



Variability in the bottom-up and top-down controls of bacteria on trophic and temporal scales in the middle Adriatic Sea

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ABSTRACT: Variability in the bottom-up (BU) and top-down (TD) regulation of bacteria was analysed on trophic and temporal (seasonal and inter-annual) scales in the middle Adriatic Sea during 1997–2006 using 3 empirical models. The analyses showed the tendency for bacteria to be TD controlled in oligotrophic open sea stations, and BU controlled in more eutrophic coastal stations. However, temporal variability in BU and TD controls was much stronger, with periods of both strong BU and strong TD controls being observed at all studied stations, independently of their trophic status. Decomposition of the time series was performed to identify seasonal and inter-annual changes in the relative importance of the BU and TD controls of bacteria. At all stations, BU control dominated during colder periods of the year, whereas TD control dominated during warmer periods. Non-seasonal fluctuations in the relative importance of BU and TD controls of bacteria pointed to a few periods when one or the other type of control was very strong. These periods coincided with some specific meteorological and hydrographic conditions—the strong influence of North Adriatic Dense Water in 1997, the strong Levantine Intermediate Water ingression in 2004, and the extremely warm winter and the Po River runoff in 2000–2001.

KEY WORDS: Bacteria · Bottom-up · Top-down · Trophic scale · Temporal scale · Adriatic Sea

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INTRODUCTION

Since bacteria are recognized as a very important component of pelagic food webs (in terms of biomass, heterotrophic production and activity), the mechanism of their ecological regulation is a matter of considerable interest to aquatic microbiologists. Two types of bacterial abundance, production and activity regulation are recognized: regulation by the availability of resources or the so-called bottom-up (BU) control, and regulation by predation or the so-called top-down (TD) control.

BU control refers to the limitation of bacteria by organic and inorganic nutrients, particularly nitrogen and phosphorus, but also by micronutrients, derived from allochthonous inputs, primary production and

heterotrophic production. TD regulation refers to the limitation of bacteria below levels supportable by resources alone, because of the number of bacterial predators, particularly nanoflagellates and ciliates, but also filter-feeding and gelatinous zooplankton. Among bacterial predators, heterotrophic nanoflagellates (HNF) are commonly found to be responsible for most of the bacterial mortality due to grazers. Some authors have reported high contribution of mixotrophic flagellates to total flagellate grazing (Unrein et al. 2007, Zubkov & Tarran 2008). Another possible source of bacterial mortality is viral lysis, although viruses seem to be more important in regulating community diversity (Thingstad & Lignell 1997). Comparative studies generally confirm the importance of BU regulation of bacteria (Billen et al. 1990, Sanders et al. 1992, Gasol et

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al. 2002). Conversely, a tight relationship between bacterial and HNF abundance has been found in a wide-range of marine environments (Sanders et al. 1992), suggesting the importance of TD control of bacteria. In general, bacterial production is considered to be controlled by the rate of nutrient supply, while bacterial abundance and specific growth rate (SGR) could be determined by both substrate supply and predation pressure (Wright & Coffin 1984, Thingstad & Lignell 1997). However, the data sets that have so far been analysed have not indicated clear conclusions about the relative importance of the BU and TD controls of bacteria.

Data set analyses showed that HNF abundance positively correlates with bacterial abundance across a wide range of aquatic environments (Sanders et al. 1992). The numerical relationship between bacterial and HNF abundance is often simplified to an ideal ratio of $10^3:1$. In reality this ratio shows variability of up to 2 orders of magnitude (from $10^2:1$ to $10^4:1$; Sanders et al. 1992). Bacterial abundance has often been observed to be less variable, but HNF abundance shows marked seasonal fluctuations. In addition, bacteria and HNF show changes in their abundances with a lag phase due to predator–prey interaction. Based on these facts, Gasol et al. (2002) proposed a new approach in studying the BU and TD regulation of bacteria that is focused on uncoupling between bacterial and HNF abundance (due to imbalances between bacterial growth and losses), which often occurs in empirical data. The degree of coupling between predators and bacteria is a measure of grazing pressure, which is thus of importance to the TD regulation of bacteria.

Most studies on BU and TD control of bacteria have been concentrated on trophic scales or comparisons of ecologically contrasting ecosystems, and much less on temporal scales. Several studies with very large data sets, which were performed over a large range of aquatic environments, suggest that bacteria seem to be more BU controlled in eutrophic systems, and more TD controlled in oligotrophic systems (Billen et al. 1990, Gasol 1994, Gasol et al. 2002). Conversely, other studies suggest opposite conclusions (Sanders et al. 1992, Dufour & Torréton 1996). However, it seems that the importance of the BU and TD regulation of bacteria may vary seasonally (Ducklow 1992) and even daily (Psenner & Sommaruga 1992). Therefore, switches between the 2 types of control may follow changes in environmental conditions which occur on both spatial and temporal scales.

Previous studies in the Adriatic Sea showed that coupling between bacteria and HNF generally exists (Šolić & Krstulović 1994). Moreover, small HNF ($<8 \mu\text{m}$) have been found to be the most important grazers of bacteria, controlling bacterial abundance

and production. Grazing experiments in the coastal Adriatic Sea showed that HNF accounted for 72 to 96 % (mean 80 %) and ciliates for 4 to 28 % (mean 20 %) of total grazing on bacteria (Šolić & Krstulović 1994); the contribution of HNF to total grazing was maximal during the warmer period of the year (June to October), while that of ciliates was maximal during the colder period (November to May). On the other hand, HNF was strongly controlled by ciliate grazing, which has a strong influence on bacteria via trophic cascade (Bojanić 2001, Bojanić et al. 2006). Moreover, the same authors reported that ciliates of 20 to 40 μm length are the most important grazers of HNF, and that this size fraction of ciliates dominated during the colder period of the year. Further, the importance of temperature changes in affecting microbial dynamics and interactions within microbial food webs was also documented in the Adriatic Sea (Šolić & Krstulović 1994). Finally, the role of water mass dynamics in controlling bacterial and HNF abundance was reported for the open middle Adriatic (Šolić et al. 2008).

However, the role of trophic and temporal scales in the mechanisms of bacterial abundance control has been sparsely studied in the Adriatic Sea. Therefore, the present study aimed to determine the relative importance of BU and TD controls of bacteria on trophic and temporal (seasonal and inter-annual) scales. We analysed 10 yr data sets (1997–2006) consisting of monthly values of bacterial abundance, biomass, production and specific growth rate, as well as HNF abundance, at 6 sampling stations located in the coastal toward the open middle Adriatic Sea. The relationships between temporal variation in BU and TD controls of bacteria and temperature, salinity, HNF and ciliate abundance, concentration of nutrients (phosphorus and nitrogen) and water mass dynamics were examined in order to determine possible differences in the underlying mechanisms responsible for the 2 types of bacterial control.

MATERIALS AND METHODS

Study area. The measurements were conducted in surface waters at 5 stations located in the coastal toward the open middle Adriatic Sea, and at 1 deep station (100 m deep) located in the open sea area (Fig. 1).

Two stations, one in the central part (KB) and the other in the eastern part (VB), are located in Kaštela Bay. The bay (61 km² surface area, 15 km long and 6 km wide, 23 m average depth) communicates with the adjacent channel through a 1.8 km wide and 40 m deep inlet. The river Jadro, which discharges into the eastern part of the bay, is the most important freshwater source, with an average annual inflow of 10 m³

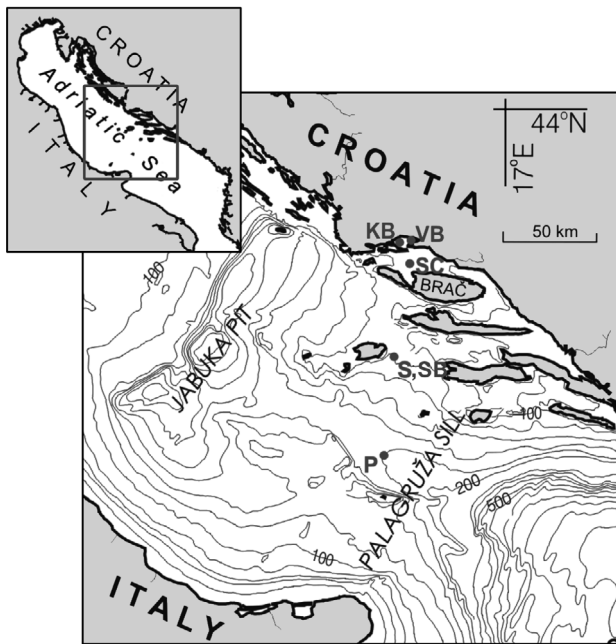


Fig. 1. Study area showing sampling stations. VB: Vranjic Bay, KB: Kaštela Bay, SC: Split Channel, S: Stončica, SB: Stončica, bottom layer, P: Palagruža

s^{-1} (Orlić et al. 2007). The eastern part of the bay also receives municipal and industrial effluents. Wide oscillations of chemical and physical parameters in this area are the result of strong land influence. Water circulation in the bay is generated mostly by local winds, which are related to the passage of mid-latitude cyclones over the area. The average water renewal time is ~ 1 mo, but can be as short as 5 d under strong wind conditions. During the warm period of the year (July to September), wind forcing is relatively weak and freshwater inflow is low, making renewal time rather long.

One station (SC) is located in Split Channel (the channel area between mainland Croatia and Brač Island). In general, productivity in the channel area of the middle Adriatic Sea is higher than in the open sea. However, karstic rivers which drain into channel areas contain rather low phosphate concentrations (very high N/P ratio), which does not favour phytoplankton growth. Besides, the coast slopes rather steeply towards the sea, making channel areas relatively deep (Stn SC is 50 m deep). These channels are characterised by relatively rapid ventilation, including the fast intrusion of open ocean water masses and, therefore, permitting rapid dispersion of loaded nutrients and pollutants, which further considerably reduces their impact on coastal ecosystems. Therefore, based on their chemical and biological characteristics, channel waters are more similar to those in the open sea.

Another 2 stations (S and P) are located in the open sea. They are exposed to the northwestern East Adriatic Current (Orlić et al. 2007) that brings saline waters from the Ionian and Levantine Seas. These waters, known as the Levantine Intermediate Water (LIW, Malanotte-Rizzoli et al. 1997), can flood the Adriatic in some years (so-called ingressions years; Buljan 1953) increasing salinity, temperature and nutrients in intermediate layers (Tziperman & Malanotte-Rizzoli 1991, Vilibić & Orlić 2001). In addition, Stn S, which is located just outside the middle Adriatic island coastal waters, is influenced by the largest east Adriatic river (Neretva river, Raicich 1996); this station gets fresh waters in its surface layer during large freshwater inputs. Stn P which is located in the area of Palagruža Sill, is rarely flooded by the low salinity West Adriatic Current (Vilibić et al. 2009) that flows along the western shore toward the southeast.

Finally, the deep-sea station (SB, 100 m depth) is a good indicator of the water mass dynamics in the open Adriatic Sea. Deep layers of the open Adriatic are periodically flooded by the LIW and the North Adriatic Dense Water (NAdDW), resulting in fluctuations in nutrient concentrations and, consequently, in different bacterial abundances and production (see Šolić et al. 2008 for details).

Therefore, surface layers of the chosen stations form a trophic gradient from richer coastal seas towards more oligotrophic open seas. Bacterial and HNF abundance (Fig. 2A,B), bacterial production (Fig. 2C), bacterial activity (measured as a percentage of high nucleic acid bacteria) (Fig. 2D), and dissolved inorganic nitrogen (DIN) and phosphate concentrations (Fig. 2E,F) decreased from coastal to open sea stations. The deep-sea Stn SB showed higher average concentrations of nutrients than the surface layers of the open sea; this was accompanied by higher values of bacterial production and activity, but not of bacterial and HNF abundance (Fig. 2).

Data collection. Several biotic (bacterial abundance and production, percent of high nucleic acid (HNA) bacteria, HNF and ciliate abundance) and abiotic (temperature, salinity, dissolved oxygen (DO), DIN and phosphorus) parameters were monitored throughout the study. All parameters were collected at all stations on a monthly basis throughout the 1997–2006 period, except ciliates which were sampled only at the coastal stations (VB, KB and SC), and HNA which were analysed only for the 2004–2006 period.

Niskin bottles (5 l) were used for sampling and samples were immediately processed on board. All samples for bacterial and HNF cell counts were preserved with formaldehyde that was filtered in $0.2 \mu\text{m}$ polycarbonate membranes (2% final concentration), and were processed in the laboratory within 3 h after collection.

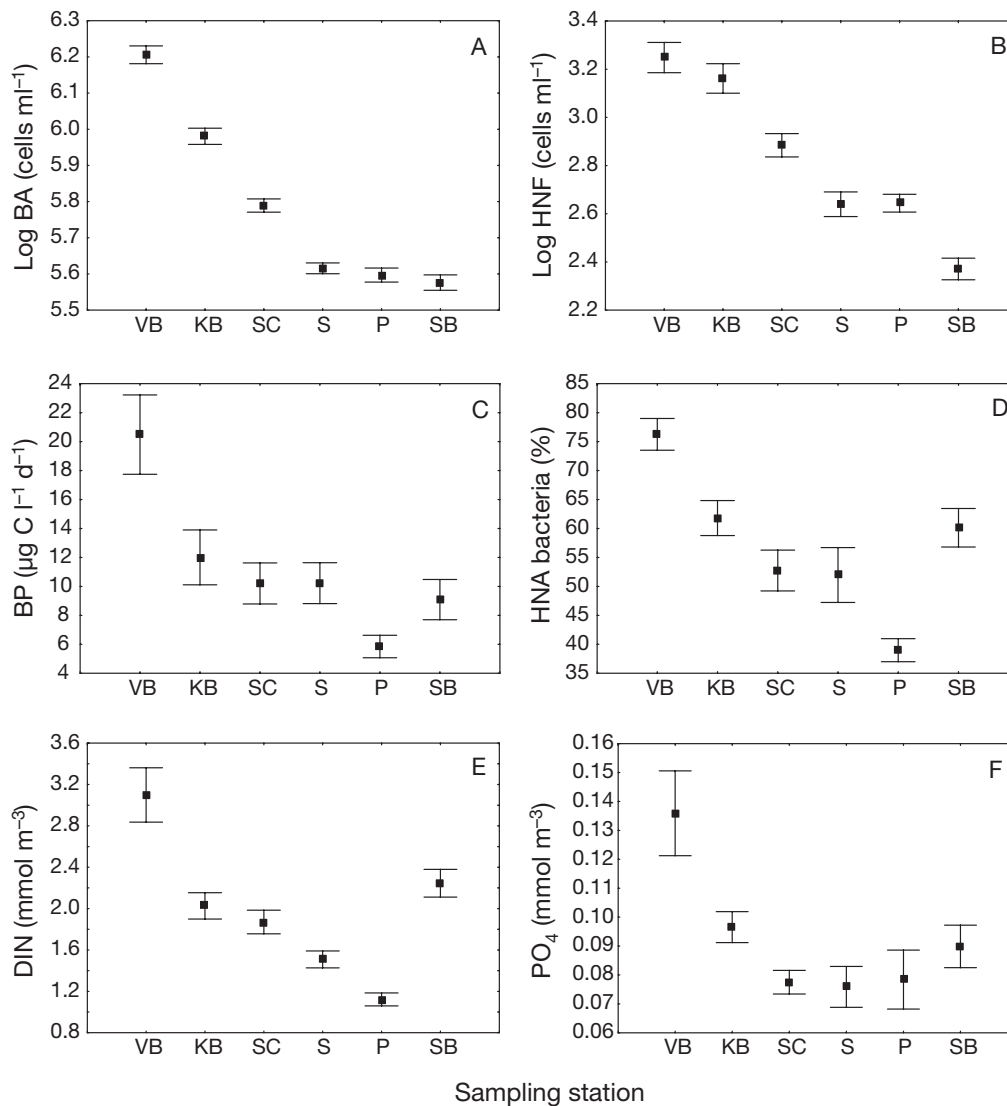


Fig. 2. Mean values (\pm SE) of (A) bacterial abundance (BA), (B) heterotrophic nanoflagellate (HNF) abundance, (C) bacterial production (BP), (D) percentage of high-nucleic acid (HNA) bacteria, (E) concentration of dissolved inorganic nitrogen (DIN), and (F) concentration of phosphate (PO₄) in the different sampling stations (see Fig. 1) for the period 1997–2006 (2004–2006 for %HNA)

The same sampling and processing methods were used throughout the investigation period.

Bacteria and HNF were enumerated by epifluorescence microscopy (Olympus BX50, 1000 \times magnification), using the standard DAPI staining technique (Porter & Feig 1980). For biovolume estimates, lengths and widths of bacterial and HNF cells were measured with an eyepiece graticule (New Porton G12, Graticules). Biovolume was then converted to carbon biomass, assuming 0.220 $\mu\text{g C } \mu\text{m}^{-3}$ for bacteria (Bratbak & Dundas 1984) and HNF (Borsheim & Bratbak 1987). Bacterial cell production was measured from DNA synthesis based on incorporation rates of ³H-thymidine (Fuhrman & 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 h. The incubations were stopped with formaldehyde (final concentration: 0.5%). The thymidine samples were extracted with ice-cold trichloroacetic acid (TCA) according to Fuhrman & Azam (1982). The TCA-insoluble fraction was collected by filtering the samples through a 0.2 μm pore size Sartorius filter. The percentage of thymidine that was 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): $CF = (N_2 - N_1)/^3H$, where N_1 and N_2 are the numbers of bacteria at the beginning and at the end of the experiment, and 3H is the integrated ^3H -thymidine incorporation rate during the experiment. From the estimates of bacterial production (BP) and bacterial biomass (BB), bacterial specific growth rate (SGR, d^{-1}) was computed as: $SGR = \ln(1 + BP/BB)$.

Cell abundance was estimated using flow cytometry after nucleid acid staining with SYBR Green I (Molecular probes) (Marie et al. 1997). Flow cytometry determined 2 bacterial subpopulations called high nucleic acid (HNA) and low nucleic acid (LNA) bacteria that were distinguished by their DNA content (Marie et al. 1997, Troussellier et al. 1999).

Samples were analysed on a flow cytometer (Beckman Coulter, Epics XL-MCL) at a flow rate of 1 to 1.2 $\mu\text{l s}^{-1}$. SYBR DNA fluorescence was detected in both the green FL 1 (505 to 545 nm) and the red FL 3 (605 to 635 nm) fluorescence channels. Side-angle light scatter (SSC) served as a proxy for bacterial cell size (Servais et al. 1999, Troussellier et al. 1999). All signals were normalized to that of the 1 μm beads (Level-II Epics Division, Coulter) that we made at the beginning of each analysis.

Microzooplankton samples were collected with 5 l Niskin bottles and preserved in CaCO_3 buffered formaldehyde–seawater solution (2.5%). Ciliate samples were sedimented (Utermöhl 1958) for 48 h in plastic containers and decanted down to a volume of 2 l. This volume was poured into a cylinder and further sedimented for another 48 h. The excess volume was then reduced to 200 ml. Prior to microscopic analysis, this volume was reduced to 20 ml. Decantation was carried out using a vacuum pump and a slightly curved pipette that removed water from the surface. The organisms were counted in a glass chamber ($76 \times 47 \times 6$ mm) using an inverted microscope (Olympus CK40) at 100 \times , 200 \times and 400 \times magnifications.

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

Time series analysis. In time series analysis, it is assumed that data consist of a systematic pattern (usually a set of identifiable components) and random noise (error) which usually makes the pattern difficult to identify. Most time series analysis techniques involve some form of noise filtering in order to make the pattern more salient. Most time series patterns can be described in terms of 2 basic classes of components:

trend and seasonality. The former represents a general systematic linear or (most often) nonlinear component that changes over time and does not repeat (at least within the time range captured by our data; e.g. a plateau followed by a period of exponential growth). The latter may have a formally similar nature (e.g. a plateau followed by a period of exponential growth), but repeats itself in systematic intervals over time. These 2 general classes of time series components may coexist in data. However, in practice, data patterns are unclear, individual observations involve considerable error, and we still need to not only uncover the hidden patterns in the data but also generate forecasts. The ARIMA methodology developed by Box & Jenkins (1976) allows us to do just that.

Time series analyses were performed using the statistical package STATISTICA for Windows version 6 (StatSoft), and are based on the ARIMA procedure (Box & Jenkins 1976). The purpose of the seasonal decomposition method is to decompose the series into the trend effect, seasonal effects, and the remaining variability. The ‘classic’ technique designed to accomplish this decomposition is known as the Census I method that is discussed in detail in Makridakis & Wheelwright (1989). In general, a time series like the one described above can be thought of as consisting of 4 different components: (1) a seasonal component (denoted as S_t , where t stands for the particular point in time); (2) a trend component (T_t), (3) a cyclical component (C_t), and (4) a random error, or irregular component (I_t). The difference between the cyclical and the seasonal component is that the latter occurs at regular (seasonal) intervals, while cyclical factors usually have a longer duration that varies from cycle to cycle. In the Census I method, the trend and cyclical components are customarily combined into a trend–cycle component (TC_t). The specific functional relationship between these components has the following form:

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

where X_t is the original time series, TC_t is the trend–cycle component, S_t is the seasonal component and I_t is the irregular component. The separation procedure has 6 steps (discussed subsequently) which are shown in Fig. 3 as exemplified by the time series of bacterial abundance.

Step 1. Elimination of intra-annual variability. The removal of intra-annual variability from the time series is based on the computation of a 12 mo centred moving average of the time series (MA_t) (Fig. 3B)

Step 2. Isolation of the seasonal plus irregular component. In the moving average series, all seasonal (within-season) variability is eliminated; thus, the ratio (R_t) of the observed to the smoothed series (X_t/MA_t) presents a seasonal plus an irregular compo-

ment (ratio values are additionally multiplied by 100%) (Fig. 3C)

Step 3. Isolation of the seasonal component. The seasonal component (S_t) is then computed as the medial average (the mean of a set of values after the smallest and largest values are excluded) for each point in a

season. The resulting values represent the average seasonal component of the series (Fig. 3D)

Step 4. Isolation of the seasonally adjusted original series (ADJ_t). The original series is adjusted by dividing it by the seasonal component (X_t/S_t). The resulting series is detrended for seasonal patterns (i.e. the sea-

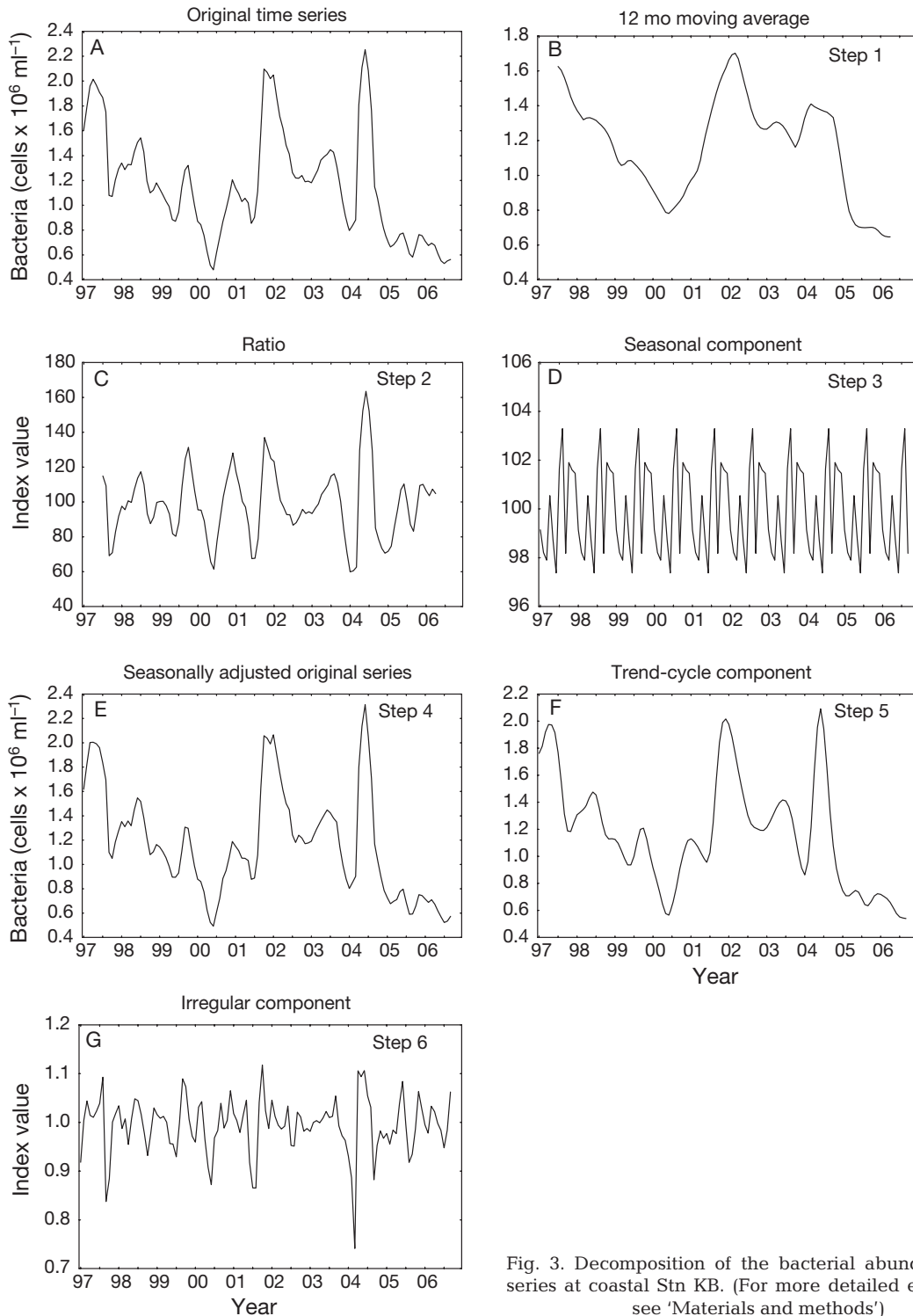


Fig. 3. Decomposition of the bacterial abundance time series at coastal Stn KB. (For more detailed explanation see 'Materials and methods')

sonal component is removed). Seasonally adjusted values are additionally multiplied by 100% (Fig. 3E)

Step 5. Isolation of the trend–cycle component (TC_t). The combined trend and cyclical component is approximated by applying a 5 point centred, weighed (weights of 1, 2, 3, 2 and 1), moving average smoothing transformation to the seasonally adjusted series, with the largest weight being attributed to the actual value and then decreasing forward and backward in time (Fig. 3F)

Step 6. Isolation of the irregular component (I_t). Finally, the random or irregular (error) component is isolated by dividing the adjusted series by the trend–cycle component (ADJ_t/TC_t) (Fig. 3G).

Methods for discriminating the relative importance of BU and TD regulation of bacteria. To examine the regulation of bacteria by substrate availability (BU control) and by predation (TD control), data were analyzed using 3 different approaches based on empirical comparative data analyses, which are briefly explained in Table 1 (see Gasol et al. 2002 for details).

The simultaneous observations of bacterial and HNF abundance are plotted on a graph which includes empirically determined values of maximum attainable abundance (MAA) and mean realized abundance

(MRA) of HNF according to the framework proposed by Gasol (1994) (Fig. 4A). The points close to the MAA line indicate strong coupling between bacteria and HNF, which according to Gasol (1994) could be interpreted as a strong TD control on bacteria. The points which lie well below the MRA line indicate conditions when bacterial abundance was not controlled by HNF grazing (i.e. weak coupling between bacteria and HNF), which suggest domination of the BU control on bacteria. Therefore, D values (differences between the maximum and realized HNF abundances at different bacterial concentrations) could be a good indicator of the importance of HNF predators in controlling bacterial abundance (higher D, lower predation pressure).

To examine if high D and low D points really represent periods of strong BU and TD controls respectively, we formed high D (D values which were >1 SD above the mean value) and low D (D values which were >1 SD below the mean value) data sets and checked them using 2 other models (Fig. 4B,C). The high D data set showed a strong relationship between bacterial production and bacterial biomass ($r = 0.774$; $b = 0.802$; $p < 0.001$) (Fig. 4B), and a significant negative correlation between bacterial abundance and SGR ($r = -0.577$; $b = -0.802$; $p < 0.001$) (Fig. 4C), which suggests

Table 1. Empirical models used to discriminate the relative importance of bottom-up (BU) and top-down (TD) regulation of bacteria. HNF: heterotrophic nanoflagellate, MAA: maximum attainable abundance, MRA: mean realized abundance, SGR: specific growth rate

Empirical model	Basic idea	Interpretation in light of BU and TD regulation of bacteria
Coupling between bacterial and HNF (main bacterial predators) abundance (Gasol 1994, Gasol et al. 2002)	Simultaneous observations of bacterial and HNF abundance plotted on a log-log graph give information about coupling between bacterial and HNF abundance. Graph includes an empirically determined (based on a large database from a variety of systems) MAA line depicting the HNF abundance that could be attained at a given bacterial abundance, and an MRA line. The distance (D) between the maximum and the actual measured HNF abundance at each bacterial abundance value shows the degree of uncoupling between bacteria and their predators, and is a surrogate for the grazing pressure of HNF on bacteria (higher D = lower grazing pressure).	Low D values mean strong coupling between the abundance of bacteria and HNF; that is, strong TD control of bacteria. High D values mean no or low coupling between bacteria and HNF, suggesting that bacterial abundance was not controlled by predation but by resources (domination of BU control).
Relationship between bacterial biomass and production (Billen et al. 1990, Ducklow 1992)	Billen et al. (1990) argued that bacterial production serves as a surrogate for nutrient supply, which is difficult to measure. If predation mortality is low, all bacterial production can be converted into bacterial biomass. Alternatively, if grazing on bacteria is very high, bacterial biomass does not increase with increasing bacterial production. Moreover, Ducklow (1992) suggested that the slope of the log-log regression between bacterial production and biomass would indicate the strength of BU control.	A strong relationship (with high slope) between bacterial production (independent variable) and bacterial biomass (dependent variable) suggests domination of BU control of bacteria. Conversely, no relationship or low slope indicates domination of TD control.
Relationship between bacterial SGR and abundance (Wright & Coffin 1984)	Density-dependent logistic growth implies that bacterial SGR is low when bacterial abundance is close to the carrying capacity (the maximum abundance of bacteria that a particular environment can sustain), and bacterial SGR is conversely high when bacterial abundance is lower than the carrying capacity. Therefore, when bacterial abundance is close to the carrying capacity, bacteria are limited by resource availability, but they could be limited by predators when their abundance is less than that value.	A negative relationship between bacterial SGR and abundance suggests that bacteria are resource-limited (domination of BU control). No relationship between bacterial SGR and bacterial abundance indicates that bacteria are dominantly controlled by predators (domination of TD control).

a strong BU control. Conversely, the low D data set showed a weak relationship (low regression slope) between bacterial production and bacterial biomass ($r = 0.603$; $b = 0.343$; $p < 0.001$), and no correlation between bacterial abundance and SGR. Therefore,

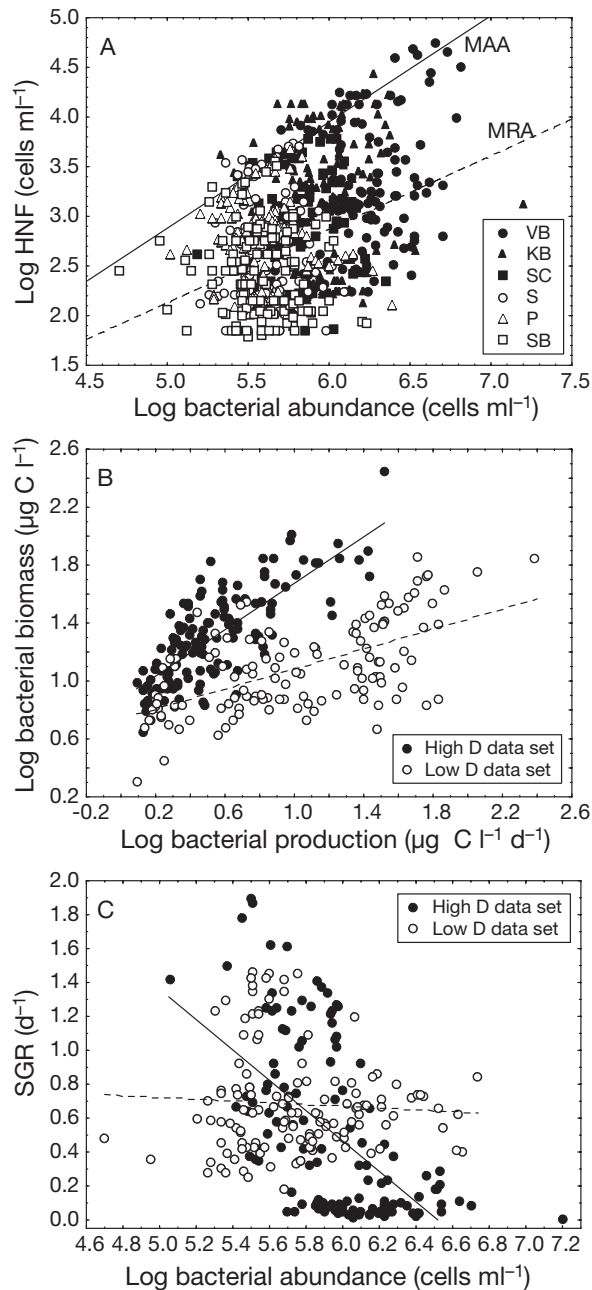


Fig. 4. (A) Relationship between bacterial and heterotrophic nanoflagellate (HNF) abundance at the studied stations (see Fig. 1), plotted in a theoretical model (Gasol 1994). MAA: maximum attainable abundance, MRA: mean realized abundance. (B) Relationship between bacterial production and bacterial biomass, and (C) between bacterial abundance and bacterial specific growth rate (SGR) for high D (D: the difference between the maximum and realized HNF abundance at each bacterial concentration) and low D data sets

both empirical models confirmed that D values could be a good indicator of the relative importance of the BU and TD controls of bacteria, promoting D as an appropriate parameter for further analyses of trophic and temporal scales.

RESULTS AND DISCUSSION

All pooled data showed that the average value of D increased with increasing bacterial abundance (Fig. 5A), suggesting better coupling between bacteria and HNF under conditions (sites or periods) of low bacterial abundance. Besides total bacterial abundance, the percentage of HNA bacteria also increased along the oligotrophic–eutrophic gradient (Fig. 5B). HNA bacteria can be interpreted as active bacteria in the community, and their percentage contribution (%HNA) is a simple indicator of the activity structure of the bacterial community (del Giorgio et al. 1996, Servais et al. 1999, Lebaron et al. 2001). Therefore, our results suggest that the contribution of active bacteria in the community increased in conditions when bacteria were dominantly controlled by substrate availability. Pooled data also showed the D values to be significantly positively correlated with the abundance of ciliates ($r = 0.552$; $b = 0.608$; $p < 0.001$) that are important HNF predators in the Adriatic Sea (Šolić & Krstulović 1994) (Fig. 5C). This suggests that uncoupling between bacteria and HNF could be due to strong grazing pressure on HNF by ciliates.

BU and TD control of bacteria on a trophic scale

When data were pooled according to the trophic gradient, the average D values showed a decreasing trend from coastal areas toward the open sea (Fig. 6A). This pattern suggests stronger coupling between bacteria and HNF (domination of the TD regulation of bacteria) at oligotrophic open sea sites than at more eutrophic coastal sites, where bacterial abundance was not controlled by HNF predation as much as by BU control. However, the variability of D was high at all studied stations (Fig. 6A). Looking at Fig. 4A, we can find points that fall close to the MAA line and points that are well below the MRA line for all studied stations, regardless of their trophic status. Therefore, we can conclude that there is a marked temporal variability in the mechanisms of bacterial abundance regulation.

The results showing the variability of the BU and TD controls of bacteria on a trophic scale, which were obtained using the empirical model proposed by Gasol (1994), were checked against 2 other models (Fig. 6B,C). Statistically significant positive correlation

between bacterial biomass and production, as an indicator of the BU control of bacteria (Billen et al. 1990, Ducklow 1992), was obtained only for the 2 coastal sites (VB: $r = 0.519$; $b = 0.282$; $p < 0.001$, and KB: $r = 0.364$; $b = 0.212$; $p < 0.001$) (Fig. 6B). However, using the slope of the log-log regression as a criterion to

assess the BU control strength (Ducklow 1992; $>0.6 =$ strong; $0.4-0.6 =$ moderate; $<0.4 =$ weak; $<0.2 =$ none) indicates the observed relationships to be weak at both coastal sites. No significant regressions were noted at oligotrophic open sea sites, suggesting domination of the TD control of bacteria. These results are consistent

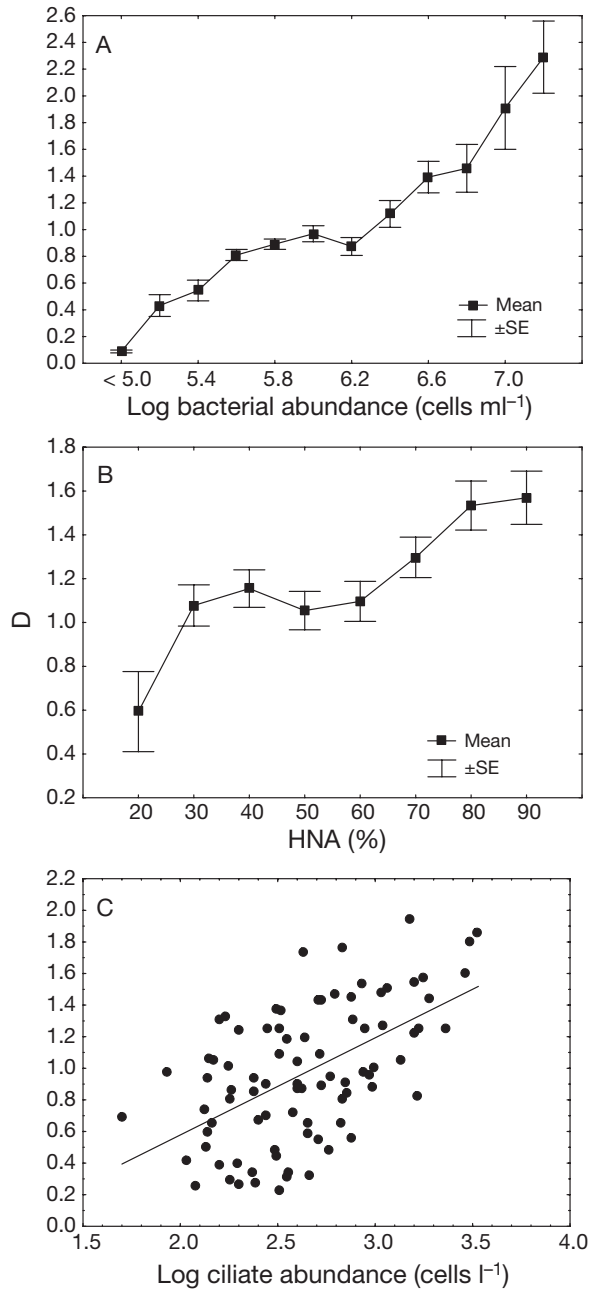


Fig. 5. Relationships between D values (the difference between the maximum and realized heterotrophic nanoflagellate (HNF) abundance at each bacterial concentration) and (A) bacterial abundance (log values grouped in equidistant discrete categories), (B) percentage of high-nucleic acid (HNA) bacteria (%HNA values grouped in equidistant discrete categories), and (C) ciliate abundance

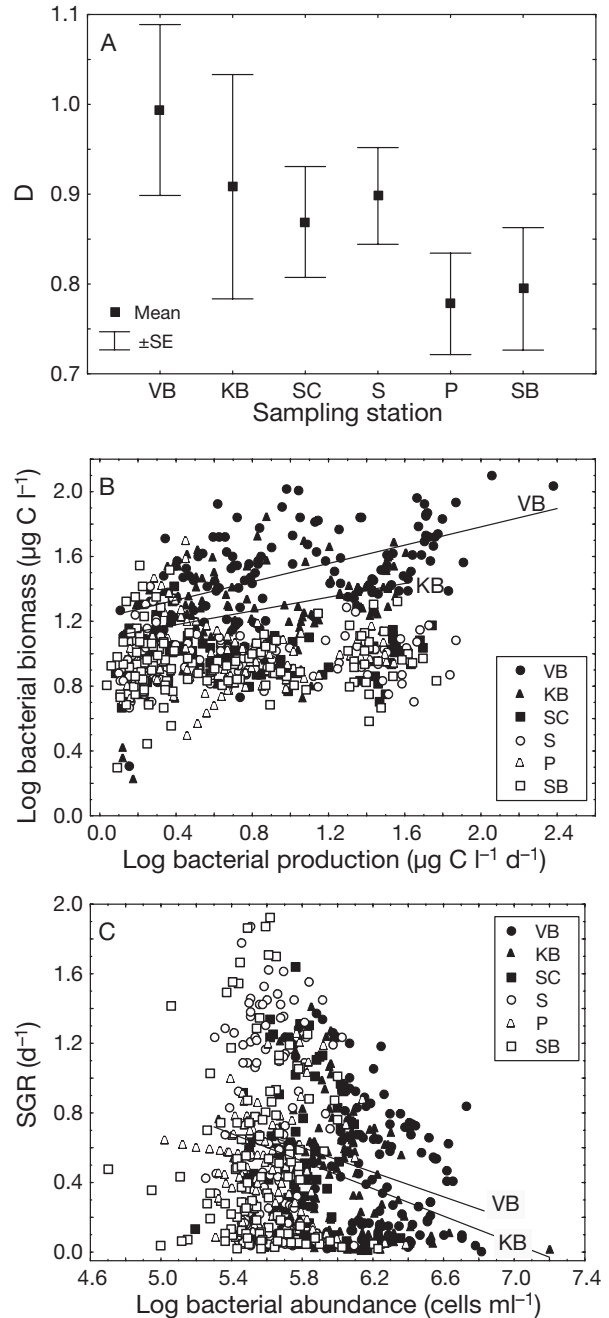


Fig. 6. (A) Mean values of D (the difference between the maximum and realized heterotrophic nanoflagellate (HNF) abundance at each bacterial concentration), and relationships between (B) bacterial production and biomass, and (C) bacterial abundance and specific growth rate (SGR) at the studied stations (see Fig. 1). Only significant regressions are plotted

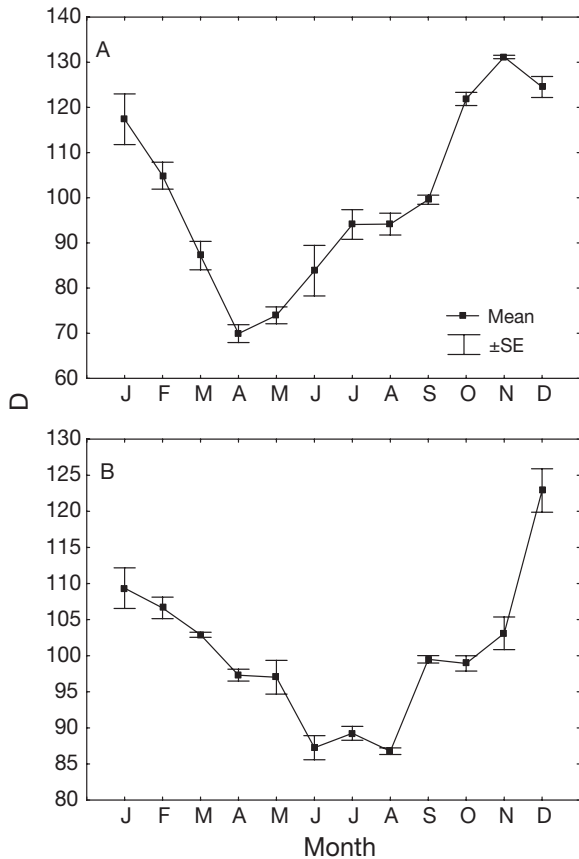


Fig. 7. Seasonal components of the D value (the difference between the maximum and realized heterotrophic nanoflagellate (HNF) abundance at each bacterial concentration) at (A) a coastal and (B) an open sea station

with results obtained using the third model (Fig. 6C). Significant negative regression between bacterial SGR and bacterial abundance, which indicate BU control of bacteria (Wright & Coffin 1984), was again observed only at the 2 more eutrophic coastal sites (VB: $r = -0.256$; $b = -0.354$; $p < 0.01$, and KB: $r = -0.302$; $b = -0.395$; $p < 0.001$).

Based on the results obtained by all 3 models, it could be concluded that there is a tendency for bacteria to be TD controlled in oligotrophic open seas, and BU controlled in more eutrophic coastal waters. However, due to the low slopes of the log-log regressions between bacterial biomass and production, which were established even at richer coastal sites, our results could be interpreted in another way. It seems that the entire studied area in the middle Adriatic could be considered as oligotrophic with weak BU control and domination of the TD control in comparison to the more eutrophic northern Adriatic where ~45% of the variability in the bacterial biomass could be explained by the BU model and only 20% could be explained by the TD model (Fuks 1995).

BU and TD control of bacteria on a temporal scale

The high variability in D values found at all studied stations suggests a noteworthy temporal variability in the relative importance of the BU and TD controls of bacteria. This is the reason why we decomposed the D value time series to identify seasonal (seasonal component) and non-seasonal (trend-cycle component) patterns of fluctuation at each studied station.

Seasonal patterns of fluctuation

The dominant pattern of seasonal fluctuations (expressed as the seasonal component after time series decomposition) in the relative importance of the BU and TD controls of bacteria, which is common to all studied stations (except the deep-sea station), can be seen in Fig. 7 (as an example, 1 coastal and 1 open sea station are shown). In all stations, the BU control dominated during the colder period of the year, while the TD control dominated during the warmer period.

In the coastal stations, the seasonal pattern of HNF fluctuations, which is characterised by high values during the warmer period of the year (May to August), was better coupled with bacterial production than with bacterial abundance which reached a maximum in September (Fig. 8A). In contrast, bacterial abundance was relatively high during the colder period from December to February. However, HNF did not reach such abundance as might have been expected according to bacterial abundance. The increase in bacterial abundance during the colder periods of a year could be due to both increasing availability of nutrients (particularly nitrogen) and low grazing on bacteria. A stronger increase in total inorganic nitrogen (TIN) and lower increase in phosphorus during the period between December and February (Fig. 8C) coincided with precipitation and the Jadro River inflow maxima which typically occur in this period (Kušpilić 2001, Orlić et al. 2007). Conversely, the coupling between bacteria and HNF was very weak during the colder part of the year, suggesting low grazing on bacteria by HNF. This is consistent with the evidence that bacterivory is highly responsive to water temperature, with the highest grazing on bacteria being observed in summer and the lowest being observed in winter (Vaqué et al. 1994). Moreover, the increased abundance of ciliates (both nonloricate and tintinnid ciliates as well as total ciliate abundance showed the same seasonal pattern) suggests that HNF themselves could also be TD controlled by ciliates during the colder period of a year (Fig. 8E).

Very similar patterns were also established in the open sea. Maximum HNF abundance was noted in the warmer period of the year and relatively high bacterial

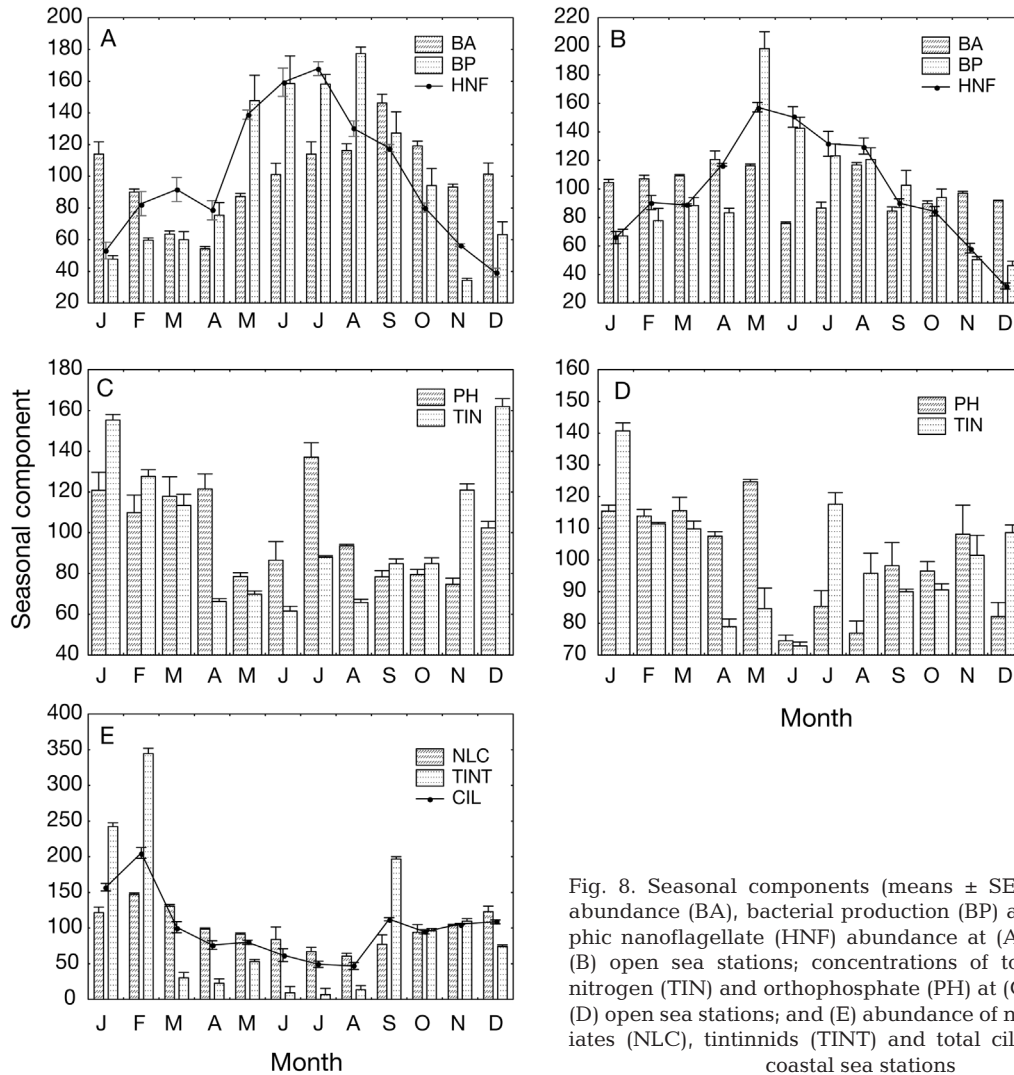


Fig. 8. Seasonal components (means ± SE) of bacterial abundance (BA), bacterial production (BP) and heterotrophic nanoflagellate (HNF) abundance at (A) coastal and (B) open sea stations; concentrations of total inorganic nitrogen (TIN) and orthophosphate (PH) at (C) coastal and (D) open sea stations; and (E) abundance of nonloricate ciliates (NLC), tintinnids (TINT) and total ciliates (CIL) at coastal sea stations

abundance during the colder period (uncoupling with HNF), which coincided with increased nutrients (Fig. 8B,D).

Non-seasonal patterns of fluctuation

To determine periods of strong BU and TD controls of bacteria, D values were standardised as z-values which are computed as: $z = (D - \mu) / \sigma$, where μ and σ are the mean and SD of D values, respectively. Thus, z-values tell us how many SDs above or below the mean each D value is (positive z-values are above the mean, negative z-values are below the mean). In Fig. 9, we extracted and plotted the z-values that were below the 5th and above the 95th percentile only, to determine the periods characterised by exceptional TD or BU control conditions. At all studied stations, strong TD

and BU controls were observed in 1997 and 2002, respectively. During 2004, strong TD control was observed in the open sea (in both surface and bottom layers), while strong BU control was observed in the coastal stations (Fig. 9). Standardised values of all other parameters are likewise shown in Fig. 10.

In coastal stations, the period of strong TD control of bacteria (found to be established in 1997) was characterised by a marked increase in TIN and bacterial abundance, followed by an increase in HNF abundance (Fig. 10A). Similarly, bacterial abundance was also strongly coupled with nitrogen during both periods of strong BU control (2002 and 2004) but, contrary to 1997, the response of HNF predators failed to occur. Limitation by nutrients in Kaštela Bay can be explained by the rapid rate of nutrient accumulation into particulate forms, as well as its removal from the water column by the settling of organic matter to the

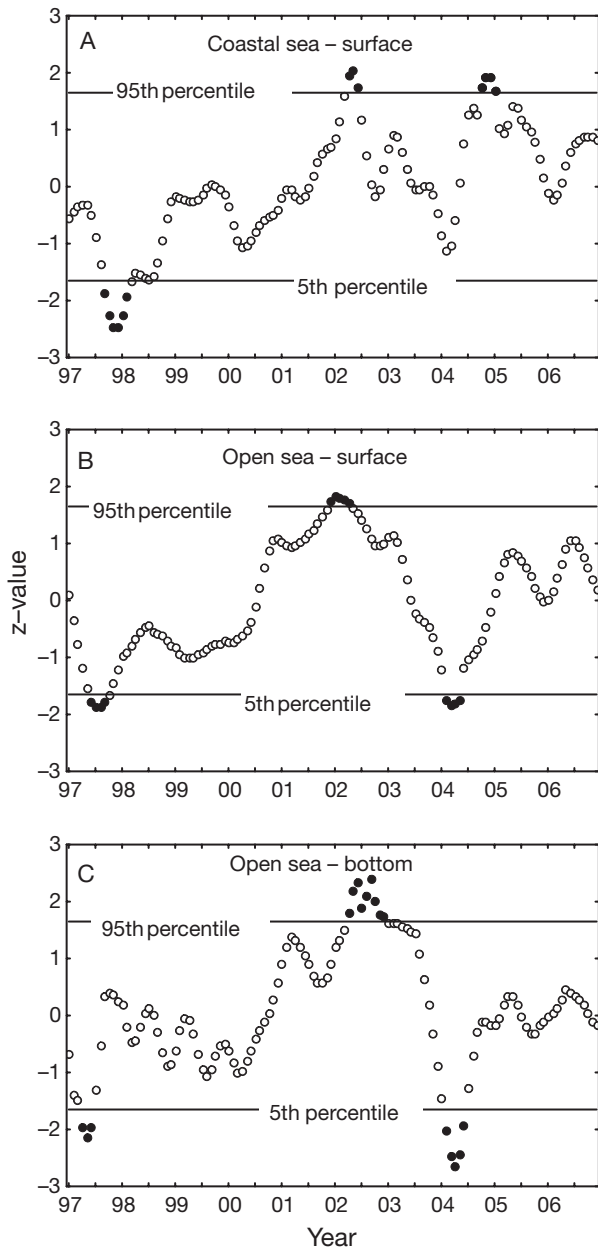


Fig. 9. Periods of strong bottom-up (dots above 95th percentile line) and strong top-down (dots below 5th percentile line) controls of bacteria as quantified by z-values at (A) coastal, (B) open sea and (C) deep-sea stations

seabed (Marasović et al. 2005). The loss of part of the nitrogen and phosphorus to the sediment is likely to be due to the processes of denitrification and phosphorus adsorption, and accumulation in sediments (Kušpilić 2001). The strong BU control in 2004 coincided with low salinity during the winter/spring period resulting from high precipitation and the Jadro River inflow maximum (Kušpilić 2001, Orlić et al. 2007).

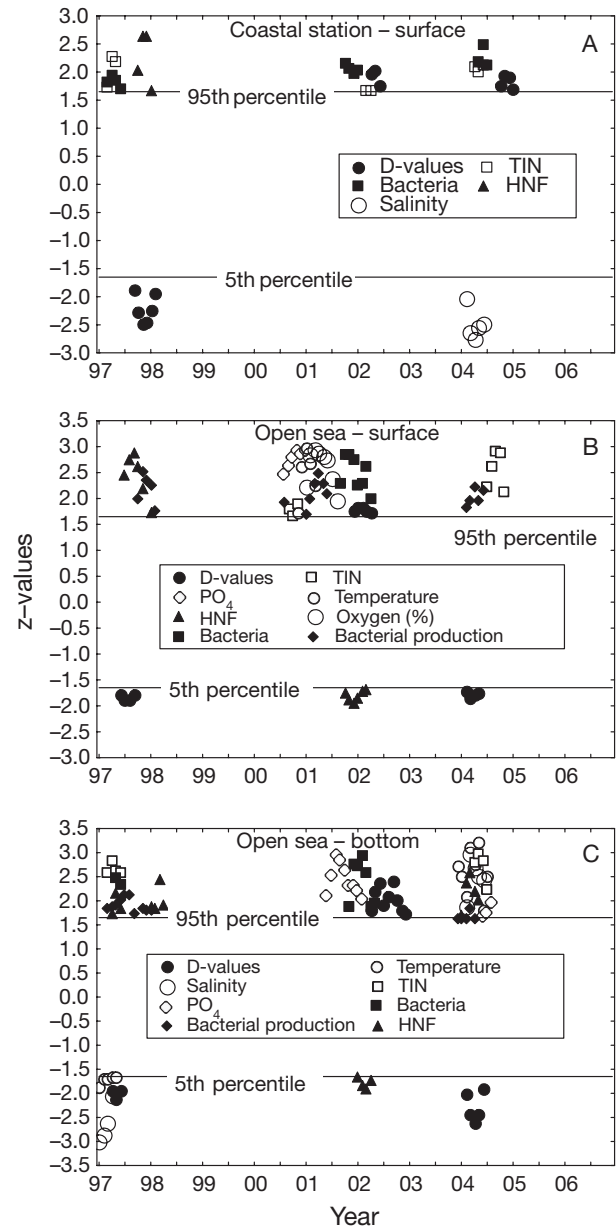


Fig. 10. Periods of strong bottom-up (symbols above 95th percentile line) and strong top-down (symbols below 5th percentile line) controls for different measured parameters standardised as z-values. Note the coincidence in exceptionalities between standardised D-values and other parameters. TIN: total inorganic nitrogen, HNF: heterotrophic nanoflagellates

In the open sea, similar fluctuations in the relative importance of the BU and TD controls of bacteria were found in both surface and bottom layers (Fig. 9B,C). In the surface layer, a first period of strong TD control was established during 1997. This period was characterised by a high abundance of HNF which was strongly coupled with bacterial production (Fig. 10B). Another period of strong TD control was established in 2004. A

marked increase in nitrogen during this period coincided with an increase in bacterial production, but no coupling between bacteria and HNF was found (Fig. 10B). Some authors hypothesised that sudden availability of nutrients would uncouple bacteria from their predators, allowing growth towards grazing resistant forms or fast-growing phylotypes (Jürgens & Güde 1994) which protozoans would not be able to crop down to steady-state abundance. Finally, the period of strong BU control, which was established to be during winter 2001–2002, was characterised by high bacterial abundance and very low HNF abundance (Fig. 10B). This winter was preceded by a marked increase in nutrients (particularly phosphorus), which peaked in winter 2000–2001, and was followed by peaks in DO saturation and bacterial production (Fig. 10B). A marked peak in DO saturation also suggests an increase in primary production during this period. Further, this period was characterised by an exceptional non-seasonal increase in temperature due to the extremely warm winter in 2000–2001 (Russo et al. 2005). In addition, an extremely large runoff from the Po River was measured between October 2000 and May 2001 (Grilli et al. 2005), this runoff being the second largest discharge in the last 200 yr (Zanchettin et al. 2008). Consequently, a rather large nutrient load was introduced into the Adriatic, presumably affecting surface waters of the middle Adriatic.

In the bottom layer, the strong TD control in 1997 was characterised by a high concentration of TIN and high bacterial abundance and production throughout 1997, which was followed by a peak in HNF abundance in the beginning of 1998 (Fig. 10C). Another characteristic of this period was very low temperature and salinity. A cold, less saline and nitrogen rich bottom layer in 1997 could be a footprint of the NAdDW generated during strong and long-lasting wintertime bora outbreaks (Dorman et al. 2007). During spring and summer, it moved downslope along the western Adriatic shelf reaching the middle and the south Adriatic. Another period of strong TD control was established in 2004. A marked increase in nutrients (both nitrogen and phosphorus) coincided with bacterial production peak, which was strongly coupled with HNF abundance (Fig. 10C). This period was characterised by extremely high bottom layer temperature and salinity (15°C and 38.9, respectively) for the investigated region (Vilibić & Orlić 2001), suggesting strong inflow of the LIW which is (besides having high salinity) also characterised by higher nutrient concentrations. Finally, the period of strong BU control in 2002 was characterised by high bacterial abundance and low HNF abundance (Fig. 10C). Bacterial abundance increase was preceded by an increase in phosphate concentration that was observed in 2001.

CONCLUSIONS

The present study showed the tendency for bacteria to be TD controlled in oligotrophic open seas, and BU controlled in more eutrophic coastal seas. The percentage contribution of HNA bacteria increased along a trophic gradient, suggesting that in conditions when bacteria are dominantly controlled by substrate availability, the contribution of active bacteria in the community increased.

However, temporal variability in the BU and TD controls was much stronger, and periods of both strong BU and strong TD controls were found at all studied stations, independently of their trophic status. We clearly showed that switching between BU and TD regulation happened on both seasonal and inter-annual scales. In general, an increase in bacterial abundance and/or production was established during periods of both strong BU and TD controls, and these increases were coupled with increases in nutrient concentrations (in coastal sea waters more often with nitrogen, and in the open sea with phosphorus). On the other hand, an increase in bacterial abundance and/or production was followed by an increase in HNF abundance during TD control periods, whereas coupling between bacteria and HNF failed to occur during BU control periods. Therefore, it seems that the input of nutrients initiated the increase in bacterial growth, whereas realised bacterial abundance was dependent on predation pressure. This occurred in both oligotrophic open sea stations and in more eutrophic coastal sea stations, and the balance between bacterial growth and loss changed over time. On a seasonal scale, BU control of bacteria dominated during the colder period of the year, while TD control dominated during the warmer period. Non-seasonal fluctuations in the relative importance of the BU and TD controls of bacteria pointed to a few periods when one or the other type of control was very strong. These periods coincided with some specific meteorological and hydrographic phenomena (the strong influence of NAdDW in 1997, the strong LIW ingression in 2004, or the extremely warm winter and the Po River runoff in 2000–2001).

However, there are many potentially important factors that were not monitored in this study. The role of viruses, mixotrophic flagellates as well as trophic and temporal changes in species composition of bacterial and protozoan assemblages were not monitored; thus, their effects on observed switches between BU vs. TD controls remain unknown. The role of viruses could be particularly important in eutrophic environments where they could significantly contribute to bacterial mortality (Guixa-Boixereu et al. 1999). Further, it seems that mixotrophic flagellates could have high contribution to total flagellate grazing on bacteria

(between 30 and 95%), particularly in the euphotic layer and under nutrient limited conditions (Unrein et al. 2007, Zubkov & Tarran 2008). Finally, protozoans might control community composition rather than bacterial abundances (del Giorgio et al. 1996, Jürgens et al. 1999).

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