

## HETEROTROPHIC BACTERIA ABUNDANCES IN RODRIGO DE FREITAS LAGOON (RIO DE JANEIRO, BRAZIL)

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### ABSTRACT

The Rodrigo de Freitas Lagoon (RJ, Brazil) is an important coastal ecosystem that has been submitted to an accelerated degradation process. The aim of this work was to determine the abundance and the spatial distribution of total heterotrophic (by flow cytometry) and cultivated bacteria ("pour plate" method on R<sub>2</sub>A agar). Another objective was to evaluate the lagoon's influence on water quality of Ipanema and Leblon beaches. Physical and chemical data were acquired too. Sub-superficial water samples were taken monthly, from December 1999 to October 2000. On lagoon, the cultivated bacteria abundance varied from 6.9x10<sup>5</sup> to 5.0x10<sup>7</sup> CFU.100 mL<sup>-1</sup>. On Ipanema and Leblon beaches, this parameter yielded 1.4x10<sup>5</sup> and 2.8x10<sup>6</sup> CFU.100 mL<sup>-1</sup>, respectively. Total bacterial abundance varied from 2.9x10<sup>7</sup> to 3.2x10<sup>7</sup> cells.mL<sup>-1</sup> on lagoon. On Ipanema and Leblon, this parameter yielded 8.7x10<sup>6</sup> and 1.1x10<sup>7</sup> cells.mL<sup>-1</sup>, respectively. Two sub-groups were determined with dominance of HNA cells. Samples were added latter to better understand the bacteria present on these environments. Bacterial abundance were analyzed only by flow cytometry and the results varied from 8.3x10<sup>6</sup> to 2.5x10<sup>7</sup> cells.mL<sup>-1</sup> on lagoon. On the beach, this parameter yielded 6.9x10<sup>6</sup> cells. mL<sup>-1</sup>. Two bacterial sub-groups were also observed, with dominance of HNA on lagoon and LNA on the beach. The results showed that the Rodrigo de Freitas Lagoon is an eutrophic ecosystem where the bacterial populations and the physical and chemical parameters do not differ spatially. The data also confirmed that the outflow of the lagoon's polluted waters affect the sanitary conditions of Ipanema and Leblon beaches.

**Key words:** bacterioplankton, flow cytometry, eutrophication, water pollution, Rodrigo de Freitas Lagoon

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### INTRODUCTION

After the establishment of the "microbial loop" concept (2,19), heterotrophic bacteria were widely regarded as an important component of aquatic ecosystems. They contribute to the structure and dynamics of the aquatic food webs and biogeochemical cycles. Increases on heterotrophic bacteria numbers can be used to monitor increases in the environmental organic pollution (13). Accurate determinations of bacterial numbers and its fluctuations are needed to evaluate more precisely the changes in the community after discharges of pollutants and coastal run-offs. We made use of heterotrophic

bacteria abundances by flow cytometry and cultivation methods to address the eutrophication of Rodrigo de Freitas Lagoon (RFL).

RFL is located at Rio de Janeiro city, Brazil, between 22°57'02" - 22°58'09"S and 043°11'09" - 043°13'03"W. This important coastal ecosystem is used for reproduction, nutrition and growth of several species. Also represents an area used for comercial fishing, turism, leisure and water sports activities. RFL has an irregular polygonal shape, with 7.2 Km of perimeter and 2.5x10<sup>6</sup> m<sup>2</sup> of total area (18). It has 2 artificial islands, 16 Km<sup>2</sup> of watershed (7), 4.3 m of average depth and 6.5x10<sup>6</sup> m<sup>3</sup> of total water volume (18). This ecosystem is connected to the Atlantic

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Ocean by the Jardim de Alah Channel, one of the main pollution source of Ipanema and Leblon beaches (15,20). The Jardim de Alah Channel has 835 m of length, 9.8 m of width and 0.70 m of depth (22). According to oceanography principles, shallow and extensive channels, such as the Jardim de Alah Channel, allows only a sub-superficial water circulation on ecosystems connected to the open seas. In these channels, the water flow are not sufficient to promote the deeper layer renewal, that remain stagnated and with high organic matter levels (22).

RFL has been losing water quality, mainly due to the effects of an inadequate city planning (16). Human interferences, in terms of unregulated development and pollution, have caused serious damage both in ecological and social-economical senses. Garbage, oil and domestic sewage are disposed with no previous treatment. The anthropic fills have also changed the water circulation, contributing to degradation process. The high availability of nutrients and organic matter allows the bacterial proliferation (15).

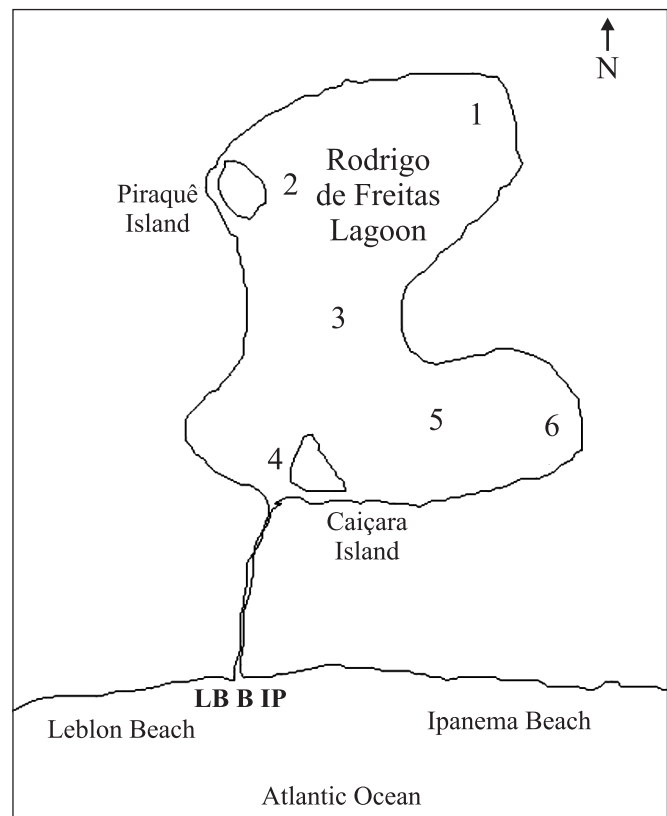
The aim of this work was to determine the abundance and distribution of total and cultivated heterotrophic bacteria at RFL in order to contribute to a better management and suitability of this ecosystem. Another objective was to evaluate the lagoon's influence on nearby oceanic beaches: Ipanema and Leblon. The results showed that the RFL is an eutrophic ecosystem where the bacterial populations not differ spatially.

## MATERIALS AND METHODS

### Sampling

Sub-surface water samples were collected monthly from December 1999 to October 2000 (RFL1). Based on previous studies (15,16), 5 different areas were selected: Rebouças Tunnel, Piraquê Island, Central Channel, Caiçara Island and Calombo Curve (Fig. 1).

The Rebouças Tunnel station is located furthest away from the entrance of lagoon and receives untreated clandestine domestic sewage. This area is also characterized by a difficult water renewal and by the presence of storm galleries. The Piraquê Island point lies on northwest region and receives the discharges from 3 rivers (4). The Central Channel station is located in the circulation channel of this ecosystem. The Caiçara Island point lies near the entrance of Jardim de Alah Channel that connects the RFL to the Atlantic Ocean, making possible a greater water renewal (22). Finally, the Calombo Curve station lies in the southeast area and receives discharges of unauthorized domestic waste. This region is relatively next to the Jardim de Alah Channel and can receive its influence. On Ipanema and Leblon beaches, the points were located 10 meters from Jardim de Alah Channel. Chemical and microbiological determinations were carried out, respectively, in the Chemical Oceanography and Microbiology Laboratories of Santa Úrsula University. Few sparsely taken samples were added latter (3/20/2003 and 5/16/



**Figure 1.** Sampling points: Rebouças Tunnel (1), Piraquê Island (2), Central Channel (3), Caiçara Island (4), Calombo Curve (5), Cantagalo Park (6), Ipanema (IP), Leblon (LB) and beach in front of the Jardim de Alah Channel (B).

2003) (RFL2) to better understand the heterotrophic bacteria at RFL system and the surrounding beaches. At RFL2, water samples were also collected in two other points: the Cantagalo Park station, situated in the southeast area of the lagoon, and in the region of the beach located in front of the Jardim de Alah Channel. These samples were analyzed only by flow cytometry at Universidade Federal do Rio de Janeiro.

### Bacterial abundance

Total bacterial abundance (TB) was determined after nucleic acid staining with Sybr Green II (RFL1) and Syto 13 (RFL2) (Molecular Probes) in samples fixed with sterile paraformaldehyde 2% (final concentration) (6,9,14). For detection, a B-D FACSCalibur flow cytometers equipped with argon laser were used. Counts were made at low flow ( $12 \mu\text{L}\cdot\text{min}^{-1}$ ) for 30 s, and data acquired in logarithmic mode. Bacteria were detected by their signature in a plot of side scatter (SSC) versus green fluorescence (FL1). Fluorescent latex beads were added (Polysciences, 1.0 diameter) and used as an internal standard. Based on optics and fluorescence signals, 2 distinct

sub-groups were differentiated: low (LNA) and high (HNA) nucleic acid content bacteria (6,9).

Cultured heterotrophic bacteria (CB) were analyzed by “pour plate” method on R<sub>2</sub>A agar (Difco 1826-17.1) with NaCl adjusted to 1.5%. The number of colony forming units (CFU) in Petri dishes were enumerated after incubation at  $26 \pm 1^\circ\text{C}$  for 5-7 days.

### Physical and chemical analysis

Abiotic parameters were analyzed according to standard oceanographic methods (21). Temperature and pH were determined in the field, respectively, with a thermometer and a pHmeter (HANNA HI8424). Salinity and Dissolved Oxygen (DO) were analyzed, respectively, by Chlorinity and Winkler-azide methods. Chlorophyll *a* determinations were carried out after vacuum filtration (0.45  $\mu\text{m}$  Whatman GF/C). The filters were extracted overnight in 90% acetone at  $4^\circ\text{C}$ , and the readings done with HITACHI U-2000 spectrophotometer. Determination of Suspended Particulated Matter (SPM) was made by gravimetry on HA cellulose acetate filters. Inorganic nutrients were analyzed: ammonia by indophenol, nitrite by diazotation, nitrate by reduction in a Cd-Cu column followed by diazotation, and orthophosphate by reaction with ascorbic acid.

### Statistical analysis

Data of all variables obtained at RFL1 were transformed to log and tested by non-parametric statistics. A Pearson matrix correlation between the parameters was established by the *Statistica* program (23).

## RESULTS AND DISCUSSION

No significant spatial differences were found on TB results obtained on RFL1 (Table 1). On average, the data of Rebouças Tunnel were higher than that of Calombo Curve. On the beaches, the highest and lowest data were found, respectively, in Ipanema and Leblon. The same distribution pattern was noted in RFL2 (Table 2), with the highest and lowest values being registred, respectively, on Rebouças Tunnel and Cantagalo Park. The last one lies next to Calombo Curve and have the same environmental characteristics. The strong influence on the beach was also noted. High values of TB were registred on area directly affected by the Jardim de Alah Channel.

Two principal sub-groups were determined in RFL1 by cytometric analysis (Table 1). An uniform distribution was noted with dominance of HNA cells. Highest and lowest average values of LNA were obtained, respectively, on Rebouças Tunnel and

**Table 1.** Mean (X), standard deviation (SD) and coefficient of variation (CV%) of Total Bacteria (TB), bacteria with low apparent nucleic acid content (LNA), bacteria with high apparent nucleic acid content (HNA) and Cultured Bacteria (CB) obtained on RFL1.

	TB (cells.mL <sup>-1</sup> )	LNA (cells.mL <sup>-1</sup> )	HNA (cells.mL <sup>-1</sup> )	CB (CFU.mL <sup>-1</sup> )
Rebouças Tunnel	$3.2 \times 10^7 \pm 2.1 \times 10^7$ (65%)	$8.0 \times 10^6 \pm 7.4 \times 10^6$ (93%)	$1.5 \times 10^7 \pm 9.8 \times 10^6$ (64%)	$1.9 \times 10^6 \pm 3.5 \times 10^6$ (184%)
Piraquê Island	$3.0 \times 10^7 \pm 2.2 \times 10^7$ (73%)	$7.5 \times 10^6 \pm 7.7 \times 10^6$ (102%)	$1.6 \times 10^7 \pm 1.3 \times 10^7$ (79%)	$8.1 \times 10^5 \pm 6.4 \times 10^5$ (79%)
Central Channel	$3.1 \times 10^7 \pm 2.0 \times 10^7$ (64%)	$6.6 \times 10^6 \pm 5.4 \times 10^6$ (82%)	$1.7 \times 10^7 \pm 1.3 \times 10^7$ (75%)	$7.4 \times 10^5 \pm 5.6 \times 10^5$ (76%)
Caiçara Island	$3.0 \times 10^7 \pm 2.0 \times 10^7$ (66%)	$5.9 \times 10^6 \pm 5.7 \times 10^6$ (96%)	$1.7 \times 10^7 \pm 1.3 \times 10^7$ (75%)	$5.0 \times 10^7 \pm 1.6 \times 10^8$ (325%)
Calombo Curve	$2.9 \times 10^7 \pm 2.0 \times 10^7$ (68%)	$5.9 \times 10^6 \pm 5.6 \times 10^6$ (95%)	$1.6 \times 10^7 \pm 1.2 \times 10^7$ (73%)	$6.9 \times 10^5 \pm 5.6 \times 10^5$ (81%)
Ipanema	$8.7 \times 10^6 \pm 6.9 \times 10^6$ (79%)	$5.4 \times 10^5 \pm 3.5 \times 10^5$ (65%)	$5.2 \times 10^6 \pm 4.2 \times 10^6$ (81%)	$1.4 \times 10^5 \pm 2.3 \times 10^5$ (161%)
Leblon	$1.1 \times 10^7 \pm 6.6 \times 10^6$ (61%)	$9.6 \times 10^5 \pm 7.3 \times 10^5$ (76%)	$6.3 \times 10^6 \pm 4.1 \times 10^6$ (66%)	$2.8 \times 10^6 \pm 6.1 \times 10^6$ (219%)

X  $\pm$  SD (CV%).

**Table 2.** Mean (X), standard deviation (SD) and coefficient of variation (CV%) of Total Bacteria (TB), bacteria with low apparent nucleic acid content (LNA) and bacteria with high apparent nucleic acid content (HNA) obtained on RFL2.

	TB (cells.mL <sup>-1</sup> )	LNA (cells.mL <sup>-1</sup> )	HNA (cells.mL <sup>-1</sup> )
Rebouças Tunnel	$2.5 \times 10^7 \pm 3.0 \times 10^7$ (118%)	$9.0 \times 10^6 \pm 1.1 \times 10^6$ (123%)	$1.6 \times 10^7 \pm 1.9 \times 10^7$ (115%)
Piraquê Island	$1.4 \times 10^7 \pm 1.1 \times 10^6$ (7%)	$4.9 \times 10^6 \pm 8.5 \times 10^5$ (17%)	$9.3 \times 10^6 \pm 1.8 \times 10^6$ (20%)
Central Channel	$9.0 \times 10^6 \pm 5.0 \times 10^6$ (56%)	$4.4 \times 10^6 \pm 3.2 \times 10^6$ (74%)	$4.6 \times 10^6 \pm 1.7 \times 10^6$ (38%)
Caiçara Island	$1.8 \times 10^7 \pm 2.5 \times 10^7$ (136%)	$9.7 \times 10^6 \pm 1.0 \times 10^7$ (108%)	$1.1 \times 10^7 \pm 9.8 \times 10^6$ (85%)
Cantagalo Park	$8.3 \times 10^6 \pm 3.7 \times 10^6$ (45%)	$4.0 \times 10^6 \pm 2.7 \times 10^6$ (67%)	$4.3 \times 10^6 \pm 1.1 \times 10^6$ (26%)
Beach	$6.9 \times 10^6 \pm 2.5 \times 10^6$ (37%)	$4.3 \times 10^6 \pm 3.1 \times 10^6$ (74%)	$2.6 \times 10^6 \pm 6.1 \times 10^5$ (23%)

X  $\pm$  SD (CV%).

Caiçara Island. The opposite was verified for HNA bacteria. In spite of this uniformity, the highest and lowest percentuals of LNA were found, respectively, in Rebouças Tunnel (24%) and Caiçara Island (19%). HNA cells yielded 55% of TB on Calombo Curve and 48% on Rebouças Tunnel. On average, LNA cells found on Leblon were higher than Ipanema. The same distribution pattern was noted for HNA whose dominance confirmed the influence of RFL on the beaches. The percentuals of LNA and HNA were, respectively, of 6% and 59% on Ipanema, and of 9% and 58% on Leblon.

On RFL2, LNA and HNA abundances found on Caiçara Island and Rebouças Tunnel were higher than that of Cantagalo Park (Table 2). The highest and lowest percentuals of LNA were noted, respectively, on Piraquê (35%) and Caiçara Island (53%). HNA cells yielded 66% of TB on Piraquê Island and 51% on Central Channel. Different to RFL1, LNA were more abundant on the beach. The percentuals of LNA and HNA cells, were respectively, of 62% and 38%.

The occurrence of different bacterial sub-groups was also described in others aquatic environments (1,17). LNA bacteria were characterized by low size cells with low nucleic acid content. These characteristics indicate the low cellular activity of these microorganisms suggesting that they would be dead, dormant or inactive (8). The elevate metabolic activity of HNA is corroborated by the high nucleic acid content.

The spatial uniformity observed in RFL, with dominance of HNA cells, indicated that the bacterial populations presented in all sampling areas were maybe controlled by the same environmental factor. Studies about bacterial regulation (3)

demonstrated the influence of nutrients availability on bacterial growth. This work showed the dominance of HNA bacteria in environments with elevated nutrients content. Despite this, a negative correlation between nutrients and bacterial sub-groups were observed in RFL1 (Table 3). These data suggest that bacterial populations were regulated by protist predation or viral lyses meaning a “top-down” control. Also high size and metabolically active bacteria are preferentially fed by protists (6,10).

Table 1 also shows the data of CB obtained in RFL1. An uniforme spatial pattern was observed. On average, the highest and lowest values were found, respectively, in Rebouças Tunnel and Calombo Curve. The data were in agreement with those of Lutterbach *et al.* (2001) (15), who found a range of  $10^3$  to  $6.5 \times 10^7$  CFU.100 mL<sup>-1</sup> over a period of 4 years. According to these authors, the little spatial difference observed between the areas may be explained by the specific characteristics of each one. The values found in Ipanema were higher than those observed at Leblon, confirming the high influence of lagoon on these beaches.

The abiotic parameters were also uniformity distributed and confirmed the eutrophication process (16). They explained the results of bacterial abundances, evidentiaded by the positive correlation observed between TB and temperature (0.53,  $p < 0.05$ ), and negative correlation with salinity (-0.88,  $p < 0.05$ ) (Table 3). The negative correlation found between TB and nutrients suggest the use of these elements on metabolic activity. The high TB numbers, the elevate chlorophyll contents and the positive correlation observed between them (0.39,  $p < 0.05$ ),

**Table 3.** Pearson correlation established between Total Bacteria (TB), bacteria with low apparent nucleic acid content (LNA), bacteria with high apparent nucleic acid content (HNA), Cultured Bacteria (CB), temperature (Temp), salinity (Sal), pH, dissolved oxygen (DO), ammonia (AN), nitrite, nitrate, ortophosphate (Ortho), chlorophyll *a* (Clh) and suspended particulate matter (SPM) in RFL1 ( $p < 0.050$ ).

	TB	LNA	HNA	CB	Temp	Sal	pH	DO	AN	Nitrite	Nitrate	Ortho	Clh	SPM
TB	1.00	0.84*	0.95*	0.14	0.53*	-0.88*	0.28	0.31*	-0.21	-0.15	0.07	-0.33*	0.39*	0.13
LNA	0.84*	1.00	0.68*	0.11	0.17	-0.81*	0.23	0.38*	-0.24	-0.01	0.24	-0.06	0.50*	0.07
HNA	0.95*	0.68*	1.00	0.17	0.63*	-0.83*	0.24	0.17	-0.12	-0.17	-0.04	-0.41	0.30*	0.20
CB	0.14	0.11	0.17	1.00	-0.16	-0.13	-0.36*	-0.42*	0.36*	0.18	0.13	0.14	-0.26	-0.15
Temp	0.53*	0.17	0.63*	-0.16	1.00	-0.43*	0.58*	0.29	-0.33*	-0.49*	-0.43*	-0.63*	0.44*	0.40*
Sal	-0.88*	-0.81*	-0.83*	-0.13	-0.43*	1.00	-0.33*	-0.31*	0.37*	0.31*	0.10	0.08	-0.51*	-0.02
pH	0.28	0.23	0.24	-0.36*	0.58*	-0.33*	1.00	0.76*	-0.83*	-0.44*	-0.27	-0.34*	0.79*	0.27
DO	0.31*	0.38*	0.17	-0.42*	0.29	-0.31*	0.76*	1.00	-0.81*	-0.12	0.11	-0.19	0.70*	0.05
AN	-0.21	-0.24	-0.12	0.36*	-0.33*	0.37*	-0.83*	-0.81*	1.00	0.38*	0.17	0.10	-0.79*	-0.13
Nitrite	-0.15	-0.01	-0.17	0.18	-0.49*	0.31*	-0.44*	-0.12	0.38*	1.00	0.84*	-0.04	-0.27	-0.33*
Nitrate	0.07	0.24	-0.04	0.13	-0.43*	0.10	-0.27	0.11	0.17	0.84*	1.00	-0.11	-0.16	-0.40*
Ortho	-0.33*	-0.06	-0.41*	0.14	-0.63*	0.08	-0.34*	-0.19	0.10	-0.04	-0.11	1.00	-0.10	-0.01
Clh	0.39*	0.50*	0.30*	-0.26	0.44*	-0.51*	0.79*	0.70*	-0.79*	-0.27	-0.16	-0.10	1.00	0.36*
SPM	0.13	0.07	0.20	-0.15	0.40*	-0.02	0.27	0.05	-0.13	-0.33*	-0.40*	-0.01	0.36	1.00

indicated that bacterial and phytoplankton populations of RFL grow in response to the same environmental factor, probably the nutrients. Although a positive correlation had been noted between TB and CB (0.14), data obtained by flow cytometry were one or two orders of magnitude higher than plate counts. Flow cytometry is better than traditional cultivation methods to determine bacterial abundances with more precision. The inhibition of natural microorganisms in nutrient rich-medium, previously developed for clinical analysis, and the presence of viable but not cultivable cells in the environment may justify this results (12). A percentual ratio between 0.0019 and 0.5107 was in agreement with Joux & Lebaron (1995) (12), who observed that cultured bacteria represents only approximately 1% of total bacterial abundances.

According to Azam *et al.* (1983) (2) and Kolm *et al.* (1997) (13), the heterotrophic bacteria are more abundant in association with suspended organic matter. This was corroborated by the positive correlation noted between CB and nutrients (Table 3). The elevated levels of ammonia, nitrite, nitrate and ortophosphate agreed with Machado *et al.* (1998) (16), confirming the high organic pollution of the lagoon. According to Hoch & Kirchman (1993) (11), the organic matter produced by phytoplankton can be used as an energy supply by heterotrophic bacteria. The negative correlation found between CB and chlorophyll *a* ( $-0.26, p < 0.05$ ) indicated that the bacteria fraction was probably more abundant after the decline of phytoplankton. The high phytoplanktonic biomass noted can be explained by the nutrients availability. The use of these nutrients by phytoplankton was corroborated by the negative correlation observed between them (Table 3). The average chlorophyll *a* data ranged from  $128.9 \pm 129.8 \mu\text{g.L}^{-1}$  (101%) in Rebouças Tunnel to  $84.7 \pm 75.6 \mu\text{g.L}^{-1}$  (89%) in Calombo Curve. The high phytoplanktonic activity was also confirmed by the positive correlation noted between chlorophyll *a* and pH ( $0.79, p < 0.05$ ) and DO ( $0.70, p < 0.05$ ).

The great variability observed in DO levels ( $1.7$  to  $11.6 \text{ ml.L}^{-1}$ ) is characteristic of eutrophic environments with high phytoplanktonic production (16). The low values found in some dates of sampling can may be explained by the presence of high levels of organic matter. This was probably due to the aerobic bacterial metabolism, which consume the oxygen of the water during decomposition activity (5). The negative correlations noted between DO and nutrients confirmed this.

The continental influence on lagoon was confirmed by the results of temperature, salinity and pH. Temperature ranged from  $18^{\circ}\text{C}$  in Caiçaras Island to  $31^{\circ}\text{C}$  in Piraquê Island, Central Channel and Calombo Curve. Salinity varied from  $5.2 \text{ S}$  in Caiçara Island to  $9.8 \text{ S}$  in Central Channel. Minimum and maximum values of pH were obtained in Caiçara Island ( $7.2$ ) and Central Channel ( $9.2$ ), respectively. The negative correlation observed between temperature and salinity ( $-0.43, p < 0.05$ ), and pH and salinity ( $-0.33, p < 0.05$ ) confirmed the antropic influence and the difficult

water renewal on RFL. This can be explained by the dimensions of Jardim de Alah Channel, which allows only a very superficial circulation (22). Beside this, the Jardim de Alah Channel is frequently blocked by silt and alluvial deposits, difficulting the oceanic water inflow (22). The results are in agreement with those obtained by Machado *et al.* (1998) (16), who working in RFL during March 1991 to February 1995 observed values of temperature, salinity and pH of  $20$  to  $31^{\circ}\text{C}$ ,  $2$  to  $26 \text{ S}$  and  $4$  to  $12$ , respectively.

Our data confirmed the degradation process previously described for RFL (15,16). This process restricts its multiple uses and has serious public health implications. The lagoon has turned into a site with sludge outcrops in some of its margins, turbid waters and accelerated eutrophication, with episodes of high fish mortality and bed smell. To avoid the eutrophication process, the authorities should restringe the sewage inflow to RFL closing the clandestine waste galleries and dredging the Visconde de Albuquerque interceptor channel. This channel was constructed to collect the RFL catchment basin water transferring it directly to the ocean. Although, it is normally halted by alluvial deposits which limit its role. Dredging and the increase of the Jardim de Alah Channel depths are mechanisms which may also contribute to avoid the lagoon's pollution. This will increase the water circulation and renewal, principally of deeper layers, reducing the residence time and promoting its oxygenation. Low levels of organic matter, low production of  $\text{H}_2\text{S}$  by sulphate-reduction bacteria present in bottom sediments, and high oxygenation are adequate conditions to preserve the local biota and avoid the constant fish mortality. The present data also showed that the outflow of the lagoon's polluted waters affect the sanitary conditions of Ipanema and Leblon beaches, which becomes unfit for bathing over long periods of time.

## CONCLUSIONS

The results indicated that the RFL is an eutrophic ecosystem where the bacterial populations and the physical and chemical parameters do not differ spatially. The strong influence of the lagoon on nearby oceanic beaches was also noted. The spatial difference between them is probably due to the presence of currents that flow predominantly from Ipanema to Leblon transporting the lagoon's polluted waters to this beach. This is the first study that used flow cytometry to evaluate heterotrophic bacteria distribution in Brazilian lagoons. The dominance of HNA cells indicated that microbial community present in RFL is metabolically active.

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## RESUMO

### Abundância bacteriana heterotrófica na Lagoa Rodrigo de Freitas (Rio de Janeiro, Brasil)

A Lagoa Rodrigo de Freitas (RJ, Brasil) é um importante ecossistema que vêm sendo submetido a um acelerado processo de degradação. O objetivo desse estudo foi determinar a abundância e a distribuição espacial das bactérias heterotróficas totais (citometria em fluxo) e cultivadas (“pour plate” em ágar R<sub>2</sub>A). Outro objetivo foi avaliar a influência da lagoa na qualidade das águas de Ipanema e do Leblon. Dados físico-químicos também foram adquiridos. Amostras de água sub-superficiais foram coletadas mensalmente, de dezembro de 1999 a outubro de 2000. Na lagoa, a abundância de bactérias cultivadas variou de  $6,9 \times 10^5$  a  $5,0 \times 10^7$  UFC.100 mL<sup>-1</sup>. Em Ipanema e no Leblon, esse parâmetro atingiu  $1,4 \times 10^5$  e  $2,8 \times 10^6$  UFC.100 mL<sup>-1</sup>, respectivamente. A abundância bacteriana total variou de  $2,9 \times 10^7$  a  $3,2 \times 10^7$  células.mL<sup>-1</sup> na lagoa. Em Ipanema e no Leblon, este parâmetro alcançou, respectivamente,  $8,7 \times 10^6$  a  $1,1 \times 10^7$  células.mL<sup>-1</sup>. Dois sub-grupos bacterianos foram observados nas estações, com predomínio de células HNA. Amostras foram posteriormente coletadas visando um maior entendimento sobre as bactérias presentes nesses ambientes. A abundância bacteriana foi analisada apenas por citometria em fluxo e os resultados oscilaram entre  $8,3 \times 10^6$  e  $2,5 \times 10^7$  células.mL<sup>-1</sup> na lagoa. Na praia, esse parâmetro atingiu  $6,9 \times 10^6$  células.mL<sup>-1</sup>. Dois sub-grupos bacterianos também foram observados, com predomínio de células HNA na lagoa e LNA na praia adjacente. Os resultados indicaram que a lagoa é um ecossistema eutrofizado, onde as bactérias e os parâmetros físico-químicos distribuem-se de modo uniforme. Os dados ainda indicaram que as águas poluídas da lagoa afetam a qualidade das praias de Ipanema e do Leblon.

**Palavras-chave:** bacterioplâncton, citometria em fluxo, eutrofização, poluição de água, Lagoa Rodrigo de Freitas

## REFERENCES

- Andrade, L.; Gonzalez, A.M.; Araujo, F.V.; Paranhos, R. Flow cytometry assessment of bacterioplankton in tropical marine environments. *J. Microbiol. Meth.*, 55: 841-850, 2004.
- Azam, F.; Fenchel, T.; Field, J.G.; Gray, J.S.; Meyer-Reil, L.A.; Thingstad, F. The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.*, 10, 257-263, 1983.
- Billen, G.; Servais, P.; Becquevort, S. Dynamics of bacterioplankton in oligotrophic and eutrophic aquatic environments: bottom-up or top-down control? *Hydrobiologia*, 207: 37-42, 1990.
- Brito, I.M.; Lemos, E.E. Evolução geológica e fauna da Lagoa Rodrigo de Freitas, Rio de Janeiro. *An. Acad. Brasil. Ciênc.*, 54(1): 143-164, 1982.
- Carmouze, J.P.; Bernardes, M.; Domingos, P. Asfixia das lagoas costeiras do Estado do Rio de Janeiro. *França-Flash Meio Ambiente*, 4: 2-3, 1995.
- Del Giorgio, P.A.; Bird, D.F.; Prairie, Y.T.; Planas, D. Flow cytometric determination of bacterial abundance in lake plankton with the green nucleic acid stain syto 13. *Limnol. Oceanogr.*, 41(4), 783-789, 1996.
- Fundação Estadual de Engenharia do Meio Ambiente. *O controle de poluição das águas do estado do Rio de Janeiro*. Cadernos FEEMA, FEEMA, Rio de Janeiro, 24p, 1980.
- Gasol, J.M.; del Giorgio, P.A.; Massana, R.; Duarte, C.M. Active versus inactive bacteria: size-dependence in a coastal marine plankton community. *Mar. Ecol. Prog. Ser.*, 128, 91-97, 1995.
- Gasol, J.M.; Zweifel, U.L.; Peters, F.; Fuhrman, J.A.; Hagström, A. Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. *Appl. Environ. Microbiol.*, 65(10): 4475-4483, 1999.
- González, J.M.; Sherr, E.B.; Sherr, B.F. Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. *App. Environ. Microbiol.*, 56(3): 583-589, 1990.
- Hoch, M.P.; Kirchman, D.L. Seasonal and inter-annual variability in bacterial production and biomass in a temperate estuary. *Mar. Ecol. Prog. Ser.*, 98: 283-295, 1993.
- Joux, F.; Lebaron, P. Applications en écologie bactérienne des sondes oligonucléotidiques fluorescentes par les techniques d'hybridation et de cytométrie. *Océanis*, 21(1): 125-138, 1995.
- Kolm, H.E.; Giamberardino Filho, R.E.; Korman, M.C. Spatial distribution and temporal variability of heterotrophic bacteria in the sediments of Paranaguá and Antonina Bays, Paraná, Brazil. *Rev. Microbiol.*, 28, 230-238, 1997.
- Lebaron, P.; Parthuisot, N.; Catala, P. Comparison of blue nucleic acid dyes for flow cytometric enumeration of bacteria in aquatic systems. *Appl. Environ. Microbiol.*, 64(5), 1725-1730, 1998.
- Lutterbach, M.T.S.; Vasquez, J.C.; Pinet, J.A.; Andreatta, J.V.; da Silva, A.C. Monitoring and spatial distribution of heterotrophic bacteria and fecal coliforms in the Rodrigo de Freitas Lagoon, Rio de Janeiro, Brazil. *Braz. Arch. Biol. Technol.*, 44(1), 7-13, 2001.
- Machado, M.C.; Andreatta, J.V.; Marca, A.G. Impacto humano avaliado pela hidrobiologia de uma lagoa costeira: Lagoa Rodrigo de Freitas, Rio de Janeiro. VIII Seminário Regional de Ecologia, São Carlos, SP, 1998, vol. III, p.1423-1438.
- Marie, D.; Partensky, F.; Jacquet, S.; Vaulot, D. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Appl. Environ. Microbiol.*, 63(1): 186-193, 1997.
- Oliveira, J.A. *Contribuição ao conhecimento da fauna da Lagoa Rodrigo de Freitas*. Rio de Janeiro, Sudepe/RJ, 11p, 1976.
- Pomeroy, L.R. The ocean's food web, a changing paradigm. *Bioscience*, 24(9), 499-504, 1974.
- Secretaria Municipal de Meio Ambiente. *Programa de monitoramento dos ecossistemas costeiros urbanos do município do Rio de Janeiro*, 48p, 1998.
- Strickland, J.D.H.; Parsons, T.R. *A practical handbook of seawater analysis*. Fisheries Research Board of Canada, Ottawa, 1972, 310p.
- Torres, J.M. Lagoa Rodrigo de Freitas. *Rev. Municipal de Engenharia*, vol. XLI (1-4): 31-53, 1990.
- Zar, J.H. *Biostatistical Analysis*. 2<sup>nd</sup> ed., Prentice-Hall, New Jersey, 1984, 469p.