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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Spring bloom of the raphidophycean *Heterosigma akashiwo* in the Golden Horn Estuary at the northeast of Sea of Marmara

Marmara Denizi'nin kuzeydoğusundaki Haliç'te rafidofit *Heterosigma akashiwo*'nun ilkbahar aşırı çoğalması

Fuat Dursun[1] • Seyfettin Taş[1*] • Tufan Koray[2]

[1] *Institute of Marine Sciences and Management, Physical Oceanography and Marine Biology Department, Istanbul University, 34134, Vefa-Fatih, Istanbul-Turkey*
[2] *Ege University, Faculty of Fisheries, 35100 Bornova, Izmir, Turkey*
** Corresponding author: stas@istanbul.edu.tr*

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Abstract: In the period of February-May 2012, harmful algal blooms (HABs) along with environmental factors were investigated biweekly in the Golden Horn Estuary at the northeast of the Sea of Marmara and a dense bloom of the raphidophyceaen *Heterosigma akashiwo* (Y.Hada) Y.Hada ex Y.Hara & M.Chihara was observed in late May. *H. akashiwo* abundance increased gradually from the lower estuary to the upper estuary. The dense bloom of *H. akashiwo* in late May occurred at the temperature of 20.2°C and salinity of 16.4 psu and its abundance reached to 10.4×10^6 cells L^{-1}. When compared to mid-May, the mean temperature of surface water increased 4.50°C, while salinity decreased 2.30 psu in late May. Secchi depth values decreased rapidly from the lower to the upper estuary (6.00 m to 0.50 m). It is considered that a rapid increase in temperature, a decrease in salinity, and high nutrient concentrations in combination with low water circulation at the upper estuary were the causes of the bloom of *H. akashiwo*. Fish mortality or other harmful effects in environment were not observed during the *H. akashiwo* bloom. But, these events may create a potentially toxic risk for the study area in the future.

Keywords: *Golden Horn Estuary, Harmful algal blooms, Heterosigma akashiwo, nutrients, Sea of Marmara*

Öz: Marmara Denizi'nin kuzeydoğusunda bulunan Haliç'te Şubat ve Mayıs 2012 arasındaki dönemde, iki haftada bir olmak üzere çevresel faktörlerle birlikte zararlı alg aşırı üremeleri incelendi ve Mayıs sonunda rafidofit *Heterosigma akashiwo* (Y.Hada) Y.Hada ex Y.Hara & M.Chihara türünün yoğun bir aşırı çoğalması gözlendi. *H. akashiwo* bolluğu Haliç'in aşağı bölümünden yukarı bölümüne doğru giderek artmıştır. Mayıs sonundaki *H. akashiwo*'nın yoğun aşırı çoğalması 20.2°C sıcaklık ve 16.4 psu tuzlulukta meydana gelmiş ve bolluğu 10.4×10^6 hücre L^{-1}'ye ulaşmıştır. Mayıs ortası ile karşılaştırıldığında Mayıs sonunda ortalama yüzey suyu tuzluluk değerleri 2.30 psu, sıcaklık değerleri ise 4.50°C artış göstermiştir. Seki derinliği aşağı bölümden yukarı bölüme doğru hızlı bir şekilde azalmıştır (6.00 m'den 0.50 m'ye). Ani bir sıcaklık artışı, tuzlulukta düşüş ve düşük su sirkülasyonu ile birlikte yüksek besin elementleri konsantrasyonu yukarı Haliç'te *H. akashiwo*'nın aşırı çoğalmasının nedenleri olarak düşünülmektedir. *H. akashiwo* aşırı çoğalması boyunca balık ölümü veya ortamda diğer zararlı etkiler gözlenmemiştir. Ancak bu olaylar gelecekte bu çalışma bölgesi için potansiyel toksik bir risk oluşturabilir.

Anahtar kelimeler: *Besin elementleri, Haliç, Heterosigma akashiwo, Marmara Denizi, Zararlı alg aşırı üremeleri*

INTRODUCTION

Heterosigma akashiwo (Y.Hada) Y.Hada ex Y.Hara & M.Chihara (Raphidophyceae) has been known to cause to harmful algal blooms (HABs) and to fish kills around the world (Rensel, 2010). HABs of *H. akashiwo* caused to the large economic losses in British Columbia salmon farms (Haigh and Esenkulova, 2013). In a bloom occurred in Cowichan Bay, Canada, in mid-summer of 2014, high concentrations of *H. akashiwo* (max. 4.00×10^4 cells mL^{-1}) caused to lethargic behaviour in salmon species (Esenkulova and Luinenburg, 2014). The harmful effects of *H. akashiwo* blooms on the fish are associated with physical clogging of fish gills by mucus excretion and gill damage by haemolytic substances (Shimada et al. 1983; Chang et al. 1990). Some species and strains of raphidophytes such as *Chattonella*, *Heterosigma* and *Fibrocapsa* may contain brevetoxin-like neurotoxins (Khan et al. 1996; Bridgers et al. 2002, Hallegraeff et al. 2003) and *H. akashiwo* have been considered as the causative organisms of the massive fish mortalities (Mendez et al. 2010). Allelopathic

effects of *H. akashiwo* are also known for marine invertebrates such as copepods, shrimps and scallops (Keppler et al. 2005; Wang et al. 2006; Yu et al. 2010).

The previous studies on phytoplankton carried out in this study area, when it was extremely polluted, demonstrated that phytoplankton abundance was very low and even they were absent particularly at the upper estuary (Uysal and Unsal, 1996; Tas and Okus, 2003; Tas et al. 2009). During rehabilitation of the GHE (after 2000), phytoplankton abundance increased and dense blooms of phytoplankton occurred in spring and summer (Tas et al. 2009; Tas and Okus, 2011; Tas, 2015). In the previous years, *H. akashiwo* has been recorded in Turkish Seas e.g. in the eutrophic coastal waters of Izmir Bay (Aegean Sea) (Bizsel and Bizsel, 2002; Koray, 2004) and at the near shore of the Sea of Marmara (Deniz and Tas, 2009). The previous study carried out in the Golden Horn Estuary reported summer and autumn blooms of *H. akashiwo* and no harmful effect on the ecosystem caused by this species was recorded (Tas and Yilmaz, 2015).

The main goal of this study is to evaluate a dense bloom of the raphidophycean *Heterosigma akashiwo* in relation to environmental parameters in the Golden Horn Estuary at the northeast of the Sea of Marmara.

MATERIALS AND METHODS

Study area

The Golden Horn Estuary (GHE) is located in southwest of the Strait of Istanbul and is approximately 7.50 km long and 700 m wide, with a surface area of 2.60 km^2 (Figure 1). The study area is divided in three parts: Lower estuary (LE), middle estuary (ME) and upper estuary (UE). The LE is the deepest section (40.0 m) and it has a strong interaction with the Strait of Istanbul (Bosphorus). The depth rapidly decreases to 14.0 m in the ME, where a bridge operating on buoys limits the upper layer circulation. The UE has a depth of 4.00 m due to a high degree of sedimentation (Figure 1). Although the two streams as known Alibey and Kağıthane carry freshwater to the GHE, the amount of the water runoff is generally low. Therefore, the main source of the freshwater flowing into the GHE is rainfall (Sur et al. 2002). The LE is characterized by a two-layered structure similar to the neighboring Strait of Istanbul where upper layer waters has a salinity of ~18 psu, originated from Black Sea and lower layer waters has a salinity of ~38 psu, originated from the Mediterranean Sea.

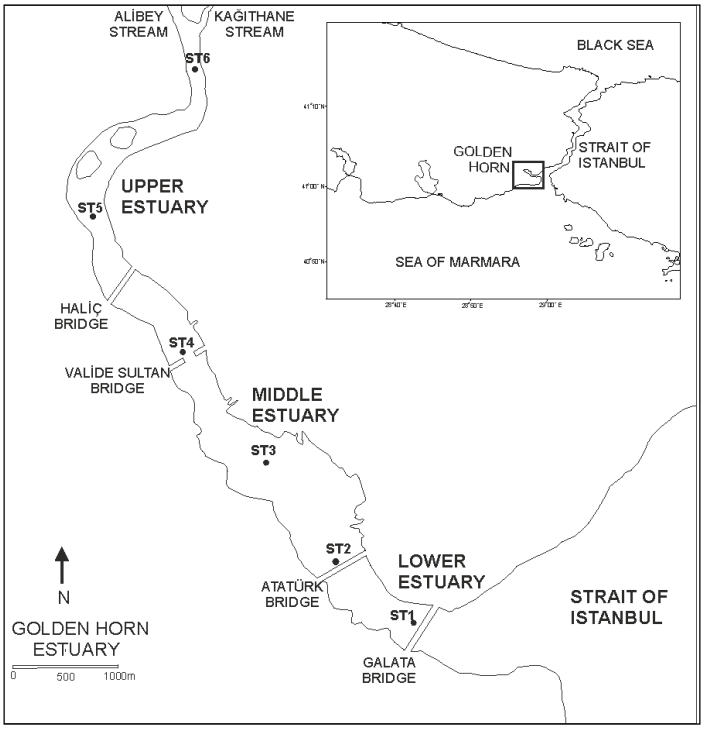

Figure 1. Study area and sampling stations

Sampling and analyses

Seawater samples were biweekly collected between 21 February and 31 May 2012 in surface water using a Niskin bottle at the six sampling sites. Temperature, salinity, dissolved oxygen (DO) and pH were measured by a multi-parameter probe (YSI Professional Pro Plus). Light transparency was measured using a Secchi-disc. Inorganic nutrients (NO_3+NO_2

and PO_4) were analyzed according to methods APHA (1999). Chlorophyll a (Chl-a) analyses were carried out by acetone extraction method according to Parsons et al. (1984). For phytoplankton analyses, 250 mL seawater was taken by Niskin bottles and preserved with acidic Lugol (2.00%). 50 mL subsamples were settled according to Utermöhl Sedimentation Method (Utermöhl, 1958). *H. akashiwo* cells were counted at 200× magnification under a Leica DM IL LED inverted microscope equipped with phase contrast.

Morphological analyses of *H. akashiwo* cells were carried out using a Leica DM IL LED inverted phase contrast microscope equipped with Image Leica Application Suite software, LAS version 3.8.0 (Leica Microsystems Limited). Taxonomic identification was based on morphological characteristics such as cell shape and size, number, colour and shape of chloroplasts according to Hallegraeff et al. (2003).

RESULTS

Environmental factors

The spatial fluctuations in main environmental variables in the three parts (LE, ME and UE) of the GHE are given in Table 1. Relationships between abundance of *H. akashiwo* and environmental variables are presented in Figures 2 and 3.

Table 1. The mean values and standard deviations (SD), minimum and maximum values of environmental factors in surface water of three parts of the GHE during the study period

Parameters	LE		ME		UE	
	Mean ± SD	Min-Max	Mean ± SD	Min-Max	Mean ± SD	Min-Max
Temperature (°C)	9.30±4.91	4.10-16.8	10.6±5.05	4.50-19.3	11.3±5.39	5.30-20.7
Salinity (psu)	18.9±0.43	18.4-19.5	17.2±1.60	15.5-19.1	14.9±2.32	6.40-19.0
Secchi depth (m)	7.20±1.38	6.00-9.00	2.70±1.49	1.00-5.00	1.30±0.64	0.50-3.00
NO_3+NO_2-N (µg L^{-1})	5.30±2.94	1.80-10.3	11.0±9.50	0.30-25.6	11.9±8.35	0.10-21.6
PO_4-P (µg L^{-1})	0.60±0.46	0.30-1.60	1.70±0.91	0.40-5.20	3.10±0.99	0.80-11.5
Chl-*a* (µg L^{-1})	1.90±1.19	0.60-3.70	9.70±31.5	0.50-86.0	8.00±23.9	0.60-65.9
DO (mg L^{-1})	10.9±1.63	9.40-13.4	9.10±2.36	5.90-11.5	5.20±2.78	0.20-11.1
pH	7.80±0.27	7.30-8.10	7.90±0.17	7.30-8.20	8.00±0.15	7.70-8.30

Temperature showed the minor differences between the three parts of the estuary, increasing relatively from the LE to the UE. The mean temperatures in three parts of the GHE for sampling period varied between 9.30 °C (LE) and 11.3 °C (UE). But, *H. akashiwo* bloom formed at 20.2 °C. Surface salinity was always lower in the UE and the mean values ranged between 14.9 (UE) and 18.9 psu (LE). But, salinity was 16.4 during the bloom (Figure 2).

Secchi depth decreased significantly from the LE to the UE. The maximum Secchi depth was measured as 9.00 m at the LE in mid-May, whereas its minimum value was measured as 0.50 m at the UE in late May (Figure 2).

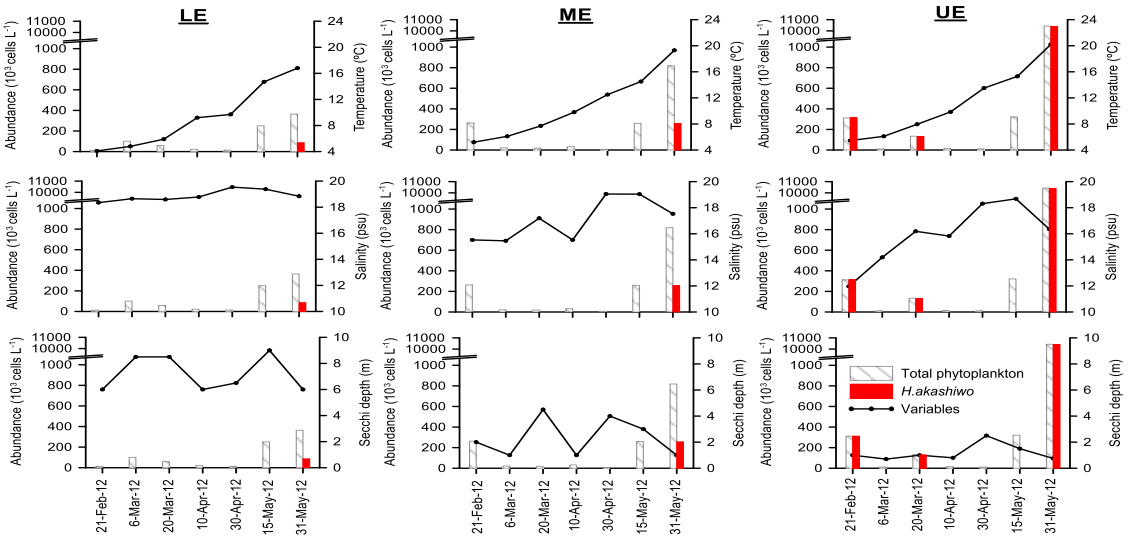

Figure 2. Relationships between *H. akashiwo* abundance and physical factors in the GHE

Inorganic nutrient concentrations increased remarkably from the LE to the UE due to high amount of nutrient carrying by two streams. While the mean values of NO_3+NO_2-N varied between 5.30 (LE) and 11.9 µg L^{-1} (UE), PO_4-P concentrations ranged between 0.60 (LE) and 3.10 µg L^{-1} (UE) (Figure 3).

Chl-*a* mean values varied between 1.90 and 8.00 µg L^{-1} from the LE to the UE. The highest chl-*a* (86.0 µg L^{-1}) was measured in the ME in late May (Figure 3). DO concentrations were often higher at the LE than two other parts of the GHE due to strong interaction with the Strait of Istanbul. The mean DO concentrations changed between 5.30 (UE) and 10.9 mg L^{-1} (LE) (Figure 3). The highest DO concentration (13.4 mg L^{-1}) was measured in February at the LE, while the lowest DO value (0.2 mg L^{-1}) was measured at the UE in late May (Figure 3). pH values varied between 7.30 (March, LE) and 8.30 (late May, UE). The mean pH values were slightly increased (7.80-8.00) from the LE to the UE (Figure 3).

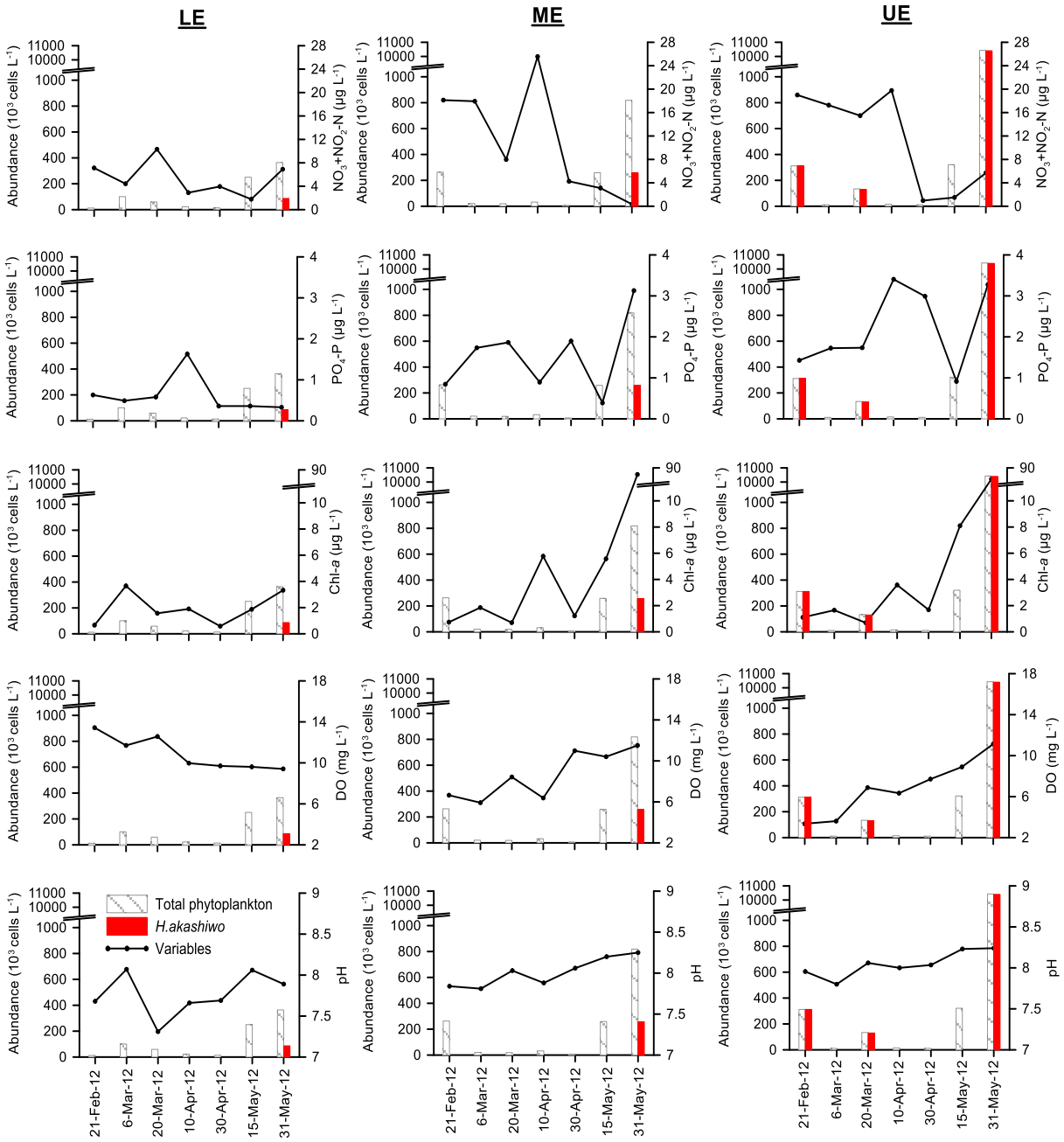

Figure 3. Relationships between *H. akashiwo* abundance and chemical factors in the GHE

Morphology of *H. akashiwo* cells

Although cells of *H. akashiwo* were observed as solitary sometimes (Figure 4A), they were observed as masses of immobile spherical cells surrounded by mucilage in some cases (Figure 4B-C). Cell shape varied from ovoid to elliptical, compressing slightly dorso-ventrally, in the length of 8.70-16.20 µm (average 13.3 µm, n=20) and in the width of 7.20-13.20 µm (average 9.90 µm, n=20), with two subequal heterodynamic flagella inserted in anterior subapical depression.

Chloroplasts typically varied from yellowish-brown to greenish yellow and the cells contained 8-16 discoid chloroplasts (average 12, n=20) situated at the cell (Figure 4C).

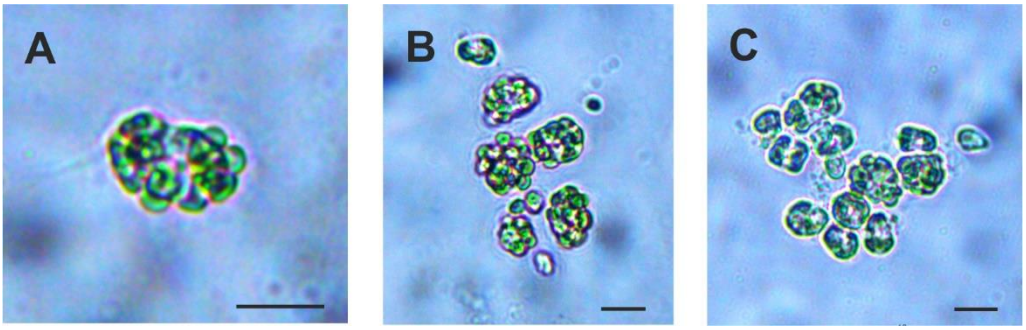

Figure 4. Light micrographs of *Heterosigma akashiwo* cells in bloom samples in late May in the GHE (A-C). A: Single cell of *H. akashiwo*; B-C: *H. akashiwo* cells in mucilage colony (Scale bars: 10µm)

Cell abundance in relation to environmental parameters

Total phytoplankton abundance including diatoms, dinoflagellates and other flagellates was generally low during the study. However, the abundance began to increase in mid-May. While phytoplankton was dominated by diatoms in March, it was dominated by euglenophycean flagellates in early April. It was observed again a diatom increase in late April and this situation lasted in mid-May. Thereafter, *H. akashiwo* formed a dense bloom in late May following the diatom increase in mid-May. During the bloom, the contribution of *H. akashiwo* to total phytoplankton increased gradually from the LE to the UE (from ~24% to ~100%). Fluctuations of *H. akashiwo* and total phytoplankton abundance in the study area are shown in Figures 2 and 3.

H. akashiwo was firstly found at the UE in February and its cell density was ~0.30×10^6 cells L^{-1}. It decreased to ~0.20×10^6 cells L^{-1} in March at the UE. *H. akashiwo* cells were not identified in the LE and ME in these periods. Among April and mid-May, when a diatom increase occurred, *H. akashiwo* cells were not observed throughout the study area. The bloom of *H. akashiwo* occurred at the UE in late May in parallel to a significant temperature rise (~4.50°C) (Figure 2). *H. akashiwo* cells were observed at a wide range of water temperatures from 5.30 (February) to 20.2 °C (late May) and the highest *H. akashiwo* bloom density occurred at 20.2 °C. The relationship between *H. akashiwo* abundance and temperature levels can be clearly followed in Figure 2. Salinity values were generally lower at the UE than the LE and *H. akashiwo* showed the highest bloom in the UE where salinity values were measured in interval of 14.2 - 16.4 psu in late May. However, in mid-May when the salinity was in 18.7 psu in the UE, there was no bloom caused by *H. akashiwo* in this part of the GHE (Figure 2).

DO concentrations were generally lower in the UE than the other parts of the GHE and they increased to 11.1 mg L^{-1} during the *H. akashiwo* bloom. The highest chl-*a* value (~66 µg L^{-1}) was recorded in the highest bloom period of *H. akashiwo*. Inorganic nutrient concentrations were higher between February and March than between April and mid-May, probably due to their high uptakes by other phytoplankton groups in middle and late spring periods. However, NO_3+NO_2-N concentrations rapidly decreased (1.80 µg L^{-1}) in the ME in late May, when diatom abundance was high. But, it was higher (5.70 µg L^{-1}) in the UE at the same period. PO_4-P concentration decreased (0.80 µg L^{-1}) in mid-May, but it increased to 3.38 µg L^{-1} in the UE in late May, probably high amount of nutrient input by the streams (Figure 3).

DISCUSSION

Identification of fixed cells of *H. akashiwo* based on morphological characters under the light microscope is not easy. When preserved with fixatives, their cell membrane shrinks tightly around the cell giving a view similar to a blackberry. Cell morphology, the number and colour of chloroplasts of *H. akashiwo* observed in the GHE were in agreement with the previous descriptions and illustrations in the literature (Tomas, 1997).

Extensive *H. akashiwo* blooms have been observed following the initial spring diatom blooms in the Fraser River (Rensel et al. 2010). Also, in the GHE, the bloom of *H. akashiwo* occurred following a diatom increase as similar to in Fraser River. Moreover, it is known that *H. akashiwo* was reported from

the eutrophic waters of the Izmir Bay, the Aegean Sea (Bizsel and Bizsel, 2002; Koray, 2004). The first record of *Heterosigma* cf. *akashiwo* in the Sea of Marmara was given by Deniz and Tas (2009). As similar to the period of the maximum cell density of *H. akashiwo* in the GHE, Shikata et al. (2008) reported that the maximum cell density of this species (1.20×10^6 cells L^{-1}) was at the end of May in the Hakata Bay, Japan. In the previous study carried out in the GHE, there were two blooms of *Heterosigma* cf. *akashiwo* in the ME in June and September 2010. The bloom in June (13.9×10^6 cells L^{-1}) caused water discoloration and the abundance pattern of the species was highly related to the temperature (Tas and Yilmaz, 2015). In this study, the dense bloom of *H. akashiwo* was observed almost at the same time period (late May) and same part (UE) of the estuary (GHE). Therefore, late May and early June provide the favorable conditions to form dense blooms caused by *H. akashiwo* in the GHE.

Germination of Heterosigma cysts is known to be successful above 15.0 °C temperature (Rensel, 2007). In the study area, the blooms of *H. akashiwo* occurred following a temperature increase reached to the 15.0 °C which is the lower limit of the water temperature required for activation of the cysts (Taylor and Haigh, 1993) and the bloom reached to the maximum levels in late May. Kempton et al. (2008) reported the dense bloom of *H. akashiwo* (19.5×10^6 cells L^{-1}) in the temperature level of 22.7 °C. In a *H. akashiwo* bloom (1.20×10^6 cells L^{-1}) occurred in the Hakata Bay of Japan, water temperature was measured over 20.0 °C (Shikata et al. 2008). Orlova et al. (2010) reported that the dense bloom of *H. akashiwo* (1.00×10^8 cells L^{-1}) occurred in the east coastal area of Vladivostok, Russia at a temperature level of 22.0 °C in June. In the period of *H. akashiwo* bloom occurred in this study area in June 2010, water temperature was 21.7 °C (Tas and Yilmaz, 2015). Both previous studies and this study revealed that there is a significant positive relationship between temperature and bloom formation of *H. akashiwo*, when it was taken into account the temperature rise (from 15.8 to 20.2 °C) between middle and late May.

H. akashiwo is a euryhaline raphidophycean and can grow at low salinity values under the 10.0 psu, but it grows faster at salinities over 10.0 psu (Rensel et al. 2010). Kempton et al. (2008) stated that *H. akashiwo* bloom related to a rapid decrease in salinities (from 31.4 to 21.3 psu). The water salinity was measured as 16.9 psu during the dense bloom of *H. akashiwo* occurred in the GHE in June 2010 (Tas and Yilmaz, 2015). Similar to previous studies, also in the current study, lower salinity values were measured in the upper estuary (UE) in late May compared to middle May. This shows a little negative relationship between salinity and *H. akashiwo*. During this study, the excessive bloom of *H. akashiwo* occurred in the salinity level of 16.4 psu, compatible with the tolerance of this species to low salinities previously reported by various researchers (Branco et al. 2014; Taylor and Haigh, 1993). Salinity levels between 14.0 and 16.0 psu appears to be more appropriate for excessive *H. akashiwo* bloom as shown also in this study area and salinitiy levels higher than 17.0 psu may considerably limit the growth of the *H. akashiwo*. Some authors reported that *H. akashiwo* is a superior competitor at low inorganic nutrient levels (Zhang et al. 2006) and some suggested that it has relatively high inorganic nutrient requirements compared with other flagellates (Smayda, 1998) and *H. akashiwo* bloom occurred together with an increase in DIP and DIN concentrations (Shikata et al. 2008). Nutrient concentrations decreased in mid-May probably due to high diatom uptake, but they increased again in late May probably due to nutrient input by two streams, when *H. akashiwo* bloomed.

The environmental conditions and the ability to form cysts in *H. akashiwo* play an important role in the population increase and germination of cysts is known to be successful above 15°C (Rensel, 2007). A rapid temperature rise (15.7 to 20.2°C) in the GHE in late May might be caused to the activation of *H. akashiwo* cysts. Thus, one of the most important factors causing the dense bloom of *H. akashiwo* is temperature rise. This ability allows to *H. akashiwo* more competitive against other phytoplankton species. Demirel (2015) stated that density and diversity of fish eggs and larvae gradually decrease from the LE towards the UE, due to the deteriorated environmental conditions. No fish mortality was observed caused by Heterosigma akashiwo during the bloom in this study probably due to the scarce of fish assemblages as mentioned above. Although any fish mortality or other harmful effects caused by *H. akashiwo* dense bloom were not observed, these events should be noted for the potential harmful risk in the near future.

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