

## Volume, abundance and biomass of sediment bacteria in the eastern mid Adriatic Sea

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*Bacterial abundance, biomass, cell volume, and morphological diversity were studied in sediment samples collected from the coastal area of the eastern mid Adriatic Sea. Samples from the topmost 10 cm of sediment were taken monthly from January to December 2002. The number of bacteria varied from  $3.54 \times 10^9$  cells  $g^{-1}$  in July to  $8.08 \times 10^9$  cells  $g^{-1}$  in September. Biomass was lowest in May ( $78 \mu g C g^{-1}$ ) and highest in December ( $378 \mu g C g^{-1}$ ). The volume of the bacteria varied markedly with season, fluctuating from  $3.87 \times 10^8 \mu m^3 g^{-1}$  in May to  $3.12 \times 10^9 \mu m^3 g^{-1}$  in December. Cells with a volume smaller than  $0.1 \mu m^3$  accounted for the largest number of bacteria (62%) and cocci comprised 93% of these cells. More than 80% of all rod cells had a volume smaller than  $0.5 \mu m^3$ . The majority of filamentous cells (63%) belonged to the  $0.32 \mu m^3$  volume class. Rod-shaped cells were dominant in terms of volume (45-72%) and biomass (42-72%) and cocci in terms of abundance (52-70%). Cells smaller than  $0.5 \mu m^3$  made up 88-94% of the bacteria and 68-84% of the biomass in the summer and 6-44% of the bacteria and 21-81% biomass in the winter.*

**Key words:** bacteria biomass, bacteria abundance, bacteria volume, sediment

### INTRODUCTION

Sediment bacteria play an important role in benthic ecosystems (DEMING & BAROSS, 1993; COWAN et al., 1996; KUWAE & HOSOKAWA, 1999). They may comprise a large fraction of the total benthic biomass, contributing significantly to the turnover of organic matter within the sediment (BILLEN *et al.*, 1990; SCHALLENBERG & KALFF, 1993). Bacteria are basic components of the benthic food chain, therefore, they represent an important food resource for benthic fauna (PACE, 1988; BAK & NIEUWLAND, 1997; EPSTEIN, 1997). High production rates of benthic bacteria indicate that bacteria play an important role in

organic matter degradation (DUYL & KOP, 1994; EPSTEIN, 1997; KUWAE & HOSOKAWA, 1999).

The quantification of bacterial roles requires accurate measurement of parameters such as bacteria biomass, abundance, and production. Bacteria biomass is commonly derived by calculating biovolume (BRATBAK & DUNDAS, 1984; BRATBAK, 1985; FRY, 1988). A basic step in biomass estimations is measurement of the length and width of individual bacteria cells. After measuring their dimensions, cells can be categorized by morphological form (rods, cocci, filamentous, vibrios) and size class. Bacteria biomass can be estimated more accurately by taking the size distribution into account and

calculating the biomass of each size category individually.

Many authors have reported on the cell volumes of pelagic bacteria (e.g. NAGATA, 1986; NAGATA & WATANABE, 1990; ŠIMEK *et al.*, 1997) and that volumes significantly vary (ALBRIGHT & MCCRAE, 1987; SIME-NGANDO *et al.*, 1991; NAKANO & KAWABATA, 2000). However, studies on cell volumes of benthic bacteria are scarce (MOHAMMADI *et al.*, 1993; KUWAE & HOSOKAWA, 1999), especially those conducted on an annual basis. Factors that affect cell volume of bacteria in aquatic environments have not yet been fully investigated. Cell volumes seem to be mostly controlled by nutrient supply (PSENNER & SOMMARUGA, 1992) and predation (SHIKANO *et al.*, 1990; SHERR *et al.*, 1992; ŠIMEK *et al.*, 1997). Inorganic N and P limit growth and the cell size of bacteria (HOLMQUIST & KJELLEMBER, 1993). A lack of C can cause a decrease in cell size (MARDEN *et al.*, 1985; TROUSSELLIER *et al.*, 1997) and lead to the formation of ultramicrocells (HOLMQUIST & KJELLEMBER, 1993; TROUSSELLIER *et al.*, 1997). Heavy protozoan grazing may change the distribution of cell types, with a greater proportion of more resistant filamentous cell forms (SHIKANO *et al.*, 1990; JÜRGENS & GÜDE, 1994; ŠIMEK *et al.*, 1997).

Our aim was to study annual changes in cell volumes and morphological diversity of sediment bacteria. We determined the proportion

of different morphological forms and size classes within the total bacteria volume, abundance, and biomass. Cells were classified into forms and size classes that served as units for calculating total biovolume according to the distribution pattern.

Information about sediment bacteria in the Adriatic Sea is available only from studies carried out on its western coast (ALBERTELLI *et al.*, 1999; LUNA *et al.*, 2002). This study provides the first data on abundance, biomass, and biovolume of benthic bacteria along the eastern coast of the mid Adriatic.

## MATERIALS AND METHODS

Cores of undisturbed sediment were collected monthly from January to December 2002 (except in March and October) at one coastal station in Kaštela Bay in the middle Adriatic Sea (Fig. 1). Kaštela Bay is a semi-enclosed bay with a total area of 60 km<sup>2</sup> and average depth of 23 m. The bay receives great quantities of untreated or partially treated effluents from industrial and urban wastewater outlets. Despite the large quantities of nutrients that enter the bay, nutrient concentrations are very low due to intense biological activity (KUŠPILIĆ *et al.*, 1991). The estimated sedimentation rate in the area where the sediment samples were taken is 3.6 mm year<sup>-1</sup> (BOGNER, 1996).

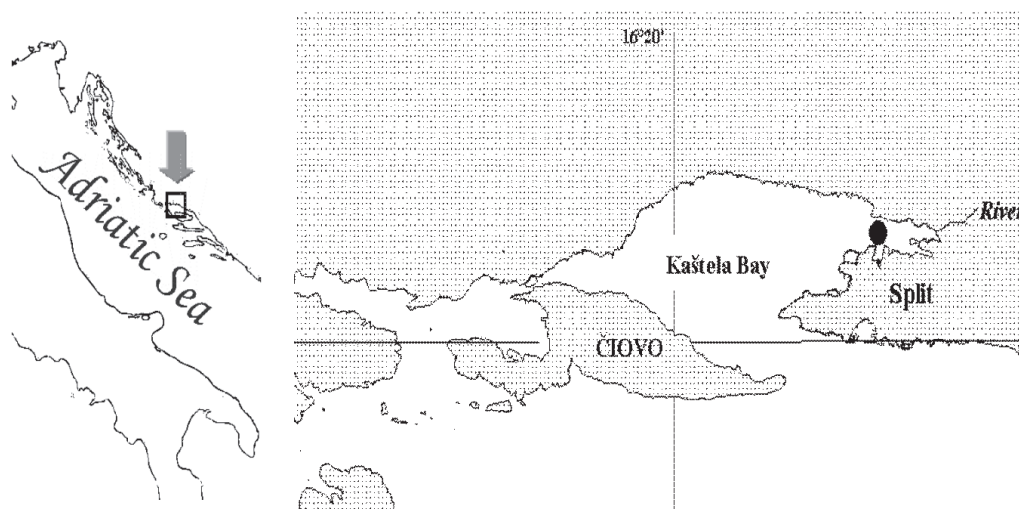


Fig. 1. Location of the sampling station in the Kaštela Bay (mid Adriatic Sea)

Samples were taken with a piston corer from the sediment surface to a depth of 10 cm. Immediately after sampling, sediment cores were vertically sectioned into ten 1 cm layers, transferred to sterile 15 ml polypropylene tubes, fixed with formaldehyde to a 4% final concentration, and refrigerated at 4°C until further processing (within one week). Prior to dislodgement treatment, samples were placed in a water bath filled with ice to prevent denaturation of nucleic acids during sonification. Bacteria were dislodged by processing with an Ultrasonic processor with a 3-mm miniprobe three times for 60 s, each time. Preliminary studies showed that this protocol results in a minimum number of destroyed cells. Samples were allowed to cool for 1 min between dispersion treatments. After sonification, the samples were vigorously vortexed, and sediment particles were allowed to settle for 5 s before dilution. The dilutions ( $\times$  2000-2666) were made with sterile prefiltered (0.2  $\mu$ m pores) distilled water.

Diluted subsamples were stained for 4 min with acridine orange, filtered on black Nucleopore polycarbonate filters (0.2  $\mu$ m pores; HOBBIÉ *et al.*, 1977) and examined under an epifluorescent microscope. At least 250 cells per sample were counted and measured using an eyepiece micrometer. Bacteria were classified into three morphological categories: cocci, rods, and filamentous bacteria (cells with a length more than five times greater than the width). Within each category cells were further classified by volume into micro classes using predefined sizes on an eyepiece grid. Cocci were classified into six, rods into 14, and filamentous bacteria into 13 micro classes (Table 1).

The volume of each cell was calculated from its length and width, assuming a spherical shape for cocci and a cylindrical shape for rods and filamentous cells, as follows:  $V = (\pi/4) \times W^2 \times (L - W/3)$ , where  $V$  is the cell volume,  $W$  is the cell width, and  $L$  is the cell length (BRATBAK, 1985). Cell volumes were converted to bacterial carbon content using the equation given by NORLAND (1993).

Biomass was calculated from the cell carbon content and number of cells. Biovolume was

Table 1. Size and volume classes of cocci, rods, and filamentous bacteria

	Dimension ( $\mu$ m)	Volume ( $\mu$ m <sup>3</sup> )
Cocci	0.257	0.009
	0.363	0.025
	0.513	0.071
	0.726	0.200
	1.026	0.565
	1.450	0.596
Rods	0.416 x 0.625	0.066
	0.588 x 0.625	0.116
	0.625 x 0.831	0.191
	0.416 x 1.25	0.151
	0.588 x 1.25	0.286
	0.831 x 1.25	0.528
	1.176 x 1.25	0.932
	1.25 x 1.663	1.529
	0.588 x 2.5	0.626
	0.831 x 2.5	1.206
	1.176 x 2.5	2.290
	1.663 x 2.5	4.226
	0.831 x 3.75	1.884
	1.176 x 3.75	3.647
Filamentous	1.176 x 5.0	5.005
	0.416 x 2.5	0.321
	0.416 x 3.75	0.491
	0.588 x 3.75	0.965
	0.416 x 5.0	0.661
	0.588 x 5.0	1.304
	0.831 x 5.0	2.562
	0.416 x 6.25	0.831
	0.588 x 6.25	1.644
	0.831 x 6.25	3.239
	1.176 x 6.25	6.363
	0.416 x 7.5	1.001
	0.588 x 7.5	1.983

calculated by multiplying the number of cells by the cell volume. Biovolume, abundance, and biomass were calculated separately for each morphological type and for each size category and then combined to calculate the total biovolume, abundance, and biomass of the sample.

Biovolume, abundance, and biomass were calculated for each 1-cm layer of sediment, then pooled to determine the values for the whole 10

cm sediment sample. Parameters were expressed in grams of sediment dry weight after drying for 48 h at 60°C.

Additional replicate cores were collected and vertically sectioned into ten 1-cm layers to determine the size distribution of sediment grains. Granulometric composition was determined by sieving the sediment through successive sieves with diameters of 4000 to 63  $\mu\text{m}$  and by using the standard hydrometric method for particles under 63  $\mu\text{m}$ . Granulometric parameters (mean grain size, sorting, skewness, and kurtosis) were calculated from cumulative curves (FOLK & WARD, 1957). For grain size nomenclature, a triangular diagram of FOLK (1954) was used.

Water samples were collected with 5 L-NISKIN sampling bottles for measurement of the temperature and salinity of the bottom layer. Temperature and salinity values were obtained by CTD probe (Idronaut).

## RESULTS

### Sediment grain size

The granulometric properties of the sediment samples are presented in Table 2. The sediment was predominantly composed of silt (Fig. 2). The fine-grained fraction (<63  $\mu\text{m}$ ) was relatively high and varied 74.5-87%. Surface sediments (0-1 cm) had a gravel content of 7% and consisted of gravelly mud, while samples from greater depths (1-9 cm) had a gravel content of less than 4.5% and were slightly gravelly sandy mud. All subsamples were poorly sorted.

### Bottom water temperature and salinity

The seasonal changes in bottom temperature and salinity are shown in Fig. 3. The temperature gradually increased from 12.42°C in January to 23.29°C in August. In September, the temperature

Table 2. Granulometric properties of sediment samples, by 1 cm layers

Depth (cm)	Mean grain size ( $\mu\text{m}$ )	Mean diameter ( $\mu\text{m}$ )	Sorting (%)	Skewness	Kurtosis
0-1	11.31	11.05	3.807	-0.081	1.256
1-2	10.55	9.62	3.439	-0.072	1.311
2-3	8.47	8.97	3.391	0.000	1.337
3-4	7.12	7.81	2.967	0.057	1.218
4-5	8.57	9.29	2.994	0.051	1.264
5-6	17.74	27.20	2.598	0.325	1.655
6-7	6.88	7.55	2.949	0.023	1.224
7-8	10.55	9.62	3.465	-0.102	1.363
8-9	8.87	8.67	3.430	-0.059	1.410
9-10	8.47	8.37	3.261	-0.041	1.316

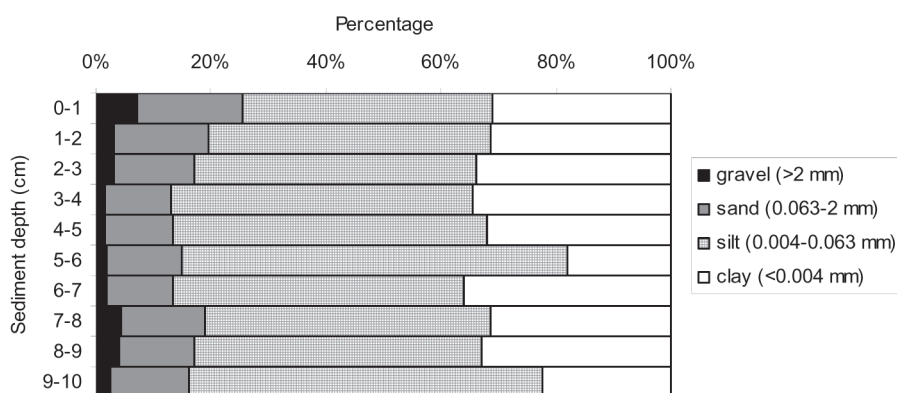


Fig. 2. Grain size analysis of sediment samples from depths of 0-10 cm in the Kaštela Bay during 2002

started to fall. During November and December, the average temperature was 16.9°C. Salinity fluctuated 37.15-38.32 psu. The lowest values were recorded in February and November as a result of higher precipitation. Salinity increased with the beginning of spring heating.

**Proportion of different bacterial morpho- types in bacteria counts (abundance)**

The number of bacteria varied from 3.54 to 8.08 x 10<sup>9</sup> cells g<sup>-1</sup>, with apparent seasonal pattern (Fig. 4). The highest values were recorded during summer months (6.74 ±8.6 x 10<sup>9</sup> cells g<sup>-1</sup> in June, and 8.81±10.1 x 10<sup>9</sup> cells g<sup>-1</sup> in July), and the lowest in September (3.54 ±4.9 x 10<sup>9</sup> cells g<sup>-1</sup>). Towards the colder months, bacterial numbers were found to increase again (from 4.01±5.5 x 10<sup>9</sup> cells g<sup>-1</sup> in November to 5.61± 6.6 x 10<sup>9</sup> cells g<sup>-1</sup> in January).

The proportion of each bacterial form in the total bacterial abundance is shown in Fig. 5. Except in December, cocci were the most numerous bacterial type, comprising 52-70% of the total abundance with no seasonal variation. Rods contributed 27-44% to the abundance, while filamentous bacteria usually constituted less than 15%. Rods and filamentous bacteria were most numerous during December, when they constituted 63% and 11% of the total abundance, respectively.

Among the cocci, 97.8-99.8% of the cells were smaller than 0.5 μm<sup>3</sup> (Fig. 6). A significant amount (60-79%) had cells with volumes of 0.01-0.5 μm<sup>3</sup>. Among rods, 50-96% of the cells were smaller than 0.5 μm<sup>3</sup>. Except in January and December, filamentous bacteria cells were smaller than 1 μm<sup>3</sup>. In January and December, there was a noticeable change in the morphological composition and size distribution

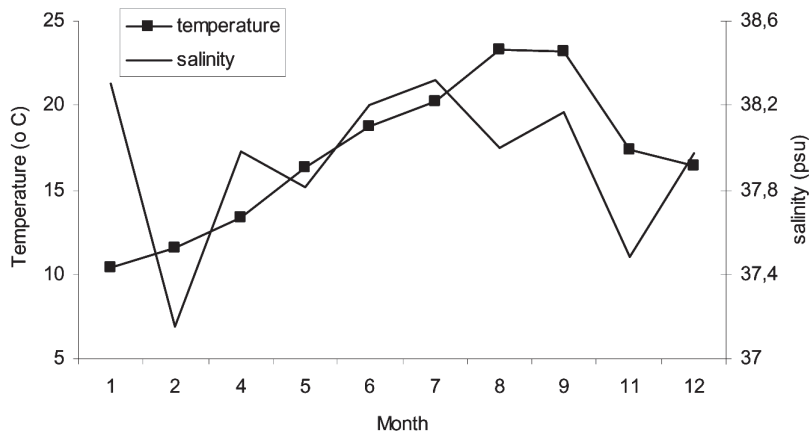


Fig. 3. Monthly temperature and salinity in the Kaštela Bay in 2002

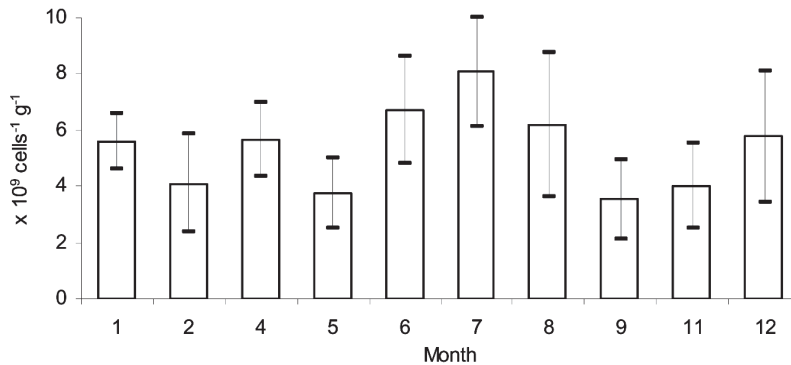


Fig. 4. Number of bacteria (mean±SD) in sediment samples

Fig. 5. Frequency of cocci, rods, and filamentous bacteria in the total abundance

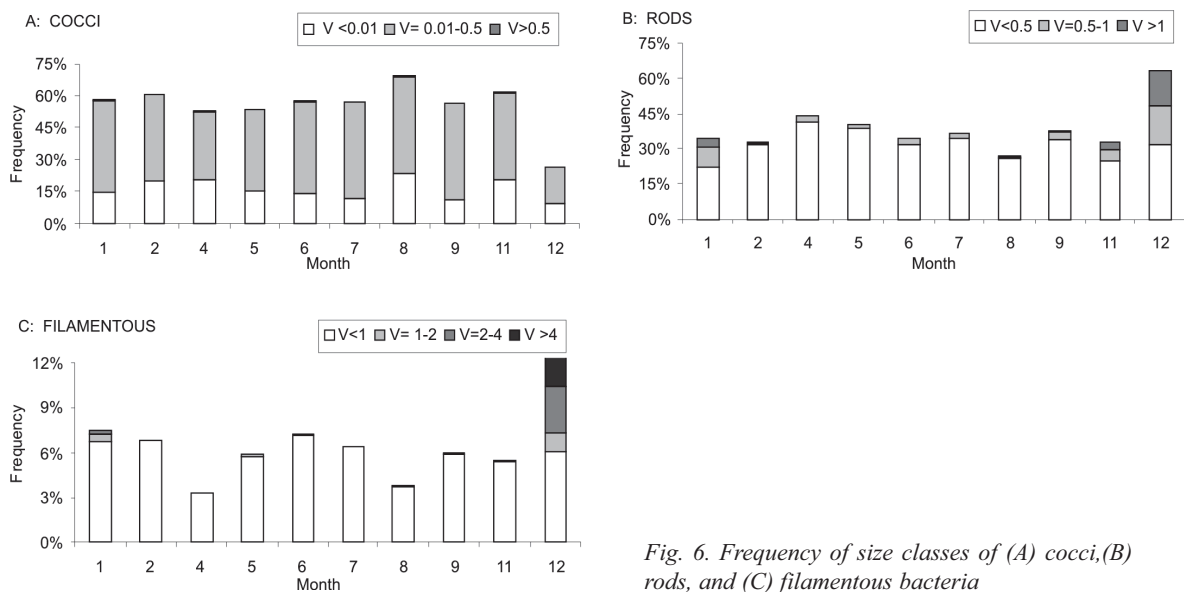
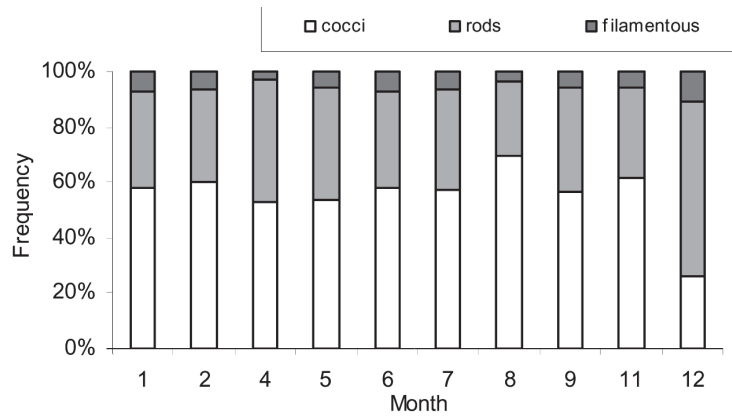


Fig. 6. Frequency of size classes of (A) cocci, (B) rods, and (C) filamentous bacteria

of the cells. During these months, the dominance of smaller cocci shifted towards larger rods and filamentous cells and the importance of rods larger than  $0.5 \mu\text{m}^3$  sharply increased (to 50% in December). Filamentous bacteria larger than  $1 \mu\text{m}^3$ , that represented no more than 7% of the filamentous cells during the rest of the year, comprised over 44% of this cell type during winter.

#### Proportion of different bacterial morphotypes in total volume of bacteria

Bacteria biovolume fluctuated  $3.87 \times 10^8$ - $3.12 \times 10^9 \mu\text{m}^3 \text{g}^{-1}$  (mean =  $9.12 \pm 8 \times 10^8$ ; Fig. 7). The biovolume rapidly decreased from  $1.17 \times 10^9 \mu\text{m}^3 \text{g}^{-1}$  in January to  $4.28 \times 10^8 \mu\text{m}^3 \text{g}^{-1}$  in

February and  $3.87 \times 10^8 \mu\text{m}^3 \text{g}^{-1}$  in May. In June and July, the biovolume rose to  $9.16 \times 10^8 \mu\text{m}^3 \text{g}^{-1}$  but, at the end of the summer, it dropped to values similar to those recorded during spring. In December, a sharp peak was recorded ( $3.12 \times 10^9 \mu\text{m}^3 \text{g}^{-1}$ ) as a result of the presence of large *Beggiatoa* sp. cells. The mean cell volume averaged  $0.168 \pm 0.141 \mu\text{m}^3$  and varied from  $0.086 \mu\text{m}^3$  in August to  $0.514 \mu\text{m}^3$  in December. Fluctuations of bacterial biovolume were higher during the colder part of the year (November-February; coefficient of variation; CV = 64.4%) than the warmer (April-October; CV = 37.6%).

The most numerous volume class was  $0.025 \mu\text{m}^3$  (21.35% of the total), followed by  $0.009 \mu\text{m}^3$  (15.30%; Fig. 8). Cells smaller than  $0.1 \mu\text{m}^3$  accounted for the greatest number of all

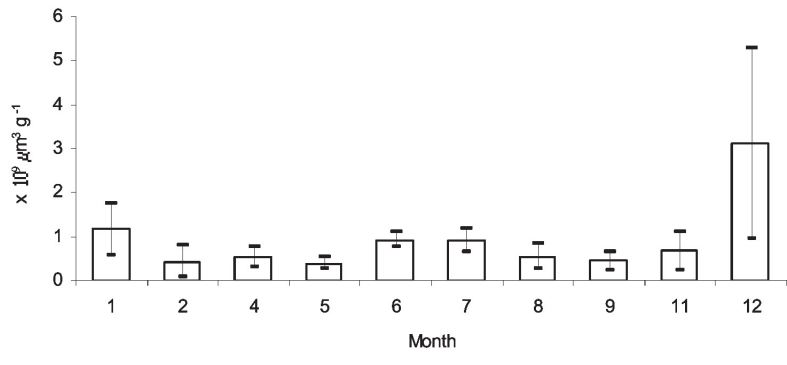


Fig. 7. Total volume of bacteria (mean ± SD) in the sediment samples

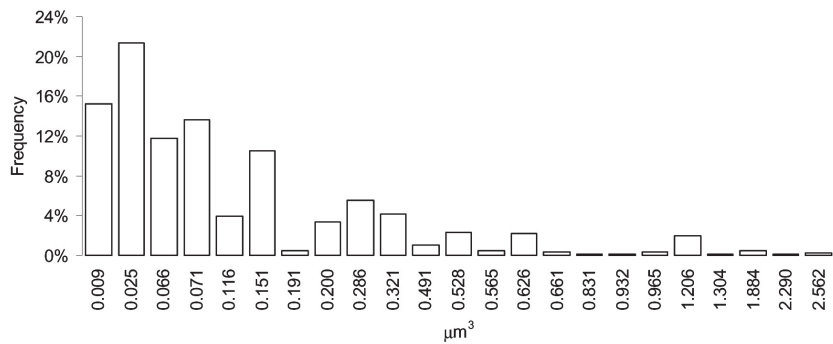


Fig. 8. Frequency of bacteria according to micro class of volume (μm<sup>3</sup>)

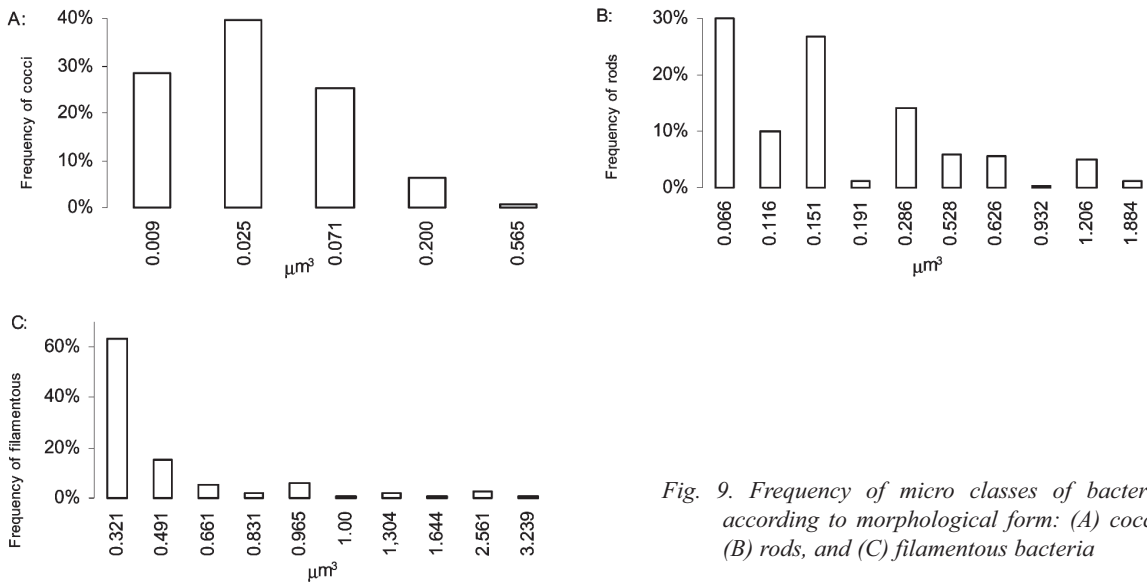


Fig. 9. Frequency of micro classes of bacteria according to morphological form: (A) cocci, (B) rods, and (C) filamentous bacteria

bacterial forms (62%). Only 38% of the bacteria had cells larger than 0.1 μm<sup>3</sup>.

Most cocci (93%) were smaller than 0.1 μm<sup>3</sup> (Fig. 9). Most rods belonged to the 0.066 μm<sup>3</sup> (30%) and 0.151 μm<sup>3</sup> (27%) classes and over 80% were smaller than 0.5 μm<sup>3</sup>. Most filamentous bacteria (63.2%) belonged to the

0.321 μm<sup>3</sup> volume class and only 7.3% were larger than 1 μm<sup>3</sup>.

Rods accounted for the greatest proportion (45-72%) of the total bacterial volume throughout the year (Fig. 10). Cocci and filamentous forms fluctuated 2-36% and 12-26%, respectively. The relative importance of cocci increased to more

than 30% during late spring and summer, but decreased during winter to 2% in December and less than 13% in January.

Cocci with volumes of 0.01-0.5  $\mu\text{m}^3$  accounted for the greatest proportion of the total cocci volume throughout the year (Fig. 11). The contribution of cells smaller than 0.01  $\mu\text{m}^3$  and larger than 0.5  $\mu\text{m}^3$  was insignificant, as they represented only 0.4-6% of the total cocci volume. The relative importance of rods increased during the colder months (representing up to 72% of the total volume), when larger rods ( $>0.5 \mu\text{m}^3$ ) prevailed, accounting for 40-62% of the total rod volume. The importance of smaller rods ( $<0.5 \mu\text{m}^3$ ) rose during the warmer part of the year (April-September), when they comprised 31.6-54.3% of the total rod volume. Filamentous forms represented a minor part of

the total bacteria volume (12-26.6%). Except in December, they were represented by cells smaller than 1  $\mu\text{m}^3$ , accounting for 76-100% of this cell type. In December, the size distribution of filamentous cells shifted with larger cells ( $>1 \mu\text{m}^3$ ) accounting for 76% of the filamentous volume.

**Proportion of different bacterial morphotypes in bacteria biomass**

Biomass ranged 78-378  $\mu\text{g C g}^{-1}$  (average  $149 \pm 0.9 \mu\text{g C g}^{-1}$ ; Fig. 12), with the highest value in December. In January, a value dropped to 182  $\mu\text{g C g}^{-1}$ . Biomass began to drop in the beginning of spring and was lowest in May. The biomass rose in summer (169-179  $\mu\text{g C g}^{-1}$ ) but dropped again in autumn (84-112  $\mu\text{g C g}^{-1}$ ).

Fig. 10. Proportions of cocci, rods, and filamentous bacteria in total cell volume, by month

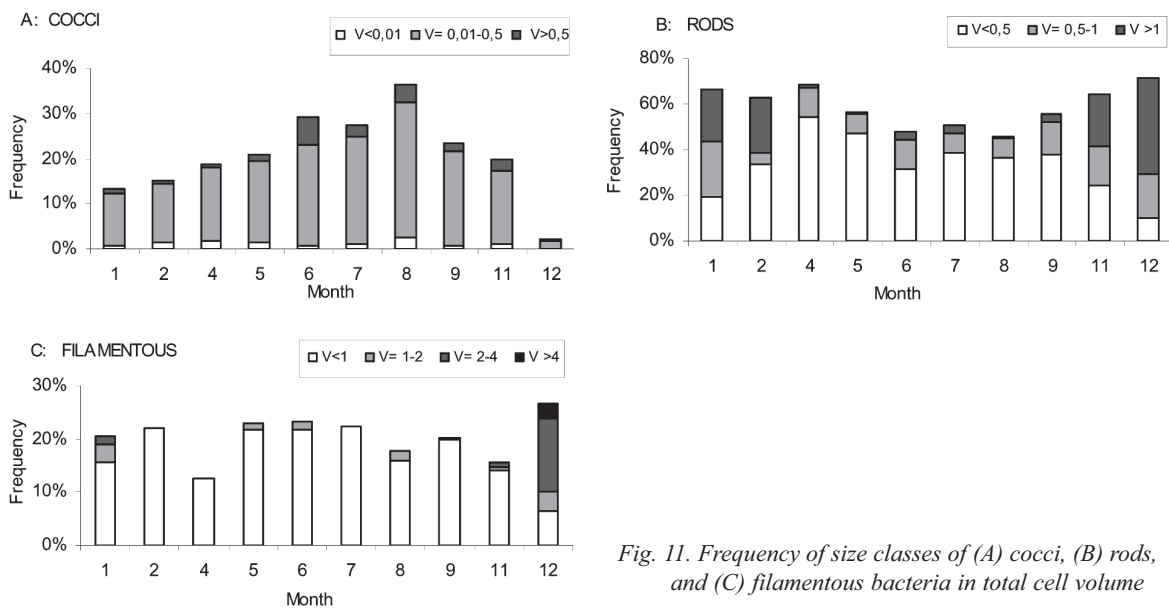
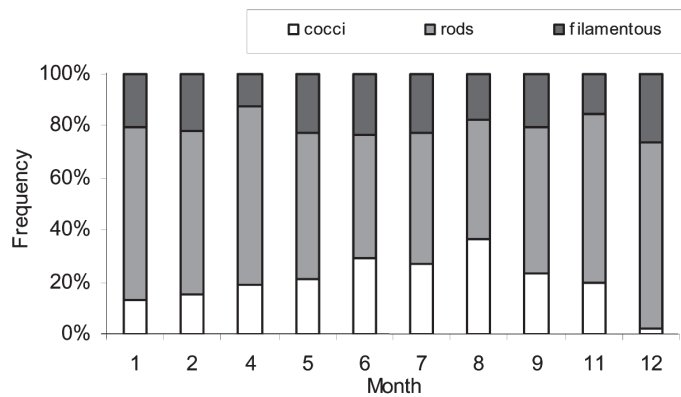


Fig. 11. Frequency of size classes of (A) cocci, (B) rods, and (C) filamentous bacteria in total cell volume



The proportion of each morphotype in the total biomass followed a similar pattern as in volume (Fig. 13). Rod shaped cells dominated throughout the year except in summer, when they accounted for 53.4-71.5% of the total biomass. Cocci comprised 4-45% of the biomass, with increasing importance in the warmer months.

Filamentous bacteria was not seasonal and varied 10-24%.

The majority of cocci biomass (76-89%) consisted of cells of 0.01-0.5  $\mu\text{m}^3$ , while smaller and larger cells represented no more than 16% (Fig. 14). Among rods, large cells ( $>1\mu\text{m}^3$ ) dominated during winter (15-36%) and small

Fig. 12. Bacterial biomass (mean $\pm$ SD)

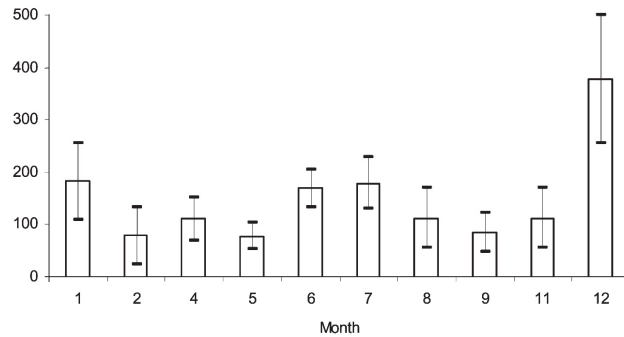
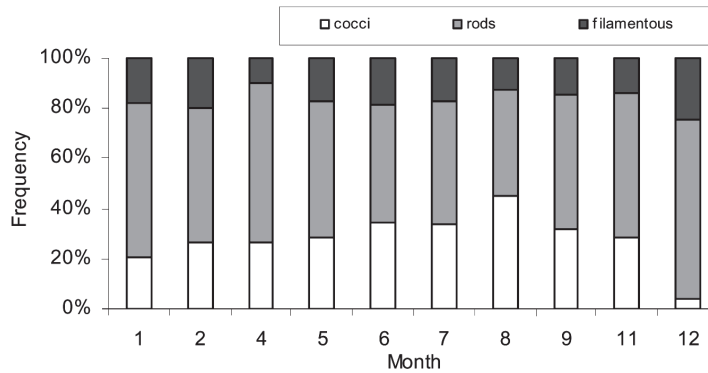
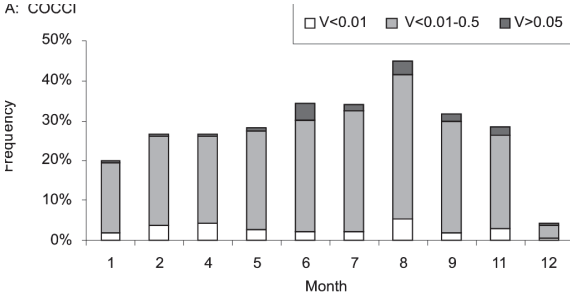


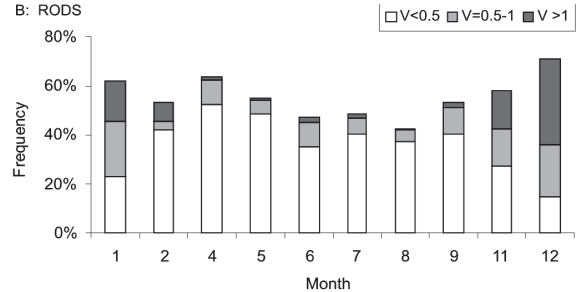
Fig. 13. Contribution of bacterial morphotypes in total biomass



A: COCCI



B: RODS



C: FILAMENTOUS

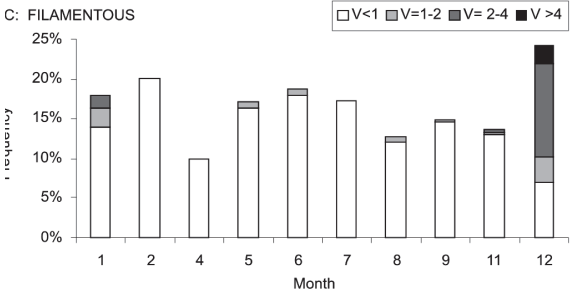


Fig. 14. Frequency of size classes of (A) cocci, (B) rods, and (C) filamentous bacteria according to biomass

cells ( $<0.5 \mu\text{m}^3$ ) the rest of the year (76-89%). The filamentous bacteria biomass was generally dominated by cells smaller than  $2 \mu\text{m}^3$  (42-100%), except during January and December when larger cells ( $>2 \mu\text{m}^3$ ), that were absent in samples from other months, comprised 2% and 58% of the filamentous biomass.

## DISCUSSION

This study provides the first results on number, volume and biomass of sediment bacteria along the eastern coast of the mid Adriatic Sea. Bacteria density and biomass were one order of magnitude higher than values reported for coastal areas of the Mediterranean Sea (DANOVARO *et al.*, 2000; MIRTO *et al.*, 2000) but within the range reported for more productive areas such as fish farms (VEZZULI *et al.*, 2002; CHELOSSI *et al.*, 2003; GOUGH & STAHL, 2003). The mean volume ranged  $0.086\text{-}0.514 \mu\text{m}^3$ , similar to values noted in other studies (KIRSCHNER & VELIMIROV, 1999; BUESING & GESSNER, 2002), however, individual bacteria cells fluctuated  $0.009\text{-}6.363 \mu\text{m}^3$ , a much wider range than noted previously (COLE *et al.*, 1993; MOHAMMADI *et al.*, 1993; BAK & NIEUWLAND, 1997).

Our study provides clear evidence of annual patterns of bacteria volume. Contrary to bacteria in pelagic environments (ALBRIGHT & MCCRAE, 1987; SERVAIS, 1989; KIRSCHNER & VELIMIROV, 1999), bacteria in coastal sediments of the Adriatic are larger during winter than in spring and autumn. Throughout the year, most bacteria were small cocci and rod-shaped cells with volumes under  $0.5 \mu\text{m}^3$ , representing 58% of the total number of bacteria in winter and 94% during the rest of the year. The higher proportion of small cells during the non-winter period was reflected in their high proportion of the bacteria biomass (80-90%). During winter, however, the community size structure changed and, although cells smaller than  $0.5 \mu\text{m}^3$  still represented 61-89% of the number of bacteria, their proportion of the biomass was minor (22-53%). Instead, less numerous (only 39% of the bacteria) but larger rods and filamentous

bacteria ( $>0.5 \mu\text{m}^3$ ) accounted for up to 77% of the bacterial biomass.

The size of bacteria cells depends on two processes: growth and frequency of cell division. The balance between these processes depends on environmental conditions. The quality and quantity of available organic matter, the temperature, and variations in biotic parameters (e.g., flagellate and ciliate grazing, primary production, organic matter sedimentation) can provoke changes in cell morphology (ALBRIGHT & MCCRAE, 1987; CARON *et al.*, 1988; NAGATA & WATANABE, 1990; SHIKANO *et al.*, 1990; SIME-NGANDO *et al.*, 1991; JUGNIA *et al.*, 1998).

Size-selective grazing seems to be one of the main parameters that determines the size-distribution of bacteria populations in pelagic environments where bacteria obtain refuge from grazing by reducing their size and developing complex morphological forms (JÜRGENS & GÜDE, 1994; PERNTHALER *et al.*, 1996; ŠIMEK *et al.*, 1997; HAHN & HÖFLE, 1999). In more productive environments such as coastal waters, however, the development of long filamentous or aggregated forms seems to be a common way to avoid grazing (GÜDE, 1982, 1989; JÜRGENS *et al.*, 1994; HAHN *et al.*, 1999). Previous studies showed that under strong grazing pressure, the contribution of resistant filamentous cells to the total bacteria biomass can reach 20-80% (SIME-NGANDO *et al.*, 1991; JÜRGENS & STOLPE, 1995; SOMMARUGA & PSENNER, 1995; PERNTHALER *et al.*, 1996). This study showed that filamentous bacteria accounted for a greater part of the bacteria biomass in the eastern Adriatic Sea during winter.

As opposed to studies conducted in the water column, most studies that measure grazing on bacteria in sediments revealed that, for a large part of the year, grazing does not have a considerable impact on bacterial dynamics (EPSTEIN & SHIARIS, 1992; STARINK *et al.*, 1996; HAMELS *et al.*, 2001). One possible explanation for the minor effect of grazing on sediment bacteria was proposed by HAMELS *et al.* (2001). They speculated that, in pelagic environments, increased productivity of a system is accompanied by increased bacteria and flagellate populations while in

sediments, the main parameter that determines the distribution of bacteria and flagellates is the size of the sediment grains: smaller grains are accompanied by lower bacteria production and higher flagellate biomass. Therefore, flagellates have greater control over bacterial dynamics only when bacterial production is minimal.

Besides adaptation to grazing, other causes of the elongation of bacteria include a high growth rate due to enhanced availability of substrates (HAHN & HÖFLE, 1998; HAHN *et al.*, 1999; JÜRGENS & SALA, 2000; MATZ & JURGENS, 2003), a shift in species composition of the bacteria assemblage, and sedimentation from the water column (BOSTRÖM *et al.*, 1989; PFANNKUCHE, 1993).

In this study, temperature was not a dominant factor in determining cell size as there was no significant correlation between mean bacteria volume and temperature of the bottom layer ( $r = -0.201$ ;  $P = 0.58$ ). This finding agrees with ALBRIGHT & McCRAE (1987) and DUYL & KOP (1994) who also failed to find a direct relationship between temperature and bacteria cell volume. These authors suggested that temperature acts simultaneously with other factors (organic nutrient quality and quantity, phytoplankton activity, flagellate grazing) in controlling bacteria dynamics.

Despite the non-existence of a significant relationship between temperature and average bacteria cell volume, our results show that warming was associated with an increase of

bacterial abundance in the sediment, as in the water column (ŠOLIĆ *et al.*, 2001). During summer, the water column is characterized by enhanced bacteria production, primary production, and chlorophyll *a* concentration (NINČEVIĆ, pers. comm.), as well as higher rates of sedimentation (UJEVIĆ, 2002) which could have stimulated the bacteria populations in the sediment to increase in number. Besides autochthonous organic matter derived from phytoplankton, high inputs of industrial and municipal waste enter the bay each year (BARIĆ, 1992), probably also acting as important food sources for benthic bacteria. Therefore, a correlation between seasonal sedimentation of organic matter and microbial processes is to be expected.

## CONCLUSIONS

Our study yielded basic information about bacterial communities in coastal sediments of the eastern Adriatic Sea. These first results show that bacteria abundance, biomass, and volume fluctuate according to seasonal patterns, as does the morphological composition of the bacteria assemblage. However, factors that provoke these changes have not yet been investigated. To understand the role of sediment bacteria along the Adriatic coast, it is important to study changes in composition of the organic matter in the sediment, abundance of bacterivorous protozoa, and production of benthic bacteria. Future investigations will focus on these goals.

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## Volumen, abundancija i biomasa bakterija u sedimentu srednjeg Jadrana

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### SAŽETAK

Promjene bakterijskog volumena, abundancije i biomase, kao i morfološka raznolikost bakterijskih stanica proučavane su u uzorcima sedimenta sakupljenih u obalnom području srednjeg Jadrana. Uzorci gornjih 10 cm sedimenta sakupljeni su mjesečno od siječnja do prosinca 2002. godine. Broj bakterija varirao je od  $3.54 \times 10^9$  st  $g^{-1}$  u srpnju do  $8.08 \times 10^9$  st  $g^{-1}$  u rujnu. Najniža vrijednost bakterijske biomase zabilježena je u svibnju ( $78 \mu g C g^{-1}$ ), a najviša u prosincu ( $378 \mu g C g^{-1}$ ). Vrijednosti volumena bakterijskih stanica pokazivale su sezonske promjene, varirajući između  $3.87 \times 10^8 \mu m^3 g^{-1}$  u svibnju i  $3.12 \times 10^9 \mu m^3 g^{-1}$  u prosincu. Stanice volumena manjeg od  $0.1 \mu m^3$  obuhvaćale su 62% svih bakterija, dok su stanice volumena većeg od  $0.1 \mu m^3$  činile svega 38% bakterijskog broja. Kokoidne stanice volumena manjeg od  $0.1 \mu m^3$  obuhvaćale su 93% svih koka. Štapićaste stanice volumena manjeg od  $0.5 \mu m^3$  činile su više od 80% svih štapićastih stanica. Većina nitastih stanica (63%) je pripadala veličinskoj kategoriji volumena  $0.32 \mu m^3$ . Štapićasti oblik stanica u ukupnom je volumenu sudjelovao sa 45-72%, a u ukupnoj biomasu sa 42-72%. U bakterijskom broju kokoidne stanice bile su najbrojnije (52-70%). Tijekom ljeta, stanice manje od  $0.5 \mu m^3$  sačinjavale su 88-94% broja svih stanica, te 68-84% biomase stanica. Zimske mjesece obilježavale su stanice veće od  $0.5 \mu m^3$  koje su sačinjavale 6-44% broja svih stanica, te 21-81% biomase. U bakterijskom volumenu i biomasu prevladavali su štapićasti oblici koji su činili 45-72% bakterijskog volumena, te 42-72% bakterijske biomase. Najbrojnije su kokoidne stanice (52-70%). Tijekom ljeta stanice manje od  $0.5 \mu m^3$  obuhvaćale su 88-94% broja svih stanica, te 68-84% biomase stanica. Za vrijeme zime došlo je do promjene u strukturi veličinskih kategorija stanica, pa su stanice manje od  $0.5 \mu m^3$  sa vrlo malim udjelom sudjelovale u ukupnoj biomasu. Nasuprot tome, manje brojne stanice volumena iznad  $0.5 \mu m^3$  činile su i do 77% bakterijske biomase.

**Ključne riječi:** bakterijska biomasa, bakterijska abundancija, bakterijski volumen, sediment, Jadransko more