UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE TECNOLOGIA E GEOCIÊNCIAS
DEPARTAMENTO DE OCEANOGRAFIA
PROGRAMA DE PÓS-GRADUAÇÃO EM OCEANOGRAFIA

AS COMUNIDADES MACROBENTÔNICAS NA AVALIAÇÃO DA QUALIDADE AMBIENTAL DE ÁREAS ESTUARINAS DE PERNAMBUCO

ANA PAULA MARIA CAVALCANTI VALENÇA

RECIFE
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Dissertação apresentada ao Programa de Pós-Graduação em Oceanografia, da Universidade Federal de Pernambuco - UFPE, como um dos requisitos exigidos para obtenção do grau de Mestre em Oceanografia.

Orientador:

Prof. Dr. Paulo Jorge Parreira dos Santos

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À minha família.

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Agradecer é mistério..."

Fernando Pessoa.

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"Superação é poder fazer acontecer com as ferramentas que temos em mãos, superação é trabalhar da melhor forma possível independentemente do que pensem ou falem. Superação é irmos além do que os outros acham que somos capazes."

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RESUMO

Apesar de sua reconhecida importância em termos de complexidade e biodiversidade, os estuários vêm sofrendo considerável processo de degradação gerado pela ação antrópica. Diversos estudos têm enfatizado o papel do macrobentos como indicador da qualidade ambiental desses ecossistemas, o que tem levado ao desenvolvimento de ferramentas e métodos baseados em seus atributos (abundância, biomassa, composição específica, etc.). Dentre esses, os índices bióticos têm sido preferidos em termos de precisão e de custo-benefício, sendo o AMBI (Índice Biótico Marinho) o mais aplicado, com sucesso, em várias áreas geográficas submetidas a diferentes fontes de impacto. Contudo, poucas informações a respeito da ecologia das comunidades macrobentônicas estão disponíveis para a costa atlântica da América do Sul, estando concentradas nas regiões sudeste e sul do continente. Dessa forma, o presente estudo teve como objetivos descrever a composição e distribuição do macrobentos de áreas estuarinas de Pernambuco, testando o efeito do tamanho da abertura da peneira (1,0mm x 0,5mm) e da profundidade de amostragem (0-10cm x 0-20cm) na descrição das associações da fauna e examinar a eficiência do AMBI na avaliação da qualidade ambiental desses estuários. As coletas ocorreram em Outubro-2007, em 14 pontos situados na costa de Pernambuco, sendo amostrados além do macrobentos, parâmetros físico-químicos de água (salinidade, temperatura, oxigênio dissolvido, amônia) e de sedimento (matéria orgânica, granulometria, potencial redox, nitrogênio-total), além do microfitobentos. Um total de 14.257 indivíduos distribuídos em 78 táxons e com uma biomassa total de 83,64g foram observados. Em geral, as diferenças na retenção das peneiras foram importantes para a abundância total (já que a peneira de 1,0mm reteve apenas 28% dos indivíduos), sendo pouco evidente para a biomassa total (92% da biomassa). Por outro lado, em termos de profundidade de amostragem, o estrato de 0-10cm compreendeu quase todos os indivíduos (94% da abundância total), mas contribuiu apenas com 39% da biomassa total. Foram encontradas apenas relações significativas para o fator "peneiras" tanto para a diversidade N_1 (ANOVA 2-way: $F_{1;13}$=5,17; p=0,02) quanto para a estrutura das comunidades (ANOSIM 2-way: R_{global}= 0,142; p=0,002). Correlações significativas foram estabelecidas entre as variáveis nitrogênio total (r=0,860; p<0,0001), matéria orgânica (r=0,801; p<0,001) e microfitobentos (r=0,749; p=0,005 and r=0,795; p=0,002, para clorofila-*a* e feopigmentos, respectivamente) contra a abundância da fauna retida nas peneiras de 1,0mm e de 0,5mm. De modo geral, o macrobentos dos estuários estudados é composto por pequenas espécies (1,0-0,5mm), de modo que o uso da peneira de 0,5mm permite uma interpretação mais precisa dos dados; além disso, para a composição da fauna e abundância, a camada superficial (0-10cm) é claramente mais importante enquanto que, para biomassa, a camada de fundo (10-20cm) deve ser considerada. Para a avaliação da qualidade ambiental, o índice AMBI mostrou que todas as áreas estudadas apresentaram algum nível de distúrbio, variando de 2,395 (pouco poluído, Ariquindá) a 5,236 (fortemente poluído, Capibaribe). Em geral, todas as áreas estiveram dentro dos limites de pouco a moderadamente poluído, devido à grande proporção dos táxons Oligochaeta e Nematoda (espécies tolerantes, grupo ecológico III). O índice provou ser eficiente na detecção da qualidade desses estuários, embora sua aplicação para águas tropicais requeira algumas adaptações na classificação ecológica das espécies.

Palavras-chave: Macrobentos, tamanho da peneira, profundidade de amostragem, índice AMBI, qualidade ambiental.

ABSTRACT

Estuarine environments are known by its importance in terms of complexity and biodiversity; however, anthropogenic activities continue to have a detrimental effect on its biodiversity levels on a worldwide-scale. Several studies have emphasized the role of benthic macroinvertebrates as indicators of environmental quality status in these ecosystems, which have lead a growing number of tools and methods based on macrofauna attributes (abundance, biomass, species composition). Among them, biotic indices has been chosen in terms of accuracy and cost-benefit, being the AMBI (AZTI Marine Biotic Index) the most successfully applied to different geographic areas and under different impact sources. However, few studies regarding ecological characteristic of soft-bottom macrobenthic communities on the Atlantic coast of South America are available and thoroughly concentrated in the south and southeast coasts. Hence, the main objectives of this contribution were to describe composition and distribution of macrobenthos from estuarine areas of Pernambuco and tests if particular sieve mesh sizes (1.0mm x 0.5mm) and sampling depth (0-10cm x 0-20cm) alter the macrobenthic association descriptors; in addition, to examine the efficiency of AMBI index to evaluate the environmental quality of these estuaries. Samples for abiotic variables of water (salinity, temperature, dissolved oxygen and ammonia-N) and sediment (organic matter, granulometry, redox potential, total-N), microphytobenthos and also macrofauna were collected at fourteen estuarine sites located in Pernambuco coastline, in October 2007. A total of 14,257 individuals, from 78 taxa and with a biomass of 83.64gAFDW were observed. In general, the differences between 1.0mm and combined sieves retention are hardly evident for biomass but it is very important for the abundance (1.0mm sieve retained only 28% of total individuals but, at the same time, was responsible for 92% of total biomass). Regarding sampling depth, the top layer (0-10cm) presented almost all individuals (94% total abundance); however, contributed with only 39.06% of the overall biomass. Significant interactions were observed only for “mesh-sieve” factor in terms of diversity N_1 (two-way ANOVA: $F_{1;13}$=5.17; p=0.02) and community structure (two-way ANOSIM: R_{global}= 0.142; p=0.002). Pearson’s correlation have selected the variables total-N (r=0.860; p<0.0001), organic matter (r=0.801; p<0.001) and microphytobenthos (r=0.749; p=0.005 and r=0.795; p=0.002, for chlorophyll-*a* and phaeopigments, respectively) as the most fit for explaining the “changes” in fauna from 1.0mm to 0.5mm. The results showed that these estuarine macrobenthos were mainly composed by small species (1,0-0.5mm), thus the use of the 0.5mm sieve will permit a more accurate interpretation of the data; besides for taxa composition and abundance, the top layer (0-10cm) is clearly the most important whereas for biomass the bottom layer (10-20cm) presents this function. In terms of environmental quality, the AMBI index indicated that all ecosystems presented some level of disturbance, ranging from 2.395 (slightly disturbed, in Ariquindá) to 5.236 (heavily disturbed, in Capibaribe). According to the index, most sites were situated between the slightly-moderate disturbed boundaries, due to higher proportion of Nematoda and Oligochaeta, both assigned here as ecological group III. In summary, AMBI proved to be efficient in evaluating the quality status of Pernambuco estuaries, although its applicability in tropical waters requests some adaptations in species’ ecological groups.

Keywords: macrobenthos, sieve mesh-size, sampling depth, AMBI index, environmental quality status.

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GENERAL INTRODUCTION

Estuaries and coastal marine waters are ranked among the most important aquatic systems on Earth in terms of ecological and economic significance (Nalesso et al., 2005). Estuaries are ecotones that play a fundamental role in enriching, by nutrient loads, the adjacent areas (Christian et al., 1991; Howarth and Marino, 2006; Lacerda et al., 2006; Boynton et al., 2008); besides, they have singular characteristics according to their geomorphology, water circulation, salinity and temperature variations (Yáñez-Arancibia, 1987; Branco, 2001). These ecosystems comprise approximately 8-15% of the coastal regions in the world (Kennish, 1992; Bonecker et al., 2007) and are important areas for reproduction, nursing and feeding of at least one stage of the life-cycle of several invertebrates and fishes, including high commercial value species (Muniz and Venturini, 2001; McLusky and Elliot, 2004; Joyeux et al., 2004).

However, a major scientific conclusion of the past century is that human population is changing Earth's ecosystems in fundamental ways and the population has been increasing rapidly in the coastal zones, resulting in a multitude of ecological stresses on aquatic systems, especially estuaries (Flemer and Champ, 2006). Anthropogenic activities, such as habitat modification, pollution and overexploitation of living resources, continue to have a detrimental effect on global biodiversity levels and subsequent provision of estuarine resources (Jackson et al., 2001; Loreau et al., 2001); moreover, many estuaries are being used as repositories for industrial effluents and domestic wastes (Ramos et al., 2006).

As coastal zone management strategies become more imperative, objective and interdisciplinary, studies are necessary to understand the ecological boundaries of its ecosystems, in order to protect – and conserve – its resources (Bonetti et al., 2004). Therefore, it is essential to realize how ecosystems function, since this type of information is crucial in the elaboration of conservation strategies (Pagliosa and Barbosa, 2006).

The benthic environment is a fundamental compartment within any aquatic ecosystem. Bottom sediments constitute a source of nutrients for the water column above having an important position in the benthic-pelagic coupling (Jørgensen, 1996; Venturini et al., 2004). There, benthic organisms alter the physical and chemical conditions at the sediment-water interface, promote the decomposition of organic matter, recycle nutrients for photosynthesis and transfer energy to other food-web components (Gaston et al., 1998). Therefore, they may be regarded better pollution indicators than the instantaneous water quality measurements (Nalesso et al., 2005).

Benthic faunal organisms are considered excellent tools to monitor the environmental health of estuaries (Caeiro et al., 2005). Among them, macrobenthic communities provide an ideal measure of its response to disturbance (natural or man-induced), which made them known as effective indicators of pollution impacts in estuarine ecosystems (Engle et al., 1994; Weisberg et al., 1997; Borja et al., 2000). Their advantages as pollution indicators may be pointed out: (i) rapidly response to anthropogenic and natural stress in both spatial and temporal scales (Pearson and Rosenberg, 1978; Dauer, 1993; Fleischer et al., 2007); (ii) restricted mobility avoiding unfavorable conditions on water/sediment (Weisberg et al., 1997; Borja et al., 2003a; Reiss and Krönche 2005); (iii) important function in cycling nutrients and materials between the underlying sediments and the overlying water column (Borja et al., 2003a); and (iv) taxonomic diversity that can usually be classified into different functional response groups (Smith et al., 2001).

The biological integrity of macrobenthic communities had traditionally been characterized by measures of abundance, diversity, or the presence of pollution indicators (Engle et al., 1994) since disturbances promote changes on community structure. The analysis of changes in these communities, using univariate and multivariate methods has taken an important part in the assessment and monitoring of marine and coastal environments (Warwick and Clarke, 1993; Muniz et al., 2005). Single community attribute measures or individual-species data combinations, including species diversity, evenness or abundance/biomass ratios are used to sum up data beyond the level of individual species (Caeiro et al., 2005), however these measures have proven to be of not great help for estuarine environments monitoring which have highly variable natural conditions (Engle et al., 1994). Conversely, multivariate methods are more sensitive in characterizing benthic patterns (Warwick and Clarke, 1993), but the results obtained are usually too complex to be understood by non-scientists (Smith et al., 2001; Muniz et al., 2005).

In more recent studies, biotic indices were developed as easily-understood tools of ecological changes, which have clear practical application in the achievement of environmental quality (Borja, 2006). Such indices estimate macrobenthic community disturbance level and establish the ecological status of soft-bottom benthos (Weisberg et al., 1997; Borja et al., 2000; Simboura and Zenetos, 2002; Llansó et al., 2003; Rosenberg et al., 2004), in addition, most are based in the concept of sensitive/tolerance taxa (Carvalho et al., 2006). According to Diaz et al. (2004), the majority of indices currently available are designed for use in freshwater streams, rivers and/or lakes (58%), but a significant number of indices are on hand for the assessment of estuarine and coastal marine systems (33%). Of these, the scale of applicability ranges from local (Xu et al., 2001) to regional (Benthic Index of Biotic Integrity, B-IBI, Llansó et al., 2003) and global (AZTI Marine Biotic Index, AMBI, Borja et al., 2000, 2003a) areas.

One of the indices extensively applied with good results is the AZTI Marine Biotic Index – AMBI (Borja et al., 2000). The index is based on the classification of macrobenthic species in ecological groups corresponding to different sensitivity levels, making it suitable to all data sets including the small ones (Bigot et al., 2008). It has been tested under different stress sources such as submarine outfalls, heavy metals, harbour construction, dredging, diffuse pollution (mines, agriculture) and river inputs (e.g. Borja et al., 2003a,b; Muxika et al., 2003; Muxika et al., 2005) and has been applied not only in Europe (Borja et al.,,2000; Borja et al., 2003a,b; Muxika et al., 2003; Salas et al., 2004; Caeiro et al., 2005; Muxika et al., 2005; Albayrak et al., 2006; Carvalho et al., 2006a,b; Sanz-Lázaro and Marin, 2006; Aguado-Giménez et al., 2007; Borja et al., 2007; Chainho et al., 2007; Dauvin et al., 2007; Fleischer et al., 2007; Muxika et al., 2007a,b; Pontil et al., 2007; Pravoni et al., 2007; Simboura and Reizopoulou, 2007; Teixeira et al., 2007; Zettler et al., 2007; Afli et al., 2008; Blanchet et al., 2008; Munari and Mistri, 2008; Puente et al., 2008; Simboura and Reizopoulou, 2008; Teixeira et al., 2008a,b; Pinto et al., 2009) but also in Asia (Cai et al., 2003), northern Africa (Bazairi et al., 2005; Bakalem et al., 2009), North America (Borja et al., 2008), South America (Muniz et al., 2005) and the southwest Indian Ocean (Bigot et al., 2008), allowing correct assessment of the ecosystem's conditions. One negative aspect of the index is that since the list of species was created based on ecological characteristics from temperate benthic fauna, the ecological role of one (or more) specie(s) may differ with the environmental context and area (Labrune et al., 2006), which implies in reviewing the assignment of ecological groups. Moreover, appropriate assignments are neither necessarily available (Muniz et al., 2005; Flaten et al., 2007) nor easy to achieve outside European coasts, where AMBI was first developed (Bigot et al., 2008). This index presents some limitations when the number of species is very low or when studying low-salinity locations (e.g. the inner part of estuaries) (see Borja and Muxika, 2005).

Biotic indices have been proposed as synthetic methodologies and its attributes should be precision and cost-effectiveness (Pontil et al., 2007), consequently it is important to verify whether one of the main source of sampling variation – specifically sieve mesh-size – affects the accuracy of benthic community based on indices calculation (Pinto et al., 2009). Commonly, 1.0 and 0.5mm sieves are used for macrobenthic pollution monitoring (Eleftheriou and Holme, 1984; Bachelet, 1990; Schlacher and Wooldridge, 1996; Rodrigues et al., 2007) however, most surveys have shown that small species associated to organic pollution are usually not retained by 1.0mm mesh sieve and due to that biased results may arrive from different methodologies (Thompson et al., 2003; Teixeira et al., 2007).

Despite tropical and subtropical coastal ecosystems comprise more than one third of world's continental shelf area, not only basic information on ecology of soft-bottom benthic communities is still lacking but also their response to disturbance, especially in coastal areas of some countries submitted to intense and unplanned anthropogenic activities (Muniz et al., 2005). According to Pagliosa and Barbosa (2006), studies concerning the ecology of soft-bottom benthic macrofauna communities in estuarine systems in the South America Atlantic coast are recent and in most cases there is no historical data or long-term monitoring programs. Besides, such studies are concentrated in south and southeast coasts, where the impacts on estuarine systems only have been registered in obvious cases of severe pollution (Venturini et al., 2004).

In Pernambuco State, northeast coast of Brazil, estuarine zones are the most affected by anthropogenic influences (e.g. sewage, removal of native mangrove forests for sugar-cane monocropping, industries such as chlor-alkali, fertilizers, agricultural defensives, paper mills, aluminum, and others) (CPRH, 2006). As a consequence, it is necessary to propose instruments and methodologies which permit to express the structural and functional quality of the benthic communities in order to achieve sustainability and conservation of these tropical ecosystems.

This study attempted to fill some of the scarcity concerning ecological information of tropical macrobenthic communities through two chapters, the first one focus on comparative surveys of macrobenthos from estuarine areas of Pernambuco and test if particular sieve mesh sizes and sampling depths alter the interpretation of macrobenthic assemblages' descriptors; the second one intend to examine the efficiency of AMBI to evaluate the environmental quality of these estuaries.

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CHAPTER I. Macrobenthic community structure in tropical estuarine ecosystems (Northeast, Brazil): the effect of sieve mesh-size and sampling depth on abundance, biomass and composition.

I.1. Introduction

The description of the patterns of species distribution, biomass and abundance with accuracy is crucial, since it provides the basis for most ecological research (Benedetti-Cecchi et al., 1996). In benthic communities, it can be directly influenced by sampling procedures such as number of samples, taxonomic resolution, sieve screen (Bachelet, 1990; James et al., 1995; Tanaka and Leite, 1998) and sampling depth (Schaffner, 1990; Flach and Heip, 1996).

Sieves mesh-sizes commonly used for macrobenthic studies are 0.5mm and 1.0mm (Eleftheriou and Holme 1984; James et al., 1995). Depending on the survey's purpose (e.g. population dynamics studies), it is imperative to determine the adequate sieve size before initiating any scale-sampling exercise (Mahadevan and Patton, 1979). In monitoring programmes, the assessment of macrobenthic community structure and productivity is a long and costly process that involves sorting, identifying and measuring large numbers of small organisms from the material collected (Gruenert et al., 2007). Therefore, many pollution-monitoring studies adopt the 1.0mm sieve as the most cost-effective method for balancing logistic constraints while maintaining the capacity to detect ecological changes (Lampadariou et al., 2005). Nevertheless, sieve efficiency varies with taxonomic group and for individual species in the same taxonomic group (Rodrigues et al., 2007); moreover, it is known that small species related to organic enrichment are able to pass through the 1.0mm mesh sieve (Schlacher and Wooldridge, 1996a,b; Teixeira et al., 2007), determining the use of finer sieves. Therefore, the choice of a particular mesh size may establish the reliability of results and, consequently, the detection of an impact (Thompson et al., 2003; Lampadariou et al., 2005).

Comparative studies on the influence of different sieve sizes on macrobenthos composition have been done especially on communities of temperate marine and coastal areas, such as Mediterranean (Benedetti-Cecchi et al., 1996; Lampadariou et al., 2005, 2008), North America (Ferraro and Cole, 1990; Ferraro et al., 1994, 2006; Gruenert et al., 2007), Iberian Peninsula (Rodrigues et al., 2007), Australia (James et al., 1995), South Africa (Schlacher and Wooldridge, 1996a,b) and Antarctica (Thompson et al., 2003) and also deep-sea zones (Gage et al., 2002). Information concerning the effect of sieves on tropical macrobenthic communities is still restrict (e.g. macrophytes-associated invertebrate assemblages), face of number and diversity of surveys on the theme (Tanaka and Leite, 1998).

The importance of sediment sampling depth for the macrobenthos community descriptor is focused in most literature dealing with benthic vertical distribution (Schaffner, 1990; Weston, 1990; Flach and Heip, 1996; Kumar, 1997; Mannino and Montagna, 1997; Flach et al. 1998; Ingole et al., 1999; Wei, 2006). All these surveys showed some trend for abundance (sampling the upper centimeters of sediment column corresponded to higher proportion of macrofauna individuals) but not for biomass profiles; in addition, as for sieves' information, the subject is mainly explored in temperate areas (and also deep-sea zones) in contrast to tropics (e.g. Gutiérrez et al., 2000).

This study investigates the role of sieve mesh-size and sampling depth on the quality and interpretation of data obtained, in order to contribute to a better knowledge of such effects when describing distribution and composition of tropical macrobenthic communities.

I.2. Material and Methods

I.2.1. Description of studied areas

The study was undertaken at estuarine areas situated along the 187km coastline of Pernambuco State, Brazil's northeast (fig.01). Great part of these estuaries is designated as Environmental Protection Area by the State Law n.9.931, December 1986 (Noronha, 2008).

The Santa Cruz Channel constitutes of a U-shaped estuarine channel situated 50 km north from Recife, capital of Pernambuco State. This estuarine system separates the Itamaracá Island from the mainland; the whole area (730km²) has an extension of 22 km, variable widths (of 0.6 to 1.5 km) and depths (from 4-5m to less than 2m). From north to south, the channel receives continental influence through a number of rivers (Flores-Montes et al., 2002), being Botafogo and Igarassu rivers the major freshwater sources (Medeiros and Kjerfve, 1993) and also the most polluted (Silva et al., 2003). The region presents high ecological importance (Murolo et al., 2006), however is exposed to agro-industrial and urban activities, mainly the sugar cane monoculture and intensive fisheries. Two sites were chosen, one close to the Itapissuma Bridge, named Itapissuma (07°46'31.20''S and 34°53'26.76''W), and the other, more to the north, Sta Cruz Channel (07°46'13.12''S and 34°52'58.19''W) (fig.02A).

The Paripe river site (07°48'38.76''S and 34°51'23.28''W) (fig.02A) is located in southern Itamaracá Island; the river has 4km of extension and 1.6km length. The mangrove is composed by *Rhizophora mangle*, *Avicennia schaueriana, Laguncularia racemosa* and *Conocarpus erectus* (the last three in smaller proportion). The anthropic action is still at a minimum, the local population living basically of subsistence agriculture and fishing (Santos and Coelho, 2001).

The Timbó river site (07°51'18.72''S and 34°50'33.96''W) (fig.02B) is located in the north coast of Pernambuco, about 35km from Recife. The hydrographic basin comprises 92.46km², representing 6.8% of the north coast; its depth ranges from 2 to 8m (Figueiredo et al., 2007). The estuarine area exhibits expressive mangrove vegetation and for a long period was considered as non-polluted (Costa and Macêdo, 1989). Nowadays, it is under strong anthropogenic pressure as a result of intensive fishery activities, domestic sewage and industrial effluents (mainly steel mill and textile) and tourism (Grego et al., 2004).

The Paratibe river site (07°57'37.44''S and 34°49'48.54''W) (fig.02B) is situated between Paulista and Olinda cities, in the north coast of Pernambuco, about 15km from Recife.

The hydrographic basin occupies 118.54km² and a total extension of 16km (Pereira et al., 2007). The estuarine area was relatively reduced as a consequence of rapid human occupation and mangrove deforestation (CPRH, 2006). Other sources of pollution consist of hospital and industrial effluents, domestic sewage (Pereira et al., 2007) and also slaughterhouse (CPRH, 2006).

The Capibaribe river site (08°3'52.98''S and 34°52'27.06''W) (fig.02C) lies on the coast of Recife. The hydrographic basin comprises 7716km²; maximum depth 8m; tidal entry around 15km from the Atlantic Ocean. Water temperature ranges from 26.2°C (rainy season) to 31°C (dry season); salinity varies considerably (0.05 to 36.0); surface and bottom dissolved oxygen saturation ranges from supersaturation to very low levels, with significant oxygen depletion during low tide (Travassos et al., 1993). Extensive mangrove areas have been destroyed, even though small patches of *Rhizophora mangle*, *Laguncularia racemosa* and *Avicennia schaueriana* can be found (Paranaguá et al., 2005). This estuarine system is eutrophic: water temperature and nutrient inputs from direct sewage effluents stimulate algal and bacterial growths. Discharges into the river include wastes from small industries (milk, detergents, soap, and leather, among others), inputs from polluted tributaries, namely Tejipió, Jordão, Pina and Beberibe rivers as well as direct sewage outfalls (Fernandes et al., 1999).

The Pina Basin is a complex estuarine area placed at the coast of Recife and separated from the ocean by a natural reef dyke, into which discharge the south arm of Capibaribe, Tejipió, Jiquiá, Jordão and Pina rivers (CPRH, 2006). The basin is about 3.6km with a total area of 2.02km². Salinity varies from 0.4 to 37.0 and water temperature from 27ºC to 32ºC. Because of the constant influx of nutrients from the rivers (especially domestic sewage), the Pina basin is hypereutrophic and organically polluted; even though its great biological potential has been exploited by the low-income population (Sommerfield et al., 2003). Two sites were selected, named Pina Basin1 (08°4'38.7''S and 34°52'29.7''W) and Pina Basin2 (08°5'27.0''S and 34°53'11.64''W) (fig.02C).

The Jaboatão river site (08°14'24.43''S and 34°56'43.20''W) (fig.02D) is placed about 20km from Recife, in the south coast of Pernambuco. The river is part of a hydrographic basin with 413km² in area and 75km in length. Due to the fact of its basin draining six great cities, the river receives high load of pollutants such as untreated sewage, mill industry and cellulose manufacture (Souza and Tundisi, 2003).

The Pirapama river site (08°14'35.52''S and 34°56'46.80''W) (fig.02D) is also in the south coast of Pernambuco; together with Jaboatão river and its tributaries they formed the "Barra das Jangadas" estuarine system, being considered one of the major estuarine systems of Pernambuco State (Branco, 2001). According to Cavalcanti et al. (2008), the development of industries (especially sugar cane mills) and irregular human occupation along the margins constitute the main problems of its hydrographic basin (about 600km² and 70km length).

The estuaries of rivers Ipojuca and Merepe jointly with Tatuoca and Massangana used to discharge in the Suape Bay system (south coast of Pernambuco). However, with the implementation of an Industrial Port Complex (created in 1980) the two former rivers had their connection with the bay interrupted by the intensive embankment to build the port (Silva et al., 2004a). As a result, more than 600 hectares of mangrove have been deforested, affecting the ecological balance and resilience of several species (Koening et al., 2002). One site was chosen in the junction of these rivers, named Ipojuca-Merepe (08°24'39.66''S and 34°58'28.62''W) (fig.02E).

Maracaípe river site (08°32'21.42''S and 35°00'21.72''W) (fig.02E) lies on Ipojuca city, in the south coast of Pernambuco. This area, formed by dense forests of *Laguncularia racemosa*, has been exploited especially by ecotourism (Mendonça and Almeida-Cortez, 2007).

Ariquindá river site (08°41'22.74''S and 35°06'08.22''W) (fig.02F) is placed nearly Carneiros beach, also in the south coast of Pernambuco (Silva et al. 2004b). The river integrates the estuarine system of Rio Formoso which presents a great level of preservation, despite some agricultural enterprises, tourism activities and lack of sanitation in the urban centers from the region (Medeiros, 2005).

Mamucabas river site (08°46'41.81''S and 35°06'27.46''W) (fig.02F) stands in Tamandaré city, about 110km from Recife, in the south coast of Pernambuco. The river (>30km of extension) originates in the interior of the Saltinho Biological Reserve (15,5km from Tamandaré bay), borders its urban area and crosses either agricultural or forested areas (Araújo and Costa, 2006).

I.2.2. Sampling design

Samples for geochemical variables and macrofauna were collected at fourteen sites in October 2007. Preliminary investigations were undertaken in these estuarine areas, being the sites chosen with base on sediment characteristics, water salinity and level of disturbance. In each site, five benthic quantitative replicates for macrofauna analyses were sampled with a cylindrical corer (area: 40.71cm², depth: 20cm), and separated in two layers: top (0-10cm) and bottom (10-20cm). All biological samples were preserved in 10% formaldehyde. Subsequently, samples from both layers were washed through 1.0 and 0.5mm mesh sieves and the material retained in each one was fixed in 4% formaldehyde and stained with Rose Bengal. Sorting and counting were performed under stereomicroscope and specimens were identified to the lowest possible taxonomic level.

Total abundance and biomass per replicate were calculated for the macrofauna retained on 1.0mm mesh sieve and combined sieves (1.0+0.5mm), in top layer (0-10cm) and combined layers (0-20cm) and results were compared for mesh-sieve (1.0mm x combined sieves) and sampling depth (0-10cm x 0-20cm) effects. For biomass, organisms were preserved in formaldehyde prior to weighting (wet weight); values were averaged into major taxonomic groups and then estimated as ash-free dry weight (g AFDW) from conversion factors according to Ricciardi and Bourget (1998). For the polychaete *Laeonereis* sp., however, the biomass (in AFDW) was obtained by measuring the 5^{th} setiger diameter, in accordance with the growth models established by Florêncio (2000).

As for macrofauna, five replicates were obtained for sediment environmental descriptors, which include, granulometry, organic matter, nutrients-total-N (cylindrical corer - area: 16.62cm², depth: 2cm) and redox potential (Eh), measured in field, in top (~2cm) and bottom (~20cm) layers. Microphytobenthos was also sampled at each site, with a smaller corer (area: 1.13cm², depth: 2cm). One single measure was carried out for water variables: salinity and ammonia-N. All methodologies applied to determine such parameters are presented in table 01:

Table 01. Methodologies applied for environmental parameters.

Parameter	Method/Equipment	References
Microphytobenthos pigments (µg/cm²)	Spectrophotometer	Modified from Colijn and Dijkema (1981) and Lorenzen (1967) equations
Total nitrogen sediment (g/kg)	Kjeldahl method	EMBRAPA (1997)
Organic matter (%)	Incineration	Wetzel and Likens (1990)
Granulometry (%)	Rot-up procedure	Suguio (1973)
Redox potential sediment (mV)	Platinum electrodes	APHA (1989)
Salinity water (psu)	Refractometer	Littlepage (1998)
Ammonia-N water (µmol/L)	Spectrophotometer	Grasshoff et al., 1983

I.2.3. Statistical analysis

Analyses were performed using univariate and multivariate techniques. The distribution and composition of macrobenthic communities in relation to mesh sieve and sampling depth were ascribed in terms of abundance (N), biomass (g AFDW) and density (individuals/m²). Univariate indices such as number of species (S), Shannon diversity index (H' $\log_e$) and evenness (J') were also presented using the PRIMER v6.0 software package (Clarke and Gorley, 2006).

Analysis of Variance (two-way ANOVA) was carried out to determine whether diversity (as measured by Hill's diversity index N_1 or exp=H') varied with the sieves (1.0mm x combined sieves) and also with the sampling depths (0-10cm x 0-20cm) in the macrobenthic samples. N_1 values were chosen instead of H' by the fact that the Hill's index seems to allow a better distribution of diversity values when compared to Shannon index, which "is so narrowly constrained in most circumstances can make interpretation difficult" (Magurran, 2004). Results for each interaction (mesh-sieves x sites and sampling depths x sites) were treated separately. In order to check the assumption of homoscedasticity, Bartlett's tests were applied. When significant differences among sites in relation to each factor (sieves/sampling depths) were found, *a posteriori* comparisons were performed using LSD tests (Sokal and Rohlf, 1997). The analyses of variance, Bartlett's and LSD tests were calculated using STATISTICA v5.0 computer program.

For macrofauna comparison and descriptive purposes, multivariate techniques followed the standard methods according to Clarke and Warwick (1994). In order to reduce the clumping effect of some numerically dominant species, abundances were weighted by means of dispersion index (Di) of each species per sample (Clarke et al., 2006). Weighted abundance data were log (x+1) transformed and resemblances were calculated by Bray-Curtis similarity measure. Multidimensional scaling (MDS) and Analysis of Similarity (two-way ANOSIM) were used to plot differences among sites for quantitative data of macrofauna and to test for “mesh-sieve” and “sampling depth” factors effects (also separately) respectively. When significant differences among sites for the macrobenthos communities in relation to each factor (sieves/sampling depths) were found, the Similarity Percentages procedure (two-way SIMPER) (Clarke, 1993) was applied to point out the taxa which contribute to distinguish each factor within the sites. MDS, ANOSIM and SIMPER were also conducted with PRIMER v6.0 software (Clarke and Gorley, 2006). Pearson correlation analysis was used to evaluate the association between sedimentological parameters and “changes” in the community structure due to the factors (only when there were significant differences). To verify these “changes” the following procedure was made: for each factor (sieves/sampling depths), the similarity matrix was obtained and values of similarity for each replicate (from each site) between factor levels (sieves: “1.0mm” and “combined sieves”/ sampling depths: “0-10cm” and “0-20cm”) were extracted from the matrix. After that, the average dissimilarity for each site was calculated, being used in Pearson correlation analysis (Average Dissimilarity = 100-Average Similarity). Pearson correlation analysis was performed in BIOESTAT v5.0 (Ayres et al., 2007).

All statistics statements were based on a significance level of $\alpha = 5\%$.

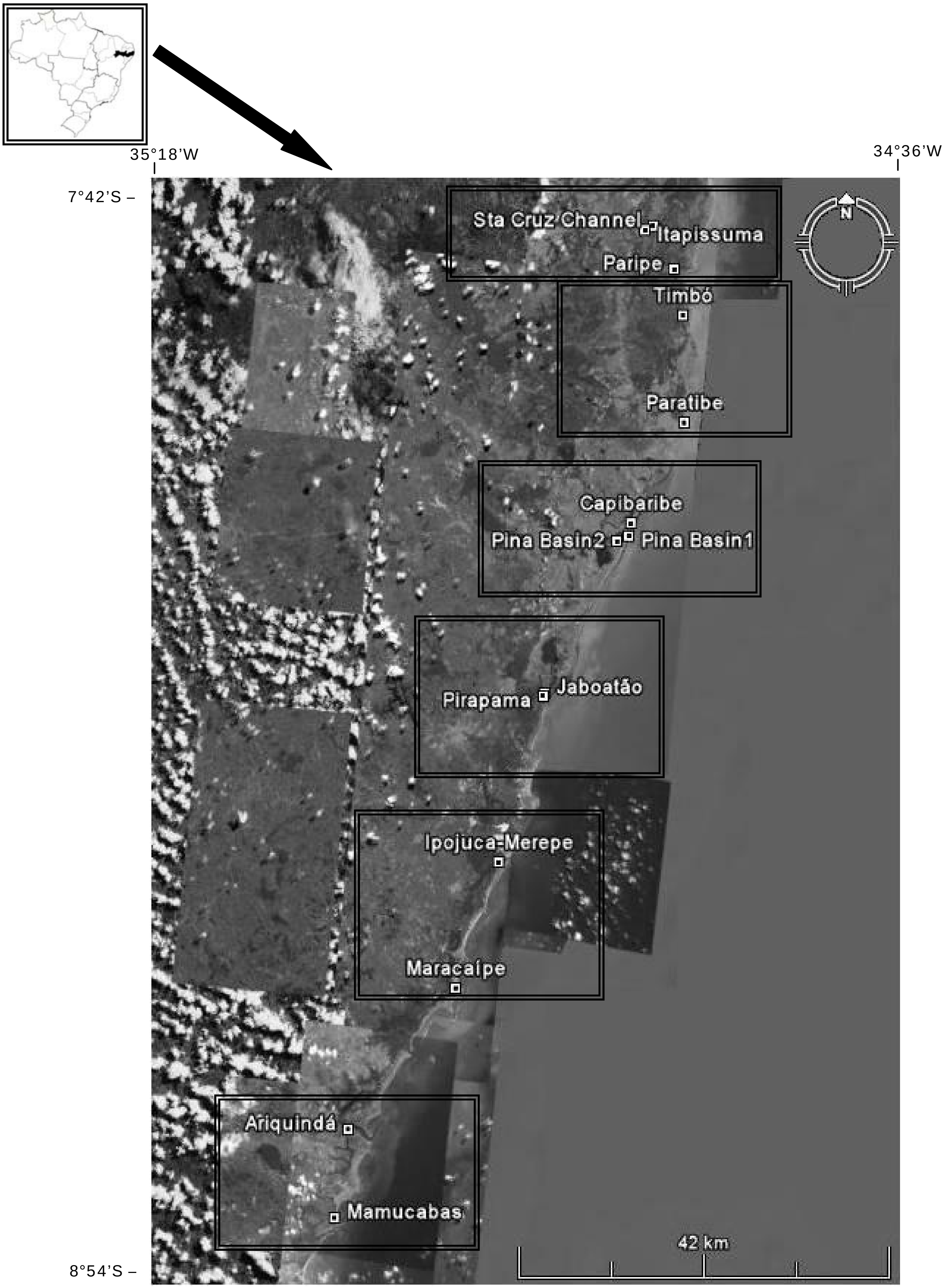
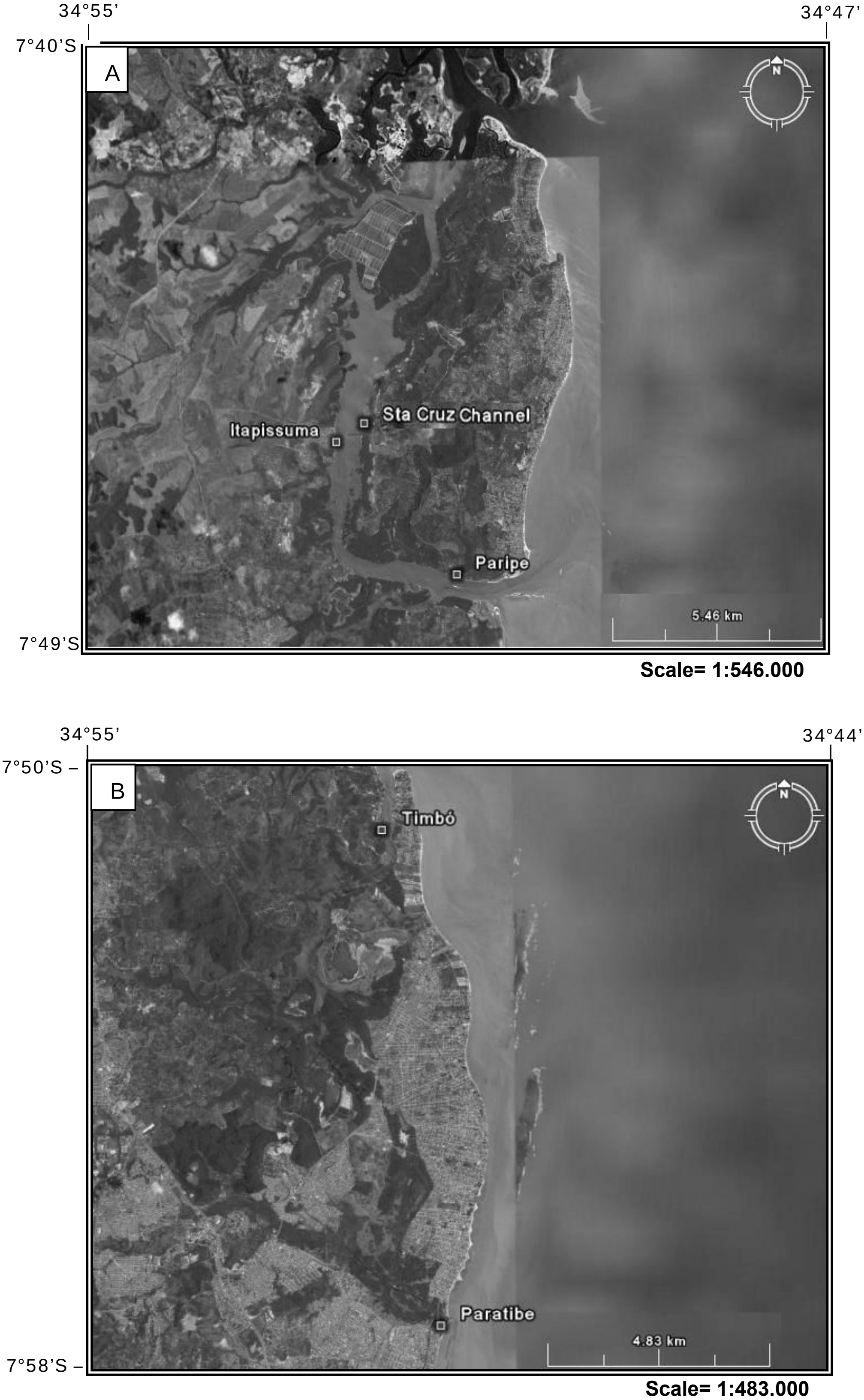


Figure 01. Location of studied areas (sites) along Pernambuco coastline (Northeast Brazil). Scale= 1:4.200.000



34°55'
34°47'
7°40'S
A
N
Itapissuma
Sta Cruz Channel
Paripe
5.46 km
7°49'S
Scale= 1:546.000
34°55'
34°44'
7°50'S
B
N
Timbó
Paratibe
4.83 km
7°58'S
Scale= 1:483.000

Scale= 1:285.100

Scale= 1:182.300

Figure 02. Studied areas (sites) represented in a more close view (letters A-F).

I.3. Results

I.3.1. Environmental data

The fourteen sites presented variations in all abiotic data (tab.02): Salinity values ranged between 8 (in Mamucabas) and 40 psu (in Maracaípe), being the majority of sites sampled in polyhaline/euhaline zones. Nitrogenous compounds were differently distributed in water and sediment: Paratibe and Jaboatão showed high ammonia-N values in their waters while, in sediment, the Total-N concentrations were superior in Pina Basin2 and Sta Cruz Channel. Although the sand fraction have predominated in sediment composition of all sites, the fine fraction (%silt-clay) was important in Timbó estuary; despite that, sediment properties (represented as median grain size) presented homogeneous distribution among sites. Negative values were registered for redox potential in all sites, ranging from -169mV (in Itapissuma) to -378mV (in Pina Basin2). In the latter area, organic matter content values were one of the highest, together with Capibaribe and Sta Cruz Channel.

Table 02. Mean values (±SD) of environmental data registered for the studied areas.

Studied areas (sites)	**Water parameters**		**Sediment parameters**					
	Salinity (psu)	Ammonia-N (µmol/L)	Total-N (g/kg)	Sand fraction (%)	Fine fraction (%)	Median grain size (phi)	Organic matter (%)	Redox potential (mV)
Itapissuma	35	0.440	0.48 (±0.36)	97.59 (±0.83)	1.61 (±0.83)	1.84 (±0.07)	1.96 (±1.27)	-169.1 (±92.90)
Sta Cruz Channel	33	0.127	3.08 (±0.98)	98.38 (±1.49)	2.37 (±1.44)	2.17 (±0.32)	14.91 (±3.72)	-320.8 (±28.72)
Paripe	35	0.059	1.34 (±0.51)	97.50 (±0.52)	2.48 (±0.50)	2.23 (±0.18)	8.22 (±2.23)	-255.7 (±84.04)
Timbó	37	1.843	0.74 (±0.32)	78.71 (±1.36)	21.01 (±1.37)	3.27 (±0.13)	5.93 (±1.91)	-300.7 (±82.34)
Paratibe	15	7.292	1.38 (±1.45)	97.55 (±1.14)	2.42 (±1.12)	1.89 (±0.08)	7.42 (±6.68)	-300.8 (±100.06)
Capibaribe	18	3.350	2.46 (±0.35)	94.91 (±1.60)	4.82 (±1.49)	1.65 (±0.15)	15.05 (±1.26)	-271.1 (±105.99)
Pina Basin1	27	3.997	1.16 (±0.09)	94.39 (±0.73)	5.54 (±0.72)	2.69 (±0.06)	8.13 (±1.40)	-240 (±67.96)
Pina Basin2	20	2.839	3.04 (±0.82)	97.11 (±1.63)	2.83 (±1.58)	1.66 (±0.44)	14.89 (±3.11)	-378.1 (±31.95)
Jaboatão	18	6.311	0.84 (±0.36)	95.87 (±2.74)	3.99 (±2.54)	2.27 (±0.11)	3.69 (±2.17)	-345.3 (±60.25)
Pirapama	12	1.219	1.58 (±0.31)	95.85 (±1.97)	4.11 (±1.96)	2.29 (±0.29)	7.58 (±1.98)	-272.5 (±81.59)
Ipojuca-Merepe	29	0.0001	1.00 (±0.46)	95.00 (±2.66)	4.94 (±2.69)	1.99 (±0.28)	6.95 (±2.84)	-283.2 (±57.06)
Maracaípe	40	0.165	0.98 (±0.58)	95.76 (±1.75)	4.16 (±1.73)	2.64 (±0.47)	4.91 (±1.96)	-304.6 (±74.71)
Ariquindá	35	0.056	1.48 (±0.29)	89.30 (±2.24)	10.59 (±2.19)	2.96 (±0.18)	9.69 (±2.66)	-296.4 (±84.19)
Mamucabas	08	0.183	0.60 (±0.57)	97.72 (±1.86)	2.25 (±1.85)	1.73 (±0.12)	8.52 (±13.23)	-285.6 (±109.11)

I.3.2. Microphytobenthos

Sediment chlorophyll-*a* and phaeopigments concentrations presented both the highest values in Pina Basin2. Among the sites, the sediment chlorophyll-*a* biomass varied between 6.06 (in Jaboatão) and 70.21µg/cm² whilst phaeopigments, from 5.86 (in Mamucabas) to 98.75µg/cm² (fig.03).

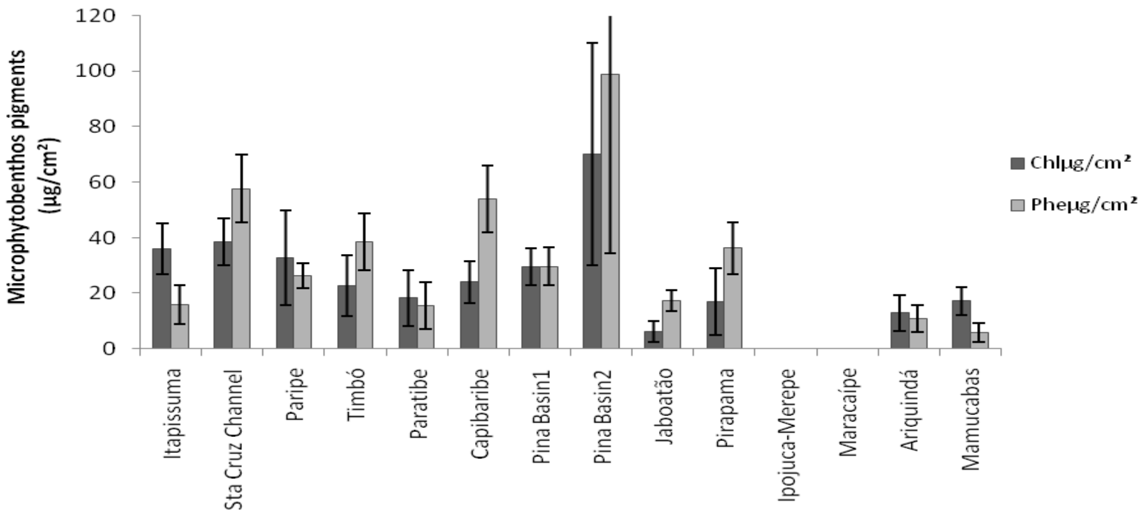


Figure 03. Mean values (±SD) of sediment chlorophyll-*a* (Chlµg/cm²) and phaeopigments (Pheµg/cm²) biomass registered in the studied areas (data for Ipojuca-Merepe and Maracaípe sites are missing).

I.3.3. Macrobenthic distribution and composition

In the present study, a total of 14,257 individuals comprising 78 taxa were identified mainly belonging to annelids. Polychaeta was the most abundant (60% in 1.0mm and 48% in combined sieves) followed by Oligochaeta (31% and 35%, respectively) and Nematoda (6% and 15%), being the other groups represented by less than 3%. Despite their recognized numerical dominance in meiobenthos, Nematoda retained in “macrobenthic sieves” (1.0+0.5mm) were responsible for almost 50% of Paripe and Sta Cruz Channel community abundances; besides, the presence of this taxon was registered in Paratibe, Capibaribe, Pina Basin1, Jaboatão, Pirapama and Mamucabas only with the use of the finer mesh sieve. The relative contribution of taxonomic groups within each site differed depending on the considered mesh size especially in Ipojuca-Merepe and Maracaípe, where the high dominance of Polychaeta observed in 1.0mm was replaced by Oligochaeta with the combined sieves (fig.04).

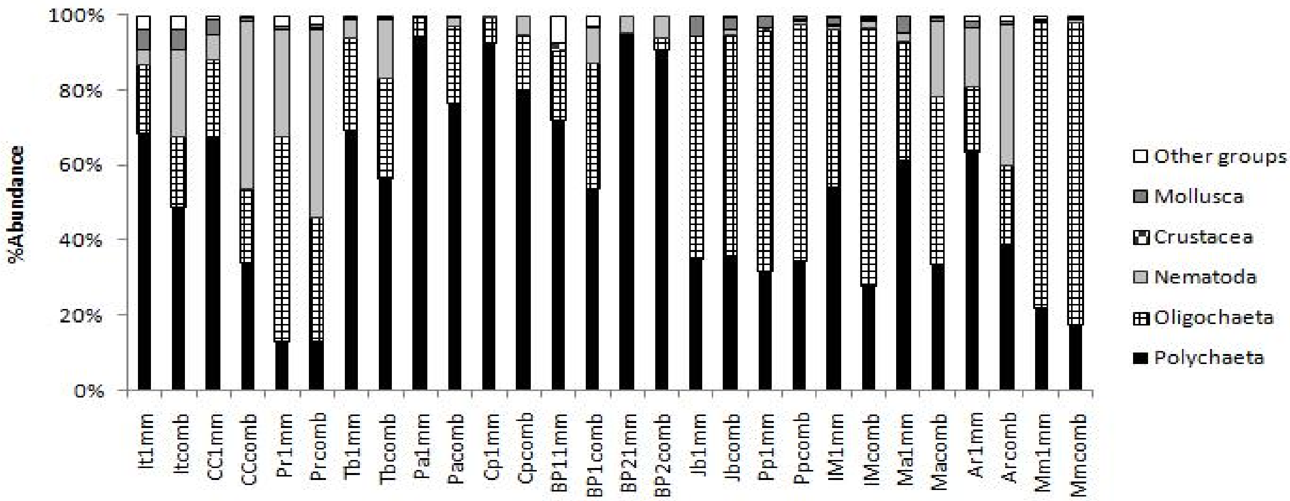


Figure 04. Distribution of major taxonomic groups (%abundance) of macrobenthic fauna data in studied areas considering the sieve mesh sizes. Legend: Sieves 1.0mm (1.0mm) and combined (comb). Sites: Itapissuma (It); Sta Cruz Channel (CC); Paripe (Pr); Timbó (Tb); Paratibe (Pa); Capibaribe (Cp); Pina Basin1(BP1); Pina Basin2 (BP2); Jaboatão (Jb); Pirapama (Pp); Ipojuca-Merepe (IM); Maracaípe (Ma); Ariquindá (Ar); Mamucabas (Mm).

Tubificidae species, nematodes, the polychaetes *Streblospio* sp., *Laeonereis* sp., *Capitella* sp., *Mediomastus* sp. and unidentified Nereididae made up over than 90% of the total abundance. In general, densities ranged from three to five times more for both sieves compared to 1.0mm, except from Pina Basin1, where the difference increased almost by an order of magnitude. Considering the studied areas, it was observed that most macrobenthic animals in combined sieves were mainly concentrated in four sites: Paripe (12.85%), Timbó (12.56%), Paratibe (11.73%) and Capibaribe (11.45%). In these areas, densities attained values equal or superior to 80,000 ind/m²; by contrast, Ariquindá was the only estuary that registered less than 10,000 ind./m² (fig.05A).

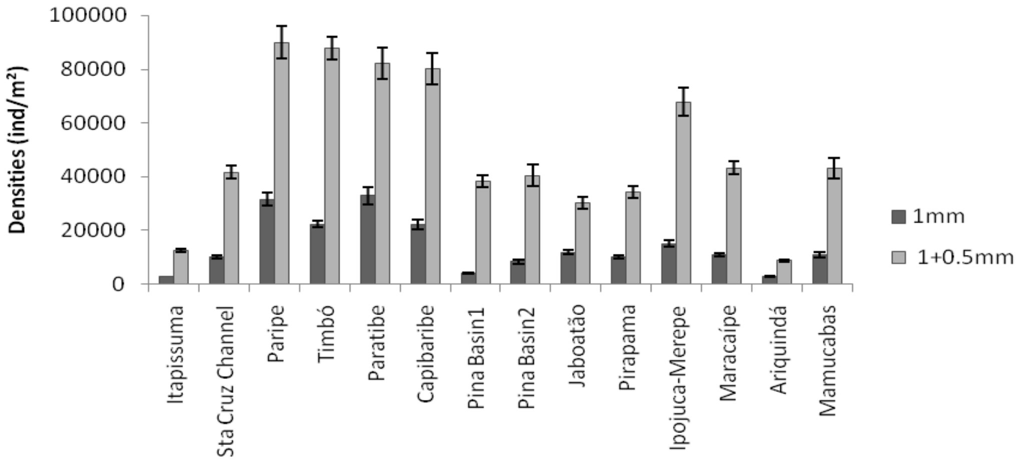


Figure 05A. Density values (±SD), expressed in number of individuals/m², of macrobenthic fauna data in studied areas considering the sieve mesh sizes.

Conversely, most areas showed little evidence of shifts in the vertical distribution of densities (fig.05B) and individual species, excepting in Mamucabas, where Tubificidae species were well-represented in both 0-10cm and 10-20cm strata.

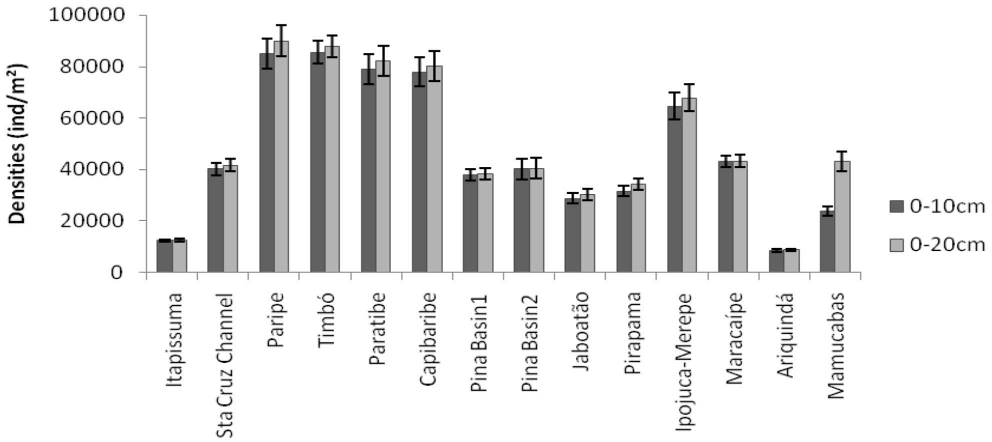


Figure 05B. Density values (±SD), expressed in number of individuals/m², of macrobenthic fauna data in studied areas considering the sampling depths.

Concerning biomass, Mollusca composed more than 73% of overall (total biomass = 83.64g AFDW), especially due to the high contribution of bivalves *Macoma* sp., *Lucina* sp. and *Anomalocardia brasiliana*, being the values of total biomass also concentrated in four sites: Jaboatão (25.67%), Ipojuca-Merepe (23.17%), Timbó (21.22%) and Pirapama (8.92%). Values ranged from 0.17g (1.0mm) – 0.30g (combined sieves) in Pina Basin2 to 21.24g (1.0mm) – 21.47g (combined sieves) in Jaboatão (fig. 06A). Even though the great dominance of mollusks' biomass has been evident in half of sites, in Capibaribe, Pina Basin1, Pina Basin2, Itapissuma, Mamucabas and Ariquindá polychaetes' biomass values were higher and little affected by the mesh size.

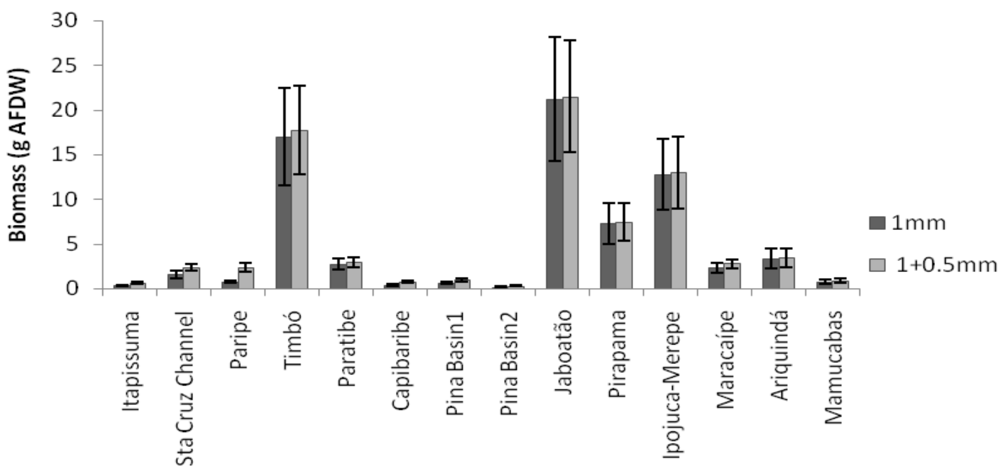


Figure 06A. Biomass values (±SD), expressed in gAFDW, of macrobenthic fauna data in studied areas considering the sieve mesh sizes.

The vertical macrofauna biomass-data stayed nearly constant in some sites, but in Timbó, Paratibe, Jaboatão, Pirapama and Ariquindá increased in different proportions with the depth (fig.06B).

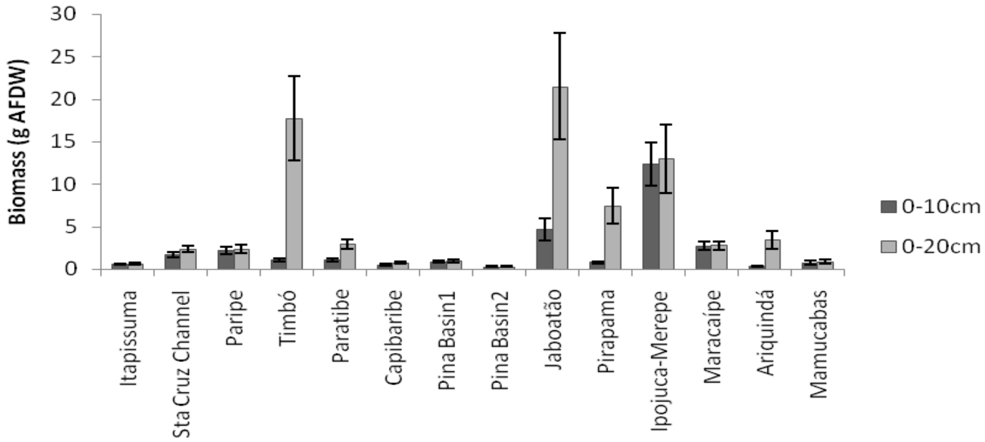


Figure 06B. Biomass values (±SD), expressed in gAFDW, of macrobenthic fauna data in studied areas considering the sampling depths.

In terms of univariate measures, evenness (J') and diversity (H') (fig.07A,B) followed a similar pattern of distribution among the sites, however, in Itapissuma, Paripe and Jaboatão this trend wasn't maintained when sieves were considered and in Itapissuma, Timbó, Paratibe and Pina Basin1, when compared the layers (0-10cm and 0-20cm). High values of diversity and evenness were found in Ariquindá (sieve:1.0mm; depth:0-20cm) and Itapissuma (combined sieves; 0-20cm) and, the minima, in Paratibe (sieve:1.0mm; depth:0-10cm) and Pina Basin2 (combined sieves; 0-10cm), respectively, which corresponded to about three times less than those recorded for the former.

As might be expected, richness increased down to the 0.5mm mesh, but remained invariable in Capibaribe (fig.07C). Since no major changes were reported in macrofaunal numbers regarding the sampling depth, its effect had little influence in these univariate indices, especially richness.

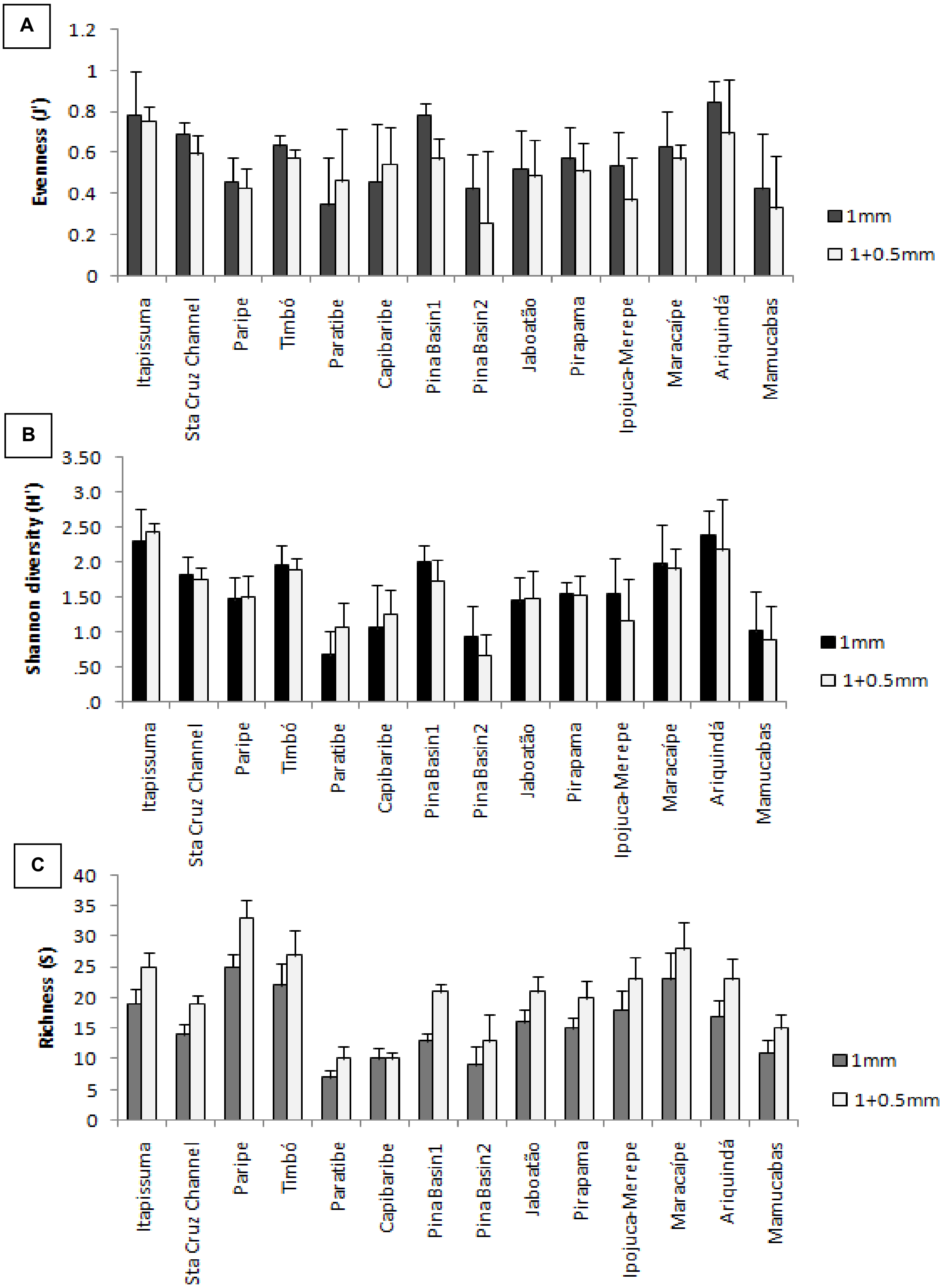


Figure 07. Univariate measures: [A] evenness(J'), [B] Shannon diversity(H'loge) and [C] richness(S) of macrobenthic data in studied areas considering the sieve mesh sizes.

I.3.4. Macrobenthic community pattern: sieve mesh x sampling depth

The overall retention efficiency showed that differences between 1.0mm and combined sieves are hardly evident for biomass but they are very important for the abundance. The 1.0mm sieve retained only 28% of total individuals but, at the same time, was responsible for 92% of total biomass. Among the sites, retention of 1.0mm varied from 10.69% in Pina Basin1 to 39.98% in Paratibe for abundance and from 33.35% in Paripe to 99.72% in Ariquindá for biomass (fig. 08).

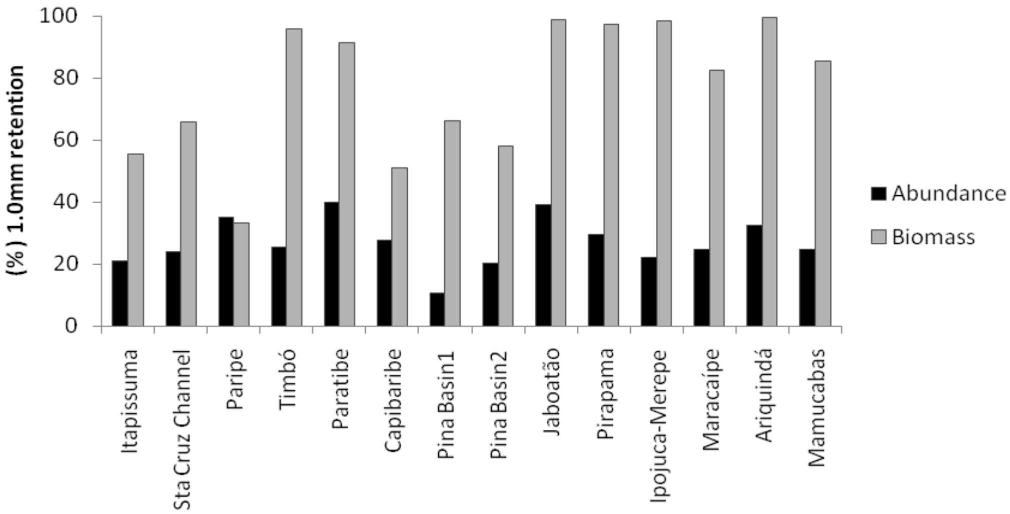


Figure 08. Percentages of abundance and biomass considering the 1.0mm sieve's retention in the studied areas.

High retention efficiency in 1.0mm sieve was recorded for crustaceans (>87%) and mollusks (>60%); on the contrary, retention was relatively low for polychaetes (35%); oligochaetes (24.75%), nemertines (41.38%), sipunculans (45.71%) and phoronides (16.67%) (fig.09).

Regarding sampling depth, the top layer (0-10cm) presented almost all individuals (94%); however, contributed with only 39.06% of the overall biomass. Among sites, except for Mamucabas, this layer comprised more than 92% of the abundance in each area whereas biomass values in 0-10cm ranged from 6.04% in Timbó to 97.69% in Maracaípe (fig. 10).

The bottom layer (10-20cm) showed to be important in the biomass values of mollusks (79.67%), sipunculans (86.76%) and phoronides (65.91%). Conversely, crustaceans, chironomids, small flatworms and the majority of nematodes (97%) were found in the first ten-centimeter layer (fig.11).

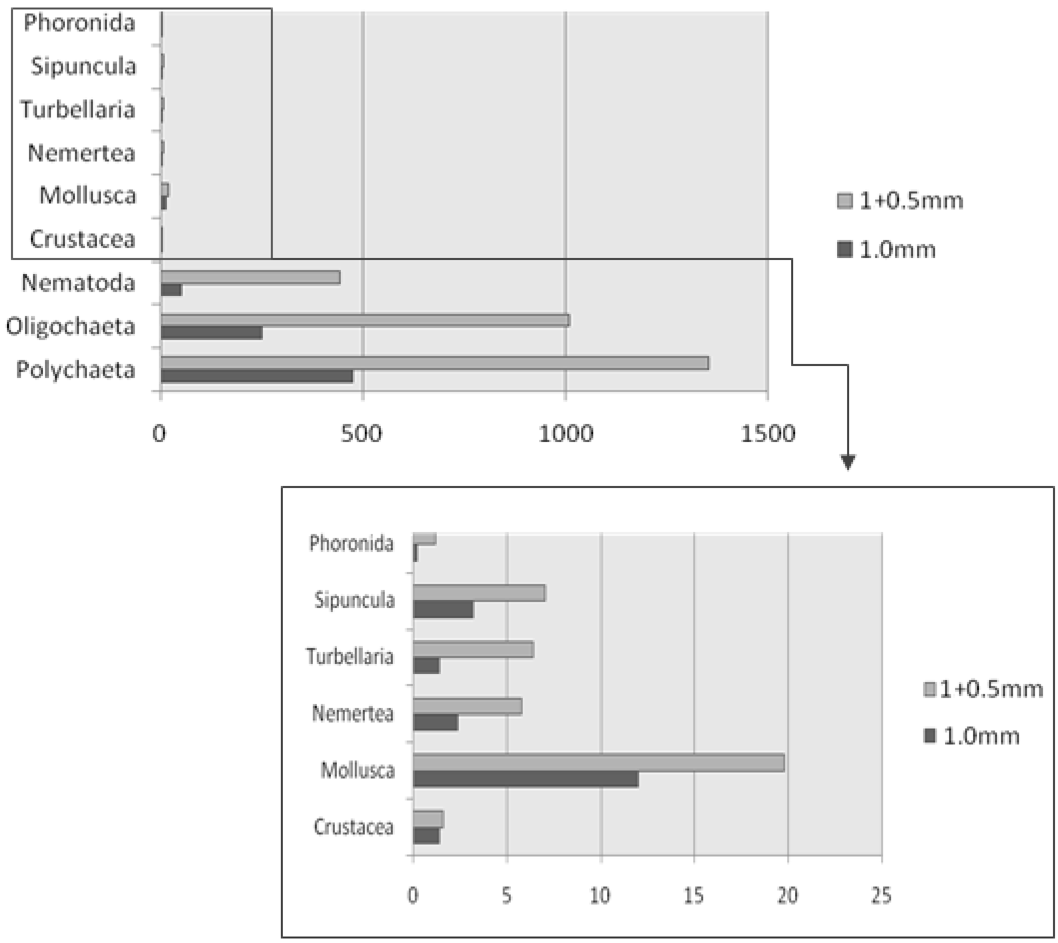


Figure 09. Abundance values (number of individuals) of major taxonomic groups considering sieves retention (1.0mm and 1+0.5mm) in the studied areas.

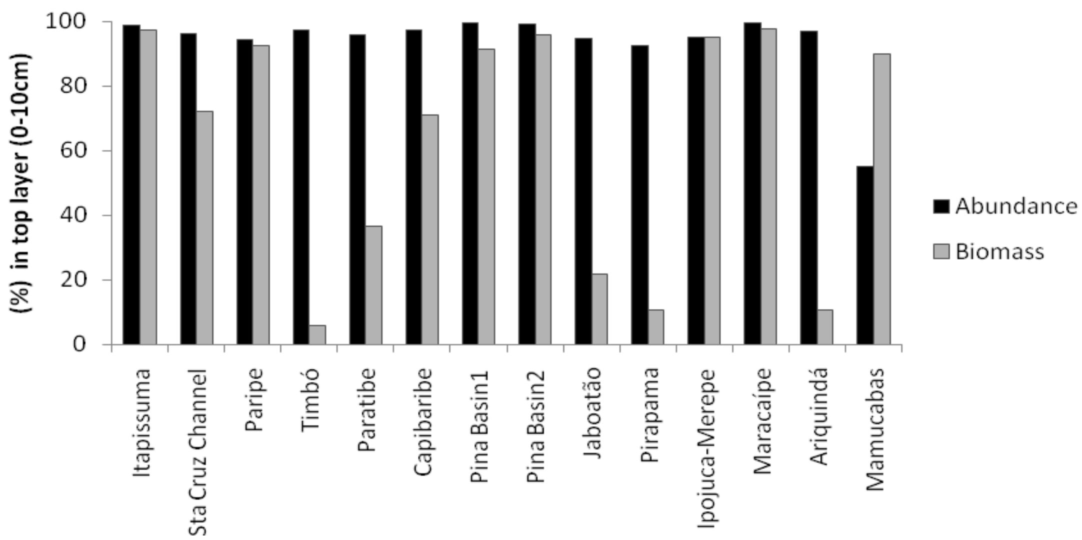


Figure 10. Percentages of abundance and biomass values considering the top layer (0-10cm) fauna in the studied areas.

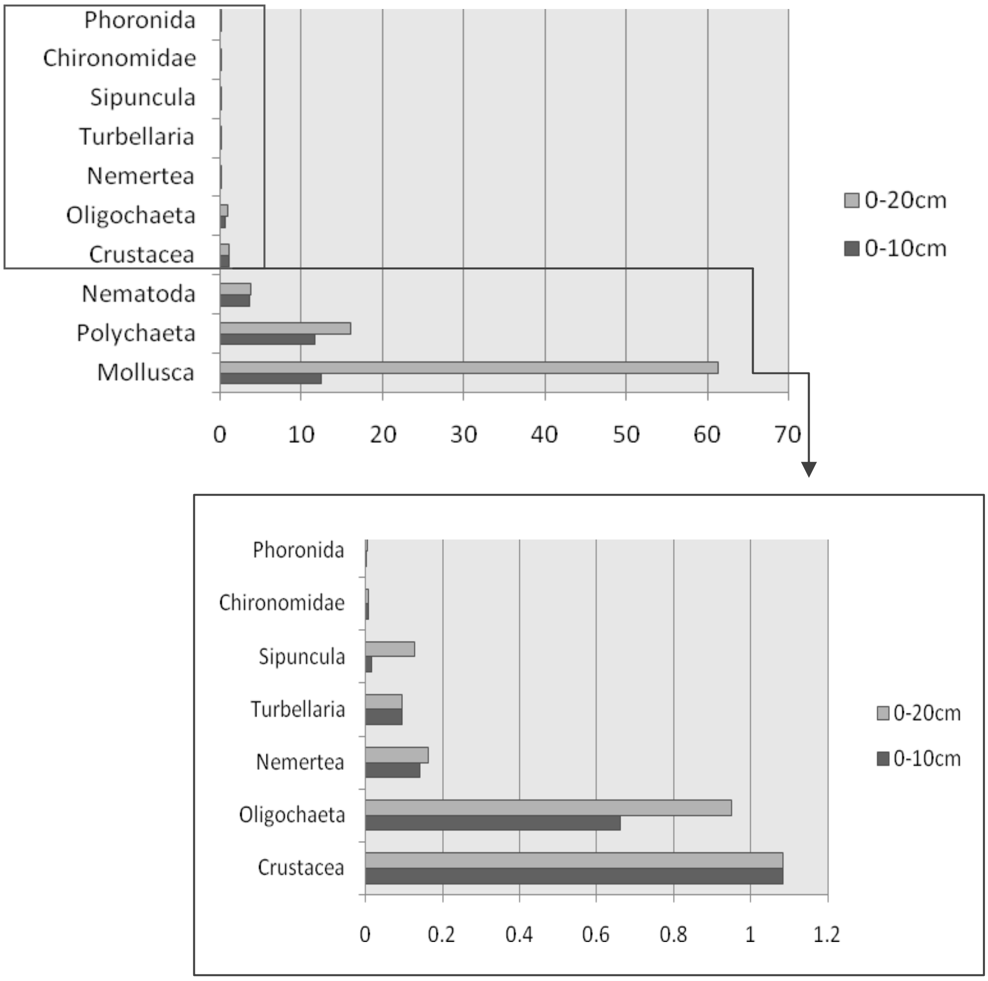


Figure 11. Biomass values (gAFDW) of the major taxonomic groups considering sampling depth (0-10cm and 0-20cm) in the studied areas.

Although significant interaction between sites and sieves have been revealed by the two-way ANOVA for Hill's index N_1 ($F_{1;13}$=5.17; p=0.02), with the exception of Itapissuma, the diversity of macrofauna retained in 1.0mm did not differ when compared to combined sieves (LSD test between sieves for each site, tab. 03).

Following diversity, similarity analysis (two-way ANOSIM) pointed out differences for community between the sieves (R_{global}= 0.142; p=0.002) and among sites (R_{global}= 0.654; p=0.001), well-represented in MDS (fig.12). Conversely, within the sites, it was observed that the use of 0.5mm brought no significant alteration for communities' structure in most sites, excluding Paratibe (R_{global}= 0.456; p=0.024) and Sta Cruz Channel (R_{global}= 0.664; p=0.008). In Paratibe, SIMPER analysis (tab.04) demonstrated that differences between sieves were due to the polychaetes *Laeonereis* sp, *Capitella* sp and *Neanthes* sp; oligochaetes Tubificidae; nematodes and the crustacean *Uca* sp; whilst in Sta Cruz Channel, apart from nematodes, the main taxa which contributed to distinguish the fauna retained in the sieves were the polychaetes *Exogone* sp, *Podarke* sp and *Sigambra* sp; the oligochaete *Tectidrilus* sp and the bivalve *Corbula* sp.

Table 03. Hill's diversity index (N_1) of each site in 1.0mm and combined sieves and significance levels of the two-way ANOVA testing the effect of sieve in diversity values (LSD test post hoc comparisons). Bold *p* values: significant differences.

Studied areas (sites)	**Diversity (N_1)**		**Two-way ANOVA (post-hoc LSD test)**
	1.0mm	Combined sieves	*p* values
Capibaribe	3.122	3.255	0.8866
Pina Basin1	4.593	4.690	0.9165
Pina Basin2	1.694	1.683	0.9908
Paratibe	2.009	2.691	0.4644
Timbó	5.450	5.930	0.6062
Jaboatão	3.601	3.993	0.6739
Pirapama	3.730	3.935	0.8258
Sta Cruz Channel	4.704	5.372	0.4736
Paripe	3.792	3.708	0.9285
Itapissuma	4.621	8.040	**0.0004**
Mamucabas	3.264	3.089	0.8511
Ariquindá	4.131	5.565	0.1255
Ipojuca-Merepe	3.701	3.537	0.8602
Maracaípe	4.549	5.378	0.3743

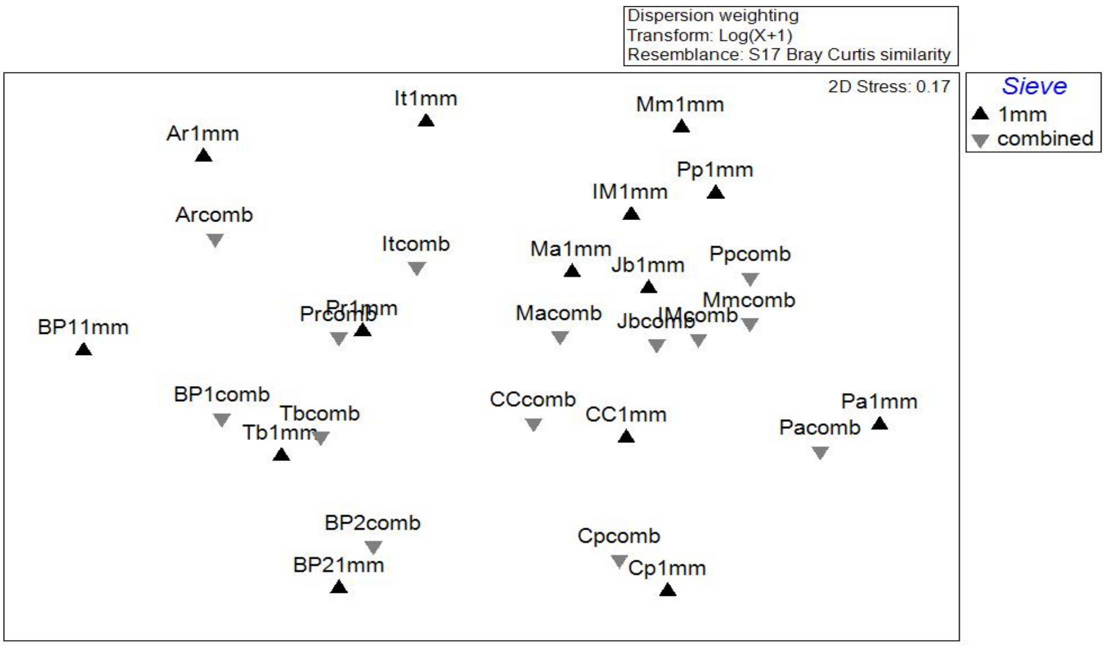


Figure 12. Multidimensional scaling (MDS) plot of sampling similarities in each site in relation to the sieves. Legend: Sieves 1.0mm (1.0mm) and combined (comb). Sites: Itapissuma (It); Sta Cruz Channel (CC); Paripe (Pr); Timbó (Tb); Paratibe (Pa); Capibaribe (Cp); Pina Basin1(BP1); Pina Basin2 (BP2); Jaboatão (Jb); Pirapama (Pp); Ipojuca-Merepe (IM); Maracaípe (Ma); Ariquindá (Ar); Mamucabas (Mm).

Table 04. SIMPER analysis with taxa contribution to dissimilarity within the sites considering the use of 1.0mm (1.0mm) and combined sieves (comb).

Studied Areas	Taxa	%Contribution	%Cumulative Total
Paratibe	*Laeonereis* sp.	24.21	24.21
($Pa_{1.0mm} \rightarrow Pa_{comb}$)	*Capitella* sp.	21.82	46.03
	oligochaetes Tubificidae	19.87	65.90
	Nematoda	11.19	77.09
	Neanthes sp.	8.34	85.44
	Uca sp.	6.21	91.65
Sta Cruz Channel	Nematoda	20.69	20.69
($CC_{1.0mm} \rightarrow CC_{comb}$)	*Exogone* sp.	12.40	33.09
	Tectidrilus sp.	8.89	41.98
	Corbula sp.	8.51	50.49
	Podarke sp.	7.21	57.50
	Sigambra sp.	6.80	64.50

No significant difference was observed for diversity values (N_1) regarding the sampling depth for the studied areas ($F_{1;13}$=0.068; p>0.05). The same pattern was observed for the whole community, according to two-way ANOSIM (R_{global}= -0.188; p>0.05) and MDS plot (fig.13) results. The analysis also indicated that the sites became more distinct for the factor "sampling depth" (R_{global}= 0.743; p=0.001) when compared to "sieves" (R_{global}= 0.654; p=0.001) for abundance, being the opposite for biomass ("sampling depth" R_{global}= 0.367; p=0.005; "sieves" R_{global}= 0.935; p=0.001). Due to the low number of variables (major taxonomic groups) used to construct the similarity matrices, the results for biomass have not separated the individual sites for each factor.

The factor "sieves" was the only which showed significant differences for the macrobenthic community and Pearson's correlation between the average dissimilarity from each site and the subset of environmental variables have selected the total-N (r=0.860; p<0.0001), organic matter (r=0.801; p<0.001) and microphytobenthos (r=0.749; p=0.005 and r=0.795; p=0.002, for chlorophyll-*a* and phaeopigments, respectively) as the most fit for explaining the "changes" in fauna from 1.0mm to combined sieves.

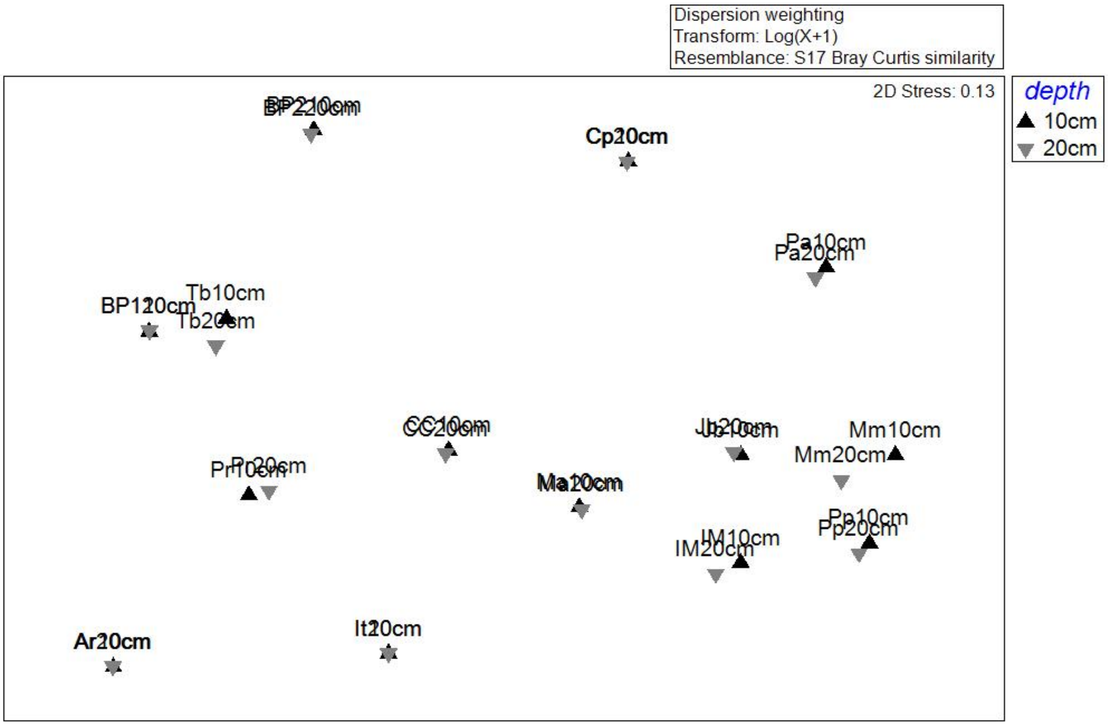


Figure 13. Multidimensional scaling (MDS) plot of sampling similarities in each site in relation to the sampling depth. Legend: sampling depth 0-10cm (10cm) and 0-20cm (20cm). Sites: Itapissuma (It); Sta Cruz Channel (CC); Paripe (Pr); Timbó (Tb); Paratibe (Pa); Capibaribe (Cp); Pina Basin1(BP1); Pina Basin2 (BP2); Jaboatão (Jb); Pirapama (Pp); Ipojuca-Merepe (IM); Maracaípe (Ma); Ariquindá (Ar); Mamucabas (Mm).

I.4. Discussion

In this study, different procedures for describing spatial structure patterns of tropical estuarine macrobenthos were tested and revealed that biological material processed with different mesh sizes and sampling depths can influence estimates of parameters from these communities. Indeed, the use of 1.0mm or 0.5mm mesh had a more significant effect on distribution rather than composition of taxa. In both sieves, macrobenthic communities from the studied areas were numerically dominated by annelids – particularly polychaetes – which is commonly the more abundant and important taxon in tropical and subtropical estuarine macrofauna, as reported by several authors (Maurer and Vargas, 1984; Alongi, 1990; Frouin, 2000; Dittmann, 2001; Paiva, 2001; Ingole et al., 2002; Lancelloti and Sotz, 2004; Bigot et al., 2006; Jayaraj et al., 2008). Besides, the presence of the polychaetes *Streblospio* sp., *Laeonereis* sp., *Capitella* sp. and *Mediomastus* sp. together with Tubificidae oligochaetes and nematodes were shown to be typical of estuarine environments in Brazilian estuaries (e.g. Bemvenuti et al., 2003; Nalesso et al., 2005; Pagliosa and Barbosa, 2006; Rosa-Filho et al., 2006; Barros et al., 2008).

Conversely, biomass data showed that Mollusca dominated the communities' composition. Biomass values derived from both sieve fractions within sites were similar, but the occurrence of deep burrowing adults of *Macoma* sp., *Lucina* sp. and *Anomalocardia brasiliana* in the 10-20cm stratum made important the differences between the sampling depths in some areas. Moreover, these species not only represented together less than 1% of total abundance (30 individuals), but they also accounted for over than 60% of total biomass, which indicated that macrofauna was clearly dominated in numbers by small specimens.

Some studies have shown that, for comparison of communities with univariate indices, differences in species richness and diversity can be found between samples processed with 1.0mm and 0.5mm (Bachelet, 1990; Tanaka and Leite, 1998). In Pernambuco estuaries, the 1.0mm mesh retained more than 70% of species richness and 63% of diversity values, indicating that this is not a general trend. Besides, other authors suggest that the mesh size used would not interfere, as both sieves would sample similarly in terms of species composition (James et al., 1995; Schlacher and Wooldridge, 1996b).

Considering the mesh efficiencies for macrobenthos sampling, the extra information added by sieving with both mesh sizes (1.0+0.5mm) had importance only for total abundance. Retention efficiency had been reported for 1.0mm sieve when compared to abundances retained by the combination of sieves (tab.05) and will diverge depending on the geographic area, habitat and benthic assemblage sampled.

Regardless of study area or benthic samples, comparative surveys on retention efficiency indicate that crustaceans, polychaetes and bivalves usually show decreasing selection, in this order, for the same mesh sieve (Bachelet, 1990; Tanaka and Leite, 1998; Lampadariou et al., 2005). In this study, while the few crustaceans present (<10 individuals) were observed in the 1.0mm sieve, the bivalves (which corresponded to 92% of mollusks) were more retained in this sieve rather than polychaetes, following a different order of selection (crustaceans>bivalves>polychaetes). Such results are in accordance with those from Rodrigues et al. (2007) for Tagus estuary (Portugal), where high abundance of *Streblospio shrubsolii* was responsible for polychaetes' lower retention in 1.0mm sieve. In the present study, the same occurred due to the species of *Streblospio* sp., *Capitella* sp., *Mediomastus* sp. and juveniles of *Laeonereis* sp.

Table 05. Retention efficiency (%) registered for macrobenthic community studies using 1.0 and 1.0+0.5mm (combined) sieves.

Area	Habitat	%Retention efficiency	References
Gironde Estuary (France)	estuarine zone	15-30%	Bachelet, 1990
Southern California Bight (USA)	coastal zone	40%	Ferraro et al., 1994
East coast of New South Wales (Australia)	shelf sand bodies	54%	James et al., 1995
Gamtoos Estuary (South Africa)	estuarine zone	8%	Schlacher and Wooldridge, 1996b
Northern coast of São Paulo (SE Brazil)	rocky shores (macrophyte-associated macrofaunal assemblage)	27%	Tanaka and Leite, 1998
Feni Ridge, Rockall Trough (NE Atlantic)	deep-sea zone	19%	Gage et al., 2002
Casey Station (Antarctica)	coastal zone	70%	Thompson et al., 2003
Eastern Mediterranean	coastal zone (marine cage fish farms)	62%	Lampadariou et al., 2005
Pernambuco (NE Brazil)	**estuarine zone**	**28%**	**present study**

Even though significance has been established for mesh sieves and sites in relation to diversity N_1 and overall community structure, no important alteration was observed in macrobenthic samples for most sites, which meant that in these areas the great contribution of the 0.5mm was only in the addition of more specimens rather than more species. Other observations seem to corroborate the above findings (e.g. James et al., 1995; Schlacher and Wooldridge, 1996b; Gage et al., 2002; Rodrigues et al., 2007). In some cases, given that relatively higher costs are associated with finer screens, sampling protocols employing 1.0mm sieves can be more cost-effective for characterizing the macrobenthic community (Ferraro et al. 1994, 2006).

On the other hand, the macrobenthic communities were mainly composed by smaller and pollution indicator species, thus it is possible that losses of information necessary to detect impacts in pollution assessment would occur if 1.0mm sieve was used instead of a 0.5mm. Considering that most sites presented evidence of disturbance (from different sources, see description of studied areas in the Material and Methods section), and that the sediment parameters (organic matter, total-N and microphytobenthos biomass) selected in the correlation analyses are frequently associated to organic enrichment conditions, it becomes fundamental using the material retained in the 0.5mm sieve in studies focusing this theme.

Concerning the sampling depth, the importance of the upper centimeter layers for the taxa composition and abundance structure of benthic communities is widely reported (Weston, 1990; Flach and Heip, 1996; Dauwe et al., 1998; Flach et al., 1998; Ingole et al., 1999; Wei, 2006). In fact, over 90% of the macrofauna specimens and taxa were presented in top layer (0-10cm). Flach and Heip (1996) and Flach et al. (1998) showed that in deep-sea areas of NE Atlantic, macrobenthic fauna concentrated in the first centimeter (40-80% and 25-59%, respectively); Gutiérrez et al (2000) observed in the continental shelf of Central Chile that 40-80% of macrofauna was in 0-2cm stratum while 20-40%, in 2-5cm. In estuaries, the proportion can be even higher: Mannino and Montagna (1997) pointed out in Nueces estuary (USA) that more than 70% of total abundance was in the 0-5cm stratum while Rodrigues et al. (2007) registered 87% in Tagus estuary (Portugal).

A different pattern was shown in the vertical profile for total biomass, which presented variations in terms of distribution within the sediment strata among areas. In five sites, highest biomass values (63 to 94%) were found bellow the first 10cm, as a result of large deep-dwelling bivalves, sipunculans and phoronides. In these areas, sampling only the upper centimeters of sediment would have missed many large deep-burrowing species that are few in number, but account for most of the total biomass. Weston (1990) demonstrated the same dilemma: 90% of the macrofauna individuals were found in the 0 to 5cm stratum, but 40 to 90% of the biomass was situated in 5-20cm.

The importance of estimation total macrobenthic biomass must be taken into account for pollution-monitoring programmes since most methods for detecting anthropogenic stress include those centered on the primary community structural variables (abundance, species richness and biomass) and its derivation, such as diversity indices, abundance (A/S) and biomass (B/A) ratios (Elliot and Quintino, 2007). Warwick (1986) had suggested that the relation between species abundance and species biomass curves can show pollution-induced condition, known as Abundance-Biomass-Comparisons (or ABC curves). According to Dauer (1993), healthy benthic communities can be characterized by high biomass estimates, dominated by long-lived, often deep-dwelling (e.g. bivalve mollusks, Maldanidae polychaetes, etc.) species and this might indicate a past history of good water/sediment quality situations. Recently, Lampadariou et al. (2008) proposed the size fraction of macrofaunal biomass (Biomass Fractionation Index – BFI) as a monitoring tool to discriminate between impacted and unimpacted sites. This index has advantage in relation to the above metrics since it incorporated measures of total macrofaunal biomass rather than biomass of specific (i.e. sensitive or non-sensitive to disturbance) taxa/groups, although further studies are still necessary.

Macrofauna descriptors (diversity and community structure) identified for total fauna (0-20cm) were similar to those obtained with the analyses (ANOVA, ANOSIM and MDS) based only on the top layer (0-10cm). These results support the importance of top layer for the taxa composition and abundance structure of the benthic community, however, for studies based on biomass, the inclusion of the bottom layer seems to be essential (Rodrigues et al., 2007).

The soft-bottom macrobenthic communities investigated in this study showed that even if sampling with a 1.0mm mesh appears to be adequate to describe biota's composition (based on some sort of cost-benefit analysis), in these estuaries - where macrofauna is mostly composed by small specimens - the use of the 0.5mm sieve will permit a more accurate interpretation of the data. In addition, for taxa composition and abundance, the top layer is clearly the most important whereas for biomass the bottom layer should be considered.

I.5. Conclusion

The requirement for adequate description of benthic communities attributes (abundance, biomass, diversity) has serious implications in making a reasonable assessment of environmental impacts and, consequently, in monitoring programmes. Although the present study is based on a single sampling period and spatially limited, the results shows the influence of both mesh-sieve and sampling depth in characterizing the tropical estuarine macrofauna. It must be highlighted that the investigator needs to decide which and how many response measures will be taken in order to achieve a particular objective. No single protocol will perform optimally for all geographic areas, habitat types and communities, thus, considerable attention should be devoted to methodological planning, including the criteria of choosing a particular sieve or sampling depth as for obtaining better accurate information on ecological interpretation of biota and ecosystems.

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CHAPTER II. Macrobenthic communities in estuarine health assessment on tropical areas (Northeast, Brazil): applying the AZTI Marine Biotic Index (AMBI).

II.1. Introduction

Estuarine and coastal environments are known by their importance in terms of complexity and biodiversity; however, human activities (pollution, tourism, commercial fisheries, eutrophication, sediment discharges, sand extraction and marine transport) have been affecting, directly or indirectly, the biodiversity of these ecosystems on a worldwide-scale (Dauer et al., 2000; Ellingsen, 2001; Kimerer, 2002; Andersen et al., 2006; Borja, 2006; Borja and Dauer, 2008).

The study of marine benthic communities allows the assessment of environmental health of marine and coastal areas (Muniz et al., 2005). Macrobenthic infauna has a fundamental role on sediment processes, providing an important measure of the response of a community to environmental perturbations (Warwick, 1986; Engle et al., 1994; Baldó et al., 1999; Frouin, 2000; Ysebaert and Herman, 2002; Figueroa et al., 2003) and exhibiting the greatest potential for monitoring conditions in a site (Pagola-Carte et al., 2002). Given its inherent ability of incorporating sediment quality (MacFarlane and Booth, 2001; Quintino et al., 2006), a growing number of tools and methods relies on macrobenthic attributes such as species composition, abundance (Labrune et al., 2006), biomass (Lampadariou et al., 2008) and ecological function (Pearson, 2001).

Within this context, biotic indices are very useful tools in decision-making processes (Pinto et al., 2009) since they synthesize complex scientific data into the most straightforward and easy interpretation (Labrune et al., 2006; Chainho et al., 2007; Pontil et al., 2007). One of them, AZTI Marine Biotic Index – AMBI, developed by Borja et al., (2000), is based upon the proportion of species gathered into sensitivity groups to increasing levels of disturbance, from very sensitive to opportunistic (Borja et al., 2008). Although this index was designed to assess the ecological quality of European water ecosystems, its applicability has extended to other geographic areas as Asia (Cai et al., 2003), northern Africa (Bazairi et al., 2005; Bakalem et al., 2009), North America (Borja et al., 2008), South America (Muniz et al., 2005) and southwest Indian Ocean (Bigot et al., 2008), allowing correct evaluation of the ecosystem's conditions.

The use of biotic indices presents limitations, mainly inside coastal and estuarine ecosystems of tropical and subtropical areas, where basic information regarding ecological characteristic of soft-bottom macrobenthic communities is still scarce (Frouin, 2000; Gray, 2002; Muniz et al., 2005; Bigot et al., 2008). The ecology of soft-bottom benthic macrofauna communities in estuarine systems on the Atlantic coast of South America is recent and without previous data or long-term monitoring programs (Pagliosa and Barbosa, 2006). Besides, they have been thoroughly concentrated in the south and southeast coasts (subtropical and temperate areas), where the impacts on estuarine systems only have been registered in obvious cases of severe pollution (Venturini et al., 2004), so extensive gaps in the north and northeast regions remain (Couto et al., 2003).

In Pernambuco State, northeast coast of Brazil, estuarine zones are the most affected by anthropogenic influences (e.g. sewage, removal of native mangrove forests for sugar-cane monocropping, industries such as chlor-alkali, fertilizers, agricultural defensives, paper mills, aluminum, and others) (CPRH, 2006). As a consequence, it is necessary to propose instruments and methodologies which permit to express the structural and functional quality of the benthic communities in order to achieve sustainability and conservation of these tropical ecosystems.

The present study aimed to examine the AMBI efficiency to evaluate with adequacy and accuracy the environmental and ecological status of the estuarine ecosystems of Pernambuco in view of extending its use to other tropical areas. The comparison between urbanized and relatively conserved areas may allow recognizing the ecological performance of AMBI in estuaries with non-intense levels of pollution or where there is a lack of historical data. This work also assessed among environmental variables those which best reflect the responses of macrobenthic communities to anthropogenic stressors and tested the AMBI index classification dependence on sieve-mesh (1.0mm and 0.5mm) and sampling depth (0-10cm and 0-20cm).

II.2. Material and Methods

II.2.1. Studied areas and sampling design

The same fourteen sites presented in Chapter 01 (see pages 17 and 18) were selected in order to evaluate their ecological condition and integrity. Although most of these estuaries are defined as Environmental Protection Areas (through the State Law n.9.931, December 1986; Noronha, 2008), they are subjected to different sources (tab.01), as shown by monitoring reports (CPRH, 2006).

The sampling procedure, number of replicates collected for environmental variables, microphytobenthos and macrofauna as well as the methods applied for abiotic parameters analyses followed the descriptions in Chapter 01 (see tab.02 and 03). Granulometric composition was determined according to Wentworth scale (1922): coarse sand (0.5mm); medium sand (0.25mm); fine sand (0.125mm); very fine sand (0.062mm) and mud (<0.062mm).

For macrofauna, the total abundance per replicate was used to examine AMBI efficiency; afterwards, abundance was separated by sieve-mesh (1.0mm and 1.0+0.5mm or combined sieves) and sampling depth (0-10cm and 0-20cm) to test the contribution of these factors for the index diagnosis.

Table 01. Sites location and summary of their main disturbance sources (according to CPRH, 2006). *Sites placed in urbanized estuaries.

Studied areas/ sites (geographic coordinates)	Sources of disturbance
Itapissuma (07°46’31.20’’S/ 34°53’26.76’’W)	Inputs from polluted tributaries; sugar cane monoculture and intensive fisheries
Sta Cruz Channel (07°46’13.12’’S/ 34°52’58.19’’W)	See Itapissuma
Paripe (07°48’38.76’’S/ 34°51’23.28’’W)	anthropic action is minimum (subsistence agriculture and fishing)
Timbó* (07°51’18.72’’S/ 34°50’33.96’’W)	Fisheries activities; domestic sewage; industrial effluents (mainly steel mill and textile) and tourism
Paratibe* (07°57’37.44’’S/ 34°49’48.54’’W)	Mangrove deforestation; hospital and industrial effluents; domestic sewage and slaughterhouse
Capibaribe* (08°3’52.98’’S/ 34°52’27.06’’W)	Small industries (milk, detergents, soap, leather, etc.); inputs from polluted tributaries and domestic sewage
Pina Basin1* (08°4’38.7’’S/ 34°52’29.7’’W)	Domestic sewage and inputs from polluted tributaries
Pina Basin2* (08°5’27.0’’S/ 34°53’11.64’’W)	See Pina Basin1
Jaboatão* (08°14’24.43’’S/ 34°56’43.20’’W)	Domestic sewage; mill industry and cellulose manufacture
Pirapama* (08°14’35.52’’S/34°56’46.80’’W)	Cane sugar monoculture and irregular human occupation
Ipojuca-Merepe (08°24’39.66’’S/34°58’28.62’’W)	Mangrove deforestation and construction of Suape Industrial Port Complex
Maracaípe (08°32’21.42’’S/35°00’21.72’’W)	Tourism
Ariquindá (08°41’22.74’’S/35°06’08.22’’W)	Agricultural enterprises and tourism
Mamucabas (08°46’41.81’’S/35°06’27.46’’W)	Agricultural activities

Table 02. Sampling procedure and number of replicates for biotic (macrofauna and microphytobenthos) and abiotic parameters. *Measures were performed both twice in the Rainy and Dry seasons with results treated as replicates.

Parameter	Replicates	Sampling strategy
Macrofauna	5	cylindrical corer (area: 40.71cm², 0-20cm)
Microphytobenthos	5	cylindrical corer (area: 1.13cm², 0-2cm)
Total nitrogen sediment	5	cylindrical corer (area: 16.62cm², 0-2cm)
Organic matter	5	cylindrical corer (area: 16.62cm², 0-2cm)
Granulometry	5	cylindrical corer (area: 16.62cm², 0-2cm)
Redox potential sediment	5	measured in field, in top (~2cm) and bottom (~20cm)
Salinity water	1(4*)	measured in field, refractometer
Ammonia-N water	1	collected with 200mL plastic bottles
Dissolved oxygen and water temperature	4*	measured in field, oximeter (~2cm)

Table 03. Methods applied for environmental parameters.

Parameter	Method/Equipment	References
Microphytobenthos pigments (μg/cm²)	Spectrophotometer	Modified from Colijn and Dijkema (1981) and Lorenzen (1967) equations
Total nitrogen sediment (g/kg)	Kjeldahl method	EMBRAPA (1997)
Organic matter (%)	Incineration	Wetzel and Likens (1990)
Granulometry (%)	Rot-up procedure	Suguio (1973)
Redox potential sediment (mV)	Platinum electrodes	APHA (1989)
Salinity water (psu)	Refractometer	Littlepage (1998)
Ammonia-N water (μmol/L)	Spectrophotometer	Grasshoff et al., 1983
Dissolved oxygen (mg/L) and temperature water (°C)	Oximeter	APHA (1989)

The ecological quality *status* of the sites was determined using the AMBI index (Grall and Grémarec, 1997; Borja et al., 2000). This index relies on the distribution of individual abundances of the soft-bottom communities into five ecological groups (EG) according to a gradient of pollution: Ecological group I (EG_I) - Species very sensitive to organic enrichment and present under unpolluted conditions; Ecological group II (EG_{II}) - Species indifferent to enrichment, always present in low densities with non-significant variations with time; Ecological group III (EG_{III}) - Species tolerant to excess organic matter enrichment. Populations stimulated by slight unbalanced situations; Ecological group IV (EG_{IV}) - Second-order opportunistic species (slight to pronounced unbalanced situations); Ecological group V (EG_V) - First-order opportunistic species (pronounced unbalanced situations).

AMBI values (also referred as biotic coefficient or BC) are computed as the sum of products of the proportion of each ecological group by an arbitrary value (0; 1.5; 3; 4.5; 6) attributed to each EG (Bigot et al., 2008) and vary continuously from 0 (undisturbed) to 6 (extremely disturbed)(tab. 04):

$$AMBI = 1/100 \times (0 \times \%EG_I + 1.5 \times \%EG_{II} + 3 \times \%EG_{III} + 4.5 \times \%EG_{IV} + 6 \times \%EG_V)$$

In the present study, AMBI was computed for each individual replicate and then averaged for each site as recommended by Borja et al. (2003), Borja and Muxika (2005a), Muniz et al. (2005) and Bigot et al. (2008). The assignment of the identified species into one of the five ecological groups proposed by the index was based upon the list available in the AMBI v.4.0 program (http://www.azti.es). Species not considered in the list or for which the classification was not in accordance with knowledge on their ecological distribution for tropical areas were assigned or re-assigned to an EG based on pollution condition monitoring reports of Pernambuco estuaries (CPRH, 2006), on literature about local ecological characteristics of macrobenthic communities of these ecosystems (e.g. Silva, 2003; Carvalho, 2004; Lima, 2006; Botter-Carvalho, 2007; Valença, 2007) and on IndVal coefficient methodology (see below).

Table 04. Summary of the ecological quality classification of sites and benthic communities according to AMBI and BI (biotic index) values (modified from Borja et al., 2000).

AMBI	BI	Dominating ecological group	Site classification	Benthic community health
0.0 < AMBI ≤ 0.2	0	I	Undisturbed	Normal
0.3 < AMBI ≤ 1.2	1		Undisturbed	Impoverished
1.3 < AMBI ≤ 3.3	2	III	Slightly disturbed	Unbalanced
3.4 < AMBI ≤ 4.3	3		Moderately disturbed	Transitional to pollution
4.4 < AMBI ≤ 5.0	4	IV-V	Moderately disturbed	Polluted
5.1 < AMBI ≤ 5.5	5		Heavily disturbed	Transitional to heavy pollution
5.6 < AMBI < 6.0	6	V	Heavily disturbed	Heavy polluted
AMBI = 6.0	7		Extremely disturbed	Azoic

II.2.2. Data analysis

For this study, the following main faunal parameters which could contribute to qualify the ecological status of the sites are presented: species richness (S), evenness (J') and AMBI index, the last one computed using the AMBI program (v.4.0). The first two indices results were described in chapter 01. In order to assess whether species were properly classified and propose new ecological assignments, before calculating AMBI, the sites were gathered into groups considering previous information on their pollution condition (CPRH, 2006) and environmental parameters. The groups of sites resulting from these associations were characterized by their indicator species, through the Indicator Value (IndVal) coefficient, developed by Dufrêne and Legendre (1997). According to the authors, a species is indicator of a group if it occurs on most of the samples from this group (specificity) and if it is poorly represented on the other groups (fidelity). The IndVal coefficient combines both the species relative abundance with its relative frequency of occurrence in the defined groups of sites. The statistical significance of the species indicator values was estimated using a random reallocation procedure (1000 randomizations) of sites among site groups, through the Monte Carlo test, thus a species is considered as a group indicator if the results are significant to a level of 0.05 (Zintzen et al., 2008). In general, groups are defined by categorical environmental parameters, disturbance levels, experimental treatments or types of habitat (Wetzel et al., 2002). In this study, categorical dissolved oxygen and disturbance levels were adopted, separating the sites into two groups: (1) undisturbed or low disturbance conditions, where dissolved oxygen values were superior to 5.0mg/L (reference concentration for Brazilian's estuarine waters, according to CONAMA Resolution n.357/2005) and (2) medium to high disturbance conditions, where dissolved oxygen values were lower than 5.0mg/L. The IndVal coefficient was calculated using the PC-ORD v.4.0 Windows program (McCune and Mefford, 1999).

On the basis of the macrofaunal parameters (AMBI index, S and J'), Multidimensional scaling (MDS) and Analysis of Similarity (one-way ANOSIM) were used to point out differences among sites in view of the ecological quality of macrobenthic community (see AMBI classification of benthic community health, tab.04). Simultaneously, the BEST/BIOENV routine was performed to associate environmental variables to the set of indices (AMBI, species richness and evenness), while Spearman's rank correlation coefficient was carried out in order to determine correlations between each index and the abiotic variables. To account for multiple simultaneous Spearman's rank correlations, Bonferroni correction was applied and results were significant at $p<0.0045$. The multivariate analyses (MDS, ANOSIM and BEST/BIOENV) were performed with PRIMER v6.0 software (Clarke and Gorley, 2006) and Spearman's correlation coefficient was calculated using the BIOESTAT v5.0 program (Ayres et al., 2007).

Finally, Wilcoxon matched-pairs test was used to compare results from AMBI classification of estuarine systems considering the factors "mesh-sieve" and "sampling depth". In this case, the significance level adopted was 0.025, after Bonferroni correction. In addition, a two-tailed t-test was applied to verify the relation between index values found (for mesh-sieve and sampling depth factors) and the presence or absence of urbanization around these estuaries. The Wilcoxon test was performed using the STATISTICA v5.0 software and the t-test was calculated with the BIOESTAT v5.0 program (Ayres et al., 2007).

All (but not Wilcoxon or Spearman's rank correlation) statistics statements were based on a significance level of $\alpha = 5\%$.

II.3. Results

II.3.1. Environmental data

Water parameters: Mean temperature ranged from 25.7 (in Mamucabas) to 30.5°C (in Maracaípe) with little variation between seasons (fig. 01). As for salinity, mean values were akin to those obtained in the period of macrofauna samplings (October-2007). In most sites, dissolved oxygen levels were found to be outside the normal limits for estuarine systems (tab.05); on the other hand, only Pina Basin1 (3.997µmol/L), Jaboatão (6.311µmol/L) and Paratibe (7.292µmol/L) presented ammonia-N concentrations (fig.02) higher than the reference value (3.89µmol/L) established by Brazilian laws. Significant correlations were registered between salinity values in October-2007 and both mean temperature (r=0.752; *p*=0.002) and dissolved oxygen (r=0.716; *p*=0.004) averaged along the seasons.

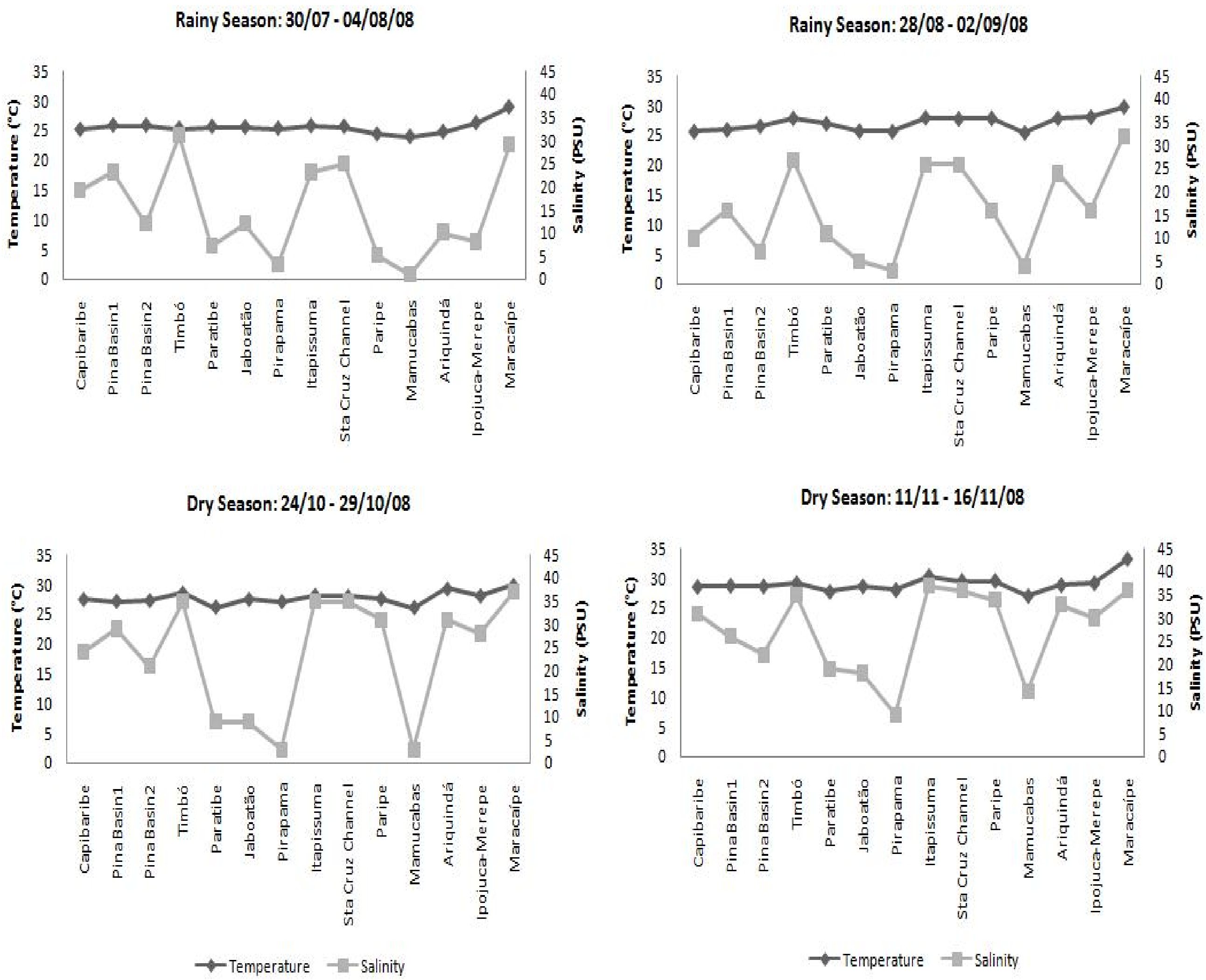

Figure 01. Water temperature (°C) and salinity (psu) values measured in both Rainy and Dry seasons in the studied areas.

Table 05. Dissolved oxygen concentrations measured during macrofauna samplings (October-2007) in comparison to the Rainy and Dry seasons. Legend: Rainy season (1): 30/07-04/08/08; Rainy season (2): 28/08-02/09/08; Dry season (1): 24/10-29/10/08; Dry season (2): 11/11-16/11/08. Bold values are outside the reference condition. *Sites placed in urbanized estuaries.

Sites	October-2007	Rainy season (1)	Rainy season (2)	Dry season (1)	Dry season (2)
Capibaribe*	6.4	7.6	**2.6**	**2.2**	**3.3**
Pina Basin1*	**4.1**	**3.9**	**3.9**	5.2	**4.0**
Pina Basin2*	**3.8**	**2.4**	**2.5**	**3.6**	**1.7**
Timbó*	**3.8**	9.1	6.7	7.9	**4.8**
Paratibe*	**2.2**	**3.4**	**3.3**	**2.2**	**2.1**
Jaboatão*	missing value	6.0	**2.6**	**3.8**	6.0
Pirapama*	missing value	**4.4**	5.0	**2.8**	**3.8**
Itapissuma	missing value	6.6	6.2	6.9	6.5
Sta Cruz Channel	missing value	6.4	5.7	5.7	6.4
Paripe	missing value	5.2	5.7	**3.9**	**4.6**
Mamucabas	missing value	5.4	**4.6**	**4.3**	5.2
Ariquindá	missing value	5.6	5.4	5.6	5.9
Ipojuca-Merepe	missing value	**4.7**	5.6	**4.0**	**4.5**
Maracaípe	missing value	5.8	7.0	5.5	5.4

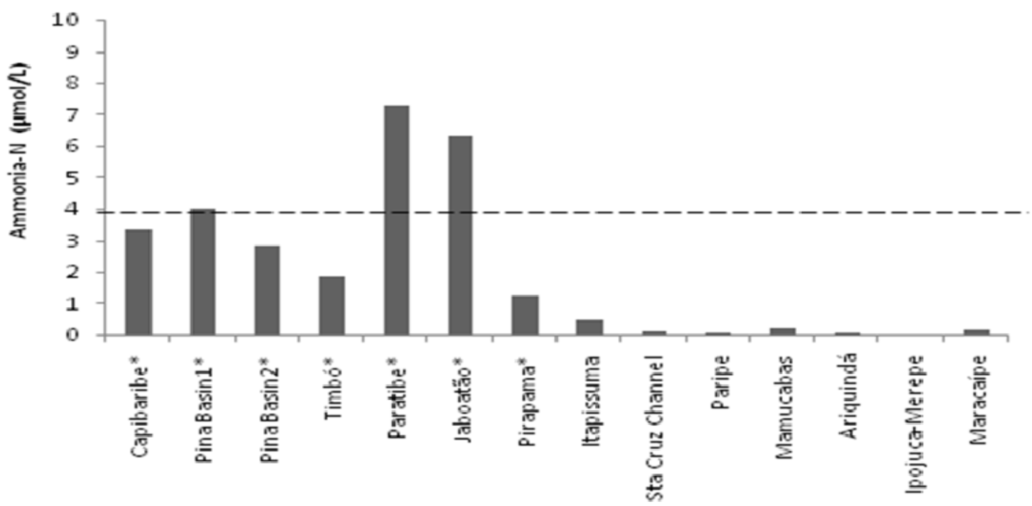

Figure 02. Ammonia-N concentration (µmol/L) measured in the studied areas. Reference concentration for Brazilian estuarine waters (CONAMA Resolution n.357/2005) is represented by (-). *Sites placed in urbanized estuaries.

Sedimentological parameters: The granulometric composition showed that the sediment at the sampling areas is mainly composed by sand fractions, especially medium sand (in Paratibe, Itapissuma, Mamucabas and Maracaípe) and fine sand (in Pina Basin1, Jaboatão, Pirapama, Paripe and Sta Cruz Channel). The very fine sand fraction dominated in Timbó, Ariquindá and Ipojuca-Merepe and the two formers presented high proportion of mud (or silt-clay) fraction. In contrast, Capibaribe and Pina Basin2 sediments were represented by coarse sand (fig.03). In these sites, surface sediment was characterized by yellow-brown color (probably diatoms) and green (cyanobacteria) color compact mats that might have interfered during the granulometric analyses. The algal mats also might contribute to the high chlorophyll-*a* and phaeopigments concentrations observed (fig.04).

Sediment presented strongly reduced conditions along sites and the redox potential (Eh) in the bottom layer (~20cm) was generally more negative than in the surface (~2cm) (fig.05).

A strong correlation between the organic matter content (fig.06) and total-N (fig.07) variables ($r=0.911$; $p<0.001$) in the sediments was registered.

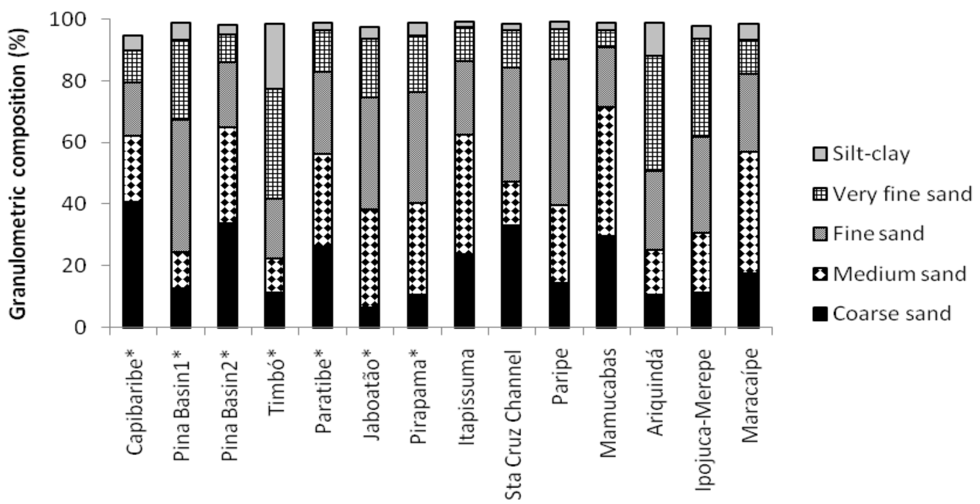

Figure 03. Granulometric composition (%) registered in the studied areas. *Sites placed in urbanized estuaries.

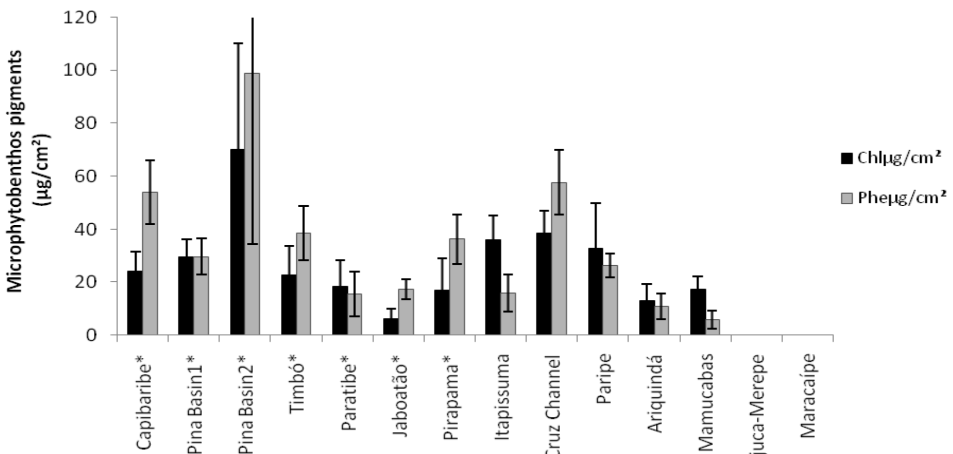

Figure 04. Mean values (±SD) of sediment chlorophyll-*a* (Chlμg/cm²) and phaeopigments (Pheμg/cm²) biomass registered in the studied areas (data for Ipojuca-Merepe and Maracaípe sites are missing). *Sites placed in urbanized estuaries.

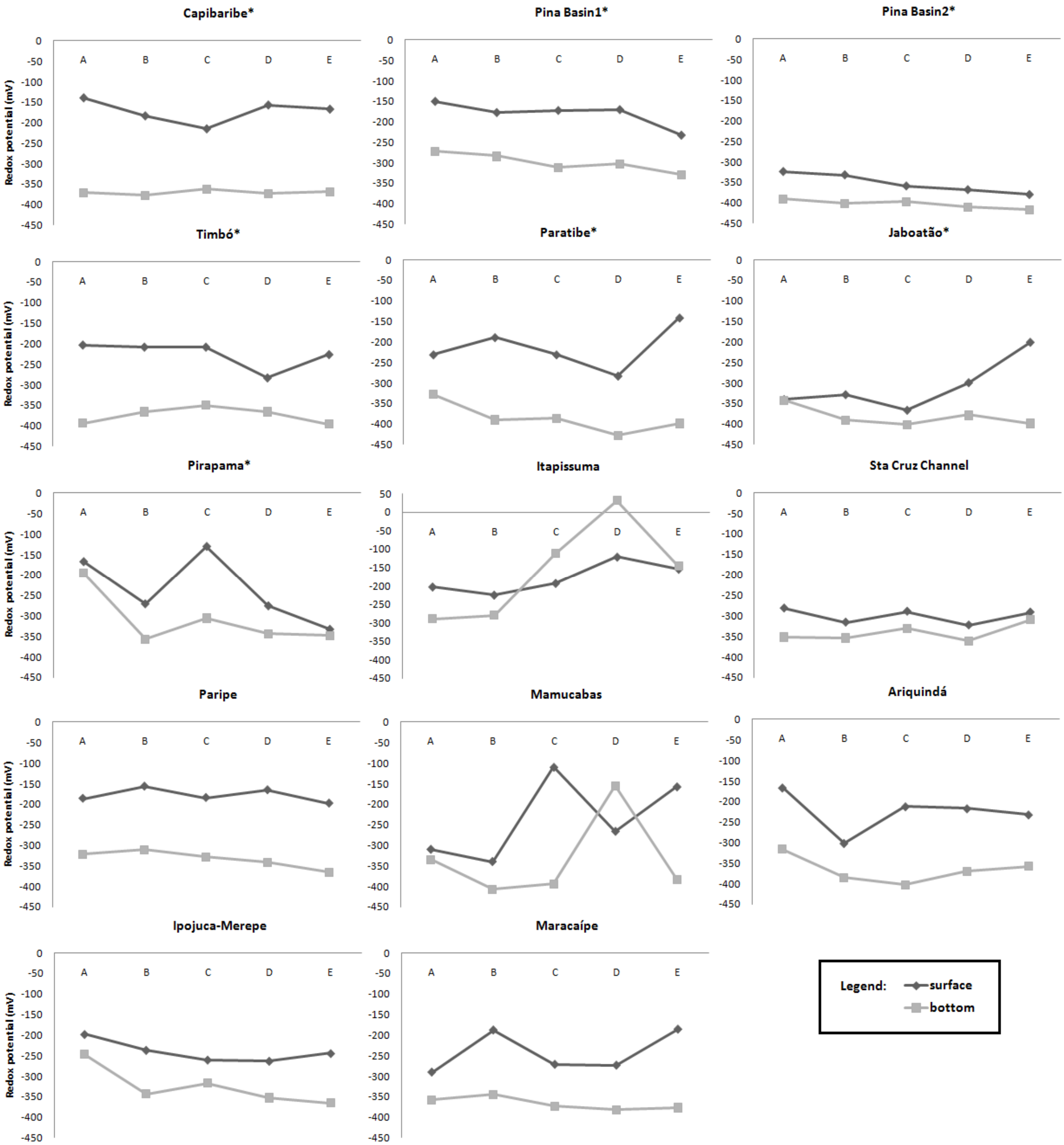

Figure 05. Redox potential (mV) for each replicate (A-E) measured in surface (~2cm) and bottom (~20cm) strata in the studied areas sediments. *Sites placed in urbanized estuaries.

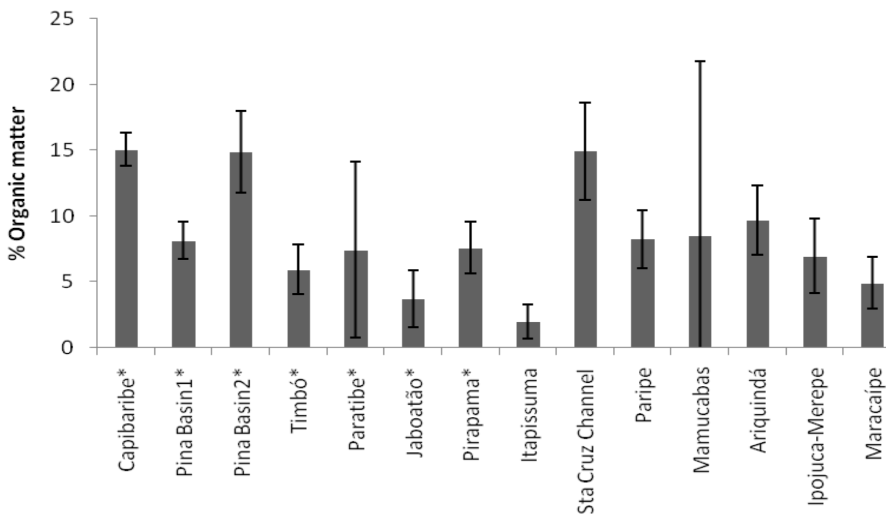

Figure 06. Mean values (±SD) of sediment organic matter content (%) registered in the studied areas. *Sites placed in urbanized estuaries.

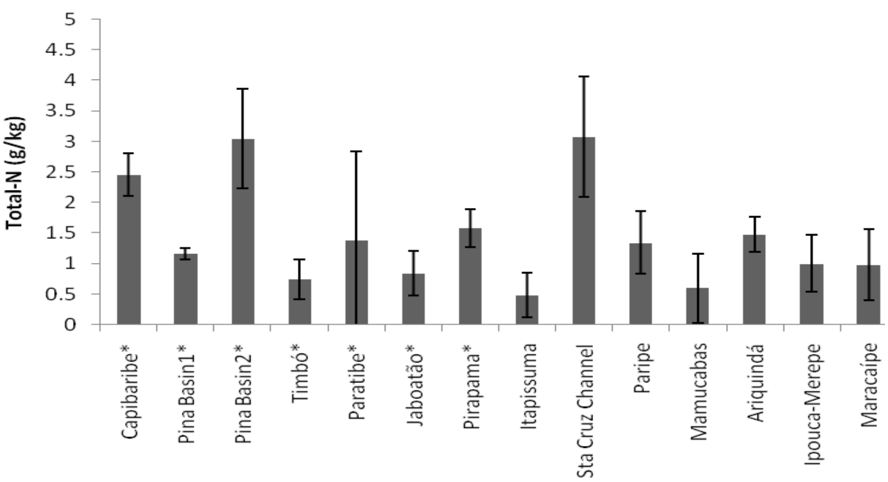

Figure 07. Mean values (±SD) of sediment total-N concentrations (g/kg) registered in the studied areas. *Sites placed in urbanized estuaries.

II.3.2. Ecological group assignments and sites classification

Among all macrofauna taxa, over 30% were not listed in any ecological group (EG) of AZTI database, being the majority (79%) ascribed an EG based on the classification for the same genus. For the following species, ecological groups were attributed according to AZTI list for higher taxonomic levels (>Family): *Anomalocardia brasiliana* (I), *Barantolla* sp. (V), *Capitellides* sp. (V), *Fabrisabella* sp. (I), *Neomediomastus* sp. (V), *Pseudobranchiomma* sp. (I) and *Timarete* sp. (IV). Due to the lack of ecological information for tropical regions, eighteen taxa still remained without classification, known as not assigned.

Considering the definition of sites into groups using the variables dissolved oxygen and disturbance levels, the Indicator Value (IndVal) coefficient revealed the presence of four significant indicator species (tab. 06). In terms of ecological interpretation, since these species presented high indicator values (>50%), they were associated to sensitive and opportunistic groups of AMBI (EG I and V, respectively). Consequently, *Magelona* sp., *Megalomma* sp. and *Sternaspis* sp. were reclassified to EG (I) and *Streblospio* sp., to EG (V).

Table 06. Indicator Value (IndVal) coefficient for significant species related to the categorical groups. Groups: (1) sites undisturbed or low disturbance conditions (dissolved oxygen concentration >5.0mg/L) and (2) sites disturbed (dissolved oxygen concentration <5.0mg/L).

Group	Indicator Species	Indicator Value (IV)	Significance level (p)%
1	*Magelona* sp.	66.2	3.1
1	*Megalomma* sp.	51.3	4.1
1	*Sternaspis* sp.	60.0	2.3
2	*Streblospio* sp.	89.1	2.8

Although Oligochaeta was not selected as an indicator species by the IndVal coefficient, this study suggests its change from ecological group V (first-order opportunistic) to III (tolerant), in view of the fact that most oligochaetes identified belong to Tubificidae which can be observed not only in impacted sites but also at low salinity unimpacted sites within estuaries (see Paul et al., 2001; Pinto et al., 2009).

The final list, including new ecological group assignments and re-assignments, is given in the following table 07:

Table 07. List of taxa registered in sites, the ecological group (EG) classification on the AZTI list and on this study. Bold taxa represented changes in relation to the AZTI database. **IndVal results were similar to the AZTI classification; n.a. = not assigned. Taxonomic Groups: BIV: Bivalvia; CHI: Chironomida; CNI: Cnidaria; CRU: Crustacea; CTE: Ctenophora; ECH: Echinodermata; GAS: Gastropoda; NEM: Nematoda; NEM': Nemertea; OLI: Oligochaeta; PHO: Phoronida; PLA: Platyhelminthes; POL: Polychaeta; SIP: Sipuncula. Sites: Capibaribe (Cp); Pina Basin1 (BP1); Pina Basin2 (BP2); Timbó (Tb); Paratibe (Pa); Jaboatão (Jb); Pirapama (Pp); Itapissuma (It); Sta Cruz Channel (CC); Paripe (Pr); Mamucabas (Mm); Ariquindá (Ar); Ipojuca-Merepe (IM); Maracaípe (Ma).

Sites	Taxonomic Group	Species/taxa	EG AZTI list	EG used for this study
Tb, Ma.	BIV	*Anomalocardia brasiliana*	I (for family)	I
CC, Pr.	BIV	*Corbula* sp.	IV (for genus)	IV
Jb, Pp, It, Pr, Ar, IM, Ma.	BIV	*Lucina* sp.	I (for *L. pectinata*)	I
Tb, Jb, Pp, It, IM.	BIV	*Macoma* sp.	n.a.	n.a.
Jb, Pp, It, CC, Pr, Ma.	BIV	*Tagelus* sp.	III (for *T. plebeius*)	III
Jb, Pp, IM, Ma.	BIV	*Tellina* sp.	I (for genus)	I
Mm	CHI	undeterm. chironomids	III	III
Jb	CNI	undeterm. cnidarians	I	I

Table 07 (continued)

Sites	Taxonomic Group	Species/taxa	EG AZTI list	EG used for this study
BP1, Pr, Mm.	CRU	*Alpheus* sp.	II (for genus)	II
IM	CRU	undeterm. amphipods	n.a.	n.a.
Pa, Pp.	CRU	*Uca* sp.	n.a.	n.a.
BP1	CTE	undeterm. ctenophore	n.a.	n.a.
Pr	ECH	undeterm. echinoderm	I	I
Ma	GAS	*Neritina* sp.	n.a.	n.a.
Pr	GAS	undeterm. Nudibranchia	n.a.	n.a.
Tb	GAS	*Solariorbis* sp.	n.a.	n.a.
All sites	NEM	nematodes	III	III
Tb, It, Pr, Mm, Ar, IM.	NEM'	underterm. nemertines	III	III
BP1, Pp, It, CC, Mm, Ar, Ma.	OLI	**undeterm. oligochaetes**	**V**	**III**
Cp, BP1, BP2, Tb, Pa, It, CC, Pr, Ar, Ma.	OLI	***Tectidrilus* sp.**	**V**	**III**
All sites	OLI	**Tubificidae**	**V**	**III**
Tb, Pr.	PHO	undeterm. phoronides	II	II
BP1, Tb, It, CC, Pr, Ar, Ma.	PLA	undeterm. turbellarians	II	II
It, Ar.	POL	*Aedicira* sp.	n.a.	n.a.
BP2	POL	*Ancistrosyllis* sp.	III (for genus)	III
Ma	POL	undeterm. Arabellidae	n.a.	n.a.
Pp	POL	*Barantolla* sp.	V (for family)	V
Cp, Tb, Pa, Jb, Pp, Mm.	POL	*Boccardia* sp.	I (for genus)	I
Tb, Pr.	POL	*Branchiomma* sp.	I (for genus)	I
All sites (except for BP1)	POL	*Capitella* sp.	V (for genus)	V
Cp, Pa, Jb, Pp, CC, Mm, IM, Ma.	POL	*Capitellides* sp.	V (for family)	V
Pp, CC.	POL	*Capitomastus* sp.	V (for genus)	V
Ar	POL	*Caulleriella* sp.	IV (for genus)	IV
Mm	POL	*Ceratonereis* sp.	II (for genus)	II
It	POL	*Dispio* sp.	III (for genus)	III
Tb	POL	*Eusyllis* sp.	II (for genus)	II
CC, Pr, Ar.	POL	*Exogone* sp.	II (for genus)	II
Pr	POL	*Fabrisabella* sp.	I (for family)	I
It	POL	*Glycera* sp.	II (for genus)	II
BP1, BP2, Tb, It, Pr, Ar.	POL	*Glycinde* sp.	II (for genus)	II
Pr	POL	*Gyptis* sp.	II (for genus)	II
BP1	POL	*Hemipodus* sp.	n.a.	n.a.
BP1, BP2, Tb, Jb, Pp, It, Pr, Mm, Ar, IM, Ma.	POL	*Heteromastus* sp.	IV (for genus)	IV

Table 07 (continued)

Sites	Taxonomic Group	Species/taxa	EG AZTI list	EG used for this study
Pr, IM.	POL	*Isolda* sp.	n.a.	n.a.
All sites (except for Ar)	POL	*Laeonereis* sp.	IV (for both *L. acuta*/ *L. culveri*)	IV
BP1, Tb, Pr, Ar.	POL	*Leitoscoloplos* sp.	IV (for genus)	IV
Tb	POL	*Lepidasthenia* sp.	I (for genus)	I
IM, Ma.	POL	*Lumbrineris* sp.	II (for genus)	II
BP1, Tb, Pr, Ar, Ma.	POL	***Magelona* sp.**	**I (for genus)**	**I****
BP1, BP2, Tb, Jb, Pp, It, CC, Pr, Ar, IM, Ma.	POL	*Mediomastus* sp.	III (for genus)	III
Tb, Pr, Ar, Ma.	POL	***Megalomma* sp.**	**I (for family)**	**I****
Pa	POL	*Neanthes* sp.	III (for genus)	III
BP1, Tb, It, IM, Ma.	POL	*Neomediomastus* sp.	V (for family)	V
BP2, It, Pr, IM, Ma.	POL	undeterm. Nereididae	n.a.	n.a.
Tb	POL	*Ophiodromus* sp.	II (for genus)	II
BP1	POL	*Ophioglycera* sp.	n.a.	n.a.
Pr	POL	*Ophiostosyllis* sp.	n.a.	n.a.
Jb, IM.	POL	undeterm. Orbiniidae	n.a.	n.a.
Ar	POL	*Paradoneis* sp.	III (for genus)	III
BP1	POL	*Parandalia* sp.	n.a.	n.a.
Jb, Mm, IM.	POL	*Paraprionospio* sp.	IV (for *P. pinnata*)	IV
It	POL	*Phyllodoce* sp.	II (for genus)	II
CC	POL	*Podarke* sp.	II (for genus)	II
Cp, BP2, Jb, Pp, IM.	POL	*Polydora* sp.	IV (for genus)	IV
Jb, Ar, Ma.	POL	*Potamilla* sp.	II (for genus)	II
Jb	POL	*Prionospio* sp.	IV (for genus)	IV
Pp	POL	*Pseudobranchiomma* sp.	I (for family)	I
BP1, Ar, Ma.	POL	*Scoloplos* sp.	I (for genus)	I
Cp, BP1, BP2, Tb, Jb, It, CC, Pr, Mm, IM, Ma.	POL	*Sigambra* sp.	n.a. (for genus)	n.a.
CC, Pr, Ma.	POL	*Sphaerosyllis* sp.	II (for genus)	II
It, Ar, Ma.	POL	***Sternaspis* sp.**	**III (for genus)**	**I**
Cp, BP1, BP2, Tb, Jb, Pp, It, CC, Pr, IM, Ma.	POL	***Streblospio* sp.**	**III (for genus)**	**V**
BP1, Tb.	POL	undeterm. Syllidae	n.a.	n.a.
Pr	POL	*Tharyx* sp.	IV (for genus)	IV
Ar	POL	*Timarete* sp.	IV (for family)	IV
CC, Pr.	SIP	undeterm. sipunculans	I	I

The AMBI values showed that all estuaries presented some level of disturbance, ranging from 2.395 (slightly disturbed, in Ariquindá) to 5.236 (heavily disturbed, in Capibaribe).

According to the index, most sites were situated between the slightly-moderate disturbed boundaries, due to the higher proportion of Nematoda and Oligochaeta (including Tubificidae species), both them assigned here as ecological group III (60-80%). The mean percentage of each ecological group and AMBI score per site are given in figures 08 and 09, respectively.

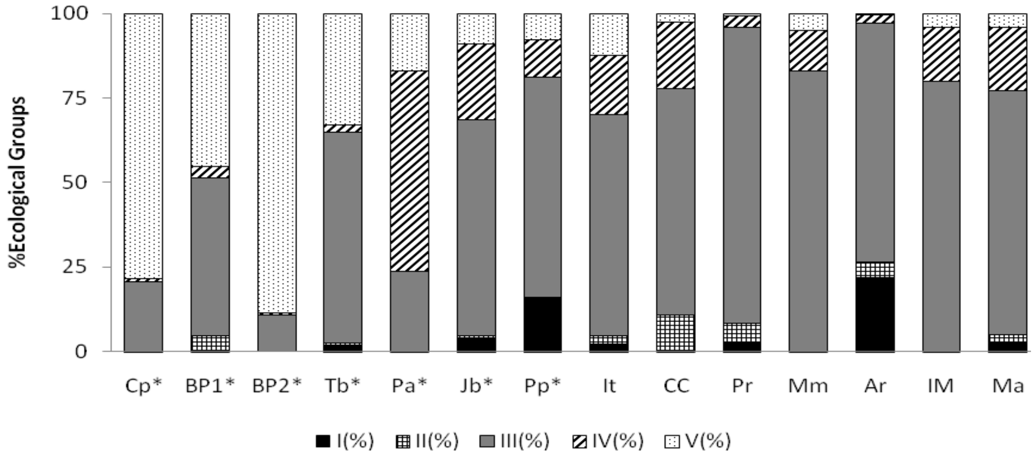

Figure 08. Percentage of ecological groups (%I→V) registered in the studied areas. Sites: Capibaribe (Cp); Pina Basin1 (BP1); Pina Basin2 (BP2); Timbó (Tb); Paratibe (Pa); Jaboatão (Jb); Pirapama (Pp); Itapissuma (It); Sta Cruz Channel (CC); Paripe (Pr); Mamucabas (Mm); Ariquindá (Ar); Ipojuca-Merepe (IM); Maracaípe (Ma). *Sites placed in urbanized estuaries.

Although classified as slightly disturbed, Ariquindá exhibited the major abundance of sensitive species (21.8%), composed by *Lucina* sp., *Magelona* sp., *Megalomma* sp., *Scoloplos* sp. and *Sternaspis* sp. In Capibaribe, on the other hand, numerical dominance of opportunistic species *Capitella* sp., *Capitellides* sp. and *Streblospio* sp., which accounted for 78.6% of total abundance, was associated to the heavily disturbed classification (fig.09).

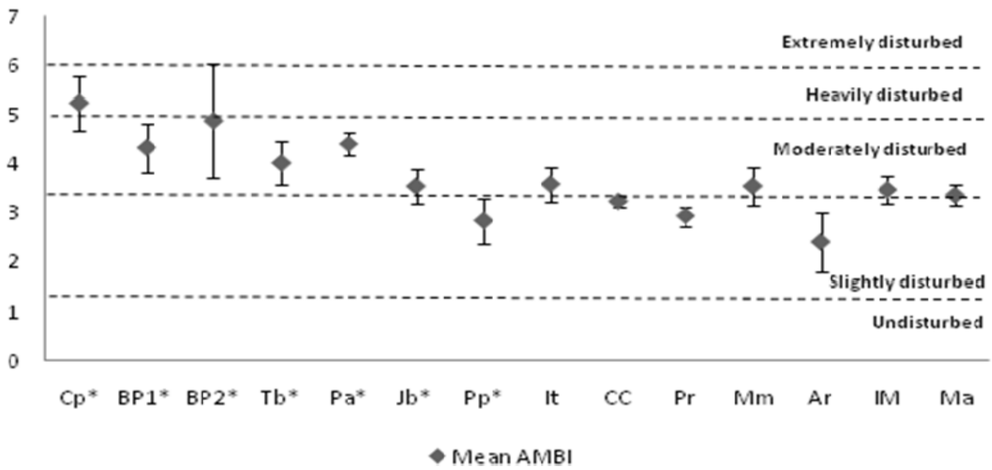

Figure 09. Mean AMBI values (±SD) registered in the studied areas. Sites: Capibaribe (Cp); Pina Basin1 (BP1); Pina Basin2 (BP2); Timbó (Tb); Paratibe (Pa); Jaboatão (Jb); Pirapama (Pp); Itapissuma (It); Sta Cruz Channel (CC); Paripe (Pr); Mamucabas (Mm); Ariquindá (Ar); Ipojuca-Merepe (IM); Maracaípe (Ma). *Sites placed in urbanized estuaries.

In terms of benthic community health, the estuarine areas of Pernambuco were classified as unbalanced (for Pirapama, Sta Cruz Channel, Paripe, Ariquindá and Maracaípe), transitional to pollution (for Pina Basin1, Timbó, Jaboatão, Itapissuma, Mamucabas and Ipojuca-Merepe), polluted (for Pina Basin2 and Paratibe) and transitional to heavy pollution (for Capibaribe).

The ordination diagram (MDS) and cluster analyses applied to AMBI, richness (S) and evenness (J') showed the presence of two distinct groups following the ecological quality of benthic community (fig.10 and 11) proposed by the index (tab.04): Group (A) includes sites where macrobenthos was classified as polluted/transitional to heavy pollution, whereas Group (B) involves sites with the benthic fauna considered as unbalanced/transitional to pollution. Significant differences were established among the sites in relation to benthic community health (R_{global}=0.363; *p*=0.001) but not within the two groups (tab. 08).

Table 08. Similarity analysis (ANOSIM) of sites considering the ecological quality of macrobenthic community. Bold p% values represent significant differences.

Similarity analysis (ANOSIM)	Rglobal	Significance level (p)%
Unbalanced → Transitional to pollution (Group B)	0.064	6.0
Unbalanced → Polluted	0.761	**0.1**
Unbalanced → Transitional to heavy pollution	0.747	**0.1**
Transitional to pollution → Polluted	0.626	**0.1**
Transitional to pollution → Transitional to heavy pollution	0.510	**0.1**
Polluted → Transitional to heavy pollution (Group A)	-0.119	80.1

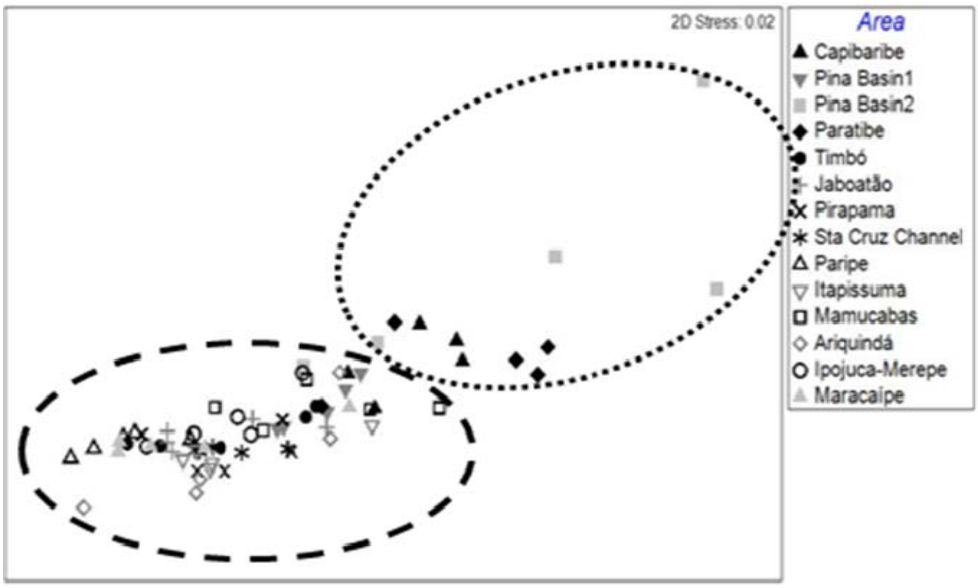

Figure 10. MDS ordination of sites (replicate values) produced with AMBI, richness (S) and evenness (J') data. Group A – dotted line; Group B – traced line.

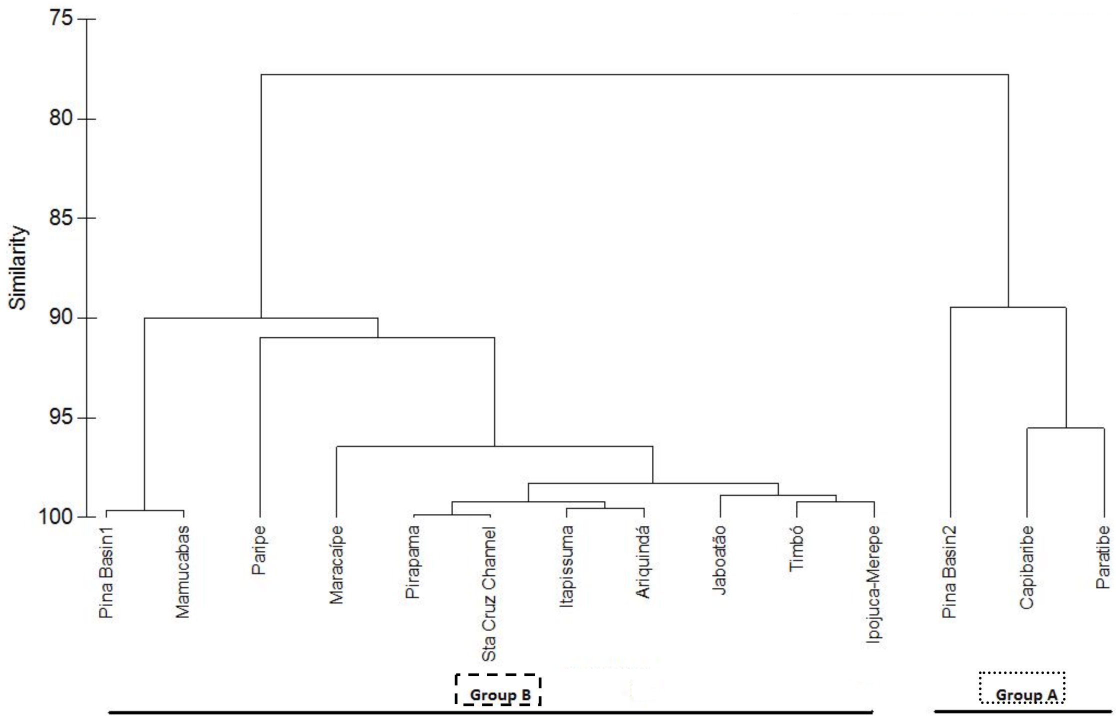

Figure 11. Cluster analysis of sites (average values) produced with AMBI, richness (S) and evenness (J') data. Groups: (A) macrobenthos classified as polluted/transitional to heavy pollution; (B) macrobenthos classified as unbalanced/transitional to pollution.

BIO-ENV routine discriminated the variables ammonia-N, dissolved oxygen and organic matter (r_s=0.376) or ammonia-N, dissolved oxygen and chlorophyll-*a* (r_s=0.451) when excluding Ipojuca-Merepe and Maracaípe sites (since microphytobenthos was lost for these estuaries) as the environmental factors best related to the similarity matrix based on the suite of indices (AMBI, species richness and evenness). Individually, significant correlations were observed for AMBI and ammonia-N (r_s=0.714; p=0.004) and between evenness (J') and grain sorting (r_s=0.749; p=0.002).

II.3.3. AMBI comparative approach: mesh size and sampling depth x urbanization

A comparison of the index results for factors "mesh-sieve" and "sampling depth" in sites' final score suggested the influence of the sieves in AMBI classification, with significant differences found between 1.0mm and combined sieves (1+0.5mm) in 0-10cm (*p*=0.0003) and in 0-20cm (*p*=0.00029) sampling depths. Using the 1.0mm sieve alone, mean AMBI ranged between a minimum of 2.134 and a maximum of 6.000, both in the 0-10cm. In the combined sieves, the values were 2.368 and 5.377, respectively, also in the first ten-centimeter layer. The classification differences observed for sieves were perceived in three sites: Pina Basin2 (in 0-20cm), Pirapama (in 0-10cm) and Sta Cruz Channel (in both layers) (tab.09).

Table 09. Disturbance level classification of each site (mean AMBI values) as a function of the type of mesh screen and sampling depth. Sites: Capibaribe (Cp); Pina Basin1 (BP1); Pina Basin2 (BP2); Timbó (Tb); Paratibe (Pa); Jaboatão (Jb); Pirapama (Pp); Itapissuma (It); Sta Cruz Channel (CC); Paripe (Pr); Mamucabas (Mm); Ariquindá (Ar); Ipojuca-Merepe (IM); Maracaípe (Ma). *Sites placed in urbanized estuaries. Bold text: significant differences.

Site	0-10cm & 1.0mm	0-10cm & combined sieves	0-20cm & 1.0mm	0-20cm & combined sieves
Cp*	Heavily disturbed (5.489)	Heavily disturbed (5.245)	Heavily disturbed (5.487)	Heavily disturbed (5.236)
BP1*	Moderately disturbed (4.038)	Moderately disturbed (4.328)	Moderately disturbed (4.065)	Moderately disturbed (4.331)
BP2*	Heavily disturbed (6.000)	Heavily disturbed (5.377)	Heavily disturbed (6.000)	**Moderately disturbed (4.876)**
Tb*	Moderately disturbed (3.904)	Moderately disturbed (4.022)	Moderately disturbed (3.894)	Moderately disturbed (4.014)
Pa*	Moderately disturbed (4.717)	Moderately disturbed (4.446)	Moderately disturbed (4.694)	Moderately disturbed (4.403)
Jb*	Moderately disturbed (3.593)	Moderately disturbed (3.550)	Moderately disturbed (3.594)	Moderately disturbed (3.535)
Pp*	Moderately disturbed (3.303)	**Slightly disturbed (2.805)**	Slightly disturbed (3.259)	Slightly disturbed (2.835)
It	Moderately disturbed (3.756)	Moderately disturbed (3.581)	Moderately disturbed (3.757)	Moderately disturbed (3.579)
CC	Moderately disturbed (3.898)	**Slightly disturbed (3.247)**	Moderately disturbed (3.821)	**Slightly disturbed (3.226)**
Pr	Slightly disturbed (3.060)	Slightly disturbed (2.935)	Slightly disturbed (3.065)	Slightly disturbed (2.932)
Mm	Moderately disturbed (3.856)	Moderately disturbed (3.811)	Moderately disturbed (3.537)	Moderately disturbed (3.537)
Ar	Slightly disturbed (2.134)	Slightly disturbed (2.368)	Slightly disturbed (2.150)	Slightly disturbed (2.395)
IM	Moderately disturbed (3.942)	Moderately disturbed (3.472)	Moderately disturbed (3.915)	Moderately disturbed (3.463)
Ma	Moderately disturbed (3.724)	Moderately disturbed (3.362)	Moderately disturbed (3.685)	Moderately disturbed (3.353)

Although significant differences were noticeable between 0-10cm and 0-20cm for combined sieves (*p*=0.0024), most areas presented no changes in the final condition assessment concerning the sampling depth. In Pirapama, the given classification of “Moderately disturbed” in 1.0mm/0-10cm for “Slightly disturbed” in both combined/0-10cm and 1.0mm/0-20cm seemed to be more related to the sieve effect (1.0mm x combined sieves) rather than the depth (0-10cm x 0-20cm). On the other hand, in Pina Basin2, both sieves and total depth (0-20cm) contributed to classify the site’s environmental status, with AMBI values varying from 4.876 (“Moderately disturbed”) to 6.000 (“Heavily disturbed”). From an overall perspective, better system conditions were achieved using the combined sieves (1.0+0.5mm) and total layer (0-20cm) (tab. 09), except for Mamucabas, where the lowest score was the same for 1.0mm and combined sieves, in 0-20cm.

The ecological quality of sites (given by mean AMBI values for combined sieves/0-20cm) was found to be significantly different in urbanized versus conserved areas (two-tailed t-test; *p*=0.016); however, when evaluating the urbanization effects on these environments using data from 1.0mm/0-10cm, AMBI did not differ significantly ($p<0.05$).

II.4. Discussion

The development of biotic indices for assessing the ecological integrity of benthic communities in estuaries and coastal areas has progressed in recent years (Engle et al., 1994; Nilsson and Rosenberg, 1997; Weisberg et al., 1997; Roberts et al., 1998; Engle and Summers, 1999; Borja et al., 2000; Paul et al., 2001; Smith et al., 2001; Simboura and Zenetos, 2002; Paul, 2003; Rosenberg et al., 2004; Dauvin and Ruellet, 2007; Muxika et al., 2007; Hale and Heltshe, 2008). Although new indices are continuously being created (Diaz et al., 2004), a general consensus has been emphasized on assessing the suitability of indices that already exist (Borja et al., 2008) in order to improve their performance in different environments.

In this context, AMBI was chosen among other available indices due to its wide applicability within the European Water Framework Directive (WFD) in relation to the use of macrobenthos as a source of information regarding marine environment quality (Borja et al., 2000; Marín-Guirao et al., 2005; Muxika et al., 2005; Reiss and Kroncke, 2005; Carvalho et al., 2006; Labrune et al., 2006; Dauvin et al., 2007; Simboura and Reizopoulou, 2007; Zettler et al., 2007; Bigot et al., 2008). Since this index is based on relative proportion of sensitive/tolerant taxa, the assignment of dominant species into one of the five ecological groups (EG) constitutes a prerequisite for adequate evaluation of the ecosystems, as described by Bigot et al. (2008). However, appropriate assignments are neither necessarily available (Muniz et al., 2005; Flaten et al., 2007) nor easy to achieve outside European coasts, where AMBI was developed (Borja et al., 2000; Bigot et al., 2008). In the present study, the overall pattern of ecological quality status in the sites would be different depending on the assignment of selected species/taxa (see tab.07). For instance, if these taxa were submitted to AZTI database without modifications of its ecological group, the number of sites classified as “Heavily disturbed” would increased from one (Capibaribe) to five (Capibaribe, Paratibe, Jaboatão, Mamucabas and Ipojuca-Merepe), besides, Pirapama, Sta Cruz Channel, Paripe and Ariquindá should be considered as “Moderately disturbed” instead of “Slightly disturbed”. Other surveys also highlighted the necessity of checking the assignment of dominant species to prevent contradictory results from the AMBI as the classification of a certain area may diverge when allocating species to an unsuitable group (Salas et al., 2004; Marín-Guirao et al., 2005; Muniz et al., 2005; Labrune et al., 2006; Albayrak et al., 2006; Bigot et al., 2008).

Another important observation concerning the contributions mentioned above is that new ecological group assignments and re-assignments are attributed in relation to previous data from monitoring programs or author experience on ecological characteristics of faunal communities in the studied ecosystems. According to Salas et al. (2004) this implies on an important exercise of subjectivity and leads to impossibility of establishing fixed reference values. The use of a simple method as the Indicator Value (IndVal) coefficient permits that reviews of assignments of particular taxa to an ecological group might be made in a more objective form and less dependent on the historical information, especially in areas where the knowledge on ecological and environmental characteristics is restricted or unavailable. In this study, despite the limited number of studied sites, significant Indicator Values enabled the objective re-assignments of two important species, *Sternaspis* sp. and *Streblospio* sp.

In general, from the results of this study, the AMBI index worked satisfactorily in disturbance detection, especially in urbanized estuaries, with recognized pollution condition, as showed by monitoring reports (CPRH, 2006). This index was also sufficiently robust to discriminate spatio-temporal effects of organic enrichment in Botafogo and Sirigi, two estuarine systems of north coast of Pernambuco subjected to different sources of pollution (probably aquaculture and sugar cane activities, respectively), as outlined by Valença (2007). Nevertheless, some inconsistencies were found in the classification of estuaries placed far from urban centers and with some level of preservation as Mamucabas, Paripe, Ariquindá and Maracaípe. Previous studies already indicated that most of Biotic Indices displayed limitations in ecosystems with low salinity habitats (Borja and Muxika, 2005a; Muxika et al., 2007) or where sediment organic matter is naturally high (Borja et al., 2003; Salas et al., 2004; Muxika et al., 2005; Albayrak et al., 2006; Lavesque et al., 2009), as the case of tropical estuaries. The species adapted to these habitats typically occur in high numbers and are listed as tolerant or opportunistic (Borja et al., 2000; Dauvin, 2007; Elliot and Quintino, 2007; Simboura and Reizopoulou, 2008). In these sites, macrofauna was dominated by Tubificidae oligochaetes (including *Tectidrilus* sp.) and Nematoda, both taxa assigned here as ecological group III (tolerant species). A similar situation was reported by Blanchet et al. (2008), during the assessment of three French Atlantic coastal areas (Arcachon Bay, Marennes-Oléron Bay and the Seine estuary). These authors observed that some sites, considered healthy and well vegetated (see Blanchet et al., 2004) were classified in moderate or worse status due to the numerical abundance of tolerant and opportunistic species. In the mentioned cases, Borja and Muxika (2005b) recommend the use of AMBI together with other metrics, in order to obtain a more comprehensive view of the environmental condition.

Indeed, a combination of AMBI, richness (S) and evenness (J') seemed to be the best approach in comparison to the index individually, although the three indices together could distinguish the sites in terms of benthic community health (as demonstrated by MDS and ANOSIM analyses) in relation to disturbance levels. These indices are known as measures of biotic integrity of macrobenthic community structure and underline species identity and diversity, which are thought to be intrinsically important features of the benthos (Díaz et al., 2003; Borja et al., 2008). The results from MDS/ANOSIM suggested that benthic communities are separated as a function of ecological quality, being in agreement with the multivariate correlation between the indices and environmental parameters, which pointed out variables associated to pollution condition, such as ammonia-N, dissolved oxygen, organic matter or chlorophyll-*a*. On the other hand, individual AMBI showed high significant correlation simply with ammonia-N. In general, the highest values of AMBI were related to sites with high concentration of ammonia-N, normally representative of hypoxic/anoxic environments or urbanized areas submitted to organic enrichment such as sewage, wastewater outfalls and agricultural runoffs (Pinckney, 2006). In coastal systems, AMBI has been tested against different anthropogenic sources, which includes anoxia and hypoxia, sediment toxicity (metals, PAH), and others (Borja et al., 2003; Muxika et al., 2005), presenting correlation with total organic carbon (TOC), sediment type (including silt-clay content), dissolved oxygen concentration (Borja et al., 2000; Muniz et al., 2005; Carvalho et al., 2006; Borja et al., 2007; Blanchet et al., 2008; Borja et al., 2008; Munari and Mistri, 2008) and also organic matter and pigments (chlorophyll-*a*/phaeopigments) content (Dauvin et al., 2007).

Recently, Dauvin et al. (2007) and Pinto et al. (2009) have tested the effect of mesh-sieve on the values of several indices, and consequently in its classification. Comparing biological samples from Bay of Seine and Seine estuary, sieved with both 2.0mm and 1.0mm, Dauvin et al. (2007) showed that AMBI, BQI (Benthic Quality Index; Rosenberg et al., 2004) and BOPA (Benthic Opportunistic Polychaeta/Amphipoda Index; Dauvin and Ruellet, 2007) exhibited few changes in the scores, being more evident in the last two indices. In Mondego estuary, Pinto et al. (2009) compared the performance of BCI (Benthic Condition Index; Engle and Summers, 1999), B-IBI (Benthic Index of Biotic Integrity; Weisberg et al., 1997) with the proposed P-BAT (Portuguese-Benthic Assessment Tool, an integration of Shannon-Wiener index, Margalef index and AMBI) in relation to two meshes sizes (1.0mm and 1+0.5mm) and observed that when both sieves were used, the indices exhibited a decrease in environmental conditions at the polyhaline sand stations but best conditions in the euhaline estuarine area; moreover the combined sieves presented robustness to determine the overall system classification. Concerning this study, no pattern was found between AMBI classification and salinity, but the use of different sieves (1.0mm x combined meshes) performed a surprising result. Once small species associated to organic enrichment usually passe through coarser sieves (>1.0mm; Schlacher and Wooldridge, 1996; Thompson et al., 2003; Teixeira et al., 2007), it was expected that the use of 0.5mm could provide a worse pollution condition (e.g. Pinto et al., 2009) of the sites. In fact, the combined sieves increased the retention of small specimens (mainly Capitellidae polychaetes, *Streblospio* sp., Tubificidae oligochaetes and nematodes) assigned here as ecological groups III and V, but the higher proportion of tolerant species in contrast to the opportunistic ones (for both sieves compared to 1.0mm) was responsible for the balance of intermediate levels of disturbance, reflecting better system conditions in most sites. On the other hand, the sampling depth seemed to have less influence on AMBI classification. Despite the fact that differences between 0-10cm and 0-20cm were in terms of few individuals, the biotic index account for the abundance and taxa composition and thus was susceptible to these parameters, even though it has not affected the overall community assessment. Furthermore, the separation of sites considering the location from urban centers revealed that pollution condition assessment of urbanized and conserved estuaries given by this index is clearly related to both factors – mesh-sieve and sampling depth

In summary, AMBI proved to be efficient in evaluating the quality status of Pernambuco estuaries, although its applicability requests some adaptations in species' ecological groups. It also demonstrated that the investment in tools as the IndVal coefficient might be essential in establishing fixed references for a particular or a group of indicator species, without depending on prior information on the taxa of the area of study. Moreover, the influence of mesh-sieve and sampling depth are visible on benthic indices' performance considering the effect of urbanization in these estuaries.

II.5. Conclusion

In order to determine the biological integrity of macrobenthic communities, every method must incorporate biotic responses through the evaluation of processes from individuals to ecosystems. Therefore, the mutual use of some metrics allows the integration of biological features, providing a more accurate diagnosis of the ecosystem's overall condition. Several studies reveal that none of the available indices applied solely should be considered ideal to measure biological effects of pollution, because each index was originally developed for one or few stressors. Thus, the complementary use of different indices or methods based on different ecological principles is highly recommended for the assessment of environmental quality. It is suggested that prior to the application of AMBI index, attention must be taken with the pre-established assignment of an ecological group to each of the sampled species, particularly the dominant ones. In addition, the present study emphasizes the need for careful methodological procedures, including the choice of an appropriate mesh-sieve or sampling depth in characterizing ecological quality of tropical estuarine ecosystems with biotic indices (such as AMBI) based on macrobenthos relative abundance and composition.

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