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Survival mechanisms of phytoplankton in conditions of stratification-induced deprivation of orthophosphate: Northern Adriatic case study

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Abstract

Phytoplankton abundance in the northern Adriatic during the summer 2008 indicated that the system was highly productive, in spite of low orthophosphate (PO_4) concentrations. Mechanisms by which phytoplankton adapted to PO_4 deprivation during the summer stratification were studied. In upper, more productive waters, phytoplankton induced high alkaline phosphatase activity (APA) to obtain phosphorus (P) from the dissolved organic pool, and the P turnover time mediated by phytoplankton APA was very short (2 min to 1.5 h). High-affinity enzymatic activity combined with high hydrolysis rates enabled metabolic flexibility to the phytoplankton in this heterogeneous and fluctuating environment. Another possible mechanism of adaptation to the PO_4 deficit during the summer was a shift toward smaller cells. The smaller nanophytoplankton, supported by higher surface : volume ratios, were presumably able to produce more alkaline phosphatase, an exoenzyme bound to the cell surface. Progressive decrease of large cells and increase of smaller cells in the phytoplankton community during summer supported this hypothesis. In upper waters with low PO_4 concentrations, phytoplankton reduced their P demand by a preferential synthesis of non-phospholipids. In bottom waters, phytoplankton abundance was markedly lower than in upper waters and growth was probably light limited. In these deeper waters with higher PO_4 concentrations, phytoplankton cells did not use APA to obtain P and were able to synthesize more phospholipids. In deeper waters, growth of bigger cells was favored.

Growing evidence suggests that phosphorus (P) is the limiting nutrient in several coastal systems (Thingstad et al. 1993) and oligotrophic oceans (Cotner et al. 1997; Karl and Yanagi 1997). Many phytoplankton species react to this limitation by accessing organic P using the enzyme alkaline phosphatase (AP; Hoppe 2003). Therefore, the importance of alkaline phosphatase activity (APA) with regard to the mobilization, transformation, and turnover of organic compounds in marine environments has been investigated with growing attention during the last decade (Nausch 1998; Xu et al. 2008). Another possibility to overcome P limitation is the ability of phytoplankton to lower their physiological P demand by as much as 50% (Geider and La Roche 2002; Bertilsson et al. 2003; Krauk et al. 2006). In oligotrophic regions of the ocean where PO_4 is scarce, phytoplankton reduces their cellular P requirements by substituting phospholipids with non-P membrane lipids (Van Mooy et al. 2009).

The northern Adriatic Sea is a shallow (up to 50 m) coastal sea under an alternating influence of freshwater from the western coast, mainly from the Po River, and advection of central Adriatic water along the eastern coast. These two water bodies, characterized by different nutrient content, influence the biological cycle in the region (Socal et al. 2008). In late spring and summer, the formation of gyres reduces exchange with the southern part of the Adriatic and retention of riverine water in the northern Adriatic area (Hopkins et al. 1999; Russo et al. 2005). At this time, the water column is stratified due to intensive heating of the sea surface and significant quantities of retained freshwater. In this period with optimal light and temperature for phytoplankton growth, nutrients in upper waters are consumed by intensive assimilation. Nutrients regenerated in deeper waters cannot reach upper waters due to the establishment of strong seasonal thermo-haline stratification. The main sources of nutrients in the upper productive waters are recycling within these waters and freshwater nutrient input (Gilmartin et al. 1990). However, although in the Po River waters both nitrogen (N) and P concentrations (total P 4.8 μmol L^{-1} and total N 263 μmol L^{-1}; Cozzi and Giani 2011), are more than one order of magnitude higher than in the Adriatic waters, the inorganic N : P atomic ratio (about 84 : 1; Cozzi and Giani 2011) provides a strongly unbalanced N vs. P supply for phytoplankton requirements (balanced N : P = 16 : 1; Redfield et al. 1963).

The objectives of this investigation are to evaluate the mechanisms by which phytoplankton in the northern Adriatic adapts to PO_4 deprivation induced by summer stratification and unbalanced N vs. P riverine supply. In this area where inorganic N supply greatly exceeds inorganic P supply, productivity in upper waters depends on the ability of phytoplankton to overcome P limitation. Previous studies have shown that in the investigated area AP was important for providing P for phytoplankton growth in upper waters during the stratification period (Ivančić et al. 2010). Consequently, the biological cycling of P controls its availability in the system, and could actively influence phytoplankton biomass and production, as well as changes in phytoplankton community compositions. For

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this purpose the P recycling within the phytoplankton fraction was studied and compared with changes in the phytoplankton composition. Furthermore, production of non-P lipids, as an alternative and/or additional mechanism to overcome P shortage, was considered.

Methods

Sampling strategy—Measurements were carried out at two stations (SJ101, SJ107) in the northern Adriatic (Fig. 1) during five cruises performed from June to October 2008, encompassing periods of stratification (June–August), as well as the onset of mixing in the water column (October). These stations were specifically chosen as they are in areas with different nutrient regimes. SJ101 (bottom depth 32 m), situated in the western area, is permanently under riverine nutrient pressure. SJ107 (bottom depth 37 m) is situated in the eastern area, which only intermittently experiences freshwater influence. Further, SJ101 is usually under more direct freshwater nutrient influence, whereas freshwater spreading toward SJ107 is already impoverished with nutrients until it reaches the station. During all cruises, sea temperature and salinity were determined with an SBE 25 conductivity–temperature–density probe (Sea-Bird Electronics), while water samples for the analysis of nutrients, APA, lipids, and phytoplankton were collected with 5-liter polyvinylchloride Niskin bottles at three depths (surface, 10 m, and 2 m over the bottom). Data for the daily Po flow mean measured at Pontelagoscuro, Italy, were kindly supplied by Assessorato Programmazione, Pianificazione e Ambiente of the Emilia Romagna region (Italy).

Analytical protocol—Inorganic nutrient analyses were performed onboard, immediately after sample collection, using methods described earlier (Strickland and Parsons 1972; Ivančić and Degobbis 1984). Samples for total dissolved P were filtered (Whatman GF/C, pre-combusted at 500°C) and stored in polyethylene tubes at −30°C. In the laboratory ashore, analyses were performed using a chemical combustion method with persulfate (Menzel and Corwin 1965). Dissolved organic P (DOP) was calculated by subtracting PO_4 from the total dissolved P. Dissolved inorganic N (DIN) was calculated as the sum of nitrate, nitrite, and ammonia.

Determination of APA was performed aboard the research vessel immediately after sample collection. Seawater was first filtered through a 200-μm mesh to remove mesozooplankton. Pre-filtrated water was then filtrated through 0.2-, 3-, or 20-μm filters. All filtrations were carried out gently and manually by Millipore filter units directly from samplers. Determined activity in the 0.2-μm filtrate was very low and in upper waters (surface and 10-m depth) often below the detection limit, thus indicating that gentle filtration did not cause rupture of cells. Filters of 3 μm were preferred (passing through both bacteria and picocyanobacteria) instead of 2 μm at which an unknown part of picocyanobacteria is retained. The nanophytoplankton fraction was retained on the 3-μm filter as confirmed by microscopic measurements, whereas the microphytoplankton fraction was retained on the 20-μm filter. The difference of activity between the 200-μm and 20-μm filtrate was assigned to microphytoplankton, whereas difference between the 20-μm and 3-μm filtrate was assigned to nanophytoplankton.

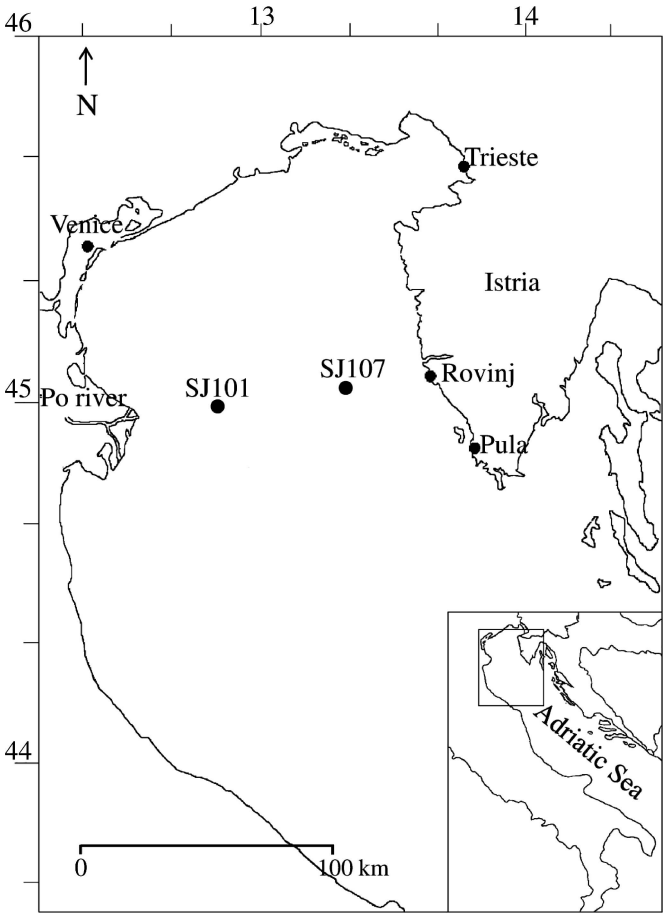

Fig. 1. Research area and sampling stations in the northern Adriatic Sea.

All APA measurements were performed with the fluorogenic substrate analogue using methyllumbelliferyl-phosphate (MUF-P) dissolved in methylcellosolve and diluted with water immediately before addition, following the procedure of Hoppe (1983). Aliquots of 5 mL of all the filtrates, in duplicate, were used for APA measurements, and the final concentration of substrate in the samples was 50 μmol L^{-1} (Ivančić et al. 2010). Additionally, at the surface, single measurements were performed using various MUF-P concentrations: from 0.5 μmol L^{-1} to the saturation concentration usually reported in the literature (250 μmol L^{-1}). Incubation was performed in the dark at the in situ temperature and pH. Fluorescence was measured immediately after substrate addition and after ~ 1 h of incubation using a Turner Designs-700 fluorometer with excitation at 365 nm and emission at 460 nm. APA (μmol L^{-1} h^{-1}) was calculated as the difference between those measurements divided by the incubation time after calibration of the fluorometer with methyllumbelliferone. Results are presented as the mean value of duplicates (mean coefficient of variation = 7.4%). Kinetic parameters

(half-saturation constant, K_m, and maximum activity, V_{max}) were calculated using the Lineweaver–Burk linearization. P turnover time (T) was estimated by the $K_m : V_{max}$ ratio (Labry et al. 2005).

For lipid class determination, 3 liters of seawater were passed through a 200-μm stainless steel screen to remove microzooplankton and larger particles. Immediately after sampling, seawater was filtered through Whatman GF/F filters pre-combusted at 450°C for 5 h. Filters were stored in liquid N until particulate lipid extraction. Filtrates containing dissolved lipids were stored in dark bottles until extraction by liquid–liquid extraction with dichloromethane (twice at pH 8 and twice at pH 2) that was performed within 24 h. Particulate lipids were extracted by a modified one-phase solvent mixture of dichloromethane–methanol–water procedure (Blight and Dyer 1959). Ten micrograms of internal standard *n*-hexadecanone were added to each sample before the extraction for the estimation of lipid recovery. Extracts were concentrated by rotary evaporation under a nitrogen atmosphere and stored at −20°C until measurements.

Lipid classes were separated on Chromarods SIII and quantified with an external calibration using a mixture of standard lipids by a thin-layer chromatograph–flame ionization detector Iatroscan Mark-VI (Iatron), using a hydrogen flow of 160 mL min^{-1} and an airflow of 2000 mL min^{-1} (Penezić et al. 2010). For the analysis, 2-μL aliquots of seawater extract in 20–100 μL solution of dichloromethane were spotted on Chromarods with a semiautomatic sample spotter.

Samples for the determination of phytoplankton composition and relative abundance were filtered through a 200-μm mesh to remove zooplankton, and filtrates were preserved with Lugol solution (2% final concentration) buffered with sodium acetate. After 38-h sedimentation of 50 mL of filtrate, cell counts were performed on an inverted Axiovert 200 microscope (Zeiss GmbH) following the method of Utermöhl (1958). During counting, phytoplankton cells were attributed to microplankton or nanoplankton fractions, based on observed cell dimensions (Sieburth et al. 1978) and counted at 200× and 400× magnification, respectively. Phytoplankton cells were identified at the lowest possible taxonomic rank.

Canonical correspondence analysis (CCA), a multivariate method elucidating the relationships between biological assemblages of species and their environment, was used to provide insight into the structure of microphytoplankton communities. CCA was conducted using canonical community ordination (CANOCO) program version 4.5 (Braak and Šmilauer 2002) by using two matrices, containing physicochemical variables and abundance data of 31 microphytoplankton species (> 2% relative abundance). Species abundances were log transformed before analysis to obtain normal distribution. Resulting species points on the ordination diagram were used as a basis for grouping the microphytoplankton community by assemblages (Muylaert et al. 2006).

Specific APA in phytoplankton fractions was calculated as the ratio between APA and carbon content in the respective fraction. Carbon content of phytoplankton species was obtained from cell bio-volume using the conversion formulae proposed by Menden-Deuer and Lessard (2000). Micro- and nanophytoplankton cell sizes were measured on micrographs using AxioVision software version 4.8.1.0 (Zeiss). For each taxon at least 33 different specimens were measured, and averaged values of cell dimensions were used in the calculation. Bio-volume approximation was made from calculations of species cell shapes from those proposed by Sun and Liu (2003).

Results

Hydrological conditions and nutrient status—During the second part of May and in June the Po River flow was high (about 3000–6000 m^3 s^{-1}) with maximal impulses at the beginning of June (Fig. 2A). At the end of June the Po flow decreased to typical summer values and remained constant until October (700–1200 m^3 s^{-1}), with somewhat higher impulses in the middle of July, August, and September (up to 2300 m^3 s^{-1}).

In the June–August period, temperatures at the surface were high (22.3–30.5°C) and decreased toward the bottom (10.6–14.8°C) with the establishment of thermal stratification at both stations investigated (Fig. 2B). During October, cooling of the surface (18.4–18.9°C) allowed mixing in the water column, and a nearly homogenous layer extended down to 20 and 30 m at SJ101 and SJ107, respectively (Fig. 2B). At SJ101, freshwater influence was considerable during the whole summer, as well as in October (surface salinity 27.07–36.52) extending down to 10–15 m (Fig. 2C). At SJ107, freshwater influence was detected at the beginning of June and in July (surface salinity 34.99–35.94), extending down to 5–15 m, while in other summer months and in October it was low (surface salinity 37.17–37.76). In deeper layers of both stations, the contribution of riverine water was low, and more saline water was present during the whole investigation period (salinity 38.05–38.21).

PO_4 concentrations in upper waters were always low (0.00–0.06 μmol L^{-1}) at both stations, even during the marked freshet at the end of June (Fig. 3A). An accumulation of PO_4 was observed only in bottom waters with maximal values in October (up to 0.32 μmol L^{-1}). During the summer months, DOP concentrations in upper waters were low (0.09–0.29 μmol L^{-1}; Fig. 3B) although always exceeding those of PO_4 (Fig. 3A), whereas markedly higher DOP concentrations (up to 0.71 μmol L^{-1}) were found in October. In bottom waters, PO_4 concentrations usually exceeded DOP concentrations (Fig. 3A,B).

In surface waters, DIN concentrations (Fig. 3C) were high during June and October at SJ101 (6.28–23.85 μmol L^{-1}), and only in June at SJ107 (1.04–2.97 μmol L^{-1}). In these waters, nitrate (NO_3) strongly predominated (up to 94%) among the inorganic N species, indicating the input of new freshwater. In July, and at SJ107 also in August, DIN concentrations in less saline waters were low (0.19–0.43 μmol L^{-1}), indicating "old" freshwater in which nutrients were consumed by assimilation. Concomitant to a small Po flow pulse in August, DIN concentration at SJ101 was two times higher than in July (Fig. 3C). Usually, in bottom waters accumulation of DIN occurred with maximal values in October at both stations (3.37–6.80 μmol L^{-1}).

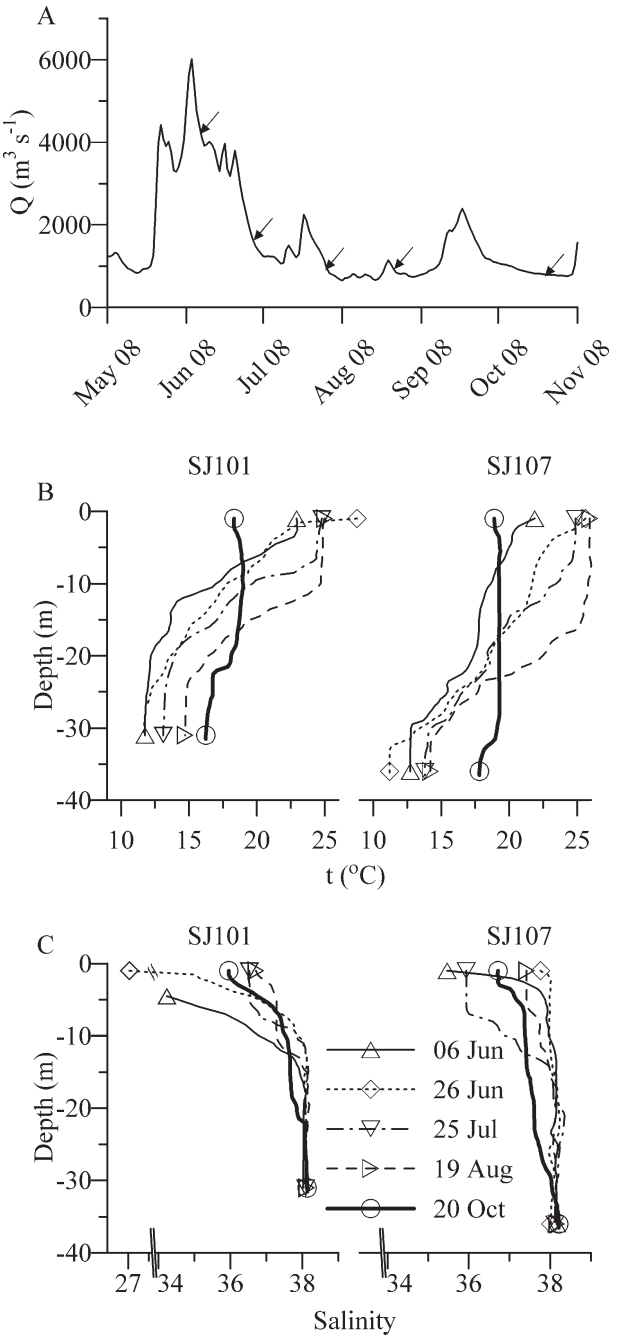

Fig. 2. (A) Daily mean of the Po River discharge rate (Q) with cruise dates denoted by arrows. (B) Temperature (t) and (C) salinity (S) profiles at SJ101 and SJ107 during the year 2008.

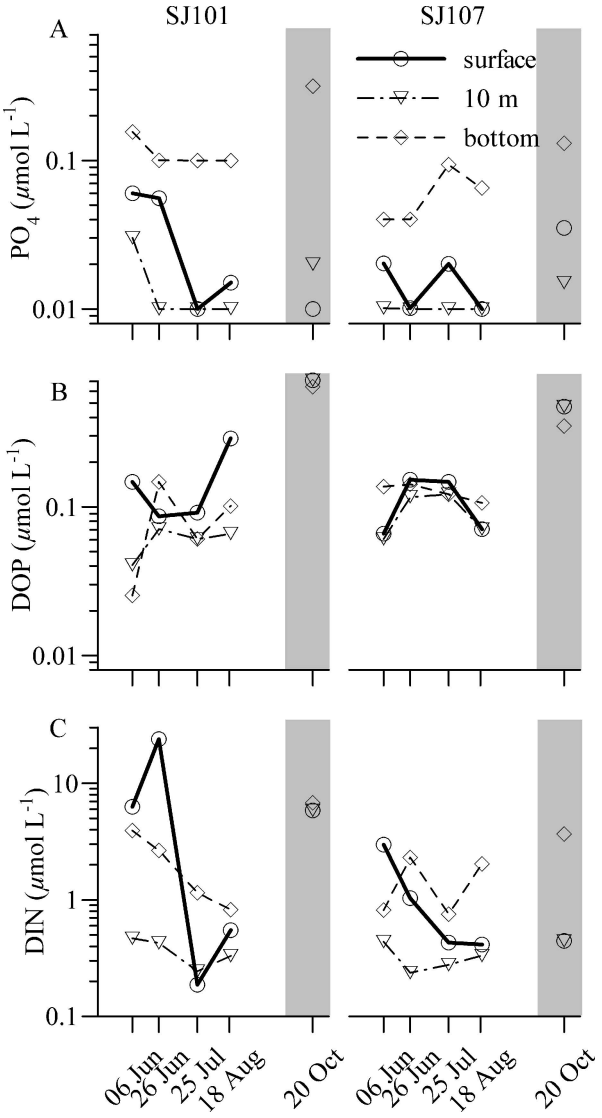

Fig. 3. Changes of (A) orthophosphate (PO_4), (B) dissolved organic phosphorus (DOP), and (C) dissolved inorganic nitrogen (DIN) at SJ101 and SJ107 during the year 2008.

At the surface and 10-m depth, DIN was present in marked surplus with respect to PO_4 (median N : P ~ 72 and 30, for the respective depths on both stations). In bottom waters, DIN and PO_4 were generally more balanced for phytoplankton requirements (median N : P ~ 21) during the entire investigation period.

Phytoplankton abundance and community composition—At the surface phytoplankton abundance was in the range of ~ 10^5–10^6 cells L^{-1} (Fig. 4A). The highest abundance at the surface of SJ101 was found during the freshet at the end of June and in August (3.6 and 1.7 × 10^6 cells L^{-1}, respectively), whereas minimal abundance was found in July (3.8 × 10^5 cells L^{-1}). At the surface of SJ107, the abundance was generally lower than at SJ101. The highest abundance at SJ107 was found in July (7.7 × 10^5 cells L^{-1}) and the lowest at the end of June (2.9 × 10^5 cells L^{-1}). In intermediate waters (10-m depth) of both stations, phytoplankton abundance (8.8 × 10^4 to 5.5 × 10^5 cells L^{-1}) was lower than at the surface (Fig. 4A). Only in July abundance in intermediate waters approached those at the surface. In contrast to the summer situation, in October phytoplankton abundance in intermediate waters (9.3 × 10^5 to 1.0 × 10^6 cells L^{-1}) approached values from the surface at both stations. October values in the upper 10 m were markedly higher than during the summer at SJ107 and in range with the highest summer values at SJ101. At the bottom,

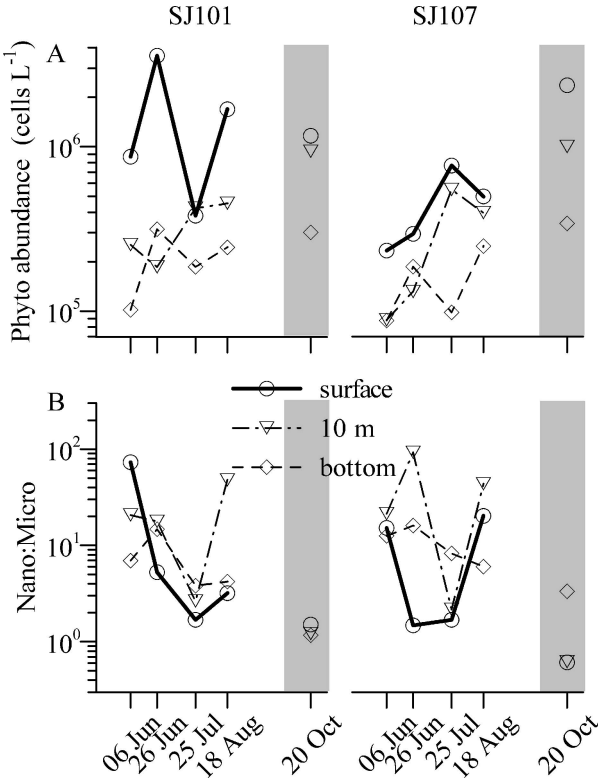

Fig. 4. Changes of (A) total phytoplankton abundance (Phyto abundance) and (B) nano : micro phytoplankton ratio (Nano:Micro) during the year 2008.

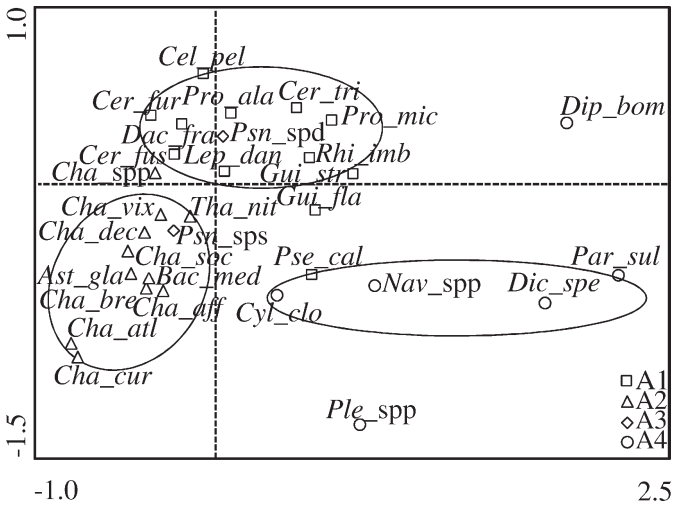

Fig. 5. Ordination diagram of microphytoplankton assemblages at SJ101 and SJ107, the first (horizontal) and second (vertical) ordination axes are presented (dashed lines). Species abbreviations: *Asterionellopsis glacialis*, *Ast_gla*; *Bacteriastrum mediterraneum*, *Bac_med*; *Cerataulina pelagica*, *Cer_pel*; *Ceratium furca*, *Cer_fur*; *C. fusus*, *Cer_fus*; *C. tripos*, *Cer_tri*; *Chaetoceros* spp., *Cha*_spp.; *C. affinis*, *Cha_aff*; *C. atlanticus*, *Cha_atl*; *C. brevis*, *Cha_bre*; *C. curvisetus*, *Cha_cur*; *C. decipiens*, *Cha_dec*; *C. socialis*, *Cha_soc*; *C. vixvisibilis*, *Cha_vix*; *Cylindrotheca closterium*, *Cyl_clo*; *Dactyliosolen fragilissimus*, *Dac_fra*; *Dictyocha speculum*, *Dic_spe*; *Diploneis bombus*, *Dip_bom*; *Guinardia flaccida*, *Gui_fla*; *G. striata*, *Gui_str*; *Leptocylindrus danicus*, *Lep_dan*; *Navicula* spp., *Nav*_spp.; *Paralia sulcata*, *Par_sul*; *Pleurosigma* spp., *Ple*_spp.; *Proboscia alata*, *Pro_ala*; *Prorocentrum micans*, *Pro_mic*; *Pseudosolenia calcar-avis*, *Pse_cal*; *Pseudo-nitzschia delicatissima* complex, *Psn*_spd; *P. seriata* complex, *Psn*_sps; *Rhizosolenia imbricata*, *Rhi_imb*; *Thalassionema nitzschioides*, *Tha_nit*.

phytoplankton abundance (8.7×10^4 to 3.4×10^5 cells L^{-1}) was lower than in upper waters (Fig. 4A).

Nanophytoplankton was more abundant than microphytoplankton, with a higher nano : micro ratio during the summer than in October when in upper waters at SJ107 microphytoplankton dominated (Fig. 4B). During the summer, the highest nano : micro ratio at the surface was found during the freshet at the beginning of June at SJ101 (73.2; Fig. 4B). Generally, in surface waters the nano : micro ratio was higher during the freshwater input (3.2–10.2) than when freshwater input was low (1.5–1.7; Fig. 4B). The only exception was a high surface ratio (20.2) found in August at SJ107 in conditions of low freshwater influence. In intermediate and bottom waters, the nano : micro ratio was generally higher (2.2–21.3; Fig. 4B). Exceptionally high values were found in intermediate waters in August at both stations, and at SJ107 also at the end of June (up to 92.7). In these waters, values had generally the same trend as at the surface.

In the microphytoplankton fraction, diatoms and dinoflagellates were the two most diverse and abundant groups with 56 and 43 taxa, respectively. Thus, the phytoplankton community was characterized on the basis of their species composition (*see* Web Appendix, www.aslo.org/lo/toc/vol_57/issue_6/1721a.html). Figure 5 shows the first two axes of CCA ordination of the microphytoplankton community. Axes 1 and 2 had eigenvalues of 0.242 and 0.191, respectively, and together explained 50% of the species–environment relation. The ordination revealed the presence of three relatively distinct microphytoplankton communities. Assemblage 1 was characterized by large species, from the genus *Rhizosolenia* and related genera (*Dactyliosolen*, *Guinardia*, *Neocalyptrella*, *Proboscia*, and *Pseudosolenia*) as well as *Cerataulina pelagica* and dinoflagellate species. Assemblage 2 consisted of cells of various sizes, and was dominated by species from the genus *Chaetoceros* as well as *Asterionellopsis glacialis*, *Leptocylindrus danicus*, and *Thalassionema nitzschioides*. An abundant small-cell-size genus, *Pseudo-nitzschia*, was additionally separated as assemblage 3 due to its cryptic species diversity and high abundances. Assemblage 4 was characterized by different species, including those with bentho-pelagic lifestyle (*Cylindrotheca closterium*, *Diploneis* spp., *Navicula* spp., *Pleurosigma* spp.).

These four assemblages had varying contributions throughout the period investigated (Fig. 6). At the surface, assemblage 1 was completely dominating (almost 100%) the phytoplankton community at the beginning of June (Fig. 6A). It was succeeded by small-cell-sized assemblage 2 at SJ107 and even smaller sized assemblage 3 at SJ101 at the end of June. In July and August, assemblage 3 persisted on both stations. In the intermediate waters, microphytoplankton succession on both stations was similar as at the surface, but the assemblage 2 domination was absent at the

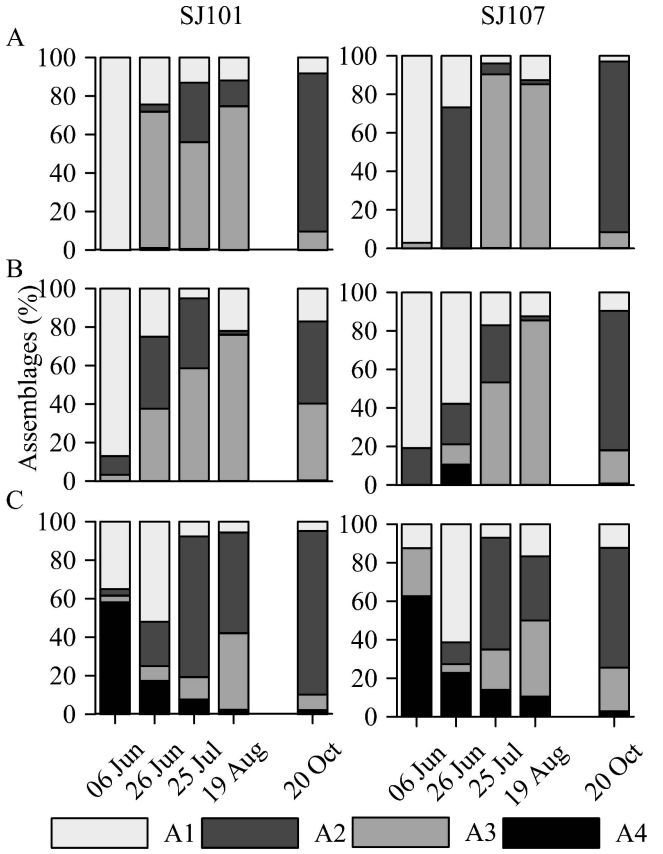

Fig. 6. Contribution of different assemblages in microphytoplankton community (Assemblages) at the (A) surface, (B) 10 m, and (C) bottom during the year 2008. For an accurate overview of the taxa belonging to the diverse assemblages *see* Results and Web Appendix.

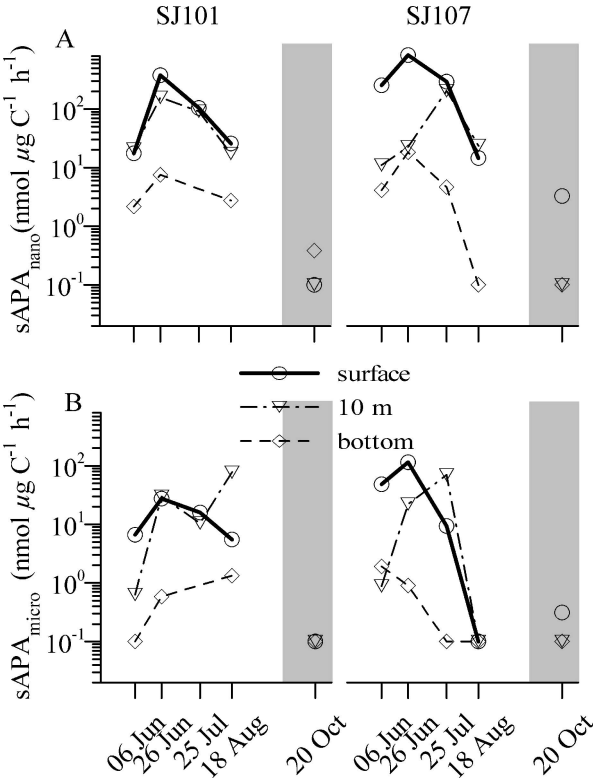

Fig. 7. Changes of specific APA (A) in nanophytoplankton fraction (sAPA$_{nano}$) and (B) in microphytoplankton fraction (sAPA$_{micro}$) during the year 2008.

end of June at SJ107 (Fig. 6B). The bottom layer was greatly differing during summer from the above layers, with more significant contributions of assemblage 4 (Fig. 6C). October, in contrast, was dominated by assemblage 2 throughout the whole water column.

Specific phytoplankton APA and P turnover time—During summer months, specific phytoplankton APA at the surface of both stations was high, except in August at SJ107 (Fig. 7A,B). Extraordinarily high values in both nano and micro fractions were measured at the end of June and in July (up to 838 and 115 nmol μg C^{-1} h^{-1}, respectively). Activity of the nano fraction was up to one order of magnitude higher than that of the micro fraction. In intermediate waters, specific phytoplankton APA had generally similar trend as at the surface, except low values in the micro fraction at the beginning of June at both stations (0.63–0.88 nmol μg C^{-1} h^{-1}). In October, specific APA in the nano- and microphytoplankton fractions in upper waters (undetectable to 3.29 and 0.31 nmol μg C^{-1} h^{-1}, respectively) was more than one order of magnitude lower than that during summer months (Fig. 7A,B).

During summer months, the half-saturation constant (K_m) at the surface ranged from 0.42–1.40 μmol, being generally similar for both stations (Table 1). P turnover time mediated by phytoplankton APA at the surface was short (< 1 h 30 min; Table 1), with exceptionally short values during the freshet at the end of June (only 2 min). Only at SJ107, P turnover time was long in August (about 16 h). Opposite, in October very long P turnover (99 h) time and higher K_m value (8.93 μmol) were found in the surface waters at SJ101. At SJ107, calculation of kinetic parameters in October was not possible since linearization was not statistically significant ($r = 0$, $n = 10$), contrary to all other ($r = 0.856-0.999$, $n = 10$, $p = 0.000$).

In bottom waters, specific phytoplankton APA in both the nano and the micro fractions was low during the entire period investigated (undetectable to 7.55 and 1.92 nmol μg C^{-1} h^{-1}, respectively; Fig. 7), except higher values in the nano fraction (18.27 nmol μg C^{-1} h^{-1}) at SJ107 in June.

Production of P and non-P lipids—Two classes of lipids were measured—phospholipids: phosphatidylglycerols and diphosphatidylglycerols (PG + DPG), and non-phospholipids: mono- and di-galactosyldiacylglycerols (MGDG + DGDG) (Table 2). Much lower ratio of PG + DPG : MGDG + DGDG was observed in the dissolved (0.16 to 0.75) than in the particulate fraction (0.43 and 1.51; Table 2). While the ratio in the dissolved fraction did not show significant variation with depth, in the particulate fraction the lowest values were always found at the surface and at 10-m depth, and higher values were characteristic for the bottom waters. The PG + DPG : MGDG + DGDG ratios showed significant positive relationships with inorganic P ($r = 0.71$, $n = 21$, $p = 0.00095$).

Table 1. Kinetic parameters for phytoplankton AP (V_{max} [μmol L^{-1} h^{-1}] and K_m [μmol]) and phosphorus turnover time (T, estimated from V_{max} and K_m in the phytoplankton fraction) at the surface of SJ101 and SJ107 during the year 2008. Dashes indicate not calculated (*see* explanation in the text).

	SJ101			SJ107		
Date	V_{max}	K_m	T	V_{max}	K_m	T
06 Jun	0.93	0.41	27 min	0.56	0.65	1 h 10 min
26 Jun	36.43	1.08	2 min	3.15	1.13	21 min
25 Jul	1.23	1.50	1 h 13 min	1.62	2.55	1 h 35 min
19 Aug	0.49	0.64	1 h 24 min	0.09	1.41	16 h
20 Oct	0.09	8.93	99 h	—	—	—

Table 2. Ratio of phospholipids (PG + DPG) and glycolipids (MGDG + DGDG) in the particulate (Part) and the dissolved (Diss) fraction at SJ101 and SJ107 during the year 2008.

		SJ101		SJ107	
Date	Depth	Part	Diss	Part	Diss
06 Jun	Surface	0.86	0.32	0.61	0.45
	10 m	1.11	0.21	1.01	0.31
	Bottom	1.51	0.40	1.08	0.63
19 Aug	Surface	0.81	0.33	0.43	0.31
	10 m	0.71	0.28	0.76	0.17
	Bottom	1.15	0.27	1.71	0.21
20 Oct	Surface	0.93	0.37	0.75	0.16
	10 m	1.02	0.43	1.08	0.75
	Bottom	1.01	0.44	1.11	0.56

Discussion

The phytoplankton abundance during the year 2008 was in the range of usual values for this area (Bernardi Aubry et al. 2006; Marić et al. in press). In the western area, summer phytoplankton abundances were one order of magnitude higher than summer values reported for oligotrophic regions of the Mediterranean (Totti et al. 2000; Aktan 2011). This relatively high productivity of the northwestern Adriatic was due to alimentation by nutrient-rich freshwater practically during the entire summer. In stratified conditions and during closed circulation, even small impulses were sufficient to increase phytoplankton abundance at the western area, as observed in August. At the eastern area, surface phytoplankton abundance was higher only after significant spreading of the Po plume, compared to abundances in the plume absence. This was most evident after the strong event of bora wind at the end of July, which pushed the Po plume over the entire northern Adriatic (I. Janeković pers. comm.). During the summer, the freshwater influence and its enriching effect on nutrients in the intermediate waters were markedly lower than at the surface, resulting in lower phytoplankton abundance. The only situation when phytoplankton abundance in intermediate waters approached those at surface was found at the end of July when the bora wind homogenized upper parts of the water column. In October, mixing in the water column enriched upper waters with nutrients regenerated in bottom waters during the previous months, although high concentrations were recorded only for DIN since the uptake of PO_4 was fast. Resuspension of bottom layer sediment represents an important source of nutrients for the water column in this period (Boldrin et al. 2009). The higher level of regenerated nutrients is a potential trigger for the autumn phytoplankton bloom in the northern Adriatic (Boldrin et al. 2009; Solidoro et al. 2009). Consequently, in October high phytoplankton abundance was observed at the surface and in intermediate waters, far exceeding summer abundances at the eastern area. In bottom waters phytoplankton abundance was lower than in upper waters, in spite of relatively rich nutrient conditions and probably constricted by light limitation.

Although the two areas had a different productivity, both exhibited P limitation, and mechanisms by which phytoplankton overcame this P deprivation were similar in both areas.

Adaptation by organic P utilization—The high specific phytoplankton APA indicated that phytoplankton during the summer in the surface and intermediate waters used DOP as a source of P. At the surface, the P turnover time mediated by phytoplankton APA was very short in the entire area investigated (2 min to 1 h and 35 min) and characteristic for P-limiting conditions. P turnover time calculated from APA is used to compare the rate of P recycling in different situations since it agrees with ^{33}P turnover times, and P limitation of a system is characterized by a value shorter than about 5 h (Nausch et al. 2004; Xu et al. 2008). During the summer in the surface waters of the northern Adriatic, a combination of high V_{max} (0.5–36.4 μmol L^{-1} h^{-1}) and low K_m (0.41–2.55 μmol L^{-1}) was estimated. The summer values in the adjacent area of the Gulf of Trieste were up to 70 μmol L^{-1} for K_m and up to 0.55 μmol L^{-1} h^{-1} for V_{max} (Istituto Nazionale di Oceanografia e di Geofisica Sperimentale unpubl. data). This high-affinity enzymatic activity with high hydrolysis rates, according to Azam and Ammerman (1984) provides metabolic flexibility to the phytoplankton in the heterogeneous and fluctuating environment of the northern Adriatic. The similar kinetic behavior at the more productive western and the less productive eastern area implies similar fluctuations in substrate availability in both areas. Considering the relatively high phytoplankton biomass at the western area during the summer, a lot of organic matter was produced. Due to the closed circulation, produced organic matter was retained in the area and further extended to the east. Consistently low surface DOP concentrations in waters of both areas were due to its fast recycling. In fact, in October high concentrations of DOP remained in waters of both areas. Although in October PO_4 concentrations at the surface dropped to undetectable levels and high DIN concentrations could support further phytoplankton growth, phytoplankton did not use DOP to obtain P. It seems that during this month phytoplankton was not P limited due to the constant supply of PO_4 from the bottom. Throughout the period investigated it was evident that in bottom waters with more balanced N : P ratios for phytoplankton requirements, AP was

probably not important in P supply for the phytoplankton community.

Adaptation by size reduction—A predomination of the nano fraction was more evident during freshwater inputs that created nutrient-rich conditions (nano : micro ratio 3–70), which is contrary to an expected dominance of larger cells (Macias et al. 2010). The unbalanced nutrient freshwater supply induced the PO_4 deficit. The nanophytoplankton exhibit markedly higher APA than microphytoplankton, suggesting higher production of AP. This was supported by higher surface : volume ratios, since AP is an exoenzyme bound to the cell surface; consequently, nanophytoplankton was more successful in overcoming the P limitation. In a strict sense, particle size may not necessarily correspond exactly to the organism's size, e.g., particle-associated bacteria. However, it is expected that different size fractions would show different responses to the change in P availability because various physiological processes and functions in planktonic communities are size dependent (Wen et al. 1997; Timmermans et al. 2004). When the freshwater input was low, the nano : micro ratio was much lower (about 1.5), since microphytoplankton have acquired an array of adaptive strategies that compensate for the competitive disadvantages arising from their larger cell size. These include variability in intracellular quotas and cell shape (Thingstad et al. 2005), cell motility (Kiørboe 1993), nutrient storage (Raven 1998), and tradeoffs between pigment concentration and light absorption (Agustí 1991). Only the high nano : micro ratio found in August at the surface of the eastern area not alimented with freshwater nutrients for a long period could reflect oligotrophic conditions. Low specific phytoplankton APA and long P turnover (16 h) in that period suggested that the community was not only P limited. In this situation, free enzymes could also contribute to P supply to the community since their higher contribution in total activity (26.5%, data not shown) was found during the investigation period (usually $< 5\%$, data not shown).

In more oligotrophic intermediate waters, the nano : micro ratio was higher than at the surface. However, the community was P limited to a similar extent as at the surface and predomination of small cells was probably primarily due to the P deficit. In fact, in October when nutrients were supplied from the bottom waters with a more equilibrated N : P ratio for phytoplankton growth, microphytoplankton abundances approached those of nanophytoplankton.

The PO_4 deficit during the summer could also contribute to changes in the microphytoplankton community, next to the seasonal succession of species. At the beginning of June, the microphytoplankton community in upper waters was dominated by assemblage 1, consisting of large diatoms and dinoflagellates. At the end of this month, the community was dominated by small cells of the genus *Chaetoceros* (at the eastern area) and by similarly sized species from the genus *Pseudo-nitzschia* (at the western area). Since noteworthy DIN concentrations remained in the water, the observed shift to smaller cells was probably related to an increased need of alkaline phosphatase. In fact, highly stimulated enzyme production was observed, indicating strongly P-limited conditions as suggested by Nausch (1998). After June in both areas the microphytoplankton was dominated by small species of assemblage 3, composed of *Pseudo-nitzschia*. A progressive decrease in the contribution of large cells and an increase of smaller sized diatoms was observed, probably due to a restricted nutrient supply.

In October in surface and intermediate waters of both areas, a shift toward bigger cells and a domination of assemblage 2 was observed. This assemblage in October consisted of species of various sizes, generally larger than those of summer assemblage 2 (consisting of smaller sized *Chaetoceros*) and even larger than those of summer assemblage 3. This shift toward bigger cells most likely occurred due to a more favorable PO_4 supply.

In bottom waters, the nano : micro ratio was similar to that in intermediate waters. Reduced oxygen saturation (60–90%; Centre for Marine Research unpubl. data) indicated that regeneration prevailed over assimilation, and probably the major part of phytoplankton in these waters sank from the upper waters and maintained similar compositions. In fact, the contribution of assemblage 4 with bentho-pelagic lifestyle in the microphytoplankton community was generally low, except for the beginning of June. The same species as in upper waters were present, although with different contributions. The markedly decreased contribution of small cells and increased contribution of bigger ones indicated that in conditions of relatively high DIN and PO_4 concentrations and their more balanced ratio for phytoplankton growth, growth of bigger cells was favored.

Adaptation by synthesis of non-phospholipids—Phospholipids and glycolipids (non-phospholipids) are two dominant lipid classes that are found in phytoplankton. Phosphatidylglycerols and diphosphatidylglycerols (phospholipids) are phytoplankton membrane lipids (and heterotrophic bacteria lipids, to a much lesser extent) while mono- and di-galactosyldiacylglycerols (non-phospholipids) are only phytoplankton photosynthetic membrane lipids (Guschina and Harwood 2009). The ratio between those two lipid classes allows conclusion whether phytoplankton preferentially synthesize non- or phospholipids. In fact, in seas where PO_4 is scarce, phytoplankton reduces its cellular P requirements by substituting P membrane lipids with non-phospholipids (Van Mooy et al. 2009). Our results showed that during the summer a preferential synthesis of non-phospholipids (low phospho : non-phospho lipid ratio; 0.4–0.7) was observed in surface and intermediate waters with low PO_4 concentrations and high APA. Probably APA provided P for sustaining the rate of primary production, whereas synthesis of non-phospholipids served to increase phytoplankton biomass. In this biomass, cyanobacteria, which can also produce non-phospholipids, contributed on average 10–20% (Centre for Marine Research unpubl. data); therefore, probably most of the non-phospholipids were synthesized by nano- and microphytoplankton. It was observed that in the Adriatic Sea synthesis and accumulation of non-P

glycolipids are enhanced during PO_4 exhaustion (Frka et al. 2011). The preferential synthesis of non-phospholipids did not stop in October, indicating that the system was still P stressed. Only during the following winter months a preferential synthesis of phospholipids was observed (lipid ratio about 1.5; Division for Marine and Environmental Research unpubl. data). However, the ratio was still low when compared to those in systems with markedly higher PO_4 concentrations (about 3; Penezić et al. 2010). This suggests that the northern Adriatic is under constant P stress, although with maximal extent during the summer. As the northern Adriatic is a system of low PO_4 content and high N : P ratio, phospholipids were much more efficiently and/or preferentially recycled than non-phospholipids, and consequently the lipid ratio in the dissolved fraction was very low (on average 0.37), implying an effective activity of alkaline phosphatase on phospholipids. Furthermore, in bottom waters with higher PO_4 concentrations, phytoplankton was able to synthesize more phospholipids and the lipid ratio was markedly higher than in upper waters and similar to winter values.

AP production by phytoplankton and its characteristics combined with the lipid composition proved to be good descriptors of adaptive mechanisms of the phytoplankton communities to PO_4 deficit. The metabolic flexibility of the phytoplankton is enabled by high-affinity enzymatic activity with high hydrolysis rates, which allows phytoplankton to promptly react to fluctuating availability of substrate. This could occur in many other heterogeneous environments with fluctuating availability of DOP. A major selective pressure on the phytoplankton species during the P limitation could be related to AP production, rather than to the availability of nutrients. The synergy between production of AP and synthesis of non-phospholipids enables phytoplankton to sustain the rate of primary production (induction of AP) and to increase their biomass (non-phospholipids synthesis) when other nutrients are available. To discern the triggers of individual strategies, further laboratory and field studies are needed. Recent studies in the Sargasso Sea also suggest that there may be further important species-specific responses to P stress embedded within a bulk phytoplankton community response (Lomas at al. 2004). The answer of biological communities to various perturbations is a key to understanding the ecosystem's response to future changes. In this context, conclusions from this study have to be taken into account when climatic (inducing changes in vertical mixing) or anthropogenic (inducing changes in nutrient loadings) effects on phytoplankton biodiversity and ecosystem functioning are studied.

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