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A new recorded of red tide forming species; *Heterocapsa triquetra*, *Gymnodinium impudicum*, *Heterosigma akashiwo* and *Thalassiosira rotula* in Alexandria Waters, Egypt

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ABSTRACT

The current study is based on daily monitoring observations to follow phytoplankton blooms in the Eastern Harbor (Alexandria) within a year cycle. The harbor ecosystem was harsh and characterized by various dynamics and variable conditions of a multitude of environmental factors that ultimately were considered strong stressors on phytoplankton development. Three red tide blooms reach extremely high biomass leading to water discoloration triggered in late summer-early autumn, and during the last week of December as well. These blooms were considered unique as represented by newly reported red tide causative species of different groups never previously reported in the Egyptian Mediterranean waters as a red tide bloom species; *Heterocapsa triquetra* and *Gymnodinium impudicum* (Dinophyceae), *Heterosigma akashiwo* (Raphidophycea) and the centric diatom *Thalassiosira rotula*, and under influence of characterized environmental conditions. The highest bloom peak of *H. triquetra* occurred on 9 August (12.97x 10^6 cells L^{-1}), *H. akashiwo* on 26 August (13.91x10^6 cells L^{-1}), *G. impudicum* on 16 September (7.12x10^6 cells L^{-1}) and *T. rotula* on 28 December (3.25 x 10^6 cells L^{-1}). The blooms of the first three species maintained much higher temperature (30.4 - 32.9 ^{0}C) and lower salinity (28.6 - 29.3) compared with the winter bloom in December, while nutrient concentrations exhibit considerable variations and the N/P ratio falls down to a minimum with the bloom peak days. The significant contribution of physical forcing rather than chemical on bloom developments was statistically confirmed but failed to define specific controlling factor/s. The winter bloom of *Thalassiosira rotula* was a surprising and unique first winter red tide bloom in the Eastern Harbor, under minimum annual temperature (18.8°C - 19.6 °C), stresses the strong effect of NO_3, NO_2 and OOM on the bloom development. These blooms maintained higher OOM relative to inorganic nutrients. No fish mortality occurred. The present work offers persuasive evidence for the increased number of newly recorded red tide bloom-forming species in Alexandria waters.

INTRODUCTION

Eutrophication as an environmental problem affects marine ecosystems processes, the natural dynamic equilibrium, and the biotic composition of the respective ecosystems, leading to increased production of phytoplankton and development of visible algal blooms of serious impacts (e.g. **Yang *et al*., 2008**). Since the mid-1970s, Alexandria coastal ecosystems experience high anthropogenic stress creating numerous ecological challenges; degradation of water quality is the major problem (**Sultan, 1975; Mikhail and Halim, 2009**) since coastal discharges of untreated and partially treated discharged effluent became a common feature of the coastline in Alexandria (**EEAA, 2008**). Despite remedial action took place by Alexandria Governorate between 1993-1997 to improve the marine environmental status, and prevent direct discharge input, particularly into the harbor, and legislation issued eutrophication due to the development associated with increased in population, industrialization, the expansion of irrigated agriculture, coastline construction and others, goes on rise and gradually intensified and widespread over new areas (**Mikhail and Labib, 2013**). Such modification impacts on coastal waters have been discussed everywhere (e.g. **Sánchez *et al*., 2007**). In the harbor, water exchange with adjacent open sea areas and the dump of large quantities of different wastes of the fishing and sailing boats anchoring inside the harbor, prone to its eutrophication. The progressive heavy eutrophication is associated with problems created by red tides and other noxious blooms of undesirable and negative impacts; its outbreaks have been at the forefront of coastal management concerns. The recurrent red tide blooms of different duration, magnitude and composition were the subject of intensive studies in the Eastern Harbor since the start of 1990s (e.g. **Labib, 1994b; 1998; Mikhail, 2002; 2003; 2007; Mikhail *et al*., 2007; 2008**). Several incidents of fish and invertebrate mortality were reported in the harbor (**Mikhail *et al*., 2005**). The results of the previous studies stressed the importance of some points summarized as follows; - the harbor suffers from algal blooms of repeated events occurred frequently in the warm seasons between April and October; - a variety of biological and environmental factors were implicated in the initiation, maintenance and decline of recorded red tide blooms in Alexandria waters;- the complexity of the problems surrounding the blooms and the numerous species involved hindered prediction and modeling efforts;- the harbor ecosystem is harsh and characterized by various dynamics and variable conditions of multitude of factors that ultimately are considered strong stressors on phytoplankton development;- specific physical and chemical conditions make it particularly vulnerable to annually reoccurring red tides and granted newly reported species to achieve dense blooms; -blooms as long-term chronic problem are considered one facet of complex ecosystem interactions with anthropogenic effect.

The current research, based on short-term sampling collection is a part of the monitoring program conducted in the Eastern Harbor (Alexandria) throughout a year cycle. The program was designed to follow up events of outbreaks of phytoplankton blooms at intermittent periods in such an eutrophic marine basin. The study offers persuasive evidence for the increased number of newly recorded red tide bloom-forming species in Alexandria waters and increasing knowledge and understanding of red tide dynamics in Alexandria waters that seem essential to face the need for effective monitoring and predictive capability.

MATERIALS AND METHODS

Study area and sampling procedure

The Eastern Harbor is a semi-enclosed marine basin, located in the central part of Alexandria (longitudes 29°53' - 29°54'-E and latitudes 31°12' - 31°13'-N, Fig. 1) with an area of about 2.53 km^2, average depth about 6.5 m and water volume of 15.2×10^6 m^3 The harbor is subjected to amounts of wastewater from different land sources, mainly from the sewer of Alexandria at its western vicinity (Qaitbey area) which receive discharged water from El-Mex Bay; the most eutrophic marine basin (**Mikhail, 2005**).

Sampling stations and collections

During the monitoring program, a weekly sampling collection was conducted at a fixed station (St. I, **Fig.1**) throughout a year cycle (March 2015- March 2016). This station was occupied daily during red tide periods, besides sites entire the harbor (Sts. II-VI) of dense water discoloration. The blooms were also followed at the other three stations along Alexandria Coast (El-Silsilla, El-Shatiby and Gelim, Fig.1). Seawater physical, chemical and biological characteristics were determined at the surface and above the bottom (3m.depth).

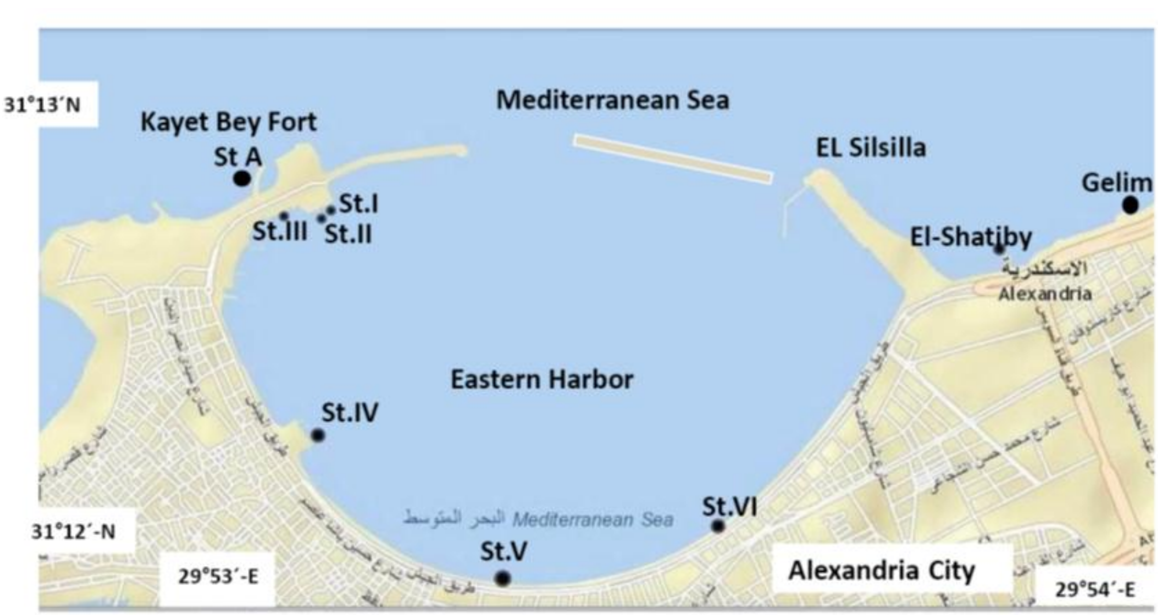

Fig. 1. Eastern Harbor and location of sampling stations

Water temperature (°C), conductivity, and pH were measured in situ using Hydro lab (HANA, Model HI9828-USA), transparency depth (ZSD) by a Secchi disc (30 cm diameter), fixed dissolved oxygen (**Strickland and Parsons, 1972**), and salinity by a refractometer. Variations in temperature and salinity were used to calculate water column stability (**Mamayev and Sergeyev, 1975**). The stored seawater at -4^{o}C was filtered through 0.45µm membrane filter for chemical analyses of dissolved inorganic nutrient concentrations (nitrite NO_2, nitrateNO_3, silicate SiO_4, and phosphate PO_4) applying the procedure of **Strickland and Parsons (1972)**, oxidizable organic matter (OOM) (**FAO, 1975**), fixed ammonia NH_4, (**Grasshoff, 1976**), and estimation of chlorophyll-*a* (**Jeffrey and Humphrey, 1975**). Surface water samples (20 litters) were filtered through the plankton net (mesh size 20 µm.) to determine the standing crop. The samples were first examined using a light inverted microscope to identify living flagellates, and then preserved by the addition of 4% neutral formalin and few drops of Lugol's solution (**Throndsen, 1978**). Phytoplankton cell counting expressed as cells.L^{-1} was performed (**Utermöhl, 1958**), and identification of species followed principally **Tomas (1997)** and **Hallegraeff *et al*. (2003)**.

Statistically, Pearson's correlation coefficient (r) was used to define the degree of the linear relationship between physical, chemical and biological variables using SPSS 13.0 Statistical software, and stepwise multiple regression analysis was also calculated using Hintze model (**Hintze *et al.*, 1993**).

RESULTS

The monitoring program, which conducted in the Eastern Harbor throughout a year cycle to follow up events of outbreaks of phytoplankton blooms, revealed several repeated outbreaks of phytoplankton blooms caused by numerous species of different groups. However, the current research focuses on the occurrence of some species never known before red tide bloom-forming species in Alexandria waters; the dinoflagellates *Heterocapsa triquetra* (Ehrenberg) Stein and *Gymnodinium impudicum* (Fraga and Bravo) Hansen and Moestrup, the raphidophycian *Heterosigma akashiwo* (Hada) Hada ex Hara and Chihara and the centric diatom *Thalassiosira rotula* Meunier. The first three species dominated the community in late summer-early autumn, while the latter one in winter under different environmental conditions. The bloom peaks severely reduced Secchi depth to vary between 0.75m and 1m and raised chlorophyll-*a* to extremely high values (**Tables 1 and 2**). Due to our daily observation, their blooms seem to be transferred into the harbor from the adjacent area.

In general, the massive occurrence of *H. triquetra*, *H. akashiwo* and *G. impudicum* maintained higher temperature, and much lower salinity, nutrient concentrations and OOM compared with that of *T. rotula*, while pH was unchanged.

Light brown colored water developed in the first week of August, spreads over the entire harbor after a couple of days and it became denser in the second week of August signaling existence of a heavy bloom. The bloom lasted for a weak. The population size with the onset of the bloom on 5 August (1.59 ×10^{6} cells.L^{-1}) declared *H. triquetra* and *H.*

akashiwo insignificant contributors (4.8 and14.42%, respectively). However, the bloom developed so rapidly and the community changed dramatically on the bloom peak day; *H. triquetra* become the major constituent (12.97x 10^6 cells L^{-1}, 66.49%), and *Heterosigma akashiwo* ranked the second (2.22x10^6 cells L^{-1}, 11.39%). This peak raised the produced DO and biomass to extremely high values (16.8 mg l^{-1} and 17.19 µgl^{-1}, respectively). Others species as *Eutreptiella* (A. da Cunha) species. (8.2%), *Pyramimonas* (Schmarda) species. (4.2 %), *Aplanochytrium* (Bahnweg and Sparrow) species (2.7 %), *Chattonella antiqua* (Hada) Ono (2.3%) and *G. impudicum* (0.6%) succeeded to achieve a noticeable occurrence. Despite the dramatic decrease in *H. triquetra* density on the next day, it was still the leader (1.97x10^6 cells L^{-1}, 54.67%). Beside the peak day in August, *H. triquetra* as a co-contributor shared actively another red tide peak on 16 September (1.71x10^6 cells L^{-1}, 10.69%).

The species *H. akashiwo* peaked again in late August during the longest bloom period that was never previously recorded during the study. The species culminated at the highest of 13.91 x 10^6 cells L^{-1} (90.06 % of the total), raising Chl *a* to 8.67 µg l^{-1} and DO to 10.08 mg l^{-1}. This peak occurred under relatively high NO_3 (6.56 µM), and reduced PO_4 (0.15 µM), and NO_3/PO_4 ratio at 18.75. *Gymnodinium impudicum* was still of limited contribution (0.41%). Other accompanying species include *Aplanochytrium* sp. (3%) and *Heterocapsa triquetra* (2.8 %).

Table 1. Physical and chemical parameters during the red tide bloom periods in the Eastern Harbor

Periods	T℃	Salinity	pH	DO (O_2 L^{-1})	OOM (µM)	NO_3 (µM)	NO_2 (µM)	PO_4 (µM)	SiO_4 (µM)
5-12 Aug.	31.9-32.9	28.6-28.9	8.3-8.8	6.6-16.8	7.4-16.6	1.3-3.8	0.25-0.7	0.1-1.15	0.1-7.9
17Aug.-20 Sep.	30.4-31.1	28.75-29.3	8.3-8.6	5.1-12	7.7-24	2.3-6.56	0.28-0.7	0.15-0.55	1.6-9
24-29 Dec.	18.8-19.6	31.9-32.5	8.3-8.5	10.6-16.8	17.9-33.3	10.37-19.8	0.85-2	0.15-0.9	4.6-8.8

DO- Dissolved Oxygen; OOM- Oxidizable Organic Matter

Table 2. Species abundance and biomass of the different bloom periods in the Eastern Harbor

Periods	Peak day	Causative species	St.crop (cellx10^{6} L^{-1})	% to St.crop	Chl.*a* (μ g L^{-1})
5-12 Aug.	9 Aug.	*Heterocapsa triquetra* *Heterosigma akashiwo*	19.51	68.37 11.71	17.19
17Aug.- 20 Sep.	26 Aug.	*Heterosigma akashiwo*	15.44	90.06	18.67
	16 Sep.	*Gymnodinium impudicum*	16	44.47	19.35
24-29 Dec.	28 Dec.	*Thalassiosira rotula*	4.73	68.65	14.26

St. crop - Standing crop; Chl.*a*- Chlorophyll *a*

By mid-September, another dense bloom was developed which turned the water reddish. This peak occurred in physical and chemical conditions similar to late August. However, *G. impudicum* became the chief component of the community (7.12x10^{6} cells.L^{-1}), and it was followed by *H. akashiwo* (4.9x10^{6} cells.L^{-1}, 30.7 %), *H. triquetra* (1.7x10^{6} cells.L^{-1}, 10.6 %) and *Prorocentrum micans* (0.4x10^{6} cells.L^{-1}, 2.57 %). The species association between *G. impudicum* and *H. akashiwo* seems to be strong ($r = 0.291$, $p< 0.05$). The blooming peak raised DO to 12 mg.l^{-1}.

A unique monospecific winter bloom of the centric diatom *Thalassiosira rotula* Meunier (61.26% - 89.92%) was observed during four days in cold winter at water temperatures near to the annual minimum (18.9 °C - 19.6 °C), never has been reported as red tide species in the harbor, and under relatively high salinity with limited variations. The bloom commenced on 24 December with high NO_3 (10.37 µM). Its peak on 28 December maintained plenty of NO_3 (19.75 µM) significantly higher compared with the previously mentioned summer-early autumn red tide bloom, intermediate concentrations of SiO_4 and extremely high OOM. The NO_3/PO_4 ratio decreased gradually with the bloom development to reach its minimum (21.9) with the bloom peak. *Thalassiosira rotula* culminated its peak of 3.25 x 10^{6} cells L^{-1}, 68.65 %, raising the produced DO to 16.84 mg.l^{-1}. The dinoflagellate *Prorocentrum cordatum* Ostenfeld J. D. Dodge succeeded to grow well under the bloom conditions, (1.08x10^{6} cells.L^{-1}, 22.45%) on the bloom peak day. The annual survey declares *T. rotula* was responsible for other two blooms during January and March 2016.

DISCUSSION

Genus *Hetrocapsa* was previously represented in the harbor by *H. circularisquama* Horiguchi, T. with its first record (0.3x10^{6} cells.L^{-1}) in May 2001 (**Mikhail, 2003**), and with densities around 0.4x10^{6} cells.L^{-1} in August 2004-2005 (**Labib, 2004; Mikhail., 2007; Mikhail *et al.*, 2008**). Regular prolonged blooms of the relatively small armored dinoflagellate *H. triquetra* are common in the coastal and estuarine waters around the world (e.g. **Tillman *et al*., 2017**), including the Mediterranean (**Kim *et al.*, 1990; Litaker *et al*., 2002a; Baek *et al*., 2011; Tas, 2011; 2015**). The present temperature and salinity ranges seem suitable for the massive growth of *H. triquetra* in contrarily with the result of **Tas (2015)** who reported the disappearance of this species in May at 23°C. The significant positive correlation between temperature and the abundance ($r = 0.285$, $p \leq 0.05$) signals its importance. Meanwhile, the yearly pattern of *H. triquetra* occurrence showed its first appearance in mid-April, its highest abundance in summer and autumn, and it was lowest in January 2016 (0.002×10^6 cells.L^{-1}) under a wide temperature and salinity range (15.3-32.9°C and 28.46-34.12, respectively). Ecologically, *H. triquetra* is often classified as a mesohaline species (**Marshall and Alden, 1990**), even though it is functionally eurythermal, euryhaline species (**Yamaguchi *et al*., 1997; Baek *et al*., 2011**). The stepwise multiple regression analysis based on temperature and salinity variations explains 37% of the species variability. The variations in temperature and salinity certainly affected water stability property, which seems a prerequisite factor for the *H. triquetra* accumulation during its massive bloom periods; a significant positive correlation was found ($r = 0.168$, $p \leq 0.05$). The strong positive correlation between the density of *H. triquetra* and pH values ($r = 0.34$, $p \leq 0.01$) emphasizes the conclusion of **Havskum and Hansen (2006)** for the strong link between the high pH values observed with the highest abundance of the species. In laboratory experiments, the growth rate of *H. triquetra* was highest at pH 8.8 to 8.9, (**Hansen, 2002)**, and its blooms can increase the pH to above 9.0 (**Olesen 2001**), thereby disrupting ecosystem function (**Park *et al*., 2018**). The bloom maintained relatively low NO_2 and NO_3, and intermediate NH_4 concentration, and the harbor seems to be P-limited. It is reported that *H. triquetra* has the capability to produce high levels of the extracellular enzyme alkaline phosphatase, allowing it to escape P limitation (**Hansen, 2002**). The high amounts of organic substances with the bloom days relative to inorganic nutrient concentrations represent alternative nutrient sources that may favor the bloom formation (**Legrand *et al*., 1998; Steidinger *et al*., 1998; Tas and Yilmaz, 2015**). Meanwhile, *H. triquetra* can feed mixotrophically to supplement its nutritional requirements and reducing competition from co-occurring other dinoflagellates (**Litaker *et al*., 2002a**). It is worth to mention that high OOM and low salinity accompanying the highest abundance of *H. triquetra* reflect arrival influx of freshwater, which reported an important bloom triggering factor (**Litaker *et al*., 2002a; Park *et al*., 2018**).

The genus *Heterosigma* includes only the species *H. akashiwo* (**Yamaguchi *et al*., 2010**), which causes prodigious red tide blooms that widely distributed in subpolar to subtropical eutrophic coastal waters (**Smayda, 1998; 2006; Shikata *et al*., 2008**). *Heterosigma akashiwo* seems to vary among different ecotypes and is environmental conditions dependent (**Rensel, 2007; Rensel *et al*., 2010**). This cosmopolitan alga exhibits a wide geographical distribution in the eutrophic waters of the Mediterranean

Sea (**Bizsel and Bizsel, 2002; Tas and Yilmaz, 2015; Dursun and Tas, 2019**), first reported as a few scattered cells in Alexandria waters (**Mikhail, 2001**). During the present monitoring, *H. akashiwo* presented throughout most of the year and detected in 75.4% of the collected samples. The species in the context of weekly variation showed the highest abundance in August and September (average >2.5 x 10^6 cells.L^{-1} & maximum 13.91 x 10^6 cells.L^{-1} on 26 August), and it was traced in March 2016. The present temperature range (Table 1) is relatively higher than the range (24.4 to 30.9°C) reported by **Branco *et al*. (2014)** for its massive occurrence. However, this species was previously registered eurythermal and euryhaline (**Strom *et al*., 2013; Branco *et al*., 2014**), offers some advantages to be a strong competitive member of the phytoplankton species assemblage, signaling its resilient ability to survive in different habitats (**Bronicheski, 2014**). Measured temperature seems among the most critical environmental factors that regulate the bloom dynamics; significant positive correlation found with abundance ($r = 0.353$, $p \leq 0.01$), supporting other results (**Li and Smayda, 2000**). Salinity range with the present bloom of *H. akashiwo* agrees with others reported (**Honjo, 1993; Martinez *et al*., 2011**); salinity negatively, significantly correlated with abundance ($r = 0.306$, $p \leq 0.05$). Subsequently, water stability represents a prerequisite factor governing *H. akashiwo* bloom; a significant positive correlation found between Δ sigma-*t* and abundance (0.34, $p \leq 0.01$). *Heterosigma akashiwo* reached its highest abundance with relatively high NO_3 (5-6.5 µM) and PO_4 (up to 3 µM) concentrations; the latter parameter severely dropped to 0.35 µM with the progress of the bloom that reflects its ability for high uptake of phosphate (**Watanabe *et al*., 1989**). The plenty of ambient nitrogen and phosphorus concentrations and high requirement of *H. akashiwo* (**Watanabe and Nakamura, 1984a**) stresses the close link between its massive occurrence and cultural eutrophication confirming others (**Honjo, 1993; Shikata *et al*., 2008**). The present massive bloom of *H. akashiwo* caused a sharp drop in the number of accompanied other species, and for example, the centric diatom *Skeletonema costatum* (Greville) which contributes significantly to the total standing crop in the harbor most of the year-round was totally excluded. Among proposed factors, allelopathic interaction between these two species may help explanation; *H. akashiwo* upon its present concentrations is able to cease and/or inhibit the growth of *S. costatum* (**Pratt, 1966; Honjo *et al*., 1978**). Except for the bloom effect on species diversity, no other harmful effects as fish mortality were observed. The relation between such conditions and the species composition is a focus point that prompted intensive research.

The stepwise multiple regression analysis reflects the significant contribution ($p< 0.05$) of the combined temperature, NH_4, and PO_4 as explain about 60% of the species variability.

Identification and quantification of *G. impudicum* have been done using a light microscope during its massive occurrence. This species closely resembles *Cochlodinium polykrikoides* Margalef based on morphological features (**Lee *et al*., 2001**). Originally, *G.*

impudicum athecate autotrophic species (**Yahia-Kéfi *et al*., 2005**) was described *Gyrodinium impudicum* as non-toxic species of no associated harmful effects, and a red tide bloom-forming species during June and September in different Mediterranean areas with high densities between $2x10^5$-$6.3x10^5$ cells.L^{-1} (**Fraga *et al*., 1995**). However, there is a possibility that *G. impudicum* might cause fish deaths due to the production of a mucous exopolysaccharide (**Kim *et al*., 1999**). However, no significant toxic or biologically hazardous compounds were detected in its extract (**Kim *et al*., 2010**). It is the first red tide bloom of *G. impudicum* never previously recorded in Alexandria coastal waters. The present monitoring shows a few scattered cells of *G. impudicum* from May to July at an increased temperature range of 21.8-29.8°C and relatively low salinity between 29.2 and 32.1. Its intensive occurrence at the measured high temperature and low salinity correspond well with previous data (**Chang *et al*., 2001**), and the species seems dependent on water stability (Δ sigma-*t* value at 120.11). The relatively high nitrate and low phosphate accompanied the bloom peak are in contrary to the observations of **Giacobbe *et al*. (1996) and Yahia-Kéfi *et al*. (2005)** that maximum abundance of *Gyrodinium impudicum* occurs under high phosphate levels versus nitrogen (nitrogen-limited conditions), and to the general assumption that dinoflagellates require higher phosphorus to face their higher contents of nucleic acids (**Costas and Lopez-Rodas, 1991**). In a culture experiment, the phosphate critical point for the growth of *G. impudicum* stands between 1.35 and 4.05 µM (**Chang *et al*., 2001**). The limited PO_4 and increased OOM might offer some advantage to *G. impodicum* to dominate and form blooms since it utilizes a wide variety of dissolved organic phosphorus compounds in addition to DIP (**Oh *et al*., 2010**), and maybe attributed to its potential mixotrophic character as described by **Jeong *et al*., (2005)**. Its restricted occurrence in summer hindered prediction and modeling efforts. These results provide important information for understanding the mechanism of *G. impodicum* blooms and developing technology to predict blooms of this organism in Alexandria waters.

Genus *Thalassiosira* in Alexandria waters is well-diversified, includes 6 species; *T. rotula*, *T. oestrupii, T. pseudonana, T. subtilis, T. angulata and T. anguste-lineata*, representing important components of the community (e.g. **Labib 2000a; b**), as well as in temperate to polar regions worldwide (**Karentz and Smayda, 1984; Lange *et al*., 1992; Degerlund and Eilertsen, 2010**). The present harmless monospecific bloom of the centric diatom *T. rotula* lasted for a few days in cold winter under minimum annual temperature is considered unique as first reported under such reduced temperature, and as a red tide event in the harbor. The annual monitoring reveals *T. rotula* a perennial species observed in 73.85% of the samples collected and with increased frequencies in summer-early autumn and winter-early spring. Such high productive winter periods under minimum annual temperature and low light fluxes detected from the measured Secchi disc readings are previously reported elsewhere (**Krawiec, 1982; Litaker *et al*., 2002a**). Several authors explain *T. rotula* a commonly-occurring cosmopolitan diatom that can dominate phytoplankton assemblages across diverse marine habitats under a wide variety of environmental conditions, achieving blooms in spring (**Ehrenhauss *et al*., 2004; Lunven *et al*., 2005; Whittaker *et al*., 2012; Boyd *et al*., 2013**). Experimentally, this species is proved eurythermal and moderately euryhaline (**Krawiec, 1982**); its optimal growth temperature at 16ºC (**Baars, 1981**), and growth rates inversely correlated with increased temperature (**Boyd *et al*., 2013**). The present bloom of *T. rotula* in winter supports others (e.g. **Glé *et al.*, 2007; Carstensen *et al.* 2014**) who pointed out winter community essentially composed of diatoms larger than 20µm (*Lauderia* spp., *Thalassiosira* spp., *Chaetoceros* spp., *Skeletonema costatum*), and such large dominate cells grow under rich nutrient conditions and mixed condition, and can only develop when the temperature is below a critical value and disappear where planetary warming increases temperature beyond their critical threshold (**Cloern, 2018**). The abrupt increase in nutrient concentrations with the bloom certainly announces the arrival of new water mass with high concentrations and/or resulted from mixing conditions during winter. The significant positive correlation between NO_3, NO_2 and OOM and the abundance of *T. rotula* gives clear evidence of their strong effect on the bloom abundance ($r = 0.307$, $p \leq 0.05$, $r = 0.403$ $p \leq 0.01$ and $r = 0.423$, $p \leq 0.01$, respectively), supporting **Harris *et al*. (1995)** and **Gayoso (1998)** that wide geographical distribution and massive blooms of *Thalassiosira* associate with eutrophic water. Meanwhile, the bloom peak occurred with high pH (8.48) that corresponds well with the experimental results of **Chen and Durbin (1994)**. The stepwise multiple regression analysis indicates variations in NO_2 and SiO_4 might explain 57% of the species variability.

Although neither fish kill events or apparent human health problems were witnessed during these dense blooms, further studies are still needed to observe water discoloration periods; the harbor with rapidly changing environmental conditions, increasing number and magnitude of potentially harmful species is a risk area, that might affect the delivery of ecosystem services to society, conservation objectives and public health.

CONCLUSION

The current study is based on daily monitoring observations to follow phytoplankton blooms and red tide in the Eastern Harbor within a year cycle. Four species were recorded as new red tide forming species; *Heterocapsa triquetra Gymnodinium impudicum*, *Heterosigma akashiwo* and *Thalassiosira rotula*, and added to the list of red tide species in Egypt. The latter species was a surprising and unique, as a first winter red tide bloom in the Eastern Harbor, under minimum annual temperature. The significant contribution of physical forcing rather than chemical on bloom developments was statistically confirmed but failed to define specific controlling factor/s. The present work offers persuasive evidence for the increased number of newly recorded red tide bloom-forming species in Alexandria waters. Neither fish kill events or apparent human health problems were witnessed during these dense blooms.

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