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Short-Term Variability of Bacterioplankton in the Maximum Turbidity Zone in the Paranaguá Bay, Southern Brazil, and its Relationship with Environmental Variables

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ABSTRACT

In this work, the density of bacterioplankton, bacterial biomass and environmental variables were monitored in two seasons (summer and winter), two times each month (spring tide and neap tide sampling), over a 12 h period, comprising a tidal cycle (semidiurnal), from subsurface and bottom waters, in a fixed station in the Estuarine Turbidity Maximum Zone (ETMZ) of Paranaguá Bay, Brazil. The data were treated with multivariate analyses methods in order to indentify the key controlling factors of the bacterial community dynamics. The microbial community seemed to be structured by a close relationship with the nutrients concentration, mainly by total phosphorous and nitrate. Regardless of variations throughout the tidal cycles, free-living bacteria had a dominant role on the Paranaguá's Bay ETMZ.

Key words: Bacterioplankton, Estuarine Turbidity Maximum Zone, free-living bacteria, attached bacteria, Paranaguá Bay

INTRODUCTION

Estuaries are complex ecosystems and important ways of organic matter transport from its origin in the continent to coastal zones (Servais and Gamier, 2006). The mixing of freshwater and seawater promotes strong biological and chemical gradients, that are, on its turn, modified by the autochthonous biological activity (Crump et al., 2004), making the dynamics of these environments not easy to predict (Bacelar-Nicolau et al., 2003). Many of the organic and inorganic matter present in the estuaries exist in the form of aggregates (Zimmermann-Timm et al., 1998), which constitutes important way of exportation of the particulate organic carbon to the oceans. This process is highly influenced by the microbial community (Azam, 1998; Hyun et al., 1999), as the microbial activity seems to be the major mechanism of organic matter degradation present on these environments (Daí and Sun, 2007). The aggregates might influence the heterotrophic turnover of organic matter (Zimmermann, 1997), creating an enriched micro-environment, where the microbial respiration could lead to a rapid cycling of the particulate organic carbon.

The Estuarine Turbidity Maximum Zone (ETMZ) has a pronounced impact on the microbial community's structure and function (Goosen et al., 1999), and its physical and chemical changes can

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dictate the ecological role of the microbial pool on the estuary (Hyun et al., 1999). Kolm et al. (2002) had studied the distribution of several bacterial groups in superficial waters along the Paranaguá's Bay and didn't find any positive correlation between the suspended matter and bacteria. The authors suggested that the most part of the microbial community of this region should be free-living. The same suggestion was made by Siqueira and Kolm (2005) for a Paranaguá's Bay tidal creek mouth.

This survey was carried out on the Paranaguá Bay, part of the Paranaguá's Estuarine Complex, with the purpose of analyzing the dynamics of the microbial community of the Estuarine Turbidity Maximum Zone (ETMZ), comprising attached and free-living bacteria, in association with the physical-chemical variables obtained concomitantly through four distinct tide cycles.

MATERIALS AND METHODS

Studied area

The Paranaguá's Estuarine Complex (25º16'34''S; 48º17'42''W) is the largest estuary of the Paraná State, with an area of 601 km^2 and a volume of 2.10^9 m^3 (Knoppers et al., 1987), being classified as a coastal plain estuary (Mantovanelli, 1999). The freshwater discharge is 178 $m^3.s^{-1}$ in the raining season (summer) and 47 $m^3. s^{-1}$ in the dry season (winter) (Mantovanelli, 1999). The estuary is formed by five main basins: Paranaguá and Antonina on de East-West axis and Laranjeiras, Guaraqueçaba and Pinheiros on the North-South axis (Fig. 1A).

The Paranaguá Bay is classified as a partially mixed estuary. The water circulation on the basins (Paranaguá and Antonina Bays) is driven by the tide currents in association with the seasonal influence of the riverine flow (Mantovanelli, 1999). The tides show a semidiurnal pattern, with diurnal differences (Marone and Jamiyanaa, 1997). The mean high spring tide of 1.7 m and the mean high neap tide of 1.3 m were registered at Galheta´s Island and 2.7 m on the spring tide and 2.0 m on the neap tide in the inner section of the estuary complex (Marone and Jamiyanaa, 1997). Previous studies had identified the presence of an Estuarine Turbidity Maximum Zone (ETMZ) between the Teixeira Island and the Paranaguá harbor (Fig. 1B), having its position changed over the semidiurnal tidal cycle (Mantovanelli, 1999; Noernberg, 2001; Mantovanelli et al., 2004).

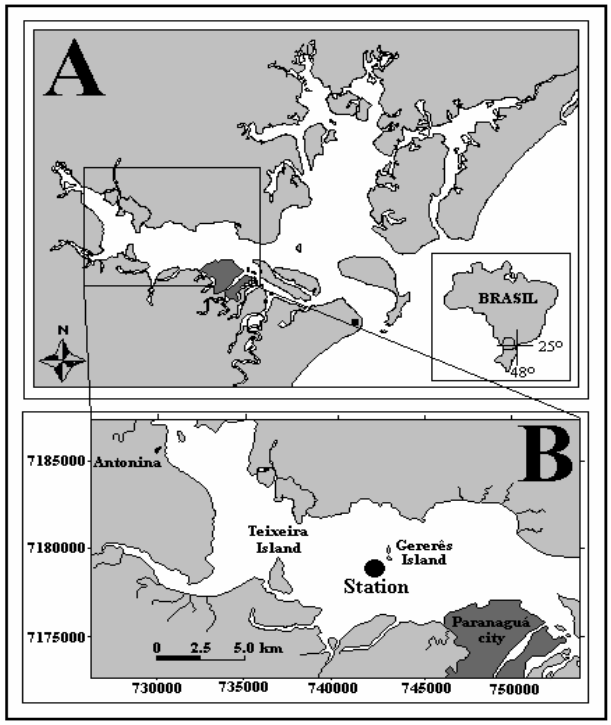


Figure 1 - A) Paranaguá Estuarine Complex; B) Part of Paranaguá's Bay, situated on the Paranaguá's Estuarine Complex. Samples was taken on the Estuarine Turbidity Maximum Region.

The concentrations of the suspended particulate material (SPM) may fluctuate between 80 and 250 $mg.l^{-1}$, being related to cyclical processes of erosion, resuspension and sedimentation, driven by tidal currents (Mantovanelli, 1999) and sediments discharge by river's flux (Noernberg, 2001).

Sampling design and analysis

In December 2005 (summer) and August 2006 (winter), water samples were collected using clean plastic bottles (from subsurface and bottom waters), two times each month (spring tide and neap tide sampling), every 2 h over a 12 h period, comprising a tidal cycle (semidiurnal). The sampling was carried out at a fixed point on the presumable position of the ETMZ, defined by the previous studies (Noernberg, 2001). The water samples used for total heterotrophic bacteria abundance and bacterial biomass counting were fixed *in loco* with formaldehyde to a final concentration of 5%. For the counting of total coliform bacteria and *Escherichia coli*, 100 ml of water was placed in sterile water bottles and kept on ice until the arrival in the laboratory.

The retention of the suspended particulate matter (SPM) was made using GF-C filters (Schleicher and Schüell). The filters containing the SPM were placed in Erlenmeyer flasks with 150 ml of distilled water and then agitated for 5 minutes at 17,000 rpm in an ultra-agitator QUIMIS. The contents were filtered through a 30μm mesh. For the heterotrophic bacteria and bacterial biomass counting, 20 μl were separated and treated with formaldehyde to a final concentration of 5%. For the total coliforms and *E. coli* counting, 100 ml were separated. Except for this step, all analyses for the attached and free-living bacteria were conducted in the same way.

For the counting of total heterotrophic bacteria, the samples were filtered using a darkened NUCLEOPORE membrane (0.22 μm of pore size). After staining with acridine orange, the counting of bacteria was conducted on an epifluorescence microscope (NIKON, Labophot model), following the methodology described by Parsons et al. (1984). The bacterial biomass was quantified by the biovolume obtained by approximated geometric forms following the methodology described by Kolm et al. (2002), utilizing a conversion factor of 0.4 $pgC.\mu m^{-3}$ (Bjørnsen and Kuparinen, 1991). To analyze the total coliforms and *E. coli*, a chromogenic substrate was used, basicly comprised by ortho-nitrofenil-ß-d-galactopyranoside (ONPG) (specific for total coliforms) and 4-metil-umberifenil glucoronide (MUG) (specific for *E. coli*), as described in the "Standard Methods for the Examination of Water and Wastewater" (Eaton et al., 1995). Colilert products were used following the methodology indicated by Idexx Laboratories, Inc. Counting was conducted by ultra-violet light (365 nm) for *E. coli* and through the natural light for total coliforms. The most probable number (MPN) was calculated by the table supplied by the manufacturer.

The measurement of the dissolved oxygen was performed as described by Grasshoff et al. (1983). pH was measured by pH-meter (ANALION) and salinity by a refractometer (QA Supplies, LLC model MT-110ATC). The suspended material was determined by the gravimetric analysis using GF-C (Schleicher and Schüell) filters.

The analyses of chlorophyll *a* and suspended material kept in the membrane was done following Strickland and Parsons (1972) and nutrients by the method of Grasshoff et al. (1983). Total nitrogen and phosphorus was measured by the oxidizing the sample with potassium persulphate in an autoclave. The concentration of total nitrogen and phosphorus was then measured by the colorimetric method described by Grasshoff et al. (1999). The method of Strickland and Parsons (1972) was used for the analysis of particulate organic carbon by the potassium dichromate oxidation of the organic carbon present in the suspended particulate matter in an acidic medium. The suspended particulate matter filters were used for the analysis of particulate phosphorus and nitrogen. Particulate phosphorus and nitrogen analysis was similar to that for total nitrogen and phosphorus analysis in the water column, except for the direct use of water and oxidizing solution, and also the use of white ribbon Whatman filter.

All the variables were normalized by logarithmic transformation. Since the data attended the pre-requisites for the statistical analysis, the Principal Component Analysis (Statistica 6.0) was carried out for the identification of relationships in the dynamics of the bacterial community and the physical-chemical variables, such as rain, tide, chlorophyll *a*, dissolved oxygen, silicate, total phosphorus, total nitrogen, suspended particulate matter, pH, phosphate, N:P ratio, and particulate organic carbon. The Stepwise Multiple Regression Analysis (Statistica 6.0) was utilized to refine the

correlations among the biological and physical-chemical variables.

RESULTS

As is evident from the oscillation patterns through the tidal cycles (Fig. 2), the density of free-living total heterotrophic bacteria present in the subsurface waters, with a maximum of 2.55 x 10^6 bacteria.ml^{-1}, was higher than the bottom density for the majority of the samplings throughout all the tidal cycles. Taking into account all the samplings (from all the tidal periods), the abundance of free-living total heterotrophic bacteria was higher in 95.83% of the samplings for subsurface communities, and in 70.83% for the bottom communities (Fig. 2). The same pattern was found for the bacterial biomass, with a higher free-living than attached carbon content found on 100% of the samplings for the subsurface and on 83.3% for the bottom samplings (Fig. 3). Most maximum values of the free-living bacteria were found in the subsurface samplings, while minimum values were found in the bottom samplings. The opposite pattern was detected for the attached bacteria, where the majority of highest values were found on the bottom samplings. The oscillation patterns of the bacterial biomass (free-living and attached) were very similar to the patterns detected for the total heterotrophic bacteria density. The free-living heterotrophic bacteria sampled in the summer, neap tide, showed negative correlation with the higher tide levels. The others samplings did not revealed such apparent correspondence to the oscillatory tidal pattern, leading to the conclusion that other parameters had greater influence on the bacterial density, as shown by the multiple regression tables (Tables 1-4). The negative correspondence pattern of the heterotrophic bacteria versus the tidal levels was detected by the Principal Components Analysis (Fig. 4 – A/B), as well as by the negative correspondence between the salinity and free-living heterotrophic bacteria density detected by the multiple regression (Table 1). It was important to note that the free-living bacterial biomass also exhibited negative correlation with pH and salinity, revealing the signs of dilution through the appearance of nutritionally poor waters, as tide became higher.

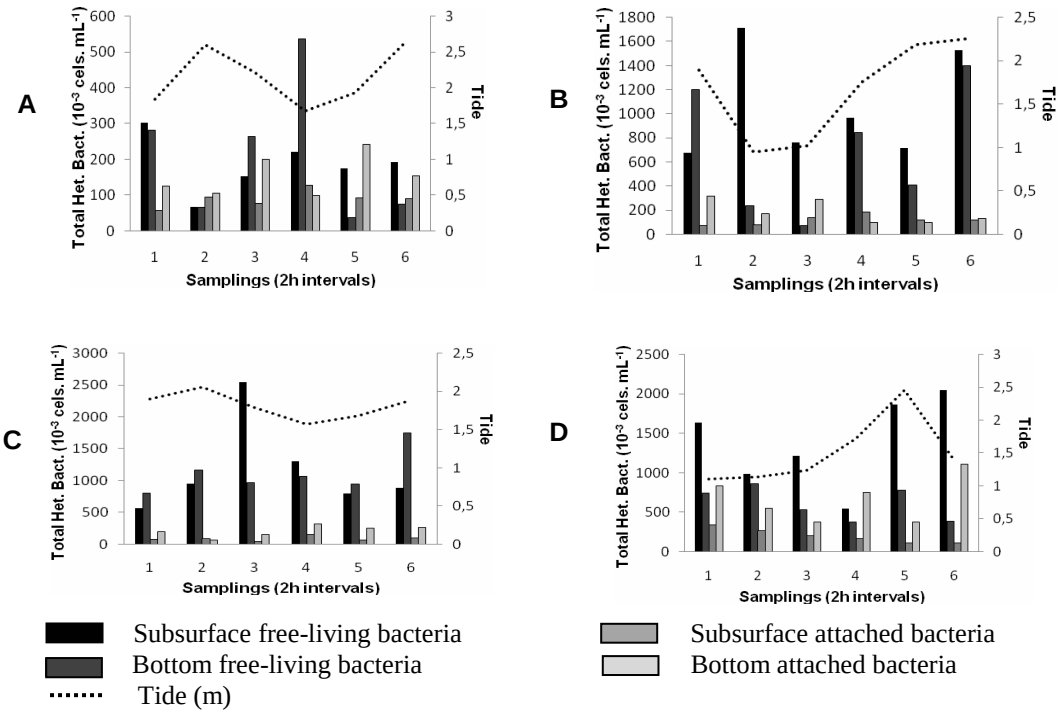


Figure 2 - Dynamics of total heterotrophic bacteria density over the tide cycle, on quadrature-summer (A); spring-summer (B); quadrature-winter (C) and spring-winter (D).

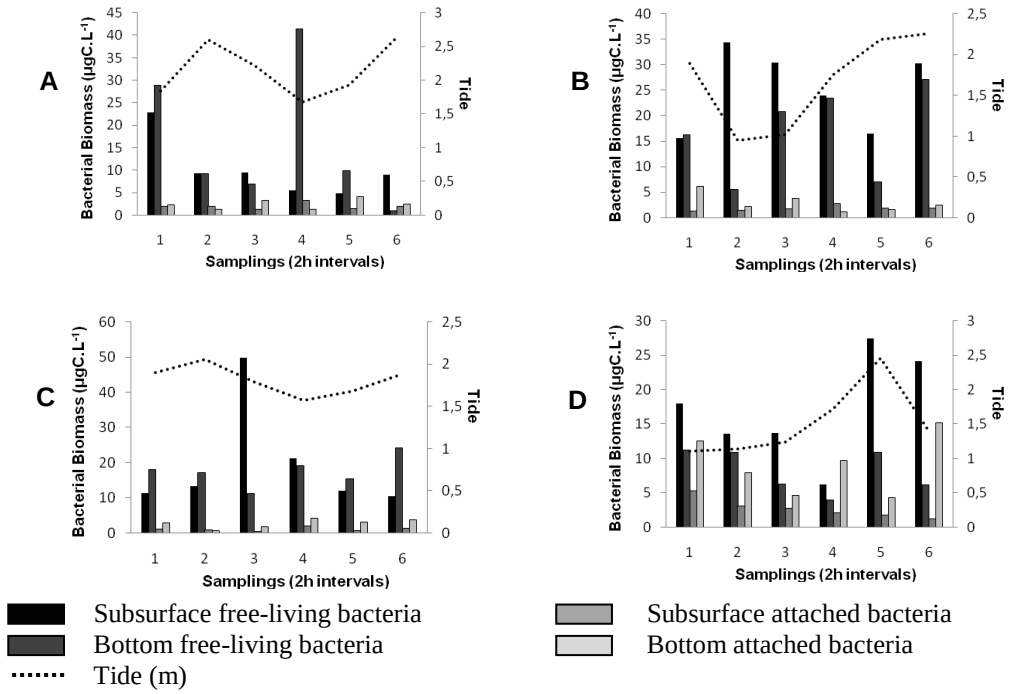


Figure 3 - Dynamics of bacterial biomass over the tide cycle, on quadrature-summer (A); spring-summer (B); quadrature-winter (C) and spring-winter (D).

Table 1 - Variables in the equation of stepwise multiple regression for the variation of the abundance of free-living total heterotrophic bacteria for all samplings; *r*: 0.8500; r^2: 0.7225; *p<0.05; ** p<0,01.

Variables	Regression coefficient	Standard error of the regression coefficient
Ammonium	0.352*	0.167723
Total phosphorous	-0.483**	0.133639
Phosphate	-0.677 **	0.149864
Nitrate	1.019 **	0.215113
Salinity	-0.343*	0.147732
Silicate	0.240 *	0.114404
Suspended particulate matter	-0.129	0.108355

Table 2 – Variables in the equation of multiple regression for the variation of the abundance of attached total heterotrophic bacteria for all samplings; *r*: 0.7778; r^2: 0.6050; * p<0.05; ** p<0,01

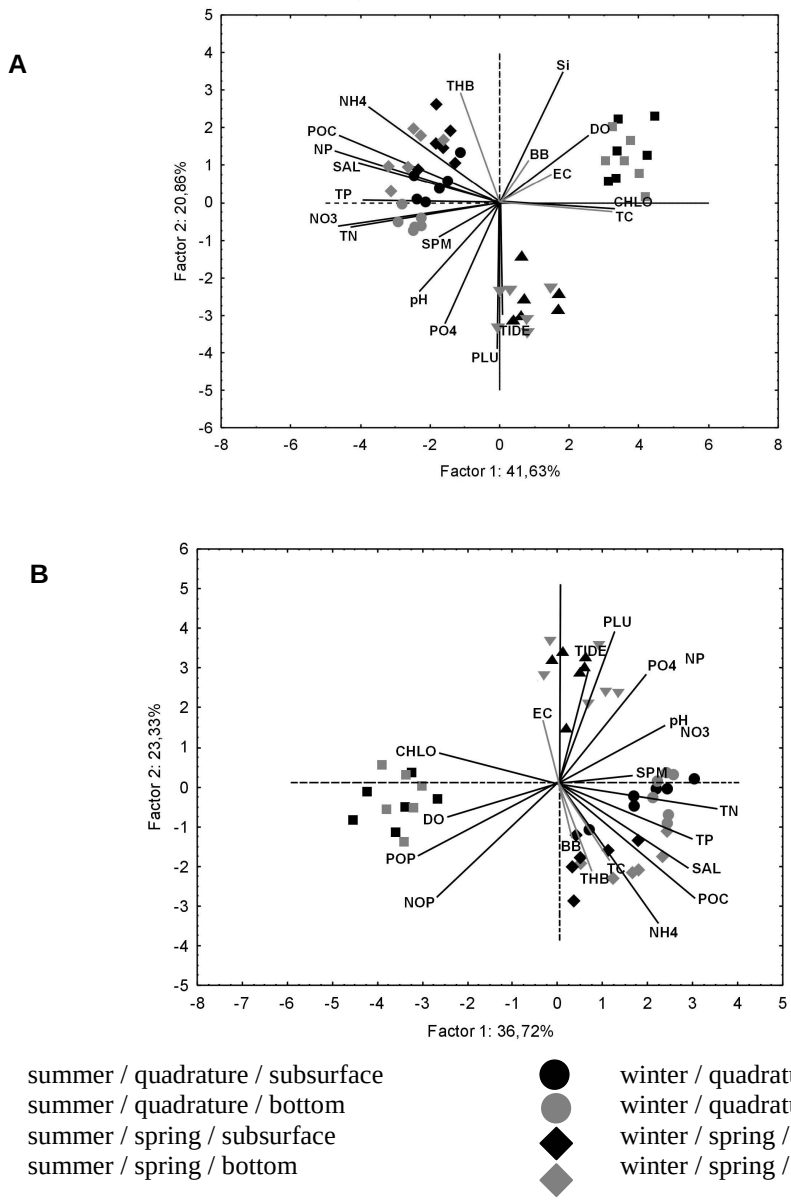
Variables	Regression coefficient	Standard error of the regression coefficient
Total phosphorous	0.432 **	0.131674
Pluviosity	-0.482 **	0.118218
Chlorophyll *a*	0.236 *	0.103951
Suspended particulate matter	0.187	0.109779
Dissolved oxygen	-0.138	0.120580

Table 3 - Variables in the equation of multiple regression for the variation of free-living bacterial biomass for all samplings; *r*: 0.5979; r^2: 0.3575; * p<0.05; ** p<0,01.

Variables	Regression coefficient	Standard error of the regression coefficient
Silicate	0.392*	0.159788
Total phosphorous	-0.558 **	0.181921
Nitrate	0.826 **	0.286138
Salinity	-0.345	0.203831

Table 4 - Variables in the equation of multiple regression for the variation of attached bacterial biomass for all samplings; *r*: 0.7307; r^2: 0.5339; * p<0.05; ** p<0,01

Variables	Regression coefficient	Standard error of the regression coefficient
Total phosphorous	0.546 **	0.133256
Chlorophyll *a*	0.229	0.146200
Pluviosity	-0.468 **	0.156416
Ammonium	-0.271	0.175410



Descriptors: Salinity (SAL); Tide (TIDE); pH (pH); Suspended Particulate Matter (SPM); Dissolved Oxygen (DO); Chlorophyll *a* (CHLO); Particulate Organic Carbon (POC); Pluviosity (PLU); Total Phosphorous (TP); Total Nitrogen (TN); Particulate Organic Phosphorus (POP); Particulate Organic Nitrogen (PON); Phosphate (PO4); Nitrate (NO3); Silicate (Si); Ammonium (NH4); N:P ratio (NP).
Supplementary variables **(A) (Free-living bacteria); (B) (Attached bacteria)**: Total Heterotrophic Bacteria (THB); Bacterial Biomass (BB); Total Coliforms (TC); *E. coli* (EC).

Figure 4 - Projection of the principal components.

As shown by Figure 4B, the attached bacterial biomass presented great correspondence with the winter-spring sampling, mainly with the bottom samples. These samples were also responsible for the major levels observed of total phosphorus, and this positive correlation was emphasized by the multiple regression analysis (Table 4).

The contrasting pattern shown by the free-living and attached bacteria in relation to total organic phosphorus suggested that the groups had inverse responses to this variable (Tables 3-4), leading to the conclusion that phosphorus components were potential key-factors of the local microbial community structure.

As shown by the Tables 2 and 4, attached total heterotrophic bacteria and bacterial biomass demonstrated negative correlation with the pluviosity, which was emphasized by PCA (Fig. 4B). This pattern could be related to the input of dissolved organic matter in rainy periods, leading to the growth of free-living bacteria instead of attached bacteria.

DISCUSSION

Siqueira and Kolm (2005), in a study in the Maciel Tidal Creek mouth on Paranaguá's Bay, indicated that the bacterioplankton of the region was dominated by the free-living bacteria. These results agreed with the present study, as shown by the total heterotrophic bacteria density graphics. The differences observed between the subsurface and bottom data for the total heterotrophic bacteria abundance and bacterial biomass could be explained by the fact that in the Estuarine Turbidity Maximum Zone, both the depths represented distinct micro-environments, exposed to different physical-chemical influences. These micro-environments could enhance or reduce the bacterial growth in different ways. The fact that the majority of the maximum values for the attached bacterial density and biomass were obtained mainly on the bottom samplings, and mostly on the ebb tide, this could be related to the input of organic matter from inner sections of Paranaguá's Bay, associated with the re-suspension activity promoted by the tidal flux.

In the ETMZ of an estuary, tidal effects (producing changes on particle's sedimentation and resuspension), presence of exopolimers producing organisms, and abundant organic matter, may develop the continuous formation of new aggregates (Zimmermann-Timm et al., 1998). This phenomenon might have a greater influence near to ETMZ's bottom, accentuating differentiation between subsurface and bottom data. Painchaud and Therriault (1989) and Crump et al. (1998) had previously described negative correlations between the free-living heterotrophic bacteria and salinity. Higher tidal levels generally represent the influx of nutritionally poor water for the bacterioplankton. The dilution effect on the heterotrophic bacteria density, motivated by the tidal upcoming, was described previously by Bacelar-Nicolau et al. (2003) in the Mondego Estuary, Portugal. Kolm and Absher (1995) investigated an annual variation on the number of halophobic and halophilic saprophytic bacteria in the Paranaguás's Bay surface waters, and found an inverse relationship between the salinity, pH and the number of cultivable aerobic heterotrophic bacteria, as well as the formation of a gradient across the inner and external portion of the estuary. Kolm et al. (2002) reported a gradient with the maximum values of total heterotrophic bacteria density and the biomass reached in the inner parts of the bay, emphasizing the high influence of this region on the microbial community structure of the ETMZ of Paranaguá's Bay, mainly by the activity of tidal fluxes.

The high positive correlation found between the nitrate and density of free-living total heterotrophic bacteria, as well as with the free-living bacterial biomass shown by the multiple regression tables disagreed with Bacelar-Nicolau et al. (2003) study. However, the levels of nitrate were positively correlated with the density of free-living heterotrophic bacteria. The presence of high levels of particulate matter could have motivated this pattern. Taking into account that the samplings were carried out on a region with characteristically higher suspended particulate matter, it was possible that this material had an impact on the limitation of light for the growth of the phytoplankton. Because of its high requisition of phosphorus, bacteria might be limited by these nutrients in an aquatic system where inorganic phosphorus limited the primary production (Cotner et al., 2000). Strong negative correlations between the free-living heterotrophic bacteria, phosphate and total phosphorus, and between the free-living bacterial biomass and total the phosphorus appeared to be related with bacteria's ability to

acquire the inorganic phosphorus in low phosphate concentration environments. The bacteria:phytoplankton ratio of ammonium and phosphate uptake rises as the concentration of these nutrients becomes lower (Rivkin and Anderson, 1997). It implies that, when these nutrients are scarce, bacteria might assimilate them more efficiently than phytoplankton.

That may be the cause of the negative relationships mentioned above. The high positive correlations observed between the attached heterotrophic bacteria, bacterial biomass and total organic phosphorus shown by the multiple regression analysis, indicated a possible limitation of attached bacterial growth by those nutrients, but mainly enlightened the different responses shown by the free-living and attached bacteria to the fluctuation of environmental parameters.

The ammonium is the result of the protein degradation into organic compounds. One probable indication of the heterotrophic process activity in the study area was the positive correlation between the ammonium and free-living total heterotrophic bacteria, shown by the multiple regressions tables and confirmed through the PCA. The samplings with highest ammonium levels were collected in the winter through the spring tide which was a period characterized by the stronger hydrodynamic process. Furthermore, some studies about the incorporation of nutrients by the bacteria observed that the increase in the marine bacterial density was enhanced by the inorganic nitrogen enrichment (Elser et al., 1995). Since nutrients consumption by the phytoplankton was strictly dependent on the light, it was possible that the obstruction of luminous energy penetration by the suspended particulate matter had altered the potential competitive relationships between the bacteria and phytoplankton for resources. In some marine environments, bacteria may be responsible for even 75% of the planktonic assimilation of ammonium (Rivkin and Anderson, 1997). The discharge of freshwater planktonic communities through the estuarine system may be the cause of the positive correlation observed between the attached bacteria and chlorophyll *a*. The death and lysis of phytoplanktonic cells by saline stress facilitate the formations of new aggregates, and by doing so, raises its nutritional value for the attached bacterial communities. The positive correlation between the bacterial density and chlorophyll *a* has been described by Painchaud and Therriault (1989) and Bacelar-Nicolau et al. (2003).

Attached heterotrophic and particulate organic carbon (POC) were directly related with the winter spring samplings on PCA. The positive correlation between the POC and attached bacteria in ETMZ´s were previously described by Painchauld and Therriault (1989) and Crump et al. (1998). The data suggested that the attached and free-living bacterial densities were driven by the dissimilar factors, supporting the theory that they represented different populations (Painchaud and Therriault, 1989; Crump et al., 1998) consequently, models of estuarine bacterial dynamics should consider these populations separately.

The microbial community seemed to be structured by a close relationship with nutrient's concentration fluctuation, mainly by the total phosphorous and nitrate. Regardless of variations throughout the tidal cycles and between the seasons, free-living bacteria had a dominant role on Paranaguá's Bay ETMZ. The structure of the microbial community in the study area (ETMZ) seemed to undergo a great influence of the inner sections of the estuarine system, mainly by the input of organic matter, mostly over spring tides.

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