Vol.51, 2026, 14-32

Research Article

Physicochemical Factors Influencing Blooms of the non-toxic HABs species *Chaetoceros* spp. and *Neoceratium lineatum* in the Bizerte Lagoon (SW Mediterranean)

Sondes MELLITI BEN GARALI[1,2], Kaouther MEJRI KOUSRI[1], Inès SAHRAOUI[1,2] & Asma SAKKA HLAILI[*1,2]

[1]University of Carthage. Faculty of Sciences of Bizerte. Laboratory of Phytoplanktonology and Plant Biology. Bizerte, Tunisia

[2]University of Carthage. Faculty of Sciences of Bizerte. Laboratory of Plant Toxicology and Microbiology. LR18ES38, Bizerte, Tunisia

***Correspondence:** asma.sakka@fsb.ucar.tn

Received: 08/12/2025; **Accepted:** 05/03/2026; **Published:** 13/03/2026

Abstract: An intense phytoplankton bloom occurred in the Bizerte Lagoon in early spring, following heavy rainfall and pronounced freshwater supply that was accompanied by nutrient enrichment. This algal outbreak was associated with spectacular discolored water event.

A weekly sampling was conducted from March to April 2012 at each depth from the surface down to 9 m in order to investigate the dynamics of this bloom in relation to physicochemical factors. During the sampling period, the development of a clear halocline and the high nutrient availability were favorable for the phytoplankton proliferation, reaching very high biomass (13 - 20 µg Chl *a* L^{-1}) and abundance (> 10^7 cells L^{-1}).

The phytoplankton bloom was mainly caused by three species that succeeded one another over time. Chain-forming diatoms *Chaetoceros* (*C. holsaticus* and *C. constrictus*) showed firstly a dominance ($6 – 10 \times 10^6$ cells L^{-1}) coinciding with low salinity (14-20 PSU) and high nutrient concentrations, particularly of N and Si. Subsequently, the decrease in Si availability and the increase in salinity (30°PSU) resulted in a decline in diatom species in favor of the dinoflagellate *Neoceratium lineatum*, which gained the advantage (7 10^6 cells L^{-1}) when N and P levels increased. Although the blooming species were non-toxic, they had harmful effects, as they were involved in fish and cuttlefish mortality.

The study indicated that the Bizerte Lagoon is highly vulnerable to hydrological variability and nutrient enrichment, conditions that can trigger the proliferation of harmful phytoplankton species. This underscores the importance of implementing continuous monitoring programs to detect and manage harmful algal blooms at an early stage.

Keywords: Diatoms; dinoflagellates; *Chaetoceros* spp*; Neoceratium lineatum;* HABs; Nutrient enrichment; Halocline stratification; Bizerte Lagoon; Tunisia; Mediterranean.

https://doi.org/10.71754/instm.bulletin.v51.1781

1. Introduction

Phytoplankton constitutes the basis of food web structure and functioning, accounting for 95% of marine primary productivity (Nielsen, 1975; Wetzel, 2001). It plays crucial roles in nutrient cycles and biological carbon pump in marine waters (Zehr and Kudela 2011; Tréguer et al., 2018).

The development, the composition and the succession of phytoplankton organisms are directly related to environmental conditions, such as light, temperature, salinity and nutrient availability (Drake et al., 2010; Abdenadher et al., 2012; Huang et al., 2012). Under certain conditions, phytoplankton populations proliferate vigorously forming pronounced blooms, with very high chlorophyll concentrations (>100 µg Chl *a* L^{-1}) and cell densities (>10^5-10^7 cells L^{-1}) (Hallegraeff, 2003; Bhat et al., 2006).

These blooms are often associated with apparent impacts on the environment (red-tides, anoxia, mucilage production, toxicity, reduction in the aesthetic value of water, etc.) which cause economic threats for fisheries, aquaculture and tourism (Vidal et al., 1999; Rodger et al., 2011; D'Silva et al., 2012). Moreover, specific composition of phytoplankton is sensitive to environmental changes and therefore phytoplankton is a good indicator of the ecological state of marine ecosystems and the level of their alteration (Paches et al., 2019; Parsons et al., 2021; Song et al., 2022).

Around 300 phytoplankton species, belonging to diverse taxonomic groups, are known to form harmful algal blooms (HABs). Among them, dinoflagellates account for 75% of these harmful species. In addition, several diatom species also contributed significantly to the occurrence of HABs (Smayda, 1997; Glibert et al., 2018; Hallegraeff et al., 2021). Both groups contain a great diversity of toxic species that can harm economic activities and human health, since they are responsible for several poisoning, which can sometime be fatal (Jasprica et al., 2005; Bratbak et al., 2011; Seoane et al., 2012). However, some dinoflagellate and diatom species, although they do not produce toxins, can form harmful blooms capable of disrupting ecosystems and impairing socio-economical services (Sunda, 2006; Trainer and Yoshida, 2014) Indeed, non-toxic harmful species can degrade water quality and trigger hypoxic or anoxic conditions, leading to large-scale mortality of invertebrates, fish and mollusks, with substantial economic consequences. A representative example is the red-tide dinoflagellate *Neoceratium lineatum*, which frequently develops dense blooms (1.5-8.9 106 cells L-1). Such proliferations are often associated with severe oxygen depletion, resulting in mass mortalities of fish, oysters and shrimp larvae (Okaichi, 2003; Vargas-Montero and Freer, 2004).

Blooms of this species are observed in the Eastern Mediterranean during late summer, associated with high temperature, salinity and nutrient levels (Spatharis et al., 2009). In addition, species of *Neoceratium*, including *N. lineatum*, characterize marine regions with specific temperature conditions and they may provide ecological indicators for global warming (Tunin-Ley and Lemée, 2013).

The chain-forming diatoms *Chaetoceros* are also known as red-tide organisms that can reach very high densities (1.2- 8 106 cells L-1) associated with fish and shellfish mortalities (Xin et al., 2023; McGlone et al., 2024). When these diatoms are filtered on gill lamella of fish, their setae are easily broken, and large quantities can stay in the inter-lamellar space, provoking irritation of gill goblet-cells that secrete mucus in excess, thus leading to fish suffocation

(Yang and Albright, 1992; Albright et al., 1993). Fish mortalities due to *Chaetoceros* species (such as *C. salsugineus* and *C.*

socialis) are already reported from aquaculture farms (Treasurer et al., 2003; Smayda, 2006; Hiagh, 2010). *Chaetoceros* is considered as the main primary producers in upwelling waters and coastal regions (Rines and Theriot, 2003; Draredja et al., 2019), where it contributed approximately 20-25% of primary production (Jensen and Moestrup, 1998). These diatoms show high peaks density often linked to an increase of temperature, a decrease in salinity and a high availability of nutrients (Orive et al., 1998; Trigueros et al., 2002; Wang et al., 2006).

In last decades, HABs have been increasing in coastal waters, as these ecosystems are exposed to several constraints (eutrophication, pollution, warming, etc.). Besides, HABs occurrence was used as an indicator to assess the environmental change by the legislation in Japan (Suzuki et al., 2016; Lv et al., 2023). Coastal lagoons are land-sea transitional areas, with high productivity and biodiversity, providing numerous ecosystem services. Unfortunately, these ecosystems face intense anthropogenic and natural pressures, leading to significant ecological disturbances, which result in biodiversity loss, depletion of vital resources and adverse impacts on human health (Xian et al., 2025; El Mahrad et al., 2022). This has prompted extensive scientific efforts to better understand the functioning of these vital ecosystems, with the aim of ensuring their effective protection and promoting sustainable management strategies.

The Bizerte Lagoon has an ecological and socio-economic importance. Numerous studies conducted in this lagoon have reported a high occurrence of harmful species, characterized by marked seasonal patterns and recurrent intense blooms occurring at different times of the year (Bouchouicha et al., 2012; Turki et al., 2014; Melliti Ben Garali et al., 2016, 2019). The lagoon supports intense fishing and shellfish farming activities, therefore, proliferations of non-toxic species can still have severe consequences for both ecosystem functioning and the economic sectors that depend on these resources. The goal of this study was to investigate the dynamics of non-toxic HABs species (such as *Chaetoceros* and *Neoceratium lineatum*) in relation to environmental factors in the Bizerte Lagoon.

2. Materials and methods

2.1. Study area

The Bizerte Lagoon, located on the northern coast of Tunisia (37°8′-37°14′ N, 9°48′-9°56′ E, Figure 1), is the third largest Tunisian Lagoon. It has an average depth of 8 m, a volume of 851.2 106 m3 and an area of 121.6 km2. It is a restricted lagoon since water exchanges with the Mediterranean Sea are limited to a channel that has a width of 800 m and a length of 7 km. The lagoon receives fresh water from several surrounding rivers, mainly from Lake Ichkeul *via* the Tinja River (Harzallah, 2003).

The hydrodynamics of the lagoon are mainly driven by winds that exhibit a defined seasonal pattern, with a dominance of strong winds from the north-west (8 m s^{-1} in autumn and winter) and from the east (5 m s^{-1} in summer). The major portion of the annual rainfall occurs during November to February. The system receives runoff from human activities such as industrial effluents, wastewater and solid waste disposals. has an ecological and socio-economic importance.

This ecosystem supports a high fishing activity (100 tons $year^{-1}$, in 2014) and constitutes the first Tunisian shellfish farming area a, with high production of mussels (*Mytilus galloprovincialis*) and oysters (*Ostrea edulis*) (>120 tons $year^{-1}$, DGPA, 2013)

The lagoon watershed holds other socio-economic activities, including intensive agriculture (mainly cereal farming), livestock, tourism and several industries (such as cement factories, steel and leather industries, oil refinery and electric circuit industry). The lagoon is characterized by high nutrient availability (NO_3^- > 5 µM; NH_4^+ > 12 µM; PO_4^{3-} > 1 µM; $Si(OH)_4$ > 11 µM; Sakka Hlaili et al., 2008; Meddeb et al., 2018; Mejri Kousri et al., 2023) leading to high comparison to the adjacent Mediterranean waters (Sahraoui et al., 2012; Meddeb et al., 2018).

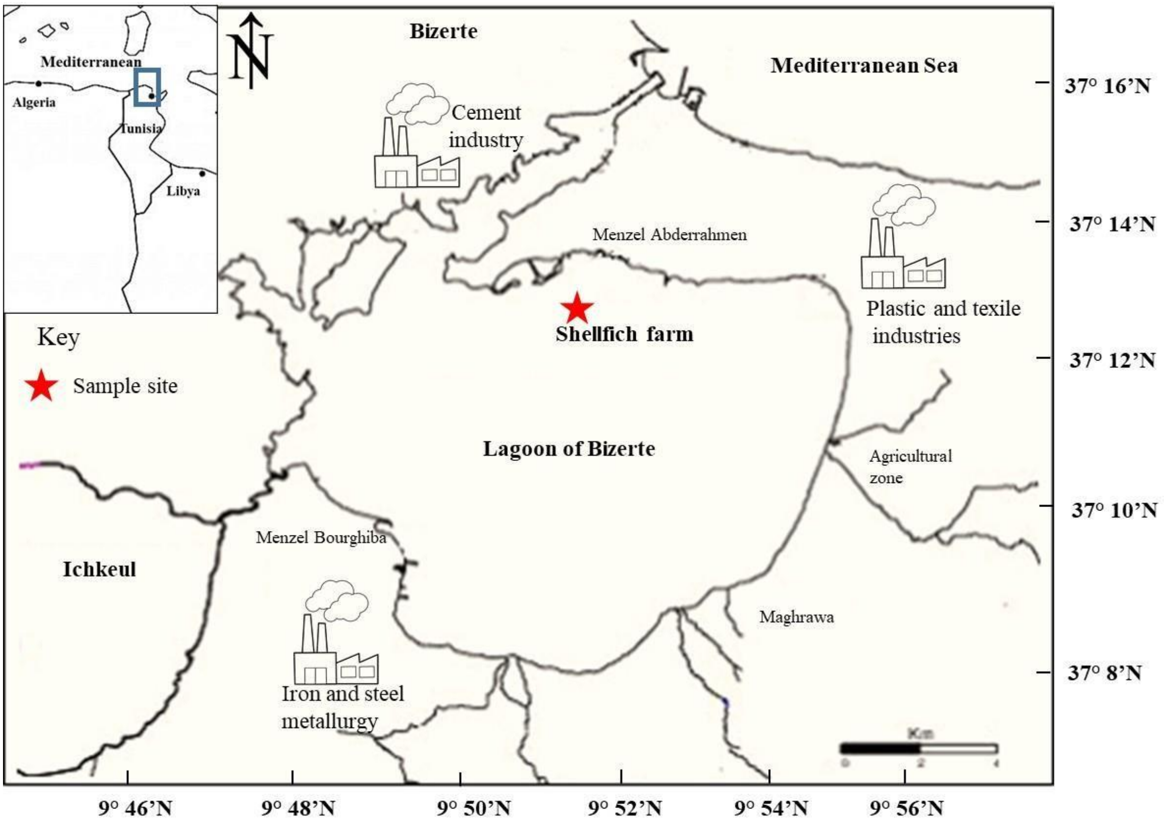

Figure 1. Bizerte Lagoon: location of sampling station

However, there is a wide spatial and temporal variability of physical and chemical factors due to the seasonality of nutrient enrichment, temperature, rivers inputs and marine inflows (Sahraoui et al., 2013; Melliti Ben Garali et al., 2019). This had led to pronounced spatial and seasonal dynamics of phytoplankton communities.

2.2. Sampling

The sampling was carried out in a station located in shellfish farm (37°13'55' 09°51'58', maximal depth of 12.5 m, Figure 1) from March to April 2012, a period characterized by a spectacular discolored water event that corresponded to a pronounced phytoplankton bloom.

In order to investigate the dynamic of this algal outbreak, the sampling was conducted at high frequency (i.e. 14, 20, 27 March and 03, 21 April) and at each meter of depth from the surface down to 9 m.

During the same period, data of weather (atmospheric temperature, rain fall and wind speed) were obtained from the National Meteorological Institute (INM, 2012).

Water samples were collected in triplicate using a water sampler (Hydrobios, 2 L), then filtered through 200 µm mesh screen (to remove large plankton) and finally stored in isothermal containers and processed within 2-4 h after sampling.

These samples were used for nutrient, Chl *a* and phytoplankton enumeration and determination. Water temperature, salinity, pH and dissolved oxygen were recorded *in situ* using a multi parameter (WTW, Multi1970i).

2.3. Laboratory analyses

Water samples for nutrients (1000 mL) were filtered through 0.2 µm polycarbonate filters. The filtrates were collected in acid-washed vials and stored at −20°C until analysis. Nutrient concentrations were determined by spectrophotometric methods, using a spectrophotometer (JENWAY 6400). Nitrite and nitrate were analyzed according to the methods of Wood et al., (1967) and ammonium following the procedure of Aminot and Chaussepied (1983). Phosphorous levels were determined as described by Murphy and Riley (1962). Reactive dissolved silicate was analyzed according to the methods of Strickland and Parsons (1968). Detection limits of the analytic methods were 0.01, 0.02 and 0.1 µM for nitrite, phosphate and silicate, respectively, and 0.05 µM for nitrate and ammonium.

Chl *a* concentrations were determined on 1000 mL samples that were filtered through 0.2 µm pore-size polycarbonate filters (Millipore). Chl *a* was extracted in 90% acetone for 30 h in darkness at 4°C in the dark. Pigment concentrations were estimated following the procedure given by Parsons et al., (1984).

For phytoplankton analysis, water samples (150 mL) were fixed with acid Lugol solution (3% final concentration) and then stored at 4°C in the dark (Parsons et al., 1984). The identification and the enumeration of phytoplankton were determined under an inverted microscope (CETI, 100x objective) on 10-100 mL settled volumes (Utermöhl, 1931). At least 500 cells were counted in each sample to ensure statistical reliability and the representativeness of the phytoplankton community.

2.4. Statistical analyses

An analysis of variance (ANOVA) was used to test the significance of temporal and vertical variation of physicochemical factors, nutrient levels, Chl *a* concentrations and phytoplankton abundances. Finally, the relationships between physicochemical factors and phytoplankton were determined using spearman correlation coefficient. Statistical analyses were performed on SPSS software.11.0 for Windows.

3. Results

3.1. Weather factors

During the period from March to April 2012, the atmospheric temperatures ranged from 10ºC to 20.5ºC (INM, 2012). The dominant winds were from the northwest and had an average speed varying from 2-11 m s^{-1}. Precipitations were distributed throughout the period of sampling. In terms of water balance, the winter 2012 was the wettest (213.55 mm, INM). Following the intense rainfall, the Bizerte Lagoon received during March 2012 an exceptional freshwater flow (55 m^3 s^{-1}, CRDA, 2013) that came mainly from Lake Ichkeul, the main source supplying this ecosystem.

3.2. Hydrological factors

Water temperature mainly displayed vertical variation in some dates ($p<0.05$, ANOVA). Indeed, a weak thermocline was developed (at 3/4 – 6 m), where surface waters were a little warmer (17.1-18.7ºC) than deeper layer (13.8-16.1) (Figure 2a).

From March 14 to April 03, salinity did not vary among dates ($p>0.05$, ANOVA), but water column was clearly stratified ($p<0.01$, ANOVA, $N = 27$), with a clear halocline development between 2 and 6 m. Salinity was low (14-18 PSU) in the surface water layer (0 - 2 m), but increased at the halocline to reach higher values (20 - 32 PSU). Beyond 6 m, salinity was vertically homogeneous (30 - 34 PSU) (Figure 2b).

On April 21, salinity increased and the halocline was absent, since the vertical profile of salinity was homogeneous over all depths (29 – 30.5 PSU).

Water pH and dissolved oxygen (DO) concentrations showed weak temporal and vertical variations. The surface layer (~2/3 m) had a pH around 8.6-8.7 and DO levels of 8.5-9.5 mg L^{-1}. These factors decreased slightly until they reached the lower limit of the halocline (6 m) and then remained stable with depth (pH: 7.8-8.3, OD: 5.9-7.7 mg L^{-1}) (Figure 2c, d).

3.3. Nutrients

Concentrations of all nutrients displayed no significant vertical variations ($p>0.05$, ANOVA) but were significantly different among sampling dates ($p<0.01$, ANOVA) (Figure 3).

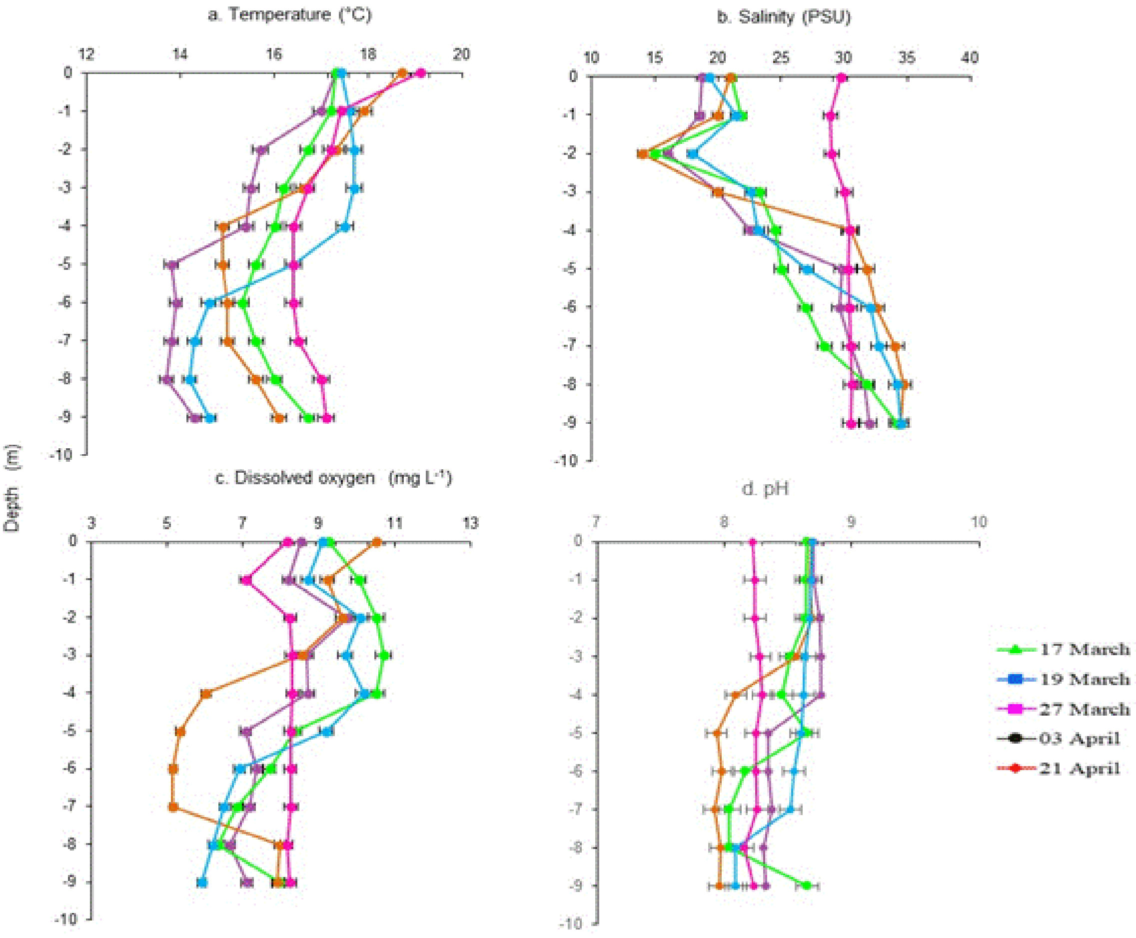

Figure 2. Vertical distributions of water temperature, salinity, dissolved oxygen and pH during the phytoplankton bloom period (March- April 2012) (Average ± SD).

On March 14, concentrations of nitrogen nutrients ($NO_2^- + NO_3^- + NH_4^+$) varied from 35 – 45 µM and then gradually increased until the end of the bloom, reaching 50 - 65 µM (Figure 3a). Concentrations of phosphates were low at the beginning of the bloom (≅ 1 µM) and then progressively increased until the end of the phytoplankton outbreak (5 µM) (Figure 3b).

From March 14 to March 27, silicate concentrations were high and almost similar among dates (6 - 8 µM), then significantly decreased (2 - 4 µM) during the rest of the period (Figure 3c).

3.4. Phytoplankton biomass and abundance

Chl *a* showed pronounced vertical variability ($p<0.01$, ANOVA). Indeed, for all dates, concentrations significantly increased from the surface (≈14 µg L^{-1}) to depth, reaching sub-surface maxima at around 2-3 m (≈ 18-20 µg L^{-1}), then gradually decreased to 9 m (≈13-15 µg L^{-1}) (Figure 4a). Chl *a* concentrations varied also among dates ($p<0.01$, ANOVA), with higher values from March 14 to March 27 than during the other dates.

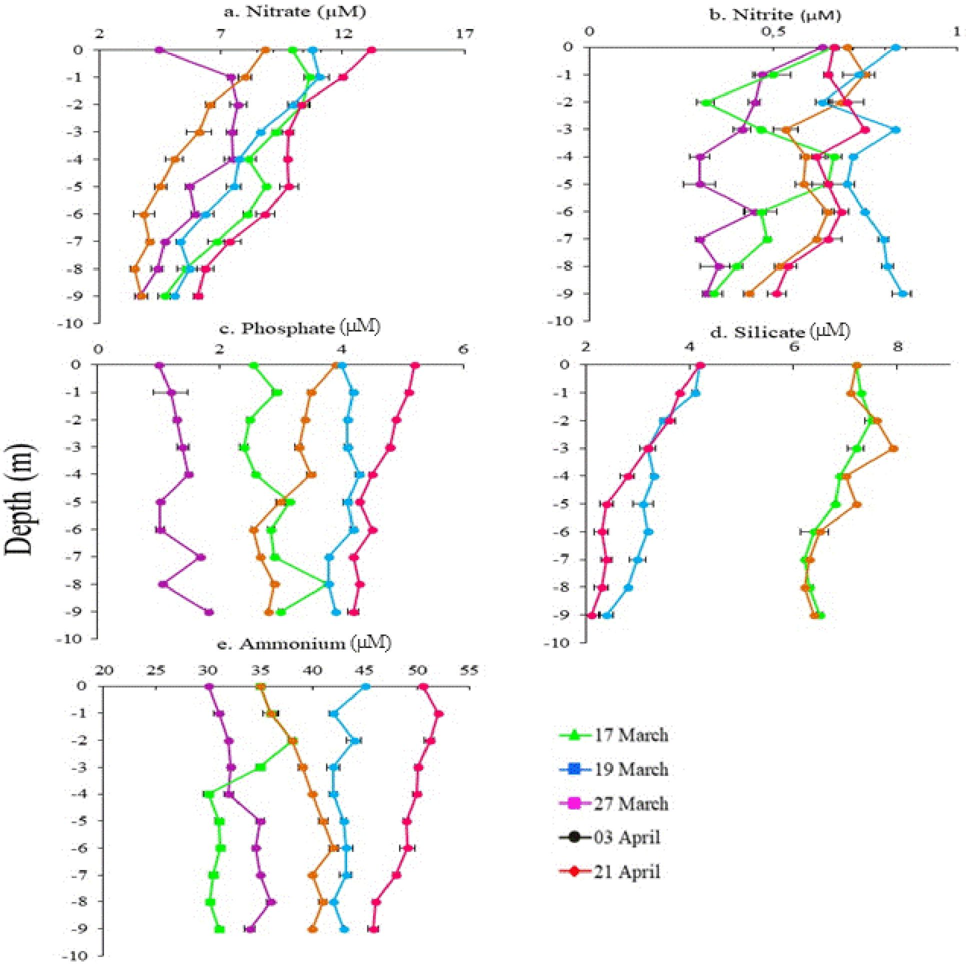

Figure 3. Vertical distributions of nutrient concentration during the phytoplankton bloom period (March - April, 2012) (Average ± SD).

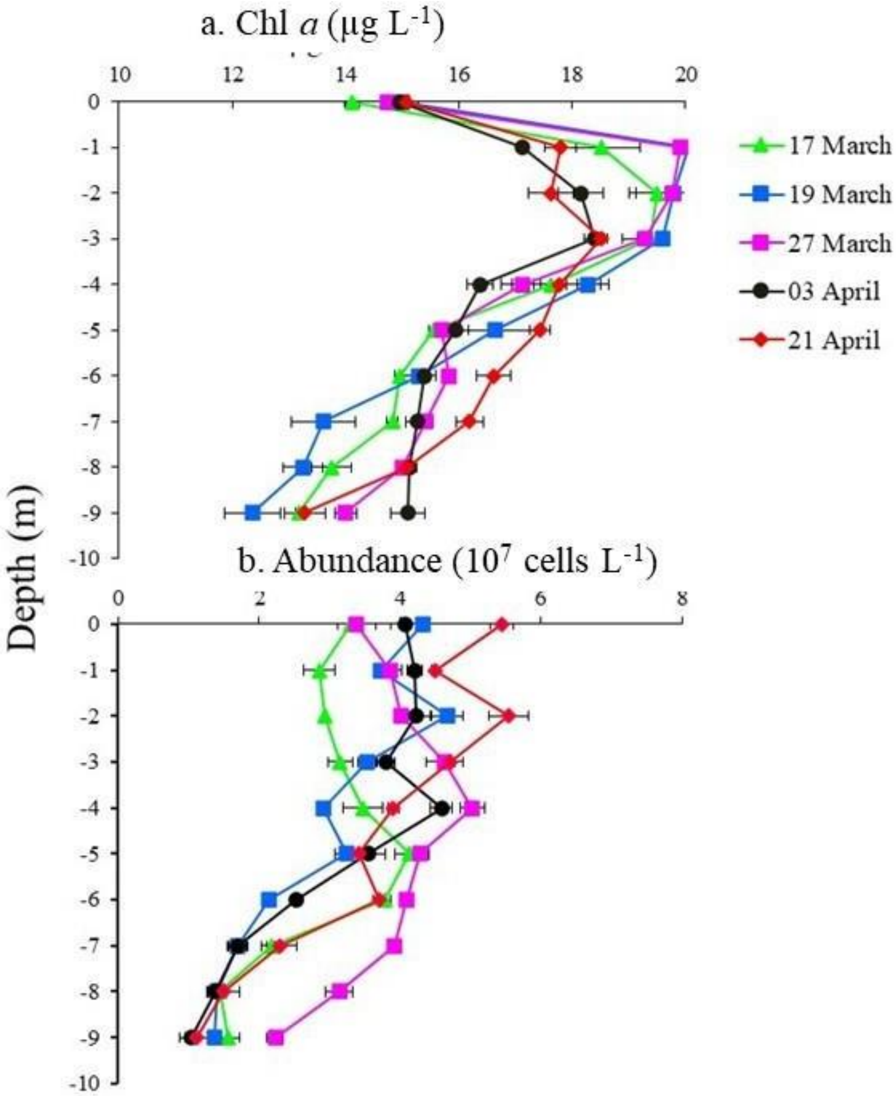

Figure 4. Vertical distribution of biomass (a) and phytoplankton abundance (b) during the phytoplankton bloom period (March - April 2012). (Average ± SD)

Phytoplankton displayed high abundances (10^7 cells L^{-1}) characteristic of blooms. Similar to Chl *a*, the vertical distribution of phytoplankton abundance was heterogeneous for each date ($p<0.01$, ANOVA). Cell abundances increased from the surface (2.8 – 4.1 10^7 cells L^{-1}) and reached higher values between 2 and 4 m (3.5 – 5 10^7 cells L^{-1}) than decreased with depth (1 - 2 10^7 cells L^{-1}) (Figure 4b).

3.5. Phytoplankton composition

The phytoplankton bloom, observed from March to April 2012, was caused by diatoms which dominated the phytoplankton community at almost all depths during this period (Figure 5). These microalgae were remarkably abundant, especially from March 14 to April 03, when they contributed up to 62-89% of the phytoplankton. Dinoflagellates showed low contributions from March 14 to April 3 (3-19%), but they were more abundant on April 21, contributing 21-30% of phytoplankton community. Phytoflagellates, including (Prasinophyceae, Cryptophyceae, Chlorophyceae and Euglenophyceae), showed variable contributions throughout the sampling period (3-25%) (Figure 5).

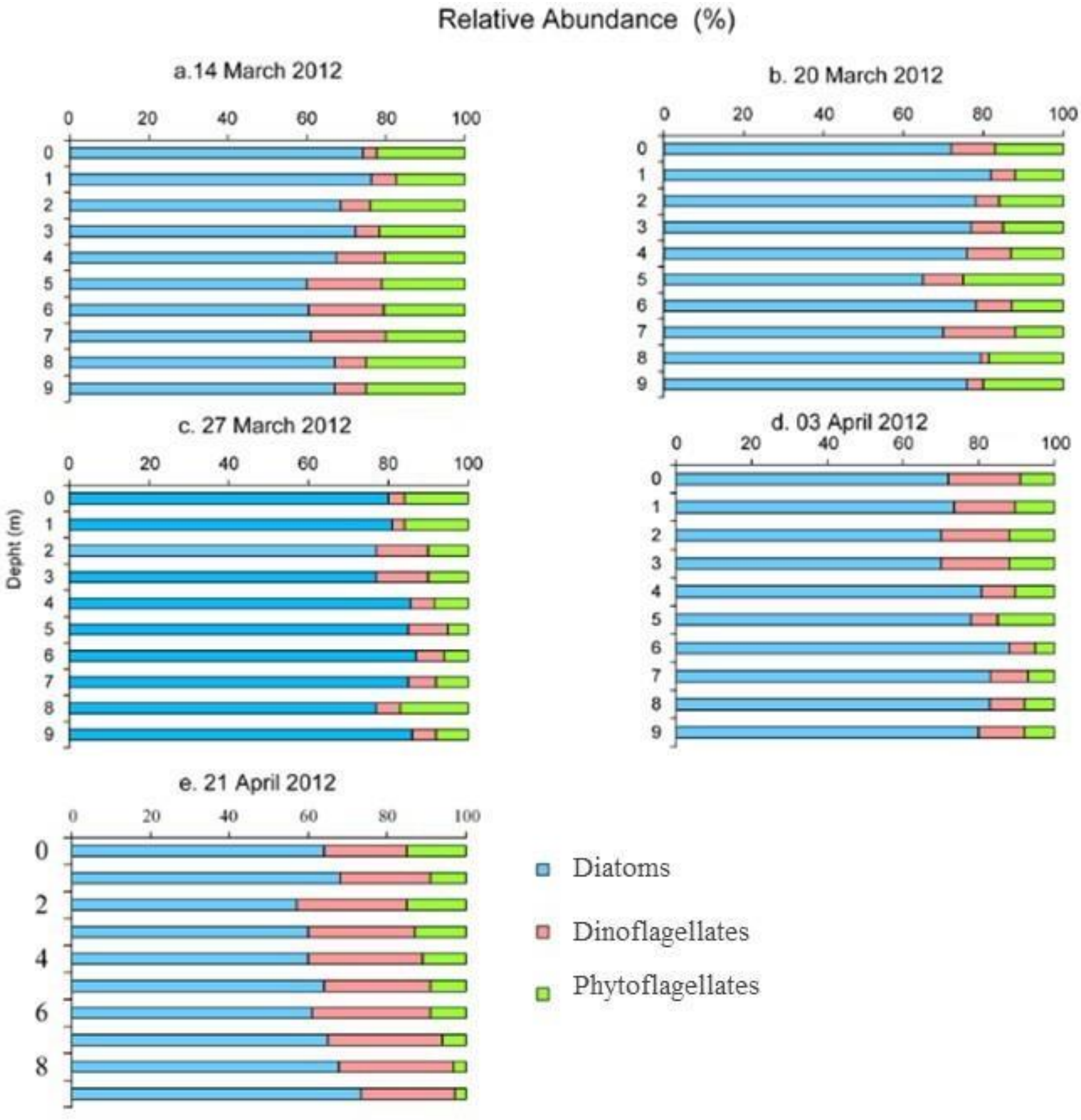

Figure 5. Vertical distribution of relative abundances of diatoms, dinoflagellates and phytoflagellates

In fact, throughout the sampling period, a succession of three species was revealed, the two *Chaetoceros* species (*C. holsaticus* and *C. constrictus*) and one dinoflagellate species, *Neoceratium lineatum* (Figure 6). *C. holsaticus* (Figure 7a) was present during all sampling period with high concentrations (5.9 - 10.37 × 106 cells L-1) and was the main responsible species of the phytoplankton bloom. *C. constrictus* occurred from March 14 to April 03 and participated to the bloom with concentrations ranging from 5.62 to 7.13 × 10^6 cells L^{-1}. On March 27, *Neoceratium lineatum* (Figure 7b) appeared with a relatively low abundance (1.51 × 106 cells l-1) which gradually increased to reach a high concentration (8 × 106 cells L-1) exceeding that of C. holsaticus on April 21 (Figure 6).

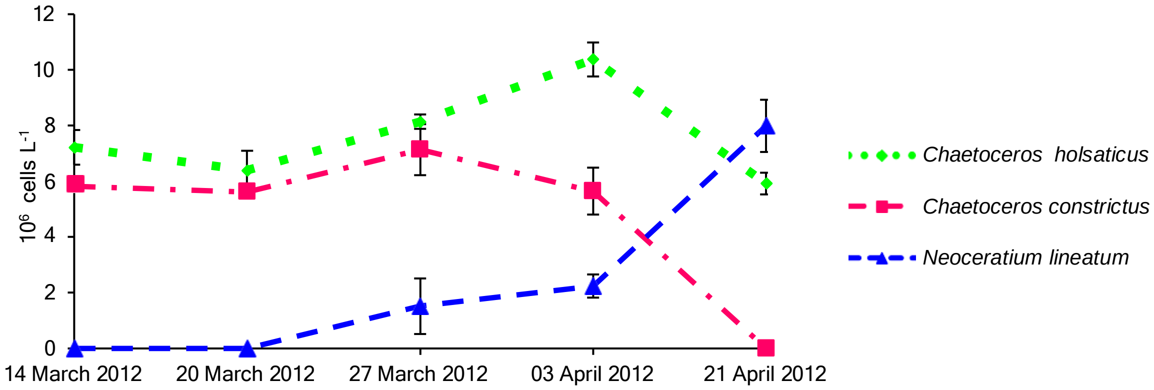

Figure 6. Succession of the three microalgal species during the phytoplankton bloom period (March - April 2012) (Average ± SD).

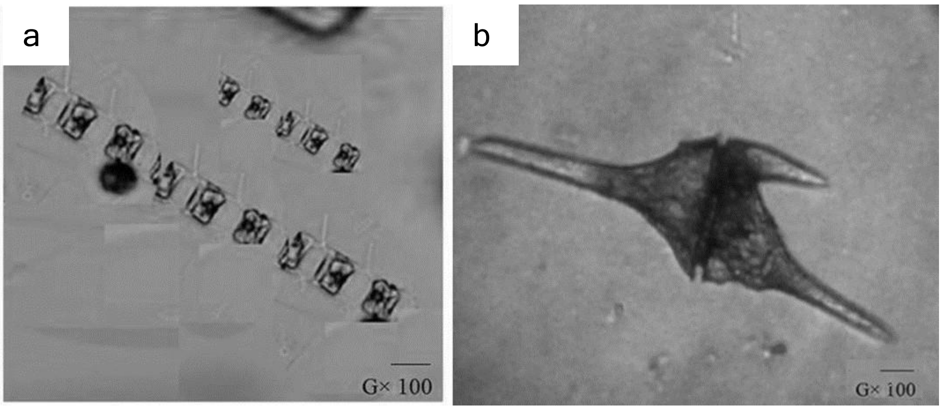

Figure 7. Photos of *Chaetoceros holsaticus* (a) and *Neoceratium lineatum* (b) sampled in Bizerte Lagoon (View under an inverted light microscope)

The abundances of both species of *Chaetoceros* were positively correlated with temperature and concentrations of nitrogen and phosphorous. Cell concentration of *N. lineatum* showed negative correlation with temperature but was positively correlated with N and P nutrients (Table 1).

4. Discussion

The Bizerte Lagoon, a site of intense fishing and shellfish farming activity, is threatened by eutrophication due to population, agricultural and industrial growth. Eutrophication most often leads to phytoplankton blooms, which could be harmful and toxic. Phytoplankton blooms have increased in frequency and intensity over the last decade in the Lagoon (Sahraoui et al., 2012; Bouchouicha-Smida et al., 2014; Turki et al., 2014; Bellakhel et al., 2014; Melliti Ben Garali et al., 2016, 2019), which can cause an imbalance in the functioning of the ecosystem with environmental and sometimes economic risks. Although much interest has been given to blooms of toxic species (Bouchouicha-Smida et al., 2014; Melliti Ben Garali et al., 2016, 2019; Sahraoui et al., 2012; Turki et al., 2014; Bellakhel et al., 2014) information remains scare regarding the non-toxic species, which blooms could also have environmental impacts and economic threat. Our study investigated the dynamics of an intense phytoplankton bloom in the Bizerte Lagoon. We attempted to depict the relationships between the responsible HAB species, i.e. the non-toxic species *Chaetoceros* and *Neoceratium lineatum*, with environmental factors.

During the entire sampling period, very high concentrations of nutrients, particularly of nitrogen (35 – 45 µM), were measured il all depths (Figure 3). The leaching of agricultural land following the heavy rains of winter 2012 could explain the high nutrient enrichment of the Lagoon during the sampling period. Our results revealed that the nutrient-rich waters of Bizerte Lagoon are suitable for phytoplankton blooms, as was previously reported in the study site (Sakka Hlaili et al., 2007; Grami, 2009; Sahraoui, 2010; Bouchouicha-Smida, 2013; Bellakhal, 2003) and for other eutrophic areas, such as coasts of Greece (Tsikoti and Genitsaris, 2021) and Levrier bay in Mauritanian coast (Ndiaye et al., 2025). Previous studies, conducted in the Bizerte Lagoon, have reported HABs at different seasons and with concentrations generally ranging from 105 to 106 cells L^{-1} (Bouchouicha-Smida et al., 2014; Melliti Ben Garali et al., 2016, 2019; Sahraoui et al., 2012; Bellakhal et al., 2014). During spring 2012, a very pronounced bloom occurred, with cell abundance > 107 cells L-1 and high Chl *a* concentrations (13-20 µg L^{-1}), indicating high eutrophic conditions in the lagoon. These findings are in agreement with those reported by Bejaoui et al. (2016) in Bizerte Lagoon, where elevated Chl *a* levels were recorded during periods of increased nutrient inputs and marked hydrological stratification. This consistency highlights the recurrent trophic enrichment of the lagoon and underscores the high sensitivity of this ecosystem to nutrient-driven phytoplankton proliferation (Figure 4).

This spring algal outbreak coincided with favorable hydrological factors. Indeed, the intense rainfall and the pronounced freshwater supply to the lagoon was followed by an exceptional decrease in salinity of surface water with a development of a clear halocline and a stratification of water column (Figure 2b). Thus, the phytoplankton was found in a stable, well-lit water layer (limited by the halocline at around 6 m) that was very rich in nutrients (Figure 3). Under these conditions of high availability of nutrients and light, phytoplankton increased its growth to reach a maximum, resulting in an intense spring bloom.

Table 1. Spearman's correlation coefficients of cell abundance of *C holsaticus*, *C constrictus*. and *Neoceratium lineatum* with environmental factors.

Species	Temp.	Salinity	pH	O_2	NO_2^-+NO_3^- +NH_4^+	PO_4^{3-}	$Si(OH)_4$
C. constrictus	0.32*	ns	ns	ns	0.42**	0.54**	ns
C. holsaticus	0.30*	ns	ns	ns	0.48**	0.52**	ns
Neoceratium lineatum	-0.55**	ns	ns	ns	0.39**	0.49**	ns

ns: not significant; * Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level

Diatoms were dominant during the spring bloom (Figure 5), as was previously reported in the Bizerte Lagoon (Meddeb et al., 2018). The high proliferation of diatoms during spring is likely a common feature of the coastal Mediterranean waters, as the Gulf of Gabès, Gulf of Naples and Gulf of Lions (d'Alcala et al., 2004; Leblanc et al., 2018; Mejri Kousri et al., 2023). In fact, diatoms are known to dominate phytoplankton community in eutrophic waters, particularly those enriched by N and Si (Sakka Hlaili et al., 2006; Chikhaoui et al., 2008).

From March 14 to April 03, the high availability of N and Si nutrients (Figure 3) associated with other favorable conditions, would result in the diatoms outbreak. This was mainly caused by two species of *Chaetoceros* (*C. holsaticus* and *C. constrictus*), the most diverse and abundant genus of cosmopolitan marine diatoms. *Chaetoceros* are marine planktonic centric diatoms that reproduce mainly by asexual cell division, while sexual reproduction occurs when a critical minimum size is reached, producing an auxospore that restores the original size. Many species are also capable of forming resting spores under unfavorable environmental conditions (Montresor et al., 2016).

These species are primarily autotrophic organisms that perform photosynthesis, playing a major role in primary production and frequently dominating phytoplankton blooms in nutrient-rich coastal and eutrophic waters, where they constitute an important food source for zooplankton, bivalves, and fish larvae (Jensen and Moestrup, 1998; Rines and Theriot, 2003; Draredja et al., 2019).

Chaetoceros, thanks to its rapid growth, can form intense blooms in coastal areas, with the appearance of brown waters (Alvain et al., 2008; Sahraoui et al., 2012), as observed during the 2012 spring bloom in the Bizerte Lagoon.

The spring outbreak of *C. holsaticus* and *C. constrictus* (from March 14 to April 03, Figure 6) coincided with the decreased salinity in surface water (14 - 20 PSU, Figure 2). In fact, Chaetoceros blooms were often linked to low salinity in several coastal ecosystems. Furthermore, Trigueros et al., (2002) reported that the most important blooms of *Chaetoceros* in Biscay Bay are observed in areas with low salinity (10.5–15.8 PSU). Our results showed that the proliferation of *Chaetoceros* species was also linked to temperature and nutrient concentrations (Table 1). This is consistent with other findings showing that temperature and nutrients are the main drivers of *Chaetoceros* dynamics (Wang et al., 2006).

High abundances of *Chaetoceros* in Bizerte Lagoon were observed at temperatures varying from 15 to 19ºC, and at high concentrations of all nutrients. This was in line with the result of Trigueros et al. (2002) reporting that important blooms of *Chaetoceros* spp. in Bisca Bay are observed at temperatures ranging from 18 to 19°C and when phosphate, ammonium and nitrate concentrations were extremely high (PO_4^{3-}: 4.3 - 17.7 µM, NH_4^+: 67 - 232.4 µM, NO_3^-: 4.3 - 17.7 µM).

The genus *Chaetoceros* is characterized by colonial and chain-forming species that have spine setae, like the blooming species *C. holsaticus* (Figure 7a). Although species of *Chaetoceros* are not known to produce any toxic compound, their high abundance (0.5 × 106 cells L-1) are often associated with fish and shellfish mortalities (Albright et al., 1993; Yang and Albright, 1994). The killing of fish results from irritation of the gills followed by capillary bleeding and excess mucus production (Tester and Mahoney, 1995). During our study, *C. holsaticus* and *C. constrictus*, which reached very high concentrations (6-10 × 106 cells L-1, Figure 6), were also involved in mortalities of fish and cuttlefish (perso. com. with fishermen).

From April 03, the abundance of both *Chaetoceros* species began to decrease, with a total decline in *C. constrictus* and a marked drop in *C. holsaticus* on April 21 (Figure 6), although N and P nutrients have increased during both dates (Figure 2a, b). So, the decline of diatoms on April 03 and 21 could be caused by the low availability of Si (Figure 2c), which is essential for the formation of their siliceous frustules. The decline of diatoms was followed by a development of dinoflagellates (Figure 5), particularly *Neoceratium lineatum* (formerly *Ceratium lineatum*) (Figure 7b), which began to grow on March 27, then gradually increased and peaked (7 106 cells L-1) on April 21 (Figure 6). In fact, the succession of phytoplankton species occurred because the species do not all have different preferences with respect to environmental conditions. The replacement, over time, of one dominant species by another is obviously related to changes in physicochemical factors.

During our study, proliferation of *Neoceratium lineatum*, coincided with a net salinity increase of the surface waters (30 PSU) (Figure 2b). Baek et al. (2007) found also that the rapid growth of Ceratium fusus in the coastal area of Sagami Bay (Japan) occurred with high salinity (24 - 30 PSU). Besides, the abundance of *N. lineatum* was positively correlated with nutrients and negatively the temperature (Table 1). On the Greek coast, the presence of this species has also been detected at high nutrients and salinities (Spatharis et al., 2009). *N. lineatum* is a marine planktonic dinoflagellate commonly found in temperate coastal waters, including the Mediterranean Sea. In Mediterranean lagoons, its life cycle is predominantly characterized by vegetative reproduction, while cyst formation has been suggested but remains far less documented than in many other dinoflagellate taxa (Baek et al., 2008). *Neoceratium lineatum* is a harmful dinoflagellate that can cause discoloration of water (Gilbert et al., 2002). In our study, this species reached very high concentrations (7.9 106 cells L^{-1}), exceeding those previously recorded in the Bizerte Lagoon (2 105 cells L^{-1}, Bouchouicha-Smida, 2013) and in other Mediterranean coasts (3.6 - 18 103 cells L^{-1}, Weaver, 1978; Spatharis et al., 2009). This high concentration also far exceeded the safety thresholds for bivalve molluscs (>106 cells L-1, IFREMER, 2006). Although the species is non-toxic, it can lead to the death of marine invertebrates, fish and even shellfish by asphyxiation because it induces a sharp decrease in available oxygen. Moreover, the mixotrophic nature of this dinoflagellate may enhance its competitive advantage under variable environmental conditions, contributing to the decline of co-occurring phytoplankton species through both resource competition and phagotrophic interactions (Stoecker et al., 2017; Flynn et al., 2013). Mixotrophy is increasingly recognized as a key functional trait influencing bloom dynamics and trophic transfer in marine ecosystems (Mitra et al., 2014; Ward and Follows, 2016).

5. Conclusion

In early spring 2012, salinity levels in the Bizerte Lagoon exceptionally dropped to very low values (14 - 20 PSU) that occurred in response to a massive influx of fresh water, caused by heavy rainfall during the winter-spring of 2012. This event proved the development of clear halocline and nutrient enrichment of seawater, resulting in a pronounced spring phytoplankton outbreak with a discoloration of seawaters. The dynamics of the species responsible for the bloom have been mainly linked to salinity and nutrient availability. At the beginning of the bloom, diatoms were the main proliferating species, when salinity was low and nutrients (particularly N and

Si) were largely available. The diatom bloom, which declined when Si levels fell, was followed by a proliferation of dinoflagellates that was linked to the increase of salinity and N and P concentrations. This pattern represented a natural succession of phytoplankton in response to shift in physicochemical factors. It also revealed the vulnerability of the Bizerte Lagoon to any change in hydrological features and in nutrient inputs. The blooming species (*C. holsaticus*, *C. constrictus* and *N. lineatum*), although non-toxic, have harmful effects and were involved in mortalities of fish and cuttlefish.

While our data on harmful phytoplankton are over 13 years old, long-term records remain invaluable for identifying historical trends, baseline conditions, and the ecological sensitivity of the Bizerte Lagoon. Although, environmental conditions have undoubtedly evolved, these data provide a crucial reference point for assessing changes in bloom frequency, intensity, and community composition over time. Comparing historical and contemporary observations can therefore reveal patterns of ecosystem response to nutrient inputs, hydrological shifts, and human activities, supporting informed management and monitoring strategies.

Acknowledgements

We thank anonymous reviewers for useful suggestions.

References

1. Abdenadher M., Hamza A., Fekih W., Hannachi I., Zouari Bellaaj A., Bradai M.N., & Aleya L. (2012). Factors determining the dynamics of toxic blooms of Alexandrium minutum during a 10-year study along the shallow southwestern Mediterranean coasts; *Estuarine, Coastal and Shelf Science,* 106, 102-111. https://doi.org/10.1016/j.ecss.2012.04.029
2. Albright L., Kenny D.A., & Malloy T.E. (1988). Consensus in personality judgments at zero acquaintance. *Journal of Personality and Social Psychology*, 55(3), 387-395.https://doi.org/10.1037//0022-3514.55.3.387
3. Alina T. L., & Rodolphe L. (2013). The Genus *Neoceratium* (Planktonic Dinoflagellates) as a Potential Indicator of Ocean Warming. *Microorganisms*, 1(1), 58-70. https://doi.org/10.3390/microorganisms1010058
4. Amaro-Seoane P., Aoudia S., Babak S., Binétruy P., Berti E., Bohe A., & Petiteau A. (2012). Low-frequency gravitational-wave science with eLISA/NGO. *Classical and Quantum Gravity*, 29 (12), 124016. https://doi.org/10.1088/0264-9381/29/12/124016
5. Aminot A., & Chausspied M. (1983). Manuel des analyses chimiques en milieu marin. Centre national pour l'exploitation des océans. Brest, (France) CNEXO.
6. Amorri J., Boufahja F., Mahmoudi E., Ounissi M., & Aissa P. (2005). Réponse des copépodes benthiques à la pollution ponctuelle par les métaux lourds et les hydrocarbures dans la lagune de Bizerte (Automne 2002). *Actes du 6ème Congrès Maghrébin des Sciences de la Mer, Monastir Tunisie* (18-22 décembre 2005).
7. Anne-Claire P., & Imran C. (2012). Social Entrepreneurs as Institutionally Embedded Entrepreneurs: Toward a New Model of Social Entrepreneurship Education. *Academy of Management Learning and Education*, 11(3), 494-510. https://doi.org/10.5465/amle.2011.0019
8. Baek S.H. , Shimode S., Han S.O., & Kikuchi T. (2008). Growth of dinoflagellates, Ceratium furca and Ceratium fusus in Sagami Bay, Japan:

The role of nutrients. *Harmful Algae*, 7(6), 729-739. https://doi.org/10.1016/j.hal.2008.02.007

9. Bhatt R.S., Sharma S.R., Singh U., Kumar D., & Risam K.S. (2005). Effect of substituting groundnut cake for different levels of rice bran on growth and wool production of German Angora rabbits. *World Rabbit Science*, 13, 179-187. https://doi.org/10.4995/wrs.2005.519
10. Bouchouicha D. (2008). *Suivi des diatomées Pseudo-nitzschia et des autres microalgues potentiellement nuisibles dans la lagune de Bizerte durant l‟été et l‟automne (2006).* [Mémoire de Mastère, Université de Carthage, Fac. Sciences de Bizerte].
11. DGPA - Direction générale de la pêche et de l'aquaculture (2013). *Plan Directeur de l'Aquaculture en Tunisie*. Tunis: DGPA, PNUD.
12. Draredja M.A., Barour C., Hocine F., Boualleg C., & Laabir M. (2019). Diatoms diversity and dynamics in a southern Mediterranean lagoon (Mellah, Algeria) *Biodiversity Journal,* 10(2), 127-140. https://doi.org/10.31396/biodiv.jour.2019.10.2.127.140
13. Flynn K.J., Stoecker D.K., Mitra A., Raven J.A., Glibert P.M., Hansen P.J., Granéli E., & Burkholder J.M. (2013). Misuse of the phytoplankton-zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. *Journal of Plankton Research* 35(1), 3-11. https://doi.org/10.1093/plankt/fbs062
14. Hallegraeff G. (2003). Harmful algal blooms: A global overview. In: Hallegraeff, M., Anderson, D.M. and Cembella, A.D. (Eds), *Manual on harmful marine microalgae* (2nd Edition, IOC-UNESCO), 25-49.
15. Hallegraeff G., Enevoldsen H., & Zingone A. (2021). Global harmful algal bloom status reporting. *Harmful Algae,* 102, 101992.https://doi.org/10.1016/j.hal.2021.101992
16. Harzallah, A. (2003). Transport de polluants dans la lagune de Bizerte simulé par un modèle de circulation de l'eau. *Bulletin de l'Institut National des Sciences et Technologie de la Mer*, 30, 121-134. https://doi.org/10.71754/instm.bulletin.v30.804
17. High W.M.J., Briones-Galang M., Clark J.A., Gilkison C., Mossberg K.A., Zgaljardic D.J., Masel B.E., & Urban, R.J. (2010). Effect of growth hormone replacement therapy on cognition after traumatic brain injury. *Journal of Neurotrauma,* 27(9), 1565-1575. https://doi.org/10.1089/neu.2009.1253
18. Huang Y.T., Churchman J., Lowe D.J., Rawlence N.J., Schipper L., & Cooper A. (2012). Evaluating the character and preservation of DNA within allophane clusters in buried soils on Holocene tephras, northern New Zealand. In: *Australian Regolith and Clays Conference, Mildura 7-10 February 2012*, 121-124.
19. Jensen K., Moestrup Ø. (1998). The genus Chaetoceros (Bacillariophyceae) in inner Danish coastal waters. *Nordic Journal of Botany,* 18(1), 88. https://doi.org/10.1111/j.1756-1051.1998.tb01103.x
20. Lv X., Liu X., Hu X., Geng R., Tang C., & Xing Q. (2023). The Red Tide Organism Chaetoceros sp. Responding to Exposure to Oil and Dispersant. *Sustainability,* 15(2), 1103. https://doi.org/10.3390/su15021103
21. Martins da Silva P., Berg M.P., Serrano A.R.M., Dubs F., & Sousa J.P. (2012). Environmental factors at different spatial scales governing soil fauna community patterns in fragmented forests. *Landscape*

Ecology, 27, 1337-1349. https://doi.org/10.1007/s10980-012-9788-2

22. McGlone M., Yñiguez A.T., Garry B., Lum L.W., Lim P. (2024). Mortalité massive de poissons liée à des proliférations d'algues nuisibles en Asie du Sud-Est. *Sustainability*, 15(2), 1103. https://doi.org/10.20944/preprints202406 1223.v1
23. Meddeb M., Grami B., Chaalali A., Haraldsson M., Niquil N., Pringault O., & Sakka Hlaili A. (2018). Plankton food-web functioning in anthropogenically impacted coastal waters (SW Mediterranean Sea): An ecological network analysis. *Progress in Oceanography*, 162, 66-82. http://dx.doi.org/10.1016/j.pocean.2018.02.013
24. Melliti Ben Garali S., Sahraoui I., Ben Othman H., Kouki A., de la Iglesia P., Diogène J., Lafabrie C., …& Sakka Hlaili A. (2021). Capacity of the potentially toxic diatoms Pseudo-nitzschia mannii and Pseudo-nitzschia hasleana to tolerate polycyclic aromatic hydrocarbons. *Ecotoxicology and Environmental Safety*, 214, 112082. https://doi.org/10.1016/j.ecoenv.2021.112082
25. Melliti Ben Garali S., Sahraoui I., de la Iglesia P., Chalghaf M., Diogène J., Ksouri J., Sakka Hlaili A. (2020). Factors driving the seasonal dynamics of Pseudo-nitzschia species and domoic acid at mussel farming in the SW Mediterranean Sea. *Chemistry and Ecology*, 36(1), 66-82. https://doi.org/10.1080/02757540.2019.1676417
26. Melliti Ben Garali S., Sahraoui I., de la Iglesia P., Chalghaf M., Diogène J., Ksouri J., & Sakka Hlaili A. (2016). Effects of nitrogen supply on Pseudo-nitzschia calliantha and Pseudo-nitzschia cf. seriata: field and laboratory experiments. *Ecotoxicology*, 25, 1211-1225. https://doi.org/10.1007/s10646-016-1675-1
27. Montresor M., Vitale L., D'Alelio D., Ferrante M.F. (2016). Sex in marine planktonic diatoms: insights and challenges. *Prespectives in Phycology*, 3(2), 61-75, https://doi.org/10.1127/pip/2016/0045
28. Murphy J., & Riley J.P. (1962). A modified single solution method for the determination of phosphate in naturel waters. *Analytica Chimica Acta*, 27, 31-36. https://doi.org/10.1016/S0003-2670(00)88444-5
29. Parsons T.P., Maita Y., & Lalli C.M. (1984). *A manual of Chemical and Biological Methods for Seawater analysis*. Pergamon Press. https://doi.org/10.1016/C2009-0-07774-5
30. Parsons L.A., Jung J., Yuta J.M., Kroeger T., Battisti D.S., Spector J.T. (2021). Tropical deforestation accelerates local warming and loss of safe outdoor working hours, *One Earth*, 4(12), 1730-1740. https://doi.org/10.1016/j.oneear.2021.11.016
31. Patricia M.G., Adnan A.J., Carus A., Alexander F., Arthur H.W., Beusen, M.A., Burford P.J., Mingjiang Z. (2018). Harmful Algal Blooms and the Importance of Understanding their Ecology and Oceanography. In: Glibert, P., Berdalet, E., Burford, M., Pitcher, G., Zhou, M. (eds), *Global Ecology and Oceanography of Harmful Algal Blooms*. (Ecological Studies, 232). Springer. https://doi.org/10.1007/978-3-319-70069-4_2
32. Rines J.E.B. & Theriot E.C. (2003). Systematics of Chaetocerotaceae (Bacillariophyceae). I. A phylogenetic analysis of the family. *Phycological Research,* 51(2), 83-98.

https://doi.org/10.1046/j.1440-1835.2003.00297.x

33. Rodger S., Fitzgerald C., Davila W., Millar F., & Allison H. (2011). What makes a quality occupational therapy practice placement? Students' and practice educators' perspectives. *Australian Occupational Therapy Journal,* 58(3), 195-202. https://doi.org/10.1111/j.1440-1630.2010.00903.x
34. Sahraoui I., Bates S.S., Bouchouicha D., Hadj Mabrouk H., & Sakka Hlaili A. (2011). Toxic and potentially toxic *Pseudo-nitzschia* populations from Bizerte Lagoon, Tunisia, southwest Mediterranean, and first report of domoic acid production by *P. brasiliana*. *Diatom Research,* 26(3), 293-303. http://dx.doi.org/10.1080/0269249X.2011.597990
35. Sahraoui I., Grami B., Bates S.S., Bouchouicha D., Chikhaoui M.A., Hadj Mabrouk H., & Sakka Hlaili A. (2012). Response of potentially toxic *Pseudo-nitzschia* (Bacillariophyceae) populations and domoic acid to environmental conditions in a eutrophied, SW Mediterranean coastal lagoon (Tunisia). *Estuarine, Coastal and Shelf Science,* 102-103, 95-104. https://doi.org/10.1016/j.ecss.2012.03.018
36. Sakka Hlaili A., Grami B., Haj Mabrouk H., Gosselin M., & Hamel D. (2007). Phytoplankton growth and microzooplankton grazing in a restricted Mediterranean lagoon (Bizerte Lagoon, Tunisia). *Marine Biology,* 151, 767-783. https://doi.org/10.1007/s00227-006-0522-y
37. Sakka Hlaili A., Grami B., Niquil N., Gosselin M., Hamel D., Troussellier M. & Hadj Mabrouk H. (2008). The planktonic food web of the Bizerte lagoon (South-Western Mediterranean) during summer: I. Spatial distribution under different anthropogenic pressures. *Estuarine, Coastal and Shelf Science*, 78(1), 61-77. https://doi.org/10.1016/j.ecss.2007.11.010.
38. Sakka Hlaili A., Sahraoui I., Bouchouicha-Smida D., Melliti Ben Garali S., Ksouri J., Chalghaf M., Bates S.S., Lundholm N., Kooistra W.H.C., De la Iglesia P., Diogène J. (2016). Toxic and Potentially Toxic Diatom Blooms in Tunisian (SW Mediterranean) Waters: Review of Ten Years of Investigations. *Advances in Environmental Research*, 48, 51-69.
39. San Diego-McGlone M.L., Yñiguez A.T., Benico G., Lum W.M., Hii K.S., Leong S.C.Y., Leaw C.P., Iwataki M., & Lim, P.T. (2024). Fish Kills Related to Harmful Algal Bloom Events in Southeast Asia. *Sustainability*, 16(23), 10521. https://www.mdpi.com/2071-1050/16/23/10521
40. Smayda T.J. (2007). Reflections on the ballast water dispersal-harmful algal bloom paradigm. *Harmful Algae,* 6(4), 601-622. https://doi.org/10.1016/j.hal.2007.02.003
41. Smayda T.J. (1997). Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnology and Oceanography, 42,* 1009-1305. https://doi.org/10.4319/lo.1997.42.5_part_2.1137
42. Spatharis S., Dolapsakis N.P., Economou-Amilli A., Tsirtsis G., & Danielidis D.B. (2009). Dynamics of potentially harmful microalgae in a confined Mediterranean Gulf-Assessing the risk of bloom formation. *Harmful Algae*, 8 (5), 736-743. https://doi.org/10.1016/j.hal.2009.03.002
43. Steemann N.E. (1975). *Marine photosynthesis with special emphasis*

on the ecological aspects. Elsevier, 142 p.

44. Suzuki N., Hanmoto T., Yano S., Furusawa Y., Ikegame M., Tabuchi Y., Kondo T., ... & Hattori A. (2016). low-intensity pulsed ultrasound induces apoptosis in osteoclasts: fish scales are a suitable model for the analysis of bone metabolism by ultrasound. *Comparative biochemistry and physiology Part A, Molecular & integrative physiology,* 195, 26-31. https://doi.org/10.1016/j.cbpa.2016.01.022
45. Tazumi T., & Okaichi H. (2003). The effects of lesions in the central nucleus of the amygdala on appetitive classical conditioning in rats. *Japanese Psychological Research*, 45(3), 202-208. https://doi.org/10.1111/1468-5884.t01-2-00047
46. Tian X.L.V., Chengfang Z., Qianqian P., Haorong X., Hongda F., Mengcen W., Haruna M. (2023). Plant pathogenesis: Toward multidimension a understanding of the microbiome. *iMeta,* 2(3), e129. https://doi.org/10.1002/imt2.129
47. Tréguer P., Bowler C., Moriceau B., Dutkiewicz S., Gehlen M., Aumont O., Bittner L., & Pondaven P. (2018). Influence of diatom diversity on the ocean biological carbon pump. *Nature Geoscience,* 11, 27-37. https://doi.org/10.1038/s41561-017-0028-x
48. Trigueros R., Aguilar-Parra J.M., Cangas A.J., Bermejo R., Ferrandiz C., & López-Liria R. (2019). Influence of Emotional Intelligence, Motivation and Resilience on Academic Performance and the Adoption of Healthy Lifestyle Habits among Adolescents. *International Journal of Environmental Research and Public Health,* 16(16), 2810. https://doi.org/10.3390/ijerph16162810
49. Turki S., (2004). Suivi des microalgues planctoniques toxiques dans les zones de production, d'élevage des mollusques bivalves et d'exploitation des oursins du Nord de la Tunisie. *Bulletin INSTM*, 31, 83-96. https://doi.org/10.71754/instm.bulletin.v31.760
50. Turki S., Harzallah A., Sammari C. (2006). Occurrence of harmful dinoflagellates in two different Tunisian ecosystems: the lake of Bizerte and the gulf of Gabès. *Cahiers de biologie marine, 47(3), 253-259*. https://dx.doi.org/10.21411/CBM.A.75D44573
51. Vidal L., Schneider R.R., Marchal O., Bickert T.S., Thomas, F., & Wefer, G. (1999). *Ages of isotopic events corresponding to global signal in the benthic $\delta^{18}O$ records from sediment core GeoB1711-4* [dataset]. PANGAEA. https://doi.org/10.1594/PANGAEA.734356
52. Wang S., Abbas J., Sial M.S., Álvarez-Otero S., & Cioca L.I. (2022). Achieving green innovation and sustainable development goals through green knowledge management: Moderating role of organizational green culture. *Journal of Innovation & Knowledge*, 7(4), 100272. https://doi.org/10.1016/j.jik.2022.100272
53. Wood E.D., Armstong F.A.J., Richards F.A. (1967). Determination of nitrate in sea water by cadmium-copper reduction to nitrite. *Journal of the Marine Biological Association of the United Kingdom*, 47(1), 23-31. https://doi.org/10.1017/S002531540003352X
54. Yang C.Z., & Albright L.J. (1992). Effects of the harmful diatom *Chaetoceros concavicornis* on respiration of rainbow trout *Oncorhynchus mykis*. *Diseases of*

Aquatic Organisms, 14(2), 105-114 https://doi.org/10.3354/dao014105

55. Zehr J.P., & Kudela R.M. (2011). Nitrogen Cycle of the Open Ocean: From Genes to Ecosystems. *Annual Review of Marine Science*, 3, 197-225. https://doi.org/10.1146/annurev-marine-120709-142819
56. Zmerli T.H. (2016). Etude des Kystes du dinoflagellés dans la lagune de Bizerte-Ecophysiologie et cycle de vie du dinoflagellé toxique *Alexandrium pseudogonyaulax*. [Thèse de Doctorat, Université de Carthage, INAT].