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Spatial Variation of Bacteria in Surface Waters of Paranaguá and Antonina Bays, Paraná, Brazil

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ABSTRACT

Spatial variability in the concentration of total bacteria, saprophytes and coliforms abundance was investigated in surface waters of Paranaguá and Antonina Bays. Six points along a profile from the entrance of Paranaguá Bay to the innermost part of Antonina Bay, were sampled on February 26, 1997. Temperature, salinity, dissolved oxygen, pH, particulate organic carbon, water transparency and seston were also measured. Determination of total bacterial abundance and biomass were made with the epifluorescent method. Saprophytic bacteria counts were conducted with ZoBell 2216E culture medium with fresh and 32‰ water and the coliform MPN was enumerated with the chromogenic substrate method. Principal component analysis of biotic and abiotic data showed a gradient from the innermost bay to the baymouth bar with an increase in total and halophobic aerobic cultivated bacteria concentration at Antonina Bay. Greatest values of halophilic aerobic cultivated bacteria were found at the bay's middle part. The greatest fecal coliform number was observed near Paranaguá City. We concluded that regarding bacterial spatial distribution the outer region of the estuary was highly influenced by the adjacent ocean and that the inner part had typical estuarine characteristics.

Key words: Bacteria, hydrographic variables, estuary, Paranaguá and Antonina Bays

INTRODUCTION

In marine environments, bacteria are found free or attached to organic, and inorganic particles. They occur in all marine regions, including the abyssal zone and thermal vents. The number of bacteria is greater in the photic zone of the water column, due to a greater accumulation of dissolved organic matter produced by phytoplankton, and in the region located immediately above the sediment (Rheinheimer, 1985). Although, they occur in all marine environments, the importance of bacteria is greater in coastal regions (deltas, estuaries, bays and river outlets). This on one hand, is due, to the input of organic matter and nutrients (Gunkel, 1966; Gocke, 1977; Rheinheimer, 1985) and on the other hand, to the anthropic influence (cities, towns, ports, etc.) that alter environmental characteristics and consequently the bacteria in a direct, or indirect, quantitative and qualitative way.

Halophilic, halotolerant and halophobic bacteria also occur in coastal regions, primarily in bays and estuaries (Rheinheimer, 1971). These are brought to the marine environments mainly by rivers. Halophobic bacteria develop only in fresh- water cultures and in the sea-water they survive for a limited time (Weyland, 1967).

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Marine bacteria have an important function in the turnover of the organic matter produced by the phytoplankton and transformed in several ways by animals. Moreover, heterotrophic bacteria and, to a smaller extent, the fungi are responsible for the re-mineralization of particulate and dissolved organic matter in the sea. In favorable conditions these microorganisms are capable of decomposing almost all the natural organic components, as well as many artificial substances (Wright, 1978; Rheinheimer & Gocke, 1994).

The study of aquatic microorganisms ecology was significantly enhanced when new methodologies such as epifluorescence microscopy, allowed a better and faster visualization of bacteria and flagellates (Wright, 1978). The accumulation of information modified the effective paradigm that bacteria were only re-mineralizing agents. It was found that biomass of these microorganisms was greater than previously supposed and represented a source of alternative food for aquatic food chains (Azam *et al.* 1983).

In coastal regions, besides the general studies on autochthonous and allochthonous microflora, knowledge about the occurrence of total and fecal coliforms is important, mainly due to the discharge of domestic sewers from cities and towns, directly into the water. In Paranaguá Bay and adjacent regions (State of Paraná), Kolm & Corrêa (1994) analyzed the number of saprophytic bacteria in the sediment along to the perpendicular axis of the sandy beach tide line in front of Pontal do Sul. The authors concluded that the greatest number of saprophytes occur in the region of the last high tide. Kolm & Absher (1995) studied the number of saprophytes along the bays of Paranaguá and Antonina and its relationship with environmental factors. Kolm & Lesnau (1997), studying the variation of saprophytic bacteria in two water columns, one close and another distant from the mangroves, concluded that in spite of both being influenced by waters from the continental shelf, variations existed among the natural conditions of the bacteria.

The aim of the present research was to study the relationship between bacteria and physical-chemical factors in surface water along a transect from Paranaguá to Antonina bays.

STUDY AREA

Paranaguá Estuarine Complex (25°16’34’’S; 48°17’42’’W) is the largest estuary of the State of Paraná and extends for approximately 50 km towards the interior (Fig. 1). It is divided into two main sections, based on the terrestrial drainage system: a) northern section, formed by the Laranjeiras, Guaraqueçaba, Pinheiros, Benito, and Itaqui Bays and b) western section, formed by the Paranaguá and Antonina Bays (Müller, 1984; Absher, 1989).

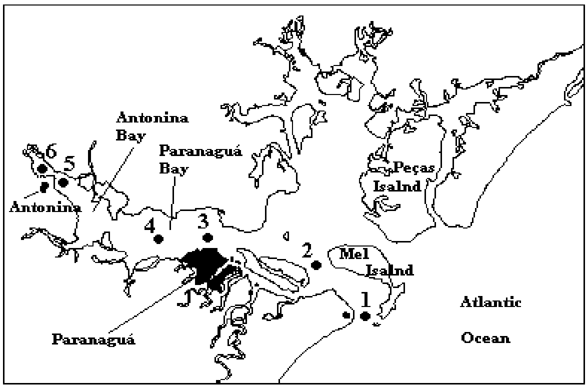

Figure1 - Paranaguá Estuarine Complex with the six sampling stations.

Paranaguá Bay is prolonged towards the west and is located between the Mel and Teixeira Islands. Upriver from Teixeira Island receives the name of Antonina Bay.

Paranaguá is the most important city of the region (Fig.1) with approximately 116.000 inhabitants. Although most of the city is located on the continent, there is a low income population located on Valadares Island. The system of sewers in the city is very faulty. Most of the sewage waters are discharged into Itiberê and Emboguaçú rivers or directly into Paranaguá Bay. Besides Paranaguá, Antonina (17.500 inhabitants) (Fig. 1) has a certain importance in the region. Its characteristics, with respect to the sewer system are similar to that described for Paranaguá. In the whole estuarine complex, sandbanks and islands of intermediary and small size can be found. The whole border of the estuary, as well as the rivers and tidal creeks, are bordered by mangroves made up of *Rhizophora mangle*, *Laguncularia racemosa* and *Avicennia schaueriana*. In the calmer regions of the estuary there are banks of *Spartina alterniflora*. According to Rebello & Brandini (1990), these floristic formations provide the enrichment of the region by producing organic detritus.

In the entrance to the Paranaguá Estuarine Complex are the Mel and Galheta Islands. The most important links of the estuary with the continental shelf are the constantly dredged Galheta Channel, which allows access to ships entering the Port of Paranaguá, and the Sueste Channel. During high tides, part of the water that enters through the Sueste Channel flows to Laranjeiras Bay and the remaining water flows to Paranaguá Bay. In the low tides, most of the water from Laranjeiras Bay flow towards the ocean by the Sueste Channel, but some water is mixed with water from Paranaguá Bay and then flows to the sea through the Galheta Channel (Knoppers et al. 1987; Disaró, 1995).

The mean temperature of the region is 21.1°C (± 7.9°C) and the annual precipitation is 2.000 mm. The climate can be defined as transitional tropical (Maack, 1981).

MATERIAL AND METHODS

In February, 26, 1997, surface water was collected at six stations between the entrance to Paranaguá Bay and the interior of Antonina Bay (Fig.1). Temperature (standard thermometer), pH (Digimed pH-meter), and water transparency (Secchi disk) were measured at the site. The remaining samples were conditioned and taken to the laboratory, where the following physical-chemical analysis were conducted: salinity (Atago refractometer), dissolved oxygen (Winkler technique), seston (filtration with Whatmann filters, GF/F) and particulate organic carbon (ignition method). The tide data was obtained from the tide tables of the "Departamento de Hidrografia e Navegação (DHN)" (Hydrography and Navigation Department).

For the quantification of the total heterotrophic bacteria, the water samples were placed in formaldehyde "in loco", at a final concentration of aproximately 5%, and taken to the "Laboratório de Microbiologia Marinha do Centro de Estudos do Mar". The counts followed the methodology described by Parsons *et al.* (1984), using the fluorochrome acridine orange. For the quantification of the bacterial biomass, bacteria in ten optic fields at the epifluorescence microscope were counted (1000x magnification) and divided in three categories - coccus (0.5 x 0.5 µm), small bacillus (0.5 x 1.0 µm) and big bacillus (> 0.5 x 1.0 µm) and its biovolume was determined, starting from approximate geometric figures and using the conversion factor of 5.6 x 10^{-7} µgC. μm^{-3} (Bratbak, 1985).

The samples used for the quantification of aerobic heterotrophic bacteria (halophobic and halophilic) and coliforms were transported to the laboratory in ice. The counts of the bacteria were made using the methodology described by Kolm & Corrêa (1994) and Kolm & Absher (1995). The culture medium used was ZoBell 2216E, modified by Gunkel (1964). For the coliform analysis, a chromogenic substratum composed basically of salts, ortho- nitrophenyl- β-D- galactopyranoside (ONPG) specific for total coliforms and 4-methyl-umbilliferyl β-D glucuronide (MUG) specific for fecal coliforms (*Escherichia coli*) was used, as described in "Standard Methods for the Examination of Water and Wastewater" (1995). The product used was Colilert, Idexx Laboratories, Inc, and the accompanying methodology was followed. To evaluate the main variation tendencies, the data were centered, reduced and assessed by a principal component analysis (PCA), obtained by the variance-covariance matrices Q and R (Bouroche & Saporta, 1982; Legendre & Legendre, 1983).

RESULTS

The results of the abiotic values are showed in Fig. 2. Figure 2A showed that the highest salinity value (33‰) was recorded at Station 1 and the smallest value (1‰), at Station 6, while the temperature of the water remained constant between 27 and 29°C. Figure 2B showed that the pH values varied from 7.6 at Station 2 to 7.0 at Station 6 and that the dissolved oxygen presented maximum values of 6.43 mg.l^{-1} at Station 4 and minimum values of 5.14 mg.l^{-1} at Station 1. The highest transparency (458 cm) was registered at Station 1 and the lowest (30 cm) at Station 6 (Fig. 2C). To make the data analysis possible, the tide variations were given hypothetical numbers according to the following outline: 1 - empty; 2 – ebb tide; 3 - flood tide, and 4 - full. During the collection period, the tide varied from full at Station 1, ebb tide at stations 2, 3 and 4 and flood tide at stations 5 and 6.

With regard to the seston, the largest values (30.78 mg.l^{-1}) were registered at Station 6 and the smallest ones (13.32 mg.l^{-1}) at Station 3. The largest values of particulate organic carbon (POC) (5.65 mgC.l^{-1}) occurred at Station 6 and the smallest ones (1.37 mgC.l^{-1}), at Station 1. Due to the very close values of dissolved oxygen and saturation point, and the little variation of pH and temperature values throughout the whole transect, these data were not included in the principal components analysis.

Figure 3 shows the results of the biotic factors. Figure 3A showed that the smallest number of total heterotrophic bacteria (2.300. 10^3 units. ml^{-1})

was found at station 1 and the smallest value of bacterial biomass (86 $gC.l^{-1}$) at station 2. The largest values ($12.720.10^3$ units. ml^{-1} and 698 $gC.l^{-1}$) were recorded at Station 6. The smallest values for the halophilic bacteria (4.070 $UFC.ml^{-1}$) (Fig.3B) were recorded at Station 3 and the greatest ones (20.386 $UFC.ml^{-1}$) at Station 4. With regard to the halophobic bacteria (Fig. 3B), the highest value (10.169 UFC. ml^{-1}) was found at Station 6 and the lowest value (410 $UFC.ml^{-1}$) at Station 4.

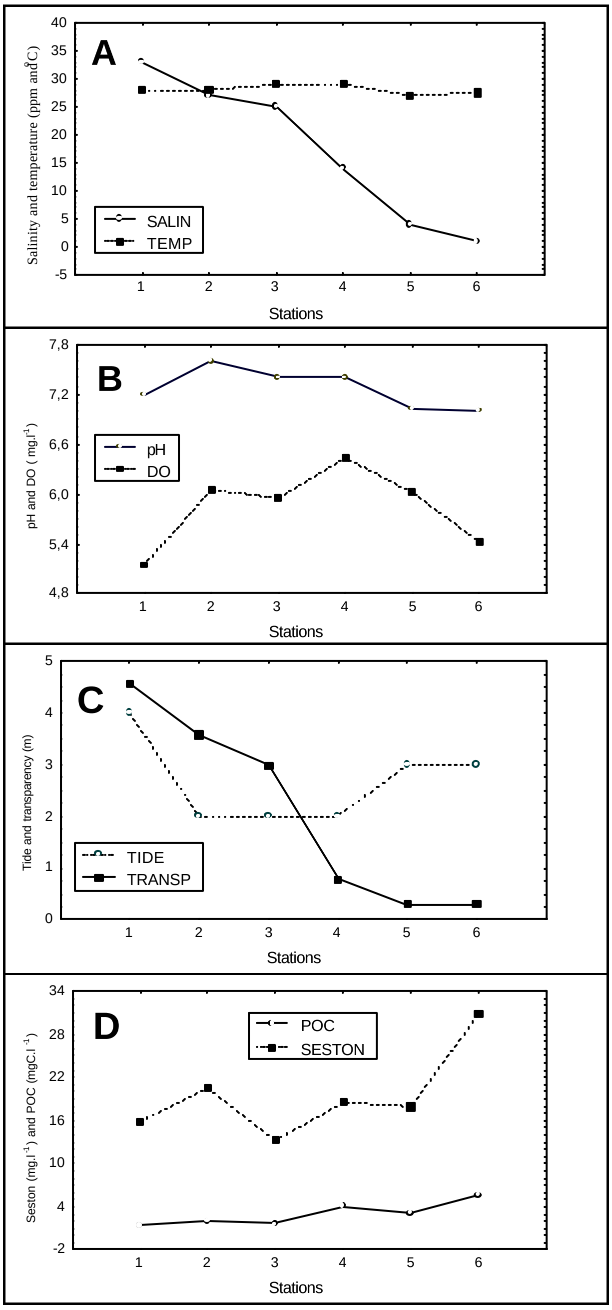

Figure 2 - A) Salinity and temperature; B) pH and dissolved oxygen; C) Tide and transparency and D) Seston and organic particulate carbon in surface waters.

Due to the difficulty in differentiating the halophilic bacteria from the halotolerant bacteria, they were all considered halophilic in this study.

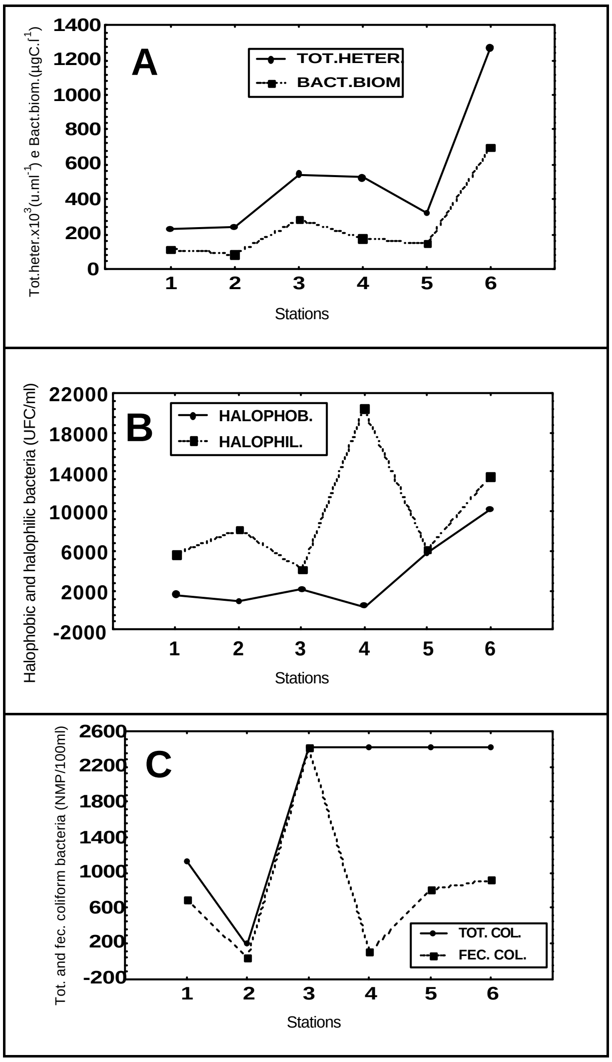

Figure 3 - Frequency of: A) Total heterotrophic bacteria and bacterial biomass; B) Aerobic halophilic and halophobic bacteria and C) Total and fecal coliform bacteria.

Regarding the total coliforms (Fig.3C), the smallest value (186.0 units) was recorded at Station 2. In the interior of the bay, values above 2419 units were found. The smallest values of fecal coliforms (Fig. 3C) were 35.0 units and these were recorded at Station 2. The largest values (> 2419.0 units) were recorded at Station 4.

The linear correlation matrix between the environmental data and the bacteria was used to make the Principal Component Analysis (PCA). As shown in Table 1 and Fig. 4 the first component accounted for 55.37% of the variation in the sample and is positively related to the salinity and transparency and negatively related to the seston, POC, total heterotrophic bacteria, bacterial biomass, halophobic bacteria and total coliforms.

Table 1 - Variance of five first principal components of PCA.

Component number	Percent of variance	Cumulative percentage
1	55.37435	55.37435
2	17.95223	73.32658
3	13.73000	87.05658
4	8.28519	95.34117
5	4.65823	100.00000

The second component accounted for 17.95% of the variation in the sample and is positively related to the fecal coliforms and negatively to the halophilic bacteria.

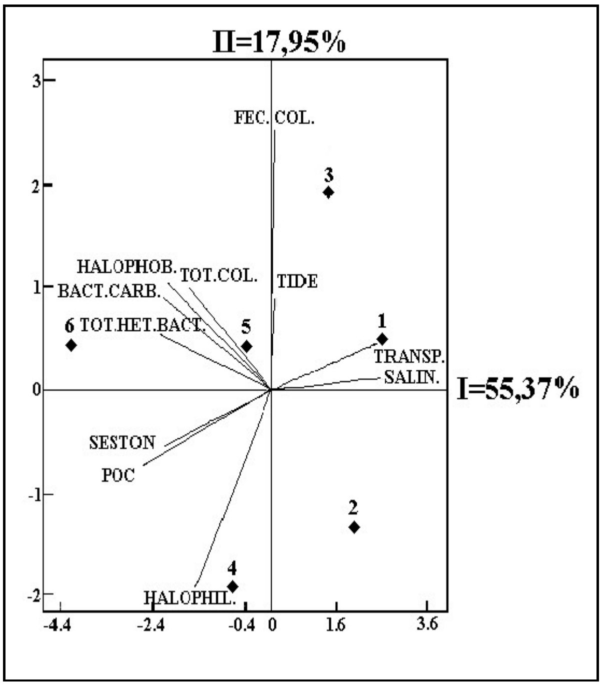

Figure 4 - First two components axes of principal component analysis of variables and stations.

DISCUSSION

In the last few years, emphasis has been given to the importance of estuaries in the global cycles of the planet, as regions of great biological activity, as sedimentation sites of continental and marine matter, as great producers of CO_2 and as regions of nutrient export (Largier, 1993; Smith & Hollibaugh, 1993; Hiep *et al.*, 1995).

Microbiological studies in these environments are of primordial importance, due to the large variation in physical-chemical and biological parameters and to their relationship with adjacent environments, mainly the terrestrial and freshwater ones. The high values of salinity and transparency, and low values of seston and particulate organic matter, found at the entrance of the Bays of Paranaguá and Antonina showed that in this region, in general, there was direct influence of marine waters which were more saline, more basic and poor in nutrients originating from the continental shelf. The opposite values, registered in the interior of the bay, characterized a typicall estuarine environment.

Kolm & Absher (1995) described an annual variation in the number of halophilic and halophobic bacteria in superficial waters of Paranaguá Bay and found an inverse relationship between the salinity and pH and the number of aerobic heterotrophic bacteria that could be cultivated with the formation of a gradient between the internal and external part of the bay. The present research confirmed the presence of a gradient with greatest values not only of halophobic bacteria, but also of total heterotrophic bacteria and of bacterial biomass in the internal section of the bay (Figs. 3 and 4).

Although the values of total heterotrophic bacteria found in the present research agreed, in general terms, with those described by Rheinheimer (1994) for coastal waters (0.5 to 10.10^6 bacteria ml^{-1}), the values of bacterial biomass differed greatly. Kennesh (1990) suggested that there could be, in coastal waters, values superior to 10 ($\mu gC.l^{-1}$). Rheinheimer (1994) recorded values between 1 and 50 ($\mu gC.l^{-1}$) for coastal waters. The very superior values (between 86 and 698 $\mu gC.l^{-1}$) found in the present study suggested a high local bacterial productivity or a lack of grazing.

In spite of the high seston values found at the inner part of the bay (St. 6) the majority of heterotrophic bacteria were free living. Montovani & De Moraes Novo (1996) found that dissolved organic matter (MOD) can interact with algae and bacteria as source of energy or organic carbon.

Kolm & Lesnau (1997) supposed that in nutrient-rich regions occured the existence of a free living bacterial life form feeding on dissolved organic matter (DOM). The high amount of mangroves that surrounded the inner part of the bay could be responsible for the production, besides great part of seston and of particulate organic matter (POM) of big quantities of DOM, propitiating this way the development of free living bacteria.

While the highest values of total heterotrophic bacteria and of halophobic cultivable bacteria were registered on the innermost part of the bay (St.6), the greatest values of halophilic and/or halotolerants were in the region close to the Island of Gegerês (St. 4).

Despite the difficulties found on the differentiation between the halophilic and halotolerants bacteria in the culture medium, this study seemed to be of great importance in coastal regions since, according to Hoppe (1986), the aerobic heterotrophic bacteria answer faster to the environmental variations and to the organic pollution than do the total heterotrophic ones. These observations have been confirmed by Andretta & Kolm (*in prep*.) which studied the tide influence over the bacteria in the Perequê tidal creek on the entrance of the Paranaguá Bay Estuarine Complex.

The intermediary values of salinity (14‰) are ideal for the development of halotolerant bacteria. Therefore, the relatively high values of seston and POC in the water recorded at the end of the ebb tide at Station 4, were probably responsible for the elevated values of halophilic (and/or halotolerant) bacteria found in this intermediary region of the bay. On the other hand, Station 4 showed the smallest values of halophobic bacteria. Thence it follows that this region is a transition zone between the shelf water and estuarine regions.

Total coliforms are made up of several enterobacteria that can occur not only in the intestinal tract of homothermal animals but also in soils and waters (Schlegel, 1993). Although the population density was small, the large amount of total coliforms found in the internal part of the estuary leaded us to believe that many of these enterobacteria occured as autochthonous microflora in this environment or develop in soils and waters and were taken to the interior of the estuary mainly by rivers. On the other hand, the repression of water inside the bay caused by the flood and full tides could also propitiate an increase in the number of these organisms in this region.

The fecal coliforms (*Escherichia coli*), on the contrary are organisms that are obligatorily symbiotic with homothermal animals and they occur exclusively in their intestinal tract. They can survive for more or less time in aquatic environments. At Station 3, in front of Paranaguá Port, there was a large number of fecal coliforms that decreased rapidly in the direction of the bay's interior.

Despite the entrance of big amounts of sewer on this region, the dissolved oxygen stayed close to the saturation point and similar to the values of the other stations. It is possible to suppose that there is a narrow relation between the high phytoplankton values found by Brandini (1985) and Brandini et al. (1988) in this region and the high dissolved oxygen values.

The results of the present research showed that the sewers from the city of Paranaguá were causing pollution in this region indicating that the total coliforms should not be used as indicators of organic pollution in the region, but the fecal coliforms should. In conclusion, high variations in the bacterial ecology were observed along Paranaguá Bay, in spite of the short sampling period. We suggest that more prolonged studies of this environment be carried out.

RESUMO

A variabilidade espacial da concentração de bactérias heterotróficas totais e cultiváveis, assim como de coliformes totais e fecais foi investigada em águas superficiais das baías de Paranaguá e Antonina. Para tanto foram efetuadas coletas ao longo de seis pontos em um transecto entre a entrada da Baía de Paranaguá e a parte interna da Baía de Antonina. Também foram obtidos dados de temperatura, salinidade, oxigênio dissolvido, pH, matéria orgânica particulada, transparência e seston da água. Para a contagem das heterotróficas totais e a avaliação da biomassa bacteriana foi usada a microscopia de epifluorescência. A contagem de heterotróficas aeróbicas foi efetuada com o meio de cultura ZoBell 2216E, preparado com água destilada (0‰) e água salina (32‰). O número mais provável de coliformes totais e fecais foi avaliado através de meio de cultura cromogênico. A ACP dos dados abióticos e bióticos mostrou um gradiente da parte interna da Baía de Antonina para a parte externa da Baía de Paranaguá, com um aumento da concentração de bactérias totais e de heterotróficas cultiváveis halófobas na Baía de Antonina e valores máximos de halófilas na parte mediana da Baía de Paranaguá. O número máximo de coliformes fecais foi observado próximo à cidade de Paranaguá. Concluiu-se que, no que se refere à

distribuição bacteriana, a parte externa do estuário é altamente influenciada pelo oceano adjacente e a parte interna tem características tipicamente estuarinas.

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