YU ISSN: 0001-5113 AADRAY

Original scientific paper UDC 577.475 (262.3)

# PHYTOPLANKTON RESPONSE TO CONCENTRATION OF NUTRIENTS IN THE CENTRAL AND SOUTHERN ADRIATIC SEA

### FITOPLANKTON I KONCENTRACIJA NUTRIENATA U SREDNJEM I JUŽNOM JADRANU

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Annual frequency distribution histograms of nutrient concentrations and phytoplankton volume values provide valuable data in the ecological evaluation of seven Adriatic ecosystems. The most frequent, minimum and maximum values of analyzed parameters are proposed as indications of eutrophication and communication among different pelagic environments. The applicability of N/P ratios as indications of limiting nutrient conditions is discussed.

#### INTRODUCTION

The main natural sources of nutrients in the coastal and offshore Adriatic waters are: 1. the rivers which discharge along the eastern Adriatic coast. 2. the northern Adriatic rivers, and 3. the ingressions of the eastern Mediterranean waters through the strait of Otranto, as already indicated in previously published papers (Buljan, 1964; Pucher-Petković and Zore-- Armanda, 1973). Along the eastern Adriatic coast there are several karst rivers, like Zrmanja and Krka, which are relatively poor in nutrients and suspended particles because they flow through a scarcely inhabited hinterland. The intensive sedimentation processes in these rivers take place especially in the lakes formed by travertine barriers of biologic origin (Juračić and Prohić, 1986). The concentration of phosphorus salts in offshore waters in the 0-200 m layer decreases from the southern towards central Adriatic, and than increases again in the northern Adriatic (Stojanoski, 1975). If compared to the main part of deep Adriatic waters, the karst rivers are poorer in all nitrogen compounds. The maximum nitrate concentration registered in the South Adriatic Pit is slightly lower than in the central Adriatic Jabuka Pit (Vukadin, 1972; Buljan et al., 1975). Higher nitrate con-

centrations were recorded in deeper Adriatic layers, which may be regarded as a significant nitrogen source for primary production both in the offshore and the coastal euphotic layers. Nitrogen compounds in deeper offshore layers probably come from the northern Adriatic waters, especially during autumn and winter, when the assimilation processes slow down (Degobbis and Gilmartin, 1981). Significant sources of eutrophication are urban sewage waters. Valuable data and discussions on nutrients in the central and southern Adriatic have already been published by Buljan (1964), Buljan et al. (1975), Buljan and Zore-Armanda (1976, 1979), Zore-Armanda and Pucher-Petković (1976), Škrivanić and Barić (1979).

The investigated stations situated in Šibenik harbour, Kaštela Bay and Gruž Bay (Dubrovnik harbour) are strongly influenced by the urban sewage waters. Šibenik harbour being situated in the river Krka estuary accounts for its increased natural and anthropogenic eutrophication. In Mali Ston Bay investigation was performed at Station Usko. The Bay is closed between Pelješac peninsula and the land, the coastline strongly indented, and the region scarcely inhabited. Washing out of surrounding sediments is the main source of eutrophication with the sporadic increased influence of the Neretva river waters and underwater springs. The stations in Privlaka and Župa Bays are markedly influenced by central and southern Adriatic offshore waters ( $Z \circ r e - A r m a n d a$  and D a d i ć, 1984;  $G a \check{c} i \acute{c}$  and  $D a d i \acute{c}$ , 1984). The only one offshore station is situated SW from Dubrovnik, above the 100 m isobath, and is strongly influenced by the oligotrophic South Adriatic Pit waters, which are carried coastward by the inflowing Adriatic current.

The relationship between nutrients and phytoplankton has been analyzed mainly in Kaštela Bay (Pucher-Petković, 1976; Pucher-Petković and Marasović, 1980; Marasović and Vukadin, 1982; Marasović and Pucher-Petković, 1983), and less in other regions of the central and southern Adriatic (Buljan et al., 1973; Pucher-Petković, 1974, 1979; Marasović and Pucher-Petković, 1981; Majić, 1984).

The aim of this paper is to present a spectrum of the nutrient concentration and phytoplankton biomass values recorded at seven differently eutrophicated stations along the eastern Adriatic coast. This has been the first attempt to compare the phytoplankton biomass expressed as a total volume of cells (fresh weight) with the concentration of nutrients in the Adriatic.

#### MATERIALS AND METHODS

Samples for phytoplankton and nutrient analyses were collected at seven stations along the eastern coast of the central and southern Adriatic Sea (Fig. 1). Six stations were neritic and one was offshore.

Samples for phytoplankton analyses were collected during the period from 1979 to 1984. During a one year study Stations 1, 2, 3 (1981/82), 6 (1983/84) and 7 (1979/80) were sampled, while during a two year study Stations 4 and 5 (1979/80, 1981/82). Phytoplankton samples were collected once a month throughout the year, while at Station 6 seasonally. Samples for the quantitative analysis of the phytoplankton, salinity and temperature were



Fig. 1. Position of Stations. 1 — Privlaka Bay, 2 — Šibenik harbour, 3 — Kaštela Bay, 4 — Mali Ston Bay (Usko), 5 — Gruž Bay, 6 — Župa Bay, 7 — Offshore station (isobath 100 m)

taken by Nansen reversing bottles from the depths of 1 m, 5 m, and near the bottom at nearshore stations, and from 1, 20 and 50 m at open sea station. All samples for phytoplankton analyses were preserved in a two per cent neutralized formaldehyde solution. The cell counts were obtained by the inverted microscope method (Utermöhl, 1958). Samples of 25, 50 or 100 ml were analyzed microscopically after a sedimentation period of 24 hours. The phytoplankton cells with a maximum length between 2 and 15  $\mu$ m were designated as nanoplankton, and cells longer than 15 µm as microplankton. Cells smaller than 15  $\mu$ m, but which made longer colonies, as well as cells whose length was longer than 15  $\mu$ m due to the presence of hair-like spines, were included with microplankton. The counting of microplankton cells was performed at the magnifications of 200 X and 80 X. For the smaller, more abundant microplankton cells, the transects across the central part of the counting chamber base plate were made at 200 X. Nanoplankton cells were counted in 20-50 randomly selected fields of vision along the counting chamber bottom plate, at the magnification of 320 X. Precision of the counting method was about +/-10 per cent. Assuming that the biomass was equal to the total volume of cells, the latter was calculated according to Smayda

(1978). Cell density and cell size were determined simultaneously (in the same sample) from measurements obtained using the inverted microscope. Descriptions of the method and the cell volume data on dominant phytoplankton species obtained at the same stations have already been published (Viličić, 1985b). The frequency distribution of phytoplankton biomass at the stations sampled is presented in Fig. 2, where total cell volume is fractionized into logarithmic classes. One decade of phytoplankton volume logarithmic scale is divided into two parts ( $10^n$ ,  $10^{n+0.5}$ ,  $10^{n+1}$ ).

The nutrient samples were taken seasonally, during the period from 1975 to 1986: Station 1 in 1975, 1976 and 1986; Station 2 and 5 in the period from 1977 to 1982; Station 3 in 1977, 1981 and 1982; Station 4 in 1980, 1981, 1985 and 1986; Station 6 in 1983, and 1984; Station 7 in the period from 1979 to 1981. Samples were taken by Nansen reversing bottles from the depths of 0.5, 5, 10, 20, 30 and 50 m. Determination of nutrients was performed using Technicon autoanalyzer. Standard methods were used for determination of amonia (H e a d, 1971), nitrates (W o o d et al., 1967) and nitrites (A r m strong et al., 1967). Phosphates were determined after extraction with isobutanol according to Strickland and Parsons (1968). Silicates were determined according to Grasshof (1965).

N/P ratio was calculated according to Redfield et al. (1963). In this ratio numerator was expressed as a summ of nitrate-, nitrite- and ammonia--nitrogen, and denominator as phosphate-phosphorus.

### RESULTS AND DISCUSSION

Nutrient concentrations in seven investigated ecosystems are listed in Table 1. The range between minimum and maximum concentration of phosphates was equal to 0.034-0.324 µmol 1-1, nitrates 0.16-18.21 and silicates 2.00-62.00 µmol 1-1, respectively. Salinity and temperature extreme values at the investigated stations can be found elsewhere (Viličić, 1985b). Phytoplankton volume at all investigated stations ranged between  $5 \times 10^6$ and  $1.03 \times 10^{10} \ \mu m^3$  l<sup>-1</sup>. Maximum microplankton and nanoplankton volumes ranged from  $10^8$  to  $10^{10}$  and  $4 \times 10^7$  to  $1.6 \times 10^9 \ \mu m^3 l^{-1}$ , respectively. The highest values of phosphate, nitrate and silicate concentrations were determined at Šibenik harbour station and were followed by maximum microphytoplankton, nanophytoplankton and total phytoplankton volumes. Considering the decreasing values of maximum nitrate concentration and phytoplankton volume, Kaštela Bay ranks second, after Šibenik station. In general, maximum concentrations of nutrients are proportional to maximum phytoplankton volumes. If compared with the most eutrophicated Sibenik station, maximum microplankton, nanoplankton and total phytoplankton volumes at oligotrophic offshore station are 50, 40 and  $100 \times$  smaller, respectively.

Nutrient concentrations and phytoplankton biomass values are presented by the annual frequency distribution histograms (Fig. 2). To determine class with the most frequent values provides more information than to calculate annual arithmetic or geometric mean value. Phosphate concentrations in the study area were most frequently found to be between 0.05 and 0.10  $\mu$ mol l<sup>-1</sup>, and nitrate concentrations between 0.5 and 1.0  $\mu$ mol l<sup>-1</sup>. The minimum phyto-

Stations	µmol l-1 PO4—P NO3—N SiO3—Si			$\mu$ m <sup>3</sup> $\times$ 10 <sup>8</sup> l <sup>-1</sup> PHYTO MICRO NANO		
	min—max	min—max	min—max	min—max		max
Privlaka Bay (1)	0.047-0.140	0.67-1.54	2.50-15.10	0.80- 9	9	3
Sibenik-harbour (2)	0.051-0.324	0.35 - 18.21	2,96-62.00	1.10-103	100	16
Kaštela Bay (3)	0.038-0.153	0.16- 2.51	2.00-15.43	1.30 - 26	25	3
Mali Ston Bay (4)	0.043-0.111	0.53 - 1.92	4.82-30.86	1.10-18	17	9
Gruž Bay (5)	0.034-0.119	0.27- 2.51	2.50 - 15.10	0.40-19	17	10
Zupa Bay (6)	0.038-0.085	0.35-0.96	2.25 - 12.86	0.20- 3	3	1.8
Offshore station (7)	0.051-0.137	0.35- 3.81	2.25 - 12.86	0.05- 2	1	0.4

Table 1. Minimal and maximal values of phosphate, nitrate and silicate concentrations, total phytoplankton (PHYTO), microphytoplankton (MICRO) and nanophytoplankton (NANO) volume, at the investigated stations.

plankton quantity may be the result of the unfavourable ecological factors that slow down the photosynthesis and division rate of phytoplankton cells: the currents rapidly transporting cells from more to less favourable environmental growth conditions, and the intensive zooplankton grazing (data on zooplankton and currents are not presented in this work). Relatively low annual minima of phytoplankton volume were recorded in deeper euphotic layers of enlarged bays and estuaries, which communicate well with offshore waters (Stations 2, 5).

Minimum, maximum and the most frequent phytoplankton volume (fresh weight biomass) and nutrient concentration were considered to be characteristic values in the ecological characterization of the pelagic environment. To obtain such a distribution of data, temporal dynamics of phytoplankton growth at the investigated stations was not necessary to be known. Minimum, maximum and the most frequent values might be accurately estimated only if samples were taken continuously and frequently enough at each investigated station, at the same depths, throughout the year. In this study the phytoplankton sampling was performed at monthly intervals, although a more conventional bimonthly sampling intervals might have provided a greater spectrum of quantity data. Although nutrient concentrations were not measured monthly, the sampling was performed over several years, and all seasons were equally covered.

Similarly to other subtropical seas, the favourable growth conditions in the Adriatic are usually reestablished in spring and autumn. If sampling is performed frequently enough, phytoplankton bloom may be registered as an indicative maximum. The trophic grade of the ecosystem may also be evaluated according to the highest phytoplankton biomass, making it possible to plan its utilization or protecting activity. In the vicinity of urban centres or other areas with more advanced eutrophication, the explosive development of phytoplankton (\*red tide\*) may occur. Relatively high phytoplankton volumes recorded at Šibenik harbour and Kaštela Bay stations did not correspond to red tide. However, this phenomenon was described in Kaštela Bay in September 1980 by Marasović and Vukadin (1982). According to the frequency distribution of data presented, the minimum and maximum phytoplankton volume is increased in more eutrophic waters, i. e. moved toward the right side of the graphical presentation. The most frequent annual

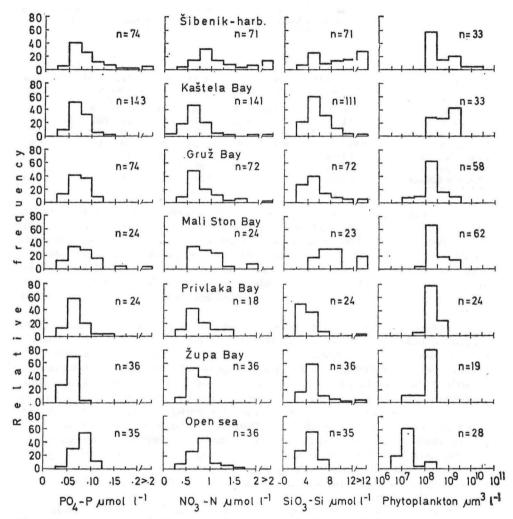


Fig. 2. Frequency distribution of nutrient concentrations and phytoplankton volume at the investigated stations

phytoplankton volumes are more uniform at all investigated stations, excepting Kaštela Bay and open sea, i.e. belong to the class  $10^8 - 3 \times 10^8 \ \mu m^3 \ l^{-1}$ . In Kaštela Bay phytoplankton volume values were most frequently determined in the class  $10^9 - 3 \times 10^9 \ \mu m^3 \ l^{-1}$ , but at the same time the larger class values are evidently lacking. At oligotrophic offshore station, values ranging from  $10^7$  to  $3 \times 10^7 \ \mu m^3 \ l^{-1}$  were most frequently recorded.

The maximum annual phytoplankton biomass occurs when concentration of nutrients become optimal or nearly optimal for phytoplankton growth. N/P ratio may be indicative of the main limiting nutrients. According to Redfield et al. (1963), optimal phytoplankton growth appears if the atomic

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ratio between inorganic nitrogen ( $\Sigma N$ , i. e.  $NO_3 - N + NO_2 - N + NH_3 - N$ ) and phosphate-phosphorus ( $PO_4 - P$ ) concentrations in the environment equals 16. If this ratio exceeds 16, phytoplankton production is probably limited by phosphorus. If an ecosystem is surrounded by a dense urbanization,  $\Sigma N/PO_4 - P$  ratio will be low and N will be limiting, as already indicated by many authors (Z d a n o w s k i, 1982; S a k s h a u g and Olsen, 1986).  $\Sigma N/PO_4 - P$  ratio is discussed at four differently eutrophicated stations (Fig. 3). It ranged most frequently from 16 to 28. Offshore waters are characterized also by relatively frequent >36 ratios. However, at Šibenik station ratio values between 36 and 44 were most frequently determined. Šibenik harbour and

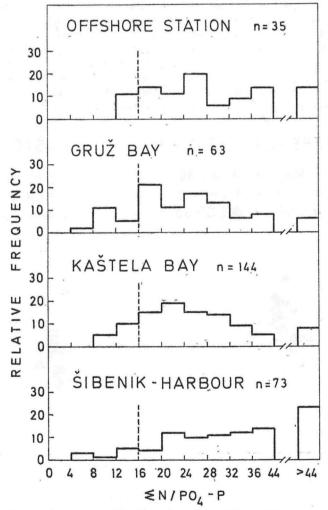


Fig. 3. Frequency distribution of Redfield's N/P ratios determined at four differently eutrophicated stations

partly Gruž Bay being well eutrophicated stations, but strongly influenced by offshore waters (estuarine circulation), resulted in more frequent  $\rangle$ 20 ratios. Gruž Bay, Kaštela Bay and Šibenik stations, were also characterized by relatively low ratio values, indicating a large inflow of pfosphorus rich sewage waters, or a maximal nitrogen depletion probably during intensive phytoplankton blooms. Phosphorus limiting primary production is a commonly occuring process in the Adriatic, as might be concluded after previously done estimations (Pojed and Kveder, 1975; Buljan et al., 1975).

Considering  $\Sigma N/PO_4 - P$  ratio, the actual role of nitrogen or phosphorus as limiting elements, cannot be assessed with certainty, probably due to a significant quantity of organic nitrogen and phosphorus compounds not being taken into consideration. The manipulation with organic N and organic P components would probably give different total - N/total - P ratios than are the  $\Sigma N/PO_4$  - P ratios presented in Fig. 3. Phytoplankton is well known to be directly using many of organic nitrogen and phosphorus compounds during photosynthesis. Iv a n č i ć and D e g o b b i s (1987) have highlighted the potential importance of organic phosphorus compounds in the biological cycle of the northern Adriatic. The organic phosphorus concentrations were shown to exceed orthophosphate concentrations through most of the year and

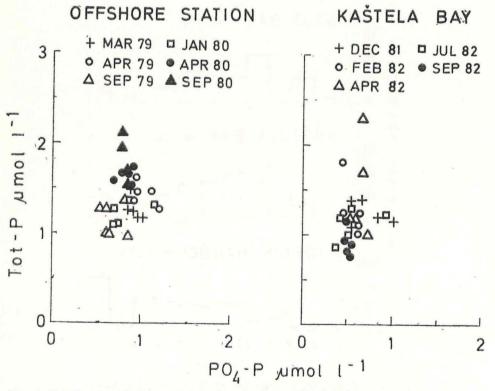


Fig. 4. Relationship between total phosphorus and phosphate phoshorus at the offshore and Kaštela Bay station

represented an important source of this element for phytoplankton requirements. Correlations between total phosphorus and phosphates, were not always positive at two investigated Adriatic stations (Fig. 4). Such a correlation corroborate the fact that organic phosphorus and probably organic nitrogen contribute significantly to the total amount of nutrients in the investigated ecosystems. A positive total —  $P/PO_4$  — P correlation was evident in January and April 1980 at offshore station. However, it was more frequently negative, indicating the presence of organic phosphorus compounds as important substances to be used by phytoplankton during photosynthesis. The most evident total —  $P/PO_4$  — P negative correlations were established in March, April, September 1979 and September 1980 at the offshore station, as well as in December 1981, February, April and September 1982 in the Kaštela Bay.

If the proportion of the different nitrogen and phosphorus forms is remarkably constant from one pelagic environment to the other, and if oxydized forms  $(NO_3 - N, PO_4 - P)$  dominate the dissolved inorganic fractions, the ratio  $NO_3 - N/PO_4 - P$  may also indicate the most probable limiting nutrient (Vaulot and Frisoni, 1986). However, ammonia may be preferently used by algae as a short circuit in protein synthesis, and the nutrient picture is incomplete without ammonia, dissolved organic nitrogen and phosphorus data (Saunder and Moore, 1979). Taking into account a variable contribution of organic phosphorus and organic nitrogen in total budget of these elements, it is obvious that total - N/total - P ratio could better indicate most probable limiting nutrient than does  $NO_3 - N/PO_4 - P$ , or  $(NO_3 + NO_2)$  $+ NH_3) - N/PO_4 - P$  ratios. However, during the investigation in the relationship between inorganic and organic nutrients in the sea water, Butler et al. (1979) have found the relative constancy of the total - N/total - P ratio throughout the year. Nevertheless, they are of the opinion that total - N//total — P ratio may demonstrate the difference existing between ecosystems dominated by heterotrophic activity and those in which autotrophs dominate. As they suggest, it is also possible that this ratio may be used in differentiating between areas rich in naturally occurring N and P and those with the high N and P levels being due to pollution.

During the forthcoming investigations, organic nitrogen will have to be measured as an additional parameter in order to establish the total — N//total — P ratio. Correlations among various forms of N, P and phytoplankton biomass (production), which would provide an additional view of ecosystem function, will have to be determined.

#### CONCLUSIONS

Annual frequency distribution histograms of nutrient concentrations and phytoplankton volume make possible the ecological evaluation of Adriatic ecosystems. In addition to the position of class with the most frequent values, class position including minimum and maximum values may also be considered as indication of eutrophication and communication between two pelagic environments. Maximum nutrient concentrations are proportional to maximum phytoplankton volume.

 $(NO_3 + NO_2 + NH_4) - N/PO_4 - P$  atomic ratio ranged most frequently from 16 to 28, indicating mainly by phosphorus limited primary production. Offshore waters are characterized by relatively frequent >36 ratios. In urban areas more frequent <12 ratios indicated a large inflow of phosphorus rich sewage waters or nitrogen depletion during intensive phytoplankton blooms.

Variable concentrations of organic phosphorus were indicated at the same investigated stations. The forthcoming investigations should aim at providing an additional view of ecosystem function by establishing the correlations among various forms of nitrogen, phosphorus and phytoplankton biomass (production).

#### ACKNOWLEDGEMENTS

The work was supported by the Self-Management Community of Interest for Scientific Research of SR Croatia.

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Accepted: December 18, 1987

#### FITOPLANKTON I KONCENTRACIJA NUTRIENATA U SREDNJEM I JUŽNOM JADRANU

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#### KRATKI SADRŽAJ

Analizirana je raspodjela učestalosti koncentracija glavnih nutrienata i vrijednosti staničnog volumena fitoplanktona (svježe težine), na sedam postaja uz istočnu obalu Jadranskog mora. Najčešće, minimalne i maksimalne vrijednosti analiziranih parametara ukazuju na intenzitet eutrofizacije ili povezanost obalnog ekosistema s otvorenim morem, pa su korištene kod ekološkog vrednovanja ekosistema.

Omjer dušika i fosfora (Redfieldov omjer) najčešće varira između 16 i 28. Fosfor češće limitira fotosintezu fitoplanktona na otvorenom moru nego uz obalu. Omjer (12 češće je utvrđen u područjima koja su pod utjecajem otpadnih voda gradske kanalizacije, kao rezultat povećane koncentracije fosfata ili brže potrošnje dušika za vrijeme cvjetanja fitoplanktona. Organski spojevi su značajan izvor dušika i fosfata za primarnu produkciju, pa se tu činjenicu ne smije zanemariti u budućim istraživanjima.