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Full Length Research Paper

Phytoplankton yield changes after enrichment in microcosm experiments: Applications for predicting progressive eutrophication in a mesotrophic lake, South Africa

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Established microcosms containing surface water from the mesotrophic Lake Mokolo were subjected to enrichment of different concentrations with phosphorus. The microcosms were sampled through a 20 day succession period to determine the net effects of increased concentrations of phosphorus in the water column on the phytoplankton community structure during the winter and summer seasons. A significant increase in chlorophyll *a* (chl-*a*) was observed when treatments of 30, 40 and 60 µg L^{-1} P were compared to the controls. On day zero in both the winter and summer microcosm experiments, all four phosphorus treatments had similar species diversity of phytoplankton of the specific seasoned tested (winter: Margalef index = 223; summer: Margalef index = 347). However, 13 days after the addition of 40 and 60 µg L^{-1} P, the phytoplankton community exhibited a strikingly different species richness (winter: Margalef index = 123; summer: Margalef index = 114). In the winter microcosm experiments, the green alga *Scenedesmus armatus* dominated the phytoplankton composition at enrichment levels of 40 and 60 µg L^{-1} P up to day 20. The biovolume of the dinophyceae *Ceratium hirundinella* declined rapidly after the addition of 40 µg L^{-1} P in the different summer microcosms. In the summer microcosms, *Spondylosium secedens* and *Microcystis aeruginosa* dominated the 40 and 60 µg L^{-1} P microcosm enrichment experiments.

Key words: *Scenedesmus armatus*, southern Hemisphere lake, enrichment, cyanobacteria, threshold phosphorus concentrations, chlorophyll *a* (chl-*a*) concentration.

INTRODUCTION

The excessive input of nutrients influences the structure and dynamics of the phytoplankton community of rivers and lakes by increasing primary production and altering species composition (De Jonge et al., 2002). Nitrogen (N) and phosphorus (P) exports from point and nonpoint sources can have profound effects upon the quality of receiving streams or lakes (Correll, 1998). The degradation of water resources by eutrophication can result in losses of their component species, as well as losses of services that these systems provide (US EPA, 1996). Eutrophication is the most widespread water quality problem in the USA and many other countries (Carpenter et al., 1998). In South Africa, 58% of the reservoirs are under threat of cyanobacterial bloom formation due to eutrophication, while 11% of the major reservoirs have severe problems with cyanobacterial

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blooms due to enrichment because of anthropogenic pollution (Carpenter et al., 1998; Oberholster and Ashton, 2008). Hence, assessing the phytoplankton assemblage from a reservoir by increasing the different concentrations of phosphorus in outdoor microcosms can give possible answers to trophic changes that may occur due to anthropogenic pollution.

In addition, microcosms provide biotic simplicity, isolation and replication, all of which are necessary in determining the net effects that different stressors have on a community (Fliedner et al., 1997). Furthermore, it is difficult to document the pathways leading to changes in the structure of natural communities since environmental variables cannot be controlled. Previous studies by Brettum (1989) and Willen (2000) presented phytoplankton species as indicators at different nutrient levels for oligotrophic waters, as well as for waters with increased anthropogenic pollution. An earlier study by Lepistø et al. (2004) showed that anthropogenic impacts manifested as eutrophication, was reflected as changes in phytoplankton assemblages and in type-specific taxa composition. Associated with key variables (for example, pH and water temperature) are threshold nutrient concentrations in an aquatic system and these nutrient concentrations determine the trophic states of such a system. If an aquatic system consistently moves beyond a critical threshold (for example, above a certain maximum concentration of nutrient inputs), it may start to behave in a different way, often with unforeseen or undesirable consequences (for example, toxic cyanobacterial blooms resulting in massive fish mortalities due to a lack of dissolved oxygen). This aquatic ecosystem will now exist in a new state, also referred to as a 'regime' (Scheffer and Carpenter, 2003).

Within a new regime, the controls or feedbacks and identity (basic structure and function) of the aquatic system are different from the original (Fellows et al., 2006; Marti et al., 2004; Scheffer and Carpenter, 2003). Once such a threshold has been crossed and a regime shift has taken place, it is usually difficult to cross back or return to the previous regime, and in most cases this can only be accomplished through remediation. Therefore, an aquatic ecosystem's resilience can be determined by its relative distance from its respective pollutant threshold or guideline values (for example, total phosphorus) and their impact on the biodiversity of the aquatic system. The closer the system is located to its threshold, the smaller the required change to alter the system (for example, a sudden increase of nutrients) (Fellows et al., 2006; Marti et al., 2004; Scheffer and Carpenter, 2003). The greater the nutrient load, the more likely it is that a switch to bloom forming cyanobacteria dominance will take place. Although long-term experiments which include the whole lake ecosystem will provide perhaps the most reliable predictors, such experiments can be problematic for replication of treatment (Moss et al., 2003). A compromise, therefore, is the use of replicated microcosms. However, microcosms can not mimic the precise flushing time or turbidity expressed as the light extinction coefficient under natural lake conditions (Moss et al., 2003).

There are several studies that have empirically described changes of phytoplankton community structures and cyanobacterial biomass after enrichment in northern hemisphere temperate lakes (Correll, 1998). However, little is known about similar quantitative relationships of phytoplankton community structures in southern hemisphere temperate lakes using microcosm studies. Publications from the 1970 and 1980's (Taylor et al., 1984; Thornton and Walmsley, 1982; Walmsley and Butty, 1980; Toerien, 1977) assume implicitly or directly that phosphorus is the yield-controlling nutrient in South African man-made lakes. Therefore phosphorus was selected in the microcosm study as the major limiting nutrient.

Cyanobacterial blooms as a symptom of eutrophication have become an increasing problem in South African freshwater bodies over the last three decades (Oberholster et al., 2005a). A survey conducted by Botha and Oberholster between 2004 to 2007 in South Africa, using polymerase chain reaction (PCR) technology to distinguish toxic cyanobacteria *Microcystis* strains bearing the *mcy* genes, which correlate with their ability to synthesize the cyanobacterial biotoxin (microcystins), revealed that 99% of South Africa's major impoundments contained toxigenic strains of *Microcystis* (Botha and Oberholster, 2007; Oberholster and Botha, 2007). The massive proliferation of these organisms is largely caused by over nutrient enrichment (eutrophication) of water bodies, which is due to progressive increase in anthropogenic pollution (Oberholster et al., 2005b, 2008).

Cyanobacteria produce some of the most potent toxins known and antidotes are not available. These biotoxins fall into three categories namely neurotoxins, hepatotoxins and lipopolysaccharides. The biotoxins in the first two groups can produce severe reactions in animals and humans, while the third group appears to be less virulent (Oberholster et al., 2005a). However the latter have been less intensively studied. Any release of these biotoxins into surrounding water can present a significant hazard to human health and the ecosystem (Oberholster et al., 2005a). The existence of gastrointestinal disorders linked to the ingestion of cyanobacterial biotoxins, as well as the chronic risks posed by hepatotoxins make these toxins a serious threat to human health when they are present in drinking water supplies (Falconer, 2005). Thus, the objectives of the present study were; (1) to determine the different phosphorus threshold values that may cause phytoplankton composition changes in the mesotrophic Lake Moloko through the use of outdoor microcosm studies and (2) to determine the critical phosphorus threshold that will cause the aquatic system to become a

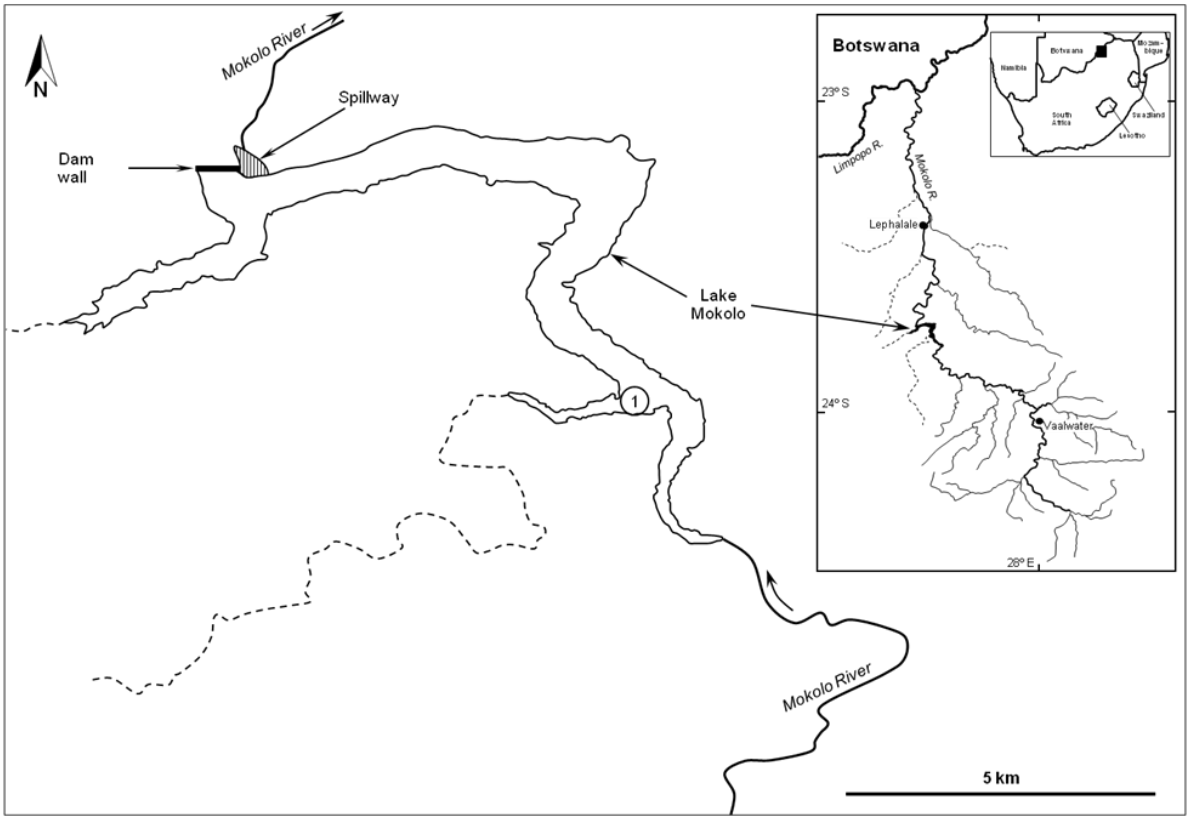

Figure 1. Map of Lake Mokolo showing the location of the sampling site, and the position of inflowing Mokolo River. Inset shows the location of the map area in South Africa.

cyanobacterial dominant system during the summer or winter months.

MATERIALS AND METHODS

Background on study area

Lake Mokolo is situated in the north-western part of the Waterberg Biosphere Reserve, approximately 50 km north-west of the town Vaalwater in the Limpopo Province of South Africa. The area surrounding the lake is characterized by densely wooded mountains which mainly comprise of sandstone and surrounding cliffs. Lake Mokolo was constructed in the late 1970s primarily to supply water to the Matimba coal-fired power station near the town of Lephalale, as well as for irrigation downstream of the dam wall (DWAF, 2004). An area of 4,500 ha was proclaimed as a provincial nature reserve in 1993. The reservoir is fed by the Mokolo River and has an area of 914 ha and a volume of 145.0 Mm^3 at full supply capacity (Figure 1). Mean annual rainfall is relatively low (560 mm $year^{-1}$) and most rainfall is received as thunderstorms during the austral summer months (Oberholster et al., 2010). High annual average rates of evaporation (1,900 mm $year^{-1}$) exceed rainfall and the area is considered to be arid (Schulze, 1997).

In the light of abundance of mineral resources in the Limpopo Valley, the mining sector and coal mining in particular, promises to become a strong economic driver in the area. South Africa produces an average of 224 million tonnes of marketable coal annually of which 25% is exported and 53% is used for electricity generation. In the face of severe power shortages, during 2007, South Africa's state-owned utility, Eskom awarded contracts for the construction of the Medupi coal fired plant in the Lephalala area within the Limpopo Province in South Africa (Fouilloux and Otto, 2009). Although the Waterberg coalfield was discovered back in the 1920s, it was always too far from the economic heartland of the country to justify further development. However, faced with the looming electricity crisis and with enough coal for the next 150 to 200 years to supply eight power stations, the region is set for dramatic change (Creamer, 2009). With these future developments, a projected continuing in-migration of people to this area will lead to increasing urbanization in coming years. Associated with urbanization, an increase in point and non point source pollution will play an important role in progressive eutrophication of the water of Lake Mokolo which is the main reservoir in this river system. Although point source discharges (sewage treatment works) are usually controllable, diffuse (for example agriculture) and atmospheric sources (coal-fired plants) in the study area will be more difficult to control in the future (Whithers and Jarvie, 2008).

Water quality parameters of Lake Mokolo

Two sampling trips were undertaken, namely during winter (July 2009) and during summer (November 2009). Surface water (0 > 20 cm depth) from the lake for the outdoor microcosm experiments were collected in 25 L plastic containers at a

Table 1. The average combined bottom sediment characteristics and physical-chemical parameters (mean ± standard deviation) of water collected for the microcosm experiment in summer and winter from Lake Moloko (n = 2).

Bottom sediment sample	Sediment (0 to 5 cm depth)
Particle diameter (µM	
Sand	220 to 231
Silt	32 to 45
Clay	1 to 3
pH	8.7
Organic matter (% dry weight)	2.65 ± 0.40
Total nitrogen (mg kg^{-1})	187 ± 11
Total phosphorus (mg kg^{-1})	1.67 ± 0.32
Water chemistry samples	
Potassium (K, mg L^{-1})	1.0 ± 0.5
Sodium (Na, mg L^{-1})	4.0 ± 1.0
Calcium (Ca, mg L^{-1})	2.2 ± 0.72
Magnesium (Mg, mg L^{-1})	1.41 ± 1.0
Ammonia (N, mg L^{-1})	0.072 ± 0.030
Sulphate (SO_4, mg L^{-1})	5 ± 1.3
Chloride (Cl, mg L^{-1})	5.1 ± 0.9
Alkalinity ($CaCO_3$, mg L^{-1})	15.5 ± 3
Total phosphate (P, µg L^{-1})	26.6 ± 2
Total nitrogen (N, µg L^{-1}	354 ± 21
Fluoride (F, mg L^{-1})	0.07 ± 0.02
Silica (Si, mg L^{-1})	2.63 ± 2.1
Conductivity mSm^{-1} (25°C)	5.5 ± 1.1
pH (25°C)	7.9 ± 3.0
Total dissolved solids (Calc) (mg L^{-1})	35 ± 9.0
Hardness ($CaCO_3$, mg L^{-1})	11 ± 2.0
Aluminium (Al, mg L^{-1})	0.010 ± 0.002
Cadmium (Cd, mg L^{-1})	0.011 ± 0. 001
Iron (Fe, mg L^{-1})	0.020 ± 0.010
Manganese (Mn, mg L^{-1})	0.009 ± 0.001
Zinc (Zn, mg L^{-1})	0.010 ± 0.001

permanent sampling station (23° 96' 93.6" S, 27° 72' 66.7" E) in the littoral zone of Lake Mokolo. These plastic containers were transported to the premises of the Council of Scientific and Industrial Research where the outdoor microcosms were set up. During the winter and summer months, surface water and sediment were collected from Lake Mokolo for these different sets of outdoor microcosms.

The categorization of the trophic states of Lake Mokolo was based on the classification of Carlson (1977). Freshwater lakes all over the world exists from eutrophic (rich in nutrients), to oligotrophic (low nutrient levels), while intermediate states also exist for example, mesotrophic. In general, the amount of chlorophyll *a* (chl-*a*), cell density, transparency as an indicator of phytoplankton biomass and/or type of algal species present can be useful indicators of the trophic status of lakes, so long no toxic compounds that inhibit algal growth are present. However, other factors that also needs to be taken into account for microcosms are temperature and pH (Premazzi and Chiaudani, 1992).

Phosphorus enrichment and time scale

During the set-up of the outdoor microcosm experiments, care was taken to closely match the ambient conditions measured at the sampling location during the winter and summer season in Lake Mokolo to determine the physical and chemical characteristics of the bottom sediment. Bottom sediment was collected at the same place as water samples (0.5 m depth) in the littoral zone of Lake Mokolo with a grab sampler for use as bottom sediment in the different microcosms. Sediment samples (250 g) were dried to constant weight (105°C), cooled and sieved to obtain particle size, while organic matter content was determined gravimetrically from50 g test portions of un-sieved material after ashing at 500°C for 8 h. The physical-chemical data of the bottom sediment and surface water column of Lake Molopo used in the microcosm study are given in Table 1.

The outdoor microcosms were set up on the premises of the Council of Scientific and Industrial Research in triplicate glass

aquariums containing 10 L of lake water and 1 kg/wet weight bottom sediment from the littoral zone of Lake Mokolo. Separate sets of outdoor microcosms were set up for the winter and summer months to mimic the environmental variables, for example, temperature, day length and rainfall of each specific season. After the bottom sediment was spread evenly on the bottom of each microcosm, 10 L of lake water (collected in winter and summer) was pored gently through a glass tube into each microcosm. All microcosms were left for one week to stabilize after which a subsample of 1 L water was taken for chemical and phytoplankton composition analyses.

Four P enrichment levels were set by adding a final concentration of 20, 30, 40 and 60 µg L^{-1} P (NaH_2PO_4) to each microcosm in triplicate containing winter or summer phytoplankton depending on season. Microcosms with no added nutrients served as nutrient controls. To ensure that P remained as limiting nutrient in the microcosm, inorganic N (as NH_4NO_3) was added to the microcosms to reach a N:P molar ratio of 30 based on P concentrations measured the day before starting the experiment. Each microcosm was gently mixed by aeration (20 air bubbles per minute) and sampled five times for each P enrichment (day 0, three, six, 13 and 20). We decided on these sampling intervals since most field observations show more than one to three dominant species at any phase of seasonal development as predicted by the competitive exclusion theory of Hardin (1960). The reasons are found in the different responses of phytoplankton to the frequency of disturbances or changes in abiotic resource conditions at different scales (Reynolds, 1984). These different scales are: (1) shorter than one generation time induces physiological responses, (2) frequencies between 20 and 200 h interact with the phytoplankton growth rate, and (3) disturbances at up to 10-day intervals can initiate a successional sequence in phytoplankton development (Reynolds, 1984).

In addition, the total time (days) taken by a specific phytoplankton species to dominate the algal community at the different P concentrations were calculated from the beginning of the experiment until ≥ 50% total algal abundance was reached. The moment of enrichment was designated as day 0 of the experiment and all changes were related to conditions immediately after this time. Water losses due to evaporation and sub-sampling for phytoplankton identification were replaced by deionized water throughout the duration of the experiment. An average of 250 ml water of each 10 L microcosm was replaced by deionized water over the study period of 20 days.

Phytoplankton community analyses

Species composition and community structure before and after enrichment with different concentrations of P were assessed from 50 ml aliquots sampled from each microcosm, then fixed with buffered 5% (v/v) formaldehyde. Phytoplankton cells were identified and counted from transects in a 1 ml Sedgewick-Rafter sedimentation chamber. All identifications were made by using a compound microscope with 1,250 x magnification (Truter, 1987; Van Vuuren et al., 2006). Strip counts were made until at least 300 individuals with protoplasm of each of the dominant phytoplankton species were counted. Algae abundance in the samples was evaluated by counting the presence of each species as cells in a filament or equal number of individual cells. Additional aliquots from each algal sub-sample were removed for diatom identification (Taylor et al., 2007). Diatom frustules were cleaned with concentrated H_2SO_4 and $K_2Cr_2O_7$ to facilitate accurate identification (Patrick and Reimer, 1966). Algal biovolume was calculated by measuring the corresponding dimensions using the geometric formulas given by Willen (1976). Diversity (d_s) was calculated, as described in Margalef (1979), by computing the equation $d_s = (s-1)/\log N$; where s is the number of species and N is the number of individuals. Species richness was determined by using the number of different taxonomic units that could be identified within 300 cells from each sample.

Physicochemical and chl-*a* parameter

Nutrients [total nitrogen (TN) and total phosphorus (TP)] were analyzed in the water column of each outdoor microcosm before enrichment with the different concentrations of P, using the spectrophotometric method according to the procedures published by the American Public Health Association, American Water Works Association, and Water Pollution Control Federation (1980). Water temperature, pH, dissolved oxygen and electrical conductivity were measured with a Hach Sension™ 156 portable multiparameter (Loveland, USA). Water transparency was measured with a 25 cm Secchi disk with black and white quadrants.

Chl-*a* was measured spectrophotometrically at 664 and 750 nm, respectively and calculated according to Porra et al. (1989). In brief, sub-samples for chl-*a* were filtered onto glass fiber filters (1.0 µM average pore size). Chl-*a* was removed from each filter by leaching in cold (4°C), buffered 90% (v/v) acetone for 18 h under subdued lighting, after which chl-*a* were measured. We calculated mean and standard error for each treatment followed by Tukey's multiple comparisons of the means to check for differences between the treatments and the controls ($P \leq 0.05$) using the SYSTAT ® 7.0.1 software package (SYSTAT, 1997).

RESULTS

The application of the Carlson (1977) trophic state index to data collected during the winter and summer water collection trips showed that Lake Mokolo was oligotrophic in terms of chl-*a* measurements, but mesotrophic in terms of P levels. No significant changes in phytoplankton composition and chl-*a* concentration were observed between the controls and the addition of 20 µg L^{-1} P in the different winter and summer microcosm experiments (Tables 3 and 4). Post-enrichment with P in these experiments were associated with several small-celled species, such as *Dinobryon divergens* and *Oocystis rupestris*. These phytoplankton species compositions agree with observations of an assemblage typical for oligo-mesotrophic waters except for the occurrence of *Ceratium hirundinella*. The median of chl-*a* in the winter and summer microcosm experiments after the addition of 20 µg L^{-1} P was 2.6 and 3.1 µg L^{-1}, respectively. The blue-green algae group Cyanophyceae contributed 6% to the post enrichment total biovolume in winter and 8% in summer, while the proportion of diatom (Bacillariophyceae) biovolume were 42% at a concentration of 20 µg L^{-1} P in the winter microcosm (Table 2 and Figure 2).

A positive correlation ($P \leq 0.05$) between an increase in chl-*a* and the addition of 30 µg L^{-1} P was observed in both the winter and summer microcosm experiments. In the

Table 2. Changes in mean seasonal (winter and summer) biovolume of major algal groups (μm^3 ml^{-1} x 10^3) after post-enrichment with 40 µg L^1 P over a period of 20 days.

Post-enrichment with 40 µgL^{-1} P	Winter			Summer		
	Mean	SD	%	Mean	SD	%
Algal group						
Chlorophyceae	7560	1260	51	421	137	10.1
Bacillariophyceae	5430	860	43	628	128	17.1
Chrysophyceae	28	9	0.6	219	36	1.1
Cryptophyceae	1,7	0.81	0.03	39	7	0.7
Cyanophyceae	1.61	0.76	0.02	8670	1410	55.1
Dinophyceae	29	8.8	0.2	563	111	14.5
Euglenophyceae	311	81	2.1	493	87	11.2

The standard deviation (SD) and percentage composition (%) for winter and summer are indicated.

different winter microcosms, the phytoplankton biovolume was dominated by the green algae species *Scenedesmus armatus* (27%) and the diatom *Melosira varians* (33%) after the addition of 30 µg L^{-1} P (Table 4 and Figure 2). The summer microcosms after post-enrichment with 30 µg L^{-1} P was dominated by *Trachelomonas intermedia* (32%) and *Euglena sociabilis* (24%). The median of total phytoplankton biomass as chl-*a* in the winter and summer microcosm experiments after the addition of 30 µg L^{-1} P was 55.2 and 69.1 µg L^{-1}, respectively. The minimum water temperature in the microcosm experiments in summer was 12°C (± 2), while the winter minimum temperature was 8°C (± 1). In the winter microcosm experiments, the green algal *S. armatus* dominated the phytoplankton composition at enrichment levels of 30, 40 and 60 µg L^{-1} P up to day 20, while the biovolume of the algae group Bacillariophyceae with the dominant N-heterotrophic diatom species *M. varians* increased from 11 to 23% after post-enrichment concentrations of 30 µg L^{-1}P.

Furthermore, the biovolume of the algae group Dinophyceae with *C. hirundinella* as the dominant species declined from 22 to 14.5% after the addition of 40 µg L^{-1} P in the different summer microcosms (Table 3). In the summer microcosms, the species *T. intermedia* and *E. sociabilis*, which dominated the phytoplankton composition at an enrichment level of 30 µg L^{-1} P was replaced as dominant species by the desmid *Spondylosium secedens* and the cyanobacteria *Microcystis aeruginosa* in the 40 and 60 µg L^{-1} P microcosm experiments (Table 3). The median of chl-*a* in the summer microcosm experiments after the addition of 40 and 60 µg L^1 P were 75.2 and 89.2 µg L^{-1}, respectively. At post-enrichment concentrations of 40 and 60 µg L^{-1} P, cyanobacteria respectively contributed 55.1 and 59.4% of the total biovolume at day 20 in the summer microcosms. The dominance of cyanobacteria ≥ 50% was evident from day 13 to 20 in the summer microcosm experiments enriched with 40 and 60 µg L^{-1} P (Figure 2). The proportion of diatom species (Bacillariophyceae) in the summer enrichment experiments with 40 and 60 µg L^{-1} P were 17.1 and 14.6%, respectively (Table 3 and Figure 2).

On day zero of our winter and summer microcosm experiments, all four treatments in triplicate had similar species richness of algae (winter: Margalef index = 223; summer: Margalef index = 347), but 13 days after the addition of 40 and 60 µg L^{-1} P, the phytoplankton community exhibited a strikingly different species richness (winter: Margalef index = 123; summer: Margalef index = 114).

DISCUSSION

From the data generated in the different outdoor microcosm experiments, it seemed that the aquatic system may reach it critical threshold for P in the summer to change to a dominant cyanobacteria system with the addition of 40 µg L^{-1} P (Table 3). Hence, although cyanobacteria showed a conspicuous stepped increase with increasing P concentrations over the entire range of data sets in summer, it seems that lower water temperature may have hampered its dominancy (≥ 50%) or prevented bloom formation in the winter microcosm experiments (Kruger and Eloff, 1978; Oberholster and Botha, 2009). Numerous studies have shown that phytoplankton taxonomic composition and species diversity changes with increasing nutrient levels (Smith, 1990; Watson et al., 1997) and that the changes can be correlated with differences amongst taxa in nutrient uptake or growth (Reynolds, 1984).

In similar microcosm experiments in which 1,000 to 4,000 L of water from Lake Michigan were enclosed in clear plastic bags, Schelske et al. (1974) found that when P was added, silica was reduced to levels that

Table 3. Composition and changes of phytoplankton community after post-enrichment in a set of summer experimental microcosms.

Division	Major species in microcosms at day 0	Species abundance in microcosms at day 20 (20 µg L^{-1}) enrichment	Species abundance in microcosms at day 20 (30 µg L^{-1}) enrichment	Species abundance in microcosms at day 20 (40 µg L^{-1}) enrichment	Species abundance in microcosms at day 20 (60 µg L^{-1}) enrichment
Chromophyta					
Cryptophyceae	*Cryptomonas* sp.	+ +	+ +	+ +	+ +
Chrysophyceae	*Dinobryon divergens*	+	+	+	+
Bacillariophyceae	*Aulacoseira muzzanensis*	+	+	+	+
	Diatoma vulgaris	+ +	+ +	+ +	+ + +
	Fragilaria ulna	+ +	+ +	+ + +	+ + +
	Gomphonema affine	+	+ + +	+ + +	+ + +
	Pinnularia viridus	+ +	+ +	+ +	+ +
	Melosira varians	+ +	+ ++	+ + +	+ + +
Dinophyceae	*Peridinium africana*	+ + +	+ + +	+ + +	+ + +
	Ceratium hirundinella	+ + +	+ +	+ +	+ +
Chlorophyta					
Chlorophyceae	*Closterium lineatum*	+ +	+ +	+ +	+ +
	Spondylosium secedens	+	+ +	+ + + +	+ + + +
	Cosmarium pseudopraemorsium	+ +	+ +	+ +	+ +
	Chlamydomonas africana	+ +	+ +	+ +	+ +
	Pediastrum duplix.	+ +	+ +	+ +	+ +
	Oocystis rupestris	+ +	+ +	+ + +	+ + +
Euglenophyta					
Euglenophyceae	*Trachelomonas intermedia*	+ + +	+ + + +	+ + ++	+ + + +
	Phacus pleuronectes	+ +	+ + +	+ + + +	+ + + +
	Euglena sociabilis	+ +	+ +	+ + +	+ + +
Cyanoprokaryota					
Cyanophyceae	*Oscillatoria princeps*	+	+ +	+ + +	+ + +
	Microcystis aeruginosa	+ + +	+ + +	+ + + + +	+ + + + +

+, Rare; + +, scarce; + + +, common; + + + +, abundant; + + + + +, predominant. The relative abundance of each phytoplankton taxa was grouped into: 1, ≤ 50 (rare); 2, 51 to 250 (scarce); 3, 251 to 1000 (common); 4, 1001 to 5000 (abundant); 5, 5001 to 25 000 (predominant) $cells^{-1}$.

limited algal growth but N concentrations were not effected. In their study, they concluded that P was the limiting nutrient in Lake Michigan, but that silica was becoming limiting for diatoms. In temperate zone lakes, oligotrophic systems supported minimal phytoplankton biomass with

Table 4. Composition and changes of phytoplankton community after enrichment in a set of winter experimental microcosms.

Division	Major species in microcosms at day 0	Species abundance in microcosms at day 20 (20 µg L^{-1}) enrichment	Species abundance in microcosms at day 20 (30 µg L^{-1}) enrichment	Species abundance in microcosms at day 20 (40 µg L^{-1}) enrichment	Species abundance in microcosms at day 20 (60 µg L^{-1}) enrichment
Chromophyta					
Cryptophyceae	*Cryptomonas* sp.	+ +	+ +	+ +	+ +
Chrysophyceae	*Dinobryon divergens*	+ +	+ +	+ +	+ +
Bacillariophyceae	*Aulacoseira muzzanensis*	+ +	+ +	+ + +	+ + +
	Craticula cuspidate	+ +	+ + +	+ + +	+ + +
	Cymbella kappi	+ + +	+ + +	+ +	+ +
	Diatoma vulgaris	+ +	+ +	+ +	+ + +
	Fragilaria ulna	+ +	+ +	+ + +	+ + +
	Gomphonema affine	+	+ + +	+ + +	+ + +
	Nitzschia reversa	+	+	+	
	Nitzschia linearis	+	+ + +	+ + +	+ + +
	Rhopalodia gibba	+ + +	+ + +	+ +	+
	Pinnularia viridus	+ +	+ +	+ +	+ +
	Pinnularia subcapitata	+ +	+ +	+	
	Surirella ovalis	+ +	+ +	+	+
	Melosira varians	+ + +	+ + +	+ + +++	+ + +++
	Stephanodiscus hantzschii		+	+ + ++	+ + ++
Chlorophyta					
Chlorophyceae	*Closterium lineatum*	+ +	+ +	+ +	+ +
	Spondylosium secedens	+ +	+ +	+ +	+ +
	Cosmarium pseudopraemorsium	+ +	+ +	+ +	+ +
	Chlamydomonas africana	+ +	+ +	+ +	+ +
	Pediastrum diplex				
	Scenedesmus armatus	+ +	+ +	+ + + + +	+ + + + +
	Oocystis rupestris	+ +	+ +	+ + +	+ + +
Euglenophyta					
Euglenophyceae	*Trachelomonas intermedia*	+ + +	+ + +	+ + +	+ + +
	Phacus pleuronectes	+ +	+ +	+ + +	+ + +
Cyanoprokaryota					
Cyanophyceae	*Oscillatoria princeps*	+ +	+ +	+ + +	+ + +
	Anabaena flos-aquae	+ + +	+ +	+	+

The relative abundance of each phytoplankton taxa was grouped into: 1, ≤ 50 (rare); 2, 51 to 250 (scarce); 3, 251 to 1000 (common); 4, 1001 to 5000 (abundant); 5, 5001 to 25 000 (predominant) cells L^{-1}. +, Rare; + +, scarce; + + +, common; + + + +, abundant; + + + + +, predominant.

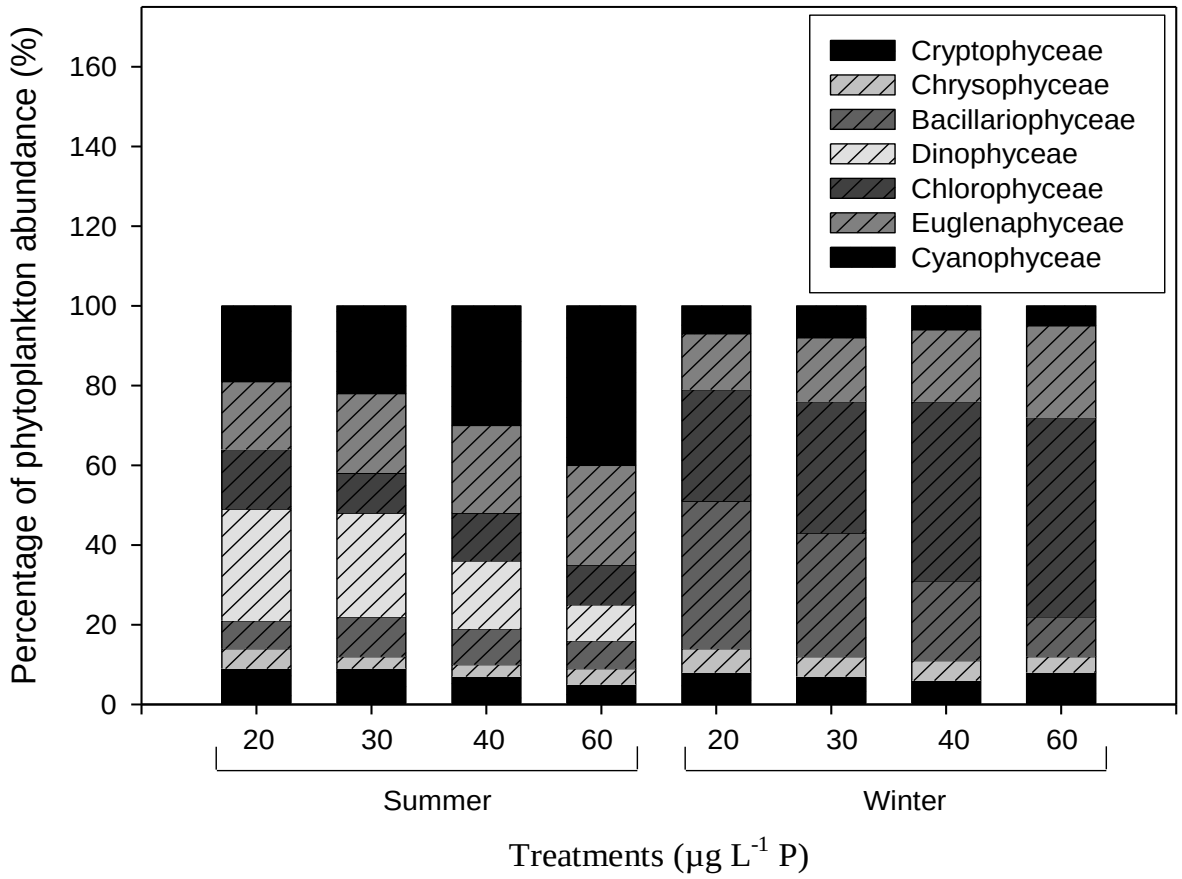

Figure 2. Percentage of phytoplankton abundance (%) during the different summer and winter treatments of phosphorus.

low species diversity, while eutrophic and hypereutrophic lakes sustain very high average algal biomass often dominated by very few taxa, usually cyanobacteria, diatoms or in some water bodies, dinoflagellates (Jensen et al., 1994). The increase in biomass of the centric diatoms *M. varians* and *Stephanodiscus hantzschii* after enrichment concen-trations of 40 and 60 µg L^{-1} of P indicates that these species were indicators of eutrophic to hypertrophic water conditions which is in association with a previous study by van Dam et al. (1994). From our study it is clear that these species typically have greater total P optima than do N-autotrophs.

The negative relationship observed in this study between the biomass of the dinophyte *C. hirundinella* which is a slow-growing inedible late summer algae and the higher tested phosphate concentrations indicate that the higher abundance of this species was associated with low concentrations (30 µg L^{-1}) of P enrichment (Willen, 1991, 2000; Reynolds et al., 2002). Similar observations are provided by Pollingher (1988). Padisak (1985) reported that at higher concentrations of phosphorus, *C. hirundinella* abundance is suppressed by blue-green algae. This is concurrent with the observations in our summer microcosm study, where the cyanobacterial species *M. aeruginosa* did replace *C. hirundinella* to become the dominant species after enrichment concentrations of 40 µg L^{-1} P. According to a study by Watson et al. (1997), dinoflagellates have low average biomass in oligotrophic systems and increased rapidly with an increase in P in meso-eutrophic lakes. This implied a threshold nutrient requirement for growth as observed in our study at a concentration of 30 µg L^{-1} of P after which their biomass generally decline at higher concentrations of P.

The conspicuous stepped increase of *M. aeruginosa* with increasing P enrichment concen-trations over the entire data set range in the summer microcosm, can possibly be related to higher water temperatures since phosphorus recycling (number of phosphorus molecules recycled per unit time) is more intensive in warmer waters, while processes of phos-phorus release from lake sediment and mineralization are highly temperature dependent (Hamilton et al., 2001). Furthermore it is a known fact that cyanobacterial blooms usually occur during warm periods at temperatures above 20°C (Robarts and Zohary, 1987).

Although diatoms are the most abundant group in oligotrophic systems and supply most of the phytoplankton biomass in lakes with low to moderate nutrient levels (10 to 30 µg L^{-1} P), they are generally subdominant to cyanobacteria in eutrophic lakes (> 100 µg L^{-1} P). The negative correlation observed between the decline in biomass of certain diatom species and the increase of phosphorus concentrations during the winter and summer microcosm experiments indicated that their dominance were associated with low phosphorus concentrations and that they are better competitors for phosphorus than other algae. This observation was also confirmed in laboratory studies by Smith and Kalh (1983). Their competitive superiority can be explained by the catalytic property of silica in cell walls of these specimens which act as adsorbent for low phosphorus concentrations (Werner, 1977). In our study, pennate diatoms were favoured at 20 µg L^{-1} P, while centric diatoms for example, *M. varians* with higher growth rates and lower sinking losses was favoured up to 40 µg L^{-1} P (Corbelas and Rojo, 1994). Unlike cyanobacteria, diatoms did not show a significant change in their rate of increase with TP. It is known that the growth of diatoms can be inhibited by a low supply of silica. However, Willen (1991) reported that Si concentrations as low as 0.2 mg L^{-1}, much lower than the concentrations measured (2.5 mg L^{-1} Si) in our study, should be sufficient for diatom reproduction.

According to a study by Happy-Wood (1988), Chlorophytes are a diverse group and rarely dominates temperate lake phytoplankton communities except at nutrient extremes. Nevertheless, in our study Chlorophytes dominated the phytoplankton community during the winter microcosm, suggesting that the absence of major effects of zooplankton grazing may have caused their sustained dominance. This data is not in relationship with previous lake studies of Watson et al. (1997). They showed in their study on 91 northern hemisphere temperature lakes a slight increase in Chlorophytes biomass with considerable variance at intermediate TP levels of 30 µg L^{-1}. On the other hand, Duarte et al. (1992) found in their study that green algae was the dominate species in shallow oligotrophic Florida lakes, but that the algal assemblage of these lakes was highly diverse. In addition, a study conducted by Peterson and Stevenson (1989) on the Ohio River and six Kentucky tributaries indicated that the abundant diatom *M. varians* and the green algae *S. armatus,* that was dominant in our winter microcosm, correlated positively with lower surface water temperatures.

Moreover, chrysophytes which initially increased with increased P concentrations, showed a smaller average biomass at P levels of 40 and 60 µg L^{-1}. According to Caron et al. (1990), there exists a comparatively poor relationship between P and chrysophyte biomass, suggesting that this algal group is more influenced by factors other than phosphorus, such as pH, alkalinity or iron. Data generated from our study indicated that species richness declines with higher nutrient loading, which is consistent with earlier studies by Reynolds (1984). It is generally accepted that the N:P ratio is an important determinant of the species composition of natural populations in lakes (Takamura et al., 1992). Studies show shifts from green algae and diatoms to blue-green algae as the N:P ratio in the lakes decrease (Schindler, 1977, 1978; Kotak et al., 2000). In Lake Hartbeespoort (South Africa), a somewhat deeper reservoir than Lake Mokolo, the absence of *Microcystis aeruginosa* during 1988 and 1989 was ascribed to the low epilimnetic phosphate concentration and the increasing N:P ratios, that is from about four to 10 (Chutter, 1989). In addition to atmospheric and agriculture sources of N and P in the study area of Lake Moloko, the increase in human population density to this area will have a strong influence on the water resources downstream of the developing areas, since humans used flowing waters as convenient wastewater disposal systems (Proulx et al., 1996; Smith et al., 1997).

Furthermore, a change in the trophic states of Lake Mokolo from a mesotrophic to eutrophic state dominated by cyanobacterial blooms due to over enrichment by nutrients can have detrimental effects for irrigation farmers downstream of Lake Mokolo. The exposure of edible crop plants to cyanobacterial toxins may cause these toxins to accumulate in plant tissues (Codd et al., 1999). The introduction of these toxins into the human food chain is therefore a strong possibility, which may pose great concern for human health if these crops were ingested. However whether spray irrigation promotes cyanobacterial biotoxin release on crops due to cell breakdown via sheer stress, is not yet known. At this stage strong evidence exist that cyanobacterial biotoxins inhibited the germination of pollen which could have an adverse effect on crop yield (Metcalf et al., 2004).

Conclusion

From our study it is evident that in order to predict the effects of P inputs on receiving reservoir waters and phytoplankton community structure of Lake Mokolo, it is necessary to be able to predict how water body P concentrations and phytoplankton community structure vary as the external anthropogenic inputs change over time. Moreover, our study indicated that the critical threshold loading value for a southern hemisphere temperate mesotrophic lake is reached with an increase of 40 µg L^{-1} P in winter and 30 µg L^{-1} P in summer, and that beyond these thresholds any additional P inputs will cause significant changes in phytoplankton diversity. The data generated in this study can play an important role in managing progressive eutrophication in southern

hemisphere temperate mesotrophic lakes.

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