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(SUBJECT TO REVISION)

Algal Nutrient Addition and Pure Culture Bioassay Studies - on Six Lakes in the Okanagan Basin British Columbia

PREPARED FOR THE OKANAGAN STUDY COMMITTEE

CANADA - BRITISH COLUMBIA OKANAGAN BASIN AGREEMENT

TASK 119

Algal Nutrient Addition and Pure Culture Bioassay Studies on Six Lakes in the Okanagan Basin, British Columbia

by

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NOTICE

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ABSTRACT

Detailed laboratory studies were carried out in 1970 and 1971 to test the effects of the nutrient elements phosphorus, nitrogen, and carbon, in regulating algal growth, and to identify if possible the specific nutrients or trace elements limiting phytoplankton populations in the Okanagan mainstem lakes.

The results of this study will be used along with other limnology task findings to provide an overall assessment of the current ecology and trophic state of these lakes. They will also be used as a basis for estimating algae growth given certain projected concentrations of nutrients in the lakes to the year 2020.

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SUMMARY - BIOASSAY PROGRAM

Results from four different experiments conducted on the six main lakes in the Okanagan Basin permitted an evaluation of the role of nutrients in regulating algal growth. Further information was gained on the causes of eutrophication of localized areas within lakes that are currently exhibiting nuisance conditions.

Kalamalka Lake and the main watermass of Okanagan Lake are currently in a nutrient deficient state. This was indicated by results from both the nutrient enrichment (NEB) and pure culture bioassay (PCB)experiments. In these lakes NO_3(N) and PO_4(P) when added together stimulated the greatest algal growth. When each nutrient was added alone, little algal growth occurred. Results from the PCB experiments indicated a paucity of available nutrients, for little growth of the test algae was noted when added to filtered water.

Certain localities of Okanagan Lake exhibited nutrient-rich characteristics, namely in the Vernon Arm, the Armstrong Arm, and the near-shore water mass in the vicinity of Kelowna and Summerland. At these localities, NO_3(N) when added alone was in most instances stimulatory to algal growth, while PO_4(P) additions were not. These results indicate a sufficient supply of PO_4(P) and a deficiency of NO_3(N). The growth of test algal in the PCB experiments was moderate to high at all these localities, again indicating a 'residual' nutrient supply.

Skaha lake appeared to be limited more by NO_3(N) than PO_4(P), for most additions of NO_3(N) were stimulatory while PO_4(P) additions

were not. Currently, the most productive region of Skaha lake is in the north end off the mouth of the Okanagan River, where yields of the test algae (PCB experiments) were the highest recorded among lakes tested. Much of the main water mass of Skaha Lake exhibited nutrient-rich characteristics with no apparent PO_4(P) limitation.

Vaseux and Osoyoos Lakes appeared to be limited by both NO_3(N)and PO_4(P), for the addition of both nutrients together produced the greatest algal yield. The noted yield was considerably higher than that observed in Kalamalka and Okanagan Lakes, largely attributable to a much higher standing stock of phytoplankton in Vaseux and Osoyoos Lakes. The station located off the mouth of the Okanagan River in Osoyoos was more productive than the station located in the central portion of the lake, showing a greater response to NO_3(N) than to PO_4(P) additions. Results of the PCB experiments also indicated that the most productive region of Osoyoos Lake was off the mouth of the Okanagan River, where moderate to high yields of the test algae were obtained. Vaseux Lake showed moderate yields of algae, indicating some nutrient availability at the time of the experiments.

Results from experiments conducted on Wood Lake water substantiated present evidence indicating it to be one of the most productive (eutrophic) lakes in the Valley. Additions of PO_4(P) had no effect whatsoever, while NO_3(N) additions promoted an excellent algal growth. response. Results from the PCB experiments showed that an ample supply of available nutrients are present in Wood Lake throughout much of the growing season.

Results from the sewage enrichment experiments (SEE) strikingly illustrated the fertilizing capacity of domestic wastes when discharged to lakes in the Okanagan Valley. Preliminary results indicated that biological treatment of wastes simply increases the availability of plant nutrients, and hence does very little to ameliorate an algal nuisance problem. Increasing the amount of sewage added to lake water simply changed the direction of algal succession toward a blue-green algae dominance.

The trace metal experiments (TME) gave some clues as to the possible role of trace metals and a chelator in regulating phytoplankton growth, but no definitive conclusions can be drawn at this time from these preliminary experiments. Further experiments should be conducted to determine the importance of iron and molybdenum in the Okanagan lakes, for some stimulation of algal growth was noted with the addition of these metals. Boron appeared to have little effect on algal growth.

TABLE OF ABBREVIATIONS

1. General

Con.	-	Control
F.R.B.	-	Fisheries Research Board
N.E.B.	-	Nutrient Enrichment Bioassay
P.C.B.	-	Pure Culture Bioassay
S.E.E.	-	Sewage Enrichment Experiments
S.T.P.	-	Sewage Treatment Plant
St.	-	Study
T.M.E.	-	Trace Metal Experiments

2. Chemical Symbols

C	-	Carbon
CO_2	-	Carbon Dioxide
EDTA	-	Ethyl-ethylene diamine triacetic acid
N	-	Nitrogen
NO_3(N)	-	Nitrate Nitrogen
Na_2CO_3	-	Sodium Carbonate
P	-	Phosphorus
PO_4(p)	-	Phosphate Phosphorus
T.O.C.	-	Total Organic Carbon

3. Weights and Measures

c.p.m.	-	counts per minute
T.C.P.M.	-	Total counts per minutes (measure of radioactivity)
g.	-	gram
mg.	-	milligram
mg/l	-	milligrams per liter
mu	-	millimicrons
ug/l	-	micrograms per liter
ml	-	milliliter
u	-	micron
uci	-	microcuries (unit of radioactivity)

TABLE OF ABBREVIATIONS

(continued)

4. Algal Species

S	-	Selenastrum
a	-	Anabaena
M	-	Microcystis

Acknowledgments

The authors wish to acknowledge the assistance of many people who through their efforts made this study possible. The staff of the South Okanagan Health Unit were most helpful in so many ways. Mr. Martin Pomeroy and Mr. David Evans provided all algal counts and Miss Linda Battelier and Mr. David Findlay ably assisted us in the 1971 experiments. Miss Linda Gardner, FRB Vancouver Laboratory, did all ^{14}C counts. Mr. Gary McKenzie and Mrs. Reid Morton of the Okanagan Basin Studies office provided excellent services and put up with various problems that developed in the summer of 1971. Mr. A.M. Thomson, Study Director, provided the excellent laboratory facilities and arranged for much of our equipment installation. Mr. Mike Stainton assisted in computing the final concentrations of chemical additions and in calibrating the fluorometer. To these people and many others we extend our most sincere appreciation.

INTRODUCTION

An adequate understanding of the role of nutrients in regulating algal growth in the Okanagan Basin lakes is an essential part of the limnology program of the Joint Federal-Provincial Okanagan Basin Study Agreement. With increasing deterioration of water quality evident in some lakes it was essential that causitive factors be identified and control measures recommended. One means of accomplishing these objectives was through detailed laboratory studies designed to test the effects of the algal nutrients PO_4(P), NO_3(N) and CO_2 known to be of importance in regulating algal growth. Furthermore, it was important to determine, if possible, the present trophic state of the Okanagan lakes, or the ability of each lake to produce algae. It was also informative to compare the fertility of water masses within lakes.

It must be borne in mind that extrapolation from changes observed in flasks kept at constant temperature and light conditions to processes actually occurring within lakes is difficult and often subject to a wide range of errors. Nonetheless, such an approach does enable an investigator to gather an appreciation of the influence of various chemicals both alone and in combination, in regulating natural phytoplankton production. With suitable caution in interpretation, this technique does offer the only means of obtaining valuable information that could not be gathered by any other means.

Thus, the overall object of this two-year laboratory study was to provide answers to many of the aforementioned problems associated with the growing eutrophication of the Okanagan Lakes. These studies were carried out at the South

Okanagan Health Unit and Okanagan Basin Study office in Penticton during 5 month periods, commencing May, 1970 and 1971, respectively.

MATERIALS AND METHODS[1.]

Nutrient enrichment experiments,

Field procedure, 1970, Surface water samples were taken from midlake stations on five Okanagan Basin lakes: Skaha, Okanagan, Osoyoos, Wood, and Kalamalka (Figs. 1-5). The samples included 500 ml for the identification of phytoplankton (Lugol's solution added immediately for preservation), 1 liter for chemical analysis (alkalinity, conductivity, nutrients, pH, T.O.C. and turbidity)[2.], and 6 liters for laboratory bioassay and nutrient enrichment studies.

Field procedure, 1971. The field procedure was conducted in a manner comparable to the previous year except that chemical analyses of water samples was eliminated, since these data were available from the 1971 "Monitor Cruises". A study of Vaseux Lake was incorporated into the program in 1971 (Fig. 6).

Laboratory procedure, 1970. The pH of the water was recorded with an Orion portable pH meter (model 401). A 500 ml sample was taken from the 6 liter carboy, filtered through an 87u mesh net to remove Zooplankton, and filtered again through a Whatman, 4.25 cm, GF/C glass fiber paper for chlorophyll-a analyses. The filters were dried in a dessicator overnight, stored in the dark, and at biweekly intervals sent to FRB, Freshwater Institute, Winnipeg, for analysis.

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1. In Appendix I pp 48, each experiment is listed, together with the experimental time period.

2. Canada Department of Energy, Mines and Natural Resources, Water Quality Control Lab, Calgary, Alberta,

Figure 1. LOCATION OF SURFACE WATER SAMPLES
FOR BIOASSAY EXPERIMENTS.

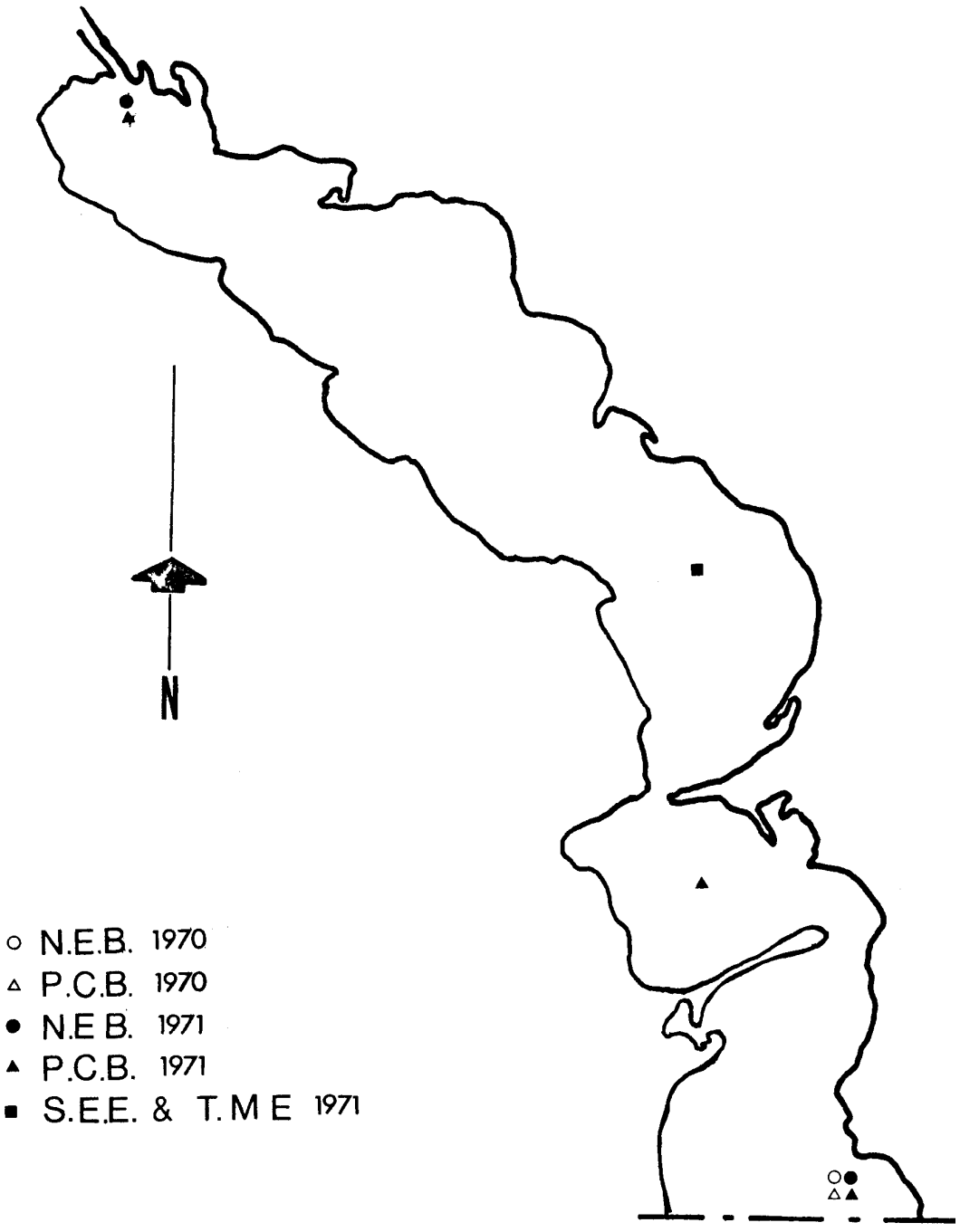


Figure 2. LOCATION OF SURFACE WATER SAMPLES FOR BIOASSAY EXPERIMENTS.

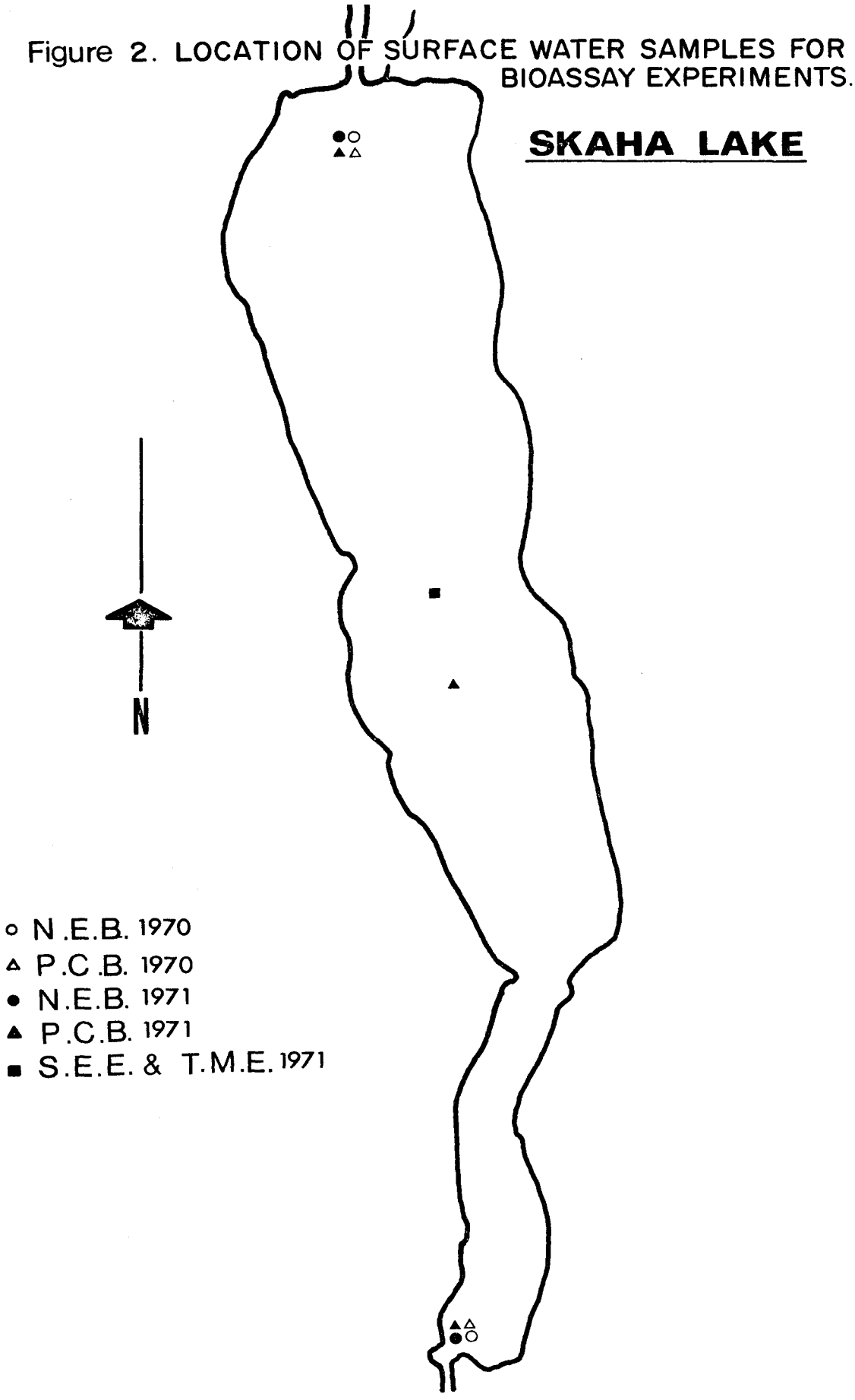


Figure 3. LOCATION OF SURFACE WATER SAMPLES FOR BIOASSAY EXPERIMENTS.

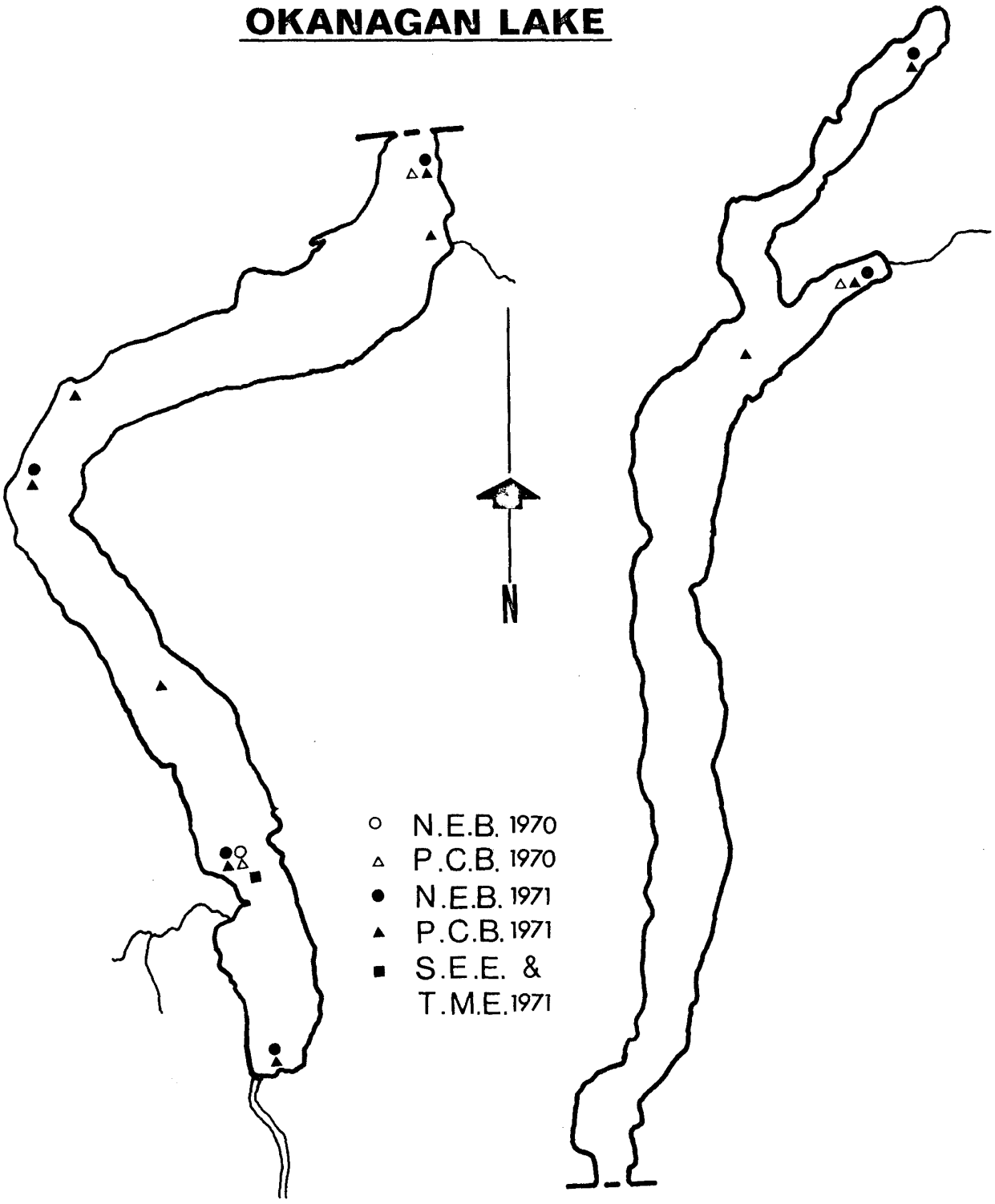


Figure 4. LOCATION OF SURFACE WATER SAMPLES FOR BIOASSAY EXPERIMENTS.

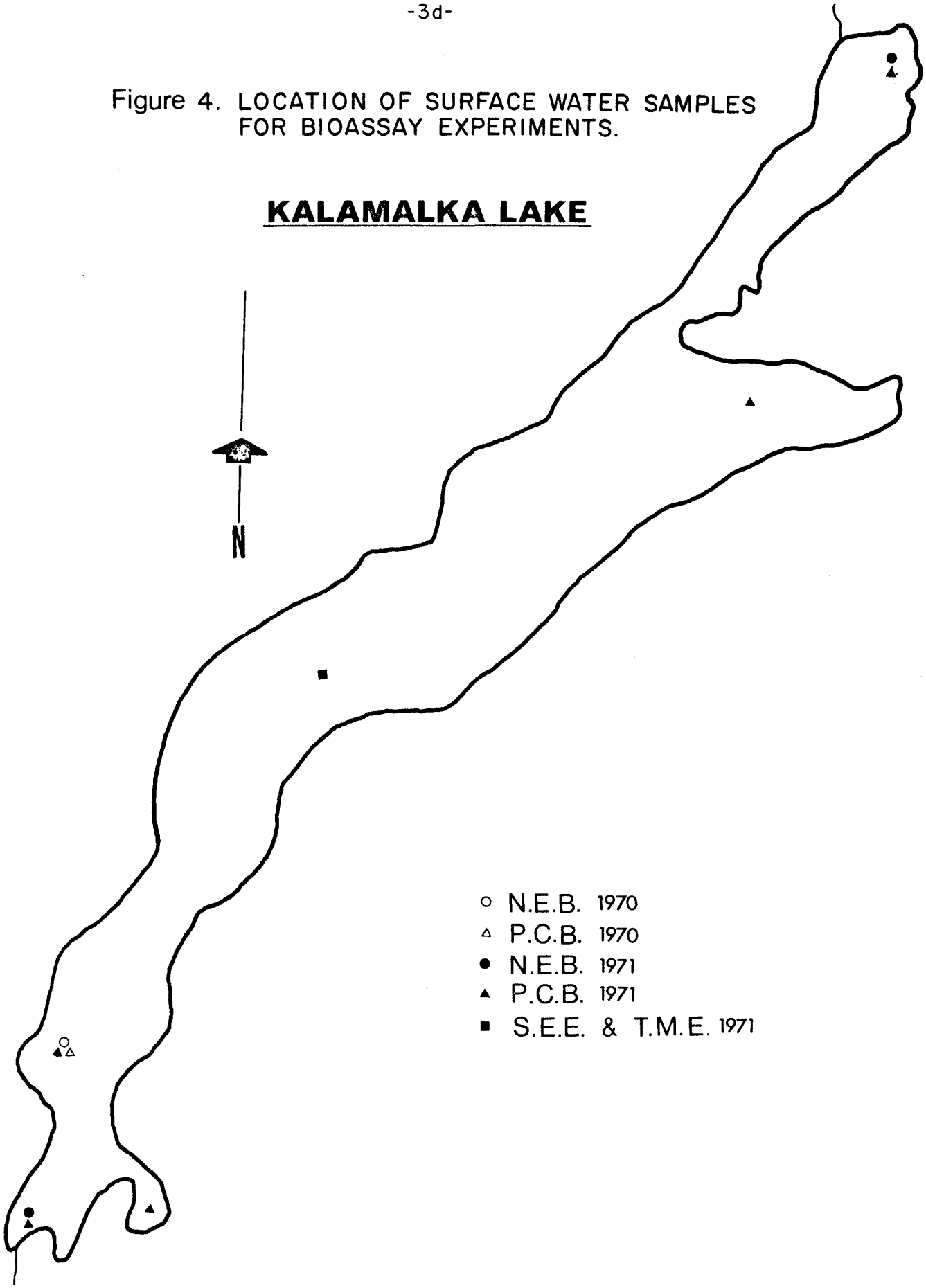


Figure 5. LOCATION OF SURFACE WATER SAMPLES FOR BIOASSAY EXPERIMENTS.

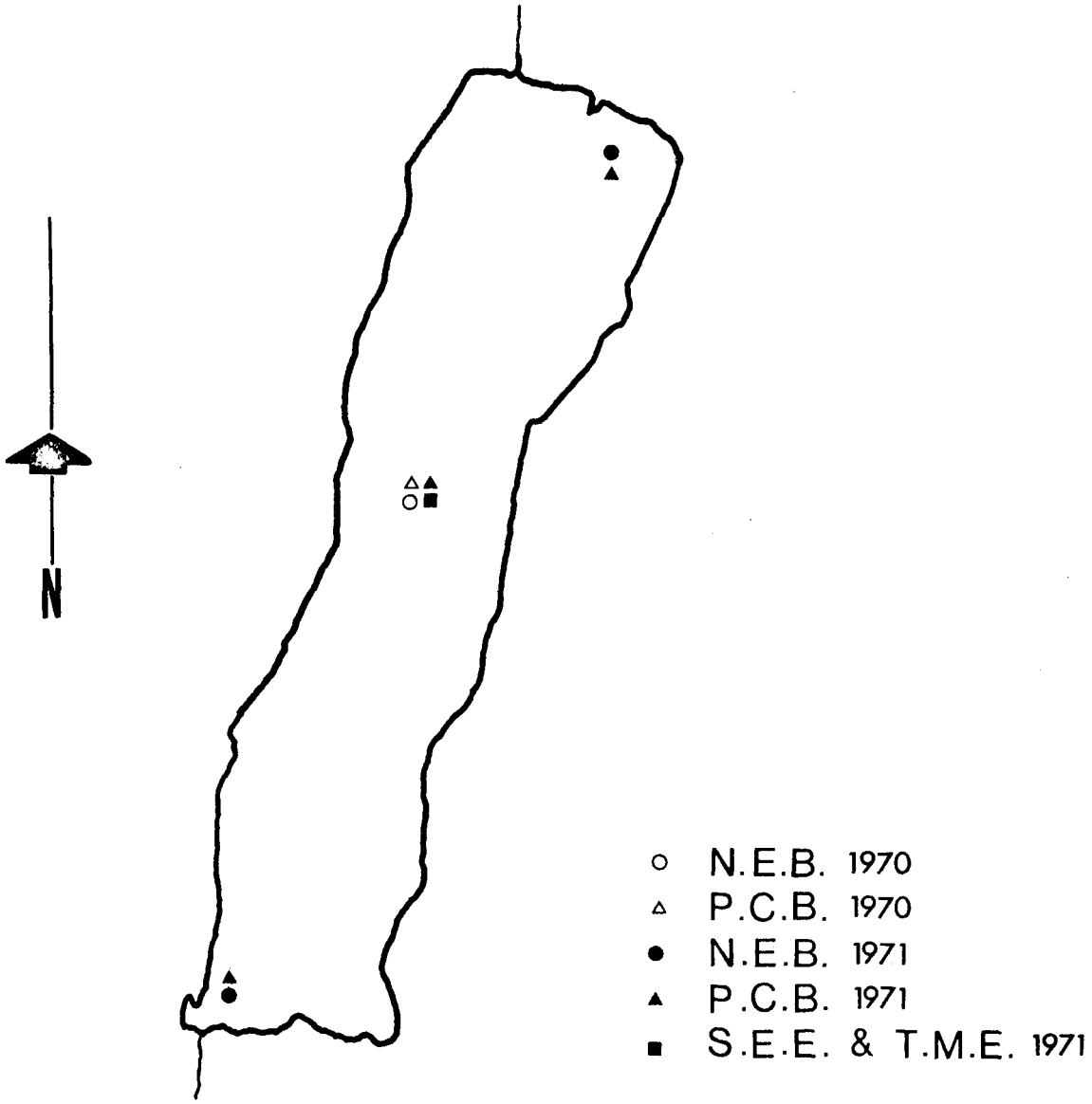
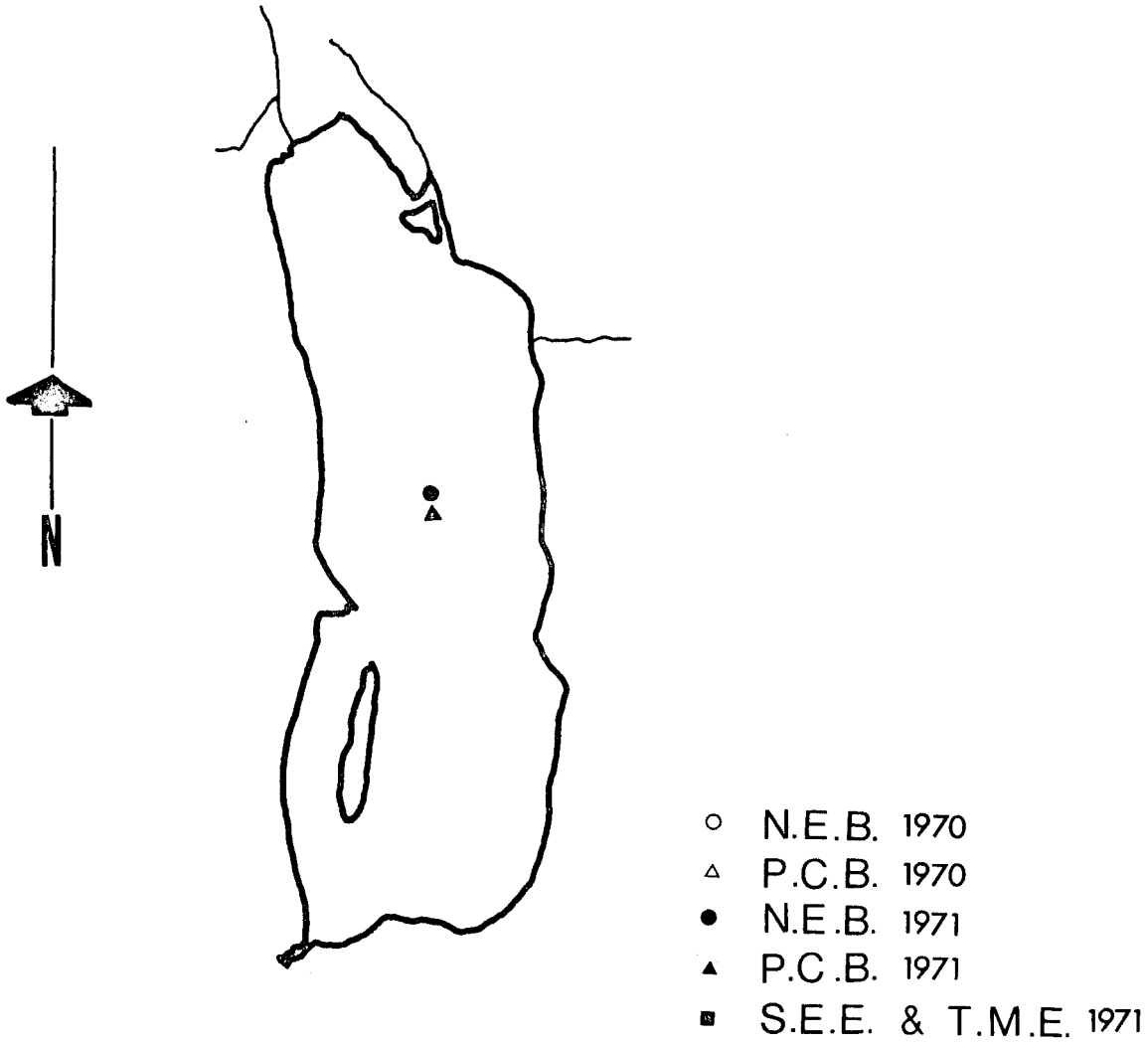


Figure 6. LOCATION OF SURFACE WATER SAMPLES FOR BIOASSAY EXPERIMENTS.



The 6 liter water sample was subdivided into 100 ml aliquots after filtering out the Zooplankton, and placed in sterile 250 ml Erlenmeyer flasks. Nutrient additions were made with sterile micropipettes in concentrations outlined in Table 1.[1] A one uci of $Na^{14}C0_3$ was added to each flask to determine the relative photo synthetic carbon uptake. The cultures were placed on a light bank (General Electric, Cool White, Mainlighter, F4OCW) at 1,750 foot candles, (18,830 lux), and illuminated from below for 15 days. The culture temperature in the first experiments (May-June) was not kept constant and varied between 25-33°C.

The cultures were gently swirled twice daily and subsampled every 5 days. At each sampling interval the pH of the cultures was recorded and 10 ml removed and filtered through a 45u Millipore filter and washed once with distilled water. The filters were then placed in scintillation vials containing 20 ml of scintillation fluid - 1 liter 1, 4-dioxane, 80 g. napthalene, 4 g. PPO, 0.4 g. dimethyl POPOP (Schindler and Holmgren, 1971). Photosynthetic carbon uptake for each culture was recorded as counts per minute (cpm) by means of the Packard Tricarb Scintillation counter at FRB laboratories, Vancouver, B.C. The relative growth rates monitored in this way provided a measure of activity for comparison among cultures in each experiment.

After 15 days growth the experiments were terminated and the remaining portion of the cultures were sampled as follows: 10 ml for measurement of carbon uptake as cpm, 20 ml filtered through a glass filter for chlorophyll-a, 20 ml placed in a vial with Lugol's solution

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1. Tables 1 to 10 on Laboratory Procedures and Results are included as Appendix II pp 52.

for algal identification, and the remaining 30 ml filtered through a 4.5u Millipore filter and dried between Parafilm sheets. These filters were later photographed for a pictorial representation of the relative effects of the various nutrient additions on algal growth.

Results of ^{14}C measurements were calculated using the following formula: cpm x (10-x) + cumulative cpm. As the subsample was 10 ml the cpm was multiplied by 10 to give the total cpm of the culture (TCPM). However, after the first subsample (10-x) was used, x being the total number of 10 ml samples removed. The cumulative cpm was the total of all radioactivity removed from the culture in earlier samples.

Commencing August 12, the nutrient enrichment experiments were repeated under more strictly controlled conditions. All field and lab procedures were identical to those previously described with the following exceptions: Only Okanagan, Skaha, and Kalamalka Lakes were sampled and tested the light bank was illuminated from below at an intensity of 400 foot candles (4,304 lux); and the culture temperature was kept at a constant 24 ± 1°C by means of a room air conditioner. Flask Nos. 2, 6, 7, 8, 9, 10, 12, 17, and 22 (Table l) were eliminated from this second run so as to accommodate all flasks from the three lakes on one light bank.

Laboratory procedure, 1971

The laboratory work followed a similar procedure to that outlined above, but with some modifications. The 6 liter water sample was subdivided into 150 ml aliquots, and to better appreciate the variability among flasks,

duplicate series were run. Concentrations of nutrients were similar to the 1970 runs but did differ in some respects (Table 2). Slightly more ^{14}C was added (1.5 uci) to compensate for the increased volume of water added to each flask, and the duration of the experiment was shortened to 9 days because results from 1970 experiments reached optimum levels after approximately 7-9 days. Samples were withdrawn at two-day intervals and the pH was not monitored since 1970 results showed little variability in readings after nine days. Fifteen mls were filtered through a 0.45u Millipore filter and placed in vials containing 15 ml of Aquasol scintillation flor. On the ninth day experiments were terminated in the following manner: 90 ml for Chlorophyll-a determination, 20 ml with Lugol addition for algal counts, and the remaining 70 ml filtered for photographic interpretation. Counts of ^{14}C were again done at the FRB laboratories, Vancouver.

Two runs of the nutrient enrichment experiments were conducted one in the spring, the other in the fall. The fall ran was on a slightly reduced scale, involving only 5 stations in 5 lakes (Figs. 1-5), and included the addition of a chelator and some trace metals (discussed later).

Pure culture bioassay

Production of inocula (Paap. 1969). The following organisms were used as inocula:

1. Selenastrum capricornutum[3]. (Chlorophyta).
2. Anabaena flos-aquae (Nitrogen fixing Cyanophyta).
3. Microcystis aeruginosa (non-nitrogen-fixing Cyanophyta),

The algae were transferred every 7 days to defined algal nutrient media (Paap, 1969 p. 10), 1 ml of inocula in 30 ml of media. These cultures were then placed on a light bank at 400 foot candles and were swirled at least four times a day for 7 days and kept at a constant temperature of 24 + 1°C. Growth was monitored daily with absorbance measurements using a Bausch & Lomb, Spectronic 70, spectrophotometer.

The pure culture bioassay in 1971 went into more depth than the experimentation of the year before. It followed the same basic procedure, except the light intensity and temperature were decreased to 300 ft. candles and 21.1°C, respectively. No spectrophotometric readings were taken, as the uptake of ^{14}C was thought to be a sufficient monitor of rotative growth among cultures.

Field procedure (Paap tests) 1970. Water samples were collected from various locations in each of the five Okanagan Basin lakes (Figs. 1-5). One liter was obtained for chemical analyses and another for testing the latent fertilizing capacity of each lake station i.e. Paap test.

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3. Obtained from the National Eutrophication Research Program, Pacific Northwest laboratory, 200 South 35th Street, Corvallis, Oregon, 97330, U.S.A.

Field procedure, 1971. Surface water samples were collected from 26 stations in the six Okanagan Basin lakes tested (Figs. 1-6). No chemical analyses were performed on these samples.

Laboratory procedure (Paap tests), 1970. The water samples were filtered through O.45)u Millipore filters, and 100 ml of the filtered water was then placed in a 250 ml sterilized Erlenmyer flask. To this, 2 ml of synchronous 7 day-old culture inocula of each algal species was added plus 1.0 uci of $Na^{14}CO_3$. The experiment was set up in duplicate and the pH monitored in selected flasks.

Duplicate flasks of each test organism in defined algal nutrient media were Included as controls for algal growth comparison. These flasks contained 50 ml of algal nutrient media, 1.0 ml of culture inocula, and 1.0 uci of $Na^{14}CO_3$.

All cultures were placed on a light bank (400 foot candles) for 9 days at 24° + 1°C and swirled at least four times daily. Every second day the absorbance and transmittance at 600 mu was measured, and every third day photosynthetic carbon uptake was monitored using ^{14}C techniques previously described.

The pure culture bioassay experiment was repeated one month later, with some modifications: Fewer stations in only Okanagan, Skaha, and Kalamalka Lakes; only one flask per test organism per station; and the cultures were continuously shaken at 80 oscillations/minute with overhead illumination at 400 foot candles.

Laboratory procedure (Paap test), 1971. The procedures were identical to those outlined above, except that the incubation period was only 6 days and spectrophotometric measurements were excluded.

Subsampling was at two-day intervals with termination procedures including an analysis for Chlorophyll-a (35 ml) and photography (35 ml). ^{14}C was used to monitor the relative algal growth among flasks.

Sewage effluent experiments.

In 1971 a sewage effluent experiment was conducted to determine the effects of sewage enrichment on natural phytoplankton populations of five lakes in the Okanagan Basin (Vaseux Lake was excluded). It was designed to test the effectiveness of tertiary treatment facilities, currently in operation at the Penticton sewage treatment plant. Since sewage addition to the lakes in the past has led to many of the current water quality problems, such experiments were more than justified.

Field procedure, 1971. Surface lake water from each lake was obtained from areas free of the direct influence of effluent discharge (Figs. 1-5). Sewage was collected from each of six different stages of treatment at the Penticton plant: 1) raw sewage; 2) after primary treatment; 3) mixed liquor; 4) final after 2' treatment (non-chlorinated); 5) final after 2' treatment (chlorinated); 6) final after 3' treatment (chlorinated). Removal of PO_4(P) at time of sampling the 3' effluent was estimated to be between 40 - 50%.

Laboratory procedure, 1971. The procedure was identical to that of the nutrient enrichment experiment, 1971, except that varying concentrations of sewage were added to each flask in place of defined concentration of nutrients (Table 3). One uci of ^{14}C was added, and different volumes of sample were filtered for Chlorophyll-a analysis (50 ml), algal counts (10 ml), and photography (35 ml).

Trace metal experiments, 1971.

These experiments were designed to test the effects of the nutrients NO_3(N) and PO_4(P), in combination with some trace metals and the chelator EDTA on the growth of natural phytoplankton populations in five of the Okanagan Basin lakes.

Field procedure. Samples were obtained from the surface waters of the five major lakes, Vaseux Lake excluded (Figs. 1-5).

Laboratory procedure. Sixty-three flasks, which included seven for the fall run of the nutrient bioassay, were set up for each lake. The procedure was identical to that of the spring nutrient enrichment experiment, except that nutrients and trace metals were added in slightly different concentrations and combinations (Table 4.).

Flasks were placed on the light bank at a light intensity of 300 ft. candles. Growth was terminated after 9 days, with 4 subsamples taken within that period. Samples at termination included: 35 ml for Chlorophyll-a, 10 ml for algal counts, and 45 ml for photography.

RESULTS AND DISCUSSION

Introduction.

The format selected for this report is that of a general summary, presenting broad interpretations of experimental results. Detailed findings in graphical form with all supplimentary data are presented in an appendix to this report. This type of presentation will allow the general non-scientific reader to better appreciate the general conclusions drawn from the bioassay program without the encumbrance of minute detail and masses of data. For the reader seeking a more in-depth appreciation of the study, the appendix will serve his purposes well.

The initial study objective was to determine what nutrients were limiting phytoplankton growth in the Okanagan Lakes. The results of the nutrient enrichment experiments provided tentative answers to this question. Pure culture bioassay experiments provided information about what regions or specific water masses in each lake contained "residual" nutrients, stimulatory to the test algae. In 1971, a few selected trace metals and one chelator were added to natural phytoplankton populations to determine their role in regulating algal growth. Since sewage discharge to the Okanagan lakes is one of the primary sources of nutrients, additional experiments were run to determine the fertilising potential of various types of raw and treated sewage on natural phytoplankton populations.

In each of the aforementioned experiments ye used several techniques to monitor growth response, i.e. Chlorophyll a, ^{14}C activity, species succession. Each method had inherent limitations, but in union a better appreciation of mechanisms involved in regulating algal growth in each lake were obtained. It must be borne in mind that extrapolation from changes observed in flasks kept at constant temperature and light conditions to processes actually occurring within lakes is difficult and often subject to a wide range of errors. Nonetheless, such an approach does enable an investigator to gather an appreciation of the influence of various chemicals, both alone and in combination, in regulating natural phytoplankton production. With suitable caution in interpretation, this technique does offer the only means of obtaining valuable information that could not be gathered by any other means.

The time limitations imposed by the study presented another problem; that of studying in enough detail six lakes with four types of experimental procedure. Ideally, it would have been better to concentrate efforts on one or two lakes, noting seasonal variability in nutrient concentration and relating these data to observed phytoplankton succession, then correlating these data with results from flask experiments. Within the time and budgetary constraints of this study this could not be achieved.

A. NUTRIENT ENRICHMENT BIOASSAY (NEB).

Results of each experiment will be discussed on a lake-to-lake basis followed by a discussion of salient findings from each lake for that particular experiment. Immediately following the discussion for the last experiment, a summary is presented. Any reader wishing to eliminate the discussion of each experiment may go directly to this summary.

Osoyoos Lake

Based on a number of biological and chemical observations, Osoyoos, the most southern lake in the Okanagan chain, appears to currently exhibit characteristics of a meso-eutrophic lake. Its most obvious nutrient source appears to be the sewage effluent of the Oliver S.T.P. which discharges into the Okanagan River, a few kilometers above the lake. Agricultural runoff also provides a moderate supply of nutrients to the lake.

Results of the 1970 NEB showed phytoplankton growth in Osoyoos could be stimulated by adding a small amount of phosphorus (0.03 mg/l as PO_4(P)) and increasing the nitrogen concentration from 0.21 to 7.0 mg/l NO_3(N). Growth in most experiments was directly proportional to the amount of nitrogen added. However with increasing concentrations of phosphorus alone or with low NO_3 alone, there was no stimulation. The highest algal yield (greatest growth) occurred in flasks enriched with small amounts of NO_3(N) and PO_4(P), 0.07 and 0.03 mg/l, respectively,

and increasing concentrations of CO_2. At the higher concentrations of CO_2 growth was inhibited due to a very high pH.

In repeat experiments of the NEB in 1971, increasing concentrations of NO_3(N) alone or PO_4(P) alone did not stimulate growth much beyond that observed in controls (Fig. 8). However, NO_3(N) and PO_4(P)when added together at the highest concentrations, 0.7 and 0.09 mg/l, promoted growth about ten times greater than the control (Fig. 8). In every NEB experiment in both years, NO_3(N) and PO_4(P) added together was most stimulatory to algal growth.

Vaseux Lake

Vaseux is a small lake located between Osoyoos and Skaha. From cursory observation, it appears that this lake acts as a nutrient sink, effectively utilizing nutrients that enter it via the Okanagan River and from various diffuse sources around the lake. The lake is very shallow, and supports a very dense growth of rooted macrophytes and periphyton in its broad littoral zone. No evidence of nuisance algal "blooms" have been observed. Vaseux was tested with the NEB in 1971 only.

Additions of NO_3(N) alone at both concentrations produced algal growth slightly greater than observed in the control (Fig. 9). PO_4(P) when added alone promoted more growth than NO_3(N) alone, with a yield about twice that noted in the control (Fig. 9). NO_3(N) and PO_4(P) added together at the lowest concentration had little stimulatory effect (Fig. 9), but at the highest concentrations, growth was about ten times that of the control (Fig. 9).

Figure 8. NUTRIENT ENRICHMENT BIOASSAY RESULTS

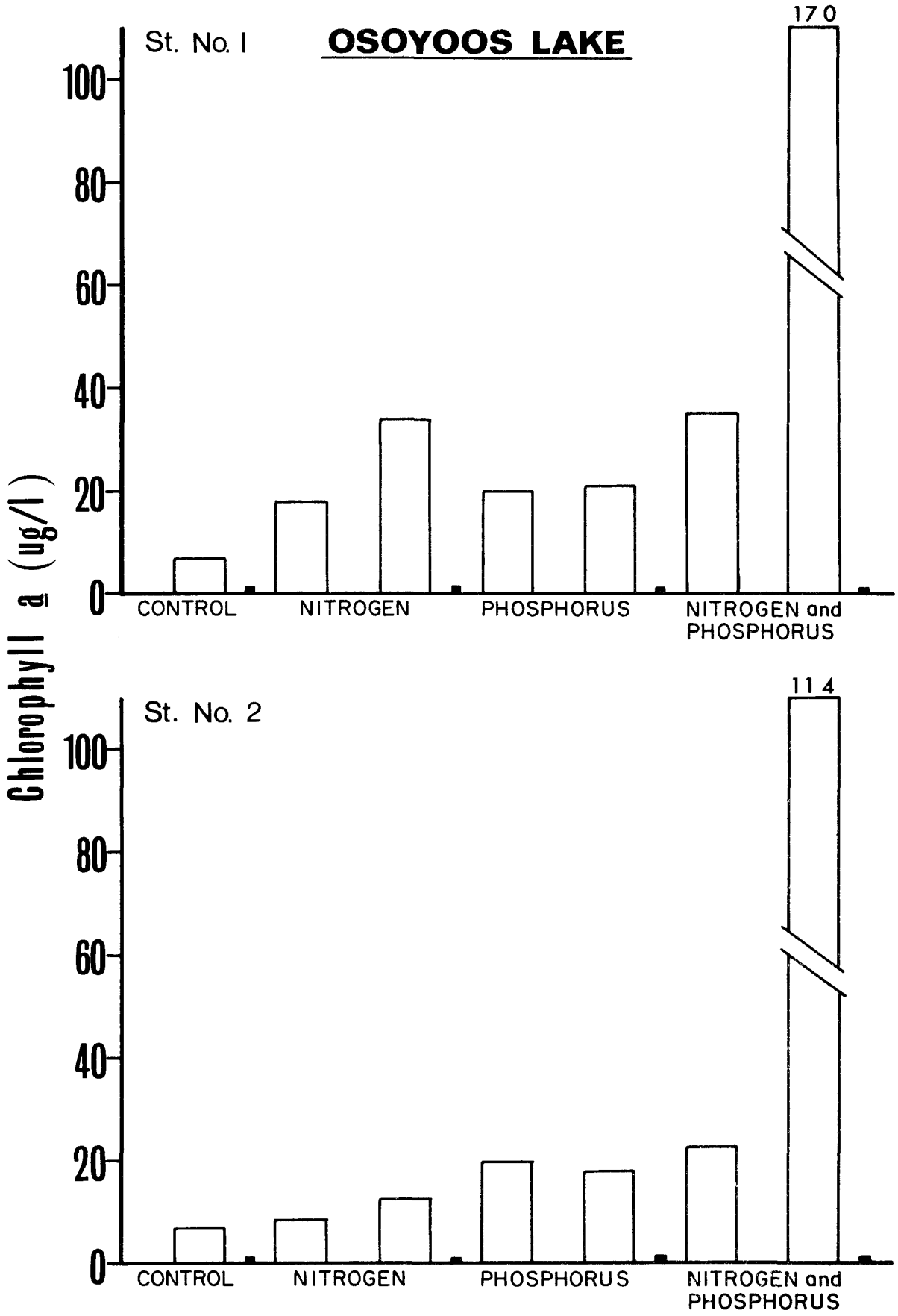

Figure 9. NUTRIENT ENRICHMENT BIOASSAY RESULTS