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Internal iron loading and warm temperatures are preconditions for cyanobacterial dominance in embayments along Georgian Bay, Great Lakes

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Abstract: Previous work suggests that a high rate of internal ferrous iron (Fe²⁺) loading from anoxic sediments into overlying waters favours cyanobacteria dominance (>50% of the phytoplankton biomass) over eukaryotic algae. This Cyanobacteria–Ferrous conceptual model was assessed along the Georgian Bay coastline of Lake Huron, Ontario, in one meso-eutrophic and three oligotrophic embayments that experience natural hypolimnetic anoxia. Cyanobacteria dominated all embayments in the relatively warmer summer of 2012 but not in the much cooler summer of 2014, although hypolimnetic anoxia and internal Fe²⁺ loading were observed in both summers in all embayments. A cyanobacteria bloom large enough to turn the lake visibly green was observed only in warmer 2012 in the meso-eutrophic embayment. Results show that warm summer temperatures and internal Fe²⁺ loading are necessary preconditions for cyanobacteria dominance, while high nutrient levels are needed to form large blooms. There were no consistent patterns between dominance and total and dissolved phosphorus (P), total nitrogen, ammonium, and nitrate. Internal P loading was not a necessary precondition for dominance. While P removal programs will decrease phytoplankton biomass in eutrophic waters, oxidized surficial sediments must be maintained throughout an aquatic system to prevent cyanobacteria dominance.

Résumé : Des travaux antérieurs portent à croire qu'une forte charge en fer ferreux (Fe²⁺) interne issu de sédiments anoxiques dans les eaux sus-jacentes favorise la prédominance de cyanobactéries (>50 % de la biomasse de phytoplancton) sur les algues eucaryotes. Ce modèle conceptuel reliant les cyanobactéries au fer ferreux a été évalué le long des côtes de la baie Georgienne du lac Huron (Ontario) dans une baie mésoeutrophe et trois baies oligotrophes qui sont le site d'anoxie hypolimnétique naturelle. Les cyanobactéries étaient prédominantes dans toutes les baies durant l'été relativement chaud de 2012, mais non durant l'été beaucoup plus frais de 2014, bien qu'une anoxie hypolimnétique et une charge en Fe²⁺ interne aient été observées pendant les deux étés dans toutes les baies. Une efflorescence de cyanobactéries assez importante pour rendre le lac visiblement vert n'a été observée qu'en 2012, année plus chaude, dans la baie mésoeutrophe. Les résultats montrent que des températures estivales chaudes et une charge en Fe²⁺ interne sont des conditions préalables nécessaires à une prédominance de cyanobactéries, alors que de fortes concentrations de nutriments sont nécessaires à la formation d'importantes efflorescences. Aucun motif cohérent n'a été observé entre la prédominance et le phosphore (P) total et dissous, l'azote total, l'ammonium et le nitrate. Une charge en P interne n'était pas une condition préalable nécessaire à la prédominance. Si les programmes de retrait du P réduiront la biomasse de phytoplancton dans les eaux eutrophes, des sédiments de surface oxydés doivent être maintenus à la grandeur du système aquatique pour prévenir la prédominance de cyanobactéries. [Traduit par la Rédaction]

Introduction

Some of the primary goals of eutrophication management include lowering the risk of algal and cyanobacteria bloom formation and maintaining deepwater oxygen levels above critical levels for fish communities. These goals are typically accomplished at the watershed scale by lowering productivity through nutrient loading controls. The link between excessive phosphorus (P) loading and higher productivity has been known since the 1960s (Sakamoto 1966) with conclusive experimental confirmation published over 40 years ago (Schindler and Fee 1974; Schindler et al. 2008), providing the basis for widespread and often successful management actions to lower productivity by decreasing total P (TP) loading (Dolan 1993; Schindler et al. 2016). Regardless of whether eutrophic systems are nitrogen (N), P, or light-limited, point-source P controls are viewed as one of the primary means to induce nutrient limitation, thereby lowering productivity and, hence, phytoplankton biomass. New nutrient reduction targets for Lake Erie are focused on P loading only (International Joint Commission 2013). Some researchers advocate removing both N and P as a means of mitigating freshwater cyanobacteria blooms (Paerl et al. 2011; Gobler et al. 2016), an idea that has been vigorously disputed (Schindler 2012; Schindler et al. 2008, 2016). In spite of these criticisms, support exists for dual nutrient controls (e.g., US EPA 2015).

One of the hallmark characteristics of eutrophic systems is the high risk of developing potentially harmful cyanobacteria blooms. Here, we use the term "dominant" to describe cyanobacteria populations comprising >50% of the total phytoplankton biomass. Fur-

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ther, the phrase "cyanobacteria bloom" is used in this paper to describe cyanobacteria populations with visible surface accumulations or in which the surface waters are clearly green regardless of whether cyanobacteria are dominant. Empirical among-lakes data show that the risk of cyanobacteria dominance increases with increasing average summer epilimnetic TP and total N (TN) concentrations (i.e., with increasing productivity) and decreasing TN/TP ratios; nevertheless, dominance can occur at most TP concentrations, TN concentrations, and TN/TP ratios (Downing et al. 2001), including systems that are not eutrophic (Chen et al. 2007, 2009; Carey et al. 2008; Vareli et al. 2009). Clearly, TN and TP are important risk factors for cyanobacteria dominance, but other factors must be investigated to consistently explain cyanobacteria dominance over their eukaryotic algal competitors along a trophic gradient.

Molot et al. (2014) hypothesized that much of the unexplained variance and the increased risk of cyanobacteria dominance with nutrient concentrations in Downing et al.'s (2001) meta-analysis might be accounted for by variation in internal ferrous iron (Fe²⁺) loading, a process dependent on development of sediment anoxia. Anoxia is generally correlated with productivity (i.e., with high nutrient levels as demonstrated by the relationship between TP and anoxic factor; Nürnberg 1995). Molot et al. (2014) presented a conceptual model (referred to here as the Cyanobacteria-Ferrous or Cyano-Fe model) describing cyanobacteria bloom formation as a function of sediment anoxia, P, N, Fe²⁺, and sulfate (SO₄²⁻). The central thesis of this conceptual model is that the magnitude of summer phytoplankton biomass is typically controlled by macronutrient concentrations (usually P or N but sometimes light in hypereutrophic systems), but dominance by eukaryotic algae or cyanobacteria is influenced by the availability of Fe²⁺, regardless of what limits total phytoplankton biomass.

The unknown link between TP and TN and risk of cyanobacteria dominance across a macronutrient concentration gradient (Downing et al. 2001) may be explained to some extent by an increasing risk of surface sediment anoxia and internal Fe2+ loading with nutrient enrichment (Molot et al. 2014). Cyanobacteria have high Fe requirements relative to eukaryotic photoautotrophs and can only transport Fe2+ directly across the inner membrane (e.g., Kranzler et al. 2013; see review in Molot et al. 2014; C. Powe and L. Molot, unpublished data). In contrast, eukaryotic algae have lower Fe requirements and can transport Fe³⁺ (ferric Fe) directly across their inner membranes (C. Powe and L. Molot, unpublished data). Cyanobacteria demand for Fe is to some extent mitigated by Fe³⁺ scavenging mechanisms such as siderophores and extracellular capsular polysaccharides (Wilhelm 1995; Rose et al. 2005; Li et al. 2016; Sorichetti et al. 2016), but they are unable to transport Fe³⁺ across their cell membranes without prior reduction. While Fe²⁺ can be generated by biological and photoreduction of Fe³⁺, Molot et al. (2014) hypothesized that rapid reoxidation to Fe³⁺ before transport across the inner membrane lowers cell supply rates. Thus, Molot et al. (2014) hypothesized that large cyanobacteria populations that turn surface waters green (i.e., large blooms) must be supported by high external supply rates of Fe²⁺, and the most likely source of this would be internal Fe²⁺ loading from anoxic sediments. Buoyancy-controlling cyanobacteria can migrate to at least 10-12 m below the surface to acquire nutrients (Camacho et al. 1996, 2000; Head et al. 1999; Gervais et al. 2003), suggesting that in eutrophic systems they may migrate into or adjacent to anoxic bottom waters to acquire Fe²⁺. Thus, diffusion of Fe²⁺ from anoxic sediments into overlying waters may play a role in the success of these taxa. If, on the other hand, the external supply rate is low, then the Cyano-Fe model predicts that a system with a low supply rate of Fe²⁺ will be dominated by eukaryotic species.

The Cyano-Fe model suggests that the timing of a cyanobacteria bloom may in part be related to the seasonal onset of internal Fe²⁺ loading, which in turn is controlled by anoxia, reducible Fe con-

tent of surface sediments, and SO_4^{2-} reduction rate (Molot et al. 2014) in waters with suitable temperatures and light. Evidence to date from eutrophic systems supports the Cyano-Fe model in thermally stratified lakes (Molot et al. 2010, 2014), with more limited evidence from polymictic systems (Smith et al. 2011).

What constitutes a suitable temperature for cyanobacterial bloom formation during the summer is not clear. Cyanobacteria exceeded 20% of the phytoplankton biovolume in eutrophic Lake St. George, Ontario, only when the temperature at 1 m exceeded 21 °C (McQueen and Lean 1987). Several studies have examined the effects of temperature on photosynthesis and growth rate in laboratory cultures of cyanobacteria. The results are difficult to compare because of different growing conditions (e.g., culture media, irradiance, photoperiods, mixing conditions), but in general they describe maximum growth rates between 20 and 30 °C with much lower growth rates below 15 °C (Konopka and Brock 1978; Robarts and Zohary 1987; Butterwick et al. 2005). Paerl and Huisman (2009) suggested that maximum growth rates for eukaryotic algae are lower than those for cyanobacteria in warm waters and that cyanobacteria dominance is favoured in eutrophic waters above 25 °C. However, others have reported that some, but not all, eukaryotic species grow as rapidly as cyanobacteria at 25 to 30 °C in culture (Cole and Christian 2000; Butterwick et al. 2005). Tropical eukaryotic species are inherently adapted to high water temperatures, and transplanted species may not survive cold winters. Confounding our understanding of the impact of temperature on cyanobacterial bloom formation is the impact of climate warming on sediment anoxia via higher respiration rates and longer icefree seasons (Stainsby et al. 2011; North et al. 2014), which promote internal Fe²⁺ loading, thereby favouring cyanobacteria (Molot et al. 2014). Therefore, the temperature-mediated effects on cyanobacterial growth must be separated from those on sediment anoxia and internal Fe²⁺ loading.

In this study, the objective was to examine relationships among water level, temperature, sediment anoxia, nutrient levels, and internal Fe²⁺ and P loading on cyanobacteria dominance and bloom formation in one meso-eutrophic and three oligotrophic embayments along Georgian Bay (northeast Lake Huron in Ontario; Fig. 1). These embayments offer an excellent opportunity to test the validity of the Cyano-Fe model, since they span a range of nutrient regimes and some experience natural hypolimnetic anoxia during the ice-free season that is not linked with anthropogenic nutrient loading (TP concentrations are very low, <10 µg·L⁻¹). Incomplete mixing during early spring means that deep waters begin the stratification season with a dissolved oxygen (DO) deficit, which can lead to anoxia even in oligotrophic systems (Molot et al. 1992). Naturally occurring anoxia is not uncommon in small, oligotrophic forested lakes with a maximum fetch <1.4 km in central Ontario (Molot et al. 1992).

In addition, one meso-eutrophic embayment in particular (Sturgeon Bay) has experienced frequent blooms of *Anabaena* since 1990 (Schiefer 2003). Water level was included in this study because there is a public perception that declining water levels in the Great Lakes in recent years (Georgian Bay Association 2016) has somehow been responsible for the increasing incidence of cyanobacteria blooms.

We hypothesized that warm temperatures, high nutrient levels, and internal Fe^{2+} loading are necessary preconditions for a bloom to form, while warm temperatures and internal Fe^{2+} loading are required for dominance across a trophic range. The study was conducted in a relatively warm (2012) and a much cooler (2014) summer, allowing us to distinguish between the potential impact of temperature and Fe^{2+} availability on cyanobacteria dominance. Seasonal time series data were collected to assess whether anoxia and internal Fe^{2+} loading preceded cyanobacterial dominance, as required by the Cyano-Fe model. If dominance occurs in the absence of either anoxia or internal Fe^{2+} loading, then the Cyano-Fe model must be either modified or rejected. **Fig. 1.** Location of study sites along Georgian Bay, Lake Huron. Inset shows Great Lakes region. Sturgeon Bay is meso-eutrophic, while the other embayments are oligotrophic. (Figure made from Natural Earth: http://www.naturalearthdata.com/.)



Materials and methods

Study sites

Four embayments along the Georgian Bay coast (Lake Huron, Ontario) were the focus of this study. Three of these included the north basin of Sturgeon Bay (45°36'51.1236"N, 80°26'1.7052"W), Deep Bay (45°23'41.3982"N, 80°13'25.7916"W), and Twelve Mile Bay (45°5'0.927"N, 79°56'44.088"W) (Fig. 1). In 2014, North Bay (the north bay of Honey Harbour; 44°53'7.1"N, 79°48'39.8"W) was sampled instead of Twelve Mile Bay. These embayments are located between Honey Harbour in the south and Sturgeon Bay Provincial Park near Pointe au Baril in the north, a distance of about 100 km. Physical characteristics of the study sites are listed in Table 1. The embayments are described in more detail in Schiefer (2003; Sturgeon Bay), Schiefer and Schiefer (2010; Twelve Mile Bay and North Bay), and Schiefer (2005; Deep Bay). The study area is located on the Precambrian Shield, with largely undeveloped forest catchments and wetlands. Many of the embayments along the Georgian Bay coast, including those studied here, have cottage development with numbers, size, and usage increasing over the past few decades from small summer occupancy units to multi-use, yearround establishments (Schiefer et al 2007; Schiefer and Schiefer 2010).

Deep Bay, Twelve Mile Bay, and North Bay are oligotrophic, while the north basin of Sturgeon Bay is meso-eutrophic. Late summer blooms of filamentous cyanobacteria have been reported intermittently for many years in the north basin of Sturgeon Bay (Schiefer 2003). Reports of Sturgeon Bay blooms have increased from relatively infrequent occurrences prior to 1990 to annual events over the past few years. While Sturgeon Bay's relatively high TP concentration and blooms are unusual for the region where most embayments are oligotrophic, there is concern among community members that blooms may spread to other embayments currently unaffected.

Water sample collection

Sampling was biweekly. Sturgeon Bay and Deep Bay were sampled between mid-June and early September in 2012 and between mid-June and early October in 2014. Twelve Mile Bay was sampled between mid-June and early September in 2012, and North Bay was sampled between mid-June and early October in 2014. Embayments were sampled at their deepest points (Table 1). Profiles of conductivity, temperature, and DO were taken using a YSI 6600v2

Table 1. Physical characteristics of the study embayments along theGeorgian Bay, Lake Huron coast.

	Mean depth (m)	Max. depth (m)	Surface area (km²)	Watershed area (km ²)
Sturgeon Bay (north basin)	6	14	2.95	21.9
Deep Bay	10	21	2.85	26.8
Twelve Mile Bay	12	24	2.98	27.2
North Bay		22	2.20	11.2

Sonde connected to a YSI 650 display and data logging system (YSI Inc., Ohio). Prior to recording these measurements, the sonde was allowed time to stabilize between each 0.5 m vertical sampling interval.

Water samples were collected using a horizontal sampler without metal parts at 2 m intervals to a depth of 0.5 m above the sediment. Equal sample volumes from discrete depths were pooled to create integrated epilimnetic and metalimnetic samples, with the depths of the layer boundaries identified each time from temperature profiles; boundaries between the epilimnion, metalimnion, and hypolimnion were defined by the intersection of straight lines drawn through the temperature-depth profile of adjacent layers. To minimize the risk of contamination from previously collected samples, samples were collected from top to bottom of the water column, since surface samples generally have lower nutrient concentrations than deeper waters. Epilimnetic and metalimnetic samples were filtered through 400 µm acid-washed mesh to remove large zooplankters and debris and stored in 4 L acid-washed carboys before pooling. To minimize oxygen contamination after collection, hypolimnetic samples were transferred directly to an acid-washed syringe from the horizontal sampler through a customized T-fitting, which allowed samples to be carefully drawn into and flushed out of the syringe repeatedly until air bubbles were no longer visible in the apparatus. Five millilitres of sample was then used to flush the filter of possible contaminants. The syringe was then filled and filtered into acid-washed 50 mL snap cap vials through 0.2 µm syringe-tip filters (Sartorius cellulose acetate with glass fibre prefilter).

Integrated epilimnetic, metalimnetic, and discrete hypolimnetic samples were subsampled for ammonium (NH_4^+ measured as $NH_3 + NH_4^+$), nitrate (NO_3^- measured as $NO_2^- + NO_3^-$), SO_4^{2-} , total dissolved Fe, and total dissolved P (DP) and filtered through 0.2 μ m syringe-tip filters into acid-washed vials. Dissolved inorganic N (DIN) is defined here as NO₃⁻ + NH₄⁺. Subsamples were also collected for TN and TP and stored unfiltered in acid-washed vials.

Fe²⁺ was not measured directly because of the difficulty of quantifying this form accurately in remote locations and the likelihood of autoreduction of Fe³⁺ to Fe²⁺ by reagents in the presence of dissolved humic matter (Verschoor and Molot 2013). Instead, we assumed that where water column profiles showed large, increasing concentrations of dissolved Fe with increasing depth in anoxic waters relative to metalimnetic and epilimnetic concentrations, such large increases indicated release of Fe2+ from anoxic sediments similar to internal loading of P from anoxic sediments (Nürnberg and Dillon 1993; Molot and Dillon 2003). While some dissolved Fe may include other forms such as Fe sulfide, settling ferric hydroxides, and organic Fe compounds (e.g., Cook 1984; Rickard and Morse 2005), for the purposes of this study it was not necessary to quantify Fe²⁺ accurately, only to confirm that an internal loading profile in anoxic waters was present. We also assumed that increasing DP profiles with depth in anoxic waters was generated by internal P loading (Nürnberg 1984; Nürnberg et al. 1986). Internal P and Fe loading was assumed present in anoxic water if concentrations at the top of the anoxic profile were at least 50% higher than the highest of the metalimnetic or epilimnetic value. Groundwater influxes to these Precambrian Shield systems were assumed confined to the nearshore epilimnetic sediments and thus negligible contributions to the hypolimnetic zone.

Subsamples for phytoplankton identification and enumeration were collected from the integrated epilimnetic and metalimnetic samples and preserved with Lugols iodine (4 mL per 96 mL of sample) in screw-cap centrifuge tubes (Jasprica 2002). Samples were gently mixed and stored in the dark until analysis.

Water chemistry methods

In 2012, TN, NO₃⁻, NH₄⁺, and SO₄²⁻ were analyzed at the University of Alberta Biogeochemical Analytical Service Laboratory in Edmonton. NH₄⁺ (in 2012 and 2104) was analyzed using the Berthelot reaction method (Method 4500-NH₄ F; American Water Works Association 1999) with a detection limit of 2 μ g·L⁻¹. NO₃⁻ was measured colourimetrically after reduction to nitrite (Method 4500-NO₃ I; American Water Works Association 1999) with a detection limit of 1 μ g·L⁻¹. TN was determined by combustion-oxidation to nitrite with chemiluminescence detection (conformed to method D5176; American Society for Testing Materials 2008) with a detection limit of 10 μ g·L⁻¹. SO₄²⁻ was measured using ion chromatography with a detection limit of 0.04 mg·L⁻¹.

In 2012 total and DP were analyzed at the Trent University laboratory in Dorset, Ontario, using ascorbic acid–molybdate colourimetry after autoclave digestion in sulfuric acid (Method 4500-P F; American Water Works Association 1999) with a detection limit of 1 μ g·L⁻¹. Total dissolved Fe was measured at the Water Quality Centre at Trent University in Peterborough, Ontario, with inductively coupled plasma – mass spectrometry (ICP-MS; Thermo XSeries-2) with a detection limit of 1 μ g·L⁻¹.

In 2014, NO_3^- and SO_4^{2-} were analyzed at the University of Waterloo with ion chromatography on a Dionex ICS-2100, total DP and total dissolved Fe were measured at the Trent University Water Quality Centre with ICP-MS (Agilent 8800 Triple Quadrupole), and TN and NH_4^+ were analyzed at the University of Alberta Biogeochemical Analytical Service Laboratory.

Phytoplankton analyses

Phytoplankton samples were identified at the genus level where possible and counted using the Utermöhl method (Lund et al. 1958) and an inverted microscope (Nikon DIAPHOT-TMD, Mississauga, Ontario) with phase-contrast optics. One millilitre of a well-mixed sample was pipetted into a 10 mm diameter, 1 cm deep Utermöhl slide and settled for a minimum of 8 h. Large individual and colonial eukaryotic phytoplankton and colonies and filaments of cyanobacteria were enumerated by counting the contents of the entire chamber at 100× magnification. Very small (\sim 10 µm greatest axial linear dimension) individual eukaryotes and cyanobacteria were enumerated in 20-50 uniformly distributed fields with a minimum of 200 total individuals counted. All phytoplankton were classified by shape and size (Prescott 1970; Wehr and Sheath 2003). Measurements from 10-20 individual cells or colonies for each phytoplankton size-shape classification were taken at the highest practical magnification (up to 1600x) and averaged. Data were organized into as many as 50 distinct size-shape classifications, and the cyanobacterial and eukaryotic phytoplankton biovolumes were calculated by multiplying cell count values by their respective geometric cell volumes (Hillebrand et al. 1999; Olenina et al. 2006; Vadrucci et al. 2007). Biovolume was converted to biomass by assuming an approximate cell density of 1 g·mL⁻¹. Population growth rate was calculated as the slope of ln(biomass) versus time.

Water level and meteorological data

Monthly water level data for Lake Michigan – Lake Huron were available online from Fisheries and Oceans Canada at http:// www.tides.gc.ca/C&A/network_means-eng.html. Mean monthly water levels were derived from observations at six monitoring stations: Thessalon and Tobermory in Ontario; and Milwaukee (Wisconsin), Ludington, Mackinaw City, and Harbor Beach (Michigan) in the US. Levels are referenced to the International Great Lakes Datum 1985. Mean daily air temperatures at Muskoka A station (44°58′00.000″N, 79°18′00.000″W) between 1939 and 2004 were available online at http://climate.weather.gc.ca/historical_data/ search_historic_data_e.html.

Statistical analyses

Redundancy analysis (RDA) was used to determine statistically significant relationships between phytoplankton community composition and environmental factors (epilimnetic and metalimnetic temperature, dissolved Fe at 10 m, redox potential at 10 m, DP at 10 m, epilimnetic TP, epilimnetic NH₄⁺, and epilimnetic NO₃-). Transformations were applied to environmental variables (z score standardization) and phytoplankton composition data (Hellinger transformation). Phytoplankton were excluded when their abundance was less than 10% of the total biomass to avoid a cluttered and unreadable diagram and because the focus of the study was on taxa that were dominant. Only data from Sturgeon in 2012 were used because this was the only lake-year to experience a bloom and cyanobacteria dominance. RDA modeling based on forward selection was performed in R using "rda" in the "vegan" package and "forward.sel" functions in the "packfor" package (R Core Team 2015; http://www.R-project.org). Pearson correlation coefficients were calculated using Microsoft Excel.

Results

Runoff to the embayments is soft water (generally with conductivity <0.08 mS·cm⁻¹), but there are occasional intrusions of harder water through narrow channels connecting the embayments to the open waters of Georgian Bay where the conductivity is typically >0.18 mS·cm⁻¹ (Schiefer et al. 2007; Schiefer and Schiefer 2010). During the summer of 2012, surface conductivity was 0.14–0.18 mS·cm⁻¹ in Twelve Mile Bay, 0.09–0.11 mS·cm⁻¹ in Deep Bay, and 0.08–0.10 mS·cm⁻¹ in the north basin of Sturgeon Bay, suggesting varying mixtures of Georgian Bay water and Precambrian Shield runoff. Surface conductivity in 2014 ranged from 0.12 to 0.13 mS·cm⁻¹ in North Bay. These measurements indicate that Sturgeon Bay and Deep Bay are less susceptible to water exchange with Georgian Bay than Twelve Mile Bay and North Bay.

 SO_4^{2-} concentrations in the embayments reflect a mixture of soft water Precambrian Shield inputs and Georgian Bay water.

Fig. 2. (A) Mean June–August water level (m) in Lake Michigan – Lake Huron between 1918 and 2014 and (B) frequency distribution of mean summer air temperatures for 1939–1989 and 1990–2014 at Muskoka A weather station.



Concentrations in nearby Lake Huron are 15.9 mg·L⁻¹ (Chapra et al. 2012), while in 2012 epilimnetic SO_4^{2-} ranged from 6.8 to 8.6 mg·L⁻¹ in Sturgeon Bay, 7.5 to 8.9 mg·L⁻¹ in Deep Bay, and 10.9 to 12.9 mg·L⁻¹ in Twelve Mile. The higher SO_4^{2-} concentrations in Twelve Mile correspond to higher conductivity in this embayment than in the others, reflecting a greater water exchange rate with Georgian Bay. In June 2012, hypolimnetic SO_4^{2-} concentrations in all embayments, but hypolimnetic concentrations began to decline in July probably because of SO_4^{2-} reduction, with differences of more than 1.5 mg·L⁻¹ from top to bottom in mid-July in Sturgeon Bay and Twelve Mile and in late July in Deep Bay. SO_4^{2-} reduction rates were not high enough to prevent internal Fe²⁺ loading (latter is discussed below).

Mean summer (June–August) water levels in Lake Michigan – Lake Huron between 1918 and 2014 ranged from a low of 175.76 m in June 1964 to a high of 177.39 m in July and August 1986, a difference of 1.63 m (Fig. 2). The mean summer water level for the 25 year periods between 1964 and 1989 and 1990–2014 were 176.8 and 176.4 m, respectively, a difference of only 0.4 m. Therefore, lake levels in the period after 1990, which were associated anecdotally with a high incidence of blooms, were on average only slightly lower than the preceding period. However, water levels after 1999 were consistently low, averaging 176.2 m.

Mean summer air temperatures between 1939 and 2004 at Muskoka A station near Bracebridge, Ontario (the nearest weather station with a continuous record but ending in 2004) ranged from 15.1 °C in 1992 (about 1 year after Mount Pinatubo erupted in the Philippines) to 19.5 °C in 1955, with a mean of 17.4 °C. The anecdotal period of blooms (1990–2004) was slightly warmer than the 15-year prebloom period preceding it (1975–1989); means were 17.6 and 17.3 °C and medians were 17.8 and 17.0 °C, respectively. An alternative way of looking at temperatures is to consider the frequency of a warm years; 41% of the summers were warmer than 17.5 °C during 1939–1989 compared with 67% during 1990–2004 (Fig. 2). The mean summer air temperature was 1.7 °C cooler in 2014 compared with 2012 at nearby Parry Sound Canadian Coast Guard meteorological station (the Parry Sound station lacks a long-term record).

The temporal sequences of chemical and biological changes are an important aspect of the Cyano-Fe model and are examined next.

Sturgeon Bay (2012 and 2014)

The difference in summer air temperatures in 2012 and 2014 had a major effect on surface water temperatures in Sturgeon Bay. The number of days when the top 3 m of the water column were above 20 °C was twice as high in the latter half of June and three times higher in July in 2012 compared with 2014 (Figs. 3A and 3b). In both years, the north basin was thermally stratified when sampling began in mid-June and remained stratified until sampling ended in early September, although the hypolimnion disappeared by mid-July in 2012 and mid-September in 2014, having been entrained into the metalimnion.

Although anoxia is in principle defined to be 0 mg·L⁻¹ DO, internal loading of Fe²⁺ and P was observed above 0 mg·L⁻¹. Indeed, the lowest value recorded in Sturgeon Bay with the YSI was 0.045 mg·L⁻¹, perhaps because of YSI calibration issues. Since the redox potential declined sharply below 0.5 mg·L⁻¹ (Fig. 3C), we used the redox–DO relationship to operationally define the top boundary of the anoxic zone as 0.5 mg DO·L⁻¹. The top of the anoxic zone moved upwards from the bottom (10–12 m depending on where the anchor was dropped) to a depth of 7 m towards the end of July in both years (Fig. 3D).

Dissolved Fe concentrations at 10 m (2 m above the bottom) ranged more than 20-fold from 45 to 2290 $\mu g \cdot L^{-1}$ in 2012 and 147 to 3053 µg·L⁻¹ in 2014 (Table 2). Dissolved Fe concentrations increased between 9 and 12 m after these strata became anoxic. Concentrations reached 1000 μ g·L⁻¹ at 10 m by the end of July in both years compared with epilimnetic concentrations <60 µg·L⁻¹ in 2012 and <150 μ g·L⁻¹ in 2014 (Figs. 4A and 4B). When the 9 m stratum became oxic in August, dissolved Fe concentrations declined from 535 µg·L⁻¹ to much lower levels similar to those found in the epilimnion due to dilution with low Fe water and oxidation of dissolved Fe²⁺ to Fe³⁺ and precipitation. DP also increased in the hypolimnion by late July in both years when anoxia developed, but the increases were not as large as the increases in dissolved Fe (Table 2; Figs. 4C and 4D). Epilimnetic DP levels ranged from 6 to 10 μ g·L⁻¹ in 2012 and 6 to 12 μ g·L⁻¹ in 2014. Hypolimnetic DP reached 55 μ g·L⁻¹ at 10 m in 2012 and 44 μ g·L⁻¹ in 2014, exceeding 180 μ g·L⁻¹ at 12 m near the sediments in both years.

In 2012 cyanobacteria dominated from first half of July through the summer, reaching over 80% of the phytoplankton biomass in the epilimnion by late July. In contrast they did not become dominant in 2014, although they had reached 33% of the biomass by mid-September (Figs. 5A and 5C). Growth of cyanobacteria began after sediment anoxia developed in early summer in both years, reaching "bloom" proportions in 2012 (i.e., the lake was visibly green) but not in 2014 (Figs. 5B and 5D). The epilimnetic biomass peaked at 9300 μ g·L⁻¹ in early September 2012 when it was predominantly *Dolichospermum*, a filamentous N₂ fixer. The most common cyanobacteria in 2014 were *Woronichinia* and *Dolichospermum*. The net summer growth rates for the epilimnetic populations were 0.054 day⁻¹ in 2012 and 0.016 day⁻¹ in 2014 when it was cooler (Fig. 5E).

Mean epilimnetic and metalimnetic nutrient concentrations ranged from 9 to 17 μ g·L⁻¹ TP (Table 2) and 372 to 432 μ g·L⁻¹ TN. TN/TP ratios by mass in the metalimnion and epilimnion ranged from 18 to 73 in 2012 (means 37 and 38, respectively) and 20 to 56 in 2014 (means 29 and 36, respectively), which are generally indicative of P deficiency (Healey and Hendzel 1979). However, while TN/TP ratios were high, epilimnetic and metalimnetic NH₄⁺ and **Fig. 3.** Degree-days above 20 °C in the top 3 m in (A) 2012 and (B) 2014, (C) dissolved oxygen versus redox (dashed line is $0.5 \text{ mg} \cdot \text{L}^{-1}$) in 2012, and (D) the depth at which $0.5 \text{ mg} \cdot \text{L}^{-1}$ dissolved oxygen occurred in Sturgeon Bay in 2012 (solid line, squares) and 2014 (dashed line, circles).



 $\rm NO_3^-$ concentrations were occasionally undetectable, especially immediately before cyanobacteria growth began, indicating that the bulk of the N was present in dissolved organic or particulate form, which may have favoured diazotrophs such as *Dolichospermum*.

Twelve taxa exceeding 10% of the phytoplankton biomass on at least one occasion (excluding picophytoplankton) were used in the RDA of Sturgeon Bay data in 2012: *Dolichospermum, Coelomoron*, Microcvstis, Planktothrix, Woronichinia, Peridinium, Ceratium, Asterionella, Fragilaria, unknown coccoids, unknown diatom "centrics", and unknown algal "medium rounds". Dolichospermum, the most abundant of the cyanobacteria, was closely associated with high dissolved Fe at 10 m and to a lesser degree high epilimnetic NH4⁺ during late summer as well as low redox at 10 m and low epilimnetic NO₃⁻ (Fig. 6). Variance inflation factors for epilimnetic TP, epilimnetic NO₃⁻, and redox potential at 10 m depth were high (45.8-84.9), indicating a high degree of multicollinearity, while variance inflation factors for dissolved Fe and DP at 10 m and epilimnetic NH₄⁺ were relatively low (2.0–7.2). The proportion of variance explained by RDA axis 1 was 49.3%, and the proportion explained by axis 2 was 27.5%. Epilimnetic NH₄⁺ was removed without loss of adjusted R², which was 70%. Global tests of the RDA were significant (p < 0.001), and the first two canonical axes were significant (p < 0.01) with and without epilimnetic NH₄⁺ included.

Deep Bay (2012 and 2014)

The anoxic zone in Deep Bay extended from the sediments upwards to a depth of 7.2 m in early August 2012, while in 2014 it had only extended upwards to 14.5 m by late August (Fig. 7). Dissolved Fe responded to development of anoxia in both years (Table 2) but increased over time at a slower rate than in Sturgeon Bay. In comparison, DP did not increase as bottom waters became anoxic in 2012 but increased by about 7 μ g·L⁻¹ in 2014 (Table 2; Fig. 7).

Cyanobacteria populations were small in this oligotrophic embayment: <400 μ g·L⁻¹ in both years (Fig. 8). Cyanobacteria growth began after the appearance of anoxic sediments. Cyanobacteria dominated the epilimnion at the end of the summer in 2012 but did not dominate the metalimnion in 2012 or either layer in 2014 (Fig. 8). In 2012, the metalimnetic population consisted primarily of the non-N₂-fixing colonial cyanobacteria, *Microcystis*. The epilimnetic population consisted primarily of *Dolichospermum*, which also was the most important cyanobacterium in both layers in 2014.

Mean epilimnetic and metalimnetic TP and TN concentrations ranged from 4 to 20 μ g·L⁻¹ TP (Table 2) and 270 to 480 μ g·L⁻¹ TN. TN/TP ratios by mass in the metalimnia and epilimnia ranged from 19 to 74 in 2012 (means 38 and 45, respectively) and 19 to 49 in 2014 (means 32 and 42, respectively).

Twelve Mile Bay (2012)

Anoxia was first detected in bottom waters (10.5 m) in late June 2012 in Twelve Mile Bay (Fig. 9A). As the summer progressed, the top of the anoxic zone gradually extended upwards to 9 m by early August but was never more than 2 m from the sediments, in contrast with the anoxic zones in Sturgeon Bay (Fig. 5) and Deep Bay (Fig. 7), which were thicker. Internal Fe²⁺ loading was clearly detectable at 10 m in early August (Fig. 9A), having increased to 141 from 48 μ g·L⁻¹ 2 weeks earlier, but the increase at 9 m was much smaller (Table 2). The maximum difference in DP between the bottom and the top of the hypolimnion on any given day was quite small: <2.3 µg·L⁻¹ (Table 2). This small concentration gradient indicates that internal P loading and contributions from mineralization of settling particulate P in the hypolimnion were minimal after anoxia developed. Epilimnetic TP ranged from 5 to 11 μ g·L⁻¹ (Table 2), epilimnetic TN ranged from 208 to 267 μ g·L⁻¹, and epilimnetic TN/TP ratios ranged from 19 to 44.

Clear differences in epi- and metalimnetic cyanobacteria biomass emerged by late August after anoxia and internal Fe loading developed (Fig. 9B). The non-N₂ fixer *Planktothrix* was the dominant taxon in metalimnetic samples collected in late August, reaching 1600 μ g·L⁻¹ in early September (89% of phytoplankton biomass; Fig. 9C). The cyanobacteria biomass was less than 60 μ g·L⁻¹ in the epilimnion during the summer, never exceeding 8% of phytoplankton biomass (Fig. 9C).

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	Sturgeon		Deep		Twelve Mile	North				
	2012	2014	2012	2014	2012	2014				
TP	13.1±2.9 (9.2–16.9)	12.8±3.0 (9.6–17.1)	7.5±2.0 (3.5–9.6)	15.4±4.5 (10.9–20.3)	9.0±2.4 (5.2–11.2)	10.9±3.3 (6.2–14.8)				
NH4+	18±18 (1–49)	19±12 (1–31)	33±68 (1–186)	29±12 (13-41)	20±26 (1-70)	21±28 (1-63)				
NO ₃ -	4±5 (0.5–15)	87±71 (0.5–197)	7±5 (0.5–15)	92±45 (30-145)	10±12 (1-35)	132±50 (7-202)				
DP	18.7±17.6 (6.5-54.7)	19.4±16.6 (6.7-43.8)	5.2±0.6 (4.5-6.0)	12.9±7.3 (5.0-23.8)	4.2±0.6 (3.6-5.2)	6.8±2.4 (4.2–9.7)				
Dissolved Fe	701±838 (45-2290)	1249±1321 (147-3053)	1065±1736 (88-4803)	792±237 (162-669)	37±8 (27–52)	163±209 (49-537)				

Table 2. Mean nutrient concentrations ± 1 standard deviation in the epilimnion (total phosphorus (TP), NH₄⁺, and NO₃⁻) and 2 m off the bottom (dissolved P (DP), dissolved Fe) in the embayments June–September.

Note: Range is in parentheses. Units are μ g·L⁻¹. Depths 2 m off the bottom are as follows: Sturgeon Bay, 10 m; Deep Bay, 16 m in 2012 and 15 m in 2014; Twelve Mile Bay, 9 m; North Bay, 14 m. Values for NO₃⁻ were set at 0.5 μ g·L⁻¹ when below detection limit of 1 μ g·L⁻¹; values for NH₄⁺ were set at 1 μ g·L⁻¹ when below detection limit of 2 μ g·L⁻¹.

Fig. 4. Dissolved Fe (panels A and B) and dissolved P (panels C and D) in Sturgeon Bay epilimnion and several hypolimnetic depths and the depth at which 0.5 mg·L⁻¹ dissolved oxygen occurred in 2012 and 2014.



North Bay (2014)

DO exceeded 0.5 mg·L⁻¹ throughout the water column to the bottom at 16 m until the end of July (Fig. 10A). The top of the anoxic zone reached its shallowest depth of 8 m at the end of August and then began to move downwards. There was a small increase in dissolved Fe of 9 and 20 $\mu g \cdot L^{-1}$ at 14 and 15 m between late July and late August (Fig. 10A), then much larger increases occurred at these depths (463 and 739 µg·L⁻¹, respectively) by mid-September (Table 2). There was no evidence of internal P loading after anoxia was detected with hypolimnetic concentrations less than 10 µg P·L⁻¹ throughout the sampling period except very close to the sediments late in the season in September and October (Table 2). Epilimnetic TP ranged from 6 to 15 μ g·L⁻¹ (Table 2), epilimnetic TN ranged from 316 to 450 µg·L⁻¹, and epilimnetic TN/TP ratios ranged from 29 to 73. Epilimnetic NH₄+ was below the detection limit throughout the study period, while epilimnetic NO₃⁻ was low in mid-June (7 µg·L⁻¹), increasing to higher levels in July and August (184–202 µg·L⁻¹).



C. Sturgeon 2012

Cyanobacteria growth began between late July and late August coincident with development of anoxia and small increases in internal Fe²⁺ loading (Fig. 10B). Cyanobacteria biomass was low and never dominated the phytoplankton community in the epilimnion and the metalimnion (Fig. 10C). Maximum biomass and proportions were reached in the epilimnion in mid-September (244 μ g·L⁻¹, 23% of total phytoplankton biomass) and in the metalimnion in early October (309 μ g·L⁻¹, 41% of total phytoplankton biomass). Cyanobacteria consisted primarily of *Dolichospermum* and *Aphanizomenon*.

TN and TP correlations with biomass

Mean epilimnetic and metalimnetic cyanobacteria biomass was highly correlated with mean epilimnetic and metalimnetic TN (r = 0.91) and mean TP (r = 0.94) (n = 5, (3 epilimnia and 2 metalimnia)) in 2012 but not in 2014 (r = -0.02 for TP and -0.26 for TN, n = 6, (3 epilimnia and 3 metalimnia)).

Fig. 5. Cyanobacteria percent (panels A and B), biomass (panels C and D), and summer growth (panel E) in Sturgeon Bay in 2012 and 2014. The horizontal dashed line in panels A and B is the 50% criterion for defining cyanobacteria dominance. The net growth rates, μ , are shown in panel E with the regression R².





DIN

In 2012, epilimnetic NO_3^- and NH_4^+ concentrations were depleted (i.e., below the detection limit) by the end of June in Sturgeon Bay and Deep Bay (Fig. 11; Table 2), and subsequently, the dominant cyanobacteria in both lakes was N_2 -fixing *Dolichospermum*. In Twelve Mile Bay, metalimnetic NH_4^+ was depleted by early July, while NO_3^- was available until the end of July. The metalimnion was dominated by non- N_2 -fixing *Planktothrix*. NH_4^+ peaked briefly in Sturgeon Bay and Twelve Mile Bay as N_2 -fixing cyanobacteria became dominant and peaked 1 month before dominance in Deep Bay. NO_3^- and NH_4^+ concentrations were higher in Sturgeon Bay and Deep Bay in 2014 than in 2012 (Fig. 11). The positive relationship between *Dolichospermum* and epilimnetic NH_4^+ illustrated in the RDA diagram (Fig. 6) was influenced in large part by the build up in midseason of $\rm NH_4^+$ from leakage or senescence associated with the large population of Dolichospermum (Fig. 11).

Discussion

Do the observations support the Cyano-Fe model?

An important feature of this study is that it interprets ecological and biogeochemical observations from several naturally anoxic, unproductive embayments as well as a meso-eutrophic embayment within a novel framework (the Cyano-Fe model) to explain cyanobacteria dominance and blooms across the productivity gradient. The framework tells us that the factors that control dominance operate in both oligotrophic and eutrophic lakes and that cyanobacteria dominance in oligotrophic lakes differs from blooms

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Fig. 6. Redundancy analysis (RDA) triplot of Sturgeon Bay 2012 phytoplankton response variables, environmental predictor variables, and sampling dates (objects).

RDA1

in more productive lakes only by the size of the populations. We cannot ignore N and P, but the framework forces us to look beyond them.

Two preconditions, warm temperatures and sediment anoxia with internal Fe^{2+} loading, were necessary for cyanobacteria dominance in all embayments regardless of TP, DIN, and TN concentrations. Cyanobacteria did not dominate in 2014 when temperatures were cooler although anoxia and internal Fe^{2+} loading occurred (but reached 30% of phytoplankton biomass in Sturgeon Bay). Three preconditions were needed to generate both cyanobacteria dominance and a large, visibly blooming population: high P levels, internal Fe^{2+} loading, and warm temperatures. These three preconditions were present only in Sturgeon Bay in 2012. These results support the Cyano-Fe conceptual model described by Molot et al. (2014) but modified to include the impact of water temperature on cyanobacteria growth rate.

Mean epilimnetic and metalimnetic cyanobacteria biomass was highly correlated with mean epilimnetic and metalimnetic TN and mean TP in 2012 but not in 2014. These correlations are consistent with the fact that higher nutrient levels support more biomass when temperatures are warm but cannot be used to infer that either N or P concentrations were responsible for cyanobacteria dominance in light of cyanobacteria dominance of the oligotrophic embayments in 2012.

There was no consistent pattern between cyanobacteria dominance and DIN in the embayments. Negative correlations between low NO_3^- and cyanobacteria biomass have been reported before (McQueen and Lean 1987; Nürnberg 2007) and have been interpreted by some as a causal relationship in which cyanobacteria purportedly have a competitive advantage at low NO_3^- and higher NH_4^+ (Hyenstrand et al. 1998). However, in 2012 both NH_4^+ and NO_3^- were depleted in the weeks preceding cyanobacteria popu-

lation growth (conditions during this critical period determine the outcome of cyanobacteria versus eukaryotic algae completion) in Sturgeon Bay and Twelve Mile Bay, and in Deep Bay, $NO_3^$ was higher than NH_4^+ so it seems unlikely that a low NO_3^- /high NH_4^+ ratio regulated the outcome of competition between cyanobacteria and eukaryotic algae.

High TN/TP ratios suggest that the embayments were P-limited, but they could have been N-limited for short periods of time when DIN was undetectable in the euphotic zone, especially in Sturgeon Bay and Deep Bay, which were dominated by N₂-fixing *Dolichospermum*. DIN depletion may have contributed to growth of N₂ fixers in 2012. The NH₄⁺ spike during the cyanobacteria growth periods may have been leakage from N₂ fixation or senescence. N₂ fixation is an Fe-rich process (see review of Fe requirements in Molot et al. 2014), so it is not surprising that growth of N₂ fixers occurred during periods of anoxia accompanied by internal Fe²⁺ loading.

Among the embayments and years, there were no consistent patterns in P concentration before initiation of cyanobacteria population growth and during the periods of cyanobacteria growth. For example, internal P loading occurred in Sturgeon Bay but not in Twelve Mile Bay or Deep Bay in 2012, although all three embayments were dominated by cyanobacteria and had anoxic hypolimnia. In Sturgeon Bay, cyanobacteria did not exceed 50% of the phytoplankton biomass in 2014 even in the presence of internal P loading.

The time series monitoring used in this study reinforces the conclusions of other studies (e.g., the experimental removal of Fe^{2+} in Lake 227 (Molot et al. 2010) and observations in Lake 227 and Hamilton Harbour (Molot et al. 2014)) that show an important role for internal Fe^{2+} loading in the competitive outcome between eukaryotic algae and cyanobacteria.

C. Deep 2012

Fig. 7. Dissolved Fe (panels A and B) and dissolved P (panels C and D) in the epilimnion and several hypolimnetic depths and the depth at which 0.5 mg·L⁻¹ dissolved oxygen occurred in Deep Bay in 2012 and 2014.

0 18 29-Jun 24-Aug 1-Jun 27-Jul 21-Sep Episodic anoxia and internal Fe²⁺ loading may promote cyanobacteria bloom formation in shallow polymictic lakes. The sediment-water interface in eutrophic Missisquoi Bay, Lake Champlain, was hypoxic (~1 mg·L⁻¹) at the beginning of a cyanobacteria bloom on 27 and 28 July 2008 (Smith et al. 2011). Fe2+ was detected 2-3 mm below the sediment surface, although the very high detection limit of the voltammetric method (~1100 µg·L⁻¹ for Fe²⁺ compared with 1.0 µg·L⁻¹ for total Fe using ICP-MS) does not exclude the possibility that biologically relevant concentrations occurred much closer to or even above the sediment surface. In late August, the full bloom in Missisquoi Bay was coincident with anoxic conditions and relatively high concentrations of Fe²⁺ (1100–2200 $\mu g \cdot L^{-1}$) at the sediment-water interface, suggesting that winds were light and water column mixing was not turbulent, which would allow an anoxic boundary layer to form at the sediment-water interface. In eutrophic, polymictic Clear Lake in California, where an Aphanizomenon bloom occurred each spring, the sediment surface was frequently anoxic but often of short duration (Wurtsbaugh and Horne 1983).

The Baltic Sea is a eutrophic, brackish water system with a long history of cyanobacteria blooms (Kahru et al. 2007; Funkey et al. 2014). In a paleolimnological study in the Baltic Sea, hypoxia and cyanobacteria abundance were correlated during an 8000-year period (Funkey et al. 2014). The increased abundance may have been caused by higher P concentrations from internal P loading. Phosphate release is typically coupled to reduction of ferric hydroxides that release Fe²⁺ when sediments become anoxic (Jensen and Andersen 1992), so if internal P loading occurred during episodes of water column hypoxia, it is possible that internal Fe²⁺ loading also occurred.

Favourable conditions for cyanobacterial dominance may exist in other naturally anoxic oligotrophic embayments along the Georgian Bay coast, although TP concentrations in these embayments are not high enough to cause large surface blooms. Favourable growth conditions for cyanobacteria in these embayments include a combination of natural anoxia caused by incomplete spring turnover (Molot et al. 1992), an adequate source of easily reducible Fe in sediments, and SO_4^{2-} concentrations low enough not to completely inhibit internal Fe²⁺ loading via formation of Fe sulfides (Loh et al. 2013).

Water level, warmer waters, and incidence of cyanobacteria blooms

The publically perceived increase in cyanobacteria blooms in Sturgeon Bay after 1990 (Schiefer 2003; Georgian Bay Association 2016) may be related to a warming trend rather than to changes in land use or water level in Georgian Bay (Fig. 2). Water levels were consistently at the low end of the historical range between 1999 and 2014 but were at the higher end between 1990 and 1998 (Fig. 2) (Gronewold and Stow 2014); hence, it appears unlikely that changes in water levels alone explain all of the blooms reported after 1990. Diatom-inferred TP concentrations in Sturgeon Bay have been stable and relatively high (20–24 μ g·L⁻¹) since about 1800, suggesting that the TP-enriched state is natural (Cumming et al. 2006) and thus not a result of cottage development in recent decades.

Cumming et al. (2006) also reported increases in pigment concentrations of total diatoms and colonial cyanobacteria in Sturgeon Bay occurred after approximately 1950, which are consistent with public reports of increased incidence of cyanobacteria blooms. It was speculated that shifts in diatom species composition in recent decades to those that prefer more stable thermal stratification can be attributed to climate change and warmer surface waters. The Muskoka A weather records show a warming trend between 1950 and 2004, but the record does not go back far enough to compare periods before and after 1950. An analysis of Canadian weather records by Zhang et al. (2000) between 1900 and 1998 revealed a summer warming trend in the Georgian Bay area





epilimnion

Fig. 8. Cyanobacteria percent (panels A and B) and biomass (panels C and D) in Deep Bay in 2012 and 2014. The horizontal dashed line in panels A and B is the 50% criterion for defining cyanobacteria dominance.



400 0 epilimnion 0.5 mg L⁻¹ O₂ depth, m 3 Cyanobacteria, µg 300 metalimnion 6 DO depth 200 9 12 100 15 0 18 1-Jun 29-Jun 27-Jul 24-Aug 21-Sep 0 400 D. Deep 2014 Cyanobacteria, µg L⁻¹ ε 3 epilimnion depth, 300 metalimnion 6 - DO depth 200 9 ဝ် 12 0.5 mg (100 15 0 18 01-Jun 29-Jun 27-Jul 24-Aug 21-Sep 19-Oct

C. Deep 2012

of about 1 °C per 99 years in daily maximum air temperature and over 2 °C per 99 years in daily minimum air temperature, most of which seem to have occurred after 1950. While the effect of warmer maximum daily air temperatures during the summer on lake warming is well understood, the increase in daily minimum air temperatures is no less important if it decreases overnight cooling (Lehman 2002).

While the extent of anoxia, nutrients, and implied availability of Fe²⁺ (from dissolved Fe profiles) were similar in 2012 and 2014 in Sturgeon Bay, temperatures were not, and we conclude that cyanobacteria growth was probably influenced by temperature. The net cyanobacteria growth rate in cooler 2014 was about 30% of the net growth rate in 2012. The warming regional climate is consistent with the public perception of increased incidence of cyanobacteria blooms in Sturgeon Bay and in Ontario in general (Winter et al. 2011).

While cyanobacteria may respond to warmer summers with higher growth rates, other climate-related mechanisms might also favour their dominance, such as longer ice-free seasons and thermal stratification periods (Stainsby et al. 2011). These longer periods will lengthen the extent of the hypolimnetic oxygen depletion season, which in turn could lead to an increase in the spatial and temporal extent of anoxia (Foley et al. 2012; North et al. 2014) and internal Fe²⁺ loading, especially in lakes that are susceptible to development of anoxia. Analysis of long-term oxygen and temperature records from Lake Simcoe in central Ontario shows that the lengthening of the ice-free season since 1980 by 30 days was accompanied by a lengthening of the oxygen depletion period in the hypolimnion (Li 2016).

Implications of the Cyano-Fe model for management

To prevent cyanobacteria blooms, the amount of substances contributing to exhaustion of dissolved oxygen must be lowered to levels that maintain surface sediments in an oxidized state. If a P removal program in a eutrophic system does little to improve sediment redox, then blooms will continue although productivity might be lower under the new loading regime.

The Cyano-Fe model can contribute to improved eutrophication management by leading us to design TP concentration and loading targets that prevent low redox levels in surficial sediments. For example, the current guideline for maximum acceptable TP concentration in Ontario is 20 µg·L⁻¹ (Ontario Ministry of Environment and Energy 1994), which is assumed to prevent cyanobacteria blooms based on historical data. However, increasing incidences of blooms in Lake Erie (Conroy et al. 2005; Michalak et al. 2013) and in inland oligotrophic and mesotrophic lakes in recent years (Carey et al. 2008; Winter et al. 2011) suggest that this target is not universally applicable, and more stringent targets may now be required for some lakes. The relationship between sediment redox and bloom formation suggests that nutrients (and perhaps organic matter loading, especially to polymictic systems) should be managed with explicit objectives to decrease productivity and DO consumption to nutrient levels that will maintain DO concentrations above a critical level at the sediment-water interface.

If external TP loading criteria were based on a mechanistic understanding of the relationship among external P loading, anoxia, and internal Fe²⁺ loading for a given lake and climate regime, lake-specific loading targets could be developed to decrease the spatial and temporal extent of anoxia. Similarly, in situ aeration might be more effective at preventing cyanobacteria blooms if systems were designed to deliver oxygen at a rate that leaves surficial sediments with a sufficiently high redox over a larger lake area, including shallow bays, than is currently considered necessary.

Schindler and his colleagues argued that N removal would have little impact on bloom formation in eutrophic lakes because **Fig. 9.** The depth at which $0.5 \text{ mg} \cdot \text{L}^{-1}$ dissolved oxygen occurred and dissolved Fe concentration (A), cyanobacteria biomass (B), and cyanobacteria percent (C) in Twelve Mile Bay in 2012. The horizontal dashed line in panel C is the 50% biomass criterion for defining dominance.

Fig. 10. The depth at which 0.5 mg·L⁻¹ dissolved oxygen occurred and dissolved Fe concentration (A), cyanobacteria biomass (B), and cyanobacteria percent (C) in North Bay in 2014. The horizontal dashed line in panel C is the 50% biomass criterion for defining dominance.







cally costly (Stam et al. 1987). Scott and McCarthy (2010) argued that N₂ fixation rates are, in general, too low to alleviate N deficiency; however, N₂ fixation was clearly important in Clear Lake, California, providing over 40% of the annual N budget (Horne and Goldman 1972), even though N₂ fixation was at times Fe-limited (Wurtsbaugh and Horne 1983). The evidence to date and the Cyano-Fe model suggest that if managers want to shut off N₂ fixation, they will have to shut off internal Fe²⁺ loading.

Laboratory studies indicate that N removal will have a negligible impact on cyanobacteria growth in natural systems as long as sufficient micronutrients, including Fe²⁺, are available. Growth rates and maximum biomass yields of heterocystous N₂ fixers in nutrient-replete, single-species laboratory cultures using only N₂ as a source of N were only slightly different (approximately 10%) than when grown with NH₄⁺ and NO₃⁻ (Allen and Arnon 1955; Bagchi et al. 1985; Schlangstedt et al. 1987; Vargas et al. 1998). A lower growth rate caused by partial or whole dependence on N₂

Fig. 11. NH_4^+ and NO_3^- (i.e., dissolved inorganic nitrogen, DIN) and cyanobacteria biomass (μ g·L⁻¹) in 2012 in (A) Sturgeon Bay (epilimnion), (B) Deep Bay (epilimnion), and (C) Twelve Mile Bay (metalimnion). Circles denote when cyanobacteria biomass first exceeded 50% of total phytoplankton biomass.



implies that it will take longer for an N₂-fixing cyanobacteria population to reach its maximum yield. It is also possible that the lag phase of the growth curve might be longer in situ, which could affect the timing of bloom onset and maximum biomass. Nevertheless, all of the $\rm NH_4^+$ and $\rm NO_3^-$ had to be removed from these cultures to achieve a modest difference in maximum population size.

There is, however, one exception regarding the effectiveness of N removal. The Cyano-Fe model predicts that cyanobacteria dominance will cease with nutrient removal only if sediment redox is maintained at a level that suppresses reduction of Fe³⁺ to Fe²⁺. In aquatic systems where P controls have not been able to sufficiently diminish internal Fe²⁺ loading rates (i.e., systems in which sediment redox remains low) and additional P controls are not feasible, nitrification of NH₄⁺ without N removal in wastewater treatment plants (WWTP; i.e., no denitrification step) might be beneficial in cases where external N loading to watersheds is dominated by WWTP point sources because in situ nitrification by

nitrifying bacteria can be a major oxygen sink in eutrophic lakes (Müller et al. 2012; Clevinger et al. 2014). WWTP nitrification will lower in situ oxygen demand and could raise the sediment redox level sufficiently in watersheds where point source N loads are relatively large. In addition, subsequent in situ NO₃⁻-based respiration can raise sediment redox, thicken the oxidized layer in surficial sediments, and suppress internal P and Fe2+ loading (Jensen and Andersen 1992; Kleeberg and Kozerski 1997; Hemond and Lin 2010). Hence, WWTP nitrification before effluent discharge might be a beneficial course of action in some situations as long as aquatic health standards for NO₃⁻ are not exceeded. The emphasis here is on the impact that WWTP nitrification has on sediment redox, not on phytoplankton macronutrient supply, since the total external N supply will not be affected (although internal loading of N and P might be lowered by a higher sediment redox). Nutrient export from diffuse urban and agricultural sources is acknowledged as very important in many watersheds, but discussion of management of diffuse sources is beyond the scope of this paper.

If N removal fails to suppress a bloom because of a failure to improve sediment redox, it will shift cyanobacteria species composition to N_2 fixers if it shifts a lake from P to N deficiency. It may also affect cyanobacteria toxin production, although the impact on toxin production is unclear (Pick 2016). Low N appears to mitigate toxin production in non- N_2 -fixing *Microcystis* (Harke et al. 2015; Gobler et al. 2016); however, N_2 -fixing *Aphanizomenon* produces more toxin under moderate N stress (Gagnon and Pick 2012). These studies suggest that toxin production varies unevenly along an N gradient from strongly excessive N relative to P to severe N depletion.

A final management consideration is that WWTP N removal (both nitrification and denitrification) might be needed in some locations to help mitigate marine coastal eutrophication (i.e., reduce coastal productivity), especially in riverine systems with large point source loads and low N retention rates.

This study represents the first test of the Cyano-Fe conceptual model in natural systems. This and other studies (Molot et al. 2010, 2014) support the model modified to include the impact of water temperature on cyanobacteria growth rate. The collective evidence from contrasting oligotrophic and meso-eutrophic freshwater systems suggests that warm temperatures, anoxia, and internal Fe²⁺ loading are critical preconditions for cyanobacteria dominance, and these preconditions along with high P are needed for relatively large, visible blooms to form.

The Cyano-Fe model could be useful for improved management of cyanobacteria blooms, but more work is needed to test validity of this conceptual model that couples the oxidation state of surficial sediments and geochemical cycles to cyanobacteria bloom formation under a range of real conditions, especially in polymictic and brackish waters. Paying close attention to the period prior to bloom onset will be critical to improving our mechanistic understanding of how blooms form.

A warming climate will increase the risk of sediment anoxia and internal Fe²⁺ loading as well as increase water temperatures into a more optimal range for cyanobacteria growth in temperate regions. A warming climate, therefore, has implications for greater incidence of cyanobacteria dominance along a trophic gradient and bloom formation in P-rich waters.

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