

Research Article

Phytoplankton Monitoring of Discovery Lake (San Marcos, California) in 2022

Clarivel Cedillo*¹, Sergio Jeronimo¹, Rosalina Stancheva¹

¹ Department of Biological Sciences, California State University San Marcos

* Correspondence: cedil005@csusm.edu

Abstract: Discovery Lake is a small shallow man-made recreational lake located in the suburbs of the City of San Marcos and used as field sampling station for the CSUSM undergraduate course Biol 387L Aquatic Ecology Laboratory since 2011. We studied the phytoplankton dynamics of Discovery Lake from March to November 2022 as a community engagement project mastering our new field and lab research skills developed during the Spring 2022 class of Biol 387L. Our study provides the first data on phytoplankton composition and dynamics of Discovery Lake. Dinoflagellate species belonging to *Ceratium* are the most characteristic for the lake phytoplankton and determine the brownish color of the lake water. They grow continuously since the beginning of phytoplankton monitoring in 2011, probably favored by the increased water salinity (492-1204 ppm in 2022). *Ceratium* forms extensive and prolonged blooms in the fall months, recently continuing until late November in 2022. In the summer months (July and August), filamentous N₂-fixing cyanobacteria belonging to *Dolichospermum* and *Aphanizomenon* form dense blooms, co-dominated by the chrysophyte colonial flagellate *Dinobryon* in 2022. Although summer cyanobacterial blooms are not confirmed to be toxic, they deplete the oxygen, sometimes causing fish kills.

Introduction

Current climate change causes warming of aquatic environments and seasonal dynamics of the temperatures are strongly affected. Climate change can provide warmer water temperatures and, when in combination with elevated nutrient conditions, promote harmful algal blooms (HAB) dominated by cyanobacteria (Paerl et al., 2012) or eukaryotic algae, such as some green algae, diatoms, chrysophytes and dinoflagellates. Cyanobacteria are the oldest oxygen producing autotrophic organisms with available fossils dating back over 3.5 billion years (Paerl et al., 2012). Algal blooms have various contributing factors that range from: temperature, light, pH, to nutrient availability (Zangett et al., 2013). An already developed bloom can be interrupted by strong winds, increasing salinity, decreasing temperature, reduced light availability phosphorus and nitrogen attainability (Cairns, 2016; Galat et al., 1989; Kahru et al., 1993; Kahru et al., 2000; Kanoshina et al., 2003; Lehtimäki et al., 1994; Kononen et al., 1996; Lehtimäki, 1997).

Most algal blooms have serious ecological and human health consequences in marine, brackish, and freshwater systems throughout the world (Watson et al., 2015). Some cyanobacteria species can produce toxins as consequence of anthropogenic eutrophication. HABs can affect the ecosystem's structure and function, drinking and irrigation water sources, recreational activities, tourism and land value (Watson et al., 2015). In California, warning signs are placed near water bodies that are experiencing cyanobacterial blooms and voluntary bloom reports can be viewed on an interactive map provided by the Freshwater and Estuarine Harmful Algal Bloom program (FHAB, 2022). The warnings serve as reminders to the public and pets to exude caution near the water. Effects of toxins can range from irritation and nausea to liver failure and death (Lyon-Colbert et al., 2018).

Citation: Cedillo, C.; Jeronimo, S.; Stancheva, R. Phytoplankton Monitoring of Discovery Lake (San Marcos, California) in 2022. *Cougar JUGR* 2023, 2.

Academic Editor: Dennis Kolosov

Copyright: © 2023 by the authors.

Regardless of whether they are toxic or not, blooms of cyanobacteria can cause adverse effects such as a shift in light, nutrient and oxygen availability for other organisms.

We studied Discovery Lake, which is a 4.9-acre, shallow man-made lake located in the suburbs of the City of San Marcos. The lake serves as a recreational area for surrounding residents and provides an environment for the local wildlife and waterfowl. However, residential development runoff drains into the lake. Irrigation runoff from surrounding development most likely contains trace amounts of pollutants and nutrients from plant fertilizers that may significantly impact on aquatic resources, including algae, fish, insects, etc. The visual turbidity of the water, shallow depth of the lake, poor water circulation suggests possible algae blooms, and oxygen depletion are indicated by records of fish-kills. Human recreational use of the lake, specifically feeding of waterfowl, probably contributes nutrients and organic compounds left from the seeds and man-made food.

The city of San Marcos oversees the maintenance, quality and beneficial uses of the lake system. The city launched water quality improvement program of Discovery Lake in 2020 (<https://www.san-marcos.net/Home/Components/News/News/5115/24>). There is no data on phytoplankton species composition and seasonal dynamics, nor on its potential toxicity, which is important for selecting the best management practice for the lake. Due to its proximity to the CSUSM campus, the lake has been continuously used as a field sampling station for the CSUSM undergraduate course Biol 387 Aquatic Ecology Laboratory. In the spring and the fall each year since 2011 Drs. Rosalina Stancheva Christova and Robert Sheath have taken students to sample the plankton of Discovery Lake as part of the course. During the COVID pandemic students performed the first seasonal study of the phytoplankton dynamics of the lake (from March to October 2020). This pilot study detected severe cyanobacterial bloom caused by potentially toxigenic cyanobacterium *Aphanizomenon* in early August. Students from the Spring 2022 class of Biol 387 Lab were highly motivated to continue monitoring the phytoplankton dynamics during the summer months and a research contract was established with the city of San Marcos.

This report presents the results from the 2022 phytoplankton study of Discovery Lake and compare the bloom events in 2020 and 2022, which could be helpful for informed sustainable management of the lake. This project provided students with the opportunity for community engagement, as this lake is near California State University San Marcos. With limited field experiences during the COVID pandemic, the study afforded students the opportunity to gain valuable sampling techniques, sample processing, and taxonomy in real-world environmental study. The study showed the conditions under which cyanobacterial blooms form and the impact they may have, on the lake and the community.

Materials and Methods

Sampling Site

Discovery Lake is located in a residential neighborhood in San Marcos, California. The maximum depth is 2.8 m but may vary depending on rainfall. The lake is fed by storm flow from the neighboring watershed. Littoral zone aquatic vegetation includes Broadleaf cattails (*Typha latifolia* Linnaeus), California bulrush (*Scirpus californicus* (C.A. Mey.) Soják) and Duckweed (*Lemna* sp.). Riparian native vegetation includes Western sycamore (*Platanus racemosa* Nutt), Willow (*Salix* spp.), California coastal live oak (*Quercus agrifolia* Née), and Pacific poison oak (*Toxicodendron diversilobum* (Torr. & A. Gray) Greene). Local fauna includes Ruddy duck (*Oxyura jamaicensis* (J. F. Gmelin), Mallard duck, American

coot (*Fulica americana* Gmelin), Red-winged blackbird (*Agelaius phoeniceus* Linnaeus), and Great Egret (*Ardea alba* Linnaeus).

Sample and Environmental Data Collection

Whole water samples were collected monthly in 2022 (March to November) from the dock of Discovery Lake and more frequently during the summer months during plankton blooms. The water samples were collected from 50 cm depth, 10 am in the morning with a Van Dorn Sampler (Fig. 1 C). A similar sampling schedule was used in 2020 (no samples were collected in July 2020). The water clarity was measured with a Secchi disk and water temperature with a thermometer. In addition, in 2022 we measured pH, conductivity, salinity, and total dissolved solids with Extech EC500 Waterproof ExStik® II pH/Conductivity Meter (Wilsonville, OR). Phytoplankton samples were preserved with Lugol's solution and transported to CSUSM Algae Lab. Data for monthly average air temperature and precipitation measured at the closest NOAA location, Carlsbad McClellan Palomar Airport, CA US, were obtained from NOAA <https://www.ncdc.noaa.gov/cdo-web/datasets/LCD/stations/WBAN:03177/detail>.

Phytoplankton Analysis

The original samples were concentrated four times by settling for at least 48 hours and decanting. Phytoplankton were analyzed using Palmer-Maloney counting cells and Olympus BX41 compound microscope. Using a high dry microscope objective (40x objective, 400x total system magnification), at least 300 natural algal units were identified, and cell numbers recorded in known sample volume (e.g., field of view), either horizontally or vertically across the Palmer-Maloney cell. Algal identifications were done to the lowest possible taxonomic level, i.e. typically genus or species when taxonomically important features are present, such as akinetes for some cyanobacteria. The diatoms were reported as whole group, due to method limitations for genus/species identification. However, genus level identification was obtained, when possible, for diatoms. The average dimensions of the algal cells were obtained for each taxon by measuring at least 20 cells for the purpose of cell biovolume calculation. Species biovolume was determined by multiplying the cell density (cells/ml) by the average biovolume of each cell (μm^3). The standard methods for the examination of water and wastewater were used for the identification of aquatic organisms (APHA, 2017).

During cyanobacterial blooms, samples were collected for quantification of total cyanobacterial toxins (anatoxin-a, cylindrospermopsin, microcystin/nodularin and saxitoxin) by enzyme linked immunosorbent assay (ELISA) by Bend Genetics.

Results

Physicochemical Conditions

We visited Discovery Lake for sample collection (Fig. 1B, C) every month from March to November 2022 at consistent times around 10 am, to eliminate the effects variations in sunlight may have on watercolor. Watercolor changes during the sampling of Discovery Lake from March to November 2022 are illustrated in Figure 1. In late July, the water was blue-green in color with golden hues (Fig. 1A-C). During the fall, the water was a brown murky color (Fig. 1D).

Physicochemical parameters for salinity, conductivity and total dissolved solids (TDS) in Discovery Lake, 2022 are detailed in Figure 2. Salinity increased steadily from 492 ppm in March and peaking in September at 1204 ppm (Fig. 2A). Conductivity showed a similar

inclining trend from 982 to 1824 $\mu\text{S}/\text{cm}$ in October (Fig. 2B). TDS ranged from 685 to 1262 ppm with the maximum in October (Fig. 2C). The pH ranged from 7 to 8.5, and water temperature from 13 to 26.8°C (Fig. 3B).

Comparison between 2020 and 2022 of Secchi disk depth, water temperature, and average monthly precipitation is demonstrated in Figure 2. Secchi disk measurements for 2020 ranged from 52.5 cm to 200 cm, no recordings were taken during July and November (Fig. 1C, Fig. 2 A). The water clarity in 2020 was generally lower from March at 91.44 cm to July at 94 cm. On June 4, 2020, a dinoflagellate bloom decreased the water clarity to 60.96 cm. The highest water clarity occurred on October 8, 2020, at 200 cm (Fig. 2A). In contrast, on October 3, 2022, we observed low water clarity at 65 cm due to the dinoflagellate bloom. Water temperatures in 2020 ranged from 15°C to 28.3°C and in 2022 ranged from 13°C to 26.8°C (Fig. 3B). There is a similar increasing trend in water temperature for both years from March to November, although no measurements were taken during July 2020 (Fig. 3B). Precipitation in 2020 ranged from 0 in to 6.44 in, the peak occurred during April, followed by a decline to 0.04 in May (Fig. 3C). During 2022, precipitation ranged from 0 in to 1.65 in, the peak occurred in March at 1.65 in and decreased to 0.04 in April (Fig. 3C). The average precipitation for 2022 was generally lower when compared to 2020 (Fig. 3C).



Figure 1 A-D. Sampling of Discovery Lake during the algal blooms in 2022. A-C. Summer cyanobacterial bloom (July 22, 2022). A. Note the dull blue-green watercolor with golden hues due to cyanobacterial and chrysophyte cell accumulations. B. Water sample collected in Van Dorn Bottle. C. Student S. Jeronimo measures water clarity with Secchi Disk. D. Fall bloom caused by the dinoflagellate *Ceratium furcoides*. Note the brown murky watercolor (October 3, 2022).

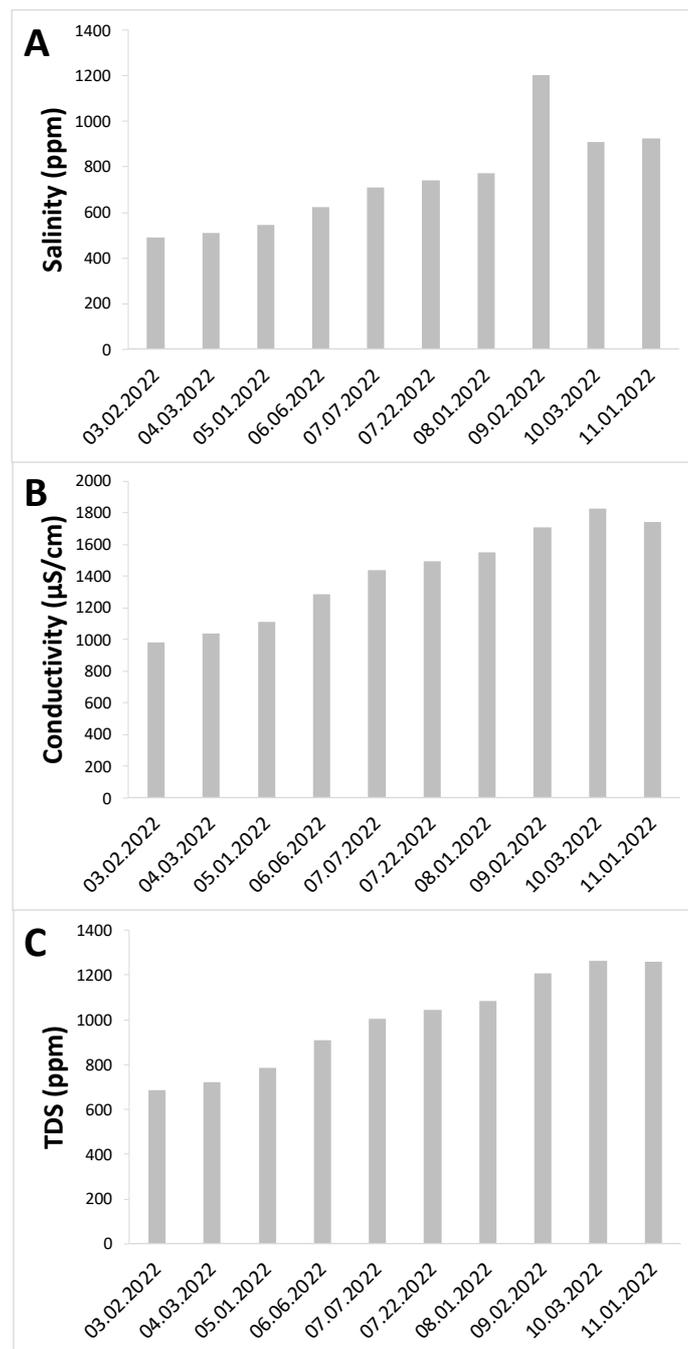


Figure 2 A-C. While the salinity of the lake peaked in September 2022 (Fig. A), conductivity (Fig. B) and total dissolved solids (Fig. C) were highest in October and November 2022.

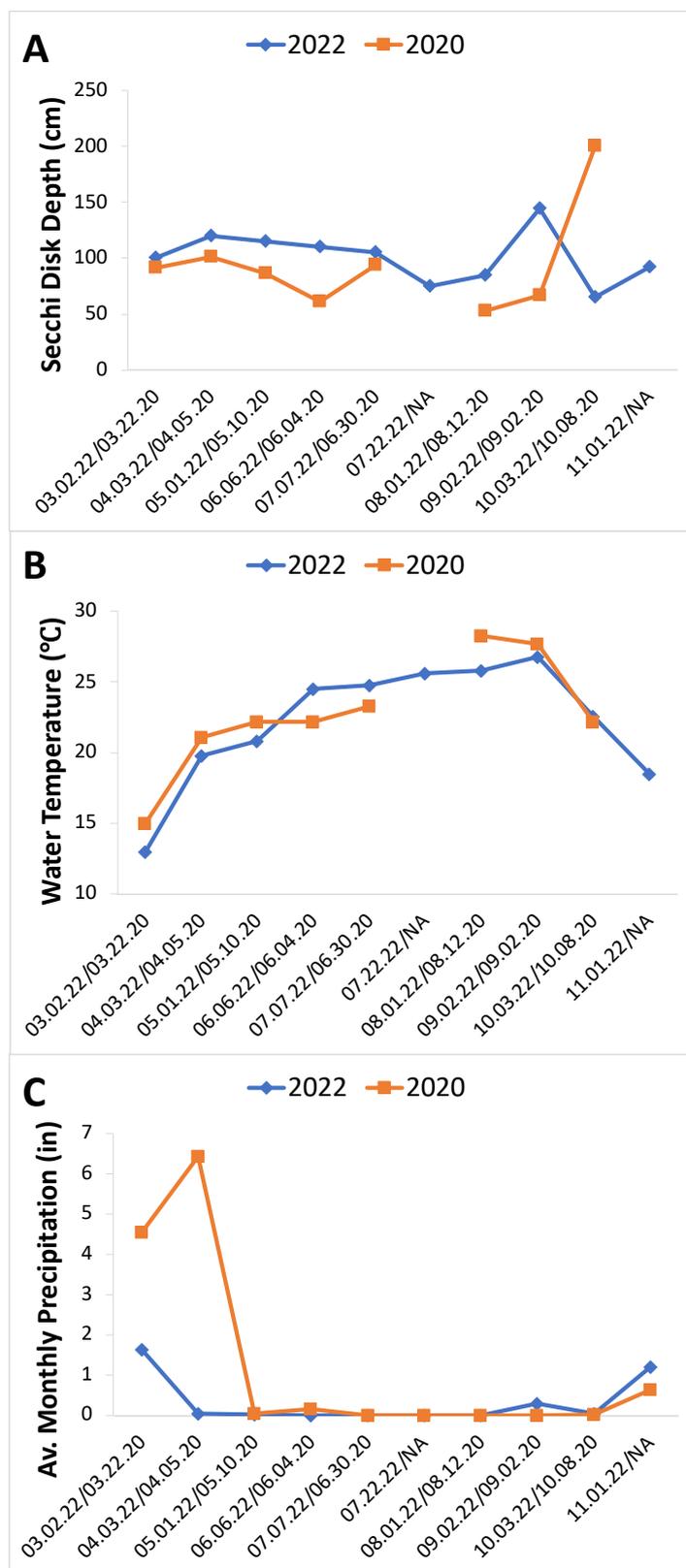


Figure 3 A-C. While the water temperature was similar in 2020 and 2022 (Fig. B), precipitation was higher in the spring of 2020 (Fig. C), and water clarity of the lake was variable between both years (Fig. A).

Phytoplankton Seasonal Dynamics

We identified 39 phytoplankton algal species in the Discovery Lake in 2022. The bloom-forming species are illustrated in Figure 4, and the small-sized species in Figure 5. The phytoplankton algae belonged to Dinophyta (*Ceratium hirundinella* (O.F.M.) Dujardin

and f. *piburgense* (Zederbauer) Bachmann (Fig. 4A-C), *Ceratium furcoides* (Levander) Langhans (Fig. 4D-H), *Peridinium* sp. 1), Cryptophyta (*Chroomonas acuta* Utermöhl, *Chroomonas* sp. 2, *Cryptomonas* cf. *marsonii* Skuja, *Cryptomonas* sp. 1, *Cryptomonas* sp. 2 (Fig. 5E-F), Euglenophyta (*Phacus acuminatus* A. Stokes, *Phacus caudatus* Hübner), Chrysophyta (*Chrysophyte* flagellate 1, *Chrysophyte* coccoid, *Dinobryon divergens* O.E.Imhof (Fig. 5C), *Mallomonas* sp. 1 (Fig. 5B), Bacillariophyta (*Aulacoseira* sp. 1, *Cyclotella* sp. 1 (Fig. 5A), *Epithemia adanata* (Kütz.) Bréb., *Navicula* sp. 1, *Nitzschia acicularis* (Kütz.) W. Smith, *Nitzschia* sp. 1, *Synedra ulna* (Nitzsch) Ehrenberg), Cyanobacteria (*Aphanocapsa* cf. *delicatissima* W. West & G. S. West, *Aphanocapsa parasitica* (Kützing) Komárek & Anagnostidis, *Cyanogranis ferruginea* (F.Wawrik) Hindák ex Hindák, *Dolichospermum flosaquae* (Bréb. ex Bornet et Flahault) P. Wacklin, L. Hoffm. et Komárek (Fig. 4I-J), *Spirulina major* Kützing ex Gomont, *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg, *Synechococcus nidulans* (Pringsheim) Komárek) and Chlorophyta (*Ankyra*, *Asterococcus limneticus* G. M. Smith (Fig. 5D), *Carteria* sp. 1, *Chlorella* sp. 1, *Closterium acutum* Brébisson, *Monoraphidium contortum* (Thuret) Komárková-Legnerová, *Monoraphidium minutum* (Nägeli) Komárková-Legnerová, *Quadrigula*, *Scenedesmus* spp., *Sphaerocystis schroeteri* Chodat, *Tetrastrum komarekii* Hindák). Most species were flagellates, actively swimming in the water (Fig 4 A-H, 5 E-C). We present here the complete phytoplankton composition and abundance data from the 2022 survey, and only comment on the major differences in comparison with 2020 observations.

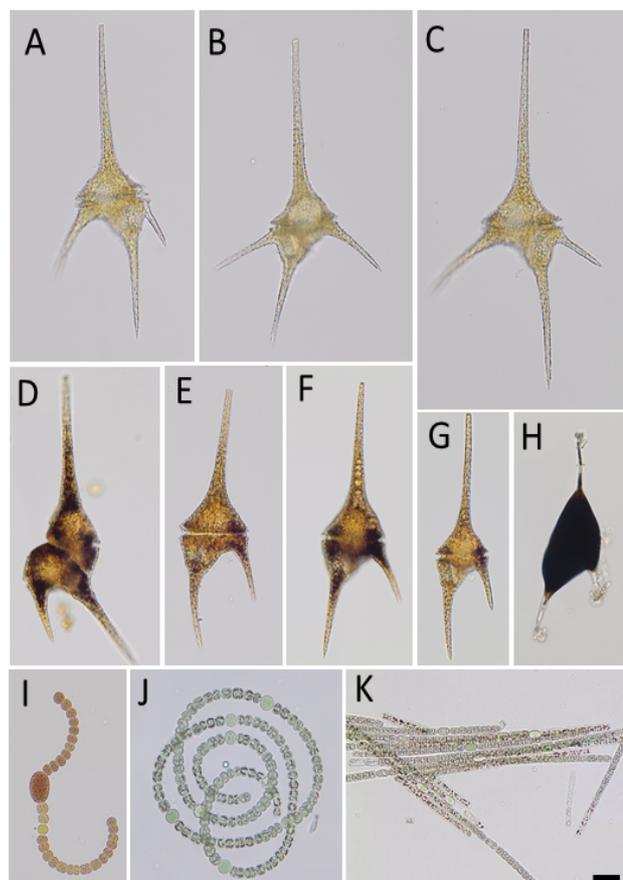


Figure 4 A-K. Main bloom-forming dinoflagellates and cyanobacteria in Discovery Lake. A. *Ceratium hirundinella* with three posterior horns directed downward (June 6, 2022). B, C. *Ceratium hirundinella* f. *piburgense* with three widely played posterior horns (June 6, 2022). D–H. *Ceratium furcoides*. D. Cell division by oblique binary fission (August 1, 2022). E, F, G. Cells with two posterior horns directed downward (September 2, 2022). H. Mature hypnozygote (cyst) (October 2, 2022). I–J. Cyanobacterium *Dolichospermum flosaque*. I. Short filament with a spherical heterocyte and a large elliptical akinete distant from the heterocyte (August 1, 2022). Long coiled filament with heterocytes and cells with gas vesicles (July 22, 2022). K. Cyanobacterium *Aphanizomenon flosaque*: long straight filaments arranged in fascicle-like colonies; filaments contain cylindrical heterocytes and cells with gas vesicles (August 12, 2020). All images from Lugol’s preserved samples, except for Figs J and K. Scale bar for all images is 20 µm.

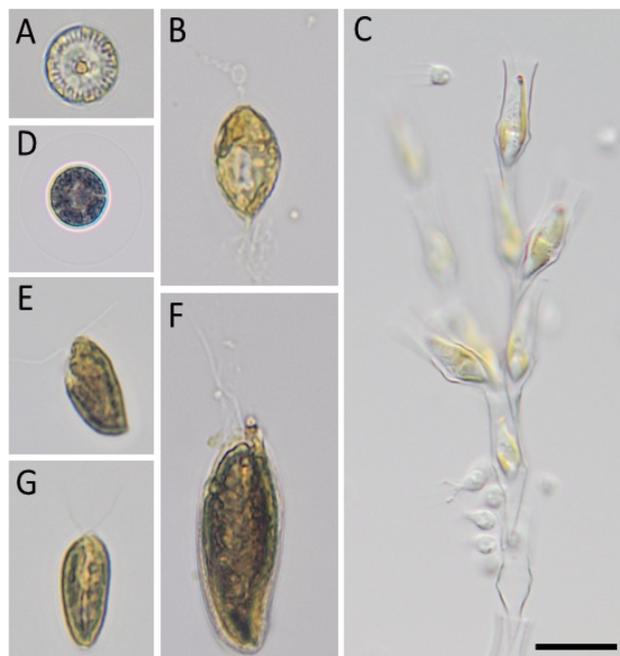


Figure 5 A-G. Small-sized planktonic algae in Discovery Lake. A. Single cell of non-motile diatom *Cyclotella* (March 2, 2022). B. Single cell of chrysophyte flagellate *Mallomonas* (March 2, 2022). C. Colonial chrysophyte flagellate *Dinobryon* (July 22, 2022). D. Single cells of green non-motile alga *Asterococcus* (March 2, 2022). E-F. Distinct species of cryptophyte flagellate *Cryptomonas* (May-June 2022). All images are from Lugol's preserved samples, except for Fig. C. Scale bar for all images is 20 μm .

The seasonal dynamics of Discovery Lake phytoplankton in 2022 is presented in Figure 6, including Secchi disk measurements (Fig. 6A), total cell density (Fig. 6B), total cell biovolume (Fig. 6C), total cell density of *Ceratium hirundinella* and *Ceratium furcoides* (Fig. 6D), proportion of cell density of each phylum (Fig. 6E) and proportion of cell biovolume for each phylum (Fig. 6F). The water clarity in 2022 ranged from 65 to 145 cm (Fig. 6A). The highest water clarity was reached in September at 145 cm. The lowest clarity readings were in July at 75 cm and October at 65 cm (Fig. 6A) caused by a cyanobacterial bloom in the summer and dinoflagellate bloom in the fall. The total cell density of all algal species was very high at the end of July and in August (Fig. 6B) due to extensive growth of cyanobacterial cells (Fig. 6E). The highest cell density correlates with the lower water clarity measured in late July (Fig. 6A). The total cell biovolume of all algal species showed a different trend; a steady increase from March to November (Fig. 6C). The highest total cell biovolume was recorded in November (138521999 $\mu\text{m}^3/\text{mL}$). This could be explained by the consistent increase in cell density of *Ceratium* from March to November, which resulted in a *Ceratium* bloom, causing the lowest water clarity in October (Fig. 6A). It is important to notice that *Ceratium* is a large unicellular dinoflagellate with cells nearly 300 times larger than the cyanobacterial cells, causing the summer bloom in July/August. We observed two morphotypes of *Ceratium*, identified as *C. hirundinella* with three posterior horns and a long apical horn (Fig. 4A) and *C. furcoides* with two posterior horns and a tapering apical horn (Fig. 4D-H). *C. hirundinella* was present in the spring from April to June and then in November, while *C. furcoides* cell density increased from July to November (Fig. 6D).

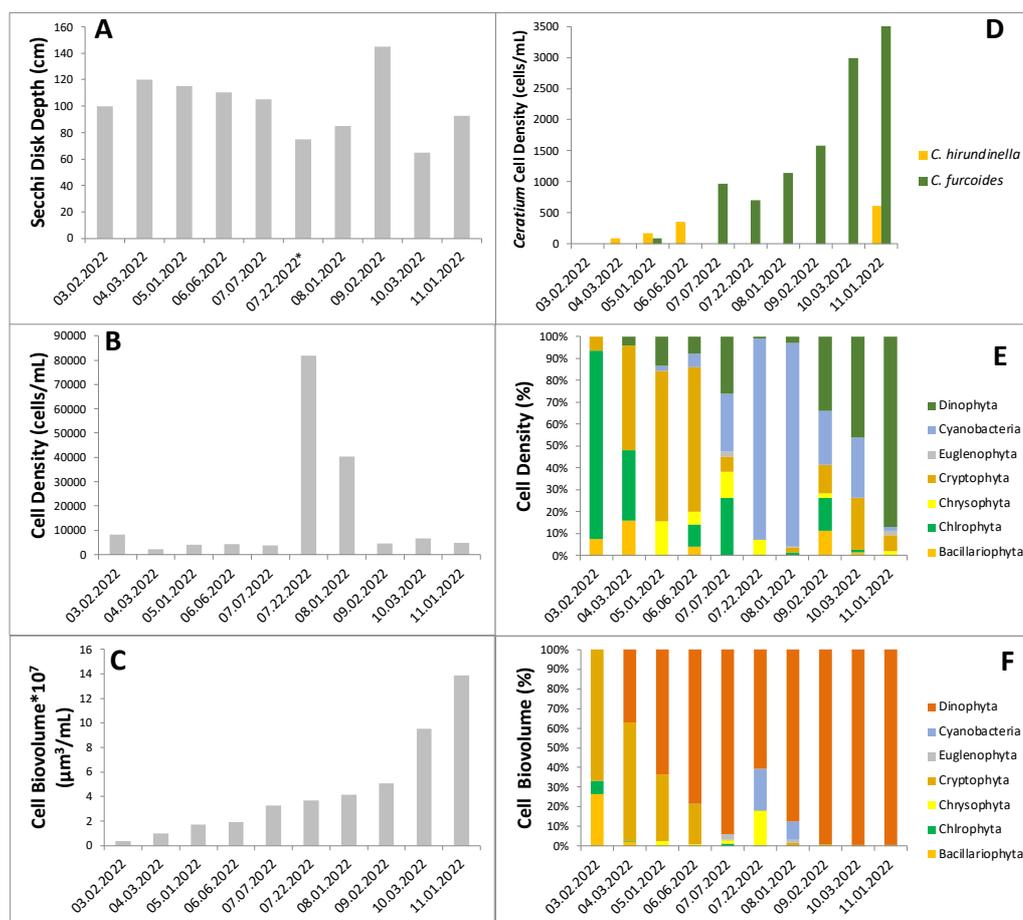


Figure 6 A-F. The reduced water clarity in the summer of 2022 measured by Secchi Disk Depth (Fig. A) correlated with a pick in the cyanobacterial cell density in July and August (Figs B, E), while the low water clarity in the fall corresponded to a peak in cell biovolume (Fig. C) due to dominance of large-sized dinoflagellates *Ceratium hirundinella* and *Ceratium furcoides* in October and November (Figs E, F).

Spring phytoplankton communities (March - June)

The spring phytoplankton community in 2022 was dominated by green unicells (*Chlorella*, *Asterococcus*, *Ankyra*), which are non-motile and non-toxic (Fig. 6E), followed in abundance by the flagellates *C. hirundinella* and cryptophytes (Fig. 6F). Dinoflagellate *C. hirundinella* was distributed from March to June 2022 (Fig. 6D-E). Although the cell density was low, the size of this species, which is much larger than any other alga recorded in the lake, results in a greater cell biovolume (Fig. 6F). Cyanobacteria and diatoms were rare in the spring. Cyanobacteria were present in May and June and diatoms in March, April and June (Fig. 6E-F). The photosynthetic pigments of the dinoflagellates, diatoms, and cryptophytes are golden-brownish, which determines the brownish color of the lake during the spring. The total algal cell density and biovolume was relatively low in the spring, with algal biovolume consistently increasing over time, but at a stagnant pace (Fig. 6B, C).

The spring phytoplankton community recorded in 2020 differed by high abundance of non-motile centric diatoms, *Cyclotella* and *Thalassiosira*. In June 2020, both *C. hirundinella* and *C. furcoides* were observed, with prevalence of *C. furcoides* which reached a density peak in June, reducing the water clarity to 60.9 cm (Fig. 3B). The higher precipitation in spring 2020 could be a key factor influencing algal composition and growth (Fig. 3C).

Summer cyanobacterial blooms (July - August)

Signs of cyanobacterial growth started to appear on July 7, 2022, by decreasing water clarity and changing the watercolor to greenish (Fig. 1 A-C, Fig. 6 E-F). By July 22, 2022, a dense bloom with odor developed due to high abundance of cyanobacterium *Dolichospermum flosaque* and colonial chrysophyte flagellate *Dinobryon divergens* (Fig. 6 E). The water clarity reduction is supported by the Secchi disk measurement, which was low on July 22, 2022 at 75 cm (Fig. 6 A). Precipitation was at 0 in during this time, but water temperature began to warm from 24.8 to 25.6°C (Fig. 2 B-C), favorable for blooms. Cyanotoxins were tested, but not detected. Fish mortality was not recorded. The bloom decreased in August and *Dolichospermum* cells were not detected in September. The total cell density reached a peak on July 22, 2022 (81838.96 cells/mL), and algal biovolume increased (Figs. 6 B-C).

In contrast, in 2020, only single filaments of *Dolichospermum* were recorded but an extensive cyanobacterial bloom was formed by *Aphanizomenon flosaque*, though not toxic. The cyanobacterial bloom observed on August 12, 2020 decreased the water clarity to its minimum of 52.5 cm, followed by fish kills due to oxygen depletion. Both *Aphanizomenon* and *Dolichospermum* have heterocytes, specialized cell types that fix atmospheric N₂. The buoyancy of both planktonic cyanobacteria is supported by gas vesicles (Fig. 4I-K). The presence of these blooming cyanobacteria able to fix atmospheric nitrogen, indicates nitrogen limitation in the lake, and high phosphorus to nitrogen ratio.

Fall phytoplankton communities (September - November)

After the disruption of the cyanobacterial bloom at the end of August 2022, a community of cryptophytes, dinoflagellates and rare diatoms developed. The fall morphotype of *Ceratium furcoides* reached high density in October, due to intense cell division in August (Fig. 4D) and sexual reproduction in September with zygote formation in October (Fig. 4H). The water clarity in October 2022 dropped to 65 cm (Fig. 6A) and the watercolor was deep brown due to *Ceratium* bloom (Fig. 1D). The *Ceratium* chloroplast colors caused a brown murky watercolor during their bloom. *C. furcoides* reached its highest cell density in October and November (Fig. 6D), much larger when compared to the rest of the algal species (Fig. 6 E) and dominated the total algal biovolume. The total cell number decreased in November, but the total phytoplankton biovolume reached its peak of 94942905 $\mu\text{m}^3/\text{m}$ (Fig. 6 E, F). During 2022, the months of October and November had one of the lowest Secchi disk readings, correlating with the high cell density and biovolume recordings of *C. furcoides* (Fig. 6 A, D). The water temperature dropped in October and November (Fig. 3B), but the salinity and conductivity reached their maximum (Fig 2) creating favorable conditions for the dinoflagellate bloom.

In contrast, the 2020 fall phytoplankton community was dominated by cryptophytes, which reached their peak in September. The phytoplankton density declined in October, which resulted in the maximum water clarity of 2 m recorded in the lake (Fig. 2 A).

Discussion

Bloom-forming phytoplankton taxa: biology, ecology and toxicity

Dinoflagellates

Marine dinoflagellates cause the toxic blooms “red tides”, including in the Pacific Ocean. However, they are not distributed in freshwater lakes, where mainly species of *Ceratium* are known to form blooms, typically not toxic (Carty & Parrow, 2015). However,

Ceratium blooms in lakes and ponds may de-oxygenate the lower water layer and cause fish kills (Meichtry de Zaburlín et al., 2016).

We recorded two species of freshwater *Ceratium* in Discovery Lake, which differ by the number and shape of posterior horns, and position of the apical plates (Fig. 4A-H) (Carty, 2014, Moreira et al., 2015). However, the cell morphology, and particularly the horn count and length can vary by season. Various factors such as seasonal changes in temperature, nutrient availability (Gligora et al., 2003, Carty, 2014), particularly nitrogen (Carty 2014), and predation by planktivorous fish are thought to contribute to the morphological variation found in *C. hirundinella* species complex (Bertolo et al., 2014). Both *C. hirundinella* and *C. furcoides* have large cells with lengths over 100 µm, two flagella and yellowish green chloroplasts (Fig. 4A-H). The large size ensures that even when *Ceratium* cell density is low, they will still dominate the biovolume when compared to smaller species.

Ceratium reproduces both sexually and asexually. Asexual reproduction results through oblique binary fusion, while sexual reproduction occurs through the female gamete enveloping the smaller male gamete, producing three to four horned cysts (hypnozygotes) which settle on the bottom and function a resting stage (Carty, 2014). We documented intense binary fusion of *C. furcoides* (Fig. 4D) in August 2022, followed by sexual reproduction in September and formation of benthic cysts in October (Fig. 4H), similarly to previous observations of the life cycle of this species blooming in a small pond in Greenport, NY (Hickel, 1988). *Ceratium hirundinella* is ubiquitous in various aquatic ecosystems during the spring and summer months when the water temperature is warmer (Gligora et al., 2003). *C. furcoides* has been observed in North and South America, Europe, Asia, Africa, and Oceania (Moreira et al., 2015). Both *C. furcoides* and *C. hirundinella* have been previously recorded in California (Carty, 2014). *C. furcoides* could be the dominant species in lakes, forming blooms from June to October (Hickel, 1988), in agreement with its 2022 dynamics in Discovery Lake.

A bloom of *Ceratium hirundinella* in Albert Falls Reservoir (Hart and Wragg, 2009) was caused by a decrease in N:P due to an increase in total phosphorus attributed to a wastewater treatment plant. Similarly, discharge of untreated sewage promoted a bloom of *Ceratium furcoides*, considered an invasive species in Furnas Reservoir, Brazil (da Silva et al., 2012). Another bloom of *C. furcoides*, an invasive species in Brazil, was recorded in high altitude temporary oligotrophic lake with low temperatures and low soil pH (Moreira et al., 2015). *C. furcoides* is ecologically similar to *C. hirundinella*. It is a S-strategist that increases its density through the warmer seasons under eutrophic and mineralized conditions, low light conditions and stable elevated temperatures (Bustamante et al., 2012). In environments of high-water turbidity, *C. hirundinella* has been observed to manage considerable diel vertical migration. This mobility enhances *C. hirundinella*'s ability to seek light and nutrients distributed throughout the water column (Gligora et al., 2003). During a bloom, especially *Ceratium* due to its size, the water clarity will decrease and thus limit the light availability to other organisms. *C. furcoides* is capable of growth under dark conditions, heterotrophic nutrition and phagotrophy are possible due to its ability for vertical and horizontal migration (Bustamante et al., 2012).

Cyanobacteria

Dolichospermum is a summer bloom-forming cyanobacterium recorded in Discovery Lake in the summer of 2022. The large filaments are typically coiled (Fig. 4J) and contain heterocytes (Fig. 4I). *Dolichospermum* strains can produce microcystins, cylindrospermopsin, anatoxins, saxitoxins, lipopolysaccharides, BMAA (beta-Methylamino-L-alanine) (Li et al., 2016). These toxins can affect humans and animals by

inhibiting protein synthesis, hypovolemic shock. Excess blood accumulation in the liver and promoting tumors due to chronic exposure may also occur (Zanchett et al., 2013). *Dolichospermum* blooms recorded in Junipers Reservoir located in southeastern Oregon, caused acute liver disease and microcystin-LR presence in cattle exposed to the water, causing the death of thirty-two steers (Dreher et al., 2018). *Dolichospermum* blooms have been recorded across North America in freshwater and brackish bodies of water (Li et al., 2015). *Dolichospermum* tolerates low nitrogen levels due to its ability to fix atmospheric nitrogen. However, it can be sensitive to turbidity and low light (Li et al., 2015). Gas vesicles function as buoyancy regulators, allowing for greater probabilities for optimal growth (Li et al., 2015). Blooms are thought to be attributed to eutrophication due to anthropogenic activities, as well as global warming. Blooms are most frequent during late spring and middle autumn (Li et al., 2015). A *Dolichospermum* bloom was recorded in Lake Superior on August 9, 2018, posing potential risks to commercial and recreational fisheries, tourism and water activities, potable and industrial water supplies (Sheik et al., 2022). Lake Erie experienced a *Dolichospermum* dominating bloom during October 2022, an unusual occurrence as this strain is typically seen for a few weeks in July, but not as a dominating species (Ellison 2022). Water temperatures and rainfall were not significantly higher during the month of October 2022 (Ellison 2022). More rainfall would facilitate water runoff containing phosphorus. It is hypothesized that the bloom could be due to phosphorus found in the sediment becoming resuspended (Ellison 2022). Resuspension of phosphorus and other sediments is an important direction for future studies on Discovery Lake, especially considering the fact that the blooming cyanobacteria are indicative of nitrogen limiting conditions, because they are able to fix atmospheric nitrogen.

Aphanizomenon is a summer blooming cyanobacterium, recorded in Discovery Lake in the summer of 2020. Trichomes contain heterocytes and form fascicle-like colonies (Fig. 4 K). Toxins produced by *Aphanizomenon* are paralytic shellfish poisoning (PSP) compounds. These include saxitoxins (SXTs), microcystins (MCs), cylindrospermopsins (CYNs) and anatoxins (Park et al., 2018). Some of the potential effects of cyanobacterial hepatotoxins and neurotoxins on mammals are liver failure, respiratory failure and paralysis, abdominal pain, headache, sore throat, vomiting, numbness and drowsiness (Lyon-Colbert et al., 2018). *Aphanizomenon* blooms have been recorded in Pinto Lake, a hyper-eutrophic lake, located in California (Kudela et al., 2015). Concern grows due to the frequency and ubiquity of toxins in various watersheds (Kudela et al., 2015). The increase in salinity, conductivity and temperature could have promoted favorable conditions for blooms during the summer. Persistent cyanobacteria blooms in an artificial recreational lake found that the presence of *Aphanizomenon* and *Dolichospermum* was linked with high-temperature and electric conductivity of water (Nowicka-Krawczy et al., 2022).

The 2022 summer cyanobacterial bloom in Discovery Lake did not result in fish kills. However, fish kills were reported during the 2020 bloom. Although the cyanobacterial strains in Discovery Lake are non-toxic, blooms can have detrimental effects on the ecosystem. Cyanobacterial blooms may lead to the depletion of oxygen and nutrients, and reduce the availability of light, affecting the growth of other organisms. The odor from the blooms can make it unpleasant for citizens near the lake, reducing the quality of the lake as a recreational location.

Chrysophytes

Several chrysophyte algae were recorded in Discovery Lake, but only *Dinobryon* have persisted since 2011, and particularly abundant in July of 2022, co-dominating the cyanobacterial bloom (Fig. 6 E, F). *Dinobryon* is a colonial flagellate alga (Fig. 5C) with

golden chloroplasts, adding golden hues to the watercolor when grown in large masses (Fig. 1A).

Dinobryon species are typically observed in the summer plankton community in predominantly low-nutrient lakes (Heinonen, 1980), sometimes forming 50% of the phytoplankton biomass (e.g., Canadian Shield lakes (Ostrowsky & Duthie, 1975). *Dinobryon* has an effective phosphate-uptake mechanism and ability to obtain nutrients from phago-trophic feeding on bacteria and other microscopic particles (Nicholls & Wujek, 2015), that contribute to its success in low-phosphorus supply situations. *Dinobryon* is a non-toxic alga, but it is well known as a taste and odor producer, which may explain the odor during the 2022 summer bloom in Discovery Lake. The influence of environmental factors (such as light intensity, temperature, nitrogen, and phosphorus) on population density and odor-compound production by *Dinobryon* is poorly understood (Nicholls & Wujek, 2015).

Phytoplankton Control by Grazers

Copepods and rotifers are the main grazers which control the phytoplankton density in Discovery Lake. However, they may graze the phytoplankton selectively as the main bloom-forming algae are protected against grazing by their large size (appr. 100-250 μm length) of their swimming single cells (*Ceratium*) or colonies (*Dinobryon*), and long coiled filaments (*Dolichospermum*) or clumps of filaments (*Aphanizomenon*). In addition, *Ceratium* is protected by long rigid horns. Probably the main food source for the zooplankton are the small single non-motile cells (appr. 7-20 μm in diameter) of green algae and diatoms developing in large quantities during the spring, and swimming single cells of cryptophytes abundant all year round (Bertolo et al., 2014).

Pollutants in the lake and their mitigation

High phosphorus concentrations in the sediments may have promoted the bloom of N₂-fixing cyanobacteria (e.g., *Dolichospermum* and *Aphanizomenon*) in July and August 2020 and 2022 similarly to the bloom in Lake Erie (Ellison 2022). Citizens may contribute to the eutrophication of Discovery Lake when feeding the local birds; nutrients not eaten sink and add to the sediment layer. Educating citizens about detrimental effects that are caused due to abundance of nutrients and placing warning signs around the lake could help reduce excess eutrophication of the lake and reduce algal blooms.

Conclusion

Our study provides the first data on phytoplankton composition and dynamics of Discovery Lake. Dinoflagellate species belonging to *Ceratium* are the most characteristic for the lake phytoplankton and determine the brownish color of the water. They have grown continuously since the beginning of phytoplankton monitoring in 2011, probably favored by increased water salinity. *Ceratium* forms extensive and prolonged blooms in the fall months, recently continuing until late November in 2022. Filamentous N₂-fixing cyanobacteria (e.g., *Dolichospermum*, and *Aphanizomenon*) form dense blooms in the summer months only (July and August), in 2022, co-dominated by the chrysophyte colonial flagellate *Dinobryon*. Although summer cyanobacterial blooms are not confirmed to be toxic, they deplete the oxygen, sometimes causing fish kills. Continued monitoring of the phytoplankton dynamics will be beneficial for selecting the best lake management practices to improve the water clarity and lake ecosystem balance.

Acknowledgements

We thank CSUSM student Sophia Grace from Biol 387L (Spring 2022) for the help with the sampling and microscope work. Nate Kristan helped with the 2020 field sampling of Discovery Lake and preparations for the virtual teaching of Biol 387L in the Fall of 2020. We thank Dr. Betsy Read for the critical review which improved the quality of the manuscript. We appreciate the collaboration with Reed Thornberry, Watershed Program Manager at the City of San Marcos, who supported this research. The project was funded by the City of San Marcos.

References

1. APHA, 2017. Standard Methods for the Examination of Water and Wastewater: Method 10200 Plankton, Method 10900 Identification of aquatic organisms Protocol P-13-52 for Analysis of USGS NAWQA Program Phytoplankton Samples, 23rd ed. American Water Works Association, Denver, CO.
2. Bertolo, A., G. Lacroix, F. Lescher-Moutoué & J. Hjelm, 2010. Relationship between fish and the number of horns in *Ceratium Hirundinella* (Dinophyceae): A food-web-mediated effect on algal morphology? *Journal of Phycology* 46: 33-40.
3. Bustamante, C., J. Restrepo, A. Boltovskoy & A. Vallejo, 2012. Spatial and temporal change characterization of *Ceratium furcoides* (Dinophyta) in the Equatorial Reservoir Riogrande II, Colombia. *Acta Limnologica Brasiliensia* 24: 207-219.
4. Carty, S. & Parrow, M. W. 2015. Dinoflagellates. In: Wehr, J., R. Sheath & P. Kociolek (eds). *Freshwater Algae of North America: Ecology and Classification*. 2nd edition. Academic Press, San Diego, CA: 773-802.
5. Carty, S. 2014. *Freshwater Dinoflagellates of North America*. Cornell University Press, Ithaca, NY: 92-95.
6. da Silva, L.C., Leone, I.C., dos Santos-Wisniewski, M.J., Peret, A.C., Rocha, O., 2012. Invasion of the dinoflagellate *Ceratium furcoides* (Levander) Langhans 1925 at tropical reservoir and its relation to environmental variables. *Biota Neotropica* 12: 1-7.
7. Dreher, T.W., LP. Collart, R.S. Mueller, K.H. Halsey, R.J. Bildfell, P. Schreder, A. Sobhakumari & R. Ferry, 2018. *Anabaena/Dolichospermum* as the source of lethal microcystin levels responsible for a large cattle toxicosis event. *Toxicon X* 1: 100003.
8. Freshwater and Estuarine Harmful Algal Bloom (FHAB) Program. 2022, December 19. https://www.waterboards.ca.gov/water_issues/programs/swamp/freshwater_cyanobacteria.html.
9. Galat DL & Verdin JP, 1989. Patchiness, collapse and succession of a cyanobacterial bloom evaluated by synoptic sampling and remote sensing. *Journal of Plankton Research* 11: 925-948.
10. Gligora, U. M., A. Plenković-Moraj & I. Ternjej, 2003. Seasonal Distribution and Morphological Changes of *Ceratium hirundinella* in two Mediterranean shallow lakes. *Hydrobiologia* 506: 213-220.
11. Hart, R.C., Wragg, P.D., 2009. Recent blooms of the dinoflagellate *Ceratium* in Albert Falls Dam (KZN): history, causes, special features and impacts on a reservoir ecosystem and its zooplankton. *Water SA* 35: 455-468.
12. Heinonen, P., 1980. Quantity and composition of phytoplankton in Finnish inland waters. Publication No. 37, Water Resources Institute, National Board of Waters, Helsinki, Finland. 91 p.
13. Hickel, B. 1988. Sexual reproduction and life cycle of *Ceratium furcoides* (Dinophyceae) in situ in the lake Plussee (F.R.G.). *Hydrobiologia* 161: 49-54.
14. Kahru M., J.M. Leppänen & O. Rud, 1993. Cyanobacterial blooms cause heating of the sea surface. *Marine Ecology Progress Series*, 101, 1-7.
15. Kahru M., J.M. Leppänen, O. Rud & O.P. Savchuk, 2000. Cyanobacteria blooms in the Gulf of Finland triggered by saltwater inflow into the Baltic Sea. *Marine Ecology Progress Series*, 207: 13-18.
16. Kanoshina I., U. Lips & J.M. Leppänen, 2003. The influence of weather conditions (temperature and wind) on cyanobacterial bloom development in the Gulf of Finland (Baltic Sea). *Harmful Algae* 2: 29-41.
17. Kononen K., J. Kuparinen, K. Mäkelä, J. Laanemets, J. Pavelson & S. Nömmann, 1996. Initiation of cyanobacterial blooms in a frontal region at the entrance to the Gulf of Finland, Baltic Sea. *Limnology and Oceanography* 41: 98-112.
18. Kudela, R.M., S.L. Palacios, D.C. Austerberry, E.K. Accorsi, L.S. Guild, & J. Torres-Perez, 2015. Application of hyperspectral remote sensing to cyanobacterial blooms in inland waters, *Remote Sensing of Environment* 167: 196-205.
19. Lehtimäki J., K. Sivonen, R. Luukkainen & S.I. Niemelä, 1994. The effects of incubation time, temperature, light, salinity, and phosphorus on growth and hepatotoxin production by *Nodularia* strains. *Archiv für Hydrobiologie* 130: 269-282.
20. Lehtimäki J., P. Moisander, K. Sivonen & K. Kononen, 1997. Growth, nitrogen fixation, and nodularin production by two Baltic Sea cyanobacteria. *Applied and Environmental Microbiology* 63: 1647-1656.
21. Li, X., T.W. Dreher & R. Li, 2016. An overview of diversity, occurrence, genetics and toxin production of bloom-forming *Dolichospermum* (Anabaena) species. *Harmful Algae* 54: 54-68.
22. Lyon-Colbert, A., S. Shelley & C. Curtis, 2018. A Systematic Literature Review for Evidence of *Aphanizomenon flos-aquae* Toxicity in Recreational Waters and Toxicity of Dietary Supplements: 2000-2017. *Toxins* 7: 254.
23. Meichtry de Zaburlín, N, R.E. Vogler, M.J. Molina & V.M. Llano, 2016. Potential distribution of the invasive freshwater dinoflagellate *Ceratium furcoides* (Levander) Langhans (Dinophyta) in South America. *Journal of Phycology* 52: 200-208.

24. Moreira, R.A., O. Rocha, R.M. Santos, R. Laudares-Silva, E.S. Dias & E.M. Eskinazi-Sant'Anna, 2015. First record of *Ceratium furcoides* (Dinophyta), an invasive species, in a temporary high-altitude lake in the Iron Quadrangle (MG, Southeast Brazil). *Brazilian Journal of Biology* 1: 98-103.
25. Nicholls, K. J. & Wujek, D. E. (2015) Chrysophyceae and Phaeothamniophyceae. In: Wehr, J. D., Steath, R. G. & J. P. Kociolek (eds.) *Freshwater algae of North America. Ecology and classification*. Elsevier Academic Press, Amsterdam. 537–586 pp.
26. Nowicka-Krawczy, K.P., J. Żelazna-Wieczorek, I. Skrobek, M. Ziulkiewicz, M. Adamski, A. Kaminski, & P. Żmudzki, 2022. Persistent cyanobacteria blooms in artificial water bodies - an effect of environmental conditions or the result of anthropogenic change. *International Journal of Environmental Research and Public Health* 12: 6990.
27. Ostrofsky, M.L., Duthie, H., 1975. Primary productivity and phytoplankton of lakes on the Eastern Canadian Shield. *Int. Ver. Theor. Agnew. Limnol.* 19: 732–738.
28. Paerl, H.W. & V.J. Paul, 2012. Climate change: links to global expansion of harmful cyanobacteria. *Water Research* 5: 1349-1363.
29. Park, H.K., M.A. Kwon, H.J. Lee, J. Oh, S.H. Lee & I.S. Kim, 2018. Molecular Verification of Bloom-forming *Aphanizomenon flos-aquae* and Their Secondary Metabolites in the Nakdong River. *International Journal of Environmental Research and Public Health* 8: 1739.
30. Sheik, C. S., K. E. Natwora, E.E. Alexson, J.D. Callaghan, A. Sailer, K.M. Schreiner, B.A. Steinman, M.S. Finkenbinder, C.T. Filstrup & A.J. Bramburger, 2022. *Dolichospermum* blooms in Lake Superior: DNA-based approach provides insight to the past, present and future of blooms. *Journal of Great Lakes Research* 5: 1191-1205.
31. Watson, S. B, Whitton, B.A., Higgins, S. N., Paerl, H. W., Brooks, B. W., J. D. Wehr, 2015. Harmful Algal Blooms. In: Wehr, J. D., Sheath, R.G., Kociolek, J. P. (Eds.), *Freshwater Algae of North America, Ecology and Classification*. Academic Press. pp. 873-920.
32. Zanchett G. & E.C. Oliveira-Filho, 2013. Cyanobacteria and cyanotoxins: from impacts on aquatic ecosystems and human health to anticarcinogenic effects. *Toxins* 10: 1896-917.