OECOLOGIA BRASILIENSIS
Tenenbaum. D.R.; Villac, M.C.; Gomes, E.A.T.; Cupelo, A.C. & Santos, V.S. 2001. A new "sight" on microbial plankton ecology: Coastal *x* Oceanic System in Brazil. pp. 133-152. In: Faria, B.M.; Farjalla, V.F. & Esteves, F.A. (eds). *Aquatic Microbial Ecology in Brazil.* Series Oecologia Brasiliensis, vol. IX. PPGE-UFRJ. Rio de Janeiro, Brazil.

A NEW "SIGHT" ON MICROBIAL PLANKTON ECOLOGY: COASTAL x OCEANIC SYSTEM IN BRAZIL

TENENBAUM, D.R.; VILLAC, M.C.; GOMES, E.A.T.;
CUPELO, A.C. & SANTOS, V.S.

Resumo

A ecologia microbiana planctônica sob uma "nova óptica": sistemas costeiros *x* oceânicos no Brasil.

Dados da baía de Guanabara, Rio de Janeiro, Brasil (PRONEX), e da região oceânica situada na costa central do Brasil (REVIZEE) foram selecionados para uma comparação entre os componentes auto e heterotróficos do picoplâncton (bacteria, 0,2-2,0 μm), nanoplâncton (flagelados e diatomáceas, 2.0-20 μm) e microplâncton (fito e protozooplâncton, >20 μm). O objetivo é contribuir com uma visão preliminar da estrutura microbiana de dois ambientes marinhos distintos e também com recomendações metodológicas necessárias para ajustar técnicas a particularidades dos ambientes em estudo. As variações quantitativas (densidade celular e biomassa em carbono) e qualitativas (componentes auto e heterotróficos) evidenciaram um nítido gradiente trófico entre a baía de Guanabara (região mais interna: ponto Ramos; entrada: ponto Urca) e o REVIZEE (7 estações, da região mais próxima à mais distante da costa). Os sistemas são caracterizados pela menor salinidade e eutrofização da região costeira, em oposição a águas mais salgadas e oligotróficas da região oceânica da Corrente do Brasil. A densidade celular total foi maior no ponto Ramos (10^{11} cel.L^{-1}), seguido pelo ponto Urca (10^{10} cel.L^{-1}) e REVIZEE (10^{9} cel.L^{-1}), decrescendo de acordo com a classe de tamanho analisada: picoplâncton, dominado por heterotróficos, contribuíram com mais de 99%, enquanto nano e microplâncton foram menos representativos. A biomassa picoplanctônica na baía de Guanabara (10^{2}-10^{4} μgC.L^{-1}) foi duas ordens de magnitude superiores à do REVIZEE. O nanoplâncton não apresentou um padrão definido. Os autotróficos dominaram o microfitoplâncton na baía (>80%, sendo em sua maioria diatomáceas e euglenofíceas) e na região oceânica (>60%, dominando os dinoflagelados heterotróficos). A maior similaridade da comunidade microbiana entre o ponto Urca e REVIZEE reforça a hipótese da recuperação da Baía de Guanabara, especialmente em áreas com circulação induzida pela maré, apesar do seu avançado estado de deteriorização devido ao impacto humano.

Palavras-chave: ecologia microbiana plânctônica, densidade, biomassa, águas costeiras e oceânicas

Abstract

Data from Guanabara Bay, Rio de Janeiro, Brazil (PRONEX), and from a region offshore the Brazilian central coast (REVIZEE) were selected for comparison between the auto- and heterotrophic components of picoplankton (bacteria, 0.2-2.0 μm), nanoplankton (flagellates and diatoms, 2.0-20 μm) and microplankton (phyto- and protozooplankton, >20μm). Our goal is to provide a preliminary view of the microbial structure of two contrasting marine environments, as well as methodological recommendations necessary to adjust techniques to particularities of the environments under study. The quantitative (density and carbon biomass) and qualitative (auto- and heterotrophic components) variations indicated a trophic gradient between Guanabara

Bay (inner reaches: site Ramos; entrance: site Urca) and REVIZEE (7 stations, from closer to farther from shore). These systems are characterized by the lower salinity and man-induced eutrophication in the coastal area, as opposed to the more saline and oligotrophic waters of the Brazil Current offshore. Total cell density was highest at site Ramos (10^{11} cel.L^{-1}), followed by site Urca (10^{10} cel.L^{-1}) and REVIZEE (10^{9} cel.L^{-1}). Total cell density decreased according to the size class analyzed: picoplankton, dominated by heterotrophs, contributed with more than 99%, while nano- e microplankton were less representative. Picoplankton biomass at Guanabara Bay (10^{2}-10^{4} µgC.L^{-1}) was two orders of magnitude higher than at REVIZEE. The nanoplankton did not show a defined pattern. Autotrophs dominated the microphytoplankton in the bay (>80%, mostly diatoms and euglenophytes) and offshore (>60%, with more heterotrophic dinoflagellates). The greater similarity of the microbial community between site Urca and REVIZEE strengthens the belief in the recovery of the Guanabara Bay ecosystem, especially in areas with tide-induced circulation, despite its advanced state of deterioration due to human impact.

Key-words: microbial plankton ecology, density, biomass, coastal and oceanic waters.

Introduction

The concept of the phytoplankton as the sole and most important component of the basis of the food chain in aquatic systems has been challenged since the 70's (Pomeroy, 1974; Azam *et al.*, 1983). The term food web, rather than food chain, became more appropriate to describe the processes of production, transfer and recycling of organic matter and energy in pelagic environments because of the complex trophic interactions between microbes and metazoans. The microbial food web that supports the metazoan food web is therefore formed not only by phytoplankton, but also by auto- and heterotrophic bacteria, small heterotrophic flagellates and ciliates, and larger protozoans (Sherr & Sherr, 1988).

The development of techniques that allowed for the assessment of small and delicate organisms such as bacteria and flagellates, as well as for the differentiation between auto- and heterotrophs, were key to a better estimate of aquatic microbial populations (Porter & Feig, 1980; Azam *et al.*, 1983; Booth, 1987; Martinussen & Thingstad, 1991). Epifluorescence microscopy is one of the widely used techniques for quantitative studies of aquatic microbes as, despite the time effort necessary to generate data, it provides complementary information on species composition (e.g., Chavez *et al.*, 1996; Kemp *et al.*, 1993).

From the same water sample, microbial populations can be analyzed by size fraction of the organisms, in the case of the present study: picoplankton (auto- and heterotrophic bacteria of 0.2 - 2.0 µm), nanoplankton (auto- and heterotrophic flagellates and diatoms of 2.0 - 20 µm) and microplankton (> 20µm), which includes the microphytoplankton and the protozooplankton. The fraction which has been traditionally studied as microphytoplankton, however, includes a large number of species known to be mixotrophs and absolute heterotrophs, that is, "microphytoplankton" corresponds to organisms with a wide spectrum of nutritional strategies, even within the same genus (Schnepf & Elbrächter, 1992; Jones,

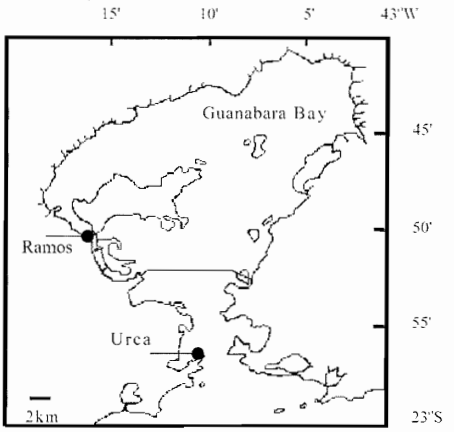
1994). Therefore, to understand the trophic status of microbial plankton communities, it is necessary to distinguish between the auto- and the heterotrophic components of all and each one of the fractions under study.

There have been few initiatives that apply this type of approach to the understanding of plankton ecology for Brazilian marine systems. The abundance and distribution of the bacterioplankton has been investigated for the coastal waters of Ubatuba, São Paulo (Mesquita, 1993), for the estuarine waters of Cananéia, São Paulo (Mesquita & Perez, 1985), and in the region of the Patos Lagoon and adjacent coastal and offshore waters, Rio Grande do Sul (Abreu *et al.*, 1992; Abreu, 1998; Abreu & Odebrecht, 1998). As for the protozooplankton, there are numerous publications that focus on checklists and taxonomical aspects of some groups, especially those that have a hard theca as tintinnids, forams and radiolarians, with few studies of quantitative aspects (reviewed in Brandini *et al.*, 1997). The most comprehensive study in terms of discriminating between the auto- and heterotrophic components of different fractions (pico-, nano- and "microphytoplankton") is that by Ribeiro (1996), which presents the abundance, composition and distribution of microbial plankton in the photic zone of offshore waters between latitudes 25-32°S and 38-50°S. This state of art reflects the need for more studies about the microbial plankton ecology of Brazilian marine systems.

Traditionally working with community structure of the "microphytoplankton", the Laboratory of Phytoplankton, Instituto de Biologia, Universidade Federal do Rio de Janeiro, established a multidisciplinary work group to study all fractions of microbial plankton in 1997. Our ultimate goal is to have a more comprehensive approach to allow for the understanding of plankton trophodynamics. This chapter contributes with a preliminary view of microbial structure of two contrasting marine environments, as well as with methodological recommendations necessary to adjust techniques to particularities of the environments under study. For the sake of comparison, data sets from two areas were selected for case studies because they contrast in terms of their trophic states: Guanabara Bay (coastal system), located in the State of Rio de Janeiro, Brazil, and a portion of the South Atlantic (oceanic system), located offshore from the central Brazilian coast.

The study areas

Guanabara Bay (Fig. 1), the postcard of Brazil abroad, is known for its beauty and for its historic and socio-economic importance. The water quality of this estuarine bay, however, has been undergoing increasing deterioration. Since the early 80's, severe pollution problems can be traced by the increase of fecal material and of ammonia levels, and the decrease of dissolved oxygen concentrations (Lavrado *et al.*, 1991; Paranhos *et al.*, 1995). This man-induced eutrophication is caused by the input of sewage and industrial wastes that are unevenly distributed along the margins of the bay. Therefore, the degree of pollution varies from place to place, so that the best water quality is found where circulation is more efficient, while the



SOCIAL-ECONOMICAL CHARACTERISTICS

population: ..... 10 million
fisheries: ..... 280 t.month^{-1}
industrial units: 6,000
port authorities: ... 2
shipyards: 12
oil refineries: 2
oil terminals: 16

PHYSICAL CHARACTERISTICS

watershed: . 4,000 km^2
area: ..... 381 km^2
volume: ..... 2 x 109 m^3
35 rivers (18 m^3. s^{-1})
tidal: semi-diurnal, maximum height of 1,4m
estuarine caracter - SALINITY (average values: 1980-1987):

	ENTRANCE	INTERIOR
	surface: 32-37	surface: 13-27
	bottom: 32-37	bottom: 27-29

depth (m): ENTRANCE: 20-50 INTERIOR: 10-20

Figure 1. Guanabara Bay. Study area and sampling station localization.

worst conditions are detected where landfills have hampered the diluting capacity of the tide-driven circulation (Mayr *et al.*, 1989).

Since 1985, the Instituto de Biologia, Universidade Federal do Rio de Janeiro, Brazil, has been involved in the study of the plankton of Guanabara Bay. Space-time variations in the structure of the phytoplankton and zooplankton communities are summarized in Valentin *et al.* (1999). At present, this study is part of a comprehensive program called "Man´s Impact on the Biota of Guanabara Bay – RJ", the goal of which being to contribute to the monitoring of the "Program for Recovery of the Guanabara Bay Ecosystem" established by the State Environmental Agency (FEEMA). Part of the data set generated by the new techniques/approaches applied since 1997 have been selected for discussion in this chapter.

The data set chosen to discuss the offshore system (Fig. 2) is part of a program that aims to assess the living resources of the Economic Exclusive Zone of the Brazilian coast, called REVIZEE. The hydrography and circulation of this

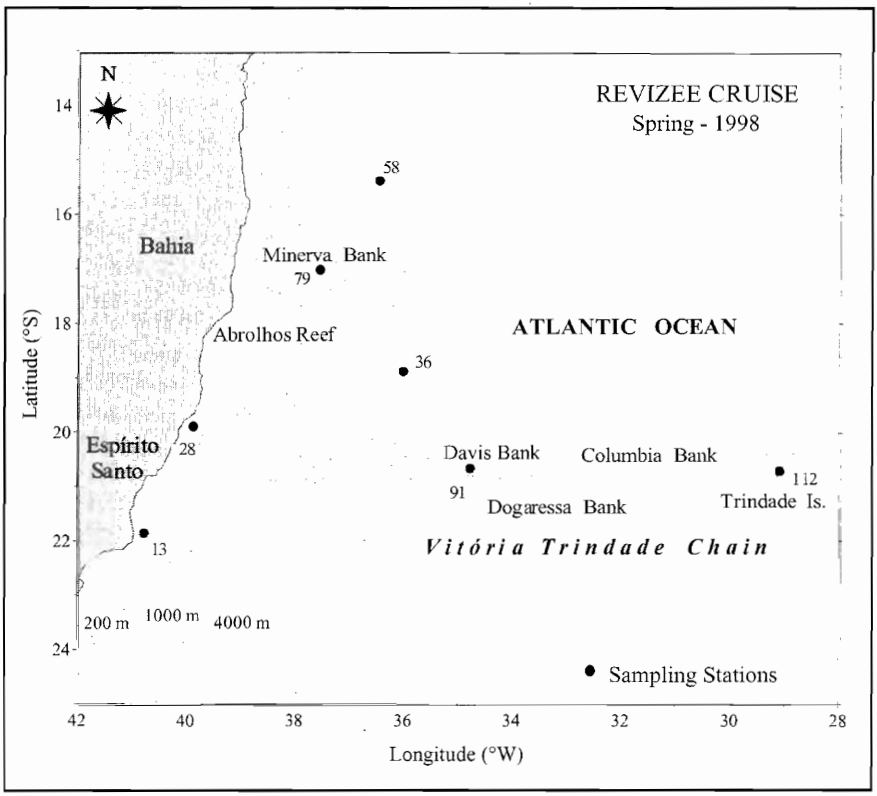


Figure 2: REVIZEE Cruise. Study area and selected oceanographic sations localization.

area is summarized in Brandini *et al.* (1997). This region is influenced by the Brazil Current, which travels parallel to the coast and transports the Tropical Water (TW >20°C, >36 salinity) at the border of the continental shelf. The South Atlantic Central Water (SACW: <18°C, <35 salinity) lies below the TW, and thus a permanent thermocline is established (Gaeta, 1997). The Abrolhos Bank (19°S) and the Vitória-Trindade submarine chain (20° 30'S) form a barrier to the flow of the Brazil Current, which becomes narrower and turns left at this point, in such a way as to allow for the formation of eddies and deep upwelling (Ekau & Matsuura, 1996; Evans *et al.*, 1984 *apud* Ribeiro Jr., 1998). Therefore, the stability of the water column can, at times, be disrupted, so that the typically warmer and oligotrophic waters of this region are affected by the contribution of colder and nutrient-rich SACW. Despite the distance from the coast which characterizes the Abrolhos region as offshore, primary productivity in this area is normally considered as mesotrophic (Gaeta, 1997), which favors the abundance of demersal fish populations (Ekau & Matsuura, 1996).

Material and methods

Methodological tests were carried out to adjust what is recommended in the literature to our laboratory conditions, according to the objectives of each of the two programs in question. The monitoring of Guanabara Bay follows a weekly sampling program at two sites on shore (samples can be immediately prepared and analyzed. The survey of the REVIZEE is carried out by 2- to 3 month cruises, samples of which can be initially prepared on board, with, however, actual analysis done on land. Moreover, the characteristics of each environment, the highly eutrophic Guanabara Bay as opposed to the oligotrophic offshore waters of the REVIZEE, are also important to sample preparation and counting procedures. Species composition and number of organisms present in each of these widely different environments, as well as time span between sampling and analysis, pose constraints on what and how the plankton can be analyzed, as discussed below. The procedures used for sampling, preservation, and analysis are summarized in Table 1, considering particularities of the group and/or fraction of organism analyzed, the differentiation between auto- and heterotrophic cells, and counting procedures in terms of estimation of cell density and conversion to biomass.

The analysis of all fractions (pico-, nano-, "microphytoplankton", and microzooplankton) was performed from splits of the same water sample (Sournia, 1978; Gomes & Godinho, *in press*): 250 mL from a 3-L Van Dorn bottle for Guanabara Bay and 2 L from a 10-L Niskin bottle for the REVIZEE Program. The pico- and nanoplankton were analyzed on slides that must be prepared and refrigerated (5°C, in the dark) immediately after sampling, to guarantee the autofluorescence of the organisms and the fluorescence of the stain used (Sherr & Sherr, 1993). The microplankton was analyzed directly from the water samples.

The samples were analyzed with light microscopy, using a combination of bright field, phase contrast and epifluorescence. Bright field gave the best results for the identification and cell estimates of coccolitophorids, dinoflagellates, and tintinnids, while phase contrast worked best for the visualization of the more delicate and transparent diatoms. Epifluorescence was necessary to count the pico- and nanoplankton, as well as to distinguish the autotrophs from the heterotrophs of all fractions, through chlorophyll autofluorescence under blue light (Booth, 1987, 1995; MacIsaac & Stockner, 1993).

The organisms were fixed with buffered formaldehyde (final concentration 2%) for two reasons: first, samples can be stored at room temperature (important for REVIZEE); and second, our tests demonstrated that fluorescence and best resolution of the organisms were maintained for a longer period of time. Other fixatives can be recommended, depending on the objective of the study (Kemp *et al.*, 1993).

The use of a dye is necessary for optical estimates of pico- (free bacteria) and nanoplankton cells. The fluorochrome DAPI (4'6-diamidino-2-fenilindol) was therefore added to the preserved samples at a final concentration of 0.01 $\mu g.L^{-1}$ (Porter & Feig, 1980). The samples were concentrated onto a black Nuclepore® filter (0.2 μm for pico- and 1.0 μm for nanoplankton), mounted on a slide, placed in a refrigerator (4-10°C) for one week to avoid the formation of crystals, and finally stored in a freezer (-20°C). Storage time of well-preserved material is controversial, but some studies indicate that analysis should be done within 12 months (Booth, 1995). The number of heterotrophs was calculated based on the total count with DAPI less the number of autotrophs analyzed by autofluorescence.

The microplankton (phyto- and zooplankton) was analyzed by the settling technique (Utermöhl, 1958). Small settling volumes (5-10 mL) were appropriate for the eutrophic waters of Guanabara Bay, but, for the REVIZEE, a 2 L sample was concentrated by gravity to about 250 mL, the excess gently removed, and this concentrate was used for analysis. In regard to the maintenance of the fluorescence of the cells of this larger fraction, it is recommended that the water samples be analyzed within 20 days after sampling and that the time of analysis should not be longer that 3 hours (Santos, 1999). Therefore, it was not possible to distinguish between the auto- and the heterotrophs in the REVIZEE Program, because of fluorescence attenuation with time. In this case, this information was taken from the literature. In regard to the microzooplankton, the oceanic areas presented a population with a large number of heavily tecate species that could be identified, counted, this data being presented here. Guanabara Bay, however, has a large number of naked species whose identification requires live samples, so that this analysis is still underway.

Biomass was estimated from the cell counts, by the calculation of biovolumes from the dimensions of cells and an approximation of standard geometric shapes such as cylinders, cones, spheres, and/or a combination (Sorokin & Kadota, 1972; Edler, 1979). The biovolume was then converted to carbon by the following conversion factors that were experimentally derived: 380 $fgC.\mu m^{-3}$ for the picoplankton (Lee & Fuhrman, 1987) and 101 $pgC.\mu m^{-3}$ for the nano- and microplankton (Montagnes *et al.*, 1994).

The case studies

Guanabara Bay

From July 1998 to January 1999, samples were taken from two sites that presented distinct water quality: site Urca - more saline, cleaner water and site Ramos - closer to riverine and waste discharges (Fig. 1). Six dates were selected based on particularities of the "microphytoplankton" such as extremes in terms of species richness, of relative abundance of auto- and heterotrophs, or the dominance of a species.

Total cell density (auto- and heterotrophs, for all fractions) varied from 4.10^9 to 2.10^{11} cells.L^{-1} (Fig. 3A, 3B). At site Urca, cell density was one order of magnitude lower than that at site Ramos, considering the sum of all fractions or each fraction individually. The high cell concentrations of picoplankton (Urca: 10^9-10^{10} cel.L^{-1}; Ramos: 10^{10}-10^{11} cel.L^{-1}) represented 99% of total cell density, which was 3 to 4 orders of magnitude higher than the density of the nanoplankton (Urca: 10^6-10^7 cel.L^{-1}; Ramos: 10^6-10^8 cel.L^{-1}) and of the "microphytoplankton" (Urca: 3.10^5-1.10^6 cel.L^{-1}; Ramos: 5.10^5-7.10^6 cel.L^{-1}).

Heterotrophs dominated (>90%) the picoplankton fraction at both sites, except in 3 samples from Urca (14 Aug 98, 18 Sep 98, and 22 Jan 99), in which autotrophs achieved 17-31% of the cell counts (Fig. 3C, 3D).

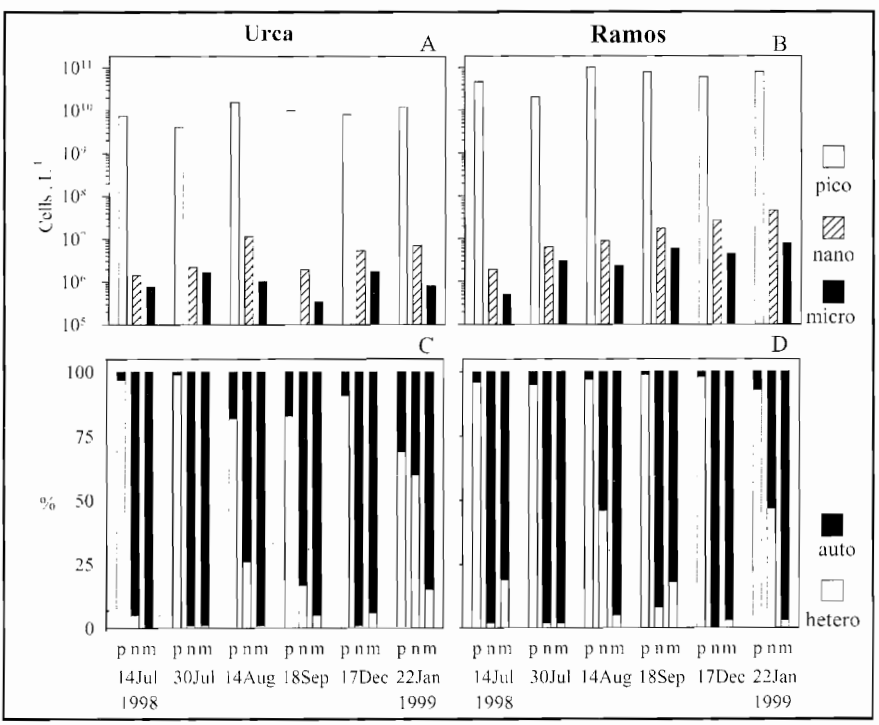


Figure 3. Guanabara Bay (selected dates): cell density of (A) site Urca and (B) site Ramos, and (C-D) respective proportions of the autotrophic and heterotrophic fractions (p=picoplankton, n=nanoplankton, m=microphyplankton).

At present, nanoplankton data include only the cells from between 10 and 20 μm in size; analysis of the remaining organisms of this fraction is underway. Although the contribution of nanoplankton autotrophs was more significant (> 40%), there were inversions to this trend (Fig. 3C, 3D). The composition of this fraction

was characterized by a great variety of organisms (various classes of flagellates, diatoms), which have a wide range of preferences and tolerances to environmental changes. For instance, the accidental sewage input that took place close to site Urca at the time of the 22 Jan 99 sampling led to an increase of heterotrophs that reached 60% of the nanoplankton populations. By contrast, if better water quality conditions occur at site Ramos, such as the contribution of cleaner and more saline waters during the flood tide of 17 Dez 98, the diatom *Skeletonema costatum* can become the major component of the nanoplankton.

The "microphytoplankton" was mostly composed of autotrophs (>80%), at all times (Fig. 3C, 3D). The species composition of the autotrophs, however, varied between sampling sites. Populations of several species of diatoms (40 taxa) dominated at site Urca, while small diatoms (14 taxa of about 20 μm) and euglenophytes were found as co-dominant at site Ramos.

The result of the conversion of cell density to biomass (Fig. 4A, 4B) confirmed the difference of trophic states between site Urca and site Ramos observed from cell counts (Fig. 3A, 3B), but provided new insights on the relationships between fractions and the relative abundance of autotrophs to heterotrophs (Fig. 3C, 3D in comparison to Fig. 4C, 4D).

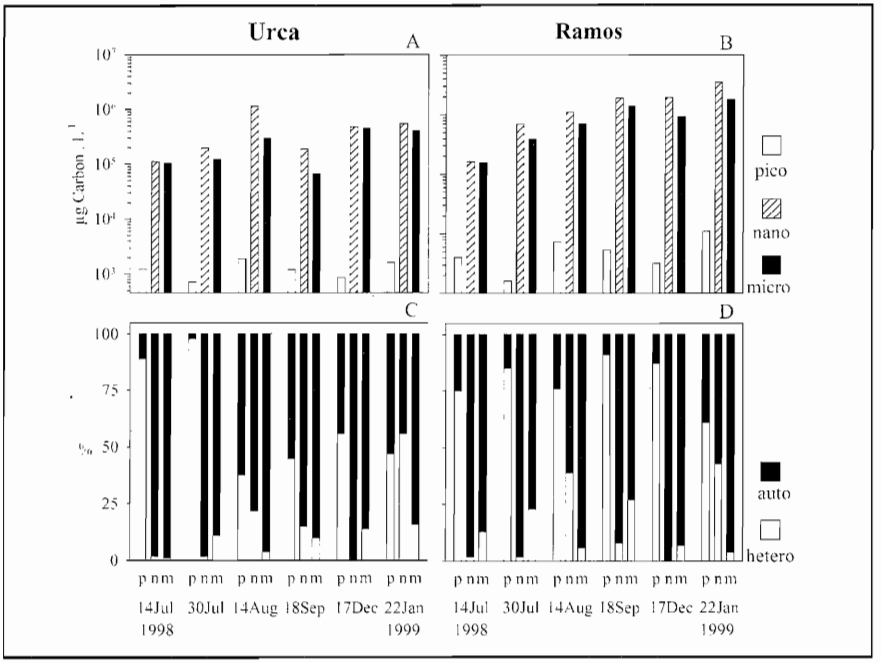


Figure 4. Guanabara Bay (selected dates): biomass in carbon of (A) site Urca and (B) site Ramos, and (C-D) respective proportions of the autotrophic and heterotrophic fractions. (p=picoplankton, n=nanoplankton, m=microphyplankton).

Total biomass (auto- and heterotrophs, for all fractions) varied from 2.10^5 to 1.10^6 µgC.L^{-1}. At site Urca, biomass was one order of magnitude lower than at site Ramos, considering the sum of all fractions or each fraction individually. Picoplankton represented only 1.5% of total biomass (Urca: 10^2-10^3 µgC.L^{-1}; Ramos: 10^3-10^4 µgC.L^{-1}), since the cell size of this fraction was the smallest. Nanoplankton biomass varied between 10^5 and 3.10^6 µgC.L^{-1}, the highest values observed at site Ramos. "Microphytoplankton" biomass remained around 10^5 µgC.L^{-1}, but carbon concentration at site Ramos was 4-7 times higher than at site Urca.

Table 1. Summary of methods used for each of the case studies.

	GUANABARA BAY	REVIZEE CRUISE
System	Coastal	Oceanic
Stations	2, from pier	7, from cruise (*R/V* Astro Garoupa)
Sampling depth	subsurface: 0.3 - 0.5 m	10 - 152 m
Sampler	Van Dorn bottle - 3 L	Ninskin bottle - 10 L
Sampled volume	250 mL	2000 mL
Fixative	Buffered formalin 2%	Buffered formalin 2%
Picoplankton	0.2 - 2.0 µm	0.2 - 2.0 µm
Filtered volume	2 mL	30 - 50 mL
Membrane	Nuclepore black, 0.22 µm	Nuclepore black, 0.22 µm
Microscopy (upright)	Epifluorescence	Epifluorescence
Magnification	1000 x	1000 x
Counting	Random fields	Random fields
Cells counted	300	400
Biomass (conversion factor)[1]	380 fgC.µm^{-3}	380 fgC.µm^{-3}
Nanoplankton	10 - 20 µm	2.0 - 20 µm
Filtered volume	5 mL	30 - 50 mL
Membrane	Nuclepore black, 1.0 µm	Nuclepore black, 1.0 µm
Microscopy (upright)	Epifluorescence	Epifluorescence
Magnification	400 x	1000 x
Counting	Random fields or transect	Random fields
Cells counted	300	200
Biomass (conversion factor)[2]	101 pgC.µm^{-3}	-
"Microphytoplankton"	>20 µm	>20 µm
Settled volume	5 - 50 mL	100 mL, concentrated
Microscopy (inverted)	Transmitted light Epifluorescence	Transmitted light
Magnification	200 x	200 x
Counting	Random fields or transect	½ to whole chamber
Cells counted	300	-
Biomass (conversion factor)[2]	101 pgC.µm^{-3}	-
Microzooplankton	-	>20 µm
Settled volume	-	100 mL concentrated
Microscopy Inverted	-	Light
Magnification	-	200 x
Counting	-	whole chamber

For the picoplankton, there was an increase in the relative abundance of autotrophic biomass (up to 44%), in comparison to cell counts, due to the fact that the autotrophic cells were larger than the heterotrophic ones. Similarly, for the nanoplankton, there was a very subtle increase in the contribution of autotrophs when cell counts were converted to carbon content. By contrast, for the "microphytoplankton", there was a decrease in the contribution of autotrophs in all samples from site Urca and in the sample of 30 Jul 98 from site Ramos. In all these samples, the predominant autotrophs were larger diatoms (>40μm) that, because of the presence of vacuoles, have a smaller plasma content than the other organisms of this fraction such as dinoflagellates and euglenophytes. In all other samples from site Ramos, the relative abundance of autotrophs of the "microphytoplankton" remained roughly the same, whether based on cell counts or on carbon content.

Revizee Cruise

Water samples were collected during a 3-month cruise (October - December 1998), from a grid of 116 stations located between 20 to 670 N.M. away from the coastline. Seven stations were selected for this discussion according to the distance from the coast and local depth, both being aspects of ecological importance in offshore environments (Table 2). These samples were taken at the depth of maximum chlorophyll, which varied from 10 to 152 m.

Table 2. Characteristics of selected sampling sites for the REVIZEE Program.

Stations	Distance from the coast (N.M.)	Local depth (m)	Sample depth (m)	Characteristics
R13	20	20	10	nearshore, shallow
R28	20	46	30	nearshore, shallow
R79	94	48	41	offshore, shallow (Minerva Bank)
R91	340	63	53	offshore, shallow (Davis Bank)
R112	670	4,200	152	offshore, deep (close to Trindade Is.)
R36	220	4,000	126	offshore, deep
R58	150	4,100	130	offshore, deep

Cell density of the picoplankton fraction (3.10^8-2.10^9 cells.L^{-1}) was three orders of magnitude higher than that of the nanoplankton (10^5-10^6 cells.L^{-1}) which was, in turn, four times higher than that of the microplankton ("microphytoplankton": 100-1,400 cells.L^{-1}; microzooplankton: 13-200 cel.L^{-1}) (Fig. 5A). All fractions showed the smallest cell count in stations 36 and 58, both stations being offshore and in deep waters. Protozooplankton varied from 102 to 1,380 cells.L^{-1} and followed the same pattern observed for the other fractions.

The relative abundance of the auto- and heterotroph components varied between fractions (Fig. 5B). The heterotrophs dominated in the picoplankton fraction (>96%) at all stations. In the nanoplankton, heterotrophs were a minor component (4%),

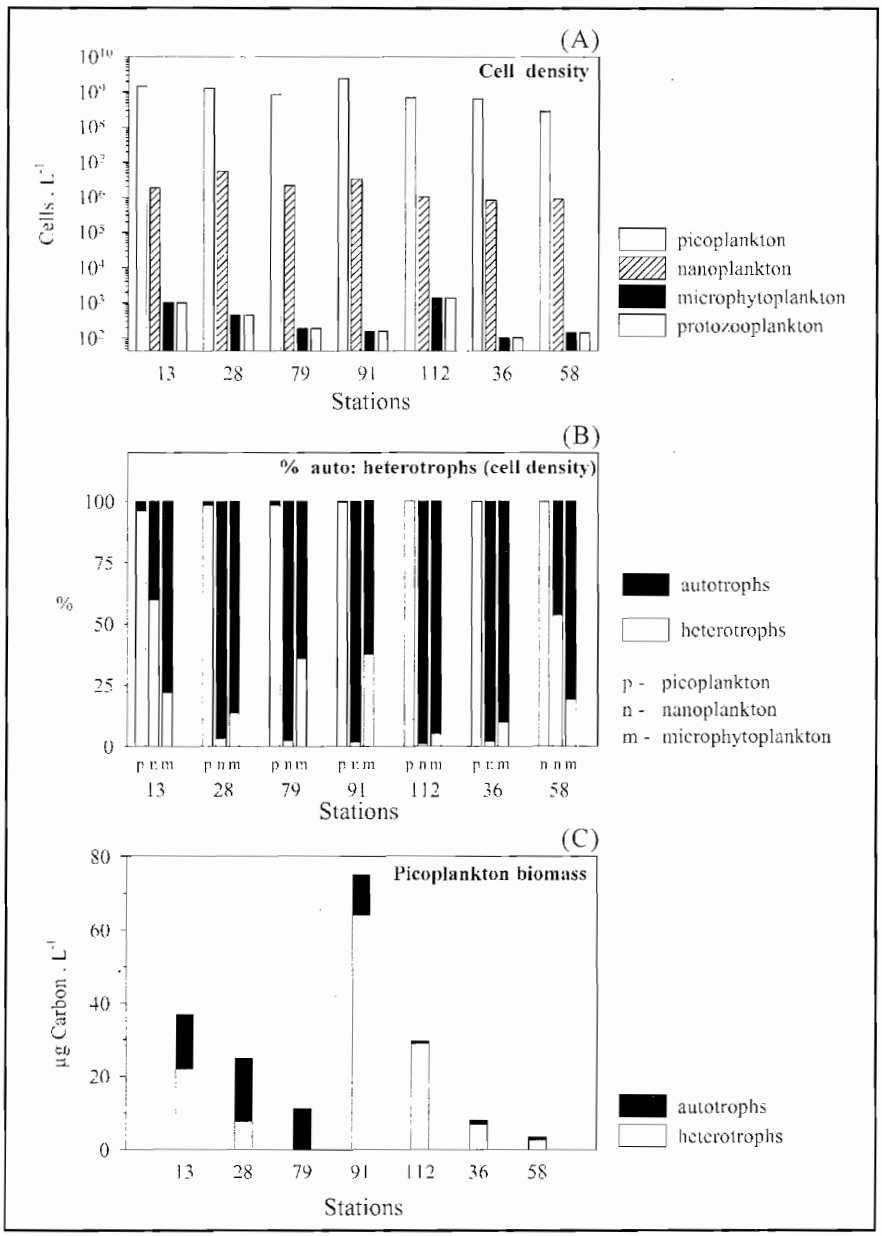


Figure 5. REVIZEE Cruise (selected stations): (A) total cell density, (B) proportions of autotrophs and heterotrophs, based on cell density, and (C) total concentration and proportions of autotrophs and heterotrophs of the picoplankton, based on the biomass.

except at stations 13 (nearshore, shallow) and 58 (offshore, deep), which had 60% of heterotrophs. The autotrophs dominated the "microphytoplankton" fraction at all stations (>62%). The contribution of autotrophs to the "microphytoplankton", calculated from literature data, is probably underestimated by 5 to 38% (error calculated based on uncertainties in species identification, especially in the order Gymnodiniales).

Biomass data is available for the picoplankton fraction only (Fig. 5C). Picoplankton biomass varied from 4 to 80 $\mu gC.L^{-1}$. Heterotrophs dominated (>85%) at the offshore, deep stations (R112, R36, R58), while autotrophs dominated (68 and 98%) in some of the shallow stations (R28 and R79, respectively). Considering that heterotroph abundance did not vary significantly between stations, this higher contribution of autotrophs when cell count is converted to biomass is due to the larger size of the autotrophic cells.

Discussion

The abundance and composition of microbial plankton of the study areas showed that there was a clear gradient of trophic states between Guanabara Bay and the REVIZEE Cruise, that is, from the highly eutrophic waters of site Ramos to the oligotrophy that characterized the offshore, deep stations of the South Atlantic. These are extremes in environmental conditions due to man-induced organic pollution as opposed to the nutrient-depleted waters of the Brazil Current.

This trend was confirmed by two types of multivariate analysis: a principal component analysis based on environmental variables (temperature, salinity, suspended particulate matter, ammonia, phosphate, silicate, and chlorophyll *a*) and a cluster analysis based on cell density of the auto- and heterotrophic components of each of the fractions analyzed (Fig. 6). Since this is a small data set (n=19 observations), the value of these analysis is more of an exercise to summarize trends.

The factorial plan I-II (Fig. 6A) shows the importance of salinity (on the negative portion of axis I) as a variable that can clearly differentiate these environments. While the samples from the REVIZEE Cruise and site Urca are projected on the left side of axis I (higher salinity), the samples from site Ramos are distributed on the right side, along with the variables that indicate a higher degree of eutrophication (higher nutrients, suspended particulate matter, and chlorophyll *a*). Samples from REVIZEE and site Urca form each a cluster of points, while the samples from site Ramos are spread out, indicating that environmental conditions at the latter are much more variable than at the other two. The effect of such variations has been shown by changes in the composition of the nanoplankton populations, which is either dominated by autotrophic flagellates (worst water quality) or by diatoms (improved water quality).

Surprisingly, site Urca seems to have more in common with the REVIZEE stations than with site Ramos. This is shown by the principal component analysis and the cluster analysis (Fig. 6B). The dendrogram shows three clusters: one for site Ramos only (labelled 1), the second for site Urca+Ramos (labelled 2a), and a third for site Urca+REVIZEE (labelled 2b). In this last group, REVIZEE samples were closest, forming an expected outgroup for the oceanic community only. The fact

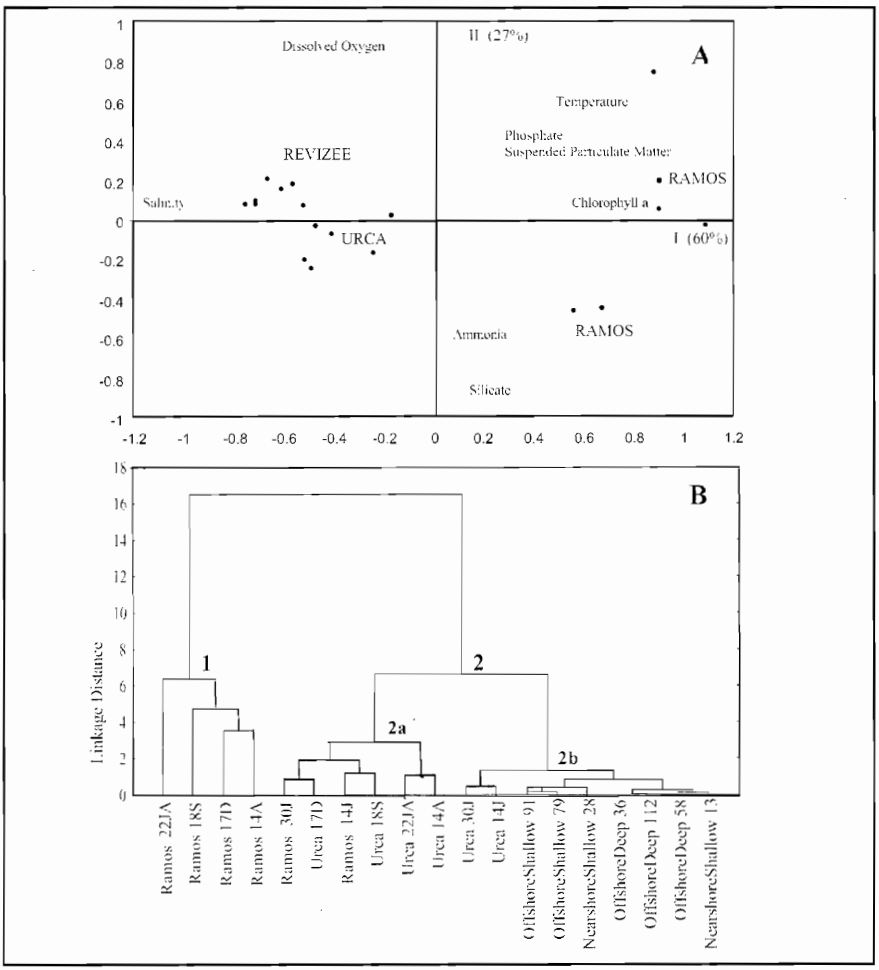


Figure 6. Comparison between the coastal and the oceanic systems. (A) Principal component analysis - distribution of the environmental variables and (·) samples on the I-II factorial plane. (B) Dendrogram for classification of samples based on cell density of the auto and heterotrophic components of each of the fractions analyzed (Euclidian distance, clustering by Ward's method). Environmental data provided by Laboratório de Hidrobiologia, Instituto de Biologia/ UFRJ.

that site Urca presents a microbial community that has similarities with that of the oceanic samples corroborates the idea that, despite the advanced state of deterioration, the recovery of the Guanabara Bay ecosystem is a true possibility, especially in areas that can be flushed by its tide-induced circulation (Mayr *et al.*, 1989).

In comparison to other areas on the shores of Brazil, picoplankton abundances in Guanabara Bay were higher than those found for the coast of Ubatuba

(Mesquita, 1993), or even higher than the concentrations found for eutrophic estuarine regions such as Cananéia (Mesquita & Peres, 1985) and the Patos Lagoon (Abreu *et al.*, 1992; Abreu & Odebrecht, 1998). As for the REVIZEE, picoplankton concentration was within the ranges found by Ribeiro (1996), but higher than those found for the colder waters investigated by Abreu (1998).

Picoplankton abundance varied less than that of the other fractions. The dominance of heterotrophic bacteria in both environments is probably the result of its adaptability due to a great metabolic diversity (Azam *et al.*, 1983). At the same time that high organic matter content is associated with high abundances of heterotrophic bacteria (Atlas & Bartha, 1993), these can also thrive on very low concentrations of organic matter (Amon & Benner, 1996). This homogeneity is further enhanced by the equilibrium established between bacterial production and grazing, especially by nanoplankton (Caron *et al.*, 1999).

If attached bacteria had been analyzed, perhaps a sharper difference would have been found between Guanabara Bay and the REVIZEE data, since the concentration of suspended particulate matter offshore was much smaller than in the bay (refer to Fig. 6A). Our values for picoplankton are underestimated because the contribution of attached bacteria can be higher than that of free bacteria, as shown for the estuary of Lagoa dos Patos and adjacent coastal and offshore regions (Abreu, 1998; Abreu & Odebrecht, 1998).

Nanoplankton can include small diatoms and a large number of flagellates whose precise identification often requires live samples and electron microscopy. Absolute and relative abundances of each of these groups depend on a large spectrum of responses to environmental conditions, according to their preferences and tolerances. Little is known, however, about these ecological requirements at the species level. Thus it seems that, more than with any of the other fractions, the nanoplankton (2-20 μm) is an arbitrary category. The interpretation of nanoplankton data is, therefore, rather complex.

The nanoplankton data from Guanabara Bay indicates that the organisms analyzed (between 10-20 μm) followed more closely a trend found for the "microphytoplankton", that is, it presented a dominance of autotrophs whose ecological requirements were better understood. This was shown, for instance, by the alternance of the dominating autotrophs at site Ramos between euglenophyceans and the diatom *Skeletonema costatum* (improved water quality). We speculate that the "behavior" of the smaller fraction of the nanoplankton (2-10 μm), once this data becomes available, might resemble the patterns observed for picoplankton, that is, with a larger contribution of heterotrophs.

Cell density of the nanoplankton from the REVIZEE was higher than that observed for the same offshore region (Ribeiro, 1996) and the oceanic Equatorial Pacific (Chavez *et al.*, 1996). In contrast to what was found for the REVIZEE, the contribution of the autotrophic component was higher at these other locations; the analysis of the other samples from REVIZEE will determine whether this difference is a true trend or not.

Of all fractions, the "microphytoplankton" presented the highest variations between Guanabara Bay and REVIZEE. Autotrophs dominated at all sites, but with decreas-

ing proportions from the coastal to the oceanic system (>80% in the bay, >60% offshore). These variations are a reflex of the well-defined ecological requirements of the most important components of this fraction. Euglenophyceans and small diatoms were best adjusted to the highly eutrophic, less saline, shallow and turbid waters of site Ramos, as found elsewhere (Cloern, 1987; Gardiner & Dawes, 1987). The conditions at site Urca, still eutrophic, but with improved water quality, favored the development of a more diverse community dominated by chain-forming diatoms, typical of coastal areas worldwide (Hulburt, 1963; Margalef, 1978). Nutrient-depleted conditions offshore, which are restrictive to autotrophs, favored the development of dinoflagellates, several of them mixotrophs and absolute heterotrophs (as reviewed in Schnepf & Elbrächter, 1992). The quantitative dominance of dinoflagellates and the overall low concentration of the "microphytoplankton" found for REVIZEE are comparable to other warm, offshore areas (Iriarte & Fryxell, 1995; Ribeiro, 1996). The same was observed in the cell concentration of protozooplankton for REVIZEE, similar to the findings of Leakey *et al.* (1996) in the coastal waters of the Indian Ocean. In spite of this likeness, the naked ciliates, which are known to dominate in warm oceanic waters (Ciotti, 1990 *apud* Odebrecht, 1998), are not well preserved in formaldehyde. The use of other fixatives (Gomes & Godinho, *in press*) would probably give better estimates of protozooplankton populations.

Whereas the same water sample can be the starting point of the study of microbial plankton communities, this research requires the use of a multitude of methods, as well as several types of expertise to properly analyze each fraction. The understanding of the microbial food web has not only changed some fundamental paradigms in plankton ecology, but it has also gathered bacteriologists, phycologists and protistologists, all working under the realm of Ecology.

Acknowledgments

We are indebted to all those who made countless hours of field work possible and enjoyable. We appreciate the possibility to use unpublished environmental data provided by the Laboratório de Hidrobiologia, Instituto de Biologia/UFRJ. We thank the following agencies for funding the programs that provided data discussed in this chapter: MCT/MEC - PRONEX to the Guanabara Bay Program, SeCirm/MMA to the REVIZEE Program, as well as Conselho Nacional de Desenvolvimento Científico e Tecnológico, and Fundação Universitária José Bonifácio/UFRJ.

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Addresses:

TENENBAUM[1,2], D. R.; VILLAC[1], M. C.; GOMES[1], E. A. T.; CUPELO[1], A. C. & SANTOS[1], V. S.

[1] Departamento de Biologia Marinha, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Cidade Universitária, CCS-A, 21944-970, Rio de Janeiro, RJ, Brasil

[2] Departamento de Oceanografia, Instituto de Geociências, Universidade do Estado do Rio de Janeiro, 20550-013, Rio de Janeiro, RJ, Brasil