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Research Article

Bioinvasion of Benthic Invertebrates in a Eutrophicated Tropical Estuarine System in South America (Santos, Brazil)

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Abstract: The economic losses due to bioinvasion in Brazil were estimated to be over USD 100 billion since 1984; however, these values are mostly related to terrestrial invasive species, whereas the financial losses in the marine realm continue to be ignored. The bioinvasion of benthic fauna was evaluated in the estuarine channel of Santos Harbor, Brazil. Three recruitment plates were spread along the estuarine channel to assess their colonization progress during two periods: April until July and August until September in 2019. Inner stations presented haline stratification, and low values of dissolved oxygen were registered as signs of eutrophication. Among the identified taxa, six species were considered exotic to the Brazilian coastline: *Branchiomma luctuosum, Hydroides elegans, Bugula neritina, Styela plicata, Clavelina oblonga, and Botrylloides giganteus.* The diversity was low due to the monospecific dominance of *B. luctuosum*, which occupied 73.4% and 42.4% of the recruitment plates in the first and second campaigns, respectively. It was possible to observe a gradient pattern in the biovolume averages between the monitoring points, which tended to be higher at the station closer to the sea, despite the lack of significant differences among them. The recruitment plate technique offered reliable results for the identification of invasive species regarding fouling communities along the Santos Harbor channel.

Keywords: benthic macrofauna, bioinvasion, estuaries, Santos Harbor

1. Introduction

Over the last few decades, the world trade has grown significantly, creating strong pressure on the infrastructure networks of exporting countries. The increase in shipping volumes and diversification of global trade networks have opened biogeographical boundaries, promoting the spread of non-indigenous species through transportation by ballast water or hull fouling and its associated species [1, 2]. The negative impacts of bioinvasion on local ecosystems have been studied globally for decades [3-8], identifying non-indigenous species as a significant component of global change [9]. The detection of invasive species depends on monitoring and adequate knowledge of the native fauna, including genetic, taxonomic, biological, and ecological data. The establishment of invasive species is rarely observed and hardly recorded [10]. The early detection of exotic species when the population is still small makes management and

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eradication more effective [11]. The lack of previous information about a species complicates the determination of its invasive status, and, in this case, the origin of the species is considered uncertain and it is classified as cryptogenic [12].

The processes of ecological succession in estuaries are influenced by natural factors; however, the influence of commercial activities in coastal environments has a direct impact on this process. Since ships are the major vector of bioinvasion, non-indigenous species are usually established in harbor areas and their surroundings, as the presence of a large number of artificial structures, such as buoys and mooring devices, facilitates their settlement [13], providing a favorable environment for the fixation and colonization of non-indigenous larval species [14, 15]. After recruitment and establishment, the next step in the bioinvasion process is the spreading of the non-indigenous species.

The understanding of impacts caused by bioinvasion is key for environmental management and for raising public awareness; however, the effects of biological invasions remain undervalued by the general public, stakeholders, and decision-makers, and therefore, effective mitigation policies towards prevention, control, and eradication are not efficient worldwide [16]. Despite the negative impacts of marine bioinvasions on biodiversity, ecosystem services, human health, and the economy, the marine realm has historically received less attention compared to terrestrial and freshwater habitats [17, 18]. Not only have the economic costs of bioinvasion in Brazil hardly been quantified, but most evaluations have focused on terrestrial ecosystems despite the high relevance of aquatic environments in this country. A minimum of USD 105.53 billion in economic impact was estimated in a study that considered only 16 invasive species out of the 460 considered invasive in Brazil [18].

Estuaries are transitional environments between the continent and the oceans that usually present large hydrological variations, particular morphologies, and peculiar chemical patterns [19]. Macrobenthos represent a fundamental stage of the food chain and, therefore, have major importance in the balance of estuaries [20-23]. Furthermore, the benthic infauna plays an important role in estuarine ecological balance since they act by revolving the sediment, promoting aeration, and stimulating biogeochemical processes [24]. Estuarine organisms are often related to environmental gradients, resulting in well-developed distribution patterns usually associated with salinity variation [25-29]. Previous studies pointed out the importance of tidal range and wave fetch distance in determining the community structure of macrobenthos [30, 31]. The knowledge of spatial distribution patterns of macrobenthos along estuarine gradients helps identify the linkages between species distributions and ecological processes, providing insights concerning the functioning of estuarine ecosystems [32], which is essential for the implementation of integrated estuarine management.

Non-indigenous species invasion research is a relatively new topic on the Brazilian coast [33, 34]. The Santos-Cubatão Estuarine System in southeastern Brazil has a history of contamination since the 1950s, when the largest industrial complex in Latin America was established [35]. The levels of chemical pollution in this area have been very well-documented [36-39].

In the present study, the dynamics of benthic colonization and the distribution of the subtidal macrobenthic community, as well as the potential bioinvasion process resulting from harbor activity in the Santos Estuarine System, were evaluated along with other ancillary variables.

2. Materials and methods

2.1 *Study area*

Santos Bay is located in the state of São Paulo in southeast Brazil (Figure 1), and it includes the touristic cities of São Vicente and Santos [40]. For the past decades, Santos Harbor has been the largest commercial harbor in South America, and it is still among the six largest in the continent. The Cubatão industrial complex, in the surroundings of Santos Harbor, is one of the most important industrial areas in Brazil, comprising around 1,100 industries. Overall, the Santos Estuarine System presents many kinds of pollution issues that have been well-documented over the last few decades [36, 41-44].

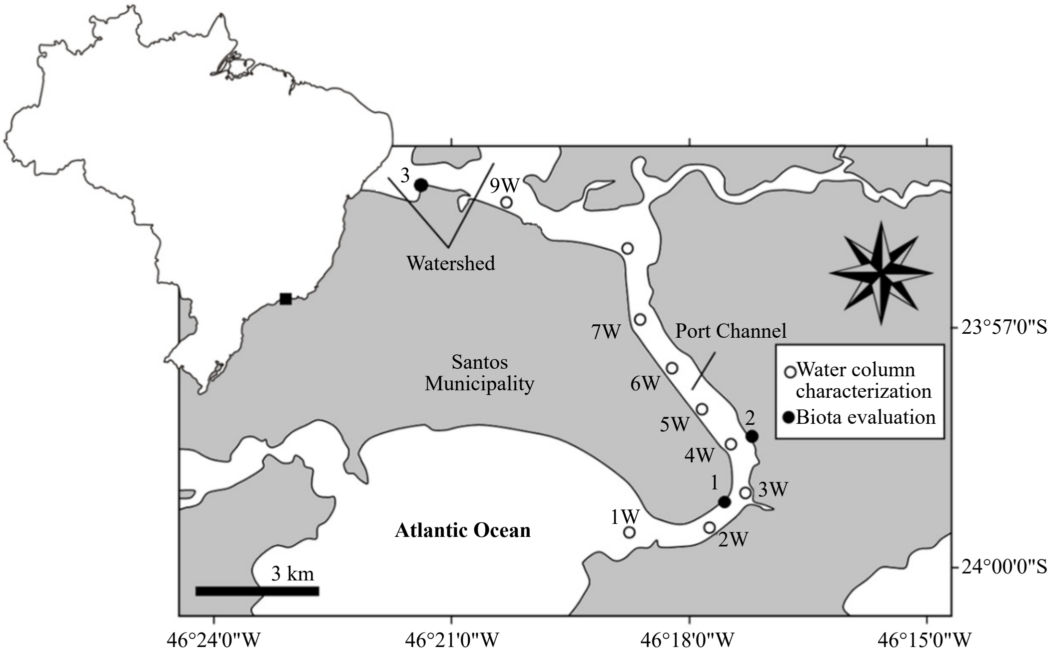

Figure 1. Santos-São Vicente estuarine system and sampling stations in the Santos Channel

2.2 *Sampling*

2.2.1 *Water column*

Nine stations were distributed along the Santos Harbor channel to characterize the water column. Temperature, pH, dissolved oxygen (DO), salinity and redox potential (ORP) were recorded *in situ* using a multi-parameter probe (Horiba U-51). For each station, physicochemical variables were measured from top to bottom, every 0.5 m up to approximately 5 m, and after that depth, every 1 m until reaching the bottom. The transparency of the water column was measured with a Secchi disk, and the results were used in the calculation of euphotic zone thickness (Z_{eu}), according to Poole et al. [45].

2.2.2 *Biota*

The recruitment plates were set up at three stations in the Santos Channel during two field surveys. These structures consist of three PVC plates measuring 15.0 cm × 15.0 cm (Figure 2), at different depths (surface, middle and bottom depths), with the experimental surface facing downward and parallel to the seafloor to mimic floating docks, an approach similar to that used by Marraffini et al. [46]. These structures remained submerged for two periods (April until July and August until September in 2019). At the end of each period, the recruitment plates were recovered and taken to the laboratory to identify the organisms.

Figure 2. Recruitment plates before installation

2.3 *Laboratory analysis*

In the laboratory, the recruitment plates were photographed and examined under a stereomicroscope (Zeiss Stemi V6), using a 15 cm x 15 cm grid with 1 cm x 1 cm subdivisions to estimate the coverage area of each taxon present on the plate. The classification of the species found on the plates was divided into native, exotic or cryptogenic according to their origin based on the literature review and criteria previously developed by taxonomists [10, 12, 33, 47-57].

For the experiment with recruitment plates, the presence of a multilayered community was considered [58]. Thus, the deployment and retrieval of recruitment plates were carried out in two stages, considering two strata, the upper and the lower. At first, the coverage estimate was performed with all the encrusted organisms in the upper stratum. After this first screening, the organisms of the upper stratum were removed, and the deployment of the recruitment plates was carried out again to obtain data from the lower stratum. The total percentage of organism coverage on recruitment plates was calculated by adding the coverage of the upper stratum (with the total biovolume) and the lower stratum (after removing the upper biovolume). In this way, the percentage of coverage on recruitment plates reached values around 100% due to the effect of multiple layers [58].

Biovolume was estimated using the volumetric variation technique. The biomass of organisms was placed in a graduated cylinder and covered with alcohol 70% (v/v) until 1 L of volume was reached. Then the alcohol was removed and its volume was measured. The volume of biomass was calculated by dividing the subtraction of the volume of alcohol 70% from the total volume of 1 L by the area of the plate in meters.

2.4 *Statistical analysis*

The coverage of the different taxa present on recruitment plates were analyzed with multivariate statistics to show spatial and temporal distribution patterns. Canonical correspondence analysis was used to verify the possible relationships between the environmental variables measured at the time of removing the plates, the depth and location of the monitoring point, and the coverage and occurrence of the different taxa.

The significance of the differences observed in the volume between monitored points, between depths, and between sampling periods were analyzed with univariate statistics. For the first sampling period, the sampling stations were used as an independent variable in an analysis of variance (ANOVA), and depth was used as an independent variable in another ANOVA. This was done to see if there were any spatial differences.

3. Results and discussion

3.1 *Characterization of the water column*

The euphotic zone thickness, Z_{eu}, presented a maximum of 5.6 m at station 2W at the entrance of Santos Channel and decreased towards the inner channel, where it reached 2.97 m in the most inner station (Figure 3). The Santos Channel presented a trend of decreasing temperature and salinity towards the head of the estuary (Figure 4). Higher temperatures toward the head are justified by the lowering of depths and darker waters inside the estuary, which corroborate with the Z_{eu} results. The mean temperature values varied from 28.20 ± 0.41 °C (1W) to 28.96 ± 0.05 °C (8W). The temperature difference between surface and bottom reached a maximum of 1.5 °C in station 1W, whereas in the other stations, these differences were kept below 1 °C, decreasing considerably towards the head of the estuary.

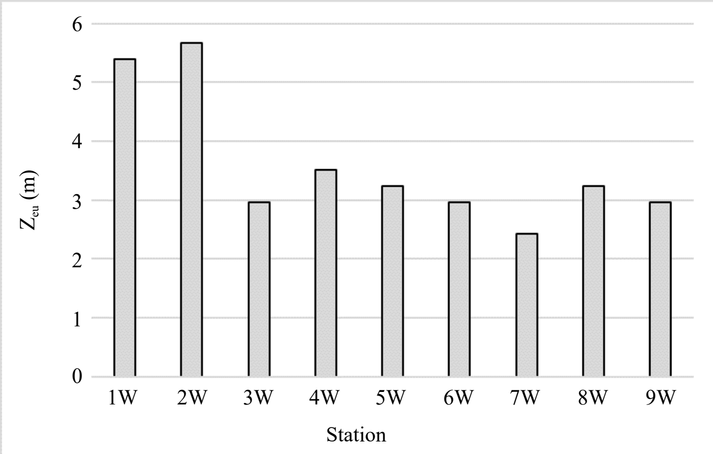

Figure 3. Euphotic zone (Z_{eu}) along the estuarine channel of Santos Harbor

Salinity variation in estuaries depends on the balance between the discharge of freshwater and saline waters from the open ocean [59]. This salinity variation drives hydrodynamic processes and makes estuaries able to shelter unique species communities and populations, some of which may decline or disappear while others begin to develop due to these changes. In the present study, salinity presented a marked decrease from the mouth to the head of the estuary, with mean values between 24.58 ± 1.78 (9W) and 33.46 ± 0.30 (1W) showing the influence of marine waters at the entrance of the channel (Figure 4). In the Santos Estuarine System, fresh water is discharged from a complex drainage basin with several rivers (Piaçaguera, Cubatão, Quilombo and Mogi). Thus, less dense river waters tend to remain on the surface while denser marine waters remain at the bottom. Results revealed vertical stratification, especially in the stations closer to the head, where differences between surface and bottom reached five units at stations 8W and 9W. At the other stations, salinity differences between surface and bottom varied between one and three units. The present results corroborate the findings of a previous study [36], which also described haline stratifications in the most inner portion of Santos Channel with differences of three to six units between surface and bottom waters. Miranda et al. [60], found a weak vertical stratification of the water column, both in spring and at neap tides, in the Santos estuary, suggesting that the hydrodynamics and mixing processes of this estuarine system directly impact the water properties.

Regarding DO, the same horizontal gradient recorded for salinity was observed, with decreasing values toward the head of the estuary (Figure 4). DO concentrations were highest at the channel's entrance (1W), with a mean value of 5.72 ± 0.44 mg/L, influenced by the more oxygenated waters from Santos Bay. On its way towards the head, from station 4W, DO maintained mean concentrations below 5 mg/L. The lowest mean value of 2.76 ± 0.58 mg/L was registered at station 9W. The concentrations of DO tend to decrease from surface to bottom at every station. From stations 6W to 9W, concentrations of DO at the bottom were around 2 mg/L, close to hypoxic conditions. This is justified by the elevated consumption of DO in the process of organic matter degradation. Oxygen-depleted conditions in estuarine areas are often attributed to anthropogenic nutrient enrichment delivered by terrestrial-fluvial pathways, which elevates primary production and consequently increases the demand for DO [61]. Concentrations of DO in the present study corroborated the findings of Aguiar et al. [36], who also found low-oxygen waters in the Santos Channel.

A decreasing gradient of pH towards the head of the estuary was also observed (Figure 4), with surface values higher than the bottom ones. With low pH values, the influence of marine waters was observed at the outer stations 1W and 2W. A pH value of around 8.1 is typical for marine waters. The decrease of pH toward the head can be justified by the influence of fluvial waters as well as the degradation of organic matter, which lowers pH values. Estuarine acidification and carbonate chemistry result from biogeochemical processes. Apart from biogenic processes, CO_2-acidification can occur allochthonously through non-carbonate sources originating in freshwater and land ecosystems [62].

ORP is strongly influenced by DO, usually presenting values between 300 mV and 500 mV in well-oxygenated waters (DO > 5 mg/L), and this value is maintained if the waters are not under hypoxia (DO < 2 mg/L) or anoxia (DO ~ 0) [63]. In the Santos Channel, ORP presented positive values all along the water column in the sampled stations (Figure 4). However, ORP values were under 300 mV, certainly influenced by the low oxygenation of the water column in the estuary. Despite that, results suggest a satisfactory water circulation capacity, keeping the water in oxidizing conditions.

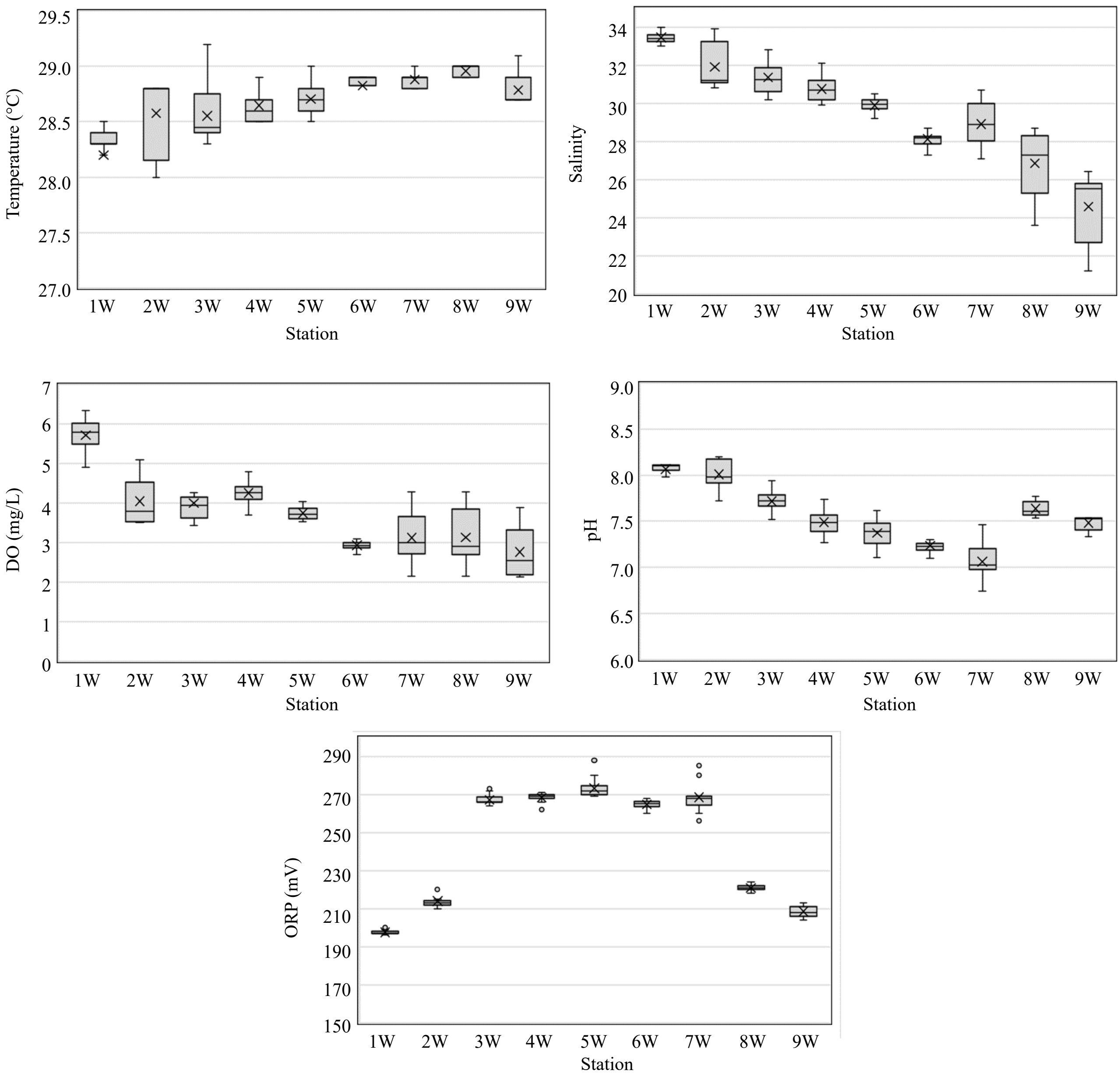

Figure 4. Basic statistics for physicochemical variables in the water column along the estuarine channel of Santos Harbor. Note: x = mean; -- = median; whisker = minimum and maximum; and box = quartiles

3.2 *Identification of benthic fauna*

The increase of invasive species in the surrounding areas of harbors and marinas is an environmental issue in coastal areas around the world, with loss of biodiversity as the main consequence. Most exotic species are able to avoid their natural predators, which would otherwise prevent them from expanding and thriving in their native habitats [34]. The presence of invasive species in the estuarine system of Santos has been documented in previous studies [34], which found the invasive fish species *Opsanus beta* in the study area. In Santos Bay, the mussel *Isognomon bicolor*, considered an invasive species, was found by Henriques et al. [64]. On the southern Brazilian coast, Melo et al. [65] found *Crassostrea gigas*.

Regarding the identified benthic fauna present on recruitment plates, a relatively low diversity of organisms was detected. The presence of 18 taxa distributed in six phyla, in addition to functional groups such as filamentous algae and invertebrate egg-laying, was found (Figure 5). This relatively low diversity of organisms recorded in the present study may be linked to the method used, since the occupation by benthic organisms is closely linked to the surface in the process of settlement [66].

Figure 5. Recruitment plates already colonized

Among the identified taxa, two species (*Xanthidae sp*. and *Symplegma rubra*) were detected only in the first studied period (April until July 2019), whereas six others were detected only in the second studied period (August until September 2019). Out of the total number of species found, six are considered exotic to the Brazilian coast, and two are cryptogenic; however, none of these occurrences is newly recorded in Brazil (Table 1).

It is worth mentioning the dominance of *Branchiomma luctuosum*, responsible for almost three-quarters of the total plate coverage in the first sampling period (April until July 2019) and just under half of the total plate coverage for the second sampling period (August until September 2019). This taxon was also responsible for most of the biovolume analyzed in both periods. Monospecific dominance may also be one of the factors explaining the low diversity of organisms sitting on the plates. In general, exotic organisms thrive especially well in polluted estuarine environments. It is noteworthy that the presence of any species of sun coral was not detected, including *Tubastraea coccinea* and *Tubastraea tagusensis*, which are already established in several locations along the Brazilian coast [67].

Among the exotic species, two of them (*Clavelina oblonga* and *Botrylloides giganteus*) were only detected in the second period (August until September) (Table 1). Ascidians are usually the dominant members of marine fouling communities in the subtidal zones of natural and artificial substrates. Their distribution is affected by physicochemical factors, such as temperature, salinity, light intensity and hydrodynamics. Alterations in physicochemical conditions due to natural or anthropogenic causes can affect the occurrence, diversity and distribution of ascidians [68]. According to da Rocha et al. [69], *C. oblonga* is restricted to the southeastern and southern cooler waters of Brazil, where it has been found on both natural and artificial substrates but never on boat hulls.

Table 1. Taxonomic identification, total percentage of coverage on the recruitment plates, and classification according to origin

Phylum	Class	Order	Family	Species	Percentage of coverage from April until July 2019 (%)	Percentage of coverage from August until September 2019 (%)	Origin
Annelida	Polychaeta	Sabellida	Sabellidae	*Branchiomma luctuosum*	73.4	42.4	E
	Polychaeta	Sabellida	Serpulidae	*Hydroides elegans*	3.3	4.7	E
Bryozoa	Gymnolaemata	Cheilostomatida	Bugulidae	*Bugula neritina*	1.2	2.3	E
	Stenolaemata	Cyclostomatida	Crisiidae	*Crisia pseudosolena*	6.8	9.1	N
	Gymnolaemata	Cheilostomatida	Bugulidae	*Crisularia guara*	0.0	2.6	N
Arthropoda	Maxillopoda	Sessilia	Balanidae	*Amphibalanus improvisus*	7.0	2.8	N
	Malacostraca	Decapoda	Potamidae	*Hexapanopeus paulensis*	0.9	0.3	N
	-	-	Xanthidae	-	0.2	0.0	-
Mollusca	Bivalvia	Ostreida	Ostreidae	*Crassostrea sp.*	4.4	1.8	-
		-	-	Clam egg-laying	0.0	5.0	-
Chordata	Ascidiacea	Stolidobranchia	Styelidae	*Styela plicata*	2.6	1.7	E
			Styelidae	*Symplegma rubra*	0.1	0.0	N
		Aplousobranchia	Didemnidae	*Trididemnum orbiculatum*	0.1	0.4	N
			Clavelinidae	*Clavelina oblonga*	0.0	1.4	E
			Polycitoridae	*Cystodytes dellechiajei*	0.0	1.4	C
		Phlebobranchia	Ascidiidae	*Phallusia nigra*	0.0	0.5	N
		Stolidobranchia	Styelidae	*Botrylloides giganteus*	0.0	5.6	E
		Aplousobranchia	Holozoidae	*Distaplia bermudensis*	0.0	6.3	C
Others	Prostista (Kingdom)	-	-	Filamentous algae	0.0	9.0	-

Note: N = native; E = exotic; and C = cryptogenic

Next, each of the exotic taxa found is presented, including information on their place of origin, ecology, and geographic distribution, among others.

3.2.1 *Hydroides elegans* (Haswell, 1883)

Despite the uncertainty surrounding its origin, *H. elegans* was first described in Australia and is considered an invasive species in many parts of the world [70]. This species produces limestone tubes and generally forms dense populations in artificial structures, such as pier piers and ship hulls [70]. It presents rapid sexual maturity, a short larval period [71], and the ability to rapidly colonize clean substrates [72]. Currently, it has a wide distribution and is considered introductory in the Adriatic, Aegean, Mediterranean, Japan, the North, the Caribbean, the Celtic Coast of the United Kingdom, New Zealand, the Hawaiian Islands, the Gulf of California, Guinea, and Mexico [73]. In Brazil, *H. elegans* was first registered on the southeast coast in the state of Rio de Janeiro, in Guanabara Bay [74].

3.2.2 *Branchiomma luctuosum* (Grube, 1870)

The sabellid polychaete *B. luctuosum* was first described in the Red Sea, where it was found attached to corals and sponges. It has since been introduced and is now well-established in the Mediterranean Sea [74]. It is usually found in shaded and low-hydrodynamic locations [74], such as harbors and other areas with high anthropogenic activity [75]. In harbor areas, it is commonly observed in rocky substrates forming aggregates of specimens. Although they do not have a preferred substrate angle for occurrence, particularly high densities are reached on vertical substrates, reaching up to 370 specimens/m^2, making them a species with high recruitment and expansion potential [75]. In fact, *B. luctuosum* seems to thrive in harbor structures, and dense populations of this invading sabellid have been reported in the Valencia harbor on Spain's Mediterranean coast [75]. It is primarily recognized for forming brownish membranous tubes that house the living part of the organism. The shape of the body is dorsally flat and ventrally convex. The branchial crown is usually violet or orange, with yellow transverse bands. The body reaches up to 12 cm long and 1.2 cm wide. The branchial crown reaches approximately 4.5 cm in length, containing 40 pairs of radioles distributed in a semicircle and fused to a short membrane that occupies approximately one-tenth the length of the crown. Seven ventral radioles are without stylods; the others present 25 to 40 pairs of filiform stylods, arranged in equidistant pairs in the radioles. There are two pairs of compound eyes, brown or red, in each pair of styles [76]. They are hermaphrodite organisms that shed their gametes into the water. Fertilization results in a lecithotrophic larva with a short pelagic development of three days before settlement [77]. In Brazil, *B. luctuosum* was first registered in 2003 on rocky shores in the state of São Paulo, on the southeast Brazilian coast [76].

3.2.3 *Bugula neritina* (Linnaeus, 1758)

Attached to any type of hard substrate, the *B. neritina* bryozoan typically forms colonies in harbors and heaps to a depth of approximately 5 m [78]. Its place of origin is unknown; however, it is established in several coastal areas of the planet and is considered a successful invasive species. It is present in the Mediterranean Sea as well as the Atlantic, Pacific, and Indian oceans. The species is tolerant of high levels of pollution when its potential for establishment tends to be greater, and this feature has already been described as a competitive advantage [79]. It can colonize a variety of artificial substrates, including harbor structures and ship hulls [80]. It is found in euryhaline and polyhaline environments, with salinities between 18 and 30 [81]. The colonies of this bryozoan are flexible, measure up to 10 cm, and are formed by a biserial branch with a brownish appearance when alive. Its zooids are white and globular [82]. Zooids are large, measuring an average of 0.97 mm x 0.28 mm, and differ from other species of the genus in the presence of avicularia and the absence of thorns. The lophophore measures an average of 0.76 mm in diameter [81]. The group has a lecithotrophic larva that can settle and undergo metamorphosis in a few days. In Brazil, its presence was reported in Espírito Santo [1], Rio de Janeiro and São Paulo [83], all located on the southeast coast.

3.2.4 *Styela plicata* (Lesueur, 1823)

Styela plicata is a solitary ascidian found in shallow and protected environments in tropical and warm temperate regions. It has a wide distribution around the world, and its place of origin is uncertain [84]. However, de Barros et al. [85] describe the Pacific Ocean as its potential source. The species was first found adhered to a ship's hull in the USA, and currently there are several records of its occurrence in the vessel's live work in parts of the world [85]. Its current distribution includes several areas of the Pacific and Atlantic oceans, in addition to the Mediterranean Sea [86]. The species thrives in sheltered and polluted waters and is often found in estuarine environments [87]. It is found in different types of substrates, particularly artificial ones. The body shape of the specimens is ovular, with a color ranging from grayish to white and adult specimens ranging from 40 mm to 70 mm. It has a siphon for conducting water into the body, where the food particles are filtered, in addition to an exhaling siphon. The siphons have red or purple lines on the inside and four lobes. It is a eurythermal tunicate capable of tolerating great thermal variation (10 °C to 30 °C) and salinities between 22 and 34 [88]. On the Brazilian coast, it has been discovered in Rio de Janeiro and São Paulo, both on the southeast coast, as well as in the south, along the Paraná and Santa Catarina coastlines [85, 89].

3.2.5 *Clavelina oblonga* (Herdman, 1880)

The colonial ascidium *C. oblonga* is a well-known species from the tropical waters of the Atlantic. It was first described in Bermuda, and its distribution includes the USA (South Carolina and Florida) and the Caribbean Sea [90]. It is considered introduced in areas of the eastern Atlantic such as the Azores and Senegal. The species can be found on different types of substrates, particularly artificial ones. The colony's body shape varies with its developmental stage and may even form a dense globular colony up to 15 cm in diameter [91]. Individuals have an elongated cylindrical shape without coloring and are commonly transparent. In Brazil, it was first described in the 1940s as an "old introduction" [92] and currently has a known occurrence from the north coast of São Paulo (the southeast Brazilian coast) to Ilha do Arvoredo in Santa Catarina (the south Brazilian coast) [70].

3.2.6 *Botrylloides giganteus* (Lesueur, 1823)

This species of ascidia was first described in Senegal in 1941 [93], but its native distribution is unknown. It has now been found in several coastal regions of the world, having invaded most of the current areas of occurrence in the last 20 years [94]. This species forms fouling colonies with thicknesses that range from 0.5 cm to 1.5 cm. Colors range from orange to red to violet to brown to dark brown. The colonies can present an organization of the zooids in meandering lines covering the substrate. In Brazil, the occurrence of the species is not new, having been reported for the first time in the 1980s [95].

3.3 *Statistical analysis*

The spatial distribution evaluation through the Canonical Correspondence Analysis considered the coverage percentages of the different organisms, the different environmental variables measured, and the depth and distance from the sampling point to the sea. It was able to distinguish and explain the few patterns of organisms' distribution (Figure 6). This is evidenced by the low eigenvalues found (Axis 1 = 0.036 and Axis 2 = 0.032).

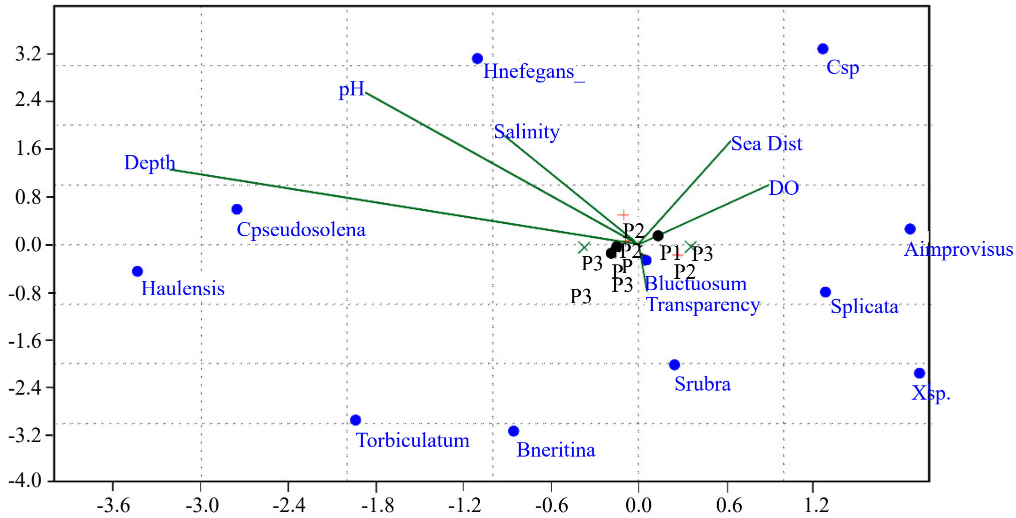

Figure 6. Graphical representation of the result of the canonical correspondence analysis considering the coverage of the different taxa and the measured environmental variables, in addition to the depth and distance in relation to the sea. Eigenvalues are 0.036 for axis 1 and 0.032 for axis 2, with an explanation percentage of 43.29% and 39.04%, respectively

Crisia pseudosolena and *Hexapanopeus paulensis* showed a trend toward greater representativeness with increasing depth. *H. elegans* was positively related to pH and higher salinities, factors related to a greater influence of marine water. *Crasostrea sp*. showed that its representativeness is related to the distance from the sea, consistent with its typical occurrence in estuarine environments. Despite the few spatial patterns found and the relationship with physicochemical variables, this may be due to the specific nature of the variables used in the analysis, measured only during the recovery of recruitment plates. Due to the relatively long period of permanence of the recruitment plates in the stations and the presence of fast-growing organisms in a eutrophic environment, a large biovolume of organisms was found, especially in the first monitoring period. These formed a layer of approximately 2 cm or 3 cm, predominantly composed of *B. luctuosum*. According to El Haddad et al. [75], this species has a high potential for recruitment and expansion. The introduction of non-indigenous species can be favored in places where the local biota is under stress caused by anthropogenic activities, opening vacant niches for colonization. The study area has long been the stage for anthropogenic actions, mainly of industrial origin, due to the presence of industrial poles in its surroundings and port activities. Santos Harbor channel has been characterized as highly eutrophic, with detection of hypoxia towards the head of the estuary, high turbidity waters, and large contents of organic matter [36, 40]. de Matos Nogueira et al. [76], who first detected *B. luctuosum* in Santos Bay in 2004, highlighted the anthropogenic impact of Santos Harbor channel as a favoring condition for the development of invasive species in this estuary with dense aggregations of sabellids on its rocky shores, dominated by *B. luctuosum*.

Regarding the biovolume distribution pattern, it did not show statistically significant differences between the different monitoring points (ratio between variances [F] = 3.816; degrees of freedom [df] = 2; p-value [p] = 0.085) or between the monitored depths (F = 0.097; df = 2; p = 0.909). However, it was possible to observe a gradient pattern in the biovolume averages between the monitoring points, tending to be greater at the proximity of the sea (Figure 7), where higher salinities, DO and pH were registered, as well as a higher euphotic zone, favoring primary production (Figures 3 and 4). On the other hand, the lowest biovolume was observed in the inner estuary, with darker waters, lower salinity, lower pH, and very low concentrations of DO almost reaching hypoxia conditions (Figure 4).

A similar pattern was not observed for the biovolume found at different depths (Figure 8). Biovolume for the first campaign was also higher (Figure 9), probably a reflection of the longer exposure time of the recruitment plates.

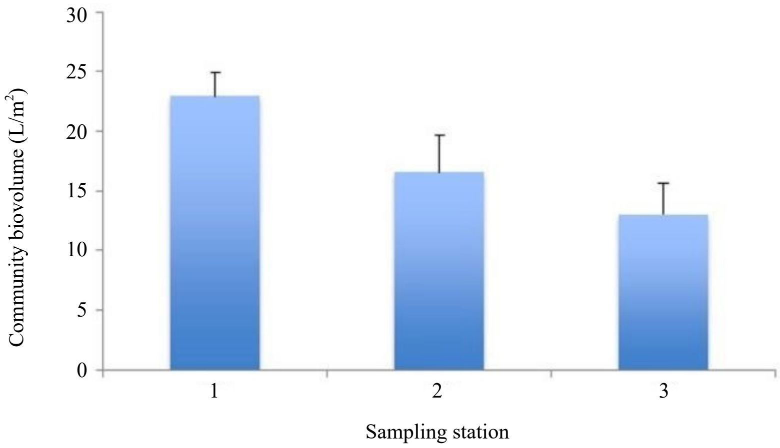

Figure 7. Biovolume averages of organisms found on recruitment plates at the different points monitored, including both sampling periods

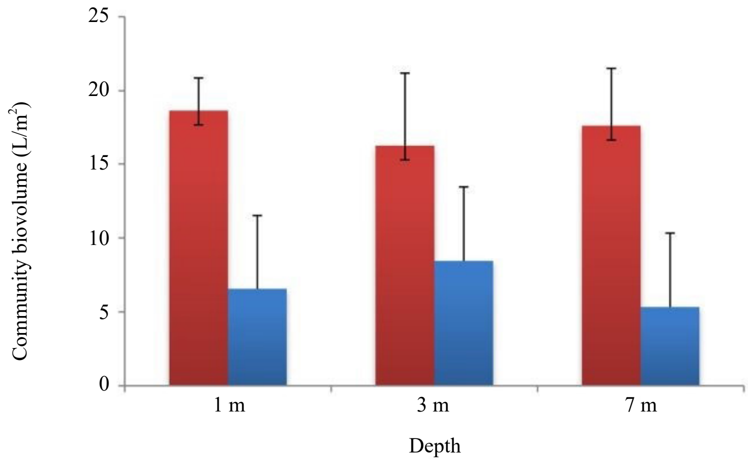

Figure 8. Biovolume averages of organisms found in the recruitment plates at the different depths monitored in the two sampling periods. Note: red bars = Survey 1 (April to July 2019); and blue bars = Survey 2 (August to September 2019)

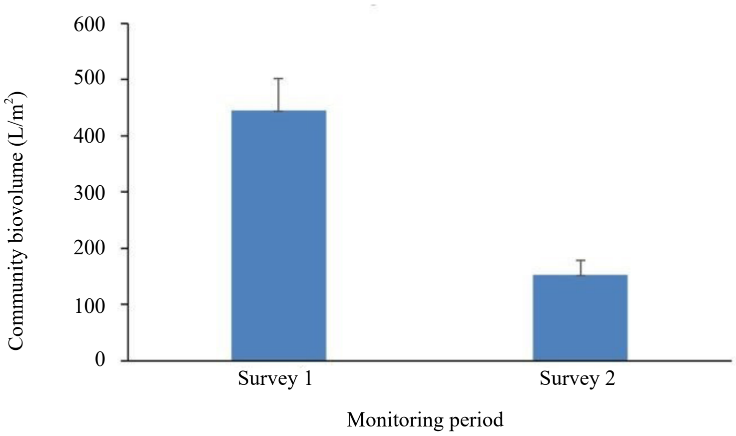

Figure 9. Biovolume averages of organisms found in recruitment plates, considering the monitoring points P-01 and P-02, in the two sampling periods. Note: Survey 1 is from April to July 2019 and Survey 2 is from August to September 2019

4. Conclusions

The method used for the detection of exotic species was effective. From the 17 taxa identified in recruitment plates, six were considered exotic in Brazil: *H. elegans, B. luctuosum, Bugulaneritina, Styelaplicata, C. oblonga* and *B. giganteus*. Despite being exotic to the Brazilian coast, all the exotic species encountered had been found before in different locations in Brazil. The results, however, presented low diversity due to monospecific dominance by *B. luctuosum*, which was responsible for 73.4% of plate coverage in the first campaign and 42.4% in the second, revealing the adaptation of this species to environments impacted by anthropogenic disturbance, as is the case of the Santos Estuarine Channel. No statistical differences were observed concerning biovolume with regards to sampling stations or depths. However, a very clear pattern of decreasing biovolume towards the head of the estuary was observed, probably related to the more extreme conditions of the water column in the inner stations, with lower salinity and oxygen levels. Data produced by this study may serve as a base for future mitigation programs to control bioinvasion in the Santos Harbor area since this is an issue that needs further understanding in terms of its ecological and financial costs to the country.

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Conflict of interest

The authors declare no conflict of interest for this study.

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