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A MECHANISM TO MAINTAIN A DINOFLAGELLATE RED TIDE IN THE NERITIC WATERS OF ALEXANDRIA (EGYPT)

CONJUNCTION OF THE DIEL VERTICAL MIGRATION OF PROROCENTRUM TRIESTINUM WITH ITS IN SITU AND IN VITRO NUTRIENT UPTAKE KINETICS AND PHASED CELL DIVISION

BY

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Key Words: Nutrients uptake kinetics, phased cell division, diel vertical migration

ABSTRACT

*The ability of **Prorocentrum triestinum** to undergo diel vertical migration crossing a distinct pycnocline, its relatively higher migration ambit as well as growth rate and corresponding doubling time represent crucial strategic factors for its massive occurrence.*

*Aspects of the species reproduction facilitate the use of the phased cell division method. Cells in different division stages were seen most of the time. Yet, the main division gate extended for about 4-hrs before sunrise and there was a lag time of about 3-4 hrs behind the formation of cells with 2 nuclei and those paired together cells. The phased division of **P. triestinum** occurred predominantly in the migrating cells and there was a depth differentiation of divided cells.*

The ability of this species to take up nitrate and phosphate in darkness has been considered a special adaptation, which might work in conjunction with the diel vertical migration to foster its development in nature. In situ, > 60 % and 70 % of nitrate and phosphate over the bottom disappeared between midnight and early morning of the next day, with the descent-partial ascent movement of

P. triestinum. *In vitro, the dark/light uptake rates were estimated at 42–88% nitrate and 33–100% phosphate.*

INTRODUCTION

The vertical movement of dinoflagellate species is a complex phenomenon. Variations in the vertical migration patterns (positive or negative phototactic behaviour) have been studied by considering external environmental factors (Heaney & Eppley 1981, Kamykowski 1981, Labib & Halim 1995). The range of vertical migration of dino-flagellates is frequently variable. Kiefer & Lasker (1975), and Heaney & Talling (1980), found that populations of the same species may either undertake appreciable diel vertical migration or form relatively stable sub-surface maxima. The presence of endogenous or circadian rhythms has also been proved (Kohata & Watanabe 1986).

According to Lehman ***et al.*** (1975) the uptake rate of nutrients by phytoplankton, which varies from one species to another, is important in understanding competition, succession and dominance within natural populations of phytoplankton. Among the factors that influence nutrient uptake are cell quota (Gotham & Rhee 1981), nitrogen sources and their relative concentrations (e.g. Paasche & Kristiansen 1982), temperature (French & Smayda 1995) and light (Rivkin & Swift 1982).

There is some understanding of the dynamics of nitrogen nutrition in a few bloom-forming species, such as ***Ceratium tripos*** (Conway & Whitledge, 1979), ***Gonyaulax polyedra*** (e.g. Heaney & Eppley, 1981), ***Gymnodinium splendens*** (e.g Dortch & Maske, 1982), ***Heterosigma akashiwo, Chattonella antiqua*** (Watanabe ***et al.***,1983), ***Amphidinium carterae, Gymnodinium galatheanum, Gyrodinium aureolum, Heterocapsa triquetra, Prorocentrum micans, Prorocentrum minimum, Scrippsiella trochoidea*** (Paasche ***et al.***, 1984), and ***Heterocapsa niei*** (Cullen ***et al.***, 1985). Yet, to our knowledge, no such information is available neither on nitrogen uptake by the bloom-forming dinoflagellate, ***Prorocentrum triestinum*** nor on phosphate uptake.

When only a portion of the cells in a population divides each day, but does so in a restricted gate, the population is termed "phased". The mechanisms by which this alignment is achieved and maintained and the extent to which it

varies among and between various phytoplankton species are critical aspects of the adaptive physiology of phytoplankton (Sournia 1974).

Several hypotheses have been proposed concerning the ecological significance of phased cell division; a valuable tool for assessing calculations of species-specific growth rates (see Weiler, 1978), in the context of grazing (Chisholm ***et al.***, 1978), nutrient uptake capacity, competitive interaction among the phytoplankton communities (Chisholm & Nobbs 1976), and species composition (Doyle & Poore 1974). However, these hypotheses cannot be evaluated until more information on the temporal patterns of various processes among a number of taxonomic groups has accumulated.

In view of the fact that marine dinoflagellates differ widely in their response to regulating parameters: swimming behavior, phototaxis, geotaxis and circadian rhytm; and the ability to take up nutrients (Paasche ***et al.***, 1984), beside the limited number of species that have been investigated and the seemingly contradictory results in some cases, it was of interest to study the *in situ* and *in vitro* uptake of nitrate and phosphate by ***P. triestinum***, its phased growth division, timing and calculated *in situ* growth rate, in conjunction with diel vertical migration during its bloom. Such information could represent an attempt to identify some behavioral mechanisms responsible for its repeated bloom outbreaks in Alexandria waters (Zaghloul & Halim 1992, Labib 1996).

MATERIAL AND METHODS

The present study was carried out at a fixed station (4.5 m depth), in the western part of the Eastern Harbour (E.H) of Alexandria during July 1998.

The E. H is a shallow, semi-enclosed embayment (area 2.5 km^2, average depth 5m, and volume 15.2×10^6 m^3), located in the central part of Alexandria City (Fig. 1). During the last 25 years, the harbour received directly municipal wastewater estimated, annually, at 35.2×10^6 m^3. It is also affected, intermittently, by additional volume of a raw sewage from the main outlet of Alexandria in its western vicinity.

The harbour water turned reddish in color during the period from 2 to 8 July 1998, indicating the development of a heavy red tide bloom. The dinoflagellate ***Prorocentrum triestinum*** was the causative organism.

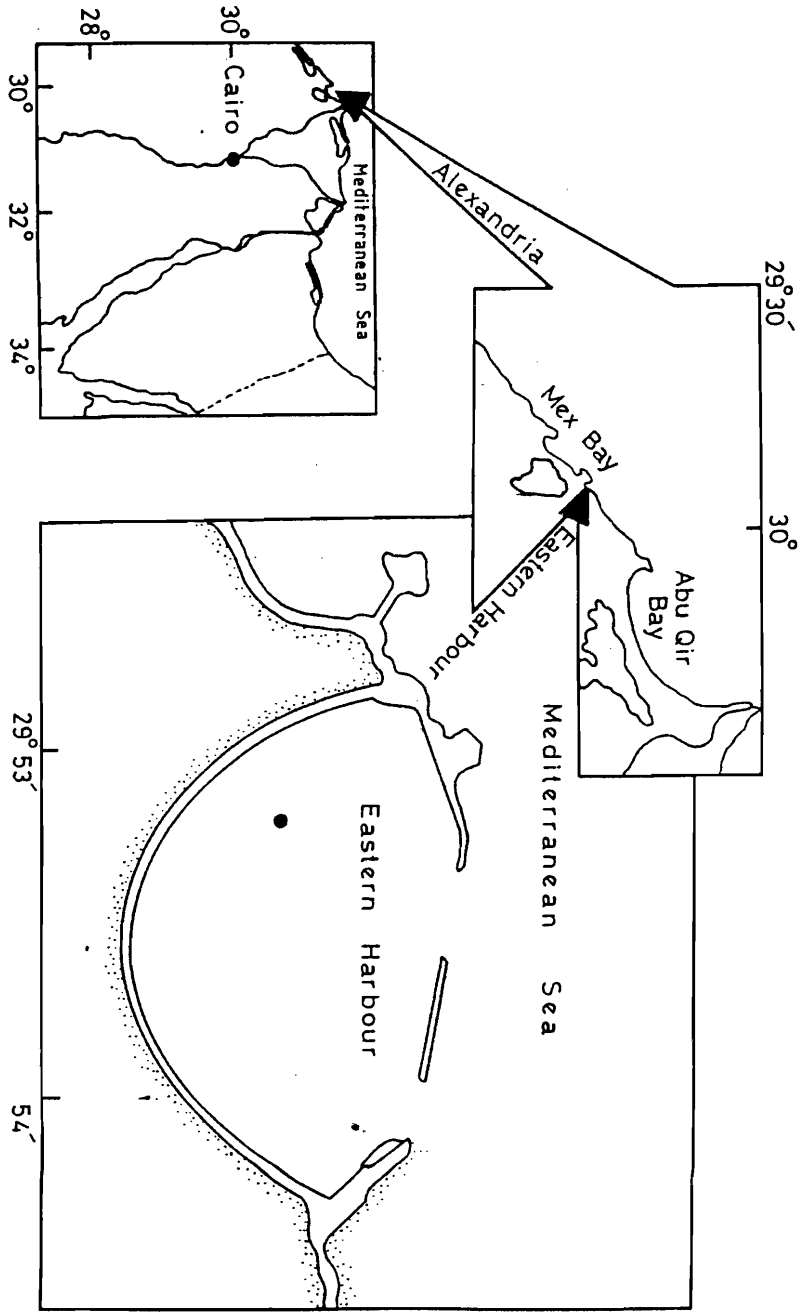

Fig. 1 - The Eastern Harbour of Alexandria and location of the sampling station (•).

On 4-5 July, the diel vertical migration pattern of ***P. triestinum*** was recorded. Samples were collected at each meter level from the surface to the layer over the bottom, during a 20-hrs cycle at 2-5-hrs intervals. The vertical profiles included water temperature (using standard thermometer, ± 0.1°C), salinity (salinity refractometer), dissolved inorganic nitrate, phosphate, chlorophyll *a* (Strickland & Parsons 1972) and cell concentrations (Utermöhl 1958). Nutrient samples were filtered, frozen and analyzed at a later time. Living phytoplankton samples were first examined with a research microscope, then preserved and counted.

The determination of the phased cell division, the timing and calculation of the growth rate of ***P. triestinium*** were based on both the vertical migration samples during the 20-hrs cycle, as well as hourly samples collected between 02.00h A:M of 4 July and 08.00h A:M on the following morning for all depths. The samples were stained following Lewin & Rao (1975). After this treatment nuclei stained bright red were very distinct. Cells with single nucleus (not including those of paired together, dividing cells), cells with paired nuclei and recently divided pairs of cells were separately counted. The growth rates of ***P. triestinum*** (μ_2) were determined from the maximum daily frequency of division and from the increase in cell number following the equations of Weiler & Eppley (1979):

$$\mu_2 = 1/t \, \mathrm{Ln} \, (F_{max}+1) \qquad (1)$$

where: F_{max} = maximum $(b+c) / (a+b+c)$ and a = no. of cells with one nucleus (not including those of paired-together recently divided cells); b = no. of cells with paired nuclei; c = half of the recently divided cells (paired-together cells); μ_2 = specific growth rate (day^{-1}) and t = 1 day.

$$\mu_2 = 1/t \, \mathrm{Ln} \, (N_1/N_0); \quad \mathrm{D.T} \; d^{-1} = \mathrm{Ln}2 / \mu_2 \qquad (2)$$

Where N_1 and N_0 are the cell numbers at t_1 and t_0 and D.T is the doubling time in days

A culture experiment was designed to determine the timing of the division gate, percentage of dividing cells and uptake rates of nitrate and phosphate.

P. triestinum was isolated from the harbour during September 1998, when this species represented an important contributory of a multi-species red tide

bloom with a maximum density of 1.1 x 10^6 cell. l^{-1}. The culture was obtained by the micropipette washing method, inoculated with 250-300 living cells and grown in a capped conical flask (1000 ml). The inoculum was bacteria-free. The f/2 medium (Guillard & Ryther 1962) was the basal medium after sterilization by autoclaving (120°C, 20 min). The culture maintained 25° C under a light intensity of 170μE.m^{-2}.s^{-1} provided by "white fluorescence", under 12:12 LD cycle. After 5 days from inoculation periodical monitoring of cell density (3-hrs interval) and chlorophyll *a* content (Strickland & Parsons 1972) was carried out and the timing and percentage of dividing cells over a 36-hrs cycle were estimated. The nitrate and phosphate uptake experiments were conducted after 14 days from inoculation when the ambient nitrate and phosphate concentrations (Strickland & Parsons 1972) were almost undetectable, and when the cell concentration reached about 15 x 10^3 cell. l^{-1} Twenty 30 ml sub-samples of nitrate and phosphate depleted culture were dispensed into 200 ml Erlenmayer flask and diluted with 70 ml of both nitrate-limited and phosphate-limited H medium (Nakamura & Watanabe 1983). Changes in ambient nitrate and phosphate were determined every 3-hrs from light on through a 27 –hrs cycle. Samples (10 ml) were filtered using Whatman GF/C fiber filter and analysed as previously mentioned. The cell counts were also determined. All experiments were done in duplicate.

The nitrate and phosphate uptake rate (v) was calculated following the equation of Watanabe ***et al***. (1983):

$$v = - dS.(dt)^{-1}/N,$$

where N is the cell count, and S is nitrate or phosphate concentration.

RESULTS

The present study was performed under bloom conditions for ***P. triestinum*** (93-98.8% of the total population).

The vertical profiles of ***P. triestinum*** cell density, chlorophyll *a* concentrations, and associated physico-chemical parameters during the 20-hrs cycle on 4-5 July are shown in Fig. 2.

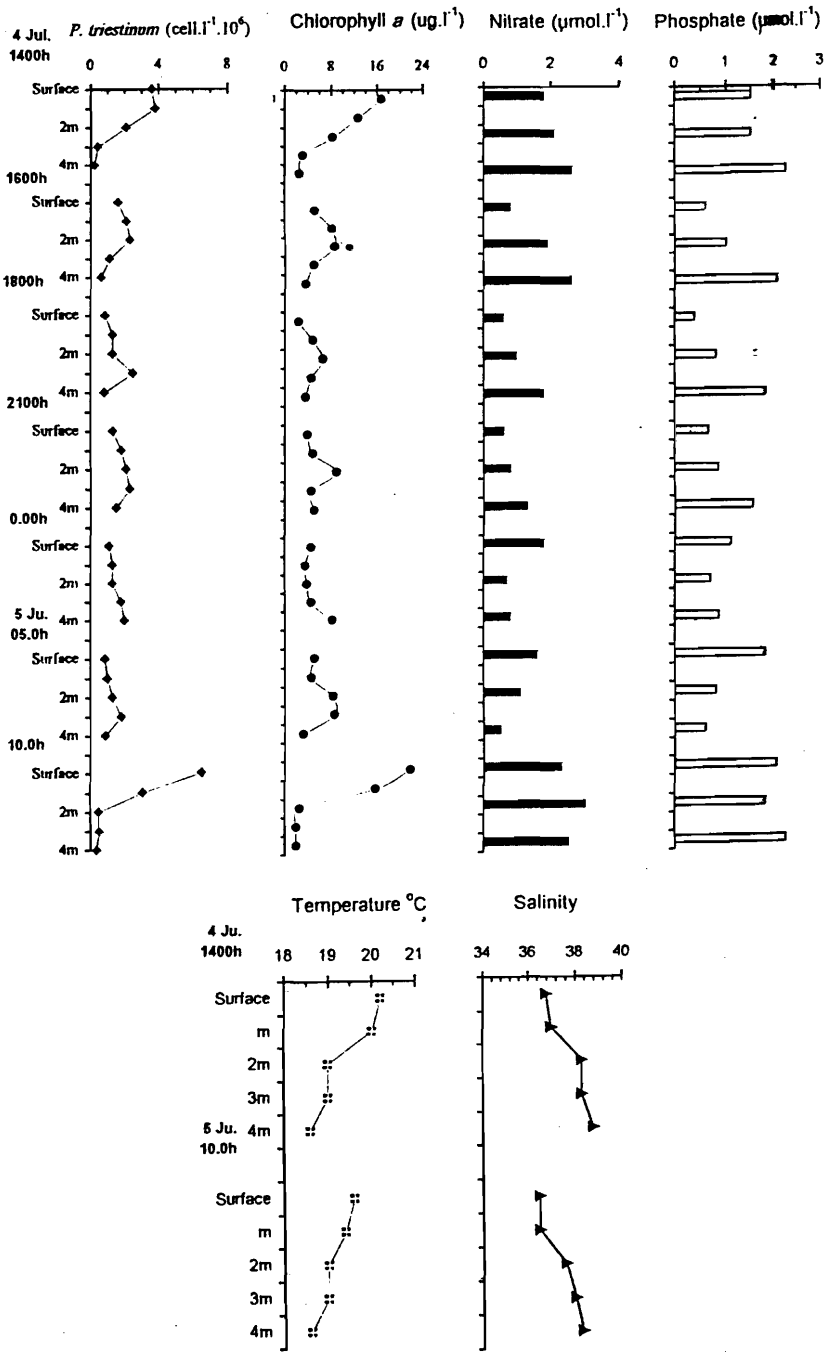

Fig. (2): Vertical profiles of the physico-chemical parameters measured, chlorophyll a and the density of *Prorocentrum triestinum* during a 20-hrs cycle.

Physical conditions

Surface water temperature was around 20°C and salinity was relatively low (36.5-36.8). The surface water was always warmer and less saline than the above-bottom layer. Temperature and salinity differences between surface and 4 m ranged between 1-1.6°C and 1.6-2, respectively. Subsequently, the water column was well density stratified.

Diel vertical migration

As evidenced from the vertical distribution of the population density of ***P. triestinum*** there was a clear periodicity, indicating a vertical migration pattern. As the bloom was mono specific, chlorophyll *a* concentrations also revealed its diel vertical migration. The species was able to migrate crossing the established thermo-haline stratification. The ascent-descent movements did not exactly coincide with the light regime. The species started its massive descent after noon, density between $2.1x10^6$–$2.3x10^6$ cell. l^{-1} in the sub-surface layer (1-2 m, 1600h). Relatively higher concentrations were recorded in the middle layer (2-3 m), during the next 5 hrs (1800-2100h). This was followed by almost homogenous vertical distribution at midnight, with increased numbers over the bottom ($2 x10^6$ cell. l^{-1}). The upward movement started before sunrise. The maximum accumulation of ***P. triestinum*** was observed at the surface before noon, at 10.00h, on 5 July ($6.5x10^6$ cell. l^{-1}), showing no avoidance of the surface layer at times of maximum irradiance.

Using the depth and time of the maximum cell and chlorophyll *a* concentrations, the mean ascent and descent rates were nearly similar (0.55 - 0 60 mh^{-1}).

Nitrate and phosphate uptake kinetics

In situ observations

The diel movement of *P. triestinum* clearly influenced the corresponding variability of nitrate and phosphate. Their surface concentrations were lower than above the bottom in the after noon – early nighttime. Nitrate and phosphate concentrations being 4.5 and 4.7 fold higher at 4 m, at 1800h. However, the reverse was true from midnight to the following early morning on 5 July. Intermediate concentrations were detected in the mid- water layer with the massive downward movement. Their concentrations over the bottom decreased drastically, reaching a minimum before dawn.

In vitro observations

The L:D culture experiment lasted for 27-hrs (Fig. 3, Table 1) and showed that ***P. triestinum*** had the ability to take up nitrate and phosphate both in light and dark. Their ambient concentration with time decreased almost linearly. A sharp drop coincided with the main time of cell division. The dark/light uptake rates were estimated at 42–88% nitrate and 33–100% phosphate.

Table (1): *In vitro* light (L) and dark (D) nitrate (v_N) and phosphate (v_P) Uptake rates of *Prorocentrum triestinum*

Time (hrs)	L or D	v nitrate (v_N) f mol. cell^{-1}. Hr^{-1}	N phosphate (v_p) f mol. cell^{-1}. hr^{-1}
0-3	L	0.33	0.23
3-6	L	0.29	0.22
6-9	L	0.16	0.11
9-12 (0)	L	0.30	0.33
0-3	D	0.29	0.33
3-6	D	0.27	0.17
6-9	D	0.22	0.17
9-12 (0)	D	0.14	0.11
0-3	L	0.14	0.08

Phased cell division

The L:D culture experiment during 36 hrs (Fig 4A.) indicated a parallel relation between the cell density and chlorophyll *a* content. Cell division could be seen to occur in both light and dark periods. However, it was noticeable with the light off, extending successfully during the dark period. The maximum percentage of cells undergoing division (ca. 14% of the total count) occurred 3-hrs before the next light phase, indicating that the main division gate was at night.

The percentage to the total and the mean number of cells of ***P. triestinum*** in different division stages during 20-hrs cycle is given in Fig 4 B&C.

The field population showed that cells of ***P. triestinum*** in processes of division were seen during most of the day and night. However:

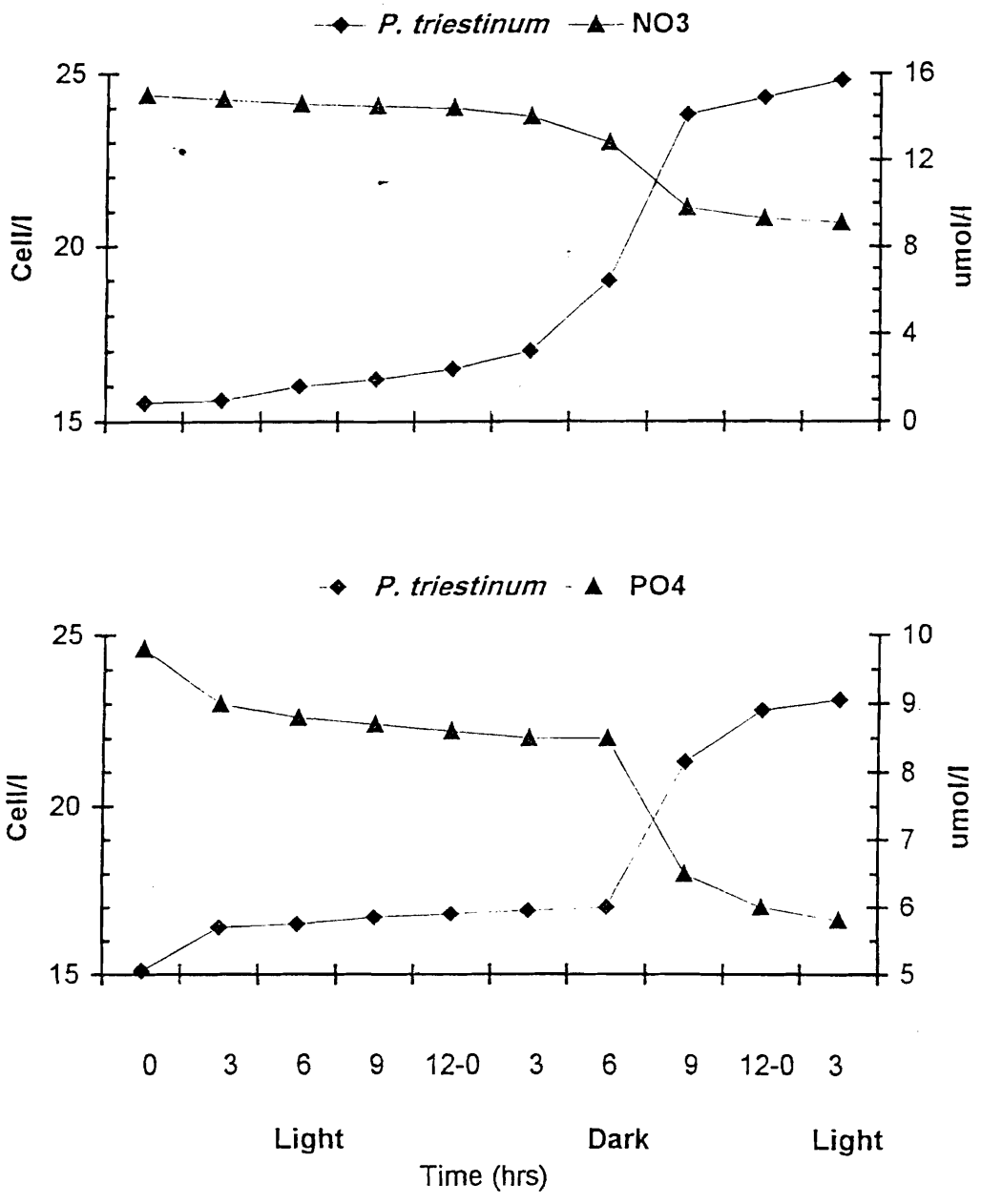

Figure 3. ***Prorocentrum triestinum*** **population and ambient nitrate and phosphate concentrations throughout a 27 hrs L:D cycle.**

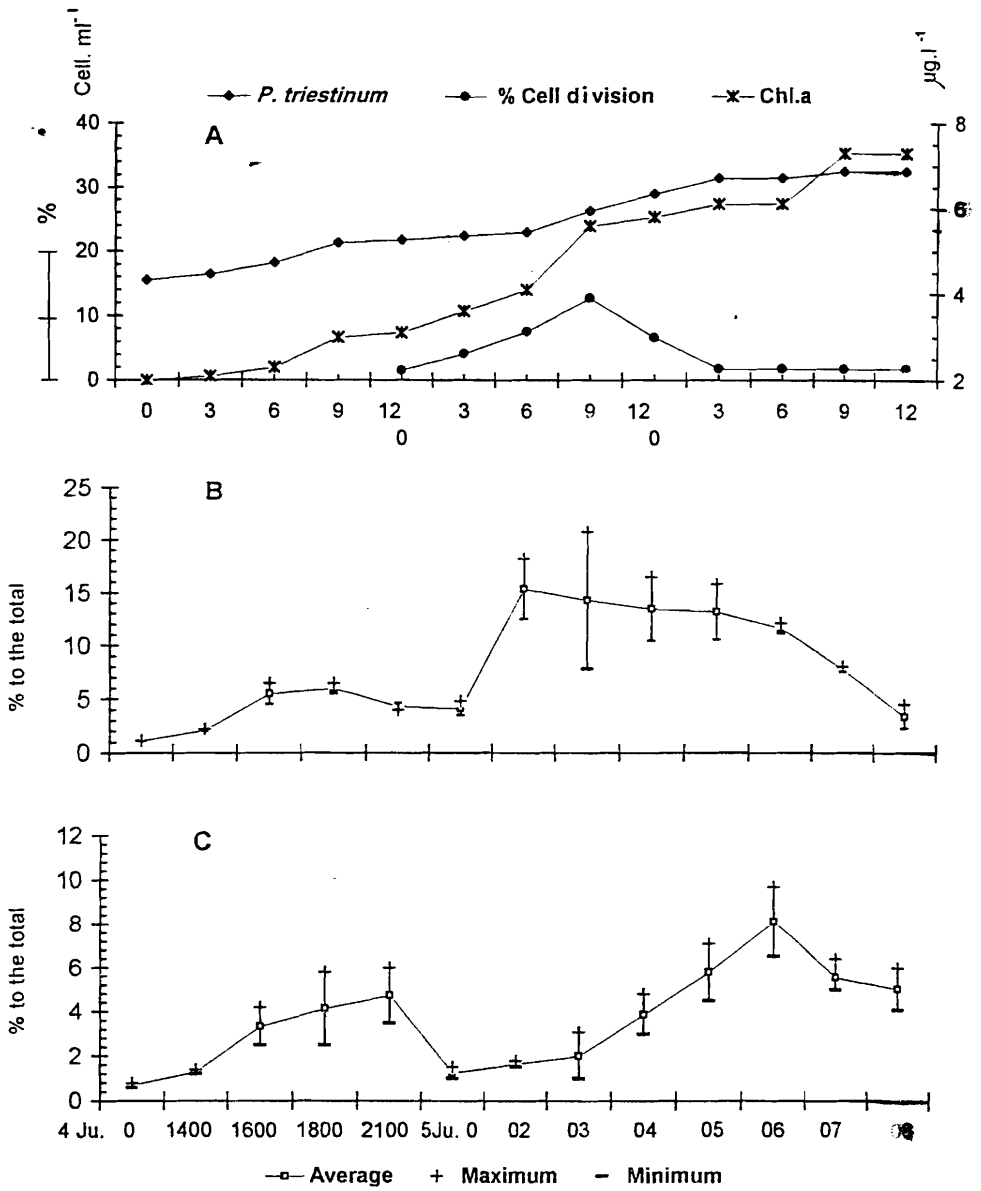

Figure 4. *In vitro* percentage of divided cells during 36 -hrs cycle (A) and *in situ* frequency percentage of cells with 2 nuclei (B), and paired cells (C) of *Prorocentrum triestinum* during 20-hrs cycle.

- despite the broad division time, two distinct periods of noticeable division stages were detected; a minor one in the afternoon, with the descent migration, to be continued after sunset, and the other, the main gate, extended for about 3-4 hrs before sunrise on the following day.

- cells undergoing division in the first period were observed occupying mainly the 2-3 m layer above the pycnocline where the maximum cell concentration existed. It was slightly higher at the 1-2 m layer, within the main gate before dawn, during the ascent movement.

- the maximum fraction of cells with 2 nuclei (15.35%), occurred consistently at 02.00h, while that of recently divided cells (8.1%), at 06.00h.

- although cells in different division stages could be seen most of the time, and it making hard to estimate exactly the time between the formation of cells with 2 nuclei to the formation of paired cells, it seems that there was a lag time of about 3-4 hrs.

- much lower frequency of division was detectable at midday and midnight.

Based on the analyses of the data collected during the diel vertical migration course and the hourly samples, it is concluded that the calculated mean value of $\mu 2$ of ***P. triestinum*** was 0.45 d^{-1}, which corresponds to a D.T of 1.38 days.

DISCUSSION

Similar to previous reports on other ***Prorocentrum*** spp. (Tangen 1980), ***P. triestinum*** blooms are characteristically found in semi-closed areas. This species has ofter. formed blooms in the E. H from late spring to early autumn (Zaghloul & Halim, 1992, Labib 1994 a,b), giving abnormally heavy blooms in April 1993 with a population peak of $71x10^6$ cell. l^{-1} (Labib 1996). The discussion of such blooms has included consideration of development and maintenance in a well-stratified density water column and low surface nutrients. A culture experiment (Labib 1995) stressed the significance of the combination of nitrate and phosphate for its growth rates, rather than their individual addition.

similar to that previously reported for other dinoflagellate species (Eppley *al.*, 1968; Weiller & Karl, 1979; Kamykowski, 1981; Cullen & Horriga 1981).

The present data show no avoidance of ***P. triestinum*** of the surfa maximum irradiance at noon under nitrate and phosphate limitation, contrary ***Gymnodinium splendens*** (Cullen & Horrigan 1981) and ***Gonyaula tamarensis*** (Anderson & Stolzenbach 1985). ***Alexandrium minutum*** a exhibited similar behavior in Alexandria waters, but with plenty of nutrien (labib & Halim, 1995). Light and ambient nutrients strongly control the vert movement patterns, causing ceasing at times (e.g. ***Gonyaulax polyedra***, Epp ***et al.***, 1968; ***Peridinium quinquecorne***, Horstmann 1980; ***Ceratium fur*** Heaney & Eppley 1981).

The swimming speed of ***P. triestinum*** was higher than that of ***P. minimu*** (0.3 mh^{-1} or less, Tyler & Seliger 1981), but lower than *in situ* speeds of ***redfieldi***, 1.2 mh^{-1} and ***P. micans***, 2.2 mh^{-1} (Staker & Bruno 1980). On other hand, Hasle (1954) estimated a velocity speed of 0.44 mh^{-1} for ***P. micar*** from *in situ* results. The rates of vertical movement for ***Heterocapsa triquet*** and ***Gonyaulax tamarensis*** were 0.6-1 mh^{-1} (Anderson & Stolzenbach 19 similar to the range reported of other dinoflagellates (Hand ***et al.***, 1965; Epp ***et al.***, 1968; Heaney & Eppley 1981).

The sampling station, being shallow, we cannot exactly determine

phosphate was fast, following their daily injection from land-based sources. This result also seems of ecological importance since many dinoflagellates cease their vertical movement under steep nitrate reduction as previously mentioned.

The present study shows that the division of ***P. triestinum*** occurred predominantly in the dark, and thus the ascent of cells during the day-light usually results in more rapid increase in cell concentration in the surface layer than could be accounted for the cell division.

The observed dark/light uptake ratio of nitrate and phosphate (42 – 88 % nitrate and 33–100 % phosphate) was compared to that of some other species. It was 41-100 % nitrate and 43-100 % phosphate for ***Heterosigma akashiwo*** and 49-100 % nitrate and 75-100 % phosphate for ***Chattonella antiqua*** (Watanabe ***et al.***, 1983). According to Harrison (1976) the range 50-100 % of the daily requirement of ***Gonyaulax polyedra*** could be obtained from dark uptake and assimilation. In general, nutrient uptake rate in the dark is less than 65 % than the light (Rivkin & Swift 1982).

Phosphate was shown to be taken up faster than nitrate, particularly in dark periods in both *in situ* and *in vitro* observations. There are seemingly contradictory results for some dinoflagellate species (e.g. ***Prorocentrum minimum***, Paasche ***et al.***, 1984). The latter authors emphasized the variability of dinoflagellate nitrogen nutrition and illustrated the difficulty of associating mass occurrence of dinoflagellates in nature with any particular nutritional mode. The important role played by phosphate limitation for the growth of ***P. trietinum*** and others in the neritic waters of Alexandria was discussed by Zaghloul & Halim (1992) and Labib (1996), as well as in culture experiment (Fukazawa ***et al.***, 1980). It has been claimed to be a controlling growth and production factor for phytoplankton in the Mediterranean (Stirn 1988). It is also important to notice, even considering variations between different species of the same genus, that principally phosphate controlled the growth of ***Prorocentrum micans*** in vitro, followed by nitrate (Mingazzini ***et al.***, 1992).

The aspects of reproduction of ***P. triestinum*** facilitate the use of the applied phased cell division method beside the long duration time of cell division. This method used to offset losses it incurs through predation, water exchange and movement of cells out of the sampled column.

Whereas division in marine diatoms occurs at varying species-specific times through the 24-hrs cycle (see Paasche 1968, Smayda 1975), division in photosynthetic marine dinoflagellates, of diverse origin is generally late at night or early morning under laboratory conditions (see Weiler 1978), and field (see Weiler & Chisholm 1976; Chisholm 1981). Yet there appear to be at least some exceptions and this generalization might not hold true.

The present data showed the main division gate to extend for about 3-4 hrs before sunrise and there was a lag time of about 3-4 hrs behind the formation of cells with 2 nuclei to the formation of paired-together cells. Comparing with others, the width of the division gate in genus *Ceratium* ranges from 2-hrs (Doyle & Poore 1974), to about 12-hrs (Heller 1977), but are commonly of the order of 5-7 hrs (Weiler & Eppley 1979).

Although the division peak occurred before dawn (15.35 %, cells with 2 nuclei), cells in different division stages were seen most of the day light and dark times. This is similar to that reported for 6 ***Ornithocercus*** species by Weiler & Eppley (1979), but contrary to other species of restricted division gates; ***Ornithocercus magnificus*** (maximum 8 %), only at 05.00h; ***Peridinium*** spp, 03.00h; and ***Ceratocorys horrida***, at 04.00h (Doyle & Poore 1974), and ***Prorocentrum micans***, 07.00h, as well as ***Ceratium furca***, 04.00-07.00h (Eppley ***et al***. 1984). The maximum population of *Ceratium* cells observed dividing, which reflects the population growth rate, has been found to range from 1 to 40%, but usually between 10-20 % (Elbrächter 1973).

Other different times were reported for ***Pyrocystis noctiluca***, and ***P. fusiformes*** (maximal production during the later part of the night, but the gate appears to span the entire dark interval, Swift & Durbin 1972), and for the fast-growing field population, ***Dinophysis fortii***, which was noted to span the entire day, with the maximum frequency of paired cells occurring soon after sunrise (Weiler & Chisholm 1976). On the other hand, in several dinoflagellate species, the division processes span the dark period; ***Gymnodinium splendens*** (Hasting & Sweeney 1964), ***Scrippsiella trochoidea*** (Nelson & Brand 1979) and ***Amphidinium carteri*** (Chisholm & Brand 1980). ***Prorocentrum micans*** exhibited different division patterns: division at night (Hasle 1954); division begins in the middle of the light period and is completed by the onset of darkness (Hastings & Sweeney 1964); or at 07.00h (Eppley ***et al***., 1984).

phosphate was fast, following their daily injection from land-based sources. This result also seems of ecological importance since many dinoflagellates cease their vertical movement under steep nitrate reduction as previously mentioned.

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The calculated mean growth rate of ***P. triestinum*** and its corresponding mean doubling time was higher than that of ***Hetrocapsa triqueter***, ***Gonyaulax tamarensis*** (Watras ***et al.***, 1982), and ***Ceratium tripos*** (Eppley *et al.*, 1984). However, it is proved that the growth division rate could vary in relation to environmental conditions. There was a 10 fold increase for the latter species at light saturation (Malone 1978). Temperature also affected its growth (maximum 0.3 d^{-1} at 16°C, Eppley ***et al.***, 1984).

Although both cell division and the patterns of the diel vertical migration of ***P. triestinum***, were phased separately and individually, they synchronized well with each other. The high proportion of cells undergoing division at 2-3 m depth above the pycnocline and the maximum cell concentrations during the descent movement, as well as at 1-2 m below the surface during the upward migration may shows depth differentiation of divided cells. This result indicates that dividing cells actively maintained no position, i.e., phased division occurred predominantly in migrating cells. Meanwhile, the maximal fraction of divided cells during ascent movement agrees with the observations of Frempong (1982), that phased cell division of ***Ceratium hirundinella***, occurred among cells not undergoing downward movement during night. Eppley ***et al*** (1968) commented that the ***Cachonina*** cells might undergo division during migration in the morning. Kohata & Watanabe (1986) discussed the relation between cell division cycle of the Rhaphidophcean, ***Heterosigma akashiwo***, which is growing and vertically migrating simultaneously in a tank experiment. They found that non-migrating cells were only old or deactivated.

Knowledge of phytoplankton standing stock is of limited value unless it is coupled with estimate of the rate at which new material is produced. Estimations of primary production are generally restricted to trophic level measurements for the entire phytoplankton community, and yet many ecological questions require information on growth dynamic of the species.

Information on the temporal patterns of division in additional species under various environmental conditions would provide insight into the pressures of different environmental factors governing the time of division. Further experiments using non-photosynthetic, as well as photosynthetic dinoflagellate species should be conducted to determine whether generalities might be made concerning the timing of division in this group.

The interaction between diel vertical migration, nutrition and phased growth of dinoflagellate species may be more complex than thought originally.

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