

Egyptian Journal of Botany http://ejbo.journals.ekb.eg/



Co-varying Environmental Conditions Control the Spatiotemporal Dynamics of *Microcystis aeruginosa* and *Botryococcus braunii* in a Freshwater Ecosystem



Shimaa Hosny[#], Wagdy Labib

National Institute of Oceanography and Fisheries (NIOF), Egypt.

NOZHA lake (South of Alexandria City, Egypt), an artificial freshwater basin, receives its water supply from the Nile River and from the surrounded urban area, which affect the ecological balance. Results during a year cycle (December 2017 to November 2018) revealed scums and dispersive blooms of *Microcystis aeruginosa* Kützing (Cyanophyta) and *Botryococcus braunii* Kützing (Chlorophyta), in no particular season, under a wide range of environmental conditions. *Microcystis aeruginosa* was a persistent form during the whole period, culminating in visible blooms in December, June, and September. The distinctive role of temperature was evidenced during the transient periods in spring and summer. The inorganic dissolved nitrate, ammonia, and phosphate might affect the variability in the community structure and abundance. Stoichiometric analyses indicated major potential nitrogen limitation (63.89% of the total observations), whereas 15.28% of the total observations were P limiting, and 20.83% of the total observations were either N or P limiting. Hence, the reduction of only one type of nutrients can lead to an imbalance in the ratio. The major bloom peaks of *M. aeruginosa* and *B. braunii* detected in June and September raised chlorophyll *a* content to its extreme values (16.5 and 21.8mg L⁻¹, respectively). The blooms of *B. braunii* influenced the accompanied phytoplankton species diversity with different degrees to explain the interaction with eutrophic fast-growing *Chlorella vulgaris* green algae.

Keywords: Algal blooms, Freshwater ecosystem, Nozha lake.

Introduction

Lake eutrophication refers to the enrichment of nutrients that can lead to prominent symptoms as the increase of phytoplankton biomass and the occurrence of algal blooms (Tang et al., 2010) and algal toxins (Shi et al., 2008). In general, harmful algal blooms (HABs) cause undesirable changes in aquatic resources such as reduced water clarity, quality, hypoxia (dissolved oxygen below 2-4mg L^{-1}), fish kills, loss of biodiversity, and an increase in nuisance species (Wolfe & Patz, 2002). The expected nitrogen (N) and phosphorous (P) concentrations in lakes and reservoirs as annual average in surface water (0.5m) indicate water quality. The annual average of total nitrogen and phosphorus can fluctuate between 0.07 and 1.00mg L⁻¹ and between 0.005 and 0.1mg L^{-1} (website of Ministry of the Environment Government of Japan, https://www. env.go.jp).

Cvanobacteria and Chlorophyta blooms have adverse impacts on the environment and human health causing a variety of water quality problems in systems used for human activities, loss of biodiversity, integrity in food webs, and negative health effects on humans and animals (Codd et al., 2005), which commonly lead to a wide range of economic consequences (Berg & Sutula, 2015). Such blooms represent major challenges for the management of aquatic ecosystems. The blooms of *Microcystis* spp. (Chroococcales and Cyanobacteria) are common in freshwater bodies worldwide, and they are of great concern because of the production of microcystin toxins (Khairy & El-Sheekh, 2019). The genus *Botryococcus* (Trebouxiales and Chlorophyta) was cosmopolitan described (Ranga Rao et al., 2007; Demura et al., 2014). It has been widely known for its ability to produce and accumulate long-chain hydrocarbon, lipids, and polysaccharides (Metzger & Largeau, 2005). As a result of the production of lipids, *Botryococcus* may rise to the surface of water where

*Corresponding author email: shimaasea@yahoo.com Received 29/08/2021; Accepted 17/02/2022 DOI: 10.21608/ejbo.2022.93159.1769 Telephone:+201224568371

Edited by: Prof. Dr. Mostafa M. El-Sheekh, Faculty of Science, Tanta University, Tanta, Egypt. ©2022 National Information and Documentation Center (NIDOC)

they float in a thick layer depending on the growth conditions (Qin, 2005). In addition, the colonial green algae *B. braunii* has been considered as a good source of biofuels, and it converts 3% of solar energy into hydrocarbons and reduces CO₂ emissions up to 1.5×10^5 tons/year (Brennan & Owende, 2010). This species can produce allelopathic substances (a mixture of free fatty acids) that inhibit the growth of other phytoplankton species (Chiang et al., 2004). However, no study has reported that this species is toxic to aquatic organisms, and the occasionally occurred incidents of fish mortality (Papa et al., 2008) might be attributed to a variety of ways as the oxygen stress (Labib et al., 2014).

The available information on the phytoplankton dynamics in Nozha lake (Helal et al., 2020), particularly HABs (Labib et al., 2014), was insufficient to fully describe the threats to ecosystem services and function, which was among the decisive targets to conduct the current study. However, previous studies reported signs of eutrophication. The present study, which was operated during a year cycle, aims to reveal the major environmental factors affecting bloom developments of *M. aeruginosa* and *B. braunii* and the impact of these blooms on the cooccurred phytoplankton community.

Materials and Methods

Nozha lake is an artificial freshwater basin located at the south of Alexandria City (31 19°N, 29 97°E), which has an area of 5.5 km² and an average depth of 2.5–3.0m. This lake is used as a fish farm (200–250ton·y⁻¹). The lake receives its water supply from the Nile River via Mahmoudia Canal and through other minor sources from the surrounded urban area. Sampling was operated monthly from December 2017 to November 2018; however, the Nile water input ceased from July to September 2018 because of the ongoing operation of the infrastructure project. This study might represent a part of the proposed survey to assess the ecosystem management of the lake, within the cooperation strategy between the National Institute of Oceanography and Fisheries and Al-Nozha fish farm (Helal et al., 2020).

Samples were collected before mid-day below the water surface at six sampling stations (Fig. 1). Water parameters viz. temperature, salinity, and alkalinity were measured in the sampling sites using the water physical parameter device HANA, Model HI 9828. Water samples were collected in a plastic bottle (1L), cleaned with diluted hydrochloric acid, and washed well with lake waters. Inorganic dissolved nutrient concentrations (NO3, NO2, NH4, and PO4) were determined following the procedures of Parsons (1984). Chlorophyll a content (Chl. a) was measured on the basis of the method of Jeffrey & Humphrey (1975). Nutrient limitation was calculated on the basis of the resultant N:P ratios according to Forsberg & Ryding (1980). A ratio of N:P above 17 indicated P limitation, below 10 indicated N limitation, and between 10 and 17 indicated that either of these nutrients may be limiting. Living phytoplankton samples were first examined to identify species that could be morphologically affected by preservation using a light microscope connected with a digital camera system. Cell and colony sizes were measured using a ruler in one of the eyepieces. The water samples (1L) at each station were fixed by neutral formalin (4%), and a few drops of Lugol's acid solution were used for phytoplankton quantitative estimation by the sedimentation method (Utermöhl, 1958), followed by counting (Hasle, 1978). The average abundance (colony L⁻¹) was based on three replicates (2mL each). Reference consulted for the identification of the main groups included Needham & Needham (1962). Moreover, vertical hauls using a plankton net (20µm) were collected and preserved for further identification of other species that might be escaped from quantitative samples. Furthermore, abundance was expressed as cell L⁻¹.



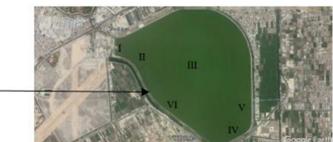


Fig. 1. Nozha lake map showing sampling stations

Statistical analysis

Pearson correlation was applied to measure the existence (given by a P value) and strength (given by the coefficient r between -1 and +1) of a linear relationship between two variables. The results were tested at significance levels of 0.01 and 0.05 using the *P* test (Microsoft Excel, Version 2016).

Results

Physico-chemical characteristics of lake water

Lake water was characterized by distinct physical and chemical properties. Water temperature indicated clear seasonality and varied normally between a minimum in winter (16°C) and a maximum in summer (32°C). No distinct regional difference was observed among the stations during the same month. Early spring warming in March increased water temperature by approximately 3°C compared with late winter. Salinity exhibited no seasonal trends (mean 4.013±1.51 PSU). Values between 3 and 5 PSU represented the major fraction of the total measurements (50.6%). Salinity correlated significantly with temperature (r=0.71, P < 0.01, n= 72) and negatively with NO₂ (r= -0.31, P > 0.05) and NO₃ (-0.37, P > 0.05). The total nitrogen (Sum of NH₄, NO₂, and NO₃) exhibited relatively higher levels in winter (0.90±0.11mg L⁻¹), and it was lowest in early autumn (0.02mg L⁻¹), showing no particular seasonal trend. Nitrate represented the main component of N sources (71.8%), followed by almost equal sharing of NH₄ and NO₂ (12.6% and 15.6%, respectively). Spatiotemporal concentrations varied greatly,

particularly in March (0.16mg L⁻¹, St. VI; 2.3mg L⁻¹, St. III). Phosphate also reached the highest levels in March and April (0.99 and 1.25mg L⁻¹, respectively), followed by a sharp drop in the next 2 months (0.13 and 0.29mg L⁻¹, respectively). Stoichiometric analyses indicated a major potential for N limitation (63.89% of the total observations), whereas 15.28% of the total observations were P limiting, and 20.83% of the total observations were either N or P limiting (Table 1).

Community structure

The phytoplankton community structure comprised five groups, arranged by magnitude as cyanophytes (63.09% of the community), dinoflagellates (12.76%), diatoms (11.36%), chlorophytes (10.63%), and euglenophytes (2.16%). Their relative contribution differed seasonally, but cyanophytes showed predominance all the year round. The community of cyanophytes and chlorophytes included 45 species, mostly from Chlorophyceae spp. The highest species diversity (number) of the two groups was registered in January (10 and 18 spp., respectively) and the lowest in June (3 and 7 spp.). The two groups were significantly correlated with each other (r=0.69, P at<0.01), and temperature and salinity might represent explanatory variables influencing the species diversity of the two groups (Table 2). Meanwhile, the variability of cyanophyta species diversity might be affected by varied $NH_4(-)$, NO₂, and PO₄ concentrations, whereas NO₂ and TN were positively correlated with chlorophytes (Table 2).

TABLE 1. Measured physical and chemical	properties (mean±S.D)
---	-----------------------

	Temperature °C	D. Oxygen mg O, L ⁻¹	Salinity PSU	NH_4	NO ₂	NO ₃	T.N	PO ₄	TN/P ratio	
		0 1		$mg L^{-1}$						
Mean	24.2	6.38	4.13	0.09	0.07	0.39	0.64	0.11	11.73	
Standard error	0.61	0.20	0.18	0.01	0.01	0.05	0.06	0.01	2.04	
Range	16.0	7.30	5.30	0.37	0.45	2.00	2.28	0.45	117.3	
Minimum	16.0	2.40	1.90	0.02	0.01	0.02	0.02	0.01	0.36	
Maximum	32.0	9.70	7.20	0.39	0.46	2.02	2.30	0.46	117.6	
Confidence level (95.0%)	1.2	0.39	0.36	0.02	0.02	0.09	0.11	0.02	4.1	

Group	Chlorophyta	Temprature	D.Oxygen	Salinity	Alkalinity	NH4	NO ₂	NO ₃	Total N	PO ₄	TN/P ratio
Cyanophyta	0.69	-0.68	0.17	-0.51	0.04	-0.23	0.06	0.21	0.12	0.23	-0.02
Chlorophyta	1.00	-0.48	0.00	-0.50	-0.29	0.09	0.28	0.45	0.29	0.02	0.14

 TABLE 2 Correlation coefficient (r) values of Cyanophyta and Chlorophyta species diversity (number) and the measured physical and chemical variables

Occurrence of M. aeruginosa and B. braunii

Microcystis aeruginosa contributed the major fraction of Cyanophyta annual abundance (75.29%-96.07%), whereas В. braunii contributed 11.37%-93.12% of Chlorophyta. The annual distribution, based on the mean values, exhibited remarkable regional fluctuations in all stations. Microcystis aeruginosa (3203.72×10³ colony L⁻¹) achieved its highest abundance at St. II and IV (525.37×10^3 and 946.9×10^3 colony L⁻¹), whereas *B. braunii* (378.51×10³ Col. L^{-1}) gained its highest abundance at St. III and V (82.5×10³ and 93.33×10^3 colony L⁻¹). On the contrary, St. I sustained the lowest abundance for both species $(321.94 \times 10^3 \text{ and } 33.36 \times 10^3 \text{ colony } L^{-1}$, respectively). Their occurrences were related to each other (r=0.30, P>0.05). The major bloom peak of M. aeruginosa was recorded in June, whereas that of B. braunii in September, with the increase of concomitant Chl. *a* to 16.5 ± 7.11 and 21.8 ± 7.45 mg L⁻¹, respectively. *Microcystis* aeruginosa was a persistent form, which formed visible blooms (Fig. 2) of monospecific and/ or multispecific nature in December, June, and September, whereas its lowest densities were restricted from July to August and from October to November (Chl. a < 5.4mg L⁻¹; Fig. 3). The

blooms during December and June appeared as scattered dispersed visible patches primarily concentrated toward the North-eastern area of the lake, whereas in September, the accumulative colonies turned the surface water into a foam-like dense green layer that occupied the whole water body. The blooms with different frequencies maintained a wide range of temperature (17.5°C-31°C), salinity (2.4-6 PSU), elevated pH values reaching the highest of 8.9 in June, well-oxygenated water, changeable nutrient concentrations, and relatively high NO₂ values with dispersive bloom in December (Table 3 A). Defining the principal factor/s influencing the annual occurrence of M. aeruginosa is difficult; however, the negative relation among NH₄, TN, and PO₄ is effective. Meanwhile, the change in nutrient ratios had no significant contribution to its seasonal abundance variation (Table 4). Some other species progressed actively within the bloom periods in June, including the diatom Cyclotella meneghiniana; the chlorophyceans Chlorella vulgaris and Scenedesmus bijuga; and the dinoflagellates Gymnodinium and Scrippsiella trochoidea. Moreover, Scenedesmus quadricauda, the cyanophycean Merismopedia glauca, and Gymnodinium were recorded in September.

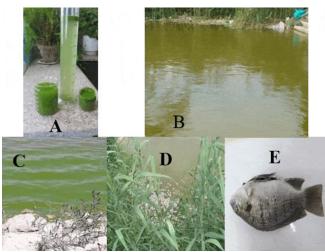
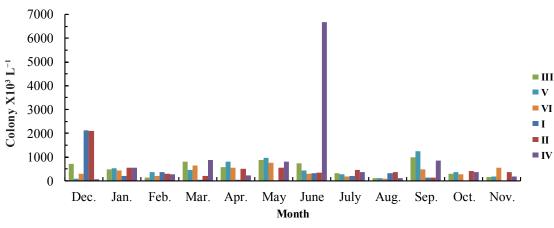


Fig. 2. September bloom photos: (A) Laboratory image, (B–C) Field images, (D) Fish mortality, and (E) Dead *Tilapia* sp.



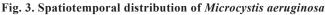


 TABLE 3. Accompanied physical and chemical parameters with regional Microcystis aeruginosa (A) and Botryococcus braunii (B) bloom peaks

	Α				В	
-		Month			Mont	h
-	Dec. 2017 June Sep.		April	Sep.		
		Station			Statio	n
Parameter	I, II, III	V	IV, V, VI	Parameter	IV	VI
Temperature	17.5	31	22	Temperature	22	22
Salinity	2.4-2.6	5.3	5.8-6	Salinity	4.3	5.8
pН	7.9-8.3	8.9	8.5	pH	8.5	8.5
D.Oxygen	5.4-7.6	8.4	6.6-7.4	D. Oxygen	6.3	6.6
NH_4	0.3-0.5	0.04	0.03-0.1	NH_4	0.017	0.06
NO ₂	0.1-0.3	0.03	0.03-0.2	NO_2	0.044	0.03
NO ₃	0.15-1.18	0.251	0.06-0.16	NO ₃	0.4	0.12
Total N	0.33-1.26	0.32	0.3-0.75	Total N	0.46	0.48
PO ₄	0.28-0.32	0.04	0.05-0.24	PO_4	0.22	0.05
TN/P ratio	6.22-45.0	8	1.67-8.89	TN/P	2.09	8.89
Colony. l ⁻¹ x10 ³	726-2133	6666.6	850-1250	Colony. 1-1x10 ³	300	380
Bloom type	Dispersive	Dispersive	Scums	Bloom type	Dispersive	Scums

 TABLE 4. Pearson's correlation coefficient (r) values between environmental factors and Microcystis aeruginosa and Botryococcus braunii annual abundance

Species	Temperature	D.Oxygen	Salinity	Alkalinity	NH4	NO ₂	NO ₃	T.N	PO ₄	TN/P
M. aeruginosa	0.06	0.11	0.01	-0.12	-0.19	-0.13	-0.06	-0.15	-0.14	-0.04
B. braunii	0.23	-0.07	0.20	-0.04	-0.23	-0.12	-0.10	-0.17	0.10	-0.06

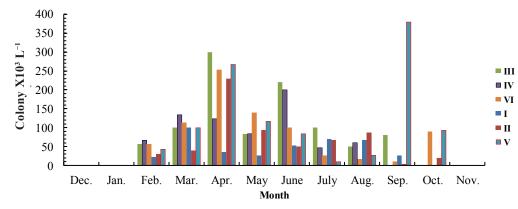
The microscopic examination of *B. braunii* revealed irregularly, indefinitely shaped aggregated colonies of various sizes (70μ m to 300μ m in diameter), attached by thread-like mucilaginous connections. The limited number of individual cells appeared ovoid to pyriform in shape, with

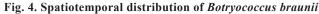
size length between 6–11 μ m and width 5–8 μ m. 72% of the measured cell length ranged from 6 to 9 μ m. This species was first seen in February (20×10³–66.7×10³ Colony L⁻¹) with increased water temperature by approximately 4 °C and relatively higher NH₄ concentrations (1.77mg L⁻¹)

compared with that observed in January (0.6mg L⁻¹). The species culminated two major peaks in April (St. IV) and September (St. VI; Fig. 4, Table 3 B); the April bloom was monospecific. The highly buoyant-dwelling aggregates found in September accumulated as visible thick scums or scattered spots (Fig. 2). Furthermore, several minor peaks $(>100\times10^{3}-270\times10^{3} \text{ colony } L^{-1})$ were observed between March and July. The density declined between late summer and mid-winter. Temperature, salinity, and NH₄ in reverse correlation with the annual abundance of B. braunii were important regulators of its monthly occurrence, and the effect of TN/P changes was comparably weaker than that of absolute nutrient element concentrations (Table 4). The accompanied community composition showed no significant changes at all stations irrespective of varied B. braunii density. The co-existing species, which peaked in April, were composed primarily of a well-diversified Cyanophyta (16 spp.) and a few growing diatoms, including Nitzschia longissima $(357 \times 10^3 \text{ cell } \text{L}^{-1})$, Cyclotella meneghiniana (40×10³ cell L⁻¹), and dinoflagellate Gymnodinium sp. (163×10³ cell L⁻¹). The blooms of *B. braunii* with different frequencies could affect the relatively reduced densities of other species, particularly chlorophycean Chlorella vulgaris, decreasing from 440×10³ cell L⁻¹ at St. I to 3.3×10^3 cell L⁻¹ at St. IV.

Discussion

In general, the temporal and regional physicochemical variations were parallel with previously reported results in the lake (Labib et al., 2014; Helal et al., 2020) and elsewhere (Campbell et al., 1996). Relatively higher salinity values and reduced accompanied phytoplankton abundance in July–August might be attributed to the cease of Nile freshwater input into the lake. Data showed the effects of NO₃, NH₄, and PO₄ on the community structure and abundance, commonly known in lakes and water reservoirs (Maberly et al., 2020), stressing the importance of nutrient concentration reduction to avoid the severity of noxious algal blooms. Meanwhile, the nutrient ratios showed relatively less effect. The distinctive role of temperature in conjunction with algal occurrence was clear during the transient period in spring and summer, thereby showing the highest production; the role of temperature as an effective factor was previously documented (Paerl et al. 2014). The water turbidity during the winter mixed period (Secchi values at <30cm) and subsequent relatively high nutrient concentrations might be used as agents to alter the growth and community structure. These conditions favored Cyanophyta, rather than Chlorophyta, species diversity at 22°C. Considering that the impact is species-specific, previously reported data regarding the role of winter conditions in a harsh eutrophic environment are inconsistent (Malazarte et al., 2017). The current study indicated the great ability of Chlorophyta and Cyanophyta to tolerate a wide temperature range, forming monospecific and/or multispecific dense blooms in no particular season. However, notably, the maintenance of cyanobacteria as perennial and predominant forms and the overall occupation of the lake during the bloom periods might be due to its dynamic nature caused by the presence of gas vesicles, which keep these mobile organisms in the water column, assisting them in getting light and nutrients (Scheffer et al., 1997). The contribution of combined M. aeruginosa and B. braunii to September heavy bloom might be attributed to the similarity in the mean optimum growth temperatures of approximately 29°C. According to Demura et al. (2014), B. braunii tolerates desiccation and extreme temperatures, which allows its global dispersion in different environments.





Egypt. J. Bot. **62**, No. 2 (2022)

Nutrient concentrations were always above limiting thresholds of chlorophytes and cyanophytes for optimum growth, and the excess inputs of nutrients can cause harmful blooms (Paerl et al., 2018). The N/P ratio changed sufficiently with Chlorophyta bloom in September and with Cyanophyta in May and June, elucidating symptoms of heavy eutrophication in the lake. Although N limitation was dominant, the present data indicated the importance of P for the development of over algal growth, which was consistent with other work (Figueiredo et al., 2004). Therefore, the data provide evidence that the continuous supply of nutrients with different quantity and specific composition might hinder the study of nutrients as decisive limiting growth factors; consequently, the interaction between seasonality in nutrient supply and algal nutrient limitation remains poorly understood (Andersen et al., 2020). However, this finding does not mean that a massive bloom can persist without an ample supply of nutrients. In general, Cyanophyta blooms maintained relatively higher N/P ratios than Chlorophyta blooms, which was inconsistent with the results of Mischke (2003), who reported low ratio levels along with other factors (thermal stratification, reduced transparency, and an increase in water temperature and pH), and frequently enhanced the occurrence of Cyanobacteria blooms. In addition, the pioneering study by Jensen et al. (1994) indicated that Cyanobacteria lost its high degree of dominance at low TN:TP ratio or low inorganic N concentrations. Andersen et al. (2020) found that cyanobacteria represented a proportionally larger fraction of the phytoplankton assemblage under N-limiting conditions. Although reducing external nutrients loading has been widely recommended as the first step to control such blooms in eutrophic ecosystems (Conley et al., 2009; Lewis et al., 2011), the present data indicate that the reduction of only one type of nutrients can lead to an imbalance in the N:P ratio of the water column, thereby leading to the worsening of Cyanophyta and/or Chlorophyta problems, particularly at high concentrations of nutrients. Thus, dual nutrient management strategies must incorporate N and P. The scientific consensus is limited on the importance of N:P ratios as a driver for freshwater algal blooms and the mitigation of eutrophication by controlling limiting nutrients (Schindler & Hecky, 2009). Present statistical analyses revealed a close relation between absolute concentrations and production rather than

with N:P ratios. However, considering that the environmental parameters work simultaneously in concert, separating the impact of nutrient concentrations and ratios from other co-occurring environmental variables to quantify the specific effect on the development of Cyanobacteria and Chlorophyta blooms in the lake is difficult. Considering the increasing populations and human activity in Alexandria City, the increase in N and P will exceed the assimilative capacity of the resident phytoplankton. Therefore, the changes in the N: P ratio have a little effect on the growth of a particular taxon, and the more enriched the system in N and P, the greater the total Cyanobacteria biomass (Davidson et al., 2012).

437

The lack of information on *M. aeruginosa* in the lake causes difficulty in conducting a detailed comparative study for its occurrence. On the contrary, B. braunii received a limited attention as its major peaks have been previously reported during January and February 2012 (maximum at 55.3×10³ colony L⁻¹), causing brown water discoloration and fish mortality (Labib et al., 2014). The current data showed higher densities; however, B. braunii can reach 7.4×106 colony L⁻¹ (Teng et al., 2021). The measured dimensions of B. braunii cells might nominate the present strain as L race. Based on the hydrocarbons present in this alga, Metzger & Largeau (2005) identified three different races with different sizes, where the cells of race L were smaller (8 to 9 μ m × 5 μ m) compared with 13 μ m × 8 to 9µm of race A and race B (Metzger et al., 1988; Dayananda et al., 2007). However, cell shape and size vary on different environmental or growth conditions (Tran et al., 2010). The present study demonstrated different recurrent blooms of B. braunii of diverse growth frequencies irrespective of varying seasonal temperatures. This result might deny the assumption stated by Labib et al. (2014), that is, this strain was only cold adapted in the lake water. Several studies have proven that this species can tolerate temperature between 20°C and up to 33°C (Qin, 2005) and salinity (Ranga Rao et al., 2007) and may require low levels of NO3 and PO4 to produce the highest hydrocarbon content (Raj et al., 2016). Blooms of B. braunii can release free fatty acids to the ambient environment (Qin, 2005) depending on its magnitude and ability. This finding can affect the phytoplankton diversity to different degrees, which in turn disturb the ecological stability in

the lake. The observed population interactions between B. braunii and the small, fast-growing green algae, Chlorella vulgaris, indicated that the regional distribution of the bloom causative species deeply affected C. vulgaris abundance. Song et al. (2012) found that the high density of B. braunii reduced algal competition. The observed multispecific bloom in September caused the mortality of hundreds of fish, primarily Tilapia sp. However, the fish-killing mechanism was not identified, and several factors might be involved such as gill clogging and oxygen stress. However, the gut content of dead Tilapia fish, characterized by its yellow body color, indicated the remains of Cyanobacteria and Chlorophyta species in the gut components (about 70%), whereas Euglenophyta, Bacillariophyta, and Dinophyta species showed less abundance by an order of magnitude.

Conclusion

Nozha Lake is a harsh eutrophic environment with clear symptoms indicated by monospecific and/or multispecific dense harmful blooms of a few Chlorophyta and Cyanophyta species in no particular season.

Dual N and P input reductions are required to combat the potential noxious blooms in the future. Future studies must include other biological factors, grazing, species interaction, metrological data, and the presence of toxins assessed through allelopathic tests to obtain a comprehensive understanding of the system function. Therefore, developing a risk plan to support policymaking is necessary.

Conflicts of interest: No conflicts of interest have been declared.

Authors' contribution: Shimaa Hosny: Field work, Collect samples, taxonomic identification of phytoplankton samples, draft the manuscript and editing of it. Wagdy Labib: Participated in the design of the study and approved the final version of manuscript.

Ethical approval: Not applicable

References

Andersen, I.M., Williamson, T.J., González, M.J., Vanni, M.J. (2020) Nitrate, ammonium, and phosphorus drive seasonal nutrient limitation of Chlorophyta, Cyanobacteria, and diatoms in a hyper-eutrophic

Egypt. J. Bot. 62, No. 2 (2022)

reservoir. *Limnology and Oceanography*, **65**(5), 962-978.

- Berg, M., Sutula, M. (2015) Factors affecting growth of cyanobacteria with special emphasis on the Sacramento-San Joaquin Delta. Southern California Coastal Water Research Project Technical Report 869.
- Brennan, L., Owende, P. (2010) Biofuels from microalgae a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and* Sustainable *Energy Reviews*, 14, 557-577.
- Campbell, N., D'Arcy, B., Forst, A., Novotny, V., Sansom, A. (1996) Diffuse pollution—an introduction to the problems and solutions. International Association on Water Quality, Tunbridge, Wells: IWA 322 pages.
- Chiang, I.Z., Huang, W.Y., Wu, J.T. (2004) Allelochemicals of *Botryococcus braunii* (Chlorpphyceae). *Journal of Phycology*, **40**, 474-480.
- Codd, G.A., Morrison, L.F., Metcalf, J.S. (2005) Cyanobacterial toxins: risk management for health protection. *Toxicology and Applied Pharmacology*, 203, 264-272.
- Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., et al. (2009) Controlling Eutrophication: Nitrogen and Phosphorus. *Science*, **323**(5917), 1014-1015.
- Davidson, A.D., Boyer, A.G., Kim, H., Pompa-mansilla, S., Hamilton, M.J. (2012) Drivers and hotspots of extinction risk in marine mammals. *Proceedings* of the National Academy of Sciences of the United States of America, **109**, 3395-3400.
- Dayananda, C., Sarada, R. Kumar, V., Ravishankar, G.A. (2007) Isolation and characterization of hydrocarbon producing green alga *Botryococcus braunii* from Indian freshwater bodies. *Electronic Journal of Biotechnology*, **10**, 78-91.
- Demura, M., Ioki, M., Kawachi, M., Nakajima, N., Watanabe, M.M. (2014) Desiccation tolerance of *Botryococcus braunii* Trebouxiophyceae, Chlorophyta, and extreme temperature tolerance of dehydrated cells. *Journal* of *Applied Phycology*, 26, 49-53.

- Environmental quality standards (EQS) (Established in 1997 and are in effect) Ministry of the Environment Government of Japan <u>https://www.env.go.jp</u>. Environmental quality standard for waters, nitrogen and phosphorus standards for lakes and reservoirs, website of Ministry of the Environment Government of Japan.
- Figueiredo, D.R., Azeiteiro, U.M., Esteves, S.M., Goncalves, F.J.M., Pereira, M.J. (2004) Microcystin-Producing Blooms A Serious Global Public Health Issue. *Ecotoxicology and Environmental Safety*, 59(2), 151-163.
- Forsberg, C., Ryding, S.O. (1980) Eutrophication parameters and trophic state indices in 30 Swedish waste-receiving lakes. *Archiv für Hydrobiologie*, 89, 189-207.
- Hasle, G.R. (1978) The inverted-microscope methods. In: "Phytoplankton Manual, United Nations Educational, Scientific, and Cultural Organization", Sournia, A. (Ed.), pp. 88-96. Paris.
- Helal, A.M., Abdelaty, B.S., Elokaby, M.A., Mustafa, M.M., Hosny, Sh., Heneash, A.M.M. (2020) Ecosystem management of al-nozha airport farm lake, Egypt utilizing TSI model. *International Journal of Fisheries and Aquatic Studies*, 8(2), 137-145.
- Jeffrey, S.W., Humphrey, G.F. (1975) New spectrophotometric equations for determining chlorophyll a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen*, **167**, 191-194.
- Jensen, J.P., Jeppesen, E., Olrik, K., Kristensen, P. (1994) Impact of nutrients and physical factors on the shift from cyanobacterial to chlorophyte dominance in Shallow Danish Lakes. *The Journal* of Foot & Ankle Surgery, **51**(8), 1692-1699.
- Labib, W., Mikhail, S.K., Kassem, A.M., El Kassas, M., Ahmed, M.M. (2014) 'Blooms of the colonial green algae *Botryococcus braunii* Kützing associated with massive fish mortality in Nozha lake, Alexandria, Egypt', In: "*Harmful Algae 2012*", H.G. Kim, B. Reguera, G.M. Hallegraeff, C.K. Lee, M.S. Han & J.K. Choi (Eds.), pp. 188-191.
- Khairy, H., El-Sheekh, M. (2019) Toxicological studies on microcystin produced by *Microcystis* aeruginosa: Assessment and management.

Egyptian Journal of Botany, 59(3), 551-566.

- Lewis, W.M., Wurtsbaugh, W.A., Paerl, H.W. (2011) Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of inland waters. *Environmental Science & Technology*, 45(24), 10300-10305.
- Maberly, S.C., Jo-Anne, P., Sian, D.P., Laurence, C. (2020) Nitrogen and phosphorus limitation and the management of small productive lakes. *Inland Waters*, **10**(1), 1-14.
- Malazarte, J.M., Hakyoung, L., Hyun-Woo, K., Yongsik, S. (2017) Spatial and temporal dynamics of potentially toxic cyanobacteria in the Riverine Region of a temperate Estuarine System altered by Weirs. *Water*, 9, 819.
- Metzger, P., Casadevall, E., Coute, A. (1988) Botryococcene distribution in strains of green alga *Botryococcus braunii*. *Phytochemistry*, **27**, 1383-1388.
- Metzger, P, Largeau, C. (2005) Botryococcus braunii: A rich source for hydrocarbons and related ether lipids. Applied Microbiology and Biotechnology, 66(5), 486-496.
- Mischke, U. (2003) Cyanobacteria associations in shallow polytrophic lakes: Influence of environmental factors. Acta Oecologica, 24, 11-23.
- Needham, J.G., Needham, P.R. (1962) "A Guide to the Study of Freshwater Biology". 5th ed. Holden–Day Inc. San Francisco., USA, 106p.
- Papa, R.D., Wu, J.T., Baldia, S., Cho, C., Cruz, M.A., Saguiguit, A., Aquino, R. (2008) Blooms of the colonial green algae, *Botryococcus braunii* Kützing, in Paoay lake, Luzon island, Philippines. *Philippine Journal of Systematic Biology*, 2(1), 21-31.
- Paerl, H.W., Xu, H., Hall, N.S., et al. (2014) Controlling cyanobacterial blooms in hypertrophic Lake Taihu, China: will nitrogen reductions cause replacement of non-N₂ fixing by N₂ fixing taxa? *PLoS One*, 9(11), 113-123.
- Paerl, H.W., Otten, T.G., Kudela, R. (2018) Mitigating the expansion of harmful algal blooms across the freshwater-tomarine continuum. *Environmental Science and Technology*, **52**, 5519-5529.

- Parsons, T. (1984) "A Manual of Chemical and Biological Methods for Seawater Analysis". 1st ed., Pergamon, 173 Pp. eBook ISBN: 9781483293394.
- Qin, J. (2005) Bio-Hydrocarbons from Algae: Impacts of temperature, light and salinity on algae growth. Rural Industries Research and Development Corporation Report. 26p.
- Raj, G.P., Arone, Soul, S., Elumalai, T., Sangeetha, D., Roop Singh, G., Kanna, R. (2016) *Botryococcus braunii* as a potential candidate for the wastewater treatment and hydrocarbon accumulation. *International Journal of Scientific Engineering and Research*, 7(6), 917-929.
- Ranga Rao, A., Dayananda, C., Sarada, R., Shamala, T.R., Ravishankar, G.A. (2007) Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. *Bioresource Technology*, **98**, 560-564.
- Scheffer, M., Rinaldi, S., Gragnani, A., Mur, L.R., Van Nes, E.H. (1997) On the dominance of filamentous cyanobacteria in shallow, turbid lakes. *Ecology*, 78, 72-82.
- Schindler, D.W., Hecky, R.E. (2009) Eutrophication: More nitrogen data needed. *Science*, **324**, 721-722.
- Shi, H.X., Qu, J.H., Liu, H.J., et al. (2008) Study of the role of nitrogen isotope tracer in Microcystins produce process (in Chinese). *Chinese Science Bulletin. (Chin Ver)*, **53**, 407-412.

- Song, L., Qin, J.G., Su, S.Q., Xu, J.H., Clark, S., Shan, Y.C. (2012) Micronutrient requirements for growth and hydrocarbon production in the oil producing green alga *Botryococcus braunii* (Chlorophyta). *PLoS One*, 7, e41459. https://doi.org/10.1371/ journal.pone.0041459.
- Tang, X.M., Gao, G., Chao, J.Y., et al. (2010) Dynamics of organic-aggregate associated bacterial communities and related environmental factors in Lake Taihu, a large eutrophic shallow lake in China. *Limnology and Oceanography*, **55**, 469-480.
- Tran, H.L., Kwon, J.S., Kim, Z.H., Oh, Y., Lee, C.G. (2010) Statistical optimization of culture media for growth and lipid production of *Botryococcus braunii*. *Biotechnology and Bioprocess Engineering*, **15**, 277–284.
- Utermöhl, H. (1958). Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Int Ver Theor Angew Limnol Verh*, **9**, 1-40.
- Teng, S.T., Law, K., Hanifah, A.H., Bojo, O.B., Idrus, F.A., Mannaf, A.H.N.A., et al. (2021) Bloom of a freshwater green alga *Botryococcus braunii* (Botryococcaceae, Trebouxiophyceae) and the associated mass fish mortality in a man-made lake, Sarawak, Malaysia. *Plankton and Benthos Research*, 16(1), 59-68.
- Wolfe, A.H., Patz, J.A. (2002) Reactive nitrogen and human health: acute and long-term implications. Ambio: A *Journal of the Human Environment*, **31**(2), 120-125.

تحكم الظروف البيئية المختلفة في ديناميكية التواجد الزماني والمكاني للMicrocystis Botryococcus braunii ، aeruginosa في بيئية المياه العذبة

شيماء حسني، وجدي لبيب

المعهد القومي لعلوم البحار والمصابد (FOIN) - مصر.

تعتبر بحيرة النزهة (جنوب مدينة الإسكندرية، مصر) حوض مياه عذبة اصطناعي، تتلقى إمداداتها المائية من نهر النيل ومن المنطقة الحضرية المحيطة مما يعرضها لارتفاع نسب المغذيات ولذك تعتبر البحيرة بيئة قاسية، مما يؤدي إلى از دهار الطحالب بصورة متكرره على مدار العام ومن هنا يؤثر على التوازن البيئي للبحيرة. وكشفت النتائج من خلال التقييم البيئي للبحيرة من ديسمبر 2017 إلى نوفمبر 2018 عن از دهارات مشتئة لكلا من Botryococcus brauni و هو نوع من الطحالب الخضراء المزرقة و Microcystis aeruginosa وهو من الطحالب الخضراء، وذلك تحت ظروف بيئية ومواسم مختلفة. وكان لل Botryococcus brauni الدور وهو من الطحالب الخضراء، وذلك تحت ظروف بيئية ومواسم مختلفة . وكان لل *Botryococcus aeruginosa و* تواجد ثابتًا خلال الفترة بأكملها، وبلغ ذروته في الإز هار المرئي في ديسمبر ويونيو وسبتمبر. تم إثبات الدور والفوسفات على التباين في بنية المجتمع ووفرته. كما تم رصد قمم التكاثر الرئيسية ل المضوية، و الأمونيا والفوسفات على التباين في بنية المجتمع ووفرته. كما تم رصد قمم التكاثر الرئيسية ل Botryococus عنوب الموني والفوسفات على التباين في بنية المجتمع ووفرته. كما تم رصد قمم التكاثر الرئيسية المادور المونيا والموسفات على التباين في بنية المجتمع ووفرته. كما تم رصد قمم التكاثر الرئيسية المونيا المونيا على تتوع أنواع العوالق النباتية المحتمع ووفرته. كما تم رصد قمم التكاثر الرئيسية المونيا المونيا على تنوع أنواع العوالق النباتية المصاحبة بدرجات مختلفة كما في الطحالب الخصراء وزدهار المحاربة على تنوع أنواع العوالق النباتية المصاحبة بدرجات مختلفة كما في المحارب الخصراء الذهار المونيا مسريعة النمو. ومن هنا يتطلب الأمر إلى العمل على انخفاض نسبة النيتروجين والفوسفات الداخل البحيرة لمحاربة تكرار الاز دهار وكثرته بالاضافه إلى زياده نسبة الدراسات البيولوجية وتفاعل الأنواع مع بعضاي البحيرة المحاربة الناتجه من التفاعل الأليلوباثي لبعض الأنواع مما يدعم ويطور من سياسة خطة تجنب المخاطر والمشاكل قبل حدوثها.