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Role of single-celled organisms in mucilage formation on the shores of Büyükada Island (the Marmara Sea)

NESLİHAN BALKIS[1], HAKAN ATABAY[2], IRFAN TÜRETGEN[1], SERHAT ALBAYRAK[1], HÜSAMETTİN BALKIS[1] AND VİLDAN TÜFEKÇİ[2]

[1]Istanbul University, Faculty of Science, Department of Biology, 34134 Vezneciler- Istanbul, Turkey, [2]TUBITAK, Marmara Research Center, Environment Institute, 41470, Gebze-Kocaeli, Turkey

*This study was implemented to determine the environmental factors and causative organisms of the recent mucilage formation in the Marmara Sea. Samples were taken during the study from 7 different depths (0.5–30 m) of one sampling point of the Büyükada Island shore between January and June 2008. As a result, 62 phytoplankton species belonging to 5 different groups were identified. Dinoflagellates were dominant in terms of species number, and diatoms in terms of cell number. In January and February, mucilage formation was very dense, where 5 phytoplankton species (*Clindrotheca closterium, Pseudo-nitzschia *sp.,* Skeletonema costatum, Thalassiosira rotula *(Bacillariophyceae) and* Gonyaulax fragilis *(Dinophyceae)) were reported as dominant organisms. Among them,* Gonyaulax fragilis *has never been reported in the Marmara Sea previously, thus that organism appeared firstly with the formation of dense mucilage and then when the mucilage decayed in May and June 2008,* G. fragilis *disappeared. Autofluorescent single-celled organisms were classified in three groups depending on their cell sizes (>20 μm, >2 μm, >0.2 μm) by membrane filtration and total count of bacteria were determined by epifluorescence microscope after dying with DAPI. The highest total bacteria was recorded in April at 25 m depth (6655 ± 44.4 cells ml^{-1}) while the lowest count was in June at 0.5 m depth (1077 ± 26.1 cells ml^{-1}). The seawater temperature ranged between 7.0 and 21.5°C, salinity between 20.9 and 37.4 ppt and dissolved oxygen amount between 2.75 and 12.75 mg l^{-1}. The chlorophyll-*a *amount ranged between 0.10 and 6.35 μg l^{-1}, the higher values were recorded in January at 15 m depth (6.35 μg l^{-1}) and in April at 10 m depth (4.89 μg l^{-1}). Among the nutrients, the amounts of nitrite + nitrate-N varied between 0.02 and 7.67 μg-at N l^{-1}, phosphate-P between 0.11 and 0.96 μg-at P l^{-1} and silicate-Si between 0.37 and 8.93 μg-at Si l^{-1}. The highest values were determined at a deeper layer where nutrients are accumulated. On the other hand, the N:P ratio interval was found as 0.1–11.3, Si:P ratio as 2.92–52.33 and N:Si ratio as 0.01–1.10 during the sampling period. Nitrogen was the limiting nutrient and the silica amount was enough to enable the development of diatoms.*

Keywords: mucilage, single-celled organism, ecological factors, the Marmara Sea

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INTRODUCTION

Seasonal changes and global warming considerably affects the biological structure of seas (Goffart *et al.*, 2002). Mucilage formation in seas is the aggregation of organic substances that are produced by various marine organisms under special seasonal and trophic conditions (Innamorati *et al.*, 2001; Mecozzi *et al.*, 2001). Having serious effects on such human activities as fisheries, tourism and aquaculture, mucilage formation was reported to occur in the Adriatic Sea since the 17th Century (Totti *et al.*, 2005). The correlation between mucilage formation and phytoplankton was demonstrated in the studies carried out in the late 19th Century and early 20th Century (Mingazzini & Thake, 1995). Rinaldi *et al.* (1995) stated that diatoms, known to produce extracellular polysaccharide substance, are effective on mucilage formation, and bacteria were reported to participate in this formation (Herndl *et al.*, 1999; Azam & Long, 2001). Subsequent studies showed that dinoflagellates also produce extracellular mucilages (MacKenzie *et al.*, 2002) and it was also stated that the dinoflagellate *Gonyaulax fragilis* (Schütt) Kofoid, 1911 takes part in mucilage formation in the Adriatic Sea (Pompei *et al.*, 2003; Pistocchi *et al.*, 2005). In addition, mucilage aggregates are the result of a hyperproduction as a pathological response of the algae to a microbial infection and this hypothesis was supported by some laboratory experiments (Innamorati *et al.*, 2001).

In Turkish territorial waters, mucilage formation began to be observed firstly in İzmit Bay in the Marmara Sea in October 2007 and mainly fisheries and tourism have been damaged seriously (Tüfekçi *et al.*, in press). The Marmara Sea is a small basin with an approximate size of 70 km in width and 250 km in length, a surface area of 11,500 km^2 and a maximum depth of 1.390 m. Situated between the European and Asian continents, the Marmara Sea forms the Turkish Straits System along with the Bosphorus and the Dardanelles (Beşiktepe *et al.*, 1995). It is connected to the

Corresponding author:
N. Balkis
Email: neslbalk@istanbul.edu.tr

Black Sea through the Bosphorus in the north-east and to the Aegean Sea through the Dardanelles in the south-west and, as a result, possesses two different water masses. The surface layer of the Marmara Sea is composed of brackish waters (22–26 ppt) originating from the Black Sea and the bottom layer of Mediterranean originated saline waters (38.5–38.6 ppt) (Ünlüata *et al.*, 1990; Tuğrul & Polat, 1995).

In this study, it was aimed to find and demonstrate the species and environmental factors causing mucilage event in the Marmara Sea, and temporal changes in the abundance of total bacteria, pico-, nano- and microphyoplankton were identified.

MATERIALS AND METHODS

Sampling was conducted monthly in a single station determined in the coastal waters of Büyükada Island between January and June 2008 (Figure 1). Samples necessary for water analyses were collected from 7 different depths (0.5, 5, 10, 15, 20, 25 and 30 m) using a water sampler with 3 l capacity, and the temperature of the samples were measured with the thermometer on the water sampler. The salinity was determined by the Mohr–Knudsen method (Ivanoff, 1972) and the dissolved oxygen by the Winkler method (Winkler, 1888). The samples necessary for nutrient analyses were put into polyethylene containers of 100 ml, and frozen in a deep freezer at -20°C and measured with an autoanalyser (APHA, 1999).

1 l of water, collected for chlorophyll-*a* analyses used for determining phytoplankton biomass, was filtered through Whatman GF/F filter papers, kept in 90% aceton solution overnight, centrifuged, and then measured with a spectrophotometer at different wave lengths (Parsons *et al.*, 1984). Trophic index (TRIX) values were calculated in order to determine the eutrophication level of the sampling area and the quality of waters (Vollenweider *et al.*, 1998). The index is given by:

$$\mathrm{TRIX} = [\log_{10}(\text{Chl-a} \cdot \text{D\%O} \cdot \text{N} \cdot \text{P}) + 1.5]/1.2$$

Chl-a = chlorophyll-*a* (μg l^{-1}), D%O = oxygen as an absolute deviation (%) from saturation, N = dissolved inorganic nitrogen N-NO_3 + NO_2 (μg-at l^{-1}), P = total phosphorus P-PO_4 (μg-at l^{-1}). Calculated TRIX values were not compared with other regions since NH_4, as dissolved inorganic nitrogen, in the original formula was not used.

In order to determine the qualitative and quantitative situation of phytoplanktonic organisms responsible for mucilage formation, an additional 1 l of water was collected from the aforementioned depths with the water sampler; it was preserved by adding 2.5 ml of Lugol's iodine solution and left for sedimentation in the laboratory for a week. The overlying excess water was siphoned until 15 ml subsamples and then the subsamples were fixed by neutral formaldehyde with a final concentration of 2–4% (Throndsen, 1978). Cell counting was carried out under an inverted phase-contrast microscope (Olympus CK2) in a Sedgwick–Rafter cell.

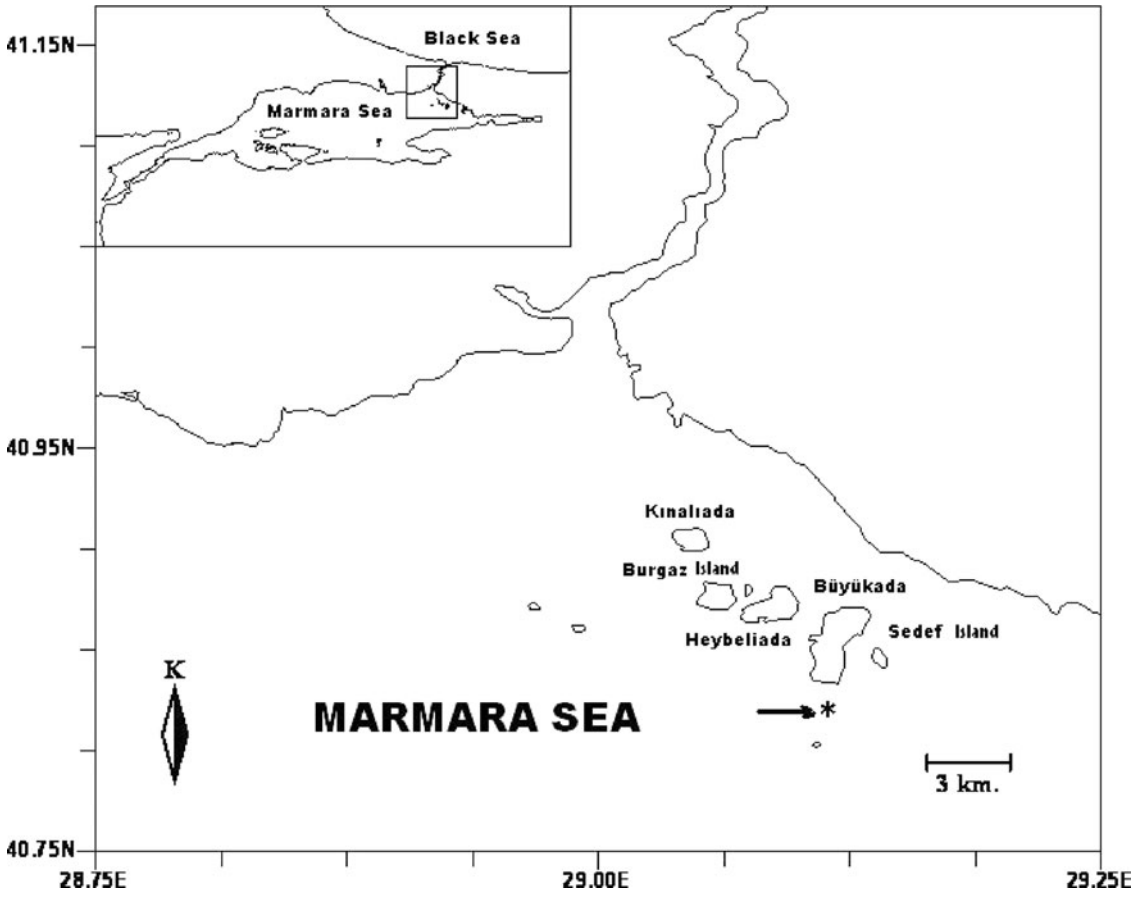

Fig. 1. Sampling station in the coastal zone of Büyükada Island.

DAPI (4′,6-diamidino-2-phenylindole) stain was used for determining total bacteria in water samples. 100 µl DAPI stain was added to 900 µl seawater taken from the sample, kept at 28°C for one hour, 3 ml of sterile bidistilled water was added to terminate the reaction and ensure homogenization, stirred and then filtered through polycarbonate filter (Millipore) with 0.2 µm pore diameter. The filter surface was observed under a microscope with epifluorescent attachment (Nikon 80i) and UV-2A filter cube, and photographs were taken from 10 different microscopical fields using 1000X magnification. The signals obtained from the photographs were counted and the mean values were calculated. Then these values were multiplied with their coefficients and the number of bacteria per millilitre was determined (Rodriguez *et al.*, 1992).

Single celled organisms in the water samples that show autofluorescent properties were analysed in three groups according to their dimensions. Water samples were filtered through a 20 µm pore size membrane filter to separate microphytoplankton, then through a 2 µm filter for capturing nanophytoplankton and finally through a 0.2 µm filter to detect picophytoplankton. The autofluorescent plankton that were divided by sequential filtration according to their size were analysed with a relevant filter cube and photographs were taken from 10 different microscopical fields using 1000X magnification. The signals obtained from the photographs were counted and the mean values were calculated. Then these values were multiplied with relevant coefficients and the number of autofluorescent microorganisms per millilitre was determined (Totti *et al.*, 2005).

The Spearman's rank-correlation coefficient was used to detect any correlation among biotic and abiotic variables (Siegel, 1956). The density of mucilage was assessed under seven categories according to the observations with an underwater camera (1 no mucilage, 2 rare, 3 less dense, 4 dense, 5 filamentous, 6 small masses and 7 big masses).

RESULTS

Environmental variables

During the sampling period, temperature values ranged between 7.0 and 21.5°C. The highest value was observed in June (21.5°C, 0.5 m) and the lowest in January (7.0°C, 0.5 m, 5 m) (Figure 2A). In January, February and March temperature values showed an increasing pattern from the surface to 30 m depth. In April, an irregular temperature differentiation by depth was observed. On the other hand, in May and June generally a decreasing pattern was observed from the surface to 30 m depth.

Salinity ranged between 20.9 and 37.4 ppt and showed an increase from the surface to deeper layers in all months (Figure 2B). The highest salinity (37.4 ppt) was recorded at 30 m depth in March and the lowest (20.9 ppt) at 0.5 m depth in June. After 15–20 m depth, salinity showed a sudden increase due to the saline Mediterranean waters.

Dissolved oxygen values ranged from 2.75 mg l^{-1} to 12.75 mg l^{-1} (Figure 2C). The highest value was recorded in January in the surface layer that is in contact with the atmosphere and the lowest at 25 m depth in April. A decreasing pattern was observed in oxygen values from the surface to the bottom along the water column.

Of nutrients $NO_2 + NO_3$-N values ranged between 0.02 µg-at l^{-1} and 7.67 µg-at l^{-1}, PO_4-P between 0.11 µg-at l^{-1} and 0.96 µg-at l^{-1} and SiO_4-Si between 0.37 µg-at l^{-1} and 8.93 µg-at l^{-1} (Figure 2D–F). The highest nutrient values were recorded generally at the bottom layer where there is aggregation. The N:P ratio ranged between 0.1 and 11.3 (Figure 2G). The minimum value (0.1) was recorded at the depths of 5 and 10 m in January and the maximum (11.3) at 25 m depth in May. The increase of nitrogen in proportion to phosphorus in May caused the N:P ratio to increase more remarkably compared to other months. The N:Si ratio ranged between 0.01 (January, 5 m and 10 m) and 1.10 (January, 20 m) (Figure 2H) while the Si:P ratio ranged between 2.92 (June, 0.5 m) and 52.33 (May, 15 m) (Figure 2I).

During the sampling period, chlorophyll-*a* amounts ranged between 0.10 µg l^{-1} and 6.35 µg l^{-1}. Two remarkable peaks were observed, one (6.35 µg l^{-1}) at 15 m depth in January and the other (4.89 µg l^{-1}) at 10 m depth in April (Figure 3A).

Trophic index (TRIX) values ranged between 1.06 (January, 10 m) and 3.30 (January, 20 m) and all values obtained were between 0 and 4 which indicates a state of high quality but low trophic level (Figure 3B).

Pico-, nano- and microphytoplankton and total bacteria

Picophytoplankton abundances during the study periods ranged from 64 ± 3.8 cells ml^{-1} to 2254 ± 29.5 cells ml^{-1} at 30 m depth in May and 10 m depth in January, respectively. Especially, at 5–20 m depth in the winter period (January and February), having intense aggregate appearance, picophytoplankton abundances were higher than in any other periods (Figure 4). Nanophytoplankton was at its lowest level (44 ± 1.9 cells ml^{-1}) at 30 m depth in May as in the case of picophytoplankton and its highest density (1562 ± 22 cells ml^{-1}) at 15 m depth in January (Figure 4). During the sampling, the abundance of microphytoplankton ranged between 33 ± 2.4 cells ml^{-1} (June, 30 m) and 712 ± 18.2 cells ml^{-1} (January, 15 m) (Figure 4). The highest value of total bacteria (6655 ± 44.4 cells ml^{-1}) was recorded at 25 m depth in April and the lowest (1077 ± 26.1 cells ml^{-1}) at 0.5 m depth in June. During all sampling periods, total bacteria values generally reached the highest density at the middle layer (10–20 m).

Phytoplankton composition, succession and abundance

During the mucilage formation on the shores of Büyükada Island, a total of 62 species from 5 different microalgae groups were identified: 24 diatoms (38.7%), 34 dinoflagellates (54.9%), two dictyochophyceans (3.2%), one euglenophycean (1.6%) and one prasinophycean (1.6%). During the winter period, with dense mucilage formation, diatoms were dominant over dinoflagellates in terms of the number of species and individuals.

In January, mucilages were observed most densely at 15–20 m depth. In this month, the dominant group in terms of individual number was diatom (80.99–97.79%; Figure 5A), and this group reached the highest individual number at 15 m depth (4.75×10^5 cells l^{-1}, 96.54%). Especially

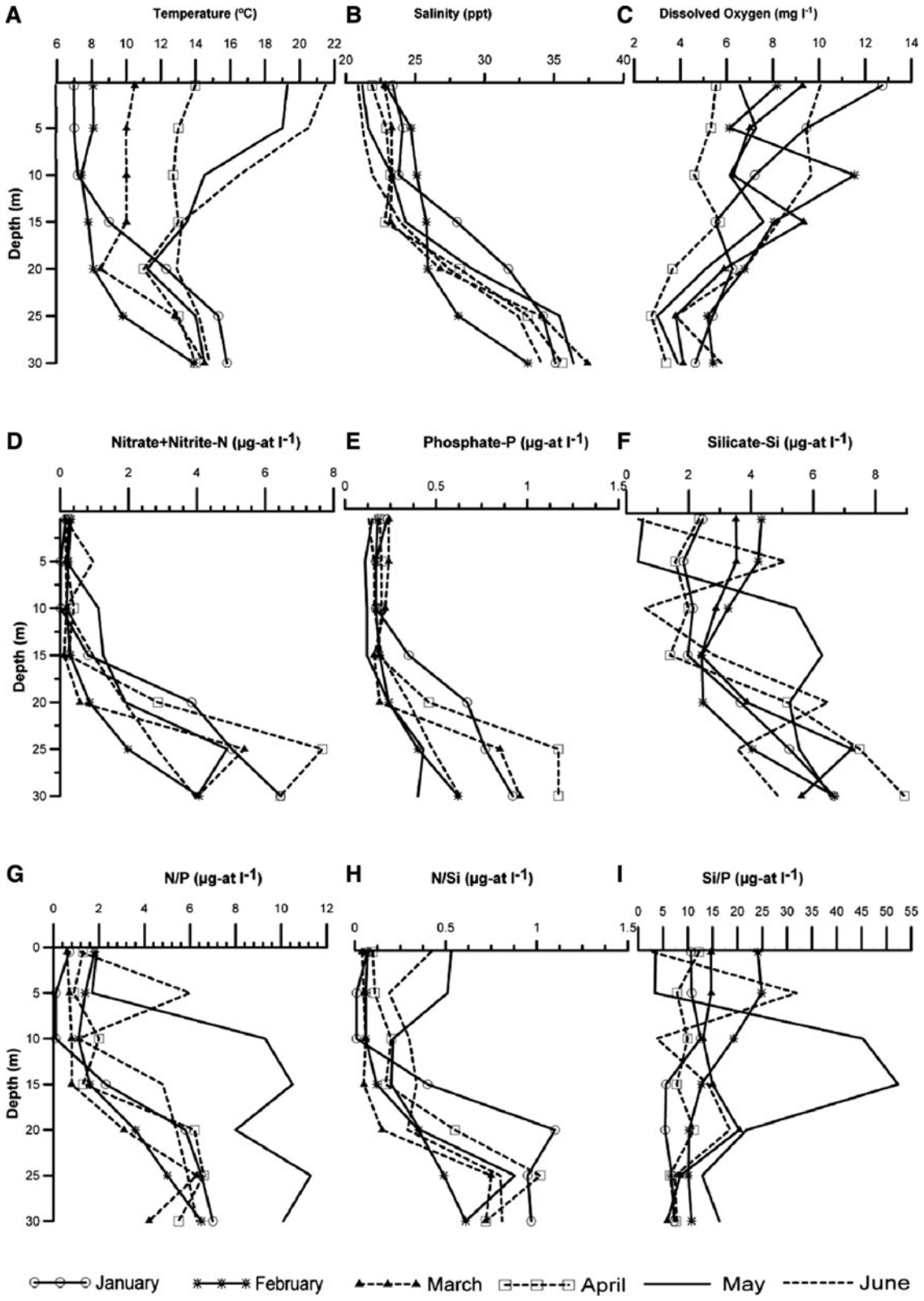

Fig. 2. Monthly variations of environmental variables: (A) temperature; (B) salinity; (C) dissolved oxygen; (D) nitrogen; (E) phosphorus; (F) silica; (G) N/P ratio; (H) N/Si ratio; (I) Si/P ratio along the water column at the sampling station.

Skeletonema costatum (Greville) Cleve, 1878 played an important role in this increase (1.60×10^5 cells l^{-1}). This species was followed by *Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin, 1964 (8.86×10^4 cells l^{-1}), *Thalassiosira rotula* Meunier, 1910 (7.62×10^4 cells l^{-1}) and *Pseudo-nitzschia* sp. (5.22×10^4 cells l^{-1}). In addition, *Thalassiosira anguste-lineata* (Schmidt) Fryxell & Hasle, 1977 (2.44×10^4 cells l^{-1}), *Dactyliosolen fragilissimus*

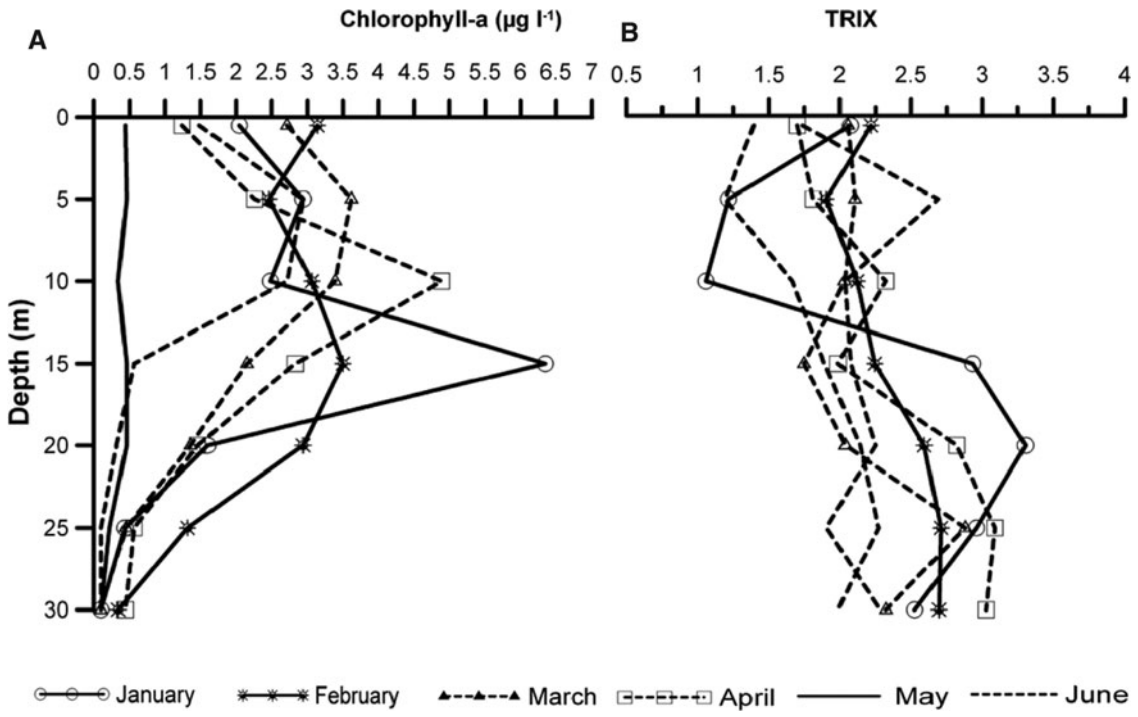

Fig. 3. Monthly variations of (A) chlorophyll-*a* and (B) trophic index along the water column at the sampling station.

(Bergon) Hassle, 1991 (1.80×10^4 cells l^{-1}) and *Leptocylindrus danicus* Cleve, 1889 (1.62×10^4 cells l^{-1}) were among the important species observed during mucilage formation at 15 m depth. Dinoflagellates were found more abundantly at the depths of 10 m (1.92×10^4 cells l^{-1}) and 15 m (1.48×10^4 cells l^{-1}). Within this group, the dominance of *G. fragilis*, a species that produces mucilage, compared to other species is important (maximum abundance at 10 m depth: 1.82×10^4 cells l^{-1}). The highest individual number (4.92×10^5 cells l^{-1}) at 15 m depth was determined according to total phytoplankton values.

In February, mucilage formation was observed very densely at 15–25 m depth and large masses were formed at these depths. The dominance of diatoms continued (60.37–100%; Figure 5B), and diatoms reached the highest individual number at the surface (1.34×10^5 cells l^{-1}, 86.69%). *Cylindrotheca closterium* played a significant role in this increase with its individual number of 1.14×10^5 cells l^{-1}. It was followed by *Pseudo-nitzschia* sp. (1.06×10^4 cells l^{-1}) and *T. rotula* (6.70×10^3 cells l^{-1}). Besides, *C. closterium* showed considerable increases at the depths of 5 m (4.02×10^4 cells l^{-1}) and 20 m (4.13×10^4 cells l^{-1}). Dinoflagellates reached the highest individual number (1.84×10^4 cells l^{-1}) at the surface (0.5 m) and were found more densely at the depths of 5 m (1.75×10^4 cells l^{-1}) and 10 m (1.79×10^4 cells l^{-1}) compared to other layers. Within this group *G. fragilis* (1.58×10^4 cells l^{-1}, 10 m) and *Protoperidinium paulseni* (Pavillard) Balech, 1974 (1.23×10^4 cells l^{-1}, 0.5 m) showed higher increases in comparison to other species. According to total phytoplankton values, the highest individual number was recorded at the surface layer (1.55×10^5 cells l^{-1}).

In March, while there was no finding about mucilage until 15 m depth, signs were seen very densely at 20–25 m depth, large masses of this depth transformed into filamentous structures at 30 m depth. While dinoflagellates were dominant in terms of species number, diatoms were dominant at some depths (26.97–92.96%; Figure 5C) and dinoflagellates at others (6.74–71.50%; Figure 5C) in terms of individual number. Dinoflagellates reached the highest individual number at 10 m depth (6.60×10^4 cells l^{-1}, 67.84%). Besides, they were dominant at 5 m depth compared to other depths (4.50×10^4 cells l^{-1}, 71.50%). *Gymnodinium* sp. (2.43×10^4 cells l^{-1}, 10 m), *Scrippsiella trochoidea* (Stein) Loeblich III, 1976 (2.42×10^4 cells l^{-1}, 5 m; 1.76×10^4 cells l^{-1}, 10 m) and *G. fragilis* (1.13×10^4 cells l^{-1}, 10 m) played a significant role in this increase in dinoflagellates. On the other hand, diatoms reached the highest individual number at 20 m depth (5.76×10^4 cells l^{-1}, 79.34%) and this depth was followed by 25 m (3.80×10^4 cells l^{-1}) and 10 m (3.02×10^4 cells l^{-1}). Within this group, the dominance of *C. closterium* at 20 m depth was important (4.26×10^4 cells l^{-1}). Moreover, this species reached a significant density also at 25 m depth (3.26×10^4 cells l^{-1}). According to total phytoplankton values, the higher individual numbers were recorded at 10 m depth (9.74×10^4 cells l^{-1}) due to the effect of dinoflagellates and at 20 m depth (7.26×10^4 cells l^{-1}) due to the effect of diatoms.

In April, the effect of mucilage began to decline and filamentous structures were observed at 20–25 m depth. In this month, dinoflagellates were the dominant group in terms of species number and diatoms in terms of individual number (94.17–98.32%; Figure 5D). Diatoms reached the higher individual numbers at the depths of 20 m (1.35×10^5 cells l^{-1}, 97.42%), 5 m (1.16×10^5 cells l^{-1}, 96.52%) and 10 m (1.09×10^5 cells l^{-1}, 95.63%). *Cylindrotheca closterium* played a significant role in the increase in diatoms. This species reached the highest individual number at 20 m depth (7.20×10^4 cells l^{-1}). The individual number of dinoflagellates decreased considerably in this period despite the warming of waters (1.40–5.83%; Figure 5D). Like diatoms,

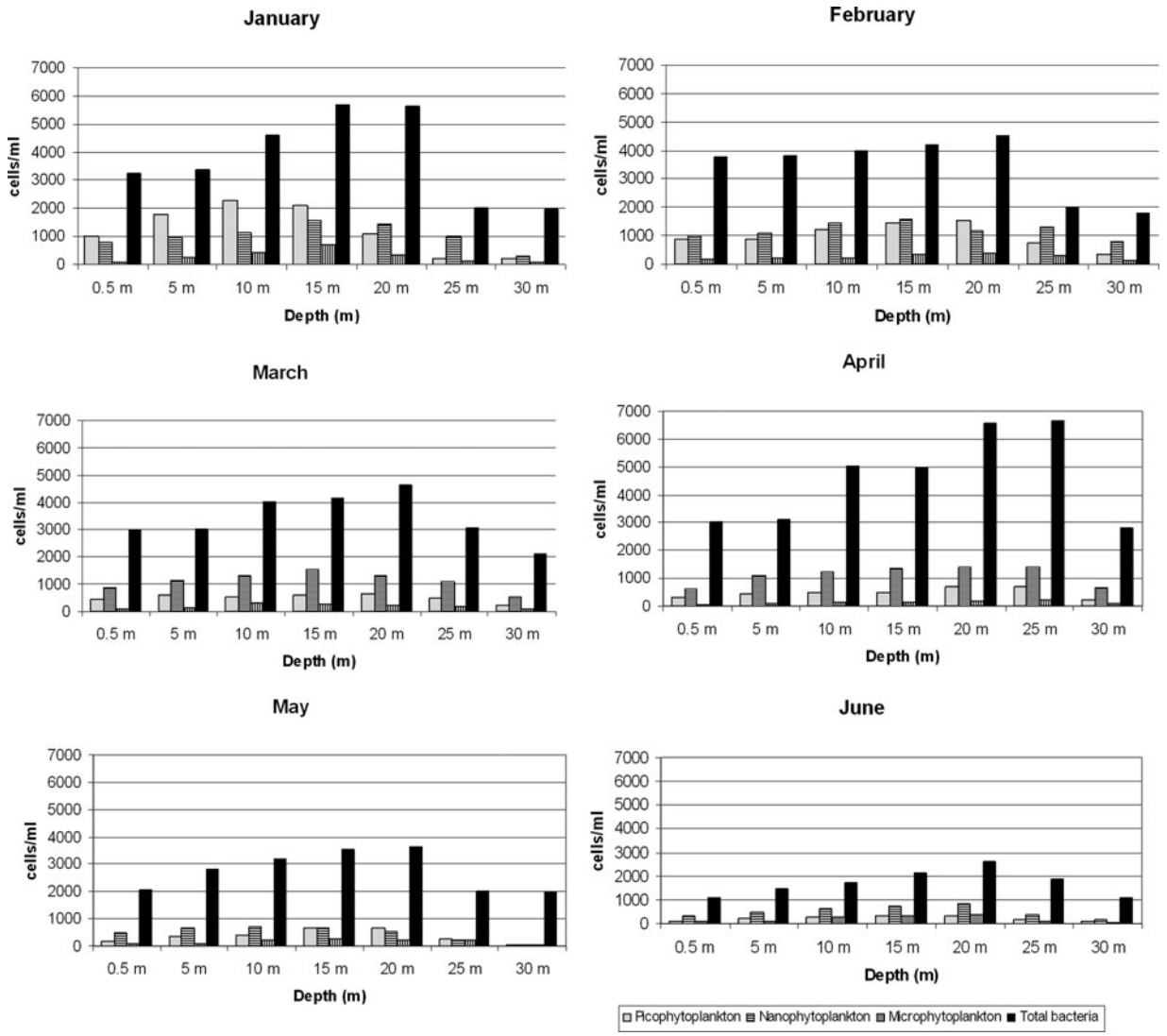

Fig. 4. Monthly distribution (cells ml^{-1}) of pico-, nano-, microphytoplankton and total bacteria abundance at the sampling station.

the higher individual numbers of dinoflagellates were recorded at the depths of 5 m (4.00×10^3 cells l^{-1}) and 10 m (4.70×10^3 cells l^{-1}). The dominant species of this group were *Prorocentrum micans* Ehrenberg, 1834 and *S. trochoidea* but they did not reach a significant individual number. According to total phytoplankton values, the higher individual numbers were recorded at the depths of 20 m (1.39×10^5 cells l^{-1}), 5 m (1.20×10^5 cells l^{-1}) and 10 m (1.14×10^5 cells l^{-1}).

In May, filamentous structures were observed at 10–20 m depth. In May, like in April, the dominant groups were dinoflagellates in terms of species number and diatoms in terms of individual number (71.79–95.17%; Figure 5E). Diatoms reached the higher individual numbers at the depths of 15 m (4.25×10^5 cells l^{-1}, 95.17%), 0.5 m (3.99×10^5 cells l^{-1}, 89.95%) and 5 m (3.01×10^5 cells l^{-1}, 82.34%). *Pseudo-nitzschia* sp. was the most abundant species of diatoms and it reached the highest individual number at 15 m depth (4.23×10^5 cells l^{-1}). The individual numbers of other species belonging to this group were considerably low. The species number of dinoflagellates increased compared to other months but a remarkable increase was not seen in terms of individual numbers (4.83–28.21%; Figure 5E). This group reached the higher individual numbers at the depths of 5 m (6.47×10^4 cells l^{-1}) and 0.5 m (4.46×10^4 cells l^{-1}) and *P. micans* was responsible for the increase. According to total phytoplankton values, the higher individual numbers were recorded at the depths of 15 m (4.46×10^5 cells l^{-1}) and 0.5 m (4.44×10^5 cells l^{-1}).

In June, the mucilage event lost its effect considerably, and it was observed very rarely at 10–30 m depth. Dinoflagellates were seen dominant over diatoms in terms of the number of species and individuals (19.51–80%; Figure 5F) while only at 0.5 m depth diatoms showed a considerable increase compared to dinoflagellates (1.72×10^4 cells l^{-1}, 80.49%). While *C. closterium* was responsible for the increase in diatoms at this depth (1.48×10^4 cells l^{-1}), dinoflagellates reached the highest individual number at 15 m depth (1.32×10^4 cells l^{-1}) and *P. micans* (4.10×10^3 cells l^{-1}) and *S. trochoidea* (5.00×10^3 cells l^{-1}) were the species responsible for this increase. According to total phytoplankton values, the higher individual numbers were recorded at the depths of 10 m (2.35×10^4 cells l^{-1}), 0.5 m (2.13×10^4 cells l^{-1}) and 15 m (2.03×10^4 cells l^{-1}).

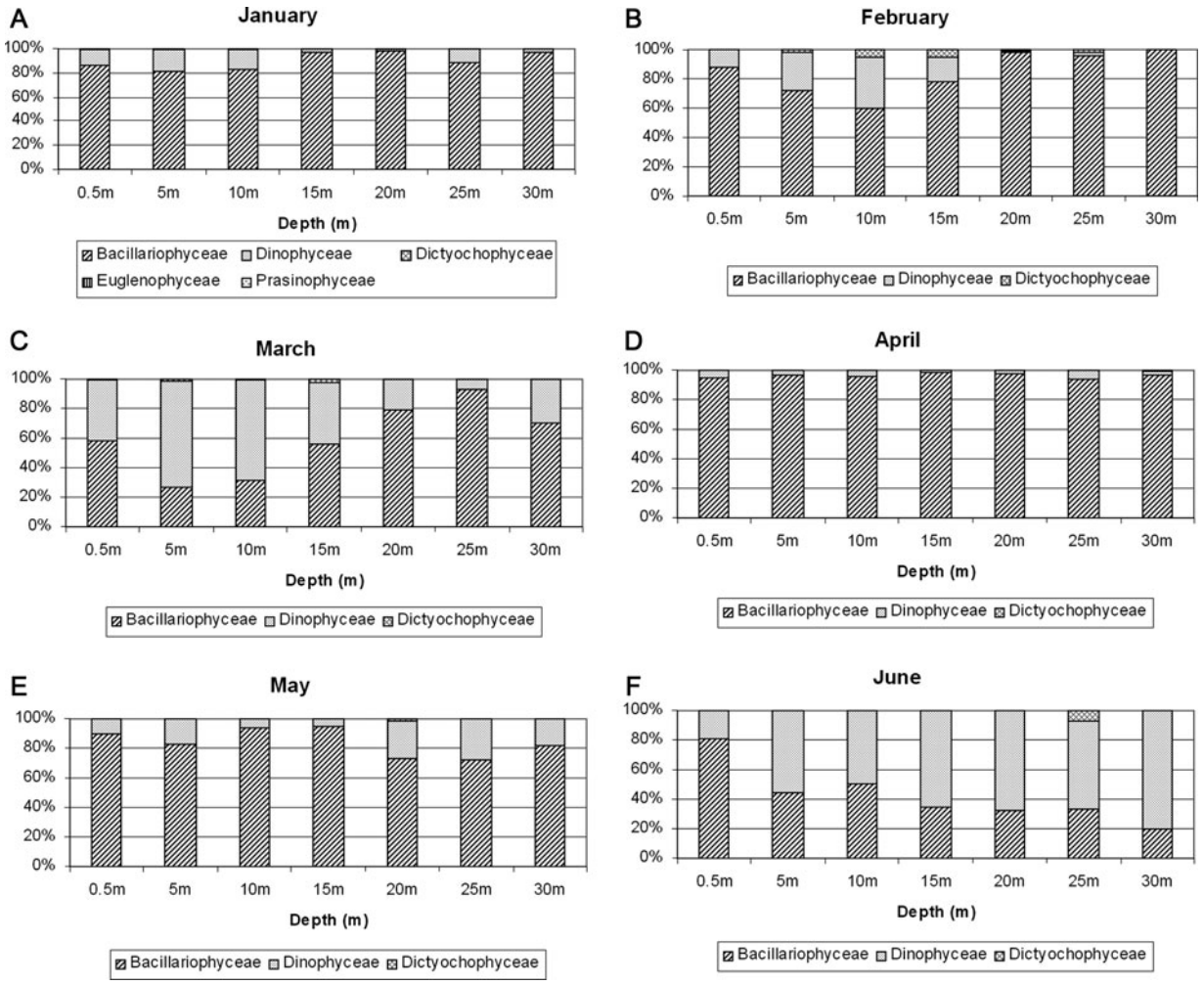

Fig. 5. Percentage composition of phytoplankton categories according to abundance in sampling months.

Distribution of individual numbers of species known to cause mucilage formation and dominant in this study and diatom:dinoflagellate ratio

The changes by month and depth observed in this study in the individual numbers of the species known to cause mucilage formation (*C. closterium, Pseudo-nitzschia* sp., *S. costatum, T. rotula* and *G. fragilis*) are shown in graphics below (Figure 6).

As can be seen in Figure 6, of diatoms *C. closterium* is the most dominant species and it is followed by *S. costatum*. Peaks are remarkable especially at 15 m depth in January, at 0.5 m depth in February and at 20–25 m depth in March, when mucilage was observed. In April, increases were seen in both upper and lower water column. Of dinoflagellates, *G. fragilis* was observed in all months until April while it was not seen in May and June with the decrease in mucilage. Of diatoms, *Pseudonitzschia* sp. played the most important role in the increase in May. In June, a considerable decrease was observed in the individual numbers of the species.

The diatom:dinoflagellate ratio ranged between 0.3 (June, 30 m) and 109.4 (February, 20 m). No dinoflagellate species was recorded at 30 m depth in February. In January and February, when mucilage formation was observed densely, especially at 20 m depth this ratio was found to be very high (44.2–109.4). In June, when mucilage formation disappeared completely, this ratio was measured to be very low (0.3–4.1) since the individual numbers of species were low and dinoflagellates showed increase compared to diatoms.

Statistical analysis

Spearman's rank-correlation analysis determined some positive and negative relations between some biotic and abiotic variables (Table 1). The density of mucilage increased meaningfully in correlation with nitrogen ($P < 0.01$), phosphorus, silica ($P < 0.05$) and also with the N:P ratio ($P < 0.01$). It was determined that the increase in the percentage of individual numbers of 5 species (*C. closterium, Pseudo-nitzschia* sp., *S. costatum*, *T. rotula* and *G. fragilis*) known to cause mucilage formation and found dominantly in this study ($P < 0.05$) and the increase in the percentage of individual number of diatoms ($P < 0.01$) were in positive correlation with mucilage formation. Especially at 15 m depth in January, when mucilage formation was observed most densely, a meaningful correlation was observed between *S. costatum* and *T. rotula*, which increased excessively, and mucilage formation ($P < 0.05$).

Of diatoms, the percentage of *C. closterium* was found to be in correlation with only phosphorus ($P < 0.01$) while the individual numbers of the species belonging to dinoflagellates

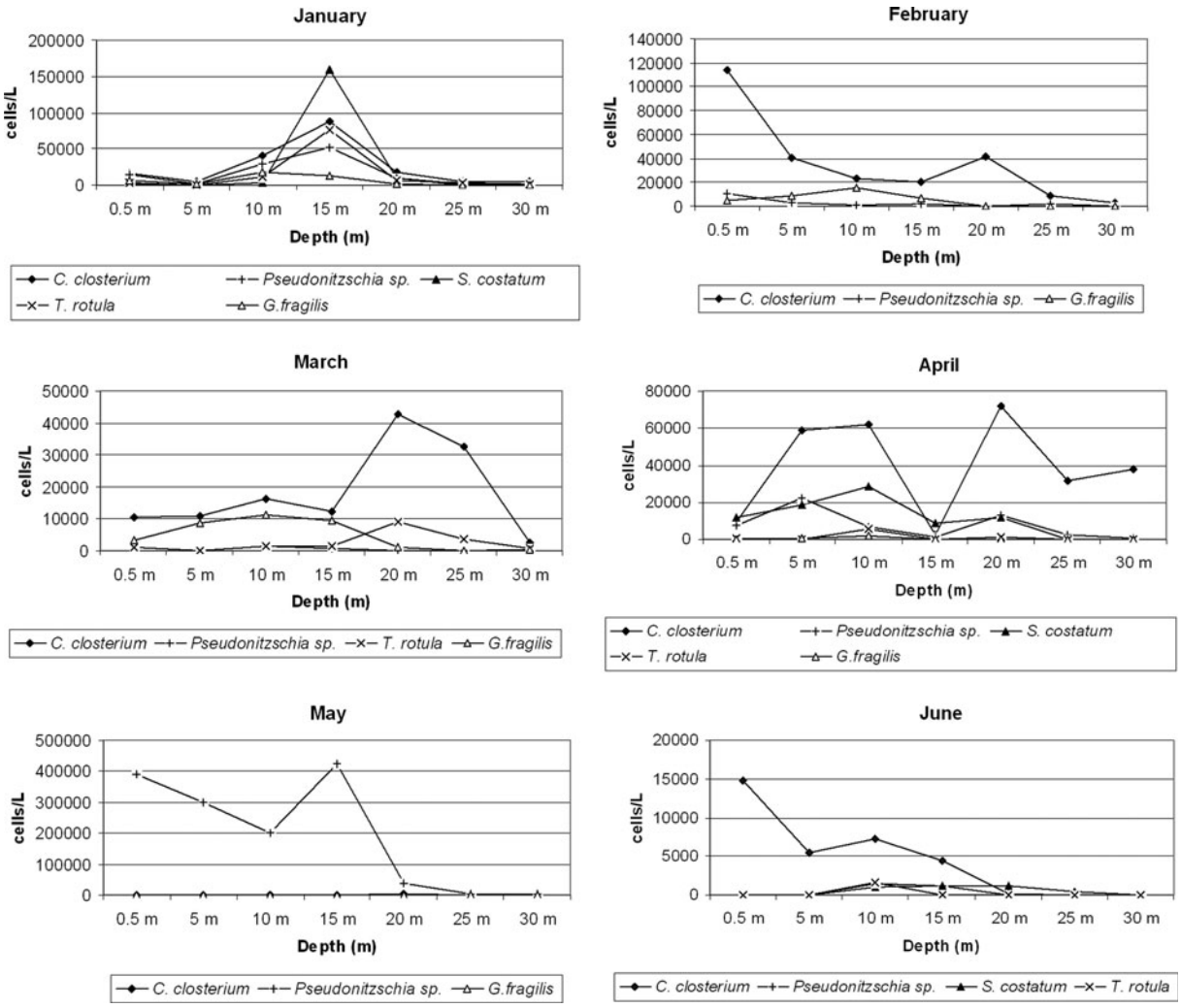

Fig. 6. Monthly distribution of abundance (cells l^{-1}) of species causing mucilage according to depth.

were negatively affected by nitrogen, phosphorus ($P < 0.01$), silica ($P < 0.05$) and also the N:P ratio ($P < 0.01$). In addition, the abundance of diatoms ($P < 0.05$) and dinoflagellates ($P < 0.01$) was negatively correlated with salinity.

Pico-, nano-, microphytoplankton and total bacteria were negatively correlated with temperature ($P < 0.01$) and positively correlated with chlorophyll-*a* ($P < 0.01$). Picophytoplankton was negatively correlated with nitrogen ($P < 0.05$) and pico- and

Table 1. Spearman's rank-correlation matrix (r_s) to correlate some biotic (DM, density of mucilage; DA, diatom abundance; DFA, dinoflagellate abundance; PI5, percentage of individual numbers of five species causing to mucilage; PDI, percentage of individual numbers of diatoms; PPHY, picophytoplankton; NPHY, nanophytoplankton; MPHY, microphytoplankton; TB, total bacteria; TPHY, total phytoplankton) and abiotic (T, temperature; S, salinity; DO, dissolved oxygen; D, depth; N, nitrogen; P, phosphorus; Si, silica) variables in the study area ($^{**}P < 0.01$, $^{*}P < 0.05$; ns, not significant; N = 42).

	T	S	DO	D	N	P	Si	N:P	Chl *a*	TRIX	DM	TPHY
DM	ns	0.514^{**}	-0.381^{**}	0.560^{**}	0.473^{**}	0.308^{*}	0.340^{*}	0.423^{**}	ns	0.439^{**}	–	ns
DA	-0.298^{*}	-0.312^{*}	ns	-0.395^{**}	ns	-0.351^{*}	-0.259^{*}	ns	0.352^{*}	ns	ns	0.958^{**}
DFA	-0.258^{*}	-0.558^{**}	0.525^{**}	-0.646^{**}	-0.589^{**}	-0.653^{**}	-0.355^{*}	-0.426^{**}	0.451^{**}	-0.463^{**}	-0.267^{*}	0.697^{**}
PI5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.306^{*}	0.456^{**}
PDI	ns	ns	-0.365^{**}	ns	0.275^{*}	ns	ns	ns	ns	0.278^{*}	0.453^{**}	0.332^{*}
PPHY	-0.887^{**}	ns	0.267^{*}	ns	-0.354^{*}	ns	ns	-0.363^{**}	0.664^{**}	ns	0.366^{**}	0.524^{**}
NPHY	-0.709^{**}	ns	ns	ns	ns	ns	ns	-0.359^{**}	0.647^{**}	ns	0.379^{**}	0.447^{**}
MPHY	-0.572^{**}	ns	0.277^{*}	ns	ns	ns	ns	ns	0.431^{**}	ns	0.433^{**}	0.289^{*}
TB	-0.690^{**}	ns	ns	ns	ns	ns	ns	ns	0.555^{**}	ns	0.535^{*}	0.620^{**}
TRIX	ns	0.613^{**}	-0.452^{**}	0.595^{**}	0.702^{**}	0.765^{**}	0.562^{**}	0.483^{**}	ns	–	0.439^{**}	ns
TPHY	-0.330^{*}	-0.401^{**}	ns	-0.484^{**}	-0.342^{*}	-0.429^{**}	-0.313^{*}	ns	0.459^{**}	ns	ns	–

nanophytoplankton with the N:P ratio ($P < 0.01$). In addition, the density of mucilage positively correlated with pico-, nano-, microphytoplankton ($P < 0.01$) and total bacteria ($P < 0.05$).

Trophic index (TRIX) values were positively correlated with depth, salinity, all nutrients, the N:P ratio and the density of mucilage ($P < 0.01$) and negatively correlated with the abudance of dinoflagellate ($P < 0.01$).

DISCUSSION

The chemical oceanography of the Marmara Sea is remarkably affected by the Black Sea and the Aegean Sea, and the basin includes two different water masses. One of these masses consists of the low salinity waters originating from the Black Sea and observed as a relatively thin layer (10–15 m) on the surface, and the other of waters with higher salinity originating from the Mediterranean Sea, flowing at the bottom and separated by a sharp intermediate layer with a thickness of about 10–20 m (pycnocline) (Ünlüata *et al.*, 1990; Tuğrul & Polat, 1995). In this study, after 15–20 m depth an increase was observed in the salinity values and the effect of the Mediterranean waters on this increase was remarkable. In addition, the increase in temperature at 30 m depth during cold periods indicates the effect of the Mediterranean waters. On the other hand, the increase in surface waters during warm periods is due the effect of light and the contact of this layer with the atmosphere.

A decrease was observed in oxygen values generally from the surface to the bottom along the water column. The main reasons for this decrease, remarkable especially at 25–30 m depth, are bacterial decomposition and less occurrence of phytoplankton activity (photosynthesis) due to insufficient light at these depths.

The highest nutrient values were recorded generally at the bottom layer where there was aggregation. The reason for the determination of low amounts in the upper water column compared to the bottom layer is the consumption of nutrients due to the increase in phytoplankton at these depths. Especially, the increase in nitrogen amount at 20–30 m depth is remarkable since this element comes out due to the bacterial decomposition of the organic substances aggregated at the bottom. The N:P ratios recorded during the study are below 16:1 and N is the limiting element in the sampling area. The increase in the N:P ratio is remarkable at lower layers in comparison to the surface water, which shows that phosphorus increases in proportion to nitrogen in the surface water while nitrogen increases in proportion to phosphorus in lower layers. Mucilage formation in the North Adriatic Sea and Tyrrhenian Sea is observed more densely especially in the deficiency of P and less densely in the deficiency of N (Innamorati *et al.*, 2001; Pompei *et al.*, 2003). According to Innamorati *et al.* (2001), in nature the deficiency of P can be considered as a necessary condition for the appearance of mucilages, but not the only factor.

During the sampling period, the N:Si ratio was below 1 except for two months of the period and two depths, which showed that silica is not a limiting element for the sampling area. These values allowed sufficient development of diatoms. The main reason for the high N:Si ratio especially in the lower layer waters is that in the upper water column silica is consumed by diatoms in building cell wall. According to the Spearman's rank-correlation analysis, the density of mucilage increased meaningfully with the N:P ratio besides nitrogen, phosphorus and silica in seawater. This shows that mucilage is closely correlated with the increase in the amount of nutrition element in seawater.

Diatoms are found densely in waters rich in nutrients and with a good mixture (Haris, 1986) and are one of the groups playing an essential role in mucilage formation (Rinaldi *et al.*, 1995). High nutrient concentrations accelerate cell division and the mixture decelerates the sinking of cells to the bottom (Arin *et al.*, 2002). Since the levels of nutrients are high in coastal areas, diatoms might reach considerably high levels in comparison to dinoflagellates in terms of density in these areas. While silica, which plays a role especially in building the cell wall of diatoms, was found in high amounts in the study area, it did not limit growth. That nutrient values were at sufficient levels for phytoplankton growth provided adequate conditions for the increase of diatoms which needs high nutrient levels. The recorded diatom:dinoflagellate ratios also indicated the dominance of diatoms. In January and February, when mucilage formation was observed densely, at especially 15–20 m depth this ratio was found to be very high (44.2–109.4). In June, when mucilage formation was not observed, this ratio was measured to be rather low (0.5) since the individual numbers of species was low and dinoflagellates increased in comparison to diatoms. Besides, in January, when increase in chlorophyll-*a* was observed, at 15 m depth (6.35 $\mu g\ l^{-1}$) and in April at 10 m depth (4.89 $\mu g\ l^{-1}$) an increase was observed in the abundance of phytoplankton, and diatoms played a significant role in this increase.

In this study conducted on Büyükada Island mucilage formation was observed most densely in January, February and March, and the density decreased gradually in April and May, and in June almost no mucilage was observed. A meaningful correlation was found between *C. closterium, T. rotula* and *S. costatum*, main species among those known to cause mucilage formation, and a mucilage event according to the Spearman's rank-correlation analysis. These species were also found dominantly in the studies carried out in İzmit Bay (Tüfekçi *et al.*, in press). However, although the dinoflagellate *G. fragilis* had not been recorded until 2007, it appeared with the beginning of mucilage formation in the Marmara Sea, increased in some periods and disappeared in May and June, when mucilage begins to disappear. These observations indicate that this species is also effective on mucilage formation as was reported in the previous studies conducted in the Adriatic Sea. Pompei *et al.* (2003) detected the capacity of this organism to produce large amounts of extracellular carbohydrates in culture and stated that a few thousand *G. fragilis* cells release the same amount of carbohydrates as that produced by tens of millions of *C. closterium* cells. In August 2005, the maximum individual number of this species on Greek coasts was determined to be 7.92×10^3 cells l^{-1} and the species was stated to cause mucilage formation (Nikolaidis *et al.*, 2006). In this study, the maximum individual number of *G. fragilis* at 10 m depth in January was determined to be 1.82×10^4 cells l^{-1}.

While the mucilage event was in positive correlation with all nutrients, it was in negative correlation with the individual number of dinoflagellates. Dinoflagellates were in negative correlation with nutrients, which indicates that dinoflagellates are not so effective on the mucilage event. The real reason

responsible for the mucilage event is the species belonging to diatoms that grow with the increase in nutrients. In addition, it is also known that diatoms grow better in mucilage mass in comparison to dinoflagellates (Pompei *et al.*, 2003; Tinti *et al.*, 2007). In the mucilage event that occurred in the Adriatic Sea in 1988 and 1999, Revelante & Gilmartin (1991) found an enrichment of species, such as *Nitzschia longissima* and *C. closterium*, at higher levels than in the surrounding seawater. The aggregates represent a microenvironment very suitable for the development of a rich community of microorganisms which is separated from the surrounding water (Simon *et al.*, 2002), but phytoplankton communities associated with mucilage aggregates have been shown to vary, with different dominating species, depending on sampling area and period (Revelante & Gilmartin, 1991; Cabrini *et al.*, 1992; Totti *et al.*, 2005).

A positive increase was observed in the number of pico-, nano-, microphytoplankton ($P < 0.01$) and total bacteria ($P < 0.05$) by the density of mucilage. Negro *et al.* (2005) determined that exopolysaccharides in mucilage aggregates could meet the carbohydrate need of bacteria and bacteria that are in relation with mucilage aggregates are metabolically more active according to enzyme analyses. As a result, it might be stated that the presence of mucilage could lead to an increase in the bacteria in water. In April, when the number of total bacteria is the highest at 25 m depth (6655 cells ml^{-1}), the dissolved oxygen value is the lowest (2.75 mg l^{-1}) due to the bacterial activity.

It is our opinion that the mucilage formation in the Marmara Sea is mainly due to the excretory activity of some diatoms together with bacteria, the dinoflagellate *G. fragilis*, the presence of sharp pycnocline and thermocline caused by the two-layered water system of the Marmara Sea; besides which, the weather conditions and the status of currents during that time are effective on this formation.

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Correspondence should be addressed to:
N. Balkis
Istanbul University, Faculty of Science
Department of Biology
34134 Vezneciler-Istanbul
email: neslbalk@istanbul.edu.tr