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The "nutrient pump:" Iron-poor sediments fuel low nitrogen-to-phosphorus ratios and cyanobacterial blooms in polymictic lakes

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Abstract

Several lines of evidence from a eutrophic lake show how polymixis enables phosphorus (P) released from anoxic, iron (Fe)-poor sediments to lower nitrogen-to-phosphorus (N : P) ratios and stimulate cyanobacterial blooms. Detailed sediment analyses revealed extensive formation of Fe sulfides, which suppressed porewater Fe levels and prevented sequestration of P in Fe minerals. Experimental additions of Fe significantly decreased the flux of dissolved P from warm, anoxic sediments, increasing N : P ratios in porewater and overlying water. The net midsummer effect of polymixis and P release from Fe-poor sediments quickly doubled the total P in the euphotic zone during a period of very low external P loading. This internal "nutrient pump" decreased N : P in surface waters and led to a cyanobacterial bloom comprised primarily of diazotrophic *Anabaena* and *Aphanizomenon* spp. along with nonheterocystous and potentially toxic *Microcystis icthyoblabe* and *Woronichinia naegelianum*. Concentrations of the cyanotoxin, microcystin, in this lake were typically elevated during, or shortly after, episodes of internal P loading. Our study demonstrates an important mechanism underlying the increasing cyanobacterial dominance of weakly stratified eutrophic north temperate lakes, and warns of further increases under a warming climate.

Despite the global acceleration of harmful cyanobacterial blooms in eutrophic lakes, effective management of such phenomena remains constrained by the lack of a mechanistic understanding of the causal factors (Hudnell and Dortch 2008). Here, we propose that low iron (Fe) availability in sediments, combined with the polymictic nature of many lakes, provide ideal conditions for cyanobacterial blooms (Fig. 1). Specifically, Fe-poor sediments and polymixis interact to cause episodic releases of highly bioavailable phosphorus (P) from sediments, lowering nitrogen-to-phosphorus (N : P) ratios in the euphotic zone, and stimulating the proliferation of harmful cyanobacteria. Below, we provide our rationale for each component of this hypothesis.

First, Fe-poor sediments enhance the recycling of P back into the water column. Iron has long been known to play a role in P transfer across the sediment-water interface, although its implicit role in sequestration of P in sediments by adsorption to an oxidized surface layer of ferric oxyhydroxides is flawed (Hupfer and Lewandowski 2008). Instead, the long-term sequestration of P by Fe likely results from the authigenic formation of ferrous-phosphate minerals, such as vivianite ($[Fe_3(PO_4)_2{\cdot}8H_2O]$), in deep anoxic sediments (Gächter and Müller 2003; Katsev et al. 2006). However, bacterial reduction of sulfate (SO_4) is expected to interfere with P retention in sediments by dissolving vivianite (Murphy et al. 2001), and depleting Fe in porewaters by precipitating insoluble minerals (e.g., pyrite, FeS_2) (Smolders et al. 2006).

Second, polymixis functions as an internal "nutrient pump," delivering bioavailable P and other nutrients from Fe-poor sediments to the euphotic zone of lakes. Polymictic lakes are found at all latitudes and are more common than dimictic lakes (Cooke et al. 2005). Polymixis is usually associated with shallow lakes but also occurs in deep lakes with large fetches. In polymictic lakes, multiple ephemeral stratification events can occur annually, leading to the accumulation of P in hypoxic bottom waters (Wilhelm and Adrian 2008; Lehman 2011). Thereafter, destratification in polymictic lakes results in upward mixing of previously hypolimnetic P through the photic zone.

Third, episodes of P release from Fe-poor sediments lower N : P ratios, conferring a competitive advantage to diazotrophic cyanobacteria that fix atmospheric nitrogen (N_2), leading to pulses of fixed N that may stimulate toxic cyanobacterial blooms. Studies have shown that low N : P ratios lead to the dominance of N_2-fixing cyanobacteria (e.g., Schindler et al. 2008). Importantly, new N inputs from N_2

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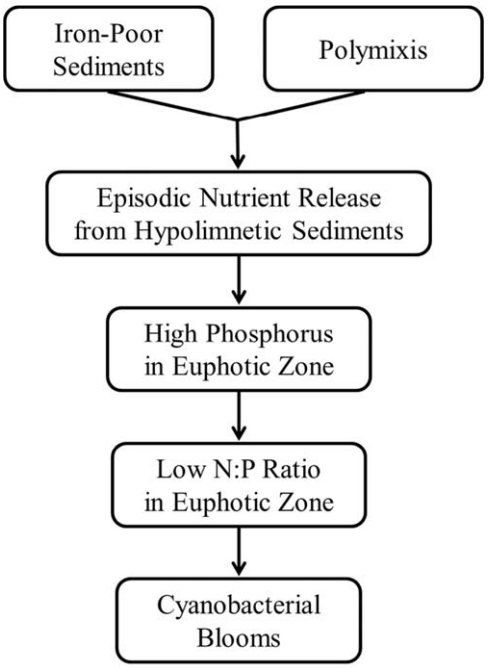

Fig. 1. Conceptual model for how iron-poor sediments and polymixis interact to create ideal conditions for cyanobacterial blooms in eutrophic lakes.

fixation may then support the growth of potentially toxic, nonheterocystous cyanobacteria (Beversdorf et al. 2013). Although severe N limitation limits the production of the N-rich cyanotoxins (Van de Waal et al. 2014), lower N : P ratios are associated with higher concentrations of the potent hepatotoxin microcystin (Orihel et al. 2012; Harris et al. 2014).

Our conceptual model for cyanobacterial bloom formation (Fig. 1) was developed based on a multifaceted study of a hypereutrophic Canadian lake. We conducted a suite of chemical and mineralogical analyses to determine the availability of Fe in sediments and to probe for P-containing minerals, as well as a series of experiments to test whether Fe-poor sediments show high P flux that results in low N : P ratios in overlying lakewater. We also quantified thermal structure, water chemistry, phytoplankton community, and microcystin dynamics over a one-year period to determine if an internal "nutrient pump" lowers N : P, thereby stimulating diazotrophic species, and potentially toxic, nonheterocystous cyanobacteria.

Methods

Study site

Nakamun Lake (53°52′58.42″N, 114°12′14.16″W) is a hardwater lake situated on the Horseshoe Canyon Formation in Alberta, Canada (Fig. 2a). Its characteristics are typical of many lakes in Alberta and have been previously described (Mitchell and Prepas 1990). Briefly, the drainage basin

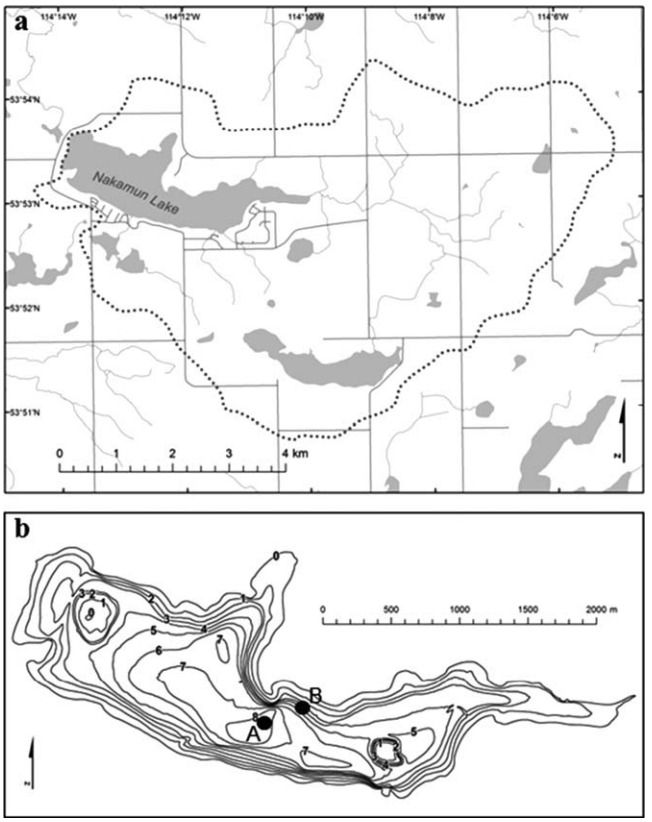

Fig. 2. Watershed (a) and bathymetry (b) of Nakamun Lake, Alberta, Canada. The watershed is outlined with a dotted line in panel a, and the locations of Stations A and B are shown in panel b.

(49 km^2) is nearly 14 times larger than the lake (3.5 km^2), and ephemeral streams flow into the lake from the east and south while the northwest outflow runs only during wet periods, resulting in a long residence time (~ 21 yr). The surficial geology of the watershed consists of fluted and stagnant ice moraine, and forest in the catchment has largely been replaced by cattle operations and cereal production. Over 300 residences are situated along the south shore and a large youth camp is located on the north shore. In most years, Nakamun Lake is hypereutrophic (sensu OECD 1982); over the last decade, mean concentrations of total P and N ranged from 55 $\mu g\ L^{-1}$ to 114 $\mu g\ L^{-1}$ and 2.0 mg L^{-1} to 2.5 mg L^{-1}, respectively, mean and maximum concentrations of chlorophyll *a* (Chl *a*) ranged from 21 $\mu g\ L^{-1}$ to 67 $\mu g\ L^{-1}$ and 59 $\mu g\ L^{-1}$ to 157 $\mu g\ L^{-1}$, respectively, and minimum secchi depth ranged from 0.3 m to 0.8 m (May–October in the euphotic zone; Alberta Environment and Sustainable Resource Development, unpubl.). Large blooms of cyanobacteria occur frequently, at times causing fish kills and exceeding safe drinking water and recreational contact guidelines. Stations (Stn.) for this study were positioned at the maximum (8.0 m; Stn. A) and mean (4.5 m; Stn. B) depths of the lake (Fig. 2b).

Sediment characterization

In March 2010, sediment cores for chemical and mineralogical analyses were collected from Stn. B using a Glew gravity-driven sediment corer. High-resolution profiles of dissolved oxygen (DO), pH, and H_2S across the sediment-water interface were measured using 50-μm tip microelectrodes (OX50, H2S50, and PH50; Unisense A/S, Denmark), calibrated following procedures in Unisense A/S manuals. To account for drift, H_2S values were rezeroed for each profile using the average zero signal, then ΣH_2S ($H_2S + HS^- + S^{2-}$) was calculated based on temperature and pH equilibria. Cores were extruded at 2.5 cm intervals to a depth of 20 cm, after which samples were centrifuged ($\sim 2500 \times g$) to separate porewaters from sediments. Porewater samples were filtered (Target GL Microfiber; 0.7 μm), acidified with analytical grade HNO_3, and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) on an Elan6000 quadrupole ICP-MS (Perkin Elmer), and other parameters as described under "Standard water analyses." Surface sediments (0–2.5 cm) were analyzed in duplicate for loss-on-ignition at 550°C and 950°C (Heiri et al. 2001), total N, and total carbon (C) by the Dumas Combustion Method on a 4010 Elemental Analyzer System (Costech Analytical Technologies), a suite of elements by ICP-MS (after HF-HNO_3 digestion), and mineral content on a Powder X-Ray Diffractometer (Rigaku).

We also conducted additional analyses to investigate interactions among P, Fe, and sulfur (S) in sediments. First, sediments were sequentially extracted to determine P speciation based on the method of Ruttenberg (1992) but modified to incorporate an extra alkaline step as proposed by Baldwin (1996). Extractions were done in duplicate at room temperature, and solutions were continuously agitated during extraction. Suspensions were centrifuged ($\sim 1700 \times g$) and supernatant decanted using syringe needles. Orthophosphate (PO_4) was measured on a UV-VIS (Thermo Evolution 260) using the molybdate blue method (Murphy and Riley 1962). The reactive PO_4 in the citrate-bicarbonate-dithionite extracts was analysed by the isobutanol method (Watanabe and Olsen 1962), after reacting the solution with 1% v/v $FeCl_3$ to minimize the interference of citrate with reduction of the molybdate complex (Ruttenberg 1992). Second, element maps of Fe, P, and S were produced by performing stage scans with a JEOL JXA-8900 electron microprobe. Prior to analysis, intact freeze-dried samples were mounted in epoxy pucks and polished using $Al(OH)_3$ powder, then sputter coated with carbon. Microanalysis was performed using wavelength dispersive spectrometry at a 15 kV accelerating voltage, a beam current of 15 nA, a dwell time of 10 ms, with a 0.2–1.0 μm step size. Colocalization analyses (Manders et al. 1993) were performed using Fiji software (ImageJ v1.47), and statistics were generated using pixel intensities above threshold limits.

Microcosm experiments

In Experiment (Exp.) 1, Fe was added to sediments to examine its effect on nutrient concentrations and ratios in water above the sediment-water interface (hereafter, "overlying water") under simulated spring and summer conditions. In August 2008, cores ($n = 12$) were retrieved from Stn. A, adjusted to the same volume of overlying water (0.5 L), and randomly assigned to treatments: 0 g Fe m^{-2}, 0.25 g Fe m^{-2}, 0.50 g Fe m^{-2}, 0.75 g Fe m^{-2}, 1.00 g Fe m^{-2}, or 1.25 g Fe m^{-2}. After doses of standard grade $FeCl_3$ (13.8% Fe; Kemira Water Solutions, Canada) were added to overlying water, cores were incubated in a cold (4°C) room, while being aerated by aquarium bubblers. After three days, cores were bubbled with N_2 gas and sealed closed, then incubated for nine days on the lake bottom (Stn. A) in a "Sediment Core Lander" (Orihel and Rooney 2012). Water temperatures during the in situ incubation ranged from 14.4°C to 16.3°C, with an average of 15.2°C. Overlying water was sampled for chemical analyses on days 0, 3, and 12.

In Exp. 2, we quantified the impact of Fe on porewater chemistry and P flux from sediments under ambient summer conditions. Cores ($n = 12$) were collected in July 2009 from Stn. B and randomly assigned to one of four treatment levels: 0 g Fe m^{-2}, 2 g Fe m^{-2}, 12 g Fe m^{-2}, or 42 g Fe m^{-2}. After cores were adjusted to achieve 0.5 L of overlying water, they were treated with Fe the same day in the field. To compare P flux among cores, overlying water was replaced with lake water of a known P concentration (0.02 mg L^{-1}) after the added Fe precipitated from the water column. Cores were incubated in a Sediment Core Lander at Stn. B, and overlying water and porewater (0–5 cm) were sampled for chemical analyses after two weeks. Water temperatures ranged from 15.8°C to 18.0°C, with an average of 17.2°C.

In Exps. 3 and 4, overlying water from incubated sediments (from Exp. 2) was used as a culture medium for growing phytoplankton. Surface water from Stn. A (passed through a 150-μm mesh to remove zooplankton) was used as phytoplankton stock in these experiments. In Exp. 3, algal growth in surface water collected from Stn. A (SW) was compared to growth in overlying water harvested from control cores (OW-0). Both SW and OW-0 were passed through Whatman GF/C filters (1.2 μm), then aliquots (100 mL) were placed in autoclaved glass flasks. Cultures ($n = 12$) were inoculated with 50 mL of phytoplankton stock and incubated in a growth chamber for 20 d under constant light at 17°C. Exp. 4 followed a similar procedure as Exp. 3 but also included media from Fe-treated sediments. Cultures ($n = 15$) for this bioassay included: SW, OW-0, and overlying water from cores treated with 2 g Fe m^{-2} (OW-2), 12 g Fe m^{-2} (OW-12), or 42 g Fe m^{-2} (OW-42). Cultures contained 180 mL of filtered medium and 25 mL of phytoplankton stock and were incubated for 30 d under constant light at 17°C (however, the temperature was unexpectedly turned down to 10°C). Cultures were sampled for Chl *a* at the end of each bioassay.

Lake sampling

We monitored lake stratification, water chemistry, phytoplankton biomass and species composition, and microcystins in Nakamun Lake from June to October 2009, and continued sampling water chemistry under ice from January to March 2010. Profiles of temperature, DO, pH, conductivity, and oxidation–reduction potential (ORP) were performed using a multiparameter water quality sonde (Hydrolab DataSonde® 5 Multiprobe; Campbell Scientific Canada). Data loggers (HOBO Pendant® Temperature/Light Data Logger 64K-UA-002-64) were installed every 0.5 m to measure temperatures hourly. Integrated samples for chemical, phytoplankton, and microcystin analyses were collected biweekly at Stn. B by sampling the top four meters of the water column using a polyvinyl chloride tube fitted with a foot valve. On alternate weeks, discrete samples (0 m, 4 m, and 7.5 m) were collected from Stn. A using a Van Dorn sampler. Samples were kept cool and dark during transport back to the laboratory. Between sampling events, equipment was soaked overnight in 10% HCl and rinsed six times with Milli-Q water.

Standard water analyses

Analyses were conducted in the Biogeochemical Analytical Service Laboratory at the University of Alberta (URL: http://www.biology.ualberta.ca/basl). All porewater and overlying water samples, and all samples analyzed for soluble reactive P (SRP), inorganic N species, or dissolved inorganic C (DIC), were filtered by syringe (Target GL Microfiber; 0.7 μm). Other samples were filtered under vacuum (Whatman GF/F; 0.7 μm) in a filtration tower, and the filtrate and filter were collected for "dissolved" and "particulate" analyses. Samples for SRP and Chl *a* were frozen (−20°C), those for N species and Fe were acidified with analytical grade H_2SO_4 and HNO_3, respectively, and all other samples were stored at 4°C until analysis. Phosphorus was determined following method 4500-P F (American Water Works Association 1999) using a QuikChem 8500 FIA Automated Ion Analyzer (Lachat Instruments). Samples for total P in unfiltered water (TP), total dissolved P (TDP), or particulate P (PP) were digested with $K_2S_2O_8$ in an autoclave prior to analysis, whereas samples for SRP were not digested. Ammonium (NH_4; measured as N-NH_4) and nitrate–nitrite (NO_3 + NO_2; measured as N-NO_3 plus N-NO_2) were determined by methods 4500-NH_3 F and 4500-NO_3 I (American Water Works Association 1999) on the QuikChem 8500 FIA Automated Ion Analyzer. The sum of NH_4 and NO_3 + NO_2 is referred to as dissolved inorganic N (DIN). Samples for total N in unfiltered water (TN) and total dissolved N (TDN) were analyzed using the same method as NO_3 + NO_2 after digestion with $K_2S_2O_8$. Dissolved organic C was determined following EPA methods 415.1 (United States Environmental Protection Agency 1983) on a 5000A TOC analyzer (Shimadzu, Japan); DIC was also analyzed on this instrument following protocols supplied by the manufacturer. Particulate C was measured by CE440 Elemental Analyzer (Exeter Analytical). Sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and Fe were measured by Inductively Coupled Plasma-Optical Emission Spectrometer ICAP6300 (Thermo Scientific), while SO_4 and chloride (Cl) were determined by EPA method 300.1 (United States Environmental Protection Agency 1983) using DX600 Ion Chromatography (Dionex). Other analyses included: total alkalinity (Standard Method 2320 Alkalinity), pH (Standard Method 4500-H^+ pH value), total dissolved solids (Standard Method 2540 B), and total suspended solids (Standard Method 2540C; American Water Works Association 1999). Chl *a* was measured fluorometrically after extraction in ethanol on a RF-1501 Spectrofluorophotometer (Shimadzu, Japan).

Phytoplankton and microcystin analyses

Phytoplankton for pigment analyses were concentrated on Whatman GF/F filters (0.7 μm) under low light and kept frozen (−80°C) until analysis. Pigments were extracted by soaking freeze-dried filters in a mixture of acetone : methanol (80 : 20, by volume) for 24 h in darkness at −80°C. Extracts were filtered through a 0.2 μm membrane filter (Millipore Millex-FG Hydrophobic PTFE), then dried under a stream of N_2 gas, shielded from direct light. Dried extracts were dissolved into a precise volume of injection solvent (70% acetone: 25% ion-pairing reagent: 5% methanol). Ion-pairing reagent consisted of 0.75 g tetra-butyl ammonium acetate and 7.7 g ammonium acetate in 100 mL water. Pigment concentrations were quantified by standard reversed-phase high performance liquid chromatography (HPLC) (Vinebrooke and Leavitt 1999). Briefly, a model 1100 HPLC unit (Hewlett-Packard Canada Ltd., Canada) equipped with a Varian Microsorb-100Å C-18 column (10-cm length, 5-μm particle size), an in-line model 1046A fluorescence detector (435-nm excitation wavelength, 667-nm detection wavelength), and a model 1100 scanning photodiode array spectrophotometer (435-nm detection wavelength) were used to quantify pigment concentrations based on mass-specific absorption calibrations of commercially available standards (DHI Water and Environment, Denmark).

Samples for phytoplankton enumeration were fixed with Lugol's solution and formaldehyde-acetic acid. After aliquots of preserved samples were gravity-settled for 24 h, counts were performed using the Utermöhl technique as described by Findlay and Kling (1998). Cell counts were converted to wet weight biomass by approximating cell volume for each taxon, which was obtained by measurements of up to 50 cells and applying the geometric formula that best fit the shape of the cell and assuming a specific gravity of one for cellular mass. Samples for microcystin determination were stored frozen (−20°C) and in darkness until analysis. Microcystin concentrations (expressed in LR toxicity equivalents) were determined by protein phosphatase inhibition assays based on the work of An and Carmichael (1994) on a Bio-

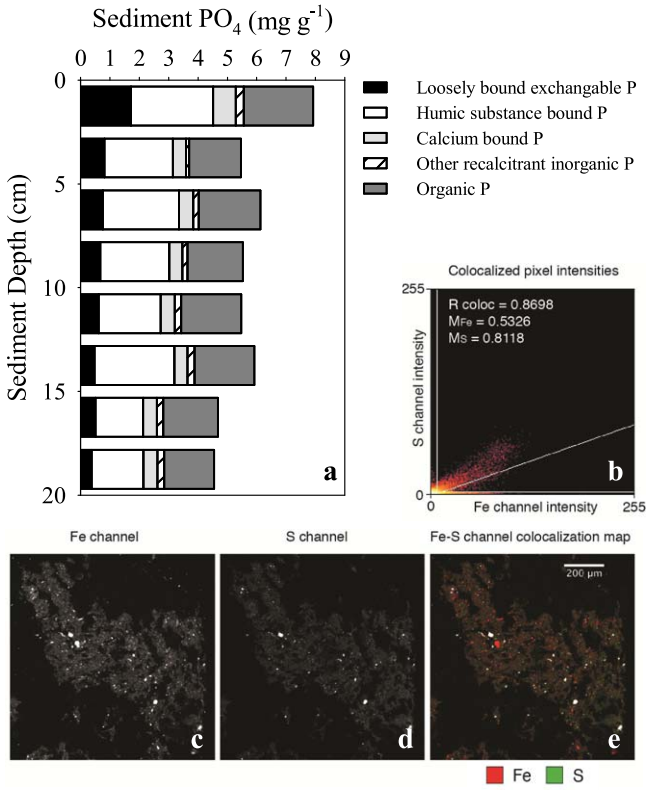

Fig. 3. Phosphorus fractions in sediments at discrete depths below the sediment surface (a) and element and colocalization maps of iron and sulfur in surface sediments (b–e). Iron bound phosphorus was below detection limits in all samples, and thus is not shown in panel a. Pearson (R coloc) and Manders coefficients for iron (M_{Fe}) and sulfur (M_S) are shown on the scatterplot of colocalized pixels intensities in panel b.

Tek ELx800TM Absorbance Microplate Reader (Fisher Scientific, Canada).

Statistical analyses

Normality and homoscedasticity of data was assessed prior to performing parametric tests, and data were log-transformed as required. Linear regression (Exp. 1) or one-way ANOVA (Exp. 2) was used to model relationships between Fe dose and nutrient concentrations or ratios. *T*-test (Exp. 3) or one-way ANOVA (Exp. 4) were used to test for differences in Chl *a* among treatments. The Holm–Sidak method was used to determine significant differences between controls and treatments following ANOVA (Exps. 2 and 4). All graphs and statistical tests were performed with SigmaPlot for Windows (Version 12.3).

Results

Sediment characterization

Sequential P extractions revealed that the majority of P in the lake sediments was organic or associated with humic substances, and not bound to Fe (Fig. 3a). Surface sediments were of high organic matter (loss-on-ignition at 550°C = 46%), poor in carbonates (loss-on-ignition at 950°C = 3%), and contained pyrite, quartz, carbonates (calcite; dolomite), feldspars (albite; orthoclase), and clays (montmorillonite; kaolinite; illite). Concentrations of selected elements in surface sediments are provided in Table 1. Notably, concentrations of Fe and P were 1.7% and 0.2% (by weight), respectively. The Fe : P ratio in surface sediments was eight by mass. Element mapping of surface sediments showed a high degree of spatial colocalization of Fe and S (Fig. 3b–e).

Table 1. Chemistry of surface sediments in Nakamun Lake

Element	Concentration* (mg g^{-1} dry weight)
C	242.0
Al	37.5
N	30.5
Ca	25.4
Fe	17.1
K	9.26
Mg	6.94
P	2.12
Ti	1.78
Mn	0.81
Sr	0.16

*Average of duplicate sediment samples (0–2.5 cm) collected at Stn. B in March 2010.

Microelectrode analysis of the sediment-water interface revealed no oxygen below two millimeter, pH decreased by more than one unit to 7.5, and ΣH_2S reached 2 mmol L^{-1} in the top three centimeter of the sediments (Fig. 4a–c). Chemical analyses of discrete porewater samples from a 20-cm core confirmed that the accumulation of high concentrations of nutrients corresponded with the transition to anaerobic respiration (Fig. 4d–l). Nitrate and SO_4 were rapidly consumed near the interface, and peaks in Mn and Fe occurred within the top five centimeters. Concentrations of Fe reached a maximum of 0.45 mg L^{-1}, and remained under 0.07 mg L^{-1} below 10 cm. Porewater P and NH_4 reached concentrations in excess of 0.5 mg L^{-1} and 5 mg L^{-1}, respectively, which was an order of magnitude greater than those in water above the sediments. The P in sediment porewaters was almost exclusively (90–100%) present as SRP.

Experiment 1: Effect of Fe on overlying water chemistry of sediment cores

Low doses of Fe (0.25–1.25 g Fe m^{-2}) precipitated P from overlying water of sediment cores under in vitro cold, oxic conditions, and modestly slowed P release from sediments under warm, anoxic conditions in Nakamun Lake. At the end of the incubation, TDP in overlying water was inversely related to the amount of Fe applied (Fig. 5a; $R^2 = 0.57$,

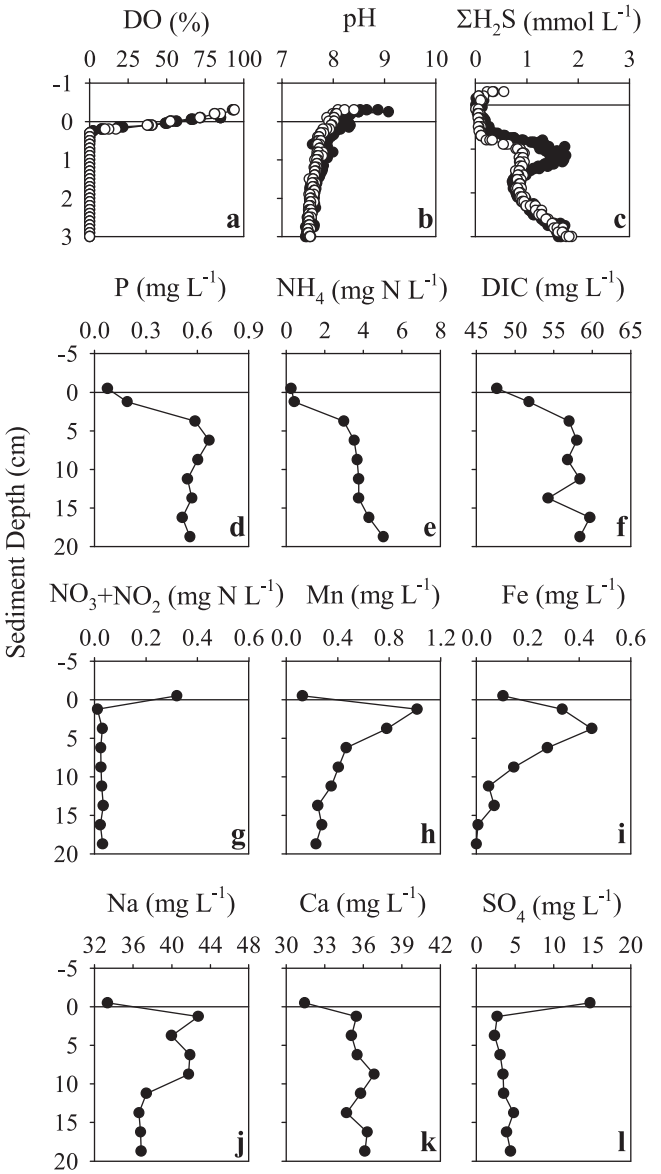

Fig. 4. Fine-scale duplicate sampling of dissolved oxygen, pH, and total sulfide near the sediment-water interface (a–c), and 20-cm profiles of nutrients, metals, and major ions in sediment porewaters (d–l). Abbreviations as in Table 3.

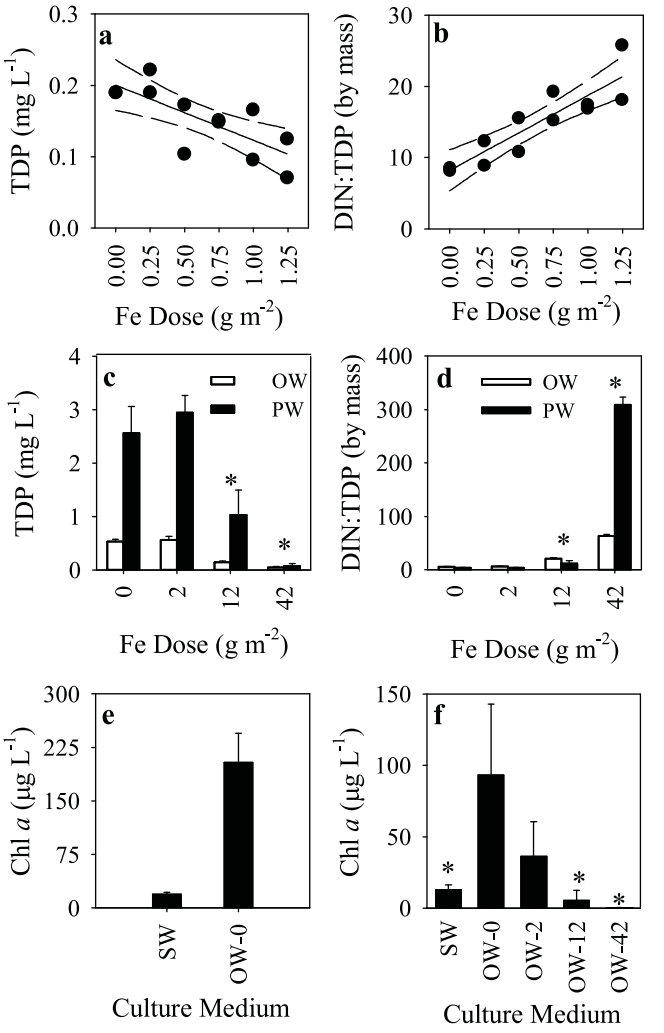

Fig. 5. Results from Experiments 1–4. Relationships between iron dose and phosphorus concentration (a) and nitrogen-to-phosphorus ratio (b) in overlying water of cores (Exp. 1). Mean (± SD; $n = 3$) phosphorus concentration (c) and nitrogen-to-phosphorus ratio (d) in overlying water (OW) and porewater (PW) of cores treated with different doses of iron (Exp. 2). Chlorophyll *a* (Chl *a*) (mean ± SD; $n = 3$) of phytoplankton grown in surface water (SW) or overlying water harvested from sediments treated with 0 g Fe m^{-2} (OW-0), 2 g Fe m^{-2} (OW-2), 12 g Fe m^{-2} (OW-12), or 42 g Fe m^{-2} (OW-42) in Exps. 3 (e) and 4 (f). Asterisks denote treatments that differ significantly from 0 g Fe m^{-2} in (c–d) or OW-0 in (f). Abbreviations as in Table 3.

$F_{1,10} = 13$, $p = 0.004$). In most samples, the majority of TDP in overlying water was present as SRP. Concentrations of SRP also decreased with increasing Fe dose ($R^2 = 0.49$, $F_{1,10} = 10$, $p = 0.01$), but DIN (which was mainly in the form of NH_4) was not affected by Fe addition ($R^2 = 0.14$, $F_{1,10} = 2$, $p = 0.2$). Consequently, the mass ratio of DIN : TDP varied from 8 to 26 and was positively related to Fe dose (Fig. 5b; $R^2 = 0.83$, $F_{1,10} = 48$, $p < 0.001$). Return of Fe from control sediments was low under anoxic conditions, and little of the Fe in treated cores precipitated under oxic conditions was recycled back to overlying water under anoxia.

Experiment 2: Effect of Fe on porewater chemistry and nutrient flux from sediments

The broad range of Fe dosages (2–42 g Fe m^{-2}) to sediment cores showed that high Fe availability can strongly decrease TDP in overlying water ($F_{3,8} = 128$, $p < 0.001$) and porewater ($F_{3,8} = 53$, $p < 0.001$; Fig. 5c). Concentrations of TDP in overlying water of sediments treated with 0 g Fe m^{-2} and 2 g Fe m^{-2} increased 30-fold over the two-week incubation to over 0.5 mg L^{-1}. In contrast, average concentrations of TDP in the 12 g Fe m^{-2} and 42 g Fe m^{-2} treatments only

Table 2. Phosphorus flux from Nakamun Lake sediments treated with different amounts of iron

Fe dose (g m^{-2})	TDP flux* (mg m^{-2} d^{-1})
0	6.8±0.6
2	6.7±0.8
12	1.2±0.3
42	0.2±0.2

*Mean ± standard deviation ($n = 3$) flux from sediment cores in Experiment 2.

reached 0.12 mg L^{-1} and 0.03 mg L^{-1}, respectively. Most (> 80%) of TDP was present as SRP in the 0 g Fe m^{-2} and 2 g Fe m^{-2} treatments, but SRP was undetectable at higher Fe doses. Iron additions reduced fluxes of TDP from 6.8 mg m^{-2} d^{-1} in control cores to 0.2 mg m^{-2} d^{-1} in cores treated with 42 g Fe m^{-2} (Table 2). Concentrations of DIN (> 99% NH_4) ranged from 2 mg L^{-1} to 4 mg L^{-1} in overlying water and 9 mg L^{-1} to 16 mg L^{-1} in porewater, and were not significantly different among treatments in overlying water ($F_{3,8} = 1$, $p = 0.4$) although differences were detected in porewater ($F_{3,8} = 4$, $p = 0.05$). The increase in DIN : TDP caused by Fe addition was significant in overlying water ($F_{3,8} = 767$, $p < 0.001$) and porewater ($F_{3,7} = 1265$, $p < 0.001$; Fig. 5d). Concentrations of Fe differed among treatments in overlying water ($F_{1,10} = 481$, $p < 0.001$) and porewater ($F_{3,8} = 77$, $p < 0.001$). Iron remained below 0.5 mg L^{-1} in controls, but reached 4.5 mg L^{-1} and 21.6 mg L^{-1} in overlying water and porewater, respectively, in treated cores.

Experiments 3 and 4: Response of algae to overlying water from Fe-treated sediments

In Exp. 3, algae cultured in overlying water harvested from incubated control sediments contained final total Chl *a* concentrations of an order of magnitude greater than cultures grown in surface water (Fig. 5e; $t = -24$, $p < 0.001$, $df = 10$). In Exp. 4, Chl *a* of algal cultures grown in surface water, overlying water harvested from control sediments, or overlying water harvested from sediments treated with Fe were significantly different (Fig. 5f, $F_{4,10} = 22$, $p < 0.001$). Concentrations of Chl *a* of phytoplankton grown in overlying water from sediments treated with 12 g Fe m^{-2} and 42 g Fe m^{-2} were significantly lower than that of cultures grown in overlying water from control sediments. Also, Chl *a* of cultures grown in overlying water from control sediments was sevenfold higher than that of cultures grown in surface water.

Polymixis

In summer of 2009, Nakamun Lake experienced several periods of thermal stratification, although only one prolonged period in late July/early August caused a substantial change in the redox potential of bottom waters. During a brief stratification event in June, DO was depleted in the hypolimnion, but ORP remained high throughout the water column (Fig. 6a–e, 19 June). A major storm occurred in early July, but stratification set up again in late July and persisted for more than two weeks (Fig. 6f) while air temperatures remained high and wind speeds low. In early August, DO was absent below five meters, which coincided with a sharp drop in ORP and an increase in conductivity (Fig. 6a–e, 6 August). High wind speeds in mid-August again destratified the lake and replenished DO in bottom waters (Fig. 6b, 20 August). A few calm days at the end of August with air temperatures around 30°C resulted in another brief stratification event which led to bottom water anoxia but little change in ORP (Fig. 6a–e, 3 September). Soon after, temperatures cooled and the lake was well-mixed for the remainder of the fall.

Water chemistry

In the summer of 2009, concentrations of TDP in the water column of Nakamun Lake increased to over 100 μg L^{-1}, corresponding to the prolonged stratification event in late July/early August. During this event, the total mass of P in the lake more than doubled to over 800 kg (Fig. 6g), and TDP in bottom waters increased by an order of magnitude to over 300 μg L^{-1} (Fig. 6h,i). External P loading to the lake was near zero at this time. After the lake mixed in mid-August, TDP in bottom water decreased, but then increased again during the next stratification event in early September (Fig. 6j,k). The majority of TDP in bottom waters was in the form of SRP (66–78%), in contrast to surface waters, where SRP accounted for less than 15% of TDP. Concentrations of TP during the ice-free season ranged from 24 μg L^{-1} to 100 μg L^{-1}, of which less than half (18–54%) was usually in the dissolved phase (Fig. 7a). Total N ranged in concentration from 1.5 mg L^{-1} to 2.5 mg L^{-1}, and was primarily (46–92%) in the dissolved phase, except for during cyanobacterial blooms (Fig. 7b). Concentrations of SRP remained below 5 μg L^{-1}, while DIN ranged from 21 μg L^{-1} to 107 μg L^{-1} (66–100% as NH_4; Fig. 7a,b). From June to August, TP increased more than fourfold, whereas TN increased about 1.5 fold, causing the TN : TP ratio to decrease from > 60 to 25 (Fig. 7c). Similarly, the ratio of DIN : SRP decreased from 21 to 6, while DIN : TDP remained below 2 in summer, then increased to 6 by October, and 14 by March.

Under ice, TP increased slightly from 33 μg L^{-1} to 44 μg L^{-1}, as a result of an increase in TDP, while TN was fairly stable around 2 mg L^{-1} and was almost entirely (> 96%) dissolved (Fig. 7a,b). All of the increase was from internal loading, as inflows were frozen. In March, DO dropped to 1–2 mg L^{-1} in water above the sediments, and SRP (17 μg L^{-1}) and DIN (454 μg L^{-1}) were at their maximum concentrations. In contrast to the summer, most (> 94%) of the DIN in winter was present as NO_3 + NO_2. Ratios of TN : TP under ice were high in comparison to midsummer (Fig. 7c). Average concentrations of chemical parameters in summer and winter are shown in Table 3.

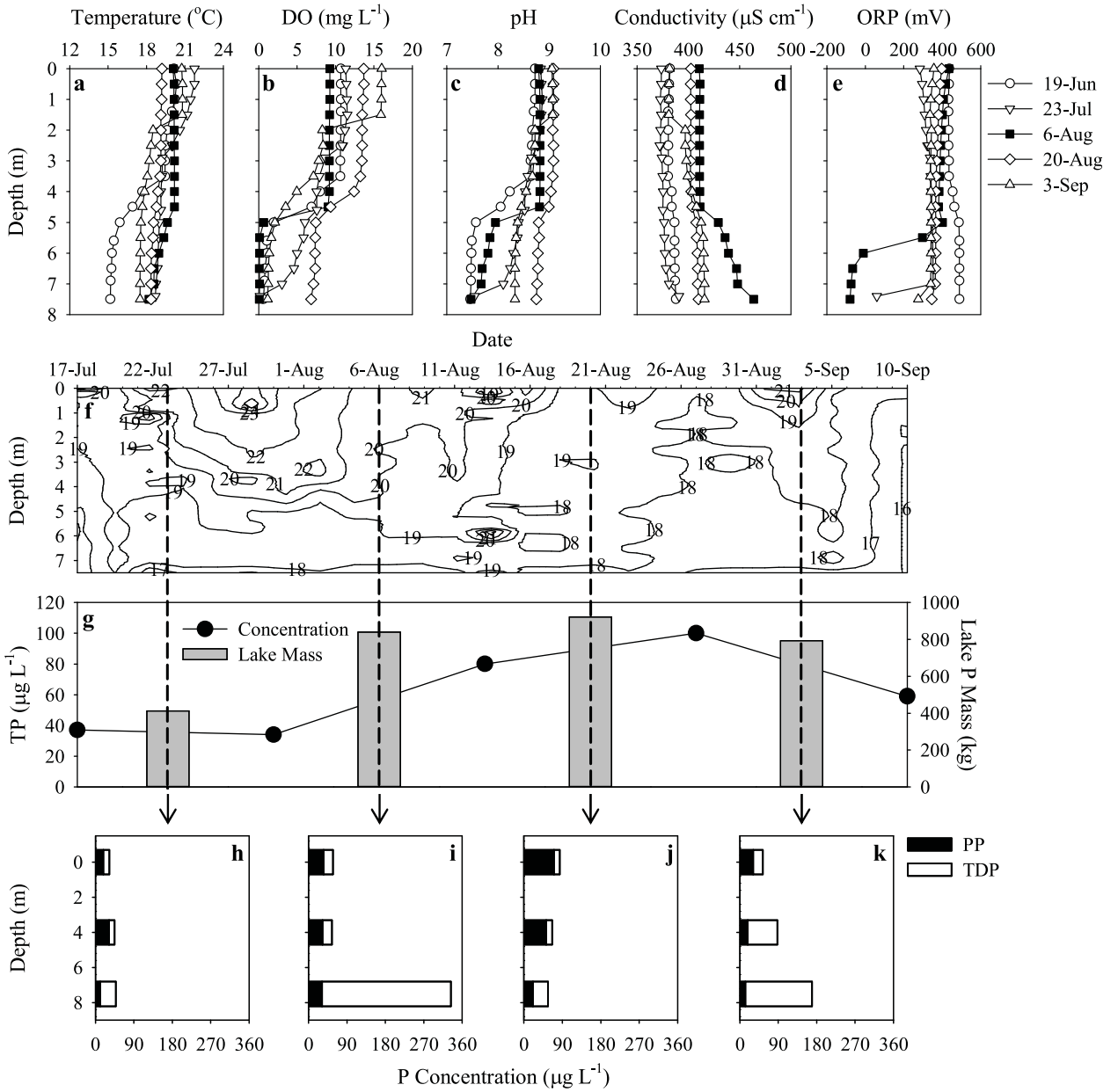

Fig. 6. Polymixis in Nakamun Lake during 2009. Vertical profiles of temperature, dissolved oxygen, pH, conductivity, and oxidation-reduction potential (a–e). High-resolution profiles of water temperature, in degrees C (f), shown with changes in total phosphorus concentration in the upper (0–4 m) water column and whole-lake phosphorus mass (g). Dissolved and particulate phosphorus at discrete depths on 23 July, 6 August, 20 August, and 3 September 2009 (h–k).

Phytoplankton

A taxonomic shift occurred during 2009 as eukaryotic algae in the spring were superseded by cyanobacteria in summer. Concentrations of Chl *a* peaked in late summer, and again, to a lesser extent, in late fall (Fig. 8a). The large peak in Chl *a* corresponded to the seasonal TN : TP minimum (Fig. 7c). The pigments canthaxanthin (filamentous cyanobacteria) and myxoxanthophyll (colonial cyanobacteria) increased through the summer and were at maximum values in August (Fig. 8b,c). A second, smaller peak in myxoxanthophyll occurred in early October. In contrast, fucoxanthin and diadinoxanthin (chrysophytes, diatoms, and dinoflagellates) and alloxanthin (cryptophytes) were highest in June and decreased through the summer, and were low or undetectable in the fall (Fig. 8d,e). Chlorophyll *b* (chlorophytes) peaked in late July/early August, and again in late September (Fig. 8f).

Taxonomic shifts deduced through changes in pigments were confirmed by light microscopy-based estimates of species biomass (Fig. 8, bars). In late June, the community

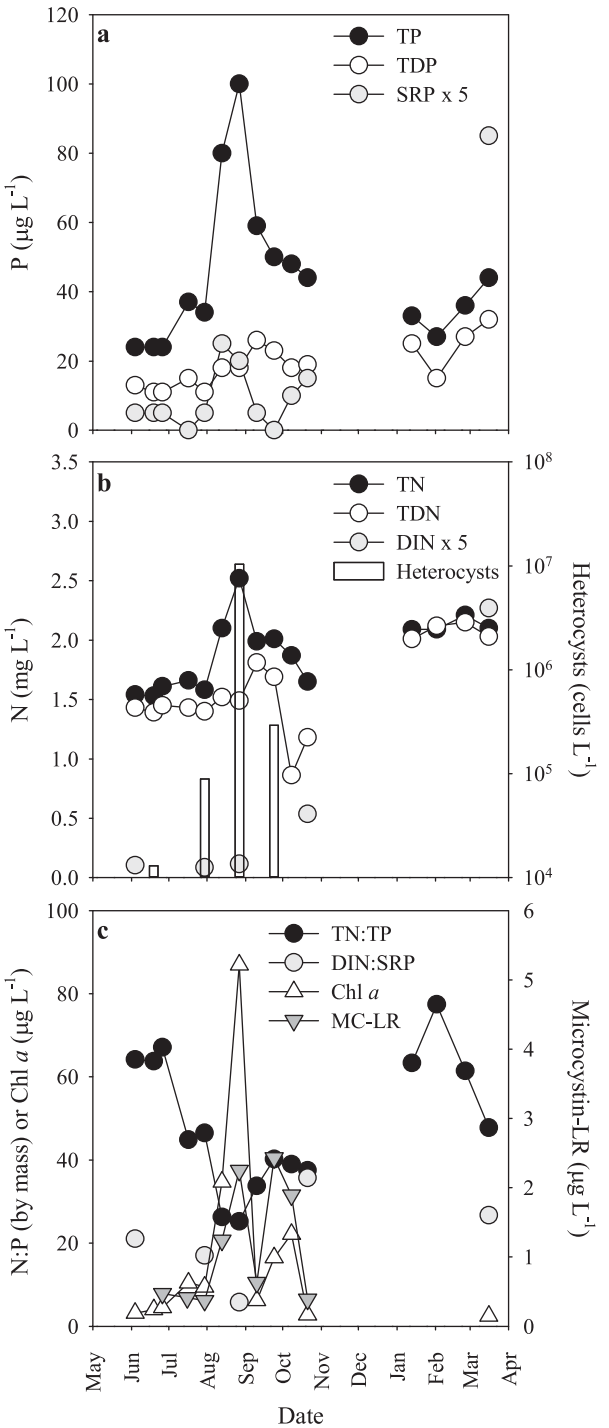

Fig. 7. Seasonal dynamics in Nakamun Lake in 2009 and under ice in 2010. Phosphorus concentrations (a) nitrogen concentrations and heterocyst counts (b), and nitrogen-to-phosphorus ratios, Chl *a* concentrations, and microcystin-LR concentrations (c). Data are from integrated (0–4 m) water column samples. Abbreviations as in Table 3. Note that SRP and DIN concentrations were multiplied by a factor of 5.

Table 3. Water chemistry of Nakamun Lake in 2009–2010

			Open water†		Under ice‡	
Parameter	**Abbrev.***	**Units**	**Mean**	***n*§**	**Mean**	***n***
Total phosphorus	TP	$\mu g\ L^{-1}$	48	11	35	4
Particulate phosphorus	PP	$\mu g\ L^{-1}$	29	11	14	1
Total dissolved phosphorus	TDP	$\mu g\ L^{-1}$	17	11	25	4
Soluble reactive phosphorus	SRP	$\mu g\ L^{-1}$	2	11	17	1
Total nitrogen	TN	$mg\ L^{-1}$	1.8	11	2.1	4
Particulate nitrogen	PN	$mg\ L^{-1}$	0.33	4	0.12	1
Total dissolved nitrogen	TDN	$mg\ L^{-1}$	1.4	11	2.1	4
Ammonium	NH_4	$\mu g\ L^{-1}$	33	4	27	1
Nitrate and nitrite	NO_3+NO_2	$\mu g\ L^{-1}$	10	4	427	1
Particulate carbon	—	$mg\ L^{-1}$	2.0	4	0.9	1
Dissolved organic carbon	—	$mg\ L^{-1}$	21	4	22	1
Dissolved inorganic carbon	DIC	$mg\ L^{-1}$	39	4	45	1
Sodium	Na	$mg\ L^{-1}$	37	4	38	1
Potassium	K	$mg\ L^{-1}$	15	4	17	1
Calcium	Ca	$mg\ L^{-1}$	23	4	25	1
Magnesium	Mg	$mg\ L^{-1}$	12	4	13	1
Chloride	Cl	$mg\ L^{-1}$	6	4	7	4
Sulfate	SO_4	$mg\ L^{-1}$	13	4	13	4
Total alkalinity	—	$mg\ L^{-1}$ $CaCO_3$	177	4	196	1
pH	—	—	8.6	4	7.8	4
Total dissolved solids	—	$mg\ L^{-1}$	239	4	312	1
Total suspended solids	—	$mg\ L^{-1}$	4	11	2	4
Chlorophyll *a*	Chl *a*	$\mu g\ L^{-1}$	18	11	2	1
Secchi depth	—	m	1.75	13	—	—

*Abbreviations for chemical parameters used throughout this manuscript.
†Integrated samples (0–4 m) collected between June and October 2009.
‡Integrated samples (0–4 m) collected between January and March 2010.
§Number of samples used in calculation of mean values.

consisted primarily of *Chrysochromulina parva* (chrysophyte), *Ceratium hirundinella* (dinoflagellate), and *Rhodomonas minuta* (cryptophyte). Total biomass increased fivefold by the end of July, largely as a result of the *C. hirundinella* and the cyanobacterium *Anabaena flos-aquae*. The community consisted almost exclusively (98%, in biomass) of cyanobacteria in late August. At this time, filamentous N_2-fixing species (*A. flos-aquae, Aphanizomenon klebahnii*, and *Anabaena crassa*) were the most abundant algae (Fig. 8b), and heterocysts exceeded 10 million cells L^{-1} (Fig. 7b). Colonies of non-N_2-fixing cyanobacteria, mainly *Microcystis ichthyoblabe*, were also present in the August bloom. In late September, the community

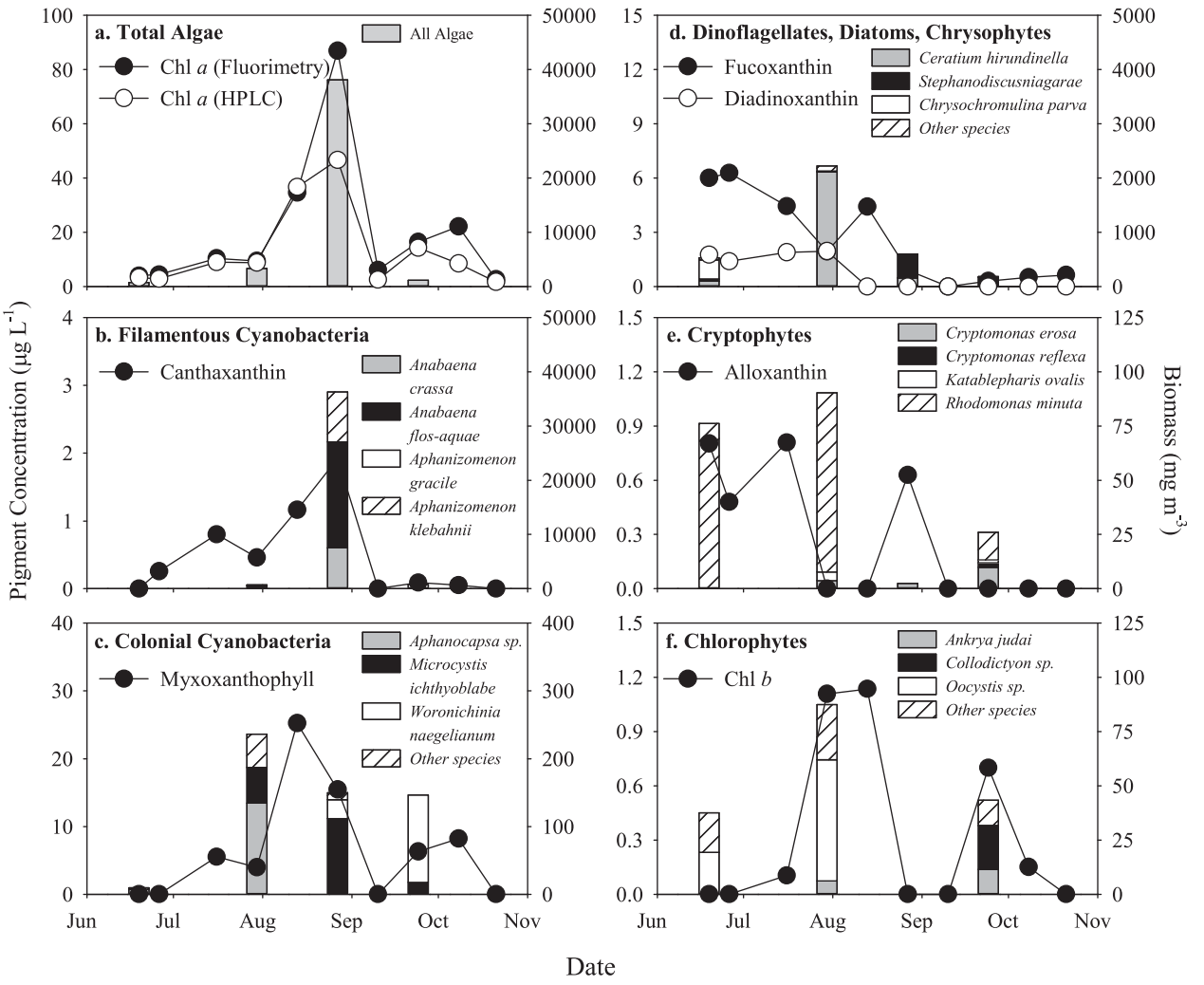

Fig. 8. Phytoplankton biomass and community composition in Nakamun Lake in 2009. Seasonal trends in pigment concentrations representative of various algal groups (circles; left axes), shown with biomass estimates of individual algal species on four dates (bars; right axes).

remained dominated by cyanobacteria, largely by *A. klebahnii*, and to a lesser extent, by *Woronichinia naegelianum*.

Microcystin

Concentrations of microcystin in the lake were low (< 0.5 μg L^{-1}) in June and July, increased to 2.25 μg L^{-1} by the end of August, then decreased and peaked a second time in September. The first peak in microcystin corresponded to the seasonal low in TN : TP ratio and the seasonal high in Chl *a* (Fig. 7c). The main species of cyanobacteria present that are known to produce microcystin were the filamentous, N_2-fixing species *A. crassa* and *A. flos-aquae*, and the colonial, non-N_2-fixing species *M. ichthyoblabe* and *W. naegelianum* (Fig. 8). During the first peak in microcystin in August, there were dense populations of *A. crassa* and *A. flos-aquae*, and some large colonies of *M. ichthyoblabe*. During the second peak in fall, *A. crassa* and *A. flos-aquae* were rare, but colonies of *M. ichthyoblabe* and *W. naegelianum* still persisted.

Discussion

Our empirical investigations of a hypereutrophic Canadian lake revealed how low-Fe sediments and polymixis stimulate cyanobacterial blooms. Here, we consider how our results support the main hypotheses of our conceptual model (Fig. 1), as well as discuss their implications for the management of eutrophic lakes.

Hypothesis 1

The hypothesis that low-Fe sediments increase internal P loading in eutrophic lakes was supported by geochemical analyses of sediments and experiments manipulating Fe levels in sediment cores. Solid phase sediment Fe concentrations in Nakamun Lake (17 mg g^{-1}) are comparable to other eutrophic lakes across the prairie provinces (7–28 mg g^{-1}) (Manning et al. 1999; Burley et al. 2001; Loh et al. 2013), but less than in boreal lakes (25–100 mg g^{-1}) (Cook 1984; Laforte et al. 2005; Couture et al. 2010), whereas porewater

Fe concentrations in our study lake ($< 0.5\,mg\ L^{-1}$) are among the lowest measured in Canadian lakes (0.02–111 mg L^{-1}) (Orihel 2013, Table 4-4). The accumulation of Fe in porewaters was limited by high H_2S production in sediments (Fig. 4c) and subsequent formation of Fe sulfides (Fig. 3b–e), including pyrite. Extensive pyrite formation has previously been observed in other eutrophic lakes in Alberta (Manning et al. 1999).

The effect of this Fe shortage in the sediments was apparent from the results of sequential P extractions, where Fe-bound P was not detectable, and most P was associated with humic and organic substances (Fig. 3a). We also confirmed the lack of Fe–P minerals through X-ray diffraction. Although some of this organic P pool can be recalcitrant, it also contains labile forms that play an important role in P cycling (Baldwin 2013). Our finding that P in Nakamun Lake sediments exists in forms that are susceptible to recycling, rather than in insoluble Fe minerals that are a more stable sedimentary sink, explains the lake's propensity for internal P loading. Fluxes of P from anoxic sediments in our study were substantial ($6.8 \pm 0.6\,mg\ m^{-2}\ d^{-1}$), but similar to earlier measurements in the lake (Riley and Prepas 1984) and within the range reported for eutrophic lakes and reservoirs (1–28 mg $m^{-2}\ d^{-1}$; Carter and Dzialowski 2012).

Importantly, addition of Fe to sediments greatly inhibited P release during warm, anoxic incubations, decreasing P flux down to $0.2\,mg\ m^{-2}\ d^{-1}$ at the highest dose (Table 2). As Fe inhibited P release under highly reducing conditions, Fe sequestered P in sediments by a mechanism other than adsorption to redox-sensitive Fe(III) oxyhydroxides. Although we did not examine the mineralogy of Fe-treated sediments, Fe(II) phosphate phases can account for a significant fraction of the P pool in lake sediments (Li et al. 2012), and evidence of the formation of vivianite can be found in other studies (e.g., Manning et al. 1999).

Hypothesis 2

As hypothesized, we observed that polymixis acts as an internal "nutrient pump": setting up conditions for P release from sediments, and then transferring this bioavailable P to phytoplankton in the euphotic zone. Support for this hypothesis comes from two lines of evidence.

First, when sediment cores were held under cold, oxic conditions in the laboratory, P levels were relatively stable in overlying water; however, after cores were purged of oxygen and incubated at the bottom of the study lake, high concentrations of TDP were observed in overlying water of the cores (Fig. 5). Although the classic explanation for this observation is that anoxia triggers the reduction of Fe(III) oxyhydroxides thereby releasing any adsorbed P (Wetzel 2001), this cannot be the case in Nakamun Lake. We showed in this study that Fe is largely sequestered in pyrite and Fe-bound P phases do not occur, as a result of the high H_2S production in anaerobic sediments. Although we cannot confirm this, it is possible that the P release we observed was mobilized from the organic P pool. Sediment bacteria can contribute directly to the uptake and release of P from sediments (Gächter and Meyer 1993). Bacteria that store P in their cells in the form of polyphosphate may affect P fluxes between the sediment and the overlying water by redox-dependent changes of their physiology (Hupfer et al. 2007). Further, lysis of aerobic bacteria caused by the onset of anoxia can also be responsible for P release from sediments (Ricciardi-Rigault et al. 2000).

Second, a large accumulation of dissolved P in bottom waters of Nakamun Lake during a prolonged stratification event led to a doubling of whole lake P mass, and on destratification, a doubling of TP concentrations in surface waters (Fig. 6). This key finding reinforces the important contribution of polymixis to the development of cyanobacterial blooms. Specifically, stratification periods in polymictic lakes lower the redox potential at the sediment-water interface enabling redox-sensitive processes that mobilize P at the sediment surface, whereas destratification allows for P accumulated in bottom waters during stratification to be efficiently transported to the euphotic zone in summer when warm temperatures promote cyanobacterial blooms. This is in contrast to dimictic lakes, where P is largely retained beneath the thermocline in summer, and thus, is less readily available to phytoplankton. In agreement with our study, the transient build-up of P in the hypolimnion and its subsequent transport to the euphotic zone has been observed in Lake Müggelsee, Germany (Wilhelm and Adrian 2008) and Lake Rotorua, New Zealand (Burger et al. 2008), among other polymictic lakes.

Our study demonstrated that polymixis delivered bioavailable P to the euphotic zone that was rapidly incorporated into the PP pool (Fig. 7a), a proxy for phytoplankton-bound P (Read et al. 2014). Much of the P in the "dissolved" phase (i.e., TDP) of sediment porewaters, overlying water of anoxic sediment cores, and hypolimnetic waters during stratification was present as SRP. SRP is a valuable metric of bioavailable P even though this operationally defined form typically overestimates concentrations of PO_4 (Effler and O'Donnell 2010), the preferred P substrate for algae and bacteria (Cotner and Wetzel 1992). In contrast, SRP usually comprised a small fraction ($< 20\%$) of TDP in the water column (except under ice), which was presumably dominated by dissolved organic P (Read et al. 2014). Despite the fact that some components of the dissolved organic P pool can be used by plankton (Bentzen et al. 1992), there was little evidence of this in our study, as has been reported by others (e.g., Elser and George 1993).

Polymictic lakes and reservoirs are very common across central Canada. Polymixis has been observed in this region over several orders of magnitude in lake size, ranging from small, prairie pothole lakes to large lakes that cover hundreds of square kilometers (e.g., Lac La Biche, Alberta: 234 km^2 in area and 21 m deep; D. Schindler, unpubl.). We

would like to emphasize that because the frequency and duration of quiescent conditions and mixing events in polymictic lakes are stochastic, the magnitude of algal blooms in these lakes is variable and difficult to forecast. The random nature of polymixis may explain why it is more difficult to link land-use practices to eutrophication of polymictic than dimictic lakes, as reported by Taranu et al. (2010).

Hypothesis 3

Our third hypothesis, that episodes of P release from Fe-poor sediments rapidly lower the N : P ratio in surface waters of polymictic lakes, was supported by results from core incubations and monitoring of Nakamun Lake. Whereas the low N : P ratios frequently observed in eutrophic lakes are believed to result from the nutrient stoichiometry of sewage effluent and agricultural runoff (Downing and McCauley 1992) or atmospheric pollution (Elser et al. 2009), we found that Fe-poor sediments can also lower N : P ratios in surface waters.

Porewater and overlying water of untreated Nakamun sediment had DIN : TDP ($\approx$ NH_4 : SRP) ratios of 4 and 6, respectively, by mass (Fig. 5d). Similarly, NH_4 : SRP ratios released under anoxic conditions from incubated sediments collected from two eutrophic reservoirs in U.S.A. were 4 (Lehman 2011) and 2 (Nowlin et al. 2005). The ratio of NH_4 : PO_4 released from anoxic sediments in Lake Kinneret was above 70 during the 1970s, when the lake was dominated by *Peridinium* (Serruya et al. 1974), whereas today, this ratio is around four and the lake is dominated by N_2-fixing cyanobacteria (Orihel et al. 2013). Notably, DIN : TDP in porewater and overlying water of Nakamun Lake sediments treated with Fe were much higher, up to 309 and 62, respectively, than control sediments (Fig. 5d). Consistent with our findings, DIN : SRP ratios of 61 and 366 have been observed in porewaters of surficial littoral and profundal sediments, respectively, in Fe-rich eutrophic Lake 227 of the Experimental Lakes Area, Ontario (I. Lehnherr and D. Orihel, unpubl.). Therefore, we suggest that Fe availability in sediments is a major factor governing the stoichiometry between N and P in lakes.

In response to episodes of internal P loading in Nakamun Lake, we observed a decrease in N : P from spring to summer in 2009 (Fig. 7), and in the last three years the lake was monitored by a provincial government agency (Orihel 2013, Figs. 4–8). Likewise, TN : TP ratios drop dramatically, as much as fivefold, in July and August in Ford Lake (Lehman 2011), and similar seasonal dynamics in N : P have been observed in shallow Swedish and Danish lakes (Søndergaard et al. 2005; Vrede et al. 2009). Further, Burger et al. (2008) determined that the mass ratio of TN : TP was lower for internal loads (5 : 1) than external loads (15 : 1) in Lake Rotorua, New Zealand, supporting our argument about the important influence of sediments in determining nutrient stoichiometry of surface waters of eutrophic lakes. Although we focused on the release of inorganic P from sediments, recruitment of resting stages of cyanobacteria from sediments to the water column may also contribute to internal P loading (Barbiero and Welch 1992) and may cause sudden decreases in N : P in surface waters (Xie et al. 2003).

Effects on phytoplankton community

Our study demonstrated the important coupling between sediments and phytoplankton in a polymictic lake. Stimulation of algal growth by sediment-derived nutrients was shown by the bioassays we performed using overlying water from incubated sediments (Fig. 5e,f), as well as algal blooms in Nakamun Lake in response to internal P loading in late summer (Fig. 7).

Most notably, we observed a shift in the phytoplankton community from a mixed assemblage composed of chrysophytes, dinoflagellates, and cryptophytes to one dominated by N_2-fixing cyanobacteria (Fig. 8), coincident with a substantial decrease in N : P from spring to summer (Fig. 7c). This observation is consistent with the hypothesis that low N : P favors the dominance of cyanobacteria that was advanced by Schindler (1977), which assumes habitats deficient in inorganic N confer a competitive advantage to N_2-fixing cyanobacteria. This hypothesis has been corroborated by experiments manipulating N : P loading to mesocosms and whole lakes (Schindler et al. 2008; Vrede et al. 2009), and monitoring of eutrophic lakes after reductions in N loading, such as Lakes Peipsi and Võrtsjärv, Estonia (Nõges et al. 2008). Nonetheless, cyanobacteria may not always dominate the phytoplankton community at low N : P because this relationship is modulated by other factors, such as light (de Tezanos Pinto and Litchman 2010) and different niches of cyanobacterial species (Dolman et al. 2012).

Our results also support the hypothesis that low DIN stimulates N_2 fixation by diazotrophic species, which in turn, facilitates the growth of nonheterocystous, potentially toxic cyanobacteria (Beversdorf et al. 2013). The large cyanobacterial bloom that occurred in our study lake in August was mainly comprised of *Anabaena* and *Aphanizomenon* spp. (Fig. 8b) with large numbers of heterocysts (Fig. 7b), as expected from the drawdown of DIN to less than 0.02 mg L^{-1} in early summer. However, this cyanobacterial bloom also included nonheterocystous species, such as *M. icthyoblabe* and *W. naegelianum* (Fig. 8c). Importantly, both *M. icthyoblabe* and *W. naegelianum* likely produce microcystin (Via-Ordorika et al. 2004; Bober et al. 2011), and concentrations of this toxin in Nakamun Lake can exceed Canada's guideline for drinking water (1.5 μg L^{-1}; Fig. 7c) and the proposed guideline for recreational contact (20 μg L^{-1}; Orihel 2013, Fig. 4–8).

Relationship between N : P and microcystin

Orihel et al. (2012) found a strong inverse threshold relationship between N : P ratios and microcystin concentrations across a broad spectrum of Canadian lakes. These

authors concluded that the probability of microcystin exceeding water quality guidelines increases when TN : TP ratios in surface waters are lower, which raised the question of whether low N : P ratios cause, or are simply spuriously correlated with, high microcystin concentrations. Our current study lends insight into this question. Here, we show how low N : P ratios in a eutrophic Canadian lake are part of a sequence of biogeochemical events that can lead to blooms of harmful cyanobacteria, and therefore, enable the potential for microcystin production. As Nakamun Lake is typical of many lakes across the prairie provinces, we believe the mechanisms described in this study contribute to the inverse relationship between N : P ratios and microcystin concentrations reported by Orihel et al. (2012) for Canadian lakes, and subsequently by Harris et al. (2014) for lakes worldwide.

Fe as a micronutrient and nutrient sequestrator

In this study, we examined the role of Fe in sequestering P in sediments, but Fe itself is an essential nutrient required by all phytoplankton. Cyanobacteria have an especially high Fe demand for their physiological processes, including N_2 fixation. Recently, Molot et al. suggested Fe(II) loading from sediments is a critical factor for cyanobacterial bloom formation (Molot et al. 2014). Superficially, this idea may seem to contradict with our conceptual model (Fig. 1), but it is complementary. It is in low-Fe lakes, such as Nakamun Lake, where Fe limitation of cyanobacteria is most likely to occur. Concentrations of total dissolved Fe in Nakamun Lake are usually below detection limits ($< 0.004\,mg\ L^{-1}$) throughout the summer when cyanobacterial blooms occur. Moreover, the physical and biogeochemical processes that lead to internal P loading and low N : P ratios in polymictic lakes are also the same processes that lead to Fe reduction in sediments and Fe(II) loading to overlying water. Nakamun Lake sediments release small quantities of dissolved Fe to overlying water under reducing conditions (Orihel 2013, Fig. S4-1). Therefore, stratification events in low-Fe, polymictic lakes not only result in high P and low N : P in surface waters, but may also provide a pulse of Fe(II) to Fe-starved cyanobacteria.

Implications

Our study highlights the synergy between Fe-poor sediments and polymixis and its effect on cyanobacterial blooms in eutrophic lakes and reservoirs. Although climate change is considered a catalyst for the worldwide expansion of cyanobacterial blooms (Paerl and Huisman 2008) because of their responsiveness to higher temperatures (Kosten et al. 2012), the importance of increased variation in lake mixing is gaining recognition (Jöhnk et al. 2008; Wilhelm and Adrian 2008). Based on our study, we expect that more pronounced series of mixing and thermal stratification events will enhance internal P loading and harmful cyanobacterial blooms, especially in eutrophic lakes lacking adequate concentrations of Fe. Thus, our findings also attest to the need for further research into Fe-based remediation strategies. Although Fe treatments have been used in Europe and U.S.A. (reviewed by Cooke et al. 2005), the application of Fe to control cyanobacterial blooms is in its infancy in Canada and most other countries.

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