

## RELATIVE IMPORTANCE OF THE SIZE-FRACTIONATED PHYTOPLANKTON POPULATION IN TEMPERATE WATERS, ALEXANDRIA (EGYPT)

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### ABSTRACT

The composition and the contribution of the 4 different cell size classes ( $>100$ , 50-100, 20-50 and  $<20$   $\mu\text{m}$ ) of the phytoplankton population in the neritic water of Alexandria were studied over an annual cycle. Their abundance exhibited considerable variability in times. Nanoplankton contributed numerically most of the total production (58.32%, range of 4.34-95.7%), culminating its peaks during the warm seasons, while the centric diatom, *Skeletonema costatum* and the microflagellates, *Pyramimonas* sp. and *Micromonas* sp. were its major constituents. Netplankton dominated at high nutrient levels. However, seasonal shifts were evident. The floristic data indicated the predominance of large diatoms and dinoflagellate cells at times, influencing their relative importance to the over all standing crop population. Temperature was significantly correlated with all the size classes, except that of  $>100$   $\mu\text{m}$ , which seems a phosphate dependent. Salinity and silicate concentrations seem affecting the variability of the smaller size classes.

### INTRODUCTION

Size-fraction is a way of separating the phytoplankton assemblages into various taxonomic groups. Traditionally, phytoplankton has been categorized according to size as either netplankton (i.e.,  $> 20\mu\text{m}$ ) or nanoplankton (i.e.,  $< 20\mu\text{m}$ ), that permits more complete

evaluation of ecosystem ecology and phytoplankton autoecology (Malone, 1971). According to Platt (1989) and Yentsch & Campbell (1991), that the recovery of bulk properties from the details of the constituents is more desirable goal in phytoplankton research.

Factors regulating the cell size of phytoplankton in the sea have been previously discussed (Parsons & Takahashi, 1973; Herbrand *et al.*, 1985).

The size of primary producers represents a basic factor to understand the ecological attribute of marine environment and the processes of succession in marine phytoplankton which require evaluation of the biological differences between species within the community (Smayda, 1973).

Size-fractionation has been used to explore the trophic interactions in marine ecosystem (Conover, 1978), as well as the contribution of phytoplankton size classes and their constituent floristic groups to bloom dynamics and primary production (Durbin *et al.*, 1975; Malone, 1980; Hallegraef 1981; Furnas, 1983).

Various metabolic parameters, biomass, sinking rate, buoyancy (Malone 1980) and nutrient uptake kinetics (Walsh 1976), are cell size dependency. Most of chlorophyll-bearing cells in the oligotrophic waters of the eastern Mediterranean Sea are small,  $<10\mu\text{m}$  (Rainbault *et al.*, 1988; Li *et al.*, 1993).

The chemical and physical character of a given ecosystem is reflected in the size of its initial energy fixers (Turbin & Harrison 1980). The experimental data show a general trend of decreasing photosynthetic activity with increasing cell size (Takahashi & Beinfang, 1983).

Size-fractionation potentially influences grazing pressure and food chain dynamics (Chervin 1978, Jackson 1980, Durbin & Durbin 1981, Sournia, 1982). According to Nival & Nival (1976) the filtration

efficiency of copepods is very low under 3 $\mu$ m size class even for first copepodite stages of the small *Acartia*.

The present study describes the temporal variability in size-fraction of a natural phytoplankton population in Alexandria (Egypt) waters, a way to declare the relative importance of the different parts of the community to the overall phytoplankton standing stock for a given particular set of environmental conditions. The results are expected to modify our concepts on the biological structure and dynamics of a temperate ecosystem and lay the foundation enabling an understanding of physical and chemical factors that probably control the production cycle in this system. Such study in Alexandria waters is so far very limited in space and time.

### MATERIALS AND METHODS

The Eastern Harbour (E.H) of Alexandria is a shallow, semi-enclosed basin located in the central part of Alexandria City. It has an area of about 2.53 km<sup>2</sup>, average depth of 5 m and a water volume of about 15.2x10<sup>6</sup>m<sup>3</sup>.

A surface water sample was collected at a fixed station every second week from March 1996 to January 1997, except for December and January, when it was once per each. The measured physico-chemical parameters include temperature, salinity (using salinity refractometer, S/Mill, after calibration), dissolved inorganic nutrients; nitrate, silicate and phosphate (following Strickland & Parsons 1972). In the laboratory, this water sample was prefiltered through 200  $\mu$ m mesh net to remove the larger zooplankton, following Herbland *et al.*, 1985. Then it was filtered successively using 100, 50 and 20 $\mu$ m mesh net, with gentle swirling to fractionate it. The contents were poured into a measuring cylinder and made to the desirable volume with filtered seawater. Cell numbers of the different species of each size fraction were determined using inverted

research microscope by counting 1ml sample. Lugol's iodine solution was added as a preservative.

The floristic authorities employed for the identification of the different species were Cupp (1943), Hendey (1974), Park & Dixon (1976) and Taylor (1976).

Because many species of diatoms form long chains or have long setae, or both, the cells retained by the net filters do not necessarily have dimensions greater than their mesh size. Thus, cell dimensions (length, width and thickness) were measured for dominant phytoplankton species to overcome such problem, helping determination their actual class structure.

The statistical analysis (*t* test) and the multiple regression analysis (NCSS, Hintze 1993) were computed to correlate the numbers of the different size fractions and the measured physico-chemical variables at a given condition.

## RESULTS

The surface water temperature, salinity, nutrient concentrations and the relative importance of the different size fractions to the total standing crop are shown in Fig. (1); the composition and abundance of the different phytoplankton size classes are given in Table (1) and the correlation matrix in Table (2).

The following is an account of the seasonal variability of the recorded phytoplankton size classes and associated water characteristics.

### **The >100 $\mu\text{m}$ size fraction**

This size class represented the lowest contributory to the total standing crop (4.31%, range 0-18.24%, and annual average of 97.1 cell. ml<sup>-1</sup>). Very low numbers were counted in spring, it was absent in the second week of April, forming about 0.12 % to the total during May. A tendency to a noticeable increase was seen by July (43.76 cell.ml<sup>-1</sup> on 8 July about 7-fold increase compared with late June). Such relatively

higher numbers extended till late November. The major peak occurred on 11 October ( $1037 \text{ cell.ml}^{-1}$ , 18.24%) at  $23^{\circ}\text{C}$ , salinity 38.6 and relatively low nutrient concentrations (1.8, 1.55 and  $0.6 \mu\text{M.l}^{-1}$ , for nitrate, silicate and phosphate, respectively). The pennate diatom, *Nitzschia seriata* ( $1100 \text{ cell.ml}^{-1}$ , length of valve falls within  $90\text{-}120\mu\text{m}$ , 25 measured cells) was the causative species. A minor peak for the same species followed this by the end of the month. Another increased number was also observed in late August ( $179 \text{ cell.ml}^{-1}$ ), attributed mostly to the pennate diatoms, *Rhizosolenia setigera*, *R. stolterfothii*, and the dinoflagellates, *Oxytoxum sceptrum* and *Ceratium furca*.

Negative correlation (Table 2) was found between the counts of the  $>100\mu\text{m}$  size class and the ambient nutrient concentrations, significantly ( $P < 0.05$ ), with phosphate; positively with temperature and salinity. The correlation matrix shows:

**The counts of the  $>100 \mu\text{m}$  size class = - 690.71-13.16.  $\text{PO}_4$  - 16.32.  $\text{SiO}_4$ -52.19.  $\text{NO}_3$ +21.16.  $\text{T}^{\circ}\text{C}$  +178.15.  $\text{S}\text{‰}$   $\text{R}^2 = 0.28$**

### **The 50-100 $\mu\text{m}$ size fraction**

This size class occupied the third level of the components comprising the total productivity ( $226 \text{ cell.ml}^{-1}$ , 14.55%, range 0.49-74.59%). Its numbers fluctuated between 2.4 and  $90.14 \text{ cell.ml}^{-1}$  during the period from the middle March and early June. This was followed by remarkable increased counts during late June, extending in the next month. The major peak on 28 July ( $1809.5 \text{ cell.ml}^{-1}$ , 38.73% to the total) was due to the proliferation of the dinoflagellate, *Prorocentrum triestinum* ( $1500 \text{ cell.ml}^{-1}$ , length  $38\text{-}47\mu\text{m}$ , 50 cells) and less so *Euglena* spp. This bloom took place at  $28.8^{\circ}\text{C}$ , low salinity 37.5 and intermediate nutrient concentrations ( $2.4, 2.4$  and  $1.35 \mu\text{M.l}^{-1}$ , for nitrate, silicate and phosphate, respectively). Yet, the significant contribution of the 50-100  $\mu\text{m}$  size class was recorded during the last week of June, due to *P.*

*triestinum*, and *Thalassionema nitzschioides*. Again, despite the low numbers in winter, this class formed 22.12 and 37.18% to the total during December and January, respectively, when *Biddulphia aurita* and *Asterionella glacialis* became dominant.

There is an inverse correlation with the measured physico-chemical parameters, except for temperature, significant ( $P < 0.05$ ), with temperature, salinity and silicate. The equation of the multiple regression analysis is:

$$\text{The counts of the 50-100 } \mu\text{m size class} = - 8654 + 141.22 \cdot \text{PO}_4 - 20.1 \cdot \text{SiO}_4 - 83.49 \cdot \text{NO}_3 + 68.76 \cdot \text{T}^\circ\text{C} + 197.15 \cdot \text{S}\text{‰} \quad R^2 = 0.39$$

### The 20 - 50 $\mu\text{m}$ size fraction

This is the second important contributory class (22.8%, range 3.3-53.9%). A minor peak (775.85 cell.ml<sup>-1</sup>, 12%) was recorded in the second week of May, due to the chain-forming diatom, *Skeletonema costatum* (532 cell.ml<sup>-1</sup>, valve diameter 2.5-7.5 $\mu\text{m}$ , length of valve 5-17.5 $\mu\text{m}$ , 70 cells) sharing with the dinoflagellate, *Gymnodinium catenatum* (103 cell.ml<sup>-1</sup>, length 40-50 $\mu\text{m}$ ). Yet, the major peak of the 20-50 $\mu\text{m}$  size class occurred on 8 July (2418 cell.ml<sup>-1</sup>, *S. costatum* dominated) and less so by the end of this month (929 cell.ml<sup>-1</sup>, 20% to the total, the dinoflagellate, *Prorocentrum minimum*, length 20-30 $\mu\text{m}$ , 15 cells with *S. costatum* were the responsible species). This bloom on 8 July maintained 28.2°C, low salinity 37 and relatively high nutrient concentrations (3.6, 4.2 and 2.5 $\mu\text{M.l}^{-1}$ , for nitrate, silicate and phosphate, respectively). Then, its numbers started to decline till November, when the diatom, *Melosira granulata* (336 cell.ml<sup>-1</sup>, diameter 3.75-7.5 $\mu\text{m}$ , length 15-45 $\mu\text{m}$ , 25 cells) and *S. costatum* (347.52 cell.ml<sup>-1</sup>) became leaders on 9 and 24 November, respectively. Despite the low number

during January, this class contributed about 52.61% to the total standing crop.

Temperature, salinity and silicate significantly correlated with the counts of the 20-50 $\mu\text{m}$  size class, positively with the first variable. The regression matrix shows:

$$\text{The counts of 20-50}\mu\text{m size class} = -7757 + 305.59 \cdot \text{PO}_4 - 52.37 \cdot \text{SiO}_4 - 105.89 \cdot \text{NO}_3 + 81.57 \cdot \text{T}^\circ\text{C} + 168.68 \cdot \text{S}\text{‰} \quad \text{R}^2 = 0.48$$

### The < 20 $\mu\text{m}$ size fraction

This comprised numerically most of the total production (average 2091.49 cell.ml<sup>-1</sup>, 58.32%, range 4.34-95.7%). Its seasonal distribution exhibited very low densities during the cold periods in early spring, December and January. Several distinct peaks were recorded. The first massive occurrence of this class was seen about the middle of May (5598 cell.ml<sup>-1</sup>) attributed to unidentified microflagellates, associated with *S. costatum*. A sharp drop was then observed till early July when the latter species contributed its intensive existence all over the year (13043 cell.ml<sup>-1</sup> on 8 July) maintaining, as previously mentioned high nutrient levels. This size class dominated till November. However, the succession progressed differently. The microflagellates, *Pyramimonas* sp. and *Micromonas* sp. were overwhelmingly dominant in the second week of both September and October, while *S. costatum* ranked the second. This latter species regained its dominance during November. The common feature of the two microflagellate blooms on May and September was the very low levels of the nutrient concentrations, silicate falling its year minimum (0.8 $\mu\text{M.l}^{-1}$ ). Salinity was unchanged.

There is an inverse significant correlation between the counts of the <20  $\mu\text{m}$  size class and salinity and silicate, but positively with temperature. A very weak correlation was found with phosphate.

The multiple regression analysis shows:

The counts of the < 20  $\mu\text{m}$  size class = - 45303+133.15.  $\text{PO}_4$  - 326.31.  
 $\text{SiO}_4$ -636.36.  $\text{NO}_3$ +422.43.  $\text{T}^\circ\text{C}$ +1032.44.  $\text{S}\%$   $\text{R}^2 = 0.37$

## DISCUSSION

The seasonal patterns in the size frequency distribution demonstrated considerable variations. Although the nanoplankton represented the main component of the community, the degree of dominance changed at times. The floristic data indicated *Skeletonema costatum*, *Pyramimonas* sp. and *Micromonas* sp. to comprise principally the main bulk of the nanoplankton. These species are numerically important constituents of the community in the E. H, the former species is a well known red tide species, with inflow of land (Labib, 1994).

Various geographical studies have reported that the nanoplankton is often responsible for 80-90% of the observed phytoplankton productivity in both temperate (McCarthy *et al.*, 1974; Malone 1977; Takahashi & Beinfang 1983) and tropical waters (Ibbara 1978). The nanoplankton (<20 $\mu\text{m}$ ) were the most important, accounting for 46.6% of the annual biomass as chlorophyll *a* and 50.8% of the total production in Narragansett Bay, USA (Durbin *et al.*, 1975). There are frequent reports that nanoplankton turn over faster than netplankton (see Malone, 1971a) under conditions favorable for diatom growth. The very low nutrient concentrations accompanied the present different massive occurrence of nanoplankton supports the conclusion of Beinfang & Takahashi (1983) that nutrient uptake rates of the small cells are more rapid than those of the larger population components. On the other hand, netplankton dominated at high nutrient levels, in agreement with the findings of Malone (1980) that a bloom of this class develops in response to large input of nutrients. Results to explain the relationship between algal size and quantitative differences in nitrogen utilization illustrated that the

highest specific ammonium uptake rates by phytoplankton have been shown to be almost exclusively in the nanoplankton (Gilbert *et al.*, 1982 a). However, seasonal shifts were evident at times. In nutrient uptake kinetics cell-surface to volume consideration predict that large cells are less able to absorb nutrients from low nutrient waters (Eppley *et al.*, 1969, Friebele *et al.*, 1978). According to Takahashi & Beinfang (1983) nanoplankton have negligible settling rate, indicating that there should be virtually loss of this small size fraction from photic zone due to sedimentation. This could represent a crucial reason for the maintenance of this class, in agreement with the present study for the predominance of nanoplankters during warm seasons with expected water density stratification. The quantitative importance of nanoplankton has been described for a variety of environments (Taguchi 1980, Beinfang & Szyper 1981, Maita & Odate 1988).

The floristic data indicated the predominance of large cells at times, affecting in clear way the relative importance of their different size classes. The dinoflagellate species (*Gymnodinium catenatum*, *Prorocentrum triestinum*, *P. minimum*, *oxytoxum sceptrum*, *Ceratium furca*), the diatoms, *Nitzschia* spp (*N. seriata*, *N. longissima*, *N. closterium*), *Rhizosolenia* spp. (*R. setigera*, *R. stolterfothii*), *Thalassiosira rotula* and *Thalassionema nitzschioides* were principally the responsible species for such variations. All of these species were previously recorded in the E.H as major components of the community, some of them contributed red tide occurrence (Labib 1994 b, 96, 98).

~~It is well known that temperature is an allogenic limiting growth factor, phytoplankton blooming, periodicity of different groups and algal succession (Tilman *et al.*, 1986). The present study stressed the importance of temperature to be a crucial controlling factor of the development of different phytoplankton size structure. The >100µm size-fractionated class seems phosphate dependent, while the abundance of~~

the smaller size classes was deeply affected by salinity and silicate variability.

In conclusion, the present study declared the need to consider the contribution of the different cell size structure and measured cell dimensions to fully describe the variability of the phytoplankton stock in Alexandria waters, where there is a paucity of such information. The separation of the phytoplankton on the basis of size permitted more complete evaluation of temperate ecosystem ecology.

For floristic research, focus on an extended size range, particularly to the ultraplankton organisms ( $<5\mu\text{m}$ ) is desirable to understand the size fractionation-ecological process relationships. Detailed analysis in natural population of phytoplankton at sub-community levels of organisms can provide valuable insight into the structure and functioning of the pelagic food web, as well as the development and decay of the phytoplankton blooms.

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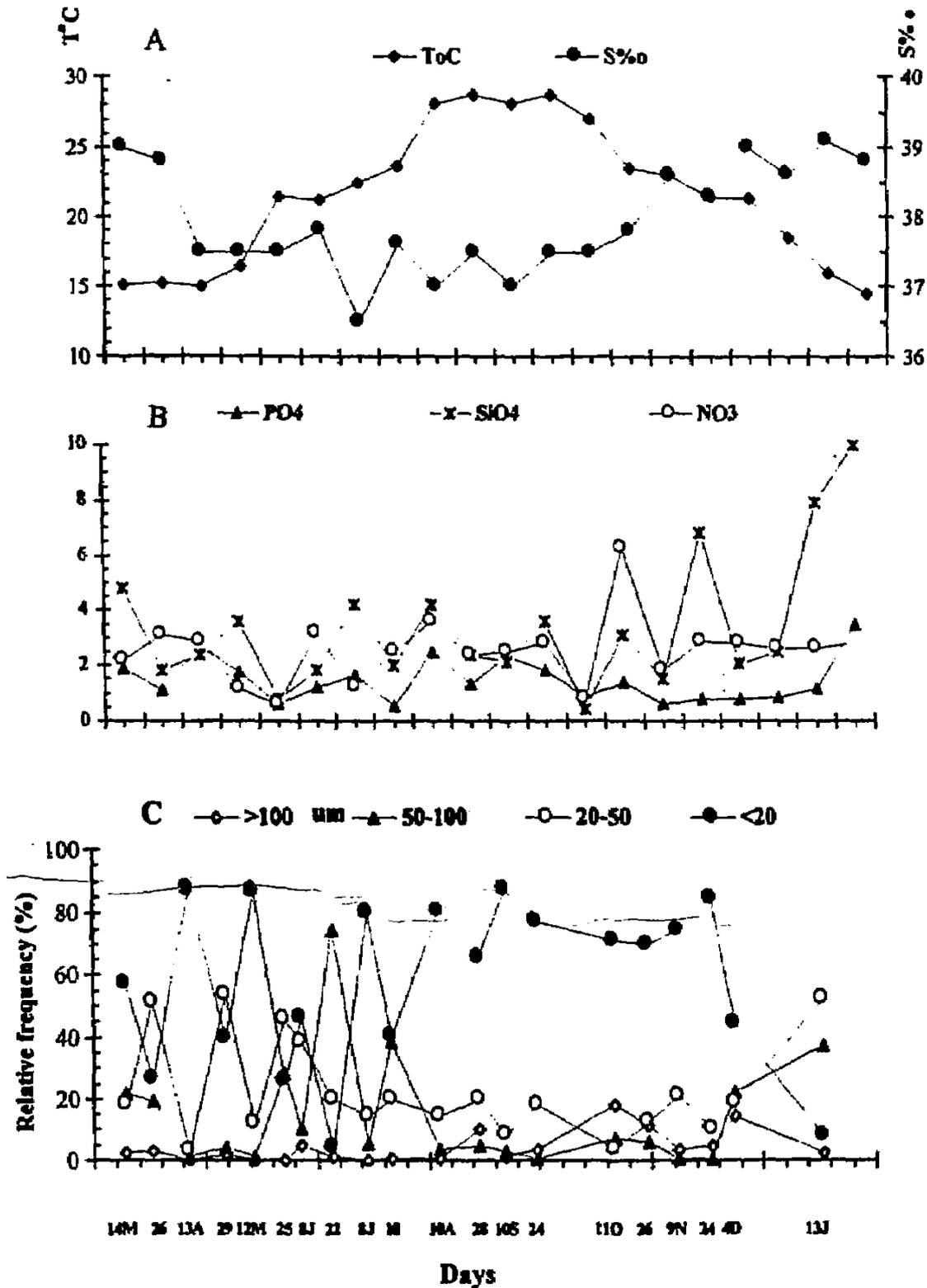


Figure 1. Surface temperature, salinity (A), nutrient concentration (B) and the relative abundance of the phytoplankton size structure (C).







Table 1. To be continued.

Species	4 December				13 January			
	>100 µm	50-100	20-50	<20	>100 µm	50-100	20-50	<20
<i>Alexandrium minutum</i>	0	0	0	0	1.26	23.9	0	0
<i>Bellarochea malleus</i>	0	0	2.4	0.13	0	0	23.9	1.26
<i>Biddulphia aurita</i>	0	11.27	0	0	0	11.8	0	0
<i>Chaetoceros affine</i>	0	0	0	0	0	0	21.4	3.78
<i>Chaetoceros decipiens</i>	0	0.05	0.44	0	0	0.1	0.9	0
<i>Coscinodiscus centralis</i>	1.27	0	0	0	0	0	0	0
<i>Cyclotella meneghiniana</i>	0	0	0	0	0	0.15	0	0
<i>Euglena acus</i>	0	0	0	0	0	0.15	0.02	0
<i>Grammatophora marina</i>	0	0.07	0.57	0	0	0	0	0
<i>Lauderia undulatum</i>	0	0	0	0	0	0	2.5	0
<i>Leptocylindrus danicus</i>	0	0	0	0	0	0.1	0.4	0
<i>Nitzschia closterium</i>	0	0	1.3	0	0	0	0.17	0
<i>Nitzschia longissima</i>	0.46	0	0	0	0	0	0	0
<i>Pleurosigma decorum</i>	0.64	0	0	0	0	0	0	0
<i>Protoperdinium</i> spp.	0	0	0	0	0.35	0	0	0
<i>Prorocentrum micans</i>	0	0	0	0	0	0	0	0
<i>Prorocentrum minimum</i>	0	0	0.17	0	0	0	0	0
<i>Prorocentrum triestinum</i>	0	0.16	0	0	0	0.34	0	0
<i>Rhizosolenia setigera</i>	0	0	0	0	0.34	0	0	0
<i>Skeletonema costatum</i>	0	0	4.1	23.3	0	0	2.57	4.64
<i>Scrippsiella trochioda</i>	0	0	0.32	0	0	0	0	0
<i>Thalassiosira rotula</i>	0	0.01	0.85	0	0	0.04	0.3	0

Table 2. The correlation matrix

	PO <sub>4</sub>	SiO <sub>4</sub>	NO <sub>3</sub>	T°C	S‰	>100µm	50-100	20-50	<20
PO <sub>4</sub>	1.00								
SiO <sub>4</sub>	0.44	1.00							
NO <sub>3</sub>	0.04	0.05	1.00						
T°C	-0.23	-0.46	0.08	1.00					
S‰	-0.17	0.39	0.17	-0.61	1.00				
>100µm	-0.34	-0.18	-0.13	0.16	0.21	1.00			
50-100	-0.05	-0.24	-0.09	0.56	-0.26	0.10	1.00		
20-50	0.11	-0.30	-0.11	0.59	-0.42	-0.05	0.58	1.00	
<20	0.01	-0.32	-0.13	0.53	-0.34	0.17	0.38	0.92	1.00