Picoplankton community structure before, during and after convection event in the offshore waters of the southern Adriatic Sea

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Abstract

This paper documents the picoplankton community’s response to changes in oceanographic conditions in the period between October 2011 and September 2012 at two stations belonging to South Adriatic Pit (SAP). The recorded data include the community’s abundance, composition, prokaryotic production rates and bacterial metabolic capacity. The aforementioned interval included an intense sea cooling with formation of exceptionally, record-breaking dense water. We documented an especially intense winter convection episode that completely diluted the core of Levantine intermediate waters (LIW) in a large area encompassing the SAP’s center and its margin. During this convection event the whole picoplankton community had significantly higher abundances with a recorded picoeukaryotic peak at the SAP margin. In the post-convection phase in March prokaryotic heterotrophic production strongly increased in the entire SAP area (up to 50 times; 456.8 nM C day\(^{-1}\)). The autotrophic biomass increase (up to 5 times; 4.86 µg L\(^{-1}\)) and a disruption of a close correspondence between prokaryotic heterotrophic biomass production and cell replication rates were observed only in the center of the SAP, which was not under the influence of LIW. At the SAP’s margin such an effect was attenuated by LIW, since the waters affected by LIW were characterized by decreased concentrations of dissolved inorganic nitrogen, decreased autotrophic biomasses and by increased bacterial biomass production balanced with cell replication rates as well as by the domination of *Synechococcus* among autotrophic picoplankton. Metabolic capacity was the lowest in spring when autotrophic biomass largely increased, while the highest levels found in the pre-convection phase (October 2011) suggests that the system was more oligotrophic before than after the convection event. Furthermore, we showed that metabolic capacity is a trait of bacterial community independent of environmental conditions and tightly linked to cell replication and substrate availability. On the other hand the bacterial community composition appears to be strongly influenced by physico-chemical characteristics of waters (e.g. temperature and nutrients) and environmental forcing (e.g. convection and LIW).
1 Introduction

The southern Adriatic includes the deepest part of the Adriatic basin, the South Adriatic Pit (SAP ~ 1243 m). The area above the pit is characterized by a quasi-permanent cyclonic gyre, the South Adriatic Gyre (Gačić et al., 1997). In winter period, due to convection processes, dense water (Adriatic Dense Water, AdDW) is formed as a mixture of Adriatic fresher and Ionian more saline waters. The convection rarely reaches the bottom layers of the pit which are mainly under influence of colder and denser water formed in northern Adriatic (North Adriatic Dense water; NadDW; Gačić and Civitarese, 2012). The East Adriatic Current, which partly belongs to South Adriatic Gyre, brings waters from the Ionian Sea into the Adriatic (Artegiani et al., 1997). Depending on the circulation in the Ionian sea it brings to Adriatic high salinity waters of Levantine origin (LIW) or lower salinity waters from Atlantic (Civitarese et al., 2010). The interaction between the Adriatic and the Mediterranean have been found to resemble the Bimodal Oscillating System (BiOS) that changes the circulation of the North Ionian Gyre (NIG) from cyclonic to anticyclonic and vice versa. This interaction has an important influence on the SA physical properties (Gačić et al., 2001). Due to the BiOS mechanism thermohaline properties in the Southern Adriatic exhibit a quasi-periodic variability, while nutrients have an opposite phase (Civitarese et al., 2010). LIW, brought by the cyclonic NIG circulation, is characterized by salinity above 38.75, temperatures higher than 14°C and poor nutrient content (Civitarese et al., 1998; Vilibić and Orlić, 2002; Civitarese et al., 2010).

The SA, being highly oligotrophic is dominated by picophytoplankton community. Microphytoplankton pulses are limited to low salinity surface layers, deep chlorophyll maximum depths (DCM) and winter convection (Cerino et al., 2012). During winter convection event (e.g. in 2008) the area of maximal phytoplankton abundance appeared below euphotic zone (Batistić et al., 2012). Winter convective mixing transports nutrients from the deep reservoir into the upper layer thus making them available for autotrophs that in spring triggers phytoplankton blooms (Gačić et al., 2002). The in-
tensity of the winter convection and spring blooms influences the downward fluxes of particulate matter to the deepest part of the basin (Boldrin et al., 2002; Turchetto et al., 2012). Due to high interannual variability of the winter convection a marked variability in the phytoplankton abundance and composition was observed (Cerino et al., 2012), downward particle fluxes (Turchetto et al., 2012) as well as differences in prokaryotic metabolism in comparison to the years when no convective process occurred (Zaccone et al., 2003; Azzaro et al., 2012).

Metabolic capacity is one of the components of bacterial community structure that influence the overall community performance in terms of carbon and nutrient metabolism (Comte and del Giorgio, 2009). Biolog plates were first used by Garland and Mills (1991) for evaluation of the metabolic potential of microbial community. It was shown that both marine (Sala et al., 2006, 2008) and freshwater bacteria (Comte and del Giorgio, 2009, 2010) express high metabolic plasticity in order to exploit changing sources for growth and also that metabolic plasticity is an intrinsic emerging property of bacterial community (Comte et al., 2013).

In our study we explored the link between prokaryotic heterotrophic productions, metabolic capacities (MC) and bacterial community composition (BCC) to document the picoplankton community’s response to changes of oceanographic conditions. The investigated interval (October 2011–September 2012) in the area of South Adriatic Pit included an intense sea cooling with formation of exceptionally, record-breaking dense water (Mihanović et al., 2013).

2 Material and methods

2.1 Sampling and environmental parameters

Temperature \( (T) \) and salinity \( (S) \) were measured continuously throughout the water column during the downcasts of SBE25 (SEA-Bird Electronics Inc., USA) CTD probe. Seawater samples (for nutrients, chlorophyll \( a \) (Chl \( a \)), picoplankton abundance and compo-
position, prokaryotic heterotrophic production (PHP) and metabolic capacities (MC) were taken with 5 L Niskin bottles at 12 or 15 depths (0, 5, 10, 20, (35 m for Chl a in March), 50, 75, 100, 150, 200, 300, 400, 600 and 800 m, 1000, 1200) at station P-1200 and 9 depths at station P-300 (0, 5, 10, 20, 50, 75, 100, 200 and 300 m). Dissolved inorganic nutrients; nitrates (NO$_3$), nitrites (NO$_2$), ammonia (NH$_4$), phosphates (PO$_4$) and silicates (SiO$_4$) were analyzed in unfiltered water immediately after collection (Strickland and Parsons, 1972). Dissolved inorganic nitrogen (DIN) was calculated as the sum of NO$_3$, NO$_2$ and NH$_4$. Chl a concentrations were determined by filtration of 500 mL on Whatman GF/F filters. Filters were frozen (−18 °C) and analyzed within a few days by fluorometric procedure after Holm-Hansen et al., (1965).

2.2 Picoplankton abundance

Samples for picoplankton counts were preserved on board in 0.5 % glutaraldehyde for 10 min, frozen in liquid nitrogen, and stored at −80 °C until analysis. Samples were analyzed on a Partec PAS III (Germany) flow cytometer, equipped with an Argon laser (488 nm). Instrumental settings were standardized for all parameters using fluorescence polystyrene calibration beads of 1, 3 and 10 µm diameter.

The different populations of picophytoplankton, namely *Synechococcus* (SYN), *Prochlorococcus* (Pro) and picoeukaryotes (pEu) were distinguished by the red autofluorescence of the chlorophyll content of their cells (FL3) and the cells’ forward-angle light scatter (FSC) as a proxy of their size as well as the orange fluorescence (FL2) of their phycoerythrin-rich cells. Heterotrophic bacteria (HB) were enumerated after staining with the DNA dye, SYBR Green I (Marie et al., 1997).

The final abundance of each group was obtained by true volumetric absolute counting. The precision of the volume measurement is defined by a fixed mechanical design, eliminating errors related to varying beads’ concentrations usually used.
2.3 Prokaryotic heterotrophic production

The rate of $^3$H-leucine and $^3$H-thymidine incorporation into macromolecules was measured for estimation of prokaryotic heterotrophic production (Smith and Azam, 1992; Fuhrman and Azam, 1982; Kirchman et al., 1985). Triplicates (1.7 mL aliquots) of samples were incubated with L-[4,5-$^3$H] leucine (spec. activity > 100 Ci mmol$^{-1}$, 20 nM final conc.) or methyl-$^3$H-thymidine (spec. activity > 70 Ci mmol$^{-1}$, 20 nM final conc.) in sterile 2.0 mL microcentrifuge tubes for 1 h in dark at in situ temperature. Samples with 100 % TCA added prior to the addition of isotopes served as blanks. After incubation finished with 100 % TCA samples were centrifuged, supernatant discarded and labeled material was extracted consecutively with cold 5 % TCA, 80 % ethanol and collected by centrifugation. Specific leucine (LeuC) and thymidine (TdRC) incorporation rates were obtained by dividing the average bulk rates per liter (LeuB, TdRB) by bacterial abundance per liter.

2.4 Bacterial community metabolic capacity (MC)

Bacterial carbon substrate utilization profiles, determined with BIOLOG Ecoplates, were used as a proxy of bacterial community MC (Comte and del Giorgio, 2009). Ecoplates contain 96-wells with 31 different carbon sources (in triplicates) plus a tetrazolium salt, which is reduced to a colored compound by active bacteria (Garland and Mills, 1991). Each well was inoculated with 150 µL of unfiltered natural samples. Immediately upon inoculation, the zero time-point absorbance of each plate was read. Changes in color development were measured using microplate reader (Multiscan Ascent, Lab Systems) at 595 nm. The time-course of color development was followed for 3–7 days until maximum color development was reached. The overall color development of each plate was expressed as average well color development (AWCD) and computed as $\left[\Sigma(R - C)\right]/93$, where $R$ is the absorbance of each response well, $C$ is the average of the absorbance of the control wells.
2.5 Bacterial community composition

For DGGE analysis 5 L of seawater was collected to 0.2 µm pore diameter filters (Nuclepore PC) with peristaltic pump. Filters were placed in cryo-vials, filled with 1.8 mL of lysis buffer (40 mM EDTA, 50 mM Tris-HCl, 0.75 M sucrose) and stored at −80 °C. DNA was extracted as previously described (Boström et al., 2004). Briefly, cells were treated with lysozyme, proteinase K, and sodium dodecyl sulfate followed by phenol-chloroform isoamyl alcohol extraction. Extracted DNA was desalted and treated with concentrated ethanol and 5 M sodium acetate. DNA was diluted in 50 mL of MQ water. Microbial DNA (1 ng) was used as template for PCR (polymerase chain reaction) amplification of bacterial 16S rDNA using the bacterium-specific primer 358f, with a 40-bp GC clamp, and the universal primer 907r. The reaction mixture volumes were 50 µL, containing 200 µM of each of the deoxynucleoside triphosphate, 0.3 µM of each of the primers, 1.5 mM MgCl₂, 1 × PCR buffer and 1 U Taq DNA polymerase (Applied Biosystems). The PCR and DGGE preparation and analysis were performed as described in Orlić et al. (2013).

3 Results

3.1 Environmental conditions

The presence of LIW was recorded at station P300 during the whole research period (with exception of February cruise), while in May and September LIW was also detected at offshore station P1200. The intense vertical convection occurred in February, and was detected on station P1200 and station P300, in upper 500–600 m and upper 200 m layer, respectively. Temperature (T) of the water column between 10 and 500 m at the station P1200 became uniform, 13.7 °C. Vertical convection that took place in a large cyclonic gyre around the SAP, was denoted by lower surface concentration of Chl a at the satellite image (Fig. 3). In March, due to heating, stratification started in
a thin surface layer. In October 2011, May and September 2012 both stations were characterized with deeper thermocline. In February advection of lower salinity ($S$) and colder waters was observed in the layer below 200 m at P300. An important drop in $S$ and $T$ that could be ascribed to arrival of NadDW occurred in thin layer (810–850 m) during March at P1200. During May the influence of NadDW strengthened causing additional decrease in $S$ and $T$ in much thicker layer, 800–1200 m (Fig. 2).

During the convection episode in February, nutrients were generally homogenously distributed throughout the mixed layer, as shown for DIN (Fig. 4). In this layer Chl $a$ was also homogeneously distributed (Fig. 4). During other months nutrient concentrations were lower in upper < 200 m layers and increased in deeper layers. The highest Chl $a$ values (up to 4.86 $\mu$g L$^{-1}$ at 35 m) were observed in March, while in February, and less productive months (May and September 2012 and October 2011) had pretty low values (Fig. 4). Satellite image (Fig. 3) shows that Chl $a$ was enhanced in surface layer of large area around the SAP. During all cruises (except in February) deep Chl $a$ maximum (DCM) was observed at 75–100 m depth and in that layer the highest (HB) abundances were observed (Fig. 4).

The productive layer (PL), defined as layer where Chl $a > 0$, at both stations was defined from surface to 200 m in October 2011, May and September 2012. During February and March PL reached 600 m and 200 m at station P1200, respectively, while at P300 it reached to 300 m in both months. During the investigated period within the entire PL the waters around the stations P1200 and P300 differed significantly in $S$ (Supplement Table S1) caused by inconsistent presence of LIW in October 2011 and March 2012 (Fig. 2; Supplement Table S2). The area affected by the LIW (LIW area; $S$: 38.83 ± 0.04; $T$: 17.61 ± 4.20 $^\circ$C) was warmer, with significantly lower Chl $a$, DIN (primarily nitrates) and silicates in comparison with non-affected areas (which we name the South Adriatic Waters (SAW) area; $S$: 38.65 ± 0.15; $T$: 15.83 ± 3.25 $^\circ$C). The phosphates in both LIW and SAW areas had similar values (Fig. 5; Supplement Table S1). However, due to higher concentrations at P300 during February, waters between the
P1200 and P300 were, on average, significantly different in that parameter (Supplement Table S2).

$T$, DIN, silicates and Chl $a$ in the PL of both stations were generally homogenously distributed with expressed seasonality. $S$ and $T$ in the PL were significantly lower in February ($S$: 38.62 ± 0.23, $T$: 13.71 ± 0.23°C), while continuously increasing during March ($S$: 38.77 ± 0.08, $T$: 14.60 ± 0.69°C), May ($S$: 38.76 ± 0.09, $T$: 16.59 ± 2.17°C) and September ($S$: 38.78 ± 0.11, $T$: 19.57 ± 4.72°C). During October 2011, $S$ and $T$ ($S$: 38.75 ± 0.08, $T$: 19.62 ± 4.38°C) were similar to values recorded in September 2012. DIN and silicates were higher in February than in other months. The phosphates did not differ significantly between seasons (Fig. 6, Supplement Table S1).

The aphotic layers where Chl $a$ was under the detection limit, were defined as deeper layers (DL). At both stations in October 2011, May and September 2012 DL comprised depths below 200 m. During February and March 2012 as DL were considered depths below 600 m and 200 m at P1200, respectively. In general, DL were significantly colder (DL: 13.71 ± 0.28°C < PL: 16.65 ± 3.8°C) and richer in DIN, silicates and phosphates than waters in PL (Fig. 6, Supplement Table S1). On the seasonal basis DL differed in DIN, being the highest in October 2011.

### 3.2 Picoplankton abundances

Within the PL, HB had the lowest abundances in February and the highest in March while during other months were similar (Fig. 6). HB abundances were significantly higher in PL ($3.6 ± 1.9 \times 10^8 \text{cells L}^{-1}$) than in DL ($1.7 ± 0.7 \times 10^8 \text{cells L}^{-1}$). In DL no seasonality in HB were observed (Supplement Table S1). HB significantly positively correlated with Chl $a$, and negatively with DIN, PO$_4$ and SiO$_4$ (Table 1).

SYN, Pro and pEu were detected only in PL. SYN was the most abundant in October 2011. During 2012 SYN abundances increased from February to September (Fig. 6). Pro appeared in May and reached the highest abundances in September (Fig. 6). In October 2011 Pro abundances were also high, varying in narrower range than in September 2012 (Fig. 6). During October 2011, May and September 2012 the
depths of SYN and Pro maxima corresponded closely to and below of DCM, respectively. In October 2011 pEu had the lowest abundances while the highest one was recorded in February (Fig. 6). The whole picoplankton community had significantly higher abundances at P300 during February (Supplement Table S2).

SYN and Pro were more abundant in LIW than in SAW, while for pEu and HB more uniform distribution was observed (Figs. 6 and 7, Supplement Table S1). SYN and Pro were significantly negatively correlated with DIN, while pEu had positive correlation with DIN. Significant positive correlations were observed between NO$_2$ and pEu, NO$_2$ and Pro (Table 1).

3.3 Prokaryotic heterotrophic production

Bulk and cell-specific prokaryotic production rates of biomass (LeuB, LeuC) and cells (TdRB, TdRC) varied largely throughout the year in PL (Supplement Table S1) being the lowest in February (Fig. 7). During March both rates extremely increased and significant differences in LeuC and L/T ratios between stations were observed (Supplement Table S2). Namely, around P300 LeuB ($49.66 \pm 28.59$ pMh$^{-1}$) and LeuC ($124.55 \pm 72.03$ zmol cell$^{-1}$ h$^{-1}$) were combined with moderate TdRB ($5.09 \pm 2.94$ pMh$^{-1}$) and TdRC ($13.87 \pm 10.99$ zmol cell$^{-1}$ h$^{-1}$) giving higher L/T ratios ($24.6 \pm 43.9$). At DCM depth (75 m) L/T ratio was extremely high ($140.6$). In contrast, at P1200 TdRB ($16.59 \pm 20.38$ pMh$^{-1}$) and TdRC ($32.33 \pm 34.02$ zmol cell$^{-1}$ h$^{-1}$) were much higher and combined with lower LeuB ($35.64 \pm 43.10$ pMh$^{-1}$) and LeuC ($54.57 \pm 62.40$ zmol cell$^{-1}$ h$^{-1}$) giving lower L/T ratio ($5.1 \pm 6.8$). Within Chl a rich layers (20–100 m) at P1200 L/T ratios were extremely low ($0.24 \pm 0.17$). In May TdRB and TdRC were still high, while in September both the rates and differences between stations decreased (Fig. 7, Supplement Table S2). During October 2011 both rates were lower and L/T ratios higher than in September 2012 (Fig. 7). In DL only TdRB and TdRC largely varied seasonally (Supplement Table S1) being very similar in March and May and much higher from the rates determined in September and October 2011 (Fig. 7).
LeuB and TdRB significantly positively correlated with HB abundances through entire water column and Chl a in PL. LeuB and LeuC significantly positively correlated with $T$ and $S$ while LeuB negatively correlated with DIN (Table 1). LeuB and LeuC were significantly higher in LIW than in SAW. Also LeuB was higher in PL than in DL (Supplement Table S1).

3.4 Bacterial community composition (BCC) in PL and DL

Cluster analysis of DGGE gel generally separated bacterial communities (BC) sampled in PL from those sampled in DL (Fig. 8). BC from PL formed 3 subclusters of which the first contained of mostly BCs from P300, the second and the third from P1200. BC from DL formed 2 subclusters: the first grouped BCs from February, March and September and the second BCs from October 2011. BC sampled in March at P1200 (10 m) remained unclustered. DGGE banding pattern of all samples and separately for PL and DL was analyzed by principal component analysis (PCA). The resulting scores of principal components (PC1 and PC2) were then analyzed for correlations with environmental parameters, prokaryotic productions and substrate utilization data. The analysis showed that only PC1 scores of DGGE banding pattern of BCs sampled in PL (28.3% of variability) significantly correlated with DIN ($p = 0.004$) and $T$ ($p = 0.037$). Also, only PC1 scores of DGGE banding pattern of BCs sampled in DL (28.6% of variability) significantly correlated with silicates ($p = 0.037$) and phosphates ($p = 0.05$).

3.5 Utilization of substrates (metabolic capacity of heterotrophic bacteria, MC)

Average utilization of substrates (AWCD) showed no significant differences between stations, PL and DL, LIW and SAW (Supplement Table S1). During the year similar variations of AWCD were observed in PL and DL (Fig. 9a). The variations were more pronounced in PL (Supplement Table S1), with maximum in October 2011 and minimum in March, while during February, May and September AWCD was similar.
In PL the highest utilization of carbohydrates (C) and carboxylic acids (CA) were observed in March and September and the lowest in May and March, respectively. Polymers (P) and amides (AMI) were mostly utilized in May although the difference with other months was not significant. The uniform utilization through the seasons was observed for amino acids (AA) and phosphorylated compounds (PC) (Fig. 9b). Between two stations differences in percent utilization of substrate groups (C and AMI) observed in March became enlarged and significant in May. In October 2011 and September 2012 important differences were observed in AA and C utilization, respectively (Fig. 9c, Supplement Table S2).

In DL differences were observed only in percent utilization of AA, being significantly higher in February in comparison to March and May (Fig. 9d). The pattern of utilization of other groups of substrates, primarily C and P, was similar to that in PL. In general, PL differed from DL only in utilization of PC (PL < DL).

AWCD was significantly negatively correlated to TdRB and TdRC and positively to L/T ratio. In PL AWCD also negatively correlated with Chl a (Table 1). Significance of this relation derived primarily from relationship between C and Chl a \( (r = -0.329, p = 0.017) \) and CA and Chl a \( (r = -0.299, p = 0.031) \).

4 Discussion

The picoplankton community of the Southern Adriatic offshore waters during the investigated period was strongly affected by two important oceanographic phenomena taking place in the region; an especially intense winter convection episode in February followed by an outbreak of the new production in March, and intrusions of saline and nutrient poor LIW.

Due to variations in the winter climatic conditions the intensity of open-sea convection and vertical mixing in the area varies strongly on a year-to-year basis (Gačić et al., 2002; Civitarese et al., 2005). The February 2012 was characterized by convection event that homogenized thermohaline properties above the SAP including its marginal
part around station P300. It was presumably induced by a long lasting Bora episode (~ 3 weeks), blowing occasionally with hurricane force. Due to extreme cooling during that bora event one of the densest water ever measured in the Adriatic Sea ($\sigma_t > 30$), was documented (Mihanović et al., 2013). Since we did not detect LIW during February, we hypothesize that intensive mixing diluted LIW core residing in the SAP during fall and probably winter 2011. Similar convection episode was recorded in the same area in February 2008, but during that event vertical mixing was less intensive, and LIW remained unperturbed in the intermediate layers (Batistić et al., 2012).

Vertical mixing brought dissolved inorganic nutrients into the upper layer largely extending the productive layer of the SAP where the occurrence of pEu and SYN was documented down to 400 m and 600 m, respectively. Occurrence of autotrophs in deep layers could be linked to convection or to downslope transport facilitated by NAdDW (Vilibić and Šantić, 2008). The abundances of autotrophs in winter 2012 were lower than previously reported (Azzaro et al., 2012; Batistić et al., 2012) plausibly ascribable to much lower amount of available DIN. Generally lower salinity and nutrient content in winter 2012 suggested involvement of several combined processes that most probably contributed to dilution of SA basin in 2012.

Although in winter 2011/2012 the eastern coastal zone was under strong impact of LIW (Mihanović et al., 2013), the amount of LIW was obviously not large enough to induce significant rise of salinity values in the entire water column in the SAP. Thus salinity values remained relatively low when compared to February 2008. Since the motions of the cyclonic gyre around the SAP are presumably highly intensified during winter mixing events larger quantity of waters from the western side of the Adriatic, can thus be entrapped within the gyre (Fig. 3). Drifter data documented that waters from western coastal area are declined towards east and can be included into the cyclonic gyre around the SAP (Taillandier et al., 2008). The cooling makes these Chl $a$ rich waters dense, forcing it to sink, whereas surface layer of the cyclonic gyre around the SAP becomes poor in Chl $a$ (Fig. 3, February). During less intense vertical mixing in
the water column, Chl $a$ rich waters might remain at the surface whereby surface gyre waters of the SAP are marked with high Chl $a$ (Fig. 3, March).

The most intense growth of pEu observed in February was supported by generally the highest DIN in PL in comparison to the rest of the year. Although thermohaline properties around two stations were similar, the autotrophic biomass was much higher at P300 in spite of much lower DIN in waters around this station in comparison to P1200. While lower DIN most likely indicated that LIW resided at P300 only short time before water mixing and cooling have started, the larger growth of pEu was probably stimulated by higher concentration of easily accessible nitrites in these waters. The marked decrease of SYN towards open waters was regularly observed in south Adriatic (Cerino et al., 2012) as well as its dominance in picophytoplankton population (Šilović et al., 2011).

In the post-convection phase during March the autotrophic biomass largely increased primarily in the area of the SAP (in avg 5 times in respect to February) than on its margin (in avg very similar to February) where LIW occupied entire water column. The onset of new production was very pronounced on P1200. In contrast, SYN quickly responded to decreased nutrients due to LIW arrival and increased in abundance at P300. The increase in abundance of SYN and the appearance of Pro; coincided with spreading of LIW into entire PL during May and September when oligotrophy of the area gradually increased. The dynamics and vertical distribution of two genera appeared to be tightly coupled with inorganic nutrients, with preference of lower DIN. However, while SYN achieved higher abundances in NO$_3$ and SiO$_4$ absolute minima, Pro accumulated along NO$_2$ maxima. Therefore, the decrease in nutrient availability in the thick subsurface layer in May induced the bloom of SYN, whereas Pro appeared in deeper layer. Pro accumulated at pronounced nitricline in September, in area of increased temperature. The co-occurrence of these two genera during summer and fall and deeper maximum of Pro than SYN appeared as characteristic feature for oligotrophic environments (Mella-Flores et al., 2011), including SA waters (Šilović et al., 2011; Cerino et al., 2012).
Coupling of bacterial and phytoplankton activities found in this study were apparent and generally supports the idea that under oligotrophic conditions co-variation between HB and DOC production occur (Gasol et al., 1998; Ruiz-Gonzáles et al., 2012). Moreover, the increase or accumulation of autotrophic biomass highly stimulates PHP (Van Wambeke et al., 2004; Baltar et al., 2009, 2010; Orlić et al., 2013). Concomitantly with autotrophic biomass, PHP increased markedly in March (209.5 ± 158.7 nMCday$^{-1}$) in comparison to February (3.7 ± 3.5 nMCday$^{-1}$). In contrast, during 2007 when winter convection was lacking the PHP values were similar (February: ~ 50 nMCday$^{-1}$; April: ~ 40 nMCday$^{-1}$) (Azzaro et al., 2012). Apparently vigorous mixing and heat loss that occurred in February 2012 strongly but unfavorably influenced PHP, whereas introduced nutrients and enhanced new production enabled enormous substrate supply in PL indirectly fueling PHP in March 2012.

Although both PHP rates increased significantly in March with respect to February, the relationship between the rates quite differed between stations. At P300, the increase in both PHP rates was more tightly coupled (L/T ~ 20), whereas at P1200 more frequently unbalanced due to much higher rates of cell replication than biomass production (L/T < 1). Consequently, at both stations HB followed the increase in respective autotrophic biomasses that were far more expressed around the SAP. Generally, our results showed that T has greater effect on bacterial biomass production (Leu) while Chl a (taken as a measure of substrate supply) affected more strongly cell replication rates (TdR). Since temperature and resource supply are principal factors that influence bacterial growth and reproduction (Shiah and Ducklow, 1997), the higher biomass production rate at P300 might be additionally induced by increased temperature of LIW and partly by much higher abundances of SYN. Cyanobacteria have been reported to take up leucine thus shifting the L/T ratio toward higher values (Hietanen et al., 2002). These two factors in addition to generally reduced substrate supply probably led to significantly higher prokaryotic biomass production in waters influenced by LIW. Similar increase in both, L/T ratio and the abundance of cyanobacteria were recorded in the
Northern Adriatic during the period of 2003–2008 which coincided with overall increase in S and T in that area (Ivančić et al., 2010).

Our result showed that bacterial MC decrease when substrate supply increase, both being in close correspondence with cell replication rates increase. This is in agreement with the finding that MC consistently mediates the link between substrate supply and bacterial community metabolism (Comte and del Giorgio, 2009). In March the lowest MC suggested that bacteria utilized freshly produced DOM released by phytoplankton. Such relation was also observed for surface NW Mediterranean waters (Sala et al., 2006). DOM produced in situ with high amino acid and amino sugar content is an important source of bioreactive organic matter favoring the growth of bacteria specialized in the use of carbon sources derived from phytoplankton (Davis and Benner, 2005; Sala et al., 2008). Clear positive correlation of Chl a with TdR in PL suggested that bacteria rapidly responded to the increased input of fresh organic matter by adapting numbers of specialist bacteria.

According to decreased input of fresh organic matter in May, the remaining organic matter from the bloom in March became less bioreactive but more complex. The conditions in May became more oligotrophic, therefore MC significantly increased. This is in accordance with ecological theory of high metabolic diversity in oligotrophic areas (Frontier, 1985) where bacteria expresses higher plasticity in metabolic pathways in order to be able to exploit the changing and limiting carbon sources for growth (Sala et al., 2006, 2008). Although autotrophic biomass significantly decreased cell replication rate was kept on the levels found for March. Apparently, such increase in MC requires adaptation of bacterial community composition with increased numbers of bacteria with versatile metabolic capabilities. During September, when system was more stable, high MC levels were sustained, while PHP rates were decreased but balanced. The highest MC levels found in October 2011 suggested that the system was more oligotrophic in 2011 than in 2012.

The differences in percentage substrate utilization between the two stations evident in March and May suggested that the composition of DOM pool differed among
these waters. These differences might be introduced by variable quality of exudates attributable to phytoplankton origin and/or amount and quality of grazing-derived DOM (Ghiglione et al., 2008; Ruiz-Gonzalez et al., 2012). Very high TdR rates and low L/T ratio at P1200 in March suggested that bacteria were more active at station P1200 than at station P300. This higher bacterial metabolic activity might be explained by more intense grazing activity on P1200, since protozoa preferentially graze on the actively dividing cells (del Giorgio et al., 1996; Gasol et al., 2009). High grazing activity was probably sustained in May when due to change in picophytoplankton composition grazers might changed their prey, additionally contributing to differences in DOM quality.

Co-variations of MC in DL and PL suggested that the quality and amount of exported particles to DL depended on the intensity of autotrophic production in PL. Such relation derived from positive coupling between downward fluxes of organic carbon and productivity in the same area (Boldrin et al., 2002; Turchetto et al., 2012). Since these two layers matched also in TdR regulation of bacterial function by the same factors, i.e. resource quality and availability across entire water column is suggested. As the changes in bacteria metabolic potential are often linked to changes in bacterial community composition (BCC) (Kirchman et al., 2004; Sala et al., 2006), the observed differences in utilization of substrates groups between the layers and between the stations might be associated with the differences in BCC.

The BCC of PL differed evidently from BCC of DL where mineralization processes took place and appears to be influenced by physico-chemical characteristics (DIN, T, SiO_4, and PO_4) of the related layers. Accordingly, in PL, BCCs of waters influenced by LIW were more similar to each other (mostly belonging to station P300), whereas BCCs at P1200 were more different, followed by apparent phytoplankton composition changes (data not shown). Also, in DL the increase in depth-related divergence between BCC within seasons increased from winter to summer, being the highest in October 2011. These results are in accordance with studies that explained the vertical distribution of bacterial community by synergy of environmental parameters and inter-
action of bacteria with other plankton (Acinas et al., 1997; Ghiglione et al., 2008). An analysis employing the complex environmental dataset in combination with microbial community structure will undoubtedly give more detailed insight into BCC changes with depth in this area.

5 Conclusions

Our results showed that winter convection event and LIW have different roles in the distribution and function of the picoplankton community. Although the winter convection induced strong increase in prokaryotic heterotrophic production in March, an enormous increase in the autotrophic biomass followed by a disruption of a close correspondence between Leu and TdR incorporation rates (L/T < 1) occurred only in the center of the SAP beyond the reach of LIW. At the SAP margin LIW attenuated winter convection effect. In general, the waters affected by LIW were characterized by decreased DIN and autotrophic biomasses and by increased abundances of SYN and prokaryotic biomass production rates balanced with cell replication. Furthermore, we showed that metabolic capacity is a trait of the bacterial community independent of environmental conditions and strongly linked to cell replication rate and substrate supply. Metabolic capacity indicated general increase in trophic state after winter convection event although autotrophic biomasses in comparable periods, October 2011 and September 2012, were similar. On the other hand, bacterial community composition appeared to be strongly influenced by physico-chemical characteristics of waters and environmental forcing. Our results reinforce the significance of two oceanographic phenomena of SA, which might have a key role in control of the total production of the Adriatic Sea as both can change the relative importance of the River Po freshwater input in this vital process.
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References


Supplementary material related to this article is available online at http://www.biogeosciences-discuss.net/10/17859/2013/bgd-10-17859-2013-supplement.pdf.


Picoplankton of the Southern Adriatic Pit
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### Table 1. Standardized correlation coefficients ($r$) for correlations between abundances of picoplankton, bulk cell specific prokaryotic heterotrophic production and environmental parameters. Number of data is given in parenthesis. All data were log or log($x + 1$) transformed and significant correlations (in bold) are at $^{a}p < 0.05$, $^{b}p < 0.001$. pEu – picoeukaryots, SYN – *Synechococcus*, Pro – *Prochlorococcus*, HB – heterotrophic bacteria, bulk (B) and cell-specific (C) prokaryotic rates for; leucine (Leu) and thymidine (TdR) incorporation.

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<th>pEu</th>
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<th>Pro</th>
<th>HB</th>
<th>Chl a</th>
<th>LeuB</th>
<th>LeuC</th>
<th>TdRB</th>
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Fig. 1. Sampling stations in the Southern Adriatic.
Fig. 2. Vertical distribution of salinity and temperature at stations P300 and P1200 during the cruises (11 October, 12 February, 12 March, 12 May, 12 September).
Fig. 3. Satellite image of surface chlorophyll $a$ (µg L$^{-1}$) distribution in the Adriatic Sea on the two dates in February and March 2012.
Fig. 4. Vertical distribution of dissolved inorganic nitrogen (DIN), chlorophyll a (Chl a) and heterotrophic bacteria abundance (HB) at stations P300 and P1200 during the cruises (11 October, 12 February, 12 March, 12 May, 12 September).
Fig. 5. Dissolved inorganic nitrogen (DIN), phosphates (PO$_4$), silicates (SiO$_4$), chlorophyll $a$ (Chl $a$), abundances of picoeukaryotes (pEu), Synechococcus (SYN), Prochlorococcus (Pro), heterotrophic bacteria (HB), bulk and cell-specific prokaryotic rates for; leucine (LeuB and LeuC) and thymidine (TdRB and TdRC) incorporation and their ratios (L/T) in LIW affected and non-affected waters (SAW).
Fig. 6. Dissolved inorganic nitrogen (DIN), phosphates (PO$_4$), silicates (SiO$_4$), chlorophyll $a$ (Chl $a$), abundances of picoeukaryotes (pEu), Synechococcus (SYN) and Prochlorococcus (Pro) in productive (PL) and deeper layers (DL) during the cruises (11 October, 12 February, 12 March, 12 May, 12 September).
Fig. 7. Heterotrophic bacteria (HB), bulk and cell-specific prokaryotic rates for; leucine (LeuB and LeuC) and thymidine (TdRB and TdRC) incorporation and their ratios (L/T) in productive (PL) and deeper layers (DL) during the cruises (11 October, 12 February, 12 March, 12 May, 12 September).
Fig. 8. Cluster analysis dendrogram of DGGE banding pattern (Gel Compare, v. 4.1, Applied Maths, Kortrijk, Belgium) performed calculating the Pearson correlation similarity coefficient for BC sampled in PL and DL (October 2011–September 2012).
Fig. 9. Changes of MC (mean AWCD) in productive and deeper layers (A); percentage utilization of substrate groups (AA – amino acids, AMI – amides, C – carbohydrates, CA – carboxylic acids, P – polymers, PC – phosphorylated compounds) at stations P1200 and P300 (B); in productive layer (C); and deeper layers (D) during the cruises (11 October, 12 February, 12 March, 12 May, 12 September).