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## **Spatial and seasonal variability of fractionated phytoplankton biomass and primary production in the frontal region of the Northern Adriatic Sea**

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### **Abstract**

*Spatial and seasonal patterns of variation of fractionated phytoplankton biomass and primary production and their relationships with nutrient concentrations were analyzed along an inshore - offshore gradient and in relation to the presence of a frontal system in the Northern Adriatic Sea. Sampling was carried out in winter and summer during four oceanographic cruises (June 1996 and 1997, February 1997 and 1998) as part of the PRISMA II project. Water samples for determining nutrient concentrations, phytoplankton biomass (as Chla) and primary production (as <sup>14</sup>C assimilation) were collected at five optical depths. Sampling stations were located along 2 or 4 parallel transects arranged perpendicularly to the shoreline and the frontal system. The transects were located at such a distance from the coast that the frontal system crossed them at their half-way point. Total dissolved nitrogen (TDN) and total dissolved phosphorus concentrations (TDP) were  $12.41 \pm 3.95 \mu\text{M}$  and  $0.146 \pm 0.070 \mu\text{M}$ , respectively. The values in the two seasonal periods were similar, decreasing along the inshore-offshore gradient. Values for phytoplankton biomass and primary production were higher in the winter than the summer cruises, and decreased, in both seasonal periods, along the inshore-offshore gradient. Moreover, in both seasonal periods, picophytoplankton dominated both biomass and productivity, (56% and 44%, respectively) at stations beyond the frontal system, while microphytoplankton was more important at stations inside it (44% and 44%, respectively). Total phytoplankton biomass and primary production were directly related to nutrient concentrations. Regarding size classes, significant patterns of variation with nutrients were observed particularly for biomass. The results indicate that the size structure and function of phytoplankton guilds seem to be mediated by nutrient inflow, as well as by competitive interaction among size fractions.*

**Keywords:** Phytoplankton biomass; Phytoplankton size structure; Primary production; Nutrient concentrations.

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## Introduction

The northern sub-basin of the Adriatic sea is characterized by extremely shallow depth (about 30m) with a weak bathymetric gradient along its major axis and is affected by abundant river run-off (about 3000 m<sup>3</sup>s<sup>-1</sup>), deriving from the Po and other northern Italian rivers, which represent about 20% of the total Mediterranean river run-off (RUSSO & ARTEGANI, 1996). The Northern Adriatic Sea is characterized by seasonal variation in the density structure of the water column, from complete mixing in winter to clear stratification during summer, and by the dynamic separation of the basin proper from the coastal zone due to the presence of a coastal frontal system. In the Northern Adriatic Sea, the coastal frontal system represents the boundary of the Po river plume (GRANCINI & CESCONE, 1973). Due to seasonal variation in density structure and water circulation, the frontal system is closer to the coast in winter than in summer (JUSTIC *et al.*, 1995). The two areas separated by the frontal system differ in both their physical and biogeochemical processes (FRANCO & MICHELATO, 1992; JUSTIC *et al.*, 1995). The frontal system is assumed to be responsible for the strong spatial gradient of resources that characterise the Northern Adriatic (MANN & LAZIER, 1996). Indeed, due to high nutrient concentrations in the freshwater inflow, regions inside the frontal system are characterised by higher trophic levels than regions outside.

This investigation is based on four oceanographic cruises carried out in the Northern Adriatic Sea from June 1996 to February 1998 in the framework of the PRISMA II project. In this investigation, the spatial and seasonal variations of total and fractionated phytoplankton biomass and primary production were studied. There is increasing evidence that size differences determine ecosystem structure and function (NOGUEIRA *et al.*, 2000) and many studies seem to suggest the existence of hierarchical competition among pico, nano and

microphytoplankton size classes in aquatic ecosystems (WATSON & KALFF, 1992; FOGG, 1995; COTTINGHAM, 1999; YEW-HOONG GIN K. *et al.*, 2000). Resource availability and size-dependent energy requirements of organisms have been proposed to play a major role in determining the size structure of phytoplankton guilds, although zooplankton grazing may be also important (FOGG, 1995; COTTINGHAM, 1999). The trophic state has been shown to affect size-fractionated phytoplankton biomass and primary production; accordingly, picophytoplankton dominate in oligotrophic conditions while microphytoplankton are more important in eutrophic conditions (SUTTLE *et al.*, 1988; MAGAZZÙ & DECEMBRINI, 1995; FRENETTE *et al.*, 1996; MAGAZZÙ *et al.*, 1996). Previous studies carried out in the Adriatic basin have shown that in the more oligotrophic waters of the Southern Adriatic, picophytoplankton accounted for almost 80% of phytoplankton biomass and primary production (BASSET *et al.*, 2000 and SAGGIOMO, pers. comm.), whereas in the Northern Adriatic the contribution of larger organisms was greater; in particular, microphytoplankton were found to dominate above all in areas closer to the coast (FONDA-UMANI, 1996) and in proximity to the subsurface chlorophyll maximum (REVELANTE & GILMARTIN, 1995).

The aim of this investigation was to analyze the variation of size structure and functions along a steep trophic gradient determined by the presence of a frontal system.

Specific objectives were:

a) to describe the patterns of variation of biomass and primary production of phytoplankton guilds and the absolute and relative contribution of the different size fractions (picophytoplankton: cell size 0.5-2 µm; nanophytoplankton: cell size 2-10 µm; microphytoplankton; cell size >10 µm);

b) to analyze the relationships between nutrient concentrations (N and P) and phytoplankton size structure and function

along an inshore-offshore gradient and in relation to the presence of the frontal system.

## Materials and Methods

### *Sampling and measurements of chemical parameters*

Sampling was carried out in winter and in summer during four oceanographic cruises (February 1997, February 1998, June 1996 and June 1997) aboard the R/V CNR-Urania. Water samples were collected in two sampling areas across the frontal system in order to estimate nitrogen and phosphorus concentrations, total and size-fractionated biomass (as chlorophyll  $\alpha$ ) and primary production (as  $^{14}\text{C}$  assimilation): one area was close to the river Po mouth (Northern area), the other close to the Conero promontory (Southern area). In each area, sampling was carried out along 2 or 4 parallel transects which crossed the frontal system. There were 4 stations in each transect: two in the area inside the frontal system and two in the area outside. The geographical position of the transects and sampling stations varied among cruises according to the position of the frontal system, but in each cruise the transects spanned the frontal system, which bisected them at their mid-point. Samples were collected using 10 l Niskin bottles at five optical levels corresponding respectively to 100, 30, 12, 4, and 1% of the surface active photosynthetic radiation (surface PAR). Dissolved inorganic nitrogen ( $\text{DIN} = \text{nitrite} + \text{nitrate} + \text{ammonium}$ ) and dissolved inorganic phosphorus (DIP) measurements were carried out directly on board, after filtration with GF/C filters in accordance with standard spectrophotometric methods (COZZI *et al.*, 2002). Dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) were determined as nitrate + nitrite and reactive phosphorus after photo-oxidation (UV + hydrogen peroxide) following the Walsh method (1989).

### *Total and size fractionated biomass*

Phytoplankton size fractions considered were  $> 10 \mu\text{m}$ ,  $2-10 \mu\text{m}$  and  $0.2-2 \mu\text{m}$ .

Water samples for chlorophyll  $a$  (Chl $a$ ) determination were filtered, immediately after collection, using GF/F and  $2 \mu\text{m}$  and  $10 \mu\text{m}$  porosity Nuclepore polycarbonate filters in order to separate the three size fractions according to MAGAZZÙ *et al.*, 1996. Filters were stored at  $-20^\circ\text{C}$  until analysis, carried out in most cases one month later. Chl $a$  was extracted in 90% acetone, for 24 hours, at  $4^\circ\text{C}$ , in the dark. Chl $a$  was determined with a spectrofluorimeter SHIMATZU-1051 before and after acidification with hydrochloric acid 0.5N (VADRUCCHI *et al.*, 2002).

### *Total and size fractionated primary production*

Primary production was assessed with the standard  $^{14}\text{C}$  radioisotopic method according to the procedure indicated in VADRUCCHI *et al.*, 2002. Immediately after water sample collection at each station, a dark and a light polycarbonate bottle was filled with water and 1 ml of sodium bicarbonate solution marked with  $^{14}\text{C}$  (activity density about  $20 \mu\text{Ci ml}^{-1}$ ) was added. The samples were incubated on deck in a continuous flow deck incubator, covered by nickel optical screens (Stork Veco International), in order to simulate the original depth light intensities. After 4 hours of exposure, samples were filtered as described above. Filters were transferred to 20 ml scintillation vials and radioactivity was estimated on a Beckman LS-1801 scintillator using 10 ml 'Aquasol' scintillation cocktail.

## Treatment of data

The average value for the five depths was considered for each station.

In order to analyse the pattern of variation along the inshore-offshore gradient, the values for the stations were pooled in accordance with their distance from the coast; since there were four stations on each transect, this yielded four groups of stations. The average value was

calculated for each group, in the two seasons and in the two areas. Analysis of variance was used to evaluate the statistical power of potential sources of variation, such as sampling area, seasonal period and distance of sampling stations from the coast. Regression analysis was used to evaluate the statistical power of (a) inshore-offshore patterns of variation of total and fractionated phytoplankton biomass and primary production, and (b) their relationships with nutrient concentrations. Multivariate regression analysis (software SPSS 11 for Windows) was used for evaluating the relative importance of each size fraction in the patterns of variation of total phytoplankton biomass and total primary production.

## Results

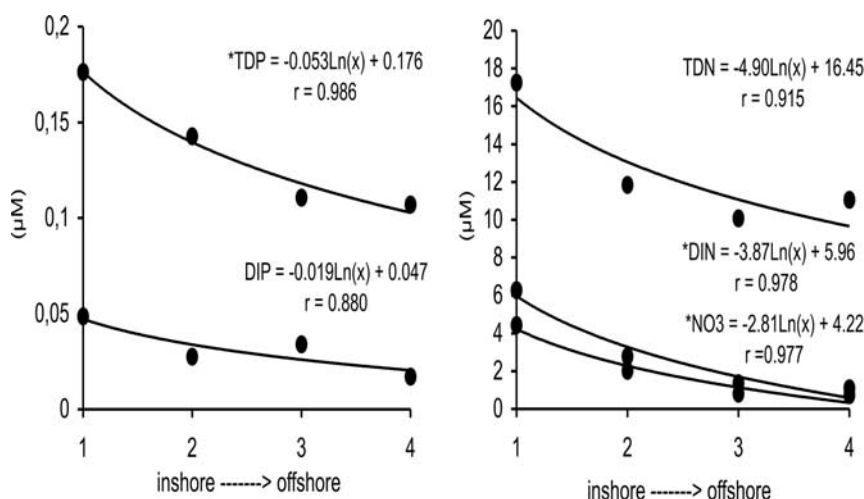
### Chemical characteristics

Total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) concentrations as an average value of all sampling stations in the two areas and in the two seasons were  $912.41 \pm 3.95 \mu\text{M}$  and  $0.146 \pm 0.07 \mu\text{M}$ , respectively, and did not differ significantly between seasons and sampling areas. The inorganic fraction was 25% of the total

nitrogen concentration and 19% of the total phosphorus concentration measured in the ecosystem. The concentrations of TDN and TDP, and of their inorganic fractions ( $\text{NO}_2 + \text{NH}_3 + \text{NO}_3 = \text{DIN}$  and  $\text{PO}_4 = \text{DIP}$ ), varied along the inshore-offshore gradient in accordance with a negative logarithmic equation (Fig. 1). Stronger variations were observed for TDP and inorganic nitrogen, particularly for the nitrate.

### Spatial and temporal variability of total and fractionated phytoplankton biomass and primary production

Phytoplankton biomass and primary production averaged  $1.38 \pm 1.52 \text{ mg(chl}a\text{)m}^{-3}$  and  $4.12 \pm 4.27 \text{ mgCm}^{-3}\text{h}^{-1}$  respectively. There were no significant differences between cruises carried out in the same seasonal period, either for biomass or primary production; therefore, the two years were considered as replicates in the data analysis. Three-way ANOVA showed that phytoplankton biomass and primary production did not vary significantly between sampling areas, but varied considerably depending on the season and the distance of sampling stations from the coast (Tab. 1).



**Fig. 1:** Inshore-offshore patterns of variation of total dissolved phosphorus (TDP), dissolved inorganic phosphorus (DIP), total dissolved nitrogen (TDN), dissolved inorganic nitrogen (DIN) and nitrate ( $\text{NO}_3$ ). (\*)  $p < 0.05$

**Table 1**  
**Three way analysis of biomass (mg(chl $\alpha$ ) m<sup>-3</sup>) and primary production (mgCm<sup>-3</sup>h<sup>-1</sup>)**  
**in the Northern Adriatic Sea in relation to three sources of variation: areas of sampling, position**  
**of sampling stations and seasonal period.**

Biomass			
Source of variation	df	F	p
Areas	1	0.064	0.80
Stations	3	54.108	<0.001
Seasons	1	16.289	<0.001
Areas*Stations	3	1.375	0.25
Areas*Seasons	1	0.646	0.42
Stations*Seasons	3	16.704	<0.001
Areas*Stations*Seasons	3	1.210	0.31
Errors	66		
Primary Production			
Source of variation	df	F	p
Areas	1	4.151	0.045
Stations	3	7.866	<0.001
Seasons	1	7.296	0.008
Areas*Stations	3	0.875	0.483
Areas*Seasons	1	8.753	0.004
Stations*Seasons	3	2.970	0.024
Areas*Stations*Seasons	3	1.037	0.393
Errors	66		

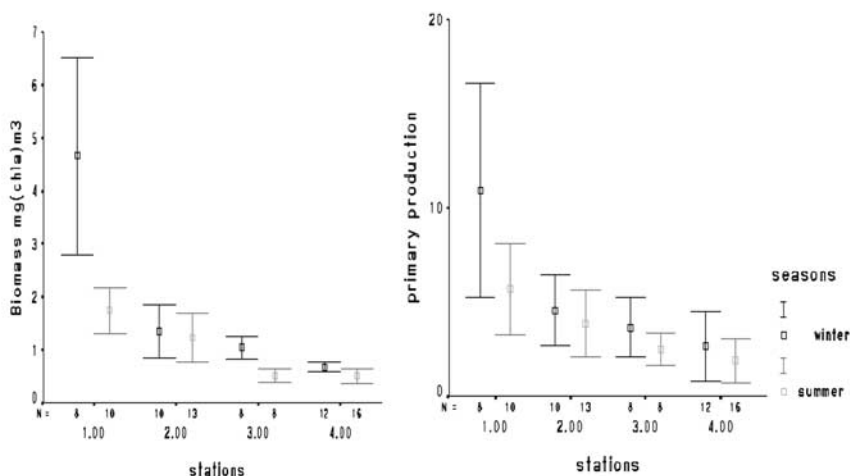
Higher values for both parameters were observed in winter than in summer (phytoplankton biomass: winter =  $1.82 \pm 2.01$  mg(chl $\alpha$ )m<sup>-3</sup>; summer  $1.05 \pm 0.870$  mg(chl $\alpha$ )m<sup>-3</sup>; primary production: winter,  $5.84 \pm 5.56$  mgCm<sup>-3</sup>h<sup>-1</sup>; summer  $3.43 \pm 3.14$  mgCm<sup>-3</sup>h<sup>-1</sup>). Moreover, both biomass and primary production exhibited significant spatial heterogeneity along the inshore–offshore gradient, with values decreasing progressively with distance from the coast (Fig. 2). The patterns of variation along the inshore–offshore gradient were described by negative logarithmic equations ( $p < 0.01$ ) and did not differ significantly between seasons (test of parallelism: Chl $\alpha$   $F_{1,4} = 0.565$  ns; primary production  $F_{1,4} = 0.01$ , ns), although higher values were observed at each station in winter than summer.

In winter, phytoplankton biomass varied from  $4.70 \pm 2.60$  mg(Chl $\alpha$ )m<sup>-3</sup> at stations closest to the coast to  $0.673 \pm 0.144$  mg(Chl $\alpha$ )m<sup>-3</sup> at stations located furthest offshore; in summer,

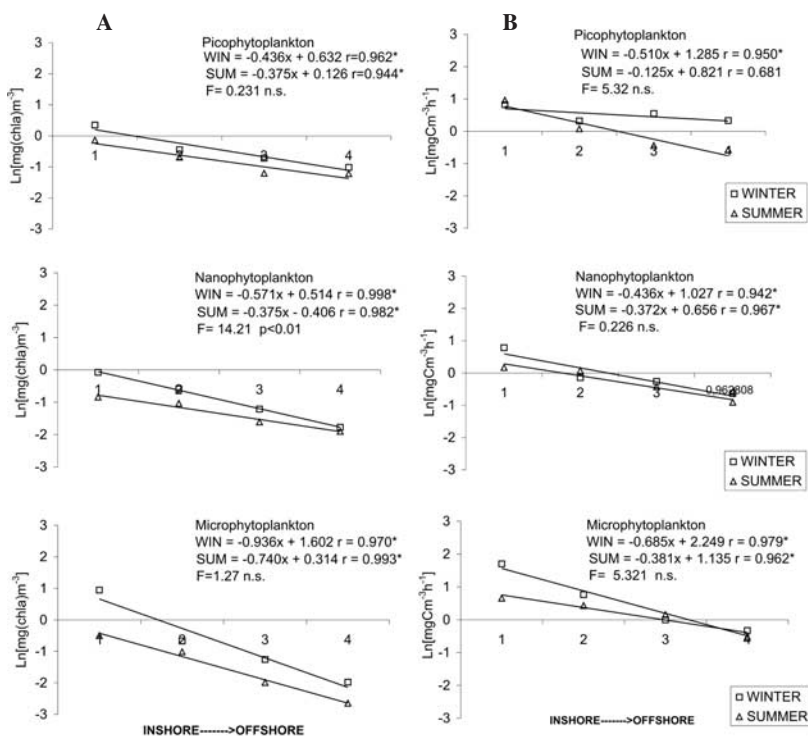
it varied from  $1.74 \pm 0.68$  to  $0.488 \pm 0.239$  mg(Chl $\alpha$ )m<sup>-3</sup> (Fig. 2).

In winter, primary production varied from  $11.05 \pm 8.04$  mgCm<sup>-3</sup>h<sup>-1</sup> at stations closest to the coast to  $2.65 \pm 3.19$  mgCm<sup>-3</sup>h<sup>-1</sup> at stations located furthest offshore; in summer, it varied from  $5.69 \pm 3.80$  to  $1.55 \pm 1.17$  mgCm<sup>-3</sup>h<sup>-1</sup> (Fig. 2).

The different phytoplankton size fractions also varied along the inshore–offshore gradient, exhibiting co-variation with total biomass and productivity. Both the biomass and primary production of each of the three size fractions of phytoplankton were inversely and statistically related to the distance from the coast (Fig 3). For each size fraction, the patterns of variation did not differ statistically between seasons (5 out of 6 comparisons showed no significant difference in the test of parallelism). The differences related only to absolute values, which for both total phytoplankton biomass and primary production were always higher in winter than



**Fig. 2:** Inshore – offshore patterns of variation of biomass and primary production in the study area in winter and summer (integrated water column values). Average values of the stations located at the same position along inshore off-shore gradient in the two sampling areas are reported. The vertical bars are  $\pm 1$  standard deviation, N = number of stations averaged.



**Fig. 3:** Patterns of variation of biomass (A) and primary production (B) of phytoplankton size classes in winter and in summer along inshore-offshore gradient (logarithm of integrated water column values). The results of comparison of the two regression lines are also reported as F values resulting from the test of parallelism.  
 (\*)  $p < 0.05$

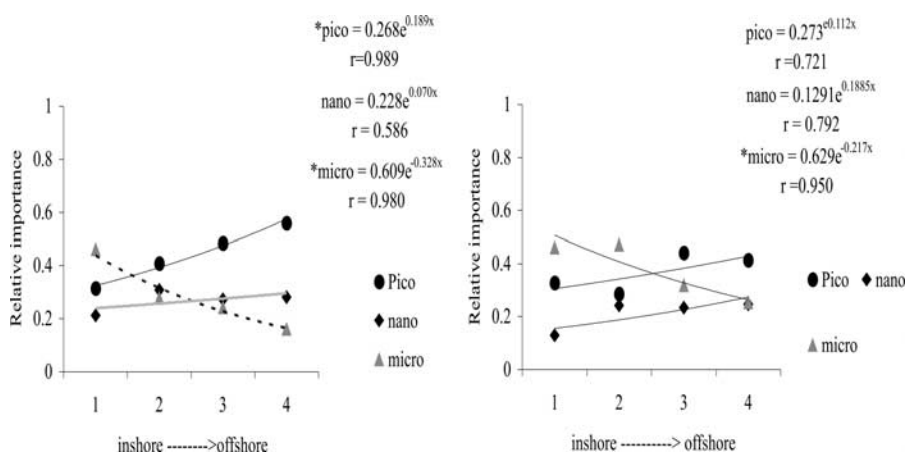


summer. In contrast, the relative contribution (% of total) of each size class did not differ statistically between seasons. The microphytoplankton component accounted for most of the variation of biomass and primary production in both seasons. Multiple regression analysis indicated that the variation of microphytoplankton accounted for 80% and 65% of the variation of total biomass and primary production respectively ( $R^2 = 0.80$  and  $0.65$ ). Accordingly, the slope of microphytoplankton biomass and primary production was steeper than the slopes of picophytoplankton and nanophytoplankton (Fig. 3), although the difference between slopes was significant only for phytoplankton biomass (micro vs pico  $F_{1,12} = 5.38$ ;  $p < 0.05$ ; micro vs nano:  $F_{1,8} = 4.38$   $p < 0.05$ ; nano vs pico  $F_{1,12} = 4.96$   $p < 0.05$ ). For both parameters, the relative contribution of picophytoplankton varied directly with distance from the coast, whereas that of microphytoplankton varied inversely, although the patterns of variation were significant only for phytoplankton biomass (Fig. 4). Picophytoplankton dominated phytoplankton guilds in the offshore stations (56% of total biomass and 44% of total primary

production), while microphytoplankton was more important at the stations inside the frontal system (46% of the total biomass and 46% of total primary production).

#### *The relationship between phytoplankton biomass and primary production and nutrient concentrations*

The relationship between nutrient concentrations and total and fractionated biomass and primary production were analysed for the whole data-set of the four oceanographic cruises. Moreover, the relationships were estimated using linear regression. The overall phytoplankton biomass and its size fractions (in absolute values) were directly related to nutrient concentrations. The phytoplankton biomass was more closely related to DIN. On the other hand, pico and microphytoplankton in relative terms, showed different patterns of variation with increasing nutrient concentrations. The relative contribution of picophytoplankton to overall phytoplankton biomass decreased with increasing nutrient concentrations, whereas that of microphytoplankton increased (Tab. 2). Similarly, overall primary production was



**Fig. 4:** Inshore-offshore patterns of variation of the relative importance of pico, nano and microphytoplankton size classes to phytoplankton biomass (A) and primary production (B). (\*)  $p < 0.05$



**Table 2**  
**Values of correlation coefficient (r of Pearson) between structural and functional characteristic of phytoplankton guild and nutrient concentrations in the study area. df = 84, (\*) p<0.05; (\*\*) p<0.01. All data concern the four oceanographic cruises.**

			TDN	TDP	NO3	DIP	DIN
Biomass	Total	$mg(chla)m^{-3}$	0.405**	0.546**	0.666**	0.218*	0.699**
	Pico		0.263*	0.362**	0.548**	0.448**	0.573**
	Nano	$mg(chla)m^{-3}$	0.109	0.501**	0.409**	0.094	0.412**
	Micro		0.429**	0.488**	0.562**	0.039	0.588**
	Pico		0.068	-0.345*	-0.239*	0.053	-0.299*
	Nano	(%)	-0.395**	0.206	-0.184	-0.066	-0.212*
	Micro		0.238*	0.349*	0.424**	-0.029	0.495**
	Total	$mgCm^{-3}h^{-1}$	0.249*	0.143	0.484**	0.354**	0.502**
	Pico		0.177	-0.114	0.269*	0.138	0.314*
Primary production	Nano	$mgCm^{-3}h^{-1}$	0.115	-0.041	0.220*	0.345	0.255*
	Micro		0.204	0.270*	0.520*	0.414**	0.513**
	Pico		0.034	-0.161	0.026*	-0.040	0.041
	Nano	(%)	0.047	-0.214*	-0.308*	-0.037	-0.284*
	Micro		-0.162	0.141	0.120	0.128	0.093

directly related to nutrient concentration, but the contribution of the various size fractions showed clear patterns of co-variation only in the case of microphytoplankton (Tab. 2).

## Discussion

In this study we did not consider depth as a potential source of variation of phytoplankton biomass and primary production. A previous study carried out on the same data set (VADRUCCHI *et al.*, 2002) showed that depth was a significant source of variation only for total primary production. Moreover, since the analysis of variance did not show significant variations between sampling areas, the values for these were considered together in the data analysis, and stations occupying the same position with respect to distance from the coast in the two areas were averaged for each seasonal period.

Many studies have addressed the structure and functions of phytoplankton guilds in the Northern Adriatic (FRANCO & MICHELATO, 1992; FONDA-UMANI, 1996). The values for phytoplankton biomass and primary production shown here are in the range of data

published for the area, although the averaged values were generally lower than those observed by other authors (ALBERIGHI *et al.*, 1997; ZOPPINI *et al.*, 1995). This may be due to the distance of the sampling stations from the coast, generally greater than that observed in others studies (in our study, the average distance from the coast of the first stations along the transects was 5 nautical miles). Finally, the results obtained in this investigation seem to support the important role of nutrient concentrations and competitive interactions among size fractions on both the structure and the function of phytoplankton guilds in the study area.

First of all, this was supported by the existence of patterns of variation of phytoplankton biomass and primary production with distance from the coast. Moreover, the higher phytoplankton biomass and primary production values and the steeper gradients observed in winter compared to summer may depend on the increased resource availability, due to the greater discharge of freshwater and the reduced area bounded by the frontal system (FRANCO & MICHELATO, 1992).

It was also supported by the direct relationships observed between phytoplankton biomass, primary production and nutrient concentrations. Indeed, the spatial variation of nutrient concentrations with distance from the coast is due to the fact that nutrients are important components of the Po river discharge (JUSTIC *et al.*, 1995). Nevertheless, the direct relationship observed between nutrients and phytoplankton biomass and primary production ( $r$  values in table 2 are positive and highly significant for both parameters) suggests that phytoplankton species were not able to take up nutrients to a point where concentrations were so low as to be limiting near the coast. Moreover, the correlations observed for DIN and TDP with respect to DIP indicated that biomass and primary production of phytoplankton guilds in the Northern Adriatic Sea were probably more dependent on phosphorus than nitrogen concentration, in agreement with other data reported in the literature regarding Mediterranean areas (COZZI *et al.*, 2002).

On the other hand, given that phytoplankton are able to decrease phosphorous concentration and increase its biomass linearly, as theoretically predicted (DE ANGELIS, 1992), the fact that dissolved inorganic phosphorus did not show significant patterns of variation along the inshore-offshore gradient (while biomass increased in proximity to the shore) would suggest phosphorus limitation in the coastal areas.

This was also supported by the patterns of variation of the different size fractions along a resource gradient: microphytoplankton were more important at the stations inside the frontal system (46.2% of biomass and 46.4% of primary production) and showed greater variation along the inshore-offshore gradient than nanophytoplankton and picophytoplankton (co-variation analysis). Picophytoplankton dominated phytoplankton guilds at the offshore stations (56.0% biomass and 44.1% primary production). These results are in

accordance with previous studies in which microphytoplankton increased with nutrient loading (PEREZ-RUZAFA *et al.*, 2002).

On the other hand, the size structure and size function of the phytoplankton guilds cannot be explained simply with reference to the dependence on size of the organisms' energy requirements. Indeed, picophytoplankton have lower energy requirements and higher photosynthetic efficiency than microphytoplankton, and are the best competitors when the availability of resources is low. However, for the same reasons and because the picophytoplankton fraction has higher growth rates than microphytoplankton, picophytoplankton should be the superior competitor even in conditions of high resource availability. Microphytoplankton species have high energy requirements, because of their mass. They are more abundant in conditions of high resource availability, and their population densities seem to increase with increasing resource availability faster than those of the pico- and nanophytoplankton size fractions, even though in general terms the smaller size fractions are likely to be more efficient and competitively superior. The importance of nanophytoplankton, in terms of relative biomass and primary production, did not vary along the spatial gradient, despite the variation in resource availability and the shift in guild dominance from microphytoplankton to picophytoplankton.

Therefore, hierarchical competition for nutrients among size fractions (micro > nano > pico) seems to play a role in the organization of phytoplankton guilds, although the causal mechanism remains unclear. However, other factors, such as zooplankton grazing, may also play a role in phytoplankton guild size structure, possibly mediated by size differential grazing (COTTINGHAM, 1999).

In conclusion, the results obtained in this study accord well with literature expectations concerning the consistency and predictability of qualitative changes in size structure and

function following perturbation of nutrient input (COTTINGHAM, 1999); accordingly, size structure may be viewed as a good descriptor of the trophic status of ecosystems.

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