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HELIO HERMINIO CHECON

INTERAÇÕES DA FAUNA BENTÔNICA COM O SUBSTRATO NÃO CONSOLIDADO NA BAÍA DO ARAÇÁ (SÃO SEBASTIÃO, SP)

BENTHIC FAUNA INTERACTIONS WITH SOFT BOTTOM SEDIMENTS AT ARAÇÁ BAY (SÃO SEBASTIÃO, SP)

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Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do Título de Doutor em Ecologia

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Prof. Dr. Paulo César de Paiva

Prof. Dr. Paulo Yukio Gomes Sumida

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Resumo

A fauna bentônica de fundos não consolidados é composta de uma grande diversidade de grupos taxônomicos. No entanto, o conhecimento dessa diversidade na costa brasileira ainda é escasso, em especial na área do sublitoral raso. Essa fauna é estreitamente relacionada com as condições do sedimento, fazendo com que modificações nessa variável implique em mudanças na comunidade, permitindo a investigação do efeito de variações ambientais. Da mesma forma, a baixa mobilidade faz com que a fauna bentônica seja comumente empregada em estudos de contaminação e qualidade ambiental. Nesse contexto, visamos avaliar a interação da fauna com as variações nas condições ambientais, em um ecossistema altamente influenciado pela atividade antrópica. A tese se encontra dividida em três capítulos. No primeiro capítulo, discutimos os efeitos da vegetação de manguezal sobre a comunidade infaunal (poliquetas). Embora os efeitos descritos na literatura sejam positivos para a epifauna, a comunidade de poliquetas na área de estudo apresentou decréscimo na abundância de espécies, ainda que a riqueza de espécies não seja afetada. É provável que alterações sedimentares condicionadas pela presença das raízes cause efeito negativo sobre a abundância de poliquetas. No segundo capítulo, investigamos a qualidade ambiental do sistema bentônico da baía utilizando o índice AMBI, amplamente empregado em costas europeias, porém subutilizado e testado na costa brasileira. Foi encontrada uma boa condição ambiental no entremarés da baía, com predomínio de espécies sensíveis. Na região supralitoral e sublitoral externo, a condição ambiental é baixa, pela presença de espécies oportunistas, especialmente no supralitoral. O índice foi correlacionado com a presença de contaminantes, em especial hidrocarbonetos e carbono orgânico total, fortalecendo a importância do seu uso na caracterização da qualidade de ambientes bentônicos. Discutimos ainda o efeito do pré-tratamento dos dados (transformações e exclusão de amostras) no desempenho do índice. O terceiro capítulo focou em fornecer subsídios para futuros projetos de monitoramento da área, atráves da caracterização dos habitats sedimentares, e das espécies indicadoras das condições em cada habitat. A baía foi caracterizada em três habitats, influenciados pela posição em relação a maré e características sedimentares. Quatro espécies foram sugeridas para monitoramento, e a relação de cada espécie com as variáveis características de cada habitat foi discutida. Esperamos que os resultados apresentados nos capítulos possam servir de subsídios para o conhecimento do funcionamento do ecossistema, como parte do projeto Biota-Fapesp, além de auxiliar no melhor conhecimento das interações da fauna bentônica com o seu ambiente.

Abstract

The benthic soft-bottom fauna is composed of a great diversity of taxonomic groups. However, knowledge regarding their diversity on the Brazilian coast is scarse, especially in the shallow sublittoral area. This fauna is strictly related to sedimentary conditions, and modifications in this variable result in community changes, allowing the investigation of the effects of environmental variation. Likewise, the low motility makes the benthic fauna a suitable group for contamination and environmental quality studies. In this context, we aimed to evaluate the interaction between the benthic fauna with changes in environmental conditions, in a highly antrophogenically affected ecosystem. The thesis is divided into three chapters. In the first, we discuss the effects of mangrove vegetation on the polychaete infaunal community. Despite the effects on the epibenthic community reportedly on literature being positive, the polychaete abundance was negatively affected by mangrove presence, although no changes were seen in species richness. It is likely that sedimentary changes caused by presence of vegetation hinder the abundance of polychaete. In the second chapter, we investigated the environmental quality of the benthic system of the bay, using the AMBI index, widely used in European waters, but under utilized and tested in the Brazilian coast. Good ecological quality was found along the intertidal area, with predominance of sensitive species. The worse ecological quality was found at the upper intertidal and external sublittoral, with presence of first-order opportunists, especially at the upper intertidal. The index was correlated with the presence of contaminants, especially hydrocarbons and organic carbon, strengthening the importance of the AMBI use in the characterization of environmental quality of benthic systems. We also discussed the effects of data pre-treatment (transformation and sample exclusions) on the performance of the index. The third chapter focused on generating subsidies to the implementation of monitoring plans in the area, through the characterization of sedimentary habitats, and indicator species of each habitat condition. The bay was characterised by three habitats, influenced by tidal position and sedimentary characteristics. Four species are suggested for monitoring purposes, and their relationship with each the corresponding characterizing variables is described. We hope that the results presented in the chapters can aid the unrevealing of the functioning of the Araçá Bay ecosystem, as a part of Biota-Fapesp/Araçá project, and help a better knowledge of the interactions of the benthic fauna with its environment.

Sumário

Introdução

A fauna bentônica de fundos inconsolados apresenta uma alta diversidade de espécies, composta principalmente por invertebrados de diferentes grupos taxonômicos. Devido a essa diversidade, uma variedade de formas, tamanhos e modos de vida são encontrados. Quanto ao tamanho, essa fauna é comumente dividida entre macrofauna (organismos com tamanho corpóreo maior que 0,5 mm); meiofauna (organismos menores que 0,5 mm e maiores que 0,044 mm); e microfauna (organismos menores que 0,044 mm) (McLachlan & Brown 2006, Giere 2009).

A importância da fauna bentônica para a estabilidade de seu habitat é reconhecida na literatura. São organismos que desempenham papel na decomposição de microalgas, mineralização da matéria orgânica, e no fluxo marinho de compostos quimícos (Andersen & Kristensen 1992, Heilskov & Holmer 2001, Nascimento et al. 2012). O hábito tubícola de algumas espécies pode facilitar o recrutamento de outras, exercendo papel na sucessão da comunidade (Gallagher et al., 1983). São ainda importantes elos da teia alimentar, servindo como alimento para outros grupos, especialmente peixes (Edgar & Shaw, 1995; Majdi et al., 2014; Schmid-Araya et al., 2016). Dessa forma, o conhecimento da diversidade e ecologia da fauna no ambiente bentônico é de extrema importância para a compreensão do funcionamento tanto do ambiente bentônico como planctônico.

A fauna bentônica é fortemente relacionada às características do ambiente, em especial nas regiões costeiras, onde a hidrodinâmica exerce grande influência sobre essas variáveis, e consequentemente sobre a fauna. Considera-se nesses ambientes que a influência das variáveis ambientais é muito mais determinante nas características das espécies do que as interações biológicas entre essas, com diversas hipóteses utilizadas para explicar os padrões de distribuição da fauna, sendo a maioria relacionada a hipótese auto-ecológica (Noy-Meir 1973, McLachlan et al. 1993, Defeo & McLachlan 2005). O sedimento é a principal variável relacionada a distribuição e ocorrência da fauna bentônica, e essa associação é amplamente discutida, em especial pela estreita relação do sedimento com outras variáveis ambientais como matéria orgânica, teor de oxigênio e produção primária microfitobentônica (Snelgrove & Butman 1994, Miller et al. 1996, Anderson 2008).

Os estudos abordando a ecologia das comunidades bentônicas foram primariamente direcionados à zona entremarés. As dificuldades logísticas das amostragens no sublitoral, como necessidade de mergulho e/ou uso de embarcações e amostradores especiais, é a provável responsável por esse direcionamento primário ao ambiente entremarés (Snelgrove 1999, Eleftheriou & Moore 2013). Entretanto, mesmo as amostragens de sublitoral ocorriam em áreas mais profundas, sendo que amostras em áreas rasas eram escassas. Clark & Milne (1955) pontuaram tal fato, citando as dificuldades na compatibilidade dos grandes amostradores da época com os pequenos barcos, necessários para amostrar áreas rasas, sendo esses autores um dos primeiros a amostrar nessas regiões. Com o avanço nas técnicas de amostragem, outros estudos se sucederam no sublitoral raso, alguns cobrindo grandes extensões da plataforma (Day et al. 1971, Valderhaug & Gray 1984, Morin et al. 1985, Kendall & Widdicombe 1999, Veas et al. 2012), incluindo estudos em regiões polares (Filgueiras et al. 2007).

No Brasil, existem vários estudos abordando a macrofauna dos ambientes sublitorâneos, entre estes: Amaral (1980); Lana (1986); Paiva (1993); Muniz & Pires (1999); Pires-Vanin (2008) e Amaral et al. (2011). Ainda assim, a fauna bentônica na costa brasilieira é pouco conhecida, sendo que a maioria dos estudos está concentrado no eixo Sul-Sudeste (Amaral & Jablonski 2005), e mesmo nessa região, o conhecimento da comunidade em profundidades rasas (5-10m) continua escasso (Amaral & Migotto 2011).

A fauna bentônica também é um importante componente em ambientes de manguezais. As características particulares desses ambientes, como a alta taxa de decomposição, grande quantidade de detritos, e complexidade estrutural, abrigam uma alta diversidade de organismos bentônicos (Lee 2008). De fato, a presença dos manguezais é conhecida por aumentar a riqueza de diversos táxons da macrofauna epibentônica, fornecendo recursos como abrigo, alimentação ou sustentação (Nagelkerken et al. 2008). Apesar disso, o conhecimento da fauna bentônica em manguezais é ainda muito escasso (Elisson 2008), em especial de organismos infaunais (Nagelkerken et al. 2008). Considerando a importância desse ecossistema, e sendo o Brasil um dos países com maior perda de áreas de manguezal (Wilkie & Fortuna 2003), o conhecimento da biodiversidade bentônica faz-se necessário para compor mais uma ferramenta na conservação desse ecossistema.

De fato, o conhecimento sobre a fauna bentônica, sua distribuição espaço-temporal e relação com o ambiente, é essencial para que estudos de monitoramento e qualidade ambiental sejam implantados de forma efetiva. Em especial, o uso de espécies bentônicas como indicadoras das condições ambientais é difundido em programas de monitoramento (Bilyard 1987, Kennedy & Jacoby 1999, Dean 2008). Espécies indicadoras são aquelas que apresentam uma resposta previsível em relação a um determinado gradiente ambiental

(Carignan & Villard 2002). Existem diferentes métodos para apontar quais espécies devem ser utilizadas como indicadoras, embora o método deva considerar táxons que possuem uma resposta forte a um grupo de locais ou condição, ao invés de múltiplas respostas a diferentes grupos (Fonseca & Gallucci 2016). Embora diferentes espécies sejam já conhecidas pelo seu papel de indicadora (p.ex. *Capitella capitata*; *Heteromastus filiformis, Scolelepis fuliginosa*), planos de manejo devem considerar dados locais para determinação dessas espécies, devido a variações na tolerância das espécies com fatores como latitude e salinidade (Zettler et al. 2013).

O uso de índices bentônicos é outra forma mais difundida de avaliar a qualidade do ambiente bentônico. O AZTI' Marine Benthic Index (AMBI) (Borja et al. 2000) é um dos mais utilizados para esse fim. O índice se baseia em dados da comunidade bentônica, calculado pela proporção de espécies classificadas em cinco grupos ecológicos (EG), de acordo com seu grau de tolerância, indo de sensíveis (EG I) à oportunistas de primeira ordem (EG V) (Grall & Glémarec 1997). Esse índice segue o modelo de sucessão ecológica frente ao enriquecimento orgânico, sugerido por Pearson & Rosenberg (1978). No entanto, a validade do AMBI frente a diferentes fontes de poluição, como hidrocarbonetos e metais, tem sido corroborada (Muniz et al. 2005, Muxica et al. 2005, Riera et al. 2011). Apesar da importância desse índice no cenário mundial, seu uso na costa brasileira ainda é recente, e mais estudos são necessários para a validação do seu funcionamento em regiões tropicais e subtropicais.

O Brasil é um dos países com uma das mais extensas linhas costeiras, tendo a população fortemente concentrada na região litorânea. Com isso, as pressões ambientais na área se tornam intensas e variam ao longo da costa, como por exemplo, supressão de manguezais, exploração comercial de espécies, maricultura, atividade portuária, e outras atividades decorrentes da urbanização. Com isso, áreas de grande importância para a biodiversidade se encontram sob ameaça.

A Baía do Araçá, localizada no Litoral Norte do Estado de São Paulo, Munícipio de São Sebastião, é um dos exemplos dessa condição. Com um histórico de transformações ambientais devido a impactos decorrentes da construção e atividade do Porto de São Sebastião e instalação de emissário submarino, a área ainda sofre com pressões recentes da expansão portuária (Amaral et al. 2010, Mani-Peres et al. 2016). Apesar disso, a baía possuí uma alta diversidade de espécies e de características ambientais, incluindo um dos poucos remanescentes de manguezais do Litoral Norte (Amaral et al. 2010, Amaral et al. 2016).

Nesse contexto, o presente estudo teve como objetivo preencher as lacunas do conhecimento da biodiversidade de sublitoral raso e manguezais na costa brasileira, tendo como objeto de estudo a Baía do Araçá. Com o conhecimento produzido da biodiversidade, o presente manuscrito foi dividido em três partes, de forma a avaliar diferentes aspectos da macrofauna bentônica, em específico: 1) influência da vegetação sobre a densidade, riqueza e estrutura trófica de uma assembleia de poliquetas infaunais; 2) cálculo de índices bentônicos para avaliar a qualidade ambiental do sistema bentônico, e sua relação com variáveis de contaminação; 3) determinação da dinâmica espaço-temporal de habitats dentro da baía e espécies indicadoras com o intuito de prover ferramentas para futuros monitoramentos na área. A estrutura da tese segue a sequência desses objetivos, com cada capítulo discutindo hipóteses e resultados relacionados.

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Capítulo 1

Mangrove vegetation decreases density but does not affect species richnenss and trophic structure of intertidal polychaete assemblages

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Abstract

The positive influence of mangrove vegetation on macrobenthic communities has been widely investigated, but studies mainly focused on epibenthic assemblages. Given the contrasting characteristics between epifauna and infauna, we expected that mangrove vegetation would not exert the same positive effect on infaunal assemblages. To test this hypothesis, we investigated polychaete assemblages in mangrove stands in a tidal flat in Southeast Brazil. Specifically, we focused on (a) whether polychaete assemblages (i.e. density, richness, community and feeding guild composition) are different inside and outside mangrove stands, and (b) if changes are related to root biomass. Our results showed that mangrove areas have lower polychaete density than adjacent sandflats, and polychaete density is negatively related to root biomass. Species richness was not affected by the presence of vegetation, but the number of exclusive species was higher inside the mangrove. Changes in composition of polychaete assemblages were mainly attributable to reductions in species density rather than species replacement. Trophic structure was not influenced by mangrove vegetation, as subsurface-feeders dominated inside and outside mangrove zones. Our results contrasts with the richness enhancement found for epibenthic fauna inside mangroves, and highlights that mangrove vegetation does not exert the same influence on epibenthic as on infaunal assemblages.

Introduction

Mangrove forests are essential to society, providing an array of ecosystem services such as sediment trapping, nutrient processing, protection of shorelines, and socio-economic goods (Schaeffer-Novelli 1990, Ewel et al. 1998; Glaser 2003, Alongi 2008). Their pneumatophores, belowground root system and litter fall, also enhance habitat heterogeneity and primary production in coastal environments and enable the occurrence of a rich and diverse benthic fauna (Kathiresan & Bingham 2001, Nagelkerken et al. 2008).

It is widely known that the three-dimensional structure created by mangrove stands on an almost flat environment enhances macrobenthic abundances and species richness by providing shelter from predators, food and protection from desiccation (Frith et al. 1976, Edgar 1990, Nobbs 2003, Kon et al. 2010). This knowledge, however, has been developed mostly around epibenthic species, especially crustaceans and gastropods (Nagelkerken et al. 2008), and the influence of mangrove vegetation on infaunal organisms such as polychaetes is far less studied (Dittmann 2001, Nagelkerken et al. 2008).

Thus far, the few studies that investigated infaunal communities in mangrove soft-bottoms have reported abundant and diversified assemblages (Lee 2008, Metcalfe & Glasby 2008); however, they did not compare areas inside and outside the mangrove. This lack of data prevents any conclusion about the effects of mangrove vegetation on infaunal assemblages. It is possible that the high abundance and diversity values reported are simply related to a rich regional pool of species and individuals rather than a result of the presence of mangroves itself. Furthermore, given that mangrove roots reduce the available space for infaunal organisms, it is also possible that infaunal assemblages are less diversified and abundant inside mangrove stands. Therefore, while a positive relationship between mangroves and epibenthic fauna is observed, a decrease in species density and richness may occur for infaunal species.

Another unanswered question is how mangrove vegetation affects the trophic relationships of infaunal assemblages. It is known that subsurface deposit feeders commonly dominate low hydrodynamic sandflats, however, mangrove roots might limit space for this group and favor the occurrence of other feeding groups, especially suspension feeder, which are otherwise limited by the bioturbation activities of subsurface feeder (Rhoads & Young 1970, Pillay et al. 2007). The studies that dealt with the influence of vegetation cover and biomass on polychaete assemblages so far only focused on non-mangrove vegetation (i.e., hard-bottoms and salt-marshes) and achieved no consensus. While some investigations found positive relationship between species richness and vegetation cover (e.g., Sardà 1991, Whitcraft & Levin 2007), others reported no effect (e.g., Netto & Lana 1997, Pagliosa & Lana 2005). The influence that mangroves may have on polychaete assemblages, however, is still poorly understood (Nagelkerken et al. 2008).

To assess the influence of mangrove vegetation on infaunal organisms, we investigated polychaete assemblages inside and outside three mangrove stands on the Southeast coast of Brazil. Specifically, we focused on examining (a) whether density, richness and feeding guild composition of these assemblages are different inside and outside mangrove stands, and (b) if any possible difference is related to the presence of belowground root biomass. Our main hypothesis is that mangrove vegetation would negatively affect polychaete assemblages due to the reduced space caused by the presence of roots. We therefore predicted (i) lower density and number of species inside mangroves and (ii) a negative relationship between these parameters and root biomass. We also hypothesized that different polychaete feeding strategies would be favoured by the different environmental features inside and outside mangroves. We predicted that the limited space caused by root biomass could limit the occurrence of subsurface feeders, and favour the establishment of other feeding guilds, such as suspension and surface deposit feeders.

Materials and Methods

Study area

This study was done at Araçá Bay, located in the municipality of São Sebastião, on the northern coast of the State of São Paulo, Brazil. The area is a sheltered bay and has been subjected to anthrogenic actions due to its proximity with the urban area and the São Sebastião Port. The intertidal zone is a sedimentary heterogeneous sandflat with patches ranging from very fine to coarse sands, a reflection of past disturbances in the area (Amaral et al. 2010).

Three major mangrove stands are found in the area, located more than 100 m apart from each other (Fig. 1). These are one of the few mangrove remnants on the Northern coast of the State of São Paulo (Amaral et al. 2010):

- Mangrove stand 1 (N1): The largest stand, with an area of 2.380 m², located at the upper intertidal area. Mature forest, with predominance of *Avicennia schaueriana*

(Stapf & Leechman ex Moldenke) and a few individuals of *Laguncularia racemosa* (L.) Gaertn. A small rocky shore is located at the stand's edge.

- Mangrove stand 2 (N2): Area of 459 m², located at the upper intertidal area. Forest composed of individuals of *A. schaueriana* and *L. racemosa*.
- Mangrove stand 3 (IP): Area of 456 m², located at the lower intertidal area, close to the Island of Pernambuco (hence the mangrove stand coding). Here, a lower stand density is found, probably due to the substrate characterized by many rocky fragments, gravel and by many shells.

Mangrove stand coding followed the standards set by other studies inside the Project "BIOTA/FAPESP – Araçá", with which this study is associated.

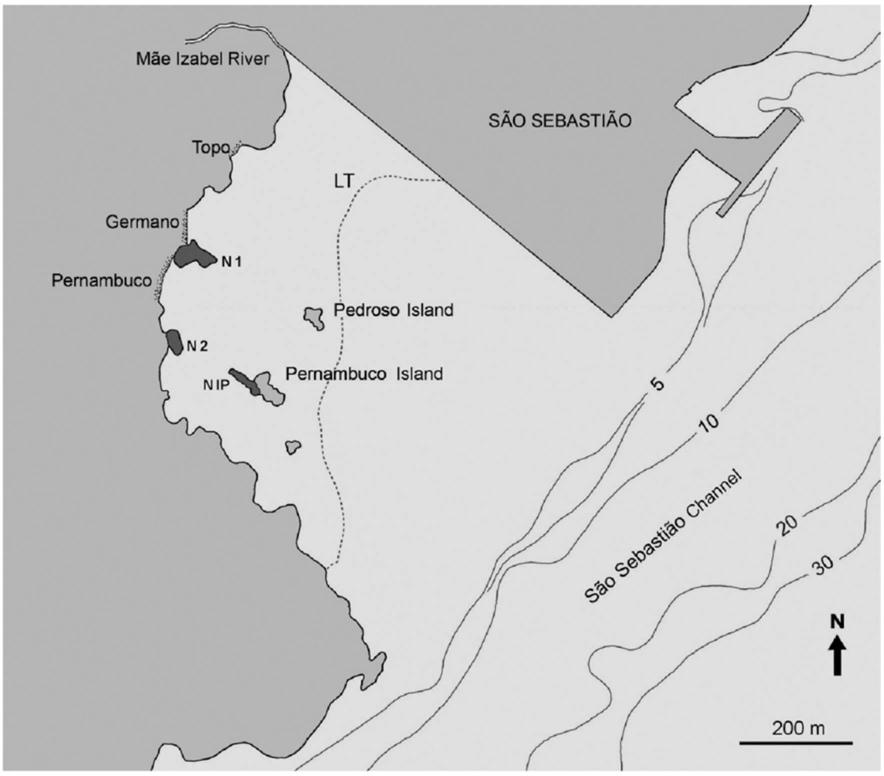

Fig. 1 Map of the study area, showing the locations of the three mangrove stands. N1, N2 and NIP are the mangrove stand codes, and LT stands for low tide.

Sampling design

All three mangrove stands were sampled twice (March and July/2014), during low tide periods. At each mangrove stand, three parallel transects were placed, 5 m apart from each other. The position of the first transect was randomly chosen. At each transect, eight sampling sites were placed, 1 m apart from each other. Transects placement was done to create three zones, forming a gradient from the exposed sandflat to the mangrove interior, with pneumatophore surface cover (mainly an *A. schaueriana* dominated zone). Thus, three sampling sites were considered as "Outside" (Sandflat), two as "Transition", and the last three as "Inside" (Mangrove). As our objectives focus on the differences between inside x outside zones, the transition zone was not included into the analysis.

At each sampling site, a PVC cylindrical corer (10 cm diameter and 20 cm deep) was used to sample polychaete fauna. Each sample was placed in a separate plastic bag and all were immediately transported to the Centro de Biologia Marinha, University of São Paulo (Cebimar – USP), where they were washed on sieves (mesh size = 0.5 mm) and the retained fauna was fixed on 70% alcohol. Taxonomic identification was done to the lowest possible level. During sediment processing, roots of each sample were separated to evaluate plant belowground biomass. Root samples were dried and biomass was expressed as g/0.015 m^3.

Two samples were taken at both the inside and outside zones of each mangrove stand to evaluate granulometric distribution and total organic carbon (TOC). These variables were chosen to characterize the sedimentary environmental at each stand and zone, which could vary due to spatial variability (e.g. intertidal position) and the conditions inside and outside mangrove (e.g. vegetation presence, detritus). The granulometric analysis was done by sieving the dried samples in twelve granulometric fractions, which were individually weighed (Suguio et al. 1973). Sediment parameters were calculated using SysGran software, version 3.0 (Camargo 2006) in accordance with the classifications of Folk & Ward (1957). Total organic carbon was determined by the widely used modified Walkley-Black titration method, as described in Gaudette et al. (1974).

Data Analysis

Differences in root biomass among stands were verified using analysis of variance with post-hoc Tukey HSD tests, on both sampling periods. Mixed-linear models were used to investigate the relationship of total polychaete density and number of species with root biomass (g of dry weight) and zones (inside/outside mangrove). Poisson distribution was applied to richness (count data) and negative binomial to density data (due to overdispersion - residual deviance >> degrees of freedom) (O'Hara & Kotze 2010). Mangrove stands and transects nested within stand, as well as their interactions with zones and roots, were considered as random factors in the model. To reduce model complexity, and given the limitation of estimating variance for random factors with few levels (Bolker et al. 2009), we accounted for sampling periods variability by running models separately for each period. Estimation of random variances was often approximately zero in the models. This does not indicate a lack of variability in the random term, but rather that this term does not add information to the model (Bates 2010). Fixed and random effects significance was tested using log-likelihood comparison between the full model with reduced models excluding the variable of interest (Bates 2010)

To detect the contribution of each individual species to the differences between zones (inside and outside mangroves), negative-binomial regressions were carried out. This framework is based on running regression for each taxon and comparing the likelihood ratio statistics to check the influence of each individual species, and is less biased than the commonly applied Similiarity Percentage analysis (Warton et al. 2012). Analyses of variance were done to test the effect of zone on each individual species density, with p-values adjusted for multiple comparisons (Wang et al. 2012). Community data was not transformed for this analysis, as negative-binomial distribution corrects for the existence of extreme values of distribution (O'Hara & Kotze 2010).

Feeding guild composition was assessed by grouping polychaete species following classification by Fauchald & Jumars (1979), with updates found in Jumars et al. (2015) and references therein. Abundance and richness for each feeding guild was calculated to compare the relative guild dominance between mangrove and sandflat zone.

Analysis were carried out using the R Software 3.3.1 (R Core Development Team, 2016), with additional packages *lme4* (Bates et al. 2015) and *glmmADMB* (Fournier et al. 2012) for mixed models, and *mvabund* (Wang et al. 2014) for negative-binomial regression models.

Results

Environmental characterization

Root biomass differed among mangrove stands, regardless of sampling period (N2 > N1 > IP, March: $F_{2,18} = 18.359$, $p < 0.001$; July: $F_{2,18} = 16.206$, $p < 0.001$) (Fig. 2). No comparisons were made between zones, as root biomass outside mangrove was always zero.

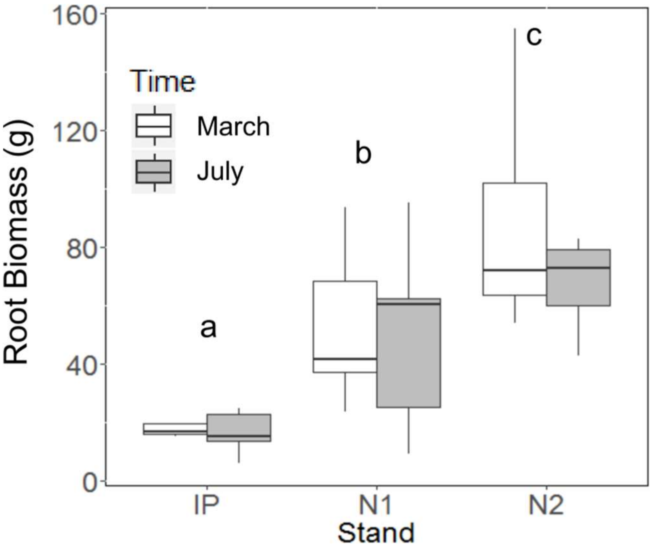

Fig. 2 Root biomass (g) at each mangrove stand during each sampling period. Different letters (a,b,c) indicate statistical differences among stands on Tukey HSD post-hoc tests. Boxes represent mean, first and third quartiles. Whiskers indicate the dataset range. No comparisons were made between zones, as biomass outside mangrove was always zero.

Sediment characteristics differed between zones (Table 1), but these results were dependent of location. Mangrove stands IP, and to a lesser degree N2, had more pronounced distinct sedimentary composition between zones. In these stands, sediment from the adjacent sandflat was finer than that from the mangrove. This was especially true at stand IP, where the inside zone was characterized as coarse sand with high contribution of gravel while the sediment outside the mangrove was characterized as very fine sand. Differences in total organic carbon (TOC) were stronger between sampling periods than between zones (Table 1).

Macrofaunal community

A total of 7827 individuals were sampled during the survey, 4086 were sampled in March, and 3741 in July. The polychaete community was composed of 22 species (March: 20 species; July: 14 species), and was mainly dominated by *Capitella* sp.C, *Capitella aciculata* (Hartman, 1959), *Heteromastus* sp. A, *Laeonereis culveri* (Webster, 1879), *Scoloplos (Leodamas)* sp.A., *Isolda pulchella* Müller in Grube 1858, *Perinereis anderssoni* Kinberg, 1866 and *Marphysa sebastiana* Steiner & Amaral, 2000. Other species were less frequent and always occurred in low densities.

Table 1 Environmental characterization of mangrove and nearby sandflat at each stand. Mean values are given. (M.D.: Sediment mean diameter; TOC: Total organic carbon).

N1	**Mean Diameter (φ)**	**Sediment Type**	**Gravel (%)**	**Sands (%)**	**Silt/Clay (%)**	**TOC (%)**
Summer						
Sandflat	3.87	Very Fine Sand	0.03	69.92	30.01	0.65
Mangrove	3.77	Very Fine Sand	0.26	75.46	24.48	0.73
Winter						
Sandflat	3.54	Very Fine Sand	0	88.11	11.89	3.02
Mangrove	3.77	Very Fine Sand	0	88.07	11.93	5.24
N2						
Summer						
Sandflat	3.83	Very Fine Sand	0.55	64.2	35.24	0.75
Mangrove	2.59	Fine Sand	5.27	82.22	12.5	0.42
Winter						
Sandflat	3.24	Very Fine Sand	0	85.84	14.16	2.13
Mangrove	3.01	Very Fine Sand	0	90.77	9.23	3.17
IP						
Summer						
Sandflat	3.42	Very Fine Sand	0.93	76.74	22.32	0.59
Mangrove	1.1	Coarse Sand	13.52	82.81	3.66	0.33
Winter						
Sandflat	3.1	Very Fine Sand	0	95.34	4.66	2.48
Mangrove	2.85	Fine Sand	0	94.32	5.68	5.7

Community descriptors x zones

Polychaete density was higher outside mangroves compared to inside in both periods (March: $X^2(1) = 2.412$, $p = 0.012$; July: $X^2(1) = 7.161$, $p < 0.001$) (Fig. 3). No interaction was found between stands or transects with zones, although polychaete density differed among stands and transects in July (Table 2). Polychaete density was negatively related to root biomass, although this result was significant in only one of the periods; (March: $X^2(1) = 4.712$, $p = 0.041$; July: $X^2 = 2.193$, $p = 0.138$) and contingent of stands ($X^2(3) = 10.400$, $p = 0.015$) (Table 3).

Table 2 Relationship between transect zone and polychaete density and richness. Values in bold indicate that the term is significant (α = 0.05) under comparisons between full and reduced models. d.f. indicate the degrees of freedom of the model comparison for each term.

March	**Richness**					**Density**				
	Estimate	sd	X^2	d.f.	p-value	Estimate	sd	Deviance	d.f.	p-value
Zone	0.296	0.205	3.289	1	0.066	1.348	0.338	2.412	1	**0.012**
Stand	0	0	0	1	1	0.374	0.611	0.012	1	0.912
Zone*Stand	0.041	0.203	1.175	3	0.758	0.08	0.092	2.412	3	0.299
Transect(Stand)	0	0	0	1	1	0.418	0.646	2.376	1	0.123
Zone*Transect(Stand)	0	0	0	3	1	0.052	0.225	0.952	3	0.328

July	**Richness**					**Density**				
	Estimate	s.d.	X^2	d.f.	p-value	Estimate	s.d.	Deviance	d.f.	p-value
Zone	0.171	0.261	0.658	1	0.404	1.188	0.912	7.161	1	**<0.001**
Stand	0	0	0	1	1	0.274	0.523	4.232	1	**0.032**
Zone*Stand	0	0	0	3	1	0.008	0.094	4.291	3	0.111
Transect(Stand)	0	0	0	1	1	0.164	0.401	2.148	1	**<0.001**
Zone*Transect(Stand)	0.094	0.306	1.597	3	0.663	0.072	0.268	4.261	3	0.118

Table 3 Relationships between root weight and polychaete density and richness. Values in bold indicate that the term is significant (α = 0.05) under comparisons between full and reduced models. d.f. indicate the degrees of freedom of the model comparison for each term.

March	**Richness**					**Density**				
	Estimate	sd	X^2	d.f.	p-value	Estimate	sd	Deviance	d.f.	p-value
Root	-0.001	0.003	0.047	1	0.827	-0.019	0.005	4.712	1	**0.041**
Stand	0	0	0	1	1	0	0	0	1	1
Root*Stand	0.048	0.021	2.175	3	0.536	1.05	1.025	10.4	3	**0.015**
Transect(Stand)	0	0	0	1	1	0	0	0	1	1
Root*Transect(Stand)	0	0	0	3	1	0	0	0	3	1

July	**Richness**					**Denisty**				
	Estimate	s.d.	X^2	d.f.	p-value	Estimate	s.d.	Deviance	d.f.	p-value
Root	0.001	0.002	0.005	1	0.921	-0.047	0.027	2.193	1	0.138
Stand	0	0	0	1	1	0	0	0	1	1
Root*Stand	0	0	0	3	1	0.001	0.032	5.494	3	0.139
Transect(Stand)	0	0	0	1	1	0	0	0	1	1
Root*Transect(Stand)	0	0	0	3	1	0	0	0	3	1

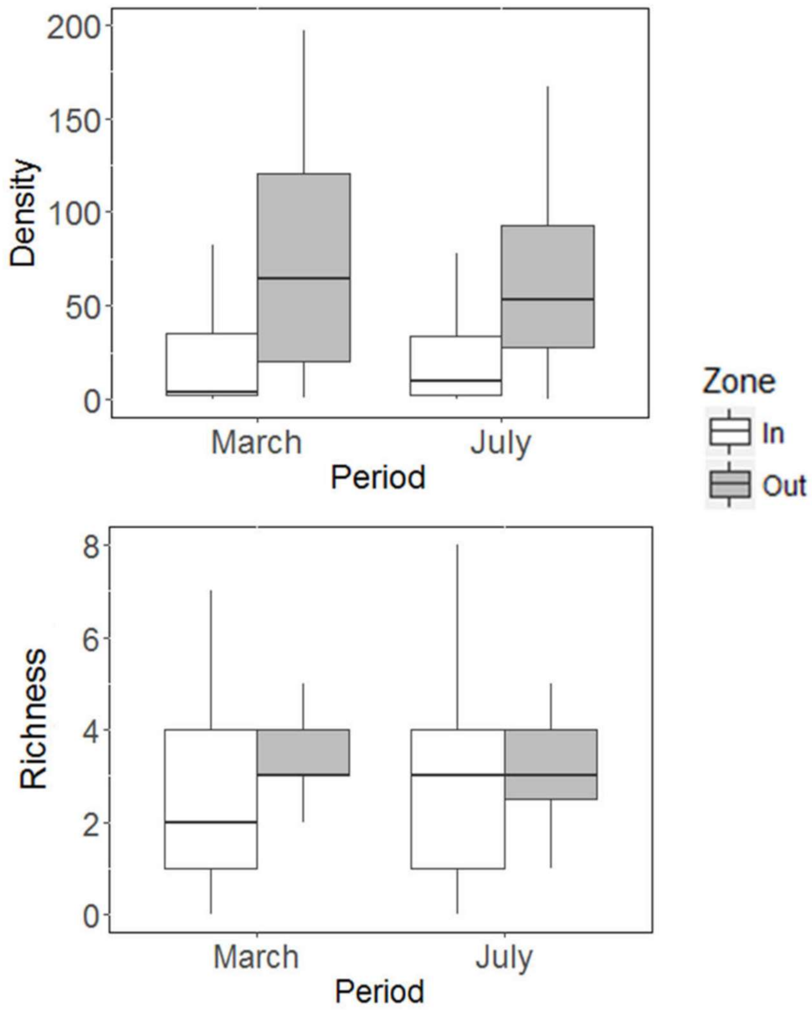

Fig. 3 Polychaete density (ind/0.015 m³) and richness at each zone (Inside/Outside) during the two sampling periods.. Boxes represent mean, first and third quartiles. Whiskers indicate the dataset range.

No difference in species richness was found between inside and outside zones, and no relationship was found with root biomass. Similarly, no effect from the random factors was found for these relationships. The number of exclusive species, however, was higher inside mangrove (*Branchiomma* sp., *Capitella* sp. H, *Cirriformia filigera* (Delle Chiaje 1928), *Diopatra aciculata* Knox & Cameron, 1971, *Marpyhsa formosa* Steiner & Amaral, 2004, *Sigambra grubei* Müller in Grube, 1858) than outside (*Aricidea (Aricidea*) *fragilis* Webster, 1879, *Leitoscoloplos fragilis* (Verrill 1873), and *Prionospio steenstrupi* Malmgren, 1867). Nevertheless, these species were found with very low densities. Thus, dissimilarity between zones was found mainly due to contrasting densities instead of exclusive occurrences.

The infaunal species that contributed most to differences between zones are shown in Table 4. *Capitella* sp.C was the species of major contribution to all fragments, occurring with higher densities outside the mangrove. Most species that contributed to differences between zones were more abundant outside the mangrove, such as *Capitella* sp. F, *Hetermosatus* sp.A and *Laeonereis culveri*. The most abundant species inside the mangrove were *Perinereis anderssoni*, *Capitella* sp. G, *Scoloplos (Leodamas)* sp.A and *Isolda pulchella,* but differences in their density between zones were less pronounced. Therefore, most abundant species inside the mangrove usually contributed less to the overall dissimilarity (Table 4).

Table 4 Summary of the species which most contributed to diferences between inside and outside zone. Mean (± sd) density values are given (ind/0.015 m^3).

Species	**Density Inside**	**Density Outside**	**2* Log-Likelihood**	**Deviance**	**p-value**
Capitella sp. C	20.6 ± 35.7	51.6 ± 48.7	-914.114	7.947	**0.046**
Heteromastus sp. A	0.8 ± 2.1	11.6 ± 19.4	-423.244	23.823	**0.002**
Laeonereis culveri	1.1 ± 3.0	3.1 ± 4.0	-350.799	5.609	0.200
Capitella sp. F	1.1 ± 3.0	3.5 ± 6.0	-343.648	5.606	0.200
Capitella sp. G	1.1 ± 2.1	0.03 ± 0.19	-158.526	20.161	**0.002**
Scoloplos (Leodamas) sp. A	1.0 ± 4.2	0.2 ± 0.5	-149.159	5.101	0.236
Perinereis anderssoni	0.6 ± 1.2	0.1 ± 0.4	-143.979	8.444	**0.043**
Marphysa sebastiana	0.3 ± 0.6	0.1 ± 0.3	-110.794	3.684	0.416
Isolda pulchella	0.2 ± 1.0	0.1 ± 0.5	-80.919	0.534	0.472
Aricidea (Aricidea) fragilis	0	0.1 ± 0.2	-28.288	5.545	0.200

Feeding guild composition did not differ between mangroves and sandflats. Subsurface deposit feeders dominated all areas in both richness and density. The relative contribution of each group shows that ~90% of individuals were subsurface deposit feeders, regardless of zone and mangrove stand. The relative contribution of omnivores was slighlty higher in mangroves, mainly due to the occurrence of the omnivore *Perinereis anderssoni* and decrease of subsurface deposit feeders *Capitella* sp. C and *Heteromastus* sp. A. Relative contributions of other feeding guilds were generally very low (< 1%) (Table 5).

Table 5 Feeding guilds richness and relative abundance at each mangrove stand.

Inside	**n**	**N1 (%)**	**N2 (%)**	**IP (%)**	**Total**
Subsurface deposit feeder	7	87.3	97.2	87.3	89.3
Surface deposit feeder	2	2.2	0.9	0.1	1.1
Omnivore	4	9.7	5.9	12.5	9.2
Herbivore	1	0.1	0	0	0.1
Carnivore	1	0	0.1	0.1	0.1
Filter-feeder	2	0.1	0.4	0	0.2
Outside	**n**	**N1 (%)**	**N2 (%)**	**IP (%)**	**Total**
Subsurface deposit feeder	9	91.5	93.4	98.7	94.4
Surface deposit feeder	1	0	0.1	0.3	0.1
Omnivore	3	8.5	6.4	0.9	5.3
Herbivore	0	0	0	0	0
Carnivore	1	0	0.1	0	0.1
Filter-feeder	2	0	0	0.1	0.1

Discussion

In accordance to our expectations, polychaete density was lower inside mangroves and negatively related to root biomass. Moreover, composition of polychaete assemblages differed between inside and outside mangrove, mainly due to a decrease in density of dominant species and increase of a few rare taxa. Contrary to expected, no difference in feeding guild composition was found, and subsurface deposit feeder dominated both mangrove and the adjacent sandflat zones.

The negative relationship between root biomass and polychaete density varied during time and was stronger during March. This result suggests that other factors, such as changes in sediment features, are also responsible for differences in polychaete density inside and outside mangroves.. Mean grain size was overall larger inside mangroves, and sediment coarseness is known to negatively affect overall polychaete density, especially in a subsurface feeder dominated assemblage (Pinedo et al. 1997, Amaral et al. 2003, Pagliosa 2005), which could explain the decrease in density in those areas.

The changes in sediment composition among zones are probably a result of hydrodynamic conditions and the presence of mangroves. The presence of pneumatophores of *Avicennia schaueriana* enhances sediment trapping and accretion, modifying the sedimentary environment and causing elevation on sediment surface (Young & Harvey 1996, Krauss et al.

2003). Thus, mangrove vegetation is likely to trap sediments that would otherwise end deposited on non-vegetated areas. Changes in sediment composition, however, were only seen consistently in stand IP. This stand is the only one located at the low intertidal area, which is subject to different hydrodynamics and sediment transport than upper intertidal areas (Bassoulet et al. 2000, Le Hir et al. 2000). The coupling of these factors is likely to cause changes in sediment composition that, together with the presence of roots, influence the density of infaunal polychaetes at the area.

Our results show that the infaunal polychaete density does not increase inside mangroves, a result that contrasts with those found for epifaunal taxa (Kathiresan & Bingham 2001, Nagelkerken et al. 2008). However, this fact may not be true for every infaunal taxon. Bosire et al. (2004) found that restored and natural mangroves had higher densities of sediment-infauna than bare sites. Nevertheless, they included class-identified taxa such as Ostracoda, Bivalvia, Gastropoda, and Isopoda, which were absent on bare sites. This result is important to stress that polychaetes may respond differently to root density than other infauna taxa.

The presence of mangrove is known to enhance the richness of macrofaunal groups, especially epifaunal mollusks and crustaceans (Frith et al. 1976, Edgar 1990, Kathiresan & Bingham 2001, Nagelkerken et al. 2008). For polychaetes, however, mangrove does not seem to enhance species richness. Nevertheless, it may have an effect on species turnover, as demonstrated by the higher number species found exclusively in mangrove than in the adjacent sandflat areas. Despite being important to the species pool, these exclusive species showed low density. Consequently, overall changes inside and outside the mangrove were more related to densities differences than the occurrence of different species.

Capitella sp.C was by far the most abundant species found regardless of zone. Three other species from the *Capitella* complex were also registered, further highlighting the abundance of this genus. These polychaetes are frequently found associated with mangroves and salt marshes (Sheridan 1997, Dittmann 2001, Netto & Gallucci 2003, Chapman & Tolhurst 2004). Dittmann (2001) observed *Capitella* species restricted to mangrove areas, and suggested that the muddy and organically rich sediments from mangroves would favor their occurrence, given the opportunistic nature of the species (Pearson & Rosenberg 1978, Weston 1990). This contrast with our results may be explained by two factors: first, although TOC was higher inside the stands during July, it is likely originated from mangrove detritus, which have a low nutritional quality and palability, and unlike to drive infauna density (Alongi &

Christensen 1992, Bouillon et al. 2002, Netto & Gallucci 2003); second, sediment composition differed between zones, with coarser fractions being registered inside some mangrove stands. Distribution of subsurface deposit feeders, such as *Capitella* spp., is negatively influenced by increasing sediment coarserseness (Snelgrove & Butman 1994, Pagliosa 2005). This also agrees with Lana et al. (1997), who found that polychaetes are likely to be more influenced by environmental changes than by the capacity to distinguish between vegetated and non-vegetated habitats.

Contrasting with previous studies that found higher trophic guild richness of polychaetes in vegetated sites (e.g., Summerson & Patterson 1984, Mattos et al. 2013), trophic group richness was not different between inside and outside mangrove at Araçá Bay. Dominance of subsurface feeders was found in both zones, probably a consequence of the low hydrodynamics conditions and finer sediments found at the study area. The three species that were most commonly found inside mangrove belong to distinct feeding guilds, *Scoloplos (Leodamas)* sp.A as a subsurface deposit feeder, *Isolda pulchella* as a surface deposit feeder, and *Perinereis anderssoni* as an omnivore (Richoux & Froneman 2008, Jumars et al. 2015, Checon et al. 2017). This result suggests that mangrove vegetation does not enhance the occurrence of a specific feeding strategy, neither increase trophic group richness, as observed for other types of vegetation such as seagrass beds (Mattos et al. 2013).

Conclusion

Our results showed that polychaete density is lower inside mangroves than at adjacent sandflats and negatively related to root biomass. Species richness and trophic group composition, however, were not affectedby the presence of vegetation. This outcome contrasts with previous results found for epibenthic fauna and highlights that mangrove vegetation may not positively affect infaunal species richness and density, as registered for epifaunal assemblages. Therefore, caution is needed and studies should not directly extend results from one group to the other. As little is known regarding the influence of mangrove on polychaete fauna, further studies could aim to improve this knowledge and investigate whether patterns found in our study can be extended to mangroves from other areas.

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Capítulo 2

Unraveling the performance of the benthic index AMBI in a South American Bay: the effects of data transformations and exclusion of low-reliability sites.

Abstract

We investigated the relationship between the AMBI index and different contamination proxies in an urbanized bay in South America (SE Brazil), and the effect of (a) abundance data transformation and (b) exclusion of low-reliability sites (high SD; low N) on the index' performance. Poor ecological quality and opportunistic species were related to an increase in contaminants concentrations and mud content. Good ecological status and sensitive species (EG I) were mainly related to increased hydrodynamics. Data transformation caused minimal changes to the overall relationships, but exclusion of low-reliability sites improved the relationship between ecological groups and contamination proxies. Our results show that AMBI is robust in detecting effects of different contaminants in the area and reinforce the importance of the index as a tool for coastal management, but local joint efforts are needed to improve and adjust local species classification in ecological groups to improve the index' performance.

Introduction

Marine ecosystems are under high pressure due to anthropic activities in coastal areas around the world (Halpern et al., 2008; Borja et al., 2016). Owing to this situation, many efforts have been made to understand the extent of these impacts and to aid management practices in marine areas. Among such efforts, several biotic indices have been developed to evaluate the ecological quality and degree of disturbance of estuarine and marine ecosystems (Borja et al., 2000; Simboura and Zenetos, 2002; Muxica et al. 2007). These indices are advantageous due to the simple calculation and interpretability, easing the communication to managers and implementation of monitoring programs (Borja and Muxica, 2005).

The AZTI Marine Benthic Index (AMBI) (Borja et al., 2000) is one of the most widely applied indices and has been used as a metric of benthic quality in many areas. The index is based on the degree of tolerance of benthic marine species to organic enrichment (Grall and Glémarec, 1997; Pinto et al., 2009), and relies on the community succession model developed by Pearson and Rosenberg (1978). In this model, increasing organic enrichment gradually shifts the benthic community towards a low richness condition, with the dominance of only a few tolerant species. Thus, AMBI was preliminarly used to assess the effects of organic matter accumulation in bottom sediments. However, researchers have also suggested its potential usefulness in detecting impacts from different sources such as heavy metals, outfalls and hydrocarbons contamination (e.g., Borja et al., 2003; Muxica et al., 2005; Muniz et al., 2005; Riera et al., 2011).

Additionally, researchers have argued that AMBI’s effectiveness and suitability might be dependent on pre-treatment of the biological data (Teixeira et al., 2014; Gillet et al., 2015). The index is usually calculated based on the raw abundance data, but different data transformations have been suggested to enhance the index performance by down-weighting dominant species (Warwick et al., 2010; Muxica et al., 2012b). To date, however, the number of studies that have assessed this topic is still small, and no consensus has been achieved about the best data treatment (Warwick et al., 2010; Muxica et al., 2012b; Teixeira et al., 2012; Gillet et al., 2015). AMBI’s effectiveness and suitability have also been shown to be affected by samples with high standard deviation (SD >2) and/or low abundance (< 3 individuals per replicate) (Borja and Muxica, 2005; Muxica et al., 2007).

Another concern about the effectiveness of AMBI is that the index was originally developed for European waters. Although it has been applied in different areas, its suitability in areas with a different species pool and climate regime still depends on calibration for better results (Teixeira et al., 2014; Gillet et al., 2015; Hutton et al., 2015). As many other economically developing countries, Brazil has a densely populated coastline with intense industrial and port activities. Given this intense pressure, there is a high potential for the use of biotic indices, but few studies have been carried out to test and validate AMBI's performance along the Brazilian coast (i.e. Muniz et al., 2005; Omena et al., 2012; Valença and Santos, 2012; Brauko et al., 2015; 2016), and only one of these studies (Muniz et al., 2005) evaluated the relationship among AMBI, hydrocarbons and heavy metals. Consequently, assessments of the index in the region are still in early stages, and further studies are necessary to better establish the validity of the index in South America.

In order to provide information and to achieve a better use of the AMBI in areas under different pressures and in a distinct region, we assessed the performance of the index in a biodiverse subtropical ecosystem impacted by different anthropogenic stressors. In particular, we verified: (1) the spatio-temporal distribution of the ecological quality in the study area according to the AMBI classification; (2) the relationships between AMBI estimates and different proxies of anthropogenic activities (i.e. total organic carbon, heavy metals and hydrocarbon); and (3) how different data transformations and exclusion of low reliability sites (i.e, high SD and low N) affect AMBI performance. Additionally, we discuss our results in regards to the implications for further studies along the Brazilian coast, which can be extended to tropical and subtropical areas where the use of benthic indices is still in its early stages.

Methods

Study area

The study was done at Araçá Bay, located on the North coast of the State of São Paulo, Southeast Brazil. The bay covers an area of 534.500 m², with a gentle slope. It is composed of a wide intertidal flat, four beaches, two rocky islands and three mangrove stands (Amaral et al., 2016; Gorman and Turra, 2016; Checon et al., 2017). The intertidal and the shallow (internal) sublittoral (up to 3 m) extend into the São Sebastião Channel, to a maximum depth of 30 m. The tides range from average levels of -0.04 to 2.06 m (Gubitoso et al., 2008).

Araçá Bay is one of the most biodiverse ecosystems on the Brazilian coast and has great ecological and social importance (Amaral et al., 2016). Nevertheless, the area has been experiencing significant impacts since the mid-1930s, when the construction of a large and structured harbor (São Sebastião Harbor) was initiated (Amaral et al., 2016). Activities such as dredging and the placement of a marine outfall followed the harbor installment and added impacts to the area. Nowadays, Araçá Bay is still under severe threat. A wide range of anthropogenic impacts, such as high organic enrichment of sediments (Gubitoso et al., 2008) and habitat loss (Mani-Peres et al., 2016), affects the area, and there is an ambitious project aiming to place pillars along the bay in order to raise a platform and expand São Sebastião Port. This platform would cover almost the entire area, and the lack of sunlight would likely hinder primary production and affect the trophic web (Amaral et al., 2016; Pardal-Souza et al., 2016). Thus, it is necessary to understand the current benthic status of the area as a baseline for conservation or management programs.

Sampling procedure

Fieldwork was performed during spring tides in October 2012 and February, May and September 2013. During each sampling event, field work was done in the early morning of two consecutive days, collecting material from 37 geo-referenced sites arranged in an irregular sampling grid, from intertidal to sublittoral areas of approximately 25 m deep (Fig. 1). Seventeen sampling sites were located in the intertidal area and twenty in the sublittoral. Four replicates (corer: 10 cm inner diameter, 20 cm depth) were collected per site and event (N = 148) for biological analyses. Sampling was performed manually at shallow sites and using a multi-corer equipment for deeper areas. At each site, sediment samples were also taken for granulometric analysis, and microphytobenthic biomass, total organic carbon (TOC), heavy metals, faecal steroids and hydrocarbon analyses.

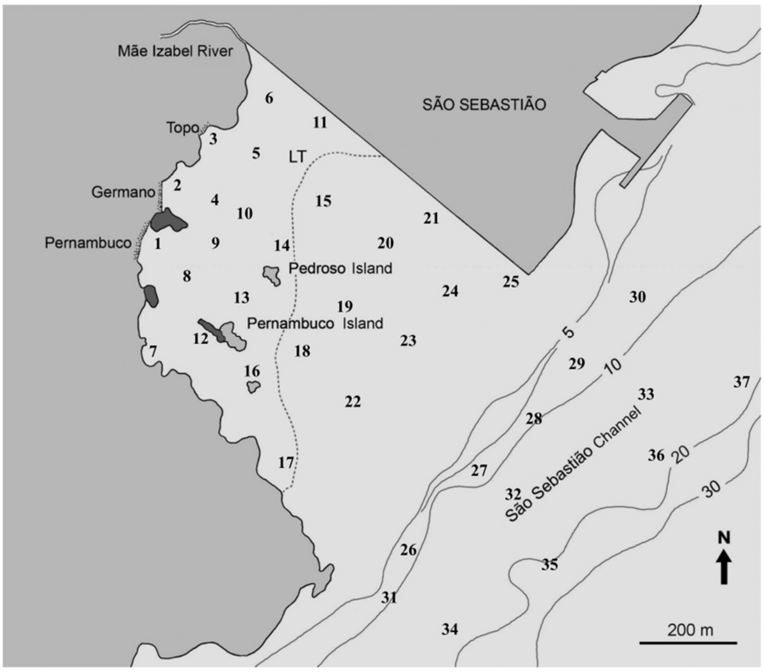

Figure 1. Map of the study area showing the 37 sampling stations along the intertidal (1 to 17) and internal sublittoral (18 to 25) at Araçá Bay, and external sublittoral at the São Sebastião Channel (26 to 37).

Biological samples were placed in plastic bags and taken to the Marine Biology Center from University of São Paulo (CEBIMar – USP) for analysis. Sediment samples for environmental analyses were placed in appropriate containers to avoid contamination and immediately taken to the laboratory. Heavy metal and hydrocarbon analyses were only carried out during the first and last sampling events. Due to financial and logistic constraints, heavy metal and hydrocarbon analyses were only carried out during the first and last sampling events, representing eventual strong temporal differences between them. As such changes were not observed, the remaining sampling events were not evaluated in regards to contamination parameters. Faecal steroids were quantified only in the first sampling period due to financial constraints and it was only used as a characterization variable.

Samples processing

Macrofaunal samples were sieved through a 0.3 mm mesh on the same day of collection. The retained fauna was sorted, fixed in 70% ethanol and posteriorly identified to the lowest possible taxonomic level.

Granulometric analysis was performed using the routine sieving and pipetting techniques described by Suguio (1973). Sediment parameters were obtained using SysGran software, version 3.0 (Camargo, 2006). The spatial distribution of wave generated bottom orbital velocities (given in $m.s^{-1}$) was obtained through the application of a wave propagation numerical model for the Araçá bay. The Delft 3D wave module (Deltares, 2014) has been applied based on high resolution morphology of the area using the measured wave climate at the entrance of the bay as a boundary condition. Based on the nearshore wave propagation, orbital velocities have been defined for each sampling point. Microphytobenthic biomass was estimated from phaeopigments and chlorophyll a concentrations according to Plante-Cuny (1973).

Organic contaminant analysis was done using methodology described in detail in Bícego et al. (submitted). Determination of hydrocarbons followed the methodology described in UNEP (1992). About 20 g of lyophilized samples were extracted via the Soxhlet process, with n-hexane/dichlorometane. The resulting extract was separated in two fractions (F_1 for aliphatics and F_2 for polycyclic aromatics) by column chromatography using alumina and silica-gel. Concentration of aliphatic hydrocarbons (AH) were determined using a gas cromatograph with flaming ionization detector (GC-FID); whereas polycyclic aromatic hydrocarbons (PAHs) were determined using a gas cromatograph equipped with a mass spectrometer (GC-MS). Certified standards from AccuStandard (USA) were used to build analytical curves and blanks, while reference material from the National Institute of Standards and Technology NIST (SRM1944) was used for surrogates. Analysis was validated using values from certified reference material 1941b, from NIST.

Total faecal steroids and coprostanol analysis was based on a method described by Kawakami & Montone (2002). Part of the F2 fraction was derivatized using Bis (trimethylsilyl*)* trifluoroacetamide + 1% chlorotrimethylsilane, and concentrations were determined using a gas cromatograph equipped with a mass spectrometer (GC-MS). Coprostanol values were evaluated following guidelines by Gonzalez-Oreja and Saiz-Salinas (1998), considering 0.5 $\mu g\ g^{-1}$ as cutoff values to indicate sewage contamination. The coprostanol/(coprostanol+cholestanol) ratio was also calculated to check the influence of sewage input, which is suggested when ratios are higher than 0.7 (Grimalt et al., 1990).

The Carbon Preference Index (CPI) was calculated in order to estimate the relative contribution of biogenic sources to AH estimates, based on the concentrations of *n-alkanes* (Aboul-Kassim and Simoneit, 1996). CPI values close to 1.0 can indicate contamination from petroleum hydrocarbons, whereas values higher than 1.0 indicate predominance of hydrocarbons from biogenic sources (Aboul-Kassim and Simoneit, 1996). Unresolved

Complex Mixture (UCM) was checked for the detection of oil sources for the hydrocarbons present in the sediment (Farrington et al., 1977). The concentration of PAHs, whose main sources are from the combustion of fossil fuels, direct input of oil and derivatives, and release of domestic/industrial wastes (Colombo et al., 1989), were evaluated using cut-offs established by Notar et al. (2001), which considers values between 250 and 500 ng g^{-1} as moderately polluted and above 500 ng g^{-1} as contaminated.

Heavy metals concentration was determined after protocols established by the United States Environmental Protection Agency (USEPA). Method 3050b (USEPA 1996) was applied to digestion and method 6010c (USEPA 2007) for analysis of concentrations under optical emission spectrometry (ICP-OES). The following metals were evaluated: aluminum (Al), arsenic (As), cadmium (Cd), chrome (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), scandium (Sc), tin (Sn) and zinc (Zn). The analysis was tested using certified reference materials SS1 and SS2 (EnviroMAT^{TM}). Values obtained for every element was within the tolerance interval from the reference materials. Concentrations were compared with reference values from threshold effect level (TEL), below which adverse effects to fauna are not likely to occur (MacDonald et al., 1996).

Total organic carbon (TOC) content was evaluated following the methodology described by Gaudette et al. (1974), based on the (exothermic) oxidation using Potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid (H_2SO_4). TOC was obtained following titration of the exceeding dichromate with Ammonium Iron Sulfate (Mohr's Salt).

Data analysis

Benthic quality indicated by AMBI

AZTI Marine Biotic Index (AMBI) is based on the proportions of taxa belonging to each of the five ecological groups, divided according to their sensitivity to organic pollution: EG I (Sensitive), EG II (Indifferent), EG III (Tolerant), EG IV (Second-order opportunists) and EG V (First-order opportunists). The index was calculated using the software provided at AZTI's web page (www.azti.es), following the guidelines of Borja & Muxica (2005).

Ecological grouping of taxa was done following the latest update of the AMBI database (November/2014) downloaded from the website. However, since this list is mostly based on taxa found in European waters, some species found in our study area were not yet included in the database. To achieve a better performance of the index, we used similar classification criteria as those made in previous studies in Brazil (i.e., Muniz et al., 2005; Omena et al., 2012; Valença and Santos, 2012; Brauko et al., 2015). When species were not classified, they were assigned to the most common group found for the *genus*. In the absence of the *genus*, species were classified as "not assigned". Index values were calculated for each replicate within site and sampling period individually. Site-specific variation in benthic status indicated by the index between periods was checked for temporal consistency.

Relationships between AMBI, ecological groups of taxa and contaminants

A predictive model was used to relate AMBI values as a function of the contamination parameters using data from the first and last sampling periods. Faecal steroids were not included because they were measured only in the first sampling period. Heavy metals had a high correlation among every pair (> 0.7). Thus, a single variable was created using the sites scores from the first axis of a principal component analysis. Due to the expected high collinearity between environmental variables (e.g. sediment parameters), an ordinary multiple linear regression could result in biased coefficients. Therefore, we used Partial Least Square (PLS) regression (Wold, 1975) to correct for such bias. This procedure consists of generating linear combinations of predictors, via principal component rotation, in order to best explain variance in the dependent variable (Carrascal et al., 2009). The components generated by this procedure were then related to the distribution of AMBI. Correlation between variables and the PLS component was used to assess interpretability of each component.

Redundancy analysis (RDA) was used to explore the correlation between the five ecological groups of AMBI (EGI to EGV), sites and contamination variables. Community data was log (x+1) transformed to reduce the effects of extreme values and the distance matrix was calculated using the Hellinger function (Legendre & Gallagher, 2001). The selection criteria for the inclusion of variables in the model was the double stoppage rule, which uses both adjusted R^2 and p-values, reducing type I-error (Blanchet et al., 2008a).

Pre-treatment of data

PLS regression was carried out multiple times to evaluate the influence of (a) abundance data transformation, and (b) exclusion of low-reliability sites. Low-reliability sites were defined as sites where samples standard deviation (SD) is higher than 2 and the number of individuals (N) is less than 3 per replicate. We used square-root, fourth-root, and ln (x+1) transformations and compared the explained variation between each transformed vs untransformed model. We also compared a model with all sites (total model) against a model with the exclusion of low-reliability sites (robust model) to check whether a better fit with chemical proxies would be achieved by suppressing these sites. Redundancy analysis (RDA) was also carried out twice to compare the influence of exclusion of low-reliability sites on the relationship between ecological groups and contamination proxies, using the data transformation that better fitted the relationship with the index on PLS regression models.

All statistical analyses were conducted using the R Software v 3.0.2 (R Core Team, 2016), using the packages *vegan* (Oksanen et al., 2013), *packfor* (Dray et al., 2013) and *plsdepot* (Sánchez, 2012).

Results

We recorded 7266 individuals from 147 taxa during the study. Polychaetes were the dominant taxa, both in number of species and abundance of individuals. Mollusca, Crustacea and Echinodermata were also commonly found. Conversely, Anthozoa and Cephalochordata were far less common. Forty-seven species (31.97%) were classified as EG I, 46 species (31.29%) assigned to EG II, 21 species (14.28 %) assigned to EG III, 14 species (9.5%) assigned to EG IV and 5 species assigned to EG V (3.4 %) (Annex 1). In terms of abundance, however, EG II and EG V dominated, due to the high densities of *Monokalliapseudes schubartii* and *Capitella nonatoi,* respectively. Forty-four taxa were classified according to genus (29.9%), whereas 14 species (9.4 %) were unassigned to the AMBI list.

Contamination parameters

Total organic carbon content was generally lower in intertidal sites, ranging from 0 % to 0.88 %, with an overall mean of 0.33 %. Values increased with depth, with shallower sublittoral values progressively increasing towards deeper sites and reaching a maximum of 2.77 % (Site 37, first sampling period) (Table 1).

Total faecal steroids concentrations ranged from <0.010 to 76.35 $\mu g\ g^{-1}$. The highest values were registered at sites 3 (intertidal) and 27 (10 m deep). Coprostanol concentrations reached values above 0.10 µg g^{-1} on almost half the sites at Araçá Bay, suggesting sewage contamination in the sediments. Four sampling sites (3, 6, 11 and 27) had values higher than 0.5 µg g^{-1}, indicating strong contamination by sewage-derived material. The coprostanol/(coprostanol + cholestanol) ratio indicated a similar result, with many sites in intertidal and internal sublittoral areas with ratios higher than 0.7 (0.71 – 0.98 range), especially at sites 3, 6, 8, 11, 19 and 20.

Table 1. Summary of contamination proxies found at Araçá Bay. Mean values are given for variables measured in more than one sampling period.

St.	TOC (%)	Total Metals (µg g^{-1})	TAH (µg g^{-1})	Cpi	UCM	PAH (µg g^{-1})	Steroids (µg g^{-1})	Coprostanol (µg g^{-1})	Ratio
1	0.05	10131.24	10.65	1.6	7.665	298.71	2.58	0.25	0.71
2	0.10	16080.98	5.52	1.4	3.32	21.65	0.35	0.01	-
3	0.52	11118.545	31	4.2	27.55	93.05	34.54	6.32	0.8
4	0.44	20755.77	16.5	1.7	13.75	14.95	0.01	0.01	-
5	0.56	12676.705	15.25	-	13.45	3.915	23.1	2.29	0.7
6	0.55	16676.11	23.95	2.3	21.6	22.065	45.8	18.9	0.89
7	0.51	15647.9	17.9	4.6	15.1	31	1.06	0.01	-
8	0.34	24032.58	27.15	14.3	23.55	34.4	5.95	1.81	0.95
9	0.40	22787.61	23.95	2.2	21.1	67	0.01	0.01	-
10	0.26	20448.41	12.7	-	10.475	3.035	1.91	0.1	0.71
11	0.30	25286.25	18.8	4.85	15.5	69.5	20	9.03	0.93
12	0.36	19491.2	16.4	-	14	25.4	0.23	0.01	-
13	0.52	28893.06	26.65	4.7	23.3	82.6	4.01	2.05	0.79
14	0.18	28225.045	12.2	-	10.05	115.2	3.38	0.02	-
15	0.18	19701.39	10.38	-	8.7	85.95	0.01	0.01	-
16	0.26	21451.065	19.1	10.25	16.9	287	23.8	2.48	0.75
17	0.26	18227.52	6.345	-	4.76	5.3	0.01	0.01	-
18	0.18	17439.92	1.275	-	-	0.5	0.4	0.01	-
19	0.12	16296.64	5.12	-	3.525	4.305	1.31	0.82	0.93
20	0.43	20141.735	7.335	18.5	5.25	59.9	5.94	1.61	0.98
21	0.12	20398.975	2.04	-	-	43.85	11.4	0.01	-
22	0.10	11242.63	1.415	-	-	2.1	0.01	0.01	-
23	0.24	20127.425	2.085	-	-	3.425	0.82	0.27	0.69
24	0.22	23508.215	2.335	-	-	55.05	0.99	0.43	-
25	0.41	28981.04	6.435	-	3.345	57.85	8.3	1.85	0.71
26	1.44	61354.635	26.95	5.3	15.9	523.5	0.01	0.01	-
27	1.59	77567.305	31	5.4	16.9	433.5	76.35	9.3	0.63
28	1.77	68911.99	33.45	5.6	17.1	521	36.7	0.01	-
29	1.98	62174.445	25.55	5.65	15.65	469	0.01	0.01	-
30	2.16	73041.695	26.6	5.6	16.7	365	2.02	0.91	0.91
31	1.28	49231.99	20.45	6.45	13.28	267	15.1	0.01	-
32	2.34	75367.495	33.45	5.75	20.25	391	31.7	0.01	-
33	1.76	63245.135	26	5.65	15.5	375.5	3.79	0.01	-
34	0.98	78245.19	18.5	5.6	4.9	190	0.13	0.01	-
35	2.28	62055.59	22.9	5.75	13.95	356	24.2	0.28	0.11
36	1.94	64262.765	24.25	5.9	14.6	402.5	2.21	0.11	0.13
37	2.01	66470.98	22.2	5.4	13	366.5	0.97	0.01	-

Ratio = Coprostanol/(Coprostanol/Colestanol)

The concentration of total aliphatic hydrocarbons was higher at the external sublittoral and upper intertidal areas, ranging from 1.27 µg g^{-1} (site 18) to 33.45 µg g^{-1} (site 28). With CPI values close to 1 in the sites 1, 2 and 4, a petroleum contamination is possible, but the UCM at these sites were above detection limit. In contrast, sublittoral hydrocarbons are likely derived from biogenic sources, with CPI values much higher than 1. The PAH concentration indicates moderate to contaminated sites at Araçá Bay, especially at the deeper sites (e.g. 523.5 and 521 ng.g-^{1} at sites 26 and 28, respectively) and in the upper intertidal area (298.1 ng.g-^{1} at site 1). Heavy metal concentrations were markedly higher in sublittoral than intertidal sites, but no individual metal value exceeded the threshold effect level. The first axis of principal component analysis of heavy metal data explained 90% and 88% of the variation for the total and robust model, respectively. The sites scores were used in further analysis, with increasing scores representing higher metal concentrations.

Benthic quality indicated by AMBI

According to AMBI, the majority of sites in Araçá Bay was classified within the "good" and "moderate" status (49 % and 27 %, respectively). A range of 6 to 16% of the sites was classified within the “high” ecological status throughout the sampling periods. Nevertheless, 13 to 19 % of sites were ranked as heavily disturbed or “poor” and 3 to 11% as extremely disturbed or “bad”.

AMBI values showed spatial consistency between sampling events, but temporal variability was observed in some areas. Deeper sublittoral sites were generally more disturbed than shallower and lower intertidal sites. These deeper sites, however, were the ones with a higher number of low-reliability sites. Upper intertidal sites attained heavy to extremely heavy levels of disturbance. The remaining intertidal and sublittoral areas showed overall good conditions (Fig. 2). Sites with poorer conditions had the highest temporal variation, with sites varying from good to poor ecological conditions throughout the study. On the other hand, sites located at the lower intertidal area had the highest temporal consistency, with small variations from slightly disturbed to undisturbed.

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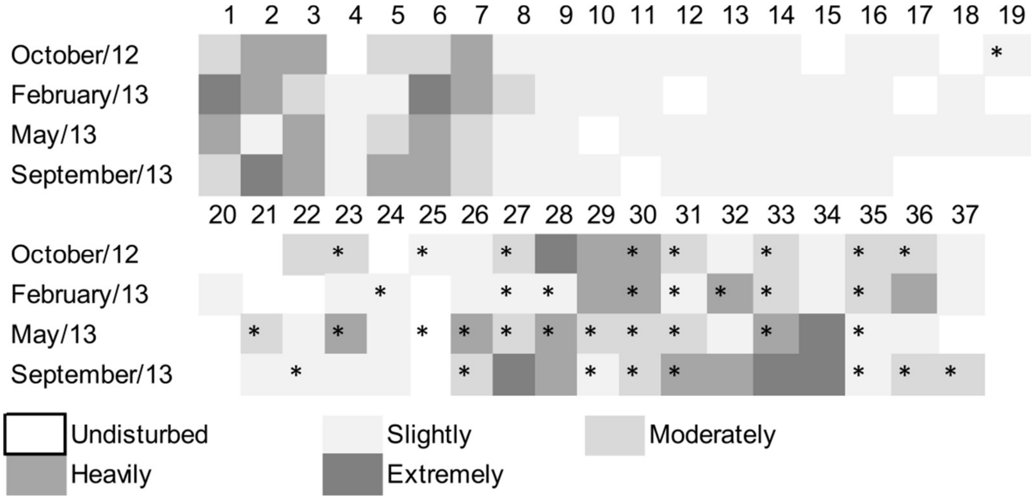

Figure 2. Classification of ecological status based on AMBI calculation. (*) denotes sites with high standard deviation (S > 2) and low abundance (< 3 individuals/replicate). Intertidal (1-17), Internal Sublittoral (18-25) and External Sublittoral (26-37) stations.

Relationship between benthic index and contamination proxies

AMBI disturbance classification showed positive correlations with all contamination proxies. Nevertheless, the correlation was lower for heavy metals (Fig. 3).

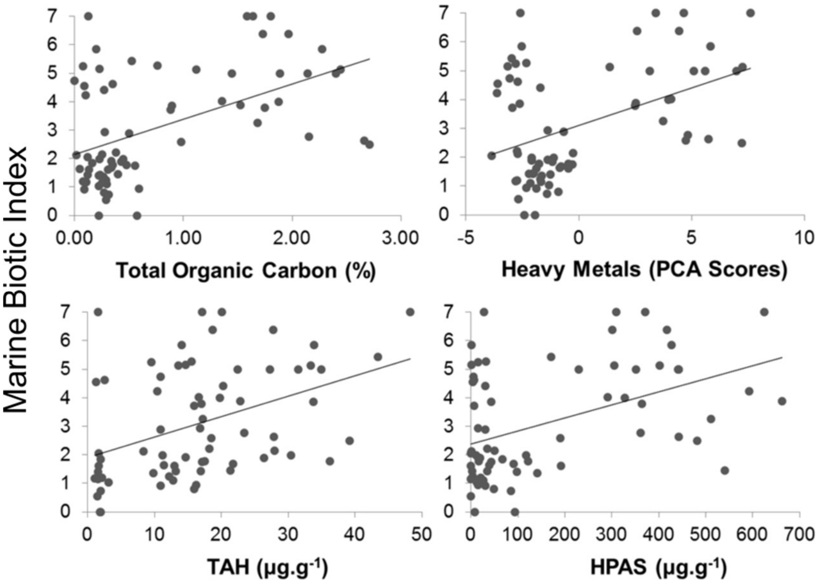

Figure 3. Distribution of contamination proxies and AMBI. Spearman pairwise rank correlations: TOC: r = 0.40, p < 0.001; Heavy Metals: r = 0.25, p = 0.041; TAH: r = 0.41, p < 0.001; PAHS: r = 0.37, p = 0.002.

The first component generated by PLS was positively correlated to contamination parameters and silt/clay content, explaining 34.78 to 37.46 % of the total variance of AMBI values. Conversely, this component was negatively correlated to orbital velocity, fine sand percentage and chlorophyll a (Table 2). The relationship between AMBI values and the latent variable (PLS component) was positive and significant for total and robust (without low reliability sites) models (Fig. 4).

Table 2. Correlation values between contaminants/environmental variables and AMBI with the PLS generated variable for each model. Only the first component of each model was included. Total model refer to the inclusion of all stations, and the robust model to the suppression of low reliability stations.

	Total				Robust			
	AMBI	2√ AMBI	4√ AMBI	Ln AMBI	AMBI	2√ AMBI	4√ AMBI	Ln AMBI
Clorophyll A	-0.630	-0.632	-0.632	-0.631	-0.521	-0.522	-0.521	-0.52
Orbital velocity	-0.940	-0.937	-0.936	-0.937	-0.92	-0.917	-0.916	-0.917
TOC	0.912	0.917	0.919	0.917	0.866	0.873	0.877	0.874
Peebles	0.143	0.124	0.117	0.122	0.255	0.241	0.237	0.239
Coarse sands	0.018	-0.001	-0.1	-0.003	0.172	0.154	0.144	0.152
Fine sands	-0.813	-0.805	-0.802	-0.804	-0.84	-0.832	-0.827	-0.831
Silt/clay	0.716	0.726	0.73	0.727	0.8	0.81	0.816	0.812
Mean diameter	0.657	0.672	0.678	0.673	0.476	0.492	0.501	0.494
Sorting	0.828	0.823	0.822	0.823	0.799	0.796	0.795	0.795
Metals	0.911	0.916	0.919	0.917	0.851	0.857	0.862	0.857
TAH	0.688	0.689	0.689	0.691	0.664	0.662	0.663	0.634
PAHS	0.811	0.815	0.816	0.815	0.757	0.76	0.762	0.761
R^2	33.81%	34.41%	36.74%	34.84%	36.67%	37.57%	40.06%	38.13%

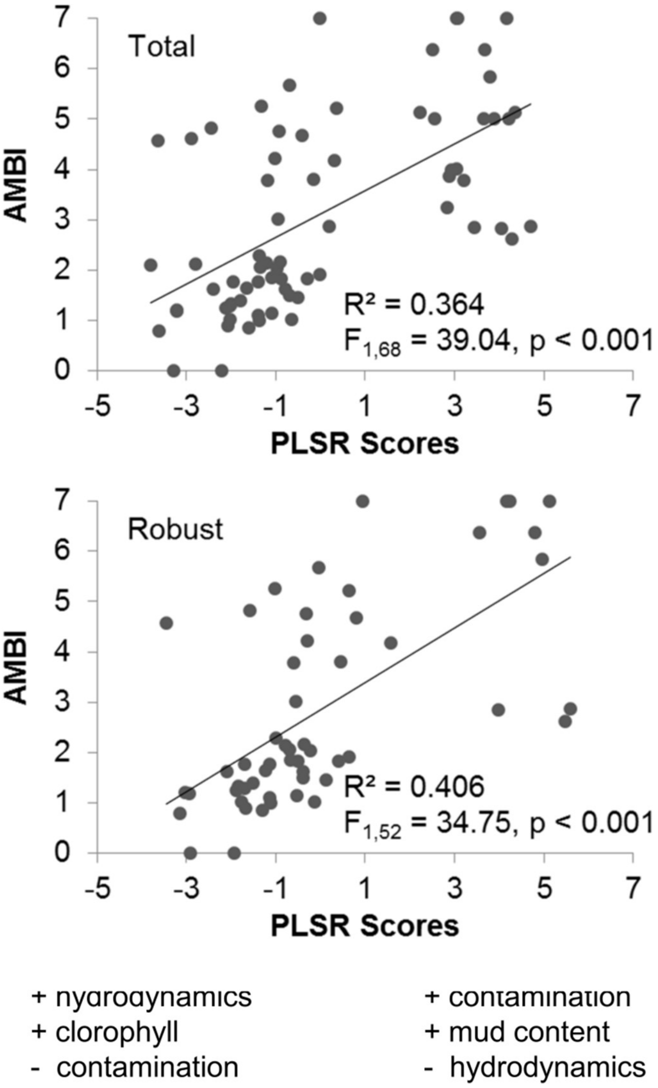

Figure 4. Relationship between AMBI values and Partial least square scores from the first component for each model (total vs reduced).

In both models (total and robust), data transformation added very little power to the explained variance, the fourth-root transformation being the model with slightly higher adjusted R^2. Similarly, the exclusion of low reliability sites did not increase much explanation to AMBI variation. The interpretation for each model did not change, as the variables correlated to the first component were generally the same (Table 2).

Ecological groups distribution and contamination proxies

Redundancy analysis models indicated that contamination and granulometric parameters explained most of the variance on ecological group distributions. The exclusion of sites with low reliability improved the adjusted R^2 value from the model, and better fitted the relationships between ecological groups and contamination proxies (Table 3). These low-reliability sites were mainly located at the sublittoral and the low ecological status of these sites were not driven by opportunistic species (EG IV-V), which were more related to intertidal areas (Fig. 5A). Ecological group I (EG I), composed of the most sensitive species, was mainly found in the intertidal and internal sublittoral, subjected to higher wave orbital velocities and predominance of fine sands. In both models, EG I was negatively correlated with contamination proxies, especially in the robust model. Contamination proxies (aliphatic hydrocarbons, heavy metals and total organic carbon) were related to opportunistic groups, especially EG IV, and the relationship was stronger in the robust model (Fig. 5B).

Table 3. Redundancy analysis results showing the explained variance, adjusted R^2 and selected variables for each model for the relationships between environmental/contamination variables, ecological groups and sites.

Model A (All stations)				
Expained Inertia	26.30			
Adj R^2	21.46			
RDA 1	0.149			
RDA 2	0.104			
		Adj R^2 Cum	F	p
TAH		0.108	8.912	0.001
Metal		0.197	5.862	0.001
Coarse sand		0.206	3.739	0.011
Fine sand		0.237	2.677	0.033
Orbital velocity		0.243	2.317	0.047
Model B (Robust stations)				
Explained Inertia	45.53			
Adj R^2	36.21			
RDA 1	0.229			
RDA 2	0.124			
		Adj R^2 Cum	F	p
Fine sands		0.116	7.453	0.001
Metal		0.173	4.360	0.004
TAH		0.222	3.947	0.005
Cloro/Phaeop.		0.274	3.613	0.010
TOC		0.314	2.984	0.021
Orbital Velocity		0.355	3.124	0.023

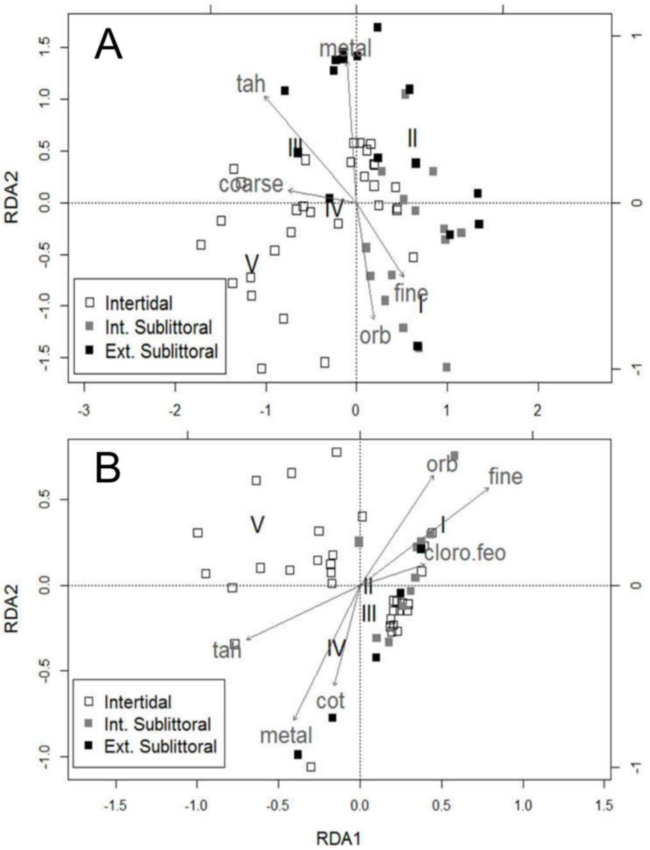

Figure 5. Redundancy analysis triplot (scaling = 2) representing the relationships between contamination/environmental variables (arrows), ecological groups (I-V) and sites (squares). Only significant variables are shown for each model (A: All stations), (B: Robust stations). (tah: total aliphatic hydrocarbons; ; orb: orbital velocity; cot: total organic carbon).

Discussion

Spatio-temporal distribution and relationship with contaminants

Our results showed that the AMBI is positively related to increases in organic matter, hydrocarbons and heavy metals content, reinforcing its importance to detect the effects of different contamination sources (Borja et al., 2003; Muxica et al., 2005; Muniz et al., 2005; Albano et al., 2013; Hutton et al., 2015). Contaminant content was negatively related to sensitive species, whereas the opposite relationship was found for opportunistic species. The contrasting distribution of opportunistic (EG IV/V) and sensitive species (EG I) shows a pattern of low co-occurrence at the bay, corroborating the divergent response of these species. Overall, the ecological status derived from the marine benthic index AMBI indicates that areas with moderate to extreme disturbances were found mainly at the upper intertidal and deeper sublittoral areas.

Good ecological status and sensitive species (EG I) were found in areas with high values of wave orbital velocity. High hydrodynamics are associated with lower deposition of organic carbon (Giles et al., 2009). Thus, these areas are expected to have higher resistance to organic enrichment (Harsten and Rowden, 2004; Keeley et al., 2013). The occurrence of mostly sensitive species in these areas resulted in most stations in the intertidal and internal sublittoral being classified as undisturbed/slightly disturbed. Poor ecological conditions were found mainly in the upper intertidal and external sublittoral stations, in areas with higher content of contaminants. However, given the low-reliability of many sites in the deeper areas, the upper intertidal is the area where low ecological quality can be more confidently inferred. The poor conditions found in this area are likely a consequence of domestic and industrial sewage inputs by riverine transport (Mãe Izabel River) and discharges from nearby houses (Carrilho, 2015; Gorman et al., 2017). This was confirmed by faecal steroid results, which indicated a strong sewage contamination in this area, with values higher than previous reports on disturbed areas of the South American coast (Martins et al., 2008; Martins et al., 2010; Albano et al., 2013), and similar to those found in areas subjected to direct sewage contamination (Venturini et al., 2015). Coprostanol values were very high in some sites (e.g. 18.9 $\mu g.g^{-1}$ at site 6, 9.3 $\mu g.g^{-1}$ at site 27), found at much higher concentrations than the 1.00 $\mu g.g^{-1}$ threshold suggested by Martins et al. (2007), and surpassing values from contaminated areas around the world (Readman et al., 2005; Tolosa et al., 2014). This result confirms previous reports of contamination in the area, highlighting the impact of the nearby marine outfall (Gubitoso et al., 2008; Muniz et al., 2015).

Temporal variations in ecological quality were observed throughout the study period, especially in areas with poorer conditions. AMBI has been shown to be stable to natural temporal variability in previous studies (Salas et al., 2004; Reiss and Kröncke, 2005; Hutton et al., 2015). However, in areas subjected to anthropic interferences, the index might temporally oscillate (Borja et al., 2003; Chainho et al., 2007). The upper intertidal area is the closest to the urban area, and may be susceptible to more direct and constant interference from human settlements (Carrilho, 2015; Amaral et al., 2016), which could explain the local temporal instability in the area. The deeper stations also concentrated most of the low-reliability sites, which likely play a role in the temporal variability. Temporal variability is an important issue to be addressed in monitoring programs (Paiva, 2001), and the uses of benthic indices need to account for this variation. Therefore, we suggest that investigations on the ecological quality of marine benthic environments should not be based on only one sampling event. This would be important to address possible seasonal changes in benthic communities (e.g. recruitment or mortality pulses) or contaminants content (e.g. increase of pollution during summer holydays) which could compromise reliable results in the long-term.

AMBI, as well as second-order opportunists (EG IV), were correlated with total organic matter, as expected due to the ecological grouping relationship with organic enrichment succession. The correlation, however, was generally lower than those reported in previous studies (Borja et al., 2000; Muniz et al., 2005; Warwick et al., 2010; Brauko et al., 2015), and is a possible consequence of the overall low values of total organic carbon at the study area. The index was also correlated with silt/clay fractions. AMBI is considered biased in muddy areas due to the natural accumulation of organic matter in this type of sediment (Blanchet et al., 2008b). Aside from organic matter increasing with mud fractions in the area, hydrocarbons and heavy metals also increased with presence of silt/clay. Thus, the relationship of the index with silt/clay fractions may also be an indicative of higher contamination, as increases in these sediment fractions enhance the surface area for the adsorption of pollutants (Thompson and Lowe, 2004; Muniz et al., 2015; Cardoso-Silva et al., 2016).

AMBI and opportunistic groups were positively correlated with hydrocarbons, reinforcing the usefulness of the index to detect impacts from different sources of contamination other than organic enrichment (Muniz et al., 2005; Muxica et al., 2005; Riera et al., 2011). Contamination by petroleum derived material at Araçá Bay is found mainly at the upper intertidal area, as shown by CPI and UCM values (Volkmann et al., 1992; Bouloubassi et al., 1997; Muniz et al., 2004). This contamination is likely to arise from port activities and domestic run-off due to the increasing urbanization in the surrounding area. On the other hand, aliphatic hydrocarbons from the deeper stations are mainly originated from higher plants wax (Bouloubassi et al., 1997). This may be a reason for the better correlation between opportunistic species and hydrocarbons with the exclusion of low-reliability sites (mainly located at the external sublittoral).

Heavy metal content was negatively related to sensitive ecological groups (EG I) and positively related to second-order opportunists (EG IV). This result agrees with Josefson et al. (2008), who found opportunistic species increasing in abundance with heavy metal content. Despite the consistent relationship between heavy metal content and the benthic index, it was not as strong as with the other contamination proxies. This is probably related to the low values of metals at Araçá Bay, which were below the threshold effect level and values reported at contaminated areas (Muniz et al., 2004; Muxica et al., 2012; Hutton et al., 2015). In fact, previous studies associating the benthic index with heavy metal contamination were carried in more polluted areas (Josefson et al., 2008; Ryu et al., 2011; Muxica et al., 2012). The low correlation found here can be appointed as an ability of the index to detect the degree of contamination by heavy metals.

Impact of data pre-treatment

Our results suggest a better relationship of AMBI with a contamination gradient (higher R^2) using abundance transformation, but the improvement was very small. Several authors have suggested that data transformations may improve AMBI performance, however, there is no consensus about which one should be used. For example, Warwick et al. (2010) found improvement with square root transformation, while Teixeira et al. (2012) advocated the use of fourth-root transformation. Gillet et al. (2015) suggested natural logarithmic transformation, but this same method has been found to decrease relationship with contaminants (Warwick et al., 2010). Our results showed that data transformation had little effect on the relationship between AMBI and contamination proxies, indicating that no transformation can yet be considered as ideal. Local differences, such as species dominance and dispersion of abundance data, are likely to influence the outcome of data transformation. Considering that we found no evidence of changes in the interpretability using transformed data, our results strengthen the comparability of different studies, regardless of pre-treatment of data (Muxica et al., 2012).

The exclusion of low reliability sites in the analyses (i.e. the application of the reduced model) also showed little improvement in the relationship between AMBI and contamination gradient. AMBI is calculated as a mean value from all replicates in a given station (Borja and Muxica, 2005). Thus, areas with high standard deviations are those where ecological quality varies greatly among replicates, generating unreliable results. That may be especially troublesome in areas with natural low abundances, which can wrongfully suggest azoic conditions or disturbance even without the occurrence of an opportunistic group. Also, these low abundances may mask the EG's composition. This is likely the reason for the improvement of the relationship between contaminants and ecological groups with the exclusion of these stations. This highlights the importance of following the guidelines, especially for the case of standard deviation, which can directly affect the results more strongly than data transformation.

AMBI use in tropical/subtropical environments

The AMBI has mainly been developed for European waters, but it is widely applied in benthic studies worldwide. Given that different regions are expected to have different species pools with contrasting tolerance (Grémare et al., 2009), it is important that the index is calibrated and tested before being applied in different areas; otherwise, its effectiveness may be seriously compromised. For example, AMBI has performed unsatisfyingly in US waters, and researchers highlight that different studies are needed to validate and improve its performance (Teixeira et al., 2012; Gillet et al., 2015). One of the reasons for this poor performance is the extrapolation of the classification of ecological groups from other areas.

So far, studies evaluating the AMBI as a measure of ecological quality on the Brazilian coast show promising results (e.g. Muniz et al., 2005; Valença and Santos, 2012; Brauko et al., 2015), including the ones presented here. However, to achieve a better performance, it is important that AMBI should be used with modifications from local experts, especially in regards to ecological grouping (Teixeira et al., 2012; Gillet et al., 2015). Species tolerances and response to contamination may change in the existence of a secondary major gradient, such as salinity or dissolved oxygen, or geographical location (Bustos-Baez and Frid, 2003; Zettler et al., 2013). This highlights the need to address the contrasting tolerances of species between locations. Gillet et al. (2015) have shown that the AMBI has improved local performance, on the US coast, when ecological grouping is adapted to local conditions.

In the present study, we used similar classification criteria as those made in other studies in Brazil (Muniz et al., 2005; Omena et al., 2012; Valença and Santos, 2012; Brauko et al., 2015) to allow comparability, but many species commonly found on the Brazilian coast are still not included in the database, resulting in generalization when classifying species. In fact, 30% of species were classified according to genera and 10% were not found in the database. Many of these species are indeed rare, and their inclusion is not likely to have a strong impact on the index performance. However, when more abundant species are considered, the impact is likely to be clearly identifiable. For instance, *Monokalliapseudes schubartii*, an EG II species in the database, is suggested as opportunistic in some areas on the Brazilian Coast (Leite et al., 2003; Pennafirme and Soares-Gomes, 2009), mainly by its reproductive behavior rather than relationship with organic enrichment. Also, *Capitella* species are considered first-order opportunists, but studies have shown that species within the genera have contrasting tolerances to pollution effects (Bach et al., 2005). It is important therefore that local experts in South America try to carry out similar efforts as those described by Gillet et al. (2015). Although a complete assessment of benthic fauna is not feasible, addressing the ecological tolerance of abundant species is a first step to improve the regional use of benthic indices.

Conclusion

Our study shows that AMBI correlated positively with all contamination gradients, but it was more efficient in detecting organic matter and hydrocarbons impacts. Data transformation and exclusion of low-reliability sites had very little effect on this relationship of AMBI with contamination proxies. Exclusion of sites, however, improved the relationship between opportunistic species and contamination proxies, and further studies should be aware of the issue of low-reliability. Overall, our results, together with previous studies, are promising in regard to the use of AMBI on the Brazilian coast; however, many local species remain unassigned in AMBI’s database. Therefore, it is important that a joint effort of researchers would be conducted in order to recognize regional differences in species pool and ecological tolerances. This would greatly improve the ecological group classifications in the area, thereby increasing AMBI effectiveness and providing an important tool to monitor and preserve our coastal ecosystems.

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Annex 1. Ecological group classification of species registered at Araçá Bay

Ecological Group 1	
Anomalocardia brasiliana	Bivalvia
Crassinella sp.	Bivalvia
Ctena pectinella	Bivalvia
Nucula semiornata	Bivalvia
Tellina gibber	Bivalvia
Tellina lineata	Bivalvia
Tellina martinicensis	Bivalvia
Tellina sp.	Bivalvia
Tellina trinitatis	Bivalvia
Olivella minuta	Gastropoda
Paradentalium dispare	Scaphopoda
Aricidea (Aricidea) fragilis	Polychaeta
Aricidea (Aricidea) wassi	Polychaeta
Armandia agilis	Polychaeta
Armandia hossfeldi	Polychaeta
Chaetopterus sp.	Polychaeta
Clymenella brasiliensis	Polychaeta
Clymenella dalesi	Polychaeta
Diopatra aciculate	Polychaeta
Magelona papillicornis	Polychaeta
Magelona posterolongata	Polychaeta
Magelona nonatoi	Polychaeta
Magelona variolamellata	Polychaeta
Naineris bicornis	Polychaeta
Naineris setosa	Polychaeta
Pholoe sp.	Polychaeta
Poecilochaetus australis	Polychaeta
Poecilochaetus perequensis	Polychaeta
Poecilochaetus sp.	Polychaeta
Scoloplos (Leodamas) sp.A	Polychaeta
Terebellides anguicomus	Polychaeta
Albunea paretti	Crustacea
Ampelisca sp.	Crustacea
Apseudes sp.	Crustacea
Callinectes cf. danae	Crustacea
Maera sp.	Crustacea
Ogryides alphaerostris	Crustacea
Ecological Group 1	
Pinnixa sayana	Crustacea
Pinnixa sp.	Crustacea
Processa sp.	Crustacea
Upogebia brasiliensis	Crustacea
Upogebia sp.	Crustacea
Hemipholis elongata	Echinodermata
Amphiodia pulchella	Echinodermata
Amphipholis squamata	Echinodermata
Sipunculus nudus	Sipuncula
Sipunculus sp.	Sipuncula
Ecological Group 2	
Chione cancellata	Bivalvia
Cooperela atlantica	Bivalvia
Diplodonta punctata	Bivalvia
Pitar fulminatus	Bivalvia
Solen tehuelchus	Bivalvia
Tagelus divisus	Bivalvia
Tagelus plebeius	Bivalvia
Trachycardium muricatum	Bivalvia
Anachis obesa	Gastropoda
Bulla ocidentalis	Gastropoda
Bulla striata	Gastropoda
Cerithium atratus	Gastropoda
Cylichna discus	Gastropoda
Nassarius vibex	Gastropoda
Dorvillea sp.	Polychaeta
Eunice sp.	Polychaeta
Eunoe serrata	Polychaeta
Eunoe tuerkayi	Polychaeta
Exogone breviantennata	Polychaeta
Glycinde multidens	Polychaeta
Goniada littorea	Polychaeta
Goniada maculate	Polychaeta
Grubeulepis cf. geayi	Polychaeta
Gymnonereis crosslandi	Polychaeta

Ecological Group 2		**Ecological Group 4**	
Hemipodia simplex	Polychaeta	*Carycorbula caribaea*	Bivalvia
Hermundura tricuspis	Polychaeta	*Corbula* sp.	Bivalvia
Malmgreniella sp.	Polychaeta	*Aphelochaeta marioni*	Polychaeta
Marphysa sebastiana	Polychaeta	*Cirriformia sp.*	Polychaeta
Mooreonuphis lineata	Polychaeta	*Heteromastus* sp.A	Polychaeta
Owenia fusiformis	Polychaeta	*Laeonereis culveri*	Polychaeta
Phyllodoce cf. arenae	Polychaeta	*Paraprionospio pinnata*	Polychaeta
Scoletoma tetraura	Polychaeta	*Prinospio dayi*	Polychaeta
Sthenelais cf. limicola	Polychaeta	*Prinospio malmgreni*	Polychaeta
Sthenelais sp.	Polychaeta	*Prinospio streenstrupi*	Polychaeta
Syllis cf. cornuta	Polychaeta	*Protocirrineris* sp.	Polychaeta
Alpheus sp.	Crustacea	*Sigambra grubei*	Polychaeta
Ambidexter symmetricus	Crustacea	*Sigambra tentaculata*	Polychaeta
Idunella nana	Crustacea	*Timarete filigera*	Polychaeta
Monokalliapseudes schubartii	Crustacea	**Ecological Group 5**	
Protankyra benedeni	Echinodermata	*Capitella aracaensis*	Polychaeta
Amphiura kinberg	Echinodermata	*Capitella biota*	Polychaeta
Microphiopholis atra	Echinodermata	*Capitella neoaciculata*	Polychaeta
Microphiopholis subtilis	Echinodermata	*Capitella nonatoi*	Polychaeta
Ophiactis lymani	Echinodermata	*Capitella* spp.	Polychaeta
Thysanocardia catarinae	Sipuncula		
Edwardsia sp.	Anthozoa		
Ecological Group 3		**Not Assigned**	
Abra sp.	Bivalvia	*Felaniella candeana*	Bivalvia
Macoma uruguayensis	Bivalvia	*Juliacorbula acquivalves*	Bivalvia
Mediomastus sp. A	Polychaeta	*Strigilla camaria*	Bivalvia
Haploscoloplos sp. A	Polychaeta	*Strigilla product*	Bivalvia
Harmothoe sp.A	Polychaeta	*Tivela mactroides*	Bivalvia
Isolda pulchella	Polychaeta	*Ophellina alata*	Polychaeta
Laonice branchiata	Polychaeta	*Ophellina* sp.	Polychaeta
Laonice cirrata	Polychaeta	*Acantholobulus schmitti*	Crustacea
Laonice sp.	Polychaeta	*Ampelisciphotis* sp.	Crustacea
Neanthes bruaca	Polychaeta	*Persephona crinite*	Crustacea
Notomastus hemipodus	Polychaeta	*Phoxocephalopis* sp.	Crustacea
Polydora websteri	Polychaeta	*Polyonyx gibbesi*	Crustacea
Prinospio multibranchiata	Polychaeta	*Sicyonia* sp.	Crustacea
Protoaricia sp.A	Polychaeta	*Ophiotella danae*	Echinodermata
Rashgua lobatus	Polychaeta		
Scolelepis squamata	Polychaeta		
Scyphoproctus sp. A	Polychaeta		
Spiophanes duplex	Polychaeta		
Sternaspis capillata	Polychaeta		
Automate cf. rugosa	Crustacea		

Capítulo 3

Defining soft bottoms habitats and indicator species as tools for monitoring of a coastal system

Abstract

Defining habitats in soft-bottom environments is challenging given their spatio-temporal dynamics, yet it is an important tool to aid monitoring and conservation programs. Additionally, identifying indicator species representing conditions present in a given habitat can also help detect environmental changes. In light of this, the objective of this study is to introduce a methodological framework to (i) depict the spatio-temporal dynamics of habitats, in order to define habitats based on the species-environment relationship instead of using only abiotic surrogates, and (ii) to identify indicator species and understand their relationships with the environmental variables of each habitat. As a case study, we used data of macro and meiofauna of Araçá Bay, a biodiverse coastal ecosystem in Southeast Brazil which is under severe anthropogenic pressure. Three main habitats were identified in the area, defined by the relationship of species with water depth and granulometric composition.: 1) the upper intertidal area, with higher contributions of coarse sand fractions and *Capitella nonatoi*; 2) intertidal and internal sublittoral, with higher microphytobenthic production and fine/very fine sands and occurrence of *Monokalliapseudes schubarti*, *Olivella minuta, Comesoma* sp. 1 and *Viscosia* sp. 1; and 3) the external sublittoral, with higher contents of silt/clay and total organic carbon, and occurrence of *Terschellingia* sp. 5, *Sabatieria* sp. 1 and *Neanthes bruaca*. Macro and meio faunal assemblages were influenced by the same variables, but macrofauna was more sensitive to changes in sediment composition, especially coarse sands, at the scale studied. Indicator species were selected with high specificity, with fidelity being overall higher for nematode species. Species were positively related to environmental variables, but optimum estimates varied among taxa. Precision of such estimates can be used as a reliability indicator of sensitivity to changes. The framework proposed was able to identify habitats and indicator species using data commonly collected in benthic studies. The habitats' current distribution, along with the indicator species can be used by monitoring programs in the area, to understand and detect changes arising from natural and human induced impacts.

Introduction

All marine ecosystems are to some extent currently affected by human activities (Halpern et al. 2008), and it is estimated that almost half of the marine environment is already impacted by a combination of stressors such as ocean acidification, coastal hypoxia, and pollution (Gray et al., 2002; Defeo et al., 2009; Halpern et al., 2015). This unprecedented level of anthropogenic threats to marine systems has increased the need for biomonitoring and conservation programs (Crain et al., 2008; Halpern et al., 2008; Stelzenmüller et al., 2010).

Nowadays, the success of conservation efforts is highly dependent on the identification and protection of natural habitats which can act as biodiversity reservoirs, are important to ecosystem functioning and stability, and can be used to understand ecosystem dynamics (Stevens and Connoly, 2004; Cogan et al., 2009). Once defined, a habitat can be used to plan monitoring programs. So far, the definition of habitats in marine benthic ecosystems usually relies on physical attributes and biogenic structures such as seagrass, rocky shores, and mussel beds (Banks and Skilleter, 2002; Seitz et al., 2014). Defining such habitats (and thereby developing a habitat-based monitoring program), however, is particularly challenging in highly dynamic and apparently homogeneous systems such as marine soft-bottoms (MacArthur et al., 2010).

The definition of habitats in marine soft-bottoms is usually linked to the less conspicuous variation in sediment properties (e.g. mud content, peebles, and sorting coefficient) (Gray and Elliot, 2009). Such classification is normally done *a posteriori* to the data acquisition and largely based on the multivariate structure of the environmental conditions. Nevertheless, the use of abiotic surrogates alone to map coastal habitats may generate unreliable results (Diaz et al., 2004; Stevens and Connolly, 2004). The organisms inhabiting the matrix of sediments exhibit complex interactions with the physical and environmental characteristics and greatly influence the habitats conditions (McArthur et al., 2010). The consideration of such complex species-environment interaction is therefore crucial for properly delimiting each potential habitat (Diaz et al., 2004).

A complementary method to the habitat-based approach in conservation programs is the selection of indicator species (Carignan & Villard 2002, De Cáceres et al. 2010, Siddig et al. 2016). Indicator species show predictable responses to various environmental variations and can be used to assess the habitat conditions (Dufrêne and Legendre, 1997; Carignan and Villard, 2002; Niemi and McDonald, 2004). An appropriate indicator species has to respond strongly to a particular group of conditions, to which it will serve as an indicator (De Cáceres et al., 2010; Fonseca and Gallucci, 2016), and these need to be based on local conditions, given that species responses to environmental variables may change within variations on a secondary gradient (e.g. salinity, temperature) (Bustos-Baez and Frid, 2003; Zettler et al., 2013). The selection of indicator species for soft benthic communities is particularly important since the sedimentary habitat is dynamic, species rich and identification of benthic biodiversity to species level is a major time consuming activity (Warwick, 1993).

The aim of this study was to introduce a methodological framework to define and monitor soft-bottom habitats based on the species-environment relationship. We first identified potential habitats based on responses of species assemblages to the environmental characteristics. Then, we selected potential indicator species which can be used to assess future changes in each habitat. The congruence between methods was used to delineate each potential habitat of soft-bottoms. We applied this methodological framework to the main groups of marine benthic fauna (meio- and macrofauna) in a biodiverse benthic ecosystem which is under recent threats due to the planned expansion of the neighboring port (Amaral et al., 2010; 2016) which reinforces the critical need to recognize and understand the local environmental dynamics in order to monitor and manage the area. The study area is also a typical example of many threatened parts of the Brazilian coast (Gorman et al., 2017), and, as such, the outcomes of the present study can be relevant for other regions.

Materials and Methods

Study area

This work was done at Araçá Bay (23º 49'S, 45º 24'W), a coastal ecosystem (534.500 m^2) located in the central area of the São Sebastião Channel, state of São Paulo, Southeast Brazil (Fig. 1). The area is environmentally heterogeneous, with a number of distinct features, such as patches of different sedimentary textures, mangroves and rocky shores (Amaral et al., 2016; Checon et al., 2017). The intertidal area has a gentle slope, with a maximum depth of 5 m, while further the bay reaches 30 m deep towards the channel. Araçá Bay is located within the Marine Environmental Protection Area of the Northern Litoral (APA Marinha do Litoral Norte), a conservation unit which aims to preserve biodiversity and natural processes, and is recognized as one of the areas with the highest marine biodiversity on the Brazilian Coast (Amaral et al., 2010; 2016).

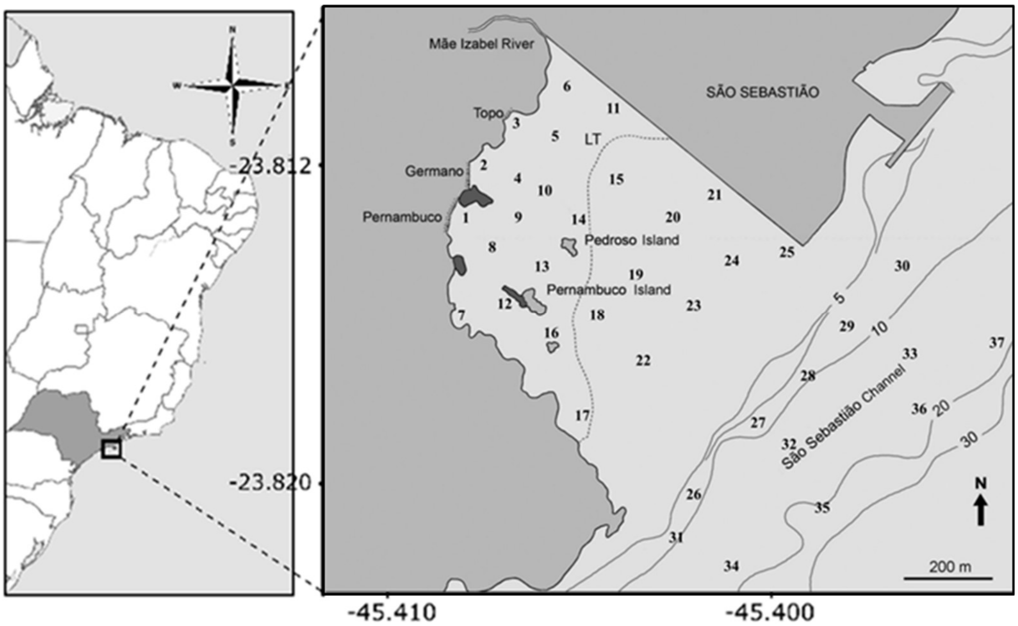

Figure 1. Map of the study area showing the 37 sampling stations along the intertidal (1 to 17) and internal sublittoral (18 to 25) at Araçá Bay, and external sublittoral at the São Sebastião Channel (26 to 37).

Sampling design

Sampling was performed during four periods (October 2012, February, June and September of 2013). Thirty-seven sampling stations were determined from the intertidal and shallow sublittoral area at the bay (< 5 m deep) to a depth of 25 m (São Sebastião Channel). Sampling stations were positioned to a) encompass habitat diversity (i.e. different sediment types and depths), and b) achieve a reasonable dispersion and spatial coverage (Fig. 1). The same locations (+/- 1 m) were sampled during each sampling period using a GPS. Sampling was done manually at the intertidal and shallow sublittoral (< 3 m deep), and with the use of a multi-corer sampler for deeper sites. At each sampling site, four samples were collected using a corer of 10 cm diameter and 20 cm depth for the evaluation of macrofauna, and one sample of 2.5 cm in diameter and 5 cm depth for meiofauna. Additional samples were taken at each station to evaluate environmental parameters: Five samples of the top 1 cm of the sediment were taken using a corer measuring 2 cm in diameter to evaluate microphytobenthic primary production; and one sample of sediment was taken for granulometric analysis using a corer of 3 cm diameter and 20 cm depth.

Samples processing

Macrofauna samples were stored in plastic bags and posteriorly sieved with a 0.3 mm mesh. The fauna retained was sorted in taxonomic groups and fixed in 70% ethanol. All individuals were identified to the species level.

Meiofauna samples were immediately fixed in 4% formaldehyde, and posteriorly washed through a 45 µM mesh sieve and extracted by flotation with Ludox TM 50 (specific density 1.18) (Heip et al., 1985). The retained material was stored in formaldehyde 4% and stained with Rose bengal. Meiofauna counting and identification was done under a stereomicroscope. We selected only the nematode assemblage for further study, as they were the most abundant in the area. Nematoda were identified to genus level and further separated into morphospecies. From each sample, a total of 100 nematodes were randomly chosen, evaporated slowly in anhydrous glycerol and mounted on permanent slides for identification.

Microphytobenthic biomass was estimated from phaeopigments and chlorophyll a concentrations according to Plante-Cuny (1973). Margalef pigment diversity index (Margalef, 1967), a ratio of total green pigments, was calculated. The index ranges from 2 to 8, increasing from young microphytobenthic communities to mature, oligotrophic ones. The granulometric analysis was carried out using the routine sieving and pipetting techniques described by Suguio (1973) and sediment parameters were obtained using SysGran software, version 3.0 (Camargo, 2006) following the classifications of Folk & Ward (1957). Total organic carbon was evaluated using a modified Walkley-Black titration method, described by Gaudette et al. (1974).

Data analysis

We combined two statistical techniques to define the potential habitats at Araçá Bay: (i) First, we performed a redundancy analysis (RDA) to evaluate the influence of environmental variables on community structure; then, (ii) we used a two-dimensional kernel density analysis to generate a contour map from the density distribution of points in RDA space and determine the habitats (i.e., areas with similar environmental characteristics and species composition). Due to the different sampling protocols, these analyses were performed separately for meiofauna and macrofauna. Given that marine meiofauna can reach extremely high densities, only a random fraction of the nematode assemblages was identified. The total number of nematodes of each species per sample was calculated by multiplying the total number of individuals per sample and the proportion of each morphospecies. For macrofauna, all individuals were identified. Both macrofaunal and nematode data were transformed using Hellinger function to minimize the importance of rare species (Legendre and Gallagher, 2001). Environmental data was checked for correlation and multicollinearity, using Spearman correlation and Variance Inflation Factor (VIF), respectively. The final model included 10 environmental variables with low collinearity: chlorophyll a, Margalef pigment diversity index, depth, total organic carbon, mean grain size, pebbles, coarse sands (as sum of very coarse, coarse and medium sands), fine sands, very fine sands and sorting coefficient.

The environmental gradient associated with each identified habitat was determined by checking the environmental variables ordination scores towards respective clustering of sites. Indicator species were determined using Indicator Values (IndVal) (Dufrêne and Legendre, 1997). This is a widely used tool to identify indicator species, which uses not only the species exclusive occurrence (i.e. specificity), but also the distribution in the sampling sites at a particular habitat (i.e. fidelity). From the species with high IndVal, we selected the ones with higher fidelity for each habitat for further evaluation (mapping and relationship with environmental variables). The relations between the indicator species and correlated environmental variables were assessed using a non-linear quantile regression approach. Variables were selected based on their correlation with the habitats. Following the method proposed by Anderson (2008), abundances were plotted against the environmental variable of interest and quantile regression spline models were constructed for the 95th percentile (i.e., the value below which 95% of the abundances are expected to fall). The degree of the polynomial used on each model was calculated using the Akaike Information Criteria (AICc, Burnham and Anderson, 2002). Polynomials of degree 2, 3, 4 and 5 were created for each taxon with the best-fit model having the smallest AICc value (Anderson, 2008; Koenker, 2011). For each model, the value predicting the maximum abundance for a given environmental factor was taken as a measure of the estimated optimum. These values were subject to 999 sample pair bootstrapping and re-modelled from the original chosen model using bias-corrected percentiles. Confidence intervals of ninety-five percent were obtained from the distribution of bootstrapped sample pairs (Anderson, 2008).

Smoothed maps were generated to illustrate the distribution of species and habitats throughout the sampling periods. Maps were made using the inverse distance weighting

(IDW) method. Interpolation of data was reached using inverse distance weighting power equal to 2. The distribution of species and their representative habitats were contrasted to check for concordance in spatial and temporal distribution.

All analyses were ran using R Software 3.3.1 (R Development Team, 2016), using the packages *vegan* (Oksanen et al., 2013), *mixtools* (Benaglia et al., 2009), *MASS* (Venables and Ripley, 2002), *gstat* (Pebesma, 2004), *raster* (Hijmans, 2016), *maptools* (Bivand and Lewin-Koh, 2017), *sp* (Pebesma and Bivand, 2005) and *quantreg* (Koenker, 2016).

Results

Community characterization

A total of 11270 individuals of 158 macrobenthic species, and an estimation of 16296 individuals of 195 nematode morphospecies were identified throughout the sampling periods. Macrobenthic assemblages were dominated by polychaetes, both in terms of abundance and species richness, and the tanaidacean *Monokalliapseudes schubarti* (Mañé-Gárzon, 1949). *Comesoma* sp.1 and *Dorylaimopsis* sp.1 were the most abundant species of nematodes.

Habitats characterization

Redundancy analysis models showed that the environmental variables were responsible for 22 % and 32% of the variation in species distribution of macrofauna (Model, $F_{10,130} = 3.723$, $p < 0.001$) and nematode (Model, $F_{10,130} = 6.105$, $p < 0.001$) assemblages, respectively. With the exception of pebble percentages, all individual variables significantly explained macro and nematode distribution. Depth, coarse sands, chlorophyll a and very fine sands were, in order of importance, the most important variables for both groups. A summary of the values obtained for each variable is presented in Table 1.

Table 1. Summary of environmental characterization of Araçá Bay in each campaign. Total organic carbon (TOC) and sedimentary fractions are given as percentages. Sorting coeficient and mean grain size are given on phi (ϕ) scale.

	Campaigns				Range
	1st	2nd	3rd	4th	
Clorophyll a	82.6 ± 46.4	79.27 ± 54.9	75.59 ± 55.7	96.09 ± 72.1	10.1 - 264.2
Phaeopigments	91.82 ± 77.3	87.16 ± 79.5	79.08 ± 75.8	121.13 ± 132.9	2.4 - 515.8
Margalef	3.09 ± 0.45	3.43 ± 0.8	3.42 ± 0.8	3.16 ± 0.7	2.4 - 5.8
Depth (m)	-4.13 ± 7.7	-4.09 ± 7.5	-3.35 ± 6.7	-3.66 ± 6.8	0.8 - (-23.2)
TOC	0.8 ± 0.8	0.75 ± 0.6	0.81 ± 0.5	0.71 ± 0.7	0 -2.71
Peebles	2.47 ±7.7	1.62 ± 3.6	3.67 ± 7.3	1.52 ± 3.5	0 - 44.5
Very Coarse Sand	2.41 ± 4.6	2.6 ± 5.6	3.08 ± 4.8	2.41 ± 6.9	0 - 37.3
Coarse Sand	3.43 ± 8.57	3.36 ± 6.3	3.73 ± 5.9	3.33 ± 8.8	0 - 47.79
Medium Sand	5.2 ± 7.52	5.22 ± 7.9	10.5 ± 14.9	7.35 ± 15.4	0 - 62.88
Fine Sand	11.4 ± 13.35	8.95 ± 9.2	16.99 ± 15.4	10.3 ± 14.3	0.32 - 56.62
Very Fine Sand	46.39 ± 25.3	50.62 ± 26.3	56.71 ± 26.9	45.85 ± 26.4	3.52 - 99.15
Silt/Clay	28.59 ± 27.1	27.5 ± 28.2	5.30 ± 6.6	29.16 ± 28.7	0 - 84.95
Sorting	1.43 ± 0.7	1.21 ± 0.6	1.78 ± 0.6	1.51 ± 0.6	0.27 - 3.43
Mean Grain Size	3.87 ± 1.5	3.57 ± 1.1	3.77 ± 1.2	3.97 ± 1.7	(-0.09) - 6.85

Based on the ordination results, three habitats types were recognized for macrofauna and two habitat types for meiofauna (Fig. 2). For macrofauna, Habitat 1 was characterized by few sites, with a high contribution of coarse sands, and mostly restricted to the upper intertidal area of the bay. Habitat 2 was characterized by most of the sites in the shallower area, with higher primary production and predominance of fine and very fine sand fractions. Lastly, Habitat 3 was characterized mainly by sites located on the external sublittoral area, from 5 m depth to the deeper areas of the channel, with higher percentages of silt/clay and total organic carbon content. Transition sites are found between Habitats 2 and 3 (Fig 3). Nematode habitats were the same as those found for macrofauna, however Habitat 2 (nematode) extended from the upper intertidal to the internal sublittoral area of the bay, and thus Habitat 1 was not found for nematodes (Fig. 4). For both groups, few sites were not categorized in any habitat and were considered as “no habitat” sites (Figs. 3, 4).

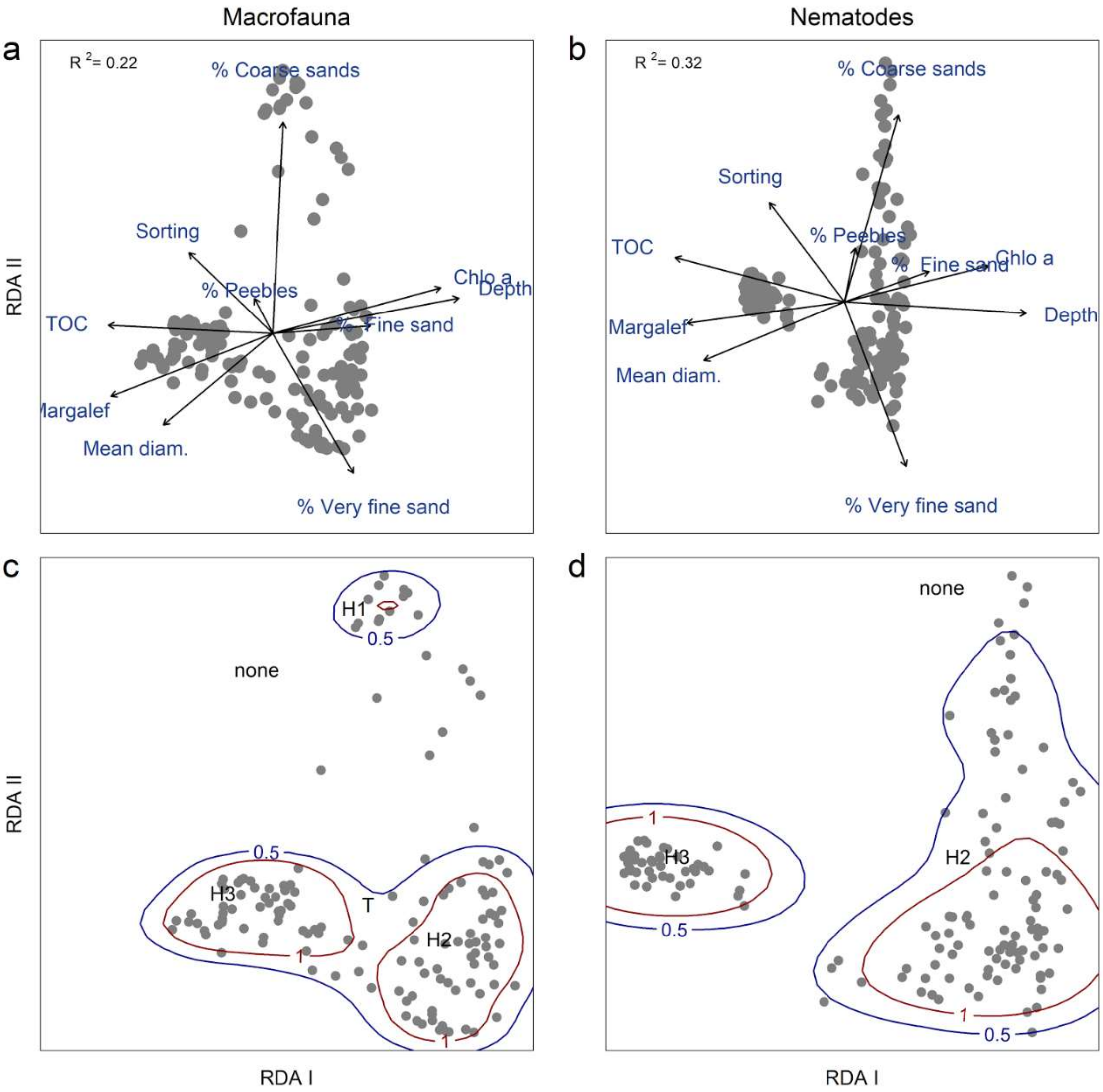

Figure 2. Redundancy analysis and kernel density analysis. (a, b) RDA results for macrofauna and nematodes**; (c, d)** Kernel density plots showing the three main macrofaunal habitats (H1, H2 and H3) and the two main nematodes habitats (H2 and H3) . "T": Transition between groups. "none": non-grouped sites

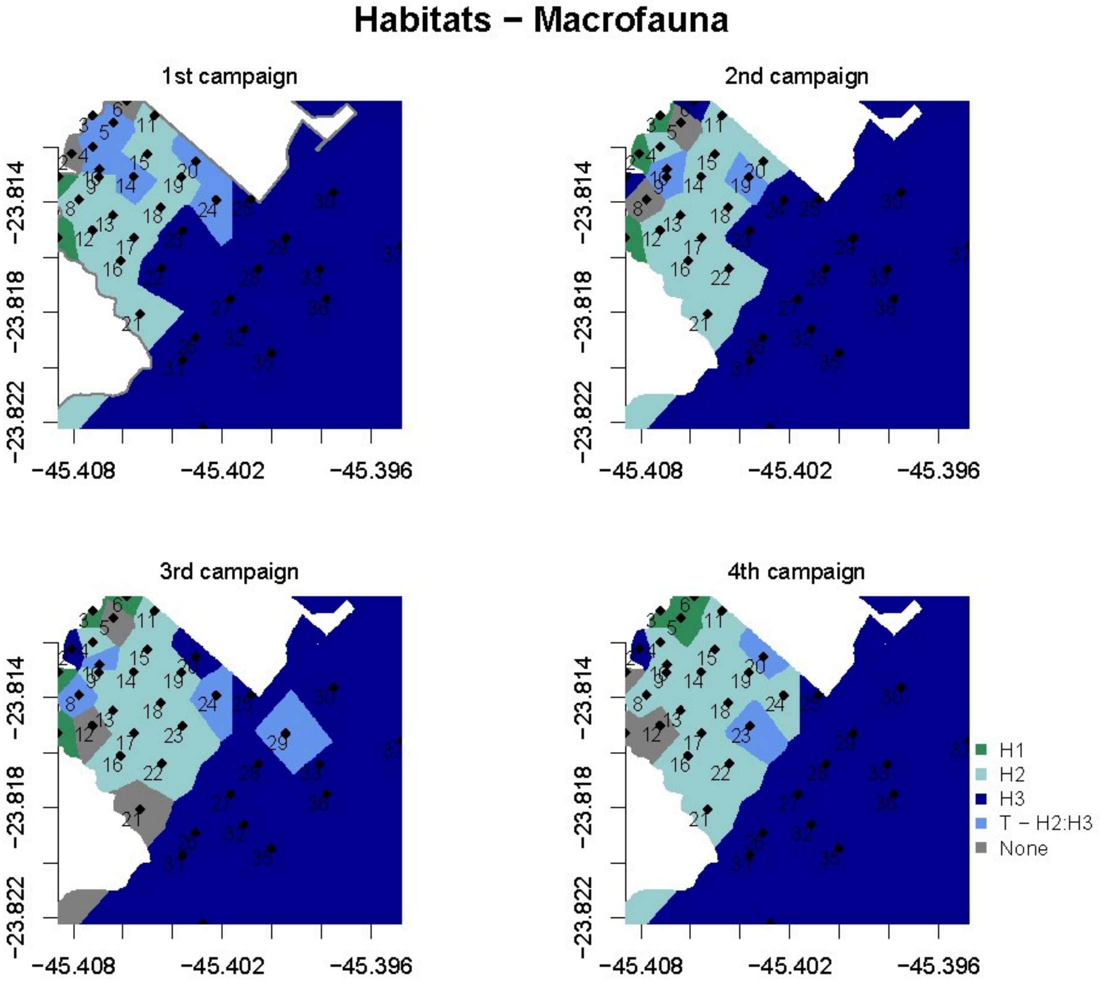

Figure 3. Macrofauna habitats. Spatial interpolation maps showing distribution of the three macrofaunal habitats (H1, H2, and H3) found at Araçá Bay during each sampling campaign. T: sites with intermediate characteristics between H2 and H3. None: unclassified sites.

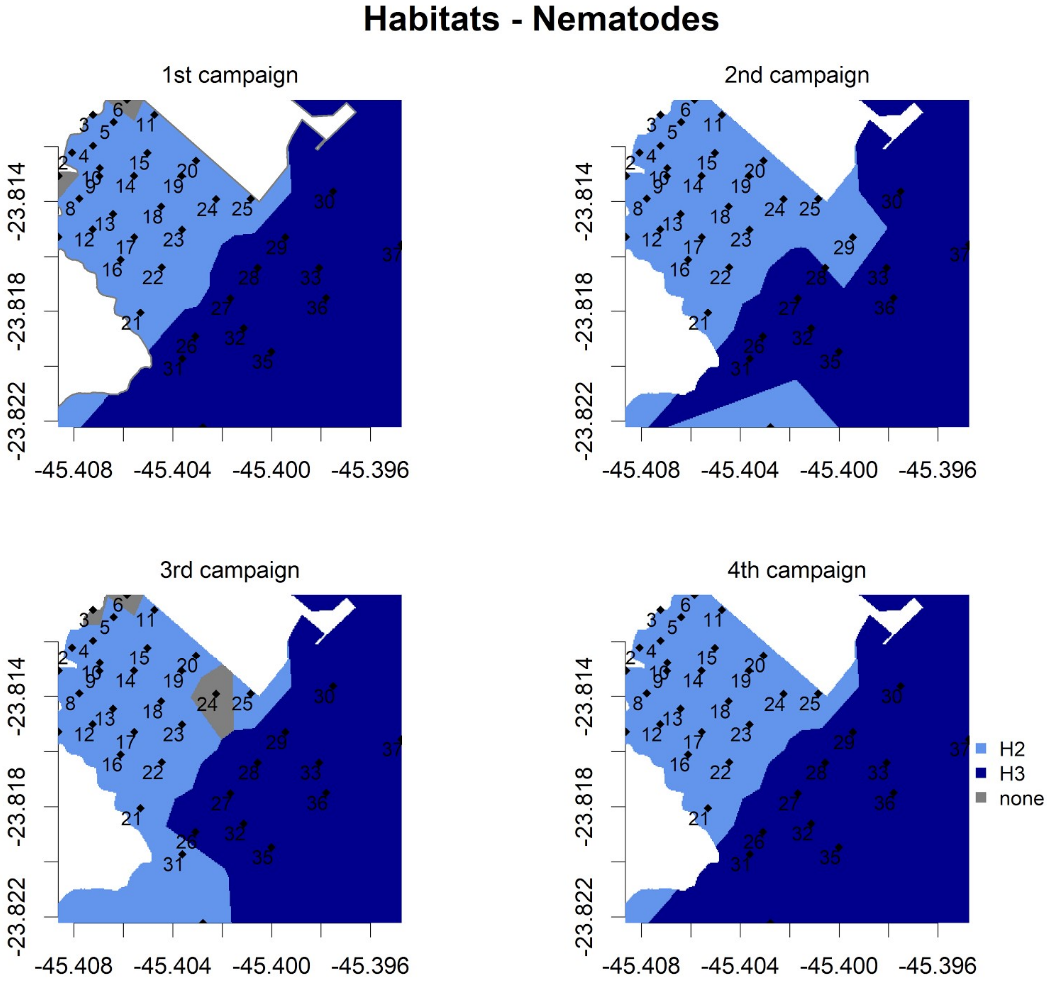

Figure 4. Nematodes habitats. Spatial interpolation maps showing distribution of the two nematodes habitats (H2 and H3) found at Araçá Bay during each sampling campaign. None: unclassified sites.

Indicator species

For each habitat, IndVal identified species with high specificity and fidelity (Table 2). For macrofaunal species, Habitat 1 was represented by the polychaetes *Capitella nonatoi* (Silva and Amaral, 2017) and *Laeonereis culveri* (Webster, 1879), whereas Habitat 2 was represented by the gastropod *Olivella minuta* (Link, 1807) and the tanaidacean *Monokalliapseudes schubarti*. For Habitat 3, the cnidaria *Protankyra benedeni* (Östergen, 1898) and the polychaete *Neanthes bruaca* (Lana and Sovierzovski, 1987) had a high specificity, but low fidelity, meaning they are found at Habitat 3, but do not have a broad distribution in the area. For meiofaunal species, *Comesoma* sp. 1, *Viscosia* sp. 1 and to a lesser degree *Subsphaerolaimus* sp. 1 were found to be strongly associated with Habitat 2. At

Habitat 3, *Terschellingia* sp. 5, *Sabatiera* sp. 1 and *Aponema* sp. 1 were found with very high specificity and fidelity.

Table 2. Indicator values (IndVal) of species with highest proportion of specificity (A) and fidelity (B) at each habitat. p-values are given by permutational analysis (n=999).

	IndVal Components		
Macrofauna	A	B	P-Value
Habitat 1			
Capitella nonatoi	0.99	1.00	<0.001
Laeonereis culveri	0.99	0.83	<0.001
Habitat 2			
Monokaliapseudes schubarti	0.97	0.66	<0.001
Olivella minuta	0.96	0.64	<0.001
Habitat 3			
Neanthes bruaca	0.94	0.24	0.02
Protankyra benedeni	1.00	0.18	0.05
Nematodes			
Habitat 2			
Comesoma sp. 1	0.94	0.83	<0.001
Viscosia sp. 1	0.98	0.72	<0.001
Subsphaerolaimus sp. 1	0.98	0.67	<0.001
Habitat 3			
Terschellingia sp. 5	0.97	1	<0.001
Sabatieria sp. 1	0.99	0.97	<0.001
Aponema sp. 1	0.99	0.9	<0.001

Species mapping showed that the indicator species distribution matches those found for their respective habitats. *Capitella nonatoi* distribution was concordant with temporal fluctuation of Habitat 1, found only in the upper intertidal. *Olivella minuta* and *M. schubarti* distribution was also temporally stable, occupying a large area of Habitat 2. In Habitat 3, however, *N. bruaca* had a strong temporal variation, being restricted to a few stations during the 2nd and 4th campaign, reflected in the low fidelity encountered for species at this habitat (Fig. 5). For meiofaunal species, *Terschellingia* sp. 5 and to a lesser degree *Sabatiera* sp. 1, occurred with little temporal variation between the sites at Habitat 3. *Comesoma* sp. 1 and *Viscosia* sp. 1 were well represented at Habitat 2 sites, but some temporal variation was observed, and distribution of both species was more restricted during the 3rd and 4th campaign, respectively (Fig. 6).

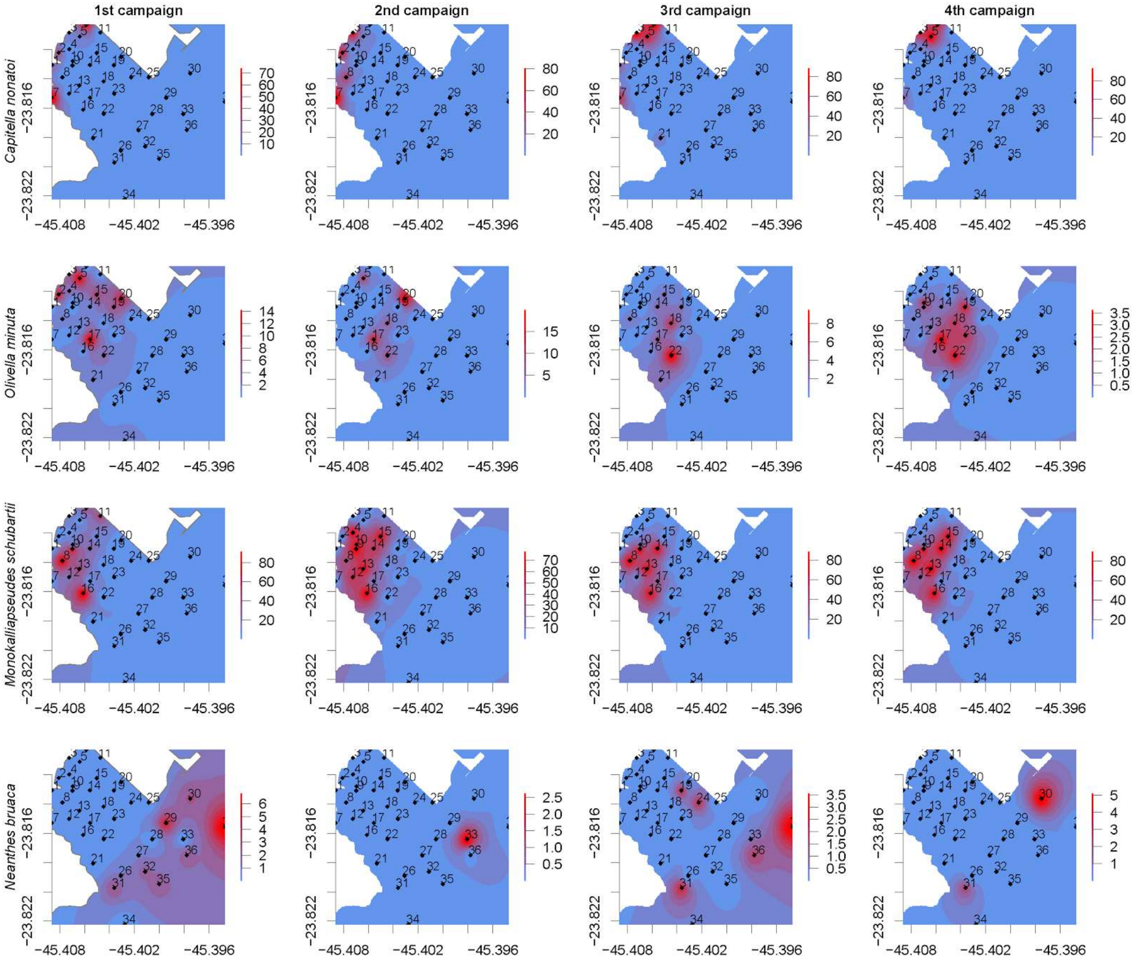

Figure 5. Macrofauna indicator species. Spatio-temporal distribution of the habitat indicator species at Araçá Bay.

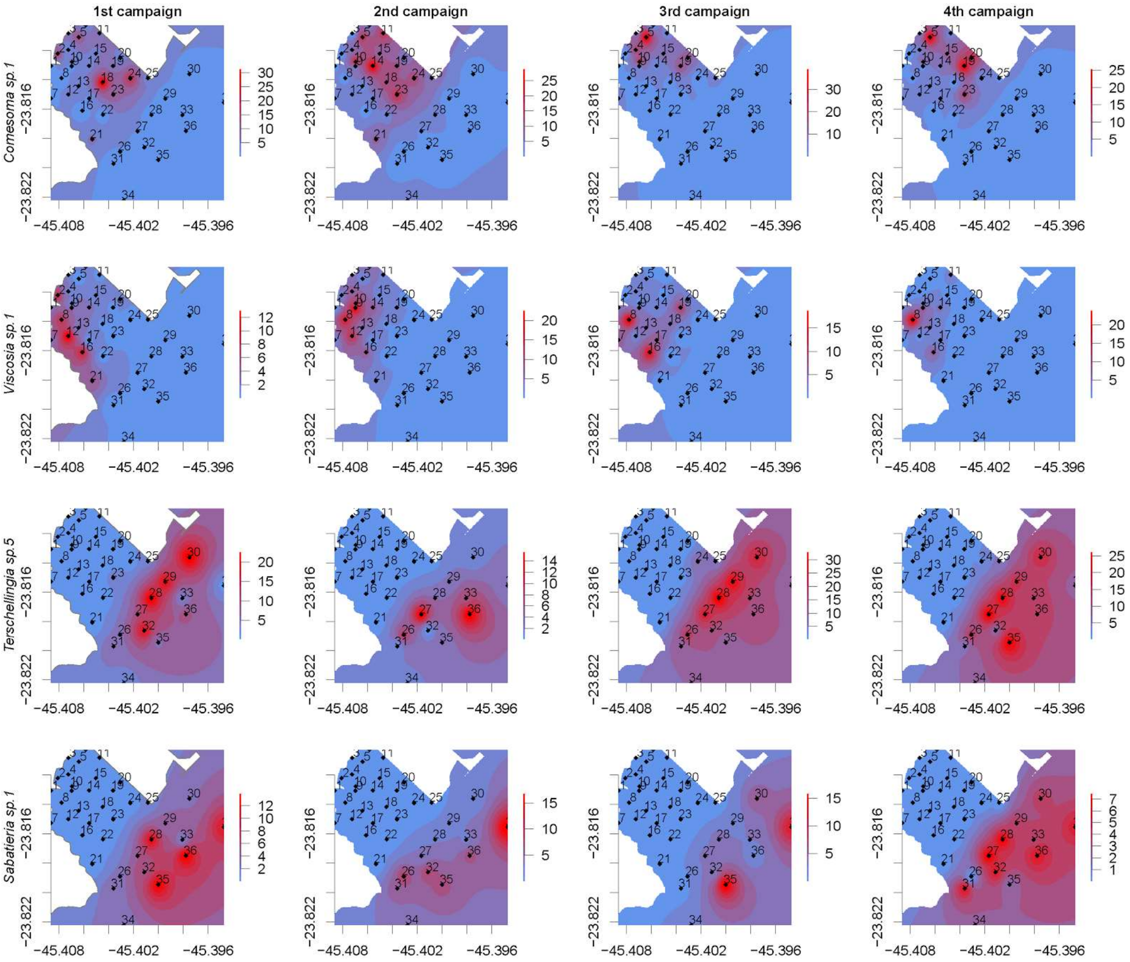

Figure 6. Nematodes indicator species. Spatio-temporal distribution of the habitat indicator species at Araçá Bay.

Based on the ordination scores for species and variables, three environmental variables were selected to investigate their relations with the respective indicator species distributions (i.e. coarse sands; chloropyll a and total organic carbon). All species showed a unimodal relationship with the most characteristic environmental variables of each habitat. Estimated optimal interval, however, was very high for some species, especially macrofaunal ones. *Capitella nonatoi,* for example, had an estimated optimum of ~45% of coarse sands at Habitat 1, but with a high uncertainty due to the wide confidence interval (23 – 75%). At Habitat 2, the tanaidacean *M. schubarti*, the gastropod *O. minuta* and the nematodes *Comesoma* sp. 1 and *Viscosia* sp. 1 had a unimodal positive relationship with clorophyll a, although a high precision was only obtained for *Comesoma* sp. 1. At Habitat 3, indicator species had a unimodal positive relationship with total organic carbon, but a high precision of estimated optimum was found only for *Terschellingia* sp. 5 (Fig. 7).

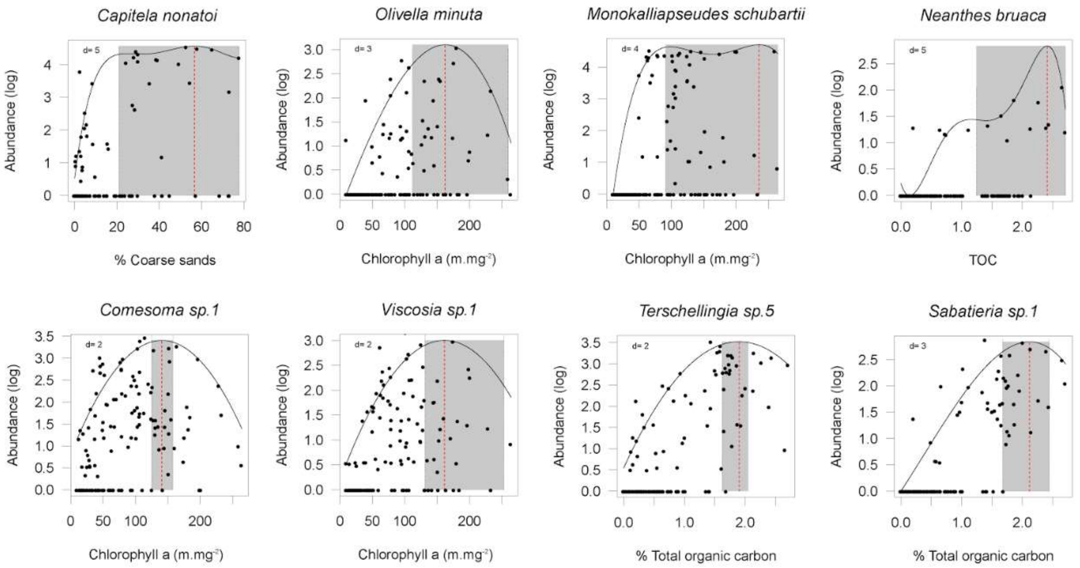

Figure 7. Quantile regressions. Relationship between indicator species abundance (log y + 1) and environmental variables characteristic of each habitat. The red line represents the estimated optimum value for the species, and the gray area illustrates their 95% confidence interval. d: polynomial degree

Discussion

The main goal of this study was to provide a more complete way to define and monitor marine soft-bottom habitats based not only on abiotic characteristics, but also taking into account the local biodiversity. The proposed framework helped us to identify the major habitats of Araça Bay and select a set of indicator species for each habitat. Additionally, the methodological framework allowed us to check which environmental variables were the most important to explain the distribution of benthic assemblages and to estimate the optimal conditions for each indicator species.

Overall, three main habitats, mainly determined by the relations of benthic species with water depth and sedimentary composition, are found at Araçá Bay. Habitat 1 is located on the upper intertidal area of Araçá Bay, and defined by a high percentage of coarse sands and the presence of *Capitella nonatoi* and *Laeonereis culveri*. A high contribution of coarse sediment fractions is not usually characteristic for tide dominated environments such as Araçá Bay. The high percentage of coarse sediments found here is likely related to sediment resuspension and or intrusion due to past anthropic activities (Amaral et al., 2010; Mani-Peres et al., 2016) and the influence of nearby rocky shores in the area. The internal area of the bay is largely homogeneous forming a single habitat (Habitat 2). This shallow sublittoral area is characterized by a gradual decrease in water depth and a higher content of microphytobenthic pigments and fine and very fine sands, in comparison to the other two habitats. This sedimentary property is characteristic for tide dominated sandflats (Dyer et al., 2000; Le Hir et al., 2000). Habitat 2 is also defined by the presence of the gastropod *O. minuta* and the tanaidacean *M. schubarti*, and the nematodes *Comesoma* sp. 1 and *Viscosia* sp. 1. Finally, at the external sublittoral area, an additional habitat is recognized (Habitat 3). This habitat is mainly characterized by high silt/clay content and total organic carbon and the predominance of nematode species, especially *Terschellingia* sp. 5, *Sabatiera* sp. 1 and *Aponema* sp. 1., and to a lesser degree, macrofaunal species such as the polychaete *Neanthes bruaca* and the cnidarean *Protankyra benedeni*. This mud deposition and organic carbon content is expected due to the hydro and morphological characteristics of the continental side of the São Sebastião channel, such as weak currents, low channel depth and natural inputs (i.e. riverine) of anthropogenic sources (i.e domestic and industrial sewage inputs) (Barcellos and Furtado, 2006). The predominance of silt fractions is a result of the low availability of sand transport in the area (Alcantará-Carrió et al., 2017). The content of organic carbon may also be attributed to the sewage outfall in the area, although the impact is higher on total nitrogen and phosphorus than organic carbon itself (Teodoro et al., 2010).

As expected, due to the close relationship between benthic fauna and habitat characteristics, water depth and sedimentary composition were the most important environmental variables explaining the distribution of macro- and meiofaunal (nematoda) benthic assemblages at Araçá Bay. The importance of these variables in structuring marine benthic communities is well-known and has been highlighted in previous studies (e.g. Flach et al., 2002; Defeo and McLachlan, 2005; Corte et al., 2017). The water depth gradient is associated with other environmental factors that are known to exert strong influence on benthic assemblages such as wave disturbance, changes in hydrodynamics, sediment characteristics, stability of physico-chemical factors, and primary production (MacIntyre et al., 1996; Le Hir et al., 2000; Wiberg and Sherwood, 2008). Sedimentary features, in turn, are a major factor structuring marine benthic assemblages because they directly affect the spatial and structural conditions of the interstitial matrix and indirectly determine the physical and chemical environment of the sediment (Gray et al., 2002; Gray and Elliott, 2009; Corte et al., 2017).

Although both groups were influenced by similar environmental variables, the presence of coarse sands at the upper intertidal area creates a habitat for macrobenthic but not for nematode assemblages. Coarse sand fraction has been shown to also modify nematode species composition (Vanaverbeke et al., 2011; Patricio et al., 2012); however, our results suggest that this effect may not be as strong as the one found for the macrofauna. In this sense, our results show that macrofauna is more affected by spatial variability of habitat characteristics than nematode species, a result similar to that found by Semprucci et al. (2013). It is worth noting, however, that nematodes are found to have a higher variability than macrofauna at smaller scales (within < 0.1 km) (Schartzberger et al., 2008), and thus studies at different scales may found contrasting results.

Indicator species analyses found eight species as potential indicators of habitat conditions in the area based on their specificity (i.e probability of a species being exclusively found at a given habitat) and fidelity (i.e. probability of a species being found at every site of a given habitat) (Dufrêne and Legendre, 1997; De Cáceres et al., 2012). Among those species, four were macrofaunal species (*Capitella nonatoi*, *Olivella minuta*, *Monokalliapseudes schubarti* and *Nenathes bruaca*) and four meiofaunal morphotypes (*Comesoma* sp. 1, *Viscosia* sp. 1, *Terschellingia* sp. 5 and *Sabatiera* sp. 1). Some of these species are associated with opportunistic behavior. For instance, *Capitella nonatoi* is part of the *Capitella capitata* complex (Silva et al., 2017), a group of species kwown as *r*-strategists and early colonizers of disturbed environments (Tsutsumi, 1987) usually associated to organically enriched sediments (Pearson & Rosenberg, 1978; Rivero et al., 2005). *Monokalliapseudes schubarti* is also suggested as being an opportunistic species due to its high fecundity and fast growth (Leite et al., 2003). In regards to nematodes, there is a more limited knowledge regarding indicator species (Kennedy and Jacoby, 1999; Semprucci & Balsamo, 2012). Nonetheless, *Terschellingia* species have been suggested as being tolerant to stressful conditions, especially hypoxia (Armenteros et al., 2010; Boufahja et al., 2016), and are commonly found in areas with organic enrichment processes (Moreno et al., 2008; Armenteros et al., 2010). The occurrence of established disturbance indicator taxa in the area suggests that further impacts may results in an expansion of the distribution of these species. They should not, however, be used as proxies or to monitor changes in biodiversity, which is a common application of indicator species (Bustos-Baez and Frid, 2003, Siddig et al. 2016). Given the framework used to establish indicator species, we recommend they should only be used to monitor changes in the conditions associated with each habitat.

All indicator taxa showed high specificity, however, fidelity was higher for meiofaunal taxa, whose distributions were overall in agreement with habitat spatial mapping. For macrofauna, aside from *Capitella nonatoi* at Habitat 1, fidelity was overall lower than meiofauna. At Habitat 2, the two indicator species, *Monokalliapseudes schubarti* and *Olivella minuta,* have a distinct, yet complementary pattern. Both species occurred in the intertidal and internal sublittoral area, but *M. schubarti* was more present in the intertidal area, likely due to the higher content of very fine sand at the internal sublittoral, known to restrict their distribution (Leite et al., 2003). In contrast, *O. minuta* occurred preferentially at the internal sublittoral and lower levels of intertidal, a result compatible to its zonation on sheltered beaches, which is suggested to be caused by lower desiccation tolerance (Arruda and Amaral, 2003). This complementary distribution shows that both species should be considered in order to monitor and understand changes in Habitat 2. In Habitat 3, fidelity of macrofauna was low, as *Neanthes bruaca* had a strong temporal variation. This result suggests that its use as an indicator in Habitat 3 is limited. Macrofauna abundance is much lower than meiofauna at the external sublittoral of Araçá Bay (Corte et al., 2017), and as such, we suggest that meiofauna taxa are more reliable indicators for this area.

The use of quantile regression allowed us to estimate the optimum values between species and environmental variable characteristics of a given habitat, information which can be used as guideline values to monitor changes in the environment (Anderson, 2008; Keeley et al., 2012). Every indicator species had a unimodal positive relationship with the environmental variables assessed. However, the precision of the estimated optimum was better for meiofaunal taxa than macrofauna, with the exception of *O. minuta*. This precision can be seen as a measure of reliability between changes in environmental variables and species response (Anderson, 2008). The lower precision found for macrofaunal taxa may be due to unmeasured variables playing a role on the species distribution (Anderson, 2008) or low abundances (Keeley et al., 2012), as is the case for *N. bruaca*.

It is important to highlight that the three main habitats observed at Araçá Bay showed relatively stable spatio-temporal distribution throughout the sampling campaigns. Habitat 1, despite presenting temporal fluctuations, was always restricted to the upper intertidal area. The consideration of temporal patterns is an important feature for management of coastal areas, especially considering the dynamic nature of these ecosystems (Paiva, 2001; Arkema et al., 2006), and the relative stability on the distribution of habitats confers reliability to the monitoring of the area over different temporal scales. The selected indicator species were also persistent in the landscape and showed a relative temporal stability, which is a desirable feature of an indicator species (Hilty and Merenlender, 2000; Siddig et al., 2016).

Conclusion

The proposed framework was able to identify habitats based on species-environment relationships, instead of using only abiotic surrogates, and defining indicator species and their optimum relationship with environmental variables. The results presented here have implications for management not only at the local scale (Araçá Bay) but also to other areas. At the local scale, we could better understand the environmental dynamics, to identify the main habitats, and to indicate species related to each identified habitat. These species can then be used in monitoring programs as indicators of possible environmental changes, especially considering the expected anthropic interference at the area. In a broader aspect, we presented a methodological framework which combines an array of statistical techniques (i.e., multivariate analysis, kernel density, spatial mapping and quantile regression) and that can be used to investigate ecosystem dynamics and identify soft-bottom habitats. The new aspect of this framework is that, differently from commonly used methods, the habitat is identified not only by abiotic features, but by the relationship between species and environment. One advantage of this method is that it can be employed with data commonly collected in benthic community studies. We believe this approach can give a more complete assessment of habitat dynamics and environmental conditions, including the identification of potential indicator species, and therefore provide critical information necessary in conservation and management efforts.

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Considerações Finais

Os resultados aqui apresentados mostram que a Baía do Araçá possui uma alta diversidade de organismos bentônicos. As amostras no sublitoral da baía revelaram uma composição contrastante em relação ao entremarés, com variação não só nas espécies, como também na dominância dos grupos, sendo que moluscos, crustáceos e equinodermos são mais epresentados nessa área, enquanto o entremarés é dominado por poliquetas Esses resultados ressaltam a necessidade da conservação do ambiente entremarés e sublitoral para a manutenção da diversidade na área, especialmente considerando a relação e conectividade entre eles.

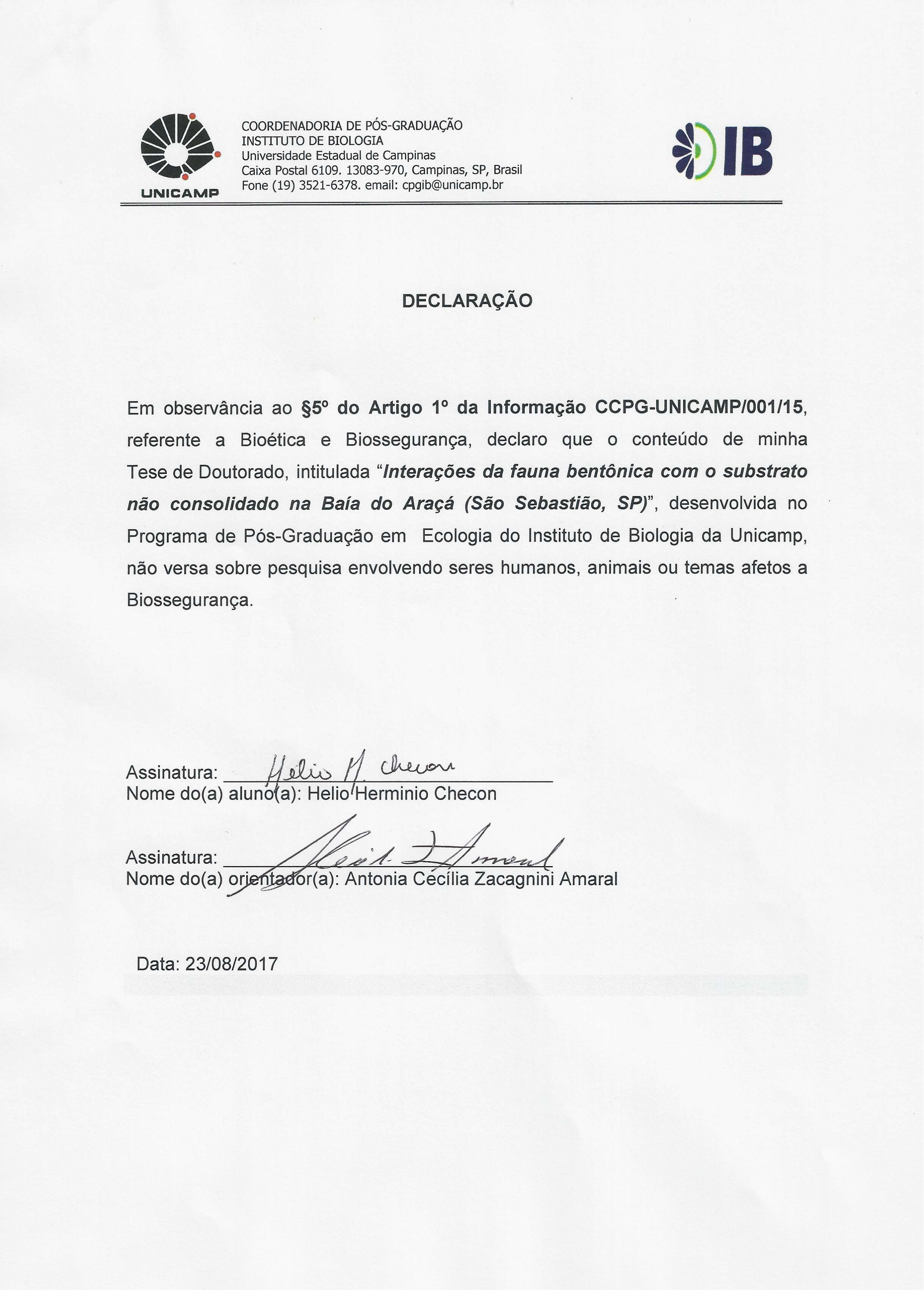
O capítulo 1 mostrou como a presença do manguezal pode alterar a comunidade endofaunal na área. A densidade de poliquetas diminuiu no interior do manguezal, em comparação à planicie de maré, mostrando que a presença da vegetação gera uma resposta nos organismos infaunais. No entanto, não foi observada mudanças em relação à riqueza de espécies. Esse resultado contrasta com o previamente encontrado para organismos epibentônicos, onde a vegetação exerce uma influência positiva na riqueza. Um estudo em andamento irá verificar a validade desse padrão para a Baía do Araçá. A detecção de tal padrão para organismos epifaunais, somado a existência de espécies similares de poliquetas nos diferentes núcleos de manguezais, pode fortalecer o papel estruturador desse sistema na área. O reconhecimento da importância dos manguezais soma-se aos resultados apresentados no capítulo 2 e 3 como subsídio para o manejo e conservação da biodiversidade bentônica na Baía do Araçá.

Os resultados do capítulo 2 mostram que as regiões do entremarés superior e o sublitoral externo (canal) são os ambientes com pior qualidade ecológica na baía. O desempenho do índice foi satisfatório, dada à relação positiva com os proxies de contaminação avaliados. Esses resultados, além da importância local, mostram que o uso de índices na costa brasileira, com fins de indicar a qualidade ecológica do ambiente bentônico, deve ser encorajado, em especial dada a necessidade de aperfeiçoamento e calibração da classificação de grupos ecológicos, necessária-indispensável para um melhor desempenho do AMBI na costa brasileira. Futuros estudos são encorajados para testar a validade do AMBI em outros locais da costa brasileira, em especial na Região Norte e Nordeste, onde apenas um estudo foi realizado com tal finalidade.

O capítulo 3 revelou a dinâmica espaço-temporal das variáveis ambientais na baía, identificando três principais habitats, estruturados pela zonação do ambiente. Esses habitats apresentam características distintas principalmente na composição sedimentar e hidrodinâmica. As espécies indicadoras sugeridas podem ser utilizadas para monitorar futuras alterações na área.

Dadas as pressões sob as quais a Baía do Araçá se encontra, esperamos que os resultados aqui apresentados possam auxiliar, somado à demais esforços, no conhecimento da biodiversidade e serem utililizados como subsídios à futuros programas de manejo e monitoramento na área. Os resultados apresentados nos capítulos 2 e 3 mostram as condições atuais em relação a qualidade ambiental e dinâmica espaço-temporal dos habitats sedimentares. Tais condições podem ser futuramente utilizadas como um comparativo em estudos futuros, para avaliar o impacto de mudanças naturais ou antrópicas.

Anexos



UNICAMP
COORDENADORIA DE PÓS-GRADUAÇÃO
INSTITUTO DE BIOLOGIA
Universidade Estadual de Campinas
Caixa Postal 6109. 13083-970, Campinas, SP, Brasil
Fone (19) 3521-6378. email: cpgib@unicamp.br
IB

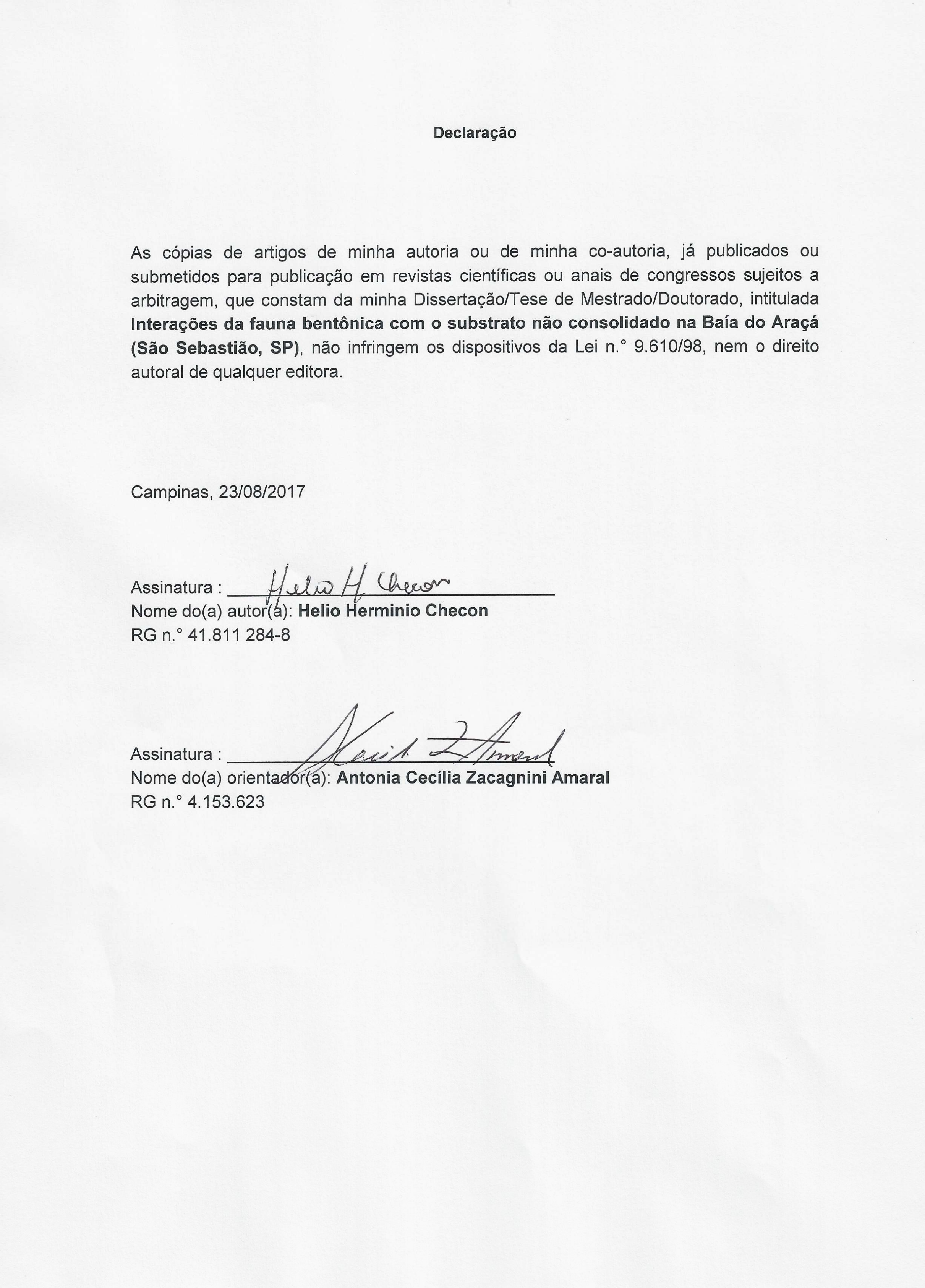
DECLARAÇÃO

Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "***Interações da fauna bentônica com o substrato não consolidado na Baía do Araçá (São Sebastião, SP)***", desenvolvida no Programa de Pós-Graduação em Ecologia do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

Assinatura: Helio M. Checon
Nome do(a) aluno(a): Helio Herminio Checon

Assinatura:
Nome do(a) orientador(a): Antonia Cecília Zacagnini Amaral

Data: 23/08/2017



Declaração

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada **Interações da fauna bentônica com o substrato não consolidado na Baía do Araçá (São Sebastião, SP)**, não infringem os dispositivos da Lei n.° 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 23/08/2017

Assinatura : Helio H Checon
Nome do(a) autor(a): **Helio Herminio Checon**
RG n.° 41.811 284-8

Assinatura : ______________________
Nome do(a) orientador(a): **Antonia Cecília Zacagnini Amaral**
RG n.° 4.153.623