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Potentially harmful microalgae and algal blooms in a eutrophic estuary in the Sea of Marmara (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 (Smayda, 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). Climate 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. The GHE eventually became a severely polluted estuary, where heavy metal concentrations were very high in the sediment (Tuncer *et al*., 2001) and water column was characterized by frequent anoxia episodes (Kıratlı & Balkıs, 2001). 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

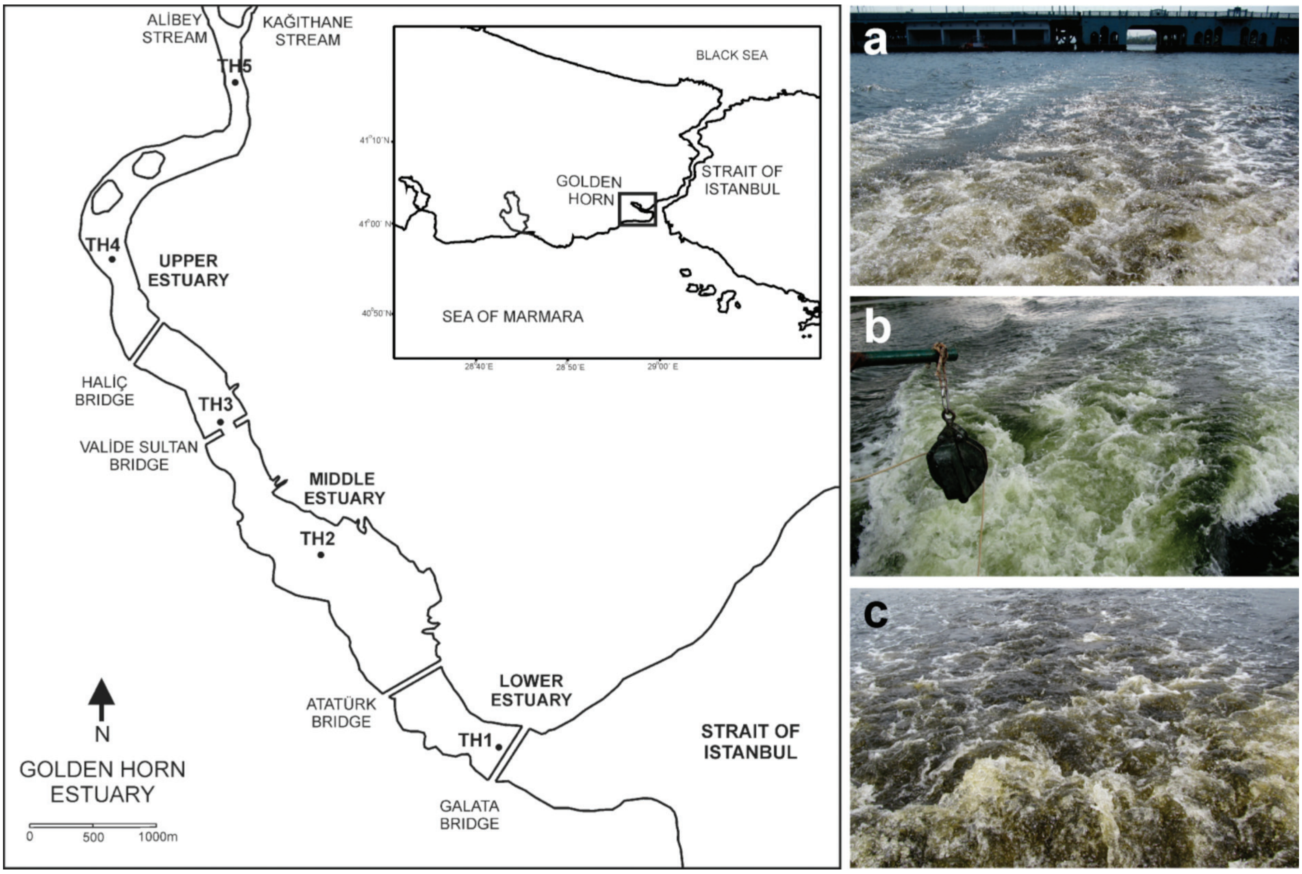

***Fig. 1*:** Location of sampling stations and photos of water discolorations during bloom events: (a) *Scrippsiella* cf. *trochoidea* (August 2010); (b) *Euglena viridis* (July .2010; (c) *Heterosigma* cf. *akashiwo* (June 2010).

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*) (5×10^6 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 (7×10^7 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* (8.12×10^6 cells L^{-1}) in March 2001 and *Thalassiosira allenii* (4×10^6 cells L^{-1}) in June 2001. In July 2001, a dense *P. cordatum* bloom was recorded in the upper estuary (>3.6×10^7 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 5m 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_x) and total nitrogen (TN) were measured according to the cadmium colon reduction method, ammonia to the Berthelot reaction method, ortho-phosphate to the ammonium molibdate 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%) (Throndsen, 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.

Cupp (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 Gómez (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

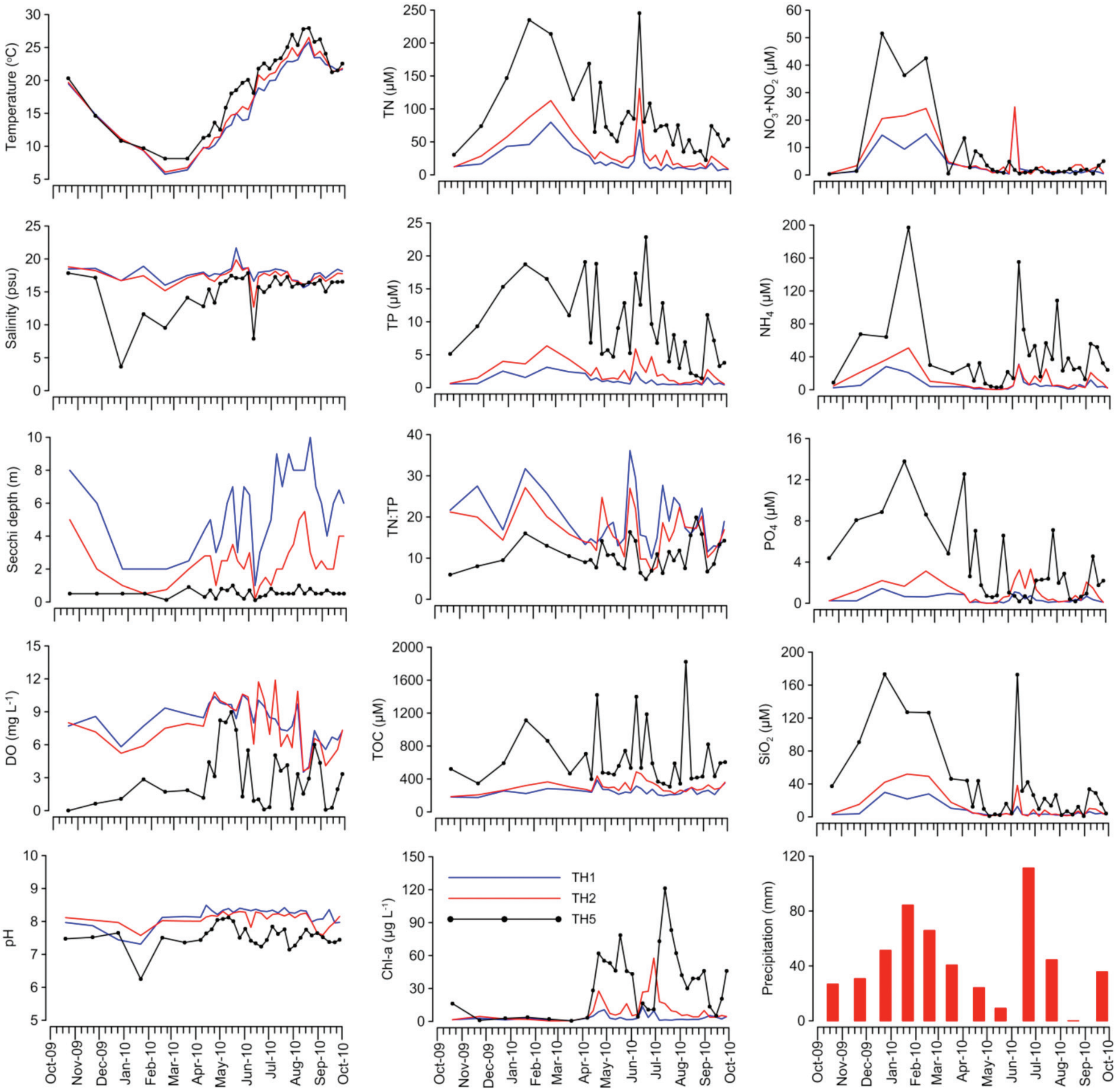

***Fig. 2*: Fluctuations in physical and chemical parameters. Precipitation data is monthly total rainfall for Istanbul.**

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).

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 belonging 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^{7} 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^{7} cells

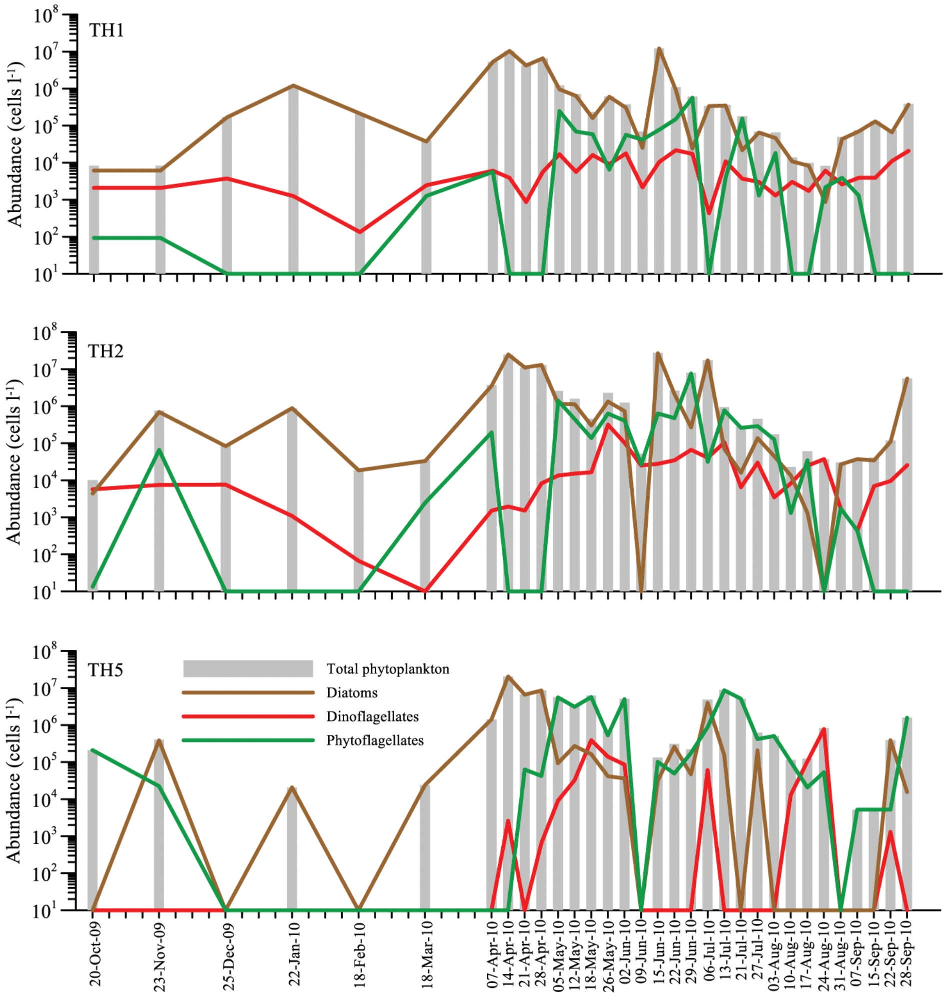

***Fig. 3*:** Spatial and temporal variations in phytoplankton community during the study period.

L^{-1}), prasinophyte *Pyramimonas* cf. *grossii* (1.2×10^6 cells L^{-1}) and raphidophyte *Heterosigma* cf. *akashiwo* (1.4×10^7 cells L^{-1}) blooms occurred successively. The blooms of *Scrippsiella* cf. *trochoidea* (2.3×10^6 cells L^{-1}) in August, *Thalassiosira* sp. (1.6×10^7 cells L^{-1}) and *Heterosigma* cf. *akashiwo* (1.6×10^6 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 ~2×10^6 cells L^{-1} in January at TH1 and ~3×10^6

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.

Species	Harmful effect	Seasonality	Locality	Max.density (×10^{3}cells L^{-1})
Bacillariophyceae				
Pseudo-nitzschia cf. *delicatissima* (Cleve) Heiden	ASP[1]	winter-spring	LE,ME	2,030
Pseudo-nitzschia cf. *pungens* (Grunow ex Cleve) Hasle	ASP[1]	winter-spring	LE,ME	1,040
Skeletonema cf. *marinoi* Sarno & Zingone	Discoloration	April	ME,UE	54,000
Thalassiosira sp.	Discoloration	September	ME	15,600
Dinophyceae				
Dinophysis acuminata Claparède & Lachmann	DSP[1]	spring	LE	1.3
Dinophysis acuta Ehrenberg	DSP[1]	spring-autumn	LE,ME	2.6
Dinophysis caudata Saville-Kent	DSP[1]	autumn	LE	2.6
Dinophysis fortii Pavillard*	DSP[1]	May	LE,ME	-
Gonyaulax fragilis (Schütt) Kofoid	Mucilage formation[2,3]	December	ME	0.8
Heterocapsa triquetra (Ehrenberg) Stein*	Discoloration/ Fish kills[4,5]	May	ME,UE	403.0
Lingulodinium polyedrum (Stein) Dodge	Homoyessotoxin producer[6]	spring	LE	-
Noctiluca scintillans (Macartney) Kofoid & Swezy*	Ammonia iritation[6]	spring-summer	LE,ME	-
Phalacroma rotundatum (Clap. & Lac.) Kofoid & Michener	DSP[1]	common	LE,ME	1.3
Prorocentrum micans Ehrenberg	Fish kills[5]	September	ME	23.4
Prorocentrum cordatum (Pavillard) Schiller	Toxic to marine fauna[7]	July	ME,UE	187.0
Scrippsiella cf. *trochoidea* (Stein) Balech ex Loeblich	Discoloration/ Fish kills[5,6]	August	ME,UE	2,340
Tripos furca (Ehrenberg) Gómez	Fish kills[5]	June	LE,ME	5.2
Tripos fusus (Ehrenberg) Gómez	Fish kills[5]	September	LE,ME	13.0
Raphidophyceae				
Heterosigma cf. *akashiwo* (Hada) Hada ex Hara & Chihara	Discoloration/ Fish kills[1,7]	June	ME,UE	13,900
Cryptophyceae				
Plagioselmis prolonga Butcher ex Novarino, Lucas & Morrall	Discoloration	May	ME,UE	7,800
Prasinophyceae				
Pyramimonas cf. *grossii* Parke	Discoloration	June	ME,UE	1,600
Euglenophyceae				
Euglena viridis (Müller) Ehrenberg	Discoloration	July	ME,UE	11,400
Eutreptiella marina da Cunha	Discoloration	July	ME,UE	3,400

Abbreviations: ASP: Amnesic Shellfish Poisoning; DSP: Diarrethic Shellfish Poisoning; LE: Lower estuary; ME: Middle estuary; UE: Upper estuary. (*): Species detected only in net samples. References: [1]Moestrup *et al.*, 2009; [2]Pompei *et al.*, 2003; [3]Pistocchi *et al.*, 2005; [4]Tas, 2011; [5]Lu & Hodgkiss, 2004; [6]Hallegraeff, 2002; [7]Heil *et al.* 2005..

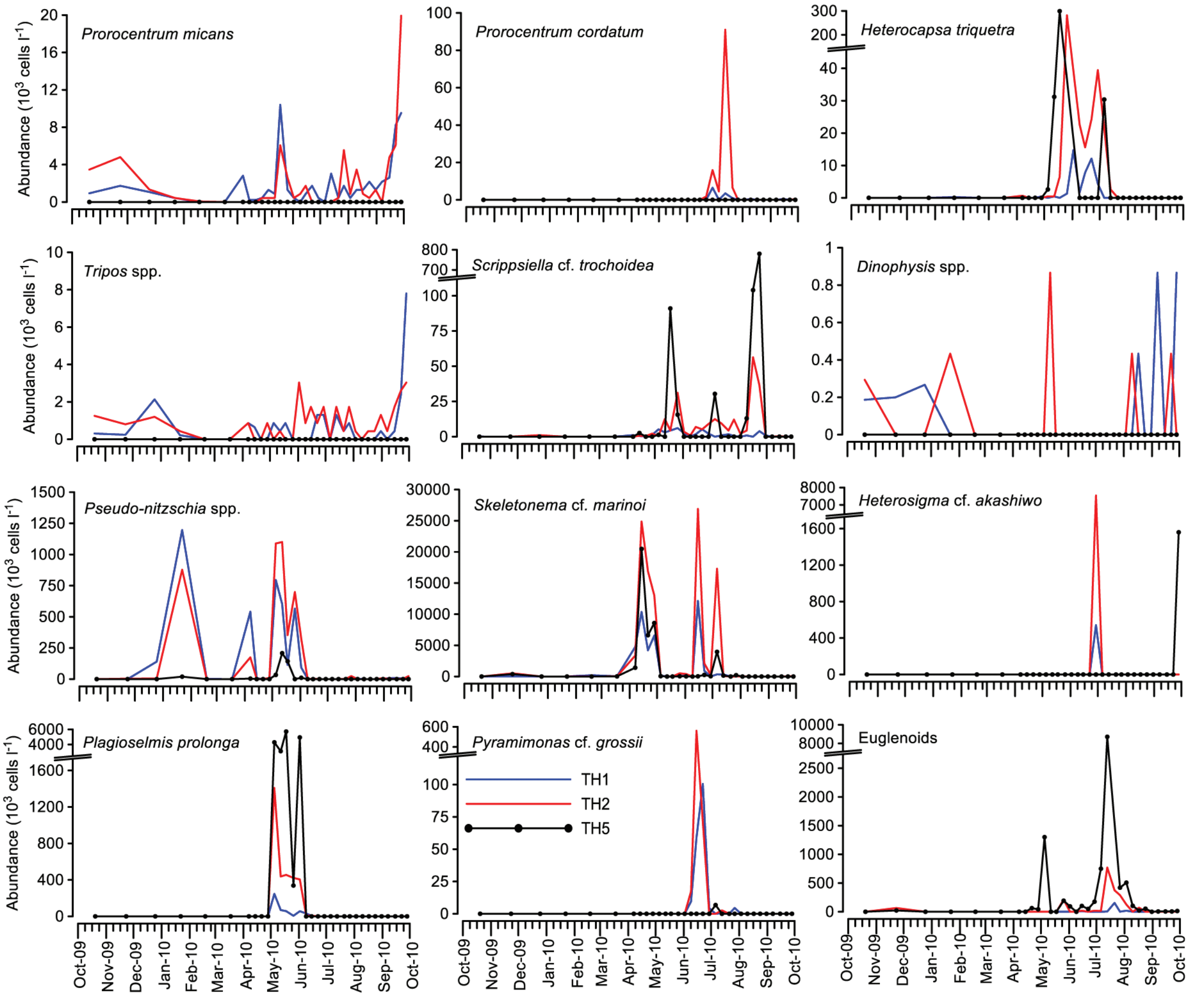

***Fig. 4*:** Temporal and spatial distribution of the potentially harmful and bloom-forming microalgal species.

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 (5.4×10^{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 (3.7×10^{7} cells L^{-1}) and July (2.3×10^{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. (1.6×10^{7} cells L^{-1}) occurred at TH2 in late September (21.9 °C, 17.7 psu).

A total of 14 potentially harmful dinoflagellates were detected in the GHE. Of these, *Dinophysis fortii, Lingulodinium polyedrum* and *Noctiluca scintillans* were only observed in net samples in the middle estuary. The potentially toxic dinoflagellates *Dinophysis acuminata, D. acuta, D. caudata, D. fortii* and *Phalacroma rotundatum* were observed in the lower and middle estuary. Abundances of *Dinophysis* species were generally low (<10^{3} cells L^{-1}). The highest *Dinophysis acuta* cell density was observed in May (8.7×10^{2} cells L^{-1}, TH2), while *D. caudata* abundance reached the maximum in September (8.7×10^{2} cells L^{-1}, TH1) (Fig. 4). *Dinophysis caudata* was related to SiO_2 and Secchi depth (Fig. 5).

Prorocentrum cordatum was frequently detected in the middle estuary in July and reached an abundance of 9.1×10^{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 (23.4×10^{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 (2.3×10^{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

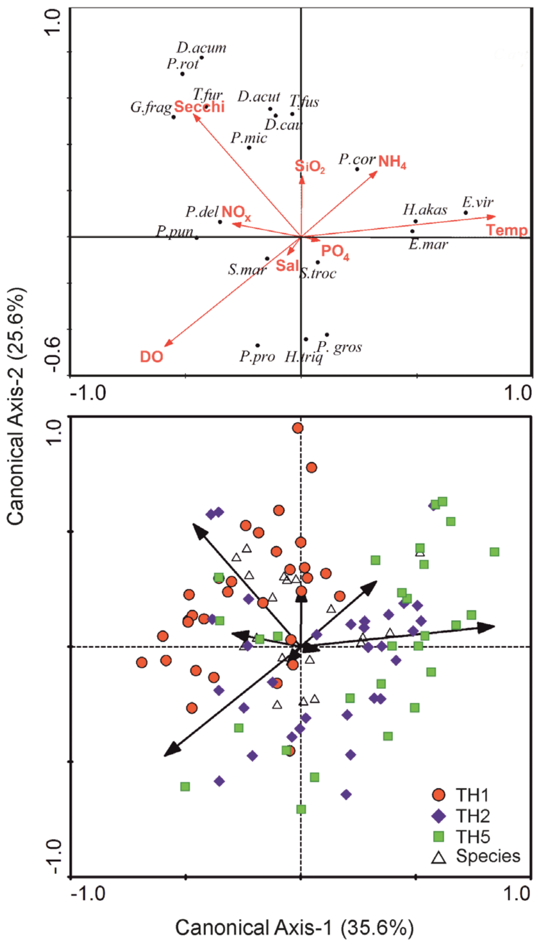

Fig. 5: Canonical correspondence analysis ordination of potentially harmful phytoplankton species and environmental factors (A) Projection of species and environmental factors; (B) Projection of samples, species and environmental factors. Percentages in parenthesis are the cumulative percentage variance of species-environment relation explained by the axis.

Abbreviations: *D.acum= Dinophysis acuminata*; *D.acut= Dinophysis acuta*; *D.cau= Dinophysis caudata; G.frag= Gonyaulax fragilis; H.triq= Heterocapsa triquetra; T.fur= Tripos furca; T.fus= Tripos fusus; P.rot= Phalacroma rotundatum*; *P.mic= Prorocentrum micans; P.cor= P. cordatum; S.troc= Scrippsiella* cf. *trochoidea; P.del= Pseudo-nitzschia* cf. *delicatissima; P.pun= Pseudo-nitzschia* cf. *pungens; S.mar= Skeletonema* cf. *marinoi; H.akas= Heterosigma* cf. *akashiwo; P.pro= Plagioselmis prolonga*; *P.gros= Pyramimonas* cf. *grossii; E.vir= Euglena viridis; E.mar= Eutreptiella marina;* Temp= temperature; Sal= salinity.

TH5 (4.0×10^5 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* (5.2×10^3 cells L^{-1}) and *T. fusus* (1.3×10^4 cells L^{-1}) were detected at TH1 in June and September and they were positively correlated with Secchi depth (Fig. 5) The raphidophycean *Heterosigma* cf. *akashiwo* was first observed in June and formed a dense bloom at TH2 (1.4×10^7 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 (1.6×10^6 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 (7.8×10^6 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 1.6×10^6 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* (1.1×10^7 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 NH_4-N and *Pseudo-nitzschia* spp. was associated with NO_3+NO_2-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, Pyramimonas* 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) (Türkoğlu & Koray, 2002; Lundholm *et al*., 2005; Liefer *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 1.0×10^6 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 (Balkıs, 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).

Prorocentrum 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. *Prorocentrum 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). *Prorocentrum micans* blooms, reported to cause fish mortality due to anoxia (Koray, 2004; Lu & Hodgkiss, 2004), are commonly described in the Turkish seas (Balkıs, 2003; Aktan *et al*., 2005; Deniz & Taş, 2009; Tas *et al*., 2011) where they can form high biomass blooms producing discolorations (reddish brown) (Eker & Kıdeyş, 2000). *Prorocentrum 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 5×10^3 cells L^{-1} (Tas *et al*., 2009). *Prorocentrum* 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 (Türkoğlu & 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; Balkıs, 2003). *Scrippsiella* abundance increased considerably in Sinop Bay (Black Sea) in June (Türkoğlu & 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* cf. *trochoidea* was not detected before the rehabilitation of the GHE, probably due to extreme pollution (Taş & Okuş, 2003), but following the rehabilitation the species was commonly observed in summer months, with bloom in August (Tas *et al*., 2009). Similarly, *Scrippsiella* cf. *trochoidea* formed a bloom in the upper estuary in August in this study period.

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 ($<1.9\times10^7$ 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*.

Blooms 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 İzmir 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 & Taş, 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 & Ögüt, 2006). Some of them formed a very dense bloom on the eastern Black Sea coasts of Turkey in spring (Feyzioğlu & Ögüt, 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 *Plagioselmis prolonga* 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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