

USE OF ARTIFICIAL COLLECTORS TO OBTAIN OYSTER SEEDS IN BABITONGA BAY, SANTA CATARINA, BRAZIL

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ABSTRACT

The aim of this study was to evaluate oyster seed settlement pattern in artificial collectors affixed along Babitonga Bay (26°28'S – 48°50'W), Santa Catarina state, Brazil. Artificial collectors were installed in the upper tidal range (supralittoral), in the line of intermediate variation of the tide (mesolittoral), and below the limit region of the low tide (infralittoral). A total of 7,579 seeds were obtained over a year, presenting clear variations of quantities and species according to the seasons, depths and collection sites. Among the total number of seeds collected, 284 were submitted to molecular analyses for species identification, among which 5.63% belonged to *Crassostrea gasar*, 16.9% to *C. rhizophorae*, 0.35% to *C. gigas*, 24.29% of *Ostrea* sp. and 52.81% to *C. talonata*. Species identification through morphological analysis is difficult because of the inherent polymorphism of shells. Results demonstrate that artificial collectors present a higher performance for the settlement of oyster species inappropriate for farm cultivation (*C. talonata* and *Ostrea* sp.). Moreover, *C. talonata* is an exotic species from Indo-Pacific seas recently confirmed along Brazilian coasts, and it already possesses a high recruitment rate in the estuarine region. This raises environmental and commercial concerns about the maintenance of the natural oyster population in Babitonga Bay.

Keywords: mangrove oyster; native oyster; *Crassostrea talonata*; recruitment; cultivation; ostreiculture

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USO DE COLETORES ARTIFICIAIS PARA OBTENÇÃO DE SEMENTES DE OSTRAS NA BAÍA BABITONGA, SANTA CATARINA, BRASIL

RESUMO

O objetivo deste trabalho foi avaliar o padrão de povoamento de sementes de ostras em coletores artificiais afixados na baía Babitonga (26°28'S – 48°50'W), Santa Catarina, Brasil. Os conjuntos de coletores foram instalados na linha superior de alcance da maré (supralitoral), na linha de variação intermediária da maré (mesolitoral) e abaixo da região limite da baixa-maré (infralitoral). Foram obtidas 7.579 sementes ao longo de um ano, apresentando claras variações de quantidades e espécies nas estações do ano, nas profundidades e nos locais de coleta. Do total de sementes coletadas, 284 foram submetidas a análises moleculares para a identificação da espécie, dentre as quais 5,63% pertencem a espécie *Crassostrea gasar*, 16,9% a *C. rhizophorae*, 0,35% a *C. gigas*, 24,29% a *Ostrea* sp. e 52,81% a *C. talonata* sp. Os resultados mostram a impossibilidade da identificação das espécies por meio da análise morfológica em função do polimorfismo inerente das conchas, bem como demonstram que os coletores artificiais propiciam maior predominância para o assentamento de espécies de ostras impróprias para o cultivo comercial (*C. talonata* e *Ostrea* sp.). Além disso, *C. talonata* é uma espécie exótica dos mares indo-pacíficos recentemente confirmada nas costas brasileiras e já possui alta taxa de recrutamento na região estuarina, o que levanta preocupação ambiental e comercial sobre a manutenção da população de ostras naturais na baía Babitonga.

Palavras-chave: ostra do mangue; ostra nativa; *Crassostrea talonata*; recrutamento; cultivo; ostreicultura.

INTRODUCTION

The collection of native oyster seeds in the natural environment for cultivation purposes has already been used in some Brazilian regions such as the estuarine complex-lagoa de Iguape-Cananéia-Paranaguá (Pereira et al., 2003). Recent studies conducted in the northern region of Brazil have determined the period of the year for seed collection, defined types of collectors as well as environmental conditions that maximize the recruitment of seeds (Garduncho et al., 2012; Funo et al., 2019). However, more than one species of native oyster is expected to occur sympatrically among our littoral zone (Carranza et al., 2009). Each one possesses different growth rates, but similar continuum of morphological variation. This complicates the organization and cultivation of seeds and adults (Absher, 1989; Nascimento, 1991; Pereira et al., 2003; Galvão et al., 2009; Melo et al., 2010b; Lazoski et al., 2011).

Several authors have demonstrated the technical viability of Brazilian native oyster cultivation, highlighting plasticity in growth rates (Lima and Vazzoler, 1963; Singarajah, 1980; Nascimento, 1983; Ramos et al., 1986; Pereira et al., 1988; Absher, 1989; Pereira and Soares, 1996; Pereira, 1997; Pereira et al., 2001; Maccacchero et al., 2005; Galvão et al., 2009; Lopes et al., 2013; Legat et al., 2017).

One native oyster species known as *Crassostrea gasar* (Dautzenberg, 1891) (= *C. brasiliiana/C. paraibanensis*; see Lazoski et al., 2011) has well-known commercial potential as it is a preferable species for ostreiculture in tropical areas (Legat et al., 2017) where the cultivation of *C. gigas* (Thunberg, 1793) is impractical (Gomes et al., 2014). Although hatchery protocol for production of seeds is available, their attainment through artificial collectors can be a viable practice as in Senegal ecosystems (Diadhiou and Ndour, 2017), or in Brazil, Guaratuba Bay/Paraná state (Christo et al., 2016) and Curuçá/Pará state (Funo et al., 2015, 2019), where only *C. gasar* is expected to settle in the plate structures. Evaluation of potential oyster larvae settlement per species is, then, a requirement to test the feasibility of obtaining seeds in artificial collectors in the environment. In particular, it is critical that the correct molecular identification of species in the seed phase be made as a fundamental tool for the development of mangrove oyster farming activities.

For decades, studies have suggested the existence of at least two species of oysters of the genus *Crassostrea* in Brazilian estuaries (Absher, 1989): *C. rhizophorae* (Guilting, 1828) and *C. gasar*, a fact later confirmed by genetic marker analysis (Ignacio et al., 2000; Lapègue et al., 2002; Lazoski, 2004; Pie et al., 2006).

However, more recently, the number of invasive oyster species in Brazilian natural stocks has increased substantially. Varela et al. (2007) reported to Ilha Canela in northern Brazil (Bragança, State of Pará) an exotic species of the genus *Crassostrea* with a strong phylogenetic relationship to Indo-Pacific seas (Wu et al., 2013). Such species was also noted in other areas of the Brazilian coastline (Galvão et al., 2013; Melo et al., 2013), the western coast of Peru (Li et al., 2017), and now in Argentina and southern Brazil, having recently been identified as *C. talonata* Li and Qi (1994) (Cavaleiro et al., 2019). Melo et al. (2010b) detected the first banks of *C. gigas* in the natural environment of southern

Brazil. Galvão et al. (2017) confirmed the invasion of a lineage of *Saccostrea* Dollfus and Dautzenberg (1920) in the Itaguapé River, São Paulo. Therefore, based on the variation in the number of species, the correct identification of oysters in regions with potential for cultivation has fundamental importance for the development of aquaculture, especially in tropical estuarine.

Molecular markers have been used successfully in the delimitation of native oyster stocks. Thus, it has been demonstrated that *C. rhizophorae* is present from the southern Caribbean to Uruguay, usually found in mangrove roots and rocks in upper mesolithic regions, while *C. gasar* is generally found in the infralittoral regions from northern South America to the southern region of the state of Santa Catarina, Brazil (Ignacio et al., 2000; Lapègue et al., 2002; Lazoski, 2004; Pie et al., 2006; Varela et al., 2007; Melo et al., 2010a; Lazoski et al., 2011; Galvão et al., 2013) and on the African continent from Mauritania to Angola (Carpenter and De Angelis, 2016).

In southern Brazil, one of the sites with potential for cultivation of native oysters from the genus *Crassostrea* is Babitonga Bay (26° 28'S - 48° 50'W) where artisanal farmers observed that these animals settle themselves on several natural and artificial substrates. However, the difficulty lies in separating species with growth potential, a problem which stems from the morphological similarities, mainly in the seed phase.

Thus, the present work aimed to identify the recruitment pattern of seeds of oysters in Babitonga Bay as a way to gauge the possibility of obtaining seeds at low cost and also infer the best condition and places where the attainment of seeds of commercial oyster species is maximal.

MATERIAL AND METHODS

The experiment was carried out in Babitonga Bay, which is located on the northern coast of the state of Santa Catarina (26°28'S - 48°50'W) (Figure 1). The bay possesses 160 km² with extensive mangrove areas and significant natural oyster stocks (IBAMA, 1998).

The artificial collectors were installed at three points inside the estuary: Iperoba (26°12'12.42"S - 48°34'11.72"W), Vila da Glória (26°14'50.44"S - 48°41'42.12"W) and Linguado (26°22'12.44"S - 48°39'51.75"W) (Figure 1).

At Iperoba, the collectors were installed in a region characterized by sand-muddy shoals under the influence of a small river. At Linguado, the collectors were installed in the margins of a channel 15 m in depth (actual max. depth of 2 m owing to siltation) characterized by intense variation in salinity (IBAMA, 1998). Vila da Glória is located in the central portion of Babitonga Bay and receives fresh water from the rivers relative to the other sampling points, as well as a strong influence of seawater from the effect of tides.

The collectors were made of PVC plates (polyvinyl chloride) with a rough texture (3 mm thick), comprising a set of 5 plates of 10 × 25 cm fixed inside a PVC pipe frame. At each collection point, three (03) sets of plates were distributed from the bottom

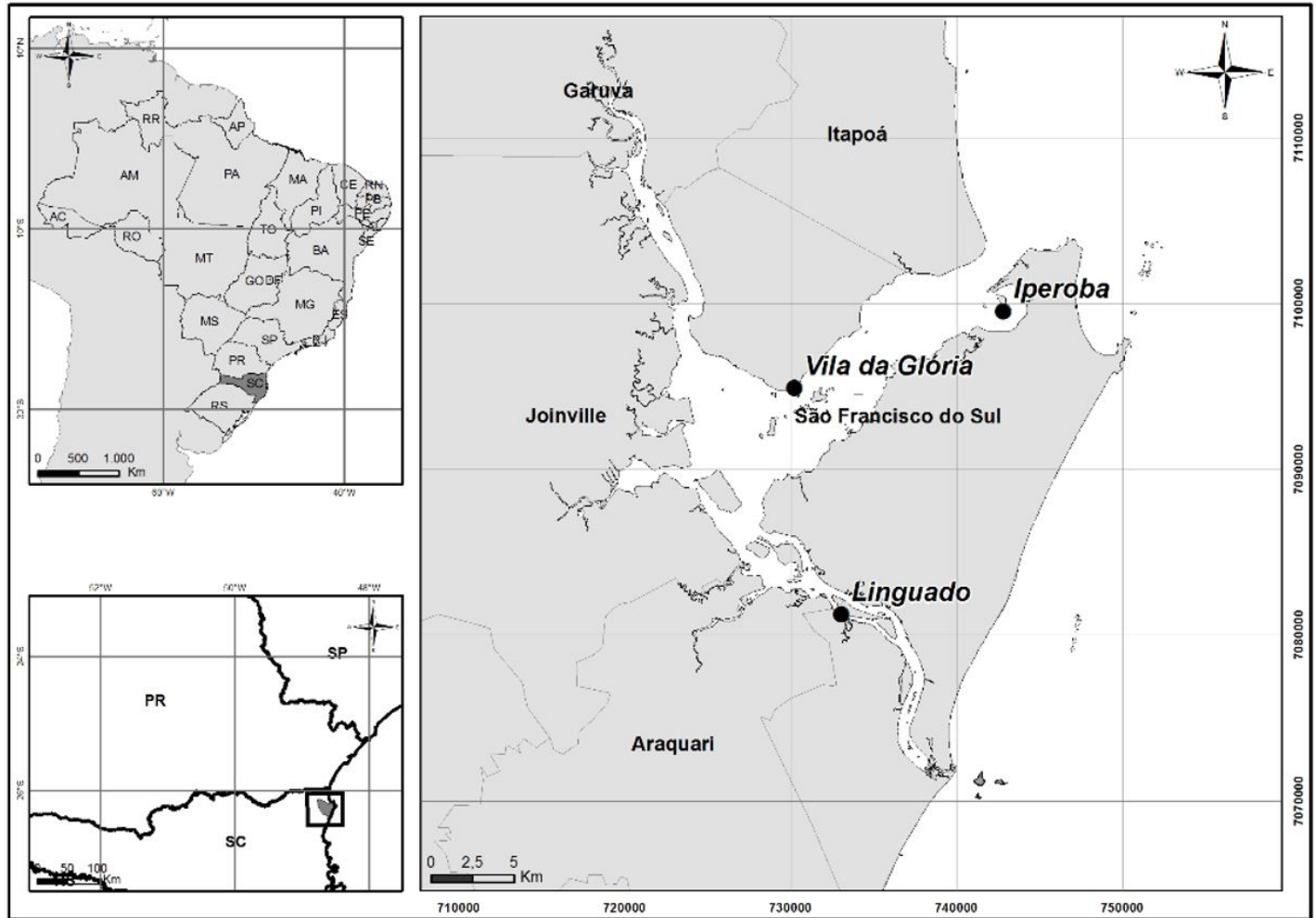


Figure 1. Location of artificial collector installation points (Iperoba, Vila da Glória and Linguado) at Babitonga Bay, Santa Catarina. Source: Centro de Cartografia Digital e Sistema de Informação Geográfica – Universidade da Região de Joinville – UNIVILLE. Datum SAD 69, (july, 2013).

to the surface of the water column, tied along two bamboo sticks previously buried in the sediment.

The collectors were installed on the surface, immersed only in the high tides, and denominated Surface (supralittoral), in the range of the tide and denominated Middle (mesolittoral) and below the low-water line and denominated Bottom (infralittoral). At each sampling point, structures were installed in triplicate, totaling 45 plates per point or 15 plates in each range of tidal variation. The collection period lasted 12 months. Temperature and salinity were recorded weekly at 30 cm below the surface. Averages and standard deviations were calculated monthly to characterize the summer, autumn, winter and spring seasons. The collection structures were placed and removed at each season of the year and then packed in separate plastic bags, identified and frozen. Once thawed, each plate was analyzed, and the oyster seeds were detached and counted.

The numbers of seeds obtained among the sites (Iperoba, Linguado and Vila da Glória), seasons (summer, autumn, winter and spring), deep level (surface, middle and bottom) at each sampling point and among the points were compared using non-parametric *t*-test using the PROC MULTITEST (Westfall et al., 1999) with SAS.

The Principal Component Analysis (PCA) was performed using the set of variables and linear combinations that were ordered and placed in descending order by their variances. Pearson Correlation was adopted to obtain the maximum possible information contained in the original variables. For PCA analysis, the XLSTAT 2014 statistical package was used. From the total number of seeds collected, 353 individuals were photographed, and tissue samples were collected for molecular analyses. The mitochondrial 16S sequence was used in the PCR/RFLP process for identification of species.

RESULTS

During the study period, water temperature showed a similar and characteristic seasonal variation in the three sites evaluated with means and standard annual deviations of 23.6 ± 3.07 °C in Iperoba, 24.1 ± 3.73 °C in Vila da Glória, and 24.5 ± 2.68 °C in Linguado (Figure 2). For salinity, a greater variation was observed, according to the place and time of the year with averages of 27.34 ± 3.0 in Iperoba, 26.8 ± 2.94 in Vila da Glória and 27 ± 6.2 in Linguado (Figure 2). The maximum and minimum values of salinity occurred in Linguado and ranged from 2 to 34 gL⁻¹.

A total of 7,579 oyster seeds attached in the artificial collectors throughout the experimental year (Table 1) with height varying from 0.5 to 3.0 cm.

Table 1. Percentage and absolute number of seeds observed at Iperoba, Linguado and Vila da Glória; in winter, autumn, spring and summer seasons; and in the surface, middle and bottom strata.

Location	Number of seeds	% of seeds
IPEROBA	5,355	74.85
LINGUADO	117	1.64
VILA DA GLÓRIA	1,675	23.5
Seasons	Number of seeds	% of seeds
WINTER	3,260	45.61
AUTUMN	1,497	20.94
SPRING	386	5.4
SUMMER	2,004	28.03
Deep level	Number of seeds	% of seeds
BOTTOM	3,480	48.7
MIDDLE	2,551	35.69
SURFACE	1,116	15.61

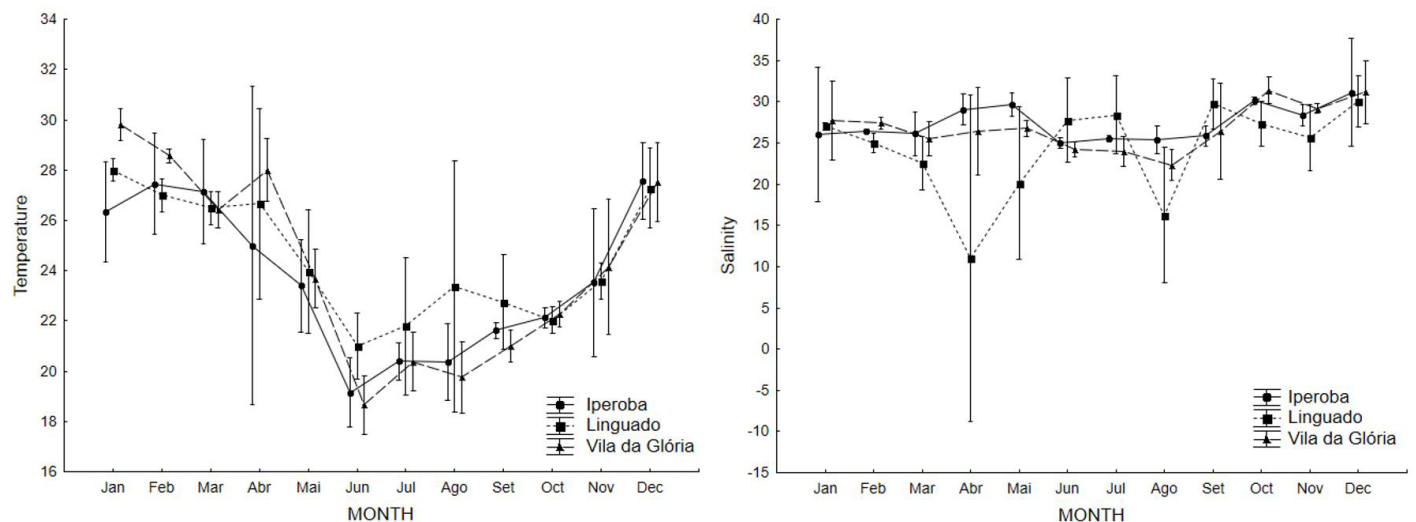


Figure 2. Monthly averages of temperature (left) and salinity (right) in the study sites Iperoba, Vila da Glória and Linguado sites.

A total of 284 seeds were analyzed genetically, and 52.81% of them were identified as *Crassostrea talonata*, 16.9% as *C. rhizophorae*, 5.63% as *C. gasar*, 24.29% as *Ostrea* sp. and 0.35% as *C. gigas* (Table 2). The diversity of external aspects of shells of different species can be seen in Figure 3. Analyzing the results of oysters settled by season, it could be seen that differences in the number of specimens occurred when compared to other seasons. Similarity in recruitment was noticed during autumn and spring (Table 2). The number of oysters attached to the collectors was higher for *C. rhizophorae* and *Ostrea* sp.

In the analyses of total seed quantities attached to artificial collectors per climatic season, significant differences were observed between spring and winter and between autumn and winter (Table 3). No recruitment of *C. gasar* occurred during winter.

Total seed quantities obtained in artificial plates placed at the surface, middle and bottom strata in all sites showed values with significant differences only between surface and bottom. However, when the total amount of seeds per site was analyzed separately, significant differences were detected only between the surface and bottom strata at the Linguado and Vila da Glória sites (Table 4).

PCA analysis demonstrated a high correlation between the distribution of *C. talonata* (CT) with bottom stratum (B) in the summer season (SU) at Iperoba (IP). Such grouping of variables was responsible for the formation of component 1, sustaining 78.94% of the total variability of the distribution. The influence of the component was also detected, albeit with less intensity, in the middle (M) and surface (S) strata, though not presenting a significant difference between both strata. These descriptors had a higher correlation with Vila da Glória (VG), which, together with winter (W), were the main factors responsible for the variability of component 2 with 12.67%. For *C. gasar* (BR), a higher occurrence was noted at the bottom (B) stratum (Figure 4).

In winter (W) and autumn (A), no significant differences were seen in species fixation. The low fixation rates of *C. rhizophorae*

Table 2. Absolute number and percentage of seeds collected by species and seasons

Season	Species					# Total of seeds	% of seeds
	<i>C. gasar</i>	<i>C. rhizophorae</i>	<i>Ostrea</i> sp.	<i>C. talonata</i>	<i>C. gigas</i>		
SUMMER	9	9	23	61	0	102	36
AUTUMN	4	9	6	46	0	65	23
WINTER	0	20	34	23	0	77	27
SPRING	3	10	6	20	1	40	14
Total of seeds	16	48	69	150	1	284	-
% of seeds	5.63	16.9	24.30	52.82	0.35	-	100

**Figure 3.** External aspect of shells submitted to genetic identification: A = *Crassostrea gasar*, B = *C. rhizophorae*, C = *C. talonata*. e D = *Ostrea* sp.(= 1cm)

Table 3. Mean number (\pm SD) and p-value of seeds per collection plate by season.

Season	Mean \pm SD	p-value
Spring	0.61 \pm 2.16	0.0700
Summer	1.95 \pm 7.37	
Spring	0.61 \pm 2.16 ^a	0.0010
Winter	2.32 \pm 10.89 ^b	
Spring	0.61 \pm 2.16	0.7930
Autumn	1.41 \pm 4.71	
Summer	1.95 \pm 7.37	0.2550
Winter	2.32 \pm 10.89	
Summer	1.95 \pm 7.37	0.5020
Autumn	1.41 \pm 4.71	
Autumn	1.41 \pm 4.71 ^a	0.0140
Winter	2.32 \pm 10.89 ^b	

Means with different letters in column between season were significantly different (P<0.05) by t-test.

Table 4. Mean number (\pm SD) and p-value of seeds per collection plate by site and stratum.

Site	Deep level	Mean \pm SD	p-value
Sum of all sites	Surface	1.01 \pm 4.29 ^a	0.0030
	Bottom	2.12 \pm 8.68 ^b	
	Surface	1.01 \pm 4.29	0,5560
	Middle	1.77 \pm 8.07	
	Bottom	2.12 \pm 8.68	0.0900
	Middle	1.77 \pm 8.07	
Iperoba	Surface	1.98 \pm 6.30	0.9350
	Middle	3.39 \pm 12.29	
	Surface	1.98 \pm 6.30	0.1530
	Bottom	3.65 \pm 12.76	
	Middle	3.39 \pm 12.29	0.2900
	Bottom	3.65 \pm 12.76	
Linguado	Surface	0.08 \pm 0.42 ^a	0.0320
	Bottom	0.28 \pm 1.17 ^b	
Vila da Glória	Surface	0.08 \pm 0.42	0.0850
	Middle	0.11 \pm 0.60	
	Bottom	0.28 \pm 1.17	0.9350
	Middle	0.11 \pm 0.60	
	Surface	0.74 \pm 3.14 ^a	0.0230
	Bottom	1.37 \pm 3.84 ^b	
	Surface	0.74 \pm 3.14	0.1600
	Middle	0.94 \pm 2.45	
	Bottom	1.37 \pm 3.84	0.6920
	Middle	0.94 \pm 2.45	

Means with different letters in column between deep level were significantly different (P<0.05) by t-test.

(RH) and *Ostrea* sp. (OS) in autumn was conditioned and limited by the distribution of *C. talonata*. However, a greater trend of occupation could be observe during winter (W) in Vila da Glória (VG) (Figure 4).

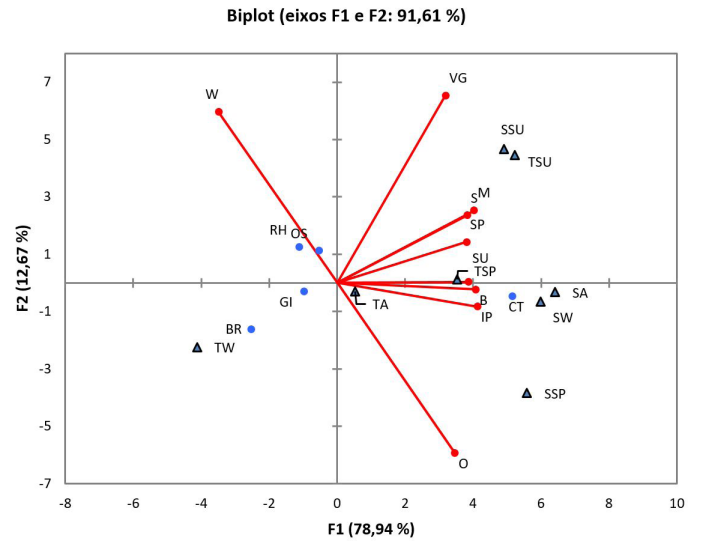


Figure 4. Principal Component Analysis (PCA) with a distribution of the *Crassostrea talonata* (CT), *C. gasar* (BR) *C. rhizophorae* (RH), *C. gigas* (GI) and *Ostrea* sp. (OS) relative to the deep level descriptors of surface (S), middle (M) and bottom (B); seasonal descriptors of summer (SU), autumn (A), winter (W), and spring (SP); and site descriptors of Iperoba (IP) and Vila da Glória (VG); temperatures of summer (TSU), autumn (TA), winter (TW), and spring (TSP) and salinity of summer (SSU), autumn (SA), winter (SW), and spring (SSP).

DISCUSSION

Differences in the rate of seed attainment in *Crassostrea* and *Ostrea* were quite evident among collector sites. Such differences are related to the oceanographic characteristic of each location, following the environmental gradient of the estuary. The physicochemical data at Babitonga Bay presented salinity variation at each site, which is a limiting parameter for the distribution of oyster larvae (Gosling, 2003; Nalesso et al., 2008).

The results showed a higher intensity of larvae recruitment at Vila da Glória, a collection point in the median section of the bay with greater density of individuals during the hotter times of the year. We found the polypropylene plate collectors to be suitable for the recruitment of oysters in the region.

Although Iperoba is closer to the ocean, it has a cove conformation and is strongly influenced by the Monte de Trigo River. Vila da Glória is located in the central body of the bay, and it is characterized by a strong current with a large water flow from both mountain and sea. Linguado exhibits the greatest variation of salinity, as recorded in the present study.

When evaluating the mechanism of dispersal and retention of larvae in estuarine environments, studies suggest the existence of an interaction among local conditions, water circulation and larval behavior (Galtsoff, 1964; Andrews, 1983; Mann, 1988; Deksheniets et al., 1996). Environmental factors that determine the horizontal transport of larvae are mainly temperature, salinity, wave action, and the velocity of currents and tides.

In their previous studies with oyster larvae in Paranaguá Bay/Paraná state, Brazil, Boehs and Absher (1997) suggest that settling individuals maintain active locomotion in order to persist in the interior of the estuary. On the other hand, studies involving *Crassostrea virginica* larvae imply that transportation of larvae in estuaries can either occur actively or passively owing to currents and tides, which explains the heterogeneity in larvae distribution in this environment (Mann, 1988; Newell et al., 2000).

In the present study, continuous seed recovery through the seasons is correlated with the reproductive pattern observed for some species of oyster collected. During winter, the number of specimens collected differed from other seasons of the year, showing a different recruitment pattern throughout the year. The gamete production of some species, such as *C. gasar* and *C. gigas*, is strongly affected by temperature pattern when they occur in places where the temperature is more pronounced according to season (Gomes et al., 2014); therefore, these species are mostly recruited in warmer seasons. Otherwise, *C. rhizophorae* and *Ostrea* sp. were the most recruited species during the winter. *C. rhizophorae* concentrated the recruitment of larvae during winter, while *Ostrea* sp. presented high larvae recruitment during both summer and winter. It is also important to note that settlements during winter involved specimens of little interest in aquaculture.

More than fifty percent of this recruitment is from *C. talonata*, a species described for the Indo-Pacific regions. In the last decade, this species expanded its distribution to the coastal zone of Peru (Li et al., 2017), Brazil and Argentina, forming large populations in estuaries, thus impacting natural stocks of native species (Cavaleiro et al., 2019). In fact, Canela Island/Pará state, where the species was first reported in Brazil (Varela et al., 2007), the recruitment of oysters through collectors was completely compromised since *C. talonata* was the only ostreid found in those structures (Garduncho et al., 2012).

Castilho-Westphal et al. (2013) also account for a species denominated *Crassostrea* sp. alongside native mangrove oyster species. This species is, most likely, *C. talonata* since it is now the exotic species expected to occur in estuaries of Brazil, and its confirmation to Brazil's littoral zone came much later than the study in question. Under the worst set of circumstances, this occurrence signals that *C. talonata* is probably present in most impacted Brazilian estuaries.

The potential compromise of the natural recruitment of oysters of Babitonga Bay for cultivation as a result of competition from *C. talonata* with native species is expected (Ruesink et al., 2005) since the species is considered rare in its natural distribution in China (Wang et al., 2008), while, in Babitonga Bay, it has the highest abundance among other ostreids. Even the fact that *C. rhizophorae* and *C. gasar* present a continuous spawning and recruitment of larvae throughout the year in Guaratuba Bay (Christo and Absher, 2006) and Paranaguá Bay/Paraná state (Absher, 1989), nearby regions with conditions similar to those of Babitonga Bay, cannot overcome the larvae settlement rate of *C. talonata*.

The preponderance of settlement of *C. talonata* in Babitonga Bay, especially in the warmer seasons, is a new concerning fact as the seeds of Crassostrean oysters possess the same morphological

conformation (Figure 3), hindering the possibility of visual identification of the species. Although the spawning cycle in this species is yet to be evaluated, it is possible to determine the potential continuous partial spawning of individuals in the warmer seasons with a high recruitment rate of seeds.

When it comes to evaluating the native mangrove oyster species *C. gasar* and *C. rhizophorae*, several studies suggest that the species possess a continuous reproduction during the whole year in the tropical littoral zone of Brazil with spawning concentrated in warmer seasons (Wakamatsu, 1973; Nascimento and Lunetta, 1978; Lenz and Boehs, 2011).

In the present study, a higher settlement of *C. gasar* seeds in Babitonga Bay was also observed in the warmer seasons. *C. gasar* is mainly associated with the tropical Atlantic (Carpenter and De Angelis, 2016), inhabiting regions significantly influenced by freshwater inflow possessing large variations in salinity (Castilho-Westphal, 2013), and Santa Catarina state (area of the present study) is the southern boundary of its distribution.

A limiting colder temperature of the water acts as a barrier for the species distribution, and different from tropical zones, it reduces the maturation of gonadal tissues completely as adult individuals build glycogen reserves, especially in the winter (Gomes et al., 2014; Ramos et al., 2014), reducing the density of *C. gasar* larvae in this season.

Such pattern is also corroborated by Christo and Absher (2006) and Castilho-Westphal et al. (2013) for the same species in Guaratuba Bay. Both estuaries are located in the subtropical area of Brazil and possess well-defined seasons; thus, the reproduction process of this species under such circumstances presents a direct correlation with an enhanced spawning process in bivalves as temperature rises in the summer season, according to Quayle (1980), Moura et al. (2008), Pereira et al. (2003), and Gomes et al. (2014), implying a higher density of seeds in this period.

However, in tropical areas, where the temperature is rather constant, salinity is the major factor that triggers spawning in *C. gasar*, as well as maturation (Paixão et al., 2013).

C. rhizophorae demonstrated a higher seed settlement rate occurring in winter than in the warmer seasons. This conformation points out the wider physiological plasticity of such species as *C. rhizophorae* which can maintain its reproduction process in several partial spawnings throughout the whole year in tropical Atlantic seas without any retractions (Lennz and Boehs, 2011), with peaks suggested to occur between March (autumn) and October (spring) (Menzel, 2018) or July (winter) and December (summer) (Velez, 1977). Since the species is distributed in southern regions up to Uruguay (Carranza et al., 2009), gonadal tissue maturation and spawning process are maintained, even in colder seasons.

The detection of one specimen of *C. gigas* through genetic analysis in the artificial collector may be a reflection of an unexpected settlement resulting from a few surviving larvae that came from spawned cultivated adults from farms located inside Babitonga Bay. Natural stocks of *C. gigas* are reported for Santa Catarina state where sea conditions probably do not permit the establishment of a viable population. Although recruitment has been observed in some places in Florianópolis Bay (Melo et al.,

2010b), the environmental factors in estuaries, in particular low salinity and high turbidity, can interfere with the development and maturation of the species, preventing its reproduction or adaptation of larvae (Akaboshi, 1979; Christo and Absher, 2006).

The settlement of oysters in our artificial collectors showed a tendency for affixation in deep level quadrants (inferior quadrants). Frequently, this pattern is related to the preference of larvae for shady substratum for occupation (Siqueira, 2008), suggesting that artificial dark collectors should be test for *C. gasar*.

In field and laboratory studies, Baker (1997) has shown that gravity is a factor for the primary settlement of *Crassostrea* larvae. According to the author, the larvae preferentially settle on the lower surfaces of shells. This strategy is more advantageous for nesting of larvae in coastal and estuarine regions with high sediment input, as the lower surfaces of the substrates are probably free of sediment (Buitrago and Alvarado, 2005).

Furthermore, previous studies report negative phototaxis (Wakamatsu, 1973; Akaboshi and Pereira, 1981; Absher, 1989). Indeed, some authors also discuss the influence of luminosity on the distribution of oyster larvae (Hidu and Haskin, 1978; Baker, 2003).

The distribution of the larvae in the water column during their cycle contributes to settlement configuration. Larvae of ostreids are abundant near the surface during the early stages of larval development, but, often, they are more abundant near the bottom at the end of the larval cycle when they seek a substrate for settlement (Dinamani, 1973; Andrews, 1983; Absher, 1989; Boehs and Absher, 1997; Baker, 2003; Finelli and Wetthey, 2003; Christo and Absher, 2006).

Diadhiou and Ndour (2017), otherwise, suggest that settlement of larvae was more efficient when plates were positioned in a sagittal way as water currents carry larvae laterally at the plates, expanding the contact surface of oysters to the substratum.

In the recruitment process of larvae, it should be considered that the interaction between genetic and environmental factors may also contribute to the definition of the substrate and the level of seed affixation (Connel, 1985; Mann et al., 1991; Baker, 2003; Finelli and Wetthey, 2003).

Thus, within this context, several factors need to be considered in the selection of areas for oyster larvae collection, among them materials, salinity, temperature, tidal level, flow, wave action, turbidity, epibionts, and predators. Therefore, the monitoring of environmental variables is fundamental in the search for the best locations for each stage of the crop, since they will directly influence the biological cycle of the species (Ramos and Castro, 2004).

Further studies are needed to assess which factors are decisive for obtaining oyster seeds in Babitonga Bay, within a set of factors such as larval flow, ecological issues, predation, and oceanographic aspects for each species. Up to the present study, no information had been available regarding the efficiency of artificial collectors from oyster seeds originating in Babitonga Bay. Artisan producers in the region observe that there are several materials with which seeds can be obtained. In the present study, however, the difficulty in the separation of the species with real

potential for cultivation became clear, making this method of capture of seeds impracticable for commercial needs.

Galvão et al. (2009) note at least more than one species in an artificial collection plate placed in the Cananéia region of São Paulo, making it difficult to perform a precise evaluation of growth performance of oyster species. Nalesso et al. (2008) and Christo et al. (2016) also reported the same difficulties in correctly identifying oyster seeds attached to artificial collectors in the littoral zone of Espírito Santo state and Guaratuba Bay/Paraná state, respectively. Additionally, the sympatric occurrence of *C. gasar*, *C. rhizophorae* and, now, most recently, *C. talonata* in estuaries configures a common assembly along the Brazilian littoral, increasing, still more, the difficulty of attaining suitable oysters for commercial purposes at low cost.

Getting economically important oyster seeds in artificial collectors is still possible in some localities, especially where the variation of salinity is higher, with values reaching the limits of the freshwater condition in rainy seasons. These areas, most of them located in northeastern and northern municipalities of Brazil, are denominated “sementeiras”, or oyster sowing, in which oyster farmers place artificial collectors with a higher chance of getting only the target species for cultivation. Indeed, Funo et al. (2015) showed through genetic analysis that such practice is possible as 100% of oysters obtained in one of those areas situated in Curuçá/Pará state belonged to *C. gasar*.

The results of this research certainly contribute to the planning of oyster crops in the Babitonga Bay region. Thus, despite the ability to get oyster seeds with the tested system, it should still be considered that most of the specimens belong to *C. talonata*, in addition to *Ostrea* sp. and *C. rhizophorae*, i.e., animals that reach small size and are not suitable for commercial farming in the molds currently used in southern Brazil. Therefore, profitable oyster cultivation may rely on seeds provided by hatcheries, discounting the fact that hatchery techniques are not well developed for getting *C. gasar* seeds (FAO, 2014).

CONCLUSIONS

Genetic analyses indicated the presence of *Crassostrea gasar*, *C. rhizophorae*, *C. gigas*, *C. talonata*, and *Ostrea* sp., in the collectors arranged along Babitonga Bay.

It was not possible to identify, by morphological analysis, the species of the genus *Crassostrea* in the seed phase, mainly owing to the enormous plasticity of shells.

Seeds of the genus *Crassostrea* were obtained mainly in the summer season, followed by spring and autumn. In winter, the highest amounts of seeds were observed for *C. rhizophorae* and *Ostrea* oysters.

The results of this work showed the current impossibility of obtaining seed quantities of oyster species with zootechnical potential to supply the commercial farms by artificial collectors in the study region since *C. talonata*, a highly invasive species in the Brazilian estuaries with no commercial value, now corresponds to 52% of the total settlement rate in the these collectors.

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REFERENCES

- Absher, T.M. 1989. Populações naturais de ostras do gênero *Crassostrea* do litoral do Paraná: desenvolvimento larval, recrutamento e crescimento. São Paulo, Brasil. São Paulo. 143p. (Tese de doutorado. Instituto Oceanográfico, USP). Available from: <<https://bdpi.usp.br/item/000722681>> Access on: 29 aug. 2019.
- Akaboshi, S. 1979. Notas sobre o comportamento da ostra japonesa *Crassostrea gigas* (Thunberg 1785) no litoral do estado de São Paulo, Brasil. Boletim do Instituto de Pesca, 6: 93-104.
- Akaboshi, S.; Pereira, O.M. 1981. Ostricultura na região lagunar de Cananea, São Paulo, Brasil; 1. Captação de larvas da ostra *Crassostrea brasiliana* (Lamarck 1819) em ambiente natural. Boletim do Instituto de Pesca, 8: 87-104.
- Andrews, J.D. 1983. Transport of bivalve larvae in James River, Virginia. Journal of Shellfish Research, 3(1): 29-40.
- Baker, P. 1997. Settlement site selection by oyster larvae, *Crassostrea virginica*: evidence for geotaxis. Journal of Shellfish Research, 16: 125-128.
- Baker, P. 2003. Two species of oyster larvae show different depth distributions in a shallow, well-mixed estuary. Journal of Shellfish Research, 3(3): 733-736.
- Boehs, G.; Absher, T.M. 1997. Distribuição de larvas de ostras do gênero *Crassostrea* Sacco 1897. (Ostreoida: Ostreidae) na Baía de Paranaguá, Paraná. Arquivos de Biologia e Tecnologia, 40(1): 39-45.
- Buitrago, E.; Alvarado, D. 2005. A highly efficient oyster spat collector made with recycled materials. Aquacultural Engineering, 33(1): 63-72.
- Carpenter, K.E.; De Angelis, N. 2016. The living marine resources of the Central Eastern Atlantic. Bivalves, gastropods, hagfishes, sharks, batoid fishes and chimaeras. FAO Species Identification Guide for Fishery Purposes, Rome: FAO. v. 2.
- Carranza, A.; Defeo, O.; Beck, M. 2009. Diversity, conservation status and threats to native oysters (Ostreidae) around the Atlantic and Caribbean coasts of South America. Aquatic Conservation, 19(3): 344-353. <http://dx.doi.org/10.1002/aqc.993>.
- Castilho-Westphal, G.G.; Magnani, F.; Ostrensky, A. 2013. Gonad morphology and reproductive cycle of the mangrove oyster *Crassostrea brasiliana* (Lamarck, 1819) in the baía de Guaratuba, Paraná, Brazil. Acta Zoologica, 96(1): 99-107. <https://doi.org/10.1111/azo.12055>.
- Cavaleiro, N.P.; Lazoski, C.; Tureck, C.R.; Melo, C.M.R.; Amaral, V.S.; Lomovasky, B.J.; Absher, T.M.; Solé-Cava, A.M. 2019. *Crassostrea talonata*, a new threat to native oyster (Bivalvia: Ostreidae) culture in the Southwest Atlantic. Journal of Experimental Marine Biology and Ecology, 511: 91-99. <http://dx.doi.org/10.1016/j.jembe.2018.11.011>.
- Christo, S.W., Ferreira-Jr., A.L.; Cruz-Kaled, A.C.; Absher, T.M. 2016. Recruitment of oysters of *Crassostrea* genus (Bivalvia, Ostreidae) in Guaratuba Bay, PR, Brazil. Pan-American Journal of Aquatic Sciences, 11(4): 301-308.
- Christo, S.W.; Absher, T.M. 2006. Reproductive period of *Crassostrea rhizophorae* (Guilding 1828) and *Crassostrea brasiliana* (Lamarck 1819) (Bivalvia: Ostreidae) in Guaratuba Bay, Paraná Brazil. Journal of Coastal Research, 39: 1215-1218.
- Connel, J.H. 1985. The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. Journal of Experimental Marine Biology and Ecology, 93: 11-45.
- Dautzenberg, P. 1891. Voyage de la Goélette Melita aux Canaries et au Sénégal, 1889-1890. Mollusques Testacés. Mémoires de la Société Zoologique de France, 4: 16-65.
- Deksheniaks, M.M.; Hofmannl, E.E.; Link, J.M.; Powel, E.N. 1996. Odeling the vertical distribution of oyster larvae in response to environmental conditions. Marine Ecology Progress Series, 136(1-3): 97-110.
- Diadhiou, H.D.; Ndour, I. 2017. Artificial capture of mangrove oyster spat *Crassostrea gasar* (Mollusca, Bivalvia) in Casamance estuary (Senegal). Aquaculture, Aquarium, Conservation & Legislation, 10(1): 48-55.
- Dinamani, P. 1973. Embryonic and larval development in the New Zealand rock oyster *Crassostrea glomerata* (Gould 1850). The Veliger, 15: 295-299.
- FAO – Food and Agriculture Organization of the United Nations. 2014. The State of World Fisheries and Aquaculture, Rome: FAO. 223p.
- Finelli, C.M.; Wethey, D.S. 2003. Behavior of oyster (*Crassostrea virginica*) larvae in flume boundary layer flows. Marine Biology, 143(4): 703-711.
- Funo, I. C.S.A.; Antonio, I.G.; Marinho, Y.F.; Galvez, A.O. 2015. Influence of salinity on survival and growth of *Crassostrea gasar*. Boletim do Instituto de Pesca, 41(4): 837-847.
- Funo, I. C.S.A.; Marinho, Y.F.; Monteles, J.M.; Lopes, R.G.P.S.; Galvez, A.O. 2019. Recruitment of oyster in artificial collectors on the Amazon macrotidal mangrove coast. Ciência Rural, 49(3). <http://dx.doi.org/10.1590/0103-8478cr20180482>.
- Galtsoff, P.S. 1964. The american oyster *Crassostrea virginica* (Gmelin). US Fish and Wildlife Service Bulletin, 64: 1-480.
- Galvão, M.S.; Alves, P.M.F.; Hilsdorf, W.S. 2017. First record of the *Saccostrea* oyster in Bertioga, São Paulo, Brazil. Boletim do Instituto de Pesca, 43(4): 638-645. <http://dx.doi.org/10.20950/1678-2305.2017v43n4p638>
- Galvão, M.S.; Pereira, O.M.; Hilsdorf, A.W. 2013. Molecular identification and distribution of mangrove oysters (*Crassostrea*) in an estuarine ecosystem in Southeast Brazil: implications for aquaculture and fisheries management. Aquaculture Research, 44(10): 1589-1601. <http://dx.doi.org/10.20950/1678-2305.2018.44.4.377>.
- Galvão, M.S.N.; Pereira, O.M.; Machado, I.C.; Pimentel, C.M.M.; Henriques, M.B. 2009. Desempenho da criação da ostra de mangue *Crassostrea* sp. A partir da fase juvenil, em sistema suspenso, no estuário de Cananéia e no mar de Ubatuba (SP, Brasil). Boletim do Instituto de Pesca, 35(3): 401-411.
- Garduncho, D.C.L.; Gomes, C.P.; Tagliaro, C.H.; Beasley, C.R. 2012. Settlement of an unidentified oyster (*Crassostrea*) and other epibenthos on plastic substrates at a northern Brazilian mangrove island. Brazilian Journal of Aquatic Science and Technology, 16(1): 41-51.
- Gomes, C.H.A.M.; Silva, F.C.; Lopes, G.H.; Melo, C.M.R. 2014. The reproductive cycle of the oyster *Crassostrea gasar*. Brazilian Journal

- of Biology = Revista Brasileira de Biologia, 74: 967-976. <http://dx.doi.org/10.1590/1519-6984.04912>.
- Gosling, E. 2003. Bivalve molluscs: biology, ecology and culture. Oxford: Fishing News Books. 443p.
- Guilding, L. 1828. Observations on the zoology of the Caribbean Islands. Journal of Zoology, 3: 542.
- Hidu, H.; Haskin, H.H. 1978. Swimming speeds of oyster larvae *Crassostrea virginica* in different salinities and temperatures. Estuaries, 1(4): 252-255.
- IBAMA – Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. 1998. Proteção e controle de ecossistemas costeiros: manguezal da Baía da Babitonga. Brasília: Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. 145p. (Coleção Meio Ambiente. Série Estudos: Pesca, 25).
- Ignácio, B.L.; Absher, T.M.; Lazoski, C.; Solé-Cava, A.M. 2000. Genetic evidence of the presence of two species of *Crassostrea* (Bivalvia: Ostreidae) on the coast of Brazil. Marine Biology, 136(6): 987-991. <http://dx.doi.org/10.1007/s002270000252>.
- Lapêgue, S.; Boutet, I.; Leitão, A.; Heurtebise, S.; Garcia, P.; Thiriot-Quiévreux, C.; Boudry, P. 2002. Trans-Atlantic distribution of mangrove oyster species revealed by 16S mtDNA and karyological analyses. The Biological Bulletin, 202: 232-242.
- Lazoski, C. 2004. Sistemática molecular e genética populacional de ostras brasileiras (*Crassostrea* spp.), Rio de Janeiro, Brasil. Rio de Janeiro. 145f. (Tese de Doutorado, UFRJ). Available from: <http://objdig.ufrj.br/50/teses/d/CCS_D_CristianoLazoski.pdf> Access on: 28 mar. 2020.
- Lazoski, C.; Gusmão, J.; Boudry, P.; Solé-Cava, A.M. 2011. Phylogeny and phylogeography of Atlantic oyster species: evolutionary history, limited genetic connectivity and isolation by distance. Marine Ecology Progress Series, 426: 197-212. <http://dx.doi.org/10.3354/meps09035>.
- Legat, J.F.A.; Puchnick-Legat, A.; Fogaça, F.H.S.; Tureck, C.R.; Suhnel, S.; Melo, C.M.R. 2017. Crescimento e sobrevivência da ostra de fundo, *Crassostrea gasar*, cultivada no nordeste e sul do Brasil. Boletim do Instituto de Pesca, 43: 172-184. <http://dx.doi.org/10.20950/1678-2305.2017v43n2p172>.
- Lenz, T.; Boehs, G. 2010. Ciclo reproductivo del ostión de manglar *Crassostrea rhizophorae* (Bivalvia: Ostreidae) en la Bahía de Camamu, Bahia, Brasil. Revista de Biología Tropical, 59(1): 137-149.
- Li, C.; Qi, Z. 1994. Studies on the comparative anatomy, systematic classification and evolution of Chinese oysters. Studia Marina Sinica, 35: 143-173.
- Li, C.; Wang, H.; Guo, X. 2017. Classification and Taxonomic Revision of Two Oyster Species from Peru: *Ostrea megodon* (Hanley, 1846) and *Crassostrea talonata* (Li & Qi, 1994). Journal of Shellfish Research, 36(2): 359-364. <http://dx.doi.org/10.2983/035.036.0208>.
- Lima, F.R.; Vazzoler, A.E.A.M. 1963. Sobre o desenvolvimento das ostras e possibilidade da ostreicultura nos arredores de Santos. Boletim do Instituto Oceanográfico USP, 13(3): 20.
- Lopes, G.R.; Gomes, C.H.A.M.; Tureck, C.R.; Melo, C.M.R. 2013. Growth of *Crassostrea gasar* cultured in marine and estuary environments in Brazilian waters. Pesquisa Agropecuária Brasileira, 48(7): 975-982.
- Maccacchero, G.B.; Guzinski, J.; Ferreira, J.F. 2005. Allometric growth on mangrove oyster *Crassostrea rhizophorae* (Guilting 1828) cultured in Southern Brazil. Ciência Agronômica, 36(3): 400-403.
- Mann, R. 1988. Field studies of bivalve and their recruitment to the benthos: a commentary. Journal of Shellfish Research, 7(1): 49-64.
- Mann, R.; Campos, B.M.; Luchenbach, M.W. 1991. Swimming rate and responses of larvae three mactrid bivalves to salinity discontinuities. Marine Ecology Progress Series, 68: 257-269.
- Melo, A.G.C.; Varela, E.S.; Beasley, C.R.; Schneider, H.; Sampaio, I.; Gaffney, P.M.; Reece, K.S.; Tagliaro, C.H. 2010a. Molecular identification, phylogeny and geographic distribution of Brazilian mangrove oysters (*Crassostrea*). Genetics and Molecular Biology, 33(3): 564-572. <http://dx.doi.org/10.1590/S1415-47572010000300030>.
- Melo, C.M.R.; Silva, F.C.; Gomes, C.H.A.M. Solé-Cava, A.M.; Lazoski, C. 2010b. *Crassostrea gigas* in natural oyster banks in southern Brazil. Biological Invasions, 12(3):441-449. <http://dx.doi.org/10.1007/s10530-009-9475-7>.
- Melo, M.A.D.; Silva, A.R.B.; Beasley, C.R.; Tagliaro, C.H. 2013. Multiplex species-specific PCR identification of native and nonnative oysters (*Crassostrea*) in Brazil: a useful tool for application in oyster culture and stock management. Aquaculture International, 21(6): 1325-1332. <http://dx.doi.org/10.1007/s10499-013-9635-8>
- Menzel, W. 2018. Estuarine and marine bivalve mollusk culture. CRC Press, 374p.
- Moura, P.; Gaspar, M.B.; Monteiro, C.C. 2008. Gametogenic cycle of the smooth clam *Callista chione* on the south-western coast of Portugal. Journal of the Marine Biological Association of the United Kingdom, 88(1): 161-167. <http://dx.doi.org/10.1017/S0025315408000337>.
- Nalesso, R.C.; Paresque, K.; Piumbini, P.P.; Tonini, J.F.R.; Almeida, L.G.; Nickel, V.M. 2008. Oyster spat recruitment in Espírito Santo state, Brazil, using recycled materials. Brazilian Journal of Oceanography, 56(4): 281-288.
- Nascimento, I.A. 1983. Cultivo de ostras no Brasil: problemas e perspectivas. Ciência e Cultura, 35(7): 871-876.
- Nascimento, I.A. 1991. *Crassostrea rhizophorae* (Guilting) and *C. brasiliana* (Lamarck) in South and America Central. In: Menzel, W. (Ed.). Estuarine and marine bivalve mollusk culture. Boca Ratón: CRC Press. p.125-134.
- Nascimento, I.A.; Lunetta, J.E. 1978. Ciclo sexual da ostra de mangue e sua importância para o cultivo. Boletim de Zoologia da Universidade de São Paulo, 2: 63-93.
- Newell, R.I.E.; Alpach, G.S.; Kennedy, V.S.; Jacobs, D. 2000. Mortality of newly metamorphosed oysters (*Crassostrea virginica*) in mesohaline Chesapeake Bay. Marine Biology, 136: 665-676.
- Paixão, L.; Ferreira, M.A.; Nunes, Z.; Fonseca-Sizo, F.; Rocha, R.A. 2013. Effects of salinity and rainfall on the reproductive biology of the mangrove oyster (*Crassostrea gasar*): Implications for the collection off broodstock oysters. Aquaculture (Amsterdam, Netherlands), 6(12): 380-383. <http://dx.doi.org/10.1016/j.aquaculture.2012.11.019>.
- Pereira, O.M. 1997. Ostreicultura: situação atual e contribuição do Instituto de Pesca para o seu desenvolvimento. In: Stempniewski, H. L. (Ed.). Retrospectiva dos serviços de pesca da Secretaria de Agricultura e Abastecimento e o jubileu de prata do Instituto de Pesca. São Paulo: Instituto de Pesca, Coordenadoria da Pesquisa Agropecuária, Secretaria de Agricultura e Abastecimento. p. 119-124.
- Pereira, O.M.; Akaboshi, S.; Soares, F.C. 1988. Cultivo experimental de *Crassostrea brasiliana* (Lamarck 1819) no Canal da Bertioiga, São

- Paulo (23°54'30"S, 44°13'42"W). Boletim do Instituto de Pesca, 15(1): 55-65.
- Pereira, O.M.; Henrique, M.B.; Machado, I.C. 2003. Estimativa da curva de crescimento da ostra *Crassostrea brasiliiana* em bosques de mangue e proposta para sua extração ordenada no estuário de Cananeia, SP, Brasil. Boletim do Instituto de Pesca, 29(1): 19-28.
- Pereira, O.M.; Machado, I.C.; Henriques, M.B.; Yamanaga, N. 2001. Crescimento da ostra *Crassostrea brasiliiana* semeada sobre tabuleiro em diferentes densidades na região estuarina-lagunar de Cananeia-SP (25°S, 48°W). Boletim do Instituto de Pesca, 27(2): 163-174.
- Pereira, O.M.; Soares, F.C. 1996. Análise da criação de ostra *Crassostrea brasiliiana* (Lamarck 1819), no sítio de Guarapari, na região lagunar-estuarina de Cananeia-SP. Boletim do Instituto de Pesca, 23: 135-142.
- Pie, M.R.; Ribeiro, R.O.; Boeger, W.A.; Ostrensky, A.; Falleiros, R.M.; Angelo, L. 2006. A simple PCR-RFLP method for the discrimination of native and introduced oyster species (*Crassostrea brasiliiana*, *C. rhizophorae* and *C. gigas* (Bivalvia: Ostreidae) cultured in Southern Brazil. Aquaculture Research, 37: 1598-1600.
- Quayle, D.B. 1980. Tropical oysters: culture and methods. Ottawa: IDRC. 80 p.
- Ramos, C.D.O.; Gomes, C.H.A.M.; Magalhães, A.R.M.; Dos Santos, A.I.; Melo, C.M.R. 2014. Maturation of the mangrove oyster *Crassostrea gasar* at different temperatures in the laboratory. Journal of Shellfish Research, 33(1): 187-194. <http://dx.doi.org/10.2983/035.033.0118>.
- Ramos, M.I.S.; Gomes, C.H.A.M.; Magalhães, A.R.M.; Dos Santos, A.I.; Melo, C.M.R. 1986. The comparative growth survival of the Pacific oyster (*Crassostrea gigas* Thunberg, *Crassostrea gigas* var. Kumamoto and mangrove oyster *Crassostrea rhizophorae*) in Todos os Santos Bay, Brazil. Ciência e Cultura, 38: 1604-1615.
- Ramos, R.S.; Castro, A.C.L. 2004. Monitoramento das variáveis físico-químicas no cultivo de *Crassostrea rhizophorae* (Mollusca) (Guilding 1828) no estuário de Paquatua – Alcântara/MA, Brasil. Boletim do Laboratório de Hidrobiologia, 17: 19-27.
- Ruesink, J.L.; Lenihan, H.S.; Trimble, A.C.; Heiman, K.W.; Micheli, F.; Byers, J.E.; Kay, M.C. 2005. Introduction of non-native oysters: ecosystem effects and restoration implications. Annual Review of Ecology and Systematics, 36: 643-689.
- Singarajah, K.V. 1980. On the taxonomy, ecology and physiology of the giant oyster *Crassostrea paraibensis*, new species. Bulletin of Marine Science, 30: 833-847.
- Siqueira, K.L.F. 2008. Avaliação do sistema de cultivo de ostras do gênero *Crassostrea* (SACCO, 1897) no estuário do Rio Vaza-barris (Sergipe), Aracaju, Brasil. Aracaju. 77f. (Dissertação de Mestrado, UNIT). Available from: <<http://openrit.grupotiradentes.com:8080/xmlui/handle/set/3001>> Access on: 28 mar. 2020.
- Varela, E.S.; Beasley, C.R.; Schneider, H.; Sampaio, I.; Marques-Silva, N.D.S.; Tagliaro, C.H. 2007. Molecular phylogeny of mangrove oysters (*Crassostrea*) from Brazil. Journal of Molluscan Studies Advanced, 73(3): 229-234.
- Velez, A. 1977. Ciclo anual de reproducción del ostion *Crassostrea rhizophorae* (Guilding) de Bahia de Mochima. Boletim do Instituto Oceanográfico, 16: 87-98.
- Wakamatsu, T. 1973. A ostra de Cananeia e seu cultivo. São Paulo: Superintendência do Desenvolvimento do Litoral Paulista/Instituto Oceanográfico USP. 141p.
- Wang, H.; Zhang, G.; Liu, X.; Guo, X. 2008. Classification of Common Oysters from North China. Journal of Shellfish Research, 27(3): 495-503. [http://dx.doi.org/10.2983/0730-8000\(2008\)27\[495:COCOFN\]2.0.CO;2](http://dx.doi.org/10.2983/0730-8000(2008)27[495:COCOFN]2.0.CO;2).
- Westfall, P.H.; Tobias, R.D.; Wolfinger, R.D. 1999. Multiple comparisons and multiple tests using the SAS System. Cary, NC: SAS Institute Inc. 397 p.
- Wu, X.; Xiao, S.; Yu, Z. 2013. Mitochondrial DNA and morphological identification of *Crassostrea zhanjiangensis* sp. nov. (Bivalvia: Ostreidae): a new species in Zhanjiang, China. Aquatic Living Resources, 26: 273-280. <http://dx.doi.org/10.1051/alr/2013065>.