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Factors influencing spatial patterns of molluscs in a eutrophic tropical bay

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Samples were collected from 10 stations distributed through three sectors in Guanabara Bay during two consecutive years, in order to determine factors that influence the spatial pattern of molluscs and to describe the structure and composition of this community in a eutrophic estuarine system on the Brazilian coast. Although only one species, the gastropod Heleobia australis, *comprised 77% of mollusc abundance, 59 species were identified in the bay. In addition to* H. australis, *three other species were dominant: the gastropod* Anachis isabellei *and the bivalves* Americuna besnardi *and* Ervilia concentrica. *The mollusc communities were significantly influenced by the spatial gradient; the outermost sector has marine conditions, and the other sectors are typically estuarine, leading to differences in the composition and abundance of molluscs. The outermost sector showed the highest diversity, which gradually decreased towards the innermost sector where the dominance of a few opportunistic species is favoured by highly organic mud sediments. Sediment type was strongly correlated with mollusc occurrence in the bay. Guanabara Bay showed two indicator species: the bivalve* E. concentrica *of the outer sector, and the gastropod* H. australis *of the intermediate sector. Our results suggest that benthic molluscs in Guanabara Bay show characteristics related to levels of environmental impact. A monitoring programme based on this community is needed to evaluate the effects of human impacts on this community and to monitor changes in its biodiversity in Guanabara Bay.*

Keywords: indicator species, multivariate analysis, Guanabara Bay, Brazil

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INTRODUCTION

Soft-sediment benthic communities have a critical role in the functioning of estuaries. They are a food resource for vertebrates and invertebrates, and act in nutrient cycling by the consumption and incorporation of organic matter deposited in the sediment (Snelgrove *et al.*, 1997; Weslawski *et al.*, 2004). Alterations in the physical and chemical characteristics of the sediment can affect the biology of benthic communities (Margalef, 1983). An evaluation of distribution patterns is the first step to understand the set of processes that structure the community, and thus to formulate predictive models (Underwood, 2000). Scale-dependent patterns of distribution may be particularly important in coastal areas, since they are characterized by variability in abiotic conditions. For benthic fauna, patterns of distribution are related to depth, salinity (Atrill, 2002; Attrill & Rundle, 2002; Ysebaert *et al.*, 2003), sediment characteristics (Day *et al.*, 1989; Teske & Wooldridge, 2003; Anderson *et al.*, 2004) and other factors.

Spatio-temporal variability in benthic macrofauna has been studied in estuaries and bays throughout the world (Morrisey *et al.*, 1992; Ieno & Bastida, 1998; Biles *et al.*, 2003; Giberto *et al.*, 2004). This variability is dependent on physical and chemical factors and biological interactions. Salinity fluctuations in estuarine systems have a strong influence on temporal patterns and daily and inter-annual variations; sediment characteristics have more influence on the spatial structure of benthic assemblages. Other factors should not be disregarded, since they can act in synergy (Bemvenuti & Colling, 2010). Benthic macrofauna in estuarine systems forms a mosaic of patches, which are maintained by a variety of disturbances and other biotic and abiotic factors (Morrisey *et al.*, 1992); and a seasonal pattern that may be related to the temporal variability of the water column.

The distribution of benthic communities in Guanabara Bay (GB) varies significantly in space and time, whereas the spatial pattern is the most prominent. Species diversity and abundance of benthic groups (molluscs, crustaceans, polychaetes and foraminifera) differ among the areas of the bay, increasing from the inner to the outer sector as a function of the bay's gradient (Lavrado *et al.*, 2000; Eichler *et al.*, 2003; Mendes *et al.*, 2004; Van der Ven *et al.*, 2006; Santi & Tavares, 2009). Molluscs constitute one of the largest phyla of invertebrates, in both numbers of living species and numbers of individuals (Gomes *et al.*, 2004). The classes Gastropoda and Bivalvia are the best represented in benthic systems, and their species have been used to characterize benthic associations (Diaz & Puyana, 1994).

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This characterization could suggest means of sustainable exploitation and appropriate management of commercially exploited species (Silva *et al.*, 2005), as well as providing important data for biodiversity evaluations.

The objectives of this study were to: (a) determine the spatial patterns of mollusc communities; (b) describe the structure and composition of this community; and (c) identify indicator species in a eutrophic estuarine system on the Brazilian coast. The study contributes to the understanding of the distribution of this mollusc assemblage and its relationship with environmental variables, since few studies have treated the mollusc communities in this tropical estuarine system (Oliveira, 1950; Batalha *et al.*, 1998; Mendes *et al.*, 2004, 2007). This work may also contribute to monitoring programmes in GB, since it can be used as a reference point in a long-term perspective.

MATERIALS AND METHODS

Study area

Guanabara Bay in the State of Rio de Janeiro is one of the largest bays in Brazil. The drainage covers an area of 4080 km^2, with 45 rivers and streams, although only six rivers are responsible for 85% of the total mean annual freshwater discharge of 100 m^{3}s^{-1} (JICA, 1994). The local climate is humid tropical, with a rainy period during the summer (December–April) and a dry period during the winter (June–August). Freshwater discharge varies during the year, from 33 m^{3}s^{-1} in winter to 186 m^{3}s^{-1} in summer (Kjerfve *et al.*, 1997). The estuary has a semi-diurnal tidal regime, with tidal amplitude ranging from 1.4 to 0 m (Amador, 1997). GB is a semi-enclosed water body surrounded by urban areas, with more than nine million people living in the immediate surroundings of the bay (IBGE, 2009). The bottom sediments are soft, ranging from sand in the outer sector to muddy in the intermediate and inner sectors; there is a strong influence of marine intrusion from the central channel (Mayr *et al.*, 1989). Human impacts including river canalization and landfills, inputs of untreated domestic sewage and industrial residues, ship-source oil pollution, and urban wastes were related to the disappearance of some species, mainly echinoderms, and reduction in crab abundance (Oliveira, 1958). In general, sediments are toxic, with high levels of heavy metals, mainly wastes from industries and oil refineries, organic pollution, petroleum derivatives and pathogenic organisms (Pereira *et al.*, 2007).

Water exchange is mainly tidally driven, through the central channel that allows the Atlantic water to extend far into the bay. Regarding pollution and water exchange, three macro-sectors have been defined in the bay (Nogueira *et al.*, 1989; Wandeness *et al.*, 1997): (A) outer sector, close to the central channel and under the strong influence of the Atlantic Ocean; (B) intermediate sector, seasonally influenced by the inner or outer sector; and (C) inner sector, more influenced by the river drainage (Figure 1). The outer sector is less submitted to anthropogenic stress, with the exception of point sources of sewage (Icaraí submarine sewage outfall) and heavy metals at Jurujuba Sound (close to station 03: Baptista-Neto *et al.*, 2000; Maranho *et al.*, 2010). Intermediate sectors are predominantly submitted to organic inputs with high contamination of sediments, indicating severe sewage contamination (mainly at station 10: Carreira *et al.*, 2004). Inner sectors are submitted to sewage inputs and heavy metals derived from combustion of fossil fuels together with some direct petrogenic input, moderately to highly contaminated area, an oil refinery is located close to station 28 (Carreira *et al.*, 2004; Silva *et al.*, 2007; Borges *et al.*, 2009).

Data collection

Ten sampling sites (stations) were chosen based on previous studies concerning biological and physico-chemical data on GB. Stations were distributed in shallow areas from the mouth to the inner area; three stations were located in the outer sector, three in the intermediate sector, and four in the inner sector (Figure 1). Six seasonal samples were taken during two consecutive years: winter (July 2005); summer (December 2005); autumn (April 2006); winter (July 2006); summer (December 2006); and autumn (April 2007). From each site, each sample was taken in ten replicates, with a gravity corer (0.008 m^2) in mud sediments, and by divers with the same corer in sandy sediments. Samples were sieved (0.5 mm) and individuals were preserved with 70% ethanol *in situ*. In the laboratory, individuals were identified and counted with the aid of a stereoscopic microscope.

Water temperature was measured with a graduated thermometer. Salinity and dissolved oxygen were evaluated by the chlorinity and Winkler methods respectively (Grasshoff *et al.*, 1999).

Sediment samples (50 g fresh weight) were collected to determine the sediment type and to analyse organic matter in the sediment (OM). The latter was calculated from the weight loss after ignition (LOI) at 500°C for 4 hours, after previously drying for 48 hours at 60°C (APHA, 1998). Sediment was categorized by major fraction (mud/sand), based on previous results (Baptista-Neto *et al.*, 2006).

Statistical analysis

The following ecological parameters were assessed to describe the structure and composition of molluscs: frequency (Fr) of organisms as % of occurrence; abundance and relative abundance (Ab, %); taxa richness (S; total number of taxa per site); taxonomic diversity expressed in Shannon–Wiener index (H′); and Pielou's evenness (J).

Canonical correspondence analysis (CCA) was performed as an exploratory multivariate method to evaluate possible associations among species and to identify the environmental factors most influential to species distribution. Tolerance values ($=1-r^2$, inverse of variance inflation factor) were calculated for each environmental variable; all values were >0.10 indicating a stable model with no collinearity between independent variables (Quinn & Keough, 2002). A forward multiple regression was performed using the score samples on significant axes of CCA (as dependent variable integrating spatial distribution of molluscs) and environmental variables (as explanatory variables), for both Bivalvia and Gastropoda.

Indicator species analysis (ISA) was applied to identify indicator species, using the sector as categorical variable and a minimum indicator value of 60. Statistical significance was analysed with the Monte Carlo test using non-restricted permutations ($N = 999$ permutations, $P < 0.05$). Ordinations were performed using the PC-Ord v.4 statistical package (MjM software: Legendre & Legendre, 1998).

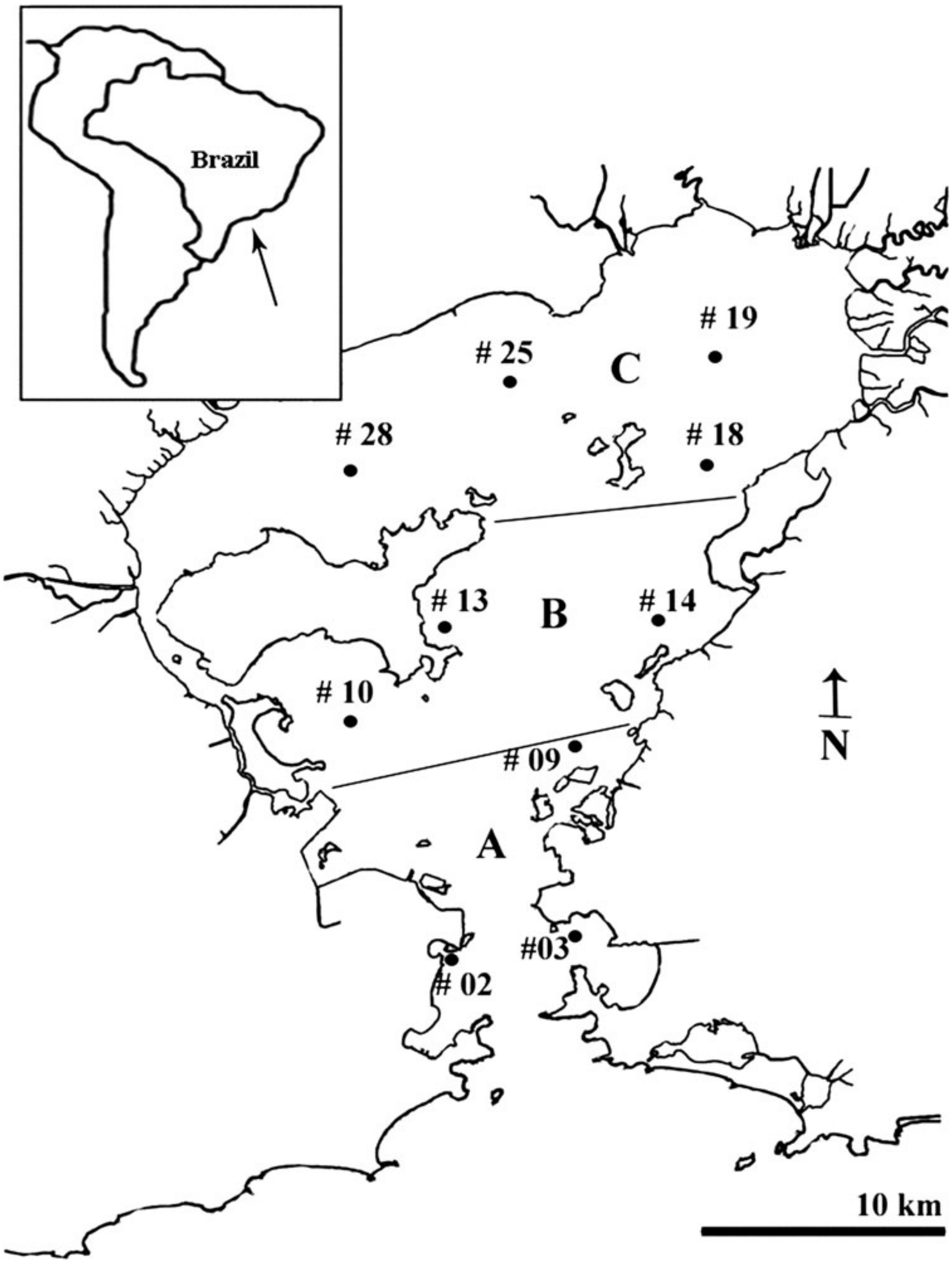

Fig. 1. Sampling sites in Guanabara Bay, Rio de Janeiro, Brazil: three macro-sectors are indicated: (A) outer sector; (B) intermediate sector; (C) inner sector.

Spatial variation in environmental variables was tested by one-way analysis of variance (ANOVA). A partly-nested ANOVA was undertaken to evaluate the influence of different factors on molluscs density, with the fixed variables Sectors and Sampling crossed, and the variable Stations nested within Sectors and crossed with Sampling. This statistical test was performed using R statistical environment (R Development Core Team, 2008), with corrections for each error-term, as described by Quinn & Keough (2002). Normality and variance homogeneity test (Levene, $P > 0.05$) was applied to the data, as assumptions for the parametric test. When the statistical test showed a significant result, the Tukey test was applied *a posteriori*.

Table 1. Environmental variables range in each sector: (A) outer, (B) intermediate and (C) inner.

Sector	T	DO	S	OM
A	21.0–26.0	3.14–8.11	29.6–35.1	0.18–1.86
B	21.0–26.25	1.07–4.68	27.55–34.96	2.65–8.16
C	18.0–25.62	1.26–4.85	27.02–33.35	4.45–9.49
GB	23.06 ± 1.81	3.17 ± 1.28	31.97 ± 2.20	4.68 ± 3.73

RESULTS

Physical and chemical characteristics

The environmental results indicated spatial differences among sectors and the natural trend of estuarine systems, increasing in salinity and dissolved oxygen content from the inner to outer bay (Table 1).

Temperatures were highest in the inner sector (23.48°C ± 1.77), with a significant difference between outer and inner sectors (23.76°C ± 1.88; $P = 0.004$). There were significant differences in temperature among sampling periods ($P = 0.05$), although these were not well marked. Dissolved oxygen content was highest in the outer sector (4.16 $\mathrm{mgl^{-1}}$ ± 1.31), decreasing significantly towards the intermediate (3.02 $\mathrm{mgl^{-1}}$ ± 1.03; $P = 0.008$) and inner sectors (2.86 $\mathrm{mgl^{-1}}$ ± 1.12; $P < 0.001$). It was not possible to detect significant temporal differences in dissolved-oxygen content. Salinity was highest in the outer sector (33.8 psu ± 1.57) and decreased significantly towards the intermediate (31.9 psu ± 2.13; $P = 0.009$) and inner sectors (31 psu ± 1.7; $P < 0.001$). There were also significant differences in salinity among sampling periods ($P = 0.05$), although the differences were not well marked. Organic matter in the sediment was lowest in the outer sector (0.78% ± 0.8) and increased significantly towards the intermediate (4.78% ± 1.73; $P = 0.04$) and inner sectors (7.56% ± 1.78; $P < 0.001$). Sediments were dominated by sand (0.062–0.016 mm) in the outer sector, and by mud, fine silt (0.008 mm) and clay (0.002 mm) in the intermediate and inner sectors. According to rainfall data for Rio de Janeiro State obtained from the National Institute of Meteorology (INMET, 2005–2007; Echeverría *et al.*, 2010), the first sampling year followed the historical average of rainfall amount (about 95 mm); however, the second year was drier than expected (< 40 mm), showing a significant difference and absence of pattern, both seasonally and between sampling years ($P = 0.02$).

Mollusc assemblages

SPECIES COMPOSITION AND PATTERN OF SPATIAL DISTRIBUTION

A total of 59 mollusc species were found in the study area: 31 bivalves, belonging to 15 families, and 28 gastropods in 17 families. Most of the bivalves occurred sporadically, and only *Nucula semiornata* occurred occasionally (46.7%). Most gastropods also occurred sporadically; only *Anachis isabellei* was common (53.3%) and *Heleobia australis* very common (81.7%). *Heleobia australis* was the most abundant species in GB, comprising 77% of total mollusc abundance, followed by 23% bivalves and 2% other gastropods. The bivalves *Americuna besnardi* and *Ervilia concentrica*, and the gastropod *Anachis isabellei* were also abundant. Bivalve abundance was higher in the outer (2.538 individuals (ind)) and intermediate sectors (521 ind), and lowest in the inner sector (13 ind). Gastropod abundance was higher in the intermediate (7.299 ind) and inner sectors (3.283 ind), and lowest in the outer sector (149 ind).

Species richness, diversity index and evenness gradually decreased towards the inner sector (Table 2). Modes of feeding in molluscs were different between bivalves and gastropods; most of the bivalves are suspension-feeders, whereas the gastropods show diverse feeding modes. The mollusc composition was different amongst sectors. Only eight species were found in all sectors: the bivalves *Ervilia concentrica* (Fr 20%), *Nucula semiornata* (Fr 46.67%), *Tellina exerythra* (Fr 6.67%), and *Transennella stimponi* (Fr 20%); and the gastropods *Acteocina bidentata* (Fr 11.66%), *Anachis isabellei* (Fr 53.33%), *Heleobia australis* (Fr 81.67%), and *Natica pusilla* (Fr 13.33%). There were differences in species number and composition between sampling years, as well as differences in the occurrence of rare or sporadic species. Six bivalves and two gastropods occurred only in the first year, when 44 species were found; five bivalves and 11 gastropods occurred only in the second year, when 51 species were found. These species are listed in Table 2.

Four species are dominant in GB: the bivalves *Americuna besnardi* and *Ervilia concentrica*, and the gastropods *Anachis isabellei* and *Heleobia australis*. According to their distribution, dominant bivalves were most numerous in the outer sector, whereas dominant gastropods were distributed throughout all the sectors. *Anachis isabellei* was most numerous in the intermediate sector, and *H. australis* in the intermediate and inner sectors.

Bivalves were significantly influenced by all sources of variation (Table 3). Significant differences were apparent among sampling occasions ($P = 0.001$) and sectors ($P = 0.05$). Contrarily, it was not possible to detect significant differences in sampling occasion ($P = 0.24$) and sectors ($P = 0.07$) for gastropods.

A significant interaction between sampling occasion and sector for mollusc assemblages, bivalves ($P = 0.01$) and gastropods ($P = 0.04$), indicates variation in sectors behaviour at different samplings (snap shots). The spatial pattern shows a dynamic behaviour within sectors and among stations (Figure 2). Despite variations within each sector through sampling occasions, variations among sectors are greater, being indicative of heterogeneity, which determines the significant difference for molluscs among sectors at GB ($P = 0.001$, station (sectors); Table 3).

The highest bivalve density was found at the outer station in autumn (4.462 ind $\mathrm{m^{-2}}$); the same pattern was found in the intermediate sector, with a lower density (1.830 ind $\mathrm{m^{-2}}$). The highest gastropod density was found in the intermediate sector in winter (10.184 ind $\mathrm{m^{-2}}$), which was the highest density recorded. Mollusc densities showed nearly inverse patterns (Figure 2): the density of bivalves decreased towards the inner sector, where they were nearly absent; contrariwise, the gastropods showed the lowest density in the outer sector and the highest in the intermediate sector. Peaks in density of bivalves could be seen in the intermediate (station 13) and inner sectors (station 19) and, simultaneously, abrupt decline of gastropods in the same stations.

ENVIRONMENTAL INFLUENCE ON SPECIES DISTRIBUTION

Two CCAs were applied separately to the bivalve and gastropod data collected at ten stations for six periods (600 samples). Temperature (T), dissolved oxygen (DO), salinity (S), organic matter (OM) and sediment type (G) were the environmental variables used in the analysis. In both analyses, only the first canonical axis was significant ($P = 0.001$), explaining 19.8% and 20.9% of total variance for bivalves and gastropods respectively, against only 3.9% and 2.1% explained by the second canonical axis. The results showed a high correlation among bivalve and gastropod species and the environment on the first axis ($r = 0.82$ and 0.80, respectively), and only 0.58 and 0.56 on the second axis. CCA (axes I–II) allowed us to evaluate species associations in different sectors of GB, which were confirmed later by ISA.

Table 2. Species abundance, feeding modes (FM), relative abundance (Ab (%)) and relative abundance without the dominant species (Ab$_{\text{hel}}$ (%)), total relative frequency (Fr (%)), richness (S), evenness (J) and taxonomic diversity (H′) in each sector of GB (A, outer sector; B, intermediate sector; C, inner sector). Feeding modes: (ND) not determined; (S) suspension-feeder; (D) deposit-feeder; (C) carnivore; (G) grazer; and (O) omnivore.

	FM	Sector			Ab	Ab$_{\text{hel}}$	Fr
		A	B	C			
Bivalvia							
Abra-cf.-uruguayensis (Pilsbry, 1897)**	ND	1	0	0	0.01	0.03	1.67
Americuna besnardi (Klappenbach, 1962)	S[1]	768	0	0	5.54	22.16	11.67
Anomalocardia brasiliana (Gmelin, 1791)	S[2]	53	2	0	0.39	1.59	5.00
Botula fusca (Gmelin, 1791)*	S[3]	1	0	0	0.01	0.03	1.67
Carditamera micella (Penna, 1971)	S[1]	201	1	0	1.50	6.00	13.33
Chione cancellata (Linnaeus, 1767)	S[2]	20	0	0	0.14	0.58	11.67
Corbula cubaniana (d'Orbigny, 1842)	S[2]	4	11	0	0.11	0.43	15.00
Crassinella marplatensis (Castellanos, 1970)	S[1]	260	0	0	1.87	7.50	15.00
Crassinella martinicensis (d'Orbigny, 1842)	S[1]	199	0	0	1.43	5.74	13.33
Ctena pectinella (Adams, 1852)**	ND	2	10	0	0.08	0.34	8.33
Ctena sp. (Adams, 1852)	ND	11	1	0	0.08	0.34	10.00
Ervilia concentrica (Holmes, 1860)	S[4]	374	2	1	2.72	10.88	20.00
Gouldia cerina (Adams, 1845)	S[3]	119	5	0	0.90	3.58	15.00
Hiatella arctica (Linnaeus, 1767)*	S[5]	0	71	0	0.51	2.05	1.67
Lasaea adansoni (Gmelin, 1791)	S[6]	2	0	0	0.01	0.06	3.33
Limopsis sp. (Sasso, 1827)**	S[1]	3	0	0	0.02	0.09	3.33
Lucina pectinata (Gmelin, 1791)	S[2]	1	0	0	0.01	0.03	1.67
Modiolus carvalhoi (Klappenbach, 1966)*	S[7]	0	1	0	0.01	0.03	1.67
Modiolus sp. (Lamarck, 1799)**	S[7]	0	1	0	0.01	0.03	1.67
Musculus lateralis (Say, 1822)	S[1]	1	69	0	0.50	2.02	3.33
Mytilidae sp. (Rafinesque, 1815)	S[3]	36	241	0	2.52	10.1	11.67
Nucula semiornata (d'Orbigny, 1846)	D[1]	89	18	6	0.81	3.26	46.67
Pinctada imbricata (Roding, 1798)*	S[3]	0	42	0	0.30	1.21	1.67
Semele nuculoides (Conrad, 1841)	D[8]	16	0	4	0.14	0.58	16.67
Semele purpurascens (Gmelin, 1791)	D[3]	202	0	0	1.44	5.77	10.00
Semele sp. (Schumacher, 1817)*	S/D[3,9]	4	0	0	0.03	0.12	1.67
Tellina exerythra (Boss, 1964)	ND	2	1	1	0.03	0.12	6.67
Tellina sp. (Linnaeus, 1758)	S/D[2,10]	1	0	0	0.01	0.03	1.67
Thracia similis (Couthony, 1839)*	ND	1	0	0	0.01	0.03	1.67
Transenella cubaniana (d'Orbigny, 1842)**	S[11]	128	1	0	0.93	3.72	16.67
Transenella stimpsoni (Dall, 1902)	ND	33	44	1	0.53	2.11	20.00
Gastropoda							
Acteocina bidentata (d'Orbigny, 1841)	C[12]	7	11	5	0.17	0.66	11.66
Acteocina bullata (Kiener, 1834)*	ND	1	1	0	0.01	0.06	3.33
Aesopus stearnsii (Tryon, 1883)**	ND	1	0	0	0.01	0.03	1.67
Alvania faberi (Jong & Coomans, 1988)**	ND	3	0	0	0.02	0.09	1.67
Anachis isabellei (d'Orbigny, 1841)	ND	10	84	25	0.86	3.43	53.33
Bittiolum varium (Pfeiffer, 1840)	G[13]	10	0	0	0.07	0.29	8.33
Caecum brasilicum (Folin, 1874)	ND	13	2	0	0.10	0.43	8.33
Caecum ryssotitum (Folin, 1867)	ND	2	2	0	0.03	0.12	6.67
Caecum someri (Folin, 1867)	ND	4	0	0	0.03	0.12	6.67
Cerithiopsis sp. (Forbes & Hanley, 1851)**	C[12]	1	0	0	0.01	0.03	1.67
Chrysallida sp. (Carpenter, 1857)	ND	15	0	0	0.11	0.43	5.00
Epitonium sp. (Roding, 1798)**	C[12]	2	0	0	0.01	0.06	1.67
Finella dubia (d'Orbigny, 1842)	ND	8	0	1	0.06	0.26	10.0
Gabrielona sulcifera (Robertson, 1973)*	ND	2	2	0	0.03	0.12	5.00
Heleobia australis (d'Orbigny, 1835)	D[14]	15	7.139	3.249	75.0	-	81.67
Melanella arcuata (Adams, 1850)**	ND	3	0	0	0.02	0.09	1.67
Melanella sp. (Bowdich, 1822)	S[10]	8	0	0	0.06	0.23	6.67
Natica pusilla (Say, 1822)	C[15]	7	7	1	0.11	0.43	13.33
Natica sp. (Scoploi, 1777)**	C[10]	1	0	0	0.01	0.03	3.33
Nassarius vibex (Say, 1822)**	C[2]	0	1	1	0.01	0.06	3.33
Odostomia sp. (Fleming, 1813)	S[10]	8	47	0	0.40	1.59	8.33
Olivella minuta (Link, 1807)	C[2]	9	1	0	0.07	0.29	13.33
Olivella sp. (Swainson, 1831)**	C[2]	2	0	0	0.01	0.06	3.33
Parviturboides interruptus (Adams, 1850)**	O[16]	3	0	0	0.02	0.09	5.00
Rissoina sp. (d'Orbigny, 1840)	D[17]	1	1	0	0.01	0.06	3.33
Teinostoma cocolitoris (Pilsbry & McGinty, 1945)**	ND	3	0	0	0.02	0.09	5.00
Turbonilla sp. 1. (Risso, 1826)	S[10]	9	1	0	0.07	0.29	13.33

Continued

Table 2. Continued

	FM	Sector			Ab	Ab$_{hel}$	Fr
		A	B	C			
Turbonilla sp. 2. (Risso, 1826)**	S[10]	1	0	1	0.01	0.06	1.67
S		54	30	12			
J		0.63	0.14	0.04			
H′		2.50	0.49	0.10			

*, occurrence only in the first year; **, occurrence only in the second year. Ab$_{hel}$: *Heleobia australis* was excluded from the calculations, in order to detect important species for the community structure that were present in lower densities. [1]Soares-Gomes & Pires-Vanin (2005), [2]Arruda *et al.* (2003), [3]Cerridwen & Jones (1991), [4]Hauser *et al.* (2007), [5]Petersen *et al.* (2003), [6]Tyler-Walters (2008), [7]Mohan *et al.* (1986), [8]Rakocinski *et al.* (1997), [9]Pohlo (1969) and Ólafson (1986), [10]Macdonald *et al.* (2010), [11]Rehder (1981), [12]Marenzi *et al.* (2006), [13]Cote *et al.* (2001), [14]Rios (1994), [15]Livingston (2002), [16]Barnes (1987), [17]Dowgiallo (2004).

For bivalves, the CCA clearly distinguished samples of the outer sector (positive coordinates on the first axis) from samples of the intermediate and inner sectors (negative coordinates on the first axis: Figure 3). Only two vectors are significant explanatory variables, G is the most important factor explaining 51% ($P < 0.001$) and S explains 21% ($P = 0.003$) of the distribution of bivalves. The strong positive direction of vectors G, S and DO (0.7%, $P = 0.53$) confirms the environmental characteristics of the outer sector; the sediment is dominated by sand with less organic matter, high salinity and dissolved oxygen, and low temperature. The positive projection of bivalve species on the first axis, mainly the significant variables G and S, shows their preferences for the outer sector in GB. The major examples are *Ervilia concentrica* at the extremity of axis I; and a species association constituted by *Americuna besnardi*, *Carditamera micella*, *Crassinella marplatensis*, *Crassinella martinicensis*, *Gouldia cerina* and *Semele purpurascens*. Two other species, *Nucula semiornata* and *Transennella cubaniana*, were also present in this area, although they were positively influenced by axis II.

Contrarily, on the negative side of the first canonical axis, samples from the intermediate and inner sectors were superimposed and joined with vectors OM (4%, $P = 0.15$) and T (1.4%, $P = 0.38$). The canonical analysis confirmed the environmental characteristics of these sectors, with a dominance of fine-grained sediment (muddy) with high organic matter, low salinity and dissolved oxygen in deep water, and high water temperature. Numerous bivalves had a projection on the negative side of axis I. An example is the species association *Abra*-cf.-*uruguayensis*, *Anomalocardia brasiliana*, *Botula fusca*, *Hiatella arctica*, *Lasaea adansoni*, *Limopsis* sp., *Lucina pectinata*, *Modiolus carvalhoi*, *Modiolus* sp., *Musculus lateralis*, *Pinctada imbricata*, *Semele* sp., *Tellina exerythra*, *Tellina* sp. and *Thracia similis*; as well as the species *Corbula cubaniana*, *Ctena pectinella* and *Mytilidae* sp.

Chione cancellata, *Ctena* sp. and *Semele nuculoides* had a central projection and likelihood of occurrence in the different areas in GB.

The CCA results for the gastropod assemblage were similar to those for bivalves. Three vectors are significant explanatory variables: G (50%, $P < 0.001$); OM (23%, $P < 0.001$); and DO (8%, $P = 0.039$). Samples from the outer sector (positive coordinates on axis I) were distinguished by the first canonical axis, and the sector was characterized by the vectors G, S (0.6%, $P = 0.58$), and DO, in contrast to samples from the intermediate and inner sectors (negative coordinates on axis I) characterized by the vectors OM and T (3%, $P = 0.18$) (Figure 4).

Gastropod species with different affinities occur in this environmental context, such as the species association *Aesopus stearnsii*, *Alvania faberi*, *Bittiolum varium*, *Caecum brasilicum*, *Caecum ryssotitum*, *Caecum someri*, *Cerithiopsis* sp., *Chrysallida* sp., *Epitonium* sp., *Finella dubia*, *Melanella arcuata*, *Melanella* sp., *Natica* sp., *Olivella* sp.,

Table 3. Results of the partly nested analysis of variance testing for differences among Sampling occasion (fixed factor), bay's Sector (fixed factor) and Stations (random and nested within Sector) in densities (log-transformed) of bivalves and gastropods at Guanabara Bay. Statistically significant *P* values are indicated with boldface type.

Source—Bivalvia	df	SS	MS	F	*P*
Sampling	5	422.48	84.50	9.74	**0.001**
Sector	2	1541.95	770.97	8.25	**0.05**
Sampling*sector	10	323.27	32.33	3.72	**0.01**
Station (sector)	7	654.40	93.49	24.0	**0.001**
Station (sector)*sampling	35	303.93	8.68	2.23	**0.001**
Residuals	540	2103.77	3.90		
Source—Gastropoda					
Sampling	5	175.53	35.11	1.43	0.24
Sector	2	1710.71	855.36	3.88	0.07
Sampling*sector	10	536.24	53.62	2.18	**0.04**
Station (sector)	7	1542.39	220.34	40.18	**0.001**
Station (sector)*sampling	35	861.13	24.60	4.49	**0.001**
Residuals	540	2961.14	5.48		

df, degrees of freedom; SS, sums of squares, MS, mean squares; F, Fisher's F.

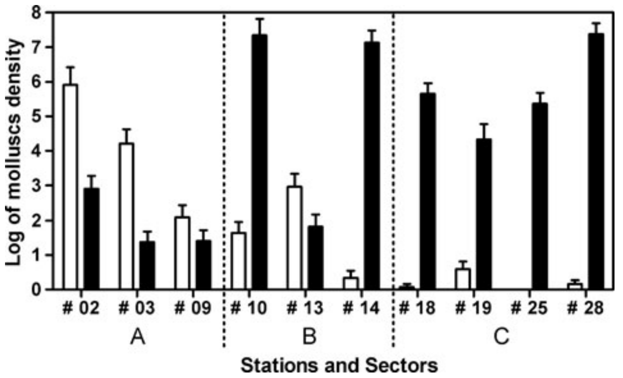

Fig. 2. Spatial distribution (ln ± standard error) of molluscs in stations at Guanabara Bay: Bivalvia (white bar) and Gastropoda (black bar). The bay sectors are represented by: (A) outer, (B) intermediate and (C) inner. The density is given on a logarithmic scale, to facilitate visualization.

Parviturboides interruptus, *Teinostoma cocolitoris* and *Turbonilla* sp. 2 related to the outer sector; and the species *Olivella minuta* and *Turbonilla* sp. 1, which were positively coordinated with axis I.

The strong negative coordinate of *Heleobia australis* on axis I, confirms its dominance and higher densities in the intermediate and inner sectors where fine-grained, highly organic mud sediment predominates. *Anachis isabellei* also tended to dominate these two sectors.

Natica pusilla had a distinct position on the positive side of axis II, reflecting a strong positive projection for the vector DO. *Acteocina bidentata*, *Olivella minuta* and *Turbonilla* sp. 1 also had a positive projection for this vector; however, *Anachis isabellei*, *Heleobia australis* and *Nassarius vibex* had a negative projection for the vector DO.

The other species were grouped in associations with a central position in relation to axis I, reflecting their wide distribution in different areas in GB, with no specific affinity.

The ISA was applied to the molluscs, using the bay's sectors as grouping variables. Two species were indicators in GB, with an indicator value greater than 60 and statistical significance ($P < 0.05$): the bivalve *Ervilia concentrica* ($P = 0.0002$; indicator value = 66.2) in the outer sector and the gastropod *Heleobia australis* ($P = 0.004$; indicator value = 70.3) in the intermediate sector. The inner sector did not show indicator species, which is probably related to the low frequency and dominance of species in this area.

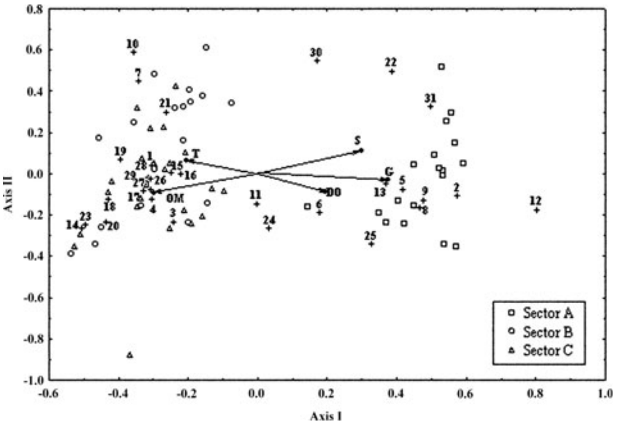

Fig. 3. Biplot of ecological preferences of bivalves in Guanabara Bay: first and second axes of an ordination diagram produced by canonical correspondence analysis. Quantitative environmental variables are indicated by arrows and shown as codes: (T) temperature; (OM) organic matter; (DO) dissolved oxygen; (G) sediment type; and (S) salinity. Bay sectors are indicated by symbols: (☐) A, outer; (○) B, intermediate; (Δ) C, inner. Species are represented by numbers (1–31): (1) *Abra*-cf.-*uruguayensis*; (2) *Americuna besnardi*; (3) *Anomalocardia brasiliana*; (4) *Botula fusca*; (5) *Carditamera micella*; (6) *Chione cancellata*; (7) *Corbula cubaniana*; (8) *Crassinella marplatensis*; (9) *Crassinella martinicensis*; (10) *Ctena pectinella*; (11) *Ctena* sp.; (12) *Ervilia concentrica*; (13) *Gouldia cerina*; (14) *Hiatella arctica*; (15) *Lasaea adansoni*; (16) *Limopsis* sp.; (17) *Lucina pectinata*; (18) *Modiolus carvalhoi*; (19) *Modiolus* sp.; (20) *Musculus lateralis*; (21) *Mytilidae* sp.; (22) *Nucula semiornata*; (23) *Pinctada imbricata*; (24) *Semele nuculoides*; (25) *Semele purpurascens*; (26) *Semele* sp.; (27) *Tellina exerythra*; (28) *Tellina* sp.; (29) *Thracia similis*; (30) *Transennella stimpsoni*; (31) *Transennella cubaniana*. Only the first axis was significant (*P*: 0.001).

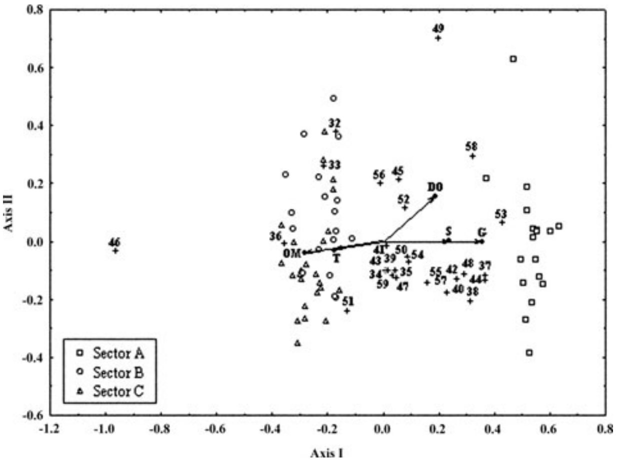

Fig. 4. Biplot of ecological preferences of gastropods in Guanabara Bay: first and second axes of an ordination diagram produced by canonical correspondence analysis. Quantitative environmental variables are indicated by arrows and shown as codes: (T) temperature; (OM) organic matter; (DO) dissolved oxygen; (G) sediment type; and (S) salinity. The sectors of the bay are indicated by symbols: (☐) A, outer; (○) B, intermediate; (Δ) C, inner. Species are represented by numbers (32–59): (32) *Acteocina bidentata*; (33) *Acteocina bullata*; (34) *Aesopus stearnsii*; (35) *Alvania faberi*; (36) *Anachis isabellei*; (37) *Bittiolum varium*; (38) *Caecum brasilicum*; (39) *Caecum ryssotitum*; (40) *Caecum someri*; (41) *Cerithiopsis* sp.; (42) *Chrysallida* sp.; (43) *Epitonium* sp.; (44) *Finella dubia*; (45) *Gabrielona sulcifera*; (46) *Heleobia australis*; (47) *Melanella arcuata*; (48) *Melanella* sp.; (49) *Natica Pusilla*; (50) *Natica* sp.; (51) *Nassarius vibex*; (52) *Odostomia* sp.; (53) *Olivella minuta*; (54) *Olivella* sp.; (55) *Parviturboides interruptus*; (56) *Rissoina* sp.; (57) *Teinostoma cocolitoris*; (58) *Turbonilla* sp. 1.; (59) *Turbonilla* sp. 2. Only the first axis was significant (*P*: 0.001).

DISCUSSION

Species composition and pattern of spatial distribution

The mollusc assemblage in GB was composed of 59 species, most of them with low frequency and abundance. The present study found more species than previous studies of molluscs in the bay (56 species in Oliveira, 1950; 18 species in Batalha *et al.*, 1998; 48 species in Mendes *et al.*, 2004). In the pioneer published description of the fauna of GB, Oliveira (1950) found a total of 56 mollusc species, of which only 2 families of bivalve were also encountered in the present study: Mytilidae and Lucinidae. Comparing our species richness with results from the last study (Mendes *et al.*, 2004), we noted differences in species composition and community structure. These differences might be related to differences in experimental design; our study collected samples from shallow areas (4–7 m) and samples were sieved through a 0.5-mm mesh net; whereas Mendes *et al.* (2004) collected over a wide depth-range (3–31.4 m) and sieved the samples through a 1.0-mm mesh net.

The spatial distribution of mollusc communities was significantly different within sectors, with differences in composition and density of species among sectors, reflecting the influence of the environmental gradient (heterogeneity) on the benthic molluscs. It was not possible to detect an isolated influence of sampling occasion and sector on gastropods; however, a significant interaction between spatial (station nested within sectors) and different sampling occasions corroborates the migration dynamics among sectors (Echeverría *et al.*, 2010).

The dominance of molluscs in GB had changed over time, which might be related to the increase in human pressure.

Three species were dominant in GB (Mendes *et al.*, 2004): *Anachis obesa* (frequency of 36.8%), *Olivella minuta* (frequency of 9.2%), and *Nucula semiornata* (frequency of 23.6%). The gastropod *A. obesa* was dominant in all sectors during the wet season and only in the inner and intermediate sectors in dry season (Mendes *et al.*, 2004); this species is absent in the present study. The gastropod *Olivella minuta* was dominant in outer sector only in the dry season (Mendes *et al.*, 2004); this species was found previously (Batalha *et al.*, 1998) and in the present study, mainly in outer sector in low abundance and frequency. The bivalve *Nucula semiornata* was dominant in intermediate and inner sectors; this species was found previously (Batalha *et al.*, 1998) and in the present study, mainly in outer and intermediate sectors in low abundance but high frequency (see Table 2).

Four species are currently dominant in GB; none of them were dominant previously indicating a replacement in the dominance probably related to changes in environmental characteristics. The mollusc assemblages are dominated by few species with high abundances, and each species was closely associated with the environmental characteristics of the sector or locale in GB. This pattern is typical of systems with contaminated sediments, organically enriched and with low dissolved-oxygen content; such systems show low richness and changes in community composition, with the establishment and dominance of opportunistic species (Dauer, 1993; Bemvenuti *et al.*, 2005) such as the gastropod *Heleobia australis*. In GB, this species is totally dominant, comprising 77% of all mollusc specimens collected. Our results agree with those of other studies that found that *H. australis* increases its density in response to eutrophication in estuaries and coastal lagoons of South America (Muniz & Venturini, 2001; De Francesco & Isla, 2003; Venturini *et al.*, 2004; Fonseca & Netto, 2006; Bemvenuti & Colling, 2010). *Heleobia australis* was found in a taphonomic study in GB by Senra (2003), who concluded that the colonization of this gastropod was favoured by environmental conditions in recently deposited sediments. However, *H. australis* was absent from some areas and in others occurred only in low densities, in the last mollusc study in GB (Mendes *et al.*, 2004). The abrupt increase in density of *H. australis* in five years might indicate changes in the mollusc pattern, probably induced by human interventions since the regions where it is most numerous are contaminated by domestic sewage (40 μg of coprostanol g^{-1} dry sediment: Carreira *et al.*, 2004), hydrocarbons (Mendonça-Filho *et al.*, 2003) and heavy metals, mainly Zn, Cu, Pb and Cr (Baptista-Neto *et al.*, 2006). The bivalve *Americuna besnardi* is restricted to the outer sector; this species shows geographical distribution associated with sandy sediments (Rios, 1994), probably related to its morphological characteristics (short siphon or asiphonate) depending on the sediment porosity and permeability to oxygen supply (Absalão *et al.*, 1999). The bivalve *Ervilia concentrica* was found previously in GB (Batalha *et al.*, 1998; Mendes *et al.*, 2004) as a frequent species (33% in dry season and 42% in wet season) in the outer sector; this species is widely distributed in the American continent and commonly related to sandy sediments (Rios, 1994). This is an infaunal asiphonate species that feeds on organic matter in suspension (Hauser *et al.*, 2007), and considered an important prey in sandy sediments (Sedberry, 1985; Caregnato *et al.*, 2009). The gastropod *Anachis isabellei* occurred in all sectors, being most numerous in the intermediate sector. Despite its association with beds of the scallop (*Euvola ziczac*: Klein *et al.*, 2001), coralline turfs (Kelaher *et al.*, 2007) and aggregates of tubiculous worms (*Phyllochaetopterus socialis*: Albano & Obenat, 2009), the occurrence is commonly related to sandy sediments where the species is widely used as food source (Caregnato *et al.*, 2009).

Mollusc densities showed nearly inverse patterns related to the influence of environmental gradients on dominant bivalves and gastropods. Density of bivalves decreased towards the inner sector, since dominant bivalves are related to marine conditions found in outer sector. Gastropods showed the lowest density in the outer sector and the highest in the intermediate sector, describing exactly the behaviour of the dominant *H. australis* related to estuarine conditions. The peaks in density of bivalves in the intermediate (station 13) and inner sectors (station 19) followed by the abrupt decline of gastropods in the same stations are possibly related to the influence of the marine water entrance from central channel (higher salinity and dissolved oxygen) on these stations.

Mollusc diversity followed the environmental gradient, with the highest diversity in the outer sector which is most influenced by marine intrusion, and decreasing towards the inner sector, where in estuarine conditions the density and biomass increase sharply. This diversity pattern observed for molluscs also occurs in other benthic communities in GB (Lavrado *et al.*, 2000; Van Der Ven *et al.*, 2006; Mendes *et al.*, 2007; Santi & Tavares, 2009), and in bays throughout the world. Although other factors are involved, community composition gradually changes according to the salinity gradient in estuarine systems (Gaston *et al.*, 1998; Giberto *et al.*, 2004; Giménez *et al.*, 2006). Similar patterns were found in other South American estuaries, where salinity gradient and sediment type strongly influence the spatial distribution and diversity of benthic fauna (Paranaguá Bay: Lana *et al.*, 1989, Boehs *et al.*, 2004; Patos Lagoon: Bemvenuti *et al.*, 1992; Todos os Santos Bay: Venturini *et al.*, 2004; Samborombón Bay: Ieno & Bastida, 1998; Rio de la Plata Estuary: Giberto *et al.*, 2004).

Environmental influence on species distribution

The close relationship between benthic organisms and the sediment where they live is well established. However, some studies have suggested little correspondence between sediment and the benthic community (Newell *et al.*, 2001) and have found relatively strong influences of other variables, such as depth and salinity, on mollusc community structure (Absalão *et al.*, 1999). Sediment type is a limiting factor for the spatial occurrence and abundance of organisms (Passadore *et al.*, 2007; Bemvenuti & Colling, 2010), and can be important for the structure of benthic communities (Teske & Wooldridge, 2001; Ysebaert & Herman, 2002). Sediment characteristics are strongly correlated with the benthic community in Patos Lagoon (Rosa & Bemvenuti, 2006), Arroio Solís Grande Estuary (Uruguay: Muniz & Venturini, 2001), Arcachon Bay (France: Blanchet *et al.*, 2005), Mar del Plata (Argentina: Arrighetti & Penchaszadeh, 2010) and Tinto-Odiel Estuary (Spain: Sánchez-Moyano *et al.*, 2010), and also with molluscs in GB ($P < 0.001$). The spatial distribution of benthic molluscs is mainly determined by the sediment characteristics in each sector of GB; this variable explains 51% of the distribution of bivalves and 50% of gastropods. However, not only the sediment but other natural factors, such as salinity (for bivalves) and dissolved oxygen (for gastropods), determine significantly the general differences between the bay's marine and estuarine areas.

Organic matter in sediment also strongly influences the structure of gastropods ($P < 0.001$). Organic matter and fine-grained sediments are deposited under the same energetic conditions, and are therefore positively related to sediment sorting (Lana, 1982). Organic matter is important for the consumption of benthic species, increasing the influence of this variable on the community. GB shows a high percentage of organic matter in sediments from the inner sector, even higher than in other highly eutrophic systems such as Laguna (Fonseca & Netto, 2006), Patos Lagoon (Rosa & Bemvenuti, 2006), and Rocha Lagoon and Samborombón Bay in Argentina (Ieno & Bastida, 1998; Giménez *et al.*, 2006). This sector receives greater organic matter input from continental sources (Baptista-Neto *et al.*, 2006), mostly from rivers draining into this area, resulting in higher organic-matter contents. The intermediate sector also has high organic-matter content in the sediments, similar to the high percentages found in eutrophic bays. Despite the natural source of organic matter, the intermediate and inner sectors of GB concentrate the input of industrial waste and domestic sewage (Paranhos *et al.*, 1998) and are regions with low or moderate energy, favouring the deposition of organic matter in sediments of these areas more affected by anthropogenic interventions. The percentage of organic matter in the outer sector is less than 1%. The lower deposition rate of organic matter in the outer sector results from the circulation pattern, which favours efficient water renewal, and higher energy because of seawater intrusion and the absence of nearby sources of organic matter (Carreira *et al.*, 2004).

The environmental pattern in GB determines the differentiation in species composition and abundance between the outer sector and the other sectors. The outer sector resembles marine environments: salinity close to 34, high dissolved-oxygen content in deep water, low water temperature, and sandy sediment with low organic matter. The indicator species of the outer sector is commonly found in sandy sediments (Rios, 1994): the dominant species being *Americuna besnardi*. The intermediate and inner sectors showed high temperatures in the water column, low dissolved oxygen and salinity in deep water, and muddy sediment with high organic matter. The indicator species of the intermediate sector is typically estuarine (Bemvenuti *et al.*, 1992), adapted to wide salinity variations, and inhabits green algae, macrophytes, and soft bottoms. *Heleobia australis* feeds on deposited organic matter (deposit-feeder) and on the bacterial biofilm on macroalgae and macrophytes (Rios, 1994), and is commonly found in eutrophic systems. There is an intense predation on *H. australis* by fish and estuarine crustaceans in bays and coastal lagoons, where the species is an important item in trophic interactions.

The mollusc distribution pattern in GB shows differences between the regions for marine and typically estuarine species. A similar pattern is seen in other bays, including Paranaguá Bay (Lana *et al.*, 1989), Tunis Bay (Afli *et al.*, 2009) and the Tinto-Odiel Estuary (Sánchez-Moyano *et al.*, 2010). Species composition and distribution are directly conditioned by environmental gradients, mainly salinity or dissolved oxygen and sediment characteristics, altering functional groups, mobility strategies and feeding among different sectors with distinct environmental characteristics (Lana *et al.*, 1989). This is consistent with the distributional pattern proposed by Sanders (1958), which describes the dominance of filter feeders or suspension feeders on sandy sediments (Rhoads & Young, 1970). Suspension feeders are dominant in the outer sector (sandy sediments); however, this sector showed the most diversified trophic structure (see Table 2). The diversity in feeding modes is a consequence of the taxonomic diversity in this sector, more than four times greater when compared to other sectors. Muddy sediments are also dominated by suspension feeders, in contrast to the expected since the dominant gastropod in muddy sectors is a deposit feeder. Nevertheless, it was not possible to analyse the complete trophic status in GB in order to explain the absence of data about feeding modes of molluscs, mainly gastropods.

The distributional pattern of molluscs in GB seems to be related mainly to the natural estuarine gradient, where there are morphological and/or sedimentary differences among regions, resulting in different sediment composition, and variations in salinity and dissolved oxygen produced by seawater input from the central channel. Nevertheless, anthropogenic factors are indirectly related to molluscs pattern since the history of regional pollution may determine complex effects on biological communities in degraded aquatic ecosystems (Clements, 1997; Wiegner *et al.*, 2003; Goto & Wallace, 2010), especially in areas with multiple sources of human stressors (Hewitt *et al.*, 2005), where the dominance of few species may indicate systems subjected to organic enrichment and contaminated sediments (Gray & Mirza, 1979; Dauer, 1993; Azrina *et al.*, 2006). GB is an estuarine system with a history of chronic pollution, mainly in intermediate and inner areas that receive organic and industrial discharges, and acute pollution in areas of recent contamination, with gradients of organic pollution (Paranhos *et al.*, 1998; Ribeiro & Kjerfve, 2002; Baptista-Neto *et al.*, 2006), hydrocarbons (Michel, 2000; Silva *et al.*, 2007) and heavy metals (Perin *et al.*, 1997; Borges *et al.*, 2007; Pereira *et al.*, 2007; Maranho *et al.*, 2010).

Significant long-term changes in molluscs communities (species disappearance, and shifts in composition, densities and frequencies) besides the dominance of *Heleobia australis* in the most degraded areas and the increase in its density in the last five years indicate that molluscs show characteristics associated with levels of environmental impact in GB, possibly resulting from the increasingly anthropogenic disturbances during past decades. This situation necessitates measures to improve biological conditions, such as reduction of nutrient input, mainly from untreated sewage, and treatment of chemical and domestic wastes. The restriction of nitrogen and phosphorus, residue treatment, and reduction of nutrient inputs originating from fertilizer industries in bays and estuaries are accompanied almost immediately by significant improvements in water quality and consequently in the local community (Paerl, 2006). Implementation of a monitoring programme together with actions for environmental improvement will allow the mollusc assemblages to recover, with the reappearance of some possible sensitive species and changes in community structure. This will also permit an evaluation of human impacts on benthic molluscs in GB.

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