

Response of soft-bottom macrobenthic assemblages to artisanal trawling fisheries in a subtropical estuary



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ABSTRACT

Bottom trawling is one of the most harmful and widespread activities affecting benthic habitats and fauna. In this study, we analyzed the impact of shrimp trawling fisheries on the densities, assemblage structure and vertical stratification of the benthic macrofauna in shallow mudflats of the Patos Lagoon Estuary (Brazil). Experimental trawls of different intensities were performed during three shrimp fishing seasons (2015–2017), comparing macrofauna among before and after trawling, and a control zone. The changes in macrofaunal assemblages were more consistently related to natural variability than to trawling impact, being mostly influenced by sediment structure and salinity variation. The trawling impact was mainly detected in the area with higher percentages of fine sediments, with different macrofaunal responses in each month and stratum. Some non-significant decreases on total densities after trawling were observed, mainly on the superficial stratum, and signs of burial activities. The response to trawling disturbance of each species was different and it highly depended on their natural variability. Only five species showed significant variation to the trawling treatment (*Erodona mactroides*, *Heleobia australis*, *H. charruana*, *Heteromastus similis* and *Laeonereis acuta*), with temporally different responses. Decreasing densities were more related to the high impact treatment. In some seasons, decreases on the abundance of *Monokalliapseudes schubarti* after high impact trawls were up to 60% of the seasonal mean densities. Trawling fisheries may reduce macrobenthic densities but not their vertical stratification. The impact of trawling on key species may compromise the ecosystem function, as benthic macrofauna provide food sources for many aquatic resources. This study highlights trawling impacts in an estuarine nursery area, which should be fully considered since they may be reflected in the trophic webs.

1. Introduction

Estuaries are very productive ecosystems, providing important ecological services but different human activities have compromised their ecological integrity (Alves et al., 2015; Rehitha et al., 2017). Trawling fisheries are one of the most harmful anthropogenic impacts on seabed (Kaiser et al., 2002; Collie et al., 2016; Hiddink et al., 2017). The impact of bottom fisheries on the seafloor result in harmful effects on benthic flora and fauna, but also on the physical environment (Kaiser et al., 2006, 2016; Collie et al., 2016; Hiddink et al., 2017). These gears can promote sediment suspension; decreasing macrofaunal bioturbation processes; remove, injure, or kill a wide range of benthic organisms, and induce evolutionary changes on population

demography and on the ecosystems structures and functions, among other consequences (Engel and Kvitek, 1998; Brown et al., 2005; Foden et al., 2010; Mangano et al., 2013, 2014; Sciberras et al., 2016).

The widespread use of bottom gears is controversial, leading to calls for bans of trawling in order to minimize ecosystem deterioration (Cinner et al., 2005; Watling, 2013; European Parliament, 2016). The studies regarding fishing impact on bottom communities have focused on large-scale or industrial fisheries, giving little attention to small-scale or artisanal fisheries, especially in estuaries (Costa and Netto, 2014). Despite of its apparently small-scale catches, artisanal fisheries contribute 50% of the world fish gross capture and two-thirds of world catches destined for direct human consumption (FAO, 2017).

A method to evaluate the impact of fisheries is the Ecosystem

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Approach to Fisheries (EAF). This method requires knowledge of how target species may be affected, but also takes into consideration the impact of the fisheries in the whole ecosystem, including trophic web disruption and the effects of multiple and potentially interacting pressures, as natural variability and climate change (Garcia and Cochrane, 2005; Bolam et al., 2014). The EAF aims to safeguard both function and biodiversity, therefore, trawling impacts on benthic community functioning needs to be understood prior to the enforcement of a given management action (Bolam et al., 2014). The natural variability generally is known for decades, but in practice it is not taken into account when evaluating impact, mainly because it is difficult to predict. When this variability is considered, it generally improves management performance (Garcia and Cochrane, 2005).

Coastal lagoons are one of the most productive ecosystems in the world, providing services to humans and acting as nursery grounds to marine and estuarine species, some of which has commercial interest (Miththapala, 2013; Sheaves et al., 2015). Artisanal trawling is widely performed in the Patos Lagoon Estuary (southern Brazil) targeting the pink shrimp *Farfantepenaeus paulensis* (Benedet et al., 2010; Kalikoski and Vasconcellos, 2012). Legal regulations for the estuarine fisheries ban the use of bottom trawling in the area (MMA, 2004). However, due to the lack of effective control, it is widely used during shrimp season. This fishery starts in the austral summer and ends in autumn (February to May), allowing only the use of passive fyke-nets (D'Incao, 1991; Benedet et al., 2010). The period of intense trawling matches a critical recruitment period for the macrobenthos at the Patos Lagoon Estuary. There is an increase of macrofauna reproduction and recruitment in late spring and summer (Bemvenuti and Netto, 1998; Rosa and Bemvenuti, 2006), which can be extended to autumn, influenced by large-scale climate events, such as ENSO (Colling et al., 2007). Therefore, it may magnify the impact due to the synchronicity of trawling and macrobenthos reproduction and recruitment seasons.

This potential impact on the macrobenthos may affect the whole estuarine ecosystem, due to the important role of these assemblages in the nutrient cycling, sediment dynamics and food webs, since act as both consumers and prey (Mola and Abdel Gawad, 2014; Piló et al., 2015; Dauvin et al., 2017). Due to the limited mobility and short life span, benthic macrofauna is considered as a good biological indicator of impact, being capable of responding to disturbances in the water column and sediment (Dauvin et al., 2017).

This study evaluates the potential impact of trawling fisheries on the grain size composition and macrofaunal densities, species richness, diversity, evenness, assemblage structure and vertical stratification in a subtidal shallow mudflat of the Patos Lagoon estuarine region. We hypothesized that trawls should reduce the percentage of very fine sediments (very fine sand, silt and clay), reduce the macrofaunal densities, richness, diversity, alter the assemblage structure and modify the previous vertical stratification of the macrobenthos.

2. Materials and method

2.1. Study area

The Patos Lagoon Estuary (PLE) is located in the southern Brazilian coastal plain (Kjerfve, 1986) (Fig. 1). Five major tributary rivers constitute significant freshwater sources to the PLE from an extensive drainage basin (201,626 km²) (Fernandes et al., 2005). The freshwater discharge and the action of predominant winds (from NE during the warm seasons and from S during the cold seasons) tightly control the hydrodynamics of the PLE (Fernandes et al., 2002). In the western margin, it has a shallow (1–4 m depth) inlet called 'Saco do Arraial', characterized as a dynamic environment concerning the erosion processes, sediment transportation, and deposition (Colling et al., 2007; Souza and Hartmann, 2008), which is our study region.

2.2. Experimental design

Two areas commonly used by artisanal fishermen in the Saco do Arraial were selected: 1) the sheltered area near Pólvora Island-Area 1; and 2) the more exposed area near Cavalos Island -Area 2 (Fig. 1). Depth was measured in every sampling, ranging between 0.9 and 2.5 m. Three zones of 20 × 20 m were marked in each area with wood sticks: the first one without any impact (Control zone), the second where two consecutive experimental tows were performed (Low-impact zone), and the third where five consecutive experimental tows were conducted (High-impact zone) (Fig. 1). In each sampling, trawling followed an imaginary line randomly selected into the marked square. Trawls were performed using a typical artisanal fishing boat of 8 m in length at a speed of approximately 2 knots (3.7 km/h), equipped with an otter trawl net for shrimp capture, with 12 mm between opposite knots and 15 kg boards.

2.3. Sampling

Sampling was carried out monthly between January and June 2015–2016, and in January, March and May 2017, resulting in five sampling campaigns in 2015, six in 2016 and three in 2017. In each sampling, three replicates of sediment samples were collected for macrofauna analysis and two for granulometric and organic matter analyses in the control zones. In the impacted zones, three samples were collected before and after trawling for fauna analysis and two for granulometric and organic matter analyses. The post-trawling samplings were immediately collected after the induced impact. Samples were collected with a *van Veen* grab, and from each grab, one subsample was taken with a 10 cm diameter core (0.0078 m²). This subsample was sectioned into 0–5 cm and 5–10 cm strata. The benthic macrofauna was sieved with a 300 µm mesh net and preserved in 4% formaldehyde. Water temperature was measured with a mercury thermometer (°C) and salinity with a refractometer. Granulometric analyses were performed by dry mechanical sieving through a column of sieves of different mesh sizes from 4 mm to 0.063 mm and by pipetting analysis for grain sizes < 0.063 mm. The granulometric procedures followed the Wentworth classification system in intervals of one phi (Suguio, 1973). The grain size composition was expressed as the percentage of the total sample weight. Organic matter content was calculated by differences on weight before and after calcination for 2 h at 375 °C.

2.4. Statistical analysis

In order to characterize and evaluate seasonal differences on the studied area, a Principal Component Analysis (PCA) was performed using all measured parameters (salinity, temperature, depth, organic matter percentage and all granulometric percentages). Similarity Percentages (SIMPER) of the species contributions were calculated to identify the main species responsible for the differences on faunal composition between the studied areas. A canonical correspondence analysis (CCA) was performed to evaluate possible relations between the macrofaunal species and environmental parameters.

The fishing impact was analyzed using a nested design in order to evaluate differences in total densities, species richness, diversity, evenness and assemblage structure between trawling periods (before and after trawling) and vertical macrofaunal stratification for each area along the three sampled years. The tested factors were Treatment (fixed- Control, Before high impact, After high impact, Before low impact and After low impact), Month (random, nested in Season), Season (random, nested in Year), Year (random), and Stratum (fixed- 0–5 cm, 5–10 cm). All fixed factors were orthogonal among themselves. The possible variability among factors was tested through a permutational multivariate analysis of variance (Permanova, Anderson et al., 2008).

For the univariate macrofaunal data (total densities, species

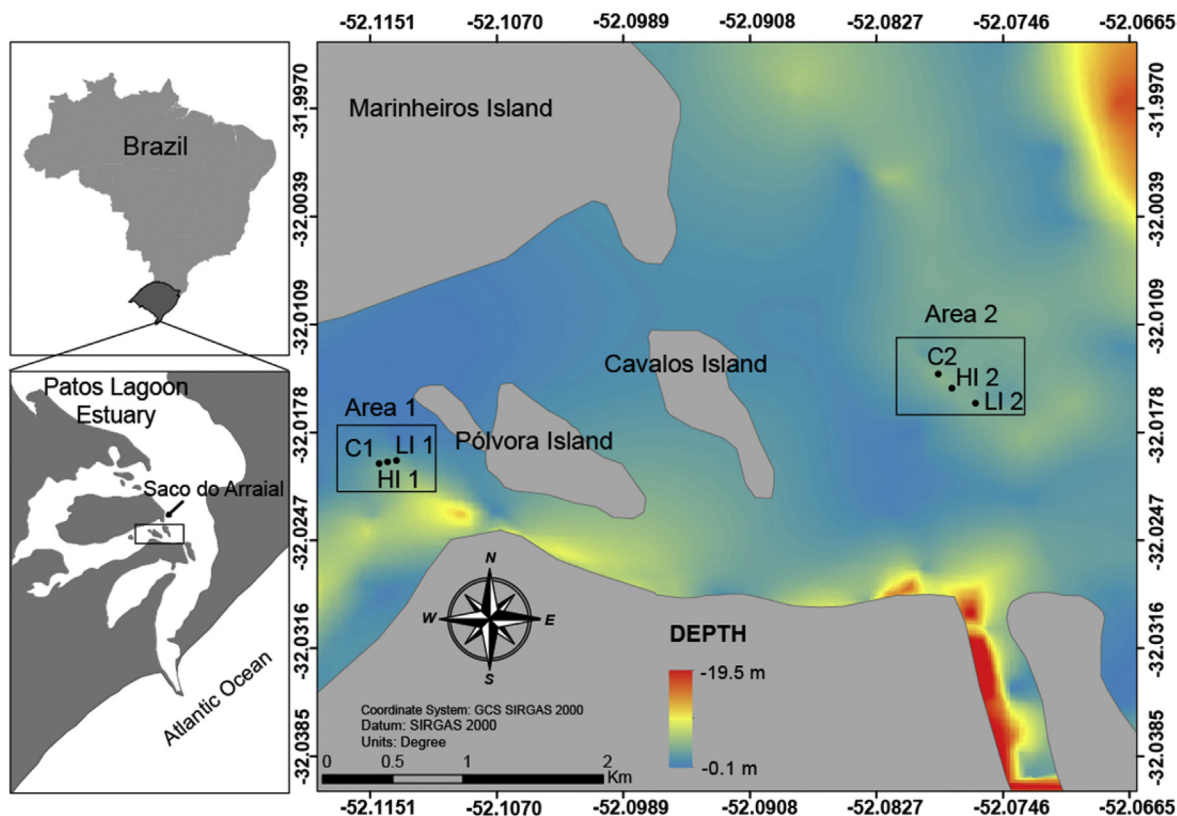


Fig. 1. Map of the study area: Saco do Arraial, Patos Lagoon Estuary, Brazil. C1 and C2: control zones, HI1 and HI2: high-impacted zones, LI1 and LI2: low-impacted zones.

richness, diversity and evenness), a Permanova with 4999 permutations of raw data units was used to analyze the possible variability among factors (Anderson, 2005). We used the Monte Carlo permutation test to obtain P-values ($P < 0.05$) (Anderson and Robinson, 2003; Anderson, 2005). This test assumes not only exchangeability, but also a linear model on dissimilarities that is appropriate for choosing reasonable statistical tests analogous to those used in univariate Anova (Anderson and Millar, 2004). Permanova was performed using a Euclidian distance matrix. The significant results were evaluated with a *posteriori* pairwise comparisons, which also used 4999 random permutations to obtain P-values through Monte Carlo correction.

For multivariate macrofaunal data (assemblage structure), the Permanova was based on the Bray-Curtis similarity matrix constructed from the square-root-transformed abundance data (ind.m^{-2}). Transformation was used to down-weight the importance of the dominant species. Dummy values of one were added to reduce the effect of the absence of individuals in some samples (Clarke et al., 2006). The statistical significance of the relationships was evaluated using Monte Carlo permutation test. Pairwise tests were run for those factors that resulted significant, using the Monte Carlo correction. The same statistical approach was applied to grain size data, but instead of a Bray-Curtis similarity matrix, the Euclidean distance matrix was employed.

3. Results

3.1. Environmental variables

Salinity varied between 0 and 34, presenting higher values at late autumn (May and June) and lower at early summer (January–February). Temperature varied between 10 and 27 °C, with the early summer presenting the highest values and at late autumn the lowest values. Organic matter varied between 0 and 8.24% per sample (minimum and maximum determined values), with higher mean values

recorded in Area 1 (approx. 2% in Area 1 and 1.5–1.16% in Area 2). Fine sands constitute the main sediment fraction for both areas. Zones from Area 1 were characterized by more than 14% of silt and clay, and less than 3% of medium sand, while the zones from Area 2 presented less than 9% of silt and clay and more than 10% of medium sands (Table 1).

The Principal Component Analysis (PCA) explained approx. 51% of variance: the axis 1 explained 37% of variation while axis 2 the 14.5% of the variation. The first axis separated spatially both areas, and the second one was related to the temporal variability (Fig. 2). The Area 1 exhibited higher percentages of silt, clay and very fine sands (fine sediments), and organic matter, while Area 2 was slightly deeper and composed by higher percentage of fine sands, medium sands, coarse sands, very coarse sands, granules and pebbles (coarser sediments) (Fig. 2; Table 1). Temperature and salinity correlated negatively and their relationship was higher than those related to sediment characteristics, characterizing a temporal trend.

3.2. Trawl impact on grain size composition

The sediment texture varied within the interaction of the factors month, stratum and treatment in both areas (Pseudo-F Area 1 = 2630.5, Pseudo-F Area 2 = 1.23E+05, $P = 0.001$), indicating a high variability on sediment response. Pairwise tests showed that differences on treatment occurred among zones (Control, High and Low-impact zones) and not between before and after trawling ($p < 0.001$). For Area 1, the Control zone exhibited approximately $39 \pm 9\%$ (mean \pm standard deviation) of very fine sediments, while High-impact zone had $35 \pm 6\%$ and Low-impact zone $31 \pm 6\%$. For the Area 2, the Control zone had approximately $13 \pm 2\%$ of very fine sediments, High-impact zone had $15 \pm 6\%$ and Low-impact zone $21 \pm 6\%$. Differences between strata were subtle, with higher percentages of very fine sediments in Stratum 0–5 for both areas (differences of $\pm 2\%$ between strata).

Table 1
Mean and standard deviation (SD) of the environmental variables in both studied areas.

Area	1				2			
	Summer		Autumn		Summer		Autumn	
Variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Temperature (°C)	23	3	17	4	24	2	17	4
Salinity	4	4	19	8	3	6	21	11
Depth (m)	1.30	0.17	1.34	0.20	1.70	0.24	1.84	0.27
Organic matter (%)	2.07	0.82	1.85	0.74	1.51	1.00	1.16	0.66
Pebbles (%)	0.003	0.015	0.005	0.022	0.008	0.024	0.017	0.046
Granules (%)	0.018	0.033	0.015	0.028	0.088	0.095	0.072	0.053
Very coarse sand (%)	0.040	0.041	0.035	0.038	0.092	0.058	0.069	0.041
Coarse sand (%)	0.224	0.124	0.215	0.097	0.293	0.426	0.200	0.090
Medium sand (%)	2.839	4.205	2.530	1.840	10.432	3.166	11.790	2.604
Fine sand (%)	61.963	7.088	62.995	6.008	71.925	6.136	71.078	4.606
Very fine sand (%)	20.369	5.214	19.039	4.048	8.702	3.926	7.406	2.462
Silt (%)	5.266	2.138	5.682	1.496	3.169	1.299	3.417	1.268
Clay (%)	9.176	2.973	9.400	2.977	5.239	2.138	5.900	3.287

3.3. Macrobenthic structure

A total of 45529 specimens were quantified, comprising 9 major groups (Amphipoda, Isopoda, Tanaidacea, Cumacea, Decapoda, Bivalvia, Gastropoda, Polychaeta and Nemertea). The most abundant and frequent species in both zones was the tanaid *Monokalliapseudes schubarti* (Table 2). Juveniles from the gastropod genus *Heleobia* were grouped as *Heleobia* sp. due to the lack of identification structures.

The assemblage structure was significantly different between both areas (Permanova, Pseudo-F = 17.842, P = 0.001). The dissimilarity percentage between both, Area 1 and Area 2, was of 62%. Seven species were the responsible for 94% of differences: the peracarids *Monokalliapseudes schubarti* (30.63%) and *Kupellonura* sp. (7.34%), the mollusks *Erodona mactroides* (14.68%) and *Heleobia australis* (9.52%), with higher abundances on Area 2; and the polychaetes *Heteromastus similis* (15.07%), *Nephtys fluviatilis* (11.36%) and *Laeonereis acuta* (5.68%) with higher abundances on Area 1.

The CCA explained 80.32% of the variance (54.47% at CCA1 and 25.85% at CCA2). Polychaetes were more correlated with the fine sediments, which were present in higher percentages on Area 1. The gastropods were predominant in Area 2 and more correlated with higher abundances of coarser sands. The tanaid *M. schubarti* correlated positively with salinity and higher percentages of medium sands, which

were predominant on Area 2 (Fig. 3).

3.4. Trawl impact on macrofauna

3.4.1. Macrobenthic total densities

The mean total density at Area 1 was $909.0 \pm 1305.8 \text{ ind.m}^{-2}$ (Mean \pm Standard deviation). The response of faunal total densities to treatment varied between strata (Pseudo-F = 4.4103, P = 0.0308). The pairwise test detected no statistically significant differences between strata for any treatment, nor differences between treatments for each stratum, although we detected more signals of impact on Stratum 0–5, with decrease on densities after trawling in some months (marked as descending arrows on Fig. 4). In addition, densities increased in some months after trawling on Stratum 5–10 cm, indicating the burial of organisms (marked as ascending arrows on Fig. 4). The greatest differences in densities between treatments were observed between years 2016 and 2017 when faunal abundances were higher. Significant differences in the stratification by month (Pseudo-F = 4.1255, P = 0.0002) were found, where higher densities occurred in Stratum 0–5 in almost all months (Fig. 4). The Permanova also detected significant differences in total densities by month (Pseudo-F = 28.383, P = 0.0002), with higher increments at late summer and early autumn (Fig. 4).

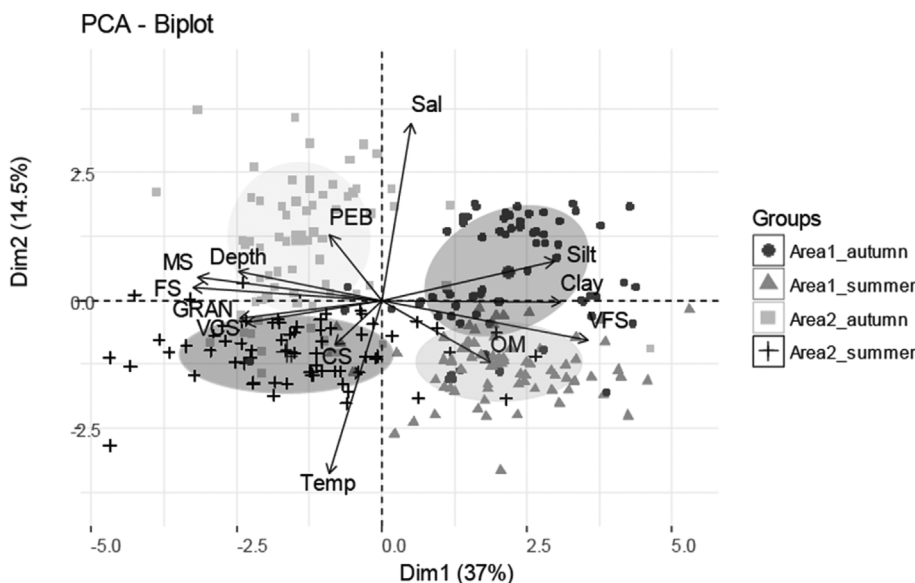


Fig. 2. Principal Component Analysis Plot (Dim1/Dim2) used to separate areas and seasons by the environmental variables. PEB: pebbles, GRAN: granules, VCS: very coarse sand, CS: coarse sand, MS: medium sand, FS: fine sand, VFS: very fine sand, Temp: water temperature, Sal: water salinity. Ellipses grouped 50% of points from each centroid.

Table 2
List of species found on the Saco do Arraial with their mean density and frequency of occurrence by treatment. C: Control zone, LI: Low-impact zone, HI: High-impact zone, HII: High-impact zone. FO: frequency of occurrence.

Group	Specie	Epifauna/ Infauna	C1		% FO		LI1 Be		% FO		LI1 Af		% FO		HII Be		% FO		HII Af	
			Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)	Mean density (ind.m ⁻²)
Amphipoda Cumacea	Gammaridea	Epifauna	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Diatylis symperygiae</i>	Infauna	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Isopoda	<i>Kupellonura</i> sp.	Infauna	254.6	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	254.6	0.3	0.0	0.0	0.0
	<i>Sphaeromopsis</i> sp.	Epifauna	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tanaidacea	<i>Uromunna peterseni</i>	Epifauna	127.3	1.6	1.5	1.5	127.3	1.5	1.5	1.5	233.4	1.8	200.1	2.1	200.1	141.5	2.1	141.5	141.5	141.5
	<i>Monokalliapseudes schubarti</i>	Infauna	1294.3	35.2	34.3	34.3	992.7	34.3	34.3	1212.3	35.3	1328.9	34.3	1328.9	1254.3	34.3	1254.3	1254.3	1254.3	1254.3
Decapoda Bivalvia	<i>Stanfordini</i>	Epifauna	127.3	0.3	0.3	0.3	127.3	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Panopeidae</i>	Epifauna	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gastropoda	<i>Erodona macroides</i>	Infauna	297.1	3.9	6.4	6.4	497.7	6.4	6.4	583.0	5.6	369.2	6.1	369.2	245.6	6.1	245.6	245.6	245.6	245.6
	<i>Heleobia australis</i>	Epifauna	418.4	4.5	4.9	4.9	516.8	4.9	4.9	617.0	3.9	1077.4	4.0	1077.4	262.1	4.0	262.1	262.1	262.1	262.1
Polychaeta	<i>Heleobia australis</i>	Epifauna	127.3	0.3	1.2	1.2	159.2	1.2	1.2	127.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Heleobia sp.</i>	Epifauna	0.0	0.0	0.3	0.3	127.3	0.3	0.3	254.6	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nemertea	<i>Heteromastus similis</i>	Infauna	1232.0	21.9	20.3	20.3	1396.9	20.3	20.3	1230.2	22.8	1247.0	20.8	1247.0	1114.5	20.8	1114.5	1114.5	1114.5	1114.5
	<i>Laeneris acuta</i>	Infauna	405.1	10.6	11.9	11.9	437.9	11.9	11.9	474.3	11.9	445.6	13.5	445.6	364.4	13.5	364.4	364.4	364.4	364.4
Nemertea	<i>Nephtys fluviatilis</i>	Infauna	714.4	17.4	17.2	17.2	606.4	17.2	17.2	731.5	16.3	712.5	15.9	712.5	675.1	15.9	675.1	675.1	675.1	675.1
	<i>Poliqeta n. i</i>	Infauna	127.3	0.6	0.6	0.6	127.3	0.6	0.6	127.3	0.3	127.3	0.6	127.3	127.3	0.6	127.3	127.3	127.3	127.3
Amphipoda Cumacea Isopoda			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			254.6	1.7	1.7	1.7	191.0	1.7	1.7	127.3	0.5	127.3	0.5	127.3	145.5	1.7	145.5	145.5	145.5	145.5
Tanaidacea			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			152.8	2.2	2.3	2.3	178.3	2.3	2.3	178.3	1.1	169.8	1.7	169.8	37.0	1.7	37.0	37.0	37.0	37.0
Decapoda Bivalvia Gastropoda			303.6	34.8	40.2	40.2	323.1	40.2	40.2	3392.5	38.2	3392.5	37.2	3392.5	3269.3	37.2	3269.3	3269.3	3269.3	3269.3
			169.8	0.6	1.8	1.8	350.1	1.8	1.8	350.1	1.8	254.6	1.3	254.6	534.8	1.3	534.8	534.8	534.8	534.8
Polychaeta			2136.7	11.9	9.7	9.7	4168.3	9.7	9.7	4526.4	9.0	4391.3	9.6	4391.3	3169.3	9.6	3169.3	3169.3	3169.3	3169.3
			1612.8	8.4	7.1	7.1	1199.3	7.1	7.1	2432.8	6.3	1733.6	8.6	1733.6	1571.4	8.6	1571.4	1571.4	1571.4	1571.4
Nemertea			2546.5	1.3	1.6	1.6	1491.5	1.6	1.6	1344.0	2.0	1909.9	1.7	1909.9	1888.6	1.7	1888.6	1888.6	1888.6	1888.6
			588.4	16.0	14.0	14.0	534.3	14.0	14.0	484.6	15.2	596.6	14.9	596.6	487.7	14.9	487.7	487.7	487.7	487.7
Nemertea			159.2	0.9	1.1	1.1	127.3	1.1	1.1	191.0	1.1	152.8	1.1	152.8	200.1	1.1	200.1	200.1	200.1	200.1
			532.7	10.6	10.1	10.1	494.8	10.1	10.1	390.3	10.4	543.8	10.2	543.8	368.1	10.2	368.1	368.1	368.1	368.1
Nemertea			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

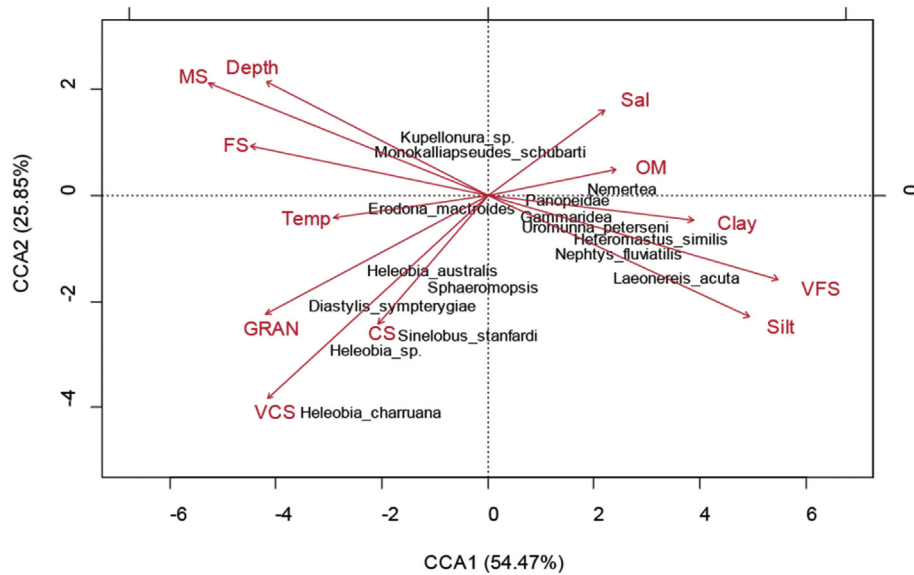


Fig. 3. Canonic Correspondence Analysis of fauna sampled on the Saco do Arraial in correlation with environmental variables. GRAN: granule, VCS: very coarse sand, CS: coarse sand, MS: medium sand, FS: fine sand, VFS: very fine sand, OM: organic matter, Sal: water salinity, Temp: water temperature.

At Area 2, the mean total density was $1910.9 \pm 3709.4 \text{ ind.m}^{-2}$. The response of total densities to treatment varied monthly (Pseudo-F = 1.611, P = 0.0236). Pairwise tests evidenced some months with differences between zones, and only in March (2017) there were differences between before and after trawling in the Low-impact zone ($t = 14.775$, P = 0.002). Although there were no-significant differences, a decrease in densities was observed in some months in both strata (marked as descending arrows on Fig. 5), as well as increases in densities on Stratum 5–10 (marked as ascending arrows on Fig. 5).

Stratification also differed monthly (Pseudo-F = 9.2454, P = 0.0002). In almost all months, the total densities were significantly higher at Stratum 0–5, with exception of January of 2015. There were also significant differences within months (Pseudo-F = 18.117, P = 0.0002). The highest faunal densities were observed at the end of summer and autumn.

3.4.2. Species richness

Species richness ranged from 0 to 7 by sample at Area 1. The

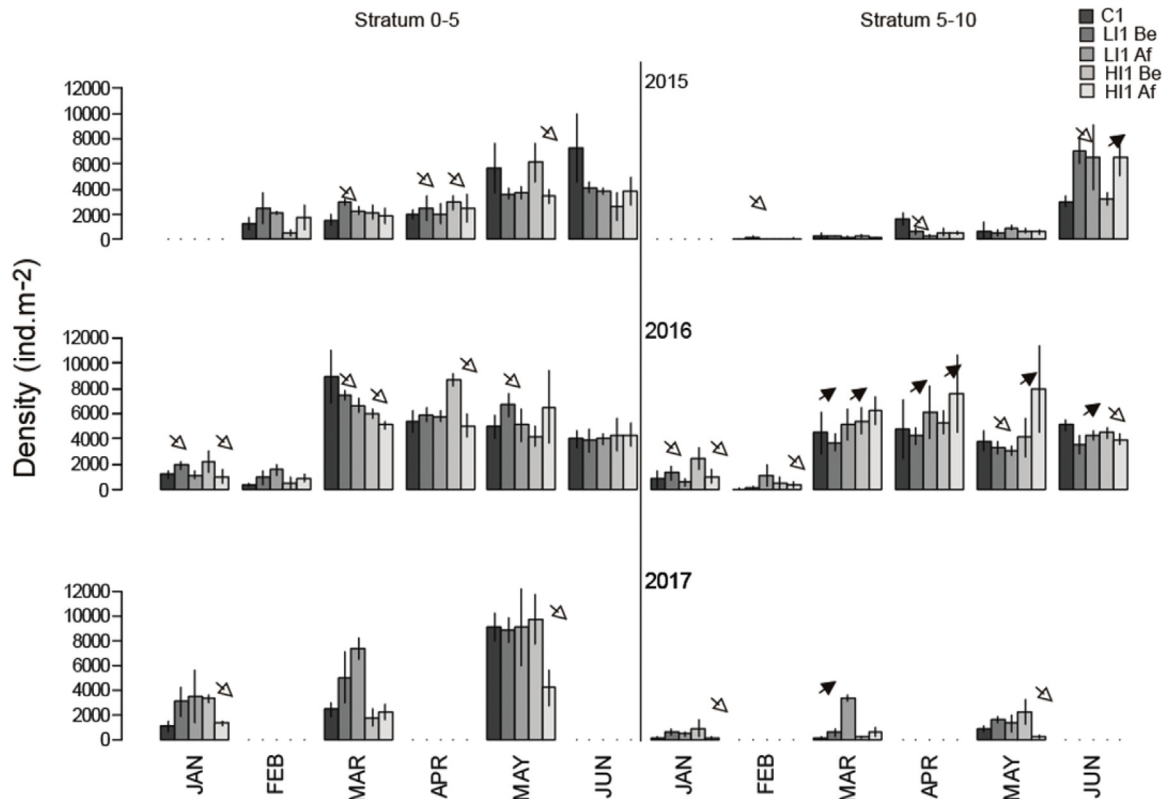


Fig. 4. Monthly variation on mean density (ind.m^{-2}) of macrobenthic organisms in each treatment for Area 1. Vertical bars indicate standard error. C1: Control zone, HI1 Be: High-impact zone before trawling, HI1 Af: High-impact zone after trawling, LI1 Be: Low-impact zone before trawling, LI1 Af: Low-impact zone after trawling.

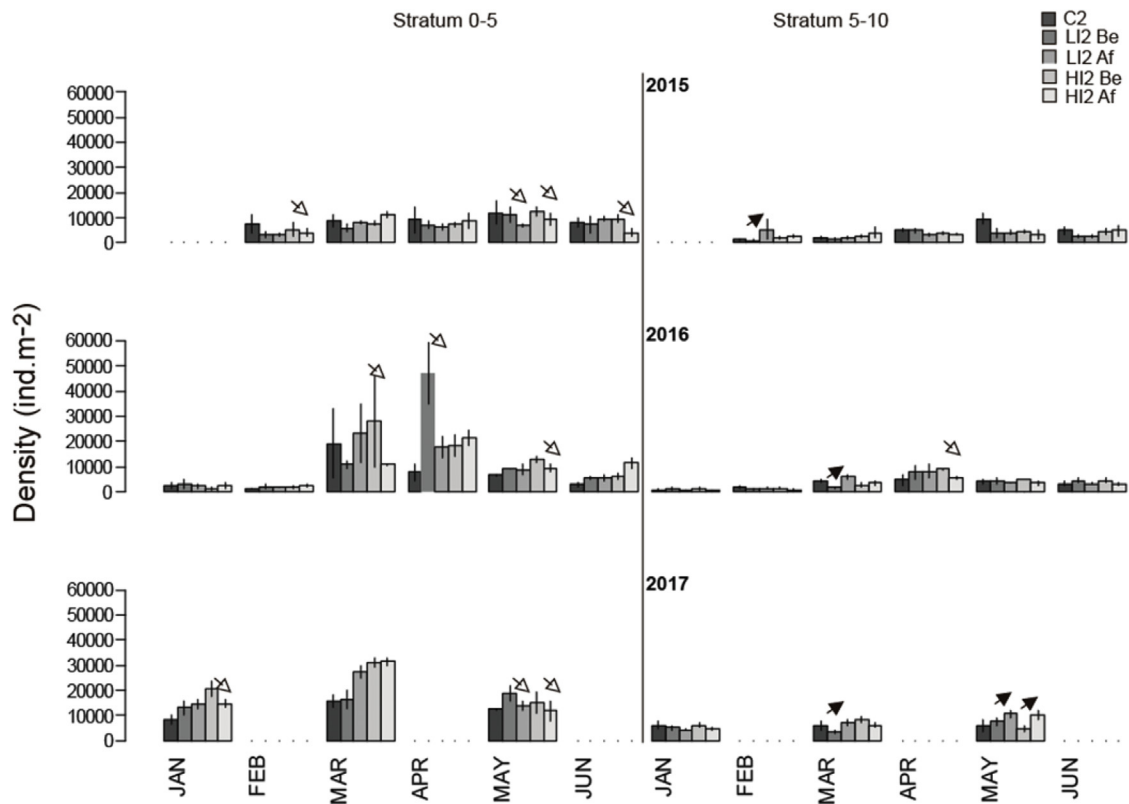


Fig. 5. Monthly variation on mean density (ind.m^{-2}) of macrobenthic organisms in each treatment for Area 2. Vertical bars indicate standard error. C2: Control zone, HI2 Be: High-impact zone before trawling, HI2 Af: High-impact zone after trawling, LI2 Be: Low-impact zone before trawling, LI2 Af: Low-impact zone after trawling.

distribution of species between strata varied differently in each month (Pseudo-F = 1.9402, $P = 0.0472$) (Fig. 6). In addition, the richness varied differently for each treatment at each month (Pseudo-F = 1.7806, $P = 0.0088$). The Stratum 0–5 presented higher richness and was more variable within months than within treatments. The Control zone tended to exhibit less or equal richness than the impacted ones. In 36% of sampling events along the three years, richness decreased after high impact trawls on the Stratum 0–5, losing between 1 and 3 species, and at 29% of experiment replicates on Stratum 5–10 (Fig. 6).

Species richness was higher at Area 2 and ranged from 0 to 10 species per sample, although it was less variable than Area 1. Higher richness occurred generally in the Stratum 0–5, with lower richness in 2015 (Pseudo-F = 885.39, $P = 0.0002$). Significant differences were detected regarding the interaction of treatment, stratum and season (Pseudo-F = 2.612, $P = 0.0126$). Differences after trawling occurred mainly in autumn, with decreases on species richness after a high impact trawling in few occasions: four in Stratum 0–5 (May 2015, January 2016, April 2016, June 2016) and five on Stratum 5–10 (April 2015, May 2015, January 2016, April 2016, March 2017) (see numbers above columns on Fig. 7).

3.4.3. Species diversity and evenness

No differences among years for both diversity and evenness were observed. Shannon diversity index presented no significant differences between treatments for any area (Area 1 mean $H' = 0.85 \pm 0.45$; Area 2 mean $H' = 0.86 \pm 0.41$). The diversity was always higher in Stratum 0–5 in the Area 1 (Pseudo-F = 21.174, $P = 0.0398$; $H' = 1.08 \pm 0.32$) when compared to the Stratum 5–10 ($H' = 0.62 \pm 0.45$). The same trend was observed for the Area 2, with higher values in the Stratum 0–5 (Pseudo-F = 57.694, $P = 0.0118$; $H' = 1.09 \pm 0.30$) and lower in the Stratum 5–10 ($H' = 0.63 \pm 0.37$). No significant differences were recorded between seasons in Area 1 (Summer $H' = 0.71 \pm 0.5$;

Autumn $H' = 0.99 \pm 0.34$). In Area 2, significant differences within seasons (Pseudo-F = 8.69, $P = 0.0078$) were observed (Summer $H' = 0.93 \pm 0.42$; Autumn $H' = 0.79 \pm 0.39$).

The evenness presented no significant differences between treatments for any area (Area 1 mean $J' = 0.68 \pm 0.31$; Area 2 mean $J' = 0.60 \pm 0.23$). There were no differences in the stratification at the Area 1 (Stratum 0–5 $J' = 0.79 \pm 0.31$; Stratum 5–10 $J' = 0.56 \pm 0.31$) and Area 2 (Stratum 0–5 $J' = 0.67 \pm 0.23$; Stratum 5–10 $J' = 0.53 \pm 0.23$). No differences were observed for seasons at the Area 1 (Summer $J' = 0.62 \pm 0.31$; Autumn $J' = 0.74 \pm 0.31$), and Area 2 (Summer $J' = 0.64 \pm 0.23$; Autumn $J' = 0.56 \pm 0.23$).

3.4.4. Macrobenthic assemblage structure

At Area 1, the Cluster analysis highlighted similarities between the High-impact and Low-impact zones before trawling, grouping those treatments with the Low-impact zone after trawling. A second group was formed by the samples of the Control zone and High-impact zone after trawling. At Area 2, the Control zone separated from the Low-impact and High-impact zones, which formed a cluster with high similarity between before and after trawling (Fig. 8).

A marked seasonal variability was observed for Area 1 (Pseudo-F = 2.2681, $P = 0.027$), with the highest abundances occurring in autumn. The treatment response of the assemblage structure (Pseudo-F = 1.2338, $P = 0.028$) and the stratification (Pseudo-F = 3.6269, $P = 0.001$) exhibited a monthly variation. The temporal variability of the dominant species presented different responses considering the treatments (Fig. 6). Only 4 of the 10 species in this area presented significant differences within treatment at some level: *Erodona mactroides* showed differences in abundances between treatment, month and strata (Pseudo-F = 2.5043, $P = 0.0002$), with differences restricted to the Stratum 0–5. In June 2015 *E. mactroides* densities increased in both impacted zones after trawling, but this increment in the Low-impact zone implied in significant differences with the Control zone

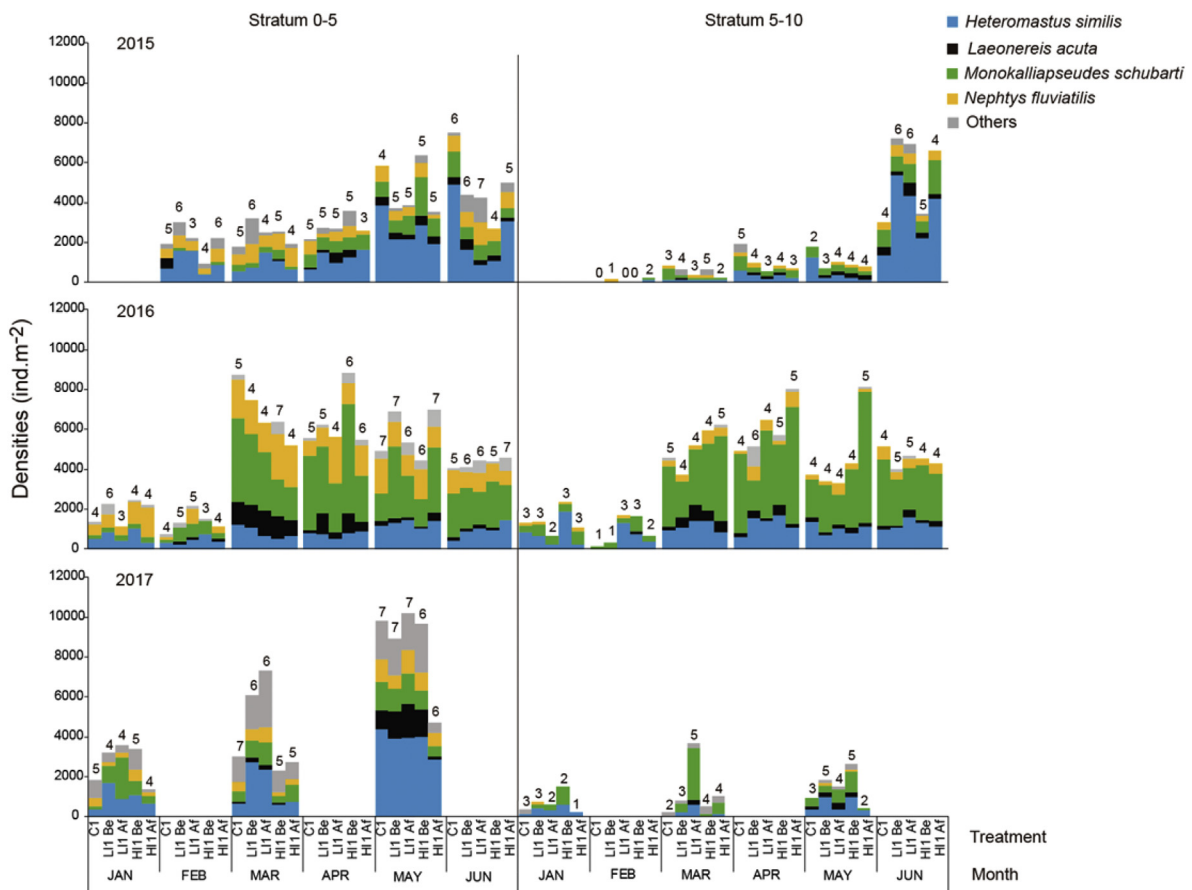


Fig. 6. Monthly mean density of species found in each treatment along the three sampled years in the Area 1. Numbers above columns indicate species richness. C1: Control zone, HI1 Be: High-impact zone before trawling, HI1 Af: High-impact zone after trawling, LI1 Be: Low-impact zone before trawling, LI1 Af: Low-impact zone after trawling.

($t = 3.7262$, $P = 0.021$). In January 2017, densities decreased significantly after high-impact trawls ($t = 4.0508$, $P = 0.0154$). *Heleobia australis* showed different responses to treatment by season (Pseudo- $F = 5.9964$, $P = 0.0002$), with significant decreases of densities in autumn of 2017 after both high-impact ($t = 2.6737$, $P = 0.0268$) and low-impact ($t = 9.1924$, $P = 0.0002$) trawling. Also, responses in the interaction of season, treatment and stratum were detected (Pseudo- $F = 5.4449$, $P = 0.0002$), with differences occurring only in the Stratum 0–5. Similarly, significant differences in *Heleobia charruana* densities for the interaction of treatment and season were estimated (Pseudo- $F = 2.1867$, $P = 0.0388$). The species was recorded only on summer of 2015 and decreased its densities after low-impact trawling. *Laeonereis acuta* also showed different responses to trawling treatment in each stratum by season (Pseudo- $F = 3.0158$, $P = 0.0078$). Even when pairwise tests did not detect significant differences, decreases on densities were observed on both seasons of 2015 and 2017, and in summer of 2016 on Stratum 0–5 after high-impact trawls. We observed marked decreases of *Monokalliapseudes schubarti* densities on autumn of 2015 (15%) and 2017 (60%) after high-impact trawling, but non-significant effects of this treatment were detected.

Monthly variation in each stratum was detected at Area 2 (Pseudo- $F = 1.9864$, $P = 0.001$). Higher densities and richness were observed on the Stratum 0–5, excepting April and May of 2015 (Fig. 7). The assemblage structure and densities of the dominant species presented variability between months and a marked seasonal variability. When analyzing species separately, significant differences on densities were detected of *Heteromastus similis* within treatment (Pseudo- $F = 4.9073$, $P = 0.0126$), with decreases after high-impact trawling, remaining significantly different from Control zone ($t = 4.6444$, $P = 0.015$).

4. Discussion

Estuarine assemblages are adapted to deal with a very dynamic ecosystem in which physicochemical characteristics vary in time and space, often presenting unpredictable environmental scenarios (Elliott and Quintino, 2007; Alves et al., 2015; Rehitha et al., 2017). The present study highlights the role of environmental conditions on the macrofauna of a subtropical estuary, which can promote variations in benthic assemblages through time and space. The responses of the macrofauna to different intensities of trawling impact, when detected, were related to the temporal trends of the species composition, abundance, and granulometric characteristics, but also on their vertical distribution.

In the shallow estuarine zones assessed in this study, the benthic macrofauna is subjected to a dynamic habitat, where the sedimentation process related to winds and currents may change in scale of days. The variability of topographic profiles of this shallow mudflat is sometimes up to 10 cm, associated to erosion and deposition processes of mud and sand sediments, with autumn being the more dynamic season (Colling et al., 2007). These erosional and depositional processes can be intensified depending on the wind speed and direction (Souza, 2002), with higher dynamic in the Area 2. Moreover, the occurrence of ENSO-El Niño events in 2015 and 2016 (NOAA, 2017) resulted in a long rainy period in southern Brazil. In consequence, a greater discharge of freshwater drains from de Patos Lagoon drainage basin to the estuarine region. These events of higher freshwater discharge imply limnetic scenarios and a greater input of silt and clay to the estuary (Tomazelli and Villwock, 2000), increasing the mud content in the sediments of shallow soft bottoms. Considering the important role of these natural

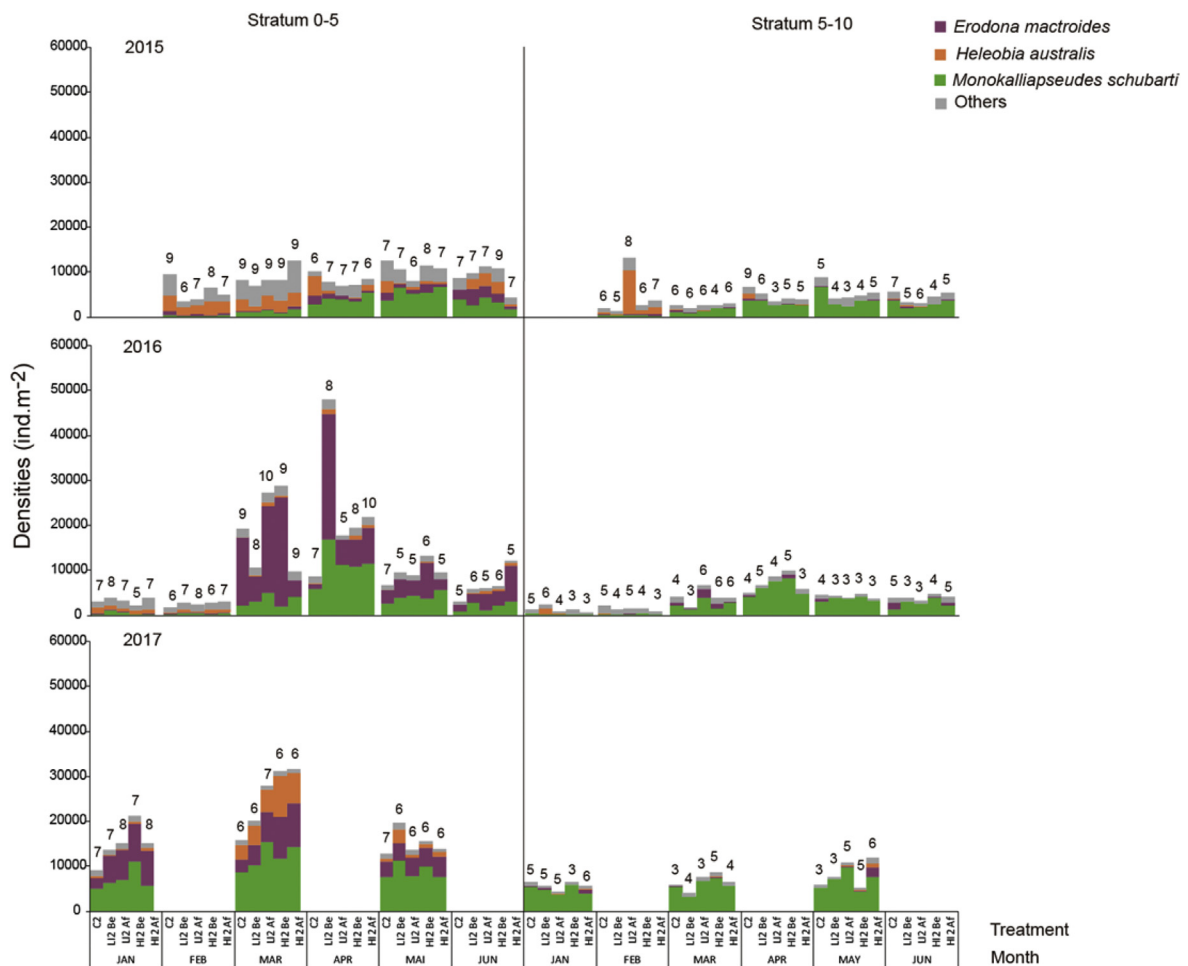


Fig. 7. Monthly mean density of species found in each treatment along the three sampled years on Area 2. Numbers above columns indicate species richness. C2: Control zone, HI2 Be: High-impact zone before trawling, HI2 Af: High-impact zone after trawling, LI2 Be: Low-impact zone before trawling, LI2 Af: Low-impact zone after trawling.

disturbances, it is necessary to recognize a possible overlap between the effects of trawling impact and the natural physic disturbances, both contributing to the temporal variation of benthic richness and diversity.

Trawling activities impacted the total densities and assemblages structure mainly in the sheltered area near the Pólvara Island (Area 1).

Furthermore, the physical disturbance caused by trawling varied markedly between both areas and seems to be primarily related to the sediment structure: Area 1 presented higher contents of silt, clay and very fine sands, while Area 2 was characterized by a coarser granulometry. As seen by using a side-scan sonar (Fig. A1), marks in the soft

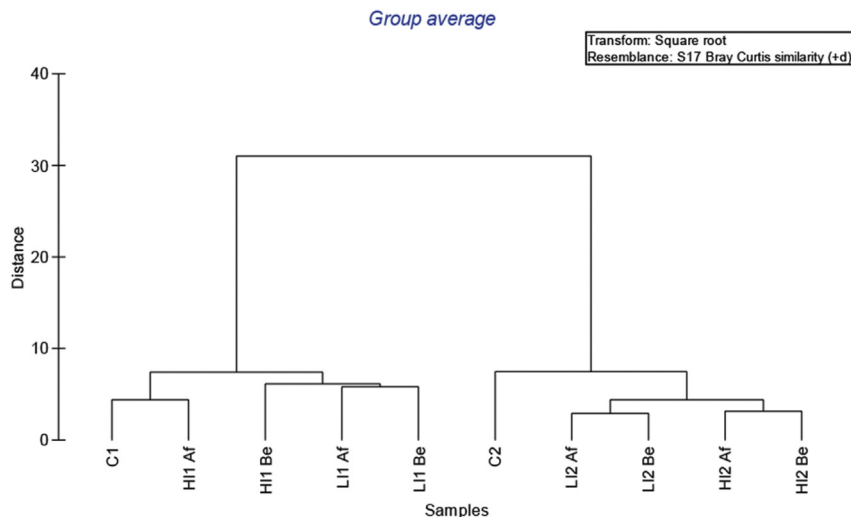


Fig. 8. Dendrogram showing differences between treatments for both areas.

bottom left by otter trawls remained visible in the majority of cases at Area 1. Conversely, the tracks left by otter trawls were immediately filled by neighboring sediments at Area 2. Similar patterns of higher impacts of trawling on zones characterized by lower levels of natural disturbance were also observed by Brylinsky et al. (1994), Jennings et al. (2001), Hiddink et al. (2006) and Prantoni et al. (2013). For instance, in the Bay of Fundy, Brylinsky et al. (1994) detected that direct impacts on benthic fauna were caused mainly by doors rather than rollers, with marks up to 5 cm depth made by trawl doors of 180 kg. We could not measure the depth of trawl marks, however, if a 180 kg door left marks of 5 cm depth, we suppose that our 15 kg doors will impact a more superficial sediment layer. These observations could imply that the sediment resuspension visually observed during trawling action could not be enough to result in granulometric changes in the analyzed strata. This may also explain the higher signals of impact over the macrobenthos of the Stratum 0–5 and the maintenance of vertical stratification.

Evaluating the effects of dredging in soft bottoms of the PLE has highlighted the efficient strategies of resilience performed by the dominant estuarine species. Macrobenthic invertebrates performed rapid recolonizations after dredging impacts in a scale of months (Bemvenuti et al., 2005). The same pattern was observed after the impact of a sulphuric acid spill on macrobenthic fauna of PLE, which presented clear recovering after six months (Bemvenuti et al., 2003). This evidence of fast macrofaunal recovering suggest an absence of cumulative effects in our experiments through subsequent years, as well as on intra-annual scales.

The faunal response to the trawling disturbance depends on the biology and behavior of each species. Trawling activities can carry or displace macrofauna as a result of the physical disturbance created by the trawl gear (Dell et al., 2013; Johnson et al., 2014; Collie et al., 2016). Thus, epifaunal weightless species, such as peracarid crustaceans probably are more susceptible to resuspension and displacement by currents than heavier organisms such as mollusks. Tube-builders such as *Laeonereis acuta* and *Monokalliapseudes schubarti*, that build tubes deeper than 5 cm may sense the approach of doors and move down into their tubes avoiding the impact, as suggested by Brylinsky et al. (1994) for tube-dwelling polychaetes. This evasive behavior may explain increasing densities on the Stratum 5–10 after trawling. Considering the importance of the deposit feeder *L. acuta* and deposit/suspension feeder *M. schubarti* as a main prey for fishes, crustaceans and birds in the PLE (Bemvenuti and Colling, 2010), any anthropogenic impact over their populations and therefore, food availability to food webs need to be carefully evaluated.

The gastropod *H. australis* and the bivalve *E. mactroides* presented similar responses to trawl fisheries, with impact related to periods of their higher abundances. The gastropod showed decreasing densities after the low and high impact trawling of 2017, while the bivalve presented increasing and decreasing densities after both trawling intensities during some months of 2015 and 2017. The temporal overlapping of fisheries activity and higher abundances of *H. australis*, for

example, which presents recruitments and faster growth during warming months (Carcedo and Fiori, 2012), represents a key point for the sustainability of the estuarine food webs. The importance of this fauna as food items for crustaceans and fishes was recognized for *H. australis* (Carcedo and Fiori, 2012) and *E. mactroides* (Oliveira et al., 2006), with these species being the main prey among all macrobenthic species for the crab *Callinectes sapidus* (Oliveira et al., 2006).

Despite the lack of a consistent pattern on the response of macrobenthic species to trawling fisheries, we showed some evidence of trawling impact in the macrozoobenthos and bottom. These impacts were shown through the application of experimental tows with a single boat once in a month. Extrapolating these observations to the whole fisheries scenario of the PLE, where Kalikoski and Vasconcellos (2012) suggest that at last 370 boats (30% of the total number of motorized boats in the estuary) are equipped to conduct trawls, the environmental impacts will be exponentially higher.

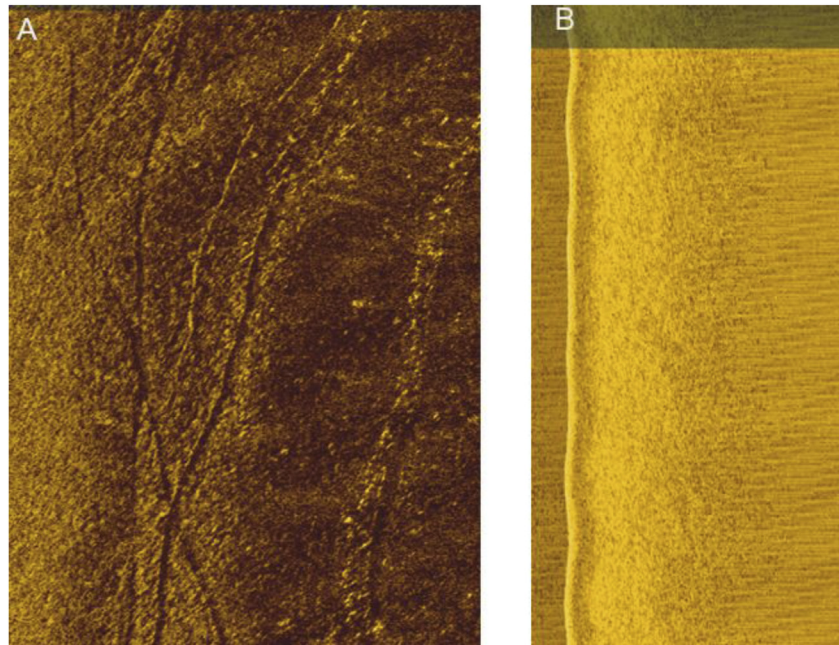
Trawling on shallow estuarine areas that are recognized nursery grounds for fishes (Costa et al., 2016) and shrimps (Noieto-Filho et al., 2017) may not affect only the target species, but the whole estuarine communities. This potentially affects the food availability for this delicate food web. A better understanding of the impacts caused by trawling fisheries and the ecosystem variability is an important tool for the improvement of fisheries management. Therefore, this assessment of ecological impacts and adequate dimensioning of anthropogenic activities is vital information for addressing effective governmental policies for the protection and conservation of estuarine nursery areas. For instance, our results may be used to determine priority areas for conservation, establishing closed areas based on their susceptibility to fisheries activities.

The different responses of macrobenthic assemblages highlight the relation between the detection of anthropogenic pressure and the natural variability of the ecosystem. The detection was closely related to the granulometric properties of the sediment which suggest a demand of studies evaluating trawling impact in different estuarine habitats, taking into account the hydrodynamics and the spatio-temporal variability of fauna and environment.

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Appendix Figure A1



Imagens of the side scan sonar over the fishing experimental zones of the Patos Lagoon estuary. A) Tracks left by trawl gears on Area 1 and B) absence of tracks on Area 2.

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