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The role of water mass dynamics in controlling bacterial abundance and production in the middle Adriatic Sea

M. Šolić, N. Krstulović, I. Vilibić, G. Kušpilić, S. Šestanović, D. Šantić
and M. Ordulj*

Institute of Oceanography and Fisheries, POB 500, 21000 Split, Croatia

* Corresponding author:

Mladen Šolić

Institute of Oceanography and Fisheries

POB 500, 21000 Split

Croatia

Phone: 385-21-408 006

Fax: 385-21-358 650

e-mail: solic@izor.hr

ABSTRACT

Month-to-month fluctuations in the abundance of bacteria and heterotrophic nanoflagellates (HNF) and bacterial production, as well as various chemical (nutrients, oxygen) and physical (salinity, temperature) parameters were analysed at a station located in the open middle Adriatic Sea during one decade (1997-2006). Being influenced by both coastal waters and open Adriatic circulation in the surface layer, and by the deep Adriatic water masses in the deep layers (100 m), this station is quite suitable for detecting the environmental changes occurring in the open Adriatic Sea with respect to the circulation of its water masses and their long-term changes and anomalies. Multivariate methods were used to identify seasonal and interannual changes of the investigated parameters, associating observed changes to the changes in Adriatic water masses and circulation regimes. The analyses showed that bacterial abundance and production were controlled by different water mass dynamics during 1997-2001 compared to 2002-2006 period, particularly noticeable in different seasonal patterns of biological parameters. The interplay between North Adriatic Dense Water (NAdDW) and Levantine Intermediate Water (LIW) resulted in a change in available nutrients (NAdDW is poor in orthophosphates), and as a consequence different bacterial abundance and production. A few periods were examined in detail, such as 2004, when LIW inflow was particularly strong and was accompanied by an increase of bacterial and HNF abundances, as well as of bacterial production.

Key words: data sampling, bacteria, heterotrophic nanoflagellates, water masses, multivariate analysis, Adriatic Sea.

1. INTRODUCTION

The Adriatic Sea is the northernmost basin in the Mediterranean, with length of 800 km and width of 200-250 km. According to the bathymetry, the Adriatic Sea may be divided into three parts: a broad northern Adriatic shelf with an average depth of 40 m, the middle Adriatic with depressions up to 280 m, connected to the southern Adriatic circular basin (depths up to 1230 m) over the Palagruža Sill (deepest parts of 170 m). As the circulation of the Adriatic is cyclonic due to estuarine conditions regulated by the northern Adriatic rivers (Cushman-Roisin et al., 2001) resulting in outflowing West Adriatic Current, eastern Adriatic region is exposed to the northwestern East Adriatic Current (Orlić et al., 2007), bringing saline waters from the Ionian and Levantine Seas along its pathway. These waters, known as Levantine Intermediate Water (LIW, Robinson et al., 1992; Malanotte-Rizzoli et al., 1997), can flood the Adriatic in some years (so-called ingression years, Buljan, 1953), increasing salinity, temperature and nutrients in intermediate layers (Buljan and Zore-Armanda, 1979; Tziperman and Malanotte-Rizzoli, 1991; Vilibić and Orlić, 2001, 2002).

The impact of LIW inflow on the biogeochemical cycles in the Adriatic Sea is substantial, and fluctuation of a number of physical, chemical and biological parameters in the Adriatic Sea was attributed to the LIW ingression. The year 1967/68 is considered to be a classical ingression year, resulting in large LIW fraction in the surface and intermediate layers of the south Adriatic (Vilibić and Orlić, 2001). Increases in salinity and nutrient levels were recorded in the middle Adriatic as well (Zore-Armanda et al., 1987), resulting in maximum phytoplankton populations in 1968 (Pucher-Petković and Marasović, 1980) and maximum primary production in 1969 (Pucher-Petković et al., 1987). Maximum quantities of sardine eggs were also recorded there in 1968/1969 and 1969/1970 (Karlovac, 1973). The next primary production maximum was reported for the year 1980, which was also characterized by a strong LIW inflow (Vilibić and Orlić, 2001) and by an increase of nutrient concentrations (Pucher-Petković et al., 1987). Maximal total bacterial counts in the middle

Adriatic were found in the same year, as well as maximal bacterioplankton production (Krstulović et al., 1995). Also, two pronounced maxima in the 23-year time series of heterotrophic bacteria, primary production and zooplankton abundance were recorded in 1968 and 1980 (Šolić and Krstulović, 1991a,b; Baranović et al., 1993; Šolić et al., 1997). Brankart and Pinardi (2001) proposed that at the beginning of the 1980s larger quantities of east Mediterranean water entered the south Adriatic, which caused a decrease in the salinity in the Eastern Mediterranean and an increase in the north Aegean Sea and south Adriatic Sea.

In some years LIW intrusion may reach northern Adriatic, and during wintertime it serves as a preconditioning for the generation of another deep Adriatic water mass. North Adriatic Dense Water (NAdDW, Zore-Armanda, 1963; Artegiani et al., 1997) is the densest Mediterranean water mass (density excess up to 30 kg m^{-3} , Vilibić, 2003), generated during strong and long-lasting wintertime bora outbreaks (Dorman et al., 2007). During spring and summer it moves downslope along the western Adriatic shelf, strongly hits and fills up deep layers of the Jabuka Pit (Vilibić et al., 2004). Its less dense component goes over the Palagruža Sill (Vilibić and Supić, 2005; Wang et al., 2007) and sinks rapidly towards the bottom of the South Adriatic Pit through the mixing and channelling processes in the Bari Canyon (Manca et al., 2002; Vilibić and Šantić, 2007). As it is generated on the open northern Adriatic shelf, and not influenced by coastal waters rich in nutrients (Tedesco et al., 2007), NAdDW brings a small amount of orthophosphates to the deep near-bottom Adriatic waters, being somewhat regenerated there through the oxidation processes (Krasakopoulou et al., 2005). However, NAdDW nutrients in the 100-150 m layer above the near-bottom water masses show even lower nutrient concentrations, as this zone of the water column is not influenced by the regeneration but rather with nutrient consumption through the primary production. NAdDW generation does not occur every winter, and it is enabled by a number of factors (heat flux preconditioning, low river discharges, wintertime cooling intensity). These

conditions are being driven by climate and hemispheric processes such as North Atlantic Oscillation (Hurrell, 1995), Mediterranean Oscillation (Grbec et al., 2003) and others, having various temporal and spatial changes in their patterns and intensity. Rather strong anomalies may occur in the climate regimes, as in 2003 (Grbec et al., 2007), influencing the Adriatic water masses and their kinetics and dynamics (Supić et al., 2004). Consequently, the changes in various biogeochemical parameters may be attributed to these regimes (Dulčić et al., 2004; Kršinić and Grbec, 2006), including the LIW ingressions and their consequences in the cycle already described.

Temporal changes of different hydrographic, chemical and biological parameters from different periods between 1960 and 1996 have been reported for Adriatic Sea (Šolić and Krstulović, 1991a; Šolić et al., 1997; Marasović et al., 2005). In this study, month-to-month fluctuations in the abundance of bacteria and heterotrophic nanoflagellates (HNF), as well as bacterial production during a decade, from 1997 to 2006, were analysed at a reference station. Multivariate methods were used to identify the main patterns of year-to-year changes of some biological parameters in relation to physical and chemical parameters, emphasizing the observed changes in seasonal patterns, long-term changes and their conjunction to the changes in Adriatic water masses and circulation regimes.

2. MATERIALS AND METHODS

2.1. Data collection

The station where data were collected for this study is located southeast of Cape Stončica on Vis Island (43°00' N; 16°20' E), about 50 km offshore, and is 100 m deep (Fig. 1). Being located just out of the middle Adriatic island coastal waters, influenced by the largest east Adriatic river (Neretva river, Raicich, 1996), this station feels these freshened waters in

its surface layer but also the open Adriatic circulation and water masses during dryer periods (Grbec and Morović, 1997). In contrast, the deep layers are flooded by the open Adriatic water masses, mostly LIW and NAdDW, which are characterized by smaller oscillations of chemical and physical parameters in comparison to coastal area (Buljan and Zore-Armanda, 1976). Therefore, this station is quite suitable for detecting the environmental changes in the open Adriatic Sea in relation to its circulation, water masses and their long-term changes and anomalies.

Another aspect favourable for the choice of this station is the excellent temporal coverage of the measurements of various environmental parameters, from physical (temperature, salinity), chemical (oxygen, nutrients) towards biological (bacteria) ones. Systematic measurement at the Stončica Cape started already in 1950s' (Buljan and Zore-Armanda, 1966), and a large amount of data has been collected till the present. For the period of this study (1997-2006), monthly coverage with the measurements of all studied parameters (temperature, salinity, nutrients, bacteria) was larger than 75%, with no gap longer than three consecutive months. Samples were collected at standard oceanographic depths (0, 5, 10, 20, 30, 50, 75 and 100 m), processed on board immediately after sampling, and by the same methods of sampling and analyses throughout the investigated period.

Bacteria and heterotrophic nanoflagellates (HNF) were enumerated by epifluorescence microscopy ("Olympus" BX50 at a magnification of x1000), using the standard DAPI staining technique (Porter and Feig, 1980). Bacterial cell production was measured from DNA synthesis based on incorporation rates of ^3H -thymidine (Fuhrman and Azam, 1982). (Methyl- ^3H -) thymidine was added to 10 ml samples at a final concentration of 10 nmol (specific activity 86 Ci mmol $^{-1}$). Triplicate samples and a formaldehyde killed adsorption control (final concentration 0.5%) were incubated for 1 hour. The incubations were stopped with formaldehyde (final concentration 0.5%). The thymidine samples were extracted with ice-cold

TCA according to Fuhrman and Azam (1982). The TCA-insoluble fraction was collected by filtering the sample through a 0.2 μm pore size Sartorius filter. The percentage of thymidine converted to protein was not checked. Conversion factors for bacterial cell production were calculated from bacterial cell number and ^3H -thymidine incorporation during bacterial growth in 1 μm -prefiltered seawater (Riemann et al., 1987): $\text{CF} = (\text{N}_2 - \text{N}_1) / ^3\text{H}$, where N_1 – number of bacteria in the beginning of the experiment, N_2 – number of bacteria at the end of the experiment, ^3H – integrated ^3H -thymidine incorporation rate during the experiment.

Dissolved oxygen concentration was determined by Winkler titration (Strickland and Parsons, 1968). Nutrients (NO_3 , NO_2 , NH_4 , PO_4) were analysed on Bran+Luebbe AutoAnalyser (II and III) models, using modified automated methods (Grasshoff, 1976). The temperature and salinity were measured with CTD multiparameter probes (Idronaut and SeaBird), with accuracy larger than $\pm 0.01^\circ\text{C}$ and ± 0.02 , respectively.

2.2. Analysing procedures

All statistical analyses were performed using the statistical package StatSoft Inc. STATISTICA for Windows version 6. (<http://www.statsoft.com>). The major idea supporting the analyses was to separate various processes at various temporal scales, so all of time series were decomposed first into the seasonal, trend-cycle and random or irregular components by Census Method 1 and X-11 Census Method II procedures using multiplicative model (Makridakis and Wheelwright, 1989). As such procedure requires continuous time series, the missing data were estimated and gaps were filled through linear interpolation from the surrounding values.

The separation of the components was performed by the following assumption:

$$X_t = TC_t * S_t * I_t \quad (1)$$

151

152 where X_t is original time series, TC_t is trend-cycle component, S_t is seasonal component and I_t
153 is irregular component. The separation procedure is as follows:

154

- 155 1. The removing of seasonal variability from the time series is based on the 12-month
156 centred moving average scheme: as intra-annual variability was eliminated in the
157 smoothed moving average series, the difference between the observed and the smoothed
158 series gives the seasonal plus irregular (intra-annual) component.
- 159 2. The seasonal component S_t was then computed as the medial average for each month
160 during the respective period, resulting in the average seasonal series and annual course
161 of the seasonal component.
- 162 3. The seasonal component S_t was removed from the original series by dividing with it.
163 Then, trend-cycle component TC_t was estimated by applying a 5 point centred weighted
164 moving average smoothing transformation with the weights of 1, 2, 3, 2 and 1, with the
165 largest weight attributed to the actual value and then decreasing forward and backward
166 in time. Finally, the random or irregular component I_t was estimated by dividing the
167 seasonally adjusted series by the TC_t component.

168

169 It should be pointed out that the separation between various temporal components can
170 be done in a number of different ways, but we choose such a procedure as it gives the results
171 which can be interpreted and discussed in the light of different processes in the sea.

172 In addition, Principal Component Analysis (PCA) was used to identify the main patterns
173 of trend-cycle and seasonal components. The analyses were all based on correlation matrices
174 involving the standardization of each variable to zero, mean and unit variance. That was done

to eliminate differences in the values between parameters, leaving only the relative seasonal or month-to-month changes in the values.

3. RESULTS AND DISCUSSION

3.1. Decomposition of time-series

Time-series of bacterial abundance (B), abundance of heterotrophic nanoflagellates (HNF), total bacterial production (TBP) and specific bacterial production or production per bacterial cell (SBP) were decomposed to identify seasonal (seasonal component) and non-seasonal (trend-cycle) patterns of fluctuations. Similar patterns of components were computed throughout the first 50 m depth, while the patterns at 75 and 100 m were somewhat different. Therefore, decomposition of time-series were performed separately for “surface layer” (mean values integrated for the water column from surface to 50 m depth) and for “bottom layer” (mean values integrated for the water column from 75 to 100 m depth).

Principal Component Analysis (PCA) was used to identify the main patterns of non-seasonal and seasonal fluctuations of examined parameters. To identify the main patterns of non-seasonal fluctuations, PCA was carried out on arrays consisting of the trend-cycle component data sets for bacterial abundance, HNF abundance, total bacterial production and bacterial production per cell, separately for surface and bottom layers (Fig. 2A). The first principal component (PC1), which can be regarded as the best possible single representation of the non-seasonal fluctuations for all studied parameters, explains 44% of variability, and PC2 explains another 26% of variability. PC Analysis showed similar patterns of fluctuations between surface and bottom layers for bacterial and HNF abundance, and different patterns for both total and specific bacterial production. On the other hand, the differences in fluctuations between examined parameters are more clearly expressed in Fig. 2B-E. Bacterial

abundance is fairly well represented by PC1 ($r = 0.79$ in surface layers, $r = 0.72$ in bottom layers; $P < 0.001$) (Fig. 2B). Joint maxima between PC1 and bacterial abundance were recorded in winter 1998, winter 2002 and late spring 2005, and joint minima in spring 1997, autumn 2000, throughout the all 2004 and in autumn/winter 2005. Significant negative correlation ($r = -0.91$; $P < 0.001$) was found between PC1 and bacterial production (both, total and per cell) in surface layers. All marked maxima in bacterial production coincide with marked minima in PC1 (Fig. 2C). On the other hand, bacterial production in bottom layers is well represented by PC2 ($r = 0.88$ for total bacterial production, $r = 0.82$ for bacterial production per cell; $P < 0.001$) because of the joint maxima in spring 2002, winter 2005 and winter 2006, as well as joint minima in autumn 1999 and winter 2004 (Fig. 2D). Finally, abundance of HNF showed significant negative correlation with PC2 ($r = -0.87$ in surface layers, $r = -0.86$ in bottom layers; $P < 0.001$) (Fig. 2E).

To identify the main patterns of seasonal fluctuations, PCA was carried out on arrays consisting of the seasonal component data sets for all studied parameters, separately for surface and bottom layers (Fig. 3A). Analysis showed similar seasonal patterns between surface and bottom layers for all parameters, except HNF abundance which showed very different patterns between surface and bottom layers. PC1 explain 53% of variability and is significantly negatively correlated with bacterial production (total and per cell) in both surface and bottom layers (Fig. 3B,C), and with HNF abundance in surface layers (Fig. 3B) (coefficients of correlation ranged between -0.74 and -0.94 ; $P < 0.001$). The main characteristics of the seasonal components for these parameters are higher values during the warmest part of the year (May to September), and lower values during the coldest part of the year (PC1 showed opposite pattern). Bacterial abundance (in surface and bottom layers) is fairly well represented by PC2 which explain 24% of variability ($r = 0.95$ in surface layers, $r = 0.81$ in bottom layers; $P < 0.001$) (Fig. 3D). Characteristics of this pattern is spring maximum

(in April or May) and another maximum in August (not expressed in bottom layers), while the minima occurs in June or July and throughout autumn-winter period.

To test variability in seasonal and non-seasonal fluctuations of examined parameters throughout the studied period, PCA were carried out on arrays consisting of the seasonal component and trend-cycle component data sets for all examined parameters for each year, separately for surface and bottom layers (Fig. 4). The results suggest no consistency in either seasonal and non-seasonal patterns throughout the studied period. However, fluctuations of these components were not random, but were characterised by systematic changes and grouping in time. PCA showed a clear separation between 1997-2001 and 2002-2006 period for both seasonal and trend-cycle components, as well as for both surface and bottom layers (Fig. 4). Due to the above results, we found it useful to separate the analysis into two periods: 1997-2001 and 2002-2006, as a significant shift in annual time-course happened at some stage in 2001/2002.

3.2. Comparison between 1997-2001 and 2002-2006 periods

Comparison of seasonal components between 1997-2001 and 2002-2006 periods shows substantial changes in seasonal patterns between the two periods, keeping their consistency within both periods. No statistically significant correlation ($P > 0.5$) has been found between 1997-2001 and 2002-2006 periods for all studied parameters in both surface and bottom layers (Figs. 5, 6); surprisingly, they show even an opposite behaviour (negative correlations) for some months and parameters. Namely, bacterial abundance showed higher values during winter-spring, with maximum values in May, than during the summer of 1997-2001. Similar patterns were established in both surface (Fig. 5A) and bottom layers (Fig. 6A). In contrast, bacterial abundance was higher in spring-summer than in winter, with maximum

values in April during 2002-2006 period (Figs. 5E, 6E). A different pattern was observed for HNF abundance in the surface layer; characterized by high values during the warmer part of the year (from April to August) and very low values during winter in the 1997-2001 period (Fig. 5B). Conversely, HNF abundance was high only during July-August during the 2002-2006 period, maintaining very low values from April to June and with rather high values recorded in January (Fig. 5F). In the bottom layers a substantial maximum was recorded in September-October and minimum in April-May during 1997-2001 period (Fig. 6B). In contrast, the highest values were established in January-February and the lowest values in October-November during 2002-2006 period (Fig. 6F). To summarize, bacterial production (both total and per cell) followed a similar seasonal pattern during 1997-2001 period, in both surface and bottom layers. This pattern was characterized by high values during the warmest part of the year (from April to September), and low values during the coldest part of the year (from November to January) (Figs. 5C-D, 6C-D). During the 2002-2006 period, however, low values were established from July to September, with much higher values during winter and a peak in May (Figs. 5G-H, 6G-H). All of these findings suggest a major shift in biological parameters between the two periods, presumably related to the environmental parameters and circulation changes, which will be discussed in Section 3.3.

As previously reported, the variance of trend-cycle and seasonal components has substantially changed over time for most of the parameters, particularly in the bottom layer (the best examples are presented in Fig. 7). For example, an average contributions of trend-cycle and seasonal components in explaining of the total time series variability were equal for bacterial abundance during 1997-2001 period (both contributions were about 32%), but during 2002-2006 period trend-cycle component increased to 48% and seasonal component decreased to 25% (Fig. 7A). Similar patterns were established for HNF (Fig. 7B), and SBP (Fig. 7C).

Correlation analyses between biological parameters were made separately for 1997-2001 and 2002-2006 periods in both surface and bottom layers (Tab. 1). There are significant positive correlations between TBP and SBP in both periods and both layers. In the surface layer the correlations are quite high ($P < 0.001$) in 2002-2006 period (bacteria were negatively correlated with HNF, TBP and SBP, and HNF were positively correlated with TBP and SBP), while they were not significant in the 1997-2001 period, presumably related to the changes in environmental conditions (see next section). In the bottom layer, significant positive correlations were established between bacteria and TBP, as well as between HNF and both TBP and SBP during the 1997-2001 period. On the other hand, during the 2002-2006 period, significant negative correlations of bacteria with HNF and SBP were established (Tab. 1). HNF are the most important bacterial grazers in the Adriatic Sea (Šolić and Krstulović, 1995; Šolić et al., 1998), therefore the higher correlation between HNF and bacterial production compared with bacterial abundance suggests that HNF control bacterial standing stock by direct cropping of bacterial production. Similar results were reported for the coastal Adriatic Sea (Šolić and Krstulović, 1994). Gonzalez et al. (1990) and Sherr et al. (1992) suggest that bacterivores crop the production rather than affecting the standing stock of bacteria because of a higher grazing pressure on more actively growing and dividing cells. This is supported by the fact that HNF abundance was strongly correlated with SBP (better indicator of bacterial reproductive activity) than with TBP, which is function not only of individual cell activity but also of bacterial abundance (Tab. 1).

3.3. Relation to the environmental factors

In order to explain the dynamics of biological parameters observed during the examined period, the association between their fluctuations and fluctuations in a number of environmental factors was analysed. Very few significant positive correlations were found

between biological and environmental factors throughout the whole 10-year study period, such as correlations between bacterial production and different nitrogen compounds. The reason for this is a shift observed in the seasonal cycle sometime in 2001/2002, which reduced the significance of the relationship between the examined parameters. In contrast, separate analyses of 1997-2001 and 2002-2006 periods brought out much more significant correlations between these factors (Tab. 2). Therefore, the environmental factors showed a significant shift between these two periods. In the surface layer, strong correlation between bacterial production and nitrate concentration was found during 1997-2001 period (Fig. 8A and Tab. 2), but not during 2002-2006. Other chemical and physical parameters were mostly not correlated to the bacterial parameters, as the environmental parameters are strongly affected by the processes which are not taken into account in the analyses, such as air-sea interaction, precipitation, etc. Their influence at the Stončica station is usually irregular and occurring on lower time scales (hourly or daily processes, e.g. synoptic perturbations, storms, etc.) and therefore producing undersampled and aliased time-series. Compared to the surface, the bottom layer is less affected by short-term variability and therefore the number of positive significant correlation is much higher in the bottom layer, as the fraction of aliased (periods less than 2 months) processes is significantly lower, and bacterial dynamics is mostly under the influence of stable deep Adriatic water masses (LIW, NAdDW). In general, all biological parameters showed positive correlation with nitrogen compounds (nitrate, ammonia, DIN) and consequently with N/P ratio (Tab. 2, Fig. 8B,C), which initially might suggest nitrogen limited conditions. However, this is contrary to the general perception of the Adriatic Sea and the eastern Mediterranean being poor with orthophosphate (Buljan and Zore-Armanda, 1976; Krom et al., 1991; Vukadin and Stojanoski, 2001). P-limitation in these basins are usually considered in relation to primary production. Nevertheless, Thingstad et al. (2005) established in an *in situ* mesoscale enrichment experiment in the eastern Mediterranean a positive

correlation between phosphorus and bacterial production, with a rapid transfer of phosphorus from bacteria to mesozooplankton throughout the microbial food web (bypassing the phytoplankton).

In the surface layers, year-to-year fluctuations of temperature and salinity are primarily under the influence of surface heating/cooling processes and the interplay between coastal/open Adriatic waters. Namely, temperature in 2001 had the lowest seasonal signal (as a result of higher winter and lower summer temperatures than usual), but a rather broad range of salinity values (Fig. 9A,B), as a result of warmer winter and coastal waters being dominated by large river runoffs. The years 2004 and 2005 were characterised by higher winter and lower summer temperature, respectively. Both years were also characterized by a wide range of salinities, as a result of high precipitation and large river runoff (river Neretva), which during some periods reached the investigated area (Fig. 9A,B). In the surface layers, the temperature time series has been predominantly determined by seasonal component (Fig. 10A). The trend-cycle component showed a strong non-seasonal increase in temperature during January-February 2001, which is presumably the result of a warmer-than-average winter and low surface cooling (Russo et al., 2005). This increase in temperature was followed by an increase in bacterial production, as well as oxygen saturation, which suggests an increase in primary production (Fig. 10B,C).

In the bottom layers, year-to-year fluctuations of the parameters showed two interesting periods characterized by marked non-seasonal changes of temperature and salinity. Salinity time-series showed lower values throughout the 1997-1998 period, and an upward trend until 2004 (Fig. 9D), when it surpassed 38.8. Low salinity in 1997 and 1998 was accompanied with lower-than-average temperatures, whereas high salinity in 2004 was accompanied with higher-than-average temperatures (Fig. 9C). The patterns of abiotic and biotic parameter fluctuations could be partly explained by the dynamics of the water masses,

causing high correlations for the bottom layer; however, they differ to some extent between two analysed periods (Tab. 2). The correlation of HNF, TBP and SBP with nitrate and DIN concentrations in the period 1997-2001 is strongly positive, but with phosphate, temperature and salinity is strongly negative. It follows that the largest bacterial production is supported by the largest supply of nitrogen components brought by the coldest and less saline waters. These latter features are a footprint of NAdDW, which is relatively rich in nitrogen nutrients, but poor in orthophosphate. The lack of phosphates compared to nitrogen compounds is a usual situation in the NAdDW generation area (northern Adriatic) during wintertime (Degobbis, 1990; Zavatarelli et al., 1998). Therefore, NAdDW carries the nutrients on its way towards the deep layers in the middle and south Adriatic, reaching deep layers of the eastern Palagruža Sill during the absence of the LIW inflow.

The period between 1997 and 1999 may be used as a case study (Fig. 11). Temperature and salinity increased over time, denoting the increase in LIW inflow in the middle Adriatic, already observed in the south Adriatic in that period (Manca et al., 2002). As LIW flow is usually occurring in the eastern and central parts of the Palagruža Sill (Vilibić et al., 2004), it confines the NAdDW arrival to the western shelf and slope, thus not reaching the area of Stončica station. In addition, it seems that NAdDW was not generated or generated in small amount in 1997 (Supić and Vilibić, 2006) as the Po River flows were higher-than-average during winter 1997, enhancing the estuarine Adriatic circulation and consequently enhancing LIW inflow in the middle Adriatic. Therefore, such cold and less saline NAdDW observed in early 1997 (January-February) was presumably generated a year before, during winter 1996. Simultaneously, the eastern Adriatic river Neretva was also higher-than-average, pushing the coastal waters even to the Stončica station (surface salinity less than 37.8), and influencing the deeper layers during the wintertime vertical convection and mixing phase (Buljan and Zore-Armanda, 1976). In other words, a mass of colder and less saline water influenced the

bottom of the Stončica station, decreasing bottom salinity and temperature in January-February 1997 (Fig. 11A). All nitrogen compounds showed a decrease in concentration (Fig. 11B) and phosphate an increase (Fig. 11C), revealing a change in water masses in the investigated region. In association with higher nutrient concentrations brought by northern Adriatic waters, later substituted by LIW inflow, this period is characterized by a decline in bacterial abundance and production as well in abundance of HNF (Fig. 11D), starting with an increased abundance/production in 1997 and then decreasing over time.

The situation was somewhat different in the following 5-year interval (2002-2006). The correlations are weaker, although still significant between some biological and environmental parameters (Tab. 2). For example, TBP and SBP are still highly correlated to N/P ratio, but HNF changed the sign of its correlation with temperature, salinity and oxygen parameters that identify Adriatic deep water masses. The salinity and temperature were generally higher than between 1997 and 2001 (Fig. 9C,D), reflecting the larger LIW inflow in the middle Adriatic. LIW inflow was particularly strong in 2004 (Fig. 12A), when bottom temperature and salinity reached 15.2°C and 38.9, which were quite extreme for the investigated region (Vilibić and Orlić, 2001). All nutrients increased in that period, meaning that they were advected by LIW from the deep south Adriatic/Ionian layers, below the euphotic zone (Fig. 12B). A contrasting situation was observed during 1997 (Fig. 11B), when nutrients were brought by less saline NAdDW. LIW inflow was accompanied by bacterial abundance and production increase, as well as a decline in oxygen saturation, as a result of intensified bacterial activity and oxygen consumption (Fig. 12C,D).

The recorded seasonal changes in biological parameters (Figs. 5 and 6) found a consistent explanation when considered in relation to environmental parameters. As the changes occurred in the shape of the seasonal cycles, one would expect similar changes in the cycles of the environmental parameters. Fig. 13 shows the time-course of seasonal surface

and bottom temperature and salinity values, in which some changes in thermohaline regimes may be encountered. Firstly, at the surface the flooding of less saline coastal waters over the Stončica station is substantially changed between the two periods: the primary salinity minimum (May-July) was much more pronounced in 2002-2006 period (Fig. 13D) versus 1997-2001 period (Fig. 13C), while the secondary minimum (January-February), existing in the first period, totally disappeared in the 2002-2006 period. The changes also occurred at the bottom; the inflow of saline LIW waters starts to recover earlier (in April-May) between 2002 and 2006, with positive seasonal values (>100) established from May to November (Fig. 13H). Differently, the positive seasonal salinity values encompassed much narrower period (June-October) between 1997 and 2001, denoting different dynamics in LIW entrance in the investigated area (Fig. 13G). That is also visible in bottom temperature; a large jump between December and January and significantly lower seasonal component between January and March during 2002-2006 period (Fig. 13F) are a footprint of large cooling events and generation of cold and dense NAdDW. The stronger leakage of such NAdDW triggered earlier LIW entrance in the middle Adriatic through the Adriatic-Ionian thermohaline cell, as observed and modelled in 2003 (Orlić et al., 2007). That supports an earlier phytoplankton production in surface layer and consequently shifts the maximum in bacterial production towards the spring instead of spring-summer.

4. CONCLUSIONS

This paper highlights the potential of bacterial parameters (abundance and production) as good indicators of the temporal changes in the Adriatic water mass dynamics, especially in its deeper parts which are not directly influenced by the processes on the surface. The data obtained from decadal series of observations focusing on temporal rather than spatial variability, provided evidence of the important role that circulation and water mass dynamics

has in controlling bacterial activity and planktonic communities (as fluctuation of HNF abundance clearly showed). Analyses showed an increasing importance of non-seasonal components of environmental parameters (expressed as trend-cycle component), and their enlarged weight in controlling bacterial activity between 1997 and 2006. On the other hand, a sharp shift in seasonal cycles has sometimes been observed between 1997-2001 and 2002/2006, presumably related to the shift in water mass (LIW and NAdDW) dynamics in the middle Adriatic Sea during 2001/2002. In the surface layers, year-to-year fluctuations of temperature and salinity are predominantly under the influence of surface heating/cooling processes and the interplay between coastal/open Adriatic waters which influenced the patterns of bacterial fluctuations. In the deep layers, the bacterial abundance and production were predominantly controlled by NAdDW during 1997-2001, due to the absence of LIW inflow at that time. NAdDW brings colder and less saline waters over the eastern Palagruža Sill, rich in nitrogen nutrients but poor in orthophosphate. In the successive period (2002-2006), the deep waters in the eastern middle Adriatic were predominantly influenced by warmer and more saline LIW which brings higher concentrations of all nutrients, as NAdDW, although generated, has been confined to the western Adriatic.

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Tab. 1. Coefficients of Spearman rank order correlation between trend-cycle components within biological parameters (HNF – heterotrophic nanoflagellates; TBP – total bacterial production; SBP – specific bacterial production or bacterial production per cell).

Correlated parameters	Surface layers		Bottom layers	
	1997-2001	2002-2006	1997-2001	2002-2006
Bacteria : HNF	n.s.	- 0.545***	n.s.	- 0.543***
Bacteria : TBP	n.s.	- 0.589***	0.404**	n.s.
Bacteria : SBP	n.s.	- 0.736***	n.s.	- 0.379**
HNF : TBP	n.s.	0.645***	0.410**	n.s.
HNF : SBP	n.s.	0.771***	0.618***	n.s.
TBP : SBP	0.966***	0.964***	0.923***	0.950***

N_{97/01} = 60; N_{02/06} = 57; *P < 0.05; **P < 0.005; ***P < 0.001

n.s. – no significant correlation

Tab.2. Coefficients of Spearman rank order correlation between trend-cycle components of biological parameters and different environmental factors (HNF – heterotrophic nanoflagellates; TBP – total bacterial production; SBP – specific bacterial production or bacterial production per cell; DIN – dissolved inorganic nitrogen).

Correlated parameters	Surface layers		Bottom layers	
	1997-2001	2002-2006	1997-2001	2002-2006
Bacteria : Nitrate	n.s.	n.s.	0.369**	- 0.389**
Bacteria : Ammonia	- 0.336*	n.s.	n.s.	n.s.
Bacteria : N/P ratio	n.s.	- 0.288*	n.s.	- 0.460***
Bacteria : Temperature	n.s.	0.309*	n.s.	n.s.
Bacteria : Salinity	- 0.302*	0.276*	- 0.521***	- 0.452***
HNF : Oxygen(%)	- 0.418***	n.s.	- 0.723***	0.466***
HNF : Nitrate	0.326*	n.s.	0.371**	n.s.
HNF : Ammonia	- 0.407**	0.292*	- 0.350**	0.289*
HNF : Phosphate	n.s.	n.s.	- 0.539***	n.s.
HNF : N/P ratio	n.s.	n.s.	0.518***	n.s.
HNF : Temperature	- 0.306*	n.s.	- 0.389**	0.482***
HNF : Salinity	n.s.	n.s.	- 0.267*	0.705***
TBP : Oxygen(%)	n.s.	n.s.	- 0.351*	n.s.
TBP : Nitrate	0.431***	n.s.	0.817***	n.s.
TBP : Ammonia	n.s.	n.s.	n.s.	0.309*
TBP : DIN	n.s.	n.s.	0.724***	0.343*
TBP : Phosphate	n.s.	n.s.	- 0.430***	n.s.
TBP : N/P ratio	n.s.	n.s.	0.596***	0.427***
TBP : Temperature	n.s.	n.s.	- 0.645***	- 0.262*
TBP : Salinity	n.s.	n.s.	- 0.691***	n.s.
SBP : Oxygen (%)	n.s.	n.s.	- 0.421***	n.s.
SBP : Nitrate	0.357**	n.s.	0.716***	0.297*
SBP : DIN	n.s.	n.s.	0.709***	0.395**
SBP : Phosphate	n.s.	n.s.	- 0.416***	n.s.
SBP : N/P ratio	n.s.	n.s.	0.601***	0.530***
SBP : Temperature	n.s.	n.s.	- 0.696***	n.s.
SBP : Salinity	n.s.	n.s.	- 0.533***	n.s.

N_{97/01} = 60; N_{02/06} = 57; *P < 0.05; **P < 0.005; ***P < 0.001

n.s. – no significant correlation

FIGURE CAPTIONS

- Fig. 1. A scheme of the circulation and major water masses pathways, together with the position of Stončica station (EAC – East Adriatic Current, WAC – West Adriatic Current, LIW – Levantine Intermediate Water, NAdDW – North Adriatic Dense Water).
- Fig. 2. (A) Principal Component Analysis plot of trend-cycle components of bacteria (B), heterotrophic nanoflagellates (HNF), total bacterial production (TBP) and specific bacterial production (SBP) for both surface (S) and bottom (B) layers. Graph shows ordination of first two component weights (PC1 explains 44% and PC2 explains 26% of variability). (B-E) Long-term fluctuations of biological parameters (reported with trend-cycle components) and PCA series (PC1, PC2) which best represented certain biological parameter fluctuations.
- Fig. 3. As in Fig. 2, but for seasonal component (PC1 explains 53% and PC2 explains 24% of variability).
- Fig. 4. Principal Component Analysis plots based on the seasonal and trend-cycle components data sets of the all examined biological parameters (bacteria - B, heterotrophic nanoflagellates - HNF, total bacterial production - TBP and specific bacterial production - SBP) for each year, separately for surface and bottom layers. (A) Seasonal component in the surface layer (PC1 explains 82% and PC2 explains 14% of variability); (B) Seasonal component in the bottom layer (PC1 -76%; PC2 – 18%); (C) Trend-cycle component in the surface layer (PC1 -64%; PC2 – 24%); (D) Trend-cycle component in the bottom layer (PC1 -69%; PC2 – 22%)
- Fig. 5. Averaged seasonal components of bacteria (B), heterotrophic nanoflagellates (HNF), total bacterial production (TBP) and specific bacterial production (SBP) in the surface layer, estimated separately for 1997-2001 (A-D) and 2002-2006 (E-H) periods.
- Fig. 6. As in Fig. 5, but for the bottom layer.
- Fig. 7. Percentage time series of trend-cycle and seasonal components of the total time-series variability (fitted linear functions are plotted too) for bacteria (A), heterotrophic nanoflagellates (B) and specific bacterial production (C) in the bottom layer.
- Fig. 8. Trend-cycle components of total bacterial production (TBP), specific bacterial production (SBP) and nitrogen concentrations (nitrate, ammonia or dissolved inorganic nitrogen - DIN) in the surface (A) and bottom (B) layers during 1997-2001 period, and in the bottom layer during 2002-2006 period (C).
- Fig. 9. Temperature and salinity annual time series in the surface (A, B) and bottom layers (C, D).
- Fig. 10. Temperature time-series in the surface layer (measured, seasonal component and trend-cycle component) (A); and total bacterial production (TBP), specific bacterial production (SBP), temperature and oxygen saturation (B, C) between June 2000 and October 2001.
- Fig. 11. Time series of temperature and salinity (A); nitrate, ammonia, dissolved inorganic nitrogen (DIN) and N/P ratio (B); orthophosphate (C); bacteria, heterotrophic

nanoflagellates (HNF), total bacterial production (TBP) and specific bacterial production (SBP) (D) observed in the bottom layer throughout the 1997-1999 period.

Fig. 12. Time series of temperature and salinity (A); nitrate, ammonia, dissolved inorganic nitrogen (DIN) and orthophosphate (B); bacteria, total bacterial production (TBP) and specific bacterial production (SBP) (C); oxygen saturation (D) observed in the bottom layer throughout 2004-2005.

Fig. 13. Averaged seasonal components of temperature and salinity in the surface (A-D) and bottom (E-H) layers, estimated separately for 1997-2001 and 2002-2006 periods.

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