The study of bacterioplankton dynamics in the Berlengas Archipelago (West coast of Portugal) by applying the HJ-biplot method

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The relationship between bacterioplankton and environmental forcing in the Berlengas Archipelago (Western Coast of Portugal) were studied between February 2006 and February 2007 in two sampling stations: Berlenga and Canal, using an HJ-biplot. The HJ-biplot showed a simultaneous display of the three main metabolic groups of bacteria involved in carbon cycling (aerobic heterotrophic bacteria, sulphate-reducing bacteria and nitrate-reducing bacteria) and environmental parameters, in low dimensions. Our results indicated that bacterial dynamics are mainly affected by temporal gradients (seasonal gradients with a clear winter versus summer opposition), and less by the spatial structure (Berlenga and Canal). The yearly variation in the abundance of aerobic heterotrophic bacteria were positively correlated with those in chlorophyll *a* concentration, whereas ammonium concentration and temperature decreased with increasing phosphates and nitrites concentration. The relationship between aerobic heterotrophic bacteria, chlorophyll *a* and ammonium reveals that phytoplankton is an important source of organic substrates for bacteria.

Key words: Berlengas Natural Reserve, aerobic heterotrophic bacteria, sulphate-reducing bacteria, nitrate-reducing bacteria

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The Berlengas Natural Reserve (BNR) is an archipelago formed by 3 groups of islands (Berlenga, Estelas and Farilhões) located 5.7 miles from Cabo Carvoeiro (Peniche). It was created in 1981, aiming to preserve a rich natural heritage and to ensure sustainable development of human activities in the area. The Reserve has a total area of 9560 hectares, 9456 of which are marine. The fauna is characteristic, very rich, diverse and peculiar (Rodrigues et al. 2008).

Despite its known biodiversity, the few existing marine scientific studies were carried out only after the establishment of the BNR. Azeiteiro et al. (1997) and Pardal & Azeiteiro (2001) studied the macrozooplankton distribution and several studies addressed the macrobenthos communities (Almeida 1996; Bengala et al. 1997a, 1997b, 1997c; Metelo 1999; Neto 1999; Neto et al. 1999).
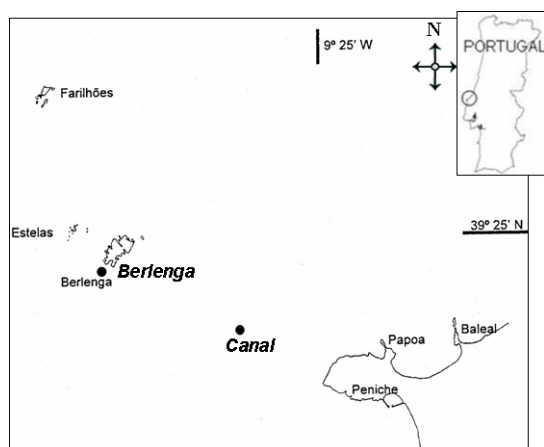

Fig. 1. Berlengas Archipelago with its 3 groups of islands and the location of the sampling stations (●).

Microbes are a key component of the structure and function of aquatic ecosystems, through their involvement in the various nutrient biogeochemical cycles namely in coastal areas (Wetz et al. 2008), and in the NW Iberian margin upwelling system (Barbosa et al. 2001).

Until now, no studies have been carried out to assess this component of the ecosystem in the BNR. Because of bacterial major role in planktonic microbial food webs (they comprise an important share of plankton biomass) and their activities impact on ecosystem metabolism and function (Gasol & Duarte 2000) this preliminary study in the BNR has a clear ecological relevance.

The present study aimed to investigate the annual dynamics (February 2006 – February 2007) of three main metabolic groups of heterotrophic bacteria: aerobic heterotrophic bacteria (AHB), sulphate-reducing bacteria (SRB) and nitrate-reducing bacteria (NRB), and to identify the key factors (among various physical and chemical parameters) that control the annual dynamics of the bacterioplankton in the Berlengas archipelago area, by an HJ-biplot (Galindo 1986; Galindo & Cuadras 1986). The HJ-biplot analysis is an extension of the classical biplots introduced by Gabriel in 1971 (Gabriel 1971, 1972; Gabriel & Odoroff 1990). The usefulness of HJ-biplot has been showed in many fields; for example in Biology (Martínez-Ruiz et al. 2001; Martínez-Ruiz & Fernández-Santos 2005; Cabrera et al. 2006; Martínez-Ruiz et al. 2007); in Geology (García-Talegón et al. 1999) and Medicine (Pedraz et al. 1985), etc.

MATERIALS AND METHODS

STUDY SITE AND FIELD PROGRAM

The BNR is an archipelago formed by 3 groups of islands (Berlenga, Estelas and Farilhões) located 5.7 miles from Peniche (Portugal). The sea floor consists primarily of irregular hard bottom substrate (i.e. rocks covered with sessile biota, including a variety of algae, sponges, hydrozoans, anemones, crustaceans, sea urchins and tunicates).

The two sampling sites used were Berlenga (inside BNR, 39º24.70' N, 9º31.22' W) and Canal (39º24.56' N, 9º26.70' W) (Fig. 1). Water samples were collected monthly from subsurface water (0.2 to 0.4 m depth) between February 2006 and February.

DETERMINATION OF PHYSICAL AND CHEMICAL PARAMETERS

Samples were analysed *in situ* for salinity and temperature (WTW MultiLine P4 portable meter). Water samples for chemical analyses and chlorophyll *a* quantification were collected and immediately stored in the dark at low temperature (4º C), until further processing was possible. In the laboratory these water samples were filtered through GF/C filters (1.2 μm pore diameter), for quantification of photosynthetic pigments. Filtrates were used for determination of nutrient contents. Samples were also analysed in the laboratory (in triplicate) for their content in silica, nitrate, nitrite, ammonium, phosphate and chlorophyll *a* (Strickland & Parsons 1972).

SAMPLING PROCEDURE AND ENUMERATION OF
VIABLE BACTERIA

Water samples (1 L) for the enumeration of viable
bacteria were collected in parallel to those
obtained for physical and chemical analyses.
Samples were collected in sterile glass flasks,
filled to capacity, sealed with gas-tight rubber
stoppers and immediately placed on ice until
processing in the laboratory (3 h later). Numbers
of viable AHB, SRB and NRB were estimated by
MPN method (Bacelar-Nicolau et al. 2003). Eight
replicate ten-fold dilution series of water samples
were prepared in appropriate selective liquid
media (below) in multiwell plates (Bacelar-
Nicolau et al. 2003). These were incubated at
room temperature, in the dark, for 2 weeks
(aerobes) or 4 weeks (anaerobes). The presence of
bacteria was scored positive on the basis of
turbidity or precipitate development, and
confirmed by microscopic observation. Growth of
SRB and NRB required strict anaerobic
procedures that were used at all times (Strickland
& Parsons 1972). YPG, VMN and the saltwater
Widdel and Back media were used, respectively
for AHB, SRB and NRB (Bacelar-Nicolau et al.
2003). In all media the concentration of NaCl was
adjusted to 30 g.L^{-1}, and pH was adjusted to 7.

STATISTICAL ANALYSIS

Data were organized in a matrix: eight
environmental variables and the abundance of the
three metabolic groups of bacteria studied (AHB,
SRB and NRB) at the two sites over the 12
months forming a data matrix of size 24 x 11 (the
data were normalized by columns to homogenize
the table).

The HJ-biplot is an exploratory data analysis
method that looks for hidden patterns in the data
matrix. The method is in some ways similar to
correspondence analysis but is not restricted to
frequency data. The HJ-biplot is a joint
representation, in a low dimensional vector space
(usually a plan), of the rows and columns of X,
using markers (points/vectors), for its rows and
for its columns. The markers are obtained from
the usual singular value decomposition (SVD) of
the data matrix. The rules for the interpretation of
the HJ-biplot are a combination of the rules used
in other multidimensional scaling techniques,
correspondence analysis, factor analysis and
classical biplots: 1) the distances among row
markers are interpreted as an inverse function of
similarities, in such a way that closer markers
(individuals) are more similar. This property
allows for the identification of clusters of
individuals with similar profiles; 2) the lengths of
the column markers (vectors) approximate the
standard deviation of the variables; 3) the cosines
of the angles among the column vectors
approximate the correlations among variables in
such a way that small acute angles are associated
with variables with high positive correlations;
obtuse angles near to the straight angle are
associated with variables with high negative
correlations and right angles are associated with
non-correlated variables. In the same way, the
cosines of the angles among the variable markers
and the axes (Principal Components) approximate
the correlations between them. For standardized
data, these approximate the factor loadings in
factor analysis and 4) the order of the orthogonal
projections of the row markers (points) onto a
column marker (vector) approximates the order of
the row elements (values) in that column. The
larger the projection of an individual point onto a
variable vector is, the more this individual
deviates from the average of that variable.

Several measurements are essential for a
correct HJ-biplot interpretation (Galindo 1986;
Galindo & Cuadras 1986). For instance, the
relative contribution of the factor to the element is
the relative variability of the variable explained
by a factor or dimension. Also, for a point (row or
column) on a factorial plan, the quality of
representation can be defined by adding the two
relative contributions of these factors to the
element. Only points with a high quality of
representation can be properly interpreted. These
indices will be indicated, for the rows and for the
columns of the data matrix. HJ-biplot has the
advantage that it is a simultaneous representation
and achieves an optimum quality of
representation for both for rows and columns,
both represented on the same reference system.

Calculations and graphs shown in this work
were done using Classical Biplot software
(Villardón [cited 2009; see Acknowledgements]).

RESULTS

The variation patterns of the environmental and biological parameters, at sites Berlenga and Canal are shown in Figure 2. Water temperature showed a similar unimodal pattern at both stations, with max.in summer (23.5 ℃) and min.in winter (13 ℃) (Figs. 2a and 2d). The concentration of chlorophyll *a* (Chl *a*) shows three peaks at both sites (Figs. 2a and 2d). At Berlenga, two moderate peaks of Chl *a* (May 2006 and January 2007) and broad maxima from July to October 2006 (Fig. 2a). At Canal, one moderate peak appeared in April 2006, and two higher peaks of Chl *a* appeared in October 2006 and December 2006 (Fig. 2d).
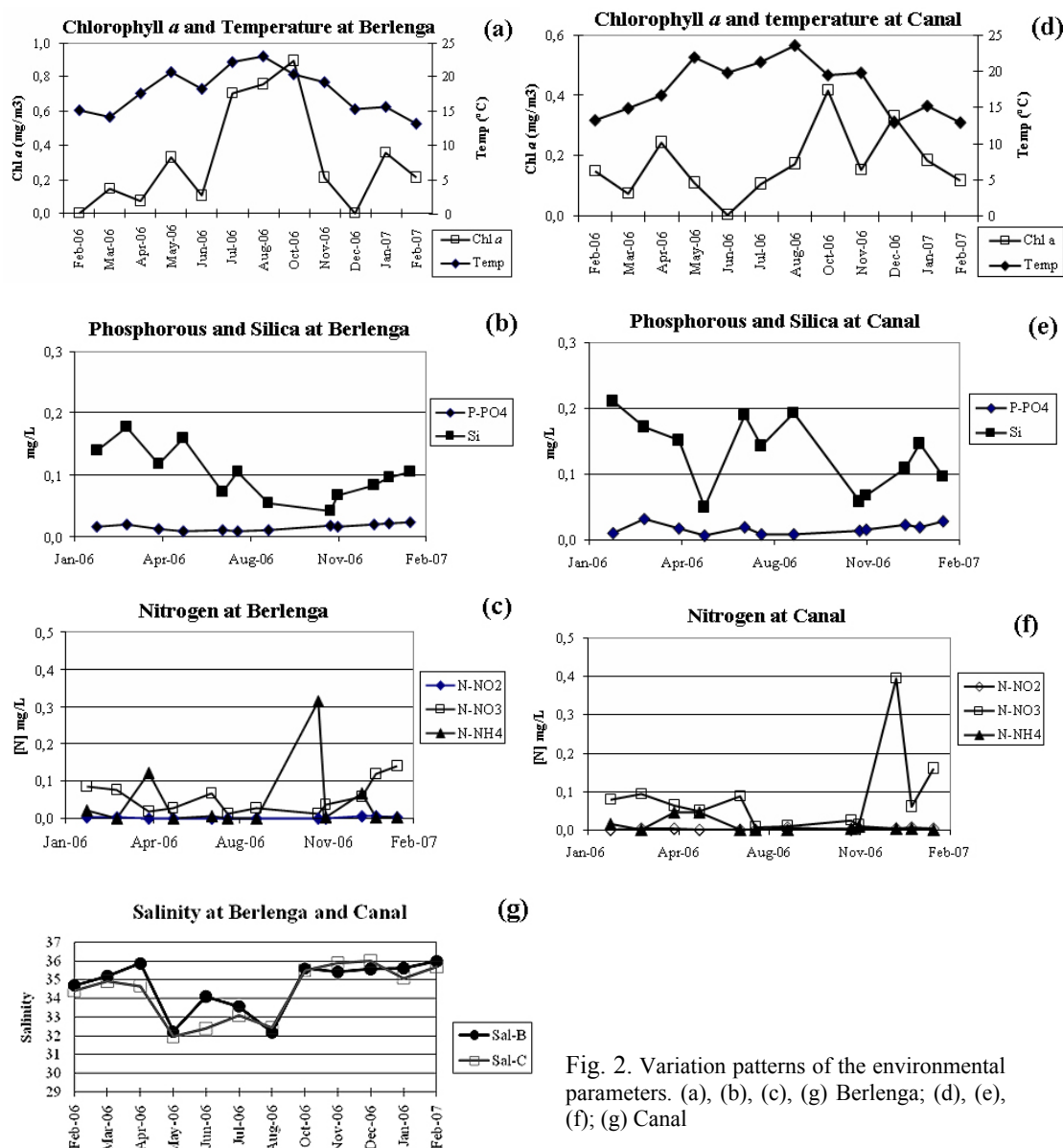

Fig. 2. Variation patterns of the environmental parameters. (a), (b), (c), (g) Berlenga; (d), (e), (f); (g) Canal

The concentration of silica showed different patterns in the two sites (Figs. 2b and 2e). At Berlenga, minimum values coincide with summer and autumn 2006, while at Canal there are two minimum values in May and October-November 2006. Phosphate concentration was fairly constant throughout the year and similar in the two sites, reaching maxima values in March 2006 (Figs. 2b and 2e). Salinity (Fig. 2g) varied between 32 and 36 with maxima in April 2006 and October to December 2006. Nitrogen dynamics are shown in Figs. 2c and 2f. Nitrite concentration was low and fairly similar at Berlenga and Canal. Nitrate and ammonium showed similar variation patterns, at the two sites, from January to September 2006, with a peak of ammonium concentration in April which coincided with a decrease of nitrate concentration. Between September and December 2006 patterns were different, with a peak of ammonium concentration at Berlenga in October, a peak of nitrate at Canal in November.

All metabolic groups were detected throughout the year at both sites (Fig. 3). AHB were, in average, present in greater numbers than SRB or NRB and showed greater number and fluctuations at Berlengas than Canal, with maxima values in February and August 2006 as well as between November and December 2006. SRB were, in average, present in greater numbers than NRB, at Berlengas, but the opposite was observed in Canal, where average numbers of NRB were greater than those of SRB. NRB showed similar patterns (and average numbers as well as standard deviation) and at both sites, although greater numbers at Canal, except for winter 2007 when numbers were greater at Berlengas.
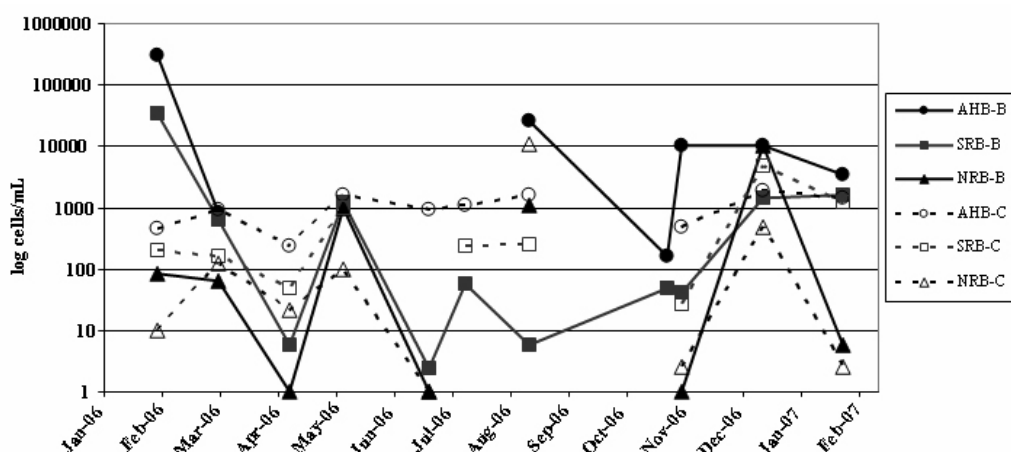

Fig. 3. Variation of physicochemical and biological parameters at Berlenga and Canal sampling sites during the annual cycle. AHB - aerobic heterotrophic bacteria, SRB - sulphate-reducing bacteria, NRB - nitrate-reducing bacteria. Points "B" and "C" correspond to the sites Berlenga and Canal.

The HJ-biplot analysis of environmental and biological parameters indicates that the system dynamics was independent of location (Berlenga, Canal) and mainly controlled by a seasonal gradient – spring/summer versus autumn/winter (Fig. 4a). The first three axes of the HJ-biplot analysis explained 63.52% of data variability (Fig. 4c and Table 1). The first axis (32.54%) revealed a strong opposition between samples collected in spring/summer and those collected in autumn/winter, independently of site of origin (Fig. 4a).

Table 1. Eigenvalues and Explained Variance (EV)

Axis	Eigenvalue	E.V. (%)	Cum. (%)
Axis 1	9.269	32.54	32.54
Axis 2	6.886	17.96	50.50
Axis 3	5.862	13.02	63.52
Axis 4	5.308	10.67	74.20

Fig. 4. HJ-Biplot representation. Projection of samples (points) and variables (arrows) onto plan of maximum inertia. Points "B" and "C" correspond to the sites Berlenga and Canal, the first three letters signify the month and the two second numbers indicate the year (2006 or 2007); AHB: aerobic heterotrophic bacteria, SRB: sulphate-reducing bacteria, NRB: nitrate-reducing bacteria; Si: silica, P-PO$_4$: phosphate, N-NO$_2$: nitrite, N-NO$_3$: nitrate, N-NH$_4$: ammonium, Chl a: chlorophyll a, S: salinity, T: Temperature. 4a) plan 1-2; 4b) plan 1-3.
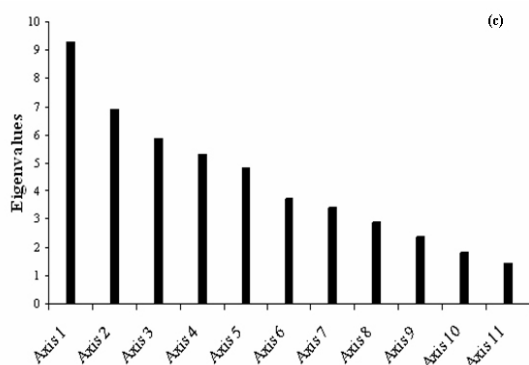
Fig. 4c). Eigenvalues barplot from HJ-Biplot analysis.

Moreover, this axis is characterized by phosphate, nitrate, nitrite, salinity and mostly by temperature. Therefore, the temperature characterizes the positive side of axis 1 (spring/summer samples), while phosphate, nitrate, nitrite and salinity appeared to be characteristic of the opposite side (autumn/winter samples at the left extreme of this axis). Hence, this is the gradient which defines axis 1. The temperature presented a negative correlation with those variables: higher values of temperature imply lower values of phosphate, nitrite, nitrate and salinity. Together, however, phosphate, nitrate, nitrite and salinity were positively correlated. The second axis of variability (17.96%) is mainly characterized by silica, chlorophyll *a* and ammonium (Fig. 4a). Silica defines the top of this axis and presented a negative correlation with the other two variables, chlorophyll *a* and ammonium (which are positively correlated). Furthermore, it was observed that chlorophyll *a* and ammonium are independent of nitrate and phosphate. The same relationship occurred between the pairs of salinity and chlorophyll *a*, silica and nitrate and salinity and phosphate. For the first principal plan AHB, NRB and SRB showed lower quality of representation (Table 2). This fact justifies the weak significance of these variables on the plan 1-2 (Fig. 4a). Particularly, in the plan 1-3 it is possible to observe that axis 3 is characterized by NRB (Fig. 4b and Table 2). Moreover, only in plan 1-4 and 3-4 (graphic representation not showed), AHB had a superior quality of representation (Table 2). These plans revealed that AHB increases with temperature and

chlorophyll *a*, but decreases with the increase of phosphate, nitrite and ammonium concentration.

Specific factorial axes that most contributed are summarized in Table 2 (as a function of the relative contribution of the factor to the element, both for the samples as for the environmental and biological variables). Shaded cells indicate the different factorial axes where the greatest relationship with the samples (Table 2a) and environmental and biological variables (Table 2b) were observed.

Table 2. Relative contributions of the factor to the element. The number refers of its maximum quality of representation on each axis (on a scale of 1000):

Table 2a) Samples

Samples	Axis 1	Axis 2	Axis 3
BFeb06	37	3	65
BMar06	607	169	5
BAbr06	3	90	137
BMay06	541	330	25
BJun06	152	19	115
BJul06	715	3	2
BAug06	468	77	73
BOct06	154	499	15
BNov06	73	188	3
BDec06	35	64	723
BJan07	493	84	186
BFeb07	821	97	0
CFeb06	42	304	190
CMar06	615	44	3
CAbr06	80	104	293
CMay06	500	29	97
CJun06	0	517	158
CJul06	439	404	92
CAug06	72	336	441
COct06	362	178	4
CNov06	31	16	108
CDec06	604	95	0
CJan07	520	77	136
CFeb07	831	54	6

Table 2b) Variables

Variables	Axis 1	Axis 2	Axis 3	Axis 4
Si	99	593	1	23
P-PO4	652	78	5	1

Table 2b). Variables (continuation)

Variables	Axis 1	Axis 2	Axis 3	Axis 4
N-NO2	**508**	4	395	3
N-NO3	**553**	33	1	74
N-NH4	57	**381**	16	367
Chl *a*	231	**420**	27	10
Sal	**435**	327	0	24
Temp	**840**	14	12	2
AHB	148	95	185	**403**
SRB	36	0	37	190
NRB	19	29	**752**	78

DISCUSSION

The Portuguese west coast is affected by weak westerly winds during winter, alternating with relatively strong northerly winds during spring and summer. Because of the north-south orientation of the coast, the increase and steadiness of these northerly along-shore winds support the occurrence of coastal upwelling that, in this area, has its maximum intensity from April to September (Fiúza 1983; Moita et al. 2003; Resende et al. 2007). The period between August and October (late summer–early autumn) is generally characterized by a relaxation of the upwelling events (Fiúza 1983). During the upwelling period, primary production is enhanced in shelf areas (Tenore et al. 1995). The fate of this new dissolved and particulate matter largely depends upon the recycling efficiency within the pelagic microbial food web (Barbosa et al. 2001). Heterotrophic bacteria play a pivotal role in the biogeochemical cycles of marine ecosystems and are the major consumers of dissolved organic carbon (Barbosa et al. 2001). Besides the heterotrophic AHB, NRB and SRB two other metabolic groups were targetted (ammonium-oxidising bacteria and nitrite-oxidising bacteria) by the MPN method in the present study, although unsuccessfully, possibly due to their low numbers. Also carbon and sulphate were not determined, which would have been interesting, as they are central to understanding the biogeochemical cycles in which AHB, NRB and SRB are involved. It would have also been important to determine total bacterial numbers, in addition to the performed viable counts by MPN, however this was not possible to accomplish. Culturable viable bacterial numbers are lower than total bacteria and each set of data allows different analysis of information. In this work we chose to study dynamics of the three main metabolic bacterial groups involved in biogeochemical cycles which would have not been possible if considering the total number of bacteria.

In this study, AHB showed a general increase during late spring indicating that the main factors regulating their abundance in the studied aquatic systems are temperature, organic and inorganic substrates. In fact, the increase in abundance of AHB and also NRB during the annual period was mainly associated with increasing temperature and chlorophyll *a* and, to a lesser degree, ammonium, as found by other authors (Ducklow & Shiah 1993; Hoch & Kirchman 1993; Cunha et al. 2000; Bacelar-Nicolau et al. 2003). Moreover, globally AHB numbers decrease with the increase of phosphate, nitrite and nitrate concentrations. The relation between AHB and chlorophyll *a* and ammonium suggests that phytoplankton is an important source of organic substrates for bacteria (e.g. Barbosa et al. 2001; Bacelar-Nicolau et al. 2003). The chlorophyll *a* content is widely used as a measure of the organic carbon available to support oxygen consuming processes (Jonas & Tuttle 1990), where degradation of phytoplankton-derived organic matter has an essential ecological role for carbon and nitrogen biogeochemistry in coastal ecosystems (Wetz et al. 2008). The negative correlation existing between AHB numbers and silica concentration also supports the importance of phytoplankton to AHB. Phosphorus (as phosphate) appeared as a limiting resource both to the bacterioplankton and phytoplankton communities, as found by other authors (Currie 1990; Rivkin & Anderson 1997; Bacelar-Nicolau et al. 2003). In fact this may also be due to the fact that N:P ratio was above the Redfield only ratio value during November 2006 at Berlenga and December 2006 at Canal, and slightly below that value in April 2006 and May 2006 (not shown). At both sites, when N:P ratio was slightly below or above the Redfield value there was an peak in ammonium concentration, and a peak of nitrate in Canal.

The quality of representation (Table 2) of NRB and SRB was very weak on the plan 1-2, similar in plan 1-3 for the SRB, and NRB showed the best representation in axis 3. However, the observed opposition between SRB and both NRB, and AHB in the two first plans may be attributed to competition for carbon, as suggested for the Mondego estuary by Bacelar-Nicolau et al. (2003). NRB dynamics, besides being suggestively related to increasing temperature and chlorophyll *a* (upwelling months) also appeared to a great extent correlated with increasing nitrite concentrations (particularly in December and August 2006), a direct product of microbial denitrification (e.g. Herbert 1999) and with increasing silica concentrations (indicating decreasing diatom phytoplankton). This latter relationship is not yet understood.

The present work points to the strong involvement of AHB, NRB and SRB in the biogeochemical cycling of carbon and nitrogen, through the water column. Even though sites were not oxygen depleted (data not shown) anaerobic metabolisms may occur in particular microniches such as marine snow particles (e.g. Azam 1998). Future objectives will be to investigate the different proportions of bacterial mortality attributable to viral lysis or bacterivory at different stages of a phytoplankton bloom and to characterize bacterioplankton functional assemblages that transform specific components of the coastal seawater dissolved organic carbon. Future work also aims to further identify and investigate some of the viable cultivable bacteria particularly in relation to their relevance for biogeochemical cycles at the studied sites.

ACKNOWLEDGEMENTS

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