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First harmful algal bloom record of tytoplanktonic dinoflagellate *Prorocentrum lima* (Ehrenberg) F. Stein, 1878 in the Dardanelles (Turkish Straits System, Turkey)

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ABSTRACT

This study focused on daily variations and harmful algal bloom features of toxic dinoflagellate *Prorocentrum lima* (Ehrenberg) F. Stein (*P. lima*), 1878 in middle summer period (9th July–6th August 2013) in the Dardanelles. Harmful algal bloom of *P. lima* was recorded for the first time in the Turkish Straits System. Density of *P. lima* reached to 2.40×10^6 cells/L and exhibited four excessive blooms over 1.0×10^6 cells/L during the study. The contribution of *P. lima* to both *Prorocentrum* spp. and dinoflagellates reached to 100%, particularly at the moment of the harmful algal bloom attested by regression ($R \geq 0.70$) and correlation findings ($R \geq 0.80$). Nutrient concentrations were lower than previous levels due to excessive blooms. Concentrations of $\text{NO}_2 + \text{NO}_3$, PO_4^{3-} and SiO_4 varied between 0.20 and 0.78 $\mu\text{mol/L}$ [$(0.44 \pm 0.17) \mu\text{mol/L}$], 0.08 and 0.18 $\mu\text{mol/L}$ [$(0.12 \pm 0.03) \mu\text{mol/L}$] and 0.25 and 0.65 $\mu\text{mol/L}$ [$(0.41 \pm 0.09) \mu\text{mol/L}$] respectively. During the bloom, nutrient ratios were more different than redfield ratios due to eutrophication ($\text{NO}_2 + \text{NO}_3/\text{PO}_4^{3-} = 4.04 \pm 1.74$; $\text{SiO}_4/\text{PO}_4^{3-} = 3.79 \pm 1.24$; $\text{SiO}_4/\text{NO}_2 + \text{NO}_3 = 1.04 \pm 0.36$). Chlorophyll a concentration reached to 8.52 $\mu\text{g/L}$ (average: $4.82 \pm 2.29 \text{ mg/L}$) in the bloom period. Temperature [$(24.70 \pm 0.44) ^\circ\text{C}$], salinity [$(22.9 \pm 0.49) \text{ ppt}$], pH (8.23 ± 0.15) and dissolved oxygen levels ($7.35 \pm 0.60 \text{ mg/L}$) were approximately constant. The compact bloom of *P. lima*, similar to excessive blooms of other dinoflagellates and diatoms, was associated not only with eutrophication, but also with ocean warming interactions. Results revealed that it will be possible to reach to millions of cell number of *P. lima* (2.40×10^6 cells/L) in eutrophied waters characterized by high chlorophyll a biomass (8.52 $\mu\text{g/L}$).

1. Introduction

Phytoplankton which is single celled microscopic plants is the base of the life in aquatic environments[1,2]. Harmful algal blooms (HABs) with phytoplankton origin generally grow in all eutrophic aquatic environments[1,2]. HABs dramatically increase and cause serious environmental and economic losses in worldwide. In coastal habitats of the Mediterranean Sea, dramatic changes based on anthropogenic influxes occurred in the past a few decades as a result of high industrial and tourism activities on coastlines[3-8]. Aquatic systems such as bays, harbours, estuaries and lagoons are more affected by HABs due to their limited water exchanges[9,10]. One of the most important factors for the formation and toxicity of HABs is higher nutrient concentrations than their background levels[7,8,11]. The other most important factor is average temperature increase in the oceans as a result of climate change having impact on the increasing levels of HABs[12-14]. Over the past decade, various

studies reported possible relationships between HAB events and climate change[3,7,8,15,16].

Generally, great amounts of *Prorocentrum* spp. which includes harmful species such as *Prorocentrum compressum*, *Prorocentrum lima* (*P. lima*) and *Prorocentrum micans* can accumulate in high dense usually in summer period in eutrophic marine systems[17,18]. Dense blooms of *Prorocentrum* spp. grow not only during the summer period, but also in late winter and early spring periods in such systems[19-21]. Marine dinoflagellate *P. lima* (Ehrenberg) F. Stein, 1878 frequently occurs excessive blooms in tropical and subtropical systems[22]. On the other hand, due to the fact that it is cosmopolitan dinoflagellate species (Steidinger), *P. lima* occurs also often similar blooms in cold temperate areas[22-24]. *P. lima* is not only prevalent in more salty marine systems, but also common in some brackish water systems such as Turkish Straits System[3,19], and the Black Sea[25,26] where salinity values are lower (18–26 ppt) than any other marine system (35–40 ppt). *P. lima* generally lives in neritic and estuarine zones as benthic, epiphytic and tytoplanktonic and reveals important worldwide variations in size as well as shapes (30–50 μm in length; 20–30 μm wide)[23,27]. The species produces a colorless resting cyst as part of its life cycle under not suitable conditions to grow[23,28,29]. It is known that *P. lima* produces some toxins such as okadaic acid[30] and various

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dinophysistoxins (DTX-1, DTX-2, DTX-4)[30-32]. These toxins are responsible for diarrhetic shellfish poisoning in humans. Even low cell concentrations of this species produce neurotoxins transported via the food-web and these neurotoxins affect and potentially kill the higher life forms from zooplankton to humans[33,34]. Concentrations of the neurotoxins vary according to different *P. lima* strains[33,34]. Almost every culture of *P. lima* produces okadaic acid and its analogs in different concentrations[35].

Since evaluation of quantitative amounts or bloom concentrations of *P. lima* is difficult due to different habitats of the firstly benthic species[22]. The study was carried out using a bottle sampling method to determine both tytoplanktonic levels of *P. lima* and phytoplankton groups along with environmental characteristics such as temperature, salinity and pH (CTD), inorganic nutrients, and chlorophyll a during July to August 2013. This period also coincided with excessive blooms of various phytoplanktonic organisms, especially diatoms in the Dardanelles, a part of the Turkish Straits System.

2. Materials and methods

2.1. Study area

The Dardanelles, which is a part of the Turkish Straits System is located between the Sea of Marmara and Aegean Sea (Figure 1). It has two flow systems, while the surface inflow carries the brackish waters of the Black Sea into the northern Aegean basin, the counterflow carries saline Mediterranean waters into the Black Sea via the Bosphorus[19,36-38]. The brackish Black Sea inflow is relatively rich in nitrate and phosphate in winter, these nutrients dropping to minimum levels in late summer and autumn. Biologically unstable nutrients of the Black Sea origin are utilized by phytoplankton due to photosynthetic processes in the Sea of Marmara and are partly exported to lower layer waters. Ultimately, the brackish Black Sea waters reach the Dardanelles Strait with modified biochemical properties. The Mediterranean saline

waters lower in nutrient enter the deep basin of the Sea of Marmara. Throughout their 6–7 years temporary stay in the Marmara basin, the saline deep waters enrich in nitrate and phosphate due to the oxidation of planktonic particles sinking from the Marmara surface layer. The annual nutrient input from the Black Sea to the Marmara was about 8.17×10^8 moles of dissolved inorganic nitrogen and 4.25×10^7 moles of dissolved inorganic phosphate, which are much less than the import from the Marmara lower layer via the Bosphorus undercurrent[39]. Saline Mediterranean waters carry nearly 6.13×10^8 moles of dissolved inorganic nitrogen and 2.79×10^7 moles of dissolved inorganic phosphate into the Marmara deep basin. The estimated outflow of dissolved inorganic phosphate is nearly half the quantity imported from the Sea of Marmara via the Dardanelles Strait[39].

The sampling station ($40^{\circ}06'50''$ N and $26^{\circ}24'10''$ E) is located in the south east part of the strait (Figure 1). The strait has a width between 1.35 and 7.73 km. While the average depth of the strait is a proximately 60 m and the maximum depth is just over 100 m[36-39], sampling station which has coastal character is about 5 m. Some bends and contractions in some sections of the Dardanelles lead to different surface temperatures and salinities in the northeast and southwest parts of Cape Nara[19].

2.2. Sampling period and measurements

The study was carried out between 9th July and 6th August 2013 in the Dardanelles, Turkey. All samples which contain also phytoplankton for analyses were daily collected with a hydrobios Niskin sampling bottle (5 L) from surface (0.50 m).

2.3. Probe (CTD) measurements

Some CTD values such as temperature, salinity, pH, and dissolved oxygen were measured with YSI 556 Model Multiple Probe System (YSI Incorporated Company, Yellow Springs, USA) *in situ*.

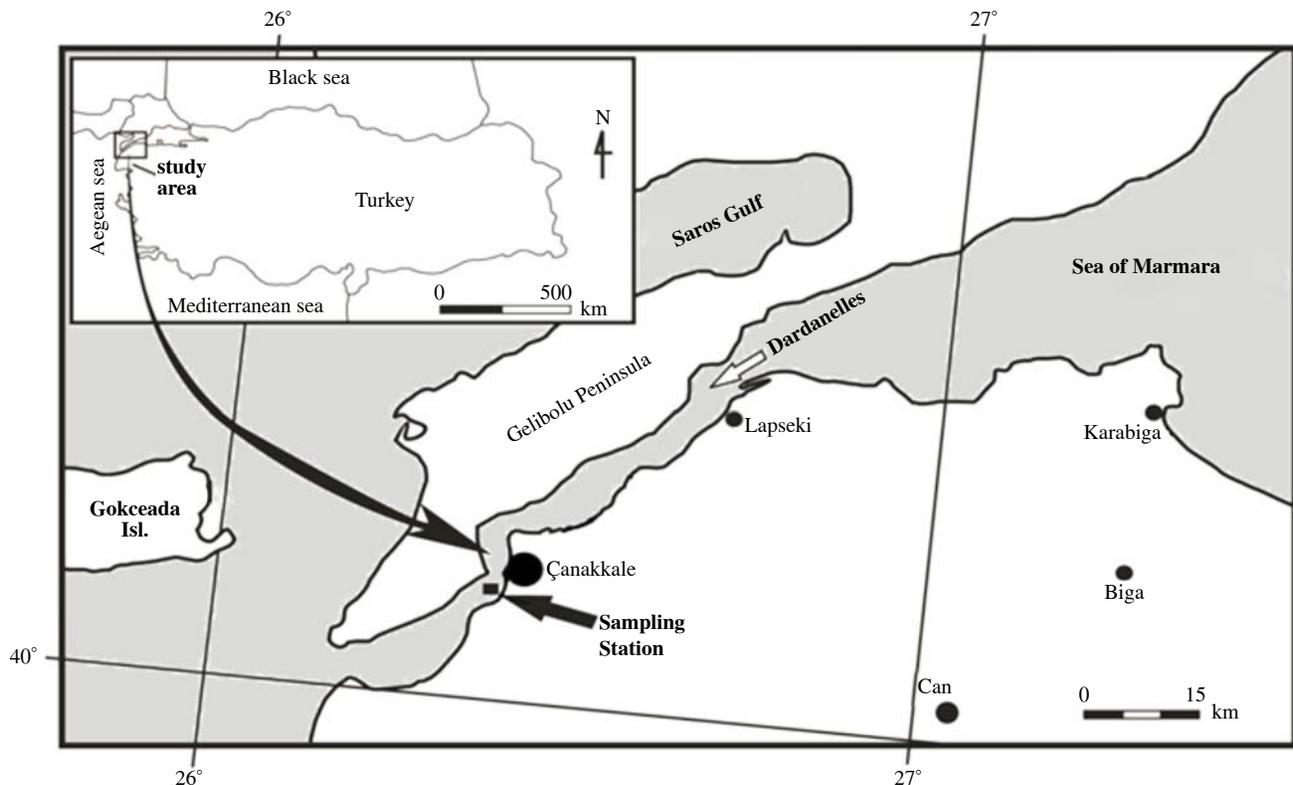


Figure 1. Dardanelles (Çanakkale Strait, Turkey) and sampling station.

2.4. Nutrient measurements

Unfiltered seawater samples for nutrient analyses were kept frozen until analysis. PO_4^{3-} , $\text{NO}_2^- + \text{NO}_3^-$, and SiO_4 were analysed using a Technicon model autoanalyser (Technicon Corporation, Cleveland, USA)[40].

2.5. Chlorophyll a

Chlorophyll a samples were filtered through GF/F glass fibre filters. The filters were folded into aluminium foil and immediately frozen for analysis. Chlorophyll a was analysed spectrophotometrically after 90% acetone extraction[40].

2.6. Quantitative analysis of phytoplankton

For quantitative analysis of phytoplankton, samples were preserved with 2.50% buffered Lugol's solution. Microscopic phytoplankton analyses were conducted within a week of collection. Sampling glasses, sedimentation chambers and Sedgwick Rafter counting slides were used for cell density of thicoplanktonic *P. lima* and other phytoplankton[41-43].

3. Results

3.1. Species description

Toxic dinoflagellate *P. lima* cells in the HAB period in the Dardanelles roughly like eggs and so anterior part is narrower than the posterior. The cell sizes of the species are nearly 40–45 μm long

and 25–28 μm wide. The cells compose of two valves and laterally compressed. There are numerous pores in the valves, except central area, where a large pyrenoid is located. There is an order of marginal pores at the valve periphery. The nucleus is located in the posterior of the cell, and the species is photosynthetic, with a number of small brown chloroplasts (Figure 2).

3.2. Daily variations of *P. lima* and other phytoplankton

Literature records revealed that the HAB of tytoplanktonic dinoflagellate *P. lima* was recorded first time in the Turkish Straits System. Descriptive statistical analysis results showed that *P. lima*, *Prorocentrum* spp., dinoflagellates, diatoms, other groups, and total phytoplankton varied between 0 and 2.40×10^6 cell/L [$(5.02 \times 10^5 \pm 5.72 \times 10^5)$ cell/L], between 0 and 2.40×10^6 cell/L [$(7.63 \times 10^5 \pm 5.82 \times 10^5)$ cell/L], between 0 and 6.16×10^6 cell/L [$(1.34 \times 10^6 \pm 1.40 \times 10^6)$ cell/L], between 4.67×10^6 and 6.24×10^7 cell/L [$(2.10 \times 10^7 \pm 1.38 \times 10^7)$ cell/L], between 0 and 3.67×10^6 cell/L [$(5.85 \times 10^5 \pm 1.01 \times 10^6)$ cell/L], and between 4.67×10^6 and 7.17×10^7 cell/L [$(2.29 \times 10^7 \pm 1.54 \times 10^7)$ cell/L] respectively during the HAB period (Tables 1 and 2). During the study, although the contribution of *P. lima* to total phytoplankton was lower (6.61%) due to the high diatom contribution (min-max: 82.2–100.0; mean: 91.90 ± 5.47), its contribution to both *Prorocentrum* spp. (min-max: 0–100; mean: 56.80 ± 34.70) and dinoflagellates (min-max: 0–100; mean: 35.40 ± 24.80) were higher (Tables 1 and 2).

During the study, daily variations of *P. lima* and its rational contributions to other phytoplankton groups showed that the cell density of *P. lima* reached to 2.40×10^6 cells/L at 19 July 2013. In addition to the biggest bloom, there were also three excessive blooms of

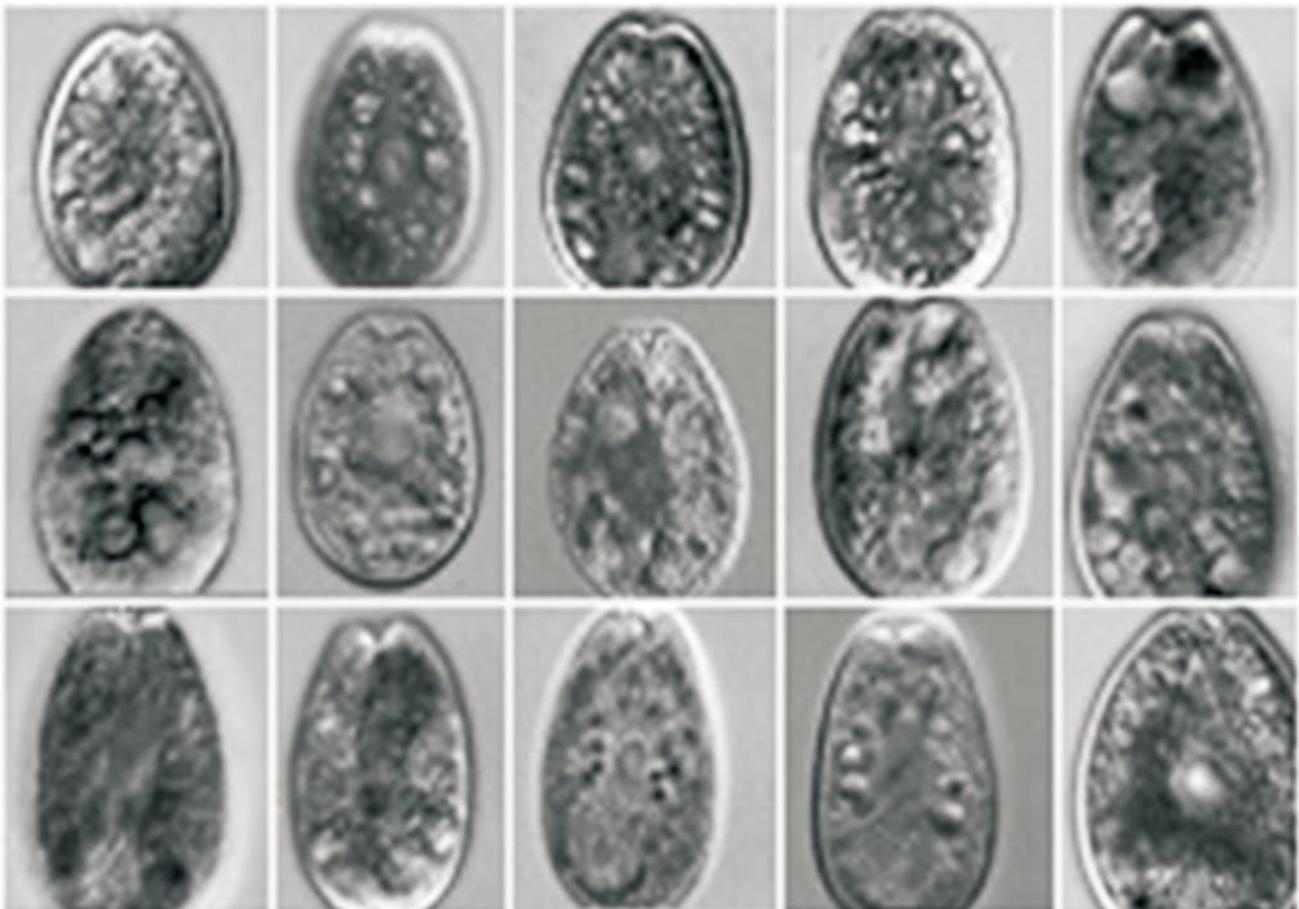


Figure 2. Morphological characters of the toxic dinoflagellate *P. lima* in the HAB period in the Dardanelles.

P. lima over 1.00×10^6 cell/L in different time periods (Figure 3). The contribution of *P. lima* to both *Prorocentrum* spp. and dinoflagellates reached to 100%, particularly in the intervening period of the excessive bloom time (Tables 1 and 2 and Figure 3) and it was attested by regression ($R^2 = 0.70-0.80$) (Figure 4) and correlation findings ($R =$

0.80-0.90). However, daily variations of contributions of *P. lima* to total phytoplankton were lower than the contribution to the dinoflagellates in the study period.

On the other hand, Bray-Curtis cluster similarity analysis results showed that both *Prorocentrum* spp. and *P. lima* were similar to

Table 1

Descriptive statistics of phytoplankton cell densities in the HAB period of the toxic dinoflagellate *P. lima* in the Dardanelles.

Phytoplankton cell density (cell/L)	Number	Minimum	Maximum	Mean	SD
<i>P. lima</i>	21	0.00E + 00	2.40E + 06	5.02E + 05	5.72E + 05
<i>Prorocentrum</i> spp.	21	0.00E + 00	2.40E + 06	7.63E + 05	5.82E + 05
Dinoflagellates	21	0.00E + 00	6.16E + 06	1.34E + 06	1.40E + 06
Diatoms	21	4.67E + 06	6.24E + 07	2.10E + 07	1.38E + 07
Other groups	21	0.00E + 00	3.67E + 06	5.85E + 05	1.01E + 06
Total phytoplankton	21	4.67E + 06	7.17E + 07	2.29E + 07	1.54E + 07

Table 2

Descriptive statistics of phytoplankton ratios in the HAB period of the toxic dinoflagellate *P. lima* in the Dardanelles.

Ratios (%)	Number	Minimum	Maximum	Mean ± SD
Contribution of <i>P. lima</i> to <i>Prorocentrum</i> spp.	21	0.00	100.00	56.80 ± 34.70
Contribution of <i>P. lima</i> to dinoflagellates	21	0.00	100.00	35.40 ± 24.80
Contribution of <i>P. lima</i> to total phytoplankton	21	0.00	6.61	2.14 ± 2.05
Contribution of dinoflagellates to total phytoplankton	21	0.00	16.10	5.92 ± 4.39
Contribution of diatoms to total phytoplankton	21	82.20	100.00	91.90 ± 5.47
Contribution of other groups to total phytoplankton	21	0.00	14.10	2.11 ± 3.26

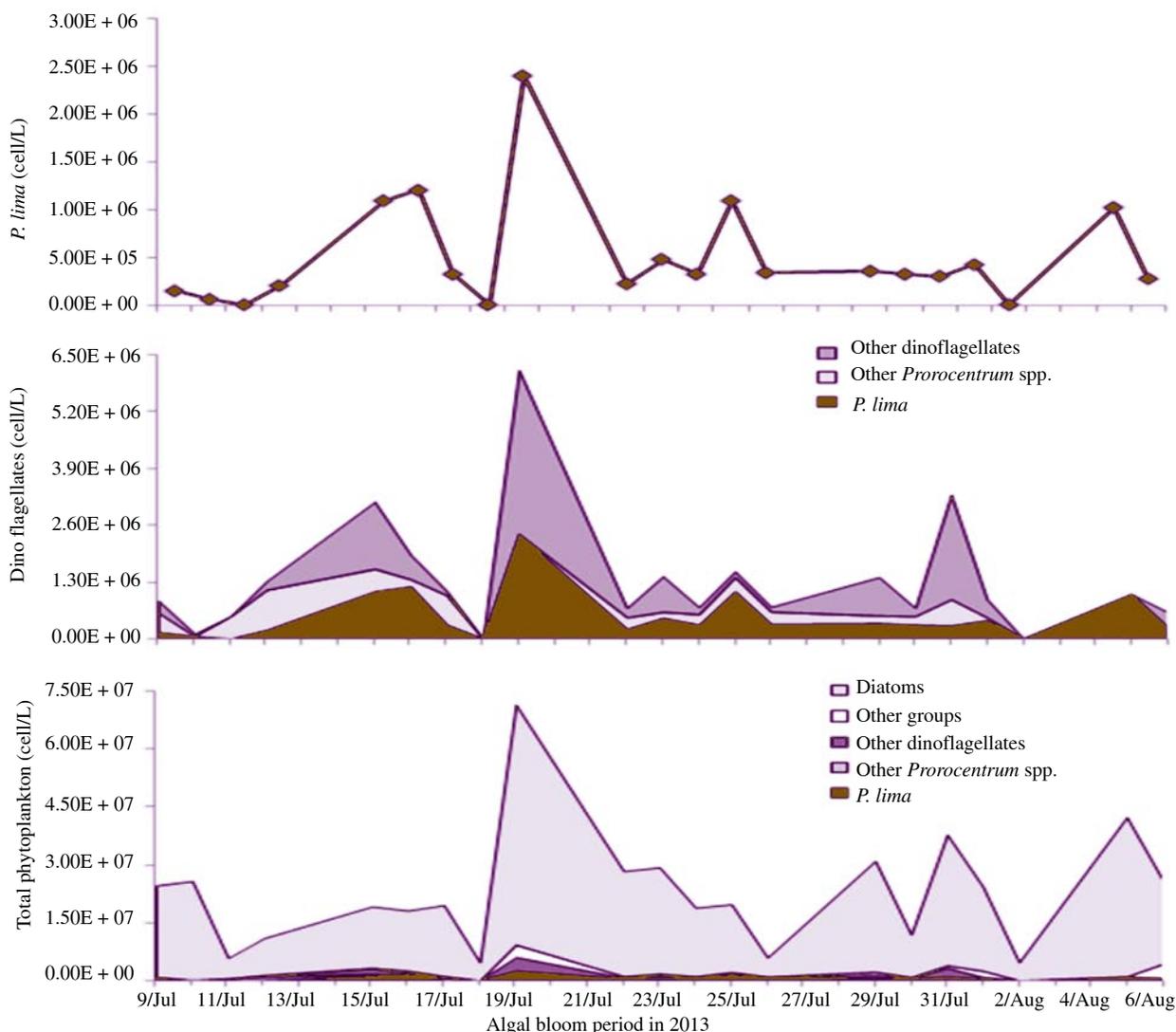


Figure 3. Daily variations of toxic dinoflagellate *P. lima* and its rational contributions to *Prorocentrum* spp., dinoflagellates, diatoms, other groups except for dinoflagellates and diatoms and total phytoplankton in the HAB period of the toxic dinoflagellate *P. lima* in the Dardanelles.

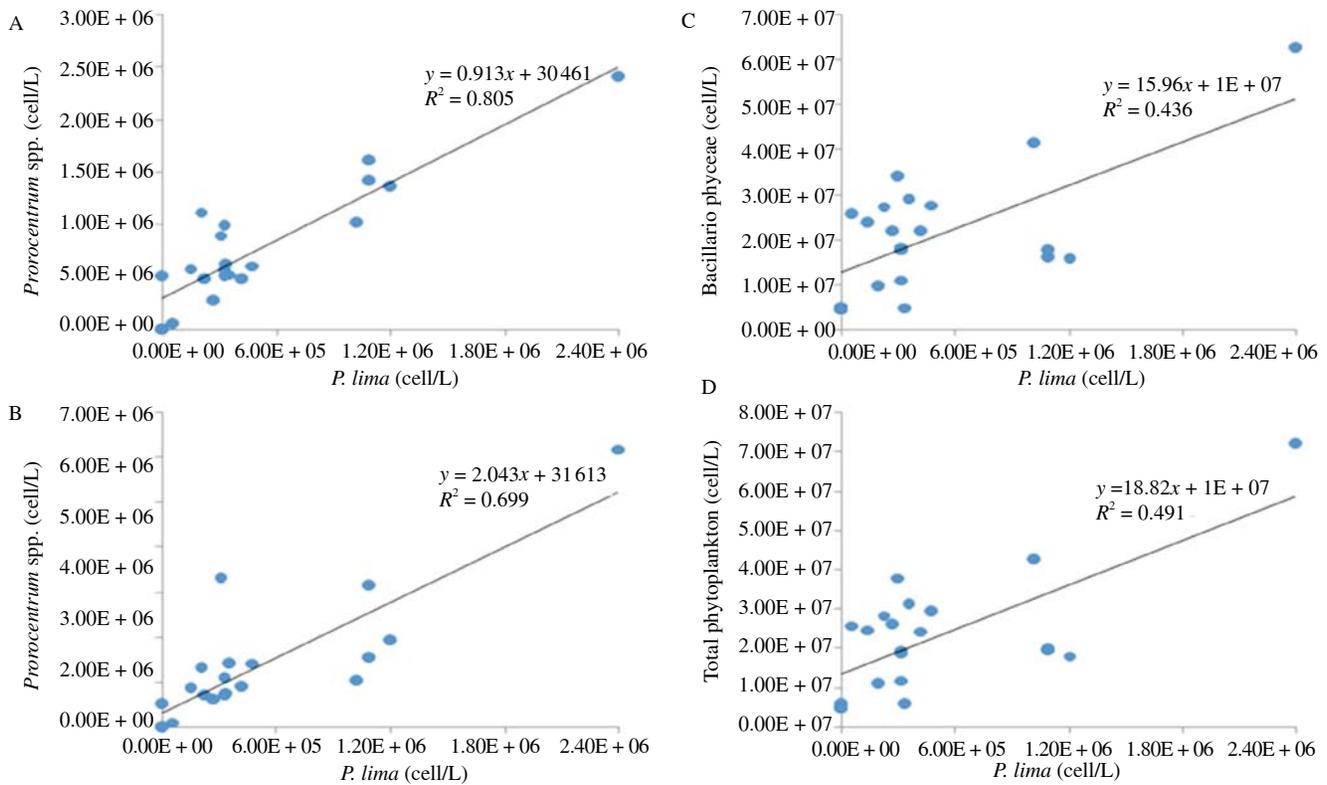


Figure 4. Relationships between cell densities of *P. lima* and *Proocentrum* spp. A: Dinoflagellates; B: Bacillariophyceae; C: Total phytoplankton; D: In the HAB period of the toxic dinoflagellate *P. lima* in the Dardanelles. The coefficients of determination (R^2) and the equating process (y) were shown for each regression.

dinoflagellates at rates in excess of 70.0%. For instance, while dinoflagellates were similar to *Proocentrum* spp. at the rate of 72.5%, *Proocentrum* spp. was similar to *P. lima* at the rate of 79.4% (Figure 5). This similarity index results confirmed that the biggest contributions to both *Proocentrum* spp. and dinoflagellates came from *Proocentrum* spp., especially from *P. lima*. However, due to the excessive diatom abundance (Figure 3) total phytoplankton abundance was more similar to diatoms (95.6%) than dinoflagellates (11.1%) (Figure 5). In other words, the biggest contribution to total phytoplankton came from diatoms (Tables 1 and 2).

Bray-Curtis cluster analysis (single link)

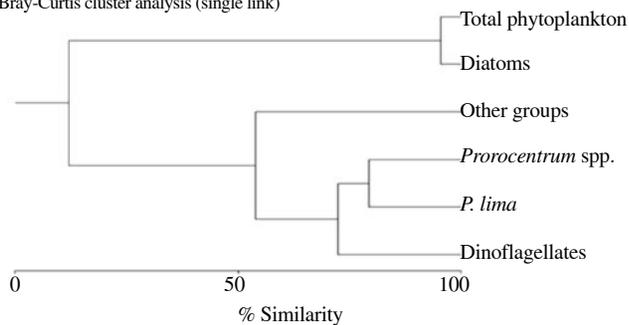


Figure 5. Bray-Curtis cluster analysis results between *P. lima*, *Proocentrum* spp., dinoflagellates, diatoms, other groups out of dinoflagellates and diatoms and total phytoplankton cell density in the HAB period of the toxic dinoflagellate *P. lima* in the Dardanelles.

3.3. Satellite images in the HAB period

Some satellite images from National Aeronautics and Space Administration (NASA) in the HAB period in the Dardanelles revealed that although there were important developments of the *P. lima* and other phytoplankton blooms in the Dardanelles[44], there wasn't any

apparent color change in the system in summer period because of turbidity (9th July and 6th August, 2013). However, satellite images from NASA confirmed the phytoplankton blooms which were not noticeable to the eye in the Turkish Straits System in July, 2013 (Figure 6).

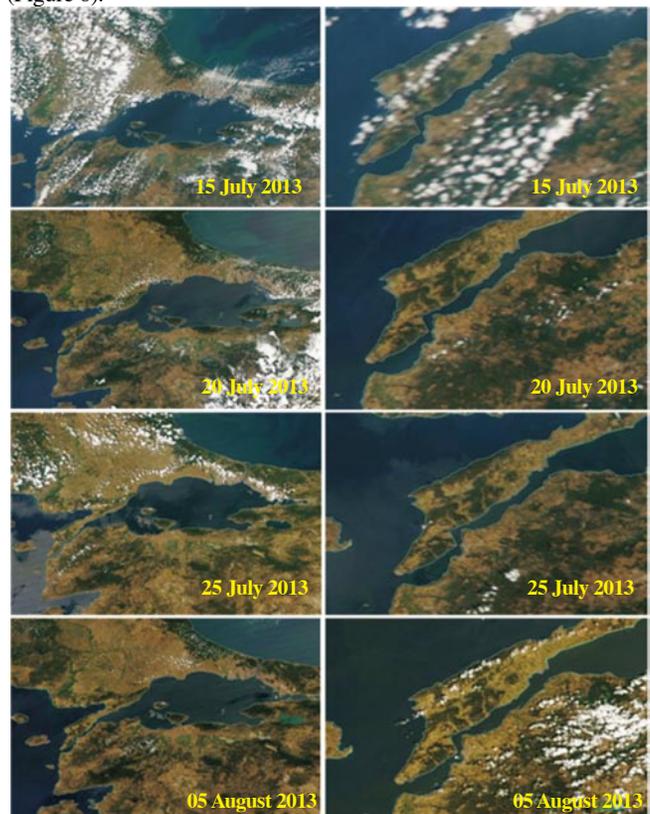


Figure 6. Some satellite images[45] in the HAB period of the toxic dinoflagellate *P. lima* in the Dardanelles.

3.4. CTD variations in the HAB period

Descriptive statistical analysis results showed that CTD, and dissolved oxygen varied between 24.0 and 25.0 °C [(24.70 ± 0.44) °C], between 21.4 and 23.5 ppt [(22.90 ± 0.49) ppt], between 8.01 and 8.54 (8.23 ± 0.15), and between 6.05 and 8.62 mg/L [(7.35 ± 0.60) mg/L] respectively, during the HAB period (Table 3).

Table 3

Descriptive statistics of CTD parameters in the HAB period of the toxic dinoflagellate *P. lima* in the Dardanelles.

CTD parameters		Number	Minimum	Maximum	Mean ± SD
Physical parameters	Temperature (°C)	21	24.00	25.00	24.70 ± 0.44
	Salinity (ppt)	21	21.40	23.50	22.90 ± 0.49
	pH	21	8.01	8.54	8.23 ± 0.15
	Dissolved oxygen (mg/L)	21	6.05	8.62	7.35 ± 0.60

Results (Table 3 and Figure 7) indicated that during the HAB period, study area was generally stable with regard to all environmental parameters except for dissolved oxygen. There was a variation in dissolved oxygen concentrations probably due to the various algal blooms sourced from *P. lima* and others, especially diatoms.

3.5. Nutrient and chlorophyll a variations in the HAB period

Descriptive statistical analysis results showed that $\text{NO}_2^- + \text{NO}_3^-$, PO_4^{3-} , SiO_4 , and chlorophyll a varied between 0.20 and 0.78 $\mu\text{mol/L}$ [(0.44 ± 0.17) $\mu\text{mol/L}$], between 0.08 and 0.18 $\mu\text{mol/L}$ [(0.12 ± 0.03) $\mu\text{mol/L}$], between 0.25 and 0.65 $\mu\text{mol/L}$ [(0.41 ± 0.09) $\mu\text{mol/L}$], and

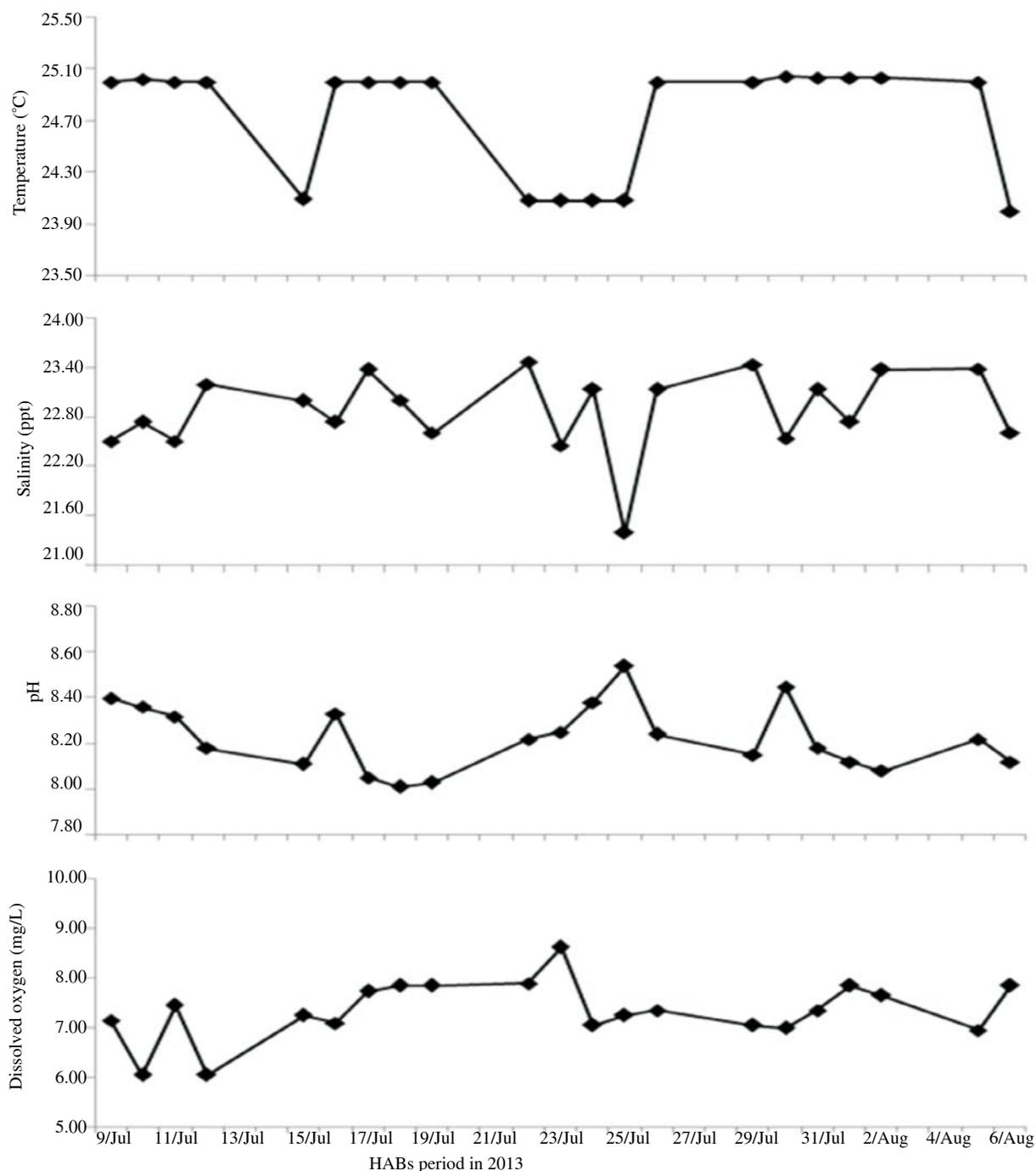


Figure 7. Daily variations of temperature, salinity, pH and dissolved oxygen in the HAB period of the toxic dinoflagellate *P. lima* in the Dardanelles.

between 1.57 and 8.52 $\mu\text{g/L}$ [(4.82 \pm 2.29) $\mu\text{g/L}$] respectively during the HAB period (Table 4).

Table 4

Descriptive statistics of nutrient and chlorophyll a concentrations in the HAB period of the toxic dinoflagellate *P. lima* in the Dardanelles.

	Environmental factors	Number	Minimum	Maximum	Mean \pm SD
Nutrients ($\mu\text{mol/L}$)	$\text{NO}_2^- + \text{NO}_3^-$	21	0.20	0.78	0.44 \pm 0.17
	PO_4^{3-}	21	0.08	0.18	0.12 \pm 0.03
	SiO_4	21	0.25	0.65	0.41 \pm 0.09
Chlorophyll	Chlorophyll a ($\mu\text{g/L}$)	21	1.57	8.52	4.82 \pm 2.29

Variations of $\text{NO}_2^- + \text{NO}_3^-$, PO_4^{3-} and SiO_4 concentrations were lower than previous concentrations probably due to the excessive utilizations of nutrients by *P. lima* blooms and other phytoplankton blooms, especially diatoms during the HAB period (Figure 8). Due to comprehensive blooms, there were high chlorophyll a levels (1.57–8.52 $\mu\text{g/L}$) during the study in the Dardanelles (Figure 8). Satellite images of chlorophyll a (NASA, 2015) in chlorophyll a minimum (July 25, 2013 and August 5, 2013) and maximum periods (July 18, 2013 and August 03, 2013) confirmed high levels of chlorophyll a during the study (Figure 9).

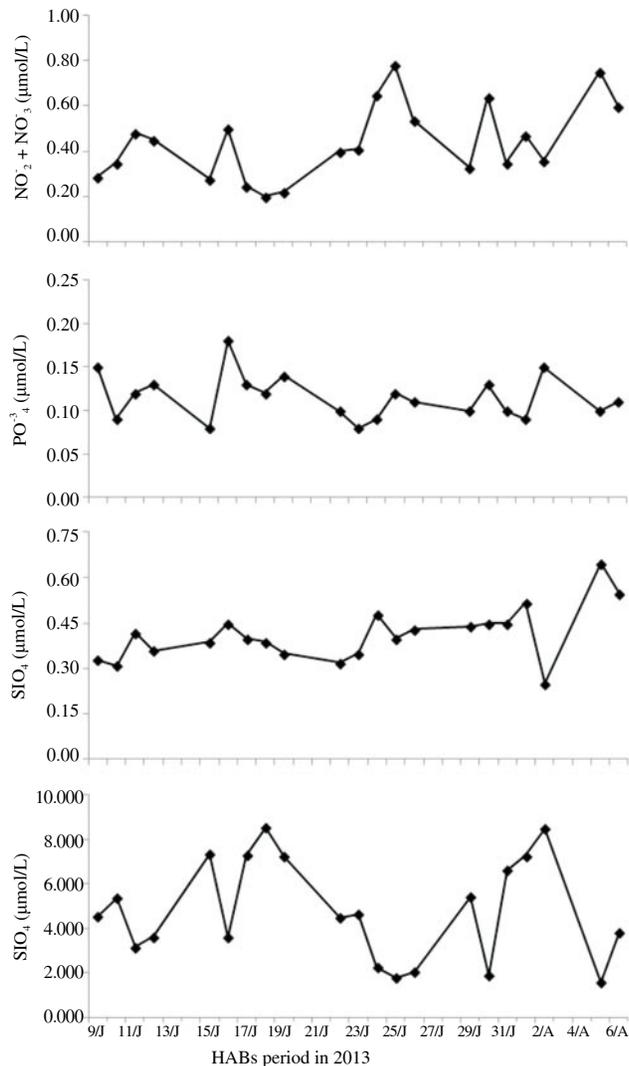


Figure 8. Daily variations of nutrient ($\text{NO}_2^- + \text{NO}_3^-$, PO_4^{3-} and SiO_4) and chlorophyll a concentrations in the HAB period of the toxic dinoflagellate *P. lima* in the Dardanelles. J: July; A: August.

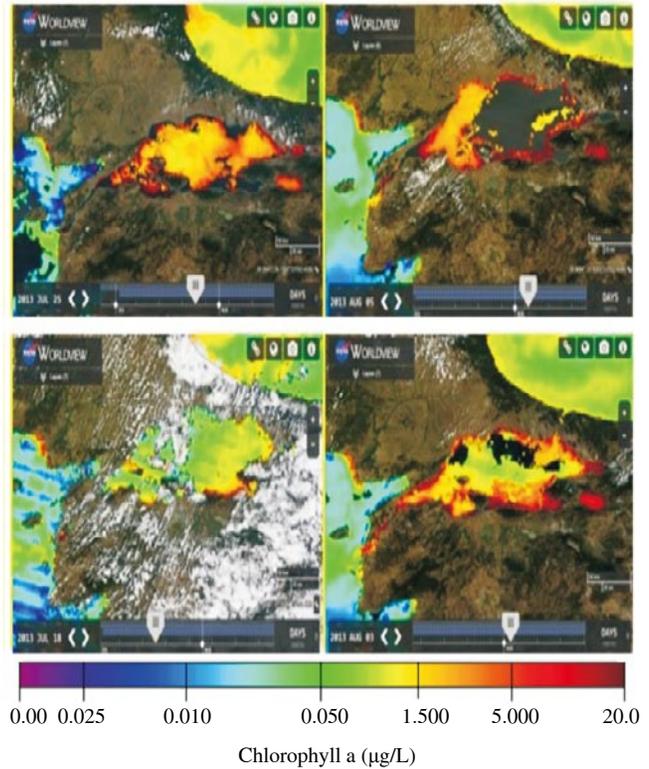


Figure 9. Satellite images of chlorophyll a (NASA, 2015) in chlorophyll a minimum (July 25, 2013 and August 5, 2013) and maximum periods (July 18, 2013 and August 3, 2013) in the HAB period of the toxic dinoflagellate *P. lima* in the Dardanelles.

3.6. Nutrient ratios in the HAB period

Descriptive statistical results on nutrient ratios showed that $\text{NO}_2^- + \text{NO}_3^-/\text{PO}_4^{3-}$, $\text{SiO}_4/\text{PO}_4^{3-}$, and $\text{SiO}_4/\text{NO}_2^- + \text{NO}_3^-$ varied between 1.57 and 7.50 (4.04 \pm 1.74), between 1.67 and 6.50 (3.79 \pm 1.24), and between 0.51 and 1.95 [(1.04 \pm 0.36) $\mu\text{mol/L}$] respectively during the HAB period (Table 5).

Table 5

Descriptive statistics of nutrient ratios in the HAB period of the toxic dinoflagellate *P. lima* in the Dardanelles.

Nutrient ratios	Number	Minimum	Maximum	Mean \pm SD
$\text{NO}_2^- + \text{NO}_3^-/\text{PO}_4^{3-}$	21	1.57	7.50	4.04 \pm 1.74
$\text{SiO}_4/\text{PO}_4^{3-}$	21	1.67	6.50	3.79 \pm 1.24
$\text{SiO}_4/\text{NO}_2^- + \text{NO}_3^-$	21	0.51	1.95	1.04 \pm 0.36

Nutrient ratios, especially $\text{NO}_2^- + \text{NO}_3^-/\text{PO}_4^{3-}$ and $\text{SiO}_4/\text{PO}_4^{3-}$ were lower than Redfield ratios ($\text{NO}_2^- + \text{NO}_3^-/\text{PO}_4^{3-}$: 16/1; $\text{SiO}_4/\text{PO}_4^{3-}$: 15/1; $\text{SiO}_4/\text{NO}_2^- + \text{NO}_3^-$: 15/16). Daily variations of nutrient ratios in the HAB period indicated that study area was more limited in view of phosphate than in view of nitrogen due to the eutrophication in the Turkish Straits System (Figure 10).

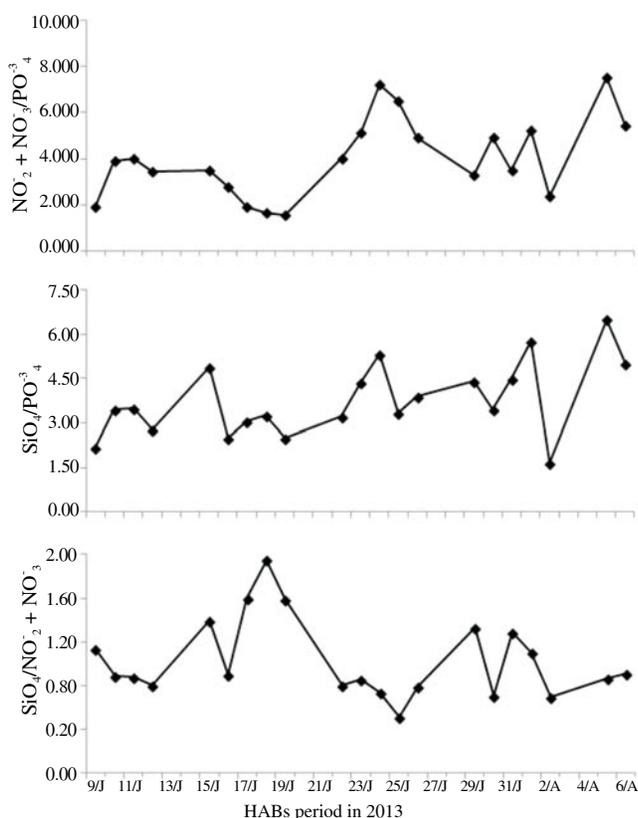


Figure 10. Temporal distribution of nutrient ratios ($\text{NO}_2 + \text{NO}_3/\text{PO}_4^{3-}$, $\text{SiO}_4/\text{PO}_4^{3-}$ and $\text{SiO}_4/(\text{NO}_2 + \text{NO}_3)$) in the HAB period of the toxic dinoflagellate *P. lima* in the Dardanelles. J: July; A: August.

4. Discussion

Our findings indicated that during the HAB period the system was generally stable with regard to temperature, salinity, and light duration. The role of these factors on the phytoplankton blooms have been discussed earlier [3,7,16,21,45]. During the course of this study, the cell density of *P. lima* reached a maximum of 2.40×10^6 cells/L on July 19th 2013 and a total of four blooms with cell concentrations exceeding 1.00×10^6 cells/L were recorded. In the intensive bloom periods the contribution of *P. lima* to both *Prorocentrum* spp. and dinoflagellates reached to 100% which was attested by regression ($R \geq 0.70$) (Figure 4), correlation findings ($R \geq 0.80$) and similarity index results ($> 70\%$). However, during the study, phytoplankton community was dominated by diatoms (95.6%). During the HAB period, morphological structure of *P. lima* in the Dardanelles was substantially similar to the morphological structure observed in other marine systems [22,33,46]. However, the cell sizes of *P. lima* in the study area were bigger (nearly 40–45 μm long and 25–28 μm wide) than in any other marine system [46].

Spatio-temporal worldwide distributions indicated that *P. lima* which is a holotype species of genus *Prorocentrum* is eurythermal and euryhaline dinoflagellate species similar to *Prorocentrum micans* which is a prototype species of genus *Prorocentrum* [23,29]. Therefore, dense blooms of these species grow not only during the summer period, but also in winter and spring periods especially in

eutrophic marine systems [3,19,21] due to the increasing temperature trend from climate change [8]. However, while *P. lima* blooms under 10^6 cells/L mostly occur from late winter to early spring (< 20.0 °C) (Sahraoui *et al.*, 2013), the blooms over 10^6 cells/L from early summer to late summer (> 20.0 °C) [9,21,25,26]. During the study, the average temperature value [(24.70 ± 0.44) °C] was over the 20.0 °C in the Dardanelles. The average salinity was (22.9 ± 0.49) ppt, which is lower than any other marine system (35–39 ppt). The study indicated that *P. lima* was the most important species among *Prorocentrum* spp., which is an agent of diarrhetic shellfish poisoning in various regions of the world ocean systems [1,34,47–50]. During the study, although the contribution of *P. lima* to total phytoplankton was lower (6.61%) due to the high diatom contribution over 80.0% [average: (91.90 ± 5.47) %], its contribution to both genus *Prorocentrum* (min-max: 0–100; mean: 56.8 ± 34.7) and dinoflagellates (min-max: 0–100; mean: 35.4 ± 24.8) were higher. Regression coefficient and Bray-Curtis cluster analysis results revealed that it will be possible to reach to millions of cell number of *P. lima* (2.40×10^6 cells/L) in eutrophied waters characterized here by high chlorophyll a biomass (8.52 $\mu\text{g/L}$). Our findings also indicated that *P. lima* seemed more likely to proliferate in the low saline waters with high concentrations of inorganic nutrients.

Nutrient concentrations ($\text{NO}_2 + \text{NO}_3$: 0.44 ± 0.17 $\mu\text{mol/L}$, PO_4^{3-} : 0.12 ± 0.03 $\mu\text{mol/L}$ and SiO_4 : 0.41 ± 0.09 $\mu\text{mol/L}$) in the study period were lower than previously reported concentrations [3,7,16–20,36,51] probably due to excessive nutrient utilizations of by *P. lima* and other phytoplankton blooms such as diatoms during the HAB period. Both high nutrient concentrations and lower average values of nutrient ratios ($\text{NO}_2 + \text{NO}_3/\text{PO}_4^{3-}$: 4.04 ± 1.74 , $\text{SiO}_4/\text{PO}_4^{3-}$: 3.79 ± 1.24 , and $\text{SiO}_4/(\text{NO}_2 + \text{NO}_3)$: 1.04 ± 0.36) with respect to Redfield ratios ($\text{NO}_2 + \text{NO}_3/\text{PO}_4^{3-}$: 16/1; $\text{SiO}_4/\text{PO}_4^{3-}$: 15/1; $\text{SiO}_4/(\text{NO}_2 + \text{NO}_3)$: 15/16) during the HAB period indicated that primary productivity in the study area was more limited by nitrogen rather than phosphate, due to the fact that the system was exposed to hypereutrophication. It is known that primary productivity is primarily more limited by phosphate than nitrogen due to phosphate scarcity in oligotrophic systems such as open Mediterranean waters [52–54]. However, eutrophic systems such as the Sea of Marmara and Black Sea are primarily limited by nitrogen due to phosphate abundance [3,7,16, 17,25,26,37,51,55]. Due to the phosphate abundance, the study area was limited by nitrogen ($\text{NO}_2 + \text{NO}_3/\text{PO}_4^{3-}$: 4.04 ± 1.74). The system was very productive as a result of algal blooms which are supported by average phytoplankton chlorophyll a level of (4.82 ± 2.29) $\mu\text{g/L}$. On the other hand, chlorophyll a levels reached to 8.52 $\mu\text{g/L}$ in late summer period (August 2, 2015). Previous studies indicated that the Turkish Straits System has been eutrophic since 1980 [3,7,8,10,16–20,39,54–59].

Based on the nutrient concentrations, nutrient ratios, chlorophyll a, and HABs, the Turkish Straits System are exposed to heavy pollution due to the urban waste waters of Istanbul and polluted North West Black Sea surface waters coming through the Bosphorus (Istanbul Strait). Reoccurring excessive *Prorocentrum*

blooms in the Dardanelles may potentially indicate possible intensification of future diarrhetic shellfish poisoning events in the region. Moreover, the current study takes attention to not only a possible intensification of diarrhetic shellfish poisoning, but also possible densifications of other shellfish poisonings events looking at the highest cell concentration of *P. lima* and other *Prorocentrum* species in the Dardanelles.

Conflict of interest statement

I declare that we have no conflict of interest.

Acknowledgments

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