



HAL
open science

Phytoplankton and bacteria alkaline phosphatase activity in the northern adriatic sea

Ingrid Ivancić, Dragica Fuks, Tomislav Radić, Daniel M. Lyons, Tina Šilović, Romina Kraus, Robert Precali

► **To cite this version:**

Ingrid Ivancić, Dragica Fuks, Tomislav Radić, Daniel M. Lyons, Tina Šilović, et al.. Phytoplankton and bacteria alkaline phosphatase activity in the northern adriatic sea. *Marine Environmental Research*, 2010, 69 (2), pp.85. 10.1016/j.marenvres.2009.08.004 . hal-00563096

HAL Id: hal-00563096

<https://hal.science/hal-00563096>

Submitted on 4 Feb 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Phytoplankton and bacteria alkaline phosphatase activity in the northern adriatic sea

Ingrid Ivancić, Dragica Fuks, Tomislav Radić, Daniel M. Lyons, Tina Šilović, Romina Kraus, Robert Precali

PII: S0141-1136(09)00108-1
DOI: [10.1016/j.marenvres.2009.08.004](https://doi.org/10.1016/j.marenvres.2009.08.004)
Reference: MERE 3365

To appear in: *Marine Environmental Research*

Received Date: 13 May 2009
Revised Date: 17 August 2009
Accepted Date: 24 August 2009

Please cite this article as: Ivancić, I., Fuks, D., Radić, T., Lyons, D.M., Šilović, T., Kraus, R., Precali, R., Phytoplankton and bacteria alkaline phosphatase activity in the northern adriatic sea, *Marine Environmental Research* (2009), doi: [10.1016/j.marenvres.2009.08.004](https://doi.org/10.1016/j.marenvres.2009.08.004)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



1 PHYTOPLANKTON AND BACTERIA ALKALINE PHOSPHATASE ACTIVITY IN THE
2 NORTHERN ADRIATIC SEA

3

4 Ingrid Ivančić^{*a}, Dragica Fuks^a, Tomislav Radić^b, Daniel M. Lyons^a, Tina Šilović^a, Romina
5 Kraus^a, Robert Precali^a

6

7 ^a Center for Marine Research, Ruđer Bošković Institute, G. Paliaga 5, 52210 Rovinj,
8 Croatia

9 ^b Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, 21000 Split, Croatia

10

11 **Key words:** APA, nutrients, DOP, phytoplankton, bacteria, northern Adriatic

* Corresponding author: Ingrid Ivančić, tel. 00385 52 804 743; fax. 00385 52 813 496; email

ingrid@cim.irb.hr

12 ABSTRACT

13 The importance of bacterial, phytoplankton and dissolved alkaline phosphatase
14 activity (APA) in the northern Adriatic was investigated during 2006. In upper waters total
15 APA increased from early spring ($0.05\text{-}0.08 \mu\text{mol l}^{-1} \text{h}^{-1}$) to late spring (up to $4.64 \mu\text{mol l}^{-1}$
16 h^{-1}) and remained relatively high during the summer ($0.46\text{-}0.71 \mu\text{mol l}^{-1} \text{h}^{-1}$), due to an
17 increase in specific phytoplankton (up to $30 \mu\text{mol } \mu\text{g C}^{-1} \text{h}^{-1}$) and bacterial APA (up to 23
18 $\mu\text{mol } \mu\text{g C}^{-1} \text{h}^{-1}$). Activity of free enzymes was not important. During late spring and
19 summer both communities exploited dissolved organic phosphorus although, taking into
20 account biomass, phytoplankton activity usually dominated over bacterial. In autumn an
21 extra P supply from deeper waters drastically reduced phytoplankton APA, though not
22 bacterial APA, in upper waters. Probably in these months bacteria that were degrading
23 phytoplankton-produced organic matter were P limited. In deeper waters APA was low and
24 mainly due to the activity of free enzymes.

25 1. INTRODUCTION

26 The northern Adriatic is characterised by significant freshwater input, mainly from
27 the Po River. In Po River waters the average total phosphorus and total nitrogen
28 concentrations in the 1999-2002 period ($5.16 \mu\text{mol l}^{-1}$ and $285.71 \mu\text{mol l}^{-1}$, respectively;
29 Milan et al., 2003), were more than an order of magnitude higher than in northern Adriatic
30 waters (Giani, 2003). Consequently, riverine waters increase nutrient content in seawater
31 leading to high microbial activity and eutrophication of the western area of the northern
32 Adriatic (Gilmartin and Revelante, 1983; Gilmartin et al., 1990; Karner et al., 1992).
33 However, in river waters inorganic nitrogen concentrations (DIN) were markedly higher
34 than orthophosphate (PO_4) concentrations, resulting in a strongly unbalanced N/P atomic
35 ratio (about 100/1; Milan et al., 2003) for microbial requirements (balanced N/P=16/1 for
36 phytoplankton, 9/1 for bacteria; Redfield et al., 1963; Goldman et al., 1987). As a
37 consequence P limitation is expected in productive northern Adriatic waters influenced by
38 freshwater. It was observed that in these waters organic phosphorus concentrations
39 markedly exceeded PO_4 concentrations, representing an important source of P for
40 microbial communities (Ivančić and Degobbis, 1987). In the presence of low PO_4
41 concentrations microbes can induce extracellular alkaline phosphatase (AP), which
42 enables them to use organic phosphorus esters as a source of this element (see Hoppe,
43 2003). A study carried out during 2004 showed that in the concerned area AP was
44 important for providing P for microbial growth, particularly during phytoplankton blooms

45 induced by freshwater imported nutrients (Ivančić et al., 2009). Alkaline phosphatase
46 activity (APA) studies in the marine environment have been focused mainly on algae and
47 have rarely dealt with bacterial activities even though bacteria are also known to have
48 significant APA (Martinez and Azam, 1993; Labry et al., 2005). Recently, Zacccone et al.
49 (2002) showed that most of the bacterial strains in the northern Adriatic are capable of
50 expressing APA.

51 The objectives of the present paper are to evaluate the importance of
52 phytoplankton and bacteria in expressing APA in one of the most productive areas of the
53 northern Adriatic. In this area where inorganic N supply greatly exceeds inorganic P
54 supply, productivity depends on the ability of microorganisms to obtain P from dissolved
55 organic phosphorus (DOP). For this purpose total APA and parameters characterising
56 microbial communities were measured seasonally at two stations mostly influenced by Po
57 River runoff. Phytoplankton and bacterial APA were determined during blooms induced by
58 freshwater imported nutrients, as well as during minimal external nutrient input in summer.
59 Even if size fractionation by filtration does not completely separate groups of
60 microorganisms (overlapping size) it does however give a useful indication as regards the
61 major microorganisms contributing to APA.

62 2. MATERIAL AND METHODS

63 2.1. *Sampling strategy*

64 Measurements were carried out at two stations (SJ101, SJ108) in the northern
65 Adriatic (Fig. 1) during 7 cruises performed from March to October 2006. These stations
66 were specifically chosen as they are in one area permanently under riverine nutrients
67 pressure, unlike other areas along the Rovinj-Po Delta profile which only intermittently
68 experience freshwater influence. Further, these stations represent two different regimes
69 where SJ108 is under direct freshwater nutrient influence while freshwater spreading
70 toward SJ101 is more impoverished with nutrients. During all cruises conventional
71 parameters (sea temperature, salinity, nutrients, DOP, chl *a*, bacteria and
72 picocyanobacteria counting and phytoplankton determination) and total APA were
73 measured at three depths within the water column (surface, 10 m, and 1 m from the
74 bottom: 30 m). In addition, APA fractions were determined during blooms in different
75 seasons (May, September and October), as well as during minimal phytoplankton biomass
76 in June.

77

Fig. 1

78 **2.2. Analytical protocol**

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

81 Inorganic nutrient analyses were performed onboard, on unfiltered water
82 immediately after sample collection, using methods widely used in oceanography
83 (Strickland and Parsons, 1972; Ivančić and Degobbis, 1984). Samples for total dissolved
84 phosphorus were filtered (Whatmann GF/C, precombusted at 500 °C) and stored in
85 polyethylene tubes at -30 °C. In the laboratory ashore analyses were performed using a
86 chemical combustion method with persulphate (Menzel and Corwin, 1965). DOP was
87 calculated by subtracting PO₄ from the total dissolved phosphorus. DIN was calculated as
88 the sum of nitrate, nitrite and ammonia. The N/P ratio was calculated by linear regression
89 between PO₄ and DIN.

90 Determination of APA was performed aboard the research vessel immediately after
91 sample collection. Measurements were carried out in unfiltered water (total APA) and two
92 pre-filtered fractions: <0.22 µm and <3 µm. Picocyanobacteria and heterotrophic bacteria
93 overlap in size ranges, and for this reason filters of 3 µm were preferred (retaining bacteria
94 and picocyanobacteria) instead of 1 µm, which do not retain all bacteria, or 2 µm at which
95 an unknown part of picocyanobacteria is retained. The abundance of picoeukaryotes
96 which are not retained on the 3 µm filters was three orders of magnitude lower than
97 picocyanobacteria abundance (Fuks, unpublished data). The nanophytoplankton fraction
98 was retained on the 3 µm filter, as confirmed by microscopic measurements. The 0.2-3
99 µm fraction contained heterotrophic bacteria and picocyanobacteria, mainly
100 *Synechococcus* (Fuks, unpublished data), and is subsequently referenced in the text as
101 the bacterial fraction. The >3 µm fraction contained nano- and microphytoplankton and is
102 subsequently referenced as the phytoplankton fraction.

103 All APA measurements were performed in duplicate with a fluorogenic substrate
104 analogue using methylumbelliferyl-phosphate (MUF-P) dissolved in methylcellosolve and
105 diluted with water immediately before addition following the procedure of Hoppe (1983).
106 The final concentration of substrate in sample was 50 µmol l⁻¹. This concentration was
107 chosen since it was observed that in seawater with various microbial activities saturation

108 with substrate was always achieved (Ivančić et al., 2009). In addition, kinetic parameters
109 (half saturation constant K_m , maximum activity V_{max}) were determined at the surface at
110 SJ108 in May, June and September using various MUF-P concentrations (0.5, 5, 10, 50,
111 100, 150, 200, 250 $\mu\text{mol l}^{-1}$). Incubation was performed in the dark in an insulated water
112 bath using water collected from the same depth as the sample to maintain the *in situ*
113 temperature and pH. Fluorescence was measured immediately after substrate addition
114 and after ~1 h of incubation using a Turner TD-700 fluorometer with excitation at 365 nm
115 and emission at 460 nm. APA was calculated as the difference between these two
116 measurements divided by the incubation time after calibration of the fluorometer with
117 methylumbelliferone. Results are presented as the mean value of duplicates. K_m and V_{max}
118 were calculated using Woolf-Hanes linearization. Although several methods are available,
119 P turnover time (T_n) was estimated by the K_m/V_{max} ratio as, for example, in Labry et al.
120 (2005). An alternative method for determining turnover time as described by Xu et al.
121 (2008) gave nearly identical results when DOP concentration was used as the natural
122 substrate concentration (organic phosphorus esters). However, results using the latter
123 method are not reported here since DOP concentration is not always a good
124 approximation of the natural substrate concentration which is not measured.

125 The samples for chl *a* determination (Strickland and Parsons, 1972) were filtered
126 onboard through Whatmann GF/C filters and stored at -30 °C. Extractions with 90%
127 acetone and fluorometric analyses were performed in the onshore laboratory within a few
128 days.

129 Samples for microphytoplankton determination (200 ml) were filtered through a 300 μm
130 mesh plankton net to remove zooplankton, and filtrates were preserved with Lugol solution
131 (2% final concentration) and buffered with sodium acetate. Microphytoplankton abundance
132 and composition were determined in the filtrate at 200x magnification by a Zeiss inverted
133 microscope after 40 hours of sedimentation of a 50 ml subsample using the Utermöhl (1958)
134 settling technique.

135 Samples for bacteria abundance (BA) and picocyanobacteria abundance (CBA)
136 were preserved with formaldehyde (2% final concentration) and stored at 4 °C. BA was
137 determined by cell counting using an epifluorescence microscope after staining with 4',6-
138 diamido-2-phenylindole (Porter and Feig, 1980). CBA was also determined by
139 epifluorescence microscopy and distinguished by orange autofluorescence under green
140 excitation (Takahashi et al., 1985).

141 Specific APA in different fractions was calculated as the ratio between APA and the
142 carbon content in each respective fraction. BA was converted to carbon content by a
143 conversion factor of 20 fg C/cell (Lee and Fuhrman, 1987) and CBA by a factor of 250 fg
144 C/cell (Kana and Gilbert, 1987). Phytoplankton C content was obtained by converting chl *a*
145 using a factor of 50 µg C/µg chl *a* (Antia et al., 1963) followed by the subtraction of C
146 content in picocyanobacteria. The bacterial and phytoplankton C content calculated from
147 the abovementioned factors are commonly used to calculate their respective specific APA
148 (Sala et al., 2001, Nausch et al., 2004; Labry et al., 2004) and data presented here are
149 comparable with literature data.

150 Statistical analyses were performed using ANOVA and t-test for groups (differences
151 between seasons) and for pairs (differences between stations) on log transformed data.

152 3. RESULTS

153 3.1. Hydrological conditions and nutrient status

154 In March the entire water column at both stations was cold (7.6-8.1 °C) and
155 homogenous (Fig. 2a). In the April-July period temperatures increased (16.9- 27.8 °C at
156 the surface and 8.7-13.4 °C at the bottom) with the establishment of thermal stratification.
157 During September cooling of the surface started mixing in the water column and in
158 October a nearly homogenous layer extended down to 20 m (18.1-21.6 °C) with increased
159 values in the bottom waters (16.2-18.4 °C; Fig. 2a). Haline stratification started from April
160 and persisted during the entire investigated period, except at SJ101 in June (Fig. 2b). At
161 SJ108 the Po River plume (salinity <36) was detected in April, May and September to a
162 depth of about 2-6 m. At this station freshwater influence was weak in June, July and
163 October (surface salinity 36.6-37.1; Fig. 2b). At SJ101 the Po River plume was detected in
164 May, September and October to a depth of about 1-6 m, while in April, June and July
165 freshwater influence was weak (surface salinity 36.4-37.9; Fig. 2b). It should be noted that
166 salinity was usually lower at SJ108 than at SJ101 though differences were not significant
167 at a probability level $p < 0.05$. At a depth of 10 m and below the contribution of riverine
168 water at both stations was always low and salinity was > 37 .

169 Fig. 2

170 PO_4 concentrations were always low (0.00-0.07 µmol l⁻¹) in the entire water column
171 at both stations, even in the Po River plume, except for higher values (0.23 µmol l⁻¹) at the

172 bottom in October (Fig. 3a). DOP concentrations were always several times higher than
173 PO_4 concentrations. In March and October DOP (generally $0.3 - 0.5 \mu\text{mol l}^{-1}$) was
174 considerably higher than from April to July (generally $0.05 - 0.25 \mu\text{mol l}^{-1}$) when somewhat
175 higher values (up to $0.3 \mu\text{mol l}^{-1}$) were periodically found at the bottom (Fig. 3b). Periods
176 with low DOP coincided with high APA, and vice versa (See next section). At the surface
177 and 10 m depth DIN was present in surplus with respect to PO_4 , resulting in a high N/P
178 ratio (28/1; data not shown). At the surface high DIN concentrations ($3.5 - 14.0 \mu\text{mol l}^{-1}$)
179 were found during freshets (April, May, September, October), while minimal values ($0.58 -$
180 $1.18 \mu\text{mol l}^{-1}$) were found during minimal freshwater influence (June and July; Fig. 3c). At
181 10 m depth values were lower than at the surface, but showed basically the same trend
182 (Fig. 3c). At the bottom minimal DIN concentrations were found during June and July
183 (generally about $1 \mu\text{mol l}^{-1}$), and maximal during October (up to $8.68 \mu\text{mol l}^{-1}$). In these
184 waters DIN and PO_4 were generally balanced for microbial requirements (N/P 9/1; data
185 not shown).

186 Fig. 3

187 It should be noted that nutrients' concentrations at the surface were generally
188 higher at SJ108 than at SJ101 due to better nutrient supply by freshwater though
189 differences were not significant at a probability level $p < 0.05$.

190 3.2. Microbial biomass evolution and total APA

191 The Po River plume was observed in April, May and September at SJ108, and in
192 May, September and October at SJ101. In these waters freshwater imported nutrients
193 increased phytoplankton biomass ($102 - 667 \mu\text{g C l}^{-1}$) while during the period of low
194 freshwater influence phytoplankton biomass in the surface layer was markedly lower
195 (about $1 - 24 \mu\text{g C l}^{-1}$; Fig. 4a). Bacterial and picocyanobacterial biomass in the surface
196 layer was not affected by freshwater, being generally higher in the July-October period
197 ($29 - 60 \mu\text{g C l}^{-1}$) than in the first part of the year (typically $14 - 18 \mu\text{g C l}^{-1}$; Fig. 4a). During
198 blooms, phytoplankton biomass markedly exceeded bacterial and picocyanobacterial
199 biomass, while in other situations they were either similar or bacterial and
200 picocyanobacterial biomass exceeded that of phytoplankton. The highest total APA ($2.59 -$
201 $4.64 \mu\text{mol l}^{-1} \text{h}^{-1}$) was observed during phytoplankton blooms, except at SJ101 in
202 September and October when the increase in surface phytoplankton biomass did not
203 result in increased total APA (Fig 4a). During June and July, as well as in October at

204 SJ108, periods characterised by low phytoplankton biomass, surface total APA (0.28-0.71
205 $\mu\text{mol l}^{-1} \text{h}^{-1}$) was markedly lower. Minimal surface total APA (0.05-0.08 $\mu\text{mol l}^{-1} \text{h}^{-1}$) was
206 found in March when the water column was mixed, even though the microbial biomass
207 was not lower than during the summer months (Fig. 4a).

208 Fig. 4

209 The phytoplankton blooms did not extend to intermediate waters (10 m depth)
210 where phytoplankton biomass was generally low (6-34 $\mu\text{g C l}^{-1}$) and seasonal changes
211 much less pronounced (Fig. 4b). In these waters bacterial and picocyanobacterial biomass
212 was also generally lower (8-38 $\mu\text{g C l}^{-1}$) than at the surface, though usually higher than
213 that of phytoplankton (Fig. 4b). In these waters total APA was lower than in upper waters
214 (Fig. 4b). The lowest APA was found in March and at SJ101 also in October (down to 0.02
215 $\mu\text{mol l}^{-1} \text{h}^{-1}$). In other months APA was generally 0.2-0.5 $\mu\text{mol l}^{-1} \text{h}^{-1}$.

216 At the bottom phytoplankton biomass (14-40 $\mu\text{g C l}^{-1}$) was somewhat higher, while
217 bacterial and picocyanobacterial biomass had similar ranges as in intermediate waters
218 (Fig. 4c). In these waters phytoplankton biomass was generally similar to those of bacteria
219 and picocyanobacteria. Total APA was lower than in upper waters and generally <0.15
220 $\mu\text{mol l}^{-1} \text{h}^{-1}$ (Fig. 4c).

221 3.3. APA fractions

222 The overlap of the size spectra of bacteria, picocyanobacteria and phytoplankton
223 could affect their APA estimation. However, in surface and intermediate waters the ratios
224 APA in 0.2-3 μm fraction/total APA and APA >3 μm fraction/total APA were significantly
225 correlated ($r^2=0.579$, $p<0.001$) with the ratios bacterial biomass/total biomass and
226 phytoplankton biomass/total biomass, respectively. This suggests that APA in the 0.2-3
227 μm fraction represented the bulk of bacterial and picocyanobacterial, while APA in the
228 >3 μm fraction the bulk of phytoplankton, activity. Calculations were not made for the
229 bottom layer where APA was not related to microbial biomass, but mostly due to free
230 enzymes.

231 At the surface enzymatic activity was usually due to microbes, while activity of free
232 enzymes was negligible (generally 0.0-4.2% of total activity; Fig. 5a). During the
233 phytoplankton bloom in May practically all surface activity at both stations was due to the
234 phytoplankton fraction (93.0-94.3%). In other months activity of the bacterial fraction also

235 became important (30.8-47.9%), and in September (at SJ101) and October (at SJ108)
236 most of the enzymatic activity was due to this fraction (64.8-79.1%; Fig. 5a). At 10 m
237 depth the contribution of free enzymes (7-20%) was higher than at the surface, though still
238 much less important than that of the microbial fraction (Fig. 5b). In this layer the
239 contribution of phytoplankton to total APA was generally lower than at the surface, while
240 the bacterial contribution increased (Fig. 5b). At both stations in May and at SJ101 in
241 June, most of the activity was due to the phytoplankton fraction (about 49-66%), while in
242 September and October, and also in June at SJ108, the contribution of the bacterial
243 fraction was more important (52.8-91.0%, Fig. 5b). At the bottom APA was mostly due to
244 free enzymes (54-100%, Fig. 5c), except at SJ101 in June when the low activity was
245 mostly due to bacteria.

246 Fig. 5

247 3.4 Specific APA and composition of the microbial communities

248 Statistical analysis (ANOVA) showed that at the surface and in intermediate waters
249 specific phytoplankton and specific bacterial APA were significantly higher than at the
250 bottom ($p < 0.02$ and $p < 0.001$, respectively), while differences between surface and
251 intermediate waters were not significant. At the surface and in intermediate waters both
252 phytoplankton and bacterial specific APA were high in May and June ($7.64 \rightarrow 30 \text{ nmol } \mu\text{g C}^{-1} \text{ h}^{-1}$
253 and $5.83 \rightarrow 23.76 \text{ nmol } \mu\text{g C}^{-1} \text{ h}^{-1}$, respectively; Fig. 6a,b). It should be noted that due to
254 the very low phytoplankton biomass in June there was greater uncertainty in the
255 calculation of specific phytoplankton APA, hence these data were simply denoted as >30
256 $\text{nmol } \mu\text{g C}^{-1} \text{ h}^{-1}$ (in statistical analyses a value of 30 was used). In September and October
257 specific phytoplankton APA dropped to low values ($1.11 \rightarrow 4.52 \text{ nmol } \mu\text{g C}^{-1} \text{ h}^{-1}$), while
258 specific bacterial APA did not show such a drastic decrease in these months. Statistical
259 analyses (t-test for groups) confirmed that in surface and intermediate waters specific
260 phytoplankton APA during May and June (stratification period; mean value $17.11 \text{ nmol } \mu\text{g}$
261 $\text{C}^{-1} \text{ h}^{-1}$) was significantly higher than during September and October (period when mixing in
262 the water column started; mean value $2.03 \text{ nmol } \mu\text{g C}^{-1} \text{ h}^{-1}$; Table 1), while differences in
263 specific bacterial APA for those two periods were much smaller (mean values 9.91 and
264 $7.27 \text{ nmol } \mu\text{g C}^{-1} \text{ h}^{-1}$, respectively) and were not significant (Table 1). At the surface
265 specific bacterial APA in October was lower ($3.78 \rightarrow 6.07 \text{ nmol } \mu\text{g C}^{-1} \text{ h}^{-1}$) than in other
266 months, while in intermediate waters a decrease in October was observed only at SJ101
267 (Fig. 6b).

268

Fig. 6

269 In surface and intermediate waters specific bacterial APA was always higher at
270 SJ108 than at SJ101 (Fig. 6b; Table 1) while, to the contrary, specific phytoplankton APA
271 was higher at SJ101, though only during the stratification period (Fig 6a; Table 1).
272 Differences between stations for specific bacterial APA were statistically significant while
273 those for specific phytoplankton APA were not significant.

274

Table 1

275 At the bottom the specific APA of both fractions was always low (generally $<2 \text{ nmol}$
276 $\mu\text{g C}^{-1} \text{ h}^{-1}$; Fig 6a,b) and no statistical differences between stations and periods were found
277 (data not shown).

278 Since observed seasonal and spatial variation in specific phytoplankton and
279 bacterial APA in the surface and intermediate waters could be due to changes in the
280 respective communities' composition, data characterising communities composition were
281 analysed and compared with changes in their respective specific APA. At both stations the
282 micro- and nanophytoplankton contributions alternated in importance irrespective of high
283 or low specific phytoplankton APA (Table 2). At both stations and in all months diatoms
284 predominated in the microphytoplankton fraction, except in June at the surface at SJ101.

285

Table 2

286 Dominant species during the period of high specific phytoplankton APA (*Pseudo-*
287 *nitzschia delicatissima*, *Nitzschiella* sp., *Skeletonema* sp.) were also abundant during the
288 period of low specific phytoplankton APA (Table 3). During the period of high specific
289 phytoplankton APA species composition at the surface (Table 3) and in intermediate
290 waters (data not shown) in June differed at the studied stations. However, in May at both
291 stations *Pseudo-nitzschia delicatissima* strongly dominated in the microphytoplankton
292 fraction. Further, in May the abundance of *Nitzschiella* sp was similar at the surface at
293 both stations. Moreover, while in June at SJ101 *Prorocentrum triestinum* was the
294 dominant species at the surface (Table 3) and *Pseudo-nitzschia delicatissima* in
295 intermediate waters (data not shown), both of these species were however present in
296 much higher abundance during the bloom in May at SJ108.

297

Table 3

298 The bacterial and picocyanobacterial contributions to the bacterial fraction biomass
299 alternated in importance at both stations and no relation with the level of specific bacterial
300 APA was observed (Table 4).

301 Table 4

302 3.5. Kinetic parameters of APA

303 Total APA as a function of substrate concentration measured in May, June and
304 September at the surface of SJ108 fitted the Michaelis-Menten model (Fig. 7). Activity
305 increased up to a substrate concentration of $50 \mu\text{mol l}^{-1}$, where V_{max} was reached, and
306 then remained constant or slightly decreased. In May and September V_{max} (3.89 and 3.83
307 $\mu\text{mol l}^{-1} \text{h}^{-1}$, respectively) was similar and markedly higher than in June ($0.54 \mu\text{mol l}^{-1} \text{h}^{-1}$).
308 The highest K_m was calculated for June ($7.97 \mu\text{mol l}^{-1}$), while in May and September (1.22
309 and $0.62 \mu\text{mol l}^{-1}$, respectively) was significantly lower. Turnover time of phosphorus
310 estimated from V_{max} and K_m was very short in May and September (0.31 and 0.16 h,
311 respectively) and longer in June (14.79 h; Fig. 7).

312 Fig. 7

313 DISCUSSION

314 The seasonal evolution of total APA during 2006 was similar to that found in 2004
315 (Ivančić et al., 2009) and 2005 (Ivančić, unpublished data). Furthermore a decrease of
316 DOP concentrations as a result of high APA was again observed. This indicates that the
317 same seasonal pattern of APA occurs year by year and that DOP is an important source
318 of P in the region. In March 2006 the investigated area was not influenced by freshwater,
319 and hence the freshwater import of nutrients was minimal. At the time a modest
320 phytoplankton and bacterial biomass developed on nutrient reserves regenerated in the
321 water column during winter. Very low total APA, as well as specific APA (total APA/ total
322 microbial carbon content, $1.46\text{-}1.89 \text{ nmol } \mu\text{g C}^{-1} \text{ h}^{-1}$; data not shown), showed that
323 microbes were not P limited. As a consequence of the microbial activity during early
324 spring, regenerated nutrients in the surface layer were consumed. The phytoplankton
325 blooms that developed in April and May in the low saline waters were stimulated with
326 freshwater imported nutrients. Since freshwater P supply is markedly lower when
327 compared to N supply (see Introduction), significant DIN remained in the water while PO_4
328 was exhausted and DOP became the largest reservoir of dissolved P available to

329 microbes. Consequently, a large increase of total APA (up to 40 times) occurred. As
330 temperature differences of about 10 °C were noted, APA would be expected to only
331 double (Pettersson and Jansson, 1978). Therefore, temperature was considered to be a
332 less important factor, and the increased APA was the response of the microbial
333 communities to P limitation. APA was practically all due to microbial expression, and
334 activity of free enzymes was not important. A major part of the surface activity was due to
335 the phytoplankton fraction, mainly due to a markedly greater algal than bacterial biomass.
336 The dominance of algal APA during the blooms was also observed in the Bay of Biscay
337 (Labry et al., 2005). In the present study specific APA was high in both the phytoplankton
338 and bacterial fractions, indicating that both communities were P limited. At SJ108 P
339 turnover time in surface waters, estimated from AP kinetic parameters, was very short
340 (about 20 min). Such a calculated P turnover time could be used to compare the rate of P
341 recycling in different situations since it agrees with ³³P turnover times (Nausch et al., 2004;
342 Xu et al., 2008). Short AP mediated P turnover times (3-16 h) in P depleted conditions
343 were also found in many other areas, compared to much longer (<30-4585 h) in P
344 repleted conditions (Nausch et al., 2004; Labry et al., 2005; Xu et al., 2008).

345 In June and July the freshwater influence was weak and persistent stratification
346 hindered the replenishment of nutrients from deeper waters. Due to low external nutrient
347 input, phytoplankton biomass dropped to low values, while the decrease in bacterial and
348 picocyanobacterial biomass (smaller cells more efficiently use low nutrient concentrations)
349 was markedly less pronounced. In these conditions a marked decrease of surface total
350 APA was observed, mainly due to the decrease of phytoplankton APA, as shown by
351 fractionation in June. Surface APA was again practically all due to microbial expression,
352 although in contrast to May, in conditions of low phytoplankton biomass, bacteria
353 significantly contributed to total APA (30-44%). In June specific APA of both the
354 phytoplankton and bacterial fractions was even higher than in May, suggesting strong P
355 limitation. However, in conditions of low N supply this element could also limit microbial
356 growth and this is probably the reason for markedly longer P turnover times in surface
357 waters of SJ108 in June (about 15 h) compared to those during the bloom in May (about
358 20 min).

359 During autumn months total APA at the surface was low, irrespective of high or low
360 microbial biomass, except for a high value in September in an area where a large
361 microbial bloom was observed. Activity of free enzymes at the surface was not important.
362 While bacteria significantly contributed to total APA during phytoplankton blooms (31-

363 48%), their contribution was dominant in the absence of phytoplankton blooms (65-78%).
364 The low specific phytoplankton APA found in autumn may be caused by a different
365 percentage of phytoplankton actively expressing the enzyme due to less severe P
366 limitation and/or by different phytoplankton species composition. However, inter-seasonal
367 changes of size structure and composition of phytoplankton were not consistent with
368 changes in specific phytoplankton APA. It is more probable that in autumn P limited
369 phytoplankton growth less than during late spring and summer. In these months mixing of
370 the water column started enriching upper waters with nutrients from deeper waters where
371 they were regenerated in a close to balanced ratio for microbial requirements, as already
372 observed during a previous study (Degobbi, 1990). Thus phytoplankton growth was
373 stimulated not only by freshwater nutrients, but also by the nutrient flux from bottom
374 waters. As a result PO_4 imported into the surface layer might be at higher concentrations
375 and less deficient with respect to DIN than during spring and summer. Nausch (1998)
376 found that PO_4 concentrations $<0.2 \mu\text{mol l}^{-1}$ stimulated production of enzymes leading to
377 very high specific APA, while at PO_4 concentrations between $0.2-1 \mu\text{mol l}^{-1}$ APA was a
378 linear function of biomass and specific APA remained at the same level. Furthermore, a
379 threshold for the regulatory function of PO_4 on APA was found at concentrations of $0.05-$
380 $0.1 \mu\text{mol l}^{-1}$ (Labry et al., 2005; Sebastian et al., 2004). In the present study it was difficult
381 to compare PO_4 concentrations and enzyme production since imported PO_4 uptake was
382 very fast and its concentrations in water were usually below the detection limit. However,
383 specific phytoplankton APA indicated highly stimulated enzyme production during late
384 spring and summer, while during autumn APA was simply a function of biomass, and
385 specific APA remained at the same low level. In contrast, specific APA of the bacterial
386 fraction did not show a significant decrease during autumn. While it may be noted that
387 bacterial AP has a more complex function than that of phytoplankton, i.e. AP could also
388 serve to provide C (see Hoppe, 2003), it is unlikely that bacteria were C limited. It is
389 important to note that changes in specific bacterial APA were not related to the
390 contribution of picocyanobacterial biomass to the total biomass of this fraction. It seems
391 more probable that phytoplankton exhausted imported PO_4 and picocyanobacteria and
392 bacteria were P limited. At the surface the highest specific APA in this fraction was
393 observed at SJ108 during a large phytoplankton bloom in September. The high quantity of
394 organic matter produced supported a large increase of bacteria. Most likely bacteria
395 degrading the produced organic matter were P limited. During this bloom P turnover was
396 fast (about 10 min) not only due to high bacterial activity, but also due to a high level of
397 phytoplankton enzymes since phytoplankton biomass was very high.

398 In intermediate waters (10 m depth) total APA was markedly lower than in surface
399 waters, in contrast to 2004 when activity at this depth did not differ noticeably from the
400 surface (Ivančić et al., 2009). In 2006 Po River flow during spring and summer was
401 considerably lower than in 2004 (unpublished data provided by Ministero dei Lavori
402 Pubblici, Servizio Idrografico, Parma, Italy) and did not notably influence intermediate
403 waters. As a consequence microbial biomass in these waters, especially phytoplankton,
404 was low resulting in low total APA. In these waters the importance of free enzymes
405 increased (9-30%), although the major part of APA was still due to microbes. In conditions
406 where bacterial biomass was similar or somewhat higher than phytoplankton biomass,
407 there was an increase of the bacterial contribution to enzyme production (up to 91%).
408 Specific APA of both fractions followed the same pattern as in surface waters suggesting
409 a similar seasonal evolution of P limitation in both communities.

410 A disproportion between specific phytoplankton APA and specific bacterial APA
411 observed in surface and intermediate waters at the two stations was not related to
412 differences in phytoplankton size and species composition or to the contribution of
413 picocyanobacteria to the bacterial fraction, and was probably due to different nutrient
414 content in those waters. Station SJ108 was generally better supplied with freshwater
415 nutrients than SJ101. Phytoplankton superiority in PO_4 uptake was observed at high and
416 bacterial superiority at low PO_4 concentrations (Thingstad et al., 1993; Xu et al., 2008).
417 Therefore, at SJ108 phytoplankton probably took up most of the imported PO_4 and
418 bacteria, degrading the phytoplankton produced matter, were even more P limited than
419 phytoplankton. On the contrary, at SJ101 bacteria used low concentrations of PO_4 more
420 efficiently and were less P limited than phytoplankton.

421 At the bottom total APA was low (generally $<0.1 \mu\text{mol l}^{-1} \text{h}^{-1}$). In these waters low
422 enzymatic activity was due mainly to free enzymes (up to 100%), while particulate activity
423 was low. As a consequence phytoplankton and bacterial enzymatic activity was usually
424 lower than expected from their biomass and thus specific APA was always low (generally
425 $<2 \mu\text{g C}^{-1} \text{h}^{-1}$) confirming that, in these waters where regeneration predominates over
426 assimilation, microbes were not significantly P limited.

427 CONCLUSIONS

428 Both phytoplankton and bacteria use APA to obtain P, though with different
429 seasonal patterns. During late spring phytoplankton blooms the major part of APA was

430 due to the phytoplankton fraction, mainly as a result of markedly greater algal biomass
431 than bacterial biomass. In summer, when phytoplankton and bacterial biomass were
432 similar, both communities significantly contributed to total APA. Strong P limitation of both
433 communities was observed during the stratification period when the phosphorus supply in
434 upper waters depended on phosphorus recycling in these waters or freshwater input. A
435 more balanced P supply during the period when mixing in the water column started
436 drastically reduced phytoplankton, though not bacterial, AP production in upper waters. In
437 this period the strongest P limitation of bacteria was observed during the massive
438 phytoplankton bloom in September when they were degrading a high quantity of
439 phytoplankton produced organic matter. In upper waters activity of free enzymes was not
440 important, while in bottom waters enzymatic activity was due mainly to free enzymes.

441 ACKNOWLEDGEMENTS

442 The authors thank Dr. T. Đakovac, S. Dujmović, M. Buterer and the crew of RV "Vila
443 Velebita" for help during sampling and analyses. Two anonymous reviewers are thanked for
444 their constructive advice. This work is part of the scientific projects „Structure and physiology
445 of microbial communities in northern Adriatic fronts“(098-0982705-2729), “Mechanism of long-
446 term changes in the northern Adriatic ecosystem” (098-0982705-2731) and Project “Jadran”
447 funded by the Ministry of Science, Education and Sport of the Republic of Croatia.

448 REFERENCES

- 449 Antia, N.J., McAllister, C.D., Parsons, T.R., Stephens, K., Strickland, J.D.H., 1963. Further
450 measurements of primary production using a large-volume plastic sphere.
451 *Limnology and Oceanography* 8,166-183.
- 452 Degobbis, D., 1990. A stoichiometric model of nutrient cycling in the northern Adriatic Sea
453 and its relation to regeneration processes. *Marine Chemistry* 29, 235-293.
- 454 Giani, M. (Ed.), 2003. Rapporto Finale (Adriatico) Processi di Formazione delle Mucillagini
455 nell'Adriatico e in Tirreno (MAT), Istituto Centrale per la Ricerca Scientifica e
456 Tecnologica Applicata al Mare, Roma, 749 pp.
- 457 Gilmartin, M., Revelante, N., 1983. The phytoplankton of the Adriatic Sea: standing crop
458 and primary production. *Thalassia Jugoslavica* 19,173-178.
- 459 Gilmartin, M., Degobbis, D., Revelante, N., Smolaka, N., 1990. The mechanism
460 controlling plant nutrient concentrations in the northern Adriatic Sea. *Internationale*
461 *Revue der gesamten Hydrobiologie und Hydrographie* 75, 425-445.

- 462 Goldman, J. C., Caron, D. A., Dennet, M.R., 1987. Regulation of gross growth efficiency
463 and ammonium regeneration in bacteria by substrate C:N ratio. *Limnology and*
464 *Oceanography* 32, 1239-1252.
- 465 Hoppe, H.G., 1983. Significance of exoenzymatic activities in the ecology of brackish
466 water: measurements by means of methylumbelliferyl-substrates. *Marine Ecology*
467 *Progress Series* 11, 299-308.
- 468 Hoppe, H.G., 2003. Phosphatase activity in the sea. *Hydrobiologia* 493, 187-200.
- 469 Ivančić, I., Degobbi, D., 1984. An optimal manual procedure for ammonia analysis in natural
470 waters by the indophenol blue method. *Water Research* 18, 1143-1147.
- 471 Ivančić, I., Degobbi, D., 1987. Mechanisms of production and fate of organic phosphorus
472 in the northern Adriatic Sea. *Marine Biology* 94, 117-125.
- 473 Ivančić, I., Radić, T., Lyons, D.M., Fuks, D., Precali, R., Kraus, R., 2009. Alkaline
474 phosphatase activity in relation to nutrient status in the northern Adriatic Sea.
475 *Marine Ecology Progress Series* 378, 27-35.
- 476 Kana, T.M., Gilbert, P.M., 1987. Effect of irradiance up to 2000 $\mu\text{E m}^{-1}\text{s}^{-1}$ on marine
477 *Synechococcus* WH7803: I. Growth, pigmentation and cell composition. *Deep-Sea*
478 *Research* 34, 479-495.
- 479 Karner, M., Fuks, D., Herndl, G., 1992. Bacterial activity along a trophic gradient. *Microbial*
480 *Ecology* 24, 243-257.
- 481 Labry, C., Delmas, D., Herbland, A., 2005. Phytoplankton and bacterial phosphatase
482 activities in relation to phosphate and DOP availability within the Gironde plume
483 waters (Bay of Biscay). *Journal of Experimental Marine Biology and Ecology* 318,
484 213-225.
- 485 Lee, S., Fuhrman, J.A., 1987. Relationships between biovolume and biomass of naturally
486 derived marine bacterioplankton. *Applied and Environmental Microbiology* 53,
487 1298-1303.
- 488 Martinez, J., Azam, F., 1993. Periplasmic aminopeptidase and alkaline phosphatase
489 activities in marine bacterium: implications for substrate processing in the sea.
490 *Marine Ecology Progress Series* 92, 89-97.
- 491 Menzel, D.W., Corwin, N., 1965. The measurement of total phosphorus in seawater based
492 on the liberation of organically bound fractions by persulfate oxidation. *Limnology*
493 *and Oceanography* 10, 280-182.
- 494 Milan, C., Trentini, P., Ascanelli, M., Bignami, S., Barbieri, C., Libono, L., Malfato, M.G.,
495 2003. Valutazione dei carichi dei nutrienti del Po a Pontelagoscuro, in: Giani, M.

- 496 (Ed.), Rapporto Finale (Adriatico) Processi di Formazione delle Mucillagini
497 nell'Adriatico e in Tirreno (MAT). Istituto Centrale per la Ricerca Scientifica e
498 Tecnologica Applicata al Mare, Roma, pp. 727-749.
- 499 Nausch, M., 1998. Alkaline phosphatase activities and the relationship to inorganic
500 phosphate in the Pomeranian Bight (Southern Baltic Sea). *Aquatic Microbial*
501 *Ecology* 16, 87-94.
- 502 Nausch, M., Nausch, G., Wasmund, N., 2004. Phosphorus dynamics during the transition
503 from nitrogen to phosphate limitation in the central Baltic Sea. *Marine Ecology*
504 *Progress Series* 266, 15-25.
- 505 Petersson, K., Jansson, M., 1978. Determination of phosphatase activity in lake water-a
506 study of methods. *Verhandlungen der Internationalen Vereinigung für*
507 *theoretische und angewandte Limnologie* 20, 1226-1230.
- 508 Porter, K. G., Feig, Y. S., 1980. The use of DAPI for identifying and counting aquatic
509 microflora. *Limnology and Oceanography* 25, 943-948.
- 510 Redfield, A.C., Ketchum, B.H., Richards, F.A., 1963. The influence of organisms on the
511 composition of seawater, in: *The Sea*, vol 2. Interscience, New York, pp. 27-77.
- 512 Sala, M.M., Karner, M., Arin, L., and Marrasé, C. 2001. Measurement of ectoenzyme
513 activities as an indication of inorganic nutrient imbalance in microbial communities.
514 *Aquatic Microbial Ecology*, 23, 301-311.
- 515 Sebastian, M., Aristegui, J., Montero, M.F., Escanez, J., Niel, F.X., 2004. Alkaline
516 phosphatase activity and its relationship to inorganic phosphorus in the transition zone
517 of the North-western African upwelling system. *Progress in Oceanography* 62, 131-
518 150.
- 519 Strickland, J.D.H., Parsons, T.R., 1972. A practical handbook of seawater analysis. *Bulletin of*
520 *the Fisheries Research Board of Canada* 167, 310 pp.
- 521 Takahashi, M.K., Kikuchi, T.R., Hara, Y., 1985. Importance of picocyanobacteria
522 (unicellular blue-green algae) in the phytoplankton population of the coastal waters
523 off Japan. *Marine Biology* 89, 63-69.
- 524 Thingstad, T.F., Skjodal, E.F., Bohne, R.A., 1993. Phosphorus cycling and algal-bacterial
525 competition in Sandsfjord, western Norway. *Marine Ecology Progress Series* 99,
526 239-259.
- 527 Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik.
528 *Internationale Vereinigung für theoretische und angewandte limnologie. Mitteilung*
529 *No.9*, 17, 47-71.

- 530 Zaccone, R., Caruso, G., Cali, C., 2002. Heterotrophic bacteria in the northern Adriatic Sea:
531 seasonal changes and ectoenzyme profile. *Marine Environmental Research* 54, 1-19.
- 532 Xu, J., Yin, K., He, L., Yuan, X., Ho, A.Y.T., Harrison, P.J., 2008. Phosphorus limitation in the
533 northern South China Sea during late summer: Influence of the Pearl River. *Deep Sea*
534 *Research I* 55, 1330-1342.

ACCEPTED MANUSCRIPT

535 CAPTIONS

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

537 Fig. 2. (a) Temperature and (b) salinity profiles in March (○●), April (△▲), May (◇◆), June
538 (▽▼), July (▷▶), September (◁◄) and October (□■) at SJ101 (open symbols and thin
539 line) and SJ108 (filled symbols and thick line) in 2006.

540 Fig. 3. (a) PO₄, (b) DOP and (c) DIN profiles in March (○●), April (△▲), May (◇◆), June
541 (▽▼), July (▷▶), September (◁◄) and October (□■) at SJ101 (open symbols and thin
542 line) and SJ108 (filled symbols and thick line) in 2006.

543 Fig. 4. Seasonal evolution of phytoplankton (○, thin line) and bacterial and
544 picocyanobacterial (△, dashed line) biomass and total APA (◇, thick line) at (a) the surface,
545 (b) 10 m depth and (c) the bottom at sampling stations in 2006.

546 Fig. 5. Contribution of phytoplankton (grey), bacteria and picocyanobacteria (white) and free
547 enzymes (black) to total APA at (a) the surface, (b) 10 m depth and (c) the bottom at
548 sampling stations in 2006.

549 Fig. 6. (a) Specific phytoplankton and (b) bacterial APA at the surface (●), 10 m depth (◇,
550 dashed line) and the bottom (△, dash dot line) at sampling stations in 2006.

551 Fig. 7. Michaelis-Menten kinetics of total APA in May (●, thick line), June (△, dashed line)
552 and September (◇, thin line) at the surface at station SJ108. Values of V_{max} ($\mu\text{mol l}^{-1} \text{h}^{-1}$), K_m
553 ($\mu\text{mol l}^{-1}$) and T_n (h) are also reported.

Table 1. Mean values (\bar{x}), standard error (SE) and number of data (N) for specific phytoplankton APA ($sAPA_{\text{phyto}}$) and specific bacterial APA ($sAPA_{\text{bact}}$). (a) Probability level (p) for differences between stratification and beginning of mixing periods at the surface and 10 m depth and (b) probability level for differences between stations at the surface and 10 m depths.

| A | Stratification | | Beg. of mix. | | p |
|-----------------------|----------------|---|----------------|---|-------|
| | \bar{x} (SE) | N | \bar{x} (SE) | N | |
| $sAPA_{\text{phyto}}$ | 17.10 (3.07) | 8 | 2.03 (0.66) | 8 | 0.000 |
| $sAPA_{\text{bact}}$ | 9.91 (2.60) | 8 | 7.27 (2.00) | 8 | 1.000 |

| B | SJ108 | | SJ101 | | p |
|-------------------------|----------------|---|----------------|---|-------|
| | \bar{x} (SE) | N | \bar{x} (SE) | N | |
| $sAPA^*_{\text{phyto}}$ | 12.13 (1.80) | 4 | 22.10 (4.91) | 4 | 0.165 |
| $sAPA_{\text{bact}}$ | 12.46 (2.37) | 8 | 4.71 (1.16) | 8 | 0.006 |

*data for the stratification period only

Table 2. Size composition of phytoplankton expressed as the % of micro fraction (Micro) in total chlorophyll *a* (chl *a*) and % of Diatoms in micro fraction during the period of high and low specific phytoplankton APA at the surface and 10 m depth.

| Station | Depth m | Date | <u>High specific APA</u> | | Date | <u>Low specific APA</u> | |
|---------|------------|----------|--------------------------|--------------|----------|-------------------------|--------------|
| | | | Micro % | Diatoms % | | Micro % | Diatoms % |
| SJ108 | 0 | 15.05.06 | 64 | 60 | 21.09.06 | 48 | 98 |
| | 10 | | 41 | 95 | | 13 | 98 |
| SJ101 | 0 | | 85 | 98 | | 15 | 95 |
| | 10 | | 62 | 96 | | 18 | 100 |
| SJ108 | 0 | 12.06.06 | 53 | 99 | 17.10.06 | 24 | 98 |
| | 10 | | 28 | 96 | | 19 | 100 |
| SJ101 | 0 | | 33 | 25 | | 54 | 100 |
| | 10 | | 30 | 79 | | 27 | 97 |

Table 3. List of the most abundant species of microphytoplankton at the surface during periods of high and low specific phytoplankton APA.

| Station | Date | High specific APA Species | Cell l ⁻¹ | Date | Low specific APA Species | Cell l ⁻¹ |
|---------|----------|---------------------------------------|----------------------|----------|---------------------------------------|----------------------|
| SJ108 | 15.05.06 | <i>Pseudo-nitzschia delicatissima</i> | 205860 | 21.09.06 | <i>Chaetoceros</i> sp. | 306900 |
| | | <i>Prorocentrum balticum</i> | 102930 | | <i>Thalassiosira</i> sp. | 244200 |
| | | <i>Diplopsalis lenticula</i> | 18980 | | <i>Nitzschiella</i> sp. | 244200 |
| | | <i>Nitzschiella</i> sp. | 14600 | | <i>Asterionellopsis glacialis</i> | 86900 |
| | | <i>Prorocentrum triestinum</i> | 9460 | | <i>Pseudo-nitzschia delicatissima</i> | 55000 |
| | | <i>Chaetoceros</i> sp. | 8760 | | <i>Skeletonema</i> sp. | 49500 |
| | | <i>Prorocentrum micans</i> | 8760 | | <i>Diatoma</i> sp. | 13200 |
| | | <i>Glenodinium</i> sp. | 5110 | | <i>Chaetoceros affinis</i> | 7700 |
| | | <i>Ceratium furca</i> | 5110 | | <i>Gyrodinium</i> sp. | 6600 |
| SJ101 | | <i>Pseudo-nitzschia delicatissima</i> | 273750 | | <i>Nitzschiella</i> sp. | 52800 |
| | | <i>Nitzschiella</i> sp. | 13140 | | <i>Asterionellopsis glacialis</i> | 34600 |
| | | <i>Bacteriastrum delicatulum</i> | 6570 | | <i>Leptocylindrus danicus</i> | 15900 |
| | | <i>Chaetoceros</i> sp. | 5110 | | <i>Pseudo-nitzschia delicatissima</i> | 15400 |
| | | | | | <i>Chaetoceros</i> sp. | 9350 |
| SJ108 | 12.06.06 | <i>Skeletonema</i> sp. | 8140 | 17.10.06 | <i>Pseudo-nitzschia delicatissima</i> | 51150 |
| | | <i>Cerataulina pelagica</i> | 3700 | | <i>Chaetoceros</i> sp. | 50600 |
| | | <i>Prorocentrum balticum</i> | 2960 | | <i>Chaetoceros affinis</i> | 13200 |
| | | <i>Cyclotella</i> sp. | 2960 | | <i>Nitzschiella</i> sp. | 9900 |
| | | <i>Prorocentrum micans</i> | 2220 | | <i>Leptocylindrus danicus</i> | 7700 |
| SJ101 | | | | | <i>Cerataulina pelagica</i> | 7700 |
| | | | | | <i>Guinardia striata</i> | 6050 |
| | | <i>Prorocentrum triestinum</i> | 2220 | | <i>Chaetoceros</i> sp. | 649000 |
| | | <i>Hemiaulus hauckii</i> | 1110 | | <i>Nitzschiella</i> sp. | 101200 |
| | | <i>Glenodinium</i> sp. | 1110 | | <i>Chaetoceros affinis</i> | 53900 |
| | | <i>Asterionellopsis glacialis</i> | 16500 | | | |
| | | <i>Chaetoceros costatus</i> | 6600 | | | |

Table 4. Specific bacterial APA (bAPA) and % of picocyanobacteria (CB) in the bacterial fraction at the surface and 10 m depth.

| Date | Station | Depth m | bAPA nmol $\mu\text{g C}^{-1} \text{h}^{-1}$ | CB % |
|----------|---------|------------|---|---------|
| 15.05.06 | SJ108 | 0 | 9.55 | 26 |
| | | 10 | 6.77 | 53 |
| | SJ101 | 0 | 5.83 | 12 |
| | | 10 | 0.10 | 58 |
| 12.06.06 | SJ108 | 0 | 17.11 | 51 |
| | | 10 | 23.76 | 30 |
| | SJ101 | 0 | 9.75 | 35 |
| | | 10 | 6.40 | 28 |
| 21.09.06 | SJ108 | 0 | 17.94 | 27 |
| | | 10 | 5.19 | 61 |
| | SJ101 | 0 | 6.99 | 58 |
| | | 10 | 4.77 | 55 |
| 17.10.06 | SJ108 | 0 | 6.07 | 56 |
| | | 10 | 13.27 | 40 |
| | SJ101 | 0 | 3.78 | 25 |
| | | 10 | 0.46 | 68 |

Figure1



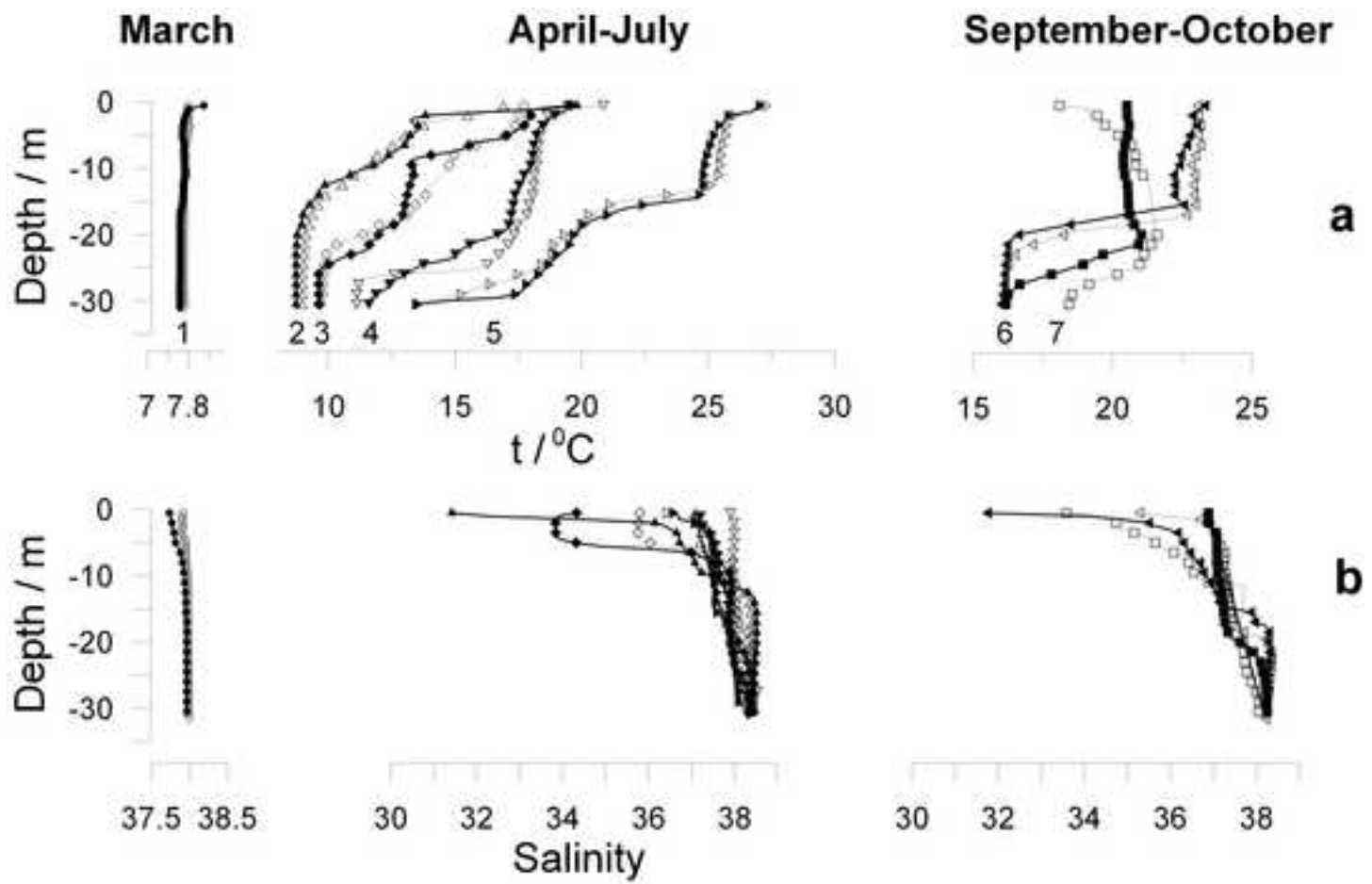


Fig. 2

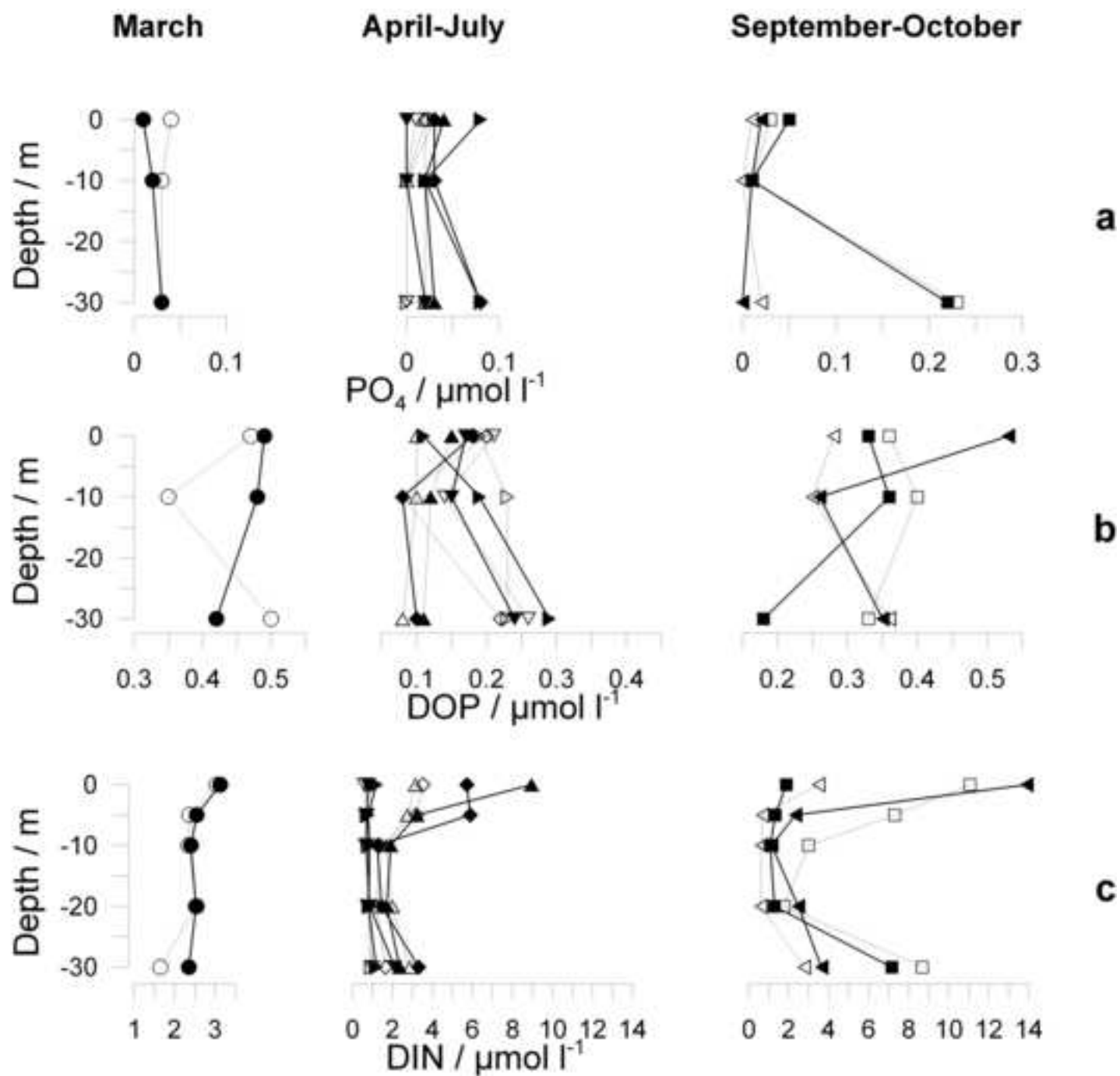


Fig.3

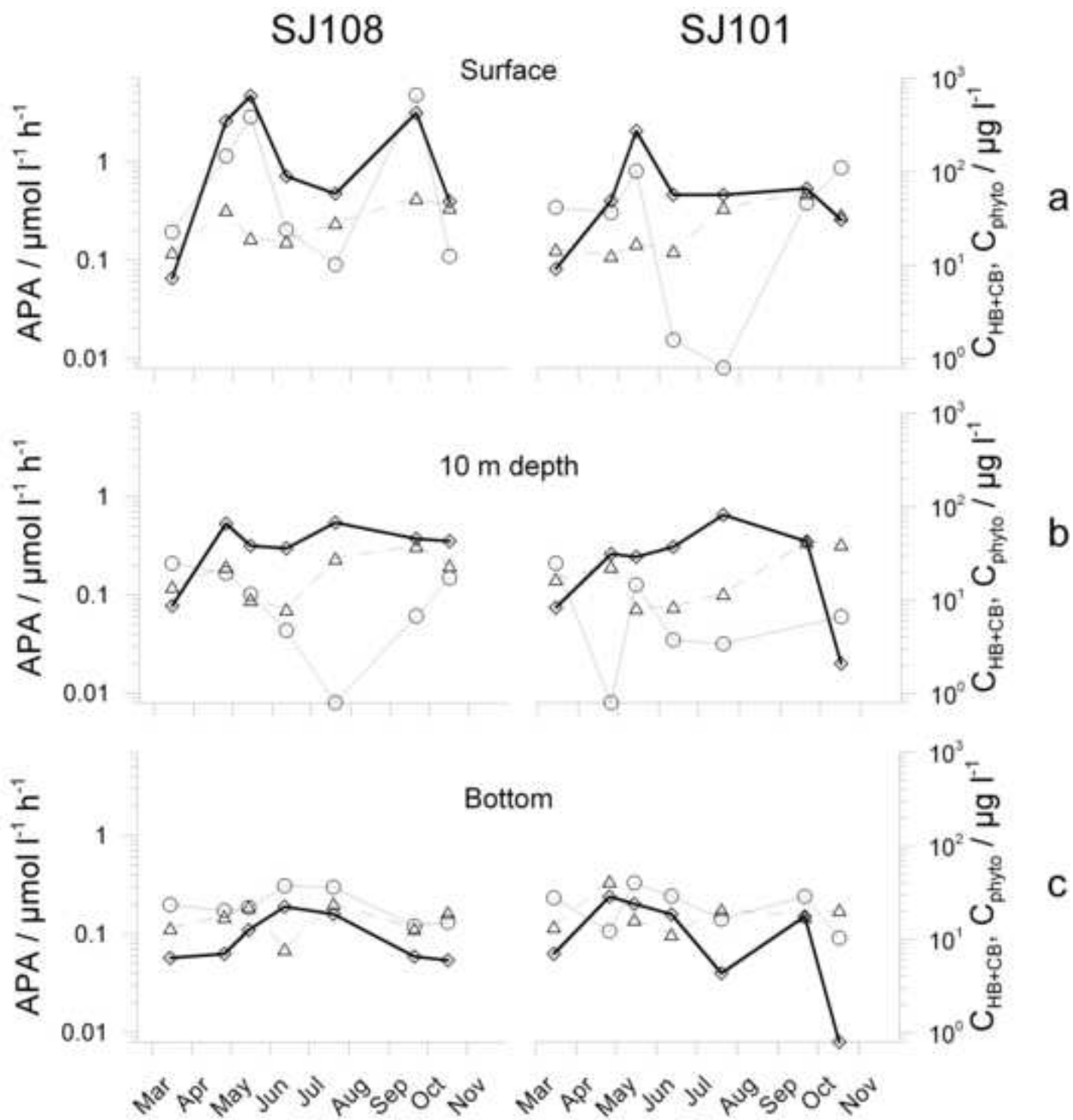


Fig. 4

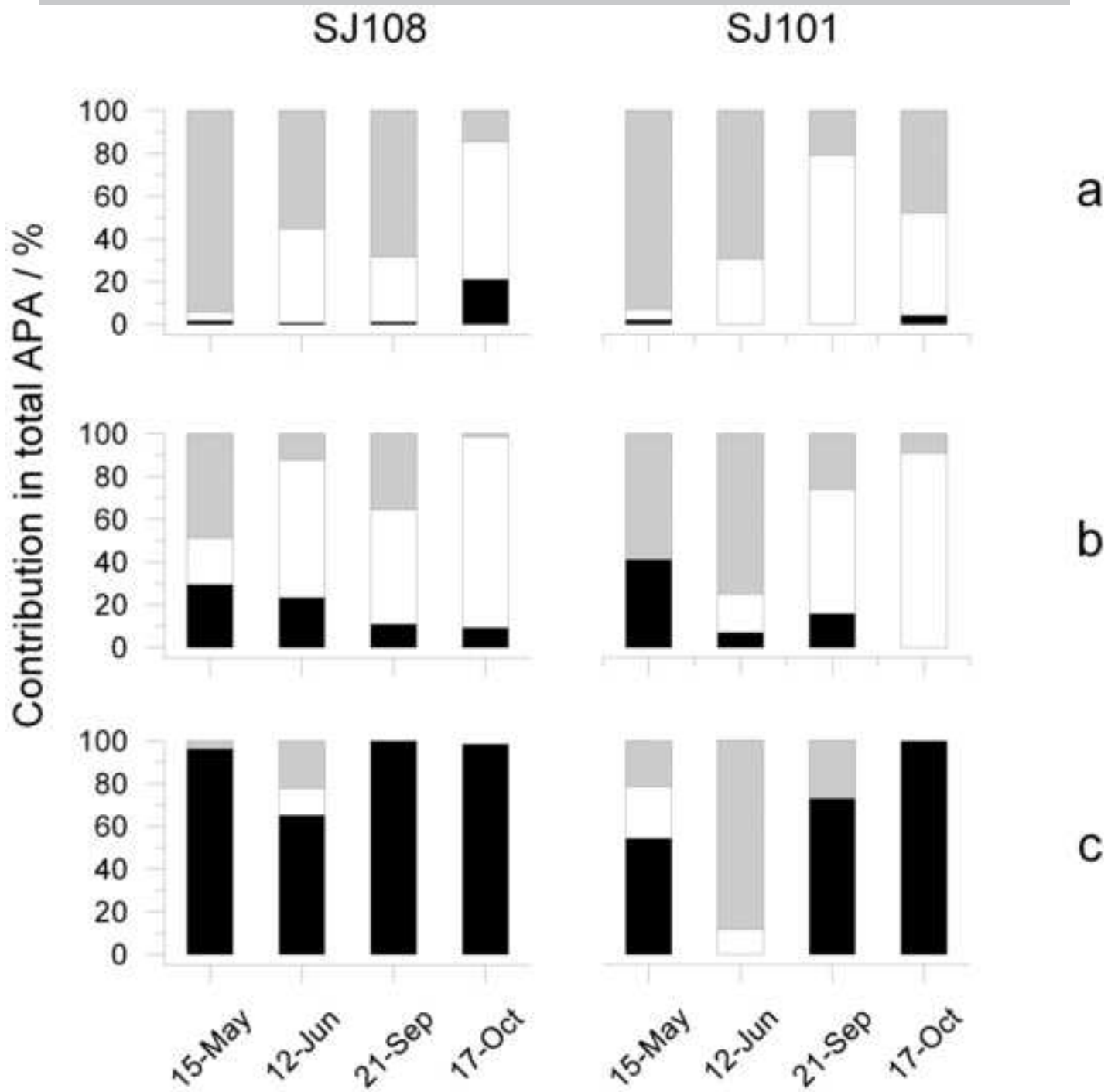


Fig.5

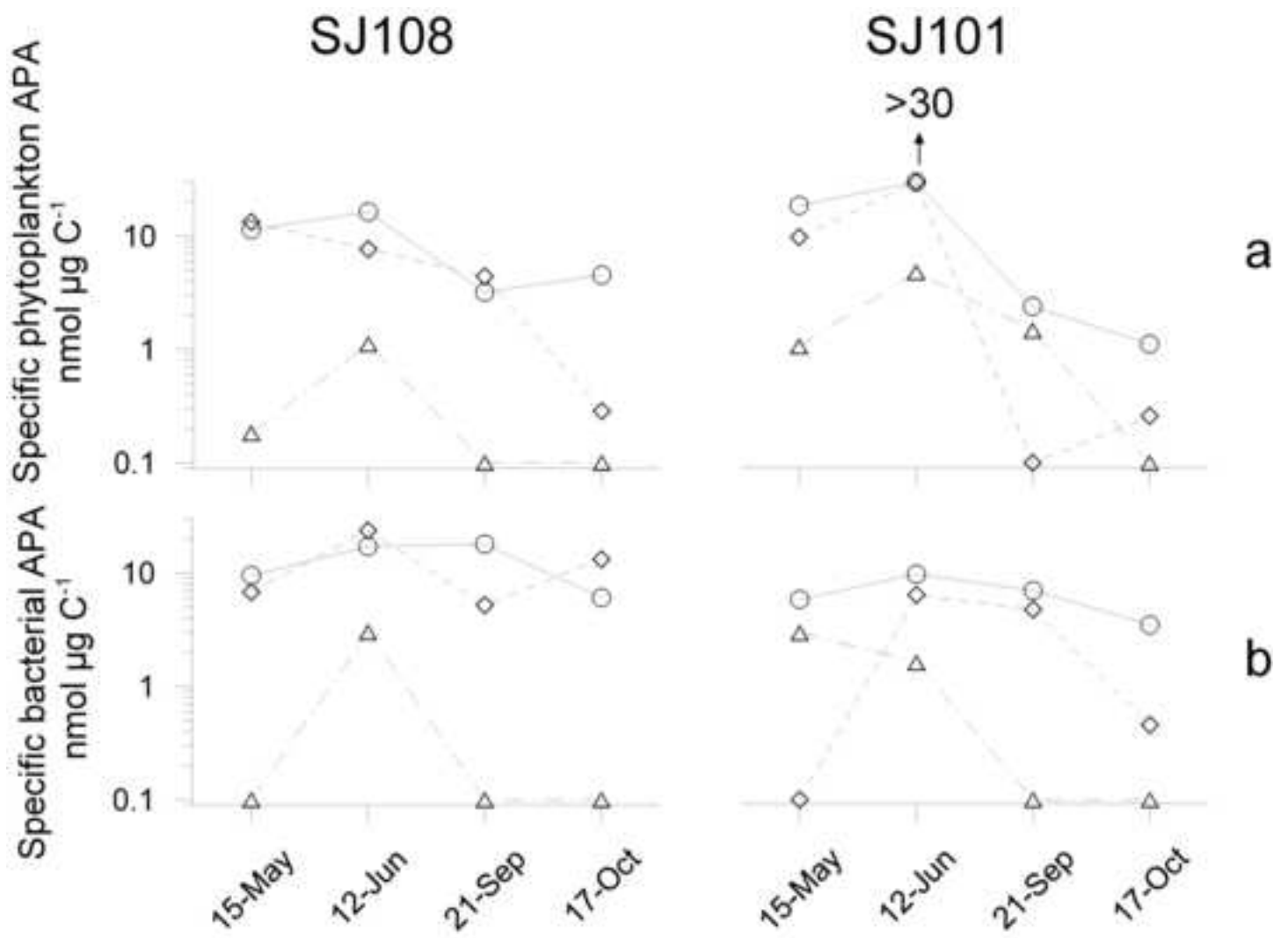


Fig. 6

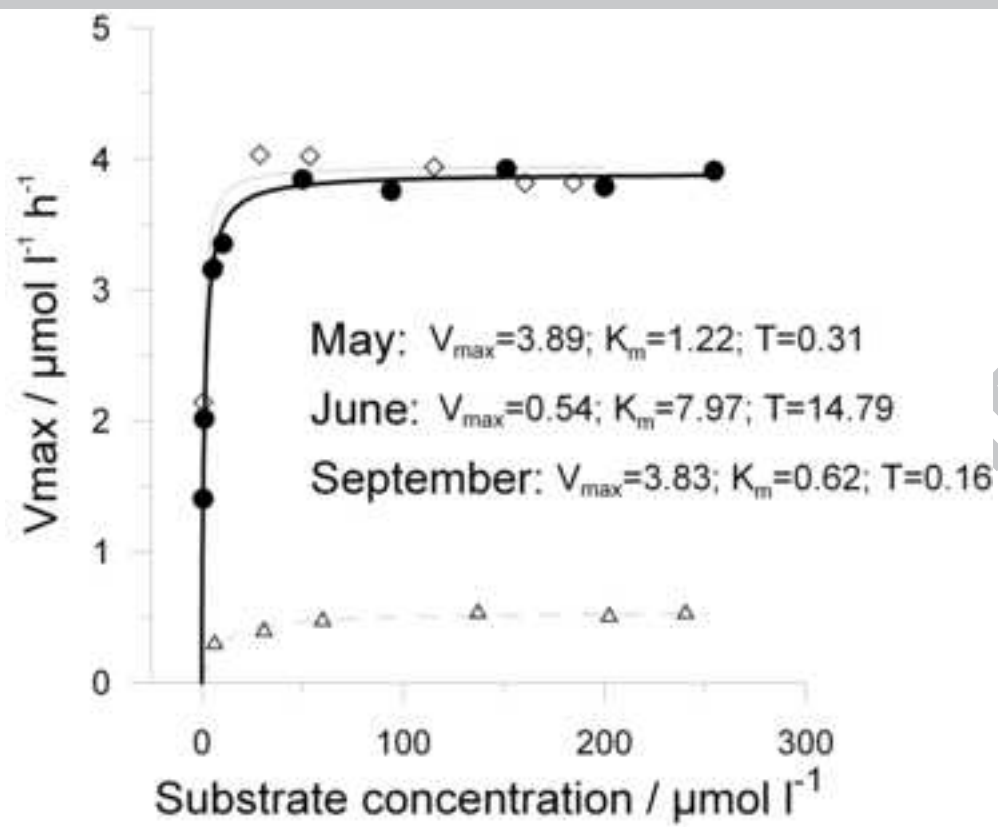


Fig.7