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Anthropogenic and climate-induced change favors toxic cyanobacteria blooms: Evidence from monitoring a highly eutrophic, urban Mediterranean lake

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ABSTRACT

Cyanobacteria Harmful Algal Blooms (or CyanoHABs) represent one of the most conspicuous waterborne microbial hazards in aquatic environments mostly due to the production of harmful secondary metabolites, known as cyanotoxins. In this paper we assessed cyanobacteria blooms in a shallow, Mediterranean, highly eutrophic lake (Lake Pamvotis, NW Greece) in relation to critical environmental parameters using a multi-approach methodology. Microscopic (identification of cyanobacteria), immunological (ELISA), and molecular techniques (PCR) combined with physico-chemical parameters were used to monitor cyanobacteria blooms and the associated cyanotoxin production for 14 months on a monthly basis. Cyanobacteria were the main phytoplankton component, representing more than 75% of the total phytoplankton abundance throughout the study period; dominant species belonged to *Microcystis*, *Anabaena* and *Aphanizomenon*. Microcystins (MCs) were detected throughout the year in all sampling stations in concentrations ranging from 0.16 to 27 $\mu g\ L^{-1}$, indicating that toxic *Anabaena* and *Microcystis* strains are persistent and dominant. Saxitoxins (STXs) found in two samples (concentrations 1.3 and 2.1 $\mu g\ L^{-1}$) in the warm period, are reported here for the first time. The total MC concentration was positively correlated with temperature, nitrate nitrogen, ammonia nitrogen, and soluble reactive phosphorus, suggesting that high-temperatures and eutrophic conditions promote the growth of MC-producing genotypes. We also investigated a *Microcystis* strain specific growth rate, which reached its maximum at 30 °C. Our data, combined with long-term data comparison of key limnological features, spanning a 25-year period, suggest that water temperature and nutrient loads may act synergistically to promote cyanobacterial dominance and persistence in Lake Pamvotis.

1. Introduction

Over the past centuries accelerated land use change and over-enrichment of nutrients mainly associated with urban, agricultural and industrial activities have promoted eutrophication of freshwater ecosystems. Recent research suggests that eutrophication and climate change are two processes that increase rates of primary production, shifting algal community toward bloom-forming and cyanobacterial species (O'Neil et al., 2012; Paerl and Paul, 2012). Cyanobacteria Harmful Algal Blooms (or CyanoHABs) represent one of the most conspicuous waterborne microbial hazards to human and agricultural water supplies, fisheries production, and freshwater and marine ecosystems (Codd et al., 2005; Paerl et al., 2011). This hazard results from the production of cyanotoxins, harmful secondary metabolites, such as microcystins, saxitoxins, and cylindrospermopsins, which can have deleterious effects within reservoirs and in downstream receiving water systems during releases (Paerl and Otten, 2013).

There are over 40 species representing 20 genera from three cyanobacterial orders known to produce cyanotoxins which include both cyclic peptides and alkaloids (Stewart and Falconer, 2008). Elucidation of the biosynthetic pathways of the toxins has paved the way for the development of molecular techniques for the detection and quantification of the producing cyanobacteria in different environments (Dittmann et al., 2013). Thus, several studies have applied molecular methods for monitoring the presence of harmful toxic cyanobacteria and the genes involved in the biosynthesis of microcystins (e.g. Hisbergues et al., 2003; Ouahid et al., 2005), cylindrospermopsins (Mankiewicz-Boczek et al., 2012) and saxitoxins (Ballot et al., 2010, Cirés et al., 2014).

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The production of cyanotoxins is thought to be influenced directly or indirectly by environmental conditions. The information on what environmental conditions are most likely to result in higher concentrations could be valuable (Chorus, 2001; Paerl and Otten, 2013) in order to proceed with effective management practices. Furthermore changes in nutrient loading also result in changes in biological communities' structure, including cyanobacteria, thus causing multiple concerns for water quality, including different cyanotoxins (Briand et al., 2003).

The warm Mediterranean climate favors cyanobacteria blooms in eutrophic waters, which may start in spring and last until December (Cook et al., 2004) or in hypertrophic lakes throughout the year (Moustaka-Gouni et al., 2007). The presence of microcystins (MCs) has been already documented in Greek freshwaters (Gkelis et al., 2005a; Papadimitriou et al., 2010). However, the increasing occurrence of species, such as *Cylindrospermopsis raciborskii* (Moustaka-Gouni et al., 2009), *Aphanizomenon ovalisporum* (Gkelis et al., 2005b), *Aphanizomenon flos-aquae* (Kormas et al., 2011) indicates that other cyanotoxins such as cylindrospermopsins (CYNs) or saxitoxins (SXTs) may also occur (Gkelis and Zaoutsos, 2014). In Mediterranean countries the knowledge on the occurrence and distribution of cyanotoxins in waterbodies becomes necessary because of the facing water shortage (Cook et al., 2004) and thus the possible use of surface freshwater bodies as drinking water sources. In Greece, for example, the Athens (population > 3,000,000) Water Company draws drinking water from some surface water sources, such as the eutrophic Lake Yliki and Marathonas; the Thessaloniki (population ca. 1,000,000) Water Company draws water from the River Aliakmonas which is linked to the outflow of freshwaters (e.g. Polyphytos Reservoir, Lake Kastoria) in which toxic cyanobacterial blooms (e.g. *Microcystis aeruginosa*) are known to occur (Cook et al., 2005).

In this work our goal was to assess cyanobacteria blooms in the Mediterranean, shallow, highly eutrophic Lake Pamvotis (NW Greece) in relation to critical environmental parameters using a multi-approach methodology. Physico-chemical parameters combined with microscopic (identification of cyanobacteria), immunological (ELISA), and molecular techniques (PCR) were used to monitor cyanobacteria blooms and the associated cyanotoxin production on a monthly basis for 14 months.

2. Materials and methods

2.1. Study area

The study was conducted in Lake Pamvotis, NW Greece (39°40′ N, 20°53′ E). It is a Mediterranean, shallow (mean depth 4.3 m, maximum depth 6.5 m) urban lake and occupies an area of 22.8 km^2. Lake Pamvotis is 470.25 m above sea level. The lake has a great recreational value (e.g. venue of national and international rowing competitions) and it also supports local agriculture, tourism and fisheries. During the last three decades, the lake was exposed to multiple activities such as irrigation, discharge of domestic sewages and sediment deposit. In the year 1995, sewage diversion to a treatment plant began resulting in a significant decrease of the external nutrient load (Kagalou et al., 2008). Urban pollution comes from the city of Ioannina with a population about 115,000 (Greek Statistics Authority) situated in the western shoreline (Fig. 1). These pressures affected its trophic status, and the lake is now classified as eutrophic to hypertrophic (Kagalou et al., 2003, 2008).

2.2. Water samples collection, preparation and chemical analysis

Water samples were collected, by filling 1 L polyethylene bottles 10–20 cm below the water surface, from two inshore sampling stations (station 1 and station 2 with depths ranging from 0.5 to 0.8 m) and two offshore ones (station 3 and station 4 with depths of 2 m and 6 m, respectively) (Fig. 1). Sampling was taking place monthly during a 14 month survey (January 2008–February 2009).

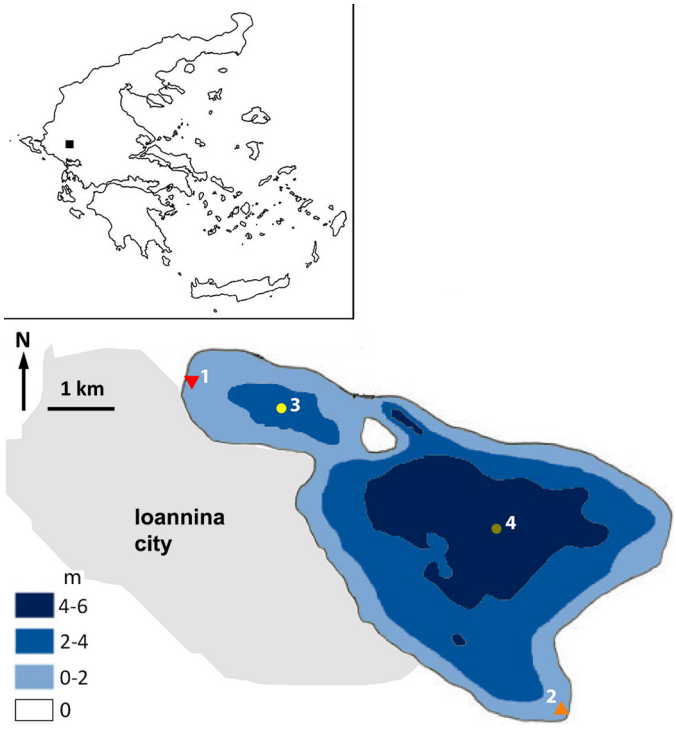

Fig. 1. Map of Lake Pamvotis showing the inshore (1, 2) and offshore (3, 4) sampling stations, indicated with triangles and circles, respectively. Inset, map of Greece (solid square indicates the location of Lake Pamvotis).

Temperature, pH and dissolved oxygen were measured in situ by electrode probes (YSI, USA). Concentrations of nitrate (NO_3-N), nitrite (NO_2-N), ammonia (NH_4-N) and soluble reactive phosphorus (SRP) in lake water were analyzed according to standard methods (APHA, 2005).

Plankton samples were taken using a plankton net (55 μm mesh size). Phytoplankton biomass was estimated as chlorophyll-*a* (chl*a*, μg L^{-1}). Sub-samples (500 mL in clear water state and 200–300 mL for turbid water state) were filtered through Whatman GF/C filters and 1 mL of magnesium carbonate added to the filter. Chl*a* was extracted from the filter with 95% (v/v) acetone solution and quantified spectrophotometrically as outlined in APHA (2005).

For cyanotoxin analysis 100 mL sub-samples were filtered onto Whatman GF/C fiberglass filters. Filter papers and filtrates were stored at −20 °C for subsequent cyanotoxin analysis.

2.3. Identification of cyanobacteria

Lugol and formaldehyde preserved samples were examined using an inverted microscope (Olympus IX71) with phase-contrast. Species were identified using Komárek and Anagnostidis (1999, 2005) and the classification system described therein. Phytoplankton abundance was determined with the Utermöhl method (Utermöhl, 1958) and was used to assess the percentage of cyanobacteria to the total phytoplankton abundance.

2.4. DNA extraction and molecular analyses

In order to identify potentially toxic cyanobacteria, different primer pairs, previously described in the literature, were used to detect different gene targets known to be involved either in the biosynthesis of MC, CYN or STX. DNA was extracted using the