Potential effects of mechanically removing macrophytes on the phytoplankton community of a subtropical reservoir

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ABSTRACT

Intensive growth of aquatic macrophytes interferes with water quality and ecosystem dynamics worldwide. Although mechanically removing macrophytes is the most commonly used method for their eradication, it can also cause undesirable disturbances in aquatic reservoir communities. We performed laboratory incubations of phytoplankton sampled before and after macrophytes were mechanically removed from the Piraquara II reservoir, South Brazil. We analyzed changes in growth and composition of the main phytoplankton groups with respect to nutrient shifting. Prior to removing the macrophytes, the phytoplankton community was dominated by low cell abundances of diatoms and flagellates. In contrast, growth rates of cyanobacteria (mainly *Cylindrospermopsis raciborskii*, *Pseudanabaena* sp., and *Geitlerinema* sp.) and of colonial chlorophytes were favored after macrophyte removal, while the abundances of diatoms and flagellates decreased. Our results suggest that removing macrophytes causes dramatic changes in phytoplankton composition and biomass and selects for toxigenic species of cyanobacteria. These changes were probably associated with the disturbance caused by removing the macrophytes, which immediately created new environmental conditions prone to species competition. These findings indicate that the use of mechanical techniques to manage macrophytes should be carefully considered, along with monitoring of harmful species and changes of limnological parameters.

Keywords: chlorophytes, cyanobacteria, diatoms, macrophytes, mechanical removal, phytoplankton, reservoir management

Introduction

Intensive growth of aquatic macrophytes can significantly interfere with multiple uses of reservoirs, e.g., electric power generation and water supply (Thomaz *et al*. 1999). The most common effect of such excessive proliferation is the accumulation of organic matter, which increases dispersion of pathogenic agents (e.g., malaria) and vectors and impacts local navigation, fishing, and recreational uses (Thomaz *et al*. 2008). A number of methods aimed at controlling and

removing undesirable macrophytes have been employed involving physical, biological, or chemical control (Dodds & Whiles 2010). Among them, physical methods are widely used, usually by motorized machinery to physically remove or cut the vegetation (Carpenter & Adams 1978; Hussner *et al*. 2017). The main advantages of these methods are the increased water quality for consumption purposes and the elimination of toxic substances (Samecka-Cymerman & Kempers 1996) and excessive nutrients accumulated by plants (Scheffer 2004; Kansiime *et al*. 2007). These

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substances compromise water use while promoting the increase in harmful organisms (Carpenter & Adams 1978).

Several studies have reported significant effects of mitigating and lowering nutrient inputs in reservoirs by regularly removing macrophytes, but this is insufficient to eradicate the problem (Carpenter & Adams 1978; Scheer *et al.* 2016). In contrast, macrophytes should be carefully disposed of to avoid contaminating adjacent water bodies with plant propagules or nutrients (Quilliam *et al.* 2015), as well as to minimize the impact on the indigenous fauna associated with the macrophytes (Booms 1999; Greer *et al.* 2012; James 2013). Depending upon the method used, the area of removal activity, and its intensity, the associated environmental changes can extend over the entire reservoir.

Studies that investigated the impacts of removing macrophytes have recorded limnological changes, such as shifts in the composition and abundance of zooplankton (Maceina *et al.* 1992; Choi *et al.* 2014), oxygen depletion, increases in carbonic acid gas and turbidity, oscillations in pH, and export of nitrogen and phosphorus generated in the lake bottom (Granéli & Solander 1988; Young *et al.* 2004; Crossetti & Bicudo 2008; Waterman *et al.* 2011). The main impacts of using excavators to mechanically manage macrophytes are oscillations in light intensity, sediment disturbances, and increased nutrients in the water column (Kuiper *et al.* 2017). Resuspending sediment promotes the bioavailability of phosphorus and nitrogen from the bottom; thus, providing an additional nutrient source for phytoplankton (Shaw & Prepas 1990). This process has significant implications in subtropical water bodies, such as the Piraquara II reservoir, as phosphorus is the main factor promoting phytoplankton growth at these latitudes (Aubriot *et al.* 2000; Downing *et al.* 2001; Perkins & Underwood 2001; Horn 2003; Figueredo *et al.* 2016). Phosphorus concentrations in sediments can reach up to 100 times that in the water column (Sondergaard *et al.* 2003), and harmful cyanobacteria usually constitute the most favored group (Bakker & Hilt 2015), posing a serious threat to aquatic organisms and human beings, either by producing secondary metabolites, such as toxins and allelopathic compounds (Leflaive & Ten-Hage 2007; Pearl & Huisman 2009) or by drastically changing the physical and chemical parameters, including the sediment-water interface (Zhu *et al.* 2013). Algal accumulations also interfere with water treatment, making the elimination of organic matter and toxins more time consuming and expensive (Hitzfeld *et al.* 2000; Villacorte *et al.* 2015). The most common genera of cyanobacteria that usually dominate the freshwater blooms around the world are *Microcystis, Cylindrospermopsis,* and *Dolichospermum* (Fernandes *et al.* 2005; Fonseca & Bicudo 2010; Soares *et al.* 2013; Antunes *et al.* 2015; Li *et al.* 2015; Harke *et al.* 2016).

Despite the negative consequences described above, few studies (Faria *et al.* 2013; present study) have focused on the effects of mechanically removing macrophytes on the phytoplankton community. In this study, we investigated the potential effects of mechanically removing macrophytes on the composition and cell abundance of phytoplankton in the Piraquara II reservoir, South Brazil. We performed phytoplankton incubations using water sampled before and after the macrophytes were removed and analyzed the compositional changes in the main phytoplankton groups. We hypothesized that cyanobacterial species would take advantage of the increased nutrients that appeared after a hydraulic excavator operation to remove macrophytes and would eventually outcompete the phytoplankton.

Materials and methods

Study Area

Lake Ingleses is a small 40 hectare arm of Piraquara II, a subtropical 560 hectare reservoir located in South Brazil (Paraná state). The reservoir has an average depth of 3.8 m and a maximum depth of 15 m (Consórcio Paranasan 2000). The macrophytes were removed from this short arm (Fig. 1). The macrophyte beds occupied a 22 hectare area in Lake Ingleses. The main aquatic species were rooted emergent *Cyperus luzulae* (L.) Retz and *Paspalum exaltatum* J. Presl, and free floating *Eichhornia crassipes* (Mart.) Solms, *Salvinia auriculata* Aubl., and *Salvinia minima* Baker, which move around under the influence of local wind.

The study area is located at 25°30'54''S–25°30'50''S 49°04'28''W–49°04'22''W, with an altitude of 892 m. The climate is Temperate Oceanic (Cfb) according to the Koppen system, with average temperatures of 12–20 °C and annual precipitation of 1450–1500 mm (Caviglione *et al.* 2000).

Mechanical removal of macrophytes in Lake Ingleses

The macrophytes were removed during August 5–9, 2013 using a Caterpillar 320C 17L/h hydraulic excavator equipped with a long reach boom (15 m); a drain bucket was operated to remove the aquatic macrophytes (see Scheer *et al.* 2016). The transport and disposal of the plant material from the reservoir to other areas nearby were made by two trucks (Mercedes Benz 2423, 6 × 4 traction) with 14 m3 dumping beds.

Total phosphorus and nitrogen concentrations in the water

Samples for analysis of total phosphorus (TP) and total nitrogen (TN) were collected before, during, and after the excavator operation, which lasted 5 days. One sample was taken on August 2, 2013, just before the operation activities started, and these samples are called "BEFORE" hereafter. Samples were collected 30 min after the operation stopped (total = 12) in the evening; these samples are labeled "AFTER" hereafter. Additional

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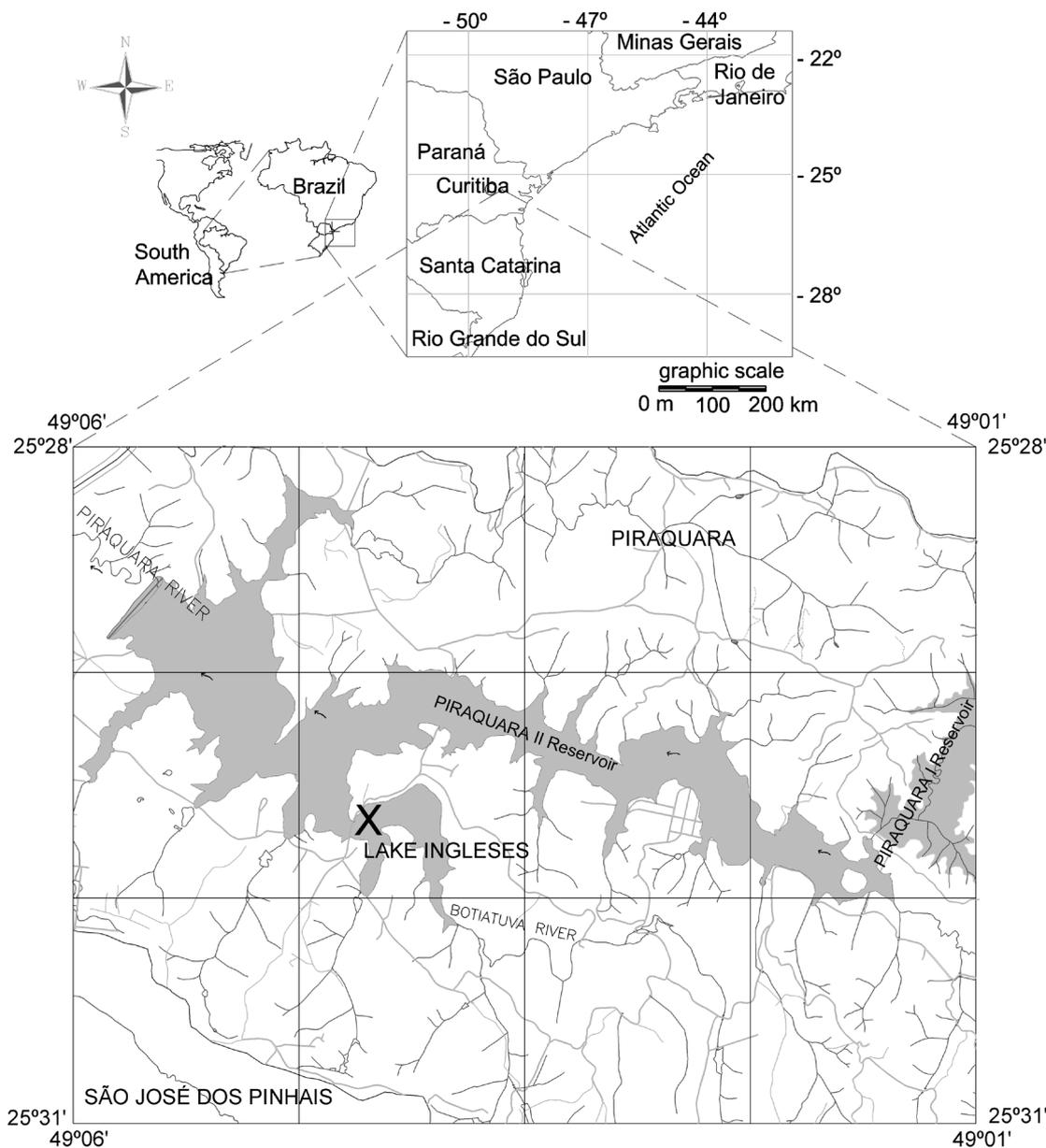
Figure 1. Piraquara II reservoir, showing the location of Lake Ingleses, Piraquara, Paraná. Adapted from: Consórcio Paranasan 2000.

AFTER samples were collected on August 13, 14, and 16. TP concentrations were determined according to the APHA 4500-P-E analytical method prescribed in APHA (2012). This method was carried out in acid medium with orthophosphate reacting with ammonium molybdate and potassium antimonyl tartarate to form phosphomolybdic acid, which was reduced by ascorbic acid in molybdenum blue. The resulting compound absorbance was measured in a spectrophotometer at 880 nm. TN concentrations were determined by the oxidative digestion method with sulfate peroxide based on DIN EN ISO 11905-1. This method allows for free ammonia, ammonium, nitrate, nitrite, and organic nitrogen compounds to be converted to nitrate under oxidative conditions (DIN 1998).

Phytoplankton sampling, experimental procedures, and counting

Two water samples (1 L each) were taken: one before macrophyte removal (August 5, 2013) and another one five days after removal (August 14, 2013). Aliquots of 300 mL of the BEFORE and AFTER samples were used for the incubation experiment.

The incubations were performed in 500 mL Erlenmeyer flasks, at 25 °C, with a light intensity of 150 µmol the photon·m^{-2}·s^{-1}, and a 12 h:12 h photoperiod. On days 1 and 20 of the incubation, subsamples of 6 mL were taken from each incubation and placed in Eppendorf tubes. The supernatant (5 mL) was removed, and the remaining

1 mL was preserved with Lugol's solution to be used for the phytoplankton analysis. The classes of phytoplankton species were identified and the densities (number of cells in 6 mL converted to cell mL^{-1}) of cyanobacteria, chlorophytes, and diatoms were estimated using a Sedgewick–Rafter chamber on an Olympus IX-71 inverted microscope at 400× magnification (Tokyo, Japan). At least 400 cells were counted to achieve a 10 % statistical error (APHA 1998).

Data analysis

Phytoplankton growth rates (μ day^{-1}) were estimated for each group during the exponential growth phase according to the Andersen (2005) equation: $(Ln\ N_t - Ln\ N_0)/\Delta t$; where $Ln\ N_t$ is the natural logarithm of the final instant of time (in days), $Ln\ N_0$ is the natural logarithm of the initial instant of time, and Δt is the time interval (Δt = final time – initial time). The *t*-test was used to compare the first-day densities to the 20-day densities of each group (cyanobacteria, chlorophytes, and diatoms) at each treatment (BEFORE and AFTER), with a significance level of 95 % (P = 0.05). Data were log transformed (log X+1) to support normality and homogeneity of variance. All analyses were performed using R software ver. 3.0.2 (R Development Core Team 2013).

Results

Concentrations of phosphorus and nitrogen in the macrophyte removal area

Levels of phosphorus and nitrogen were below the detection limits (< 0.001 and < 0.5 mg L^{-1}, respectively) in all of the BEFORE samples. Phosphorus levels increased 100 times (0.49 ± 0.08 mg L^{-1}; n = 12) during macrophyte removal, whereas nitrogen was about 90 times as much (2.3 ± 0.91 mg L^{-1}; n = 3). Nutrient levels returned to undetectable values 30 min after each day of the operation and one week (August 13, 16, and 19) after the end of the field excavator operation.

Phytoplankton experiment: cell abundances before macrophyte removal

Mean phytoplankton densities (estimated from three replicates) of the samples taken BEFORE almost doubled after 20 days of incubation, varying from 2.9 × 104 cell mL^{-1} to 5.9 × 104 cell mL^{-1} (Tab. 1). These samples were dominated by cyanobacteria on day 1. However, chlorophytes were the main group at the end of the incubation period, with a growth rate (0.08 day^{-1}) that was significantly higher ($t4$ = 3.274; P = 0.031) than that of cyanobacteria. The densities of diatoms and cyanobacteria decreased, and lower growth rates of 0.02 and 0.002 day^{-1}, respectively were observed (Tab. 1). The most common chlorophyte species were *Pediastrum simplex*, *Desmidium* sp., *Dictyosphaerium* sp., *Scenedesmus ecornis*, *Closteriopsis* sp., *Staurodesmus* sp., and *Staurastrum* sp.. Among cyanobacteria, *Geitlerinema* sp. and *Pseudanabaena* sp. were the most dominant species throughout the incubation.

Phytoplankton experiment: cell abundances after macrophyte removal

The incubation of water collected after mechanical removal of macrophytes resulted in increases in phytoplankton abundance compared to the BEFORE treatment (Tab. 1). Mean phytoplankton density varied from 0.4 × 10^4 cells mL^{-1} on day 1 to 20.9 × 10^4 cells mL^{-1} on day 20; an increase of 50 times. Cyanobacteria was the dominant group (t_4 = 3.6864; P = 0.021). The density of cyanobacteria on day 1 was low (0.8 cells mL^{-1}) in this treatment. However, there was an increase of 240 times by day 20, reaching 20.6 x 10^4 cells mL^{-1} (Tab. 1). The estimated growth rate (0.29 day^{-1}, Tab. 1) was relatively higher compared to the other phytoplankton groups. *Pseudanabaena* sp. was the most common species at the beginning of the incubation, while *Cylindrospermopsis raciborskii* and *Geitlerinema* sp. were the most common at the end of incubation. The abundance of chlorophytes decreased until day 20 of the experiment in this treatment.

Table 1. Cell abundances (cells mL^{-1}) of main phytoplankton groups in the samples taken from the Piraquara II Reservoir (Lake Ingleses), BEFORE and AFTER the mechanical removal of macrophytes in August 2016. The values refer to the initial day and the end (20 days after) of each incubation experiment. Data are presented as average (minimum – maximum). Statistically significant values (P < 0.05) between the start and the end of incubation are in bold. DF = 4; μ = growth rate (day^{-1}); t = t-test value.

	BEFORE					AFTER				
	Day 1	Day 20	μ	t	P	Day 1	Day 20	μ	t	P
Chlorophytes	5583 (4800 - 6600)	27617 (13200 - 52450)	0.08	3.2739	**0.0307**	1824 (1310 - 2483)	1228 (50 - 2534)	-0.04	-1.6116	0.1824
Cyanobacteria	21233 (18950 - 22650)	29333 (14550 - 39500)	0.02	1.0587	0.3494	869 (350 - 1430)	206117 (14650 - 575050)	0.29	3.6864	**0.0211**
Diatoms	2433 (2200 - 2900)	2533 (750 - 4250)	0.002	0.0964	0.9278	1550 (1080 - 1980)	2692 (925 - 5950)	0.03	0.3638	0.7344
Total	29259 (26500 - 32150)	59483 (51400 - 67750)	0.04	-	-	4244 (4010 - 4330)	209700 (15850 - 582800)	0.21	-	-

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Discussion

We observed a dramatic change in the composition and biomass of the phytoplankton from Lake Ingleses incubated in water sampled after mechanically removing the macrophytes. The species composition changed from low cell densities of slow-growing colonial non-flagellate chlorophytes to high densities of filamentous cyanobacteria (*Pseudanabaena*, *Geitlerinema*, and *Cylindrospermopsis*). *Cylindrospermopsis raciborskii* is a widespread toxigenic species that occurs in Brazilian reservoirs, with the potential to cause a serious threat to human health. The high levels of saxitoxins from this taxon lead to interruptions in water supply (Fernandes *et al.* 2005; Wojciechowski *et al.* 2016). Here, we demonstrated under appropriate conditions of light and temperature that removing the macrophytes can cause intensive growth of harmful algae, which could have downstream consequences.

Cell densities of chlorophytes increased significantly from 5,583 to 27,617 cells mL^{-1} in the BEFORE treatment on day 20 of the incubation. At this time, the phytoplankton community was dominated by *Pediastrum simplex* and *Scenedesmus ecornis*. Chlorophytes are more high-light adapted than other algal groups, which is one of the reasons why they have higher growth rates when exposed to this condition (Schwaderer *et al.* 2011). The high-light condition was likely due to the absence of macrophytes during the incubation.

After 20 days of incubating the AFTER treatment, the increase in cell density was higher and the phytoplankton community changed to cyanobacteria-dominated cells (from 869 to 206,117 cells mL^{-1}). Toxigenic cyanobacteria, such as *Geitlerinema* sp. and *Pseudanabaena* sp., dominated the phytoplankton, most likely favored by the new habitat conditions, with a new environment for competition, and increases in nutrient concentrations recorded soon after the macrophyte removal operations commenced. These macronutrients reached 100 times as much at the study area immediately after the macrophyte removal procedure.

Remarkable changes in phytoplankton composition and abundance have been recorded in other subtropical and tropical lakes and reservoirs after removing macrophytes, and which also lead to massive blooms of cyanobacteria (e.g., Bicudo *et al.* 2007; Crossetti & Bicudo 2008). Studies that have investigated the effect of resuspended sediment on phytoplankton growth suggest that nutrient inputs from the bottom are the major vector regulating development of these blooms, which are usually dominated by cyanobacteria (Mur *et al.* 1999; Faithfull & Burns 2006; Niemistö *et al.* 2008; Zhu *et al.* 2015). Although no sediment analysis was performed in the present study, the high levels of P and N detected after removing the macrophytes might be related to nutrients that accumulated on the bottom of the lake. A reservoir becomes temporarily turbid and nutrient rich after removing the macrophytes (Mur *et al.*

1999). Many species of cyanobacteria are unable to take-up phosphorus and nitrogen faster than other species, but several filamentous species have evolved adaptations to store phosphorus by producing the extracellular enzyme alkaline phosphatase (Isvánovics *et al.* 2000; Cottingham *et al.* 2015). This mechanism, allied to efficient positioning in the water column by means of gas vesicles (Sharma *et al.* 2013), the ability to produce resting cells (akinetes) and photochromatic adaptation to optimize light absorption (Reynolds 1998) confers competitive advantages over other phytoplankton groups.

Among the cyanobacterial species reported here, *C. raciborskii* stands out due to its high invasiveness, efficient dispersal capacity, and production of neurotoxins, such as saxitoxins and cylindrospermopsin (Padisák 1997; Antunes *et al.* 2015). In the last 20 years, blooms of this cyanobacteria have been reported progressively worldwide, raising serious concerns regarding water treatment for human consumption (Piccini *et al.* 2011). Since 2002, *C. raciborskii* has been the main factor responsible for recurrent blooms in reservoirs used to publically supply the Paraná state (Fernandes *et al.* 2005; 2011; IAP 2009). Two other cyanobacteria recorded in high abundance during the laboratory incubations were *Pseudanabaena* sp. and *Geitlerinema* sp.. Several *Pseudanabaena* species produce microcystins, with hepatotoxic action (Rangel *et al.* 2014), and are frequently associated with blooms (Kim *et al.* 2006; Willame *et al.* 2006). The genus *Geitlerinema*, though not yet recorded in blooms, has recently been reported to produce harmful toxins (Dogo *et al.* 2011).

Our laboratory results indicate potential significant changes in the composition and biomass of the main phytoplankton groups in Lake Ingleses (Piraquara II reservoir) as a consequence of removing the macrophytes. The phytoplankton species responded to the disturbance under laboratory conditions and shifted from colonial non-flagellate chlorophytes to fast growing filamentous cyanobacteria (*Pseudanabaena, Geitlerinema*, and *Cylindrospermopsis*), with the concomitant increase in phytoplankton biomass.

Therefore, we recommend that any attempts to remove macrophytes mechanically should be planned and executed carefully. Moreover, the operations must be accompanied by a monitoring program of harmful phytoplankton and limnological parameters. These precautions will minimize the harmful effects resulting from the mechanical removal of macrophytes and prevent an increase in costs associated with water treatment for human consumption.

Acknowledgements

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