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Benthic Foraminifera and Bacterial Activity as a Proxy for Environmental Characterization in Potengi Estuary, Rio Grande do Norte, Brazil

Foraminíferos Bentônicos e Atividade Bacteriana como Ferramenta para Análise Ambiental no Estuário do Rio Pontegi, Rio Grande do Norte, Brasil

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Abstract

The aim of this study was to identify possible zonation patterns and assess the environmental impact on the Potengi River Estuary, Rio Grande do Norte State, through the distribution of benthic foraminifera associated to bacterial activity and abiotic parameters. Six sediment samples were collected from locations that presented clear signs of pollution. The environment was predominantly anaerobic and fermentation occurred at all sites. Forty-two species of foraminifera were identified. The dominant species were *Ammonia tepida* and *Arenoparrella mexicana*, which are known to be opportunistic, and able to adapt to rapidly changing conditions. CCA analyses showed that salinity and organic matter, followed by bacterial carbon, were more strongly linked to organism distribution in the Potengi River Estuary. Dissolved oxygen concentration, temperature and total organic matter were higher at the estuary mouth than at the other sites, creating favorable conditions for foraminiferal growth and allowing the faunistic succession on the upper estuary. As foraminifera assemblages when associated to environmental parameters can be used as efficient proxies for environmental diagnosis, these results suggest that the Potengi Estuary is under great stress from the surrounding urban development.
Keywords: Foraminifera; bacteria activity in sediment; mesotidal estuary; environmental diagnoses

Resumo

Este estudo teve como objetivo estabelecer um modelo de zonação e avaliar o impacto ambiental sofrido pelo estuário do rio Potengi, estado do Rio Grande do Norte, através da distribuição das assembléias de foraminíferos associados à atividade bacteriana e a parâmetros físico-químicos. Seis amostras de sedimento foram coletadas em regiões que apresentavam algum tipo poluição aparente. Predominaram os processos bacterianos anaeróbicos principalmente sulfatoredução no estuário. Foram identificadas quarenta e duas espécies de foraminíferos. As espécies dominantes foram *Ammonia tepida* e *Arenoparrella mexicana,* que são conhecidas como oportunistas, pois se adaptam com facilidade as variações ambientais. A análise em CCA mostrou que a salinidade e a matéria orgânica, seguidos pelo carbono bacteriano, conduziram a distribuição dos organismos no estuário. A concentração de oxigênio, temperatura e matéria orgânica total foram mais altas na foz do que em outras estações, criando condições favoráveis ao crescimento de foraminíferos e permitiu uma sucessão faunística em direção ao estuário superior. Assembléias de foraminíferos quando associadas a parâmetros ambientais podem ser usadas como eficientes indicadores para o diagnostico ambiental. Os resultados sugeriram que o estuário do rio Potengi encontra-se sobre condições de grande estresse ambiental provocado pelo desenvolvimento urbano a sua volta.
Palavras-chaves: foraminíferos; atividade bacteriana em sedimento; estuários de mesomaré; diagnóstico ambiental

1 Introduction

Estuaries are highly dynamic environments and, in their natural state, the most productive ecosystems (Sumich & Morrissey, 2004). As a rule they have high oxygen input in the sediment which leads to high nutrient production, serving as feeding and nursery grounds for a great number of economically significant fish, crustacean and mollusc species (Primavera, 1993).

World population growth has been rapid especially in coastal areas, resulting in great ecological stress on aquatic systems, particularly estuaries (Flemer & Champ, 2006). Because they constitute attractive areas for human activities the estuaries receive a large amount of domestic and industrial effluents (Frazão, 2003). The local effects on the biota depend on the effluent types and whether they are thrown directly into the estuary at a particular spot or indirectly by a river system. In addition, the geomorphological and hydrographical settings also determine the types of impacts and their consequences on the environment (Alve, 1995).

Monitoring the introduction of pollutants in estuaries and the resulting local changes is important, since they show the integrated perception of environmental reality, serving as a support for the control of polluting activities (Batista *et al.,* 2003) and for future projects of conservation and management of these areas.

The use of organisms as proxies in marine environments has been intensified in recent years because environmental changes reflect directly on the trophic levels, modifying the food chain. Over the last decades, benthic foraminifera and their response to environmental changes have been studied in much more detail (Yanko *et al*., 1994; Bonetti *et al*., 2000; Laut, 2003; 2007). They are considered great bioindicators for pollution due to their high sensitivity to any natural or anthropogenic changes in the environment (Scott *et al.,* 2002).

Most foraminifera in coastal areas feed on algae, bacteria and larvae playing a fundamental ecological role due to their place in the food web, associated to the microbial loop. They allow bacterial carbon to reach higher levels of the chain, linking the classic chain to the microbial loop. Foraminifera and other microorganisms assimilate a great portion of the energy produced by bacteria, which are responsible for part of the primary production on marine environments, and they pass this energy on to the next trophic levels. Without them, the transference of energy in trophic chain would be much less inefficient (Laut, 2007).

Changes in the environment reflect on the foraminiferal population directly or indirectly, and consequently, on the whole trophic web, generating changes in food diet, extinction of some species, changes in the reproduction and size of organisms, deformation of shells, etc (Laut, 2005).

Studies based on foraminifera assemblages, bacterial respiratory activity, and environmental and sedimentological parameters using multivariate analyses can be efficient proxy for carrying out zonation and environmental diagnoses of coastal areas.

Potengi Estuary (Figure 1), where the Natal Harbor (an oil terminal) and shrimp farms are located, is important both to Rio Grande do Norte State and to northeastern Brazil as a whole. However, studies of its zonation and environmental diagnoses are lacking. This study aims to reduce this gap and to provide new data on estuarine environments in tropical areas.

2 General Settings

The Potengi River Estuary (5º40’S - 5º55’S, 35º05’W - 35°25’W) is one of the largest estuaries on the Brazilian northeastern coast, located in Rio Grande do Norte State. The Potengi Estuary extends inland for about 20 km and is bordered by the municipal districts of Macaíba, São Gonçalo do Amarante and Natal. It is 5 km wide and has a maximum depth of 15 m at the main navigation channel (Silva *et al*., 2001). It receives the discharge of the Jundiaí, Doce and Potengi rivers, the latter boasting the largest drainage area among them (Figure 1).

The predominant climate, according to the classification of Köppen, is AS’, hot and humid, with annual average temperature of 26.8°C and with two very well defined seasons during the year. The summer (from October to December) is dry, and the winter (from April to June) features high rainfall 2,000 mm/year (Ramos e Silva e*t al*., 2006). The tide is semidiurnal with two high tides and two low tides in a lunar day, with maximum height of 2.83 m.

The city of Natal has 778,000 inhabitants and 1500 industries, mostly associated to the integrated center of the clothing and textile industry (IBGE 2007). About 60% of the sewage is thrown directly into the Potengi River without any treatment, and several sources of organic effluents exist along the estuary (Frazão, 2003). The port and industrial activities are well developed and also contribute with effluents into the estuary. Silva *et al.* (2001) showed that the estuary is contaminated with heavy metals, such as Mn, Fe, Cu, Ni and Pb, and that Zn, Cd and Cr were present in concentrations higher than the permitted levels.

The mangrove forests have been removed to give place to shrimp farming, an activity that has been growing in Northeast Brazil (Diegues, 2006).

3 Materials and Methods

Six sediment samples were collected along the main channel of the estuary, with an Ekman grab, ranging from the inner limit of the mangrove forest to the outer area. These stations were distributed so that estuarine gradients and/or environmental stresses could be identified (Figure 1). Station PT 01 was located on the estuary's outer part, where there is a channel that receives the residues from the shrimp farms; station PT 02 was located near an oil terminal; station PT 03 is opposite to Baldo channel, which drains most of the untreated sewage of Natal city; station PT 04 was at a site where mangrove vegetation was well preserved; station PT 05 was placed on the margin of the mangroves, where low-income populations live; and station PT 06 was located in the estuary's innermost area, at the confluence of the Potengi River and a small channel.

At each station a 50 ml sample was collected for foraminiferal analysis, 100 g of sediment for grain size and organic matter analyses, and 10 ml for bacterial respiratory activity and bacterial carbon analyses. Physiochemical parameters of the water, such as salinity, temperature, pH and dissolved O_2 were measured in situ and formaldehyde at 4% was added to the samples to preserve them from microorganism attacks. Rose Bengal was added to stain cell

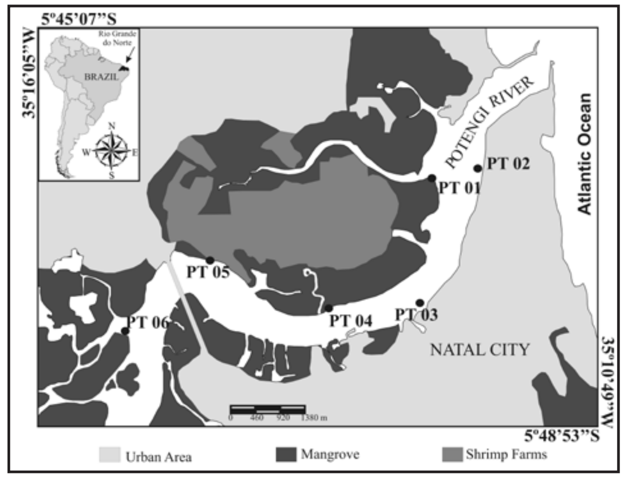

Figure 1 Location map of the samples in Pontengi Estuary, Rio Grande do Norte State.

plasma, to help identification of living individuals at the laboratory.

Grain size analyses were accomplished according to the methodology described in Suguio (1973), where humid samples pass through a 0.062 mm sieve. Sand is thus separated from the sediment. The finest fractions were analyzed using the pipette method and the textural classification adopted followed Flemming (2000).

The total organic matter (TOM) was determined through calcination of 50 g of dry sediment at 500ºC for 4h (Byers & Stewart, 1978)

Bacterial respiratory activity such as aerobic activity, fermentation, denitrification and sulfate reduction was analyzed using the methodology described in Alef & Nannipieri (1995). Aerobic, fermentation and denitrification growth media and sulfate-reduction growth medium contained peptone (0.2g/l) and sodium lactate (0.2g/l), respectively. Methylene blue solution (0.03% final concentration) and resazurin solution (0.0003% final concentration) were used as redox indicators in fermentation and sulfate-reducing growth media. Durham vials and $NaNO_2$ (0.687g/l) were utilized in denitrification growth medium. The results were described as positive or negative.

Heterotrophic bacteria were enumerated by epifluorescent microscopy (Axiosp 1, Zeiss, triple filter Texas Red – DAPI – fluorescein isotiocianate, 1,000 x magnification) and using the fluorochrome fluorescein diacetate and UV-radiation (Kepner & Pratt, 1994). Carbon biomass (mg C/g) data were obtained using the method described by Carlucci *et al*. (1986).

Foraminifera samples were processed following the methodology in Boltovskoy (1965). The material was washed through 0.5 mm and 0.062 mm sieves, the fractions above and below being discarded. After drying, foraminifera were separated by flotation on carbon trichloride (C_2HCl_3). Counts were carried out under a stereomicroscope at 80 x magnification.

Classification at the phylum level was based on Margulis et al. (1999), at class and order level on Sen Gupta (1999), at genus level on Loeblich & Tappan (1988) and at the species level on several authors (Cushman & Bronniman, 1948; Boltovskoy et al., 1980; Todd & Bronnimann, 1957).

The species identified in Pontegi Estuary were picked out from the samples and were mounted on stubs using a carbon conductive adhesive tape. The stubs were then coated with two layers of palladium gold and examined at the Electronic Microscope ZEISS DMS 960 (Figure 2).

Ecological indexes such as abundance, richness, constancy, diversity, equitability or evenness and dominance were used in the interpretation of the data. Abundance is the percentage of individuals of a given species in relation to the total population. Richness is the number of species found in one sample (live + dead). Constancy is the relationship between the species and the samples, expressed as a percentage according to the formula: C=100p/P, where p is the number of samples that contain a given species and P is the total number of samples. Species are considered constant when they are present in over 60% of the samples; they are accessory if they occur in 25% - 59% of samples; and they are considered accidental or rare if they occur in less than 25% of the samples (Tinoco, 1989). Dominance is an index proposed by Simpson (1949) which presents an advantage over other indices in that it does not only consider the number of species and organisms, but also the proportion of total occurrence of each species.

Diversity (*H'*) is an index proposed by Shannon-Wiener (1949), which is appropriate for random samples of a community of species or sub-community of interest, and it is estimated by: $H' = \Sigma p_i Ln p_i$, where pi represents the portion of *i*-th species in the sample and Ln is the natural (base e) logarithm. For calculation of the diversity (*H*') index the software MVSP 3.1 was used.

Equitability (*J'*) refers to the individuals' distribution among species, being proportional to the diversity and inversely proportional to the dominance. It compares the Shannon-Wiener diversity index with the distribution of the observed species, maximizing diversity. This index is obtained through $J'=H'/Ln\ (S)$, where S is the richness of species, *H*' is the Shannon diversity index and Ln the natural (base e) logarithm. The software MVSP 3.1 was used for this calculation.

Cluster analysis in Q-mode (Euclidean distance and Ward linkage method) and R-mode (r-Pearson linear correlation coefficient and Ward linkage method) were used to define the foraminiferal assemblages in the estuary.

Canonical Correspondence Analysis (CCA) was used to show which environmental data influence the community structure the most. By means of a graph CCA shows how environmental variables influence species distribution.

4 Results

4.1 Abiotics

Temperature of the water varied from 25.6ºC at PT 05 to 26.9ºC at PT 01. The pH varied very little, with the highest pH being found at PT 01 (6.84) and the lowest at PT 02 (6.75). Oxygen concentration remained low, between 2.8 $ml.l^{-1}$ at PT 03 and 3.2 $ml.l^{-1}$ at PT 01. Salinity was 30 at PT 06 and 37 at PT 04 (Table 1).

Organic matter percentages varied from 0.5% at PT 05 to 3.6% at PT 03. Muddy sediment was predominant on the sediment samples analyzed in this study, except at stations PT 01 and PT 05, where samples were classified as silty sand (Table 1).

4.2 Biotics

In all six stations the sediments were in anaerobiosis, with presence of fermentative processes. At station PT 01 denitrification also occurred and sulfate-reduction occurred at PT 02, PT 04, and PT 05. Bacterial carbon varied between 0.006 μC.g^{-1} at PT 06 and 0.382 μC.g^{-1} at PT 01. At station PT 06, microbiological analyses were not performed (Table 1).

Forty-two species of foraminifera were identified (Figure 2). Station PT 01 was sterile of foraminifera. The station that presented the smallest amount of organisms was PT 05, with only 49 organisms, while the station that presented the largest amount was PT 04, with 485. The PT 05 site presented the lowest species richness, with only 3 species, and the PT 02 station presented the highest species richness with 24 species (Table 2).

Station PT 02 was the one that presented the largest percentage of stained tests (29%) and PT 05 was the one that presented the smallest percentage of them (4%). Station PT 05 did not present tests with abnormalities tests, station PT 06 had the smallest percentage of abnormalities test (7%) and PT 02 had the largest percentage (15%). *Ammonia tepida* was the species that presented more abnormalities test.

Ammonia tepida was the dominant species in samples PT 02, PT 03 and PT 04, as was *Arenoparella mexicana* in samples PT 05 and PT 06. In the areas closest to the ocean (PT 02, PT 03 and PT 04) calcareous species prevailed, while at the innermost points (PT 05 and PT 06) agglutinant species predominated.

The species *A. tepida*, *Elphidium discoidale*, *Elphidium excavatum*, *Quinqueloculina laevigata*, *Textularia earlandi and Trochammina macrescens* were constant (> 60%), the highest percentage (80%) being related to *A. tepida*. *Ammobaculites dilatatus, Arenoparrella mexicana, Bolivina translucens, Fissurina lucida, Fursenkonia pontoni, Miliolinella subrotunda, Quinqueloculina seminula* and *Uvigerina peregrina* were accessory and the majority of species (27 species) were accidental.

The highest diversity index was found at stations PT 02 (1.8) and PT 06 (1.6). The equitability and dominance indexes follow the pattern shown by the diversity index (Table 02).

4.3 Statistical Analysis

Cluster analysis in R-mode showed the existence of three foraminiferal assemblages at the Potengi River Estuary (Figure 3).

A – *A. dilatatus, A. mexicana, S. lobata, A. exiguus, A. salsum, T. inflata, T. ealandi, T. macrescen* and *U. peregrina.*

B – *A. tepida, E. excavatum, B. traslucens, Q. seminula, A. cassis, B. doniezi, B. spatulata, H. germanica, Quinqueloculina* spp., *Bolivina* spp.*, B. elegantíssima, D. williansonii, Q.* cf. *tenagus* and *Spiroculina* sp.

C – *B. inflata, B. compacta, C. incerta, C. planorbis, L.laevis, L.spirialis, N.opima, O. vilardeboana, P. atlanticum, Q. lamarckiana, Rutherfordoides* sp.*, F. lucida, F. pontoni, Q. laevigata* and *M. subrotunda.*

Stations	T	S	pH	O_2	TOM	Sand	Silt	Mud	Classification	Respiratory Bacterial Activity				Bacterial Carbon
	(ºC)			(ml.L^{-1})	(%)	(%)			(Flemming, 2000)	AR	FER	DES	SUL	(μg C.g^{-1})
PT 01	26.9	35	6.84	3.2	2.4	60	30	10	Very silty sand	N	P	P	N	0.382
PT 02	26.7	35	6.75	3.1	2.6	10	43	47	Very clayey slightly sandy mud	N	P	V	P	0.007
PT 03	26.2	34	6.82	2.68	3.6	1	54	45	Clayey silt	N	P	V	V	0.381
PT 04	26.4	37	6.83	2.8	1.8	10	55	35	Silty slightly sandy mud	N	P	V	P	0.01
PT 05	25.6	32	6.78	2.8	0.5	77	17	6	Slightly silty sand	N	P	V	P	0.006
PT 06	25.7	30	6.78	2.9	1.2	43	39	18	Silty sandy mud	-	-	-	-	-

Table 1 Abiotic, sedimentological and microbiological parameters from Potengi River Estuary. T – temperature; S – salinity; TOM – total organic matter; (P) – growth presence; (N) – growth absence; (V) – growth variable; AR – aerobiosis; FER – fermentation; DES – denitrification; SUL – sulfate-reduction.

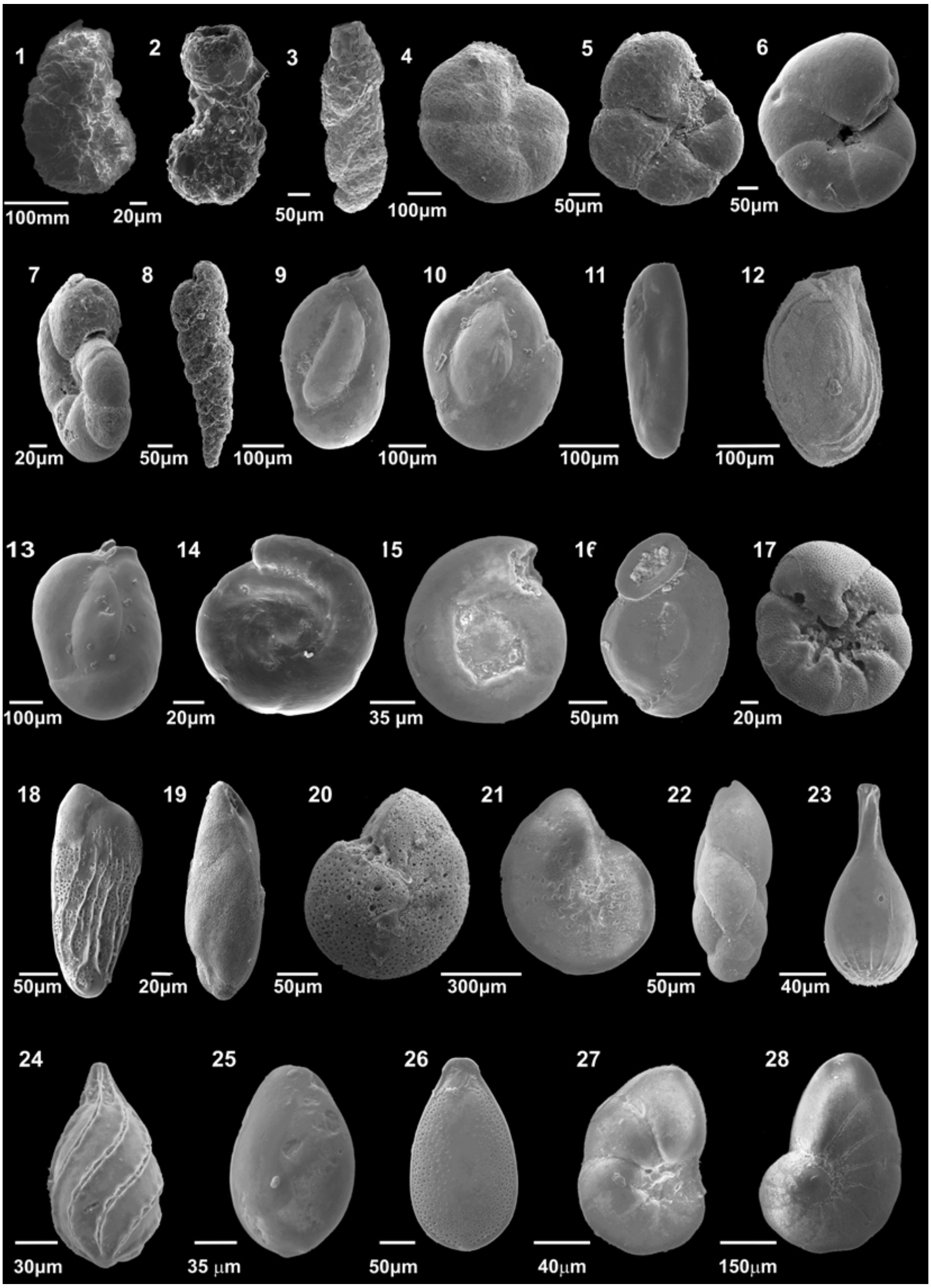

Figure 2 1 - *Ammobaculites dilatatus,* 2 - *Ammobaculites exiguus,* 3 - *Ammotium salsum,* 4 - *Arenoparrella mexicana,* 5 – *Siphotrochammina lobata,* 6 – *Trochammina inflata,* 7 – *Trochammina macrescens,* 8 – *Textularia earlandi,* 9 – *Quinqueloculina seminulum,* 10 – *Quinqueloculina lamarckiana,* 11 – *Quinqueloculina laevigata,* 12 – *Quinqueloculina costata,* 13 – *Miliolinella subrotunda,* 14 – *Cornuspira incerta,* 15 – *Cornuspira planorbis,* 16 – *Wisnerella auriculata,* 17 – *Ammonia beccarii,* 18 – *Bolivina striatula,* 19 – *Buliminella elegantissima,* 20 – *Elphidium discoidale,* 21 – *Elphidium excavatum,* 22 – *Fuserkoina pontoni,* 23 - *Lagena laevis,* 24 – *Lagena spirialis,* 25 – *Oolina viladeboana,* 26 - *Fissurina lucida,* 27 – *Haynesina germanica,* 28 – *Peseudononion atlanticum.*

SPECIES	PT02	PT03	PT04	PT05	PT06
Number of specimens (50 ml)	304	116	485	49	57
Number of species (50 ml)	24	13	15	3	10
Diversity (*H'*)	1.8	1.3	0.8	0.4	1.6
Equitability (*J'*)	0.6	0.5	0.3	0.3	0.8
Dominance	0.7	0.5	0.3	0.2	0.7
Number of stained tests	90	18	94	2	10
Test abnormalities	45	14	47	0	4
Ammobaculites dilatatus	-	-	-	8.2	6.9
Ammobaculites exiguus	-	-	-	-	3.4
Ammonia tepida	52.9	68	83.1	-	3.4
Ammotium cassis	-	-	0.4	-	-
Ammotium salsum	-	-	-	-	3.4
Arenoparrella mexicana	-	-	-	89.8	55.2
Bolivina doniezi	-	-	1.4	-	-
Bolivina inflata	0.6	-	-	-	-
Bolivina compacta	0.3	-	-	-	-
Bolivina spatulata	-	-	0.2	-	-
Bolivina spp.	-	3.4	1.4	-	-
Bolivina translucens	1	-	1.0	-	
Buliminella elegantissima	-	3.4	0.2	-	-
Cornuspira incerta	0.6	-	-	-	-
Cornuspira planorbis	1.6	-	-	-	-
Discorbis williamsonii	-	0.9	-	-	-
Elphidium discoidale	3	4.3	0.2	-	
Elphidium excavatum	5	8.5	8.7	-	-
Fissurina lucida	0.3	0.9	-	-	-
Fursenkoina. pontoni	1.3	0.9	-	-	-
Haynesina germanica	-	-	0.4	-	-
Lagena laevis	0.3	-	-	-	-
Lagena perlucida	0.3	-	-	-	-
Lagena spiralis	0.3	-	-	-	-
Lagena striada	0.3	-	-	-	-
Miliolinella subrotunda	20	2.6	-	-	-
Nonionella opima	0.3	-	-	-	-
Oolina vilardeboana	0.3	-		-	-
Pseudononion atlanticum	2.3	-	-	-	-
Quinqueloculina laevigata	3.2	1.7	0.2	-	-
Quinqueloculina lamarckiana	2.2	-	-	-	-
Quinqueloculina polygona	-	0.9	-	-	-
Quinqueloculina seminula	1.3	-	1.0	-	-
Quinqueloculina spp.	-	-	0.2	-	-
Rosalina bradyi	0.3	-	-	-	-
Rutherfordoides sp.	0.3	-	-	-	-
Siphotrochammina lobata	-	-	-	2.0	-
Spiroculina sp.	-	3.4	-	-	-
Textularia earlandi	0.3	-	0.2	-	5.2
Trochammina macrescens	1	-	1.2	-	15.5
Trochammina inflata	-	-	-	-	1.7
Trochamminita salsa	-	-	-	-	3.4
Uvigerina peregrina	-	0.9	-	-	1.7
Wiesnerella aviculata	1	-	-	-	-

Table 2 Ecological parameters and relative frequency of species from Potengi River Estuary. Station PT 01 was devoid of foraminifera.

Cluster analysis in Q-mode showed the existence of three groups of stations in the Potengi River Estuary (Figure 2): Group I comprising the station closest to the ocean, PT 02; Group II comprising stations PT 03 and PT 04; and Group III comprising the estuary's innermost stations, PT 05 and PT 06 (Figure 3).

Analyzing both cluster techniques, it is possible to verify that: Group I was composed of Assemblage C and the same species from Assemblages A and B; Group II was dominantly composed of Assemblage B, and showed some species of Assemblages A and C; and Group III was composed of Assemblage A, with some species from Assemblage B (Figure 4).

The variation coefficient found in axis 1 in CCA analyses was 98%, and in axis 2, 21%. The largest vector with smallest angle in relation to axis 1 was organic matter and salinity, followed by bacterial carbon. Stations PT 03 and PT 04 were the ones that suffered most influence, while station PT 05 was the one least influenced by those abiotic parameters (Figure 3). CCA analysis showed that abiotic parameters had great influence on the distribution of the foraminifera species in the estuary. Only *A. mexicana*, Ammobaculites *dilatatus* and *Siphotrochammina lobata* were not influenced by those parameters.

5 Discussion

The physiochemical parameters measured in the Potengi River Estuary did not show great oscillations, and the values found are similar to those found by other authors (Frazão 2003; Ramos e Silva *et al*., 2006).

Dissolved oxygen levels follow the estuarine gradient, the highest values at the mouth and decreasing landward. The lowest oxygen value found at station PT 03 was the reflection of sewage transported by Baldo Channel.

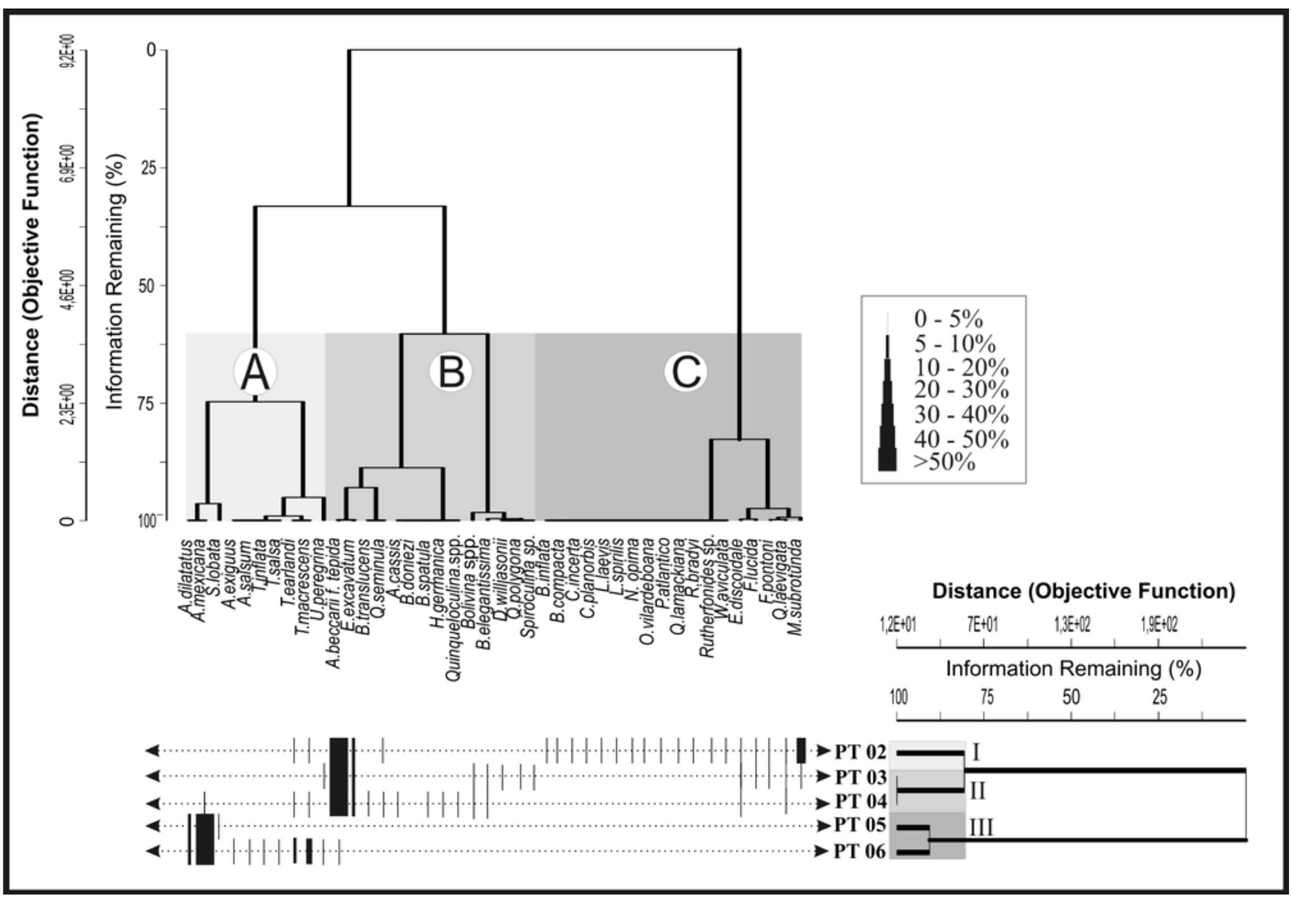

Figure 3 Cluster analyses in Q-mode and R-mode.

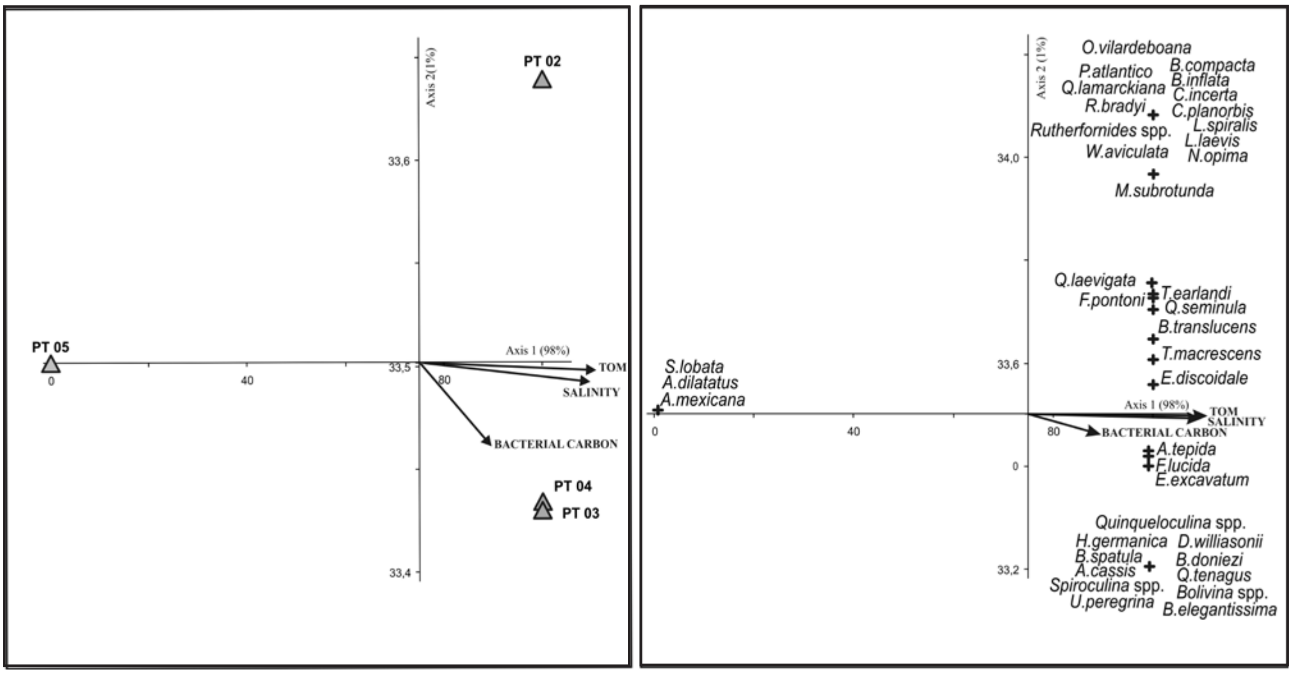

Figure 4 CCA showing proportions of parameter influence on distribution of stations and species in the Potengi River Estuary.

All these parameters are compatible with those measured in other estuaries and impacted environments, e.g. Guanabara Bay (Batista-Neto *et al*., 2000) in Southeast Brazil. Those values remained within bounds of the averages found in other eutrophic areas (Batista-Neto *et al.,* 2000; Carreira *et al.,* 2001; Vilela *et al.,* 2002).

The sand facies is predominant in the Pontengi River Estuary (Frasão *et al.,* 2003) and it was observed in the inner portion (PT 05 and 06) and mouth (PT 01) of the estuarine system. On the other hand, the central portion of the estuary had greater concentration of mud sediment and organic matter because of the sewage discharge from Baldo Channel (PT 03) and the presence of mangrove forests (PT 04).

Mangroves are usually confined areas with low energy in estuaries, and sulfate-reduction processes are normal in such areas; however, in order for the environment to be balanced several types of metabolisms must be present. At the Potengi River Estuary the predominant bacterial metabolic processes were anaerobic, implicating in loss of nitrogen to the atmosphere and sulfide production. Despite the estuary hydrodynamics, aerobic processes were absent at all stations, showing the permanence of pollutants in the sediment. In conditions with low concentrations of O_2 in the water column, organic matter is mineralized by anaerobic bacteria and the process entails formation of inorganic compounds in reduced form such as sulphidric gas, which is harmful to the environment and to human health. For the carbon and nitrogen cycles to be complete both aerobic and anaerobic processes must occur in the environment, producing oxidized and reduced substances (Bispo, 2005).

In polluted areas, the most common community structure is the decrease of species richness, by the elimination of the least resistant species and the increase of the opportunistic ones (Bonetti *et al*., 1999), as occurred at Potengi Estuary, where the species *Ammonia beccarri* and *Arenoparella mexicana* dominated. Several opportunistic or tolerant species benefit from some types of pollution or contamination: directly from the increase of nutrients (organic substances, salts, bacteria, etc.); or indirectly from the decrease of competition and predation (Alve, 1995). *A. tepida* is commonly found in polluted environments (Debenay *et al.*, 2000; 2001), being usually described in the literature as a species tolerant to pollution by heavy metals (Alve, 1991) and hydrocarbons (Yanko & Flexer, 1991). In addition, they are the calcareous species most resistant to environmental changes (Boltovskoy, 1965), being common in estuaries. *A. mexicana* is only common in estuaries when the environment is inhospitable (Laut, 2003), and it is highly adapted to stressed environments, successfully growing in such places (Laut *et al.,* 2006; Souza *et al*., 2007). The dominant presence of those species can be an indication that the environment is stressful to the sea fauna.

In hypohaline areas, the domain belongs to agglutinant individuals (Bonetti, 1995), as was observed at the stations placed further within Potengi Estuary. In those areas, organisms from Milioliina order were not found. According to Murray (1968), species of this group are stenohaline and salinity variation cause osmotic changes in their plasma, impairing pseudopod activity. As a result, they are usually absent from mangroves and estuaries. Moreover, they have little tolerance to low oxygen concentrations, and when present in estuaries they are found in the area closest to the ocean, where there is renew of oxygen (Brönnimann *et al*., 1981), as was the case in Potengi Estuary.

Salinity is clearly the most important factor in distinguishing different associations (Madeira-Falcetta, 1974). In the CCA analysis applied in this work, salinity had large influence on species distribution (Figure 4).

Oxygen plays a very important role in the distribution of foraminifera. Jorissen *et al.* (1998) showed that a potential relationship exists among areas with successive redox activities and distribution of foraminifera. In oligotrophic areas, the amount of food available is one of the most important factors influencing distribution, while in eutrophic environments oxygen level is one of the limiting factors (Jorissen *et al*., 1995). The largest concentration of stained shells at station PT 02 (Table 2) may be related to the fact that this area is located in the outermost part of the estuary, a more hydrodynamic area with water mixing and oxygen renewal, where organisms find ideal conditions to thrive.

Benthic foraminifera are some of the few eukaryotic groups that successfully inhabit anoxic and dioxic environments (Alve, 1994). According to Bernhard & Alve (1996), benthic foraminifera are optional anaerobes and most species are affected when exposed to severe lack of oxygen. Studies with foraminifera in low-oxygen environments showed that several genera are able to tolerate dysoxic and anoxic conditions for some time. Surprisingly, abundance can be high in places with low oxygen levels due to the decrease of predation (Bernhard & Sen Gupta, 1999). Genera typical of low-oxygen areas are *Ammonia, Elphidium, Bolivina, Buliminella, Ammotium* and *Trochammina* (Gustafsson & Nordberg, 2000). All of these were found at the Potengi River Estuary. Most species had test abnormalities, showing that these species may be suffering from the low oxygen levels.

Increase of equitability in polluted areas was observed by Beat & Spencer (1979) and has been interpreted as a larger capacity of resistant species to compete amongst themselves, promoting larger homogeneity among populations. Another explanation for high equitability is that, given the abundance of food and habitats available after the exclusion of the most sensitive species, competition does not exist in these areas, allowing all the resistant species to grow equally. This may have been the case in stations PT 02 and PT 06, which displayed the highest equitability among the stations.

At station PT 02, next to the estuary mouth, several foraminifea species typical of the continental shelf were found: *W. aviculata, U. peregrina, Ruterfordoides* sp., *R. bradyi*, *O. vilardebona, L. laevis, L. spirialis* (Cushman, 1969). The presence of exotic species that are transported in suspension in mesotidal estuaries is a common phenomenon, resulting in a rise in ecological indexes at the mouth of estuaries (Wang & Murray, 1983). The Potengi Estuary mouth may thus have high diversity and equitability proportioned by introduced species that are little represented in the total population. The dominance values index at this station shows a high frequency of *Ammonia tepida.* These characteristics suggest an environmental imbalance at the mouth of the Potengi River. A similar situation was observed at stations PT 05 and 06, where ecological indexes were high in spite of dominance by *Arenoparrella mexicana*. This could be why only three foraminifera species were found close to the area inhabited by low-income populations (PT 05). Besides, the smallest species richness at that station can be explained by its low oxygen and salinity concentrations and lower temperature in relation to the other points, as well as for being the site of the sulfate-reduction process.

The absence of foraminiferal tests in PT 01 could be a consequence of shrimp farm activities, because a series of untreated residues are spilled directly into the channel. Even the most resistant species tend to disappear when exposed to unfavorable conditions for a long time (Jorissen *et al*., 1995). However, more studies are necessary to assess the degree of this activity's impact over the foraminifera population.

Heavy metal pollution from oil refineries and local industries affect foraminiferal distribution and deform their shells. Abnormalities test may be considered a pollution indicator, since the proportion of abnormalities increases notably in those areas (Le Cadre & Debenay, 2005). The deformations that happen in unpolluted environments can result from regeneration of the cells and from the tests after reproduction, when a small amount of the cytoplasm containing the nucleus remains in the tests (Stouff *et al*., 1999). Anomalous forms in benthic foraminifer tests appear under natural conditions in all environments (Alve, 1991), but are usually rare (Geslin *et al*., 2002). In the Potengi River Estuary the largest concentrations of abnormalities tests were seen at the areas close to the oil terminal (PT 02) and to the sewage outfall (PT 03), the latter probably explained by the discharge of untreated sewer and the heavy metals present there. Industrial residues are rich in heavy metals and these, even at low concentrations, can also affect benthic communities (Alve, 1995). Deformations have been observed in the tests of some species in areas with high concentrations of Pb, Cd and Zn (Samir & El Din, 2001). In the Potengi River the metals Cd and Zn were found above the permitted levels (Silva *et al*., 2001; Silva *et al*., 2006).

Most of the benthic food chain depends on primary activity and on the flow of organic matter that reaches the sediment (Gooday, 1993). There are indications that specific foraminifera species depend on bacteria or on bacterial activity to survive in anoxic conditions or in other hostile conditions (Duijnstee 2001). However, the specific role that bacteria play in the control of foraminiferal occurrence is still not conclusive. Lee *et al.* (1991) suggest that many coastal foraminifera feed on algal blooms, indicating that they benefit from a food source when it is abundant, but feed and reproduce more slowly when food is scarce. This could be the situation in the estuary, *i.e*., the foraminifera species might be benefiting from the presence of bacteria and could be feeding on them. Heeger (1990) showed that some species change their food sources depending on which are available.

Zonation patterns for Potengi Estuary can be proposed based on abiotic, biotic and statistical analyses that represent estuarine/stress gradients (Figure 5):

Upper Estuary (Stations PT 05 and 06) – Characterized by dominance of *A. mexicana* in association with other mangrove agglutinant species. Sedimentary facies was sandy poor in organic matter and showed low bacterial carbon with sulfate-reduction activity (Table 1). In this region of Potengi Estuary the ecological indexes were high (Table 2).

Middle Estuary (Stations PT 03 and 04) – Characterized by dominance of *A. tepida* in association with *E. excavatum.* Bacterial metabolism is anaerobic, showing high values of bacterial carbon with fermentative anaerobic activity (Table 1). Ecological indexes decrease and some abnormalities tests are present (Table 2).

Lower Estuary (Stations PT 01 and 02) – Characterized by the dominance of *A*. *tepida* associated to marine species. It is likely that marine species were brought in suspension by tidal action. This zone presents anaerobic bacteria metabolism, sandy facies on the left margin and muddy facies on the right (Table 1) and high ecological indexes and abnormalities tests (Table 2). This area differs from the others for its high hydrodynamic discharge which tends to accumulate fewer pollutants, and higher oxygenation which contemplates the species that colonize the sediment.

6 Conclusion

The Potengi River Estuary is a classic mesotidal estuary in foraminifera assemblage distribution patterns. It shows more richness and diversity index in the mouth with a result of suspension transports of tests form the adjacent continental shelf. In the middle zone of the estuary there is a large population of calcareous species typically of estuarine regions with *Ammonia* spp. and *Elphidium*. And in the inner portion the calcareous species were substituted by agglutinated species with *Arenoparrella mexicana.*

The large number of tests with abnormalities test and the dominance of opportunistic species like *Ammonia tepida* and *Arenoparrella mexicana* suggest environmental stress in the middle estuary.

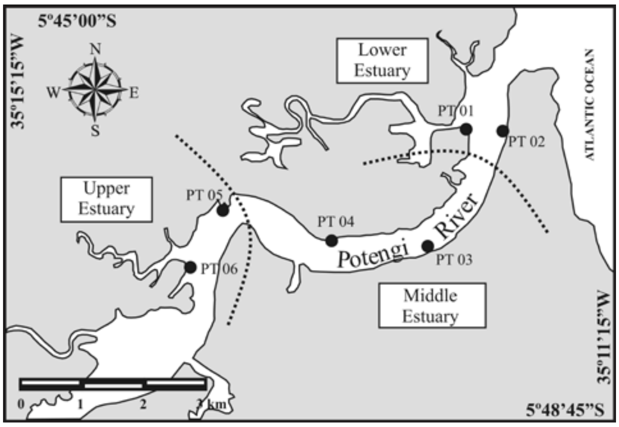

Figure 5 Potengy Estuary zonation.

CCA proved to be a good statistical tool to identify the main parameters responsible for the distribution of samples and species. Distribution of foraminifera assemblages was influenced in this estuary by salinity, organic matter and bacterial carbon.

Studies comprising foraminifera and bacterial associations and environmental parameters can be extremely helpful in the monitoring and diagnosing coastal environments.

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