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AUTO- AND HETEROTROPHIC NANOPLANKTON AND FILAMENTOUS BACTERIA OF GUANABARA BAY (RJ, BRAZIL): ESTIMATES OF CELL/FILAMENT NUMBERS *VERSUS* CARBON CONTENT

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ABSTRACT

Variations of nanoplankton (2-20 μm) and filamentous bacteria (diameter: 0.5-2.0 μm) of Guanabara Bay (RJ, Brazil) are presented, considering cell density and carbon content of auto- and heterotrophs. Our goal is to contribute to future modeling of local trophic dynamics. Subsurface water samples were taken weekly during the year 2000 at two sites: Urca (close to the entrance, more saline, eutrophic) and Ramos (inner area, less saline, hypertrophic). Microscopic analysis was done by epifluorescence and cell density was converted to biomass through cell biovolume. Total nanoplankton was about 10^8 cells.l^{-1} in most samples (>57%), and total filamentous bacteria densities varied from 10^5 to 10^8 fil.l^{-1}. Autotroph density was one order of magnitude higher at Ramos, both for nanoplankton (Md: 10^8cells.l^{-1} at Ramos and 10^7cells.l^{-1} at Urca) and for filamentous bacteria (Md: 10^6 fil.l^{-1} at Ramos and 10^5 fil.l^{-1} at Urca). The same was observed for autotrophic biomass (Md: 10^3μgC.l^{-1} at Ramos and 10^1μgC.l^{-1} at Urca for nanoplankton; Md: 28μgC.l^{-1} at Ramos and 1.4μgC.l^{-1} at Urca for filamentous bacteria). The relative contribution of autotrophs increased after conversion to biomass. Seasonal variation was conspicuous for filamentous bacteria at both sites and for nanoplankton only at Ramos, with maximum autotrophic abundances during the rainy period (spring-summer).

RESUMO

Variações do nanoplâncton (2-20μm) e bactérias filamentosas (diâmetro: 0.5-2.0 μm) da Baía de Guanabara (RJ, Brasil) são apresentadas, considerando densidade celular e biomassa de autótrofos e heterótrofos. A meta deste trabalho é contribuir para uma futura modelagem da dinâmica trófica neste sistema. Amostras subsuperficiais de água foram coletadas semanalmente durante um ano em dois pontos: Urca (próximo à entrada, mais salino, eutrófico) e Ramos (no interior, menos salino, hipertrófico). Foi feita análise por microscopia de epifluorescência, com densidade celular convertida para biomassa através do biovolume celular. A concentração do nanoplâncton total foi alta (10^8cel.l^{-1}) na maioria das amostras (>57%) e das bactérias filamentosas variou de 10^5 a 10^8fil.l^{-1}. A densidade de autótrofos em Ramos foi uma ordem de grandeza superior tanto para o nanoplâncton (Md: 10^8cel.l^{-1} em Ramos e 10^7cel.l^{-1} na Urca) quanto para as bactérias filamentosas (Md: 10^6fil.l^{-1} em Ramos e 10^5fil.l^{-1} na Urca). A biomassa autotrófica do nanoplâncton (Md: 10^3μgC.l^{-1} em Ramos e 10^1μgC.l^{-1} na Urca) e das bactérias filamentosas (Md: 28μgC.l^{-1} em Ramos e 1,4μgC.l^{-1} na Urca) seguiu o mesmo padrão. A contribuição relativa de autótrofos aumentou após a conversão para biomassa. Uma tendência temporal foi evidenciada para as bactérias filamentosas em ambos os pontos e, para o nanoplâncton autotrófico, em Ramos, com valores máximos no período chuvoso (primavera-verão).

Descriptors: Estuary, Guanabara Bay, Nanoplankton, Filamentous bacteria, Autotrophs, heterotrophs, Biomass, Cell density.
Descritores: Estuário, Baía de Guanabara, Nanoplâncton, Bactérias filamentosas, Autótrofos, Heterótrofos, Biomassa, Densidade celular.

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INTRODUCTION

Cell density, carbon content, cell volume and chlorophyll concentration have been used to determine phytoplankton abundance. Although cell density is most often used to quantify phytoplankton in community structure studies, if biovolume, biomass and/or chlorophyll are used as additional descriptors, the factors that influence phytoplankton dynamics can be more clearly understood (Jiménez *et al*., 1987). Relationships between cell carbon and cell volume were established because of difficulties in assessing carbon content of living phytoplankton in the sea, due to the presence of detritus and variability of phytoplankton carbon:chlorophyll ratios (Mullin *et al*., 1966). Thus, biomass expressed as carbon content, calculated from biovolume estimates, can better reflect cellular metabolic processes and phytoplankton standing crop because cell size differs between species and for the same species during its cell cycle (Smayda, 1978). The downside of such an approach is that it is labor intensive and, therefore, seldom applied in routine analysis, despite its ecological value in understanding phytoplankton dynamics in natural environments.

The differentiation of true autotrophs from heterotrophic flagellates and bacteria is also key to estimating phytoplankton biomass and thus revealing trophic relationships. Actually, if heterotrophs are taken into account separately, their species composition and relative abundances can also be used as water quality indicators, especially in extreme conditions such as oligotrophic or highly polluted waters, as done elsewhere (Gaines & Elbrächter, 1987). The use of epifluorescence microscopy is needed to assess the truly photosynthetic fraction of the plankton. The use of such methodology, although not a novelty (Wilde & Fliermans, 1979; Davis & Sieburth, 1982), can only provide reliable data when operational circumstances allow for the appropriate storage and quick analysis of samples to counteract fairly rapid loss of chlorophyll fluorescence (and/or the fluorescence of other accessory pigments or stains of interest).

The phytoplankton community of Guanabara Bay, a tropical estuary with a high degree of organic pollution, is well known from studies that have focused on structural aspects of the community, such as composition, cell density and species diversity (Faria & Cunha, 1917; Sevrin-Reyssac *et al*., 1979; Soares *et al*., 1981; Villac *et al*., 1991; Valentin *et al*., 1999). The majority of these studies emphasize the microplankton fraction (cells > 20 μm). Those that included nanoplankton (cells of 2-20 μm) indicated that this fraction comprised the bulk of the local phytoplankton community, contributing with up to 90% of total cell abundance (Sevrin-Reyssac *et al*., 1979; Villac *et al*., 1991; Valentin *et al*., 1999).

Filamentous cyanobacteria of Guanabara Bay have been considered as a separate fraction within the phytoplankton community for methodological and ecological reasons. The diameter of the cells (or filaments) in question vary between 1.0-2.0 μm and the differentiation of cells within each filament is not possible with the final magnification (most often 400X) used in routine analysis. Therefore, the whole filament has been considered as the functional unit of the organism. At any given sample, filamentous cyanobacteria were very abundant (10^5 - 10^8 fil.l^{-1}) and filament numbers were as high as, or even higher than, cell numbers of the nanoplankton fraction (Sevrin-Reyssac *et al*., 1979; Villac *et al*., 1991; Valentin *et al*., 1999). Their space-time distribution in the Bay has been associated with variations in freshwater input; that is, higher filament numbers were found during rainy periods (summertime), especially in the inner areas of the Bay.

More recently, a preliminary study on autotrophic phytoplankton biomass estimates in Guanabara Bay has tested and adapted protocols of fixation, storage and routine analysis for this tropical environment (Tenenbaum *et al*., 2001). Based on a restricted set of samples, this study indicated a high contribution of heterotrophs for the picoplankton and nanoplankton fractions, especially in terms of cell density. Therefore, previous phytoplankton studies have probably overestimated the contribution of what was then called nanoplankton because they included heterotrophic nanoflagellates and coccoid bacteria.

The purpose of this study was to assess the seasonal variation of auto- and heterotrophic nanoplankton and filamentous bacteria at two contrasting sites in Guanabara Bay during the year 2000. Our goal was to provide information to advance our understanding of the abundance of the truly photosynthesizing phytoplankton of the Bay, based not only on cell numbers but also on carbon content. This type of information can be valuable to future modeling of local trophic dynamics.

MATERIAL AND METHODS

Study area and Sample Collection

Guanabara Bay is known for its beauty, historic and socio-economic importance, and for its severe pollution problems caused by the input of sewage and industrial waste. It is considered a partially mixed estuary, where salinity gradients are affected by the interactions of freshwater, salt water and tidal energy (Kjerfve *et al*., 1997). The degree of pollution varies according to a tidal-driven circulation pattern, which renders the inner areas of the Bay more eutrophic (Mayr *et al*., 1989). The climate of the study area is warm and wet all year around, with a rainy

season during spring-summer (from September to March) and a drier period during autumn-winter (from April to August), a seasonality that influences the hydrobiology of the Bay (Mayr *et al.*, 1989). This pattern was observed during the study period, at both sites, with higher temperatures and precipitation in the Bay inner area (Fig. 1).

Our data reports on the findings relative to the year 2000, based on weekly field trips at two contrasting sites (n=80) according to salinity and degree of pollution (Fig. 2): (1) Urca, close to the entrance to the Bay, a less polluted site due to the contribution of more saline, cleaner and clearer coastal water; and (2) Ramos, in the Bay inner area, a less saline and more polluted site due to the proximity to rivers and waste outfalls, and where landfills have altered water circulation. Sites Urca and Ramos can represent, respectively, the best (eutrophic) and the worst (hypertrophic) water quality scenarios in Guanabara Bay (Paranhos *et al*., 2001).

Plankton Sampling and Analysis

Water samples (Van Dorn bottle) were taken from the subsurface, fixed with buffered formaldehyde (final concentration 2%), and kept cool during transport to the laboratory. The nanoplankton and filamentous bacteria were analyzed according to Booth (1987, 1995) on previously treated microscope slides (see Sherr & Sherr, 1993), as follows. Immediately after sampling, 5-ml aliquots were stained with fluorochrome DAPI (4'6-diamidino-2-fenilindol) to a final concentration of 0.01 $\mu g.l^{-1}$ (Martinussen & Thingstad, 1991), and concentrated onto a black Nuclepore® filter (1.0 µm). These were mounted on microscope slides, kept cool (4-10°C) for one week to avoid the formation of crystals and then stored at -20°C for microscopic analysis no later than 3 months after sampling (Booth, 1995). These slide mounts were analyzed under epifluorescent microscopy to distinguish autotrophs from heterotrophs, through chlorophyll autofluorescence under blue (λ = 450 – 490 ηm) and green (λ = 500 – 550 ηm) light excitation (Booth, 1987, 1995; MacIsaac & Stockner, 1993), at 1000X final magnification. The number of heterotrophs was calculated based on total count with DAPI less the number of autotrophs analyzed by autofluorescence.

At least 400 units of a single cell, a chain of cells or a filament were counted in a variable number of random fields. Each individual cell of the nanoplankton fraction (solitary or in a chain) was considered a functional unit and their abundance was thus expressed as cells per liter ($cells.l^{-1}$). The abundance of filamentous bacteria was expressed as filaments per liter ($fil.l^{-1}$) because individual cells were not clearly visible.

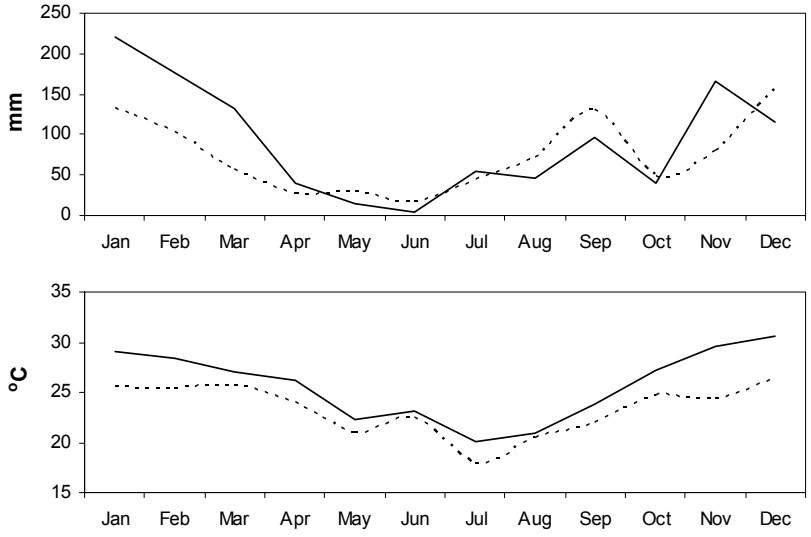

Fig. 1. Variation of (a) precipitation (cumulative) and (b) air temperature (average) at meteorological stations closer to the entrance (dotted line) and closer to the inner area (solid line) of Guanabara Bay for the year 2000. Source: GEORIO.

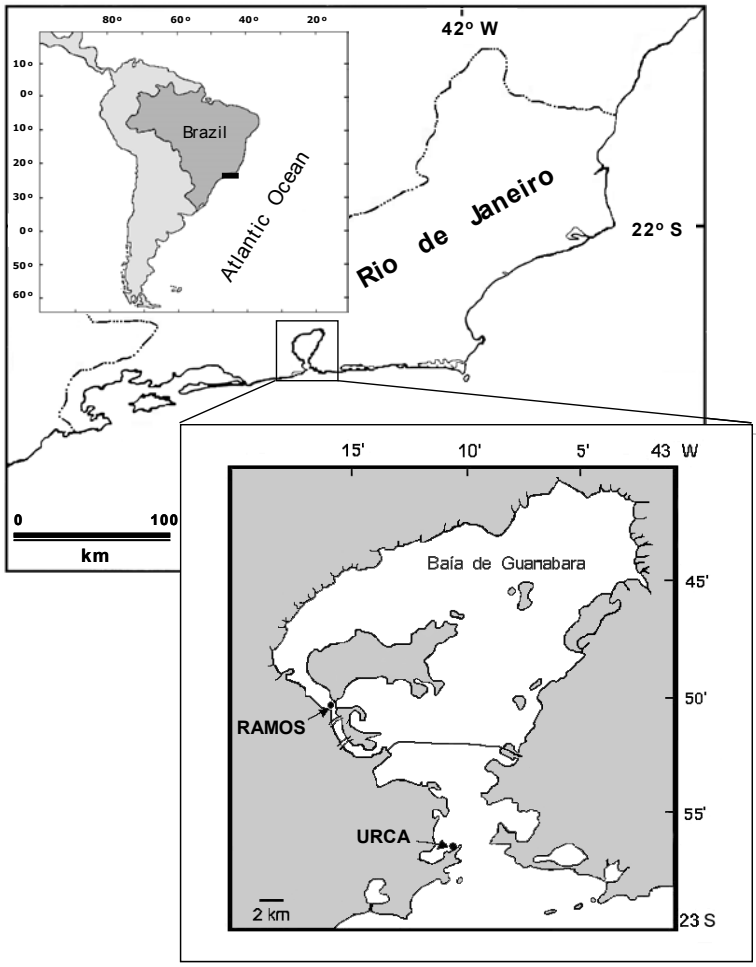

Fig. 2. Guanabara Bay, Brazil, showing site Urca and site Ramos, which were visited weekly from January to December 2000.

The distinction between the coccoid cyanobacterium *Synechocystis* (Komárek) Kovácik, the diatoms *Skeletonema* spp. Greville, and cryptophytes was possible because sub-samples were observed using transmitted light microscopy with bright field and phase contrast. Once their identities were determined, the fluorescence of each type of cell was distinguishable due to: (1) the uniform and deep fluorescence of *Synechocystis* whose pigments are not individualized in chloroplasts; (2) the chain-forming habit of *Skeletonema*; and (3) the orange fluorescence of cryptophytes excited by blue light (λ = 450-490 ηm) (Booth, 1987).

Cell bioVolume and Biomass Estimates

Cell biovolume was calculated through microscopic measurements of various cell dimensions, and an approximation of standard geometric shapes according to Edler (1979). Biovolume was then converted to cell carbon biomass using the 101pgC.μm^{-3} factor derived experimentally from the nano-size fraction (Montagnes *et al*., 1994).

Abiotic Parameters

The following analyses were performed to better characterize the water quality at each sampling site during the study period: water temperature was determined by a mercury thermometer; salinity by conductivity (Aminot, 1983); dissolved oxygen by the Winkler-azide method (Aminot, 1983); suspended particulate matter by gravimetry (Grasshoff *et al.*, 1999); total phosphorus by persulphate oxidation followed by the molybdenium blue method (Grasshoff

et al., 1999); reactive orthophosphate by the molybdenium blue method (Grasshoff *et al.,* 1999); ammonia ($N\text{-}NH_3$ + $N\text{-}NH_4^+$) by the indophenol method (Parsons *et al*., 1984); nitrite by diazotation (Grasshoff *et al.,* 1999); nitrate by reduction in a Cd-Cu column followed by diazotation (Grasshoff *et al.,* 1999); total nitrogen by alkaline persulfate oxidation (Grasshoff *et al.,* 1999); silicate by the silicomolybdic method (Grasshoff *et al.,* 1999); and chlorophyll *a* by spectrophotometry (Parsons *et al.*, 1984). Linear correlation (r-Pearson) was performed between physical, chemical and biotic parameters to help understand seasonal variations of nanoplankton and filamentous bacteria.

RESULTS

The physical and chemical settings found during the study period (Fig. 3, Table 1) confirmed that sites Urca and Ramos are significantly different. Except for salinity, dissolved oxygen and nitrate, all parameters were higher in the inner area of the Bay. Salinity, dissolved oxygen and nitrate were more variable (at least 3 times as much) at site Ramos, whilst ammonia was more variable (twice as much) at site Urca. Despite some variability, silicate, nitrogen and phosphorus forms were higher during the drier period (March to August) and the beginning of the wet period (September and October).

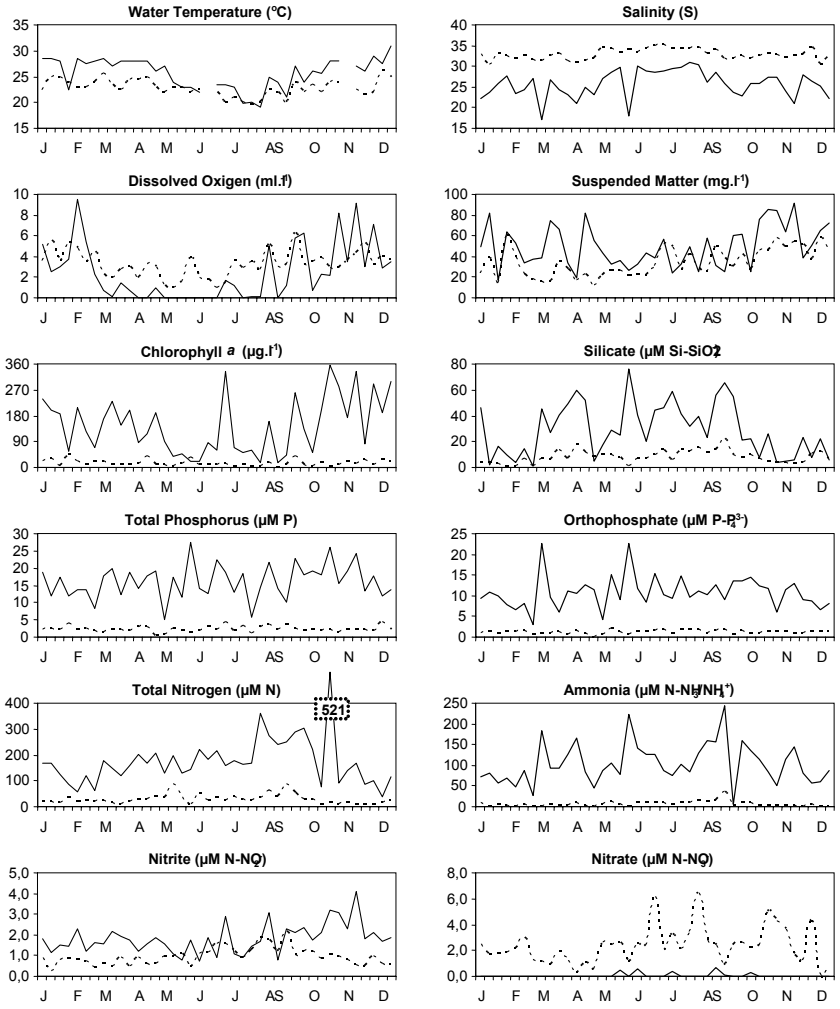

Fig. 3. Variation of physical and chemical parameters at site Urca (doted line) and site Ramos (solid line) from January to December 2000.

Table 1. Average (Avg) and coefficient of variation (CV) of physical and chemical parameters at site Urca and site Ramos (n=40 for each site). * Significant differences between sampling points ($p<0.0001$).

Parameters	Urca		Ramos	
	Avg	CV (%)	Avg	CV (%)
Temperature (^oC)	22.78	8	25.46	12
Salinity (S)	32.84	4	25.84	13
Dissolved oxygen ($ml.l^{-1}$) *	3.34	37	2.35	113
Suspended matter ($mg.l^{-1}$) *	34.04	39	49.37	40
Chlorophyll *a* ($\mu g.l^{-1}$) *	14.37	75	141.47	69
Silicate ($\mu M\ Si\text{-}SiO_2^-$) *	8.29	56	29.73	66
Total phosphorus ($\mu M\ P$) *	2.35	40	16.09	30
Orthophosphate ($\mu M\ P\text{-}P_4^{3-}$) *	1.14	37	10.69	35
Total nitrogen ($\mu M\ N$) *	28.25	62	176.59	49
Ammonia ($\mu M\ N\text{-}NH_3/NH_4^+$) *	6.30	99	105.86	45
Nitrite ($\mu M\ N\text{-}NO_2^-$)	0.95	43	1.84	38
Nitrate ($\mu M\ N\text{-}NO_3^-$) *	2.35	61	0.07	261

The presence of a variety of taxonomic groups was observed with transmitted light microscopy, such as coccoid and filamentous cyanobacteria, diatoms, dinoflagellates, cryptophytes, prasinophytes, and chlorophytes. Among the autotrophs, only the cyanobacterium *Synechocystis*, the diatoms *Skeletonema* spp., and cryptophytes could be distinguished with epifluorescence. The first two were the most abundant ones.

Total densities of nanoplankton and filamentous bacteria at site Ramos were one to two orders of magnitude higher than those found at site Urca (Fig. 4, Table 2). Nanoplankton densities were *ca.* 10^8 $cells.l^{-1}$ in the majority of the samples from site Urca (65%) and site Ramos (57%); otherwise, site Urca had lower cell densities (10^7 $cells.l^{-1}$) and site Ramos had higher cell densities (10^9 $cells.l^{-1}$). The same trend was found for the total number of filamentous bacteria (Md: 10^6 $fil.l^{-1}$ at site Urca and 10^7 $fil.l^{-1}$ at site Ramos).

At site Urca, the contribution of heterotrophic nanoplankton was high (Md = 91%) during the whole study period (Fig. 4a). At site Ramos, high relative densities of heterotrophic nanoplankton (> 90%) were found only during the winter-spring period (from June to November) (Fig. 4b). At both sites, nanoplankton was mostly composed of heterotrophic flagellates.

The autotrophic nanoplankton comprised almost 100% of the total cell density during the wet summer (from November to March) and the beginning of autumn (April and May), due to the occurrence of the coccoid cyanobacterium *Synechocystis*. Cryptophytes and diatoms (*Skeletonema* spp.) were relatively more abundant in the dry period (from June to August), when cyanobacteria cell densities decreased. Although the relative numbers of *Skeletonema* spp. in the dry period were high (10^5 to 10^6 $cells.l^{-1}$, equivalent to 90-100%), their highest absolute cell densities occurred during the wet period at both sites (Urca: 10^6 $cells.l^{-1}$; Ramos: 10^7 $cells.l^{-1}$).

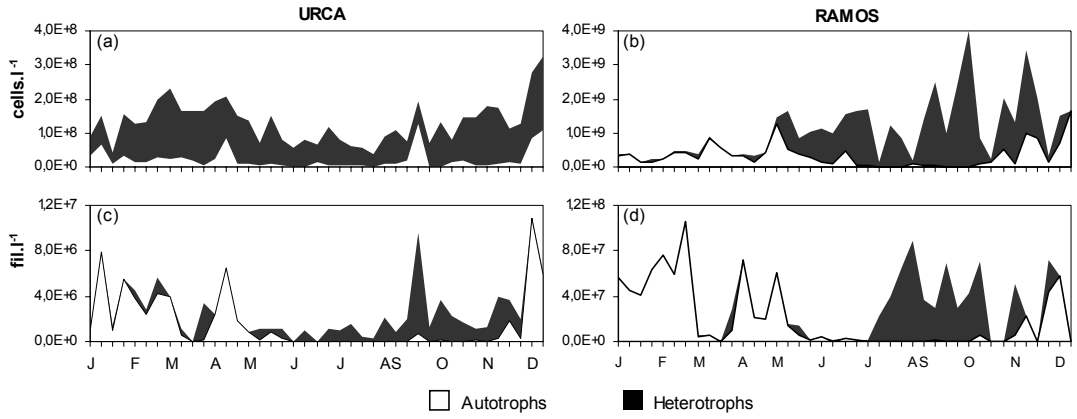

Fig. 4. Density of nanoplankton (a, b) and filamentous bacteria (c, d) from January to December 2000, differentiating the contributions of autotrophs and heterotrophs at site Urca and site Ramos.

Table 2. Minimum, maximum, average, standard deviation and median values of cell density (cells.l^{-1}) of autotrophs found at site Urca and site Ramos. (SD=Standard Deviation; Md=Median; nd=not detected).

Parameters	Category	Minimum	Maximum	Average ± SD	Md
SITE URCA					
Nanoplankton	Total	$3.8x10^7$	$3.2x10^8$	$1.3x10^8 \pm 6.3x10^7$	$1.3x10^8$
	Autotrophs	$1.5x10^6$	$1.3x10^8$	$2.2x10^7 \pm 3.0x10^7$	$1.1x10^7$
	Heterotrophs	$3.4x10^7$	$2.1x10^8$	$1.1x10^8 \pm 4.8x10^7$	$1.1x10^8$
Filamentous bacteria	Total	nd	$1.1x10^7$	$2.7x10^6 \pm 2.6x10^6$	$1.8x10^6$
	Autotrophs	nd	$1.1x10^7$	$1.6x10^6 \pm 2.6x10^6$	$2.2x10^5$
	Heterotrophs	nd	$9.0x10^6$	$1.1x10^6 \pm 1.6x10^6$	$8.5x10^5$
SITE RAMOS					
Nanoplankton	Total	$1.3x10^8$	$4.0x10^9$	$1.1x10^9 \pm 8.9x10^8$	$9.0x10^8$
	Autotrophs	$1.3x10^7$	$1.6x10^9$	$3.3x10^8 \pm 3.6x10^8$	$2.3x10^8$
	Heterotrophs	$2.3x10^6$	$4.0x10^9$	$7.6x10^8 \pm 8.9x10^8$	$5.0x10^8$
Filamentous bacteria	Total	nd	$1.1x10^8$	$3.4x10^7 \pm 3.0x10^7$	$2.9x10^7$
	Autotrophs	nd	$1.1x10^8$	$2.0x10^7 \pm 2.8x10^7$	$4.4x10^6$
	Heterotrophs	nd	$8.8x10^7$	$1.4x10^7 \pm 2.4x10^7$	nd

Temporal variation of auto- and heterotrophs was more conspicuous for filamentous bacteria at both sites (Fig. 4c, d). Maximum abundances of autotrophic filamentous bacteria were observed in the wet period and in the beginning of the dry period. Autotrophic filamentous bacteria were almost 100% of the total density in these periods, due to the presence of species of Pseudoanabaenaceae, whose diameter was 1.0-2.0 µm. Heterotrophic filaments, when present, were narrower (*ca.* 0.5 µm). The total length was highly variable (average length of ca. 50 µm).

The conversion of cell density into biomass revealed a different relative contribution of autotrophs at both sites (Fig. 5, Table 3), that is, the overall contribution of autotrophs became relatively higher than that of heterotrophs. The seasonal trend was especially modified for heterotrophs because they had a smaller biovolume than autotrophs. The winter-spring peaks in the number of heterotrophic filamentous bacteria found at both sites (Fig. 4c,d) were not very evident in terms of carbon content (Fig. 5c,d); the same was observed when cell numbers of heterotrophic nanoplankton of site Ramos (Fig. 4b) were compared with their corresponding carbon content (Fig. 5b). Though differences in temporal patterns and relative contributions of autotrophs and heterotrophs became clear when density and carbon biomass data were compared, both were higher at site Ramos.

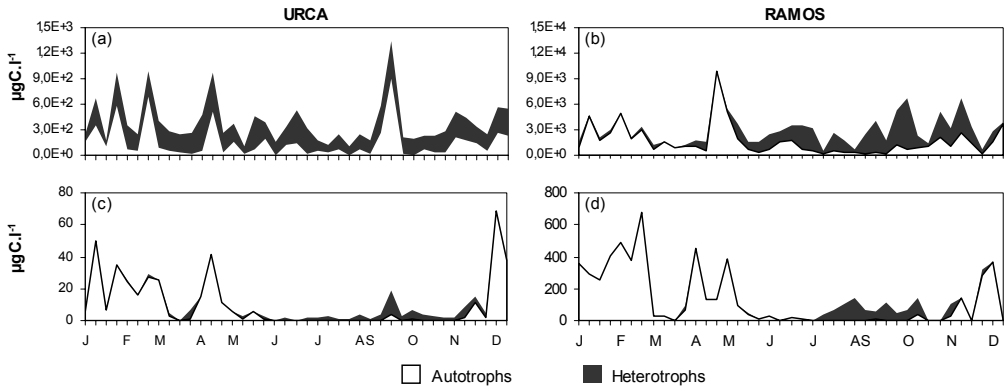

Fig. 5. Biomass of nanoplankton (a, b) and filamentous bacteria (c, d) from January to December 2000, differentiating the contributions of autotrophs and heterotrophs at site Urca and site Ramos.

Table 3. Minimum, maximum, average, standard deviation and median values of cell biomass ($\mu gC.l^{-1}$) of autotrophs found at site Urca and site Ramos. (SD=Standard Deviation; Md=Median; nd=not detected).

Parameters	Category	Minimum	Maximum	Average ± SD	Md
SITE URCA					
Nanoplankton	Total	77	1,400	390 ± 300	300
	Autotrophs	3.7	890	150 ± 200	73
	Heterotrophs	74	460	240 ± 99	220
Filamentous bacteria	Total	nd	84	12 ± 19	2.8
	Autotrophs	nd	69	9.9 ± 16	1.4
	Heterotrophs	nd	15	1.9 ± 2.6	1.4
SITE RAMOS					
Nanoplankton	Total	110	16,000	3,000 ± 3,200	2,000
	Autotrophs	110	9,900	1,600 ± 1,800	1,000
	Heterotrophs	4.2	6,000	1,400 ± 1,300	990
Filamentous bacteria	Total	nd	820	150 ± 220	28
	Autotrophs	nd	670	130 ± 180	28
	Heterotrophs	nd	150	24 ± 39	nd

The attempt to test the linear correlation (r-Pearson, ρ) between physical, chemical and biotic parameters did not give statistically significant results, except for some of the parameters at site Urca. This hindered further use of multivariate analysis, whose interpretation became of little ecological value. A more descriptive approach was thus preferred. At site Urca, chlorophyll *a* had a positive correlation with autotrophs, that is, $\rho = 0.75$ for nanoplankton biomass, $\rho = 0.63$ for nanoplankton density, and $\rho = 0.60$ for filamentous cyanobacteria density or biomass. Filamentous cyanobacteria density and biomass also had a positive correlation with water temperature ($\rho = 0.64$) and an inverse correlation with salinity ($\rho = -0.64$).

DISCUSSION

The environmental settings found during the study period confirmed that site Urca and site Ramos represent extreme conditions of the gradient of salinity and degree of pollution described for Guanabara Bay. The inner area of the Bay is hypertrophic, often anoxic, and subject to wide variations of salinity and dissolved oxygen. Nitrate levels were low, indicating that input of organic matter has rendered this area a reduction-geared environment (Paranhos *et al.*, 1998). Variations in dissolved oxygen levels, for example, took place at different cell concentrations and chlorophyll *a* levels, which illustrates the ecological instability of the area. The correlations between abiotic and biotic parameters were, therefore, of low statistical meaning. A longer time-series is probably needed to allow the understanding of ecological relationships in this case. Future studies on phytoplankton dynamics at inner areas of the Bay should take into account other variables (bacterioplankton and grazers) because there is evidence (Paranhos *et al.*, 2001) that this is a top-down plankton system.

Site Urca is a less impacted site, where plankton dynamics seems to follow a bottom-up pattern (Paranhos *et al.*, 2001). Therefore, correlations between abiotic and biotic parameters available at present provided better statistical results for this outer area of the Bay. This was especially true for cyanobacteria, whose high concentrations were associated with higher water temperature and lower salinity, as detailed further below.

Filamentous cyanobacteria in Guanabara Bay are composed of few taxa (identified in previous studies as *Oscillatoria limnetica* Lemmermann, *Oscillatoria neglecta* De Toni, and *Oscillatoria quadripunctulata* var. *unigranulata* Singh). The autotrophic nanoplankton fraction, however, may include a variety of taxonomic groups, such as diatoms, dinoflagellates, cryptophytes, prasinophytes, and chlorophytes, among others. Therefore, it was not surprising that the study of nanoplankton variations did not reveal statistically significant results. Although nanoplankton organisms, as a group, can be considered C-strategists (*sensu* Reynolds, 1988), each phylum (or even each species) can fill in a specific niche among the myriad of niches available. Filamentous cyanobacteria, as R-strategists, may also thrive in impacted environments. As a group, they have shown a more uniform space-time distribution in Guanabara Bay since the studies carried out in the 1970s.

Filamentous and coccoid cyanobacteria were best represented during rainy periods, especially in the outer Bay area. The increase in cyanobacteria during these times of the year could be related to their preference for less saline waters (Fogg, 1975; Murrel & Lores, 2004; Marshall *et al.*, 2005). In the inner area, this increase was more conspicuous probably due to its lower salinity throughout the year and its highly variable, though often low or not detected, oxygen concentrations. Photosynthetic rates of cyanobacteria can be higher at low oxygen levels (Fogg, 1975).

Large abundances of the diatom *Skeletonema* spp. occurred at both sites, but were more conspicuous at the inner site. Although these diatoms were relatively more important during the dry season, when *Synechocystis* densities were lower, higher absolute abundances of *Skeletonema* spp. occurred during the wet season, when lower concentrations of silicate can be interpreted as the uptake of this essential nutrient by diatoms. A similar pattern was reported in previous studies in Guanabara Bay (Sevrin-Reyssac *et al*., 1979; Villac *et al*., 1991), Paranaguá Bay (Brandini, 1985; Brandini & Thamm, 1994; Rezende & Brandini, 1997), Cananéia estuary (Brandini, 1982), and in temperate bays as well (Cloern & Cheng, 1981; Han *et al*., 1992).

Heterotrophs, both nanoplankton and filamentous bacteria, seem to have taken advantage of environmental conditions present during the winter-early spring period, that was characterized by higher nutrient levels. At both sites, virtually all filamentous bacteria found during this drier time of the year were the heterotrophic ones. Although rainy periods are expected to provide the Bay with large nutrient loads, it has been proposed that a dilution effect also takes place (Mayr *et al.*, 1989). Therefore, bay waters during drier months can become more eutrophic. Our biological response can be a shift from autotrophs to heterotrophs. Indeed, except for a few peaks, dissolved oxygen concentrations were lower during this time of the year.

Biomass estimates showed a lower relative contribution of heterotrophic nanoplankton and filamentous bacteria, but the seasonal trend of autotrophs was not strongly modified in relation to cell density results. Although cell density allowed the comparison of our data with that obtained in earlier studies, abundance expressed as carbon content is required to model trophic dynamics.

Previous studies of nanoplankton and filamentous cyanobacteria in Guanabara Bay recorded densities from 10^5 to 10^7 cells.l^{-1} and from 10^5 to 10^8 fil.l^{-1}, respectively (Barth, 1972; Sevrin-Reyssac *et al*., 1979; Villac *et al*., 1991). The present study showed higher total nanoplankton cell numbers (10^8 cells.l^{-1} in most samples: >57%) though densities of total filamentous bacteria remained roughly the same (10^6 fil.l^{-1} at site Urca and 10^7 fil.l^{-1} at site Ramos). Higher densities of total nanoplankton (auto- and heterotrophic forms included) are possible because: (1) Guanabara Bay has been under a severe eutrophication process since the 1970s and increasing concentrations of ammonia and faecal coliform have been reported between 1980 and 1990, especially at the inner bay areas (Lavrado *et al*., 1991; Paranhos *et al*., 1995); and (2) the methods of preservation and analysis used in the present work were better at detecting and maintaining fragile flagellates, when compared with the routine analysis adopted in previous studies, in which samples were preserved at room temperature and analyzed under transmitted light microscopy at 400X final magnification.

Despite differences in the methods used in microscopic analyses, the comparison between previous data (more comprehensive in terms of space-time coverage) and the present data set (1-year cycle at 2 sites only) can be useful. Previous data may supply valuable information for future modeling of local trophic dynamics, provided that data is considered with caution. During 2000, higher numbers of heterotrophic nanoplankton were detected all year in the outer bay area (average = 86 % ± 14) and during winter-spring in the inner area (56 – 100 %). Heterotrophic filamentous bacteria detected were narrow (0.5 μm) and autotrophic filaments of the present and previous studies were wide (1.0 – 2.0 μm). Therefore, the message is: the true autotrophic portion of what was considered nanoplankton in previous studies was overestimated, but the true autotrophic portion of the filamentous bacteria (cyanobacteria) was not. If previous nanoplankton data is used for modeling trophic dynamics, our data indicates that discrepancy in true nanophytoplankton counts can take place all year around in less eutrophic waters, and this discrepancy may vary seasonally in hypertrophic areas of the Bay. A longer time-series is needed to confirm a possible trend in this regard.

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