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An evaluation of iron bioavailability and speciation in western Lake Superior with the use of combined physical, chemical, and biological assessment

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Abstract

An iron-dependent cyanobacterial bioreporter (*Synechococcus* strain KAS101) was used in unison with size-fractionated iron content (>0.45, <0.45, <0.02 μm), and chemical characterization of iron complexation (C_{18} resin column) to elucidate the bioavailable forms of iron present in Lake Superior during periods of inverse thermal stratification (May) and strong thermal stratification (September) of the water column. The results provide evidence of organic complexation of iron in Lake Superior waters. Iron in most sampled water was complexed by organic compounds that behaved like fulvic acids, whereas some samples showed evidence for the presence of siderophore-like compounds. The presence of dissolved organic matter suppressed the cellular luminescence of the bioreporter, indicating an increased iron bioavailability. This effect could result either from the presence of siderophores forming iron complexes that are bioavailable to the bioreporter, or from more indirect effects because of the presence of other organic compounds, such as fulvic acids or polysaccharides. Model ligand additions, iron bioaccumulation, and photo-oxidation of dissolved organic matter were used to assess the bioavailability of organically complexed iron to the bioreporter. A significant fraction of the iron (40–100%) was bioavailable to the bioreporter. Iron bioavailability was high enough for the bioreporter not to be iron limited in the water collected from Lake Superior. This measure of bioavailability to picocyanobacteria is relevant because picoplankton accounted for the majority of chlorophyll *a* in Lake Superior during this study.

Introduction

Lake Superior has the largest surface area (82,100 km^2) of any freshwater lake worldwide, and is the deepest (maximum depth, 406 m) and coldest (average temperature, 4°C) of the Laurentian Great Lakes. Investigations in oligotrophic Lake Superior reveal that total dissolved iron is present at low concentrations (0.6–27 nmol L^{-1}; Nriagu et al. 1996; Field and Sherrell 2003; McKay et al. 2005). Bottle assays showed that iron-limited phytoplankton grew after phosphorus supplementation in water sampled from the western region of the lake (Sterner et al. 2004). Despite these recent advances in knowledge of iron dynamics in Lake Superior, relatively little is known with regard to the availability of iron to phytoplankton in this large lake. In oceanic surface waters, organic complexation of most iron by organic ligands increases its solubility (Rue and Bruland 1995) and constitutes chemical forms of iron with variable bioavailability to microorganisms (Hutchins et al. 1999; Mioni et al. 2005). Iron-dependent bioreporters provide a quantifiable measure of iron deficiency and thus represent a promising new technique for evaluating iron bioavailability in natural waters.

Because the iron assimilation strategies of green algae and diatoms differ from cyanobacteria (Hutchins 1995; Hutchins et al. 1999; Volker and Wolf-Gladrow 1999), we cannot speculate whether a cyanobacterial bioreporter serves as a proxy for the algal community at large. However, the use of a cyanobacterial bioreporter is relevant here given that the phototrophic picoplankton of Lake Superior can compose >50% of the pelagic chlorophyll *a* (Chl *a*; Fahnenstiel et al. 1986; McKay et al. 2005; Ivanikova et al. 2007*b*). In addition, assays of photosynthesis as a function of irradiance performed on Lake Superior surface-water samples indicated that the autotrophic plankton fraction <5 μm in size is an important contributor to lake biogeochemistry because it contributes from 30% to 100% of primary productivity, depending on the season (Ivanikova et al. 2007*b*).

The bioreporter strain used in this study, *Synechococcus* sp. PCC 7942 (KAS101; Durham et al. 2002; Porta et al. 2003), has undergone extensive physiological characterization and optimization to determine its dynamic response range and its laboratory reliability (Hassler et al. 2006). This bioreporter shows a dose-dependent response (measured as increasing cellular bioluminescence with decreasing iron availability) in the form of a sigmoid curve with a linear range over two orders of magnitude (0.45–45 nmol L^{-1}) of bioavailable iron (Hassler and Twiss 2006). Ligands with varying stability constants for Fe(III), including synthetic ligands, well-defined natural iron-binding ligands, and an aquatic fulvic acid, were used to clarify the chemical forms of iron sensed by the bioreporter (Hassler and Twiss 2006). Iron complexed by ligands with

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conditional stability constants $<10^{23.3}$ L mol^{-1} contribute to the pool of iron available to the bioreporter, and fully, partially, and non-bioavailable iron complexes can be differentiated according to their conditional stability constants (Hassler and Twiss 2006).

The objective of this study was to examine iron bioavailability across spatial and temporal scales in the western region of Lake Superior (Fig. 1) with the use of the *Synechococcus* sp. KAS101 bioreporter. We hypothesize that this bioreporter can be used as an analytical tool capable of quantifying the bioavailable fraction of iron in natural samples. Here we successfully use the iron-dependent bioreporter to examine iron bioavailability using different biological approaches: iron bioaccumulation, measurement of cellular bioluminescence in lake water, and use of ligand exchange theory to assess iron bioavailability by measuring the bioreporter response to water containing defined amounts of three model ligands of varying strength. In addition, physical (size fractionation) and chemical (organic complexation) iron speciation was measured and related to in situ Chl *a* concentrations. Most complexed iron in Lake Superior behaved like iron bound to an aquatic fulvic acid and was readily bioavailable to the iron-dependent bioreporter. One exception was from a midlake pelagic station that displayed water chemistry consistent with that of iron complexation by siderophore-like compounds. Our results showed a high degree of iron bioavailability in Lake Superior during both cold and warm surface water phases, but they also reflect a highly variable response in concert with the known complexity of iron chemistry in natural waters. Given the known abundance and importance of picoplankton in the Laurentian Great Lakes (Fahnenstiel and Carrick 1992) and the documentation of low concentrations of dissolved trace metals present in Lakes Superior, Ontario, Erie, and Huron (Nriagu et al. 1996), the approaches presented here can be expanded to provide analyses of Fe bioavailability in other large oligotrophic lakes.

Methods

Sampling—Waters from the western basin of Lake Superior (Fig. 1) were sampled with trace metal clean techniques from the R/V *Blue Heron* during periods of both inverse thermal stratification (18–22 May 2004) and strong thermal stratification (14–16 September 2004) (Fig. 2). Stations were selected to reflect a range of nearshore and pelagic characteristics. Guided by depth profiles of in vivo Chl *a* fluorescence and temperature at two pelagic stations (Castle Danger [CD1] and Western Mid-lake [WM]) during the September cruise, water was sampled from the surface 5 m (epilimnion of both Stas. WM and CD1), 15 m (epilimnion and deep chlorophyll maximum [DCM] of Stas. WM and CD1, respectively), 30 m (within the DCM of the metalimnion at Sta. WM), and 50 m (within a second DCM in the hypolimnion of Sta. CD1, Fig. 2). During the May cruise, during which the water column was mainly isothermal, water was sampled only from 5 m.

At pelagic and offshore stations, water was pumped from depth through Teflon™-lined polyethylene tubing

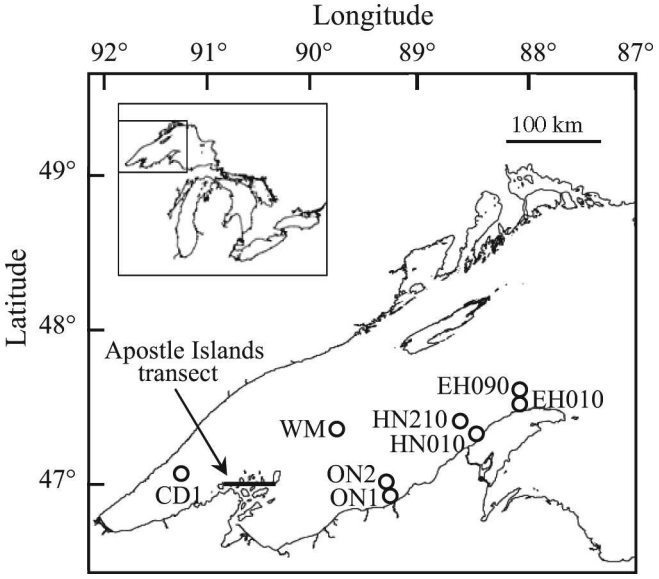

Fig. 1. Study sites on western portion of Lake Superior. Castle Danger (CD1) and Western Mid-lake (WM) are the two pelagic stations. Nearshore–offshore transects are close to Ontonagon River (ON1, ON2), Hancock North (HN010, HN210), and Eagle Harbor (EH001, EH090). The number after the abbreviation for each station stands for the distance from shore (km). In addition, a transect through the Apostle Islands (Stas. AP1–5) was performed.

with a pneumatic Teflon™ double-diaphragm pump (Husky model 307). Water was delivered directly to a HEPA laminar flow hood from which polypropylene taps provided whole (unfiltered) or filtered lake water. Filtration was done through a molded capsule filter (0.45-μm pore size; Calyx polypropylene capsule filter, GE Osmonics, GE Water Technologies) that was rinsed with 20 liters of lake water before first use and with 1 liter of lake water before sampling at each station. Before the first use, tubing and taps were acid-washed (dilute trace metal–grade HCl) and then rinsed 25 min at the station before sampling. Previous studies demonstrated that this pumping system allows sampling under trace metal–clean conditions (Field and Sherrell 2003).

Nearshore station Ontonagon 1 (ON1) and Hancock North 010 (HN010) were sampled with a Teflon-coated Go-Flo bottle deployed along a Kevlar™ line connected to a plastic-coated anchor. All material used were composed of polycarbonate, polyethylene, or Teflon soaked in 0.1–0.5% HCl overnight and rinsed sevenfold in de-ionized water (Milli-Q Gradient™, purity >18 Mohms cm^{-1}; Millipore).

Chlorophyll a *and DOC analyses*—Whole lake water was analyzed onboard for Chl *a*. A known amount of lake water was filtered in duplicate onto 0.2-, 2-, and 20-μm–pore size filters in parallel. Filters containing seston were extracted for 24 h in 90% acetone at 4°C in the dark before fluorimetric determination of Chl *a* (TD 700, Turner Designs) according to the nonacidification technique of Welschmeyer (1994).

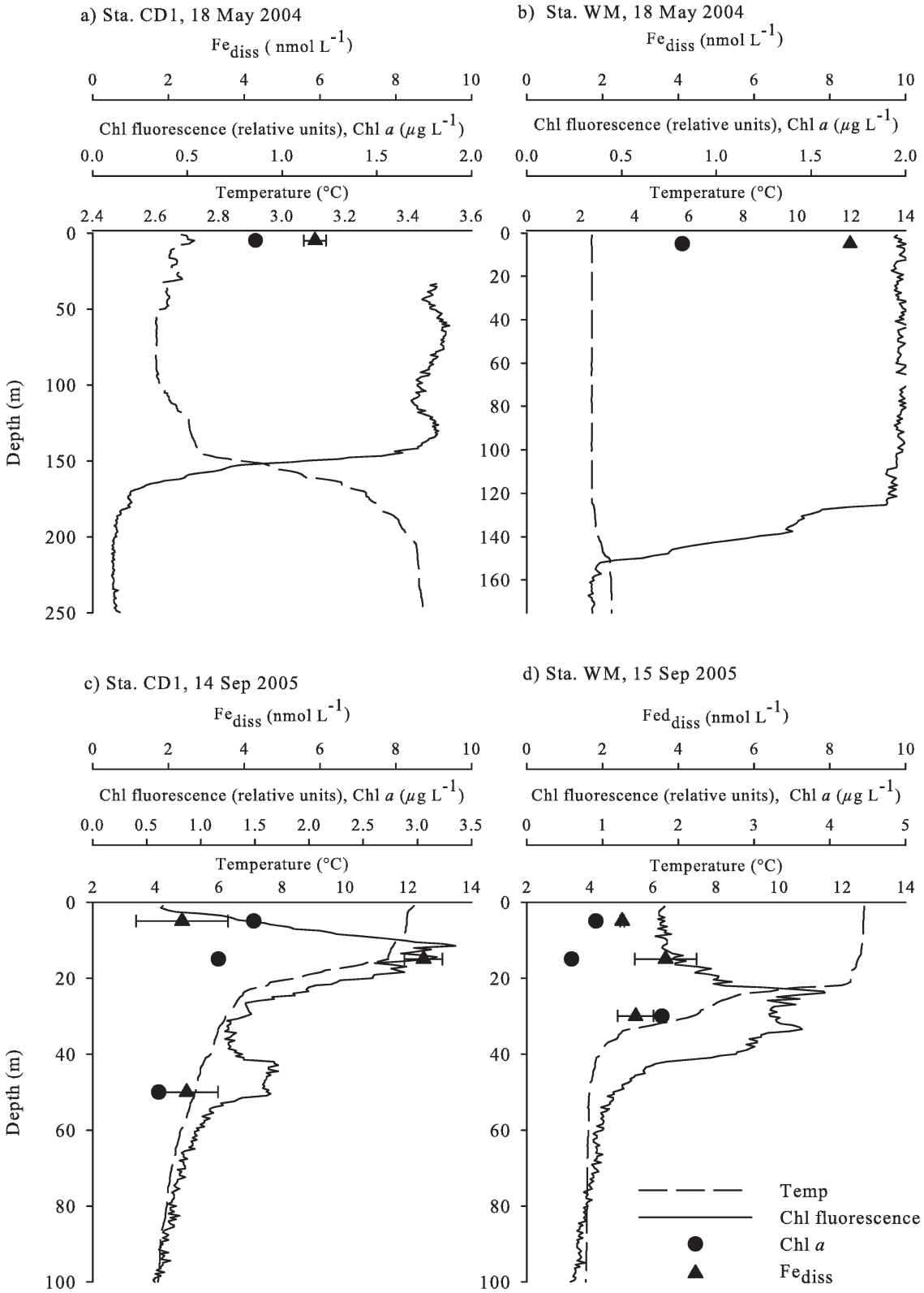

Fig. 2. Temperature and relative chlorophyll fluorescence from the CTD profiles at Stas. CD1 and WM in May and September. In May, the lake was inversely stratified with surface temperatures between 2.4°C and 2.7°C, whereas it was thermally stratified in September with surface water temperatures between 12°C and 13°C. Discrete measurement of Chl *a* and dissolved iron (Fe_{diss}) are also shown. Error bars represents data range ($n = 2$).

Amber glass bottles were completely filled with filtered (<0.4 μm) water and kept at 4°C in the dark before measurement of dissolved organic carbon (DOC) with a Rosemount Dohrmann DC-190 high-temperature total organic carbon analyzer (Hinton et al. 1997). In September, colored dissolved organic matter (CDOM) was measured fluorimetrically (TD 700) in quartz tubes on freshly collected whole lake water. The fluorometer was calibrated according to known concentrations of Suwannee River Fulvic Acid (SRFA, International Humic Substances Society).

Dissolved and particulate iron analyses—Manipulations of lake water were done in a HEPA laminar flow hood. For particulate iron determination, whole lake water (100–500 mL) was filtered onboard onto 0.4-μm–pore size polycarbonate membrane filters (Millipore) with the use of an acid-washed Teflon filtration system (Savillex™). Duplicate membranes were rinsed with low-iron artificial freshwater (modified Fraquil medium; Andersen et al. 2005) and stored frozen at −20°C. Total particulate iron (Fe_{part}) was determined from room temperature digestion of the 0.4-μm–pore size membrane filter in 1 mL of concentrated trace metal clean HNO_3 (Baseline, Seastar) for 2–3 d. This digestion would not dissolve iron within aluminosilicate matrices but would effectively liberate any iron associated with organic particles. Filtered lake water was acidified to pH 2 with HNO_3 before determination of dissolved iron (Fe_{diss}). In September, filtered (<0.45 μm) water was ultrafiltered (0.02 μm pore size) on board with an acid-washed and lake water–rinsed polypropylene syringe (HSW) and Anopore® syringe filter (Anotop 25, Whatman). Samples (60–80 mL) were manually ultrafiltered under laminar flow.

Iron concentrations were determined with a graphite furnace atomic absorption spectrophotometer (GFAAS, Perkin Elmer AAnalyst 600) with pyrolyzed tubes with L'Vov platforms and the addition of 15 μg of $Mg(NO_3)_2$ matrix modifier. The detection limit was 0.66 nmol L^{-1}. Each measurement was performed in duplicate, and accuracy (101.6% ± 3.4%, $n = 10$) was assured with SLRS-4–certified standard freshwater reference solution (National Research Council Canada).

Organically complexed iron assessment—Iron bound to organic compounds possessing hydrophobic functional groups in the lake water fraction <0.45 μm was assessed by C_{18} Sep-Pak Plus (Waters) cartridges as described in Abbasse et al. (2002). The C_{18} resin was activated by passing 10 mL of 100% methanol (ACS Reagent, JT Baker) and conditioned by sequentially passing various solvents through at a flow rate of 6–7 mL min^{-1}: 10 mL of de-ionized water (high-performance liquid chromatography–grade), 10 mL of 2 mol L^{-1} HNO_3 (ACS Regent, Fisher Scientific), 20 mL of de-ionized water, and finally, 15 mL of a mild ion-pairing agent (ammonium acetate 0.01 mol L^{-1}). A sample (10 mL) was passed through the activated C_{18} cartridge at a flow rate of 3–4 mL min^{-1}. The resin was then rinsed with 10 mL of ammonium acetate to remove nonspecifically retained iron, and retained iron was extracted with 10 mL of HNO_3 (2 mol L^{-1}) at a flow rate of 1 mL min^{-1}. Remaining organic ligands were eluted with 5 mL of 100% methanol. No appreciable amounts (<4%) of iron were extracted by a second rinse with HNO_3 or a final methanol rinse. Mass balance calculations showed good (>90%) iron recovery. The percentage of iron retention, corresponding to organically complexed iron (Abbasse et al. 2002), was calculated according to the difference of iron content before and after C_{18} treatment.

To facilitate the determination of organically bound iron at low ambient concentrations in small volumes, the samples were spiked with radioactive Fe ([^{55}Fe]–$FeCl_3$, specific activity 69.64 mCi mg^{-1}, Perkin Elmer Life Sciences) at a final activity of 1.0–1.5 nCi mL^{-1} and equilibrated for 7 d in the dark at 4°C. The radioactive iron addition correspond to a negligible iron enrichment of 0.26–0.39 nmol L^{-1}. Samples were allowed to reach room temperature for 4 h in the dark before analysis. In addition to lake water samples, C_{18} resin retention of inorganic iron (major salts of Fraquil media, pH 7.0, to which no additional iron was added—[Fe] < GFAAS detection limit) and synthetic and natural organic iron complexes with variable binding groups and affinities were evaluated. The following Fe-binding ligands (model ligands) were analyzed: ethylenediaminetetraacetic acid (EDTA, Sigma), 8-hydroxyquinoline-5-sulfonic acid (8HQS, Sigma), *N*,*N*′-di[2-hydroxybenzyl]ethylenediamine-*N*,*N*′-diacetic acid monohydrochloride hydrate (HEBD, Strem Chemicals), SRFA, DFB (desferroxamine B, Sigma), and ferrichrome (Sigma). Each model ligand solution was composed of macronutrients from Fraquil medium, 100 nmol L^{-1} iron, and 200 nmol L^{-1} ligand. Under these conditions, >97% of the iron is calculated to be chelated by the ligand (MINEQL+, version 4.5, with NIST, version 8.0, database); for SRFA, no Fe-binding constants are available, so 10 mg L^{-1} was used to complex iron. These model solutions were equilibrated for 2 d in the dark before analysis. Stock solutions of synthetic ligands were prepared at 10^{-3} mol L^{-1} and adjusted to pH 6 with ultrapure NaOH (Alfa-Aesar) and HNO_3 (Seastar), and iron contamination was checked with GFAAS.

Samples collected in May 2004 (Stas.WM, Apostle Island 3 [AP3], and ON1) were analyzed with and without ultraviolet (UV) irradiation. Samples were placed in acid-washed quartz tubes and exposed to two cycles of 17 min (separated by a 3-h cooling period) to a 1200 W mercury vapor lamp. The UV treatment resulted in a complete disappearance of CDOM (TD 700 fluorometer), and a slight decrease in the pH (0.1–0.3 units, initial pH 7.5).

Iron bioavailability assessment—Iron bioavailability was measured with the use of the iron-dependent *Synechococcus* sp. bioreporter strain KAS101 (Durham et al. 2002) at early exponential growth phase in Fraquil medium. For details regarding maintenance, low-iron acclimation, and optimization of the use of the bioreporter, see Hassler et al. (2006). *Synechococcus* sp. was isolated from the growth medium by gentle filtration (0.4-μm–pore size polycarbonate filter, <30 kPa), washed for 1 min in 10^{-5} mol L^{-1} EDTA, rinsed in Fraquil with no added trace metals, and

resuspended in 1.5 mL of the same medium. Cell counts were measured with an electronic particle counter (Multisizer Z3, Beckman Coulter), and aliquots of this cyanobacterial suspension were dispensed to obtain 10^5 cells mL^{-1} into various samples. Samples of filtered lake water (<0.45 or <0.02 μm) that were either UV-treated or not irradiated were assayed. All manipulations were done in acid-washed polycarbonate containers in a HEPA laminar flow hood.

Bioreporter assays—Under iron deficiency, the reporter strain emits light from the expression of bacterial luciferase. To provide appropriate substrate to the luciferase, 3 μL of 60 μmol L^{-1} decanal was added to 1.5 mL of sample, let stand 5 min to react in the dark, then bioluminescence was measured with a luminometer (Zylux FB14). Previous characterization of the bioreporter (Hassler and Twiss 2006; Hassler et al. 2006) showed a minimal and maximal response at >45 and <0.45 nmol L^{-1} of bioavailable iron, respectively. Between these threshold concentrations, the logarithm of the bioreporter response (i.e., the log of cellular bioluminescence; relative light units [RLU] s^{-1}) is proportional ($r^2 = 0.90$) to the logarithm of the bioavailable iron concentration in Fraquil ($\log[Fe]_{bio}$, mol L^{-1}, Eq. 1; Hassler et al. 2006).

$$\log(\text{cellular bioluminescence}) = -0.8 \log\left([Fe]_{bio} - 8.0\right) \quad (1)$$

For each sample, a positive control (addition of 100 nmol L^{-1} DFB) and a negative control (addition of 100 nmol L^{-1} Fe) were performed to verify that cellular bioluminescence was related to iron bioavailability in the sample. Variable initial biomasses of the bioreporter (5×10^4 to 5×10^6 cells mL^{-1}) were used to measure iron bioavailability from natural samples containing variable concentrations of dissolved iron.

To determine the fraction of iron that is bioavailable in Lake Superior, the bioreporter was exposed to filtered lake water in the presence of increasing concentrations of model ligands (0, 1, 10–1000 nmol L^{-1}, and 2500 nmol L^{-1} [ON1] for samples collected in May and 0, 50–1000 nmol L^{-1} for samples collected in September). Solutions were allowed to equilibrate for 24 h at 20°C in the dark. Three ligands were chosen on the basis of their bioavailability to the bioreporter (Hassler and Twiss 2006): (1) DFB, which forms iron chelates that are non-bioavailable to the bioreporter; (2) EDTA, which forms bioavailable iron chelates; and 3) diethylenetriaminepentaacetic acid (DTPA), which forms an iron complex that is slightly bioavailable to the bioreporter (Hassler and Twiss 2006). Each model ligand was selected to probe different bioreporter responses. Addition of EDTA does not affect the bioavailability of iron as sensed by the bioreporter but decreases the free concentrations of other trace metal nutrients present in the sample (Cu, Mn, Co, Zn) that could affect bioreporter response (Hassler et al. 2006). A previous study (Hassler and Twiss 2006) showed that bioavailability, if iron bound to EDTA, did not result from a significant photo- or thermal dissociation of the complexes under the experimental conditions used here. Thus, a change in the response of the bioreporter in the presence of EDTA takes into account matrix effects related to the addition of strong ligands. An excess of DFB titrates all the iron in solution (i.e., bioavailable and non-bioavailable iron), yielding maximal bioluminescence. The maximal response observed in the presence of DTPA identifies the iron pool that is potentially available to the bioreporter. In this case, iron bioavailability can be estimated by subtracting the response in presence of EDTA and comparing the two levels of the plateau of maximal response (Max) observed in the presence of DTPA and DFB (Eq. 2). The data points defining the maximal level of luminescence were determined by statistical comparison (paired *t*-test, 95% confidence interval).

$$\text{Bioavailability (\%)} = \frac{\text{Max}_{DTPA} - \text{Max}_{EDTA}}{\text{Max}_{DFB} - \text{Max}_{EDTA}} \times 100 \quad (2)$$

The response of the bioreporter was verified for each experiment with a calibration of the cellular bioluminescence to variable bioavailable concentrations of iron (0.3–450 nmol L^{-1}, Eq. 1) in Fraquil media (Hassler and Twiss 2006). Each sample was assessed in triplicate, whereas each calibration was conducted in duplicate.

^{55}Fe bioaccumulation—Iron bioavailability to the bioreporter was also measured by the bioaccumulation of ^{55}Fe. Bioaccumulation experiments were performed under identical conditions as the bioassay (*see* above). Previous characterization of the bioreporter showed a linear relationship ($r^2 = 0.99$) between the logarithm of bioaccumulation ($\log[Fe]_{int}$, mol $cell^{-1}$) and the logarithm of the concentration of bioavailable iron (<100 nmol L^{-1} in Fraquil medium; Eq. 3; Hassler et al. 2006). Note that the slope of 1.0 relates to a true linear relationship between $[Fe]_{int}$ and $[Fe]_{bio}$.

$$\log\left([Fe]_{int}\right) = 1.0 \times \log\left([Fe]_{bio}\right) - 10 \quad (3)$$

^{55}Fe was added to filtered lake water and Fraquil solutions to a final activity of 1.0 nCi mL^{-1} and allowed to equilibrate for 24 h at 20°C in the dark. Intracellular and surface-bound iron were differentiated by a 20-min contact with a mixture of ligands after 12 h exposure to ^{55}Fe (Tovar-Sanchez et al. 2003; Hassler and Schoemann in press). The recipe was slightly modified as follows: omission of NaCl, 10^{-3} mol L^{-1} citric acid, 10^{-3} mol L^{-1} EDTA, and 0.02 mol L^{-1} oxalic acid at pH 8 (Hassler et al. 2006). These surface-washed cells were isolated by gentle filtration and rinsed with non-radiolabeled Fraquil medium. ^{55}Fe content was measured by liquid scintillation (RackBeta model 1219, LKB Wallac) with quench correction. Initial and final dissolved ^{55}Fe were determined to exclude any biological consumption of iron. The nominal concentration of dissolved iron was used to determine the initial isotopic ratio and to transform ^{55}Fe disintegrations per minute into molar concentration. Intracellular iron was determined in triplicate (mol $cell^{-1}$). Iron bioavailability (%) was assessed by comparing iron bioaccumulation in Fraquil, in which 100% is bioavailable (Hassler and Twiss

Table 1. Water quality parameters from western Lake Superior in May 2004. Size fractionation of dissolved iron ($[Fe]_{diss} < 0.45$ μm) and particulate iron ($[Fe]_{part} > 0.4$ μm), Chl *a* (>20-μm microplankton [μ], 2.0–20-μm nanoplankton [n], and 0.2–2.0 μm picoplankton [p]), dissolved organic carbon (DOC), and the cellular bioluminescence of the bioreporter *Synechococcus* sp. KAS101 are presented. ND = not detected.

Station	Latitude	Longitude	$[Fe]_{diss}$ (nmol L^{-1}, mean ± SD, $n = 4$)	$[Fe]_{part}$ (nmol L^{-1}, mean ± SD, $n = 4$)	Total Chl *a* (μg L^{-1}) (μ/n/p, %)	DOC (mg L^{-1})	Bioluminescence (Lum $cell^{-1}$, $\times 10^{-4}$ RLU s^{-1}, mean ± SD, n = 3–6)
WM	47°20′01″	89°47′89″	12.0±0.0	48.8±2.3	0.82 (10/35/55)	1.2	6.8±0.9
CD1	47°03′86″	91°25′87″	5.9±0.3	64.8±1.6	0.86 (10/40/51)	1.5	9.3±3.2
EH090	47°33′04″	88°09′18″	ND	21.7±3.2	0.86 (8/38/55)	1.9	17.4±5.9
EH010	47°28′31″	88°08′00″	0.6±0.1	44.3±0.9	0.88 ND	1.4	16.0±8.8
HN210	47°24′33″	88°44′33″	ND	51.0±7.6	0.93 (11/51/39)	1.9	10.9±2.3
HN010	47°15′47″	88°35′17″	56.2±0.8	194.7±10.6	1.76 (24/28/47)	2.1	6.0±0.8
ON2	46°59′02″	89°21′55″	13.3±0.5	148.9±6.9	1.37 (12/27/67)	1.3	16.9±2.8
ON1	46°54′56″	89°17′51″	61.9±0.0	248.8±29.0	1.82 (13/45/42)	1.8	34.5±3.4
AP1	46°59′99″	90°49′04″	1.2±1.0	107.0±5.9	1.27 (13/47/39)	1.2	ND
AP2	47°00′01″	90°43′45″	1.5±0.4	86.4±17.3	1.27 (16/37/47)	1.4	ND
AP3	46°59′99″	90°34′99″	15.5±5.0	74.8±6.5	1.30 (15/38/48)	—	10.0±3.08
AP4	46°59′95″	90°30′55″	1.3±0.2	63.2±2.9	1.23 (16/33/50)	1.8	ND
AP5	46°59′96″	90°15′04″	0.7±0.5	51.9±4.9	0.98 (9/39/53)	1.9	7.4±0.8

2006), and iron bioaccumulation in the sample, for an identical dissolved iron concentration (Eq. 4).

$$\text{Bioavailability (\%)} = \frac{[Fe]_{int} - \text{Sample}}{[Fe]_{int} - \text{Fraquil}} \times 100 \quad (4)$$

Results

Limnological parameters of Lake Superior sampling stations—Western Lake Superior sampling stations were inversely thermally stratified in May and thermally stratified in September (Fig. 2). In May, Chl *a* content was higher at nearshore stations (AP1–3, ON1, HN010, EH001; Table 1) than at their corresponding offshore sites (AP5, ON2, HN210, EH090) or at pelagic stations (WM and CD1). The relatively high Chl *a* concentration at Sta. ON1 suggests that nutrients from the Ontonagon River supported greater phytoplankton biomass in this area. Size-fractionated Chl *a* showed that most of the primary producers present were in the picoplankton (0.2–2 μm) and nanoplankton (2–20 μm) size classes for offshore and nearshore stations, respectively, except for Sta. HN (Table 1).

In May, both particulate and dissolved iron were higher at nearshore stations compared with offshore stations (May, Table 1; Fig. 1) with a positive correlation observed ($r^2 = 0.86$) between particulate iron and total Chl *a* ($[Chl\ a]_{tot}$, Fig. 3a). If we assume that Chl *a* is an appropriate proxy of phytoplankton biomass, then the relationship between dissolved iron and $[Chl\ a]_{tot} \geq 1.2$ μg L^{-1} ($r^2 = 0.98$, Fig. 3b) suggests that primary productivity could be iron limited. For stations with low [Chl *a*] (<1.2 μg L^{-1}; pelagic stations and the offshore sites of nearshore–offshore transects AP5 and EH090), the Chl *a* was lower than solely predicted by the concentration of dissolved iron. In this case, another nutrient or grazing pressures might limit the standing crop of phytoplankton.

The concentration of DOC was low at all stations and no increase of DOC was observed in the coastal areas during May. The state of the lake in May was quiescent, whereas a near-gale prevented nearshore sampling in September and likely caused fine sediment resuspension at stations with maximum depths of <50 m.

Thermal stratification of the water column in September 2004 favored the development of deep chlorophyll maxima in the metalimnion at the pelagic Sta. CD1 and WM (Fig. 2). For both stations, Chl *a* was most abundant in the picoplankton size class at all sampled depths, except 15 m. In September, higher $[Chl\ a]_{tot}$ (1.7- to 2.2-fold) was observed compared with May despite a lower $[Fe]_{diss}$ (2.5- to 3.7-fold). No significant variation of CDOM was observed with depth.

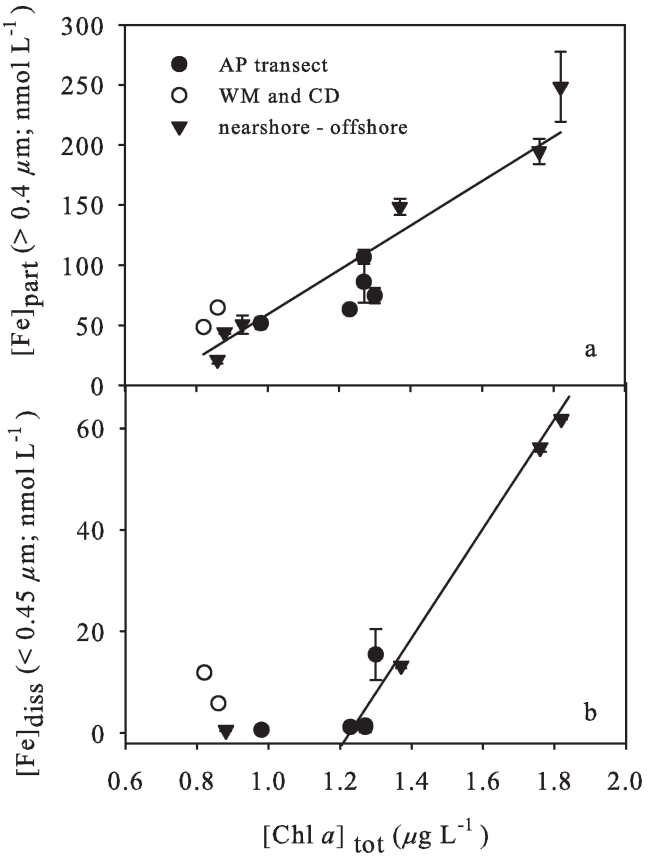

Fig. 3. Particulate Fe_{part} (a) and dissolved Fe_{diss} (b) iron at sampling stations as a function of total [Chl *a*]. Data are presented for a different type of stations: pelagic, nearshore–offshore, and Apostle Islands. The solid lines represents linear regression between total Chl *a* and particulate iron ($r^2 = 0.86$) or dissolved iron ($r^2 = 0.98$, for total [Chl *a*] ≥ 1.2 μg L^{-1}). Error bars represent standard deviations ($n = 4$).

Organic complexation of iron—Most of the inorganic forms of iron—supposedly Fe(III) as $[Fe]_{tot}$ (background contamination and ^{55}Fe addition) < 1 nmol L^{-1}—were retained by the C_{18} cartridge (Table 2). When lake water from Stas. ON1 and AP3 was UV-treated, the degree of iron retention through a C_{18} cartridge was identical to that of inorganic iron (Table 2). Because most of the dissolved iron is known to be associated with organic ligands in surface water, the difference between retention through the C_{18} cartridge with and without UV treatment is presumably due to organic complexation in the sample (Table 2). The use of model ligands provided information on the type of organic speciation likely present (Table 2). Addition of EDTA resulted in a poor degree of iron retention; for the other model ligands used, the C_{18} cartridge retained >98% of the organically bound iron. However, only 32% of iron in the presence of SRFA was retained; this low retention is attributed to the relatively high degree of hydrophilicity of SRFA, like EDTA. In this case, low retention suggested that iron is associated with ligands similar to SRFA, whereas high retention indicated a complexation of iron with more hydrophobic ligands.

For most stations sampled in May and for areas affected by the input of the Ontonagon River (Stas. ON1, HN010), retention of 39–55% was measured, suggesting that iron was organically bound with relatively hydrophilic ligands like fulvic acids. Station WM shows a higher degree (69%) of retention, consistent with iron bound by more hydrophobic organic compounds. In September, the degree of retention significantly increased at Sta. WM (80–88%), whereas it remained unchanged at Sta. CD1. Because the percentage of retention was not depth dependent (at Stas. WM and CD1), organic complexation of dissolved iron does not appear to be correlated to water stratification. Retention of organically bound iron by the C_{18} cartridge is similar for 0.45- and 0.02-μm–filtered samples from Sta. WM (Table 2), suggesting that most of the Fe–organic complexes were truly dissolved or associated with small (<0.02 μm) colloids.

Assessment of iron bioavailability: bioreporter assay—Because iron is present in low concentrations in Lake Superior waters, the use of a high bioreporter biomass could either deplete the dissolved iron, via surface sorption or cellular internalization, or contaminate the sample with

Table 2. Relative retention of iron by the C_{18} cartridge for samples collected in May and September 2004 (at 5, 15, and 30 m). All natural samples were 0.45-μm filtered, except when stated (0.02 μm). In addition, some samples collected in May (Stas. ON1 and AP3), with UV irradiation, and several characterized organic ligands were used: EDTA, 8HQS (8-hydroxyquinoline-5-sulfonic acid), HEBD (*N*,*N*′-di[2-hydroxybenzyl]ethylenediamine-*N*,*N*′-diacetic acid monohydrochloride hydrate), ferrichrome, desferrioxamine B (DFB), and Suwannee River Fulvic Acid (SRFA).

May 2004		Sep 2004		Ligand	
Sample	Retention ± SD (%, $n = 3$)	Sample	Retention ± SD (%, $n = 3$)	Sample	Retention % ± SD $n = 3$–9
WM	68.5±2.0	WM, 5 m	82.6±1.1	Inorganic Fe	80.4±2.6
CD1	55.0±3.3	WM, 5 m <0.02 μm	87.8±1.0	EDTA	9.7±3.9
EH090	42.6±5.6	WM, 15 m	80.8±0.6	8HQS	98.8±1.3
EH001	52.8±4.2	WM, 15 m <0.02 μm	81.7±1.7	HEBD	98.6±1.9
HN210	45.7±0.6	WM, 30 m	83.0±1.5	Ferrichrome	99.9±0.1
HN010	39.2±4.2	CD1, 5 m	54.9±5.6	DFB	99.9±0.0
ON2	42.7±2.3	CD1, 15 m	53.4±4.1	SRFA	31.8±6.6
ON1	39.0±4.2			ON1 + UV	79.2±3.4
AP3	55.0±4.0			AP3 + UV	80.1±1.9

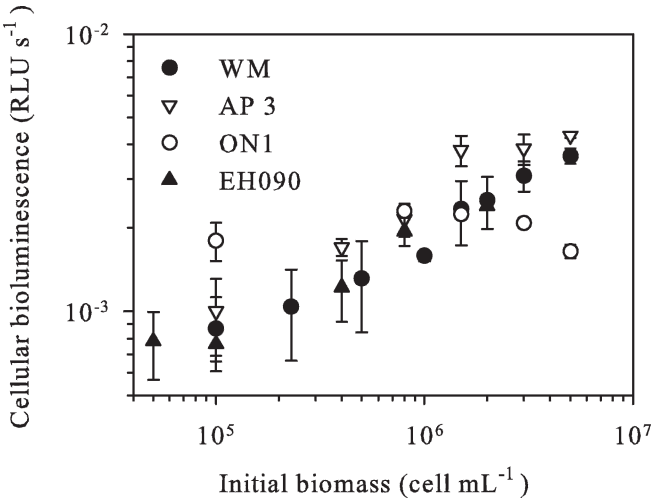

Fig. 4. Relationship between the response of the bioreporter and initial bioreporter biomass (3×10^3 to 5×10^6 cells mL^{-1}) in four natural samples. The four stations have different dissolved iron concentrations, which illustrate the variability observed in Lake Superior during May 2004 (*see* Table 1). The bioreporter was incubated for 12 h at 19°C and 60 μmol photons m^{-2} s^{-1}. Error bars represents standard deviation (n = 3–6).

iron initially contained in the bioreporter cells. The effect of variable biomass (Fig. 4) additions to natural water samples on the expression of cellular bioluminescence was assessed for stations with variable $[Fe]_{diss}$ (*see* Table 1). Whereas no effect on cellular bioluminescence was observed at high $[Fe]_{diss}$ (62 nmol L^{-1}, Sta. ON1), the use of biomass $>5 \times 10^5$ cells mL^{-1} increased the cellular bioluminescence in water sampled from all other samples ($[Fe]_{diss} < 16$ nmol L^{-1}). In this study, the bioreporter was used at an initial biomass of 10^5 cells mL^{-1}, a level at which no biomass effect was observed.

Because the bioreporter bioluminesces in proportion to iron deficiency, the bioreporter assays should indicate iron limitation, expressed as high cellular bioluminescence, in addition to an evaluation of iron bioavailability (*see* Eq. 1). Maximum bioluminescence was not observed in these assays, indicating that the bioreporter was not limited by iron in water sampled from these stations (Table 1). The response of the bioreporter did not correlate to particulate iron or total Chl *a* concentrations (Tables 1, 3). According to cellular bioluminescence, iron bioavailability was lower at the nearshore Sta. ON1 than at pelagic Stas. WM and CD1, despite the higher $[Fe]_{diss}$ measured at Sta. ON1. Seasonal variation in Fe bioavailability was witnessed at Sta. WM but not at Sta. CD1 (Tables 1, 3). In September, a depth-dependent bioluminescence was measured at Sta. WM but not at Sta. CD1.

Ultrafiltration (<0.02 μm) should result in a decrease of iron concentration (compared with the fraction <0.45 μm) because of the removal of iron associated with colloids, but not necessarily a decrease in iron bioavailability. At Sta. CD1, the ultrafiltration resulted in a decrease of iron bioavailability (i.e., an increase of bioluminescence), whereas ultrafiltration resulted in an increase of iron bioavailability at Sta. WM (Table 3).

To understand the relationship of cellular bioluminescence to dissolved iron concentrations, the response (i.e., cellular bioluminescence) of the bioreporter for all stations was plotted according to the $[Fe]_{diss}$ and superimposed on the average of all the calibrations (n = 42) performed in Fraquil (Fig. 5). The response of the bioreporter in EDTA-buffered Fraquil medium, in which all dissolved iron is bioavailable (Hassler and Twiss 2006), was compared with the response of the bioreporter in lake water samples, for which iron bioavailability should be <100%. Therefore, for an identical $[Fe]_{diss}$ in both Fraquil and natural water, the cellular bioluminescence should be higher in the lake water

Table 3. Water quality parameters for western Lake Superior in September 2004. In situ iron distribution as ultrafiltered water (<0.02 μm), dissolved iron ($[Fe]_{diss} < 0.45$ μm), and particulate iron ($[Fe]_{part} > 0.4$ μm); Chl *a* (>20 μm microplankton [μ], 2.0–20 μm nanoplankton [n], and 0.2–2.0 μm picoplankton [p]); CDOM (Suwannee River fulvic acid equivalents); and cellular bioluminescence of the bioreporter (inversely related to iron bioavailability, Lum $cell^{-1}$) are reported.

Sample	$[Fe]_{diss}$ (nmol L^{-1}, mean ± SD, n = 4)	$[Fe]_{part}$ (nmol L^{-1}, mean ± SD, n = 4)	Total Chl *a* (μg L^{-1}) (μ/n/p) (%)	CDOM (mg L^{-1})	Bioluminescence (Lum $cell^{-1}$, $\times 10^{-4}$ RLU s^{-1}, mean ± SD, n = 3)
Sta. CD1					
5 m	2.4±1.2	39.4±12.9	1.49 (30/23/46)	0.36	9.5±1.7
5 m, filtered <0.02 μm	1.6±0.0	—	—	—	34.7±12.3
15 m	8.7±0.5	51.4±0.1	1.16 (45/50/6)	0.4	8.2±1.2
15 m, filtered <0.02 μm	<0.6	—	—	—	20.9±7.3
50 m	2.5±0.8	69.7±1.7	0.61 (15/38/48)	0.37	5.7±1.4
Sta. WM					
5 m	2.5±0.1	47.30±9.95	0.91 (27/30/44)	0.26	51.5±6.8
5 m, filtered <0.02 μm	<0.6	—	—	—	23.4±1.8
15 m	3.7±0.8	67.12±1.02	0.59 (18/52/29)	0.37	36.8±10.9
15 m, filtered <0.02 μm	<0.6	—	—	—	20.3±4.7
30 m	2.9±0.5	75.35±7.02	1.78 (16/37/47)	0.32	28.3±6.2

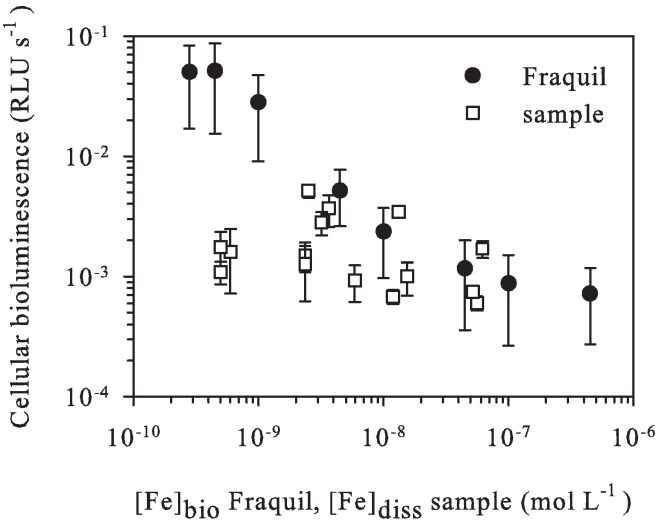

Fig. 5. Relationship between the response of the bioreporter as a function of dissolved iron concentration ($[Fe]_{diss}$) in the Fraquil medium and in the samples from Lake Superior. The bioreporter was incubated for 12 h at 19°C and 60 μmol photons m^{-2} s^{-1}. Because all the chemical forms of iron present in the Fraquil medium are bioavailable to the bioreporter (Hassler and Twiss 2006), $[Fe]_{diss}$ equals the concentration of bioavailable iron, $[Fe]_{bio}$. Error bars represent the standard deviation for the response in both the Fraquil medium (n = 42) and natural samples (n = 3–6).

sample because of lower iron bioavailability owing to Fe complexation by organic ligands. For higher $[Fe]_{diss}$ ($>10^{-8}$ mol L^{-1}), the response of the bioreporter measured in lake water is within the expected range, suggesting that enough bioavailable iron was present to satisfy bioreporter requirements (Fig. 5). However, for low $[Fe]_{diss}$, the response of the bioreporter in lake water samples was lower than that measured in Fraquil. Neither nonferrous trace metal enrichment nor macronutrient enrichment can explain such decreases in the response of the bioreporter (Hassler et al. 2006); thus, the effect of organic matter on cellular bioluminescence was further assessed.

Samples from Stas. WM, AP3, and ON1 were UV-irradiated to destroy organic ligands before assessment by the bioreporter. Assuming that most of the iron was complexed by organic ligands in the lake water, the UV treatment should result in a sample containing only fully bioavailable inorganic forms of iron. If the low observed bioluminescence was due to organic matter, then UV treatment should result in increased luminescence—to the level predicted in the Fraquil media, in which 100% of the iron is bioavailable to the bioreporter. In all cases, an increase in cellular bioluminescence was observed in all UV-treated samples (Table 4), suggesting that some organic matter–mediated suppression of bioluminescence was indeed responsible for the observed low bioluminescence in non–UV-treated filtered lake water. In addition, bioluminescence in UV-treated samples was similar (Stas. AP3, ON1) or higher (Sta. WM) than expected on the basis of the parallel calibration curves in Fraquil (Eq. 1, Table 4).

According to the theory of ligand exchange, the use of EDTA, DTPA, and DFB should provide useful information about the relative bioavailability of the iron present in natural waters (Eq. 2; Hassler and Twiss 2006). For water collected in May 2004, 42% ± 2%, 77% ± 9%, and 100% of iron was bioavailable at Stas. WM, ON1, and AP3, respectively. The addition of a large excess of EDTA (Fig. 6) resulted in only a slight increase of cellular bioluminescence (less than twofold), except for Sta. AP3 (fivefold). For Sta. ON1, in which $[Fe]_{diss}$ and other metals that could react with the added ligands were expected to be higher because of Ontanogan River inputs, higher concentrations of added model ligands were required to reach a maximal level of bioluminescence (Fig. 6). In September at Sta. WM, the smallest concentration of DTPA added (50 nmol L^{-1}) resulted in a maximal level of luminescence, identical to or slightly higher than that observed for DFB additions, indicating that this lowest level of DTPA effectively out-competed all the bioavailable iron complexes present in solution.

Maximal luminescence observed in the presence of DFB was significantly lower than the maximal luminescence obtained at calibration in Fraquil (Table 4; Fig. 6), as was observed in lake water samples (Fig. 4). If this difference is related to some effect from organic matter, then the addition of DTPA in UV-treated lake water (containing only inorganic forms of iron) is expected to bind all the iron present; therefore, the maximal level of bioluminescence should be similar to that observed in Fraquil medium. The

Table 4. Effect of UV treatment of lake water on the response of an iron-dependent bioreporter to water sampled from Lake Superior surface waters (5 m) in May in the presence and absence of DTPA. Data were compared with response in Fraquil medium at identical dissolved iron concentrations ($[Fe]_{diss}$). Considering that all the iron species in Fraquil medium contribute to iron bioavailability (Hassler and Twiss 2006), such comparison permits evaluation of the effect of UV irradiation on iron bioavailability sensed by the bioreporter.

Lake water treatment	Cellular bioluminescence (RLU s^{-1}, mean ± SD, $\times 10^{-3}$)		
	WM	ON1	AP3
Sample, n=3–6	0.68±0.09	1.7±0.3	1.0±0.3
Sample, UV, n=3	21±5	3.2±1.0	2.9±0.8
Predicted from $[Fe]_{diss}$ and Fraquil, n=2	2.0–2.2	1.6–1.7	2.2–2.8
Maximal, +DTPA, n=6–14	6.5±1.6	16±3	34±4
Maximal, UV + DTPA, n=6–8	85±8	120±10	120±20
Maximal, Fraquil medium, n=4	31±6	81±12	87±15

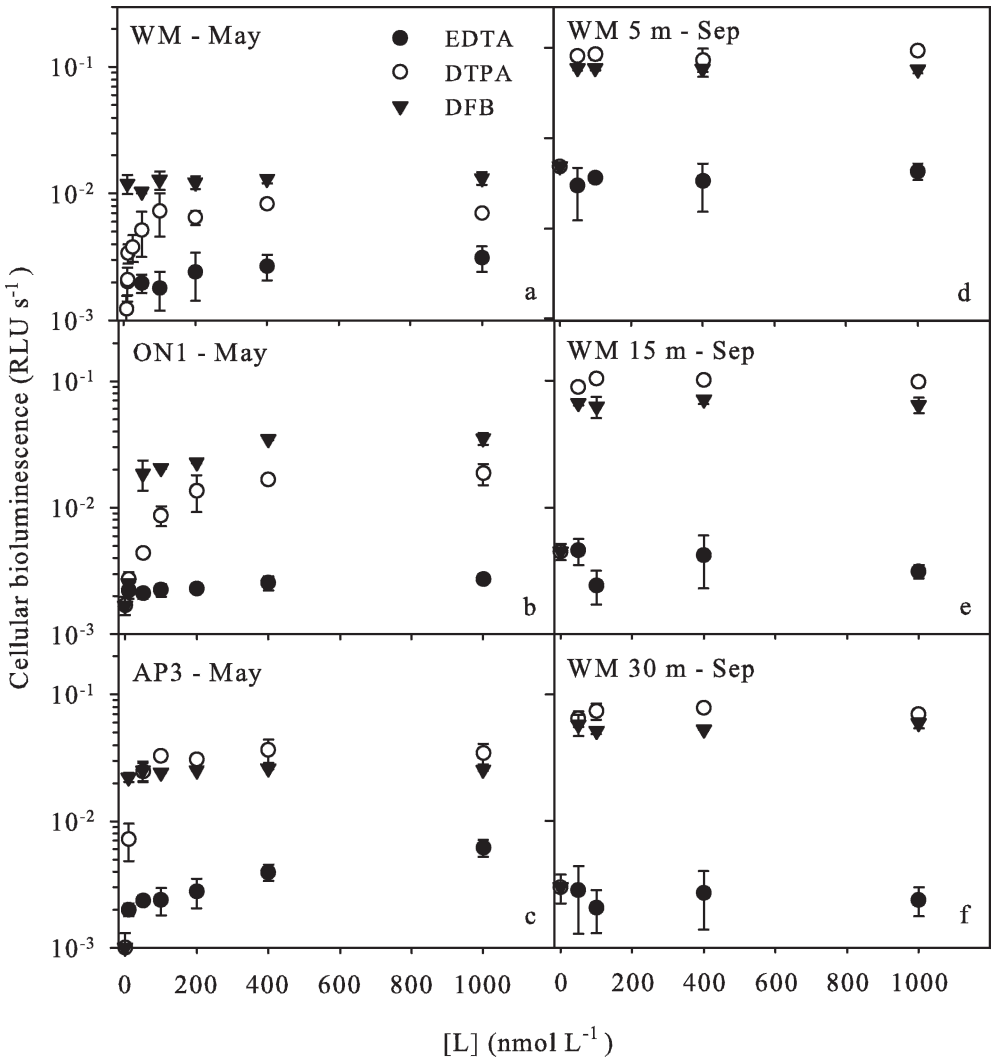

Fig. 6. Response of the bioreporter to variable addition of ligands after a 12-h exposure (19°C, 60 μmol photons m^{-2} s^{-1}). Exchange of ligands between characterized ligands (EDTA, DTPA, and desferrioxamine B [DFB]), natural ligands, and the bioreporter was performed on 0.45-μm filtered water from several stations sampled in May—(a) WM, (b) ON1, and (c) AP3—and in September—WM at (d) 5 m, (e) 15 m, and (f) 30 m. The maximal level of bioluminescence observed for the different characterized ligands is used to estimate the fraction of bioavailable iron. Error bars represent standard deviation (n = 3–6).

maximal cellular bioluminescence with DTPA addition in UV-treated samples is similar to (Sta. AP3) or higher than (Stas. WM and ON1) that observed in Fraquil (Table 4).

Assessment of iron bioavailability: bioaccumulation experiments—Iron bioaccumulation was performed in natural samples as well as in Fraquil solutions (Fig. 7). For identical $[Fe]_{diss}$, comparison of iron bioaccumulation measured in parallel with lake water and Fraquil medium, (Eq. 3) was used to assess the fraction of bioavailable iron in natural water (Eq. 4). The intracellular iron measured in natural samples was of the same order as that predicted in Fraquil, suggesting that no major synergistic or competitive effects on iron bioaccumulation because of the presence of organic matter or variable amounts of other metals are seen in natural waters as compared with Fraquil. In May, similar to bioreporter results, 40% ± 10%, 73% ± 14%, and 100% of the iron was bioavailable at Stas. WM, ON1, and AP3, respectively. In September, iron from a depth of 5 m at Sta. WM was fully bioavailable, whereas 70% ± 19% and 56% ± 7% of the dissolved iron was bioavailable in the water from the 15- and 30-m samples, respectively.

Discussion

Both dissolved and particulate iron were related to Chl *a* concentrations in most of the western Lake Superior stations observed here, which is similar to what has been observed in Lake Erie (Havens 2007). Nevertheless, phytoplankton biomass at pelagic stations (WM and

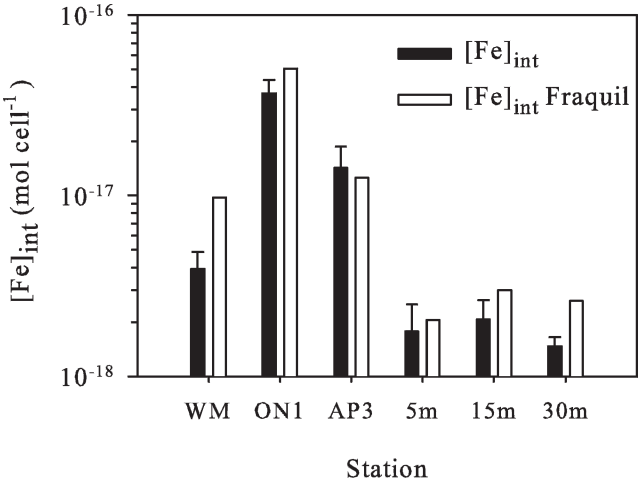

Fig. 7. Intracellular iron concentration ($[Fe]_{int}$) after a 12-h exposure (19°C, 60 μmol photons m^{-2} s^{-1}) observed for 0.45-μm filtered water from stations sampled in May (WM, ON1, AP3) and September (WM at 5, 15, and 30 m). Error bars represent the standard deviation (n = 6). To estimate the fraction of bioavailable iron, iron bioaccumulation in natural samples was compared with accumulation predicted in Fraquil. The relationship between $[Fe]_{diss}$ (equal to [Fe] bioavailable in Fraquil) and $[Fe]_{int}$ (Hassler and Twiss 2006) was verified in duplicate for each experiment. This relationship was used to calculate the average $[Fe]_{int}$ predicted in Fraquil medium at an identical $[Fe]_{diss}$ in the natural sample.

CD1) and some offshore stations (AP and EH090) seem to be limited by another factor (such as another nutrient or grazing pressure). Iron and phosphorus were shown to co-limit primary production in Lake Superior sampled during the summer period of thermal stratification (Sterner et al. 2004), which might be what is occurring at these stations. Indeed, a parallel project that used a nitrate-dependent bioreporter and shared the same water samples as in this study demonstrated interactions between P and Fe bioavailability in constraining nitrate uptake in Lake Superior (Ivanikova et al. 2007*a*).

In this study, the potential ability for iron to limit phytoplankton in western Lake Superior was assessed with a cyanobacterial iron bioreporter. Because the response of the bioreporter to variable biological, physical, and chemical conditions has been studied previously (Hassler and Twiss 2006; Hassler et al. 2006), it can also be used to assess the fraction of bioavailable iron in the water column. This bioreporter previously has been used to infer iron limitation in Lake Superior (McKay et al. 2005); however, no iron limitation was detected in the euphotic zone at Stas. WM, CD1, HN010, and HN210. As was observed here, McKay et al. (2005) measured a slight increase in luminescence (although not relating an iron-limited situation) at Stas. ON1 and ON2. In that study, strong iron limitation was only observed in the pelagic central Lake Superior.

The presence of natural organic matter (NOM) suppressed the cellular luminescence of the bioreporter and resulted in enhanced iron bioavailability. Siderophore-like compounds were observed in Lake Superior (this study). Because the bioreporter is a cyanobacterium known to produce siderophores under iron-limiting conditions (Wilhelm 1995), the presence of siderophores might promote iron bioavailability. Indeed, the slow kinetics of Fe-EDTA dissociation compared with the specialized assimilation of ferric-siderophore complexes might account for the observed low bioluminescence in natural samples (in the presence of siderophores) relative to Fraquil. Furthermore, several laboratory studies showed that organic ligands such as fulvic and humic acids, as well as polysaccharides, can promote trace metal availability above the level predicted by chemical speciation for freshwater green algae (aluminum, Parent et al. 1996; lead, Lamelas et al. 2005). This effect was recently attributed to the formation of ternary NOM–metal–algal surface complexes, depending on both NOM affinity for the trace metal and the algal surface (lead and cadmium, Lamelas and Slaveykova 2007). Finally, increased iron bioavailability as a result of both enhanced iron solubility and the formation of bioavailable forms (either because of formation of bioavailable chemical complexes or because of favored iron reduction) in the presence of (poly)saccharides was observed for model and natural Antarctic plankton (Hassler and Schoemann in press). The occasionally observed higher bioluminescence in UV-treated natural samples, relative to Fraquil, could be a result of (1) the formation and precipitation of inorganic ferric oxides, (2) the change in nutrient and trace elements (other than iron) chemical speciation and bioavailability, and (3) the formation of reactive oxygen species. Given Fe(III) solubility in freshwater, colloid formation should not be a concern for most samples in this study (except for ON1 and HN010). Iron bioavailability was assessed shortly after irradiation (<12 h), resulting in only inorganic or newly formed colloidal iron, known to be strongly bioavailable to phytoplankton (Yoshida et al. 2006). The potential effect related to the release of high concentrations of nutrients (Hassler et al. 2006) or Cu on UV treatment (which could alter membrane permeability) was rejected. The addition of 10 nmol L^{-1} Cu to Fraquil media, an enrichment shown to be toxic to Lake Superior phytoplankton (Twiss et al. 2004), did not affect the level of cellular bioluminescence for 0.4–45 nmol L^{-1} bioavailable iron. Given that the promoter P*isiAB*, on which the iron bioreporter is based, is also activated under oxidative stress (Li et al. 2004), organic compounds might act to repress luminescence by acting as free radical scavengers in solution.

Despite organic matter–mediated suppression of the bioreporter response observed here, the bioreporter can still be used to indirectly assess iron bioavailability in natural samples. For example, the percentages of bioavailable iron at Stas. WM, ON1, and AP3 in May on the basis of the ^{55}Fe bioaccumulation technique (Eqs. 3, 4) were in accordance with the percentages of bioavailable iron in these samples, evaluated with the use of the bioreporter in the presence of model ligands (Eqs. 1, 2). Although the bioreporter represents a powerful tool, allowing a relevant measurement of the natural complexity of iron bioavail-

ability, an extensive and careful characterization is required before its use as an analytical tool in the field.

We consider the concentration of the partially bioavailable model ligand DTPA, used to titrate iron in natural samples in September, to have been too high and thus to have missed the bioreporter detection window. Nevertheless, this method can still be used in future assessments with either concentrations of DTPA <50 nmol L^{-1} or with the use of model ligands with lower stability constants for Fe(III) than DTPA. The model ligand 8HQS is used to measure fractions of labile iron via competition and retention of the $Fe(8HQS)_2$ complex in a C_{18} resin (Abbasse et al. 2002) and is the only other candidate ligand we are aware for use as a model ligand with the bioreporter. The ideal ligand for use in this technique should have a conditional binding strength in Fraquil (NIST, version 8.0 database, MINEQL+ version 4.5) between that of 8HQS ($K = 10^{20.4}$ L mol^{-1}) and DTPA ($K = 10^{21.1}$ L mol^{-1}; Hassler and Twiss 2006). Additionally, the model ligand should have a greater affinity for Fe(III) than for other trace metals present in natural waters.

Here, the picocyanobacterial bioreporter was used successfully to quantify iron bioavailability across spatial and temporal scales in the western portion of Lake Superior. Several artifacts might exist in the iron bioavailability assessed with bioassays using ^{55}Fe and ligand exchange: (1) iron regeneration and recycling by microbial food web activity, known to contribute to trace metal bioavailability (Twiss and Campbell 1998; Hassler et al. 2008), is omitted because only filtered water is assessed; (2) water temperature effects are neglected because an increase in temperature (from 8°C to 19°C in this study) will likely affect iron speciation and the organic matter present; and (3) experimental solutions are assumed to be at equilibrium. Because iron is a metal slow to react, iron and ^{55}Fe exchanges with organic ligands, either naturally present or added, will be slow. A reasonable applicable equilibration time as performed here (12 and 168 h) might not be sufficient to reach equilibrium (Town and van Leeuwen 2005). Because of their higher water loss rate constant, FeOHL species are the fastest to equilibrate (Hudson and Morel 1990; Morel and Hering 1993). Incomplete equilibration might therefore result in truncated iron bioavailability, and further work addressing this question is needed to ensure proper measurement of iron uptake, bioavailability, or chemical speciation in natural systems.

In natural waters, most of the iron is associated with organic matter (Rue and Bruland 1995). Because organically bound iron can show variable bioavailability to phytoplankton (Hutchins et al. 1999; Hassler and Schoemann in press), the major organic ligand(s) or functional group(s) involved in iron chelation is of importance. To date, few have studied the relationship between iron organic speciation and its bioavailability in freshwaters. The results from this study reveal that the majority of iron in the western portion of Lake Superior was organically complexed and highly bioavailable.

The moderate retention (39–55%) of iron in lake water samples by the C_{18} resin columns suggest that the majority of the samples contained iron complexed to relatively hydrophilic ligands. Low measures of DOC and CDOM, as well as the similarity in retention of iron in the majority of the natural samples with that of iron in the presence of SRFA, suggests that a large fraction of iron was complexed by fulvic acid–like compounds. This is consistent with previous investigations in Lake Superior, wherein mercury complexation was also dominated by fulvic acids (Hurley et al. 2003). Complexation of iron by these compounds maintains iron in the dissolved state (Koenings and Hooper 1976; Sunda 1995) and is the basis for iron buffering in phytoplankton growth media. The high measures of iron bioavailability in samples displaying characteristics consistent with iron complexed by fulvic acids suggests that picocyanobacteria can access this pool of iron. This was particularly evident at Sta. CD1 (5 m), wherein iron bioavailability remained high despite a 2.5-fold decrease in dissolved iron concentrations from May to September. This suggests that the chemical speciation of iron is a better indicator of iron bioavailability than measures of total dissolved iron. Previous characterization of the bioreporter showed that a high concentration of SRFA (15 mg L^{-1}) was required to decrease iron bioavailability from 100 to 1 nmol L^{-1} (Hassler and Twiss 2006), supporting the high bioavailability of iron bound to SRFA to this bioreporter.

In contrast to all other stations, the dissolved iron at Sta. WM in September seemed to be complexed by strong hydrophobic ligands and was not readily bioavailable to picocyanobacteria. The retention of iron by the C_{18} cartridge at Sta. WM in September is consistent with the retention of either inorganic iron or iron complexed to hydrophobic ligands. A 4.8-fold decrease in dissolved iron and a corresponding increase in C_{18} retention from May to September at Sta. WM (5 m) resulted in a 7.6-fold decrease in iron bioavailability. The relatively high bioreporter bioluminescence measured at Sta. WM in September suggests that a large portion of iron at this station was likely complexed to strong hydrophobic ligands, rather than to inorganic ligands (viz. hydroxide).

The presence of strong hydrophobic Fe-binding ligands at Sta. WM, but not at other locations, might reflect a bias in our sampling regimen to nearshore locations. Although Sta. WM is characterized as a pelagic site, owing to its midlake location, two additional stations, EH090 and CD1, could also be representative of pelagic sites despite their location relatively close to shore. Sta. EH090 is isolated to some extent from nearshore influence as a result of bathymetry, the fast-moving Keweenaw Current (sweeps northeast along the Keweenaw Peninsula), and a strong thermal bar located shoreward. However, it is unclear whether spring runoff from land might have preceded formation of the thermal bar that existed during sampling in May (Auer and Gatzke 2004). An argument in favor of nearshore influence comes from sediment trap data, demonstrating that cross-margin transport of particles influences offshore sites of the Keweenaw Peninsula (Urban et al. 2004). Sta. CD1 is located within a deep trough along the north shore of the lake and has a water depth of ~250 m. A generally counter

clockwise gyre in the western arm of Lake Superior tends to bring waters from the open lake along the north shore through this region (Beletsky et al. 1999). It thus has strong open-lake characteristics, in spite of its proximity to the coast and the population and industrial centers at Duluth. However, high iron concentrations were previously reported at this station (McKay et al. 2005).

The majority of dissolved iron at Stas. WM and CD1 was associated with colloids in the 0.02- to 0.45-μm size fraction, as had been previously demonstrated for Sta. WM (McKay et al. 2005). Dissolved iron samples analyzed at these stations (with the exception of Sta. CD1 [5 m]) decreased below detection limit with ultrafiltration (Table 3). Correspondingly, these decreases in dissolved iron on ultrafiltration of Sta. CD1 samples resulted in decreases in iron bioavailability. This suggests that dissolved iron associated with small colloids in the 0.02- to 0.45-μm size fraction represented a bioavailable pool of iron dominated by fulvic acid–like compounds.

Conversely, decreases in dissolved iron concentrations to below detection with ultrafiltration at Sta. WM resulted in increased iron bioavailability, an observation consistent with our prior analysis made with water sampled from Sta. WM (McKay et al. 2005). During that study, a more sensitive high-resolution ICP-MS assay (Field and Sherrell 2003) demonstrated dissolved iron associated with the soluble ($<$0.02 μm) fraction at Sta. WM to be present at 0.34 and 0.83 nmol L^{-1} at 5 and 25 m, respectively, during late summer sampling (McKay et al. 2005). At this station, it appears that iron was bound to siderophore-like compounds that were present in the $<$0.02-μm fraction. Because the bioreporter (derived from *Synechococcus* PCC 7942) is known to produce siderophores (Wilhelm 1995), ultrafiltration might have promoted the iron bioavailability sensed by the bioreporter. Ultrafiltration might induce several artifacts in the colloidal fraction collected (such as contamination and aggregation; Guo and Santschi 2007). Manual syringe ultrafiltration, as done in this study, results in variable pressure and flow rate, which might increase the variability between samples. If iron contamination was causing the increased iron bioavailability observed at Sta. WM, it occurred at a level below our analytical detection limit (0.6 nmol L^{-1}). One other hypothesis that could explain this observation is that ultrafiltration lysed the phytoplankton cells, thereby introducing additional bioavailable iron (Poorvin et al. 2004). In this case, a similar increase of iron bioavailability would have been expected at Sta. CD1, especially given its higher Chl *a* concentration (Table 3). The decrease in iron bioavailability at Sta. CD1 with ultrafiltration signifies that the increase in iron bioavailability at Sta. WM was not likely due to ultrafiltration-induced cell lysis.

The results from this study reveal that the majority of iron in the western portion of Lake Superior was organically complexed and highly bioavailable. The results of this iron bioavailability assessment provide evidence in support of the presence of siderophore-like compounds in ambient Lake Superior waters. These putative bioavailable siderophore compounds were not as prevalent as fulvic acid–like compounds that dominated iron complexation. The model ligand technique revealed measures of iron bioavailability comparable to that in the radiolabeled iron bioaccumulation technique.

An organic matter–mediated suppression of bioluminescence is likely due to either siderophores (likely to be bioavailable to the bioreporter) or to an indirect effect of NOM (such as fulvic acids and saccharides). Future experiments, including siderophore and NOM additions to the calibration curve might help to validate this hypothesis. Despite this organic matter–mediated suppression, the bioreporter can still be used to evaluate iron bioavailability to picocyanobacteria because the presence of siderophores or NOM might similarly affect iron bioavailability to other picocyanobacteria. This measure of bioavailability to picocyanobacteria is relevant because autotrophic picoplankton accounted for the majority of Chl *a* in Lake Superior during this study and is applicable to other oligotrophic systems with similar phytoplankton size-class composition.

Given that the iron uptake strategy can differ between various group of phytoplankton (e.g., cyanobacteria and diatom; Hutchins et al 1999; Volker and Wolf-Gladrow 1999), it is not clear how bioavailability sensed by this cyanobacterial bioreporter will compare with iron limitation sensed by the natural assemblage. McKay et al. (2005) obtained a promising result given that no iron limitation was reported for both the bioreporter and immunological assay on diatoms during vernal holomixis in Lake Superior. However, given the heterogeneity of natural phytoplankton communities in freshwater systems, further comparative studies are required before one can extend the iron limitation sensed by the cyanobacterial bioreporter beyond the endemic picoplankton community.

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References

Abbasse, G., B. Ouddane, and J. C. Fischer. 2002. Determination of total and labile fraction of metals in seawater using solid-phase extraction and inductively coupled plasma atomic emission spectrometry (ICP-AES). J. Anal. At. Spectrom. **17:** 1354–1358.

Andersen, R. A., J. A. Berges, P. J. Harrison, and M. M. Watanabe. 2005. Appendix A—recipes for freshwater and seawater media, p. 429–538. *In* R. A. Andersen [ed.], Algal culturing techniques. Academic.

Auer, M. T., and T. L. Gatzke. 2004. The spring runoff event, thermal bar formation, and cross margin transport in lake Superior. J. Gt. Lakes Res. **30:** 64–81.

Beletsky, D., J. H. Saylor, and D. J. Schwab. 1999. Mean circulation in the Great Lakes. J. Gt. Lakes Res. **25:** 78–93.

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

Fahnenstiel, G. L., and H. J. Carrick. 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.

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

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

Guo, L., and P. H. Santschi. 2007. Ultrafiltration and its application to sampling and characterization of aquatic colloids, p. 159–221. *In* K. J. Wilkinson and J. R. Lead [eds.], Environmental colloids and particles. IUPAC.

Hassler, C. S., and V. Schoemann. 2009. Bioavailability of organically bound iron to model phytoplankton of the Southern Ocean. Biogeosciences Discuss. **6:** 1677–1712.

———, and ———. In press. Discriminating between intra- and extracellular metals using chemical extractions—the case of iron. Limnol. Oceanogr: Meth.

———, and M. R. Twiss. 2006. Bioavailability of iron sensed by a phytoplanktonic Fe-bioreporter. Environ. Sci. Technol. **40:** 2544–2551.

———, ———, R. M. L. McKay, and G. S. Bullerjahn. 2006. Optimization of iron-dependent cyanobacterial (*Synechococcus*, Cyanophyceae) bioreporters to measure iron bioavailability. J. Phycol. **42:** 324–335.

———, ———, D. F. Simon, and K. J. Wilkinson. 2008. Porous underwater chamber (PUC) for in-situ determination of nutrient and pollutant bioavailability to microorganisms. Limnol. Oceanogr. Methods **6:** 277–287.

Havens, S. M. 2007. Iron biogeochemistry in the Laurentian Great Lakes. M.S. thesis. Clarkson Univ.

Hinton, M. J., S. L. Schiff, and M. C. English. 1997. The significance of storms for the concentration and export of dissolved organic carbon from two Precambrian Shield catchments. Biogeochemistry **36:** 67–88.

Hudson, R. J. M., and F. M. M. Morel. 1990. Iron transport in marine phytoplankton: Kinetics of cellular and medium coordination reactions. Limnol. Oceanogr. **35:** 1002–1020.

Hurley, J. P., and others. 2003. Methyl mercury in Lake Superior: Offshore processes and bioaccumulation. J. Phys. IV **107:** 641–644.

Hutchins, D. A. 1995. Iron and the marine phytoplankton community. Prog. Phycol. Res. **11:** 1–49.

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

Ivanikova, N. V., R. M. L. McKay, G. S. Bullerjahn, and R. W. Sterner. 2007*a*. Nitrate utilization in Lake Superior is impaired by low nutrient (P, Fe) availability and seasonal light limitation—a cyanobacterial bioreporter study. J. Phycol. **43:** 475–484.

———, L. C. Popels, R. M. L. McKay, and G. S. Bullerjahn. 2007*b*. Lake Superior supports novel clusters of cyanobacterial picoplankton. Appl. Environ. Microbiol. **73:** 4055–4065.

Koenings, J. P., and F. F. Hooper. 1976. The influence of colloidal organic matter on iron and iron–phosphorus cycling in an acid bog lake. Limnol. Oceanogr. **21:** 684–696.

Lamelas, C., and V. I. Slaveykova. 2007. Comparison of Cd(II), Cu(II) and Pb(II) biouptake by green algae in the presence of humic acid. Environ. Sci. Technol. **41:** 4172–4178.

———, K. J. Wilkinson, and V. I. Slaveykova. 2005. Influence of the composition of natural organic matter on Pb bioavailability to microalgae. Environ. Sci. Technol. **39:** 6109–6116.

Li, H., A. K. Singh, L. M. McIntyre, and L. A. Sherman. 2004. Differential gene expression in response to hydrogen peroxide and the putative PerR regulon of *Synechocystis* sp. strain PCC 6803. J. Bacteriol. **186:** 3331–3345.

McKay, R. M. L., and others. 2005. Bioavailable iron in oligotrophic Lake Superior assessed using biological reporters. J. Plankton Res. **27:** 1033–1044.

Mioni, C. E., S. M. Handy, M. J. Ellwood, M. R. Twiss, R. M. L. McKay, P. W. Boyd, and S. W. Wilhelm. 2005. Tracking changes in bioavailable Fe within high-nitrate low-chlorophyll oceanic waters: A first estimate using a heterotrophic bacterial bioreporter. Glob. Biogeochem. Cycles **19:** GB4S25, doi:10.1029/2005GB002476.

Morel, F. M. M., and J. G. Hering. 1993. Principles and applications of aquatic chemistry. Wiley Interscience.

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

Parent, L., M. R. Twiss, and P. G. C. Campbell. 1996. Influences of natural dissolved organic matter on the interaction of aluminum with the microalga *Chlorella*: A test of the free-ion model of trace metal toxicity. Environ. Sci. Technol. **30:** 1713–1720.

Poorvin, L., J. M. Rinta-Kanto, D. A. Hutchins, and S. W. Wilhelm. 2004. Viral release of Fe and its bioavailaiblity to marine plankton. Limnol. Oceanogr. **49:** 1734–1741.

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

Rue, E. L., and K. W. Bruland. 1995. Complexation of iron(III) by natural organic ligands in the central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. Mar. Chem. **50:** 117–138.

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

Sunda, W. G. 1995. The influence of nonliving organic matter on the availability and cycling of plant nutrients in seawater, p. 191–207. *In* R. G. Zepp and C. H. Sonntag [eds.], Role of non-living organic matter in the earths carbon cycle. Wiley.

Tovar-Sanchez, A., S. A. Sanudo-Wilhelmy, M. Garcia-Vargas, R. S. Weaver, L. C. Popels, and D. A. Hutchins. 2003. A trace metal clean reagent to remove surface-bound iron from marine phytoplankton. Mar. Chem. **82:** 91–99.

Town, R. M., and H. P. van Leeuwen. 2005. Measuring marine iron(III) complexes by CLE-AdSV. Environ. Chem. **2:** 80–84.

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

———, K. J. Rattan, R. M. Sherrell, and R. M. L. McKay. 2004. Sensitivity of phytoplankton to copper in Lake Superior. J. Gt. Lakes Res. **30:** 245–255.

URBAN, N. R., L. XUEFEI, C. YINTAO, AND D. S. APUL. 2004. Sediment trap studies in Lake Superior: Insights into resuspension, cross-margin transport, and carbon cycling. J. Gt. Lakes Res. **30:** 147–161.

VOLKER, C., AND D. A. WOLF-GLADROW. 1999. Physical limits on iron uptake mediated by siderophores or surface reductases. Mar. Chem. **65:** 227–244.

WELSCHMEYER, N. A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol. Oceanogr. **39:** 1985–1992.

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.

YOSHIDA, M., K. KUMA, S. IWADE, Y. ISODA, H. TAKATA, AND M. YAMADA. 2006. Effect of aging on the availability of freshly precipitated ferric hydroxide to coastal marine diatoms. Mar. Biol. **149:** 379–392.

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