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The structure of the benthic macrofaunal assemblages and sediments characteristics of the Paraguaçu estuarine system, NE, Brazil

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ABSTRACT

The structure of the benthic macrofaunal assemblages of the estuarine portion of Paraguacu River, NE. Brazil, and its relationship with surface sediment characteristics (trace metals, PAHs, nutrients and grain size) and physical variables were investigated at ten stations on two contrasting occasions, summer (dry season) and winter (rainy season). A total of 1258 individuals (632 in winter and 626 in summer) and 62 taxa representing polychaetes, crustaceans, bivalves, echinoderms, bryozoans, sponges, cnidarians and cephalochordates were collected. Benthic assemblages in the upper estuary were unlike those in the lower estuary and a clear substitution of benthic taxa along the estuary was observed. Macrofaunal invertebrates in the low salinity region, composed of coarse sediments, were dominated by tellinids, venerids (bivalves), cirolanids (isopods), cyclopoids (copepods), and nereidids (polychaetes). While the high salinity region, composed of fine sediments, were dominated by nuculids (bivalves), cirratulids (polychaetes), and by amphiurids (ophiuroids). The Paraguacu estuarine system is not severely affected by anthropogenic activities. In the great majority of the study sites, concentrations of trace metals and PAHs in the sediments were near background values. Nutrients values were also low. We formulated new models of taxon distribution and suggested detailed studies on the effects of salinity variation and studies using functional approaches to better understand the processes causing the spatial patterns in tropical estuarine benthic assemblages.

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1. Introduction

The Paraguaçu River, the main tributary of the Todos os Santos Bay (BTS) is one of the most important aquatic systems of the Bahia State. This system is of high value for wildlife conservation and provides the main source of protein and income (i.e. consumption and commercialization of fish and shellfish) for the local communities.

Paraguaçu drainage basin, with more than 50,000 km², encompasses around 60 cities, totaling more than 1 million inhabitants (IBGE, 2000). Several anthropogenic activities potentially influence the system's environmental quality, including domestic effluents, solid wastes, agriculture, industrial and mining activities (CRA, 2004). Since 1985, the hydrological regime of the Paraguaçu River has been artificially controlled by the construction of the Pedra do Cavalo dam.

It is well know that sediments provide a temporally integrated indication of ecosystem condition and support a wide range of flora and fauna, which are important components of the aquatic food chain (e.g. Soares et al., 1999; Bellucci et al., 2002; Cave et al., 2005). Furthermore, sediments are strongly influenced by anthropogenic activity, which can lead to the accumulation of trace metals and organic contaminants, and might represent an environmental risk due to their potential ecotoxicity (e.g. Cruz-Motta and Collins, 2004).

Benthic organisms have an important role in mediating both physical and chemical processes near the sediment-water interface and in interstitial water, including the degradation of organic matter, metabolism and dispersion of contaminants such as trace metals and oil derivatives (Snelgrove, 1998; Wild et al., 2004a,b). Macrofaunal assemblages are very suitable for the assessment of sediment quality (Dauer, 1993; Olsgard and Gray, 1995; Lu, 2005), however, the distribution of these assemblages is not homogeneous in space (Schneider, 1994). Many different ecological processes, as well as physical and chemical characteristics of the environment (e.g. sediments characteristics) can regulate the spatial abundance

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patterns of different species (Legendre et al., 1997). Accordingly, marine benthos is subject to changing environmental conditions and shifts in spatial and temporal patterns may occur also due to anthropogenic impacts, with a possible reduction in the abundance of some species and an increase in the abundance of opportunistic taxa (Harvey et al., 1998; De Grave and Whitaker, 1999; Lenihan et al., 2003).

In spite of the ecological and economical importance of the Paraguaçu River estuary, there is no published work done in this area and very few studies have been published in other estuarine areas of BTS. Silva et al. (1997) published some general comments on the hydrocarbon contamination in BTS. Venturini and Tommasi

(2004); Venturini et al. (2004a,b) and Muniz et al. (2005) studied different aspects of the sediments and the benthic macrofaunal assemblages of an area adjacent to an oil refinery in the northeastern portion of the BTS. Hatje et al. (2006) worked on the benthos and the sediment characteristics of an estuarine portion of another tributary of this same bay.

In order to test if there were environmental impacts influencing the structure of the soft-bottom benthic assemblages in the Paraguaçu estuarine system, the present study investigated the relationships between these assemblages and the characteristics of the sediments (grain size, trace metals, nutrients, and PAHs).

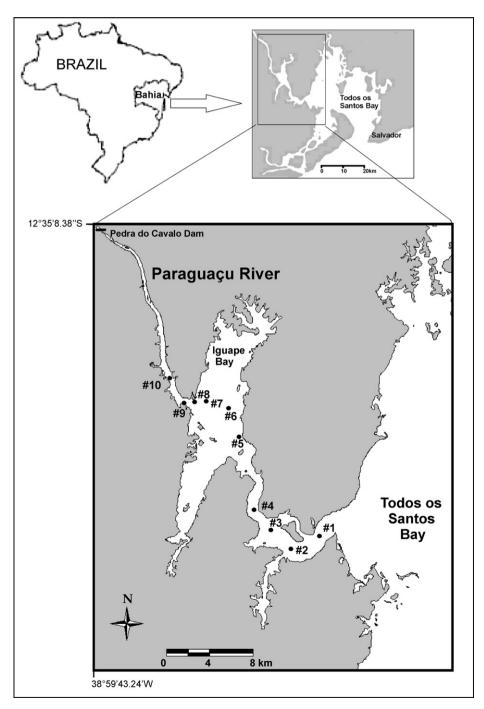


Fig. 1. The location map of the studied area with the position of the sampling stations (#1-#10) along Paraguaçu estuary.

2. Materials and methods

2.1. Sample collection and processing

Ten stations along the estuarine portion of Paraguaçu River (Fig. 1) were sampled on two different occasions, May (winter) and December (summer) of 2005. For metal analysis, surface sediment samples (0–5 cm) were collected at each station using a 0.3 m² van Veen grab. Each sediment sample was carefully taken from the central portion of the dredge with a plastic spoon and stored in plastic containers at 4 °C. In the laboratory, samples were subsequently sieved through a 63 μ m nylon sieve, dried at 55 °C, finely grounded and stored in plastic vials.

For Polycyclic Aromatic Hydrocarbons (PAH) analysis, superficial sediment (0–5 cm) samples were collected only at stations where fine particle sizes dominated (i.e. #1–#6 in summer and #1, #3–#5 in winter), using the van Veen grab. Samples were stored in aluminum boxes, previously decontaminated with acetone, and frozen until analysis.

Redox potential (Eh) and pH of the superficial sediments were measured in situ, immediately after pulling the grab, with a calibrated pH/Eh meter Thermo Orion Model 630. At each sample station depth was measured and the salinity of the superficial water at spring low tide was recorded using a Hydrolab Data Sonde.

Six replicate of the van Veen grab were used to collect the benthic fauna at each station. In the field, samples were washed through a $500\,\mu m$ mesh and preserved with 70% alcohol. In the laboratory, organisms were sorted, identified and counted. Invertebrates were grouped into families for analysis, because it has been shown that genus and species are not necessary to detect environmental impacts on soft-bottom benthic communities (Ellis, 1985; Ferraro and Cole, 1990; Vanderklift et al., 1996; Olsgard et al., 1997; Venturini et al., 2004a; Mendes et al., 2007).

2.2. Laboratory procedures

Carbon, nitrogen and sulfur in the sediments were determined using a CHNS analyzer (Flash EA 1112). For organic carbon determinations, samples were initially treated with HCl (1.0 mol $\rm L^{-1}$) for 12 h. The inorganic carbon was obtained by difference between

the total and organic contents. The available phosphate was determined by a UV-VIS-NIR Spectrophotometer (VARIAN CARY 500).

Trace metals (Ba, Cd, Cr, Co, Cu, Mn, Ni, Pb and Zn) were extracted with 1.0 mol L^{-1} HCl and determined by ICP OES (VARIAN Vista-Pro). Blanks were included in each batch of analysis. The precision and accuracy of the analytical technique were assessed by the analysis of a Certified Reference Material, MESS-2 (National Research Council of Canada) with each batch of samples. Results indicated good analytical precision, but incomplete digestion (4.6–62%). These results were not unexpected since the extraction procedure did not include HF.

The 16 USEPA priority PAHs were determined in total sediment samples. Sediments were Soxhlet extracted with dichloromethane according to USEPA 3540 (USEPA, 1996). Extracts were analyzed by GC-MS (Shimadzu QP5050A). Quantification was performed using five internal standards and the analytical program was conducted under controlled laboratory conditions, following a laboratory quality assurance protocol. These included analyses with surrogate, procedure blanks and analytical curves with determination coefficient >0.990 (Dórea et al., 2007).

Sediments particle size was determined by standard techniques (e.g. Folk, 1980).

2.3. Statistical data analyses

Bray–Curtis similarity matrices were constructed with the benthic macrofauna data, fourth root transformed to down-weight the influence of dominant species. Non-metric multidimensional scaling (nMDS) ordinations were used to investigate spatial patterns in the structure of the macrofaunal assemblages.

Principal component analyses (PCA) were performed on the abiotic sediment data which were first normalized and log(x + 1) transformed to account for the different units of the variables.

Patterns in the distribution of the benthic macrofauna were linked to variation in the environmental variables using the BIOENV procedure (PRIMER 6.0; Clarke and Warwick, 2001). These latter analyses were based on the correlation of Bray–Curtis dissimilarity matrices, fourth root transformed data, with euclidian distance matrices, $\log(x+1)$ transformed data. Separate analyses were performed for the two sampling occasions.

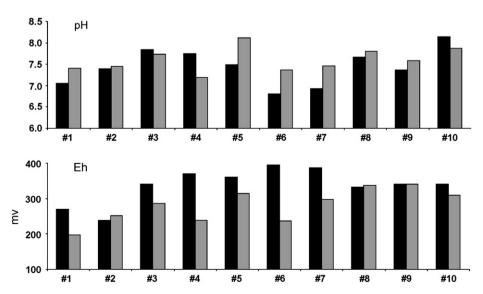


Fig. 2. Eh and pH in superficial sediments at ten stations (#1-#10) along Paraguaçu estuary on two sampling occasions (winter and summer).

3. Results

3.1. Physical and chemical variables and substrate composition

Superficial water salinity varied between 0.6 and 30.8 (winter) and 7.78 and 34.2 (summer) at stations #10 and #1, respectively, while depths varied between 2 m and 19 m. During both sampling occasions, redox potential values varied between 198 and 395 mV, indicating that superficial sediments presented oxic conditions, and pH varied between 6.8 and 8.14 (Fig. 2).

The sediments were predominantly composed of sand, with coarser sediments at upper estuary (#6–#10) while fine sand, silt and clay fractions were more abundant at the lower estuary stations (#1–#4) (Fig. 3). Comparing sampling occasions, nearly all sampled stations showed minor changes in grain size composition, the only exception to this pattern was observed at station #1.

Total carbon and organic carbon content values were relatively low (2.13–3.79%) and did not vary substantially among stations or between sampling occasions (Fig. 4). Values of carbonate, phosphate and S total were also low, but demonstrate more variability among stations on both sampling occasions. Nitrogen values were below the detection limit for the majority of the samples (Fig. 4), hence C/N ratios could not be calculated.

The results showed that for the great majority of the analyzed samples, PAH concentrations were below detection (Table 1). The only stations that presented detectable PAH levels were station #1 on both sampling occasions and stations #3 and #5 for the winter samples only.

The ranges of trace metal concentrations are presented in Table 2. Of the studied elements Mn, Ba, Pb and Zn had the highest concentrations. Because of analytical problems trace metals results obtained for the winter samples at station #8 were disregarded.

The PCA performed with the abiotic data showed that the first two principal components explained 61.1% (winter) and 57.8% (summer) of the total variance (Fig. 5). There were weak correlations (r < 0.4) between the environmental variables and the first two principal components (PCs). The first PC of the winter ordination were mainly correlated with Zn (r = -0.28) and Ba (r = -0.28) while the second component with Cr (r = -0.37) and Eh (r = 0.38). In the summer analyses, the first PC were manly correlated with depth (r = 0.31) and coarse sand (r = -0.31) while the second PC with Zn (r = -0.38) and pH (r = 0.35).

3.2. Benthic macrofauna

A total of 1258 individuals (632 in winter and 626 in summer) and 62 taxa representing polychaetes, crustaceans, bivalves, echinoderms, bryozoans, sponges, cnidarians and cephalochordates were sampled. The most abundant groups were Polychaeta, Mollusca and Crustacea. Fig. 6 shows the most abundant taxa, i.e. those that accounted for more than 80% of the total number of individuals sampled on each occasion, and a clear substitution of taxa along the estuary.

The nMDSs for both sampling occasions based on the benthic assemblages data (Fig. 7) showed that stations #8–#10, especially in summer, were dissimilar from the other stations and, on the summer ordination, station #7 also grouped with the above stations. Bivalves of the families Tellinidae, Veneridae and Donacidae, Cirolanidae isopods, cyclopoid copepods and Nereididae polychaetes were dominant in the inner portion of the estuary, characterized by low salinities and coarse sediments (i.e. #8, #9 and #10). The region with high salinity and fine sediments was dominated by Nuculidae bivalves, cirratulid polychaetes and by Amphiuridae ophiuroids (Fig. 6).

The BIOENV analyses performed with the winter data showed that the best significant correlation between the benthic fauna was

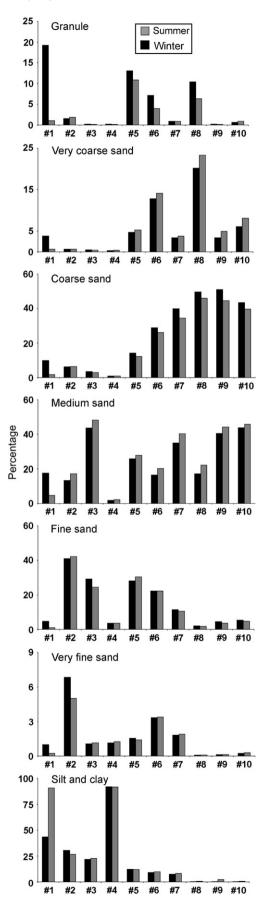


Fig. 3. Percentage of the sediment fractions at ten stations (#1–#10) along Paraguaçu estuary on two sampling occasions (winter and summer).

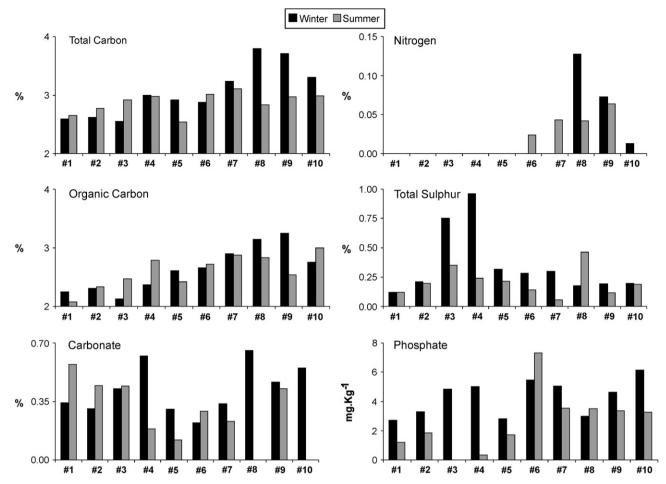


Fig. 4. Total carbon, nitrogen, organic carbon, total sulphur, carbonate and phosphate in superficial sediments at ten stations (#1-#10) along Paraguaçu estuary on two sampling occasions (winter and summer)

with total C, Carbonates, pH, Cr and Zn ($\rho=0.80$; p<0.05). However, Zn on its own also showed a significant correlation with the benthic fauna (Table 3). The greatest correlation observed for the summer survey was with three environmental variables (e.g. $\rho=0.64$ with total C, pH and Ni), but this was not significant (p>0.05).

4. Discussion

Several studies have shown that spatial distributions of aquatic organisms, such as benthic invertebrates along an estuarine gradient, are related to natural environmental factors, for instance salinity, sediment particle size, depth, and/or anthropogenic stressors. The results of this study showed that upper estuarine stations were dissimilar from the others and a clear substitution of benthic taxa was observed along the estuary. The abundances of some bivalves (Tellinidae, Veneridae and Donacidae), isopods (Cirolanidae), copepods and polychaetes (Nereididae) were higher at those stations with low salinities whereas the region with high salinity was dominated by other groups of bivalves (Nuculidae), polychaetes (Cirratulidae) and by ophiuroids.

The principal component analyses showed that some trace metals (i.e. Zn, Ba and Cr) were weakly correlated (r < 0.4) with the first two principal components. These analyses also indicated that the stations at the inner portion of the estuary were more similar than those at the outer portion of the estuary.

The results indicated that some trace metals (i.e. Zn, Cr) could negatively influence the benthic assemblages at the Paraguaçu upper estuary, a relationship reported by several authors in other places (e.g. Watzin and Roscigno, 1997; Inglis and Kross, 2000; Warwick, 2001). Nevertheless, when metal concentrations in sediments of the Paraguaçu estuary are compared to other studies, within the BTS system and worldwide (Table 2), generally, the studied area showed lower metal concentrations than those classified as contaminated sediments. Additionally, a comparison between trace metal concentrations and sediment quality guidelines (Buchman, 1999) revealed that most of the studied trace metal concentrations were below levels which adverse effects are expected to occur. Manganese was an exception and presented concentrations above AET levels (i.e. concentration above which adverse biological impacts would always be expected). Nevertheless, it has been reported that Baía de Todos os Santos (BTS) is naturally enriched with Mn (CRA, 2004; Hatje et al., 2006).

Furthermore, a significant correlation (benthic macrofauna and metals) was observed only in one of the two sampling occasions and other variables were also correlated with the structure of the benthic assemblage (i.e. total C, carbonates, very fine sand and pH).

Almost all nutrient concentrations were lower than those recorded at impacted coastal zones worldwide (e. g. Paez-Osuna et al., 1998, Monteiro and Roychoudhury, 2005; Nayar et al., 2007). In general, the nutrient levels showed little variability between sample stations and between sampling occasions. Organic carbon, C_{total} , S_{total} and Carbonate levels were similar to or smaller than those values found in more impacted areas of the BTS by other authors (CRA, 2004; Venturini and Tommasi, 2004). Several studies

Table 1PAH (ng g⁻¹ dry wt) concentrations at each station of the superficial sediments of the Paraguaçu estuary (W: winter, S: summer; -: not determined; BDL: below detection limits). Note that only stations with fine sediments were analyzed (i.e. #1-#6 in summer and #1, #3-#5 in winter). The detection limits (DL) were defined as a response three times the average height of the blank baseline noise for 10 g of sample

	DL		#1	#2	#3	#4	#5
Acenaphthene	0.01	W	BDL	_	BDL	BDL	5.9
		S	BDL	BDL	BDL	BDL	BDL
Acenaphthylene	0.01	W	BDL	BDL	BDL	BDL	BDL
		S	BDL	BDL	BDL	BDL	BDL
Anthracene	0.01	W	BDL	BDL	BDL	BDL	BDL
		S	BDL	BDL	BDL	BDL	BDL
Benzo[a]anthracene	0.05	W	BDL	BDL	BDL	BDL	BDL
		S	BDL	BDL	BDL	BDL	BDL
Benzo[a]pyrene	0.10	W	BDL	-	8	BDL	BDL
		S	BDL	BDL	BDL	BDL	BDL
Benzo[b]fluoranthene	0.05	W	BDL	-	52.7	BDL	BDL
		S	BDL	BDL	BDL	BDL	BDL
Benzo[g,h,i,]perylene	0.10	W	BDL	-	43.6	BDL	BDL
		S	BDL	BDL	BDL	BDL	BDL
Benzo[k]fluoranthene	0.05	W	BDL	-	57.3	BDL	BDL
		S	BDL	BDL	BDL	BDL	BDL
Chrysene	0.05	W	BDL	-	22.9	BDL	BDL
•		S	BDL	BDL	BDL	BDL	BDL
Dibenz[a,h]anthracene	0.10	W	BDL	-	69.9	BDL	10.7
		S	BDL	BDL	BDL	BDL	BDL
Fluoranthene	0.05	W	BDL	-	BDL	BDL	11.9
		S	BDL	BDL	BDL	BDL	BDL
Fluorene	0.01	W	BDL	-	BDL	BDL	10.7
		S	BDL	BDL	BDL	BDL	BDL
Indeno[1,2,3-cd]pyrene	0.10	W	BDL	-	59.6	BDL	BDL
1		S	BDL	BDL	BDL	BDL	BDL
Naphtalene	0.01	W	12.6	-	BDL	BDL	14.3
•		S	23.5	BDL	BDL	BDL	BDL
Phenanthrene	0.01	W	18.8	-	BDL	BDL	9.5
		S	8.1	BDL	BDL	BDL	BDL
Pyrene	0.05	W	BDL	_	BDL	BDL	4.8
		S	BDL	BDL	BDL	BDL	BDL

have associated oligotrophy with very low or an absence of measurable concentrations of nutrients in sediments (e.g. Kucuksezgin et al., 1995). Therefore, the low levels of nutrients observed in the present study suggests that Paraguaçu is an oligotrophic estuarine system, where anthropogenic nutrient inputs either are reduced or/and nutrient cycling in the water column is fast, resulting in minimal accumulation in sediments. The low nutrient levels could also be, at least in part, a result of the damming of Paraguaçu head waters in 1985, which likely promoted severe alterations of the hydrological regime (Genz pers. comm.), for instance retention of suspended material, nutrients, organic matter and changing the natural regime of freshwater input to the estuary. Unfortunately,

there are no data available for the period prior to the dam installation.

Almost all PAH analyzed were below TEL (Threshold Effects Levels, i.e. the concentration below which adverse effects are expected to occur only rarely; Buchman, 1999) values, the only exception was Dibenz[a,h]anthracene at two stations, #3 and #5 during winter sampling, and the former showed values above the ERL level (Effects Range-Low, i.e. level at which toxicity may begin to be observed in sensitive species; Buchman, 1999). Most of PAHs observed in these two stations were of low molecular weight, whereas at the other stations they were mostly of high molecular weight, indicating contribution from pirogenic sources (e.g. Prahl

Table 2
Range of trace metal concentrations (mg kg⁻¹) observed on the present survey for the Paraguaçu River Estuary and other coastal regions worldwide. bd: below detection; na: not available

	Ba	Cd	Со	Cr	Cu	Mn	Ni	Pb	Zn	Extractor	Reference
Paraguaçu River Estuary – Brazil	7.7–27.5	bd-0.21	3.64-7.25	8.27-13.0	4.25-15.2	151-1594	5.64-13.6	10.7-34.5	20.1-32.5	HCI	This study
Todos os Santos Bay – Brazil	na	<0.02-4.52	na	<0.04-28	0.16-118	16.0-1482	na	<0.09-84	<0.02-237	HCl	CRA, 2004
Santos Cubatão Estuary – Brazil	17–196	<bd-1.6< td=""><td>4-14</td><td>23-138</td><td>13-109</td><td>160-5699</td><td>10-39</td><td>9–127</td><td>53-476</td><td>HNO₃-HCl</td><td>Luiz-Silva et al., 2006</td></bd-1.6<>	4-14	23-138	13-109	160-5699	10-39	9–127	53-476	HNO ₃ -HCl	Luiz-Silva et al., 2006
Guanabara Bay – Brazil	na	na	1-209	2-41,364	2-18,840	na	1-3515	2-19,340	5-755,149	HF-HClO ₃	Neto et al., 2006
Medway Estuary – UK	9-296	na	3-16	na	9-103	219-1878	4-70	8-203	20-392	HNO ₃ -HCl	Spencer et al., 2006
Southport Broadwater – Australia	na	bd-0.6	na	bd-30.8	bd-2900	na	bd-23.5	bd-166	1.6-595	HNO ₃ -H ₂ O ₂ -HCl	Burton et al., 2004
Tagus Estuary – Portugal	na	1.7-5.9	na	na	27.6-89.1	na	na	65.2-199	168-427	HNO ₃ -HCl	França et al., 2005
Caspian Sea	70–1250	0.01-0.25	0.7-24.2	1.9–128	1.2-57.6	45-1110	1.8-68	0.69-28.6		HNO ₃ -HF	Mora et al., 2004
Background Todos os Santos Bay	na	0.02-0.04	na	33.7-51.1	10.8-22	239–449	na	10.4-26.4	55.1–86.1	HCl	CRA, 2004

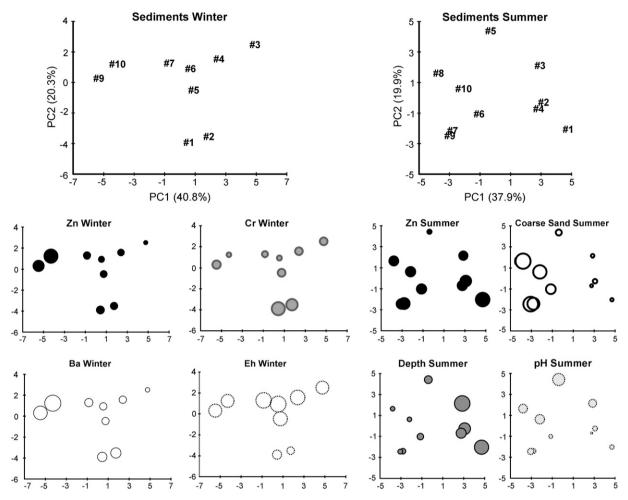


Fig. 5. Principal component analyses performed with the sediment environmental variables at ten stations (#1-#10) along Paraguaçu estuary on two sampling occasions (winter and summer).

and Carpenter, 1983). When comparing the obtained results with other studies for BTS (CRA, 2004) it was observed that levels of PAH in Paraguaçu River were similar to those reported previously for the region. Venturini and Tommasi (2004) suggested that effluents from a petroleum refinery, located in the north of BTS, is the main source of petrogenic PAHs found at different locations in BTS. The source of PAHs for the Paraguaçu estuary itself could be an oil company shipyard located nearby the sampling station #2, but this needs further investigation.

Based on benthic structural characteristics, a previous study done by CRA (2004) classified one site at the outer estuarine portion of Paraguaçu River as an extremely impacted site. The present study observed, in the same area, a much more diverse and abundant benthic assemblage. Nevertheless, the former used a 2000 μm mesh and did not properly sample benthic macrofauna (i.e. invertebrates $>\!500~\mu m$).

The identification of which variable or group of variables are the most important for the benthic fauna is not an easy task, nevertheless, it seems that salinity is an important variable influencing the species replacement along Paraguaçu estuary. However, salinity was strongly correlated with depth and our analyses indicated that the combinations of other environmental variables are more important than is depth. Additionally, the salinity measurement was taken on superficial waters at low tide and more precise measurements are needed to estimate the salinity ranges instead of absolute salinity values (e.g. Attrill, 2002).

It is well known that estuaries are remarkably variable systems and that it can be rather difficult to determine whether structural changes in benthic assemblages are due to natural or anthropogenic stresses, unless the later are severe (Elliot and Quintino, 2007). This is certainly not the case of the estuarine portion of Paraguaçu River.

Animal-sediment relationships are fairly variable and sediment type and grain-size can vary with other variables like microbial content, food supply and trophic interactions (Snelgrove and Butman, 1994). More ecological studies on Paraguaçu River estuary are strongly recommended, especially studies concerning detailed temporal and spatial patterns of benthic species distribution with salinity variation and those dealing with structural and functional characteristics of benthic assemblages.

In conclusion, this is the most comprehensive study on benthic assemblages of the Paraguaçu estuary and the results motivated the formulation of new models for further tests in other tropical estuaries (e.g. the observed pattern of species substitution will be similar at other tropical estuaries; salinity changes promoted by dam operation will affect the observed pattern of benthic invertebrates). There was no prior published work conducted to evaluate the potential impacts of developmental activities on this estuary, making this study important. Much more work is still needed on tropical estuaries, without it, there will be no effective strategies for future conservation.

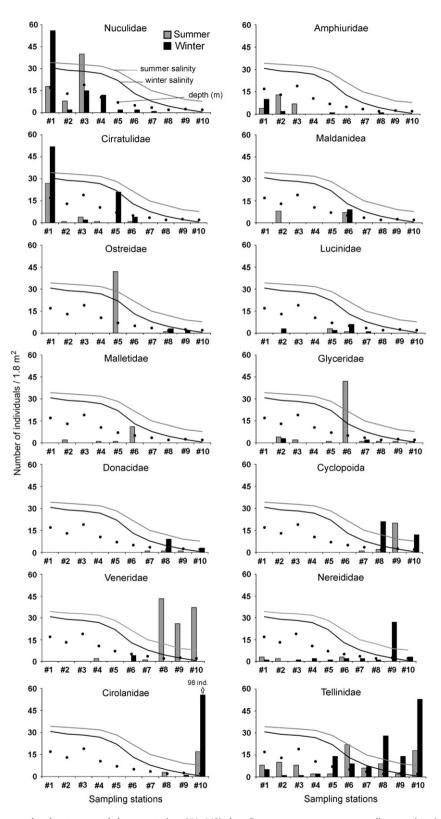


Fig. 6. Spatial distribution of the most abundant taxa sampled on ten stations (#1-#10) along Paraguaçu estuary on two sampling occasions (winter and summer). Black lines (winter) and grey lines (summer) are showing the salinity and black dots are showing the depth (m) observed at each station.

#6 #2 #7 #3 #5 #4 #9 #1 #8 #10 stress: 0.12

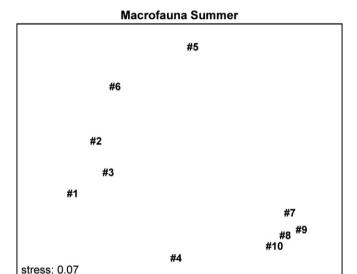


Fig. 7. nMDS performed with the benthic macrofauna sampled on ten stations (#1–#10) along Paraguaçu estuary on two sampling occasions (winter and summer).

Table 3 Summary of the results from BIOENV analyses for each sampling occasion. The best combinations of variables appear first and the following combinations were performed without those already used in the previous combination (*: p < 0.05; ns: not significant)

No. of variables	Winter	Summer			
	Variable	ρ	Variable	ρ	
1	Zn	0.62*	Ni	0.56 (ns)	
	Depth	0.54 (ns)			
2	Zn, Cr	0.72*	Ni, pH	0.61 (ns)	
	Depth, Very fine sand	0.65 (ns)	-		
3	Zn, C total, Cr	0.74*	Ni, pH, C total	0.65 (ns)	
	Depth, Very fine sand, Phosphate	0.66*	0.66*		
	Silt Clay, S total, Cu	0.55 (ns)			
4	Zn, C org, Carbonates, Cr	0.79*	Ni, pH, N total, C org	0.64 (ns)	
	Depth, Very fine sand, Phosphate, Cd	0.67*	, and the second		
	Silt Clay, S total, Co, Mn	0.55 (ns)			
5	C total, Carbonates,	0.80*	Ni, Zn, N total,	0.64 (ns)	
	pH, Cr, Zn		C org, Cd		
	Depth, Very fine sand, Phosphate, Co, Ni	0.67 (ns)	-		

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