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REVIEW

A review on the Bloom Dynamics of a Harmful Dinoflagellate *Prorocentrum minimum* in the Golden Horn Estuary

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Abstract

The potentially harmful and bloom-forming dinoflagellate *Prorocentrum minimum* (Pavillard, 1916) Schiller, 1933 was followed monthly during four years in the Golden Horn Estuary (GHE). Simultaneously measured some environmental variables including salinity, temperature, dissolved oxygen, inorganic nutrient and chlorophyll *a* concentrations were evaluated together with *P. minimum* data. The density of this species increased mostly in late spring and summer during three years and caused two significant blooms in July 2000 and July 2001. Environmental conditions which triggered these bloom events were considered during whole study period. Blooms have mostly occurred in middle and upper estuary where water movements are insufficient (salinity 15.2-18.5 psu, temperature 19.0-24.2°C). The cell density of *P. minimum* reached to 5.5×10^6 cells L^{-1} at the early stage of the first bloom, and after two weeks, it reached to highest level of 70.0×10^6 cells L^{-1}. Cell density was calculated as 36.0×10^6 cells L^{-1} at the second bloom in July 2001. Dissolved oxygen value reached to super saturation (29.0 mg L^{-1}) and chlorophyll *a* concentration was measured as 200 µg L^{-1} in July 2000. Inorganic nutrient concentrations were lower than before bloom periods. The results appeared that *P. minimum* could well develop at the high temperature and moderate salinity under the calm weather in nutrient-rich estuarine environment.

Keywords: Phytoplankton, *Prorocentrum minimum*, harmful algae, eutrophication, Golden Horn.

Haliç'te Dinoflagellatelardan Prorocentrum Minimum Zararlısının İlk Evrelerinin Dinamikleri Hakkında Bir Çalışma

Özet

Haliç'te potansiyel zararlı ve aşırı üreyen dinoflagellat *Prorocentrum minimum* (Pavillard, 1916) Schiller 1933 dağılımı dört yıl boyunca aylık olarak izlendi. *Prorocentrum minimum* verisi tuzluluk, sıcaklık, çözünmüş oksijen, inorganik besin elementleri ve klorofil *a* gibi eş zamanlı olarak ölçülen bazı çevresel değişkenlerle birlikte değerlendirildi. Bu türün bolluğu peş peşe üç yıl boyunca özellikle yaz ortası ve bahar sonunda artış gösterdi ve Temmuz 2000 ile Temmuz 2001'de iki önemli aşırı üreme olayına neden oldu. Tüm çalışma periyodu boyunca bu aşırı üreme olaylarını tetikleyen çevresel koşullar dikkate alındı. Aşırı üreme olayları genellikle su hareketlerinin yetersiz olduğu orta ve yukarı Haliç'te meydana geldi (15.2-18.5 psu, 19.2-24.2 °C). *P. minimum* yoğunluğu birinci aşırı üreme olayının başlarında 5.5×10^6 hücre L^{-1}, iki hafta sonra ise en yüksek seviyesi olan 70×10^6 cells L^{-1}'ye ulaştı. Temmuz 2001'deki ikinci aşırı üremede ise hücre yoğunluğu 36×10^6 hücre L^{-1} olarak hesaplandı. Temmuz 2000'de çözünmüş oksijen aşırı doygunluk seviyesine (29 mg L^{-1}) ulaştı, klorofil *a* konsantrasyonu da 200 µg L^{-1} olarak ölçüldü. İnorganik besin elementleri konsantrasyonu aşırı üreme olayları öncesine göre daha düşüktü. Sonuçlar *P. minimum* türünün sakin hava koşulları altında besin elementlerince zengin haliçlerde, yüksek sıcaklık ve düşük tuzlulukta iyi gelişebildiğini ortaya koymaktadır.

Keywords: Fitoplankton, *P. minimum*, zararlı algler, ötrofikasyon, Haliç.

Introduction

Prorocentrum minimum (Pavillard, 1916) Schiller, 1933 is a common, neritic, bloom-forming dinoflagellate with a wide distribution in coastal and estuarine environments. Its ecology, bloom dynamics and impacts its blooms on ecosystem effects have been well investigated by several researchers in coastal and estuarine environments (Heil *et al.*, 2005; Hajdu *et al.*, 2005; Pertola *et al.*, 2005; Tango et al. 2005; Sierra-Beltrán *et al.*, 2005). Wikfors and Smolowitz (1993) suggested that high density of *P. minimum* in natural blooms could have detrimental effects on the filtering and feeding process of oyster population. According to many researchers, *P. minimum* has been considered potentially toxic to

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humans via ingestion of toxic shellfish (Tangen, 1983; Taylor *et al*., 2003; Heil *et al*., 2005) and harmful impacts in the ecosystem including hypoxic/anoxic events and fish kills (Tango *et al*., 2005).

P. minimum is small (approximately 20 µm in length and slightly smaller in width), variably heart shaped, triangular or oval, flattened in side view, with a concavity at its wider end (Faust, 1974; Pertola *et al*., 2003). Because the cell shape of this species varies considerably (Hajdu *et al*., 2000), it needs to further taxonomic studies (Faust *et al*., 1999). In a recent review of dinoflagellate taxonomy, *P. minimum* is considered synonymous with *Prorocentrum cordatum* (Ostenfeld, 1901) Dodge, 1975 (Taylor *et al*., 2003). *P. minimum* has been described as a eurythermal and euryhaline species and can bloom under a wide range of environmental conditions (Tango *et al*., 2005) and develops at the near coasts, often in nutrient-rich bays, fjords or river estuaries (Hajdu *et al*., 2005). Grzebyk and Berland (1996) suggested that this species have optimal growth between 18.0 and 26.5°C. *P. minimum* seems to be a good competitor (Pertola *et al*., 2005) and it is capable of both autotrophic and mixotrophic nutrition and this feeding mechanism can contribute to dominate and form blooms under nutrient-limiting conditions (Heil *et al*., 2005).

The Golden Horn Estuary (GHE) is located at northeast of the Sea of Marmara and southwest of the Strait of Istanbul. Golden Horn, extending in northwest-southwest direction, is a 7.5 km length and max. 0.7 km width estuary and covers an area of about 2.6 km^2. The maximum depth of the GHE is around 40.0 m at the lower parts and it rapidly decreases to 14.0 m in the middle and upper part is < 5.0 m (Figure 1). Alibey and Kağıthane streams were the main sources of freshwater input, while the amount of freshwater influx from these streams decreased considerably by the end of 1990s, following the construction of a series of dams. At present, rainfall and coastal inputs are the main sources of freshwater input in the GHE (Sur *et al*., 2002). The GHE is characterized by a two-layered structure similar to the neighboring Strait of Istanbul, whose upper layer has salinity of ~20.0 psu and lower layer ~38.0 psu. The surface layer also contains very high amounts of suspended particulate matter (SPM), carried by local discharges, streams and precipitation (Özsoy *et al*., 1988).

As a result of unplanned urbanization and heavy industrialization, the Golden Horn Estuary (GHE) has been polluted since 1950s and became the most important environmental problem in the region of Istanbul. Domestic and industrial wastewaters gradually damaged the estuarine ecosystem. By early 1990's, the estuarine life was limited to lower and middle part of estuary, and also upper part was almost completely lifeless due to anoxic conditions and heavy sedimentation. The pollution also increased by bridges operating on buoys that limited upper layer circulation greatly. In 1997, the Golden Horn Rehabilitation Project was initiated. The surface discharges were gradually taken under control, connected to collector systems and discharged into the lower layer of the Strait of Istanbul. Also, 4.25×10^6 m^3 anoxic sediment was removed from upper estuary and at least 5 m depth was gained. As a result the highly polluted GHE ecosystem shifted from anoxic to eutrophic conditions.

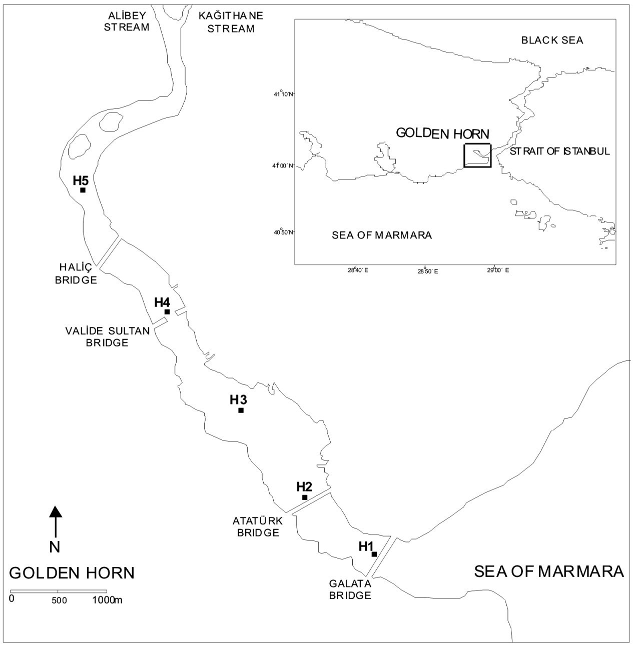

Figure 1. Study area and sampling stations

Chlorophyll *a* and inorganic nutrient concentrations display a significant difference in the lower and upper estuary and generally they increase remarkably from lower to upper parts of estuary. The shift from the highly polluted system to eutrophication resulted in a considerable increase in phytoplankton abundance. Alterations in the physico-chemical structure of GHE following the rehabilitiation studies were revealed in successive phytoplankton blooms (Taş *et al.*, 2009).

The present study aimed to determine the dynamics of the successive blooms of harmful dinoflagellate *P. minimum* with concurrent environmental variables and to discuss the results.

Materials and Methods

Samplings were performed monthly intervals at 5 stations for four years (from January 1999 to December 2002), as representatives of lower (H1), middle (H3) and upper parts (H5) of the estuary (Figure 1). Lower estuary (LE) has a depth of 40 m and is heavily influenced by the dynamic physico-chemical properties of the Strait of Istanbul. Middle estuary (ME) is maximum 14 m depth, where a bridge operating on buoys (Ataturk Bridge) also limits upper layer circulation between lower and middle estuaries. Upper estuary (UE) has a maximum 5 m depth and there is much weaker water circulation than other parts of estuary. As the physical descriptors, salinity and temperature values were recorded by a SBE-Sea Logger 25 CTD system and light transparency was measured by a Secchi disc.

Seawater samples were monthly collected using 5 L Niskin bottles from February 1999 to January 2003. NO_3+NO_2-N were detected by cadmium reduction method on a Skalar autoanalyser (APHA, 1999) and PO_4-P was detected spectrophotometrically and chlorophyll *a* analyses were carried out by acetone extraction method (Parsons *et al.*, 1984). Dissolved oxygen (DO) (Winkler Method) was measured following APHA (1999). The saturated DO (SDO) values were determined as the solubility of oxygen in sea water as a function of concurrently measured values of temperature and salinity. Percent saturation (% sat) was then calculated according to the difference between DO and SDO values.

For phytoplankton analysis were taken water samples of 1 L capacity and were immediately fixed buffered formaldehyde solution in the proportion 1:100. In the laboratory, samples were allowed to settle for a week. Then the water at the upper part is removed by siphoning and the remaining part is concentrated to 100 ml and stored in dark colored glass bottles (Sukhanova, 1978; Throndsen, 1978). The phytoplankton cells were counted with a Sedgewick-Rafter Counting Cell under a light microscope. For species identification were used the following references: Delgado and Fortuna (1991), Dodge (1985), Hallegraeff (2002), Moestrup *et al.* (2004), Hasle *et al.* (1997).

The relationship among abundance of *P. minimum* and environmental variables were analyzed by Spearman rank correlation, following transformation to natural logarithms.

Results

Physical Variables

The GHE have two bridges (Atatürk and Valide Sultan) operating on buoys which limit upper layer circulation between upper and lower part of estuary (Figure 1.). The lower estuary showed the characteristics of the neighboring Strait of Istanbul. The seasonality is clear for temperature; while salinity displayed annual and spatial variations (Figure 2). Temperature ranged from 5.6 ºC (January 2002) to 27.4 ºC (August 1999). Generally, the upper estuary had higher temperature than the lower and middle estuary. Surface salinity ranged from 1.4 to 20.7 psu in the upper estuary and from 9.9 to 20.9 psu in the lower estuary. In contrast to temperature, salinity values were always lower in the upper estuary. The annual average salinity values at H5 increased from 12.0 to 17.0 psu, following June 2000.

No significant difference was found at the salinity and temperature values in middle and upper estuary at the bloom periods. It was observed that the salinity values decreased from lower part towards upper part, while the temperature values increased (Table 1). At the beginning of the first bloom period (July 11, 2000) surface water salinity ranged from 15.2 to 18.3 psu and temperature were measured between 20.4°C and 23.4°C throughout the estuary. Two weeks later (July 26, 2000) when the cell density reached to maximum, salinities ranged from 17.5 to 18.5 psu and temperatures ranged from 19.2 to 21.8°C. In this period, the highest density of *P. minimum* occurred in conditions of 17.5 psu and 21.6°C in the middle estuary. At the second bloom period (July 10, 2001), surface water salinity ranged from 18.3 to 18.7 psu and temperature was measured between 21.7°C and 22.9°C. In this period, the highest density of *P. minimum* occurred at salinity of 18.3 psu and temperature of 22.9°C in the upper estuary. Occurrences of *P. minimum* were bounded within a salinity-temperature range of 15.2-18.7 psu and 19.2–24.1°C throughout the estuary. According to Spearman's rank correlation the abundance of *P. minimum* was a weak positively correlated with water temperature (rho= 0.35, $P<0.001$) and salinity (rho= 0.28, $P<0.005$).

Annual average Secchi disc values increased from 1.81 to 5.65 m in the lower and from 0.36 to 1.21 m in the upper estuary in four years. In general, the Secchi disc values have been gradually decreased from lower to upper part of estuary due to the

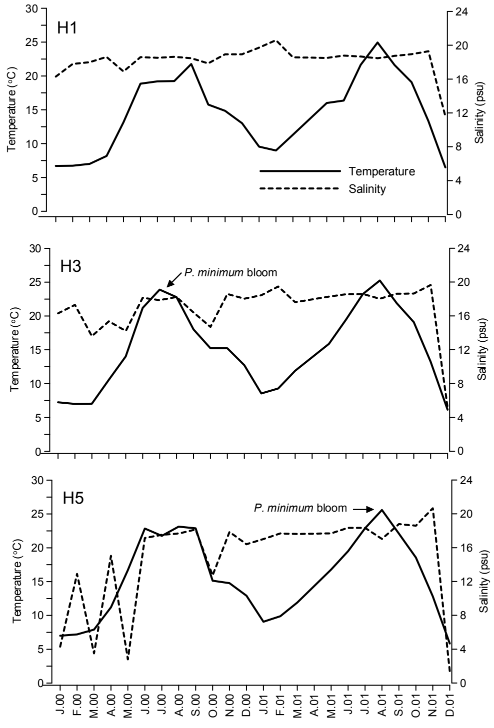

Figure 2. Monthly variations of surface temperature and salinity in the GHE.

Table 1. Mean and standard deviation values of environmental variables measured during the bloom events

Parameters	Lower part (H1)	Middle part (H3)	Upper part (H5)
Salinity (psu)	18.5±0.19	17.28±1.29	17.03±1.61
Temperature (°C)	20.4±1.23	23.15±1.33	22.6±0.77
Secchi depth (m)	4.73±2.42	2.00±2.18	0.67±0.35
NO_3 + NO_2 (μM)	1.03±0.47	0.71±0.52	1.01±0.80
PO_4 (μM)	0.27±0.27	0.71±0.61	3.58±0.92
DO (mg L^{-1})	8.84±1.33	19.6±9.4	14.6±8.74
Chl-a (μg L^{-1})	7.50±3.36	93.6±98.6	170±118

increased suspended particulate matter coming by streams. Also, mean Secchi disc values seem to decrease rapidly in mid and upper sections because of the increased cell density through the blooms (Table 1).

Inorganic Nutrients, DO and Chlorophyll *a*

Inorganic nutrients were important indicators to understand the magnitude of change in the GHE after rehabilitation. The significant difference between PO_4-P concentrations in upper and lower estuary indicates discharges to the upper estuary. NO_3+NO_2-N concentrations showed monthly fluctuations and increases in NO_3+NO_2-N values were highly associated with precipitation. In December 2001, concentrations reached 176.5 μM at H5 after the heavy rainfall and the highest NO_3+NO_2-N value was detected as 328.8 μM in May 2000 (Figure 3). In the bloom periods, NO_3+NO_2-N and PO_4-P concentrations were measured very low. During the bloom periods NO_3+NO_2-N and PO_4-P concentrations varied from 0.09 to 2.22 μM, from 0.10 to 4.14 μM, respectively (Figure 3). Nutrient concentrations are generally higher in upper estuary than other sections due to high loading to upper estuary by streams and terrestrial inputs. Average values of NO_3+NO_2-N and PO_4-P at the bloom periods were given in Table 1. NO_3+NO_2-N concentrations were measured as 0.17 μM while PO_4-P value 1.38 μM in middle part at the first bloom period (Figure 3). In general, inorganic nutrient concentrations were negatively correlated with the abundance of *P. minimum* (NO_3+NO_2-N, rho = -0.22, $P<0.005$; PO_4-P, rho = -0.32, $P<0.001$).

DO values were often higher in lower than upper part depending on water circulations except the bloom periods. However, DO values were frequently detected higher in upper and middle parts as a result of *P. minimum* blooms and it reached to super saturation values in surface water of middle and upper

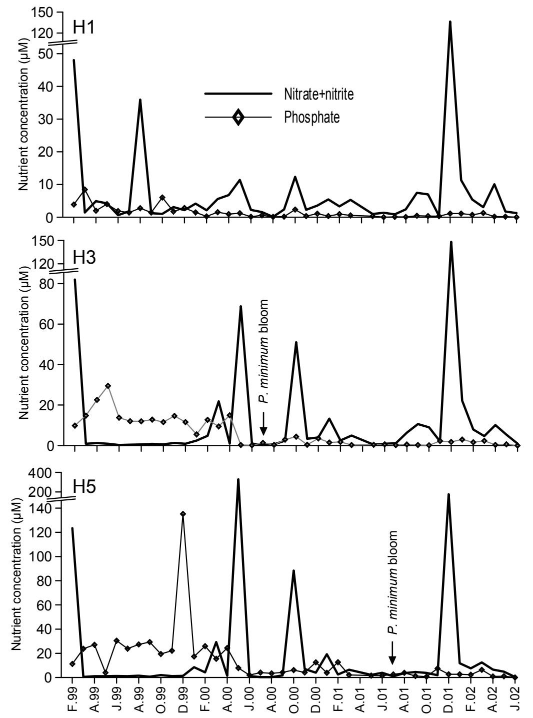

Figure 3. Temporal variations of the main nutrient concentrations in the GHE.

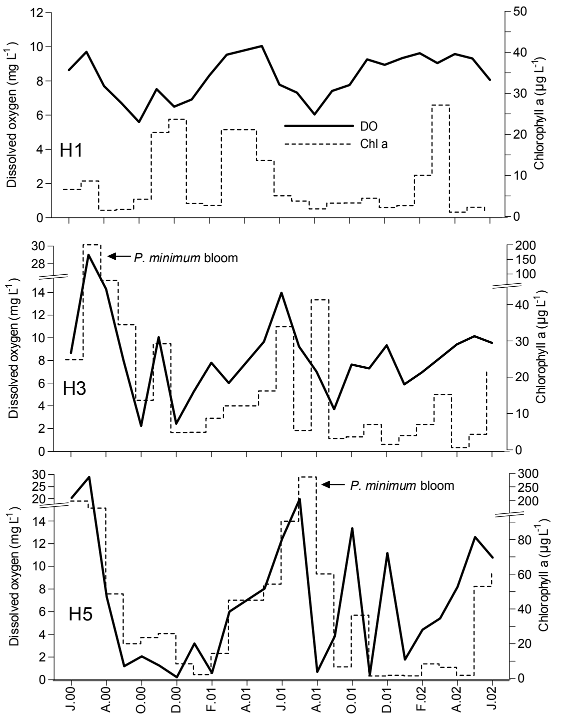

Figure 4. Temporal variations of DO and chlorophyll *a* concentrations in the GHE.

estuary (29.0 mg L^{-1}) (Figure 4). DO concentrations were a clear positively correlated with the cell density of P. *minimum* (rho = 0.37, P<0.001). In July 2001, similar to July 2000, a dense *P. minimum* bloom was recorded at H5 (36.0×10^6 cells L^{-1}) and DO reached very high super saturation level (DO= 19.86 mg L^{-1} SDO= 5.40 mg L^{-1}, 367% sat). However, DO concentration decreased to 4.96 mg L^{-1} in the bottom water at the same time period.

Chlorophyll *a* concentration, which is an indicator of phytoplankton biomass, reached to very high values depending on the *P. minimum* cell density (Figure 4). Average chlorophyll *a* values seem to considerably increase and have a very high standard deviation (SD) in the middle and upper estuary (Table 1). Chlorophyll *a* concentrations ranged from 3.74 to 10.17 µg L^{-1}, from 5.33 to 200 µg L^{-1}, from 51.0 to 286 µg L^{-1} in lower, middle and upper estuary, respectively. Middle and upper part of estuary were characterized by higher concentrations due to high biomass of *P. minimum.* The highest value of chlorophyll *a* was observed on July 10, 2001 (286 µg L^{-1}) in the upper estuary (Figure 4).

Cell Morphology and Density

The morphology of natural populations of *P. minimum* collected from GHE had a typical character. The cell shapes were generally oval, oval-round, heart-shaped and sometimes unclear shaped, but they were mostly oval-round shaped. The dimensions of *P. minimum* cells during the bloom periods are variable, and the size range seems to be 18.0-24.0 µm length and 12.0- 20.0 µm wide (Figure 5).

P. minimum was firstly detected in September 1999 in the middle part and it was in very low density (1.25×10^3 cells L^{-1}). Two significant bloom events ($>10^6$ cells L^{-1}) were observed in the GHE throughout the study period (Figure 6). The first bloom of *P. minimum* occurred on 11 July 2000. The blooms have commonly been observed in mid and upper parts of GHE, which have weak water circulations. Density of cells in these blooms showed significant differences between parts of estuary. At the beginning of the first bloom period, *P. minimum* density varied from 0.4×10^6 to 5.5×10^6 cells L^{-1} and the highest density was detected as 5.5×10^6 cells L^{-1} in the middle and upper estuary. Two weeks later from this occurrence on July 26, 2000 the density of *P. minimum* reached to maxima (70.0×10^6 cells L^{-1}) in the middle part of estuary, while cell density was 0.68×10^6 cells L^{-1} in lower part. At the same months of the following year (10 July 2001) was detected a second bloom of *P. minimum.* At this bloom, cell density varied from 0.025×10^6 to ~ 36.0×10^6 cells L^{-1} (from lower to upper part). The last occurrences of *P. minimum* were in May and June 2002 and its density reached to 0.38×10^6 cells L^{-1} and 0.024×10^6 cells L^{-1} in the upper estuary, respectively (Figure 6).

Discussion

Water movements (currents and upwelling) are important for the transport of phytoplankton and the

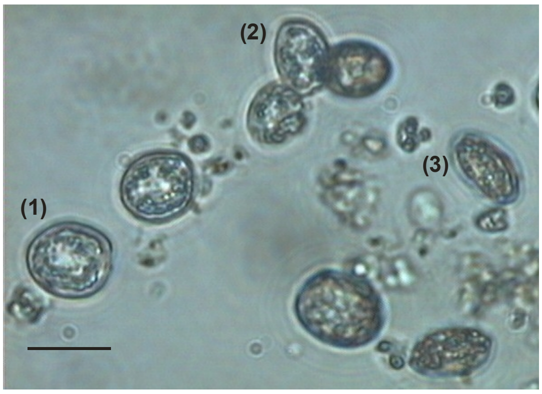

Figure 5. Light micrographs of *P. minimum* morphology which in the different cell shape (1; oval-round, 2; oval, 3; unclear cell shape) observed in the GHE. Scale bar: 20 µm.

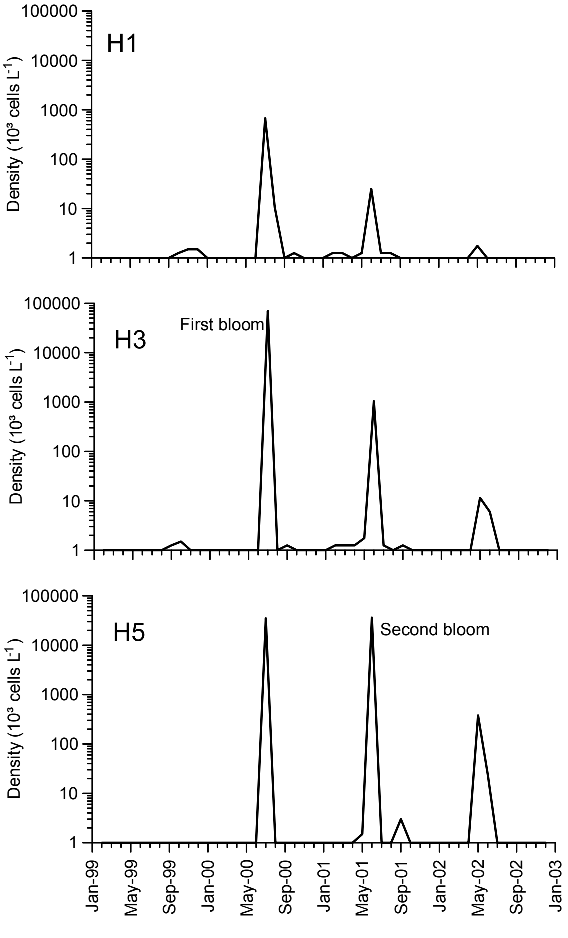

Figure 6. Cell densities of *P. minimum* during successive blooms in the GHE.

occurrence of blooms as described by Tyler and Seliger (1978, 1981). The effects of natural and artificial eutrophication on the GHE ecosystem must be evaluated as a main factor of the change in the ecosystem. Natural eutrophication of the region is mainly due to estuarine characteristics and the two streams feeding the estuary is the main reason for this process (Tas *et al.*, 2009). Blooms of *P. minimum* occurred mostly in middle and upper parts which have a weaker water movement (often upper layer circulation) due to bridges operating on buoys. Also, the calm weather might be considered as another stimulating factor to develop of *P. minimum.*

P. minimum has been described as a eurythermal and euryhaline species performed at the studies in Cheaspeake Bay (Tango *et al.*, 2005). Pertola *et al.* (2005) suggested that *P. minimum* could be adapted to relatively low temperatures-salinity environments, while Heil *et al.* (2005) stated *P. minimum* blooms occur at the high temperatures and the low to moderate salinities similar to our results. The consequences obtained in this study revealed that the growth period of *P. minimum* was from May until August but mainly occurrences were in July. The combination of moderate salinity (from 15.0 up to 20.0 psu) and high temperature (>20°C) may be consider favorable conditions to *P. minimum* in GHE. Further, the light penetration also significantly decreased, as derived from the decrease in secchi depth due to high density of *P. minimum.*

P. minimum could well utilized organic substances (nitrogen or carbon) when inorganic nutrients are not sufficient, because it is capable of both autotrophic and mixotrophic nutrition (Bockstahler and Coats, 1993; Jacobson and Anderson, 1996) and this feature contributes to the ability of *P. minimum* to dominate and to bloom under nutrient-limiting conditions (Heil *et al.*, 2005). The bloom events occurring under nitrogen-limiting conditions in the GHE showed that most likely *P. minimum* could be utilizing organic forms of nutrients. Because inorganic nitrogen constituted only a minor fraction of total nitrogen concentration (Pertola *et al.*, 2005), it might be most likely considered that organic fraction of total nitrogen could contribute to the feeding mechanism of *P. minimum* during these blooms formed in the GHE. Thus, this species is an opportunist and a competitor species in the phytoplankton community. The negative correlation between *P. minimum* abundance and inorganic nutrients indicates to utilize rapidly at the early stage of the bloom. The results obtained from nutrient data revealed that nitrogen is commonly the limiting nutrient in the GHE. In a large amount dissolved and particulate organic material enters to the estuary by streams and terrestrial inputs. Thus, it might be considered that the GHE is rich either organic or inorganic nutrient resources to thrive of *P. minimum*.

In the blooms of *P. minimum* occurred in the GHE, there were no observed detrimental organismal effects. However, in DO concentrations were detected the rapid decreases in the bottom of the bloom area. Heil *et al.* (2005) suggested that the blooms of *P. minimum* cause environmental damage due to high algal biomass and related effects (localized oxygen depletion, pH change, and significant light attenuation) in bloom areas. In general, the lower estuary has the higher DO concentration than upper part because of the strong dynamic structure. The upper estuary contains mostly low DO especially in the bottom, due to oxygen depletion. DO values decrease rapidly in the upper estuary as a result of decomposition of the high amount organic matter either originating bloom or stream. Also, a much weaker water movement in the upper part is an important factor for low DO concentrations. In addition, pH values increase significantly in surface water due to very high algal biomass. When the *P. minimum* density reached to the peak, pH value was measured as 9.20. Some researchers recognized that *P. minimum* has one of the highest tolerances for high pH (Hansen 2002; Sierra-Beltrán *et al.*, 2005) and the influence of high pH on the bloom dynamics of *P. minimum* (Martinez-López *et al.*, 2001). Thus, this tolerance for high pH indicated that *P. minimum* has a competitor species over other species in this condition of the GHE.

As the other areas around the world, the cell shape of *P. minimum* in the GHE differ from oval to oval-round and sometimes unclear shaped, but the oval-round form was more common in this area. Many factors (physiological turgor pressure, salinity, temperature and light intensity) play an important role in the cell shape variation (Pertola *et al.*, 2003). No significant differences were regionally found in the morphology of *P. minimum* cells in the GHE. Specific physical characteristics of the study area influence likely morphology of *P. minimum* cells and similar cells shapes mostly oval and oval-round have been observed in the GHE.

P. minimum was not reported from the GHE in the previous studies (Uysal, 1996; Taş and Okuş, 2003). In fact, blooms of *P. minimum* have been considered that the highly polluted GHE ecosystem with anoxic character has shifted to eutrophic. Thus, optimal conditions (high water temperature, moderate salinity, light intensity and rich–nutrient sources together with calm weather) caused to *P. minimum* blooms. The first bloom period lasted possibly at least two or three weeks, similar to stated by Sierra-Beltrán *et al.* (2005) and two weeks later reached to peak on 26 July 2000. At the same month of the following year (July 2001) *P. minimum* bloomed again. The occurrence this species continued in May 2002, but it was not at the level of previous blooms. *P. minimum* was observed at the very low densities in June 2002 as the last occurrence. These successive blooms indicated that of *P. minimum* develops well in late spring and summer in the GHE ecosystem.

The cells density of *P. minimum* shows differences in spatial distribution and so increases gradually from lower to upper part of estuary. *P. minimum* could well develop in the middle and upper part and could be a good competitor species. During the bloom events *P. minimum* dominated total phytoplankton biomass (> 90%). On the other hand, very high chlorophyll *a* concentrations (> 200 µg L^{-1}) measured at the time of bloom has been supported in a large scale the cell density. Blooms of *P. minimum* occurred in July 2000 and July 2001 followed the diatom blooms (*Skeletonema costatum* (Greville) Cleve, 1873 in June 2000 and *Thalassiosira allenii* Takano, 1965 in June 2001) and diatoms were displaced by dinoflagellates in a short time similar to the studies carried out by Heil *et al.* (2005). Thus, a seasonal variability in phytoplankton cycle such that diatoms develops generally in spring and early summer and dinoflagellates reproduces more in mid summer in the GHE.

In this study revealed that *P. minimum* could well adapt to high water temperature and moderate salinity and may cause the successive blooms in summer under the optimal conditions. These blooms might be considered a response to increasing eutrophication as result. During the bloom events were not detected any detrimental organismal effect, but the rapid oxygen depletion in the bottom, increasing pH and the reduction in light attenuation coincident with high density of *P. minimum* may cause a negative effect on the ecosystem and water quality.

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