, increased river discharge by months of monsoon high water flow rates and current wave in extreme weather events. All those factors can affect the success of the spats survival in the experimental sites (Hossain *et al.* 2013).

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Received: April 2016
Accepted: December 2016
Published: January 2017