Long-term changes in heterotrophic bacterial abundance and growth characteristics in the northern Adriatic Sea

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Abstract

Heterotrophic bacteria abundance (HB) in the northern Adriatic during the period 1990-2008 shows a substantial decrease after the 2003, evident in the entire water column, both in the generally eutrophic western and in the generally oligotrophic eastern area. HB annual cycle before 2003 was characterized by minimal values during winter months (100-134·10^7 cell l^{-1}, in average), gradually increase during spring and summer, and maximal values at the end of summer (212·10^7 cell l^{-1}, in average). After 2003 HB was low during the most part of the year (27-63·10^7 cell l^{-1}, in average), with somewhat higher values only at the end of summer and in April (up to 80·10^7 cell l^{-1} in average). Bacterial growth characteristics significantly changed after 2003 as indicated by the overall higher Leu/TdR ratio showing that bacteria were generally more increasing their biomass per cell but more slowly dividing. The most responsible factors for the observed decrease in HB and change in growth characteristics were substrate supply and its quality. As a consequence of lower HB heterotrophic flagellates abundance (HF) after 2003 (average 0.57·10^6 cell l^{-1}) generally decrease for about three times. Weakened coupling between HB and HF confirmed the minor role of grazing pressure in controlling HB after 2003.

Keywords: heterotrophic bacteria, bacterial production, cyanobacteria, heterotrophic flagellates, chl *a*, northern Adriatic Sea

1. Introduction

The northern Adriatic Sea is modeled by the alternating influence of freshwater, mainly from the Po River, and middle Adriatic waters. These two water bodies, characterized by different thermohaline properties and different nutrient content, influence the circulation and biological cycle in this region. The large freshwater discharges from the western coast and advection of middle Adriatic waters along the eastern coast generate marked west-east gradients of nutrients and enhance growth and activities of microbial communities (Gilmartin et al, 1990; Karner et al., 1992; Fuks et al., 2005; Socal et al., 2008). Influence of these two waters bodies change during the year. During winter the prevailing of cyclonic circulation brings warmer more saline oligotrophic middle Adriatic waters into the region, while in late spring and summer formation of gyres causes lower exchange with the middle

Adriatic and retention of riverine eutrophic water into the region (Franco and Michelato, 1992; Artegiani et al., 1997; Russo et al., 2005).

In natural systems temperature and substrate supply are the principal factors controlling HB and bacterial growth characteristics. Importance of temperature and phytoplankton biomass, as proxy for available substrate for bacterial growth, has been recently confirmed as main regulation factors of HB in the northern Adriatic (Fuks et al., 2005). However, in the last decade corresponding to the decrease in the Po River discharge (Zanchettin et al., 2008) a significant reduction of PO_4 and NH_4 in the northern Adriatic waters occurred (Solidoro et al., 2009). Due to lower alimentation with freshwater nutrients a decrease of phytoplankton biomass was observed in northern Adriatic waters (Socal et al, 2008; Mozetić et al., in press). Furthermore a general shifts toward smaller plankton size classes (Bernardi Aubry et al., 2006; Pugnetti et. al., 2008), and within the microphytoplankton fraction toward smaller species (Mozetič et al., in press) were observed in the area.

Reported changes could influence bacteria both in terms of abundance and growth characteristics since the substrate supply could affect the ratio between protein production and DNA synthesis (Shiah and Ducklow, 1997; Hoppe et al., 2006). For this purpose in the present study 18-year time series of microbial and environmental parameters for the northern Adriatic Sea extending from 1990 to 2008 were presented and analyzed. The main objective was to establish changes in HB and bacterial growth characteristics related to hydrographic conditions and availability of substrate in this highly dynamic area characterized by variable anthropogenic and natural influences. Factors that may be responsible for the changes in bacterial growth characteristics and abundance are discussed in detail, as well as possible consequences in microbial food loop relations.

2. Material and methods

2.1. Sampling strategy

Measurements were performed at four stations at the transect Po River delta - Rovinj (Po transect) located in the northern Adriatic (Fig. 1) in the period 1990-2008 on a monthly scale. Stations were located along the trophic gradient, covering the area from the western station SJ108 which is under direct freshwater influence during

most of the year to the eastern station SJ107 where the freshwater influence is minimal. Data for hydrographic parameters were measured continuously thought the water column, while nutrients and parameters characterizing microbial communities were measured at five depths (surface, 5 m, 10 m, 20 m, and 1 m above the bottom: 27-35 m). Measurements of parameters characterizing bacterial growth (thymidine and leucine incorporation) started in 1999.

2.2. Analytical protocol

Water samples were collected with 5 l PVC Niskin samplers. Temperature and salinity profiles were acquired during the downcasts of a SEABIRD SBE 25 CTD probe.

Inorganic nutrients were analyzed from unfiltered water immediately after collection (Parsons et al., 1985; Ivančić and Degobbis, 1984). In a few cases, when the sample was turbid, measurements were corrected by turbidity blank. Dissolved inorganic nitrogen (DIN) was calculated as the sum of nitrate, nitrite and ammonia.

Total chlorophyll *a* concentrations (chl *a*) were determined by filtration of 500 ml on Whatmann GF/C filters. Filters were frozen (–18 ºC) and analyzed within a few days by an acidification fluorometric procedure in 90% acetone (Parsons et al., 1985).

Samples for bacteria abundance (HB), picocyanobacteria abundance (CB) and heterotrophic pico- and nanoflagellates abundance (HF) were preserved with formaldehyde (2% final concentration) and stored at 4 °C. HB, CB and HF were estimated by epifluorescence microscopy (Leitz Laborlux D and Nikon Microphot-SA at a magnification of 1000x). For HB determination, 2 ml of samples were stained with 4, 6-diamidino-2-phenylindol (DAPI;1 μgmL^{-1}, final conc.) and then passed through 0.2 µm black polycarbonate filters (Porter and Feig, 1980). UV excitation of specimen was used and at least 500 single cells were counted per sample. Samples (5-10 ml) for cyanobacteria (CB) and heterotrophic pico- and nanoflagellates (HF) counts were filtered onto 0.6 µm black polycarbonate filters (Nucleopore). CB count was performed using green light excitation, with a minimum of 300 cells counted per sample (Takahashi et al. 1985). HF abundance was obtained by the primulin staining

technique, while phototrophic organisms were differentiated by their chlorophyll autofluorescence (Caron, 1983).

Bacterial bulk production was estimated by measuring incorporation of two different substrates: (i) ^{3}H- thymidine (TdR; specific activity: 70-90 Ci mmol^{-1}; 20 nM final conc.) and (ii) L-[3,4,5-^{3}H] leucine (Leu; specific activity 100-150 Ci mmol^{-1}; 20 nM final concentration). The rate of substrates incorporation into macromolecules was measured following the method described in Smith and Azam (1992).Triplicate (1.7ml aliquots) samples plus 100% TCA (trichloracetic acid)-killed blank were incubated at *in situ* temperature in the dark for 1 hour. Incubations were stopped by adding 100% TCA. Subsequently, labeled material was extracted with (i) ice-cold 5% TCA and (ii) 80% ethanol and collected by centrifugation. Collected material was dissolved in 0.5 ml of scintillation cocktail and assessed after 20 h. Specific leucine (sLeu) and thymidine (sTdR) incorporation rates were obtained by dividing the average rates per liter by bacterial abundance per liter.

Statistical analyses were performed using ANOVA, ANCOVA, and stepwise General Linear Models estimation with probability level 0.15, confidence limit 0.95, and regression technique. Statistical analyses were performed on not transformed data for parameters showing normal distribution (temperature and salinity) and logarithmically transformed data for parameters which did not present normal distribution. For parameters which displays negative logarithmic values (chl *a*, nutrients) the x+1 condition was used. The conditions of normal distribution were tested with Shapiro-Wilk test.

3. Results

In the investigated period (1990-2008) heterotrophic bacteria abundances (HB: 3-948·10^{7} cell l^{-}1) showed large spatial, seasonal and year-to-year variation, although a substantial decrease occurred after 2003, as shown for the surface layer (Fig. 2). This decrease was significant in the entire water column over all investigated area (ANOVA, $p<0.005$). Furthermore, from April to October when the water column was stratified, HB at the surface and 5 m depth was significantly different than in the rest of the water column (ANOVA, $p<0.005$). In contrast, during the water column mixing (November to March), no significant difference with depth was found. Based on these

findings, data for further analyses were grouped in two periods: a) period 1990-2002 (P1) and b) period 2003-2008 (P2). Each period was additionally subdivided in mixing and stratification seasons. During the mixing season data were analyzed for the entire water column, while in the stratification season upper (surface and 5 m) and deeper (10 m to bottom) waters were analyzed separately. All values given in brackets throughout the results are medians.

3.1. Temporal dynamics of heterotrophic bacteria abundance and activity

During P1 HB in upper waters gradually increased from April ($118 \cdot 10^7$ cell l^{-1}) and reached maximum at the end of summer (August/September; $212 \cdot 10^7$ cell l^{-1}; Fig. 3a). Although lower, HB in the deeper waters (Fig. 3b) also increased toward the end of summer ($147 \cdot 10^7$ cell l^{-1}). In the mixing season HB gradually decreased reaching minimum in February/March ($102\text{-}104 \cdot 10^7$ cell l^{-1}; Fig. 3c). In P2 HB ($28\text{-}80 \cdot 10^7$ cell l^{-1}) was markedly lower than in P1 in both seasons, as well as in upper and deeper waters (Fig. 3). In P2 a gradual increase toward the summer was not observed and somewhat higher values were found only in April and August - October periods (Fig. 3a,b). During the mixing season values decreased down to minima in March (Fig. 3c).

The changes of sTdR from February to June were markedly different in P1 and P2. During P1 a marked increase of sTdR was observed already in February ($0.264 \cdot 10^{-7}$ pmol cell^{-1} h^{-1}) and maximal values and variation occurred in March/April (Fig. 4). On the contrary, in P2 sTdR markedly increased only in June ($0.139 \cdot 10^{-7}$ pmol cell^{-1} h^{-1}), while from February to May values were markedly lower than in P1. From July to January changes in specific TdR were generally similar in both periods, although values tended to be somewhat higher in P1 (Fig. 4). In both periods minimal values and variations were recorded from September to January ($0.006\text{-}0.042 \cdot 10^{-7}$ pmol cell^{-1} h^{-1}).

The variations of sLeu were much more pronounced in P2 when values were markedly higher than in P1. In both periods seasonal changes paralleled changes in sTdR: higher values were typical for upper waters during the stratification season ($0.369\text{-}1.038 \cdot 10^{-7}$ pmol cell^{-1} h^{-1} in P1 and $0.800\text{-}2.395 \cdot 10^{-7}$ pmol cell^{-1} h^{-1} in P2; data not shown). Minimal values were found during mixing season ($0.102\text{-}0.299 \cdot 10^{-7}$ pmol

cell^{-1} h^{-1} in P1 and 0.355-0.691·10^{-7} pmol cell^{-1} h^{-1} in P2; data not shown), except for higher values (1.503·10^{-7} pmol cell^{-1} h^{-1}; data not shown) in March of P2.

Opposite changes of sTdR and sLeu in P1 and P2, resulted in distinctly higher Leu/TdR ratios in P2 (Fig. 5). During the stratification season in upper waters a tendency of ratios <10 in P1, while in P2 a tendency of ratios >20 was observed (Fig. 5a). In deeper waters during P1 ratios were <10, while in P2 mostly laid between 10 and 20 (Fig. 5b). In the mixing season ratios were <10 during both periods, except higher values in January (P2) and November-December (P1) (Fig. 5c).

Correlation between Leu/TdR ratio and chl *a* (proxy for bacterial substrate) was higher in P1 ($r=0.284$, $p=0.000$) than in P2 ($r=0.099$, $p=0.011$).

3.2. Temporal changes of factors influencing heterotrophic bacteria abundance and activity

The most important factors controlling HB in the northern Adriatic waters were temperature and chl *a*, with relatively similar influence, while the less important factors were inorganic nitrogen species (Table 1). It is important to notice that while dependence of HB on ambient parameters (temperature, salinity and nutrients) was irrespective of temporal periods, its dependence on biological parameters, proxy for bacterial substrate and predation, was different in P1 and P2. Testing slope between HB and biological parameters in P1 and P2 showed small differences for correlation with chl *a*, although correlation with CB become higher while dependence on HF became not important in P2 (Table 2). Furthermore, while in P1 correlation of HB was higher with chl *a*, contrary in P2 was higher with CB.

Concerning ambient parameters, a regular seasonal fluctuation of temperature with minimal values in the water column during February (about 9.0 ºC) and maximal in the upper waters during August was observed in both periods (about 26 ºC; Fig. 6). However, the fluctuations of salinity markedly differed between periods. As expected, the highest variability of salinity was found in upper waters during the stratification season. In P1 typical salinity seasonal trend with a general decrease in spring months and minimal values during June-July (34.5) was followed by an increase in autumn months (Fig 7a). In P2 salinity of these waters was markedly higher and seasonal trend was not so well defined. After a general decrease in April-May, an

192 increase to unusually high values in June-July (36.9-37.1) followed by a small
193 decrease only at the end of summer (August-September; Fig. 7a) was found. Salinity
194 of deeper waters during the stratification season (Fig. 7b), and of the whole water
195 column during the mixing season (Fig. 7c) was higher and variations minimal in both
196 periods. The increase of salinity during P2 was low, although very important for these
197 waters were salinity changes were minimal.

198 A typical seasonal trend was observed for inorganic nutrient concentrations in
199 both periods, as found in previous studies (Gilmartin et al., 1990). The lowest PO_4
200 and DIN concentrations were recorded in upper waters during summer (0.01-0.04
201 µmol l^{-1}, and 0.42-0.97 µmol l^{-1}, respectively; data not shown). Higher values and
202 maximal variation were found during mixing season and during late spring (up to 0.11
203 µmol l^{-1}, and up to 3.62 µmol l^{-1}, respectively; data not shown). In P2, PO_4
204 concentrations were generally lower while contrary DIN concentrations were higher
205 compared to earlier period. Exceptions were lower DIN concentrations in deeper
206 waters during stratification season.

207 Seasonal trends of chl *a* concentrations with maximal values in April/May (0.97-
208 1.61 µg l^{-1}) and October/November (0.88-1.88 µg l^{-1}), especially in the upper waters,
209 and minimal values during summer months and in deeper waters (0.19-0.76 µg l^{-1};
210 Fig. 8) were also typical for the investigated area (Gilmartin et al., 1990, Mozetić et
211 al., in press). However, it is important to note that while in P1 chl *a* generally
212 increased already in February, in P2 a general increase was observed only in April.
213 Furthermore, in P2 values were generally lower than in P1, especially in upper waters
214 during summer months.

215 CB was the highest in upper waters during the stratification season of both
216 periods (17.34-58.87·10^6 cell l^{-1} in P1 and 20.23-69.13·10^6 cell l^{-1} in P2; Fig. 9a).
217 While in P1 CB gradually increased from April to July/August and then decreased in
218 October, in P2 a constant increase from April to October was observed. In deeper
219 waters values were lower (12.71-36.28·10^6 cell l^{-1} in P1 and 16.94-31.41·10^6 cell l^{-1} in
220 P2) and in both periods maximized in August (Fig. 9b). In May and July, CB was
221 higher in P1 than in P2, contrary in October it was higher in P2 than in P1, while in
222 other months during the stratification season values did not differ between periods

(Fig. 9a,b). Minimal CB was found in the mixing season (6.78-13.26·10^6 cell l^{-1} in P1 and 7.22-11.26·10^6 cell l^{-1} in P2, Fig. 9c) when no difference between two periods was observed.

The highest heterotrophic flagellate abundance (HF) was found in upper waters during the stratification season (1.75-5.95·10^6 cell l^{-1} in P1 and 0.58-2.10·10^6 cell l^{-1} in P2; data not shown) with maximal values in May. Minimal values occurred during the mixing season (0.92-1.60·10^6 cell l^{-1} in P1 and 0.35-0.81·10^6 cell l^{-1} in P2; data not shown). HF was in average three times lower in P2 (0.57·10^6 cell l^{-1}) than in P1 (1.83·10^6 cell l^{-1}). While in P1 HF was correlated with both HB and CB in P2 correlation with HB was not significant (Table 2).

4. Discussion

After the 2003 a marked decrease of HB was observed with similar intensity along entire investigated area and thorough the whole water column. The decrease matched the reduction of the Po River discharge (Zanchettin et al., 2008) which resulted in decreased PO_4 concentrations in the northern Adriatic waters (Solidoro et al., 2009). A general decrease of PO_4 concentrations in the area after 2003 was also observed in this study, while DIN concentrations in sea water were generally higher. This enhanced accumulation of DIN could be attributed to its lower uptake by microbes due to the deficit of PO_4, which is the limiting element for microbial growth in the northern Adriatic (Pojed and Kveder, 1978; Ivančić et al., 2009). Accordingly to lower PO_4 concentrations a decreasing trend in chl *a* levels (Mozetić et al., in press) and therefore organic matter production has been emphasized to occur in the northern Adriatic area during last decade.

Moreover, comparing the bacterial annual cycle before and after 2003 some differences were observed. In P1 freshwater input in the northern Adriatic increased in May supporting intense phytoplankton blooms, typical for this month (Smodlaka, 1986; Gilmartin et al., 1990), during which large quantity of fresh autochthonous organic matter was produced. During the summer alimentation of the area with freshwater was, as usually, minimal during the year. However, closed circulation favored retention of freshwater nutrients supporting intense phytoplankton activity, though phytoplankton biomass was low due to predation (Revelante and Gilmartin,

1976; Gilmartin et al., 1990). Constant supply of organic matter and favorable temperature supported intensive growth of bacteria which abundance gradually increased during late spring and summer months (May-September). In deeper waters HB variations were similar as in upper waters, although values decreased due to reduced substrate availability and lower temperature. Phytoplankton activity in deeper waters is limited mainly by unfavorable light conditions, although relatively high availability of nutrient which accumulate in these waters. Consequently, the production of fresh organic matter is limited and settled organic matter from upper waters is probably more refractory. In P2 HB from May to September was markedly lower and the pattern was modified in comparison to that observed in P1. The typical maximum of Po River discharge in May was absent and during summer months it was far below the usual. Reduced freshwater input caused weaker intensity of gyre formation and consequently, lower transport of freshwater toward the east (Supić, personal comm.) and more intense advection of middle Adriatic waters. As a result unusually high saline waters were present in the area. Limited spreading of freshwater PO_4 decreased phytoplankton biomass and hence production of organic matter. Lower substrate availability and more intense export of produced organic matter and bacteria from the area, resulted in unusually low HB both in upper and deeper waters. A moderate increase in HB, far below than in P1, occurred only at the end of summer (August-September) when freshwater influence was somewhat higher than in previous months, as indicated by lower salinity of upper waters.

From October to February a gradual decrease in HB was observed during P1, even if abundance in October and November was still high. October and November are characterized with an increase in Po discharge and starting of mixing thorough the water column. Nutrients input by these two mechanisms stimulated phytoplankton blooms in these months and produced organic matter supported bacterial growth maintaining high HB. In the following months the activation of cyclonic circulation, which transport produced organic matter from the area, and a parallel decrease in temperature caused a gradual decrease in HB. During P2 poor freshwater supply of PO_4 and its lower transport from deeper water, due to weak accumulation during summer, resulted in decreased intensity of phytoplankton blooms and lower

production of organic matter. As a consequence HB, although showing the same pattern as in P1, also decreased.

In P1 from February to April phytoplankton blooms were, as usually, based primarily on the nutrients regenerated during autumn (Gilmartin et al., 1990). Although temperature in these months was generally low, availability of substrate stimulated bacterial division, as shown by high sTdR, but their abundance was the lowest during the year. This could be ascribed to the export of produced organic matter together with bacteria from the area by the cyclonic circulation. In P2 HB in February and March was markedly lower than in P1. Temperature in these months was similarly low in both periods, although in P2 alimentation with riverine PO_4, and its reserves regenerated during preceding autumn were reduced. Therefore, the phytoplankton biomass and production of substrate for bacterial growth were also considerably reduced. Only in April, when phytoplankton biomass was similar in both periods, HB approached values in P1.

As a consequence of HB reduction a marked decrease in HF occurred in P2. Although no predominance of heterotrophic flagellates on HB regulation was observed, top-down mechanism controlled an upper limit for HB in both periods. However, while in P1 relative importance of grazing pressure was expressed on both bacterial and cyanobacterial communities, in P2 coupling between HF and HB weakened while with CB remained still tight. Probably, after 2003 heterotrophic bacteria represented less important food resource for heterotrophic flagellates than cyanobacteria.

The changes in substrate availability in P1 and P2 did not cause only decrease in HB, but were reflected also in growth characteristics of bacteria in terms of protein production (sLeu) or DNA synthesis (sTdR). Shift of sTdR maxima from February-April in P1 to June in P2 could be linked to the decrease in magnitude of winter-early spring phytoplankton blooms. In P1 an increase of phytoplankton biomass occurred already in February and continuously higher biomass persisted until May, while in P2 a general increase in phytoplankton biomass was recorded only in April/May. In other months relative changes of sTdR as well as sLeu were similar in both periods with maximal values during spring-summer months. Similar

annual pattern of leucine incorporation was found also in a NW Mediterranean coastal region as a result of high ectoenzyme activities during spring and summer (Alonso-Sáez et al., 2008). During our study markedly higher sTdR during P1 and sLeu during P2 resulted in distinctly higher Leu/TdR ratios in the last period. This indicate that in P2 bacteria were generally more increasing their cellular biomass but more slowly dividing than in P1. Shiah and Ducklow (1997) found that changes toward less favorable environment conditions (e.g. reduction in temperature or substrate supply) might reduce bacterial protein and DNA synthesis simultaneously, although the former process may be favored to maximize survival and this might lead to a higher Leu/TdR ratio. Conversely, in favorable environment conditions both processes could be enhanced and bacteria might optimize DNA duplication over protein metabolism to maximize reproduction, resulting in lower Leu/TdR ratio. In our study temperature values and pattern in P1 and P2 did not differ, but phytoplankton biomass decreased in P2 that presumably presented less favorable conditions for bacteria reproduction. In both periods Leu/TdR ratios were within the ranges reported for marine ecosystems (Hoppe et al., 2006). Otherwise the sTdR and sLeu in specific situations were uncoupled, at the annual scale they show the same pattern indicating that globally bacteria increased simultaneously DNA and protein synthesis in both periods. Increased Leu/TdR ratios during P2 probably reflected adaptations of bacterial assemblages to more oligotrophic conditions. Oligotrophic regions are characterized by higher Leu/TdR ratios (Hoppe et al., 2006). In additions, the possible impact of cyanobacteria on Leu/TdR ratio should be considered. Although HB in P2 decreased, CB generally remained similar as in P1. Several species of cyanobacteria have been reported to take up leucine, and only to a minor extent thymidine shifting the Leu/TdR ratio toward higher values (Hietanen et al., 2002). Furthermore, decrease in PO_4 concentrations during P2 could additionally shift the ratio toward higher values. P is building element of DNA and hence it could more limit cell division than production of proteins. Measurements of alkaline phosphatase activity confirmed that in 2006 bacterial communities in upper waters were strongly P limited (Ivančić et al., 2010). In contrast to PO_4, DIN concentrations were generally higher than in P1 and could stimulate production of proteins increasing cellular biomass.

There is some evidence of different quality of produced substrate in P2. In these years a shift toward smaller size classes of phytoplankton (Bernardi Aubry et al., 2006; Mozetić et al., in press) and preferential domination of autotrophic nanoflagellates and small dinoflagellates over diatoms during winter- spring blooms (Socal et al., 2008) could indicate changes in dissolved organic matter quality. This is supported also by the findings that the dependence of HB on ambient conditions (temperature, salinity and nutrients availability) during our study did not change, while in P1 HB were better related with chl *a* and in P2 with CB. Furthermore, markedly higher correlation of Leu/TdR ratio and chl *a* in P1 than in P2 may suggest, according to Hoppe et. al. (2006), that organic matter combined with chl *a* in P1 was more nutritious than in P2.

5. Conclusion

HB decrease after 2003 was mainly caused by lower availability and different quality of primary produced organic matter. Simultaneously changes in bacteria growth characteristics were reflected in higher Leu/TdR ratio. In P2 bacteria were generally more increasing their cellular biomass but more slowly dividing that in P1as adaptations of bacterial assemblages to more oligotrophic conditions. In P2 cyanobacteria contributed more to the bacterial community than in P1, additionally increasing Leu/TdR ratio. Grazing pressure probably did not contribute to the decrease in HB during P2 since a parallel decrease in HF occurred and decoupling of HB and HF was observed. Temperature also did not contribute in observed decrease.

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474 CAPTIONS

475 Fig. 1. Research area and sampling stations in the northern Adriatic Sea.

476 Fig. 2. Heterotrophic bacteria abundance (HB) in surface layer at the Po transect (+
477 SJ107; ◆ SJ103; ▲ SJ101; ● SJ108) during the period 1990-2008.

478 Fig. 3. Box-whiskers plot of heterotrophic bacteria abundance (HB) at the Po transect
479 during 1990-2002 (white box) and 2003-2008 (grey box) periods in (a) upper and (b)
480 deeper waters during the stratification season and (c) in the water column during the
481 mixing season. Outliners (up to $945 \cdot 10^7$ cell l^{-1}) are omitted from the figure and
482 vertical bars are referred to 95% of data.

483 Fig. 4. Box-whiskers plot of specific thymidine incorporation (sTdR) at the Po transect
484 during 1990-2002 (white box) and 2003-2008 (grey box) periods in (a) upper and (b)
485 deeper waters during the stratification season and (c) in the water column during the
486 mixing season.

487 Fig. 5. Box-whiskers plot of Leucine/Thymidine incorporation ratio (Leu/TdR) at the
488 Po transect during 1990-2002 (white box) and 2003-2008 (grey box) periods in (a)
489 upper and (b) deeper waters during the stratification season and (c) in the water
490 column during the mixing season. Outliners (up to 147) are omitted from the figure
491 and vertical bars are referred to 95% of data.

Fig. 6. Box-whiskers plot temperature (t) at the Po transect during 1990-2002 (white box) and 2003-2008 (grey box) periods in (a) upper and (b) deeper waters during the stratification season and (c) in the water column during the mixing season.

Fig. 7. Box-whiskers plot of salinity (S) at the Po transect during 1990-2002 (white box) and 2003-2008 (grey box) periods in (a) upper and (b) deeper waters during the stratification season and (c) in the water column during the mixing season.

Fig. 8. Box-whiskers plot of chlorophyll *a* (chl *a*) at the Po transect during 1990-2002 (white box) and 2003-2008 (grey box) periods in (a) upper and (b) deeper waters during the stratification season and (c) in the water column during the mixing season. Outliners (up to 40.2 µg l^{-1}) are omitted from the figure and vertical bars are referred to 95% of data.

Fig. 9. Box-whiskers plot of cyanobacteria abundance (CB) at the Po transect during 1990-2002 (white box) and 2003-2008 (grey box) periods in (a) upper and (b) deeper waters during the stratification season and (c) in the water column during the mixing season. Outliners (up to up to $1008 \cdot 10^6$ cell l^{-1})) are omitted from the figure and vertical bars are referred to 95% of data.

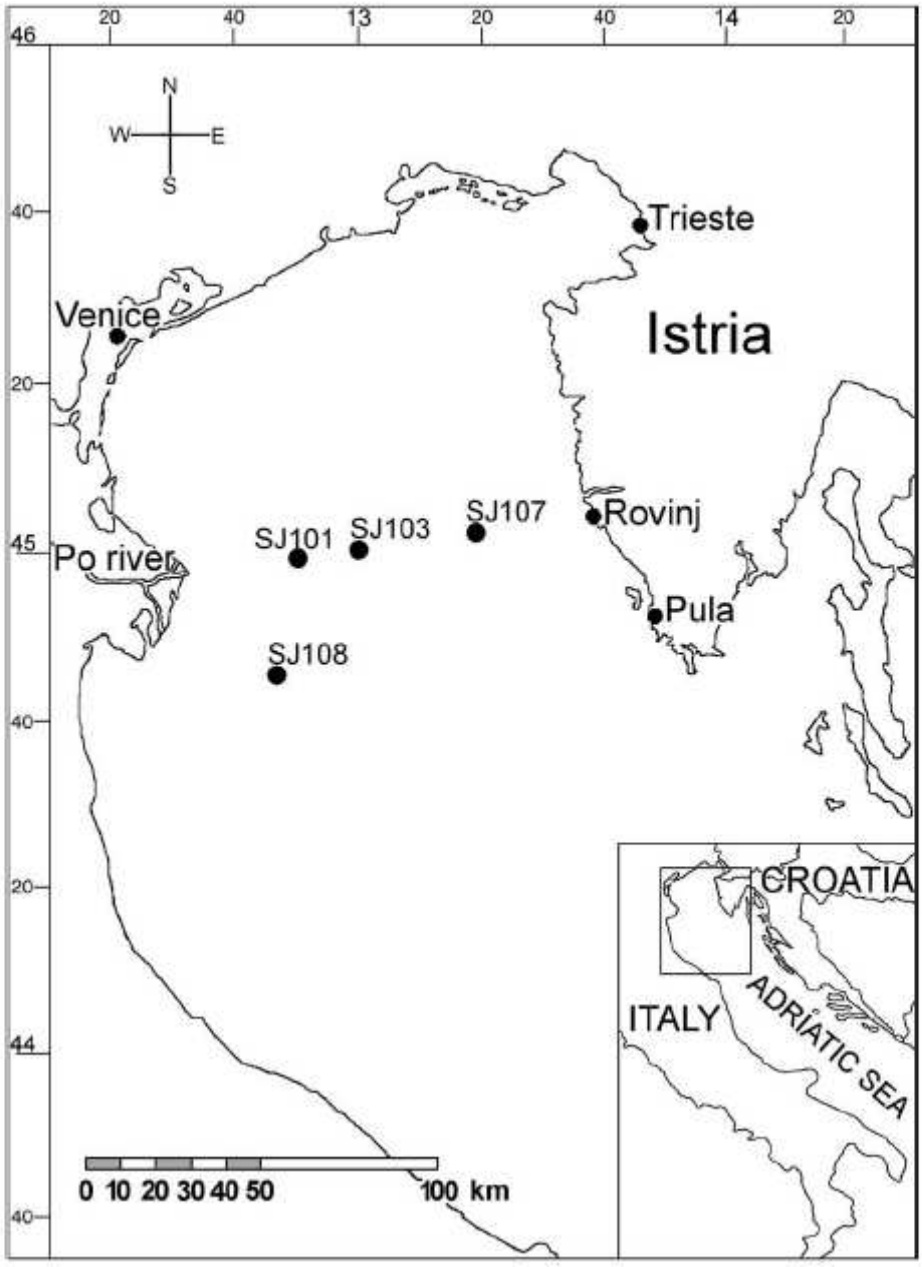

Fig. 1 Research area and sampling stations in the northern Adriatic Sea.

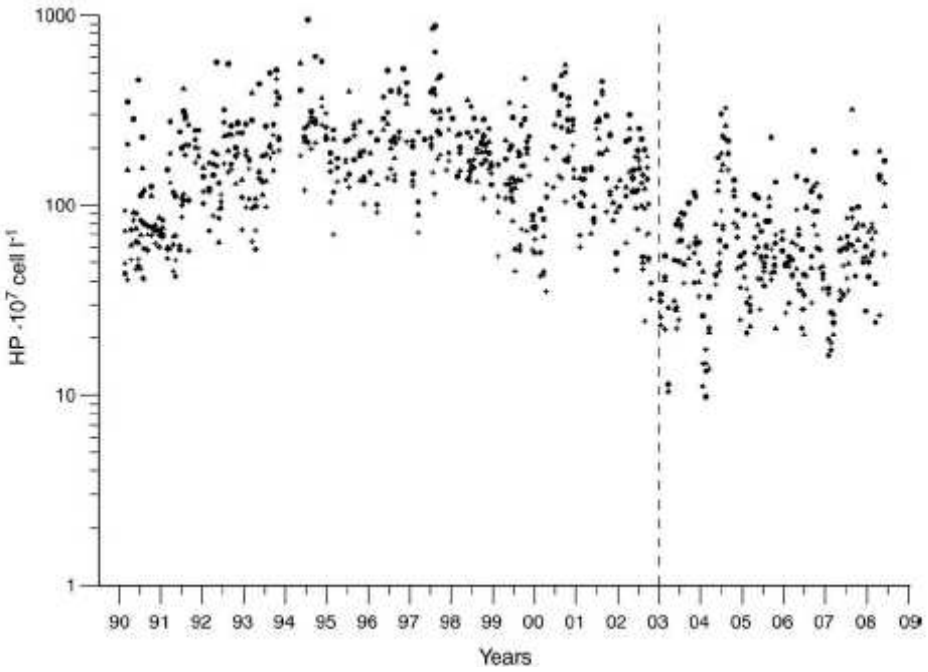

Fig. 2 Heterotrophic prokaryotes abundance (HP) in the surface layer at the Po transect (+SJ107; ♦SJ103; ▲SJ101; ●SJ108) during the 1990–2008 period.

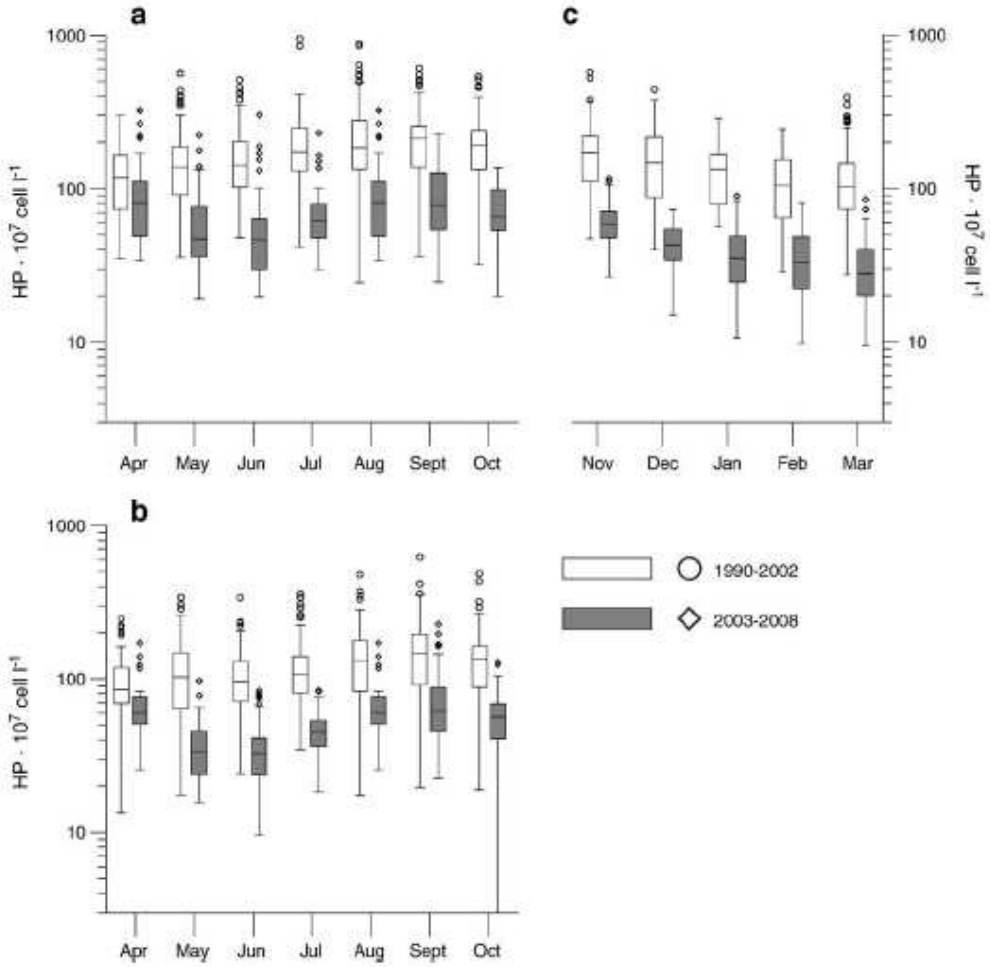

Fig.3 Box-whisker plot of heterotrophic prokaryotes abundance (HP) at the Po transect during the 1990–2002 (white box) and 2003–2008 (grey box) periods in (a) upper and (b) deeper waters during the stratification season and (c) in the water column during the mixing season.

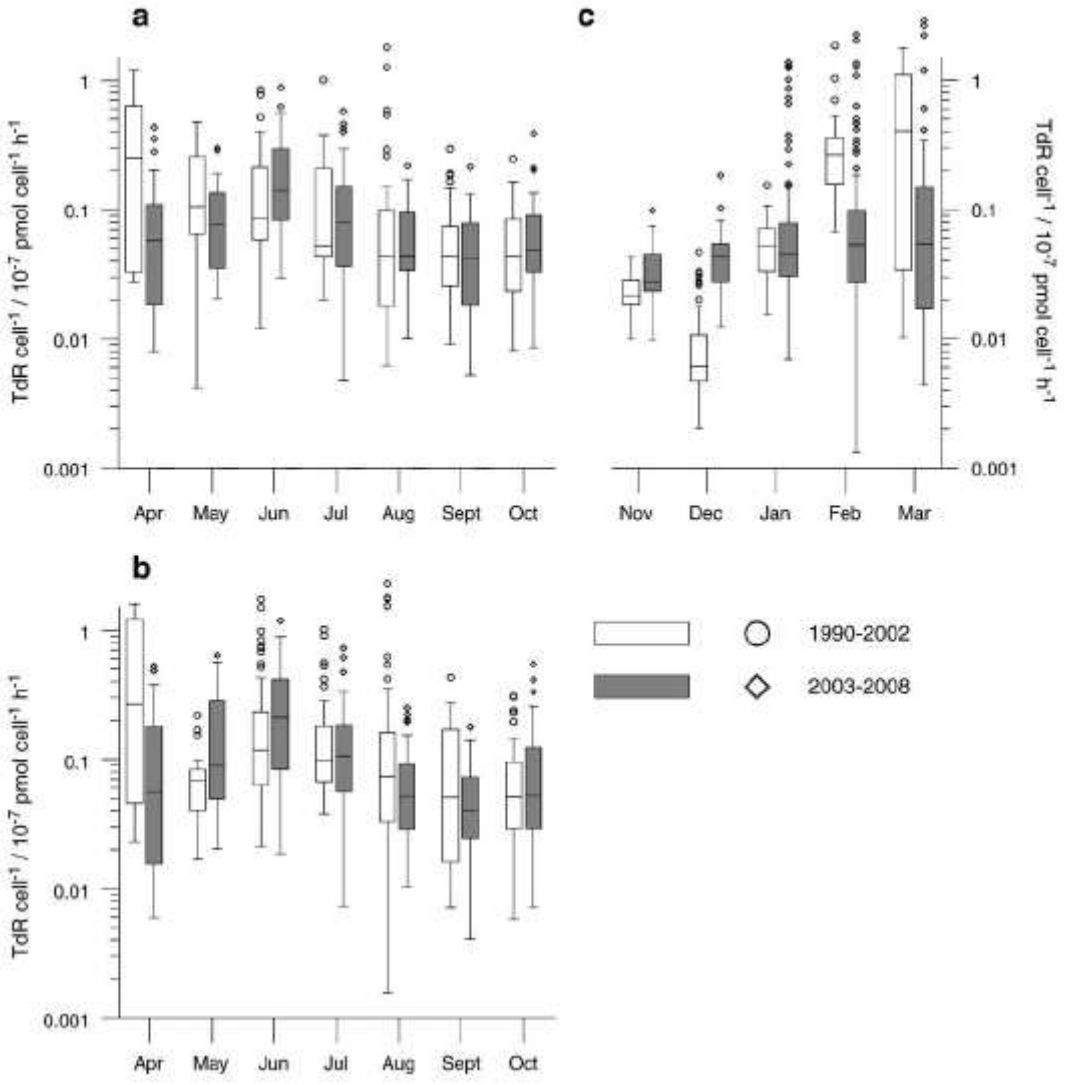

Fig. 4 Same caption as for Fig. 3 except specific thymidine incorporation (TdR cell^{-1}) shown.

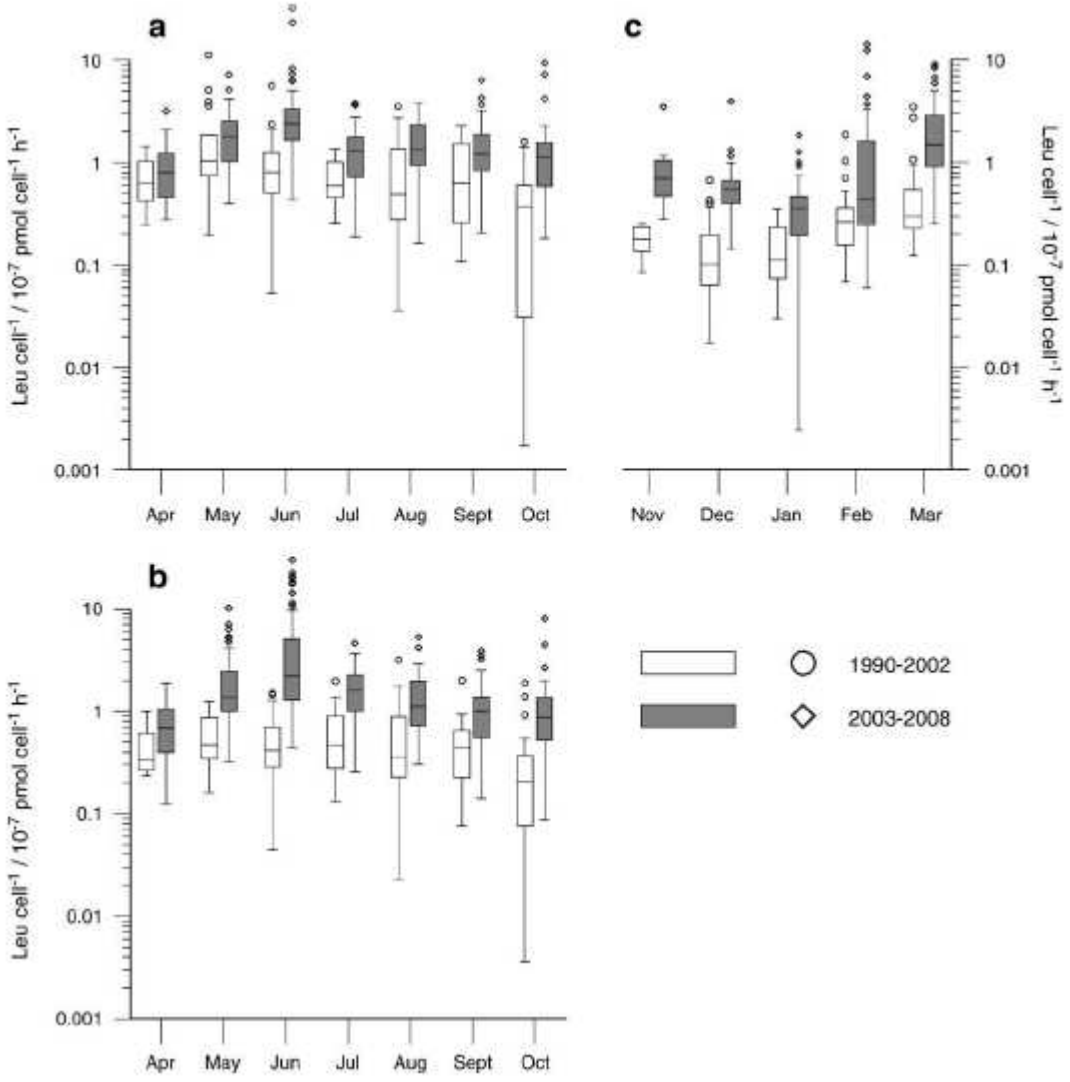

Fig 5 Same caption as for Fig. 3 except specific leucine incorporation (Leu cell^{-1}) shown.

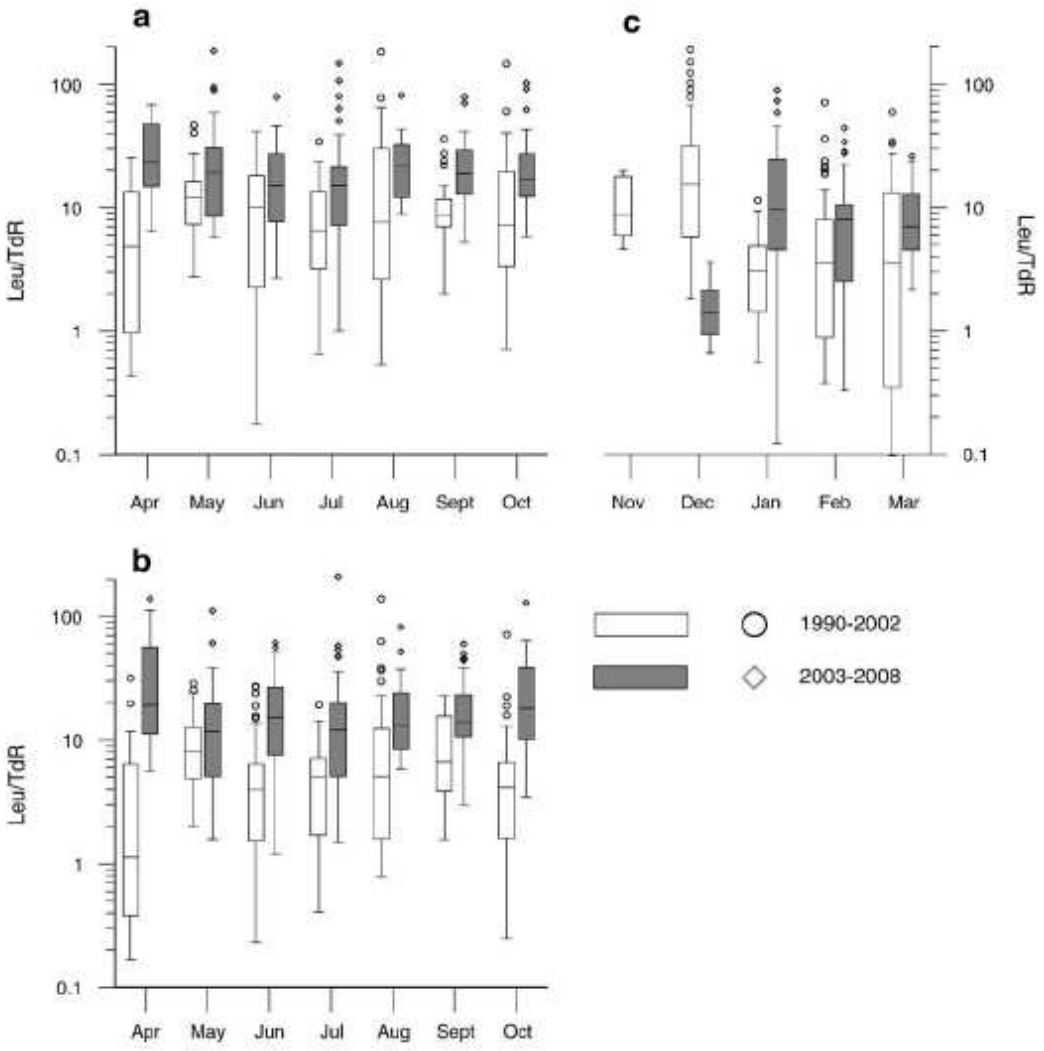

Fig 6 Same caption as for Fig. 3 except Leucine/Thymidine incorporation ratio (Leu/TdR) shown.

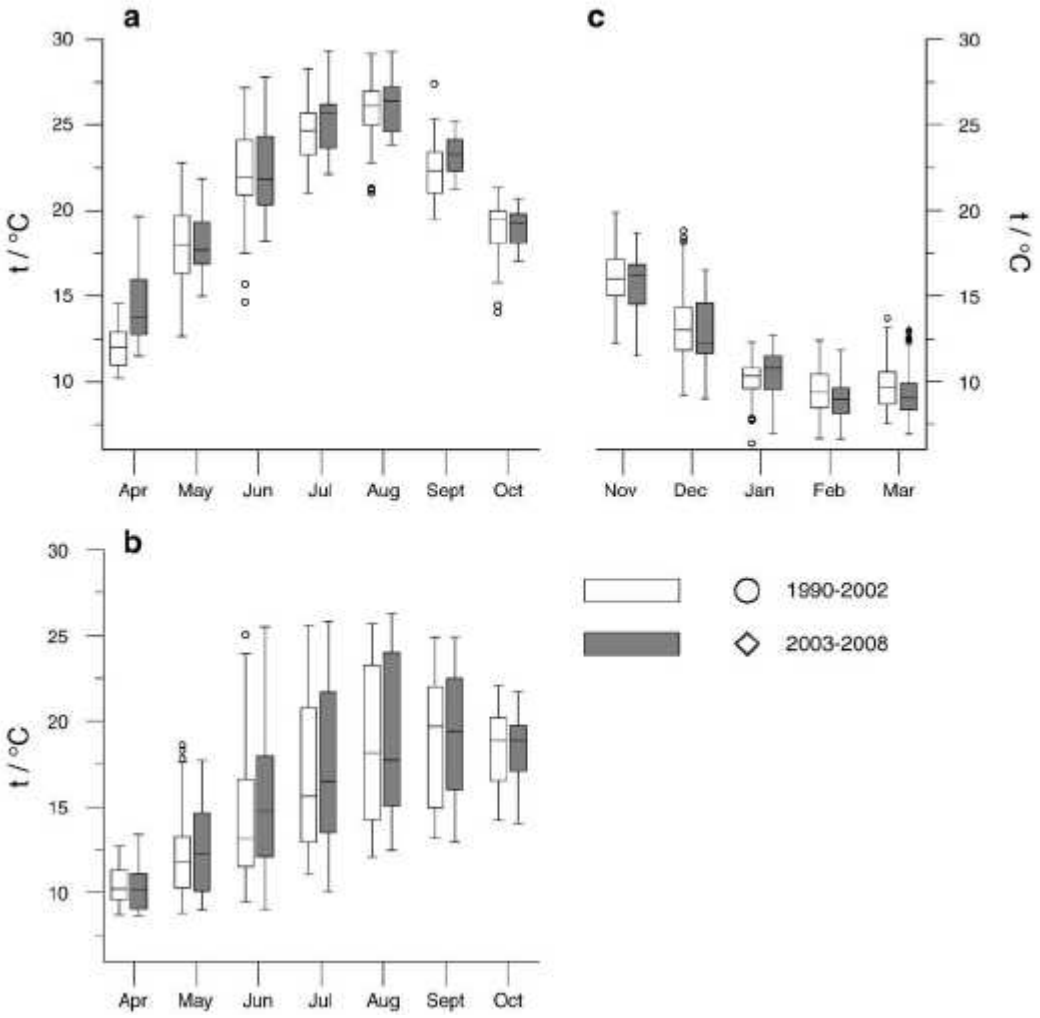

Fig.7 Same caption as for Fig. 3 except temperature (t) shown.

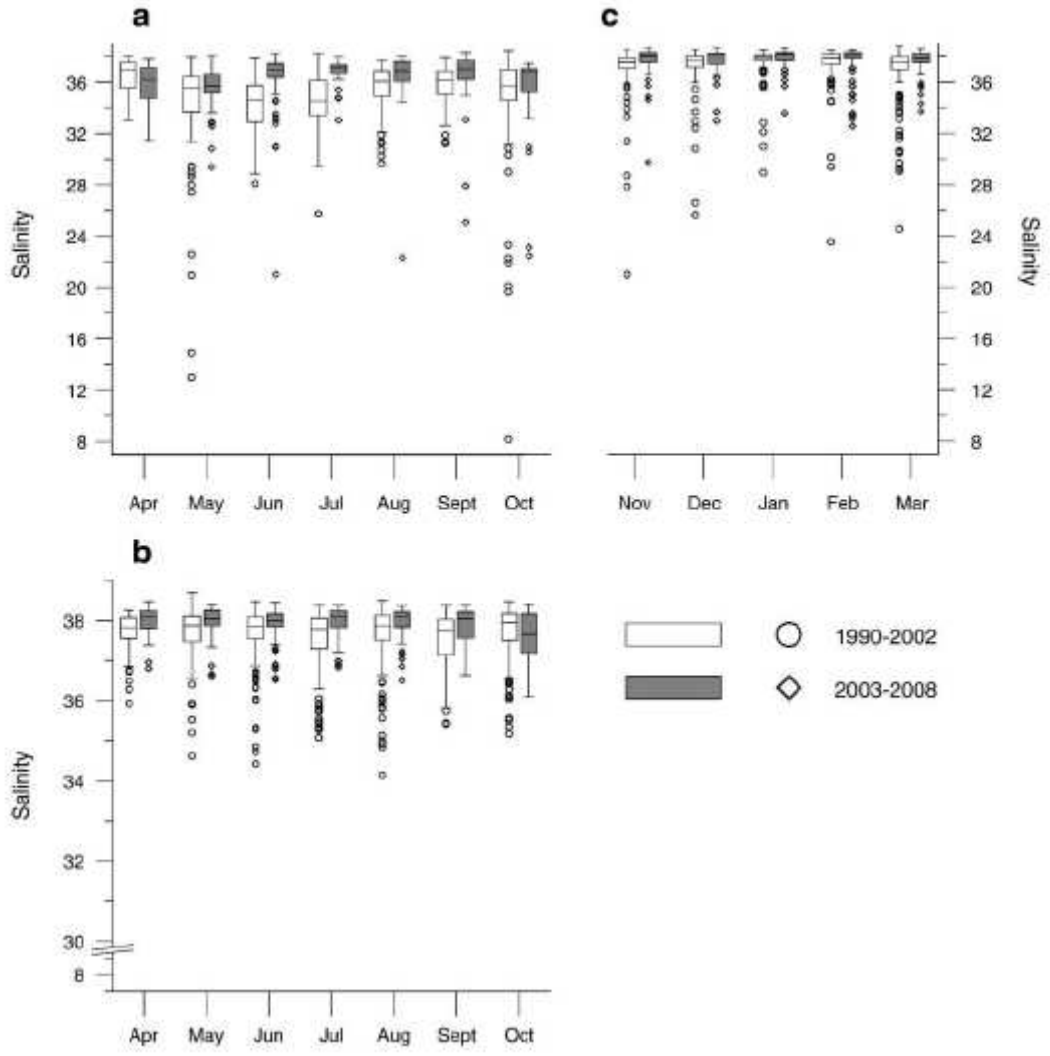

Fig. 8 Same caption as for Fig. 3 except salinity (S) shown.

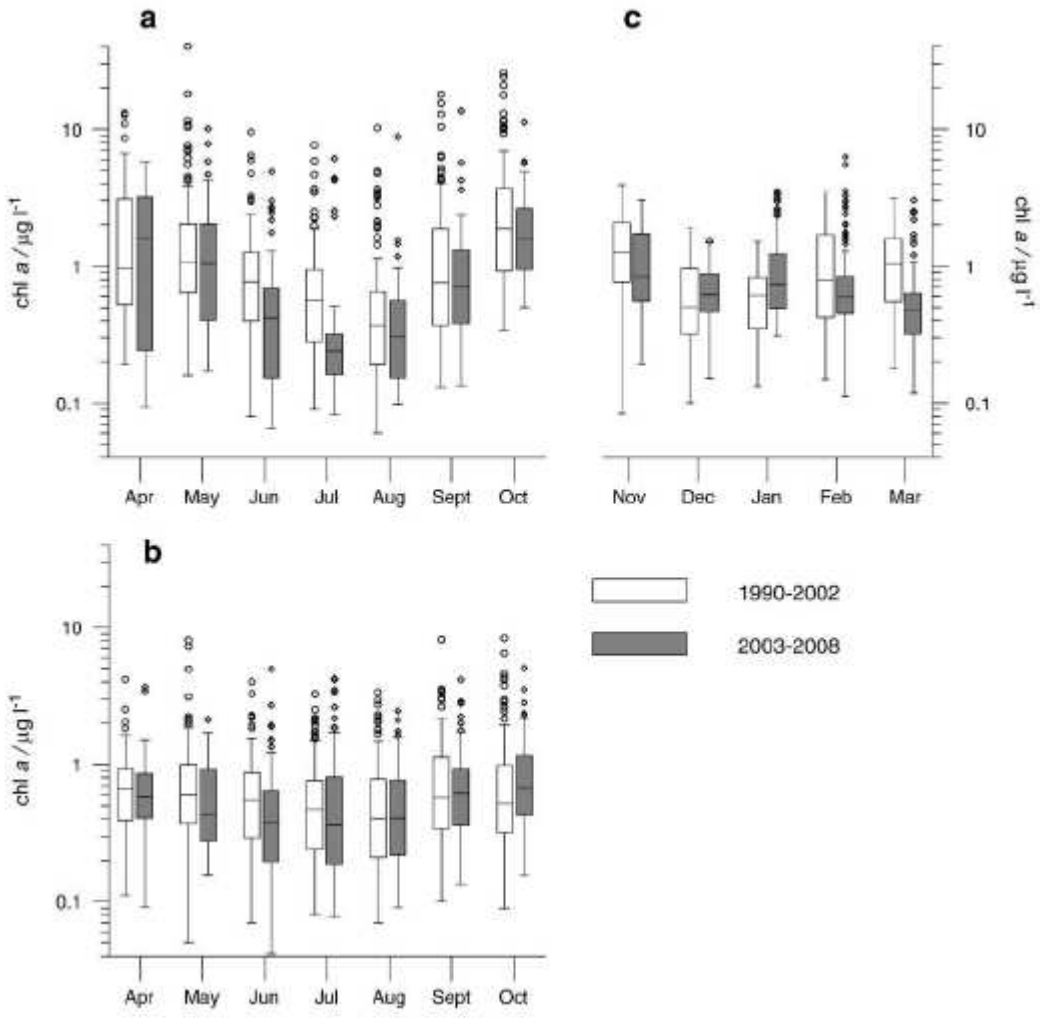

Fig. 9 Same caption as for Fig. 3 except chlorophyll a (chl a) shown.

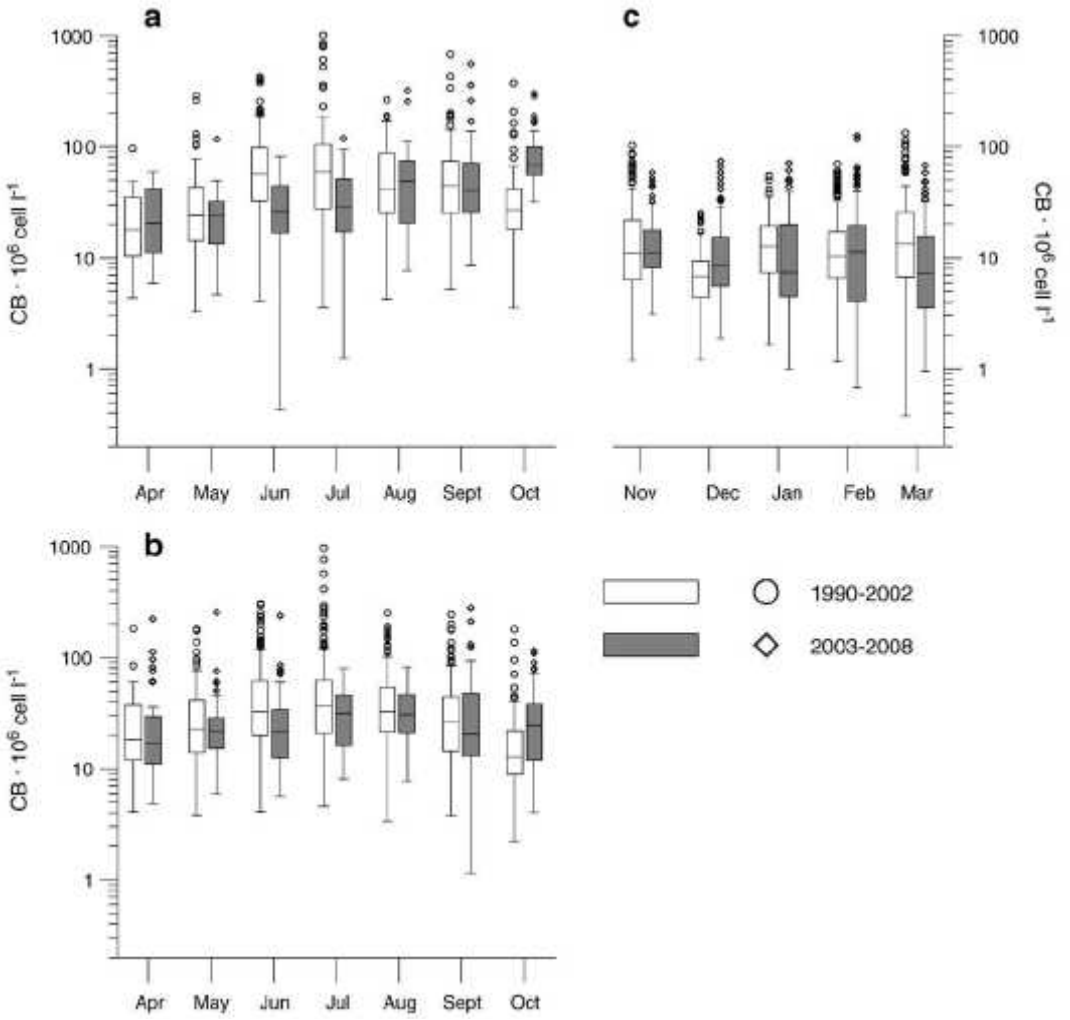

Fig. 10 Same caption as for Fig. 3 except cyanobacteria abundance (CB) shown.

Table 1. Correlation between heterotrophic bacteria abundance (HB) and environmental and biological parameters (r-standardized correlations coefficients) during the 1990-2008 period tested by multiple regression (n=4052, multiple r=0.746, p<0.000). Differences in HB dependence on listed parameters in different periods (2003-2008 *versus* 1990-2002; SP effect) were tested by ANCOVA. ++ correspond to the probability level p<0.001, and ns to not significant at p<0.05.

Parameter	r	SP effect
Period	-0.550	
Temperature (t)	0.169	ns
Salinity (S)	-0.068	ns
Orthophosphate (PO_4)	0.080	ns
Dissolved inorganic nitrogen (DIN)	0.052	ns
Chlorophyll *a* (chl *a*)	0.154	++
Cyanobacteria abundance (CB)	0.130	++
Heterotrophic flagellate abundance (HF)	0.107	++

Table 2. Standardized correlation coefficients (r) for A) correlation of heterotrophic bacteria abundance with biological parameters and B) heterotrophic flagellates abundance with cyanobacteria abundance in periods 1990-2002 (n=2731) and 2003-2008 (n=1341). ++ correspond to the probability level p<0.001, and ns to not significant at p<0.05.

	1990-2002		2003-2008	
Parameter	r	p	r	p
A)				
Chlorophyll *a* (chl *a)*	0.278	++	0.244	++
Cyanobacteria abundance (CB)	0.143	++	0.397	++
Heterotrophic flagellate abundance (HF)	0.235	++	0.025	ns
B)				
Cyanobacteria abundance (CB)	0.316	++	0.240	++