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Northern Adriatic mesocosm experiment Rovinj 2003: Changes in the microphytoplankton community

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Abstract

Background and Purpose: *The purpose of the experiment was to study species successions within the microphytoplankton community in nutrient balanced and P-deficient conditions. Analyses of macroaggregate samples and laboratory experiments indicated that some diatom species (e.g.* Cylindrotheca closterium*) could have an important role in the mucilage phenomenon in the northern Adriatic.*

Materials and Methods: *Seawater for the experiment was sampled in a frontal zone between oligotrophic and eutrophic waters developed in the open northern Adriatic. Triplicate subsamples, enriched with nutrients (N/P ratios 100:1 and 16:1), were incubated free floating at the sea surface in an anchored wooden frame. Samples were preserved with neutralised Lugol's solution and counted within a few days by a Zeiss inverted microscope, using the Utermöhl settling method.*

Results and Conclusions: *Diatom* Dactyliosolen fragilissimus, *the most abundant species in the seawater sample dominated also the microphytoplankton bloom during the major part of the experiment in both P-deficient and nutrient balanced conditions. Concomitantly, succession of some smaller diatoms occurred (*Chaetoceros affinis, Nitzschia longissima *and* Chaetoceros *sp.). At the very end of the experiment, a bloom of* Cylindrotheca closterium *developed. The larger bloom of this species was favoured by regenerated and accumulated ammonium in the nutrient balanced subsamples. Similar microphytoplankton changes in the P-deficient and nutrient balanced conditions in the experiment were due to a faster P recycling in the P-deficient conditions. Bloom of* Cylindrotheca closterium *in the experiment is another indication of her probable importance in the mucilage phenomenon.*

INTRODUCTION

The occurrence of enormous quantities of sticky mucilaginous macroaggregates, up to several meters large *(1)*, makes the organic matter continuum *(2)* in the northern Adriatic a complex problem. This event has been quite frequent since the late eighties (1988, 1989, 1991, 1997, 2000–2003), after a decrease of phosphorus compounds was noticed in the region *(3)*, as a consequence of a substantial reduction of the P load within the Po River watershed *(4)*.

It is recognized that changes in nutrient ratios and drastic nutrient limitations can greatly increase exudation of polysaccharide mucus, the mucilage matrix, in phytoplankton cultures, as well as in natural popu-

lations (*5, 6*). In particular, phosphorous limitation was found to precede excessive carbohydrate production (*7*). The role of phytoplankton in the phenomenon has been intensively investigated since the late eighties, both with the aim to identify the principal species involved in the events and to evidence the influence of the mucilage aggregation on the northern Adriatic community (e.g., *1, 8–11*). While these researches resulted in several hypotheses, evidencing the extreme complexity of the mucilage phenomenon, many important aspects still need more precise answers.

Therefore, the purpose of this paper is to contribute to the knowledge of possible role of microphytoplankton species during the fate of primary produced organic carbon through bacterial and physical transformations up to microaggregates, considered the precursors of the mucilaginous aggregates, in different initial conditions of phosphorous availability.

MATERIALS AND METHODS

Sampling station

Seawater for the experiment was sampled in the shallow pycnocline layer, at 4 m depth, (on 6th May 2003), at the station SJ105 (45°02.00'N, 13°09.30'E; 25 Nm W from Rovinj). This station was chosen because of its position within a pronounced frontal zone between oligotrophic and eutrophic waters, as indicated by satellite imagery and afterwards verified by CTD and other measurements during the sampling (*12*).

Experimental set-up

Sampled seawater was filtered through 250 μm mesh and divided into seven 20 L Nalgene bottles. One set of 3 bottles was P-deficient (supplemented with 1 μM PO_4, N/P=100) and another set of 3 bottles was nutrient balanced (supplemented with 6.3 μM PO_4, N/P=16). One bottle was left untreated (0.04 μM PO_4, 7.42 μM TIN, 3.2 μM SiO_4) as the control bottle. Seawater in P-supplemented bottles was manipulated with nutrient additions as follows: 100 μM $NaNO_3$, 100 μM Na_2SiO_3 and vitamins [Thiamine-HCl 0.02 mgL^{-1}; B12 and Biotin as in F/2 (*13*)]. All seven bottles were incubated free floating at the sea surface in the anchored wooden frame.

Microphytoplankton analysis

Aliquots from P-deficient and nutrient balanced bottles were withdrawn every other day, for the first 12 days of the experiment, but afterwards in longer intervals (Figure 1). After the 16th day, the determination was limited to five bottles only (two nutrient balanced, two P-deficient and control). Control was sampled 2–3 times a week, except on the last, 28th day of the experiment, because the water was used for other measurements (*14*). Samples for counts were preserved with Lugol's solution buffered with sodium acetate and counted within a few days by a Zeiss inverted microscope, using the Utermöhl settling method (*15*). For the calculation of cell abundances in the samples (cells L^{-1}), various multiplication factors, depending on the analysed subsample volume and area investigated, were used. For example, for the calculation of cell abundances of some samples, a smaller volume was studied and higher multiplication factor was used (to express values per liter) thus elevating some scarce species to higher importance (greater abundance) than actually was.

As microphytoplankton species were considered cells or colonies larger than 20 μm.

RESULTS

The microphytoplankton community in the seawater used in the set-up of the experiment contained equal number of diatom and dinoflagellate species (Table 1). However, the centric diatom *Dactyliosolen fragilissimus* (Berghon) Hasle / syn. *Rhizosolenia fragilissima* Berghon (64 % of the total count) and one undetermined pennate diatom (25–30 μm; 35 % of the total count) made up almost the whole initial microphytoplankton crop (99 %).

During the major part of the experiment, microphytoplankton composition and species abundances underwent mostly the same changes in both P-deficient and nutrient balanced conditions. While the initial total abundance of the microphytoplankton community ($50 \cdot 10^3$ cells L^{-1}) raised slowly during the first 5 days (Figure 1. a), a more rapid increase followed in the next few days, culminating on the 10th day and remaining stationary up to the 17th day ($150 \cdot 10^6$ cells L^{-1}), when the abundance of microphytoplankton increased up to 3 orders of magnitude. After first bloom, abundances decreased in all the bottles. While in P-deficient conditions the bloom decayed gradually up to the end of the experiment, in the nutrient balanced conditions a second bloom developed after the 21st day ($250 \cdot 10^6$ cells L^{-1}), even larger than the first one. The experiment was stopped on the 28th day, probably around the maximum of the second bloom, dominated by *Cylindrotheca closterium* (Ehrenberg) Reimann et Lewin / syn. *Nitzschia closterium* (Ehrenberg) W. Smith, as the nutrients were exhausted *(16)*.

Diatoms dominated in the total microphytoplankton abundance (98 ± 2 %) during the entire experiment, with only scarce appearance of dinoflagellates. In both P-deficient and nutrient balanced conditions as well as in the control, the taxonomic composition remained qualitatively quite similar, at least concerning the prominent species (Table 1), including those that have maintained the microphytoplankton community abundance high up to the end of the experiment.

Dactyliosolen fragilissimus, already present in very high proportion in the original seawater sample, dominated the community during almost all the experiment. From an initial of $36 \cdot 10^3$ cells L^{-1} (64 % of the total abundance), it reached a maximum on the 10th day ($170 \cdot 10^6$ cells L^{-1}), increasing greatly its contribution to the total abundance

(~95 %) and then gradually decreased to $3.7 \cdot 10^6$ cells L^{-1} (36 %) in P-deficient and $450 \cdot 10^3$ cells L^{-1} (3 %) in nutrient balanced conditions (Figure 1. b).

In contrast, undetermined pennate present in high abundance in the initial seawater sample, decreased in a first few days, becoming unimportant for the experiment.

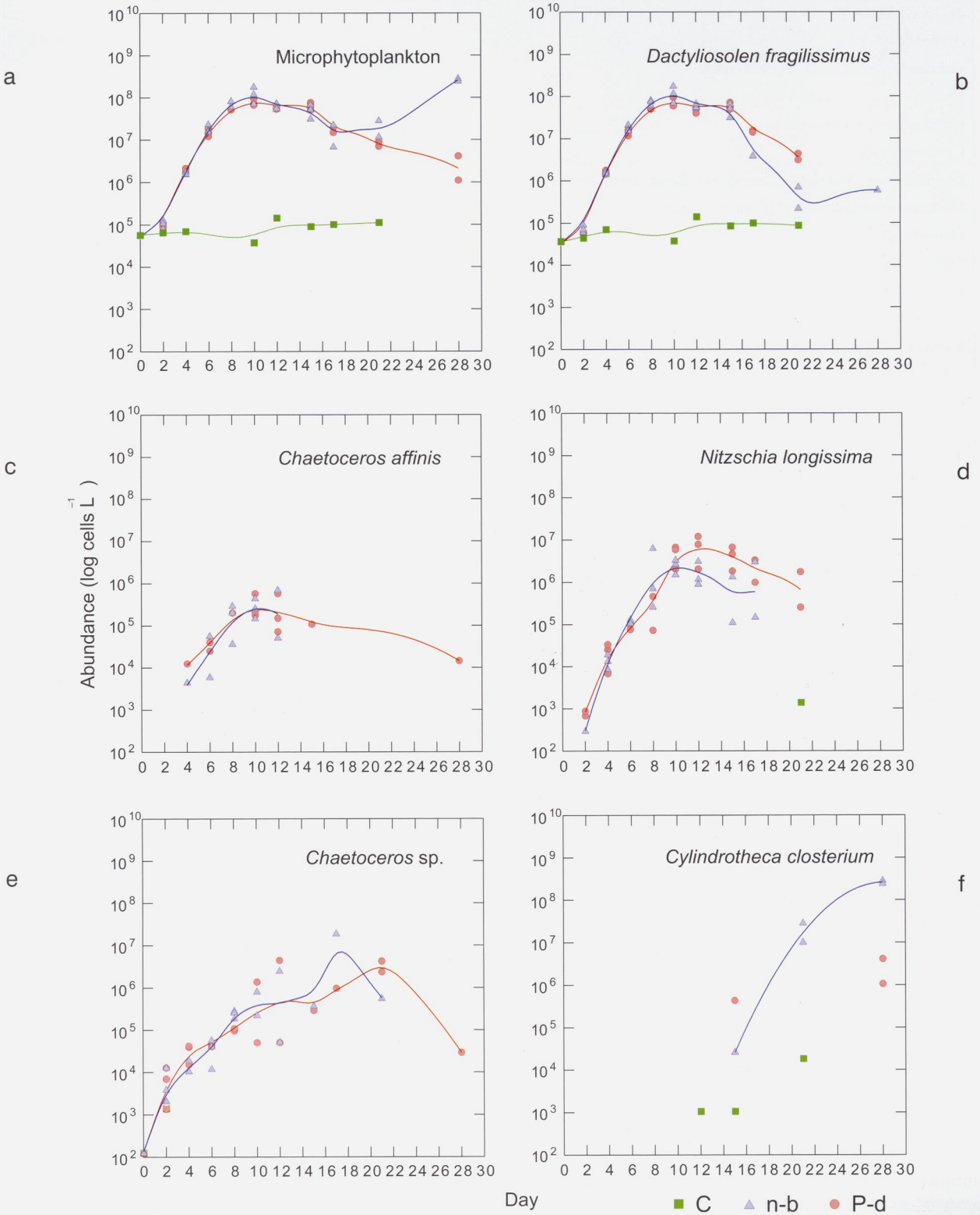

Figure 1. *Abundances of total microphytoplankton community and dominant diatoms in P-deficient (P-d) and nutrient balanced (n-b) conditions, compared with the control (C).*

TABLE 1

Taxonomic composition in the seawater sample (I) and in all 7 bottles during the experiment: control (C), P-deficient ($P\text{-}d_{1,2,3}$) and nutrient balanced ($n\text{-}b_{1,2,3}$) conditions

SPECIES	I	C	$P\text{-}d_1$	$P\text{-}d_2$	$P\text{-}d_3$	$n\text{-}b_1$	$n\text{-}b_2$	$n\text{-}b_3$
DIATOMS								
Amphora sp.								+
Cerataulina pelagica (Cleve) Hendey	+	+	+	+	+	+	+	+
Chaetoceros affinis Lauder			+	+	+	+	+	+
Chaetoceros curvisetus Cleve			+	+	+			
Chaetoceros sp.	+	+	+	+	+	+	+	+
Cylindrotheca closterium (Ehrenberg) Reimann & Lewin		+	+	+	+	+	+	+
Dactyliosolen fragilissimus (Bergon) Hasle	+	+	+	+	+	+	+	+
Diatoma sp.		+						
Leptocylindrus danicus Cleve	+	+		+				+
Licmophora ehrenbergii (Kützing) Grunow		+						
Licmophora paradoxa (Lyngbey) Agardh		+				+		
Licmophora sp.		+		+	+			
Naviculoid cells				+	+		+	+
Nitzschia longissima (Brébisson, in Kützing) Ralfs in Pritchard		+	+	+	+	+	+	+
Pseudo-nitzschia delicatissima (Cleve) Heiden in Heiden & Kolbe	+	+	+	+	+	+	+	+
Nitzschia sp.							+	
Striatella unipunctata (Lyngbye) C. A. Agardh				+				
Thalassionema sp.							+	
Thalassiosira sp.	+	+	+	+	+		+	+
Undetermined pennate diatom	+	+	+	+	+	+	+	+
DINOFLAGELLATES								
Ceratium candelabrum (Ehrenberg) Stein	+	+						
Ceratium furca (Ehrenberg) Claparède & Lachmann	+	+		+	+		+	+
Ceratium fusus (Ehrenberg) Dujardin	+	+						+
Ceratium longirostrum Gourret	+	+						
Dinophysis sp.							+	
Glenodinium sp.	+	+	+	+	+	+	+	+
Gonyaulax sp.	+	+						
Gymnodinium sp.					+		+	+
Gyrodinium sp.			+	+	+	+	+	+
Oxytoxum sp.				+	+			
Prorocentrum micans Ehrenberg				+	+			+
Protoperidinium crassipes (Kofoid) Balech			+				+	
Pyrophacus horologicum Stein	+				+			
Undetermined Dinoflagellates					+		+	+

During the period from the 10th to the 12th day, two other diatoms, *Chaetoceros affinis* and *Nitzschia longissima*, reached their own maximal abundance ($50 - 680 \cdot 10^3$ cells L^{-1} and $1–12 \cdot 10^6$ cells L^{-1}, respectively; Figures 1. c, 1. d) with a relatively low contribution to the total community (≤ 1 and 1–19 %, respectively). In contrast, *Chaetoceros* sp. in one nutrient balanced bottle reached a maximal abundance of $18 \cdot 10^6$ cells L^{-1}, which accounted for 82 % of the total microphytoplankton count and in P-deficient bottles, $2 - 4 \cdot 10^6$ cells L^{-1}, which accounted for 33–47 % of the total microphytoplankton count (Figure 1. e). After the maximal abundance of each bloom, abundance of each co-dominant diatom decreased sharply in both P-deficient and nutrient balanced conditions as well.

Almost all mentioned diatoms were present with similar abundances in both P-deficient and nutrient balanced

conditions overall the experiment. Differently, *Cylindrotheca closterium* was detected in both conditions on the 15 day in similar abundance (~10^5 cells L^{-1}), grew further considerably in the nutrient balanced conditions up to an abundance of 200 · 10^6 cells L^{-1} at the end of the experiment. This value was the highest measured overall the experiment. In the P-deficient conditions, however, the presence of this species remained around 10^6 cells L^{-1} for the rest of the experiment, but still remaining practically the only species present (Figure 1. f).

In the control bottle during the whole period (until 21st day) the abundance varied between 10^4 – 10^5 cells L^{-1}. Like in all other bottles (for the period in question) dominant was the same diatom, *Dactyliosolen fragilissimus*. Only two other species (*N. longissima* and *C. closterium*) appeared in the control bottle, toward the end of the experiment, compared to four species in nutrient added samples.

Not all of the dominant diatoms in the experiment were easy to identify. This was the case for pennate diatoms, *Nitzschia longissima* and *Cylindrotheca closterium*, that are generally difficult to distinguish with the method used. In seawater samples from the northern Adriatic those two species are routinely identified according to the shape and length of the cells, i.e. length of valves: 125 (250) – 450 μm and 25(30) – 100 (400) μm, respectively *(17, 18)*. Moreover, *N. longissima* is defined to have ends extended into very long horns, while the cells of *C. closterium* are usually slightly bent *(17)*. In the experiment, diatoms identified as *N. longissima* were 50–80 μm long and totally linear, i.e. needle like. In the seawater samples, this diatom is also linear, although longer, usually more than 150 μm. The experiment *C. closterium* cells resembled quite well the cells from seawater samples, that is, cells were slightly bent and 50–80 μm long. Even if the cell's length of *N. longissima* in the experiment was under the reported minimum value (125 μm), the species in question was with high probability *N. longissima*, because of the linearity of its cells and clear distinction from the *C. closterium* cells. It might be assumed that unfavourable conditions for the natural development and growth of *N. longissima* prevailed during the experiment.

DISCUSSION

The development of microphytoplankton in both P-deficient and nutrient balanced conditions occurred by successions of the same species, approximately with the same pattern, and, interestingly, with close changes in the abundance, except at the end of the experiment. This indicates similar nutrient availabilities have driven the phytoplankton growth, even if the initial orthophosphate concentrations in the two systems differed by almost an order of magnitude. However, measurements of relevant parameters indicated that diverse recycling mechanism occurred in the different conditions, being efficient to assure approximately equal phosphorus availabilities.

Microphytoplankton cells are able to concentrate high quantities of orthophosphate if the concentrations in the medium surpluses 0.3 μmol L^{-1} *(19)* and use the stored P if it becomes deficient in the medium *(20)*. In both P-deficient and nutrient balanced conditions, rapid uptake of orthophosphate occurred *(16)*.

Orthophosphate in water was exhausted in both systems at the first bloom maximum. While in the nutrient balanced bottles orthophosphate has been regenerated obviously by growing microheterotrophs *(21)*, and successively accumulated in the water, the medium in the P-deficient bottles remained exhausted until the end of the experiment. However, in these bottles phosphorus was kept available to phytoplankton growth, without accumulation in the water, by regeneration mechanisms involving alkaline phosphatase, which activity was significant already a few days after the experiment start *(16)*. Alkaline phosphatase is activated by bacteria and phytoplankton in P-limiting conditions *(22)*. For example, it is induced by *Chaetoceros affinis var. willei* (Gran) Hustedt when N/P ratios in the medium exceed values of 30–40 *(23)*.

Succession of several diatom blooms occurred during the first 16 days of the experiment. *Dactyliosolen fragilissimus* was highly the most abundant in this period, while concomitant blooms of *Chaetoceros affinis* and *Nitzschia longissima* occurred at levels of three and one order of magnitude lower, respectively. Successively, a bloom of *Chaetoceros* sp. occurred, although less intense than the first bloom, still being co-dominant with *D. fragilissimus*. All mentioned species are considered to be cosmopolitan *(18)* and are usual for the Adriatic Sea *(24)*.

Dactyliosolen fragilissimus was a dominant diatom in the first part of the experiment, probably being such in the seawater used in the experiment. Since the eighties, this species has been often present in the northern Adriatic, at the station SJ105, during the entire year. It was generally more abundant in the upper layers (0–10 m), and from May to October, often co-dominating the microphytoplankton community in this period.

Contrary to the findings of Carlsson and Granéli *(25)*, who concluded that *Dactyliosolen fragilissimus* prefers P-deficient conditions, its growth was not significantly different in nutrient balanced to P-deficient conditions. In an experiment carried out with northern Adriatic seawater, this species developed as a co-dominant species *(26)*. Although *D. fragilissimus* was not recognised as an indicator species of eutrophication in the northern Adriatic before *(27)*, findings from this experiment together with the fact that it often co-dominates microphytoplankton community in this region can point towards that. Moreover, changes in form of the diatom was similar in both P-deficient and nutrient balanced conditions; *D. fragilissimus* is a chain-forming diatom, and in the experiment its size distribution changed from solitary cells (4th day) to long chains (6th day), appearing afterwards in solitary form again, cells being mostly in the phase of decay (from 12th day onward). This fact indicates that this

diatom favoured these conditions, both P-deficient and nutrient balanced, for its normal development and growth, indicating its adaptability to deficient P availability in the medium. High abundances and contributions of *D. fragilissimus* in the microphytoplankton community (mostly over 90 %) during the first part of the experiment were with a slight temporal delay followed by increase of the TEP abundance *(28)*. This may indicate that this diatom can be a significant source of particles considered precursors of mucilaginous aggregates.

Since the nineties *Nitzschia longissima* has been generally found at this sampling station, being more abundant from June to December, with mean contribution of around 10 % in the total microphytoplankton abundance. *Chaetoceros affinis* was also present at this same sampling station from the eighties, although more often since the mid nineties. Mostly it accounts to 10–20 % of the total microphytoplankton cells, with higher contributions in the upper layer (up to 70 %) from July to October. In the experiment, this species was present in its usual form, but in relatively much lower contributions than in situ (rarely reaching 1 %).

A few days after the first bloom another diatom species, *Chaetoceros* sp., reached maximal abundances, appearing as single cells, 10–15 μm in size.

In nutrient balanced conditions, marked decrease of orthophosphate, nitrate and orthosilicate coincided with the rapid growth of *Cylindrotheca closterium* in the last phase of the experiment, followed by a significant increment of ammonium concentration. In the P-deficient conditions, negligible changes of the concentrations of nutrients occurred, where low phosphorous probably limited further growth of this diatom. *Cylindrotheca closterium* is also a cosmopolite and usual specie for the Adriatic Sea. It is occasionally present in the upper layer of the sampling station, with higher abundances in winter (10^4 cells L^{-1}), although major blooms can occur (e.g. 10^6 cells L^{-1} in June 2002). Laboratory experiments and samples of mucilaginous aggregates form the northern Adriatic indicated an important role of this planktonic diatom in the mucilage phenomenon *(10, 11)*.

In contrast to diatoms, no significant response of dinoflagellates to nutrient additions was observed in both P-deficient and nutrient balanced conditions. In the control bottle, abundances of total microphytoplankton remained at levels around 10^4 cells L^{-1} during the most of the experiment, with the absolute dominance of *Dactyliosolen fragilissimus* (up to 21st day, when the last control sample was analysed).

Similar growth and succession of microphytoplankton species in this mesocosm study in both P-deficient conditions and nutrient balanced conditions suggested that microphytoplankton species and community from the northern Adriatic can grow in wide range of N/P ratios, because efficient mechanisms of P regeneration are provided.

Appearance of *Cylindrotheca closterium* at the end of the experiment, together with prior analyses of samples of mucilaginous aggregates form the northern Adriatic and laboratory experiments implies the important role of this benthic diatom in the mucilage phenomenon.

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REFERENCES

1. DEGOBBIS D, MALEJ A, FONDA-UMANI S 1999 The mucilage phenomenon in the northern Adriatic. A critical review of the present scientific hypotheses. *Ann Ist Super Sanità 35*: 373–381
2. AZAM F, SMITH D C, STEWARD G F, HANGSTRÖM A 1993 Bacteria-organic matter coupling and its significance for oceanic carbon cycling. *Microbial Ecol 28*: 167–179
3. DEGOBBIS D, PRECALI R, IVANČIĆ I, SMODLAKA N, FUKS D, KVEDER S 2000 Long-term changes in the northern Adriatic ecosystem related to anthropogenic eutrophication. *Int J Environ Pollut 13*: 495–533
4. TARTARI G, MILAN C, ELLI M 1991 Hydrochemistry of the nutrients. *Quad Ist Ric Acque 92*: 6.1–6.29 (in Italian)
5. HERNDL G J 1992 Marine snow in the northern Adriatic Sea: possible causes and consequences for a shallow ecosystem. *Mar Microb Food Webs 6*: 149–172
6. OBERNOSTERER I, HERNDL G J 1995 Phytoplankton extracellular release and bacterial growth: dependence on the inorganic N:P ratio. *Mar Ecol-Prog Ser 116*: 247–257
7. FOGG G E 1995 Some speculations on the nature of the pelagic mucilage community of the northern Adriatic Sea. *Sci Total Environ 165*: 59–63
8. REVELANTE N, GILMARTIN M 1991 The phytoplankton composition and population enrichment in gelatinous »macroaggregates« in the northern Adriatic during the summer of 1989. *J Exp Mar Biol Ecol 146*: 217–233
9. DEGOBBIS D, FONDA-UMANI S, FRANCO P, MALEJ A, PRECALI R, SMODLAKA N 1995 Changes in the northern Adriatic ecosystem and the hypertrophic appearance of gelatinous aggregates. *Sci Total Environ 165*: 43–58
10. MONTI M, WELKER C, DELLAVALLE G, CASARETTO L, FONDA UMANI S 1995 Mucous aggregates under natural and laboratory conditions: a review. *Sci Total Environ 165*: 145–154
11. NAJDEK M, DEGOBBIS D, MIOKOVIĆ D, IVANČIĆ I 2002 Fatty acid and phytoplankton composition of different types of mucilaginous aggregates in the northern Adriatic Sea. *J Plankton Res 24*: 429–441
12. ĐAKOVAC T, SUPIĆ N, DEGOBBIS D, KRAUS R, PRECALI R, IVANČIĆ I, SVETLIČIĆ V, SMODLAKA N 2004 Northern Adriatic mesocosm experiment Rovinj 2003: Oceanographic conditions at the sampling station. *Period biol 106*: 7–15
13. GUILLARD R R L 1975 Culture of phytoplankton for feeding marine invertebrates, p. 29–60. *In:* Smith W C, Chanley M H *(ed.)*, Culture of marine invertebrate animals. Plenum, New York, N.Y, p 26–60
14. ŽUTIĆ V, SVETLIČIĆ V, RADIĆ T, MALFATTI F, DEGOBBIS D, AZAM F 2004 Controlled ecosystem carbon flow experiment in the northern Adriatic Sea. *Period biol 106:* 1–6
15. UTERMÖHL H 1958 Zur Verfollkommnung der quantitativen Phytoplankton-Methodik. *Mitt int Ver theor angew Limnol 17*: 47–71

16. IVANČIĆ I, DEGOBBIS D, PEČAR O, FUKS D, MANGANELLI M, KRAUS R, ĐAKOVAC T, PRECALI R, SCENATI R 2004 Northern Adriatic mesocosm experiment Rovinj 2003: Nutrient dynamics. *Period biol 106:* 17–22
17. CUPP EE 1943 Marine plankton diatoms of the west coast of North America. University of California press, Berkeley and Los Angeles.
18. HASLE G R, SYVERTSEN E E 1996 Marine diatoms. *In:* Tomas C R *(ed)* Identifying marine diatoms and dinoflagellates. Academic Press, San Diego, p 5–385
19. KUENZLER E J, KETCHUM B H 1962 Rate of phosphorous uptake by *Phaedactylum tricornutum*. *Biol Bull 123*: 134–145
20. ELGAVISH A, ELGAVISH G A, HALMAN M, BERMAN T 1980 Phosphorus utilization and storage in batch cultures of the dinoflagellate *Peridinium Cinctum* F. Westii. *J Phycol 16*: 623–633
21. FUKS D, RADIĆ J, RADIĆ T, PEČAR O 2004 Northern Adriatic mesocosm experiment Rovinj 2003: Pico- and nanoplankton dynamics. *Period biol 106:* 39–47
22. REICHARDT W, OVERBECK J, STEUBING L 1967 Free dissolved enzymes in lake waters . *Nature 216*: 1345–47
23. MØLLER M, MYKLESTAD S, HAUG A 1975 Alkaline and acid phosphatases of the marine diatoms *Chaetoceros affinis* var. willei (Gran) Hustedt and *Skeletonema costatum* (Grev.) Cleve. *J Exp Mar Biol Ecol 19*: 217–226
24. VILIČIĆ D, MARASOVIĆ I, MIOKOVIĆ D 2002 Checklist of phytoplankton in the eastern Adriatic Sea. *Acta Bot Croat 61*: 57–91
25. CARLSSON P, GRANÉLI E 1999 Effects of N : P : Si ratios and zooplankton grazing on phytoplankton communities in the northern Adriatic Sea. II. Phytoplankton species composition. *Aquat Microb Ecol 18*: 55–65
26. FILIPIĆ B, IVANČIĆ I, DEGOBBIS D 1989 Dynamics of northern Adriatic phytoplankton in enrichment experiments. *Period biol 91*: 172
27. REVELANTE N, GILMARTIN M 1985 Possible phytoplankton species as indicators of eutrophication in the northern Adriatic Sea. *Rapp Comm Int Mer Médit 29*: 89–91
28. RADIĆ T, FUKS D, RADIĆ J, LYONS D M 2004 Northern Adriatic mesocosm experiment Rovinj 2003: Dynamics of transparent organic microparticles. *Period biol 106:* 57–65