Potentially harmful microalgae and algal blooms in a eutrophic estuary in Turkey

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**Abstract**

Distribution of potentially harmful microalgae and algal blooms were investigated at monthly and weekly time scales between October 2009 and September 2010 in the Golden Horn, a eutrophic estuary in the Sea of Marmara (Turkey). Several physical and chemical parameters were analysed together with phytoplankton composition and abundance. A total number of 23 potentially harmful and/or bloom-forming microalgae (14 dinoflagellates, 4 diatoms and 5 phytoflagellates) were identified throughout this study period, of which nine taxa have been confirmed to be toxic elsewhere in the world. Most harmful species and algal blooms were observed in late spring and summer particularly in the middle and upper estuaries, and nine taxa formed dense and successive algal blooms causing water discoloration. Nutrient concentrations increased significantly from the lower to the upper estuary. Additionally, high organic matter loads in the upper estuary could also have benefited by mixotrophic species. The increasing number of potentially harmful and bloom-forming species and algal blooms indicated that the GHE is a potential risk area for future HABs.

**Keywords**: Phytoplankton, Harmful Algal Blooms, Nutrients, Estuary, Golden Horn, Sea of Marmara.

**Introduction**

Phytoplankton is an efficient and easily detectable indicator of ecological change. The information obtained from phytoplankton communities can significantly contribute to the assessment of eutrophication levels in aquatic systems, since they are sensitive to numerous environmental stressors (Paerl et al., 2007). Despite the abundance of nutrients in estuaries, other factors, such as light attenuation, salinity change and water circulation may limit the production of estuarine phytoplankton (McLusky & Elliott, 2004). Although many algal blooms may be harmless events, even blooms of non-toxic species can at times cause harmful effects such as water discolorations that limit light penetration, hypoxia and anoxia. Eutrophication (Struyd, 1990), heavy industrialization (Lam & Ho, 1989; Okaichi, 1989) and high nutrient loading of aquatic ecosystems (Rousseau et al., 2002) have been suggested as the main causes of increasing HABs in fresh, estuarine and coastal marine waters. One of the most significant problems regarding algal blooms is the harmful effect due to different types of toxins produced by some phytoplankton species (e.g. dinoflagellates) on fish, invertebrates and humans (Anderson et al., 2012). Climatic change may also be considered as a possible reason for HABs increase (Hallegraeff, 2010).

The Golden Horn Estuary (GHE) has been polluted since the 1950s as a result of unplanned urbanization and heavy industrialization, and has become the most significant area of environmental pollution in Istanbul. By the early 1990s, estuarine life was limited to the area around the Galata and Atatürk Bridges due to anoxia and heavy sedimentation. In 1997, the Golden Horn Rehabilitation Project was initiated. Surface discharges were connected to collector systems, and discharged into the lower layer of the Strait of Istanbul from two deep discharge systems. Most significantly, 4.25×10^6 m^3 of anoxic sediments were removed from the almost completely filled upper estuary and in this way at least 5 m depth was gained here. Prior to this, industrial activity around the GHE was stopped and industries on the drainage basin of streams feeding the estuary were forced to install and properly operate treatment plants for their wastewater.

Phytoplankton studies carried out in the GHE before rehabilitation clearly demonstrated that the lack of adequate upper layer circulation, extreme pollution and light limitation inhibited the growth of eukaryotic phytoplankton, particularly in the upper part of the estuary (Uysal & Unsal, 1996; Taş & Okuş, 2003). During the rehabilitation project, enhanced water circulation resulted in a rapid renewal and oxygenation...
of anoxic and highly polluted water which was followed by consecutive blooms of phytoplankton. Increased phytoplankton activity resulted in super saturated dissolved oxygen concentrations and a significant decrease in nutrient concentrations. The first bloom following the rehabilitation efforts was caused by Skeletonema cf. marinoi (reported as S. costatum) ($5 \times 10^6$ cells L$^{-1}$) in the upper estuary in June 2000, when dissolved oxygen (DO) concentration reached super-saturation levels (20.35 mg L$^{-1}$). The densest bloom ($7 \times 10^7$ cells L$^{-1}$) was caused by dinoflagellate Prorocentrum cordatum (reported as P. minimum) in the mid-estuary. Subsequent diatom blooms were caused by S. cf. marinoi ($8.12 \times 10^6$ cells L$^{-1}$) in March 2001 and Thalassiosira allenii ($4 \times 10^6$ cells L$^{-1}$) in June 2001. In July 2001, a dense P. cordatum bloom was recorded in the upper estuary ($>3.6 \times 10^7$ cells L$^{-1}$) and DO reached super-saturation levels (19.86 mg L$^{-1}$). Dense blooms continued until the end of 2001. At times, different groups such as euglenophytes dominated the phytoplankton in this period (Tas et al., 2009).

The present study reports the occurrence of harmful microalgae and algal blooms in the GHE at monthly and weekly time scales in the period between October 2009 and September 2010. We hypothesize that high nutrient concentrations in the upper estuary supported a diverse harmful microalgae community.

Materials and Methods

Study area and sampling strategy

The GHE is located at the southwest of the Strait of Istanbul, extending in a northwest-southeast direction, and is approximately 7.5 km long and 700 m wide (Fig. 1). The maximum depth is 40 m in the lower part, rapidly decreasing to 14 m in the middle part and to <4 m in the upper part, where two small streams (Alibey and Kağıthane) carry freshwater into the estuary. Following the construction of a series of dams on these streams, freshwater input considerably decreased and precipitation has become the main source of variability in freshwater for the GHE (Sur et al., 2002). The lower part of the GHE is characterized by a two-layered structure similar to the neighboring Strait of Istanbul, where the upper layer flowing from the Black Sea has a salinity of ~18 and the lower layer flowing from the Mediterranean Sea has a salinity of ~38. The study area was categorized in three regions based on the hydrographic structure, i.e. lower, middle and upper estuary (Fig. 1), and five sampling stations were distributed along the estuary. Station TH1, representing the lower estuary (LE) (40 m depth), was heavily influenced by the dynamic physico-chemical properties of the Strait of Istanbul. Station TH2 represented the middle estuary (ME) (14 m depth), where a bridge operating on buoys ( Atatürk Bridge) limits upper...
layer circulation between the lower and middle estuaries. Stations TH3, TH4 and TH5 were located in the upper estuary (UE), where the water depth is less than 4 m due to the sedimentation. The sampling period covered one year, from October 2009 to September 2010. Water samples were collected monthly (from October to March) and weekly (from April to September) using Niskin bottles. Samples were taken from depths of 0.5, 2.5 and 5 m at TH1, TH2 and TH3, 0.5, 2 and 4 m at TH4 and 0.5, 2 m at TH5, depending on the station depth.

**Environmental variables**

Salinity (according to the practical salinity scale), temperature, pH and DO were measured using a multiparameter probe (YSI Professional Pro Plus) and light transparency was measured with a 30 cm diameter Secchi disc. Precipitation data were taken from a local meteorological station in Istanbul (http://www.enka.com/weather/). Nutrient analyses were performed on a Bran+Luebbe AA3 auto-analyser according to the standard methods (APHA, 1999). Nitrate+nitrite (NO$_3$) and total nitrogen (TN) were measured according to the cadmium reduction method, ammonia to the Berthelot reaction method, ortho-phosphate to the ammonium molybdate reaction method, total phosphorus (TP) to the alkaline persulfate oxidation method and reactive silica to the molybdate method. Total organic carbon (TOC) analyses were performed on a Schimadzu TOC-V CPH/CPN Total Organic Carbon Analyser (APHA 1999). Chlorophyll a (chl-a) analyses were carried out according to the acetone extraction method (Parsons et al., 1984). All environmental data were averaged over the three depths sampled.

**Phytoplankton analysis**

Seawater samples for quantitative phytoplankton analyses were collected at the five stations at the same depths as described above using Niskin bottles, transferred into 250 ml glass bottles and were preserved with acidic Lugol’s solution (to a final concentration of 2%) (Thurston, 1978). Subsamples (50, 25, 10 or 5 ml) were settled in sedimentation chambers (Utermöhl, 1958) and observed under a Leica DM IL LED inverted microscope with phase contrast at magnifications of 100×, 200× or 400× for counting and identification. At least 300 cells in two or more transects were counted using Utermöhl sedimentation chambers. Phytoplankton abundance was calculated as cells per liter and averaged over the depths sampled. Net samples for qualitative analyses were collected using a Nansen plankton net (0.57 m diameter, 55 μm mesh size) by vertical tows from 10 m to the surface at TH1 and TH2. Net samples were transferred into PVC jars and preserved with addition of borax-buffered formaldehyde (to a final concentration of 4%). Species identification in net samples was performed under a Leica DM 2500 light microscope equipped with a Leica DFC camera at 100×, 200× or 400× magnification. Coup (1943), Drebes (1974), Dodge (1985), Delgado & Fortuna (1991) and Tomas (1997) were used for taxa identification. The identification and definition of harmful microalgae were made according to Hallegraeff (2002) and Hallegraeff et al. (2003). The IOC Taxonomic Reference List of Toxic Plankton Algae was used as the list of proved toxic species (Moestrup et al., 2009). The species name of the dinoflagellate genus Neoceratium was revised based on Gomez (2013). For the identification of the diatom genus Skeletonema, the revision of Sarno et al. (2005) was used.

**Statistical analysis**

The environmental and quantitative phytoplankton data of TH1, TH2 and TH5 were analyzed using Canonical Correspondence Analysis (CCA), in order to relate the change in potentially harmful phytoplankton species with environmental variability and to visualize relations among species. Phytoplankton data were transformed to natural logarithms prior to analyses. Temperature, salinity, DO, Secchi depth and nutrient concentrations (NO$_3$+NO$_2$-N, NH$_4$-N, PO$_4$-P, SiO$_2$-Si) were included among environmental parameters. Analyses were performed using the CANOCO 4.5 software (ter Braak & Smilauer, 2002).

**Results**

**Temperature, salinity and Secchi depth**

Surface temperature varied from 5.9 (February, TH1) to 28.4°C (August, TH5) and the highest temperature values were recorded in August. The surface salinity ranged between 2.5 (December, TH5) and 21.6 psu (May, TH1) during the study period, except for June, when the surface salinity values decreased to 0.5 in the UE following a heavy rainfall. Generally, the upper estuary had higher temperature values than the LE, while salinity values were always lower in the UE. Precipitation was the major factor affecting the change in salinity in the estuary. Secchi depth significantly decreased during rainy periods due to increased terrestrial runoffs and ranged from 0.1 (February, TH5) to 10 m (August, TH1), with a significant decreasing trend from the LE to the UE (Fig. 2).

**Nutrients**

Inorganic nutrient concentrations increased in winter due to high amounts of terrestrial inputs through the streams and precipitation, but decreased in late spring and summer depending on the phytoplankton consumption. All nutrient values increased remarkably from the LE to the UE (Fig. 2). NO$_3$+NO$_2$-N concentrations ranged between 0.2 and 51.6 μM, with a mean value of 3.3±5.2 μM for the LE and 6.4±12.4 μM for the UE. NH$_4$-N ranged between 0.4 and 196.9 μM, with a mean value of 5.9±7.3 μM for the LE and 42.1±42.0 μM for the UE, showing a remarkable increase on days of high levels
of precipitation. PO$_4$-P concentrations varied between 0.01 and 13.8 µM, with a mean value 0.39±0.36 for the LE and 3.54±3.6 µM for the UE. Reactive SiO$_2$-Si concentrations ranged between 0.5 and 172.6 µM, with a mean value of 6.2±7.0 µM for the LE and 37.1±47.2 µM for the UE. TN concentrations varied between 6.4 and 245.5 µM TP varied between 0.4 and 19.1 µM. TN and TP values increased considerably in rainy periods, while they decreased rapidly in late spring and summer. The mean annual values of TN were 19.9±17.3 µM in the LE and 88.0±56.9 µM in the UE. The mean annual values of TP were 1.1±0.7 µM for the LE and 9.3±5.8 µM for the UE. The highest nutrient concentrations (except for PO$_4$-P) were measured following heavy precipitation in early June. The mean TOC concentrations varied between 175.0 and 1,823.0 µM during the study period and increased gradually from the LE to the UE (Fig. 2).

The mean molar N:P ratios based on dissolved inorganic nitrogen-DIN (NO$_3$+NO$_2$-N and NH$_4$-N) and dissolved inorganic phosphorus-DIP (PO$_4$-P) concentrations were 26.2±20.6 for the LE and 35.3±35.6 for the UE increasing from the LE to the UE with a high deviation from the Redfield ratio (16:1). The annual mean TN:TP ratios was 19.0±6.3 for the LE and 10.7±3.6 for the UE, decreasing gradually from the LE to the UE with a little deviation from the Redfield ratio. The highest TN:TP ratio (~36) was detected in early June due to increased TN values following heavy precipitation (Fig. 2).

**Fig. 2:** Fluctuations in physical and chemical parameters. Precipitation data is monthly total rainfall for Istanbul.

**DO, pH and Chl-a**

The mean DO values ranged between 3.5 and 10.6 in the LE, 0.1 and 10.4 in the ME, 0.1 and 9.0 mg L\(^{-1}\) in the UE (Fig. 2). DO concentrations were higher than 10 mg L\(^{-1}\) in spring in the ME due to the high phytoplankton biomass, while they were 5-6 mg L\(^{-1}\) during winter. DO value oversaturated during the Skeletonema cf. marinoi bloom in July (17.6 mg L\(^{-1}\), 221%, TH2) and was at a minimum level (~0.1 mg L\(^{-1}\), 1%, TH5) in the UE following the heavy rainfall in early June. The mean pH values ranged from 6.2 (TH5) to 8.4 (TH1) at the surface and generally decreased from the LE to the UE. However, pH values increased at the UE during high algal blooms when compared to the rest of estuary.

Chl-a concentrations ranged between 0.7 and 10.6 in the LE, 0.5 and 35.4 in the ME, 0.6 and 121.4 µg L\(^{-1}\) in the UE. Chl-a values increased considerably in spring and summer due to the increasing phytoplankton biomass. The highest chl-a value at the surface was measured as ~141 µg L\(^{-1}\) during the bloom of the euglenophycean Euglena viridis in July and as ~79 µg L\(^{-1}\) during the bloom of the cryptophycean Plagioselmis prolonga that occurred in May in the UE. The chl-a value decreased considerably in the whole study area in early June due to increased suspended particulate matter load from streams during heavy rainfall (Fig. 2).

**Total phytoplankton**

155 taxa to 9 classes were registered in 512 water samples and 64 net samples collected during the sampling period. Among these, 54% were diatoms, 35.5% dinoflagellates and the remaining 10.5% were marine phytoflagellates. The number of diatom species in fall, winter and spring was higher than that of dinoflagellates, while dinoflagellate species richness was slightly higher in summer. Total phytoplankton abundance (N) was low in winter, began to increase in April, remaining high till September, with blooms of different species. The diatom increase in April was followed by dinoflagellate and phytoflagellate increases in May, phytoflagellate and diatom increases in June and July, dinoflagellate increase in August and diatom and phytoflagellate increases in September (Fig. 3).

An N increase in November was due to a Skeletonema cf. marinoi bloom (~2×10\(^6\) cells L\(^{-1}\)). Pseudo-nitzschia spp. bloomed in diatom-dominated samples in January (2×10\(^6\) cells L\(^{-1}\)). In April and May, Skeletonema cf. marinoi (4.6×10\(^6\) cells L\(^{-1}\)) Pseudo-nitzschia spp. (2.8×10\(^6\) cells L\(^{-1}\)), cryptophyte Plagioselmis prolonga (7.8×10\(^6\) cells L\(^{-1}\)) and euglenophyte Euglena viridis (1.3×10\(^6\) cells L\(^{-1}\)) blooms increased N significantly. In June, Plagioselmis prolonga (7.5×10\(^6\) cells L\(^{-1}\)), Skeletonema cf. marinoi (3.8×10\(^6\) cells L\(^{-1}\))

![Fig. 3](http://epublishing.ekt.gr) **Fig. 3:** Spatial and temporal variations in phytoplankton community during the study period.
L⁻¹), prasinophyte Pyramimonas cf. grossii (1.2×10⁶ cells L⁻¹) and raphidophyte Heterosigma cf. akashiwo (1.4×10⁷ cells L⁻¹) blooms occurred successively. The blooms of Scrippsiella cf. trochoidea (2.3×10⁶ cells L⁻¹) in August, Thalassiosira sp. (1.6×10⁷ cells L⁻¹) and Heterosigma cf. akashiwo (1.6×10⁶ cells L⁻¹) in September once again resulted in an increase in N (Fig. 3).

### Potentially harmful and/or bloom-forming microalgae

A total of 23 potentially harmful and/or bloom-forming microalgae were detected in the GHE during the study period, of which 9 species have caused confirmed toxic effects around the world (Moestrup et al., 2009). Table 1 shows the check-list of the potentially harmful and bloom-forming species detected in the GHE, their harmful effects, seasonality (most abundant period), locality (most abundant part of estuary) and maximum cell density. The distribution patterns showed significant spatial and temporal differences in both abundance and species assemblages (Fig. 4). Most harmful microalgae were observed in spring and summer, particularly in the middle and upper estuary. The abundance and species numbers of harmful microalgae increased remarkably from the LE to the UE, particularly in summer.

Among diatoms, *Pseudo-nitzschia* species (*P. cf. delicatissima* and *P. cf. pungens*) were commonly observed in both water and net samples. Their density reached ~2×10⁶ cells L⁻¹ in January at TH1 and ~3×10⁶ cells L⁻¹ in February at TH2 and TH3. *Prorocentrum micans* Ehrenberg reached ~2×10³ cells L⁻¹ in August, *Gonyaulax* spp. (1.6×10⁶ cells L⁻¹) in September once again resulted in an increase in N (Fig. 3).

Table 1. List of potentially harmful and/or bloom-forming microalgae of the Golden Horn Estuary, indicating their harmful effect, seasonality, locality and maximum cell density.

<table>
<thead>
<tr>
<th>Species</th>
<th>Harmful effect</th>
<th>Seasonality</th>
<th>Locality</th>
<th>Max. density (×10⁶cells L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillariophyceae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudo-nitzschia cf. delicatissima (Cleve) Heiden</td>
<td>ASP⁴</td>
<td>winter-spring</td>
<td>LE,ME</td>
<td>2,030</td>
</tr>
<tr>
<td>Pseudo-nitzschia cf. pungens (Grunow ex Cleve) Hasle</td>
<td>ASP⁴</td>
<td>winter-spring</td>
<td>LE,ME</td>
<td>1,040</td>
</tr>
<tr>
<td>Skeletonema cf. marinai Sarno &amp; Zingone</td>
<td>Discoloration</td>
<td>April</td>
<td>ME,UE</td>
<td>54,000</td>
</tr>
<tr>
<td>Thalassiosira sp.</td>
<td>Discoloration</td>
<td>September</td>
<td>ME</td>
<td>15,600</td>
</tr>
<tr>
<td><em>Dinophyceae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinophysis acuminata Claparède &amp; Lachmann</td>
<td>DSP⁴</td>
<td>spring</td>
<td>LE</td>
<td>1.3</td>
</tr>
<tr>
<td>Dinophysis acuta Ehrenberg</td>
<td>DSP⁴</td>
<td>spring-autumn</td>
<td>LE,ME</td>
<td>2.6</td>
</tr>
<tr>
<td>Dinophysis caudata Saville-Kent</td>
<td>DSP⁴</td>
<td>autumn</td>
<td>LE</td>
<td>2.6</td>
</tr>
<tr>
<td>Dinophysis forii Pavillard*</td>
<td>DSP⁴</td>
<td>May</td>
<td>LE,ME</td>
<td>-</td>
</tr>
<tr>
<td>Gonyaulax fragilis (Schütt) Kofoid</td>
<td>Macilage formation⁵,⁶</td>
<td>December</td>
<td>ME</td>
<td>0.8</td>
</tr>
<tr>
<td>Heterocapsa triqueta (Ehrenberg) Stein*</td>
<td>Discoloration/Fish kills⁵</td>
<td>May</td>
<td>ME,UE</td>
<td>403.0</td>
</tr>
<tr>
<td>Lingulodinium polyedrum (Stein) Dodge</td>
<td>Homoyessotoxin producer⁵</td>
<td>spring</td>
<td>LE</td>
<td>-</td>
</tr>
<tr>
<td>Noctiluca scintillans (Macartney) Kofoid &amp; Swezy*</td>
<td>Ammonia irritation⁴</td>
<td>spring-summer</td>
<td>LE,ME</td>
<td>-</td>
</tr>
<tr>
<td>Phalacroma rotundatum (Clap. &amp; Lac.) Kofoid &amp; Michener</td>
<td>DSP⁴</td>
<td>common</td>
<td>LE,ME</td>
<td>1.3</td>
</tr>
<tr>
<td>Proorocentrum micans Ehrenberg</td>
<td>Fish kills⁵</td>
<td>September</td>
<td>ME</td>
<td>23.4</td>
</tr>
<tr>
<td>Proorocentrum cordatum (Pavillard) Schiller</td>
<td>Toxic to marine fauna⁵</td>
<td>July</td>
<td>ME,UE</td>
<td>187.0</td>
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<tr>
<td>Scrippsiella cf. trochoidea (Stein) Balech ex Loeblitch</td>
<td>Discoloration/Fish kills⁵</td>
<td>August</td>
<td>ME,UE</td>
<td>2,340</td>
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<tr>
<td>Tripos furca (Ehrenberg) Gómez</td>
<td>Fish kills⁵</td>
<td>June</td>
<td>LE,ME</td>
<td>5.2</td>
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<tr>
<td>Tripos fusus (Ehrenberg) Gómez</td>
<td>Fish kills⁵</td>
<td>September</td>
<td>LE,ME</td>
<td>13.0</td>
</tr>
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<td><em>Raphidophyceae</em></td>
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<tr>
<td>Heterosigma cf. akashiwo (Hada) Hada ex Harra &amp; Chihara</td>
<td>Discoloration/Fish kills⁵</td>
<td>June</td>
<td>ME,UE</td>
<td>13,900</td>
</tr>
<tr>
<td><em>Cryptophyceae</em></td>
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<tr>
<td>Phagodiniums longo (Butcher ex Novarino, Lucas &amp; Morrell</td>
<td>Discoloration</td>
<td>May</td>
<td>ME,UE</td>
<td>7,800</td>
</tr>
<tr>
<td><em>Prasinophyceae</em></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Pyramimonas cf. grossii Parke</td>
<td>Discoloration</td>
<td>June</td>
<td>ME,UE</td>
<td>1,600</td>
</tr>
<tr>
<td><em>Euglenophyceae</em></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euglena viridis (Müller) Ehrenberg</td>
<td>Discoloration</td>
<td>July</td>
<td>ME,UE</td>
<td>11,400</td>
</tr>
<tr>
<td>Eutreptiella marina da Cunha</td>
<td>Discoloration</td>
<td>July</td>
<td>ME,UE</td>
<td>3,400</td>
</tr>
</tbody>
</table>

**Abbreviations:** ASP: Amnesic Shellfish Poisoning; DSP: Diarrhetic Shellfish Poisoning; LE: Lower estuary; ME: Middle estuary; UE: Upper estuary. (†): Species detected only in net samples. References: ¹Moestrup et al., 2009; ²Pompei et al., 2003; ³Pistocchi et al., 2005; ⁴Tas, 2011; ⁵Lu & Hodgkiss, 2004; ⁶Hallegraeff, 2002; ⁷Heil et al. 2005.
cells L$^{-1}$ in May at TH2 (Fig. 4). *Pseudo-nitzschia* spp. were more abundant from January to June (9 to 16°C) in the LE and ME and densities decreased significantly towards the UE (Fig. 4). The abundance of *Pseudo-nitzschia* spp. was correlated with NO$_3$+NO$_2$ (Fig. 5).

The bloom-forming diatom *Skeletonema* cf. marinoi was commonly observed throughout the study area and formed a dense bloom ($5.4\times10^7$ cells L$^{-1}$) at TH2 in April (10.5 °C, 16.6 psu), causing water discoloration. Other blooms of *S. cf. marinoi* occurred in June ($3.7\times10^7$ cells L$^{-1}$) and July ($2.3\times10^7$ cells L$^{-1}$) at TH2 (Fig. 4). The abundance of *Skeletonema* cf. marinoi was correlated with salinity (Fig. 5).

Another diatom bloom caused by *Thalassiosira* sp. ($1.6\times10^7$ cells L$^{-1}$) occurred at TH2 in late September (21.9 °C, 17.7 psu). *Prorocentrum cordatum* was frequently detected in the middle estuary in July and reached an abundance of $9.1\times10^4$ cells L$^{-1}$ at TH2. *Prorocentrum cordatum* occurred more frequently at temperatures above 20°C (Fig. 4) and was related both to NH$_4$ as well as to temperature (Fig. 5). *Prorocentrum micans* was commonly observed during the summer and reached the highest density at TH2 in September ($23.4\times10^3$ cells L$^{-1}$, 21.8°C, 17.9 psu). *Prorocentrum micans* abundance decreased gradually from the lower to the upper estuary (Fig. 4).

*Scrippsiella* cf. trochoidea was one of the most important bloom-forming dinoflagellate species observed in the GHE and formed a dense bloom at TH4 in August ($2.3\times10^6$ cells L$^{-1}$, 28°C, 16.3 psu), causing an orange-brown water discoloration in the upper estuary (Figs 1, 4). The abundance of *Scrippsiella* cf. *trochoidea* was related to salinity and PO$_4$ (Fig. 5).

*Heterocapsa triquetra* was frequently observed from April to July and the highest density was detected in May at
TH5 (4.0×10⁵ cells L⁻¹, 18.7°C, 16.9 psu) (Fig. 4). Tripos furca and T. fusus were commonly found throughout the study period and were more frequently observed in the LE and ME (Fig. 4). The highest cell densities of Tripos furca (5.2×10³ cells L⁻¹) and T. fusus (1.3×10⁴ cells L⁻¹) were detected at TH1 in June and September and they were positively correlated with Secchi depth (Fig. 5). The raphidophyte Heterosigma cf. akashiwo was first observed in June and formed a dense bloom at TH2 (1.4×10⁷ cells L⁻¹), causing water discoloration (Figs. 1c and 4). A second bloom of Heterosigma cf. akashiwo was detected in September, when cell density was lower than the first bloom (1.6×10⁷ cells L⁻¹) at TH4 (Table 2). The abundance pattern of the species was highly related to temperature (Fig. 5). The cryptophycean Plagioselmis prolonga formed a dense bloom (7.8×10⁶ cells L⁻¹) in May at TH3, causing a greenish-brown water discoloration (Fig. 3). Plagioselmis prolonga was frequently observed in spring throughout the estuary (Fig. 4) and abundance was significantly correlated with DO (Fig. 5). The prasinophycean Pyramimonas cf. grossii was frequently observed in June particularly in the LE and ME, and its highest density reached 1.6×10⁷ cells L⁻¹ at TH3, causing water discoloration (Fig. 4). The euglenophycean Euglena viridis and Eutreptiella marina were frequently observed in the UE in spring and summer. A dense bloom of Euglena viridis (1.1×10⁶ cells L⁻¹) caused a green discoloration in surface in July at TH5 (Figs. 1, 4). The abundance of both species was correlated with temperature (Fig. 5).

CCA provided a clear ordination of species’ distribution according to environmental parameters (Fig. 5A). First canonical axis explained the 35.6% of cumulative percentage variance of species-environment relation, while second axis explained the 25.6%. The highest separation of species was along temperature, Secchi depth and DO. The number of harmful species increased with increasing Secchi depth. Although nutrients appeared to play a minimal role in the distribution patterns of most species, Prorocentrum cordatum was highly associated with NH₄-N and Pseudo-nitzschia spp. was associated with NO₂⁻N availability. Euglenoids and raphidophyte Heterosigma cf. akashiwo were significantly associated with temperature. Projection of stations and environmental variables provided an upper to lower estuary separation of stations in the ordination plane along a diagonal transects (Fig. 5B). The variability was particularly structured along Secchi depth and temperature.

Discussion

Precipitation, which increases terrestrial runoffs, has appeared as the most important factor affecting nutrient concentrations in the GHE in recent decades (Sur et al., 2002). High nutrient concentrations in the upper estuary during rainy periods indicated that this area has an even higher risk of pollution compared to the rest of the estuary. Inorganic nutrient concentrations decreased greatly in spring in parallel to increasing phytoplankton biomass. Significant deviations from the Redfield ratio indicated that this area had a highly variable concentration of nitrogen and phosphorus. A recent study clearly highlights the variations of Redfield ratios as associated with anthropogenic nutrient, yet it questions the role of this ratio in the occurrence of harmful algal blooms (HABs) (Davidson et al., 2014). Dissolved silicate concentrations were generally high and adequate.
for supporting the diatom proliferation. The oversaturated DO and very high chl-a measured in spring and summer confirms the high abundance of phytoplankton in this estuary. Physical and chemical features such as a more stagnant body of water due to insufficient water circulation and high nutrient concentrations of the ME and UE, support and enhance phytoplankton growth, increasing the potential risk of HABs.

During monthly samplings in 1998-2002, 17 potentially harmful and/or bloom-forming species were reported by Tas et al. (2009), while 23 potentially harmful and/or bloom-forming species in the total phytoplankton community were observed in this study period. The high nutrient concentrations originating from the upper estuary supported a diverse harmful algal species in the LE together with a better mixed upper layer due to Strait’s currents and increased light penetration. Considering the seasonality of different harmful species, it appears that spring and summer poses a higher risk for the occurrence of harmful conditions, as reported in previous studies at the region (Tas et al., 2009) and elsewhere (Zingone et al., 2006). Moreover, water discolorations caused by mostly different dinoflagellates and phytoflagellates, were observed in spring and summer depending on the water column stability. The dinoflagellates Prorocentrum cordatum, Heterocapsa triquetra, Scrippsiella cf. trochoidea, the phytoflagellates Heterosigma cf. akashiwo, Plagioselmis prolonga, Pyrannimonas cf. grossii and euglenoids displayed a seasonal pattern, while no seasonality was evident in the distribution of Prorocentrum micans, Tripos spp., Dinophysis spp., Pseudo-nitzschia spp. and Skeletonema cf. marinoi.

Diatoms, the primary group in the study area, were more abundant than other groups of phytoplankton in the lower and middle part of the estuary. Dinoflagellates showed a wider distribution throughout the study area than diatoms. The third group, including all phytoflagellates, played an important role particularly in the upper part of the estuary, as reported in an earlier work (Tas et al., 2009). A wide distribution and high abundances of mixotrophic flagellates in the upper estuary indicates the suitability of trophic conditions for mixotrophy.

Blooms of Pseudo-nitzschia spp. (P. cf. delicatissima and P. cf. pungens) are generally reported to occur at low temperatures in early spring (<18°C) (Türköğlu & Koray, 2002; Lundholm et al., 2005; Liefers et al., 2009). In the present study, Pseudo-nitzschia species were observed to be more abundant in the lower and middle estuary in January and May, but they were also observed in June and August. This indicates that, although Pseudo-nitzschia species are found to be more abundant at low temperatures, they might adapt a wide temperature range and there are likely to be different species blooming in different seasons, as observed in the Gulf of Naples (Ruggiero et al., 2015). Pseudo-nitzschia species were also common in the Black Sea coast and numbers surpass 1.0×10^7 cells L^-1 in spring in Sevastopol Bay (Ryabushko et al., 2000) and near the Bulgarian coast (Ryabushko, 1991). Domoic acid was isolated from P. calliantha at Sevastopol Bay (Besiktepe et al., 2008). Further research on identification and toxicity of Pseudo-nitzschia species in the GHE is necessary in order to better understand their potentially harmful effect.

Mixotrophic Dinophysis species were observed in the LE throughout the study period. Their abundances were similar to those reported in a previous study carried out in the GHE (Tas et al., 2009). Blooms of these dinoflagellates were reported from many coastal areas and bays worldwide (Gisselson et al., 2002; Koukaras & Nikolaidis, 2004; Campbell et al., 2008). Some species of Dinophysis were commonly observed throughout the year in a coastal embayment of the Sea of Marmara (Balkis, 2003). Considering that the abundance levels are close to 1000 cells L^-1, the species should be carefully monitored in this area, as it has been reported that even these low abundance levels of Dinophysis species are sufficient to toxify mussels (Hoppenrath et al., 2009).

Proorocentrum cordatum formed dense blooms in the GHE in the last decade. An abundance peak of Prorocentrum cordatum was observed in July, in agreement with what was reported in an earlier work (as P. minimum, Taş & Okuş, 2011). However no high density blooms of this species were encountered in this study period. Proorocentrum cordatum is reported to be potentially harmful to humans via shellfish poisoning (Tangen, 1983; Taylor et al., 2003; Heil et al., 2005), although toxins have not been isolated. The species also has toxic effects on molluscs (Moestrup et al., 2009) and may cause fish kill (Hallegraeff, 2002; Lu & Hodgkiss, 2004). Proorocentrum micans blooms, reported to cause fish mortality due to anoxia (Koray, 2004; Lu & Hodgkiss, 2004), are commonly described in the Turkish seas (Balkis, 2003; Aktan et al., 2005; Deniz & Taş, 2009; Tas et al., 2011) where they can form high biomass blooms producing discolorations (reddish brown) (Eker & Kideç, 2000). Proorocentrum micans was rarely found before the rehabilitation of GHE (Taş & Okuş, 2003). In the post-rehabilitation era the species has been frequently observed, with abundance surpassing 5×10^7 cells L^-1 (Tas et al., 2009). Proorocentrum spp. were commonly observed at the eastern part of İzmit Bay, Sea of Marmara, and formed dense blooms in summer (Taş & Okuş, 2004; Aktan et al., 2005) and in Sinop Bay, Black Sea, in mid-August (Türköğlu & Koray, 2002). This study’s observation of a higher density of P. micans compared to that of past studies should be considered for future research.

Scrippsiella cf. trochoidea, a species causing fish kill through anoxia (Hallegraeff, 2002; Lu & Hodgkiss, 2004), has been commonly observed in the Turkish seas (Koray, 2004; Feyzioğlu & Ögüt, 2006; Balkis, 2003). Scrippsiella abundance increased considerably in Sinop Bay (Black Sea) in June (Türköğlu & Koray, 2002). A dense bloom of Scrippsiella cf. trochoidea with brownish-red discoloration has been also observed in Sürmene Bay, Black Sea (Feyzioğlu & Ögüt, 2006). Scrippsiella...
Heterocapsa triquetra is known to cause red tides in estuaries and coastal areas worldwide (Kim et al., 1990; Litaker et al., 2002), resulting in fish kill (Lu & Hodgkiss, 2004). Although Heterocapsa triquetra abundance was low (<5000 cells L⁻¹) before the rehabilitation of the GHE (Tas et al., 2009), during the post-rehabilitation period, cell densities increased significantly. Heterocapsa triquetra formed a prolonged and dense bloom (<1.5×10⁷ cells L⁻¹) in April 2007, causing an orange-brown water discoloration in the upper estuary (Tas, 2011). In this study Heterocapsa triquetra density also increased in the upper estuary in May. Heterocapsa triquetra red tides are considered to be an indicator of eutrophication (Lindholm & Nummelin, 1999), the phagotrophic feeding of this species providing an advantage over other species (Legrand et al., 1998). High amounts of particulate organic substances based on high TOC concentration in the GHE may favor the blooms of *H. triquetra*.

Bloom of Heterosigma cf. akashiwo in coastal and brackish waters worldwide may be both toxic and harmful for fish (Hallegraeff, 2002; Hallegraeff et al., 2003). *Heterosigma akashiwo* has been reported from the eutrophic waters of Izmir Bay, Aegean Sea (Bizsel & Bizsel, 2002; Koray, 2004). The environmental conditions and the species’ ability to form cysts play an important role in the population increase (Rensel, 2007). The first occurrence of *Heterosigma cf. akashiwo* in the Sea of Marmara was recorded in 2000 (Deniz & Tas, 2009). In this study, the species formed two blooms in the GHE in June and September.

Euglenophyceans were commonly observed in Turkish seas (Balkıs, 2003; Koray, 2004; Feyzioğlu & Öğüt, 2006). Some of them formed a very dense bloom on the eastern Black Sea coasts of Turkey in spring (Feyzioğlu & Öğüt, 2006). In the GHE, euglenophyceans were frequently observed and formed dense blooms causing dark green water discoloration in the upper estuary from May to August, similar to what was observed in previous studies (Tas et al., 2009). These microalgae have the potential to grow rapidly in the presence of high nutrient concentrations (Olli et al., 1996). High nutrient concentrations, high temperatures (>20°C) and moderate salinity appeared to provide optimum conditions for the blooms of euglenophyceans in the upper estuary. The species also benefited from particulate organic matter loads based on high TOC concentration in the upper estuary through their phagocytosis capability. The cryptophycean *Plagiocoleus prolunga* and the prasinophycean *Pyramimonas cf. grossii* formed dense blooms in the upper estuary in summer, causing green to greenish-brown water discolorations.

During this study nine species formed successive and dense blooms in the middle and the upper sections of the GHE in late spring and summer. Although neither fish-kill events nor human health problems were witnessed during these blooms, anoxia and light attenuation due to discoloration was observed. More severe effects might be observed in the future since the GHE is a potential risk area for future HABs with increase in the number of potentially harmful species and magnitude of blooms in response to rapidly changing environmental conditions.

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