original scientific paper

UDC 593.1:591.13(262.3-16)

NUTRIENT-ENRICHMENT EFFECT ON PLANKTON COMPOSITION

Patricija MOZETIČ, Valentina TURK, Alenka MALEJ
Marine Biological Station Piran, SI-6330 Piran, Fornače 41

ABSTRACT

Three enrichment experiments were conducted in order to analyze the development of plankton biomass and species composition as a response to different nutrient regimes. The additions of all nutrients had the most profound effect on phytoplankton biomass increase, whereas picoplankton abundance decreased continuously in all treatments. Initially dominating phytoplanktonic groups and species expanded significantly towards the end of experiments. Inputs of new nutrients not only stimulate phytoplankton growth in general but also affect the community structure. In such conditions the development of community structure seems to be controlled by the fast-growing opportunistic seasonally dominating species (mainly Skeletonema costatum *and* Chaetoceros *spp.).*

Key words: phytoplankton, bacteria, species composition, nutrients, enrichment experiment, Gulf of Trieste

INTRODUCTION

The Gulf of Trieste is a typical temperate coastal area where the dynamic of phytoplankton abundance and species/group composition usually follow seasonal and interannual fluctuations in relation to freshwater discharges and thereby nutrient inputs (Malej *et al.*, 1995). These seasonal fluctuations are characterized by a sequence of events from a spring diatom bloom due to high nutrient concentrations supplied by rivers towards late-spring and summer microflagellate-dominated community, supported by regenerated nutrients typical for many coastal seas and estuaries (Garcia-Soto *et al.*, 1990; Ragueneau *et al.*, 1994). In nutrient-enriched environments diatoms seem to be more successful in taking up nutrients due to their "luxury consumption" (Sakshaug & Olsen, 1986) and higher growth rates (Furnas, 1990) compared to smaller cells like microflagellates and picoplanktonic cyanobacteria. On the other hand, microflagellates and especially cyanobacteria dominate in nutrient-depleted environments (Iriarte, 1993; Fuks, 1995), where low nutrient concentrations prevent development of diatoms. However, some studies have recently indicated that this sequence might be altered by a silicate limitation of diatom blooms in coastal waters (Smayda, 1990; Conley & Malone, 1992). Namely, in many industrialized coastal areas anthropogenic enrichment by nitrogen and phosphorus compounds induced a decline in Si/N and Si/P ratios, causing modifications of phytoplankton succession (controlled not only by nutrients' amount but also by their ratios) (Fisher *et al.*, 1988; Egge & Aksnes, 1992). As a consequence, diatom-dominated spring blooms are replaced with flagellate blooms (microflagellates, dinoflagellates), among which many harmful and toxic species are found (Smayda, 1990).

The aim of this work was to analyze the development of phytoplankton biomass and species composition in nutrient-enrichment experiments. These controlled experiments were integrated in a three-year EU/Environment project PALOMA that had a principal goal to estimate the effects of different nutrient concentrations and ratios on coastal plankton dynamics and the production of organic matter (Cauwet, 1996; Malej *et al.*, 1997a). In the present contribution we focused on development of dominant phytoplankton species and groups in different nutrient regimes and tried to evaluate their dynamics relative to control conditions without nutrient additions.

MATERIALS AND METHODS

Materials

Three enrichment experiments were carried out: 27 June - 2 July 1994 (PALEX 1), 3-12 April 1995 (PALEX 2), and 22 March - 3 April 1996 (PALEX 3). Six to seven treatment regimes were set up using the natural plankton, collected in the south-eastern part of the Gulf of Trieste at the subsurface as experimental assemblage. In the first two experiments we applied the following treatments: control without any addition (A), single additions of phosphate (B: 0.6 µM l^{-1}), nitrogen as nitrate + ammonium (C: 5.1 + 1.8 µM l^{-1}), silicate (D: 10.6 µM l^{-1}), a mixture of all nutrients (G: 0.6 µM PO_4^{3-} l^{-1}, 5.1 µM NO_3^- l^{-1}, 1.8 µM NH_4^+ l^{-1}, 10.6 µM Si l^{-1}), and additions of rain water (E) and river water (F) as natural nutrient sources (15% v/v dilutions). In PALEX 3 only control (A1, A16), and treatments with the addition of phosphate (B1, B16) and all nutrients (G1, G16) were set up, using plankton assemblage from the subsurface (1 m) and from the depth of fluorescence maximum (16 m). Experimental plankton assemblages were enclosed in Nalgene containers (8 and 20 l) and incubated *in situ* at 2 m depth. All sampling procedure and set-up of the enrichment experiments are described in detail in Mozetič *et al.* (1997) and Malej *et al.* (1997a).

Samples were withdrawn daily or every second day and inorganic nutrients, particulate and dissolved organic nitrogen and carbon, particulate and dissolved carbohydrates, phytoplankton structure and pigment biomarkers, bacterioplankton abundance, and primary and bacterial production were measured. For the purpose of this work we present the initial nutrient status, and the development of phytoplankton biomass, cell abundance (phyto- and bacterioplankton) and taxonomic composition, while other data are presented in Cauwet *et al.* (1998, *in press*).

Methods

Nutrients were analyzed in filtered (NO_2^-, NO_3^-, PO_4^{3-}) and unfiltered (NH_4^+, Si) samples using standard procedures (Grasshoff, 1983).

Phytoplankton biomass was determined fluorometrically as chlorophyll *a* (Chl *a*) concentration (Holm-Hansen *et al.*, 1965). Subsamples of seawater (25 ml) were filtered onto 0.22 µm Millipore filters, extracted in 90% acetone and the fluorescence of extracts measured on a Turner fluorometer 112.

Cell counts. Samples for enumerating plankton were preserved with neutralized formalin (1.5-2% final concentration). Phytoplankton abundance (micro- and nanoplankton) and taxonomic composition were determined on an inverted microscope using the technique of Utermöhl (1958). Organisms identified on a species or genus level belonged to three algal classes (Bacillariophyceae - diatoms, Dinophyceae - dinoflagellates, Prymnesiophyceae - coccolithophores) and to one non-taxonomic group (microflagellates).

Bacterioplankton (cyanobacteria and heterotrophic bacteria) and picoplanktonic eucaryotes (2-3 µm) were counted with an epifluorescence microscope (1250x magnification). Cyanobacteria and small eucaryotic cells were counted in green excitation light according to the protocol of Takahashi *et al.* (1985) and heterotrophic bacteria in UV light using DAPI according to Porter & Feig (1980).

To study the effect of different nutrient additions on species composition, we compared species abundances in different treatments relative to control conditions at the time of biomass maximum. Firstly, we chose the most abundant species and/or groups (> 1% of total abundance) in the initial phytoplankton assemblages as well as on the day of chlorophyll maximum. Afterwards, the relative increase or decrease of the most abundant species/groups was expressed as the ratio between the abundance in different treatments and control treatment (X/Control, where X stands for single treatment).

During PALEX 3 *epifluorescence micrographs* of phytoplankton and bacterioplankton stained with primuline and DAPI, respectively, were taken with Olympus camera in UV light.

Tab. 1: Nutrient conditions and basic biological parameters of inoculated assemblages during enrichment experiments PALEX 1, 2 and 3.
Tab. 1: Hranilne razmere in osnovni biološki parametri naravne združbe na začetku obogatitvenih poizkusov PALEX 1, 2 in 3.

Parameter	PALEX 1	PALEX 2	PALEX 3	
			1 m	16 m
nitrate (µM l^{-1})	3.44	3.62	1.01	0.93
phosphate (µM l^{-1})	0.1	0.01	0.03	0.06
silicate (µM l^{-1})	8.11	1.15	1.85	0.13
N/P	60	> 100	40	33
N/Si	< 1	4	< 1	15
Si/P	80	> 100	62	2
Chl *a* (µg l^{-1})	0.68	3.39	0.94	1.23
phytoplank. (cells l^{-1})	0.4×10^6	7.3×10^6	0.7×10^6	0.8×10^6
diatoms (% phyto.)	39	88	52	49
µflagellates (% phyto.)	55	11	41	42
cyanobacteria (cells l^{-1})	2.9×10^7	1.3×10^7	9.5×10^6	7.9×10^6
hetero. bacteria (cells l^{-1})	9.3×10^8	4.8×10^8	1.5×10^8	3.4×10^8

RESULTS

Characteristics of inoculated assemblages

Nutrient concentrations and their ratios measured in field samples from which the three initial assemblages

were taken (Table 1) indicated P-limitation of phytoplankton growth (N/P >30). Initial assemblages were also Si-limited in PALEX 2, and especially in PALEX 3 phytoplankton collected from 16 m. Initial phytoplankton biomass was the highest in PALEX 2 and the lowest in PALEX 1, so was phytoplankton abundance. The dominant phytoplankton groups were microflagellates in PALEX 1 and diatoms in PALEX 2, while proportions of these two groups were rather similar in PALEX 3. Bacterial component (autotrophic cyanobacteria and heterotrophic bacteria) was higher in June/July experiment compared to spring experiments (PALEX 2 and 3).

Plankton standing stock

The duration of experiments was different for the PALEX 1, 2, 3 experiments: we finished the experiment when phytoplankton reached the stationary phase in PALEX 2 and 3. The exception was PALEX 1, where the experiment stopped when the population was still growing. In all three experiments the largest increase of the phytoplankton biomass (Chl *a*) was observed in treatment with the addition of all nutrients (G treatment), although stimulating effect of other nutrients, especially phosphate, was also notable (Fig. 1). In PALEX 1 the maximal biomass in G treatment was measured at the end of the experiment (day 6; 4.31 µg Chl *a* l^{-1}), while during PALEX 2 and 3 peak values amounting to 20.38 and 22.47 µg Chl *a* l^{-1} were measured after 5-6 days, respectively.

Likewise the Chl *a* biomass, we observed a similar pattern of phytoplankton abundance during the three experiments (data not shown). The highest cell densities were counted in treatments with the addition of all nutrients, reaching values from 1.8×10^7 and 3.6×10^7 cells l^{-1} in PALEX 1 and 3, respectively, and up to 7.2×10^8 cells l^{-1} in PALEX 2. The common feature of all three experiments was the absolute and relative growth of the dominant groups in the inoculated assemblages (see Table 1) in all treatments. At the end of the experiments the average relative proportion (determined from all treatments) of microflagellates increased up to 98% in PALEX 1, while diatoms represented 93 and 72% of total phytoplankton in PALEX 2 and 3, respectively. Dinoflagellates and coccolithophores were less important, especially in PALEX 2, where their proportions accounted for less than 1% of total phytoplankton at the beginning as well at the end of the experiment. The relative contribution of these two groups was higher in PALEX 1 and 3, with dinoflagellates being more important in PALEX 1 (4.5% at the beginning) and coccolithophores in PALEX 3 (4.7-5.4% at the beginning).

Compared to eucaryotic micro- and nanoplankton, cyanobacteria were not very successful (Fig. 2a). In PALEX 1 cyanobacteria were completely outcompeted by larger size-classes and picoplanktonic eucaryotes (data not shown), while in PALEX 2 their abundance decreased steadily in all treatments. The same was observed in PALEX 3 except for control conditions (A16).

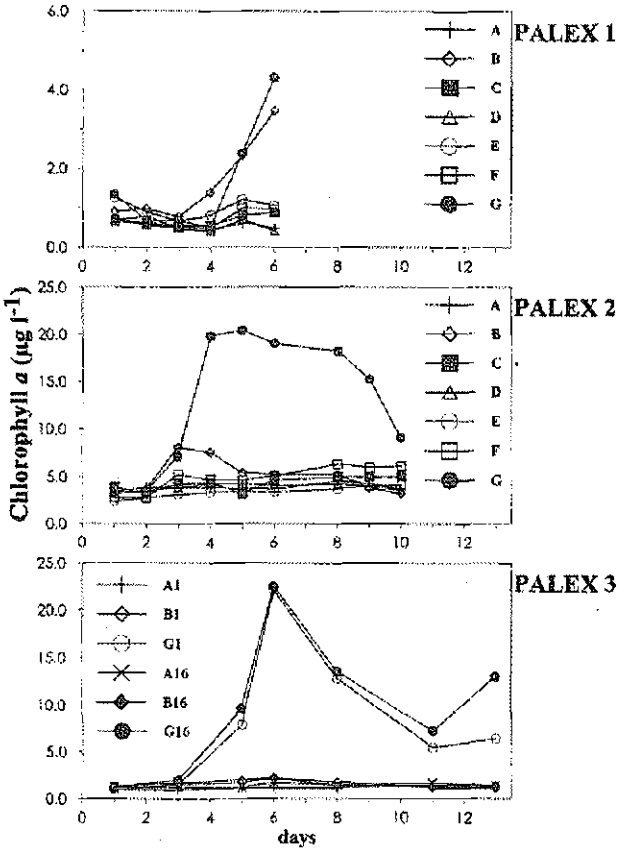


Fig. 1: Chlorophyll a biomass in different nutrient treatments during PALEX experiments 1 (27 June - 2 July 1994), 2 (3-12 April 1995) and 3 (22 March - 3 April 1996).
Sl. 1: Klorofilna biomasa v različnih hranilnih razmerah v obogatitvenih poizkusih PALEX 1 (27.6. - 2.7.1994), 2 (3. - 12.4.1995) in 3 (22.3. - 3.4.1996).

Bacterial abundance increased initially and had already reached maximal values on days 2-4 in all three PALEX experiments, preceding the autotrophic component of the system (Fig. 2b, Fig. 4a).

Species composition in relation to nutrient additions

Species composition was quite similar in the two spring experiments (PALEX 2 and 3). In both cases we collected a population during the late phase of a diatom bloom with the highest species diversity among the diatoms. The dominant diatom species were *Pseudonitzschia pseudodelicatissima, Skeletonema costatum* and *Chaetoceros* sp. 2 in PALEX 2, and *Chaetoceros* spp. (mainly *C. compressus, C. decipiens* and other larger species), *P. pseudodelicatissima, Bacteriastrum* sp. and

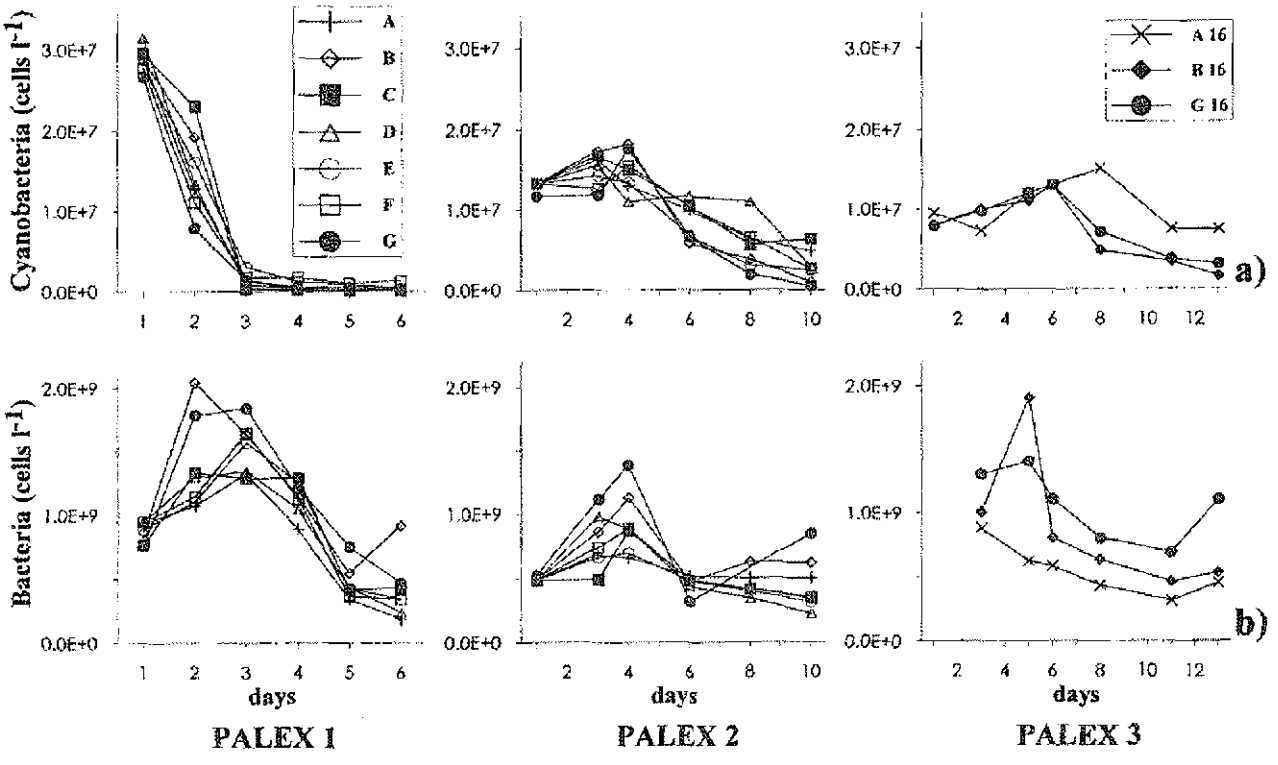


Fig. 2: Cyanobacterial (a) and bacterial (b) abundance in different nutrient treatments during PALEX experiments 1, 2 and 3. Only data for assemblage collected at the depth of fluorescence maximum (16 m) are shown for PALEX 3.
Sl. 2: Gostota cijanobakterij (a) in bakterij (b) v različnih hranilnih razmerah v obogatitvenih poizkusih PALEX 1, 2 in 3. V PALEX 3 poizkusu so prikazani rezultati le za združbo, zajeto na globini fluorescentnega viška (16 m).

Cylindrotheca closterium in PALEX 3 (Figs. 4b-f). Among dinoflagellates, *Gymnodinium* sp., *Prorocentrum minimum* and *Protoperidinium minusculum* were the most abundant, while *Emiliania huxleyi* and a non-identified species were the dominant coccolithophores. In PALEX 1 species diversity of dinoflagellates was the same as of diatoms, although the abundance of the former group was lower. The dominant species were small *Chaetoceros* sp.1, *Navicula* sp., *Cylindrotheca closterium* and a non-identified species for diatoms, and *Ceratium furca*, *Gymnodinium* sp. and various species of the genus *Prorocentrum* for dinoflagellates.

On the day of chlorophyll maximum we compared the most abundant species/groups in different nutrient regimes relative to control conditions. In all three experiments maximal Chl *a* was reached on sixth day and diatom species prevailed in PALEX 2 and 3. Microflagellates which dominated in PALEX 1 were taken as a group, likewise dinoflagellates in PALEX 1 and 2 due to very low abundance of single dinoflagellate species (<1% of total abundance). On two occasions coccolithophores and non-identified, naked, *Gymnodinium*-like dinoflagellates were also chosen.

The highest increase of species abundance relative to control we observed in treatment G (Fig. 3) along with interesting differences between species and/or groups. The highest cell densities were counted for microflagellates (up to 1.8×10^7 cells l^{-1}), *P. pseudodelicatissima* (up to 7.6×10^8 cells l^{-1}), and *Chaetoceros* sp. 3 (up to 1.3×10^7 cells l^{-1}) on day 6 (Table 2) in PALEX 1, 2 and 3, respectively. However, the most marked responses to addition of all nutrients were observed for other species: diatoms *Chaetoceros* sp.1 and *Navicula* sp. in PALEX 1 (Fig. 3a), and *S. costatum* in other two experiments. The relative increase of *S. costatum* was higher in PALEX 3, when a high growth was observed also for *Cylindrotheca closterium* (Fig. 3b & 3c, Figs. 4a, c).

In PALEX 1 a clear increase relative to control was observed also in other treatments, especially with the additions of phosphate and rain water. However, the stimulating effect of nutrient additions was much lower for other phytoplankton groups compared to diatom species (Fig. 3a). Similarly, a higher response was observed for coccolithophores compared to microflagellates and dinoflagellates. In this experiment the addition of inorganic nitrogen alone also stimulated the growth especially of coccolithophores with *Emiliania huxleyi* being the predominant species.

A similar situation was found also in PALEX 3, where among non-diatom species/groups only the coccolitho-

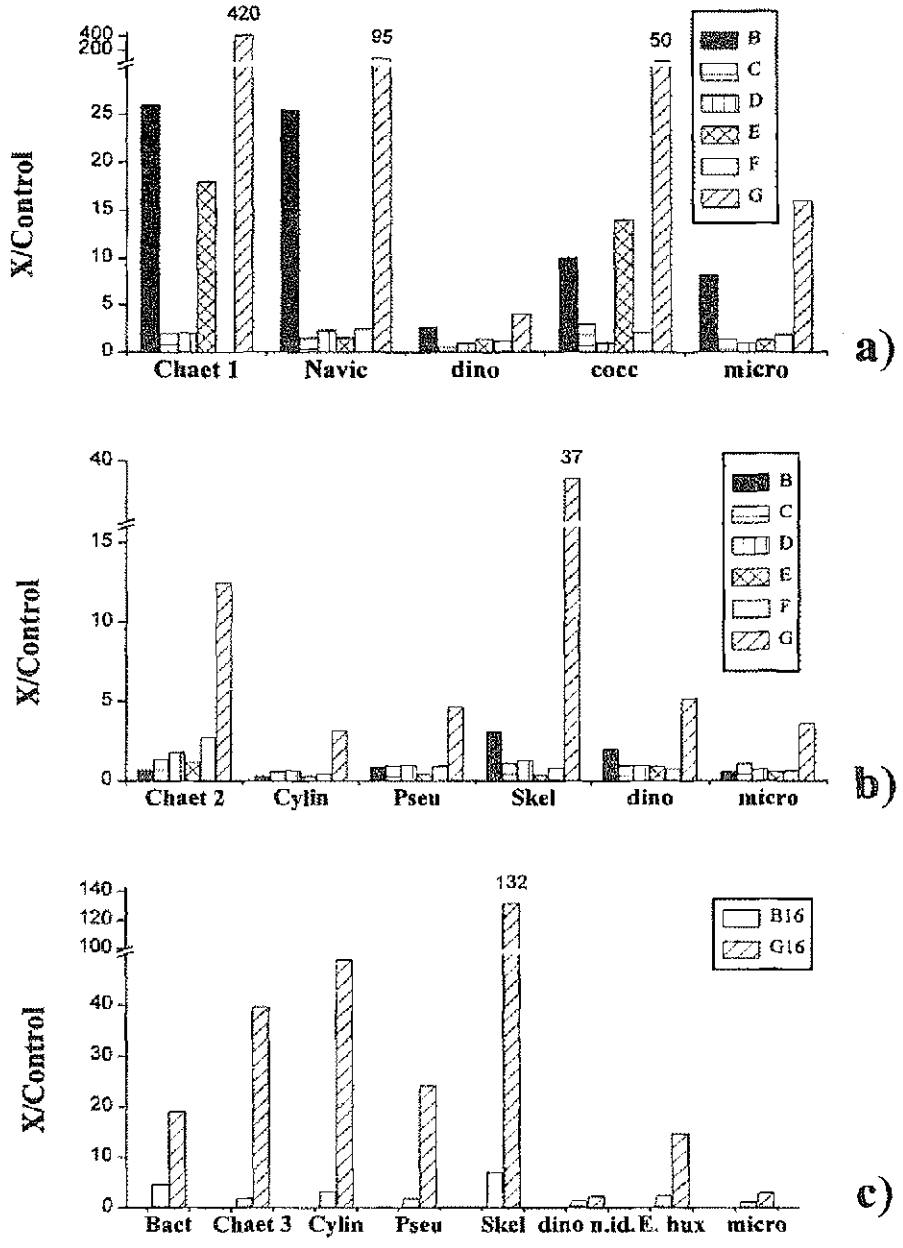


Fig. 3: Ratios between the abundance of dominant phytoplankton species/groups in different treatments (X) and the respective abundance in control conditions on day 6 during PALEX experiments 1 (a), 2 (b) and 3 (c). Only data for assemblage collected at the depth of fluorescence maximum (16 m) are shown for PALEX 3.

Legend: Chaet** (1, 2, 3) = Chaetoceros (sp. 1, spp. 2, spp. 3); **Navic** = Navicula **sp.**; **Cylin** = Cylindrotheca closterium; **Pseu** = Pseudonitzschia pseudodelicatissima; **Skel** = Skeletonema costatum; **Bact** = Bacteriastrum **sp.**; **E. hux** = Emiliania huxleyi; **dino (n.id.)** = **dinoflagellates (non identified)**; **cocc** = **coccolithophores**; **micro** = **microflagellates.

Sl. 3: Razmerja med gostoto prevladujočih fitoplanktonskih vrst/skupin v posameznih hranilnih razmerah (X) in gostoto v kontroli 6. dne obogatitvenih poizkusov PALEX 1 (a), 2 (b) in 3 (c). V poizkusu PALEX 3 so prikazani rezultati le za združbo, zajeto na globini fluorescentnega viška (16 m).

Legenda: Chaet** (1, 2, 3) = Chaetoceros **(sp. 1, sp. 2, sp. 3)**; **Navic** = Navicula **sp.**; **Cylin** = Cylindrotheca closterium; **Pseu** = Pseudonitzschia pseudodelicatissima; **Skel** = Skeletonema costatum; **Bact** = Bacteriastrum sp.; **E. hux** = Emiliania huxleyi; **dino (n.id.)** = **dinoflagelati (nedoločeni)**; **cocc** = **kokolitoforidi**; **micro** = **mikroflagelati.

phore *E. huxleyi* responded significantly to nutrient additions compared to control (Fig. 3c). The addition of phosphate alone had stimulating effect only on *S. costatum* and to lesser extent to other diatoms and *E. huxleyi*.

The highest relative increase in PALEX 2 (37x) was much lower than those in PALEX 1 (420x) and PALEX 3 (132x). Only the addition of all nutrients provoked a clear response of specific species or groups compared to control (Fig. 3b). However, a slight increase was observed also for *S. costatum* and *Chaetoceros* sp.2 in treatments with the addition of phosphate (B) and river water (F).

DISCUSSION AND CONCLUSIONS

Response of plankton assemblages to nutrient additions

In all three enrichment experiments the most marked response was observed in the plankton assemblages receiving all nutrients (G treatment), followed by the addition of phosphate (B treatment), river (F treatment) and rain (E treatment) water. The additions of the latter two in approx. natural dilutions enhanced biomass accumulation especially in PALEX 1 (early summer), suggesting the importance of these freshwater sources of nutrients in the northern Adriatic. Similar responses as in experimental conditions were observed also in the field (Malej *et al.*, 1995; 1997a; 1997b).

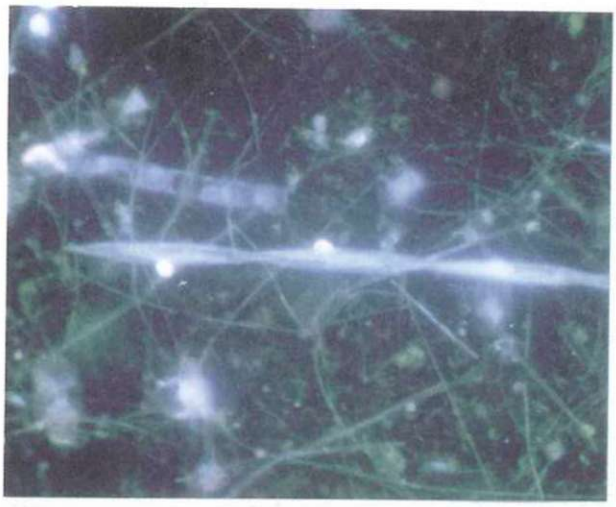
However, the development of autotrophic and heterotrophic components of planktonic assemblage was quite different. Phytoplanktonic groups, which dominated absolutely and relatively in the initial assemblages, expanded significantly towards the end of the experiments. Growth of microflagellates in PALEX 1 and diatoms in PALEX 2 and 3 was highly stimulated by the addition of nutrients, especially in the mixed treatments. This reflected not only in a large increase of their abundance (86- to 105-fold) but also in their relative proportions (for example, from 49% at the beginning to 94% at the biomass peak in G treatments).

On the other hand, cyanobacteria were less successful than larger cells (nano- and microplankton) being outcompeted in all three experiments (Fig. 2a). This was especially evident in G treatments in all PALEX experiments, where cyanobacterial abundance decreased sharply and was the lowest compared to other treatments. In areas with high nutrient supply larger cells, mainly diatoms, seem to respond faster to nutrients' input (Sakshaug & Olsen, 1986; Stolte & Riegman, 1995), whereas in oligotrophic areas smaller cells are responsible for most of the primary production. This phenomenon is in part due to different nutrient uptake strategies adopted by phytoplankton species of different size classes as algal uptake activity is finally limited by available surface area (Stolte & Riegman, 1995). However, in a recent laboratory experiment performed on the cosmopolitan cyanobacterial genus *Synechococcus* and diatom *Thalassiosira weisflogii* Donald *et al.* (1997) pointed out that differences in nutrient uptake might have originated also from different taxonomic as well as evolutionary position of the two organisms: the first being procaryotic and the second one eucaryotic. This refers to the enzymes involved in nutrient uptake or uptake kinetics that are related to taxonomic groups and not to cell size (Stolte & Riegman, 1995). As in our experiment Donald *et al.* (1997) found out that cyanobacteria *Synechococcus* grew better in P-limited conditions (A16 treatment in PALEX 3 experiment), while under P-replete conditions larger diatom species posses higher rates of nutrient uptake and a better ability to incorporate phosphate than cyanobacteria.

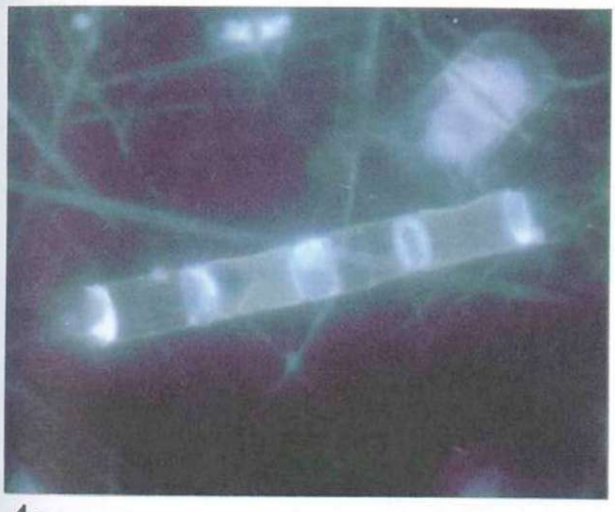
In all PALEX experiments the response of heterotrophic bacteria to nutrient addition (Fig. 2b) preceded the response of phytoplankton indicating the importance of competition for inorganic nutrients. In fact many authors observed, in freshwater systems mainly, this competitive character of bacteria for inorganic nutrients, especially when concentrations of dissolved organic matter are low (Toolan *et al.*, 1991; Coveney & Wetzel, 1992; Wehr *et al.*, 1994). Bacterial growth can be directly limited by the phosphate availability (Toolan *et al.*, 1991), regardless the phytoplankton response (Le *et al.*, 1994), suggesting that the nutrient uptake of these microorganisms can at times become a sink for nutrients within the mi-

Fig. 4a: Mixed plankton community dominated by diatom *Cylindrotheca closterium* ***and blue-stained heterotrophic bacteria in PALEX 3 experiment (400x). Epifluorescence microscopy, DAPI stained, UV light.***
Sl. 4a: Mešana planktonska združba s prevladujočo diatomejsko vrsto *Cylindrotheca closterium* ***in modro obarvanimi heterotrofnimi bakterijami v obogatitvenem poizkusu PALEX 3 (400x).***
Epifluorescentna mikroskopija, barvano z DAPI-jem, UV svetloba.
Figs. 4b-f: Dominating diatoms of the phytoplankton assemblage and bacterioplankton during PALEX 3 experiment: day 6, G treatment (mixed nutrients). Epifluorescence microscopy, primuline and DAPI stained, UV light.
Sl. 4b-f: Prevladujoče diatomeje v fitoplanktonski združbi in bakterije 6. dne obogatitvenega poizkusa PALEX 3, mešane hranilne razmere (G). Epifluorescentna mikroskopija, barvano s primulinom in DAPI-jem, UV svetloba.
b) *Pseudonitzschia sp., Chaetoceros spp.* ***(200x)***
c) *Skeletonema costatum* ***(400x)***
d) *Skeletonema costatum, Chaetoceros spp., Pseudonitzschia pseudodelicatissima* ***(200x)***
e) *Chaetoceros decipiens, Chaetoceros spp.* ***(200x)***
f) DAPI stained bacteria (filamentous, rods and coccal forms) around autotrophic diatom cell (400x).
DAPI obarvane bakterije (nitaste, paličaste in okrogle oblike) okoli avtotrofne diatomejske celice (400x).

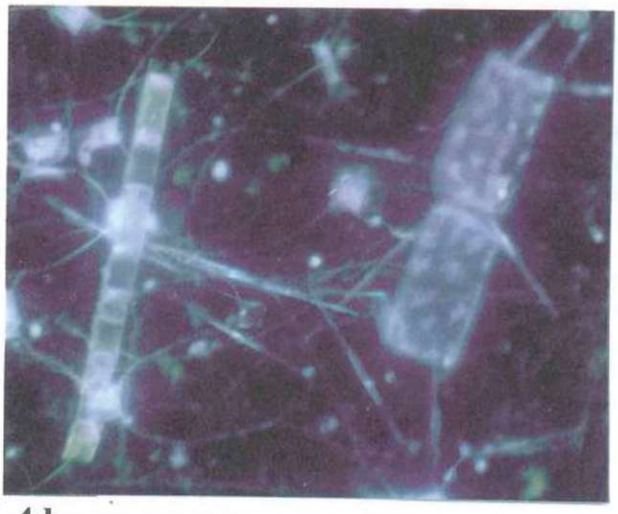
4a



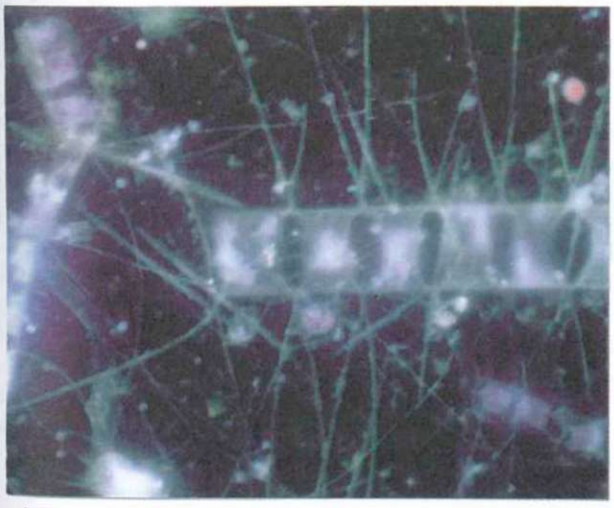
4b



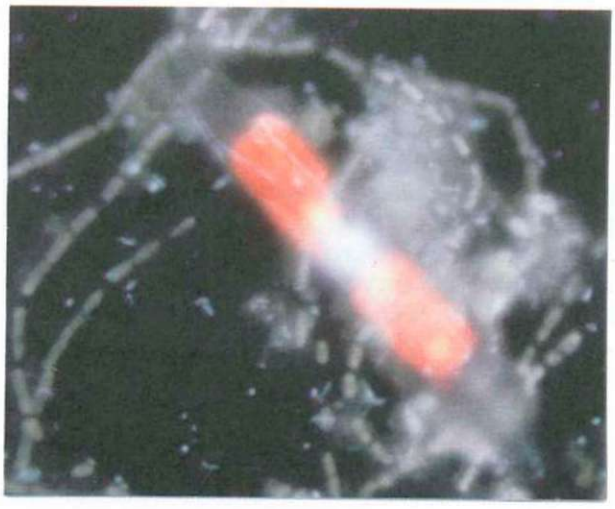
4c



4d



4e



4f

crobial loop (Toolan *et al.*, 1991; Coveney & Wetzel, 1992). Although these observations arise from freshwater environment we can find some similarities to PALEX experiments. Initial conditions in our experiments were always P-limited and a fast response of bacteria to nutrient addition followed by a sharp decrease was observed.

Cyanobacterial as well as bacterial decrease could also be due to grazing by heterotrophic nanoflagellates (HNAN). In fact HNAN abundance in PALEX 1 increased sharply (Malej *et al.*, 1997a), while for PALEX 2 and 3 data are not available. Wehr *et al.* (1994) also suggested that more rapid mineralization of organic matter occur with herbivory on autotrophic cyanobacteria, indicating that HNAN may perhaps prefer cyanobacteria to heterotrophic bacteria as a food source.

Species composition and succession of phytoplankton

Phytoplankton growth in all three enrichment experiments was limited by the availability of nutrients and unbalanced ratios. All three initial (field) assemblages were P-limited (N/P>30). With the addition of nutrients high N/P ratios changed close to the optimal Redfield ratio (N/P≈16) in the mixed treatment (G bottles) while in B treatment (phosphate addition) optimal nutrient ratios were achieved only in PALEX 1. In PALEX 2 and 3, N/P ratios in B treatments were much lower than 16, suggesting nitrogen co-limitation of phytoplankton growth. Consequently, the highest response of phytoplankton community was achieved in mixed treatments. Among other treatments only during PALEX 1 phytoplankton was significantly stimulated following phosphate addition alone (see Fig. 1).

However, phosphate and nitrogen alone are not sufficient for phytoplankton growth, especially in diatom-dominated assemblages. Besides microflagellates, diatoms were the most abundant group during our experiments, suggesting silicate as crucial nutrient as well. If we consider 2 µM silicate per liter as the limiting concentration (Fisher *et al.*, 1988; Egge & Aksnes, 1992) we can presume that silicate was co-limiting diatoms most of the time during PALEX experiments. High silicate concentrations (5-21 µM l^{-1}) were found in all enclosures of PALEX 1 experiment, where microflagellates were the most abundant and predominant group (Table 2). However, the addition of all nutrients stimulated diatom growth much more than flagellates (Fig. 3a) as indicated by treatment/control ratios (up to 420 for diatoms and 16 for microflagellates). Especially in mixed treatment and with the addition of phosphate *Chaetoceros* sp.1 and *Navicula* sp. abundance increased extremely (420- and 95-times in G enclosure, respectively), while in control their abundance decreased or did not change (Table 2). A high relative increase of two diatom species was observed also in the treatment with the addition of rain water (E), as previously reported also from the field after heavy summer storms (Malej *et al.*, 1997b). In PALEX 2 and 3 experiments, dominated by diatoms, the most evident feature was the highest relative increase of *Skeletonema costatum*, followed by the increase of *Chaetoceros* spp. and *Cylindrotheca closterium* (Figs. 3b, c). Although these species were not the most abundant (except *Chaetoceros* sp. 3 in PALEX 3; Table 2) their increase was the greatest. This was particularly true of *S. costatum* increasing up to 37- and 132-times in PALEX 2 and 3, respectively. In both experiments the most numerous species was *Pseudonitzschia pseudodelicatissima*, in all enclosures including control, but for this species a slight increase was observed only when all nutrients were added (G, C16). Beside the greatest response of selected species to mixed nutrients, the addition of phosphate slightly stimulated only *S. costatum*, and *Bacteriastrum* sp. in PALEX 3. In PALEX 2 the addition of river water (F) enhanced *Chaetoceros* sp.2 growth. No significant response of dinoflagellates and microflagellates compared to control was observed in both experiments.

Another group, which was significantly stimulated by the addition of all nutrients, rain water, as well as by the addition of inorganic nitrogen (C) and phosphate only, were coccolithophores with *Emiliania huxleyi* as the most abundant species. In PALEX 3 experiment the abundance of coccolithophore *E. huxleyi* itself accounted for more than 1% of total abundance, while in PALEX 2 coccolithophores exhibited a small increase relative to control only in G and C (nitrogen) treatments (data not shown; Mozetič, 1997).

E. huxleyi is known to form dense blooms in northeast Atlantic (Tyrrell & Taylor, 1996) and Norwegian fjords (Egge & Heimdal, 1994) usually in mid-summer when surface irradiances are high. Mesocosm experiments and modeling studies from these areas have suggested that *E. huxleyi* may have a competitive advantage over other phytoplankton, when phosphate is limiting but nitrate is abundant. *E. huxleyi* is believed to utilize better dissolved organic phosphorus than other phytoplankton (Aksnes *et al.*, 1994) due to greater activity of enzyme alkaline phosphatase. In similar experiments such as our PALEX (multi-species chemostat experiments; Riegman *et al.*, 1992) higher numbers of *E. huxleyi* were obtained at high N/P ratios rather than at low N/P ratios. Besides specific nutritional conditions high surface irradiances were the most influential parameter for bloom development (Egge & Heimdal, 1994). Our results from day 6 in PALEX 1 experiment showed indeed the highest concentrations of inorganic nitrogen in C (21.9 µM l^{-1}) and E (16.2 µM l^{-1}). In these treatments, besides G and B, the highest increase of coccolithophores compared to control were observed (Fig. 3a). Due to low phosphate concentrations (around 0.05 µM l^{-1}), N/P ratios in C and E enclosures were the highest (313 and 540, respectively). Interestingly, the in-

crease of coccolithophores/*E. huxleyi* in this early summer experiment was greater compared to PALEX 3 (Fig. 3c), although the initial abundance was 10-times higher in the later one (see Table 2).

Tab. 2: Abundances (cells l^{-1}) of dominant phytoplankton species/groups (>1% of total abundance) at the beginning of the enrichment experiments and on the day of Chl a maximum in controlled (A) and mixed conditions (G).
Tab. 2: Gostota (cel. l^{-1}) prevladujočih fitoplanktonskih vrst/skupin na začetku obogatitvenih poizkusov in na dan klorofilnega viška v kontroli (A) ter ob dodatku mešanice hranil (G).

PALEX 1	START	DAY 6	
		Control (A)	Mix (G)
Chaetoceros sp.1	$1.3x10^5$	$0.5x10^3$	$2.2x10^5$
Navicula sp.	$2.2x10^3$	$2.2x10^3$	$2.1x10^5$
dinoflagellates	$1.6x10^4$	$9.2x10^3$	$3.8x10^4$
coccolithophores	$2.7x10^3$	$0.5x10^3$	$2.7x10^4$
microflagellates	$1.9x10^5$	$1.1x10^6$	$1.8x10^7$
PALEX 2			
Chaetoceros sp.2	$1.3x10^5$	$2.9x10^5$	$3.7x10^6$
Cylindrotheca closterium	$6.5x10^3$	$7.3x10^4$	$2.3x10^5$
Pseudonitzschia pseudodelic.	$5.6x10^6$	$1.6x10^7$	$7.6x10^7$
Skeletonema costatum	$6.5x10^5$	$7.5x10^5$	$2.8x10^7$
dinoflagellates	$4.1x10^4$	$3.5x10^4$	$1.8x10^5$
microflagellates	$8.2x10^5$	$1.4x10^6$	$4.9x10^6$
PALEX 3			
Bacteriastrum sp.	$5.9x10^4$	$1.7x10^5$	$2.6x10^6$
Chaetoceros sp.3	$1.9x10^5$	$3.2x10^5$	$1.3x10^7$
Cylindrotheca closterium	$1.8x10^4$	$1.6x10^4$	$7.9x10^5$
Pseudonitzschia pseudodelic.	$1.1x10^5$	$3.1x10^5$	$7.7x10^6$
Skeletonema costatum	$6.3x10^3$	$9.4x10^3$	$1.2x10^6$
dinoflagell. non-ident.	$8.1x10^3$	$6.7x10^3$	$1.5x10^4$
Emiliania huxleyi	$2.6x10^4$	$3.5x10^4$	$5.2x10^5$
microflagellates	$3.4x10^5$	$2.9x10^5$	$8.6x10^5$

In chemostat enrichment experiments previously performed in the Gulf of Trieste Mozetič (1993) obtained similar results with *S. costatum* being generally the most stimulated by nutrient additions. Its opportunistic nature to adapt rapidly to changing nutrient regimes was observed many times in controlled conditions (Sanders *et al.*, 1987) as well as in natural environment (i.e. estuaries and coastal areas: Keržan, 1976; Garcia-Soto *et al.*, 1990; Borkman & Turner, 1993; Hama & Handa, 1994; Marshall & Nesius, 1996). Sanders *et al.* (1987) in a series of enrichment experiments over a nearly 2-year period in the Chesapeake Bay observed important changes in dominant species and patterns of species succession. Nutrient addition caused a shift from flagellate species to small centric diatoms (*S. costatum*, *Cyclotella* sp., *Cylindrotheca closterium*, *Thalassiosira* spp.) in N-enriched cultures, but only during the summer-autumn period. Their results can be compared with the situation in PALEX 1 with a significant increase of small *Chaetoceros* species and *Navicula* sp. over microflagellates, although comparison with other treatments indicated that the addition of phosphate rather than inorganic nitrogen stimulated their growth. This different phytoplankton response to specific nutrient additions can be attributed to regional (and perhaps seasonal) differences in determining the limiting nutrient (northern Adriatic vs. Chesapeake Bay subestuary).

Several authors (Turpin & Harrison, 1979; Harrison & Turpin, 1982; Kilham & Kilham, 1984) proposed that nutrient flux and nutrient ratios are important factors influencing the dominance of various taxonomic groups (diatoms vs. flagellate species), while nutrient patchiness or chemical form of the nutrient may influence the success of one particular species over another.

Our enrichment experiments confirmed that inputs of new nutrients not only stimulate phytoplankton growth in general but also affect community structure. Total phytoplankton biomass expressed as Chl *a* augmented approx. 20-times over six days period following the addition of nutrients with optimal Redfield ratio. Majority of eucaryotic phytoplankton increased their abundance; in contrast, cyanobacteria decreased. Among taxonomic groups besides diatoms, coccolithophores seemed to be stimulated most (10-50 times increase in six days) by addition of nutrients. Diatoms that were growing most successfully were *Skeletonema costatum* (increasing its abundance from 40 up to 130 times in six days) during spring experiments, and small *Chaetoceros* species (augmenting its abundance up to 420 times) in early summer experiment.

ACKNOWLEDGMENTS

We thank Dr. Branko Čermelj for technical assistance. The study was a part of Project PALOMA (Production and Accumulation of Labile Organic Matter in the Adriatic) and was financially supported by the PECO Program (Contract No. CIPD-CT94-0106) and the Ministry of Science and Technology of the Republic of Slovenia.

VPLIV VNOSA HRANILNIH SNOVI NA SESTAVO PLANKTONSKE ZDRUŽBE

Patricija MOZETIČ, Valentina TURK, Alenka MALEJ
Morska biološka postaja Piran, SI-6330 Piran, Fornače 41

POVZETEK

V prispevku avtorice opisujejo odziv planktonske združbe na različne hranilne razmere z uporabo metode obogatitvenih poizkusov. V treh poizkusih, ki so potekali v juniju/juliju 1994, aprilu 1995 in marcu/aprilu 1996, so med drugim spremljale razvoj planktonske biomase in spremembe v vrstni sestavi kot odgovor na povečane koncentracije hranilnih snovi. Naravni planktonski združbi, inkubirani in situ, so dodale štiri umetne vire hranilnih snovi (fosfat, anorganski dušik kot nitrat in amonij, silikat in mešanico vseh hranil) ter naravna vira hranil v obliki deževnice in rečne vode. Inkubacijska posoda brez dodatka hranilnih snovi je ponazarjala kontrolne razmere.

V vseh treh poizkusih so največji porast fitoplanktonske biomase opazile ob dodatku mešanice hranil, nekoliko manjši pa je bil odgovor ob dodatku fosfata. Prav nasprotno pa je gostota cijanobakterij in heterotrofnih bakterij s časom strmo upadala. Fitoplanktonske skupine in vrste, ki so prevladovale v začetnem vzorcu morske vode, so znatno narasle tudi ob koncu poizkusov (mikroflagelati v prvem poizkusu, diatomeje s prevladujočo vrsto Pseudonitzschia pseudodelicatissima *v obeh spomladanskih poizkusih). Navkljub temu so avtorice zabeležile največji porast števila v primerjavi s kontrolo pri drugih, v začetku neprevladujočih vrstah* (Skeletonema costatum, Chaetoceros *spp.*, Cylindrotheca closterium, Navicula *sp.*, Emiliania huxleyi). *Absolutno največji porast je povzročil dodatek mešanice vseh hranil pri vrstah* Skeletonema costatum *v spomladanskem času (132-kratni porast v primerjavi s kontrolo) in* Chaetoceros *sp. v poletnem poizkusu (420-kratni porast). Edina nediatomejska vrsta, ki se je močneje odzvala na dodatke mešanice hranil, deževnice in anorganskega dušika, je bila kokolitoforida* Emiliania huxleyi *v poletnem obdobju.*

Pričujoča raziskava je potrdila, da obogatitev morske vode s hranilnimi snovmi v optimalnem koncentracijskem razmerju pospeši rast celotne fitoplanktonske združbe, pomembno pa vpliva tudi na vrstno sestavo. Videti je, da so takšne hranilno obogatene razmere ugodne za hitro rastoče, oportunistične vrste (zlasti Skeletonema costatum, Chaetoceros *spp.), ki prevladujejo v določenem obdobju.*

Ključne besede: fitoplankton, bakterije, vrstna sestava, hranilne snovi, obogatitveni poizkusi, Tržaški zaliv

REFERENCES

Aksnes, D. L., J. K. Egge, R. Rosland & B. R. Heimdal, 1994. Representation of *Emiliania huxleyi* in phytoplankton simulation models: a first approach. Sarsia, 79, 291-300.

Borkman, D. G. & J. T. Turner, 1993. Plankton studies in Buzzards Bay, Massachusetts, USA. II. Nutrients, chlorophyll *a* and phaeopigments, 1987 to 1990. Mar. Ecol. Prog. Ser., 100, 27-34.

Cauwet, G. 1996. Production and Accumulation of Labile Organic Matter in Adriatic. Progress report (June 1994-June 1995), Part I. EU Environment Report, 20 pp.

Cauwet, G., S. Terzić, M. Ahel, P. Mozetič, V. Turk & A. Malej, 1998. Effect of nutrient addition on microbial plankton and dissolved organic matter variability. Part II. Biochemical Aspect. In: Ecosystem Research Report, the Adriatic Sea, EU/Environment Series, Brussels (in press).

Conley, D. J. & T. C. Malone, 1992. Annual cycle of dissolved silicate in Chesapeake Bay: implications for the production and fate of phytoplankton biomass. Mar. Ecol. Prog. Ser., 81, 121-128.

Coveney, M. F. & R. G. Wetzel, 1992. Effects of nutrients on specific growth rate of bacterioplankton in oligotrophic lake water cultures. Appl. Environ. Microbiol., 58, 150-156.

Donald, K. M., D. J. Scanlan, N. G. Carr, N. H. Mann & I. Joint, 1997. Comparative phosphorus nutrition of the marine cyanobacterium *Synechococcus* WH7803 and the marine diatom *Thalassiosira weissflogii*. J. Plankton Res., 19(12), 1793-1813.

Egge, J. K. & D. L. Aksnes, 1992. Silicate as regulating nutrient in phytoplankton competition. Mar. Ecol. Prog. Ser., 83, 281-289.

Egge, J. K. & B. R. Heimdal, 1994. Blooms of phytoplankton including *Emiliania huxleyi* (Haptophyta).

Effects of nutrient supply in different N:P ratios. Sarsia, 79, 333-348.

Fisher, T. R., L. W. Jr. Harding, D. W. Stanley & L. G. Ward, 1988. Phytoplankton, nutrients, and turbidity in the Chesapeake, Delaware, and Hudson estuaries. Estuar. Coast. Shelf Sci., 27, 61-93.

Fuks, D. 1995. Uloga bakterioplanktona u ekosustavu sjevernog Jadrana. Ph. D. Thesis, University of Zagreb, 155 pp.

Furnas, M. J. 1990. *In situ* growth rates of marine phytoplankton: approaches to measurement, community and species growth rates. J. Plankton Res., 12(6), 1117-1151.

Garcia-Soto, C., I. de Madariaga, F. Villate & E. Orive, 1990. Day-to-day variability in the plankton community of a coastal shallow embayment in response to changes in river runoff and water turbulence. Estuar. Coast. Shelf Sci., 31, 217-229.

Grasshoff, K. 1983. Methods for sea water analyses. Verlag Chemie, Weinheim, 317 pp.

Hama, J. & N. Handa, 1994. Variability of the biomass, chemical composition and productivity of phytoplankton in Kinu-ura Bay, Japan during the rainy season. Estuar. Coast. Shelf Sci., 39, 497-509.

Harrison, P. J. & D. H. Turpin, 1982. The manipulation of physical, chemical, and biological factors to select species from natural phytoplankton communities. In: Marine mesocosms (eds. Grice, G. D. & Reeve, M. R.), Springer-Verlag, New York, 275-289.

Holm-Hansen, O., C J. Lorenzen, R. W. Holmes & J. D. H. Strickland, 1965. Fluorometric determination of chlorophyll. J. Cons. Perm. Int. Explor. Mer, 30, 3-15.

Iriarte, A. 1993. Size-fractionated chlorophyll *a* biomass and picophytoplankton cell density along a longitudinal axis of a temperate estuary (Southampton Water). J. Plankton Res., 15(5), 485-500.

Keržan, I. 1976. Prispevek k poznavanju odnosov primarne pelagične bioprodukcije in fekalnega onesnaženja v slovenskem delu Severnega Jadrana. M. Sc. Thesis, University of Ljubljana, 39 pp.

Kilham, S. S. & P. Kilham, 1984. The importance of resource supply rates in determining phytoplankton community structure. In: Trophic interactions within aquatic ecosystems (eds. Meyers, D. G. & Strickler, J. R.), Westview Press, Inc., Boluder, CO, 7-27.

Le, J., J. D. Wehr & L. Campbell, 1994. Uncoupling of bacterioplankton and phytoplankton production in fresh waters is affected by inorganic nutrient limitation. Appl. Environ. Microbiol., 60, 2086-2093.

Malej, A., P. Mozetič, V. Malačič, S. Terzić & M. Ahel, 1995. Phytoplankton responses to freshwater inputs in a small semi-closed gulf (Gulf of Trieste, Adriatic Sea). Mar. Ecol. Prog. Ser., 120, 111-121.

Malej, A., P. Mozetič, V. Turk, V. Malačič, L. Lipej & J. Forte, 1997a. Production and Accumulation of Labile Organic Matter in the Adriatic. EU Environment/PECO Report, Piran, 66 pp.

Malej, A., P. Mozetič, V. Malačič & V. Turk, 1997b. Response of summer phytoplankton to episodic meteorological events (Gulf of Trieste, Adriatic Sea). P.S.Z.N.I.: Mar. Ecol., 18, 273-288.

Marshall, H. G. & K. K. Nesius, 1996. Phytoplankton composition in relation to primary production in Chesapeake Bay. Mar. Biol., 125, 611-617.

Mozetič, P. 1993. Vloga posamezniih velikostnih razredov fitoplanktona pri biomasi in produkciji južnega dela Tržaškega zaliva. M. Sc. Thesis, University of Zagreb, 94 pp.

Mozetič, P. 1997. Odziv neritičnega fitoplanktona na dodatke hranil v naravnih in kontroliranih pogojih. Ph. D. Thesis, University of Ljubljana, 184 pp.

Mozetič, P., V. Turk, A. Malej, S. Terzić, M. Ahel & G. Cauwet, 1997. Coastal plankton response to nutrient enrichment: an experimental system. In: Water Pollution IV. Modelling, Measuring and Prediction. (eds. Rajar, R. & Brebbia, C. A.), Computational Mechanics Publs., Southampton, Boston, 151-160.

Porter, K. G. & Y. S. Feig, 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr., 25, 943-948.

Ragueneau, O., E. De Blas Varela, P. Tréguer, B. Quéguiner & Y. Del Amo, 1994. Phytoplankton dynamics in relation to the biogeochemical cycle of silicon in a coastal ecosystem of western Europe. Mar. Ecol. Prog. Ser., 106, 157-172.

Riegman, R, A. A. M. Noordeloos & G. C. Cadee, 1992. *Phaeocystis* blooms and eutrophication of the continental coastal zones of the North Sea. Mar. Biol., 112, 479-484.

Sakshaug, E. & Y. Olsen, 1986. Nutrient status of phytoplankton blooms in Norwegian waters and algal strategies for nutrient competition. Can. J. Fish. Aquat. Sci., 43, 389-396.

Sanders, J. G., S. J. Cibik, C. F. D'Elia & W. R. Boynton, 1987. Nutrient enrichment studies in a coastal plain estuary: changes in phytoplankton species composition. Can. J. Fish. Aquat. Sci., 44, 83-89.

Smayda, T. J. 1990. Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. In: Toxic marine phytoplankton (eds. Graneli, E., Sundstrom, B., Edler, L. & Anderson, D. M.), Elsevier Publs., Amsterdam, 29-40.

Stolte, W. & R. Riegman, 1995. Effect of phytoplankton cell size on transient-state nitrate and ammonium uptake kinetics. Microbiology, 141, 1221-1229.

Takahashi, M., K. Kikuchi & Y. Hara, 1985. Importance of picocyanobacteria biomass (unicellular, bluegreen algae) in the phytoplankton population of the coastal waters of Japan. Mar. Biol., 89, 63-69.

Toolan, T., J. D. Wehr & S. Findlay, 1991. Inorganic phosphorus stimulation of bacterioplankton production in a meso-eutrophic lake. Appl. Environ. Microbiol., 57, 2074-2078.

Turpin, D. H. & P. J. Harrison, 1979. Limiting nutrient patchiness and its role in phytoplankton ecology. J. Exp. Mar. Biol. Ecol., 39, 151-166.

Tyrrell, T. & A. H. Taylor, 1996. A modelling study of *Emiliania huxleyi* in the NE Atlantic. Journal of Marine Systems, 9(1/2), 83-112.

Utermöhl, H. 1958. Zur Vervollkommung der quantitativen Phytoplankton-Methodik. Mit. Int. Verein. Theor. Angew. Limnol., 9, 1-38.

Wehr, J. D., J. Le & L. Campbell, 1994. Does microbial biomass affect pelagic ecosystem efficiency? An experimental study. Microb. Ecol., 27, 1-17.