9-1-1984

Evaluation of Nitrogen and Phosphorus Enrichment Using In Situ Enclosure Bags with Temporal Indigenous Phytoplankton Populations

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EVALUATION OF NITROGEN AND PHOSPHOROUS ENRICHMENT USING *IN SITU* ENCLOSURE BAGS WITH TEMPORAL INDIGENOUS PHYTOPLANKTON POPULATIONS

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Publication No. 110

September, 1984

Technical Completion Report Research Project G-829-04

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Prepared for
United States Department of the Interior

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The research on which this report is based was financed in part by the United States Department of the Interior as authorized by the Water Research and Development Act of 1978, (P. L. 95-467).

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A B S T R A C T

EVALUATION OF NITROGEN AND PHOSPHORUS ENRICHMENT USING *IN SITU* ENCLOSURE BAGS WITH TEMPORAL INDIGENOUS PHYTOPLANKTON POPULATIONS

An *in situ* experimental procedure and protocol was developed to evaluate nitrate and phosphate enrichment using isolated indigenous phytoplankton assemblages during different seasons. Results of the comparison of the parameters-temperature, pH, alkalinity, conductivity, and dissolved oxygen between the open water and enclosed systems indicated that there was no significant influence of the physicochemical factors on the isolated biological processes. Growth responses were measured by turbidity, biomass and chlorophyll-a, the most sensitive being chlorophyll-a. Additions of nitrate and phosphate were added in known concentrations and in different magnitudes of concentration based upon ambient conditions and ratio. During the fall, phosphorus influenced phytoplankton growth, whereas in the spring both nutrients effect growth response equally, and in the summer nitrate had the greatest influence. Based upon the results of these experiments a sampling regime for physicochemical parameters and growth response is recommended.

Richard L. Meyer and W. Reed Green

Completion Report to the U. S. Department of the Interior, Washington, D. C., September, 1984.

KEYWORDS--Nitrogen/ Phosphorus/ Enclosures/ Lakes/ Phytoplankton/ Algae/ Standing crop/ Limiting factors/ Eutrophication/ Bioassay.

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ACKNOWLEDGEMENTS

The able assistance of Mrs. Jeanette Shipley-Phillips and Mr. Lewis Smith is gratefully acknowledged.

Special thanks is given to Mr. & Mrs. Peter Harkins for the use of their property during the field phase of this research.

INTRODUCTION

The general awareness of the need for proper ecological effects monitoring to insure the survival, growth, and propagation of indigenous species and the maintenance of the diversity, productivity, and stability of the ecological systems has resulted in federal legislation. It is recognized that environmental data, however, can provide only correlative relationships; therefore, experimental manipulations are necessary for identification of cause-effect relationships.

With increasing stress from multiple sources of cultural eutrophication being subjected upon aquatic ecosystems, a critical need exists for effective experimental manipulation to assess the present and potential environmental changes resulting from the stresses. Effective predictive tools are needed to permit early detection and prediction of possible impacts so that sources of disruption can be detected.

Biological response analysis of the indigenous phytoplankton assemblage has been proposed as an indicator and evaluator of trophic status and water quality. As the base of the food-energy web of the aquatic ecosystem these organisms are usually the first to reflect the biotic and abiotic factors effecting the system. The reported research involves isolates of the indigenous phytoplankton assemblage, maintained under ambient conditions, with different modification of primary nutrients in order to analyse the response of the indigenous phytoplankton to divergent levels of nutrients. The re-

search directly addresses the cause-effect relationships associated with the addition of typical primary nutrients to the ambient phytoplankton population.

A. Purpose and Objectives

The research protocol was directed at an *in situ* approach to examine the role of nitrogen and phosphorus as limiting factors of the indigenous phytoplankton populations, the impact of various nitrogen-to-phosphorus ratios, and the magnitude of nitrogen-phosphorus concentration on the quantity and quality of the indemic phytoplanktors.

The developed protocol and experimental results provide a basis for measurement of the response of the phytoplankton assemblage present in a lake to the addition or removal of the principal nutrients effecting the growth of the total assemblage. With this type of knowledge the effects of management practices in restricting nutrients from aquatic ecosystems can be forecast. Thus this type of approach provides a "pilot" or "test-bed" mechanism prior to the application of larger scale techniques. The protocol also provides for seasonal management practices rather than the typical single modification technique approach. With the type of knowledge provided by the developed protocol the effects of management practices in restricting nutrients from aquatic ecosystems can be forecast. Based upon these estimates the appropriate water management and regulatory program can be applied.

The experimental design was developed to examine selected objectives relative to the applicability of the test procedure and to the annual successional events. The specific objectives of the research

are enumerated below:

a. To determine the influence of the enclosures and protocol on parameters which might substantiate the validity of the test procedures.

b. To determine the relationship of the quantity and quality of the enclosed phytoplankton assemblage to that of the surrounding or open water.

c. To follow the effects of additions of various concentrations and ratios of primary nutrients, nitrate and phosphate, through time, and

d. To determine the effectiveness of changes in turbidity, biomass and chlorophyll-a through time as a measure of the assemblage response to nutrient concentration.

B. Related Research or Activities

Adam (1982) reviewed and evaluated the several design and applications of enclosures for analyzing subunits of ponds, lakes and reservoirs. This review included consideration of the types of enclosures, advantages and limitations, design and monitoring. Adam notes that "...a critical need exists for effective monitoring to assess the present and potential environmental changes resulting from ... stress." In 1980 Morris in his treatise on the physiological ecology of algae stresses the importance of *in situ* studies of natural populations and assemblages. A similar request for in-lake studies is put forth by Round (1983).

As cited by Marvan, Pribil & Lhotsky (1979) and the previously cited authors, most of the previous research employing enclosures has been for determining the effects of toxins or nutrient loading on gross production or yield. The enclosures have also been of value

in determining the sinking rates of certain plankters. The behavior of phytoplankton in enclosures and the influence of nutrients limitation and recycling has been studied with the use of open bottom bags and/or bags incorporating bottom sediments (e.g., Moss, 1983).

The enclosure design used for this research is closed at the bottom to that neither nutrients nor phytoplankton or zooplankton are introduced during the experimental period. The experimental studies of Poppe, et al. (1980 & 1982), Wade, et al. (1981) and Marvan, et al. (1979) have demonstrated the effectiveness of using closed bags for examining the response of phytoplankton to nutrient limitation. Their studies, however, have focused only on the questions of nitrogen or phosphorus as the limiting nutrient during the summer maximum phytoplankton production period.

The research included within this report extends beyond the traditional question, "Is nitrogen or phosphorus the limiting nutrient?". It includes the major seasons of fall, spring and summer and analyzes physical and chemical parameters which influence seasonal succession. Also, the factors influencing maximum production during each season is ascertained by modifying nutrient concentration and ratio. Thus, this research brings together laboratory studies and field studies into an integrated design which is invaluable for water quality studies in the detection and prediction of environmental response to ecological modifiers.

METHODS AND PROCEDURES

The test site in which the research project was conducted was a spring-fed pond located approximately 0.2 km south of the junction between Washington Co. Hwy. 345 and Arkansas St. Hwy. 45. The pond is approximately 53 meters by 29 meters with the maximum dimension with a north-south orientation. The maximum depth is slightly greater than two meters. (Placke, 1979).

The experimental design was developed to analyze the effects of in situ nutrient enrichments applied to isolated assemblages of the indigenous phytoplankton. Three groups of experiments were developed consisting of eighteen individual tests plus a control. The first group of tests followed the design of the bottle algal assay developed by Miller, et al. (1978). This design was developed to determine nutrient limitation and consists of nutrient additions equaling 0.05 ug PO_4/1, 1.00 mg NO_3/1 and 1.00 mg EDTA/1, singly and in all combinations. This group of tests are identified by the prefix letter "L".

The second and third group of experiments were developed to analyze the enrichment effect of different concentrations of nitrate and phosphate. The second group of experiments addresses the influence of ratio of the primary nutrients. These treatments are referred to as the "Variable Ratio Test" and are designated by the prefix Letter "V". Additions of either nitrate or phosphate were added to achieve concentrations equaling 2x, 5x, or 10x that of the ambient concentration.

The third group of experiments or "Fixed Ratio Test" are identified by the prefix letter "F". Additions of both nitrate and phosphate were added to achieve concentrations equaling 2x, 5x or 10x that of the ambient concentrations. Also, certain aliquots were diluted with distilled water to achieve 0.75 and 0.50 final concentration to determine the effect of nutrient removal.

The enclosures in which the indigenous phytoplankton assemblages were isolated were composed of 0.9 x 0.6 m polypropylene bags (Autoclavable Sterilization Bags, Cat. No. 2200, Bellco Glass, Inc., Vineland, NJ). They were filled with 70 liters of test water and inoculated with nutrient stocks made from $NaNO_3$ and K_2HPO_4.

The bags were supported by wooden slates resting on rafts constructed of 10 cm polyvinyl chloride plastic thinwall pipe. The rafts measured 1 by 4 m and contained one randomly distributed test of each triplicate. The bags were distributed in a two by ten configuration within the raft with a 20 cm spacing between each enclosure. The aperature to each enclosure was ca. 20 x 20 cm and ca. 10 cm above the water line. The rafts were arranged in a triangle and were positioned in the center of the pond.

The tests were conducted during three seasons and lasted fourteen days; Fall, November 13-27, 1983, Spring, April 6-20, 1984, and Summer, June 7-21, 1984. The winter tests were cancelled due to extensive ice cover.

Field analysis included measurements of temperature, conductivity and dissolved oxygen on selected days. Temperature and conductivity

were determined using a YSI model 33 S-C-T meter and dissolved oxygen was determined using a YSI model 54 oxygen meter.

Water samples were returned to the laboratory for anlaysis of pH, alkalinity, turbidity, gravimetric biomass, chlorophyll concentration, and nutrient analysis. Turbidity and chlorophyll were measured daily with a Hack 2100A Analytical Nephelometer and a Bausch & Lomb Spectronic 70, respectively. Alkalinity and pH were determined electrometrically according to Standard Methods (APHA, 1975) with a Markson model 88 pH meter. Biomass was determined gravimetrically with a Mettler model H18 analytical balance. Nitrate was determined using the UV method (Standard Methods, APHA, 1975) with a Perkin-Elmer model 202 spectrophotometer. Phosphate was determined using the stannous chloride method (Standard Methods, APHA, 1975) in association with the Bausch & Lomb Spectronic 70 and 5 or 10 cm cuvettes.

PRINCIPLE FINDINGS AND SIGNIFICANCE

The enclosures represent an intrusion into the natural ecosystem and can possibly introduce an artificial environment. To test the impact of experimental design certain parameters were measured which might reflect the naturalness or artificiality of the procedures. Therefore, the parameters of temperature, pH, alkalinity, conductivity, and dissolved oxygen were measures to determine differences between the ambient environment of the pond and that of the test enclosures. Table 1 summarized the differences between the combined means of the control bags and the open water for the fourteen-day period for the fall, spring and summer experiments. It is clear from the table that

TABLE 1

DIFFERENCE BETWEEN THE COMBINED MEANS OF THE CONTROL BAGS AND THE OPEN WATER FOR CONSERVATIVE PARAMETERS FOR EACH SEASON

Period	°C	pH	Alk*	Cond*	DO_2*
Fall	+0.2	+0.12	+0.26	-1.3	+1.1
Spring	0.0	-0.05	-0.96	0.0	+0.4
Summer	+0.1	+0.10	-0.08	-2.6	+0.2

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*Alkalinity as mg/L $CaCO_3$.
Conductivity as umhos.
Dissolved oxygen as mg/L.

there is very minimal difference between the open water and that enclosed within the control bags. The temperature varied no greater than 0.2 degrees from the open water while the pH remained within 0.1 pH units. Alkalinity, which is strongly influenced by carbon dioxide uptake, was always less than 1 mg/L. Likewise there was minimal variation in conductivity and dissolved oxygen.

These data indicate that the design and construction of the rafts, placement of the enclosures and the materials from which the bags were constructed had minimal influence on the conservative parameters which may modify the phytoplankton assemblage structure. The temperature data suggest that there is little shading or trapping of insolation and that the sample waters are in near thermal equilibrium with the surrounding waters. The alkalinity and dissolved oxygen data indicate that the bags are adequately porous to carbon dioxide and oxygen so that neither of these biologically important gases become depleted and/or supersaturated. Therefore, the data indicates that the structural features of the experimental protocol have no significant influence on the test results.

The differences between the mean of the triplicates of the control and treated samples are compared in Table 2 for the fall, spring and summer series. The fall series shows no important change in either pH or dissolved oxygen. The only notable changes are in alkalinity and conductivity associated with nutrient addition or the dilution of the samples with distilled water. Both the buffering capacity as measured by alkalinity and the conductivity are reduced in a predict-

TABLE 2

DIFFERENCE BETWEEN THE COMBINED MEANS OF THE CONTROL AND TREATED SAMPLES FOR pH, ALKALINITY, CONDUCTIVITY, AND DISSOLVED OXYGEN

FALL

Test	pH	Alk*	Cond*	DO_2*
Control	7.21	40.0	59	8.6
LP	0.10	0.6	1	-0.1
LN	0.00	-0.2	8	-0.1
LPN	0.13	0.8	7	0.1
LE	0.01	0.8	0	0.1
LPE	0.08	0.6	2	0.2
LNE	0.04	0.0	6	0.1
LPNE	0.09	0.8	9	0.1
VN 2	0.07	1.4	2	-0.1
VN 5	0.02	0.0	3	-0.1
VN10	0.00	0.2	7	0.0
VP 2	0.04	0.2	0	-0.1
VP 5	0.07	0.4	2	0.0
VP10	0.10	0.4	2	-0.1
FNP 2	0.05	0.2	0	0.1
FNP 5	0.09	0.6	7	0.0
FNP10	0.10	0.6	8	0.0
FNP.75	-0.15	-8.4	-11	-0.2
FNP.50	-0.22	-19.4	-25	0.0

SPRING

Test	pH	Alk*	Cond*	DO_2*
Control	7.16	23.0	38	10.0
LP	-0.01	0.4	0	-0.1
LN	0.22	0.0	0	0.0
LPN	0.06	0.4	0	0.0
LE	0.05	0.0	0	-0.2
LPE	0.01	0.4	1	-0.1
LNE	0.03	0.4	0	-0.1
LPNE	0.04	0.4	2	0.0
VN 2	0.03	0.4	2	-0.3
VN 5	0.02	0.4	0	-0.1
VN10	0.06	0.8	0	-0.1
VP 2	0.00	0.4	1	0.1
VP 5	0.02	0.0	0	-0.2
VP10	0.00	0.0	1	-0.2
FNP 2	0.00	0.0	1	-0.2
FNP 5	0.00	0.0	1	-0.4
FNP10	-0.02	0.4	0	0.0
FNP.75	-0.01	-7.2	-9	-0.2
FNP.50	-0.22	-10.4	-18	-0.4

SUMMER

Test	pH	Alk*	Cond*	DO_2*
Control	7.38	34.0	72	8.1
LP	-0.07	-0.4	-1	0.1
LN	0.07	0.8	8	0.6
LPN	-0.03	0.0	10	1.2
LE	-0.01	0.0	0	-0.1
LPE	-0.08	-0.4	0	0.1
LNE	0.04	0.4	10	0.4
LPNE	-0.06	0.4	7	0.4
VN 2	-0.05	1.2	3	0.7
VN 5	0.09	0.0	20	0.7
VN10	-0.05	-0.4	32	1.1
VP 2	-0.13	-0.8	0	-0.1
VP 5	0.00	-0.4	0	-0.1
VP10	-0.06	0.0	-1	0.0
FNP 2	-0.11	-0.8	3	0.7
FNP 5	0.04	-0.4	18	0.6
FNP10	0.48	0.4	46	0.9
FNP.75	-0.12	-8.0	-15	0.2
FNP.50	-0.29	-16.0	-32	0.0

---

* Alkalinity as mg/L $CaCO_3$.
  Conductivity as umhos.
  Dissolved oxygen as mg/L.

able pattern by the dilutions. Similar responses are noted in the spring and summer series.

The differences in seasonal control data indicate the dynamics of the ecosystem throughout the annual cycle. The pH remains relatively stable through the year while alkalinity varies in response to the phytoplankton's demand for an inorganic carbon source. Similar variations are noted in the change in conductivity. The summer maximum in conductivity is probably related to runoff and possible increase in nutrient concentration associated with pond level reduction and lesser ground spring flow. The oxygen concentration, with a maximum in the spring, is associated with the spring maximum of phytoplankton both within the bags and in the open water of the pond.

The differences in primary nutrients, turbidity, biomass, and chlorophyll-a between the control bag and open water were compared through the entire test period (14 days). The data in Table 3 can be used to estimate the impact of the enclosures on the population. These data can be used to determine if the test populations continue to follow the dynamics of the open water or if they are candid representations of the algal populations at a point in time.

In general the nutrients within the control bags continued to decrease through time; whereas the nutrient levels in the pond vary over time. During each season the concentration of phosphate and nitrate in the open water is influenced by runoff and input by the springs. The bags, however, enclose water with the ambient concentration at day one and lack a supplemental nutrient source. Therefore,

TABLE 3

DIFFERENCES IN PHOSPHATE, NITRATE, TURBIDITY, BIOMASS,

AND CHLOROPHYLL-a BETWEEN CONTROL BAG AND OPEN WATER

THROUGH TIME DURING EACH SEASON*

		PHOSPHATE		NITRATE		TURBIDITY		BIOMASS		CHLORO-a	
FALL		OW	Dif	OW	Dif	OW	Dif	OW	Dif	OW	Dif
Day	1	24.4	7.7	0.76	0.04	5.7	0.1	2.0	-0.2		
	2					5.4	0.3	3.5	--.2	1.7	1.5
	3					5.2	0.2	2.3	-0.3		
	5					5.0	0.4	2.0	1.2	1.9	0.9
	7	10.4	6.2	0.83	-0.02	4.7	0.6			2.1	3.7
	8					4.6	0.4	2.0	2.5	2.5	4.7
	10					4.5	0.5			1.6	3.0
	12					6.5	-1.6	1.7	1.1		
	13							0.5	3.0	1.1	2.5
	14	26.3	-9.4	0.89	-0.19	5.9	-1.3	1.0	2.3		
SPRING											
Day	1	18.1	-5.9	0.30	0.04	6.1	0.0	3.8	-0.5		
	2									1.5	1.1
	4					6.7	-1.0	2.7	1.5		
	5									1.6	0.8
	7	20.7	-11.3	0.42	-0.14	6.0	-1.5	5.8	-0.8		
	8									2.9	1.2
	10									2.4	2.1
	11	7.8	-0.9	0.48	-0.14	5.7	-3.1	2.7	0.0		
	13									1.9	0.9
	14	8.2	-3.5	0.41	-0.19	5.6	-3.2	2.3	0.2		
SUMMER											
Day	1	8.7	-1.0	0.38	-0.06	7.6	-2.2	11.8	1.4		
	2									27.8	-3.5
	4	12.8	-7.4	0.41	0.94	4.6	-1.5	9.2	-1.9		
	5									10.8	-5.4
	7	10.9	-5.8	0.68	-0.94	5.9	-4.6	5.5	-2.0		
	8									16.4	-8.3
	10									13.8	-10.4
	11	32.0	-24.5	0.53	-0.13	8.5	-7.8	5.0	-2.7		
	13									16.6	-14.4
	14	20.5	-14.6	0.54	-0.08	9.4	-8.3	9.7	-8.0		

---

*Phosphate as ug/L. Nitrate as mg/L. Turbidity as NTU's.
Biomass as mg/L. Chlorophyll-a as ug/L.
"OW" equals "open water". "Dif" equals the difference between the control and the open water.

the differences between the open water and enclosures tends to increase over time.

A similar pattern of increasing divergence over time is noted in the data for turbidity, biomass and chlorophyll-a. The differences between the open water and controls on day one is slight and tends to increase in magnitude by day fourteen. These differences are probably influenced by the inhibition of nutrient input entering the isolates in the control bag. The bags contain a finite quantity of nutrients while the open water can be replenished from several sources. Parallel tracking of the populations continues for several days, ca. seven, after which the differences increase. This type of response can be measured by turbidity, biomass or chlorophyll-a.

Although each of the above cited parameters can be used to measure the changes in population density, chlorophyll-a appears to be the most sensitive. For example, the population isolated during the summer was in an intrinsic decline at the time of enclosure. This decline was captured and isolated within the control enclosure. As the chlorophyll-a concentration indicates the crash continues. In the open water, however, the decline extends until the middle of the experimental period and then rises again. The decline is not nutrient related because of the continuous increase of both nitrate and phosphates within the pond through time. The differences in the chlorophyll-a between the control and open water/through time results in a straight line projection with $r = 0.99$. This correlation suggests that the intrinsic decline is trapped and continues within the control. The correlation

between the turbidity or biomass data is less definitive and it is, therefore, recommended that chlorophyll-a be the measure of choice.

The preceeding data clearly indicates that the experimental design and construction of the test rafts has reduced the "bag effect" to a minimum. The "bag effect" associated with this research is limited to the capture of an isolate of the naturally occurring dynamic phytoplankton populations. That is, the test enclosures capture the phytoplankton as it is undergoing annual succession at a point in time. The enclosures only mimic the open water for a short period of time after which nutrient limitation restricts growth. This entrapment of a selected population and/or nutrient condition has the advantage of being able to determine the effects of certain manipulations on the conditions of interest.

The data reported in the following series of tables and with the associated discussions are related to the specific treatment of the enclosed water samples. The data reported is the mean value of the triplicates of the samples randomly distributed within each raft. The changes in nutrient concentrations followed by measures of phytoplankton assemblage response will be reported separately.

The mean concentration of nitrate and phosphate were measured at selected intervals during each of the three seasonal series. During the fall (Table 4) the nitrate concentrations remained relatively stable through time for each of the nutrient additions. The phosphate concentrations dropped rapidly suggesting that phosphate was rapidly utilized by the fall population. The most dramatic decreases were

TABLE 4

MEAN CONCENTRATION OF NITRATE AND PHOSPHATE

BY TREATMENT THROUGH TIME

	NITRATE*					PHOSPHATE*				
FALL Day	1	4	7	11	14	1	4	7	11	14
Control	0.7		0.8		0.7	32		17		17
LP	0.8		0.9		0.7	1085		1476		871
LN	1.4		2.0		1.5	31		17		8
LPN	1.5		1.9		1.4	1290		1092		1217
LE	0.7		0.9		0.7	31		18		10
LPE	0.7		0.8		0.6	1808		2414		1634
LNE	1.4		1.8		1.4	31		17		12
LPNE	1.5		2.0		1.6	1221		1399		1495
VN 2	0.9		1.0		0.8	29		10		15
VN 5	1.3		1.6		1.3	25		22		18
VN 10	2.0		2.1		1.7	25		11		15
VP 2	0.8		0.9		0.7	109		75		18
VP 5	0.7		0.9		0.6	636		420		239
VP 10	0.8		0.9		0.6	1336		1315		734
FNP 2	0.9		1.0		0.7	130		53		12
FNP 5	1.3		1.6		1.2	545		63		443
FNP 10	2.0		2.0		1.6	855		1036		789
FNP.75	0.6		0.8		0.6	29		22		15
FNP.50	0.5		0.5		0.5	17		8		12
Pond	0.7		0.8		0.9	24		10		26

SPRING Day	1	4	7	11	14	1	4	7	11	14
Control	0.3		0.3	0.3	0.2	12		9	7	5
LP	0.3		0.4	0.4	0.2	185		89	57	61
LN	0.4		0.4	0.4	0.2	27		21	4	4
LPN	0.4		0.4	0.4	0.2	325		107	62	76
LE	0.3		0.3	0.3	0.2	13		10	6	2
LPE	0.2		0.4	0.3	0.1	321		87	47	47
LNE	0.3		0.4	0.4	0.2	12		10	2	3
LPNE	0.4		0.4	0.3	0.2	200		80	50	43
VN 2	0.4		0.4	0.3	0.2	28		16	7	7
VN 5	0.4		0.4	0.3	0.2	30		12	7	3
VN 10	0.4		0.3	0.3	0.2	34		10	4	6
VP 2	0.3		0.3	0.3	0.2	98		40	17	20
VP 5	0.3		0.3	0.3	0.2	136		56	30	28
VP 10	0.3		0.4	0.3	0.2	408		200	151	110
FNP 2	0.3		0.4	0.3	0.3	90		19	7	6
FNP 5	0.4		0.4	0.3	0.2	128		40	27	25
FNP 10	0.4		0.4	0.3	0.2	389		142	126	101
FNP.75	0.2		0.2	0.2	0.1	14		12	3	8
FNP.50	0.1		0.2	0.2	0.1	18		11	4	4
Pond	0.3		0.4	0.5	0.4	18		21	8	8

SUMMER Day	1	4	7	11	14	1	4	7	11	14
Control	0.3	0.5	0.6	0.4	0.5	9	5	5	8	6
LP	0.3	0.4	0.5	0.3	0.4	12	14	5	6	7
LN	1.1	1.4	1.3	1.0	0.9	13	16	5	5	7
LPN	1.3	1.3	1.2	0.6	0.5	22	10	5	8	5
LE	0.3	0.4	0.6	0.4	0.5	10	6	7	8	5
LPE	0.3	0.4	0.5	0.3	0.4	10	11	5	6	8
LNE	1.2	2.0	1.5	1.0	0.8	9	7	3	5	4
LPNE	1.2	1.2	1.2	0.7	0.5	6	6	3	5	6
VN 2	0.9	0.9	0.8	0.4	0.4	10	11	5	7	4
VN 5	1.8	2.6	2.6	2.1	1.7	17	8	5	6	11
VN 10	2.6	4.4	4.7	4.2	3.9	12	11	2	7	6
VP 2	0.3	0.4	0.6	0.5	0.5	14	12	3	5	11
VP 5	0.3	0.5	0.5	0.3	0.4	14	11	7	7	6
VP 10	0.3	0.4	0.5	0.4	0.4	58	43	18	7	5
FNP 2	0.9	1.0	0.9	0.5	0.5	7	8	5	5	4
FNP 5	1.8	2.3	2.5	1.8	1.9	15	7	5	8	5
FNP 10	1.9	5.7	5.6	5.0	4.7	106	9	4	6	9
FNP.75	0.2	0.4	0.4	0.2	0.3	6	6	4	3	4
FNP.50	0.1	0.2	0.3	0.2	0.3	5	11	7	4	4
Pond	0.4	0.4	0.7	0.5	0.5	9	13	11	32	3

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*Nitrate as mg/L.
Phosphate as ug/L.

associated with the singular addition of phosphate in the "L" and "V" series.

The nitrate concentrations during the spring series only disclosed a slight reduction during the fourteen-day period. This pattern seemed to be consistent for each of the treatments. The phosphate concentrations, however, declined rapidly from day one through day eleven with a less abrupt decline through day fourteen. The nitrate concentrations during the summer series increased slightly during the middle of the test period and then declined to near initial levels by day fourteen. In contrast, the phosphate concentrations declined rapidly by the middle of the experiment and either remained at the minimal level or slightly increased. The rate of decrease during the summer was greater than that of the spring.

These data indicate that during the fall series the nutrients probably had a minimal effect on the population. Parameters other than nitrate and phosphate controlled production, for example, the physical parameters of light and/or temperature. During the spring and summer nitrate was initially taken up as was phosphate. This initial uptake suggests that the existing assemblage utilized these nutrients. The increase in nitrate, and in some instances phosphate, during the latter half of the experimental period suggests a change in the composition of the assemblage. Further interpretation of these data must await enumeration of the phytoplankton samples.

Three measurements, turbidity, biomass and chlorophyll-a, were obtained to measure the biological response to the nutrient additions.

These three parameters were chosen as procedures which are generally available. The water at the test site contained no detectable silt or other matter which was not of phytoplankton or zooplankton origin. The latter organisms are easily excluded from the measurements and therefore the NTU readings primarily reflected phytoplankton. Similiarly, the gravimeterically determined biomass was minimally effected by non-phytoplankters. The chlorophyll-a data is independent of debris and extraneous zooplanktors. It, therefore, represents the measuring instrument with the minimum interference.

At the time of isolation the phytoplankton assemblage became removed from the naturally occurring nutrient cycles and input sources which drive the ecosystem. Thus, the control bags simulate the ecosystem at the point in time at which isolation occurred and the treated tests simulate the ecosystem if the given nutrient enrichments were applied. The control enclosure population is isolated from additional inputs and should contain a depressed population after the nutrients have been utilized. The turbidity data for the control enclosures of the fall, spring and summer test series show this expected response (Table 5). The turbidity data also reflects the impact of dilution as reflected in the "FNP.75" and "FNP.50" values for the three series.

In general, the chloropyll-a and biomass data follow the same trends. The turbidity values, however, tend to be less sensitive in that greater changes in population density must occur before they are detectable by this method. This method appears to be influenced by cell size. During the summer series the turbidity and, for comparison,

TABLE 5

MEASUREMENT OF RESPONSE TO TREATMENT VIA TURBIDITY*

FALL	Day	1	4	7	11	14
	CONTROL	5.8	5.4	5.0	4.9	4.6
	LP	5.8	5.4	5.2	4.9	4.2
	LN	5.8	5.4	5.0	4.8	4.4
	LPN	5.8	5.6	5.3	4.8	3.8
	LE	5.7	5.4	5.0	4.8	4.6
	LPE	5.7	5.6	5.4	4.9	4.2
	LNE	5.7	5.4	5.1	4.8	4.3
	LPNE	5.8	5.5	5.3	4.9	4.2
	VN 2	5.8	5.6	5.2	5.1	4.8
	VN 5	5.8	5.4	5.1	4.9	4.5
	VN 10	5.8	5.4	5.1	4.9	4.6
	VP 2	5.8	5.4	5.2	4.8	4.1
	VP 5	5.8	5.5	5.1	4.7	3.9
	VP 10	5.8	5.5	5.3	4.9	4.1
	FNP 2	5.8	5.5	5.1	4.7	3.9
	FNP 5	5.7	5.5	5.3	4.9	4.1
	FNP 10	5.8	5.5	5.3	4.8	4.0
	FNP.75	4.4	4.5	4.3	4.0	3.9
	FNP.50	2.9	2.7	2.7	2.7	2.5
	Pond	5.7	5.2	4.6	6.5	5.9

SPRING	Day	1	4	7	11	14
	Control	6.1	5.7	4.5	2.6	2.4
	LP	6.1	5.6	4.5	2.5	2.0
	LN	6.2	5.6	4.3	2.3	2.0
	LPN	6.3	5.6	4.5	2.5	1.8
	LE	6.2	5.6	4.6	2.5	2.0
	LPE	6.2	5.7	4.5	2.6	2.1
	LNE	6.1	5.6	4.4	2.5	2.1
	LPNE	6.2	5.8	4.5	2.6	2.2
	VN 2	6.3	5.8	4.4	2.6	2.3
	VN 5	6.1	5.7	4.6	2.7	2.3
	VN 10	6.2	5.8	4.4	2.2	2.0
	VP 2	6.4	5.9	4.5	2.5	1.9
	VP 5	6.1	5.6	4.5	2.6	2.1
	VP 10	6.1	5.6	4.4	2.4	1.9
	FNP 2	6.2	5.8	4.5	2.4	1.8
	FNP 5	6.1	5.8	4.5	2.4	1.8
	FNP 10	6.1	5.6	4.5	2.3	1.7
	FNP.75	4.6	4.5	3.8	2.4	2.1
	FNP.50	3.1	3.1	3.0	2.3	2.0
	Pond	6.1	6.7	6.0	5.7	5.6

SUMMER	Day	1.	4	7	11	14
	Control	5.4	3.1	1.3	0.7	1.1
	LP	5.2	2.9	1.2	0.6	0.8
	LN	5.5	3.1	1.5	1.0	1.1
	LPN	5.5	3.0	1.3	1.1	1.3
	LE	5.6	2.9	1.1	0.8	1.0
	LPE	5.6	2.7	1.0	0.6	0.9
	LNE	5.4	2.6	1.0	0.9	0.9
	LPNE	5.5	2.7	1.2	1.1	1.2
	VN 2	5.4	2.8	1.2	1.0	1.2
	VN 5	5.3	2.6	0.9	0.9	1.1
	VN 10	5.7	2.6	1.4	1.1	1.2
	VP 2	5.4	2.8	1.4	0.7	0.9
	VP 5	5.6	2.8	1.0	0.7	0.7
	VP 10	5.5	2.9	1.0	0.7	0.7
	FNP 2	5.4	3.1	1.8	0.9	1.1
	FNP 5	5.4	2.7	1.0	1.0	1.3
	FNP 10	5.2	2.4	0.9	1.1	1.0
	FNP.75	4.7	2.6	1.0	0.7	0.2
	FNP.50	4.1	2.3	0.8	0.6	0.7
	Pond	7.6	4.6	5.9	8.5	9.4

*Turbidity as NTU's.

chlorophyll-a readings were elevated because the population consisted mainly of large dinoflagellates. On day eight, however, the turbidity readings were low but the chlorophyll was relatively high, as were the biomass readings. This difference is due to the composition of the phytoplankton population which consisted of numerous small coccoid green algae. Similar examples can be chosen from any of the treatments and from the three test series. On the basis of the experimental data the use of turbidity data must be used with caution not only in waters with extraneous suspended debris but in systems with low biomass or marked differences in plankton size.

The biomass data shown in Table 6 represents the dry weight of suspended materials in the test enclosures through time for each treatment as well as the pond. During the fall and spring series the biomass peaked on the seventh day and declined thereafter. The summer series followed a pattern of general decline from day one through day fourteen with little difference between days eleven and fourteen.

The additions of phosphate in the "L" and "VP" series of the fall tests suggest that this nutrient results in the increase of biomass through day seven. Addition of nitrate, either singularly or in combination ("L", "VN", & "F" series) has little effect on the mass of the population. The spring tests are less definitive as to the importance of nitrate or phosphate. The addition on the two nutrients seem to produce similar results. Only a slight increase in biomass is noted when these nutrients are used in combination (cf. "FNP" series). Data from day seven of the summer experiment indicate that phosphate

TABLE 6

MEASUREMENT OF RESPONSE TO TREATMENT VIA BIOMASS*

FALL	Day	1	4	7	11	14
	Control	1.8	2.0	4.5	2.8	3.3
	LP	0.8	2.2	5.2	3.5	3.5
	LN	1.7	2.5	3.8	3.7	3.3
	LPN	1.0	2.7	7.0	4.7	4.5
	LE	1.3	2.5	4.2	3.2	3.3
	LPE	0.5	2.3	6.7	4.8	3.8
	LNE	0.8	2.7	4.2	4.5	4.7
	LPNE	1.0	1.7	6.0	4.2	3.5
	VN 2	0.5	4.2	4.8	4.3	4.3
	VN 5	1.3	2.3	4.3	3.7	3.3
	VN 10	2.0	2.5	4.3	4.7	5.3
	VP 2	0.8	2.7	6.8	3.7	2.7
	VP 5	1.2	2.0	6.3	5.0	3.7
	VP 10	0.8	2.5	3.2	3.0	2.8
	FNP 2	1.2	2.7	5.2	4.7	4.2
	FNP 5	1.0	1.7	5.2	4.3	3.8
	FNP 10	1.0	2.0	5.2	3.7	3.8
	FNP.75	1.5	1.5	3.3	3.0	2.3
	FNP.50	0.0	0.8	2.7	1.2	1.5
	Pond	2.0	2.3	2.0	1.7	1.0

SPRING	Day	1	4	7	11	14
	Control	3.3	4.2	5.0	2.7	2.5
	LP	2.7	4.0	6.2	3.7	2.0
	LN	2.8	3.5	6.0	3.2	3.2
	LPN	2.8	3.3	6.5	4.2	2.0
	LE	2.7	2.8	5.3	2.7	2.0
	LPE	3.2	3.5	6.2	4.7	2.7
	LNE	2.5	3.2	5.3	2.8	2.2
	LPNE	3.7	3.2	6.2	3.8	3.5
	VN 2	2.2	4.5	5.3	3.2	1.8
	VN 5	2.8	3.2	5.5	2.8	3.0
	VN 10	2.2	3.2	6.8	4.2	2.8
	VP 2	2.7	4.5	6.3	3.3	1.8
	VP 5	2.2	3.8	6.0	3.8	2.2
	VP 10	3.0	2.7	5.8	3.5	2.3
	FNP 2	2.5	3.8	7.0	3.8	2.8
	FNP 5	2.7	3.3	6.8	4.0	2.2
	FNP 10	2.8	4.2	7.2	4.0	2.7
	FNP.75	2.5	2.3	4.0	2.7	1.8
	FNP.50	2.3	1.7	4.9	2.3	2.7
	Pond	3.8	2.7	5.8	2.7	2.3

SUMMER	Day	1	4	7	11	14
	Control	13.2	7.3	3.5	2.3	1.7
	LP	13.8	6.5	4.3	1.5	1.5
	LN	15.5	9.2	2.8	2.3	2.2
	LPN	11.3	7.2	3.2	2.2	1.5
	LE	11.5	6.5	2.8	2.5	2.5
	LPE	14.0	6.2	3.2	2.0	1.7
	LNE	11.2	7.7	2.5	2.3	1.8
	LPNE	10.8	5.8	2.3	2.3	1.3
	VN 2	9.2	7.7	3.0	1.5	1.3
	VN 5	13.0	8.5	3.7	3.3	2.5
	VN 10	17.3	8.7	4.8	3.3	2.5
	VP 2	12.0	7.3	3.8	2.3	1.8
	VP 5	11.8	8.0	3.2	2.7	1.8
	VP 10	9.8	7.0	3.7	2.2	2.0
	FNP 2	12.2	8.7	7.8	2.7	1.3
	FNP 5	10.8	9.2	3.3	3.2	2.8
	FNP 10	14.3	8.5	3.8	4.5	2.2
	FNP.75	9.2	3.5	2.3	1.3	1.3
	FNP.50	10.3	4.3	2.8	2.5	1.3
	Pond	11.8	9.2	5.5	5.0	9.7

*Biomass as mg/L dry wt.

has little effect on total production but that nitrate has the greatest impact. A comparison of "VP" and "VN" shows that increasing additions of phosphate resulted in insignificant change in biomass while the addition of nitrate resulted in increased biomass. The addition of combinations of nutrients produced ambiguous results which may reflect a marked change in the species present in the plankton. The analysis and interpretation of these data await the enumeration of the phytoplankton.

The biomass data can be influenced by cellular remains, e.g., empty cell walls, spores, debris, etc. Therefore, the use of this type of data must be used with caution. It is imperative that knowledge of the structure of the phytoplankton community and changes in the assemblage be known if proper interpretation of the data is to occur. As with the turbidity, the data can be biased by the presence of silt and debris from an external origin.

The concentration of chlorophyll-a in response to the several treatments is reported in Table 7. The use of chlorophyll-a as a measurement of phytoplankton response has the advantage of being a simple procedure which is free of interferences from silt and debris. Because of the lack of interferences present in the other two measuring procedures, chlorophyll-a is afforded the greatest importance.

During each of the seasonal experiments the eighth day was the time at which the chlorophyll-a was at its maximum. Following the mid-test maximum there was a general decline in concentration with few exceptions. The eight day data, therefore, will be used for com-

TABLE 7

MEASUREMENT OF RESPONSE TO TREATMENT VIA CHLOROPHYLL-a*

FALL	Day	2	5	8	10	13
	Control	3.2	2.9	7.2	4.6	3.6
	LP	3.3	4.5	11.5	7.9	4.5
	LN	2.2	3.5	7.9	5.0	3.4
	LPN	2.7	5.1	15.3	7.3	5.8
	LE	2.4	3.1	7.3	6.1	4.3
	LPE	3.0	4.9	14.3	8.6	6.2
	LNE	2.2	4.5	8.8	6.1	6.5
	LPNE	2.6	4.3	14.3	8.8	5.0
	VN 2	3.1	2.9	9.8	5.3	5.1
	VN 5	2.8	3.6	7.5	5.1	3.6
	VN 10	2.9	2.8	8.1	5.6	5.1
	VP 2	2.3	2.8	13.0	8.6	6.8
	VP 5	3.6	5.2	15.0	7.5	5.2
	VP 10	2.6	5.3	9.9	5.5	3.0
	FNP 2	2.9	4.8	13.7	8.2	5.5
	FNP 5	2.7	5.4	17.3	7.0	4.9
	FNP 10	2.9	4.2	10.8	7.5	4.9
	FNP.75	2.3	2.5	4.8	2.9	3.3
	FNP.50	1.7	2.3	4.4	2.6	3.7
	Pond	1.7	1.9	2.5	1.6	1.1

SPRING	Day	2	5	8	10	13
	Control	2.6	2.4	4.1	4.5	2.8
	LP	2.5	4.4	8.0	3.7	2.3
	LN	2.8	4.4	8.0	6.3	2.6
	LPN	2.7	4.2	8.7	6.0	2.0
	LE	2.8	2.9	2.7	3.5	2.1
	LPE	2.3	3.9	3.8	3.1	2.2
	LNE	2.5	2.9	3.2	4.4	2.7
	LPNE	2.9	4.4	7.5	7.4	2.2
	VN 2	2.9	4.6	4.3	4.7	4.0
	VN 5	2.8	2.5	3.5	4.7	2.6
	VN 10	2.6	3.7	5.2	5.3	3.2
	VP 2	2.7	4.7	5.0	3.7	2.2
	VP 5	3.0	4.0	4.0	3.4	2.0
	VP 10	2.3	4.4	3.7	2.8	2.0
	FNP 2	2.5	4.9	3.9	3.3	2.3
	FNP 5	2.5	4.2	6.0	3.7	2.1
	FNP 10	2.9	4.5	5.9	5.2	2.4
	FNP.75	1.5	2.2	2.8	2.7	2.0
	FNP.50	1.1	1.6	2.9	2.4	3.4
	Pond	1.5	2.3	2.2	2.4	1.9

SUMMER	Day	2	5	8	10	13
	Control	24.3	5.4	8.1	3.5	2.3
	LP	33.6	5.8	9.0	3.6	2.8
	LN	40.0	12.8	14.3	6.0	4.0
	LPN	35.7	16.4	16.6	8.8	4.6
	LE	31.8	5.7	7.3	3.8	3.4
	LPE	21.5	6.5	8.6	3.1	3.0
	LNE	31.9	8.4	14.4	7.8	4.2
	LPNE	39.6	11.3	17.0	7.5	3.5
	VN 2	39.7	11.3	16.6	8.8	4.6
	VN 5	46.9	10.5	17.8	9.3	4.7
	VN 10	37.5	14.0	13.9	8.1	5.3
	VP 2	29.0	7.3	9.5	4.3	3.4
	VP 5	34.4	7.6	13.4	5.8	4.9
	VP 10	28.8	4.7	7.8	3.7	2.9
	FNP 2	36.4	16.3	13.1	5.5	4.0
	FNP 5	44.8	12.8	19.0	9.0	4.7
	FNP 10	37.9	12.8	27.0	15.6	9.7
	FNP.75	20.0	5.7	6.8	3.6	2.7
	FNP.50	27.7	5.6	9.4	4.0	3.0
	Pond	27.8	10.8	16.4	13.8	16.6

*Chlorophyll-a as ug/L.

parison of the impact of enrichments.

The "L", "V" and "F" series of treatments containing phosphate additions during the fall experiment consistently resulted in greater concentrations of chlorophyll. At the highest concentrations, "VP 10" and "FNP 10",the concentration of phosphate may be great enough to depress cell growth. The addition of nitrate alone results in minor variation from the control. The combination of nitrate with phosphate results in an increased concentration greater than phosphate alone. This increase in response suggest a synergistic interaction of these nutrients.

The treatment series in the spring indicates that both nitrate and phosphate are equally important in influencing the production of phytoplankton. The "L" series data are nearly equivalent for single additions of the nutrients with only a slight increase when added in combination. Similar results are noted when the "VN" and "VP" series are compared. Slight increases in concentration are recorded for the increasing "FNP" series at the two and five times ambient concentration. The effect of nutrient reduction is clearly reflected in the reduced concentrations in the "FNP.75" and "FNP.50" tests.

During the summer treatment series there seems to be a direct relationship between growth response and nitrate additions. High concentrations of chlorophyll-a were measured on day one but because of the intrinsic population decline these declined rapidly. (Note the decline in the chlorophyll-a concentration in the pond from 27.8 ug/L on day one to only 10.8 ug/L on day five and subsequent recovery. The

isolated populations in the control enclosure extended the initial decline throughout the experimental period.) The rate of the rapid decline was moderated by the addition of nitrate alone or in combination with phosphate. The "L", "VN" and "FNP" containing nitrate each showed a reduced rate of decline. The "L" tests clearly indicated that nitrate is the limiting nutrient. The addition of 1.0 mg/L NO_3, alone or in combination with EDTA, produces an increase of 6.2 and 6.3 ug/L, respectively, over the control.

In contrast, the addition of phosphate had little or no effect. The addition of 1.0 mg/L PO_4 alone or in combination with EDTA, results in increased concentration of only 0.9 and 0.5 ug/L, respectively, over the control. Additions of both nitrate and phosphate produce greater differences than nitrate alone (8.5 and 8.9 ug/L) suggests that these nutrients might have a slight synergistic effect. They may, however, influence the development of a modified assemblage of plankters.

The two series of tests based upon the ambient condition ("V" & "F") demonstrate the same responses. The "VN" series each show increased response over the control while the "VP" series produces little, if any, increase above the control. The greatest response is in the fixed ratio test ("FNPx"). The addition of both nutrients increases the growth greater than single additions of nitrate, except for FNP-2. Again, nitrate appears to limit population growth but is synergistically influenced by phosphate. The FNP-2, FNP-5 and FNP-10 upon comparison of their concentration and that of the control have a correlation of $r = 0.93$.

The synergistic interaction of nitrate and phosphate can be implied from the summer chlorophyll-a data. Twice the concentration of nitrate produces significant growth (16.6 ug/L) while five times concentration results in even greater growth (17.3 ug/L). But at ten times ambient concentration (13.9 ug/L), growth is less than the five times concentration. The multiple additions of phosphate do not result in a significant change in growth. Only a slight increase is noted when twice the concentration of both nutrients are added. At five times addition of both nutrients the chlorophyll-a concentration (19.0 ug/L) is nearly equal to the five times addition of nitrate alone. However, at ten times the enrichment of both nutrients the chlorophyll-a concentration has increased to 27.0 ug/L. Therefore, with concentration of nitrate being greater and five times greater than the ambient, phosphate becomes limiting and any addition of phosphate will produce greater growth. From these data it can be estimated that the addition of phosphate can be added to the system with no significant change in growth. But if nitrate is added, significant growth would occur up to a concentration of approximately five times greater than ambient conditions. Additions greater than five times would have an inhibitory effect. However, if both nitrate and phosphate are added together growth would continue with a linear increase.

Three measures of growth response were chosen to track the impact of additions of single and combined primary nutrients. The data and discussions presented above clearly show that each of the measuring tools can be used to determine the response of the phytoplankton populaltions to enrichment. The data also show that the applicatication of

turbidity to measure growth responses is less sensititve than either biomass or chlorophyll-a and is subject to certain errors. Biomass is more sensitive to changes in growth than turbidity but it is subject to the same errors as turbidity. Clearly the most sensitive measuring tool with the minimum of interferences is analysis of chlorophyll-a. Because of its great sensitivity and minimal interferences chlorophyll-a is the method of choice.

CONCLUSIONS

1. The experimental design and test protocol have demonstrated that the use of in situ enclosures captures a phytoplankton assemblage at a point-in-time during the annual cycle.

2. The design permits the application of laboratory bench experiments on indemic phytoplankton assemblages. The field experiments contain controls and replicates (3) for adequate quality assurance.

Based upon the results of the fourteen day test period, it is recommended that samples for chemical parameters be collected of 1, 4, 7, 11, and 14 and that growth measurement samples be collected daily.

3. The test frame design and construction allows the enclosed waters to closely mimic the ambient temperature, pH, alkalinity, and dissolved oxygen. The data indicate that thermal shock is absent and that the primary biological gases adequately diffuse through the enclosure membrane to avoid stress. The design, therefore, eliminated certain of the problems which have limited the application of the in situ technique.

4. The test protocol examined the influence of the addition of primary nutrients, phosphate and nitrate, singly and in combination.

Single additions of nitrate and phosphate, with and without EDTA, were used to determine the limiting nutrient and the presence of heavy metal inhibition. Variable and fixed concentrations were used to determine the importance of N:P ratios and absolute concentration, including dilution.

The data derived from these experiments indicates that phosphorus was the limiting nutrient during the fall diatom dominated population while both nutrients were equally influential during the mixed spring assemblage and nitrogen was of greater influence during the summer. The impact of these treatments on the quality of the assemblage awaits enumeration of the phytoplankton samples.

5. The research protocol used three measurements of growth response: turbidity, biomass and chlorophyll-a. Turbidity was the least sensitive measure of response and can be influenced by extrensic factors. Biomass is intermediate in sensitivity but also may be influenced by the same extrensic factors as turbidity. Chlorophyll-a is the most sensitive measure and with the minimum of interferences. This latter measure is, therefore, recommended as the measurement of choice.

LITERATURE CITED

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