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Diatoms and bacteria diversity: study of their relationships in the Southern Adriatic Sea

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Abstract

In the Southern Adriatic Sea (Italian coasts) bacterial and diatom diversity was studied over an annual cycle and the relationships between these two microbial components were analyzed. As regards the culturable heterotrophic bacteria, Aeromonas was the predominant genus among the Gram-negative bacteria. The presence of Enterobacteriaceae, Cytophaga, Pseudomonas, Acinetobacter and Photobacterium was also remarkable. Flexibacter, Moraxella, Chromobacterium, Flavobacterium and Vibrio were present at less than 5%. Of the 92 diatom species determined, 16 were the most abundant representing the 96% of this phytoplankton group.

The results obtained demonstrated that several bacterial genera were significantly related to the most abundant and representative diatoms (Chaetoceros spp., Pseudo-nitzschia pseudodelicatissima and Rhizosolenia spp.).

Keywords: Bacteria, Biodiversity, Diatoms, Southern Adriatic Sea.

Introduction

Biodiversity conservation in the marine environment has long attracted the attention of scientists. While in the past the focus was on macrorganisms, these days there is on an increasing awareness of the importance of conserving microbial diversity (ATLAS 1984; HAGSTROM *et al.*, 2000). Attempts to understand diversity at the microbial level are hindered by the fact that microbial communities typically consist of a large variety of autotrophic and heterotrophic microrganisms. The different groups of bacteria, with their metabolic preferences and capabilities are involved in combined actions for the degradation of organic matter, which is a heterogeneous mixture of carbon sources (AZAM, 1998). As a result of this variability of organic matter, growth conditions are not uniform for different groups of bacteria, therefore biotic and abiotic factors may promote successive changes in the community composition (PERNTHALER *et al.*, 1998). Concerning the phytoplankton communities, they are represented by diatoms, dinoflagellates, coccolithophorids and phytoflagellates. Diatoms show a high diversity and are responsible for typical phytoplankton blooms. These blooms create a heterogeneous environment of particulate, colloidal, and dissolved organic matter (FOGG *et al.*, 1965; LANCELOT, 1979). The variation of the organic matter regime is typically accompanied by pronounced changes in bacterial abundance. Hence, it is possible that bacterial dynamics during a phytoplankton bloom reflect a succession of specialized bacterial genera related to changes in the organic environment.

Traditionally, the diversity of marine bacteria has been assessed by identification by phenotypic tests and numerical taxonomic approaches, according collections of isolates from plating on nutrient media and enrichment. Common identified types mostly belonged to the γ-*Proteobacteria, Cytophaga - Flexibacter - Bacteriodes* (CFB) and Gram-positive groups. Recent studies by 16S rDNA approaches confirmed that apart for the existence of new phylotypes such as SAR 11 (GIOVANNONI *et al.*, 1990) and JAP504 (ROCHELLE *et al.*, 1994), unable to grow on solid media, the major phylogenetic groups of bacteria most abundant in marine habitats are the γ- *Proteobacteria, Cytophaga - Flexibacter - Bacteriodes* (CFB). These groups contain many aerobic or facultative heterotrophs that are relatively easy to culture (e.g. > 60% of Proteobacteria and > 40% of CFBs are culturable; HUGENHOLTS *et al.*, 1998). On the basis of these considerations we have focused our study on the biodiversity of culturable heterotrophic bacteria.

In the Southern Adriatic Sea diatoms are the most conspicuous component of the community (44.3%) and largely contribute to phytoplanktonic diversity, furthermore they are responsible for a vernal bloom (CAROPPO *et al.*, 1999). In this framework, diatoms and culturable heterotrophic bacterial diversity was studied over an annual cycle and the relationships between these two microbial components were analyzed.

Material and Methods

Monthly sampling was performed (from April 1996 to March 1997) at four stations located at 0.3 (station 1), 1.6 (station 2), 2.7

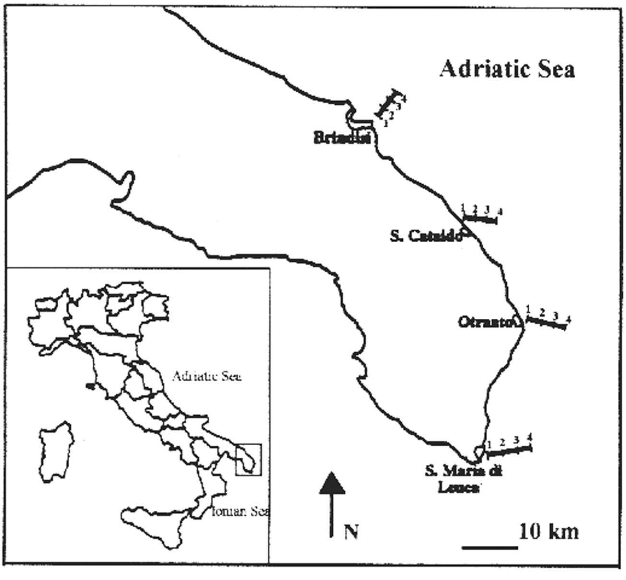


Fig. 1: Map of the Southern Adriatic Sea Italian coast showing the location of the sampling transects and stations.

(station 3) and 5.4 miles (station 4) from the coast, on four transects in the southern Adriatic Sea: Brindisi, S. Cataldo (Lecce), Otranto and S. M. Leuca (Fig. 1). Samples were taken at the surface and near the bottom using 5 - liter NISKIN bottles in sterile conditions. Diatom cell counts were obtained from 50-100 ml subsamples, preserved with Lugol‘s iodine solution, allowed to settle for 24-48 hours and examined under an inverted microscope (Labovert FS Leitz), using the method described by UTERMÖHL (1958). Identification of diatom species was based on: CUPP (1943), HASLE & SYVERTSEN (1996), HUSTEDT (1977) and RICARD (1987).

For laboratory bacteriological analyses, water samples were diluted with filtered (0.45μm) seawater. Serial dilutions of each sample were plated onto Marine agar (seeding with 0.1 ml). The plates were incubated at 22°C over 7 days and all these colonies were isolated, subcultured and identified by several morphological, biochemical and cultural methods (HOLT *et al.*, 1994).

The Shannon diversity index (H’, Shannon and Weaver, 1949) was calculated to describe the phytoplanktonic and bacterial community diversity. Statistical analyses were carried out using the pooled yearly data to evaluate relationships between diatom and bacteria occurrence: a correlation analysis was performed using diatom log transformed values. Significance was tested by the Student’s t-test. All the statistical analyses were carried out by using STATSOFT STATISTICA Version 6.0 (STATSOFT, 2001).

Results

Diatom diversity

Of the 92 diatom species determined, 13 were the most abundant representing 95.4% of this phytoplanktonic group (Table 1 and Fig. 2a). *Skeletonema costatum* was dominant, with high cell densities in winter, when reached the highest value of 2.1 cells x 10^6 l^{-1} corresponding to 92% of the phytoplankton community. Also *Chaetoceros* spp., mainly represented by *C. decipiens*, *C. convolutus*, *C. curvisetus* and *C. simplex*, contributed significantly to diatoms’ diversity. Furthermore, quite high values for *Cylindrotheca closterium*, *Pseudo-nitzschia* spp., *Proboscia alata, Rhizosolenia imbricata* and *R. styliformis* were recorded, mainly in the autumn-winter period.

The trend of the diversity index (H’) (Fig. 3), representing the evolution of diatom coenosis through the various succession stages, showed an average value typical of temperate coastal areas (H’ = 2.14 ± 1.10) (MARGALEF, 1978). In particular, the diversity index reached a rather homogeneous value during the sampling period, except for a decrease in the late spring and autumn-winter periods and a peak in April. In June, the Shannon index reached the average value of 1.38 ± 0.84 and the diatom population was dominated by *Pseudo-nitzschia delicatissima, Chaetoceros* sp. and *Thalassionema nitzschioides*. In January, the minimum value of 0.66 ± 0.33 was reached for the index, caused by the bloom of *Skeletonema costatum*.

Bacteria diversity

Regarding the culturable heterotrophic bacteria (Fig. 2b), *Aeromonas* was the predominant genus among Gram-negative bacteria. The presence of *Enterobacteriaceae, Cytophaga, Pseudomonas, Acinetobacter* and *Photobacterium* was also remarkable. *Flexibacter, Moraxella, Chromobacterium, Flavobacterium* and *Vibrio* were present at less than 5%.

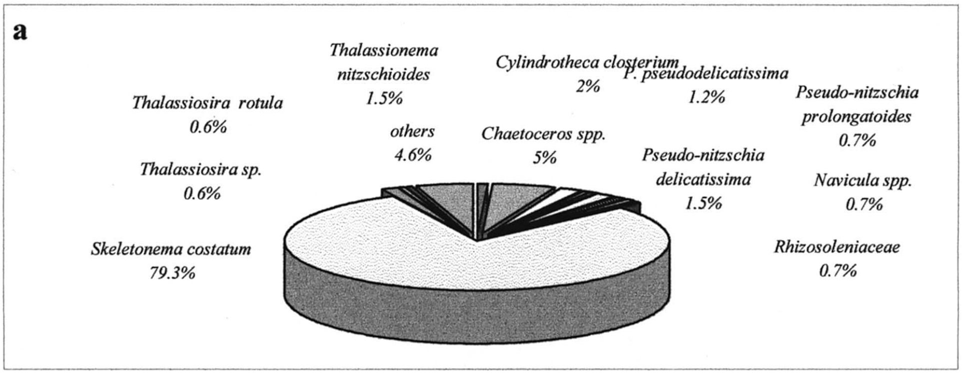
The diversity index (Fig. 3) did not show remarkable differences throughout the year with an average value of 2.13 ± 1.56.

Statistical analysis

The results of the correlation analysis demonstrate that the most abundant and representative diatoms (*Chaetoceros* spp., *Pseudo-nitzschia pseudodelicatissima* and *Rhizosolenia* spp.) were positively and negatively related to some bacterial genera (Table 2).

Table 1
List and presence of the most abundant diatoms in the Southern Adriatic Sea.

	min cells ml^{-1}	max cells ml^{-1}	presences n	presences %
Amphora spp.	0.3	2.9	51	0.1
Asterionellopsis glacialis	0.4	42.3	57	0.8
Bacteriastrum delicatulum	0.4	10.2	9	0.1
Bacteriastrum spp.	0.2	6.2	58	0.3
Cerataulina pelagica	0.4	5.8	51	0.3
Chaetoceros convolutus	0.7	11.0	12	0.1
Chaetoceros curvisetus	0.4	7.3	23	0.2
Chaetoceros decipiens	0.4	14.6	33	0.4
Chaetoceros lauderi	0.4	5.8	14	0.1
Chaetoceros simplex	0.4	7.3	26	0.1
Chaetoceros teres	2.2	7.3	4	0.1
Chaetoceros sp.	0.2	78.8	150	4.3
Cylindrotheca closterium	0.4	38.0	185	1.9
Cocconeis scutellum	0.4	7.3	45	0.1
Coscinodiscus sp.	0.3	3.7	88	0.3
Dactyliosolen blavyanus	0.4	11.7	102	0.5
Diploneis crabro	0.3	1.5	65	0.1
Diploneis sp.	0.2	1.8	26	0.1
Grammatophora spp.	0.4	2.9	24	0.1
Guinardia delicatula	0.3	3.7	40	0.1
Guinardia striata	0.4	3.7	64	0.2
Haslea wawrikae	0.4	1.1	34	0.1
Hemiaulus hauckii	0.3	5.8	64	0.2
Lauderia annulata	0.4	2.9	14	0.1
Leptocylindrus adriaticus	0.4	11.0	15	0.2
Leptocylindrus danicus	0.4	14.6	81	0.6
Leptocylindrus mediterraneus	0.4	2.9	15	0.1
Leptocylindrus minimus	0.4	3.7	40	0.1
Licmophora spp.	0.3	2.6	49	0.1
Melosira nummuloides	0.4	4.4	12	0.1
Navicula spp.	0.4	4.4	139	0.7
Pleurosigma elongatum	0.4	1.5	30	0.1
Pleurosigma spp.	0.3	3.7	75	0.2
Proboscia alata	0.2	18.3	109	0.4
Pseudo-nitzschia delicatissima	0.4	21.9	105	1.5
Pseudo-nitzschia prolongatoides	0.4	2.9	28	0.1
Pseudo-nitzschia pseudodelicatissima	0.3	27.7	98	1.2
Pseudo-nitzschia fraudolenta	0.7	23.4	22	0.7
Pseudosolenia calcar avis	0.2	1.5	32	0.1
Rhizosolenia imbricata	0.4	4.4	28	0.1
Rhizosolenia styliformis	0.4	2.2	20	0.1
Skeletonema costatum	0.4	2096.6	55	79.3
Thalassionema bacillare	0.4	20.4	52	0.5
Thalassionema frauenfeldii	0.4	10.2	39	0.2
Thalassionema nitzschioides	0.3	116.8	100	1.5
Thalassiosira rotula	0.2	14.6	58	0.6
Thalassiosira sp.	0.3	29.2	59	0.6
Thalassiothrix longissima	0.2	5.1	30	0.1
other diatoms				0.6



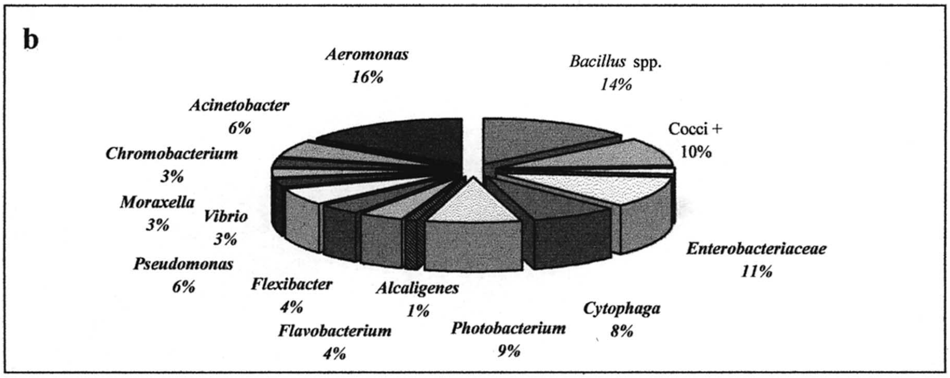


Fig. 2: Diatoms (a) and bacteria (b) annual average percentages of identification in the Southern Adriatic Sea.

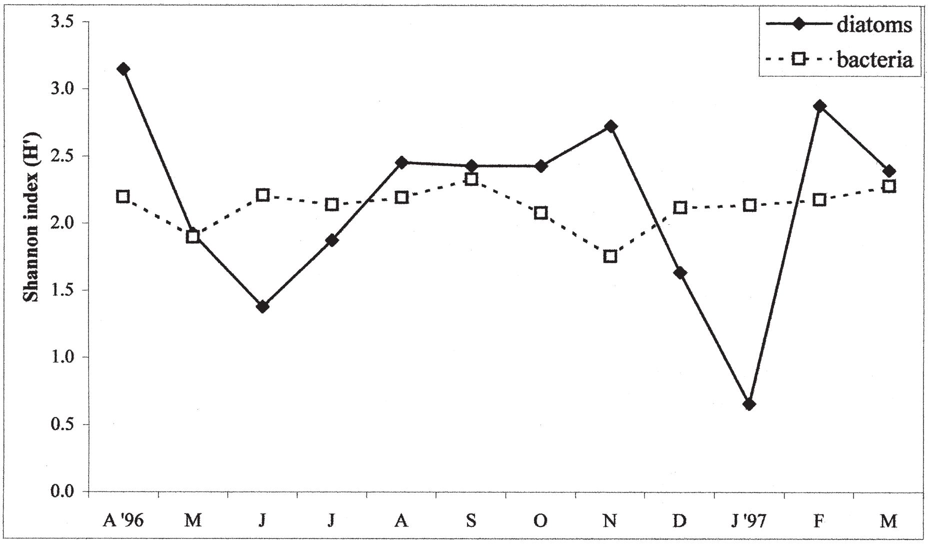


Fig. 3: Averaged values of the diatoms and bacteria Shannon-Weaver indexes.

Only some diatoms (*Asterionellopsis glacialis, Cylindrotheca closterium* and *Thalassiosira* sp.) were not correlated to bacteria densities. Usually, the relationship between the two planktonic components was inverse. The exceptions were *Navicula* spp. and *Thalassionema nitzschioides* which were positively correlated to bacterial genera like *Cytophaga, Flavobacterium, Vibrio* and *Xanthomonas.*

Table 2

Correlation coefficients between diatoms and bacteria in the Southern Adriatic Sea. N = 96. Significative values for p< 0,05 are signed in bold.

	Enterobacteriaceae	*Aeromonas*	*Cytophaga*	*Photobacterium* sp.	*Alcaligenes*	*Flavobacterium*	*Flexibacter*	*Pseudomonas*	*Vibrio*	*Moraxella*	*Chromobacterium*	*Acinetobacter*	*Xanthomonas*	Cocchi	Bacilli
Asterionellopsis glacialis	-0.09	-0.10	-0.17	-0.02	-0.10	-0.18	-0.19	-0.03	-0.11	-0.03	-0.02	0.01	-0.09	-0.11	-0.16
Chaetoceros spp.	**-0.23**	-0.21	-0.20	-0.22	**-0.25**	-0.19	**-0.29**	**-0.30**	0.06	**-0.27**	-0.08	-0.13	-0.11	-0.18	**-0.24**
Cylindrotheca closterium	-0.12	-0.17	-0.08	-0.13	-0.04	-0.05	-0.16	-0.17	-0.10	-0.15	-0.13	-0.06	0.03	-0.16	-0.12
Leptocylindrus danicus	-0.10	-0.12	-0.19	-0.10	-0.19	0.07	**-0.24**	-0.19	-0.00	-0.07	-0.06	-0.04	-0.10	-0.22	**-0.25**
Navicula spp.	-0.07	0.17	**-0.23**	0.05	0.18	-0.07	0.15	0.06	**0.27**	-0.05	-0.10	-0.10	**0.24**	0.02	0.05
Pseudo-nitzschia delicatissima	-0.19	-0.20	-0.09	-0.12	-0.09	-0.10	-0.20	-0.14	-0.07	-0.11	0.02	**-0.23**	-0.12	0.09	-0.1
P. pseudodelicatissima	-0.22	**-0.25**	-0.22	**-0.26**	-0.19	-0.19	**-0.25**	**-0.23**	-0.12	-0.22	-0.10	-0.19	-0.11	-0.21	**-0.27**
P. prolongatoides	-0.11	0.01	-0.15	0.00	-0.12	-0.21	-0.17	-0.08	**0.30**	-0.09	0.12	-0.05	-0.11	-0.04	-0.16
Rhizosoleniaceae	-0.16	**-0.24**	-0.04	-0.21	-0.10	-0.12	-0.11	**-0.33**	-0.05	**-0.26**	**-0.26**	-0.12	-0.03	-0.17	-0.08
Skeletonema costatum	-0.17	-0.13	-0.17	-0.11	-0.10	**-0.23**	-0.17	-0.10	0.02	-0.11	0.01	-0.12	-0.11	-0.12	-0.16
Thalassionema nitzschioides	0.03	-0.07	0.01	0.03	-0.09	0.26	-0.08	-0.09	-0.05	-0.08	-0.07	0.06	0.21	-0.07	-0.02
Thalassiosira rotula	-0.12	-0.10	**-0.24**	-0.03	-0.16	-0.19	-0.21	-0.09	0.09	-0.09	-0.01	-0.11	-0.14	-0.19	-0.21
Thalassiosira sp.	0.04	-0.04	0.09	-0.02	-0.10	-0.02	-0.01	-0.07	-0.11	-0.02	-0.00	0.07	-0.09	-0.07	-0.04

Discussion

The paramount importance of microbial life in marine environment attaches a key role to research on the diversity of the indigenous microrganisms. Nevertheless, very literature is available on the trophic interaction between diatoms and heterotrophic bacteria and their diversity. Furthermore, little is known about these aspects in the Southern Adriatic Sea. Thus, our work represents a preliminary contribute on developing this knowledge. The Southern Adriatic Sea can be considered as an oligotrophic ecosystem (CAROPPO *et al.*, 1999), characterized by rapid water exchange, due mainly to Adriatic Superficial Waters (A.S.W.) and Levantine Intermediate Waters (L.I.W.) (RUSSO & ARTEGIANI, 1996). In winter, the overlapping of these two water masses, are characterized by different trophic and thermohaline properties, is responsible for the seasonal enrichment of nutrients (FONDA UMANI *et al.*, 1992). In fact, the concentration of nutrient salts is usually low except for the winter months. Nitrites and nitrates reached their maximum levels, of 1.35 and 4.27 μg l^{-1} respectively, in January and February. Ammonia was present with values ranging between 0.01 and 0.84 μg l^{-1}. Phosphate showed average levels below 0.1 μg l^{-1}. Silicate concentrations ranged from 0.31 μg l^{-1} to 7.23 μg l^{-1}. Also biomass concentrations (in terms of chlorophyll *a*) proved to be very low, with values ranging between 0.05 and 1.25 μg Chl*a* l^{-1}. Phytoplankton densities showed specific oligotrophic water values and varied in a wide range from $9 \cdot 10^3$ to $2.9 \cdot 10^6$ cells l^{-1} (CAROPPO *et al.*, 1999).

In our study we have found species of diatoms typical of the Mediterranean Sea. The association *Chaetoceros-Rhizosolenia*, which is characteristic of the Levantine Basin (KIMOR 1983; KIMOR *et al.*, 1987), has been observed over the whole year as recorded also by VILICIC *et al.* (1995). Quite remarkable is the presence of the *Pseudo-nitzschia* spp., since potentially toxic algal species belong to that group (SUBBA RAO *et al.*, 1988; FRITZ *et al.*, 1992). A toxicity bioassay on *Pseudo-nitzschia delicatissima* and *P. pseudodelicatissima* opportunistically collected in the Southern Adriatic Sea proved negative (CAROPPO *et al.*, 2001). Furthermore, the winter diatom population was dominated by a typical species in the middle and higher Adriatic Sea (*Skeletonema costatum*, FONDA UMANI *et al.*, 1992). In January, the predominance of this species over other diatoms together with its high density is responsible for the Shannon Weaver index reduction. This small fast growing diatom, capable of exploiting available resources and characterized by high surface/volume ratios and reproductive rates, developed in these nutrient enriched winter waters and dominated the community. The diatom community reached its maturity in April, as shown by the high diversity index values. The population was characterized by the species typical of the last stage of the succession, such as *Chaetoceros* spp., *Leptocylindrus* spp., *Cylindrotheca closterium*, *Proboscia alata*, *Rhizosolenia* spp. and *Pseudo-nitzschia* spp.

Concerning culturable heterotrophic bacteria diversity, the genera found in this study have been frequently isolated from marine water, ocean sediments, seaweeds, surface and intestinal contents of marine animals (HOLT *et al.*, 1994). The most abundant genera identified were found also in other Mediterranean areas (CAVALLO *et al.*, 1999). Because of their different enzymatic activity, the bacterial genera could integrate for the complete degradation of the scarce resources of such an oligotrophic ecosystem. Such a trend remains throughout the year as confirmed by the low variability of the Shannon Weaver index observed for bacteria.

Consequent to algae photosynthesis, both excreted and secreted extracellular products (ECP) are released into the marine environment. Typical ECP include, but are not limited to, carbohydrated, nitrogen substances, organic acids, lipids, phosphatases, and other enzymes. The amount and nature of exuded products depends on several factors. The taxon of the algae dictates the compositions of compounds released. Environmental conditions and algal age may also significantly affect exudate release (BELL & SAKSHAUG, 1980; COLE, 1982).

The bacterioplankton may respond to different types of carbon products by altering their metabolic pathways and rates of growth and activity. Furthermore, the bacteria may be unable to use or withstand the compounds, resulting in their decline and eventual loss from the community (SERVAIS *et al.*, 1999; BERNARD *et al.*, 2000).

In our study, the diatoms *Chaetoceros* sp. and the Rhizosoleniaceae show only negative relationships with some bacterial genera, mainly *Pseudomonas* and *Moraxella*. In this case, we can hypothesize that diatoms could have an inhibitory effect on those culturable heterotrophic bacteria, for example in terms of competition for nutrients. In fact, if the concentration of nutrients, mainly N-compounds, is sufficiently low, as in oligotrophic ecosystems like the Southern Adriatic Sea, bacteria may show a net uptake of mineral nutrients from the water and so compete with diatoms (FENCHEL, 1988). Generally, phytoplankton have a greater 'capacity' to assimilate inorganic phosphorus (Pi) than bacteria (THINGSTAD *et al.*, 1993). This capacity means that, although in the short term (minutes to hours) Pi uptake rates by bacteria are higher than those by phytoplankton, more Pi can accumulate in phytoplankton than in bacteria over long time scales (SUTTLE *et al.*, 1988). Concentrations of Pi are also important in affecting bacterial uptake. That is, bacteria appear to outcompete phytoplankton at low but not high concentrations of Pi (THINGSTAD *et al.*, 1993; JACOBSEN *et al.*, 1994; KIRCHMAN, 1994; SCHLÜTER, 1998).

As regards N source, diatoms prevails over bacteria in the use of nitrates. KIRCHMAN *et al.* (1994) concluded that in the North Atlantic Ocean heterotrophic bacteria accounted for <10% of nitrate uptake. WHEELER & KIRCHMAN (1986) also observed low nitrate uptake by bacteria in the coastal waters of Georgia. These data indicate that nitrate uptake by heterotrophic bacteria is usually low.

Furthermore, some algae may produce bioactive secondary metabolites known as biotoxins. In addition to being toxic to some metazoans, such algae may be toxic or inhibitory to some bacteria. Allelopatic interactions are widespread among algal taxa and may play an important role in the structuring of planktonic communities. Such phenomena could explain the negative relationship observed in the Southern Adriatic Sea between *Pseudo-nitzschia pseudodelicatissima* (a potentially toxic diatom) and several bacterial genera (*Aeromonas, Photobacterium, Flexibacter, Pseudomonas* and *Bacillus* spp.).

Of interest also appears the positive (*Thalassionema nitzschioides – Flavobacterium* and *Pseudo-nitzschia prolongatoides – Vibrio*) and negative (*Leptocylindrus danicus – Flexibacter, Thalassiosira rotula – Cytophaga, Pseudo-nitzschia delicatissima – Acinetobacter and Skeletonema costatum – Flavobacterium*) relationship observed between some specific diatoms and bacterial genera. Some studies have shown that a significant positive relationship between *Skeletonema costatum* and *Flavobacterium* sp. exists (KOGURE *et al.*, 1979). In our study these two microrganisms were inversely related: we can thus suppose that *Flavobacterium* competes with *Skeletonema costatum* for nutrients. In such competition the diatom prevails and furnishes only secondary organic matter to the bacteria, which peaked just after the Skeletonema costatum increase. These results were confirmed by a laboratory experiment aimed at studying the coexistence under axenic conditions of these two planktonic components (CAVALLO *et al.*, 2002).

Finally, algae may produce secondary metabolites with a stimulative effects on specific organisms. In our study we observed a positive relationships between *Navicula* spp. and *Cytophaga, Vibrio* and *Xanthomonas.* We can thus suppose that such an interspecific relationship exists between these microbial components in the Southern Adriatic Sea.

On the basis of our results, we might conclude that further studies will be carried out to be evaluated the relationships between bacteria and diatoms in order to further characterize the Southern Adriatic Sea. In fact, the microbial assemblages, such as bacteria and phytoplankton, represent useful indicators of description in terms of the processes and functioning of marine environments.

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