Ocean and Coastal Research

Original Article
Special Article Collection
Di Domenico, M.; Andriguetto-Filho, J.M. and Lopes, R.M. (Eds)
An ocean of science, worms, and humaneness:
a tribute to Paulo Lana
http://doi.org/10.1590/2675-2824072.23088
ISSN 2675-2824

Navigation buoys as stepping-stones for invasive species

Rafael Metri[1]*, Cassiana Baptista-Metri[1], Yara Aparecida Garcia Tavares[1],
Mariana Baptista Lacerda[2], Elliezer Lima Correia[3], Gésica da Costa Bernardo Soares[4],
Pablo Damian Borges Guilherme[1]

[1] Programa de Pós-graduação em Ciências Ambientais – Ambientes Litorâneos e Insulares – Universidade Estadual do Paraná (Paranaguá – 83203-560 – Paraná – Brazil).
[2] Universidade Federal do Paraná (Pontal do Paraná – 83255-976 – Paraná – Brazil).
[3] Cia Ambiental (Curitiba – 80530-100 – Paraná – Brazil).
[4] Programa de Pós-graduação em Zoologia – Universidade Federal do Paraná (Curitiba – 81531-980 – Paraná – Brazil).

* Corresponding author: rafael.metri@unespar.edu.br

Abstract

The movement of ships between continents is one of the leading causes of the increased bioinvasion of benthic organisms, surpassing the geographical barriers that prevent the natural dispersal of species. Artificial floating structures, such as buoys, can also serve as secondary dispersers in the introduction of exotic species on a regional scale. This study describes the fauna associated with buoys demarcating the access channel to Paraná's ports, focusing on recording introduced invasive species. Biota samples were collected from 23 buoys from different estuary sectors and some on their fastening chains, resulting in 73 samples. A total of 88 taxa were identified, of which half were sessile organisms and half, vagile. All observed taxa were previously recorded in the region, but introduced species clearly predominate among the sessile organisms colonizing the buoys, representing over half of the taxa determined at the species level. Buoys showed differentiated communities depending on their estuary location, with introduced species accounting for a larger share of the fauna on buoys in more saline sectors, reflecting their adaptations to oceanographic factors such as marine influence and tidal-driven water exchanges or an ecophysiological barrier caused by lower salinities in the inner estuary. Considering the negative effects of non-native species in natural environments and economic activity, the facilitating action of artificial floating substrates like signaling buoys highlights the need for environmental monitoring programs and more frequent maintenance of these structures to help control bioinvasions.

Keywords: Artificial consolidated substrate, Benthic communities, Bioinvasion, Port

INTRODUCTION

The introduction of exotic species into different ecosystems is considered one of the leading causes of biodiversity loss (Carlton, 1989; Ojaveer et al., 2018). Bioinvasion can harm native species and damage the functioning of ecosystems, as well as produce economic losses (Cohen and Carlton, 1998). The movement of ships between continents is undoubtedly one of the main causes of the increase in bioinvasion processes, overcoming the geographical barriers that prevent the natural dispersal of species (Silva et al., 2004; Sardain et al., 2019). On ships, organisms can be transported in ballast water in the adult and larva/propagule

Submitted: 14-Jun-2023
Approved: 21-Apr-2024

Editor: Rubens Lopes

stage, which are introduced during deballasting at the destination port. They can also adhere to the hull while the ship is docked and then reproduce upon arrival at another port (Lopes, 2009; Afonso et al., 2020).

In addition to ships, a variety of objects installed in marine environments can function as habitats in the form of artificial surfaces, such as piers, marinas and renewable energy infrastructure (Rocha et al., 2010; Miller et al., 2013). Navigation signaling buoys are one of these artificial substrates often present in port regions. As floating objects, there is almost no surface to attach to in the intertidal zone, most of which is truly submerged and offers favorable conditions for organisms (Astudillo et al., 2009; Rocha et al., 2010).

The bioinvasion process is effective only when the organisms find favorable conditions and resources to become established in the new location. For species of consolidated substrates, the presence of suitable substrates is one of the requirements for their fixation or refuge. In this regard, artificial substrates often favor the establishment of exotic fauna (Coutts and Forrest, 2007; Cangussu et al., 2010, Sheehy and Vik, 2010). This is even more evident in urbanized areas, which are subject to more frequent arrivals of invaders (Oricchio, 2019) and other stressors that can benefit non-native species and even promote gene flow between previously unconnected areas (Alter et al., 2020). Arrival and occupation of invasive species near natural outcrops can occur through trampolines (Tyrrell and Byers, 2007; Fowler et al., 2020), which arise from the presence of adjacent artificial substrates along urbanized coastlines. Artificial floating structures such as buoys can therefore serve as secondary dispersers of these species on a regional scale (Afonso et al., 2020; Soares et al., 2020). Detached floating buoys can disseminate organisms over great distances, which also helps non-indigenous species spread (Astudillo et al., 2009). Nonetheless, access to these biological communities is quite restricted since the Brazilian Navy prohibits the manipulation of buoys to ensure navigation safety.

In addition to this potential for facilitating the establishment and dispersal of introduced species, buoys could be good biota samplers over time and throughout the estuary. Located from the innermost estuary portions to the marine sector in the open sea, buoys can be excellent monitoring points, and this study is the first to record this part of the biota.

The Paranaguá port (Dom Pedro II port) is located in a large estuary in southern Brazil and receives many ships from various parts of the world every day, making this region particularly vulnerable to bioinvasions. Several studies have noted the possibility of bioinvasions in the region and the need to monitor these events (Neves et al., 2007, Bumbeer and Rocha, 2012, Altvater et al., 2019). Dozens of maritime signaling buoys were placed in the estuary to mark the channel, indicate obstacles, and guide navigation along the access channel to the port. Since they are rigid and stable, these buoys provide a suitable substrate for the establishment of organisms (Floerl and Inglis, 2003). Additionally, they can be left for several years without maintenance or replacement, thus maintaining a well-developed fouling community and potentially promoting the colonization and dispersal of introduced species.

This study describes the fauna associated with buoys that demarcate the access channel to the Paraná ports, focusing on recording introduced fouling species, and evaluates the importance of buoys as stepping-stones for sustaining dispersal events and expansion of non-native species.

METHODS

STUDY AREA

Samples were collected from the signaling buoys in the access channel to the Paranaguá port, along the entrance and interior of the Paranaguá Estuary Complex (*Complexo Estuarino de Paranaguá* – CEP) (Figure 1). CEP is a large estuarine system with nearly 456 km^2 of waterline, extending along two main axes. On the east-west axis is a channel for access to the ports of Paranaguá and Antonina called the Galheta Channel. The waters on this route vary considerably in salinity, ranging between 12-29 psu in summer and 20-34 psu in winter, and surface temperatures of 23-30°C in summer and 18-25° C in winter (Lana et al., 2001). These oceanographic characteristics are reflected in the biotic communities of the muddy sandy bottoms: the estuary can be divided into a mesohaline sector (the furthest inland and least saline due to the greater influence of continental waters), a polyhaline sector (intermediate portion), and a euhaline sector (closer to the estuary mouth), in addition to the external oceanic sector (Lana et al., 2001). Rocky islands and coasts permeate the entire estuary, although tidal plains, mangroves, and marshes make up the greatest extent of the shores. The main artificial consolidated substrates consist of docks, ramps, and decks in the port and marina areas as well as signaling buoys. The port authority carries out biota monitoring programs on some consolidated substrates in the port's area of influence, but none focusing on non-native species or on the navigation channel buoys.

Field Procedures

In July 2011, the buoys that signal the access channel to the ports of Paranaguá and Antonina (Figure 1) were removed from the sea by the port authority and replaced thus enabling sample collection. These buoys had been on site for at least a decade, serving as substrates for benthic organisms. For safety reasons, the buoys were sampled immediately after tugboat arrival at the port pier (Figure 2), where areas of approximately 225 cm² were scrapped with spatulas. Samples about the same size were also collected from some of the fastening chains. Most of the 23 buoys removed had three samples taken, one near the waterline, one at the bottom (~1.5 m deep) and one in the middle (~1 m deep) of the buoy, grouped together to represent the biota of each buoy in each estuarine sector. Samples were taken from two buoys in the mesohaline sector (seven samples), seven in the polyhaline sector (25 samples), seven in the euhaline sector (18 samples) and seven in the oceanic sector (23 samples). The collected organisms were packed in plastic bags labeled with the collection date and number of each buoy, anesthetized with menthol, and preserved in 10% formalin.

Laboratory Procedures

In the laboratory, all samples were analyzed under a stereoscopic microscope, and the taxa were determined to the lowest possible taxonomic level. Vagile organisms such as crabs, amphipods, and polychaetes were identified in the samples; however, they were considered subsampled as the buoys were towed to the dock alongside the vessels, allowing the vagile animals to escape. Thus, the analyses assess primarily the sessile organisms, although the observed vagile species are also listed.

Data analysis

Initially, a rarefaction analysis of sessile species was performed using data from the 73 samples analyzed to evaluate whether the expected biodiversity had been sufficiently represented. Replicate data of each buoy were then merged and spatialized, pooled with chain samples for each buoy when available, allowing the description and comparison of the communities of each estuary sector: marine or oceanic, euhaline, polyhaline, and mesohaline (Lana et al., 2001). The polyhaline sector was represented by buoys located in the vicinity of the Paranaguá port. Taxa constancy was then computed by calculating the percentage of buoys with a given taxon to point out the most representative species. To characterize the faunal associations in each sector, the overall species richness and that of introduced species were compared between buoys from the different estuary sectors and evaluated in relation to their distance from the Paranaguá port and the estuary mouth. Species categorized as being introduced to the region were highlighted in the list.

To better understand the differences in species composition between the estuary sectors the components of diversity in each sector were calculated. The α-diversity was calculated from the average of observed sessile taxa in all buoys by saline sector and the γ-diversity as all observed sessile taxa using the *vegan* package (Osaken et al., 2013). The β-diversity index and its partitions, turnover (βSIM), and nesting (βNES) were calculated as proposed by Baselga (2010b, 2012), using the Sørensen dissimilarity (βSOR) with the *betapart* package (Baselga et al., 2020).

Differences in composition between the samples collected from buoys were visualized through non-metric multidimensional scaling (nMDS) ordinations using Raup-Creck dissimilarity matrices. Envfit analysis (*vegan*) determined whether distance from the estuarine mouth or port was correlated with samples' separation in the nMDS space. The ANOSIM test (similarity analysis) assessed the difference between the four groups of buoys according to their location in different estuary sectors.

A permutational multivariate analysis of variance ("adonis" function in *vegan*) tested for significant differences between groups of samples. A permuted distance-based test for the homogeneity of multivariate dispersion ("PERMDISP2" function in *vegan*) tested for significant differences in the variance between sample groupings (Osaken et al., 2013).

The univariate analyses were tested with generalized linear models (GLM) of different families depending on data distribution to test whether different saline sectors of the estuary support a greater or lesser proportion of non-native in relation to the total number of species. Plots were created in the *ggplot2* (Wickham, 2016) and *ggord* (Beck, 2020) packages. All analyses and graphs were coded in R language (R Core Team, 2020), version 4.0.2 (2020-06-22).

RESULTS

Seventy-three samples were analyzed from 23 buoys along the estuary. A total of 88 taxa were observed, of which half were sessile (n = 44) and half were vagile (n = 44). In each subgroup, 25 and 23 taxa were identified up to the species level, respectively. The rarefaction curve of sessile species (Figure 3), which approaches stability, shows that the effort employed seems sufficient to adequately record most of the expected richness in this group.

Representatives of several animal groups were found among the 44 sessile taxa, such as Cirripedia, Bivalvia, Bryozoa, Hydrozoa, Ascidiacea, Anthozoa, and Porifera. The vagile taxa primarily belonged to the Brachyura, Amphipoda, and Polychaeta groups (Supplementary Material). Of all the 25 sessile species identified, 13 (52%) are considered introduced in the south west Atlantic (Table 1), and 11 are cryptogenic—species that cannot be classified as native or introduced because they have a wide distribution and their origin site is unknown—and only one species (4%) is native (Table 1). In this study, we define invasive as organisms that have been introduced to regions outside their normal distribution area, either intentionally or accidentally, and then establish, reproduce, and efficiently disperse to new areas (Blackburn et al., 2011; Dechoum et al., 2024). All introduced species can be considered invasive in the area, as they maintain self-sustaining populations, surviving and reproducing throughout the region. Table 1 presents a categorization of invasion status according to the criteria of Blackburn et al. (2011) and some remarks about the populations based on the authors' observations in various surveys in the region. Among the 23 determined vagile taxa, 11 are native and 12 are cryptogenic.

As mentioned, we were unable to perform detailed analyses of the vagile fauna. Nonetheless, some observations of the vagile biota are presented to help catalog the estuarine biodiversity. The observed amphipod assembly shifted along the salinity gradient of the estuary, with greater species richness in the marine sector. Some species were found exclusively in the most extreme sectors: *Apolochus neapolitanus* in the innermost sector, showing its adaptation to lower salinity levels, while *Ampithoe ramondi*, *Caprella penantis*, and *C. scaura* had the opposite pattern and were only present in the marine sector, exhibiting low tolerance to large salinity ranges. *Monocorophium cf. acherusicum*, *Stenothoe valida*, *C. equilibra*, *Elasmopus pectenicrus*, *Jassa* sp., *Podocerus brasiliensis*, and *Quadrimaera* sp. occurred in all sectors. The cryptogenic and possible invasive *M. acherusicum* (Neves et al., 2007; Desiderato, 2020) was abundant in some samples. Nine species of crabs were observed (from? Brachyura and Anomura), which also appeared to have a different occurrence across sectors, with lower richness toward the estuary's interior, as only two species were found in the innermost buoys, *Mennipe nodifrons* and *Petrolisthes armatus*.

The α-diversity varied between sectors, with an average of 15.7 taxa per buoy in the marine sector, 12.8 in the polyhaline sector, 10.5 in the mesohaline sector, and 9.7 in the euhaline sector. A similar pattern was observed for the γ-diversity, with 27 taxa in the marine sector, 24 in the polyhaline, 17 in the euhaline, and 16 in the mesohaline. Results of the β-diversity index (βSOR) and its partitions, turnover (βSIM), and nesting (βNES) (Figure 4) show that the turnover was higher among the buoys of the euhaline, polyhaline, and marine sectors, with a greater relative contribution to the formation of βSOR in all the buoys analyzed except those of the mesohaline sector, where nesting had a greater contribution.

Of the sessile taxa, Bivalvia had 100% constancy, appearing in all samples in the 25 buoys analyzed, followed by Cirripedia with 92.3%. Ascidiacea, Anthozoa, and Bryozoa had between 65.4% and 73.1% constancy. Hydrozoa and Porifera were the least commonly found on the buoys. Bryozoans, hydrozoans, ascidians, anemones, and sponges, while considered common in the samples, had lower constancy compared with bivalves and cirripedes.

In the most constant group (Bivalvia), oysters appeared on 84.6% of the buoys, and the bivalve *Perna perna* had a very representative constancy, appearing on more than 80% of the buoys. Notably, the introduced bivalve *Isognomon bicolor* was present on about 40% of the buoys. The second most frequent group was Cirripedia, with nine species observed. Of these, six are considered introduced in the region. Figure 5 shows all the most constant taxa in the samples, with values greater than 40%.

The nMDS analysis of the sessile fauna illustrates the spatial distribution (PERMANOVA: $R^2 = 0.3957$; $p < 0.001$) of the species along the salinity gradient, as confirmed by “*envfit*” ($p < 5$), with the most extreme sectors showing clearer separations in the fauna assemblies (Figure 6), with typical communities of the marine sector and the euhaline and polyhaline sector of the estuary (ANOSIM, global index $R = 0.3658$; $p < 0.001$). However, we observed no clear separation for the fauna of the mesohaline sector, probably due to the low number of samples ($n = 2$). The pairwise comparison analysis of similarity only indicated significant differences when comparing the fauna of the marine and polyhaline sectors ($R^2 = 0.9875$; $p < 0.01$).

The ratio of introduced species and the total observed taxa differed between the buoys of different saline sectors (GLM: $F_3 = 6.36$; $p = 0.005$). Buoys of the mesohaline sector presented an average ratio of 0.12 (± 0.17), distant from the values of the other sectors were the introduced/total ratio was: marine sector, 0.48 ± 0.1; polyhaline sector, 0.4 ± 0.04; euhaline sector, 0.34 ± 0.15.

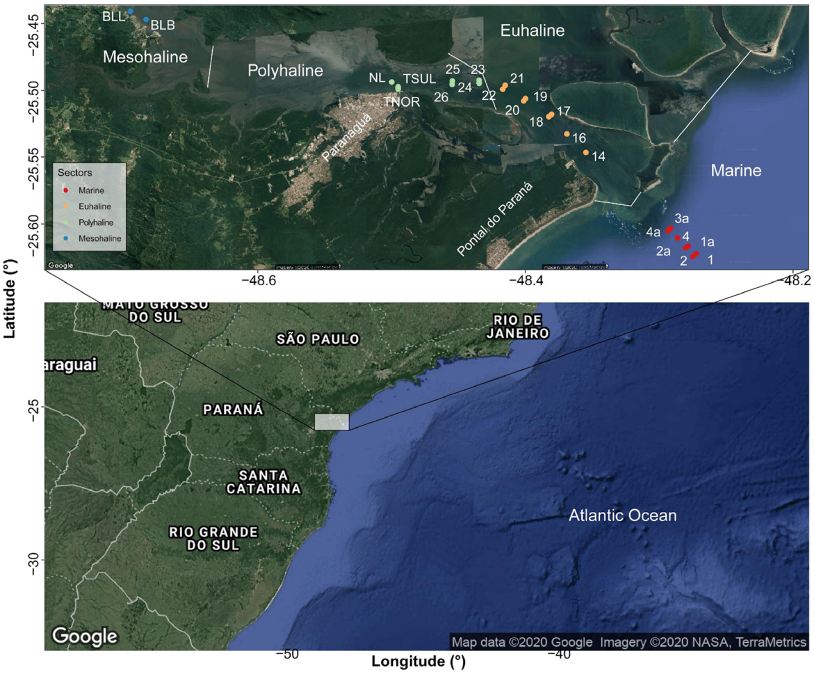

Figure 1. Paranaguá Estuary Complex, Southern Brazil. Location of the analyzed buoys from the access channel to the Paraná ports is indicated by the official acronym/number used by the Navy (numbers and/or letters to designate each buoy) and different colors for each saline sector (Google, 2020).

Figure 2. Some of the buoys sampled during the study and a snapshot of the scraping process.

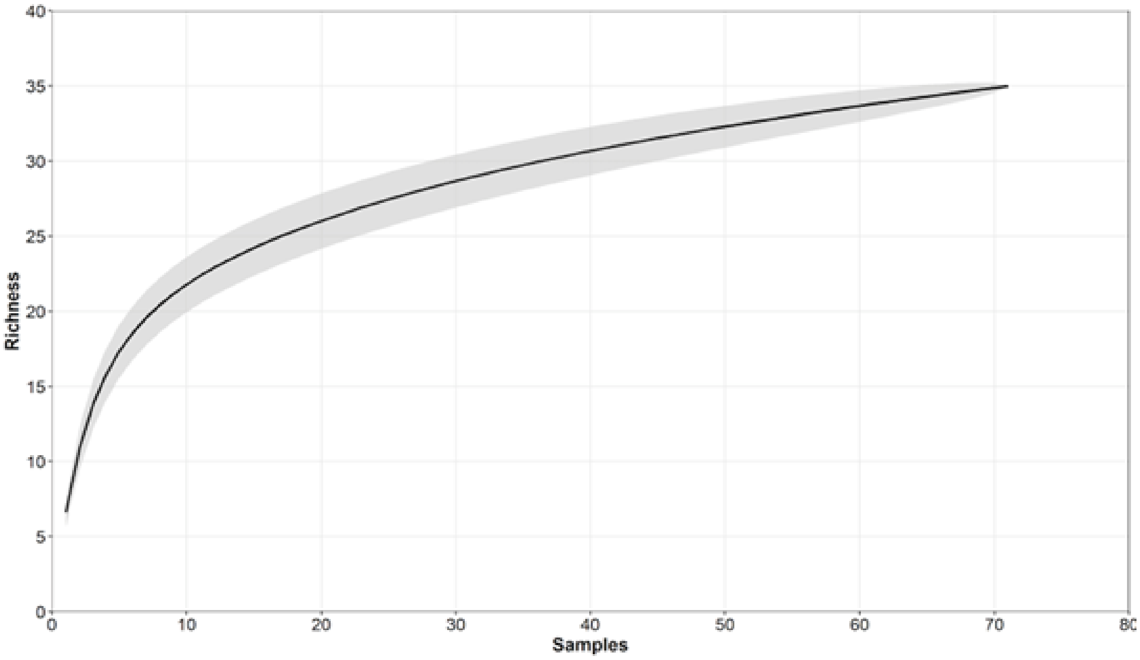

Figure 3. Species rarefaction curve, indicating the number of new occurrences per buoy sampled.

Table 1. Introduced species and invasion categories according to Blackburn et al. (2011) criteria. D2 and E are categories used for invasive species. D2 describes species with “self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction” and E is used for “fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence.“

Species	Sources	Category	Comments on the local occurrence
Amphibalanus amphitrite	Neves et al. (2007); Carlton et al. (2011)	E	Very frequent and abundant, on natural and artificial substrates
Amphibalanus eburneus	Carlton et al. (2011); Arruda (2014)	E	Very common, sometimes abundant, in natural and artificial substrates
Amphibalanus reticulatus	Neves et al. (2007); Carlton et al. (2011)	E	Very frequent and abundant, on natural and artificial substrates
Balanus trigonus	Cangussu et al. (2010); Bumber, Rocha (2012)	D2	Infrequent and non-abundant, on natural and artificial substrates
Megabalanus coccopoma	Cangussu et al. (2010); Bumber, Rocha (2012)	E	Very frequent and abundant, on natural and artificial substrates
Striatubalanus amaryllis	Neves et al. (2007); Carlton et al. (2011)	E	Very frequent and abundant, on natural and artificial substrates
Bugula neritina	Miranda et al. (2018)	E	Very frequent and abundant, on natural and artificial substrates
Hippoporina indica	Miranda et al. (2018)	E	Very frequent and abundant, on natural and artificial substrates
Sinoflustra annae	Miranda et al. (2018)	E	Very frequent and abundant, on natural and artificial substrates
Microcosmus exasperatus	Rocha, Kremer (2005); Metri et al. (2019)	E	Very frequent and abundant, on natural and artificial substrates
Podocoryna loyola	Haddad et al. (2014)	D2	Frequent, sometimes abundant, on natural and especially artificial substrates
Isognomon bicolor	Santos et al. (2005); Lopes (2009)	E	Very common, sometimes abundant, in natural and artificial substrates
Perna perna	Souza et al. (2003); Lopes (2009)	E	Very common, sometimes abundant, in natural and artificial substrates

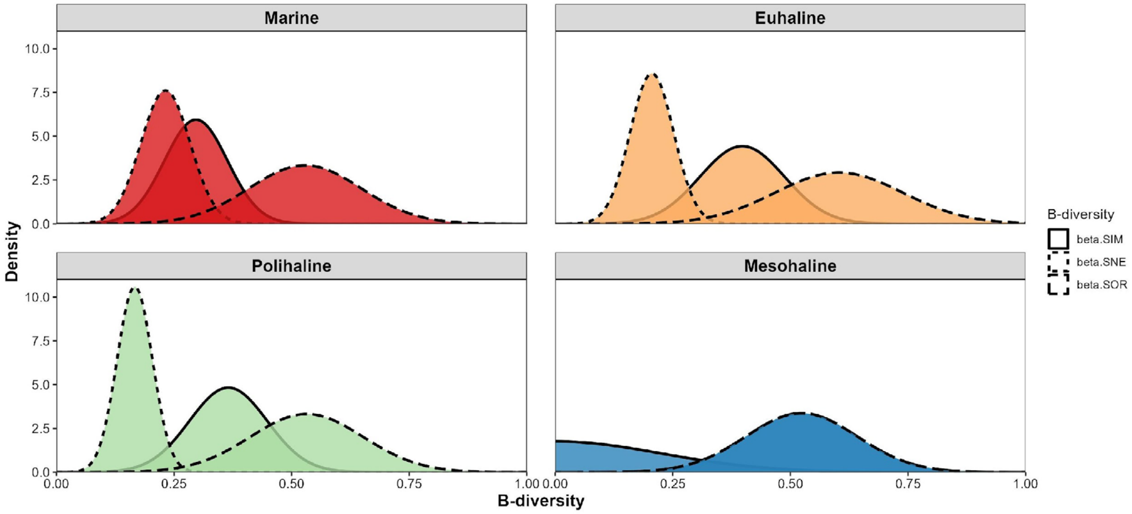

Figure 4. β -diversity index (βSOR) and its turnover (βSIM) and nesting fractions (βNES) categorized by the saline sectors of the Paranaguá Estuarine Complex.

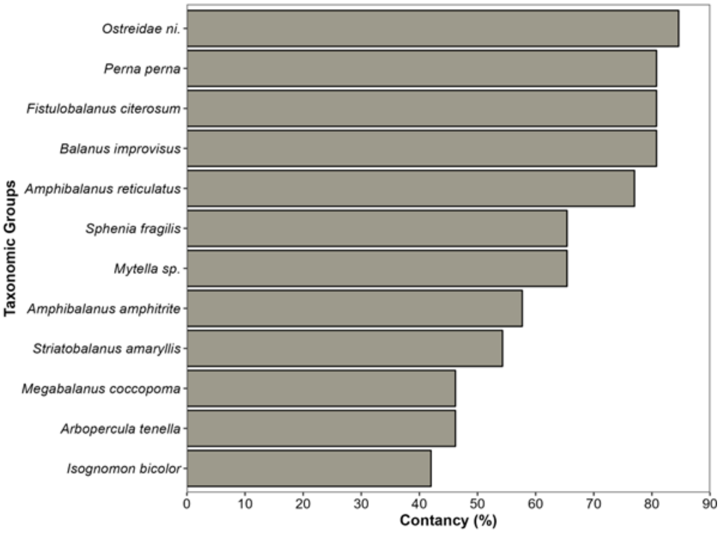

Figure 5. Most constant sessile taxa in the 25 buoys analyzed.

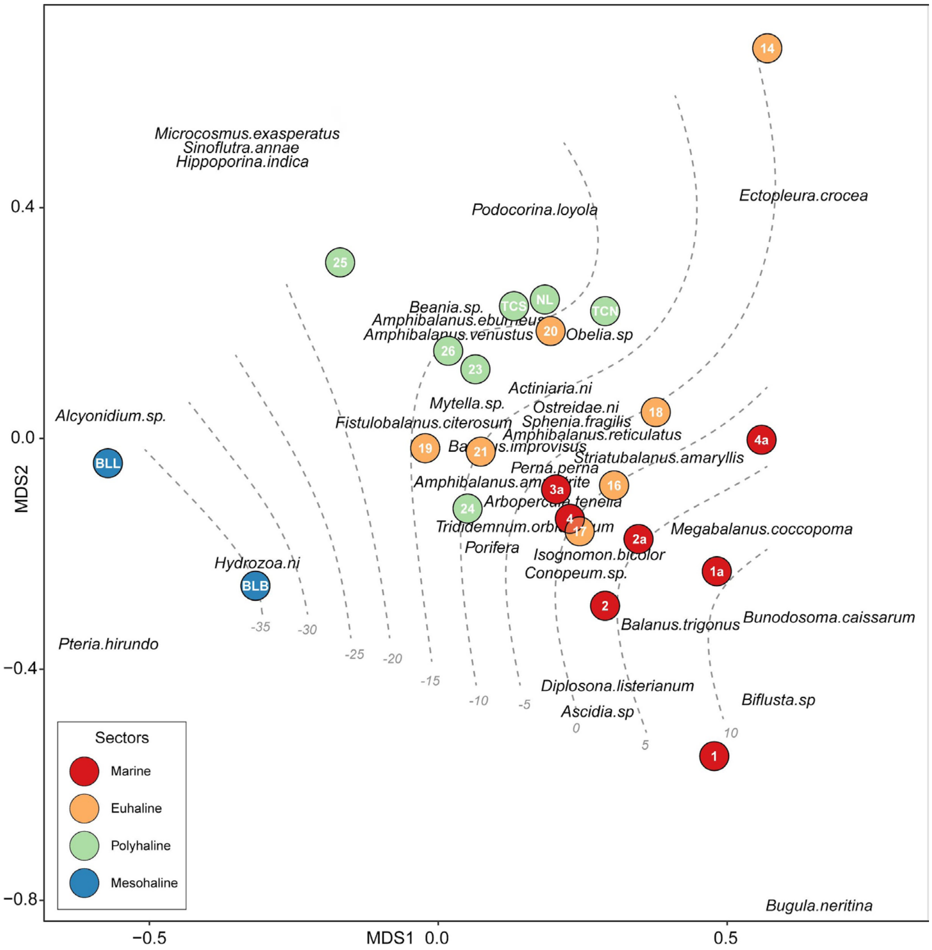

Figure 6. nMDS (0.15 stress) showing the similarity of the sessile community between the buoys of the four saline sectors of the Paranaguá Estuarine Complex. The dotted lines represent the distance (km) of the buoys from the estuary mouth. Supplementary Material. List of taxa identified on buoys and their invasion status. N = native, E = exotic, C = cryptogenic, HI = historical introduction and ni = non identified.

DISCUSSION

By describing the buoys' biota, we confirmed the estuary sectorization reflected in the faunal associations along its east-west axis, for both vagile and encrusting species. We also confirmed the clear predominance of exotic species in the colonization of the artificial structures analyzed in the case of encrusting species, representing more than half of the taxa identified at the species level. The exotic species predominate even in the buoys furthest from the port, outside the estuary, showing that the exotic fauna uses these substrates to disperse. All observed taxa have been previously recorded in the region, and most sessile taxa had their invasion status defined based on previous conducted done in the region (Neves et al., 2007; Cangussu et al., 2010; Bumbeer and Rocha, 2012; Miranda et al., 2018). In this study, we define invasive exotic species as organisms that settle, produce offspring, and disperse to new areas where they establish populations (well-established) (Blackburn et al., 2011; Dechoum et al., 2024). According to the unified framework criteria proposed by Blackburn et al. (2011) combining previous stage-based and barrier models, all our introduced species have overcome barriers (geographical, survival, reproduction, dispersal and environmental) and can thus be treated as invasive.

According to Felippe (2016), the main ecological barrier to establishing introduced species is competition with native species. Procopiak et al. (2006), Tyrrell and Byers (2007), and Mangelli and Creed (2012) discuss the high abundance of exotic faunal groups observed in artificial substrates as an important aspect of bioinvasion. Notably, native species preferentially select natural local substrates and are thus not effective competitors on artificial substrates. Tyrrell and Byers (2007) postulate that when native and invasive species are present in similar numbers on natural substrates, the native community will collapse. Although these buoys also serve as a substrate for native species, those introduced may have fewer problems with predators, parasites, and diseases in the new settlement regions and may thus direct their resources exclusively towards growth and reproduction. This allows them to compete more effectively with native species, reducing or even eliminating them (Bax et al., 2001; Xavier et al., 2023). This reinforces the role of buoys in maintaining an essentially exotic fauna, functioning as stepping-stones or facilitators for the dispersal of exotic organisms (Astudillo et al., 2009; Rius et al., 2011). Additionally, artificial substrates are homogeneous, with smooth, flat surfaces such as the plastic material of buoys or the concrete of pillars and piers, and fewer microhabitats. These characteristics facilitate the dominance of those species most adapted to this environment and reduce diversity, thereby also reducing interspecific competition, even if Rocha et al. (2010) suggest that stationary substrates would be more susceptible to exotic species than floating ones.

This greater occurrence of non-native species on artificial substrates tends to be even more evident in areas subject to various stressors. Oricchio et al. (2019) analyzed the encrusting biota at different points along a highly anthropized region and pointed out the predominance of exotic fauna at all points, especially those with greater pollution stress.

The communities present on the buoys varied by sector according to the species' adaptations to oceanographic factors such as salinity and to marine influence and tide-driven water exchanges (Afonso et al., 2020). The pattern observed for the macrofauna of floating substrates aligns with the sectorization proposed by Lana et al. (2001) for this estuary, based mainly on the fauna of unconsolidated substrates (see Methods). The greater similarity between the biota of the polyhaline and euhaline buoys in relation to the marine sector is evidence of this gradient. Environmental stress can shape patterns of biota occurrence and structuring, as pointed out by Oricchio et al. (2020), in places with greater salinity variation, such as the innermost points in the estuary, where lower species richness is observed compared to points in less selective environments. Even though non-native species are more tolerant of environmental gradients, the greater occurrence of exotic species in saltier estuarine areas seems to be a pattern (Afonso et al., 2020). Moreover, the frequency trend of introduced species in relation to the distance from the estuary mouth indicates that the much lower salinity inside the estuary represents an ecophysiological barrier for exotic marine species, decreasing their chances of establishment.

The β-diversity observed was primarily driven by turnover between marine, euhaline, and polyhaline sectors, with relatively little overlap in the species present. In the mesohaline sector, the greatest contribution came from nesting, suggesting a greater species overlap between this and the other sectors. Nesting indicates that species in one sector are a subset of species present in another, with some additional exclusive species (Baselga, 2010). Our results corroborate the findings by Barros et al. (2014) that β-diversity is higher in the lower estuary than the upper estuary, though their study focused on different components of benthic macrofauna. The higher prevalence of β-diversity in the nesting component may be related to extinction or slow recolonization of species (Dobrovolski et al. 2012). Additionally, some authors associate sites in which the nesting component dominates the partition of β-diversity with the impact of various anthropogenic activities (Barros et al., 2014). Others, such as Giberto et al. (2007), suggest that β-diversity is strongly affected by changes in salinity and that benthic assemblies show greater species turnover near the limits of salinity zones.

Various studies identify the Paranaguá port as a principal dispersal source of exotic species in the region (Neves et al., 2007; Cangussu et al., 2010). As in other anthropized port areas where environmental degradation and eutrophication are common, these areas favor the establishment of non-native species (Petersen, 2007; Marins et al., 2010). Our results indicate that the salinity gradient can be key in determining the final establishment of exotic species of consolidated substrates on this spatial scale. In other words, upon their arrival, exotic species are able to disperse easily in the region, especially toward the outermost portions of the estuary, taking advantage of tidal movements and the abundance of favorable substrates in this area. This explains the high proportion of exotic species observed even in the marine areas of the shallow shelf outside the estuary (Bumbeer and Rocha, 2012). Assessments of other natural and artificial substrates along the entire estuarine gradient and in port structures could corroborate this hypothesis and greatly assist in the environmental management associated with the risk of bioinvasions.

In the case of sessile fauna, biofouling in ship hulls is the most common mechanism leading to bioinvasion, as well as through larvae or propagules present in the ballast water of ships (Lopes, 2009). This mechanism has been repeatedly described for non-native bivalves and cirripedes (Neves et al., 2007; Cangussu et al., 2010; Bumbeer and Rocha, 2012).

Among the bivalves, *P. perna* had a very high constancy, appearing in almost all buoys, especially in sectors of higher salinity. This mussel was introduced in Brazil between the 18th and 19th centuries (Souza et al., 2003;2004). Also noteworthy is the presence of the introduced bivalve *Isognomon bicolor* in about 40% of buoys, having been recorded for the first time in mid-2005 (Santos et al., 2005). This species can compete for space with barnacles and mussels in the intertidal region, causing changes in natural communities. It even competes with *P. perna*, which, despite being a historically introduced species, can be used as food or in mariculture (Breves-Ramos et al., 2010).

The introduced cirripedes have been routinely recorded on rocky shores and marinas in CEP (Neves and Rocha, 2007; Cangussu et al., 2010; Bumbeer and Rocha, 2012). Six of the nine species observed are introduced to the region*: Amphibalanus eburneus*, *A. reticulatus*, *A. amphitrite*, *M. coccopoma*, *S. amaryllis*, and *B. trigonus*. Of these, *A. reticulatus* had a greater constancy than the others, reaching 80% of buoys, very close to *Fistulobalanus citerosum*, the only species of native barnacle observed throughout our study, and the cryptogenic *B. improvisus*. These data justify the categorization of invasive species rather than just introduced for some cirripedes that, in addition to altering the composition of communities by competing with native species, can cause losses related to fouling in vessel hulls.

The ascidians and bryozoans also include several representatives introduced to the region (Neves and Rocha, 2007; Cangussu et al., 2010; Bumbeer and Rocha, 2012; Miranda et al., 2018). Rocha and Kremer (2005) list several ascidian species for the region and identify *D. listerianum* (although considered cryptogenic in this study) and *M. exasperatus*, which were observed on the buoys, as being among the most commonly introduced ascidians worldwide. There is a clear relation between the occurrence of introduced species and port areas and evidence of eutrophication and an increase in particulate matter, which may be related to their establishment (Marins et al., 2010). Among the Bryozoa, three of the four species identified are introduced. Miranda et al. (2018) point out that these are well-established species in the region, with dispersal via fouling in vessel hulls or rafting and with a preference for artificial substrates near port areas.

Although vagile taxa were considered subsampled in this study, several species of crabs were recorded. The crabs, all native to the region, showed a trend of greater richness in buoys from higher salinity areas. The assembly of amphipods also showed an occurrence pattern related to the estuary salinity gradient, with greater species richness in the marine sector. The genus *Stenothoe* was present in virtually all buoys analyzed, as was the cryptogenic *M. acheruscium*. The latter is often considered invasive in the region, as discussed by Desiderato (2020) (kept here as cryptogenic as it seems to be a species complex), which demonstrates the contribution of this type of substrate to its maintenance and dispersion (Astudillo et al., 2009). Amphipodans are known for their ability to colonize diverse environments worldwide, probably due to their high potential for reproduction and displacement associated with substrates that can drift in the oceans since these organisms do not have a planktonic larval stage and adults have low swimming capacity (McCain, 1968; Thiel et al., 2003). Thus, they are considered invasive in much of the world, often displacing native and previously established invasive species (Kestrup and Riciardi, 2009). This can easily occur in port regions of large circulation of vessels from various regions of the globe and smaller regional or local transport vessels. However, many species still require more information about the origin site and invasion routes, reinforcing the need for studies focused on this topic.

Considering the negative effects of non-native species on natural environments and economic activities, the facilitating action of artificial floating substrates such as signaling buoys (and aquaculture enterprises as well) is worth greater attention (Astudillo et al., 2009; Rius et al., 2011). The importance of these artificial stepping-stone patches in sustaining crucial dispersal events and range expansion of invasive species are underestimated, as Soares et al. (2020) argue. In this regard, these often ignored substrates may act as stepping-stones and corridors that facilitate the spread of invasive species, resulting in negative impacts on both the environment and the economy. In any case, periodic maintenance programs of the structures are necessary. In addition to replacing buoys and using antifouling paints, periodic cleaning of structures is also important, preferably on land and in a contained area, to minimize the risk of escape of non-native species to the marine area (Coutts et al., 2010). Biofouling also leads to biocorrosion when the encrusted organisms deteriorate the substrate (Messano et al., 2008). Thus, periodic maintenance of buoys decreases their facilitation of the dispersal of non-native species while also increasing their durability and improving their functionality in guiding navigation. In short, new records of recent introductions in the region (Gernet et al., 2019; Amaral et al., 2020) highlight the importance of continuous monitoring to detect potentially invasive species in a timely manner and to monitor known species of concern. Many methods need to be combined to understand and adequately manage the risks related to bioinvasion in port areas, but prevention programs and early detection of non-native species must be a primary focus of current port policies since they are the most cost-effective strategies for bioinvasion management (Miralles et al., 2021; Tamburini et al., 2021).

CONCLUSION

The signaling buoys on the access channel to Paranaguá Port play a key role as a habitat for parts of the macrofaunal benthic community and in establishing exotic organisms, functioning as stepping-stones. Non-native species predominate in this kind of substrate, especially in the more saline portions of the estuary. Paranaguá Port is the major dispersal source of exotic species in the region. Secondary dispersion occurs in various forms in the CEP, possibly due to the natural flow of estuary water exchanges, dispersal of organisms, or even via small local vessels. Buoys can be important facilitators of this dispersal. This study is unique in the region and demonstrates the need for more frequent maintenance of these structures combined with periodic monitoring of port environmental licensing inspected by federal environmental agencies.

ACKNOWLEDGMENTS

The authors are particularly grateful to Dr. Paulo Lana, a great inspiration for all authors. RM, CBM, YGT, MBL, and PDBG were Paulo's students during undergraduate or graduate studies in Zoology and Ecology. Professor Lana also contributed directly to this study by helping us to identify Polychaeta by studying the specimens, indicating the list of taxa, and training part of the team to identify this group. Professor Lana helped us dissect and assemble slides, indicating the best bibliographies and confirming our identifications. We are grateful for the dedication, enthusiasm, and examples of the great scientist Paulo Lana. The authors are grateful to the anonymous reviewers for their suggestions and comments.

AUTHOR CONTRIBUTIONS

R.M.: Conceptualization; Investigation; Writing – original draft; Writing – review & editing.

C.B.M.: Conceptualization; Investigation; Decapod identification; Writing – original draft.

Y.A.G.T.: Conceptualization; Investigation; Writing – original draft.

M.B.L.: Amphipod identification; Writing – original draft.

E.L.C.: Collection and sorting; Methodology; Writing – original draft.

G.C.B.S.: Collection and sorting; Methodology; Writing – original draft.

P.D.B.G.: Conceptualization; Investigation; Analysis; Writing – original draft; Writing – review & editing.

REFERENCES

Afonso, E. I., Berecibar, N., Castro J. L., Costa, P., Frias F., Henriques P., Moreira P. M., Oliveira G. & Silva, P. 2020. Assessment of the colonization and dispersal success of non–indigenous species introduced in recreational marinas along the estuarine gradient. *Ecological Indicators*, 113, 106147. DOI: https://doi.org/10.1016/j.ecolind.2020.106147

Alter, S., Tariq, L., Creed, J. K. & Megafu, E. 2021. Evolutionary responses of marine organisms to urbanized seascapes. *Evolutionary Applications*, 14(1), 210–232. DOI: https://doi.org/10.1111/eva.13048

Altvater, L., Haddad, M. A. & Coutinho, R. 2019. Temporal patterns of recruitment and substrate use by the nonindigenous octocoral *Stragulum bicolor* van Ofwegen and Haddad, 2011 (Alcyonacea) in the Southern Brazilian Coast. *Aquatic Invasions*, 14(2), 206–220. DOI: https://doi.org/10.3391/ai.2019.14.2.04

Amaral, V. S., Simone, L. R., Tâmega, F., Barbieri, E., Calazans, S. H., Coutinho, R. & Spotorno–Oliveira, P. 2020. New records of the non–indigenous oyster *Saccostrea cucullata* (Bivalvia: Ostreidae) from the southeast and south Brazilian coast. *Regional Studies in Marine Science*, 33, 100924. DOI: https://doi.org/10.1016/j.rsma.2019.100924

Arruda, K. 2014. *Variação temporal da comunidade incrustante na baía de Guaratuba, Paraná: recrutamento e sucessão ecológica com ênfase em espécies introduzidas* (Mestrado em Ecologia e Conservação). Curitiba: Universidade Federal do Paraná.

Astudillo, J. C., M. Bravo, C. P. Dumont & Thiel, M. 2009. Detached aquaculture buoys in the SE Pacific: potential dispersal vehicles for associated organisms. *AquaticBiology*, 5, 219–231. DOI: https://doi.org/10.3354/ab00151

Barros, F., Blanchet, H., Hammerstrom, K., Sauriau, P., Oliver, J. 2014. A framework for investigating general patterns of benthic β–diversity along estuaries. Estuarine, Coastal and Shelf Science, 149, 223–231. DOI: https://doi.org/10.1016/j.ecss.2014.08.025

Baselga, A. 2010. Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, 19(1), 134–143. DOI: https://doi.org/10.1111/j.1466-8238.2009.00490.x

Baselga, A., Orme, D., Villeger, S., Bortoli, J., Leprieur, F. & Logez, M. 2020. betapart: Partitioning Beta Diversity into Turnover and Nestedness Components. R package version 1.5.2. Available from: https://CRAN.R-project.org/package=betapart. Access date: 2024 may 9.

Bax, N., Carlton, J. T., Mathews–Amos, A., Haedrich, R. L., Howarth, F. G., Purcell, J. E., Riese, A. & Gray, A. 2001. The control of biological invasions in the world's ocean. *Conservation Biology*, 15(5), 1234–1246. DOI: https://doi.org/10.1111/j.1523-1739.2001.99487.x

Beck, M. 2020. ggord: Ordination Plots with ggplot2. R package version 1.1.5. Available from: https://fawda123.github.io/ggord/. Access date: 2024 may 9.

Blackburn, T. M., Pyšek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarosik, V., Wilson, J. R. U. & Richardson,

D. M. 2011. A proposed unified framework for biological invasions. *Trends in Ecology and Evolution*, 26(7), 333–339. DOI: https://doi.org/10.1016/j.tree.2011.03.023

Breves–Ramos, A., Junqueira, A. O. R., Lavrado, H. P., Silva, S. H. G. & Ferreira-Silva, M. A. G. 2010. Population structure of the invasive bivalve *Isognomon bicolor* on rocky shores of Rio de Janeiro State (Brazil). *Journal of the Marine Biological Association of the United Kingdom*, 90(3), 453–459. DOI: https://doi.org/10.1017/S0025315409990919

Bumbeer, J. A. & Rocha, R. M. 2012. Detection of introduced sessile species on the near shore continental shelf in southern Brazil. *Zoologia (Curitiba)*, 29(2), 126–134. DOI: https://doi.org/10.1590/S1984-46702012000200005

Cangussu, L. C., Altavater, L., Haddad, M. A., Cabral, A. C., Heyse, H. L. & Rocha, R. M. 2010. Substrate type as a selective tool against colonization by non-native sessile invertebrates. *Brazilian Journal of Oceanography*, 58(3), 219–231. Available from: https://www.scielo.br/j/bjoce/a/pmHRP8dVvy69R4PPsbMV3pB/?lang=en. Access date: 2024 may 9.

Carlton, J. T. 1985. Transoceanic and inter–oceanic dispersal of coastal marine organism: the biology of ballast water. *Oceanography and Marine Biology: An Annual Review*, 23, 313–371.

Carlton, J. T. 1987. Patterns of transoceanic marine biological invasions in the Pacific Ocean. *Bulletin of Marine Science*, 41(2), 452–465. Available from: https://www.ingentaconnect.com/content/umrsmas/bullmar/1987/00000041/00000002/art00030. Access date: 2024 may 9.

Carlton, J. T. 1989. Man's role in changing the face of the ocean: biological invasions and implications for conservation of near-shore environments. *Conservation Biology*, 3(3), 265–273. DOI: https://doi.org/10.1111/j.1523-1739.1989.tb00086.x

Carlton, J. T., Newman, W. & Pitombo, F. 2011. Barnacle invasions: Introduced, cryptogenic, and range expanding Cirripedia of North and South America. *In*: Galil, B. A, Clark, P. F. & Carlton J. T. (Ed.). *In the Wrong Place* – Alien Marine Crustaceans: Distribution, Biology and Impacts. Dordrecht – Springer Series in Invasion Ecology (vol. 6, pp. 159–214). Dordrecht, Springer.

Cohen, N. A. & Carlton, J. T. 1998. Accelerating invasions rate in a highly invaded estuary. *Science*, 279(5350), 555–558. DOI: 10.1126/science.279.5350.555

Coutts, A. D. M. & Forrest, B. M. 2007. Development and application of tools for incursion response: lessons learned from the management of fouling pest Didemnum vexillum. *Journal of Experimental Marine Biology and Ecology*, 342(1), 154–162. DOI: https://doi.org/10.1016/j.jembe.2006.10.042

Coutts, A. D. M., Valentine, J., Edgar, G., Davey, A. & Burguess-Wilson, B. 2010. Removing vessels from the water for biofouling treatment has the potential to introduce mobile non-indigenous marine species. *Marine Pollution Bulletin*, 60(9), 1533–1540. DOI: https://doi.org/10.1016/j.marpolbul.2010.04.015

Dechoum, M. S., Junqueira, A. O. R. & Orsi, M. L. (Org.). 2024. *Relatório Temático sobre Espécies Exóticas Invasoras, Biodiversidade e Serviços Ecossistêmicos*. São Carlos, Cubo.

Desiderato, A. 2020. Amphipod fouling: diversity, biogeography and drivers of an understudied community (Doutorado em Zoologia). Curitiba: Universidade Federal do Paraná.

Dobrovolski, R., Melo, A. S., Cassemiro, F. A., & Diniz-Filho, J. A. F. 2012. Climatic history and dispersal ability explain the relative importance of turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, 21(2), 191–197. DOI: https://doi.org/10.1111/j.1466-8238.2011.00671.x

Floerl, O. & Inglis, G. 2003. Boat harbour design can exacerbate hull fouling. *Austral Ecology*, 28(2), 116–127. DOI: https://doi.org/10.1046/j.1442-9993.2003.01254.x

Fowler, A. M., Jørgensen, A. M., Coolen, J. W. P., Jones, D. O. B., Svendsen, J. C., Brabant, R., Rumes, B. & Degraer, S. 2020. The ecology of infrastructure decommissioning in the North Sea: what we need to know and how to achieve it. *ICES Journal of Marine Science*, 77(3), 1109–1126. DOI: https://doi.org/10.1093/icesjms/fsz143

Gernet, M. V., Belz, C. E., Baggio, R. A., Birckolz, C. J., Santos, E. V., Simone, L. R. L., Abatte, D. & Metri, R. 2019. Nassarius foveolatus (Gastropoda, Nassariidae), a new record of an exotic species in Brazil. *Papéis Avulsos De Zoologia*, 59, e20195955-8. DOI: https://doi.org/10.11606/1807-0205/2019.59.55

Giberto, D. A., Bremec, C. S., Cortelezzi, A., Capitulo, A. R. & Brazeiro, A. 2007. Ecological boundaries in estuaries: macrobenthic β-diversity in the Río de la Plata system (34-36S). *Journal of the Marine Biological Association of the United Kingdom*, 87(2), 377–381. DOI: https://doi.org/10.1017/S0025315407050126

Google. 2020. *Google Earth*. Available from: https://www.google.com.br/earth/ Access date: 2024 July, 12.

Haddad, M. A., Bettim, A. & Miglieta, M. P. 2014. *Podocoryna loyola*, n. sp. (Hydrozoa, Hydractiniidae): a probably introduced species on artificial substrate from southern Brazil. *Zootaxa*, 3796(3), 494–506. DOI: https://doi.org/10.11646/zootaxa.3796.3.5

Hostin, L. M., Sandrini–Neto, L. & Oliveira, V. M. 2007. Associações macrofaunais em áreas sujeitas à dragagens do Complexo Estuarino de Paranaguá (CEP). *In*: Boldrini, E. B., Soares, C. R. & Paula, E. V. (Org.). *Dragagens portuárias no Brasil: licenciamento e monitoramento ambiental* (pp. 288–299). Brasília, DF, UNIBEM.

Kestrup, A. & Ricciardi, A. 2009. Environmental heterogeneity limits the local dominance of an invasive freshwater crustacean. *Biological Invasions*, 11, 2095–2105. Available from: https://link.springer.com/article/10.1007/s10530-009-9490-8. Access date: 2024 may 9.

Lana, P. C., Marone, E., Lopes, R. M. & Machado, E. C. 2001. The subtropical estuarine complex of Paranaguá Bay. *In*: Seeliger, U. & Kjerfve, B. (Org.). *Coastal Marine Ecosystems of Latin America* (pp. 132–145). Berlin, Springer.

Lopes, R. M. (Ed.). 2009. *Informe sobre as espécies exóticas invasoras marinhas no Brasil*. Brasília, DF, Ministério do Meio Ambiente.

Mangelli, T. & Creed, J. 2012. Comparative analysis of the invasive coral *Tubastraea* spp: (Cnidaria, Anthozoa) on natural and artificial substrates at Ilha Grande, Rio de Janeiro, Brazil. *Iheringia* Série Zoologia, 102 (2), 122–130. DOI: https://doi.org/10.1590/S0073-47212012000200002.

Marins, F., Novaes, R. L., Rocha, R. M. & Junqueira, A. 2010. Non indigenous ascidians in port and natural environments in a tropical Brazilian bay. *Zoologia*, 27(2), 213–221. DOI: https://doi.org/10.1590/S1984-46702010000200009

Mccain, J. C. 1968. The Caprellidae (Crustacea: Amphipoda) of the Western North Atlantic. *United States National Museum Bulletin*, 278, 1–47. DOI: https://doi.org/10.5962/bhl.part.8960

Messano, L. V. R. Sathler, L., & Coutinho, R. 2008. Biocorrosão marinha: interface entre a bioincrustração, processos eletroquímicos e ciência dos materiais. *Revista Pesquisa Naval*, 21, 32–43.

Metri, R., Soares, G., Guilherme, P., Roveda, L. 2019. The ascidian *Microcosmus exasperatus* as bioindicator for the evaluation of water quality in estuaries. *International Journal of Advanced Research*, 7(8), 174–185. https://doi.org/10.21474/IJAR01/9491

Miller, R., Hutchison, Z., Macleod, A., Burrows, M., Cook, E., Last, K. & Wilson, B. 2013. Marine renewable energy development: assessing the Benthic Footprint at multiple scales. *Frontiers in Ecology and the Environment*, 11(8), 433–440. DOI: https://doi.org/10.1890/120089

Miralles, L., Ibabe, A., González, M., Garcia-Vászquez, E. & Borrel, Y. 2021. "If you know the enemy and know yourself": Addressing the problem of biological invasions in ports through a new NIS invasion threat score, routine monitoring, and preventive action plans. *Frontiers in Marine Science*, 8, 633118. DOI: https://doi.org/10.3389/fmars.2021.633118

Miranda, A., Almeida, A. & Vieira, L. 2018. Non-native marine bryozoans (Bryozoa: Gymnolaemata) in Brazilian waters: Assessment, dispersal and impacts. *Marine Pollution Bulletin*, 130, 184–191. DOI: https://doi.org/10.1016/j.marpolbul.2018.03.023

Neves, C. S., Rocha, R. M., Pitombo, F. B & Roper, J. J. 2007. Use of artificial substrata by introduced and cryptogenic marine species in Paranaguá Bay, southern Brazil. *Biofouling*, 23(5-6), 319–330. DOI: https://doi.org/10.1080/08927010701399174

Ojaveer, H., Galil, B. S., Carlton, J. T., Alleway, H., Goulletquer, P., Lehtiniemi, M., Marchine, A., Miller, W., Occhipinti-Ambrogi, A., Peharda, M., Ruiz, G. M.,Williams, S. L. & Zaiko, A. 2018. Historical baselines in marine bioinvasions: Implications for policy and management. *PloS One*, 13(8), e0202383. DOI: https://doi.org/10.1371/journal.pone.0202383

Oricchio, F., Marques, A. C., Hajdu, E., Pitombo, F., Azevedo, F., Passos, F., Vieira, L. M., Stampar, S., Rocha, R., M., Dias & G. M. 2019. Exotic species dominate marinas between the two most populated regions in the southwestern Atlantic Ocean. *Marine Pollution Bulletin*, 146, 884–892. DOI: https://doi.org/10.1016/j.marpolbul.2019.07.013

Osaken, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. & Wagner, H. 2013. vegan: Community Ecology Package. R package version 2.0- 10. Available from: https://cran.r-project.org/web/packages/vegan/index.html. Access date: 2024 may 9.

Petersen, J. K. 2007. Ascidian suspension feeding. *Journal of Experimental Marine Biology and Ecology*, 342(1), 127–137. DOI: https://doi.org/10.1016/j.jembe.2006.10.023

Procopiak, L., Fernandes, L. & Moreira–Filho, H. 2006. Diatomáceas (Bacillariophyta) marinhas e estuarinas do Paraná, Sul do Brasil: lista de espécies com ênfase em espécies nocivas. *Biota Neotropica*, 6(3), 1–28. DOI: https://doi.org/10.1590/S1676-06032006000300013

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.R-project.org/. Access date: 2024 may 9.

Rocha, R. M. & Kremer, L. P. 2005. Introduced Ascidians in Paranaguá Bay, Paraná, southern Brazil. *Revista Brasileira de Zoologia*, 22(4), 1170–1184. DOI: https://doi.org/10.1590/S0101-81752005000400052

Rocha, R. M., Cangussu, L. C. & Braga, M. P. 2010. Stationary substrates facilitate bioinvasion in Paranaguá bay in southern Brazil. *Brazilian Journal Of Oceanography*, 58, 23–28. Available from: https://www.scielo.br/j/bjoce/a/tcdRWHLxP4sn9RfcPLTC5NJ/?lang=en. Access date: 2024 may 9.

Rius, M., Heasman, K. G. & Mcquaid, C. D. 2011. Long-term coexistence of non-indigenous species in aquaculture facilities. *Marine Pollution Bulletin*, 62(11), 2395–2403. DOI: https://doi.org/10.1016/j.marpolbul.2011.08.030

Santos, H. F., Borzone, C. A. & Tavares, Y. A. G. 2005. *Distribuição espacial e temporal de Isognomon bicolor (C. B. Adams, 1845) (Bivalvia, Isognomonidae) no litoral Paranaense, Brasil*. Pontal do Paraná, Centro de Estudos do Mar (CEM), Universidade Federal do Paraná (UFPR).

Sardain, A., Sardain, E., & Leung, B. 2019. Global forecasts of shipping traffic and biological invasions to 2050. *Nature Sustainability*, 2(4), 274–282. DOI: https://doi.org/10.1038/s41893-019-0245-y

Sheehy, D. & Vik, S. F. 2010. The role of constructed reefs in non-indigenous species introductions and range expansions. *Ecological Engineering*, 36(1), 1–11. DOI: https://doi.org/10.1016/j.ecoleng.2009.09.012

Silva, J. S. V., Fernandes, F. C., Souza, R. C. C. L., Larsen, K. T. S. & Danelon, O. M. 2004. Água de Lastro e Bioinvasão. *In*: Silva, J. S. V.; Souza, R. C. L. De. (Org.). Água de Lastro e *Bioinvasão* (pp. 1–9). Rio de Janeiro, Interciências.

Soares M. S., Salani, S., Paiva S. V., Braga M. D. A. 2020. Shipwrecks help invasive coral to expand range in the Atlantic Ocean. *Marine Pollution Bulletin*, 158, 111394. DOI: https://doi.org/10.1016/j.marpolbul.2020.111394

Souza, R., Fernandes, F. & Silva, E. 2003. A study on the occurrence of the brown mussel Perna perna on the sambaquis of the brazilian coast. *Revista do Museu de Arqueologia e Etnologia*, 13, 3–24. DOI: https://doi.org/10.11606/issn.2448-1750.revmae.2003.109462.

Souza, R. C. C. L., Fernandes, F. C. & Silva, E. P. 2004. Distribuição atual do mexilhão Perna perna no mundo:

um caso recente de bioinvasão. *In*: Silva, J. S. V. & Souza, R. C. C. L. (Org.). Água de lastro e *bioinvasão* (p. 157–172). Rio de Janeiro, Interciências.

Tamburini, M., Keppel, E., Marchini, A., Repetto, M. F., Ruiz, G. M., Ferrario, J. & Occhipinti-Ambrogi, A. 2021. Monitoring non-indigenous species in port habitats: first application of a standardized North American Protocol in the Mediterranean Sea. *Frontiers in Marine Science*, 8, 700730. DOI: https://doi.org/10.3389/fmars.2021.700730

Thiel, M., Guerra-Garcia, J. M., Lancellotti, D. A. & N. Vásquez. 2003. The distribution of littoral caprellids (Crustacea: Amphipoda: Caprellidea) along the Pacific coast of continental Chile. *Revista Chilena de Historia Natural*, 76 (2), 297–312. DOI: http://dx.doi.org/10.4067/S0716-078X2003000200014

Tyrrell M. C. & Byers J. E. 2007. Do artificial substrates favor nonindigenous fouling species over native species? *Journal of Experimental Marine Biology and Ecology*, 342(1), 54–60. DOI: https://doi.org/10.1016/j.jembe.2006.10.014

Xavier, E., Almeida, A., Nogueira, M. & Miranda, L. 2023. Effects of substratum type and orientation on the recruitment of bryozoans in an artificial area of the Western Atlantic. *Biofouling*, 39(7), 748–762. DOI: https://doi.org/10.1080/08927014.2023.2262928

Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. New York, Springer.