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EFFECTS OF NUTRIENT ENRICHMENT ON COASTAL PHYTOPLANKTON COMPOSITION AND ABUNDANCE IN THE NORTHEASTERN MEDITERRANEAN

SEVİM POLAT

Faculty of Fisheries,
University of Çukurova, Balcalı, 01330 Adana, Turkey

Abstract

The response of coastal phytoplankton community to increased nutrient concentrations was examined over a period of 20 days in the summer period on the NE Mediterranean coast. Two different nutrient enrichments were performed. The enrichment treatments received N, P and Si at a ratio of 15 N: 5 Si: 1 P. Two different doses of nutrients (1x and 2x) were applied. The abundance and biomass of the phytoplankton community were also examined in control units to which no nutrients were added. The increase in phytoplankton abundance and biomass were observed in all treatments including the controls 3 days after the initiation of the experiment. The biomass of phytoplankton in terms of chl *a* increased to a maximum level of 10.6 μgl^{-1} on 11^{th} day of the experiment in the 2x treatment. The structure of the phytoplankton community shifted from an initial dominance of diatoms to small flagellates. Microphytoplankton abundance reached its maximum level of $33.7x10^5$ cells l^{-1} in the 1x treatment 13 days after the initiation of the experiment whereas small flagellates reached their maximum abundance as $33.4x10^5$ cells l^{-1} in the 2x treatment on day 17. From the results obtained, it can be suggested that nutrient enrichment could affect biomass of phytoplankton rather than species composition.

Introduction

The growth of phytoplankton in coastal environments is enhanced by nutrient enrichment processes such as coastal upwelling, anthropogenic and riverine inputs (Berdalet *et al.*1996; Carter *et al*., 2005). Coastal nutrient inputs may supply N and P for phytoplankton growth at proportions very different from Redfield ratio (Berdalet *et al*., 1996). Altered nutrient ratios may have play an important role for the aquatic environment by affecting the selection of particular groups (Berdalet *et al*., 1996). Furthermore, after the nutrient inputs, the proliferation of a particular phytoplankton group or form depends on different factors (Oviatt *et al*., 1989). For this reason, the increase pattern of phytoplankton in marine environments is not very predictable. The response of phytoplankton community to the enrichment of nutrient may be result in a decrease in diatoms and an increase in small and flagellated forms (Caroppo, 2000). Moreover, over increase of harmful organisms in coastal environments is linked to anthropogenic nutrient inputs.

Most studies concerning the response of the species to nutrient inputs have been obtained from experiments with pure phytoplankton culture (Berdalet *et al*., 1996). These kinds of studies provide information for the physiological properties of the species, but they can not sufficiently help to understand the ecological processes and complex effects of environmental factors. For this reason, the response of phytoplankton communities to nutrient inputs is examined through the microcosm and mesocosm experiments by using natural populations.

E-mail: sevcan@cu.edu.tr

The Mediterranean Sea is one of the less productive seas of the world and the eastern Mediterranean is the most oligotrophic part of that sea in terms of low nutrient level and low primary production (Azov, 1991; Krom *et al.*, 1991). The deep waters of eastern Mediterranean are characterized by high N:P ratios of ~28:1, indicative of P-limitation especially in winter (Krom *et al*.,1991; Zohary & Robarts, 1998). However, increased human pressure in the Mediterranean Sea causes major changes in the coastal ecosystems. The red tide events in the Mediterranean appear to have increased and deterioration of water quality has been reported (Duarte *et al*., 2000).

For the regulation of nutrient inputs into coastal areas, it is necessary to know the response of phytoplankton to increased nutrient concentrations. The coasts of the İskenderun Bay are heavily populated and there are many industries on this coastal area. Environmental perturbations resulting from human activities cause dramatic changes in coastal ecosystems. Therefore, there is a need to experimentally investigate the response of phytoplankton communities of such kinds of areas to increased nutrient levels.

The aim of this study was to investigate the response of a coastal phytoplankton community from the northeastern Mediterranean to two different levels of nutrient applications after the enclosure of natural seawater into the microcosms.

Materials and Methods

The microcosms consisted of 6 polyethylene cylindrical tanks of approximately 1 m^3. Natural seawater was collected in June 2002 from 0.5 m depth in İskenderun Bay, northeastern Mediterranean Sea. Each of the 6 tanks located at the seaside were filled immediately with 500 liter of natural seawater. The temperature of water was between 25.1 and 27.9°C during the experiment. The initial seawater contained 0.17 µM phosphate, 1.47 nitrate, 1.20 µM ammonium and 1.32 µM silicate. In the enrichment treatments, nitrogen, phosphorus and silicate were applied to maintain the ratio of ~15 N: 5 Si: 1 P. Nutrients were added in a single addition using solutions of NH_4CL, KH_2PO_4 and Na_2SiF_6 as N, P and Si sources. At the start of the experiment, two different doses of enrichment, each duplicated, were performed. In the first treatment (1x), N, P and Si at the level of 15 µM N, 5 µM Si and 1 µM P were added. In the second treatment (2x), the ratio among N, P and Si was the same, but they were added at two times concentrations of the first treatment. Two other ponds with no nutrient additions were prepared as controls for this experiment. Mixing was accomplished with an air pump located 0.1 m above the bottom. The sunlight was reduced approximately by 30% with a net placed on the tanks.

Microcosms were sampled to determine nutrients, phytoplankton biomass and abundance on alternate days between $9.^{00}$-$9.^{30}$ in the morning. Samples were immediately transported to the laboratory for analysis. Samples for chlorophyll *a* (chl *a*) analysis were filtered through GF/F filters. The filters were extracted in 90% acetone in the dark for a night. Following the extraction, absorbance was measured in a spectrophotometer according to the method provided by Parson *et al*., (1984).

The concentrations of inorganic phosphate, ammonium and silicate were measured following the methods outlined by Strickland & Parsons (1972).

Samples to investigate phytoplankton abundance were preserved in Lugol's solution. Large-sized phytoplankton (microphytoplankton) was examined and counted under an inverted microscope (Hasle, 1978). For the identification of the species, the taxonomic texts (Tregouboff & Rose, 1957; Rampi & Bernhard, 1980; Sournia, 1986; Ricard, 1987; Tomas, 1997) were used.

Small-sized flagellates which could not be identified were defined as small flagellates (mainly <10 µm). The cell counts of this fraction were conducted with a Fuchs-Rosenthal counting chamber. Magnification power of 400x was utilized during the microscopic examination. The results were expressed in cells l^{-1}.

In order to compare the experimental results of phytoplankton abundance and biomass among the treatments the analysis of one-way ANOVA was used.

Results

Nutrient concentrations were at very low levels in natural seawater at the initiation of the experiment. The nutrients added were rapidly assimilated by the community in both treatments. After the initiation of the experiment, P, Si, and N concentrations sharply decreased until day 7. Afterwards, slight increases of P and N concentrations shows recycling of these nutrients in the microcosms. At the end of the experiment, the lowest nutrient levels were 2.90 µM for ammonium in the control, 0.23 for phosphate and 0.89 µM for silicate in the 1x treatment (Fig. 1).

Chlorophyll *a* started to increase after the third day in all treatments. The chl *a* concentration reached maximum levels on day 11 in the 2x treatment and on day 13 in the 1x treatment. The maximum chl *a* concentrations were 8.6, 10.6 and 3.15 µg l^{-1} in the 1x, 2x and control units, respectively (Fig. 2). After the peak, chl *a* decreased sharply in the 2x treatment. In contrast, the decrease was slower in the 1x treatment. At the end of the experiment, chl *a* decreased to 1.67, 3.70 and 0.91µg l^{-1} in the treatment of 1x, 2x and controls, respectively. Differences were important in term of chl *a* concentrations among treatments ($p=0.013$).

During the experiment, 31 phytoplankton species were identified belonging to 5 algal classes: Bacillariophyceae, Dinophyceae, Cyanophyceae, Dictyochophyceae and Prasinophyceae. Microphytoplankton was dominated by diatoms and dinoflagellates in term of species number in the initial community. Of the diatoms, *Rhizosolenia alata* and *Thalassiothrix fraunfeldii* and of dinoflagellates *Scrippsiella trochoidea* were dominant species at the beginning of the experiment. Small flagellates (<10µm) were also present in the initial community but at low abundance. Dominant species were similar for two treatments even in control replicates during the experiment.

Phytoplankton cell numbers started to increase after day 3 in all treatments. In the 1x treatment, microphytoplankton cell number increased slowly until day 9, there was a decrease on day 11 and then it again started to increase (Fig. 3). The highest cell numbers of microphytoplankton were observed in the 1x treatment on day 13 ($33.7x10^5$ cells l^{-1}). Cell numbers decreased after day 15 and remained almost stable until the end of experiment. The abundance in 2x treatment slightly increased from day 3 until day 7 and reached its maximum on day 11 ($30.1x10^5$ cells l^{-1}). In general, microphytoplankton abundances were lower in 2x treatment (Fig. 4). However, no significant differences were found concerning microphytoplankton abundance among the treatments ($p=0.148$).

At the beginning of the experiment, diatoms and dinoflagellates were similar on account of their low abundance levels. This period was followed by a decrease in dinoflagellates and an increase of dominance of diatoms. In the last week of the experiment, the cell numbers of two dinoflagellate species viz., *Protoperidinium conicum* and *Ceratium kofoidii* slightly increased. However, of the diatoms and dinoflagellates, the abundance of neither species exceeded $2x10^4$ cells l^{-1} except a small diatom, *Cerataulina pelagica*. This species became the most abundant species in all treatments. *C. pelagica*

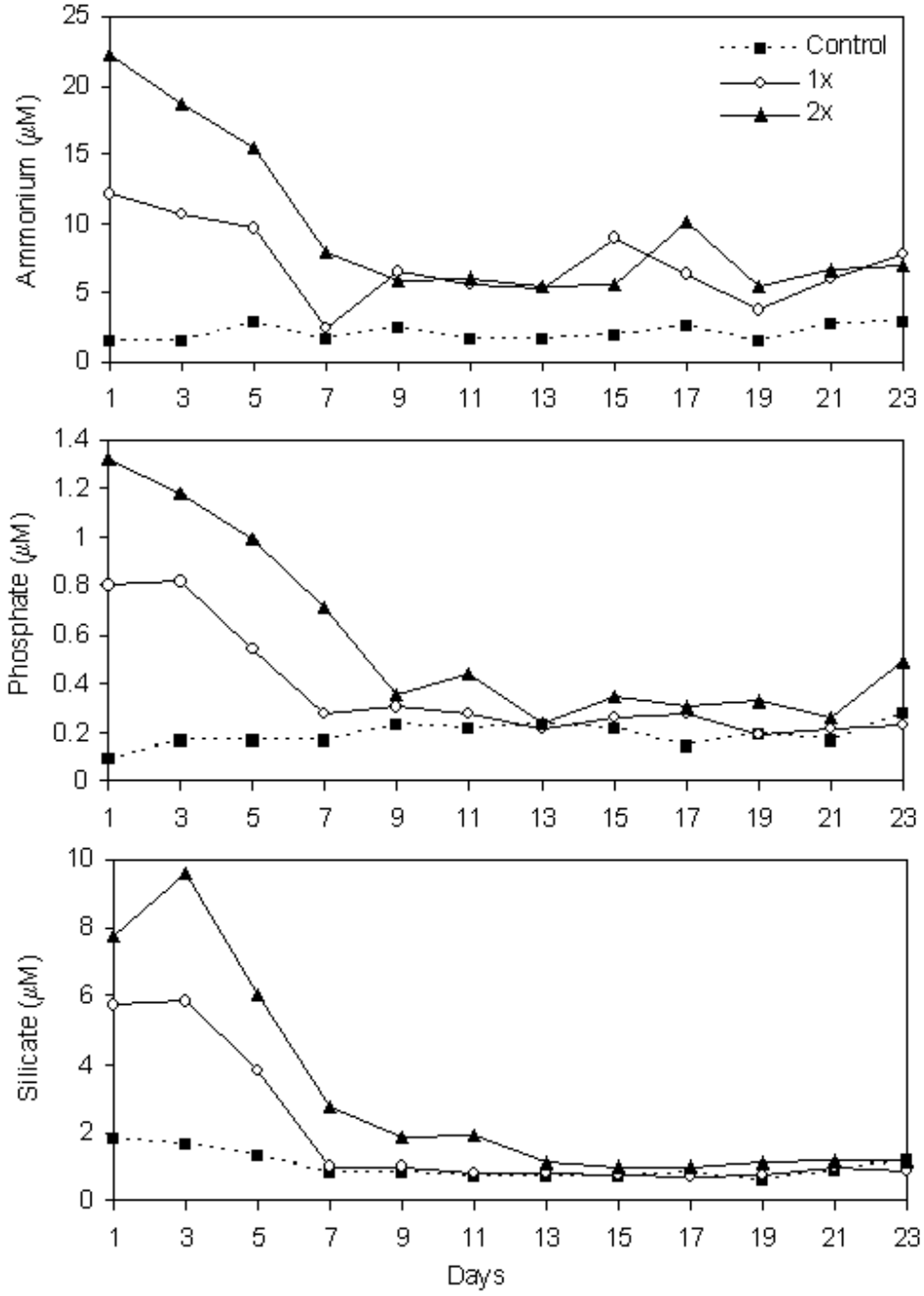

Fig. 1. Variations of nutrient concentrations in the experimental treatments.

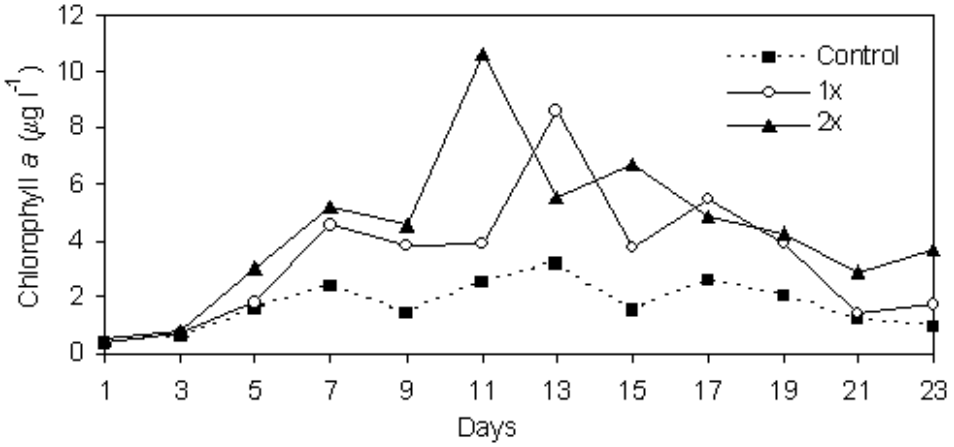

Fig. 2. Variations of chlorophyll *a* concentrations in the experimental treatments.

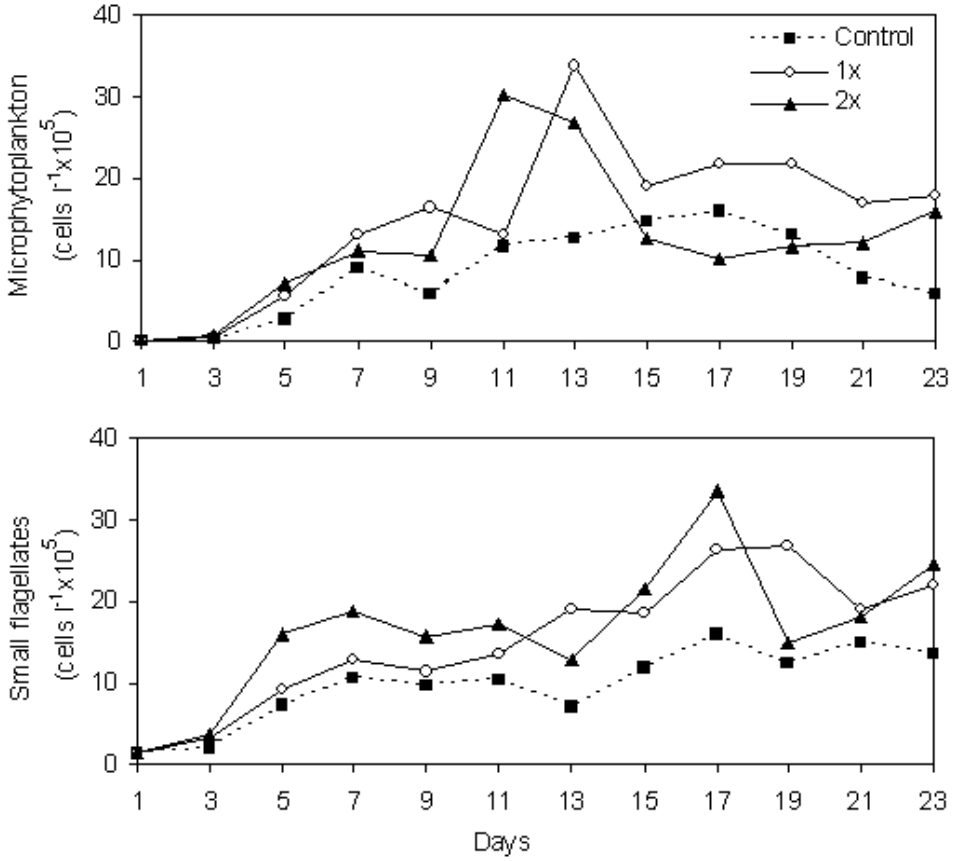

Fig. 3. Variations of microphytoplankton and small flagellate abundance in the experimental treatments.

started to increase from day 6 until day 11 in 2x treatment and day 13 in 1x treatment. The maximum cell number of *C. pelagica* reached $29.3x10^5$ cells l^{-1} on day 13 in 1x treatment. From day 13 on, diatoms decreased in treatments of 1x and 2x. The decrease of diatoms contributed to an increase of the small flagellates. From day 3 on, small flagellates (<10µm) increased in both treatments (Fig. 3). From then on, small flagellates reached their maximum concentration as $33.4x10^5$ cells l^{-1} in the treatment 2x on day 17. The small flagellates were most stimulated in the 2x treatment than the 1x treatment (Fig. 4). However, no significant differences were observed in small flagellate cell numbers among treatments ($p=0.071$). After the maximum cell number of small flagellates on day 17, values decreased sharply in 2x treatment, but towards the end of the experiment, cell numbers increased. Their cell numbers in the 1x treatment decreased after day 19, but as was observed in 2x treatment, they slightly increased at the end of the experiment.

Discussion

The enrichment of coastal phytoplankton communities with nutrients in enclosed mediums results in a phytoplankton increase (Oviatt *et al*., 1989; Berdalet *et al*., 1996). In the present study, community biomass increased after the enrichment in all treatments. Species composition was not significantly different in control and nutrient treatments. Both nutrient treatments were not dramatically different in term of phytoplankton abundance. On the other hand, phytoplankton abundance was lower in the controls than in the nutrient treatments (Fig.4). However, abundance in the controls was higher than those observed in the field in summer period (Polat *et al*., 2005). The increase even in no nutrient added controls suggests that conditions in the tanks contributed to the increase in abundance. The initial peak was caused by microphytoplankton (mainly diatoms). This result supports the view that diatoms have rapid growth rate and tend to thrive in high nutrient concentrations (Caroppo, 2000; Carter *et al*., 2005). On the other hand, dinoflagellates were low in abundance during the experiment. But, their abundance was relatively higher in the late phase of the experiment. This result is consistent with the general succession pattern in the natural environments (Margalef, 1958) as dinoflagellates could not compete with diatoms in the nutrient rich environments.

Diatoms decreased after the nutrient limitation in the treatments. A regeneration pattern of silicate was not observed during the experiment, whereas slight increases of N and P showed the regeneration of these nutrients. These conditions resulted in the relative dominance of small flagellates in the second half of the experiment. These results are similar to the findings of Officer & Ryhter (1980). They reported that silicate is the controlling nutrient in the shift of community from diatoms to flagellates. On the other hand, it is suggested that the increase in flagellates is set by phosphorus (Escavarage *et al*., 1996). Small flagellates started to increase in the first week in the presence of P, but they reached their peak on day 17 after decrease of diatoms. This situation could be attributed to a competition between small flagellates and diatoms for nutrients. Smaller size is better in competition for nutrients due to a higher surface area to volume ratio and faster growth rates (Carter *et al*., 2005). While microphytoplankton mainly depends on new nutrients, small-sized phytoplankton such as nanoflagellates can use regenerated nutrients (Ferrier & Rassoulzadegen, 1991). The peak in the second half of the experiment indicates that small flagellates outcompeted the microphytoplankton under nutrient limited conditions probably due to their advantages mentioned above.

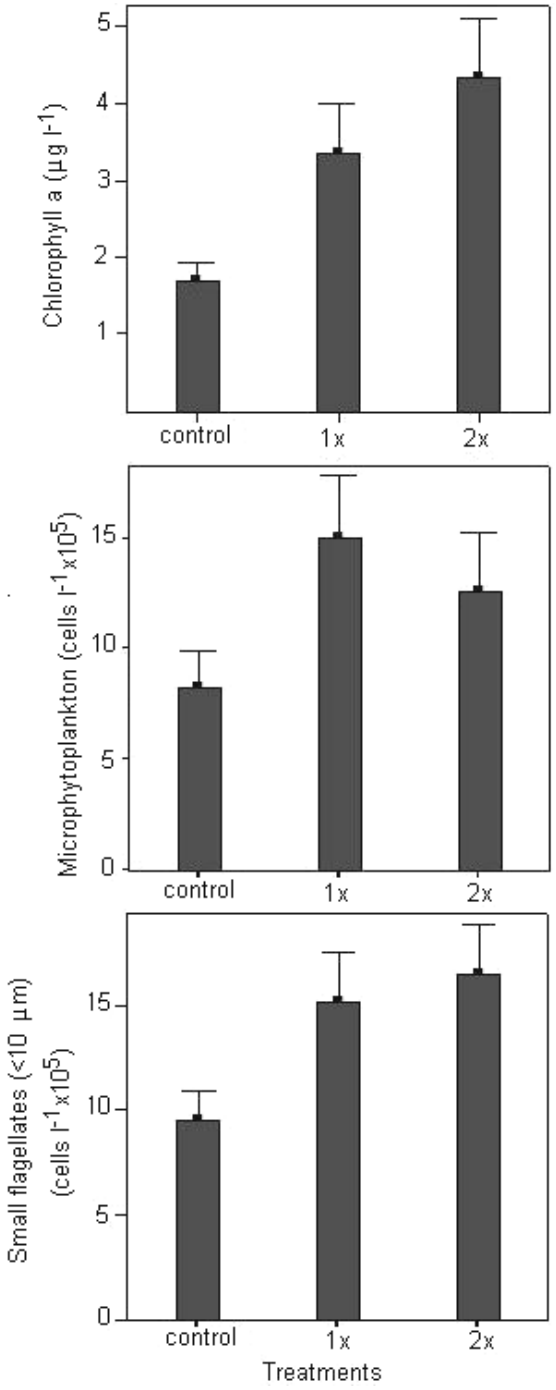

Fig. 4. The mean chlorophyll *a* concentrations, microphytoplankton and small flagellate abundances in the experimental treatments (error bars show mean ± 1 SE).

Bioassay studies in the eastern Mediterranean as well as nutrient stoichiometry have suggested that biological production in the region is limited by phosphorus (Kress *et al*., 2005). However, in a microcosm experiment conducted by Zohary *et al*., (2005) upon the addition of P alone, phytoplankton assimilated P, but the phytoplankton did not grow until N was also added. Moreover, according to Kress *et al*., (2005) the addition of P alone stimulates photosynthetic rate, but the highest photosynthetic rate occurred by adding P and N together. This situation could be attributed to the fact that the higher additions of P alone pushed the system to N-limitation. Thus, they pointed out that the phytoplankton was co-limited by N and P. In the present study, the addition of P alone was not applied. However, the results of this study and previous studies conducted by Brett *et al*., (1999) and Kress *et al*., (2005), revealed the addition of N and P together stimulated the phytoplankton increase.

In conclusion, the results were in accord with the classical model in term of the response of phytoplankton community to nutrient enrichment. The peak of microphytoplankton occurred first, after nutrient addition. On the other hand, the most prominent increase of flagellates observed after the decrease in the diatom dominated microphytoplankton. Significant differences were not found among abundance of phytoplankton in either enrichment. However, microphytoplankton maximum occurred in the 1x treatment whereas small flagellate maximum occurred in the 2x treatment. Taking these processes into account it can be suggested that, microphytoplankton abundance increased up to a special level with the increase of nutrients, but after this point, an important increase of microphytoplankton no longer occurred. It is also important to conclude that, species composition and succession patterns of coastal phytoplankton community investigated were similar in different treatments.

References

Azov, Y.1991. Eastern Mediterranean-A marine desert? *Mar. Pol. Bull*., 23: 225-232.

Berdalet, E., C. Marrase, M. Estrada, L. Arin and M. Maclean. 1996. Microbial community responses to nitrogen and phosphorus deficient nutrient inputs: microplankton dynamics and biochemical characteriazation. *J. Plankton Res.,* 18(9): 1627-1641.

Brett, M.T., F.S. Lubnow, M. Villar-Argaiz, A. Müller-Soller and C.R. Goldman. 1999. Nutrient control of bacterioplankton and phytoplankton dynamics. *Aquatic Ecol.,* 33: 135-145.

Caroppo, C. 2000. The contribution of picophytoplankton to community structure in a Mediterranean brackish environment. *J. Plankton Res*., 22: 381-397.

Carter, C.M., A.H. Ross, D.R. Schiel, C. Howard-Williams and B. Hayden. 2005. *In situ* microcosm experiment on the influence of nitrate and light on phytoplankton community composition. *J. Exp. Mar. Biol. Ecol*., 326: 1-13.

Duarte, C.M, S. Agusti and N.S.R. Agawin. 2000. Response of a Mediterranean phytoplankton community to increased nutrient inputs: a mesocosm experiment. *Mar. Ecol. Prog. Ser*., 195: 61-70.

Escavarage, V., T.C. Prins, A.C. Smaal and J.C.H. Peeters. 1996. The response of phytoplankton communities to phosphorus input reduction in mesocosm experiments. *J. Exp. Mar. Biol. Ecol*., 198: 55-79.

Ferrier, C. and F. Rassoulzadegen. 1991. Density-dependent effects of protozoans on specific growth rates in pico- and nanoplanktonic assemblages. *Limnol. Oceanogr*., 36: 657-669.

Hasle, G.R.1978. The inverted-microscope method. In: *Phytoplankton Manual*. (Ed.): A.Sournia, UNESCO, pp. 88-96.

Kress, N., T.F. Thingstad, P. Pitta, S. Psarra, T. Tanaka, T. Zohary, S. Groom, B. Herut, R.F.Z. Mantaura, T. Polychronaki, F. Rassoulzadegen and G. Spyres. 2005. Effect of P and N addition to oligotrophic eastern Mediterranean waters influenced by near-shore waters: A microcosm experiment. *Deep-Sea Res. II*., 52: 3054-3073.

Krom, M.D., N. Kress, S. Brenner and L.I. Gordon. 1991. Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. *Limnol. Oceanogr*., 36: 424-432.

Margalef, R.1958.Temporal succession and spatial heterogenity in phytoplankton. In: *Perspective in Marine Biology*. (Ed.): A.A. Buzzti-Traverso, Univ. California Press, pp. 323-349.

Officer, C.B and J.H. Ryhter. 1980. The possible importance of silicon in marine eutrophication. *Mar. Ecol. Prog. Ser*., 3:83-91.

Oviatt, C., L. Patricia, F. Franch III and P. Donaghay. 1989. Phytoplankton species and abundance in response to eutrophication in coastal marine mesocosms. *J. Plankton Res.*, 11(6): 1223-1244.

Parsons, T.R., Y. Maita and C.M.Lalli. 1984. A *Manual of Chemical and Biological Methods for Seawater Analysis*, Pergamon Press, Oxford.

Polat, S., A. Akiz and M.P. Olgunoğlu (Piner). 2005. Daily variations of coastal phytoplankton assemblages in summer conditions of northeastern Mediterranean (Bay of İskenderun). *Pak. J. Bot.*, 37(3): 715-724.

Rampi, L and M. Bernhard. 1980. *Chiave per la Determinazione delle Peridinee Pelagiche Mediterranee.* CNEN-RT/BIO (80) 8.

Ricard, M.1987. *Atlas Du Phytoplancton Marin, vol: II, Diatomophycees*, Editions Du Centre National de la Resherche Scientifique, Paris.

Sournia, A.1986. *Atlas Du Phytoplancton Marin. vol I: Introduction, Cyanophycées, Dictyochophycées, Dinophycées et Raphidophycées*. Éditions du Centre National de la Recherche Scientifique, Paris.

Strickland, J.D.H and T.R. Parsons. 1972. *A Practical Handbook of Seawater Analysis*. Bull. Fish Res. Board. Can.,167, Ottawa.

Tomas, C.R.1997. *Identifying Marine Phytoplankton*, Academic Press, New York.

Tregouboff, G and M. Rose.1957. *Manuel De Planktonologie Mediterraneenne. I, II*. Centre National de la Resherche Scientifique, Paris.

Zohary, T and R.D. Robarts. 1998. Experimental study of microbial P limitation in the eastern Mediterranean. *Limnol. Oceanogr*., 43: 387-395.

Zohary, T., B. Herut, M.D. Krom, R.F.C. Mantaura, P. Pitta, S. Psarra, F.Rassoulzadegen, N. Stambler, T. Tanaka, T.F. Thingstad, E. Malcolm and S. Woodward. 2005. P-limited bacteria but N and P co-limited phytoplankton in the eastern Mediterranean - a microcosm experiment. *Deep-Sea Res. II*, 52: 3011-3023.

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