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## A prolonged red tide of *Heterocapsa triquetra* (Ehrenberg) F. Stein (Dinophyceae) and phytoplankton succession in a eutrophic estuary (Turkey)

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### Abstract

A prolonged red tide of *Heterocapsa triquetra* and phytoplankton succession were investigated in a eutrophic estuary (Golden Horn) for a year. Runoff following the rainfall in winter supplied large amounts of nutrients and also created a mesohaline frontal zone in the middle and upper estuary. A red tide of *H. triquetra* was first observed with an orange-brownish water discoloration at the upper estuary in January. The highest cell density of *H. triquetra* was  $2.7 \times 10^6$  cells  $l^{-1}$  in January and it reached  $19.2 \times 10^6$  cells  $l^{-1}$  in April. Successive blooms continued with dinoflagellate *Prorocentrum minimum* in May and phytoflagellates *Eutreptiella marina* and *Fibrocapsa* sp. in summer. High chl-*a* and dissolved oxygen were measured in spring depending on algal biomass. Findings indicate that *H. triquetra* might be adapted to moderate salinity and low temperatures and red tide events should be considered a response to increasing eutrophication due to large amounts of nutrients. Eutrophication could cause harmful algal blooms for this area in the near future.

**Keywords:** *Heterocapsa triquetra*, dinoflagellate, phytoflagellate, red tide, Golden Horn.

### Introduction

Dinoflagellates are a nutritionally diverse group of protists with phototrophic, heterotrophic, and mixotrophic members. Heterotrophy is a nutritional mode in which an organism acquires some or all of its nutritional requirements from organic sources. Osmotrophs utilize dissolved organic matter, whereas phagotrophs feed on particulate organic matter (Lessard, 1993). Dinoflagellate blooms are currently known as being an important phenomenon in marine and estuarine ecosystems. *Heterocapsa triquetra* (Ehrenberg) F. Stein is one of the most common bloom-forming and mixotrophic dinoflagellates found in estuaries and coastal regions (Kim *et al.*, 1990; Litaker *et al.*, 2002). The uptake of inorganic carbon by phytoplankton during photosynthesis may increase pH levels. Therefore, in eutrophic estuaries, where primary productivity is high, pH may rise considerably during bloom periods. *H. triquetra* is a common bloom-forming dinoflagellate in eutrophic coastal waters and it is fast-growing, sustaining growth to high pH values (Havskum & Hansen, 2006).

The Golden Horn Estuary (GHE) has been extremely polluted since the 1950s as a result of unplanned urbanization, heavy industrialization, and domestic and industrial wastewaters that damaged the estuarine ecosystem. By the early 1990's, estuarine life was limited to the lower and middle part of the estuary. Furthermore, the upper part was almost completely abiotic due to anoxic conditions and heavy sedimentation. Additionally, floating bridges on buoys greatly limited upper layer circulation. The Golden

Horn Rehabilitation Project (GHRP) was initiated in order to remove high amounts of pollutants in 1997. The previous studies carried out prior to the GHRP clearly demonstrated that pelagic life was very limited due to heavy pollution. Phytoplankton studies before the GHRP clearly showed that insufficient water circulation, extreme pollution and high turbidity limited phytoplankton growth particularly in the upper part (Saydam, 1986; Uysal, 1996; Taş & Okuş, 2003). The GHRP resulted in a rapid change in phytoplankton composition and in dense blooms (Tas *et al.*, 2009; Taş & Okuş, 2011). As a result of the GHRP, this highly polluted ecosystem shifted from anoxic to eutrophic conditions (Tas *et al.*, 2009). After remediation of GHE waters, phytoplankton growth occurred immediately with an increase in abundance. High chlorophyll *a*, super-saturated dissolved oxygen and decreasing nutrient concentrations following rehabilitation (after June 2000) indicated high phytoplankton activity (Tas *et al.*, 2009). The main goal of this study is to investigate a prolonged red tide of *H. triquetra* and phytoplankton succession in a eutrophic estuary.

### Materials and Methods

#### Study area

The GHE is located in the northeast of the Sea of Marmara and the southwest of the Strait of Istanbul (Bosporus), extending in northwest-southwest direction. It has a length of 7.5 km and a width of 700 m covering an area of about

2.5 km<sup>2</sup>. The lower estuary (LE) is 40 m deep and is characterised by strong interaction with the Strait of Istanbul. Maximum depth in the middle estuary (ME) is 14 m where water circulation is more limited than in the lower part. The upper estuary (UE) has a maximum depth of 5 m and is characterised by insufficient water circulation (Fig. 1). The Alibey and Kağıthane streams are the main sources of freshwater input; however, the amount of freshwater influx from these streams decreased considerably by the end of the 1990s, following the construction of a series of dams. Nowadays, rainfall and coastal runoff are the main sources of freshwater input into the GHE (Sur *et al.*, 2002). The estuary is characterized by a two-layered structure; the upper layer has a salinity of ~ 18 psu and the lower layer a salinity of ~38 psu.

### Sampling design and seawater analyses

Seawater samples were taken from the surface during monthly intervals at 7 stations in 2007, representing the LE, ME and UE areas (Fig. 1). Seawater samples for chemical analysis were collected from the surface using 5 litre Niskin bottles. Inorganic nutrients and dissolved oxygen (DO) (Winkler Method) were analyzed following APHA (1999). TN (Total nitrogen) and TP (Total phosphorus) concentrations could only be measured in the three months of April, May and June, and analyses were made using a Brab+Luebbe AA3 auto-analyser, according to Grasshoff

*et al.* (1983). Chl-*a* analyses were carried out by applying the acetone extraction method (Parsons *et al.*, 1984). The physical descriptors, salinity and temperature values were recorded by a SBE-Sea Logger 25 CTD system and water transparency was measured using a secchi disc. One-litre samples were taken for phytoplankton analysis using Niskin bottles and the samples were fixed in formaldehyde (final conc. of % 0.4). Samples were allowed to settle in the laboratory for at least one week in accordance with the Utermohl technique (Utermohl, 1958). Supernatant was removed by siphoning and the bottom water was stored in dark-coloured glass bottles until microscopic examination (Sukhanova, 1978; Throndsen, 1978). A Sedgewick-Rafter Counting Cell under a light microscope was used for phytoplankton enumerations. The following references were used for species identification: Drebes (1974); Dodge (1985); Tomas (1997). The relationships between *Heterocapsa triquetra* abundance and environmental factors were analyzed by the Spearman rank correlation, following transformation to natural logarithms.

## Results

### Hydrography

The lower estuary is highly dynamic and presents the characteristics of the neighbouring Strait of Istanbul. A permanent stratification prevailed in the region, where the

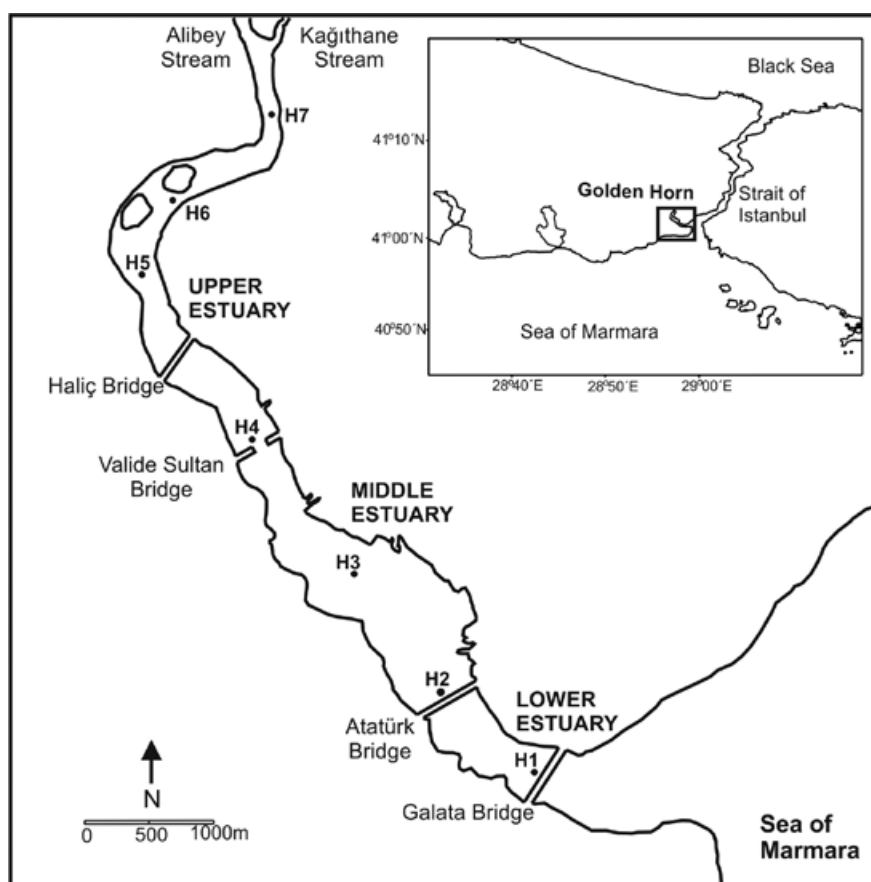


Fig. 1: The study area and sampling stations.

upper layer represented less saline waters of Black Sea origin (17-18 psu), and following a transition zone, saline waters of Mediterranean origin (38 psu) constituted the lower layer. The seasonality is clear for temperature; while salinity and Secchi disc measurements displayed annual and spatial variations. Temperature ranged from 9° C to 24° C and salinity from 15.2 to 19.7 psu (Fig 2). Temperature values in the upper estuary are higher than in the lower and middle estuary. In contrast to temperature, salinity values are always lower in the upper estuary. Water circulation is very limited in the shallow (4 m) upper estuary. Secchi depths ranged from 5.5 to 9 m in the lower part and from 0.2 to 1 m in the upper part, throughout the study period. Secchi depth decreased clearly from the lower to the upper part due to increased quantities of suspended materials originating from two streams and relatively high phytoplankton density.

### Nutrients

Inorganic nutrient concentrations gradually decreased from January to May during the dense blooms (Fig. 2).  $\text{NO}_3+\text{NO}_2$  and  $\text{PO}_4$  concentrations were relatively low between June and December (Fig. 2). During the study period,  $\text{NO}_3+\text{NO}_2$  and  $\text{PO}_4$  concentrations at the surface varied from 0.05 to 21.8  $\mu\text{M}$  (LE) and 0.05 (LE) to 7.3  $\mu\text{M}$  (UE), respectively. N:P ratios based on  $\text{NO}_3+\text{NO}_2$  and  $\text{PO}_4$  concentrations were between 0.5 (LE) and 16.6 (UE) and lower than

the Redfield ratio (16:1) during the period of prolonged red tide. The N:P ratio declined gradually from the lower to the upper part. TN and TP concentrations were generally high in the study area and, in contrast to the N:P ratio, the TN:TP ratio was higher than the Redfield ratio and ranged between 25.5 and 36.5 at the surface. Nutrient concentrations, with the exception of nitrate+nitrite, increased gradually from the lower to the upper estuary. It appears that the  $\text{NO}_3+\text{NO}_2$  concentration decreased depending on the increased abundance of *Heterocapsa triquetra* (Fig. 2).

### Chlorophyll a

Chl-*a* concentrations varied between 2 and 3.9  $\mu\text{g l}^{-1}$  in the lower estuary, while the upper estuary was characterized by very high concentrations (39.5 to 104.5  $\mu\text{g l}^{-1}$ ) during the prolonged red tide of *Heterocapsa triquetra* (from January to April). In April, chl-*a* concentration reached a high level (104  $\mu\text{g l}^{-1}$ ) due to the very high density of *H. triquetra* in the upper part; however, it was calculated as 2.8  $\mu\text{g l}^{-1}$  in the lower part, yielding a 37-fold difference in chl-*a* concentrations among the upper and lower estuaries. Chl-*a* values increased considerably in May and reached the highest level (392.5  $\mu\text{g l}^{-1}$ ) as a result of a dense bloom of dinoflagellate *Prorocentrum minimum*. In June and August, the increased abundance of *Eutreptiella marina* contributed significantly to the chl-*a* concentration in the upper estuary (Fig. 2).

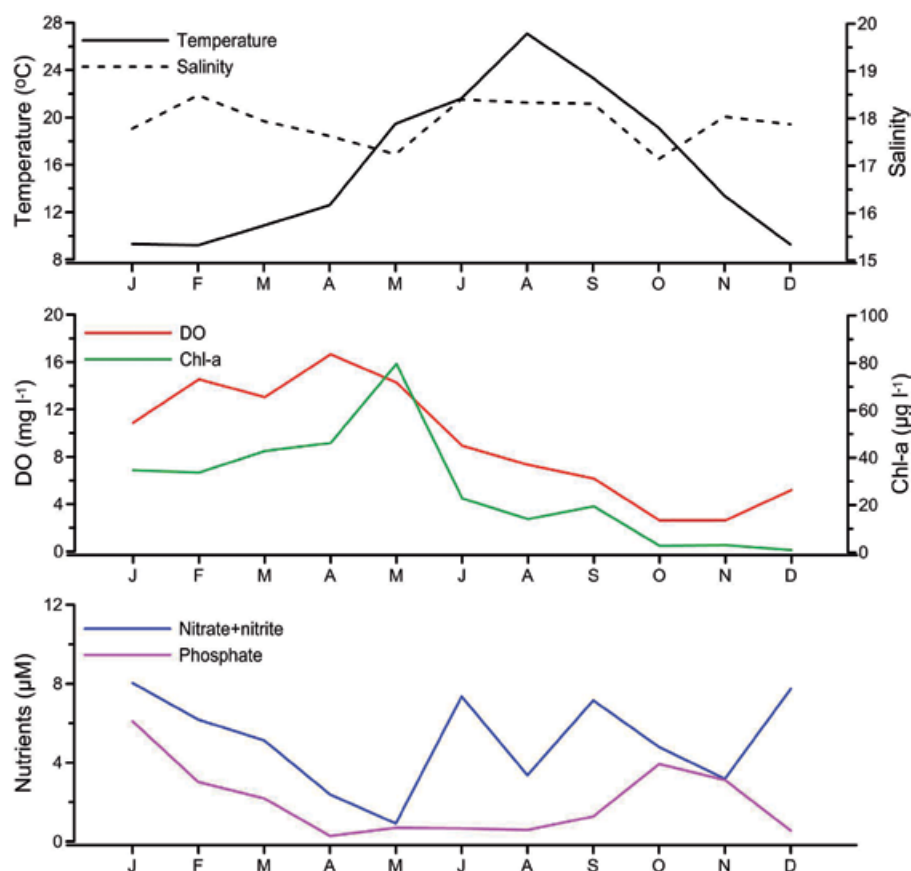


Fig. 2: Mean values of the main environmental parameters in the study area.

## Dissolved oxygen

DO values are generally higher in the lower than the upper estuary, because of strong hydrodynamics. However, one of the most important effects of the *Heterocapsa triquetra* red tide is the increase in DO concentrations in the upper estuary. The average DO values increased considerably from January (10.6 mg l<sup>-1</sup>) to April (16.6 mg l<sup>-1</sup>) due to the density of *H. triquetra* and the subsequent bloom of *Prorocentrum minimum* occurring in May (14.3 mg l<sup>-1</sup>). Thereafter, DO values began to decrease gradually until December (Fig. 2). DO values increased more than twice the saturated DO values (DO=20.4 mg l<sup>-1</sup>, SDO=9.4 mg l<sup>-1</sup>, 217% sat.) in April as a result of the high density of *H. triquetra* (>19×10<sup>6</sup> cells l<sup>-1</sup>) and about three fold its saturated DO values (DO=21.4 mg l<sup>-1</sup>, SDO=7.8 mg l<sup>-1</sup>, 273% sat.) in May during the dense bloom of *P. minimum* (32×10<sup>6</sup> cells l<sup>-1</sup>). In the upper estuary, DO values increased twice as much as in the lower part (20.4 mg l<sup>-1</sup>) in April.

## Phytoplankton succession and cell abundances

The red tide caused by *Heterocapsa triquetra* (Fig. 4) was initially observed due to an orange-brownish discoloration in the surface water of the upper estuary in mid-January when the temperature was low (9.5 °C) and salinity moderate (16.6). Cell density of *H. triquetra* was calculated as 2.7×10<sup>6</sup> cells l<sup>-1</sup>, increasing from the LE to the UE. One of the most notable aspects is the presence of the diatom *Pseudo-nitzschia* spp. in the LE and ME. They were more abundant than *H. triquetra* in the LE (Fig. 3).

In February, the cell density of *H. triquetra* increased to 6×10<sup>6</sup> cells l<sup>-1</sup> in the UE under the same temperature conditions (Fig. 3). The abundance of *Pseudo-nitzschia* spp. also increased to 729×10<sup>3</sup> cells l<sup>-1</sup> in the LE; however, they were absent in the UE. The increase of *H. triquetra* continued in March throughout the study area and cell density exceeded 10×10<sup>6</sup> cells l<sup>-1</sup> in the UE. In April, the cell density of *H. triquetra* reached the highest level (>19×10<sup>6</sup> cells l<sup>-1</sup>) in parallel with the temperature increase (13.4 °C) (16.6) (Fig. 3). *H. triquetra* was the predominant species; more than 90% throughout study area in March and April. The abundance of *H. triquetra* decreased to 22×10<sup>3</sup> cells l<sup>-1</sup> in May and the most important occurrence in May was the dense bloom of *Prorocentrum minimum*. This bloom occurred in the ME and cell density increased gradually towards the UE. The abundance of *P. minimum* reached 32×10<sup>6</sup> cells l<sup>-1</sup> in H7 under high water temperature conditions (23°C) and low salinity (15.0) (Fig. 3).

The cell density of *P. minimum* decreased to 254×10<sup>3</sup> cells l<sup>-1</sup> in the UE in June, while an increase in euglenophycean *Eutreptiella marina* was observed (Fig. 3). The cell density of *E. marina* reached 442×10<sup>3</sup> cells l<sup>-1</sup> in the UE. The increase of *E. marina* continued in August and approached the bloom level (~10<sup>6</sup> cells l<sup>-1</sup>) under high temperature conditions (27°C) (Fig. 3). In September, a bloom of raphidophycean *Fibrocapsa* sp. occurred in the UE and

cell density reached ~3.4×10<sup>6</sup> cells l<sup>-1</sup>. The density of the phytoplankton community was very low between October and December and represented by dinoflagellates *Triplos* spp. and *Prorocentrum micans* in the LE and ME, while cyanobacterial forms were dominant in the UE (Fig. 3).

## Discussion

The prolonged red tide phenomenon and successive phytoplankton blooms that occurred and are presented in this study are directly related to eutrophication and hydrographical conditions. A number of researchers suggest that the highest growth rate of *Heterocapsa triquetra* is observed when there is a combination of 15°C and 15 psu, and is well adapted to euryhaline waters (Yamaguchi *et al.*, 1997). In this study, the relationships between physical conditions and *H. triquetra* abundance show that low water temperature (9.5 to 14.5°C) and moderate salinity (15 to 16) are probably favourable for rapid growth from January to April.

The effects of eutrophication in the GHE were evaluated as a main factor of the change in ecosystem and estuarine characteristics. The two streams feeding into the estuary have been shown to be the main reason for natural eutrophication (Tas *et al.*, 2009). Nutrient concentrations were generally higher in the middle and upper estuary. Therefore, these parts of the GHE were more suitable for phytoplankton blooms during this period of study. Stream runoff following heavy rainfall in winter provides all the nutrients are critical for phytoplankton growth. A mesohaline frontal zone and the lack of water circulation in the upper part created favourable conditions for the *H. triquetra* red tide. Inorganic nitrogen and phosphorus concentrations in the upper part decreased significantly during the red tide due to uptake by *H. triquetra*. Despite low quantities of inorganic nutrients due to consumption, high TN and TP concentrations might be an indicator of the large amounts of organic substances entering this area through streams.

Mixotrophy capacity and temporary cyst production of *H. triquetra* in unialgal culture is well known (Olli, 2004). Phagotrophy in *H. triquetra* may be important in maintaining the population (Legrand *et al.*, 1998). Thus, *H. triquetra* may feed mixotrophically on organic particles, when inorganic nutrients become insufficient for growth. This feeding characteristic of *H. triquetra* may have provided a large advantage due to reduced competition with other dinoflagellates. As stated by Litaker *et al.* (2002), low grazing pressure due to low water temperature and heavy nutrient loading by rainfall and streams, and favourable hydrodynamic conditions may support the prolonged red tide of *H. triquetra*. It should be noted that these events in such ecosystems adversely affect local water quality such as increasing pH, hypoxia/anoxia events, reduced light penetration and damage to benthic organisms.

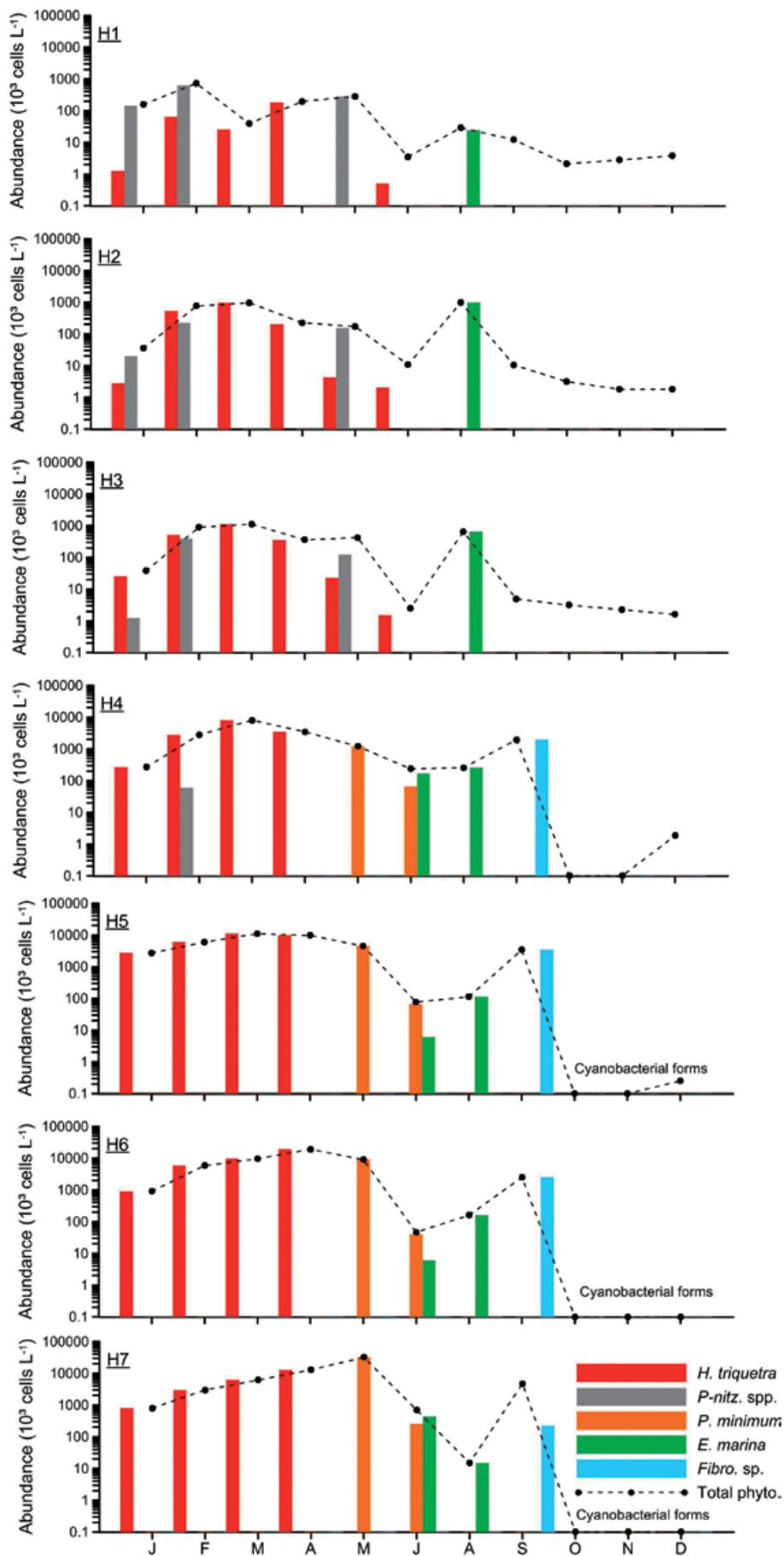


Fig. 3: Spatial and temporal variations in the phytoplankton during the study period.



**Fig. 4:** Light micrography of a single cell of *H. triquetra* in samples collected from Golden Horn estuary.

*H. triquetra* almost disappeared in May when water temperature increased to 23°C and *P. minimum* caused a dense bloom. In previous studies, *Prorocentrum minimum* was described as a eurythermal and euryhaline species (Tango *et al.*, 2005) with blooms occurring under high temperature and low to moderate salinities (Heil *et al.*, 2005). *P. minimum* caused the successive blooms in the GHE in summer. Salinity between 15 and 18 psu and high temperature (>20°C) may be considered as a favourable condition for *P. minimum* (Taş & Okuş, 2011). Inorganic nitrogen contributed to total nitrogen only at the rate of 2%, and the rest consisted of an organic form of nitrogen in May. High TN and TP concentration measured during this bloom showed that *P. minimum* may consume organic nutrients. Phytoplankton composition changed rapidly in parallel with increased temperature and a number of phytoflagellates, such as euglenophycean *Eutreptiella marina* and raphidophycean *Fibrocapsa* sp., which were dominant species in the phytoplankton community in summer. It is well known that euglenophyceans can adapt well and grow rapidly in aquatic environments that are known to be rich in organic matter. Considering that the upper estuary is rich in organic matter, we can argue that optimal conditions exist for the rapid growth of these flagellates.

Cell density of *H. triquetra* was found to be very low (<5×10<sup>3</sup> cells l<sup>-1</sup>) in the pre-remediation period (1998-2000) in the GHE, while density increased in spring and early June during post-remediation (2000-2001) (Taş, 2003). It was reported that the highest density of *H. triquetra* was found in May in the Sea of Marmara (Balkis, 2003; Aktan *et al.*, 2005) and in March in the southern Black Sea (Türkoğlu & Koray, 2002). Higher DO values were often measured in the upper part than the lower part due to the density of *H. triquetra* and reached 20.4 mg l<sup>-1</sup> in April. At the same time, SDO (saturated dissolved oxygen) was 9.4 mg l<sup>-1</sup> and the DO values were up to more than twice the SDO level (217% saturation). During the May bloom of

*Prorocentrum minimum*, DO super saturated (21.4 mg l<sup>-1</sup>) for the second time. The highest chl-*a* concentrations were measured in the upper part of the estuary depending on algal production. Moreover, it should be noted that nano-phytoplankton contribute significantly to the chl-*a* concentration of such estuarine environments (Tas *et al.*, 2009).

As a conclusion, this study showed that the prolonged red tide of *H. triquetra* was a response to increasing eutrophication and that this species can adapt well to low water temperature and moderate salinity under eutrophic conditions. High TN and TP concentrations indicate that the GHE is also rich in organic matter and this promotes a rapid growth of all flagellates that are able to feed mixotrophically. Phytoplankton succession exhibited a characteristic pattern and eutrophication can lead to harmful algal blooms in this area in the near future. Furthermore, dense blooms affect the ecosystem via rapid oxygen depletion in the bottom, increased pH and light attenuation.

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