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

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1 **ABSTRACT**

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

20  
21 **Key words:** data sampling, bacteria, heterotrophic nanoflagellates, water masses, multivariate  
22 analysis, Adriatic Sea.

23

24

25 **1. INTRODUCTION**

26           The Adriatic Sea is the northernmost basin in the Mediterranean, with length of 800 km  
27 and width of 200-250 km. According to the bathymetry, the Adriatic Sea may be divided into  
28 three parts: a broad northern Adriatic shelf with an average depth of 40 m, the middle Adriatic  
29 with depressions up to 280 m, connected to the southern Adriatic circular basin (depths up to  
30 1230 m) over the Palagruža Sill (deepest parts of 170 m). As the circulation of the Adriatic is  
31 cyclonic due to estuarine conditions regulated by the northern Adriatic rivers (Cushman-  
32 Roisin et al., 2001) resulting in outflowing West Adriatic Current, eastern Adriatic region is  
33 exposed to the northwestern East Adriatic Current (Orlić et al., 2007), bringing saline waters  
34 from the Ionian and Levantine Seas along its pathway. These waters, known as Levantine  
35 Intermediate Water (LIW, Robinson et al., 1992; Malanotte-Rizzoli et al., 1997), can flood  
36 the Adriatic in some years (so-called ingressions years, Buljan, 1953), increasing salinity,  
37 temperature and nutrients in intermediate layers (Buljan and Zore-Armanda, 1979; Tziperman  
38 and Malanotte-Rizzoli, 1991; Vilibić and Orlić, 2001, 2002).

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

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

58 In some years LIW intrusion may reach northern Adriatic, and during wintertime it  
59 serves as a preconditioning for the generation of another deep Adriatic water mass. North  
60 Adriatic Dense Water (NAdDW, Zore-Armanda, 1963; Artegiani et al., 1997) is the densest  
61 Mediterranean water mass (density excess up to  $30 \text{ kg m}^{-3}$ , Vilibić, 2003), generated during  
62 strong and long-lasting wintertime bora outbreaks (Dorman et al., 2007). During spring and  
63 summer it moves downslope along the western Adriatic shelf, strongly hits and fills up deep  
64 layers of the Jabuka Pit (Vilibić et al., 2004). Its less dense component goes over the  
65 Palagruža Sill (Vilibić and Supić, 2005; Wang et al., 2007) and sinks rapidly towards the  
66 bottom of the South Adriatic Pit through the mixing and channelling processes in the Bari  
67 Canyon (Manca et al., 2002; Vilibić and Šantić, 2007). As it is generated on the open northern  
68 Adriatic shelf, and not influenced by coastal waters rich in nutrients (Tedesco et al., 2007),  
69 NAdDW brings a small amount of orthophosphates to the deep near-bottom Adriatic waters,  
70 being somewhat regenerated there through the oxidation processes (Krasakopoulou et al.,  
71 2005). However, NAdDW nutrients in the 100-150 m layer above the near-bottom water  
72 masses show even lower nutrient concentrations, as this zone of the water column is not  
73 influenced by the regeneration but rather with nutrient consumption through the primary  
74 production. NAdDW generation does not occur every winter, and it is enabled by a number of  
75 factors (heat flux preconditioning, low river discharges, wintertime cooling intensity). These

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

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

93

## 94 **2. MATERIALS AND METHODS**

95

### 96 **2.1. Data collection**

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

101 its surface layer but also the open Adriatic circulation and water masses during dryer periods  
102 (Grbec and Morović, 1997). In contrast, the deep layers are flooded by the open Adriatic  
103 water masses, mostly LIW and NAdDW, which are characterized by smaller oscillations of  
104 chemical and physical parameters in comparison to coastal area (Buljan and Zore-Armanda,  
105 1976). Therefore, this station is quite suitable for detecting the environmental changes in the  
106 open Adriatic Sea in relation to its circulation, water masses and their long-term changes and  
107 anomalies.

108 Another aspect favourable for the choice of this station is the excellent temporal  
109 coverage of the measurements of various environmental parameters, from physical  
110 (temperature, salinity), chemical (oxygen, nutrients) towards biological (bacteria) ones.  
111 Systematic measurement at the Stončica Cape started already in 1950s' (Buljan and Zore-  
112 Armanda, 1966), and a large amount of data has been collected till the present. For the period  
113 of this study (1997-2006), monthly coverage with the measurements of all studied parameters  
114 (temperature, salinity, nutrients, bacteria) was larger than 75%, with no gap longer than three  
115 consecutive months. Samples were collected at standard oceanographic depths (0, 5, 10, 20,  
116 30, 50, 75 and 100 m), processed on board immediately after sampling, and by the same  
117 methods of sampling and analyses throughout the investigated period.

118 Bacteria and heterotrophic nanoflagellates (HNF) were enumerated by epifluorescence  
119 microscopy ("Olympus" BX50 at a magnification of x1000), using the standard DAPI  
120 staining technique (Porter and Feig, 1980). Bacterial cell production was measured from DNA  
121 synthesis based on incorporation rates of  $^3\text{H}$ -thymidine (Fuhrman and Azam, 1982). (Methyl-  
122  $^3\text{H}$ -) thymidine was added to 10 ml samples at a final concentration of 10 nmol (specific  
123 activity 86 Ci mmol<sup>-1</sup>). Triplicate samples and a formaldehyde killed adsorption control (final  
124 concentration 0.5%) were incubated for 1 hour. The incubations were stopped with  
125 formaldehyde (final concentration 0.5%). The thymidine samples were extracted with ice-cold

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

133 Dissolved oxygen concentration was determined by Winkler titration (Strickland and  
134 Parsons, 1968). Nutrients ( $\text{NO}_3$ ,  $\text{NO}_2$ ,  $\text{NH}_4$ ,  $\text{PO}_4$ ) were analysed on Bran+Luebbe  
135 AutoAnalyser (II and III) models, using modified automated methods (Grasshoff, 1976). The  
136 temperature and salinity were measured with CTD multiparameter probes (Idronaut and  
137 SeaBird), with accuracy larger than  $\pm 0.01^\circ\text{C}$  and  $\pm 0.02$ , respectively.

138

## 139 2.2. Analysing procedures

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

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

149

$$150 \quad X_t = TC_t * S_t * I_t \quad (1)$$

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

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

177

### 178 **3. RESULTS AND DISCUSSION**

179

#### 180 **3.1. Decomposition of time-series**

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

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

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

212 To identify the main patterns of seasonal fluctuations, PCA was carried out on arrays  
213 consisting of the seasonal component data sets for all studied parameters, separately for  
214 surface and bottom layers (Fig. 3A). Analysis showed similar seasonal patterns between  
215 surface and bottom layers for all parameters, except HNF abundance which showed very  
216 different patterns between surface and bottom layers. PC1 explain 53% of variability and is  
217 significantly negatively correlated with bacterial production (total and per cell) in both surface  
218 and bottom layers (Fig. 3B,C), and with HNF abundance in surface layers (Fig. 3B)  
219 (coefficients of correlation ranged between  $-0.74$  and  $-0.94$ ;  $P < 0.001$ ). The main  
220 characteristics of the seasonal components for these parameters are higher values during the  
221 warmest part of the year (May to September), and lower values during the coldest part of the  
222 year (PC1 showed opposite pattern). Bacterial abundance (in surface and bottom layers) is  
223 fairly well represented by PC2 which explain 24% of variability ( $r = 0.95$  in surface layers,  $r$   
224  $= 0.81$  in bottom layers;  $P < 0.001$ ) (Fig. 3D). Characteristics of this pattern is spring maximum

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

227

228 To test variability in seasonal and non-seasonal fluctuations of examined parameters  
229 throughout the studied period, PCA were carried out on arrays consisting of the seasonal  
230 component and trend-cycle component data sets for all examined parameters for each year,  
231 separately for surface and bottom layers (Fig. 4). The results suggest no consistency in either  
232 seasonal and non-seasonal patterns throughout the studied period. However, fluctuations of  
233 these components were not random, but were characterised by systematic changes and  
234 grouping in time. PCA showed a clear separation between 1997-2001 and 2002-2006 period  
235 for both seasonal and trend-cycle components, as well as for both surface and bottom layers  
236 (Fig. 4). Due to the above results, we found it useful to separate the analysis into two periods:  
237 1997-2001 and 2002-2006, as a significant shift in annual time-course happened at some stage  
238 in 2001/2002.

239

### 240 **3.2. Comparison between 1997-2001 and 2002-2006 periods**

241 Comparison of seasonal components between 1997-2001 and 2002-2006 periods  
242 shows substantial changes in seasonal patterns between the two periods, keeping their  
243 consistency within both periods. No statistically significant correlation ( $P > 0.5$ ) has been  
244 found between 1997-2001 and 2002-2006 periods for all studied parameters in both surface  
245 and bottom layers (Figs. 5, 6); surprisingly, they show even an opposite behaviour (negative  
246 correlations) for some months and parameters. Namely, bacterial abundance showed higher  
247 values during winter-spring, with maximum values in May, than during the summer of 1997-  
248 2001. Similar patterns were established in both surface (Fig. 5A) and bottom layers (Fig. 6A).  
249 In contrast, bacterial abundance was higher in spring-summer than in winter, with maximum

250 values in April during 2002-2006 period (Figs. 5E, 6E). A different pattern was observed for  
251 HNF abundance in the surface layer; characterized by high values during the warmer part of  
252 the year (from April to August) and very low values during winter in the 1997-2001 period  
253 (Fig. 5B). Conversely, HNF abundance was high only during July-August during the 2002-  
254 2006 period, maintaining very low values from April to June and with rather high values  
255 recorded in January (Fig. 5F). In the bottom layers a substantial maximum was recorded in  
256 September-October and minimum in April-May during 1997-2001 period (Fig. 6B). In  
257 contrast, the highest values were established in January-February and the lowest values in  
258 October-November during 2002-2006 period (Fig. 6F). To summarize, bacterial production  
259 (both total and per cell) followed a similar seasonal pattern during 1997-2001 period, in both  
260 surface and bottom layers. This pattern was characterized by high values during the warmest  
261 part of the year (from April to September), and low values during the coldest part of the year  
262 (from November to January) (Figs. 5C-D, 6C-D). During the 2002-2006 period, however, low  
263 values were established from July to September, with much higher values during winter and a  
264 peak in May (Figs. 5G-H, 6G-H). All of these findings suggest a major shift in biological  
265 parameters between the two periods, presumably related to the environmental parameters and  
266 circulation changes, which will be discussed in Section 3.3.

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

275 Correlation analyses between biological parameters were made separately for 1997-  
276 2001 and 2002-2006 periods in both surface and bottom layers (Tab. 1). There are significant  
277 positive correlations between TBP and SBP in both periods and both layers. In the surface  
278 layer the correlations are quite high ( $P < 0.001$ ) in 2002-2006 period (bacteria were negatively  
279 correlated with HNF, TBP and SBP, and HNF were positively correlated with TBP and SBP),  
280 while they were not significant in the 1997-2001 period, presumably related to the changes in  
281 environmental conditions (see next section). In the bottom layer, significant positive  
282 correlations were established between bacteria and TBP, as well as between HNF and both  
283 TBP and SBP during the 1997-2001 period. On the other hand, during the 2002-2006 period,  
284 significant negative correlations of bacteria with HNF and SBP were established (Tab. 1).  
285 HNF are the most important bacterial grazers in the Adriatic Sea (Šolić and Krstulović, 1995;  
286 Šolić et al., 1998), therefore the higher correlation between HNF and bacterial production  
287 compared with bacterial abundance suggests that HNF control bacterial standing stock by  
288 direct cropping of bacterial production. Similar results were reported for the coastal Adriatic  
289 Sea (Šolić and Krstulović, 1994). Gonzalez et al. (1990) and Sherr et al. (1992) suggest that  
290 bacterivores crop the production rather than affecting the standing stock of bacteria because of  
291 a higher grazing pressure on more actively growing and dividing cells. This is supported by  
292 the fact that HNF abundance was strongly correlated with SBP (better indicator of bacterial  
293 reproductive activity) than with TBP, which is function not only of individual cell activity but  
294 also of bacterial abundance (Tab. 1).

295

### 296 **3.3. Relation to the environmental factors**

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

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

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

328 In the surface layers, year-to-year fluctuations of temperature and salinity are primarily  
329 under the influence of surface heating/cooling processes and the interplay between  
330 coastal/open Adriatic waters. Namely, temperature in 2001 had the lowest seasonal signal (as  
331 a result of higher winter and lower summer temperatures than usual), but a rather broad range  
332 of salinity values (Fig. 9A,B), as a result of warmer winter and coastal waters being  
333 dominated by large river runoffs. The years 2004 and 2005 were characterised by higher  
334 winter and lower summer temperature, respectively. Both years were also characterized by a  
335 wide range of salinities, as a result of high precipitation and large river runoff (river Neretva),  
336 which during some periods reached the investigated area (Fig. 9A,B). In the surface layers,  
337 the temperature time series has been predominantly determined by seasonal component (Fig.  
338 10A). The trend-cycle component showed a strong non-seasonal increase in temperature  
339 during January-February 2001, which is presumably the result of a warmer-than-average  
340 winter and low surface cooling (Russo et al., 2005). This increase in temperature was  
341 followed by an increase in bacterial production, as well as oxygen saturation, which suggests  
342 an increase in primary production (Fig. 10B,C).

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

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

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

375 bottom of the Stončica station, decreasing bottom salinity and temperature in January-  
376 February 1997 (Fig. 11A). All nitrogen compounds showed a decrease in concentration (Fig.  
377 11B) and phosphate an increase (Fig. 11C), revealing a change in water masses in the  
378 investigated region. In association with higher nutrient concentrations brought by northern  
379 Adriatic waters, later substituted by LIW inflow, this period is characterized by a decline in  
380 bacterial abundance and production as well in abundance of HNF (Fig. 11D), starting with an  
381 increased abundance/production in 1997 and then decreasing over time.

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

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

400 and bottom temperature and salinity values, in which some changes in thermohaline regimes  
401 may be encountered. Firstly, at the surface the flooding of less saline coastal waters over the  
402 Stončica station is substantially changed between the two periods: the primary salinity  
403 minimum (May-July) was much more pronounced in 2002-2006 period (Fig. 13D) versus  
404 1997-2001 period (Fig. 13C), while the secondary minimum (January-February), existing in  
405 the first period, totally disappeared in the 2002-2006 period. The changes also occurred at the  
406 bottom; the inflow of saline LIW waters starts to recover earlier (in April-May) between 2002  
407 and 2006, with positive seasonal values ( $>100$ ) established from May to November (Fig.  
408 13H). Differently, the positive seasonal salinity values encompassed much narrower period  
409 (June-October) between 1997 and 2001, denoting different dynamics in LIW entrance in the  
410 investigated area (Fig. 13G). That is also visible in bottom temperature; a large jump between  
411 December and January and significantly lower seasonal component between January and  
412 March during 2002-2006 period (Fig. 13F) are a footprint of large cooling events and  
413 generation of cold and dense NAdDW. The stronger leakage of such NAdDW triggered  
414 earlier LIW entrance in the middle Adriatic through the Adriatic-Ionian thermohaline cell, as  
415 observed and modelled in 2003 (Orlić et al., 2007). That supports an earlier phytoplankton  
416 production in surface layer and consequently shifts the maximum in bacterial production  
417 towards the spring instead of spring-summer.

418

#### 419 **4. CONCLUSIONS**

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

425 has in controlling bacterial activity and planktonic communities (as fluctuation of HNF  
426 abundance clearly showed). Analyses showed an increasing importance of non-seasonal  
427 components of environmental parameters (expressed as trend-cycle component), and their  
428 enlarged weight in controlling bacterial activity between 1997 and 2006. On the other hand, a  
429 sharp shift in seasonal cycles has sometimes been observed between 1997-2001 and  
430 2002/2006, presumably related to the shift in water mass (LIW and NAdDW) dynamics in the  
431 middle Adriatic Sea during 2001/2002. In the surface layers, year-to-year fluctuations of  
432 temperature and salinity are predominantly under the influence of surface heating/cooling  
433 processes and the interplay between coastal/open Adriatic waters which influenced the  
434 patterns of bacterial fluctuations. In the deep layers, the bacterial abundance and production  
435 were predominantly controlled by NAdDW during 1997-2001, due to the absence of LIW  
436 inflow at that time. NAdDW brings colder and less saline waters over the eastern Palagruža  
437 Sill, rich in nitrogen nutrients but poor in orthophosphate. In the successive period (2002-  
438 2006), the deep waters in the eastern middle Adriatic were predominantly influenced by  
439 warmer and more saline LIW which brings higher concentrations of all nutrients, as NAdDW,  
440 although generated, has been confined to the western Adriatic.

441

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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***	<b>0.404**</b>	n.s.
Bacteria : SBP	n.s.	- 0.736***	n.s.	- 0.379**
HNF : TBP	n.s.	<b>0.645***</b>	<b>0.410**</b>	n.s.
HNF : SBP	n.s.	<b>0.771***</b>	<b>0.618***</b>	n.s.
TBP : SBP	<b>0.966***</b>	<b>0.964***</b>	<b>0.923***</b>	<b>0.950***</b>

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

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.	<b>0.369**</b>	- 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.	<b>0.309*</b>	n.s.	n.s.
Bacteria : Salinity	- 0.302*	<b>0.276*</b>	- 0.521***	- 0.452***
HNF : Oxygen(%)	- 0.418***	n.s.	- 0.723***	<b>0.466***</b>
HNF : Nitrate	<b>0.326*</b>	n.s.	<b>0.371**</b>	n.s.
HNF : Ammonia	- 0.407**	<b>0.292*</b>	- 0.350**	<b>0.289*</b>
HNF : Phosphate	n.s.	n.s.	- 0.539***	n.s.
HNF : N/P ratio	n.s.	n.s.	<b>0.518***</b>	n.s.
HNF : Temperature	- 0.306*	n.s.	- 0.389**	<b>0.482***</b>
HNF : Salinity	n.s.	n.s.	- 0.267*	<b>0.705***</b>
TBP : Oxygen(%)	n.s.	n.s.	- 0.351*	n.s.
TBP : Nitrate	<b>0.431***</b>	n.s.	<b>0.817***</b>	n.s.
TBP : Ammonia	n.s.	n.s.	n.s.	<b>0.309*</b>
TBP : DIN	n.s.	n.s.	<b>0.724***</b>	<b>0.343*</b>
TBP : Phosphate	n.s.	n.s.	- 0.430***	n.s.
TBP : N/P ratio	n.s.	n.s.	<b>0.596***</b>	<b>0.427***</b>
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	<b>0.357**</b>	n.s.	<b>0.716***</b>	<b>0.297*</b>
SBP : DIN	n.s.	n.s.	<b>0.709***</b>	<b>0.395**</b>
SBP : Phosphate	n.s.	n.s.	- 0.416***	n.s.
SBP : N/P ratio	n.s.	n.s.	<b>0.601***</b>	<b>0.530***</b>
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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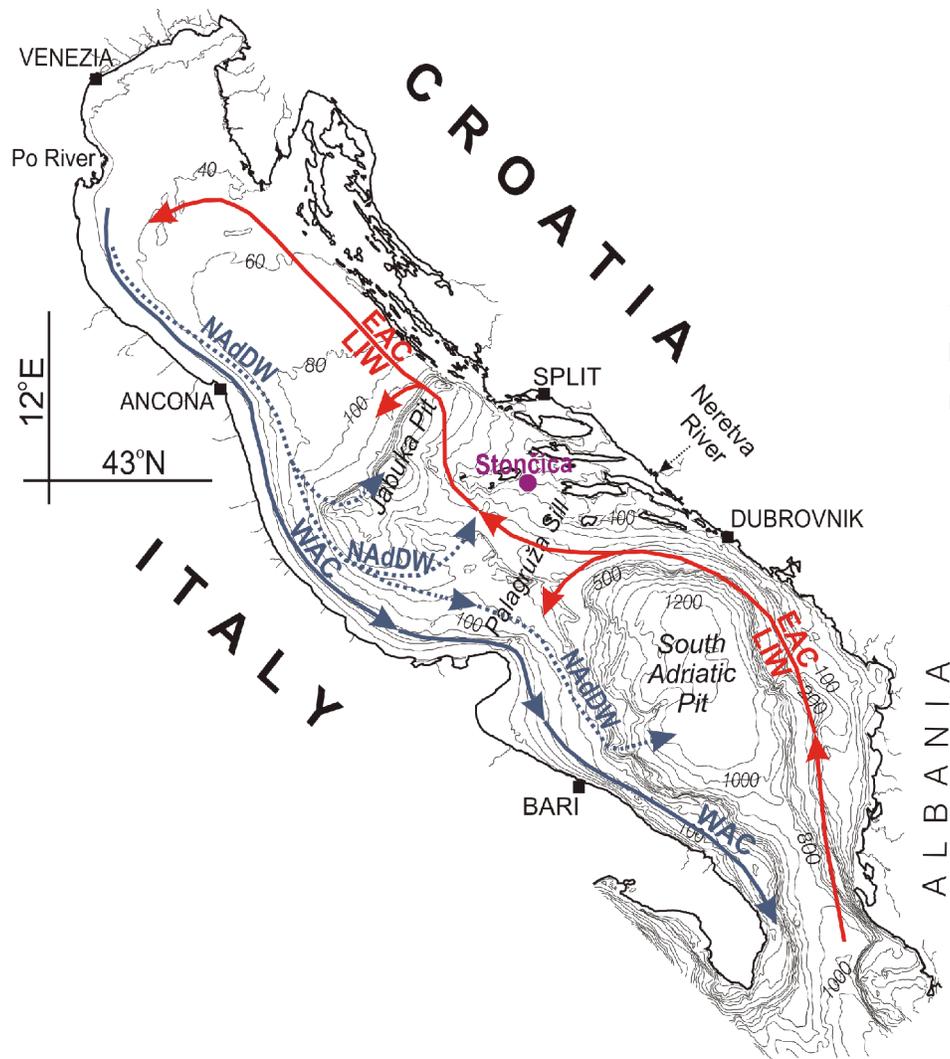


Fig.1

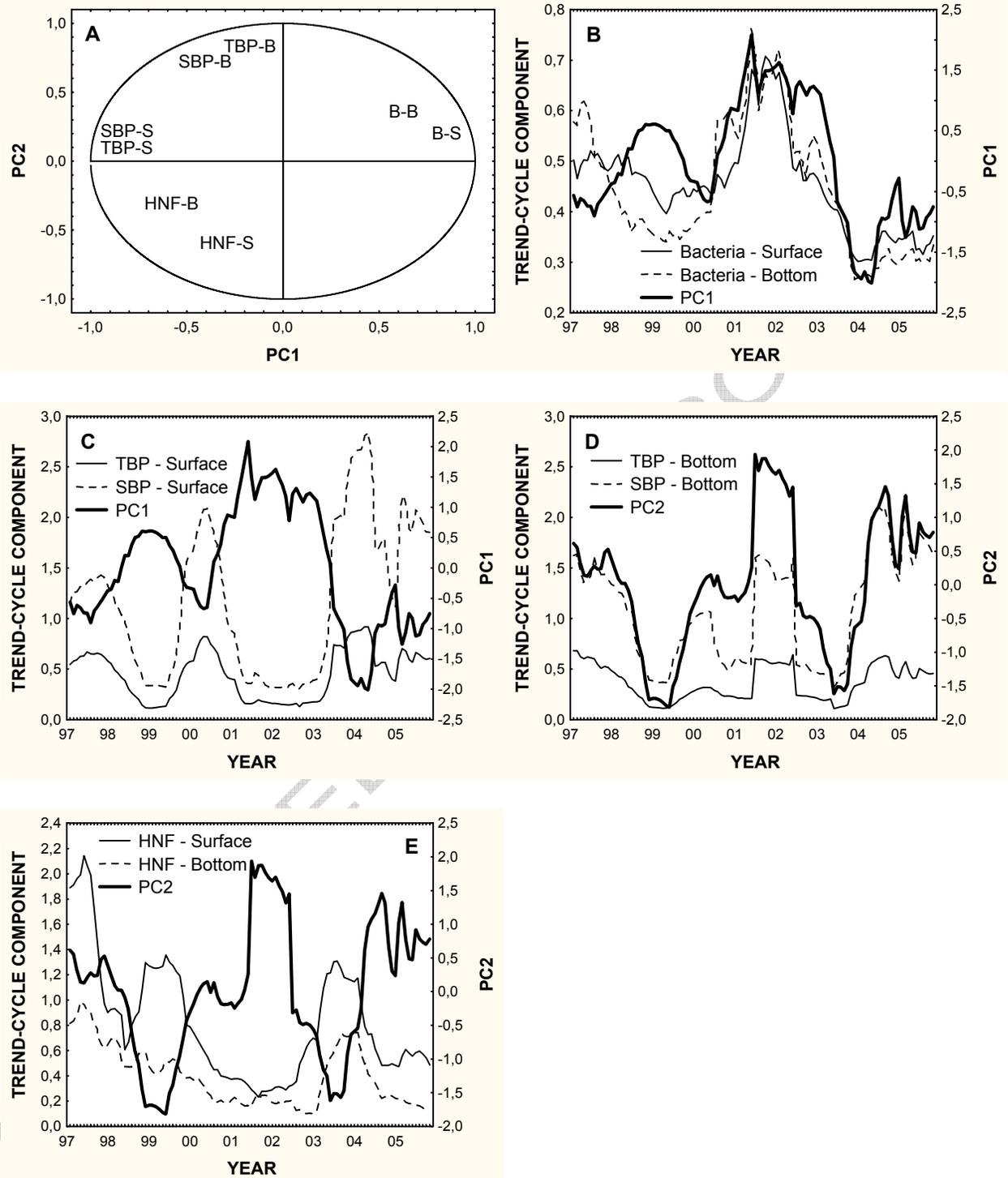


Fig. 2

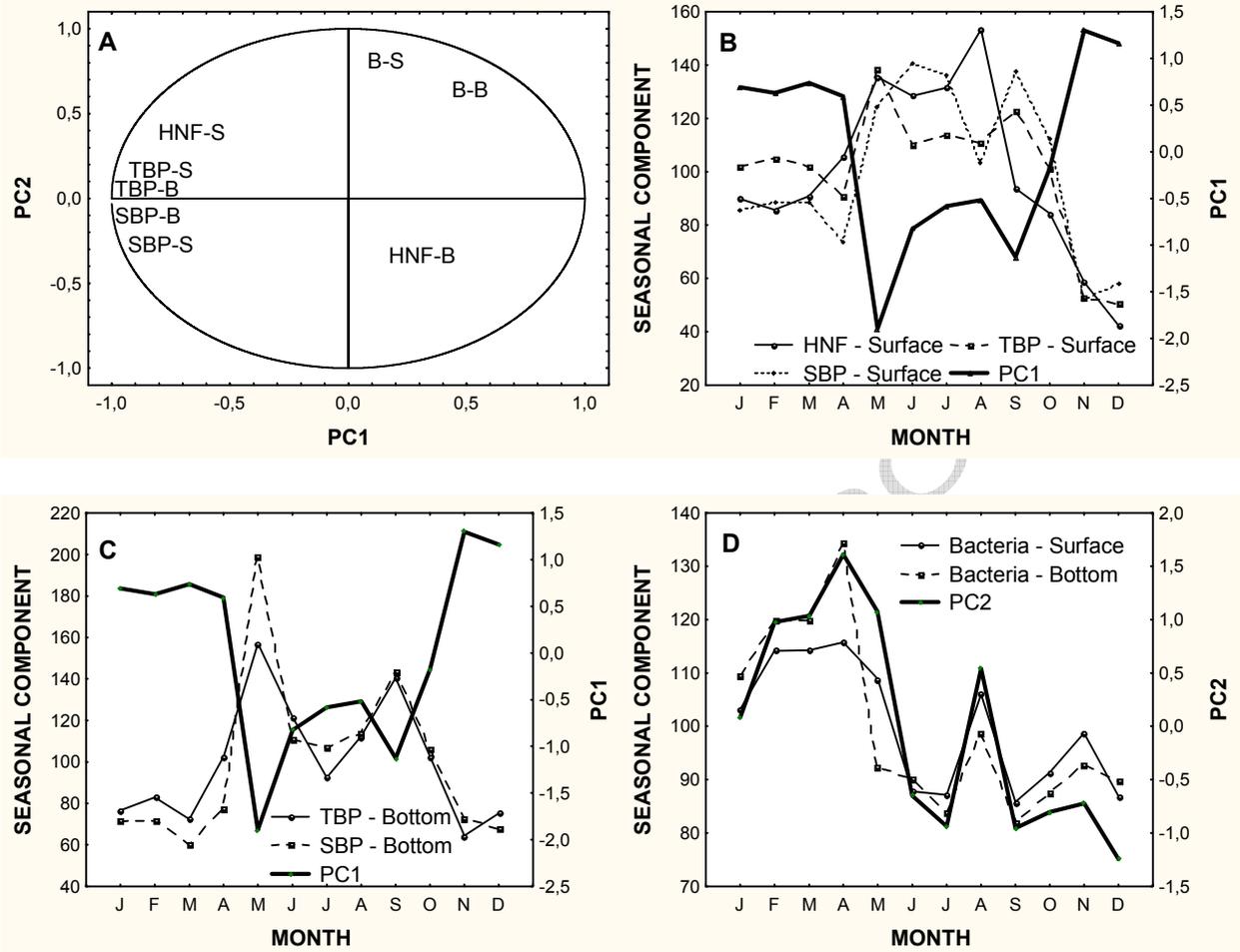


Fig.3

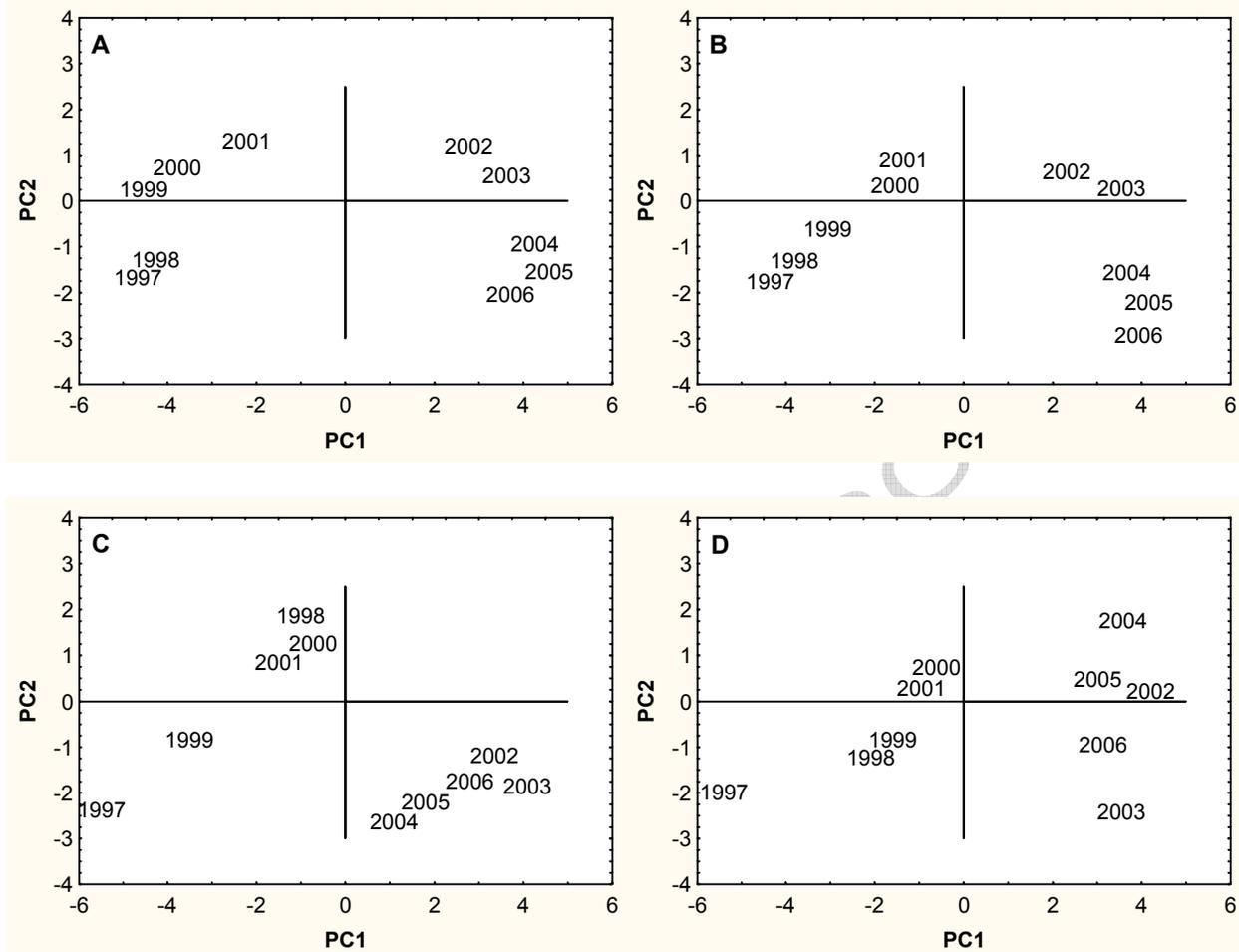


Fig.4

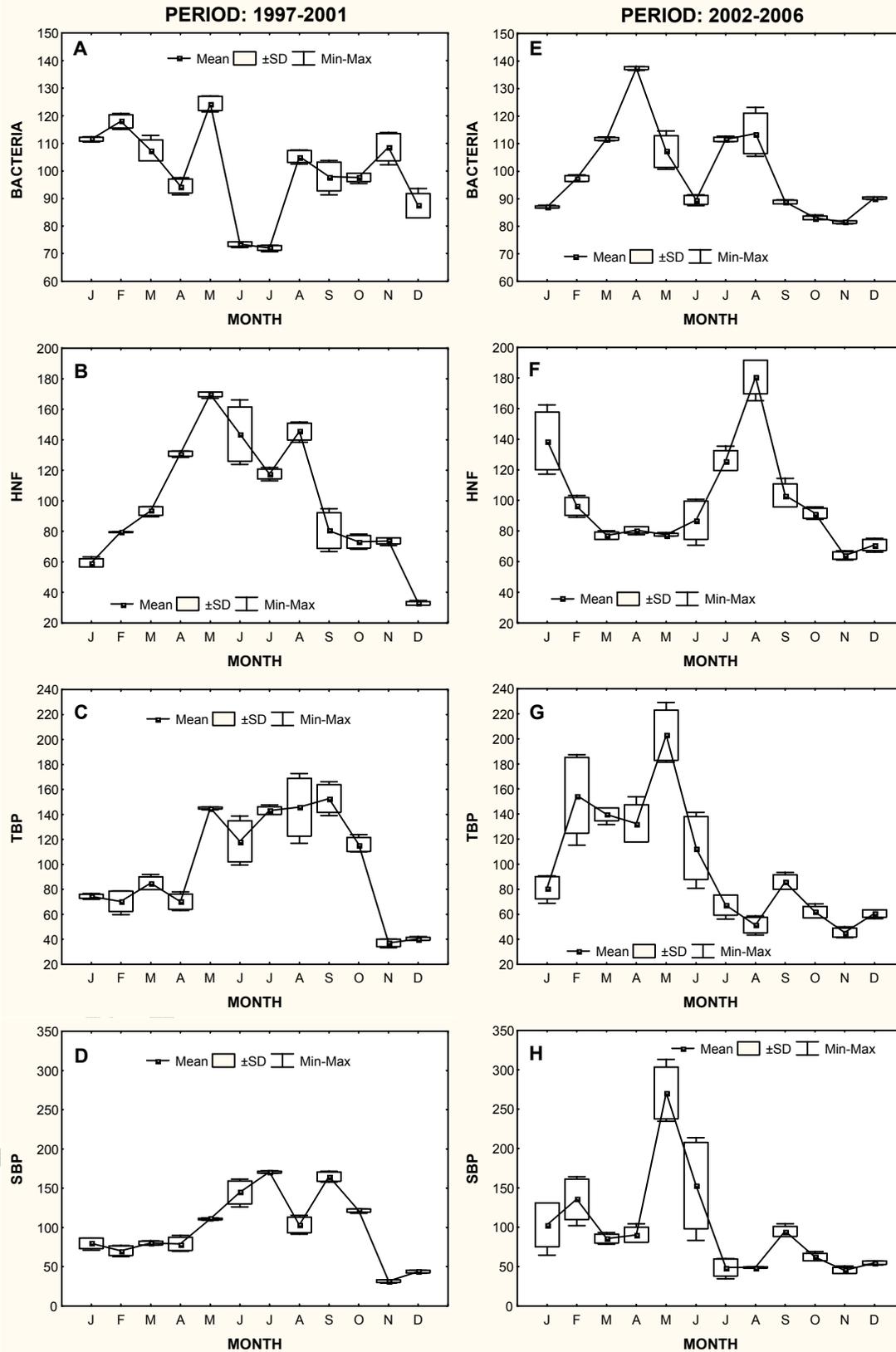


Fig.5

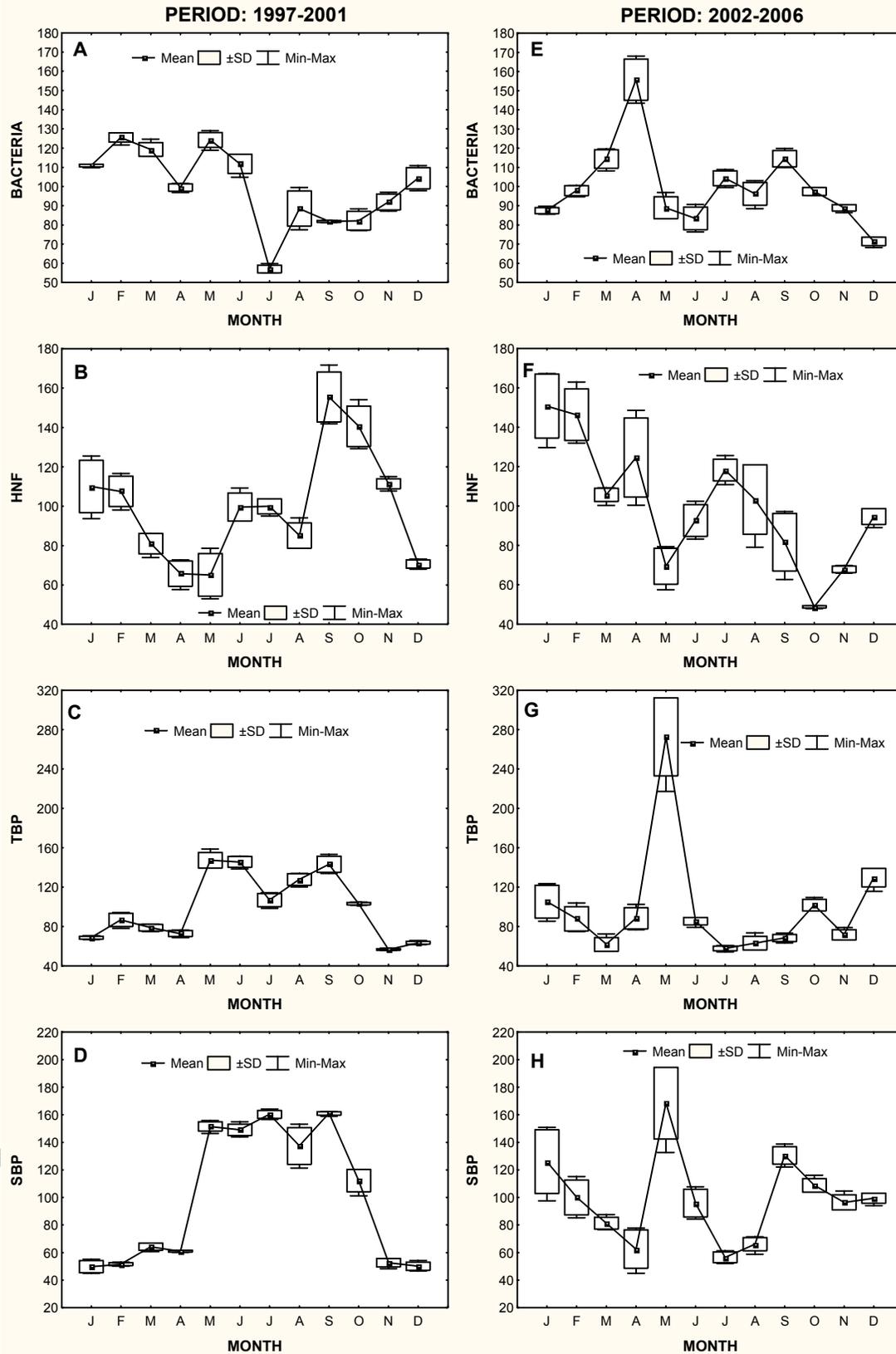


Fig.6

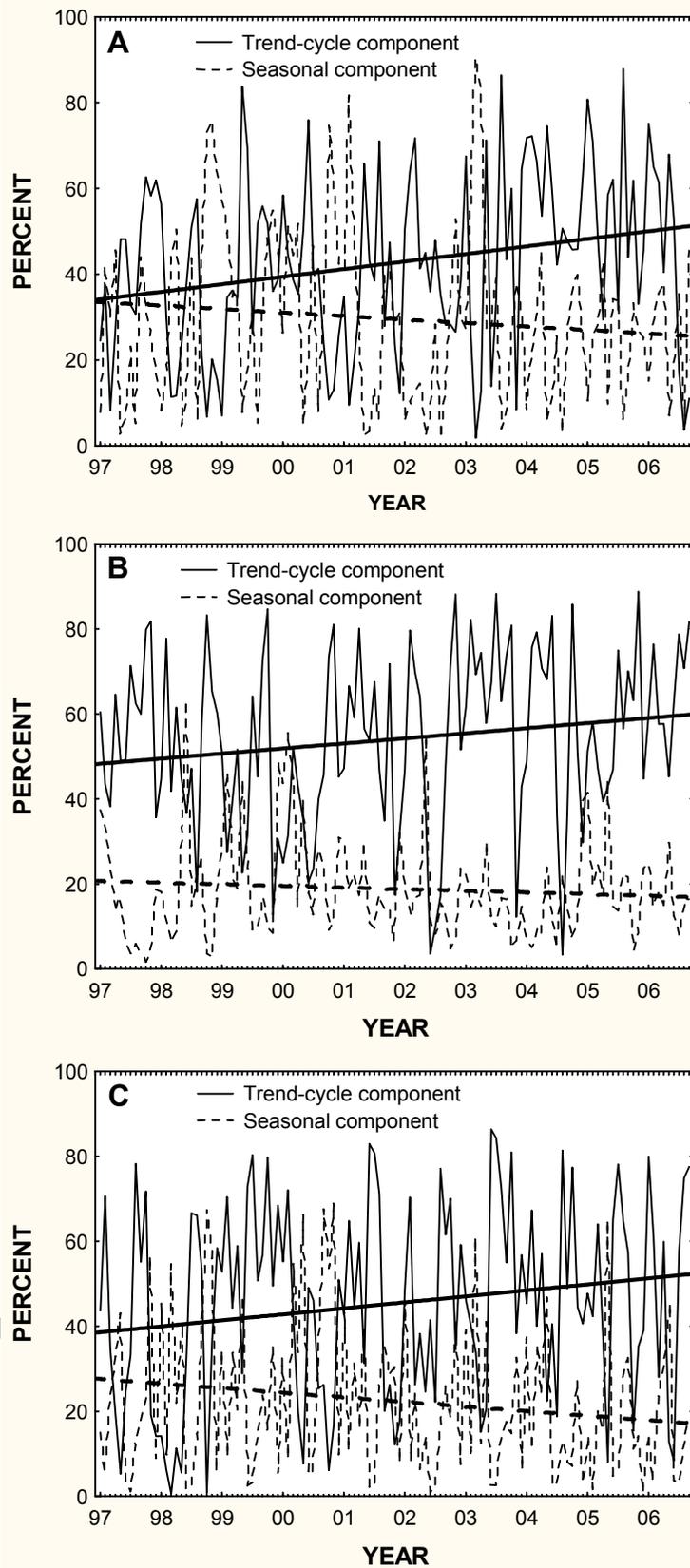


Fig.7

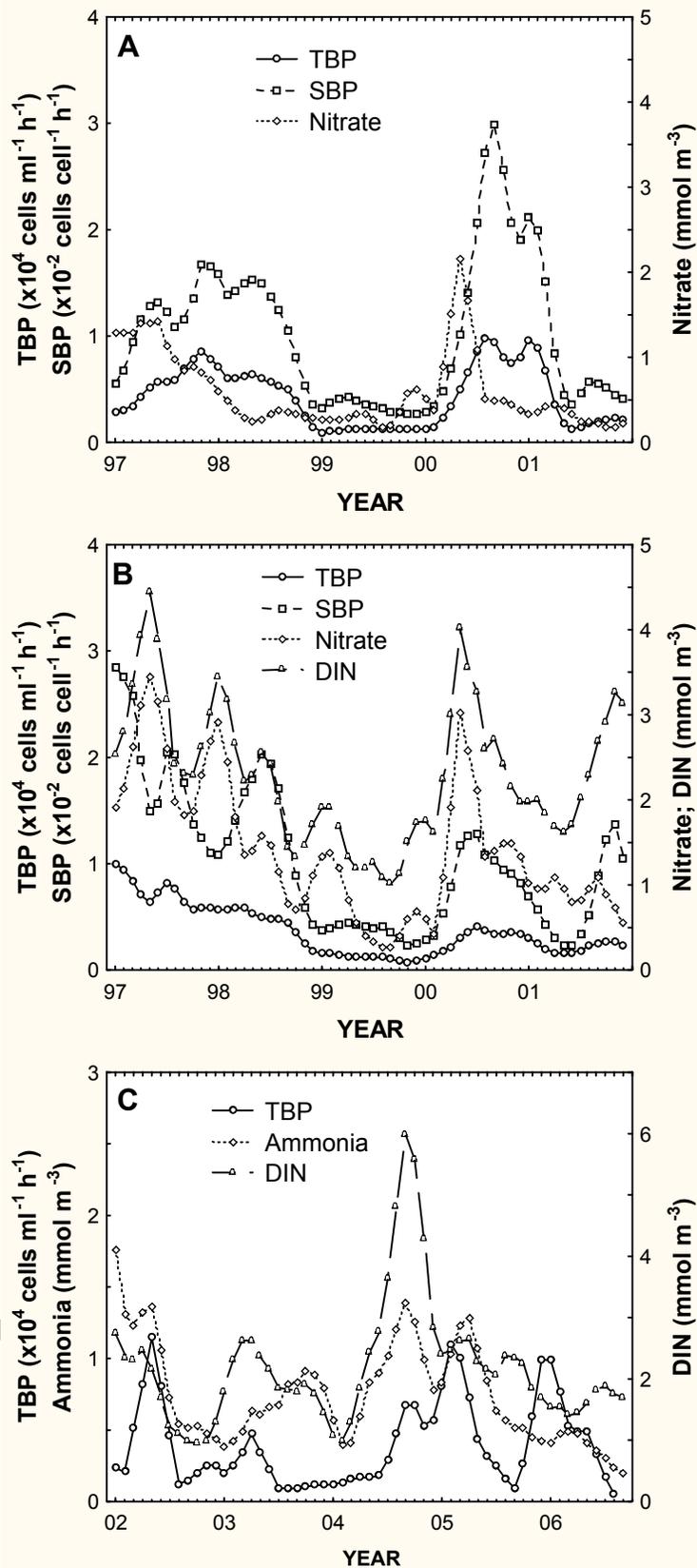


Fig. 8

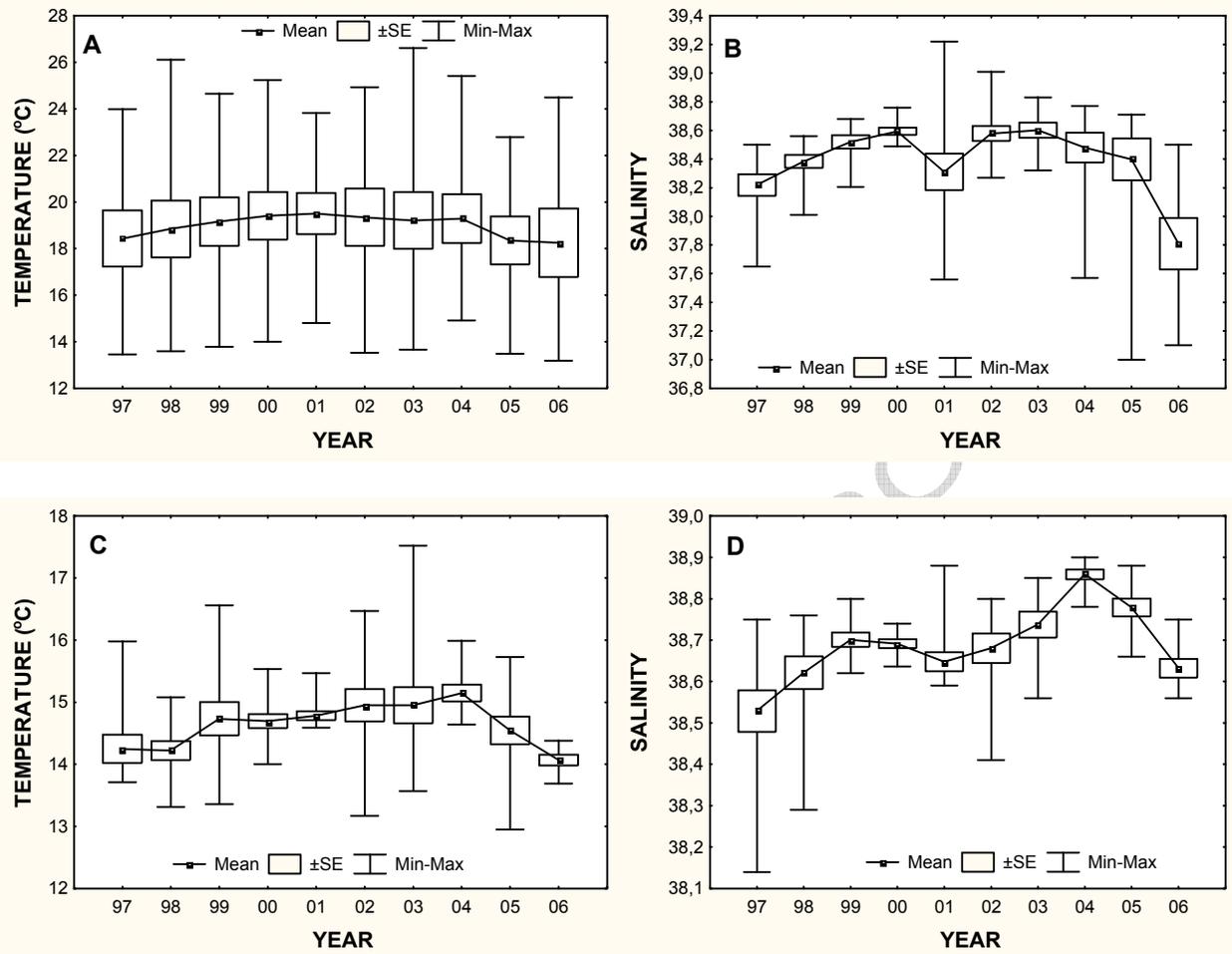


Fig.9

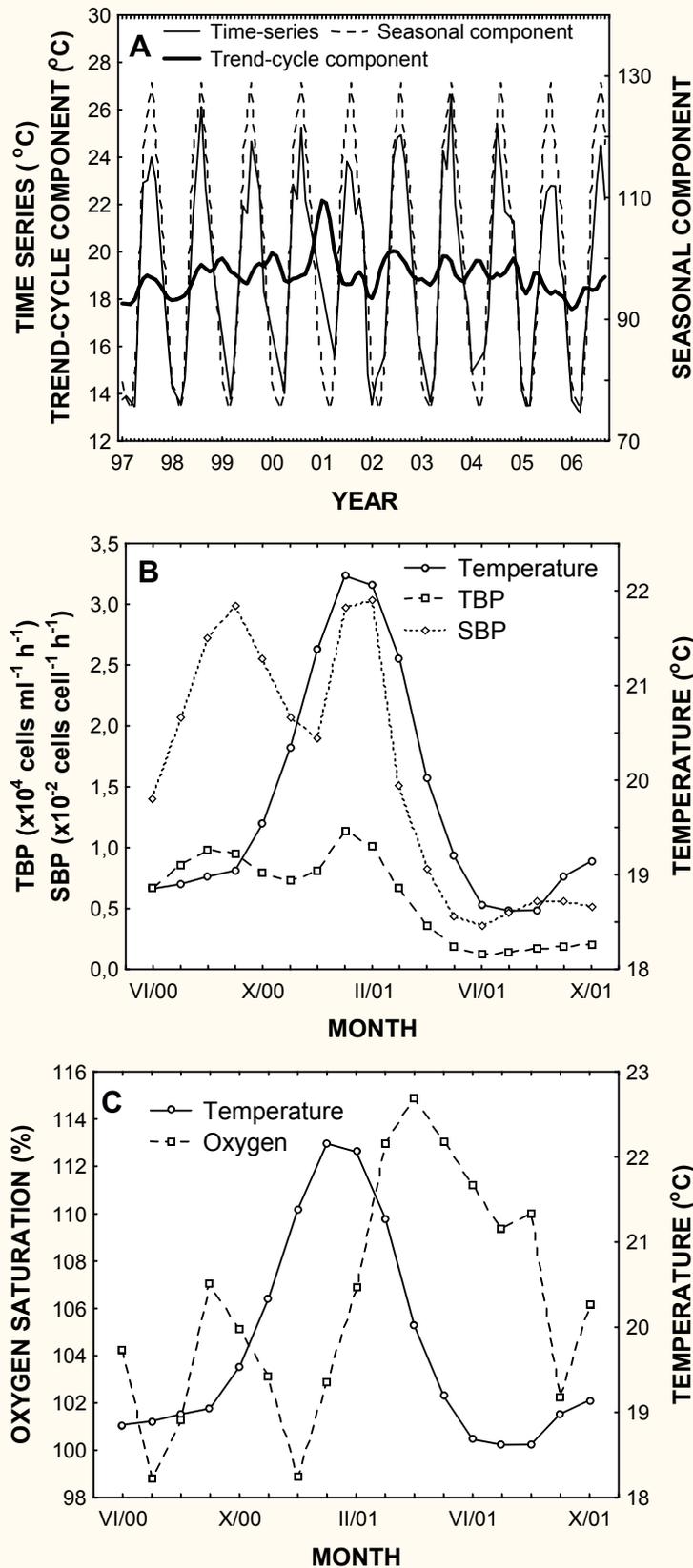


Fig. 10

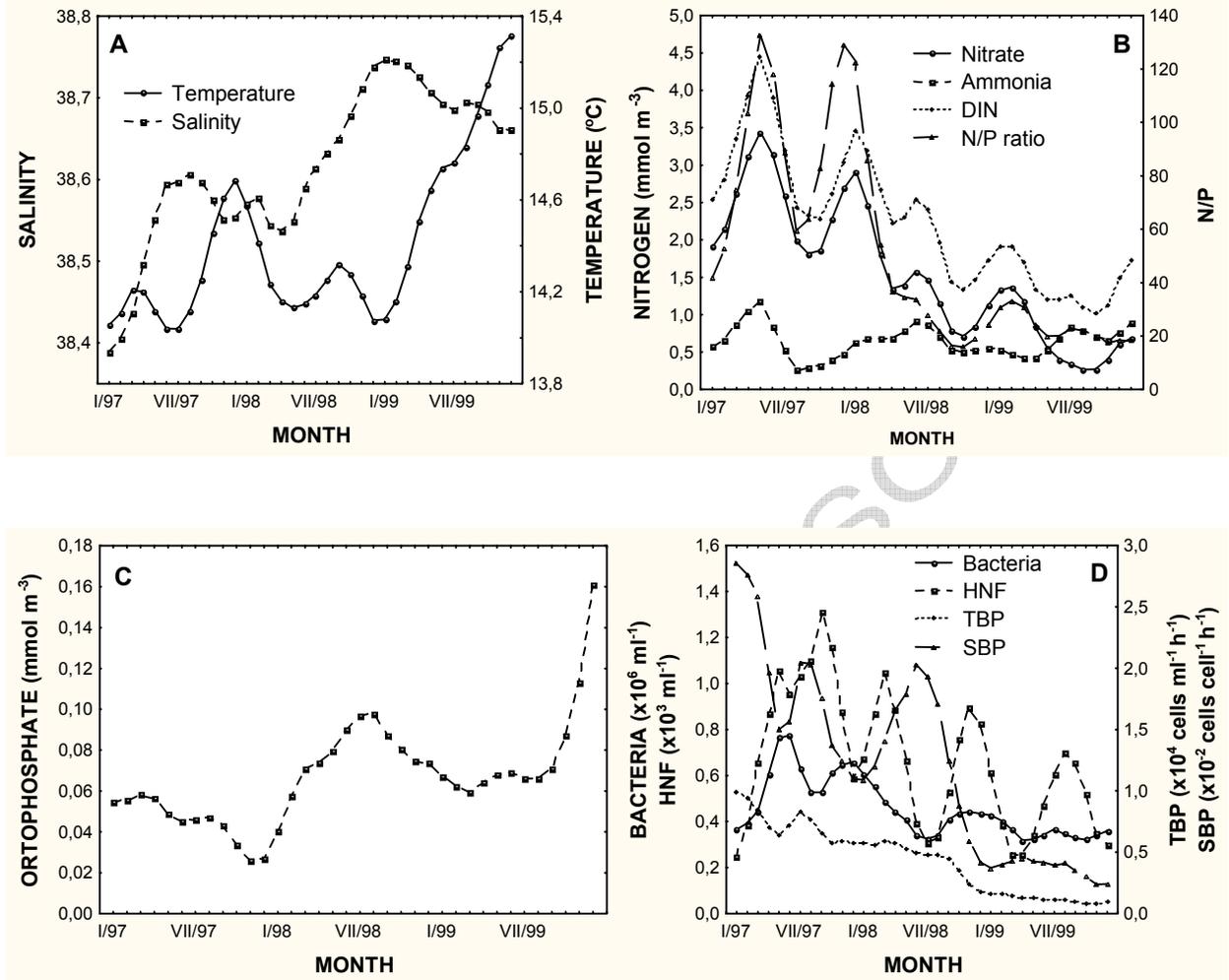


Fig.11

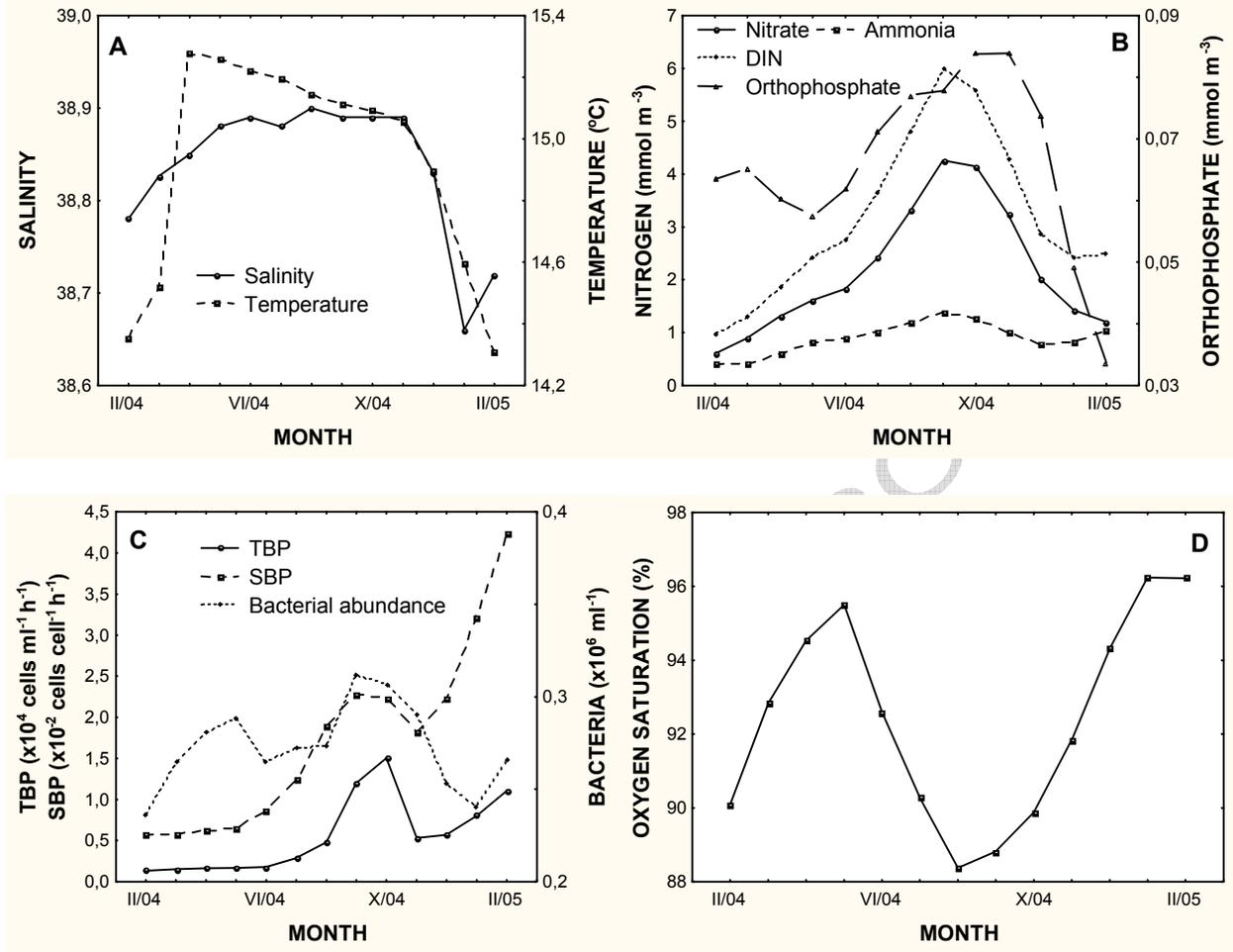


Fig.12

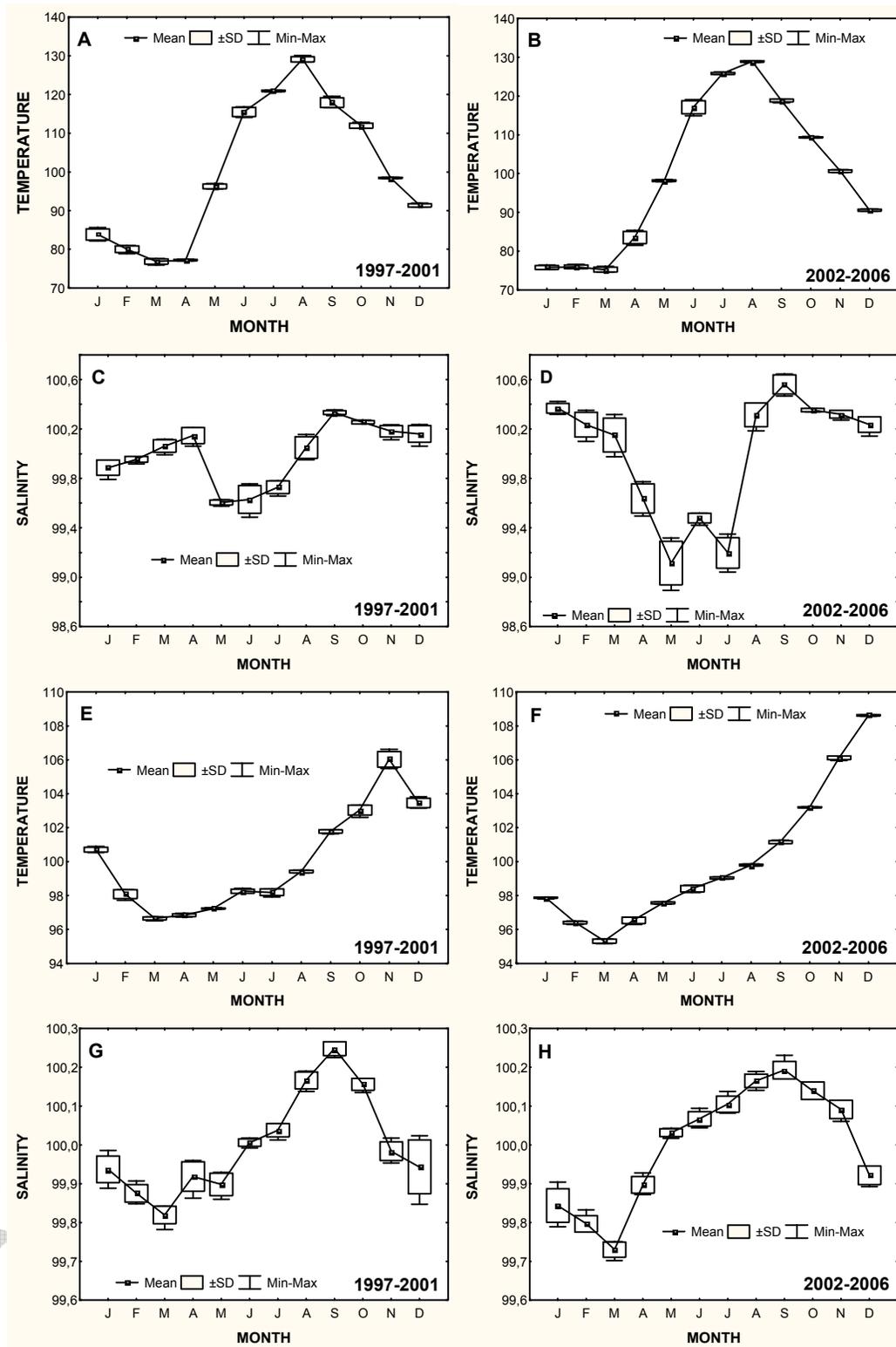


Fig.13