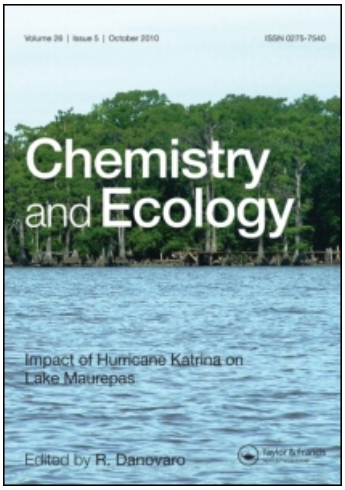

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Population dynamics of the dinoflagellate *Prorocentrum minimum* in Alexandria (Egypt) coastal waters

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RESEARCH ARTICLE

Population dynamics of the dinoflagellate *Prorocentrum minimum* in Alexandria (Egypt) coastal waters

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During an annual cycle, *Prorocentrum minimum* contributed three minor peaks, and two monospecies blooms in the Eastern Harbour of Alexandria (Egypt) following the intensive occurrence of *Skeletonema costatum*. The blooms occurred at 23.5–26.8 °C and salinity 35.5–37.2. The great variability of salinity, nutrient and oxidisable organic matter concentrations limit their contribution as regulating factors. *P. minimum* seems able to utilise organic sources and exhausts ammonia faster than nitrate. The statistical analysis during the bloom periods suggests a link between nutrient and OOM enrichment and the outbreaks of *P. minimum* in the harbour. Generally, the growth rate of *P. minimum* was manipulated by a single addition of different concentrations over several orders of magnitude of molybdenum, zinc, Fe-EDTA and vitamin B_{12}. Fe-EDTA was the most effective element; particularly at 200 μM > Mo (at 1200 nM) >Zn (18 nM) >vitamin B_{12} (0.3–1.8 nM). Generally, the growth was highly stimulated after two days of incubation. The work offers additional information to the growing understanding of the massive occurrence of *P. minimum* in a highly dynamic marine basin.

Keywords: *Prorocentrum minimum*; environmental conditions; trace metals; Fe-EDTA; vitamin B_{12}

1. Introduction

The autotrophic dinoflagellate *Prorocentrum minimum* (Pavillard) Schiller is widely distributed geographically in temperate and subtropical coastal waters, especially in estuarine areas, and it seems to be a cosmopolitan [1], eurythermal and euryhaline species [2]. The species is quite common when growth conditions are suboptimum [3] and able to grow under low light and/or nutrient stress in the field and laboratory [4]. It is known to be a potentially toxic species [5], responsible for the death of fish and shellfish [6], can cause serious impact on aquaculture [7] and to human consumers of mussels, especially during its intensive blooms [8]. Its global spread through ships' ballast water is possible [3]. *P. minimum* has been described as a mixotrophic species; for example, see [1].

Anthropogenic enrichment is considered one of the most pervasive changes altering coastal environments worldwide [9] and the input of macro- and micro-nutrients to the coastal waters stimulates phytoplankton blooms [10,11]. However, there is evidence that trace metals and

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vitamins, particularly vitamin B_{12}, might have been involved in the development of certain harmful species [12,13]. Trace metal concentrations can be an important factor in controlling the location of phytoplankton blooms [14].

Since the 1990s, an intensive concern was paid to investigate ecological factors affecting phytoplankton blooms in Alexandria waters, but mainly in the Eastern Harbour [15–20]. *P. minimum* forms monospecific/multispecific blooms in the harbour between late April and early October. Its bloom (1.8×10^6 cells l^{-1}) at the western vicinity outside the harbour in late spring was linked to drainage waters rich in nutrients [21]. The species is common in Mex Bay, 8 km west to the harbour [22] and in Abou Qir Bay, 20 km east [23].

Relatively little is known about the environmental conditions and preferences leading to the massive occurrence of *P. minimum* in the harbour. The present study during an annual cycle attempts to investigate ambient physical and chemical water characteristics with the occurrence of *P. minimum*, particularly its blooms in October 2004 and June–July 2005. To gain additional insights concerning its nutritional requirements, the effects of molybdenum, zinc, Fe-EDTA and vitamin B_{12} concentrations were tested on laboratory-grown cultures of *P. minimum* isolated from the Eastern Harbour of Alexandria.

2. Material and methods

In order to investigate inherent physical, chemical and biological factors that allow the development, maintenance and dissipation stages of harmful/harmless phytoplankton blooms in the Eastern Harbour of Alexandria (Egypt), a monitoring program based on short-term sampling collection was operated at three fixed stations (3–4 m depth) for 169 consecutive days between 5 July 2004 and 4 October 2005. The harbour, a shallow, semi-enclosed marine basin (area 2.53 km^2, water volume 15.2×10^6 m^3) connects with the adjacent Mediterranean Sea, and acted for a long time as a recipient of large volumes of domestic wastewater from Alexandria city. By 1996–1997, all outfalls of direct discharging into the harbour were closed. However, due to water circulation [24], the harbour receives additional volumes of wastewater, loaded by agricultural, industrial and municipal wastes from Mex Bay; the most highly eutrophic marine basin in Alexandria [22], altering strongly the physical and chemical properties of its water [18].

2.1. In situ *measurements*

The present study was restricted to station II (3 m depth) which showed relatively higher densities of *P. minimum* (Figure 1). Surface and the above bottom water temperature and salinity were measured using thermometers accurate to $\pm 0.1\,^{\circ}C$, and salinity refractometer (S/Mill, after calibration). Dissolved inorganic nutrients (nitrate, nitrite, ammonia, silicate & phosphate) and chlorophyll *a* samples were filtered through Whatman GF/F filters and frozen immediately. Nutrients, chlorophyll *a* [25] and oxidisable organic matter (OOM) [26] were determined. Phytoplankton samples were collected from the surface (approx. 0.5 m depth), fixed by 4% neutralised formaldehyde, and a few drops of Lugol's acid solution, identified and enumerated [27,28] using an inverted microscope.

2.2. In vitro *procedure*

Prorocentrum minimum cells were isolated from the harbour in late April 2004 using a streaking technique [29]. Stock culture was maintained in seawater enriched with f/2-Si medium [30]. The culture was incubated at 20 °C, salinity 30, and light at 300 $\mu mol\, m^{-2}\, s^{-1}$, provided by

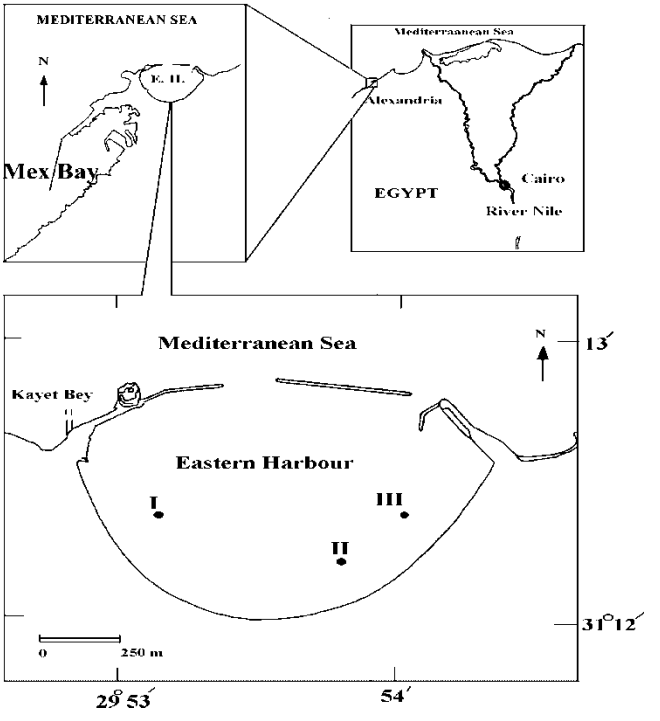

Figure 1. Eastern Harbour of Alexandria and location of the sampling points.

cool-white fluorescent and under 12 h:12 h light/dark cycle. *P. minimum* cells were inoculated (initial density of ca 200–250 cells ml^{-1}) into seawater enriched with f/2-Si (control) with the addition of six different concentrations of molybdenum and zinc (1, 20, 60, 180, 540 & 1200 nM), five concentrations of vitamin B_{12} (0.01, 0.3, 0.9, 1.8 & 3.6 nM), and four levels of Fe-EDTA (10, 50, 200 & 500 μM). All experiments were conducted independently and incubated for 12 days for the control, and about three weeks for other experiments under the same conditions of constant temperature, salinity and light as the stock culture. Duplicate sub-samples were taken for counts and average was used for calculation. The growth rate (μ) was estimated from cell counts at two-day interval in the exponential phase using the formulation of Guillard [31]:

$$\mu = (\ln C_1 - \ln C_0)/(T_1 - T_0),$$

where C_0 and C_1 indicate the cell numbers at time T_0 and T_1, respectively. The doubling time (DT), in days, was calculated using the following equation:

$$\text{D.T.} = \ln 2/\mu.$$

The counting after two days of incubation followed the observation of Wang and Dei [32] that *P. minimum* did not grow appreciably within the first day.

The correlation matrix was applied to determine the relative importance of measured environmental parameters (independent variables), and the corresponding density of *P. minimum* (dependent variable). Simple correlation was calculated between highest growth rate and added element concentration, using replicates.

3. Results

3.1. In situ *temporal variations*

Prorocentrum minimum was a perennial form with distinct temporal variability. The species was scarcely observed during the cold period that extends from mid December 2004 to late March 2005, and it was followed by a noticeable occurrence with the spring warming in April. Beside the monospecies blooms of *P. minimum* in October 2004 and June–July 2005, the species exhibited three minor peaks on 9 and 25 August 2004, and 11 August 2005. The first two peaks (*P. minimum* at 0.88×10^6 and 0.76×10^6 cells l^{-1}, respectively) occurred at 27.2 °C and 28.8 °C, salinity 33 and 36, and relatively low nutrient concentrations (except for nitrate, 9.14 μM and OOM, 10 mg l^{-1}, with the first bloom). These peaks were preceded by a visible phytoplankton bloom mainly of the dinoflagellate, *Gymnodinium catenatum*, and the small centric diatom, *Thalassiosira pseudonana*. The third minor peak of *P. minimum* on 11 August 2005 (0.63×10^6 cells l^{-1}) took place at almost unchangeable temperature and salinity, compared with the previous 2004 data, much higher phosphate (7.6 μM), and reduced nitrate and ammonia (0.6 and 2.93 μM l^{-1}), and it followed extreme high OOM (16.1 mg l^{-1}). The annual cycle showed the density of *P. minimum* to be sharply decreased in September; density never exceeding 0.14×10^6cells l^{-1}.

3.1.1. *Bloom periods*

October 2004. Inherent hydrographic conditions with the population density of *P. minimum* during the bloom period (12–24 October 2004) are given in Table 1 and Figure 2.

The bloom followed the intensive occurrence of the centric diatom, *Skeletonema costatum* (2.1×10^6–11.2×10^6 cells l^{-1}) in late September–early October. Its initiation on 9 October occurred with pulsed nitrate (9.65 μM) and intermediate ammonia (4.83 μM). The nutrient concentrations with the bloom's development exhibited no definite variability trend.

The discoloured water persisted between 18 and 24 October. *P. minimum* culminated a population size at 2.89×10^6 cells l^{-1} on 23 October at 23.5 °C, salinity 35.5, high nitrate (6.03 μM), and ammonia (4.21 μM), and much reduced phosphate (0.62 μM), which increased on the next day to 8.62, 6.11, and 3.6 μM, respectively, accompanying lower salinity at 35. Temperature, salinity, nitrate and ammonia significantly correlated with the bloom population, while, phosphate and OOM showed insignificant correlation (Table 2).

Ammonia seems to be exhausted faster than nitrate. Chlorophyll *a* content with the peak reached 37.5 μg l^{-1}. The bloom dissipated in late October when the nutrient concentrations (2.5 μM

Table 1. Physical and chemical condition with *P. minimum* bloom between 12 and 24 October 2004 (±SD).

Parameter	Mean	Minimum	Maximum
Temperature (°C)	23.9 ± 0.8	23.0	25.5
Salinity	35.7 ± 0.5	35.0	36.2
pH	8.6 ± 0.3	8.2	9.0
PO_4 (μM)	1.4 ± 1.1	3.0	3.6
SiO_4 (μM)	17.2 ± 6.5	8.7	32.2
NO_2 (μM)	0.6 ± 0.2	4.0	0.8
NO_3 (μM)	4.2 ± 2.2	1.9	8.6
NH_4 (μM)	3.9 ± 1.5	2.1	6.5
OOM (mg l^{-1})	3.3 ± 1.6	4.0	6.3
P. minimum (Cells $l^{-1} \times 10^6$)	1.4 ± 1.0	0.18	2.89

Confidence Level (95.0%).

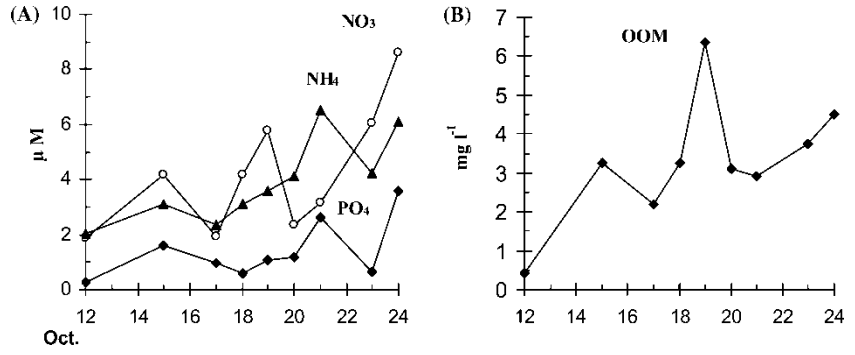

Figure 2. Nutrient (A) and oxidisable organic matter (B) concentration from 12–24 October 2004.

Table 2. Correlation matrix between the density of *P. minimum* and the physical and chemical conditions from 12–24 October 2004.

Variable	Tem.	Sal.	pH	PO_4	SiO_4	NO_2	NO_3	NH_4	OOM	*P. minimum*
Temperature	1									
Salinity	0.23	1.00								
pH	−0.74	−0.60	1.00							
PO_4	−0.24	−0.78	0.52	1.00						
SiO_4	0.04	−0.70	0.20	0.44	1.00					
NO_2	−0.53	−0.21	0.53	0.34	−0.38	1.00				
NO_3	−0.58	−0.40	0.67	0.54	−0.29	0.92	1.00			
NH_4	−0.49	−0.92	0.70	0.84	0.63	0.32	0.53	1.00		
DOM	−0.82	−0.09	0.46	0.33	−0.31	0.66	0.72	0.40	1.00	
P. minimum	−0.67	−0.70	0.87	0.35	0.29	0.44	0.59	0.71	0.38	1.00

phosphate, 6.3 µM nitrate, and 4.2 µM ammonia) were still able to push its development further. Accompanied physical condition indicated a severe drop in surface temperature by three degrees (20 °C), salinity increasing (38.9) and break-down of thermo-haline stratification of the water column.

Among the phytoplankton species within the bloom period, the dinoflagellate *Prorocentrum triestinum* achieved 0.63×10^6 cells l^{-1} on 19 October. Flourishment of the small diatom *Thalassiosira pseudonana*, and microflagellate species (2.06×10^6 and 2.57×10^6 cells l^{-1}, respectively) immediately followed the bloom dissipation.

June 2005. Inherent hydrographic conditions and the population density of *P. minimum* during the bloom period (5 June–6 July 2005) are given in Table 3 and Figure 3 (A & B).

The bloom was visible (yellow-brown) for 22 days between 7 and 28 June, and associated with limited fish mortality during its last 10 days. Again, *S. costatum* at 4×10^6–13.4×10^6 cells l^{-1} dominated the community prior to the bloom between 7 and 30 May. The bloom dynamics showed relatively high density of *P. minimum* during the last week of May (0.12×10^6–0.25×10^6 cells l^{-1}), which progressed successfully, and achieved three major peaks on 11, 19 and 26 June (3.1×10^6–4.1×10^6 cells l^{-1}), raising chlorophyll *a* to its maximum of 41.2 µg l^{-1} on the last day. The bloom maintained a temperature of 23.8–26.8 °C, salinity of 35.5–37.6, low nutrient concentrations; 0.41–1.56 µM nitrate, 0.82–3.7 µM ammonia, 0.31–2.6 µM phosphate, and extreme high OOM (5.6–15.5 mg l^{-1}). The matrix correlation stresses the contribution of the last two parameters, and the relative importance of ammonia (Table 4). The community changed after the bloom dissipation as *Skeletonema costatum* overwhelmingly dominated until 11 July (5.25×10^6–9.11×10^6 cells l^{-1}).

Table 3. Physical and chemical conditions during the *P. minimum* bloom between 5 June and 6 July 2005 (±SD).

Parameter	Mean	Minimum	Maximum
Temperature (°C)	25.0 ± 1.14	23.8	26.8
Salinity	36.8 ± 0.47	36.2	37.8
pH	8.65 ± 0.22	8.4	9.0
PO_4 (μM)	0.74 ± 0.75	0.31	2.67
SiO_4 (μM)	2.52 ± 1.37	0.57	4.92
NO_2 (μM)	0.21 ± 0.12	0.12	0.51
NO_3 (μM)	0.96 ± 0.4	0.41	1.56
NH_4 (μM)	1.77 ± 0.96	0.62	3.71
OOM (mg l^{-1})	11.0 ± 3.55	5.6	15.5
P. minimum (Cells $l^{-1} \times 10^6$)	1.76 ± 1.28	0.25	4.08

Note: Confidence level (95.0%).

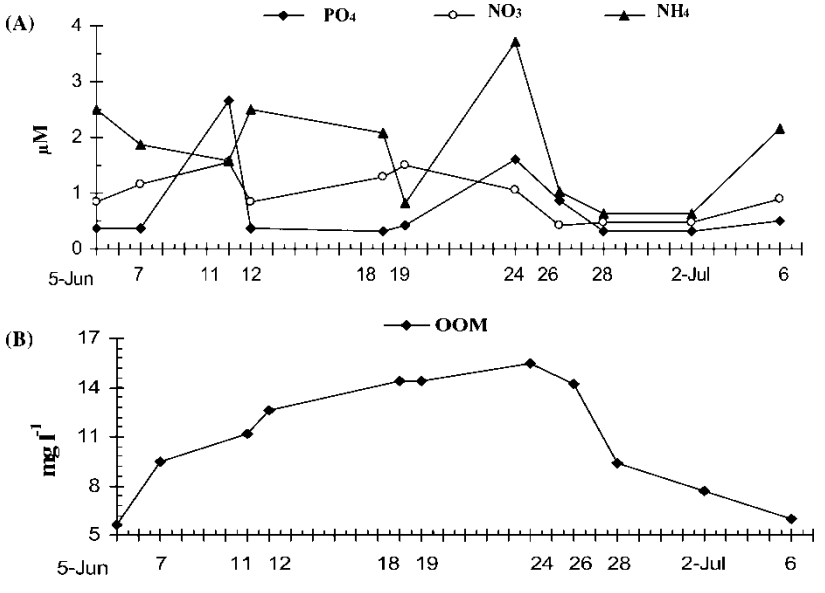

Figure 3. Nutrient (A) and oxidisable organic matter (B) concentration from 5 June–6 July 2005.

Table 4. Correlation matrix between the density of *P. minimum* and the physical and chemical conditions from 5 June to 6 July 2005.

Variable	Tem.	Sal.	pH	PO_4	SiO_4	NO_2	NO_3	NH_4	OOM	*P. minimum*
Temperature	1									
Salinity	0.8	1								
pH	−0.33	−0.27	1							
PO_4	−0.3	0.01	0.26	1						
SiO_4	−0.57	−0.18	0.27	0.65	1					
NO_2	0.19	0.03	−0.27	0.2	0.17	1				
NO_3	−0.62	−0.55	0.19	0.42	0.41	0.04	1			
NH_4	−0.41	−0.52	−0.13	0.23	0.37	0.75	0.27	1		
DOM	−0.41	−0.28	0.64	0.28	0.68	0.02	0.32	0.14	1	
P. minimum	-0.29	−0.01	0.89	0.45	0.42	−0.36	0.17	−0.3	0.66	1

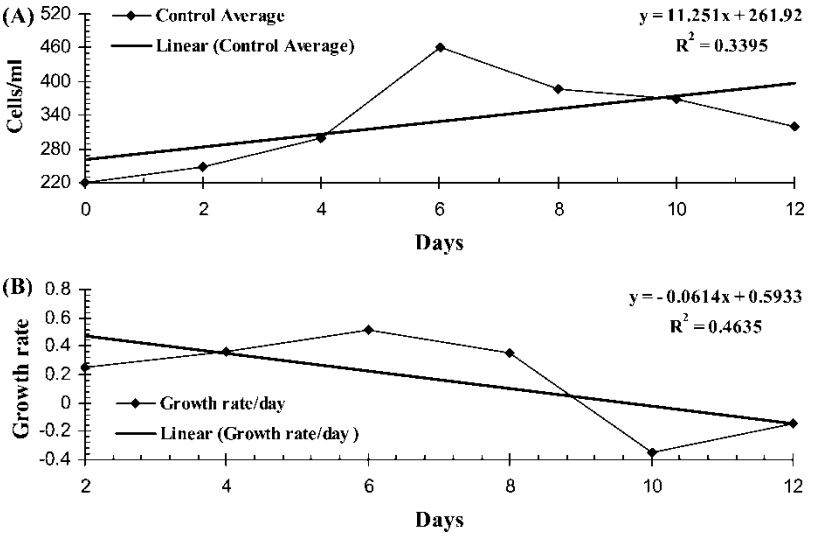

Figure 4. Population growth of *P. minimum* over a 12-day period (Control experiment), (A) Density (Cells l^{-1}) and (B) Growth rate (μ d^{-1}).

3.2. In vitro *measurements*

Population growth of the 'initiation culture' of *P. minimum* inoculating into f/2-Si medium over a 12-day experimental period is shown in Figure 4.

Starting from a cell density of 220×10^3 cells ml^{-1}, the initiation culture grew slowly after the first day and developed considerably after four days. Cell number reached its maximum on the 6th day and then started to drop. The lag phase probably lasted only for one day (the 1st day), and an exponential growth phase started on the second day (highest DT 2.77 d^{-1}). The highest growth rate (μ), i.e. 0.52 d^{-1} was calculated on the 6th day (shortest doubling time, i.e. 1.33 d^{-1}). The maximum carrying capacity was approximately 461.1 cells ml^{-1}. The effect of molybdenum, zinc, Fe-EDTA and vitamin B_{12} additions on cell density of *P. minimum* are shown in Figure 5.

3.2.1. *Molybdenum (Mo)*

A wide range of molybdenum concentrations affected the growth of *P. minimum*, and the range between 60–180 nM contributed significantly the increasing population of *P. minimum*. The highest growth rate (μ) was always observed after two days of incubation (0.69–0.94 d^{-1}, Table 5), except at 20 nM concentration, which was delayed to day six (μ 0.74 d^{-1}). It showed positive significant correlation with increasing Mo concentrations ($r = 0.91$, at 0.05 level, $n = 12$), reaching the highest at 1200 nM (0.94 d^{-1} & DT, 0.74 d^{-1}). The maximum cell densities were found on day 14 at 180 nM (12.3×10^3 cells ml^{-1}), and day 12 at 60 nM (11.3×10^3 cells ml^{-1}).

3.2.2. *Zinc (Zn)*

The highest (μ) values of *P. minimum* (0.72–0.9 d^{-1}) were found after two days, and it was delayed to day 4 at 540 nM ($\mu = 0.84$ d^{-1}, Table 5). It showed a tendency to increase by increasing Zn concentration until 180 nM, to reach 0.9 d^{-1} (DT, 0.77 d^{-1}), and decreased slightly to 0.84 and 0.75 d^{-1} at 540 and 1200 nM, respectively. The variations in cell numbers showed: the highest densities between days 10 and 14; equal numbers at the concentrations 1 and 60 nM ($1.02 \times$

Table 5. Tested trace metal and B_{12} concentrations and their maximum growth rate (μ d^{-1}).

Mo (nM)							Zn (nM)					
Conc.	1	20	60	180	540	1200	1	20	60	180	540	1200
Day	2	6	2&4	2&4	2	2	2	2	2	2	4	2
μ d^{-1}	0.69	0.74	0.80	0.80	0.85	0.94	069	0.72	0.85	0.90	0.84	0.75

Fe-EDTA (μM)					B_{12} (nM)				
Conc.	10	50	200	500	0.01	0.3	0.9	1.8	3.6
Day	4	6	2	8	2	2	2	2	2
μ d^{-1}	0.60	0.67	1.5	0.76	0.35	0.8	0.85	0.85	0.59

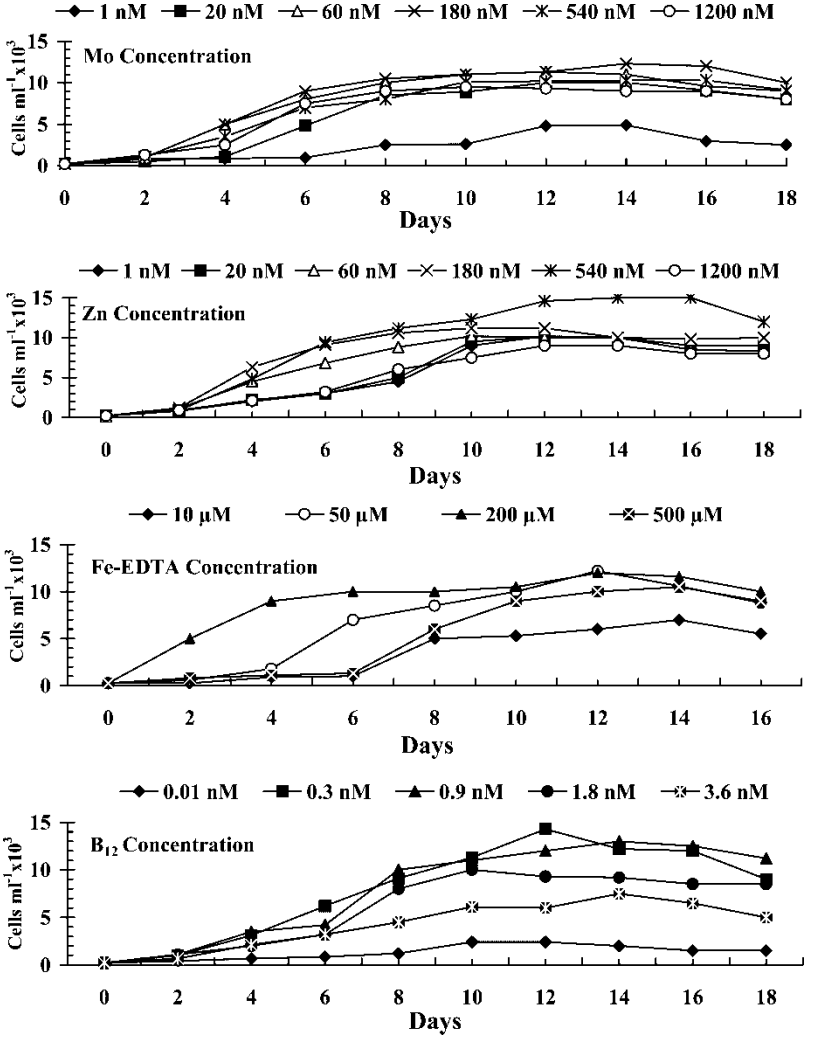

Figure 5. Effect of molybdenum, zinc, Fe-EDTA and vitamin B_{12} additions on cell density of *P. minimum*.

10^3 cells ml^{-1}); the maximum at 540 nM (1.5×10^3 cells ml^{-1}); and the lowest at 1200 nM (0.9×10^3 cells ml^{-1}).

3.2.3. *Fe-EDTA*

The maximum growth rate (1.5 d^{-1}, DT 0.46 d^{-1}) was calculated after two days at 200 μM (Table 5), while the highest μ values (0.60–0.76 d^{-1}) for other tested concentrations were seen between days 4 and 8. Insignificant correlation ($r = 0.05$, $n = 8$) was found between the highest μ values and the increasing Fe-EDTA levels. *P. minimum* reached its maximum density on the day 12 at 50 and 200 μM (ca 1.2×10^3 cells ml^{-1}).

3.2.4. *Vitamin B_{12}*

The highest growth rate was calculated for all concentrations after two days of incubation. Almost equal (μ) values (around 0.8 d^{-1}, DT, 0.81 d^{-1}) were found at 0.3–1.8 nM (Table 5). The extreme low and high concentrations (0.01 and 3.6 nM) resulted in lower growth rate (0.35 and 0.59 d^{-1}), and cell density (2.4×10^3 and 7.5×10^3 cells l^{-1}). The highest cell densities of *P. minimum* were counted between days 10–14, and the maximum at 0.3 nM (14.3×10^3 cells ml^{-1}).

4. Discussion

The present field data during an annual cycle showed the occurrence of *P. minimum* over a wide range of temperature and salinity; scarcely found at 12.4–15.5 °C, salinity >39, minor peaks at 27.2–28.5 °C, salinity 33–36, and blooms at 23–26.8 °C, and salinity 35–37.8, therefore, it is considered eurythermal and euryhaline species in accordance with Grzebyk et al. [3]. Water temperature during the year cycle seems to have an insignificant contribution on *P. minimum* variability ($r = 0.11$, $n = 169$, $p < 0.05$). However, an inverse significant correlation did exist throughout its bloom in October 2004. The remarkable salinity variations were attributed to the arrival of discharged water with water exchange, which limited its effect on the annual temporal variability of *P. minimum* ($r = -0.16$, $n = 169$, insignificant at $p < 0.05$). However, the major peaks of *P*. minimum usually accompanied relatively reduced salinity, which seems to have significantly affected its bloom dynamic in 2004. The species, as a numerically important contributor to the phytoplankton community in the harbour's previously formed blooms at 28.5–30 °C, and salinity 30–37.5 [15,18].

The blooms occurred with differences in temperature and salinity between surface and over bottom layers of 1–2.2 °C and 1.5–3, and despite the shallowness of the sampling station, the establishment of the thermohaline stratification of the water column might help to trigger development and maintenance of the blooms, and its breakdown might represent an ecological reason for the bloom dissipation (see, for example, [33]). The bloom stages and the newly additional supplies with the water exchange that were detected by sudden decreases in salinity governed the availability and variability of nutrient concentrations. The large spectra of the nutrients variability limit its contribution on the temporal variability of *P. minimum* for the whole year. However, nutrients affected significantly the bloom dynamic in 2004 and 2005. No definite trends were detected during the bloom periods, thus, the bloom in 2004 triggered under high nitrate, low phosphate and intermediate ammonia, and its peaks occurred with pulsed nitrate and ammonia, while the condition in 2005 was completely different; low levels of nutrients and extreme high OOM content; and fast exhaustion of ammonia than nitrate. Among reasons for the bloom collapse

was the breakdown of thermo-haline stratification of the water column in October 2004, and the severe reduction in nutrient concentrations in early July 2005.

The response of *P. minimum* to changing environmental conditions might explain its recurrent blooms in the harbour. The low nutrient concentrations may enhance the chance of *P. minimum* to form its bloom in June–July 2005, and gives it an advantage for competition with other species [34]. The preference for ammonia by *P. minimum* was reported [35], which could share, in part, the limited fish mortality event, since harmful algal populations that utilise ammonia could be more toxic than others grown on nitrate or urea [36]. The importance of phosphate in regulating the present abundance of *P. minimum* agrees with observations of other red tide bloom species in the harbour [20,37]. The increased concentrations of OOM relative to inorganic nitrogen and phosphorous and their great variability might face the ability of *P. minimum* to utilise both sources (see, for example [35,38,39]). According to Glibert et al. [40], inorganic nutrient concentrations have often been insufficient to maintain the high biomass of *P. minimum*. The statistical analysis during the bloom periods suggests a link between enrichment of nutrient and OOM concentrations (eutrophication), and the outbreaks of *P. minimum* in the harbour. The stimulation of a *P. minimum* bloom by addition of dissolved organic nitrogen (DON) has been shown previously [40,41]. The *Skeletonema costatum* blooms just prior to *P. minimum* massive outbreaks seem to be an important reason for the extreme high OOM (diatom decomposition). In culture experiments, the luxuriant growth of co-occurring *S. costatum* enhances the growth of *P. minimum*; *S. costatum* is probably able to produce some stimulants [42]. Such species interaction was previously reported for *Prorocentrum triestinum* bloom in the harbour [16] and for other red tide species elsewhere (see, for example, [43]).

The *in vitro* experiments showed different effects of molybdenum, zinc, Fe-EDTA and vitamin B_{12} concentrations on the growth of *P. minimum*. However, its growth rate was manipulated by a single addition of different concentrations over several orders of magnitude. Comparing with the maximum growth rate of the control experiment, the luxuriant growth of *P. minimum* was observed with the addition of Fe-EDTA (2.88-folder greater) > Mo (1.81) > Zn (1.61–1.73) > vitamin B_{12} (1.54). A parallel relationship existed between increasing (μ) values and Mo concentrations and the maximum μ calculated with the addition of 1200 nM. According to Fuse [44], the addition of $0 \sim 10^{-3}$ M Mo neither promoted nor inhibited the growth of five red tide species. Zinc had a broad concentration range affecting the growth, particularly between 60 and 540 nM. The maximum growth rate was observed at 180 nM ($0.9\,d^{-1}$), and lower or higher concentration led to reduced (μ) values. Studies on the growth and physiological response of *P. minimum* to zinc levels [45] showed its specific growth rate to be limited by low Zn ($1.4\,p\,mol\,l^{-1}$) and it was concluded that zinc concentration might be one of the key factors controlling red tide blooms in eutrophic environments. Zinc levels (dissolved phase) in the harbour during 1998 and 1999 fluctuated seasonally between 1.5 and 19.31 $\mu g\,l^{-1}$ [46].

The maximum growth rate of *P. minimum* ($1.5\,d^{-1}$) found with 200 μM Fe-EDTA confirms the important role of iron as a nutritional factor regulating the population dynamics of red tide dinoflagellate species (see, for example, [47,48]). The growth rate of *P. minimum* reached values around $0.8\,d^{-1}$ at a wide range of vitamin B_{12} concentrations (0.3–1.8 nM). About 80–90% of the Dinophyta require B_{12} for growth alone or in combination with either thiamin and/or biotin [49]. Experimentally, vitamin B_{12} is an essential element for the growth of *Prorocentrum triestinum* isolated from the harbour [37], *Prorocentrum micans* [50], and *Chattonella antiqua* and *Heterosigma carterae* [12].

The present work offers additional information to the growing understanding of the massive occurrence of *P. minimum* in a highly dynamic eutrophic marine basin. However, further laboratory studies on combination of trace metals, chelators and vitamins under different temperature, salinity, light and nutrient regimes would help to give a better insight into understanding the bloom dynamics of *P. minimum* in Alexandria waters.

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