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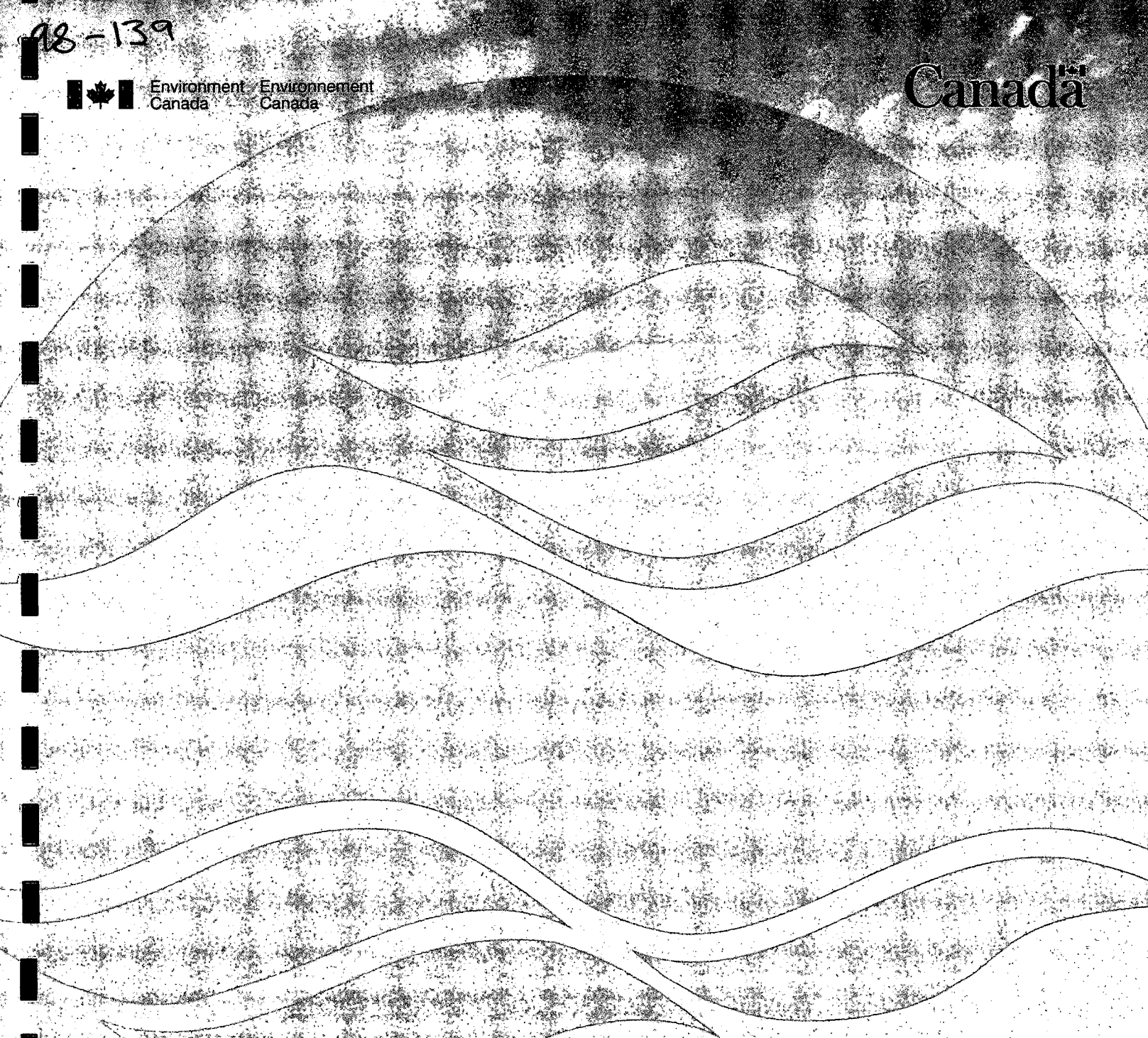

Iron-Stimulated Phytoplankton Productivity in the pelagic surface water of eastern and central Lake Erie during thermal stratification: a field investigation using trace mental clean techniques.

By:

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NATIONAL WATER RESEARCH INSTITUTE

INSTITUT NATIONAL DE RECHERCHE SUR LES EAUX

Management Perspective

Title: Iron-stimulated phytoplankton productivity in the pelagic surface water of eastern and central Lake Erie during thermal stratification: a field investigation using trace metal clean techniques

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Current Status: M.N. Charlton (AERB/NWRI) has encouraged and facilitated research partnerships at Universities to add knowledge about the changing processes in Lake Erie. Experiments with new clean techniques show that, sometimes, low iron limits phytoplankton growth as well as phosphorus. Previously it was thought that phosphorus is the only nutrient limiting phytoplankton and ecosystem productivity..

Next Steps: Continue research to find the importance in terms of extent of the phenomenon. No change in nutrient management needed at this time. Continue to work with University partners on Lake Erie.

Iron-stimulated phytoplankton productivity in the pelagic surface water of eastern and central Lake Erie during thermal stratification: a field investigation using trace metal clean techniques

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Abstract: We tested the hypothesis that phytoplankton productivity in pelagic Lake Erie is limited by low iron bioavailability during the period of thermal stratification. Iron enrichment (20 and 200 nM Fe) of water sampled from the eastern basin surface water (5 m depth) in July 1996 revealed a dramatic 180% and 30% increase in the standing crop of the picoplankton (0.2-2 μm) and nanoplankton (2-20 μm) size-fractions, respectively. Light-saturated rates of photosynthesis for picoplankton were 2.8 times that of controls within the first 24 h and then decreased to control levels within 3 d, while there was little change in the nanoplankton. Simultaneous phosphorus (200 nM phosphate) and iron (20 and 200 nM) enrichment experiments carried out in July 1997 with water samples from three pelagic stations revealed that phosphorus enrichment alone stimulated phytoplankton growth in the nanoplankton and picoplankton. However, phytoplankton yield was greater in combined phosphorus and iron amended experiments relative to phosphorus enriched treatments. Delayed water column stratification and enhanced wind-induced vertical mixing observed in July 1997 may have prevented severe iron limitation in the phytoplankton community. The results from these field experiments, and the heterogeneous distribution of dissolved iron in the pelagic surface waters, suggest that at times both iron and phosphate limit phytoplankton growth in Lake Erie during thermal stratification.

Résumé: Nous avons vérifié l'hypothèse que la production phytoplanctonique est limitée par la faible disponibilité du fer pendant la période de la stratification thermique au lac Érié. Suite à un d'enrichissement (20 and 200 nM Fe) d'échantillons de surface (5 m) provenant du bassin Est effectué en juillet 1996, nous avons observé une augmentation dramatique de 180% et 30% de la biomasse picoplanctonique et nanoplanctonique respectivement. La photosynthèse à intensité lumineuse saturante du picoplancton a augmenté d'un facteur 2.8 par rapport à l'échantillon témoin durant les premières 24 h; sur les trois jours suivant, le taux a diminué jusqu'à la valeur du témoin. Il n'y a eu que peu de changement dans le taux photosynthétique du nanoplancton sur 3 jours. En revanche, des expériences d'enrichissement simultané en phosphore et en fer effectuées en juillet 1997 à trois stations pélagiques ont révélé que seul le phosphore stimulait la croissance. Néanmoins, le rendement en biomasse a toujours été plus élevé dans les traitements combinés fer et phosphore que ceux enrichis en phosphore seulement, ce qui suggère que les deux éléments peuvent parfois limiter la croissance. Un retard dans la stratification thermique et une augmentation du mélange vertical observé en juillet 97 ont possiblement empêché le développement d'une forte carence en fer dans la communauté phytoplanctonique. Les résultats de ces expériences et l'hétérogénéité spatiale du fer dissous dans les eaux pélagiques suggèrent que parfois le fer et le phosphore pourraient limiter la croissance phytoplanctonique dans le lac Érié pendant la stratification estivale.

Introduction

Only recently have trace metal clean techniques been used to reliably and accurately measure the concentration of trace metals in surface waters of the Great Lakes (Coale and Flegal 1989, Nriagu et al. 1993). Whereas some metals reveal distributions suggesting anthropogenic sources, i.e. increasing in concentration from the upper to lower lakes, e.g. Tl (Cheam et al. 1995) and Cr (Beaubien et al. 1994), the dissolved concentrations of some physiologically essential, bioactive, trace metals approach values measured in open oceanic surface waters. For example, zinc concentrations in pelagic Lake Erie surface waters during thermal stratification have been measured in the range of 0.39-0.84 nM (Coale and Flegal 1989); a representative surface water Zn concentration in the North Pacific Ocean is 0.23 nM (Bruland et al. 1994). The concentrations of bioactive trace metals are so low in the surface waters of some regions of the Great Lakes, and in some parts of the oceans, that limitation of phytoplankton growth due to the low bioavailability of trace metals is hypothesized. Such is the case for iron.

The discovery that iron additions enhance phytoplankton growth in the equatorial Pacific Ocean (Martin et al. 1994) and in seawater from subtropical regions of the world's oceans (Martin and Fitzwater 1988) has greatly stimulated oceanographic interest in the bioavailability of this element. Given the low solubility ($K_{sp}=10^{-20}$) of Fe^{3+}, the complex aqueous redox chemistry, and the high degree of organic complexation of iron (99.97% organically bound; Rue and Bruland 1995), there may be kinetic constraints on iron bioavailability that limit phytoplankton growth (Rich and Morel 1990). It is therefore not surprising that iron limitation has been found in pelagic ocean environments where the

concentration of total dissolved Fe (0.08-0.2 nM; Martin and Gordon 1988, Rue and Bruland 1995) is a great deal lower in concentration than in pelagic Lake Erie (2-16 nM; Table 1). But it is the bioavailability of iron to phytoplankton in Lake Erie that is important.

Existing water quality data suggest that phytoplankton biomass is related to iron bioavailability. We combined data sets on dissolved iron concentrations and total chlorophyll *a* for surface waters of Lake Erie during the months of July and August, coinciding with the mid-season period of thermal stratification (Table 1). Low levels of bioavailable iron, Fe^{3+}, are positively correlated with low levels of chlorophyll *a* in the pelagic surface waters of Lake Erie during summer months ($r = 0.67$, $P < 0.01$). In fact, most of the dissolved iron is complexed to dissolved organic matter (>99%; cf. Table 1) and the calculated free-ion concentrations are within the range known to cause iron limitation in cyanobacteria (Wilhelm 1995) and eukaryotic microalgae (Brand 1991). Such a relationship may not be directly and solely due to iron limitation since other factors such as macronutrient (N, P) bioavailability and predation by zooplankton will affect phytoplankton abundance. However, in Lake Erie nitrogen concentrations are in large excess in relation to planktonic requirements and soluble reactive orthophosphate (Table 1) appears to be under-utilized compared to other nutrient-enriched lakes (Levine and Schindler 1980). Such an under-utilization of macronutrients could be due to remineralization rates attributable to grazing activity exceeding the demand for plankton growth, or growth limitation by other essential elements.

We hypothesize that iron bioavailability in Lake Erie is low enough to induce physiological limitation of phytoplankton growth during the period of thermal stratification. To test this hypothesis, trace metal clean protocols were used to conduct iron enrichment experiments designed to assess the response of autotrophic members of the picoplankton (0.4/0.2-2 μm) and nanoplankton (2-20 μm) sampled from the eastern and central basins of Lake Erie.

Materials and methods

Trace metal clean protocols

Precautions were taken to avoid contamination of sampled lake water during sampling and the manipulation steps that followed. The cleaning protocol for polycarbonate and Teflon® plasticware involved: a warm soap wash (Liqui-Nox, 1%), methanol (HPLC grade) soak, HCl (10%) soak; with a 7-fold rinse after each cleaning step using deionized water (17.5 >Mohms·cm^{-1}). A non-metallic water sampling bottle (Go-Flo, model 1080; General Oceanics, Miami, FL) was used to collect water. The Go-Flo bottle was treated similarly except that 1% HCl was used in the acid leaching step. All polycarbonate filters and nylon screens were previously soaked in 10% HCl and exhaustively rinsed with deionized water before use. Water samples collected for trace metal analysis were stored in Teflon® bottles.

Enrichment experiments

Lake water was collected from multiple 8-L Go-Flo bottle casts to a depth of 5 m at Station 23 (1996-97) and Stations 935 and 953 (1997) (Figure 1). All further manipulations were conducted in a portable clean room onboard the research vessel. Lake water from three casts was screened through a 20-μm screen-type filter, pooled in a polycarbonate carboy, and dispensed under pressure from pre-filtered (<0.2 μm), pre-purified N_2 into 2-L polycarbonate bottles. Iron, from freshly prepared stock solutions (0.72 mM $FeCl_2$ in deionized water), was added to bottles to give duplicate low (20 nM) and high (200 nM) treatments. In 1997, additional experimental treatments were conducted: a phosphate (200 nM) enriched treatment, phosphate-enriched low (200 nM P + 20 nM Fe) and high (200 nM P + 200 nM Fe) iron treatments. Phosphate (K_2HPO_4) was added from a stock solution that was previously passed through an ion-exchange column (Chelex-100, Na-form; Biorad) and irradiated by ultraviolet light (12 h, 1000 W) to eliminate trace metal and organic contaminants, respectively. All treatments were conducted in duplicate and two bottles received no Fe or P additions and thus served as controls. Bottles were incubated in an environmental chamber under simulated *in situ* conditions: 20°C, light was provided by fluorescent tubes (190 μmol photons·m^{-2}·sec^{-1} on a 12h:12h light:dark cycle). At approximately 24 h intervals, sub-samples were removed from the bottles for measurements of size-fractionated chlorophyll *a* (chl *a*) concentrations and light-saturated rates of photosynthesis.

Assessing treatment response

Chlorophyll a content

Changes in chl *a* concentration were chosen as surrogates for changes in phytoplankton biomass and used to monitor growth in the experimental bottles over time. For chlorophyll *a* determinations, one hundred mL was removed from each bottle and serially filtered onto a 2 μm-filter followed by a 0.2 μm-filter (filters were 47 mm dia. polycarbonate membrane filters; Nuclepore). Filters were immediately plunged into cold 90% acetone and allowed to extract overnight at 4°C. After centrifugation, pigment concentration was determined by fluorometric analysis (Welschmeyer 1994) aboard the research vessel.

Photosynthesis

Light-saturated rates of photosynthesis were measured to examine changes in physiological status in the phytoplankton community. If phytoplankton growth is limited by the availability of Fe then photosynthesis will also be limited by reason of reduced chl *a* production and a reduction in the levels of cytochromes and complementary proteins such as ferredoxin that are intimately involved with photosynthesis (Raven 1988).

To determine light-saturated photosynthetic rates, 125 mL sub-samples from each bottle were placed into acid-cleaned 125-mL borosilicate glass-stoppered bottles. $NaH^{14}CO_3$ was added into each bottle to give a total ^{14}C radioactivity of 141 kBq. These bottles were incubated for 2 h, adjacent to the enriched bottles, where they experienced the same irradiance field. After the incubation period the entire bottle contents were

serially filtered onto a 2 μm-filter and then a 0.2 μm-filter. Filters were rinsed with filter-sterilized lake water (<0.2 μm). Time-zero filter controls revealed that there was negligible non-photosynthetic sorption of ^{14}C by particles. All filter samples containing ^{14}C were immediately frozen for transport to the laboratory. Filters were counted by liquid scintillation spectrophotometry (Wallac Winspectral, model 1414) and quench corrected using the internal quench library.

Statistical analyses

One-way ANOVA and an a posteriori comparison of mean biomass content in treatments was conducted with the Student-Neumann-Keuls test (SigmaStat, SPSS Corp.) after verifying the normality and heteroscedasticity of the data. To stabilize the variance and increase the power of the test, the last two time points of the enrichment assays were combined at stations 935 and 23 (n=4 per treatment), while the single endpoint was used for station 953 (n=2 per treatment).

Iron accumulation by plankton

Radioactive iron was used to trace the uptake of this element from the aqueous to particulate phase in the sampled lake water. The accumulation of iron by plankton was conducted on water sampled from Stations 935, 23, and 953 in 1997. Details of this protocol are provided elsewhere (Twiss and Campbell 1998). Briefly, radiolabelled iron (12 μL of $^{59}FeCl_2$ in 0.1 M HCl) was added to triplicate bottles each containing 2 L of sampled lake water (filtered <20 μm, *see above*) to give a total radioactivity of 222 kBq

and a nominal added iron concentration of 2.2 nM. Bottles were incubated under simulated *in situ* conditions (*see above*) and at timed intervals over a period of 24-60 h, the partitioning of ^{59}Fe into the picoplankton (0.2-2 μm) and nanoplankton (2-20 μm) was determined by filtration. Two-50 mL aliquots from each bottle were placed in a polyethylene tube to which disodium ethylenediaminetetraacetic acid (Na_2EDTA; pH 8) was added to give a concentration of 10 μM EDTA. After a period of 20 minutes, the contents of each tube were filtered onto a 0.2-μm or 2-μm pore size filter (47 mm; Nuclepore). Radioactivity was measured using liquid scintillation spectrophotometry (Wallac Winspectral, model 1414). Accumulated ^{59}Fe in the picoplankton-sized fraction was determined by difference, i.e. $[^{59}Fe_{0.2\text{-}2\ \mu m}] = [^{59}Fe_{>0.2\ \mu m}] - [^{59}Fe_{>2\ \mu m}]$, and accumulation in each size fraction was expressed as the percentage of the total aqueous ^{59}Fe measured in duplicate 1 mL aliquots removed from each bottle at each sampling period.

Trace metal analyses

Total dissolved iron analysis

In 1996, lake water was filtered (<0.2 μm), and acidified to pH <2 using HCl (Merck Supra-Pur; final concentration 0.04 M). Iron was analyzed by graphite furnace atomic absorption spectrophotometry (Varian SpectraAA Model 300) using an $MgNO_3$ matrix modifier and standard additions methodologies. In 1997, lake water was filtered (<0.2 μm), and stored frozen; prior to analysis, water was acidified to pH <2 using HNO_3 (Ultrex II, J.T. Baker; final concentration 0.05 M). Analysis of Fe content was conducted

by inductively coupled plasma mass spectrometry (Finnegan Matt Element) using a standard curve and indium as an internal standard.

Iron speciation

Aqueous Fe speciation was determined using a computerized chemical speciation model (Windermere Humic Aqueous Model–Waters, Version 1.0; Tipping 1994). Calculations were based on: reported major ion concentrations in the epilimnion of Lake Erie during summer (Rockwell et al. 1989), dissolved concentrations of Fe determined in this study and those reported in the literature (*see* Table 1), a dissolved humic substance concentration of 1.6 $mg \cdot L^{-1}$ (9:1 ratio of fulvic acid to humic acid), pH 8, and 20°C. The concentration of dissolved organic carbon (DOC) is relatively constant throughout Lake Erie surface waters (3.2 ± 0.5 $mg \cdot L^{-1}$; mean ± s.d.) during summer months (July 1994 and August 1995: S. L'Italien, pers. comm., NWRI, Burlington, ON). We have assumed that humic and fulvic acids comprise 50% of the DOC (Buffle 1988) present in the surface waters of the lake.

Results

Total dissolved iron analyses

Total dissolved iron values are highly variable and show no intra-basin consistency. The range in iron concentrations measured during in the surface waters of the eastern and central basins of Lake Erie during the 1996 and 1997 survey (3-34 nM; Table 1) are close

to the range of measurements reported for these basins by other researchers using trace metal clean sampling protocols (2-17 nM; Table 1).

Dissolved iron repesented a small fraction of total aqueous iron. In 1996, total iron was determined in samples by acidifying unfiltered lake water at two stations. Total Fe was 30.6 nM (79% particulate Fe) at Station 84 (central basin) and 485 nM (96% particulate Fe) at Station 357 (western basin). A temperature/depth profile at Station 357 revealed that the surface water of the western basin was in contact with the sediment, hence the elevated iron concentration is probably due to suspended sediments. All other stations analyzed in the current study were from epilimnetic waters.

Iron enrichment assays

1996 results

In 1996, there was a profound stimulation of phytoplankton growth following the experimental addition of inorganic iron to sampled surface water. Phytoplankton biomass in the picoplankton (0.4-2 μm) and nanoplankton (2-20 μm) size fractions increased following the addition of the iron, relative to the control treatment (Figure 2A-B). In the 20 nM Fe treatment, biomass increased by 182% and 30% in the picoplankton and nanoplankton size-fractions respectively.

The photosynthetic efficiency of the picoplankton in the iron treatments increased dramatically within the first 24 h and returned to control levels within 3 d (Figure 2C), whereas there was little change in the photosynthetic efficiency of the nanoplankton in the same treatments (Figure 2D) over the 3 d incubation. The results suggest that the

increased photosynthetic capacity of the picoplankton caused by the addition of iron was rapidly translated into picoplankton biomass.

1997 results

The addition of iron alone in 1997 did not result in any significant growth of nanoplankton or picoplankton at any of the off-shore stations (Table 2). Increases of biomass in enrichment treatments were linked to phosphate additions. At Station 935, a 200 nM phosphate enrichment resulted in a significant stimulation of growth in both the nanoplankton and picoplankton size fractions; addition of phosphate and 200 nM Fe resulted in significant increased growth in the picoplankton size-fraction relative to the phosphate-enriched treatments (Table 3). In contrast, at Station 23 phosphate addition alone did not result in increased nanoplankton or picoplankton biomass; only in the phosphate-enriched low and high Fe treatments were the nanoplankton and picoplankton yields greater relative to than all other sample treatments (Table 3). Finally at Station 953, increased nanoplankton growth was observed in both phosphate-enriched and the combined phosphate and iron enrichments relative to control and phosphate treatments, respectively (Table 3). Unfortunately, at Station 953 an unusually high experimental variance among replicate samples in the picoplankton size-fraction masked the significance tests and precluded reliable statistical interpretation, although from the treatment means there appears to have been some stimulation of growth in the phosphate and the phosphate and iron-enriched treatments (Table 2).

In 1997, a marked increase in photosynthetic efficiency in the picoplankton in the iron enriched treatments was not observed, as in 1996. Nevertheless, by the 48 hour time point, the assimilation ratios of both picoplankton and nanoplankton in the phosphate and the phosphate and iron enriched treatments had doubled relative to the control and iron enriched values at stations 23 and 953 (Table 4; measurements were not conducted at Station 935). Therefore, phosphorus addition alone appeared to stimulate light-saturated rates of photosynthesis in the 1997 assays.

Iron accumulation

Despite the lack of growth stimulation by the addition of only iron in the 1997 experimental treatments, iron was in biological demand in the picoplankton and nanoplankton size fractions. Patterns of iron accumulation into the picoplankton and nanoplankton were consistent among all 3 stations assayed. Accumulation into the picoplankton was essentially complete at 15-20% of total radioactivity within 20 h, whereas accumulation into the nanoplankton increased slowly (0.44 $\%\cdot h^{-1}$) after 20 h (Figure 3). The observed pattern of non-EDTA exchangeable ^{59}Fe accumulation is consistent with biological uptake: accumulation into the picoplankton is solely by transport of dissolved iron species across the cell membrane, whereas accumulation into the nanoplankton is assumed to result from both the internalization of dissolved species and the uptake of ^{59}Fe-containing particles by the phagocytotic activity of nanoplanktonic protozoa present in the lake water (*see* Twiss et al. 1996).

Discussion

Planktonic iron demand in pelagic surface waters of Lake Erie

The results obtained from 1996 and 1997 have revealed both a profound stimulation of phytoplankton productivity following Fe enrichment and inter-annual variability. Testing for iron limitation in the Laurentian Great Lakes is not novel. The addition of chelated iron (FeEDTA) to surface water collected from southern Lake Huron during summer months caused a significant increase in phytoplankton (Lin and Schelske 1981), and the addition of unchelated iron was found to occasionally enhance photosynthesis in plankton collected from the eastern basin of Lake Erie (Storch and Dunham 1986). However, these earlier studies are compromised by the lack of a trace metal clean protocol for collecting and manipulating water samples. It is now widely accepted that rigorous attention to trace metal hygiene is a critical component of protocols designed to manipulate natural water samples for studying phytoplankton interactions with trace metals (Fitzwater et al. 1982). Moreover, the use of iron chelated by a synthetic organic ligand (e.g. EDTA; Lin and Schelske 1981) in natural waters might result, through displacement, in the complexation of toxic trace metals rendering interpretation of these experiments difficult. Recent work on iron chemistry in marine waters has shown that iron oxides are maintained in an amorphous and bioavailable state by a rapid photoreduction/oxidation cycle (Wells et al. 1991a, 1991b). Given the similar elevated pH (pH $\geq$8) and low photosynthetically active radiation extinction coefficients (ε = -0.19 m^{-1}) in Lake Erie and mid-oceanic areas, we enriched our samples with reduced iron, anticipating that within minutes it would be oxidized to amorphous iron oxide and be

bioavailable to the phytoplankton. Accordingly, our investigation utilized trace metal clean protocols and unchelated iron additions in order to test the response of the ambient phytoplankton population with minimal disturbances.

Plankton size fractions responded differently to iron additions. In 1996, the nanoplankton appeared to require much less iron than the picoplankton. Since the specific growth rates (mean ± SD; d^{-1}) of the picoplankton (0.61 ± 0.42) and nanoplankton (0.43 ± 0.26) are similar under these conditions (Twiss and Campbell 1998), it is possible that the nanoplankton required less iron than the picoplankton. Lower iron requirements may be due to a lower physiological requirement by eukaryotic phytoplankton (Brand 1991). Alternatively, nanoplanktonic mixotrophic organisms can accumulate iron through grazing of picoplankton. Some heterotrophic marine protozoa have been shown to satisfy their iron requirements through phagocytosis (Chase and Price 1997). The trophic transfer of bioactive trace metals through the microbial food web (picoplankton → nanoplankton) has been demonstrated for ^{65}Zn and ^{109}Cd in Lake Erie (Twiss et al. 1996), and for ^{59}Fe in the Equatorial Pacific (Hutchins et al. 1993). Moreover, the observed pattern of ^{59}Fe accumulation in the picoplankton and nanoplankton observed in the present study (Figure 3) suggests that organisms in the nanoplankton size class were accumulating Fe by grazing on picoplankton.

The concentration of bioavailable iron may have influenced the magnitude of the size-dependent response of the phytoplankton to iron additions. The rapid response of the picoplankton in 1996, of which cyanobacteria are a significant component, to the iron addition is consistent with the enhancement of cellular uptake mechanisms in

cyanobacteria due to iron limitation. The ambient chemical conditions in pelagic Lake Erie surface waters during thermal stratification suggests that much of the dissolved iron is not biologically available, primarily due to complexation by organic ligands (Table 1). Indeed, the values for Fe^{3+} availability (pFe 20.5 to 21.9) estimated by us for these surface waters indicates a status of iron-limitation in cyanobacteria which corresponds to the induction of high-affinity Fe transport systems (Wilhelm 1995). Our calculations of pFe omit possible enhancements due to *in situ* photoreduction or remineralization of colloidal iron oxyhydroxides by protozoan grazing activity (Barbeau et al. 1996); however, the effect of these mechanisms on the concentration of dissolved inorganic iron in the natural environment is not yet assessable.

Siderophore production is proposed as an ecological strategy wherein cyanobacteria can suppress the growth of other phytoplankton (Murphy et al. 1976). Although some eukaryotic marine phytoplankton can produce siderophores (Trick et al. 1983), the data from our experiment are consistent with siderophore production and utilization by cyanobacteria in that iron accumulation and utilization by the nanoplankton in the 1996 experiment may have been prevented by the formation of ferrisiderophores complexes available for uptake only by the cyanobacteria. If this was the case, then the ambient level of siderophores in the sampled Lake Erie surface water was able to effectively complex Fe^{3+} following the addition of iron in these treatments, thus allowing the marked short-term response of the picoplankton to the iron addition (*cf.* Figure 2A,C). A similar response by picoplankton was observed following an *in situ* iron enrichment in the equatorial Pacific ocean (Behrenfeld et al. 1996; Kolber et al. 1994).

Possible hydrographic/hydrodynamic influences on iron bioavailability in surface waters

An examination of archived hydrographic information in Lake Erie provides insight into the inter-annual differences in responses to iron enrichment at Station 23. Hydrological conditions in Lake Erie in 1997 were not comparable to those observed in 1996 and may have alleviated severe iron limitation. As a result of increased spring runoff, the lake level (1.45 m) was near its extreme historical maximum (1.5 m above chart datum; Canadian Hydrographic Service) in July 1997, while the level was considerably lower in 1996 (1.1 m). Thus, increased lake volume and colder spring temperatures in 1997 retarded the establishment of the deep seasonal thermocline, relative to 1996 (Figure 4).

The reduced response to iron enrichment in 1997 could be due to higher epilimnetic iron concentrations or reduced biological Fe demand. At Station 23, the concentration of total dissolved iron in 1997 (11.8 nM) was slightly lower than that measured in 1996 (16.2 nM). However, we do not have an analytical determination of labile Fe concentration, so it is possible that iron bioavailability was greater in 1997, despite the lower total dissolved [Fe] observed at this station in 1996. Instead, a reduced biological iron demand in 1997 may be related to the lower temperatures and weaker stratification observed at Station 23, a few weeks prior to our experiments (Figure 4). In addition, archived meteorological data from weather buoy 45142 (Lat. 42.7° N, Long. 79.3° W) near Station 23 revealed a large sub-surface entrainment event in early July 1997, as a

result of sustained westerly winds and a weakly stratified epilimnion (Figure 5A)– given the stronger stratification and thicker epilimnion (Figure 4B), this event was not observed in July 1996 (Figure 5B). Therefore, the combined influence of late stratification and increased vertical mixing would have resulted in a "younger" epilimnetic water mass at the time of our experiments in 1997 relative to the more stratified water column observed in 1996.

We can only speculate that iron speciation in 1997 may have been markedly different from that existing at this station in 1996 at the same time of year, and that iron bioavailability was greater in 1997 leading to a reduced iron demand by the phytoplankton in 1997. The fact that the plankton were actively accumulating iron (Figure 3), illustrates the physiological adaptations of the plankton to assimilate as much of this element as possible when it is available. In support of this proposed adaptative response mechanism of pelagic Lake Erie phytoplankton, phytoplankton in high nutrient/low chlorophyll regions of the Pacific have been shown to produce Fe-binding ligands for facilitating iron uptake even when iron availability was artificially enhanced by a large-scale *in situ* enrichment (Rue and Bruland 1997). It is possible that phytoplankton in a chemical environment like the thermally stratified epilimnion of the pelagic Great Lakes (pH ≥8, 2-4 mg DOC·L^{-1}) have similar physiological adaptations that enable them to accumulate this essential element whenever possible. It remains to be seen however, how often and widespread is the occurrence of plankton growth outstripping the bioavailability of iron in the surface waters of this and other Great Lakes.

Conclusions

This is the first direct demonstration of trace metal limitation of phytoplankton in the Laurentian Great Lakes using trace metal clean protocols. While iron alone greatly stimulated phytoplankton growth in 1996, phosphorus addition was required to stimulate growth in 1997. Phosphorus availability is commonly considered the single limiting nutrient for phytoplankton productivity in Lake Erie (Makarewicz and Bertram 1993), yet iron bioavailability may be a significant co-limiting nutrient during certain periods. The implications for fisheries management and water quality criteria are important – we must acknowledge that a shift from P-limitation to Fe-limitation in Lake Erie might be related to time scales of hydrodynamic forcing and investigate this effect on primary production, shifts in phytoplankton species composition, and heterotrophic plankton productivity. Similar limitations of phytoplankton may exist for other trace elements, or ratios thereof, in this and other Great Lakes.

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Table 1. Chlorophyll *a*[a], macronutrient[b], total dissolved and calculated free ion concentrations[c] of Fe^{3+} in Lake Erie surface waters.

Date	Station	Latitude	Longitude	Chl *a*[a] ($\mu g \cdot L^{-1}$)	Soluble Reactive Phosphorus ($\mu g \cdot L^{-1}$)	NO_3 ($\mu g \cdot L^{-1}$)	NH_3 ($\mu g \cdot L^{-1}$)	Fe (nM)	pFe (-log [Fe^{3+}])
8/1993	23	42°30'26"	79°53'54"	2.0	0.5	219	6	9.3*	21.26
8/1993	43	42°34'28"	80°44'01"	1.2	0.6	204	3	8.5*	21.30
8/1993	40	42°21'44"	81°26'22"	1.9	0.4	211	7	12.7*	21.12
8/1993	84	41°55'57"	81°39'35"	0.9	0.5	213	4	2.1*	21.92
8/1993	22	41°42'51"	82°10'13"	11.4	0.8	122	11	14.9*	21.05
8/1993	255	42°08'32"	80°59'19"	1.7	0.6	195	8	7.3*	21.4
8/1993	47	42°17'36"	80°18'13"	1.1	0.5	204	8	5.2*	21.5

Table 1

(cont.)

8/1993	54	42°39'11"	79°07'54"	2.8	0.9	255	7	16.8*	21.0
8/1993	357	41°49'32"	80°58'26"	4.5	0.9	254	9	47.8*	20.5
8/1993	30	41°34'00"	82°37'59"	6.5	0.6	143	9	18.3*	21.0
8/1993	18	41°31'49"	81°42'31"	4.8	0.8	263	14	49.3*	20.5
7/1994	84	41°55'57"	81°39'35"	1.65	—	—	14	21.0	20.9
7/1996	935	42°35'33"	79°27'58"	0.90	—	—	—	19.9	20.9
7/1996	23	42°30'26"	79°53'54"	0.97	0.2-1.5	—	—	16.2	21.0

Table 1

(cont.)

7/1996	946	42°09'55"	80° 38'30"	1.14	—	—	—	7.3	21.4
7/1996	953	42°11'09"	81°26'26"	—	—	—	—	21.1	20.9
7/1996	84	41°55'57"	81°39'35"	0.67	0.9-1.9	—	—	8.1	21.3
7/1996	341	41°47'11"	82°17'10"	0.61	—	—	—	3.0	21.8
7/1996	357	41°49'32"	80°58'26"	2.23	1.0	—	—	17.7	21.0
7/1997	935	42°35'33"	79°27'58"	1.47	—	—	—	33.8	
7/1997	23	42°30'26"	79°53'54"	2.76	—	—	—	11.8	
7/1997	953	42°11'09"	81°26'26"	1.41	—	—	—	5.1	

Footnotes to Table 1.

a From 1993 to 1995, Chl *a* was determined spectrophotometrically (Parsons et al. 1984) and fluorometrically (Welschemeyer 1994) in 1996 and 1997. Chl *a* measured at stations in 1996 and 1997 is ≤ 20 μm.

b Soluble reactive phosphorus, ammonium and nitrate concentrations were determined by the automated molybdenum blue, phenate and cadmium reduction methods respectively (Manual of Analytical Methods, Major ions and nutrients, Vol. 1, 1994, National Laboratory for Environmental Testing, Environment Canada, CCIW, Burlington, ON).

c Total dissolved iron data at stations marked with an asterisk (*) are from Nriagu et al. (1996).

Table 2. Final phytoplankton biomass in iron and phosphorus enrichment experiments on surface water sampled from pelagic eastern (Stations 935 and 23) and central (Station 953) basins of Lake Erie during July 29-August 1, 1997. Values are treatment means (± SD, $n = 2$).

Treatments; Station 935, 56.5 h incubation	**Picoplankton Biomass µg chl *a* $\cdot L^{-1}$ (0.2-2 µm)**	**Nanoplankton Biomass µg chl *a* $\cdot L^{-1}$ (2-20 µm)**
200 nM PO_4-P + 200 nM Fe	1.15 ± 0.07	1.53 ± 0.18
200 nM PO_4-P + 20 nM Fe	0.98 ± 0.10	1.38 ± 0.25
200 nM PO_4-P	0.83 ± 0.01	1.30 ± 0.14
200 nM Fe	0.43 ± 0.00	0.09 ± 0.21
20 nM Fe	0.49 ± 0.08	0.89 ± 0.05
Control	0.45 ± 0.07	0.84 ± 0.01
Treatments; Station 23, 48 h incubation	**Picoplankton Biomass µg chl *a* $\cdot L^{-1}$ (0.2-2 µm)**	**Nanoplankton Biomass µg chl *a* $\cdot L^{-1}$ (2-20 µm)**
200 nM PO_4-P + 200 nM Fe	0.73 ± 0.12	2.55 ± 0.35
200 nM PO_4-P + 20 nM Fe	0.77 ± 0.02	2.45 ± 0.21
200 nM PO_4-P	0.66 ± 0.08	2.03 ± 0.04
200 nM Fe	0.37 ± 0.01	1.52 ± 0.09
20 nM Fe	0.44 ± 0.01	1.65 ± 0.07
Control	0.37 ± 0.01	1.53 ± 0.04
Treatments; Station 953 45 h incubation	**Picoplankton Biomass µg chl *a* $\cdot L^{-1}$ (0.2-2 µm)**	**Nanoplankton Biomass µg chl *a* $\cdot L^{-1}$ (2-20 µm)**
200 nM PO_4-P + 200 nM Fe	0.68 ± 0.00	2.13 ± 0.04
200 nM PO_4-P + 20 nM Fe	0.76 ± 0.05	2.15 ± 0.07
200 nM PO_4-P	0.74 ± 0.19	1.90 ± 0.06
200 nM Fe	0.58 ± 0.03	1.45 ± 0.04
20 nM Fe	0.67 ± 0.05	1.50 ± 0.11
Control	0.59 ± 0.01	1.49 ± 0.01

Table 3. Treatment means (μg chl *a* $\cdot L^{-1}$) and *a posteriori* significance comparisons (SNK test) among enriched treatments at Lake Erie Station 23 in July 1996 and Stations 935, 23 and 953 in July 1997. P = PO_4-P; Nano = nanoplankton, 2-20 μm; Pico = picoplankton, 0.2-2 μm. Values in bold face show significant ($P = 0.05$) difference between the treatments compared.

Treatment:	Controls		+20 nM Fe		+200 nM Fe		+200 nM P		P+20 nM Fe		P+200 nM Fe	
versus:			Controls		Controls		Controls		+200 nM P		+200 nM P	
Size fraction	**Nano**	**Pico**	**Nano**	**Pico**	**Nano**	**Pico**	**Nano**	**Pico**	**Nano**	**Pico**	**Nano**	**Pico**
Station 23 (1996)	0.35	0.18	**0.40***	**0.49***	**0.40***	**0.52***	-	-	-	-	-	-
Station 935	0.76	0.52	0.85	0.48	0.85	0.49	**1.18***	**0.92***	1.18	0.93	1.29	**1.12***
Station 23	1.56	0.46	1.71	0.57	1.56	0.45	1.96	0.70	**2.19***	**0.87***	**2.12***	**0.82***
Station 953	1.49	0.58	1.50	0.66	1.45	0.58	**1.90***	0.74	**2.15***	0.76	**2.13***	0.68

Table 4. Photosynthetic assimilation ratios in phytoplankton biomass in iron and phosphorus enrichment experiments on surface water sampled from pelagic eastern (Station 23) and central (Station 953) basins of Lake Erie during July 29-August 1, 1997. Values are treatment means (± SD, $n = 2$) at the end of the stated incubation period.

Treatments; Station 23, 48 h incubation	P_M^B Picoplankton (0.2-2 µm) µg C· µg chl a^{-1} ·h^{-1}	P_M^B Nanoplankton (2-20 µm) µg C· µg chl a^{-1} ·h^{-1}
200 nM PO_4-P + 200 nM Fe	3.77 ± 0.62	4.86 ± 0.47
200 nM PO_4-P + 20 nM Fe	4.13 ± 0.51	5.73 ± 0.35
200 nM PO_4-P	4.02 ± 0.29	5.52 ± 0.63
200 nM Fe	1.81 ± 0.31	2.85 ± 0.56
20 nM Fe	1.96 ± 0.34	2.72 ± 0.13
Control	1.78 ± 0.015	2.52 ± 0.17
Treatments; Station 953 45 h incubation	**P_M^B Picoplankton (0.2-2 µm) µg C· µg chl a^{-1} ·h^{-1}**	**P_M^B Nanoplankton (2-20 µm) µg C· µg chl a^{-1} ·h^{-1}**
200 nM PO_4-P + 200 nM Fe	3.09 ± 0.06	2.19 ± 0.28
200 nM PO_4-P + 20 nM Fe	3.07 ± 0.53	2.76 ± 0.26
200 nM PO_4-P	2.77 ± 0.70	2.72 ± 0.67
200 nM Fe	2.16 ± 0.39	2.22 ± 0.10
20 nM Fe	2.01 ± 0.10	2.03 ± 0.03
Control	1.96 ± 0.19	2.09 ± 0.03

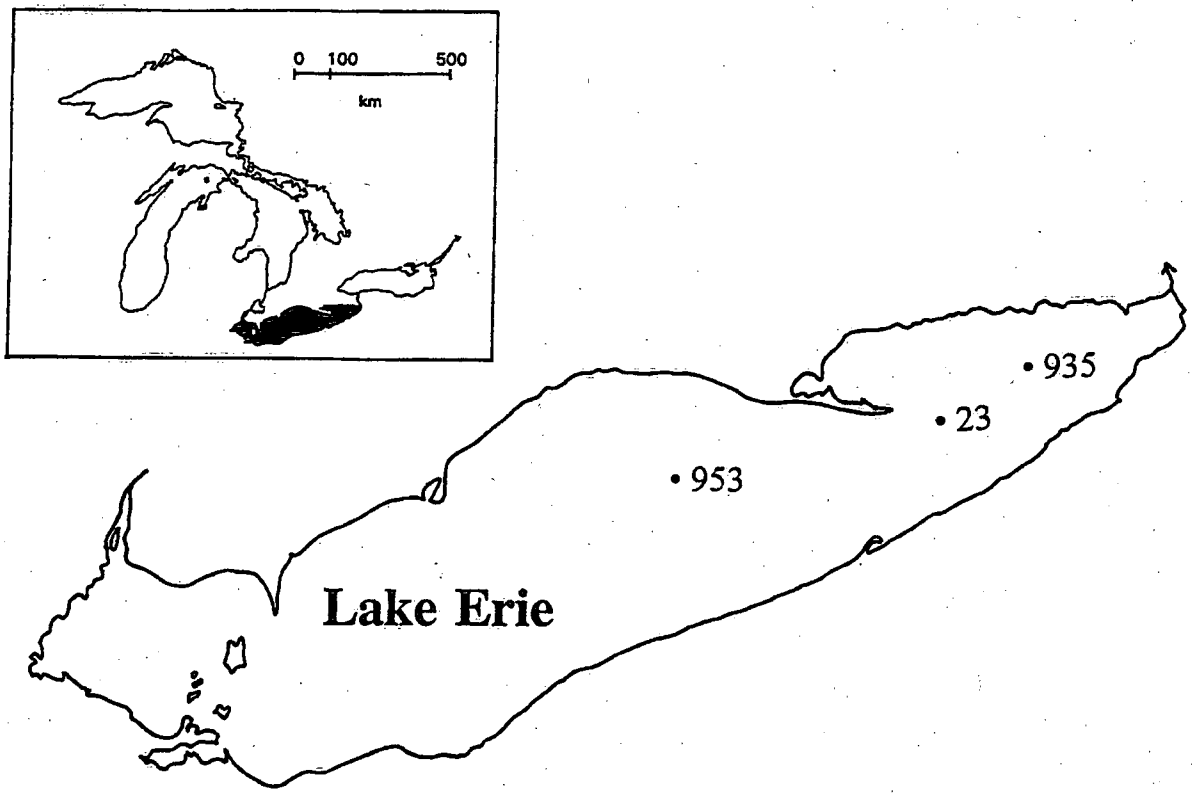
Figure 1. Pelagic study sites in the eastern and central basins of Lake Erie.

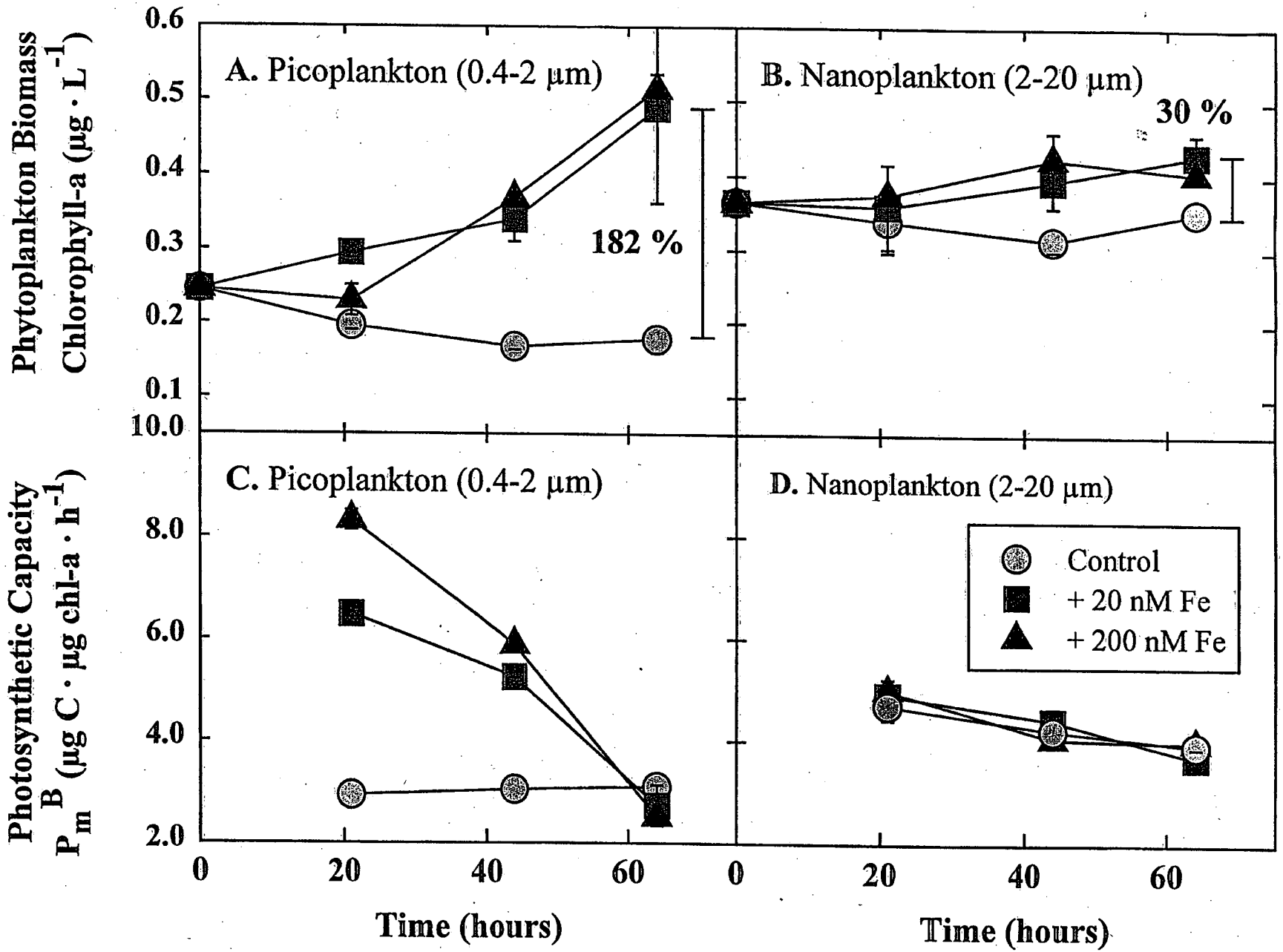
Figure 2. Changes in biomass (A, B) and photosynthetic efficiency (C, D) of pelagic nanoplankton and picoplankton sampled at Station 23 from the eastern basin of Lake Erie following the addition of iron in July 1996. Values are mean ± SD, $n = 2$.

Figure 3. Accumulation of iron into the nanoplankton and picoplankton sized particle fractions sampled from the eastern basin of Lake Erie in July, 1997. Particles were rinsed with 10 μM EDTA (pH 8) to remove extractable iron from particle surfaces. Accumulation of ^{59}Fe into nanoplankton was 31.5 ± 6.0 %, and 20.7 ± 4.8 % in the picoplankton after 25 h at Station 935. Values are mean ± SD, $n = 3$.

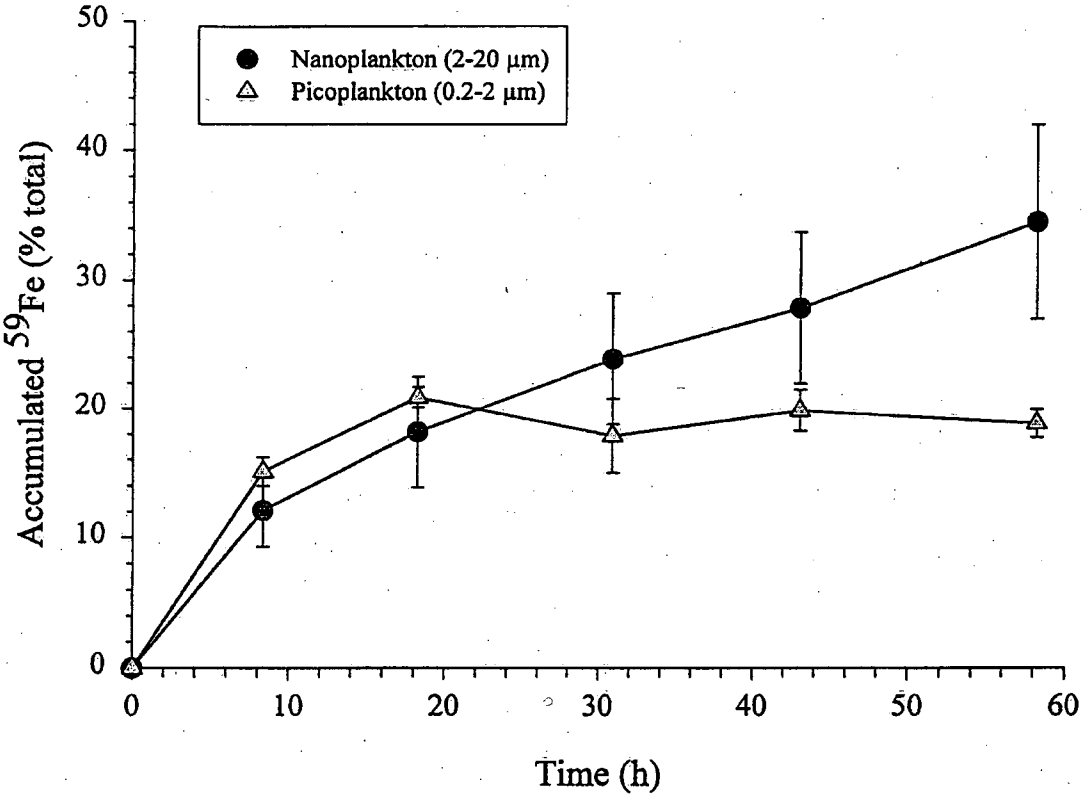
Figure 4. Evolution of thermal profiles at Lake Erie Station 23 during June and July 1996 (A) and 1997 (B).

Figure 5. Sub-surface (0.5 m) temperature variations and wind speeds measured at weather buoy 45142 (42.7° N, 79.3° W), near Station 23, (eastern basin of Lake Erie), in June and July 1996 (A) and 1997 (B).

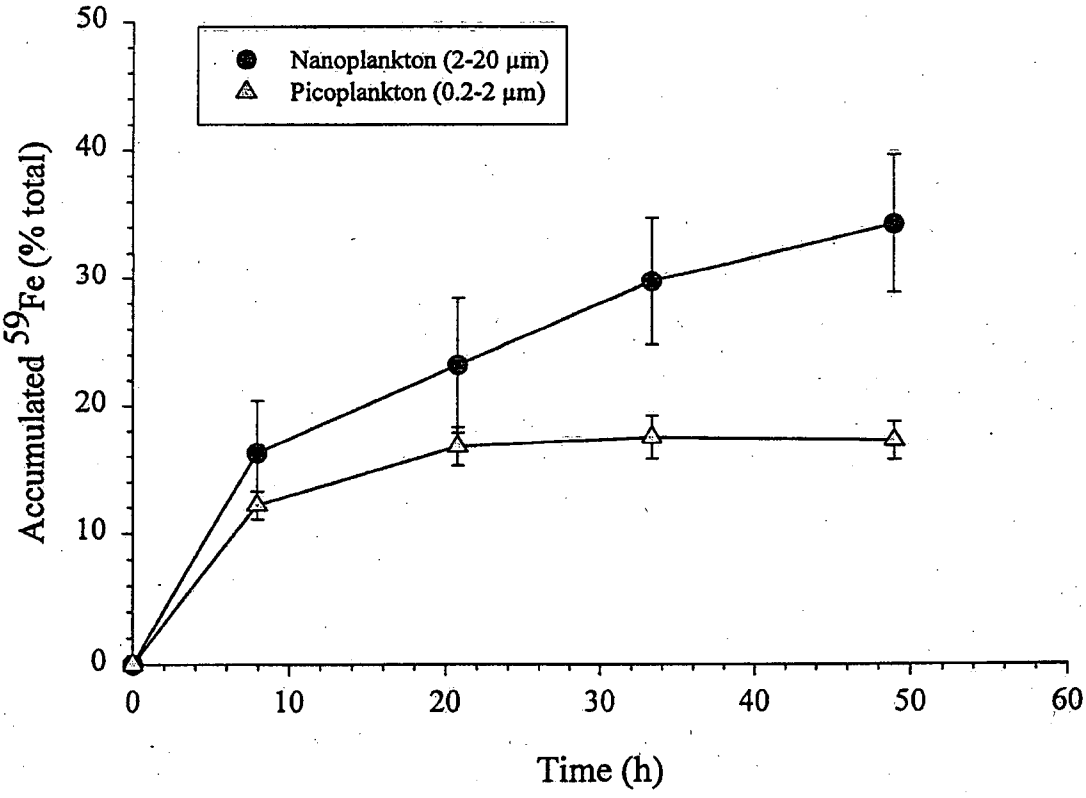

0 100 500
km
935
23
953
Lake Erie

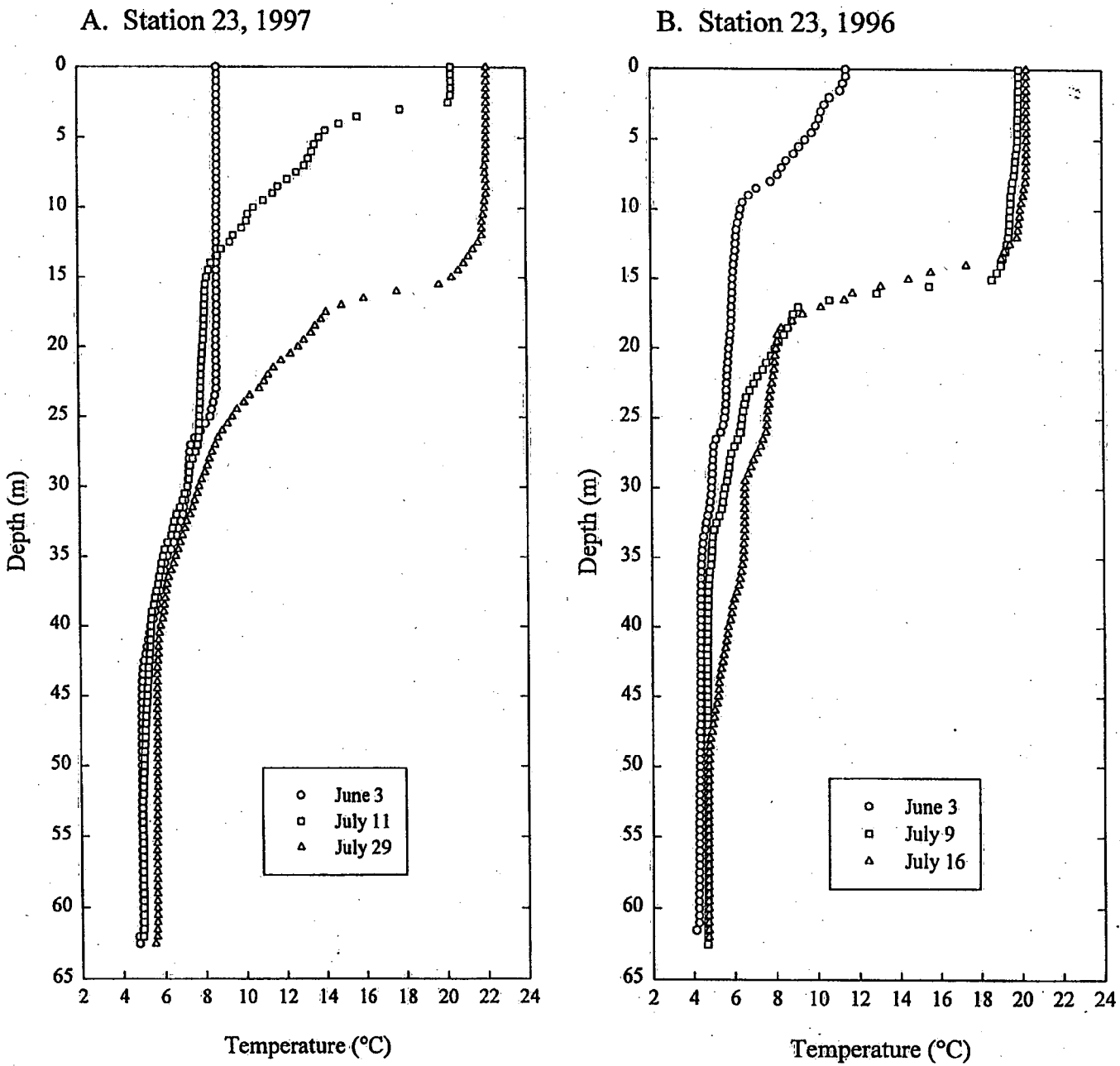

A. Picoplankton (0.4-2 µm)
B. Nanoplankton (2-20 µm)
C. Picoplankton (0.4-2 µm)
D. Nanoplankton (2-20 µm)
182 %
30 %
Phytoplankton Biomass
Chlorophyll-a (µg · L-1)
Photosynthetic Capacity
PmB (µg C · µg chl-a · h-1)
0.6
0.5
0.4
0.3
0.2
0.1
10.0
8.0
6.0
4.0
2.0
0
20
40
60
Time (hours)
Control
+ 20 nM Fe
+ 200 nM Fe

A. Station 935: Eastern Basin, Lake Erie; July 29-31, 1997

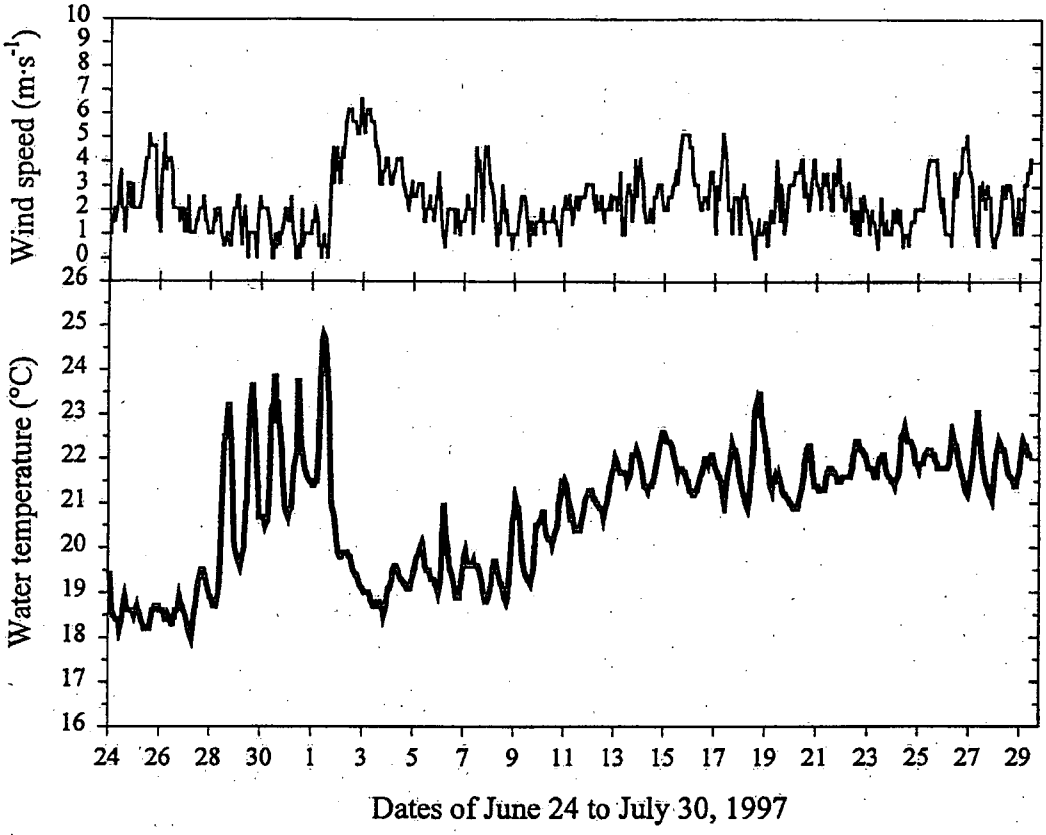


B. Station 23: Eastern Basin, Lake Erie; July 29-31, 1997

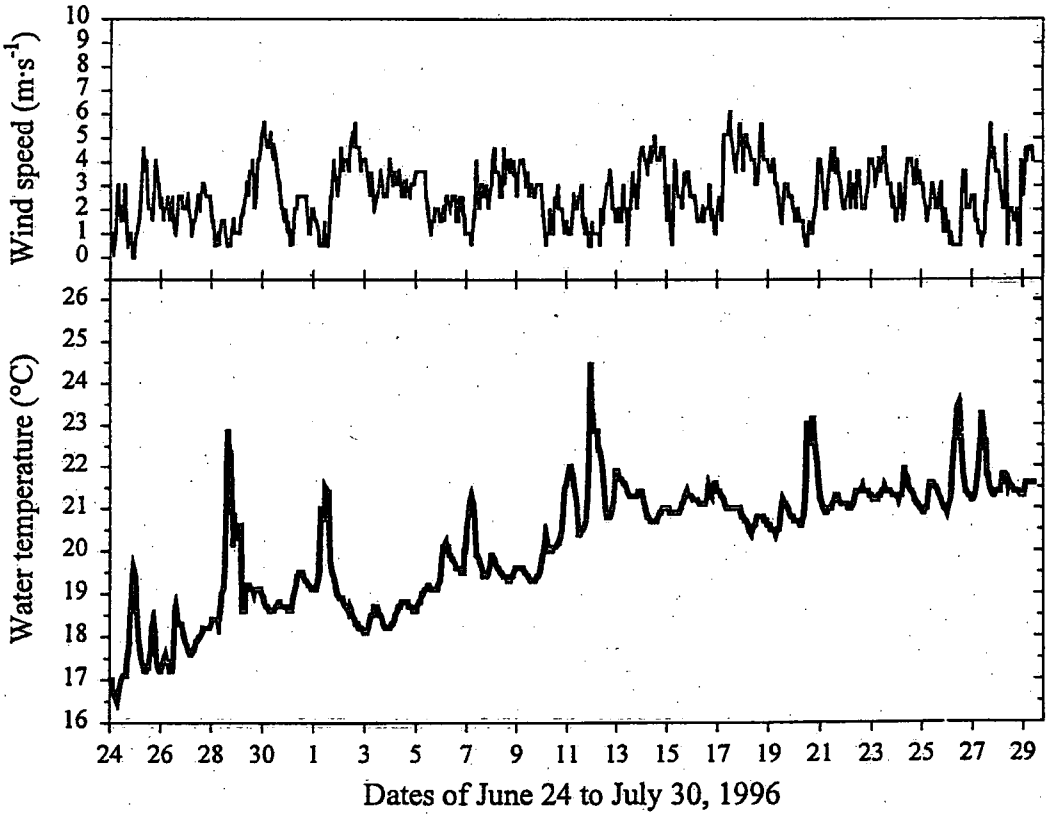



A. Station 23, 1997
B. Station 23, 1996
Depth (m)
Temperature (°C)
June 3
July 11
July 29
June 3
July 9
July 16

A. Station 23, Eastern basin, Lake Erie, 1997.



B. Station 23, Eastern basin, Lake Erie, 1996.



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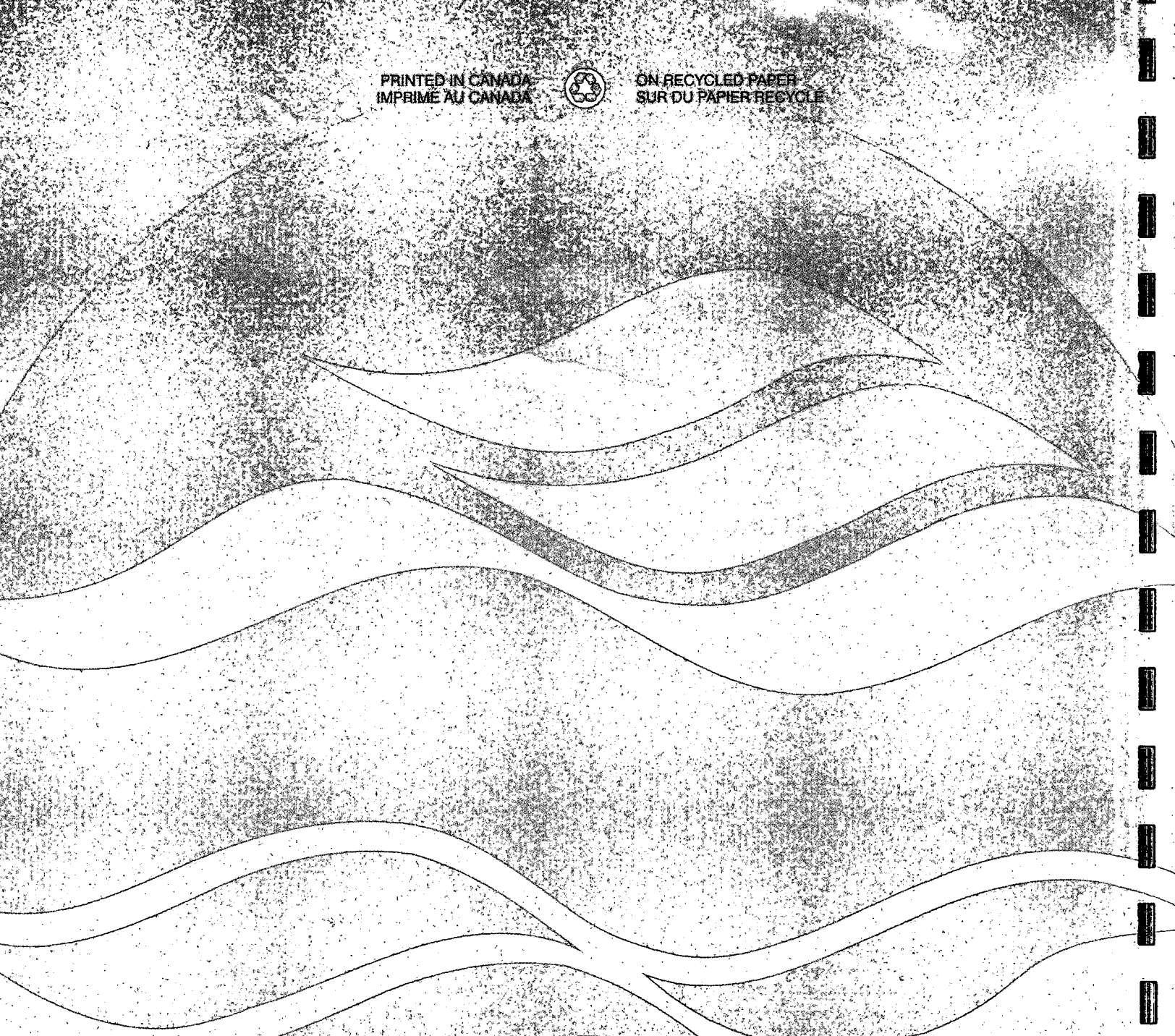

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