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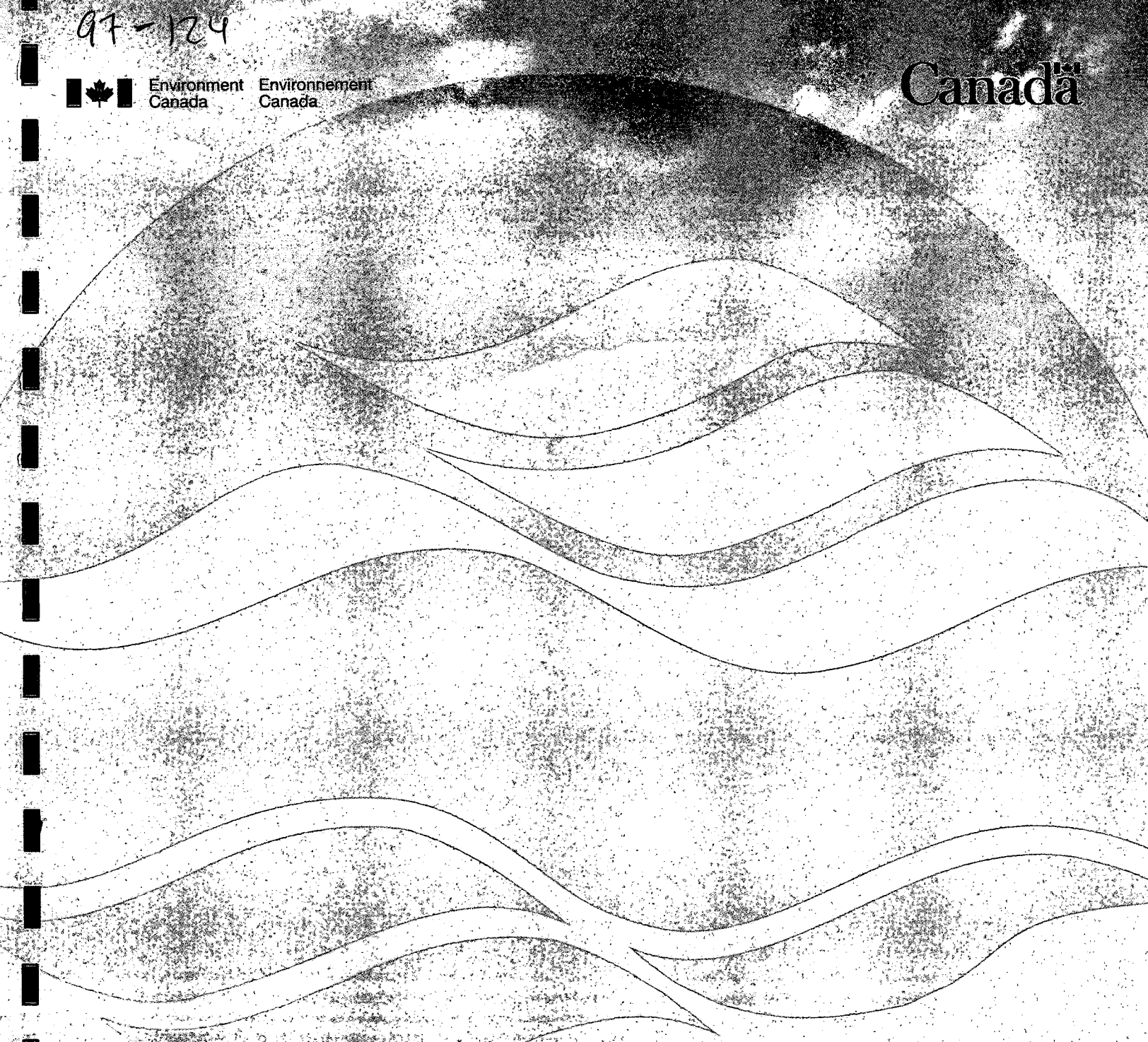

Limitation by Iron of phytoplankton from the open waters of Lake Erie

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Limitation by iron of phytoplankton from the open waters of Lake Erie

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Management Perspective

Title: Limitation by iron of phytoplankton from the open waters of lake Erie

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EC Priority/Issue: Conserving Canada's Ecosystems. Great Lakes 2000. Lake Erie LaMP

Current Status: Experiments in Lake Erie during 1996 indicated that algae growth was limited by iron availability. This is important because the algae are usually thought to be limited by phosphorus. The phosphorus limitation phenomenon is so well accepted that fishing interests are beginning to suggest that waning fish production is a sign that phosphorus controls under the Great Lakes Water Quality Agreement went too far. LaMP co-ordinator has been notified. The work results from a partnership between M. Charlton (NWRI) and the authors from U du Qébec.

Next Steps: Publish manuscript. Strengthen partnership with participation of University of Waterloo scientists in 1997 who will be conducting P limitation studies at the same time as INRS will be conducting more iron limitation studies during NWRI (Charlton) research missions on Lake Erie. Recommend to LaMP on importance of new information.

Abstract

Historically, Lake Erie has been subjected to heavy pollution stresses, primarily due to sewage and industrial effluents. However, recent measurements of low concentrations of trace metals in Lake Erie surface waters suggest that phytoplankton productivity may be limited by the bioavailability of essential trace elements. We report here experimental evidence of iron limitation in a summer Lake Erie phytoplankton community.

Research activities in regions of the world's oceans, containing high levels of macronutrients yet low chlorophyll concentration, have demonstrated that phytoplankton biomass is limited in these areas by Fe bioavailability (1). No comparable studies have been conducted in fresh waters, due mainly to the perception of ample trace metal levels in fresh waters. However, the recent use of rigorous trace metal clean protocols for sample collection and processing has radically changed the view of trace metal levels in the surface waters of the lower Laurentian Great Lakes, lakes Erie and Ontario (2). Paradoxically, the pelagic surface waters of these lakes have remarkably low concentrations of dissolved trace metals during the summer (*2*, 3) despite their proximity to land and anthropogenic sources of these metals.

In Summer

Low concentrations of trace metals in the surface waters of the Great Lakes are attributed to strong thermal stratification of the water column, which isolates pelagic surface waters from input of trace metals from the underlying hypolimnion and sediments (4). Scavenging, the sorption of trace metals from the aqueous phase by sedimentable particles, is considered to be the main mechanism responsible for the low levels of particle reactive trace elements in these (*3*, 5) and other large lakes (6).

Scavenging of essential trace metals may reduce the levels of these metals required for optimal phytoplankton growth in the Great Lakes. For example, the correlation between concentrations of dissolved Zn and Cd in Lake Erie surface water when Zn concentrations are low suggests that biota are utilizing Cd to substitute for the trace nutrient Zn (*3*), as demonstrated in culture studies of marine phytoplankton (7). 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 (8) and microalgae (*9*). Such a relationship may not be directly and solely due to iron bioavailability 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 orthophosphate (SRP; cf. table 1) appears to be under-utilized compared to other nutrient enriched lakes (*10*).

We determined if phytoplankton growth in the pelagic surface water of Lake Erie during thermal stratification is limited by the availability of dissolved essential trace metals. Trace metal clean protocols were used to test the hypothesis of Fe and Zn limitation in

autotrophic members of the picoplankton (0.4-2 μm) and nanoplankton (2-20 μm) sampled from eastern Lake Erie (see Fig. 1 caption).

Change in chlorophyll-*a* (chl-*a*) concentration was used to follow the effect of trace metal enrichment on phytoplankton biomass (11), and $^{14}CO_2$ uptake by phytoplankton was used to measure the photosynthetic efficiency of the chl-*a* (12). If Zn availability limits carbonic anhydrase activity in phytoplankton then the addition of Zn will allow more C to be fixed per unit of chl-*a* with respect to the control treatment, to which no Zn was added. Similarly, 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 (13).

The profound stimulation of phytoplankton following the experimental addition of inorganic iron to sampled surface water strongly supports the hypothesis of an iron-limited phytoplankton population in Lake Erie during summer stratification. 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 (Fig. 1A-B). For example, biomass increased in the low iron treatment by 182% and 30% in the picoplankton and nanoplankton, 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 (Fig. 1C), whereas there was little change in the photosynthetic efficiency of the nanoplankton in the same treatments (Fig. 1D) 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.

Since the specific growth rates (mean ± s.d.; d^{-1}) of the picoplankton (0.61 ± 0.42) and nanoplankton (0.43 ± 0.26) are similar under these conditions (14), it is possible that the nanoplankton required less iron than the picoplankton. Lower iron requirements may be due to a lower physiological requirements by eukaryotic phytoplankton (15) and the accumulation of iron from picoplankton grazed by mixotrophic organisms in the nanoplankton size class, as demonstrated for the trophic transfer (picoplankton → nanoplankton) of ^{65}Zn and ^{109}Cd in Lake Erie (16) and ^{59}Fe in the Equatorial Pacific (17).

However, the rapid response of the picoplankton, 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 chemistry of iron in pelagic Lake Erie surface waters suggests that much of this element 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 (*8*, 18). Siderophore production is proposed as an ecological strategy wherein cyanobacteria can suppress the growth of other phytoplankton (19). Although some eukaryotic marine phytoplankton can produce siderophores (20), the data from our experiment are consistent with siderophore production and utilization by cyanobacteria in that iron accumulation by the nanoplankton may have been prevented by the formation of ferrisiderophores complexes available for uptake only by the cyanobacteria. If this was indeed 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. Fig. 1A, 1C). A similar response by picoplankton was observed following an *in situ* iron enrichment in the equatorial Pacific ocean (21, 22).

In contrast to the effect of Fe additions, changes in phytoplankton biomass and photosynthetic efficiency were not significantly different from controls in low (0.05 nM) and high (0.5 nM) added zinc treatments (data not shown). Hence, there is no evidence of a Zn-limited phytoplankton community in the surface waters of Lake Erie at the time of this sampling.

This is the first direct demonstration of trace metal limitation of phytoplankton in the Great Lakes. An earlier study in Lake Huron showed that the addition of chelated iron, FeEDTA (iron ethylenediaminetetraacetic acid), to surface water collected during summer months causes a significant increase in phytoplankton (23). This earlier study is, however, 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 (24). Moreover, the use of iron chelated by a synthetic organic ligand (EDTA) might result, through displacement, in the complexation of toxic trace metals rendering experiment interpretation difficult.

Efficient scavenging of trace elements by plankton maintains very low concentrations of dissolved trace metals, despite high fluxes in this area (25). Similar limitations of phytoplankton may exist for other trace elements, or ratios thereof, in this and other Great Lakes. The implications for fisheries management and water quality

criteria are important – we must acknowledge that a shift from P-limitation to trace metal limitation in Lake Erie during the period of thermal stratification has likely occurred and its potential effects on primary productivity should be investigated.

Acknowledgements

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Table 1. Chlorophyll-*a* in Lake Erie surface waters in relation to calculated free ion concentrations of Fe^{3+}. Aqueous Fe speciation was determined using a computerized chemical speciation model (Windermere Humic Aqueous Model–Waters, Version 1.0; 26). Calculations were based on: reported major ion concentrations in the epilimnion of Lake Erie during summer (27), dissolved concentrations of Fe determined in this study (28) and those reported in the literature (3), a dissolved humic subtance concentration of 1.6 $mg \cdot L^{-1}$ (10:1 ratio of fulvic acid to humic acid), pH 8, and 20°C. The concentrations of dissolved organic carbon 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.), and we have assumed that humic and fulvic acids comprise 50% of the dissolved organic carbon (29).

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

8/1993	255*	42°08'32"	80°59'19"	7.3	21.37	1.7	0.6	195	8
8/1993	47*	42°17'36"	80°18'13"	5.2	21.52	1.1	0.5	204	8
8/1993	54*	42°39'11"	79°07'54"	16.8	21.00	2.8	0.9	255	7
8/1993	357*	41°49'32"	80°58'26"	47.8	20.50	4.5	0.9	254	9
8/1993	30*	41°34'00"	82°37'59"	18.3	20.96	6.5	0.6	143	9
8/1993	18*	41°31'49"	81°42'31"	49.3	20.49	4.8	0.8	263	14
7/1994	84	41°55'57"	81°39'35"	21.0	20.89	1.65	n.d	n.d	14
7/1996	935	?	935	19.9	20.92	0.90	n.d	n.d.	n.d.
7/1996	23	42°30'26"	79°53'54"	16.2	21.01	0.97	0.2-1.5	n.d.	n.d.
7/1996	946	?	946	7.3	21.37	1.14	n.d	n.d.	n.d.
7/1996	953		953	21.1	20.89	n.d.	n.d	n.d.	n.d.
7/1996	84	41°55'57"	81°39'35"	8.1	21.33	0.67	0.9-1.9	n.d.	n.d.
7/1996	341	?	341	3.0	21.76	0.61	n.d.	n.d.	n.d.
7/1996	357	41°49'32"	80°58'26"	17.7	20.97	2.23	1.0	n.d.	n.d.

*Iron measurements reported by Nriagu et al. (*3*).

n.d. = no data.

Figure 1. Changes in biomass (A, B) and photosynthetic efficiency (C, D) of pelagic nanoplankton and picoplankton sampled from the eastern basin of Lake Erie following the addition of iron. Values are mean ± s.d., n = 2. Water was collected from a depth of 5 m at station no. 23 (42°30'26 N, 79°53'54" E) in the pelagic eastern basin of Lake Erie at 1230 h on July 9, 1996, using trace metal clean techniques (*16*). All further manipulations were conducted in a Class 100 portable clean room onboard the research vessel. Lake water from three casts was screened through a 20 μm-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 low (20 nM) and high (200 nM) treatments. Treatments were conducted in duplicate. Two bottles received no trace metal 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, samples of water from the bottles were removed for measurements of photosynthesis and the determination of size fractionated chlorophyll-a concentrations and ^{14}C uptake.

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11. Chlorophyll-*a* Determinations: One hundred mL was removed from each bottle and serial filtered onto a 2 μm-filter followed by a 0.4 μm-filter (filters were 47 mm dia. polycarbonate membrane filters; Nuclepore). Shipboard fluorometric analysis (N. Welschmeyer, *Limnol Oceanogr* 42, 1994) of pigment content was conducted on chl-*a* extracted from the filters into 90% acetone at 4°C over 12 h.

12. ^{14}C Uptake: Water (125 mL) sampled from each bottle was 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. The bottles were incubated for 2 h under the simulated *in situ* conditions (see Fig. 1 caption). After the incubation period the entire bottle contents were serially filtered onto a 2 μm-filter and then a 0.4 μm-filter. Filters were rinsed with filter-sterilized lake water (<0.4 μm). Filter controls were conducted to account for the non-photosynthetic sorption of ^{14}C by particles. For these controls, the entire contents of a sample were filtered within 2 min of adding the ^{14}C to the lake water sample. All filter samples containing ^{14}C were immediately frozen for transport to the laboratory. Filters were counted by liquid scintillation spectrometry (Wallac Winspectral model 1414) and quench corrected using the internal quench library.

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28. Trace Metal Analysis: Lake water was collected from a series of pelagic stations from all major regions of Lake Erie during daylight hours from July 9-11, 1996. Lake water was filtered (<0.2 μm), and acidified to pH <2 using HCl (Merck Supra-Pur; final concentration 0.12% HCl). Sample blanks were prepared using deionized

water. Analysis of Fe content was conducted by graphite furnace atomic absorption spectrophotometry (Varian SpectraAA Model 300) using an $MgNO_3$ matrix modifier and standard additions methodologies.

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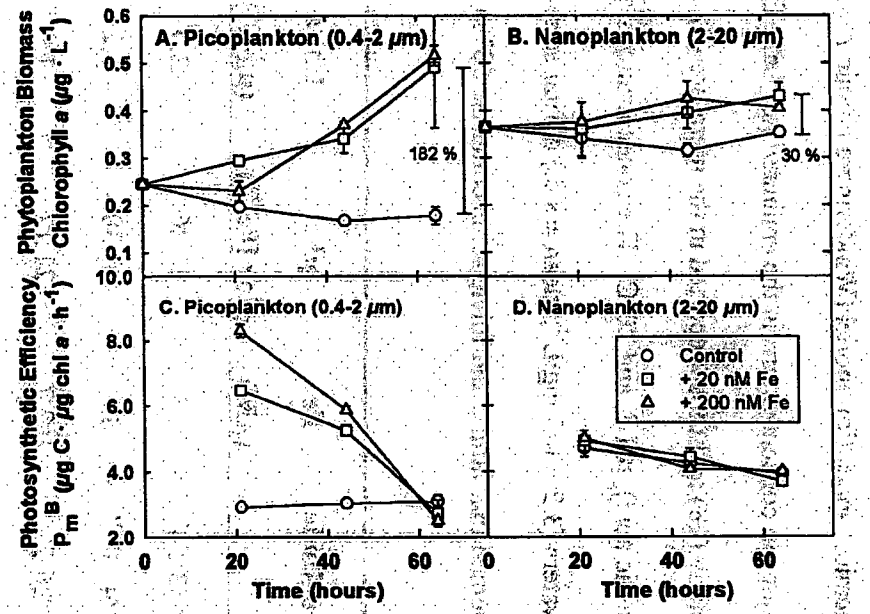
Twiss, Auclair and Charlton (Figure 1)



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