BRITISH COLUMBIA

MINISTRY OF THE ENVIRONMENT

AN INEXPENSIVE IN SITU ALGAL BIOASSAY PROCEDURE WITH SOME PRELIMINARY RESULTS BEARING ON NUTRIENT LIMITATIONS IN SKAHA LAKE

WATER INVESTIGATIONS BRANCH

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SUMMARY

An algal bioassay procedure is described which appears to be useful for in situ evaluation of nutrient effects on lake phytoplankton. The apparatus consists of a set of large (22 litre) glass bottles which are set in a flotation rack in the lake and filled with lake water and phytoplankton. Monitoring of the rates of growth under chosen conditions can give some indication of the effects of the lake phytoplankton in general. The apparatus is relatively inexpensive and has a number of advantages over the standard laboratory bottle bioassays. As a result of experiments carried out in Skaha Lake, it appears that the lake phytoplankton, which in 1971 were limited by the quantity of nitrogen available for growth, is now limited by phosphorus.

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Acknowledgements

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1. Introduction

1.1 Background

A number of situations involving watershed management or wastewater discharge management require identification of limitation factors for phytoplankton growth.

At Penticton, the Pollution Control Branch was involved in assessment of best means of disposal and treatment of effluent from the Penticion sewage treatment plant to Skaha Lake. The Penticton sewage treatment plant began operation in 1948 and by the late 1960's increases in population had made the treatment plant the major contributor of phosphorus to Skaha Lake. Deteriorating water quality (algal blooms, decreased water clarity) resulted in public pressure for government action. The federal and provincial governments began a joint study of water resources of the Okanagan Basin. The Okanagan Basin Study (Technical Supplement IV) estimated that the city discharge in the 1969-1971 period accounted for 28 and 60 percent of the total input of nitrogen and phosphorus respectively into Skaha Lake. A major recommendation of the Okanagan Basin Study was that a reduction of phosphorus loadings to the main valley lakes be undertaken. In 1971 the City of Penticton completed a tertiary treatment unit for the removal of phosphorus and the efficiency 1972-1973 was estimated at 50-60% (Okanagan Basin Study, Technical Supplement IV).

In the recent few years, the Pollution Control Branch has been involved in monitoring the plant, assessing the efficiency of the treatment (Wetter, 1978) and considering the most appropriate type of treatment when Penticton applied to increase its discharge due to increasing population. Part of the problem involved controversy over whether phosphorus or nitrogen were the limiting nutrient. A part of the lake biology investigations carried out during the Basin Study in 1971 were algal bioassays, the results of which indicated that in Skaha Lake, phytoplankton was limited by the availability of nitrogen. The decision to remove phosphorus was based on the strategy that by removing phosphorus, it could be made to be the limiting nutrient. Little information was available to deter-

mine whether this policy has succeeded in either reducing the productivity of Skaha Lake or making the algal production phosphorus dependent.

1.2 Objectives

The Water Investigations Branch was asked to give some suggestions on determining nutrient limitation in Skaha Lake. Use of an algal bioassay was suggested since the most appropriate methods of sewage treatment and disposal would depend on whether or not the removal of phosphorus had succeeded in making the lake phosphorus limited.

1.3 Methodology

A great deal of literature exists on nutrient bioassays using algae. Basically algal bioassays fall into two categories. The first type, and the most generally used, is a bioassay using pure cultures with tests being carried out under laboratory conditions. The most well known of this type is the Algal Assay Procedure Bottle Test designed by the Environmental Protection Agency (E.P.A. 1971). The other approach to bioassays is to conduct *in situ* experiments. A great deal of variation exists in this type of approach, from isolating large columns of water from the general water mass as used in the CEPEX experiements, (Grice *et al*. 1977) to the much smaller scale isolations used in the experiments described below. The *in situ* methodology has a number of inherent advantages over the laboratory method. The laboratory technique allows much more control over physical growth parameters (light and temperature) but although they are under much better control, they normally differ from the field environmental conditions. The laboratory method also uses algal cultures of a single species, normally free of bacteria to maintain strict control. The *in situ* bioassay used the natural phytoplankton population and as the experiment is designed to test an effect on a lake response, the use of the natural phytoplankton seems more appropriate especially when the experiment is carried out in the lake where the light and temperature regimes used are the normal regimes to which the lake phytoplankton are exposed.

1.3.1 Apparatus

The apparatus shown in figures 1 and 2 was used in the bioassay conducted in Skaha Lake. It consists of a metal framework holding five 22 litre glass carboys. The apparatus is floated and stabilized by plastic foam at the four corners held by outriggers. Two or three sets of apparatus can be used to provide duplicate data. In Skaha Lake, two sets were used. The bottles were stoppered to prevent entry of lake water or rainwater. Acrylic tubing of one-quarter inch outside diameter passed through the stopper to allow free gas exchange. Bottles were filled to the shoulder to allow maximum gas exchange across the water surface in the bottles.

1.3.2 Experimental Design

Nutrient additions were made in approximate proportion to the loadings to the lake from the Penticton Sewage Treatment Plant and with reference to the normal nutrient levels in the lake. The paramenters used to calculate the dosage for each treatment included lake volume ($588 \times 10^6 m^3$) and effluent discharge volume and phosphorus concentration which Wetter (1978) gives or approximately 2 million gallons per day ($9000\ m^3$ per day) and total phosphorus (mean) concentration of 870 µg/L.

The experiments were designed to be run over a 15 day period, based on the results and recommendations of Stockner and Antia (1976) indicating that algal bioassays require incubation periods of this time period to produce acceptable results.

The procedure used to maintain and sample the experiment is as follows. At the initiation of the experiment the bottles were filled with lake water from a central, open water portion of the lake from a depth of approximately one meter. The water was filtered through a #10 mesh zooplankton netting to exclude the larger grazing animals from the bottles. Appropriate amounts of nutrients were added to the other bottles carboys to test the effects of each. The phosphorus (as K_2HPO_4) and nitrogen (as $NaNO_3$) were added such that the experimental nutrient concentration was approximately twice the approximate spring time (overturn)

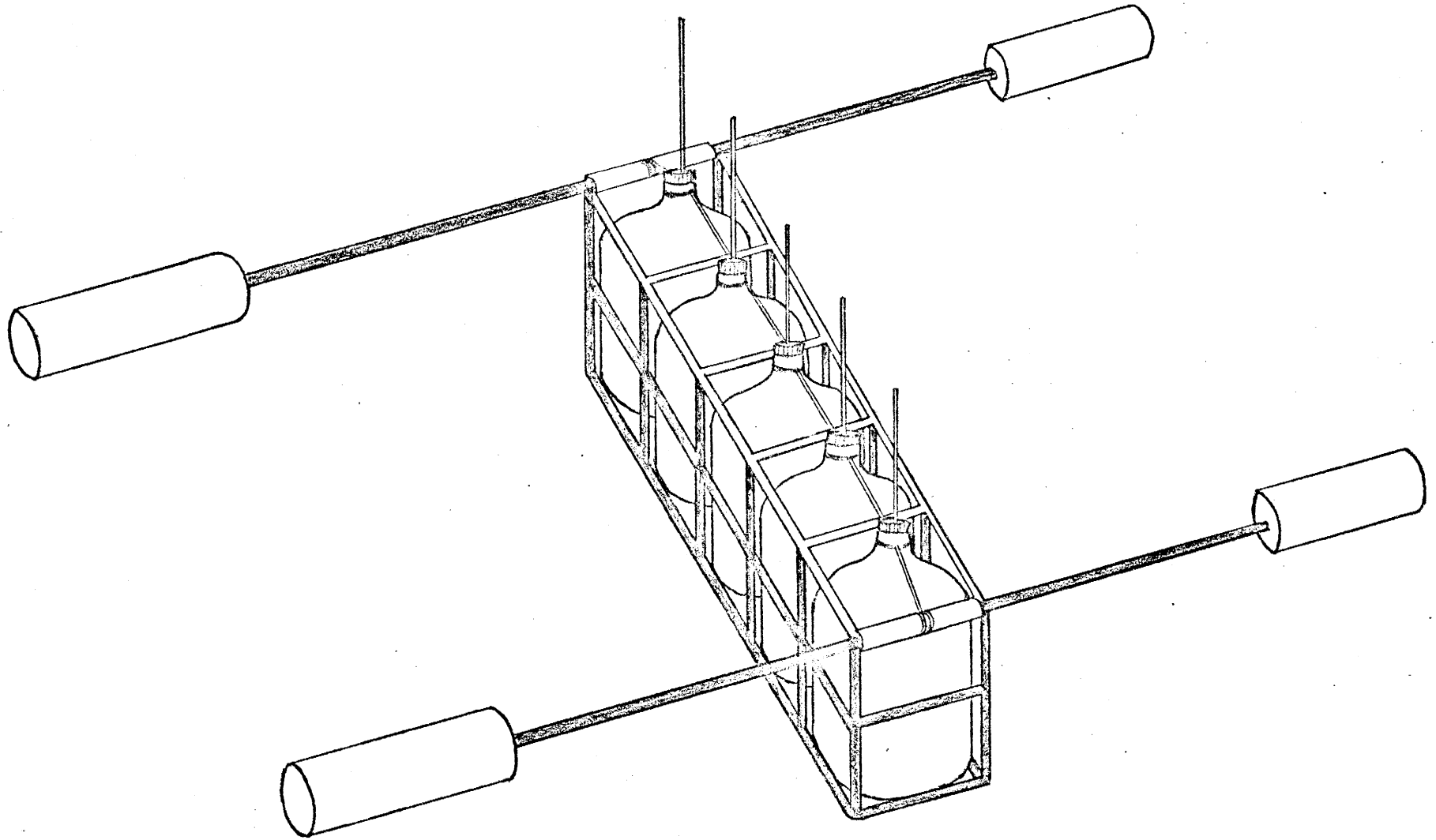

Figure 1 The algal bioassay apparatus.

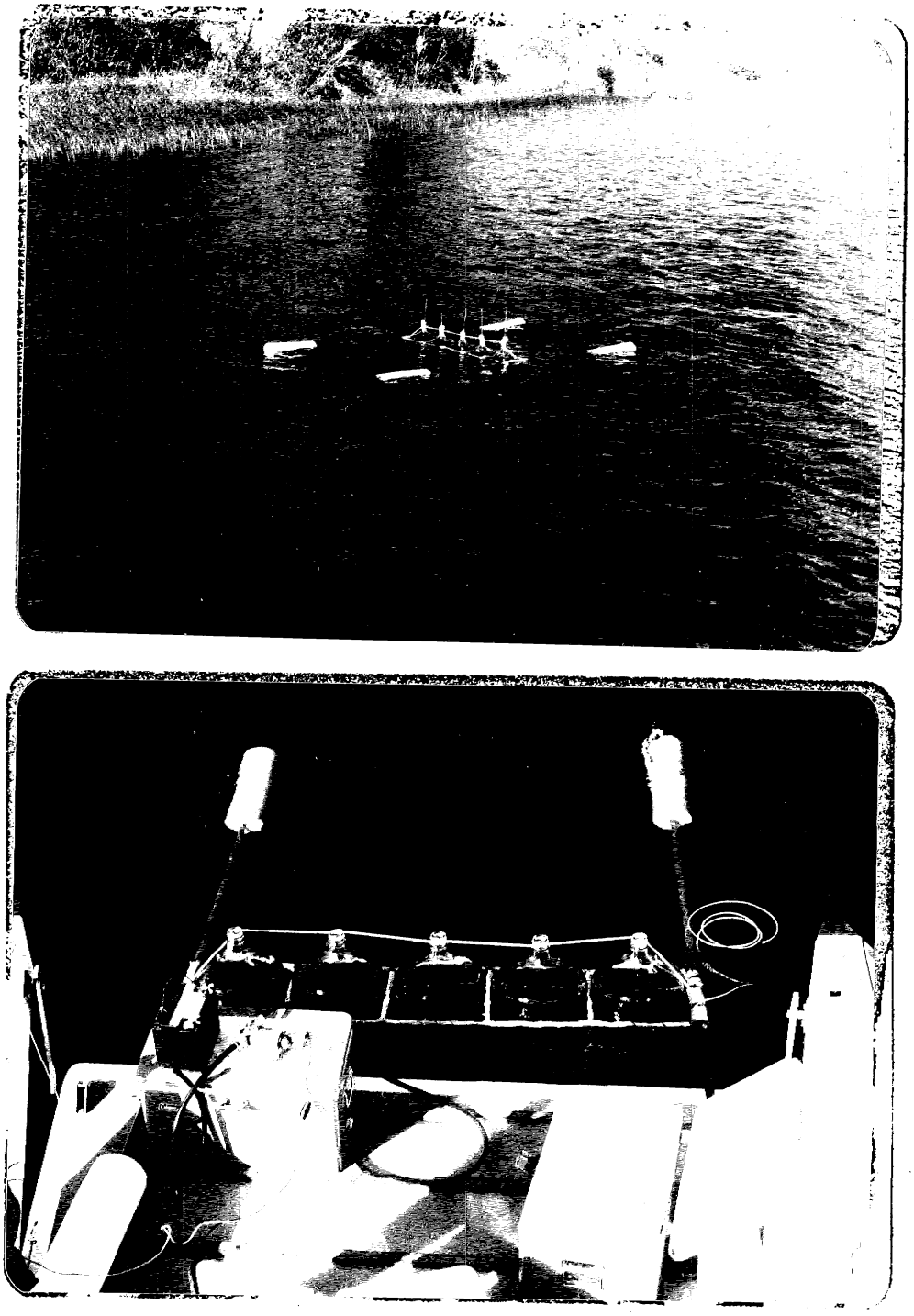

Figure 2 The apparatus installed in the lake.

concentration. In the 1975-1977 period these lake concentrations were approximately 15 µg/l (total) phosphorus and 300 µg/L (total) nitrogen. By doubling these normal lake concentrations, it would be expected that nutrient limitations could be avoided. A number of small variations to the general pattern outlined were used and are discussed in descriptions of the individual experiments.

1.3.3 Sampling

Sampling was carried out at three day intervals during the experiment and used the following procedure. From each bottle, samples were removed for monitoring algal biomass, growth rate, algal species and numbers and water chemistry. Algal biomass was measured using chlorophyll as an indicator. Three hundred millilitres of water was removed from each bottle, returned to the laboratory and filterd through 0.45 µmmembrane filters, placed on dessicant and frozen for shipment to the Ministry of the Environment Environmental Laboratory in Vancouver for analysis.

Samples were also removed and preserved with Lugol's Iodine for identification and enumeration of algal species.

Growth rate was measured by carbon 14 uptake. Glass stoppered 350 ml bottles in which 1 ml of $NaHC_{14}O_3$ (5µCi) had previously been added, were filled from appropriate carboys and placed in a plexigalss bottle holder (figure 3) and suspended from the bottle assembly at a depth of one meter. Bottles were incubated for a period of 3-4 hours during midday (1030 - 1430). After incubation bottles were removed, placed in a dark box, returned to the laboratory, filtered onto .45µmfilters and placed in Aquasol II scintillation fluid in standard vials and shipped for counting to the Canada Center for Inland Waters West Vancouver.

Water samples were pumped from each bottle for water chemistry analysis into standard bottles, kept in coolers packed in ice for shipment to the Environmental Laboratory where analyses were done. Parameters for which analyses were done included ammonia, nitrite, nitrate, organic nitrogen, Kjeldahl nitrogen,

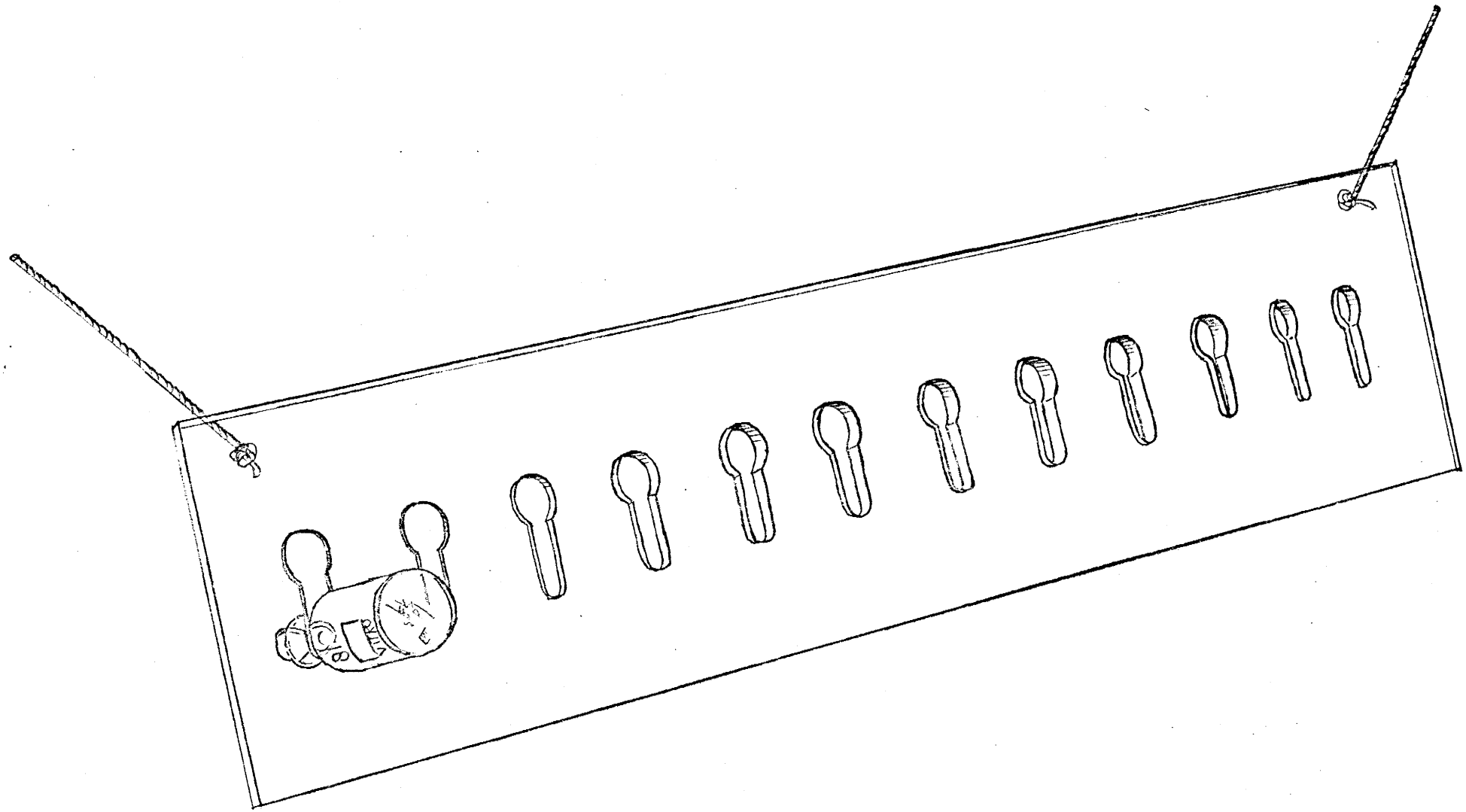

Figure 3 Rack used for incubation of carbon 14 samples.

total nitrogen, ortho phosphorus, total dissolved phosphorus, and total phosphorus.

After completion of sampling, the result was that approximately five litres of the bottle volume had been removed. The water was replaced by pumping lake water, filtered through zooplankton netting, to replace the volume lost. Nutrients were kept at the desired concentration be adding replenishing aliquots to the bottles to compensate for nutrients removed in sampling but not replaced by lake water.

2. Experiments

2.1 Results

In all, three separate experiments were carried out. The first two were of preliminary nature and were designed to serve several purposes. Firstly, to test the practicability of sampling and the use of equipment. Secondly to test the experiment itself in pilot stages, and thirdly to shed light on some related questions. The two preliminary experiments were only partially successful for reasons cited below but some explanation of these experiments and the points they made is useful.

2.1.1 First Experiment

The first concern in testing the design of the apparatus was whether it was practicable and functional in the lake situation. There were two other questions which were to be answered. Consideration was being made of the possibility of nitrogen removal at the Penticton Sewage Treatment Plant based on the earlier findings of the Okanagan study that Skaha Lake was limited by nitrogen. A third question, to which a fairly obvious answer was expected was whether the untreated sewage (influent to the plant) caused more stimulation in algal growth than the treated effluent. This aspect was expected also to give some idea of the sensitivity of the methods of monitoring used. The experiment consisted of four bottles. The first had added to it normal Penticton sewage effluent (i.e., with the phosphorus removed). To the second bottle was added Penticton sewage effluent

which was first passed through a nitrogen removal column which was composed of granular clay material used for ammonia removal in water borne effluents. To the third bottle was added untreated sewage (influent to the Penticton plant.) The fourth bottle served as a control.

Because of the normal period required by the laboratory to do analyses of water chemistry, it was not discovered until the end of the experiment that the nitrogen removal column did not function properly and that this particular variable would be impossible to evaluate. Thus the question whether nitrogen removal from the effluent had any effect on the growth of phytoplankton could not be answered. A second question to which some answer was readily apparent, was to question whether the removal of phosphorus from the effluent caused a significant decrease in phytoplankton growth. The data (Table 1) indicate the difference between the two treatments.

Table 1

Comparison of rates of phytoplankton carbon 14 uptake with different sewage additions. Units are C_{14} counts per minute.

	effluent added	influent added	control
24 Aug	367	491	301
25 Aug	712	702	573
26 Aug	427	781	642
27 Aug	408	-	338
mean	$\bar{x}$ 478	$\bar{x}$ 658	$\bar{x}$ 463

The results gave the first indication that phosphorus might be limiting since the removal of phosphorus from the effluent appeared to decrease the phytoplankton photosynthetic activity. This first experiment also indicated a number of other points. Firstly there was a great deal of variation from day to day (apparently correlating with light intensity) of the carbon-14 counts. It was decided that perhaps chlorophyll a might also be a useful parameter to measure the growth of phytoplankton in the bottles since it would be for less variable day to day and measure the biomass of phytoplankton rather than the rate of photosynthesis. It was also apparent that the C_{14} method as sensitive as it was required

a great deal of care if consistant results were to be obtained. There was a great deal of inherent variation in the C_{14} counts in the results of this first preliminary experiment and if definitive conclusions were to be drawn better data would be necessary. A final problem was the length of the experiment, which it was felt was of two short a term to provide good results.

One surprising result was the low amount of stimulation caused by addition of the phosphorus-deficient effluent. The Okanagan Basin Study, Technical Supplement IV (p. 197) indicated that during 1972-1973 the efficiency of removal was 50-60%. In the 1975-77 period the amount of removal increased but the variation from day to day and week to week is very large. Depending upon the day which the effluent was obtained it might contain 100 µg/L to 2910 µg/L (Wetter 1977). The effluent obtained in this instance (336 µg/l) happened to be very low in phosphorus. The concentrations of nutrients in the bottles and of the effluents are shown in Appendix 1.

2.1.2 Second Experiment

The second experiment was set up on March 31 1977 and was planned to be run for a period of 15 days. In this experiment five treatments and a control were used with duplicate bottles for each. The bottles consisted of (1) lake water to which Penticton Sewage Treatment Plant effluent was added, (2) lake water to which Penticton effluent which was treated with biologically active sludge containing denitrifying bacteria and which resulted in effluent with a majority of the nitrogen as well as phosphorus removed, (3) lake water to which phosphorus in the form of K_2HPO_4 was added, (4) lake water to which nitrogen in the form of $NaNO_3$ was added, (5) lake water to which both nitrogen and phosphorus were added and (6) a control-lake water only.

There were a number of objectives of this particular experiment. Firstly to investigate the effects of nutrient additions in a period when nutrients in the lake were not of limiting quantities-as opposed to mid summer when they normally are and when the crucial experiment would be run. Secondly, the practicability and logistics of sampling 12 bottles during a limited period of time were to be investigated, and the usefulness of chlorophyll a sampling evaluated.

The experiment was initiated March 31 and sampling done on April 4 and 6. On April 9 a severe storm on the lake destroyed the apparatus, and the lack of spare equipment and time prevented the experiment being set up again.

The results of this abbreviated experiment show a number of things. The carbon-14 data (table 2) shows that there is little difference between the treatments. This is also indicated by the chlorophyll a data (table 3).

Table 2

Rates of carbon-14 uptake during second experiment with different experimental additions. Units are C_{14} counts per minute.

	control	effluent added	effluent-N added	phosphorus	nitrogen	P&N
4 April	248	271	387	523	593	520
6 April	356	409	396	398	410	380

Table 3

Chlorophyll a concentrations in experimental bottles to which different experimental additions were made. Units are µg/L.

	control	effluent added	effluent-N added	phosphorus	nitrogen	P&N
31 March	2.8	2.1	2.5	2.6	2.1	2.9
4 April	2.9	2.4	3.4	4.2	2.8	2.9
6 April	3.0	2.2	2.5	2.5	2.5	2.8

There are two main reasons for the lack of stimulation of growth from the nutrient additions in this case. Firstly the length of the experiment was only half of what had been intended. Secondly, the lake at this springtime phase is relatively homogeneous, being shortly after turnover, and before the main period of phytoplankton growth, and contains phosphorus and nitrogen in sufficient quantities to not be limiting. During this period, low light levels and cold water temperatures may also be likely factors limiting growth. The water chemistry of the test bottles is shown in Appendix 2.

2.1.3 Third Experiment

After the two preliminary experiments, it was decided to carry out the last experiment using a control plus four treatments. The additions consisted of addition of (1) Penticton effluent (2) phosphorus (3) nitrogen and (4) phosphorus and nitrogen. Duplicate bottles were used and the experiment was set up on 16 August, with samples being taken 18,22,25,29 August and 1 September.

2.1.3.1 Water Chemistry

The analyses of the water quality inside the bottles reveals the changes associated with each treatment (table 4). The control bottle indicates that the total nitrogen being 300 µ/L. Phosphorus was largely in an unreactive particulate form (8 µg/L total P) with total dissolved phosphorus and ortho phosphorus levels being 3-4 µg/L and less than 3 µg/L respectively. The second set of bottles, to which Penticton effluent was added showed a higher nitrogen concentration, manifested predominantly in the ammonia fraction. Very little increase in phosphorus was apparent, again due to the fact cited in the first experiment of good phosphorus removal by the sewage treatment plant on the day when the effluent was obtained. Analysis indicated ortho phosphorus concentration of 171 µg/L, whereas the average effluent concentration between June 1975 and September 1977 was 490 and 870 µg/L of ortho and total phosphorus respectively (Wetter 1977).

The third set of bottles had phosphorus added as K_2HPO_4. When water chemistry measurements were made, usually three days after the initial addition and the replenishing additions, most of the phosphorus was evident in an unreactive particulate form, apparently due to uptake by phytoplankton during the preceeding days. The fourth set of bottles had $NaNO_3$ added to them and this was detected in increases in the nitrate concentration. Very little of the nitrate appeared to be taken up by the phytoplankton. Normal lake water nitrate concentration was less than 20 µg/L with total nitrogen being 250 - 300 µg/L. The addition raised the nitrate concentration to 400 µg/L and the total nitrogen to 550 - 600 µg/L.

The fifth set of bottles with additions of both phosphorus and nitrogen

showed an additive effect of the two treatments described above.

2.1.3.2 Growth Measurements

Two methods were used to measure the growth of the phytoplankton in the bottles. The carbon-14 uptake method is a measurement of rate of growth. Chlorophyll *a* measures biomass of the phytoplankton present.

A problem was encountered with the carbon-14 method in this instance. In the previous experiments, the ampules of carbon-14 had been opened and the contents combined so that a homogeneous stock could be used to inoculate each sample. This was not done in the third experiment and because a wide range of activity in individual ampules was later found to exist, the results obtained for the carbon-14 measurements were unusable.

The chlorophyll *a* method proved to be a very useful measurement and the results given in table 4 are a dramatic indication of the state of nutrient limitation in Skaha Lake.

Table 4

Lake phytoplankton response to additions of phosphorus and nitrogen over a period of seventeen days. Chlorophyll *a* concentration (µg/L)

treatment date	control	Penticton effluent	+P	+N	+P+N
18 August	2.0	2.1	2.0	2.6	2.1
1 Sept.	2.7	2.9	7.6	2.5	7.5
% increase over beginning concentration	35%	38%	280%	35%	257%

3. Discussion

The bioassay data indicates that Skaha Lake in the summer period, in strongly phosphorus-limited. There are also supplementary data to support this conclusion.

The spring concentration of phosphorus is used as an indication productivity in lake systems (Dillon and Rigler 1974, Sakamoto 1966). The spring concentration of total phosphorus at overturn of the lake but before biological (phytoplankton) activity begins, is also an indication of potential phytoplankton growth during the summer months. In 1970 and 1971, three sets of samples were taken during spring overturn and give some indication of the differences between the 1970-71 period and 1977. B.C. Research (1971) list a mean total phosphorus for 1970 of 33 µg/L (100 µg/L as PO_4). Stein and Coulthard (1971) note a mean concentration of 32 µg/L (96 µg/L as PO_4) for March 1970. The Okanagan Basin Study (station 2) indicate mean water column concentration of 22 µg/L (66 µg/L as PO_4) for March 1971. In contrast, for 1976 and 1977 the concentration were 11 and 13 µg/L.

The algal biomass, which is the component of water quality of most concern, and which is a result of phosphorus loading to the system shows a similar pattern. In 1971 the mean chlorophyll concentration (Tech Suppl. V) was 31.0 µg/L. In the period 1975-77 at station 250 the mean chlorophyll concentration was 4.15 µg/L.

The major change over this period of time has been the removal of phosphorous from the effluent of the sewage treatment plant at Penticton. Thus, the removal of phosphorus from effluent succeeded in achieving the intended effect, even in the case where nitrogen was, in 1971, the limiting nutrient.

The second question, and the one to which the bioassay was primarily directed, was whether the lake has changed from nitrogen to phosphorus limitation. The results of the bioassay indicate that phosphorus is presently the limiting nutrient, and there are other lake water quality data and literature information, as well, to support this suggestion.

In general the ratio of nitrogen to phosphorous required by algae is in the range 10:1 (Golterman 1975) to 12:1 (Dillon and Rigler 1974). At ratios of less than 10:1 nitrogen is the limiting nutrient at greater than 12:1 phosphorus is limiting.

In Skaha Lake the ratio of nitrogen to phosphorus in 1971 which was calculated to be entering Skaha Lake was 199:24 (metric tonnes) or approximately 8:1 indicating nitrogen limitation (Basin Study, Tech, supplement IV). This was confirmed by Stockner's algal bioassays (Okanagan Basin Study Technical Supplement V). In contrast, in April 1976 Skaha Lake had concentrations of 260 µg/L and 11 µg/L of total nitrogen and total phosphorus. In March 1977 the levels were 300 and 13 respectively. The ratio of N:P for these two years appears to be 23-24:1.

From this evidence, it would appear that Skaha Lake in 1971 when the sewage treatment plant was discharging secondarily treated sewage, (which has a low N:P ration and is condusive to producing a nitrogen-limiting stituation) was loading the lake with an excess of phosphorus. With implementation of tertiary treatment, the lake has responded and is now apparently phosphorus-limited. Obviously more evidence should be examined before it is certain that such is the case. Such information should be available shortly as the result of the monitoring programs carried out under the Okanagan Implementation Program.

One implication of this information is that the productivity of the lake under this phosphorus-limiting situation will respond directly to any increases or decreases in the loading of phosphorus. Any further decreases of loading of phosphorus would result in an increase in water quality of Skaha Lake.

4. Conclusions

1. The bioasay technique described here provides a system which

appears to be a very useful tool in evaluating the effects of nutrients, and possibly toxicants on phytoplankton communities. The apparatus provides a compromise between the large scale water column isolation studies which provide the best circumstances to evaluate effects on plankton communities, but are extremely expensive; and the laboratory bottle bioassay which contains a number of inherent disadvantages.

2. The results of this preliminary experiment indicate that the lake had changed in its nutrient limitations, from being nitrogen-limiting in 1971 to being strongly phosphorus-limiting at present. Information also indicates that the lake productivity has decreased since 1971.

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Literature Cited

B.C. Research. 1971. Sampling of Okanagan Lakes - Spring 1970. Prepared for the Water Investigations Branch. 51 pp.

Canada - B.C. Okanagan Basin Agreement, Technical Supplements IV and V. 1974.

Dillon, P.J. and F.H. Rigler. 1974. The phosphorus-chlorophyll relationship in lakes. Limnology and Oceanography 19:767-773.

Environmental Protection Agency. 1971. Algal Assay Procedure Bottle Test. National Eutrophication Research Program, Corvallis, Oregon. 82 pp.

Golterman, H.L. 1975. Physiological Limnology. Elsevier Scientific Publishing Co. Amsterdam, Oxford, New York. 489 pp.

Grice, D.G., M.R. Reeve, P. Koeller, and D.W. Menzel. 1977. The use of large-volume (1300 m^3), transparent, enclosed sea-surface water columns in the study of stress on plankton ecosystems. Helgo. Will. Meeresunters. (In press).

Sakamoto, M. 1966. Primary production by phytoplankton community in some Japanese Lakes and its dependance on lake depth. Arch. Hydrobiol. 62:1-28.

Stein, J.R. and T.L. Coulthard. 1971. A report on the Okanagan Water Investigation 1969-70. Prepared for the Water Investigations Branch. 89 pp.

Stockner, J.G. and N.J. Antia. 1976. Phytoplankton adaptation to environmental stresses from toxicants, nutrients and pollutants - a warning. J. Fish Res. Board Can. 33:2089-2096.

Wetter, R.D. 1977. Penticton phosphorous removal study. Pollution Control Branch Report. B.C. Ministry of the Environment.

Appendix 1. Preliminary Experiment Number 1.

Experiment #1

25 August 1976

parameter	*Bottle # 1	2	3	4
pH	8.4	8.4	8.3	8.5
conductivity *	254	254	254	246
N(NH_3)	704	680	660	16
N(NO_2,NO_3)	<20	<20	< 20	< 20
N(Kjeldahl)	850	960	1050	250
N(total)	850	960	1050	250
P(ortho)	3	3	72	3
P(total)	17	16	135	9

27 August 1976

pH	8.2	8.2	7.9	8.4
conductivity	254	255	256	246
N(NH_3)	820	865	880	15
N(NO_2,NO_3)	<20	<20	20	<20
N(Kjeldahl)	960	1120	1030	210
N(total)	-	-	-	
P(ortho)	3	3	123	<3
P(total)	16	14	166	7

30 August 1976

pH	8.5	8.4	8.8	8.5
conductivity	253	253	253	245
N(NH_3)	800	825	450	17
N(NO_2,NO_3)	<20	<20	< 20	<20
N(Kjeldahl)	1090	960	1080	210
N(total	1090	960	1080	210
P(ortho)	8	3	51	<3
P(total)	18	17	121	7

*Bottle 1. addition of Penticton sewage treatment plant effluent
Bottle 2. Penticton effluent passed through nitrogen removal column
Bottle 3. addition of Penticton treatment plant influent
Bottle 4. control - lake water only

all units in µg/L except pH and conductivity (µmhos/cm), < indicates 'less than'

Penticton city site water quality 23 August 1976. Values in µg/L

	ammonia	nitrate/nitrite	Kjeldahl N	total N	ortho P	total P
influent	32600	80	44000	44000	5600	7370
effluent	30800	220	33000	33220	239	336

* specific conductance

Appendix 2. Preliminary Experiment Number 2.

Experiment #2

March 31, 1977

*Bottle #

parameter	1	2	3	4	5	6	7	8	9	10	11	12
pH	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
conductivity	270	279	272	270	272	272	270	270	270	270	270	270
$N(NH_3)$	9	12	243	252	44	31	9	10	16	16	14	9
$N(NO_2,NO_3)$	<20	<20	<20	<20	20	20	<20	<20	<20	20	<20	20
$N(NO_3)$	<20	<20	<20	<20	20	20	<20	<20	<20	20	<20	20
$N(NO_2)$	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
N(organic)	190	210	220	220	250	210	200	200	310	240	230	190
N(Kjeldahl)	200	220	460	470	290	240	210	210	330	260	290	200
N(total)	200	220	460	470	310	260	210	210	350	260	260	200
P(total diss.)	5	4	5	5	9	5	19	29	4	4	15	20
P(ortho)	<3	<3	<3	<3	6	<3	17	27	<3	<3	12	17
P(total)	8	10	10	10	16	17	26	37	7	9	21	27
chlorophyll a	2.8		2.1		2.5		2.6		2.1		2.8	

*Bottles 1,2 control; 3,4 Penticton effluent added; 5,6 Penticton effluent with nitrogen removed; 7,8 phosphorus added; 9,10 nitrogen added; 11,12 phosphorus and nitrogen added.

all units in µg/L except pH and conductivity (µmhos/cm) < indicates 'less than'

Experiment #2

April 4, 1977

*Bottle #

parameter	1	2	3	4	5	6	7	8	9	10	11	12
pH	8.4	8.4	8.5	8.5	8.5	8.5	8.6	8.6	8.5	8.5	8.6	8.6
conductivity	272	271	272	272	272	272	268	269	270	268	268	270
$N(NH_3)$	13	15	216	243	30	31	10	9	11	10	10	9
$N(NO_2,NO_3)$	<20	<20	<20	<20	20	20	<20	<20	20	<20	<20	<20
$N(NO_3)$	<20	<20	<20	<20	20	20	<20	<20	20	<20	<20	<20
$N(NO_2)$	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
N(organic)	240	270	240	280	300	210	230	140	130	270	270	230
N(Kjeldahl)	250	280	460	520	330	240	240	150	140	280	280	240
N(total	250	280	460	520	350	260	240	130	160	280	280	240
P(total diss.)	4	3	3	4	6	5	28	37	6	3	6	13
P(ortho)	<3	<3	<3	<3	<3	<3	21	34	<3	<3	<3	8
P(total)	9	7	12	10	14	17	40	52	7	7	16	20
chlorophyll a	3.1	2.7	2.1	2.6	2.8	3.9	4.3	4.1	2.4	5.2	3.1	2.7

*Bottles 1,2 control; 3,4 Penticton effluent added: 5,6 Penticton effluent with nitrogen removed; 7,8 phosphorus added; 9,10 nitrogen added; 11,12 phosphorus and nitrogen added.

all units in µg/L except pH and conductivity (µmhos/cm) < indicates 'less than'

Experiment #2

April 6, 1977

	Bottle #											
parameter	1	2	3	4	5	6	7	8	9	10	11	12
pH	8.4	8.5	8.5	8.5	8.5	8.5	8.6	8.6	8.6	8.6	8.6	8.6
conductivity	272	271	272	272	272	270	270	270	270	270	270	270
$N(NH_3)$	10	16	198	214	20	23	9	10	13	11	11	9
$N(NO_2, NO_3)$	<20	<20	<20	<20	20	<20	<20	<20	20	<20	<20	<20
$N(NO_3)$	<20	<20	<20	<20	20	<20	<20	<20	20	<20	<20	20
$N(NO_2)$	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	5
N(organic)	210	300	290	230	280	470	40	220	80	150	230	240
N(Kjeldahl)	220	320	490	440	300	490	50	230	90	160	240	250
N(total)	220	320	490	440	320	490	50	230	110	160	240	250
P(total diss.)	4	5	13	11	5	4	30	40	4	3	9	12
P(ortho)	<3	<3	8	6	<3	<3	26	39	<3	<3	4	8
P(total)	8	8	25	29	14	14	46	53	9	8	19	24
chlorophyll a												

*Bottles 1,2 control; 3,4, Penticton effluent added; 5,6 Penticton effluent with nitrogen removed; 7,8 phosphorus added; 9,10 nitrogen added; 11,12 phosphorus and nitrogen added.

Appendix 3. Main Experiment, Water Chemistry Data

Experiment #3

22 August 1977

	Bottle #									
parameter	1	2	3	4	5	6	7	8	9	10
pH	8.8	8.8	8.8	8.7	8.8	8.8	8.8	8.8	8.8	8.8
conductivity	258	257	259	258	254	254	258	257	253	251
$N(NH_3)$	15	17	250	264	9	10	21	23	18	15
$N(NO_3)$	<20	<20	<20	<20	<20	<20	400	410	<20	<20
$N(NO_2)$	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
N(organic)	220	200	160	240	210	320	110	190	270	340
N(Kjeldahl)	230	220	410	500	220	330	130	210	290	350
N(total)	230	220	410	500	220	330	530	620	290	350
P(ortho)	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3
P(total diss.)	3	5	5	6	3	7	6	6	7	6
P(total)	12	12	14	14	29	28	12	12	25	34

*Bottles 1,2 control: 3,4 Penticton effluent added; 5,6 phosphorus added; 7,8 nitrogen added; 9,10 phosphorus and nitrogen added.

all units in µg/l except pH and conductivity (µmhos/cm), < indicates 'less than'.

Experiment #3

25 August 1977

	Bottle #									
parameter	1	2	3	4	5	6	7	8	9	10
pH	8.7	8.7	8.7	8.7	8.8	8.8	8.8	8.8	8.9	8.8
conductivity				261		256				
$N(NH_3)$	21	18	210	217	12	10	21	18	14	27
$N(NO_3)$	<20	<20	<20	<20	<20	<20	370	370	160	<20
$N(NO_2)$	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
N(organic)	460	310	340	280	310	390	360	320	350	390
N(Kjeldahl)	480	330	550	500	320	400	380	340	360	420
N(total)	480	330	550	500	320	480	750	110	520	420
P(ortho)	<3	<3	<3	<3	<3	<3	<3	<3	5	<3
P(total diss.)	6	4	4	4	6	6	4	4	11	6
P(total)	11	10	9	9	24	27	8	10	22	22

*Bottles 1,2 control; 3,4 Penticton effluent added; 5,6 phosphorus added; 7,8 nitrogen added; 9,10 phosphorus and nitrogen added.

all units in µg/l except pH and conductivity (µmhos/cm), < indicates 'less than'

Experiment #3

29 August 1977

	Bottle #									
parameter	1	2	3	4	5	6	7	8	9	10
pH	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	9.0	8.8
conductivity	262	260	258	260	258	258	259	260	232	234
$N(NH_3)$	36	17	193	198	13	12	14	26	15	21
$N(NO_3)$	<20	<20	<20	≤20	<20	<20	280	320	<20	<20
$N(NO_2)$	<5	5	<5	<5	<5	<5	<5	<5	<5	<5
N(organic)	240	230	320	260	280	320	330	290	320	330
N(Kjeldahl)	280	250	510	460	290	330	340	310	330	350
N(total)	280	250	510	460	290	330	620	640	330	350
P(ortho)	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3
P(total diss.)	3	4	5	3	6	4	4	3	4	4
P(total)	11	12	11	10	23	22	10	9	10	23

*Bottles 1,2 control; 3,4 Penticton effluent added; 5,6 phosphorus added; 7,8 nitrogen added; 9,10 phosphorus and nitrogen added.

all units in µg/l except pH and conductivity (µmhos/cm), < indicates 'less than'

Experiment #3

1 September 1977

	Bottle #									
parameter	1	2	3	4	5	6	7	8	9	10
pH	8.7	8.8	8.8	8.9	8.9	8.8	8.8	8.8	9.1	8.9
conductivity	260	258	256	256	254	254	256	256	227	227
$N(NH_3)$	31	13	169	181	11	12	15	15	12	19
$N(NO_3)$	≤20	<20	<20	<20	<20	<20	30	30	<20	<20
$N(NO_2)$	≤5	<5	<5	<5	<5	<5	<5	<5	<5	<5
N(organic)	350	340	370	320	380	420	380	300	370	470
N(Kjeldahl)	380	350	540	500	390	430	390	310	380	490
N(total)	380	350	540	500	390	430	690	610	380	490
P(ortho)	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3
P(total diss.)	5	5	3	5	6	4	5	5	6	7
P(total)	9	9	8	11	19	18	9	10	16	18

*Bottles 1,2 control; 3,4 Penticton effluent added; 5,6 phosphorus added; 7,8 nitrogen added; 9,10 phosphorus and nitrogen added.

all units in µg/l except pH and conductivity (µmhos/cm), < indicates 'less than'