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## Temporal variations in phytoplankton composition in the north-eastern Sea of Marmara: potentially toxic species and mucilage event

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### Abstract

Temporal variations in phytoplankton composition in the northeastern Sea of Marmara were investigated in conjunction with physico-chemical variables, from January 2004 to December 2007. The occurrence of potentially toxic species and a mucilage event was also evaluated during the study period. The confined upper layer of the Sea of Marmara is mesotrophic to eutrophic and is characterised by higher productivity compared to the neighbouring Black Sea and Aegean Sea. 132 taxa were identified in the micro-phytoplankton community, 11 of which are known to be potentially toxic. The most abundant species were the diatom *Pseudo-nitzschia* spp. and the dinoflagellate *Prorocentrum micans*. Potentially toxic species were more common at the coastal stations. The onset of a mucilage formation was observed in October 2007, and well-known mucilage producers such as *Gonyaulax hyalina* (reported as *G. fragilis*) and *Thalassiosira gravida* (reported as *T. rotula*) dominated the phytoplankton community during this event. A marked decrease in the number of species and the diversity index after June 2007, and the reported shifts in the zooplankton community during the same period point to possible cascading effects in the pelagic ecosystem of the Sea of Marmara.

**Keywords:** Phytoplankton; harmful species; mucilage; biodiversity; Sea of Marmara.

### Introduction

Phytoplankton plays an important role in the aquatic food chain and is an efficient and easily detectable indicator of ecological change. The information obtained from phytoplankton communities is very useful for the assessment of eutrophication levels and ecosystem changes due to several stressors (e.g. pollution, overfishing, climate change, invasive species) in aquatic systems, since they are very sensitive to changes in the ecosystem (Paerl *et al.*, 2007). Under certain circumstances, some microalgae species can form high biomass and/or toxic cell proliferations, thereby causing harmful effects at various trophic levels of aquatic ecosystems (Kudela *et al.*, 2015). The harmful effects of different types of phytoplankton toxins on fish, invertebrates and humans as well as hypoxia caused by decomposing algal biomass are among the most significant problems associated with algal blooms (Anderson *et al.*, 2012). An increase in the frequency and magnitude of algal blooms in parallel to ongoing climate change might enhance HAB related impacts on environment and public health (Hallegraeff, 2010).

The Sea of Marmara is a small, semi-enclosed basin (11,350 km<sup>2</sup>), connected to the Black Sea and the Aegean Sea through the Strait of Istanbul (Bosphorus)

and Canakkale (Dardanelles). The Sea of Marmara has two distinctly different water masses; the upper layer (0-25m) is brackish (~22 salinity) and originates from the Black Sea, while the lower saline (~38 salinity) layer originates from the Mediterranean Sea. These two distinctly different water masses are separated by an interface layer (Unluata *et al.*, 1990; Besiktepe *et al.*, 1994). The hydrography of the upper layer is strongly associated with the Black Sea inflow and significantly affects the chemistry of the basin (Polat & Tuğrul, 1995). In the upper euphotic zone, nutrient concentrations are relatively low with seasonal variations that reflect photosynthetic activity (Baştürk *et al.*, 1990). Primary production is always higher in the less saline upper layer, while the lower layer waters are always rich in nutrients as a result of the limitation of the euphotic zone by the intermediate layer (Polat *et al.*, 1998).

The first study on phytoplankton in the Sea of Marmara (SoM) was carried out by Artüz (1974), and studies focusing on the distribution, taxonomy, morphology and ecology of phytoplankton in the SoM increased after 2000 (Uysal, 1996; Balkis, 2004; Balkis *et al.*, 2004; Okus & Tas, 2007; Deniz & Tas, 2009; Tas *et al.*, 2011; Balkis & Toklu-Alicli, 2014; Balkis & Tas, 2016; Balci & Balkis, 2017).

A peculiar impact of some phytoplankton blooms that evolved under special environmental and trophic conditions is secretion of a vast quantity of extracellular organic substances causing mucilage (Innamorati *et al.*, 2001; Mecozzi *et al.*, 2001). These extracellular organic substances can be produced by diatoms (Rinaldi *et al.*, 1995), bacteria (Herndl *et al.*, 1999; Azam & Long, 2001), and dinoflagellates (MacKenzie *et al.*, 2002). In the autumn of 2007, a very dense mucilage event was recorded in the Sea of Marmara that caused significant economic and environmental impacts (Aktan *et al.*, 2008; Tüfekci *et al.*, 2010; Balkis *et al.*, 2011; Yilmaz, 2015).

The main goals of this study are to investigate phytoplankton community composition associated with physico-chemical factors over four years, and to evaluate the occurrence of potentially toxic species and the mucilage event recorded in the area.

## Materials and Methods

### Sampling strategy and seawater analysis

Water Sampling was carried out monthly at three stations (MY2, MKC, and MBC), and seasonally at two stations (M8, M23) in the northeastern SoM. The sampling stations were selected in view of monitoring the impact of urban discharges and the effect of inflowing Black Sea water on the SoM. The MY2, MKC, MBC and M8 stations are close to the coast and have a maximum depth of 80 m, while station M23 is located offshore, at a depth of 1100 m (Fig. 1). The sampling program covers a period of four years (from January 2004 to December 2007). No sampling was performed in the study area in July 2007.

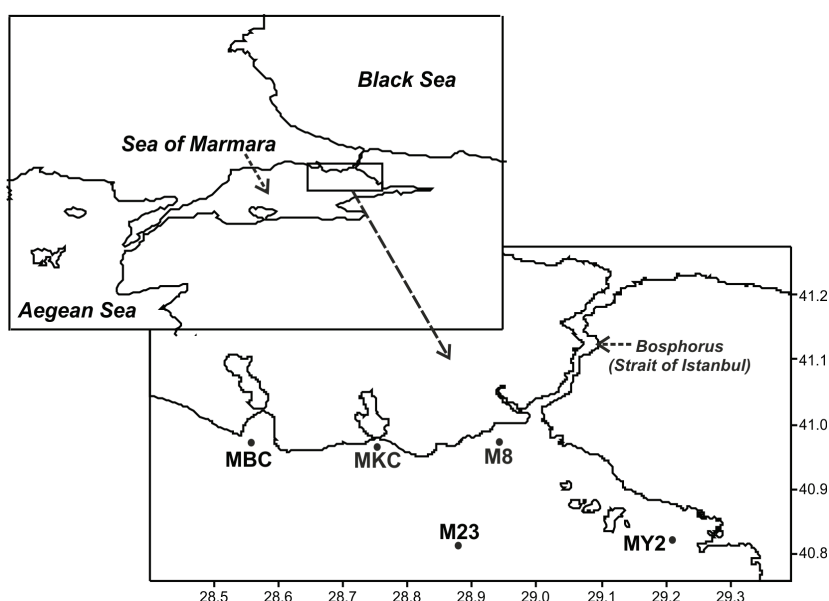
Water samples were collected using rosette operated 5-L Niskin bottles at depths of 0.5, 5 and 10 m. Temperature and salinity profiles were recorded by a Sea Bird Electronics 9/11 CTD system. Nutrient analyses ( $\text{NO}_3^- + \text{NO}_2^-$ ,  $\text{PO}_4$  and  $\text{SiO}_2$ ) were performed on a Bran+Lue-

be AA3 auto-analyzer according to standard methods (APHA, 1999). Chlorophyll *a* (Chl-*a*) analyses were carried out according to the acetone extraction method (Parsons *et al.*, 1984) and dissolved oxygen (DO) was measured according to the Winkler titration method (APHA, 1999). All environmental data were averaged over the three depths sampled.

### Phytoplankton analysis

In this study, only 20-200  $\mu\text{m}$  (microplankton) phytoplankton cells were examined. For quantitative analysis of phytoplankton, the sea water samples obtained from 0.5, 5 and 10 m were transferred to 1-L containers and then immediately fixed by the addition of a borax-buffered formaldehyde solution (0.4 %). The samples were allowed to settle for a week in the laboratory and then the upper water in the containers was removed with a 55- $\mu\text{m}$  mesh mounted pipette (Sukhanova, 1978). After that, the sub-samples were stored in darkcoloured glass bottles after the addition of 2 ml formaldehyde for longtime storage (Thronsen, 1978). Cell counts were performed using an Olympus CH-2 model light microscope combined with a Sedgewick-Rafter counting cell (1mL). All phytoplankton abundance data were averaged over the three depths sampled.

For qualitative analysis of phytoplankton, a Nansen plankton net (0.57 m in diameter, 55  $\mu\text{m}$  mesh size) was used. Net samples were collected by vertical tows, from 15 m to the surface, performed at three stations (MKC, M8, and M23). Then they were transferred to PVC containers and preserved by the addition of borax-buffered formaldehyde to a final concentration of 4 %. Subsequently, the net samples were stored in a dark and cool room until microscope examination. Phytoplankton species collected with net samples were identified under an Olympus CH-2 model light microscope. The light micrography of some important species was taken using a



**Fig. 1:** Study area and sampling stations.

digital camera system (Leica DFC camera system).

Available resources were used for species identification (Cupp, 1943; Hendey, 1964; Drebes, 1974; Dodge, 1985; Delgado & Fortuna, 1991; Tomas, 1997). Potentially toxic and/or harmful microalgae were determined according to Hallegraeff (2002), Hallegraeff *et al.* (2003), Pompei *et al.* (2003), Lu & Hodgkiss (2004), Pistocchi *et al.* (2005), and Heil *et al.* (2005), and toxic species were checked through the IOC Taxonomic Reference List of Toxic Plankton Algae (Moestrup *et al.*, 2009). The AlgaeBase (<https://www.algaebase.org/>) and WoRMs (World Register of Marine Species) database (<http://www.marinespecies.org>) were used for checking nomenclature and synonyms.

### Data analysis

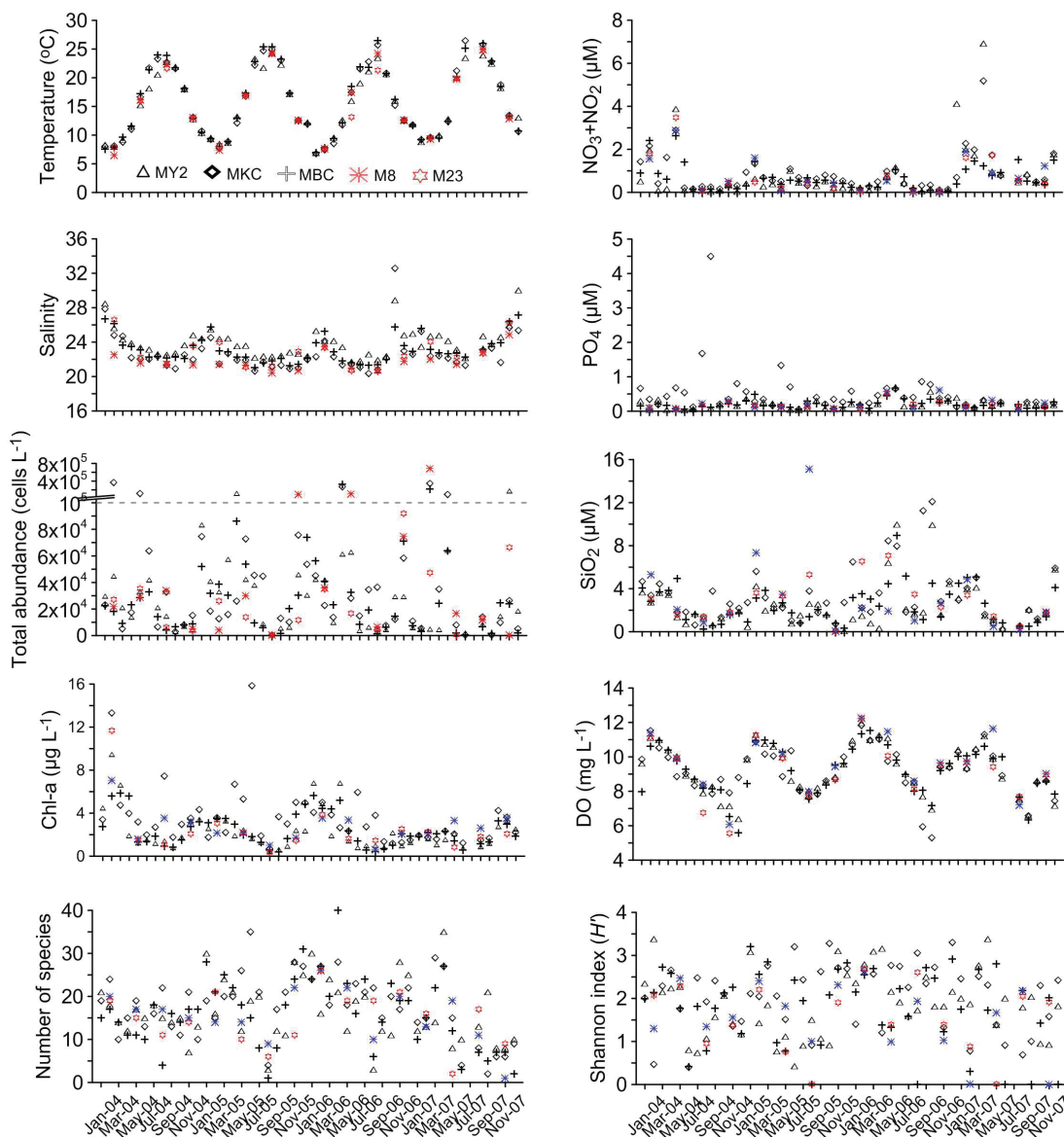
The relationships between environmental factors and phytoplankton abundance ( $N$ ), number of species ( $S$ ), Shannon diversity index ( $H'$ , bits) and Chl-*a* were analyzed by the Spearman rank correlation, following trans-

formation to natural logarithms using the SPSS program. A series of Principal Components Analyses (PCA) were used to explore the spatio-temporal variations in environmental factors and phytoplankton community structure. The dimensionality in multivariate datasets was reduced and a series of factors (axes) were extracted. These factors were used to relate community structure to environmental variability. Prior to all PCAs, data were transformed to natural logarithms to reduce the heterogeneity in the data and to normalize distribution.

## Results

### Physico-chemical variables

Temperature showed a clear seasonality, while salinity displayed annual and spatial variations (Fig. 2). The lowest temperature (6.5°C) was recorded at station M8 in February 2004 and the highest (26.5°C) at station MBC in August 2006, and it was generally higher at MBC and



**Fig. 2:** Temporal variations in the mean values of some environmental parameters during the study period.



MKC. There was a significant negative correlation between temperature and salinity/SiO<sub>2</sub>/DO ( $p < 0.01$ , Table 1). Salinity ranged from 20.4 (M8, August 2005) to 32.5 (MKC, October 2006) and decreased in summer due to the reduction in wind-induced mixing and stronger thermohaline stratification, while increases were observed in winter due to vertical mixing (Fig. 2). Maximum salinity (32.5) was due to a strong and long period of southerly winds. A negative correlation was observed between salinity and temperature and PO<sub>4</sub> ( $p < 0.01$ ) (Table 1).

Inorganic nutrient concentrations differed significantly among sampling stations. Generally, nutrient concentrations were higher at near-shore stations. The highest values of NO<sub>3</sub>+NO<sub>2</sub>, PO<sub>4</sub> and SiO<sub>2</sub> were measured at stations MY2, MKC and MBC, respectively (Fig. 2). A weak negative correlation was found between PO<sub>4</sub> and salinity ( $p < 0.05$ ) and a significant negative correlation between SiO<sub>2</sub> and temperature ( $p < 0.01$ ) (Table 1).

DO concentrations displayed a seasonal pattern and ranged between 5.3 mg L<sup>-1</sup> (MKC, October 2006) and 12.3 mg L<sup>-1</sup> (M23, February 2006), and they were higher than 10 mg L<sup>-1</sup> in late winter and spring. The lowest DO value (5.3 mg L<sup>-1</sup>) is due to increased vertical mixing of low-oxygenated lower layer water during a strong southerly gale. DO concentrations were relatively lower during the mucilage event at the end of 2007 than in previous years (Fig. 2). DO concentrations were negatively correlated with temperature ( $p < 0.01$ ), while positively correlated with NO<sub>3</sub>+NO<sub>2</sub> and SiO<sub>2</sub> ( $p < 0.01$ , Table 1).

Chl-*a* concentrations ranged between 0.35 (M23, August 2005) and 15.9 µg L<sup>-1</sup> (MKC, June-2005) and increased generally in spring and early summer due to higher primary production. Chl-*a* values were correlated negatively with temperature ( $p < 0.01$ ) and positively with PO<sub>4</sub> and DO (Table 1).

### Phytoplankton composition

A total of 132 phytoplankton taxa belonging to 6 taxonomical classes were found during the study period; 95% of these consisted of two major groups, diatoms (62 taxa) and dinoflagellates (63 taxa). The other groups consisted of silicoflagellates (3 taxa), euglenophytes (2 taxa), chrysophyte (one taxon) and chlorophyte (one taxon). The diatom *Chaetoceros* (19 taxa) and the dinoflagellates *Protoperidinium* (22 taxa) and *Triplos* (12 taxa) were the most abundant genera (Table 2). Light micrography for selected and important phytoplankton species are given in Figure 5.

The total number of taxa identified was 92 in 2004, 104 in 2005, 103 in 2006 and 90 in 2007. The number of species (*S*) was the highest (40 taxa) at MBC in April 2006, while it was the lowest (2 taxa) at MKC in September and at MBC in December 2007. In general, the number of species was higher in spring than in summer and decreased markedly after June 2007 (Fig. 2). The number of species was negatively correlated with temperature and positively correlated with DO concentrations ( $p < 0.01$ ) (Table 1).

The Shannon diversity index (*H'*) ranged from 0.0 to 3.39. The highest *H'* was 3.39, 3.31, 3.21, 2.69 and 2.63 at MY2, MKC, MBC, M23 and M8, respectively (Fig. 2). The annual average *H'* increased from 1.88 to 2.09 between 2004 and 2006; however, it decreased to 1.92 in 2007. There was a significant negative correlation between temperature and *H'* ( $p < 0.01$ ) and a significant positive correlation with DO concentration ( $p < 0.01$ , Table 1).

A seasonal variation in phytoplankton abundance (*N*) was clear during the study period and it was higher between February and May than in summer. The highest

**Table 1.** Spearman rank correlation coefficients (rho) between environmental factors and phytoplankton data and chlorophyll *a* (chl-*a*) values.

Parameters	Temperature	Salinity	NO <sub>3</sub> +NO <sub>2</sub>	PO <sub>4</sub>	SiO <sub>2</sub>	DO
Temperature	-	-.545**	-	-	-.423**	-.603**
Salinity	-.545**	-	-	-.171*	-	-
NO <sub>3</sub> +NO <sub>2</sub>	-	-	-	-	.260**	.241**
PO <sub>4</sub>	-	-.171*	-	-	.390**	-
SiO <sub>2</sub>	-.423**	-	.260**	.390**	-	.238**
DO	-.603**	-	.241**	-	.238**	-
Chl- <i>a</i>	-.570**	-	-	.194*	-	.451**
N-Dino	-	-	-	-	-	.298**
N-Dia	-.317**	-	-	.192*	.255**	.403**
N-Total	-.414**	-	-	-	-	.432**
<i>S</i>	-.394**	-	-	.215*	-	.448**
<i>H'</i>	-.331**	-	-	-	-	.280**

DO: Dissolved oxygen; N: Abundance; *S*: Number of species; *H'*: Shannon diversity index. Statistically significant correlations are indicated by symbols: – not significant ( $p > 0.05$ ); \* $p < 0.05$ ; \*\* $p < 0.01$ .

**Table 2.** Species list of phytoplankton and their occurrence in the sampling stations during this study period.

Taxa	2004					2005					2006					2007				
	MY2	MKC	MBC	M8	M23	MY2	MKC	MBC	M8	M23	MY2	MKC	MBC	M8	M23	MY2	MKC	MBC	M8	M23
<b>BACILLARIOPHYCEAE</b>																				
<i>Actinoptychus</i> sp.		+							+											
<i>Asterionellopsis glacialis</i>			+			+		+												
<i>Bacteriastrium hyalinum</i>				+	+															
<i>Cerataulina pelagica</i>									+	+										
<i>Chaetoceros aequatorialis</i>									+	+	+		+							
<i>Chaetoceros affinis</i>	+		+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Chaetoceros brevis</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+					
<i>Chaetoceros compressus</i>														+					+	+
<i>Chaetoceros constrictus</i>	+		+	+		+	+			+	+		+	+						
<i>Chaetoceros costatus</i>				+		+		+	+											
<i>Chaetoceros curvisetus</i>	+		+	+		+		+	+	+	+		+	+					+	+
<i>Chaetoceros danicus</i>												+		+						
<i>Chaetoceros debilis</i>	+		+	+						+	+									
<i>Chaetoceros decipiens</i>	+	+	+	+		+		+	+	+	+	+	+	+	+				+	+
<i>Chaetoceros diadema</i>										+									+	+
<i>Chaetoceros didymus</i>				+	+							+		+						
<i>Chaetoceros holsaticus</i>	+		+	+		+		+	+	+	+	+		+	+					
<i>Chaetoceros laciniatus</i>			+	+				+	+	+		+								
<i>Chaetoceros lauderi</i>	+			+																+
<i>Chaetoceros lorenzianus</i>	+		+	+						+		+	+	+						+
<i>Chaetoceros teres</i>	+					+					+	+	+	+		+				
<i>Chaetoceros wighami</i>	+									+	+	+	+	+	+					
<i>Chaetoceros</i> sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Coscinodiscus concinnus</i>	+	+	+	+	+	+	+	+	+	+	+				+	+	+	+	+	+
<i>Coscinodiscus radiatus</i>	+	+	+	+	+	+	+	+	+	+	+	+		+					+	+
<i>Coscinodiscus</i> sp.	+	+	+	+	+	+	+			+		+	+	+	+				+	
<i>Cylindrotheca closterium</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+
<i>Detonula confervacea</i>		+		+		+	+		+	+	+	+	+	+	+			+		
<i>Ditylum brightwellii</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+	
<i>Guinardia delicatula</i>						+				+		+								
<i>Guinardia flaccida</i>			+	+		+				+	+		+	+		+				+
<i>Guinardia striata</i>	+														+					
<i>Gyrosigma</i> sp.										+		+	+	+						
<i>Hemialus hauckii</i>	+														+					
<i>Hemiaulus membranaceus</i>						+														
<i>Leptocylindrus danicus</i>	+		+	+		+		+	+	+	+		+		+	+				
<i>Leptocylindrus minimus</i>												+								
<i>Melosira moniliformis</i>												+								
<i>Navicula</i> sp.	+	+	+	+	+	+	+				+	+	+	+	+	+				
<i>Neocalyptrella robusta</i>						+			+											
<i>Nitzschia longissima</i>	+	+	+	+	+	+	+	+	+		+	+	+	+	+				+	
<i>Pleurosigma normanii</i>																				+
<i>Pleurosigma</i> sp.	+		+	+	+	+	+				+		+	+						

Continued

Table 2 continued

Taxa	2004					2005					2006					2007					
	MY2	MKC	MBC	M8	M23	MY2	MKC	MBC	M8	M23	MY2	MKC	MBC	M8	M23	MY2	MKC	MBC	M8	M23	
<i>Proboscia alata</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
<i>Proboscia alata</i> f. <i>gracilima</i>		+		+	+		+		+	+	+	+	+	+						+	+
<i>P.-nitzschia delicatissima</i> group	+	+		+	+	+	+	+	+		+		+	+	+	+	+	+	+	+	
<i>P.-nitzschia seriata</i> group	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
<i>Pseudosolenia calcar-avis</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+	+
<i>Rhizosolenia</i> cf. <i>castracanei</i>															+						
<i>Rhizosolenia hebetata</i>	+	+				+	+	+	+	+	+	+	+	+			+	+	+	+	+
<i>Rhizosolenia setigera</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+			+	+
<i>Rhizosolenia styliiformis</i>												+									
<i>Skeletonema costatum</i>	+	+		+	+	+	+	+		+	+	+	+	+	+	+					
<i>Thalassionema frauenfeldii</i>						+		+	+	+	+		+								
<i>Thalassionema nitzschioides</i>	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+				+	+
<i>Thalassiosira anguste-lineata</i>																		+	+		
<i>Thalassiosira decipiens</i>	+	+	+	+	+	+	+	+	+							+	+	+	+	+	+
<i>Thalassiosira eccentrica</i>			+	+		+	+		+	+	+	+	+	+							
<i>Thalassiosira gravida</i>	+	+													+	+	+	+	+	+	+
<i>Thalassiosira hyalina</i>	+	+															+	+			
<i>Thalassiosira minima</i>												+		+	+	+	+				
<i>Thalassiosira nordenskiöldii</i>							+		+	+											
<i>Thalassiosira</i> sp.	+	+	+	+	+	+	+	+		+	+	+	+	+	+						
<b>DINOPHYCEAE</b>																					
<i>Archaeoperidinium minutum</i>							+		+	+											
<i>Akashiwo sanguinea</i>																		+			
<i>Dinophysis acuminata</i>							+		+					+	+						+
<i>Dinophysis acuta</i>	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
<i>Dinophysis caudata</i>		+	+	+	+	+	+	+	+	+	+	+	+	+	+					+	+
<i>Dinophysis hastata</i>												+		+	+						+
<i>Dinophysis odiosa</i>	+			+	+	+	+			+		+	+	+			+				
<i>Dinophysis sacculus</i>										+		+	+			+	+				
<i>Diplopsalis lenticula</i>		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
<i>Gonyaulax hyalina</i>												+		+	+	+	+	+	+	+	+
<i>Gonyaulax spinifera</i>			+									+	+			+	+	+	+	+	+
<i>Gonyaulax</i> sp.		+				+					+	+	+	+	+	+	+	+	+	+	+
<i>Gyrodinium spirale</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Heterocapsa triquetra</i>		+				+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
<i>Kofoidinium velleloides</i>							+		+	+		+		+	+					+	+
<i>Lingulodinium polyedra</i>	+						+	+			+	+		+	+	+	+	+	+	+	
<i>Noctiluca scintillans</i>		+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Oxytoxum scolopax</i>												+		+	+			+	+	+	+
<i>Phalacroma oxytoxoides</i>	+	+	+	+	+	+	+	+	+	+	+	+									

Continued

Table 2 continued

Taxa	2004					2005					2006					2007				
	MY2	MKC	MBC	M8	M23	MY2	MKC	MBC	M8	M23	MY2	MKC	MBC	M8	M23	MY2	MKC	MBC	M8	M23
<i>Phalacroma rotundatum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Podolompas palmipes</i>							+	+		+		+	+		+					+
<i>Prorocentrum compressum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Prorocentrum cordatum</i>	+		+	+		+	+	+	+	+	+	+	+	+		+	+	+		
<i>Prorocentrum micans</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Prorocentrum scutellum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Prorocentrum triestinum</i>	+	+		+	+	+	+	+								+				
<i>Proto-peridinium bipes</i>		+				+	+	+	+	+	+	+				+	+	+		
<i>Proto-peridinium brevipes</i>		+		+	+	+	+					+				+				
<i>Proto-peridinium brochii</i>						+	+	+						+	+					+
<i>Proto-peridinium claudicans</i>	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+
<i>Proto-peridinium cf. conicoides</i>							+			+		+			+					
<i>Proto-peridinium conicum</i>		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Proto-peridinium crassipes</i>		+																+	+	
<i>Proto-peridinium depressum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+			+	+
<i>Proto-peridinium divergens</i>		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Proto-peridinium cf. exiguipes</i>							+		+	+										
<i>Proto-peridinium grande</i>		+		+	+	+				+		+	+						+	
<i>Proto-peridinium leonis</i>									+	+										
<i>Proto-peridinium oblongum</i>		+							+										+	+
<i>Proto-peridinium cf. obtusum</i>															+					
<i>Proto-peridinium oceanicum</i>						+			+	+										
<i>Proto-peridinium pallidum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
<i>Proto-peridinium pellucidum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+			+
<i>Proto-peridinium pentagonum</i>	+		+	+	+	+			+	+	+	+	+		+					+
<i>Proto-peridinium punctulatum</i>								+												
<i>Proto-peridinium pyriforme</i>		+				+			+	+	+									
<i>Proto-peridinium steinii</i>	+	+		+	+					+	+	+	+	+	+		+	+	+	+
<i>Proto-peridinium sp.</i>	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+
<i>Pyrophacus horologium</i>													+	+					+	
<i>Scrippsiella acuminata</i>	+	+	+	+	+	+	+	+	+	+	+					+	+	+	+	+
<i>Tripes cf. declinatus</i>			+	+				+												
<i>Tripes furca</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Tripes fusus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Tripes gibberus</i>		+		+	+	+	+							+						
<i>Tripes horridus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Tripes inflatus</i>										+										
<i>Tripes lineatus</i>	+					+	+			+		+	+	+		+	+	+		
<i>Tripes macroceros</i>										+					+					
<i>Tripes pentagonus</i>									+	+		+								
<i>Tripes muelleri</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Continued

Table 2 continued

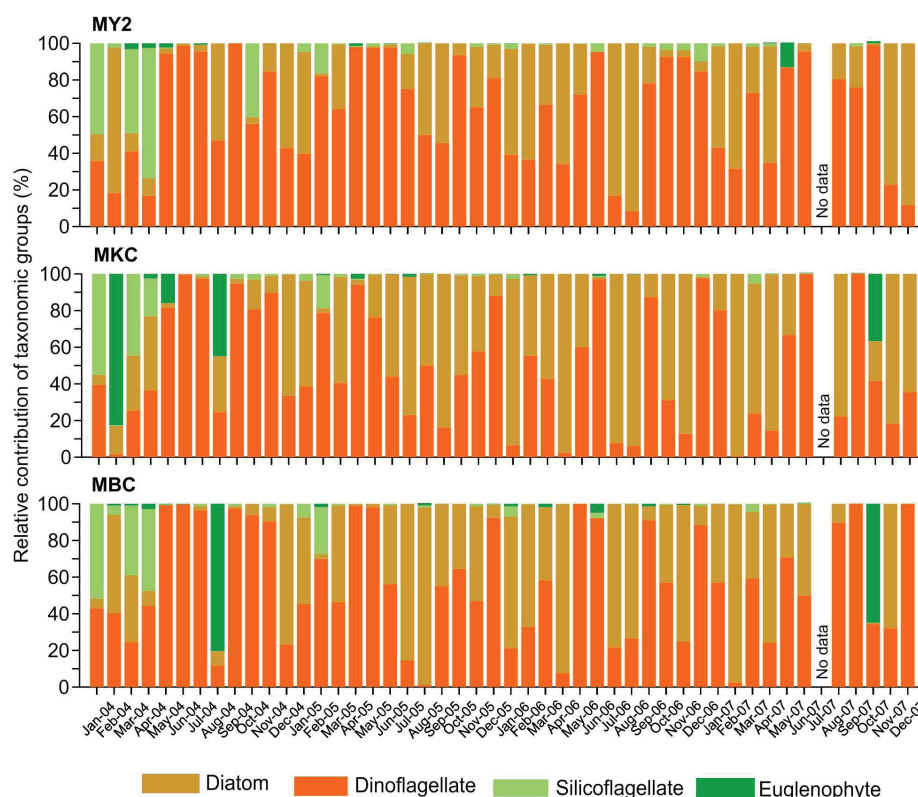
Taxa	2004					2005					2006					2007				
	MY2	MKC	MBC	M8	M23	MY2	MKC	MBC	M8	M23	MY2	MKC	MBC	M8	M23	MY2	MKC	MBC	M8	M23
<i>Triplos muelleri</i> f. <i>massiliensis</i>										+										
<i>Triplos trichoceros</i>	+	+	+	+	+	+	+	+	+	+						+	+	+	+	+
<b>DICTYOCOPHYCEAE</b>																				
<i>Dictyocha fibula</i>	+	+	+	+	+	+	+	+	+	+	+	+	+		+					
<i>Dictyocha speculum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>Octactis octonaria</i>			+	+	+	+	+	+	+	+					+	+	+	+	+	
<b>CHRYSOPHYCEAE</b>																				
<i>Dinobryon</i> sp.															+					
<b>EUGLENOPHYCEAE</b>																				
<i>Euglena</i> sp.	+	+	+	+	+	+	+	+		+	+	+	+		+					
<i>Eutreptiella</i> sp.	+	+	+	+	+	+	+						+	+	+	+	+	+		
<b>CHLOROPHYCEAE</b>																				
<i>Staurastrum</i> sp.	+	+	+																	

abundance was found at the coastal stations (MKC, MY2, M8). The highest mean cell density ( $686 \times 10^3$  cells L<sup>-1</sup>) was found at station M8 in February 2007 and the lowest (86 cells L<sup>-1</sup>) was observed at MBC in August 2005 (Fig. 2). There was a significant negative correlation between *N*-total and temperature ( $p < 0.01$ ), and a significant positive correlation between *N*-total and DO ( $p < 0.01$ , Table 1).

Diatom species were generally more abundant in winter and early spring (January to April) and their highest cell density reached  $1.133 \times 10^3$  cells L<sup>-1</sup> ( $685 \times 10^3$  cells L<sup>-1</sup>, on average), dominated by *Pseudo-nitzschia* species, at station M8 in February 2007. The contribution of dia-

atoms to *N*-total was more than the other groups between July 2005 and May 2007. The contribution of diatoms to *N*-total decreased between June and October 2007 (Fig. 3). There was a significant negative correlation between diatom abundance (*N*-Dia) and temperature ( $p < 0.01$ ), a weak positive correlation with PO<sub>4</sub> ( $p < 0.05$ ), and a significant positive correlation with SiO<sub>2</sub> and DO ( $p < 0.01$ , Table 1).

Dinoflagellate species were generally more abundant between May and June and their relative contribution to *N*-total was higher at station MY2 (Fig. 3). The highest abundance for dinoflagellates was  $181 \times 10^3$  cells



**Fig. 3:** Relative contribution of taxonomic groups to total phytoplankton abundance at the monthly-monitored stations during the study period.



L<sup>-1</sup> (124×10<sup>3</sup> cells L<sup>-1</sup>, on average) at station MY2, dominated by *Prorocentrum micans* (171×10<sup>3</sup> cells L<sup>-1</sup>). A significant positive correlation between dinoflagellate abundance (*N*-dino) and DO ( $p < 0.01$ , Table 1) was found.

As regards the other phytoplankton groups, silicoflagellates and euglenophytes were more common in the study area and they were generally more abundant between January and August 2004. The cell abundance of silicoflagellate *Dictyocha speculum* reached 18×10<sup>3</sup> cells L<sup>-1</sup> (15×10<sup>3</sup> cells L<sup>-1</sup>, in average) at MKC and euglenophyte *Eutreptiella* sp. reached 873×10<sup>3</sup> cells L<sup>-1</sup> (304×10<sup>3</sup> cells L<sup>-1</sup>, in average) at MKC (Fig. 3).

### Potentially toxic species

A total of 11 potentially harmful microalgae species (9 dinoflagellates and 2 diatoms) were detected during the study period (Table 3). Potentially harmful species were more common and frequent at stations MKC and MBC, although their abundance reached a peak level at M8 in February 2007 (Fig. 4).

*Pseudo-nitzschia* diatoms had the highest cell density among the potentially toxic species, and were separated into two groups based on cell sizediameter: the *Pseudo-nitzschia delicatissima* group (<3 μm) and the *Pseudo-nitzschia seriata* group (>3 μm). *Pseudo-nitzschia* species were frequently observed at MKC and MBC, particularly in late autumn and winter. The highest cell density of *Pseudo-nitzschia delicatissima* group reached 1,128×10<sup>3</sup> (685×10<sup>3</sup> cells L<sup>-1</sup>, on average) at station M8, and 417×10<sup>3</sup> cells L<sup>-1</sup> (322×10<sup>3</sup> cells L<sup>-1</sup>, on average) at MKC in February 2007, while for *Pseudo-nitzschia seriata* group maximum cell density reached 112×10<sup>3</sup> cells L<sup>-1</sup> (55×10<sup>3</sup> cells L<sup>-1</sup>, on average) at MBC (Table 3, Fig. 5).

Some members of the genus *Dinophysis* are known to be potentially toxic, and their cell density was found to be very low during the study period. *Dinophysis acuminata* was only observed in net samples, while the cell density of other species such as *D. acuta*, *D. caudata* and *D. sacculus* was generally low (<10<sup>3</sup> cells L<sup>-1</sup>). The cell density of the other potentially toxic dinoflagellate *Phalacroma rotundatum* reached 13.5×10<sup>3</sup> cells L<sup>-1</sup> at station MKC and was more common in the study area than the other species. *Lingulodinium polyedra* was one of the toxic dinoflagellates observed occasionally in the study area and its maximum cell density was 11.5×10<sup>3</sup> cells L<sup>-1</sup> at station MKC. *Protoceratium reticulatum* was rarely found in the study area and it reached 2.5×10<sup>3</sup> cells L<sup>-1</sup> at station MBC. *Prorocentrum cordatum* was another potentially toxic dinoflagellate species found in the study area and its maximum cell density reached 10×10<sup>3</sup> cells L<sup>-1</sup> at station M8 (Table 3, Fig. 5). No toxic or harmful effects caused by these potentially toxic species on the ecosystem or humans was detected during the study period.

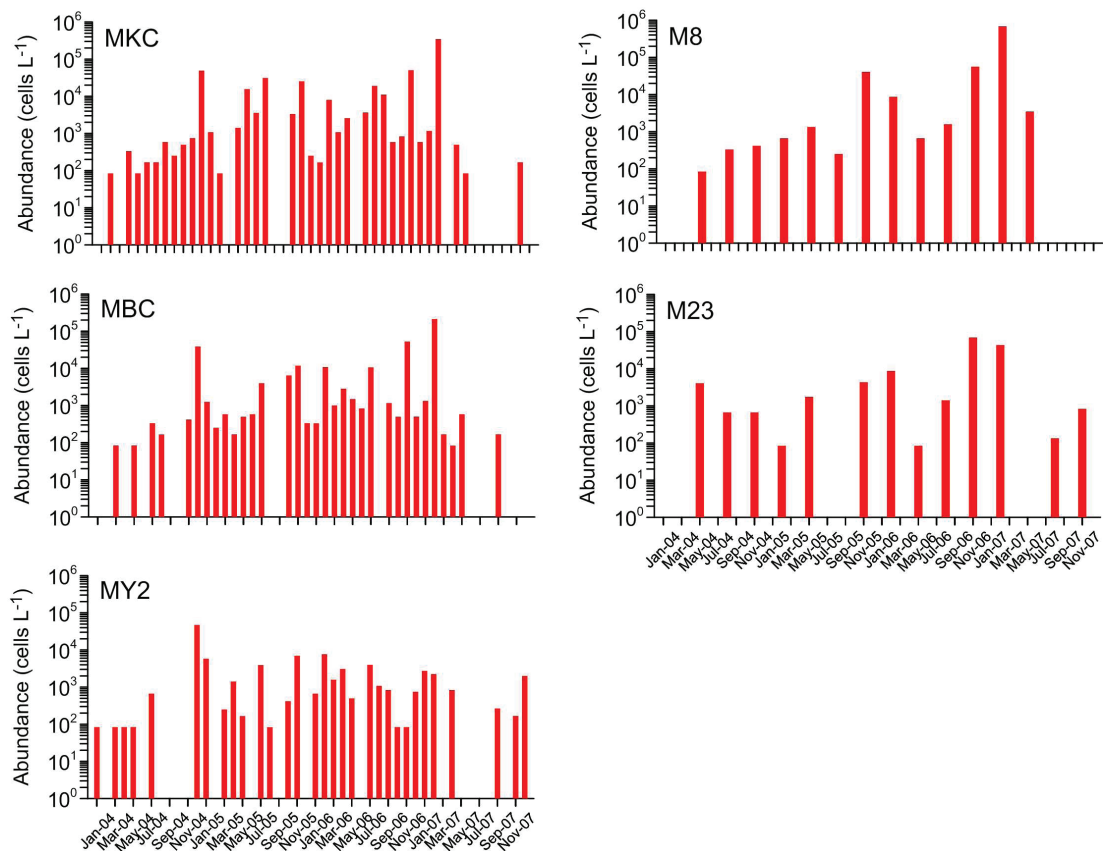
### Mucilage event

A very dense mucilage formation was recorded in the Sea of Marmara in October 2007, characterized by thick-creamy surface accumulations and aggregations of various sizes dispersed throughout the upper layer. The mucilage event attracted public attention by almost discontinuing pelagic fisheries, and the unsightly appearance of surface aggregations. Some significant changes occurred in phytoplankton during the mucilage event, including a marked decrease in the number of species and the diversity index after June 2007. The most important change was observed in the species composition of the

**Table 3.** Potentially toxic microalgae in the northeastern Sea of Marmara during the sampling period with their known harmful effects (see Moestrup *et al.*, 2009), together the month and station at which they reached highest cell density (cells L<sup>-1</sup>).

Species	Known harmful effect	Month	Station	Cell density (×10 <sup>3</sup> )
<b>Bacillariophyceae</b>				
<i>P.-nitzschia delicatissima</i> group	ASP	Feb.	M8	1,128.0
<i>P.-nitzschia pungens</i> group	ASP	Aug.	MBC	112.0
<b>Dinophyceae</b>				
<i>Dinophysis acuminata</i> *	DSP	May	-	-
<i>Dinophysis acuta</i>	DSP	July	MBC	0.5
<i>Dinophysis caudata</i>	DSP	Aug.	MY2	1.0
<i>Dinophysis sacculus</i>	DSP	Jan.	MKC	0.3
<i>Gonyaulax spinifera</i>	Other toxins	June	MKC	0.5
<i>Lingulodinium polyedra</i>	Other toxins	May	MKC	11.5
<i>Phalacroma rotundatum</i>	DSP	Aug.	MKC	13.5
<i>Prorocentrum cordatum</i>	Other toxins	May	M8	10.0

\*Species observed in net samples only. ASP: Amnesic Shellfish Poisoning; DSP: Diarrhetic Shellfish Poisoning.



**Fig. 4:** Mean abundance of potentially toxic microalgae at the sampling stations during the study period.

phytoplankton community.

The dinoflagellate *Gonyaulax hyalina* (reported as *G. fragilis*) and the diatom *Thalassiosira gravida* (reported as *T. rotula*) (Fig. 5) were only found between September and December 2007, and they dominated the phytoplankton community in the dense mucilaginous aggregations. The cell density of *G. hyalina* reached a maximum of  $49 \times 10^3$  cells L<sup>-1</sup> ( $38.7 \times 10^3$  cells L<sup>-1</sup>, on average) and the cell density of *T. gravida* reached a maximum of  $241 \times 10^3$  cells L<sup>-1</sup> ( $164 \times 10^3$  cells L<sup>-1</sup>, on average) at station MY2, in November 2007.

### Community structure

The PCA ordination reveals a different community structure in 2006, particularly significant along the first principal component (PC). As supported by increased diversity, species richness and abundance levels, more species prevailed in the community (Fig. 5). On the other hand, changes in overall phytoplankton community structure during the mucilage event observed in the autumn of 2007 were reflected in lower PC scores. However, a rapid decrease in the number of species and the Shannon diversity index, as seen in Figure 2, coincided with the beginning of the mucilage event. The PC1 of phytoplankton data was correlated with temperature and the first PC of environmental PCA; however, statistically significant correlations with parameters other than temperature were due to auto-correlation of these parameters with temperature. The PCA of environmental parameters showed that

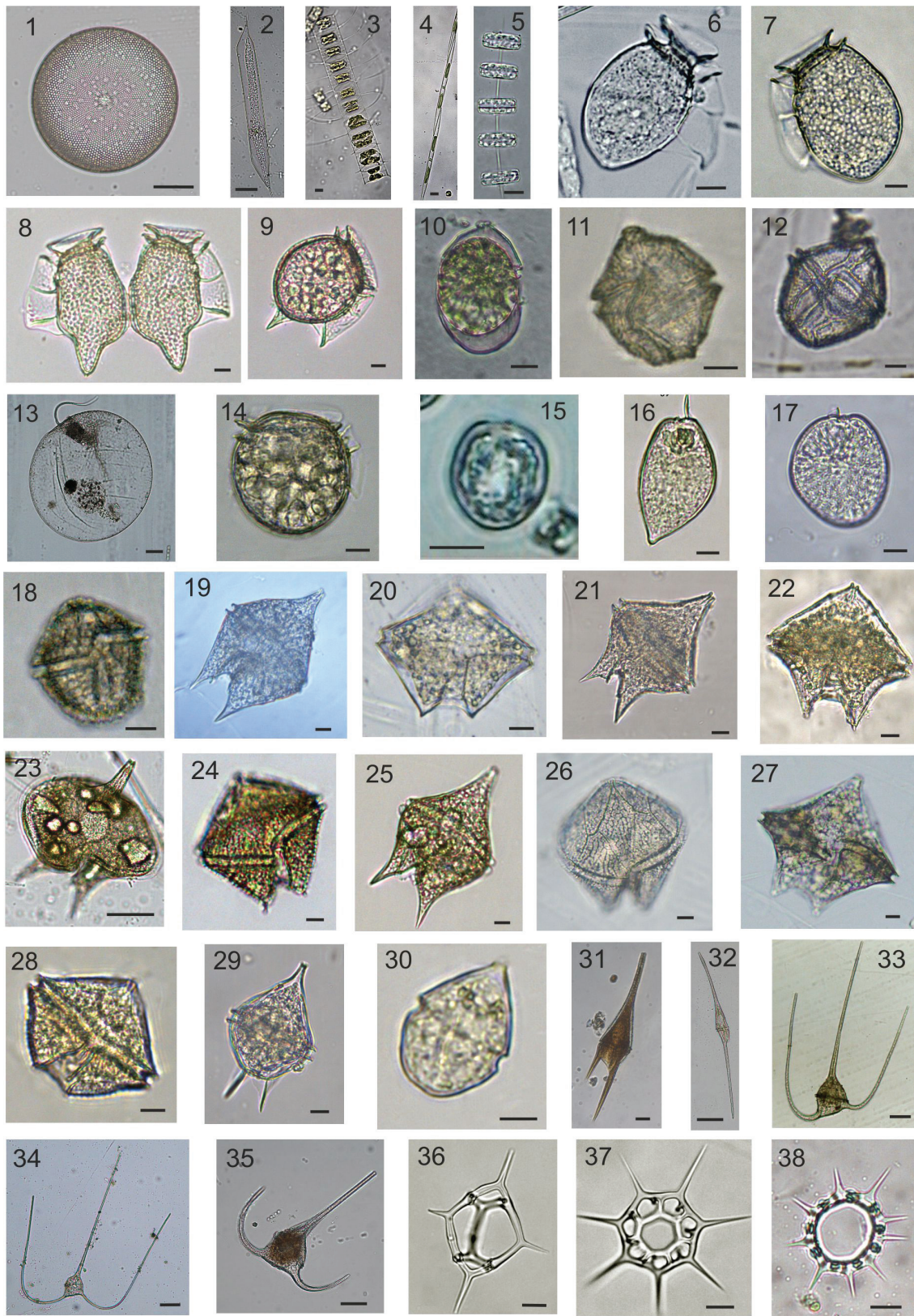
the first PC evolved significantly along with temperature, while PC2 was associated with salinity and NO<sub>3</sub>+NO<sub>2</sub> (Fig. 6).

### Discussion

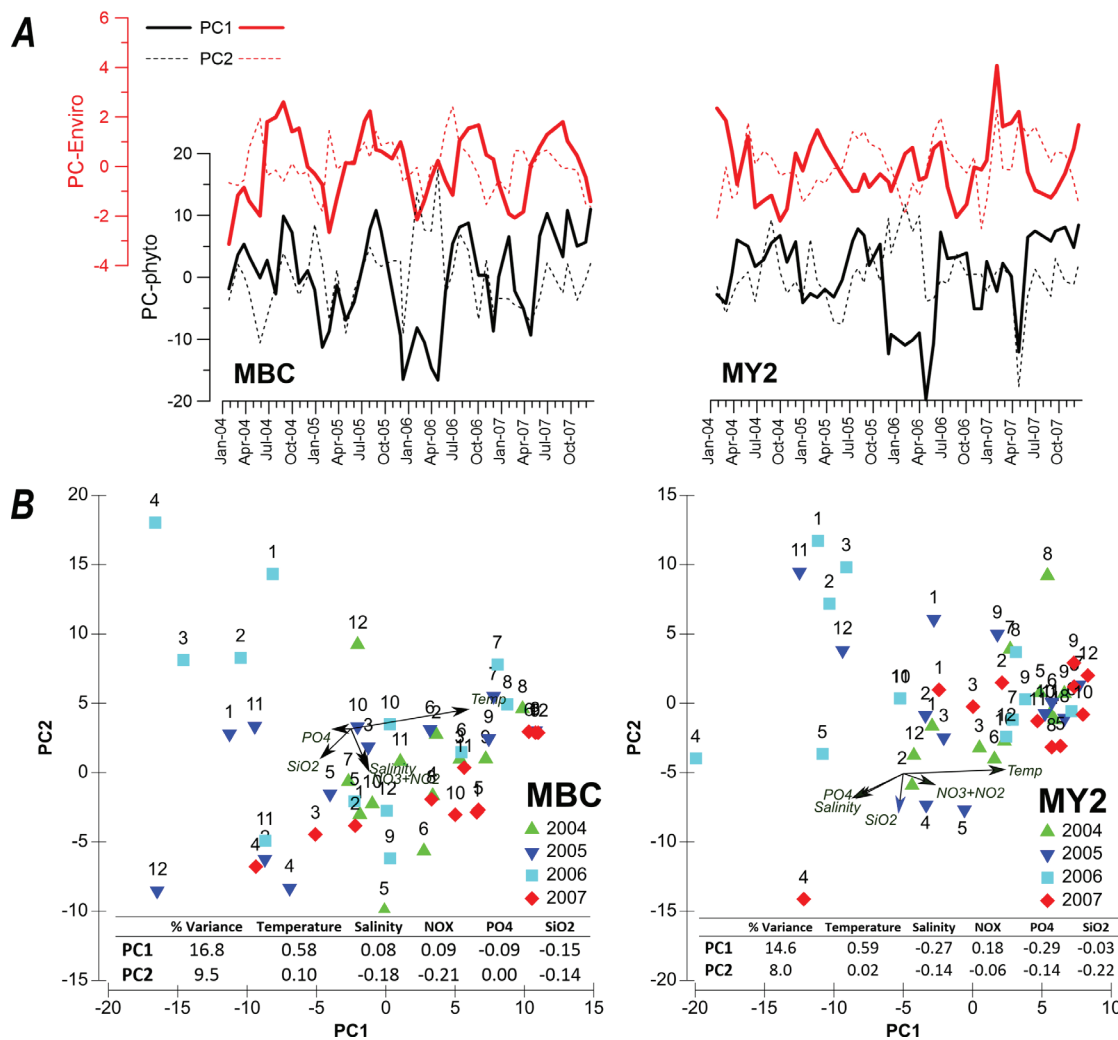
The confined upper layer of the highly stratified Sea of Marmara is characterised by very high productivity compared to the neighbouring Black Sea and Aegean Sea, as confirmed by long term and high resolution (8-days) comparisons of surface chlorophyll a in the selected grids of each basin (Fig. 7). The statistically significant correlation between *in-situ* and satellite-derived measurements ( $r^2=0.70$ ,  $p<0.001$ ) proves the validity of this method in interbasin comparisons. A recent study demonstrated the driving force of the Strait of Istanbul's jet flow to the Sea of Marmara. This jet flow supports primary production even during periods of low nutrient availability by triggering a strong upward motion that injects nutrients from the nutrient-rich lower layer to the upper layer (Oguz, 2017). This system, together with wind-induced vertical mixing, nutrient regeneration and coastal inputs, supports the highly productive SoM ecosystem (Polat & Tuğrul, 1995; Polat *et al.*, 1998).

There is a reported tendency for the development of toxic species in case of altered phytoplankton biomass at global level due to eutrophication (Smith *et al.*, 1999). The seasonality of potentially toxic species indicates increases in spring rather than late autumn, when the peak in phytoplankton abundance is generally encountered.

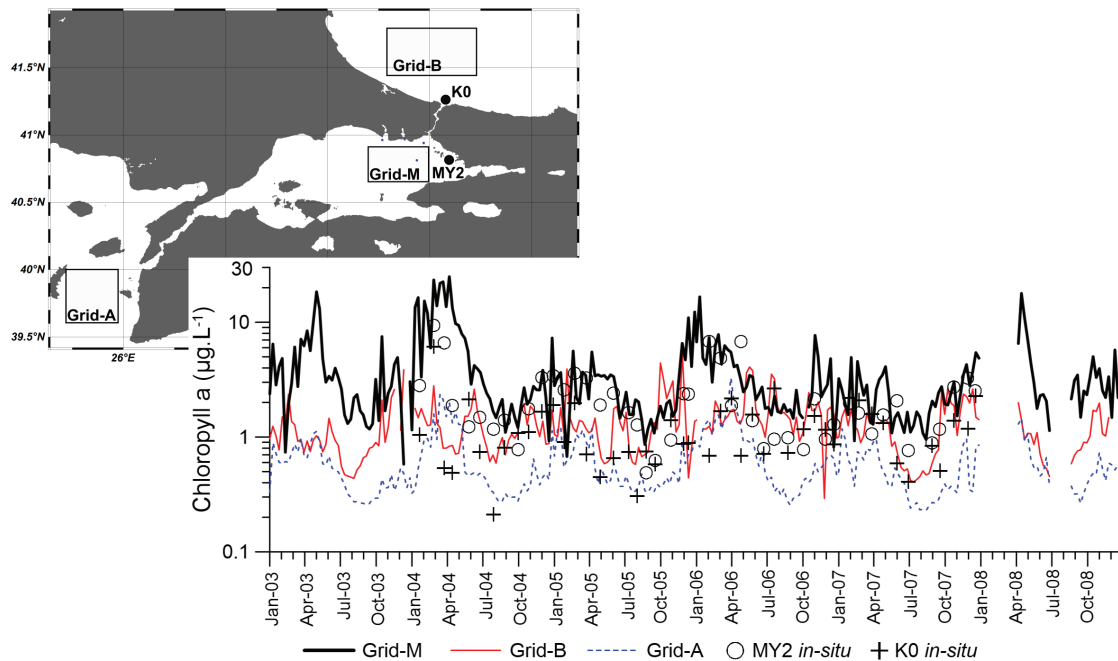




**Fig. 5:** Light micrography of selected and important phytoplankton species detected in the northeastern Sea of Marmara during the study period. 1-5: Diatoms; 6-34: Dinoflagellates; 35-37: Silicoflagellates. 1- *Coscinodiscus radiatus*, 2- *Pseudosolenia calcar-avis*, 3- *Chaetoceros constrictus*, 4- *Pseudo-nitzschia* sp., 5- *Thalassiosira gravida*, 6- *Dinophysis acuminata*, 7- *D. acuta*, 8- *D. caudata*, 9- *D. hastata*, 10- *Gonyaulax hyalina*, 11- *Gonyaulax spinifera*, 12- *Lingulodinium polyedra*, 13- *Noctiluca scintillans*, 14- *Phalacroma rotundatum*, 15- *Prorocentrum cordatum*, 16- *P. micans*, 17- *P. scutellum*, 18- *Protoceratium reticulatum*, 19- *Protoperidinium claudicans*, 20- *P. conicoides*, 21- *P. divergens*, 22- *P. cf. exiquipipes*, 23- *P. grande*, 24- *P. leonis*, 25- *P. oblongum*, 26- *P. cf. obtusum*, 27- *P. pentagonum*, 28- *P. punctulatum*, 29- *P. steinii*, 30- *Scrippsiella acuminata*, 31- *Tripos furca*, 32- *T. fusus*, 33- *T. horridum*, 34- *T. trichoceros*, 34- *T. muelleri*, 35- *Dictyocha fibula*, 37- *D. speculum*, 38- *Octactis octonaria*. Scale bars: (4)= 5  $\mu$ m; (1, 2, 15, 23, 32, 33, 34, 35)= 50  $\mu$ m; (Others)= 10  $\mu$ m.



**Fig. 6:** Time series of principle component scores of phytoplankton and environmental data (A); PCA ordination of phytoplankton community structure with vectors and Spearman correlation coefficients of environmental variables (B). Numbers above symbols indicate month of sampling.



**Fig. 7:** Interbasin comparison of surface chlorophyll *a* (chl-*a*) concentrations. Satellite chl-*a* data was acquired using the GIOVANNI platform of NASA and in-situ chl-*a* data for station K0 was obtained from Yilmaz (2015).



The contribution of potentially toxic species to total phytoplankton abundance was higher at coastal stations (MKC, MBC, MY2), as a direct consequence of pollution caused by domestic and industrial discharges. Previous studies have reported various numbers of potentially harmful species, including toxic species from the SoM (Balkis, 2003; Aktan *et al.*, 2005; Deniz & Tas, 2009).

A total of 35 phytoplankton taxa, including potentially harmful or toxic and bloom-forming species, have been enlisted in the Sea of Marmara (Tas *et al.*, 2016). Among these, 3 eukaryotic forms were potentially toxic. Harmful algal blooms (HABs) have usually been reported from the coasts of the northeastern Sea of Marmara and the Dardanelles. During the last decade, the diatom *Pseudo-nitzschia calliantha*, the dinoflagellates; *Heterocapsa triquetra*, *Noctiluca scintillans*, *Prorocentrum micans*, *Prorocentrum cordatum* and *Scrippsiella acuminata* (reported as *Scrippsiella trochoidea*) and the raphidophyte *Heterosigma akashiwo* have been reported as bloom-forming species (Turkoglu, 2008 and 2010; Tas *et al.*, 2009; Tas & Okus, 2011; Tas, 2015; Tas & Yilmaz, 2015; Dursun *et al.*, 2016; Tas *et al.*, 2016; Tas & Lundholm, 2017). In this study, we considered only the known potentially toxic species. *Pseudo-nitzschia* spp. only reached bloom level at station M8 in February 2007. The dinoflagellate *Phalacroma rotundatum* was found in relatively higher cell densities than other potentially toxic dinoflagellates, but did not reach bloom levels.

Balkis *et al.* (2016) have documented 34 dinoflagellate cysts in the SoM, with high relative cell density of potentially toxic dinoflagellates (74%-92% of total cysts cm<sup>-3</sup>). The well-known long viability of cysts in sediment and their germination during favourable environmental conditions indicate that a higher number of potentially harmful phytoplankton species might develop into HABs in the SoM in case of changes in physical conditions that could alter the permanently stratified water column of the SoM. The current density gradients limit both settlement and germination of plankton cysts.

The onset of an extensive mucilage formation was detected in October 2007 while the cell densities of *G. hyalina* (reported as *G. fragilis*) and *Thalassiosira gravida* (reported as *T. rotula*) significantly increased in September-October 2007. A recent paper on the taxonomy of *G. hyalina* and *G. fragilis* reports these two species as one (Escalera *et al.*, 2018). It is also reported that *Thalassiosira rotula* is a heterotypic synonym of *Thalassiosira gravida* with morphological evidence (Sar *et al.*, 2011).

The mucilage formation has been linked to the occurrence and dominance of well-recognized mucilage-producing species and enhanced extracellular release of polysaccharides, while the suitability of environmental conditions, including calm weather conditions and high variability in N:P ratios, were also discussed as important factors (Aktan *et al.*, 2008; Tüfekci *et al.*, 2010; Balkis *et al.*, 2011). A zooplankton study covering the same period and stations indicates an abrupt change in dominant cladoceran assemblages in summer due to the sudden occurrence and bloom of a predator hydromedusa, *Liriope tetraphylla*, causing a drastic decrease in zooplankton

cell density potentially leading to cascading effects on phytoplankton (Yilmaz, 2015). The decrease in the number of species during the mucilage event shows the response of the phytoplankton community to the changing environmental conditions in the study area. Studies from the Adriatic Sea and other regions show that diatom species *Skeletonema costatum* and *Cylindrotheca closterium* (Urbani *et al.*, 2005; Najdek *et al.*, 2005), and the dinoflagellate *G. hyalina* (reported as *G. fragilis*) (MacKenzie *et al.*, 2002; Pompei *et al.*, 2003; Pistocchi *et al.*, 2005; Nikolaidis *et al.*, 2008) were the main sources of mucilage. The recent studies carried out in the Sea of Marmara revealed that *G. hyalina* (reported as *G. fragilis*) played an important role during the mucilage event (Aktan *et al.*, 2008; Tüfekci *et al.*, 2010; Balkis *et al.*, 2011). In Izmit Bay, located in the east of the SoM, mucilage producers *G. hyalina*, *S. costatum*, and *C. closterium* were abundant and maximum cell density of *G. fragilis* was also observed in November 2007 (Tüfekci *et al.*, 2010), as found in this study. In the meanwhile, *C. closterium*, *Pseudo-nitzschia* sp., *S. costatum*, *Thalassiosira gravida* (reported as *T. rotula*) and *Gonyaulax hyalina* (reported as *G. fragilis*) were dominant organisms around the Prince Islands (Istanbul), and mucilage formation was linked to high cell density of diatoms and their excretory activity (Balkis *et al.*, 2011). However, our data do not show a significant increase in the cell density of *S. costatum* and *Pseudo-nitzschia* spp. prior to and during the mucilage formation.

The highly stratified and eutrophic Sea of Marmara is Turkey's second most important fishery ground after the Black Sea, and is a unique two layered system. As demonstrated during the mucilage formation, the eutrophic-mesotrophic upper layer is prone to anthropogenic/natural disturbances. The potential risk of an increase in the number and magnitude of phytoplankton blooms might lead to a basin-wide collapse of the system through depletion of the already scarce lower layer dissolved oxygen levels (e.g. Balkis, 2003). As an adaptation strategy to a changing climate, surface and lower layer domestic and industrial discharges should be biologically treated. The dredged material from polluted coastal areas should be carefully analyzed for cyst characterization and any disposal to deeper waters should be prohibited during high bloom periods (December-March) in order to limit the magnitude of blooms. Continuous monitoring of phytoplankton will be crucial for assessing the current status and response of the Sea of Marmara, as well as early warning of probable HAB related public health issues.

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