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NUTRIENT ENRICHMENT STUDIES

ON

LAKE HURON

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LAKE HURON

by:

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#### **ABSTRACT**

Nutrient enrichment experiments were performed on integrated water samples taken to 10M depth from Lake Huron between April and December 1971. Phytoplankton response to added N, P, N & P, Fe and Mn at maximum concentrations normally found in the lake was measured by <sup>14</sup>C uptake.

In general, statistically significant positive responses to all nutrient elements added occurred during the spring algal pulse in April and May and during the fall pulse in early October-November. Most positive results were found with manganese and fewest with phosphate.

Negative or non-significant responses compared to controls occurred mainly from June to the period of the fall pulse. This seasonal effect upon both positive and negative response appears to be explained by a combination of factors including species composition, physiological mechanisms within species, and ambient nutrient concentrations.

Our results also indicate that the concept of a "limiting factor" as developed by Liebig and others does not apply to phytoplankton communities.

#### INTRODUCTION

Lake Huron, the second largest of the Laurentian Great Lakes, is presently considered by limnologists to be oligotrophic in offshore waters with indications of mesotrophy in some nearshore waters in the southern part (Glooschenko et al., 1973). However, Saginaw Bay is one of the most highly eutrophic embayments in the Great Lakes. Thus, concerns to preserve the water quality of the lake proper will involve nutrient management schemes. In order to do this, identification of limiting nutrients in the lake needs to be made.

Two types of bioassays have been used by limnologists to identify limiting nutrients. The first of these involves additions of various presumed limiting nutrients to water samples with their inherent phytoplankton community, then measuring the response to such additions by <sup>14</sup>C uptake (Goldman, 1972). The other approach is to add a test species of algae to filtered lake water and measure its subsequent growth under controlled conditions by various methods, the so-called algal assay procedure (Forsberg, 1972). For purposes of the present study, the <sup>14C</sup> bioassay procedure was chosen in order to determine whether the elements phosphorous, nitrogen, iron and manganese were limiting photosynthesis of natural phytoplankton communities in Lake Huron.

# METHODS

Water samples were obtained during seven cruises on Lake Huron between April and December 1971. Stations investigated on each cruise are

listed in Table 1 and shown on Figure 1. The samples were obtained by taking an integrated water sample from 10M to the surface. After thorough mixing, 100 ml aliquots were placed in each of 24 125 ml glassstoppered pyrex reagent bottles. The bottles were divided into six groups of three each plus one darkened bottle for the control treatment. Thus, triplicate light bottle samples were available for statistical analysis of results of treatments compared to controls. The dark bottle uptake value was then subtracted from each treatment. The following treatments were used besides controls: N-100  $\mu g/\ell$  as NH<sub>4</sub> NO<sub>3</sub>, P-20  $\mu g/\ell$ as NaH2PO4 , N + P as 50  $\mu g/\ell$  N and 10  $\mu g/\ell$  P, Fe - 10  $\mu g/\ell$  as FeEDTA and  $M_{\rm m} = 5 \, \mu {\rm g}/{\rm k}$  as MnC1 . These concentrations were chosen to approximate maximum ambient nutrient concentrations in the open lake (Dobson et al., 1974). Such maximum ambient concentrations were used in order to avoid toxicity problems with the trace metals. Upon additions of these nutrients, 0.5  $\mu$ C Na<sub>2</sub>  $^{14}$ CO<sub>3</sub> (100  $\mu$  $^{\ell}$ ) was added to each bottle which was then incubated for 5 hours in a shipboard incubator through which surface lake water was circulated. Thus, algae were exposed to the temperature from which they were obtained. The bottles were mechanically rotated for mixing and illumination was done by fluorescent lights at an intensity of approximately 30 klux.

The samples were then filtered through a 0.45µ membrane filter which was stored in a desiccator until analysed. Filters were counted by liquid scinitillation in an intact state using a toluene - PPO - POPOP scintillation liquid by the method of Lind and Campbell (1969).

#### RESULTS

The results obtained from nutrient enrichment experiments are presented in Table 1. The p  $\leq$ .05 confidence level was chosen as the basis for determining whether a treatment was significantly different from controls.

In terms of P additions, few significant positive responses were observed; the results that were positive were mainly noticed during the April cruise. Inhibitory (negative) responses compared to controls were present during all cruises, but more of these occurred in the summer as compared to the spring or fall. Additions of N gave more significant positive results than P, especially in the spring, while fewer negative responses during the summer were noted with N compared to P. For the major part, most results did not significantly differ from controls. Both trace elements, Fe and Mn, gave more positive responses during the summer. Adding N + P together produced more significant positive responses during the summer. Adding N + P together produced more significant positive responses than N or P alone during the May and October cruises.

Summarizing results over the year (Table 2), most significant positive results occurred with Mn (26.1% significant positive stimulation), followed by N + P together (18.8%). The least number of significant positive results was with P alone (8.7%) which also gave the highest number of significant inhibitory responses (24.6%). No definite geographical trends were noted in terms of response except Saginaw Bay stations appeared to show fewer positive responses to Fe and Mn.

## DISCUSSION

Of special interest are the results obtained with phosphorous, an element that should limit Lake Huron phytoplankton due to its low concentration in Lake Huron. Dobson et al.(1974) reported surface soluble reactive phosphate values below 1  $\mu$ g P/ $\ell$  to occur during the year. We only obtained positive results during 8.7% of our experimental trials (Table 2). This could imply another nutrient was more limiting thus affecting uptake of P or lack of response of our bioassay technique during the short time interval utilized. However, Healey (1973) indicates such short time intervals can give positive results. Another possibility is that the phytoplankton are able to utilize the dissolved organic phosphorous fraction by alkaline phosphatase activity. Mean levels of this form of phosphorous average 1.9  $\mu$ g P/ $\ell$  in the lake with June values of 3.9  $\mu$ g P/ $\ell$  (Dobson et al., 1974)

Recently, some experimental evidence has been brought out that under severe phosphorous limitation there is a competition between a given nutrient such as P and  $\mathrm{CO}_2$  for ATP necessary for phosphorylation (Healey, 1973; Falkowski and Stone, 1975). Thus our data could also indicate that P was so limiting that P uptake took place inhibiting  $\mathrm{CO}_2$  (in this case  $\mathrm{H}^{14}\mathrm{CO}_3^-$ )uptake. Note that only 8.7% of our P additions gave a positive response to P while 24.6% gave a negative response. At the level added, there appears to be no other mechanism to explain inhibition of  $\mathrm{CO}_2$  uptake by P or N additions, i.e. no toxic mechanism appears possible at these low concentrations. More work is needed to explain the data in terms of the negative responses. Such negative

responses to phosphorous additions were also noted by Glooschenko (unpublished data) in the Gulf Stream offshore of North Carolina, an oceanic area of extremely low phosphorous concentrations. More work is needed in Lake Huron to determine the significance of this latter proposed mechanism.

Our lack of positive response to nitrogen additions except during the two spring cruises may indicate N is not limiting in the lake. Dobson et al. (1974) report  $NO_3^- + NH_4^+$  concentrations in surface waters of the lake to average approximately 180  $\mu g$  N/ $\ell$  in the summer and 270  $\mu g$  N/ $\ell$  in the winter. These concentrations should be non-limiting. The results obtained with Fe and Mn show these elements probably are limiting at times. Of particular interest is that Shapiro and Glass (1970) found manganese limitation in Lake Superior and Schelske et al. (1971) felt that trace metals could be limiting in that lake. However, since Fe-EDTA was used, the possibility exists that EDTA itself could have made other essential trace metals were available. In the case of Mn, caution is also needed as Mn has been shown to coprecipitate with  $H^{14}CO_3^-$  (Goldman, 1965).

Comparing our results with other Great Lakes nutrient enrichment experiments, Lange (1971) performed a bioassay study on Western Lake Erie water utilizing pure cultures of algae to determine growth responses. Out of 60 experiments, he found P to be limiting roughly 1/3 of the time, and N, 2/3's of the time. His P or N limitation was higher than our mean percentages of 8.7% and 16.5% respectively. But a difference in our <sup>14</sup>C uptake techniques and his laboratory culture study

utilizing algal biomass as a criteria of response can probably account for the differences seen. Schelske & Stoermer (1971) found evidence for N, P, & Si limitation in Lake Michigan while Schelske et al. (1972) found P limitations in Lake Superior.

Our results in terms of positive responses to nutrient additions indicate that none of the nutrients appears to be consistently limiting to Lake Huron phytoplankton. Any limitation appears to have a seasonal character in that positive responses occur mainly in the late spring, and in the fall, i.e. during periods of active algal growth. This was based upon highest values of algal biomass (chlorophyll  $\underline{a}$ ), not primary production rate. In Lake Huron, primary production was highest in the summer, not during periods of highest algal concentrations (Glooschenko and Moore, 1973; Glooschenko  $\underline{et}$  al., 1973). Such a seasonal response to nutrient enrichment has been demonstrated in several other studies. For example, Fournier (1966) studied a southern U.S. estuary and found positive nutrient responses to occur when cell numbers and metabolic rates were relatively higher, i.e. in the late spring. He found no response to added nutrients in the late summer. Kalff (1971) performed nutrient enrichment experiments in an Arctic tundra pond and found positive responses only during periods of high sustained algal growth similar to our results. In July, he either observed no effects or inhibitory responses to nutrient additions, and proposed that during periods of high light intensity, nutrient additions could lead to inhibition of photosynthesis. Glooschenko and Curl (1971) also found seasonal

responses to nutrient additions in the North Pacific Ocean, while Goldman (1960, 1969) observed seasonal nutrient enrichment effects in Alaskan Lakes and Lake Tahoe, California. Hence, interpretation of nutrient enrichment experiments must take into account the season during which the experiments were performed, especially the phase of the phytoplankton growth cycle.

Species composition of algae would also affect such results. Different species of algae vary in their response to nutrient concentrations as evidenced by half-saturation values of nutrient uptake (Healey, 1973; Lehman et al., 1975). In Lake Huron, diatoms make up over 80% of the algal biomass in the open waters of the lake throughout the year, while in Saginaw Bay, diatoms are predominant during the early spring and late fall, while cryptomonads dominate in the late spring and blue-green algae are present in large numbers in the summer (Vollenweider et al., 1974). In open waters, it would be impossible to generalize on the role of species composition due to lack of half-saturation constants for the dominant diatom species in the literature, and the fact that diatoms are able to adapt to lowered concentrations of phosphate or nitrate as evidenced by lower half-saturation constants (Healey, 1973). In Saginaw Bay (Stations 31 and 32), no clear response to species composition was noticed as positive responses to nutrients were found during all seasons, especially at station 31.

One other comment needs discussion, that of limiting factors.

Originally, Liebig and others developed the concept of a single limiting factor (see Hutchinson, 1973). However, in aquatic ecosystems, many

species of algae exist together at the same time, and even though a single species can have a single factor that is "most" limiting at a given time, another species can have a different limiting factor. Thus the possibility exists of multiple limiting factors (Goldman, 1972; Glooschenko and Alvis, 1973) and the authors feel the concept of a single limiting factor doesn't apply to an assemblage of phytoplankton species.

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TABLE 1

<sup>14</sup>C UPTAKE, % OF CONTROL BY TREATMENT

- \* Significant at P .05
- \*\* Significant at P ≤ .01

CRUISE DATE 19-28 April 1971			N 121 62* 112 84** 120* 131** 87* 123* 93 112*	12N + 12P 190** 72* 142* 84** 105 124** 78** 115 107 108	Fe 176** 66* 120 88* 110 124** 94 126* 105 124**	Mn 190** 77 117 84* 120* 127** 97 153** 84	
17-25 May	1 20 24 32 54 78 91 92 96	95 101 101 109 104 105 116* 100	142** 112 107 121** 106* 111 108 99 124	- 115* 107 122** 108** 124 131** 105 100	138** 123** 116* 112 103 119 122* 100 117	112** 121* 112* 97 104* 119 116* 95	
21-28 June	11 20 31 32 47 54 78 91 92	108 92 101 101 85* 96 57** 89**	121 89 100 97 108 100 58** 99	122 112 112** 105 109 98 74* 92* 100 90	118 110 106 98 99 98 73* 97 93	140* 95 104 100 96 92** 66* 95 93	
19-27 July	1 20 31 32 45 47 78 91 92	95 95 89** 87 143* 96 71** 71** 89*	107 100 102 73 107 97 80** 78** 85	103 96 93 83 93 105 73** 76** 80*	110 101 97 121 99 107 77** 82** 90	115* 108 94 100 93 106 74*8 88 89	

CRUISE DATE	Station Number	<u> </u>	<u> </u>	12N + 12P	<u>Fe</u>	Mn
23-30 August	1 15 31 32 52 78 91 92 98	89 - 86* 102 105 100 108 101 110	87 - 94 101 85 101 107 81 112 104	100 - 99 101 105 89* 114 94 110	85 - 79** 102 94 102 111 77 114 93	106 - 99 106 98 96 111 92 106 99
27 Sept 4 Oct.	9 11 31 32 52 78 88 91 92 96	79** 93 96 107* 91 91* 88* 90 115 79**	103 108 102 110** 117 97 99 90 114	98 93 90* 105 101 83* 90 116* 106 109	107 103 103 107* 123* 97 88* 105 107	- 116** 98 107* 114 93 91 115* 103
27 Oct 4 Nov.	1 11 31 42 47 78 91 92 96 98	91 102 79** 97 102 102 115* 89 95	92 98 91 108 109 115* 94 102 87**	90 121* 85** 127* 103 106 121** 124* 86**	94 130** 100 110 108 112 113* 95 83**	100 132** 97 127* 102 106 114* 105 80**

TABLE 2 - Significant (p  $\leq$  .05) positive and negative nutrient responses in Lake Huron.

CRUISE DATE		Р		N . 121		V + ½P		Fe		Mn	No
	+	-	+	-	+	<b>-</b>	+	-	4	_	Number of samples
19-28 April, 1971	2	2	4	3	3	3	4	2	4	1	
17-25 May	1	0	3	0	3	1	4	0	5		10
21-28 June	0	3	0	1	0	2	0	1	5 1	0	9
19-27 July	1	5	0	2	0	3	0	2	1	2	10
23-30 August	0	2	0	0	0	2	0		1	1	10
27 September-4 October	1	4	1	0	. 1	2	2	1	0.	0	10
27 October - 4 November	1	1	1	1	5	2	2	1	3 4	0	10
Yearly totals of % of all experiments (N=69)	8.7	24.6	16.5	10.0	18.8	20.3	17.4	11.6	26.1	7.3	



