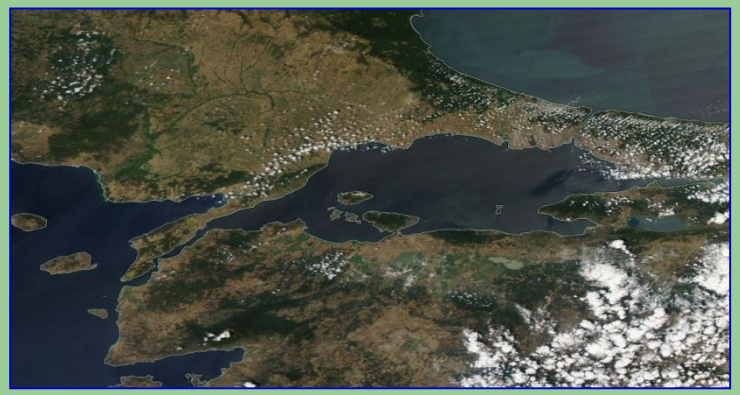
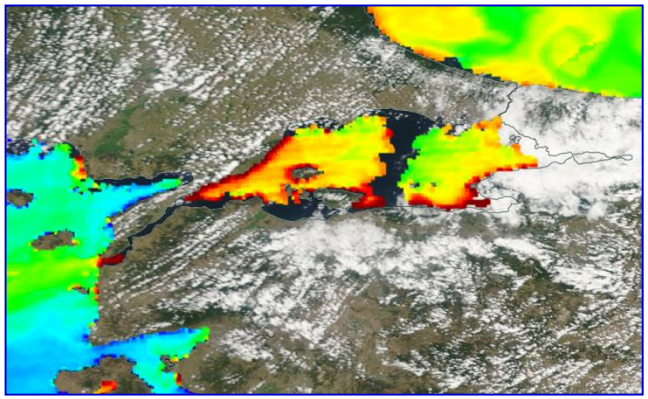
First bloom record of toxic dinoflagellate Prorocentrum lima and climate change interactions in the Dardanelles (Turkish Straits Sistem)





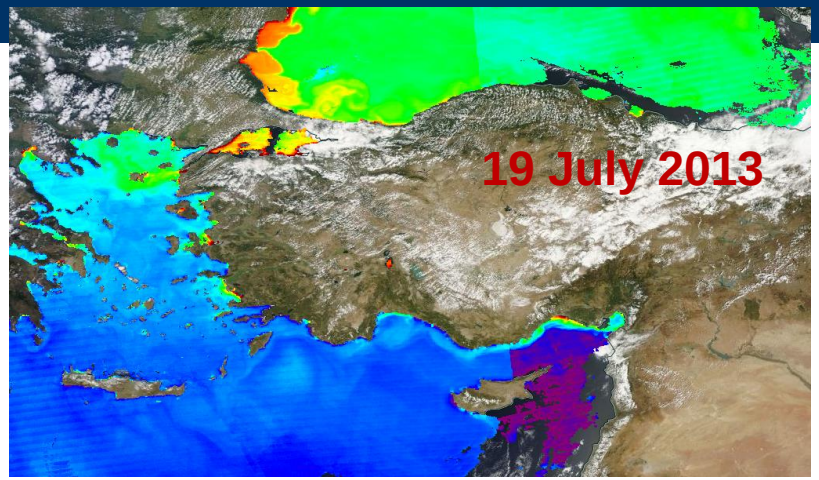


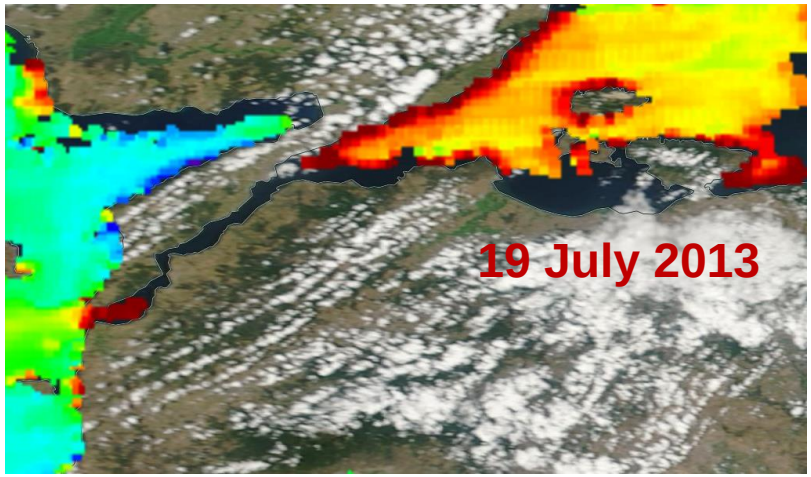


Muhammet Turkoglu (PhD)

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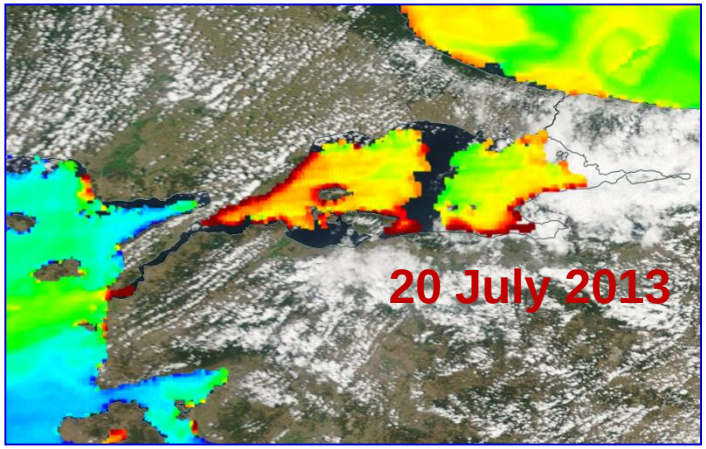
Main Scope of the Study





- The main target of this study is to exhibit the bloom circumstances and the reasons of toxic and tycoplanktonic dinoflagellate *P. lima* along with environmental characteristics, hydrographic structure, inorganic nutrients, and chlorophyll a in summer period in the Dardanelles.

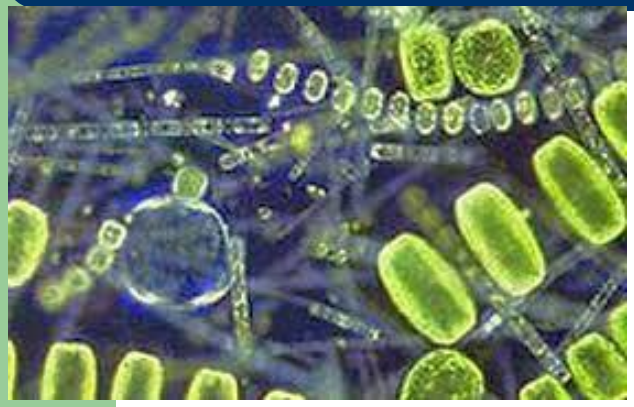
Main Scope of the Study





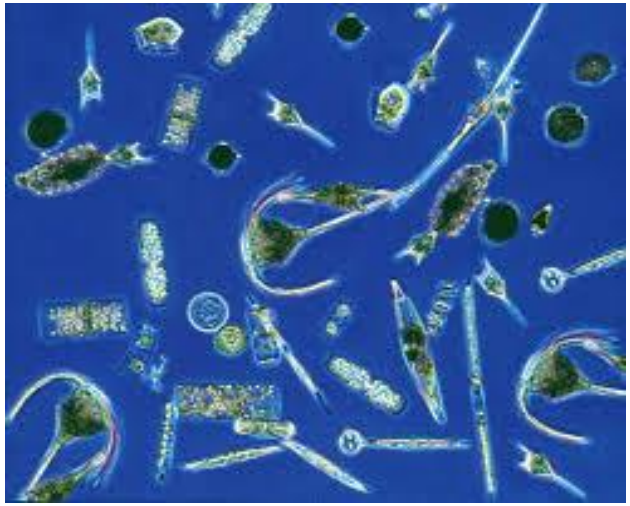
- **This study reports the first bloom of *P. lima* and interactions of this species with other phytoplankton species in response to environmental parameters for the first time in the Dardanelles .**

Description of phytoplankton and HABs



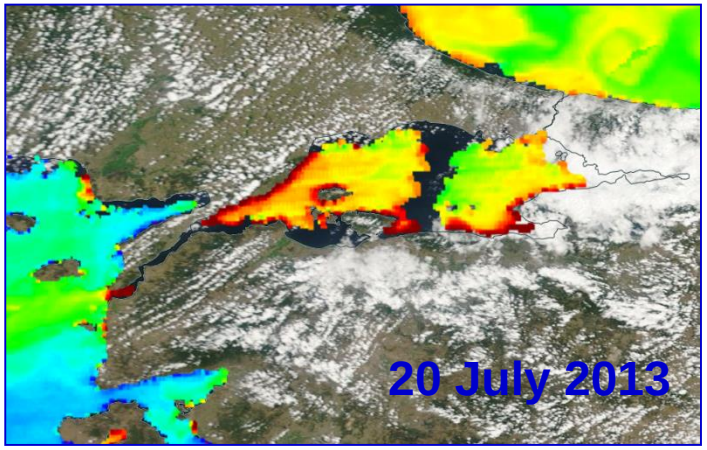
➢**Phytoplankton** species are single-celled microscopic plants (**Domain**: **Protista**) that are the base of aquatic life.
➢**Harmful algal** or **phytoplankton blooms (HABs)** develop in the marine or freshwater environment when there is an excess of growth of these organisms because of eutrophication in that environment (Anderson, 1994; Hallegraeff, 1995).

➢**Observations of HABs have been increasing intricate phenomena which cause serious environmental and economic troubles on a global scale, as are fall in with in Mediterranean, frequently in coastal waters, in relation to anthropogenic inputs and hereby habitat changes occurred during the past a few decades (Turkoglu, 2008; Collos et al., 2009; Nincevic Gladan et al., 2009; Garces and Camp, 2012; Turkoglu, 2013).**
➢Therefore, habitats most affected by HABs include bays, harbours, estuaries and lagoons affected by eutrophication.



Description of phytoplankton and HABs





- An important factor for the formation and toxicity of **HABs** is more availability of nutrients, such as nitrogen and phosphate than their background levels (Graneli et al., 1998).

- Over the past three decades, the occurrence of harmful algal blooms has increased in many marine aquatic ecosystems of the world, both in frequency and in geographic distribution (Anderson, 1989; Smayda, 1990; Hallegraeff, 1993).

Description of target species (*P.lima*)

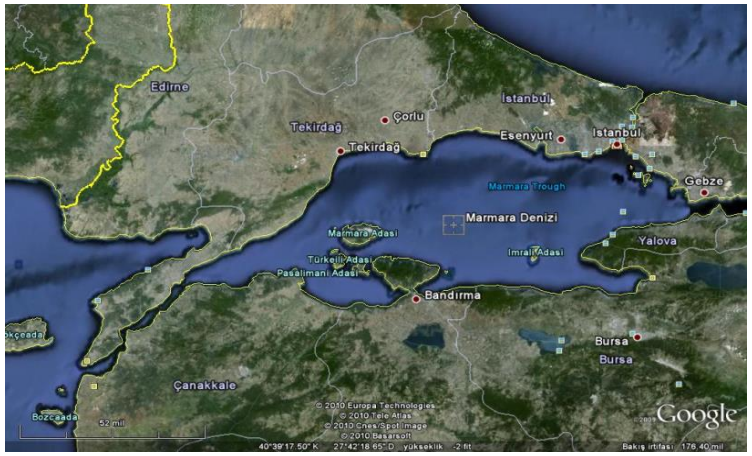


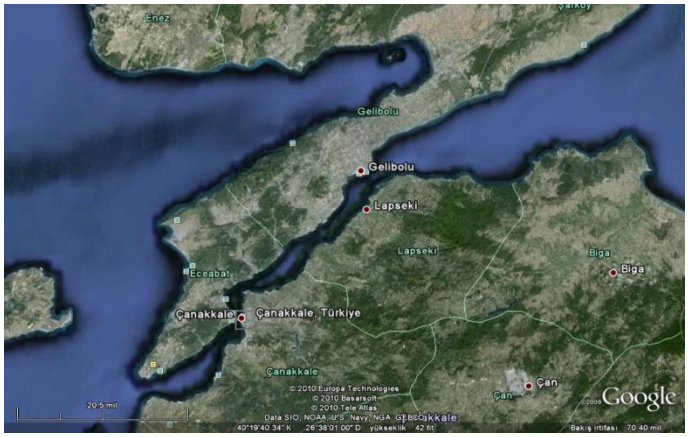




- The dinoflagellate **Prorocentrum lima** (Ehrenberg) F.Stein, 1878 is a marine photosynthetic species which contains two chloroplasts, a central pyrenoid and a large posterior nucleus and an armor plate.
- This species lives in benthic, epiphytic and tycoplanktonic with world wide distribution.
- **This species varies considerably in size as well as shape: 31-57µm in length; 20-46 µm wide. World-wide distribution, neritic and estuarine, benthic/epiphytic (Faust et al. 1999), sand dwelling, can be tychoplanktonic (Steidinger & Tangen 1996), attached to the surface of red and brown algae and floating mangrove detritus.**
- ***P. lima*** is a **toxic species**. The primary toxins are **okadaic acid (OA)** and **dinophysistoxin (DTX)** and their derivatives. These toxins are responsible for **diarrhetic shellfish poisoning (DSP)** in humans.
- **Unfortunately, a small cell density of this species produce such vigorous neurotoxins which can be transfered through the food web where they affect and even kill the higher life forms such as zooplankton, shellfish, fish, birds, marine mammals, and even humans via food web.**

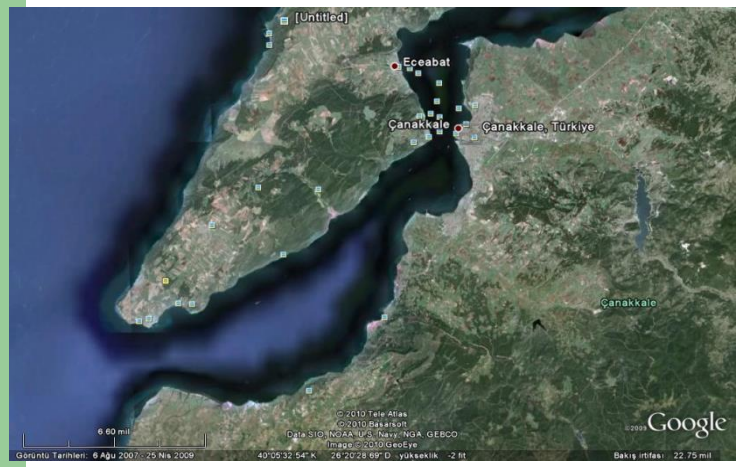
Identification of the study Area (Dardanelles)





- The Dardanelles is a part of the Turkish Strait System and located between the Aegean Sea and the Sea of Marmara.
- **It has two flow system reverse to one another; one of the currents derives from the Aegean Sea, where the water density is high (38-39 ppt), and the second one comes from the Sea of Marmara, characteristically low in density (22-26 ppt).**
- The width of the Strait varies from 1.35 to 7.73 km (average: 2.5 km)**.**
- **The average depth of the Strait is approximately 60 m with the deepest part reaching more than 100 m (Unsal *et al.* 2003; Turkoglu *et al.* 2004a, 2006; Baba *et al.* 2007).**

Identification of the study Area (Dardanelles)





- Its NE/SW trend is interrupted by a **north-south bend** between Eceabat and Canakkale**. This bend is also the narrowest part of the Dardanelles.**
- In addition to the first bend, there is a second bend called "**Nara Cape**".
- The narrowing of the Dardanelles leads to different surface temperature and salinity values in the northeast and southwest of the Nara Cape.
- **The surface waters in the southern part of the Dardanelles were also more saline especially in the spring and winter seasons compared to other seasons (Unsal et al., 2003; Turkoglu et al., 2004, 2006).**

Material and Method (Sampling Station)

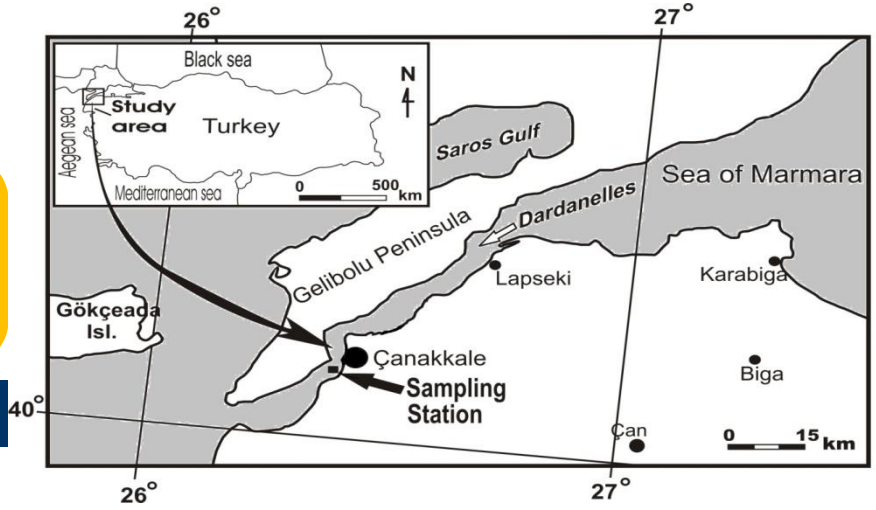
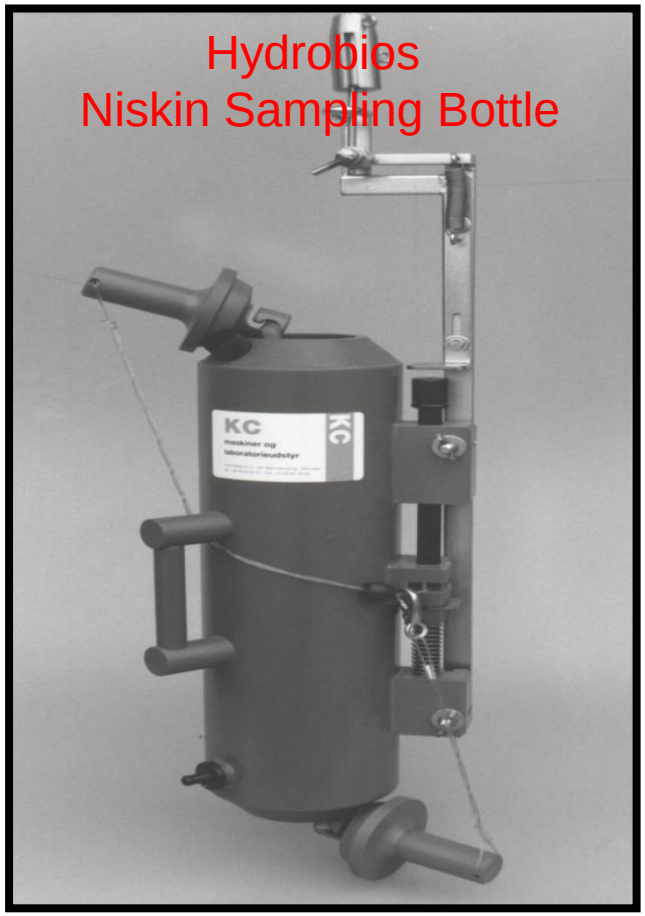




Fig. 1. Study area and sampling station

- This study was planned in the period of 09 July 2013 and 06 August 2013 coincided with excessive blooms of phytoplankton in view of both dinoflagellates and diatoms in the Dardanelles at sampling station in Fig. 1.
- The location of the sampling station (40º06'50" N–26º24'10" E) is given in Fig. 1.
- The sampling station is an topographical upwelling area due to the narrowing and ridge between Çanakkale and Kilitbahir.
- The depth of the study area in the Dardanelles is about 5.00 m.

Material and Method
(Collection of Water Samples)



- In order to collect phytoplankton, nutrient and chlorophyll-a samples, etc., Hydrobios Niskin Sampling Bottle was used.

Material and Method
(In situ Measurements – CTD)

YSI 556 Model MPS



- CTD parameters such as temperature, salinity, pH, and dissolved oxygen (DO) were measured in situ using an YSI 556 Model Multiple Probe System.

Material and Method
(Nutrient Analysis)


Auto-analyzer

- Nutrients (NO^{-}_{2}+NO^{-}_{3}, PO^{-3}_{4} and SiO_{4}) were analysed by a technicon model auto-analyzer according to Strickland & Parsons (1972).

Material and Method (Chlorophyll *a* Analysis)



Spectrophotometer

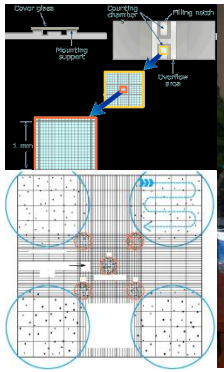
- Chlorophyll a was analyzed spectrophotometrically after extraction by 90% acetone (Strickland & Parsons 1972).

Material and Method
(Quantitative analysis of phytoplankton)





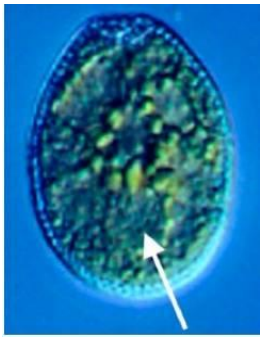




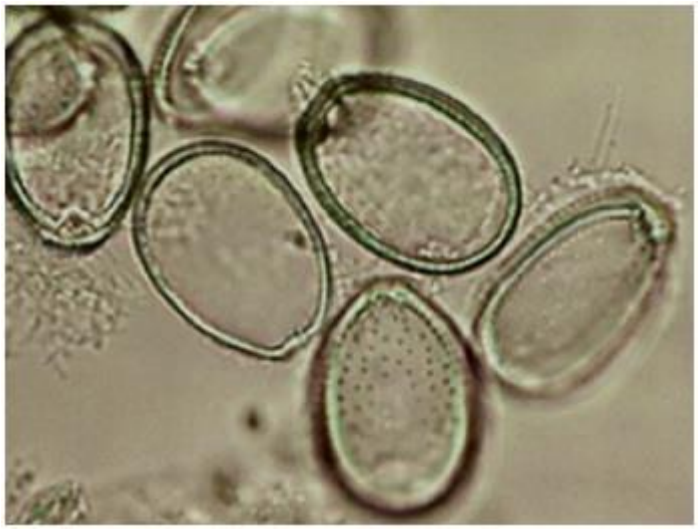


- **For quantitative analysis of phytoplankton, samples were preserved with 2% lugol fixative and microscopic analysis was conducted within a week of the collection.**
- Sampling Glass, Sedimentation Chambers, Neubauer and Sedgwick Rafter Counting Slides were used in combination for enumeration of the phytoplankton species depending on the dimensions and concentrations of the organisms (Guillard 1978; Hasle 1978; Venrick 1978).

RESULTS AND DISCUSSION
Species Description



Chloroplasts in live cell



- Cells of *P. lima* are photosynthetic approximately oval, with the anterior narrower than the posterior, thus often egg-shaped.
- The cell sizes of *P. lima* are nearly 40-45 µm long and 25-28 µm wide. The cells are laterally compressed and composed of two valves with a small cluster of eight periflagellar plates.

RESULTS AND DISCUSSION
Species Description

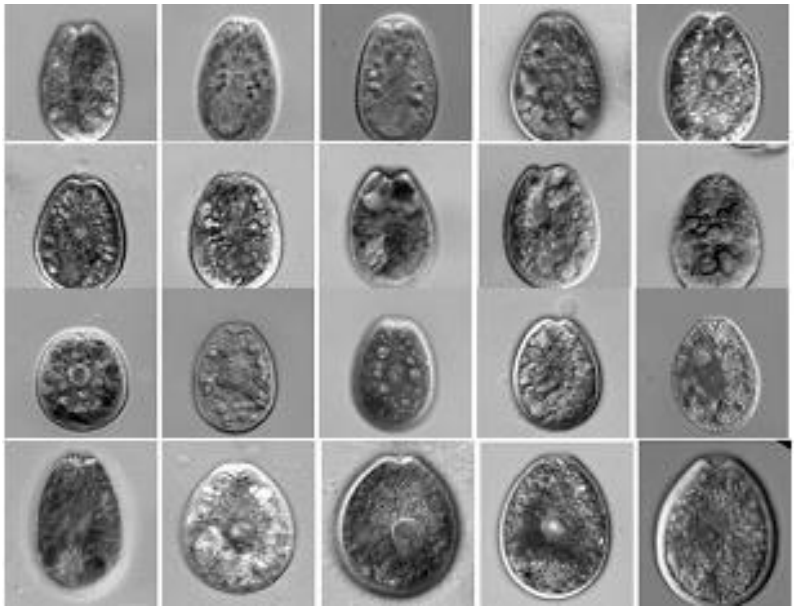


Fig.2. Morphological characters of the toxic dinoflagellate *P. lima* in the HAB period in the Dardanelles

- **During the HAB period, morphological structure of *P. lima* in the Dardanelles was substantially similar to the morphological structure in other marine systems (Morton & Tindall, 1995; Maranda et al., 2007; Nagahama et al., 2011).**
- **However, the cell sizes of *P. lima* in the study area were generally bigger (nearly 40-45 µm long and 25-28 µm wide) than in any other marine system as average values (Nagahama et al., 2011).**

RESULTS AND DISCUSSION

Succession of *P. lima* and other phytoplankton groups

Table 1. Descriptive statistics of phytoplankton cell densities and their ratios to each other in the HAB period of the toxic dinoflagellate *Prorocentrum lima* in the Dardanelles

Phytoplankton Cell Density (Cell L^{-1})	N	Minimum	Maximum	Mean	SD
Prorocentrum lima	21	0.00E+00	2.40E+06	5.02E+05	5.72E+05
Prorocentrum 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
Ratios (%)	**N**	**Minimum**	**Maximum**	**Mean**	**SD**
Contribution of *P.lima* to *Prorocentrum* spp.	21	0.00	100	56.8	34.7
Contribution of *P.lima* to dinoflagellates	21	0.00	100	35.4	24.8
Contribution of *P.lima* to total phyto.	21	0.00	6.61	2.14	2.05
Contribution of dinoflagellates to total phyto.	21	0.00	16.1	5.92	4.39
Contribution of diatoms to total phyto.	21	82.2	100	91.9	5.47
Contribution of other groups to total phyto.	21	0.00	14.1	2.11	3.26

- **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; mean: 91.9 ± 5.47), it's contribution to both *Prorocentrum* spp. (min-max: 0-100%; mean: 56.8 ± 34.7%) and dinoflagellates were higher (min-max: 0-100%; mean: 35.4 ± 24.8%) (Table 1).**

RESULTS AND DISCUSSION

Succession of *P. lima* and other phytoplankton groups

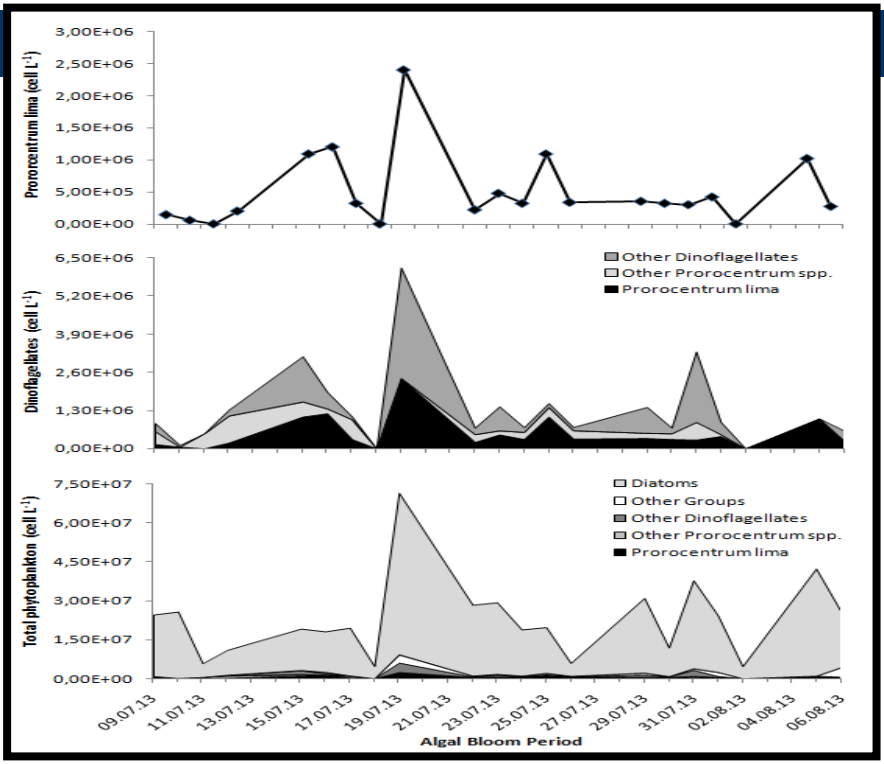


Fig. 3. Daily variations of toxic dinoflagellate *Prorocentrum lima* and it's rational contributions to *Prorocentrum* spp., dinoflagellates, diatoms, other groups except for dinoflagellates and diatoms and total phytoplankton in the HAB period of the toxic dinoflagellate *Prorocentrum lima* in the Dardanelles

- During the study the cell density of *P. lima* reached to 2.40 x 10^6 cells L^{-1} at 19 July 2013 and this species, in total, formed four excessive blooms in different time frames of summer season in excess of 1.00 x 10^6 cells L^{-1}.
- 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^2=0.70-0.80) (Fig. 4), correlation findings (R=0.80-0.90) and similarity index results (>70-80%).
- However, phytoplankton abundance was more similar to diatoms (95.6%) than dinoflagellates (11.1%) due to the excessive diatom during the study.

RESULTS AND DISCUSSION

Succession of *P. lima* and other phytoplankton groups

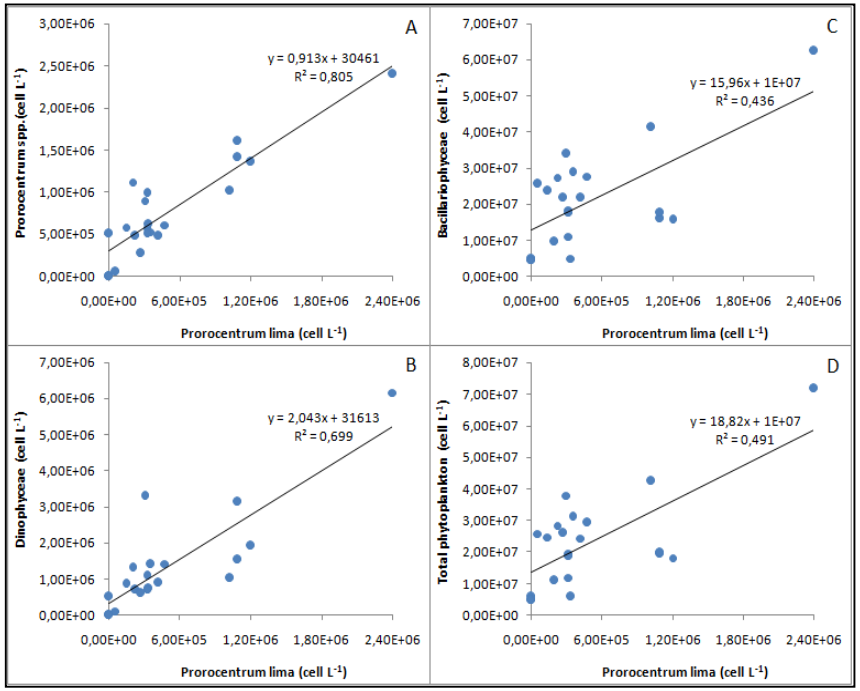


Fig. 4. Relationships between cell densities of *Prorocentrum lima* and *Prorocentrum* spp. (A), dinoflagellates (B), Bacillariophyceae (C) and total phytoplankton (D) in the HAB period of the toxic dinoflagellate *Prorocentrum lima* in the Dardanelles. The coefficients of determination (R^2) and the equating process (y) are shown for each regression.

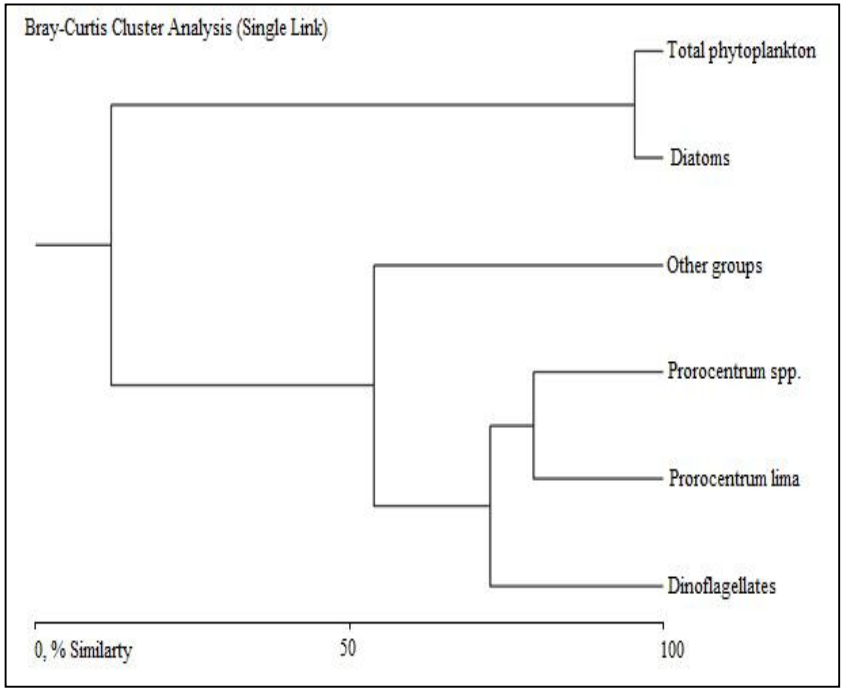


Fig. 5. Bray–Curtis Cluster analysis results between *Prorocentrum lima*, *Prorocentrum* spp., dinoflagellates, diatoms, other groups out of dinoflagellates and diatoms and total phytoplankton cell density in the HAB period of the toxic dinoflagellate *Prorocentrum lima* in the Dardanelles

RESULTS AND DISCUSSION
CTD variations in the *P. lima* bloom period

Table 2. Descriptive statistics of CTD parameters in the HAB period of the toxic dinoflagellate *Prorocentrum lima* in the Dardanelles

	CTD Parameters	N	Minimum	Maximum	Mean	SD
Physical Parameters	Temperature (°C)	21	24.0	25.0	24.7	0.44
	Salinity (ppt)	21	21.4	23.5	22.9	0.49
	pH	21	8.01	8.54	8.23	0.15
	DO (mg L^{-1})	21	6.05	8.62	7.35	0.60

- Table 2 and Fig. 6 indicated that during the HAB period, study area was generally stable in view of all environmental characters except for DO. There was a variation in DO concentrations probably due to the various algal blooms sourced from *P.lima* and others, especially diatoms (Fig. 6).
- Spatiotemporal distributions in the world and this study indicated that *P.* lima were more eurythermal and euryhaline similar to other species of *Prorocentrum* spp. than the other dinoflagellates.
- For instance, in summer period while the average temperature value in the study area was 24.7 ± 0.44 °C in the surface waters of the study area, the average salinity value was 22.9 ± 0.49 ppt was less saline than other marine systems (35-39 ppt).
- In suchc an environment, phytoplankton was highly abundant (2.29 x 10^7 ± 1.54 x 10^7 cell L^{-1}).

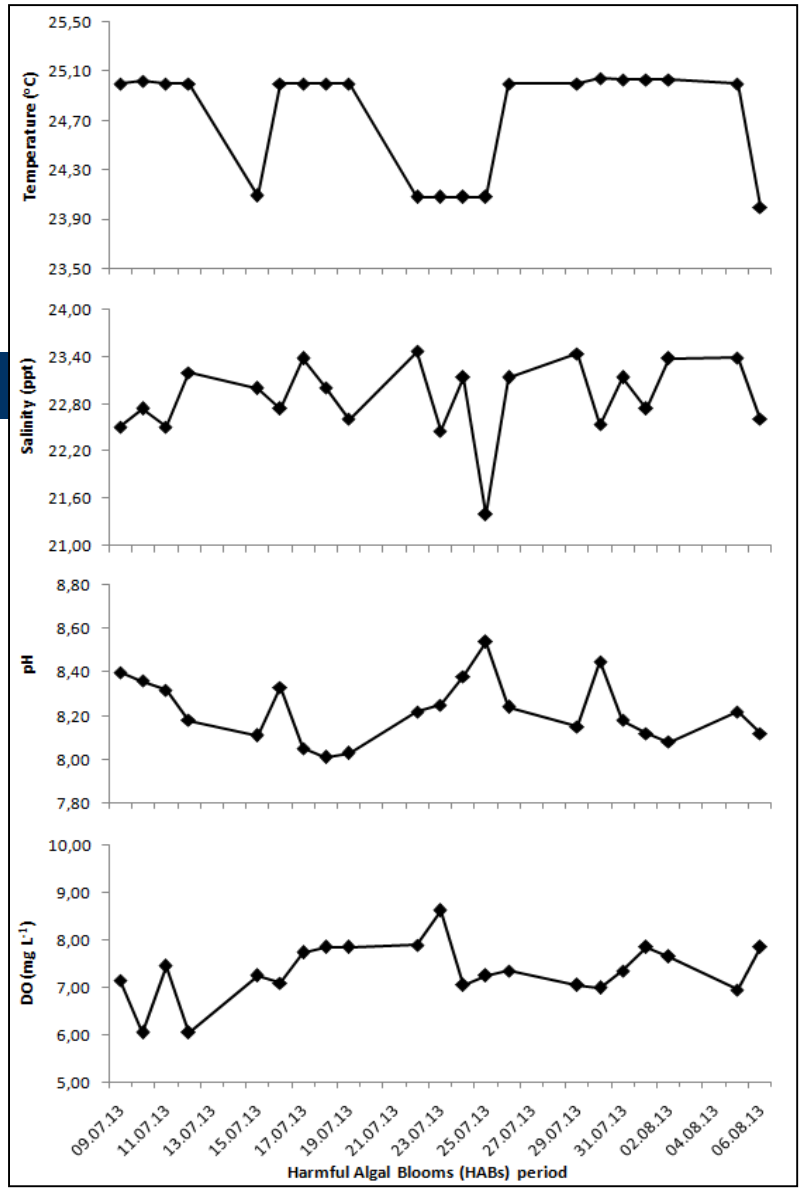


Fig. 6. Daily variations of temperature, salinity, pH and dissolved oxygen (DO) in the HAB period of the toxic dinoflagellate *Prorocentrum lima* in the Dardanelles

RESULTS AND DISCUSSION

Nutrient and chlorophyll *a* variations in the *P.lima* bloom period

Table 3. Descriptive statistics of nutrient and chlorophyll *a* concentrations in the HAB period of the toxic dinoflagellate *Prorocentrum lima* in the Dardanelles

	Environmental Factors	N	Minimum	Maximum	Mean	SD
Nutrients (µM)	$NO^-_2+NO^-_3$	21	0.20	0.78	0.44	0.17
	PO^{-3}_4	21	0.08	0.18	0.12	0.03
	SiO_4	21	0.25	0.65	0.41	0.09
Chlorophyll	**Chla (µg L^{-1})**	**21**	**1.57**	**8.52**	**4.82**	**2.29**

- Nutrient concentrations in the study were lower than previous concentrations (Unsal et al., 2003; Turkoglu, 2008, 2013) probably due to the excessive utilizations of nutrients by *P.lima* blooms and other phytoplankton blooms, especially diatoms during the HAB period.
- High nutrient levels during the HAB period indicated that primary productivity in the study area was affected by more nitrogen than phosphate due to the eutrophication in the Dardanelles.
- Due to the comprehensive blooms, there were high chlorophyll *a* levels (min-max:1.57-8.52, average: 4.82 µg L^{-1}) during the study in the Dardanelles (Fig. 7).

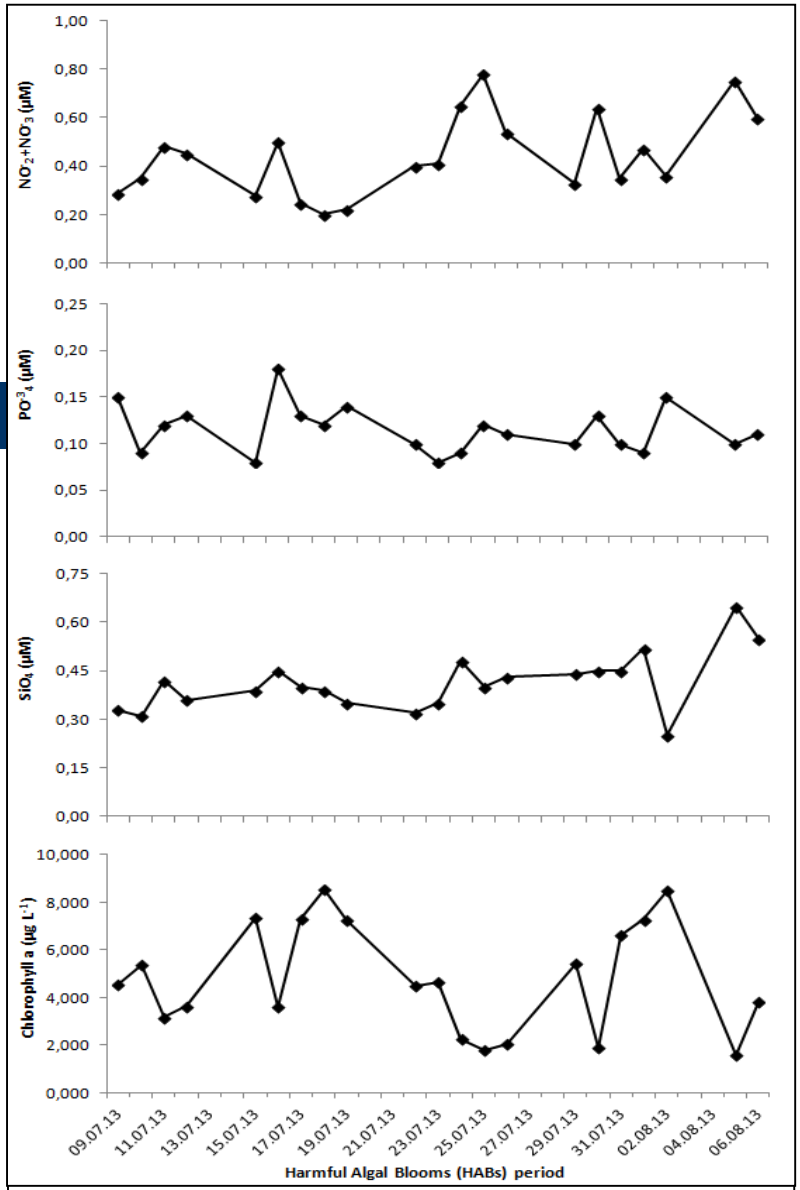


Fig. 7. Daily variations of nutrient ($NO^-_2+NO^-_3$, PO^{-3}_4 and SiO_4) concentrations in the HAB period of the toxic dinoflagellate *Prorocentrum lima* in the Dardanelles

RESULTS AND DISCUSSION

Nutrient and chlorophyll *a* variations in the *P.lima* bloom period

Table 4. Descriptive statistics of nutrient ratios in the HAB period of the toxic dinoflagellate *Prorocentrum lima* in the Dardanelles

	Environmental Factors	N	Minimum	Maximum	Mean	SD
Nutrient Ratios	N:P	21	1.57	7.50	4.04	1.74
	Si:P	21	1.67	6.50	3.79	1.24
	Si:N	21	0.51	1.95	1.04	0.36

- **Nutrient ratios**, **especially N:P and Si:P** were lower than **Redfield ratios** (N:P=6:1; Si:P=15:1; Si:N=15:16). The ratios indicated that primary productivity in the study area was affected by more nitrogen than phosphate due to the eutrophication in the Turkish Straits System (Fig. 8).
- **In the other words**, the system was exposed to hypereutrophication and so nitrate was more limiting nutrient than phosphate.

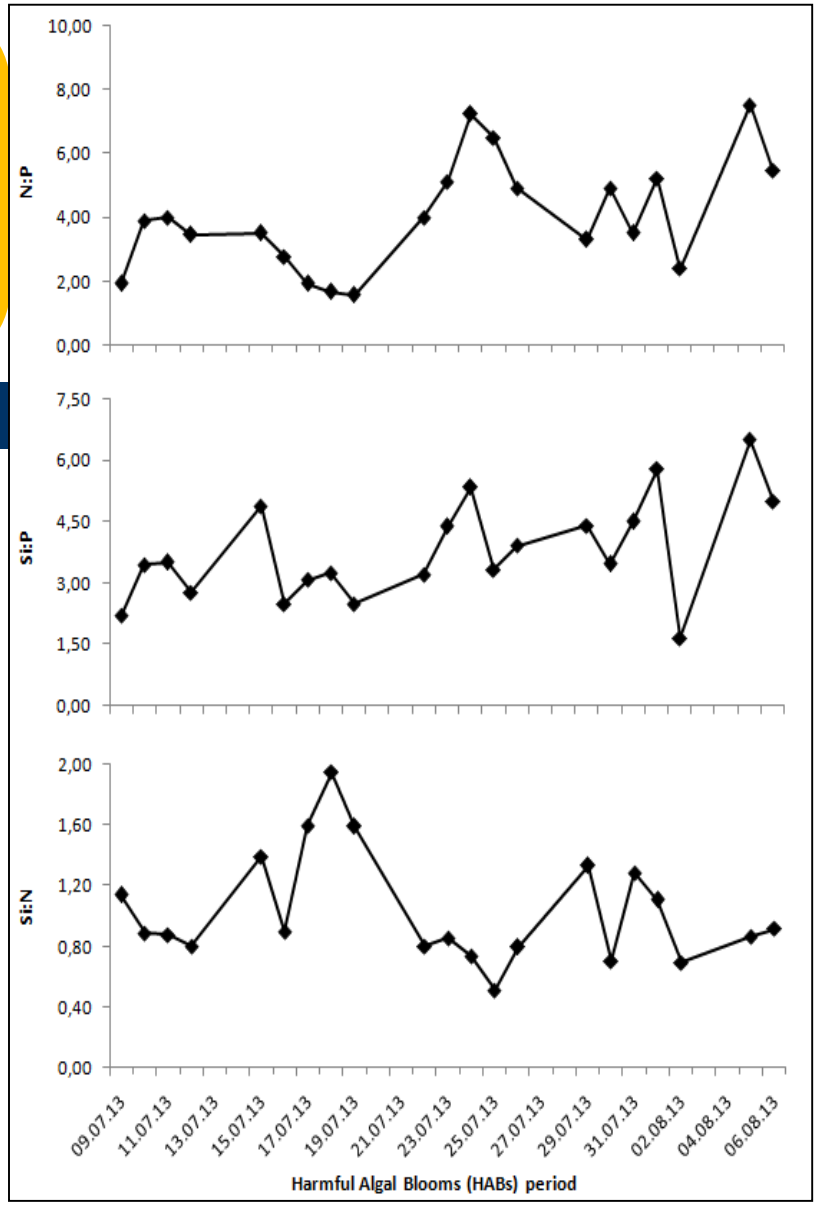
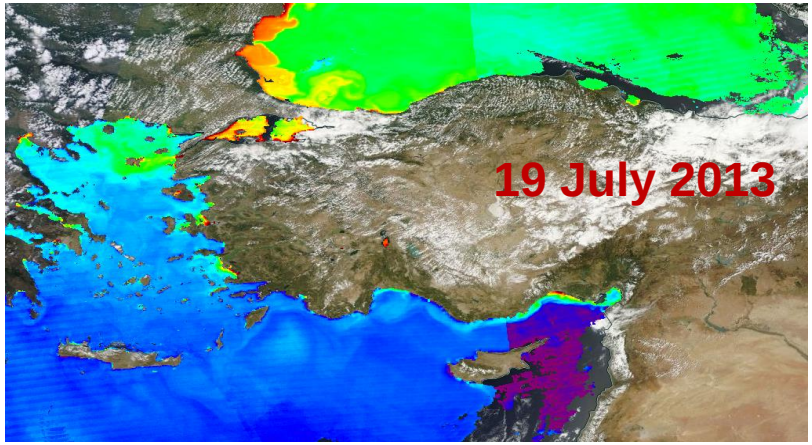
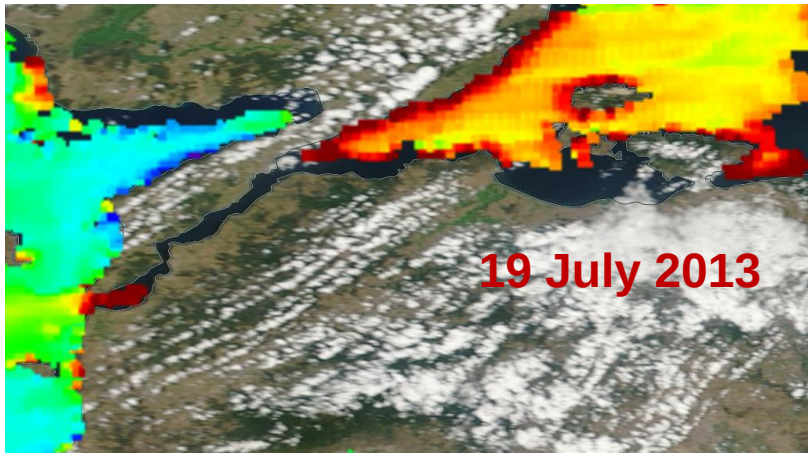


Fig. 8. Temporal distribution of nutrient ratios (N:P, Si:P and Si:N) in the HAB period of the toxic dinoflagellate *Prorocentrum lima* in the Dardanelles

RESULTS AND DISCUSSION

Succession of *P. lima* and other phytoplankton groups



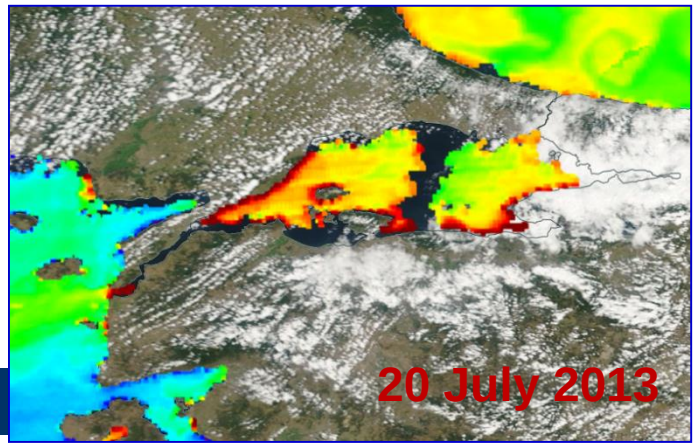


- In light of the nutrient concentrations, nutrient ratios, chla, and harmfull phytoplankton blooms (HABs), the Sea of Marmara and thereby the Dardanelles are underneath all the heavy pollution due to the urban waste waters of Istanbul and polluted North West Black Sea surface waters coming through the Bosphorus (Istanbul Strait).

- The study calls attention to a possible intensification of DSP events in the Dardanelles in light of excessive *Prorocentrum* blooms in the Dardanelles.

RESULTS AND DISCUSSION

HABs and Satellite images in real HAB time in the TSS





- Although there was an important development of the *P. lima* and other phytoplankton bloom in the Dardanelles, there wasn't any apparently color change in the system in summer (09 July and 06 August, 2013) in visible manner.
- However, satallite images from NASA confirms the phytoplankton bloms in the TSS in July, 2013.

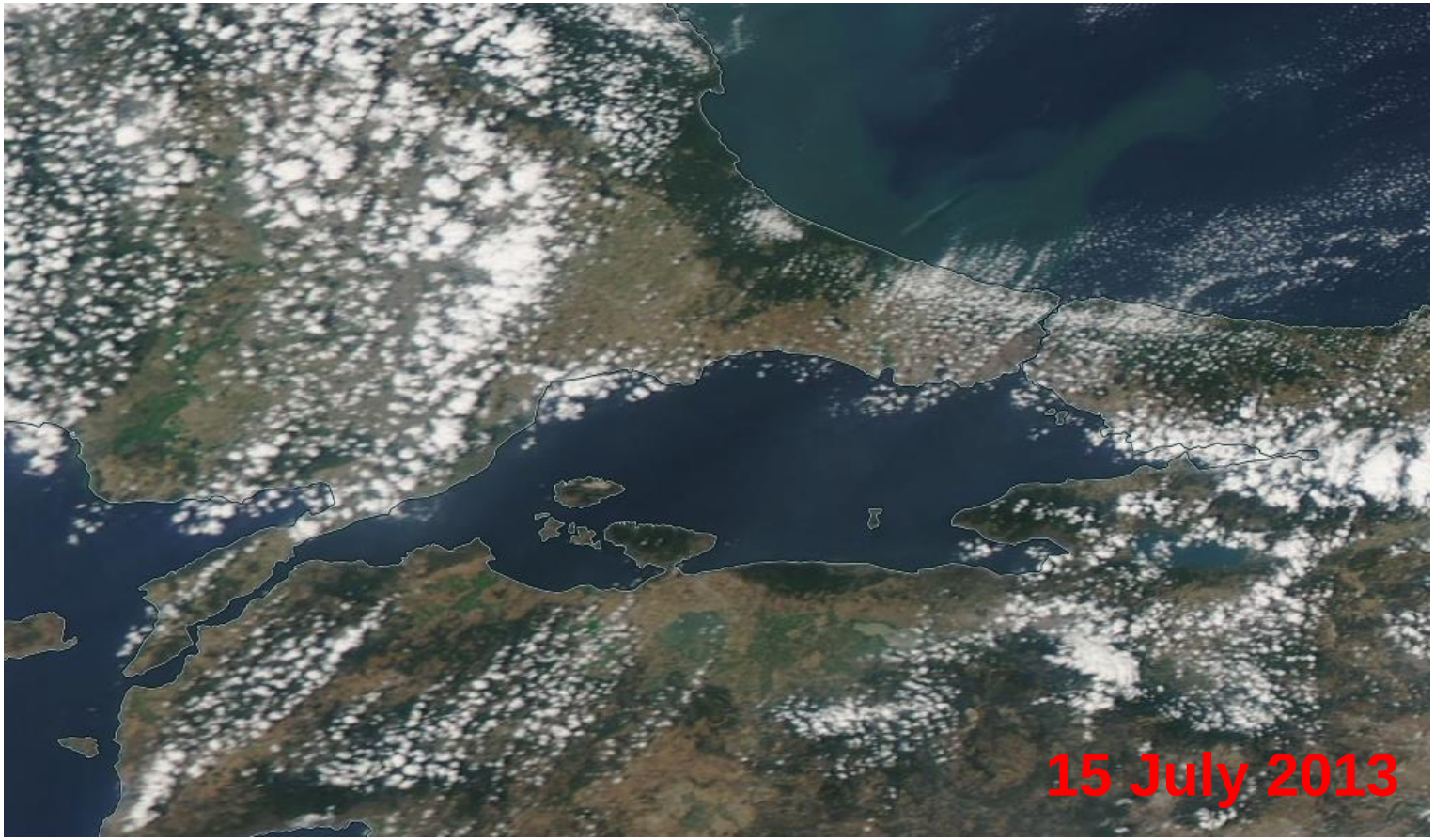
RESULTS AND DISCUSSION

HABs and Satellite images in real HAB time in the TSS



RESULTS AND DISCUSSION

HABs and Satellite images in real HAB time in the TSS



RESULTS AND DISCUSSION

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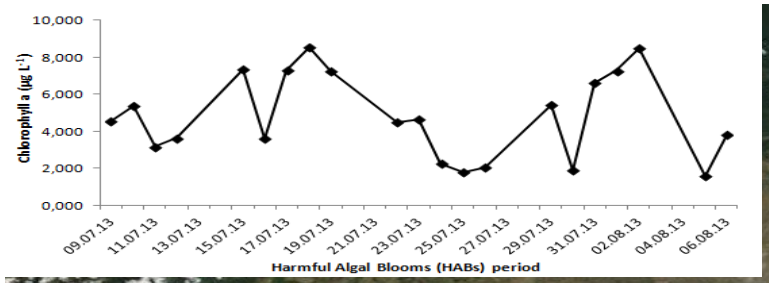
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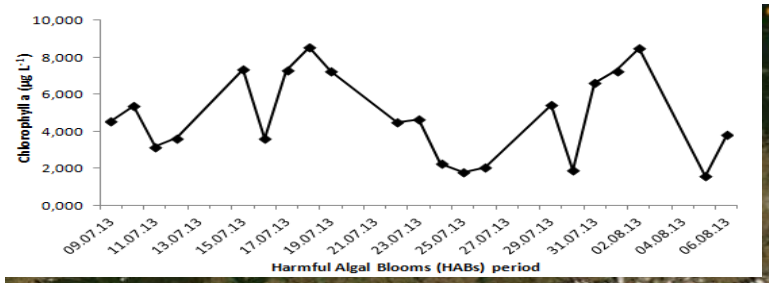
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CONCLUSIONS

- **This study may also indicate advancing of this species from the Black Sea region through the Sea of Marmara and the Dardanelles under favorable conditions. This may be due to the climate changes in addition to the dramatic eutrophication of the system since 1980s.**
- **The findings indicated that during the HAB period the system was generally stable in view of temperature, salinity, and light duration.**
- ***P.lima* bloom was reported for the first time in the Turkish Straits System and the bloom concentration reached to 2.40 x 10^6 cells L^{-1} in summer season. Unfortunately, this species seems be able to create more extensive algal blooms in the near future.**
- **In the intensive bloom periods, the contribution of *P.lima* to both *Prorocentrum* spp. and dinoflagellates reached to 100%.**
- **The strong bloom potential of *P. lima* and other phytoplankton in summer period in end of the this study and in other periods** (Turkoglu, 2004a, 2008, 2013; Turkoglu & Tugrul, 2013) **has revealed that the Dardanelles is under the hyper-eutrophication due to the fact that it is a part of the Turkish Strait System affected by the Black Sea.**
- **Both high nutrient concentrations** (NO^-_2+NO^-_3: 0.44 ± 0.17 µM, PO^{-3}_4: 0.12 ± 0.03 µM and SiO_4: 0.41 ± 0.09 µM) **and lower average values of nutrient ratios** (N/P: 4.04 ± 1.74, Si/P: 3.79 ± 1.24, and Si/N: 1.04 ± 0.36) **according to Redfield ratios** (N:P=16:1; Si:P=15:1; Si:N=15:16) **during the HAB period indicated that primary productivity in the study area was affected by more nitrogen than phosphate due to the eutrophication in the Dardanelles.**
- **Further monitoring of the system in terms of anomalies in the temperature, salinity, and nutrient changes as well as the phytoplankton species composition is needed for a better understanding of the ecological significance of this species in this system and its neighbor systems, the Black Sea and Northern Aegean Sea.**
- **The study calls attention to a possible intensification of DSP events in the Dardanelles in light of excessive *Prorocentrum* blooms in the Dardanelles.**

THANKS FOR YOUR INTEREST



