J. Great Lakes Res. 31(Suppl. 2):180–194
Internat. Assoc. Great Lakes Res., 2005

Determination of Bioavailable Fe in Lake Erie Using a Luminescent Cyanobacterial Bioreporter

David Porta[1], George S. Bullerjahn[1], Michael R. Twiss[2], Steven W. Wilhelm[3], Leo Poorvin[3], and R. Michael L. McKay[1,*]

[1]*Department of Biological Sciences*
Bowling Green State University
Bowling Green, Ohio 43403

[2]*Department of Biology*
Clarkson University
Potsdam, New York 13699-5805

[3]*Department of Microbiology*
The University of Tennessee
Knoxville, Tennessee 37996

ABSTRACT. *Low Fe bioavailability has been suggested as a potential constraint on primary production in the Great Lakes. Here we report on the use of a cyanobacterial bioreporter to assess available Fe in Lake Erie during summer and fall field seasons in 2001–02. Bioreporter luminescence was derived from a luciferase reporter controlled by iron-responsive promoter element* isiAB. *Filtered (< 0.2 μm) water sampled from the western basin during summer 2001–02 yielded low bioreporter response indicating Fe sufficient conditions [-log [free Fe^{3+}] (pFe) < 20.8]. Likewise, water collected from the eastern basin following autumnal mixing in November 2001 yielded a Fe sufficient bioreporter response. In contrast, surface water collected at pelagic stations located in central and eastern basins during summer 2002 indicated a seasonal depletion of bioavailable Fe. Whereas water sampled from these locations during July and August was characterized as Fe sufficient (pFe < 20.8), samples collected during September elicited a high luminescent response from the bioreporter (pFe > 21). Contrary to the characterization provided by the filtered samples, assay of bioreporter response in unfiltered water conducted during the September 2002 cruise indicated these samples to be Fe sufficient (pFe < 20.6). Although this suggests that the dominant pool of bioavailable Fe is contained in the particulate fraction, we cannot discount the possibility that the bioreporter was rendered Fe sufficient by Fe regenerated predominantly from bioreporter cells themselves. Thus, while it is clear that regenerative processes contribute to the pool of bioavailable Fe, it is equally clear that future efforts using the bioreporter with unfiltered water samples must account for the potential influence of Fe introduced by the added reporter cells.*

INDEX WORDS: *Bioreporter, iron,* isiAB, *Lake Erie,* luxAB, Synechococcus *sp. PCC 7942.*

INTRODUCTION

Our insights on phytoplankton production in eutrophic and mesotrophic systems such as Lake Erie have been guided largely by considerations of N and P availability (Elser *et al*. 1990, Guildford and Hecky 2000, Wilhelm *et al*. 2003). Until recently, there had been little cause to consider the role of other elements in these systems owing largely to our perception that they are plentiful, a view reinforced by several early surveys of bioactive trace metal distributions in the Great Lakes (see Sturgeon and Berman 1987). However, in light of overwhelming evidence in some marine environments suggesting that low availability of trace metals, specifically Fe, ultimately limit primary production, there is cause to reexamine the relationship between trace metals and phytoplankton nutrition in freshwater systems.

The role of Fe in basic physiological processes

*Corresponding author. E-mail: rmmckay@bgsu.edu

such as photosynthesis, respiration, and nitrogen assimilation makes it one of the most important nutritive factors for phytoplankton growth. Although Fe is abundant in the Earth's crust, numerous studies have demonstrated that low iron bioavailability limits phytoplankton growth in diverse marine environments (Martin *et al*. 1991, Coale *et al*. 1996, Boyd *et al*. 2000, Hutchins *et al*. 1998, Tsuda *et al*. 2003). This apparent contradiction is attributed to the particular features of Fe biogeochemistry in the aquatic milieu that may lead to the precipitation or complexation of Fe (McKay *et al*. 2004). These considerations combined with sporadic low input of terrestrial-derived Fe to many of these marine environments results in Fe deficiency to the endemic phytoplankton assemblage.

The results of several studies support a role for trace nutrient deficiency in freshwater environments (Schelske 1962, Wetzel 1966) including the Great Lakes (Schelske *et al*. 1972, Lin and Schelske 1981, Hartig and Wallen 1984, Twiss *et al*. 2000, Sterner *et al*. 2004). This is consistent with our more recent perspectives on the abundance of trace metals in the Great Lakes by researchers adopting metal-clean sampling techniques (Coale and Flegal 1989; Nriagu *et al*. 1993, 1996; Field and Sherrell 2003). It is now apparent that dissolved bioactive trace metals are present in low nanomolar to picomolar concentrations throughout most of the Laurentian Great Lakes system.

Lake Erie is the smallest of the Great Lakes by volume yet it supports a population base of over 11 million people and is arguably the most heavily affected by anthropogenic activity, both in terms of industry and agriculture. Despite its relative shallow bathymmetry and high coastal influence, dissolved Fe (Fe_d; < 0.2 μm) is generally low in offshore waters of the central and eastern basins of the lake (≤ 10 nM; Nriagu *et al*. 1996, Twiss *et al*. 2000, Durham *et al*. 2002, Mioni *et al*. 2003).

Although measures of Fe_d can provide a first-order estimate of the potential for Fe deficiency, it is becoming apparent that Fe_d is not synonymous with bioavailable Fe. Some particulate forms of Fe appear to be bioavailable (Mioni *et al*. 2003) and some complexed Fe associated with the operationally-defined dissolved phase (viz. < 0.2–0.45 μm) may not be available for uptake (Hutchins *et al*. 1999).

We have developed a luminescent cyanobacterial bioreporter as a tool to assess Fe bioavailability in freshwaters (Durham *et al*. 2002, Porta *et al*. 2003, McKay *et al*. 2004). The bioreporter features the Fe-regulated *isiAB* promoter (Straus 1994) fused to *luxAB* encoding bacterial luciferase. A comprehensive physiological characterization of the bioreporter demonstrated a dynamic range of response between -log [Fe^{3+}] (pFe) 21.1 and pFe 20.6. Within this range, the bioreporter emits light in inverse proportion to the amount of bioavailable Fe in solution. This provides a means to assess Fe bioavailability from the perspective of a living organism, in this case, a cyanobacterium of the genus *Synechococcus*, an ecologically important member of the phytoplankton assemblage in many lakes (Callieri and Stockner 2002) including the Laurentian Great Lakes (Caron *et al*. 1985; Fahnenstiel *et al*. 1986; Pick and Caron 1987; Fahnenstiel *et al*. 1991a, 1991b; Fahnenstiel and Carrick 1992).

The objective of this study was to use the Fe-dependent bioreporter to determine Fe bioavailability as a function of Fe_d concentrations in a wide range of water column environments in Lake Erie. The purpose was two-fold: to assess the frequency of potential Fe-limitation to phytoplankton in this system, and to examine the utility of the newly developed Fe-bioreporter.

METHODS

Strain and Culture Conditions

Bioavailable Fe was determined using a cyanobacterial *Synechococcus* strain sp. PCC 7942 p*isiAB*::*luxAB/CDE* construct (KAS101; Durham *et al*. 2002, Porta *et al*. 2003). The strain was grown in semi-continuous batch culture in trace metal-buffered Fraquil medium containing Fe at pFe 20.6. The formulation for Fraquil is available from Cyanosite (http://www-cyanosite.bio.purdue.edu/). Macronutrient stocks were treated with Chelex-100 resin prior to their addition to the medium (Price *et al*. 1988/1989). Spectinomycin and kanamycin were added to the culture medium at 20 μg/mL to preserve the integrity of the *luxAB/CDE* chromosomal construct (Durham *et al*. 2002). The cultures were maintained at 24°C under continuous illumination of *ca.* 50 μmol photons/m^2s.

Sampling Locations

Water samples were collected from 33 hydrographic stations located throughout Lake Erie and its connecting waterways and tributaries (Table 1) during six research cruises over a 2-year period extending between July 2001 and September 2002. Two research platforms were used during this

TABLE 1. Sampling stations and coordinates.

Sampling Station	Station Location	Sampling date	Depth (m)	Dissolved Fe (nM)
		Lake Erie Eastern Basin		
ER 15	42°31′00″N 79°53′36″W	18 Aug 2002	5	3.09
		17 Sep 2002	10	5.44
		17 Sep 2002	27	9.75
ER 63	42°25′00″N 79°48′00″W	17 Sep 2002	10	5.96
LE 23	42°30′41″N 79°53′24″W	10 Jul 2001	10	46.3
		10 Jul 2001	15	n.a.
		10 Jul 2001	25	9.78
		5 Nov 2001	10	137
		22 Jul 2002	10	n.a.
LE 442	42°50′28″N 79°23′35″W	9 Nov 2001	10	216
LE 449	42°45′41″N 79°59′13″W	7 Nov 2001	5	21.6
LE 450	42°42′08″N 79°55′00″W	7 Nov 2001	10	24.6
LE 451	42°38′53″N 79°53′20″W	7 Nov 2001	10	100
LE 452	42°35′04″N 79°55′20″W	6 Nov 2001	10	257
LE 453	42°47′52″N 79°34′43″W	9 Nov 2001	10	104
LE 560	42°44′07″N 79°39′54″W	7 Nov 2001	10	122
LE 561	42°36′36″N 79°46′34″W	7 Nov 2001	5	12.6
LE 933	42°49′14″N 79°35′15″W	8 Nov 2001	5	156
LE 938	42°49′00″N 80°03′30″W	6 Nov 2001	5	312
Grand River 1		9 Jul 2001	surface	1,080
Grand River 2		9 Jul 2001	surface	217
Grand River 3		9 Jul 2001	surface	45
Grand River		8 Nov 2001	surface	1,132
		Lake Erie Central Basin		
ER30	42°25′48″N 81°12′18″W	18 Aug 2002	10	5.97
		15 Sep 2002	10	7.71
ER 31	42°15′12″N 81°06′24″W	18 Aug 2002	10	15.1
		15 Sep 2002	10	6.43
ER 42	41°57′54″N 82°02′30″W	14 Sep 2002	epilimnion	10.8
ER 43	41°47′18″N 81°56′42″W	20 Aug 2002	10	2.70
		14 Sep 2002	10	19.9
ER 78	42°07′00″N 81°15′00″W	19 Aug 2002	10	8.11
		15 Sep 2002	10	3.93
LE 84	41°55′57″N 81°39′35″W	11 Jul 2001	10	4.08
		11 Jul 2001	20	n.a.
		12 Jul 2001	10	3.65
		15 Jul 2001	10	3.62
		15 Jul 2001	21	10.9
		19 Jul 2002	5	5.07
		Lake Erie Western Basin		
ER 61	41°56′48″N 83°02′42″W	17 Sep 2002	5	84.2
ER 91	41°50′27″N 82°55′00″W	21 Aug 2002	5	404
		18 Sep 2002	7	27.1
LE 357	41°48′44″N 82°58′55″W	14 Jul 2001	5	316
		17 Jul 2002	5	25.5
LE 969	41°36′29″N 82°55′34″W	14 Jul 2001	2	34.8
LE 1163	41°28′16″N 82°43′10″W	14 Jul 2001	surface	27.5
LE 1156	42°02′50″N 83°08′05″W	18 Jul 2001	surface	19.3
LE 1169	42°19′57″N 83°01′17″W	19 Jul 2001	surface	40.8
LE 1160	42°21′46″N 82°54′12″W	19 Jul 2001	surface	20.9
LE 510	42°28′57″N 82°43′14″W	19 Jul 2001	surface	11.3
LE 1164	42°39′06″N 82°30′28″W	19 Jul 2001	surface	11.3

study: the CCGS *Limnos* (July 2001, 2002; November 2001) and the R/V *Lake Guardian* (August 2002, September 2002).

Bioreporter Response from Lake Water

At each hydrographic station, water was collected using a metal-clean sampling pump as described previously (Wilhelm *et al*. 2003). Samples were pre-screened through acid-rinsed 210-µm pore size polypropylene mesh (Spectra/Mesh®; Spectrum Laboratories Inc., Rancho Dominguez, CA). Filtered samples (operationally-defined dissolved fraction) were passed through a 0.2 µm pore size Polycap AS cartridge filter (Whatman Inc., Clifton, NJ) into acid-rinsed polycarbonate bottles and immediately frozen. Unfiltered water was collected directly into polycarbonate bottles and processed on the vessel. To further minimize contamination, all materials were soaked in 10% HCl (Trace Metal Grade; Fisher Scientific, Pittsburgh, PA) for at least 24 h and rinsed thoroughly with deionized (> 18 MΩ/cm) water prior to use. All manipulations were performed in a metal-clean environment; either in a HEPA-filtered laminar flow bench (R/V *Lake Guardian*) or in a laminar flow bench contained in a portable clean van (CCGS *Limnos*). Bioreporter response was calibrated via standards run in triplicate in trace-metal buffered Fraquil medium prepared over a range of free ferric ion concentrations (Porta *et al*. 2003). Calibration yielded a dose-response curve for each experiment from which pFe in environmental samples could be extrapolated by regression analysis.

Lake water samples (20 mL) were distributed to replicate acid-rinsed polycarbonate tubes (n = 3–4) and were seeded with bioreporter cells pre-rinsed with Fe-free Fraquil medium and incubated for 6 or 12 h at 24°C under continuous illumination of *ca.* 50 µmol photons/m^2/s. Direct cell counts indicated bioreporter cell density to be *ca.* 6×10^8 cells/L, a concentration comparable to the maximum reported autotrophic picoplankton cell density reported in the Laurentian Great Lakes (Caron *et al*. 1985). Following incubation, 2-mL aliquots were transferred to glass tubes and exposed to vapors of *n*-decyl-aldehyde (decanal; Sigma Chemical Co., St Louis, MO) for 50 min as described by Porta *et al*. (2003). Luminescence was measured using a Femtomaster FB14 luminometer (Zylux Corp., Maryville, TN). Positive and negative controls were obtained by the addition of desferrioxamine mesylate (desferal; Sigma) and $FeCl_3$, respectively. For samples from the 2001 cruises, Chelex-100 (Bio-Rad, Hercules, CA) treated N and P macronutrient stocks (BG-11 medium: Allen 1968) and a Fe-free Fraquil micronutrient stock were added in order to relieve deficiency by nutrients other than Fe. Subsequent experiments run with parallel unamended water samples provided similar results (data not shown). Thus, no additional nutrients were added to filtered water samples acquired in 2002.

Direct counts of glutaraldehyde-preserved *Synechococcus* cells were conducted in order to normalize measures of luminescence. Chlorophyll (chl) *a* autofluorescence was detected using a Leica DMRXA Microscope with epifluorescence attachment (Leica Microsystems, Inc., Buffalo, NY) and Image Pro Plus software (version 4.1; Media Cybernetics, Inc., Silver Spring, MD). For assays using unfiltered water, bioreporter luminescence was normalized against *in vivo* chl *a* fluorescence measured using a TD-700 laboratory fluorometer (Turner Designs, Sunnyvale, CA).

Chemical Fe Analysis

Samples filtered (< 0.2 µm) in the field were stored in Teflon™ bottles and frozen. Iron content in lake water samples was measured by Zeeman-corrected graphite furnace atomic absorption spectrophotometery (GFAAS) (Model 4110; Perkin-Elmer, Wellesley, MA) using standard conditions. Samples were acidified with HNO_3 (Baseline; Seastar Chemicals Inc., Sidney, BC) to a final concentration of 1.6 mM HNO_3 and 40 µL sub-samples were analyzed by direct injection with 10 mg of $Mg(NO_3)_2$ matrix modifier onto transversely heated pyrolytically-coated graphite tubes containing L'Vov platforms. Three replicate measurements were made from each sample that was filtered in the field. Analytical accuracy was assured by analysis of SLRS-4 standard freshwater reference material (National Research Council of Canada) that was diluted 200-fold to 9.2 nM in order to be within the expected range of Fe_d content; values obtained for SLRS-4 were always within the range of the certified value.

Particulate Fe was determined by filtering 250 mL of lake water onto an acid-cleaned (dilute HCl) 0.2 µm pore-sized polycarbonate membrane filter (47 mm diameter; Millipore, Billerica, MA). The filter and retained seston were placed in a 7-mL round-bottomed Teflon™ jar. The seston was digested by adding 1 mL of concentrated HNO_3 (Baseline; Seastar). After 3 days of digestion at

20°C, Fe content was determined as above following an appropriate dilution.

Fe Scavenging Model

The static scavenging model of Twiss and Campbell (1998b) was run using an initial concentration of 6 nM Fe_d, the median value for eastern and central Lake Erie during thermal stratification. The ability of an organism to concentrate nutrients from the water column can be assessed using a volume concentration factor (VCF). Since no VCFs are available for Fe and freshwater phytoplankton, but the percentage of particulate Fe is known, the model was run with a range of VCFs (Fig. 1). The model suggests that at a VCF of 2.5×10^6, the contribution of total Fe (Fe_t) represented by particulate Fe (Fe_p) is 74%. The resulting modeled total Fe is 23 nM, in close agreement with values observed at Sta. 84, the Environment Canada central basin master station: 79% Fe_p, Fe_t = 30.6 nM (Twiss *et al.* 2000), and 91% Fe_p, Fe_t = 23.8 nM (Mioni *et al.* 2003).

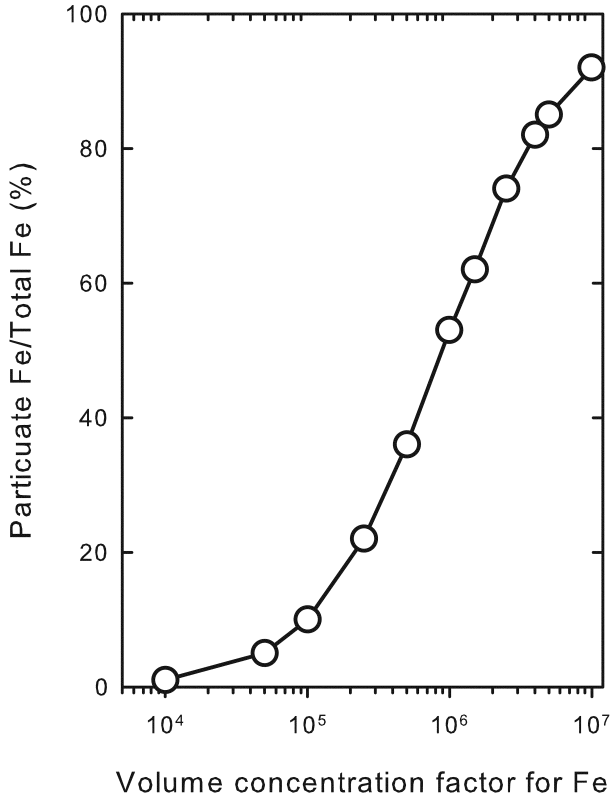

FIG. 1. Estimation of a bioconcentration factor for Fe by bioseston in Lake Erie surface water. The model is based on average bioseston abundances in the pelagic Lake Erie epilimnion (see Twiss and Campbell 1998b for details). A unique bioconcentration factor was assigned for all plankton classes (bacteria, autotrophic picoplankton, autotrophic nanoplankton and autotrophic microplankton).

RESULTS

Bioreporter luminescence was negatively correlated with the amount of Fe_d measured in each sample (Fig. 2A, B). An association between luminescence and log transformed Fe_d was tested using a Spearman Rank Correlation, a test free of

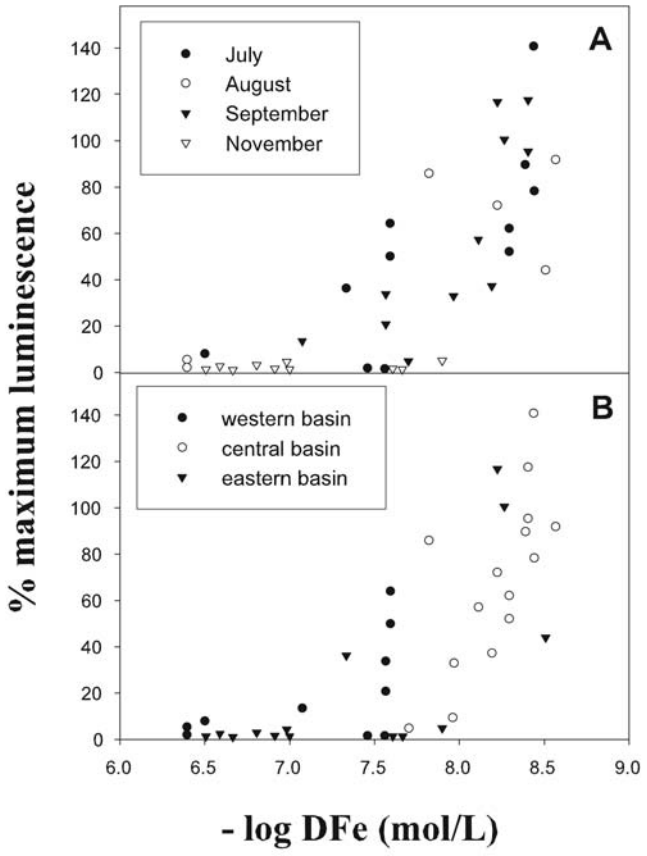

FIG. 2. Bioreporter luminescence expressed against the -log function of total dissolved iron (Fe_d) for all samples collected during 2001–02. The luminescent response of the bioreporter is reported as a percentage of maximum luminescence elicited by a series of accompanying calibration standards. (A) Seasonal resolution of Fe availability: (●) July, (○) August, (▼) September, (▽) November. (B) Basin-scale resolution of Fe availability: (●) western basin, (○) central basin, (▼) eastern basin.

assumptions about normality or constancy of error variance. The two variables were significantly correlated ($r_s = 0.778$, $p < 0.000001$, N = 40).

Considering seasonal variation in Fe bioavailability (Fig. 2A), Fe was readily available at all stations sampled in November 2001. This research cruise was restricted to the eastern basin of the lake and coincided with the period of autumnal mixing (Wilhelm *et al*. 2003). During November, Fe_d was measured in excess of 100 nM at eight of eleven stations representing a 20–60 × increase over levels existing in the epilimnion at eastern basin stations during the summer (Table 1). Coincident with elevated levels of Fe measured in November, bioreporter response was low indicating Fe-replete conditions.

Fe bioavailability during the other sampling periods was highly variable with no discernible patterns (Fig. 2A). The bioreporter response ranged full scale compared to the maximum luminescence elicited by the accompanying calibration standards. In several cases, Fe_d was low (< 10 nM), yet the bioreporter response was also low indicating Fe-replete conditions. In other instances, Fe_d was > 20 nM yet bioreporter response was high, indicative of Fe deficient conditions.

We next compared trends in Fe availability between basins of Lake Erie. Comparing central basin sites, the bioreporter response ranged full scale indicating a mix of Fe deficient and Fe sufficient sites (Fig. 2B). If we remove from consideration eastern basin stations sampled in November, then the remaining eastern basin sites represent a mix of Fe deficient and Fe sufficient sites similar to the central basin (Fig. 2B). In contrast, the bioreporter response was uniformly low when testing water sampled from the western basin with only a single site providing luminescence > 50% of the maximum response of the standards.

Iron appeared to be readily available throughout the connecting waterways between Lake Huron and Lake Erie as well as at several sites sampled in the Grand River, a tributary of the eastern basin of Lake Erie (data not shown). Low bioreporter luminescence elicited from water sampled from the Grand River and the Detroit River was consistent with elevated levels of Fe_d measured at these sites (Table 1). In contrast, Fe_d was comparatively low (10–20 nM) at locations sampled in Lake St. Clair and the St. Clair River.

Offshore master stations designated in each of the three basins of Lake Erie were occupied during July (Environment Canada) and September (US EPA-GLNPO) 2002. We also report data acquired during August 2002 (US EPA-GLNPO) where master stations in the eastern (LE 23, ER 15) and western (LE 357, ER 91) basins of the lake were assessed using the Fe bioreporter. This provided a seasonal comparison at a comparable offshore location in each of the three basins. Emerging from these assays was a similar pattern of Fe bioavailability in water sampled from the epilimnion at offshore master stations located in both the central and eastern basins of the lake where a depletion in available Fe was observed in September (Fig. 3). At the eastern basin sites, Fe bioavailability was essentially unchanged during July and August and only declined in September. Notably, the reduction in Fe bioavailability measured in September was not accompanied by a decrease in Fe_d (Table 1).

At the western basin offshore master stations (LE 357, ER 91), the bioreporter luminescence was similar between July and September where Fe_d was *ca.* 25 nM. In contrast, luminescence was below the dynamic range of response of the bioreporter in August indicating enhanced Fe bioavailability at this time. Here, the bioreporter response was consistent with corresponding elevated (> 400 nM) Fe_d (Table 1).

Figure 3 also includes a single depth-resolved profile conducted at the eastern basin offshore master station ER 15 during September 2002. A distinct difference in Fe bioavailability between the epilimnion (5 m) and the hypolimnion (27 m) was noted with the former eliciting 2 × higher luminescence than the latter indicating Fe to be more highly bioavailable with depth at this site. Consistent with this, the concentration of Fe_d in the hypolimnion was nearly double that measured in the epilimnion (Table 1).

The difference between Fe availability in the epilimnion and the hypolimnion was further reinforced by results obtained from the central basin master station LE 84 sampled over several days during the same week in July 2001 (Fig. 4). This clearly demonstrated higher Fe availability in water sampled from the hypolimnion compared to the corresponding response elicited from the epilimnion. Consistent with this trend, Fe_d measured in the hypolimnion was *ca.* 11 nM whereas it ranged between 3.6–4.1 nM in the epilimnion (Table 1).

Prior to September 2002, our analyses had been restricted to use of filtered water. In September, we included an assessment of the bioreporter challenged with unfiltered water. This provided results in dramatic contrast to assays conducted simultane-

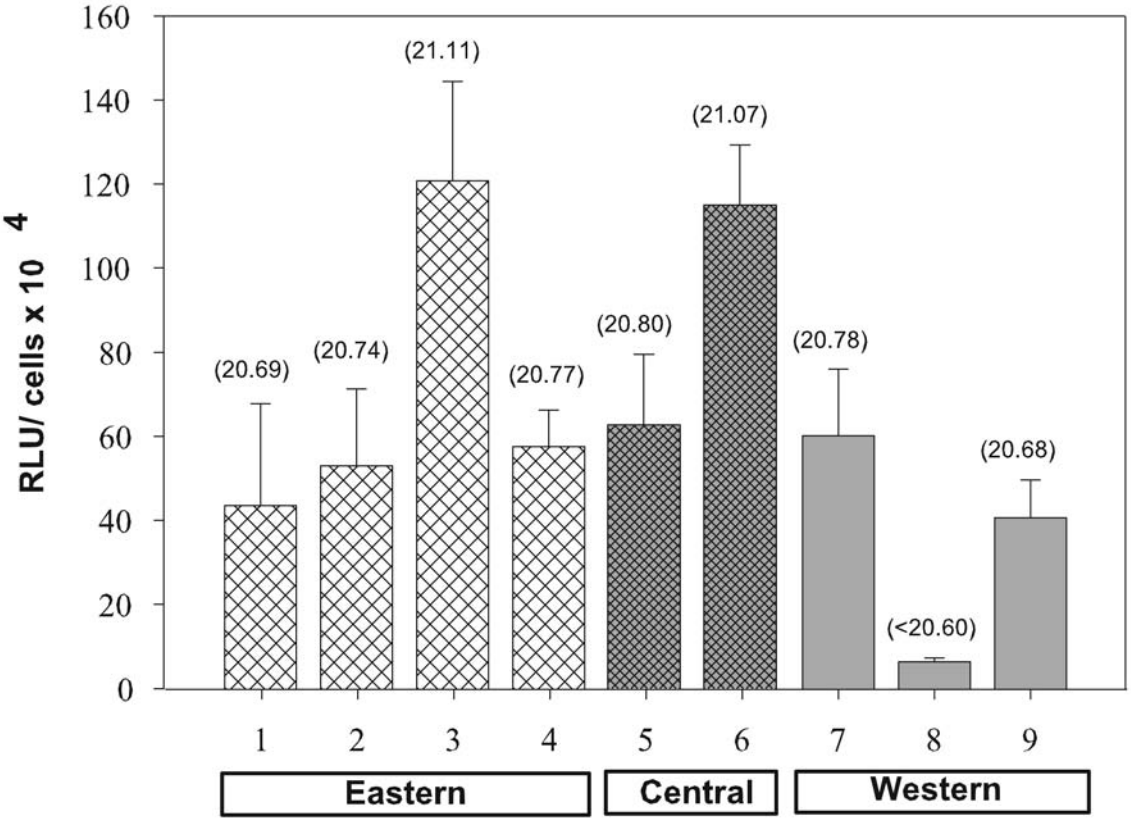

FIG. 3. Seasonal progression of Fe availability in filtered (< 0.2 μm) water measured at master stations located in each basin during 2002. Relative luminescence units (RLU) are normalized to bioreporter cell numbers. pFe equivalencies calculated for each sample are reported in parentheses. 1: LE 23, July; 2: ER 15, August; 3: ER 15, September (5 m); 4: ER 15, September (27 m); 5: LE 84, July; 6: ER 78, September; 7: LE 357, July; 8: ER 91, August; 9: ER 91, September. Master stations: 23, 84, and 357 are Environment Canada designations; 15, 78, and 91 are U.S. EPA designations.

ously using filtered water (Fig. 5). The luminescent signal measured in response to unfiltered water was below the dynamic range of response of the bioreporter indicating Fe sufficient samples (pFe < 20.6). We discount the possibility that the low bioreporter response was attributed solely to loss of *Synechococcus* bioreporter cells by grazing. Although microzooplankton were likely present in the assay tubes and substantial grazing of *Synechococcus* in microcosm assays has been reported previously (Hutchins *et al*. 1993, Twiss *et al*. 1996), positive controls to which desferal had been added and which were run simultaneously alongside the samples showed moderate- to high levels of luminescence (range: 35–74% of maximum luminescence of accompanying calibration standards) demonstrating the persistence of viable bioreporter cells following the 6 h incubation period.

DISCUSSION

Concentrations of dissolved nutrients provide a first order proxy for assessing the potential for deficiency of specific nutrients in aquatic systems. In marine systems, in particular, relating levels of dissolved nutrients to the Redfield ratio has been used in a predictive capacity to determine the limiting nutrient (Sterner and Elser 2002). In freshwater systems, the relative higher loads of allochthonous materials make comparisons to Redfield stoichiometry more tentative, although this ratio can still be held as a guideline (Sterner and Elser 2002, Wilhelm *et al*. 2003). In both instances, an important caveat to consider is that the term dissolved corresponds to an operationally-defined size fraction, usually including materials that pass through a nominal pore size filter of 0.2 to 0.45 μm. When interpreting

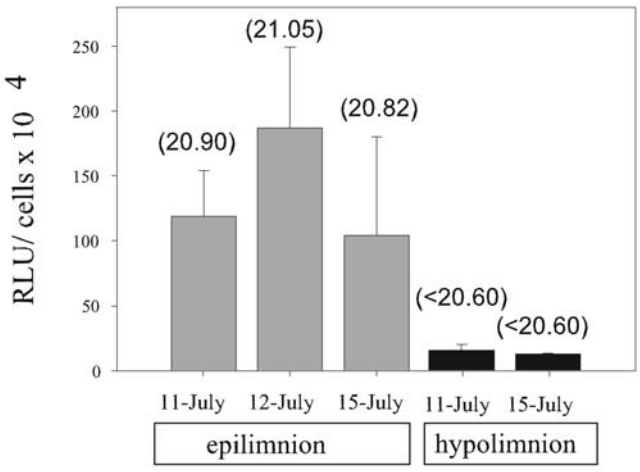

FIG. 4. Fe availability measured over a 4-day period in July 2001 at central basin master station LE 84. Bioreporter luminescence was measured using filtered water collected from both epilimnion (5 m) and hypolimnion (20 m) at this station. Relative luminescence (RLU) is normalized to bioreporter cell number. pFe equivalencies calculated for each sample are reported in parentheses.

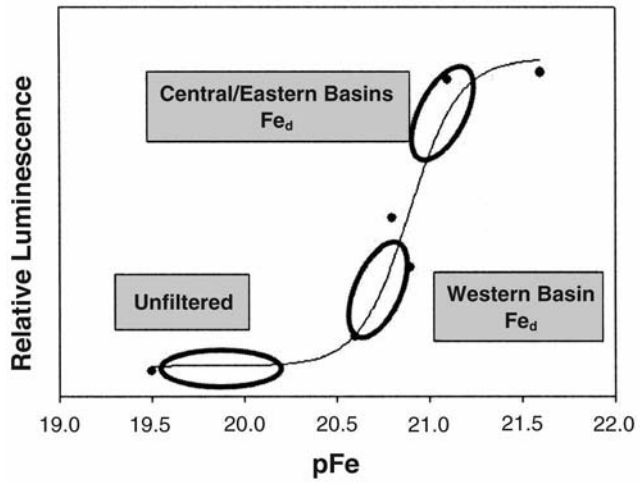

FIG. 5. A typical KAS101 Fe bioreporter dose-response curve generated using trace metal-buffered Fraquil medium of known pFe. Superimposed on the curve are ranges of luminescent response elicited by the bioreporter when challenged with filtered and unfiltered water. Samples of unfiltered water collected from each of the three U.S. EPA master stations were tested in September 2002.

these values, it is important to consider that not all of the material passing through the membrane is biologically available. Additionally, some portion of the material excluded from the operationally-defined dissolved fraction may be available to the biota.

As a means to address the bioavailability of nutrients in aquatic systems, several groups, have promoted the use of biological reporter organisms (Bachmann 2003, Belkin 2003, McKay *et al*. 2004). Bioreporters are broadly defined, although in all cases they relate some intrinsic physiological or biochemical feature of an organism to the availability of a particular nutrient. Some investigators have invoked the use of model organisms as bioreporters (e.g., Visser *et al*. 2003). Here, physiological indices that vary in response to nutrient availability are measured following incubation of the organism with an environmental sample. This approach can be informative, particularly if the organism is ecologically-relevant to the sampling site. However, prolonged incubations required to measure changes in indices, such as growth rate or photochemical efficiency, must be interpreted with caution. Increases in cell density can serve to deplete nutrients and normal metabolic activity can contribute to pH shifts, particularly in weakly buffered samples, that will modify nutrient availability. As such, a bioreporter that responds rapidly is preferable so that the response reflects the chemistry of the sample rather than the organism inadvertently serving to modify the chemistry.

In this direction, genetically modified bioreporters have been developed that exploit recent advances in genomics of ecologically-relevant organisms as well as technical advances in more broadly applying reporter genes such as those encoding luciferase (*lux* or *luc*) or the green fluorescent protein (*gfp*). Pertaining to aquatic systems, bioreporters responsive to phosphorus (Schreiter *et al*. 2001, Gillor *et al*. 2002), nitrogen (Mbeunkui *et al*. 2002, Gillor *et al*. 2003, Ivanikova *et al*. 2005), and iron (Durham *et al*. 2002, 2003; Mioni *et al*. 2003; Porta *et al*. 2003) have been developed. Several of these efforts even include immobilized bioreporters with a view toward future autonomous sensor development (Schreiter *et al*. 2001, Mbeunkui *et al*. 2002).

Bioreporter Assessment of Filtered Water

Restricting consideration to filtered water, our analysis reflected what can be described as the

highly variable nature of Fe deficiency in Lake Erie (Twiss *et al*. 2000). The most interesting trend associated with analysis of filtered water samples was the temporal decline in Fe availability observed at offshore central and eastern basin master stations during summer 2002. Whereas these stations were characterized as Fe sufficient during July (central and eastern basin) and August (eastern basin), a higher bioreporter response during September was suggestive of Fe deficiency. Based on our previous physiological characterization of the bioreporter (Porta *et al*. 2003), steady-state growth rates of the *Synechococcus* reporter would be expected to be suppressed by *ca.* 30% during September compared to July and August, based solely on considerations of bioavailable Fe. We cannot uniformly attribute this decrease in apparent Fe availability to a seasonal drawdown in Fe_d. Although levels of Fe_d measured at the central basin site decreased by 20% from July to September, Fe_d increased by 25% at the eastern basin location. This leads to consideration of the quality of Fe_d. Assuming that Fe_d is highly complexed to organic ligands in Lake Erie much like other freshwater systems (Maranger and Pullin 2003), it is reasonable to speculate that more recalcitrant ligands, be they allochthonous or autochthonous in origin, remain as the growing season progresses thereby making Fe_d less available later in the season.

Iron limitation of the autotrophic picoplankton has been observed previously in Lake Erie (Twiss *et al*. 2000). Amendment of lake water sampled from an offshore eastern basin location with 20 nM or 200 nM Fe in July 1996 resulted in increases in chl *a* standing stock and rates of light-saturated photosynthesis of endemic pico- and nanoplanktonic communities. A similar set of amendments conducted at 3 pelagic locations in July 1997 and August 1998, however, resulted in no physiological stimulation in response to added Fe (Twiss *et al*. 2000). A lack of direct stimulation by Fe in these instances was suggested to be related to turbulent mixing events recorded by meterologic buoys that preceded the sampling efforts in 1997–98. In these same trials, the endemic phytoplankton responded to addition of P, although in several cases, there was an enhanced response when both Fe and P were added. This synergistic interaction between P and Fe has been observed also in Lake Superior (Sterner *et al*. 2004) and suggests that endogenous levels of Fe in these lakes are low and is at times unable to support even modest P-stimulated growth.

Depth-resolved differences in Fe availability were apparent during the period of thermal stratification at offshore stations in both the eastern and central basins. Samples collected at depth in each instance coincided with the location of the seasonal hypolimnion and bioreporter luminescence was markedly lower when incubated with these samples compared to the corresponding surface (10 m) water samples. In each instance, the lower bioreporter response was consistent with elevated levels of Fe_d measured in the hypolimnion at these locations. Likewise, in marine systems, vertical profiles of Fe_d are generally described as nutrient-like, showing depletion in surface waters and replenishment at depth (Johnson *et al*. 1997). Hydrographic profiles conducted at each station showed no evidence of severe oxygen depletion occurring in the hypolimnion at the time of sampling (data not shown). Thus, enhanced Fe availability measured with these samples was not attributed to reducing conditions that typically accompany the onset of seasonal hypolimnetic anoxia as has been documented in other lakes (Achterberg *et al*. 1997, Reid *et al*. 1999) and as might be expected later in the season in the central basin of Lake Erie where recurrent hypoxia and anoxia are measured. Rather, elevated levels of bioavailable Fe likely resulted from bacterial remineralization of organic detritus, and in the case of the central basin station, the additional influence of sediment exchange into the adjacent shallow (< 5 m) hypolimnion.

Bioreporter Assessment of Unfiltered Water

Analysis of filtered water (< 0.2 μm) collected from central and eastern basin master stations during September 2002 indicated these sites to be Fe deficient. Parallel analysis of unfiltered water, however, provided a markedly different response eliciting a luminescent signal below the dynamic range of detection of the bioreporter and suggestive of Fe replete conditions.

This suggests that at least a portion of the Fe contained in the particulate fraction was bioavailable as recently reported by Mioni *et al*. (2003) who used a luminescent heterotrophic bacterial reporter to measure Fe availability in Lake Erie. In oligotrophic regions, regeneration of Fe from the particulate fraction represents an important source of nutrient flux (Hutchins *et al*. 1993). Although Lake Erie tends more toward mesotrophic rather than oligotrophic, regenerative processes augment nutrient flux in this system also (Heath *et al*. 2003). Regeneration of Fe may arise from grazing (Hutchins *et*

TABLE 2. A mass balance Fe budget for bioreporter incubations using unfiltered water.

Plankton group	Fe:C (μmol:mol)	Biomass (μmol C/L)	Fe in Biota (pmol/L)	Fe Turnover (grazing; pmol/L)	Fe Turnover (viral; pmol/L)
heterotrophic bacteria	8.3[1]	5.9[2]	49	7 - 18	3[3]
autotrophic picoplankton:	19[4]	2.1[5]	40	6–15	0.32–0.53[6]
endemic bioreporter	22.6	29.5	667[7]	102–242	0[8]
nanoplankton	70.8[9]	5.8[10]	411	35	1.0[11]
microplankton	31[12]	14.1[10]	439	17	1.06[11]

[1]mean taken from laboratory and field determinations for marine heterotrophic bacteria (Tortell *et al*. 1996).
[2]derived from mean cell density measured at central and eastern basin stations (DeBruyn *et al*. 2004) and bacterial cellular C quotas reported in Tortell *et al.* (1996) and Wilhelm and Suttle (2000).
[3]measured mortality of 25% for Lake Erie heterotrophic picoplankton (J. Higgins and S.W. Wilhelm, unpublished)
[4]from Brand (1991).
[5]mean biomass from two stations in Lake Erie sampled during summer (Fahnenstiel *et al.* 1998).
[6]based on mortality estimate of 5–10% (Wilhelm and Suttle 1999).
[7]derived from mean cell density of bioreporter used in the present study and from Fe and C quotas reported in Porta *et al*. (2003).
[8]we assume the bioreporter is not subject to viral lytic activity due to the short period of incubation during bioassay.
[9]mean of Fe:P ratios reported for 15 phytoplankton species (Ho *et al*. 2003) and converted to Fe:C by dividing by 106.
[10]mean total nano- and microplankton biomass estimate from Lake Erie (Twiss and Campbell, 1998b). Cell carbon biomass was assumed to be 0.24 pg C/μm^3 as reported by Verity *et al.* (1992).
[11]mortality estimate of 1% (S.W. Wilhelm, unpublished)
[12]mean from field determinations in subantarctic Pacific during austral summer 2003 (R.M.L. McKay and D.A. Hutchins, unpublished).

al. 1993, 1995; Hutchins and Bruland 1995; Barbeau *et al*. 1996; Chase and Price 1997; Maranger *et al*. 1998) or viral lysis (Gobler *et al*. 1997, Wilhelm and Suttle 1999, Poorvin *et al*. 2004). Average grazing rates of 1.42/d, 0.65/d, and 0.27/d have been measured for autotrophic pico-, nano-, and microplankton, respectively, in Lake Erie at central and eastern basin offshore stations using dilution assays (Twiss and Campbell 1998a). Considering a regeneration efficiency ([Fe excreted/Fe ingested] × 100) approximating 70% for mixotrophic nanoflagellates (Maranger *et al.* 1998) and heterotrophic microflagellates (Chase and Price 1997) and between 75–95% for metazoan grazers (Hutchins *et al*. 1995), regeneration of Fe via grazing is expected to contribute substantially to nutrient cycling in the lake.

Likewise, regeneration of nutrients as a result of viral activity may have contributed to the apparent high availability of Fe in the unfiltered samples (e.g., Gobler *et al*. 1997, Poorvin *et al.* 2004). Similar to grazing, viral lysis is expected to result in the liberation of nutrients from lysed cells, thereby simultaneously serving to short-circuit the conventional linear food chain and to sustain components of a microbial loop (Wilhelm and Suttle 1999). Virus-like particles (VLP) are abundant in Lake Erie and range from 3.7–37.9 × 10^{10} VLP/L (Wilhelm and Smith 2000, DeBruyn *et al*. 2004). This is an order of magnitude higher than many marine environments where it is estimated that between 10–20% of heterotrophic bacteria and 5–10% of cyanobacteria are lysed by viruses each day (Wilhelm and Suttle 1999). For Lake Erie, Wilhelm and Smith (2000) estimated viral lytic activity to be responsible for 12–23% of all bacterial mortality.

We compiled a mass balance Fe budget comprising the biota present in our assay in an attempt to frame our bioreporter results in the context of Fe available for redistribution via regenerative processes (Table 2). In compiling the budget, we used estimates of biomass and grazing pressure reported from previous studies on Lake Erie. Rates of viral mortality for heterotrophic picoplankton were taken from measurements recently made in Lake Erie (J. Higgins and S.W. Wilhelm, unpublished) and rates of viral mortality for other members of the community were taken from the oceanographic literature as were cellular Fe quotas, which are not available for Great Lakes plankton.

Our calculations suggest that > 40% of Fe present in our assays was contained in the introduced bioreporter cells themselves. While this accounted for < 3% of the total Fe (particulate and dissolved) contained in the sample, its potential toward contributing to the bioavailable pool is high since it is contained in an actively grazed form (Hutchins *et al*. 1993, Twiss and Campbell 1995, Twiss *et al*. 1996). Therefore, we cannot discount the possibility that the Fe sufficient response characteristic of bioreporter cells when challenged with unfiltered water in our study was at least in part the result of Fe regenerated from grazed bioreporter cells themselves. Our analysis suggests that labile Fe derived from grazing on bioreporter cells alone in these assays could have accounted for 60–75% (102–242 pmol/L) of total Fe regenerated through grazing. Application of a static plankton model of trace metal scavenging designed for Lake Erie (Twiss and Campbell 1998b) estimated that the bioreporter cells have the capacity to scavenge *ca.* 6% of ambient Fe. Using a mortality estimate of 56% for the bioreporter cells based on an average observed grazing rate for picoplankton of 1.42/d (Twiss and Campbell 1998a) and a 6 h incubation time, we calculate regenerated Fe derived from grazed bioreporter cells and scavenged by the remaining cells to range from $2–5 \times 10^{-2}$ amol/cell which represented < 5% of the Fe quota of cells at the beginning of the incubation. Augmenting this with regenerated Fe derived from grazing and viral lysis of endemic members of the community increased the total Fe scavenged only marginally to $3–6 \times 10^{-2}$ amol/cell. Further, it is unclear what proportion of this would be intracellular Fe; Hutchins *et al*. (1993) reported that < 30% of the Fe scavenged by plankton > 5-μm following regeneration of Fe contained in radiolabeled *Synechococcus* prey was internalized in cells sampled in Monterey Bay, an Fe-replete coastal region, whereas in excess of 60% of scavenged Fe was found in internal pools of the same size class at a site in the equatorial Pacific, a Fe-limited region.

An additional pool of Fe that may have contributed to the observed bioreporter response was that of the abiotic particulate fraction comprising particulate and colloidal Fe. Based on calculations used to derive our sestonic Fe budget, abiogenic particles accounted for *ca.* 95% of the Fe contained in the total particulate fraction. It has been recognized for some time that phytoplankton can use particulate colloidal Fe, although the efficacy with which they do so is thought to be dependent on the thermodynamic and photochemical stability of the various colloidal species (Rich and Morel 1990, Wells *et al*. 1995, Chen and Wang 2001). Exceptions to this are the hetero- and mixotrophic flagellates that are able to effect dissolution of inorganic colloids through phagotrophy (Barbeau *et al*. 1996, Barbeau and Moffett 2000, Nodwell and Price 2001) and possibly specialized reducing microenvironments such as diatom mats or *Trichodesmium* colonies (Rueter *et al*. 1992). By contrast, use of colloidal Fe by most obligate autotrophic phytoplankton depends on its dissolution before it is available for uptake and growth. Viewed in this context, the relatively slow dissolution of highly crystalline forms such as geothite generally preclude their use as direct Fe sources whereas the more rapid solubilization of amorphous colloids such as ferrihydrite render them more readily available for use by phytoplankton. Ferrihydrite, however, is composed of subunits *ca.* 10 nm in diameter (Barbeau *et al*. 1996), and thus was likely partitioned to the dissolved fraction in our study and available equally to bioreporter cells incubated in filtered and unfiltered water. This leaves the larger, generally more recalcitrant colloidal forms associated with the particulate fraction. Direct use of the larger colloids by the cyanobacterial bioreporter remains untested although the possibility remains that dissolution of colloidal Fe was accelerated by flagellate grazing or bacterial reductive mechanisms.

While it is not clear whether Fe acquired from regenerative processes resulted in the low bioreporter response reported from unfiltered water in this study, it is apparent that regenerated Fe derived from grazing activity on bioreporter cells was substantial and could have masked the effect of Fe regenerated from the endemic community. While regenerative processes contributed to the pool of biovailable Fe, it is clear that future efforts using the bioreporter must account for the potential influence of Fe added with the reporter cells; this might be accomplished by simply seeding the assays with fewer reporter cells thereby contributing a smaller proportion of the total Fe load. Alternatively, bioreporter cells could be separated from potential grazers by use of membranes of defined pore size allowing for the exchange of regenerated Fe but excluding direct contact with grazers.

Caveats to Consider in Using the Cyanobacterial Fe Bioreporter

The *Synechococcus* Fe bioreporter used in this study is presented as a novel tool to assess the

bioavailability of Fe in fresh water since it provides a measure of nutrient availability from the perspective of a living organism. Chemical approaches, although providing a first-order indication of nutrient status, are less flexible in discriminating between biologically available and more recalcitrant forms of a nutrient.

It is important to bear in mind some of the limitations associated with the use of the Fe bioreporter. Already discussed was the potential contribution of Fe by the bioreporter itself. Also notable is that the Fe bioreporter used in this study is a prokaryotic organism possessing an Fe acquisition strategy distinct from eukaryotes. This does not diminish the significance of our results, especially considering the sizeable allocation of biomass and production among picoplanktonic photoautotrophs in the Great Lakes (Caron *et al*. 1985; Fahnenstiel *et al*. 1986; Pick and Caron 1987; Fahnenstiel *et al*. 1991a, 1991b; Fahnenstiel and Carrick 1992). However, by relying solely on a cyanobacterial bioreporter, we cannot comment with certainty on Fe availability to eukaryotic cells in Lake Erie. Whereas cyanobacteria, including the *Synechococcus* sp. PCC 7942 strain used in the construction of our bioreporter (Kerry *et al*. 1988), generally rely upon siderophores to acquire Fe^{3+} (Wilhelm 1995), eukaryotic cells more commonly possess a cell surface ferric reductase activity used to reduce Fe, including organically-complexed species, to the ferrous form for uptake (Maldonado and Price 2000, Weger *et al*. 2002). Reflecting the inherent differences in prokaryotic vs. eukaryotic Fe acquisition, Hutchins *et al*. (1999) demonstrated distinct differences in the uptake efficiency of various molecules containing radiolabeled Fe by cyanobacteria and diatoms.

Given this dichotomy in Fe acquisition strategies between prokaryotes and eukaryotes, development of a suitable eukaryotic Fe bioreporter is desired. A diatom is arguably the best candidate for such a eukaryotic bioreporter organism. Diatoms are ecologically important members of the phytoplankton community in the Great Lakes (Munawar and Munawar 1996). Further, recent advances in stable transformation procedures for diatoms (as reviewed by Falciatore and Bowler 2002) combined with ongoing genome sequencing and annotation efforts for two diatom species (Scala *et al*. 2002, Armbrust *et al*. 2004) has cleared some of the obstacles to developing a diatom bioreporter organism.

ACKNOWLEDGMENTS

Field sampling and analysis were supported by a grant from the United States Environmental Protection Agency-Great Lakes National Program Office (Lake Erie Trophic Status Study) to RMLM and MRT. Additional field support was provided by a grant from the Natural Sciences and Research Council of Canada to MRT (while at Ryerson Polytechnic University, Toronto, ON) and by the National Water Research Institute and Environment Canada through coordination by Dr. R. Bourbonniere. Development of the Fe bioreporter used in this study was supported by National Science Foundation grant OCE-9911592 to RMLM and GSB. We thank Dr. R. Sterner (University of Minnesota) for help with statistical analyses and the captain and crews of the R/V *Lake Guardian* and the CCGS *Limnos* for assistance in the field. This is Contribution No. 101 of the Clarkson Center for the Environment.

REFERENCES

Achterberg, E.P., van den Berg, C.M.G., Boussemart, M., and Davison, W. 1997. Speciation and cycling of trace metals in Esthwaite Water: A productive English lake with seasonal deep-water anoxia. *Geochim. Cosmochim. Acta* 61:5233–5253.

Allen, M.M. 1968. Simple conditions for growth of unicellular blue-green algae on plates. *J. Phycol.* 4:1–4.

Armbrust, E.V., Berges, J.A., Bowler, C., Green, B.R., Martinez, D., Putnam, N.H., Zhou, S., Allen, A.E., Apt, K.E., Bechner, M., Brzezinski, M.A., Chaal, B.K., Chiovitti, A., Davis, A.K., Demarest, M.S., Detter, J.C., Glavina, T., Goodstein, D., Hadi, M.Z., Hellsten, U., Hildebrand, M., Jenkins, B.D., Jurka, J., Kapitonov, V.V., Kröger, N., Lau, W.W.Y., Lane, T.W., Larimer, F.W., Lippmeier, J.C., Lucas, S., Medina, M., Montsant, A., Obornik, M., Schnitzler Parker, M., Palenik, B., Pazour, G.J., Richardson, P.M., Rynearson, T.A., Saito, M.A., Schwartz, D.C., Thamatrakoln, K., Valentin, K., Vardi, A., Wilkerson, F.P., and Rokhsar, D.S. 2004. The genome of the diatom *Thalassiosira pseudonana*: Ecology, evolution, and metabolism. *Science* 306:79–86.

Bachmann, T. 2003. Transforming cyanobacteria into bioreporters of biological relevance. *Trends Biotechnol.* 21:247–249.

Barbeau, K., and Moffett, J.W. 2000. Laboratory and field studies of colloidal iron oxide dissolution as mediated by phagotrophy and photolysis. *Limnol. Oceanogr.* 45:827–835.

_____, Moffett, J.W., Caron, D.A., Croot, P.L., and Erdner, D.L. 1996. Role of protozoan grazing in relieving iron limitation of phytoplankton. *Nature* 380:61–64.

Belkin, S. 2003. Microbial whole cell sensing systems of environmental pollutants. *Curr. Opin. Microbiol.* 6:206–212.

Boyd, P.W., Watson, A., Law, C.S., Abraham, E., Trull, T., Murdoch, R., Bakker, D.C.E., Bowie, A.R., Buesler, K.O., Chang, H., Charette, M., Croot , P., Downing, K., Frew, R., Gall, M., Hadfield, R., Hall, J., Harvey, M., Jameson, G., La Roche, J., Liddicoat, M., Ling, R., Maldonado, M., McKay, R.M., Nodder, S., Pickmere, S., Pridmore, R., Rintoull, S., Safi, K., Sutton, P., Strzepek, R., Tannenberger, K., Turner, S., Waite, A., and Zeldis, J. 2000. A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization. *Nature* 407:695–702.

Brand, L.E. 1991. Minimum iron requirements of marine phytoplankton and the implications for the biogeochemical control of new production. *Limnol. Oceanogr.* 36:1756–1771.

Callieri, C., and Stockner, J.G. 2002. Freshwater autotrophic picoplankton: a review. *J. Limnol.* 61: 1–14.

Caron, D.A., Pick, F.R., and Lean, D.R.S. 1985. Chroococcoid cyanobacteria in Lake Ontario: Vertical and seasonal distributions during 1982. *J. Phycol.* 21: 171–175.

Chase, Z., and Price, N.M. 1997. Metabolic consequences of iron deficiency in heterotrophic marine protozoa. *Limnol. Oceanogr.* 42:1673–1684.

Chen, M., and Wang, W.-X. 2001. Bioavailability of natural colloid-bound iron to marine plankton: Influences of colloidal size and aging. *Limnol. Oceanogr.* 46: 1956–1967.

Coale, K.H., and Flegal, A.R. 1989. Copper, zinc, cadmium and lead in surface waters of Lakes Erie and Ontario. *Sci. Total Environ.* 87/88:297–304.

_____, Johnson, K.S., Fitzwater, S.E., Gordon, R.M., Tanner, S., Chavez, F.P., Ferioli, L., Sakamoto, C., Rogers, P., Millero, F., Steinberg, P., Nightingale, P., Cooper, D., Cochlan, W.P., Landry, M.R., Constantinou, J., Rollwagen, G., Trasvina, A., and Kudela, R. 1996. A massive phytoplankton bloom induced by an ecosystem- scale iron fertilization experiment in the equatorial Pacific Ocean. *Nature* 383:495–501.

DeBruyn, J.M., Leigh-Bell, J.A., McKay, R.M.L., Bourbonniere, R.A., and Wilhelm, S.W. 2004. Microbial distributions and the impact of phosphorus on bacterial activity in Lake Erie. *J. Great Lakes Res.* 30:166–183.

Durham, K.A., Porta, D., Twiss, M.R., McKay, R.M.L., and Bullerjahn, G.S. 2002. Construction and initial characterization of a luminescent *Synechococcus* sp. PCC 7942 Fe-dependent bioreporter. *FEMS Microbiol. Lett.* 209:215–221.

_____, Porta, D., McKay, R.M.L., and Bullerjahn, G.S. 2003. Expression of the iron-responsive *irpA* gene from the cyanobacterium *Synechococcus* sp. strain PCC 7942. *Arch. Microbiol.* 179:131–134.

Elser, J.J., Marzolf, E.R., and Goldman, C.R. 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: A review and critique of experimental enrichments. *Can. J. Fish. Aquat. Sci.* 47:1468–1477.

Fahnenstiel, G.L., and Carrick, H.J. 1992. Phototrophic picoplankton in lakes Huron and Michigan: abundance, distribution, composition and contribution to biomass and production. *Can. J. Fish. Aquat. Sci.* 49: 379–388.

_____, Sicko-Goad, L., Scavia, D., and Stoermer, E.F. 1986. Importance of picoplankton in Lake Superior. *Can. J. Fish. Aquat. Sci.* 43:235–240.

_____, Carrick, H.J., and Iturriaga, R. 1991a. Physiological characteristics and food web dynamics of *Synechococcus* in Lakes Huron and Michigan. *Limnol. Oceanogr.* 36:219–234.

_____, Carrick, H.J., Rogers, C.E., and Sicko-Goad, L. 1991b. Red fluorescing phototrophic picoplankton in the Laurentian Great Lakes: What are they and what are they doing? *Int. Revue ges. Hydrobiol.* 76:603–616.

_____, Krause, A.E., McCormick, M.J., Carrick, H.J., and Schelske, C.L. 1998. The structure of the planktonic food-web in the St. Lawrence Great Lakes. *J. Great Lakes Res.* 24:531–554.

Falciatore, A., and Bowler, C. 2002. Revealing the molecular secrets of marine diatoms. *Annu. Rev. Plant Biol.* 53:109–130.

Field, M.P., and Sherrell, R.M. 2003. Direct determination of ultra-trace levels of metals in fresh water using desolvating micronebulization and HR-ICP-MS: application to Lake Superior waters. *J. Anal. At. Spectrom.* 18:254–259.

Gillor, O., Hadas, O., Post, A.F., and Belkin, S. 2002. Phosphorus bioavailability monitoring by a bioluminescent cyanobacterial sensor strain. *J. Phycol.* 38:107–115.

Gillor, O., Harush, A., Hadas, O., Post, A.F., and Belkin, S. 2003. A *Synechococcus* p*glnA::luxAB* fusion for estimation of nitrogen bioavailability to freshwater cyanobacteria. *Appl. Environ. Microbiol.* 69: 1465–1474.

Gobler, C.J., Hutchins, D.A., Fisher, N.S., Cosper, E.M., and Sanudo-Wilhelmy, S.A. 1997. Release and bioavailability of C, N, P, Se, and Fe following viral lysis of a marine chrysophyte. *Limnol. Oceanogr.* 42:1492–1504.

Guildford, S.J., and Hecky, R.E. 2000. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? *Limnol. Oceanogr.* 45:1213–1223.

Hartig, J.H., and Wallen, D.G. 1984. Seasonal variation of nutrient limitation in western Lake Erie. *J. Great Lakes Res.* 10:449–460.

Heath, R.T., Hwang, S.-J., and Munawar, M. 2003. A hypothesis for the assessment of the importance of

microbial food web linkages in nearshore and offshore habitats of the Laurentian Great Lakes. *Aquat. Ecosyst. Health Manage*. 6:231–239.

Ho, T.-Y., Quigg, A., Finkel, Z.V., Milligan, A.J., Wyman, K., Falkowski, P.G., and Morel, F.M.M. 2003. The elemental composition of some marine phytoplankton. *J. Phycol*. 39:1145–1159.

Hutchins, D.A., and Bruland, K.W. 1995. Fe, Zn, Mn and N transfer between size classes in a coastal phytoplankton community: Trace metal and major nutrient recycling compared. *J. Mar. Res*. 53:297–313.

_____, DiTullio, G.R., and Bruland, K.W. 1993. Iron and regenerated production: Evidence for biological iron recycling in two marine environments. *Limnol. Oceanogr*. 38:1242–1255.

_____, Wang, W.-X., and Fisher, N.S. 1995. Copepod grazing and the biogeochemical fate of diatom iron. *Limnol. Oceanogr*. 40:989–994.

_____, DiTullio, G.R., and Bruland, K.W. 1998. An iron limitation mosaic in the California upwelling regime. *Limnol. Oceanogr*. 43:1037–1054.

_____, Witter, A.E., Butler, A., and Luther, G.W. III. 1999. Competition among marine phytoplankton for different chelated iron species. *Nature* 400:858–861.

Ivanikova, N.V., McKay, R.M.L., and Bullerjahn, G.S. 2005. Construction and characterization of a cyanobacterial bioreporter capable of assessing nitrate assimilatory capacity in freshwaters. *Limnol. Oceanogr.:Methods* 3:86–93.

Johnson, K.S., Gordon, R.M., and Coale, K.H. 1997. What controls dissolved iron concentrations in the world ocean? *Mar. Chem*. 57:137–161.

Kerry, A., Laudenbach, D.E., and Trick, C.G. 1988. Influence of iron limitation and nitrogen source on growth and siderophore production by cyanobacteria. *J. Phycol*. 24:566–571.

Lin, C.K., and Schelske, C.L. 1981. Seasonal variation of potential nutrient limitation to chlorophyll production in southern Lake Huron. *Can. J. Fish. Aquat. Sci*. 38:1–9.

Maldonado, M.T., and Price, N.M. 2000. Nitrate regulation of Fe reduction and transport by Fe-limited *Thalassiosira oceanica*. *Limnol. Oceanogr*. 45:814–826.

Maranger, R., and Pullin, M.J. 2003. Elemental complexation by dissolved organic matter in lakes: Implications for Fe speciation and the bioavailability of Fe and P. In *Aquatic ecosystems: Interactivity of dissolved organic matter,* eds. S.E.G. Findlay and R.L. Sinsabaugh, pp. 185–214. San Diego, CA: Academic Press.

_____, Bird, D.F., and Price, N.M. 1998. Iron acquisition by photosynthetic marine phytoplankton from ingested bacteria. *Nature* 396:248–251.

Martin, J.H., Gordon, R.M., and Fitzwater, S.E. 1991. The case for iron. *Limnol. Oceanogr*. 36:1793–1802.

Mbeunkui, F., Richaud, C., Etienne, A.-L., Schmid, R.D., and Bachmann, T.T. 2002. Monitoring of nitrate bioavailability in water by an immobilized luminescent cyanobacterial reporter strain. *Appl. Microbiol. Biotechnol*. 60:306–312.

McKay, R.M.L., Bullerjahn, G.S., Porta, D., Brown, E.T., Sherrell, R.M., Smutka, T.M., Sterner, R.W., Twiss, M.R., and Wilhelm, S.W. 2004. Consideration of the bioavailability of iron in the North American Great Lakes: Development of novel approaches toward understanding iron biogeochemistry. *Aquat. Ecosyst. Health Manage*. 7:475–490.

Mioni, C.E., Howard, A.M., DeBruyn, J.M., Bright, N.G., Twiss, M.R., Applegate, B.M. and Wilhelm, S.W. 2003. Characterization and field trials of a bioluminescent bacterial reporter of iron bioavailability. *Mar. Chem*. 83:31–46.

Munawar, M., and Munawar, I.F. 1996. *Phytoplankton Dynamics in the North American Great Lakes. Volume 1: Lakes Ontario, Erie and St. Clair.* Amsterdam, The Netherlands: SPB Academic Publishing.

Nodwell, L.M., and Price, N.M. 2001. Direct use of inorganic colloidal iron by marine mixotrophic phytoplankton. *Limnol. Oceanogr*. 46:765–777.

Nriagu, J.O., Lawson, G., Wong, H.K.T., and Azcue, J.M. 1993. A protocol for minimizing contamination in the analysis of trace metals in Great Lakes water. *J. Great Lakes Res*. 19:175–182.

_____, Lawson, G., Wong, H.K.T., and Cheam, V. 1996. Dissolved trace metals in Lakes Superior, Erie, and Ontario. *Environ. Sci. Technol*. 30:178–187.

Pick, F.R., and Caron, D.A. 1987. Picoplankton and nanoplankton biomass in Lake Ontario: Relative contribution of phototrophic and heterotrophic communities. *Can. J. Fish. Aquat. Sci.* 44:2164–2172.

Poorvin L., Rinta-Kanto, J.M., Hutchins, D.A., and Wilhelm, S.W. 2004. Viral lysis as a major source of bioavailable iron in marine ecosystems. *Limnol. Oceanogr.* 49:1734–1741.

Porta, D., Bullerjahn, G.S., Durham, K.A., Wilhelm, S.W., Twiss, M.R., and McKay, R.M.L. 2003. Physiological characterization of a *Synechococcus* sp. strain PCC 7942 iron-dependent bioreporter for freshwater environments. *J. Phycol*. 39:64–73.

Price, N.M., Harrison, G.I., Hering, J.G., Hudson, R.J., Nirel, P.M.V., Palenik, B., and Morel, F.M.M. 1988/1989. Preparation and chemistry of the artificial algal culture medium Aquil. *Biol. Oceanogr.* 6:443–461.

Reid, M.R., Kim, J.P., and Hunter, K.A. 1999. Trace metal and major ion composition of Lakes Hayes and Manapouri. *J. Roy. Soc. New Zeal*. 29:245–255.

Rich, H.W., and Morel, F.M.M. 1990. Availability of well-defined iron colloids to the marine diatom *Thalassiosira weissflogii. Limnol. Oceanogr*. 35:652–662.

Rueter, J.G., Hutchins, D.A., Smith, R.W., and Unsworth, N.L. 1992. Iron nutrition of *Trichodesmium*. In *Marine Pelagic Cyanobacteria:* Trichodesmium *and Other Diazotrophs,* E.J. Carpenter,

D.G. Capone, and J.G. Rueter, eds., pp. 289–306. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Scala, S., Carels, N., Falciatore, A., Chiusano, M.L., and Bowler, C. 2002. Genome properties of the diatom *Phaeodactylum tricornutum*. *Plant Physiol.* 129:993–1002.

Schelske, C.L. 1962. Iron, organic matter, and other factors limiting primary productivity in a marl lake. *Science* 136:45–46.

_____, Feldt, L.E., Santiago, M.A., and Stoermer, E.F. 1972. Nutrient enrichment and its effect on phytoplankton production and species composition in Lake Superior. In *Proc. 15th Conf. Great Lakes Res.,* pp. 149–165. Internat. Assoc. Great Lakes Res.

Schreiter, P.P.-Y., Gillor, O., Post, A., Belkin, S., Schmid, R.D., and Bachmann, T.T. 2001. Monitoring of phophorus bioavailability in water by an immobolized luminescent cyanobacterial reporter strain. *Biosens. Bioelectron*. 16:811–818.

Sterner, R.W., and Elser, J.J. 2002. *Ecological Stoichiometry: the biology of elements from molecules to the biosphere.* Princeton, New Jersey: Princeton University Press.

_____, Smutka, T.M., McKay, R.M.L., Qin, X., Brown, E.T., and Sherrell, R.M. 2004. Phosphorus and trace metal limitation of algae and bacteria in Lake Superior. *Limnol. Oceanogr*. 49:495–507.

Straus, N.A. 1994. Iron deprivation: physiology and gene regulation. In *The molecular biology of cyanobacteria,* D.A. Bryant, ed., pp. 731–750. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Sturgeon, R., and Berman, S. 1987. Sampling and storage of natural water for trace metals. In *Critical reviews in Analytical Chemistry* 18(3):209–244. CRC Press.

Tortell, P.D., Maldonado, M.T., and Price, N.M. 1996. The role of heterotrophic bacteria in iron-limited ocean ecosystems. *Nature* 383:330–332.

Tsuda, A., Takeda, S., Saito, H., Nishioka, J., Nojiri, Y., Kudo, I., Kiyosawa, H., Shiomamoto, A., Imai, K., Ono, T., Shimamoto, A., Tsumune, D. Yoshimura, T., Aono, T., Hinuma, A., Kinugasa, M., Suzuki, K., Sohrin, Y., Noiri, Y., Tani, H., Deguchi, Y., Tsurushima, N., Ogawa, H., Fukami, K., Kuma, K., and Saino, T. 2003. A mesoscale iron enrichment in the western subarctic Pacific induces a large centric diatom bloom. *Science* 300:958–961.

Twiss, M.R., and Campbell, P.G.C. 1995. Regeneration of trace metals from picoplankton by nanoflagellate grazing. *Limnol. Oceanogr.* 40:1418–1429.

_____, and Campbell, P.G.C. 1998a. Scavenging of ^{137}Cs, ^{109}Cd, ^{65}Zn, and ^{153}Gd by plankton of the microbial food web in pelagic Lake Erie surface waters. *J. Great Lakes Res*. 24:776–790.

_____, and Campbell, P.G.C. 1998b. Trace metal cycling in the surface waters of Lake Erie: Linking ecological and geochemical fates. *J. Great Lakes Res.* 24:791–807.

_____, Campbell, P.G.C., and Auclair, J.-C. 1996. Regeneration, recycling, and trophic transfer of trace metals by microbial food-web organisms in the pelagic surface waters of Lake Erie. *Limnol. Oceanogr.* 41:1425–1437.

_____, Auclair, J.-C., and Charlton, M.N. 2000. An investigation into iron-stimulated phytoplankton productivity in epipelagic Lake Erie during thermal stratification using trace metal clean techniques. *Can. J. Fish. Aquat. Sci.* 57:86–95.

Verity, P.G., Robertson, C.Y., Tronzo, G.R., Andrews, M.G., Nelson, J.R., and Sieracki, M.E. 1992. Relationships between cell volume and carbon and nitrogen content of marine photosynthetic nanoplankton. *Limnol. Oceanogr.* 37:1434–1446.

Visser, F., Gerringa, L.J.A., Van der Gaast, S.J., de Baar, H.J.W., and Timmermans, K.R. 2003. The role of the reactivity and content of iron of aerosol dust on growth rates of two Antarctic diatom species. *J. Phycol.* 39:1085–1094.

Weger, H.G., Middlemiss, J.K., and Petterson, C.D. 2002. Ferric chelate reductase activity as affected by the iron-limited growth rate in four species of unicellular green algae (Chlorophyta). *J. Phycol*. 38: 513–519.

Wells, M.L., Price, N.M., and Bruland, K.W. 1995. Iron chemistry in seawater and its relationship to phytoplankton: a workshop report. *Mar. Chem*. 48:157–182.

Wetzel, R.G. 1966. Productivity and nutrient relationships in marl lakes of northern Indiana. *Verh. Int. Ver. Theor. Angew. Limnol.* 16:321–332.

Wilhelm, S.W. 1995. Ecology of iron-limited cyanobacteria: a review of physiological responses and implications for aquatic systems. *Aquat. Microb. Ecol.* 9:295–303.

_____, and Smith, R.E.H. 2000. Bacterial carbon production in Lake Erie is influenced by viruses and solar radiation. *Can. J. Fish. Aquat. Sci*. 57:317–326.

_____, and Suttle, C.A. 1999. Viruses and nutrient cycles in the sea. *BioScience* 49:781–788.

_____, DeBruyn, J.M., Gillor, O., Twiss, M.R., Livingston, K., Bourbonniere, R.A., Pickell, L.D., Trick, C.G., Dean, A.L., and McKay, R.M.L. 2003. Effect of phosphorus amendments on present day plankton communities in pelagic Lake Erie. *Aquat. Microb. Ecol.* 32:275–285.

Submitted: 8 January 2004
Accepted: 17 March 2005
Editorial handling: Gerald Matisoff