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The Influence of Changes in Nutrient Ratios on Several Biological Processes in Inner Bay of Izmir

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Abstract

The dilution method was employed in Inner Bay of Izmir in order to measure the phytoplankton community potential growth rate and grazing rate in May 2005. In this study, the percentage of primary production consumed via grazing and the significance of grazing in food chain were emphasized. The dilution technique was studied on the natural sea water enriched with various Si:N and N:P ratios. Nitrogen limitation was observed in Si:N experiment series. The consumed percentage of primary production via grazing increased with the rising Si:N ratio while the grazed percentage of biomass was decreased. The net growth rate of phytoplankton was greater than the grazing rate at a Si:N ratio of 0.34 and 1.31. For greater ratios, grazing rate became higher than the net growth rate. From the data obtained, it was concluded that microplankton is able to keep the size of the algal populations around the steady state. The Si:N ratio in İzmir Bay is approximately 6 on May 18, 2005. Because of the nitrogen treatment that tends to increase this ratio in the sea water, microzooplankton grazing must limit phytoplankton blooms. The changes in Si:N ratios can account for the 83-98% of the taxon-specific grazing. *Prorocentrum gracile*, *Cylindrotheca closterium* and picoplankton were the most abundant species observed constantly both in Si:N ratio and N:P ratio series. Quite few and negative *in situ* growth rates revealed that nutrient limitation was also of great significance besides grazing on phytoplankton. The N:P ratio of 4 in the environment indicated that *Prorocentrum gracile* and *Cylindrothece closterium* would be consumed via grazing and picoplankton would be under pressure by nutrient limitation.

Key words: Si:N, N:P, primary production, growth rate, grazing.

Introduction

Because of the fact that C:N:P ratios taken up by organisms especially phytoplankton come back to the marine environment by their decomposition, these ratios represent also marine C:N:P ratios (Redfield *et al*., 1963). This ratio is known to be 106:16:1 as mean value. However, the aforementioned ratios in marine systems may vary depending on species distribution, biochemical processes, territorial discharges, atmospheric precipitation and fluxes from sediment.

The eastern basin of the Mediterranean Sea has more oligotrophic characters compared to the other oceans. Aegean Sea also exhibits similar features. In contrary to the fresh water environments (Phosphorus limitation), nitrogen limitation is the most encountered condition in marine environments. However, Eastern Mediterranean Sea is an exception. Phosphorus limitation was demonstrated by several authors (Krom *et al*., 1991; 1992; Berland *et al*., 1995; Tselopides *et al*., 2000; Ignatiades *et al*., 2002; Büyükışık *et al*., 2003). Nevertheless, some of the bays in the Aegean Sea differ from this general characteristic due to the impact of anthropogenic inputs. Nitrogen limitation for İzmir Bay was reported by Büyükışık and Erbil (1987) and Büyükışık (1986).

The nutrients from allochthonous sources enter into marine environments but with different ratios from those in sea. Thus a regional heterogeneity is observed for the distribution of nutrient concentrations and the nutrient ratios from these sources have an influence on species composition and succession pattern (Tilman *et al*., 1986; Suttle and Harrison, 1988; Estrada *et al*., 1996). Various factors such as load rates, the residence time of water, nutrient fluxes from sediment to water, atmospheric precipitation, fresh water input, thermal stratification and turbulence play important roles in maintenance of the species diversity. In these complex relationships, in order to determine what sort of changes occurred on size distribution of phytoplankton community, community growth rates and microzooplankton grazing rates under varying nutrient ratios that exist in sea water, two series of nutrient enrichment experiments were carried out by means of the dilution method.

Since the suggestion of dilution method by Landry and Hassett (1982) it has been used various researchers (Landry *et al*., 1995; Landry *et al*., 2000; Gaul and Antia, 2001; Verity *et al*., 2002) as it provides several advantages over the other techniques. One of the most important advantages is that less manipulation is required on experimental organisms. Also the other advantages are to be obtained in the phytoplankton community potential growth rate (or taxon specific potential growth rates) and grazing rates measurements. This method has been improved. Gallegos and Vant (1996) used the dilution method with 14ºC incubations in order to find out the C/Chl ratios of phytoplankton. It was also utilized for determining pigment specific grazing rates and selectivity indexes (Strom and Welschmeyer, 1991; Gaul and Antia, 2001). It is suggested by researchers that by minimizing the weak sides such as the restriction of the method by certain environmental conditions and its limitation to particular organisms and by emphasizing the strong sides of the method, its use continue until better methods are developed (Paranjape, 1990).

Materials and Methods

Izmir Bay is a natural Bay inlet that opens to the Aegean Sea on the western coast of Turkey. It covers an area of 410.3 km^2 between the latitudes 38º20' N, 38º40' N and the longitudes 26º30' E, 27º10' E.

Izmir Bay consists of three regions: the outer, middle and inner bays. Outer is bay 20 km wide between Karaburun and Foça extends 45 km in a NE-SW direction. Uzun Island and Urla Peninsula divide the outer bay into two parts with a ~6 km wide western region (Mordogan Strait and Gulbahce Bay) and ~12 km wide southern region (Outer bay). The middle bay of ~10 km long is separated from inner bay by Yenikale strait which is a quite narrow channel with a depth of 13 m. This shallow gate has emerged in the last few centuries due to delta shifts that took place at the Pelikan and Karsiyaka banks of the Gediz River. The depth of water is generally below 15 m in the inner bay (Basoglu, 1975). The study area is located in the inner bay.

The sea water sample was taken from the study station shown in the Figure 1 from a depth of 0.5 m into 20 L plastic cans, by means of a diaphragm pump on 12.05.2005 and 25.05.2005. With the help of wet white cotton wrapped around the cans, the temperature inside the cans were maintained stable until they were transferred to the laboratory. The samples taken to the laboratory were filtered through a 0.22 µ pore-sized Sartorius filter capsule and were used as organism-free sea water for dilutions. The filtration efficiency was tested by means of fluorometer by measuring the chlorophyll-a concentration. All the containers were washed prior to the experiments with 10% HCl solution prepared by ultra pure water and then they were cleaned by rinsing three times with ultra pure water. 1 L of sea water filtered for the first time was not used to remove HCl residues. Nutrients were added to the bottles and this was followed by additions of filtered sea water and natural sea water according to the dilution ratios (0.20, 0.45, 0.70, 1). Silicate was maintained at f/2 medium final concentration in order to provide diverse Si:N ratios. Nitrogen was added in various amounts as NH$_4$Cl. *In situ* nutrient concentrations were included to the estimate and Si:N ratios were determined to be 0.25, 1, 4.1, 6.9 and 13.3. The sea water sample obtained on 25.05.05 were processed as described above and the nutrient concentrations were adjusted to the N:P ratios (1.5, 4.9, 9.1, 13.5, 17.6) and Si and P were at the f/2 medium final concentrations. *In vivo* chlorophyll-a concentrations were measured for all the bottles at the onset and 1 day later by using a Turner Design 10 AU field Fluorometer.

Nutrient additions for Si:N series were constant with Si 107 µg at Si/L, P 33 µg at P/L and the final N concentrations varied from 10 µg at N/L to 400 µg at N/L. At the N:P series, P was constant and N varied from 33 µg at N/L to 660 µg at N/L.

In situ phytoplankton mean growth rate (μ_0) was calculated from the (100% natural sea water) net growth rate and grazing rate in the nutrient-free undiluted sample (Verity *et al.*, 2002). The grazing

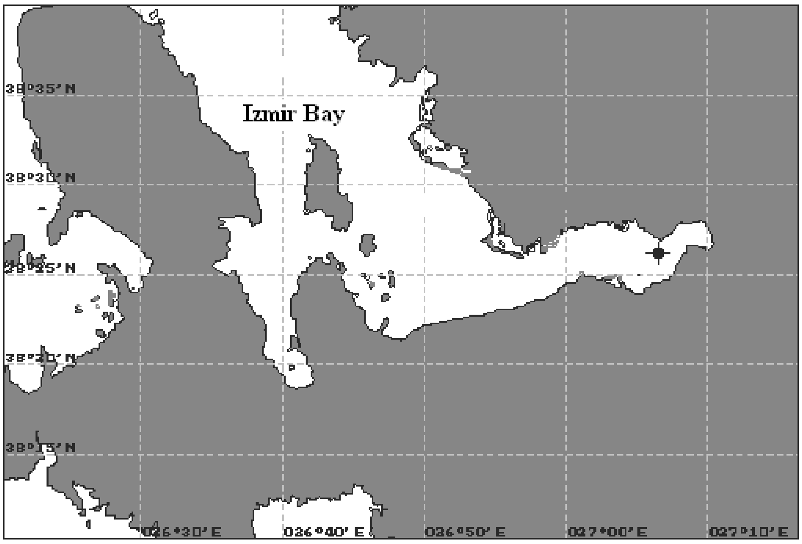

Figure 1. Sampling station (taken and modified from Sunlu *et al.*, 2006).

loss of phytoplankton (G) and biomass production (PP: Primary production) were estimated according to Landry *et al.* (2000).

Among the physico-chemical environmental parameters, the water temperature was determined by means of ATC prope of digital pH meter electronic thermometer (Hanna HI 8314) with a 0.1ºC precision and the salinity values were estimated by the Harvey method (Martin, 1972). The water sample obtained from the sampling station was taken to polyethylene sampling bottle of 1 L and transferred to the laboratory for nutrient analysis. Nutrient analyses were performed following Strickland and Parsons (1972) and expressed in terms of µg-at/L. It was followed by nitrate (NO_3^-), ammonium (NH_4^+), reactive phosphate and silicate (SiO_2) measurements performed via Bosch-Lomb Spectronic 21 UVD model spectrophotometer according to Strickland and Parsons (1972), Wood (1975) and Parsons *et al.* (1984). *In vivo* chlorophyll-a concentrations measured according to Lorenzen (1966), Madden and Day (1992) using 10 AU Model Turner Designs fluorometer. Nutrient analyses were performed following Strickland and Parsons (1972) and expressed in terms of µg-at/L.

Net growth rates were calculated via the formula $k=(1/\Delta t)*\ln(C_t/C_0)$ (Guillard, 1973) based on chlorophyll-a. The dilution graphic was evaluated according to Landry and Hasset (1982). Grazing rate and potential growth rate were estimated from the slope and intercept of the line respectively.

Results and Discussion

Dilution experiments were conducted based on *in vivo* chlorophyll-a by using the net growth rates. Mean maximum growth rates and grazing rates of the community (Figure 2-5), grazed percentage of primary production and grazing percentage of biomass were estimated. When the grazed percentage of primary production was 57.8% day^{-1} at a Si:N ratio of 0.25, it raised to 97.05% day^{-1} with the increase of the Si:N ratio. Daily grazed percentage of phytoplankton biomass was however decreased from 96.0% day^{-1} to 73% day^{-1} with the increase of the Si:N ratio. In low ranges of Si:N ratios, an increase in flagellates preferred by ciliates was reported from the Helgoland coastal waters by Pearl (1997). This was

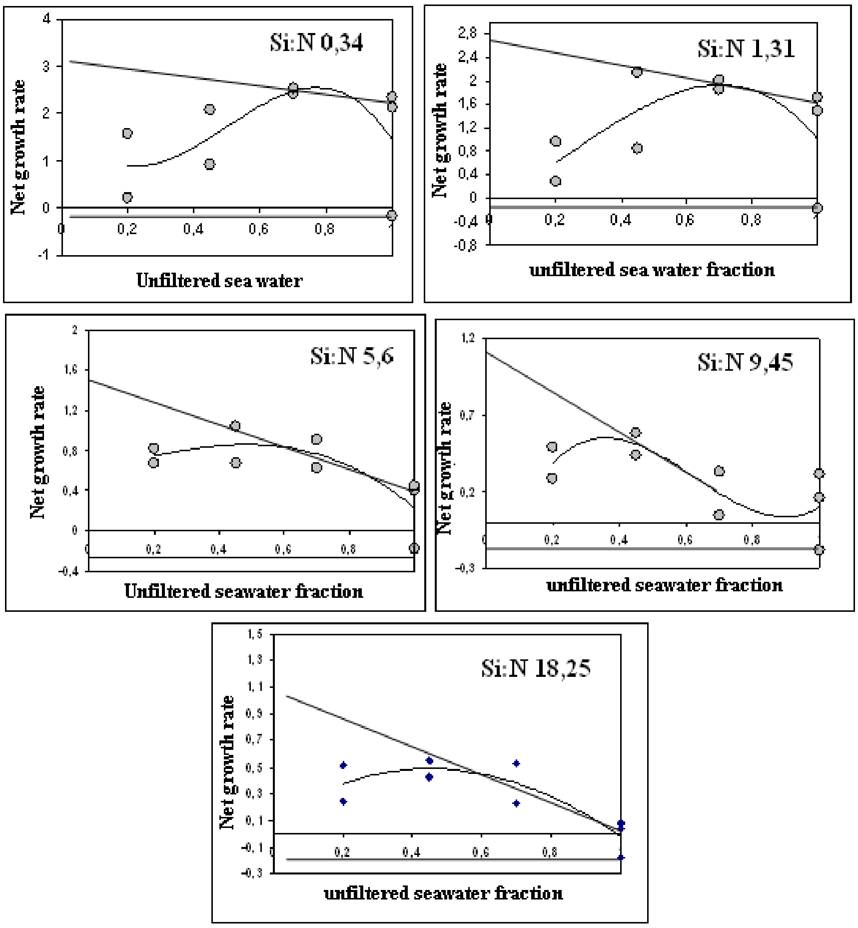

Figure 2. Dilution experiments performed via enrichment by various Si:N ratios for the water samples taken on 12.05.2005.

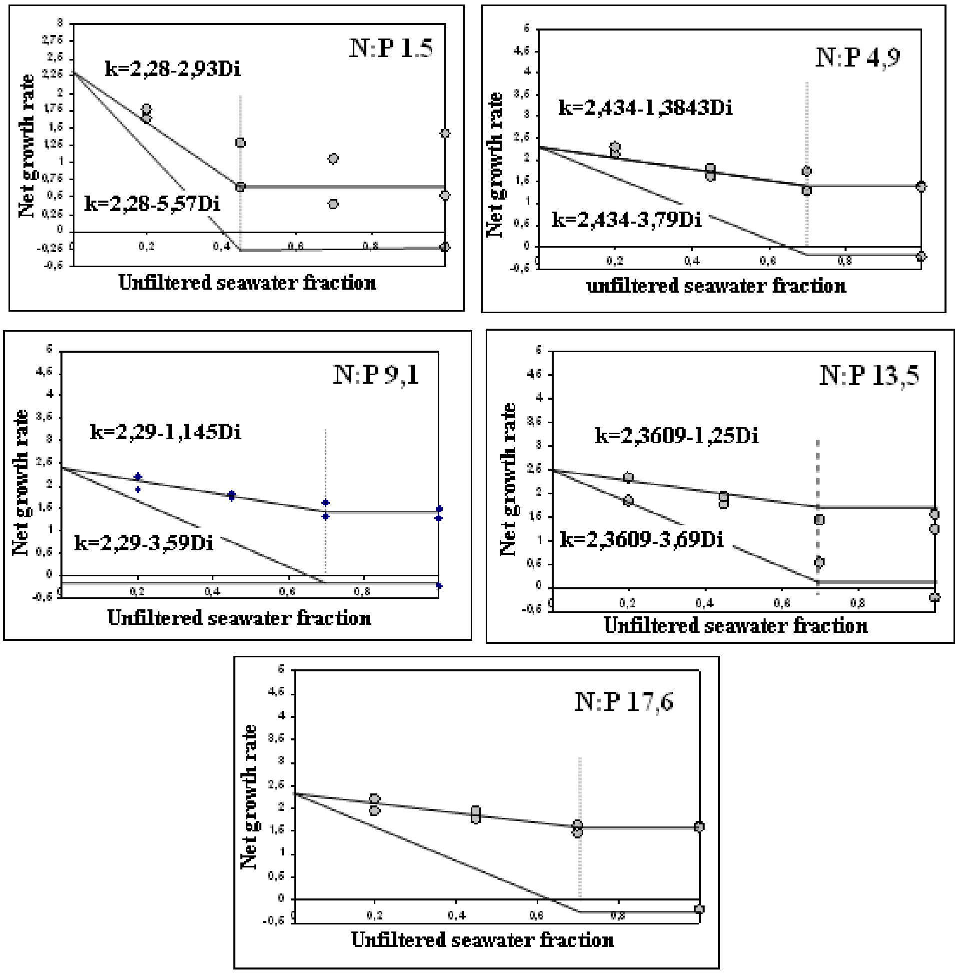

Figure 3. Dilution experiments performed on natural sea water enriched with various N:P ratios on 25.05.2005.

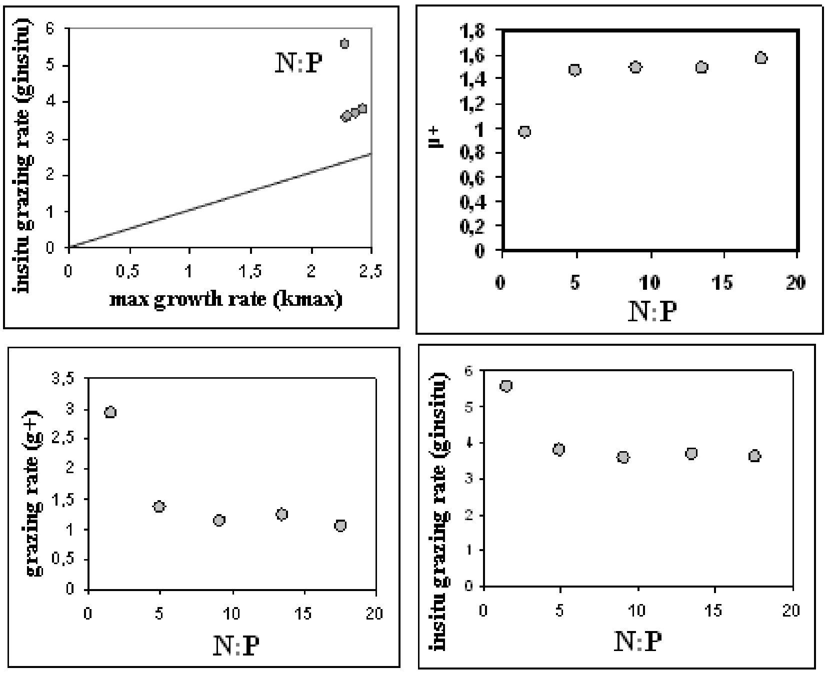

Figure 4. The changes observed in kmax, g *in situ* μ+ and g+ under different N:P ratios.

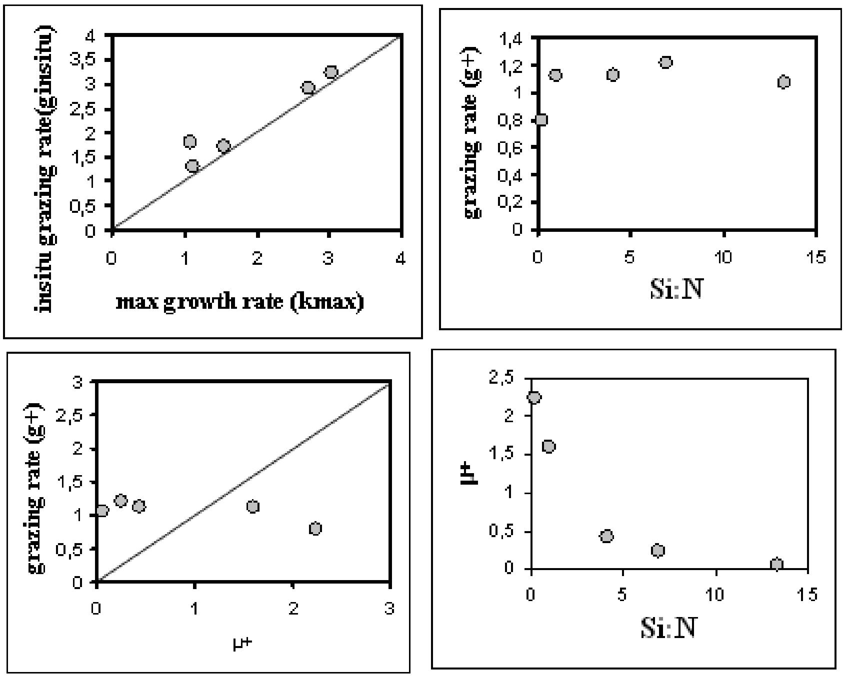

Figure 5. The changes observed in kmax, g *in situ* μ+ and g+ under different Si:N ratios.

attributed to the increase in percentage of grazed biomass to 96% day^{-1} under low Si:N conditions.

The situation, when the linear correlation disappeared may be attributed to food saturation or to that an evident microzooplankton proliferation rate was only observed under a Si:N ratio of 9.45 for the dilution ≥70%. With the increase of Si:N ratio, the maximum growth rates decreased from 3.04 day^{-1} to 1.13 day^{-1}. At Si:N ratios of 1.31 and 0.34, the net growth response (μ+) of the communities to the nutrient enrichment was more than the grazing rate (g+) (Figure 2). For greater ratios, grazing rate exceeded growth rate. However, the results just above the linear 1:1 steady state line between *in situ* grazing rate (g *in situ)* and k_{max} indicate that microplankton is able to keep the algal populations around the steady state.

Low Si:N ratios reveal to favor bloom of phytoplankton in the bay. Since silicate is stable at f/2 concentration in these Si:N ratios, increasing N concentrations will result in accumulation of phytoplankton biomass. Nitrogen removal in treatment facilities causes the ratio to increase by which the phytoplankton stock will be regulated due to grazing and N limitation. Thus, the Si:N value of the bay is approximately 6 and it may be stated that grazing exceeded the growth rate of the phytoplankton communities. Indeed, the negative μ *in situ* value demonstrates that phytoplankton populations exhibit an intensive grazing in the bay.

The condition that linear correlation disappeared at a 45% dilution may be attributed to the grazing saturation at an N:P ratio of 1.5 or to the marked microzooplankton growth rate. However, this was observed at 70% for N:P ≥ 5 (Figure 3). In case of a feeding saturation, a change in the prey species or prey size should be considered (Frost, 1972). Otherwise it will indicate the increasing microzooplankton growth rates. With the rise of the N:P ratios, the daily grazed percentage of PP declined from 81.5 % day^{-1} to 58.06 %day^{-1}. However, the grazed percentage of biomass did not reveal a significant change (91.8-92.97%). No clear change was also observed for the maximum growth rates of phytoplankton community (2.28-2.43 day^{-1}) demonstrating that no nutrient limitation exists in the environment. Whereas the net growth rates (μ+) in the nutrient enriched sea water was approximately 0.96 day^{-1} for N:P <5, it inclined to 1.5 day^{-1} at N:P >5 and remained stable (Figure 4). In compliance with our findings, Molina *et al.* (1991) reported that the growth rate of *Tetraselmis* sp. increased with the raising N:P ratio to 5 and than remained stable.

Heavy grazing at an N:P ratio of 1.5 indicates that the biomass of phytoplankton population will rapidly decrease. A positive linear relationship was evident between k_{max} and g *in situ* at the other N:P ratios. However, the experimental points were above the steady state line (1:1). The low N:P ratio of the environment is approximately 4 indicating that grazing will rapidly deplete the phytoplankton biomass. The N:P ratio of 3.5 reported by Küçüksezgin *et al.* (2005) for Inner Bay demonstrates that grazing will be dominant throughout the year. The ratio remaining below normal values is attributed to the treatment of N but not P.

The nutrient concentrations and *in situ* temperatures of the sea water samples taken on different dates are shown in Table 1. The most important difference between these two samplings is that the nitrate content of the sample taken on 25th May was 40 times greater than that of the sample obtained on 18th May. The other nutrient concentrations were almost identical.

In the dilution experiments performed without phosphate addition at different Si:N ratios, nutrient limitation was observed for low animal concentrations. The results were in consistency with the interpretations of Sterner (1985) for his modelling results. Net growth rates inclined with the increase of animal concentration. At 45-70% animal concentrations, a dilution line with an inverse correlation was obtained as proposed by Landry and Hasset (1982). The increase of animal concentration results with a rise in the nutrient excretion of the animals and reduces the limitation. However, no limitation is seen at 45-70% dilution whereas an inverse correlation is observed with the increase of the dilution factor. By extrapolating these lines, µmax values were determined. This situation demonstrates that under *in situ* animal concentrations, the ammonium and phosphate excretions of the animals and the regenerated phosphate will not be limited for phytoplankton in the bay. As comes to the dilution lines, the fact that greater net growth rates were observed for those with nutrient addition (Si and N) compared to those free of a nutrient addition reveals the limiting effect of silica and/or nitrogen. In case nitrogen is considered to be introduced to the environment by regenerating from zooplankton excretions, silicate in phytoplankton communities may be deemed as limiting in samples which did not receive additional nutrient. The predominance of diatom population in the phytoplankton community is a characteristic and the limiting effect of silica may be attributed to the occasional re-direction of water from Izmir Treatment Facility towards Foca rather than Inner Bay of Izmir. The water output of the treatment facility and the bottom waters of Inner Bay provide silica to water column in certain periods (Büyükışık and Erbil, 1987).

The results of the dilution experiments for *Prorocentrum gracile* (*P. gracile), Cylindrotheca closterium (C. closterium)* and picoplankton which are dominant in natural sea water are given in Table 2a, 2b. At Si:N ratios of 0.34 and 1.31 under 45% natural sea water conditions a grazing saturation was seen for *P gracile*. Grazing threshold was observed at Si:N 9.45 up to the 45% natural sea water fraction. For picoplankton, grazing saturation was detected at Si:N 0.34 and Si:N 1.31 under 45% and 70% natural sea water conditions, respectively.

The grazing rate on *P. gracile* was quite low at N:P 1.47 and the maximum growth rate of the species was at negative levels. Beginning from N:P 4.87 the maximum growth rate inclined from 1.32 day^{-1} to 2.24 day^{-1}. The maximum grazing rate was found to be 4.80 day^{-1} at N:P 9.12. The saturation was seen at 45% dilution and N:P 9.12 whereas it was detected at N:P 13.5 and 17.62 and 70% dilution. The maximum growth rate of *C. closterium* increased from 0.238 day^{-1} to 1.16 day^{-1} at N:P 4.87, 1.769 day^{-1} at N:P 9.12 and to 2.08 day^{-1} at 17.62. The grazing rate rose to a level of 2.86 at N:P 9.12. No grazing saturation was observed. The maximum growth rate increased beginning from -0.4683 day^{-1} at N:P 1.47 and reached to a maximum of 3.41 day^{-1} at N:P 9.12 then decreased to 1.67 day^{-1} at N:P 17.62.

The maximum growth rate of *P. gracile* is invariably lower than grazing rate (Figure 6a) and the difference of grazing rises more at lowest Si:N ratios. Experimental points significantly deviate from steady state except that at Si:N 9.45. At the lowest Si:N

Table 1. The physico-chemical parameters of sea waters

	18.05.2005	25.05.2005
NO_3^- -N	0.49	19.85
NO_2^- -N	2.06	2.35
NH_4^+	1.97	1.97
Si	30.27	34.87
PO_4^{-3} -P	5.95	5.84
T°C	21	21

Table 2. Maximum net growth rates (d^{-1})and grazing rates (d^{-1}) obtained from regression for different Si:N (a) and N:P (b) ratios

	Prorocentrum gracile			*Cylindrotheca closterium*			Pikoplankton		
Si:N	k_{max}	G+	R^2	k_{max}	G+	R^2	k_{max}	G+	R^2
0.34	1.5497	5.6236	0.8731	0.8409	1.4262	0.1323	1.7771	6.4218	0.9978
1.31	0.7060	1.9124	0.2988	0.6004	0.7800	0.0647	1.9521	3.2855	0.2245
5.6	0.2375	1.0254	0.3897	0.3363	1.1016	0.4012	0.5166	1.4985	0.3546
9.45	1.0394	1.4684	0.4133	2.1318	2.5195	0.4947	0	0	
18.25	-0.00058	0.7338	0.3078	2.5507	3.3181	0.7695	-0.2264	0.0486	0.0006
N:P									
1.47	-0.2068	0.0254	0.0004	0.2380	1.3793	0.7612	-0.4683	0.0540	0.0069
4.87	1.3272	2.5414	0.7227	1.1693	2.8650	0.6067	0.4694	0.7725	0.3462
9.12	0.9229	4.8045	0.8704	1.7963	3.0001	0.6371	3.4142	8.5760	0.9428
13.5	2.0095	4.0465	0.7985	0.9411	1.3872	0.2956	2.6719	5.6441	0.9953
17.62	2.2455	3.5163	0.9638	2.0846	1.1806	0.1104	1.6727	1.5752	0.3788

ratios, when the environment is not nitrogen-limited for this species or at least a final limiting effect of silicate is evident on diatoms, the deviations from stability reache to maximum levels.

Potential primary productivity (PPP) is observed to reach two maximums at Si:N 0.34 and 9.45. However, PPP significantly decreases at the other Si:N ratios. It may result from optimum N requirement at Si:N 9.45 whereas it may be due to their lead over diatoms at lower Si:N ratios (Figure 6b).

All the experimental points are around the line of steady state, however, indicating excessive grazing. Since Si concentrations are at f/2 concentration, it may be suggested that *C. closterium* has a grazing level around the steady state. With the change in Si:N ratio, grazing closely follows the maximum growth rate of *C. closterium* (Figure 7a, 7b).

Since Si is constant and at f/2 concentration for all the ratios, the growth rate of *C. closterium* is limited by nitrogen whereas *P. gracile* and picoplankton is not affected. In case Si:N ratio is 9.45 or more, the limiting effect of N appears to be more important than grazing pressure (Figure 8a, 8b).

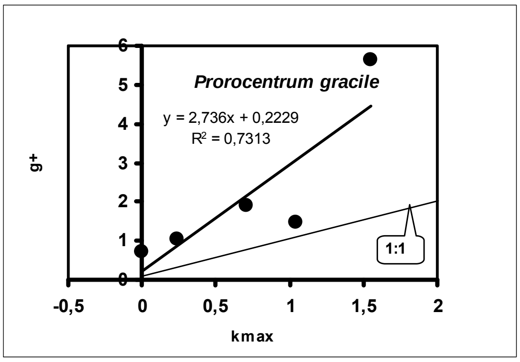

Figure 6a. The relationship between the maximum growth rate and grazing rate of *Prorocentrum gracile* at various Si:N ratios. 1:1 straight line exhibits the steady state line.

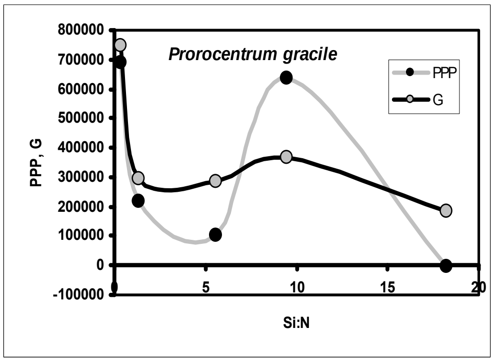

Figure 6b. Changes of potential primary productivity and grazing (*P. gracile* cells/L.day) in relation to Si:N ratios.

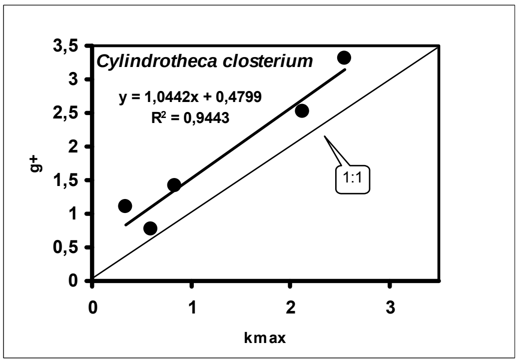

Figure 7a. Distribution of the maximum growth and grazing rates of *Cylindrotheca closterium.* The line shown with 1:1 implies the stable line.

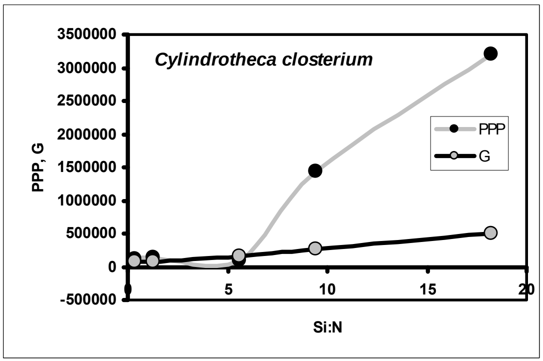

Figure 7b. Changes of potential primary productivity and grazing (*C. closterium* cells/L.day) in relation to Si:N ratios.

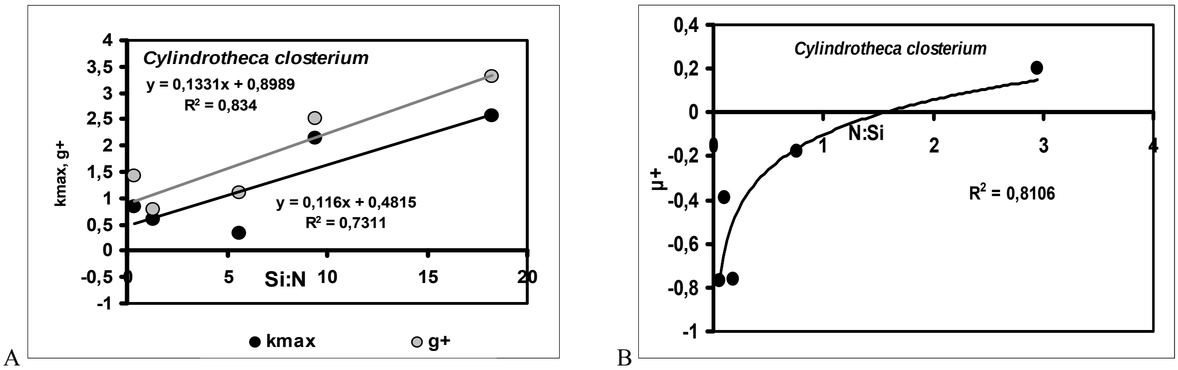

Figure 8. The changes in maximum growth rate and grazing rates of *Cylindrotheca closterium* with the changes in Si:N ratios (A). The changes in net growth rates of *C closterium* with N:Si ratios (B).

The difference between g+ and k_{max} is maximum at lowest Si:N ratios for picoplankton and gets alienated from the line of steady state in favor of grazing (Figure 9). No correlations exist between the net growth rate and N:Si ratios (Figure 10). The changes in N:Si ratios are able to account for 91% and 46% of the changes in grazing rate and maximum growth rate, respectively.

Values nearest to the steady state line are obtained at N:P 1.47 and the deviations are significantly greater in favor of grazing at higher ratios (Figure 11). Grazing reaches its maximum level at a N:P ratio of 9.12.

The negative net growth rate indicates that the species will be swept from the environment. Grazing increases more with the escalation of maximum growth rates. The differences between maximum photosynthesis and net photosynthesis are greater at higher N:P ratios (Figure 12). Grazing is observed to be effective on *P. gracile* production at N:P ratios ≤ 9.2. The parallel changes of kmax and g+ up to a N:P ratio of 13.5 for *C. closterium* fail at N:P 17.62 (Figure 13). All the maximum growth rates up to a N:P ratio of 17.62 are smaller than grazing. N:P ratio of 4 in the environment demonstrates that *C. closterium* will be exhausted by excessive grazing. Indeed, μ+ values are negative except N:P 17.62. Grazing on *C. closterium* appears to be effective at N:P ratios ≤ 13.5. The maximum photosynthesis and grazing pressure closely follows each other except N:P 17.62 (Figure14). This relationship is not observed with net photosynthesis. It is revealed that *C. closterium* will cause a bloom at N:P 17.62 despite grazing (Figure 14). Grazing occurs to be effective on picoplankton except an N:P ratio of 9.12 (Figure 15). A linear relationship exists between growth rate and grazing rate and it is close to or above the steady state line except for N:P ratios of 9.12 and 13.5. In case the potential productivity of picoplankton is significantly higher than grazing pressure at N:P 9.12, nutrient/light limited growth is implied to exist in the bay, just grazing is not sufficient (Figure 16).

Conclusion

Treatment of nitrogen has been beneficial for the bay. Nutrient limitation and dynamic grazing system is able to be maintained at steady state by treatment process. As the species approach to optimum ratios, both the growth rates and consequently the grazing rates increase. In another words, microzooplankton show a higher grazing activity on healthy populations. Microzooplankton can perceive both the maximum growth rate and the nutrient-limited growth rates and keeps net *in situ* growth rates at 0 or at slightly negative values.

During the period after treatment compared to that prior to 2002, the improvement in the clarity of the water is due to grazing and nutrient limitation (Sunlu *et al*., 2006). Although the N:P ratio of 14 in Outer Bay reveals the necessity of a change in favor of *C. closterium* in the community, increased nutrient limitation should keep the system stable.

The fall in N:P ratios from 14 to 4 by nitrogen treatment and the limited Si input due to reduction in rainfall demonstrate a potential for the proliferation of toxic species. As is known, silicate supplementations were proposed to improve undesirable impacts on the community (Officer and Ryther, 1980; Sommer, 1987). Increase of red-tides in Tolo harbor due to declined N:P ratios was reported by Hodgkiss and Ho (1997). Therefore, treatment of P with N is necessary for reducing rise of the ratios and consequently withdrawing the risks to a minimum level. Nutrient uptake of the rivers from local or non-local sources along their route and consumption of silicate by diatoms resulting in transport to river sediments, significantly reduce silicate input to the bay leading toxic dinoflagellates to find opportunity to proliferate. Consequently, we suggest that management of nutrient inputs in river basins will be beneficial for İzmir Bay.

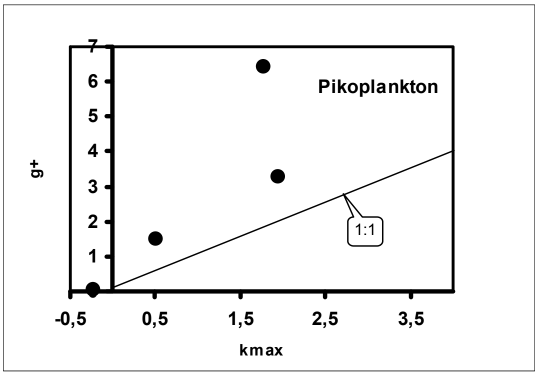

Figure 9. Distribution in maximum growth rate and grazing rate of picoplankton. The 1:1 line implies the stable line.

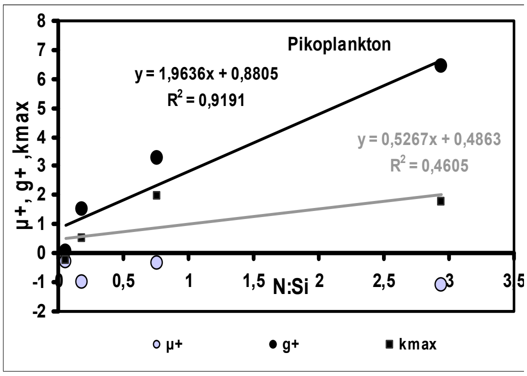

Figure 10. Changes in net growth, maximum growth and grazing rates of picoplankton at various N:Si ratios.

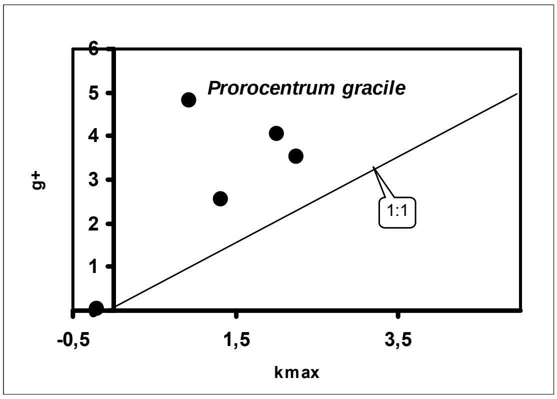

Figure 11. Correlation between grazing rate and maximum growth rate of *Prorocentrum gracile* and the steady state line (1:1).

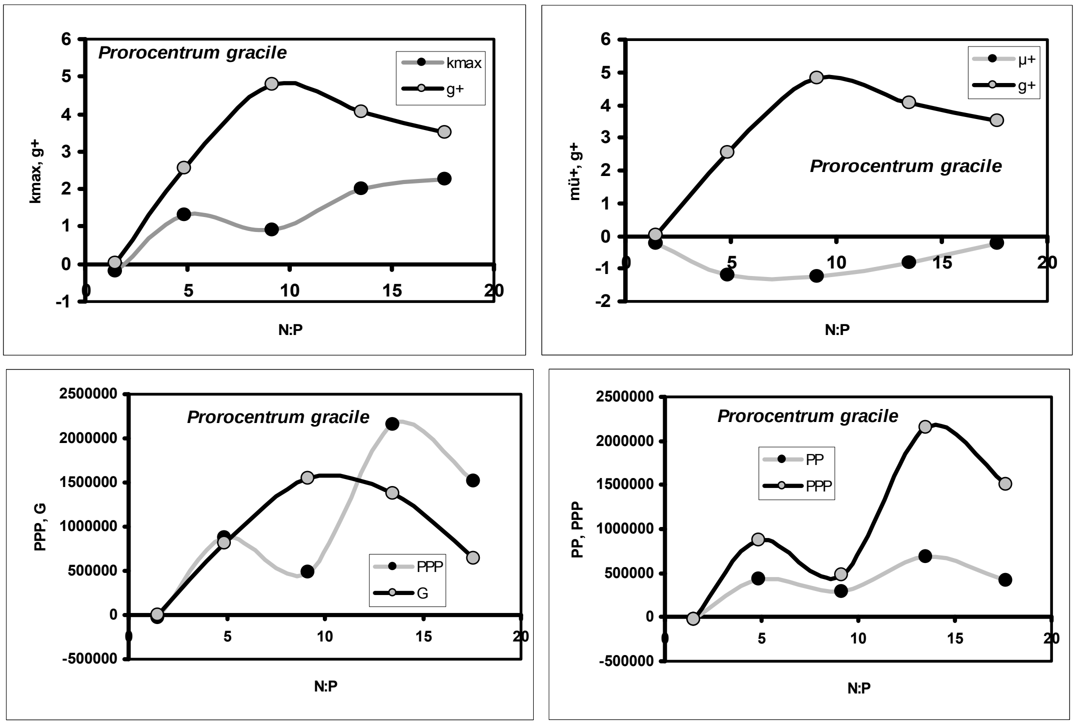

Figure 12. The changes in dynamic parameters with changes in N:P ratios for *P gracile*.

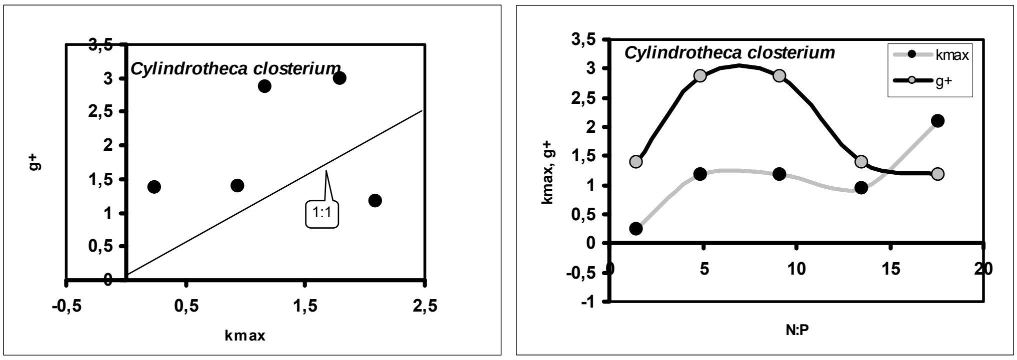

Figure 13. Changes in dynamic parameters for *Cylindrotheca closterium* with various N:P ratios.

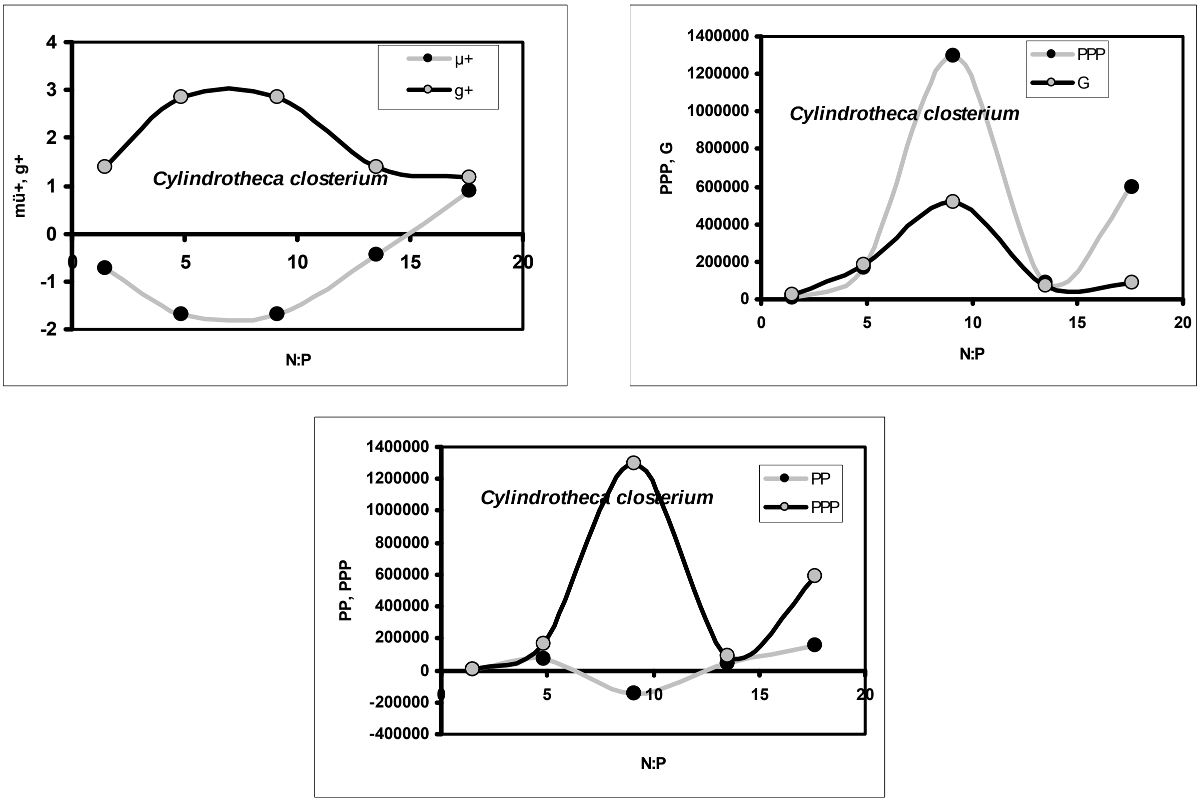

Figure 14. Changes in dynamic parameters for *Cylindrotheca closterium* with various N:P ratios.

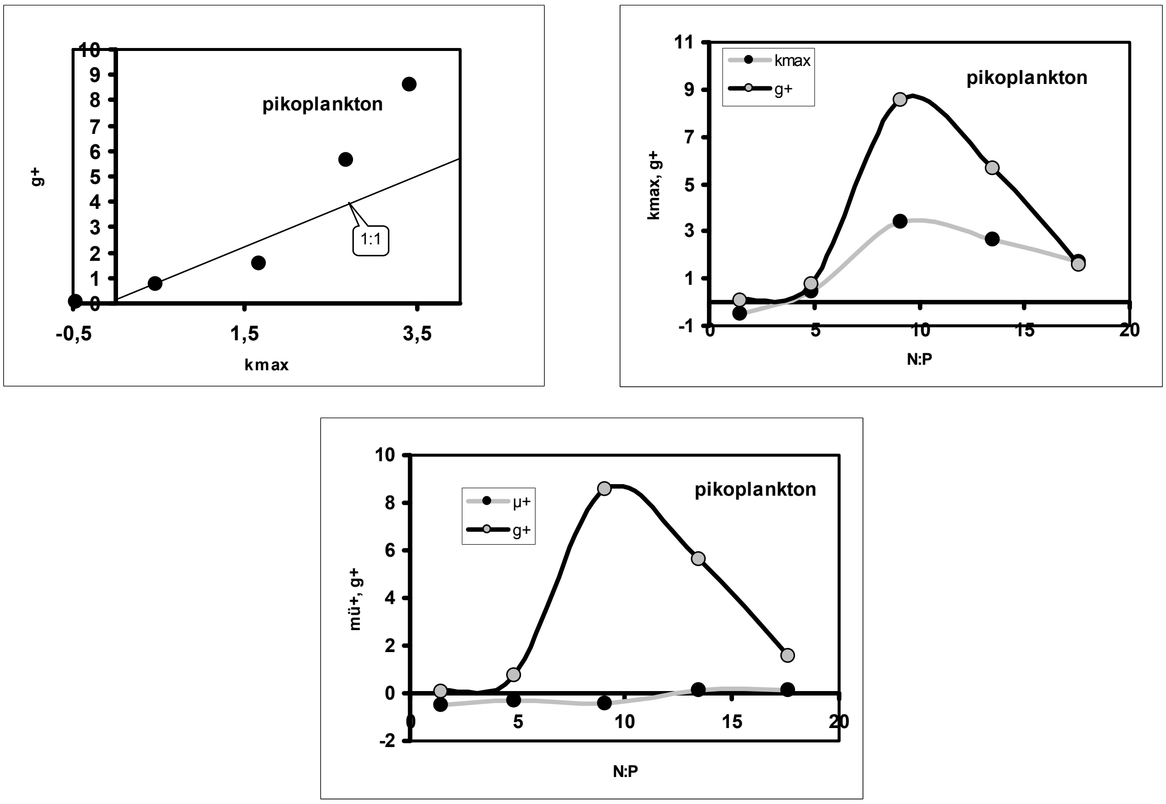

Figure 15. Changes in dynamic parameters for picoplankton at various N:P ratios.

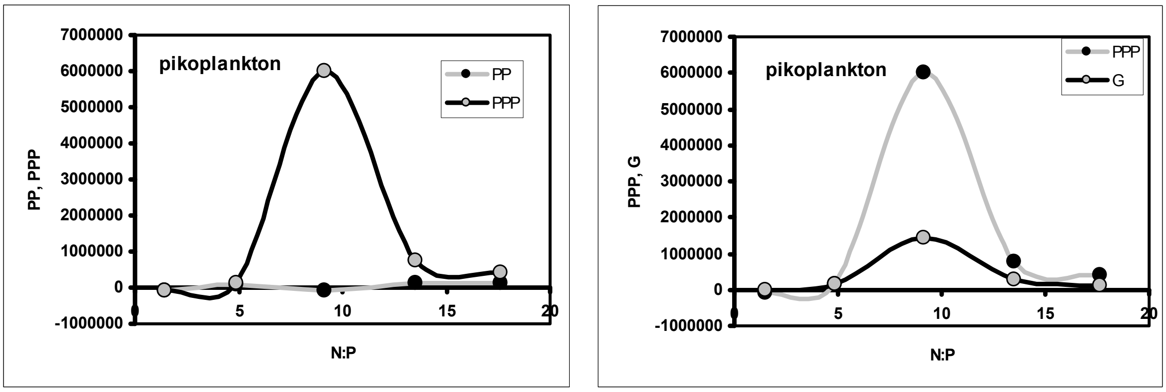

Figure 16. Changes in dynamic growth parameters for *Cylindrotheca closterium* supplied with various N:P ratios.

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