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The influence of organic matter and phytoplankton pigments on the distribution of bacteria in sediments of Kaštela Bay (Adriatic Sea)

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SUMMARY: Bacterial abundance, biomass, volume and morphological diversity were studied in sediments collected in the eastern Adriatic Sea (Kaštela Bay) in order to investigate their relationship with changes in environmental parameters. To asses the changes in the investigated parameters on a temporal scale and between sediment layers, the sediment samples were collected monthly in 2002 with a piston corer from the sediment surface to a depth of 10 cm. The concentrations of organic matter (OM), chloroplastic pigment equivalents (CPE), chlorophyll *a* (chl *a*) and phaeopigments (PHAEO) were used as indicators of substrate concentrations in sediments. Sediment depth was a significant factor influencing the distribution of bacterial abundance, biomass and volume. Granulometric properties of the sediment had no effect on the distribution of bacteria. Bacterial abundance, biomass and volume were strongly related to the indicators of substrate concentrations on both scales. The accumulation of labile OM in deeper layers also had a profound effect on the size and structure of bacteria. High amounts of OM and the low proportion of labile organic fraction (CPE; chl *a* and PHAEO) indicate that this environment acts as a sink for accumulation of detrital material.

Keywords: benthos, bacterial biomass, cell volume, chlorophyll *a*, phaeopigments.

RESUMEN: La influencia de la materia orgánica y pigmentos de fitoplancton sobre la distribución de bacterias en sedimentos de la bahía de Kaštela (mar Adriático). – La abundancia, biomasa volumen y la diversidad morfologica bacteriana fueron estudiadas en sedimentos recogidos del este del mar Adriático (bahía de Kaštela) con el objeto de investigar su relación con los cambios de diferentes parámetros ambientales. Para establecer los cambios de los parámetros investigados a escala temporal y entre capas de sedimentos, las muestras de sedimento fueron recogidas mensualmente durante 2002, con un corer de pistón desde la superficie del sedimento hasta la profundidad de 10 cm. La concentración de materia orgánica, pigmentos cloroplastídicos equivalentes (CPE), clorofila *a* (chl *a*) y feopigmentos (PHAEO) fueron utilizados como indicadores de la concentración de sustrato en el sedimento. La profundidad del sedimento era un factor significativo en la influencia de la distribución del número de bacterias, biomasa y volumen. Las propiedades granulométricas del sedimento no tenían efecto sobre la distribución de bacterias. La abundancia, la biomasa y el volumen bacteriano estaban fuertemente relacionadas con los indicadores de sustrato en ambas escalas. La acumulación de la materia orgánica lábil en las capas más profundas tambien tenían un gran efecto sobre el tamaño y la estructura bacteriana. Una alta cantidad de materia orgánica y baja proporción de la fracción orgánica lábil (CPE; chl *a* y PHAEO) indica que estos ambientes actúan como sumideros para la acumulación de material detrítico.

Palabras clave: bentos, biomasa bacteriana, volumen celular, clorofila *a*, feopigmentos.

INTRODUCTION

Shallow coastal basins like the Adriatic Sea are subject to great microbiological activity as their sediments accumulate large quantities of organic matter (OM). They can show great variations in physical, chemical and hydrodynamic properties, especially in the vicinity of large industrial and urban areas. These changes can strongly affect bacterial populations, leading to heterogeneity in their biomass,

abundance and heterotrophic activities (Stoeck and Kröncke, 2001). Sediment resuspension by currents or waves can have a profound effect on sediment bacterial populations, altering their biomass or increasing bacterial production (Stoeck and Kroncke, 2001). Mixing of sediment particles by burrowing macrofauna can also support bacterial metabolism in deeper sediment layers, allowing the downward flow of pore water that delivers particulate OM directly to the buried bacteria.

The crucial factors in controlling the distribution of bacteria in marine sediments are the quantity and quality of the sedimented organic material. The importance of OM input into the sediment is strongly determined by its availability for bacteria. Algal pigments, such as chlorophyll *a* (chl *a*) and pheopigments (PHAEO) have been shown to be indicators of available food for bacteria (van Duyl and Kop, 1994). On the other hand, detritus and allochthonous matter are degraded more slowly and mostly remain buried in the sediment. Bacteria react very quickly to the substrate changes although the relationship between the substrate and bacteria is often masked by the influence of hydrodynamic circumstances (sediment resuspension, bottom waves and currents). In coastal areas sedimented OM is derived from terrestrial sources, in situ primary production from the euphotic zone, and benthic faunal metabolites and remains. Most of the OM in sediments of the Adriatic Sea originates from terrestrial sources and degrades very slowly as it comprises refractory polymeric materials (Giordani *et al.,* 2002).

Our knowledge regarding bacteria in the sediments along the eastern coast of the Adriatic Sea is still very limited (Šestanović *et al.,* 2006). The available primary data consider the pelagic food web and the roles of bacteria within it (Šolić *et al.*, 2001; Šestanović *et al.,* 2004). There is therefore a great need to elucidate the trophic importance of bacteria in the benthic food web of the coastal Adriatic Sea. We conducted this study in the semi-enclosed Kaštela Bay basin, which is the largest bay in the central part of the eastern Adriatic coast. The intensive industrialisation and urbanisation of the bay has led to increased effluent discharges. The accumulation of allochthonous matter on the bottom of the bay over the last few decades has influenced the biological processes within it, resulting in increased pelagic primary production and occurrence of red tides, oxygen depletion in bottom waters, mortality of shellfish and fish and DSP toxicity of mussels. A remaining unknown, however, is whether these changes are reflected in the bacterial communities in the sediment. The aim of this study was to characterise the variability of benthic bacteria parameters (abundance, biomass, volume) on spatial and temporal scales, in order to identify the main factors that influence the observed variations. The research was focused on the following parameters:

1. Concentrations of sediment-bound chloroplastic pigments (chl *a* and PHAEO), which serve as indicators of the amount of substrate available for bacteria.

2. The amount of OM, as a measure of quantity of substrate in sediments.

The parameters were compared on seasonal (between the months) and vertical (between the sediment layers) scales. In addition, the relationships with some abiotic parameters (sediment grain size and depth of sediment layer) were analysed.

MATERIALS AND METHODS

Study area

Samples were collected at a station in the eastern part of Kaštela Bay (Fig. 1), a semi-enclosed shallow basin on the eastern Adriatic coast. Water circulation in the bay is generated mostly by the local winds, *sirocco* and *bora*. The mean current vector in the bottom layer of the bay interior is north-eastern, with a 2.6 cm s^{-1} speed. The most important influx of freshwater is from the River Jadro with a mean annual runoff of 10 cm^3 s^{-1}.

Vranjic station is located in the most eutrophic part of the bay, which receives great quantities of effluents from industrial and urban wastewater outlets that results in a higher content of suspended OM and trace and heavy metals in sediment. However, de-

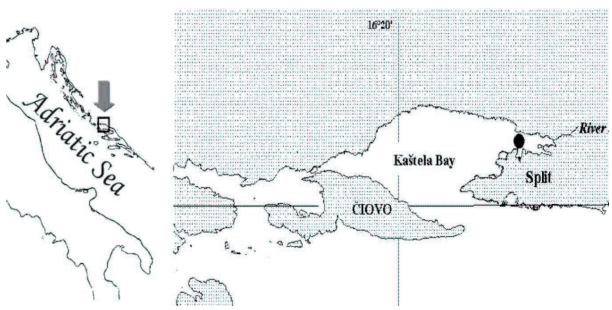

FIG. 1. – Location of the sampling station in the Kaštela Bay (central Adriatic Sea).

spite large quantities of nutrients that enter the bay, nutrient fluxes between sediments and the water column are relatively low. The estimated sedimentation rate in the area where the sediment samples were taken is 3.6 mm $year^{-1}$.

Field sampling

Cores of undisturbed sediment were collected monthly from January to December 2002 from 17 m depth at Vranjic station. All the samplings were carried out between the 1st and 5th of each month. Due to technical difficulties sampling was not conducted in March and October.

For the determination of granulometric composition sediment cores were collected only in August 2002 since previous investigations showed that granulometric properties of these sediments do not vary significantly during the year (Bogner *et al.,* 1998).

Sediment samples were taken with a piston corer from the sediment surface to a depth of 10 cm. Immediately after sampling the cores were vertically sectioned into ten 1 cm layers. Subsamples for analysis were taken from each layer using cut-off syringes and transferred to sterile polypropylene tubes. For granulometric composition, chemical and pigment analysis subsamples were frozen at -18°C and kept at this temperature until analysed. For analysis of bacterial parameters, subsamples from each layer were fixed with formalin (4% final concentration) in filtered (0.22 μm) and autoclaved seawater and then stored at 4°C in the dark until further processing in the laboratory (within one week).

Vertical temperature and conductivity profiles were measured with a Sea Bird CTD probe (Sea Bird Electronics, Inc.). Salinity was measured in practical salinity units (psu).

All the analysis was made in three replicates. The values for each parameter represent the mean of the replicates.

Sediment properties

Granulometric composition of each sediment layer was determined by sieving the sediment through successive sieves with diameters of 4000 to 63 μm. For particles smaller than 63 μm we used a standard aerometric method after Casagrande (Casagrande, 1934). For grain size nomenclature, a triangular diagram of Folk (1954) was used.

For the determination of OM a representative portion of the sediment sample (about 0.5 g) was transferred into a pre-weighed crucible and placed in an oven for 24 h at 60°C until constant weight of the samples was achieved. Sample dry weight (A) was determined using an analytical balance after cooling the crucible. The samples were then treated with an excess of 30% peroxide to remove carbonates that may interfere with OM determination, transferred to a muffle furnace and burned at 450°C for 6 hours. After cooling in a dessiccator, samples were re-weighed (B) and OM percentages were determined as follows:

$$\% \text{ OM} = [(A-B)/A] \times 100$$

OM was expressed in milligrams of OM per gram of sediment dry weight (mg g^{-1}).

Bacterial analysis

For the dislodgement of bacteria from the sediment particles the protocol of Epstein and Rossel (1995) was followed. 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. Samples were allowed to cool for 1 min between treatments.

After sonification, the samples were vigorously vortexed, and sediment particles were allowed to settle for 5 s before dilution. Dilutions (× 2000-2666) were made with sterile prefiltered (0.22 μm pores) and autoclaved seawater water. Diluted subsamples were stained for 4 min with acridine orange and then filtered on black Nucleopore polycarbonate filters (0.2 μm pores) according to the standard AODC method (Hobbie *et al.,* 1977). The filters were mounted between a slide and glass cover slip with non-fluorescent oil prior to examination by epifluorescent microscopy. The cells were counted and measured with 1000x magnification. At least 250 cells per sample were counted and measured using an eyepiece micrometer.

Cells were classified into three morphological categories: cocci, rods and filamentous bacteria (cells with the 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.

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).

For estimation of bacterial biomass a conversion of bacterial cell volume to cell carbon content is needed. For calculation of cell carbon content we applied an allometric conversion factor according to the model given by Norland (1993). This model proposes that carbon content per cell-to-volume ratio is linearly dependent on volume, so that smaller cells tend to have a higher biomass–to-volume ratio than larger ones. Therefore, the relationship between the biomass and volume of bacteria can be described by the following: $B = C \times V^a$, where B is biomass, C is the conversion factor between biomass and volume for unity volume (120 fg C μm^3), and a is the scaling factor (0.72).

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

Bacterial parameters were expressed per gram of sediment dry weight. For the determination of sediment dry weight the sediment samples were dried for 48 h at 60°C.

Pigment analysis

Chloroplastic pigment equivalents (CPE) are defined as the sum of chl *a* and PHAEO and serve as a measure of the input of phytodetrital matter to the benthos. For extraction of pigments 8 ml of 90% acetone was added per 1 cm^3 of sediment and samples were stored overnight in the dark under 4°C. After centrifugation for 20 min at 3000 x g the concentration of chl *a* was measured in the supernatant using a Turner TD 700 laboratory fluorometer according to Strickland and Parsons (1972). For the determination of PHAEO the supernatant was acidified with 1N HCl. The results were expressed in grams of sediment dry weight after drying the sediment for 48 h at 60°C.

Data analysis

We used two-way analysis of variance (ANOVA) to assess the changes in the investigated parameters with sampling dates and sediment depth as main factors. A Pearson correlation analysis was performed to test for possible relationships among the investigated variables. The relationships were tested by including all data from all sediment depths. For testing the effect of different variables on bacterial parameters a multiple regression test was performed. Statistical analysis was preformed with STATISTICA 6.0. (Stat Soft, Inc).

RESULTS

Hydrographic and granulometric properties

With respect to the vertical temperature gradient, two distinct annual periods can be observed: the colder part of the year (winter months) when the entire water column had a similar temperature, and the rest of the year when there was a distinct temperature gradient. Temperature of the bottom water showed a marked seasonality ranging between 10.42 and 23.29°C. The lowest value that was recorded in January gradually increased toward September, when the highest value was observed. In November and December the temperature of the bottom layer gradually decreased to the values of 17.4 and 16.47°C, respectively.

Throughout the year, salinity increased with depth. The lowest salinity values were recorded at the surface layer, as a result of River Jadro inflow. Bottom water salinity values ranged between 37.15 and 38.32. Higher values were present during winter and summer months. A marked decrease in bottom salinity values were recorded in spring and autumn.

The sediment showed a relatively homogeneous granulometric structure and was mainly composed of silt particles. The fine-grained fraction (<63 μm) was relatively high, varying between 74.5 and 87%. Surface sediments (0 to 1 cm) with a gravel content of 7% consisted of gravelly mud, while samples from 1 to 9 cm depth with a gravel content of less than 4.5% were characterised as slightly gravelly sandy mud.

Organic matter and chloroplastic pigments in sediment

The average OM content of dry sediment ranged from 30.1 to 98.3 mg g^{-1} (3.01-9.83%) (Fig. 2). The highest values were present in January and December in the surface layer. From January to April, the

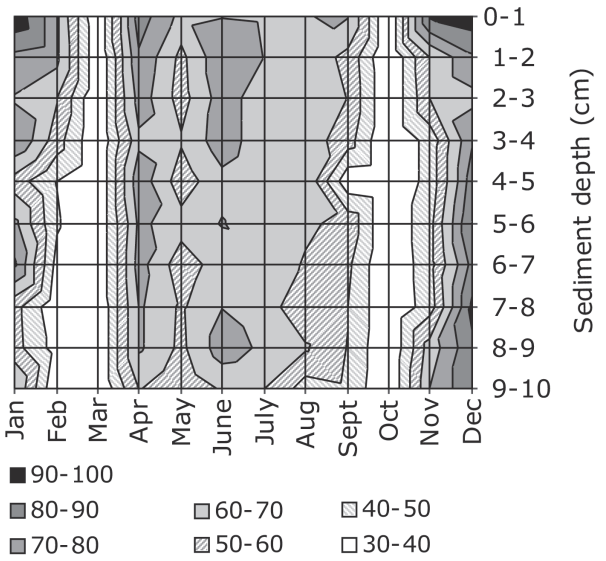

FIG. 2. – Organic matter (mg g^{-1}) of sediment samples from depths of 0-10 cm.

OM distribution decreased towards deeper sediment layers and a significant correlation between OM content and sediment depth was found, which explained between 34 and 92% of the vertical heterogeneity in OM. In December a higher OM content was found throughout the sediment core. Warmer months were characterised by higher OM concentrations than in spring and autumn.

CPE concentrations ranged from 1.78 to 13.58 μg g^{-1} (mean = 5.47 ± 2.15). CPE was always the highest in the top sediment layer, decreasing considerably towards the bottom (Fig. 3). The temporal changes of the CPE amount could only be observed in the top sediment layer from 0-2 cm. The highest values were observed during the periods January-April and November-December and the lowest in the period June-September. Deeper layers (from 2-10 cm) exhibited only moderate seasonal differences.

Chl *a* concentrations were extremely low, ranging from 0.35 to 2.14 μg g^{-1} (mean = 0.92 ± 0.76). The highest concentrations occurred in the surface layers, declining with the sediment depth (Fig. 3). About 76-90% of chl *a* variations between sediment layers from April to September could be explained by the variations in sediment depth. However, during the winter period, chl *a* did not change notably with sediment depth and deeper sediment layers from 4-10 cm exhibited very small chl *a* changes throughout the year.

The proportion of chl *a* (% chl *a*) in CPE ranged from 4.4 to 45.4%. The highest chl *a* % was present in June and August in the layer from 0 to 3 cm and then declined sharply towards the bottom. A vertical gradient of the chl *a* proportion in CPE was not observed during the rest of the year.

Chl *a*/PHAEO ratio varied between 0.05 and 0.83. The highest values were recorded in June and July (0.83 and 0.73, respectively), and the lowest in January and December (0.09). Strong temporal changes in the ratio were the result of the differences in chl *a* and PHAEO distribution between colder and warmer months. The high coefficients of variation for different sediment layers of CPE, chl *a* and PHAEO (Table 1) indicated a substantial variability that greatly exceeded the temporal variations over the entire investigated period.

Distribution of bacterial abundance, biomass and volume in sediment

Bacterial abundance in the sediment ranged from 0.19 to 1.25 x 10^{10} cells g^{-1} (mean = 5.38 ± 2 x 10^{9}).

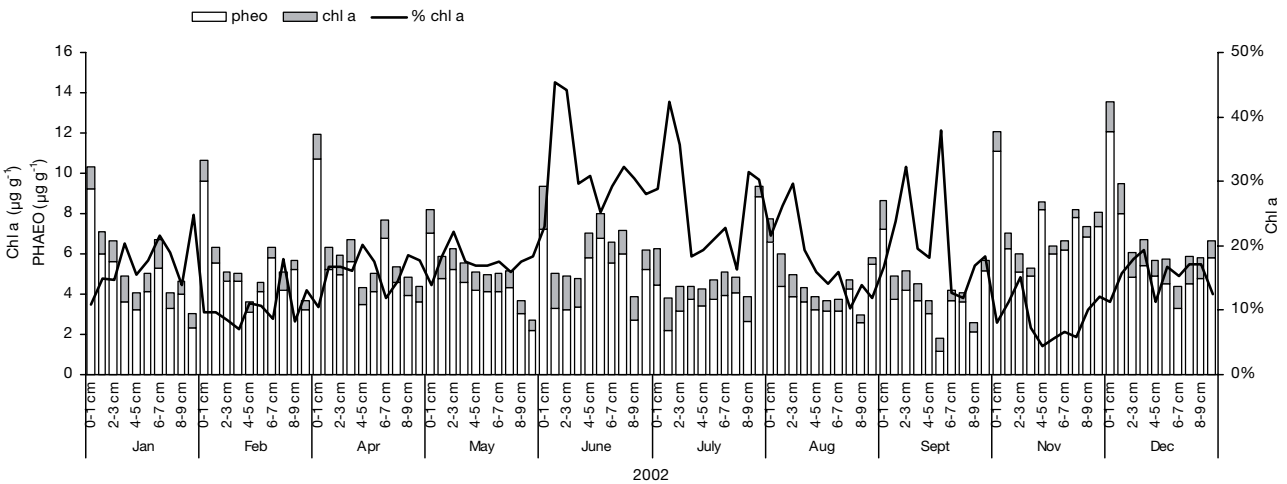

FIG. 3. – Concentrations of phytoplankton pigments (μg g^{-1}) in sediment samples from depths of 0-10 cm.

TABLE 1. – Results of two-way (ANOVA) comparing the effect of sediment depth and sampling period on the values of investigated parameters (OM, organic matter; Chl *a*, chlorophyll *a*; PHAEO, phaeopigments; CPE, chloroplastic pigment equivalents; BB, bacterial biomass; BA, bacterial abundance; BBV, bacterial biovolume; ACV, average cell volume; d.f., degrees of freedom; MS, mean square; F, ratio of between-group variation divided by within-group variation).

Variable	Source of variation	d.f.	MS	F	% of total variations	*p*-value
OM	Months	9	1192	16.43	53.24	<0.0001
	Sediment depth	9	394	5.43	17.59	<0.0001
Chl *a*	Months	9	0.236	0.22	1.01	0.99
	Sediment depth	9	13.44	12.40	57.36	<0.0001
PHAEO	Months	9	8172	5.12	13.38	<0.0001
	Sediment depth	9	38.51	24.12	63.08	<0.0001
CPE	Months	9	24.46	8.48	17.35	<0.0001
	Sediment depth	9	9056	31.41	64.24	<0.0001
BB	Months	9	70350	19.39	59.38	<0.0001
	Sediment depth	9	15480	4.27	13.06	0.0001
BA	Months	9	2.12 x 1019	12.83	41.98	<0.0001
	Sediment depth	9	1.45 x 1019	8.73	28.56	<0.0001
BBV	Months	9	8.14 x 1018	20.97	61.26	<0.0001
	Sediment depth	9	1.65 x 1018	4.26	12.45	0.0002
ACV	Months	9	0.161	8.34	73.98	<0.0001
	Sediment depth	9	0.006	0.78	2.71	0.4123

Biomass of bacteria varied between 47.1 and 715.2 μg C g^{-1} (mean = 149.4 ± 11.7). The highest values of bacterial abundance and biomass were recorded in the period from June to August (Fig. 4a and 4b). Variations in sediment depth explained, on average, 42% of variations in bacterial abundance and 48% of variations in bacterial biomass. Seasonal changes in both parameters were the most pronounced in the top layers (0-2 cm), while deeper layers exhibited moderate changes in bacterial abundance and biomass.

Surface peaks in summer bacterial biomass showed high values in the whole sediment column in January, November and December as the result of the presence of bigger cells. Bacterial cell volume exhibited very high variations through the year, ranging from 0.056 to 0.789 μm^3 (mean =0.168 μm^3; C.V. =83%). It showed a strong seasonal pattern, with the lowest values in spring and autumn and the highest in January and December (Fig. 5c). In December the mean cell volume was 3-8 times higher than was recorded in the rest of the year. The increase in bacterial volume in winter was the result of high abundance of rods larger than 0.5 μm^3 and filamentous cells larger than 1 μm^3, which were present down to the 10 cm layer.

Morphological characteristics of bacteria in sediment

Most bacteria had a volume of 0.025 μm^3 (21.35% of the total) or 0.009 μm^3 (15.30%; Fig. 5). The cells with these volumes dominated during the whole year

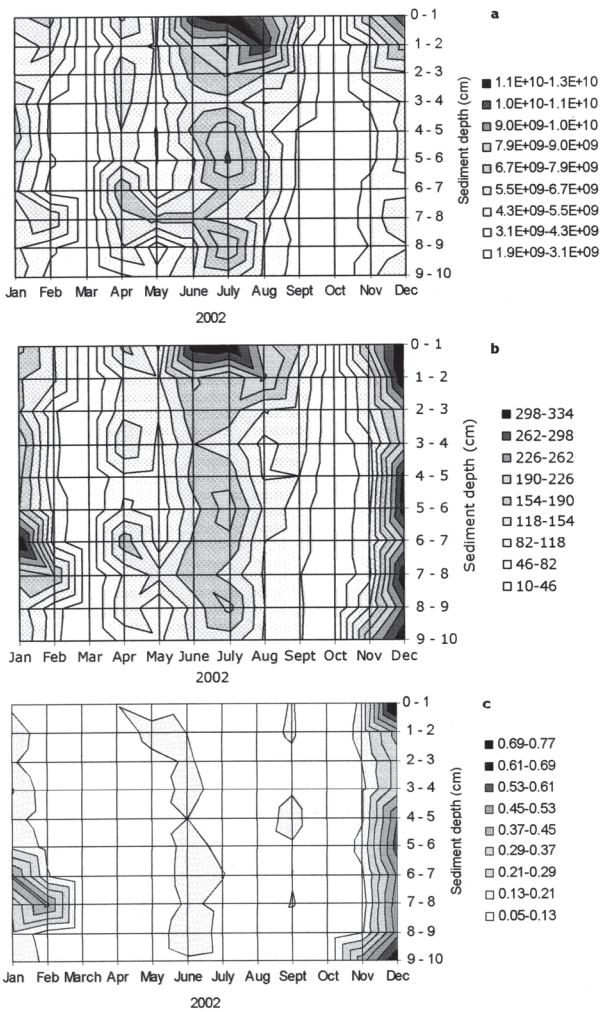

FIG. 4. – The distribution of bacterial abundance (cells g^{-1}) in sediment samples (a). The distribution of bacterial biomass (μg C g^{-1}) in sediment samples (b). The distribution of the mean cell volume (μm^3) in sediment samples (c).

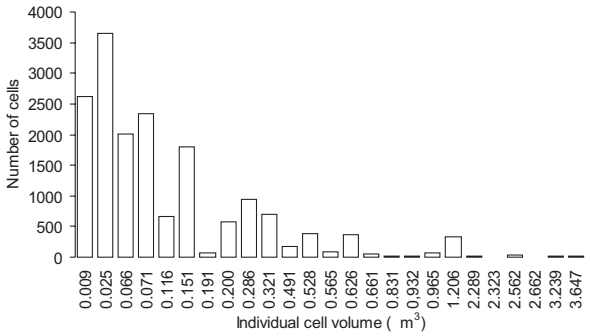

FIG. 5. – Distribution patterns of the total number of bacteria counted in all samples (n=16 976) and their measured volumes.

except in January and December, making up 80 to 91% of all bacterial abundance. In January and December the size distribution of cells shifted towards cells larger than 0.5 μm^3, which included between 24 and 44% of all cells.

During the whole year except in December, cocci were the most numerous bacterial type, comprising 52-70% of all cells (Fig. 6a) but their importance in total bacterial biomass was much lower (4-43%; Fig. 6b). The biomass of rods accounted for the highest proportions in bacterial biomass during the whole year, making up between 44 and 71% of total bio-

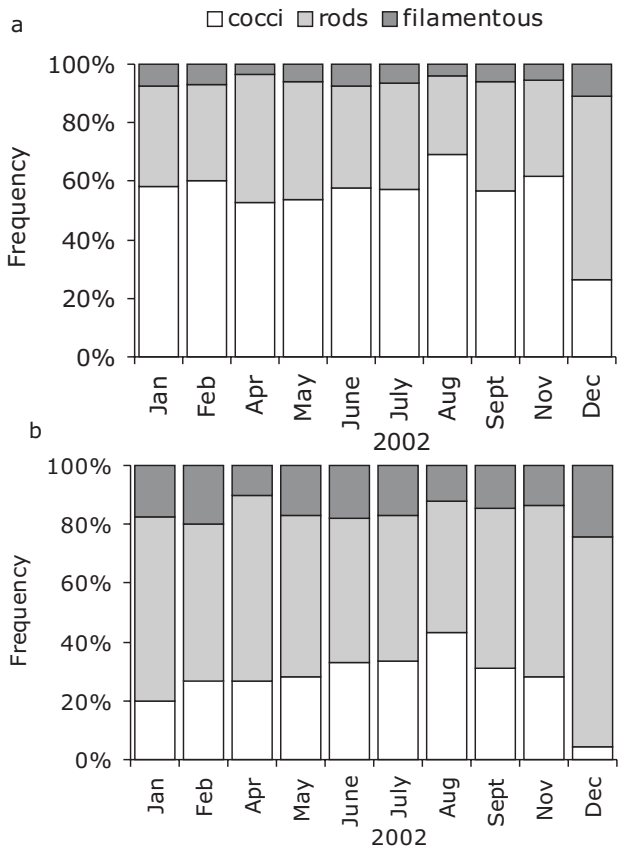

FIG. 6. – Relative importance of cocci, rods and filamentous bacteria in total bacterial number (a) and biomass (b).

mass. The importance of filamentous cells in total bacterial biomass was relatively low (9-24%).

In January and December there was a noticeable change in the morphological composition of cells. The dominance of smaller cocci shifted towards larger rods and filamentous cells. Rods and filamentous cells larger than 0.5 μm^3 comprised 89-92% of total bacterial abundance and 79-96% of total bacterial biomass during this period. Also, the importance of filamentous bacteria larger than 1 μm^3 greatly increased during these months. These cells represented no more than 7% of the filamentous cells during the rest of the year, but over 44% in winter.

The distribution of bacterial abundance covaried with the abundance of the smallest coccoid bacteria (volume <0.1 μm^3). The distribution of bacterial biomass covaried with the distribution of rods and filamentous cells larger than 0.5 μm^3.

Changes in the analysed parameters on the temporal and vertical scale

The influence of temporal and vertical scales on the variations of the analysed parameters is summarised in Table 1. The results showed that all the variables displayed significant differences among the sampling periods (between months) except chl *a*. Bacterial parameters (bacterial abundance, biomass, biovolume and mean cell volume) and the OM content displayed stronger variability between months than between different sediment layers. The temporal scale explained 42-74% of the variability in OM, bacterial abundance, biomass, biovolume and average cell volume. On the other hand, sediment pigments (CPE, chl *a* and PHAEO) displayed higher variability related to depth of sediment than to differences between months. On average, sediment depth explained 57-64% of the total variability in CPE, chl *a* and PHAEO in sediments.

Factors influencing bacterial number, biomass and volume distribution in sediment

A Pearson correlation analysis was carried out in order to examine the relationship between the different variables (Table 2). The relationships were tested by including all data from all sediment depths. The analysis revealed a high number of significant correlations. CPE was correlated to the sediment grain size. A high positive correlation was found between the amount of phytopigments and the distribution of

TABLE 2. – Results of correlation analysis between investigated parameters (BB, bacterial biomass; BA, bacterial abundance; BV, bacterial volume; ACV, average cell volume; OM, organic matter; CPE, chloroplastic pigment equivalents; Chl *a*, chlorophyll *a*; PHAEO, phaeopigments).

	BN	BV	Sediment depth	OM	CPE	Chl *a*	PHAEO/ Chl *a*	PHAEO	Gravel	Sand	Silt	Clay
BB	0.79**	0.93**	-0.38*	0.45**	0.35*	0.47**	-0.40*	-0.45**				
BA		0.60**	-0.38*	0.43**	0.21	0.47**	-0.36*	-0.49**				
BV			-0.36*	0.40*	0.36*	0.35*						
ACV												-0.43**
Sediment depth				-0.47**	-0.59**	-0.54**		-.050**	-0.26	-0.54**	0.47**	
OM					0.40*	0.34*	-0.34*					
CPE								0.40*	0.52**	0.54**	-0.48**	
Chl *a*							-0.84**	-0.79**				
PHAEO/Chl *a*								0.57**				
PHAEO												
Gravel										0.83**	-0.61**	
Sand											-0.48**	
Silt												-0.83**

** $p<0.0001$; *$p<0.001$

coarser particles (gravel and sand) and a significant negative correlation with the contribution of silt. The variations in bacterial biomass, number and biovolume were significantly correlated to the changes in the concentrations of OM and substrate indicators (CPE; chl *a* and PHAEO). The distribution of all the investigated parameters depended significantly on the sediment depth.

Bacterial biomass and biovolume were greatly affected by the changes in chl *a* and OM concentrations. Multiple linear regression was used to investigate the combined effect of concentrations of OM and chl *a*, as independent variables, on bacterial biomass and biovolume (Table 3). The coefficient of multiple regression (R), which measured the overall degree of association of bacterial biomass and biovolume with both independent variables (OM and chl *a*), was 0.70 for bacterial biomass and 0.62 for bacterial biovolume. That is, 49% of the variance of bacterial biomass and 38% of the variance of bacterial biovolume can be explained with concentrations of OM and chl *a*.

The relative importance of the two factors studied is shown by the coefficient of partial correlation and beta coefficients. The partial correlation between bacterial biomass (biovolume) and concentration of OM (correlation when the effect of chl *a* concentration was removed) was higher than partial correlation between bacterial biomass (biovolume) and chl *a* concentration (when the individual effect of concentration of OM was excluded). This suggests that variation in concentration of OM was more important than concentration of chl *a* in changing the bacterial biomass (biovolume). Beta coefficients (regression coefficients stated in terms of their standard deviation) point to the same conclusion. For example, each increase of one standard deviation (SD) in the value of OM concentration will be accompanied (if the concentration of chl *a* stays constant) by an increase of 0.48 SD in the value of bacterial biovolume. On the other hand, each increase of one SD in the value of the chl *a* concentration will be accompanied (if the concentration of OM stays constant) by an increase of only 0.19 SD in the value of bacterial biovolume (Table 3).

TABLE 3. – Multiple regression equations and statistics predicting sediment bacterial biomass (BB) and biovolume (BV) (r, Pearson's correlation coefficient; r_p, partial correlation coefficient; β, beta coefficient; R, coefficient of multiple regression; R^2 (%), coefficient of multiple determination; OM, organic matter; Chl *a*, chlorophyll *a*).

Dependent variable	Independed variable	r	rp	β	R^2
BB	OM	0.65*	0.43*	0.43	0.49*
	Chl *a*	0.62*	0.37*	0.36	
BV	OM	0.60*	0.44*	0.48	0.38*
	Chl *a*	0.49*	0.19	0.19	

*$p<0.0001$

DISCUSSION

The properties of sediments including grain size, quality and quantity of OM and nutrients considerably affect benthic bacteria colonisation. Due to higher concentrations of particulate OM and nutrients and greater particle surface area, muddy sediments generally support denser populations of bacteria. However, a simple relationship between number or biomass of sediment bacteria and the sediment grain

size cannot always be achieved. This holds true especially for the shallow coastal sediments that are subject to large variations in bottom waves and currents (Kristensen *et al.,* 1997) and and are expected to show a highly variable particle size distribution. These sediments are also subject to anthropogenic disturbances that alter the sediment organisation, thus inducing changes in bacterial community composition. The results of the present study reveal a highly variable vertical sediment grain size composition and a rather predictable bacterial vertical distribution in accordance with these statements. A typical bacterial number or biomass vs depth profile exhibits a surface maximum and a decrease towards deeper layers showing no relationships with the granulometric properties of the sediment.

The OM content in sediments in Kaštela Bay was relatively high and was comparable to that of other eutrophic sites such as the Po River (DellAnno *et al.,* 2003). The highest OM values were found in surface layers in January, November and December (from 9.2 to 9.8 mg g^{-1}). This was probably the consequence of sedimentation rates that were two times higher during these months than in the period from June to August (Ujević, 2002).

High quantities of OM were always found in the layers containing a higher percentage of fine-grained fraction (<63 μm). This suggests the importance of sediment grain size structure for the distribution of OM in sediments of Kaštela Bay. Since sediments with higher percentages of fine-grained fraction have a higher specific surface area, the surface processes such as adsorption of dissolved and colloidal particles are more intensive than those of sediments of coarse grained particles.

The information on biomass and number of sediment bacteria reported here are the first such data from the eastern coast of the mid-Adriatic Sea. Therefore, these records are quite significant for comparisons with studies from other areas of the Mediterranean Sea or broader ones (Table 4). Bacteria number and biomass in Kaštela Bay were one order of magnitude higher than the values reported for deep sea sediments and some coastal areas of the Mediterranean Sea. However, they were within the range reported for more productive areas such as fish farms and some lake sediments. Higher bacterial biomass was always found either at the sediment surface or associated with the largest amounts of OM. Statistically significant correlations between bacterial biomass (and abundance) and OM content suggest the importance of substrate concentrations for bacteria in Kaštela Bay. This is in accordance with the previous investigations that described the importance of available substrate in controlling pelagic microbial biomass and activity in Kaštela Bay (Šolić *et al.,* 2001; Šestanović *et al.,* 2004). Although the data presented indicated a significant bacterial response to OM, we consider OM content to be too crude a variable to provide insight into the availability of OM for bacteria. The literature also assigns more importance to the quality of sedimented material rather than to its quantity (van Duyl and Kop, 1994). Even though it is generally assumed that the content of OM in the surface layer is made up of degradable, mostly biogenic carbon, in the case of Kaštela Bay, the nature of OM can be expected to be quite refractory due to its allochtonous origin. We therefore used the content of chloroplastic pigments (chl *a* and PHAEO) as an indicator of the labile carbon (van Duyl and Kop, 1994). By estimating the contribution of the labile fraction of the OM from primary production we tried to get a better understanding of the relationship between bacteria and the organic substrates. Average concentrations of chl *a* and CPE reported here were much lower than those of other enclosed basins of the Tyrrhenian Sea (Puscedu *et al.,* 1999) or southern Adriatic Sea (Gambi *et al.,* 2003), but comparable to those reported for adjacent shelf sediments of the Albanian and Italian coast (Vezzuli and Fabiano, 2006) and for some oligotrophic sites (Fabiano *et al.,* 1995). Higher concentrations of chl *a* in surface sediment layers were found during in and summer at the same time when phytoplankton bloom in the

TABLE 4. – Comparison of bacterial biomass (μg C g^{-1}) and abundance (cells g^{-1}) from different areas

Area	Sediment depth (cm)	Bacterial biomass	Bacterial abundance	Authors
Deep sea sediments (North Pacific)	0-3		0.03 - 1.2 x 10^9	Smith *et al.,* 2002
River Po, northern Adriatic Sea	0-6	2.8-301.6	0.14 - 3.6 x 10^9	Danovaro *et al*., 2002
Eutrophic area (North Sea)	0-8	6.99-27.39	≈ 0.5 - 8.5 x 10^9	Stoeck and Kröncke, 2001
Deep Mediterranean sediments	0-1		0.2 - 1.3 x 10^{10}	Luna *et al.,* 2004
Fish farm sediment, Ligurian Sea	0-3	271-2602	0.31 - 3.9 x 10^{10}	Vezzulli *et al.,* 2002
Lake sediment, Canada	0-5		5 x 10^{10}	Schallenberg *et al.,* 1989

whole water column occurred (Marasović, personal observations). The accumulated chl *a* was probably not the result of enhanced sedimentation from the water column since the CPE values remained low. Moreover, due to the formation of temperature stratification in this period of the year, higher sedimentation rates were not expected. The higher chl *a*/PHAEO ratio that was observed was probably a consequence of bottom water hypoxia that slowed the degradation of chl *a*, resulting in lower PHAEO concentrations. The formation of bottom water hypoxia during the warm part of the year is a common phenomenon in shallow basins like this part of the Bay (Marasović *et al.*, 2005).

Though the quantities of CPE and chl *a* were very low, their presence in sediment was detected during the whole year. This could be the result of a continuous input from the water column and the presence of a microphytobenthic community. Since the investigations on the microphytobenthos in Kaštela Bay have not yet been done, its significance as the contributor to primary production is still unknown. However, the importance of benthic microalgae as the substrate source for benthic bacteria cannot be underestimated because their biomass can sometimes exceed that of the phytoplankton in the overlying waters. It has also been shown that microphytobenthic production which occurs at the surface of the sediments can be as high as phytoplankton production in overlying waters (Conde *et al.,* 1999).

The low chl *a*/PHAEO ratio (<0.83) observed during the whole study period suggests a greater proportion of detritus material in the labile fraction of OM. These observations may suggest either rapid chl *a* degradation or its removal (by waves and currents) before it reaches the sediment. A higher PHAEO concentration in comparison with chl *a* can be the result of different rates of pigment degradation (Leavitt, 1993) but also of algal senescence and intensive grazing. Numerous studies show that an increase in abundance of microalgal consumers could also cause a corresponding increase in PHAEO concentrations (Head and Harris, 1992; Leavitt, 1993) since the ingestion of algae by herbivores severely degrades chl *a*. The loss of pigments varies with the feeding rate and the species of algae and their consumers (Head and Harris, 1992). However, without the investigation of the benthic community structure of Kaštela Bay this hypothesis cannot be confirmed.

The occurrence of chloroplastic pigments in deeper sediment layers suggests the presence of the mechanisms that allow the burial of the labile fraction of OM. Sediment mixing by macrofauna could be a dominant mechanism for redistribution of OM in subsurface sediments (Stoeck and Kroncke, 2001). Action of the waves and bottom currents could also cause the mixing of the sediment. Variations in PHAEO content strongly depended on the chl *a* content and this finding was confirmed by a very strong correlation between these parameters (Table 2).

A very strong relationship between the indicators of the labile fraction of OM (CPE and chl *a*) and OM suggests that the photosyntheticaly produced OM is an important source of OM in the sediments of Kaštela Bay.

The variations in benthic pigments were significantly reflected by the variations in bacterial number, biomass and biovolume (Table 3). The presence of sedimented OM at the sediment surface seemed to cause the increase in bacterial biomass and number, thus supporting the idea that bacterial biomass is generally controlled by phytoplankton deposition.

The accumulation of labile OM in deeper layers in winter also had a profound effect on the size structure of the bacterial population. The shift from the dominance of smaller cocci toward larger rods and filamentous cells during the winter months could have been the consequence of the accumulated phytoplankton material. This conclusion is in agreement with the results of other studies showing that, due to its labile nature, sedimenting algal detritus can have profound effects on bacterial cell size (Goedkoop *et al.,* 2000). Variations in other parameters such as the temperature, grazing pressure by flagellates and ciliates, rates of sedimentation and primary production also casue changes in cell morphology. Previous studies demonstrated that under strong grazing pressure, the contribution of filamentous cells to the total bacterial biomass can be from 20 to 80% (Pernthaler *et al.,* 1996). This study showed that filamentous bacteria accounted for up to 52% of the total bacterial biomass in the eastern Adriatic Sea in winter, whereas in the rest of the year they made up only 11-20%. Although this study did not deal with the problem of grazing on sediment bacteria, previous studies conducted in the water column at the same sampling site revealed the importance of flagellates in controlling the number and biomass of bacteria (Šolić *et al.,* 1998).

In addition to autochthonous OM derived from phytoplankton, high inputs of industrial and municipal waste that enter the bay each year probably also acted as important food sources for benthic bacteria.

The analysis of the simultaneous effect of OM and chl *a* on biomass and biovolume of bacteria showed that 49% of the variations in bacterial biomass and 38% of the variations in bacterial biovolume can be explained by the combined influence of OM and chl *a*. These significant relationships suggest that variations in the substrate concentration may be an important factor affecting bacterial populations in sediments of Kaštela bay.

CONCLUSIONS

Significant temporal and vertical changes of all investigated parameters make the sediment of Kaštela Bay a highly dynamic ecosystem. High amounts of OM and the low proportion of the labile organic fraction (CPE, chl *a* and PHAEO) indicate that this environment acts as a sink for accumulation of detrital material. Bacterial communities in these sediments are strongly related to the concentrations of substrates. However, studies investigating the sources of OM for bacteria in sediments of Kaštela Bay are still lacking. Bearing in mind that benthic microalgae could contribute significantly to the primary production of shallow aquatic systems, it is crucial for further investigations to focus on the role of microphytobenthos as a food source for bacteria in sediments of Kaštela Bay. The importance of other factors that affect the size and activity of sediment bacteria is is also still pending investigation. Further studies should therefore include the relationships with other benthic organisms like viruses, heterotrophic flagellates and ciliates, which form a complex link with bacteria through the benthic microbial food web.

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