

Research Paper

Yearly variation of bacterial production in the Arraial do Cabo protection area (Cabo Frio upwelling region): An evidence of anthropogenic pressure

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Abstract

Arraial do Cabo is where upwelling occurs more intensively on the Brazilian coast. Although it is a protection area it suffers anthropogenic pressure such as harbor activities and sporadic sewage emissions. Short-time studies showed a high variability of bacterial production (BP) in this region but none of them evaluated BP during long periods in a large spatial scale including stations under different natural (upwelling and cold fronts) and anthropogenic pressures. During 2006, we sampled surface waters 10 times (5 in upwelling and 5 in subsidence periods) in 8 stations and we measured BP, temperature as well as the concentrations of inorganic nutrients, pigments and particulate organic matter (POM). BP was up to 400 times higher when sewage emissions were observed visually and it had a positive correlation with ammonia concentrations. Therefore, in 2007, we did two samples (each during upwelling and subsidence periods) during sewage emissions in five stations under different anthropogenic pressure and we also measured particles abundance by flow cytometry. The 12 samples in the most impacted area confirmed that BP was highest when ammonia was higher than 2 μM , also reporting the highest concentrations of chlorophyll *a* and suspended particles. However, considering all measured variables, upwelling was the main disturbing factor but the pressure of fronts should not be neglected since it had consequences in the auto-heterotrophic coupling, increasing the concentrations of non fluorescent particles and POM. Stations clustered in function of natural and anthropogenic pressures degrees and both determined the temporal-spatial variability.

Key words: bacterioplankton, heterotrophic activity, impacted area; extractive reserve, upwelling system.

Introduction

The up-flow of cold and nutrient-rich waters describes upwelling process and it disturbs ecosystem dynamics and increases environmental heterogeneity (Lehmann and Myrberg, 2008). Brazilian upwelling events are more pronounced in the Cabo Frio region (RJ); mainly on the Arraial do Cabo coast (23° S, 42° W), an environ-

mental protection area (Silva, 2004) that is directly impacted by anthropogenic activities (Ferrerira *et al.*, 2004; Lopez and Coutinho, 2010; Cury *et al.*, 2011). In the Cabo Frio region, upwelling occurs with higher frequency and intensity during austral spring and summer seasons, mainly as a function of northeast (NE) winds prevalence. During the autumn/winter season, cold fronts are more common describing a subsidence period (Valentin, 2001; Coelho-

Souza *et al.*, 2012). Upwelling events occur intensely off the Cabo Frio embayment, precisely at Focinho do Cabo (Figure 1), a fixed station for many oceanographic studies (eg., Valentin *et al.*, 1987; Guenther *et al.*, 2008).

Sampling in the Focinho do Cabo station, Valentin (1984) showed that upwelling events are the main disturbing factor of the region but did not measure bacterial activity. A few studies focused on bacterioplankton heterotrophic activity and how it was related to upwelling process (Carvalho and Gonzalez-Rodriguez, 2004; Guenther *et al.*, 2008). Measurements of bacterial production (BP) are important to evaluate the cycles of carbon and nutrients in the environment as reported in the experimental and sampling studies in the Focinho do Cabo station associating BP with primary production. These studies showed that carbon, nitrogen and phosphorous cycles were coupled with BP. Herein, we extend the analysis of bacterioplankton heterotrophic activity in space and time to include the interference of cold fronts and anthropogenic activities. Our hypotheses is that natural (upwelling and cold fronts) and anthropogenic (sewage emissions) pressures have different interference scales. We expected a regional interference of upwelling and fronts but punctual anthropogenic impact in function of water circulation.

Materials and Methods

Study area

This study was performed during 2006/7 in 8 sampling stations with different characteristics (Figure 1). Pon-

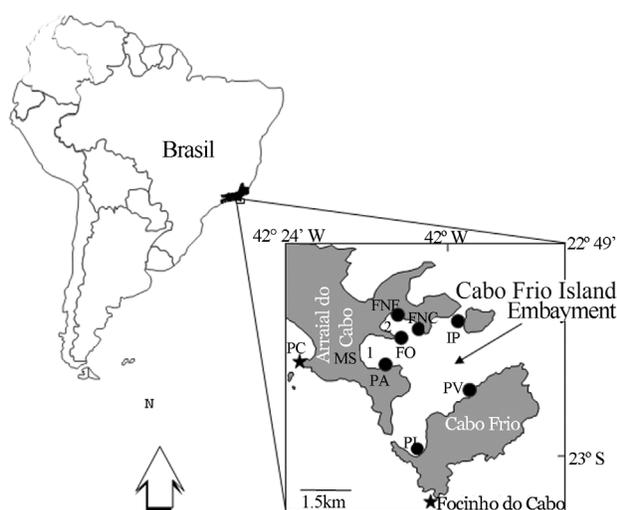


Figure 1 - Map of the study area and sampling stations in Arraial do Cabo, Cabo Frio region, state of Rio de Janeiro. Circles represent the studied stations in the Cabo Frio embayment and the stars the sites with greater influence of cold fronts and upwelling (including Focinho do Cabo, studied in previous work). IP: Ilha dos Porcos; PA: Praia dos Anjos; FO: Ponta da Fortaleza; PV: Pedra Vermelha; FNC: Forno Cultivo; FNE: Forno Flutuante; PI: Praia da Ilha de Cabo Frio; PC: Ponta da Cabeça. 1 = Anjos embayment; 2 = Forno embayment. MS = Meteorological station. Details in the text.

ta da Cabeça (PC), for instance, is outside the bay and directly influenced by upwelling events and strong waves during cold fronts. The other seven are distributed inside the Cabo Frio embayment as follows: Praia da Ilha (PI) is frequently influenced by upwelling and cold fronts; Pedra Vermelha (PV) and Ilha dos Porcos (IP) are influenced by north waters from Arraial do Cabo; In the Forno inlet (number 2 in Figure 1), upwelling influence is rare and three stations were established: one with oyster and coquille cultivation (Forno Cultivo - FNC); one more exposed to wave action (Fortaleza - FO), and another one near the beach and a floating restaurant (Forno Flutuante - FNF); The eighth site is the Praia dos Anjos (PA) station in the Anjos inlet (number 1 in Figure 1) that also has rare upwelling influence but is directly influenced by harbor activities, as well as by sporadic sewage emissions. These emissions are usually associated with intense rainfalls or with population increase during summer vacations.

Sampling procedure and water variables measurements

All stations were sampled 12 times, 6 during the spring/summer (upwelling period) and 6 during the autumn/winter (subsidence period) seasons, always between 9:00 and 12:00 at morning. At each station, 5 liters of surface water were harvested with a bucket and kept in thermo plastic bottles until laboratory analysis (~3 h). Nutrients (nitrate, nitrite, ammonia and phosphate) were determined according to Strickland and Parsons (1972). The determination of pigment concentrations (chlorophyll *a*, *b*, *c* and pheophytin) followed Richard and Thompson (1952) after filtration through cellulose acetate membranes (Millipore[®], 0.45 μm pore size) and SCOR-UNESCO (1966) equations were used for calculations. Suspended Particulate Matter (SPM) was determined using Whatman[®] glass fiber filters with ~1.2 μm mesh and Particulate Organic Matter (POM) was determined through SPM loss upon ignition at 400 °C during 24 h.

Bacterial production

Heterotrophic bacterioplankton activity was measured using the ³H-Leucine incorporation technique (Kirchman *et al.*, 1985). The use of one or two controls was tested using 100% trichloroacetic acid (5% final concentration) in 1.7 mL of sampled water, but one control was found adequate. After 1 h incubations and within a 15 days period after that (Miranda *et al.*, 2007), proteins were extracted using the micro centrifugation method described by Smith and Azam (1992). Protein radioactivity was measured on a Perkin-Elmer TRICARB 1600 liquid scintillation detector during 30 min or after the accumulation of 10000 counts (Coelho-Souza *et al.*, 2013). DPM values were obtained after quenching correction using the external standard of the equipment.

^3H -Leucine incorporation rates were calculated using DPM, the specific activity of ^3H -Leucine and a conversion rate from DPM to 1 mol of leucine (Kirchman, 1993). Bacterial activity standard error was determined using different leucine concentrations and sub-sampling (Cumming *et al.*, 2007). 5 μL of different substrate concentrations (0.1 to 32 nM) and ^3H -Leucine specific activities were used to evaluate the average error associated with sub-sampling (maximum of 7).

Leucine incorporation saturated at 10 nM, and the average errors were usually < 17%, even when adding less than 1 nM leucine. The addition of saturated leucine concentrations could inhibit amino acid biosynthesis but this was not confirmed by Kirchman (2001) and it was observed that 0.5-1.0 nM of leucine was not incorporated by cyanobacteria (Kirchman *et al.*, 1985) while when using 20 nM it was incorporated by picoeukaryotes (Michelou *et al.*, 2007). Hence, for this study we used 0.5 nM leucine and used Simon and Azam (1989) and Simon (1991) conversion factors for isotopic dilution to measure carbon production. Meanwhile, with this empiric correction there was no difference between the measurements using 0.5 or 20 nM of leucine, as used by other authors that studied the region (Carvalho and Gonzalez-Rodriguez, 2004; Guenther *et al.*, 2008).

Flow cytometry

To measure the density of particles in water, 1.5 mL samples were injected in a CytoBuoy Flow Cytometer (Woerden - The Netherlands) equipped with a solid blue laser providing 20 mW at 488 nm, one side scatter (SWS, 446/500nm) detector and three others for red (chlorophyll-a) fluorescence (FL1- 669/725 nm); orange/yellow (FL-2, 601/651 nm), and green/yellow (FL-3, 515/585 nm) fluorescence (Dubelaar *et al.*, 1999). Data acquisition was done through the Cytosiftlab software and the use of the CytoQuick software allowed us to discriminate between fluorescent and non fluorescent particles and estimate their relative size distributions. These analyses were only done two times during sewage emissions observed visually in 2007 and were not included in the statistical analysis.

Statistical analysis

Descriptive statistics were performed using the median value of all measured variables and sampling stations. The similarities between all sampling stations were assessed by cluster analysis (a dendrogram using the Ward method and Euclidean distances). In order to associate the physical-chemical-biological variables, a principal component analysis (PCA) was used (James and McCulloch, 1990). For PCA, we also used wind data from a station situated in Arraial do Cabo (Figure 1), acquired at the web page of the Instituto Brasileiro de Meteorologia (www.inmet.gov.br). The used wind variables were the mean force (speed) and direction (NE x SW) during the last

24 h before sampling. Then, we considered BP, winds, temperature, the concentrations of phosphate, nitrate, ammonia, chlorophyll *a* and POM as variables for analysis and the others (concentrations of nitrite, chlorophylls *b* and *c* and pheophytin) as supplementary variables. The number of accepted factors was determined by Eigen values ≥ 1 which justifies more than 10% of variability.

Results

Heterotrophic bacterioplankton production

BP was variable between samplings periods and stations, and its average ranged from 0.6 (PC) to 4.4 (PA). Considering all samples, BP had an average of 1.3 $\mu\text{gC}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ and a standard deviation of 2.4 ($n = 96$). Values above 99% of confidence intervals were observed in PA and FO stations (Figure 2; Table 1). When sewage discharges were visually observed in PA station, BP increased up to almost 400 times and the highest values were associated with ammonia concentrations above 2 μM (Figure 3).

Environmental variability

The dendrogram using physical-chemical-biological water variables (median values) confirms the great pressure of hydrobiological variability in the Cabo Frio upwelling region (Figure 4). The PI station had the highest concentrations of chlorophylls and POM (Table 1), indicating an intense autotrophic production in this area. POM was also high in IP and in the main impacted stations, suggesting an input of allochthonous material from coastal waters and anthropogenic activities, respectively. PC station was different from the other sites since it is under influence of both upwelling and cold fronts, as shown by its lowest minimum temperature and maximum nutrient concentrations (Table 1). Inner the Cabo Frio embayment, the minimum temperatures indicated a low pressure of upwelling, except at PI station. As observed visually, this area was also under high pressure of cold fronts.

Considering the four main components, PCA analysis justified more than 80% of the data variability (data not shown). Factor 1, for instance, accounts for 32.3% and seems to be determined mainly by the contrast between temperature and concentrations of phosphate and nitrate, indicating that upwelling events are the main factor disturbing the system. Factor 2 explained 26.4% of the variability and was associated with wind direction and intensity. As expected, NE wind was associated with upwelling and SW wind was associated with cold fronts increasing the concentrations of POM and pigments. Factor 3 explained 12.4% of the total variability and was associated with BP. On the other hand, factor 4 was associated with the concentration of chlorophylls and justified 10.2% of the data variability. Polling factors 3 and 4, it was possible to speculate on a change of trophic state in the system as another envi-

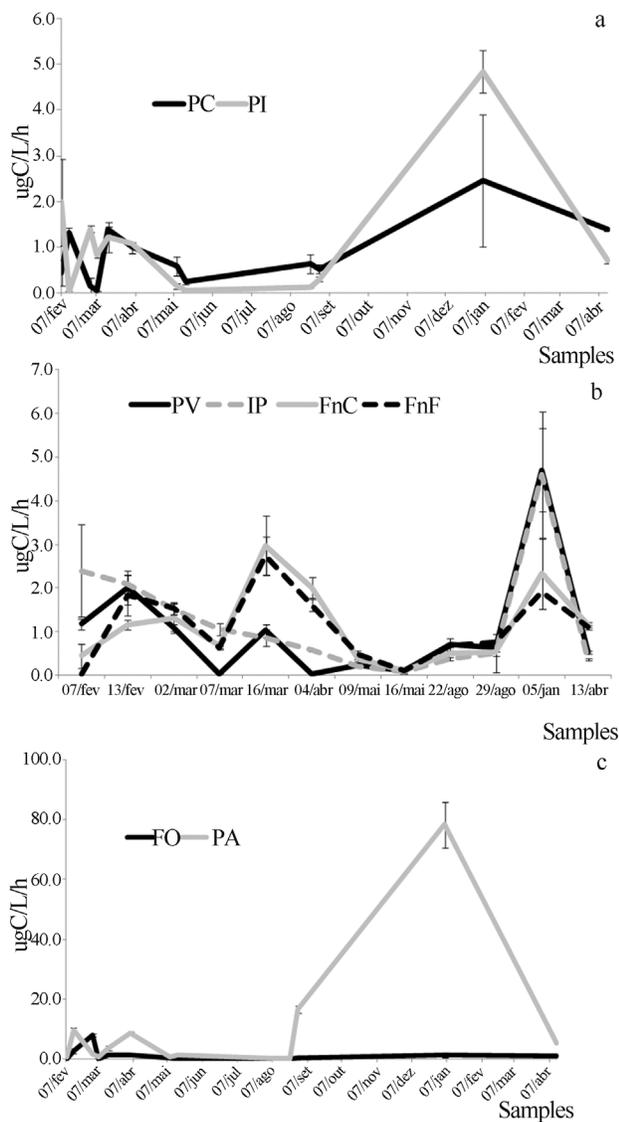


Figure 2 - Heterotrophic bacterial production ($\mu\text{gC.L}^{-1}.\text{h}^{-1}$) in surface waters sampled at: a) Ponta da Cabeça (PC) and Praia da Ilha (PI) stations; b) Pedra Vermelha (PV), Ilha dos Porcos (IP), Forno Cultivo (FnC) and Forno Flutuante (FnC) stations, and; c) Fortaleza (FO) and Praia dos Anjos (PA) stations ($n = 12$). Note the different scales of the Y-axis and the highest values in PA station.

ronmental disturbance. Projecting factors 2 and 3 (Figure 5) the association between BP and ammonia was evident.

Table 2 shows data variability in two samples realized in 2007 during sewage discharges. BP, Cl *a* and suspended particles were highest in PA station. The average of suspended particles decreased from Anjos and Forno inlets to PC station. Meanwhile, POM was highest in PI and PC areas. PC also had the highest ratios between the abundance of non fluorescent and fluorescent (NF/F) particles, as well as the highest ratios between the abundance of non fluorescent particles and the concentration of chlorophyll *a* (NFpart/Cl*a*). These data indicated that upwelling and cold fronts resuspended inorganic and organic particles with

Table 1 - Range (minimum and maximum values) of the physical-chemical-biological variables measured in surface waters from studied stations during 2006. PI - Praia da Ilha; PC - Ponta da Cabeça; PV - Pedra Vermelha; PA - Praia dos Anjos; IP - Ilha dos Porcos; FnC - Forno Cultivo; FnF - Forno Flutuante; FO - Fortaleza. Temp - Temperature; N- NO_2 - nitrite; N- NO_3 - nitrate; N- NH_4 - ammonia; P- PO_4 - phosphate; Cl - chlorophylls *a*, *b* and *c*; Pheo - pheophytin; POM - Particulate Organic Matter; BP - Bacterial Production.

Station	Temp (°C)	N- NO_2 (μM)	N- NO_3 (μM)	N- NH_4 (μM)	P- PO_4 (μM)	Cl <i>a</i> (g.L^{-1})	Cl <i>b</i> (g.L^{-1})	Cl <i>c</i> (g.L^{-1})	Pheo (g.L^{-1})	POM (g.L^{-1})	BP ($\mu\text{gC.L}^{-1}.\text{h}^{-1}$)
PI	20.2 27.0	$\leq 0.3 \leq 0.3$	$\leq 0.3 \leq 2.3$	0.9 1.8	$\leq 0.1 \leq 0.4$	$\leq 0.1 \leq 9.1$	$\leq 0.1 \leq 5.4$	$\leq 0.1 \leq 3.6$	$\leq 0.1 \leq 4.8$	9.0 19.5	$\leq 0.1 \leq 4.8$
PC	17.1 27.1	$\leq 0.3 \leq 1.2$	$\leq 0.3 \leq 6.0$	0.7 3.0	$\leq 0.3 \leq 0.8$	0.3 3.7	$\leq 0.1 \leq 0.8$	$\leq 0.1 \leq 1.5$	$\leq 0.1 \leq 2.9$	$\leq 0.1 \leq 8.7$	$\leq 0.1 \leq 2.5$
PV	22.2 27.1	$\leq 0.3 \leq 0.3$	$\leq 0.3 \leq 2.4$	0.8 1.9	$\leq 0.3 \leq 0.3$	0.3 1.8	$\leq 0.1 \leq 0.6$	$\leq 0.1 \leq 1.5$	$\leq 0.1 \leq 1.2$	0.3 8.7	$\leq 0.1 \leq 4.7$
PA	22.2 27.9	$\leq 0.3 \leq 0.3$	$\leq 0.3 \leq 2.2$	0.7 2.4	$\leq 0.3 \leq 0.3$	0.6 2.2	$\leq 0.1 \leq 2.2$	$\leq 0.1 \leq 0.7$	$\leq 0.1 \leq 1.4$	0.5 15.3	0.2 78.2
IP	22.1 27.3	$\leq 0.3 \leq 0.3$	$\leq 0.3 \leq 2.5$	0.8 3.5	$\leq 0.3 \leq 0.3$	0.3 1.6	$\leq 0.1 \leq 0.3$	$\leq 0.1 \leq 1.2$	$\leq 0.1 \leq 1.2$	$\leq 0.1 \leq 22.5$	0.1 4.6
FNC	22.1 27.5	$\leq 0.3 \leq 0.3$	$\leq 0.3 \leq 2.3$	0.9 1.9	$\leq 0.3 \leq 0.3$	0.3 1.7	$\leq 0.1 \leq 0.4$	$\leq 0.1 \leq 1.3$	$\leq 0.1 \leq 1.1$	$\leq 0.1 \leq 13.9$	0.1 3.0
FNF	22.1 27.6	$\leq 0.3 \leq 0.3$	$\leq 0.3 \leq 2.1$	0.8 2.4	$\leq 0.3 \leq 0.3$	0.4 1.4	$\leq 0.1 \leq 0.4$	$\leq 0.1 \leq 1.2$	$\leq 0.1 \leq 1.0$	0.2 16.5	$\leq 0.1 \leq 2.8$
FO	22.1 27.9	$\leq 0.3 \leq 0.3$	$\leq 0.3 \leq 2.9$	0.8 2.0	$\leq 0.3 \leq 0.3$	0.1 1.5	0.1 0.4	$\leq 0.1 \leq 1.0$	$\leq 0.1 \leq 1.0$	0.7 9.3	$\leq 0.1 \leq 8.2$

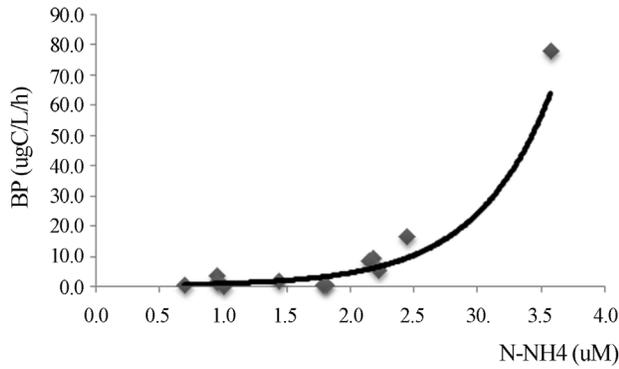


Figure 3 - Association between heterotrophic bacterial production ($\mu\text{gC.L}^{-1}.\text{h}^{-1}$) and ammonia concentration (μM) in surface waters collected at Praia dos Anjos station ($n = 12$).

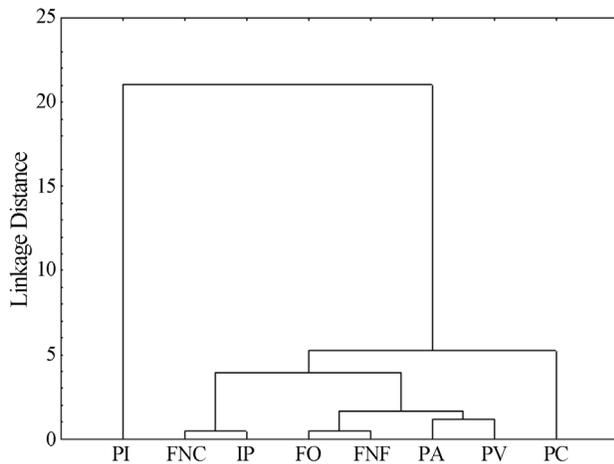


Figure 4 - Clustering of the studied stations considering the medians of all measured variables in surface waters. PI - Praia da Ilha; PC - Ponta da Cabeça; PV - Pedra Vermelha; PA - Praia dos Anjos; IP - Ilha dos Porcos; FnC - Forno Cultivo; FnF - Forno Flutuante; FO - Fortaleza. Details in the text.

size bigger than particles inputted during sewage emissions.

The abundance of small particles was highest in in-shore stations during upwelling and in the PC station during

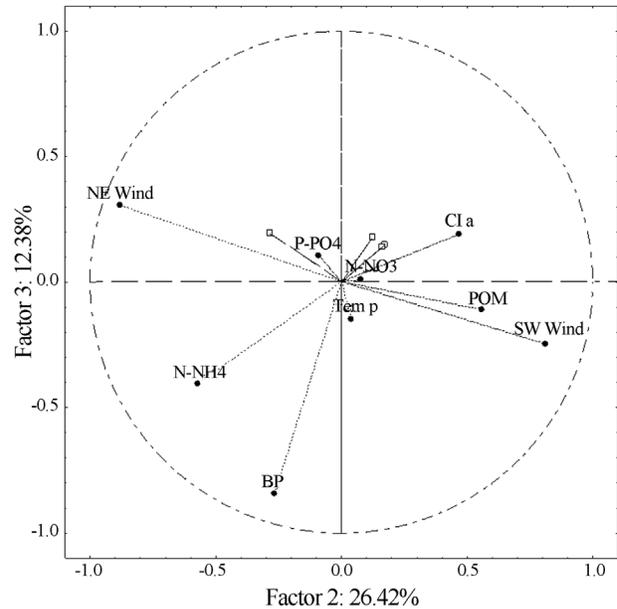


Figure 5 - Representation of factors 2 and 3 of the PCA considering active (circles) and supplementary variables (squares) from all sampling stations during 2006/7 ($n = 96$). Variables: Northeastern (NE) and Southwestern (SW) Winds; Bacterial Production (BP); Temperature (Temp); Particulate Organic Matter (POM); and the concentrations of phosphate (P-PO₄), nitrite (N-NO₂), nitrate (N-NO₃), ammonia (N-NH₄), chlorophyll (Cl a, Cl b and Cl c), pheophytin (Pheo). Details in the text.

the subsidence period. The concentration of red fluorescent particles was higher at PO/PV area. Upwelled waters had the highest NF/F ratio and the smallest concentration of particles with orange and yellow/green fluorescence (data not shown). Cold fronts events are expected to decrease the spatial variability of NF/F ratio due to their homogenizing effect in the water column.

Discussion

Our results confirmed that upwelling is the main process determining the abiotic and biotic characteristics of the surface waters in Arraial do Cabo, followed by the winds dynamics, as observed by Valentin and colleagues (1984; 1987) in a fixed station (Focinho do Cabo). Hydrological

Table 2 - Mean and standard variation (range in parenthesis) of Bacterial Production (BP), and of the concentrations of Chlorophyll *a* (Cl *a*), Particulate Organic Matter (POM) and total particles (TPart) measured in surface waters from the studied sites during a sewage emission visually observed at PA station in 2007 ($n = 2$). NF/F part represents the proportion of non fluorescent and fluorescent particles. NFpart/Cl *a* represents the proportion of non fluorescent particles measured by flow cytometry with the concentration of chlorophyll *a*. The sites were divided in Anjos embayment, Forno embayment, between Porcos Island and Pedra Vermelha area (PO/PV), Praia da Ilha area (PI) and, Ponta da Cabeça area (PC). Details in the text and in Figure 1.

Stations	BP ($\mu\text{gC.L}^{-1}.\text{h}^{-1}$)	Cl <i>a</i> (g.L^{-1})	POM (g.L^{-1})	TPart ($10^7 \text{ cells.L}^{-1}$)	NF/F part	NFpart/Cl <i>a</i>
Anjos	23.7 ± 30.7 (5-78)	1.5 ± 1.4 (1-4)	3.5 ± 3.4 (0-9)	39.0 ± 45.3 (7-71)	2.4 ± 0.8 (2-3)	8.5 ± 4.1 (6-11)
Forno	1.4 ± 0.7 (0-3)	0.7 ± 0.4 (0-1)	3.7 ± 2.8 (1-9)	25.4 ± 22.6 (10-41)	1.9 ± 0.8 (1-3)	15.8 ± 8.7 (10-22)
PO/PV	1.6 ± 1.7 (0-5)	0.8 ± 0.3 (0-1)	3.5 ± 3.1 (0-8)	21.5 ± 21.8 (6-37)	2.8 ± 2.6 (1-5)	11.3 ± 1.5 (10-12)
PI	1.4 ± 2.0 (0-5)	0.9 ± 0.2 (0.7- 1.2)	8.6 ± 9.7 (0-19)	15.5 ± 12.7 (6-25)	2.4 ± 2.0 (1-4)	8.9 ± 1.5 (8-10)
PC	1.4 ± 0.9 (0-3)	0.7 ± 0.2 (0-1)	5.4 ± 4.5 (1-13)	13.7 ± 10.8 (6-21)	7.7 ± 6.6 (3-12)	19.8 ± 15.6 (9-31)

data were variable during sampling periods and stations (Tables 1 and 2), confirming constant disturbances in the system. The average values of BP was according to previous studies in the region (Carvalho and Gonzalez-Rodriguez, 2004; Guenther *et al.*, 2008; Cury *et al.*, 2011) but its range showed that BP was higher in the stations inner the embayment (Figure 2). Wide coastal area and its extension can maintain the elevated upwelling intensity and chlorophyll concentrations in upwelling systems and the importance of Cabo Frio embayment to the system production should be evaluated in future studies (Coelho-Souza *et al.*, 2012). Furthermore, sewage discharge in the Anjos beach was the main factor increasing bacterial production (Figure 3) and it was coupled with high chlorophyll concentrations.

Abiotic factors such as temperature and nutrient availability usually determine temporal and spatial patterns in the ecosystem and are considered the first selective pressures on growth, survival and metabolic activity of microorganisms (Terrado *et al.*, 2009). BP should be inhibited by low temperatures during upwelling (Kirchman *et al.*, 1995; Tibbles, 1996), and the mixture and warming of the upwelled water is needed to activate the bacteria (Carvalho and Gonzalez-Rodriguez, 2004; Guenther *et al.*, 2008). Cury *et al.* (2011) reported higher BP values in surface than in deep waters and Zubkoz *et al.* (2004) observed that amino acid uptake was related to sampling depth and associated it with phytoplankton photosynthetic activity as well as with light intensity and quality (Seymour *et al.*, 2000; Coelho-Souza *et al.*, 2013).

On the other hand, biotic variables, such as abundance, biomass and metabolic activity are also essential for a better understanding of microbial ecology. Nevertheless, some technical limitations impose caution on the interpretation of microbial data. For example, correlations between the number of individuals, their biomass and metabolic activity are not always observed (Andrade *et al.*, 2007). This limits the projection of patterns from other directly measured variables (McArthur, 2006).

Specifically, measurements of bacterial activity are done in closed samples which can modify bacterial metabolism (Pace *et al.*, 2004). The sampling process to evaluate microorganism density and diversity is another challenge for microbial ecology studies. The aggregated distribution of communities requires sub-sampling to be statistically determined by its confidence limits. Thus, the interpretation of results, in addition to the analyzed variables, must also take into account the sampling and sample processing methods (Karl and Dore, 2001).

Bacterial production and the nitrogen cycle

Natural eutrophication by upwelling events is usually associated with inorganic nitrogen inputs (mainly nitrate) that consequently increase the biomass and size of phytoplankton (Guenther *et al.*, 2008). Artificial anthropogenic

eutrophication by sewage discharges increased the concentration of ammonia (Figure 3) and, probably, the dissolved organic nitrogen (DON) input in the system. It makes the trophic web longer (Suzuki *et al.*, 1996) since picoplankton prefers to assimilate ammonia than nitrate (Harrison and Wood, 1988).

Many authors suggested that the metabolic balance between autotrophy and heterotrophy indicates the autochthonous and allochthonous inputs in a system (*e.g.*, Cottrell *et al.*, 2006; Thottathil *et al.*, 2008). In this way, Pereira *et al.* (2010) associated the heterotrophic/autotrophic ratio and the viral abundance with upwelling events in the Cabo Frio system. These authors reported a highest concentration of heterotrophic prokaryotes in the Anjos inlet. They also observed a high abundance of phytoplankton and a highest viral/bacterial ratio close to PI station (Pereira *et al.*, 2009). Our results showed that PI had hydrological difference from all other stations (Figure 4) probably due its influence from coastal, tropical and subtropical waters (Pereira *et al.*, 2009; 2010) that increases the proportion of live cells (*e.g.*, Cl *a*) in POM (Tables 1 and 2). Therefore, PI should be considered as the main station under autochthonous production due mixing of rich and warm waters. Bacteria use carbon and nutrients from this green web but are also regulated by virus (Pereira *et al.*, 2009). As expected, PC had more pressure of upwelling and cold fronts and stations inner the embayment were divided in function of the highest frequency of north coastal waters in IP and FnC. Unexpectedly, PV grouped with the main anthropogenic impacted stations (PA, FnF and FO) suggesting that water circulation decreases punctual impacts but can disperse pollutants to adjacent areas (Ribeiro, 2002).

Our results indicated a change in the energy source associated with anthropogenic activities; confirming an autochthonous input associated with upwelling and an allochthonous input associated with sewage discharges. We suggest that autochthonous production is associated with ascension of inorganic nutrients and benthic particles from deep water during upwelling (Guenther *et al.*, 2008) that is quickly exported (Valentin *et al.*, 1987) while marine snow is precipitated on the bottom sediment. During cold fronts, carbon and nutrients are resuspended, water column is homogenized and POM as well as Cl *a* increases (Figure 5). We hypothesize a regenerated production during cold fronts as a function of the new production during upwelling. However, the equilibrium is broken during sewage discharges since there is a high input of dissolved organic matter that is consumed by autotrophic and heterotrophic microorganisms.

Comparing the lowest ($0.2 \mu\text{gC}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$) and highest ($78.2 \mu\text{gC}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$) values of BP in PA station, we observed a ~400-fold increase during sewage discharges (Tables 1 and 2). BP was well associated with ammonium concentrations (Figure 3). Kirchman (1994) suggested that nitrogen is the main element associated with marine bacterio-

plankton that can also regulate carbon and phosphorus absorption (Azam *et al.*, 1994; Berman and Bronk, 2003). Hoch *et al.* (2008) observed that organic nitrogen compounds are the main stimulus to bacterial activity. Following up, ammonification of dissolved organic nitrogen (DON) favors the uptake of inorganic nitrogen by bacteria (Kirchman, 1994). Herein, a high range in ammonia concentrations was reported in all stations, but the peaks of nitrate and nitrite were highest in the area directly influenced by upwelling (PC). We suggest different pathways of ammonia production and oxidation in the nitrogen cycling.

Following Cury *et al.* (2011), Anjos embayment had lower microbial diversity than an area directly influenced by upwelling, mainly for Bacteria and Eukarya domains. Prokaryotes and Eukaryotes are important contributors in NH_4^+ cycling since eukaryotes support prokaryotic needs, including uptake and oxidation processes (Molina *et al.*, 2005). Simulating upwelled waters mixture, Carvalho and Gonzalez-Rodriguez (2004) observed that ammonia concentration was associated with auto and heterotrophic activities, as well as with the water type predominance. Ammonia can also facilitate nitrate assimilation by primary producers (Bode and Varela, 1994) and it is in accordance with the high chlorophyll concentrations reported during sewage discharges observed visually. Blooms of opportunistic and toxic phytoplankton are another risk for the environment (Coelho-Souza *et al.*, 2006).

Ammonifying bacteria were the primary source of ammonium in the Antarctic coastal waters (Serebrennikova *et al.*, 2008). In the Chilean upwelling system, in the oxycline base, NH_4^+ was in high demand by various components of the bacterioplankton community with different metabolisms: aerobic-anaerobic, photoautotrophic, chemoautotrophic, and heterotrophic. Specifically, the aerobic ammonium oxidation was more regulated by NH_4^+ than O_2 availability and contributed to more than 30% of the total dark carbon fixation (Molina and Farias, 2009).

Some planktonic Archaea are able to take up amino acids, being considered as heterotrophic (Alderkamp *et al.*, 2006). In the Cabo Frio system, for Archaea domain, Crenarchaeota was found only in surface waters from the anthropogenic impacted area and this group uses ammonia as its sole energy source (Cury *et al.*, 2011). Following our results obtained during sewage emissions, we suggest that bacteria are producing ammonia from DON and that Crenarchaeota is consuming ammonia. A significant negative relation was observed between Crenarchaeota and NH_4^+ in the Black Sea (Stoica and Herndl, 2007).

Conclusion

The main risk of eutrophication is the establishment of dead zones due to hypoxia when microorganism growth reduces the concentration of dissolved oxygen in the water column (Diaz and Rosenberg, 2008). Though hypoxia can be observed in upwelling regions (Molina and Farias, 2009), no

oxygen depletion was observed in the Cabo Frio upwelling system (Valentin, 1984; Gonzalez-Rodriguez *et al.*, 1992; Guenther *et al.*, 2008) probably due to a high water circulation (Seymour *et al.*, 2000). Coastal ecosystems with short residence times tend to export more exogenous nitrogen (Cloern, 2001) but eutrophication can also deteriorate adjacent ecosystems (Hoch *et al.*, 2008) since the fluxes of nutrients and energy are altered (Madigan *et al.*, 2004; Garren *et al.*, 2008). In addition, harbor activities resuspend sediment and nitrogen that could be exported to the continental shelf and to the open ocean (Fulweiler *et al.*, 2007).

Embayment areas favor the growth of microorganisms but vast sewage dispersion and phytoplankton blooms are rare in the Cabo Frio embayment. On the other hand, there is no information about the compounds present in the Arraial do Cabo sewage and studies associating this discharges with toxicological consequences from domestic chemical products are needed (Schindler, 2006; Soares *et al.*, 2008). Furthermore, bacterial activity is both associated with environmental remediation and pollutants dispersion (Madigan *et al.*, 2004; Coelho-Souza *et al.*, 2006; 2011; Guimaraes *et al.*, 2006).

The view of eutrophication is biased from observations in few systems (eg., Taketani *et al.*, 2003; Araujo and Hagler, 2011). This scientific discipline is still very young to marine systems and a broader view of coastal eutrophication will consider how anthropogenic nutrient enrichment interact with other stressors such as species translocation, habitat loss, fishing, inputs of toxic contaminants, manipulation of freshwater flows, aquaculture, and climate change. Ammonium accumulation in water column should influence the communities of benthic primary producers. Consequently, the overgrowth of macroalgae could cause replacement of corals, as well as other changes in benthic invertebrate populations (Cloern, 2001).

In the Arraial do Cabo protection area, we should consider the signs of disturbances that occur before the catastrophic symptoms. These early responses include changes in communities at the species level, indirect responses, changes in the ratios of biogeochemical processes such as nutrient cycling, and shifts in seasonal patterns or in their variability (Cloern, 2001). To avoid ecosystem deterioration, an adequate coastal management is strictly necessary; especially in marine protection areas like Arraial do Cabo (Floeter *et al.*, 2001). Following Nixon (2009), scientists, regulators, politicians, and even activists need to consider coastal marine eutrophication not as a simple "pollution problem" but as a major ecological change that must be viewed through the microscope.

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