

Does Trace Metal Availability Constrain Phytoplankton Growth and Standing Crop in Lake Erie?

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Limitation of primary production by phosphorus availability is a central tenet of modern day Great Lakes limnology. Indeed, the importance of P availability in constraining phytoplankton growth and standing crop has long directed lake management decisions, perhaps most notably via the Great Lakes Water Quality Agreement which has resulted in reduced P loading and contributed to enhanced water quality in the Great Lakes system over the past two decades (Neilson et al. 1995). In light of the seeming overwhelming evidence in support of P limitation, it is tempting to overlook the often-important role played by other elements in regulating lake productivity. Specifically, the potential for trace metal, or “micronutrient” limitation in the Great Lakes has received limited attention. Compared to macronutrients (C,N,P), micronutrients (Fe, Zn, Mn, Cu, Ni, Co) are required in only nominal amounts to support cellular metabolic processes. However, recent findings in marine systems identify a need to reexamine trace metal-phytoplankton interactions. Indeed, current opinion favors Fe availability as the ultimate factor limiting productivity in the equatorial Pacific and possibly other “high nutrient, low chlorophyll” oceanic provinces (Martin et al. 1991; Coale et al. 1996; Boyd et al. 1999).

Although present consideration is limited to the bioavailability of Fe in the Great Lakes, many of the same principles and approaches can be extended to other nutritive trace metals.

Relevance of Fe deficiency to lacustrine systems

It is becoming increasingly apparent that consideration of Fe availability as a factor constraining phytoplankton growth is not limited to the open ocean. Supporting Fe limitation in “non-traditional” marine environments, Hutchins and colleagues recently described Fe limitation in terms of a “mosaic” throughout the California coastal upwelling region (Hutchins and Bruland 1998; Hutchins et al. 1998). Coastal Fe limitation has also been described for regions of the northern Japan Sea (Suzuki et al. 1995) and in a coral reef environment (Entsch et al. 1983).

Furthermore, Fe limitation is not restricted to the marine environment. It has long been recognized that some lacustrine systems are inherently subject to Fe limitation as a result of lake chemistry (e.g. hard-water calcareous lakes, Schelske 1962; Schelske et al. 1962; Wetzel 1966; prairie saline lakes of high alkalinity, Evans and Prepas 1997). Consideration of Fe limitation for many lakes, however, has received little attention. Much of this is driven by the importance placed on phosphate as a limiting element of lacustrine systems. Taken together with the extent to which the Great Lakes are subject to anthropogenic influence (e.g., Dolan et al. 1993; Nriagu et al. 1996), trace metal deficiency is rarely afforded consideration when identifying factors limiting lake productivity. This view of metals as “toxic” rather than “tonic” was further reinforced by results of available field surveys that documented relatively high levels of dissolved trace metals in the Great Lakes system. The adoption of ultraclean metal sampling protocols by geochemists working on the Great Lakes, however, has resulted in a change in perception and reveals that dissolved levels of many metals are quite low and within the range commonly reported in the open ocean (Flegal et al. 1989; Nriagu et al. 1993, 1996; Twiss et al. 1999). In general, dissolved Fe is reported to be present in low nanomolar amounts (Nriagu et al. 1996; Twiss et al. 1999).

Renewed confidence in measurements of trace metal levels in the Great Lakes sheds light on the interpretation of enrichment bioassay results reported over two decades ago by Schelske and colleagues for various locations throughout the upper Great Lakes (Schelske et al. 1972, 1978; Stoermer et al. 1978; Lin and Schelske 1981). These studies demonstrated that chelating agents and trace metals could enhance the phytoplankton growth response over that obtained by addition of phosphate alone in bioassays of natural lake water. However, because of prevailing attitudes regarding metal abundance, the positive response elicited by trace metals and chelating agents on phytoplankton growth was difficult to reconcile at the time their observations were made and it was unclear whether growth was promoted by decreasing metal toxicity or, alternatively, by increasing metal availability. Our current perspective on trace metal levels lends support to the latter interpretation.

Iron deficiency in Lake Erie?

Whereas high levels (~ 50 nM) of dissolved Fe are anticipated for, and likewise measured in surface waters of the well-mixed, shallow western basin of Lake Erie (Nriagu et al. 1996), this observation underscores the potential for Fe limitation elsewhere in the lake. Despite significant loading of metals from anthropogenic sources (Flegal et al. 1989), average concentrations of Fe in the central and eastern basins of Lake Erie remain low. In addition to anticipated complexation (organic and inorganic), trace metals are rapidly scavenged (Twiss and Campbell, 1998a) and recycled (Twiss and

Campbell, 1998b) by the seston. Low, overall concentrations of trace metals are particularly evident during periods of thermal stratification where dissolved Fe measured in pelagic waters of stations monitored in the central and eastern basins is often < 10 nM (Nriagu et al. 1996; Twiss et al. 1999). Comparing dissolved Fe concentrations in Lake Erie to the range of values found to be limiting in laboratory cultures (10-25 nM total Fe for neritic marine diatoms; McKay et al. 1997), it is evident that Fe may limit productivity in parts of the lake. The effect is further compounded if we consider organic complexation of the dissolved Fe, estimated by a chemical speciation model (WHAM) to be in excess of 99% for Lake Erie (Twiss et al. 1999). This is consistent with voltammetric measurements indicating that > 99% of dissolved Cu and Co are complexed to strong organic ligands in this lake (M. Saito and M.R. Twiss, unpublished). Moreover, the results of recent Fe enrichment assays performed using trace metal-clean techniques at various locations throughout Lake Erie report variable stimulation of phytoplankton growth and increased photosynthetic efficiency following additions of Fe to lake water (Twiss et al. 1999). These experiments demonstrated that during July 1996, picoplankton sampled from the epipelagic eastern basin responded markedly to Fe addition; biomass and photosynthetic rates were substantially elevated over that of control treatments.

Approaches to Studying Fe Deficiency

As indicated by reference to previous work by Schelske and colleagues, the enrichment bioassay approach to identifying limiting nutrients is not new. Although experiments of this design have provided investigators the means with which to manipulate nutrient composition in a controlled manner, results must be interpreted with care (Schelske, 1984). Critics of this approach argue that “bottle assays” do not adequately mimic the natural environment; grazing is disrupted, physical mixing is negated and phytoplankton are isolated at a fixed optical depth (Banse, 1991; Cullen, 1991). That biomass periodically increases, albeit to reduced levels, in unamended control bottles during enrichment experiments lends support to this view. Furthermore, despite the implementation of metal-clean protocols, concerns over contamination are always valid when applying this experimental approach to study phytoplankton-trace metal interactions.

In response to these concerns, effort has been directed in recent years toward developing chemical and biological approaches with which to directly or indirectly assess Fe bioavailability *in situ*. During periods of Fe deficiency, flavodoxin (Flvd) replaces the Fe-containing electron transfer catalyst ferredoxin (Fd) as a means of relieving the cellular Fe burden in cyanobacteria (Straus, 1994) and numerous algae (Geider and La Roche, 1994). As such, Flvd is identified as a candidate biochemical marker of Fe deficiency in phytoplankton; its detection providing an *in situ* assessment of cellular Fe

nutrition thereby avoiding the complicating factors associated with manipulation experiments. Among criteria satisfied to serve in the role of diagnostic indicator, induction of Flvd synthesis is specific in response to Fe deficit (La Roche et al. 1993; Erdner et al. 1999). Further, accumulation of flavodoxin can be related to parameters of physiological limitation, especially when expressed in terms of a Fd Index, a parameter defined as relative cellular $Fd/(Fd + Flvd)$ (Doucette et al. 1996; Erdner et al. 1999). Accumulation of Flvd and Fd in samples collected from the field is measured using HPLC (Doucette et al. 1996; Erdner and Anderson, 1999; Erdner et al. 1999) or alternatively, assessed by immunological means, either by Western blot (La Roche et al. 1993, 1995, 1996; McKay et al. 1997, 1999) or single-cell immunofluorescence (La Roche et al. 1996). Whereas HPLC detection carries no bias with respect to phylogeny and measures total community response to endemic levels of Fe, immunoassay is generally more selective, providing a measure of taxonomic resolution. This is particularly true if the immunofluorescence approach is adopted where immunoassay is combined with microscopic analysis of intact cells. The immunological approach is also generally more sensitive; depending on antibody affinity, detection limits can be 3 orders of magnitude lower than that achieved with HPLC (femtomolar vs. picomolar). This limits application of the HPLC approach when dealing with ultra-oligotrophic waters where difficulties in sampling sufficient amounts of biomass are likely to be encountered (Erdner and Anderson, 1999).

Extending the immunological approach to preliminary studies of Fe bioavailability in the Great Lakes has been greatly facilitated by availability of appropriate antisera. Despite initial concerns over the efficacy of a “marine” probe to study Fe deficiency in freshwater systems, antisera directed against flavodoxin (La Roche et al. 1995) isolated from a marine diatom cross-react with antigens of appropriate size in freshwater diatoms (Fig. 1). Antisera directed against diatom ferredoxin (McKay et al. 1999) are similarly cross-reactive with antigen from freshwater forms (R.M.L. McKay, unpublished).

LEPF-Supported Effort

Funding provided by the LEPF has been used to conduct preliminary investigations on Fe deficiency in each of the 3 basins of Lake Erie. Our efforts are presented below and generally represent collaborative endeavors with US and Canadian colleagues.

Assessing Fe Nutrition in the Western Basin of Lake Erie

During 1998, the PI held a summer fellowship with the Lake Erie Center (University of Toledo). The objective of this fellowship was to conduct research into bioremediation of PCBs in Maumee Bay and the Ottawa River. As such, sampling

opportunities were made available on which to “piggy-back” investigations of phytoplankton Fe nutrition in the western basin of the lake. Sampling was conducted on a monthly basis between June and September, 1998. On each occasion, water was collected using acid-cleaned polycarbonate carboys from a station located near the City of Toledo water intake (station coordinates: 41° 41.320” N, 83° 19.220” W). Water samples were returned to the laboratory and there they were processed for *in situ* immunological analysis of Flvd/Fd as outlined above. Despite a considerable diatom component (on the order of 50%) included in each sample, Flvd was not detected in immunoassays. Rather, a strong Fd signal was consistently observed indicating an iron-replete diatom assemblage.

During the August and September collection dates, water samples were additionally subject to nutrient enrichment bioassays whereby bottles were amended with Fe, P or N and incubated over 6 days in an environmental chamber maintained at 24°C (surface water temperature at time of sampling). Enrichment bioassays conducted in August indicated a nutrient-replete assemblage with no stimulation of growth in bottles provided either P or Fe compared to control bottles (one-way ANOVA). In contrast, addition of P to water collected during September, 1998 resulted in 33% elevated accumulation of biomass over that of control bottles (one-way ANOVA; $P < 0.05$). Addition of Fe or N afforded no enhancement over that recorded in the controls.

Immunological Analysis of Samples of Opportunity

Arrangements were made with Canadian government scientists during field seasons in 1998 and 1999 to have samples collected for subsequent immunological analysis in the PI’s lab at BGSU. During 1998, arrangements were made with Dr. Murray Charlton (Environment Canada – Canada Center for Inland Waters) and with Dr. Todd Howell (Environmental Monitoring and Reporting Branch, Ontario Ministry of the Environment) as part of regular monitoring and surveys conducted by their respective agencies on Lake Erie. In 1999, additional samples were provided by Dr. Charlton. Samples have consisted of biomass obtained using plankton net tows as well as filtered material. Unfortunately, the latter approach has not resulted in a suitable quantity of biomass for the immunoassay conducted in our laboratory. Net tow material, however, has provided an abundant source of extractable protein and subsequent evidence of Flvd expression in Lake Erie diatoms (Fig. 2). The profiles depicted in this figure come from an August/September, 1998 cruise during which samples were procured from two eastern basin stations (935 and 23) and two central basin stations (946 and 84). A map of Lake Erie showing the location of Environment Canada survey stations is provided as Figure 3. Evident from the immunoassays is accumulation of Flvd in samples collected from the eastern basin stations but not from the central basin stations. This profile may be

interpreted in several ways. It may indicate that diatoms in the eastern basin of the lake are Fe-stressed whereas those of the central basin are Fe-replete. Alternatively, it may reflect a paucity of diatoms endemic to the central basin stations at the time of sampling. In fact, the latter interpretation has some basis since an Fe-replete population would be expected to accumulate Fd, yet only a minor Fd signal was evident (Fig. 2; recall that our antisera are biased toward reaction with diatoms and not other forms). Further supporting this interpretation, diatoms were rare at station 84 during a sampling effort on which the PI participated during July, 1999 (see below). Thus, the negative Flvd staining result for central basin samples does not necessarily reflect an iron-replete assemblage.

Environment Canada – National Water Research Institute Research Cruise (July, 1999)

The PI was invited to participate in an Environment Canada sponsored cruise on board the *CCGS Limnos* (Fig. 4) during 10 days in July 1999. The cruise plan called for an extended mooring at station 84 in the central basin (Fig. 3). During the cruise, the ship also occupied station 23 in the eastern basin (located at the deepest point in the lake) and conducted a single-day (9 July) multi-station survey around the western basin.

Complementing efforts of the PI on this cruise was the participation of Dr. Michael Twiss (Ryerson Polytechnic University) and Dr. Steven Wilhelm (The University of Tennessee). Dr. Twiss is an environmental chemist who was charged with analyses of trace metal chemistry and Dr. Wilhelm is a microbial ecologist with interests in viral-mediated nutrient cycling and cyanobacterial Fe acquisition. In addition, ancillary data (macronutrients, chlorophyll, CTD profiles) were made available by Dr. Rick Bourbonniere (Environment Canada) who served as the Chief Scientist on this cruise.

Several approaches were adopted to study trace metal nutrition. At all stations occupied, samples of the endemic phytoplankton assemblage were taken for subsequent immunological analysis of Flvd and Fd (outlined above) in the laboratory of the PI. To date, a portion of this data has been analyzed. Of note, Flvd was not detected in samples collected from the central basin station. Subsequent floristic analysis of samples preserved with Lugol's iodine, however, indicates that diatoms did not form a significant component of the phytoplankton assemblage in these waters at this time. Rather, samples were dominated by various chlorophytes, the dinoflagellate *Ceratium* and the chrysophyte *Dinobryon*. As such, a negative result provided by the immunoprobe used in this instance (which is diatom-specific) cannot be extended by default to the remainder of the assemblage. To address this methodological deficiency, the PI is attempting to procure additional taxa-specific Flvd probes.

Nutrient enrichment bioassays were also employed as part of this study. As noted previously, there are problems inherent in the interpretation and applicability of

bioassays. Regardless, they are still useful, especially when used in concert with other analyses. Arrangements were made to have an Environment Canada “clean van” included on the ship (Fig. 5) which made it possible to conduct metal-clean bioassays as part of this cruise. Employing metal-clean techniques, water samples from 10-m depth were collected at stations in the central and eastern basins of the lake and amended with 50 nM Fe followed by on-deck incubation (Fig. 6). Phytoplankton sampled from the eastern basin showed no response to Fe amendment following 2 and 6 day intervals. Subsequent amendment of control and +Fe bottles with 1000 nM phosphate (P) resulted in a 4-fold accumulation of biomass with no additional stimulation in growth afforded by Fe. Likewise, addition of Fe alone had no stimulatory effect on phytoplankton sampled from a central basin station. Again, subsequent amendment of control and +Fe bottles with P resulted in a 3-fold increase in biomass. A similar result was provided from a factorial design enrichment (Fe, P) conducted at the central basin station. No additional stimulation in growth was afforded by Fe in concert with P. Despite these findings, the importance of Fe availability to phytoplankton growth and community dynamics remains tenable as highlighted in bioassay experiments conducted using water sampled from the central basin to which the Fe chelating agent deferoxamine (DFB) was added. Addition of DFB resulted in a 50% decrease in biomass, depressed rates of ^{55}Fe uptake and a shift in the composition of the phytoplankton assemblage manifested mainly as a decline in the *Dinobryon* component (40% *Dinobryon* in +Fe and control bottles compared to 10% presence in +DFB bottles).

Results obtained during the Environment Canada cruise suggest that Fe was not limiting during the period tested. Final interpretation of experiments conducted as part of this cruise await complete analysis of trace metal chemistry (concentration and assessment of metal lability) by Dr. Twiss.

Conclusions

The results of this preliminary investigation are mixed and as such, support the conclusions reached by Twiss and colleagues (1999) that Fe deficiency in Lake Erie is a patchy phenomenon and likely dependent on prevailing hydrologic and climatic patterns. It would appear that direct limitation of phytoplankton growth by low Fe availability in Lake Erie is rare. However, positive immunoassay results, at least for the eastern basin, indicate that although Fe is not likely physiologically limiting, its availability is low and may be important as a secondary limiting nutrient poised behind P. This would have implications for future management strategies in which P loads to the lake are increased – that is, the anticipated accumulation of standing crop in order to revitalize fisheries may not be realized, particularly in the central and eastern basins, as a result of Fe limitation in the face of increased P loads.

Publications/Abstracts in Which LEPF Funding is Acknowledged

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Fig. 1. Flavodoxin antiserum specificity

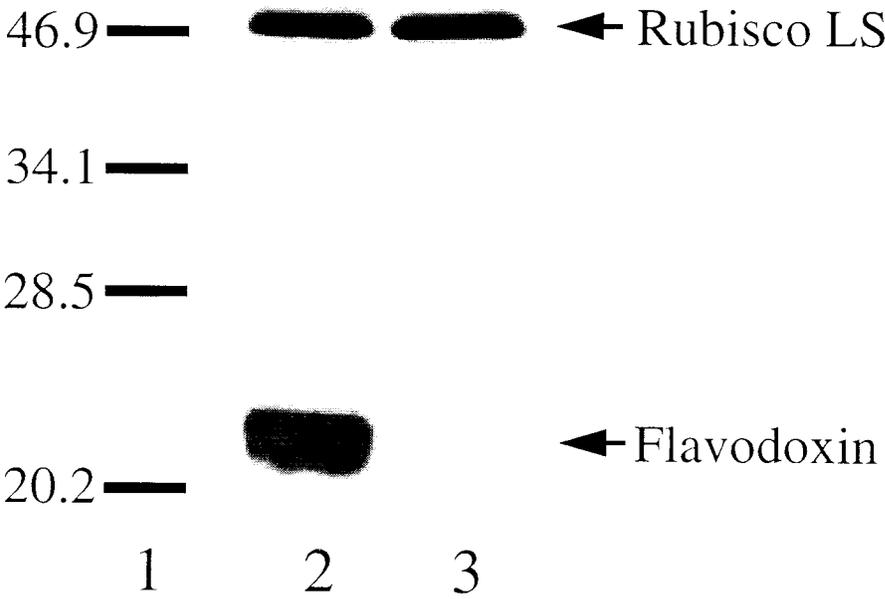
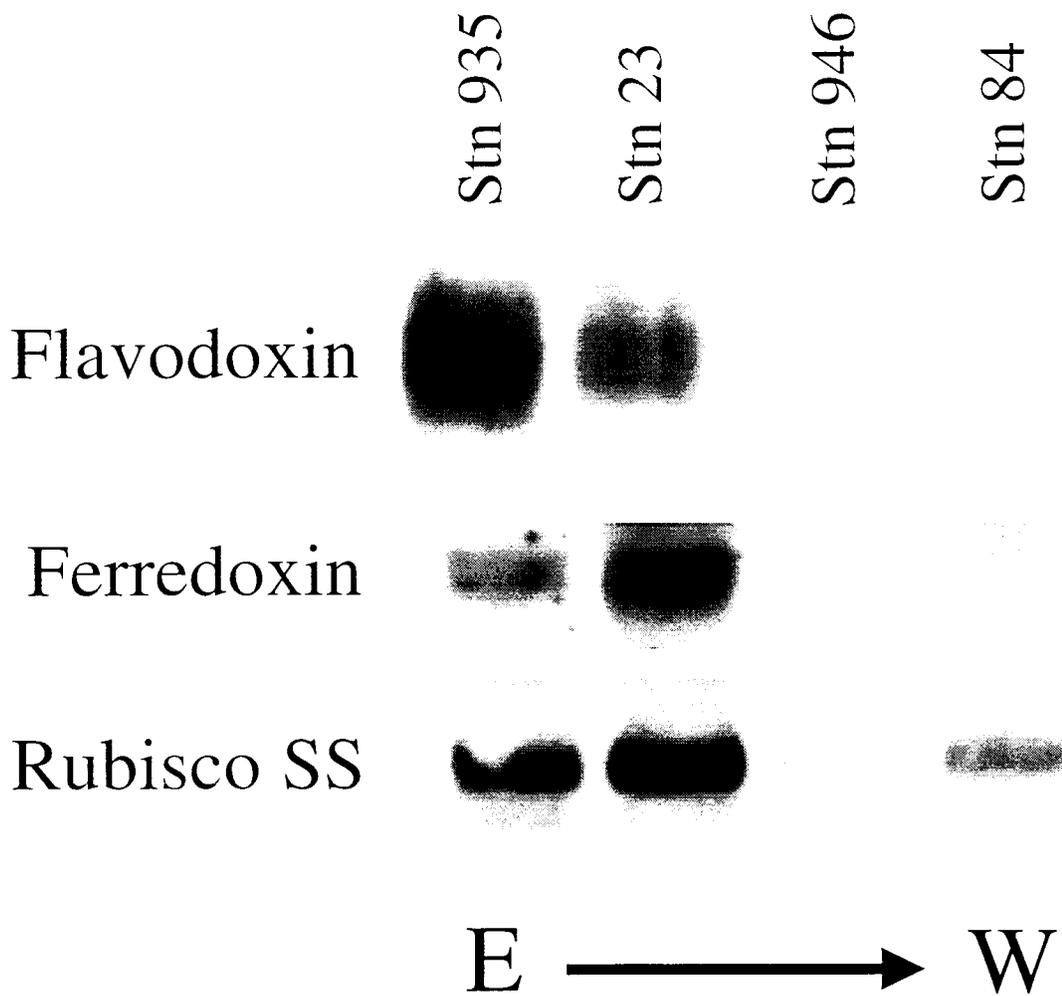


Fig. 2. Flvd/Fd Accumulation at Stations in the Central and Eastern Basins

Lake Erie Transect Net Tow (8/98)



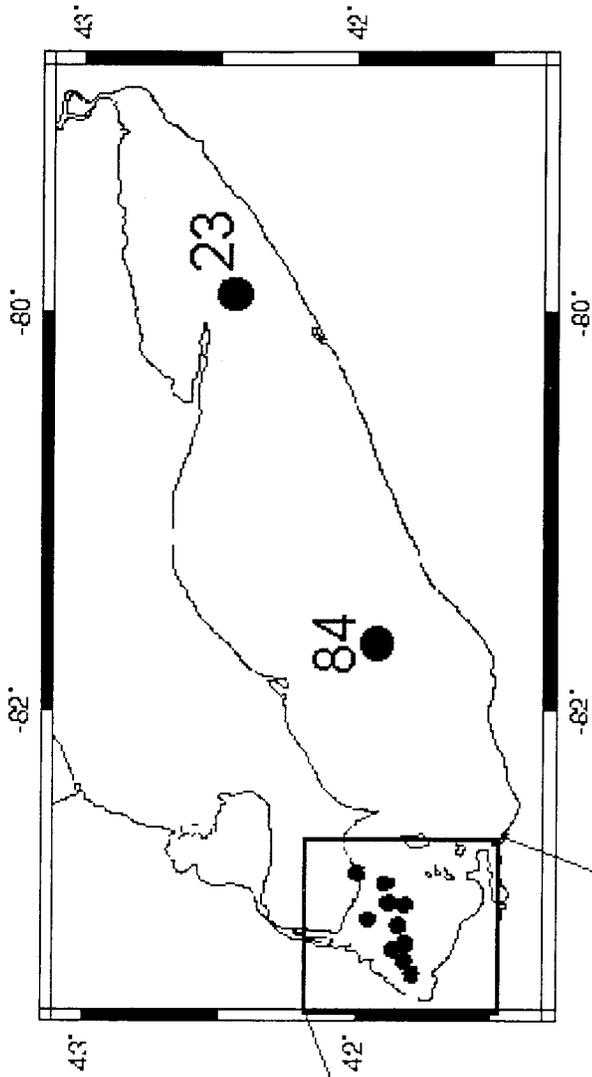
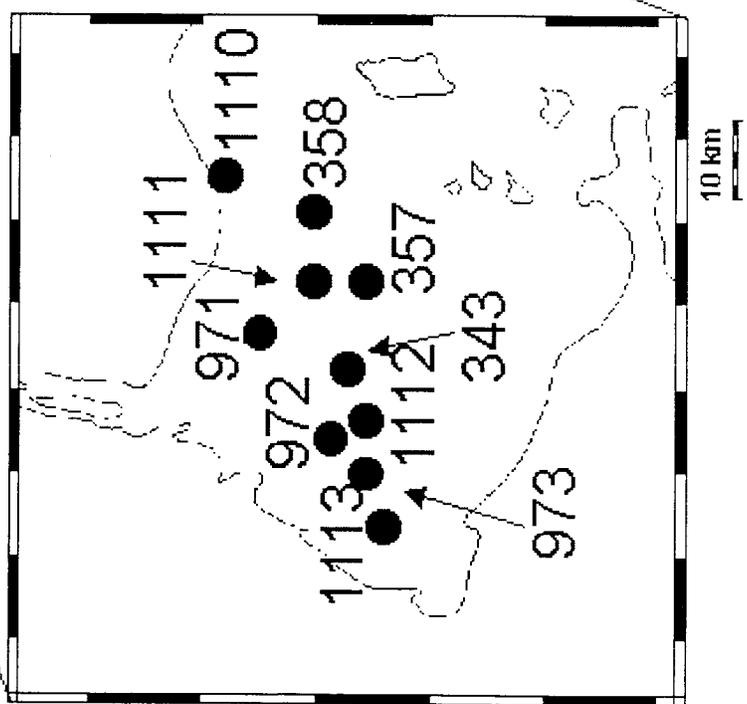


Fig. 3. Environment Canada Survey Stations



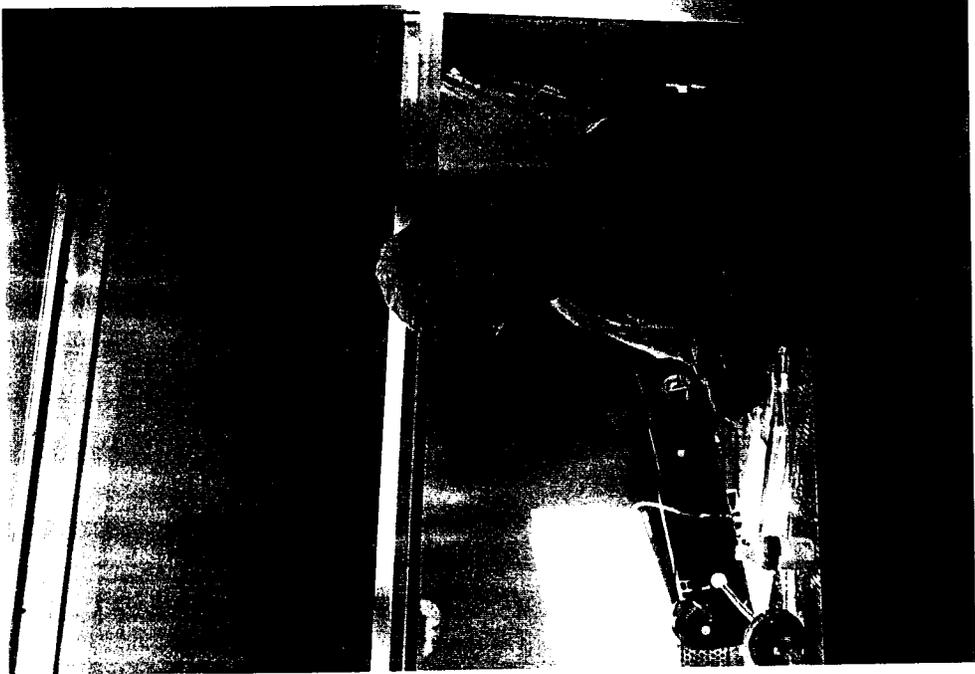


Fig. 5. Inside metal-clean laboratory on *CCGS Limnos*

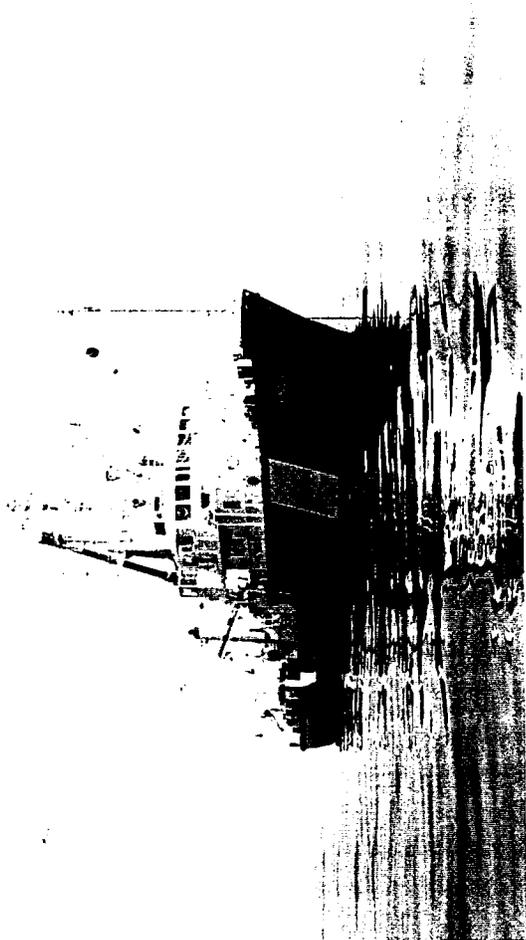


Fig. 4. *CCGS Limnos* moored at station 84

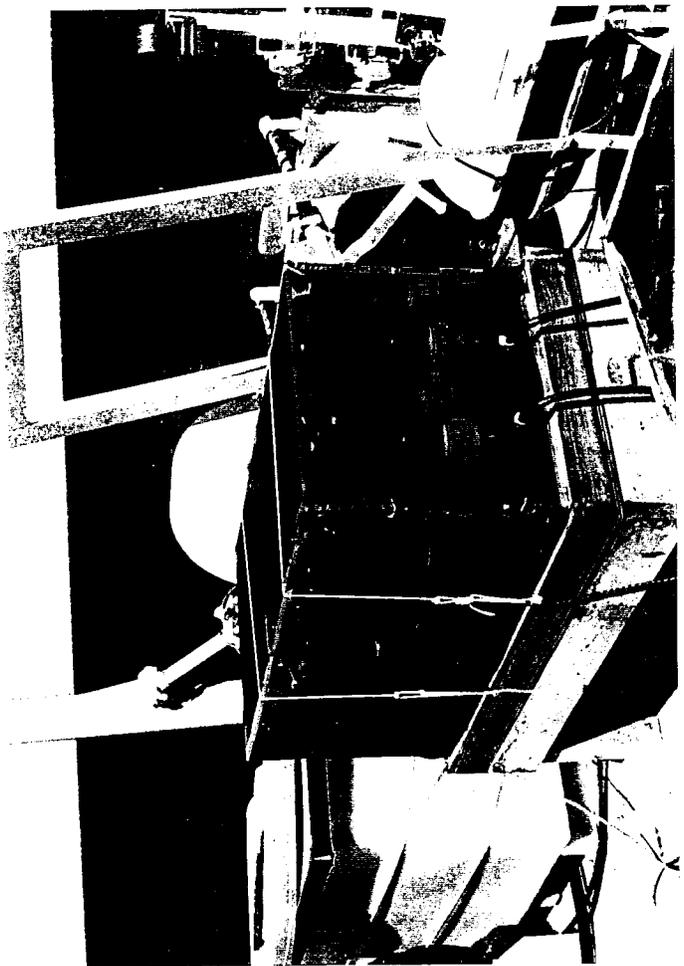


Fig. 6. On-deck flow-through incubation chamber