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Research Article

# Bacterioplankton community composition in nearshore waters of the NW Black Sea during consecutive diatom and coccolithophorid blooms

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**Abstract.** In this study we analyzed the dynamics of bacterioplankton community composition during coastal Black Sea phytoplankton blooms using a combination of whole-cell fluorescence *in situ* hybridization with rRNA-targeted oligonucleotide probes and catalyzed reporter deposition (CARD-FISH). Bacterial and algal assemblages were sampled in eutrophic shelf surface waters in Constanta Bay between May and August 2004. While diatoms dominated the spring phytoplankton bloom, the late summer bloom (16-August) involved mainly coccolithophorids and dinoflagellates. The coccolithophorid *Emiliania huxleyi* was the numerically abundant phytoplankton species in August (~1.1 x $10^6$ cells/L). The composition of bacterioplanktonic communities was dominated by members of alpha-Proteobacteria (mainly the *Roseobacter* clade) and gamma-Proteobacteria, which together accounted for up to 31 % of total prokaryotic abundance during the summer phytoplankton bloom. Our results suggest that members of gamma-Proteobacteria and the *Roseobacter* clade are associated with *Emiliania huxleyi* blooms in the Black Sea.

**Key words.** Marine bacterioplankton; phytoplankton bloom; diversity; CARD-FISH; Black Sea.

## Introduction

Around 50 % of the phytoplankton production is channelled through bacterioplankton indicating a close link between phyto- and bacterioplankton activity. Several studies have demonstrated that heterotrophic bacterioplankton have the capability of transforming dimethylsulfoniopropionate (DMSP), a sulfur compound produced by some marine phytoplankton taxa including dinoflagellates, coccolithophorids and diatoms, to dimethylsulfide (DMS), the most abundant volatile sulfur compound in the surface oceans (Charlson et al., 1987; Kiene, 1990; Simó, 2001). Bacteria associated with phytoplankton blooms dominated by DMSP-producing algal species also play an important role in DMSP assimilation and

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transformation (Burkill et al., 2002). DMSP is metabolized by members of the *Roseobacter* clade of the alpha-Proteobacteria (González et al., 2000; Moran et al., 2003; Buchan et al., 2005). However, recent studies have shown that DMSP assimilation may be wide-spread among marine bacteria (e.g. *Silicibacter pomeroyi*, SAR11, *Pelagibacter ubique*), about a third of bacteria in surface ocean waters may participate in DMSP demethylation (e.g. Zubkov et al., 2002; Malmstrom et al., 2004; Grossart et al., 2005; Pinhassi et al., 2005; Howard et al., 2006).

The Black Sea is known as the world's largest anoxic sea with substantial river discharge and has been strongly affected by anthropogenic eutrophication over the last three decades (Murray et al., 1989; Mee, 1992; Oguz, 2005). The intense eutrophication of the Black Sea between 1970's –1990's induced significant bloom events, particularly at the north-western shelf (Bodeanu, 1993). Despite the reduction in the nutrient load since the mid 1990's, nutrient levels are still fairly high in the western coastal waters (Cociasu and Popa, 2004). During the summer season, massive flagellate-dominated blooms occur (Cokacar et al., 2004) and associated with them, high production of biogenic gases such as DMS (Amouroux et al., 2002). Although major blooms of the coccolithophorid *Emiliania huxleyi*, a key phytoplankton source of DMSP (Malin et al., 1993), occur regularly in the Black Sea (Cokacar et al., 2004; Besiktepe et al., 2004), information on the specific DMSP-degrading bacterioplankton community at the taxonomic level is not yet available for the Black Sea.

In the present study we relate the species composition of phytoplankton to the composition of bacterioplankton and follow the dynamics in abundance of some specific bacterioplankton groups. Thus, we aimed at determining the dynamics of the major taxa of bacterioplankton associated with DMSP-producing algal blooms. Unfortunately, we do not have DMSP and DMS concentration measurements to relate directly with phytoplankton and bacterioplankton taxonomic composition. However, the previous studies mentioned above indicate that *E. huxleyi* and dinoflagellate blooms lead to high DMSP and DMS concentrations in coastal surface waters of the Black Sea.

## Materials and methods

### Study sites and sample preparation

Surface water samples (from 0.5 m depth) were collected from a shallow coastal Black Sea site (Constanta Bay, 44°10'N, 28°41'E, 2.5 m total water depth, 500 m off shore) (Fig. 1).

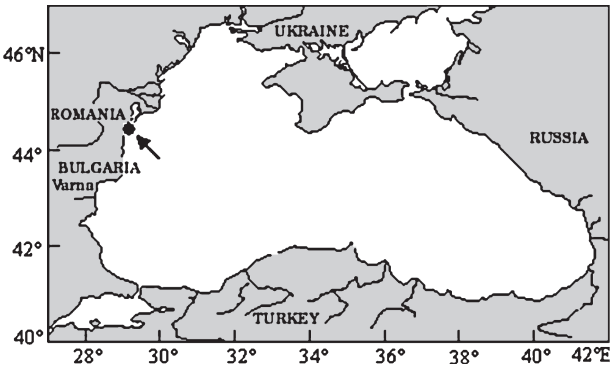


**Figure 1.** The sampling site at Constanta Bay located in the NW Black Sea (Romanian sector).

Duplicate seawater samples were taken twice a month between May and August 2004. Seawater was collected with Nansen bottles and 500 mL of this sample was fixed with formaldehyde (4% final concentration) and the particulate material including the phytoplankton allowed to settle in sedimentation chambers (Bodeanu, 2002). Phytoplankton were identified at the species level wherever possible and counted using an inverted microscope with 20× or 40× magnification. For bacterial community analysis, seawater samples were fixed with 35% paraformaldehyde at a final concentration of 2% (vol/vol) for 30 min to 1 h. A 5 mL water sample was filtered onto a polycarbonate filter (Millipore, 0.2 μm pore size, 25 mm filter diameter) with a cellulose nitrate supporting filter (Millipore 0.45 μm pore size, 25 mm filter diameter). Filters were air-dried and stored at –20 °C until further processing. Water temperature, salinity, dissolved oxygen, inorganic nitrogen, phosphate, silicate and chlorophyll *a* were also determined on separate samples collected concurrently with the samples for the enumeration of the prokaryotes using standard methods (Parsons et al., 1984).

### Catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH)

Total prokaryotic abundance was determined using DAPI (4',6'-diamidino-2-phenylindole) according to Porter and Feig (1980) and Bacteria were enumerated by catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH) by epifluorescence microscopy at 1250x magnification. CARD-FISH was performed using general and group specific oligonucleotide probes labeled with horseradish peroxidase (Eub338 targeting Bacteria, Alpha968 for alpha-Proteobacteria, Beta42A for beta-Proteobacteria, Gamma42A for gamma-Proteobacteria, Ros537 and Ros1029 probes for *Roseobacter* clade) and the hybridization conditions previously described by Pernthaler et al. (2002). After this CARD-FISH

procedure, cells were stained with a DAPI mix (5.5 part (v/v) Citifluor [Citifluor, Ltd., Leicester, U.K.], 1 part (v/v) Vectashield Vector LAboratires, Inc., Peterborough, U.K.], 0.5 parts (v/v) phosphate-buffered saline (PBS, 145 mM NaCl, 1.4 mM $NaH_2PO_4$, 4.3 mM $Na_2HPO_4$) with DAPI at a final concentration of 1 μg/ml). The microscope slides were enumerated under a Zeiss Axioplan 2 microscope equipped with a 100-W Hg lamp and appropriate filter sets for DAPI and Alexa488. At least 20 microscope fields were counted or at least 200 DAPI-stained cells.

## Results

### Environmental conditions

The water temperature was lowest (13.6°C) at the beginning of the sampling in May and steadily increased until an upwelling event occurred in July when surface water temperature dropped again to 13.6 C (Fig. 2). Maximum temperature was recorded in August with 23.6 °C. Salinity ranged from a minimum of 11.59 to a maximum of 17.86 which is the typical full strength marine water value for coastal marine Black Sea water. Dissolved oxygen concentrations varied between 257–308 μM. The mean nitrate, ammonium, orthophosphate and silicate concentrations were 8.8 μM (2.7–21.2 μM), 4.8 μM (2.1–10.1 μM), 0.05 μM (0.04–0.1 μM) and 10.1 μM (3.4–16.0 μM), respectively. The mean chlorophyll *a* concentration was 2.7 μg /L, and fluctuated during study period between 1.7 and 4.2 μg /L (Fig. 2).

### Algal numbers and phytoplankton community composition

The composition of the phytoplankton community is presented in Fig. 3. Total phytoplankton reached an abundance exceeding $1 \times 10^6$ cells /L and was generally higher in late summer than in May (Fig. 3). A diatom-dominated bloom developed in the middle of May reaching its maximum at the beginning of June. The number of phytoplankton cells declined from mid June towards the end of July when the phytoplankton community consisted almost entirely of diatoms. From May-July, diatoms dominated by *Chaetoceros curvisetus, Skeletonema costatum* and *Chaetoceros socialis*, comprised between 50 – 94 % of the total phytoplankton cell abundance (Fig. 4). In contrast, the late summer bloom (August) was dominated by dinoflagellates and other groups (primarily the coccolithophorid *E. huxleyi*) (Fig. 4).

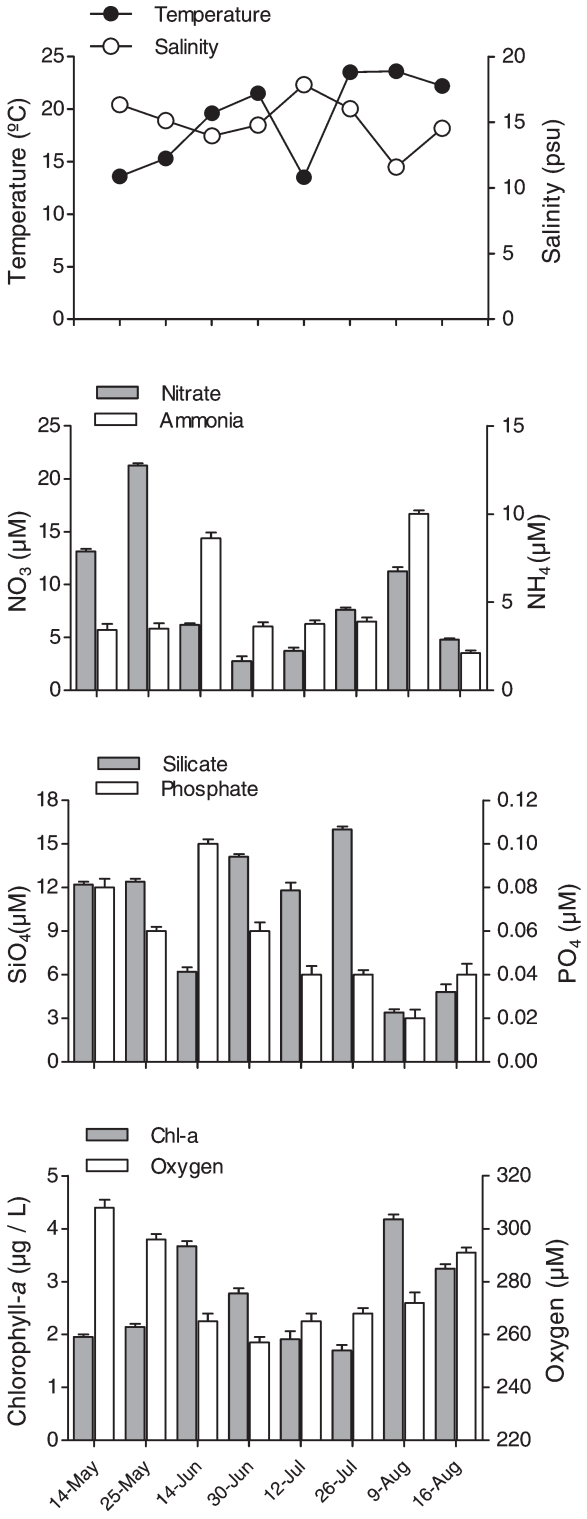


**Figure 2.** Chemical and physical parameters at 0.5 m sampling depth between May and August 2004. Bars represent the mean ± range of the duplicate samples.

### Bacterial abundance and community composition

Prokaryotic abundance enumerated after DAPI staining remained within the same order of magnitude throughout the investigation period, with two abundance peaks of about $9.5 \times 10^6$ cells/mL in June and August and low abundance in May ($5.7 \times 10^6$ cells /mL)

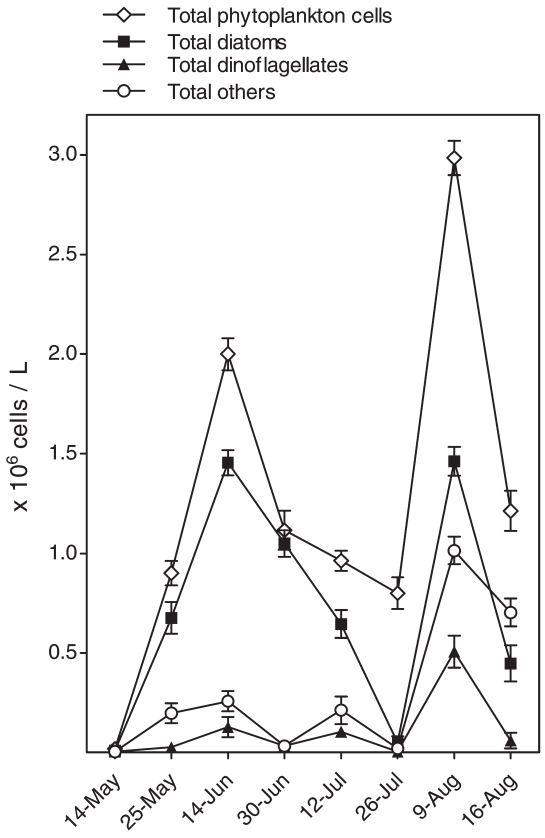


**Figure 3.** Temporal dynamics of mean total abundance (cells/L) of taxonomic groups and total phytoplankton. Bars represent the mean ± range (min-max) of the duplicate samples.

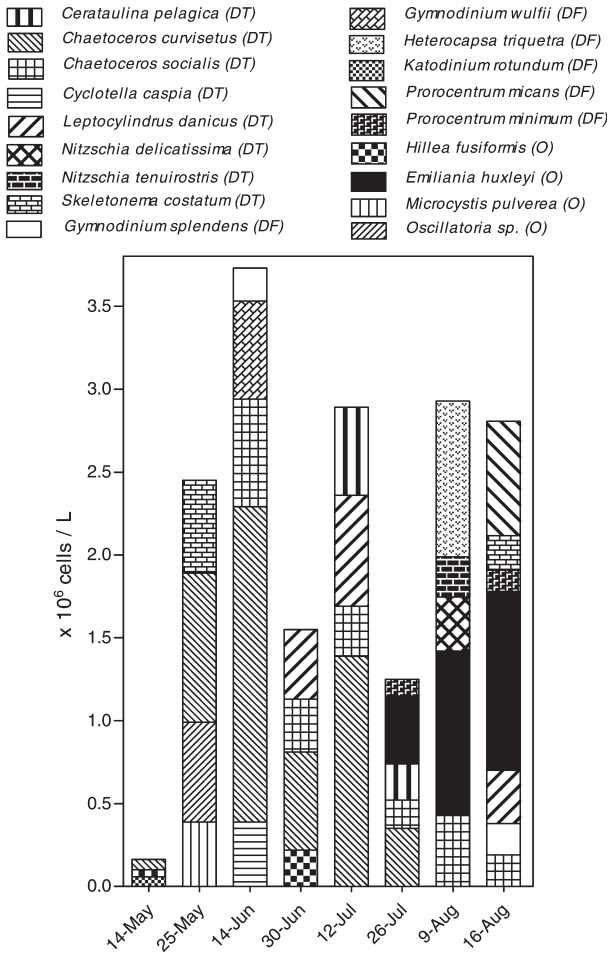


**Figure 4. M**aximum densities (cells/L) of predominating phytoplankton species of diatoms (DT), dinoflagellates (DF) and total 'others' (O including coccolithophorids, chrysophytes, chlorophytes, cyanophytes and small flagellates).

and July ($5.9 \times 10^6$ cells /mL) (Fig. 5A). CARD-FISH analysis indicated that all the targeted phylogenetic groups were present during the sampling period. The contribution of bacteria detected with the general bacterial probe (Eub338) to the total DAPI-stained cells ranged from 56.5 to 71% (Fig. 5B). Alpha-Proteobacteria (including the *Roseobacter* clade) was the largest identified fraction of the bacterial community at the sampling site, comprising between 9–27% of the total prokaryotic community. Between 2% and 12% of bacterial community were affiliated with the *Roseobacter* clade of the alpha-Proteobacteria. Gamma-Proteobacteria were the second most abundant group ranging between 8% and 23% of total prokaryotic abundance. The least abundant major phylogenetic group was the beta-Proteobacteria comprising between 3–10% of the total prokaryotic community (Fig. 5B). Bacterial community structure varied during the sampling period. In late spring (May) and June, the bacterial assemblage was dominated by members of alpha-Proteobacteria. Gamma-Proteobacteria were also an important component of bacterioplankton but less abundant than alpha-Proteobacteria (Fig. 5A). In midsummer, July and August, the abundance of alpha-Proteobacteria was highest ($1.3 \times 10^6$ to $2.3 \times 10^6$ cells/ mL) while gamma-Proteobacteria exibit the opposite trend (Fig. 5A). Beta-Proteobacteria were detected throughout the study period, but these bacteria never made up a substantial portion of community with abundances ranging from $2.48 \times 10^5$ to $9.39 \times 10^5$ cells/ mL (Fig. 5A).

## Discussion

Group-specific probe hybridization showed large contributions of alpha-Proteobacteria (mainly *Roseobacter*) and gamma-Proteobacteria to the late summer bacterial community when a flagellate-coccolithophorid bloom occurred (Fig. 5C). Our CARD-FISH results on dominant bacterioplankton groups during algal blooms are in agreement with the recent studies on bacterial community composition associated with

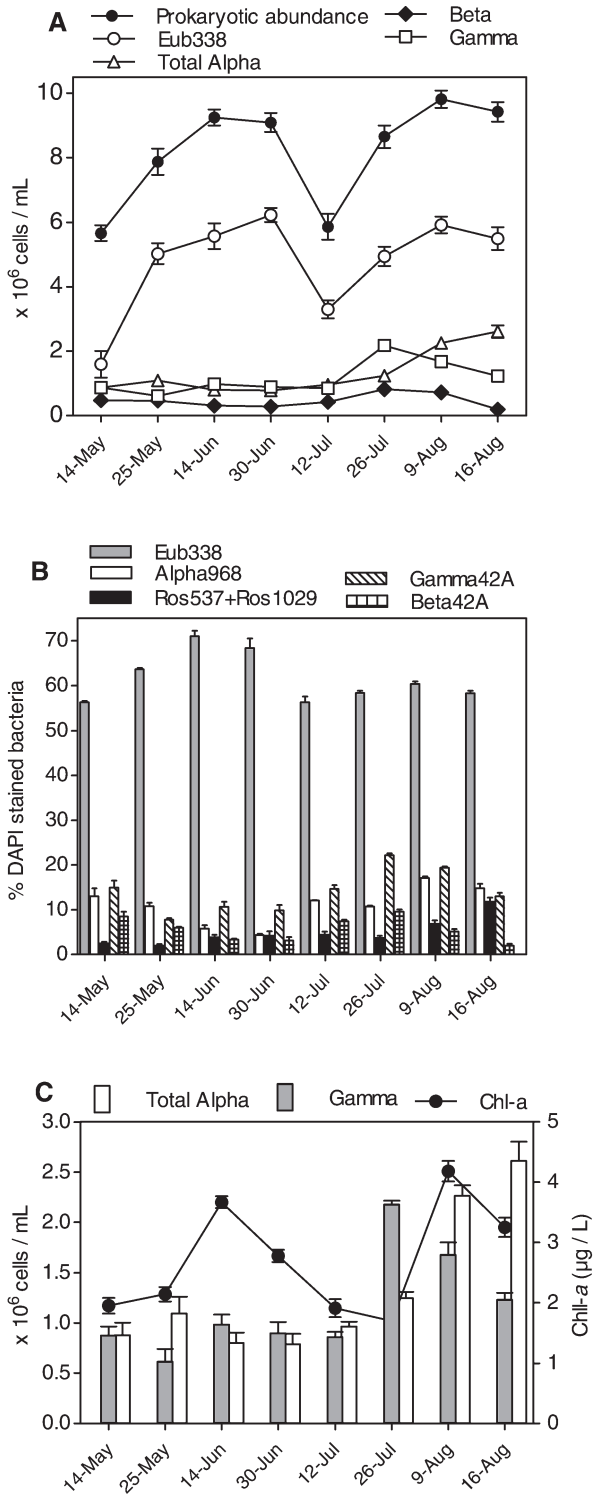


**Figure 5.** Temporal changes in (A) total prokaryotic abundance and the major bacterioplankton groups; (B) bacterioplankton composition as indicated by CARD-FISH analysis with probes for Bacteria (Eub338), alpha-Proteobacteria (Alpha968) and *Roseobacter* clade of alpha-Proteobacteria (Ros537, Ros1029), beta-Proteobacteria (Beta42A) and gamma-Proteobacteria (Gamma42A) shown as monthly mean percentage of DAPI stained bacteria; c) dynamics of gamma- and alpha-Proteobacteria and chlorophyll *a* concentrations at Constanta Bay. Bars represent the mean ± range (min-max) of the duplicate samples.

phytoplankton blooms (Gonzáles et al., 2000; Pinhassi et al., 2005; Zubkov et al., 2001). The contribution of alpha-Proteobacteria to total bacterial abundance was high during the late summer bloom. As expected, the *Roseobacter* group, a major and ubiquitous clade of marine alpha-Proteobacteria, some of which can utilize DMSP (Moran et al., 2003; Buchan et al., 2005), accounted for a significant percentage of the bacterial community during the summer bloom, with value ranging from 2% to 12% of total prokaryotic abundance (Fig. 5B). The higher temperature, nitrogen and phosphate concentrations and lower silicate level recorded in surface waters during late summer (August) led to an increase in dinoflagellates and coccolithophorids and a decrease in diatom abundance. The coccolithophorid *E. huxleyi,* one of the most prominent producers of DMSP (Malin and Kirst, 1997; Moran et al., 2003), reached its maximum abundance ($1.1 \times 10^6$ cells/L) in August, whereas its contribution to overall phytoplankton abundance was lower during the rest of year (Fig. 4). The high abundance of *E. huxleyi* coincided with the high abundance of alpha-Proteobacteria (mainly *Roseobacter*) observed in surface Black Sea waters during late summer 2004.

Apart from alpha-Proteobacteria, gamma-Proteobacteria were the second most abundant major bacterial group associated with the non-diatom algal bloom, while beta-Proteobacteria were least abundant (Fig. 5). Nevertheless, our results show that beta-Proteobacteria were consistently present during the flagellate-coccolithophorid bloom.

The bacterial groups that dominated our samples during the non-diatom bloom were also found to be components of the diatom-bloom surface samples (May-June). The comparison between bacterial communities during the seasonal phytoplankton blooms showed that alpha-Proteobacteria were also prominent during May to June but less abundant than in August 8–15% and 27% respectively. In contrast, gamma-Proteobacteria only accounted for 10% of the bacterioplankton community during the intense diatom bloom in June corresponding to the less prominent role of gamma-Proteobacteria in diatom-bacteria interactions reported by Grossart et al. (2005). Thus, following the seasonal phytoplankton blooms we found that the abundance and taxonomic composition of bacterioplankton are linked to the species composition of the phytoplankton community. Alpha-Proteobacteria (including *Roseobacter*) and gamma-Proteobacteria exhibited a greater abundance than the other groups when high DMSP-producing phytoplankton species dominated phytoplankton blooms.

In conclusion, bacterioplankton in Constanta Bay of the coastal Black Sea seem to follow the general

pattern of DMSP-producing phytoplankton-bacteria interactions previously observed in other temperate marine systems. We suggest that further detailed studies of bacterial and phytoplankton composition, alongside analysis of DMSP transformation and DMS production would be worthwhile in this shallow bay ecosystem of the Black Sea.

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