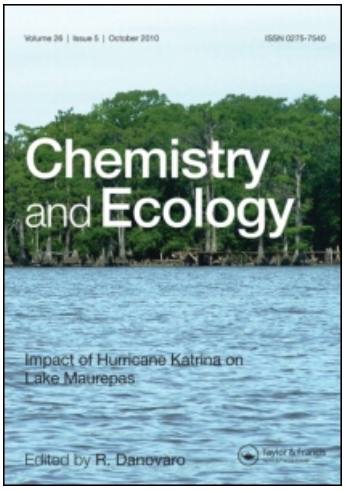

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

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To cite this Article Paoli, A. , Negro, P. Del and Umani, S. Fonda(2006) 'Temporal variability in bacterioplanktonic abundance in coastal waters of the Northern Adriatic Sea', Chemistry and Ecology, 22: 4, S93 — S103
To link to this Article: DOI: 10.1080/02757540600557769
URL: http://dx.doi.org/10.1080/02757540600557769

Chemistry and Ecology
August 2006, Vol. 22 (Supplement 1), pp. S93–S103

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Temporal variability in bacterioplanktonic abundance in coastal waters of the Northern Adriatic Sea

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(Received 15 February 2005; in final form 7 September 2005)

The purpose of this study is to analyse the heterotrophic bacterial abundance (HBA) distribution in the water column at a coastal site of the Gulf of Trieste (Northern Adriatic Sea), from 1999 to 2003, by adopting three different sampling frequencies: monthly, twice monthly, and daily. The HBA ranged from 1.6 to 54.6 $\times$ 10^8 cell l^{-1}, showing a high variability of more than two orders of magnitude over the study period, with a mean annual variation of one order of magnitude and a 60% annual mean coefficient of variation. Similar seasonal patterns were observed every year: these were characterized by low bacterial abundances during the winter which increased in the summer. Intensifying samplings from monthly to twice monthly did not provide more detailed information on the seasonal HBA distribution but allowed us to detect sporadic HBA hot spots perhaps as the consequence of micro-environmental changes. The results of a daily sampling carried out for 4 weeks, during winter 2002, did not show any significant changes in HBA, ranging from 0.8 to 2.4 $\times$ 10^8 cell l^{-1}. The highest variations between two consecutive days was of about twofold, and the CV over the period was lower than 30%. A principal-component analysis was used to compare HBA, temperature, salinity, dissolved organic carbon, and chlorophyll *a*. Different gradients related to the surface water hydrological and biochemical characteristics resulted from the long- and short-term study periods. The study of different timescales allowed us to obtain a complete view of HBA temporal distribution confirming an annual pattern that could be affected by sporadic HBA hot spots as the consequence of changes of local environmental conditions.

Keywords: Bacterioplankton; Temporal variability; Coastal waters; Adriatic sea

1. Introduction

No satisfactory theory of bacterial stock regulation in the sea has yet been formulated [1]. One facet of this problem is that we do not have a good understanding of the extent and causes of variation in heterotrophic bacterial abundances (HBA) at different scales of time and space. There are surely many parameters influencing HBA distribution, and it is not easy to foresee the space and time environmental dynamics for planning an efficient sampling strategy. Sampling for estimating HBA generally covers kilometre-scale distances over timescales of weeks to years, but to the best of our knowledge, different timescales have not been considered yet.

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Chemistry and Ecology
ISSN 0275-7540 print/ISSN 1029-0370 online
http://www.tandf.co.uk/journals
DOI: 10.1080/02757540600557769

Spatial heterogeneity in bacterioplankton abundance has been observed at centimetre scales: Duarte and Vaque [2] found a 2.5-fold variation, while Mitchell and Fuhrman [3] found a 3.5-fold variation. Muller-Niklas *et al.* [4] detected no significant bacterioplankton abundance variations among 100 nl samples, but Long and Azam [5] showed significant variations in bacterial species richness among 1 µl samples. HBA variability on a meter scale is usually observed in studies on bacterioplankton vertical distribution ([1] and references therein). Macroscale spatial variability from cross-frontal to basin scales has been mostly studied using a transect approach [6, 7].

Some authors found that marine bacteria have seasonal patterns of distribution, but they can also rapidly aggregate into small-scale patches and rapidly modify their metabolism in response to favourable micro-environmental conditions [8–10]. Studies specifically designed to analyse changes in bacterial standing stocks over timescales of days or weeks at one specific location are rare. Del Negro *et al.* [11] and Turk *et al.* [12] have already described HBA annual dynamic in the Gulf of Trieste by using a monthly sampling frequency, but there is no information about HBA variability over shorter periods. Gasol *et al.* [13] carried out a study in the north-western Mediterranean Sea and found a substantial HBA diel variability; and Solič and Krstulovič [14], during a 24 h experiment in the Kastela bay (Adriatic Sea), found a twofold higher bacterial abundance during daylight hours rather than in the night due to the effect of a strong predation by heterotrophic nanoplankton on bacteria. Also, a diel cycle with significant variations of bacterial activities and growth rates has been found in open ocean samples [15], in slope waters [16], and in coastal waters [13, 17, 18]. Sherr *et al.* [9] reported a variation in bacterial abundance over timescales of hours to days at three sites sampled off the Oregon coast at the same season.

Here, we analyse the variations in HBA distribution in the water column at a coastal site on the Gulf of Trieste by adopting three different sampling frequencies: monthly, twice monthly, and daily.

2. Material and methods

2.1 *Study area and sampling strategies*

The Gulf of Trieste (figure 1) is the northernmost part of the Adriatic Sea with a surface area of about 600 km^2 and maximum depths of 26 m [19]. The main freshwater input comes from the Isonzo River, while the rivers from the south-eastern coast are of a torrential nature. Freshwater inputs, representing the main source of organic matter, show a high interannual variability [20], which affects salinity, with values ranging from 32 to 38 at the surface [21, 22]. Water temperatures show a regular annual pattern from winter minima as low as 6 °C in February, to summer maxima >25 °C [23], and an annual medium variation of more than 20 °C. A high variability of the water column profile, due to riverine outflows and temperature variations, is enhanced by an alternance of cold winds from the north-north-east (i.e. Bora) and mild winds from the south (i.e. Scirocco).

Water samples were collected at midday ±30 min at a coastal station (C1 45° 42′ 03″ N, 13° 42′ 36″ E) in the Gulf of Trieste (figure 2) with three different timescales: monthly from January 1999 to August 2002; twice monthly from September 2002 to December 2003; daily from 12 February to 11 March 2002 (interrupted from 15 to 17 February and from 22 to 24 February because of strong northern winds). Temperature and salinity data were collected by a multiparametric probe (Idronaut Ocean Seven 316). Samples to estimate heterotrophic bacterial abundances were collected with a 10 l Niskin bottle, equipped with silicon elastic

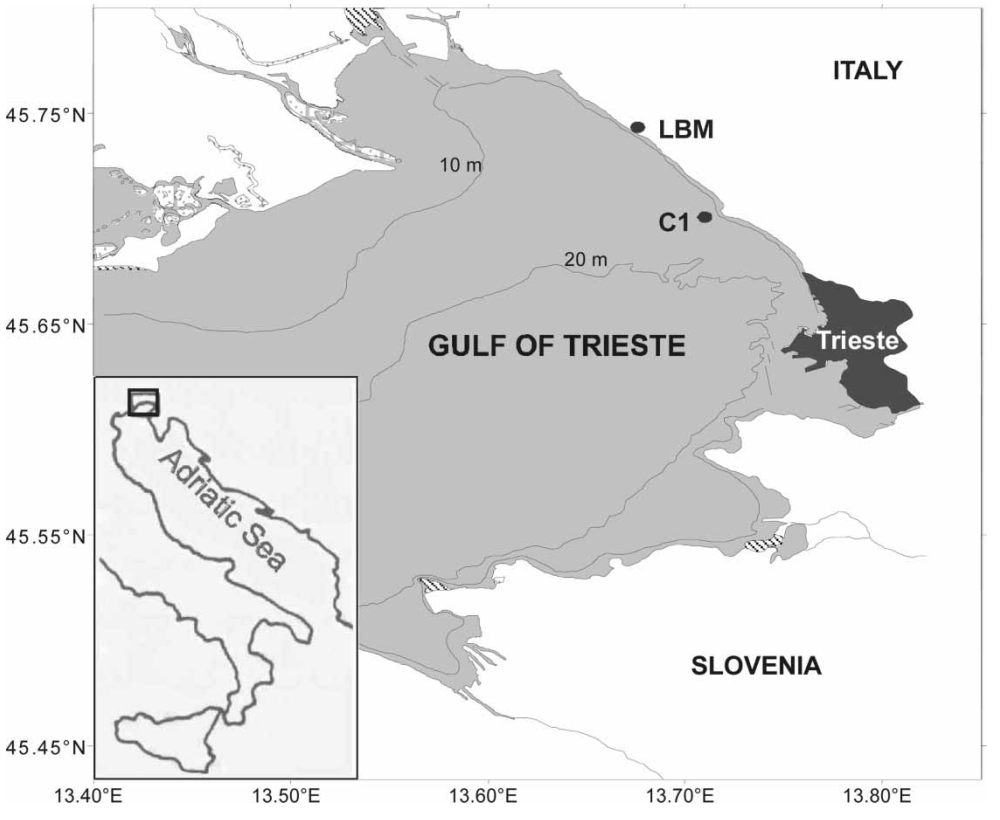

Figure 1. Location of the sampling station C1 in the Gulf of Trieste, Northern Adriatic Sea.

and red silicon O-rings, at −0.5, −5, −10, and −15 m depths during the monthly and twice monthly sampling and only at the surface (−0.5 m) and at the bottom (−15 m) during the daily sampling. Samples were stored in freezing bags (6 ± 2 °C) and processed at the Laboratory of Marine Biology (LBM) within 2 h of collection (figure 2).

Samples (1 l) for chlorophyll *a* (Chl *a*) analysis were filtered onto glass fibre filters (Whatman GF/F) and stored at −20 °C. Pigments were extracted overnight in the dark at 4 °C with 90% acetone from the homogenized filter and determined fluorimetrically according to Lorenzen and Jaffrey [24]. The Chl *a* concentration was measured in triplicate by means of a Perkin Elmer LS 50B spectrofluorometer at 450 nm excitation and 665 nm emission wavelengths. The replicate samples showed a dispersion lower than 4%.

Samples (15 ml) for dissolved organic carbon (DOC) analysis were filtered through precombusted (4 h at 480 °C), acidified (1 N HCl) glass fibre filters (Whatman GF/F), and stored at −20 °C in 20 ml glass vials (previously treated with chromic mixture and precombusted for 4 h at 480 °C). Before the analysis, samples were acidified (pH < 2) with a 6 N HCl solution and purged for 8 min with high-purity oxygen bubbling (150 ml min^{-1}). The DOC concentration was measured using a Shimadzu TOC 5000 Analyser with a 1.2% Pt on silica as catalyst [25] at 680 °C. One-hundred-microlitre samples were injected into the instrument port. Carbon concentration was determined by automatic comparison with four-point calibration curves. Standardization was carried out at every sampling using potassium hydrogen phthalate. Each value was determined from a minimum of three injections, with a variation coefficient of less than 2%. The replicate samples showed a dispersion of 1.5–4%.

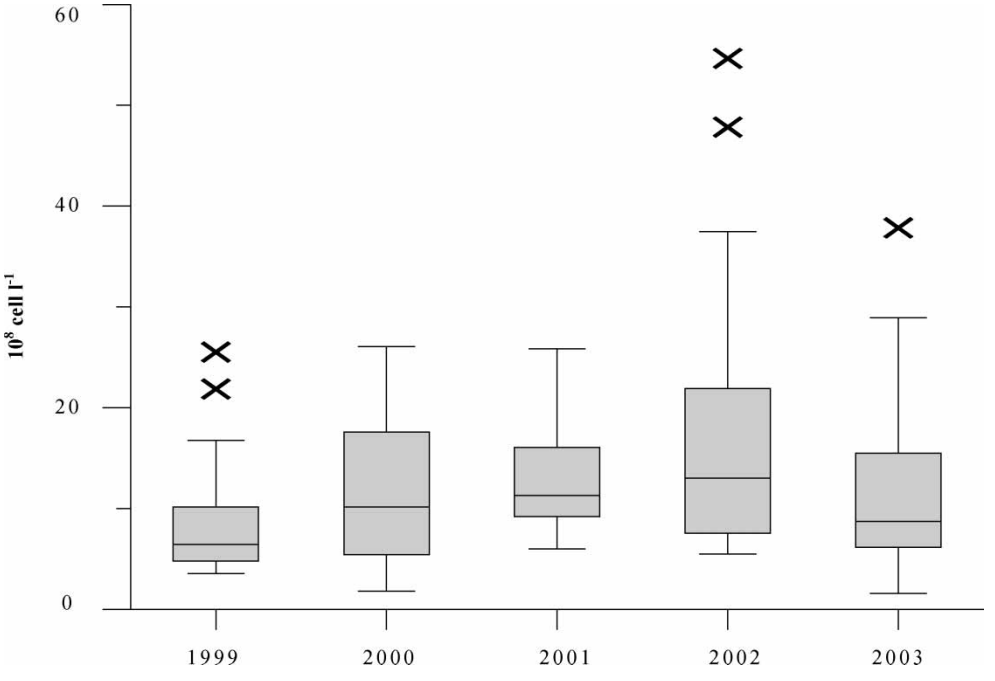

Figure 2. Boxplots of annual heterotrophic bacterial abundances from 1999 to 2003. The median values (-), the first (□) and third (□) quartile, the minimum (_) and maximum (¯) and the outlayers (x) of every year are shown.

Temperature, salinity, DOC, and Chl *a* values of the monthly and twice monthly sampling were kindly furnished by the LBM database.

2.2 *Heterotrophic bacterial abundances (HBA)*

Samples (10 ml) were fixed with 2% final concentration borate-buffered formalin (pre-filtered through a 0.2 μm Acrodisc filter) and stained for 15 min with 4′6 diamidino-2-phenylindole (DAPI, Sigma) at 1 μg ml^{-1} final concentration [26]. Subsamples (2–3 ml) were filtered in triplicate onto 0.2 μm black-stained polycarbonate filters (Nuclepore).

Filters were mounted on microscope slides, between layers of non-fluorescent immersion oil (Olympus), and counted within a few hours under a UV filter set, using an Olympus BX 60 F5 epifluorescence microscope at 1000×. At least 20 random fields and a minimum of 300 cells were counted for each filter. Each HBA value represents the mean of triplicate samples with a variation coefficient of less than 5%.

2.3 *Data analysis*

The dispersion of HBA data set over the study period was estimated by means of the coefficient of variation (CV = SD/mean × 100).

A principal-component analysis (PCA) [27, 28] was used to better understand the information of our multivariate data set considering only surface values. This multivariate analysis rotates a cloud of data points such that the maximum variability is visible to facilitate the identification of the most important gradients over the long- and short-term study periods. Temperature, salinity, DOC, Chl *a*, and HBA values were standardized for the analysis by subtracting the mean and dividing by the standard deviation.

3. Results and discussion

The HBA annual range varied every year of one order of magnitude, from 10^8 to 10^9 cell l^{-1}, with an annual CV of approximately 60% (table 1). Estimates of HBA fall within the range reported by Del Negro *et al.* [11] and Turk *et al.* [12] for the Gulf of Trieste. The annual HBA range and CV were found to be similar during the 5 yr, except for the low *CV* value in 2001 (37%) and the maximum HBA value of 54.6×10^8 cell l^{-1} in 2002, which was twice as high as that in the other years. HBA annual mean values were always slightly higher than medians, and both mean and median values showed an increasing trend from 1999 to 2002 which strongly decreased in 2003 reaching values weakly higher than in 1999. In the daily sampling period, the HBA were in the range of values detected during the whole 2002 (table 1) with almost the same median, 12.9×10^8 cell l^{-1} against 13.0×10^8 cell l^{-1}, respectively, and a slightly lower average of 13.2×10^8 cell l^{-1} against 16.0×10^8 cell l^{-1} for the whole year. The CV of all data was lower than 30%, and the threefold HBA range variation showed a monthly variability lower than the annual variability.

During 2000 and 2002, the highest HBA annual variations were observed, and HBA data sets showed a high dispersion from median values (figure 2). In these two years, mucilage events occurred in the Gulf of Trieste, and this might have affected the characteristic annual range and distribution pattern of HBA.

The temporal distribution of HBA during the 5 yr (figure 3A) showed a clear annual periodicity with the lowest and highest values in winter and summer, respectively. This seems to be mainly due to the effect of temperature which controls, possibly indirectly, the growth of the heterotrophic bacterial community. The high annual variation in water temperature, from $<6\,^{\circ}$C in winter to $>25\,^{\circ}$C in summer, determines a clear seasonal pattern in the Gulf of Trieste. The winter photo-limitation and low temperatures usually inhibit algal blooms which are mainly responsible for the production of the organic matter that represents a favourable substrate for bacterial growth [29]. Also, Gerdts *et al.* [30], in a long-term study of microbial parameters, found that bacterial counts were positively correlated to water temperature. No significant variations (lower than twofold) were observed at the four sampling depths except for a few sporadic episodes like October 2002 and May 2003 when the HBA was more than twofold higher at the surface and at the bottom than at -5 and -10 m (figure 3A). The twice monthly sampling (figure 3B) confirmed the same HBA annual pattern observed with the monthly sampling. Generally, no significant variations (lower than twofold) between two consecutive samplings were observed by using both monthly and twice a month sampling strategies. In October 2002, May 2003, and December 2003, the highest HBA variations, from twofold to more than fourfold, occurred between the first and second sampling of each month both at the surface and at the bottom depths. These differences might have been due to the

Table 1. Range, mean, median, and coefficient of variation (CV = SD/mean × 100) of heterotrophic bacterial abundances (HBA) from 1999 to 2003.

HBA	Range ($\times 10^8$ cell l^{-1})	Mean ($\times 10^8$ cell l^{-1})	Median ($\times 10^8$ cell l^{-1})	CV (%)
1999	3.6–25.5	8.1	6.5	58.2
2000	1.8–26.1	11.7	10.2	57.8
2001	6.0–25.9	12.5	11.3	37.1
2002	5.5–54.6	16.0	13.0	63.2
2003	1.6–37.8	11.0	8.7	60.8
12 Feb 2002–11 Mar 2002	8.1–23.6	13.2	12.9	27.9

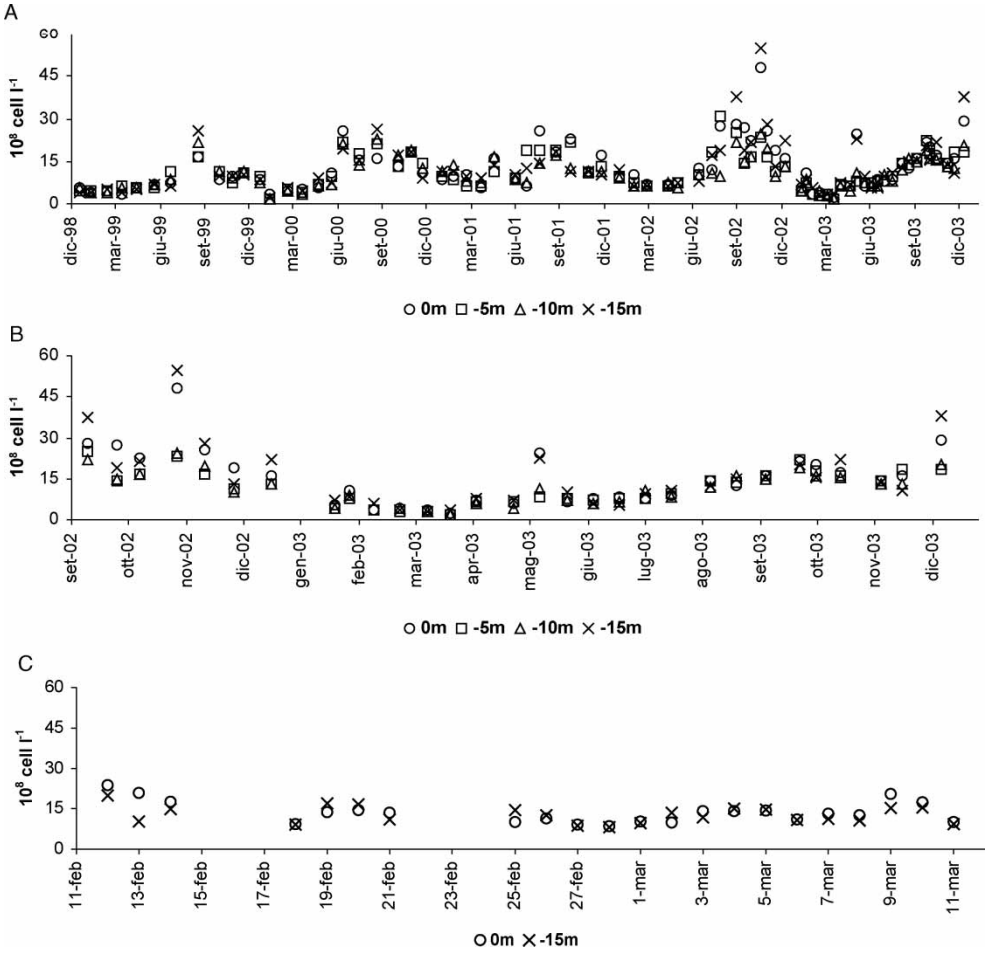

Figure 3. Heterotrophic bacterial abundance (HBA) from 1999 to 2003 (A) collected at three different timescales: monthly from January 1999 to August 2002; twice monthly from September 2002 to December 2003 (B); daily from 12 February to 11 March 2002 (C). Each point represents the mean of triplicate samples with a variation coefficient of less than 5%.

ability of marine bacteria to rapidly modify their metabolism [9, 10] and aggregate into 'hot spots' in response to favourable micro-environmental conditions [8].

During the daily sampling period, HBA ranged from 8.1 to 23.6 $\times$ 10^8 cell l^{-1} and showed the same pattern at the two sampling depths (figure 3C). From the initially HBA maxima, there was a decreasing trend until the end of February. From 1 March, HBA progressively increased. The highest variation between two consecutive samplings was about twofold and in any case lower than that observed with a lower sampling frequency.

The relation between surface values of HBA, temperature, salinity, DOC, and Chl *a* in the long- and short-term study periods is discussed here by using a PCA. This multivariate analysis showed a helpful distribution of variables in new linear combinations (PC). Only PC with eigenvalues >1 are considered. Variables with the greatest absolute magnitude or loading in each PC have the greatest influence on the sample separations or projections for that PC [31].

In the 1999–2002 period, the first two components (PC1 and PC2) together explained 62.6% of the total variance (table 2). PC1 was found to be highly positively related to temperature, DOC, and HBA, and weakly negatively related to salinity and Chl *a*. Inversely PC2 was

Table 2. Rotated variables (loadings), percentage variance, and cumulative variance for the first and second components of the PCA, considering surface values of a long-term (1999–2003) and short-term (12 Feb 2002–11 Mar 2002) study period, carried with a monthly/twice a month and daily sampling frequency, respectively.

		Temperature	Salinity	DOC	Chl *a*	HBA	Percentage variance	Cumulative
1999–2003	PC1	0.62	–0.34	0.54	−0.19	0.43	37.9	37.9
	PC2	0.21	0.61	−0.21	−0.73	0.11	24.7	62.6
12 Feb 2002–	PC1	−0.50	−0.56	0.49	−0.27	0.36	51.7	51.7
11 Mar 2002	PC2	−0.03	−0.30	−0.11	0.90	0.30	18.5	70.2

highly related to salinity and Chl *a* positively and negatively, respectively. In the diagram of PC1 vs. PC2 the resulting samplings were clearly distributed on a gradient mainly determined by the PC1 axis (figure 4A). PC1 showed a clear annual pattern of distribution (figure 4B). Temperature, DOC, and HBA varied together, always reaching minima and maxima values in winter and summer, respectively. PC2 showed a less defined gradient, but samplings seemed to reach minima (high Chl *a* and low salinity values) in early spring and weakly in autumn (figure 4C) when generally intense rainfalls and algal blooms occurred. A weak increasing trend of PC2 values suggests that freshwater input and autotrophic biomass generally decreased over the study period. According to Fonda Umani *et al.* [32], our results support the recent findings of a general oligotrophy characterizing the pelagic system in the Gulf of Trieste.

HBA seems to be positively related to the water temperature and DOC availability over the year. The spring and autumn low water salinity is due to the terrestrial freshwater inputs typical of this periods. Freshwater inputs by carrying inorganic nutrients support algal blooms. Terrestrial inputs and phytoplankton together help to increase the DOC availability for bacterial growth. Both HBA and DOC concentrations begin to rise in the late spring and reach high values during the summer until the end of the autumn. The winter period, characterized by low temperatures, reduced daylight, strong winds, and a lack of rainfall, generally inhibits the growth of autotrophic and heterotrophic planktonic organisms.

During the daily sampling period, the amount of variance explained by the PC1 and PC2 was higher than 70% (Table II). PC1 was found to be negatively related to temperature and salinity and positively related to DOC, while PC2 was highly positively related to Chl *a*. Both components showed a similar positive but weak relation to HBA. The diagram of PC1 vs. PC2 (figure 5A) showed an arch effect [33]. This is because there is a predominant gradient, determined by PC1, while PC2 showed a non-linear gradient in the sampling distribution. Samplings on PC1 showed a progressively decreasing trend suggesting an increase in temperature and salinity values and a decrease in DOC concentration (figure 5B). PC2 showed high values at the beginning of the sampling and in the first days of March, periods characterized by high Chl *a* concentrations (figure 5C). A different temporary distribution occurred between HBA (figure 3C), PC1 (figure 5B), and PC2 (figure 5C).

The daily sampling period fell within the vernal blooming season [34] when surface temperatures begin to rise, and rainfalls usually increase organic matter and nutrient availability. Despite the HBA dependence on temperature during a whole year, in the short sampling period HBA was strongly and inversely related to salinity ($r = -0.85$, $n = 14$, $P < 0.001$) within the first 3 weeks, thus confirming the role of freshwater input in increasing substrate availability for bacterial growth. At the beginning of the daily sampling, the surface water was characterized by low temperatures typical of late winter which progressively increased (PC1: figure 5B). Rainfalls which occurred in the days before the sampling period helped to increase the terrestrial input. Freshwater, which determined the initial low surface salinity (PC1: figure 5B), also carried inorganic nutrients, which sustained the development of an algal bloom responsible for the production of favourable substrate for bacteria [29].

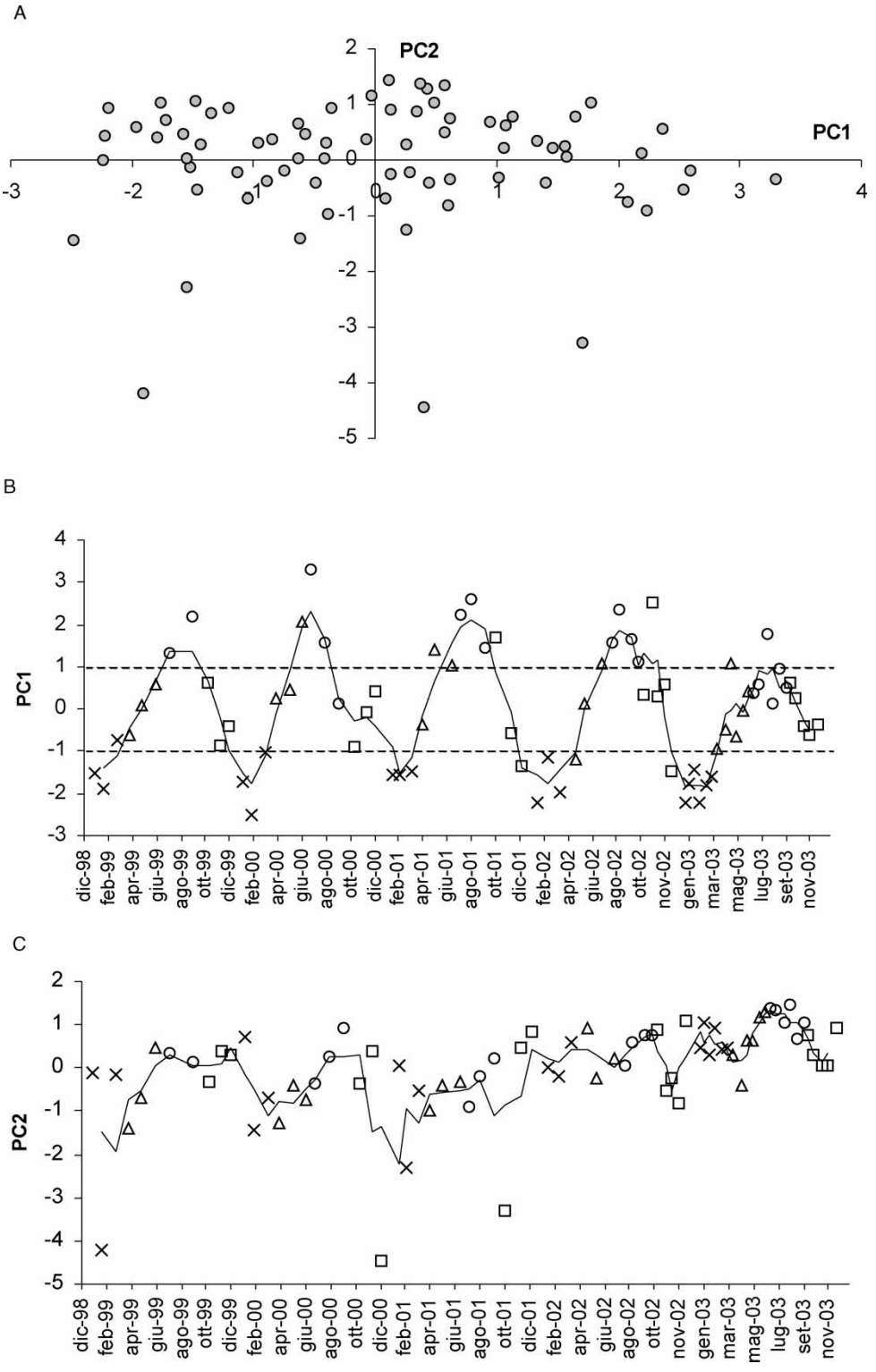

Figure 4. Diagram of PC1 vs PC2 (A) and sampling plots for the first (B) and second (C) principal components of the PCA models of a long-term study period (1999–2003). X = Jan–Mar, Δ = Apr–Jun, ○ = Jul–Sep, □ = Oct–Dec. The trend is calculated by a three-sampling simple moving average.

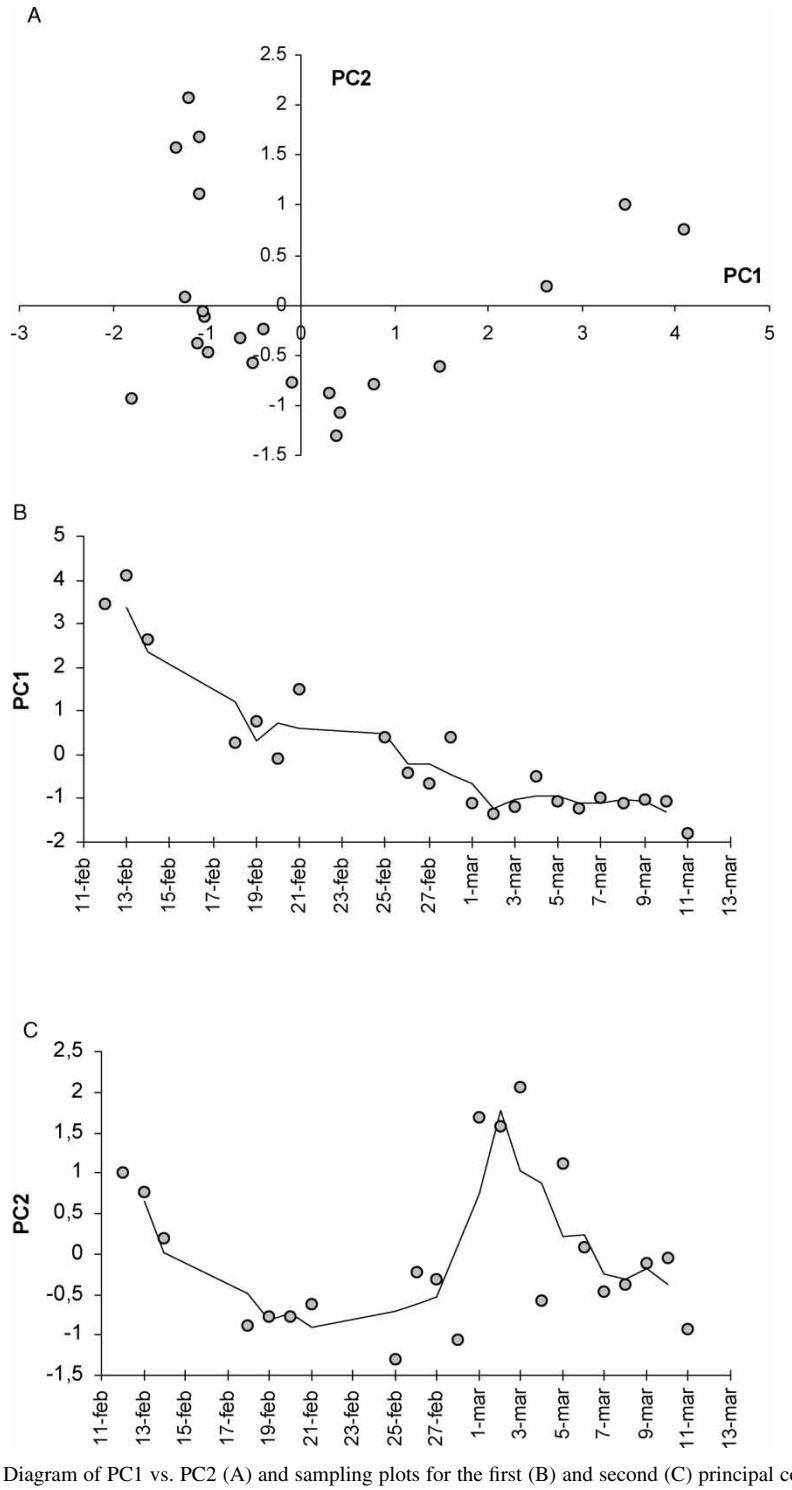

Figure 5. Diagram of PC1 vs. PC2 (A) and sampling plots for the first (B) and second (C) principal components of the PCA models of a short-term study period (12 February to 11 March 2003). The trend is calculated by a three-sampling simple moving average.

HBA (figure 3C), DOC (PC1: figure 5B) and Chl *a* (PC2: figure 5C) concentrations reached high values in the first 3 d. Stormy winds occurred from 15 to 17 February and from 22 to 24 February, helping to strongly modify the structure of the water column and corresponding to a drastic decrease in HBA. A second maximum of Chl *a* occurred at the beginning of March, suggesting that another phytoplanktonic bloom sustained the increase in HBA. The low DOC values after the second algal bloom lead us to the hypothesis that the high DOC concentration of the first days was mainly as a result of riverine outflow rather than of autochthonous origin.

4. Conclusion

The sampling scheme here utilized to detect HBA at a coastal station of the Gulf of Trieste resulted in a sufficient time resolution to assess changes due to temporal variability. There is an annual pattern of HBA distribution almost partially influenced by water temperature, rainfalls and terrestrial inputs, Chl *a* concentration (as a proxy of phytoplankton), and DOC availability. The continuation of the long-term study could confirm this first result and should provide information about the behaviour of bacterial community in response to long-term climatic changes. Intensifying our samplings from monthly to twice monthly did not yield any more information about the HBA annual pattern but did allow us to detect the sporadic HBA hot spots as a consequence of changes in local environmental conditions. In monitoring coastal waters, monthly sampling should be frequent enough to catch any anomalies in the annual HBA pattern of distribution, and the cost–benefit ratio of a more intensive sampling results extremely high. Because of the ability of HBA to rapidly modify their metabolism and abundances, the most direct way to determine the relationships with the environment is to use a short-term sampling strategy. Knowledge about short-term HBA variations could also confirm, as in our case, that annual studies, carried out with monthly or twice monthly observations, provide certain information about the annual HBA dynamics, even if a conspicuous part of the real distribution remains unknown by using this sampling strategy. Obviously, the optimal sampling strategy is to integrate the HBA analysis at different timescales, from diel, to daily, to monthly until a substantial time series is developed.

Acknowledgements

This work was supported by the European Community and the Friuli Venezia Giulia Interreg I, II, III Italy–Slovenia project. The authors would like to thank Dr C. De Vittor for DOC and Chl *a* analyses and Dr M. Celio for support in obtaining physical data.

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