APPENDICES A, B, AND C

FOR

WATER QUALITY STANDARDS

STUDY REPORT

March 1982

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APPENDIX A

UNLV Procedures Manual

METHODS FOR BIOLOGICAL, CHEMICAL, AND PHYSICAL ANALYSES IN RESERVOIRS

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INTRODUCTION

This manual contains detailed descriptions of biological, chemical and physical sampling and analytical procedures used at the Lake Mead Limnological Research Center. The manual was prepared in response to requests made by numerous individuals involved in the Las Vegas Valley Water Quality Program. This manual represents what we consider to be a reasonably complete compilation of methods useful in studying both general and specific limnological questions. While each section is sufficiently detailed to be of use to investigators with little experience in limnological methodology, the methods can be used on a wide variety of lakes and reservoirs, with relatively little modification.

SECTION I. BIOLOGICAL METHODS

Procedures for Collection, Measurement of Growth Rate (Dry Weight and Ash Free Weight), Chlorophyll-a Content, Species Diversity, Nitrogenase Activity, Productivity and Alkaline Phosphatase Activity of Periphyton

1. Collection of Samples

A. Materials

1. Artificial substrates, in situ incubation apparatus (see B., below)
2. Ice chest
3. Atomizer

B. Procedure

Rectangular pieces of fiberglass (5 cm x 15 cm) used as substrates for the collection of periphyton are supported vertically by polyvinylchloride tubing, as shown in Fig. 1. Each sample unit consists of two rectangles riveted onto opposite sides of the PVC tube. The units are then placed onto pieces of PVC tubing of smaller diameter attached to the holding racks. The holding apparatus for limnetic stations consists of PVC pipes, arranged in a double-T configuration, and is suspended by lines from a buoy at the desired depth (Fig. 1A). The holding apparatus for littoral stations consists of a cement slab which is placed on the surface of the sediment (Fig. 1B). After an approximate incubation period of two weeks, the substrate units are collected by SCUBA and returned to the laboratory in an ice chest; samples are prevented from desiccation by frequent misting with an atomizer containing lake water.

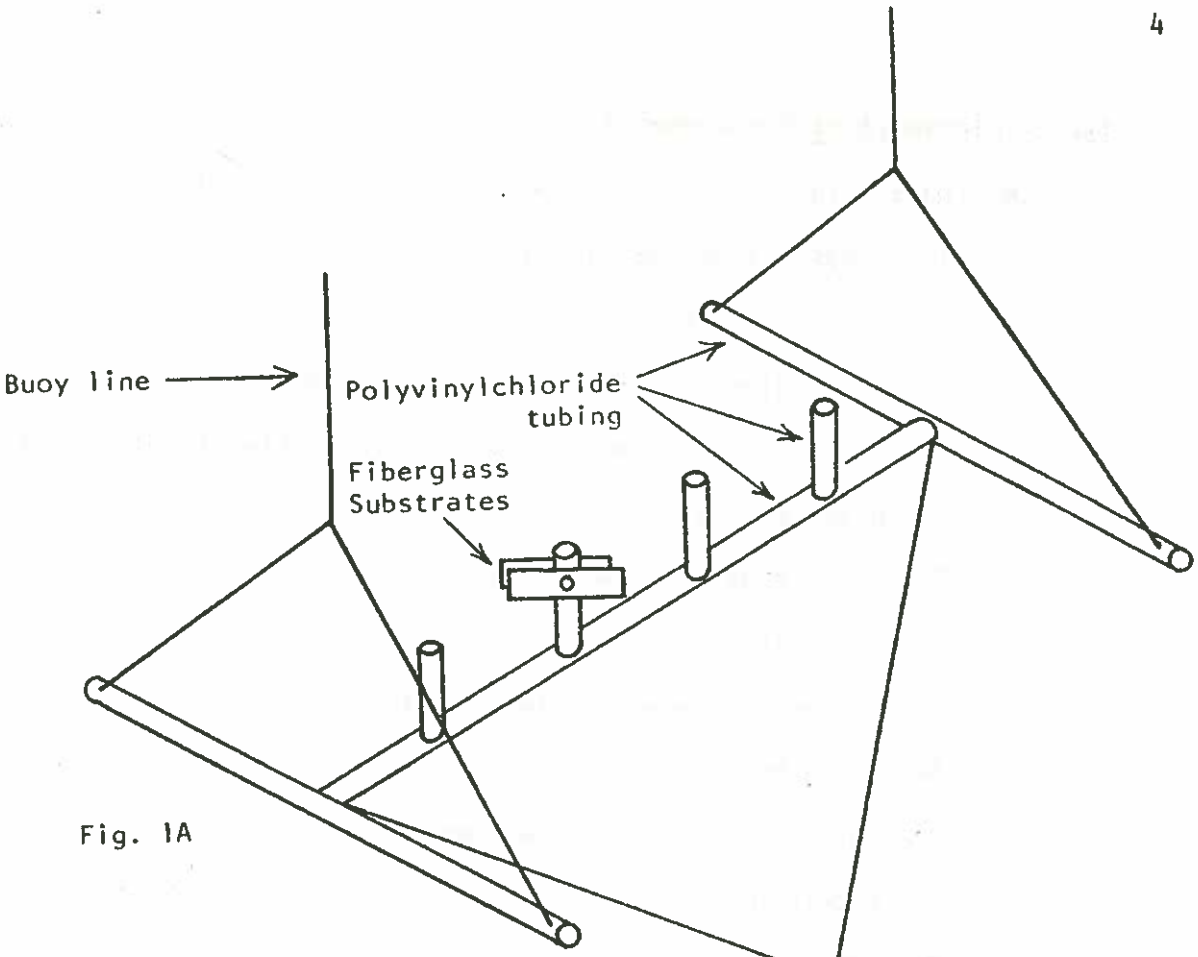


Fig. 1A

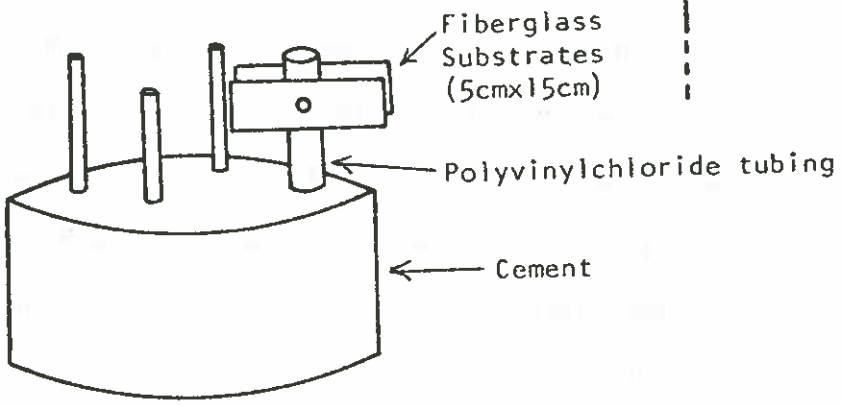


Fig. 1B

Figure 1. In situ incubation apparatus for sampling periphyton growth in the limnetic (1A) and littoral (1B) zones of reservoirs.

II. Chlorophyll-a Measurements

A. Materials

1. Glass microscope slides
2. Rubber policeman
3. GF/C, glass fiber filters, 25 mm diameter
4. Millipore^{R} filtration apparatus, capable of holding 25 mm diameter filters
5. Centrifuge tubes with caps (10 ml capacity)
6. Centrifuge
7. Methanol, reagent grade, absolute
8. 0.1 N HCl
9. Autopipet capable of delivering 105 µl volume (e.g. Pipetman^{R})

B. Procedure

1. As soon as possible after collection, 25 cm^2 areas are measured on replicate substrate units for each station and the periphyton within each area is scraped with the edge of a glass slide and a rubber policeman. The samples are then transferred to GF/C glass fiber filters and excess water removed by filtration. The filters are then transferred to capped centrifuge tubes and stored frozen until analysis, usually conducted within 24 hours.
2. At the time of analysis, the samples are brought to room temperature in the dark. A 10-ml volume of methanol is added to each tube. The tubes are placed in complete

darkness for 1 hour at room temperature to extract the chlorophyll (Holm-Hansen and Riemann 1978). Samples are then centrifuged for 10 minutes and 3.2 ml aliquots transferred to 1 cm cuvettes. Absorbances at 750 nm and 665 nm are then read against a methanol blank (used to zero the machine) on a Coleman 55 Spectrophotometer before and after acidification with 105 µl of 0.1 N HCl.

C. Chlorophyll-*a* and phaeophytin-*a* concentrations are calculated using Tett's (1975, 1977) formulas:

$$\text{Chl-}a\ (\text{mg}\cdot\text{m}^{-2}\cdot\text{da}^{-1}) = \frac{G}{1-GJ}[(E^U-E^A) - JGE^A(H-1)]\ \frac{v}{V} \div t$$

$$\text{Phaeo-}a\ (\text{mg}\cdot\text{m}^{-2}\cdot\text{da}^{-1}) = \frac{G}{1-GJ}(HE^A-E^U) \times \frac{v}{V} \div t$$

where $G = 0.029\ \text{cm}\cdot\text{mg}\cdot\text{ml}^{-1}$

$J = 5.9\ \text{ml}\cdot\text{cm}^{-1}\cdot\text{mg}^{-1}$

$H = 2.0$

E^U = absorbance at 665 nm before acidification corrected for background absorbance at 750 nm

E^A = absorbance at 665 nm after acidification corrected for background absorbance at 750 nm

v = extraction volume (ml)

V = subsample area (m^2)

t = incubation time in days

Note: G, J, and H are constants determined by Robert C. Furtek, U.S.E.P.A., EMSL-LV, Las Vegas, NV, using methods in Tett (1975).

III. Growth Rate Measurements: Dry and Ash Free Weights ($mg \cdot m^{-2} \cdot da^{-1}$)

A. Materials

1. Glass microscope slides
2. Rubber policeman
3. Aluminum weighing dishes
4. Muffle furnace (Thermolyne and Thermolyne Furnatrol I)
5. Drying oven
6. Analytical balance capable of weighing to nearest 0.1 mg

B. Procedure

As soon as possible after collection, a 25 cm^2 or 50 cm^2 area is scraped from replicate substrate units for each station with a glass slide and a rubber policeman. Subsamples are rinsed into aluminum pans which have been precombusted at 550°C for 1 hour and pre-weighed. The subsamples are dried for 24 hours to a constant weight at 105°C in a drying oven and weighed to the nearest 0.1 mg. They are then ashed for 1 hour at 550°C in a muffle furnace (Vollenweider 1969, p. 36). The ashed sample is then rewetted with distilled water to reintroduce the waters of hydration, and again dried to constant weight at 105°C, and weighed to the nearest 0.1 mg (APHA 1975, p. 1048).

C. Calculations:

Dry weight ($mg \cdot m^{-2} \cdot da^{-1}$) =

$$\frac{\text{total wt. after drying (mg) - tare wt. (mg)}}{\text{subsample area } (m^2) \cdot \text{incubation time (da)}}$$

Ash free dry weight ($mg \cdot m^{-2} \cdot da^{-1}$) =

$$\frac{\text{total wt. after drying (mg) - wt. after ashing (mg)}}{\text{subsample area } (m^2) \cdot \text{incubation time (da)}}$$

IV. Species Identifications

A. Materials

1. Polyethylene bottles
2. Acid-Lugols solution - prepared by dissolving 10 g iodine crystals and 20 g potassium iodide in 200 ml distilled-deionized water. Add 20 ml glacial acetic acid a few days prior to using (Vollenweider 1969).
3. Glass microscope slides
4. Glass coverslips
5. Compound microscope capable of 450X magnification
6. Hot plate
7. Muffle furnace (Thermolyne and Thermolyne Furnatrol I)
8. Hyrax mounting medium

B. Procedure

1. Subsamples for species identification are scraped from periphyton samplers, and brought up to a volume of 100 ml with distilled water containing 1 ml acid-lugols solution, and stored in polyethylene bottles in the dark. A subsample of this preparation is then pipetted onto a glass slide and observed at 450X magnification.
2. A separate slide for identification of diatom species is prepared as follows: several drops of the sample prepared as above are placed on a coverslip and evaporated

to dryness on a hot plate. The residue is then combusted at 500°C in a muffle furnace for 0.5 to 0.75 hours and mounted in Hyrax mounting medium on a glass slide. Microscopic observations are made at 450X magnification.

3. Proportional cell counts (at least 200 cells) are made on the wet mount.

C. Calculations

Each individual cell is measured and the volume calculated by approximation to an appropriate geometrical shape. The mean cell volume for each species is then derived. Biovolume ($\mu 3$) is obtained by multiplying the cell volume by the cell number. The dominant five species according to biovolume, thus determined, are ranked and recorded.

V. Nitrogenase Activity

The acetylene reduction assay of nitrogenase activity in periphyton communities is similar to that used in phytoplankton communities (Flett et al. 1975). Care must be taken to avoid physically disrupting the structural integrity of the periphyton community since nitrogenase activity may occur in microzones. Therefore, slight modifications in the procedure of the addition of acetylene gas are incorporated to preserve the physical structure of communities.

A. Materials

1. Acetylene gas transported in football bladders
2. Various-sized (e.g. 5, 10 and 50 ml) Luer-lok syringes (plastic disposable, Becton-Dickinson type), with 25 gauge, 3/4 in. needles

3. Mason jars (pints) fitted with Vacutainer^R stoppers and seals, through which gases are injected and withdrawn
4. Formalin (10 ml added per 400 ml lake water for blank)
5. 500 ml polypropylene graduated cylinder
6. Vacutainers^R, 4 ml, red stopper, non-sterile evacuated tubes, Becton-Dickinson
7. Steel plate apparatus (Fig. 2) fitted with holders for Mason jars

B. Procedure

1. For each station, fill 4 Mason jars of known volume with 400 ml lake water each. Add 10 ml formalin to one jar to use as a blank.
2. Seal jars tightly and inject 40 ml C_2H_2 gas through septum.
3. Shake vigorously for approximately one minute.
4. Remove lid, and carefully place substrate community (rock) into jar. Reseal lid tightly and add 7 additional ml C_2H_2 gas. Do not shake.
5. The vessels are then secured to the holder plate and returned to the site from where the sample was taken, or to an area of comparable light intensity and temperature.
6. Incubations are conducted for 4 hours.
7. At the end of the incubation period, the vessels are removed from the plate, and shaken for approximately one minute.
8. From each vessel 3 ml of gas from the headspace is withdrawn. The syringe is pumped several times into the head-

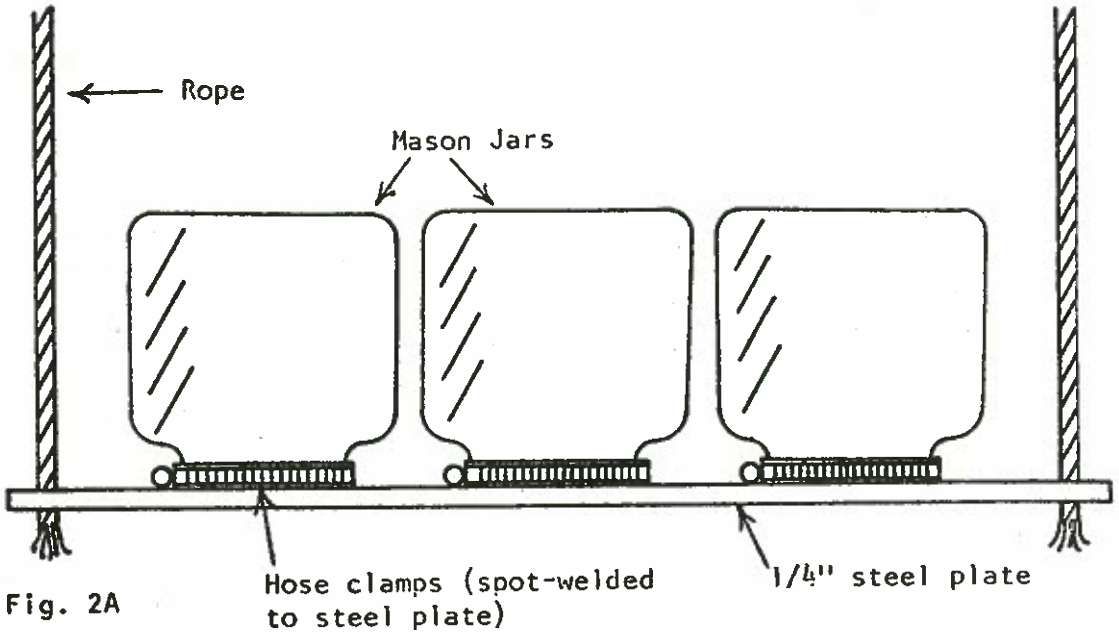


Fig. 2A

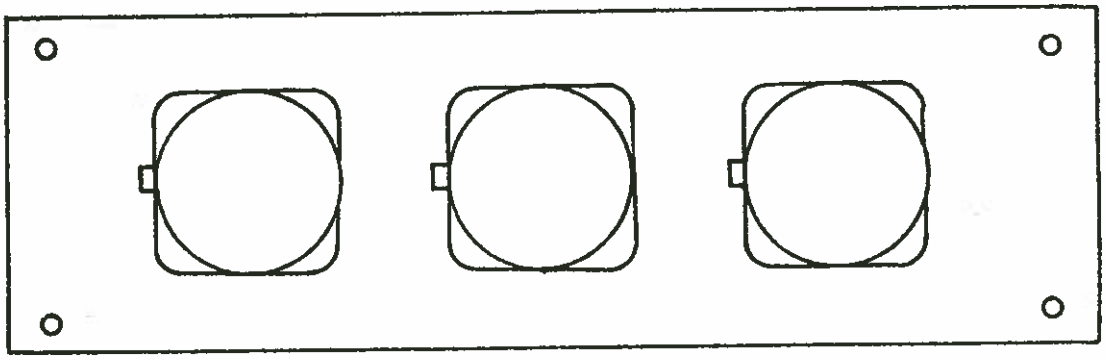


Fig. 2B

Figure 2. In situ incubation apparatus for measurement of nitrogenase activity in periphyton by the acetylene reduction method. Fig. 2A: Side view of incubation vessels in place with apparatus suspended from buoy. Fig. 2B: Top view of apparatus.

space to assure that a pure sample of headspace is obtained. The sample is then transferred to a Vacutainer^{R} which has been pre-labeled with date, location, and vessel number. Note exact time of removal of subsample and temperature of water at time of subsampling. Subsample may be replicated, but the total gas volume removed should not exceed 7 ml, in order to maintain positive or neutral pressure within the vessel and avoid contamination of the subsample with air during transfer.

9. Upon return to the laboratory, the periphyton from each rock substrate is removed by scraping and placed into a pre-weighed, disposable aluminum weighing dish. Dry weights are determined as in III., this section.

10. To estimate the surface area originally covered with periphyton, Parafilm^{R} is then stretched over the region of the rock from which the periphyton was removed. The Parafilm^{R} is placed on paper, its outline traced, and its area determined by planimetry.

C. Sample Analysis

1. Prepare gas chromatograph (see Appendix D, page 69).
2. Run standard curve (see Appendix E, page 71).
3. Allow Vacutainer to reach atmospheric pressure by placing syringe needle (without syringe) into septum for approximately 30 seconds. Do not allow needle to remain in septum for longer than 30 seconds, since the gases will leak.
4. To subsample Vacutainer, using a 1-ml plastic syringe, slowly pump plunger 2-3 times, and depress to the 0.3-ml

mark before removing needle from Vacutainer.

5. Immediately place needle into injection port of gas chromatograph, and slowly and steadily inject sample; ensure that Range and Output of gas chromatograph are appropriately set for specific compound.

D. Calculations

1. Determine concentration of C_2H_4 in subsample of Vacutainer^R by entering peak height of sample (corrected for background C_2H_4) into regression line of peak height (chart units x Range x Output) vs. amount of C_2H_4 (nmoles)

2. To calculate rate of ethylene production:

$$\text{nmoles } C_2H_4 \cdot h^{-1} = \frac{\text{nmoles } C_2H_4 \times (B-b) \times \frac{S}{s}}{v \times t \times c}$$

where nmoles C_2H_4 is determined in 1, above,

B = volume of vapor phase (ml) in incubation vessel

b = volume of rock substrate

S = volume of Vacutainer (ml)

s = volume of gas (ml) subsampled from vapor phase of incubation vessel and transferred to Vacutainer

v = volume of gas (ml) injected into gas chromatograph

t = incubation time (h)

c* = transfer coefficient for ethylene

*Refer to Appendix F, p. 74. In this case B must be corrected for b, the volume of the rock substrate. Thus the equation for ethylene transfer coefficient is:

$$\frac{X}{m} = \frac{1}{1 + (\alpha A/B-b)}$$

3. To express the rate of C_2H_4 production in terms of biomass (dry weight) and surface area, the value determined in (1.), above, is divided by biomass (dry weight), determined in B.9. (above) and by surface area determined in B.10. (above), respectively.

VI. Productivity

A. Materials

1. Jars (approximately 2 liter-capacity) fitted with screw caps and seals; 3 clear and 3 opaque, covered with black electrical tape
2. Steel plate apparatus (Fig. 2) fitted with holders for jars
3. Thermometer
4. Dissolved oxygen meter (e.g. Y.S.I.)

B. Procedure

1. Each of six replicate bottles, three light and three dark, is filled with lake water which is allowed to overflow.
2. The initial temperature and dissolved oxygen concentration of water in each jar are measured and recorded.
3. Six rocks with periphyton are taken from the sampling site and one placed in each jar.
4. The jars are held underwater to remove all air bubbles and tightly sealed.
5. The jars are inverted and secured to the steel plate apparatus and returned to the site from where they were taken, or to an area of comparable light and temperature.

6. Incubations are conducted for 4 hours.
7. At the end of the incubation period, the jars are removed from the plate, opened, and the final temperature and dissolved oxygen concentration of the water are determined.
8. Upon the return to the laboratory, the volume of water contained in each incubation vessel is measured and the periphyton from each rock substrate is removed by scraping and placed in a pre-weighed, disposable aluminum weighing dish.
9. Parafilm^{R} is then stretched over the rock to estimate the surface area covered with periphyton. The Parafilm^{R} is then placed on paper and its outline traced. The area of the figure is then determined by planimetry.
10. Data collected are reported with the date, location, and time of sampling.

C. Calculations

1. To calculate productivity per unit surface area per time:

$$\text{Productivity } (\text{mg } O_2 \cdot m^{-2} \cdot h^{-1}) = \frac{(D.O._f - D.O._i)}{S \times t} \times V$$

Where $D.O._f$ = dissolved oxygen concentration at end of incubation period ($mg \cdot l^{-1}$)

$D.O._i$ = dissolved oxygen concentration at beginning of incubation period ($mg \cdot l^{-1}$)

S = surface area (m^2) of rock covered with periphyton

t = length of incubation period (h)

V = volume (l) of water in incubation vessel

2. To calculate productivity per unit dry weight per unit time:

$$\text{Productivity (mg } O_2 \cdot g^{-1} \cdot h^{-1}) = \frac{D.O._f - C.O._i}{D.W. \times t} \times V$$

where $D.O._f$ = dissolved oxygen concentration at end of incubation period ($mg \cdot l^{-1}$)

$D.O._i$ = dissolved oxygen concentration at beginning of incubation period ($mg \cdot l^{-1}$)

D.W. = dry weight (g) of periphyton

t = length of incubation period (h)

V = volume (l) of water in incubation vessel

VII. Alkaline Phosphatase Activity

The assay for alkaline phosphatase activity in periphyton communities is similar to that used in phytoplankton communities (Perry 1972). Samples of periphyton, 1 to 2 cm^2 in area are scraped from artificial substrates (see I.B., above) and suspended in 100 ml distilled-deionized water. A 6-ml subsample is then analyzed according to the method for phytoplankton (Perry 1972).

A. Materials and Reagents

1. Turner 110 or 111 Fluorometer
2. Filters
   a. Primary 47B [Wratten gelatin filter (Kodak), cat. no. 149 5795]

b. Secondary 2A [Wratten gelatin filter (Kodak), cat. no. 164 4988]

12 [Wratten gelatin filter (Kodak), cat. no. 149 5522]

Filter 47B is used alone, placed closest to lamp. Filters 2A and 12 are used in combination and are placed near the photomultiplier tube, #12 on the inside, #2A on the outside.

3. Test tubes, Bausch and Lomb Spectronic 20 Colorimeter, 1/2"

4. Pipets

   i. Automatic (capable of delivering 1.0, 2.0, and 3.0 ml), e.g. Eppendorf or Oxford

   ii. Volumetric (1-5 ml capacity)

   iii. Serological (10 ml capacity) for pipetting water samples

5. $MgCl_2$ (3%)

   Dissolve 3.0 g analytical reagent grade $MgCl_2$ in 100 ml distilled-deionized water.

6. Hydrochloric acid (concentrated)

   Use only analytical reagent grade.

7. Tris buffer (1.0 M, pH 8.6)

   a. Dissolve 121.1 g Trizma^R Base in ~950 ml distilled-deionized water.

   b. Add 10.0 ml 3% $MgCl_2$.

   c. Add 20.0 ml concentrated HCl, slowly.

   d. Bring volume to 1000 ml with distilled-deionized water.

e. Mix well and check pH. If it is not between 8.55 and 8.65, add HCl or NaOH to adjust.

f. Autoclave at 115-120°C for 15 minutes; store in refrigerator.

8. Substrate: 3-O-methylfluorescein phosphate (5.00 $\mu g \cdot ml^{-1}$) (Sigma Chemical Co.)

a. Dissolve 12.50 mg methylfluorescein phosphate in 10 ml cold Tris buffer (made up as in 7., above) (concentration = 1.25 $mg \cdot ml^{-1}$). If stored, place in freezer in dark.

b. Dilute 1.0 ml of 1.25 $mg \cdot ml^{-1}$ methylfluorescein phosphate to 250 ml with cold Tris buffer (made up as in 7., above).

c. Transfer to acid-cleaned screw cap test tubes. Store frozen in dark. Substrate should remain frozen until immediately before use (see Note B, p. 29).

9. EDTA (0.1 M)

Dissolve 9.305 g analytical reagent grade EDTA into 250 ml distilled-deionized water. Store in refrigerator.

10. Standards: Methylfluorescein (Sigma Chemical Co.)

a. Methanol, absolute, analytical reagent grade

b. 1.0 N NaOH

Dissolve 40 g analytical reagent grade NaOH in 1000 ml distilled-deionized water. Store at room temperature in polyethylene bottle.

c. Primary standard (0.50 $mg \cdot ml^{-1}$)

Dissolve 50.00 mg methylfluorescein in 100 ml absolute, reagent grade methanol. Store in polyethylene or glass in dark at -5°C.

d. Secondary standard (0.005 $mg \cdot ml^{-1}$)

Prepare on day of analysis. Add 1.0 ml of the primary standard (made up as in 10.c., above) to ∿85 ml distilled-deionized water and 5 ml of 1.0 N NaOH in a 100 ml volumetric flask. Bring volume up to 100 ml, mix well.

e. Working standards

Prepare on day of analysis, from secondary standard (0.005 $mg \cdot ml^{-1}$).

For window 3X, dilute

$$\frac{1.0 \text{ ml of } 0.005 \text{ mg} \cdot \text{ml}^{-1}}{100 \text{ ml}} = 50 \text{ ng} \cdot \text{ml}^{-1}$$

For window 1X, dilute

$$\frac{3.0 \text{ ml of } 0.005 \text{ mg} \cdot \text{ml}^{-1}}{100 \text{ ml}} = 150 \text{ ng} \cdot \text{ml}^{-1}$$

i. To run standard curve, make dilutions of working standards (above) using volumetric pipets (1 to 20 ml capacity) and 10 ml graduated cylinder.

ii. Add appropriate amount of working standard (either 50 $ng \cdot ml^{-1}$ or 150 $ng \cdot ml^{-1}$) (see below, iv.), 1 ml EDTA, and 3 ml Tris buffer to graduated cylinder. Bring volume to 10 ml with

distilled-deionized water.

iii. Mix well and transfer to fluorometer tube.

iv. Make dilutions as follows:

For window 3X

$$\frac{1 \text{ ml of } 50 \text{ ng}\cdot\text{ml}^{-1}}{10 \text{ ml}} = 5 \text{ ng}\cdot\text{ml}^{-1}$$

$$\frac{2 \text{ ml of } 50 \text{ ng}\cdot\text{ml}^{-1}}{10 \text{ ml}} = 10 \text{ ng}\cdot\text{ml}^{-1}$$

$$\frac{3 \text{ ml of } 50 \text{ ng}\cdot\text{ml}^{-1}}{10 \text{ ml}} = 15 \text{ ng}\cdot\text{ml}^{-1}$$

$$\frac{4 \text{ ml of } 50 \text{ ng}\cdot\text{ml}^{-1}}{10 \text{ ml}} = 20 \text{ ng}\cdot\text{ml}^{-1}$$

$$\frac{5 \text{ ml of } 50 \text{ ng}\cdot\text{ml}^{-1}}{10 \text{ ml}} = 25 \text{ ng}\cdot\text{ml}^{-1}$$

For window 1X

$$\frac{1 \text{ ml of } 150 \text{ ng}\cdot\text{ml}^{-1}}{10 \text{ ml}} = 15 \text{ ng}\cdot\text{ml}^{-1}$$

$$\frac{2 \text{ ml of } 150 \text{ ng}\cdot\text{ml}^{-1}}{10 \text{ ml}} = 30 \text{ ng}\cdot\text{ml}^{-1}$$

$$\frac{3 \text{ ml of } 150 \text{ ng}\cdot\text{ml}^{-1}}{10 \text{ ml}} = 45 \text{ ng}\cdot\text{ml}^{-1}$$

$$\frac{4 \text{ ml of } 150 \text{ ng}\cdot\text{ml}^{-1}}{10 \text{ ml}} = 60 \text{ ng}\cdot\text{ml}^{-1}$$

$$\frac{5 \text{ ml of } 150 \text{ ng}\cdot\text{ml}^{-1}}{10 \text{ ml}} = 75 \text{ ng}\cdot\text{ml}^{-1}$$

v. Place tubes in dark for at least 15 minutes prior to reading, to minimize background fluorescence.

B. Procedure

1. As soon as possible after collection, measure a 1 or 2 cm^2 area on one substrate unit for each station and scrape the periphyton within each area with the edge of a glass slide and a rubber policeman. Transfer the sample to a 100 ml volumetric flask and dilute with distilled-deionized water.
2. Mix the sample well to evenly suspend the periphyton, and pipet 6 6-ml aliquots into 6 tubes.
3. Add 2 ml Tris buffer to each tube.
4. Add 1 ml EDTA to 3 tubes. These are the blanks.
5. Mix samples well by placing Parafilm^R over top of tube and inverting several times. Place all 6 tubes in the dark in the water bath (25°C) and allow sample temperature to equilibrate for approximately 15 minutes.
6. Successively add 1 ml of substrate to each tube, and again mix thoroughly by inverting several times. Read fluorescence against distilled water blank and return tube to water bath and allow reaction to proceed in the dark.
7. Mix samples and blanks thoroughly before reading; read fluorescence of blanks at 30 minutes and of samples at 15 and 30 minutes.
8. Add 1 ml EDTA to sample immediately after 30-minute reading. Mix well. Read fluorescence.

C. To calculate methylfluorescein (orthophosphate) formed

during assay period, generate regression of concentration of methylfluorescein ($ng \cdot ml^{-1}$) vs. fluorescence units. Enter above-determined fluorescence units after subtracting blanks and compute methylfluorescein concentration. To convert methylfluorescein concentrations to orthophosphate released, the following equations are used:

i. methylfluorescein ($ng \cdot ml^{-1}$) $\div$ 346.3 $g \cdot mole^{-1}$
= methylfluorescein ($nmoles \cdot ml^{-1}$)

ii. methylfluorescein ($nmoles \cdot ml^{-1}$)
= $PO_4^{\equiv}$ ($nmoles \cdot ml^{-1}$)

iii. $PO_4^{\equiv}$ ($nmoles \cdot ml^{-1}$) $\div \frac{1\ cm^{2*}}{100\ ml} \times \frac{10\ ml}{6\ ml} \div \frac{10^{-4}\ m^2}{cm^2} \div t$

= $PO_4^{\equiv}$ ($nmole \cdot m^{-2} \cdot min^{-1}$)

where $\frac{10\ ml}{6\ ml}$ = correction for dilution of sample by reagents

t = incubation time in minutes

*If 2 cm^2 are diluted, divide by 2 $cm^2 \cdot 100\ ml^{-1}$.

REFERENCES

APHA. 1975. Standard Methods for Examination of Water and Wastewater. 14th ed. American Public Health Association. Washington, D.C., 1193 pp.

Flett, R.J., R.D. Hamilton, and N.E.R. Campbell. 1975. Aquatic acetylene reduction techniques: solutions to several problems. Can. J. Microbiol. 22:43-51.

Holm-Hansen, O. and B. Reimann. 1978. Chlorophyll-a determination: improvements in methodology. Oikos 30:438-447.

Perry, M.J. 1972. Alkaline phosphatase activity in subtropical Central North Pacific waters using a sensitive fluorometric method. Mar. Biol. 15:113-119.

Tett, P., M.G. Kelly, and G.M. Hornberger. 1975. A method for the spectrophotometric measurement of chlorophyll-a and pheophytin-a in benthic microalgae. Limnol. Oceanogr. 20:887-896.

Tett, P., M.G. Kelly, and G.M. Hornberger. 1977. Estimation of chlorophyll-a and pheophytin-a in methanol. Limnol. Oceanogr. 22:579-580.

Vollenweider, R.A. 1969. A manual on methods for measuring primary production in aquatic environments. IBP Handbook No. 12. Blackwell Scientific Publications. Oxford.

Fluorometric Procedure for Assay of Alkaline Phosphatase Activity (Tentative) (After method of Perry 1972)

I. Basis of Method

Phosphatase activity is measured by the method of Perry (1972) as modified by J. Shapiro (personal communication, University of Minnesota, Minneapolis). The increase in fluorescence is measured as the substrate, 3-O-methyfluorescein phosphate, is enzymatically hydrolyzed to the more fluorescent product, methylfluorescein. Orthophosphate is concurrently released in equimolar concentration. A standard calibration curve of fluorescence units vs. methyl-fluorescein concentration permits conversion of fluorescence units to the amount of methylfluorescein, and hence orthophosphate, liberated. Alkaline phosphatase activity is then reported as phosphate concentration (molar) per volume (liter) per time (minute).

II. Materials and Reagents

A. Turner 110 or 111 Fluorometer

B. Fluorometer filters

1. Primary 47B [Wratten gelatin filter (Kodak), cat. no. 149 5795]

2. Secondary 2A [Wratten gelatin filter (Kodak), cat. no. 164 4988]

12 [Wratten gelatin filter (Kodak), cat. no. 149 5522]

Filter 47B is used alone, placed closest to lamp.

Filters 2A and 12 are used in combination and are placed near the photomultiplier tube, 12 on the inside, 2A on the outside.

C. Test tubes, Bausch and Lomb Spectronic 20 Colorimeter, 1/2"

D. Pipets

1. Automatic (capable of delivering 1.0, 2.0 and 3.0 ml),

e.g. Eppendorf^{R} or Pipetman^{R}

2. Volumetric (1-5 ml capacity)

3. Serological (10 ml capacity for pipetting lake water samples)

E. $MgCl_2$ (3%)

Dissolve 3.0 g analytical reagent grade $MgCl_2$ in 100 ml distilled-deionized water.

F. Hydrochloric acid (concentrated)

Use only analytical reagent grade.

G. Tris buffer (1.0 M, pH 8.6)

1. Dissolve 121.1 g Trizma^{R} Base in ~950 ml distilled-deionized water.

2. Add 10.0 ml 3% $MgCl_2$.

3. Add 20.0 ml concentrated HCl, slowly.

4. Bring volume to 1000 ml with distilled-deionized water.

5. Mix well and check pH. If it is not between 8.55 and 8.65, add HCl or NaOH to adjust.

6. Autoclave at 115-120° for 15 minutes; store in refrigerator.

H. Substrate: 3-O methyfluorescein phosphate (5.00 $\mu g \cdot ml^{-1}$) (Sigma Chemical Co.)

1. Dissolve 12.50 mg methylfluorescein phosphate in 10 ml cold Tris buffer (made up as in G., above) (concentration = 1.25 $mg \cdot ml^{-1}$). If stored, place in freezer in dark.

2. Dilute 1.0 ml of 1.25 $mg \cdot ml^{-1}$ methylfluorescein phosphate to 250 ml with cold Tris buffer (made up as in G., above).

3. Transfer to acid-cleaned screw cap test tubes. Store frozen in dark. Substrate should remain frozen until immediately before use (see Note B).

I. EDTA (0.1 M)

Dissolve 9.305 g analytical reagent grade Na_2EDTA into 250 ml distilled-deionized water. Store in refrigerator.

J. Standards: methylfluorescein (Sigma Chemical Co.)

1. Methanol, absolute, analytical reagent grade

2. 1.0 N NaOH

Dissolve 40 g analytical reagent grade NaOH in 1000 ml dissolved-deionized water. Store at room temperature in polyethylene bottle.

3. Primary standard (0.50 $mg \cdot ml^{-1}$)

Dissolve 50.00 mg methyfluorescein in 100 ml absolute, reagent grade methanol. Store in polyethylene or glass in dark at -5°C.

4. Secondary standard 0.005 $mg \cdot ml^{-1}$)

Prepare on day of analysis. Add 1.0 ml of the primary standard (made up as in J.3., above) to ~85 ml distilled-deionized water and 5 ml of 1.0 N NaOH in a 100 ml volumetric flask. Bring volume to 100 ml, mix well.

5. Working standards

Prepare on day of analysis, from secondary standard (0.005 $mg \cdot ml^{-1}$).

For window 3X, dilute

$$\frac{1.0 \text{ ml of } 0.005 \text{ mg} \cdot \text{ml}^{-1}}{100 \text{ ml}} = 50 \text{ ng} \cdot \text{ml}^{-1}$$

For window 1X, dilute

$$\frac{3.0 \text{ ml of } 0.005 \text{ mg} \cdot \text{ml}^{-1}}{100 \text{ ml}} = 150 \text{ ng} \cdot \text{ml}^{-1}$$

a. To run standard curve, make dilutions of working standards (above) using volumetric pipets (1 to 20 ml capacity) and 10 ml graduated cylinder.

b. Add appropriate amount of working standard (either 50 $ng \cdot ml^{-1}$ or 150 $ng \cdot ml^{-1}$) (See below, d.), 1 ml EDTA, and 3 ml Tris buffer to graduated cylinder. Bring volume to 10 ml with distilled-deionized water.

c. Mix well and transfer to fluorometer tube.

d. Make dilutions as follows:

For window 3X

$$\frac{1 \text{ ml of } 50 \text{ ng} \cdot \text{ml}^{-1}}{10 \text{ ml}} = 5 \text{ ng} \cdot \text{ml}^{-1}$$

$$\frac{2 \text{ ml of } 50 \text{ ng} \cdot \text{ml}^{-1}}{10 \text{ ml}} = 10 \text{ ng} \cdot \text{ml}^{-1}$$

$$\frac{3 \text{ ml of } 50 \text{ ng} \cdot \text{ml}^{-1}}{10 \text{ ml}} = 15 \text{ ng} \cdot \text{ml}^{-1}$$

$$\frac{4 \text{ ml of } 50 \text{ ng} \cdot \text{ml}^{-1}}{10 \text{ ml}} = 20 \text{ ng} \cdot \text{ml}^{-1}$$

$$\frac{5 \text{ ml of } 50 \text{ ng} \cdot \text{ml}^{-1}}{10 \text{ ml}} = 25 \text{ ng} \cdot \text{ml}^{-1}$$

For window 1X

$$\frac{1 \text{ ml of } 150 \text{ ng} \cdot \text{ml}^{-1}}{10 \text{ ml}} = 15 \text{ ng} \cdot \text{ml}^{-1}$$

$$\frac{2 \text{ ml of } 150 \text{ ng} \cdot \text{ml}^{-1}}{10 \text{ ml}} = 30 \text{ ng} \cdot \text{ml}^{-1}$$

$$\frac{3 \text{ ml of } 150 \text{ ng} \cdot \text{ml}^{-1}}{10 \text{ ml}} = 45 \text{ ng} \cdot \text{ml}^{-1}$$

$$\frac{4 \text{ ml of } 150 \text{ ng}\cdot\text{ml}^{-1}}{10 \text{ ml}} = 60 \text{ ng}\cdot\text{ml}^{-1}$$

$$\frac{5 \text{ ml of } 150 \text{ ng}\cdot\text{ml}^{-1}}{10 \text{ ml}} = 75 \text{ ng}\cdot\text{ml}^{-1}$$

e. Place tubes in dark for at least 15 minutes prior to reading, to minimize background fluorescence.

III. Procedure

A. Place 2 ml Tris buffer into each tube.

B. Add 1 ml EDTA to half the tubes. These are the blanks.

C. Add 6 ml lake water to each tube. Make sure lake water samples are well mixed before subsampling. If zooplankton are present, remove by straining (nitex netting). The effect on enzyme activity by zooplankton presence during the assay has not been investigated.

D. Place tubes in dark in water bath (25°C) and allow sample temperature to equilibrate for approximately 15 minutes.

E. Add 1 ml substrate to tube. Mix thoroughly by placing Parafilm^{R} over top of tube and inverting several times. Return tube to water bath and allow reaction to proceed in the dark.

F. Read fluorescence of blanks at 0 and 30 minutes. Read fluorescence of samples at 0, 15 and 30 minutes.

G. Add 1 ml EDTA to sample immediately after 30-minute reading. Mix. Read fluorescence.

H. To calculate methylfluorescein (orthophosphate) formed during assay period, generate regression of concentration of methylfluorescein ($ng\cdot ml^{-1}$) vs. fluorescence units. Subtract blank

readings at 30 minutes from readings of samples after EDTA addition (G., above) and compute methylfluorescein concentration.

To express activity in terms of orthophosphate released, the following equations are used:

1. Methylfluorescein $(ng \cdot ml^{-1}) \div 346.3\ g \cdot mole^{-1}$
   $= \text{methylfluorescein}\ (nmoles \cdot ml^{-1})$
2. Methylfluorescein $(nmoles \cdot ml^{-1}) = PO_4^{\equiv}\ (nmoles \cdot ml^{-1})$
3. $PO_4^{\equiv}\ (nmoles \cdot ml^{-1}) \times \frac{10\ ml}{6\ ml} \div t \times 1000\ ml \cdot l^{-1}$
   $= PO_4^{\equiv}\ (nmoles \cdot l^{-1} \cdot min^{-1})$

Where $\frac{10\ ml}{6\ ml}$ = correction for dilution of sample by reagents

t = incubation time in minutes

IV. Notes

A. Primary standard appears to remain stable several months. Secondary standard should be made up on day of analysis, but may remain stable for several weeks. Working standards should be made up on day of analysis. If sealed with parafilm, they may be stored in the dark, overnight.

B. Substrate background fluorescence may increase if substrate is stored frozen for extended periods of time; it should probably not be stored longer than one month. Methylfluorescein phosphate also hydrolyzes spontaneously when stored at room temperature, increasing background fluorescence.

C. The window used depends on the activity of the samples and the initial (background) fluorescence of the substrate. If substrate fluorescence is initially low and/or activity of sample is low, a more sensitive window may be used.

D. A reading half-way through the incubation period is necessary to ensure that enzyme activity remains linear during assay period.

E. All glassware should be clean and free of orthophosphate since orthophosphate inhibits enzyme activity. Tubes, flasks and pipets are cleaned with 0.1 N HCl, and thoroughly rinsed with distilled-deionized water between assays. Less frequently, acid dichromate solution is used to clean all glassware; copiously rinse with distilled-deionized water after using dichromate.

F. Samples and blanks are analyzed in triplicate.

G. If activity of sample is greater than highest standard, decrease the dilution of secondary standard, to extend range of standard curve. Do not dilute the sample.

H. All solutions (substrate, EDTA, Tris) should be at 25°C prior to addition to lake water.

I. Lake water samples should be collected in at least 1-liter quantities in acid-washed containers and stored on ice until analysis. Enzyme activity may increase if small samples (< 1 liter) are taken and stored for any length of time.

J. For standards, zero fluorometer with 6 ml distilled water, 3 ml Tris and 1 ml EDTA.

REFERENCE

Perry, M.J. 1972. Alkaline phosphatase activity in subtropical Central North Pacific waters using a sensitive fluorometric method. Mar. Biol. 15:113-119.

Procedure for Determination of Chlorophyll-*a* (After methods of Golterman 1969 and Strickland and Parsons 1972)

I. Basis of Method

The chemical determination of characteristic plant pigments, particularly chlorophyll-*a*, is a rapid method for the indirect estimation of living plant matter in water. A quantity of lake water is filtered through a GF/C filter to which a few drops of a saturated $MgCO_3$ solution have been added. The filter is then either frozen for later analysis or immediately placed in 90% acetone for pigment extraction. After appropriate extraction time, the samples are analyzed spectrophotometrically at λ = 750, 663, 645, 630, 510, and 480 nm. Absorbance readings are converted to pigment concentrations using equations from Golterman (1969, pp. 116-120) and Strickland and Parsons (1972, pp. 185-194).

II. Materials and Reagents

A. Spectrophotometer capable of readings at the above-mentioned wavelengths and holding 1 cm cells

B. Millipore^R filtration apparatus equipped for holding 4.25 cm diameter GF/C filters

C. GF/C glass fiber filters, 4.25 cm in diameter

D. Saturated $MgCO_3$ solution [made up by dissolving 1 g $MgCO_3$ (analytical reagent grade) in 100 ml distilled-deionized water]

E. Graduated cylinders (500 to 1000 ml capacity)

F. Aluminum foil (for holding filters individually if they are to be stored for later analyses)

G. Forceps for handling filters

H. Labels for identifying sample date, depth, location, and volume filtered

I. Foil covered test tubes with stoppers (greater than 10 ml capacity)

J. Acetone (90%, diluted from 100% with distilled-deionized water)

K. Centrifuge

L. Teflon tissue grinder

III. Procedure

A. Filtration

1. Turn off any bright lights. Filter 500 to 1000 ml of lake water sample, after thorough mixing, through GF/C filter to which 3 drops of saturated $MgCO_3$ solution have been added. (Note: $MgCO_3$ prevents the breakdown of chlorophyll into phaeophytin by making the sample alkaline). $MgCO_3$ solution should be shaken vigorously to suspend the particles immediately prior to use. Filters should not be handled with fingers.

2. After filtration, filter should be carefully removed from filtering apparatus with forceps and folded in half with plankton side inward. Place filter inside aluminum foil and crease to make a light-tight packet. Label filters individually with date, depth, location and volume (ml) filtered. Prolonged storage induces breakdown of chlorophyll into degradation products and hence chlorophyll will be underestimated. Best results are obtained when filters are extracted and analyzed immediately. If immediate extraction cannot be

done, filters should be frozen and analyzed as soon as possible.

B. Extraction

1. Place filter in clean, dry, stoppered test tube containing approximately 2 ml of 90% acetone. Using the teflon-tipped grinder, slowly grind sample until filter is completely broken down, taking care to insure sample and tube do not heat up. Add 90% acetone until total volume in tube is 10 ml. Shake tube.
2. Place darkened tubes in refrigerator and allow to extract for 3 hours.
3. Place tubes in centrifuge for 9 minutes at 2300 RPM.
4. Make sure all the filter fibers are centrifuged; if not, wash down with supernatant and re-centrifuge.
5. Decant the clear supernatant into 1 cm path-length spectrophotometer cell.
6. Read absorbance at 750, 663, 645, 630, 510 and 480 nm against acetone blank. If readings of samples at 750 nm are $> .005$ absorbance units, repeat centrifugation step.
7. Add 1 drop of 2 N HCl and reread at 750 and 663 nm, against acetone blank to which 1 drop of 2 N HCl has been added.

C. Analysis

1. Equations used to convert absorbance to concentration are those of Strickland and Parsons (1972):

$$\text{mg pigment} \cdot m^{-3} = P_n \times K$$

Where K = acetone volume (ml) x light path (cm^{-1})
x sample volume (l^{-1})
and P_n is determined as follows (after subtracting absorbance readings at 750 nm from each wavelength):

$$P_1 = \text{Chl.}\underline{a} = 11.6E_{663} - 1.31E_{645} - 0.14E_{630}$$

$$P_2 = \text{Chl.}\underline{b} = 20.7E_{645} - 4.34E_{663} - 4.42E_{630}$$

$$P_3 = \text{Chl.}\underline{c} = 55.0E_{630} - 4.64E_{663} - 16.3E_{645}$$

$$P_4^* = \text{ast-t} = 2.0 \times (4.45E_{510} - E_{480})$$

$$P_5^* = \text{n-ast-t} = 7.6 \times (E_{480} - 1.49E_{510})$$

$$P_6^* = \text{n-ast-1} = 4.0E_{480}$$

$$P_7^* = \text{n-ast-2} = 10.0E_{480}$$

where Chl.$\underline{a}$ = Chlorophyll $\underline{a}$

Chl.$\underline{b}$ = Chlorophyll $\underline{b}$

Chl.$\underline{c}$ = Chlorophyll $\underline{c}$

ast-t = total astacin (animal) carotenoid

n-ast-t = total non-astacin (plant) carotenoid

n-ast-1 = specific non-astacin carotenoid (Chlorophyta-Cyanophyta)

n-ast-2 = specific non-astacin carotenoid (Chrysophyta-Pyrrophyta)

E_{xyz} = absorbance at wavelength xyz

* See Portz (1973)

2. To calculate chlorophyll degradation products from absorbance readings after acidification, the following equations (after correction for blank at 750 nm) from (a) Strickland and Parsons (1972) and (b) Golterman (1969) are used.

a. $\text{Corr. Chl. } (mg \cdot m^{-3}) = 26.7\ (E^{u}_{663} - E^{a}_{663}) \times K$

$\text{Phaeophytin } (mg \cdot m^{-3}) = 26.7\ [1.7\ (E^{a}_{663}) - E^{u}_{663}] \times K$

Where E^{u}_{663} = absorbance before acidification

E^{a}_{663} = absorbance after acidification

K = acetone volume (ml) x light path (cm^{-1}) x sample volume (l^{-1})

b. $$\text{Corr. Chl. } (mg \cdot m^{-3}) = \frac{2.43[E^{u}_{663} - E^{a}_{663}]}{89} \times \frac{\text{volume extract (ml)}}{\text{volume filtrate (l)}} \times 1000$$

$$\text{Phaeophytin } (mg \cdot m^{-3}) = \frac{E^{u}_{663} - [2.43\ (E^{u}_{663} - E^{a}_{663})]}{56} \times \frac{\text{volume extract (ml)}}{\text{volume filtrate (l)}} \times 1000$$

$$\text{Total Pigment } (mg \cdot m^{-3}) = \text{Corr. Chl. } (mg \cdot m^{-3}) + \text{Phaeophytin } (mg \cdot m^{-3})$$

Where E^{u}_{663} = absorbance before acidification

E^{a}_{663} = absorbance after acidification

REFERENCES

Golterman, H.L. 1969. Method for Chemical Analysis of Fresh Waters. IBP Handbook No. 8. Blackwell Scientific Publ. Oxford and Edinburgh. 166 pp.

Portz, D.E. 1973. Plankton pigment heterogeneity in seven reservoirs of the Lower Colorado Basin. Ph.D thesis. Arizona State Univ. 168 pp.

Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis. 2nd ed., Bulletin of the Fisheries Research Board of Canada. No. 167. Ottawa. 311 pp.

Procedure for the Enumeration and Identification of Phytoplankton (After method of Utermöhl 1958)

I. Basis of Method

Phytoplankton are counted using Utermöhl's (1931, 1958) inverted microscope method. The method allows identification and enumeration of phytoplankton in all size components and enables the analysis of both rare and common species. Lake water samples are poured into sedimentation cylinders (see Fig. 1), and phytoplankton allowed to settle onto a slide containing a thin glass cover plate. The chamber containing phytoplankton is then transferred to the stage of an inverted microscope and a number of transects analyzed according to the density of cells and statistical accuracy desired. Cell volumes are obtained by measuring 25 individuals (for common species), and averaging the values. The volumes of less common species are obtained by measuring as many individuals as possible. Biovolume ($\mu m^3 \cdot ml^{-1}$) is obtained by multiplying the cell volume ($\mu m^3 \cdot cell^{-1}$) by the cell number$\cdot ml^{-1}$. This value is then converted to biomass or fresh weight ($mg \cdot m^{-3}$) assuming that the specific gravity of algae is equal to 1.0.

II. Materials

A. Inverted microscope (e.g. Olympus IMT) equipped with counting strip eyepiece

B. Sedimentation cylinders (10 and 50 ml capacity) (Fig. 1)

C. Combined plate chambers (Wild Ml, catalogue 14d X 11.69) (Fig. 2)

D. Acid-Lugols preservative (prepared by dissolving 10 g iodine

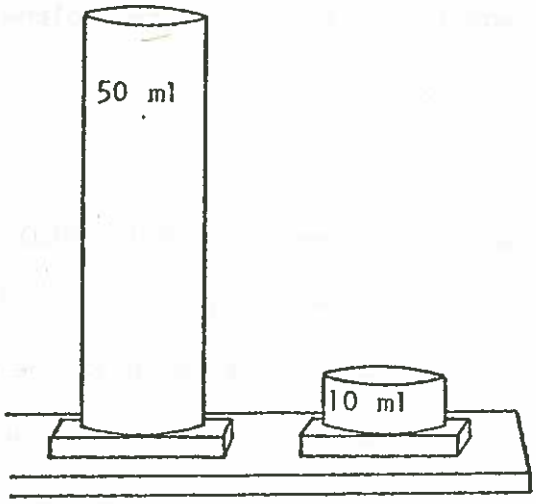


Figure 1

Sedimentation cylinders for combined plate chamber (Wild Ml, catalogue 140d XII.69).

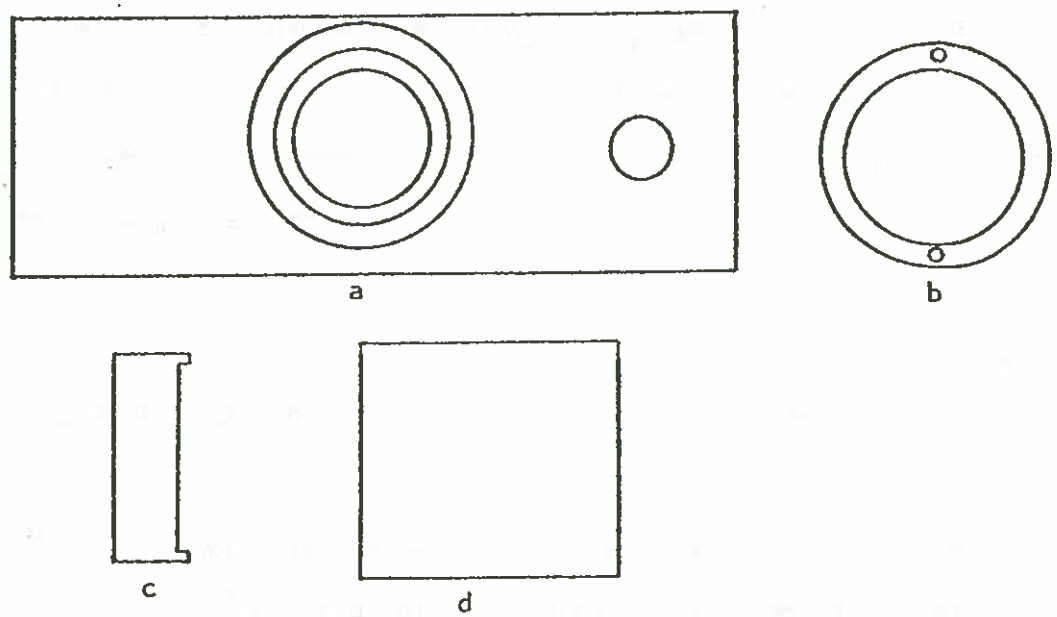


Figure 2

Plate chamber: (a) perspex plate with larger opening for top cylinder and smaller drainage hole; (b) ring for support to bottom (base) plate; (c) key to fasten ring to underside of perspex plate; (d) top plate of chamber, also to be used to remove cylinder after sedimentation is completed.

crystals and 20 g potassium iodide in 200 ml distilled-deionized water. Add 20 ml glacial acetic acid a few days prior to using (Vollenweider 1969, p. 7). One ml of Lugols solution is added to 100 ml of phytoplankton sample.

E. Glass bottles with plastic lids for storage of samples (stored in cool, dark place)

III. Procedure

A. Sample volumes of 2-50 ml may be sedimented in the 10-ml and 50-ml cylinders (Fig. 1) depending on algal density and amount of sediment.

1. Allow the samples to stand at room temperature for one or two days. This allows the algae to release their gases (O_2, CH_4 and CO_2) and reduces the intensity of water currents which are generated when the sample and sedimentation cylinder walls are at different temperatures.

2. Mix the plankton by continuous shaking in opposite directions, i.e., to and fro, up and down, and clockwise and counter-clockwise in a circular motion. Avoid prolonged and vigorous shaking which damages delicate forms. Best mixing results are obtained with bottles 3/4 to 4/5 filled. Full sample bottles cannot be properly mixed.

3. Place the sedimentation cylinder on the plate chamber (Fig. 2) directly over the bottom (base) plate (Fig. 3a). Check the bottom plate to insure that it is scrupulously clean on both the inner and outer surfaces. Hold the two sections firmly together with thumb and forefinger.

4. The mixed plankton sample is poured into the sedimentation

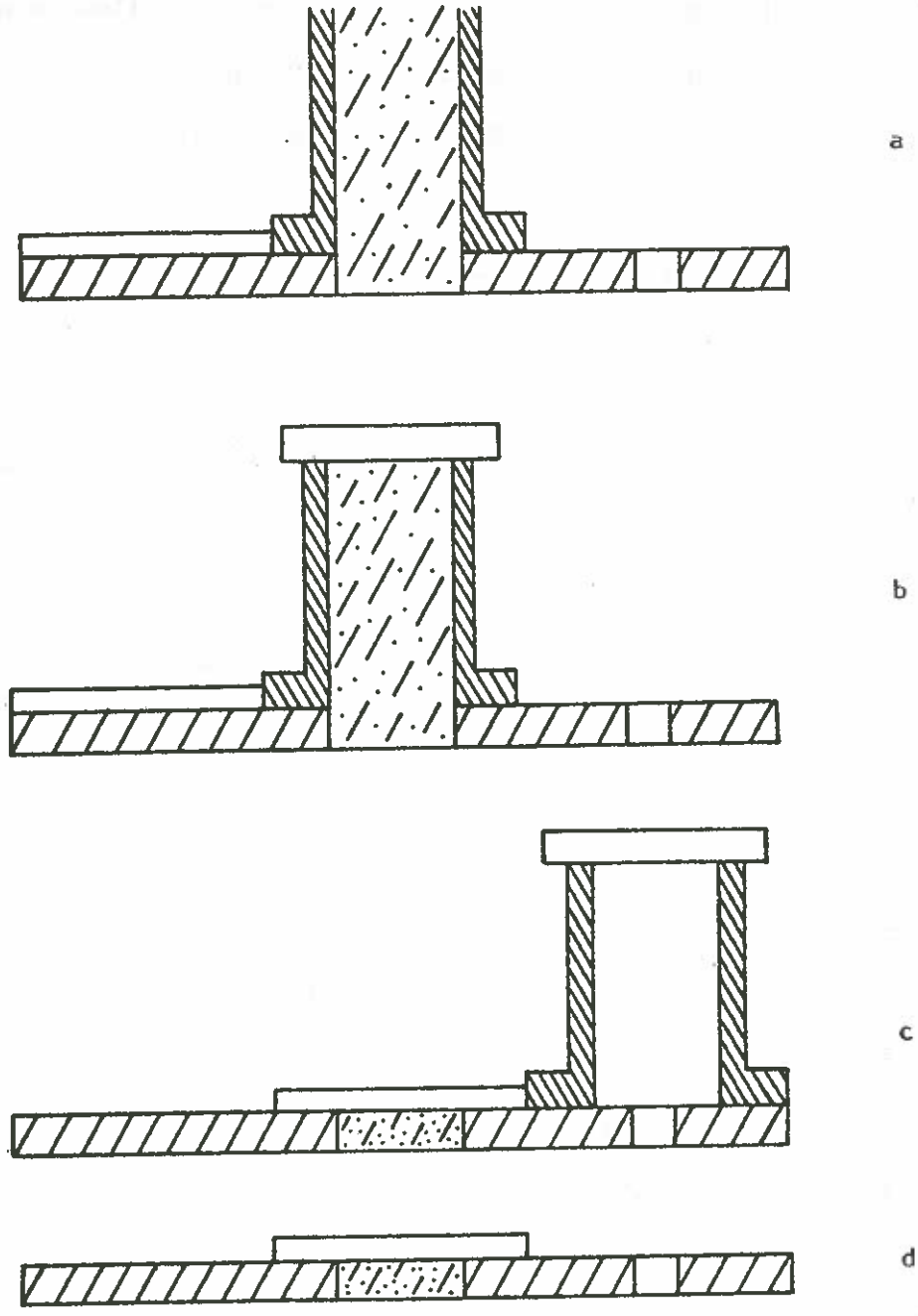


Figure 3

Sedimentation procedure for Utermohl method: (a) combined plate chamber and cylinder, in position for filling with plankton sample; (b) cover disk in place, position for sedimentation; (c) phytoplankton separated from sedimentation cylinder, position for drainage of cylinder; (d) combined plate chamber containing concentrated phytoplankton.

cylinder until it overflows. Wait until most of the air bubbles have disappeared from the arched water surface. When sedimenting a smaller volume of water than the capacity of the sedimentation cylinder, e.g., 25 ml in a 50 ml cylinder, insure that the dilution water is the same temperature as the phytoplankton sample, sedimentation cylinder, plate chamber, and ambient room temperature.

5. The cover disk is now placed on the sedimentation cylinder; avoid sliding it on. The chamber should be wiped dry. Check to insure that the sedimentation cylinder is centered over the circular opening in the plate chamber and that the cover disk is centered over the sedimentation cylinder (Fig. 3b).
6. A label with sample location, date, volume sedimented, date and time of sedimentation is affixed to the plate chamber.
7. The chamber and cylinder are left undisturbed for 24 hours (Javornicky 1958) or 4 hours per cm of chamber height (Nauwerck 1963). A level surface free from changes in ambient room temperature is critical during sedimentation.
8. The top plate is now placed on the plate chamber (side without the drainage hole) and pushed until the cylinder is directly above the drainage hole of the plate chamber (Fig. 3c). The cover disk is removed and the supernatant drained into a shallow dish. If air bubbles are formed in the chamber under the sliding plate, inspect the top plate and upper surface of the plate chamber for the presence of deposits. Also the bottom of the top plate may be moistened

and slight pressure exerted on the top of the plate with your forefinger as the sedimentation cylinder is displaced to the drainage hole.

9. The plate chamber containing the concentrated phytoplankton (Fig. 3d) is ready to be placed on the stage of an inverted microscope.

B. Enumeration

1. Operation of the counting strip eyepiece

This eyepiece must be inserted in the microscope such that its parallel thread hairs run in one of the two main directions of the adjustment of the mechanical stage. The position is clamped with the set screw.

The width of the counting strip may be varied by turning the large milled ring below the focusing eye lens of the eyepiece. The parallel lines are set a known distance apart (using a stage micrometer). This distance can be set to provide convenient conversion factors (Appendix A). To insure that this position does not move, it is advisable to secure the ring to the housing with tape.

2. Magnification and areas counted in each major size class.

   a. The nannoplanktonic species (< 64 µm) are counted at 400X magnification in one or two strips across the entire diameter of the plate chamber (25.5 mm).

   b. The larger, more common forms (excluding Ceratium hirundinella) are counted at 200X magnification in one or two strips across the diameter.

   c. The entire plate chamber is then scanned at 100X

magnification for Ceratium hirundinella and the less common large planktonic species. All species not encountered in a and b (above) are enumerated.

d. If necessary, the small forms (usually less than 5 µm) that could not be adequately enumerated at 400X are enumerated at 1000X magnification (oil immersion) in one or two strips 10 mm in length.

C. Statistical Considerations

To establish the number of individuals to count, Lund et al. (1958) and Javornicky (1958) recommend the following formula:

$$\text{error max} = \pm 2\frac{100}{\sqrt{n}}\ \%$$

where n is the number of units (colonies, filaments, etc.) counted. The formula assumes random distribution of organisms and gives a 95% confidence interval as the percentage of individuals counted. For Lake Mead, at least 300 units are counted, giving a 12% error. During certain periods of the year, this number cannot be achieved due to large amounts of sediment and low algal density.

D. Calculations (See Appendix B)

1. Cell abundance

$$\text{cells/ml} = \frac{C \times A_c}{L_1 \text{ or } L_2 \times W_1,\ W_2,\ \text{or } W_3 \times S \times V_s}$$

where C = cells counted

A_c = area of plate chamber (510 mm^2)

L_1 = length of strip (25.5 mm)

L_2 = length of strip (10.0 mm) (usually for 1000X only)

W_1 = width of counting strip at 200X (0.2 mm)

W_2 = width of counting strip at 400X (0.1 mm)

W_3 = width of counting strip at 1000X (0.04 mm)

S = number of strips counted

V_s = volume sedimented (2-50 ml)

2. Cell volume and biomass ($mg \cdot m^{-3}$) (See Appendices C and D)

The appropriate dimensions of 25 randomly selected cells are measured for each of the common species and as many as possible (not exceeding 25) for each of the uncommon species (Munawar 1972). Measurements are made for each month. The volume of each measured cell is calculated by approximation to an appropriate geometrical shape (see Appendix D). The mean cell volume for each species is then derived.

The total biovolume for each species in a sample is obtained by multiplying the cell volume by the cell number. The biovolume ($\mu m^3 \cdot ml^{-1}$) is converted to biomass or freshweight ($mg \cdot m^{-3}$) assuming that the specific gravity of algae is equal to 1.0 ($\frac{1\ \mu m^3 \cdot ml^{-1}}{1000} = 1\ mg \cdot m^{-3}$).

Calculation:

Cell volume (μm^3) x $cells \cdot ml^{-1}$ = biovolume ($\mu m^3 \cdot ml^{-1}$)

The biomass for the entire population is calculated as follows:

$$\sum_{i=1}^{n} V = (V_1)(N_1)(10^{-3}) + (V_2)(N_2)(10^{-3}) \ldots (V_n)(N_n)(10^{-3})$$

Where V is the total biomass ($mg \cdot m^{-3}$), n equals the number of species found, i represents species i (i=1, 2...n) in terms of its mean cell volume V_i (μm^3) and N_i represents the cell abundance of species i in $cells \cdot ml^{-1}$.

REFERENCES

Javornicky, P. 1958. Die revision einiger methoden zum feststellen der quantat des phytoplankton. Inst. Chem. Technol., Prague, Faculty Technol. Fuel Water. Scient. Papers 2, pt 1:283-367.

Lund, J.W.G., C. Kipling and E.D. LeCren. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11:143-170.

Munawar, M. 1972. Methods of taxonomic identification and enumeration of plankton. Report of a workshop. January 26-27, 1972 at Canada Centre for Inland Waters, Burlington, Ontario, Canada. IFYGL Bull. 3:24-32.

Nauwerck, A. 1963. Die beziehungen zwischen Zooplankton und Phytoplankton im see Erken. Symbolae Botonical Upsalienses. 17:1-163.

Utermöhl, H. 1931. Neue Wege in der quantitativen erfassung des planktons. Verh. Inter. Verein. Limnol. 5:567-596.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen phytoplankton methodik. Mitt. Int. Verein. Limnol. 9:1-38.

Vollenweider, R.A. (ed.). 1969. A manual on methods for measuring primary production in aquatic environments. IBP Handbook No. 12. Blackwell Scientific Publications, Oxford. 213 pp.

APPENDIX A. PHYTOPLANKTON CONVERSION FACTORS

OLYMPUS IMT MICROSCOPE

Factors to convert raw phytoplankton counts to cells per ml.

width of strips		0.4mm	0.2mm	0.1mm	0.04mm
Volume settled ml	number of strips counted 25.5 mm	magnifications 100X	200X	400X	1000X
10	1	5.00	10.00	20.00	×
	2	2.50	5.00	10.00	×
	3	1.67	3.33	6.67	×
	4	1.25	2.50	5.00	×
	entire chamber	0.10	0.10	0.10	0.10
	(10mm)				127.50
25	1	2.00	4.00	8.00	×
	2	1.00	2.00	4.00	×
	3	0.67	1.33	2.67	×
	4	0.50	1.00	2.00	×
	entire chamber	0.04	0.04	0.04	0.04
	(10mm)				51.00
50	1	1.00	2.00	4.00	×
	2	0.50	1.00	2.00	×
	3	0.33	0.66	1.34	×
	4	0.25	0.50	1.00	×
	entire chamber	0.02	0.02	0.02	0.02
	(10mm)				25.50

APPENDIX B. PHYTOPLANKTON BENCH SHEET

Affix sample label

PHYTOPLANKTON DATA SHEET
LAKE MEAD

SHEET____OF____

TOTAL BIOMASS_____ mg/m^3

TOTAL CELL COUNTS_____cells/ml

DATE COLLECTED:_____

STATION:_____

PHYCOLOGIST:_____

DATE ANALYZED:_____

MAGNIFICATION 100X 200X 400X 1000X

STRIP WIDTH 0.4 0.2 0.1 0.04 mm.

NUMBER STRIPS (10mm) 1 2 3 4 Entire

VOLUME SEDIMENTED_____mls.

A. CONVERSION FACTOR_____

SPECIES	COUNTS		D. TOTAL (B+C)	E. CELLS/ml. (A×D)	BIOMASS mg/m^3 ($E \times F \times 10^{-3}$)
	B. STRIP 1	C. STRIP 2			

APPENDIX C. BIOMASS SUMMARY SHEET

LAKE MEAD PHYTOPLANKTON

BIOMASS SUMMARY SHEET

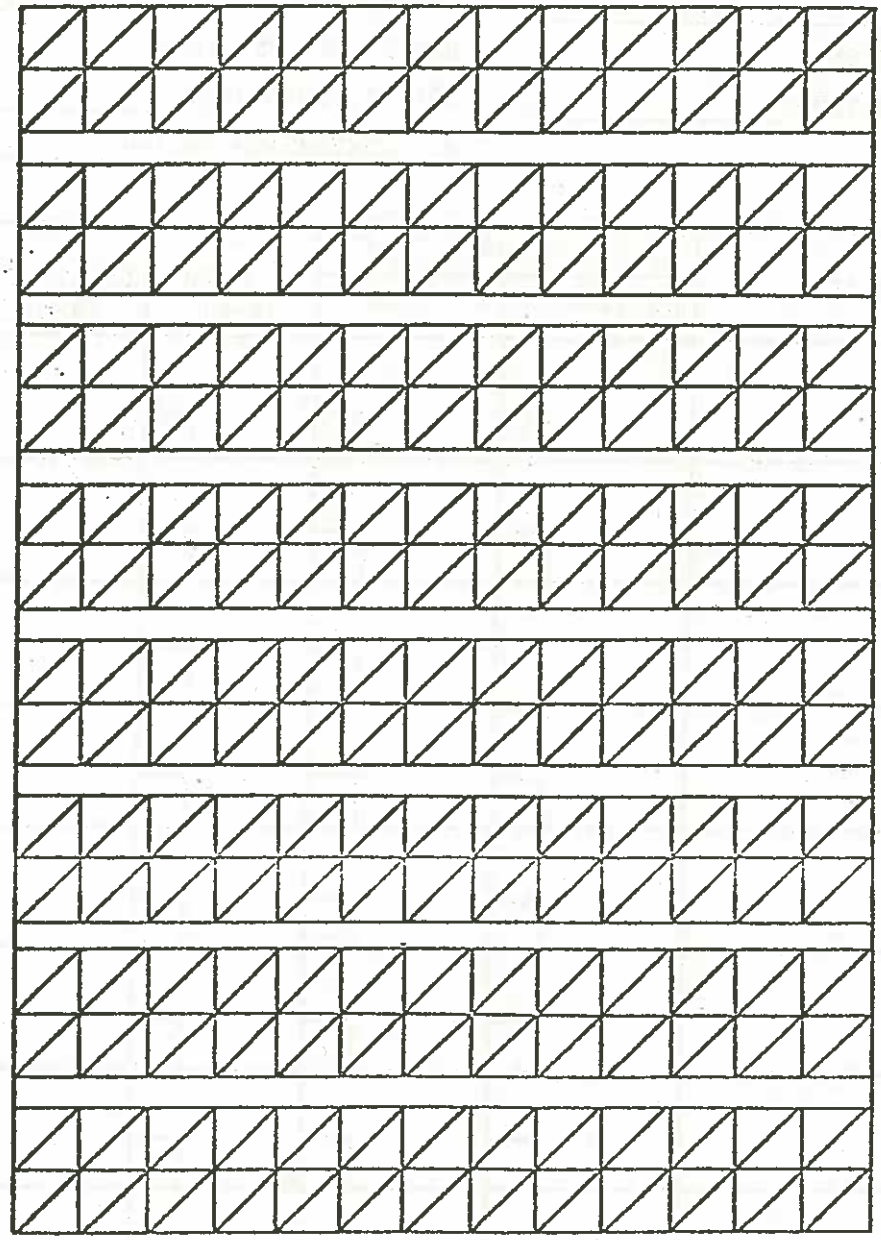
DATE COLLECTED: _____

STATION: _____

SPECIES

(F) BIOVOLUME (μm^3)

_____

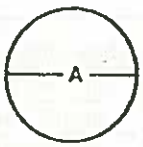
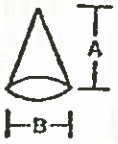


Key:

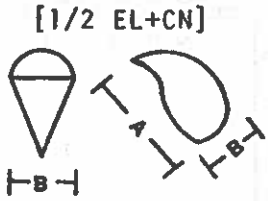
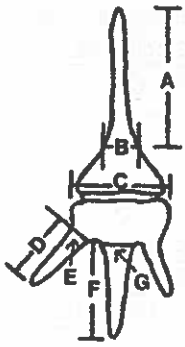
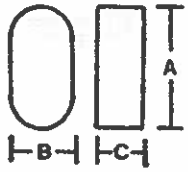
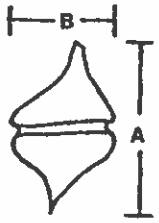
a/b a = length
b = width

c/d c = height
d = diameter

APPENDIX D. BIOVOLUME FORMULAE

SHAPE	CODE	REPRESENTATIVE SPECIES	DIAGRAM	FORMULA	NO. OF MEAS.
Sphere	Sp.	Sphaerocystis schroeteri		$\pi A^3/6$	1
Ellipsoid	EL	Scenedesmus bijuga Cryptomonas Euglena		$\pi AB^2/6$	2
Rod	Rd	Melosira granulata Cyclotella Asterionella		$\pi AB^2/4$	2
Two Cones	Cϕ	Ankistrodesmus falcatus		$\pi AB^2/12$	2
One Cone	CN	(horn of Ceratium)		$\pi AB^2/12$	2
Rectangular Box	RB			ABC	3

APPENDIX D, cont.

SHAPE	CODE	REPRESENTATIVE SPECIES	DIAGRAM	FORMULA	NO. OF MEAS.
Ellipsoid/ Cone	RM	Rhodomonas minuta Gymnodinium helveticum Mallomonas Synura		$\frac{\pi B^2(A+B/2)}{12}$	2
Irregular	CH	Ceratium hirundinella		$\frac{\pi}{12}[AB^2+C^3+2ED^2+FG^2]$	7
Irregular	AC	Achnanthes B Valve view C Girdle view		$BC(A-B+\frac{\pi}{4}B)$	3
Irregular	CT	Crucigenia tetrapedia		$A^3/4$	1
Irregular	PD	Peridinium		$\pi AB^2/9$	2

Assay for Demonstration of Nitrogen Deficiency in Phytoplankton using Ammonium Enhancement of Dark $^{14}C\text{-}CO_2$ Uptake (After method of Yentsch et al. 1977)

I. Basis of Method

In phytoplankton cultures grown under conditions of nitrogen limitation, the addition of ammonium enhances the rate of dark uptake of $^{14}C\text{-}CO_2$ relative to controls to which no ammonium is added. An increase in dark $^{14}C\text{-}CO_2$ assimilation in response to an addition of ammonium can therefore be used as an indication of nitrogen deficiency. Yentsch et al. (1977) chose an arbitrary ratio of 2 (CPM of sample with added NH_4^+:CPM of sample without added NH_4^+) as an indication of nitrogen deficiency.

II. Material and Reagents

A. Scintillation counter (e.g. Beckman LS-100)

1. Scintillation vials, clear glass with screw tops
2. Scintillation cocktail (e.g. PCS)

B. Incubation bottles (125 ml reagent bottles, wrapped with black electrical tape and painted white to exclude light)

C. ^{14}C-labeled bicarbonate purchased from Amersham, prediluted and ampoulated to proper activity working solution (See Appendix, page 84).

D. Ice chests or other light-proof boxes

E. Millipore^R filtration apparatus (25 mm or 47 mm)

1. Millipore^R filters or equivalent, 25 mm or 47 mm, 0.45 µm pore size

F. Ammonium chloride stock solution (concentration = 50 mg $N \cdot l^{-1}$)

1. Dissolve 0.3818 ± .0002 g NH_4Cl (oven-dried to constant

weight at 105°C) in 2000 ml distilled-deionized water.

2. 1 ml capacity MLA (or equivalent) pipet for adding NH_4Cl to samples

III. Procedure

A. Dilute 50 mg $N \cdot l^{-1}$ NH_4Cl stock 1:5 with distilled-deionized water as follows:

$$\frac{20 \text{ ml of } 50 \text{ mg } N \cdot l^{-1}}{100 \text{ ml}} = 10 \text{ mg } N \cdot l^{-1}$$

Note: The dilution MUST be done on day prior to use. The diluted solution is unstable and must not be stored, since loss of NH_3 through volatilization may occur. Store in reagent bottle.

B. Sample water with 0-5 m integrated sampler. Transfer to holding carboy, mix thoroughly.

1. Rinse and overfill 6 replicate bottles from carboy. Replace lids and place bottles in darkened box until ammonia and $^{14}C\text{-}HCO_3^-$ are ready to be added.
2. Remove lids, and from 3 bottles, withdraw 1.0 ml of water with MLA pipet. Add 1 ml dilute NH_4Cl (= 80 µg $N \cdot l^{-1}$, final).
3. Add appropriate amount of $^{14}C\text{-}HCO_3^-$ by syringe to all 6 bottles (see Appendix, page 55). (When adding radiocarbon immerse syringe tip below sample water surface). Immediately replace lids after injection and shake bottles to mix isotope and ammonia solution.
4. Replace bottles in light-proof boxes partially filled with lake water to maintain in situ lake water temperature.

5. Filter 6 to 24 hours following radiocarbon and ammonia additions.

   a. Filter bottle contents or subsample onto 0.45 µm pore size Millipore^{R} filter.

   b. Rinse sample filter with 0.005 N HCl to remove any unincorporated $^{14}C\text{-}HCO_3^-$.

   c. Immediately place filter into pre-labled (numbered) scintillation vial. Place vial on top of corresponding numbered lid, leaving vials uncapped overnight before dispensing scintillation cocktail into each.

   d. When filters are dry, add 20 ml of premixed scintillation cocktail (see Appendix, page 83) to each vial.

   e. Wipe off vial surface, cap tightly and place in scintillation counter overnight in dark before counting to prevent elevated counts due to fluorescence of cocktail.

   f. Count each sample for period of time sufficient to yield counts within 5%.

C. Calculations

Results are reported as percent control and are calculated as

$$\% \text{ Control} = CPM^{+} \div CPM^{-} \times 100$$

where CPM^{+} = average CPM's of 3 replicates to which ammonia was added

CPM^{-} = average CPM's of 3 replicates to which no ammonia was added

APPENDIX

SPECIFIC INSTRUCTIONS FOR INDEX STATIONS ON LAKE MEAD

STATION	ml of $^{14}C\text{-}HCO_3^-$ (1.90 $\mu Ci \cdot ml^{-1}$)	INCUBATION PERIOD (h)
2	0.5	6-24
5	0.5	6-24
8	1.0	6-24
9	1.0	6-24
10	1.0	6-24

REFERENCES

Yentsch, C.M., C.S. Yentsch, and L.R. Strube. 1977. Variations in ammonium enhancement, an indication of nitrogen deficiency in New England coastal phytoplankton populations. J. Mar. Res. 35:537-555.

Acetylene Reduction Assay of Nitrogenase Activity (After method of Flett et al. 1975)

I. Basis of Method

A. The acetylene reduction assay of nitrogenase activity is based on the fact that the enzyme, commonly called nitrogenase, which is responsible for reducing atmospherically derived dinitrogen (N_2) to ammonia (NH_3) also reduces acetylene (C_2H_2) to ethylene (C_2H_4) and hydrogen ions to hydrogen gas, according to the following equations:

(1) $N{\equiv}N + 6H^+ + 6e^- \rightarrow 2\ NH_3$

(2) $2H^+ + 2e^- \rightarrow H_2$

(3) $HC{\equiv}CH + 2H^+ + 2e^- \rightarrow C_2H_4$

Equation (1) shows that $6e^-$ are utilized during breakage of the triple bond of dinitrogen, $2e^-$ for each bond. When dinitrogen is the substrate for nitrogenase, a second nitrogenase-mediated reaction occurs, that of hydrogen production, requiring 2 additional electrons, equation (2). A total of $8e^-$ are thus transferred when dinitrogen and hydrogen ions serve as the substrates. Nitrogenase-mediated hydrogen evolution does not occur when C_2H_2 is the substrate for nitrogenase. Therefore, theoretically, the ratio of C_2H_2 reduced to N_2 fixed, based on numbers of electrons transferred, is close to 4. In practice, however, the ratio deviates from this value, usually falling between 2.5 and 6.

A quantity of acetylene gas (sufficient to fully saturate, but not inhibit, nitrogenase) (see Appendix B) is introduced by

syringe into a sealed vessel containing a vapor phase and the sample is incubated for a period of time sufficient to assure that the ethylene produced is above background (contained in the introduced acetylene gas). The length of the incubation period is determined in a preliminary experiment and is chosen such that the production of ethylene remains linear over time. Since both acetylene and ethylene can be analyzed easily using a flame-ionization gas chromatograph, the rate of nitrogenase activity can be quantified. The rate is reported as nmoles C_2H_4 produced per unit of time, or as nmoles N fixed by dividing ethylene production rate by the ratio of C_2H_2 reduced to N_2 fixed. Since the value of this ratio does vary depending on a variety of factors, it is advisable to periodically conduct parallel $^{15}N\text{-}N_2$ fixation measurements when acetylene reduction assays are performed.

II. Materials

A. Analytical gas chromatograph (Carle AGC 211), flame ionization detector

1. Strip chart recorder
2. 1 to 1.5 m x 1/8" O.D. stainless steel columns packed with Porapak T^R
3. Helium carrier gas; hydrogen gas and compressed air for flame (all should be high purity for maximum sensitivity)

B. Incubation vessels, gas tight and clear, with septa through which gases can be injected and withdrawn, with wire loops around necks to suspend from clips on incubation lines

C. High purity acetylene and ethylene gases (Matheson Gas Company)

1. Rubber bladders (such as football liners), equipped with septa, for transporting acetylene to the field and for holding ethylene to be used for standards
2. Syringes, Luer-lok, plastic, disposable, Becton-Dickinson type (1, 3, 5, 10, 20 and 50 ml) with 25 gauge, 3/4" needles
3. Vacutainers^{R}, 4 ml, red-stopper, non-sterile evacuated tubes, Becton-Dickinson for subsampling incubation vessels

D. In situ incubation apparatus

1. Lines equipped with clips at derived depths of incubation, on which to hang bottles
2. Ice chest or similar box for transporting bottles

E. Glass bottles, heavy-walled, approximately 1 liter capacity, with septa through which gases can be injected and withdrawn to use for ethylene standards

III. Procedure

A. Sample incubation

1. Fill bladder, until moderately pressurized, with pure C_2H_2 gas, flushing 2-3 times to remove residual air (bladder should be filled daily, since prolonged storage of acetylene will increase ethylene background).
2. Fill 4 190-ml capacity vessels with 150 ml of lake water (see Appendix A).
3. To one bottle, add 5 ml of 36% formalin. This will serve as an ethylene blank.
4. Seal all bottles tightly.
5. Flush a 20-ml syringe with C_2H_2 from bladder 2-3 times; fill syringe with 20 ml C_2H_2 and, before removing syringe

from bladder, slowly push plunger in to 16-ml mark (see Appendix B). Slowly inject C_2H_2 through septum of incubation vessel and shake gently to dissolve C_2H_2 completely.

6. Note injection time.
7. If bottles are incubated in situ, place bottles on clips at appropriate depths and suspend lines for suitable incubation interval (see Appendix C).
8. At the end of the incubation period, remove bottles from lines and shake vigorously to ensure that gases are equilibrated between the aqueous and vapor phase (at least 30 secs). Note exact time and water temperature to the nearest degree centigrade.
9. To withdraw subsample, place needle of syringe into septum and slowly pump plunger at least 5 times; depress plunger to 3-ml mark (Appendix B) before removing syringe from incubation vessel septum. Quickly withdraw syringe and inject sample into a pre-marked (sample date, depth and station) Vacutainer. To avoid creating positive pressure, a maximum of 3 ml should be placed into each Vacutainer.
10. Samples can be stored in a cool (<25°C) place for up to 3 months.

B. Sample Analysis

1. Prepare gas chromatograph (see Appendix D).
2. Run standard curve (see Appendix E).
3. Allow Vacutainer to reach atmospheric pressure by placing syringe needle (without syringe) into septum for

approximately 30 secs. Do not allow needle to remain in septum for longer than 30 secs, since the gases will leak.

4. To subsample Vacutainer, using a 1-ml plastic syringe, slowly pump plunger 2-3 times, and depress to 0.3 ml mark before removing needle from Vacutainer.

5. Immediately place needle into injection port of gas chromatograph, and slowly and steadily inject sample; ensure that Range and Output of gas chromatograph are appropriately set for specific compound.

C. Calculations

1. Determine concentration of C_2H_4 in subsample of Vacutainer® by entering peak height of sample (corrected for background C_2H_4) into regression line of peak height (chart units x Range x Output) vs. concentration of C_2H_4.

2. To calculate rate of ethylene production:

$$\text{nmoles } C_2H_4 \cdot ml^{-1} \cdot h^{-1} = \frac{\text{nmoles } C_2H_4 \times \frac{B}{A} \times \frac{S}{s}}{v \times t \times c}$$

where nmoles C_2H_4 is determined in 1, above

B = volume of vapor phase (ml) in incubation vessel

A = volume of aqueous phase (ml) in incubation vessel

S = volume of Vacutainer (ml)

s = volume of gas (ml) subsampled from vapor phase of incubation vessel and transferred to Vacutainer

v = volume of gas (ml) injected into gas chromatograph

t = incubation time (h)

c = transfer coefficient for ethylene (see Appendix F).

REFERENCES

Flett, R.J., R.D. Hamilton and N.E.R. Campbell. 1975. Aquatic acetylene-reduction techniques: solutions to several problems. Can. J. Microbiol. 22:43-51.

Loomis, A.G. 1928. In: E.W. Washburn, ed. International Critical Tables of Numerical Data, Physics, Chemistry and Technology. Vol. 3. McGraw-Hill, New York.

APPENDIX A

RATIO OF AQUEOUS PHASE TO VAPOR PHASE

Within the incubation vessel, it is necessary to maintain a vapor phase large enough to allow accurate subsampling. However, if the vapor phase: aqueous phase ratio is too large, the gases originally dissolved in the aqueous phase will diffuse into the vapor phase; conversely, gases in the vapor phase may dissolve into the aqueous phase, thus, alteration of the in situ conditions may occur. This may have marked effects on nitrogenase activity (Flett et al. 1975).

For Lake Mead, we have chosen an aqueous phase constituting approximately 79% of the total volume of the incubation vessel, i.e. 40 ml vapor phase: 150 ml aqueous phase in a 190-ml vessel.

APPENDIX B

AMOUNT OF C_2H_2 TO ADD TO SAMPLES

In order to measure maximum rates of nitrogenase activity, the appropriate amount of C_2H_2 must be added to the incubation vessels. If insufficient C_2H_2 is added, rates may be underestimated because the enzyme will be subsaturated; if excess C_2H_2 is added, enzyme inhibition may result and rates will also be underestimated.

The appropriate amount of C_2H_2 to add can be calculated based on literature values (Flett et al. 1975, see below) or can be determined experimentally. It is preferable to determine the amount experimentally by adding a range of concentrations and choosing that which yields the highest rates, since the amount of C_2H_2 per ml of water which saturates the enzyme in one situation may not do so in another.

1. By calculation

   a. To calculate the amount of C_2H_2 needed to saturate nitrogenase using the literature value of 0.082 ml C_2H_2/ml H_2O (Flett et al. 1975) the following equation is used:

$$0.082 = \frac{(m-[m/\{1 + (\alpha A/B)\}])}{A}$$

where m = amount of acetylene to add (ml)

α = Bunsen absorption coefficient for C_2H_2

A = volume of aqueous phase (ml)

B = volume of vapor phase (ml)

0.082 = literature value for saturation (mlC_2H_2/ml H_2O)

The computation for α, Bunsen absorption coefficient for C_2H_2, is shown in the equation below. Loomis (1928) gives values for K which are then used in the equation shown to derive α, at the appropriate temperature.

$$\alpha = \frac{17{,}032{,}400d(1 + a)}{Km}$$

where d = density of solvent (in this case H_2O, so d = 1)

$1 + a \simeq 1$

m = molecular weight of solvent (in this case, H_2O, so m = 18)

$$\therefore \alpha = \frac{946{,}244}{K}$$

K is taken from Table 1

Table 1. Variation with temperature of K and α for acetylene, when the solvent is H_2O.

T°C	$Kx10^6$	α	T°C	$Kx10^6$	α
10	0.727	1.302	21	0.935	1.012
11	0.746	1.268	22	0.955	0.991
12	0.765	1.237	23	0.974	0.972
13	0.783	1.208	24	0.993	0.953
14	0.803	1.178	25	1.01	0.937
15	0.822	1.151	26	1.03	0.919
16	0.841	1.125	27	1.05	0.901
17	0.858	1.103	28	1.07	0.884
18	0.878	1.078	29	1.09	0.868
19	0.897	1.055	30	1.11	0.852
20	0.917	1.032			

b. Example calculation of amount of C_2H_2 to add

At a water temperature of 20°C with an aqueous:vapor phase ratio of 150:40, the amount of C_2H_2 to add is determined as follows:

$$\alpha_{20°C} = \frac{946244}{0.917 \times 10^6} = 1.032$$

$$0.082 \text{ ml} C_2H_2 \cdot \text{ml} H_2O^{-1} = \frac{(m-[m/\{1+(1.032 \times 150/40)\}])}{150}$$

$$m = 15.5 \text{ ml}$$

Therefore, under the above conditions, and assuming that 0.082 $\text{ml}C_2H_2 \cdot \text{ml}$ of H_2O^{-1} saturates nitrogenase, at least 15.5 ml of C_2H_2 should be added.

For Lake Mead, we add 16 ml C_2H_2 to 150 ml lake water in a 190-ml incubation vessel.

c. Example of amount of C_2H_2 to subsample

To determine the amount of the vapor phase which can be subsampled without creating negative pressure within the incubation vessel, the amount of C_2H_2 remaining in the vapor phase after equilibrium is reached must be known.

The total amount of C_2H_2 dissolved in the aqueous phase is calculated as follows:

$$(0.082 \text{ ml} C_2H_2 \cdot \text{ml} H_2O^{-1})\ (150 \text{ ml } H_2O) = 12.3 \text{ ml } C_2H_2$$

The amount of C_2H_2 remaining in the vapor phase is thus:

$$15.5 \text{ ml} - 12.3 \text{ ml} = 3.2 \text{ ml}$$

Therefore, at least 3 ml can be withdrawn without creating negative pressure within the vessel.

2. By experimental determination

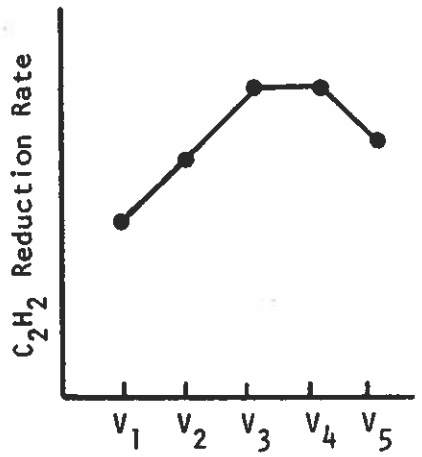
   a. Using an aqueous:vapor phase ratio of 150:40, choose 5 volumes of C_2H_2 bracketing the volume which will yield a concentration of 0.082 $mlC_2H_2 \cdot mlH_2O^{-1}$ at the given temperature (see Appendix B, section 1-b).

   b. Inject each volume into triplicate vessels with 1 blank for each volume. Allow approximately 1 h for incubation.

   c. At the end of the incubation period, subsample all vessels simultaneously.

   d. Calculate the acetylene reduction rate for each addition of C_2H_2.

   e. Plot the rate of C_2H_2 reduction vs. amount of C_2H_2 added.



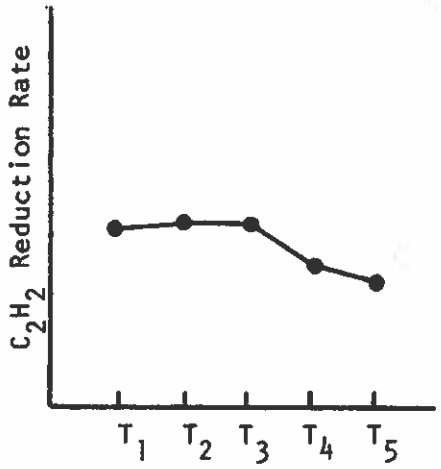
Choose the volume which yields the highest rate of C_2H_2 reduction.

APPENDIX C

LENGTH OF INCUBATION INTERVAL

The length of the incubation period should be chosen such that the production of ethylene remains linear during the entire interval. This period of linearity may vary with varying conditions, so the determination should be repeated periodically.

1. Experimental determination
   a. Add a volume of C_2H_2 known to be sufficient to saturate nitrogenase to each of 20 flasks (15 samples and 5 blanks) at T_0.
   b. Subsample 3 samples and 1 blank at hourly intervals.
   c. Determine C_2H_2 reduction rate per hour for each time interval.
   d. Make the following graph:



The theoretical graph above shows that linearity occurs only during a three-hour period, and that ethylene production

rate decreases thereafter. Thus, in this case, incubation periods should be limited to 3 hours.

2. For Lake Mead, the length of the incubation period at each site coincides with that chosen for the primary productivity measurements, i.e. 2.5 to 4 hours for stations 2 and 5; and 3 to 5 hours for stations 8, 9 and 10.

APPENDIX D

1. Operation of gas chromatograph (Carle AGC 211)

   a. Turn on helium carrier gas to ∿ 23 PSI.

   b. Turn on oven power switch, set temperature at 80°C. Oven must heat up for about 3-4 hours.

   c. Turn on recorder power switch.

   d. Measure helium flow rate through jet with teflon-tipped flow-meter provided with the instrument (caution:do not bend flow-meter tip since the quartz jet is extremely fragile); set flow rate to 100 mls·min^{-1}.

   e. After recorder is warmed up, balance it (return pen to zero) with recorder ZERO KNOB, with OUTPUT (G.C. panel) on TEST and RANGE (G.C. panel) on BALANCE.

   f. Turn OUTPUT to 1 and balance recorder again with AMPLIFIER BALANCE knob (G.C. panel).

   g. Turn hydrogen on to approximately 40 PSI. Wait approximately 5 minutes.

   h. Light flame with IGNITE button (G.C. panel); you should hear a definite pop. Check to see that flame is lit by holding a shiny surface, such as a knife blade or mirror, over flame opening and observe condensation. If flame is not lit, wait approximately 10 minutes and depress IGNITE button again.

   i. Turn on air to approximately 16 PSI, slowly.

   j. Slowly turn down hydrogen to 20 PSI. Wait approximately 5 minutes.

   k. Balance recorder by using ZERO SUPPRESSION knobs (coarse and

fine) (G.C. panel) with RANGE on 10^5 initially and OUTPUT on 1. Progressively turn down RANGE to 1 while balancing (zeroing) recorder pen with each step.

2. Shutdown of gas chromatograph

   a. Turn recorder off.

   b. Turn helium and air off.

   c. Turn helium down to approximately 5 PSI and leave oven on for daily use.

      Note: If gas chromatograph will not be used on a daily basis, the oven and helium should be turned off.

APPENDIX E

MIXING OF ETHYLENE STANDARDS

1. Techniques/suggestions

   a. Use only high purity ethylene (C_2H_4) gas from Matheson Gas Company.

   b. Fill football bladder with C_2H_4, flushing a number of times. Do not interchange bladders for C_2H_2 and C_2H_4!

   c. When making gas transfers with plastic syringes, flush syringe a number of times by slowly filling and depressing plunger at least 5 times.

   d. Depress syringe plunger to appropriate volume mark before removing needle from Vacutainer^R septum attached to football bladder.

   e. Quickly transfer syringe to clean and dry flask of known volume containing a small piece of paper to keep gas mixed. Slowly depress plunger into flask.

   f. Standards will yield best replicate injections if they are allowed to equilibrate for at least 4 h prior to subsampling.

   g. When taking samples from flasks, mix each standard prior to subsampling by shaking paper around. Again, flush syringe a number of times (this also helps mix standard) and depress plunger to 0.3 ml prior to removing syringe.

   h. Run duplicate or triplicate injections from each standard.

2. Procedure

   a. Using the Universal Ideal Gas Law, calculate the number of moles in 25 ml of pure C_2H_4 at 25°C (298°K):

$$n = \frac{PV}{RT}$$

$$n = \frac{(1\ \text{atm})(25\times10^{-3})}{(0.08205\ \text{l-atm/mol-deg})(298°\text{K})}$$

$$= 1.022\times10^{-3}\ \text{moles}$$

b. Primary Standard

Place 25 ml of pure C_2H_4 (1.022×10^{-3} moles) into 1000 ml:

$$\frac{1.022\times10^{-3}\ \text{moles}}{1000\ \text{ml}} = 1.022\times10^{-6}\ \text{moles}\cdot\text{ml}^{-1}$$

c. Secondary Standards

$$\frac{0.5\ \text{ml} \times 1.022\times10^{-6}\ \text{moles}\cdot\text{ml}^{-1}}{1000} = 0.51\times10^{-9}\ \text{moles}\cdot\text{ml}^{-1}$$

$$\frac{1.0\ \text{ml} \times 1.022\times10^{-6}\ \text{moles}\cdot\text{ml}^{-1}}{1000} = 1.02\times10^{-9}\ \text{moles}\cdot\text{ml}^{-1}$$

$$\frac{1.5\ \text{ml} \times 1.022\times10^{-6}\ \text{moles}\cdot\text{ml}^{-1}}{1000} = 1.53\times10^{-9}\ \text{moles}\cdot\text{ml}^{-1}$$

$$\frac{2.0\ \text{ml} \times 1.022\times10^{-6}\ \text{moles}\cdot\text{ml}^{-1}}{1000} = 2.04\times10^{-9}\ \text{moles}\cdot\text{ml}^{-1}$$

$$\frac{3.0\ \text{ml} \times 1.022\times10^{-6}\ \text{moles}\cdot\text{ml}^{-1}}{1000} = 3.07\times10^{-9}\ \text{moles}\cdot\text{ml}^{-1}$$

d. To run standard curve, place 0.3 ml of each secondary standard into gas chromatograph:

$0.3\ \text{ml} \times 0.51\ \text{nmoles}\cdot\text{ml}^{-1} = 0.15\ \text{nmoles}$

$0.3\ \text{ml} \times 1.02\ \text{nmoles}\cdot\text{ml}^{-1} = 0.31\ \text{nmoles}$

$0.3\ \text{ml} \times 1.53\ \text{nmoles}\cdot\text{ml}^{-1} = 0.46\ \text{nmoles}$

$0.3\ \text{ml} \times 2.04\ \text{nmoles}\cdot\text{ml}^{-1} = 0.61\ \text{nmoles}$

$0.3\ \text{ml} \times 3.07\ \text{nmoles}\cdot\text{ml}^{-1} = 0.92\ \text{nmoles}$

Generate standard curve of peak height (chart units x range x output) vs. nmoles C_2H_4.

e. If ethylene concentrations in samples fall beyond the range of

the standard curve, the primary standard should be decreased or increased accordingly, so that the secondary standards cover the range of sample activity.

APPENDIX F

CORRECTION FOR ETHYLENE SOLUBILITY

1. Since ethylene is not completely insoluble in water, a portion of that produced will remain in the aqueous phase. This amount depends on the temperature of the aqueous phase and the ratio of the aqueous phase to vapor phase. If this is not taken into consideration, the rate of ethylene production (acetylene reduction) will be underestimated.

   a. The following equation from Flett et al. (1975) is used to calculate the proportion of ethylene transferred from the aqueous phase to the vapor phase:

$$\frac{X}{m} = \frac{1}{1 + (\alpha\ A/B)}$$

   where X = the proportion of ethylene transferred to the vapor phase

   m = the total amount of ethylene in the incubation vessel (assume it is 1)

   α = Bunsen absorption coefficient for C_2H_4

   A = volume of aqueous phase (ml)

   B = volume of vapor phase (ml)

   b. The computation for α, Bunsen absorption coefficient for C_2H_4, is shown on the following page. Loomis (1928) gives values for K which are then used in the equation shown to derive α, at the appropriate temperature.

$$\alpha = \frac{17{,}032{,}400d(1+a)}{Km}$$

   where d = density of solvent (in case H_2O, so d = 1)

   $1 + a \simeq 1$

m = molecular weight of solvent (in this case H_2O, so m = 18)

$$\therefore \alpha = \frac{946,244}{K}$$

K is taken from Table 2.

Table 2. Variation with temperature of K and α for ethylene.

T°C	$Kx10^6$	α	T°C	$Kx10^6$	α
10	5.84	.1620	21	7.93	.1193
11	6.03	.1569	22	8.12	.1165
12	6.22	.1521	23	8.31	.1139
13	6.41	.1476	24	8.48	.1116
14	6.59	.1436	25	8.67	.1091
15	6.80	.1392	26	8.88	.1066
16	6.97	.1357	27	9.03	.1048
17	7.16	.1322	28	9.24	.1024
18	7.36	.1286	29	9.42	.1005
19	7.55	.1253	30	9.62	.0984
20	7.74	.1223			

c. Example of calculation of transfer coefficient for ethylene

At a water temperature of 20°C with an aqueous: vapor phase ratio of 150:40, the proportion of C_2H_4 transferred is determined as follows:

$$\alpha_{20°C} = \frac{946,244}{K} = \frac{946,244}{7.74x10^6} = 0.1223$$

$$X = \frac{1}{1 + (\alpha\ A/B)}$$

$$X = \frac{1}{1 + (.1223 \times 150/40)} = 0.686$$

The amount of ethylene determined in the vapor phase is then corrected by dividing by 0.686 to account for that remaining in the aqueous phase.

Procedure for Measuring Phytoplankton Productivity using $^{14}C\text{-}CO_2$ (After method of Steemann-Nielsen 1952)

I. Basis of Method

The radiocarbon assay of primary productivity is based on the fact that organisms which assimilate $^{12}C\text{-}CO_2$, containing the stable isotope of carbon, will also assimilate $^{14}C\text{-}CO_2$, containing the radioactive isotope, with little difference between the rate of uptake of each. A small amount of radioactive bicarbonate, relative to the total CO_2 pool, is added to the sample, contained in a clear glass bottle. After a suitable incubation period, the amount of radioactivity incorporated into the phytoplankton by photosynthesis is measured. This is done by filtering an aliquot (or the entire bottle) onto one of various filters (e.g., Millipore^R, Nuclepore^R) and counting with standard scintillation counting techniques. Knowing the total amount of carbon-12 in the sample (determined from pH and alkalinity measurements), the ratio of carbon-12 to carbon-14 available, and the isotope factor (correction for difference in the rate of uptake of carbon-12 and carbon-14), the amount of carbon-12 incorporated during the incubation interval can be determined.

II. Materials

A. Scintillation counter (e.g., Beckman LS-100)

1. Scintillation vials, clear glass with screw tops

2. Scintillation cocktail (e.g., PCS)

B. Incubation bottles (125 ml clear reagent bottles, 2 light, and

1 dark, wrapped with black electrical tape and painted white, with wire loops around necks to suspend from hooks on incubation lines)

C. Field (in situ) incubation apparatus

1. Line equipped with clips at derived depths of incubation, on which to hang bottles

D. ^{14}C-labeled bicarbonate purchased from Amersham, prediluted and ampoulated to proper-activity working solution (see Appendix, page 84).

E. Dispensing syringe (0.5 to 1.0 ml capacity) for adding $^{14}C\text{-}HCO_3^-$ to samples

F. Ice chests or other light-proof boxes

G. Millipore^R filtration apparatus (25 or 47 mm)

H. 25 or 47 mm Millipore^R filters or equivalent, 0.45 µm pore size

I. Alkalinity determination apparatus (see page 109 for necessary equipment and methods)

III. Procedure

A. Sample depths of interest with van Dorn (or similar) sampler.

1. Rinse and overfill 2 light bottles and 1 dark bottle from each depth; replace lids and place bottles in darkened box, taking care to prevent light shock by shielding from direct sunlight.

2. After all bottles are filled, remove tops sequentially and add $^{14}C\text{-}HCO_3^-$ by syringe (see Appendix D). (When adding radiocarbon, immerse syringe tip below sample water surface.) Immediately replace lids after injection, and shake

bottles to mix isotope.

3. Place bottles on clips at appropriate depths and suspend lines.

B. After suitable incubation interval (see Appendix D), place bottles immediately into light-proof boxes. Keep cool.

C. Filter as soon as possible.

1. Filter bottle contents or subsample onto 0.45 µm pore size Millipore^{R} filter.

2. Rinse sample filter with 0.005 N HCl to remove any unincorporated $^{14}C\text{-}HCO_3^-$.

3. Immediately place filter into pre-labeled (numbered) scintillation vial. Place vials on top of corresponding numbered lids and leave vials uncapped overnight before dispensing scintillation cocktail into each.

D. Prepare filters for scintillation counting.

1. When filters are dry, add 20 ml of premixed scintillation cocktail (see Appendix A).

2. Wipe all spilled cocktail off vial surface, cap tightly and place in scintillation counter overnight in dark before counting to prevent elevated counts due to fluorescence of cocktail.

3. Count each sample for period of time sufficient to yield counts within 5%. For Lake Mead, samples are counted for 10 minutes.

E. Calculations

1. Productivity (mg $C \cdot m^{-3} \cdot h^{-1}$) =

$$\frac{[(L-D) \times {}^{12}C \text{ available} \times 1.06 \times 1000]}{{}^{14}C \text{ added} \times E \times 2.22 \times 10^{6} \times t}$$

where L = CPM of light bottle (total in 125 ml)

D = CPM of dark bottle (total 125 ml)

${}^{12}C$ available = total carbon (g $C \cdot m^{-3}$), derived from alkalinity, pH and temperature (see Alkalinity, p. 109)

1.06 = correction factor for uptake of ^{14}carbon vs. ^{12}carbon

1000 = $mg \cdot g^{-1}$

${}^{14}C$ added = μC_i of isotope added to 125 ml

E = efficiency of scintillation counter

2.22×10^{6} = $DPM \cdot \mu C_i^{-1}$

t = incubation time (h)

2. Productivity (mg $C \cdot m^{-2} \cdot h^{-1}$) =

$$\sum_{i=1}^{n} \left(\frac{X_i + X_{i+1}}{2}\right) (Z_{i+1} - Z_i)$$

where X_i = productivity (mg $C \cdot m^{-3} \cdot h^{-1}$) at depth Z_i

Z_i = discrete depth in meters of ith sample

n = total depth of water column

3. Productivity (mg $C \cdot m^{-2} \cdot da^{-1}$) =

$$I \times \sum_{i=1} \left(\frac{X_i + X_{i+1}}{2} \right) (Z_{i+1} - Z_i)$$

where I = ratio of total incident radiation (langleys$\cdot da^{-1}$) to incident radiation during incubation period (langleys$\cdot h^{-1}$)

See example in Appendix E (page 91).

Note: I is determined by planimetric integration of light curve produced by pyroheliometer.

REFERENCES

Steemann-Nielsen, E. 1952. The use of radio-active carbon (C^{14}) for measuring organic production in the sea. J. Cons. Int. Mer. 18:177-140.

APPENDIX A

SCINTILLATION COCKTAIL

1. PCS, phase combining solution, is obtained from Amersham Corporation.
2. PCS is mixed with xylene (2:1, vol/vol); under these conditions, PCS will hold up to 10% aqueous volume.

APPENDIX B

DILUTION AND AMPOULATION OF ^{14}C-HCO_3^- SOLUTION

1. Sodium ^{14}C-carbonate (2.5 ml of aqueous solution, 2.0 $mCi \cdot ml^{-1}$) is obtained from The Radiochemical Center, Amersham, England.
2. Procedure
   a. Boil approximately 3000 ml of distilled-deionized water in a 5-l beaker for approximately 10 minutes.
   b. To 2300 ml boiled distilled-deionized water, add 0.1 g NaOH.
   c. Add either (1) 0.294 g $NaHCO_3$ or (2) 0.186 g Na_2CO_3 to get an alkalinity of 120 $mg \cdot l^{-1}$ (this is added so that solution will have approximate alkalinity of the lake water to which it is added).
   d. Check pH (it should be between 9.5-10.0); adjust if necessary.
   e. Add purchased ampoule of sodium ^{14}C-bicarbonate or sodium ^{14}C-carbonate.
   f. Bring volume up to 2500 ml with boiled distilled-deionized water.
   g. Mix thoroughly on magnetic stirrer with stir bar.
   h. Fill ampoules from siphon system. Seal.
   i. Check for faulty seals by inverting ampoules in pan containing water to which a suitable dye (e.g. phenolphthalein) has been added. Autoclave for 15 min at 15 PSI. Discard any ampoules containing dye.
   j. Log all information concerning batch in record book.

APPENDIX C

STANDARDIZATION OF ^{14}C-CO_2

1. Standardized solution of carbon-14 is obtained from the Radiochemical Center, Amersham, England.

   Chemical form: 0.05 M sodium carbonate solution in water containing 1 mg formaldehyde·ml^{-1}

   Activity = 1.191 μCi·ml^{-1} = 2.64 × 10^6 DPM·ml^{-1}

2. Prepared solution of carbon-14 (see Appendix B)

3. Fluor: PCS/Xylene (2:1 vol/vol)

4. Procedure for standardization

   Triplicate 1, 3 and 5 μl aliquots of a standard ^{14}C-solution of known activity (1, above) are added to the scintillation fluor with a 10 μl syringe. Aliquots of the prepared ^{14}C-solution are dispensed similarly. Both are then counted in a Beckman LS-100 Scintillation Counter. The efficiency of the scintillation counter and the activity of the prepared ^{14}C-solution can be determined from the results.

5. Calculations

   a. Efficiency of scintillation counter

$$E = \frac{CPM}{DPM}$$

   where CPM = counts per minute of the known standard

^{14}C-solution in the scintillation counter

DPM = disintegrations per minute of the known standard ^{14}C-solution calculated from the activity of the solution obtained from Amersham

b. Activity of prepared ^{14}C-bicarbonate solution ($\mu Ci \cdot ml^{-1}$)

$$\mu Ci = 2.22 \times 10^6 DPM$$

$$DPM = \frac{CPM}{E}$$

$$\therefore \mu Ci \cdot ml^{-1} = \frac{CPM}{(E)(2.22 \times 10^6\ DPM \cdot \mu Ci^{-1})(V)}$$

where CPM = counts per minute of solution

E = efficiency of scintillation counter determined in a, above

2.22×10^6 = constant converting DPM, disintegrations per minute, to μCi (microcuries)

V = volume of solution counted, in ml

c. Example calculation

The following information is known:

i. Activity of standard ^{14}C-solution from Amersham = $1.191\ \mu Ci \cdot ml^{-1}$

ii. CPM of standard ^{14}C-solution from scintillation counter = $6912\ CPM \cdot 3\mu l^{-1}$

iii. CPM of prepared ^{14}C-solution from scintillation counter = $11035\ CPM \cdot 3\mu l^{-1}$

Using the above information, the following can be calculated:

i. Efficiency of scintillation counter

^{14}C standard activity = 1.191 $\mu Ci \cdot ml^{-1}$ = 2644 $DPM \cdot \mu l^{-1}$

= 7932 $DPM \cdot 3\ \mu l^{-1}$

$$E = \frac{CPM}{DPM} = \frac{6912}{7932} = 0.87$$

ii. Activity of prepared ^{14}C-solution ($\mu Ci \cdot ml^{-1}$)

$$\mu Ci \cdot ml^{-1} = \frac{11035\ CPM}{(0.87)(2.22 \times 10^{6}\ DPM \cdot \mu Ci^{-1})(3 \times 10^{-3}\ ml)}$$

$$\mu Ci \cdot ml^{-1} = 1.90$$

Note: Possible quenching, that is, a decrease in CPM due to to some interference, may occur in some systems. Algal cells and other particulate matter, such as silt, as well as some filter types, may decrease the efficiency of the scintillation counter. It is therefore advisable to run quench curves (efficiency vs. amount of interfering substance), from which counter efficiency can then be determined. In water samples from Lake Mead no quenching has been found.

APPENDIX D

SPECIFIC INSTRUCTIONS FOR INDEX STATIONS ON LAKE MEAD

Station	ml of $^{14}C\text{-}HCO_3^-$ (1.90 $\mu Ci \cdot ml^{-1}$)	Incubation Period (h)
2	0.5	2.5 - 4
5	0.5	2.5 - 4
8	1.0	3 - 5
9	1.0	3 - 5
10	1.0	3 - 5

APPENDIX E

EXAMPLE OF CALCULATIONS

Table 1. Determination of ^{12}C available from alkalinity.

Depth (m)	Alkalinity (g $CaCO_3 \cdot m^{-3}$)	Temperature (°C)	pH	Factor[a]	^{12}C available (g $C \cdot m^{-3}$)
0	129	13.5	7.35	0.27	34.8
1	129	13.5	7.25	0.275	35.5
3	130	13.75	7.35	0.27	35.1
5	128	13.75	7.30	0.27	34.6
7	131	13.75	7.15	0.285	37.3
10	140	13.25	7.20	0.28	39.2

[a]Determined by interpolation from Table 1, p. 112

APPENDIX E, cont'd

Table 2. Productivity Calculations

Depth (m)	^{12}C available ($g\ C \cdot m^{-3}$)	Productivity[b] ($mg\ C \cdot m^{-3} \cdot h^{-1}$)	Productivity[c] ($mg\ C \cdot m^{-2} \cdot h^{-1}$)	Productivity[d] ($mg \cdot m^{-2} \cdot da^{-1}$)
0	34.8	28.8	439	4726
1	35.5	62.5		
3	35.1	72.3		
5	34.6	56.1		
7	37.3	25.5		
10	39.2	7.0		

[b]Productivity ($mg\ C \cdot m^{-3} \cdot h^{-1}$)

For depth 0 m, let:

L = 12,526 CPM

D = 335 CPM

^{12}C available = 34.8 g $C \cdot m^{-3}$

^{14}C added = 1.905 μC_i

E = 0.893

t = 4.17 h

$$\frac{[(12{,}526 - 335) \times 34.8 \times 1.06 \times 1000]}{1.905 \times 0.893 \times 2.2 \times 10^6 \times 4.17} = 28.8\ mg\ C \cdot m^{-3} \cdot h^{-1}$$

Continue calculations for each depth.

APPENDIX E, cont'd

[c]Productivity (mg $C \cdot m^{-2} \cdot h^{-1}$) =

$$\frac{(28.8 + 62.5)}{2} \times (1 - 0) + \frac{(62.5 + 72.3)}{2} \times (3 - 1) + \frac{(72.3 + 56.1)}{2} \times (5 - 3)$$

$$+ \frac{(56.1 + 25.5)}{2} \times (7 - 5) + \frac{(25.5 + 7.0)}{2} \times (10 - 7) = 439 \text{ mg } C \cdot m^{-2} \cdot h^{-1}$$

[d]Productivity (mg$C \cdot m^{-2} \cdot da^{-1}$) =

$$I \times 439 \text{ mg}C \cdot m^{-2} \cdot h^{-1}$$

Where

$$I = \left[\frac{537 \text{ langleys} \cdot da^{-1}}{208 \text{ langleys} \cdot \text{incubation period}^{-1}}\right] \times 4.17 \text{ h} \cdot \text{incubation period}^{-1}$$

Procedure for Zooplankton Collection and Enumeration

I. Sample Collection

One to three replicate vertical tows are taken at each sampling site with an 80 µm mesh-size Wisconsin net, the diameter of which is 13 cm (= 1.3273×10^{-2} m^2 sampling area). Depths of tows are as follows: at stations where depth exceeds 100 m, tows are made from 100 m to surface; where depth is between 40 and 100 m, tows are made from 40 m to surface; at shallow stations (<40 m) the net is lowered to within 1 m of the bottom and retrieved to the surface. Preliminary studies on Lake Mead (Burke, unpublished) have shown that zooplankton are uncommon below 40 m; therefore, the establishment of 40 and 100 m sampling depths should not result in appreciable underestimates of zooplankton densities in Lake Mead.

Tows are made at a constant velocity of approximately 1 $m \cdot sec^{-1}$. When the net is brought to the surface, zooplankton are rinsed from the sides of the net and bucket and are transferred to 40-ml snap-top polyethylene vials with a 20 percent formalin solution. Samples are thus fixed with a formalin concentration of approximately 5 to 10 percent. Vials are then sealed with masking tape, and marked with location, date, time, and sampling depth. Samples are stored in the refrigerator until counting (usually within one month of collection).

II. Enumeration

A. Samples are thoroughly mixed and three to five one-ml replicate subsamples are removed with a large bore one-ml pipet; each

subsample is then placed in a separate Sedgewick-Rafter counting chamber.

B. The volume (ml) of vial contents remaining is measured and corrected by the volume removed by subsampling.

C. The total number of organisms in each chamber is counted with a compound microscope at 40X magnification.

D. Microcrustacea are usually identified to species and Rotifera to genus or species. Identifications are made using the keys of Brooks (1957), Edmondson (1959) and Pennak (1953).

E. Calculations

1. Mean number$\cdot$ml^{-1} of each organism in the concentrated tow sample is calculated as follows:

$$\bar{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

where $\bar{X}$ = mean number of organisms per ml in sample

X_i = number of organisms counted in each replicate i

n = number of 1-ml replicates counted

2. Areal and cubic densities are calculated by extrapolation of the data obtained above.

a. Areal density (number of organisms$\cdot$m^{-2}):

$$D_A = \frac{\bar{X} \times V}{K}$$

where $\bar{X}$ = mean number of organisms per ml in sample

V = total volume of sample

K = 1.3273×10^{-2} m^2 = surface area of opening of Wisconsin net (13 cm diameter)

b. Cubic density (number of organisms·m^{-3}):

$$D_C = \frac{\overline{X} \times V}{K \times Z}$$

where $\overline{X}$, V, and K are as defined above

and Z = depth (m) of vertical tow

Note: D_C is useful in comparing densities between stations only when Z is equal.

REFERENCES

Brooks, J.L. 1957. The systematics of North American Daphnia. Mem. Conn. Acad. Arts Sci. 13:5-180.

Edmondson, W.T., ed. 1959. Fresh-water Biology. John Wiley and Sons, New York and London. 1248 pp.

Pennak, R.W. 1953. Freshwater Invertebrates of the United States. The Ronald Press Co., New York. 769 pp.

SECTION II. CHEMICAL METHODS

Procedure for Collection of Water Samples for Nutrient Analyses

I. Discrete samples

A three-liter opaque, non-metallic, polyvinylchloride (PVC) van Dorn water bottle suspended from a line calibrated in 0.5-1.0-meter increments is used to sample lake water from discrete depths. Depths sampled vary, but are usually representative of regions within the epi-, meta-, and hypolimnion. Subsamples are transferred, after mixing, to containers through a neoprene or tygon tube attached to bottle outlet.

II. Integrated (0 to 5 m) samples

Integrated water samples are taken from the surface to a 5-meter depth with a flexible swimming pool hose (approximately 5 cm in diameter). A lead weight and a line running to the water surface are attached to the bottom end of the hose. A rubber stopper is attached to the surface end with a line. At each station, the hose is lowered and raised without the stopper inserted, and flushed several times with lake water. The hose is then lowered at a rate slow enough to ensure that a vertical water sample from surface to 5 m is obtained. The stopper is then inserted at the surface end which is held precisely even with the surface of the water. The surface end (with stopper securely inserted) is then released and allowed to float. The sampler is retrieved by the line attached to the weighted end, which is then placed into the opening of a 3 gallon polyethylene carboy which has been rinsed thoroughly with surface water. The stopper is removed and the sample is drained into the carboy. The procedure is repeated if necessary. The sample within

the carboy is thoroughly mixed by shaking before subsamples are removed.

III. Bottles for nutrient samples

Polyethylene bottles (100- to 250-ml capacity) used for subsampling are thoroughly washed with 0.1 N HCl and distilled-deionized water, and are pre-rinsed with sample in the field. Bottles are pre-marked with sample date, location, and depth. Approximately 10% of the bottle volume is left as an airspace to prevent bottle breakage when samples are frozen. Larger samples (> 1 liter) are transferred to Cubitainers^{R}, flexible polyethylene containers. These are also washed with acid and distilled-deionized water and are rinsed in the field with the sample. Cubitainers are marked with station location only, since samples contained in them are not stored.

IV. Sample treatment after collection

After samples are obtained, they are placed on ice in Coleman coolers and transported to the laboratory for appropriate treatment.

Care Of Glassware Used in Chemical Analyses

I. All stored glassware (e.g. flasks, cylinders, etc.) is filled with 0.1 N HCl, covered with Parafilm^{R} or rubber caps, and rinsed (2-3 times) with distilled-deionized water before use.

II. All pipets and funnels are rinsed with 0.1 N HCl and distilled-deionized water prior to storing.

III. After use, all glassware is washed as follows:

A. Hot tap water (2 rinses)

B. Tap distilled water (2 rinses, include outside)

C. 0.1 N HCl

1. Fill flasks and cylinders, dry outside with paper towel.

2. Rinse pipets, funnels including outside walls of pipets (follow with distilled-deionized water rinse).

Procedure for Determination of Accuracy and Precision of Analyses (Quality Control) (After method in EPA 1979)

I. Accuracy of Instrument and Technician

This can be determined from the standard curves which are generated by reading absorbances of standards made up with reagents and distilled water and plotting against concentrations. Daily results are compared to means and standard deviations of values determined in previous routine runs. Instrument and technician are required to produce results (means) within standard deviations determined from previous routine runs; if not, sample analysis is repeated.

II. Accuracy of Method Relative to Particular Natural Water System

A. This can be assessed by determining the per cent recovery from water samples of added spikes of known concentration.

1. Percent recovery is calculated as follows:

$$\left[\frac{\text{Concentration of spiked sample} - \text{Concentration of unspiked sample}}{\text{Concentration of spike}}\right] \times 100$$

$$= \% \text{ Recovery} = P$$

2. Quality Control Chart - based on percent recovery of spike (Refer to Chapter 6.3.1 Quality Control Charts for Accuracy of EPA Handbook for Analytical Quality Control in Water and Wastewater Laboratories; March 1979).

a. Calculate average percent recovery of spike for data from initial run as follows:

$$\bar{P} = \frac{\sum_{i=1}^{n} P_i}{n}$$

Where $\bar{P}$ = average percent recovery

n = number of samples

P_i = percent recovery of individual samples

b. Calculate standard deviation of percent recovery of spike for data from initial runs as follows:

$$S_p = \sqrt{\frac{\sum_{i=1}^{n} P_i^2 - (\sum_{i=1}^{n} P_i)^2/n}{n-1}}$$

c. Calculate upper control limit (UCL), i.e. the value of the average percent recovery plus 3 standard deviations:

$$UCL = \bar{P} + 3S_p$$

All determined values for P_i must be less than the value for UCL or samples should be re-analyzed.

d. Calculate upper warning limit (UWL), i.e. the value of the average percent recovery plus 2 standard deviations:

$$UWL = \bar{P} + 2S_p$$

If determined values for P_i fall above this UWL it is a signal that problems may be developing.

e. Calculate lower control limit (LCL), i.e. the value of the average percent recovery minus 3 standard deviations:

$$LCL = \bar{P} - 3S_p$$

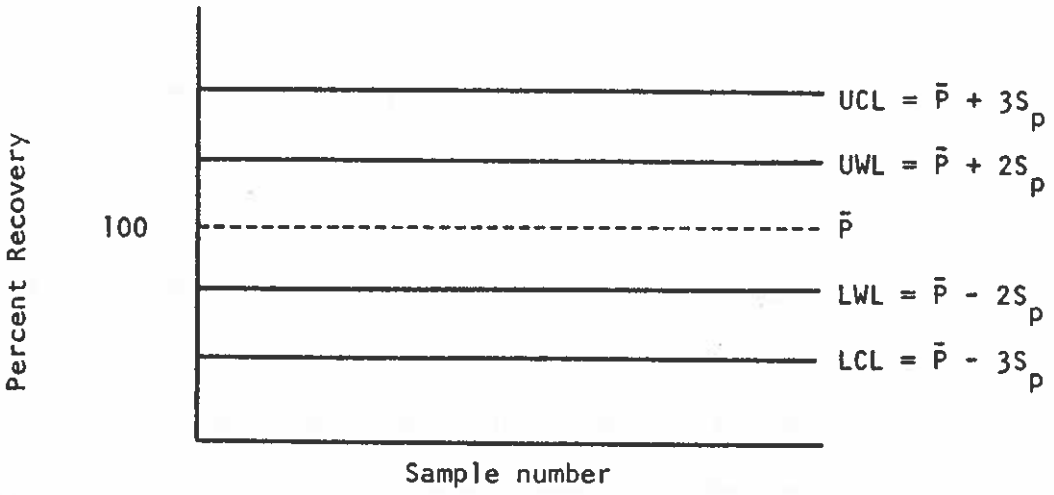
All determined values for P_i must be greater than the value of LCL or samples should be re-analyzed.

f. Calculate lower warning limit (LWL), i.e. the value of the average percent recovery minus 2 standard deviations:

$$LWL = \bar{P} - 2S_p$$

If determined values for P_i fall below this LWL it is a signal that problems may be developing.

g. Draw control chart as follows:



Note: 68% of the initializing data should fall within the range of $\bar{P} \pm S_p$; if less than 50 percent of the initializing data falls within this interval, the control chart is probably not valid.

h. Application of control chart

The following two conditions indicate an out-of-control situation:

(a) any point beyond upper or lower control limits, or

(b) seven successive points on the same side of the value $\bar{P}$ of the central line.

If either situation occurs, analyses must be terminated until problem is identified and resolved.

(Note: This method of quality control assessment is often confusing to reviewers because, for example, a recovery within 1 $\mu g \cdot l^{-1}$ of a 5 $\mu g \cdot l^{-1}$ spike is 80% while recovery within 1 $\mu g \cdot l^{-1}$ of a 10 $\mu g \cdot l^{-1}$ spike is 90%. Therefore, a more easily understood assessment of accuracy can be made using actual concentrations recovered from a spiked sample; see B., below).

B. Accuracy can also be assessed by determining the actual concentration recovered from a spiked sample.

1. Concentration ($\mu g \cdot l^{-1}$) recovered is calculated as follows: Concentration of spiked sample-Concentration of unspiked sample

2. Quality control chart-based on concentration recovered from a spiked sample (separate charts are required for each concentration of spike)

a. Calculate average concentration recovered for data from initial runs as follows:

$$\bar{C} = \frac{\sum_{i=1}^{n} C_i}{n}$$

Where $\bar{C}$ = average concentration recovered

n = number of samples

C_i = concentration recovered of individual samples

b. Calculate standard deviation of concentration recovered for data from initial runs as follows:

$$S_c = \sqrt{\frac{\sum_{i=1}^{n} C_i^2 - \left(\sum_{i=1}^{n} C_i\right)^2/n}{n-1}}$$

c. Calculate upper control limit (UCL), i.e. the value of the average concentration recovered plus 3 standard deviations:

$$UCL = \bar{C} + 3S_c$$

All determined values for C_i must be less than the value for UCL or sample should be re-analyzed.

d. Calculate upper warning limit (UWL), i.e. the value of the average concentration recovered plus 2 standard deviations:

$$UWL = \bar{C} + 2S_c$$

If determined values fall above this line it is a warning that there may be problems developing.

e. Calculate lower control limit (LCL), i.e. the value of the average concentration recovered minus 3 standard deviations:

$$LCL = \bar{C} - 3S_c$$

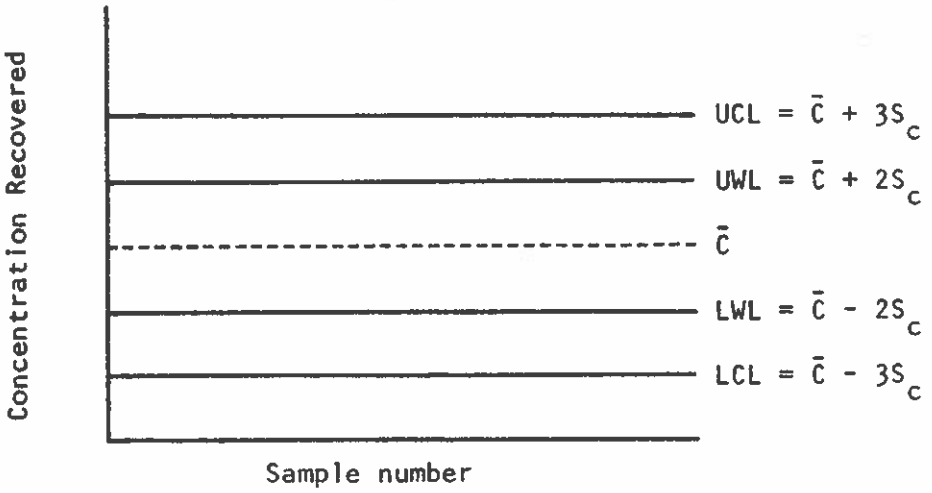
All determined values for C_i must be greater than the value for LCL or samples should be re-analyzed.

f. Calculate lower warning limit (LWL), i.e. the value of the average concentration recovered minus 2 standard deviations:

$$LWL = \bar{C} - 2S_c$$

If determined values fall below this line it is a warning that there may be problems developing.

g. Draw control chart as follows:



h. Application of control chart

The same criteria for judging acceptability of data based on percent recovery of spike (see A.h. above) apply in judging acceptability of data based on concentration recovered.

C. 95% Confidence Intervals

95% confidence intervals are calculated as follows:

$$C.I._{95\%} = \bar{X} \pm \frac{t_{.05}\ S.D.}{\sqrt{n}}$$

Where $\bar{X} = \bar{P}$ or $\bar{C}$

$\pm t_{.05}$ = value of student t for n observations and 95% confidence level

S.D. = S_p or S_c = standard deviation

n = number of samples

III. Precision of Method

A. Precision of the method is determined by analyzing replicate samples and determing R, the absolute value of the difference in concentrations.

1. The range of replicates A and B is given as follows:

$$R = |A-B|$$

Where R = range

A = concentration of replicate A

B = concentration of replicate B

2. Quality Control Tables - based on range of duplicate determinations (Refer to Chapter 6.3.2 Quality Control Charts for Precision of EPA Handbook for Analytical Quality Control in Water and Wastewater Laboratories; March 1979).

Control charts, similar to those for accuracy assessment, can be developed for R. However, since the primary concern in using such charts is whether the value R is less than the upper control limit, it is easier to develop tables of these limits.

a. Since R depends on concentration, when such tables are initially established, samples with similar concentration levels and ranges are placed into groups. The average range of each group is determined by averaging the ranges of duplicates within each group, i.e.:

$$\bar{R} = \frac{\sum_{i=1}^{n} R_i}{n}$$

Note: R refers to the range of individual sets of duplicates, determined from analysis.

$\bar{R}$ refers to average range used to establish quality control tables.

b. Upper control limits (UCL) for each concentration range are calculated by multiplying the range $\bar{R}$ for each group by the Shewhart factor, D_4, which is equal to 3.27 for duplicate analyses.

$$UCL = \bar{R} \times 3.27$$

c. The tables should also contain values for the critical range, R_c, within each group. This critical range is the value of the upper control limit rounded off to the nearest 0.5 unit for lower concentration levels and to the nearest 1 unit for higher concentration levels.

d. Application of control table

If the range of any set of duplicate analyses exceeds R_c within the appropriate concentration level, further analyses should be suspended until the problem is identified and eliminated.

IV. Sensitivity of method (minimum detection level)

Minimum detection levels are estimated from recovery of spikes of lake water samples, and the standard curve of absorbance vs. concentration of standards prepared with distilled-deionized water. The minimum detection level must be consistently higher than the distilled-deionized water-reagent blanks.

REFERENCES

U.S. Environmental Protection Agency. 1979. Handbook for analytical quality control in water and wastewater laboratories. EPA Report No. 600/4-70-019, March 1979. Environmental Monitoring and Support Laboratory, Office of Research and Development, Cincinnati, Ohio.

Procedure for Determination of Alkalinity (After APHA 1975)

I. Basis of Method

Alkalinity is the capacity of water to accept protons and is due to the presence of bicarbonate, carbonate, and hydroxyl ions. It is determined by titration of a water sample with a strong mineral acid (such as hydrochloric or sulfuric) to the successive bicarbonate and carbonic acid equivalence points, indicated either potentiometrically or by the use of acid-base color indicators (such as phenolphthalein), responding in the pH range 4-5. The pH values suggested as equivalence points for the corresponding alkalinity concentrations as calcium carbonate are as follows: pH 5.1 for total alkalinities of about 30 $mg \cdot l^{-1}$, pH 4.8 for 150 $mg \cdot l^{-1}$, and pH 4.5 for 500 $mg \cdot l^{-1}$ (APHA 1975, pp. 278-282).

II. Materials and Reagents

A. pH meter

B. Buffer (Potassium acid phthalate, pH 4.01 ± .01 @ 25°C obtained from: Harleco, Division of American Hospital Supply Corporation, Gibbstown, NJ 08027 or equivalent)

C. Standard NaOH (N/44 = 0.0227 N)

Obtained from Hach Chemical Company, cat. no. 192

D. H_2SO_4 (1 N)

Dilute 28 ml concentrated acid to 1 liter with distilled-deionized water.

E. H_2SO_4 (0.02 N)

Dilute 20 ml 1 N H_2SO_4 to 1 liter with distilled-deionized water.

F. Phenolphthalein acid-base color indicator

G. Erlenmeyer flasks (250 ml) for titrating samples

H. Buret (50 ml) for holding H_2SO_4

III. Procedure

A. Standardization of H_2SO_4

Note: Standardization should be done exactly as if the NaOH were a lake water sample.

1. Calibrate pH meter with buffer at temperature of the standard NaOH solution.
2. Place 100 ml of standard NaOH solution into Erlenmeyer flask.
3. Add 3 drops of phenolphthalein indicator.
4. Titrate with H_2SO_4 to pH 4.8 or other end points chosen on basis of the total alkalinity.
5. Calculate the normality of acid by the following equation:

$$N_A = \frac{V_B \times N_B}{V_A}$$

where N_A = normality of H_2SO_4

N_B = normality of NaOH

V_A = volume of H_2SO_4 used to titrate to endpoint

V_B = volume of NaOH

B. Sample Determination

1. Calibrate pH meter with buffer at temperature of the lake water sample.
2. Place 100 ml of lake water sample into Erlenmeyer flask.
3. Add 3 drops of phenolphthalein indicator.

4. Titrate with H_2SO_4 until solution reaches faint pink color corresponding to a pH of 8.3.
5. Note volume to 0.1 ml.

   This will equal carbonate alkalinity (pH = 8.3, for Lake Mead, total alkalinity in range of 150 $mg \cdot l^{-1}$).
6. Continue titration until pH reaches 4.8, determined by pH electrode.
7. Note volume to 0.1 ml.

   This will equal total alkalinity (pH = 4.8, for Lake Mead, alkalinity in range of 150 $mg \cdot l^{-1}$).

C. Calculations

1. Carbonate alkalinity (mg $CaCO_3 \cdot l^{-1}$) =

$$\frac{A \times N \times 50{,}000}{\text{ml sample}}$$

where A = ml of H_2SO_4 to reach phenolphthalein end point

N = normality of H_2SO_4

2. Total alkalinity (mg $CaCO_3 \cdot l^{-1}$) =

$$\frac{B \times N \times 50{,}000}{\text{ml sample}}$$

where B = ml of H_2SO_4 to reach endpoint of pH = 4.8.

3. To convert total alkalinity (mg $CaCO_3 \cdot l^{-1}$) to carbon (mg $C \cdot l^{-1}$),

Total alkalinity (mg $CaCO_3 \cdot l^{-1}$) × F = carbon (mg $C \cdot l^{-1}$)

where F is factor taken from Table 1, below, depending on temperature and pH of sample at time of alkalinity determination.

Table 1. Factors for the conversion of total alkalinity to milligrams of carbon per liter (from Saunders et al. 1962)

pH	Temperature (°C)			
	10	15	20	25
7.0	0.31	0.30	0.30	0.29
7.1	0.29	0.29	0.29	0.28
7.2	0.28	0.28	0.28	0.27
7.3	0.27	0.27	0.27	0.27
7.4	0.27	0.26	0.26	0.26
7.5	0.26	0.26	0.26	0.26
7.6	0.26	0.25	0.25	0.25
7.7	0.25	0.25	0.25	0.25
7.8	0.25	0.25	0.25	0.25
7.9	0.25	0.25	0.25	0.25
8.0	0.25	0.25	0.24	0.24
8.1	0.24	0.24	0.24	0.24
8.2	0.24	0.24	0.24	0.24
8.3	0.24	0.24	0.24	0.24
8.4	0.24	0.24	0.24	0.24
8.5	0.24	0.24	0.24	0.24
8.6	0.24	0.24	0.24	0.24
8.7	0.24	0.24	0.24	0.24
8.8	0.24	0.24	0.23	0.23
8.9	0.23	0.23	0.23	0.23
9.0	0.23	0.23	0.23	0.23
9.1	0.23	0.23	0.23	0.23
9.2	0.23	0.23	0.23	0.23
9.3	0.23	0.22	0.22	0.22
9.4	0.22	0.22	0.22	0.22

REFERENCES

APHA. 1975. Standard Methods for Examination of Water and Wastewater. 14th ed., American Public Health Association. Washington, D.C., 1193 pp.

Saunders, G.W., F.B. Trama, and R.W. Bachmann. 1962. Evaluation of a modified C^{14} technique for shipboard estimation of photosynthesis In large lakes. Great Lakes Research Division Publication No. 8. Univ. of Michigan, Ann Arbor, Michigan. 61 pp.

Procedure for Determination of Color (After APHA 1975)

I. Basis of Method

The true color of a water sample is the color of light transmitted by the solution after the suspended particles have been removed by filtration. True color is imparted by dissolved substances such as metallic ions (iron and manganese) and humic acids. Color is determined by comparing samples to known concentrations of standard color units. This method employs platinum-cobalt standards and is a modification of that given in APHA (1975, pp. 64-65).

II. Standards

A. Stock standard: Platinum-cobalt solution = 500 color units (Hach Chemical Co.)

B. Working standards

Prepare from 500 color unit platinum-cobalt standard using distilled-deionized water

a. $\frac{1 \text{ ml of } 500 \text{ units}}{500 \text{ ml}} = 1 \text{ unit}$

b. $\frac{1 \text{ ml of } 500 \text{ units}}{250 \text{ ml}} = 2 \text{ units}$

c. $\frac{1 \text{ ml of } 500 \text{ units}}{100 \text{ ml}} = 5 \text{ units}$

d. $\frac{2 \text{ ml of } 500 \text{ units}}{100 \text{ ml}} = 10 \text{ units}$

III. Procedure

A. Filter 50 ml of sample through distilled-deionized water-washed GF/C glass fiber filter into a 125 ml rubber-capped Erlenmeyer flask. Change filter between each sample.

B. Read absorbance at 350 nm (personal communication, J. Shapiro,

University of Minnesota, Minneapolis) in a 10 cm cell. Read absorbance of one set of standards prior to samples and one set after samples.

C. To calculate color units of samples, generate regression of absorbance vs. color units of standards. Enter above determined absorbance values of samples and report color in units.

IV. Notes

A. Working standards are made on day of analysis.

B. Analysis is conducted on samples which have been frozen without filtration and then thawed and filtered immediately prior to analysis.

REFERENCES

APHA. 1975. Standard Methods for the Examination of Water and Wastewater. 14th ed., American Public Health Association. Washington, D.C. 1193 pp.

Procedure for Determination of Total Dissolved Iron (After method of Gibbs 1979)

I. Basis of Method

Ferrous ions react with Ferrozine^{R} (Hach Chemical Co.) to form a magenta color with a maximum absorbance at 562 nm; the absorbance is proportional to the concentration of ferrous ions. Ferric ions do not complex with the reagent and must be reduced to ferrous to obtain total dissolved iron concentrations. Interferences by copper, cobalt, and nickel are reduced by adjusting the pH to 4.5. We use standards ranging from 0 to 100 µg $Fe \cdot l^{-1}$. A 10 µg $Fe \cdot l^{-1}$ spike to lake water samples yields a mean recovery of 9.6 µg $Fe \cdot l^{-1}$ with a standard deviation of 0.9 µg $Fe \cdot l^{-1}$ and a 95% confidence interval of 8.8 to 10.4 µg $Fe \cdot l^{-1}$. The limit of detection is approximately 5 µg $Fe \cdot l^{-1}$.

II. Reagents and Standards

A. 5.0 N HCl

Add 200 ml concentrated HCl to 480 ml distilled-deionized water. Store in glass, at room temperature.

B. Reductant

Dissolve 10 g hydroxylamine hydrochloride (analytical reagent grade) in 100 ml distilled-deionized water. Store in glass.

C. Ferrozine^{R} reagent (Hach Chemical Co.)

Dissolve 0.075 g Ferrozine^{R} in 50 ml distilled-deionized water. Add 2 ml of 5.0 N HCl and dilute to 100 ml. This is stable for over 1 month.

D. Buffer

1. Dissolve 473 g sodium acetate trihydrate (analytical reagent grade) in 500 ml distilled-deionized water, with warming.
2. Cool and add 115 ml glacial acetic acid.
3. Dilute to 1 liter and store in glass, at room temperature.

E. Standards and spike

1. 0.1 N H_2SO_4 (approximately)

   Add 5 ml concentrated H_2SO_4 to 2 liters distilled-deionized water.

2. Stock standard (100 mg $Fe \cdot l^{-1}$)

   Dissolve 0.7022 g ferrous ammonium sulfate (analytical reagent grade) in 1 liter of 0.1 N H_2SO_4.

3. Working standards

   Prepare from 100 mg $Fe \cdot l^{-1}$ stock standard, dilute with 0.1 N H_2SO_4.

   a. $\frac{0.2 \text{ ml of } 100 \text{ mg } Fe \cdot l^{-1}}{2000 \text{ ml}} = 10 \text{ µg } Fe \cdot l^{-1}$

   b. $\frac{0.5 \text{ ml of } 100 \text{ mg } Fe \cdot l^{-1}}{2000 \text{ ml}} = 25 \text{ µg } Fe \cdot l^{-1}$

   c. $\frac{1.0 \text{ ml of } 100 \text{ mg } Fe \cdot l^{-1}}{2000 \text{ ml}} = 50 \text{ µg } Fe \cdot l^{-1}$

   d. $\frac{2.0 \text{ ml of } 100 \text{ mg } Fe \cdot l^{-1}}{2000 \text{ ml}} = 100 \text{ µg } Fe \cdot l^{-1}$

4. Spike

   Prepare from 100 mg $Fe \cdot l^{-1}$ stock standard, dilute with 0.1 N H_2SO_4.

   $\frac{\text{Dilute 1 ml of } 100 \text{ mg } Fe \cdot l^{-1}}{200 \text{ ml}} = 500 \text{ µg } Fe \cdot l^{-1}$

a. Remove 1 ml of sample to be spiked.

b. Add 1 ml of 500 µg $Fe \cdot l^{-1}$ to obtain 10 µg $Fe \cdot l^{-1}$ in sample (sample size 50 ml).

$$\frac{1 \text{ ml of } 500 \text{ µg } Fe \cdot l^{-1}}{50 \text{ ml}} = 10 \text{ µg } Fe \cdot l^{-1}$$

III. Procedure

A. Samples are filtered as soon as possible through a distilled-deionized water washed GF/C. Filtrates are then acidified with 1 ml of 5 N HCl and frozen until analysis.

B. Place 50 ml of thawed sample into rubber-capped 125 ml Erlenmeyer flask.

C. Add 2 ml reductant, mix.

D. Wait 2 minutes or more.

E. Add 2 ml Ferrozine^{R} reagent, mix.

F. Add 5 ml buffer, mix.

G. Wait 1 minute.

H. Read absorbance at 562 nm in 10 cm cell. Color is stable for several months. Read absorbance of one set of standards prior to samples and one set after samples.

I. To calculate Fe concentration, generate regression of absorbance vs. concentration of standards. Enter above-determined absorbance values of samples and report Fe (total dissolved) concentrations.

IV. Notes

A. Standards and spike may be stored indefinitely, in glass.

B. If concentration of Fe in sample is greater than highest standard, it should be diluted with distilled-deionized water and re-analyzed.

C. Refiltration of samples, which have been frozen and thawed, immediately prior to analysis is unnecessary.

REFERENCES

Gibbs, M.M. 1979. A simple method for the rapid determination of iron in natural waters. Water Research 13:295-297.

Method For Determination of Ammonia (NH_3-N) (After method of Liddicoat et al. 1975).

I. Basis of Method

This method is a modification of that outlined in Liddicoat et al. (1975) and Solorzano (1969). A blue compound, indophenol, is formed by the reaction of ammonia, hypochlorite, and phenol, using potassium ferrocyanide as a catalyst.

Interference of other nitrogen compounds is negligible; calcium and magnesium interference is eliminated by complexing with sodium citrate.

The method is reportedly applicable to 500 µg $N \cdot l^{-1}$. We use standards ranging from 0 to 50 µg $N \cdot l^{-1}$, however. The limit of detection is approximately 5 µg $N \cdot l^{-1}$. A 10 µg $N \cdot l^{-1}$ spike to lake water samples yields a mean recovery of 9.1 µg $N \cdot l^{-1}$ with a standard deviation of 1.5 µg $N \cdot l^{-1}$ and a 95% confidence interval of 8.7 to 9.5 µg $N \cdot l^{-1}$.

II. Reagents and Standards

A. Phenol-ethanol solution

Dissolve 50.0 ± 0.1 g of analytical reagent grade phenol in 500 ml 95% ethanol. Store in glass at room temperature.

B. Alkaline citrate solution

Dissolve 200 ± 0.1 g of analytical reagent grade trisodium citrate dihydrate and 10.00 ± 0.05 g analytical reagent grade sodium hydroxide in 1000 ml of freshly distilled-deionized water. Store at room temperature.

C. Oxidizing solution

Mix 80 ml of alkaline citrate solution with 20 ml of sodium hypochlorite. Prepare fresh daily.

D. Catalyst

Dissolve 2.50 ± 0.01 g of analytical reagent grade potassium ferrocyanide in 500 ml freshly distilled-deionized water. Prepare fresh daily.

E. Standards and spike

1. Stock standard (50 mg $N \cdot l^{-1}$)

Dissolve 0.1909 ± .0002 g NH_4Cl (oven-dried to constant weight at 105°C) in 1000 ml distilled-deionized water.

2. Working standards

Prepare fresh daily from stock standards (50 mg $N \cdot l^{-1}$).

a. $\frac{1 \text{ ml of } 50 \text{ mg } N \cdot l^{-1}}{500 \text{ ml}} = 100 \text{ µg } N \cdot l^{-1}$

b. $\frac{20 \text{ ml of } 100 \text{ µg } N \cdot l^{-1}}{200 \text{ ml}} = 10 \text{ µg } N \cdot l^{-1}$

c. $\frac{50 \text{ ml of } 100 \text{ µg } N \cdot l^{-1}}{200 \text{ ml}} = 25 \text{ µg } N \cdot l^{-1}$

d. $\frac{70 \text{ ml of } 100 \text{ µg } N \cdot l^{-1}}{200 \text{ ml}} = 35 \text{ µg } N \cdot l^{-1}$

e. $\frac{100 \text{ ml of } 100 \text{ µg } N \cdot l^{-1}}{200 \text{ ml}} = 50 \text{ µg } N \cdot l^{-1}$

3. Spike

$$\frac{1 \text{ ml of } 50 \text{ mg } N \cdot l^{-1}}{100 \text{ ml}} = 500 \text{ µg } N \cdot l^{-1}$$

a. Remove 1 ml of sample to be spiked.

b. Add 1 ml of 500 µg $N \cdot l^{-1}$ to obtain 10 µg $N \cdot l^{-1}$

in sample (sample size 50 ml).

$$\frac{1 \text{ ml of } 500 \text{ µg N}\cdot\text{l}^{-1}}{50 \text{ ml}} = 10 \text{ µg N}\cdot\text{l}^{-1}$$

III. Procedure

A. Filtration: If glass, rather than plastic, filter frits are used, they should be pre-rinsed with distilled-deionized water and then an aliquot of sample in order to saturate the sites on the frits. Adsorption of NH_4^+ to the filter frit will cause an inconsistent error in the analysis. After pre-rinsing, filter 50 ml of sample through GF/F filter.

B. Add 2 ml phenol-ethanol, mix.

C. Add 2 ml potassium ferrocyanide, mix.

D. Add 5 ml oxidizer, mix.

E. Incubate in UV radiation hood for 50 minutes.

F. Read absorbarce at 640 nm in a 10 cm cell. Read absorbance of one set of standards prior to samples and one set after samples.

G. To calculate NH_3-N concentration, generate regression of absorbance vs. concentration of standards. Enter above-determined absorbance values of samples and report NH_3-N concentrations.

IV. Notes

A. Use fresh sodium hypochlorite.

B. Standards and spikes are made on day of analysis.

C. This method yields best results on samples containing less than 50 µg NH_3-N$\cdot l^{-1}$. If sample determination falls beyond the highest standard, it should be diluted with freshly distilled-deionized water and re-analyzed.

REFERENCES

Liddicoat, M.I., S. Tibbits, and E.I. Butler. 1975. The determination of ammonia in seawater. Limnol. Oceanogr. 20:131-132

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14:799-801.

Procedure for Determination of Nitrate (NO_3-N) (After Method of Kamphake et al. 1967)

1. Basis of Method

This method is a modification of that first described by Mullin and Riley (1955) and later modified by Kamphake et al. (1967). The reaction involves the reduction by hydrazine of nitrate ion to nitrite. The diazotization reaction between nitrite and sulfanilamide is followed by a coupling reaction with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED), producing a red azo-dye, the color of which is proportional to the concentration of nitrite.

Kamphake et al. (1967) investigated interference of nine ions with the analysis; results are shown in Table 1. Concentrations below those listed cause no interference in either the reduction or the colorimetric steps of the reaction. In their analyses, nitrite was oxidized by chlorine to nitrate, which was subsequently recovered upon reduction with hydrazine. Precipitation of calcium and magnesium salts, at concentrations higher than those shown, hindered filtration by clogging filter pores. Analytical reagent grade chloroform (1 ml:100 ml, used as a preservative) produced an interference equivalent to 0.05 mg $N \cdot l^{-1}$ which was eliminated as described in Kamphake et al. (1967).

Our modification of the method employs standards ranging from 0-100 µg $N \cdot l^{-1}$. The mean recovery of a 10 µg $N \cdot l^{-1}$ spike to lake water is 9.5 µg $N \cdot l^{-1}$ with a standard deviation of 1.4 µg $N \cdot l^{-1}$ and a 95% confidence interval of 9.1 to 9.9 µg $N \cdot l^{-1}$. The limit of detection is approximately 1 µg $N \cdot l^{-1}$.

Table 1. Ion Interference

Ion	Salt	$mg \cdot l^{-1}$ ion not producing interference	
		Nitrate Analysis	Nitrite Analysis
Cl^-	NaCl	30,000	30,000
Mg^{++}	$MgSO_4$	75	75
Free chlorine	-	200	<1.0*
NH_3-N	$(NH_4)_2SO_4$	80	80
$S^=$	Na_2S	†	†
Ca^{++}	$CaSO_4$	240	240
Fe^{+++}	$FeCl_3$	30	30
$PO_4^{\equiv}$	KH_2PO_4	50	50
ABS	-	60	60

* Chlorine oxidizes $NO_2^=$ in sample to NO_3^-.

† Apparent $NO_2^=$ and NO_3^- varied ± 10% up to 10 $mg \cdot l^{-1}$ of sulfide ion.

II. Reagents and Standards

A. Stock Hydrazine

Dissolve 1.20 g of analytical reagent grade hydrazine sulfate in 250 ml distilled-deionized water. Store in glass at room temperature.

B. Copper solution

Dissolve 0.040 g of analytical reagent grade $CuSO_4 \cdot 5H_2O$ into 100 ml distilled-deionized water. Store in glass at room temperature.

C. Reducing solution

Dilute 25 ml stock hydrazine solution and 5 ml copper solution to 50 ml with distilled-deionized water. Do not store.

D. 1.0 N NaOH

Dissolve 40 g analytical reagent grade NaOH in 1000 ml distilled-deionized water. Store at room temperature in polyethylene bottle.

E. Sulfanilamide

Add 50 ml concentrated hydrochloric acid to approximately 250 ml distilled-deionized water. Dissolve 5 g analytical reagent grade sulfanilamide in the diluted HCl. Dilute to 500 ml with distilled-deionized water. Store in glass at room temperature.

F. N-(1-naphthyl)-ethylenediamine dihydrochloride (NED)

Dissolve 0.5 g analytical reagent grade N-(1-naphthyl)-ethylenediamine dihydrochloride in 500 ml distilled-deionized water. Store in refrigerator, in dark. Discard solution if brown coloration develops.

G. Phenate buffer

Add 1.8 g analytical reagent grade phenol and 16 ml of 1.0 N NaOH to 100 ml distilled-deionized water. Place in dark bottle. Do not store.

H. Standards and spike

1. Stock standard (50 mg $N \cdot l^{-1}$)

Dissolve 0.3609 g KNO_3 (oven-dried to constant weight at 105°C) in 1000 ml distilled-deionized water. Store in glass at room temperature.

2. Working standards

Prepare fresh daily from stock standard (50 mg $N \cdot l^{-1}$)

a. $\frac{2 \text{ ml of } 50 \text{ mg } N \cdot l^{-1}}{500 \text{ ml}} = 200 \text{ µg } N \cdot l^{-1}$

b. $\frac{10 \text{ ml of } 200 \text{ µg } N \cdot l^{-1}}{200 \text{ ml}} = 10 \text{ µg } N \cdot l^{-1}$

c. $\frac{25 \text{ ml of } 200 \text{ µg } N \cdot l^{-1}}{200 \text{ ml}} = 25 \text{ µg } N \cdot l^{-1}$

d. $\frac{50 \text{ ml of } 200 \text{ µg } N \cdot l^{-1}}{200 \text{ ml}} = 50 \text{ µg } N \cdot l^{-1}$

e. $\frac{100 \text{ ml of } 200 \text{ µg } N \cdot l^{-1}}{200 \text{ ml}} = 100 \text{ µg } N \cdot l^{-1}$

3. Spike

$$\frac{1 \text{ ml of } 50 \text{ mg } N \cdot l^{-1}}{100 \text{ ml}} = 500 \text{ µg } N \cdot l^{-1}$$

a. Add while diluting sample.

b. Add 1 ml of 500 µg $N \cdot l^{-1}$ to obtain 10 µg $N \cdot l^{-1}$ in sample (sample size 50 ml)

$$\frac{1 \text{ ml of } 500 \text{ µg } N \cdot l^{-1}}{50 \text{ ml}} = 10 \text{ µg } N \cdot l^{-1}$$

III. Procedure

A. Filter 50 ml* of sample through distilled-deionized water-washed GF/C glass fiber filter into 125 ml rubber-capped Erlenmeyer flask. Change filter between each sample.

B. Place flasks into water bath at 37°C ± 1°C; bring samples to this temperature.

C. Simultaneously add 1 ml reducing solution and 2 ml phenate buffer, mix.

D. Allow reaction to proceed at 37°C for 30 minutes.

E. Remove samples from water bath.

F. Simultaneously add 1 ml sulfanilamide and 1 ml NED, mix.

G. Cool to room temperature or long enough to prevent fogging of cuvette in spectrophotometer.

H. Read absorbance at 543 nm in a 5 cm cell. Read absorbance of one set of standards prior to samples and one set after samples.

I. To calculate NO_3-N concentration, generate regression of absorbance vs. concentration of standards. Enter above-determined absorbance values of samples and report nitrate-N concentrations.

IV. Notes

A. Reducing solution and phenate buffer are made up on day of analysis.

B. Standards and spikes are made on day of analysis.

C. If concentration of nitrate in sample is greater than highest standard, it should be diluted with distilled-deionized water and re-analyzed.

*See IV. Notes, F.

D. This method includes nitrite in the analysis; therefore, if samples contain a significant concentration of nitrite, a separate nitrite analysis should be conducted and values subtracted to get nitrate only.

E. Analysis is conducted on samples which have been frozen without filtration, and then thawed and filtered immediately prior to analysis.

F. We remove some unknown interference in Lake Mead water by diluting samples at least 1:5 with distilled-deionized water immediately prior to analysis.

REFERENCES

Kamphake, L.J., S.A. Hannah, and J.M. Cohen. 1967. An automated analysis for nitrate by hydrazine reduction. Water Research 1:205-216.

Mullin, J.B. and J.P. Riley. 1955. The spectrophotometric determination of nitrate in natural waters, with particular reference to seawater. Anal. Chem. Acta 12:464-480.

Procedure for Determination of Nitrite (NO_2-N) (After method of Brown et al. 1970)

I. Basis of Method

This method is based on that of Brown et al. (1970, pp. 121-122) and involves the formation of a diazo-compound of nitrite with sulfanilamide. This compound is then coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a red azo-dye, the absorbance of which is proportional to the amount of nitrite present.

Interference by substances commonly occurring in natural waters is negligible.

We use standards ranging from 0 to 100 µg $N \cdot l^{-1}$. The mean recovery of a 10 µg $N \cdot l^{-1}$ spike to lake water is 9.9 µg $N \cdot l^{-1}$ with a standard deviation of 0.5 µg $N \cdot l^{-1}$ and a 95% confidence interval of 9.7 to 10.1 µg $N \cdot l^{-1}$. The minimum detection limit is approximately 1 µg $N \cdot l^{-1}$.

II. Reagents and Standards

A. Analytical reagent grade formic acid, 87 to 90%.

B. N-(1-naphthyl)-ethylenediamine dihydrochloride (NED)

Dissolve 0.5 g analytical reagent grade N-(1-naphthyl)-ethylenediamine dihydrochloride in 100 ml distilled-deionized water. Store in refrigerator, in dark. If brown coloration develops, discard solution.

C. Sulfanilamide

Dissolve 0.5 g analytical reagent grade sulfanilamide in 100 ml distilled-deionized water. Prepare fresh daily.

D. Standards and spike

1. Stock standard (50 mg $N \cdot l^{-1}$)

   Dissolve 0.3038 g KNO_2 (oven-dried to a constant weight at 105°C) in 1000 ml distilled-deionized water. Store in glass at room temperature.

2. Working standards

   Prepare fresh daily from stock standard (50 mg $N \cdot l^{-1}$).

   a. $\frac{2 \text{ ml of } 50 \text{ mg } N \cdot l^{-1}}{500 \text{ ml}} = 200 \text{ µg } N \cdot l^{-1}$

   b. $\frac{10 \text{ ml of } 200 \text{ µg } N \cdot l^{-1}}{200 \text{ ml}} = 10 \text{ µg } N \cdot l^{-1}$

   c. $\frac{25 \text{ ml of } 200 \text{ µg } N \cdot l^{-1}}{200 \text{ ml}} = 25 \text{ µg } N \cdot l^{-1}$

   d. $\frac{50 \text{ ml of } 200 \text{ µg } N \cdot l^{-1}}{200 \text{ ml}} = 50 \text{ µg } N \cdot l^{-1}$

   e. $\frac{100 \text{ ml of } 200 \text{ µg } N \cdot l^{-1}}{200 \text{ ml}} = 100 \text{ µg } N \cdot l^{-1}$

3. Spike

   $$\frac{1 \text{ ml of } 50 \text{ mg } N \cdot l^{-1}}{100 \text{ ml}} = 500 \text{ µg } N \cdot l^{-1}$$

   a. Remove 1 ml of sample to be spiked.

   b. Add 1 ml of 500 µg $N \cdot l^{-1}$ to obtain 10 µg $N \cdot l^{-1}$ in sample (sample size 50 ml).

   $$\frac{1 \text{ ml of } 500 \text{ µg } N \cdot l^{-1}}{50 \text{ ml}} = 10 \text{ µg } N \cdot l^{-1}$$

III. Procedure

A. Filter 50 ml of sample through distilled-deionized water-washed GF/C glass fiber filter into 125 ml rubber-capped Erlenmeyer flask. Change filter between each sample.

B. Place flasks in ice-bath for approximately 2 minutes.

C. Add 1 ml sulfanilamide and mix.

D. Add 4 ml formic acid and mix.

E. Add 1 ml NED and mix.

F. Remove from ice bath.

G. Allow color to develop for 15 minutes.

H. Read absorbance at 543 nm in a 5 cm cell. Read absorbance of one set of standards prior to samples and one set after samples.

I. To calculate NO_2-N concentration, generate regression of absorbance vs. concentration of standards. Enter above-determined absorbance values of samples and report NO_2-N concentrations.

IV. Notes

A. Standards and spikes are made on day of analysis.

B. If concentration of nitrite in sample is greater than highest standard, it should be diluted with distilled-deionized water and re-analyzed.

C. Analysis is conducted on samples which have been frozen without filtration and then thawed and filtered immediately prior to analysis.

REFERENCES

Brown, E., M.W. Skougstad, and M.J. Fishman. 1970. Methods for collection and analysis of water samples for dissolved minerals and gases. Techniques of Water-Resources Investigations of the United States Geological Survey. Book 5. Chapter A 1. U.S. Government Printing Office, Washington, D.C. 160 pp.

Procedure for Determination of Total Nitrogen (After methods of D'Elia et al. 1977 and APHA 1975)

I. Basis of Method

Total nitrogen in natural waters (particulate organic nitrogen, dissolved organic nitrogen, ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen) is oxidized by persulfate under alkaline conditions to nitrate-nitrogen (D'Elia et al. 1977). Nitrate-nitrogen is then reduced to nitrite-nitrogen by the cadmium reduction method (APHA 1975, pp. 423-427). A diazo-compound of nitrite is then formed with the addition of sulfanilamide. This compound is then coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride, forming a red azo-dye, the absorbance of which is directly proportional to the amount of nitrite present.

We use standards ranging in concentration from 0 to 200 $\mu g\ N \cdot l^{-1}$. The mean recovery of a 25 $\mu g\ N \cdot l^{-1}$ spike to lake water is 25.1 $\mu g\ N \cdot l^{-1}$ with a standard deviation of 2.2 $\mu g\ N \cdot l^{-1}$ and a 95% confidence interval of 24.4-25.8 $\mu g\ N \cdot l^{-1}$.

II. Reagents and Standards

A. Oxidizer

Dissolve 3.0 g NaOH (analytical reagent grade) in 1 liter distilled-deionized water; then add 6.7 g $K_2S_2O_8$ (analytical reagent grade). This should be made immediately before use.

B. 1 N HCl

Dilute 83 ml concentrated HCl (analytical reagent grade) to 1 liter with distilled-deionized water. Store in glass, at room temperature.

C. Ammonium chloride solution (200 g $NH_4Cl \cdot l^{-1}$)

Dissolve 100 g NH_4Cl (analytical reagent grade) in 500 ml distilled-deionized water. Store in glass, at room temperature.

D. Dilute ammonium chloride solution (5 g $NH_4Cl \cdot l^{-1}$)

Dilute 50 ml concentrated ammonium chloride solution (C., above) to 2000 ml with distilled-deionized water. Store in glass, at room temperature.

E. Amalgamated cadmium filings

"File sticks of pure cadmium metal (reagent grade) with a coarse metal hand file (about second cut) and collect the fraction that passes a sieve with 2-mm openings and is retained on a sieve with 0.5-mm openings. Stir about 300 g filings with 300 ml $HgCl_2$ solution (1 g $HgCl_2$/100 ml) for 3 minutes. (This amount suffices for six reduction columns). Let the metal particles settle and decant the liquid. Wash the amalgamated filings several times with distilled water, then several times with 1 + 99 HCl. Wash copiously with distilled water until no nitrite can be detected in the supernatant fluid. Store the filings in the dark under dilute NH_4Cl solution." (APHA 1975, p. 424).

F. Sulfanilamide

Add 50 ml concentrated analytical reagent grade HCl to approximately 300 ml distilled-deionized water. Add 5 g sulfanilamide (analytical reagent grade) and dilute to 500 ml with distilled-deionized water (APHA 1975, pp. 424-425).

G. N-(1-naphthyl)-ethylenediamine dihydrochloride

Dissolve 0.5 g analytical reagent grade N-(1-napthyl)-ethylenediamine dihydrochloride in 500 ml distilled-deionized water. Store in refrigerator, in dark. Discard solution if brown coloration develops.

H. Hydrochloric acid: 5% (1 + 19 HCl)

Dilute 1 ml to 20 ml with distilled-deionized water.

I. Standards and spike

1. Stock standard (50 mg $N \cdot l^{-1}$)

Dissolve 0.3609 KNO_3 (analytical reagent grade) (oven-dried to a constant weight at 105°C) in 1000 ml distilled-deionized water.

2. Working standards

Prepare fresh daily from stock standard (50 mg $N \cdot l^{-1}$)

a. $\frac{1 \text{ ml of } 50 \text{ mg } N \cdot l^{-1}}{500 \text{ ml}} = 100 \text{ µg } N \cdot l^{-1}$

(10 ml diluted to 100 ml = final concentration of 10 µg $N \cdot l^{-1}$)

b. $\frac{1 \text{ ml of } 50 \text{ mg } N \cdot l^{-1}}{100 \text{ ml}} = 500 \text{ µg } N \cdot l^{-1}$

(10 ml diluted to 100 ml = final concentration of 50 µg $N \cdot l^{-1}$)

c. $\frac{2 \text{ ml of } 50 \text{ mg } N \cdot l^{-1}}{100 \text{ ml}} = 1000 \text{ µg } N \cdot l^{-1}$

(10 ml diluted to 100 ml = final concentration of 100 µg $N \cdot l^{-1}$)

d. $\frac{2 \text{ ml of } 50 \text{ mg } N \cdot l^{-1}}{50 \text{ ml}} = 2000 \text{ µg } N \cdot l^{-1}$

(10 ml diluted to 100 ml = final concentration of 200 µg $N \cdot l^{-1}$)

3. Spike

$\frac{5 \text{ ml of } 50 \text{ mg } N \cdot l^{-1}}{100 \text{ ml}} = 2500 \text{ µg } N \cdot l^{-1}$

a. Add 1 ml of 2500 µg $N \cdot l^{-1}$ to obtain 25 µg $N \cdot l^{-1}$ in sample (sample size 100 ml)

$$\frac{1 \text{ ml of } 2500 \text{ µg } N \cdot l^{-1}}{100 \text{ ml}} = 25 \text{ µg } N \cdot l^{-1}$$

III. Procedure

A. Preparation of reduction column

"Pack a plug of glass wool in the bottom of a reduction column and fill the column with distilled water. Pour in sufficient amalgamated cadmium filings to produce a column 30 cm long. Use the column size specified because columns of lesser diameter and length give erratic results and show rapid deterioration. Wash the column thoroughly with dilute NH_4Cl solution. When apparatus is not in use, cover the metal in the column with dilute NH_4Cl solution." (APHA 1975, p. 425).

B. Maintenance of reduction column

"There is no need to wash columns between samples, but if columns are not to be reused for several hours or longer, pour 50 ml dilute NH_4Cl solution onto the top and let it pass through the system. Store the cadmium filings in this NH_4Cl solution and never allow them to go dry. If there are indications that the columns are becoming inactivated, as shown by a significant decrease in the color intensity produced per microgram NO_3-N in the standards, empty the columns, wash the cadmium filings briefly with 1 + 19 HCl and copiously with distilled water, air-dry at about 60°C, resieve and reamalgamate. Well-prepared columns of the correct size should be stable for many weeks before such reactivation becomes

necessary." (APHA 1975, p. 426).

C. Place 10 ml unfiltered sample in 125 ml rubber-capped Erlenmeyer flask.

D. Add 15 ml oxidizer, mix.

E. Autoclave (120°C) for 30 minutes.

F. Adjust volume to 100 ml with distilled-deionized water by transferring sample with successive washings to 100 ml graduated cylinder. Return sample to Erlenmeyer.

G. Adjust pH to 8-9 with 1 N HCl (measure with pH electrode).

H. If sample is turbid, filter through GF/C.

I. Discard 20 ml.

J. Add 2 mls concentrated NH_4Cl, mix.

K. Pass through cadmium columns, discarding first 25 ml. Collect next 50 ml.

L. Pour 50 ml sample back into Erlenmeyer to which 1 ml of sulfanilamide has been added.

M. Allow reaction to proceed for 2 to 8 minutes.

N. Add 1 ml of N-(1-naphthyl)-ethylenediamine dihydrochloride.

O. Allow color to develop at least 10 minutes but no longer than 2 hours.

P. Read absorbance at 543 nm in 1 cm cell. Read absorbance of one set of standards prior to samples and one set after samples.

Q. To calculate total N concentration of samples, generate regression of absorbance vs. concentration of standards. Enter above-determined absorbance values of samples and report total N concentration.

IV. Notes

A. Standards and spikes are made on day of analysis.

B. If concentration of total N in sample is greater than highest standard, it should be diluted with distilled-deionized water and re-analyzed.

C. Analysis is conducted on unfiltered samples which have been frozen.

D. Oxidizer should be made immediately prior to use.

E. Samples preserved with $HgCl_2$ should not be placed into cadmium columns.

F. It is unnecessary to regulate flowrate through the columns (personal communication, J. Shapiro, University of Minnesota, Minneapolis).

REFERENCES

APHA. 1975. Standard Methods for the Examination of Water and Wastewater. 14th ed., American Public Health Association. Washington, D.C. 1193 p.

D'Elia, C.F., P.A. Steudler, and N. Corwin. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. Limnol. Oceanogr. 22:760-764.

Procedure for Determination of Dissolved Phosphorus (After method of Strickland and Parsons 1972 and Goldman 1974)

I. Basis of Method

Acid hydrolysis at 120°C of filtered water samples releases orthophosphate from dissolved phosphorus compounds, such as condensed phosphate (pyro-, tripoly- and higher molecular weight species), organic phosphorus compounds, and polyphosphates (Goldman 1974, pp. 387-392). This method of analysis includes dissolved acid-hydrolyzable phosphorus and orthophosphate. The orthophosphate released is then converted to a phosphomolybdate complex which is reduced by ascorbic acid to molybdenum blue. This compound is then analyzed colorimetrically (Strickland and Parsons 1972, pp. 49-52).

We employ standards in the range between 0 and 50 µg $P \cdot l^{-1}$. The mean recovery of a 5 µg $P \cdot l^{-1}$ spike to lake water is 4.8 µg $P \cdot l^{-1}$ with a standard deviation of 0.7 µg $P \cdot l^{-1}$ and a 95% confidence interval of 4.6 to 5.0 µg $P \cdot l^{-1}$. The limit of detection is approximately 1 µg $P \cdot l^{-1}$.

II. Reagents and Standards

A. 10.8 N H_2SO_4

Dilute 300 ml of concentrated H_2SO_4 (analytical reagent grade) to 1000 ml with distilled-deionized water (add acid to water).

B. 10.8 N NaOH

Dissolve 432 g NaOH (analytical reagent grade) in 1000 ml distilled-deionized water. (Caution - this is an exothermic reaction).

C. Molybdenum-antimony

Add 244 ml concentrated sulfuric acid (H_2SO_4) to approximately 1500 ml distilled-deionized water. Cool. Add 21.00 g ammonium molybdate (analytical reagent grade) and 0.60 g antimony potassium tartrate (analytical reagent grade). Bring volume up to 2000 ml with distilled-deionized water. Store in brown bottle at room temperature.

D. Ascorbic acid

Add 3.00 g ascorbic acid (analytical reagent grade) to 100 ml distilled-deionized water. Make on day of analysis; do not store.

E. Standards and spike

1. Stock standard (100 mg $P \cdot l^{-1}$)

Dissolve 0.4393 ± 0.0002 g KH_2PO_4 (analytical reagent grade) (oven-dried to constant weight at 105°C) in 1000 ml distilled-deionized water.

2. Working standards

Prepare from 100 mg $P \cdot l^{-1}$ stock standard and store.

a. $\frac{0.1 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 5 \text{ µg } P \cdot l^{-1}$

b. $\frac{0.2 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 10 \text{ µg } P \cdot l^{-1}$

c. $\frac{0.6 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 30 \text{ µg } P \cdot l^{-1}$

d. $\frac{1.0 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 50 \text{ µg } P \cdot l^{-1}$

3. Spike

$$\text{Dilute } \frac{0.5 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{200 \text{ ml}} = 250 \text{ µg } P \cdot l^{-1}$$

a. Remove 1 ml of sample to be spiked.

b. Add 1 ml of 250 µg $P \cdot l^{-1}$ to obtain 5 µg $P \cdot l^{-1}$ in sample (sample size 50 ml).

$$\frac{1.0 \text{ ml of } 250 \text{ µg } P \cdot l^{-1}}{50 \text{ ml}} = 5 \text{ µg } P \cdot l^{-1}$$

III. Procedure

A. Filter 50 ml of sample through distilled-deionized water washed GF/C glass fiber filter into 125 Erlenmeyer flask with autoclave cap. Change filter between each sample.

B. Add 0.5 ml of 10.8 N H_2SO_4, mix.

C. Autoclave at 115-120°C for 15 minutes.

D. Cool to room temperature.

E. Add 0.5 ml 10.8 N NaOH, mix.

F. Add 5 ml molybdenum-antimony, mix.

G. Add 0.5 ml ascorbic acid, mix.

H. Allow color to develop for 30 minutes.

I. Read absorbance at 675 nm in a 10 cm cell. Read absorbance of one set of standards prior to samples and one set after samples.

J. To calculate dissolved phosphorus concentrations, generate regression of absorbance vs. concentration of standards. Enter above determined absorbance values of samples and report dissolved phosphorus concentrations.

IV. Notes

A. Standards and spikes may be stored, in glass, at room temperature.

B. If concentration of phosphorus in sample is greater than highest standard, it should be diluted with distilled-deionized water and re-analyzed.

C. Analysis is conducted on samples which have been frozen without filtration and thawed and filtered immediately prior to analysis.

REFERENCES

Goldman, C.R. 1974. Eutrophication of Lake Tahoe emphasizing water quality. EPA-660/3-74-034. U.S. Govt. Printing Office, Washington, D.C. 408 pp.

Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis. 2nd ed., Bulletin of the Fisheries Research Board of Canada. No. 167. Ottawa. 311 pp.

Procedure for Determination of Extractable Phosphorus (After method of Fitzgerald and Nelson 1966)

I. Basis of Method

Surplus or stored phosphorus compounds, as a result of luxury uptake of orthophosphate, can be detected in algal cells by boiling in Tris buffer and analyzing the extract for orthophosphate (Fitzgerald and Nelson 1966). The analysis is done on cells filtered onto GF/C glass fiber filters (personal communication, J. Shapiro, University of Minnesota, Minneapolis).

We employ standards ranging between 0 and 50 µg $P \cdot l^{-1}$ to which 1 ml of 1 $g \cdot l^{-1}$ Tris and a blank filter have been added. The limit of detection is approximately 1 µg $P \cdot l^{-1}$.

II. Reagents and Standards

A. Tris buffer

Add 0.1 g Tris to 100 ml distilled-deionized water. Make daily.

B. Molybdenum-antimony

Add 244 ml concentrated sulfuric acid (H_2SO_4) to approximately 1500 ml distilled-deionized water. Cool. Add 21.00 g ammonium molybdate (analytical grade) and 0.60 g antimony potassium tartrate (analytical grade). Bring volume up to 2000 ml with distilled-deionized water. Store in glass at room temperature.

C. Ascorbic acid

Add 3.00 g ascorbic acid (analytical grade) to 100 ml distilled-deionized water. Make on day of analysis; do not store.

D. Standards and spike

1. Stock standard (100 mg $P \cdot l^{-1}$)

Dissolve 0.4393 ± 0.0002 g KH_2PO_4 (analytical grade)(oven-dried to constant weight at 105°C) in 1000 ml distilled-deionized water.

2. Working standards

Prepare from 100 mg $P \cdot l^{-1}$ stock standard and store.

a. $\frac{0.1 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 5 \text{ µg } P \cdot l^{-1}$

b. $\frac{0.2 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 10 \text{ µg } P \cdot l^{-1}$

c. $\frac{0.6 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 30 \text{ µg } P \cdot l^{-1}$

d. $\frac{1.0 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 50 \text{ µg } P \cdot l^{-1}$

3. Spike

Dilute

$$\frac{0.5 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{200 \text{ ml}} = 250 \text{ µg } P \cdot l^{-1}$$

a. Remove 1 ml of sample to be spiked.

b. Add 1 ml of 250 µg $P \cdot l^{-1}$ to obtain 5 µg $P \cdot l^{-1}$ in sample (sample size 50 ml).

$$\frac{1.0 \text{ ml of } 250 \text{ µg } P \cdot l^{-1}}{50 \text{ ml}} = 5 \text{ µg } P \cdot l^{-1}$$

III. Procedure

A. Filter replicate water samples (100 to 500 ml) through distilled-deionized water washed GF/C glass fiber filters. Wrap filters individually in aluminum foil packets, marked with date, station and volume filtered. Freeze in airtight containers to store.

B. Add 1 ml Tris and filter to be analyzed to 50 ml of distilled-deionized water in autoclave-capped Erlenmeyer flask (125 ml).

C. Add 1 ml Tris and blank GF/C filter to 50 ml of standard in autoclave-capped Erlenmeyer flask.

D. Boil gently on hot plate for 60 minutes.

E. Cool.

F. Transfer sample or standard to 50 ml graduated cylinder and bring up to 50 ml with distilled-deionized water.

G. Filter through distilled-deionized water rinsed GF/C.

H. Analyze for orthophosphate.

1. Add 5 ml molybdenum-antimony, mix.
2. Add 0.5 ml ascorbic acid, mix.
3. Allow color to develop for 30 minutes.
4. Read absorbance at 675 nm in a 10 cm cell. Read absorbance of one set of standards prior to samples and one set after samples.
5. To calculate extractable phosphorus concentration, generate regression of absorbance vs. concentration of standards. Enter above-determined absorbance values of samples and report concentrations.

IV. Notes

A. Standards and spikes may be stored, in glass.

B. If concentration of phosphate in sample is greater than highest standard, the sample should be diluted with distilled-deionized water and re-analyzed.

REFERENCES

Fitzgerald, G.P. and T.C. Nelson. 1966. Extractive and enzymatic analyses for limiting or surplus phosphorus in algae. J. Phycol. 2:32-37.

Procedure for Determination of Orthophosphate (PO_4-P) (After method of Strickland and Parsons 1972 and Goldman 1974)

I. Basis of Method

Orthophosphate and molybdate react to form a phosphomolybdate complex which is reduced to molybdenum blue by ascorbic acid (Strickland and Parsons 1972, pp. 49-52, Goldman 1974, pp. 392-393).

We employ standards in the range between 0 and 50 µg $P \cdot l^{-1}$. The mean recovery of a 5 µg $P \cdot l^{-1}$ spike to lake water was 5.0 µg $P \cdot l^{-1}$ with a standard deviation of 0.4 µg $P \cdot l^{-1}$ and a 95% confidence interval of 4.9-5.1 µg $P \cdot l^{-1}$. The limit of detection is approximately 1 µg $P \cdot l^{-1}$.

II. Reagents and Standards

A. Molybdenum-antimony

Add 244 ml concentrated sulfuric acid (H_2SO_4) to approximately 1500 ml distilled-deionized water. Cool. Add 21.00 g ammonium molybdate (analytical grade) and 0.60 g antimony potassium tartrate (analytical grade). Bring volume up to 2000 ml with distilled-deionized water. Store in glass at room temperature.

B. Ascorbic acid

Add 3.00 g ascorbic acid (analytical grade) to 100 ml distilled-deionized water. Make on day of analysis; do not store.

C. Standard and spike

1. Stock standard (100 mg $P \cdot l^{-1}$)

Dissolve 0.4393 ± 0.0002 g KH_2PO_4 (analytical grade) (oven-dried to constant weight at 105°C) in 1000 ml distilled-deionized water.

2. Working standards

Prepare from 100 mg $P \cdot l^{-1}$ stock standard and store.

a. $\frac{0.1 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 5 \text{ µg } P \cdot l^{-1}$

b. $\frac{0.2 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 10 \text{ µg } P \cdot l^{-1}$

c. $\frac{0.6 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 30 \text{ µg } P \cdot l^{-1}$

d. $\frac{1.0 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 50 \text{ µg } P \cdot l^{-1}$

3. Spike

$$\frac{0.5 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{200 \text{ ml}} = 250 \text{ µg } P \cdot l^{-1}$$

a. Remove 1 ml of sample to be spiked.

b. Add 1 ml of 250 µg $P \cdot l^{-1}$ to obtain 5 µg $P \cdot l^{-1}$ in sample (sample size 50 ml).

$$\frac{1.0 \text{ ml of } 250 \text{ µg } P \cdot l^{-1}}{50 \text{ ml}} = 5 \text{ µg } P \cdot l^{-1}$$

III. Procedure

A. Filter 50 ml of sample through distilled-deionized water-washed GF/C glass fiber filter into a 125 ml rubber-capped Erlenmeyer flask. Change filter between each sample.

B. Add 5 ml molybdenum-antimony, mix.

C. Add 0.5 ml ascorbic acid, mix.

D. Allow color to develop for 30 minutes.

E. Read absorbance at 675 nm in a 10 cm cell. Read absorbance of one set of standards prior to samples and one set after samples.

F. To calculate PO_4-P concentration, generate regression of absorbance vs. concentration of standards. Enter above determined absorbance values of samples and report PO_4-P concentration.

IV. Notes

A. Standards and spikes may be stored, in glass, at room temperature.

B. If concentration of phosphate in sample is greater than highest standard, it should be diluted with distilled-deionized water and re-analyzed.

C. Analysis is conducted on samples which have been frozen without filtration and then thawed and filtered immediately prior to analysis.

REFERENCES

Goldman, C.R. 1974. Eutrophication of Lake Tahoe emphasizing water quality. EPA-600/3-74-034. U.S. Govt. Printing Office, Washington, D.C. 408 pp.

Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis. 2nd ed., Bulletin of the Fisheries Research Board of Canada. No. 167. Ottawa. 311 pp.

Procedure for Determination of Particulate Phosphorus

I. Basis of Method

The amount of phosphorus contained in the particulate fraction of a water sample can be analyzed by a persulfate digestion of material retained on a GF/C filter and subsequent determination of orthophosphate in the digest (personal communication, R. Prentki, University of Nevada, Las Vegas). We employ standards ranging between 0 and 100 µg $P \cdot l^{-1}$ to which a blank GF/C filter has been added.

II. Reagents and Standards

A. Ammonium persulfate $[(NH_4)_2S_2O_8]$

Add 24.0 g ammonium persulfate (analytical reagent grade per 1000 ml distilled-deionized water (1.2 g per 50 ml); this will do 20 samples. Make immediately before use; do not store.

B. Molybdenum-antimony

Add 244 ml concentrated sulfuric acid (H_2SO_4) to approximately 1500 ml distilled-deionized water. Cool. Add 21.00 g ammonium molybdate (analytical reagent grade) and 0.60 g antimony potassium tartrate (analytical reagent grade). Bring volume up to 2000 ml with distilled-deionized water. Store in brown bottle at room temperature.

C. Ascorbic acid

Add 3.00 g ascorbic acid (analytical reagent grade) to 100 ml distilled-deionized water. Make on day of analysis; do not store.

D. Standards

1. Stock standard (100 mg $P \cdot l^{-1}$)

   Dissolve 0.4393 ± 0.0002 g KH_2PO_4 (analytical reagent grade) (oven-dried to constant weight at 105°C) in 1000 ml distilled-deionized water.

2. Working standards

   Prepare from 100 $mg \cdot l^{-1}$ stock standard and store.

   a. $\frac{0.1 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 5 \text{ µg } P \cdot l^{-1}$

   b. $\frac{0.2 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 10 \text{ µg } P \cdot l^{-1}$

   c. $\frac{0.6 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 30 \text{ µg } P \cdot l^{-1}$

   d. $\frac{1.0 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 50 \text{ µg } P \cdot l^{-1}$

   e. $\frac{2.0 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 100 \text{ µg } P \cdot l^{-1}$

III. Procedure

A. Filter replicate or triplicate water samples (500 to 1000 ml, see Appendix A) through distilled-deionized water washed GF/C glass fiber filters. Wrap filters individually in aluminum foil packets, marked with date, station, and volume filtered. Freeze in airtight containers to store.

B. Add filter to be analyzed to 50 ml ammonium persulfate in autoclaved-capped Erlenmeyer flask (125 ml).

C. Add distilled-deionized water washed blank GF/C filter to 50 ml ammonium persulfate in autoclaved-capped Erlenmeyer flask (125 ml).

D. Add 1.2 g ammonium persulfate and 1 distilled-deionized water washed blank GF/C filter to 50 ml of each standard in

Procedure for Determination of Particulate Phosphorus

I. Basis of Method

The amount of phosphorus contained in the particulate fraction of a water sample can be analyzed by a persulfate digestion of material retained on a GF/C filter and subsequent determination of orthophosphate in the digest (personal communication, R. Prentki, University of Nevada, Las Vegas). We employ standards ranging between 0 and 100 μg $P \cdot l^{-1}$ to which a blank GF/C filter has been added.

II. Reagents and Standards

A. Ammonium persulfate $[(NH_4)_2S_2O_8]$

Add 24.0 g ammonium persulfate (analytical reagent grade per 1000 ml distilled-deionized water (1.2 g per 50 ml); this will do 20 samples. Make immediately before use; do not store.

B. Molybdenum-antimony

Add 244 ml concentrated sulfuric acid (H_2SO_4) to approximately 1500 ml distilled-deionized water. Cool. Add 21.00 g ammonium molybdate (analytical reagent grade) and 0.60 g antimony potassium tartrate (analytical reagent grade). Bring volume up to 2000 ml with distilled-deionized water. Store in brown bottle at room temperature.

C. Ascorbic acid

Add 3.00 g ascorbic acid (analytical reagent grade) to 100 ml distilled-deionized water. Make on day of analysis; do not store.

D. Standards

1. Stock standard (100 mg $P \cdot l^{-1}$)

   Dissolve 0.4393 ± 0.0002 g KH_2PO_4 (analytical reagent grade) (oven-dried to constant weight at 105°C) in 1000 ml distilled-deionized water.

2. Working standards

   Prepare from 100 $mg \cdot l^{-1}$ stock standard and store.

   a. $\frac{0.1 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 5 \text{ µg } P \cdot l^{-1}$

   b. $\frac{0.2 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 10 \text{ µg } P \cdot l^{-1}$

   c. $\frac{0.6 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 30 \text{ µg } P \cdot l^{-1}$

   d. $\frac{1.0 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 50 \text{ µg } P \cdot l^{-1}$

   e. $\frac{2.0 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 100 \text{ µg } P \cdot l^{-1}$

III. Procedure

A. Filter replicate or triplicate water samples (500 to 1000 ml, see Appendix A) through distilled-deionized water washed GF/C glass fiber filters. Wrap filters individually in aluminum foil packets, marked with date, station, and volume filtered. Freeze in airtight containers to store.

B. Add filter to be analyzed to 50 ml ammonium persulfate in autoclaved-capped Erlenmeyer flask (125 ml).

C. Add distilled-deionized water washed blank GF/C filter to 50 ml ammonium persulfate in autoclaved-capped Erlenmeyer flask (125 ml).

D. Add 1.2 g ammonium persulfate and 1 distilled-deionized water washed blank GF/C filter to 50 ml of each standard in

autoclaved-capped Erlenmeyer flask (125 ml).

E. Autoclave at 115-120°C for 30 minutes.

F. Cool to room temperature.

G. Filter samples through distilled-deionized washed GF/C filter to remove filter fibers. It is unnecessary to change filter between samples, but filter must be washed between samples with distilled-deionized water.

H. Bring volume of samples up to 50 ml and dilute samples with distilled-deionized water in 50 ml graduated cylinder (see Appendix A).

I. Bring volume of blanks and standards up to 50 ml and filter.

J. Analyze for ortho-phosphate.

1. Add 5 ml molybdenum-antimony, mix.
2. Add 0.5 ml ascorbic acid, mix.
3. Allow color to develop for 30 minutes.
4. Read absorbance at 675 nm in a 10 cm cell. Read absorbance of one set of standards prior to samples and one set after samples.
5. To calculate particulate phosphorus concentration, generate regression of absorbance vs. concentration of standards. Enter above-determined absorbance values of samples, and determine concentration of phosphate in samples. Using factors in Appendix A, correct values and report concentrations.

IV. Notes

A. Standards may be stored, in glass.

B. If concentration of phosphate in sample is greater than

highest standard, the sample should be diluted further with distilled-deionized water and re-analyzed.

APPENDIX A

For Lake Mead water samples taken from stations 2, 3, 4, 5 and 8, the volumes filtered, dilutions, and calculation correction factors based on volumes filtered and dilutions are given below:

Station	Volume (ml)	Dilution	Factor
2	500	1:25	$\frac{25 \times 50}{500} = 2.5$
3	500	1:10	$\frac{10 \times 50}{500} = 1.0$
4	1000	1:10	$\frac{10 \times 50}{1000} = 0.5$
5	1000	1:10	$\frac{10 \times 50}{1000} = 0.5$
8	1000	1:2	$\frac{2 \times 50}{1000} = 0.1$

Example:

If concentration of phosphate in sample digest of Station 8 determined from regression of absorbance vs. concentration of standards (as in J.5., above), is 34 µg $P \cdot l^{-1}$, the amount of particulate phosphorus in the lake water sample is:

$$34 \text{ µg } P \cdot l^{-1} \times 0.1 = 3.4 \text{ µg } P \cdot l^{-1}$$

Note: The above values for amount filtered and dilutions are approximations and should be adjusted as necessary, depending on particulate phosphorus concentrations of samples.

Procedure for Determination of Total Hydrolyzed Phosphorus (After methods of Strickland and Parsons 1972 and Goldman 1974)

I. Basis of Method

Acid hydrolysis at 120°C releases condensed phosphates (pyro-, tripoly-, and higher molecular weight species such as hexametaphosphate), organic phosphorus compounds, and polyphosphates (Goldman 1974, pp. 387-392). The ortho-phosphate released is then converted to a phosphomolybdate complex which is reduced by ascorbic acid to molybdenum blue. This compound is then analyzed colorimetrically (Strickland and Parsons 1972, pp. 49-52).

We employ standards in the range between 0 and 50 µg $P \cdot l^{-1}$. The mean recovery of a 5 µg $P \cdot l^{-1}$ spike to lake water is 4.8 µg $P \cdot l^{-1}$ with a standard deviation of 0.6 µg $P \cdot l^{-1}$ and a 95% confidence interval of 4.6 to 5.0 µg $P \cdot l^{-1}$. The limit of detection is approximately 1 µg $P \cdot l^{-1}$.

II. Reagents and Standards

A. Molybdenum-antimony

Add 244 ml concentrated sulfuric acid (H_2SO_4) to approximately 1500 ml distilled-deionized water. Cool. Add 21.00 g ammonium molybdate (analytical reagent grade) and 0.60 g antimony potassium tartrate (analytical reagent grade). Bring volume up to 2000 ml with distilled-deionized water. Store in glass at room temperature.

B. Ascorbic acid

Add 3.00 g ascorbic acid (analytical reagent grade) to 100 ml distilled-deionized water. Make on day of analysis; do not store.

C. 10.8 N H_2SO_4

Dilute 300 ml of concentrated H_2SO_4 to 1000 ml with distilled-deionized water (add acid to water).

D. 10.8 N NaOH

Dissolve 432 g NaOH in 1000 ml distilled-deionized water. (This is an exothermic reaction.)

E. Standards and spike

1. Stock standard (100 mg $P \cdot l^{-1}$)

Dissolve 0.4393 ± 0.0002 g KH_2PO_4 (analytical grade) (oven-dried to constant weight at 105°C) in 1000 ml distilled-deionized water.

2. Working standard

Prepare from 100 mg $P \cdot l^{-1}$ stock standard and store.

a. $\frac{0.1 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 5 \text{ µg } P \cdot l^{-1}$

b. $\frac{0.2 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 10 \text{ µg } P \cdot l^{-1}$

c. $\frac{0.6 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 30 \text{ µg } P \cdot l^{-1}$

d. $\frac{1.0 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 50 \text{ µg } P \cdot l^{-1}$

3. Spike

$$\frac{0.5 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{200 \text{ ml}} = 250 \text{ µg } P \cdot l^{-1}$$

a. Remove 1 ml of sample to be spiked.

b. Add 1 ml of 250 µg $P \cdot l^{-1}$ to obtain 5 µg $P \cdot l^{-1}$ in sample (sample size 50 ml).

$$\frac{1.0 \text{ ml of } 250 \text{ µg } P \cdot l^{-1}}{50 \text{ ml}} = 5 \text{ µg } P \cdot l^{-1}$$

III. Procedure

A. Place 50 ml of unfiltered lake water sample into 125 ml Erlenmeyer flask with autoclave cap.

B. Add 0.5 ml of 10.8 N H_2SO_4, mix.

C. Autoclave at 115-120°C for 15 minutes.

D. Cool to room temperature.

E. Add 0.5 ml 10.8 N NaOH, mix.

F. Add 5 ml molybdenum-antimony, mix.

G. Add 0.5 ml ascorbic acid, mix.

H. Allow color to develop for 30 minutes.

I. Read absorbance at 675 nm in a 10 cm cell. Read absorbance of one set of standards prior to samples and one set after samples.

J. To calculate total phosphorus concentrations, generate regression of absorbance vs. concentration of standards. Enter above determined absorbance values of samples and report total phosphorus concentrations.

IV. Notes

A. Standard and spikes may be stored, in glass, at room temperature.

B. If concentration of phosphorus in sample is greater than highest standard, it should be diluted with distilled-deionized water and re-analyzed.

C. Analysis is conducted on unfiltered samples which have been frozen.

C. 10.8 N H_2SO_4

Dilute 300 ml of concentrated H_2SO_4 to 1000 ml with distilled-deionized water (add acid to water).

D. 10.8 N NaOH

Dissolve 432 g NaOH in 1000 ml distilled-deionized water. (This is an exothermic reaction.)

E. Standards and spike

1. Stock standard (100 mg $P \cdot l^{-1}$)

Dissolve 0.4393 ± 0.0002 g KH_2PO_4 (analytical grade) (oven-dried to constant weight at 105°C) in 1000 ml distilled-deionized water.

2. Working standard

Prepare from 100 mg $P \cdot l^{-1}$ stock standard and store.

a. $\frac{0.1 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 5 \text{ µg } P \cdot l^{-1}$

b. $\frac{0.2 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 10 \text{ µg } P \cdot l^{-1}$

c. $\frac{0.6 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 30 \text{ µg } P \cdot l^{-1}$

d. $\frac{1.0 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{2000 \text{ ml}} = 50 \text{ µg } P \cdot l^{-1}$

3. Spike

$$\frac{0.5 \text{ ml of } 100 \text{ mg } P \cdot l^{-1}}{200 \text{ ml}} = 250 \text{ µg } P \cdot l^{-1}$$

a. Remove 1 ml of sample to be spiked.

b. Add 1 ml of 250 µg $P \cdot l^{-1}$ to obtain 5 µg $P \cdot l^{-1}$ in sample (sample size 50 ml).

$$\frac{1.0 \text{ ml of } 250 \text{ µg } P \cdot l^{-1}}{50 \text{ ml}} = 5 \text{ µg } P \cdot l^{-1}$$

III. Procedure

A. Place 50 ml of unfiltered lake water sample into 125 ml Erlenmeyer flask with autoclave cap.

B. Add 0.5 ml of 10.8 N H_2SO_4, mix.

C. Autoclave at 115-120°C for 15 minutes.

D. Cool to room temperature.

E. Add 0.5 ml 10.8 N NaOH, mix.

F. Add 5 ml molybdenum-antimony, mix.

G. Add 0.5 ml ascorbic acid, mix.

H. Allow color to develop for 30 minutes.

I. Read absorbance at 675 nm in a 10 cm cell. Read absorbance of one set of standards prior to samples and one set after samples.

J. To calculate total phosphorus concentrations, generate regression of absorbance vs. concentration of standards. Enter above determined absorbance values of samples and report total phosphorus concentrations.

IV. Notes

A. Standard and spikes may be stored, in glass, at room temperature.

B. If concentration of phosphorus in sample is greater than highest standard, it should be diluted with distilled-deionized water and re-analyzed.

C. Analysis is conducted on unfiltered samples which have been frozen.

REFERENCES

Goldman, C.R. 1974. Eutrophication of Lake Tahoe emphasizing water quality. EPA-660/3-74-034. U.S. Govt. Printing Office, Washington, D.C. 408 pp.

Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis. 2nd ed., Bulletin of the Fisheries Research Board of Canada. No. 167. Ottawa. 311 pp.

Procedure for Determination of Silica (After APHA 1975)

I. Basis of Method

At pH 1.2, ammonium molybdate reacts with silica and phosphate to form molybdosilicic acid and molybdophosphoric acid, respectively (APHA 1975, pp. 487-490). The concentration of "molybdate-reactive" silica is proportional to the yellow color of the molybdosilicic acid. An optional step unnecessary for Lake Mead samples can be used to convert any "molybdate-unreactive" silica. Interference by the presence of molybdophosphoric acid is eliminated by the addition of oxalic acid, which does not affect the silica compound. Interference from color or turbidity in the sample can be eliminated by zeroing the spectrophotometer with sample blanks containing all reagents except ammonium molybdate or by measuring the absorbance and subtracting from absorbance of samples.

We use standards ranging from 0 to 20 mg $Si \cdot l^{-1}$. Recovery of a 1 mg $Si \cdot l^{-1}$ spike to lake water samples yields a mean concentration of 0.9 mg $Si \cdot l^{-1}$ with a standard deviation of 0.2 mg $Si \cdot l^{-1}$ and a 95% confidence interval of 0.8 to 1.0 mg $Si \cdot l^{-1}$. The limit of detection is approximately 0.5 mg $Si \cdot l^{-1}$.

II. Reagents and Standards

A. Ammonium molybdate

Dissolve 10.0 g $(NH_4)_6\ Mo_7O_{24} \cdot 4H_2O$ (analytical reagent grade) in 100 ml distilled-deionized water, with stirring and gentle warming on hot plate/stirrer. Filter if cloudiness develops. Adjust pH to 7-8 with NH_4OH or NaOH to prevent precipitation.

Store in polyethylene bottle at room temperature (do not store in glass).

B. Oxalic acid

Dissolve 10.0 g $H_2C_2O_4 \cdot 2H_2O$ (analytical grade) in 100 ml distilled-deionized water. Store in polyethylene at room temperature.

C. Hydrochloric acid (50%)

Dilute concentrated hydrochloric acid 1:1 (vol:vol) with distilled-deionized water. Store in polyethylene at room temperature.

D. Standards and spike

1. Stock standard (store in polyethylene) (1000 mg $Si \cdot l^{-1}$)

   May be purchased in "Dilute-it" ampoules

2. Working standards (store in polyethylene)

   a. $\frac{1 \text{ ml of } 1000 \text{ mg } Si \cdot l^{-1}}{1000 \text{ ml}} = 1 \text{ mg } Si \cdot l^{-1}$

   b. $\frac{5 \text{ ml of } 1000 \text{ mg } Si \cdot l^{-1}}{1000 \text{ ml}} = 5 \text{ mg } Si \cdot l^{-1}$

   c. $\frac{10 \text{ ml of } 1000 \text{ mg } Si \cdot l^{-1}}{1000 \text{ ml}} = 10 \text{ mg } Si \cdot l^{-1}$

   d. $\frac{20 \text{ ml of } 1000 \text{ mg } Si \cdot l^{-1}}{1000 \text{ ml}} = 20 \text{ mg } Si \cdot l^{-1}$

3. Spike (store in polyethylene)

   $\frac{5 \text{ ml of } 1000 \text{ mg } Si \cdot l^{-1}}{100 \text{ ml}} = 50 \text{ mg } Si \cdot l^{-1}$

   a. Remove 1 ml from sample to be spiked.

   b. Add 1 ml of 50 mg $Si \cdot l^{-1}$ to 50 ml sample to get 1 mg $Si \cdot l^{-1}$ spike.

$$\frac{1 \text{ ml of } 50 \text{ mg Si} \cdot l^{-1}}{50 \text{ ml}} = 1 \text{ mg Si} \cdot l^{-1}$$

III. Procedure

A. Place 50 ml of unfiltered lake water in 125 ml Erlenmeyer flask.

B. Add in rapid succession 1 ml HCl and 2 ml ammonium molybdate, mix.

C. Wait 5 minutes.

D. Add 1.5 ml oxalic acid, mix.

E. Wait 2 minutes.

F. Read absorbance within 15 minutes at 410 nm in 1 cm cell. Read absorbance of one set of standards prior to samples and one set after samples.

G. Generate regression of absorbance vs. concentration from standards; enter above-determined absorbance values of samples and report Si concentrations.

IV. Notes

A. Standards and spike are to be stored in polyethylene containers.

B. If concentration of silica in sample is greater than highest standard, it should be diluted and re-analyzed.

C. Analysis is conducted on unfiltered samples which have been frozen.

D. It is best to avoid using soft-glass (borosilicate) containers to hold reagents since contamination with silica results. However, samples, blanks, and standards are treated identically so any possible contamination will be accounted for, during the analysis.

E. We use 125 ml Erlenmeyer flasks for reaction vessels, and not platinum crucibles (personal communication, J. Shapiro, University of Minnesota, Minneapolis).

REFERENCES

APHA. 1975. Standard Methods for the Examination of Water and Wastewater. 14th ed., American Public Health Association. Washington, D.C. 1193 pp.

Procedure for Measurement of Total and Volatile Suspended Solids (After APHA 1975)

I. Basis of Method

Suspended solids is the term used to describe residue or material contained in a water sample which is retained by a filter (in this case, a GF/C glass fiber filter). A known volume of water is filtered onto a GF/C filter that has been precombusted for 6-8 hours in a muffle furnace at 550°C, to remove any volatile inorganic and organic matter. The filter is dried for 6 to 8 hours at 105°C to a constant weight and weighed to nearest 0.1 mg (APHA 1975, pp. 96-98). The filter is combusted at 550°C in a muffle furnace for 15 minutes and weighed again (APHA, pp. 96-98). The loss in weight by this treatment is due to the combustion of residue known as volatile suspended solids.

II. Materials

A. GF/C glass fiber filters, 42.5 mm diameter

B. Forceps for handling filters

C. Graduated cylinders (200-1000 ml capacity)

D. Aluminum weighing dishes

E. Desiccator containing $CaSO_4$ indicator desiccant

F. Drying oven

G. Muffle furnace (Thermolyne and Thermolyne Furnatrol I)

H. Analytical balance capable of weighing to 0.1 mg

III. Procedure

A. GF/C glass fiber filters in aluminum weighing dishes appropriately marked with sample identification are precombusted in

a muffle furnace at 550°C for 6-8 hours.

B. Filters in weighing dishes are placed in a desiccator until they can be weighed to nearest 0.1 mg. Filters are then stored in a dry place, such as a desiccator, until use.

C. Replicate 200- to 1000-ml (depending on solids concentration) lake water samples (from 0-5 m integrated water sample) are filtered onto the precombusted GF/C filters (handled carefully with forceps to prevent filter breakage or contamination with fingers) and replaced into appropriate aluminum weighing dish.

D. Filters in dishes are then transferred to drying oven and dried at 105°C for 6 to 8 hours until constant weight is achieved.

E. Each filter is then weighed to nearest 0.1 mg; if filters cannot be weighed immediately, they should be stored in a desiccator.

F. Filters are transferred to muffle furnace and combusted at 550°C for 15 minutes, placed in a desiccator while transporting to analytical balance and reweighed to nearest 0.1 mg.

G. Calculations

Total suspended solids $(mg \cdot l^{-1})$ =

$$\left[\text{Filter}_{105°C}\text{wt. (mg)} - \text{Filter}_{\text{precombusted}}\text{wt. (mg)}\right] \times \left[\text{volume filtered (l)}\right]^{-1}$$

Volatile suspended solids $(mg \cdot l^{-1})$ =

$$\left[\text{Filter}_{105°C}\text{wt. (mg)} - \text{Filter}_{550°C}\text{wt. (mg)}\right] \times \left[\text{volume filtered (l)}\right]^{-1}$$

REFERENCES

APHA. 1975. Standard Methods for Examination of Water and Wastewater. 14th ed., American Public Health Association. Washington, D.C. 1193 pp.

Procedure for Determination of Particulate Carbon, Nitrogen, and Hydrogen in Water Samples

1. Basis of Method

Seston is analyzed for carbon, nitrogen and hydrogen content in a CHN analyzer, an automated combustion instrument for the laboratory determination of carbon, nitrogen, and hydrogen in materials dissociable at temperatures below 1100°C. The operating cycle of the instrument is divided into three stages: purging, combustion, and absorption. During purging, a stream of helium displaces atmospheric gases trapped in the combustion chamber. During combustion, the sample is converted to its elemental constituents (Eq. 1) which combine with oxygen, supplied from an oxygen stream. During absorption, the gaseous combustion products are swept in the absorption chamber where hydrogen (as H_2O), carbon (as CO_2), and nitrogen (probably as NO_2) are selectively absorbed. Detection of constituents is by differential thermal conductivity, i.e., difference between the thermal conductivity of the gas and that of the helium carrier gas. Interferences by halogens and sulfur are removed by chemicals in the combustion tube. The operating cycle of the analyzer is 13 to 15 minutes.

$$\text{Eq. 1} \quad O_2 + CNH_2O \xrightarrow{< 1100^\circ C} CO_2 + H_2O + NO_2$$

Lake water seston is filtered onto pre-combusted (550°C in muffle furnace) GF/C glass fiber filters, dried at 105°C, and combusted in the CHN analyzer at 600°C. The low combustion temperature is required to keep the filters from melting.

Note: See Appendix A for operational procedure of CHN.

II. Materials

A. Elemental analyzer (Perkin-Elmer Model 240B)

B. Muffle furnace (Thermolyne and Thermolyne Furnatrol I)

C. Drying oven (capable of maintaining temperature to within ± 1°C)

D. Analytical electrobalance (e.g. Cahn 21 Automatic Electrobalance, see Appendix B.)

E. Gases: He (99.9999% purity - from Matheson Gas Company)
O_2 (99.99% purity - from Matheson Gas Company)

F. Desiccator with dry $CaSO_4$ indicating desiccant

G. Filtering apparatus capable of holding 4.25 cm filters (e.g. Millipore^R)

H. GF/C glass fiber filters 4.25 cm (e.g. Whatman)

I. Acetanilide standard for C, H, and N (purchased from Perkin-Elmer)

J. Microspatula (Hayman, purchased from Perkin-Elmer) for transferring acetanilide standard

K. Platinum boats (purchased from Perkin-Elmer) for combusting standard

L. Cahn forceps for transferring standards to and from Cahn electrobalance

M. Brushes for cleaning platinum boats (commercial artist's brushes are adequate)

N. Ladle (made by Perkin-Elmer on special order) to transfer

filters to combustion chamber of elemental analyzer

O. Aluminum weighing dishes

III. Procedure

Note: See Appendix C for sequence of analysis of standards, standard blanks, filter blanks, samples and sample blanks.

A. Sample preparation

1. Combust GF/C glass fiber filters for samples, standards and filter blanks, if they are to be run, in aluminum weighing dishes in muffle furnace at 550°C for 6-8 h.
2. Filter samples of lake water through ashed filters in quantities sufficient to deposit an estimated 1-3 mg organic matter (in Lake Mead, usually 0.5 to 2 l). Filter in triplicate.
3. Using forceps, carefully roll filters (avoid touching filters with fingers) and place each filter in separate aluminum foil packet. Label with sample date and station, volume filtered, and replicate number.
4. Freeze filters to store.
5. Prior to CHN analysis, partially open packets and dry at 105°C in drying oven (2-24 h). Store in desiccator until analysis.
6. Dry blank, pre-combusted GF/C filters at 105°C in drying oven (2-24 h) for use with standards, and as filter blanks if needed. Store in desiccator until use.

B. Standard and standard blank determinations

1. Weight determination of standard

a. Refer to instruction manual for Cahn balance, and Appendix B (balance should remain on).

b. Replace desiccant in balance at least daily or more often if necessary.

c. Re-calibrate balance daily according to instruction manual.

d. Tare balance to 0 mg with 210 mg tare weights (this maximizes balance sensitivity in presence of 200 mg sample boats).

e. Set response power switch to 0 (on electrobalance).

f. Place an empty boat which has been stored in desiccator on clean paper positioned in front of balance. Place standard into boat with clean microspatula. Remove any particles on boat exterior with brush.

   Note: Never add more than 3 mg standard to boat since the CHN analyzer will malfunction and will not yield any data.

g. Place boat with standard back into balance. Record weight after about 1 minute.

   Note: When removing an item from balance tray, first lift item carefully with no horizontal movement, pause, and then remove item from tray. Otherwise, balance tray may be damaged.

h. Place ashed, blank GF/C filter on clean paper positioned in front of balance. Place standard from boat (g, above) onto filter. Reweigh boat and calculate weight of standard; carefully roll filter and

follow procedure outlined in 2, below.

2. CHN analysis

   a. Turn chart recorder speed switch on (chart speed 20 $cm \cdot h^{-1}$).

   b. Using forceps, place filter containing standard in ladle (contained in combustion tube of CHN analyzer), touching only the end of the ladle handle.

   c. Place ladle with filter and standard in cool zone of combustion tube.

      Note: Always store ladle in combustion tube.

   d. Press START button (it should light).

   e. When INJ-COMB light comes on (after 2 minutes and 20 seconds, START light goes off), move ladle into combustion zone with magnet provided and press INJ-COMB button (START Light comes back on).

   f. When READOUT Light comes on:

      i. confirm that chart recorder is on,

      ii. retract ladle to cool position in combustion tube,

      iii. use fine zero (on CHN) to adjust N, C, and H baselines (in that order) to between 5 and 10% of full scale.

      Note: It may be unnecessary to readjust zeroes more than once daily.

   g. About 3 to 5 minutes after READOUT light goes out, it will come on again. Attenuate the recorder signals

with N, C, and H ATTENUATION switches (on CHN) as required to keep pen on scale. Record ATTENUATION values. If samples with similar N, C, and H contents will be analyzed next, attenuation switches may be left at these positions. Otherwise, return them to lowest positions.

Note: If ladle is not moved to cool position in combustion tube at first readout, allow it to cool for at least 4 minutes before introducing the next sample, standard, or blank. Contamination of system may otherwise result.

h. The CHN analyzer must be pre-conditioned with two standards prior to recording data. Run 2 standards of approximately 2 to 3 mg and then run 2 standards of known weight. Calculate machine sensitivities using data from latter two runs (see IV. below).

i. To run standard blank, re-insert standard without opening combustion tube.

C. Filter blank determinations

1. To determine filter blanks, roll an ashed GF/C filter, place it in the ladle, and follow the CHN analysis procedure outlined in B.2, above.

2. The CHN analyzer must be pre-conditioned with two ashed, blank GF/C filters, prior to recording data. Use data only from the last two runs in calculations.

Note: Filter blanks should be analyzed in duplicate on each new box of filters.

D. Sample and sample blank analysis

1. Precondition CHN analyzer with two samples.

   Note: For Lake Mead water samples, use the third of 3 replicate filters from each of two stations closest to station from which first sample is taken.

2. Analyze 5 to 10 filters, run replicates from each station in sequence.

3. Analyze a sample blank by re-inserting the last sample in the above sequence (2) without opening the combustion tube.

4. After blank analysis, re-equilibrate machine with 2 filters (again using the third of three replicate filters from each of the two stations closest to station from which last sample was taken).

E. End-of-day shut-down of system

1. Clean all platinum boats with brush.

2. Manually rotate programmer wheel clockwise to position 9.

3. Turn off recorder and cap pen.

4. Do <u>not</u> turn off gas supplies.

IV. Calculations (see Appendices D and E)

A. Calculate sensitivities ($\mu V \cdot \mu g^{-1}$) of C, N and H based on standard (acetanilide) analysis according to instructions on Calibration Data Sheet (Appendix D).

B. Calculate µg C, µg N, and µg H in filter blanks according to instructions on Data Sheet for Samples (Appendix E).

C. Calculate µg C, µg N, and µg H in samples according to instructions on Data Sheet for Samples (Appendix E).

D. Subtract blank µg C, µg N, and µg H in filter from µg C, µg N, and µg H in samples.

E. Divide µg C, µg N, and µg H by volume filtered in liters to get µg $C \cdot l^{-1}$, µg $N \cdot l^{-1}$, and µg $H \cdot l^{-1}$.

REFERENCES

Instruction Manual for Cahn 21 Automatic Electrobalance. Cahn Instruments, Cerritos, California, U.S.A.

Instruction Manual for Perkin-Elmer Model 240B Elemental Analyzer. 1978. Perkin-Elmer, Norwalk, Connecticut, U.S.A.

APPENDIX A

OPERATION OF PERKIN-ELMER MODEL # 240B

FOR GLASS FIBER FILTERS

1. Verify gas pressure; do not change pressure settings.
   a. Helium ≅ 18 PSI, minimum 200 PSI in tank
   b. Oxygen ≅ 42 PSI, minimum 200 PSI in tank
2. Verify that DET RESET light is off. If on, press DET RESET button in quickly and release. Do not hold in depressed position. If light goes out, it is okay to proceed. If light stays on, do not proceed until problem is identified and corrected.
3. Turn on recorder power. At least 0.5 h warm-up period is needed to establish steady baseline.
4. Make following preliminary control settings
   a. PROGRAMMER SWITCH - should be on DETECT (DET)
   b. PROGRAMMER INDEX WHEEL - rotate clockwise from position 9 to position 0.
   c. Confirm DET RESET light still off.
   d. Confirm COMBUST control is at 600°C (∿2.0 on Dial).
   e. Confirm REDUCE control is at 650°C (∿4.5 on Dial).

      Note: Temperature gauge is on front panel. Actual controls are under lid, to the left of programmer wheel.

      Note: The temperature dials have more than 100°C hysteresis. DO NOT ASSUME that positioning dial where it was previously will always give previous temperature.

      Note: For filter combustion, temperatures are critical; 600°C ± 10°C is the allowable range. Analyzer should be

equilibrated at proper temperature for 16 h prior to sample run.

f. Set H and N attenuation switch on X1.

g. Set C attenuation switch on 0.

h. Set EXT-COMB on +2 minutes if samples and standards are in rolled filters, and on +4 minutes if on folded filters.

i. Confirm that CX10 blue light on (if not, push light).

j. Turn DETECTOR OUTPUT switch to A.

5. PURGE oxygen line daily for 10 minutes by first removing sample inlet fitting and installing inlet flow plug (Perkin-Elmer part no. 240-0101). Turn programmer index wheel CLOCKWISE to position 2 for 10 minutes. Turn wheel CLOCKWISE to position 1; reinstall standard fitting. Leave in this position for 3 minutes; then rotate wheel clockwise to 0.

6. Condition and calibrate.

a. Run two unweighed 2 to 3-mg acetanilide standards in ashed filters through machine (acetanilide standard has 10.36% N, 71.09% C, and 6.71% H by weight).

b. Run a standard blank by recombusting the second unweighed standard without opening combustion tube. If more than 1 blank is to be run, machine must be re-equilibrated with 2 more unweighed standards before proceeding.

Note: It is necessary to run additional blanks if C, H, and N blanks vary by more than 40 µV from previous day's runs or if no previous runs exist for comparison. Range between these standard blanks and a within-day mean should also not be greater than 40 µV.

c. After running standard blank, re-equilibrate with 2 additional unweighed standards. Then run a 2-to 3-mg weighed standard. Calculate sensitivities (See Appendix D); compare with previous day's runs. Maximum permissible deviation from previous sensitivity means are 0.30 $\mu V \cdot \mu g^{-1}$ for N, 0.17 $\mu V \cdot \mu g^{-1}$ for C, and 4 $\mu V \cdot \mu g^{-1}$ for H. If new values are outside this range run sufficient standards to establish confidence in a new mean. Use either the new mean or value for single run for a day's samples rather than previous day's mean.

Note 1: Record all standard blanks and standard data on calibration Data Sheets (See Appendix D).

Note 2: If no problems are anticipated with blanks or standards, the following sequence yields sufficient data in less time - 2 unweighed standards, 2 weighed standards, standard blank.

On first blank or standard (whichever comes first), record the time at which start light remains off with programmer wheel at approximately position 5. The light must remain off between 1 and 3 minutes. Record time on calibration data sheet and check against value for previous day's run. If it varies by more than 15 seconds or if light remains off for less than 1 minute or longer than 3 minutes, consult manual.

APPENDIX B

OPERATION OF CAHN 21 AUTOMATIC ELECTROBALANCE

A. Set-up

1. Hang identical stirrups on sample "A" or "B" and TARE hang-downs.
2. Set RANGE to highest sensitivity.

B. Zero

1. Adjust the digital readout to 0.000 with coarse and fine ZERO.

C. Calibrate

1. Set RANGE to 200 mg.
2. Place calibrating weight on sample pan.
3. Adjust the digital readout to 199.99 with CALIBRATE.
4. Remove calibrating weight.

D. Weigh

1. Select weighing range to be used.
2. Place sample on sample stirrup.
3. Read sample weight on digital readout.

Note: 1) Refer to instruction manual for complete operation details.

2) Always use Cahn forceps for moving the tare and calibrating weights. Never use sharp, pointed, or grooved forceps.

APPENDIX C

SEQUENCE OF ANALYSES

The following is the proper sequence for analysis of standards and seston samples:

1. Pre-condition machine with 2 or 3 standards of approximately 2 to 3mg.
2. Run 2 standards of known weight.
3. Run 1 standard blank by re-inserting the last standard without opening combustion tube.
4. Pre-condition machine with the third replicates from each of two stations to be analyzed.
5. Run 5 seston samples.
6. Run 1 sample blank by re-inserting the last sample without opening combustion tube.
7. Pre-condition machine with the third replicates from each of next two stations to be analyzed.
8. Run the next 10 seston samples.
9. Run 1 sample blank by re-inserting the last sample without opening combustion tube.
10. Repeat steps 7, 8 and 9 until analyses are complete.

If filter blanks are to be analyzed, it is easiest to run them at the beginning or end of the above sequence.

1. Pre-condition machine with 2 blank, pre-combusted GF/C filters.
2. Run 2 blank, pre-combusted GF/C filters.
3. Re-insert second filter above (2), without opening combustion tube.

APPENDIX D

Model 240 CALIBRATION DATA SHEET (STANDARDS)

A. Standard Name:_____ Run #:_____ By:_____

Date:_____

B. Standard Weight:_____

C. Theoretical Percentage: Acetanilide: 10.36%N, 71.09%C, 6.71%H

D. Theoretical Weight:_____µg N _____µg C _____µg H

E. Calculated Signal: (Recorder Reading) X (Attenuation) = Signal

	Nitrogen	Carbon	Hydrogen
Signal (µV)	_____X___=_____	_____X10 = _____	_____X___=_____
*C Suppression (µV)		Setting___ = _____	
-Blank (µV)	=_____	= _____	=_____
-Filter blank (µV)	=_____	= _____	=_____
-Zero (µV)	_____X___=_____	_____X10 =_____	_____X___=_____
Total	=	=	=

F. Sensitivities (K, in $\frac{\mu V}{\mu g}$):

$$K_N = \frac{\text{Total N signal } (\mu V)}{\text{Theoretical weight N } (\mu g)} = \_\_\_\_\_ \frac{\mu V}{\mu g}$$

$$K_C = \frac{\text{Total C signal } (\mu V)}{\text{Theoretical weight C } (\mu g)} = \_\_\_\_\_ \frac{\mu V}{\mu g}$$

$$K_H = \frac{\text{Total H signal } (\mu V)}{\text{Theoretical weight H } (\mu g)} = \_\_\_\_\_ \frac{\mu V}{\mu g}$$

*Values of C Suppression for C ATTENUATION Switch Setting.
(Use in Computation of C Signal Only.)

Switch Setting	0	1	2	3	4	5
C Suppression (µV)	0	10,000	20,000	30,000	40,000	50,000

APPENDIX E

DATA SHEET FOR SESTON SAMPLES AND SESTON SAMPLE BLANKS

A. Sample Identification_____ B. Analysis Date_____ by_____

C. Pretreatment_____ D. Sample Volume (l)_____

E. Sensitivities: K_N = _____ $\frac{\mu V}{\mu g}$ K_C = _____ $\frac{\mu V}{\mu g}$ K_H = _____ $\frac{\mu V}{\mu g}$

F. Total Signals:

Total Signal (μV) = Signal + C Suppression* - Sample Blank - Zero

Where:

Signal or Zero (μV) = Recorder Reading X Attenuation or 10**

Sample Blank (μV) = Blank Signal - Blank Zero

	Nitrogen	Carbon	Hydrogen
Signal (μV)	____X____=____	____X____=____	____X____=____
+C Suppression* (μV)		Setting____=____	
-Sample blank (μV)	=____	=____	=____
-Zero (μV)	=____	=____	=____
=Total Signal (μV)	=____	=____	=____

*C Suppression Values for C ATTENUATION Switch Setting - Use in C Signal computation only.

Switch Setting	0	1	2	3	4	5
C Suppression (μV)	0	10,000	20,000	30,000	40,000	50,000

**C Signals are internally attenuated by 10.

G. Calculations

Weight (μg) of N, C, and H = $\frac{\text{Total Signal (μV)}}{\text{Sensitivity K (μV·μg}^{-1})}$

	μg N	μg C	μg H
Sample + Filter	_____	_____	_____
-Filter Blank	_____	_____	_____
=Sample	_____	_____	_____

Concentration ($\mu g \cdot l^{-1}$) of N, C, or H = $\frac{\text{Weight (μg)}}{\text{Sample Volume (l)}}$

_____ μg N·l^{-1} _____ μg C·l^{-1} _____ μg H·l^{-1}

Procedure for Determination of Carbon, Nitrogen and Hydrogen in Sediment Samples

I. Basis of Method

Sediment samples are analyzed for carbon, nitrogen, and hydrogen content in a CHN analyzer, an automated combustion instrument for the laboratory determination of carbon, nitrogen, and hydrogen in materials dissociable at temperatures below 1100°C. The operating cycle of the instrument is divided into three stages: purging, combustion, and absorption. During purging, a stream of helium displaces atmospheric gases trapped in the combustion chamber. During combustion, the sample is converted to its elemental constituents (Eq. 1) which combine with oxygen, supplied from an oxygen stream. During absorption, the gaseous combustion products are swept in the absorption chamber where hydrogen (as H_2O), carbon (as CO_2) and nitrogen (probably as NO_2) are selectively absorbed. Detection of constituents is by differential thermal conductivity, i.e., the difference between the thermal conductivity of the gas and that of the helium carrier gas. Interferences by halogens and sulfur are removed by chemicals in the combustion tube. The operating cycle of the analyzer is 13 to 15 minutes.

$$\text{Eq. 1} \quad O_2 + CNH_2O \xrightarrow{<1100^\circ C} CO_2 + H_2O + NO_2$$

II. Materials and Reagents

A. Elemental Analyzer (Perkin - Elmer model 240 B)(See Appendix A)

B. Drying oven (capable of maintaining temperature to within ± 1°C)

C. Analytical electrobalance (e.g., Cahn 21 Automatic Electrobalance, see Appendix B)

D. Hot plate

E. Gases: He (99.9999% purity - from Matheson)

O_2 (99.99% purity - from Matheson)

F. Desiccator with dry $CaSO_4$ indicating desiccant

G. Acetanilide standard for C, H and N (purchased from Perkin-Elmer)

H. Microspatula (Hayman, purchased from Perkin-Elmer) for transferring acetanilide standard

I. Platinum boats for holding standards and samples - Perkin-Elmer # 240-0090

J. Cahn forceps for transferring standards to and from Cahn electrobalance

K. Brushes for cleaning platinum boats (commercial artist's brushes are adequate)

L. Mortar and pestle for grinding dried sediment sample

M. Screw cap (polyseal), glass scintillation vials, engraved with sample identification number

N. 0.5 N H_2SO_4 for cleaning vials

O. 5.0 N HCl for ridding samples of carbonates

III. Procedure

A. Sample Preparation

1. Wash glass scintillation vials with 0.5 N H_2SO_4. Rinse with distilled water. Dry at 105°C. Wash polyseal caps for vials in distilled water only; dry at room temperature.

Note: Do not dry vials in box with dividers, since divider material sticks to vials.

2. Store vials in desiccator until use.
3. Place 0.4 ± 0.1 g dry sediment into duplicate vials. Transfer to drying oven and dry 16 h at 105°C. Cool in a desiccator and weigh to nearest 0.00001 g. This is dry $\text{weight}_{105°C}$.
4. To sets of 30 samples or less, add 5 drops 5 N HCl. Shake down any foaming; wait 10 minutes. Add 5 more drops of acid; shake down again. Wait 10 additional minutes. Add 10 drops of acid, washing down foam from sides of vial with drops. Wait 30 minutes. Add 1 drop of acid, watching carefully for evolution of carbon dioxide. If additional carbon dioxide is evolved (observe vials individually) add 5 drops of acid and wait 30 minutes. Repeat this last step until carbon dioxide evolution ceases.
5. Dry vial contents on hot plate in hood. Hot plate should be set at about 200°F (94°C).
6. After drying on hot plate, transfer vials to oven and dry 16 h at 105°C. Cool in desiccator and reweigh (this is the acidified dry weight). Cap tightly with polyseal cap.
7. Scrape sample from vial with spatula; grind with mortar and pestle. Return sample to original vial, and redry 16 h in 105°C oven. Remove sample from oven and cool in desiccator; afterwards cap tightly with polyseal cap. If sample is not run within one week, redry overnight at 105°C prior to CHN analysis.

8. When weighing sample for CHN, record weight after sample has been on balance for 1 minute. Try not to remove sample from vial more than 2 minutes before it will be placed into combustion tube. Sample is now ready for analysis.

B. Sample, Standard, and Blank Run Procedure

1. Weight determination of samples and standards

   a. Refer to instruction manual for Cahn balance, and Appendix B (balance should remain on).

   b. Replace desiccant in balance at least daily, or more often if necessary.

   c. Re-calibrate balance daily according to instruction manual.

   d. Tare balance to 0 mg with 210 mg tare weights (this maximizes balance sensitivity in presence of 200 mg sample boats).

   e. Set response power switch to 0 (on electrobalance).

   f. Weigh empty boat which has been stored in combustion tube on CHN or desiccator.

   g. Record weight on Calibration Data Sheets or Data Sheets (see Appendices C, D).

      Note: Weight may be negative.

   h. Remove boat from balance (use only CHN forceps). Place on clean paper in front of balance. Place either standard or sample into boat, with clean micro-spatula. Remove any particles on boat exterior with brush provided.

Note: NEVER add more than 3 mg of the standard since it will cause machine to malfunction and will not yield data.

i. Place boat with standard or sample into balance. If acid-treated sample is being weighed, record weight after 1 minute. (This minimizes weighing error due to uptake of moisture.)

Note 1: When removing an item from balance tray, first lift item carefully with no horizontal movement, pause, and then remove item from tray. Otherwise, balance tray may be damaged.

Note 2: Do not allow acid-treated samples to remain outside sample vial for more than 2 minutes before they are placed in combustion tube.

j. Wipe spatula with tissue. Tightly cap standard and sample containers. When standard analysis is complete, return vial containing standard to desiccator.

2. CHN analysis (See Appendix A)

a. Turn chart recorder speed switch on (chart speed 20 $cm \cdot h^{-1}$).

b. Using forceps, place sample boat in ladle (contained in combustion tube of CHN analyzer), touching only the end of ladle handle.

c. Place ladle with boat in cool zone of combustion tube.

Note: Always store ladle in combustion tube.

d. Press START button (it should light).

e. When INJ-COMB light comes on (after 2 minutes and 20 seconds, START light goes off), move ladle into combustion zone with magnet provided and press INJ-COMB button (START light comes back on).

f. When READOUT light comes on:

(1) confirm that chart recorder is on,

(2) retract ladle to cool position in combustion tube,

(3) use fine zero (on CHN) to adjust N, C, and H baselines (in that order) between 5 and 10% of full scale.

Note: It may not be necessary to readjust zeroes more than once daily.

g. About 3 to 5 minutes after READOUT light goes out, it will come on again. Attenuate the recorder signals with N, C, and H ATTENUATION switches (on CHN) as required. Record ATTENUATION values. If samples with similar C, H, and N levels will be analyzed next, attenuation switches may be left at these positions. Otherwise, return them to lowest positions.

Note: If ladle is not moved to cool position in combustion tube at first readout, allow it to cool for at least 4 minutes before introducing the next sample, standard or blank. Contamination of the system may otherwise result.

3. Sequence of sample analysis in CHN analyzer

a. Condition with 2 samples. (For Lake Mead sediments,

use 20-60 mg (or as much as will fit into boat).

b. Run sample (use replicates, either 2 different subsamples or 2 aliquots from same subsample).

c. After each run of 5-10 samples, run a sample blank (empty boat). After blank runs, equilibrate machine with 2 samples of approximate weight.

d. Run all samples from any single source in sequence to maximize machine calibration. When source of samples is changed run sample blank and re-equilibrate with 2 aliquots of first sample in new sequence.

C. End-of-day shut-down of system

1. Clean all platinum boats with brush.
2. Manually rotate programmer wheel clockwise to position 9.
3. Turn off recorder and cap pen.
4. Do <u>not</u> turn off gas supplies.
5. Lower combustion and reduction tube temperatures by about 100°C (i.e., by 1 unit on each dial).

IV. Calculations

A. Calculate percent C*, N*, and H* according to instructions on sample data sheets (See Appendix D).

B. Carbon and nitrogen data, only, must be corrected for weight increase occurring during and after acidification process, since 2 Cl^- replace $CO_3^=$, and sample absorbs water. Refer to example below. Do not correct hydrogen.

	g	g	Percent†		
Replicate	Dry Weight$_{105°C}$	Acidified Dry Weight	Organic C*	N*	H*
a	0.39013	0.44033	1.119	0.1347	1.21
b	0.38795	0.43960	1.272	0.1540	0.85

†As calculated in A., above

1. Correction for weight increase due to acidification

a. $\%\ \text{Organic } C'_a = \%\ \text{Organic } C^*_a \times \frac{\text{Acidified Dry Weight}}{\text{Dry Weight}_{105°C}}$

$$\%\ \text{Organic } C'_a = 1.119 \times \frac{0.44033}{0.39013} = 1.263\%, \text{ Replicate a}$$

b. $\%\ N'_a = \%\ N^*_a \times \frac{\text{Acidified Dry Weight}}{\text{Dry Weight}_{105°C}}$

$$\%\ N'_a = 0.1347 \times \frac{0.44033}{0.39013} = 0.152\%, \text{ Replicate a}$$

Note: C' and N' are % C and % N corrected for weight increase due to acidification.

Note: Repeat calculations for Replicate b.

2. Correction of replicates to same water content for weight increase due to water absorption

Note: $\%\ H^*_a$ = percent H in subsample a, from A., above

$\%\ H^*_b$ = percent H in subsample b, from A., above

Replicate a and b defined such that "a" has higher %H; thus, [if] $\%\ H^*_a > \%\ H^*_b$

let $F_1 = [(\%\ H^*_a - \%\ H^*_b)/100\%] \times 9$

$\cong$ excess H_2O in Replicate a

and F_a or F_b = (Acidified Dry Weight/Dry Weight$_{105°C}$)$_{a \text{ or } b}$

a. For $F_a \leq F_b$,

$$\% \text{ Organic } C''_a = (\% \text{ Organic } C'_a) \times [(F_a + F_1)/F_a]$$

$$\% N''_a = (\% N'_a) \times [(F_a + F_1)/F_a]$$

$$\% \text{ Organic } C''_b = \% \text{ Organic } C'_b$$

$$\% N''_b = \% N'_b$$

$$\% H''_a = \% H''_b = \% H^*_b$$

b. For $F_a > F_b$, and

i. $F_a \geq F_b + F_1$, no further correction is needed

$$\% H'' = \frac{H^*_a + H^*_b}{2}, \quad \% H''_a = H^*_a, \quad \% H''_b = H^*_b$$

$$\% \text{ Organic } C''_a = \% \text{ Organic } C'_a$$

$$\% N''_a = \% N'_a$$

$$\% \text{ Organic } C''_b = \% \text{ Organic } C'_b$$

$$\% N''_b = \% N'_b$$

ii. $F_a < F_b + F_1$

$$\% \text{ Organic } C''_a = \% \text{ Organic } C'_a \times [(F_b + F_1)/F_a]$$

$$\% N''_a = \% N'_a \times [(F_b + F_1)/F_a]$$

$$\% \text{ Organic } C''_b = \% \text{ Organic } C'_b$$

$$\% N''_b = \% N'_b$$

$$\% H''_a = \% H''_b = \% H^*_b$$

C. Sample calculation using data given in B., above

$\%\ H_1 = 1.21$

$\%\ H_2 = 0.85$

since $\%\ H_a^* > \%\ H_b^*$, $\%\ H_1^* = \%\ H_a^*$, $\%\ H_2^* = \%\ H_b^*$

$F_1 = [(1.21 - 0.85)/100] \times 9 = 0.0324$

$F_a = (0.44033/0.39013) = 1.12868$

$F_b = (0.43960/0.38795) = 1.13314$

because $F_a \leq F_b$,

$\%$ Organic $C_a'' = [(1.12868 + 0.0324)/1.12868] \times 1.263 = 1.299$

$\%$ Organic C_b'' = $\%$ Organic $C_b' = 1.441$

$\%\ N_a'' = [(1.12868 + 0.0324)/1.12868] \times 0.152 = 0.156$

$\%\ N_b'' = \%\ N_b' = 0.175$

$\%\ H_a'' = \%\ H_b'' = \%\ H_b^*$

Note: 1) 9 = mass ratio of H_2O:2H

2) The differences in % H between replicates due to differences in organic H are negligible compared to differences in water H in Lake Mead sediments.

REFERENCES

Instruction Manual for Cahn 21 Automatic Electrobalance. Cahn Instruments, Cerritos, California, U.S.A.

Instruction Manual for Perkin-Elmer Model 240B Elemental Analyzer. 1978. Perkin-Elmer, Norwalk, Connecticut, U.S.A.

APPENDIX A

OPERATION OF PERKIN - ELMER MODEL # 240B

1. Verify gas pressure.

   a. Helium ≅ 18 PSI, minimum 200 PSI in tank

   b. Oxygen ≅ 42 PSI, minimum 200 PSI in tank

   Do not change pressure settings.

2. Verify that DET RESET light is off. If on, press DET RESET button in quickly and release. Do not hold in depressed position. If light goes out, it is okay to proceed. If light stays on, it is impossible to proceed until problem is identified and corrected.

3. Turn on recorder power. At least 0.5 h warm-up period is needed to establish steady baseline.

4. Make following preliminary control settings

   a. PROGRAMMER SWITCH - should be on DETECT (DET)

   b. PROGRAMMER INDEX WHEEL - rotate clockwise from position 9 to position 0.

   c. Confirm DET RESET light still off.

   d. Set COMBUST control to 950°C. (∿4.8 on Dial).

   e. Set REDUCE control to 650°C. (∿4.5 on Dial).

      Note: Temperature gauge is on front panel. Actual controls are under lid, to the back and left of programmer wheel. It takes 1 h for machine to re-equilibrate approximately after temperature change of 100°C. Never set temperature at or above 1000°C. Never raise temperature when machine will be untended over the next hour. The temperature dials have more than

100° hysteresis. DO NOT ASSUME that positioning dial where it was previously will always give previous temperature.

f. Set H and N attenuation switch on X1.

g. Set C attenuation switch on 0.

h. Set EXT - COMB on off.

i. Confirm that CX10 blue light on (if not, push light).

j. Turn DETECTOR OUTPUT switch to A.

5. PURGE oxygen line daily for 10 minutes by first removing sample inlet fitting and installing inlet flow plug (Perkin - Elmer part no. 240-0101). Turn programmer index wheel CLOCKWISE to position 2 for 10 minutes. Turn wheel CLOCKWISE to position 1; reinstall standard fitting. Leave in this position for 3 minutes; then rotate wheel clockwise to 0.

6. Condition and calibrate.

a. Run two unweighed 1 to 3 mg acetanilide standards through machine (acetanilide standard currently in use has 10.36% N, 71.09% C, and 6.71% H).

b. Run a blank (with ladle and boat). If more than 1 blank is needed, machine must be re-equilibrated with 2 more unweighed standards between blank runs. More blanks are necessary if:

C, H, and N blanks vary by more than 40 µV from previous day's runs or if no previous runs exist for comparison. Range between a previous-day mean and a within-day mean should also not be greater than 40µV.

c. Re-equilibrate with 2 additional standards; then

run a 1 to 3 mg weighed standard. Calculate sensitivites (see Appendix C), compare with previous day's runs. Maximum permissible deviation from previous sensitivity means are 0.20 $\mu V \cdot \mu g^{-1}$ for N, 0.085 for C, and 2.70 for H. If new values are outside this range, run sufficient standards to establish confidence in a new mean. Use either the new mean or value from single run for a day's samples rather than previous day's mean.

Note 1: Record all standard blanks and standard data on calibration Data Sheets (see Appendix C).

Note 2: If no problems are anticipated with blanks or standards, the following sequence yields sufficient data in less time - 2 unweighed standards,1 to 2 weighed standards,blank.

On first blank or standard (whichever comes first), record the time at which start light remains off with programmer wheel at approximately position 5. The light must remain off between 1 and 3 minutes. Record time on calibration data sheet and check against value for previous day's run. If it varies by more than 15 seconds or if light remains off for less than 1 minute or longer than 3 minutes, consult manual.

APPENDIX B

OPERATION OF CAHN 21 AUTOMATIC ELECTROBALANCE

A. Set-up

1. Hang identical stirrups on sample "A" and TARE hangdowns.
2. Set RANGE to 2 mg.

B. Zero

1. Adjust the digital readout to 0.000 coarse and fine ZERO.

C. Calibrate

1. Set RANGE to 200 mg.
2. Place calibrating weight on sample pan.
3. Adjust the digital readout to 199.99 with CALIBRATE.
4. Remove calibrating weight.

D. Weigh

1. Select weighing range to be used.
2. Place sample on stirrup "A".
3. Read sample weight on digital readout.

Note: 1) Refer to instruction manual for complete operation details.

2) Always use Cahn forceps for moving the tare and calibrating weights. Never use sharp, pointed, or grooved forceps.

APPENDIX C

Model 240 CALIBRATION DATA SHEET (STANDARDS)

A. Standard Name:_____ Run #:_____ By:_____

Date:_____

B. Standard Weight:_____

C. Theoretical Percentage: Acetanilide 10.36%N, 71.09%C, 6.71%H

D. Theoretical Weight:_____µg N _____µg C _____µg H

E. Calculated Signal: (Recorder Reading) X (Attenuation) = Signal

	Nitrogen	Carbon	Hydrogen
Signal (µV)	_____X____=_____	_____X10 =_____	_____X____=_____
*C Suppression (µV)		Setting___=_____	
-Blank (µV)	=_____	_____X10 =_____	=_____
-Zero (µV)	_____X____=_____	_____X10 =_____	_____X____=_____
Total	=	=	=

F. Sensitivities (K, in $\frac{\mu V}{\mu g}$) :

$$K_N = \frac{\text{Total N signal } (\mu V)}{\text{Theoretical weight N } (\mu g)} = \_\_\_\_\_ \frac{\mu V}{\mu g}$$

$$K_C = \frac{\text{Total C signal } (\mu V)}{\text{Theoretical weight C } (\mu g)} = \_\_\_\_\_ \frac{\mu V}{\mu g}$$

$$K_H = \frac{\text{Total H signal } (\mu V)}{\text{Theoretical weight H } (\mu g)} = \_\_\_\_\_ \frac{\mu V}{\mu g}$$

*Values of C Suppression for C ATTENUATION Switch Setting.
(Use in Computation of C Signal Only)

Switch Setting	0	1	2	3	4	5
C Suppression (µV)	0	10,000	20,000	30,000	40,000	50,000

APPENDIX D

DATA SHEET FOR SAMPLES

A. Sample No.:_____ B. Sample Name:_____

C. Date:_____ D. Pretreatment:_____

E. Sample + Tare Weight:_____μg

F. Tare Weight:_____μg

G. Sample Weight:_____μg

H. Sensitivities: K_N = _____ $\frac{\mu V}{\mu g}$; K_C = _____ $\frac{\mu V}{\mu g}$; K_H = _____ $\frac{\mu V}{\mu g}$

I. Total Signals:
Total Signal (in μV) = Signal + C Suppression* - Blank - Zero

Where:
Signal or Zero (in μV) = Recorder Reading X Attenuation or 10**
Blank (in μV) = Blank Signal - Blank Zero

	Nitrogen	Carbon	Hydrogen
Signal (μV)	____X____=____	____X10 =____	____X____=____
+C Suppression* (μV)		Setting___=____	
-Blank Values (μV)	=____	=____	=____
-Zero (μV)	____X____=____	____X10 =____	____X____=____
Total Signals (μV)	=____	=____	=____

*Values of C Suppression for C ATTENUATION Switch Setting:
Use in Computation of C Signal Only.

Switch Setting	0	1	2	3	4	5
C Suppression (μV)	0	10,000	20,000	30,000	40,000	50,000

**10: Use only in C Signal Computation

J. Calculated Weights and Percentages:

Weight (μg) of N, C, or H in sample = $\frac{\text{Total signal } (\mu V)}{\text{Sensitivity K } (\mu V \cdot g^{-1})}$

_____μg N :_____μg C:_____μg H

Percentages of N, C, or H in a sample = $\frac{\text{Weight } (\mu g) \times 100}{\text{Sample Weight}}$

Percentages_____% N*:_____% C*:_____% H*

Procedure for Determining Sediment Total Phosphorus (After methods of Aspila et al. 1976, and John 1970)

I. Basis of Method

Ignition at 550°C of a sediment sample converts all phosphorus compounds to inorganic phosphorus. Total phosphorus, as inorganic phosphorus, is then extracted with 1 N sulfuric acid (Aspila et al. 1976, Wildung and Schmidt 1973). In calcarious and some soft water sediments, virtually all the phosphorus is extracted by this procedure (Sommers et al. 1970). The extraction procedure outlined here is a modification of that of Aspila et al. (1976). The John (1970) modification of Murphy and Riley's (1962) technique, designed to minimize interferences in soil analysis, is used to determine inorganic phosphorus in the acid extract.

II. Materials and Reagents

A. Dry weight and ignition

1. Equipment

a. Muffle furnace (Thermolyne and Thermolyne Furnatrol I)

b. Balance with 0.00001 g resolution (e.g. Mettler)

c. Drying oven (capable of maintaining temperature to within ± 1°C)

2. Supplies

a. Glass scintillation vials, engraved with identification number

b. Polyethylene scintillation vials, marked with identification number

c. Desiccator(s)

d. Dry $CaSO_4$ indicator desiccant

Store in metal tray in drying oven; raise oven temperature daily to $\geq$ 160°C for a few hours. Open door slightly for 30 minutes; close oven and readjust to 105°C.

B. Extraction

1. Shaker (e.g. Lab-Line Model 3590)
2. Centrifuge (e.g. International Equipment Company Model CL 10407M-1)
3. 0-30 ml repipet
4. Leak-resistant plastic bags
5. Polyethylene scintillation vials with polyseal caps
6. Reagents

1 N H_2SO_4: 28 ml concentrated H_2SO_4, reagent grade, to 1 liter distilled-deionized water. Store in 1-liter volumetric flask.

C. Analysis

1. Balance with 0.01 g resolution
2. Spectrophotometer (e.g. Perkin-Elmer Model 552)
3. Automatic pipets capable of delivering 0.05, 0.10, 0.30, 1.00, and 2.0 ml volumes
4. Repipet capable of delivering 18 ml
5. Polyethylene or glass scintillation vials with polyseal caps
6. Reagents and standards
   a. Ammonium molybdate, analytical grade
   b. H_2SO_4 (10 N)

Add 280 ml concentrated analytical grade H_2SO_4 to approximately 700 ml distilled-deionized water in a 1-liter volumetric flask. Bring volume up to 1 liter with distilled-deionized water. Store in 1-liter flask.

c. Antimony potassium tartrate (0.5 percent)

Add 0.5 g of analytical grade antimony potassium tartrate to 100 ml distilled-deionized water. Make up fresh.

d. Ascorbic acid

Use analytical grade. Store in refrigerator.

e. NaOH (0.1 N)

Add 4 g NaOH to 1 liter. Store in polyethylene container. (For rinsing flow-through cell of spectrophotometer.)

f. Extraction reagent (primary)

1. Dissolve 20 g ammonium molybdate in approximately 300 ml distilled-deionized water.
2. Add 450 ml of 10 N H_2SO_4, slowly, while constantly stirring.
3. Add 100 ml of 0.5% antimony potassium tartrate; stir.
4. Bring volume up to 1 liter with distilled-deionized water. Reagent may be stored in the dark for up to 3 months.

g. Extraction reagent (secondary)

1. Add 1.5 g ascorbic acid per 100 ml of primary

extraction reagent; mix well (make sufficient reagent to analyze all standards and samples).

2. Do not make up more than 4 hours prior to starting phosphorus analysis.

h. Standards

1. Primary (50 mg $P \cdot l^{-1}$)

Dissolve 0.2195 g KH_2PO_4 (analytical grade) (oven-dried to constant weight at 105°C) in 1000 ml distilled-deionized water, to which 1 ml concentrated H_2SO_4 has been added as a preservative. Store in dark in polyethylene.

2. Using automatic pipet, add the following amounts (in triplicate) of 50 mg $P \cdot l^{-1}$ primary standard to polyethylene scintillation vials.

i. 0 ml = 0 µg P (blank)

ii. 0.05 ml × 50 mg $P \cdot l^{-1}$ = 2.5 µg P

iii. 0.30 ml × 50 mg $P \cdot l^{-1}$ = 15.0 µg P

iv. 0.60 ml × 50 mg $P \cdot l^{-1}$ = 30.0 µg P

Bring total volume in each vial up to 18 ml with repipet.

III. Procedure

A. Prepare all labware as in III A. 1 and 2, p. 183.

B. Dry weight and ignition (preparation)

1. Prior to analysis, sediment samples are dried at temperatures ranging from 25°C (room temperature) to 105°C. Drying times range from two weeks (room temperature) to

24 hours (105°C).

2. Dried samples are ground by hand with a mortar and pestle and a subsample of approximately 20 g is placed into a clean polyethylene scintillation vial with polyseal cap. These subsamples are stored in a desiccator until analyses are completed.

C. Dry weight analysis

1. Weigh empty glass scintillation vial; record to nearest 0.00001 g.
2. Add 0.4 ± 0.1 g sediment to each vial; reweigh to nearest 0.00001 g. Duplicate each sample.
3. Place vial containing dry sediment in oven at 105°C for at least 16 hours.
4. Cool in desiccator; reweigh and record weight (see Note A).

D. Ignition

1. Place vial containing sediment dried at 105°C (see B.2, above) in anterior third of muffle furnace (behind the -25°C isopleth, Fig. 1), but do not allow vials to touch back wall (see Note B).
2. Place oven temperature control setting at 300°C for 30 minutes; increase to 550°C. After 1.5 hours turn off oven.
3. Cool vials in oven or in desiccator. If vials are cooled in oven, place into desiccator immediately upon removal.

E. Phosphorus extraction

1. Add 20.0 ml 1 N H_2SO_4 to previously-ignited sample with

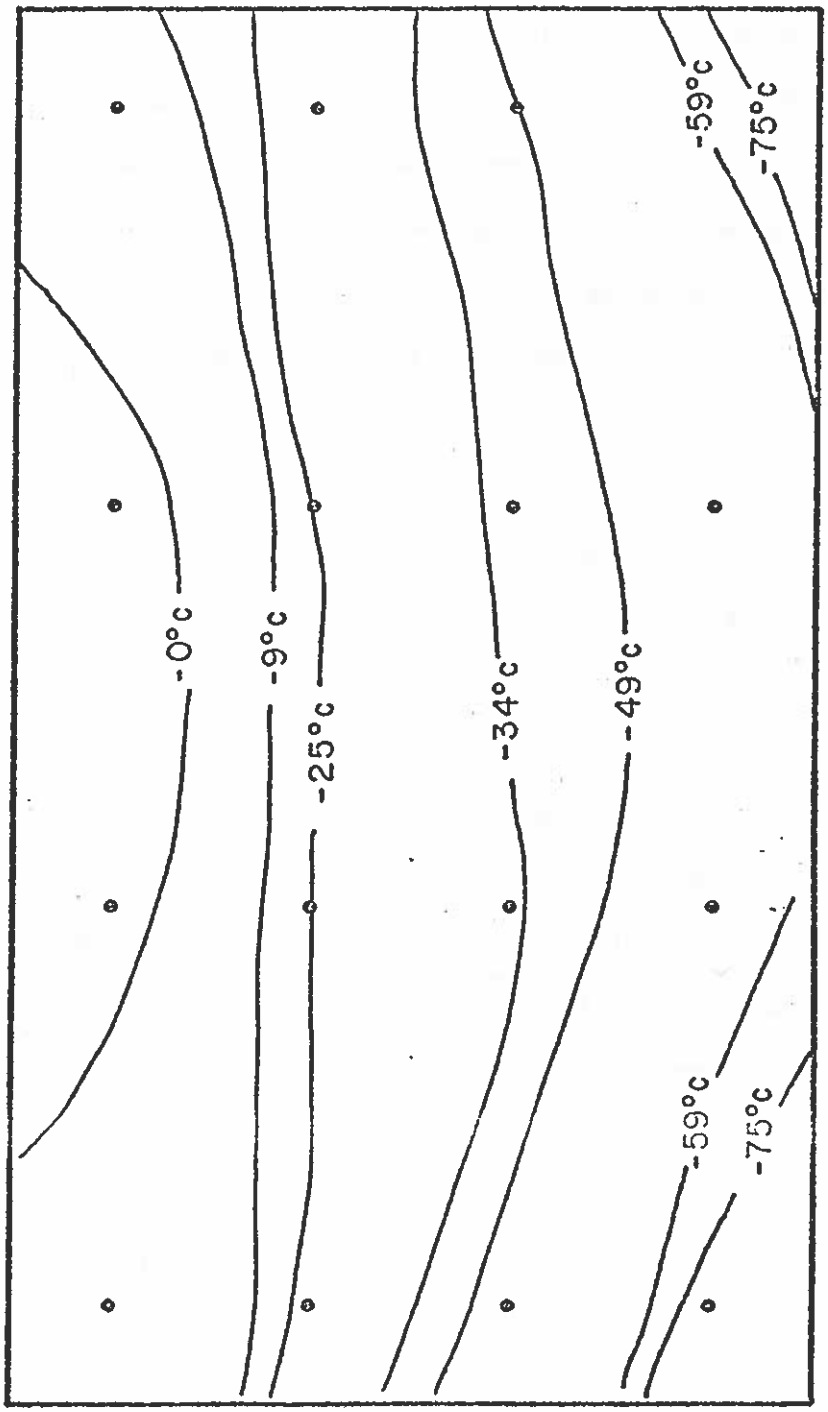


Figure 1. Error in muffle furnace temperature as a function of sample location.

Test range is 525 - 650°C for a burn time of 1.5 hours.

automatic pipet.

2. Loosely cap if gas evolution occurs. If not, proceed with step 5, below.
3. When gas evolution ceases, tighten polyseal cap, shake vial vigorously, and immediately loosen cap.
4. Repeat step 3 twice over a 1-2 hour period.
5. Tighten polyseal cap; place groups of 5-6 vials each into plactic bags, and secure to shaker.
6. Shake approximately 16 hours in room with temperature controlled to 25°C.
7. Briefly loosen and retighten caps if gas evolution occurred in step 2.
8. Centrifuge vials at approximately 2000 RPM for 5 minutes.
9. Decant into clean polyethylene scintillation vials with masking tape labels marked with waterproof ink. Cap with polyseal caps.
10. At this point, samples may be frozen until analysis.

F. Phosphorus analysis

1. If samples have been frozen, thaw overnight at room temperature or under lukewarm tap water.
2. Vigorously shake sample and place 1.00 ml of sample with automatic pipet and 17.00 ml distilled-deionized water with repipet into a clean glass or polyethylene scintillation vial.
3. At 30-second intervals, add 2.0 ml of secondary extraction reagent, in order, to:
   i. 1 set of standards

ii. first 1/2 of samples

iii. 1 set of standards

iv. remaining 1/2 of samples

v. 1 set of standards

Cap and briefly shake vials after reagent addition.

4. After 45-60 minutes begin reading absorbance, at 882 nm in a flow-through or 1 cm-cell, in order of reagent addition.

   Note: Vigorously shake each sample immediately prior to reading absorbance.

   Note: If flow-through cell is used, rinse with 1 volume 0.1 N NaOH and 2 volumes of distilled-deionized water after each sample and standard.

5. Immediately after use, disassemble automatic pipet and repipet; flush all parts with distilled-deionized water and wipe dry with tissue; place, disassembled, in a closed drawer to dry.

6. Rinse scintillation vials with 0.5 N H_2SO_4 and rinse copiously with distilled-deionized water. Dry plastic vials in closed cupboard and glass vials in drying oven at 105°C. Polyseal caps should be rinsed only with distilled-deionized water and dried in cupboard; never rinse with acid or oven-dry.

IV. Calculations

A. Plot standard curve of absorbance vs. amount of P (µg) in

standards and verify linearity. If standard curve is not linear beyond 15 µg P standard, extend the line to absorbance value of 30 µg P standard by drawing smooth curve.

B. If standard curve is linear, generate regression of absorbance vs. amount of P (µg) in standards. Determine amount of P (µg) in samples.

C. If standard curve is not linear, determine amount of P (µg) in samples from graph of smoothed curve.

D. Divide µg P in sample by dry weight (105°C) of sample = µg $P \cdot g^{-1}$.

E. Precision

1. Duplicate analyses of a single extract should be repeated if values differ by more than 50 µg $P \cdot g^{-1}$.
2. Single analyses of duplicate extracts should not differ by more than 75 µg $P \cdot g^{-1}$. If they do, repeat analyses of both extracts. If re-analysis still yields differences greater than 75 µg $P \cdot g^{-1}$, ignite and extract two new subsamples.

V. Notes

A. When cooling hot vials, avoid contact with sides of desiccator to prevent damage to desiccator and to prevent desiccator sealant from contacting vials. Likewise, cool vials should not contact sealant.

B. The rear wall of muffle furnace is hotter than the interior air or floor and can partially melt glass vials if contact is made.

C. In sediments from Lake Mead, this extraction gives results comparable to sequential oxalate (Williams et al. 1971) and 1 N H_2SO_4 extractions, indicating that single acid extraction does solubilize occluded Fe and Al phosphates from the clay-containing sediment. Other problems affecting the procedure are discussed in Dormarr and Webster (1964).

D. The specific procedure outlined here is similar to that of Aspila et al. (1976) but is slightly modified by using H_2SO_4 as the extractant and by using glass scintillation vials as the ignition and extraction vessels. The use of divalent H_2SO_4 as extractant rather than monovalent HCl is preferable because of the approximately four-fold greater desorbing capacity of sulfate, and consistency with the use of H_2SO_4 as a P analytical reagent. We combine phosphorus analyses with organic weight determinations, using the samples after ashing for P analyses.

REFERENCES

Aspila, K.I., H. Agemian, and A.S.Y. Chau. 1976. A semi-automated method of determination of inorganic, organic and total phosphate in sediments. Analyst 101:187-197.

Dormaar, J.F., and G.R. Webster. 1964. Losses inherent in ignition procedures for determining total organic phosphorus. Can. J. Soil Sci. 44:1-6.

John, M.K. 1970. Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. Soil Sci. 109:214-220.

Murphy, J., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27:31-36.

Sommers, L.E., R.F. Harris, J.D.H. Williams, D.E. Armstrong, and J.K. Syers. 1970. Determination of total organic phosphorus in lake sediments. Limnol. Oceanogr. 15:301-303.

Wildung, R.E., and R.L. Schmidt. 1973. Phosphorus release from lake sediments. Ecology Series (EPA-R3-73-024), U.S. Environmental Protection Agency, Washington, D.C.

Williams, J.D.H., J.K. Syers, S.S. Shukla, R.F. Harris, and D.E. Armstrong. 1971. Levels of inorganic and total phosphorus in lake sediments as related to other sediment parameters. Environ. Sci. Technol. 5:1113-1120.

Procedure for Determination of Sediment Dry Weight$_{105°C}$, Organic Matter, Water of Hydration (After APHA 1975) and Sediment Carbonate Content (After Method of Wetzel and Manny 1978)

1. Basis of Method

   A. Dry Weight$_{105°C}$, Organic Matter Content, and Water of Hydration

      The standard method of determining volatile and ash components of sediment is ignition at 550°C (APHA 1975, p. 95). The volatile component is often assumed to approximate organic matter. In the presence of clays and gypsum, however, a significant proportion of weight loss upon ignition may be due to loss of water of hydration. The water of hydration can be reintroduced by wetting the ashed sample with distilled-deionized water and redrying at 105°C. Reweighing the ashed sample at this point provides an estimate of the water of hydration (See Note A) and a more accurate estimate of organic matter content (APHA 1975, p. 1048) (See Note B).

   B. Carbonate Content

      Carbonate content of sediments is determined by difference in weight loss between samples combusted at 550°C and 950°C (Wetzel and Manny 1978). The weight loss between these two temperatures is due to loss of CO_2 from carbonates as they decompose to form oxides. Combustion at 550°C volatilizes organic matter, dehydrates gypsum, partially dehydrates clays, and decomposes some magnesium carbonates. Magnesite, $MgCO_3$, decomposes at 350°C (Weast 1976). Basic magnesium carbonate,

$(MgCO_3)_4 \cdot Mg(OH)_2 \cdot n\ H_2O$ (n = 4.3) decomposes between 300 and 450°C in our muffle furnace. Dolomite, $CaCO_3 \cdot MgCO_3$, decomposes between 730 and 760°C; and $CaCO_3$ phases of aragonite and calcite, decompose at 825°C and 898°C, respectively (Weast 1976). However, we have found that 72 percent of the weight loss of reagent-grade calcite occurred between 650 and 750°C in the muffle furnace. (Furnace temperatures are within 1% of values on setting.) The reason for this discrepancy from findings in the literature is unclear.

Direct organic carbon and total carbon determinations by CHN analysis (See page 182) on one sample from Lake Mead indicated that 96% of carbonates decomposed above 550°C. Only 97% of organic carbon was lost at 550°C; the additional 3% would be included as error in the carbonate fraction. However, concentrations of carbonate are much higher than concentrations of organic matter in Lake Mead sediments; therefore, for carbonate analyses, this error is negligible.

Regression of carbonate-carbon determined by the muffle furnace technique against carbonate-carbon determined by CHN analysis (a more tedious, but more exact procedure) demonstrates an almost 1:1 correspondence (Fig. 1). Therefore, the simpler muffle furnace procedure is adequate and does not create significant error from release

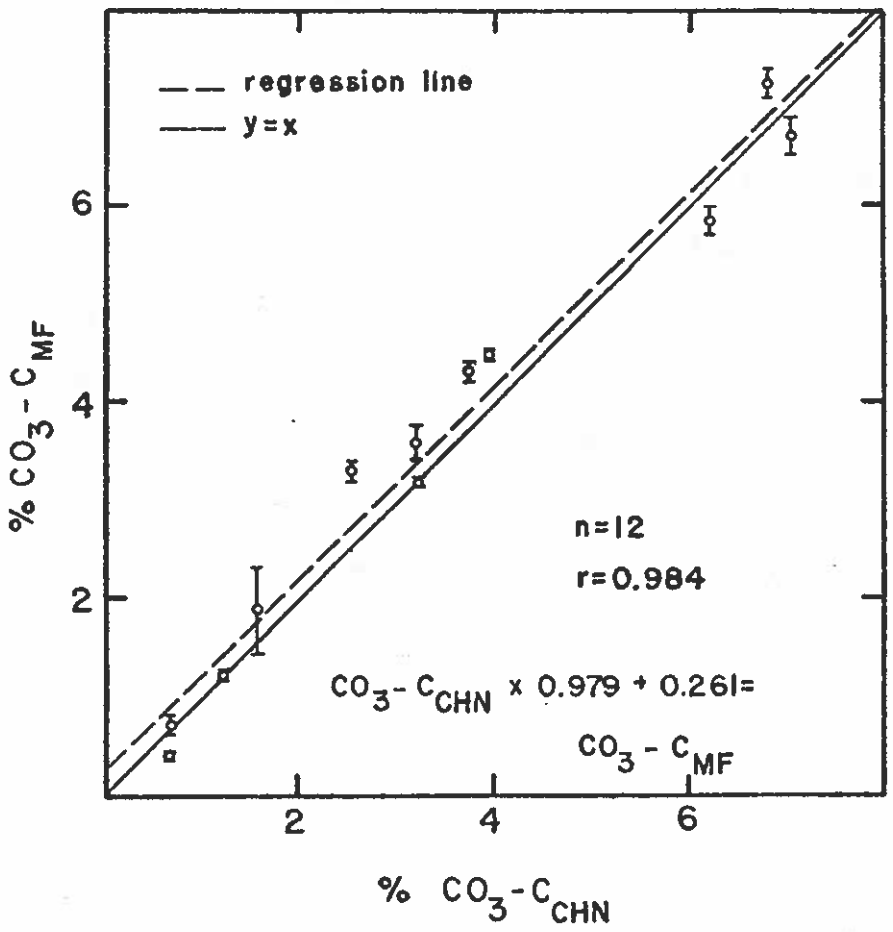


Figure 1. Regression of carbonate-carbon determined by the muffle furnace technique against carbonate-carbon determined by CHN analysis.

of water of hydration or other non-carbonate volatiles above 550°C.

II. Materials

A. Equipment

1. Muffle furnace (Thermolyne and Thermolyne Furnatrol I)
2. Balance with 0.00001 g resolution (e.g. Mettler)
3. Drying oven (capable of maintaining temperature to within ± 1°C)

B. Supplies

1. Desiccator
2. $CaSO_4$ indicator desiccant

   Store in metal tray in drying oven; raise oven temperature daily to $\geq$ 160°C for a few hours. Open door slightly for 30 minutes; close oven and readjust to 105°C.
3. Combustion vessels

   Set of 48 10-ml porcelain crucibles with engraved identification (See Note C)
4. Dropper bottle containing distilled-deionized water (for water of hydration analysis)

III. Procedure

A. Preparation of combustion vessels

1. Rinse crucibles in 0.5 N H_2SO_4 and then copiously with distilled-deionized water.
2. Dry, place in muffle furnace, and combust crucibles for 3 additional hours after temperature reaches 950°C

(6-7 hours total). Turn off furnace, allow crucibles to cool overnight in furnace, then transfer to a desiccator.

Note: Always handle crucibles with tongs or forceps, not with fingers (See Note D).

B. Preparation of balance

1. Each day, or more often if necessary, replace desiccant in balance weighing chamber with dry desiccant from oven.
2. Zero balance before, and frequently in between, sample weight determinations.

C. Preparation of sediment

1. Prior to analysis, sediment samples are dried at temperatures ranging from 25°C (room temperature) to 105°C. Drying times range from two weeks (room temperature) to 24 hours (105°C).
2. Dried samples are ground by hand with a mortar and pestle and a subsample of approximately 20 g is placed into a clean polyethylene scintillation vial with a polyseal cap. These subsamples are stored in a desiccator until analyses are completed.

D. Analysis

1. Dry Weight
   a. Weigh empty crucible; record weight to nearest 0.00001 g.
   b. Add 0.4 ± 0.1 g sediment to each crucible; reweigh to nearest 0.00001 g. Duplicate each sample.
2. Dry Weight$_{105°C}$

a. Place crucible containing sediment (See 1, above) in oven at 105°C for at least 16 hours.

b. Cool in desiccator; reweigh and record weight (See Notes E and F).

3. Ignited Weight

a. Place crucible containing sediment dried at 105°C (See 2, above) in anterior third of muffle furnace (behind the -25°C isopleth, Fig. 2 and Note G); if glass vials are used (See Note C) do not allow vials to touch back wall (See Note H).

b. Place furnace temperature control setting at 300°C for 30 minutes; increase to 550°C. After 1.5 hours turn off furnace.

c. Cool crucibles in furnace or in desiccator. If crucibles are cooled in furnace, place into desiccator immediately upon removal.

d. Reweigh crucibles as soon as possible after samples have cooled. Record weight to nearest 0.00001 g.

4. Wetted Ignited Weight

a. To each crucible containing sediment ignited at 550°C (See 3, above) add enough distilled-deionized water to wet ash (2-4 drops).

b. After 15 minutes, dry samples at 105°C, in oven for 16 hours.

c. Cool samples in desiccator; reweigh and record weight.

d. Store ashed samples if needed for additional analyses.

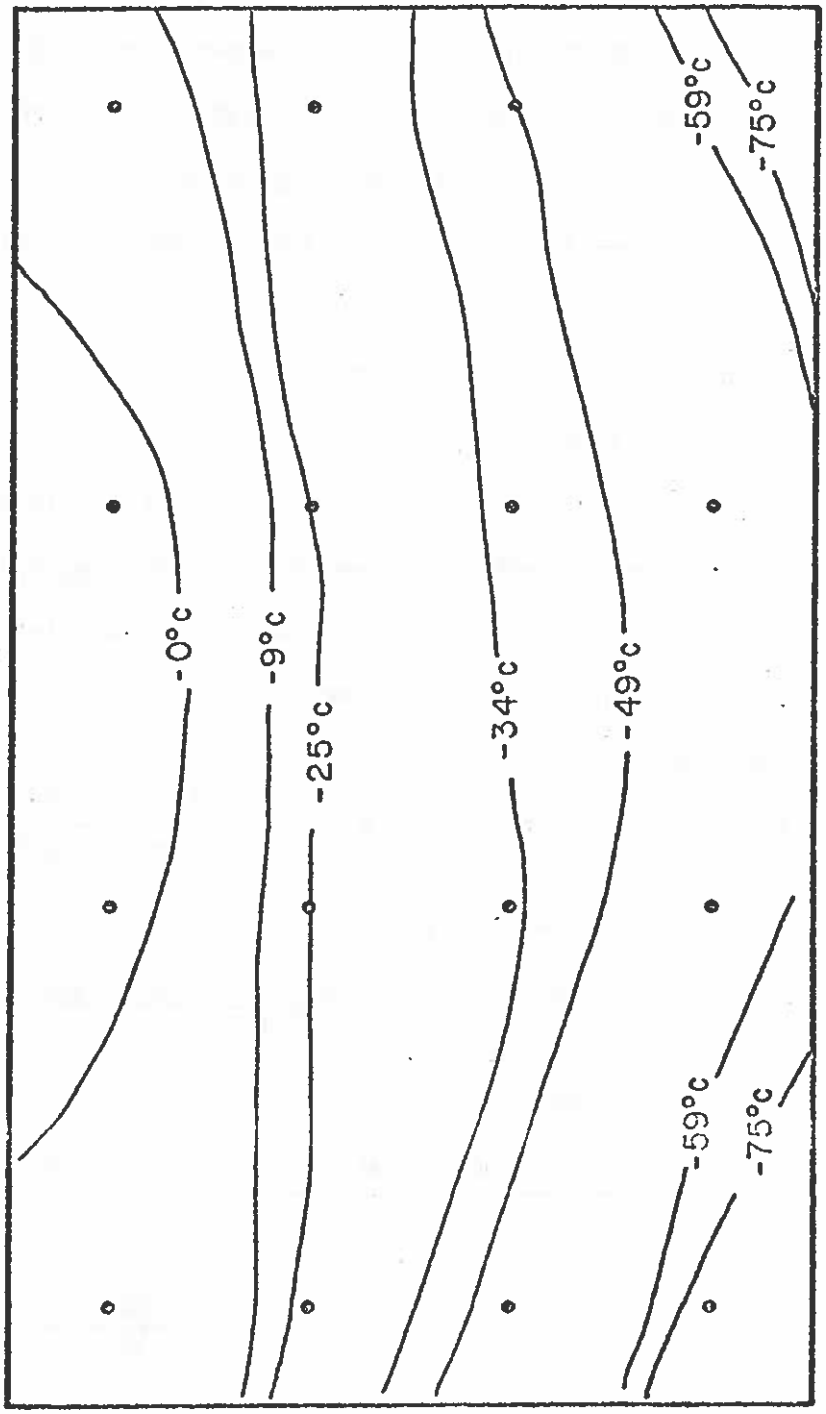


Figure 2. Error in muffle furnace temperature as a function of sample location.

Test range is 525 - 650°c for a burn time of 1.5 hours.

5. Carbonate content

   a. Place crucibles in muffle furnace within the -49°C isopleth (Fig. 2). Set temperature at 300°C for 30 minutes, then increase to 550°C for 30 minutes, and finally, increase setting to 950°C (See Note 1). After furnace reaches 950°C, combust for an additional 3 hours.

   b. Turn off furnace, and cool crucibles in closed furnace, or transfer to desiccator. If cooled in furnace, transfer to desiccator as soon as possible.

   c. Reweigh and record weight (= Weight $_{950°C}$).

   d. Discard ashed samples and rinse crucibles with tap water; clean according to III A.

C. Calculations

1. $$\text{Percent Water Content} = \frac{\text{Dry Weight} - \text{Dry Weight}_{105°C})}{\text{Dry Weight}_{105°C}} \times 100$$

2. $$\text{Percent Water of Hydration} = \frac{(\text{Wetted Ignited Weight} - \text{Ignited Weight}) \times 100}{\text{Dry Weight}_{105°C}}$$

3. $$\text{Percent Organic Matter} = \frac{(\text{Wetted Ignited Weight} - \text{Dry Weight}_{105°C}) \times 100}{\text{Dry Weight}_{105°C}}$$

4. $$F_1 = \text{Weight}_{950°C}\ \text{Loss} = \left(1 - \frac{\text{Weight}_{950°C}}{\text{Dry Weight}_{105°C}}\right)$$

5. F_2 = Percent Carbonate-CO_2 Loss = 100 F_1 - Percent Water Content - Percent Water of Hydration - Percent Organic Matter

6. Carbonate-C = $F_2 \times \frac{12}{44.01}$

7. Carbonate-CO_3 = $F_2 \times \frac{60.01}{44.01}$

8. Percent $CaCO_3$ Equivalent = $F_2 \times \frac{100.09}{44.01}$

IV. Notes

A. Correction for water of hydration is critical in analysis of Lake Mead sediments. If correction is not made, organic matter content may be overestimated by up to 100 percent.

B. The regression of organic matter on organic carbon gives a slope of 3.9 ($r = 0.798$, $N = 210$) for all Lake Mead sediment data. This value is beyond the range and 1.7X the mean value for 18 lake sediments analysed by Hansen (1961); therefore, despite our precautions, we are overestimating true organic matter content in Lake Mead sediment.

C. If carbonate analysis is not done, the use of crucibles as combustion vessels is unnecessary. In determination of Dry $Weight_{105°C}$, Organic Weight, and Water of Hydration only, the use of glass scintillation vials is preferred; the ashed samples at(550°C) can then be used in the total phosphorus analysis (See page 200).

Preparation of glass scintillation vials and caps is as follows:

1. Rinse all labware except polyseal caps with 0.5 N H_2SO_4; follow with distilled-deionized water. Rinse caps with distilled-deionized water only and allow to air dry; do not dry in oven.

   Note: Avoid drying any labware in the drying oven immediately prior to or during sediment drying, since moisture from the labware will be absorbed by the sediment.

2. Place glass scintillation vials in muffle furnace at 550°C for 1.5 hours, and store in desiccator.

D. When cooling hot vials or crucibles, avoid contact with sides of the desiccator to prevent damage to the desiccator, and to prevent desiccator sealant from contacting vials or crucibles. Cool vials or crucibles should not contact sealant.

E. Despite careful precautions during drying and weighing procedures in Dry Weight$_{105°C}$ determinations, a Lake Mead sediment sample will often increase in weight due to moisture absorption if ambient humidity has increased. If this occurs, use the Dry Weight determination as Dry Weight$_{105°C}$, and record water content as zero.

F. Although the standard drying temperature is 103-105°C, many authorities recommend lower temperatures to minimize loss of organics through volatilization (e.g. Strickland and Parsons 1972). Lake Mead sediments, however, are very hygroscopic, contain gypsum, and have water of hydration contents as large as organic matter content and greater than organic carbon content. Thus, the need to minimize water content and the variability in its measurement is more critical for accuracy and precision of the analysis than loss of volatile organics at 105°C.

G. The permissible temperature range for determination of organic matter content is 550 ± 50°C (APHA 1975). This wide range is allowable because normal weight loss between 500 and 600°C is usually insignificant. For Lake Mead sediments, however,

the rate of increase of weight loss from 500°C to 600°C is at least $0.16\% \cdot 1°C^{-1}$, i.e.

$$\frac{(W_{500°C} - W_{600°C})}{W_{Tot}} \div 100°C \times 100 = 0.16\% \cdot 1°C^{-1}$$

where $W_{500°C}$ = weight at 500°C

$W_{600°C}$ = weight at 600°C

W_{Tot} = dry weight at 105°C - weight at 950°C

To minimize the error in organic matter determination, we place our samples in the anterior third of the muffle furnace where temperatures average 537.5 ± 12.5°C (Fig. 2).

H. The rear wall of the muffle furnace is hotter than the interior air or floor and can partially melt glass vials if contact is made.

I. Use of a cool furnace and protracted time before attaining 500°C decrease volatilization of ash. Lake sediments in general (and Lake Mead sediments in particular) have sufficient inorganic matrix not to require the $MgCl_2$ addition necessary to stop loss of ash in more organic samples (Aspila et al. 1976).

J. Crucible blanks should be run with each data set as a precaution; however, our blanks have been low and not significantly different from zero. Our calculations do not contain blank corrections.

K. The % calcium carbonate equivalent parameter includes both magnesium and calcium carbonates and thus overestimates the

sum of $MgCO_3$ + $CaCO_3$. Gould (1960) reported average MgO content of 3.2% and CaO of 7.4% in bottomset deltaic sediments in Lake Mead (n = 6). If present entirely as carbonates, these numbers represent 6.8% $MgCO_3$ and 13.1% of $CaCO_3$. For these percentages, % calcium carbonate equivalent would be 106% of total magnesium + calcium carbonate percentage.

APPENDIX

CHOICE OF 550°C AS COMBUSTION TEMPERATURE IN ORGANIC MATTER DETERMINATION

Destruction of organic matter is incomplete at 550°C (Dormaar and Webster 1964, Froelich 1980), and some authorities suggest higher ignition temperatures. This suggestion was tested and rejected on the basis of total and organic carbon determinations by CHN analysis of one sample from Lake Mead (Table 1).

One subsample of the sediment was treated with HCl to remove carbonates; the other was analyzed with carbonates present. The true organic carbon content (determined by combustion at 950°C after acidification) was 1.3% of Dry Weight $_{105°C}$. Combustion of this sample at 550°C released only 97% of the organic carbon. With increasing temperature, however, losses of magnesium carbonate and calcium carbonate cause more than compensatory error. The total percent error in organic matter determination can be estimated by assuming the solid phase loss of organic carbon as CH_2O and carbonates as CO_2, i.e.:

$$\text{Percent Error in Organic Matter} = (\%\ \text{unacid}_T - \%\ \text{Acid}_T) \times \frac{\text{wt.}CO_2}{\text{wt.}CH_2O} - (\%\ \text{Acid}_{950} - \%\ \text{Acid}_T)$$

where T = combustion temperature

The values for these calculations are shown in the last column in Table 1. Thus, analytical determination of organic matter in this sediment sample should overestimate the true value by 19% at 550°C

combustion, 25% at 610°C combustion, and by 549% at 950°C combustion. Therefore, combustion at 550°C yields a more valid estimate than combustion at higher temperatures.

Table I. Comparison of organic carbon recovery by CHN analysis as a function of combustion temperature and acid treatment.

Temperature °C	Analytical organic carbon as percent of true organic carbon		Percent error in organic matter determination
	Acidified	Unacidified	
550	97	112	+19
610	100	117	+25
950	100	474	+549

Example:

$$\text{Percent Error in Organic Matter}_{550°C} =$$

$$(112-97) \times \frac{44}{30} - (100-97) = 19$$

REFERENCES

APHA. 1975. Standard Methods for the Examination of Water and Wastewater. 14th ed., American Public Health Association. Washington, D.C. 1193 pp.

Aspila, K.I., H. Agemian, and A.S.Y. Chau. 1976. A semi-automated method for the determination of inorganic, organic, and total phosphate in sediments. Analyst 101:187-197.

Dormaar, J.F., and G.R. Webster. 1964. Losses inherent in ignition procedures for determining total organic phosphorus. Can. J. Soil Sci. 44:1-6.

Froelich, P.M. 1980. Analysis of organic carbon in marine sediments. Limnol. Oceanogr. 25:564-572.

Gould, H.R. 1960. Character of the accumulated sediment, pp. 149-186. In: W.O. Smith, C.P. Vetter, G.B. Cummings, and others, Comprehensive survey of sedimentation in Lake Mead, 1948-1949. Geological Survey Professional Paper 295, U.S. Government Printing Office, Washington, D.C.

Hansen, K. 1961. Lake types and lake sediments. Verh. Internat. Verein. Limnol. 14:285-290.

Strickland, J.D.H., and T.R. Parsons. 1972. A practical handbook of seawater analysis. 2nd ed. Fisheries Research Board of Canada, Ottawa.

Weast, R.C. (ed.). 1976. Handbook of chemistry and physics. 57th ed. CRC Press, Cleveland.

Wetzel, R.G., and B.A. Manny. 1978. Postglacial rates of sedimentation, nutrient and fossil pigment deposition in a hardwater marl lake of Michigan. Pol. Arch. Hydrobiol. 25:453-469.

SECTION III. PHYSICAL METHODS

Procedure for Measuring Temperature, Dissolved Oxygen, pH and Conductivity Using Hydrolab^{R} Model IIA Surveyor and Hydrolab^{R} Model System 8000

I. Description of Instruments

Hydrolab^{R} water quality instruments consist of a surface unit from which data readings are taken, a submersible unit with multiple probes for measurements of various parameters, and an underwater cable connecting the two units.

Both the Hydrolab^{R} IIA Surveyor and Hydrolab^{R} System 8000 are equipped to measure temperature, pH, dissolved oxygen and conductivity. Specifications for parameters for each model are as follows:

Hydrolab^{R} IIA Surveyor

Parameter	Range	Accuracy
Temperature	-5°C to 45°C	± 0.25°C
pH	0 to 14	± 0.05 pH per 4 unit pH interval
Dissolved oxygen	0 to 20 $mg \cdot l^{-1}$	± 1.5% of reading ± 0.05 $mg \cdot l^{-1}$
Conductivity	0 to 1,10,100 $mmho \cdot cm^{-1}$	± 0.5% range

Hydrolab^{R} System 8000

Parameter	Range	Accuracy
Temperature	-5°C to 45°C	± 0.15°C
pH	0 to 14	± 0.05 pH per 4 unit pH interval
Dissolved oxygen	0 to 20 $mg \cdot l^{-1}$	± 1.5% of reading ± 0.05 $mg \cdot l^{-1}$
Conductivity	0 to 1,10,100 $mmho \cdot cm^{-1}$	± 0.5% range

II. Operation of Instruments

A. Calibration (performed prior to each sampling run)

1. Dissolved oxygen probe

a. Comparisons are made between Winkler determinations and probe measurements of dissolved oxygen on tap water saturated with oxygen by shaking water in an enclosed partially filled container.

b. Calibrations can be made by measuring dissolved oxygen of oxygen-saturated water with concentration determined from temperature readings and percent saturation tables.

c. Calibrations may be made in air as specified by Hydrolab^{R}.

Note: Membrane in probe should be changed when meter won't maintain calibration, when erratic readings are obtained, or when response time is abnormally slow.

2. Conductivity probe

Calibration of conductivity probe is conducted using a standard salt solution (usually KCl) of conductivity = 1200 $\mu mhos \cdot cm^{-2}$.

3. pH probe

Calibration of pH probe is conducted by using standard buffers of pH=7 and pH=10.

4. Temperature probe

The temperature probe is calibrated using a hand-held thermometer, calibrated by the National Bureau of Standards.

B. Measurements

The multiparameter probe is lowered to specific depths by a cable

calibrated in 1.0 m increments, from 0 m to within 1.0 m of sediment surface. Temperature, pH, conductivity and dissolved oxygen readings are taken at each depth. Dissolved oxygen concentrations are read last to allow probe to equilibrate.

REFERENCES

Instruction manual for use of Hydrolab^{R} Models IIA Surveyor and System 8000. Hydrolab Corporation. Austin, Texas, U.S.A.

Procedure for Measuring Photosynthetically Active Radiation (PAR)

I. Description of LI-COR Model LI-188 Photometer

Photosynthetically active radiation (400 to 700 nm) is measured with a Li-Cor Model Li-188 Integrating Quantum/Radiometer/Photometer. The instrument is equipped with a digital readout system, and is capable of providing instantaneous light measurements, provided by 1 second integrations, and integrated light measurements, provided by 10-second, 100-second, and 1000-second integrations. Integrated readings for periods of 10 seconds or longer average the effects of rapidly changing light due to cloud cover, surface waves (for underwater readings) or sensor movement (which results in spatial variation). The LI-188 measures radiation levels ranging from 0.001 microeinsteins$\cdot m^{-2}\cdot sec^{-1}$ (= 0.0001 watts$\cdot m^{-2}$ = 0.01 lux) to greater than maximum daylight, with an accuracy of 0.5 percent. The instrument can be used to measure PAR both underwater and in the atmosphere.

II. In situ Light Measurements with LI-COR Model LI-188 Photometer

The quantum sensor for the LI-188 Photometer is suspended from a line calibrated in increments of 1.0 m to a depth below 1 percent of surface value. The 1 percent light level (i.e. the depth at which radiation equals 1 percent of the surface value) can then be determined. Data are reported in microeinsteins$\cdot m^{-2}\cdot sec^{-1}$ ($\mu\varepsilon\cdot m^{-2}\cdot sec^{-1}$).

For specific operating instructions, see instruction manual.

APPENDIX

CONVERSION FACTORS FOR RADIATION MEASUREMENTS

1. Incident photon - flux density

1 microeinstein m^{-2} sec^{-1}	= 1 micromol m^{-2} sec^{-1}
	= 6.02×10^{17} quanta m^{-2} sec^{-1}
	= 6.02×10^{17} photons m^{-2} sec^{-1}

2. Irradiance

1 watt m^{-2}	= 1.433×10^{-3} cal cm^{-2} min^{-1}
	= 1.433×10^{-3} langley min^{-1}
	= 0.100 mW cm^{-2}
	= 1000 erg cm^{-2} sec^{-1}
	= 5.285×10^{-3} BTU ft^{-2} min^{-1}
	= 0.317 BTU ft^{-2} hr^{-1}

3. Radiant exposure

1 watt-hour m^{-2}	= 0.0860 cal cm^{-2}
	= 0.0860 langley
	= 3600 joule m^{-2}
	= 3.600×10^{6} erg cm^{-2}
	= 0.317 BTU ft^{-2}

4. Illumination

1 lux	= 1 lumen m^{-2}
	= .0929 lumen ft^{-2}
	= .0929 ft-candle
	= 1×10^{-4} phot

5. Luminous exposure

1 lux-hr	= 3600 lux-sec
	= 0.0929 ft-candle-hr

REFERENCES

Instruction manual for operation of LI-COR Model LI-188 Integrating Quantum/Radiometer/Photometer. Brochure A-878. LI-COR, Inc. Lincoln, Nebraska, U.S.A.

Procedure for Assessing Water Transparency

Secchi depth measurements depend on surface light and therefore, measure light attenuation only in the surface waters. The transmissometer has its own light source, which is unaffected by surface light; therefore transparency can be measured at any depth. Secchi depth and transmissometer measurements of water transparency are not directly comparable.

1. The secchi disk

   The secchi disk is a flat white metal disk approximately 20 cm in diameter which is suspended from a line calibrated in 0.25-meter increments. The measurement is conducted by lowering the disk into the water on the shaded side of the boat. The depth at which the disk just disappears is noted. It is then raised until it is just visible and depth is again noted. This procedure is repeated once or twice. Depths are then averaged, and reported to the nearest 0.25 m.

2. Transmissometer: Martek Model XMS

   The transmissometer measures the transmission of light and its degree of attenuation by both particulate and dissolved material. The instrument has its own light source and sensor, with a one-meter light path. It is calibrated in air at each sampling site by setting percent transmission to 85.5% as directed in the manual. The transmissometer is suspended from a cable, calibrated in one-meter increments, and measurements are made from the surface to within 0.5 m of bottom. Data are reported as percent transmission (0-100%). Accuracy is within ± 1.0 percent.

REFERENCES

Instruction manual. Martek model XMS in situ Transmissometer. Martek Instruments. Newport Beach, California, U.S.A.

APPENDIX B

Other UNLV Technical Reports

Lake Mead Water Quality History
Limnological Monitoring Program (1979)
Limnological Monitoring Program (1980)
Periphyton Study (1979)
Periphyton Study (1979 to 1980)
Intensive Surveys (1979 to 1980)

LAKE MEAD WATER QUALITY HISTORY

LAKE MEAD WATER QUALITY HISTORY

TECHNICAL REPORT NO. 4

Submitted to

BROWN and CALDWELL

by

John R. Baker and Larry J. Paulson
Lake Mead Limnological Research Center
University of Nevada, Las Vegas

November 1980

Introduction

Concern over the water quality in Lake Mead was first expressed in the late 1960's. At that time increased algal growth accompanied by low water transparency was noted in Las Vegas Bay. A newspaper article entitled "A Story of Pollution and Neglect" (Vincent 1967) revealed the public concern at that time stating that:

> "There is a cancer in Lake Mead. A foul green-brown growth which is eating its way through Las Vegas Wash toward the deep, blue waters of Boulder Basin, the very heart of one of the finest bodies of water in the country".....

The increased algal growth in Las Vegas Bay was attributed to high nutrient loading from Las Vegas Wash (FWPCA 1967) containing secondary treated sewage effluent from the Clark County and Las Vegas sewage treatment plants, and industrial effluent from Basic Management Industries (BMI) in Henderson.

Water Quality in Las Vegas Bay

A report by the Federal Water Pollution Control Administration (FWPCA 1967) was the first to present data documenting the higher algal growth in Las Vegas Bay as a result of the high nutrient loading from Las Vegas Wash. This was followed by a number of short term investigations which substantiated these findings (Hoffman, Tramutt and Heller 1967; FWQA 1970; EPA 1971; Hoffman, Tramutt and Heller 1971). Long term data collected by the University of Nevada, Las Vegas (Deacon and Tew 1973, Deacon 1975, 1976, 1977) added to these findings showing seasonal and yearly variations in algal growth in Las Vegas Bay. Deacon (1975, 1976) noted an improvement in water quality in Las Vegas Bay with a continual decrease in chlorophyll from 1972-1976. This was again shown in a summary

report by Goldman and Deacon (1978) which included data from Hoffman et al. (1971).

The decrease in chlorophyll in the inner bay was not related to a reduction in nutrient loading from Las Vegas Wash. Based on U.S. Geological Survey data collected at Pabco Road, sewage effluent discharges increased from 50-70 $ft^3 \cdot sec^{-1}$ over this period (Fig. 1) thus increasing nutrient loading to Las Vegas Wash. Goldman and Deacon (1978) summarized all the available nutrient data at North Shore Road and found that nitrogen loading (in all forms) did not change appreciably and that phosphorus only increased slightly over the period of 1970 to 1977. It is interesting to note that although there was a substantial increase in sewage effluent discharge into Las Vegas Wash, there was only a slight increase in the nutrient load at North Shore Road. This was due to the nutrient stripping capability of the wash which has been reported by FWPCA (1967) and Goldman and Deacon (1978). The factors resulting in the apparent decline in chlorophyll in the inner bay have not been adequately defined, but it was probably related to a decrease in nutrient loading from the Colorado River and/or to increasing water levels in Lake Mead during this time period.

Water Quality in Lake Mead

There have been two major changes or continuation of events which have had, or may have had, an effect on the water quality in Lake Mead and Las Vegas Bay. These were the construction of Glen Canyon Dam forming Lake Powell on the Colorado River upstream of Lake Mead in 1963, and a continual increase in water levels in Lake Mead after the initial filling

of Lake Powell.

Physical and chemical characteristics of the Colorado River inflow to Lake Mead were altered as a result of the construction of Glen Canyon Dam (Paulson and Baker 1980). Seasonal fluctuations in the Colorado inflow were stabilized as were seasonal fluctuations in water temperatures with higher winter and lower summer water temperatures. Suspended sediment loads to Lake Mead were also reduced. These physical changes in the Colorado inflow altered the circulation patterns in Lake Mead and completely eliminated the spring overflow of the Colorado River water in Lake Mead. Furthermore, a large percentage of the Colorado River nutrient loads are now being retained in Lake Powell reducing the nutrient load to Lake Mead. These alterations have resulted in an apparent overall reduction in the productivity of Lake Mead (Paulson and Baker 1980).

Also, related to the formation of Lake Powell, was a dramatic reduction in Lake Mead water levels (Fig. 2). Subsequent to the filling of Lake Powell, water levels in Lake Mead continually increased up until 1979 when the lake reached full capacity. The extremely low water levels in the 1960's may have aggravated water quality conditions in Las Vegas Bay and Boulder Basin because of the reduced volume of water in Boulder Basin to dilute the high nutrient load from Las Vegas Wash. As water levels increased from 1964-79, there was an increasingly greater volume of water in Boulder Basin and therefore, a greater dilution of the nutrient load from Las Vegas Wash. This also would tend to reduce algae growth and productivity in Las Vegas Bay and Boulder Basin. The combined effects of reduced nutrient loading from the Colorado River and increasing water

levels in Lake Mead have probably resulted in the decline in chlorophyll noted by Deacon (1975, 1976).

Water Quality Conditions, 1977-78 and 1979-80

Phosphorus removal at the sewage treatment plants and alterations in the Las Vegas Wash system have changed the nutrient load to Las Vegas Bay. Comparing nutrient loading at North Shore Road from September-October 1977-78 and 1979-80 we see that there was a reduction in total phosphorus loading with ortho-phosphorus loading remaining at the same level for both time periods (Table 1). There was also a reduction in nitrate loading, but ammonia loading more than doubled resulting in an overall increase in the total inorganic nitrogen load. The increase in ammonia loading was apparently due to a breakdown in the stripping capacity of the Las Vegas Wash system which had previously reduced ammonia concentrations to low levels by the time the effluent reached North Shore Road (FWPCA 1967, Goldman and Deacon 1978). The actual mechanism within the wash that reduced ammonia has not been completely determined at this time.

The change in nutrient loading from Las Vegas Wash was reflected in nutrient concentrations in the inner bay. At Station 3, mean annual ortho-phosphorus concentrations were lower and ammonia concentrations were higher in 1979-80 compared to 1977-78 (Table 2). The annual average N:P ratio increased from 4.7 (1977-78) to 13.5 (1979-80) and indicated a potentially shorter supply of phosphorus for algal growth than in previous years. Although phosphorus concentrations were reduced, chlorophyll concentrations did increase from 9 $\mu g \cdot l^{-1}$ (1977-78) to 16 $\mu g \cdot l^{-1}$ (1979-80). Monthly average chlorophyll and inorganic nutrient concentrations at

Station 3 are shown in Figure 3. Seasonly, chlorophyll was higher in 1979-80 except for the winter period. Nitrate was generally lower except for slightly higher levels in May and June, 1980. Ammonia did not show any type of seasonal pattern for either period but the peaks were much higher in 1979-80. There are any number of factors which may have resulted in the higher chlorophyll concentrations in 1979-80. If it were due to nutrients, all indications would point to the higher ammonia concentrations. The fact that there was higher chlorophyll and lower phosphorus concentrations adds support to the inner bay being nitrogen limited.

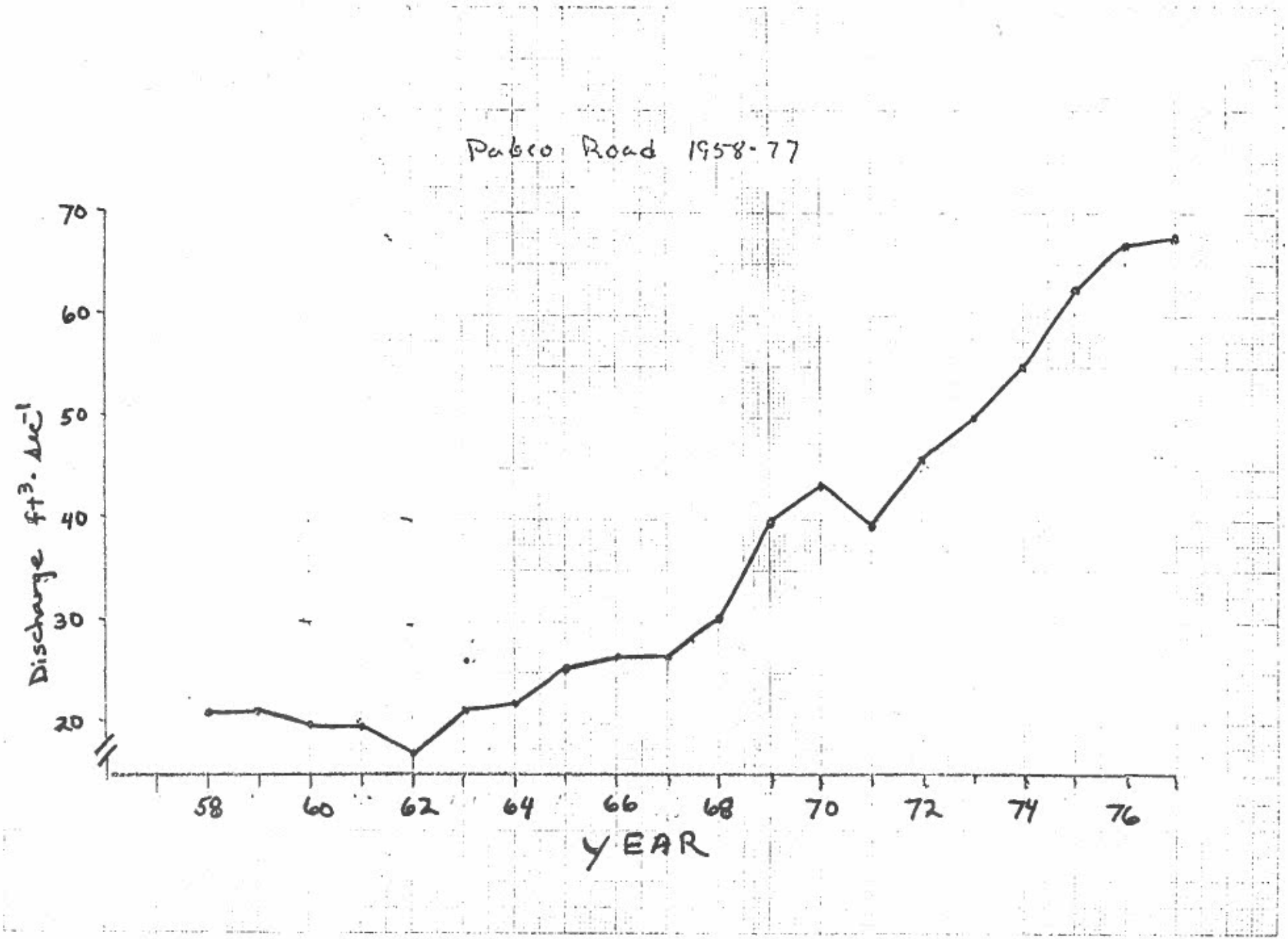


Figure 1. Mean annual discharge at Pabco Road, 1958-77.

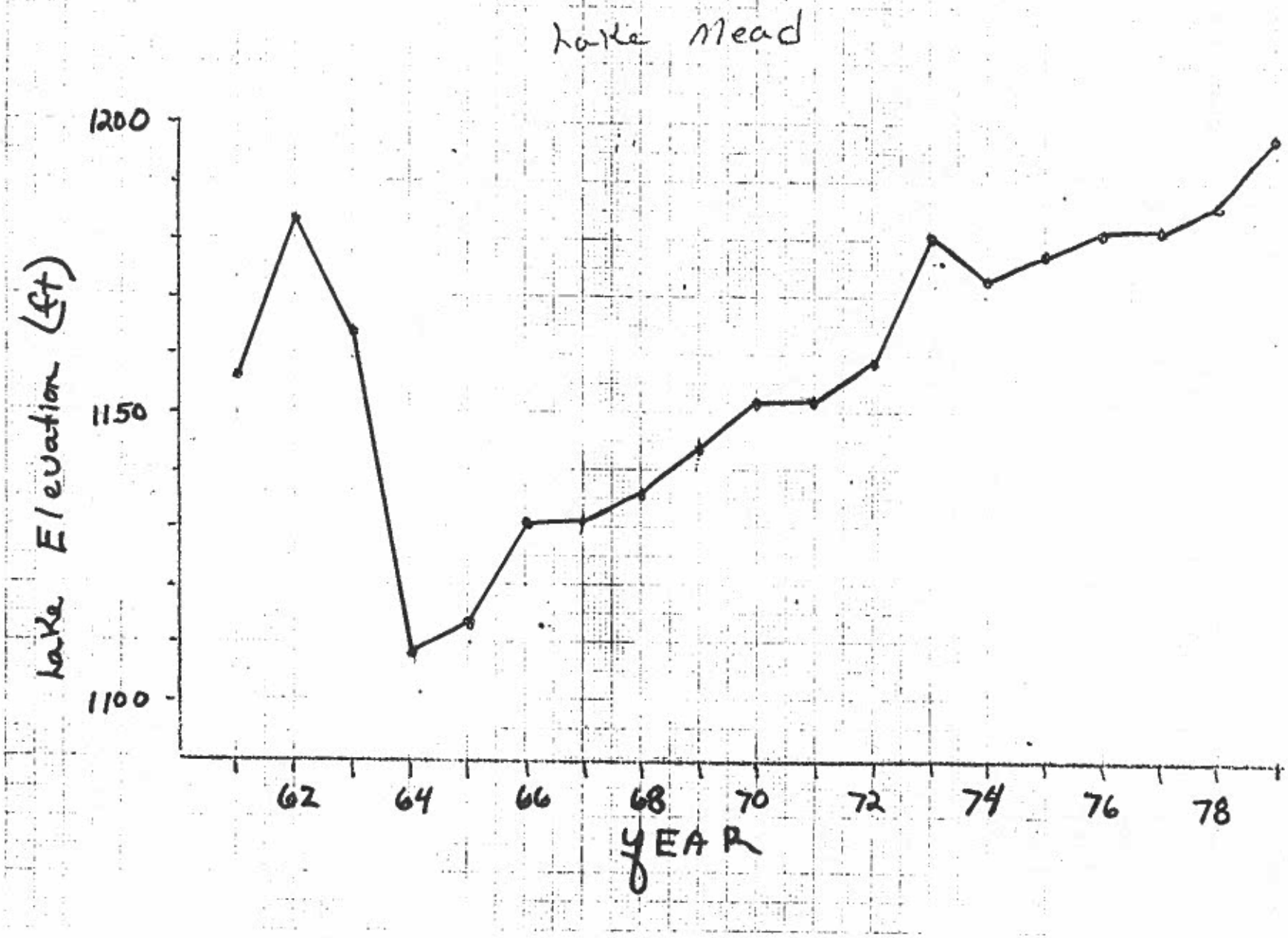


Figure 2. Lake Mead water elevations, 1961-79.

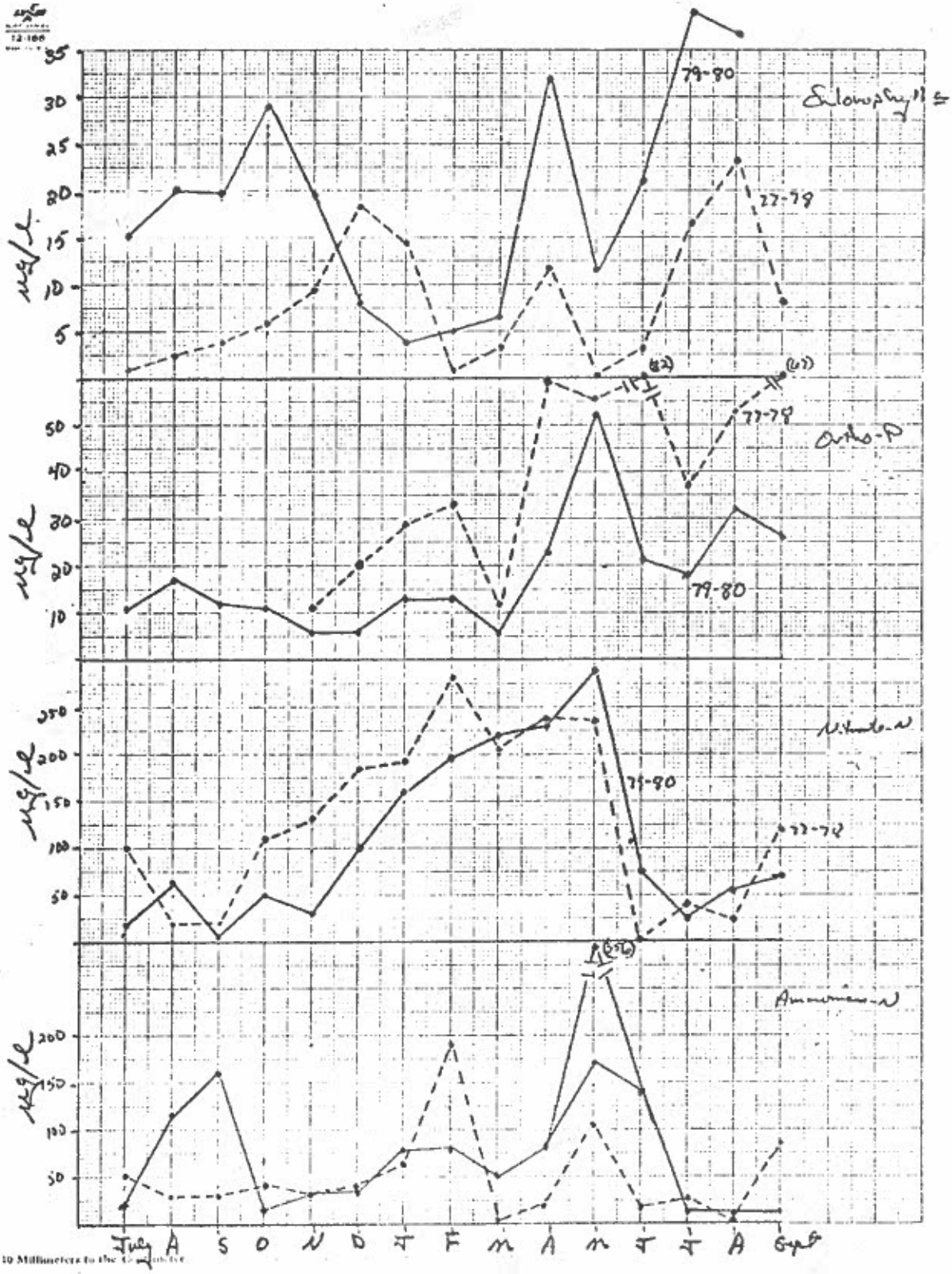


Figure 3. Monthly chlorophyll-a and nutrient concentrations at Station 3. Las Vegas Bay 1977-78 and 1979-80.

Table 1. Las Vegas Wash phosphorus and nitrogen loading at North Shore Road. Measurements from September - October.

	1977-78 $Kg \cdot yr^{-1}$	1979-80 $Kg \cdot yr^{-1}$
Total phosphorus	2.63×10^5	2.15×10^5
Ortho-phosphorus	1.37×10^5	1.37×10^5
Nitrate Nitrogen	3.49×10^5	2.70×10^5
Annonia Nitrogen	3.24×10^5	7.09×10^5
Total Inorganic Nitrogen	6.73×10^5	9.79×10^5

Table 2. Average annual nutrient and chlorophyll-a concentrations in the inner bay (Station 3) 1977-78 and 1979-80.

	1977-78* $\mu g \cdot L^{-1}$	1979-89** $\mu g \cdot L^{-1}$
Ortho-phosphorus	42	16
Nitrate nitrogen	147	120
Ammonia Nitrogen	51	96
Inorganic N:P Ratio	4.7	13.5
Chlorophyll-a	9	16

* Determined from monthly measurements October 1977-September 1978

** Determined from average monthly measurements July 1979-June 1980

REFERENCES

Deacon, J.E. 1975. Lake Mead monitoring program. Final Report to Clark County Sanitation District No. 1, Waste Treatment Physical Development Section. Univ. of Nevada, Las Vegas. 207 pp.

Deacon, J.E. 1976. Lake Mead monitoring program. Final Report to Clark County Sanitation District No. 1, Waste Treatment Physical Development Section. Univ. of Nevada, Las Vegas. 182 pp.

Deacon, J.E. 1977. Lake Mead monitoring program. Final Report to Clark County Sanitation District No. 1, Waste Treatment Physical Development Section. Univ. of Nevada, Las Vegas. 55 pp.

Deacon, J.E. and R.W. Tew. 1973. Interrelationships between chemical, physical and biological conditions of the waters of Las Vegas Bay and Lake Mead. Univ. of Nevada, Las Vegas. 186 pp.

EPA. 1971. Report on pollution affecting Las Vegas Wash, Lake Mead and the lower Colorado River. U.S. Environmental Protection Agency, Office of Enforcement, Division of Field Investigations, Denver. 52 pp.

FWPCA. 1967. Report on pollution in Las Vegas Wash and Las Vegas Bay. U.S. Department of the Interior, Federal Water Pollution Control Administration. 19 pp.

FWQA. 1970. Analysis of algal growth potential and possible discharge requirements for the lower Colorado River. Federal Water Quality Administration, Tech. Asst. Rept. to Nevada Dept. of Health, Welfare and Rehabilitation. 46 pp.

Hoffman, D.A., P.R. Tramutt and F.C. Heller. 1967. Water quality study of Lake Mead. U.S. Department of the Interior, Bureau of Reclamation Report No. CHE-70. 151 pp.

Hoffman, D.A., P.R. Tramutt and F.C. Heller. 1971. The effect of Las Vegas Wash effluent upon the water quality in Lake Mead. U.S. Department of the Interior, Bureau of Reclamation, REC-ERC-71-11. 22 pp.

Goldman, C.R. and J.E. Deacon. 1978. Recommended Water Quality Standards for Las Vegas Bay and Las Vegas Wash. Report to the Nevada Environmental Commission. 42 pp.

Paulson, L.J. and J.R. Baker. 1980. Nutrient interactions among reservoirs on the Colorado River. Symposium on Surface Water Impoundments, June 2-5, 1980. Minneapolis, Minnesota. (in press).

Vincent, B. 1967. Lake Mead, a Story of Pollution and Neglect. The Nevadan, 12 November: 4-5.

LIMNOLOGICAL MONITORING PROGRAM (1979)

LIMNOLOGICAL MONITORING PROGRAM (1979)

John R. Baker and Larry J. Paulson

INTRODUCTION

The primary objective of the routine limnological monitoring program was to develop a comprehensive set of limnological data on Lake Mead. The routine limnological monitoring program is part of the Water Quality Standards Study (WQSS) designed to answer specific questions set forth in the Consent Decree. Data from the monitoring program will be used to: identify current water quality conditions, identify any problems that may exist, and determine the cause of those problems. Because of the scientific and economic decisions that will have to be made from this data set, a Technical Advisory Board (TAB), made up of leading scientists in limnology and sanitary engineering, was established. It was the function of the TAB to design the monitoring program, such that the methodology used and the data collected would answer the specific questions in the Consent Decree, and as far as possible, be beyond scientific criticism. After a lengthy review of the monitoring program by all parties involved in the Consent Decree, the program was started in July 1979.

SAMPLING AND ANALYTICAL PROCEDURES

A total of 11 stations were established in Lake Mead with 7 stations in Las Vegas Bay, 1 in Boulder Basin, 1 in Virgin Basin, and 2 in Bonelli Bay. Parameters measured at each of the stations in 1979 are given in Table 1.

Table 1. Parameters measured in the Routine Monitoring Program.

Parameter	Sampling Depth	Sampling Stations
Physical		
Secchi depth	-	All
Light Transmittance	1 m intervals	All
Temperature	1 - 5 m intervals	All
Dissolved oxygen	1 - 5 m intervals	All
Specific conductance	1 - 5 m intervals	All
pH	1 - 5 m intervals	All
Color	Intergrated	All
Total suspended solids	Intergrated	Index*
Volatile suspended solids	Intergrated	Index
Chemical		
Total phosphorus	Int, 25,40,70 m	All
Dissolved phosphorus	Int, 25,40,70 m	All
Orthophosphate	Int, 25,40,70 m	All
Ammonia	Int, 25,40,70 m	All
Nitrate + Nitrite	Int, 25,40,70 m	All
Nitrite	Int, 25,40,70 m	All
Total nitrogen	Int, 25,40,70 m	Index, 1 + 3
Soluble iron	Int, 25,40,70 m	Index
Silica	Int, 25,40,70 m	Index
Biological		
Chlorophyll a	Intergrated	All
Pheophytin a	Intergrated	All
Phytoplankton	Intergrated	All
Zooplankton	Vertical Tow	Index
Fish distribution	-	Index
Physiological		
Nitrogen fixation	Intergrated	Index
Dark ammonia uptake	0,3,5 m	Index
Alkaline phosphatase	Intergrated	Index
Extractable phosphorus	Intergrated	~~Index~~ 1
Primary productivity	0-25 m	Index

*Stations 2,5,8,9, and 10

Physical, chemical and biological parameters were measured weekly in July - October, biweekly in November and monthly in December. Physiological parameters were measured biweekly in July - November and monthly in December. Analytical procedures are summarized in Table 2 and will be described in detail in a subsequent technical report.

Some additional tests and changes in the chemistry procedures have been made as follows:

1. Total dissolved iron samples are being filtered upon arrival from the field before the addition of HCl and freezing. This eliminates the possibility of acid release of particulate iron. This change was made starting on samples collected 23 October 1979.
2. A test was conducted on unfiltered, frozen samples and pre-filtered, frozen samples for any change in ortho-phosphate concentration. No difference was detected so we are not prefiltering these samples.
3. A test was conducted on Lake Mead samples to determine the difference between total hydrolyzable phosphorus and total persulfate digestible phosphorus. There was no significant difference so we have continued to use the total hydrolyzable method.
4. Dr. Shapiro suggested using the silica method in Standard Methods, 14th edition. Therefore, as of 1 August 1979, we are no longer using the USGS Method.
5. After extensive testing and several modifications of the total nitrogen method we could not adapt it to our existing nitrate method. Therefore, we have started using the cadmium column nitrate method in determining total nitrogen. This change was made on samples

collected after 25 February 1980.

6. As of 5 September 1979, color determinations are read at a wavelength of 350 nm rather than 487 nm as suggested by Dr. Shapiro.

7. Data reported as NO_3 are actually NO_3+NO_2.

DISCUSSION

Most of the summary data available to us was discussed in detail in the Quarterly Data Review. Chemical, physical and biological conditions in 1979 were similar to what we have observed in the past and are briefly discussed here. A complete discussion on past limnological conditions in Lake Mead is available in our recent report to the Water and Power Resources Service (Paulson, Baker and Deacon 1980).

Thermal Stratification

Water temperatures in March were nearly isothermal at 10°-12°C. Thermal stratification started to develop by the end of May when surface temperatures were 20°-23°C. In July, stratification was well developed at 5-15 m. Surface temperatures at this time were 27°-28°C. Fall overturn started in October with mixing to a depth of about 17 m. The lake was completely destratified by late December.

Oxygen Depletion

Metalimnetic oxygen depletion was first evident in July and there was a progressive depletion throughout the stratified period with the lowest oxygen concentrations occuring in the fall (Table 3). All of the stations had minimum oxygen concentrations of less than 3 $mg \cdot l^{-1}$ except Virgin Basin (Station 9) with a minimum of 4.55 $mg \cdot l^{-1}$. Metalimnetic oxygen minimums were similar in

Table 2. Analytical procedures used for nutrient determinations.

Parameter	Method
Total Phosphorus	Ascorbic acid
Dissolved Phospohorus	Ascorbic acid
Orthophosphate	Ascorbic acid
Extractable phosphorus	Tris buffer
Ammonia	Phenolhypochlorite
Nitrite	Sulfanilamide
Nitrate	Hydrazine reduction
Total nitrogen	Cadmium reduction
Soluble iron	Ferrozine
Silica	Ammonium molybdate

Table 3. Minimum oxygen concentrations in Lake Mead, 1979.

Station	1979 Minimum $mg \cdot l^{-1}$	1979 Depth m	1979 Date	1978 Minimum $mg \cdot l^{-1}$	1978 Depth m	1978 Date
1	5.85	2	7 Aug		!	
2	6.91	8.5	25 July			
3	0.05	16.5	28 Aug	0.6	12.5	24 July
4	0.00	28	26 Sept.			
5	0.10	35	16 Oct	.10	35	22 Sept
6	1.6	22	25 Oct			
7	2.5	27.5	25 Oct	1.7	30	23 Sept
8	2.8	30	16 Oct	2.6	28	3 Nov
9	4.55	40	16 Oct	5.20	43	29 Nov
10	2.8	45.5	10 Oct			
11	2.1	21	11 Oct			

1978 and 1979 (Table 3).

Nutrients

Phosphorus and nitrogen concentrations are summarized in Table 4. The high nutrient loading from Las Vegas Wash was very evident with high phosphorus and nitrogen concentrations in the inner bay (Stations 1-3). Phosphorus concentrations were extremely low in the upper basin (Stations 9-10) as a result of low phosphorus loading from the Colorado River (Paulson, et. a. 1980). Epilimnetic inorganic nitrogen concentrations in Las Vegas Bay and Boulder Basin were at extemely low levels in the summer when phytoplankton growth was high. Nitrogen concentrations in the upper basin were higher than those in Boulder Basin (Station 8) due to lower phytoplankton production in the upper basin and to high nitrogen loading from the Colorado River (Paulson, et. al., 1980).

Chlorophyll

Maximum chlorophyll concentrations in Las Vegas Bay and Boulder Basin (Stations 3-8) were in late fall with spring-summer concentrations remaining relatively low (Table 5). At Stations 1 and 2, high chlorophyll peaks occured throughout the year with no real seasonal pattern. There was also no seasonal pattern in the upper basin (Station 9-11) which had very low chlorophyll (0-3 $Mg \cdot l^{-1}$). Chlorophyll concentrations appear to be slightly higher in 1979 than those found in 1978 (Table 5).

Physiological Parameter

Nitrogen fixation - There was no nitrogen fixation during 1979.

Dark ammonia uptake - Ammonia addition did not enhance ^{14}C uptake at any time. The validity of the test for nitrogen limitation is questionable.

Alkaline phosphatase activity - APA showed a steady increase in activity from Inner Las Vegas Bay to the upper Basin reflecting the greater phosphorus limitation in the upper Basin.

Table 4 Phosphorus and nitrogen concentrations ($\mu g \cdot l^{-1}$) in Lake Mead, 1979

Station		Total Nitrogen	Inorganic Nitrogen	Total Phosphorus	Dissolved Phosphorus
1	Mean	380.8	115.8	98.2	68.1
	Range	241-868	9-203	38-426	17-339
2	Mean	231.4	181	42.0	25.9
	Range	143-368	9-1108	27-115	10-64
3	Mean	174.7	140.8	27.3	16.5
	Range	134-263	15-674	15-40	8-27
5	Mean	128.8	25.7	11.9	6.9
	Range	93-181	7-77	7-21	4-11
8	Mean	106.8	23.7	6.8	3.6
	Range	86-155	5-92	4-12	1-6
9	Mean	145.5	82.4	4.1	2.3
	Range	55-230	24-202	1-8	1-4
10	Mean	151.6	75.0	4.6	3.1
	Range	106-197	18-189	2-9	1-4

Table 5. Maximum chlorophyll concentrations in Lake Mead, 1979.

Station	1979 Maximum $\mu g \cdot l^{-1}$	1979 Date	1978 Maximum $\mu g \cdot l^{-1}$	1978 Date
1	386	25 July		
2	88	25 July		
3	29	25 July/ 10 Sept	26	2 July
4	20	29 Nov		
5	24	31 Oct		
6	13	29 Nov		
7	13	29 Nov	8	22 Aug
8	12	29 Nov	6	22 Aug
9	2	*	2	25 Sept
10	2	*		
11	3	27 Sept		

*Concentrations ranged from 1-2 $\mu g \cdot l^{-1}$ throughout the year

CHLOROPHYLL
PEAK conc.
386 ug/l
station 1

Primary productivity - Data reduction has not been completed.

RECOMMENDATIONS

We were involved in the January Quarterly Reviews meeting and made recommendations on program changes at that time. The following recommendations were made at that meeting and have subsequently been incorporated into the program:

1. Particulate nitrogen and phosphorus should be measured periodically at a number of selected stations.
2. Dark ammonia uptake should be discontinued except for one run in the spring when there was an apparent algal bloom.
3. Continue total and volatile suspended solids measurements with the addition of Stations 1 and 3.
4. A minimum number of stations are now being sampled and none of the sampling stations should be discontinued.
5. A 2.5 meter integrated sample should be collected at Stations 2 and 3 in addition to the 5 meter intergrated sample.
6. An additional water sample for nutrient analyses should be taken at Stations 3, 4 and 5 at the depth of the highest conductivity.
7. The intensive survey should be continued with the January survey being postponed until April.
8. The diurnal survey should be assessed after one more additional survey.
9. A special study on the metalimnetic oxygen depletion should be conducted to determine the cause(s) for this unusual distribution of oxygen.

REFERENCES CITED

Paulson, L.J., J.R. Baker and J.E. Deacon. 1980. The limnological status of Lake Mead and Lake Mohave under present and future powerplant operations of Hoover Dam. Lake Mead Limnological Research Center, Univ. of Nev. Las Vegas. Tech. Rept. No.1. 229 p.

LIMNOLOGICAL MONITORING PROGRAM (1980)

LIMNOLOGICAL MONITORING PROGRAM (1980)

John R. Baker and Larry J. Paulson

INTRODUCTION

The limnological monitoring program was designed to develop a comprehensive set of data on water quality conditions in Lake Mead. An intensive sampling program was established in Las Vegas Bay and Boulder Basin to evaluate and quantify the effects of sewage effluent discharged from Las Vegas Wash. Data were also collected from Virgin Basin and Bonelli Bay to establish background conditions without sewage nutrient enrichment. The program was started in July 1979. This report is a summary of data from 1980 (January-November).

SAMPLING AND ANALYTICAL PROCEDURES

A total of 11 stations was sampled in Lake Mead with 7 stations in Las Vegas Bay, 1 station in Boulder Basin, 1 station in Virgin Basin and 2 stations in Bonelli Bay. Parameters measured at each of the stations are given in Table 1. Physical, chemical and biological parameters were measured weekly during April-October, biweekly in March and November and monthly in January-February. Physiological parameters were measured biweekly in March-November and monthly in January-February. Analytical procedures are described in detail in Kellar, Paulson, and Paulson (1980).

Table 1. Parameters measured in the Routine Monitoring Program.

Parameter	Sampling Depth	Sampling Station
Physical		
Secchi depth	--	All
Light Transmittance	1 m intervals	All
Temperature	1 - 5 m intervals	All
Dissolved oxygen	1 - 5 m intervals	All
Specific conductance	1 - 5 m intervals	All
pH	1 - 5 m intervals	All
Total suspended solids	Intergrated	Index*
Volatile suspended solids	Intergrated	Index
Chemical		
Total phosphorus	Int, 25,40,70 m	All
Dissolved phosphorus	Int, 25,40,70 m	All
Orthophosphate	Int, 25,40,70 m	All
Ammonia	Int, 25,40,70 m	All
Nitrate + Nitrite	Int, 25,40,70 m	All
Nitrite	Int, 25,40,70 m	All
Total nitrogen	Int, 25,40,70 m	Index 1 + 3
Particulate phosphorus	Int	2.5 + 8
Particulate nitrogen	Int	2.5 + 8
Soluble iron	Int, 25,40,70 m	Index
Silica	Int, 25,40,70 m	Index
Biological		
Chlorophyll a	Intergrated	All
Pheophytin a	Intergrated	All
Phytoplankton	Intergrated	All
Zooplankton	Vertical Tow	Index
Fish distribution	---	Index
Physiological		
Nitrogen fixation	Intergrated	Index
Alkaline phosphatase	0,3,5 m	Index
Extractable phosphorus	Intergrated	Index 1
Primary productivity	0-25 m	Index

* Stations 2,5,8,9, and 10

DISCUSSION

Thermal Stratification

The temperature structure in Lake Mead during 1980 was similar to that observed in the past. Thermal stratification developed in June when surface temperatures were approximately 23° C. In July-September thermal stratification was well developed with the thermocline located at 10-15m. Maximum epilimnetic water temperatures occurred in late July ranging from 28.7-30.5°C and were 1-2°C higher than 1979 (Table 2). The highest water temperatures occurred in Las Vegas Bay probably because of advective heating from the Las Vegas Wash inflow. The lake started to destratify in October with mixing occurring to a depth of 30 m and epilimnetic temperatures of 18-19° C. Surface temperatures cooled to approximately 16° C by the end of November. Complete mixing of the lake probably did not occur in December or January because of unusually warm winter conditions.

Oxygen Depletion

As in previous years, oxygen depletion in the metalimnion occurred as thermal stratification developed. At the shallow stations in Las Vegas Bay (Stations 3-5) and in Bonelli Bay (Stations 10 and 11) the metalimnion intersected the bottom and a clinograde profile developed. A negative hetrograde profile occurred at the deeper stations. A progressive depletion of oxygen occurred throughout the stratified period with the lowest oxygen concentrations occurring in the fall. Minimum oxygen

Table 2. Average summer (July-September) epilimnetic (3M) temperatures in Lake Mead. Date of maximum temperature in parentheses.

Station	July-Sept. 1979	Maximum	July-Sept. 1980	Maximum
Sta 2	26.6	28.5(7/31)	27.3	30.5(7/30)
Sta 3	26.6	28.3(7/31)	27.3	30.4(7/30)
Sta 5	26.3	27.8(7/31)	27.0	29.9(7/30)
Sta 7	26.0	27.4(7/25)	26.4	28.4(7/30)
Sta 8	25.7	26.8(7/26)	26.1	28.7(7/30)
Sta 9	25.7	27.0(8/1)	26.2	28.8(7/31)
Sta 10	25.2	26.8(8/1)	25.8	28.9(7/31)
Sta 11	24.9	26.3(8/1)	25.7	28.7(7/31)

concentrations in 1980 were not as pronounced as in 1979. Again the most severe oxygen depletion occurred in Las Vegas Bay (Table 3).

Conductivity

The Las Vegas Wash inflow was evident in Las Vegas Bay by high conductivity readings because of the high TDS . In the winter the density current flowed along the bottom but, as thermal stratification developed, an interflow developed along the thermocline. The distribution of the density current has not changed appreciably and a complete description is given in Baker and Paulson 1980).

The Colorado River inflow was also evident throughout the Virgin and Boulder Basin as an interflow during the summer of 1980. Conductivity levels decreased from approximately 1100 to 900 $\mu mhos \cdot cm^{-1}$ between 10-30 m at stations 8 and 9. These low midwater conductivity levels did not occur in 1979. After making a number of vertical profiles uplake, it was determined that the areas of low conductivity were due to the Colorado River inflow.

Nutrients

Seasonal patterns and concentrations of phosphorus and nitrogen in 1980 were similar to those observed in the past. Gradients in both phosphorus and nitrogen occurred in Las Vegas Bay with concentrations decreasing from the inner to outer bay (Table 4). Phosphorus concentrations were high in Las Vegas Bay because of high phosphorus loading from Las Vegas Wash. With the

Table 3. Minimum oxygen concentration in Lake Mead, 1979 and 1980.

Station	1979	1980
2	6.91	0.30
3	0.05	0.09
4	0.00	0.31
5	0.10	0.51
6	1.60	2.75
7	2.50	3.02
8	2.8	3.76
9	4.55	4.81
10	2.80	3.81
11	2.10	5.18

Table 4. Phosphorus and nitrogen concentration ($\mu g \cdot l^{-1}$) in Lake Mead 1980.

	Station										
Parameter	1	2	3	4	5	6	7	8	9	10	11
Total-P	164	94	47	21	16	12	10	10	6	6	6
Ortho-P	114	33	24	8	6	4	2	3	1	1	2
Total-N	1414	799	516		364			338	382	362	
Nitrite + Nitrate-N	277	159	140	112	112	114	125	121	186	175	169
Ammonia-N	469	97	85	32	15	9	5	6	2	3	5
Jan-Nov TN:TP	8.6	8.5	12.2		22.8			33.8	63.7	60.3	

exception of the inner bay stations (1-3), total and nitrate nitrogen concentrations were generally higher in the upper basin because of the high nitrogen loading from the Colorado River (Paulson, Baker and Deacon 1980). Phytoplankton uptake depleted epilimnetic nitrate to nondetectable levels at stations 4-8 during July-September. In the upper basin nitrate concentrations remained relatively high (50-100 $\mu g \cdot l^{-1}$) but orthophosphorus was reduced to nondetectable levels during this period. These conditions were reflected in N:P ratios (Table 4). In Las Vegas Bay N:P ratios were low indicating the relative short supply of nitrogen whereas in the upper basin N:P ratios were high indicating a short supply of phosphorus.

Chlorophyll

Chlorophyll concentrations were high in the inner bay and progressively decreased in the middle and outer bay in relation to nutrient concentrations. In the inner bay concentrations were extremely variable from March-October due to numerous phytoplankton blooms (Fig. 1). Chlorophyll levels in the middle bay did increase in the spring (April-May) but as in previous years maximum concentrations occurred in late summer (August-September). Chlorophyll concentrations in Bonelli Bay and in the Virgin Basin were extremely low (usually not exceeding 2 $\mu g \cdot l^{-1}$) and showed no seasonal pattern (Fig. 1). Maximum chlorophyll concentrations in 1980 in the inner bay were generally higher than those found in 1979, whereas middle bay and Boulder Basin chlorophyll concentrations were lower (Table 5).

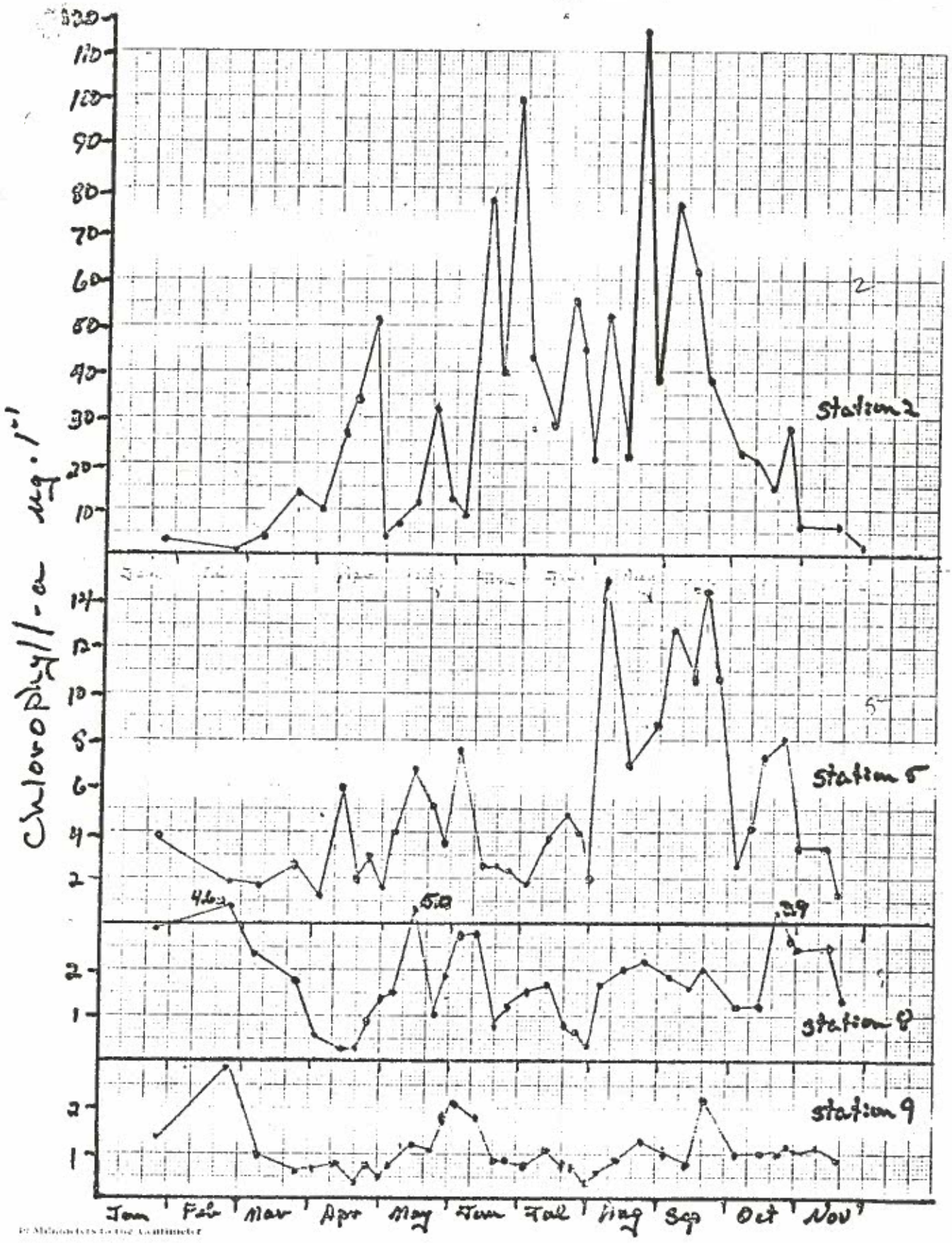


Figure 1. Chlorophyll-a concentration in Lake Mead 1980.

Table 5. Maximum chlorophyll-a concentration in Lake Mead 1979 and 1980.

Station	1979 $\mu g \cdot l^{-1}$	1980 $\mu g \cdot l^{-1}$
1	386	359
2	88	116
3	29	83
4	20	51
5	24	15
6	13	6
7	13	4
8	12	5
9	2	1
10	2	3
11	3	3

Zooplankton

Lake Mead zooplankton densities were highest in the inner Las Vegas Bay and decreased outward into Boulder Basin (Fig. 2). In general, zooplankton densities in Boulder Basin were higher than in Virgin Basin, primarily because of nutrient input from Las Vegas Wash.

Zooplankton densities throughout the lake were highest in spring and fall, except in the inner Las Vegas Bay, where high numbers of rotifers resulted in a summer maximum. Zooplankton densities throughout Lake Mead have gradually decreased since 1977-78 and have decreased by a factor of 10 since 1971 (Wilde 1981).

Alkaline Phosphatase Activity

Alkaline phosphatase activity (APA), measured at the five index stations in Lake Mead, consistently showed increases with distance from the Inner Las Vegas Bay. Patterns were generally similar to that shown in Figure 3. Levels of APA were highest during the periods from August-October 1979 and April-October 1980 at all stations, except for increases during December 1979-February 1980 in the Upper Basin. APA normalized for chlorophyll-a vs. orthophosphorus concentrations best fit a negative exponential curve for both growing seasons. As an indication of phosphorus deficiency, APA measurements generally agreed with conclusions drawn from nitrogen to phosphorus ratios.

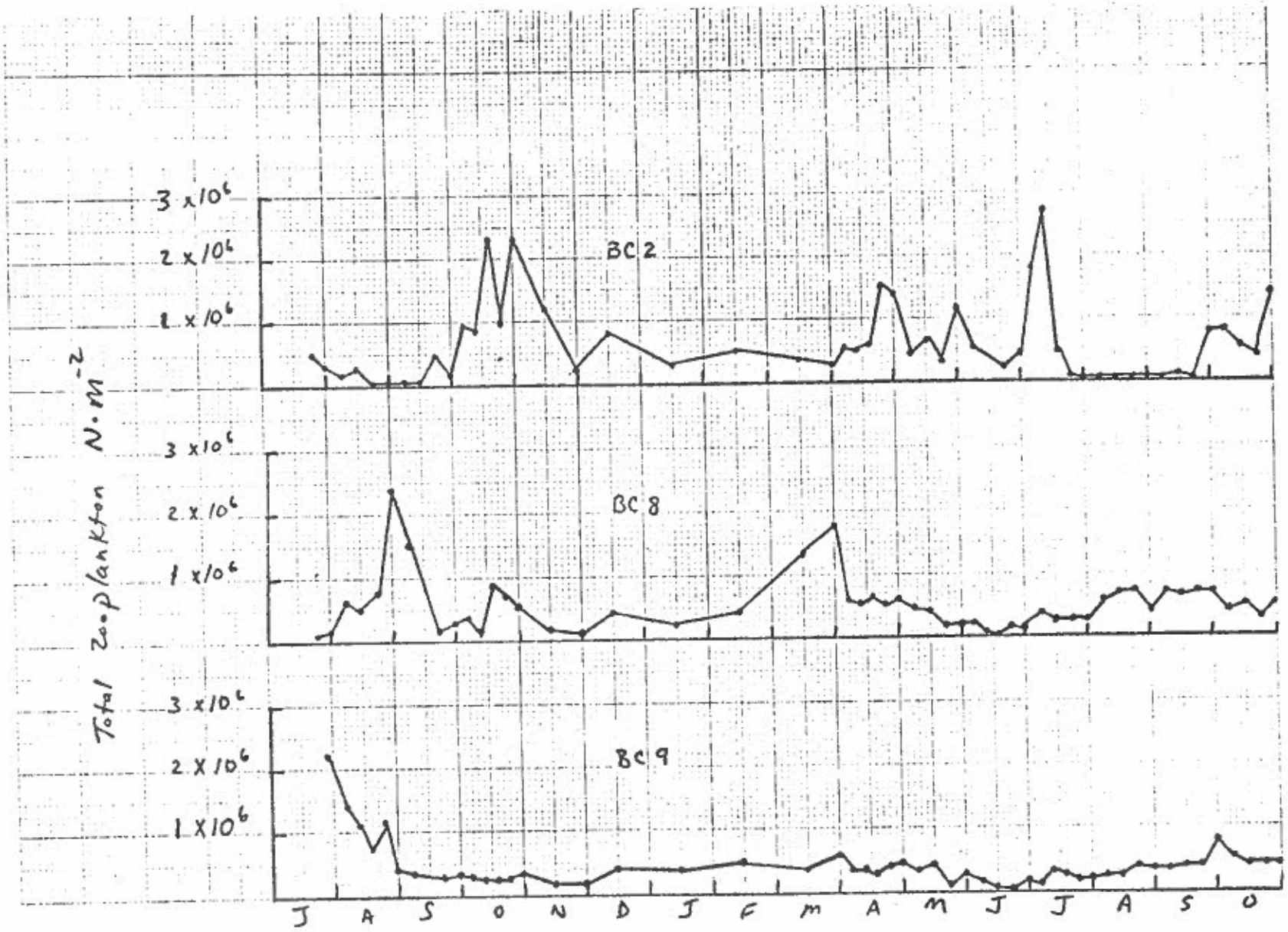


Figure 2. Seasonal zooplankton concentrations in Las Vegas Bay (Station 2) Boulder Basin (Station 8) and Virgin Basin (Station 9), 1979-1980.

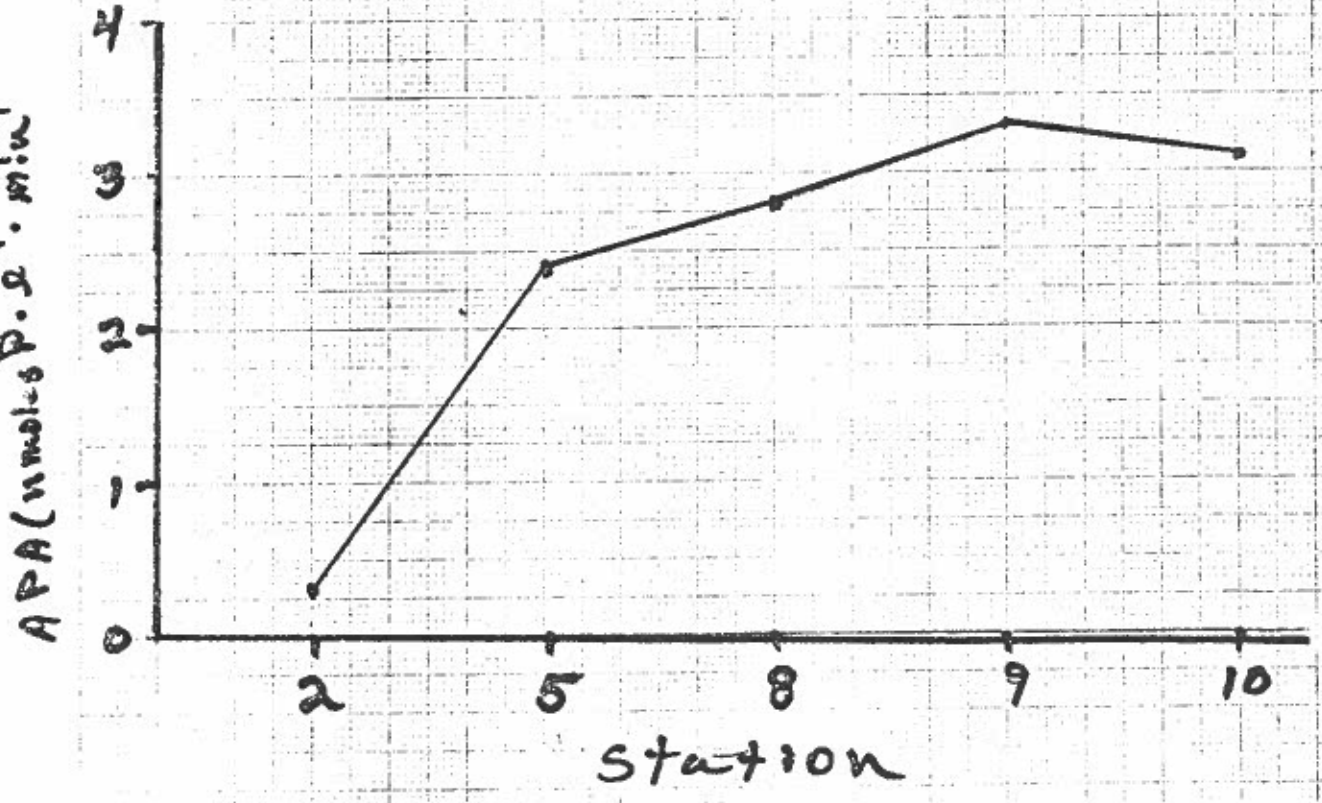


Figure 3. Alkaline phosphatase activity measured in Lake Mead, 11 September 1980.

Nitrogen Fixation

As in 1979, there was no measurable nitrogen fixation in Lake Mead during this time period.

Primary Productivity

Data reduction has not been completed.

Fish Distribution

Sonargrams taken from various areas of Lake Mead indicated that threadfin shad (Dorosoma petenense) populations dramatically decline over the past years. In 1975 large numbers of schools were detected in the epilimnion throughout Lake Mead during the stratified period (Fig. 4). In 1980 shad schools were still observed in the coves but the large number of schools in the limnetic areas were no longer present. The decline in the threadfin shad population corresponded with a rapidly increasing striped bass (Morone saxatilis) population and the decline was probably due to predation.

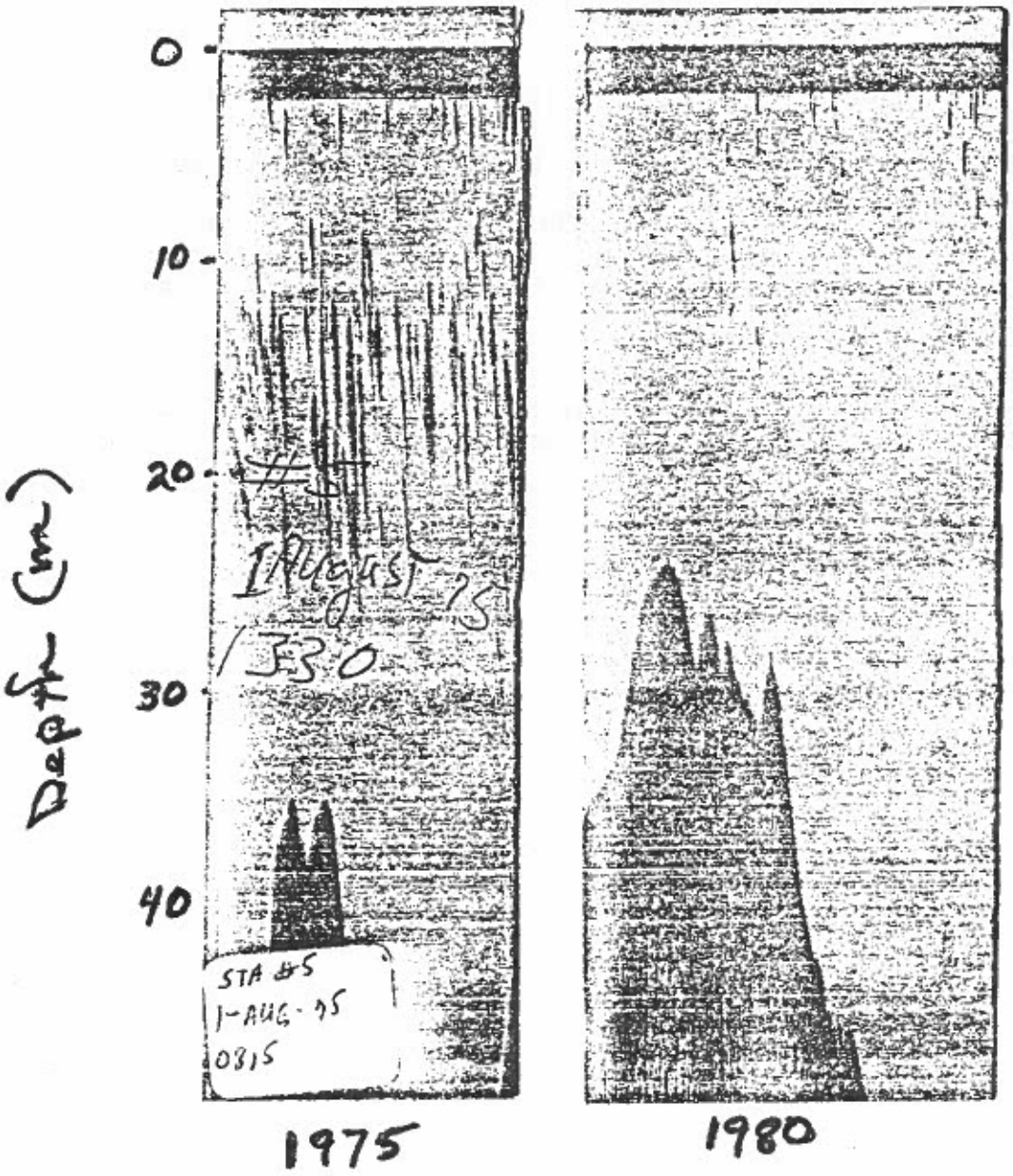


Figure 4. Sonargrams showing fish distribution in Boulder Basin, August, 1975 and 1980.

REFERENCES

Baker, J.R. and L.J. Paulson. 1980. Influence of Las Vegas Wash density current on nutrient availability and phytoplankton growth in Lake Mead, (in press). In Symposium on surface water impoundments. June 2-5, 1980. Minneapolis, Minn.

Kellar, P.E., S.A. Paulson and L.J. Paulson. 1980. Methods for biological, chemical and physical analyses in reservoirs. Tech. Rept. No. 5. Lake Mead Limnological Research Center, Univ. Nev., Las Vegas. 235 pp.

Paulson, L.J., J.R. Baker and J.E. Deacon 1980. The Limnological status of Lake Mead and Lake Mohave under present and future power plant operation of Hoover Dam. Tech. Rept. No. 1. Lake Mead Limnological Research Center, Univ. Nev., Las Vegas. 229 pp.

Wilde, G.R. (1981). Recent changes in zooplankton standing crop in Lake Mead and their probable effects on Largemouth bass fry. Ariz.-Nev. Acad. Sci. 25th Ann. Meet. May 1-2, 1981, Tucson, Ariz. (abstr.).

PERIPHYTON STUDY (1979)

PERIPHYTON STUDY (1979)

Larry J. Paulson and Marsha Morris

INTRODUCTION

Periphyton (attached algae) often form an important component of the productivity in lakes and reservoirs. Periphyton, like phytoplankton, are strongly influenced by nutrients. In water bodies that are subject to high nutrient loading periphyton growth can reach levels that impair recreational use, especially swimming.

Concern over periphyton growth in Las Vegas Bay, Lake Mead was first reported in 1967 (FWPCA 1967) when floating mats of Cladophora were sighted in the bay. Extensive growths of Cladophora were also observed in Las Vegas Wash, and it could not be determined whether those in the bay actually originated there or from inflows of Las Vegas Wash. Boat owners at Las Vegas Wash Marina have often complained about extensive algae growth on boat hulls, particularly during the early 1970's. As a result of these concerns, a periphyton study was initiated as part of the Water Quality Standards Study of Lake Mead. The objectives of the periphyton study as outlined in the work plan for the WQSS were to determine:

(i) periphyton growth (chlorophll-a, dry weight, ash free dry weight), species composition and alkaline phosphatase activity at two week intervals during the recreation season and monthly during the rest of the year on artificial substrates (fiberglass) installed at the index stations (2,5,8,9,10) and nearshore stations (lateral to the index stations) (Fig. 1).

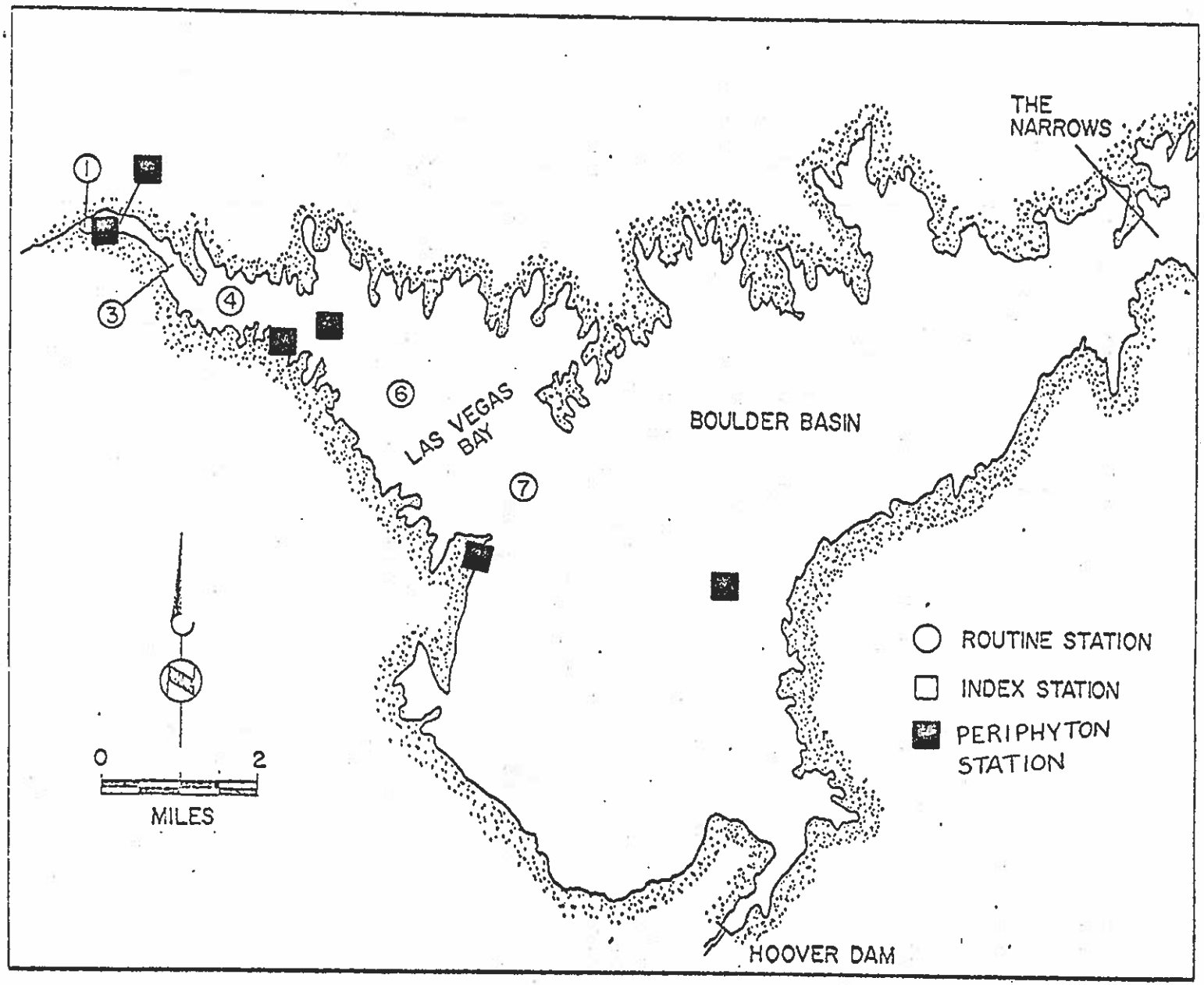


Figure 1. Location of Periphyton Samplers in the Lower Basin, Lake Mead

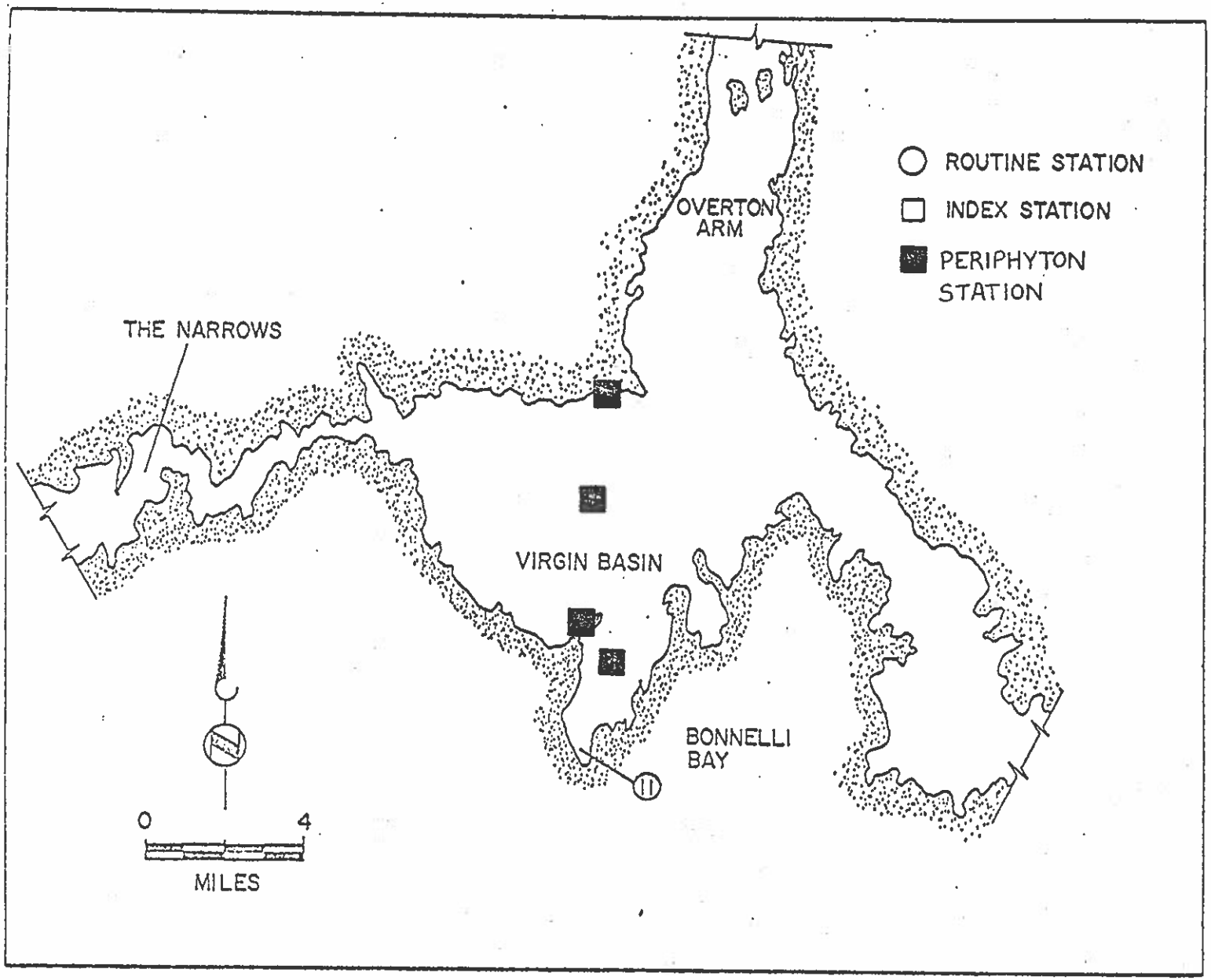


Figure 1. (cont.) Location of Periphyton samplers in the Upper Basin, Lake Mead

(ii) periphyton biomass (qualitative) and species composition at various locations in Las Vegas Bay, Bonelli Bay and other points in Lake Mead (Fig. 2) on a quarterly basis.

(iii) periphyton productivity and nitrogen fixation on natural substrates at nearshore stations in Lake Mead on a quarterly basis.

PERIPHYTON SAMPLING PROCEDURES

The littoral and limnetic artificial samplers were collected by divers at approximately two-week intervals in September and October, and monthly in November and December 1979 (Table 1).

Table 1. Periphyton Sampling Dates

September 07, 1979
September 19, 1979
October 3, 1979
October 23/24, 1979
November 21, 1979
December 20, 1979

The fiberglass samplers were stored in an ice chest and returned to the laboratory. Periphyton were scraped from a known area of each sampler and analyzed for chlorophyll-a and phaeophytin, by methanol extraction (Holm-Hansen and Reimann 1978), alkaline phosphatase activity (modified from Perry 1976) and dry and ash free weight (APHA 1975). Species identification were made on Lugol's preserved sampler. See Appendix 1 for a detailed description of the analytical procedures.

RESULTS

Data from the limnetic and nearshore samples were presented and discussed at the Quarterly Data Review held in Las Vegas in January 1980. It was noted then that trends in periphyton growth generally followed

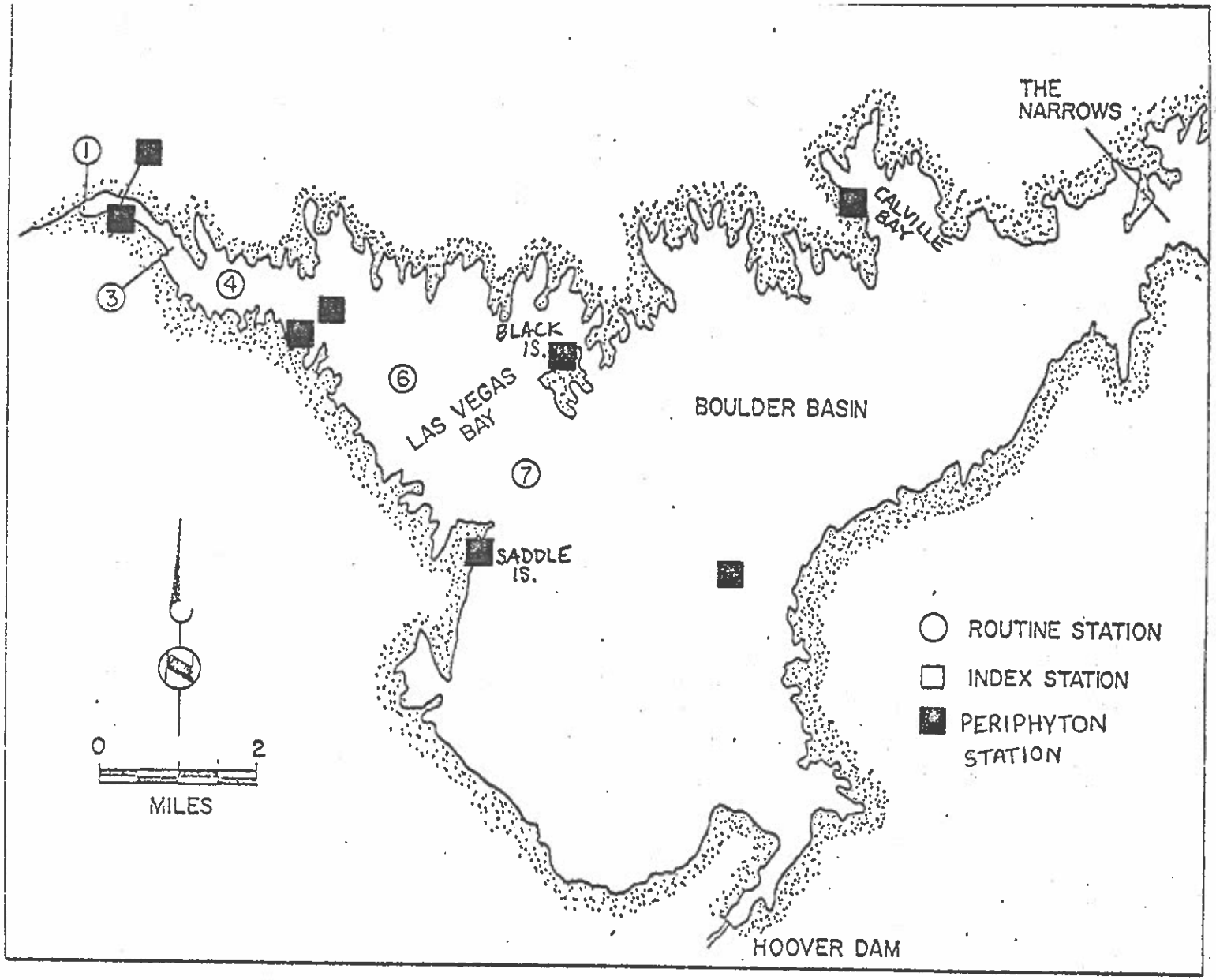


Figure 2. Location of Periphyton Stations for Quarterly Survey of the Lower Basin, Lake Mead

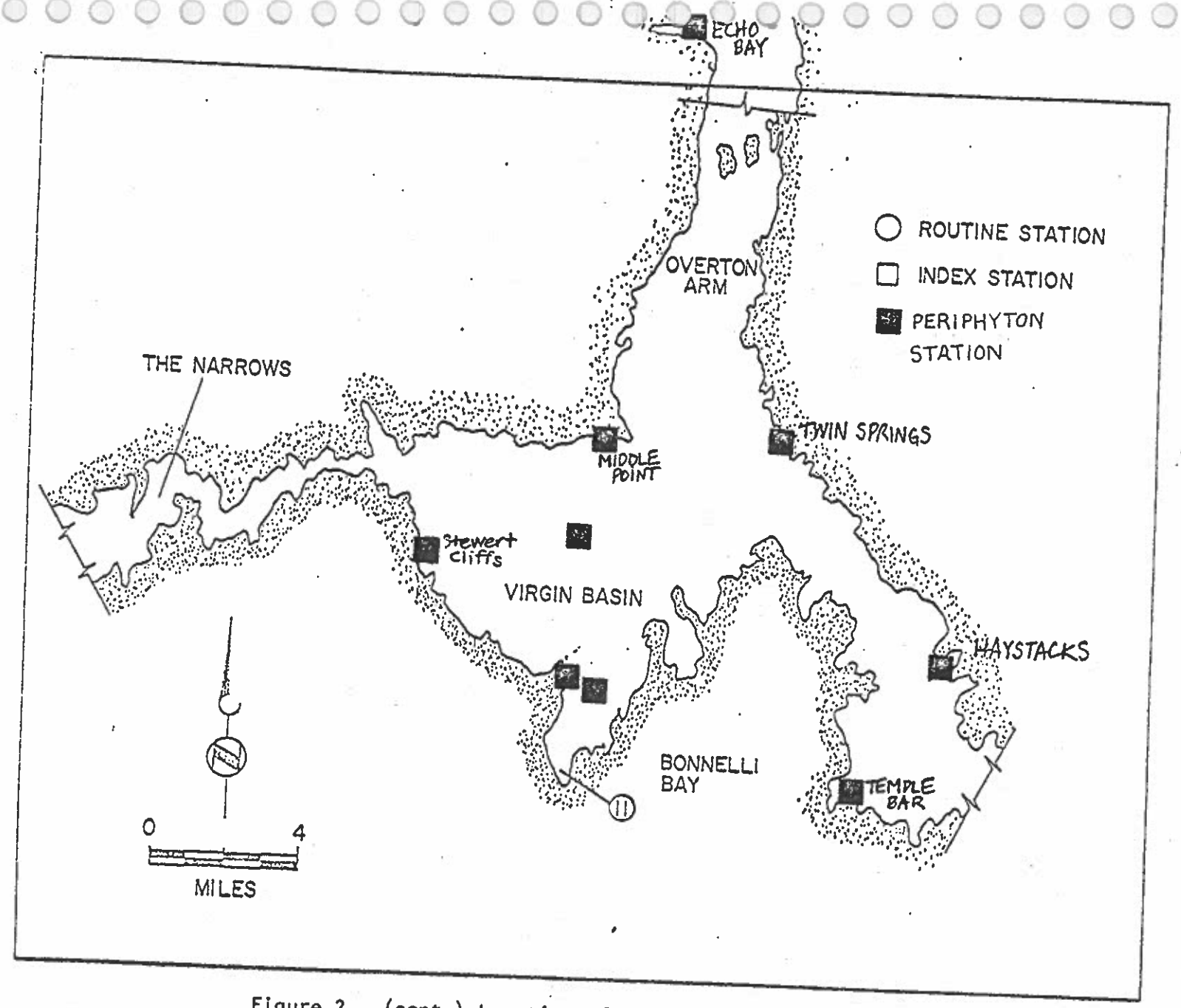


Figure 2. (cont.) Location of Periphyton Stations for Quarterly survey of the Upper Basin, Lake Mead

those observed for phytoplankton; namely, that growth was highest in the inner Las Vegas Bay and decreased with increasing distance from Las Vegas Wash. Alkaline phosphatase activity of periphyton increased with increasing distance from Las Vegas Wash reflecting more phosphorus limitation in upper portions of the lake. In Las Vegas Bay, periphyton growth was highest in nearshore samplers located on the south side of the bay. We suggested that this was probably caused by greater nutrient availability since the density current tends to flow along the south side of the bay. The periphyton community in late-summer and fall was characterized by green and bluegreen filamentous periphyton and attached diatoms. There was considerable variation in species composition at each station. Beyond these general statements, few conclusions can be drawn from the 1979 data.

PERIPHYTON SAMPLING PROBLEMS

We have experienced considerable difficulty in sampling both littoral and limnetic periphyton communities in Lake Mead. Although the fiberglass sampling devices appear to be suitable substrates for periphyton growth, the samplers are frequently subject to disturbances from swimmers, fisherman and high winds. The nearshore samplers are, by study design, located in high-use recreational areas. They are visible from the surface and, consequently, are prime targets for vandalism. This has resulted in the loss of several samplers and a considerable amount of data. The limnetic samplers are less subject to human disturbance, but high winds often result in their loss or destruction. Severe storms in the winter resulted in the loss of three limnetic samplers. Even though these samplers are relatively inexpensive, they are difficult to install because of special mooring requirements.

More important, however, is the loss of data we experience when the samplers are lost or destroyed. However, there are no alternative methods available for collecting periphyton in the deep limnetic areas.

Another problem that we have experienced with the periphyton samplers involves the constant fluctuation in lake level. Rapid increases in lake level during the winter required that we move the nearshore samplers to shallower depths and lengthen the lines in the limnetic samplers. This spring we have had to readjust the depth of the samplers to compensate for decreases in lake level. This is a problem we will be forced to deal with throughout the study.

Generally, the periphyton study has resulted in far more work than we or members of the TAB anticipated at the start of the study. Due to high light transparency, we have had to install the samplers at deeper depths to avoid light shock to the periphyton. Since these have to be installed and retrieved by divers, we were forced to purchase scuba gear which was not budgeted for in the contract. Morever, it takes three people (two divers and one observer) at least 12 hours to do the periphyton sampling. If it is windy, or if the divers have difficulty locating the samplers, we often have to go out the next day to finish one sampling run. Even though nearshore samplers on the north side of Las Vegas Bay were dropped during the Quarterly Data Review, we still have manpower deficiencies in the periphyton study. We are closely monitoring this situation and may be forced to request additional funding for the periphyton study in the data review in June.

RECOMMENDATIONS

We requested and received approval at the Quarterly Data Review to drop the nearshore periphyton stations on the north side of Las Vegas Bay.

This helped reduce our manpower deficiencies, and, consequently, we have caught up on the large sample backlog.

We further recommended that attempts be made to develop relationships between nutrients and periphyton growth. This will be required if nutrient control measures are necessary to control periphyton growth in the bay.

REFERENCES

FWPCA. 1967. Report on Pollution in Las Vegas Wash and Las Vegas Bay. Colorado River Basin Waters Quality Control Project, Federal Water Pollution Control Administration. 18 p.

Kellar, P.E., S.A. Paulson and L.J. Paulson. 1980. Methods for Biological and Chemical Analyses in Reservoirs. Lake Mead Limnological Research Center, Technical Report #5. (in press).

Appendix I (from Kellar et.al.1980)

Procedures for Collection, Measurement of Growth Rate (Dry Weight and Ash Free Weight), Chlorophyll-a Content, Species Diversity, Nitrogenase Activity, Productivity of Periphyton

I. Collection of Samples

A. Materials

1. Artificial substrates, in situ incubation apparatus (see B., below)
2. Ice chest
3. Atomizer

B. Procedure

Rectangular pieces of fiberglass (5cm x 15cm) to be used as substrates for the collection of periphyton are supported vertically by polyvinylchloride tubing, as shown in Fig. 1. Each sample unit consists of two rectangles riveted onto opposite sides of the PVC tube. The units are then placed onto smaller diameter pieces of PVC tubing attached to the holding racks. The holding apparatus for limnetic stations consists of PVC pipes, arranged in a double-T configuration and is suspended by lines from a buoy at the desired depth (Fig. 1A). The holding apparatus for littoral stations consists of a cement slab which is placed on the surface of the sediment (Fig. 1B). After an approximate incubation period of two weeks, the substrate units are collected by SCUBA and returned to the laboratory in an ice chest; samples are prevented from dessicating by frequent misting with an atomizer containing lake water.

II. Chlorophyll-a Measurements

A. Materials

1. Glass microscope slides
2. Rubber policeman
3. GF/C, glass fiber filters, 25mm diameter
4. Millipore^{R} filtration apparatus, capable of holding 25mm diameter filters
5. Centrifuge tubes with caps (>10 ml capacity)
6. Centrifuge
7. Methanol, reagent grade, absolute
8. 0.1 N HCl
9. Autopipette capable of delivering 50 µl volume

B. Procedure

1. As soon as possible after collection, 25 cm^2 areas are measured on replicate substrate unit for each station and the periphyton within each area are acraped with the edge of glass slide and a rubber policeman. The samples are then transferred to GF/C glass fiber filters and excess water removed by filtration. The filters are then transferred to capped centrifuge tubes and stored frozen until analysis usually within 24 hours.
2. At the time of analysis, the samples are brought to room temperature in the dark. A 10-ml volume of methanol is added to each tube. The tubes are then allowed to sit for one hour in complete darkness at room temperature to extract the chlorophyll (Holm-Hansen and Riemann 1978). Samples are then centrifuged for 10 minutes. Absorbances at 750nm and 650nm are then read against a methanol blank on a Coleman 55 Spectrophotometer before and after acidification with 50 µl of 0.1N HCl.

C. Chlorophyll-a and phaeophytin-a concentrations are calculated using Tett's (1975, 1977) formulas:

$$\text{Chl. } \underline{a} \ (\mu g \cdot m^{-2} \cdot da^{-1}) = \frac{G}{(1-GJ)} \left[(O_{665} - A_{665}) - JGA_{665} \ (H-1) \right] \frac{v}{V} \div t$$

$$\text{Phaeo. } \underline{a} \ (\mu g \cdot m^{-2} \cdot da^{-1}) = \frac{G \ (HA_{665} - O_{665})}{(1 - GJ)} \times \frac{v}{V} \div t$$

Where : G = 0.029

J- 5.9

O_{665} = absorbance before acidification corrected for background absorbance read at 750 nm

A_{665} = absorbance after acidification corrected for background absorbance read at 750 nm

H = 2.0

v = extraction volume in l

V = sample area in cm^2

t = incubation time in days

The above G, J, and H are unitless constants determined by Robert C. Furtek, U.S.E.P.A., EMSL-LV, Las Vegas, NV using methods in Tett (1975).

III. Growth Rate Measurements: Dry and Ash Free Weights

A. Materials

1. Glass microscope slides
2. Rubber policeman
3. Aluminum weighing dishes
4. Muffle furnace
5. Drying oven
6. Analytical balance capable of weighing to nearest 0.1 mg

B. Procedure

As soon as possible after collection, a 25 cm^2 or 50 cm^2 area is scraped from replicate substrate units for each station with a glass slide and a rubber policeman. Subsamples are rinsed into

aluminum pans which have been precombusted at 500°C for 1 h and pre-weighed. The subsamples are then dried to a constant weight at 105°C in a drying oven and weighed to the nearest 0.1 mg. They are then ashed for 1 h at 550°C in a muffle furnace (Vollenweider 1969). The ashed sample is then rewetted with distilled water to reintroduce the waters of hydration, and again dried to constant weight at 105°C, and weighed to the nearest 0.1 mg (APHA 1975).

C. Calculations:

Dry weight $(mg \cdot m^{-2} \cdot da^{-1})$ =

$$\frac{\text{wt. after drying (mg)} - \text{tare wt. (mg)}}{\text{subsample area } (m^2) \cdot \text{incubation time (da)}}$$

Ash Free dry weight $(mg \cdot m^{-2} \cdot da^{-1})$ =

$$\frac{\text{total wt. after drying (mg)} - \text{wt. after ashing (mg)}}{\text{subsample area } (m^2) \cdot \text{incubation time (da)}}$$

IV. Species Diversity

A. Materials

1. Polyethylene bottles
2. Acid-lugols solution (see Section)
3. Glass microscope slides
4. Glass coverslips
5. Compound microscope capable of magnifications of 450 X
6. Hot plate
7. Muffle furnace
8. Hydrax mounting medium

B. Procedure

1. Subsamples for species identification are scraped from periphyton

samplers, and brought up to a volume of 100 ml with distilled water containing 1 ml acid-lugols solution (see Section), and stored in polyethylene bottles in the dark. A subsample of this preparation is then pipetted onto a glass slide and observed at 450 X magnification with a compound microscope.

2. Diatom species are identified by preparing a slide as follows: several drops of the sample prepared as above are placed on a coverslip and evaporated to dryness on a hot plate. The residue is then combusted at 500°C in a muffle furnace for 0.5 to 0.75 h and mounted in Hydrax mounting medium on a glass slide. Microscopic observations at 450 X magnification are made as above.
3. Proportional cell counts (at least 200 cells of each dominant species) of both preparations are made.

C. Calculations

Each individual cell is measured and the volume calculated by approximation to an appropriate geometrical shape. The mean cell volume for each species is then derived. Biovolume (μ^3) is obtained by multiplying the cell volume by the cell number. The dominant five species according to biovolume, thus determined, are ranked and recorded.

V. Nitrogenase Activity

The acetylene reduction assay of nitrogenase activity in periphyton communities is similar to that in phytoplankton communities (Flett et al. 1975). Care must be taken to avoid physically disrupting the structural integrity of the periphyton community since nitrogenase activity may occur in microzones. Therefore, slight modifications in the procedure of the addition of acetylene gas are incorporated to

preserve the physical structure of communities.

A. Materials

1. Acetylene gas transported in football bladders
2. Various-sized (e.g. 5, 10, and 50) Luer-lok syringes (plastic disposable, Becton-Dickinson type), with 25 guage, 3/4 in. needles
3. Mason jars(pints) fitted with Vacutainer® stoppers and seals, through which gases are injected and withdrawn
4. Formalin (10 mls added per 400 mls lake water for blank)
5. 500 ml polypropylene graduated cylinder
6. Vacutainers®, 4 ml, red stopper, non-sterile evacuated tubes, Becton-Dickinson
7. Steel plate apparatus (Fig. 2) fitted with holders for Mason jars

B. Procedure

1. For each station, fill 4 Mason jars of known volume with 400 mls of lake water each. Add 10 mls formalin to one jar to use as a blank.
2. Seal jars tightly and inject 40 mls C_2H_2 gas through septum.
3. Shake vigorously for approximately one minute.
4. Remove lid, and carefully place substrate community into jar. Reseal lid tightly and add 7 additional mls of C_2H_2 gas. Do <u>not</u> shake.
5. The vessels are then secured to the holder plate and returned to the site from where the sample was taken, or to an area of comparable light intensity and temperature.
6. Incubations are conducted for 4 hr.

7. At the end of incubation period, the vessels are removed from the plate, and shaken for approximately one minute.

8. From each vessel 3 mls of gas from the headspace is withdrawn. Syringe is pumped several times into headspace to assure that a pure sample of headspace is obtained. The sample is then transferred to a Vacutainer^{R} which has been pre-labeled with date, location, and vessel number. Note exact time of removal of subsample and temperature of water at time of subsampling. Subsample may be replicated, but the total gas volume removed should not exceed 7 mls, in order to maintain positive or neutral pressure within the vessel and avoid contamination of the subsample with air during transfer.

9. Upon return to the laboratory, the periphyton from each rock substrate is remaved by scraping and placed into a pre-weighed, disposable aluminum weighing dish. Dry weights are determined as in III. A., this section.

10. To estimate the surface area orginally covered with periphyton, Parafilm^{R} is then stretched over the region of the rock from which the periphyton was removed. The Parafilm^{R} is placed on paper, its outline traced and its area determined by planmetry.

C. Calculations (see sect.)

1. The rate of C_2H_4 production in the incubation vessel is calculated as follows:

$$\frac{\text{nmoles } C_2H_4 \text{ (corr. for background)} \times \text{Vapor Pahse (ml)} \times 4\text{ml}/3\text{ml}}{0.3 \text{ ml} \times \text{Inc. Time (h)} \times \text{Transfer Coefficient}} = \frac{\text{nmoles}}{\text{hxvessel}}$$

2. To express the rate of C_2H_4 production in terms of biomass (dry weight) and surface area, the value determined in (1.), above,

is divided by biomass (dry weight), determined in B.9. (above) and by surface area determined in B.10. (above), respectively.

V. Productivity

A. Materials

1. Jars (approximately 2 liter-capacity) fitted with screw caps and seals; 3 clear and 3 opaque, covered with black electrical tape.
2. Steel plate apparatus (Fig. 2) fitted with holders for jars.
3. Thermometer
4. Dissolved oxygen meter (e.g. Y.S.I.)

B. Procedure

1. Each of six replicate bottles, three light and three dark, is filled with lake water which is allowed to overflow.
2. The initial temperature and dissolved oxygen concentration of water in each jar is measured and recorded.
3. Six rocks with periphyton are then taken from the sampling site and one placed in each jar.
4. The jars are then tightly sealed while being held underwater to remove all air bubbles.
5. The jars are then inverted and secured to the steel plate apparatus and returned to the site from where they were taken, or to an area of comparable light and temperature.
6. Incubations are conducted for 4 h.
7. At the end of the incubation period, the jars are removed from the plate and the final temperature and dissolved oxygen concentration of the water are determined.

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3. Six rocks with periphyton are then taken from the sampling site and one placed in each jar.
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6. Incubations are conducted for 4 h.
7. At the end of the incubation period, the jars are removed from the plate and the final temperature and dissolved oxygen concentration of the water are determined.
8. Upon return to the laboratory, the periphyton from each rock substrate is removed by scraping and placed in a pre-weighed, disposable aluminum weighing dish.
9. Parafilm^{R} is then stretched over the rock to estimate the surface area covered with periphyton. The Parafilm^{R} is then placed on paper and its outline traced. The area of the figure is then determined by planimetry.
10. Data collected are then reported with the date, location, and time of sampling.

C. Calculations

1. To calculate productivity per unit surface area per time:

$$\text{Productivity} = \frac{D.O._f - D.O._i}{Sxt}$$

where $D.O._f$ = dissolved oxygen concentration at end of incubation period ($mg \cdot l^{-1}$)

8. Upon return to the laboratory, the periphyton from each rock substrate is removed by scraping and placed in a pre-weighed, disposable aluminum weighing dish.

9. Parafilm^{R} is then stretched over the rock to estimate the surface area covered with periphyton. The Parafilm^{R} is then placed on paper and its outline traced. The area of the figure is then determined by planimetry.

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C. Calculations

1. To calculate productivity per unit surface area per time:

$$\text{Productivity} = \frac{D.O._f - D.O._i}{Sxt}$$

where $D.O._f$ = dissolved oxygen concentration at end of incubation period ($mg \cdot l^{-1}$)

$D.O._i$ = dissolved oxygen concentration at beginning of incubation period ($mg \cdot l^{-1}$)

S = surface area (m^2) of rock covered with periphyton

t = length of incubation period (h)

2. To calculate productivity per unit dry weight per unit time:

$$\text{Productivity} = \frac{D.O._f - D.O._i}{D.W. \times t}$$

where $D.O._f$ = dissolved oxygen concentration at end of incubation period ($mg \cdot l^{-1}$)

$D.O._i$ = dissolved oxygen concentration at beginning of incubation period ($mg \cdot l^{-1}$)

$D.W.$ = dry weight (g) of periphyton

t = length of incubation period (h)

REFERENCES

APHA. 1975. Standard methods for Examination of Water and Wastewater. 14th ed. American Public Health Association. 1193 pp.

Flett, R.J., R.D. Hamilton, and N.E.R. Campbell. 1976. Aquatic acetylene reduction techniques: solutions to several problems. Can. J. Microbiol. 22:43-51.

Holm-Hansen, O. and B. Reimann. 1978. Chlorophyll-a determination: improvements in methodology. Oikos 30:438-447.

Tett, P., M.G. Kelly, and G.M. Hornberger. 1975. A method for the spectrophotometric measurement of chlorophyll-a and pheophytin-a in benthic microalgae. Limnol. Oceanogr. 20:887-896.

Tett, P., M.G. Kelly, and G.M. Hornberger. 1977. Estimation of chlorophyll-a and pheophytin-a in methanol. Limnol. Oceanogr. 22:579-580.

Vollenweider, R.A. 1969. A manual on methods for measuring primary production in aquatic environments. IBP Handbook No. 12. Blackwell Scientific Publications. Oxford.

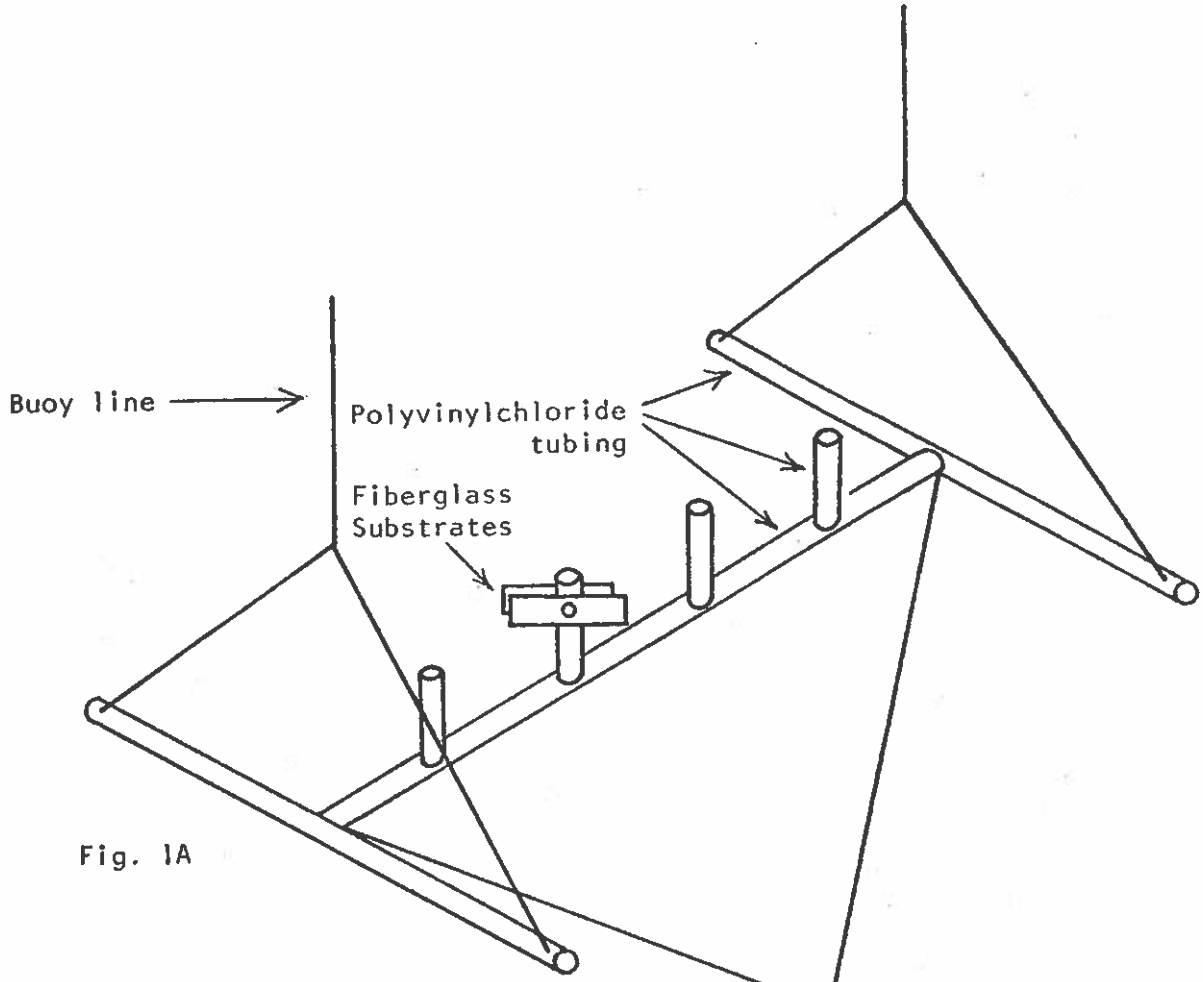


Fig. 1A

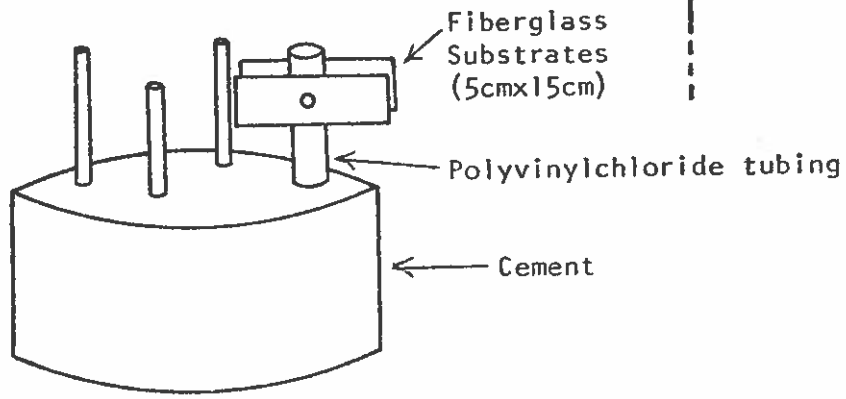


Fig. 1B

Figure 1. In situ incubation apparatus for sampling periphyton growth in the limnetic (1A) and littoral (1B) zones of reservoirs.

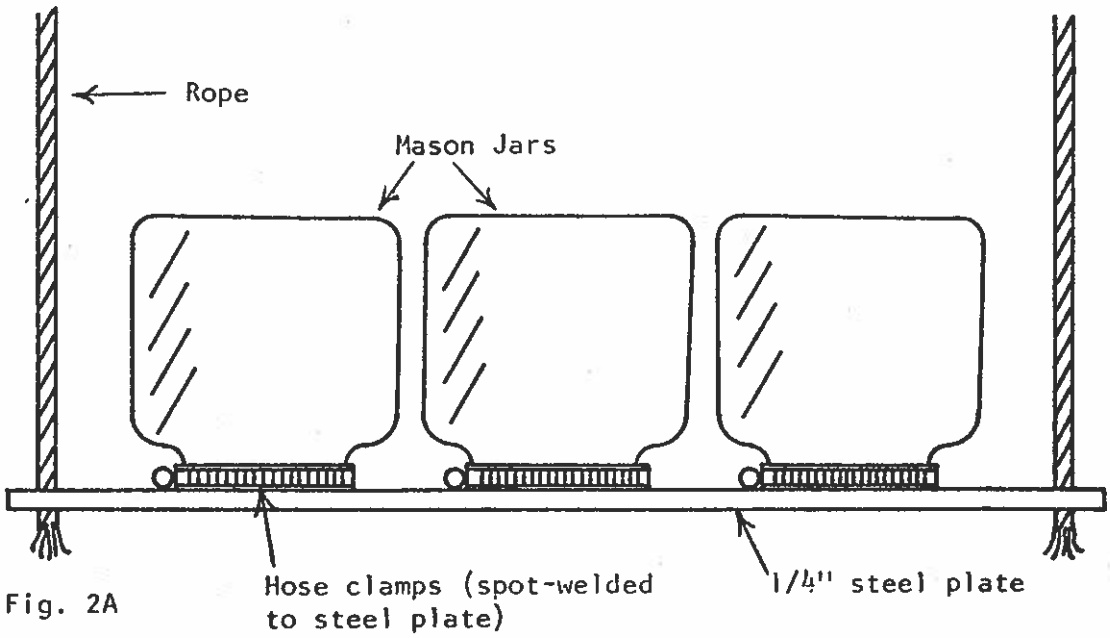


Fig. 2A

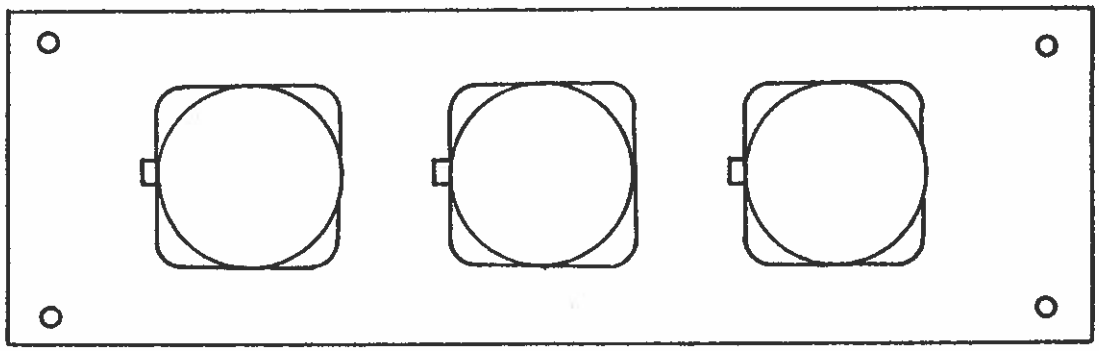


Fig. 2B

Figure 2. In situ incubation apparatus for measurement of nitrogenase activity in periphyton by the acetylene reduction method. Fig. 2A: Side view of incubation vessels in place with apparatus suspended from buoy. Fig. 2B: Top view of apparatus.

PERIPHYTON STUDY (1979 to 1980)

Periphyton Study-Lake Mead

1979-80

Penelope Kellar and Larry J. Paulson

INTRODUCTION

Growth, species diversity, and activity of alkaline phosphatase were studied on periphyton (attached algae) communities in Lake Mead at 5 index stations and 5-10 nearshore stations (lateral to the index stations) (Fig.1). Samples were collected from artificial substrates at two-week intervals during the recreation season and monthly during the rest of the year. Methods of analyses were as previously described (Paulson and Morris 1979).

In addition to the routine sampling program, 4 qualitative lake-wide surveys of periphyton biomass and species composition were conducted in 1980. Samples were collected from natural substrates at various locations in Lake Mead (Fig.2), on March 2, June 30, September 8, and November 20; representative substrates were then qualitatively analyzed for dominant species of periphyton.

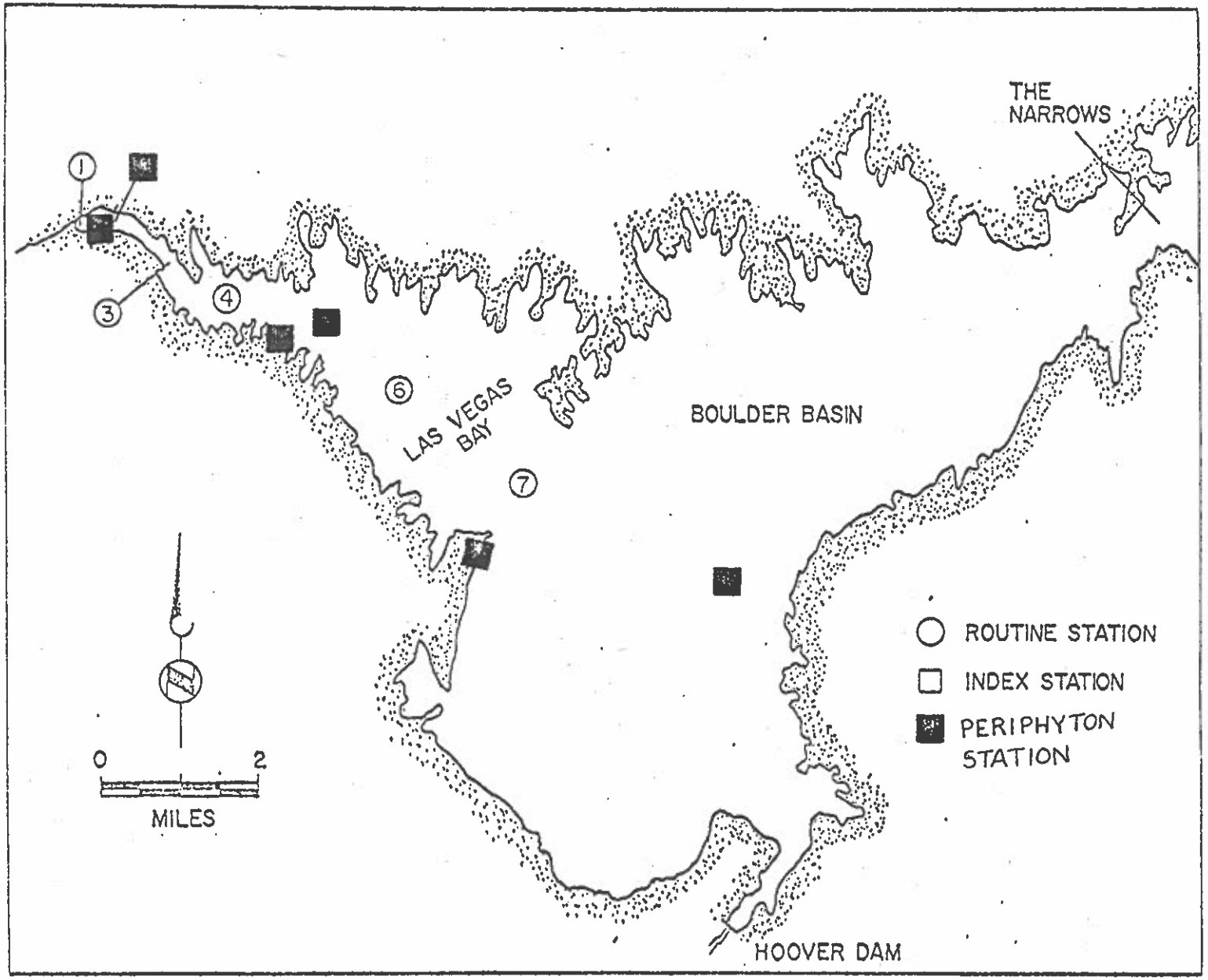


Figure 1. Location of Periphyton Samplers in the Lower Basin, Lake Mead

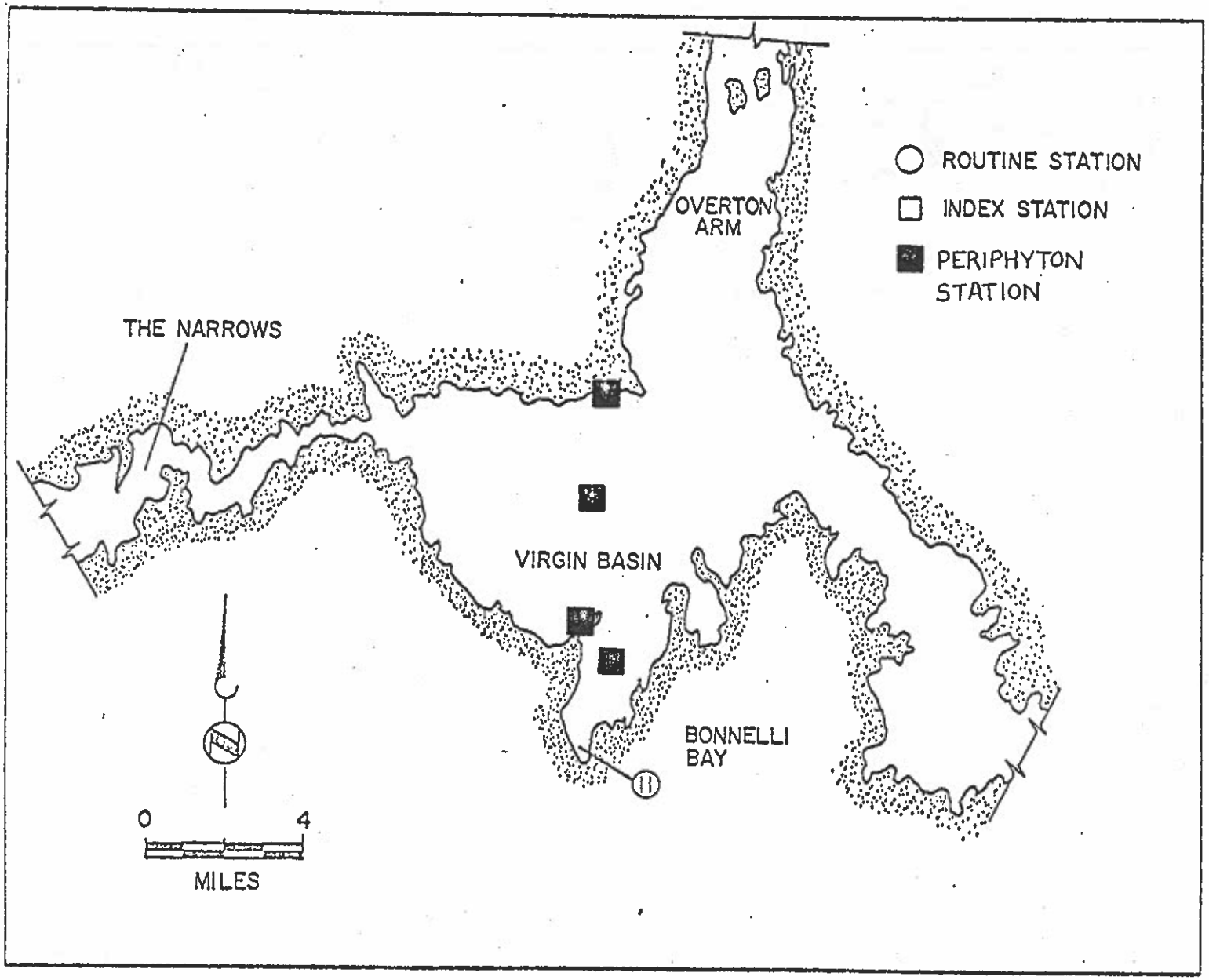


Figure 1. (cont.) Location of Periphyton samplers in the Upper Basin, Lake Mead

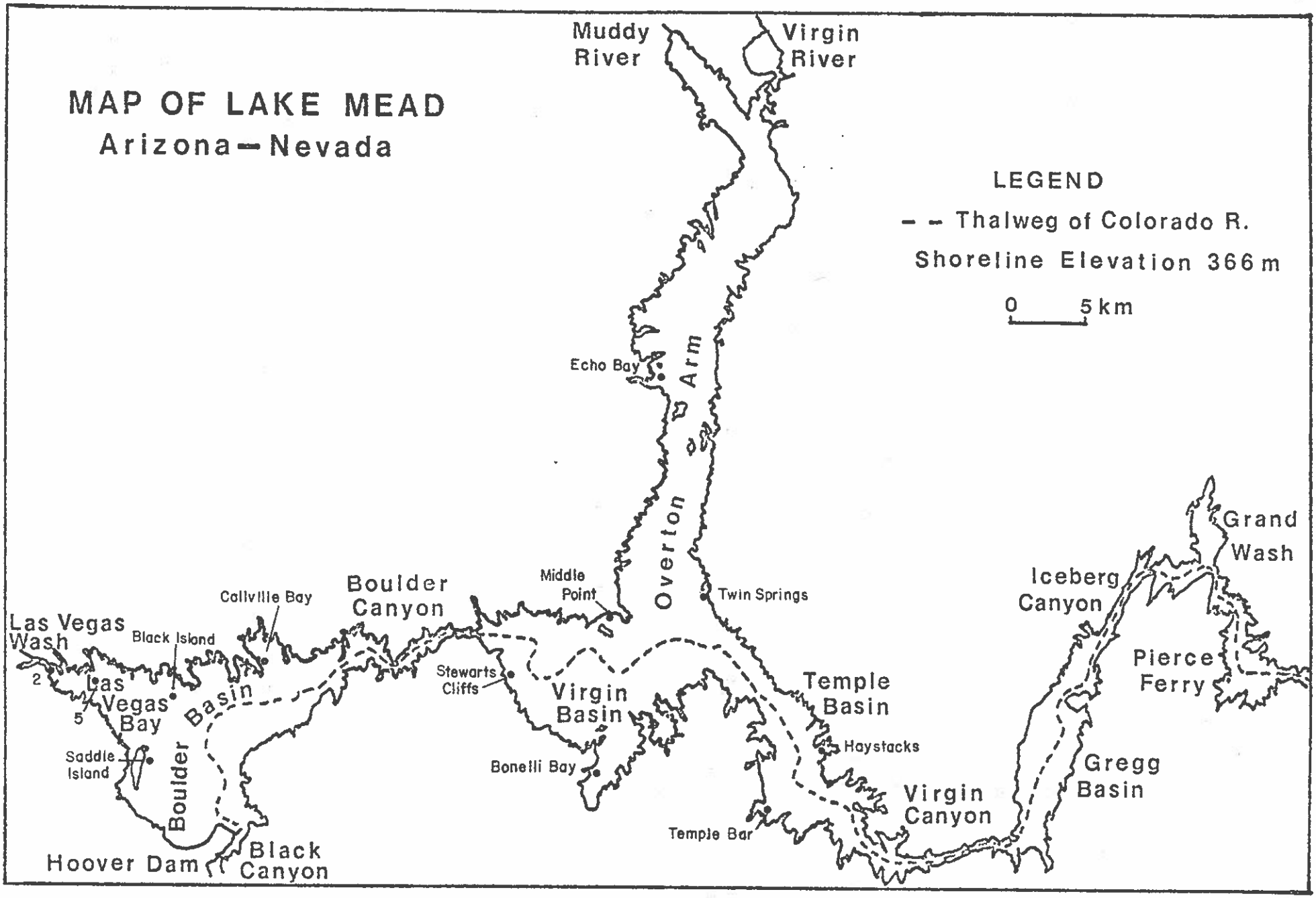


Fig. 2. Location of sampling stations for lakewide survey of periphyton in Lake Mead.

Station 2:

Limnetic

At the limnetic station, bluegreens were among the top 5 dominants during early September 1979, and April through the end of May 1980. Bluegreens were also present in late June 1980, late July 1980 and early December 1980. Diatoms were among the top 5 dominants during every sampling date except mid-May 1980. Green algae dominated the community between late October 1979 and late January 1980, and again from mid-May through mid-June. During early August through early December 1980 diatoms were dominant with greens sub-dominant.

Littoral

In general, communities at the littoral station were quite different from those at the limnetic stations. Bluegreens were among the top 5 dominants in December 1979, October 1980, and November 1980. Diatoms were the exclusive dominants in September 1979 and in general co-dominated with greens throughout the rest of the study.

Station 5:

Limnetic

Data were not collected at the limnetic station for the period from September through December 1979. Bluegreens were among the top 5 dominants in January, early and mid-May, and late July 1980; they were also present in early September, early October and early November 1980. Diatoms were among the top

5 dominant species from March through late July and September 1980. Greens were sub-dominant from late June through late September 1980 and were dominant from early October through early December 1980.

Littoral

There was slightly more agreement between the littoral and limnetic station communities than displayed at Sta.2. At the littoral station, Lyngbya martensiana, a bluegreen alga, was common from early September 1979 through November 1979 and again in March and April 1980. It was also dominant in July, August, and September 1980 and present in October 1980. Other bluegreens were present in January, and March through June 1980. Diatoms were among the top 5 dominants on all sampling dates except September and November 1979, and July and August 1980.

Station 8:

Limnetic

No data were collected from the limnetic station from September 1979 through January 1980, late August, early November and early December 1980. No algae were present in mid-May 1980. Bluegreens were among the top 5 dominants only in March and early August 1980. Diatoms were among the top 5 dominants from March through early July 1980 and late September through late November 1980. Greens were dominant in early September 1980 and were co-dominant with diatoms in early May, mid-June through late July, and late September through late November 1980.

Littoral

There was little agreement between communities at the littoral and limnetic stations. Bluegreens were among the top 5 dominants at the littoral station in late September and late November 1979, in early May 1980 and in July through December 1980. Greens were among the top 5 dominants from September through December 1979 and in June through November 1980. Diatoms were present, in general, throughout the study period and were the sole dominants in March and May 1980.

Station 9:

Limnetic

Data were not available from the limnetic station from September through December 1979 and for December 1980. No growth of algae was observed in April 1980. Bluegreens were absent throughout the period of study. Diatoms were the exclusive dominants in March, early May, June, August through early September, and early October 1980. Greens were the exclusive dominant in late November 1979. Diatoms and greens co-dominated during the remaining sampling dates.

Littoral

Communities were fairly similar between the limnetic and littoral stations. No data were collected from the littoral station from September 1979 through March 1980. No growth of algae occurred in April 1980. Bluegreens were among the top 5 dominants only in early October 1980. Diatoms were the exclusive dominants in November 1980. Diatoms and greens co-dominated throughout the rest of the study.

Station 10:

Limnetic

Data were not available from the limnetic station in early September 1979, November 1979, and in January, April, late August, early November and early December 1980. Bluegreens were present only in late September 1979. Diatoms were the exclusive dominants from May through early July, early August and early September 1980. Greens were the exclusive dominants in late October and December 1979. Greens and diatoms co-dominated on all other dates.

Littoral

Communities were similar between littoral and limnetic stations on some occasions, and dissimilar on others. Bluegreens were among the top 5 dominants September and October 1979 and in March and May 1980. Diatoms were the exclusive dominants in December 1979, and May and June 1980. Diatoms and greens were co-dominant during the rest of the study except when bluegreens were present.

GROWTH RATES

Ash Free Dry Weight ($mg \cdot m^{-2} \cdot da^{-1}$)

Ash free dry weight (organic matter content) was measured from December 1979 through December 1980 at the Index Stations, both in the limnetic and littoral zones. Growth rate measurements were obtained by dividing Ash Free Weights by the length of the incubation period.

In the Inner Bay, growth rates were higher at the limnetic station than the littoral station, possibly due to inhibition by suspended sediments in the littoral zone. In the Outer Bay and Boulder Basin, highest growth rates sometimes occurred at the littoral stations and sometimes at the limnetic stations. In the Upper Basin, growth rates were higher at the littoral stations than at the limnetic stations.

In general, decreasing trends with distance from Las Vegas Wash were observed. Values at the Outer Bay Station were about one-half those observed in the Inner Bay. Growth rates were about 10 times greater in the Inner Bay than in Bonelli Bay.

The highest growth rates in the Inner Bay occurred at the end of May 1980. In the Outer Bay, peaks were observed in mid-May 1980 and in December 1980. In Boulder Basin, peak growth occurred in December 1979 - January 1980; lesser peaks were observed at the end of June 1980 and August 1980. The peak growth rate in Virgin Basin occurred in December 1979. Highest growth rate in Bonelli Bay occurred at the end of June 1980 with a lesser peak in mid-March 1980.

Chlorophyll-a (mg chl-a·m^{-2}·da^{-1})

Growth rate (based on chl-a measurements) generally followed patterns observed with ash free dry weights, i.e. decreasing trends with distance from Las Vegas Wash. However, growth rates in the Outer Bay were often greater than in the Inner Bay, possibly reflecting differences in communities. Growth rates were approximately ten times greater in the Inner Bay than in Bonelli Bay.

The peak growth rate in the Inner Bay, based on chl-a measurements, occurred at the end of May 1980, as with ash free dry weights. A lesser peak was evident in November 1980 which was not observed with ash free dry weight. In the Outer Bay, peak growth was observed in December 1980 with two smaller peaks occurring in mid-May and early November 1980. In Boulder Basin growth rates were high from December 1979 through mid-April 1980; rates were low during the period from mid-April through the end of July 1980, and relatively high but variable from August through November 1980. In Virgin Basin the peak growth rate occurred in December 1979; throughout the remainder of the study, values were low and invariable. In Bonelli Bay very little seasonal variation in growth rate was observed; a slight peak was evident at the end of January 1980 but the peak may have actually occurred prior to this date, but data were not available.

Dry Weight (mg·m^{-2}·da^{-1})

Growth rates based on dry weight measurements were difficult to interpret because of possible sedimentation of non-periphyton origin onto samplers.

ALKALINE PHOSPHATASE ACTIVITY

Alkaline phosphatase activity at the limnetic stations was measured from December 1979 through December 1980. Values were normalized by dividing by ash-free weights. Values were consistently low at Station 2 throughout the period of study except for a small peak observed in early September 1980. In general, values were highest at Station 5, with maximum activity occurring in mid-April 1980. In Boulder Basin and Bonelli Bay the highest activity was measured in December 1979; activity at Station 9 was relatively low on this date. Lesser peaks occurred at Stations 8, 9, and 10 during fall 1980. No consistent seasonal or spatial trends were evident.

3-02-80: Bluegreen algae were present at all Lower Basin stations except Station 2, the Inner Las Vegas Bay. Bluegreens were also present in the Upper Basin at Stewart's Cliffs, Bonelli Bay and Haystacks. Diatom dominated communities occurred at all stations except Station 5, the Outer Las Vegas Bay, Saddle Island, Black Island, and Bonelli Bay. Green-dominated communities occurred on salt cedar and rock at Station 2 and Saddle Island, respectively. Growth was sparse in Las Vegas Bay and Middle Point, and totally absent in Twin Springs. Growth was moderate to abundant elsewhere in the lake (table 1).

6-30-80: Bluegreen algae occurred in small numbers at all Lower Basin stations, except the Inner Las Vegas Bay, and were also present at Stewart's Cliffs and Virgin Basin. Raphidiopsis, a bluegreen, was dominant along with diatoms at Bonelli Bay and in Temple Basin. Diatom dominated communities occurred at Outer Las Vegas Bay, Boulder Basin, Stewart's Cliffs and Virgin Basin. Green dominated communitites occurred on salt cedar at the Outer Las Vegas Bay and on rocks in Callville Bay. Growth was very sparse at Twin Springs and Echo Bay. Abundance data at other stations were unavailable (table 2).

Table 1. Dominant species and abundance data for lakewide survey in Lake Mead on March 2, 1980.

DATE: 3-02-80				
STATION	BOTTOM	SUBSTRATE	RELATIVE ABUNDANCE	DOMINANT SPECIES
Las Vegas Bay 2	silt	silt salt cedar	sparse	silt-diatom community mainly Fragilaria salt cedar-Cladophora
Las Vegas Bay 5	silt	silt	sparse	Oscillatoria mat
Saddle Island	rock silt	rock silt	moderate	rock-Cladophora silt-Oscillatoria mat
Black Island	silt	silt	moderate	Spirulina and Oscillatoria mats
Callville Bay	silt cobble	silt cobble salt cedar	abundant	Oscillatoria mat Firm, gelatinous clumps of Cymbella Floating mats observed
Stewart's Cliff	sand boulders	salt cedar rock	abundant	salt cedar-firm clumps of Cymbella rock-diverse diatom community with some Oscillatoria and Mougeotia
Bonelli Bay 10	gypsum	**	abundant	Oscillatoria mat covering old periphyton growth
Temple Bar	sand	**	moderate	Diverse diatom community primarily Nitzschia and Cymbella
Haystacks	sand gravel	**	moderate	Thin Oscillatoria mat with diatom community dominated by Nitzschia
Twin Springs	sand cobble	**	absent	
Middle Point	rock	**	sparse	Diverse diatom community dominated by Gomphonema and Achnanthes
Echo Bay	rock	**	abundant	Diatom Community dominated by Cymbella

** Substrate information not noted.

Table 2. Dominant species and abundance data for lakewide survey in Lake Mead on June 30, 1980.

DATE 6-30-80				
STATION	BOTTOM*	SUBSTRATE	RELATIVE ABUNDANCE*	DOMINANT SPECIES
Las Vegas Bay 2		stick		Cladophora mat with Phacus and some Cryptomonas
Las Vegas Bay 5		stick rock		stick-Cladophora mat with diverse diatom community; some Oedogonium rock-diatom dominated community with some green and Chroococcus
Boulder Basin 8		rock		Diatom dominated community with rare greens and bluegreens
Callville Bay		stick rock		stick-Cladophora mat with greens and diatoms rock-Oedogonium dominated community with diatoms and some bluegreens
Stewart's Cliff		**		Diatom dominated community with some greens and bluegreens
Virgin Basin 9		rock		Diatom dominated community with some greens and bluegreens, both rare
Bonelli Bay 10		**		Bluegreen (Raphidiopsis) dominated community with diatoms
Temple Bar		rock		Bluegreen (Raphidiopsis) dominated community with Nitzschia, and Oscillatoria and Stigeoclonium rare
Haystacks		rock		Bluegreen (Raphidiopsis) dominated community with Synedra, Cladophora and Chroococcus
Twin Springs		rock	sparse	Very sparse community with greens, bluegreens and diatoms
Echo Bay		**	sparse	Very sparse diatom community

* Bottom configuration and abundance data were unavailable.

** Substrate information not noted.

9-08-80: Salt cedar in Las Vegas Bay was dominated by Rhizoclonium (green). Diatoms and greens co-dominated communities at Black and Saddle Islands. Bluegreens occurred at every station surveyed and co-dominated with diatoms at all stations in the Upper Basin. Diatoms were also present throughout the lake. Growth was abundant only in Outer Las Vegas Bay and Callville Bay, and was sparse at Haystacks, Twin Springs, Middle Point and Echo Bay. Elsewhere in the lake, growth was moderate (table 3).

11-20-80: Bluegreens were either common or dominant at all stations surveyed. Green algae were common or co-dominated with bluegreens and diatoms throughout the lake except at Black Island where they were rare. Diatoms occurred at all stations. Growth was abundant throughout the Lower Basin, sparse at Stewart's Cliff and Haystacks and moderate elsewhere (table 4).

Table 3. Dominant species and abundance data for lakewide survey in Lake Mead on September 8, 1980.

DATE: 9-08-80

STATION	BOTTOM	SUBSTRATE	RELATIVE ABUNDANCE	DOMINANT SPECIES
Las Vegas Bay 2	silt	stick rock	moderate	stick-green (Rhizoclonium) dominated community rock-diatom dominated community with bluegreens rare
Las Vegas Bay 5	gravel	stick rock	abundant	stick-green (Rhizoclonium) dominated community with Synedra; bluegreens rare rock-greens, bluegreens and diatoms common
Saddle Island	rock sand	stick	moderate	stick-diatoms, greens and bluegreens common rock-diatoms common with blue-green and Glenodinium rare
Black Island	rock silt	rock silt	moderate	rock-diatom and green dominated community with blue-greens rare
Callville Bay	gravel silt	rock	abundant	stick-diatom and green dominated community diatoms and green dominated community with bluegreens rare
Stewart's Cliff	gravel silt	rock stick	moderate	rock-bluegreen and diatom dominated community with greens stick-bluegreen, green, and diatom dominated community
Bonelll Bay 10	silt gypsum	rock	moderate	Diatom dominated community with greens and blugreens
Temple Bar	gravel silt	rock stick	moderate	rock-bluegreen and diatom dominated community with greens rare stick-same as rock
Haystacks	gravel	rock	sparse	Diatom dominated community with greens and bluegreens
Twin Springs	gravel	rock stick	sparse	rock-bluegreen and diatom dominated community stick-bluegreen and diatom dominated community with greens rare
Middle point	gravel silt	rock stick	sparse	rock-bluegreen and diatom dominated community with greens common stick-bluegreen and diatom dominated community wlth green rare
Echo Bay	gravel cobble	stick rock	sparse	stick-bluegreen and diatom dominated community with greens and Peridinium rock-same as above

Table 4. Dominant species and abundance data for lakewide survey in Lake Mead on November 20, 1980.

STATION	BOTTOM	SUBSTRATE	RELATIVE ABUNDANCE	DOMINANT SPECIES
		DATE: 11-20-80		
Las Vegas Bay 2	silt rock	stick	abundant	diatom and bluegreen dominated community with greens common
Las Vegas Bay 5	rock gravel	**	abundant	bluegreen dominated community with diatoms and greens
Saddle Island	rock	stick/Najas rock	abundant	stick/Najas-diatom and bluegreen dominated community with greens common rock-diatom and bluegreen dominated community with greens common
Black Island	rock gravel	stick rock	abundant	stick-diatom dominated community with bluegreens common, greens rare rock-diatom dominated community with bluegreens common and greens rare
Callville Bay	rock bushes	rock	abundant	rock-diatom and bluegreen dominated community with greens mat-bluegreen, green and diatom dominated community
Stewart's Cliff	silt rock	stick	sparse	green and diatom dominated community with bluegreens common
Bonelli Bay	gypsum silt	stick silt/rock	moderate	stick-green and diatom dominated community with bluegreens common silt/rock-green, bluegreen, and diatom dominated community
Temple Bar	silt sand	stick Najas	moderate	stick-bluegreen and diatom dominated community with greens Najas-bluegreen and diatom dominated community with greens rare
Haystacks	sand rock	stick rock	sparse	stick-bluegreen, green, and diatom dominated community rock-bluegreen, green, and diatom dominated community mat-bluegreen, green, and diatom dominated community
Twin Springs	silt sand	rock-sediment stick	moderate	rock-green, bluegreen and diatom dominated community with Dinobryon rare stick-green, bluegreen and diatom dominated community
Middle Point	rock sand	silt rock/aquatic weeds	moderate	silt-mat of diatoms and greens, Dinobryon rare rock/weeds-bluegreen and diatom dominated community with greens
Echo Bay	cobble boulder	Najas/silt rock stick aquatic weeds		Najas/silt-diatom and bluegreen dominated community with greens rock-bluegreen mat with diatoms stick-same as on rock weeds-diatom and bluegreen dominated with greens

** Substrate information not available.

REFERENCES

Paulson, L.J. and M. Morris. 1979. Periphyton study. Technical report submitted to Brown and Caldwell, Co. 5 pp.

INTENSIVE SURVEYS (1979 to 1980)

WATER QUALITY STANDARDS STUDY

INTENSIVE SURVEYS (1979-1980)

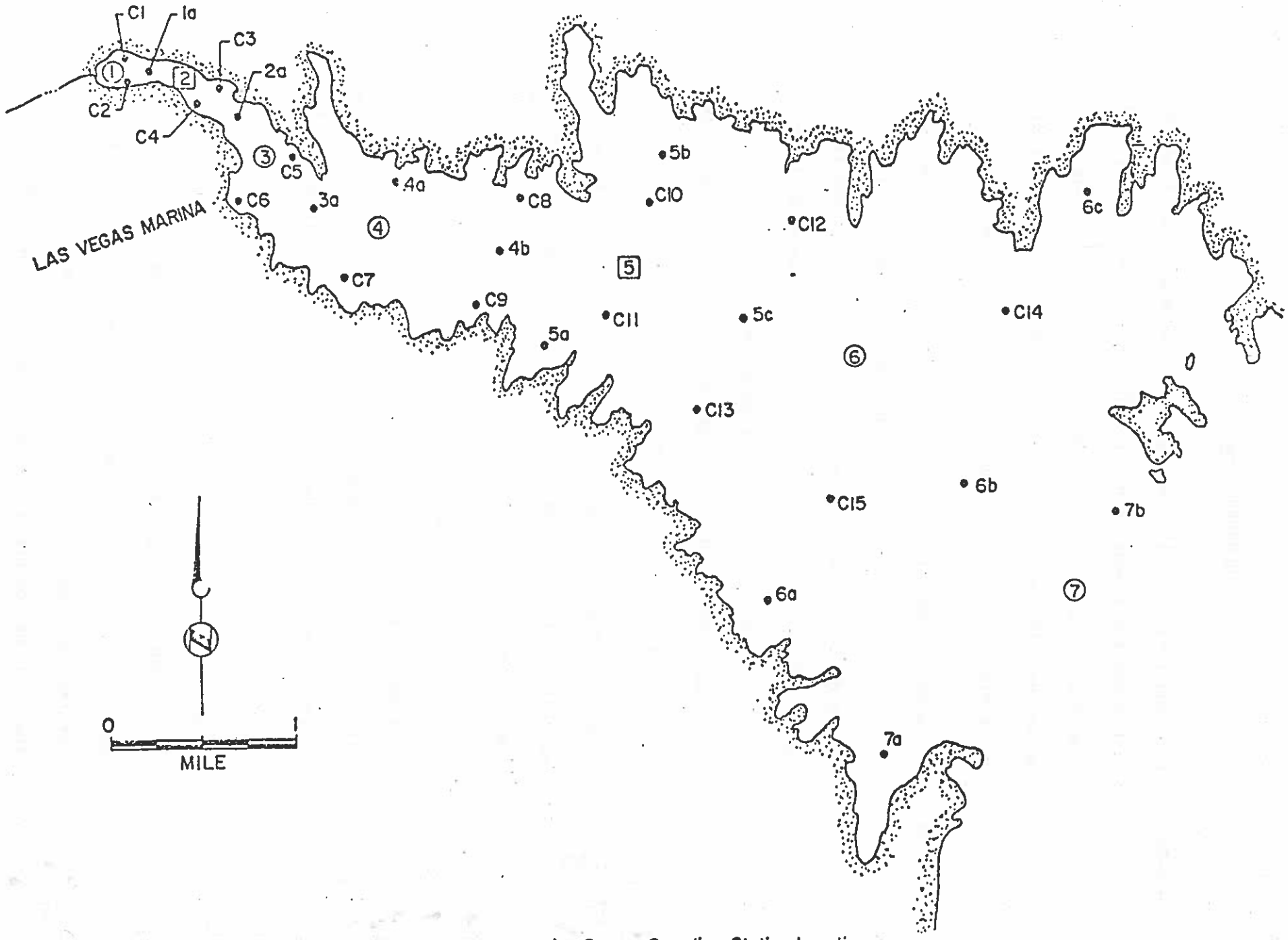
Penelope Kellar and Larry J. Paulson

INTRODUCTION

Four intensive surveys were conducted on Las Vegas Bay on September 6, 1979, April 17, 1980, July 23 and 25, 1980, and August 27, 1980 to determine spatial and temporal variations in water quality. Samples were collected at 20 stations for nutrient and chlorophyll-a analyses and at 15 additional stations for chlorophyll-a. Secchi depth measurements were conducted at all 35 stations. Nutrient samples were collected at three depths (surface, metalimnion and hypolimnion) at 18 of the 20 stations and at 8 depths at Stations 2 and 5. Results and general discussion are given below.

DEPTH PROFILES

In general, on the basis of depth profiles taken at stations 2 and 5, the 0-2.5 m intergrated sample at station 2 and the 0-5 m integrated sample elsewhere were appropriate in reflecting epilimnetic nutrient and chlorophyll-a concentrations.



Intensive Survey Sampling Station Locations

CHLOROPHYLL-a

9-6-79: Chlorophyll-a (Chl-a) showed decreases with distance from the Wash along the linear transect stations. Values sharply declined from Sta.3, outward. Levels were slightly higher along the south shore of the bay out to Sta. 3. No significant trends in lateral stations lying beyond Sta. 3 were evident.

4-17-80: Chl-a was highest at Sta. 1 and sharply fell at Sta. 1a, increased at Sta. 2 and slowly declined through Sta. 3a. Levels declined rapidly from Sta. 3a outward away from the Wash. Higher values again occurred on south shore stations out to Sta. 4; beyond 4, values were somewhat higher at the north shore than south shore stations. Values were near the limit of detection beyond Sta. 5c.

7-23 & 7-25-80: Except for Sta. 1, values declined steadily through 3a and sharply declined thereafter. No significant lateral trends were evident.

8-27-80: The decreasing trend with distance from the wash was not as consistent as on other intensives. Levels in the Inner Bay fluctuated spatially along the linear transect. No consistent lateral trends were evident. Values again sharply declined at Sta. 3a.

Overall: Highest chlorophyll-a concentrations occurred at Sta. 1 in April 1980 and at Sta. 1a in July 1980. Levels in the Inner Bay through 3a were relatively similar on each survey except for the two peak concentrations. Lowest concentrations in the Outer Bay occurred during the April and July 1980 surveys.

SECCHI DEPTH

9-6-79: Secchi depth increased with distance from the Wash at linear transect stations. Lateral stations showed similar trends; transparency increased from north to south in the Inner Bay out to the region between Stas. 4b and 5. Beyond this point, the trend was reversed with slightly more transparent water occurring on the north side of the Bay out to Sta. 6. Lateral stations beyond Sta. 6 showed deeper transparency than middle stations.

4-17-80: Secchi generally increased with distance from the Wash at linear transect stations, gradually up to 3a, and then dramatically beyond. No consistent lateral trends were evident between north and south shores of the Bay. Lateral stations also showed increasing transparency with distance from the wash.

7-23 & 7-25-80: Secchi depth increased with increasing distance from the wash. At lateral stations, transparency generally increased from north to south beyond Sta. 4b. No obvious lateral trends were shown in the Inner Bay.

8-27-80: Trends in secchi depth were similar to other intensives- transparency generally increased with distance from the wash. No obvious lateral trends were apparent in the Inner Bay. Increasing trends from south to north beyond the edge of the Inner Bay were evident.

Overall: Transparency was generally similar in September 1979 and July 1980. Transparency in the Middle and Outer Bay was greatly increased on 4-17-80; but, transparency in the Inner Bay was similar to that

on all other intensives. Transparency was markedly decreased in the Middle and Outer Bay during the August 1980 intensive, probably due to turbid inflow during storms between July and August 1980.

ORTHOPHOSPHORUS

9-6-79: Orthophosphorus (OP) generally decreased with distance from the inflow, except that levels were lower at Stas. 1, 1a, and 2 than at 2a, where the highest concentration occurred. The concentration at Sta. 3a was less than 1/2 that at Sta. 3. Values < 5 (except at Stations 5b and 7) occurred from Sta. 5 outward.

4-17-80: Generally decreasing trends were evident from 2a outward with the highest value occurring at Sta. 1. An abrupt decline occurred from Sta. 3 to Sta. 3a. Values were < 5 from Sta. 5 outward.

7-23 & 7-25-80: Generally decreasing trends occurred from Sta. 1a outward except for an increase at Sta. 5c. A marked decline occurred between Stas. 2a and 3. Values were less than 5 from Sta. 4 outward except for Sta. 5c.

8-27-80: Generally decreasing trends were noted from Sta. 1 outward, except at Sta. 2. A decline occurred between Stas. 3 and 3a; concentrations were less than 5 from Sta. 4 outward except at 6a. Levels were non-detectable at Sta. 7.

Overall: All surveys showed generally decreasing trends with increasing distance from Las Vegas Wash, with exceptions during September 1979 and April 1980 when the trend began only beyond Sta. 2a.

Relatively abrupt declines occurred on all surveys at or near Sta. 3. Values were less than 5 at most stations beyond Sta. 5 during the Fall 1979 and Spring 1980, and beyond Sta. 4 during the July and August 1980 surveys. Highest concentrations occurred in the Inner Bay (before Sta. 3) during April 1980 and lowest during September 1979. Lowest concentrations in Outer Bay occurred during August 1980.

DISSOLVED PHOSPHORUS

9-6-79: Highest concentrations of dissolved phosphorus (DP) occurred in the Inner Bay, with values declining at Sta. 3. The decreasing trend with distance was not obvious since stations 6b and 7 had levels higher than the Inner Bay. Concentrations were fairly variable in the Middle and Outer Bay.

4-17-80: Generally decreasing trends with distance from Las Vegas Wash were noted. An abrupt decline between Stas. 3 and 3a, and Stas. 3a and 4 occurred. Concentrations were generally uniform in the Middle and Outer Bay.

7-23 & 7-25-80: A generally decreasing trend with distance from the Wash was evident except for a dramatic increase at Sta. 5c in the Middle Bay. Concentrations were fairly uniform in the Middle and Outer Bay except for 5c, 6c (in littoral zone) and 7b, near Saddle Island. Levels declined sharply at 3 but at 3a increased slightly.

8-27-80: Generally decreasing trends were noted with distance from the Wash except for a slight increase at 6b in the Outer Bay. The most rapid declines occurred around Sta. 3a, and at Sta. 5c. Somewhat variable concentrations occurred in the Outer Bay.

Overall: Highest concentrations of DP occurred in Inner Bay before Sta. 3a. Although, in general, concentrations decreased with distance from the Las Vegas Wash, trends were not always consistent. Highest Inner Bay concentrations occurred during April 1980 and August 1980. Fairly abrupt declines occurred at Sta. 3a in September 1979 and April 1980 and at Sta. 4 in July and August 1980. Concentrations were quite variable on all dates in the Middle and Outer Bay.

TOTAL PHOSPHORUS

9-6-79: A generally decreasing trend with distance from the Wash was noted except for slight increases at Stas. 2a and 6b. An abrupt decline occurred at Sta. 3a. Values were less than 13 in the Middle and Outer Bay.

4-17-80: A generally decreasing trend with distance from the Wash was evident except that the concentration at Sta. 1a was slightly less than at Sta. 2. An abrupt decrease occurred between Stas. 3a and 4. Values were less than 13 in the Middle and Outer Bay.

7-23 & 7-25-80: The decreasing trend from Las Vegas Wash outward was not consistent, although values were higher in the Inner Bay. Again, an abrupt decline was evident in the area between Stas. 3a and 4. Values were quite variable in the Middle and Outer Bay; all were less than or equal to 15 except for Stas. 5a and 5c.

8-27-80: A dramatic decreasing trend with distance from the Wash was noted. Concentrations in the Outer and Middle Bay were less than 12.

Overall: Highest total phosphorus concentrations occurred in the Inner Bay during April 1980. Values were also high during the August 1980 survey. Decreasing trends were evident on all surveys, but were not always consistent except during August 1980. In the Middle and Outer Bay, values were nearly always less than or equal to 15µg $P \cdot l^{-1}$. Values rapidly declined at or near Sta. 3 on all occasions except for August 1980.

AMMONIA

9-6-79: Concentrations varied dramatically in the Inner Bay up to Sta. 3a where values became fairly consistent. With the exception of Stas. 2 and 3, concentrations at all stations were < 10. The highest concentration occurred at Sta. 3. Beyond Sta. 4, values in the limnetic stations consistently, though gradually, decreased.

4-17-80: Concentrations were extremely high in the Inner Bay, and dramatically declined with distance from Sta. 1 to Sta. 3. The value at Sta. 3a was 1/10 that at Sta. 3. From 3a outward, concentrations gradually decreased.

7-23 & 7-25-80: Concentrations were fairly low throughout the entire bay, but highest concentrations occurred in the Inner Bay. Concentrations fluctuated in the Inner Bay with the highest value occurring at Sta. 3. A rapid decrease occurred at Sta. 3a. Values were non-detectable in the Outer Bay.

8-27-80: Highest concentration occurred at Sta. 2. Values fluctuated dramatically in the Inner Bay and rapidly declined between Stas. 3a and 4, and remained low throughout the rest of the Bay.

Concentrations fell to non-detectable levels in the Outer Bay.

Overall: The highest concentrations of ammonia occurred in the Inner Bay during April 1980, when a consistent decreasing trend with distance from the Wash was noted. Concentrations fluctuated in the Inner Bay during the other 3 surveys. The most dramatic declines occurred at or near Sta. 3a on all surveys. Concentrations were non-detectable in the Outer Bay during the July and August surveys.

NITRITE

9-6-79: Nitrite was non-detectable at all stations except at Stas. 1 and 2 when concentrations were 1 $\mu g\ N \cdot l^{-1}$.

4-17-80: Nitrite was detectable throughout the entire Bay, showing a characteristic decline with distance from Sta. 1a, where the highest concentration occurred, to the Outer Bay. Concentrations declined dramatically from Stas. 3 to 3a.

7-23 & 7-25-80: Nitrite was non-detectable from Sta. 3a outward throughout the entire Middle and Outer Bay. Measurable nitrite occurred only in the Inner Bay with the highest level at Sta. 1a.

8-27-80: Nitrite was non-detectable from Sta. 3a outward, throughout the entire Middle and Outer Bay. Highest concentrations occurred at Sta. 1.

Overall: Nitrite was measurable throughout the Bay only in April 1980 survey when the highest concentrations occurred. The characteristic decline with distance was noted. Lowest levels occurred during the September 1979 survey when nitrite was detectable

in the Inner Bay only through Sta. 3a. Concentrations were higher during the August survey than during July.

NITRATE

9-6-79: Concentrations of nitrate were non-detectable at all stations except Stas. 1, 5a, and 6a.

4-17-80: The highest concentration was observed at Sta. 1 in the Inner Bay. No spatial trends were obvious. Values in the Middle and Outer Bay were as high as those in the Inner Bay.

7-23 & 7-25-80: Highest concentrations were observed at Sta. 1 in Inner Bay. No spatial trends were obvious. Concentrations were non-detectable at Stas. 4b and 5 in the Middle Bay. Concentrations in the Middle and Outer Bay were low, relative to those in the Inner Bay.

8-27-80: Values consistently decreased from Sta. 1 to Sta. 4b. Concentrations varied from non-detectable to 12µg $N \cdot l^{-1}$ in the Middle and Outer Bay where no spatial trends were obvious.

Overall: Nitrate concentrations were lowest during the September 1979 survey and highest during the April 1980 survey. Concentrations were fairly low in the Middle and Outer Bay on all surveys except April 1980 when concentrations averaged around 200 µg $N \cdot l^{-1}$. Spatial trends were evident only during the August 1980 survey.

9-6-79: Data were unavailable for all but one station (Sta. 1) since samples were not run by the cadmium reduction method.

4-17-80: Concentrations were highest in the Inner Bay and showed the characteristic decline with distance from the Wash to Sta. 5. Values were somewhat more variable in the Middle and Outer Bay and displayed no spatial trends.

7-23 & 7-25-80: Concentrations showed the characteristic decline from Sta. 1a out from the Wash. Levels showed most rapid declines in the Inner Bay and decreased more gradually in the Middle and Outer Bay.

8-27-80: Concentrations showed the characteristic decline from Sta. 1 to Sta. 4b, and increased at Sta. 5. Values fluctuated in the Middle and Outer Bay and showed no significant spatial trends.

Overall: Concentrations were highest during the April 1980 survey and lowest during July 1980. All three surveys showed characteristic declines with distance from the Wash out to the Middle Bay. Beyond the Middle Bay, no spatial trends were evident.

APPENDIX C

Bioassay Reports

Section 1--ERA Bioassay Report
Section 2--EPA Bioassay Report

SECTION 1

ERA Bioassay Report

LAS VEGAS VALLEY WATER QUALITY PROGRAM

BIOASSAY TASK—FINAL REPORT

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I. INTRODUCTION

Discharge of municipal wastewater effluent into Las Vegas Bay of Lake Mead and low water transparency of inner Las Vegas Bay, associated by the casual observer with algal abundance, has focused attention on relationships between nutrient loading to the bay and the levels of algal growth associated with this enrichment. Identifying the factor or factors limiting phytoplankton levels in Las Vegas Bay is of critical importance to establishing a rational basis for setting criteria for receiving water quality. The following narrative presents a detailed final report on the Bioassay Studies Task of the Las Vegas Valley Water Quality Program.

OBJECTIVES

The objectives of the bioassay studies were twofold. First, to identify the nutrient, or combination of nutrients, that limits the growth of the indigenous phytoplankton in Las Vegas Bay and Boulder Basin of Lake Mead. Second, to determine the temporal and spatial patterns of such nutrient limitation throughout an annual cycle.

Many complex factors are involved in the regulation of the level of algal biomass (and chlorophyll) in an aquatic system. In the Bioassay Studies Task, no evaluation of the quantitative relationships between algal growth and the nutrient availability was made. Indeed a program to increase the scope of the Bioassay Task to include such quantitative investigation was proposed during the course of the study but not implemented. Therefore, the results of the Bioassay Studies cannot by themselves be used quantitatively to set ambient nutrient levels or nutrient loading levels from the Las Vegas Wash, however, the results are useful in developing treatment strategy.

II. BACKGROUND

Nutrient enrichment bioassay experiments are based on the application of the "Law of the Minimum". In simple form, this states that growth of algal cells will be limited or regulated by the essential nutrient element present in lowest concentration relative to the their nutritional requirement. It is important to recognize that a community of algae respond to nutrient limitation in relation to the specific nutrient requirements of the various species present. One species may be more responsive to a particular nutrient addition than another species present at the same time. Thus, because of species specific requirements, more than a single nutrient may increase growth of the community at any given time. The bioassays experiments can be combined with water quality data from the limnological program in order to clearly establish the relationship between algal growth and nutrient levels in Las Vegas Bay. The bioassay program was undertaken for this purpose.

Previous studies of the nutrient status of Lake Mead phytoplankton have been performed by UNLV personnel and also by ERA (1976). A nutrient enrichment study by Deacon and Tew (1973) demonstrated that algal growth at inner Las Vegas Bay stations was most severely limited by nitrate and less so by phosphate. It was suggested that a high phosphate input from Las Vegas Wash results in initial nitrogen limitation, but that phosphate may become limiting further into the Bay as nitrogen is assimilated. Support for this hypothesis is the observed high phosphate concentrations at Station 1 and 2 and the progressive dilution (and lowered phosphate concentrations) outward from the Bay to Boulder Basin. A report by Deacon (1976) describes batch bioassays, using indigenous phytoplankton incubated in the laboratory. Reported results suggest that during the summer (May-June) N was the primary limiting nutrient at both inner Las

Vegas Bay and Boulder Basin stations. Goldman (ERA, 1976) conducted nutrient enrichment bioassays on water samples from inner Las Vegas Bay and Virgin Basin. At Virgin Basin, additions of NO_3-N and PO_4-P alone, and in combination, substantially increased algal growth. Nitrate enrichment of inner Las Vegas Bay waters was shown to stimulate algal productivity. In that study it also appeared that nitrogen was the most limiting nutrient in the inner Las Vegas Bay due to the high direct P loading from Las Vegas Wash. Goldman (ERA, 1976) presented ratios of dissolved N:P and total N:P. The data showed that a ratio of approximately 8:1 was the point where the transition from N to P limitation occurs. This general range of about 8:1 for N to P requirements of natural phytoplankton populations in lakes may be considered reasonably typical. Both dissolved N:P or total N:P ratios indicated that nitrogen was the limiting nutrient during mid-summer in the inner and middle Las Vegas Bay waters. During the same time period the outer Bay and Boulder Basin stations with their higher N:P values were more P deficient.

III. APPROACH

The complexity of physical, chemical, and biological interactions in an aquatic system makes it difficult to determine algal growth limiting factors conclusively by direct measurements. Some physiological properties of algae have been used as indicators of the presence of particular nutrient deficiencies (Healey and Hendzel, 1980). These include sestonic P:C, N:C, and P:N ratios, alkaline phosphatase activity per unit biomass, and ATP per unit biomass (Healy, 1975). Many studies which attempt to identify what factor or group of factors limits phytoplankton growth use some form of bioassay procedure in which algal growth is monitored with and without the addition of the nutrients that are thought to be limiting. Several types of bioassays are reported in the literature and have been employed in a variety of ways. Major bioassay types include:

1. Laboratory algal assays using a test species (e.g., Selanastrum capricornutum) inoculated into the water from the lake under investigation. This technique has been standardized and outlined in the Algal Assay Bottle Test (Miller, Greene and Shiroyoma, 1978).
2. Enrichments of continuous cultures of the indigenous phytoplankton from the lake in question (Barlow et al., 1973; Dunstan and Menzel, 1971).
3. Enrichment of batch cultures of the indigenous phytoplankton in glass bottles or plastic containers, incubated in the laboratory or in situ (Goldman, 1969).
4. Enrichment of large plastic enclosures incubated in situ (Goldman, 1962; Schelske and Stoermer, 1971).
5. Experimental enrichments of whole lake ecosystems (Schindler, 1971).

All of the bioassay techniques monitor in some manner the growth response of the algal population for use as an index of the effect of enrichment. Parameters monitored can include chlorophyll a or in vivo fluorescence, ^{14}C uptake (Goldman, 1969), dry weight or particulate carbon, and direct microscopic cell counts.

Two types of bioassays were conducted by Ecological Research Associates (ERA) as part of the Las Vegas Valley Water Quality Program, in order to identify the specific nutrients controlling levels of algal biomass in Las Vegas Bay and Boulder Basin of Lake Mead, Nevada. First, bioassays using the indigenous algal community incubated at the surface of pools in one liter plastic cubitainers were conducted on a regular basis, bimonthly (November-March), from July 1979 to September 1980, and monthly (April-October). These regular bioassays were standardized, and designed so as to determine the spatial and temporal patterns of nutrient limitation (primarily N and P) in Lake Mead. Algal growth in these bioassays was monitored by measuring in vivo fluorescence in a Turner 111 fluorometer and also by measuring levels of chlorophyll a. Second, in situ bioassays in large plastic enclosures (1-1.5 m^3) were conducted on two occasions to obtain an "in-lake" corroboration of the results of regular pool-cubitainer bioassays, and also to compare results of in vivo fluorescence, chlorophyll a, alkaline phosphatase activity, primary productivity (^{14}C uptake in the light), ammonium enhancement of dark ^{14}C uptake, and nutrient measurements (soluble and particulate N & P).

The study was designed so that regular bioassays coupled with the two in situ bag experiments and the AAP tests, performed for the Water Quality Program by Greene and Merwin (ERA, 1981), would compliment each other in establishing spatial and seasonal patterns of nutrient limitation in Las Vegas Bay and Boulder Basin.

The water quality standards study (WQSS) was designed with nutrient enrichment bioassays coupled with the analysis of N:P ratios and other water quality and physiological data which provide a strategy to verify experimental results seasonally and along a gradient of ratio values from the inner Bay outwards to Boulder Basin.

IV. METHODS

From July 31, 1979 through September 10, 1980, bioassays were regularly performed to delineate the temporal and spatial patterns of nutrient limitation in Las Vegas Bay (Stations 2, 4, 5) and in Boulder Basin (Station 8) of Lake Mead, Nevada. Regular bioassays were performed using indigenous phytoplankton and consisted of enriching lakewater samples with nitrogen and phosphorus (singly and in combination), and with additions of filtered Las Vegas Wash (LVW) water. The bioassay containers (1 liter plastic cubitainers) were incubated on the surface of outdoor pools on the University of Nevada, Las Vegas campus. In vivo fluorescence was used as the main index of algal growth, with frequent correlation of fluorescence values to actual extractable chlorophyll a concentration.

STANDARD PROCEDURE FOR REGULAR BIOASSAYS

A) Bioassay cubitainers were soaked in 0.1 N HCl for 24 hours to remove contaminants. If cubitainers were previously used, then the inside walls were brushed to mechanically remove any residue adhering to container walls. Each cubitainer was then rinsed four times with tap water and 4 times with double distilled water (DDW).

B) The following nutrient stock solutions were prepared as needed and stored in refrigeration:

NH_4NO_3	10 mg atoms N $liter^{-1}$
NH_4NO_3	100 mg atoms N $liter^{-1}$
KH_2PO_4	10 mg atoms P $liter^{-1}$
KH_2PO_4	100 mg atoms P $liter^{-1}$
NaH_2PO_4	10 mg atoms P $liter^{-1}$
NaH_2PO_4	100 mg atoms P $liter^{-1}$

C) All lake samples were collected in carboys rinsed with tap water, DDW, and subsequently in the field with lake water.

D) Incubation was carried out in pools maintained at a temperature approximating the ambient surface lake temperature by regulating flow of cool well water into the pools.

COLLECTION OF LAS VEGAS WASH WATER

At approximately 0600-0700 on the day the bioassay was initiated, a 3-4 liter sample was collected from the Las Vegas Wash at the North Shore Road sampling station. The sample was placed in the dark on ice, and transported to the Clark County Sanitation District Treatment Plant. One liter of the sample was given to their laboratory for same day analyses of nitrate-N, nitrite N, ammonia N, organic N, total P and soluble phosphorus. The remaining sample was returned to the University laboratory where 750 ml were filtered immediately through a 4.25 cm diameter GF/c filter. Five hundred ml of this filtrate was frozen for subsequent analysis and 250 ml was refrigerated for use that night as an "LVW" enrichment.

COLLECTION OF LAKE MEAD BIOASSAY WATER

This was initiated immediately after dusk, sampling first at Station 8 and then at Stations 5, 4, and 2 consecutively. At each station a Van Dorn Water Sampler was used to collect water from 1, 3, and 5 meter depths to obtain an epilimnetic composite sample. Each haul was filtered through 80 μ mesh netting (to remove zooplankton) into a 25L carboy. For either Station 4 or 5 an additional 20 liter composite sample was taken for assay of a similarly treated replicate set of large cubitainers. The samples were returned immediately to the UNLV Laboratory, where the enrichments were immediately implemented.

NUTRIENT ENRICHMENTS

A) Approximately one hour prior to receiving the lakewater samples, empty bioassay cubitainers were "spiked" with appropriate nutrient enrichments using a calibrated 5 ml automatic pipette fitted with disposable tips. Nutrients were added according to the standard enrichment schedule indicated on page 9.

B) In low light at the UNLV Laboratory, lake water was dispensed to the appropriate cubitainers. Prior to transfer, the water was mixed thoroughly to resuspend any phytoplankton or debris. Using a prerinsed 1000 ml graduated cylinder, the contents of each cubitainer were brought to 900 ml with lake water, capped tightly and then agitated to distribute the enrichment uniformly. Where no enrichment was made (i.e., controls) contents were similarly agitated. Upon completing all enrichments for a station, the set of 24 cubitainers was immediately transferred to the incubation pools, and the next set of enrichments initiated.

C) After completing all enrichments, the remaining lake water for each station was again mixed thoroughly and subsampled for analysis as follows:

1) 125 ml transferred to a labeled screw cap polybottle, preserved with 1 ml Lugol's Reagent and stored at room temperature for later phytoplankton enumeration.

2) 500 ml transferred to an acid-rinsed, labeled polybottle and frozen for later water chemistry.

3) 300-500 ml, depending on phytoplankton density, was treated with magnesium carbonate and filtered through glass fiber filters at 100 mmHg. The filters were folded, wrapped in foil and frozen for later pigment analysis.

Station	Replicates	Enrichment	Treatment Name	Final Concentration
2,4,5,8	3	None	Control	
2,4,5,8	3	5ml 10 mg/l N-stock	N_1	55 µg/l
2,4,5,8	3	1.5ml 100 mg/l N-stock	N_2	166 µg/l
2,4,5,8	3	5ml 10 mg/l P-stock	P_1	55 µg/l
2,4,5,8	3	1.5ml 100 mg/l P-stock	P_2	166 µg/l
2,4,5,8	3	1.5ml 100 mg/l N-stock and 5 ml 10 mg/l P-stock	$N_2 + P_1$	166 µg/l N ± 55 µg/l P
2,4,5,8	3	18ml of filtered Las Vegas Wash	LVW	1:50 dilution
2,4,5,8	3	(*see below)	N + P*	Variable
4 or 5	3	4x any enrichment above to 3600L	Lg. Cub	

* The N + P* enrichment consisted of an addition of N + P corresponding to the weight of soluble N & P contained in 18 ml of a Las Vegas Wash enrichment, as determined by C.C.S.D. analytical results. Soluble nitrogen was calculated as the concentration sum of NO_3 + NO_2 + NH_4 and soluble orthophosphorus was reported directly. This N + P* treatment was developed to yield in the bioassay cubitainers, approximately the same final concentration of N + P as in the LVW enrichments. The effect on algal growth of other constitutents in Las Vegas Wash waters (besides N + P) could then be ascertained.

4) Three 8 ml replicates transferred to optical grade tubes and in vivo fluorescence determined using a Turner fluorometer (Model 111-003) with a high sensitivity door and appropriate scale.

INCUBATION

Samples were incubated for 5-6 days floating at the surface of outdoor "doughboy" pools situated adjacent to the lab. Parachute netting was draped above the pools to reduce intense solar radiation. Constant circulation of water in the pools caused continuous rotation of the cubitainers around the pool surface. This assured equal sun exposure for each sample. In vivo fluorescence was monitored daily as an estimate of algal growth, and 300-500 ml subsamples from the large cubitainer were taken daily, treated with magnesium carbonate, GF/c filtered and frozen for later pigment extraction, to provide necessary chlorophyll-a fluorescence correlation.

FINAL TREATMENT

Bioassays were terminated following 5-6 days of incubation, by which time fluorescence had usually reached a peak and had begun to decline. After making final fluorescence measurements, all cubitainers were removed from the incubation pools and subsampled as follows:

1) 300-500 ml from the "large cubitainers" was treated with magnesium carbonate, GF-C filtered and frozen for later pigment extraction.
2) 300-500 ml from several cubitainers exhibiting a wide range of fluorescence values were treated with magnesium carbonate and handled as above.
3) 80 ml from each replicate of the control, N_2 and P_2 treatments for each station were composited in 250 ml (acid rinsed) labeled, polybottles, and frozen for later water chemistry analysis.

4) 10 ml from each of the 3 replicate treatments for each nutrient added for all stations were composited in small labeled vials, treated with 0.5 ml Lugol's Reagent, capped, and stored at room temperature for later phytoplankton enumeration.

ESTIMATION OF CHLOROPHYLL CONCENTRATION BY IN VIVO FLUORESCENCE

Chlorophyll, like many organic molecules, fluoresces. Unlike most natural substances, chlorophyll fluoresces at very long wavelengths; with maximum in vivo emission at 685 mm when excited by 436 mm radiation from a mercury lamp (Goedheer, 1964). Natural porphyrins (other than chlorophyll) and the degradation products of chlorophyll typically provide interfering fluorescence in natural waters, but these porphyrin not usually abundant (Lorenzen, 1966). In addition, humic substances can affect fluorescence measurements of chlorophyll, though this is not a likely problem in Lake Mead. In vivo fluorescence, therefore, may be used as a direct estimation of chlorophyll and phaeophyton content in water. The method is a modified application of that described by Lorenzen (1966). Subsamples of water containing phytoplankton are transferred to optic quality test tubes and fluorescence measured in a Turner 111 fluorometer. The subsample is excited with a blue fluorescent lamp #F4T5B emitting light through a blue (Corning CS 5-60) primary filter with maximum transmission at 430 mm. Fluorescent emissions pass through a red (Corning CS 2-64) secondary filter (opaque to light at wavelengths shorter than 645 mm and reaching its maximum transmission at 650 mM) and are collected at a red sensitive photomultiplier (R136).

MATERIALS

A. Turner 111 fluorometer equipped with a:

- Red sensitive photomultiplier #R136
- Blue fluorescent lamp #F4T5B
- Blue (Corning CS 5-60) primary filter
- Red (Corning CS 2-64) secondary filter

B. STD neutral density filters

C. Spectronic 20 or comparable optic quality test tubes

D. Oxford• or similar 10 ml automatic pipette (to dispense aliquots rapidly)

E. Kimwipes• tissue for cleaning tubes

F. DDW Rinse (for cleaning pipette tip between samples)

G. Millipore• filtration apparatus equipped for holding 4.25 cm Dia GF/C filters

H. Millipore• 4.25 cm Dia GF/C filters

I. Clean side armed flask

PROCEDURE

The standard procedure consisted of the following five steps:

1. Turn on both the fluorometer and the excitation lamp, allow to warm up for at least two minutes prior to use. Turn off any bright or fluorescent lights in the area of analysis.

2. Prepare a blank for zeroing the instrument. Double distilled water may be used where expected background of interfering fluorescence from molecules other than chlorophyll in the medium is low. For the bioassay studies, as a natural correction for background, a filtered lake water blank was prepared using "initial" lake water collected from either Station 5 or 8. Approximately 50 ml of lake sample were filtered gently at 5 lb in^{-2} through a 4.25 cm diameter

GF/C filter into a clean side-armed flask. The filtrate was then transferred to a clean, dry, spectronic 20 (optical grade) test tube, and capped.

3. Samples to be analyzed are agitated to suspend phytoplankton uniformly. Eight ml of sample are then withdrawn using an automatic pipette and dispensed into a clean spectronic 20 test tube. Depending upon fluorescence variation per sample through time, groupings of 20-24 subsamples may be transferred to test tubes at a time and fluorescence subsequently determined rapidly in succession.

4. The fluorometer is zeroed using the blank, with the sensitivity knobs set at the least sensitive IX position. The sample is then inserted into the fluorometer and fluorescence determined. Fluorescence is most accurately recorded when values obtained register within the range 10-90 units. Thus, samples registering less than 10 units at 1X sensitivity are generally remeasured at sensitivities of 3X, 10X or 30X if necessary to bring the value within the desired range. For example, a sample registering 9 units on the 1X scale would exhibit a value of 27 units on the 3X scale. Values registering above the desired range at IX sensitivity may be brought into conformance by using neutral density filters added either on the primary or secondary filter side. Each time the sensitivity is changed or a neutral density filter added, the instrument must be re-zeroed.

5. Fluorescence measurements were generally made in groupings of samples according to station. Values for all samples of a particular station were determined in rapid succession.

FLUORESCENCE VS. CHLOROPHYLL CORRELATION

Included in Appendix A is a plot of the correlation between in vivo fluorescence and uncorrected chlorophyll concentration, for bioassays conducted in 1980. The second figure in Appendix A shows the relationship between initial values of fluorescence and chlorophyll, and the respective values after one day of incubation in the cubitainers. It can be seen that there is a strong correlation ($r = .883$) between in vivo fluorescence and level of chlorophyll, and that the initial decline in fluorescence also reflects a decrease in pigment concentration. In spite of this fact, there is considerable scatter in the points on the fluorescence versus chlorophyll plot. This is probably due to the fact that differences in cellular fluorescence have been shown to reflect variations between unialgal cultures (Strickland, 1968), successional change in phytoplankton species (Flemer, 1969) and algal physiological condition, and nutritional deficiency (Kiefer, 1973). For the purposes of our bioassay analysis chlorophyll a concentration is most accurately reflected by measurements of fluorescence made at the time the bioassay was conducted. It is important to note that our analysis for the most part is based upon an interpretation of relative algal growth response as compared to a control, and this does not necessitate a precise knowledge of absolute chlorophyll concentration.

V. RESULTS AND DISCUSSION

The following section details the results obtained for the three principal components of the Bioassay Task--the regular and special bioassays and the in situ experiments. A graphical representation of each bioassay is included in Appendix B.

REGULAR BIOASSAYS

Results of the regular bioassays performed by ERA between July 1979 and September 1980 clearly show the character of nutrient limitation, specifically with regard to the capacity for algal growth enhancement by additions of the nutrients N and P, either singly or in combination. Table 1 lists those N + P treatments for which in each bioassay the growth response measured significantly different from the control (student's two-tailed t-test, 95% level). Statistical analysis of each bioassay is included in Appendix C and detailed methods for each in Appendix D. Figure 1 shows the maximum algal growth response to enrichment (as a fraction of the mean value for the controls) as a function of time, for N and P additions to lakewater samples from Stations 2, 4, 5, and 8 for all treatments listed in Table 1, for all regular bioassays performed. Figure 2 shows time plots of the values for dissolved N:P ratios (NO_3 + NO_2 + NH_3: Ortho P). Two major conclusions can be drawn from these data. First, there appears to be a strong seasonal determinant to the patterns of N and P stimulation. In general, nitrogen is the principal nutrient which alone stimulates algal growth in bioassay cultures during the period from mid-summer to late fall in Las Vegas Bay. After destratification of the lake in autumn, phosphorus assumes the role as the principal limiting nutrient, as evidenced by the capacity for stimulation of bioassay cultures with P alone. The establishment of stable stratification in mid-summer and the resultant depletion of available dissolved

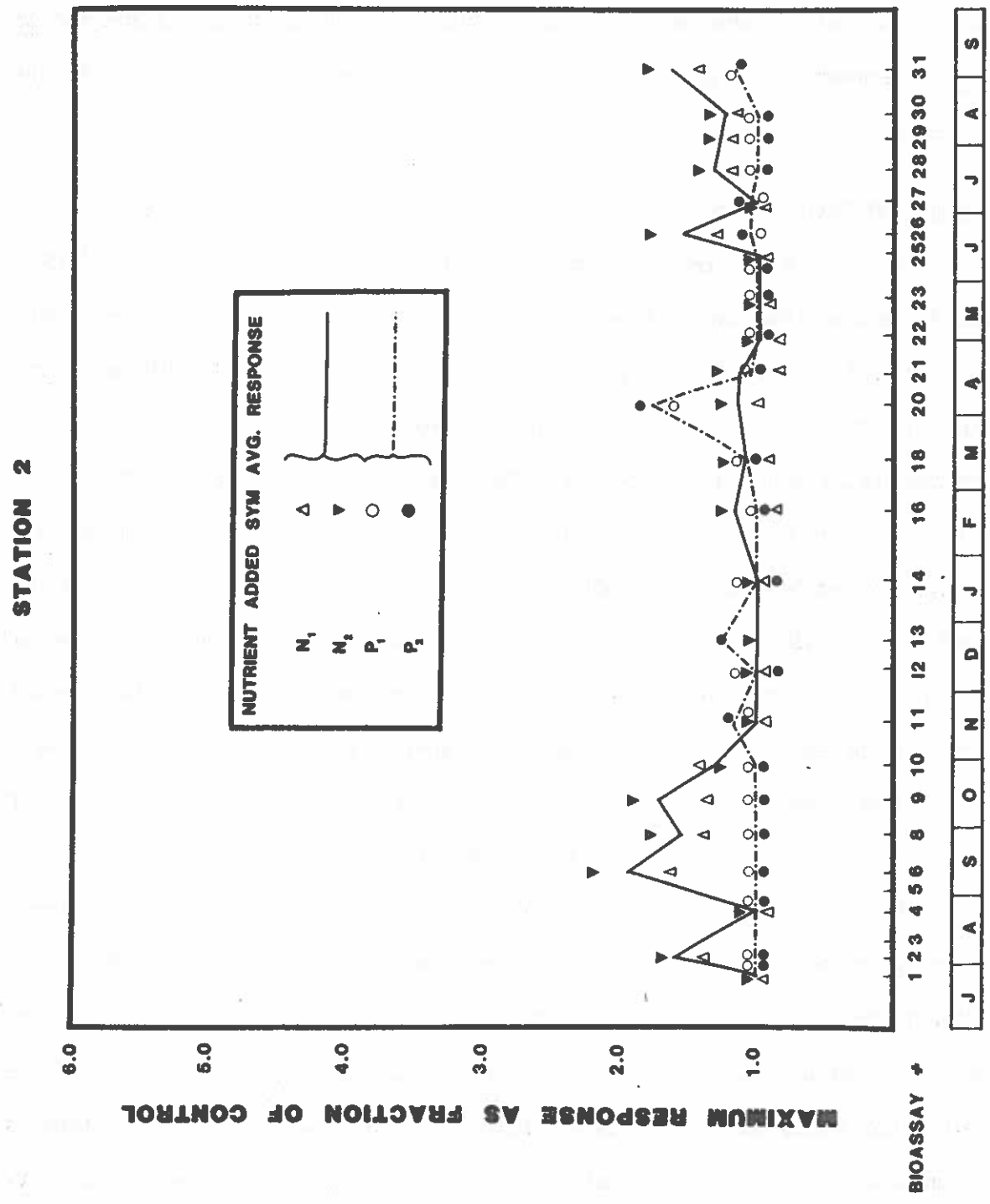


Figure 1. Maximum Response as a fraction of Control vs. Time ($N_{1,2}$, $P_{1,2}$ Treatments)

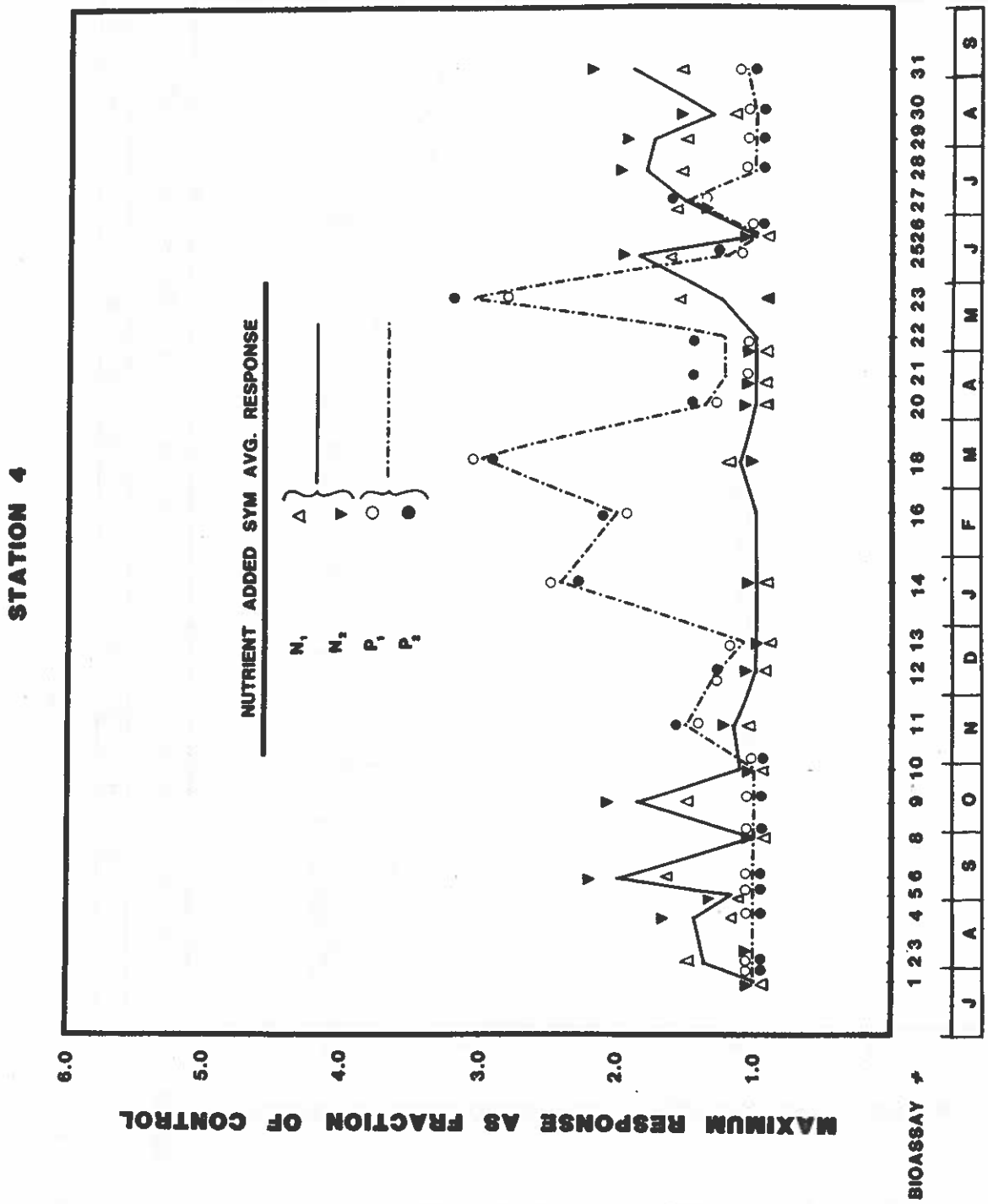


Figure 1. Maximum Response as a fraction of Control vs. Time ($N_{1,2}$, $P_{1,2}$ Treatments)

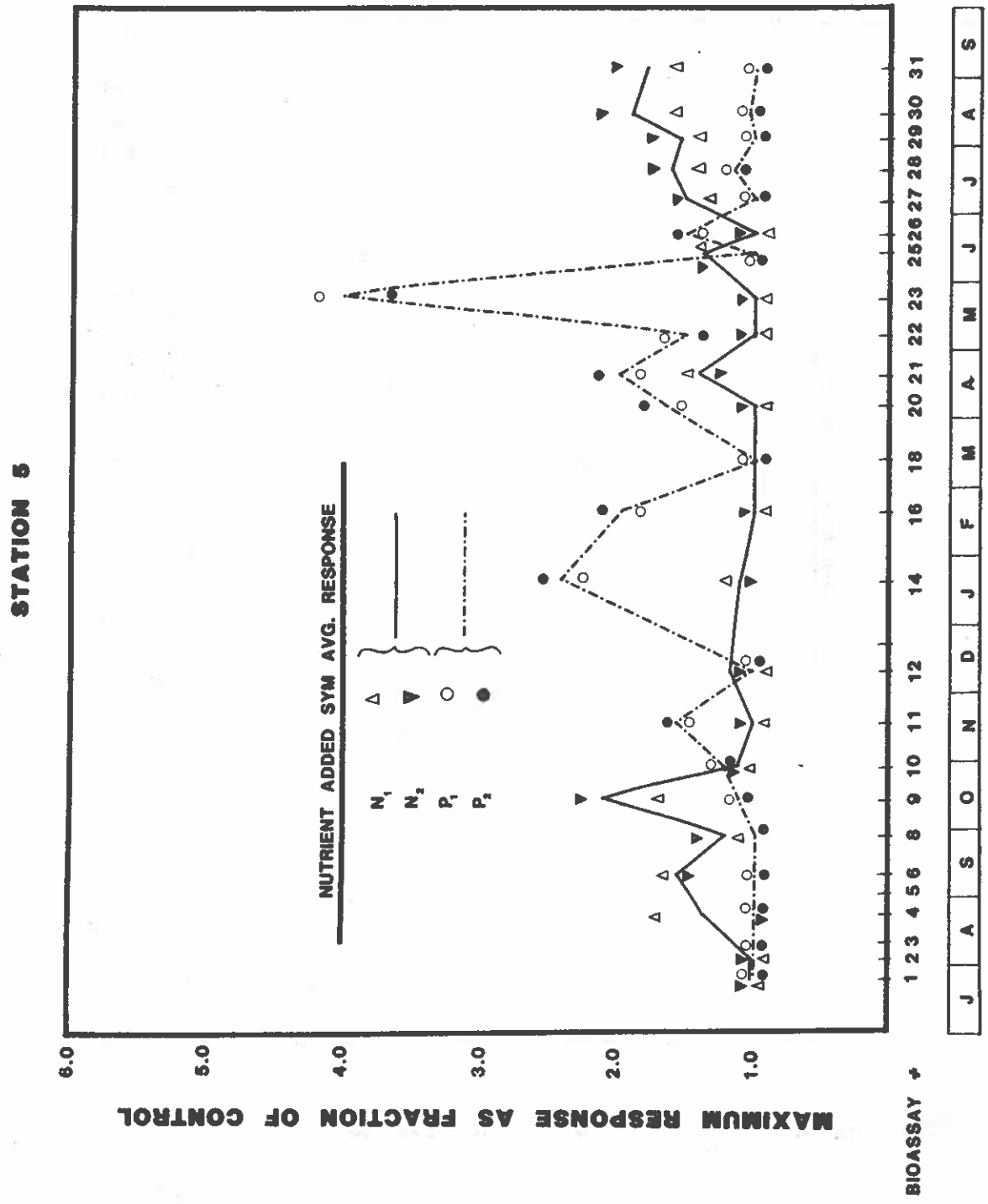


Figure 1. Maximum Response as a fraction of Control vs. Time ($N_{1,2}$, $P_{1,2}$ Treatments)

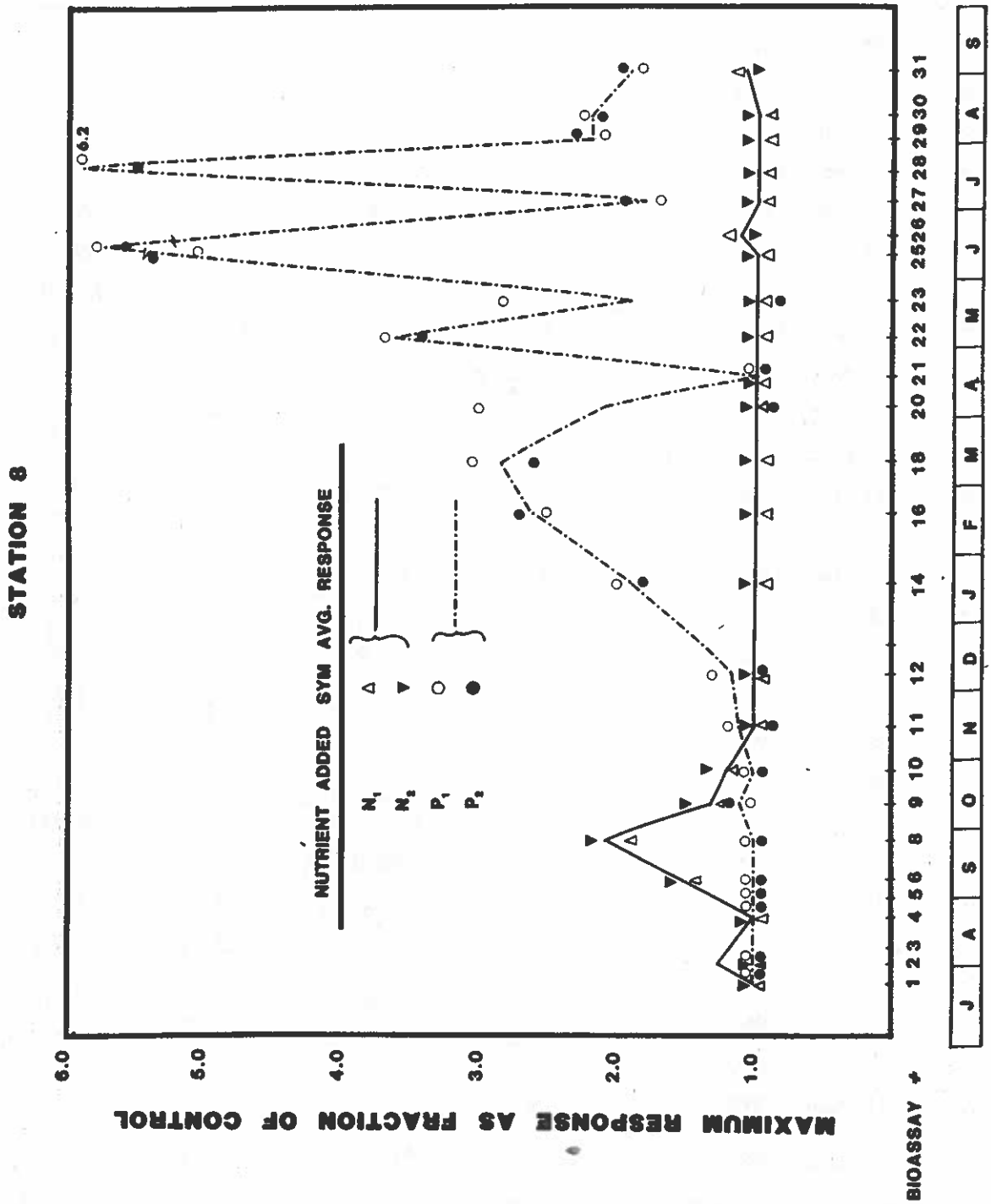


Figure 1. Maximum Response as a fraction of Control vs. Time ($N_{1,2}$, $P_{1,2}$ Treatments)

Table 1

LIST OF TREATMENTS YIELDING SIGNIFICANT* ALGAL GROWTH RESPONSE AS COMPARED TO CONTROL

Date	Bioassay No.	Station 2	Station 4	Station 5	Station 8
23 Jul - 28 Jul, 1979	1				
30 Jul - 4 Aug, 1979	2	$N_{1,2}$	N_1		N_1
21 Aug - 25 Aug, 1979	4		N_2	N_1	
30 Aug - 4 Sep, 1979	5		N_2		
6 Sep - 11 Sep, 1979	6	$N_{1,2}$	$N_{1,2}$	$N_{1,2}$	$N_{1,2}$
24 Sep - 29 Sep, 1979	8	$N_{1,2}$		N_2	$N_{1,2}$
10 Oct - 15 Oct, 1979	9	$N_{1,2}$	$N_{1,2}$	$N_{1,2}$ $P_{1,2}$	N_2 P_2
25 Oct - 1 Nov, 1979	10	$N_{1,2}$	N_1	N_2 $P_{1,2}$	N_2
15 Oct - 21 Nov, 1979	11	P_2	$N_{1,2}$ $P_{1,2}$	$P_{1,2}$	P_1
6 Dec - 13 Dec, 1979	12		$P_{1,2}$	N_1	P_1
19 Dec - 23 Dec, 1979	13	P_2	P_2		
15 Jan - 21 Jan, 1980	14		$P_{1,2}$	N_1 $P_{1,2}$	$P_{1,2}$
15 Feb - 21 Feb, 1980	16	N_2	$P_{1,2}$	$P_{1,2}$	$P_{1,2}$
13 Mar - 18 Mar, 1980	18	N_2 P_1	N_1 $P_{1,2}$		$P_{1,2}$
3 Apr - 8 Apr, 1980	20	N_2 $P_{1,2}$	$P_{1,2}$	$P_{1,2}$	P_1
17 Apr - 22 Apr, 1980	21	N_2 P_1	P_2	$N_{1,2}$ $P_{1,2}$	
2 May - 8 May, 1980	22		P_2	$P_{1,2}$	$P_{1,2}$
20 May - 26 May, 1980	23		N_1 $P_{1,2}$	$P_{1,2}$	P_1
20 May - 26 May, 1980	24		$N_{a,b}$ $P_{a,b,c}$ $Fe_{1,2}$ $Si_{1,2}$ NaEDTA		N_b $P_{b,c}$ NaEDTA
10 Jun - 14 Jun, 1980	25		$N_{1,2}$ $P_{1,2}$	$N_{1,2}$	$P_{1,2}$
19 Jun - 23 Jun, 1980	26	$N_{1,2}$ P_2		$P_{1,2}$	N_1 $P_{1,2}$
1 Jul - 8 Jul, 1980	27	P_2	$N_{1,2}$ $P_{1,2}$	$N_{1,2}$	$P_{1,2}$
18 Jul - 22 Jul, 1980	28	$N_{1,2}$	$N_{1,2}$	$N_{1,2}$ $P_{1,2}$	$P_{1,2}$
31 Jul - 4 Aug, 1980	29	$N_{1,2}$	$N_{1,2}$	$N_{1,2}$	$P_{1,2}$
7 Aug - 11 Aug, 1980	30	$N_{1,2}$	$N_{1,2}$	$N_{1,2}$ P_1	$P_{1,2}$
3 Sep - 7 Sep, 1980	31	$N_{1,2}$ $P_{1,2}$	$N_{1,2}$ $P_{1,2}$	$N_{1,2}$	N_1 $P_{1,2}$

*Student's Two-tailed T-test, 95% level

nitrogen in euphotic waters signals a return to a period of N (by itself) limitation. It follows then that periods of N and P colimitation occur in the fall as turnover proceeds and in the late spring and early summer while stratification is being developed.

Second, comparison of the profile of the maximum algal response (Figure 1) to the profile of the dissolved N:P ratios (Figure 2), indicates that the transition from N to P limitation at fall turnover parallels a relatively rapid increase in the dissolved N:P values, and in the same manner the changeover from P to N limitation in the summer occurs soon after the sharp decline of the N:P ratio as nitrogen is rapidly depleted in the surface waters.

It is of particular interest to closely examine the period of destratification where the mixing of nutrients stored in the hypolimnion into photic waters, leads to both an increase in the dissolved N:P ratios and a dramatic algal bloom which may be uniquely attributed to this pulse of nitrogen. The regular bioassays (Figure 1) showed nitrogen was the principal limiting nutrient at all Stations 2, 4, 5, and 8 in the period preceding the fall bloom in 1979. Indeed, the bloom serves as a kind of "whole-lake" bioassay which can corroborate the results of the regular bioassay program. For example, at fall turnover levels of inorganic N at Station 8 increased from 12 µg/l on 16 October to 41 µg/l on 25 October, and to 90 µg/l by November 14, 1979. This was accompanied by a bloom of algae with increases in chlorophyll a and in vivo fluorescence paralleling sharp increases in primary productivity (800 mg/m^2/day in October to nearly 1900 mg/m^2/day by November 11). During the same period, levels of dissolved P remained for the most part unchanged, below values of 2-4 µg/l. Since light and temperature regimen also did not change significantly during this time, the bloom was most very likely due to the influx of inorganic N as mixing proceeded during destratification. This in-lake "natural" bioassay did in fact show how

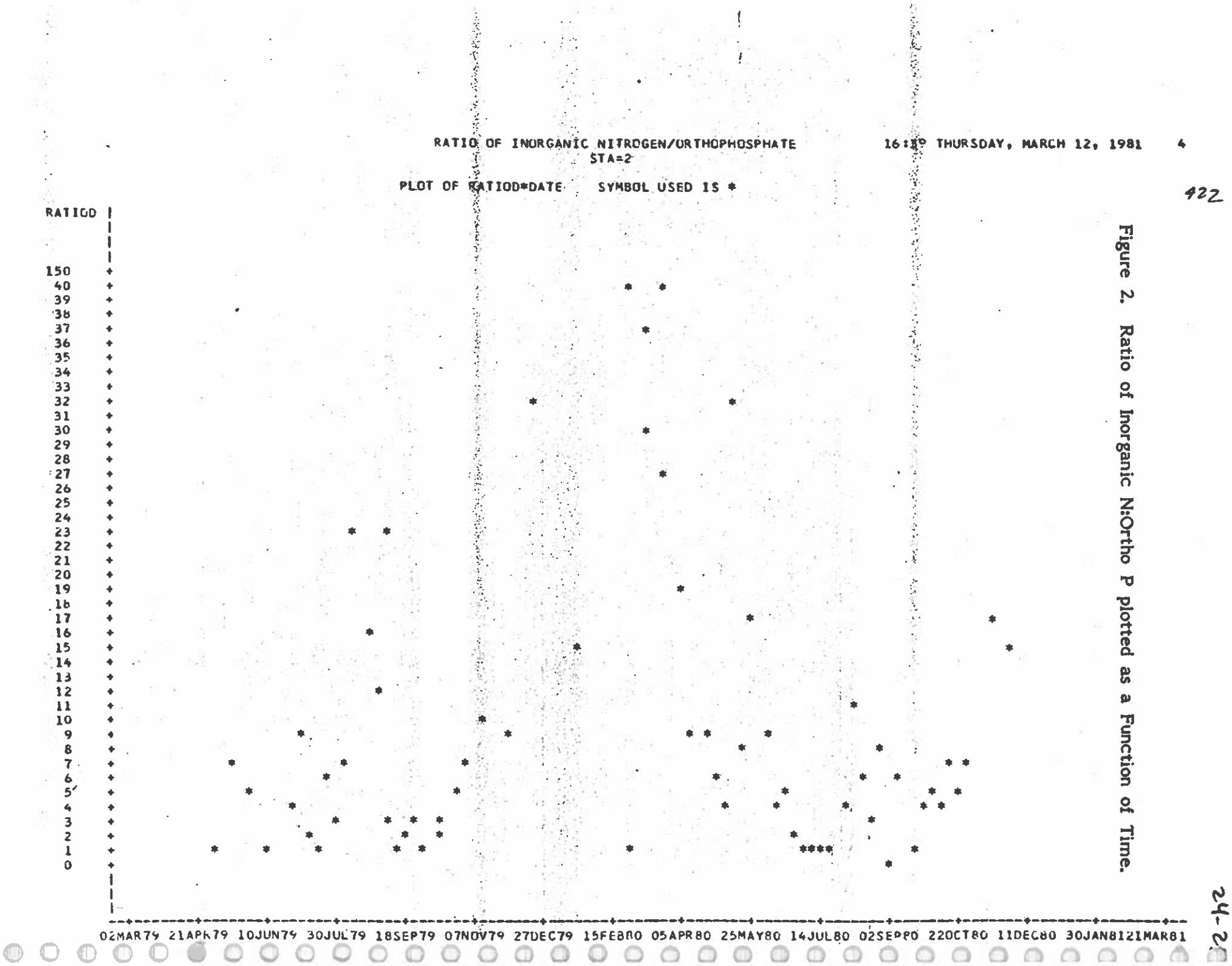


Figure 2. Ratio of Inorganic N:Ortho P plotted as a Function of Time.

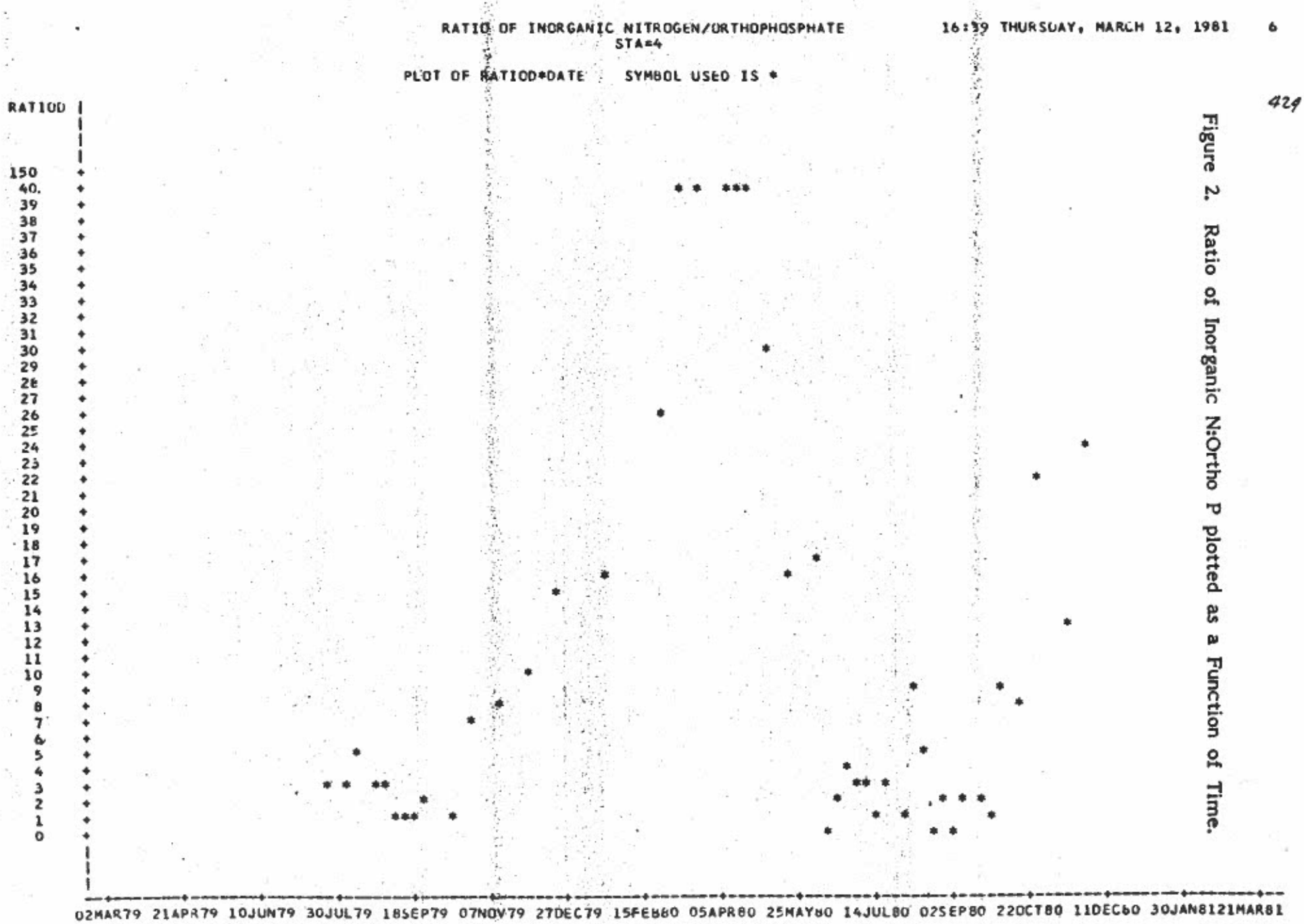


Figure 2. Ratio of Inorganic N:Ortho P plotted as a Function of Time.

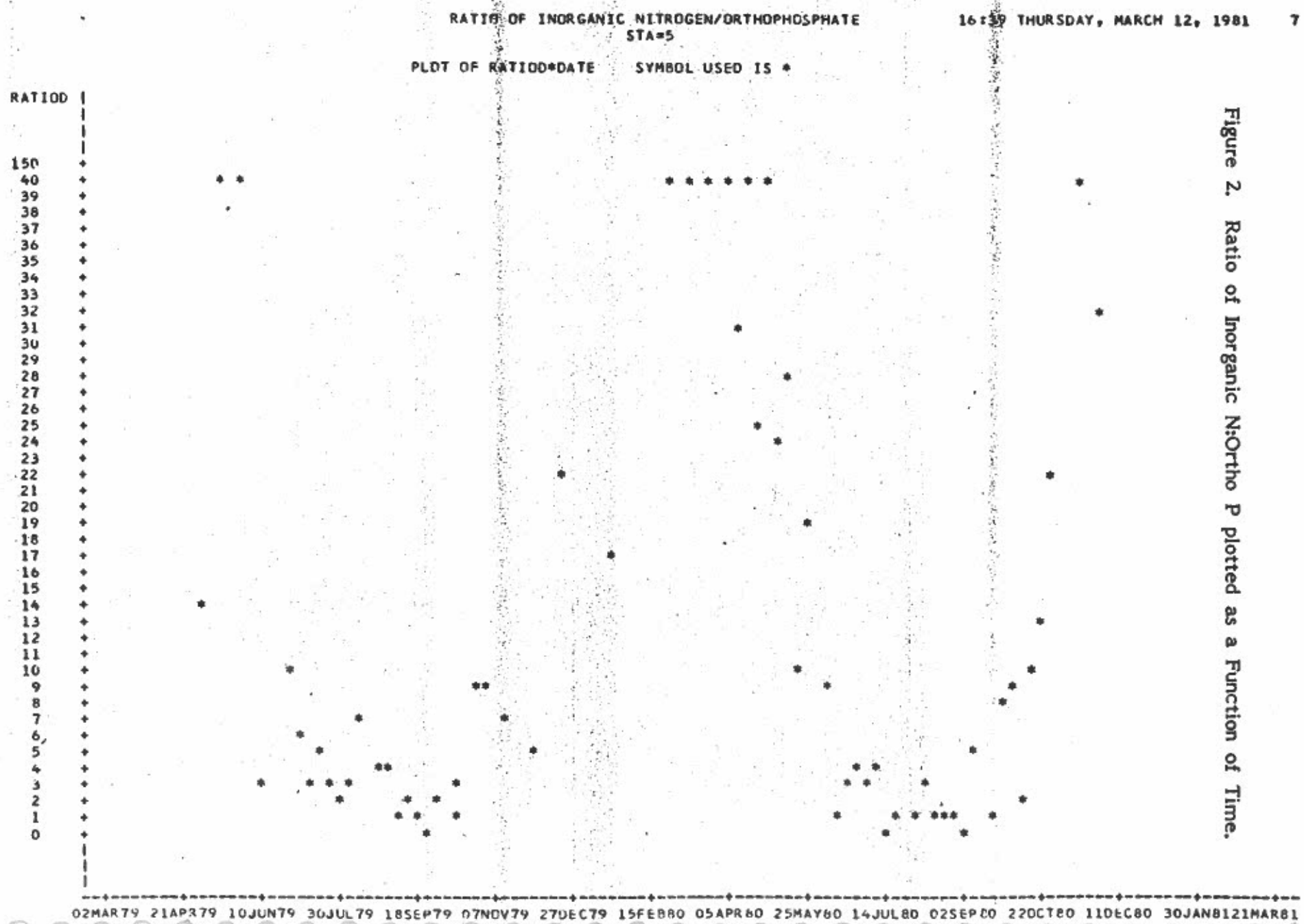


Figure 2. Ratio of Inorganic N:Ortho P plotted as a Function of Time.

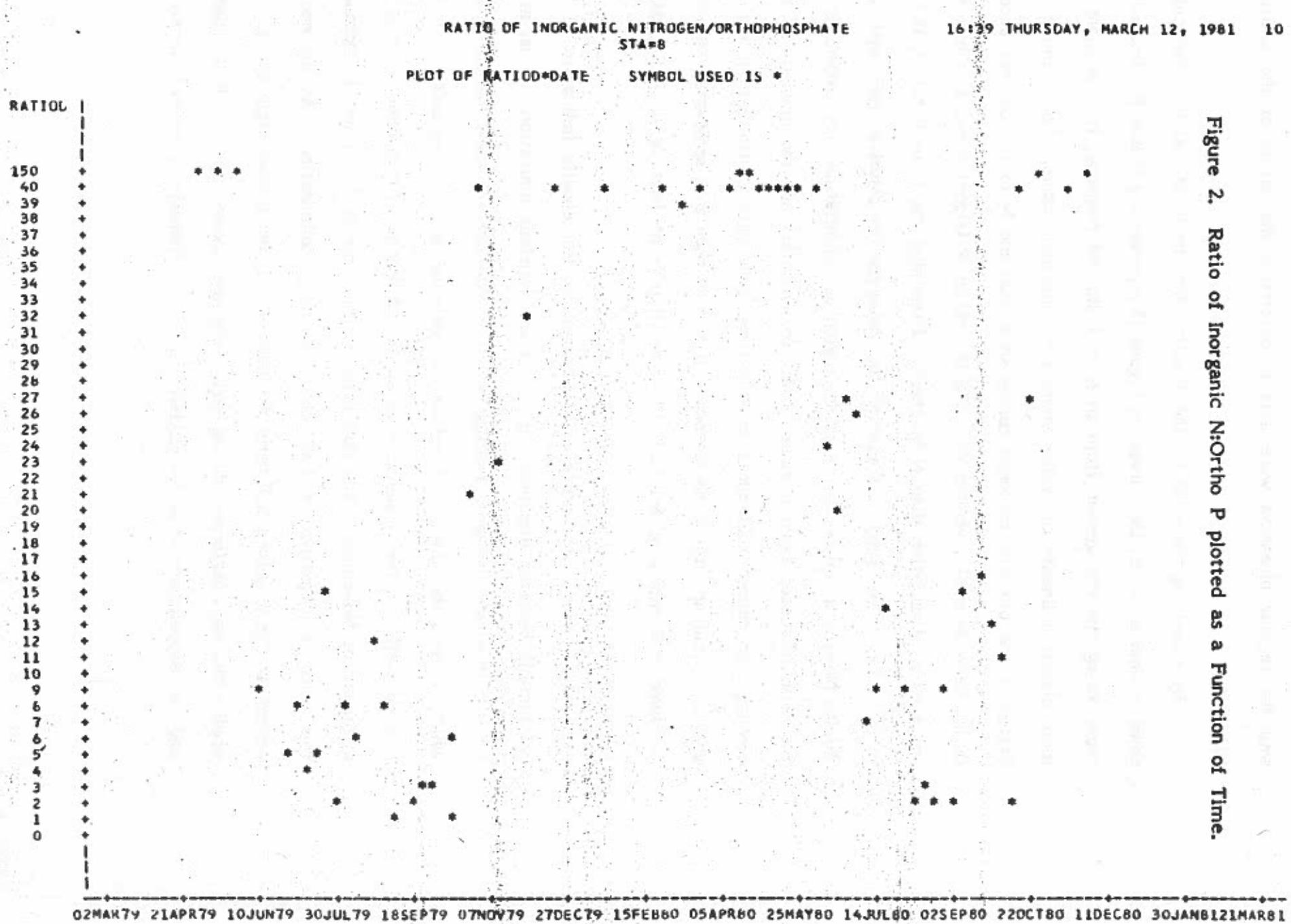


Figure 2. Ratio of Inorganic N:Ortho P plotted as a Function of Time.

well the regular bioassays were able to determine the nature of the limiting nutrient.

By examining the value for the dissolved N:ortho P ratio at that particular point in time at which the curves (in Figure 1) representing N and P stimulation cross during the changeover from an N to P limited response, it is possible to more clearly delineate the value where the transition occurs. For example, at Station 4 the bioassay response curves show that the N to P crossover occurs during early to mid-November of 1979, thereafter P (alone) shows a significant and greater stimulation than N by itself. Examining the N to P ratios, it can be seen that at the time of transition the value for the dissolved N:P ratio was 8:1 (on November 14), evidently in the region of colimitation. By December 19 the ratio increased from a value of 8:1 to over 14:1 and the majority of the bioassays conducted subsequent to this time show only P stimulation, with N additions yielding little or no response. It follows then that somewhere between dissolved N:P ratios of 8:1-13:1, the transition point from N to P limitation must occur.

At Station 5 the pattern is very similar and equally indicative of the relationship between dissolved N:P ratios and nutrient limitation. It is, after all, the dissolved inorganic fraction which is available for algal utilization and which controls the kinetics of uptake and assimilation by phytoplankton. In the case of Station 5, the N and P bioassay curves (Figure 1) crossover right at the beginning of November. The N:P ratio at this time is 9.25:1 (on 31 October), this value is indicative of a region of N and P colimitation. At the end of November the dissolved N:P ratio declined to 5:1 and interestingly enough, the regular bioassays performed during early December showed a loss of P stimulation and the reappearance of an N-deficient condition. Thereafter, the ratio increased

to 22:1 by December 19 and remained above 9:1 until June. During this latter period the bioassay responses indicated that P alone was the limiting nutrient.

The pattern is again very similar at Station 8 (Boulder Basin). In this case, the transition rate is attained in late October and the bioassay curves actually cross over (Figure 1) in early November. This dissolved N:P ratio is shown to increase from 1.3:1 on October 10, 1979 to 6:1 on October 16 and 20:1 on October 25. Regular Bioassay 11, conducted in mid-November shows that at this time (N:P ratio now at ~22.1) P (by itself) is the limiting nutrient. The ratio remains greater than 20:1 until 9 July 1980, and during this whole winter-spring period, nitrogen alone is never observed to stimulate algal growth.

At Station 2 (in the inner Bay) the response curves are seen to cross in mid-November, 1979, at which time the N:P ratio is 10.4:1. At Station 2, the absence for the most part of a prolonged period of P (alone) stimulation (Figure 1) is most probably due to the fact that in the inner Bay the influence of the Las Vegas Wash plume is strongest and the mean value of nutrient levels is high ($\bar{x}$ - 214 µg/l diss. N and 35 µg/l ortho P). Loading of total N:total P from the Las Vegas Wash (Northshore Road) to the inner Bay is at the ratio of approximately 6:1. Another important factor to be considered at Station 2, is that given the ample supply of nutrients for growth at optimal summer temperatures, constant loading from Wash should lead to constant and dense bloom conditions. However, the low secchi depths (<2 m) observed at Station 2 during most of the growing season would tend to result in a significant light limitation, since with a mixed layer depth of 8 m, algae would spend much of their time below light levels of 1% which exist below about 4m. Indeed, it is possible that the phenomenon of "self-shading" occurring as algal blooms progress in the inner Bay, may account for the extreme fluctuation of levels of biomass where algal blooms and die-offs occur rapidly.

In the regular pool-cubitainer bioassay method performed, the assay cultures were incubated at the surface of the pools; therefore, the growth response in nutrient enriched samples may be enhanced as a result of relieving the effect of light limitation. The fact that in many cases algal growth of the unenriched controls at Station 2 increases during incubation (in contrast to the mostly level control response at Stations 4, 5, and 8, see Bioassays 16, 18, 21, 23, 24, 27) points to a condition where the surplus nutrient supply in the inner Bay could support even higher algal levels were it not for light limitation.

The transition from P to N limitation occurs in the summer, soon after the precipitous decline of dissolved N:ortho P ratios, as nitrogen is depleted in the surface waters by algal assimilation. At Station 4 the ratio falls from 30:1 on May 7, 1980 to 17:1 on June 4 and 0.3:1 on June 11. The bioassay response curves are similarly indicative, showing that crossover occurs during the first week of June when the ratio drops below about 10:1. The ratio remains less than 5:1 (except for one occasion) throughout the summer. The regular bioassays (Table 1) show that both N and P (each alone) are colimiting in late May and early June, but by mid-July, N (by itself) is the main nutrient which enhances algal growth.

At Station 5 the transition from P to N limitation is equally clear. On May 24 the ratio is 28:1, decreasing to 10.5:1 on May 21, and 9:1 on June 4, and then finally falling to 0.5:1 on June 11. The bioassay response curves (Figure 1) indicate the P to N transition by crossing over during early June when the ratio is nearly 9:1. The ratio does not exceed 10:1 until late October of 1980. Regular bioassays indicate that by Bioassay 27 (in early July) N had become the principal limiting nutrient and it remained so all summer long.

At Station 8 the above pattern deviates somewhat. Data show that for the summer of 1979, N (alone) stimulation occurred at times when the dissolved

N:ortho P ratio was less than 10:1. However, although the ratios fell below 10:1 in early June of 1980, N stimulation did not appear until Bioassay 31 performed in September. An examination of the dissolved N:ortho P ratios helps to explain this phenomenon. In 1979 the ratios fell to low levels (<10:1) in mid-June and remained so until mid-October, with mean values of 5.9:1 during this whole period. By contrast, in 1980, the ratios did not drop below 10:1 until early July and then as soon as early September increased to greater than 10:1. The mean ratio value for the summer period (mid-June through mid-October) for 1980 is 11.8:1, this high value possibly explaining the delayed response to N (alone) enrichment in 1980 summer bioassays.

Figure 3 shows the temporal profiles for the algal growth response to enrichments by the nutrients N + P in combination; (N_2 + P_1), a 1:50 dilution of Las Vegas Wash (LVW) water (Northshore Road, filtered), and an addition of (N + P*) formulated to yield nitrogen and phosphorus concentrations which approximate those levels of N and P in the LVW (1:50 dilution) treatments. It can be seen by comparison of the algal growth response at the four stations 2, 4, 5, and 8, that while at Station 2 the response to the LVW and N + P* treatments was of similar magnitude, the values diverge progressively outwards from Station 4 to Station 8. At Station 8 the LVW treatment is at times several fold higher than the N + P*, but the N + P* and N_2 + P_1 treatments are usually much closer in value. This implies that at Stations 5 and 8, which are <u>not strongly influenced</u> by the LV Wash plume, there may be limiting factors other than N and P which are supplied to inner Bay waters but not to waters of Boulder Basin. Factors, such as vitamins, may be limiting algal growth and indeed in special Bioassay 24, the response of Station 8 water with a multi-vitamin addition (see Appendix D) was 8-fold that of the control.

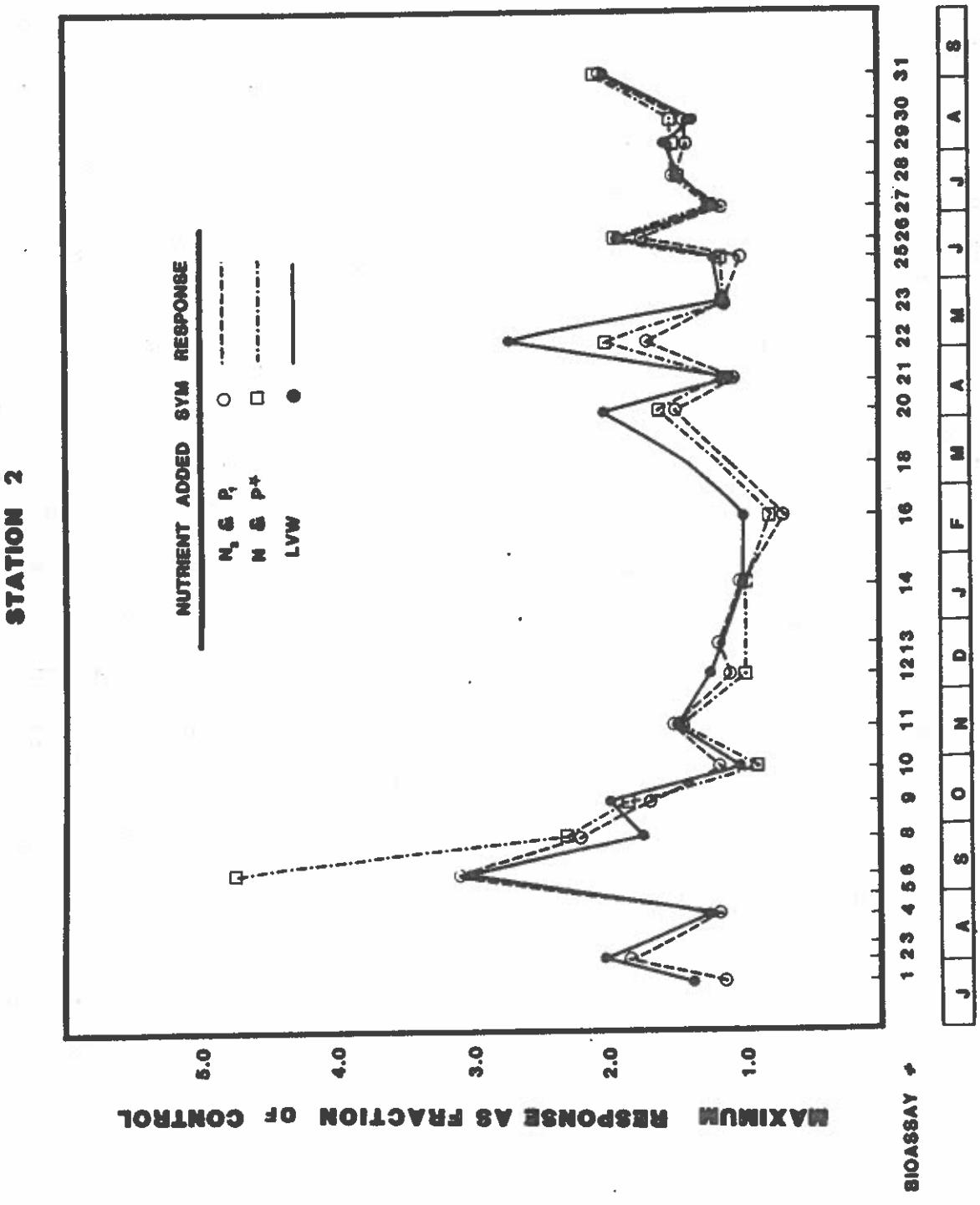
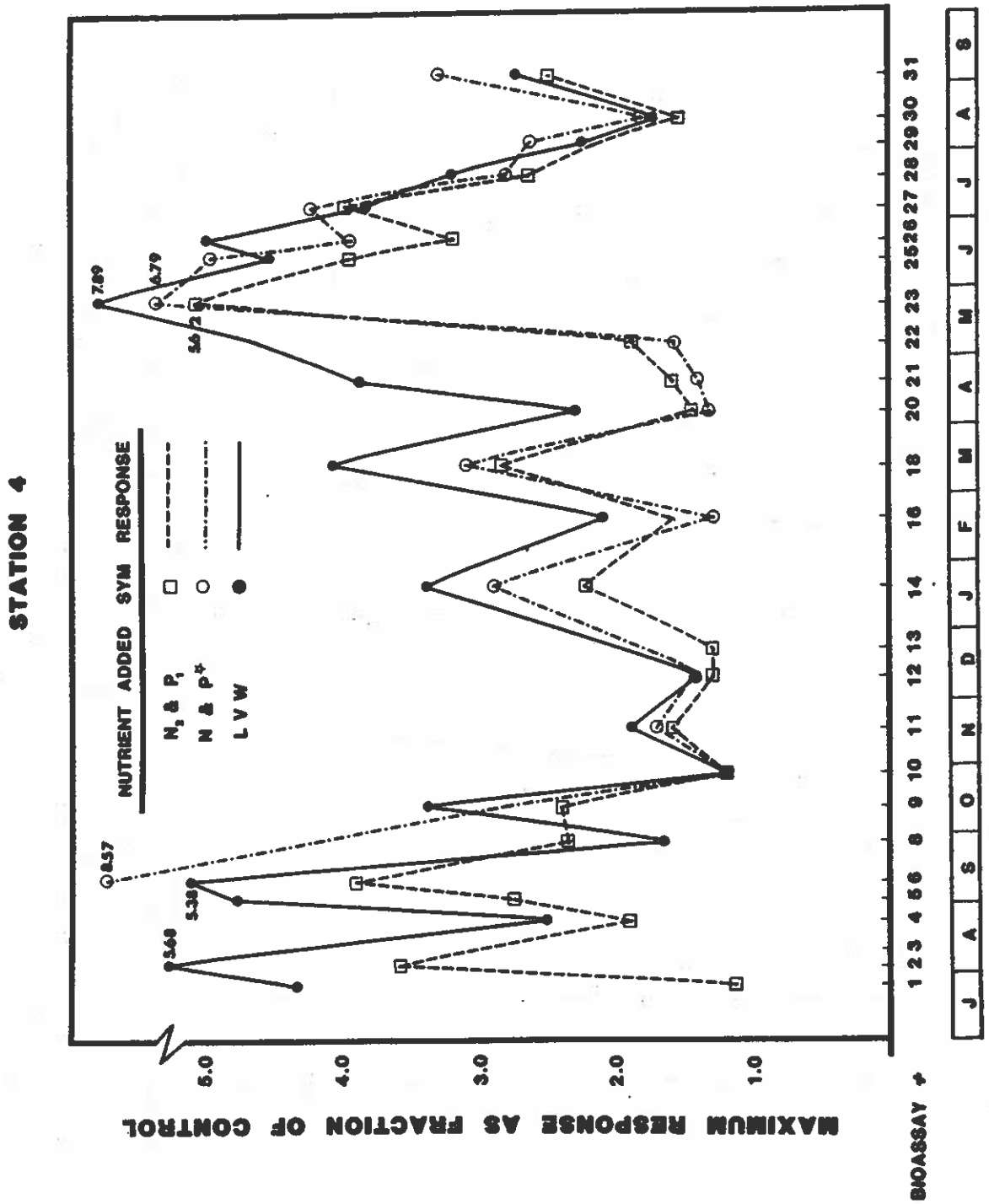


Figure 3. Maximum response as a fraction of control (N + P Combination, LVW Treatments)



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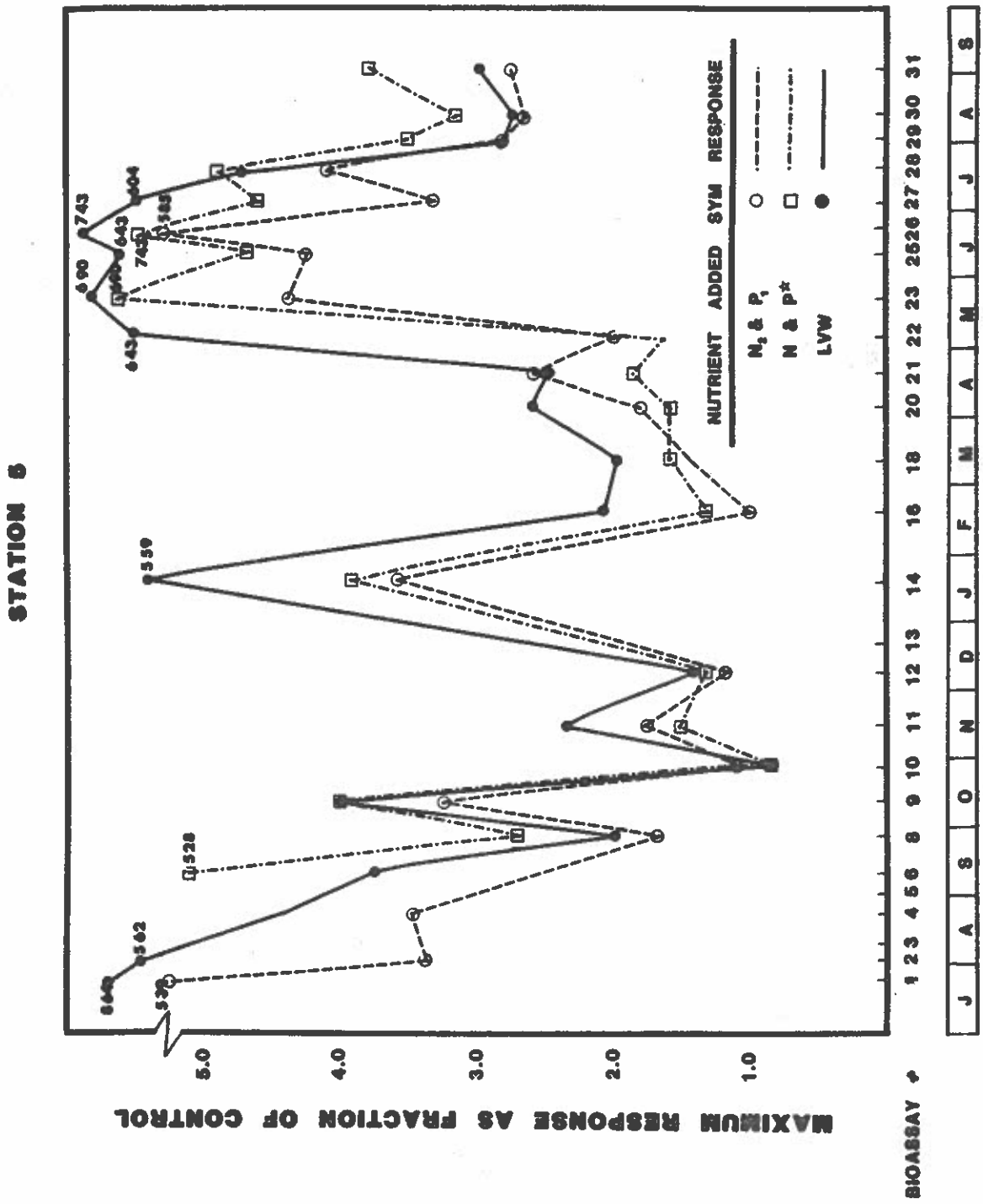

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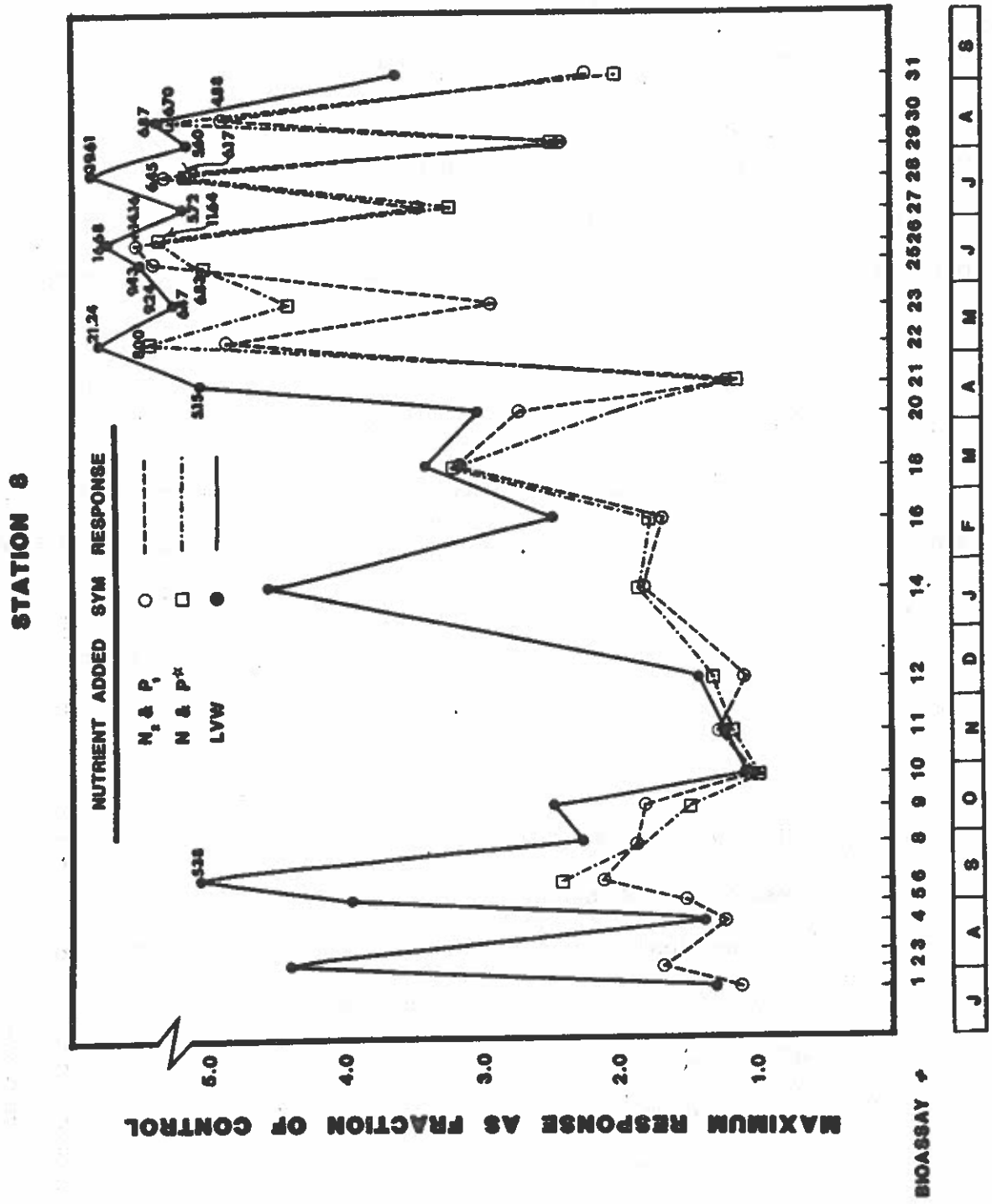


Figure 3. Maximum response as a fraction of control (N + P Combination, LVW Treatments)

It is clear from the foregoing analyses that the Las Vegas Bay Stations 2, 4, and 5 are all under the influence of the LVW plume (total N:P = 6.1) and should be considered in a different light than Boulder and Virgin Basin stations. Station 2 appears to be supplied with ample amounts of both N and P to sustain continuous blooms. In fact, when the special bioassay enrichments of untreated (secondary) effluent versus alum treated enrichments were compared, water from Station 2 seemed to behave differently than Stations 4, 5, and 8. In special Bioassays 3 and 7 (see Appendix E) at Station 2, there was no significant difference between the maximal fractional response of the treated and untreated enrichments, however, at all other stations alum treatment definitely reduced stimulation, presumably by P removal. Even in winter and spring, when the outer Bay and Boulder Basin are P limited, there is enough surplus phosphorus present at Station 2 to sustain maximal production at ambient light and temperatures. In the absence of significant non-point P inputs or high P flux from sediment exchange, it would appear then that some reduction of dissolved P concentration would first be necessary to reduce the P load (raise dissolved N:ortho P ratio) so that Station 2 would become limited by P during the growing season.

OTHER PHYSIOLOGICAL INDICES

Other physiological parameters may be examined to determine how closely these indices of nutritional status parallel the results of both the bioassay program and the trends shown by the ratios of dissolved N:ortho P.

Total N:P ratios, which represent the dissolved fraction plus that N and P actually incorporated by the cells, may in some ways be more accurate in predicting nutrient deficiencies than inorganic ratios. There is no certain consensus on what specific value of Total N:P denotes the transition point from

N to P limitation. Typically, values between 10-17 are in the region of colimitation and value >17 point to P limiting conditions (Forsberg, 1978).

At Stations 2 and 3, the mean values during the summer of 1980 (June 11 - September 11) for total N:P ratios are 10:1 and 11.3:1 respectively, these values indicate that inner Bay stations are N limited. At Station 5 the total N:P ratio during this same period is 19.5:1, a value indicating P limitation. In spite of this, our bioassays, conducted during the summer of 1980, clearly show that N (alone) is stimulating to algal growth at Station 5. This discrepancy cannot be explained, except to say that where levels of both N and P are close to the limiting threshold separate indices may yield somewhat differing results. At Station 8, during the summer of 1980, total N:P ratios of 30:1 point to P limitation and all of the bioassays conducted agree well. In Virgin Basin and Bonelli Bay the ratios increase to >60:1, this indicates definite P deficiency in the Upper Basin of Lake Mead.

The particulate N:P ratio, obtained by directly measuring the amount of these nutrients in the particulate fraction, can be a more meaningful index of nutrient status, since it is actually measuring the quantity of N and P in the algal cells. During May-October of 1980, the particulate N:P ratios (mean of monthly averages) increased from about 7:1 at Inner Bay (Station 2) to nearly 10:1 at Station 5 and 12:1 at Station 8 in Boulder Basin (Kellar and Paulson, 1981). Since our bioassays conducted during this same period indicated primarily N limitation at Stations 2, 4, and 5 and P limitation at Station 8, it therefore follows that at a particulate N:P ratio of about 10:1 the transition from N to P limitation occurs. The ratio, by weight of N to P in algal cells has been cited to vary from 4.6 to 9 (Chiaudani and Vighi, 1974) to be 7 (Richards, 1965) and to be 10 and 13.5:1 (Vallentyne, 1974; Rhee, 1978). Such values help to

explain our bioassay results, which show that at particulate N:P ratios of ~12:1 P assumes the limiting role.

Alkaline phosphatase activity (APA) may also be used as an indicator of nutrient deficiency in lakes. Kellar and Paulson (1981) report a steady increase in APA from the Inner Bay to Boulder Basin and then very little change from there to the Upper Basin. These authors also found that when APA was normalized for biomass (APA/Chla or APA/VSS) there was a significant inverse relationship between APA per unit chla and ortho P concentration, at concentrations between 5 and 15 µg/liter. The conclusion of these findings was that the phytoplankton were P limited below 5 µg/l ortho P, levels of ortho P between 5 and 15 µg/l represented a transition zone, and above 15 µP/l, phosphorus was not limiting phytoplankton growth. Using these criteria, the inner Las Vegas Bay is rarely P limited, Boulder Basin and the Upper Basin are always P limited, and the transition occurs somewhere in the middle of Las Vegas Bay. The general conclusions of the Kellar and Paulson (1981) study were consistent with the results of the experimental bioassay program conducted during the same period.

Special Bioassays

Table 2 lists the enrichments used in each special bioassay conducted as part of the Bioassay Study. A detailed description of each bioassay is included in Appendix E, with a summary of specific objectives and results. A few of the special assays were incorporated into the regular bioassays detailed in Appendix D.

Special bioassays, designed to compare the algal growth response of enrichments with regular secondary effluent to those with alum treated effluent, are summarized in Table 3. These assays are a direct measure of the capacity

of the AWT process to remove phosphorus and decrease algal growth stimulation. The data in Table 3 show that at all stations (2, 4, 5, and 8) the mean response (as a fraction of control) was higher for the untreated enrichments than for equal dilutions of treated enrichments. The former was 3.4-fold higher at Station 8 decreasing to less than two-fold at Stations 4 and 5, and only 18% higher at Station 2. This suggests that at Station 2, where P is most in excess, alum treatment and its associated P removal has less effect than at stations further away from the wash inflow. The high responses to treated, unfiltered effluent in Bioassay 7 probably reflect the stimulatory activity of nutrients bound to the particulate floc.

In Situ Bioassays

An in situ Lake Mead bioassay was conducted from 26 October to 1 November 1979. The purpose of this bioassay was two-fold. First, to obtain a corroboration of the regular fluorescence and extracted chlorophyll data by simultaneously evaluating physiological parameters (Alk. Phosphatase, dark ^{14}C uptake @ NH_4^+, and primary productivity) and nutrient concentrations in the bioassay enclosures (6 mil plastic bags, 1 m diam. x 2 m long). Second, to compare results of an in-situ bioassay in large (~1500 l) bags with results obtained in the regular pool-cubitainer bioassay conducted during the same period.

Lake water used for both bioassays was from Station 4, 1-5 m composite. The plastic bags were filled by pumping, and zooplankton were not removed. For the pool-cubitainer bioassay, Station 4 water was prefiltered through 80 micron mesh to remove zooplankton. The bags were enriched with nutrients just after filling, and then towed to the breakwater, suspended at 1-2 m from the surface, and anchored to the bottom.

The treatments were as follows:

Bag No.	1	control, no additions
	2	(31.4 ppb N - 5.9 ppb P)
N_2	3	164 ppb N as NH_4NO_3
	4	182 ppb N as NH_4NO_3
P_2	5	166 ppb P as KH_2PO_4
	6	151 ppb P as KH_2PO_4
N + P*	7	383 ppb N and 35.6 ppb P
	8	374 ppb N and 33.7 ppb P

The above nutrient concentrations are calculated from subsamples of water removed from bags just after nutrient additions, and therefore represent accurate initial values. Variance from ideal concentrations (166 ppb) reflect the degree of error of final bag volumes after filling and tying them off.

The data presented in Table 4 show the results of various analyses performed on subsamples of the plastic enclosures (Bags 1-8) from the initial day, October 25, through the final day, November 1.

The data for chlorophyll a (uncorrected for phaeophytin) on the four sample dates included show clearly that by the end of the 6-day incubation, on November 1, the amount of pigment had increased nearly three-fold from the initial value of 32.7 µg/l on October 26 to 105.4 µg/l and 110.2 µg/l in Bags 3 and 4, respectively. The next highest value of chlorophyll a (89 µg/l) was measured in the N + P* treatment, Bag #8 (Bag #7 was lost after sustained 60 mph winds on the third day of the experiment). Note that on November 1, the final chlorophyll a values for the P_2 treatments (Bags 5 and 6) end up somewhat lower than the control levels (36.5 µg/l for Bags 5 and 6 as opposed to 71.3 µg/l and 55.6 µg/l for Controls 1 and 2). These data provide a strong indication that N was the limiting nutrient for Station 4 water at this time. The levels of in vivo fluorescence in the bags as measured on a Turner 111

fluorometer follow the same general pattern as that shown by the extracted chlorophyll data. Due to certain logistical constraints, only two replicates of each treatment were utilized, making statistical analysis of the fluorescence results less than definitive; however, by November 1, fluorescence levels in the N-enriched Bags 3 and 4 were over 100 and are statistically significant from the controls at the 95% confidence level. On November 1, the fluorescence values for the controls was higher than either the P treated (5 and 6) or N + P* treated (8) bags.

During the period of these bioassay studies it was noted by ERA staff that fluorescence values were fluctuating widely for subsamples from the same container and not remaining stable as had previously been the rule. This condition leads to a greater degree of error in interpretation of fluorescence data for this particular bioassay. The cause is uncertain, but may have something to do with increased levels of particulate matter in the water due to resuspension subsequent to fall turnover. At any rate, both fluorescence data and extracted chlorophyll measurements agree in showing N stimulation versus control levels.

Levels of N and P (as measured by the UNLV water chemistry lab) indicate the nutritional status of the water in the plastic enclosures. The dissolved N:P ratios initially increase from the ambient Station 4 value of 5.3 on October 26 to 74:1 and 221:1 on October 28 in N-enriched Bags 3 and 4, respectively. In contrast, in P-enriched Bags 5 and 6, the N:P ratios are reduced to 0.06 and 0.04. Between October 28 through November 1 there is a progressive and rapid lowering of the N:P ratios in all bags. This points to the fact that N was the primary nutrient in demand by the algal community. Indeed, in P-enriched Bags 5 and 6, the phosphate concentrations first decrease, then between October 28 and October 30, increase; this effect indicating that another factor, most likely N, was limiting P utilization.

Particulate N:P ratios (total N or P minus dissolved N or P) can also serve as an indicator of the nutritional status of the algae. Data presented in the table for October 25 shows that there is considerable scatter in particulate ratios, probably due to some carry-over from the dissolved N and P enrichments. However, the particulate ratios do show high values in Controls 1 and 2 and N-enriched Bags 3 and 4 and significantly lower values in the P-enriched Bags 5 and 6, generally indicative of the N-deficiency as measured by other parameters.

The physiological parameters measured for the in-situ bioassay all confirm the results noted thus far, that is, stimulation of algal levels in Bags 3 and 4 as compared to Controls 1 and 2. The alkaline phosphatase, dark ^{14}C uptake @ NH_4^+, and primary productivity (^{14}C) data in the table are given as relative indicators of physiological status, and particularly in the case of ^{14}C, values have not been converted to absolute values of carbon uptake.

The alkaline phosphatase data clearly shows that the levels of the enzyme do increase greatly in Controls 1 and 2 and also N-enriched Bags 3 and 4, again indicating P-deficiency in the treatments; Bags 1-4 rapidly deplete ortho P, the levels dropping to below 2 ppb by October 28. On the other hand, in Bags 5 and 6 subject to P-enrichment, the alkaline phosphatase levels are about 2 orders of magnitude lower than the controls, and somewhat lower than ambient Station 4 levels of 0.025-0.086 nmoles P per liter per minute.

The dark ^{14}C uptake experiment designed to reveal N deficiency by measuring stimulation of radiocarbon uptake in NH_4^+-enriched samples are compared to controls, shows a level of N-deficiency in the controls, no significant stimulation in N-enriched Bags 3 and 4, but a three-fold stimulation in Bags 5 and 6 (P-enriched and N-deficient) by October 28. The N + P* treatment and ambient Station 4 water showed N sufficiency as noted by the similarity of control with N-enriched levels.

Table 2

SUMMARY OF SPECIAL BIOASSAYS AND SPECIAL TREATMENTS INCLUDED IN REGULAR BIOASSAYS

Special Treatment	Bioassay Identification																
	Reg no 1	Sp no 3	Sp no 7	Reg no 9	Sp no 10	Reg no 11	Sp no 13	Reg no 14	Sp no 15	Reg no 16	Sp no 17	Sp no 19	Sp no 24	Reg no 25	Reg no 26	Sp no 27	Sp no 32
Alum Treated		X	X				X		X		X	X					X
Untreated 2° Effluent		X	X				X		X		X	X					
Unfiltered Alum Treated Affluent			X														X
Artificial Alum + P Floc																	X
Artificial Alum + PO_4			X				X										
Alum Treated + N							X										
Plume Enrich (sta,2, 8 meters)	X																
Unfiltered LVW						X											
Broad Range of N + P Conc.										X			X				
Serial Additions of P								X									
Vitamins, Fe, Si, EDTA	X (Fe)												X	X (Fe)	X (Si)		
Virgin Basin Enrich.		X															
Storm Effect on Response to OVW										X							
Mechanisms of Light Shock				X		X	X										
In Situ Bioassays					X											X	

Table 3

ALGAL GROWTH RESPONSE TO ENRICHMENTS BY ALUM TREATED VS. UNTREATED 2° EFFLUENT
(ALL VALUES ARE IN PERCENT ABOVE CONTROL)

Bioassay	Enrichments	2	4	5	8
No. 3	untreated	210	735	1450	460
(Aug. 8-13, 1978)	treated	277	329	470	13
No. 7	untreated	440	680	780	440
(Sep. 18-24, 1979)	treated	430	540	410	300
	treated, unfiltered	430	1146	1180	440
	treated + P	450	680	820	390
No. 13	untreated	128	220	31	43
(Dec. 18-23, 1979)	treated	0	6	0	36
	treated + P	16	12	20	50
No. 15	untreated			260	
(Jan. 30-Feb. 5, 1980	untreated			180	
No. 17	untreated			225	
(Feb. 27-Mar. 8, 1980)	treated			192	
No. 19	untreated			0	
(Mar. 27-Apr. 3, 1980)	treated			0	
No. 32	treated	170	351	410	12
	treated, unfiltered	180	400	490	270
Mean $\bar{x}$ for all	untreated	260	480	460	310
dates:	treated	220	310	240	90

Table 4

RESULTS OF MULTI-PARAMETER IN-SITU BIOASSAY (OCTOBER 1979)

Treatment	Bag No.	Uncorr. Chl. data	Fluor	Diss N/ Ortho P	Part N/P	Alk. Phos.	Dark ^{14}C	PPR	10/26
Control	1	32.7	25.3	5.3	13.71	0.025	C-1025 N-955	41,944	
Control	2	32.7	25.3	5.3	13.71	0.025	"	"	
N	3	32.7	25.3	31.5	8.36	0.025	"	"	
N	4	32.7	25.3	33.8	9.01	0.025	"	"	
P	5	32.7	25.3	0.21	3.9	0.025	"	"	
P	6	32.7	25.3	0.18	5.3	0.025	"	"	
N+P	7	32.7	25.3	10.2	5.9	0.025	"	"	
N+P	8	32.7	25.3	10.3	7.6	0.025	"	"	

Table 4 (continued)

Treatment	Bag No.	Uncorr. Chl. data	Fluor	Diss N/ Ortho P	Part N/P	Alk. Phos.	Dark ^{14}C	PPR	10/28
Control	1	34.1	56.5	3.3	7.1	0.504	C-731 N-2221	32,018	
Control	2	33.5	41.0	3.1	7.0	0.230	C-837 N-2099	25,793	
N	3	49.4	47.5	74.1	8.3	0.767	C-781 N-806	37,137	
N	4	50.3	62.5	221.	6.7	0.841	C-884 N-815	34,028	
P	5	34.0	41.0	.04	6.0	0.014	C-794 N-2310	34,310	
P	6	28.6	45.0	.06	3.3	n.s.	C-763 N-2514	36,022	
N+P	7	50.5	47.5	16.0	5.0	0.029	C-840 N-908	40,271	
N+P	8	---	62.5	9.6	9.5	0.017	---	20,947	
sta 4		30	32.5	2.4	8.4	0.090	C-980 N-1213	9,361	

Table 4 (continued)

Treatment	Bag No.	Uncorr. Chl. data	Fluor	Diss N/ Ortho P	Part N/P	Alk. Phos.	Dark ^{14}C	PPR	10/30
Control	1	41.0	49.0	0.94	18.3	3.99	C-2121 N-4547	40,426	
Control	2	37.7	52.0	0.77	19.2	0.65	C-1566 N-2983	15,647	
N	3	63.8	66.0	3.49	22.5	2.37	C-1346 N-1465	45,407	
N	4	61.9	76.0	3.45	17.8	2.10	C-3168 N-8000	64,658	
P	5	36.3	47.5	0.06	4.3	0.048	C-1844 N-3477	7,382	
P	6	33.3	52.0	0.03	5.8	0.022	C-817 N-2836	7,732	
N+P	7								
N+P	8	66.3	54.5	9.2	11.9	0.142	C-2061 N-2514	29,445	
sta 4		25.6	25.5	6.3	12.3	0.086	C-1121 N-826	1,867	

Table 4 (continued)

Treatment	Bag No.	Uncorr. Chl. data	Fluor	Diss N/ Ortho P	Part N/P	November 1
Control	1	44.3	82.0	0.67	9.66	
Control	2	55.6	87.0	0.44	15.16	
N	3	110.2	>1.00	0.65	12.83	
N	4	105.4	>1.00	0.84	14.73	
P	5	36.6	56.0	0.08	3.44	
P	6	36.5	55.3	0.02	4.83	
	7					
N+P	8	89.0	58.0	4.75	15.75	
sta 4		24.6	20.5	5.0	14.64	

On October 30, there is a very similar pattern with regard to dark ^{14}C uptake. Bags 3 and 8 and Station 4 water show sufficient N levels. For Bag 4, there appears to be an abnormally high value for one replicate, probably due to contamination, the other replicate not much higher than control levels.

Primary productivity (^{14}C uptake in light bottles minus ^{14}C uptake in dark bottles) is a measure of the rate of photosynthesis. The effect of nutrient enrichment may be detected by noting changes in this parameter. Note that on October 28, primary productivity (PPr) measurements for all enclosures fall in the same range; however, by October 30, much lower values are obtained for P-enriched Bags 5 and 6 as compared to controls and higher values are evidenced for N-enriched Bags 3 and 4. These data again strongly support the overall outcome for the in situ experiment; that is, N stimulation of algal growth.

In the pool-cubitainer bioassay, statistical analysis of maximum fluorescence values for nutrient treatments compared to the maximum control values, reveal that of all the enrichments, only the N_1 treatments showed significant stimulation (>95% confidence level). Other treatments (including N_2, P_2, $N_2 + P_2$, and LVW) show somewhat higher levels than controls, but do not exceed the 95% confidence level, and thus cannot be differentiated from the controls. The pool-cubitainer bioassay and the in situ bioassay did compare well in that N additions alone in both bioassays yield significant stimulation of algal growth.

A second in situ bioassay was conducted from 2 July to 8 July, 1980 at Station 4 of Las Vegas Bay, Lake Mead, Nevada. The purpose of this bioassay was two-fold. First, to obtain a corroboration of in vivo fluorescence data using a multi-parameter assay in which chlorophyll a, alkaline phosphatase activity, primary productivity (^{14}C), ammonium enhancement of dark ^{14}C uptake, and nutrient (N + P) regime, are simultaneously measured. Second, to compare the

results of an in situ bioassay in large (~750 liter) plastic enclosures (6 mil polyethylene; 1 m diam. x 1 m long), with those results obtained in the regular pool-cubitainer bioassay conducted at the same time.

It was hoped that this, the second in situ bioassay, conducted during the period of summer stratification, would confirm the results of the first in situ assay which was conducted in late October during fall overturn. The first in situ experiment (in 1500 l bags) showed that the several parameters measured as indicators of nutrient limitation are well correlated, and that the pattern of growth response to N or P additions, in either bags in situ or in the pool-cubitainer assay, compared well.

Lake water used for the July in situ bioassay was from Station 4, 1-5 m composite. The plastic bags were filled by pumping and the zooplankton were removed with an 80 μ net at the inlet to the enclosure. The bags were then enriched with nutrients and were towed to the breakwater near the Las Vegas Boat Harbor where they were suspended at 1 m from the surface and tethered by anchor to the bottom.

The treatments were as follows:

	Bag #1	
	2	controls, no additions
	3	(24 ppb N and 3 ppb P)
N_2	4	94 ppb N as NH_4NO_3
	5	146 ppb N as NH_4NO_3
P_2	6	161 ppb as NaH_2PO_4
	7	159 ppb P and NaH_2PO_4
N+P*	8	159 ppb N and 20 ppb P
	9	202 ppb N and 26 ppb P

The above nutrient concentrations were calcualted for subsamples of water removed from the bags just after nutrient addition, and therefore represent accurate initial values which differ somewhat due to a certain degree of variation in final bag volumes after filling and closure.

The data presented in Table 5 show the results of various analyses performed on subsamples from the enclosures (Bags 1-9) during the period from July 2 to July 8.

The data for chlorophyll a concentration (uncorrected for phaeophytin) show that maximum pigment concentrations in all the bags were obtained 2-3 days after the start of the assay (on or about July 4); by July 6 the values for chlorophyll a concentration are nearly 50% lower than the July 4 values. On July 4, the level of pigment in the N + P* Bags 8 and 9 had increased to 35.5 and 27.7 µg/l respectively, about 5 times the value of the mean for the 3 controls (6.3 µg/l). The next highest values of 18.4 and 18.6 µg/l were measured in the N treated Bags 4 and 5. The bags enriched with P alone (6 and 7) reached maximum concentrations of 7.9 and 9.4 µg/l. All treatments, N, P, and N + P*, had chlorophyll levels significantly higher than the controls by July 4 ($p > .05$). These data provide strong support for the existence of algal growth colimitation by N and P with N yielding a greater algal response to enrichment.

The level of in vivo fluorescence has been shown to be well correlated with extracted chlorophyll a concentration. This was indeed the case in the bags. By July 4, the highest values for fluorescence were shown by Bags 8 and 9, 99.3 and 1115.6 respectively, compared to the mean of the controls of 31.8 (a level of 3.4 times the control). The next highest levels of fluorescence were for N-enriched Bags 4 and 5, 56.8 and 58.7 respectively. Both N + P* and N only treatments were significantly higher than controls at $p > .01$ level. On

July 5 the P-enriched Bags 6 and 7, had fluorescence values of 44.0 and 48.2, significantly above the controls only at the 90% level of significance. However, by July 5 in vivo fluorescence measurements for all treatment, N, P, and N + P* were significantly above the controls (all, $p > .01$). It can be seen therefore that the in vivo fluorescence response of algae in the bags, precisely parallels the chlorophyll a pigment response, that is, both N and P are costimulatory, but N enrichment yields a greater response than P alone.

Levels of N and P (as measured by water chemistry) can indicate much about the nutritional status of the water in the enclosures. The dissolved N:P ratio of 8:1 at Station 4 at the start of this experiment is in the region where both N and P may be colimiting. This ratio increases subsequent to enrichment to 31.3 and 35.4 in Bags 4 and 5, respectively; however, by July 4, after just 2 days of growth, the ratio is lowered to 4 and 3.5, as the nitrate and ammonium become rapidly depleted. A similar pattern is evidenced in N + P enriched Bags 8 and 9, where ratios of 7.95 and 7.8 on July 2 are reduced to 2.3 and 5.8 by July 4. The rate of utilization of nitrogen in Bags 4 and 5 was equal to 43.0 and 69.5 µg N per liter per day, as compared to rates of 75 and 89.5 µg N per liter per day for the N + P enriched Bags 8 and 9. In the latter bags, with both N and P in ample supply during the first few days, N:P uptake ratios are 10 and 9.4 respectively, in good agreement with literature values of the ratio of N:P required for balanced growth. The relatively high values of N uptake in N-enriched bags is somewhat surprising, since dissolved P concentrations were only 3-4 µg/l at the start (July 2) and by July 4, water chemistry indicates that only 1-2 µ/g of P/liter had been utilized; this, in spite of the fact that by July 4, chlorophyll a levels in Bags 4 and 5 had more than doubled. This tends to infer that P is rapidly recycled or that cells contain pools of stored P. Rates of P utilization in P-enriched Bags 6 and 7 were 10

and 5 µg P per liter per day from July 2 through July 6, respectively. These values probably indicate there is some "luxury consumption" of P occurring, since chlorophyll levels and primary productivity did not show any large increases during this same period. Particulate N:P ratios (total N or P minus dissolved N or P) do seem to bear this out; in P-enriched Bags 6 and 7, the ratio fell from 14.7 on July 2 to 7.9 and 6.5 by July 6, this indicative of the fact that a surplus of P had been accumulated by the algal cells.

Primary productivity (C^{14} uptake in the light minus uptake in the dark) is a measure of the rate of photosynthesis by algae. For purposes of this analysis, ^{14}C uptake has not been converted to absolute carbon uptake values. By July 4, two days into the bioassay, ^{14}C uptake in Bags 4 and 5 was 2-3 times the control and uptake in Bags 8 and 9 is 5-6 times the control. P-enriched Bags 6 and 7 evidenced no significant stimulation of algal uptake on this date. On July 6, the N or N + P bags were again much higher than control levels, however, after 4 days, the P-enriched bags also showed a significant but small stimulation of ^{14}C uptake when compared to controls. These data strongly suggest the overall conclusion derived from chlorophyll a and in vivo fluorescence measures, that is, N and P are costimulatory, but N enrichments lead to a greater algal response. Furthermore, data gathered in both in situ experiments show there is an excellent correlation between algal growth as measured by chlorophyll a, in vivo fluorescence, or ^{14}C uptake.

The dark ^{14}C uptake assay was designed to reveal N deficiency by measuring the stimulation of ^{14}C in the dark in NH_4^+-enriched samples as compared to controls. On July 2 there was no significant stimulation by NH_4^+ addition to Station 4 water. On July 4, however, the Controls 1, 2, and 3 showed a significant level of NH_4^+ enhancement as their nitrogen supply had

become depleted. N-enriched bags, as one would expect, showed no stimulation. P-enriched bags showed a >75% increase in dark ^{14}C uptake as compared to controls. Of course, the P-enriched bags were the most severely N-deficient and their response to NH_4^+ addition reflects this condition. By July 6 all of the bags (except #4) had become N-deficient as revealed by this assay. This response paralleled the rapid depletion of dissolved N levels after the onset of the experiment in all bags.

The alkaline phosphatase activity assay clearly showed that levels of enzyme activity increased significantly in N-enriched Bags 4 and 5. On July 5, these bags showed rates of 8-10 nmoles P per liter per minute compared to control levels of 0.4-1.1 nmoles P per liter per minute, the ten-fold increase indicative of a P deficiency. At the same time, N + P enriched Bags 8 and 9 showed a much lower increase above control values to 1.4-4.5 nmoles P per liter per minute. By July 7, Bags 3 and 4 still showed an elevated response, as did Bags 8 and 9. P-enriched Bags 6 and 7 showed levels much lower than the control, 0.35 nmoles P per liter per minute as compared to control level of ~2 nmoles P per liter per minute. Indeed, P addition does either suppress or inactivate the alkaline phosphatase enzyme.

In the pool-cubitainer bioassay, statistical analysis of <u>in vivo</u> fluorescence response revealed that for both Stations 4 and 4, N and P were costimulatory either by themselves, or in combination. Only July 5 all treatments in the pool-cubitainer bioassay for Station 4 ($N_{1,2}$, $P_{1,2}$, N + P* and N_2 + P_1) were significantly above controls ($p > .05$). The pool-cubitainer bioassay and the <u>in situ</u> bioassay therefore, do compare well, in that both show that N and P alone or together can yield a significant stimulation of algal growth.

VI. CONCLUSIONS

The experimental bioassay program conducted over a 14-month period found Stations 2, 4 and 5 to be N-limited most of the summer with the transition from N to P limitation occurring somewhere in outer Las Vegas Bay or in Boulder Basin. In the summer of 1979 Station 8 was shown to be N-limited, however, in the summer of 1980 P-limited conditions prevailed with higher ambient N:P ratios. After destratification of the lake in autumn, phosphorus becomes the principal limiting nutrient at all stations. Previous limnological investigations (Paulson et al., 1980; Baker and Paulson, 1980) have indicated that Las Vegas Bay and Boulder Basin are nitrogen-deficient during the growing season, whereas the remainder of the lake is phosphorus-deficient. Our studies corraborated these results.

In the inner Las Vegas Bay (Station 2) light may be an important limiting factor, given the strong influence of LV Wash on the inner Bay and the high ambient nutrient levels. When light is made to be non-limiting, as in our regular bioassays which incubate the lakewater at close to surface light levels, N is the major limiting nutrient. In the middle Bay, N appears to be the sole limiting nutrient during the growing season from July to October. For protracted periods during this time, levels of dissolved N are reduced to ~5 µg/l, with nitrate levels just at 1 µg/l. This system is obviously relying on internal regeneration of NH_4^+ (mineralization, zooplankton excretion) to sustain productivity. At the same time the concentration of ortho P varies between 2-6 µg/l. At Station 8 our bioassays demonstrate a general tendency towards P limitation, although during the summer of 1979, N was showing more significant stimulation of algal growth than was P.

The above pattern is generally consistent with trends shown by other indices of nutrient status, including dissolved N:ortho P ratio, particulate N:P

Table 5

RESULTS OF MULTI-PARAMETER IN-SITU BIOASSAY (JULY, 1980)

Treatment	Bag	Uncorr. Chl. Data	Fluor.	Diss N/P	Total N/P	Alk. Phos.	Dark ^{14}C	PPR	July 2
Control	1	14.7	62.8(3x)	8.	---	0.59	C-209 N-182	4,718	
Control	2	14.7	62.8(3x)	8.	---	0.59	"	4,718	
Control	3	14.7	62.8(3x)	8.	---	0.59	"	4,718	
N	4	14.7	62.8(3x)	31.3	---	0.59	"	4,718	
N	5	14.7	62.8(3x)	36.5	---	0.59	"	4,718	
P	6	14.7	62.8(3x)	0.09	---	0.59	"	4,718	
P	7	14.7	62.8(3x)	0.08	---	0.59	"	4,718	
N+P	8	14.7	62.8(3x)	7.95	---	0.59	"	4,718	
N+P	9	14.7	62.8(3x)	7.77	---	0.59	"	4,718	

Table 5 (continued)

Treatment	Bag	Uncorr. Chl. Data	Fluor.	Diss N/P	Total N/P	Alk. Phos.	Dark ^{14}C	PPR	July 4
Control	1	5.8	21.6	6.	18.9	1.1	C-180 N-202	960	
Control	2	7.3	36.7	3.	19.1	0.9	C-179 N-363	1110	
Control	3	7.3	37.2	3.	22.1	0.9	C-136 N-304	1275	
N	4	18.4	56.8	4.	23.7	9.5	C-330 N-282	2394	
N	5	18.6	58.7	3.5	30.6	8.8	C-340 N-268	2728	
P	6	7.9	44.0	0.02	2.4	0.28	C-188 N-349	1001	
P	7	9.4	48.2	0.02	2.0	0.98	C-255 N-466	1098	
N+P	8	35.5	99.3	2.3	16.3	1.45	C-461 N-406	5719	
N+P	9	27.7	115.6	5.8	16.0	3.6	C-312 N-225	3135	
	sta 4 amb.	4.3	46.8(3x)	9.	20.8	1.7	C-65 N-100		

Table 5 (continued)

Treatment	Bag	Uncorr. Chl. Data	Fluor.	Diss N/P	Total N/P	Alk. Phos.	Dark ^{14}C	PPR	July 6
Control	1	1.4	19.9(1x)	1.	12.0	1.81	C-106 N-296	792	
Control	2	4.2	26.0	2.	17.2	3.40	C-136 N-742	961	
Control	3	3.5	26.7	3.	16.3	1.67	C-121 N-393	807	
N	4	11.4	60.0	2.	23.1	5.19	C-282 N-294	2725	
N	5	8.8	55.7	0.7	26.2	12.96	C-228 N-509	2345	
P	6	5.0	32.5	0.03	2.1	0.33	C-154 N-548	946	
P	7	5.7	34.2	0.04	1.8	0.36	C-158 N-518	1009	
N+P	8	14.4	71.8	4.5	15.3	2.62	C-315 N-1090	3265	
N+P	9	18.6	80.2	0.75	15.3	4.10	C-65 N-89	4012	
	sta 4	2.6	13.5	5.					

Table 5 (continued)

Treatment	Bag	Uncorr. Chl. Data	Fluor.	Diss N/P	Total N/P	Alk. Phos.	Dark ^{14}C	PPR	July 8
Control	1	3.3	27.3(1x)	7.	27.2	---	---	---	
Control	2	3.5	23.1	2.	16.4	---	---	---	
Control	3	2.7	25.3	1.	19.7	---	---	---	
N	4	7.4	45.5	5.	18.0	---	---	---	
N	5	9.9	54.8	2.	26.9	---	---	---	
P	6	6.2	29.0	0.02	3.5	---	---	---	
P	7	6.2	33.3	<0.01	2.1	---	---	---	
N+P	8	13.2	64.2	0.5	10.2	---	---	---	
N+P	9	19.1	73.0	1.75	15.6	---	---	---	
	sta 4	6.6	22.4	15.	31.8				

ratio, and APA. The gradient in nutrient concentrations and algal biomass, through Las Vegas Bay outwards to Boulder Basin, offers a unique opportunity to determine at what point the transition from N to P limitation occurs. The analysis of nutrient enrichment bioassay results, and N:P ratios along this gradient, allows one final conclusion, that is, if either dissolved N:P or particulate N:P ratios can be increased in Las Vegas Bay above about 10:1 by phosphorus removal, then some reduction in the levels of algal biomass will result.

REFERENCES

Baker, J. R. and L. J. Paulson. 1980. Influence of Las Vegas Wash density current on nutrient availability and phytoplankton growth in Lake Mead. Symposium on Surface Water Impoundments. June 2-5, 1980, Minneapolis, Minnesota. (In press)

Barlow, J. P., W. R. Schaffner, F. deMoyelles, Jr., and B. J. Peterson. 1973. Continuous flow nutrient bioassays with natural phytoplankton populations. p. 299-319. In G. Glass (ed.) Bioassay techniques and environmental chemistry. Ann. Arbor Sci.

Chiaudani, G. and M. Vighi. 1974. The N:P ratio and tests with Selenastrum to predict eutrophication in lakes. Water Res. 8, 1063.

Deacon, J. E. 1976. Lake Mean Monitoring Program. Final Report to Clark County Wastewater Management Agency. 297 p.

Deacon, J. E. and R. W. Tew. 1973. Interrelationships between chemical, physical, and biological conditions of the waters of Las Vegas Bay of Lake Mead. Final Rept. to Las Vegas Valley Water District. 186 p.

Dunstan, W. M. and D. W. Menzel. 1971. Continuous cultures of natural populations of phytoplankton in dilute, treated sewage effluent. Limnol. Oceanogr. 16:623-632.

Ecological Research Associates, Goldman, C.R. 1976. A review of the limnology and water quality standards for Lake Mead. Davis, California. 101 p.

Flemer, D. A. 1969. Continuous measurement of in vivo chlorophyll of a denoflagellate bloom in Chesapeake Bay. Chesapeake Sci. 10, 99.

Forsberg, C., Rydeny, S., Claesson, A., and A. Forsberg. 1978. Water chemical analyses and/or algal assay? - Sewage effluent and polluted lake water studies. Mit. Internat. Verein. Limnol. 21, 352.

Goedheer, J. C. 1964. Fluorescence bonds and chlorophyll a forms. Biochimica et. Biophysica. Acta 88, 304.

Goldman, C. R. 1962. A method of studying nutrient limiting factors in situ in water columns isolated by polyethylene film. Limnol. Oceanogr. 7:99-197.

Goldman, C. R. 1969. In A manual on methods for measuring primary production in aquatic environments. R. A. Vollenweider (ed.). IBP Handbook No. 12. Blackwell Sci. Publ. Oxford and Edinburgh. 211 p.

Green, J. C. and E. Merwin. Algal growth potential results of tests performed on Lake Mead, Nevada waters collected October 1979 and July 1980. EPA Report, Corvallis Environ. Research Laboratory.

Healy, F. P. 1975. Physiological indicators of nutrient deficiency in algae. Fish. Mar. Serv. Res. Dev. Tech. Rep. 585.

Healy, F.P. and L.L. Hendzel. 1980. Physiological indicators of nutrient deficiency in lake phytoplankton. Can. J. Fish. Aquat. Sci. 37, 442.

Kiefer, D. A. 1973. Chlorophyll a fluorescence in marine centric diatoms: responses of chloroplasts to light and nutrient stress. Mar. Biol.

Lorenzen, C. J. 1966. A method for the continuous measurement of in vivo chlorophyll concentration. Deep Sea Res. 13, 223.

Miller, W. E., Greene, J. C., and T. Shiroyama. 1978. The Selanastrum capricornutum Printz-Algal Assay Bottle Test: Experimental Design, Application, and Data Interpretation Protocol. U.S. E.P.A., Corvallis, Oregon. EPA-600/9-78-018.

Paulson, L. J., Baker, J. R., and J. E. Deacon. 1980. The limnological status of Lake Mead and Lake Mohave under present and future powerplant operation of Hoover Dam. Tech. Rept. No. 1. Lake Mead Limnological Res. Center. Univ. of Nevada, Las Vegas. 229 p.

Rhee, T. Y. 1978. Effect of N:P atomic ratios and nitrate limitation on algal growth, cell composition and nitrate uptake. Limnol. Oceanogr. 23, 10.

Richards, F. A. 1965. In J. R. Riley and G. Skirrow (ed.) Chemical Oceanography. Vol. 1. Academic Press. New York.

Schelske, C. L. and E. F. Stoermer. 1971. Eutrophication, silica depletion and predicted changes in algal quality in Lake Michigan. Science, 173, 423.

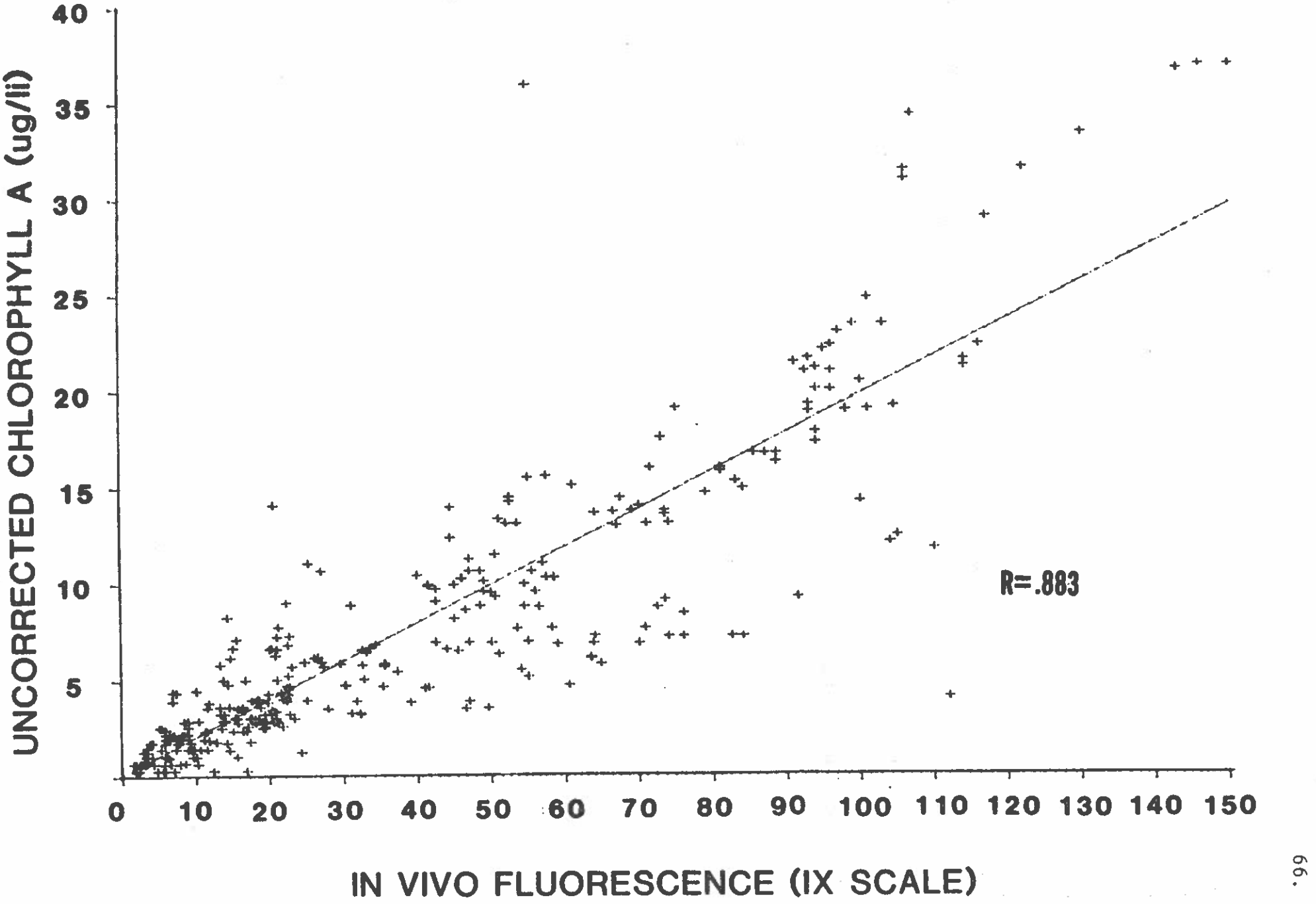
Schindler, D. W. 1971. Carbon, nitrogen, and phosphorus and the eutrophication of freshwater lakes. J. Phycol. 7:321-329.

Strickland, J. D. H. 1968. Continuous measurement of in vivo chlorophyll: a precautionary note. Deep Sea Res. 15, 225.

Vallentyne, J. R. 1974. The algal bowl. Misc. Special Pub. 22. Dept. of Environ. Fish and Marine Service, Ottawa. 186 p.

APPENDIX A

Uncorrected chlorophyll *a* vs *in vivo* fluorescence on 1X scale. Lake Mead stations 2, 4, 5, and 8 were included.

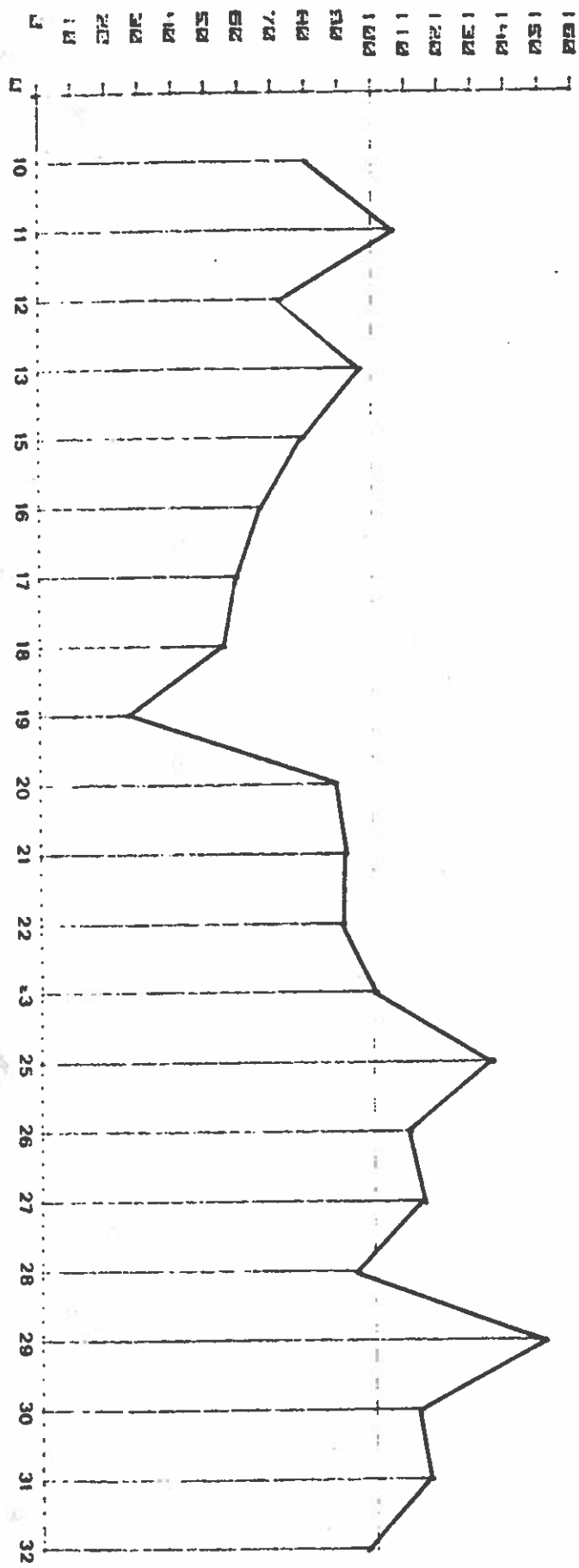

UNCORRECTED CHLOROPHYLL A (ug/li)
40
35
30
25
20
15
10
5
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
R=.883
IN VIVO FLUORESCENCE (IX SCALE)

APPENDIX A-1

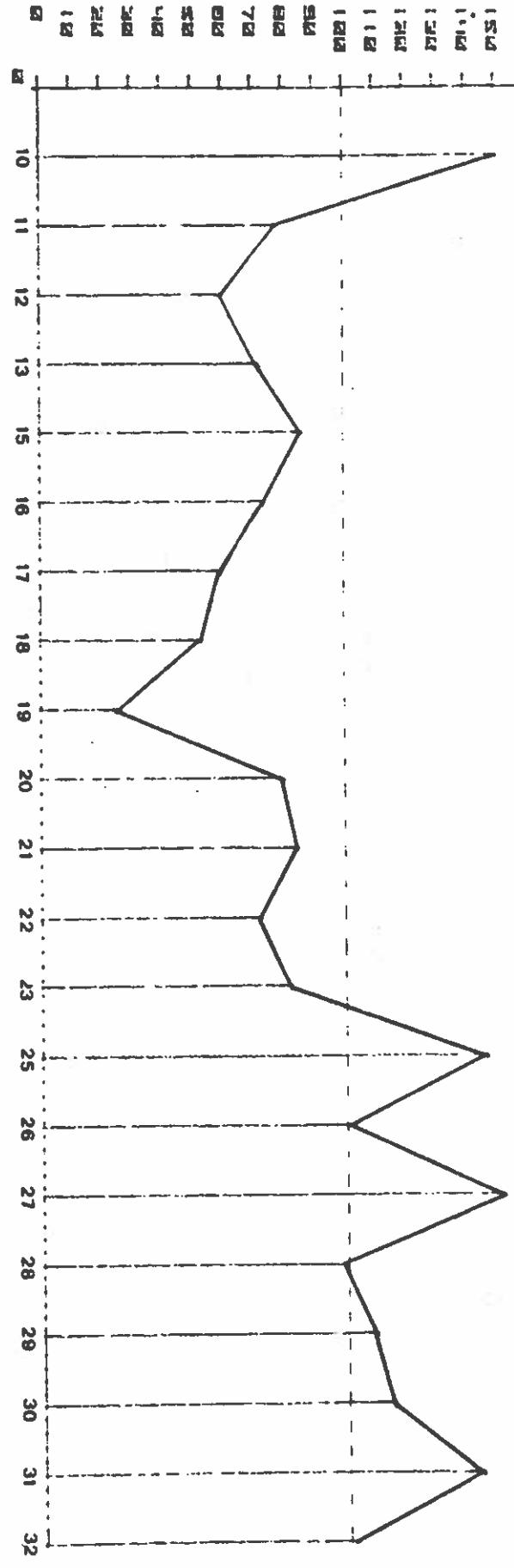
Bioassays 10 to 32. Day 1 fluorescence as a percent of initial, typically showing a decline.

Lower histogram shows uncorrected chlorophyll *a* as percent of initial. This also typically shows a decline.

DAY 1 AS % INIT (CHL A)

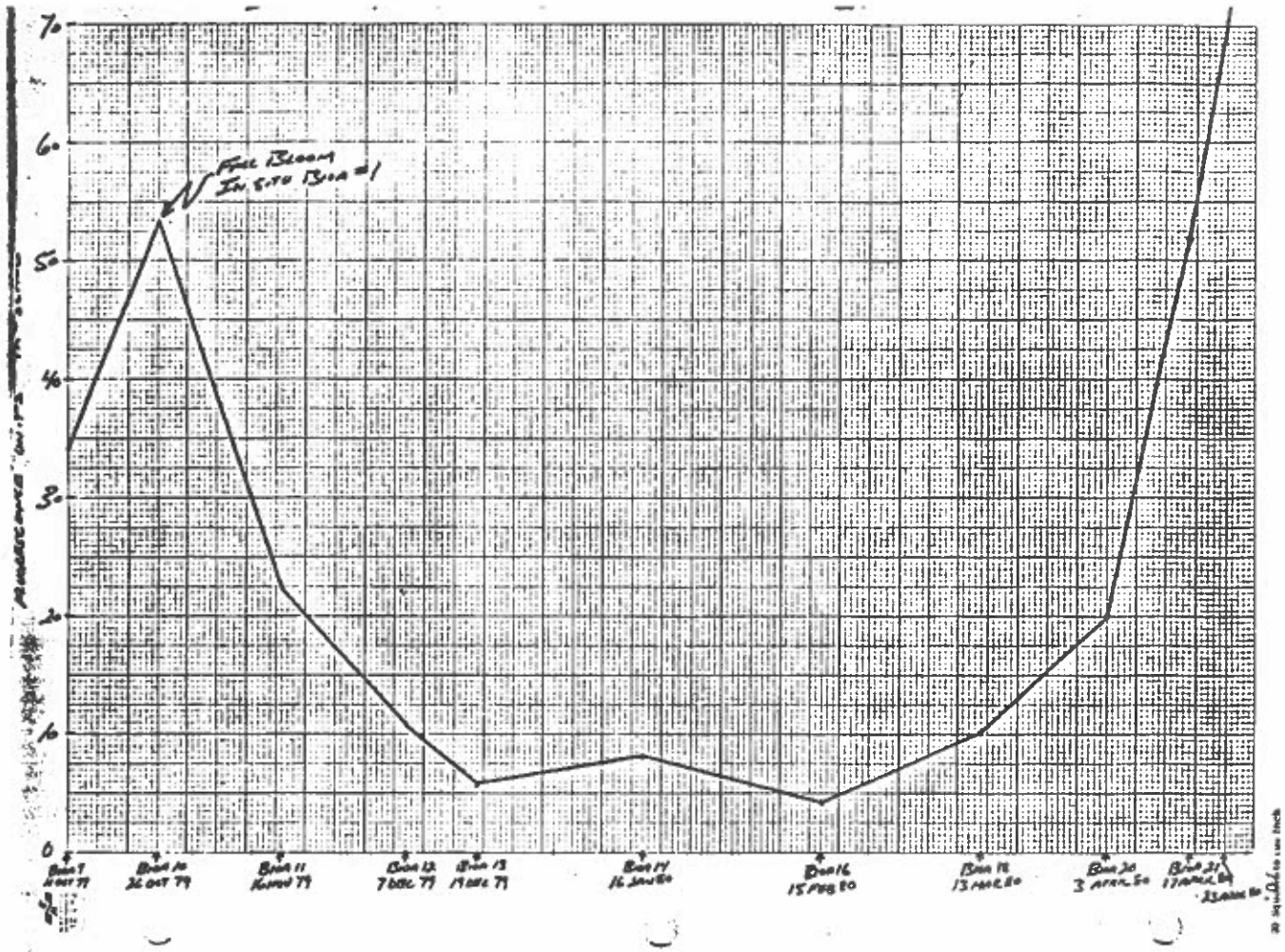


DAY 1 AS % INIT (FLUOR)

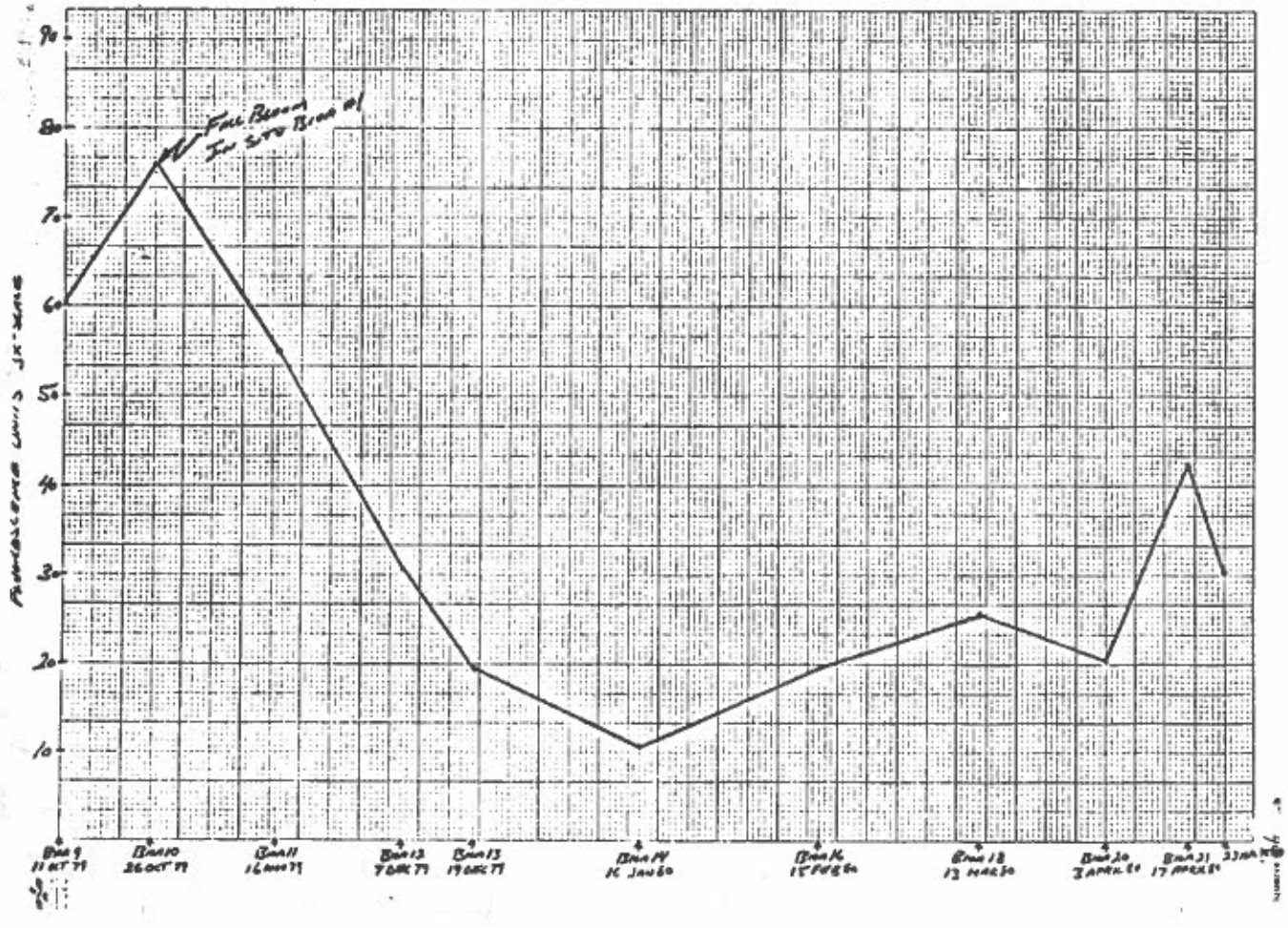


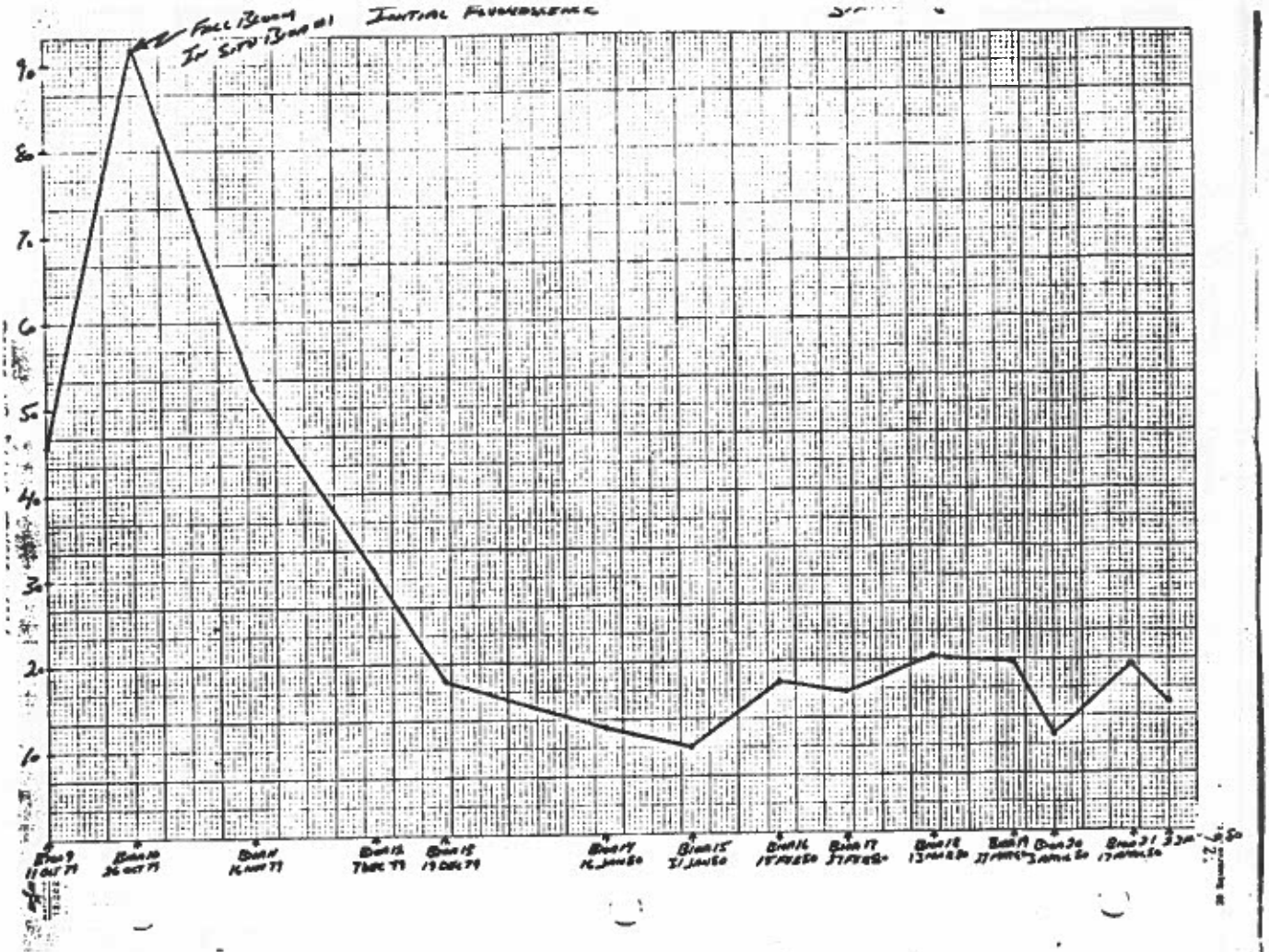
APPENDIX B

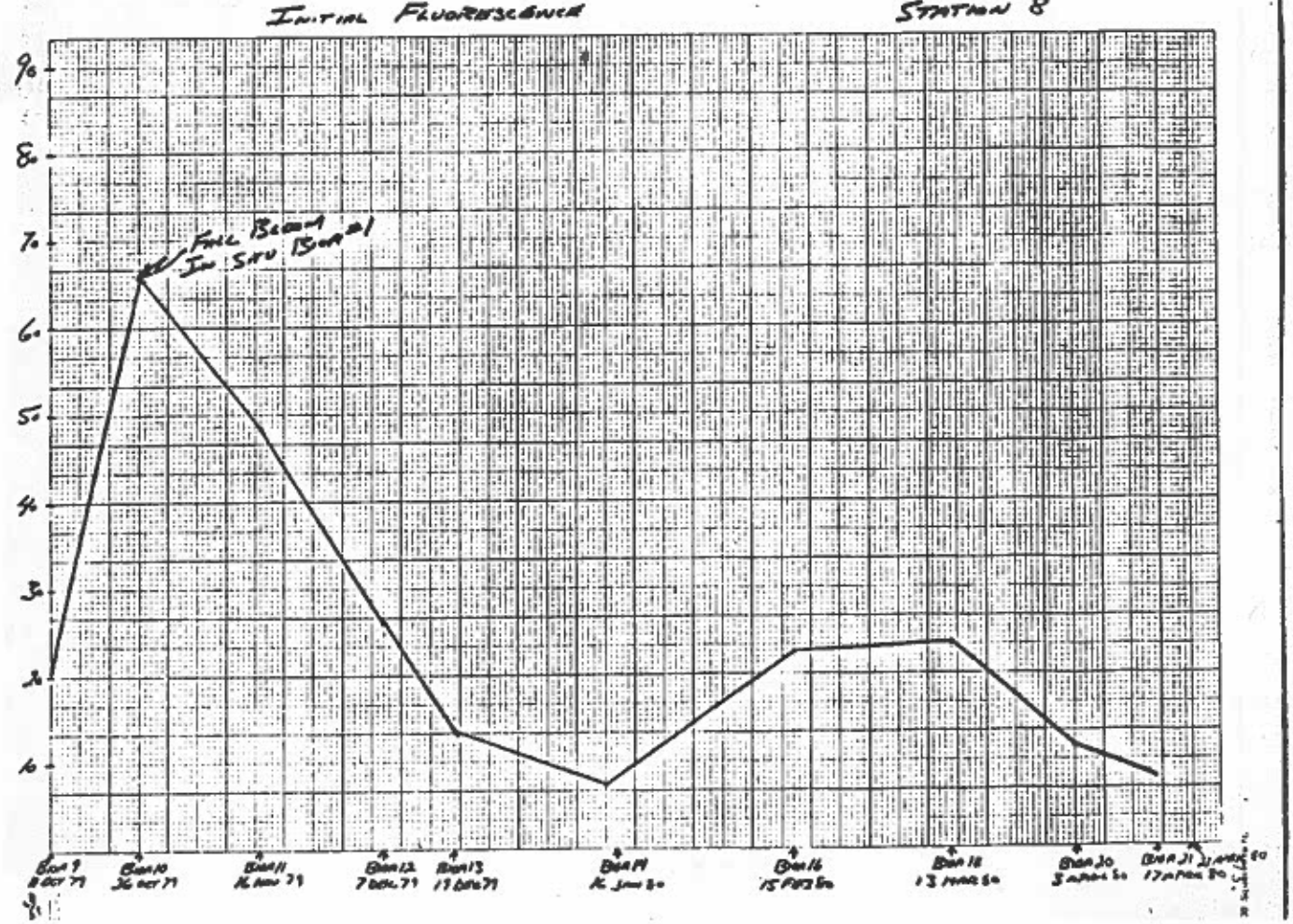
Graphic presentation of Lake Mead Bioassay experiments.



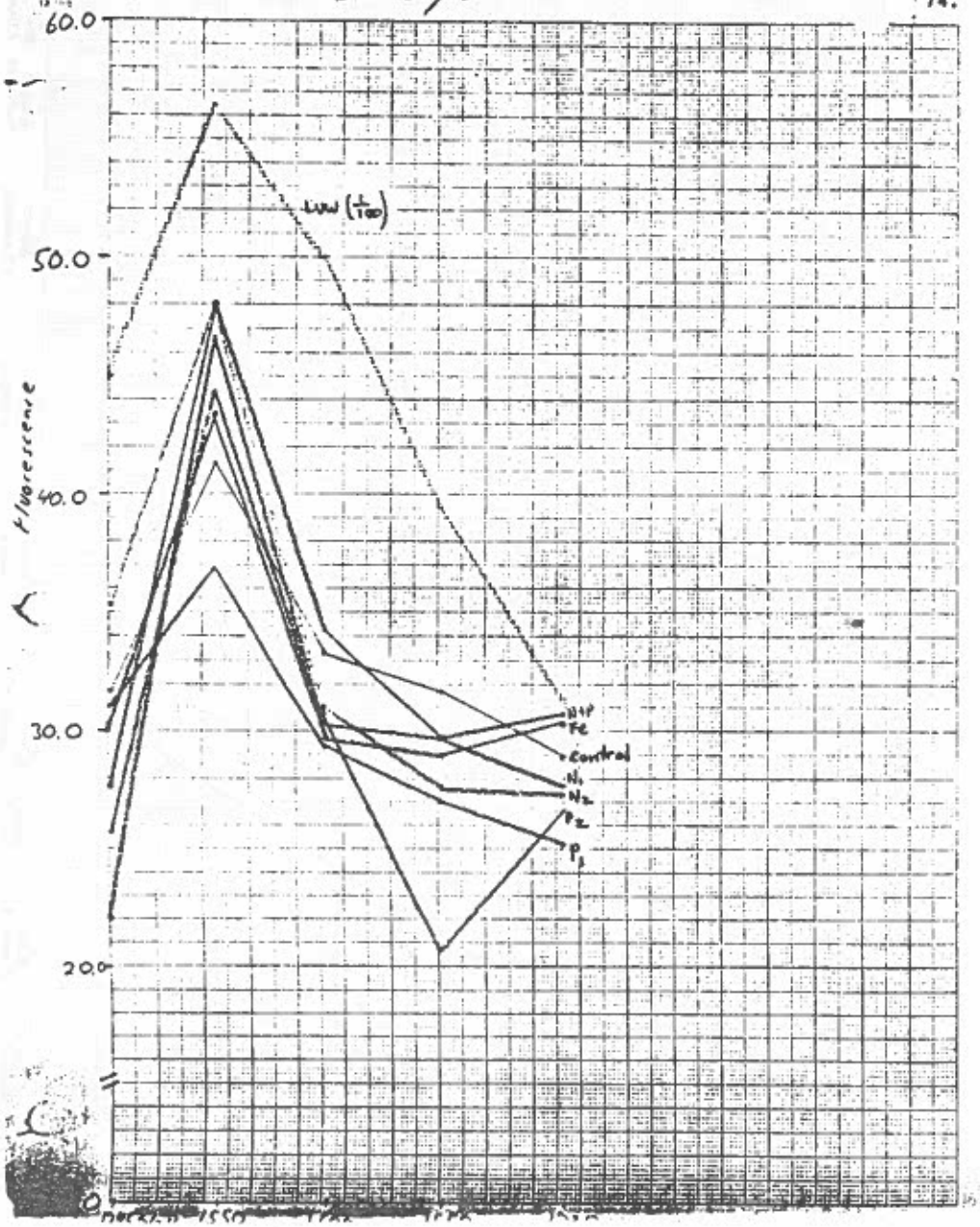
INITIAL FLUORESCENCE STATION 4



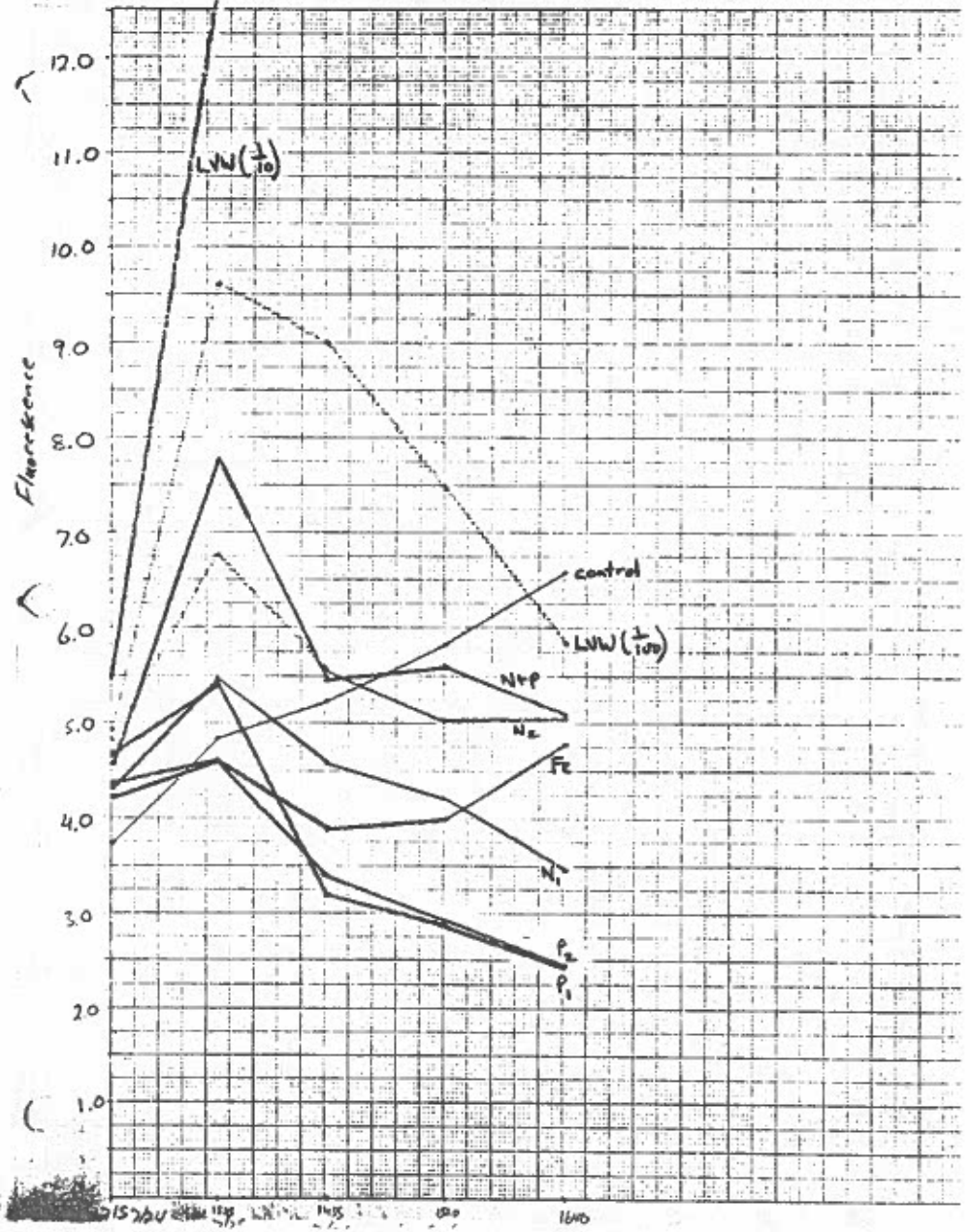

Full Bloom
In Situ Bloom #1
Initial Fluorescence
9
8
7
6
5
4
3
2
1
Bloom 9
11 Oct 79
Bloom 10
26 Oct 79
Bloom 11
6 Nov 79
Bloom 12
7 Dec 79
Bloom 13
19 Dec 79
Bloom 14
15 Feb 80
Bloom 15
3 March 80
Bloom 16
17 March 80

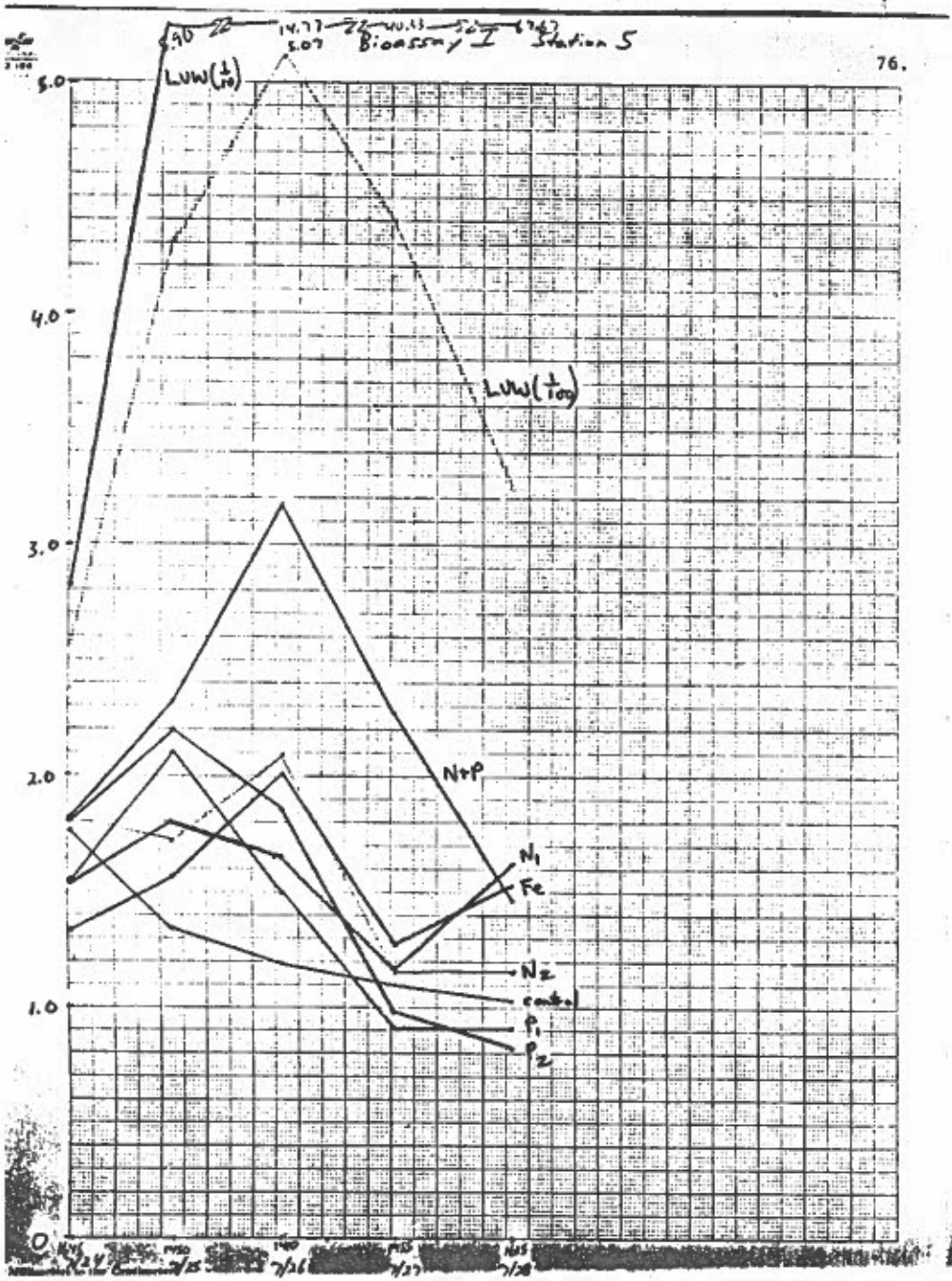

Initial Fluorescence
Station 8
Full Bloom
In Situ Bloom #1
9
8
7
6
5
4
3
2
1
Bloom 9
8 Oct 79
Bloom 10
26 Oct 79
Bloom 11
6 Nov 79
Bloom 12
7 Dec 79
Bloom 13
19 Dec 79
Bloom 14
15 Feb 80
Bloom 16
13 March 80
Bloom 18
3 April 80
Bloom 30
17 April 80

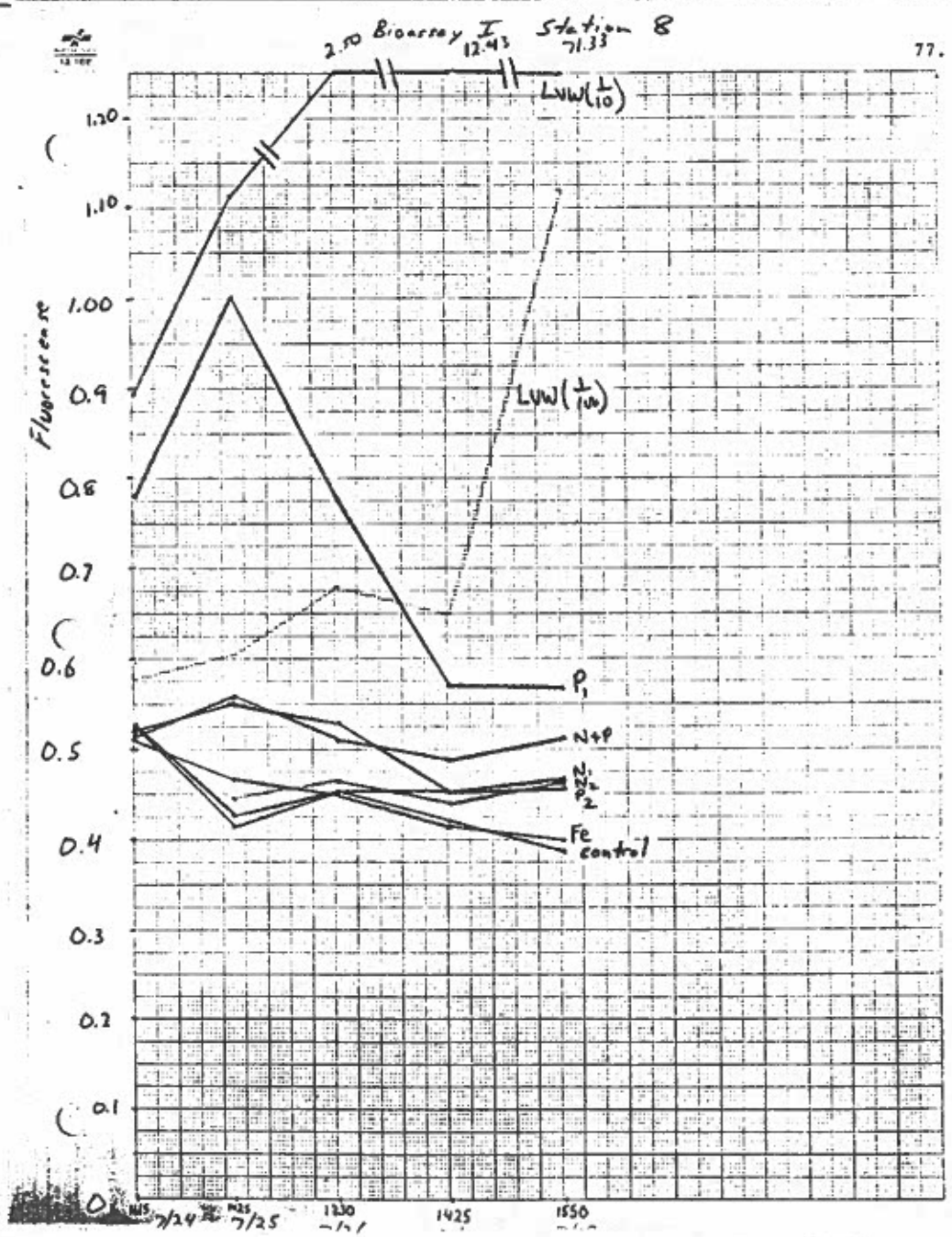
Bioassay I Station 2



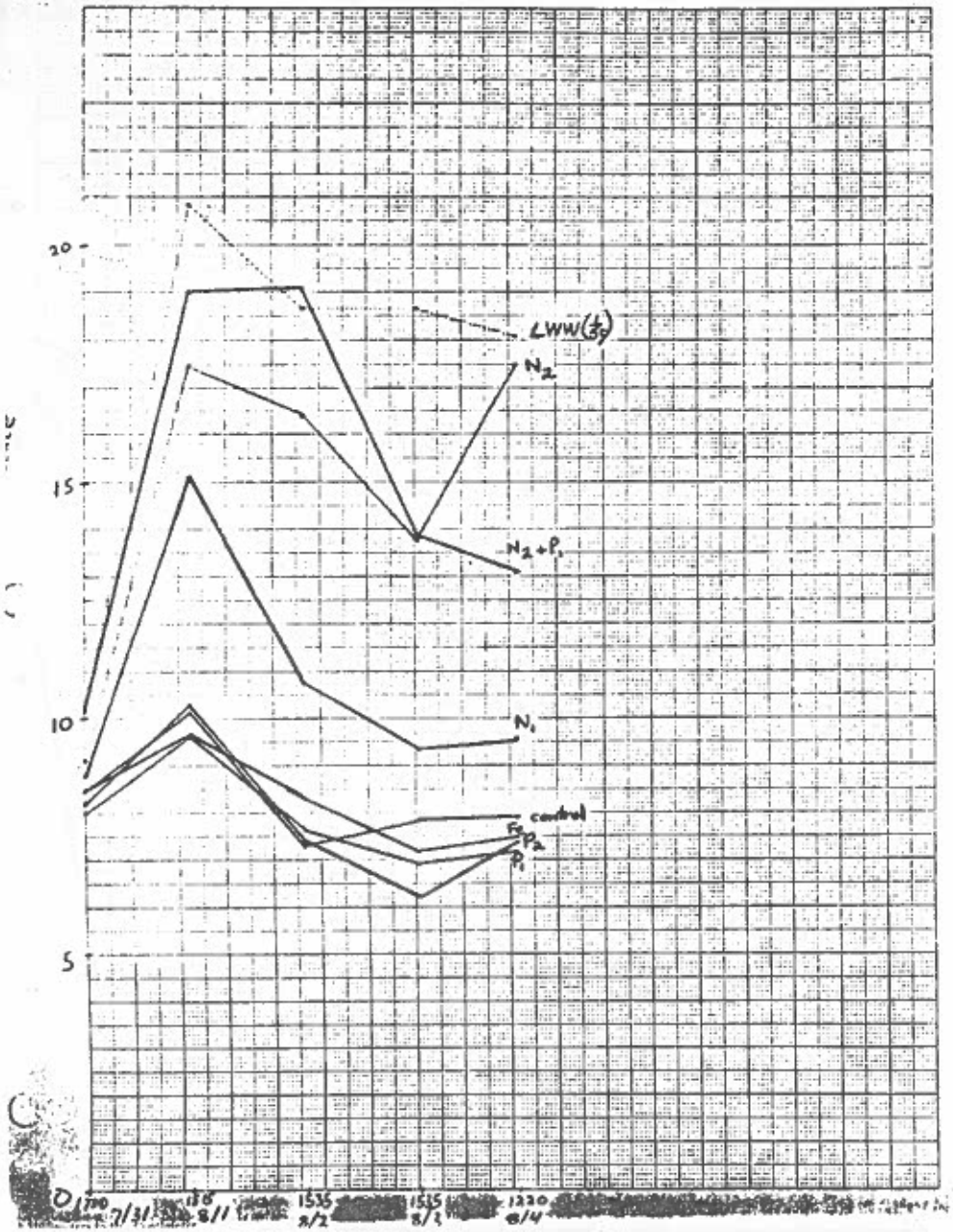
14.5 28.7 Bioassay I 64.67 Station 4 60.73 75



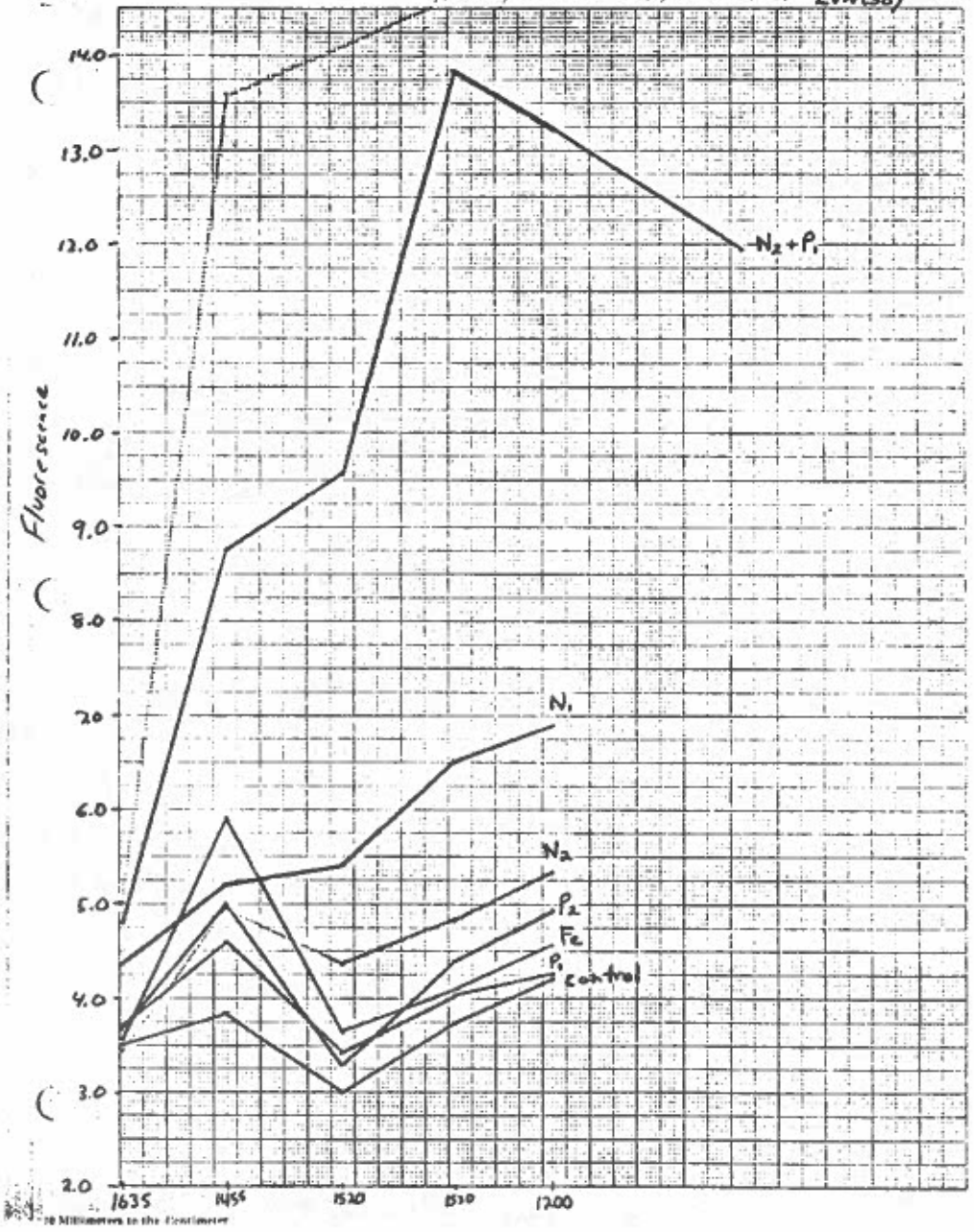

5.90
14.77
5.07
Bioassay I
Station 5
LUW(1/10)
LUW(1/100)
5.0
4.0
3.0
2.0
1.0
0
N+P
N1
Fe
N2
control
P1
P2
7/24
7/25
7/26
7/27
7/28


2.50
12.43
Bioassay I
Station 8
71.33
LUW(1/10)
LUW(1/100)
Fluorescence
1.20
1.10
1.00
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
P1
N+P
N1
N2
P2
Fe
control
7/24
7/25
1230
1425
1550

Bioassay II Station 2

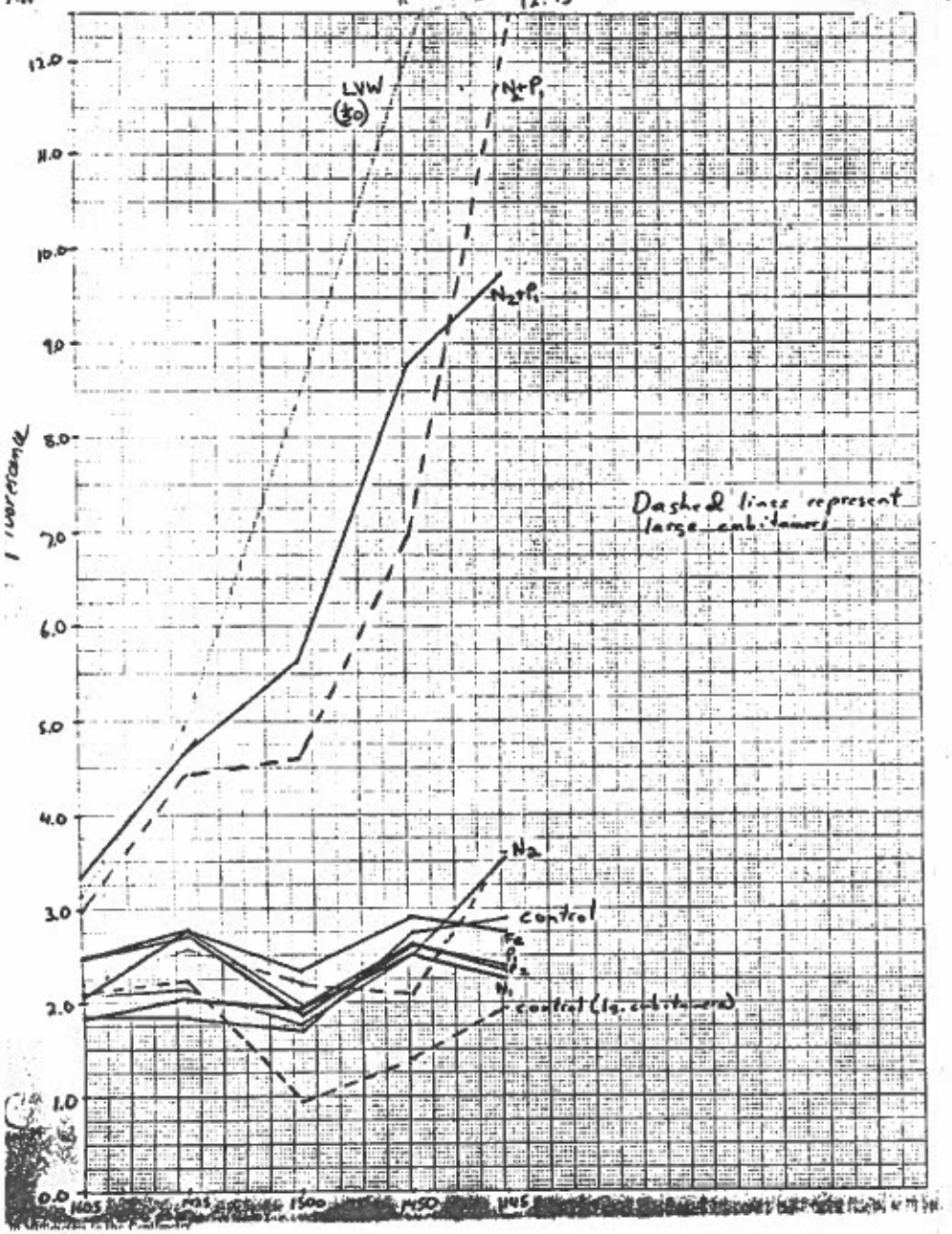


Bioassay II Station 4

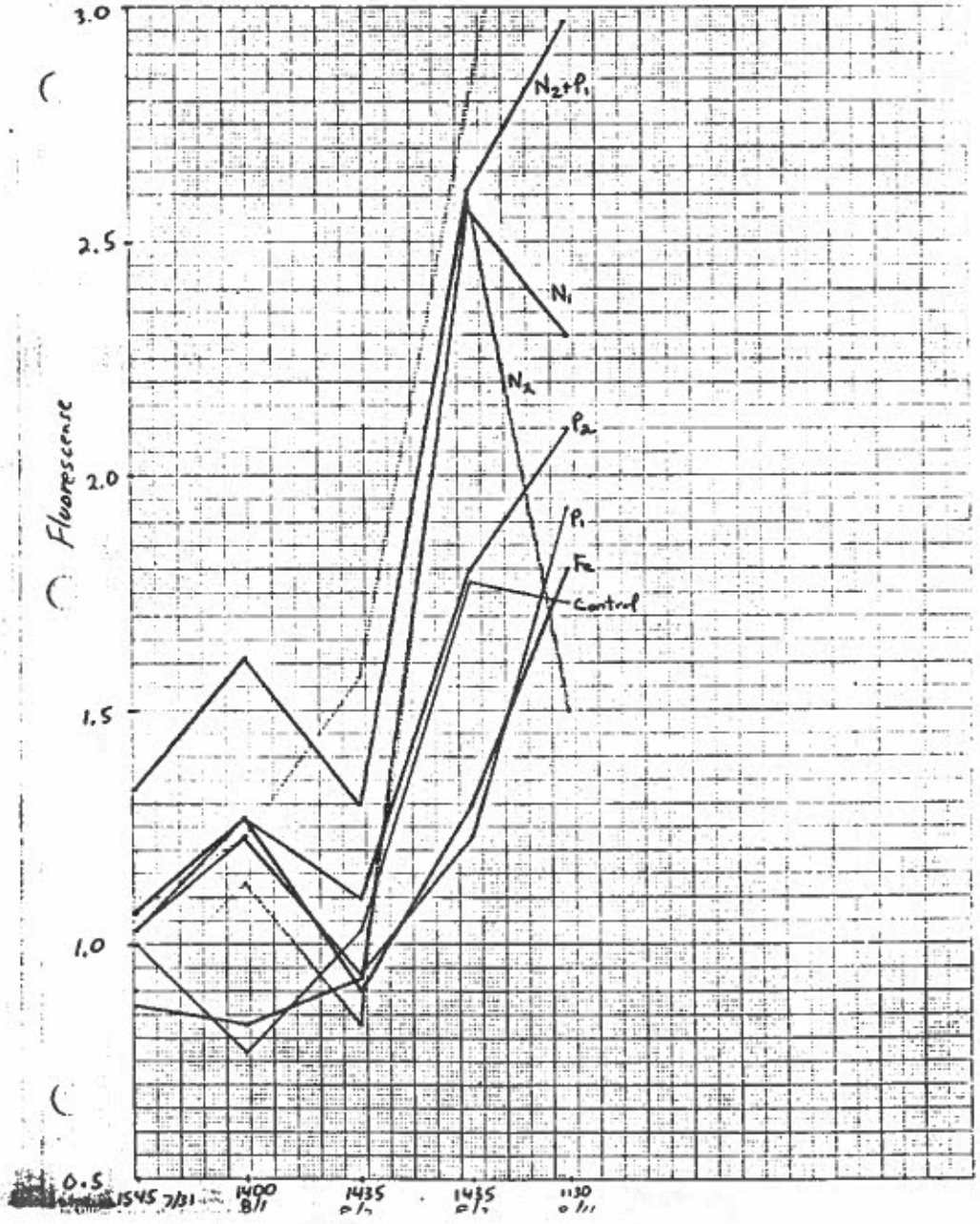


Bioassay II Station 5

12.93

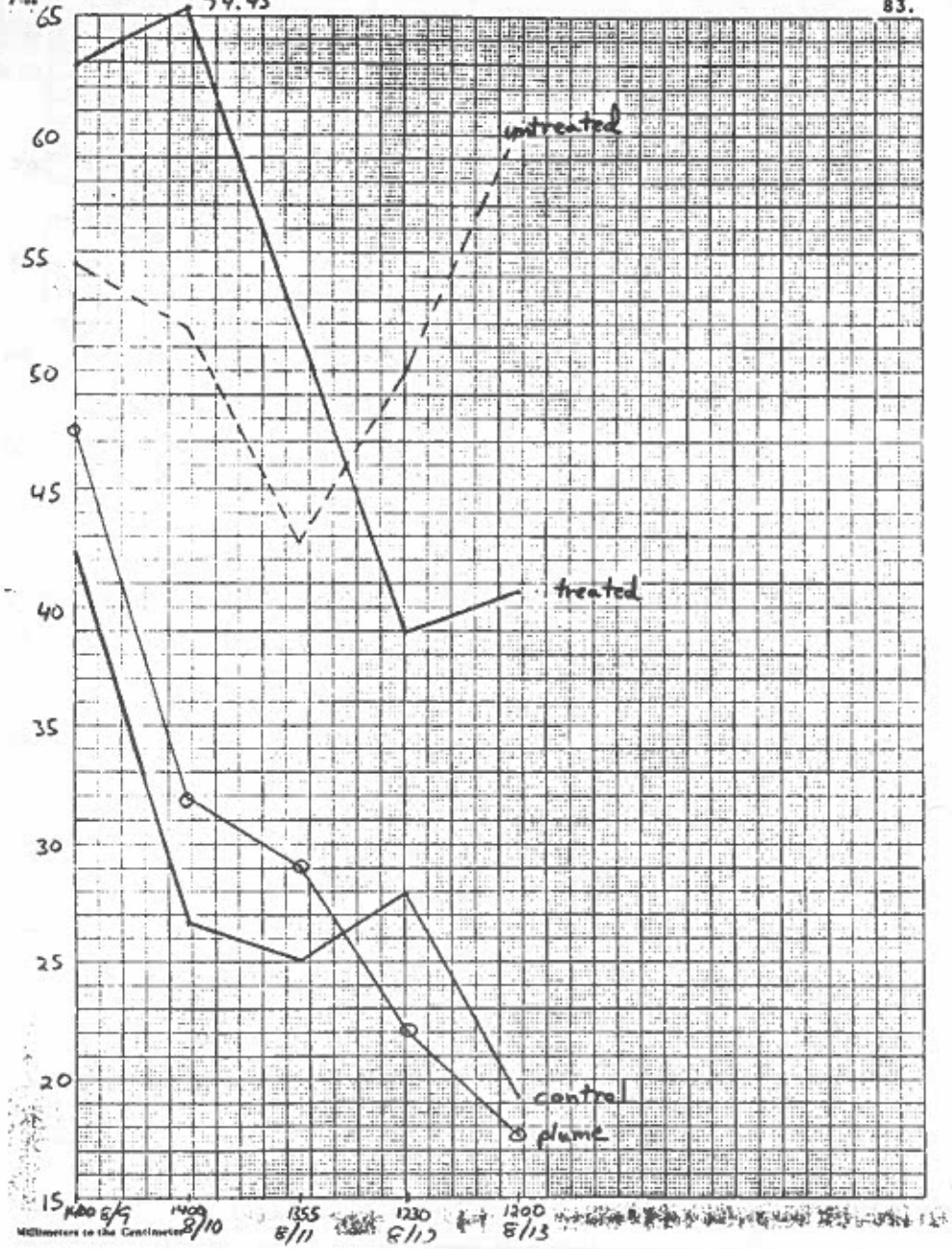


Bioassay II Station 8 LVW (1/30)



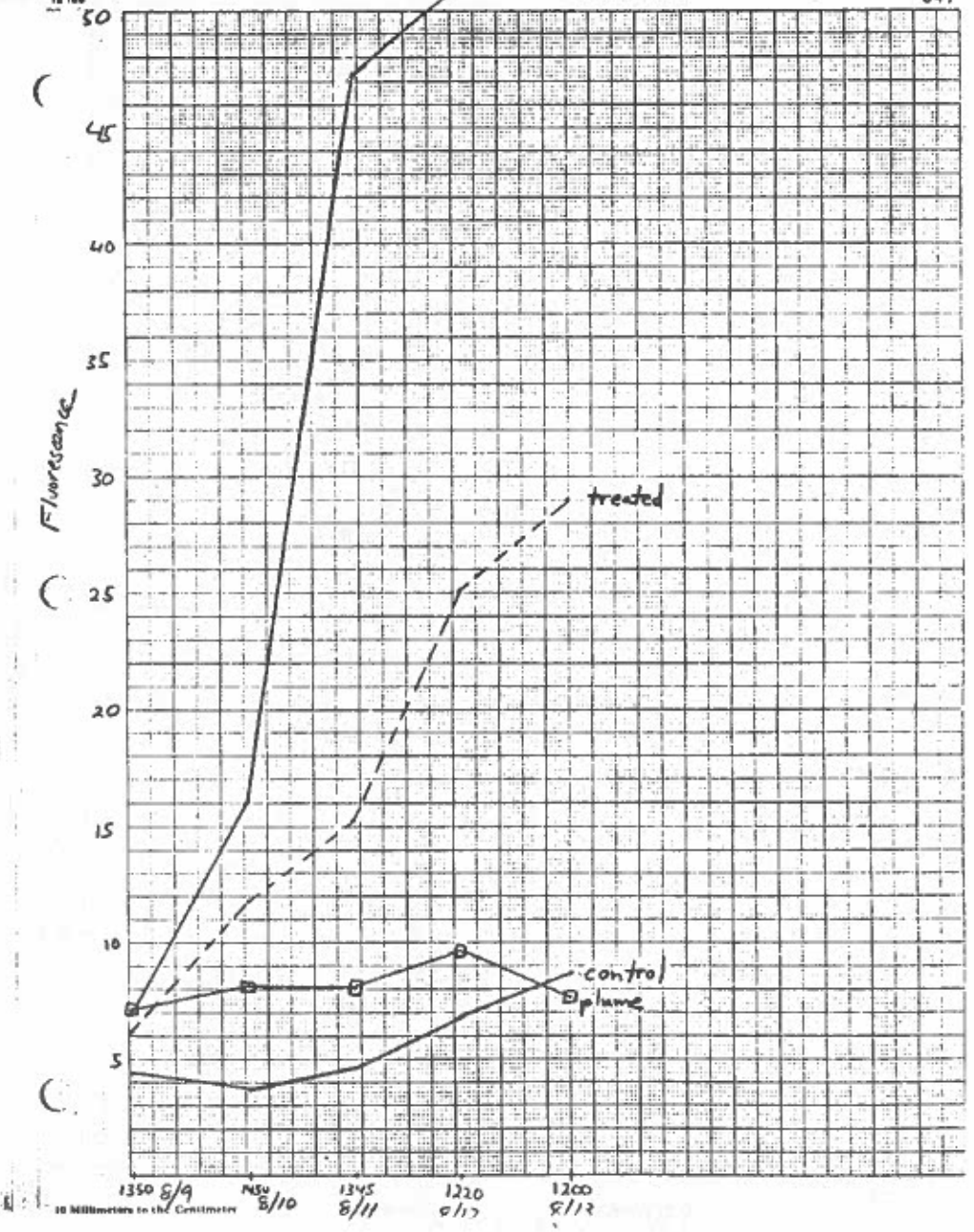
Special Bioassay III
Station 2

83.

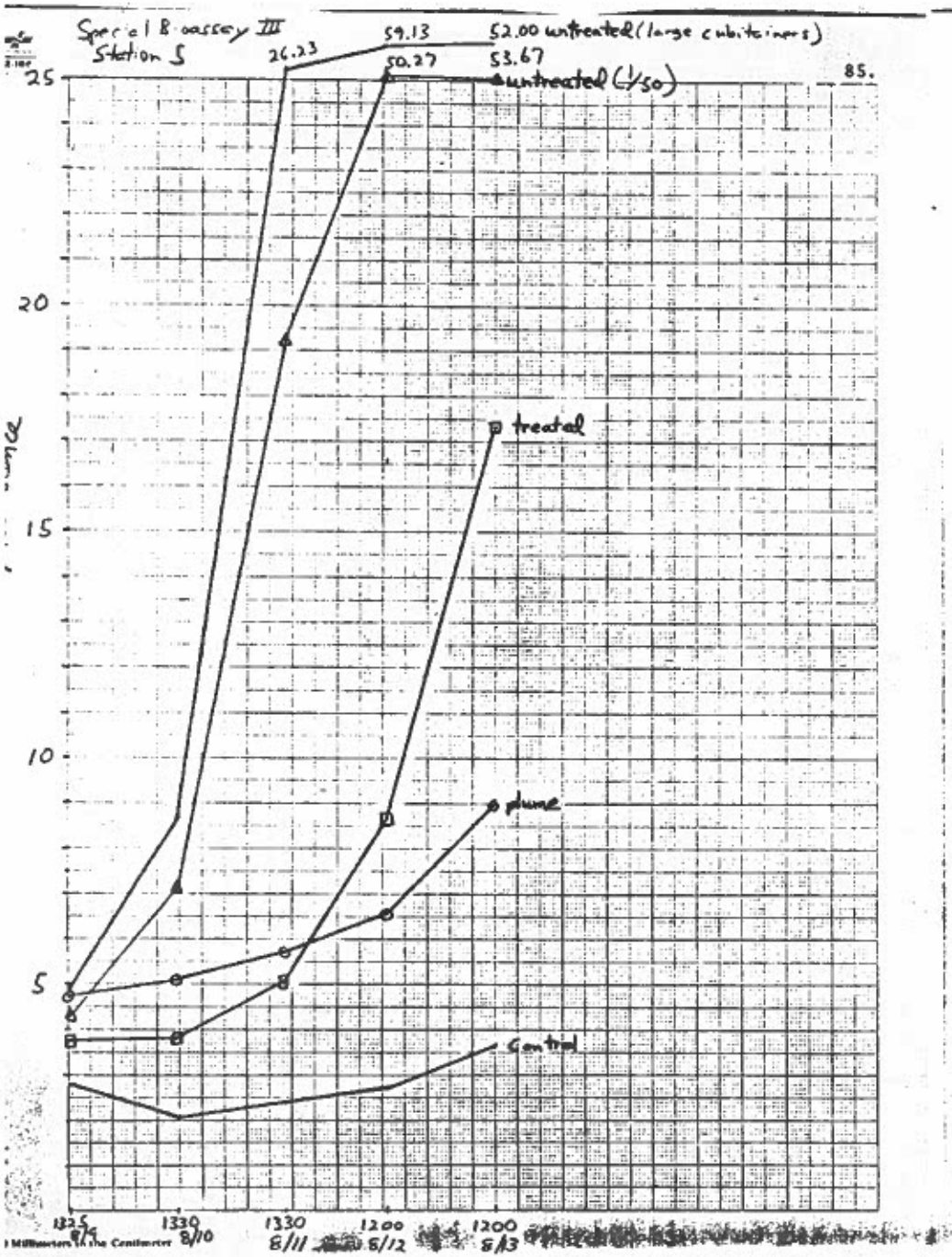


Special Bioassay III
Station 4

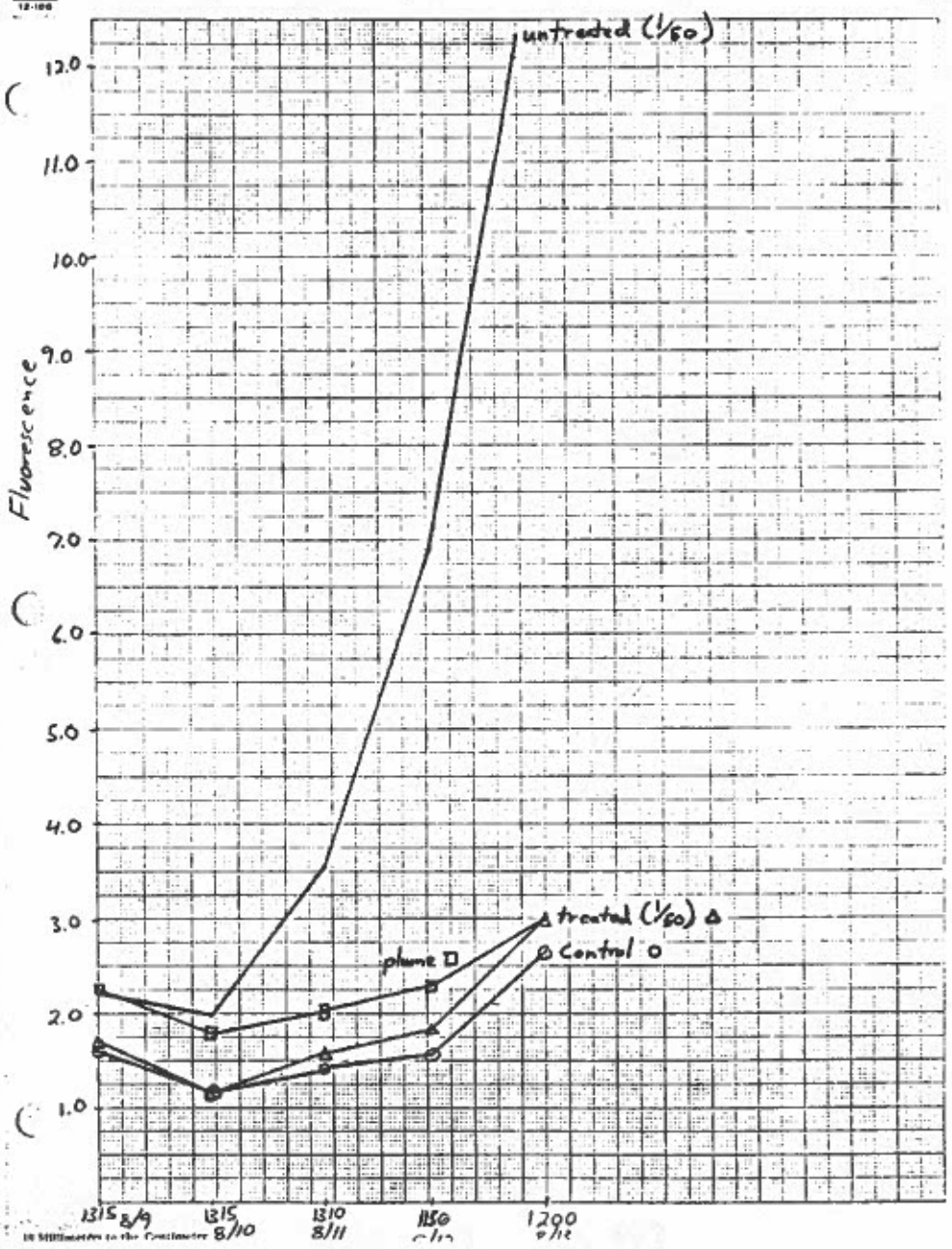
84.



Special Bioassay III
Station 5

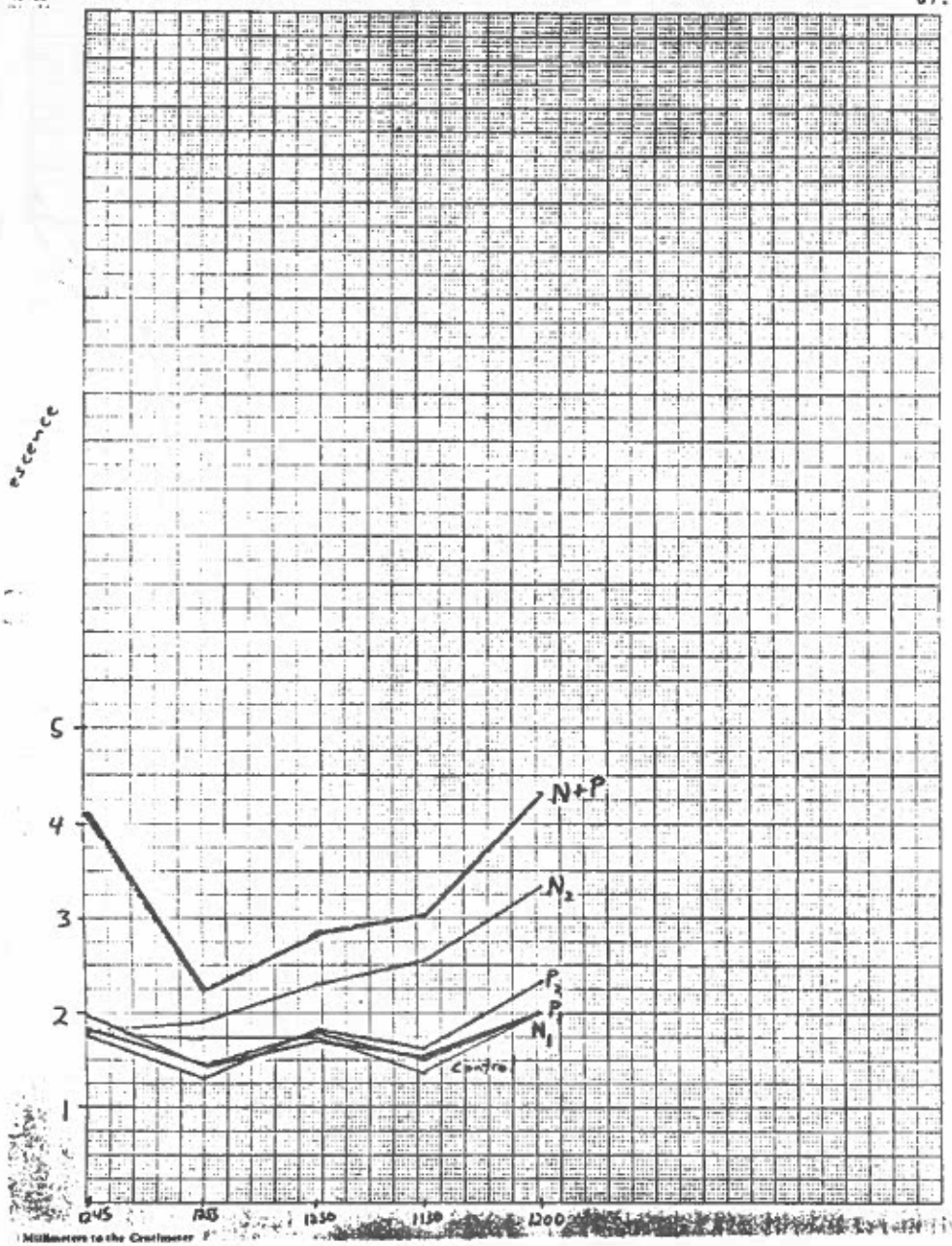


Special Bioassay III 8/9 – 8/13 Station 8



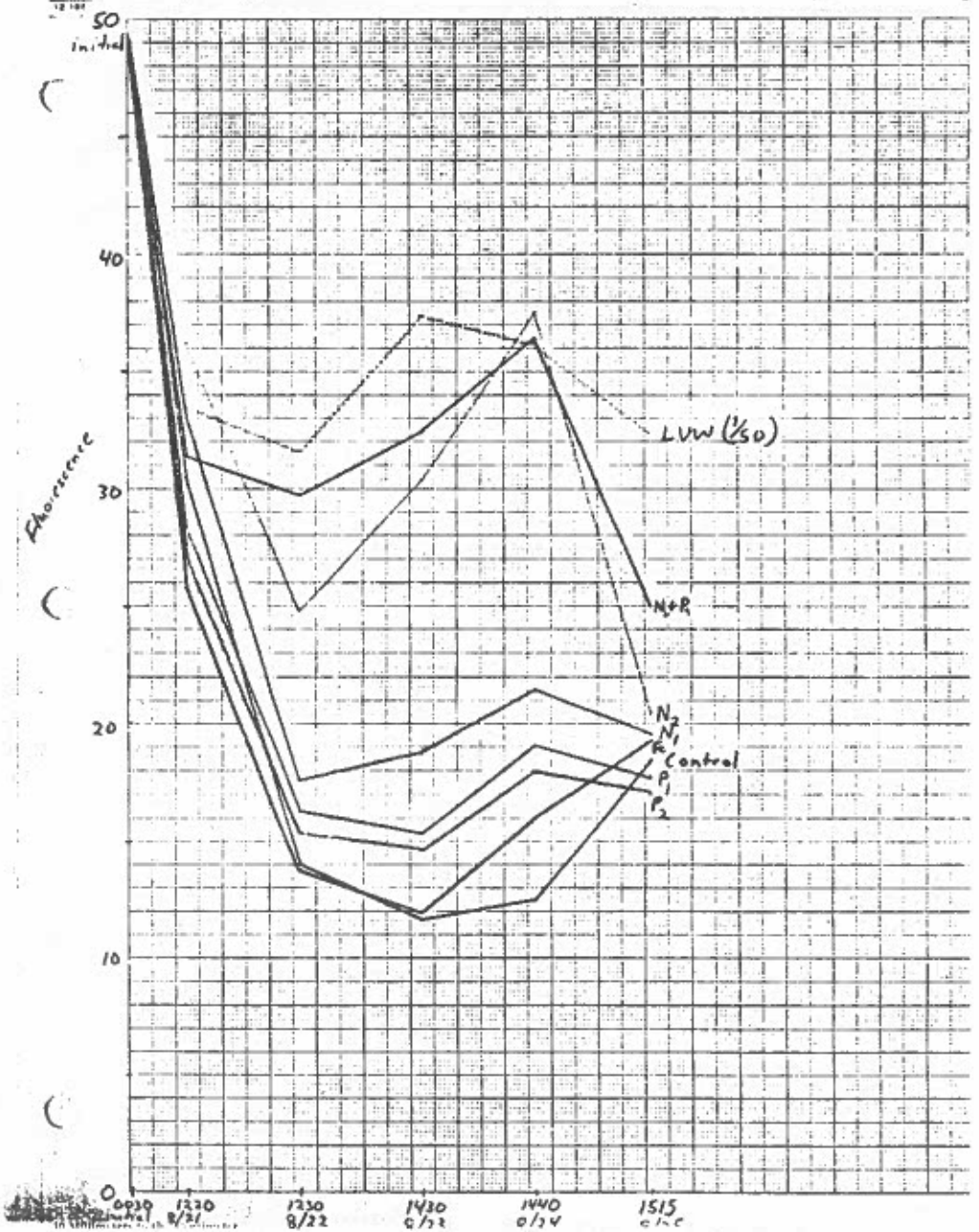
Special Bioassay III 8/9 - 8-13
Virgin Basin

87.



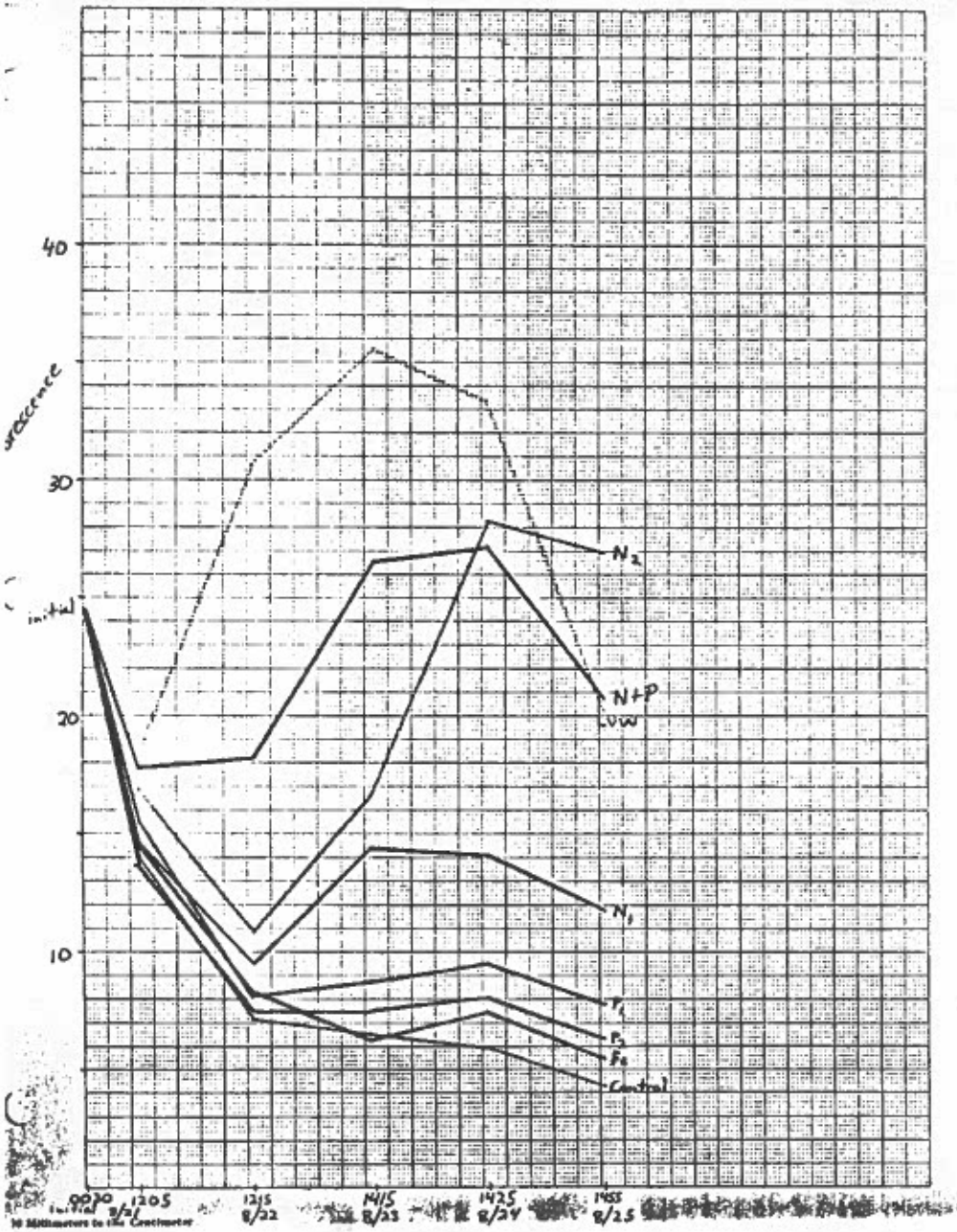
Bioassay III Station 2 8/21 - 8/25

8



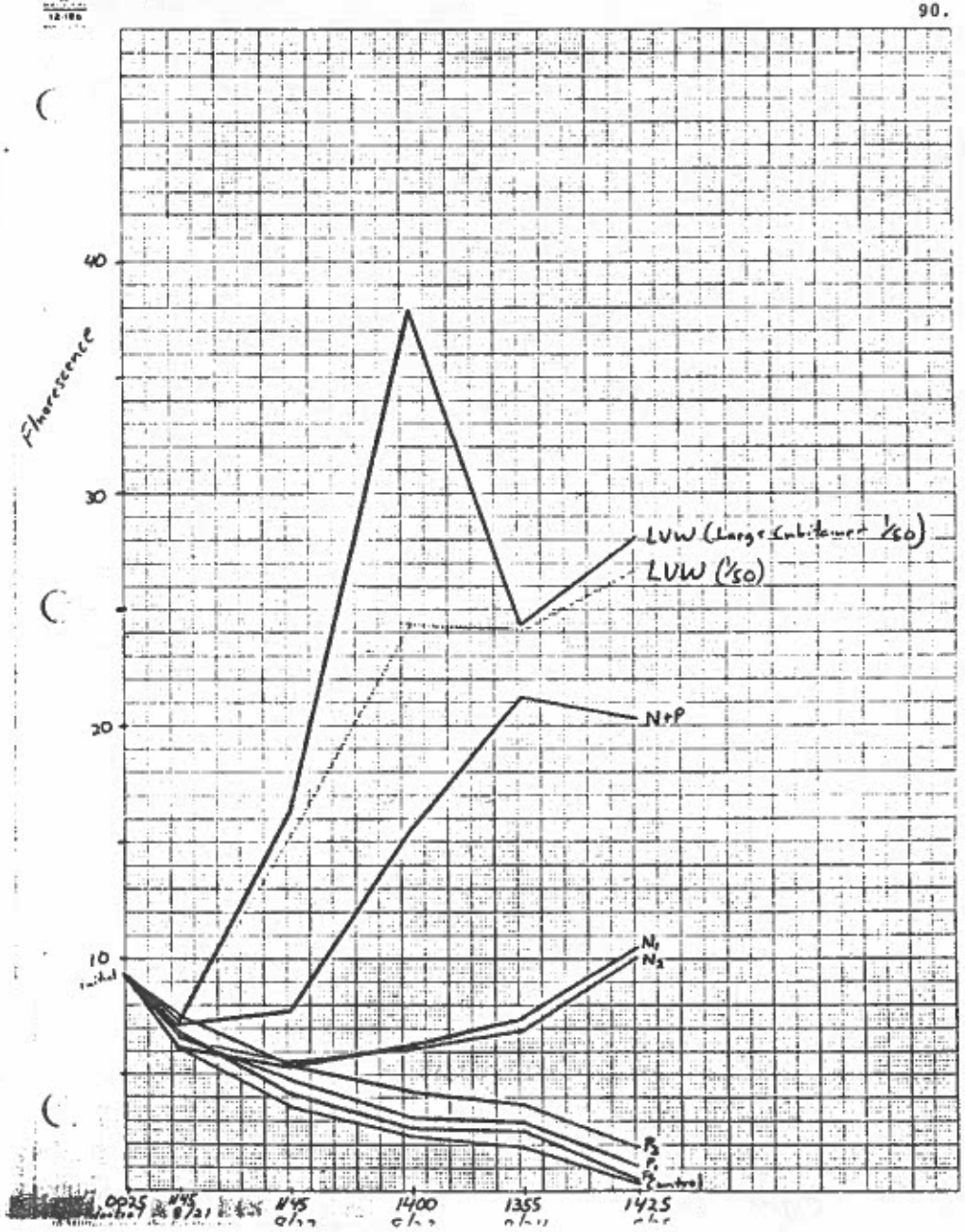
Bioassay III Station 4 8/21 - 8/25

89.



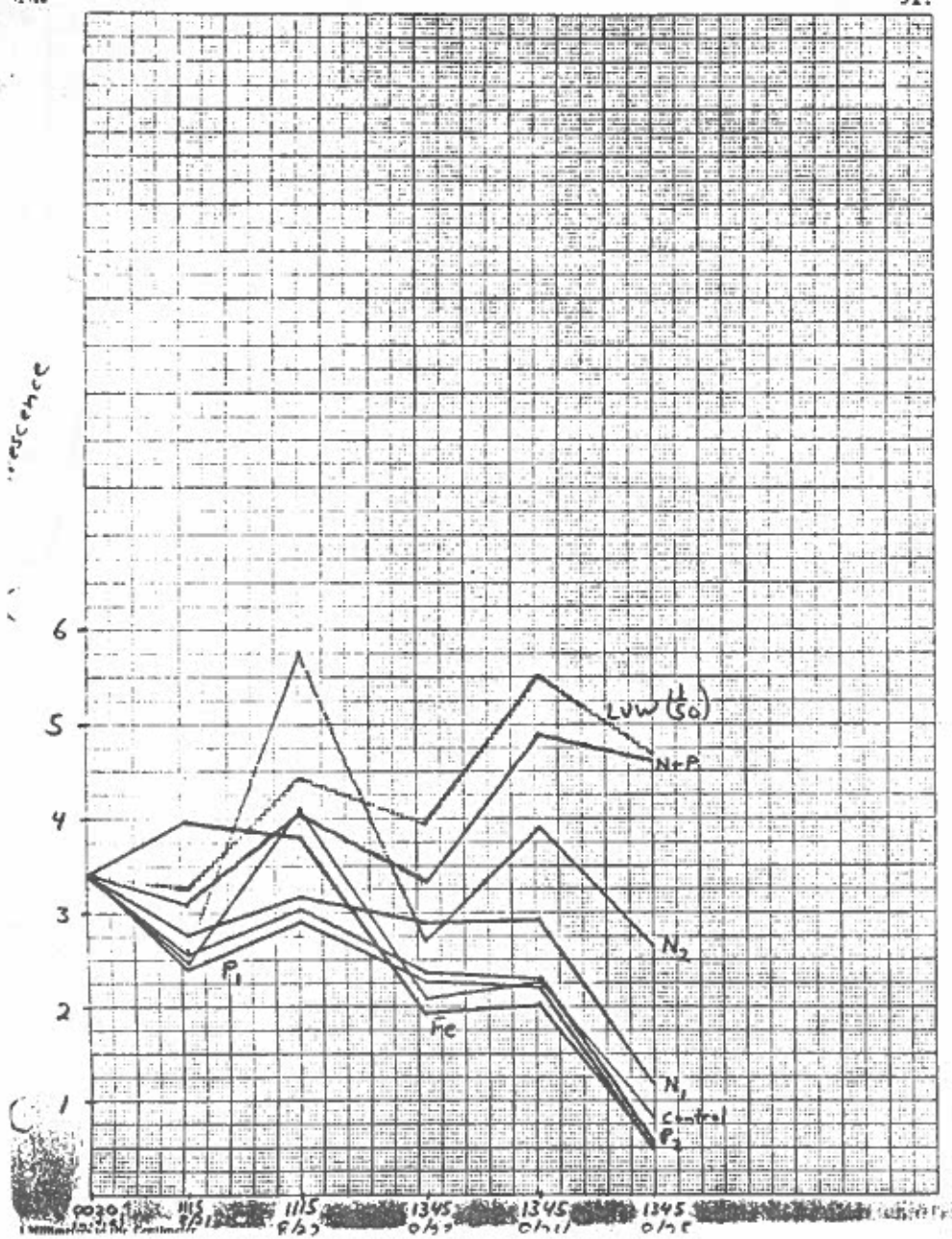
Bioassay III Station 5 8/21 - 8/25

90.



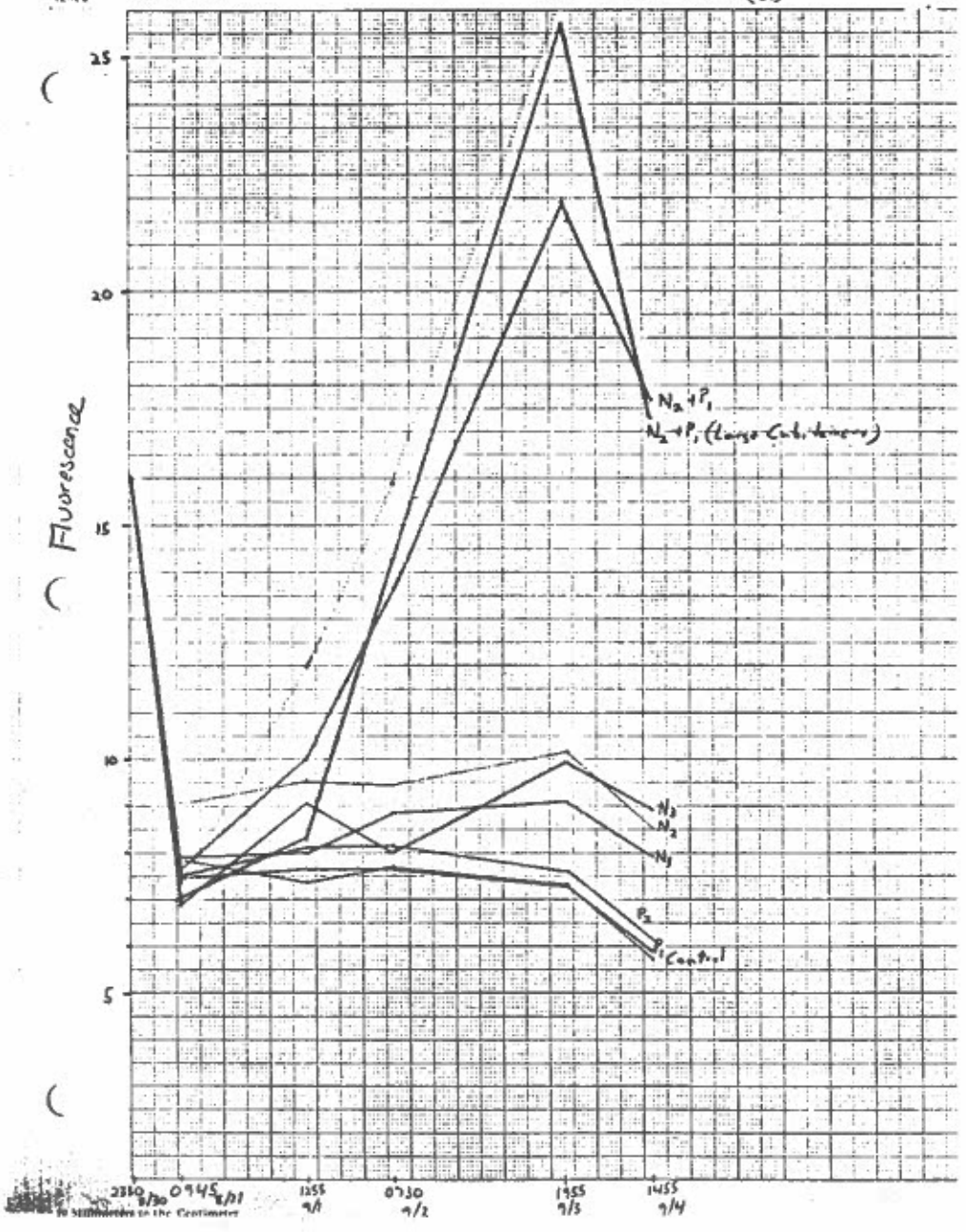
Bioassay III Station 8 8/21 - 8/25

91.



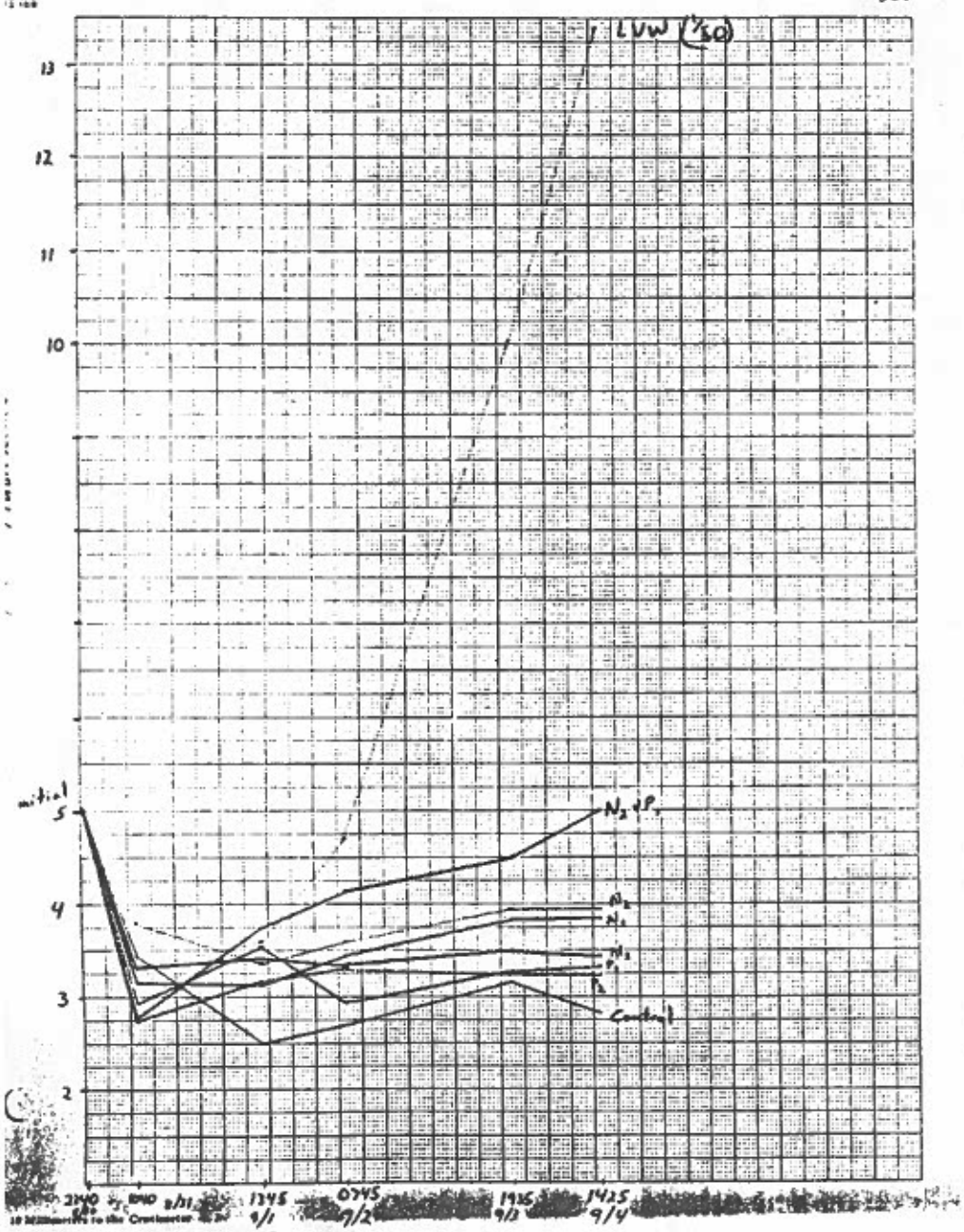
Bioassay II 8/30 - 9/4
Station 4

92



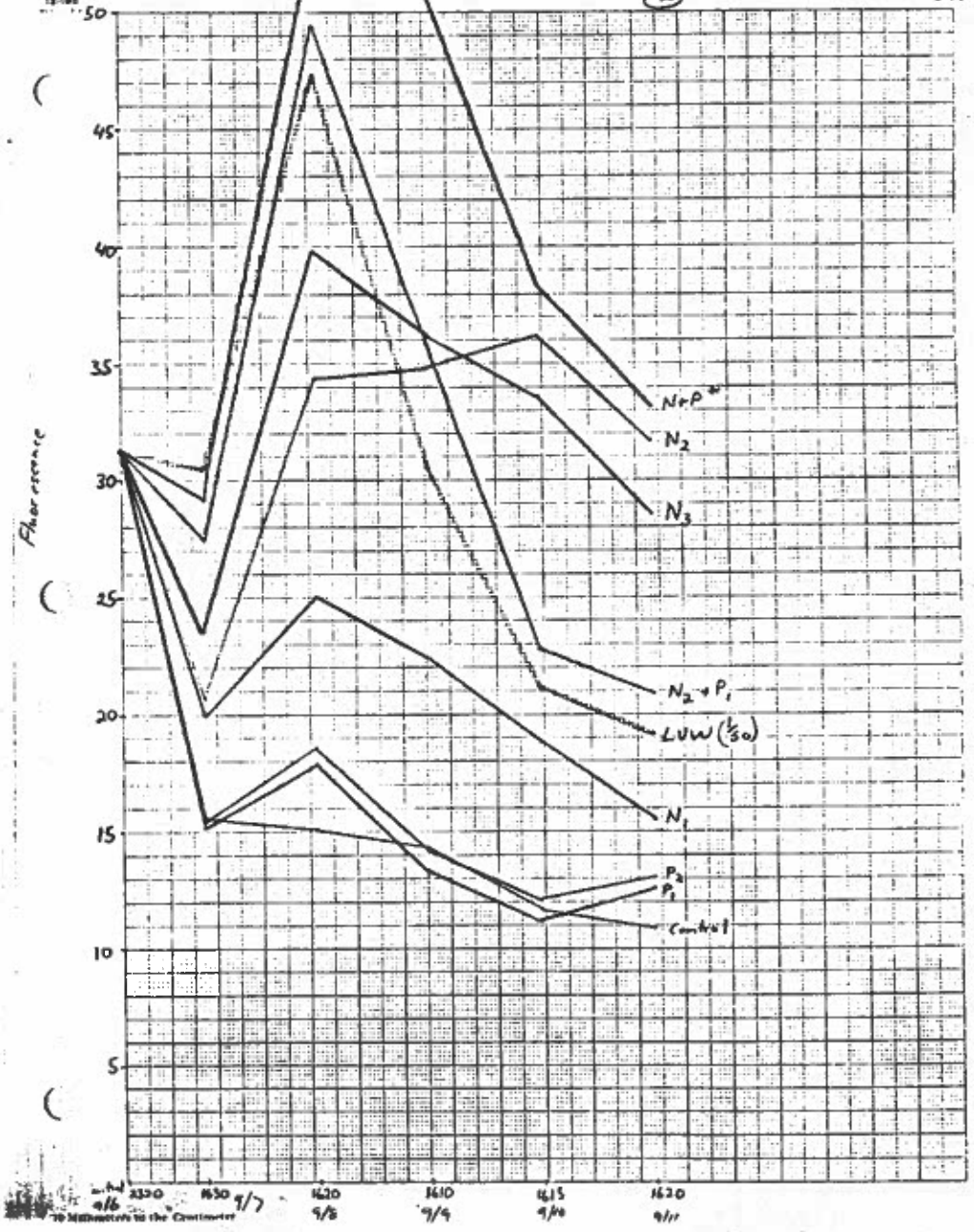
Bioassay V Station 8 8/30 - 9/4

93.

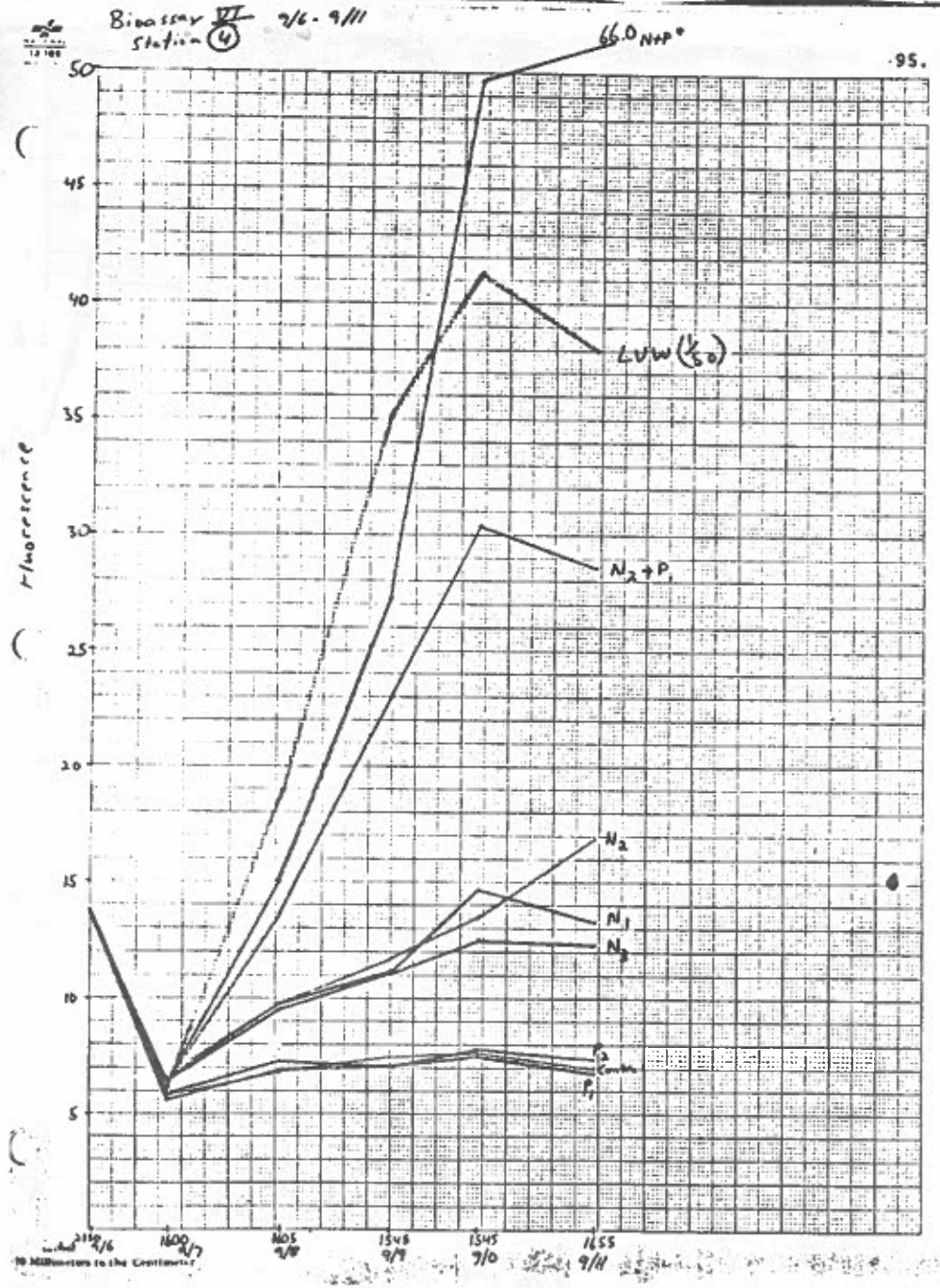


Bioassay VI Station-(2) 9/6-9/11

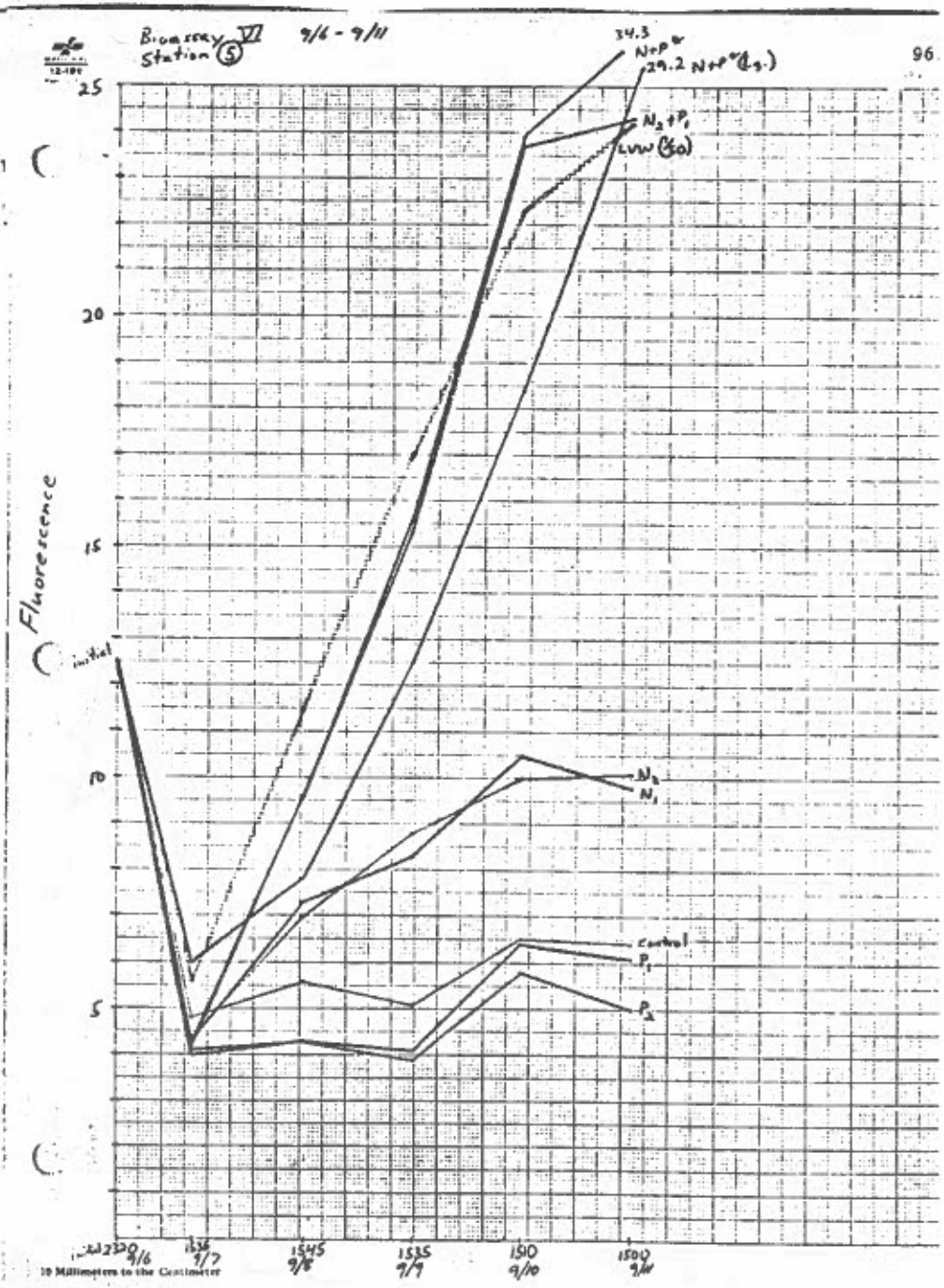
94.



Bioassay VI 9/6 - 9/11
Station (4)

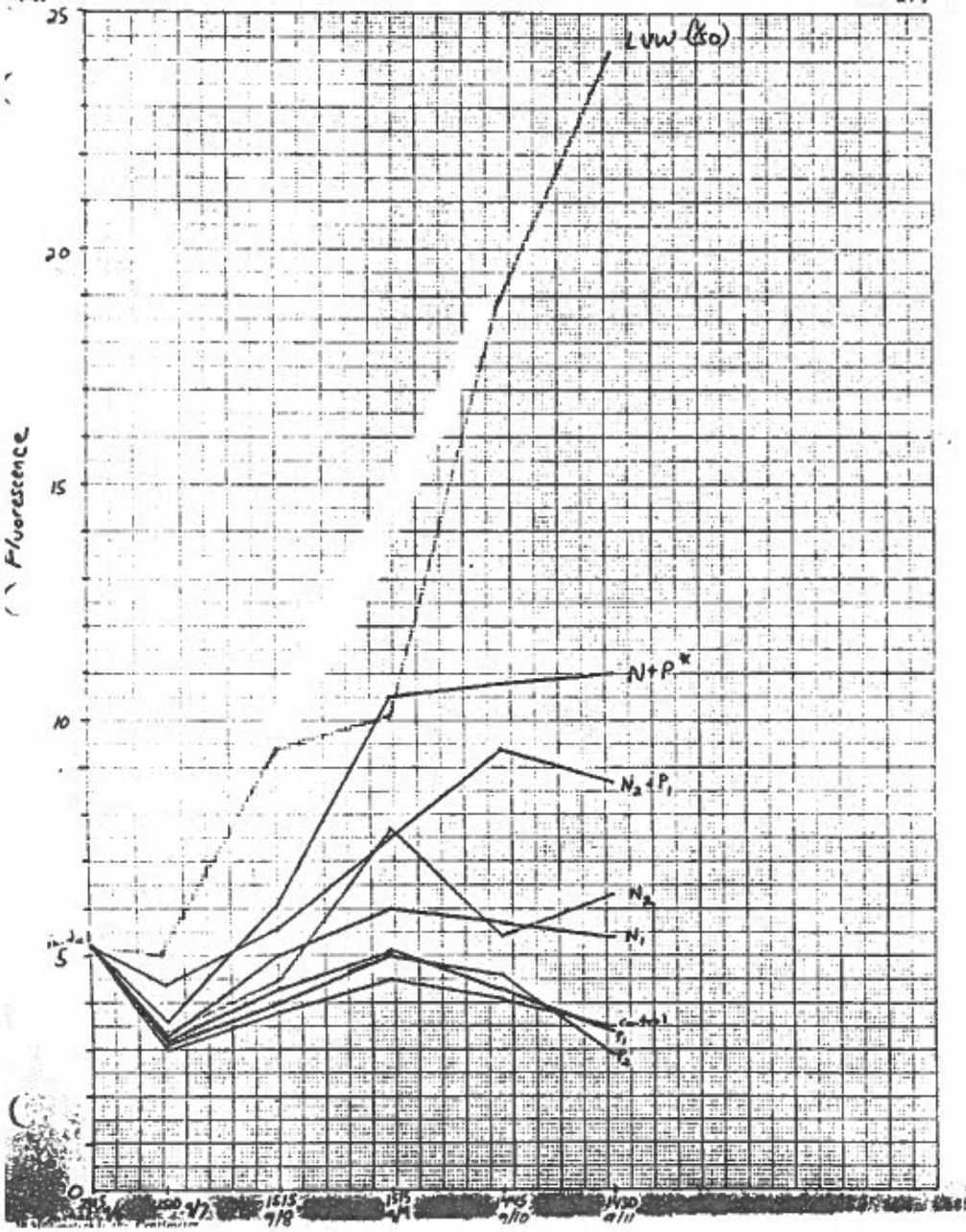


Bioassay VI 9/6 - 9/11
Station (5)



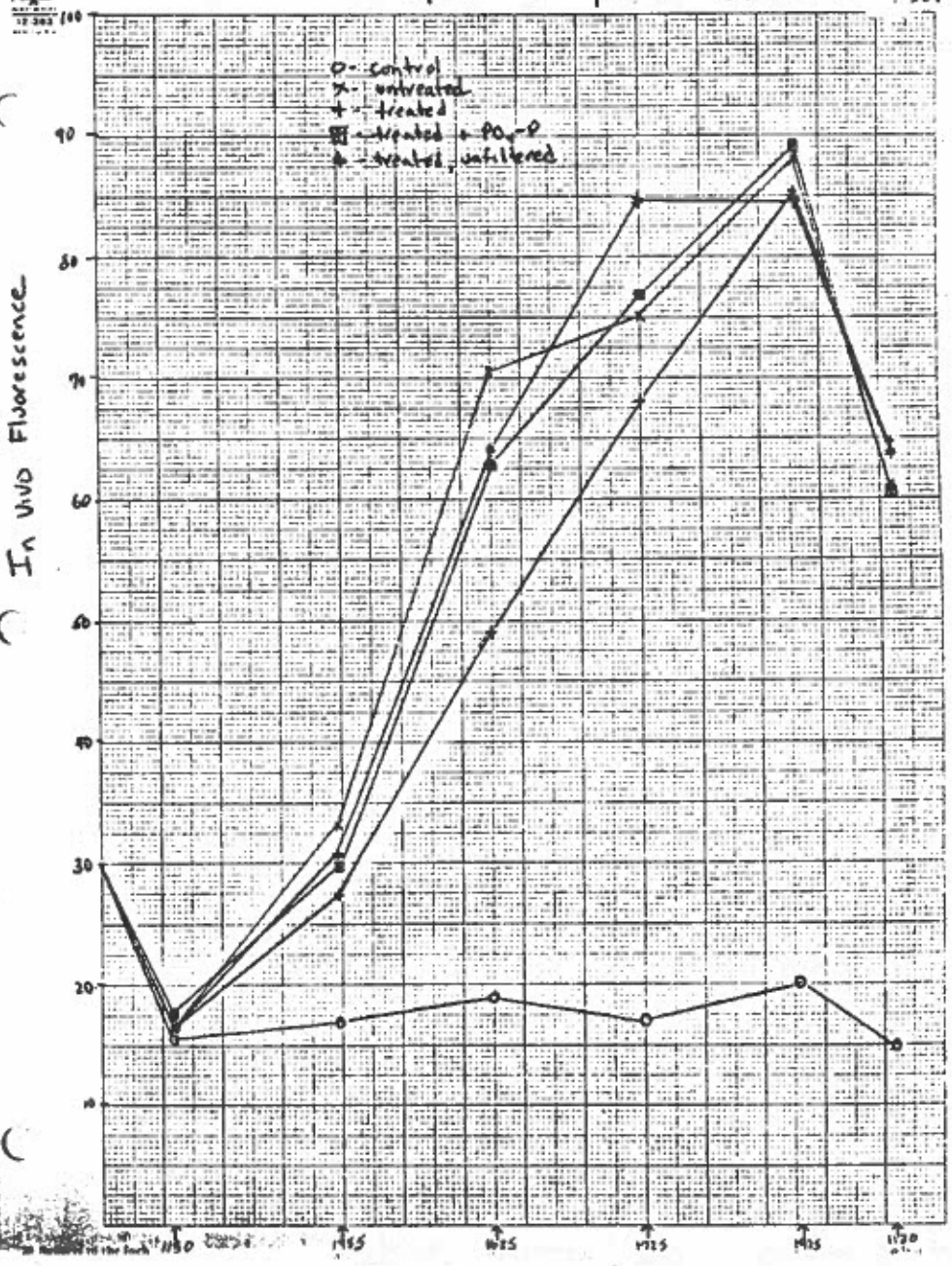
Bioassay III Station (5) 9/6 - 9/11

97.

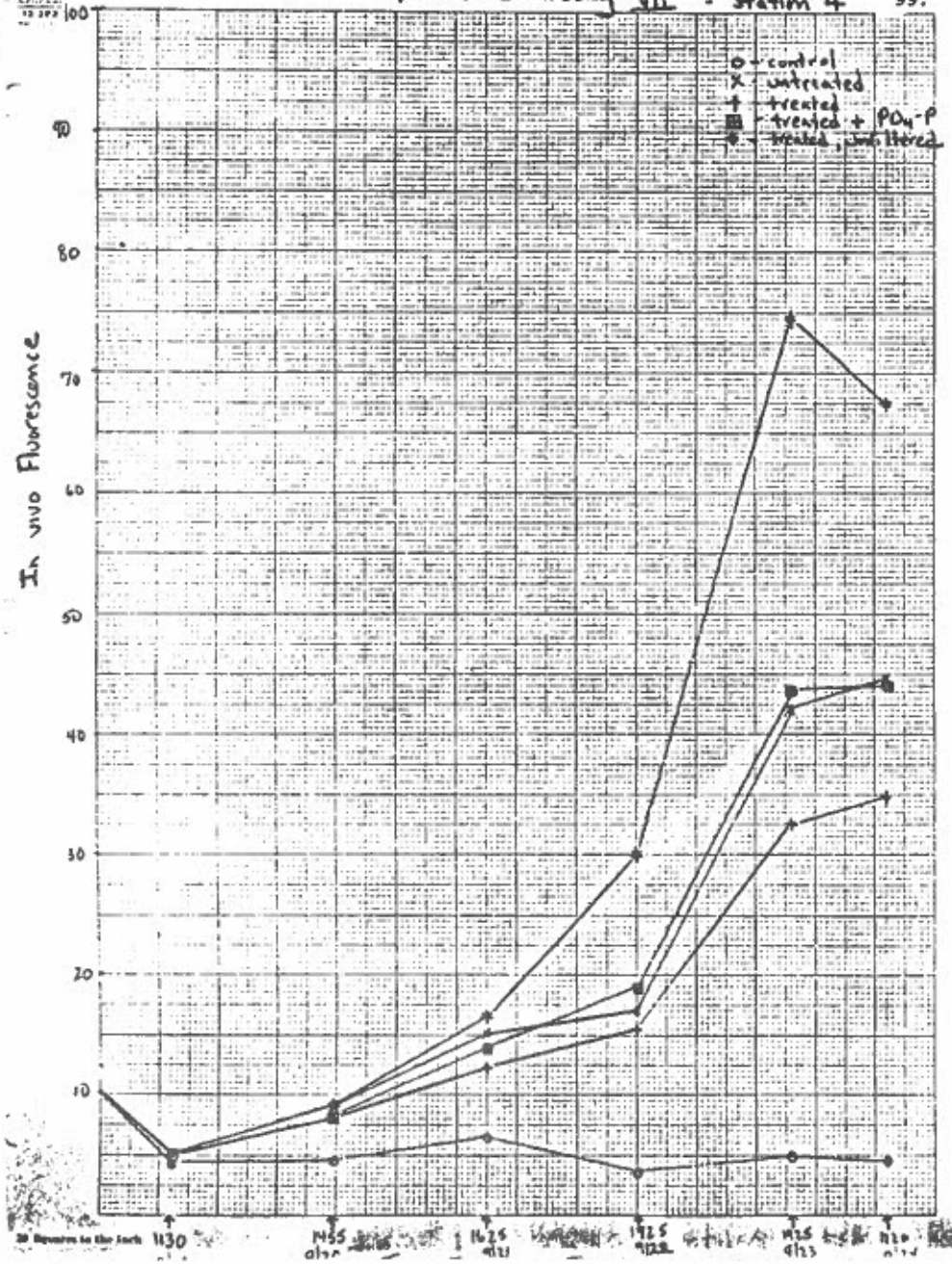


Special Bioassay VIII Station 2

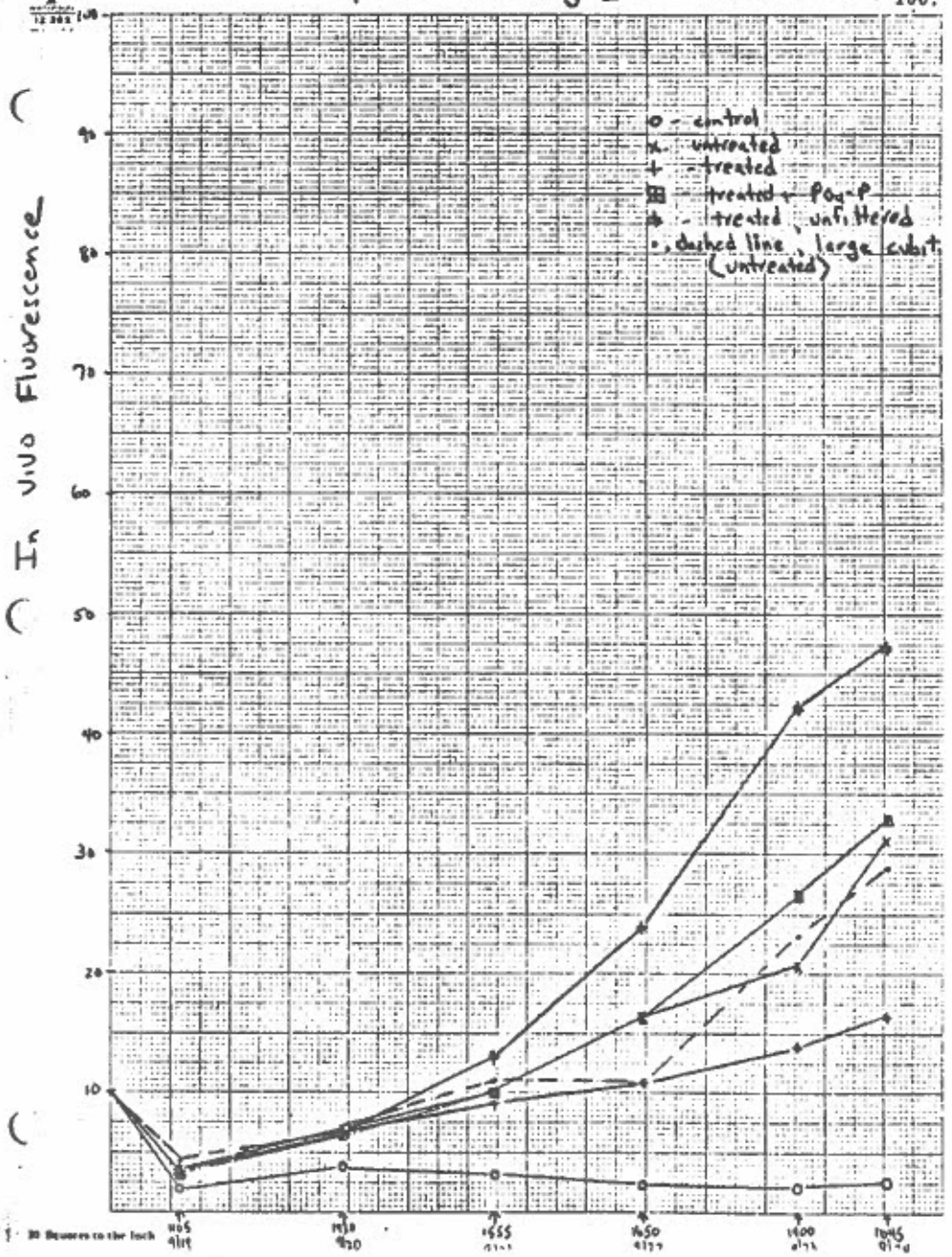
98.



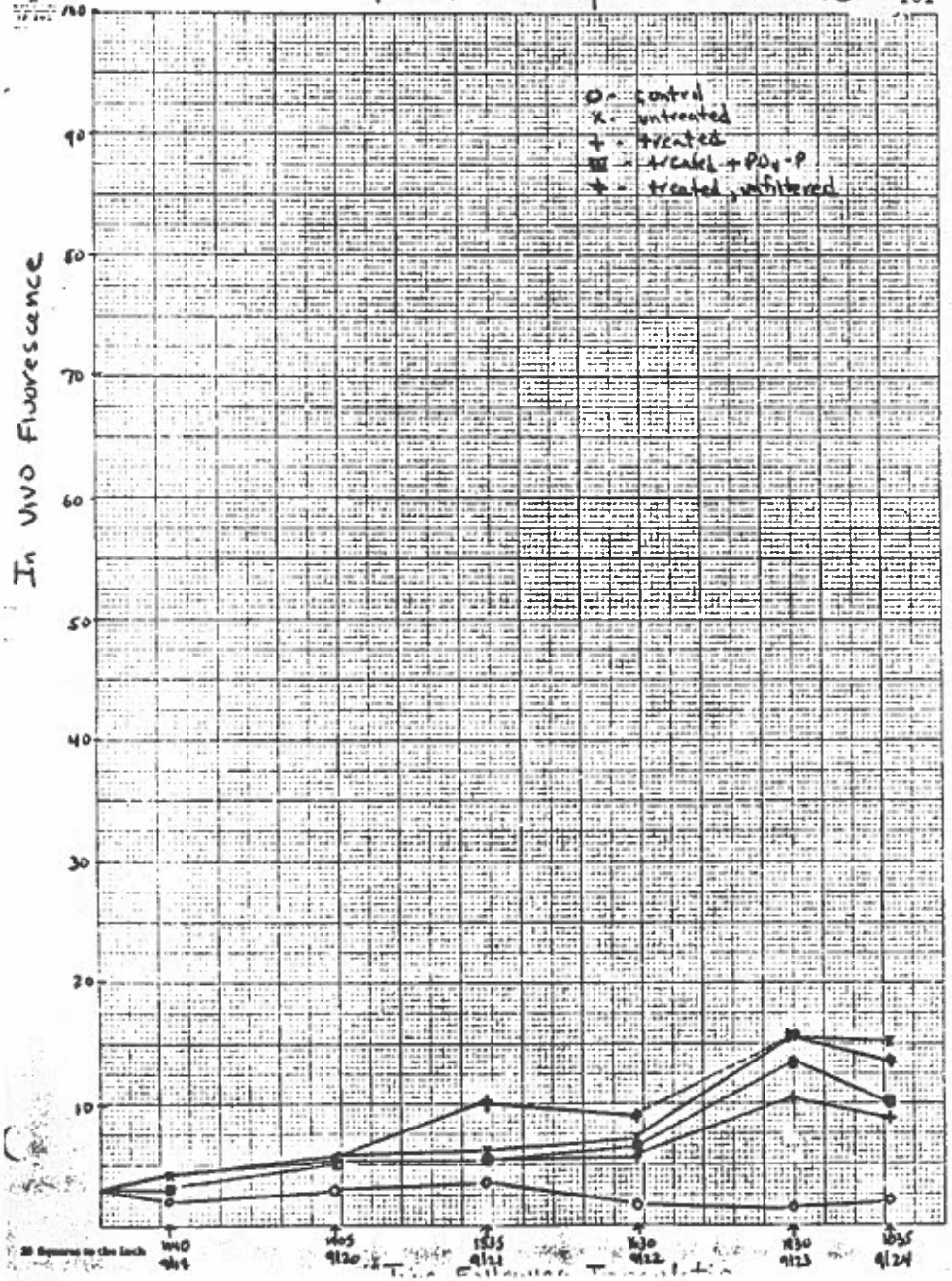
Special Bioassay VII - Station 4 99.



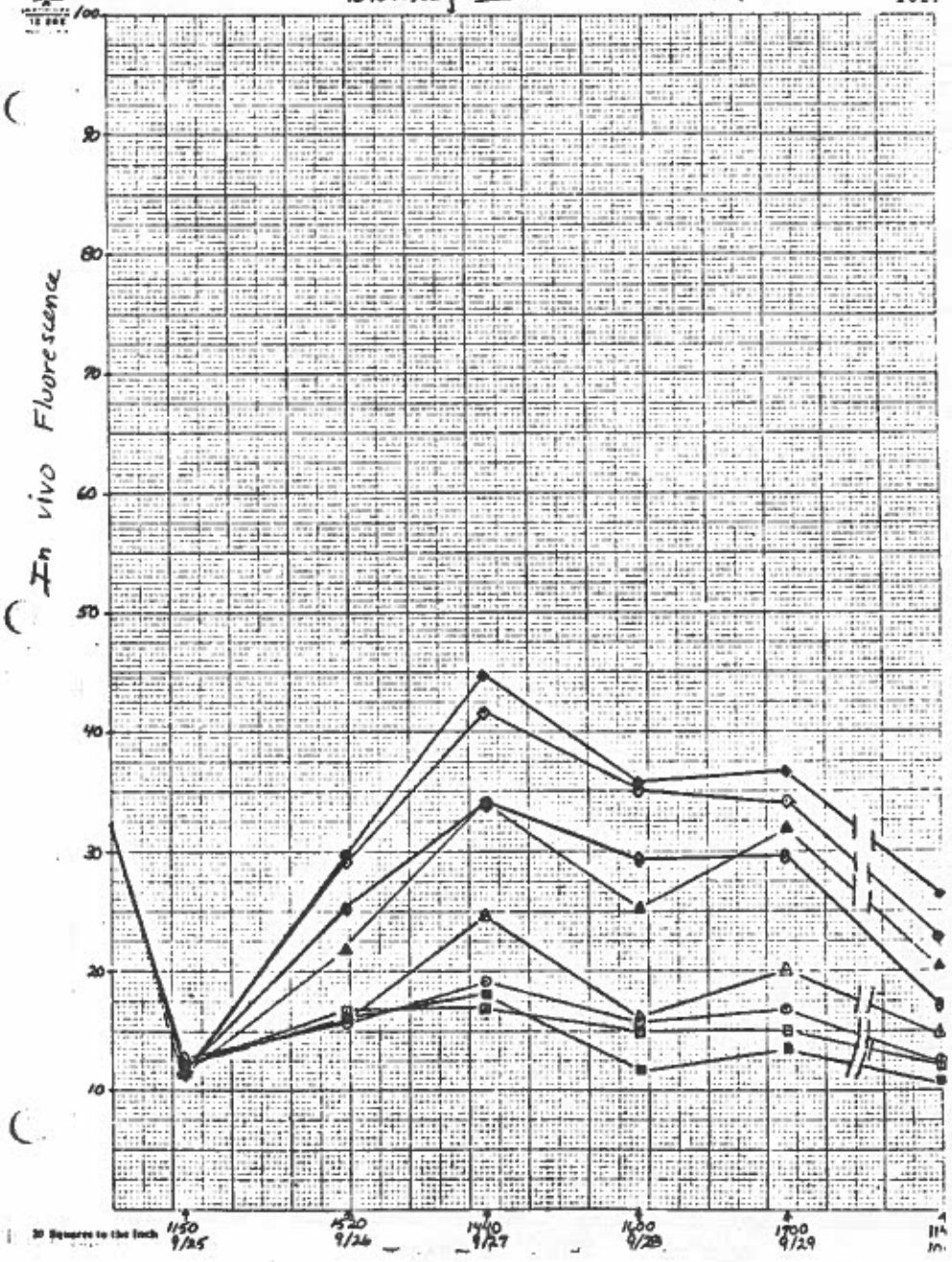
Special Bioassay VII Station 5 100.



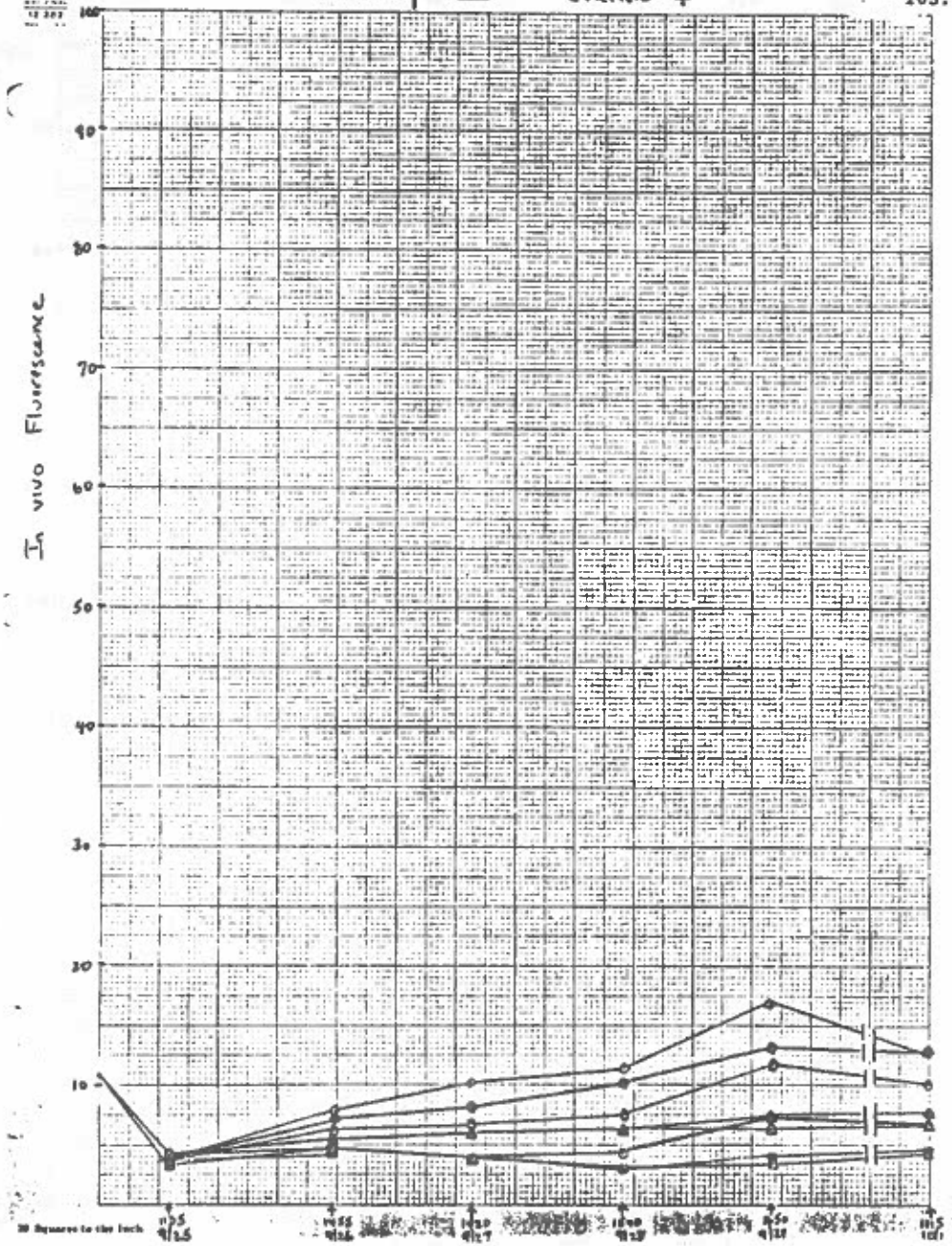
Special Bioassay VII Station 8



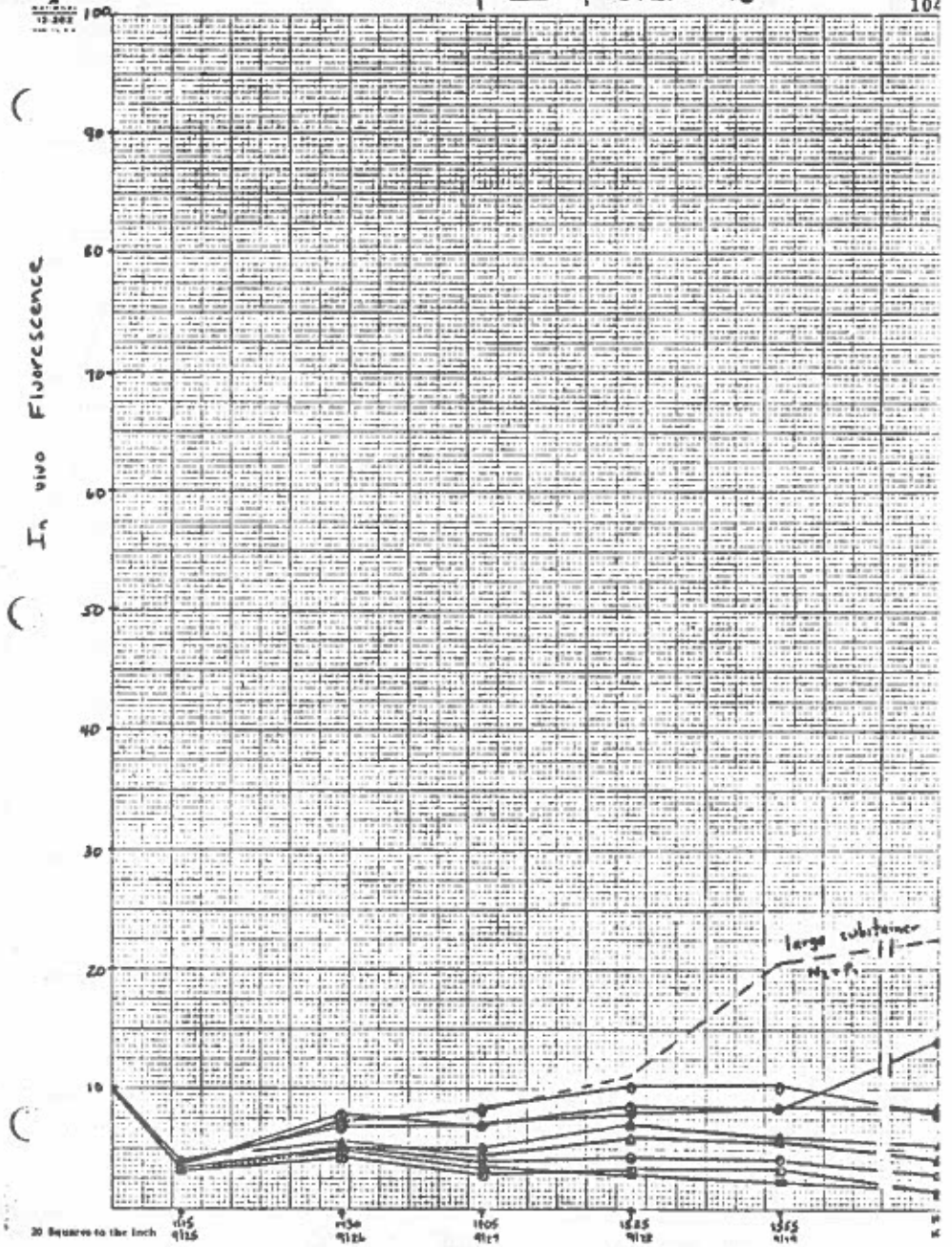
BioAssay VIII Station 2



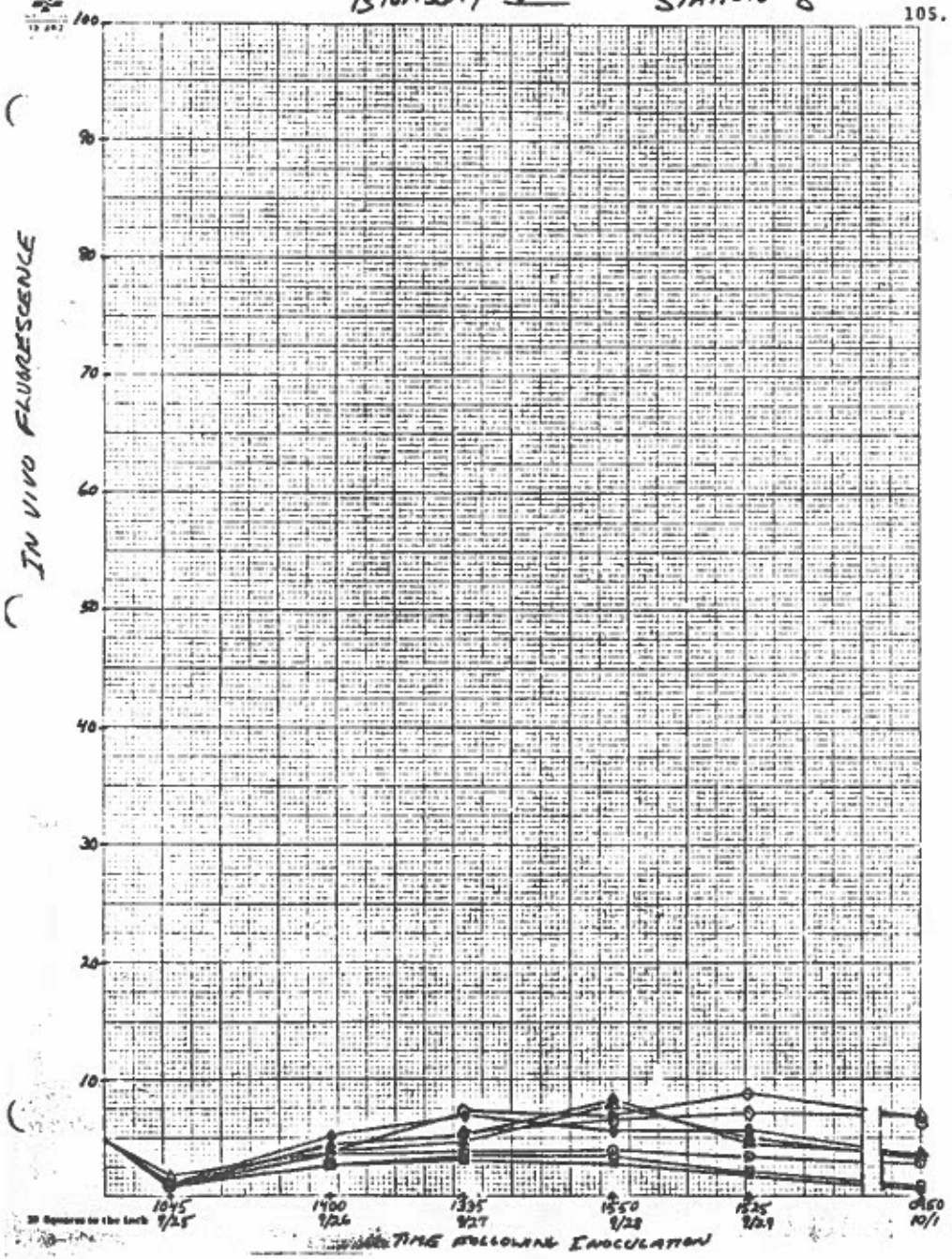
Bioassay VIII Station 4



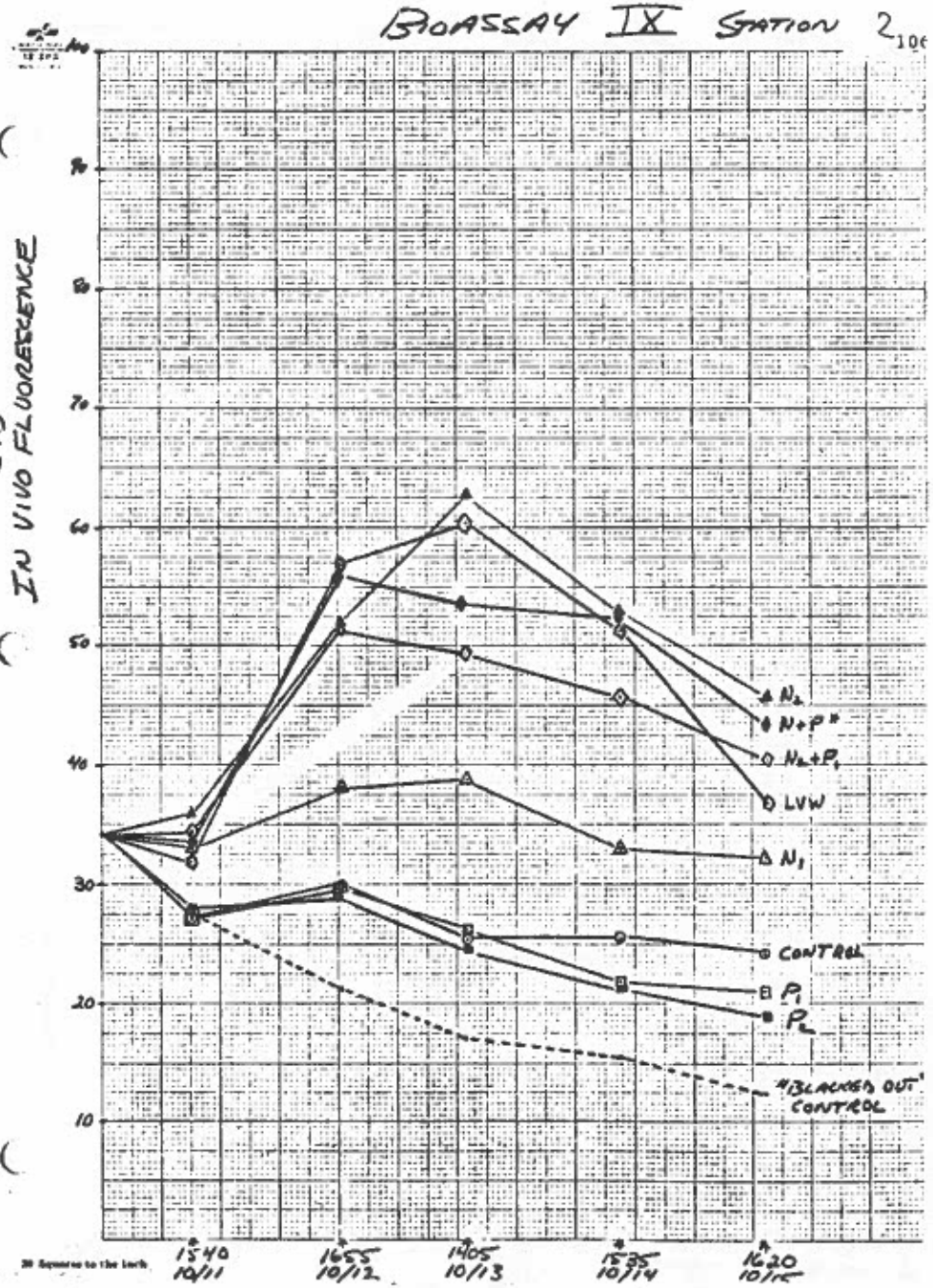
Bioassay VIII, Station 5



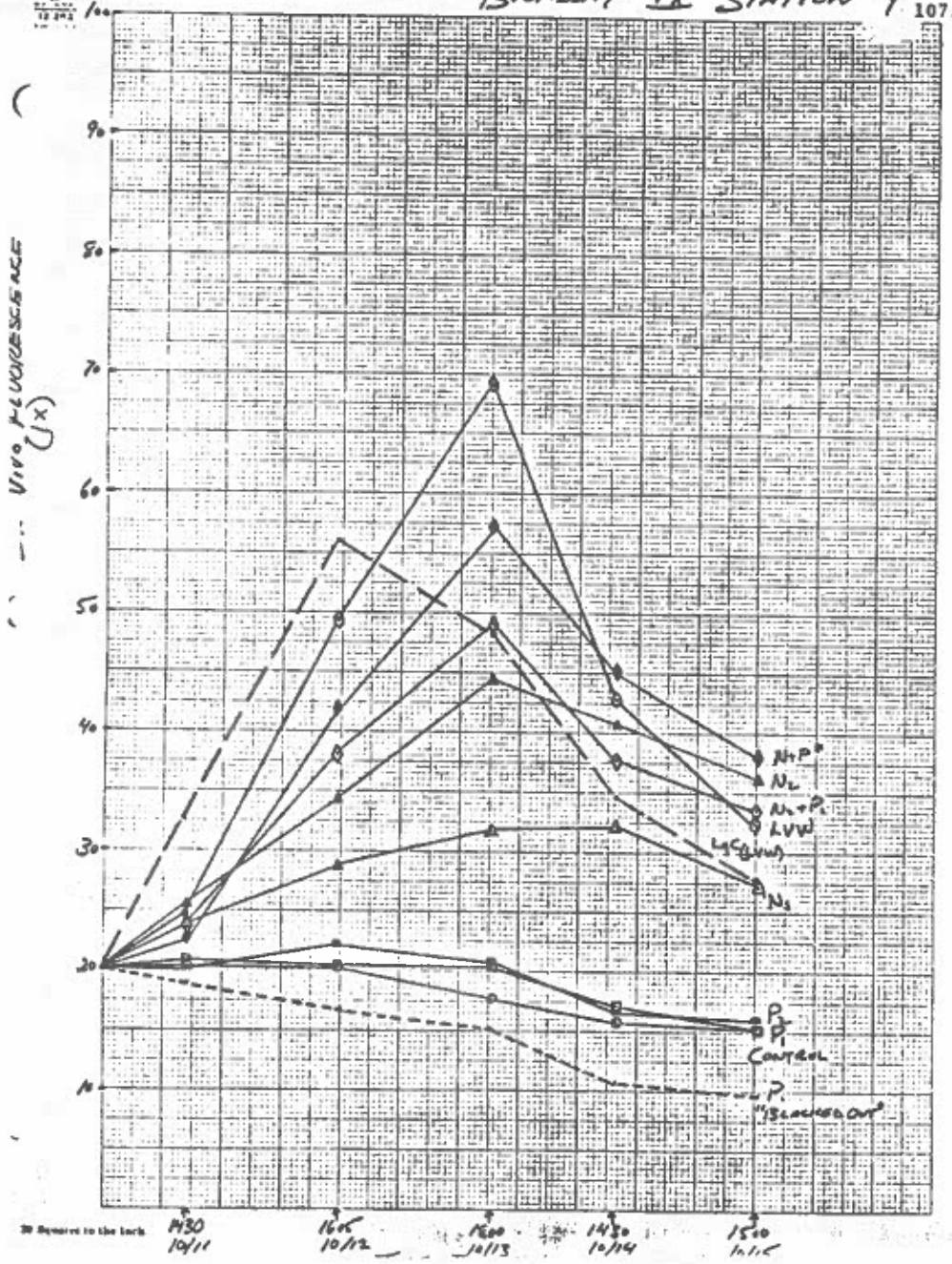
BIOASSAY VIII STATION 8



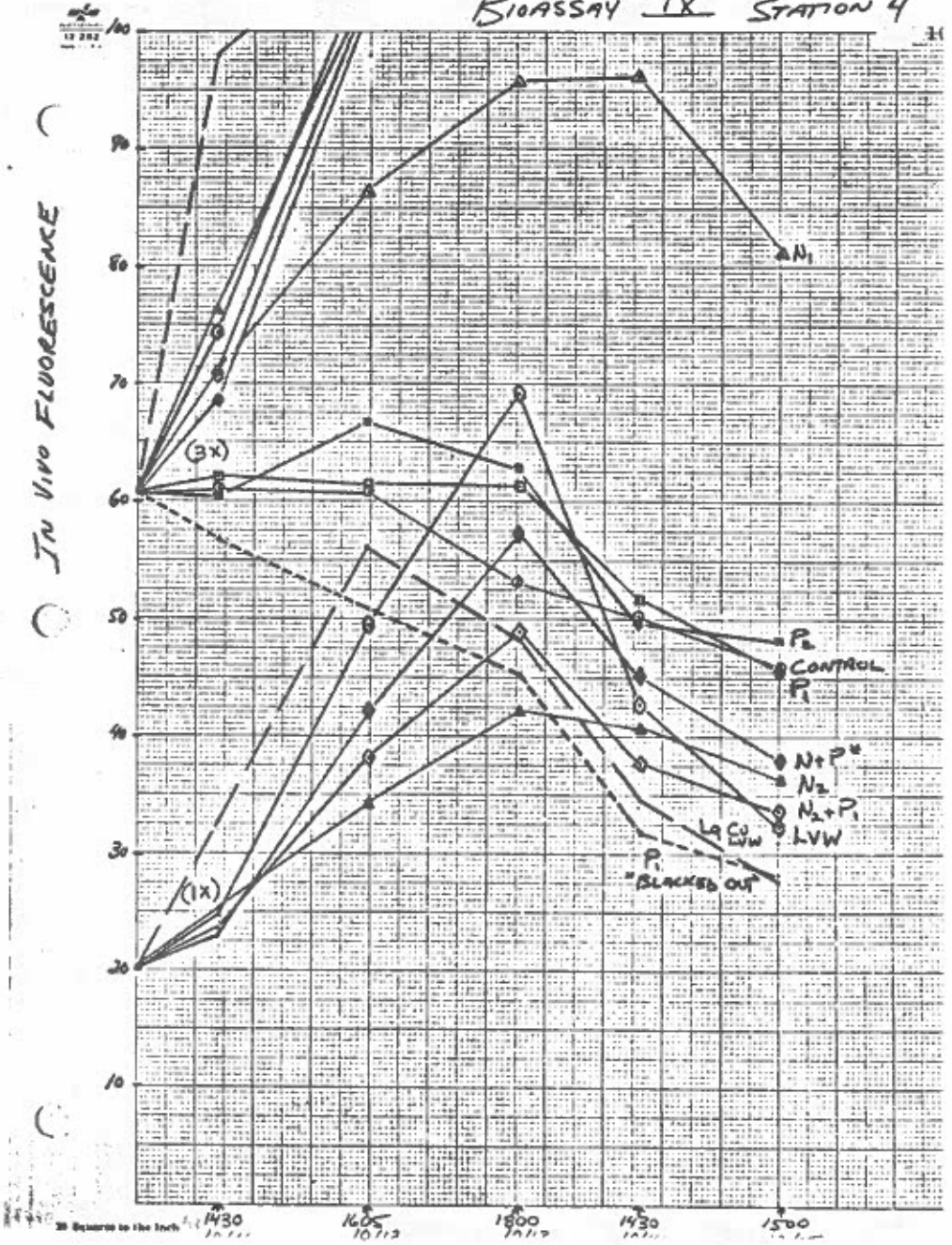
BIOASSAY IX STATION 2



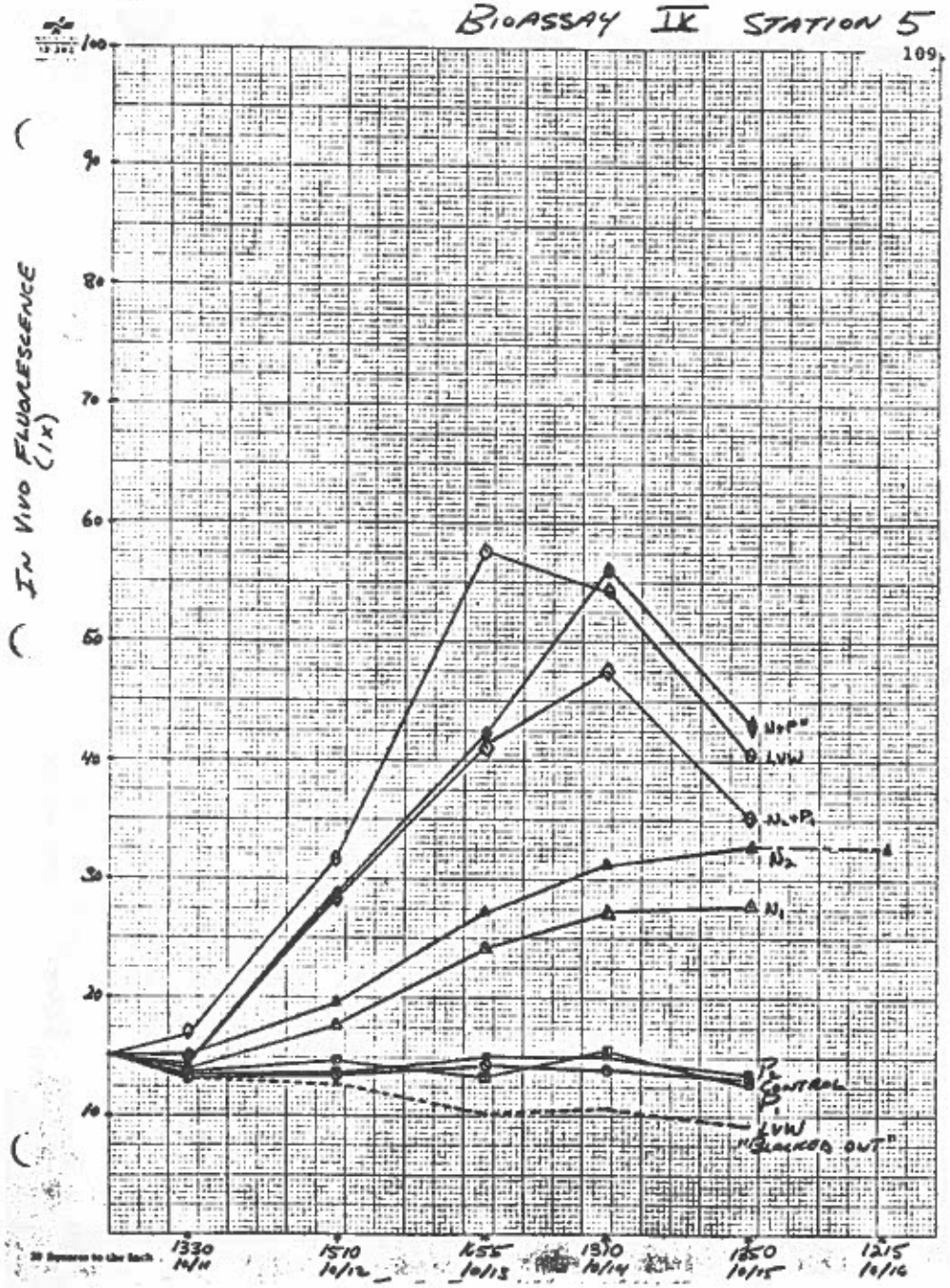
BIOASSAY IX STATION 4



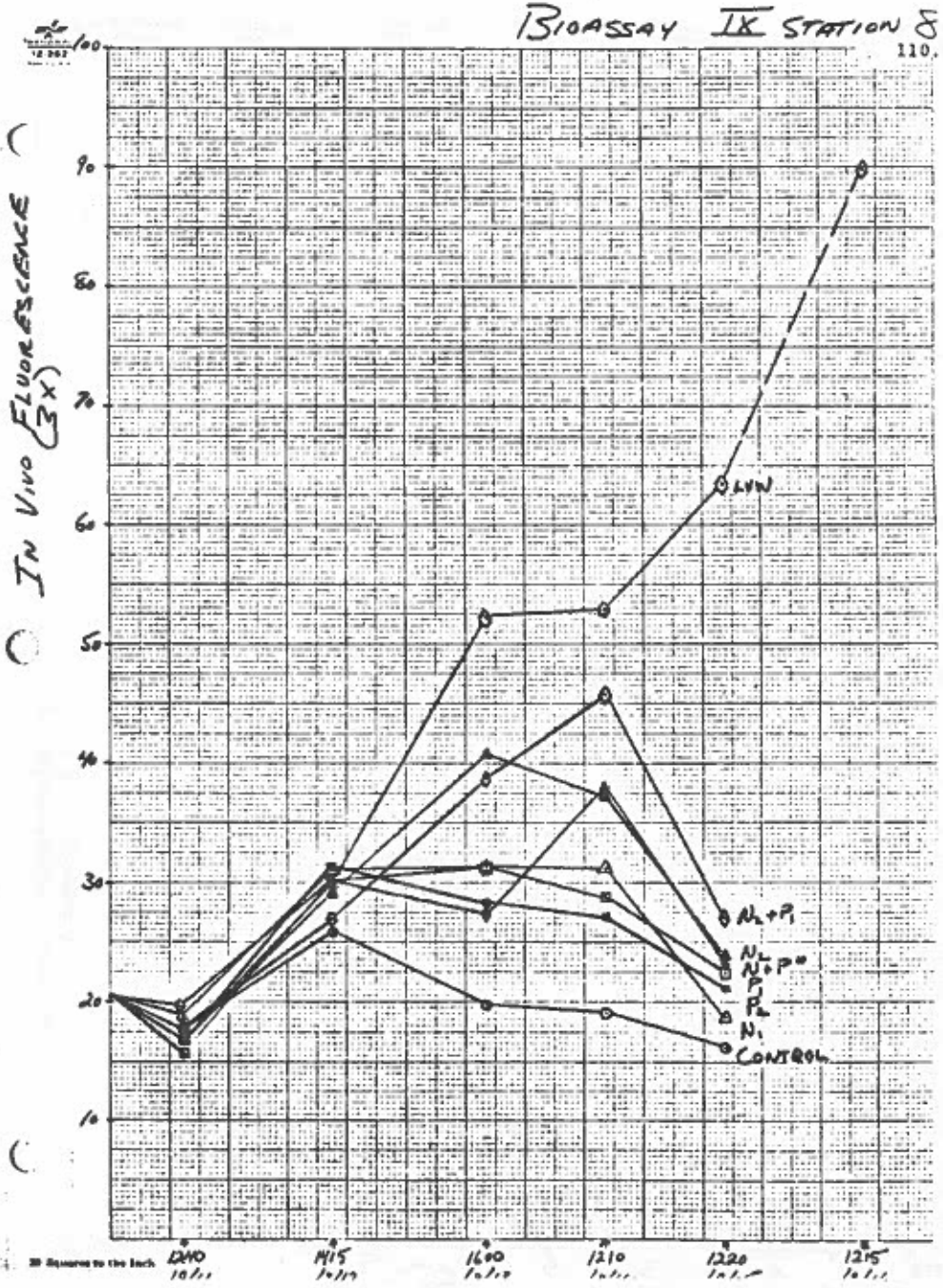
BIOASSAY IX STATION 4



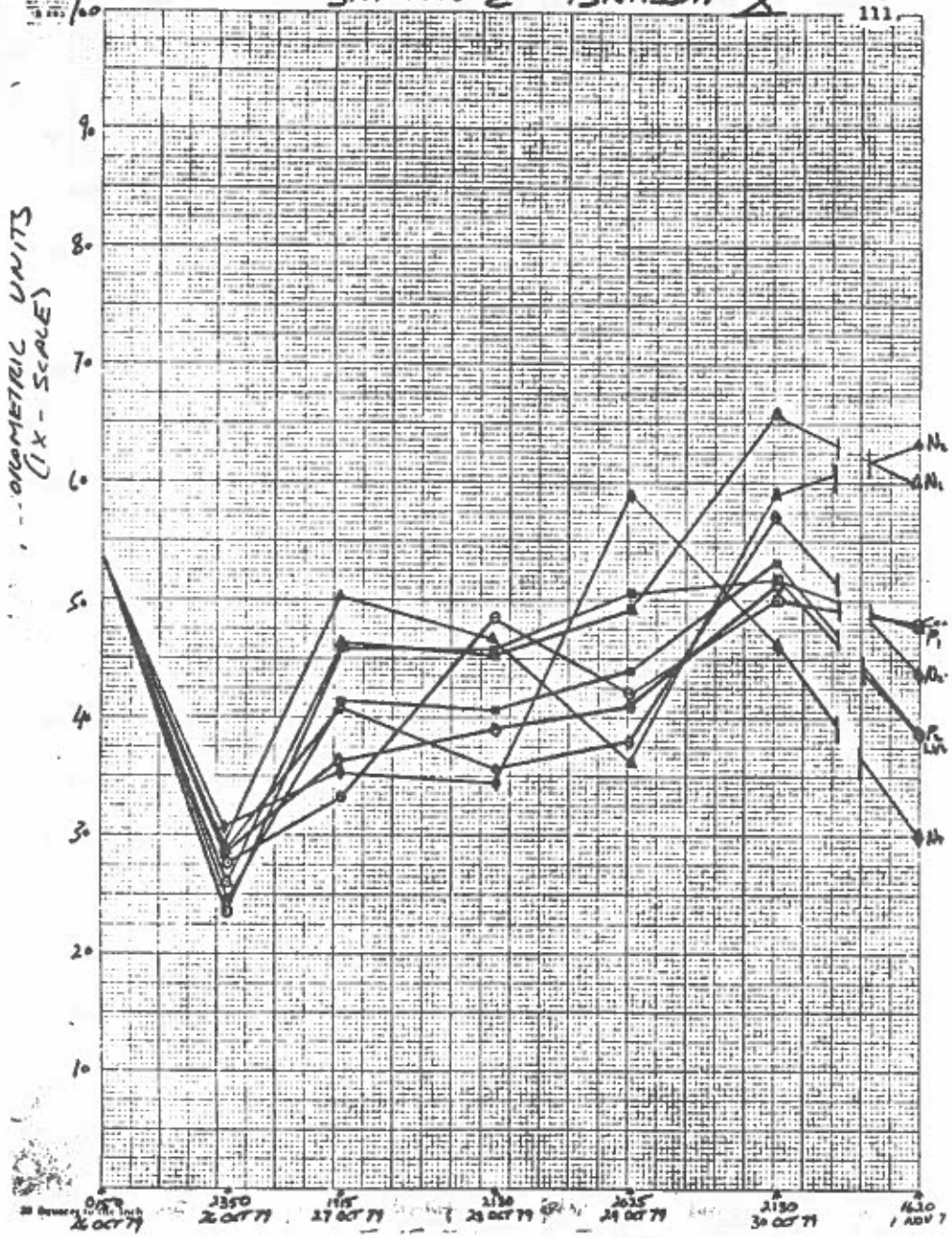
BIOASSAY IX STATION 5



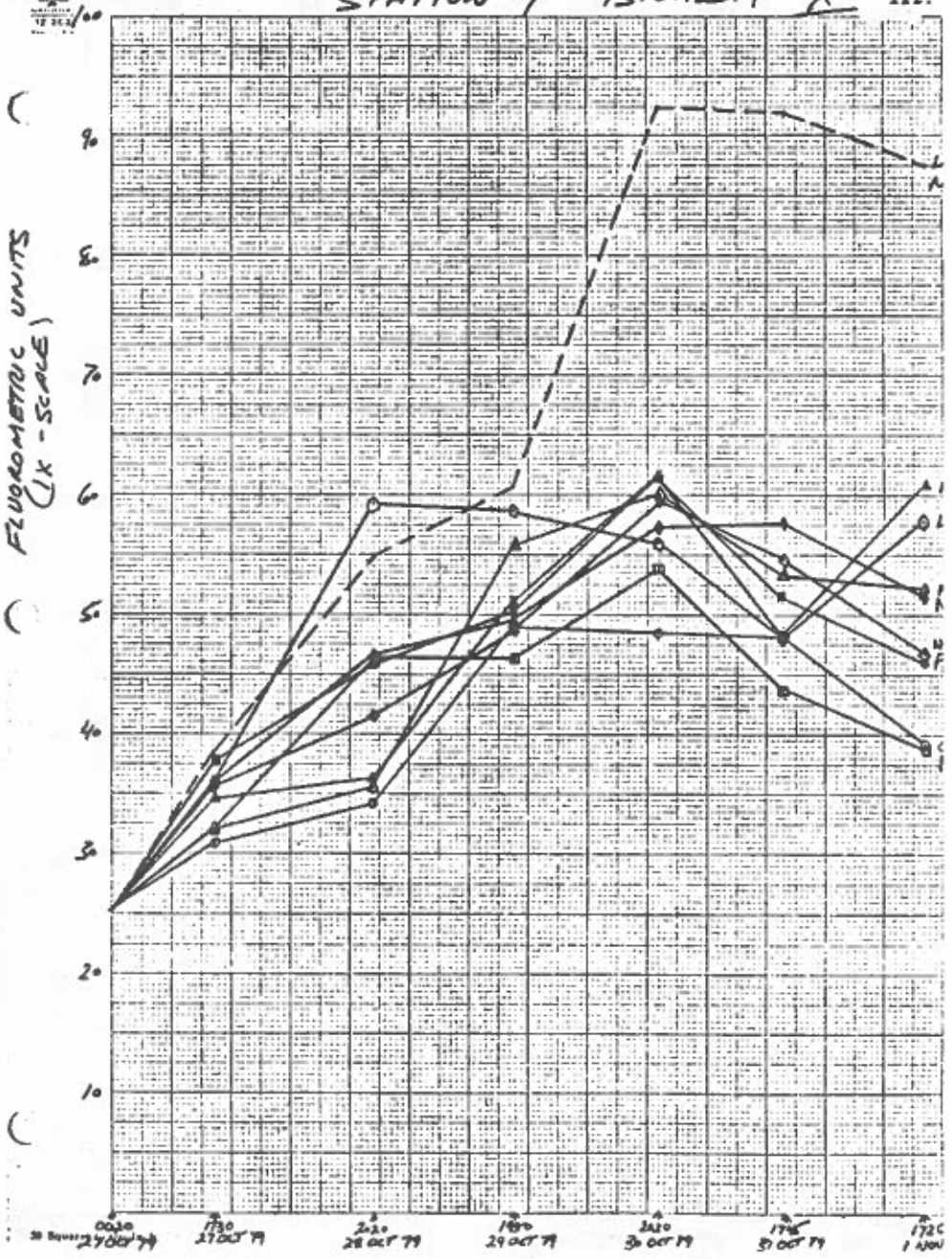
BIOASSAY IX STATION 8



STATION 2 BIOASSAY X

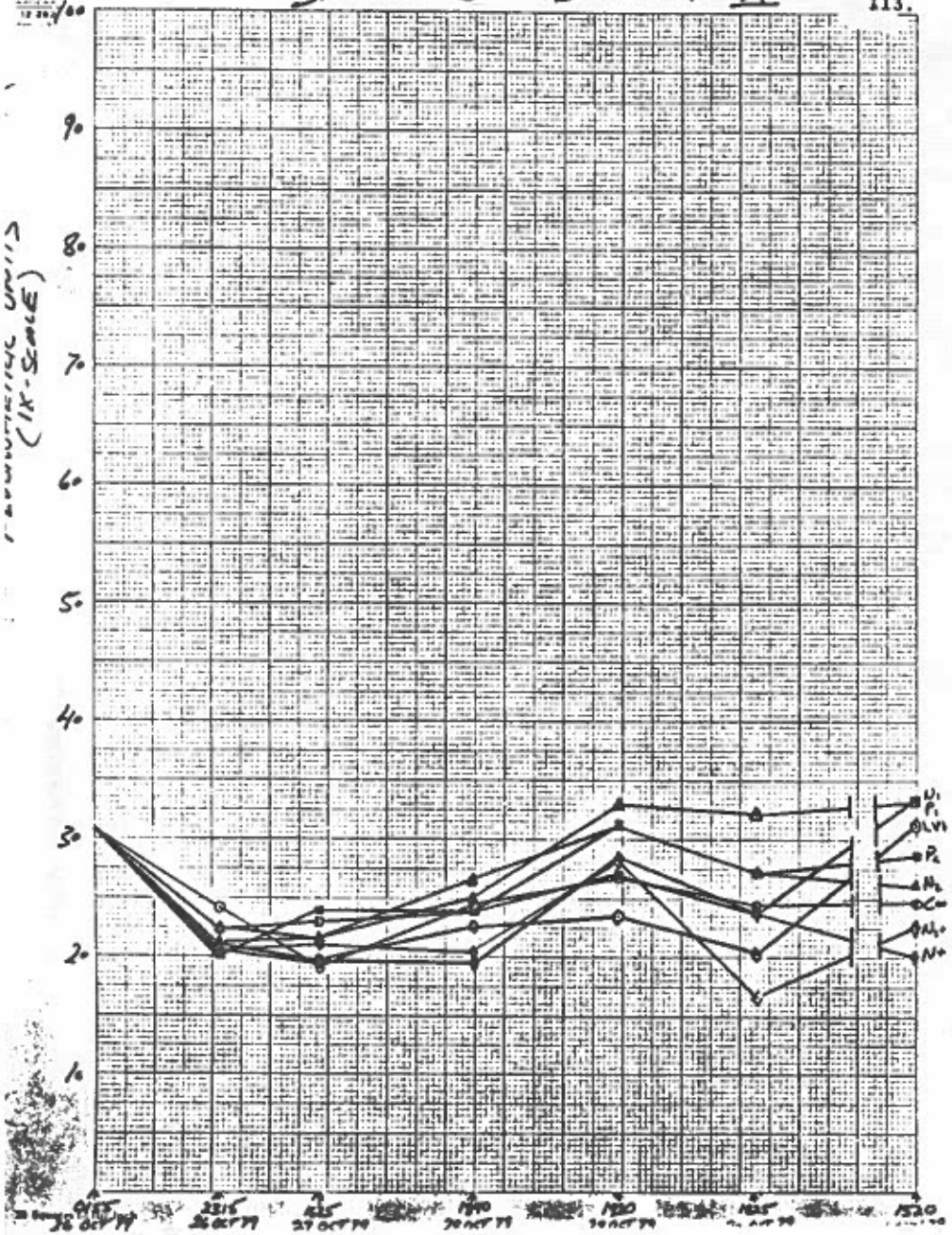


STATION 4 BIOASSAY X



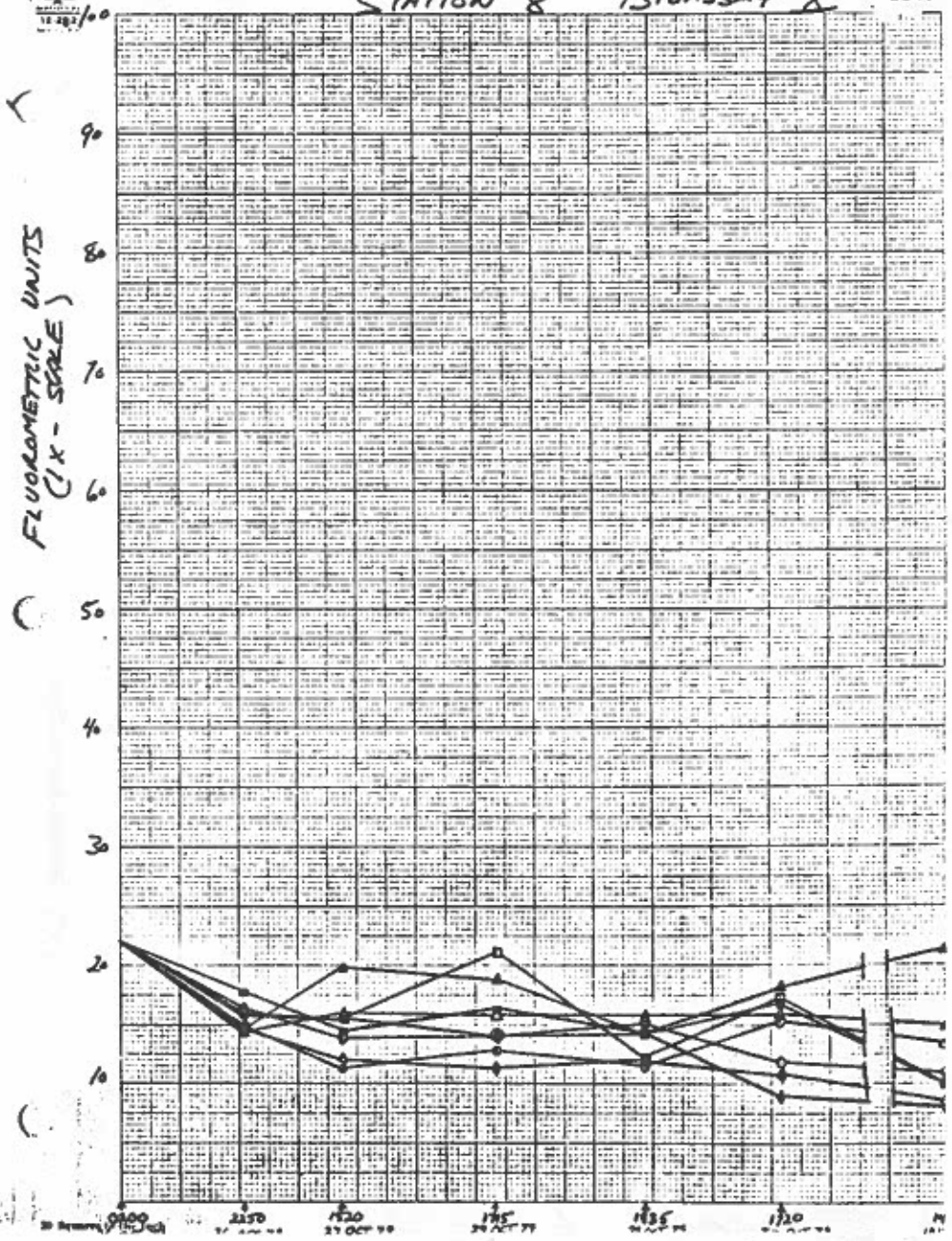
STATION 5 BIOASSAY X

113.

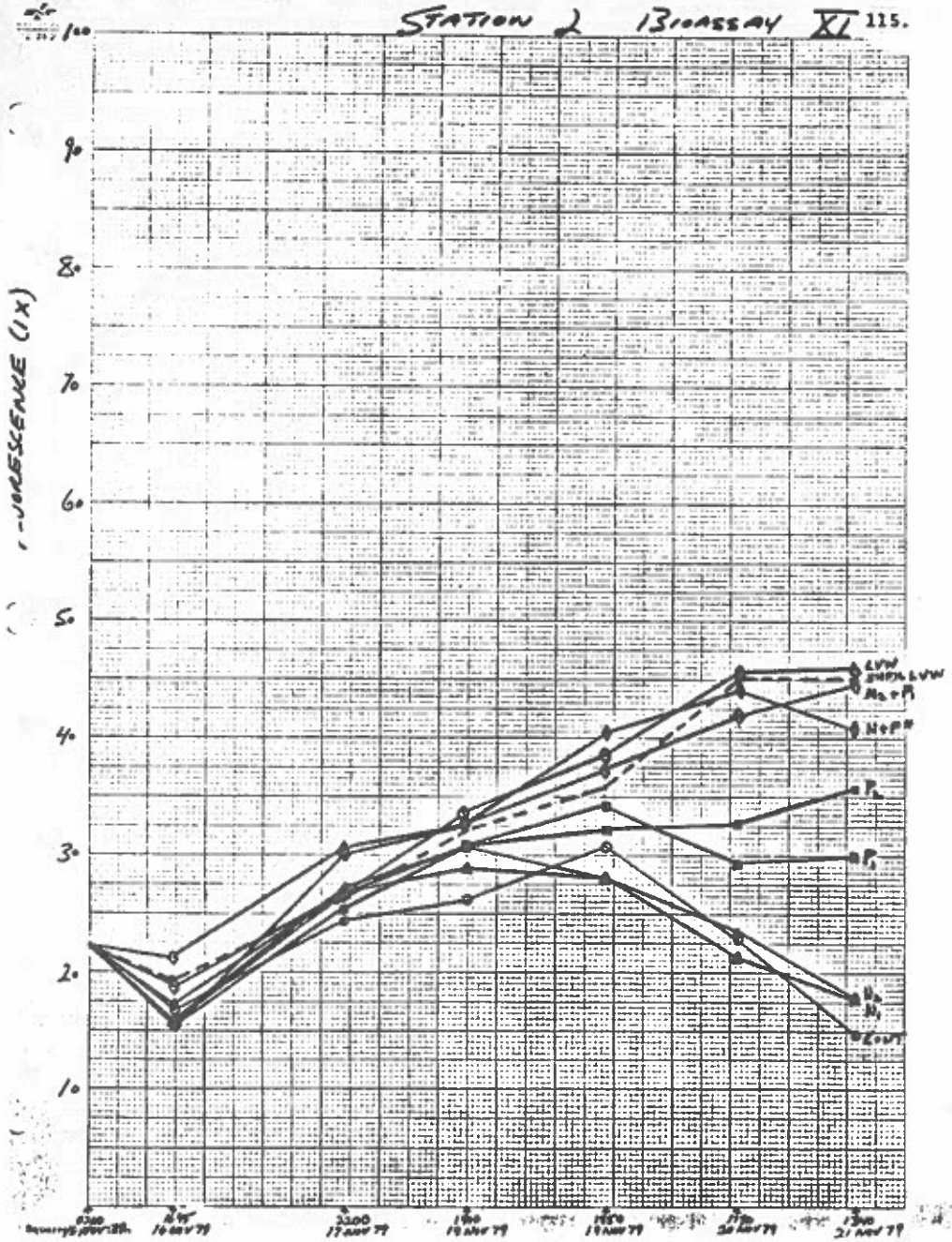


STATION 8 BIOASSAY X

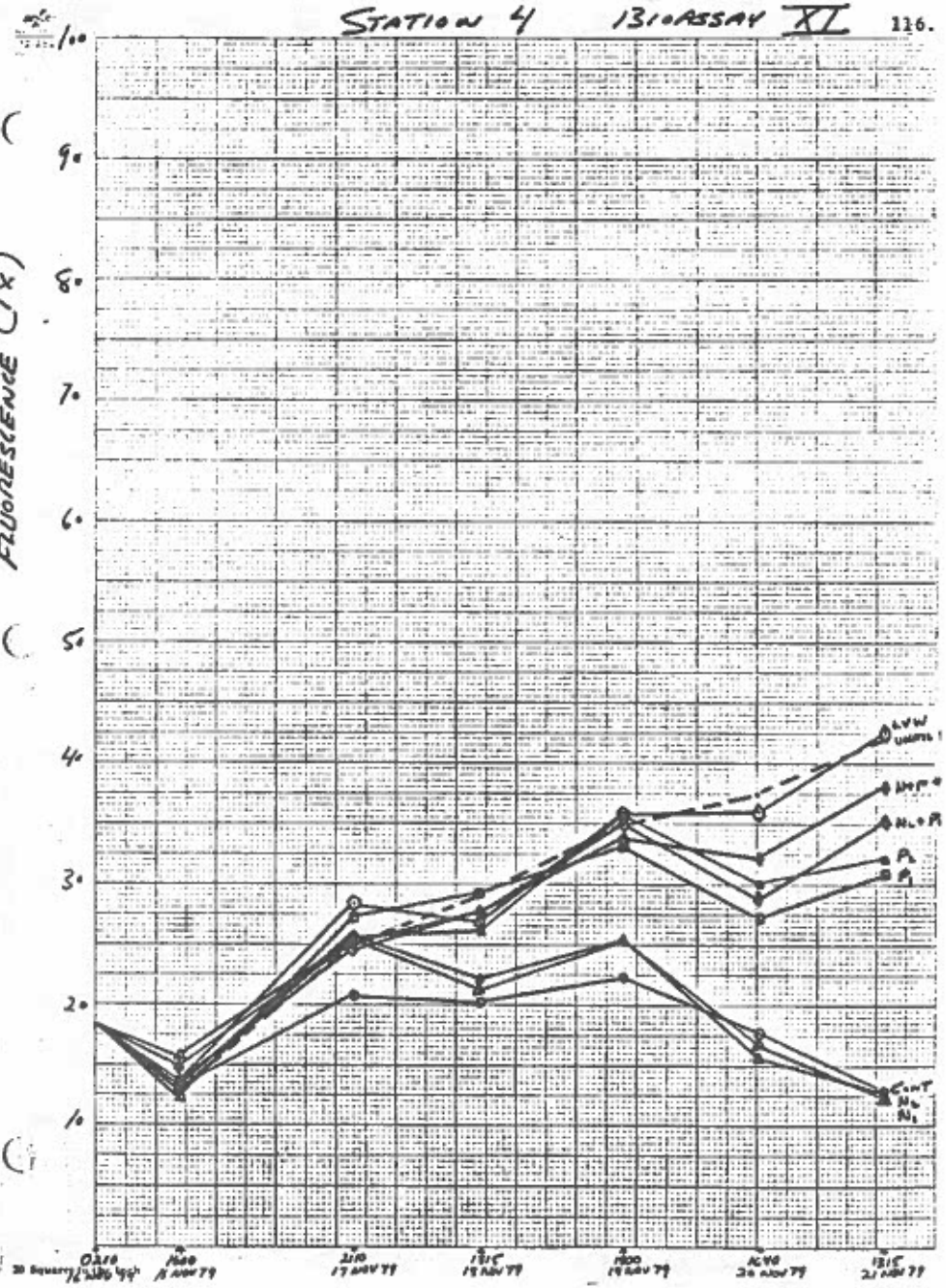
114.



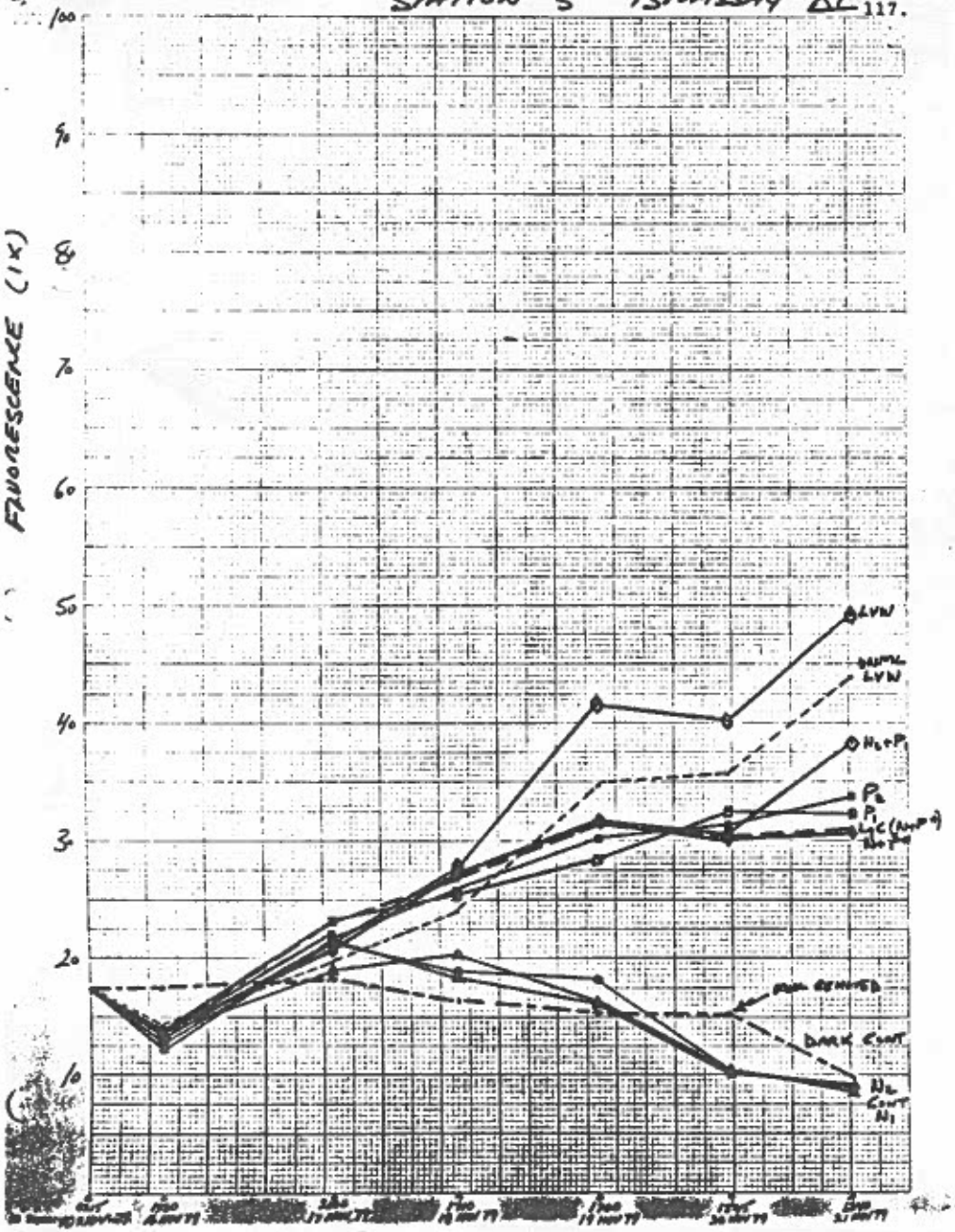
STATION 2 BIOASSAY XI



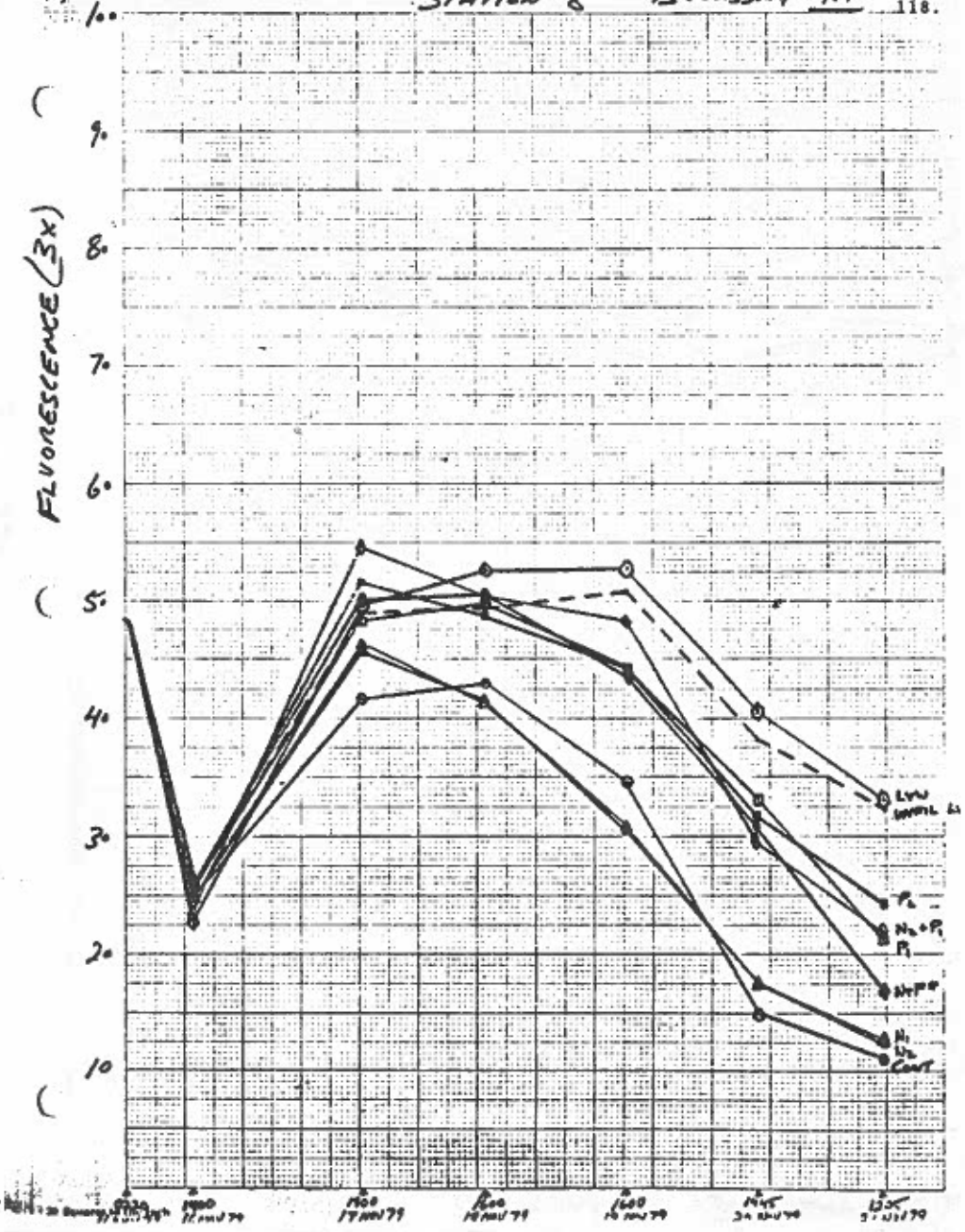
STATION 4 BIOASSAY XI



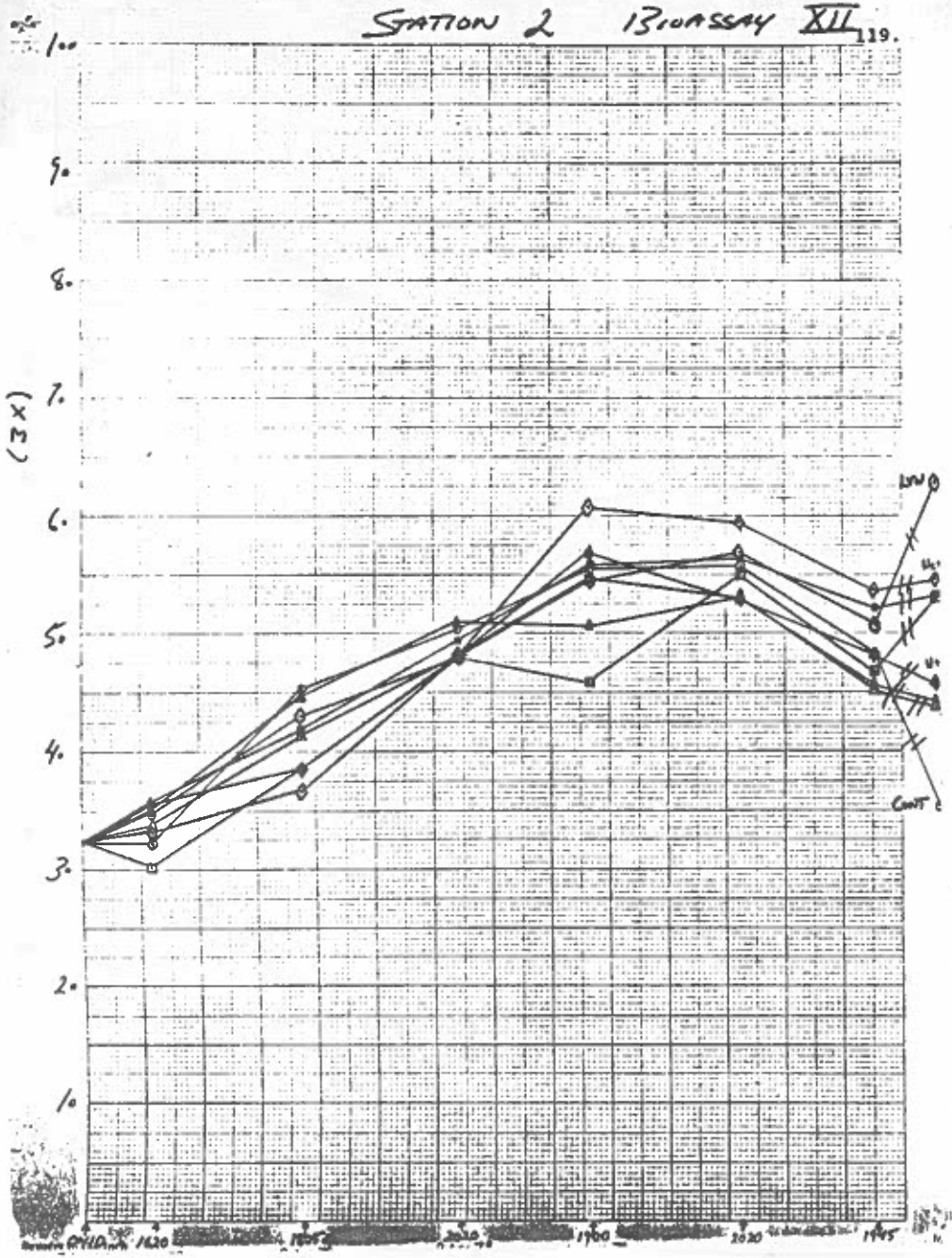
STATION 5 BIOASSAY XL



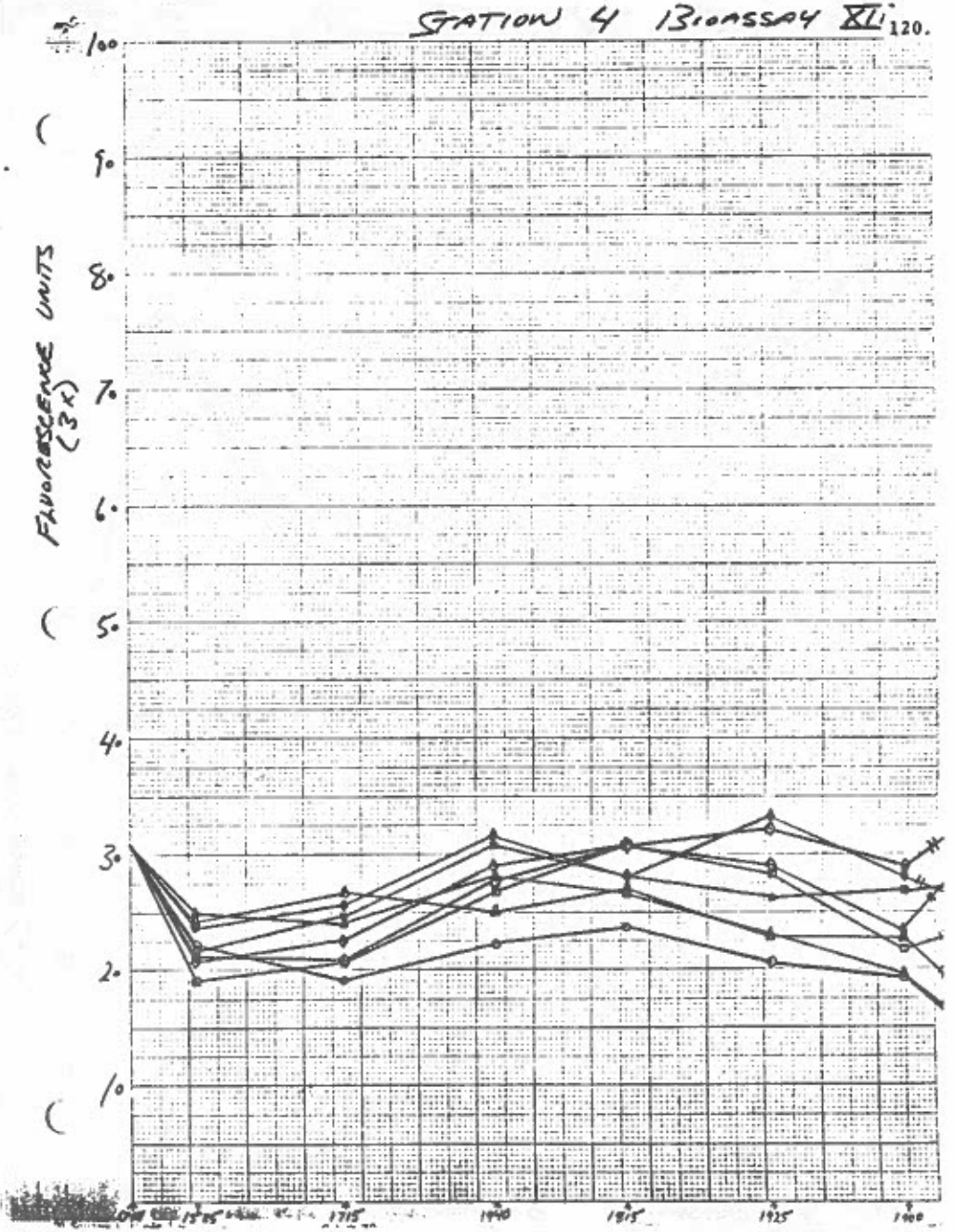
STATION 8 BIOASSAY XI



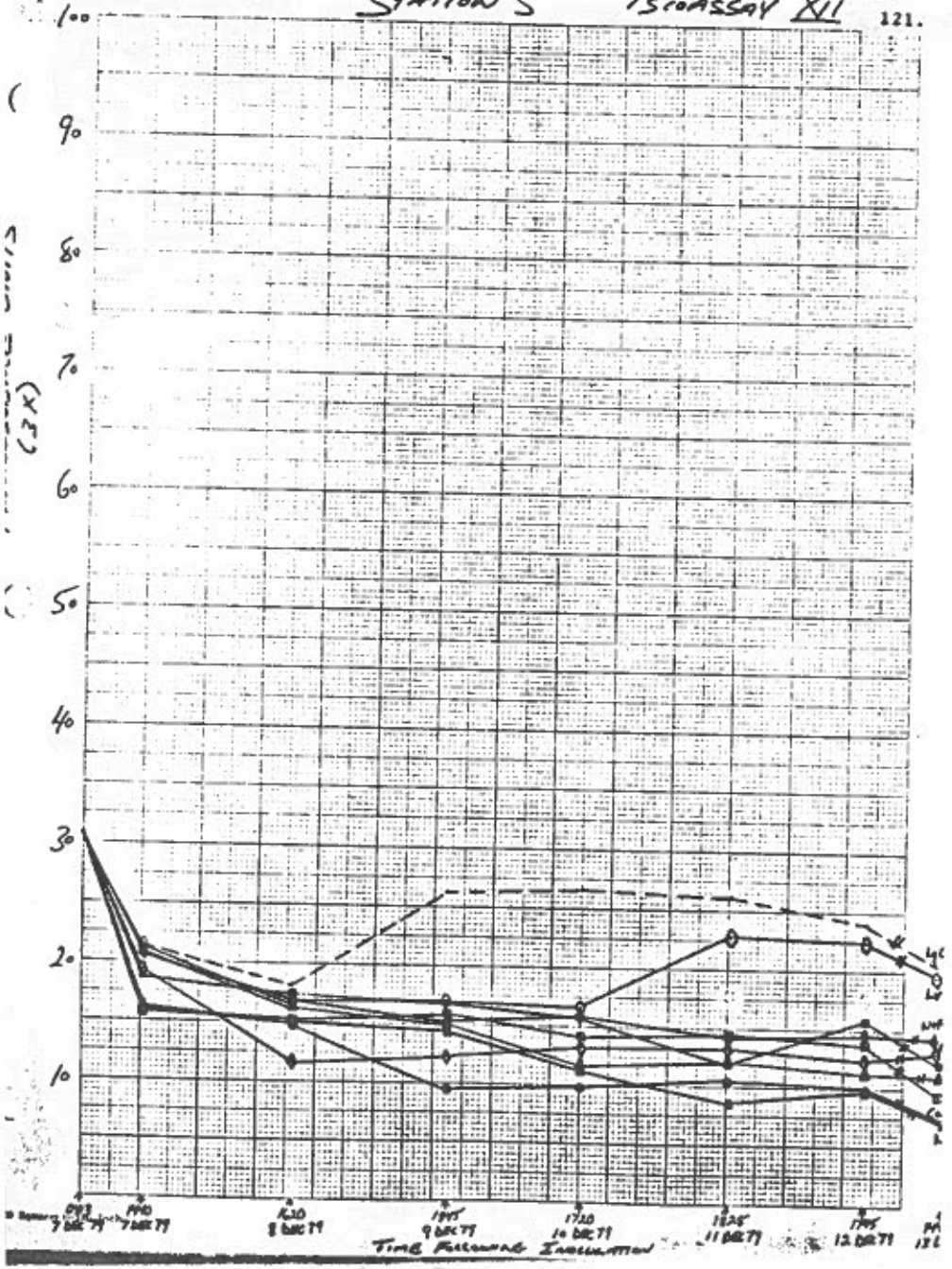
STATION 2 BIOASSAY XII 119.



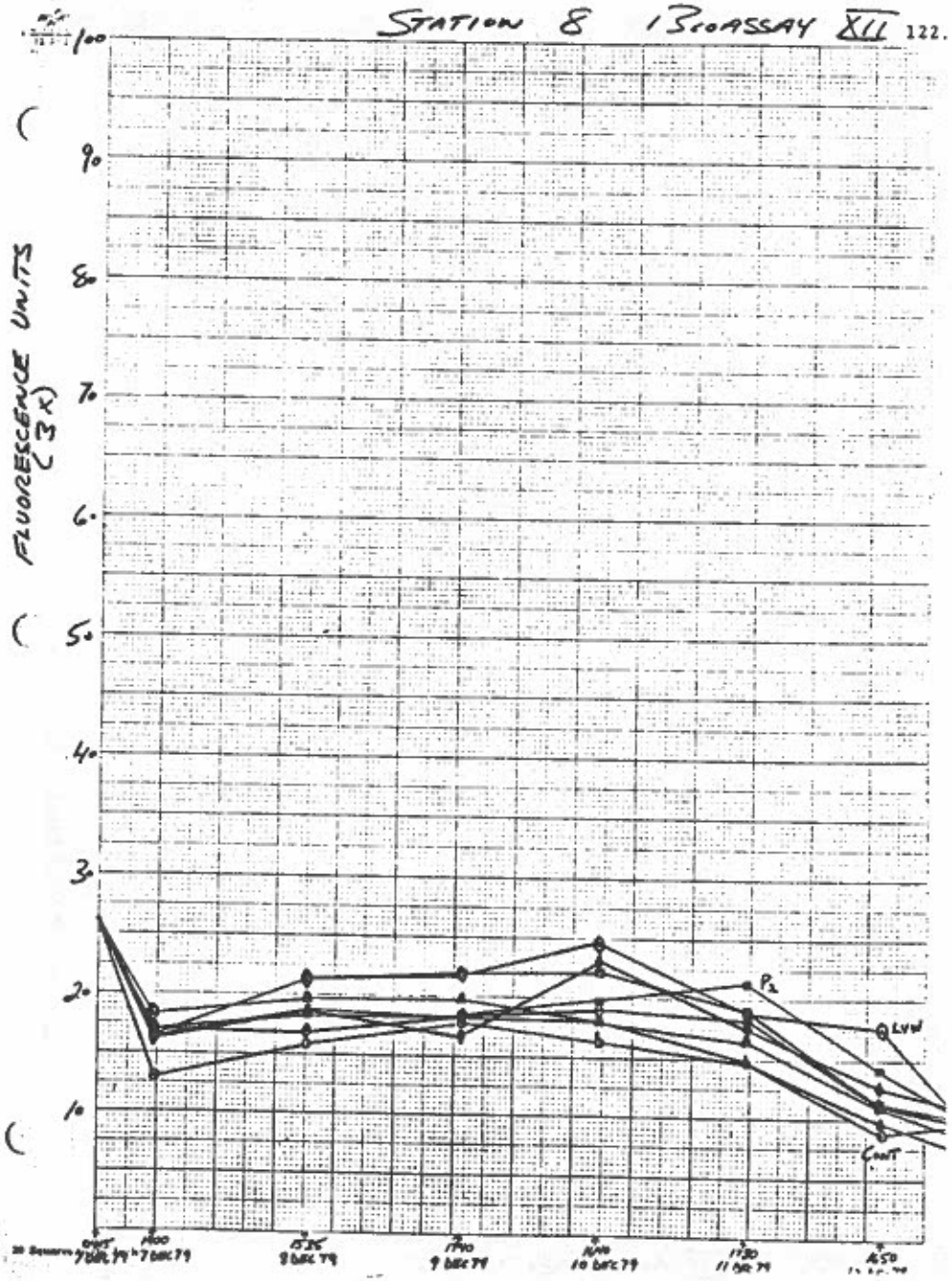
STATION 4 BIOASSAY XI 120.



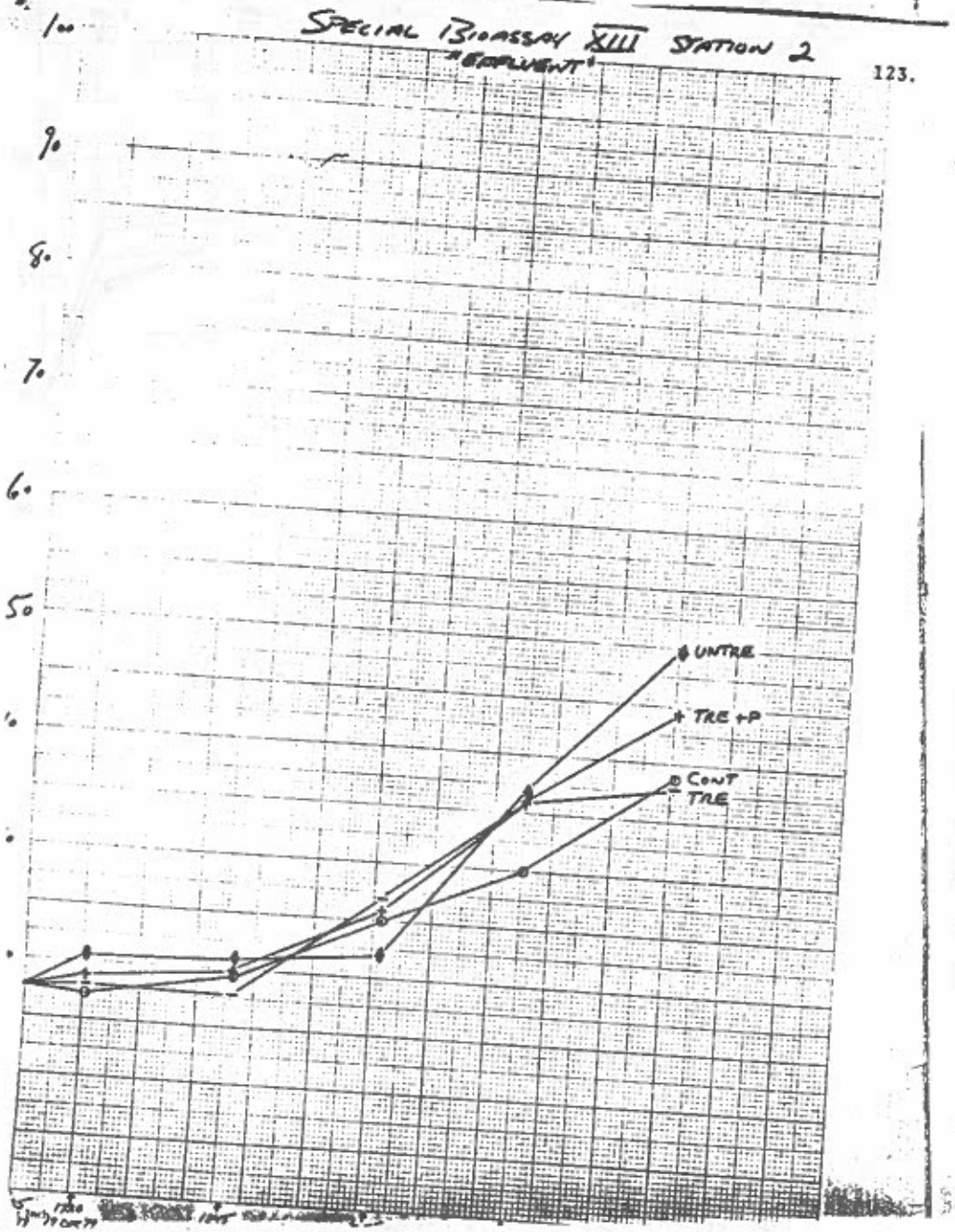
STATION 5 BIOASSAY XII 121.



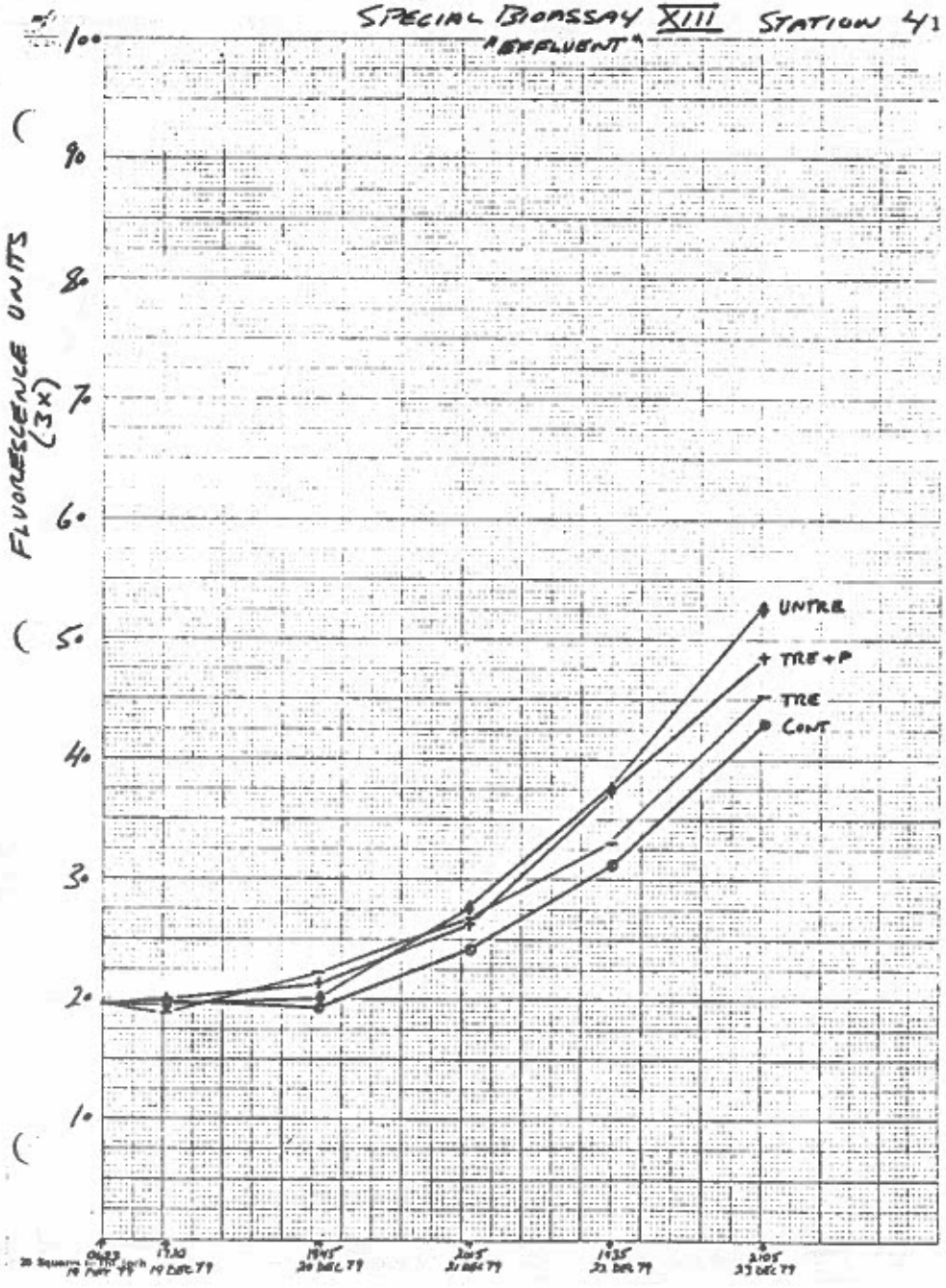
STATION 8 BIOASSAY XII 122.



SPECIAL BIOASSAY XIII STATION 2
"EFFLUENT"



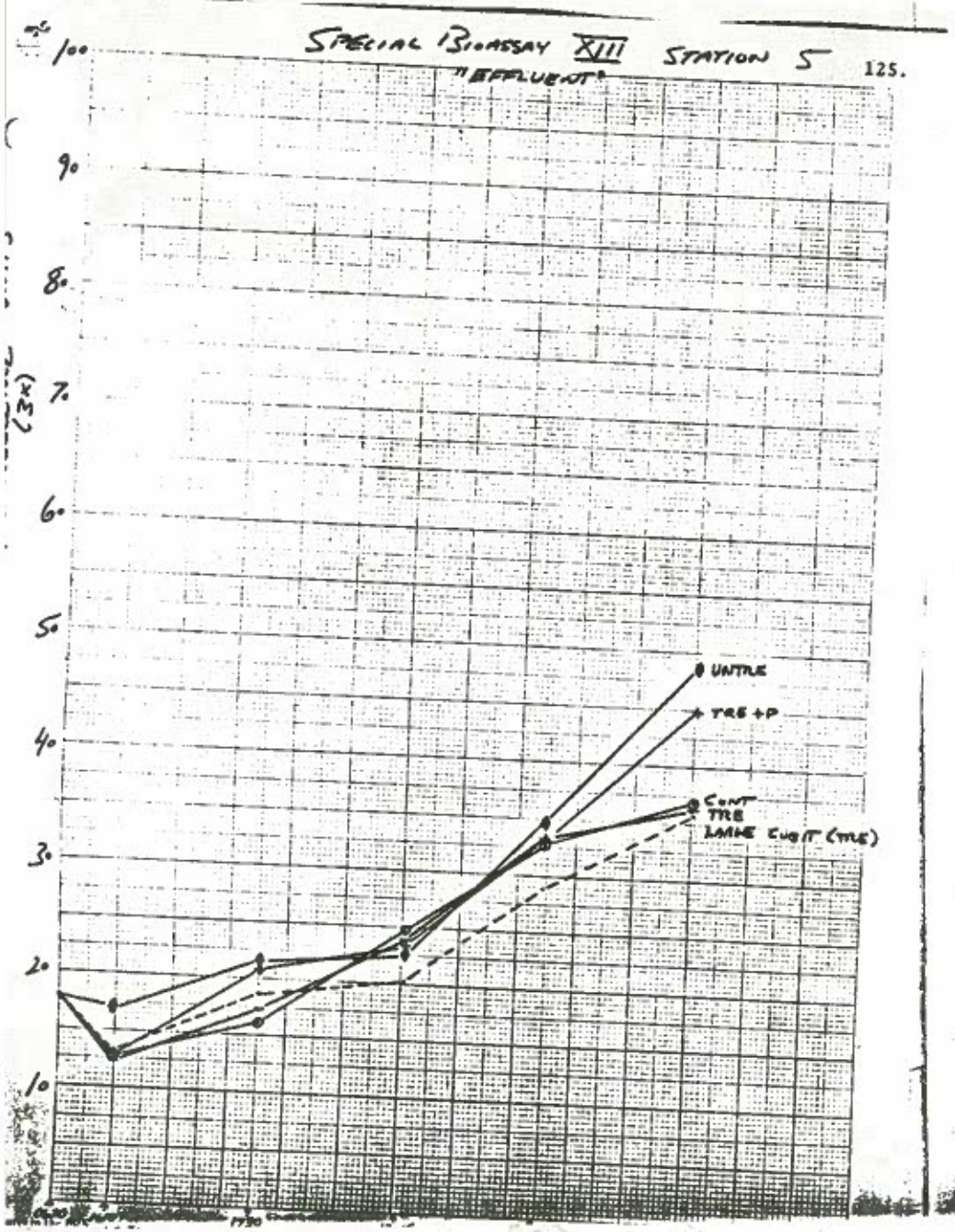
SPECIAL BIOASSAY XIII STATION 4
"EFFLUENT"



SPECIAL BIOASSAY XIII STATION 5

"EFFLUENT"

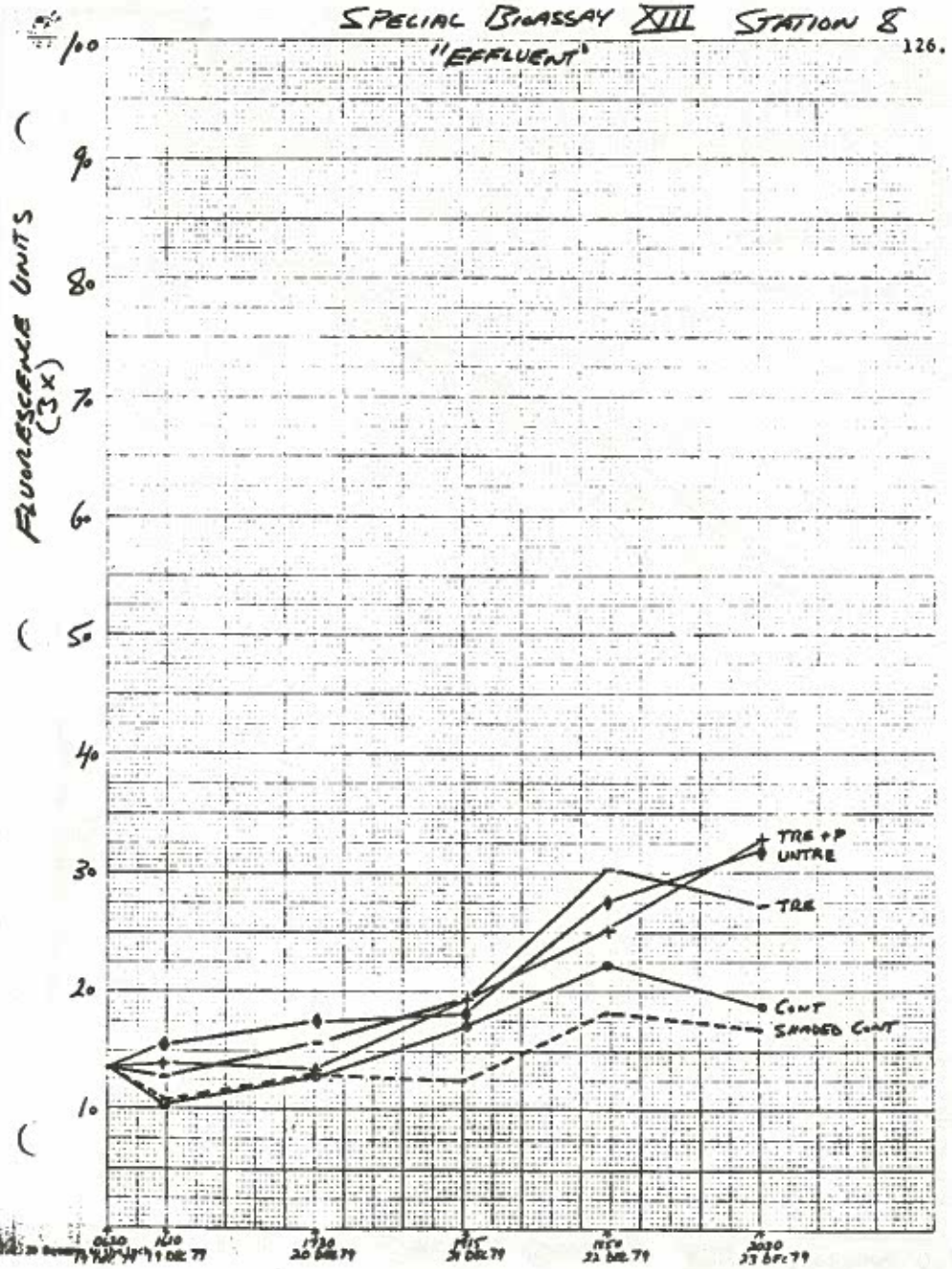
125.



SPECIAL BIOASSAY XIII STATION 8

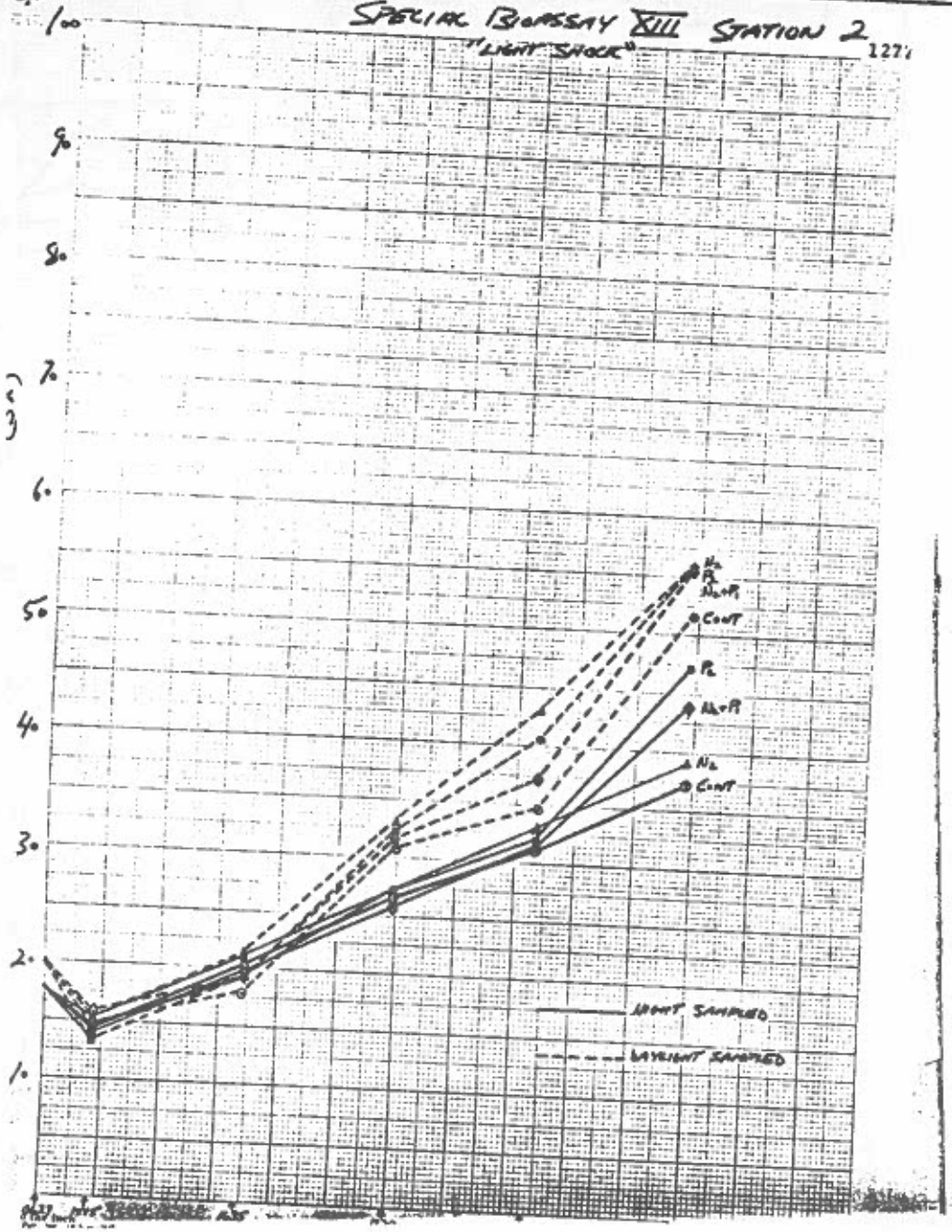
"EFFLUENT"

126.



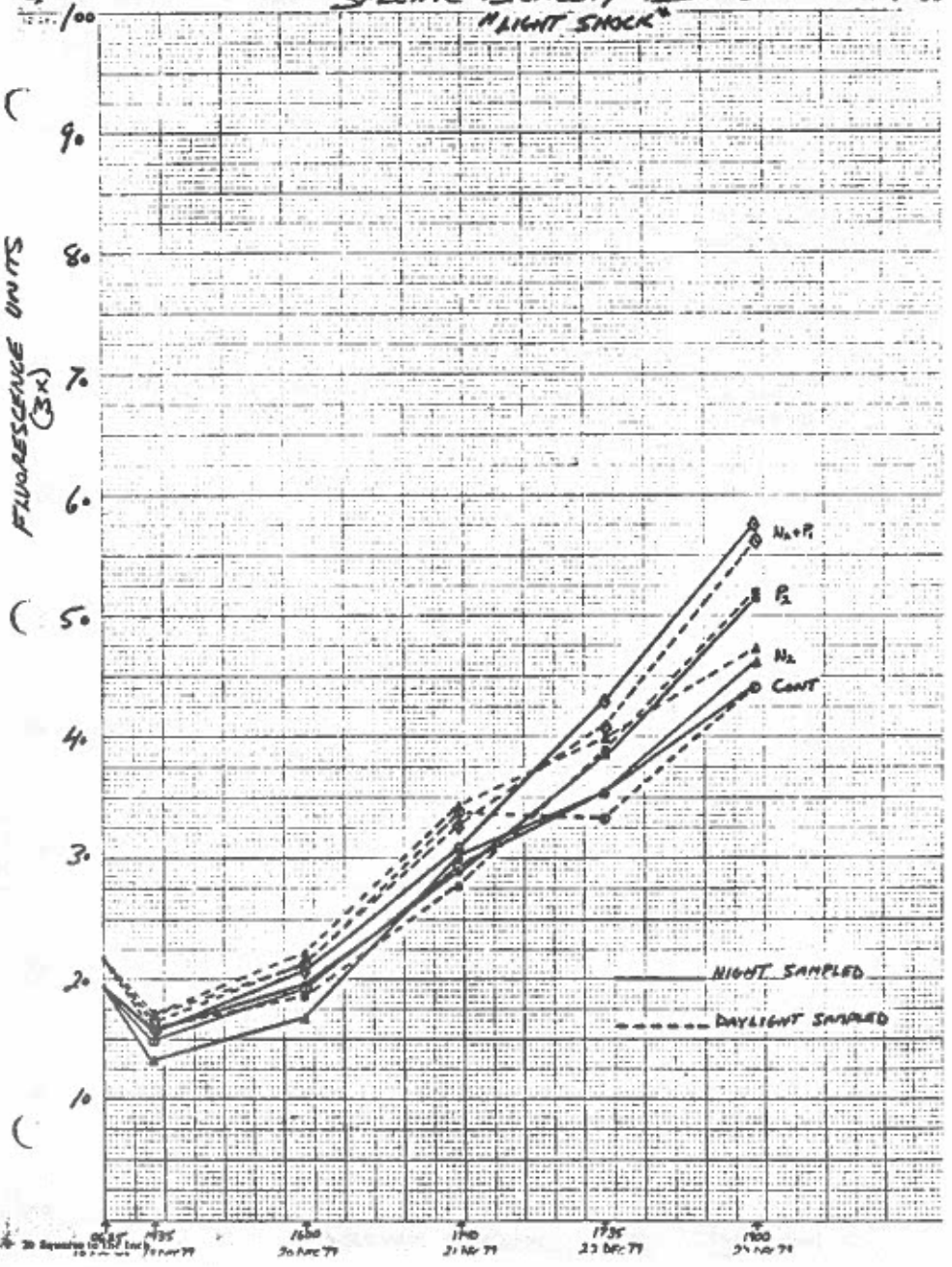
SPECIAL BIOASSAY XIII STATION 2

"LIGHT SHOCK"



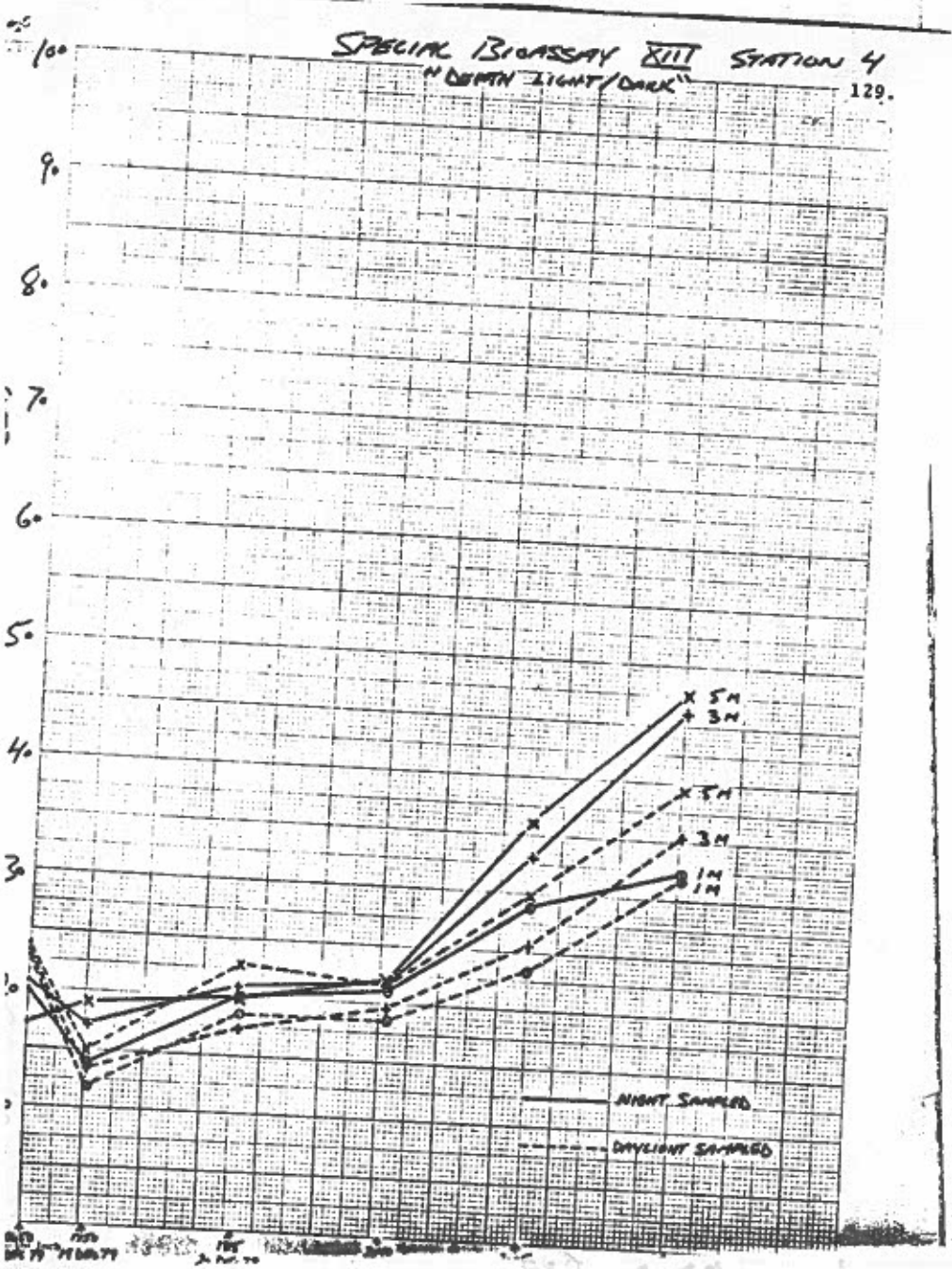
SPECIAL BIOASSAY XIII STATION 4

"LIGHT SHOCK"

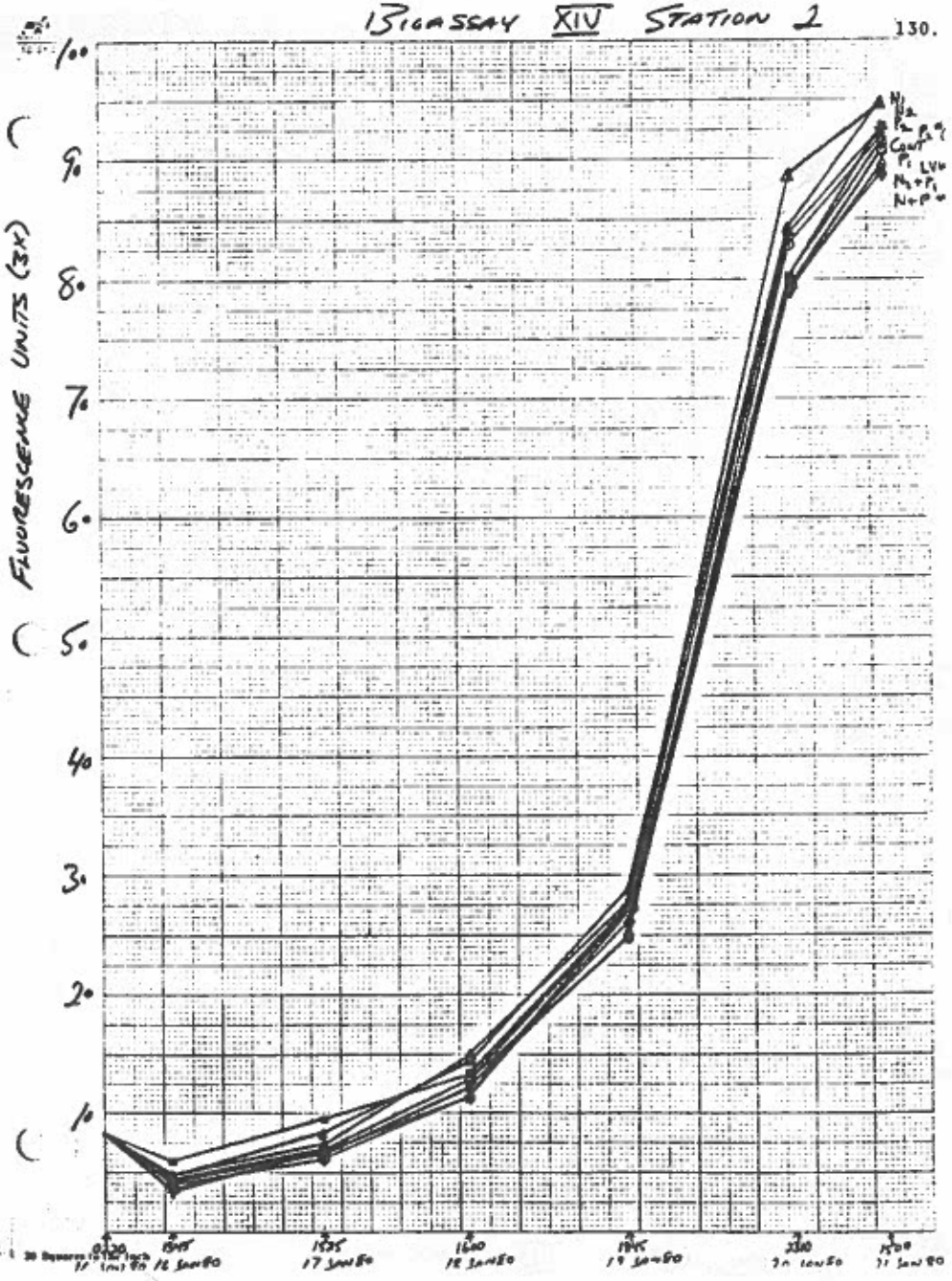


SPECIAL BIOASSAY XIII STATION 4

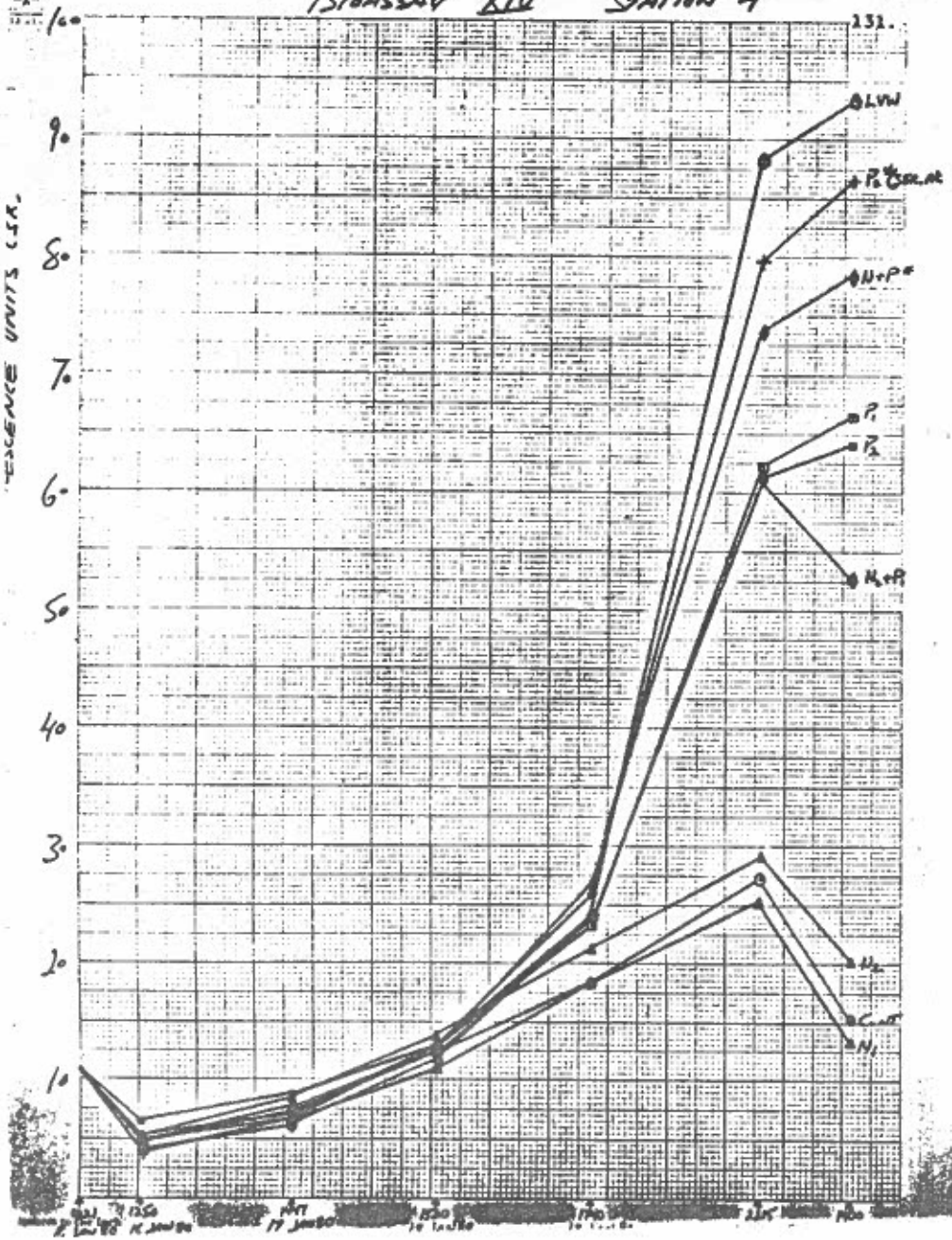
"DEPTH LIGHT/DARK"



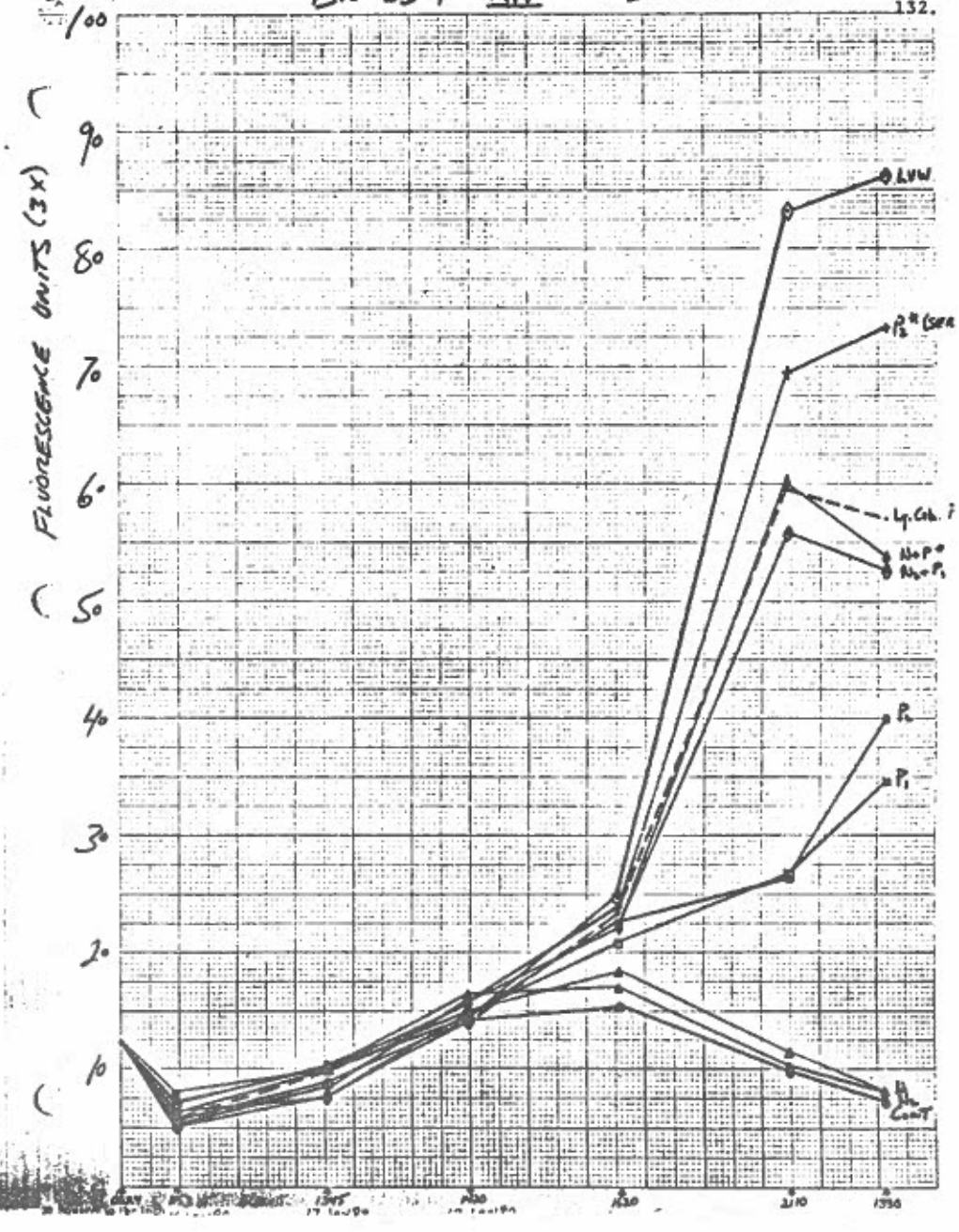
BIOASSAY XIV STATION 2

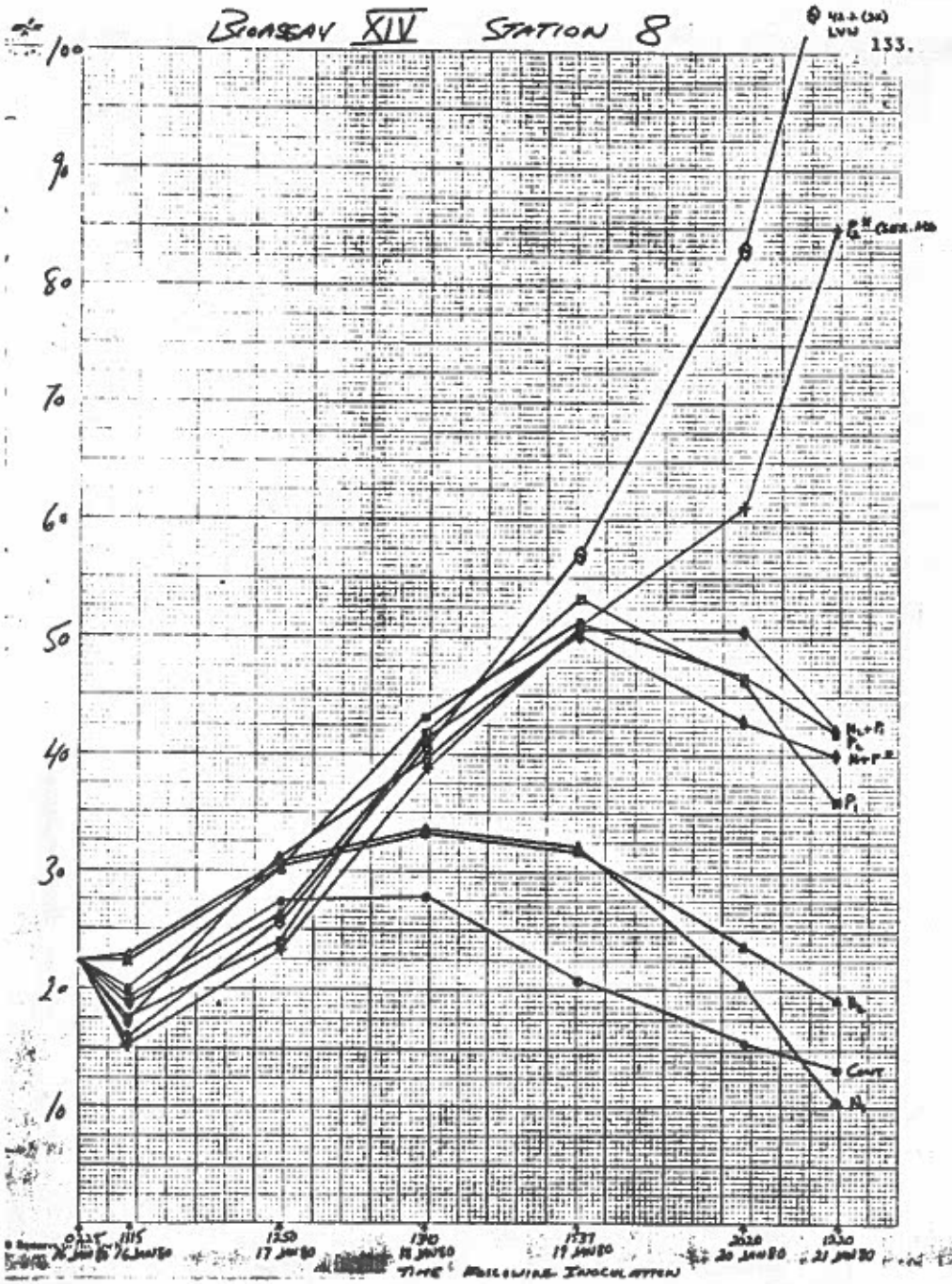


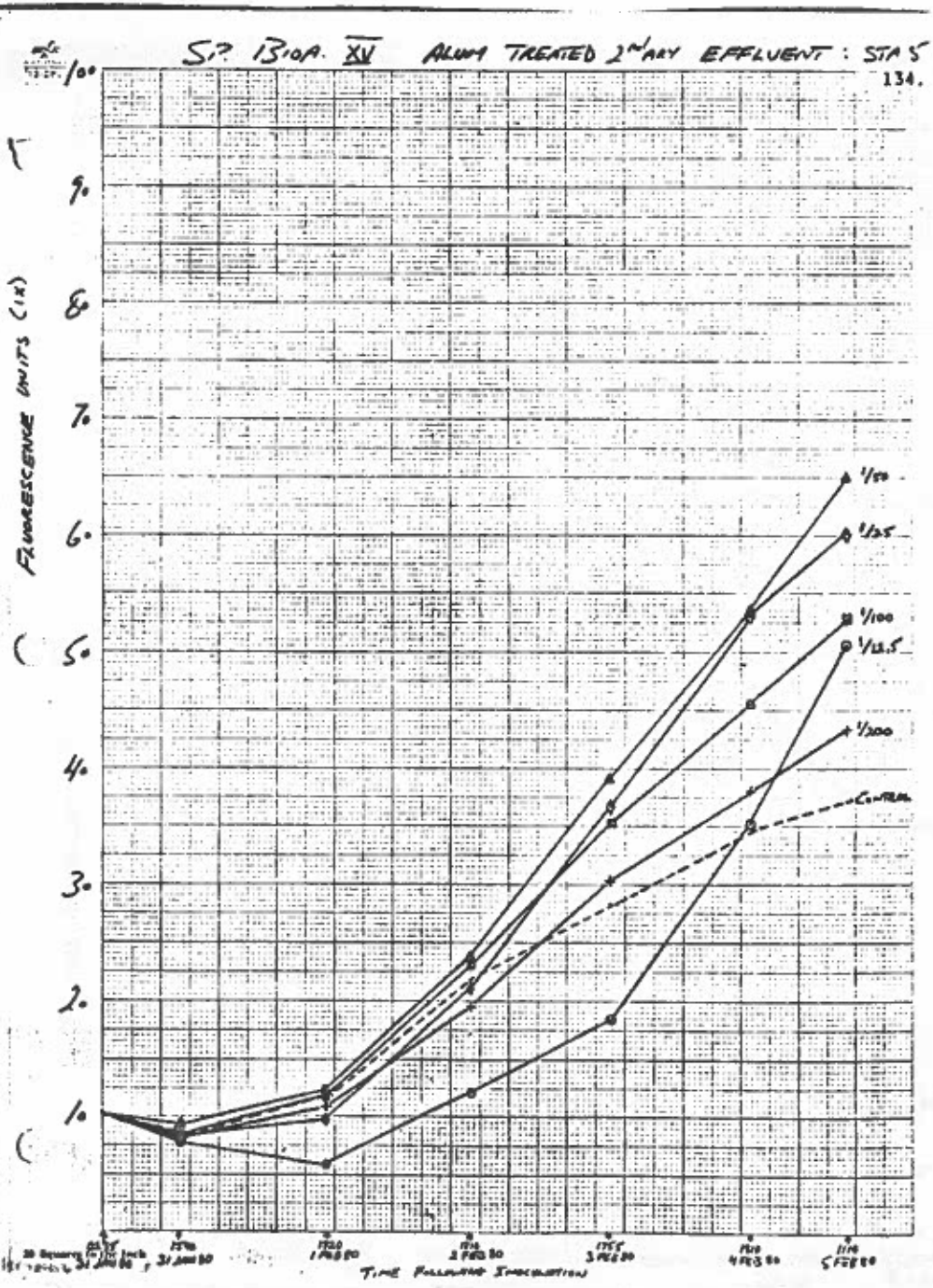
BIOASSAY XIV STATION 4



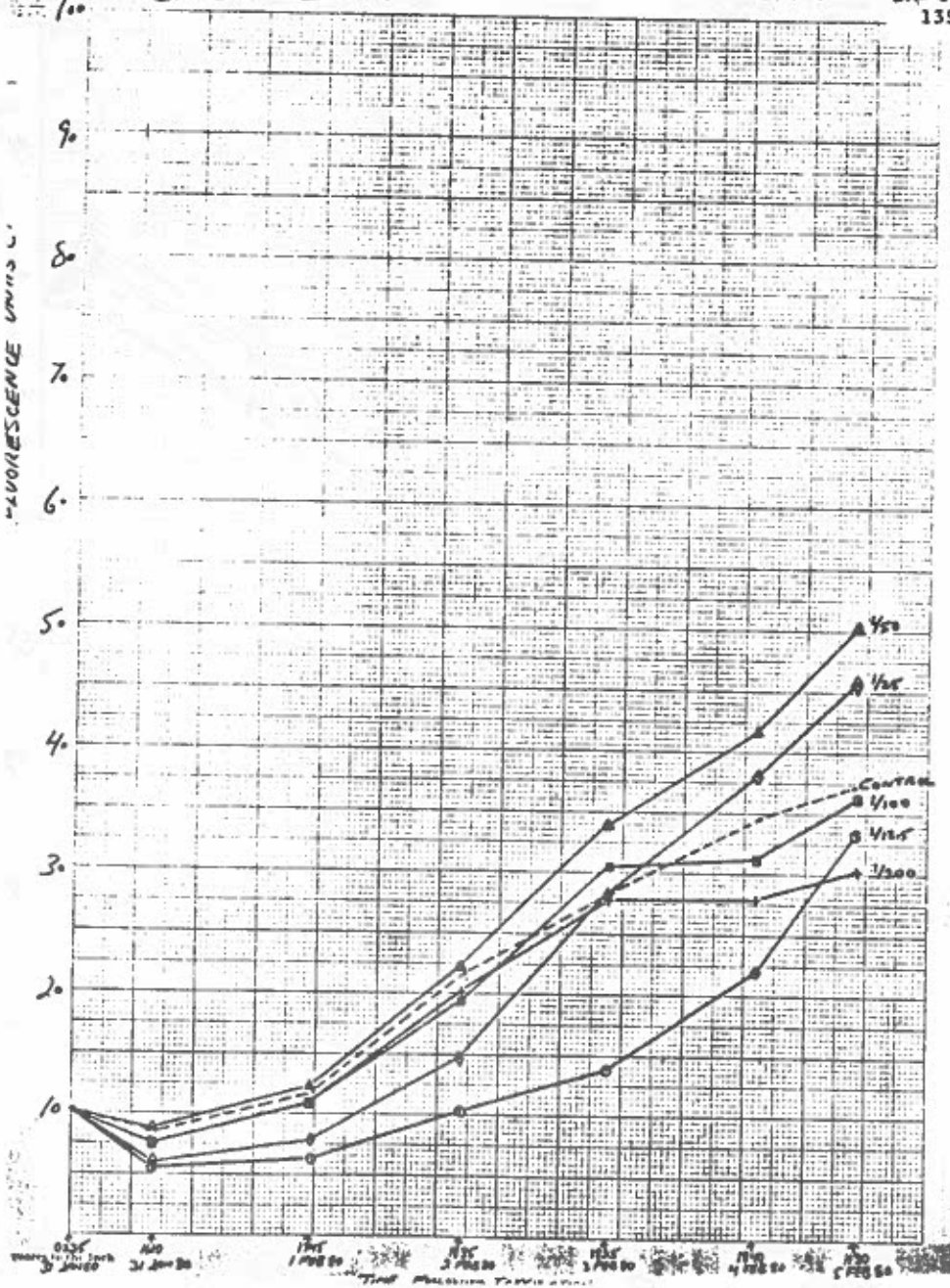
BIOASSAY XIV STATION 5




BIOASSAY XIV STATION 8
133.
100
90
80
70
60
50
40
30
20
10
0225
1115
1250
1310
1537
2020
1220
17 JAN 80
18 JAN 80
19 JAN 80
20 JAN 80
21 JAN 80
TIME FOLLOWING INOCULATION

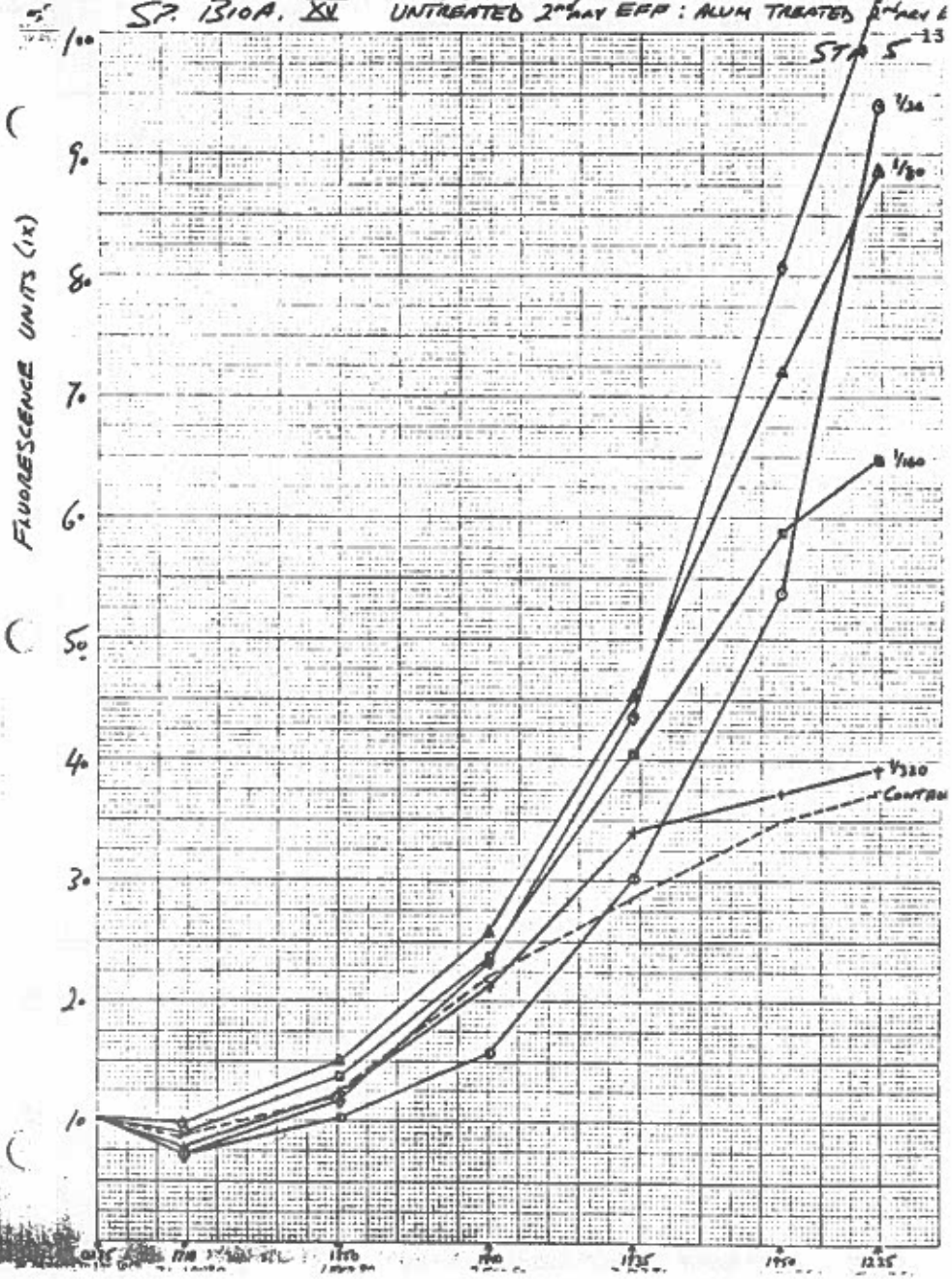

SP. BIOA. XV ALUM TREATED 2ND ARY EFFLUENT : STA 5
134.
FLUORESCENCE UNITS (1X)
100
90
80
70
60
50
40
30
20
10
1/50
1/25
1/100
1/12.5
1/200
CONTROL
TIME FOLLOWING INOCULATION

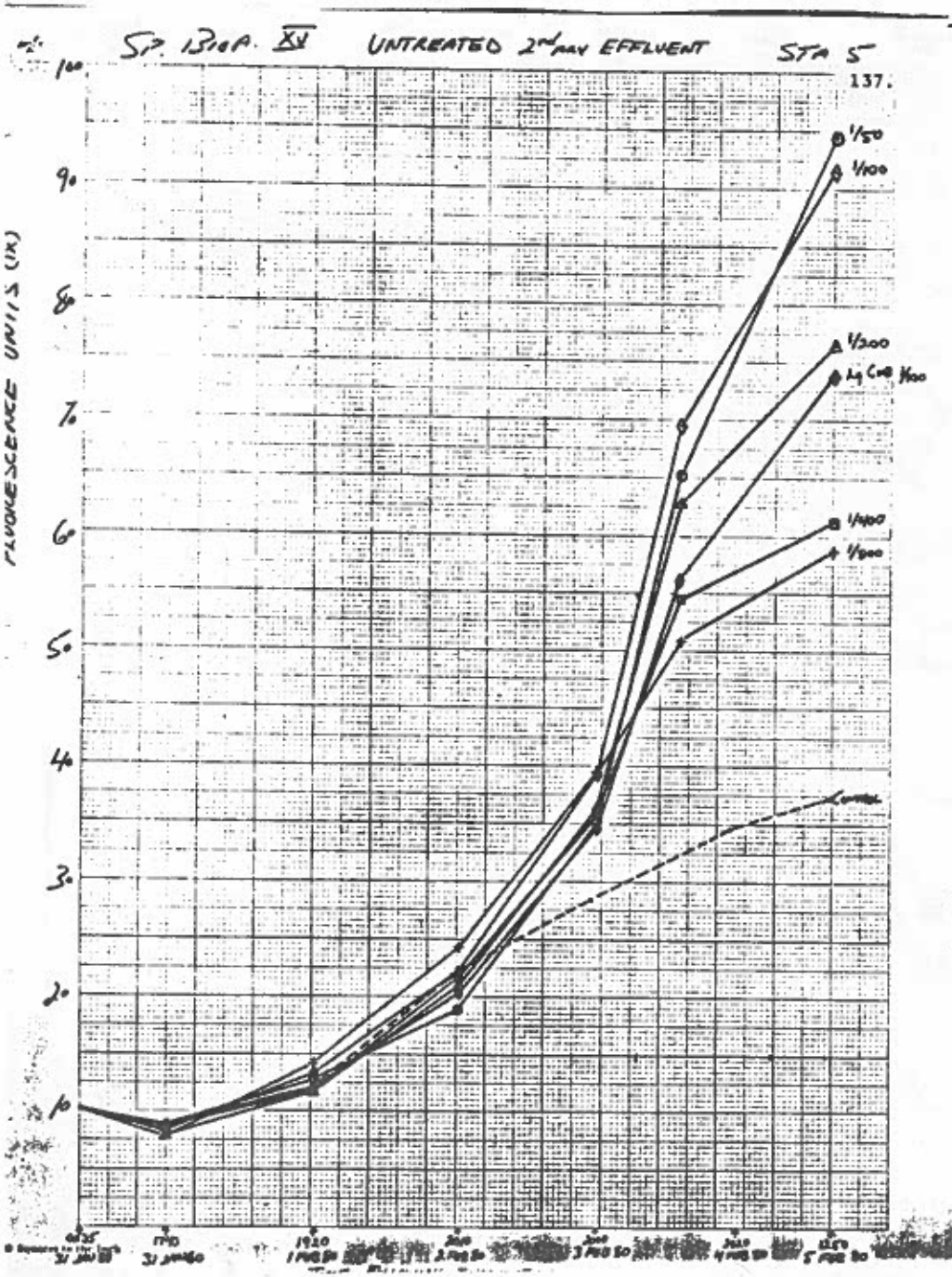
Sp. Bio A XV ALUM TREATED 2nd day EFFLUENT +N : Stp 5

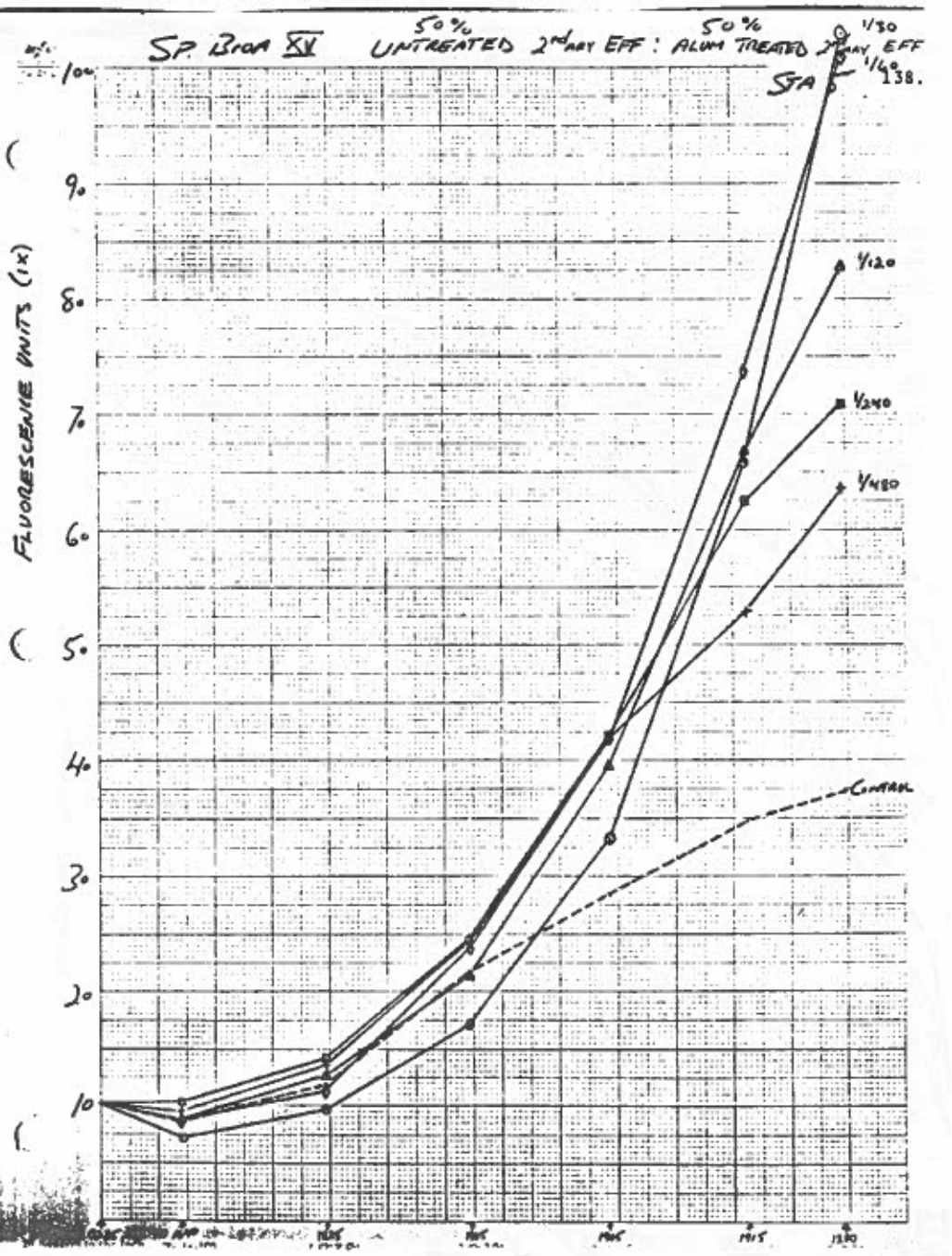


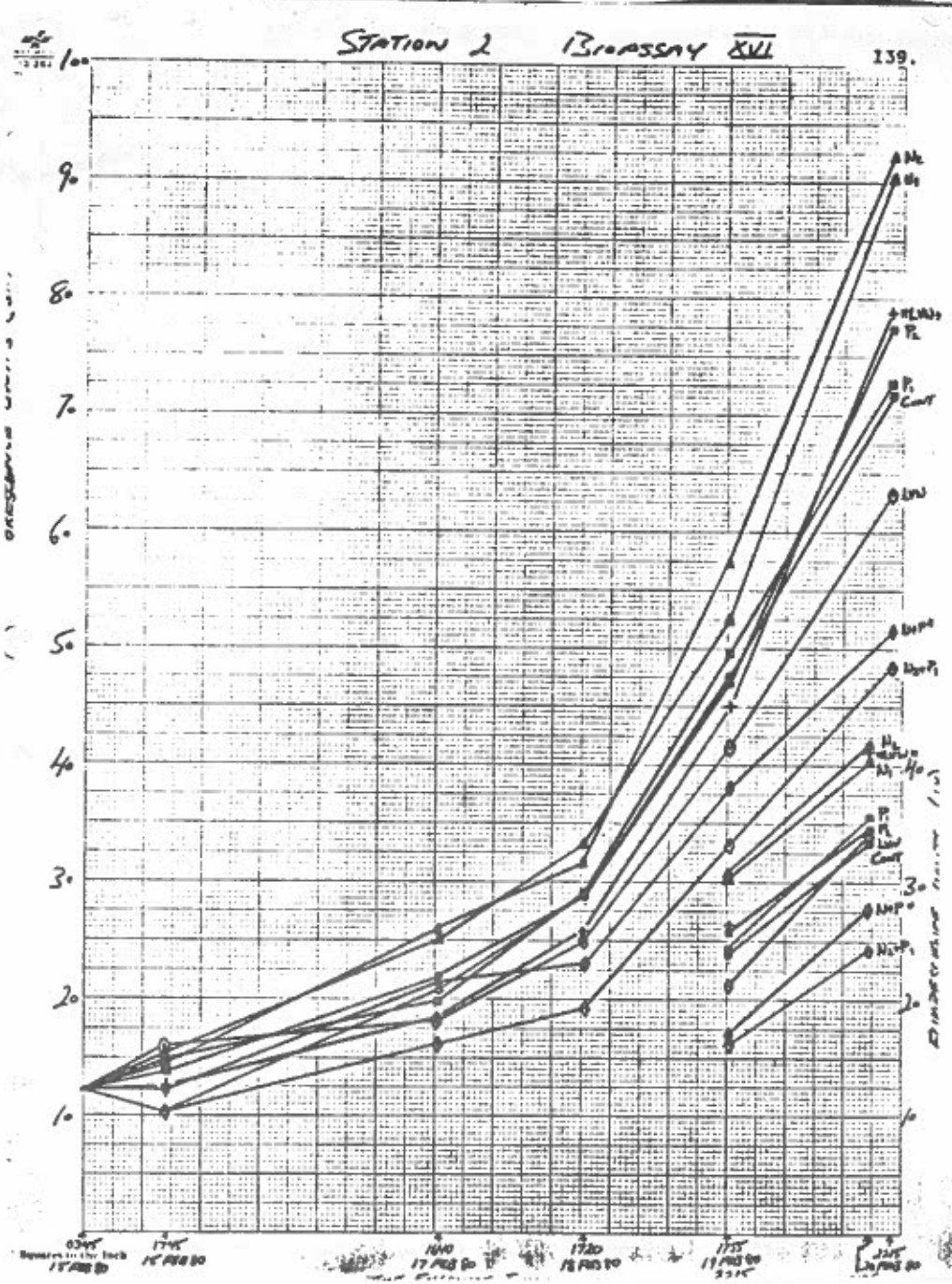
25% UNTREATED 2nd day EFF : 75% ALUM TREATED 2nd day E

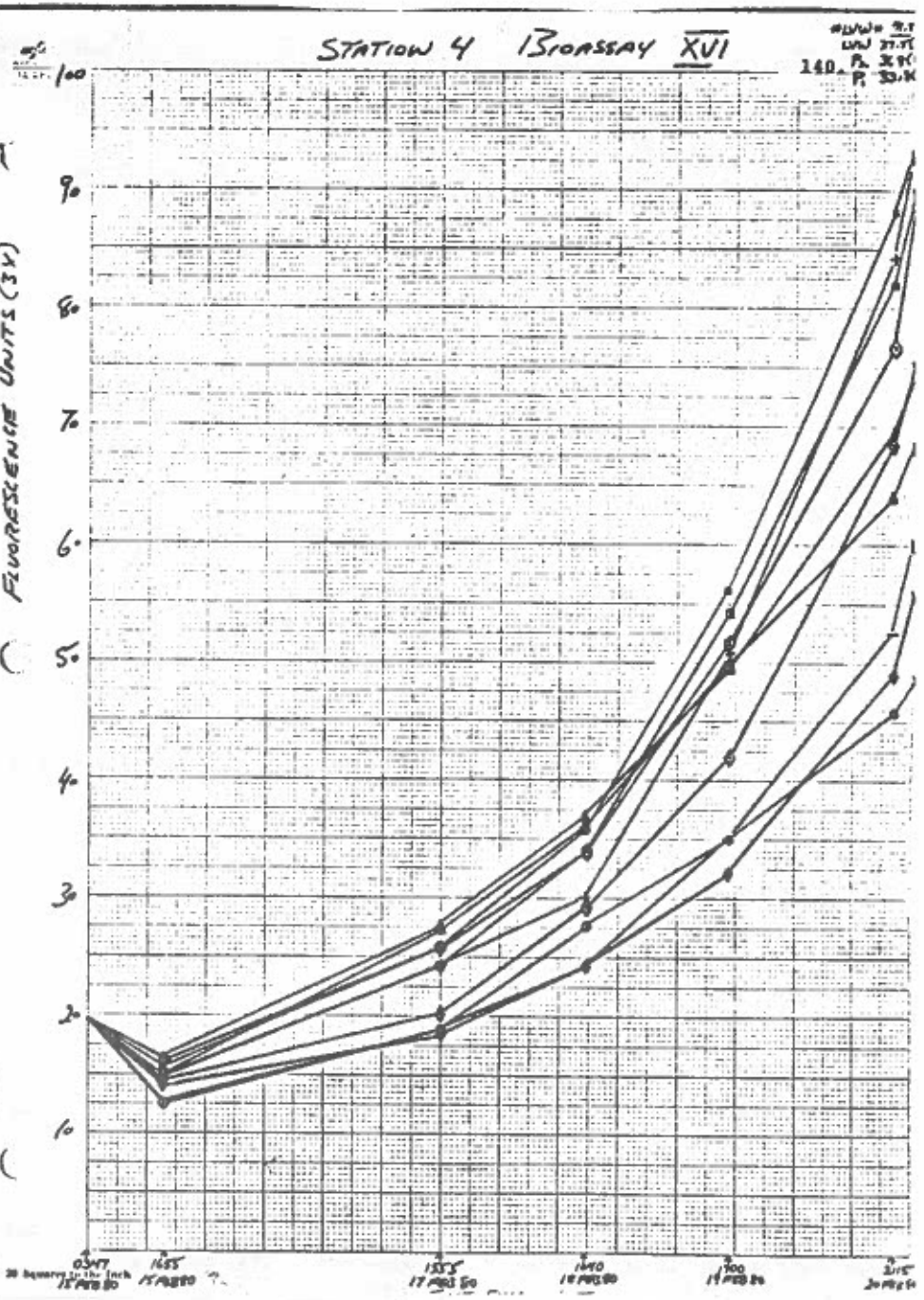
Sp. Bio A. XV



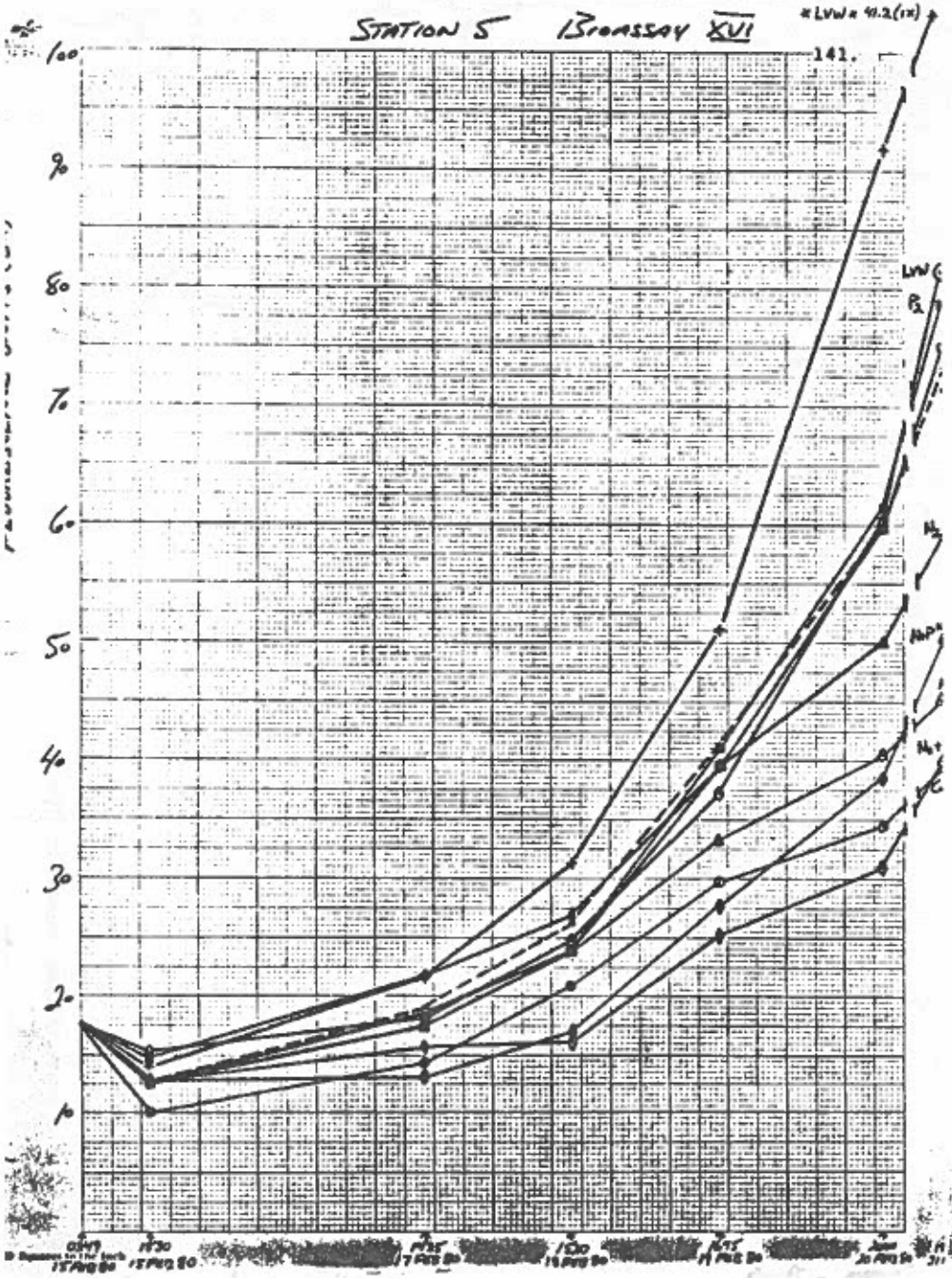

SP. BIOA. XV
UNTREATED 2nd MAY EFFLUENT
STA 5
137.
FLUORESCENCE UNITS (1X)
1/50
1/100
1/200
1/400
1/800
CONTROL


SP. BIOA XV
50% UNTREATED 2nd MAY EFF : 50% ALUM TREATED 2nd MAY EFF
STA
1/30
1/60
138.
FLUORESCEIN UNITS (1X)
1/120
1/240
1/480
CONTROL

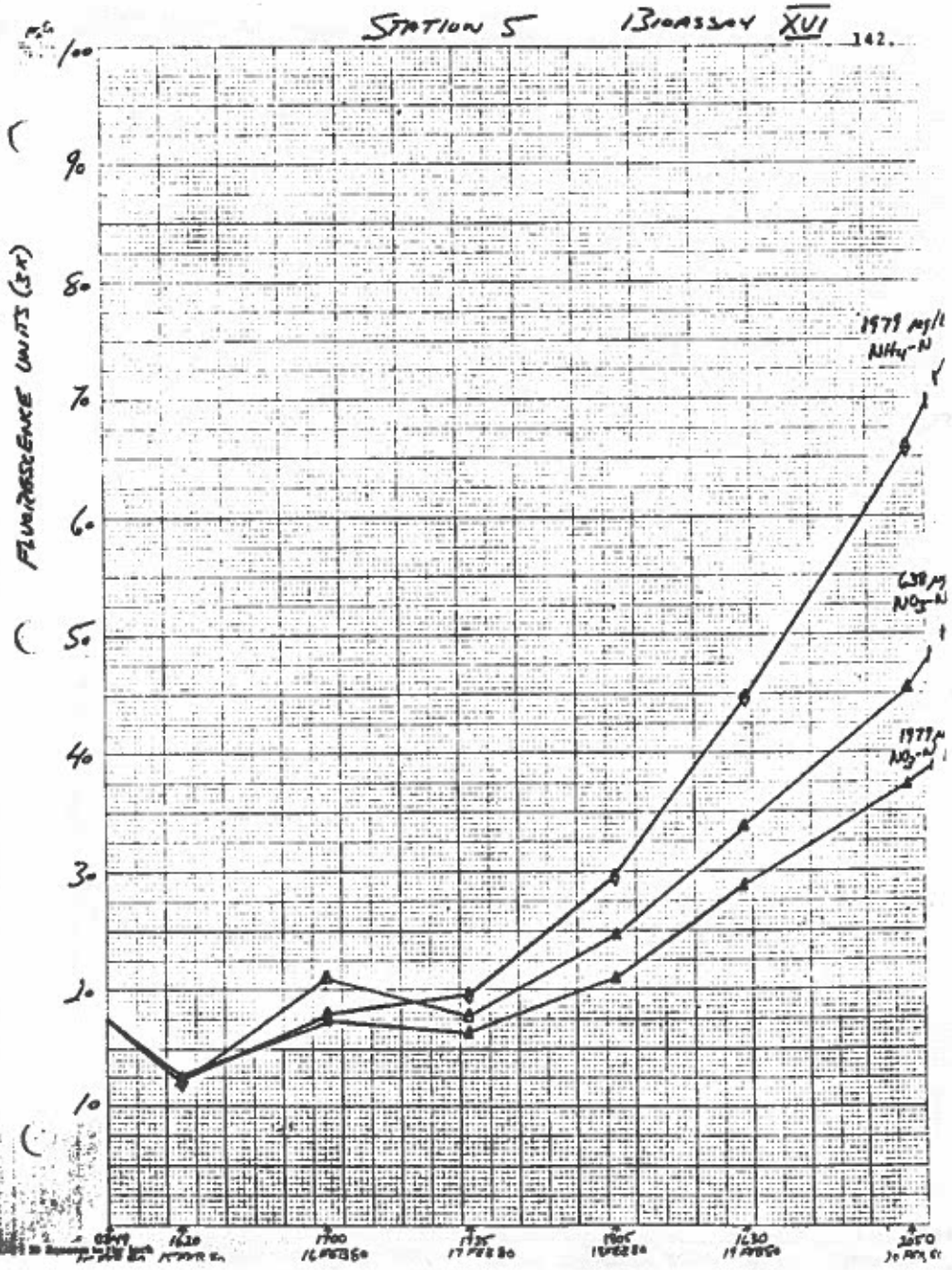

STATION 2 BIOASSAY XVI
139.
100
90
80
70
60
50
40
30
20
10
0345
1745
1640
1720
1755
2215
15 FEB 80
17 FEB 80
18 FEB 80
19 FEB 80
20 FEB 80
Squares to the Inch


STATION 4 BIOASSAY XVI
140.
FLUORESCENCE UNITS (3V)
100
90
80
70
60
50
40
30
20
10
0347
1655
1555
1640
1700
2115
15 FEB 80
17 FEB 80
18 FEB 80
19 FEB 80
20 FEB 80
Squares to the Inch

STATION 5 BIOASSAY XVI

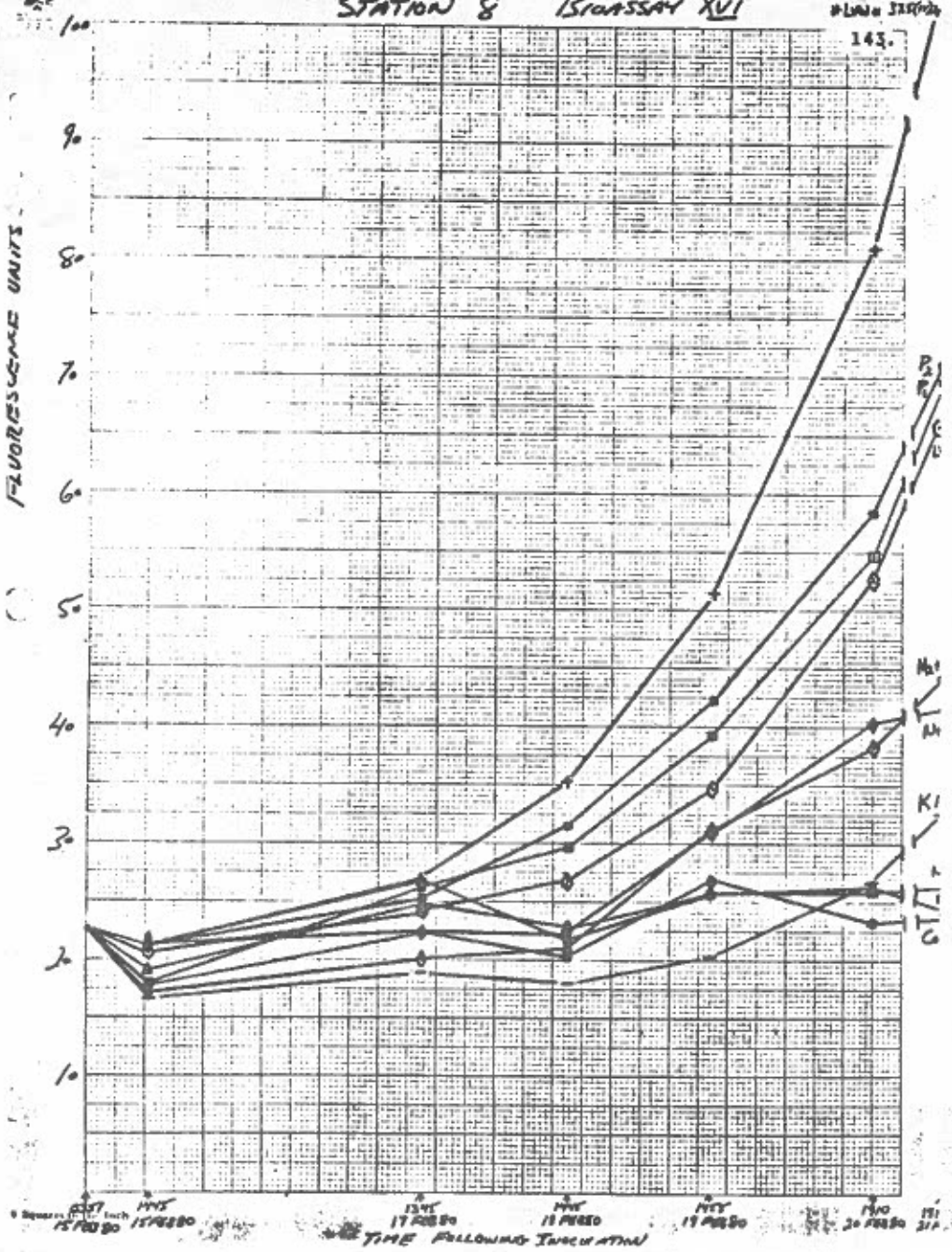


STATION 5 BIOASSAY XVI



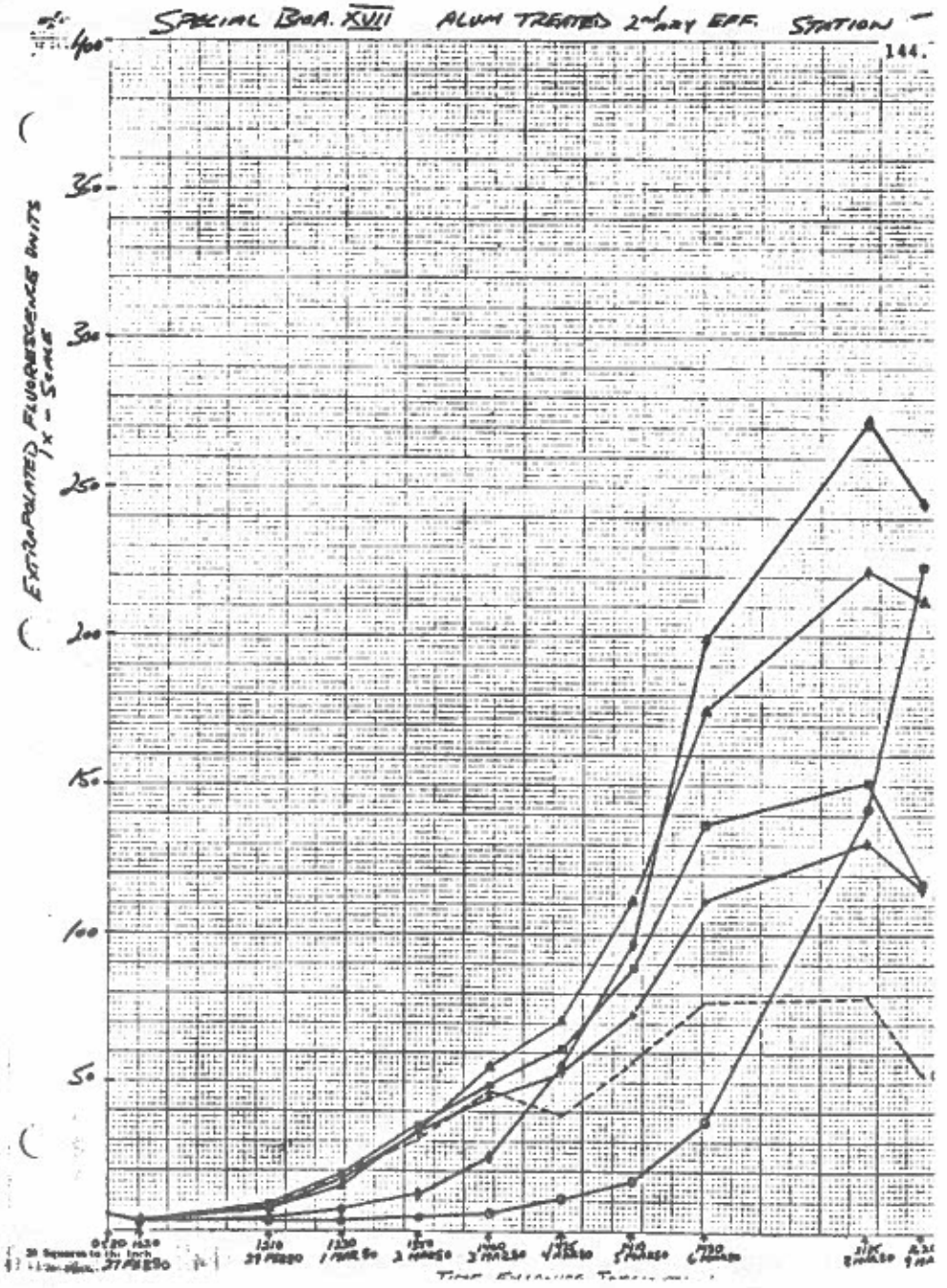
STATION 8 BIOASSAY XVI

143.

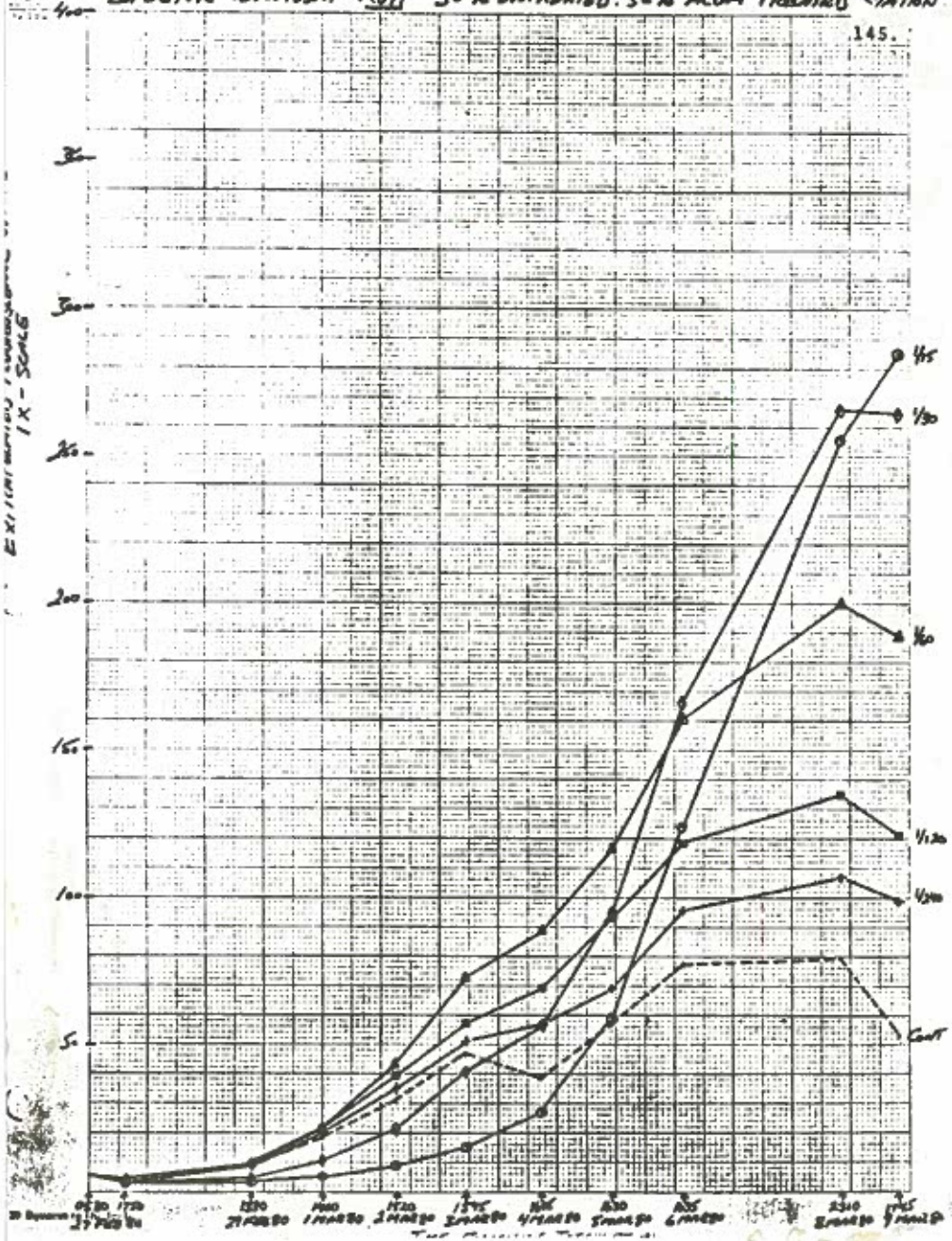


SPECIAL BIOA. XVII ALUM TREATED 2nd DAY EFF. STATION —

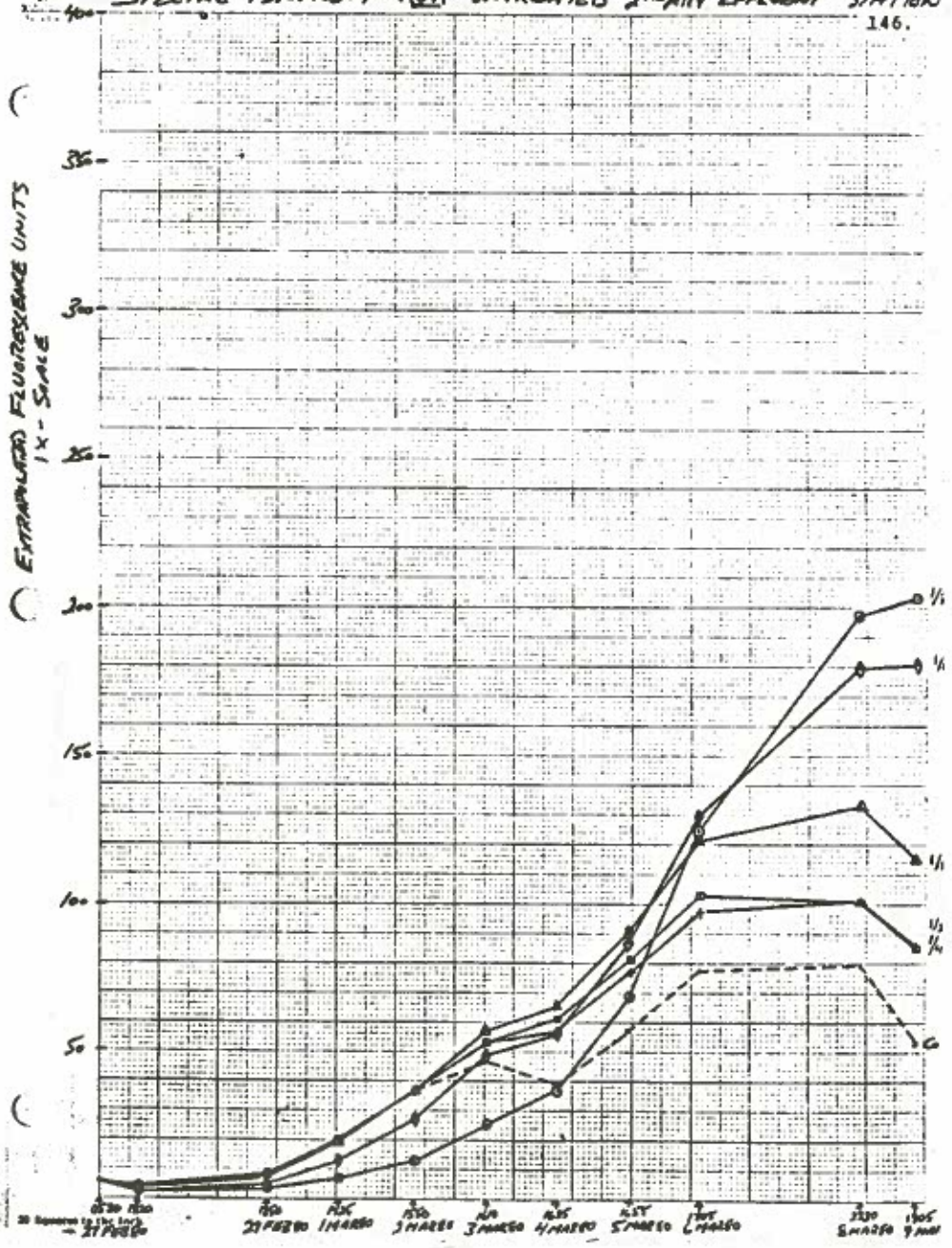
144.



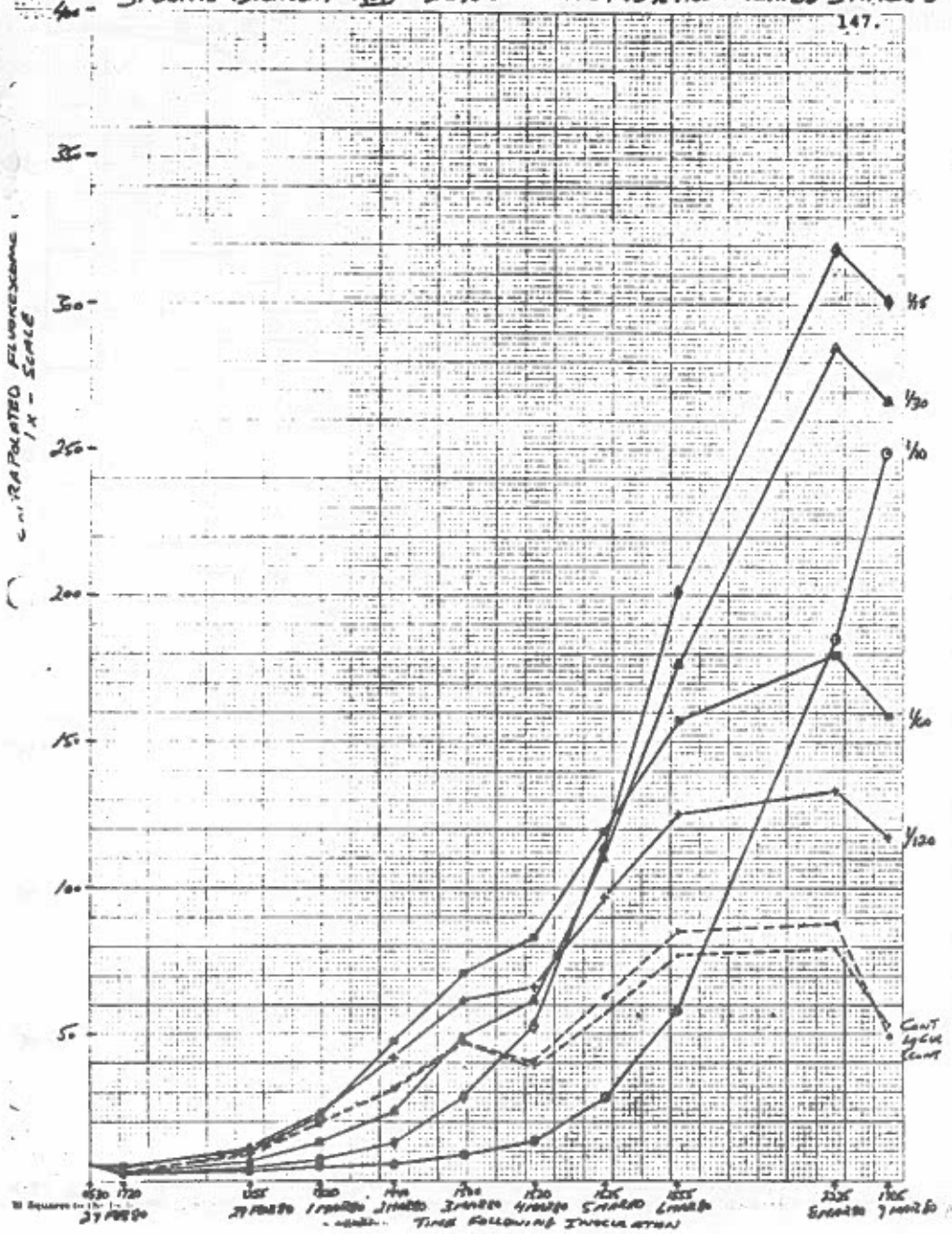
SPECIAL BIOASSAY XVII 50% UNTREATED : 50% ALUM TREATED STATION



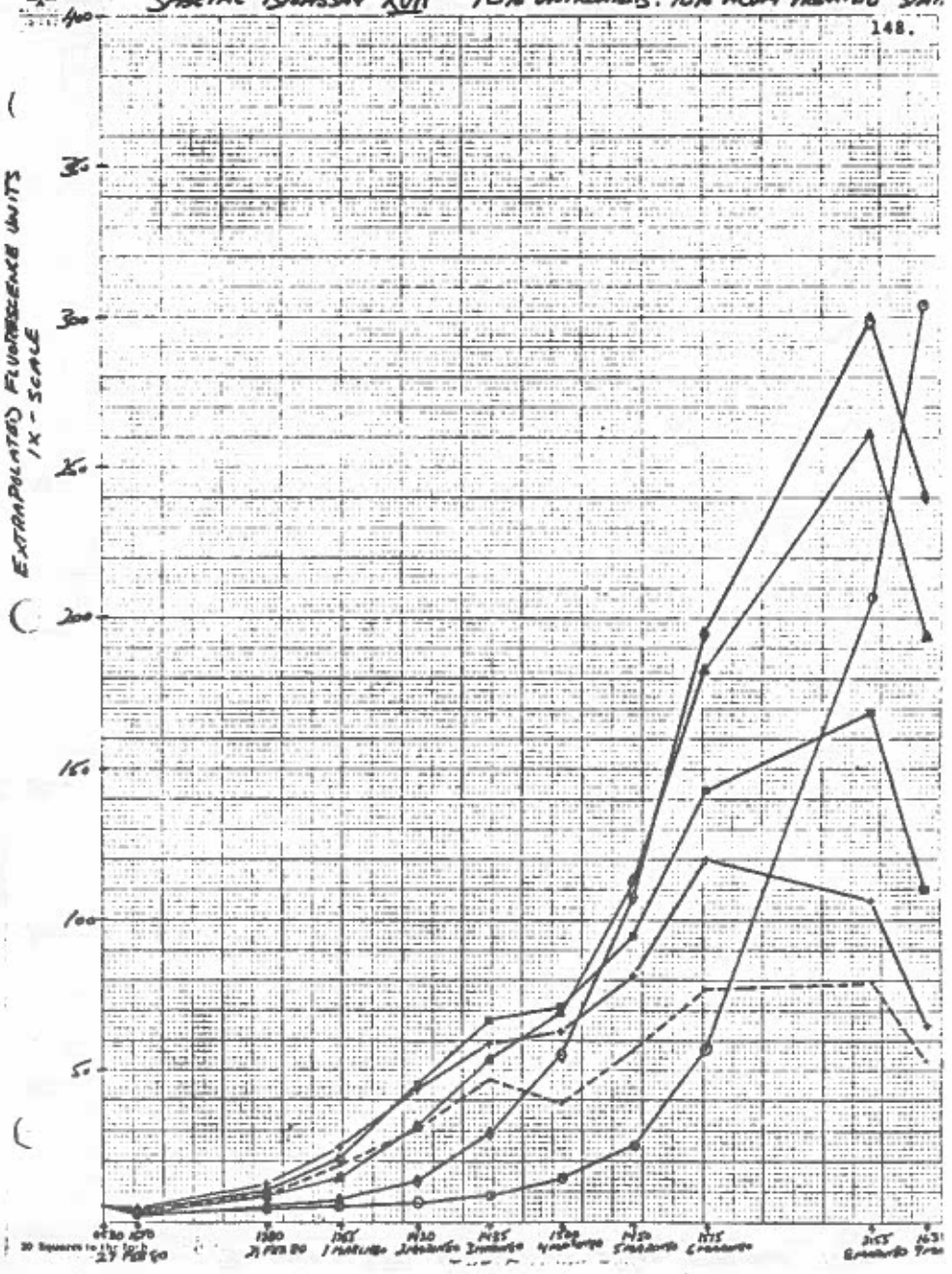
SPECIAL BIOASSAY XVII UNTREATED 2ND ARY EFFLUENT STATION

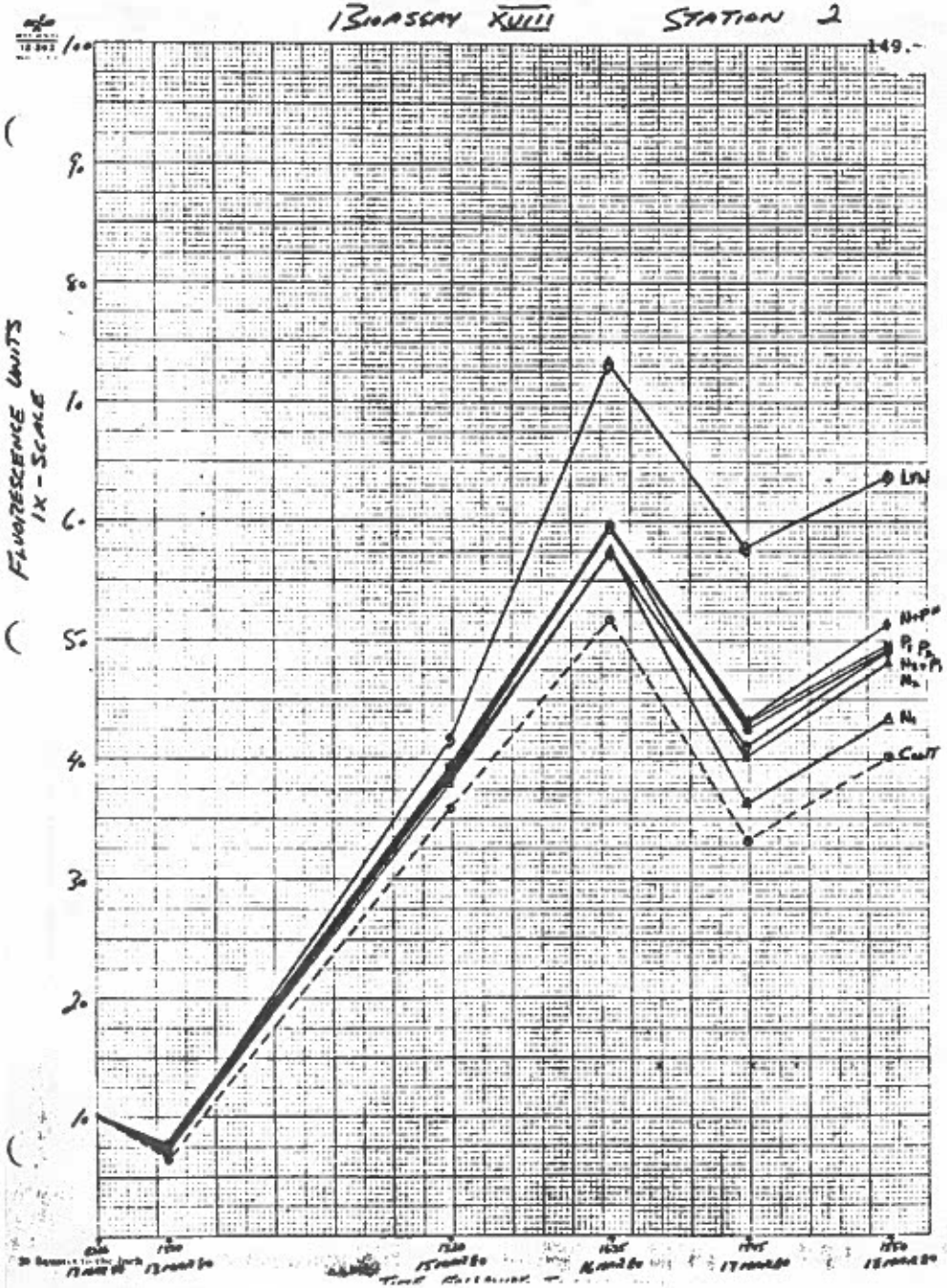


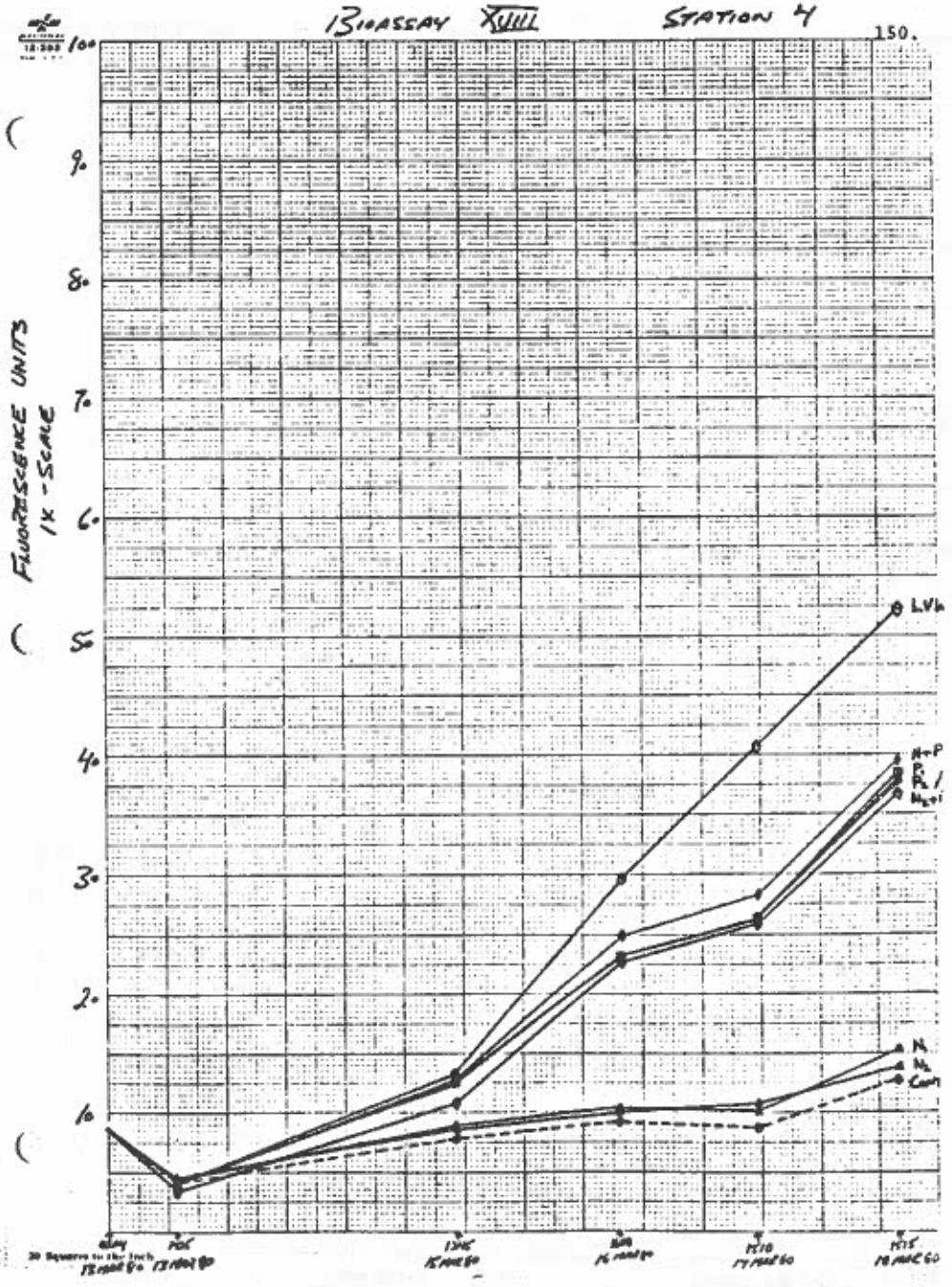
SPECIAL BIOASSAY XVII 25% UNTREATED : 75% ALUM TREATED STATION 5



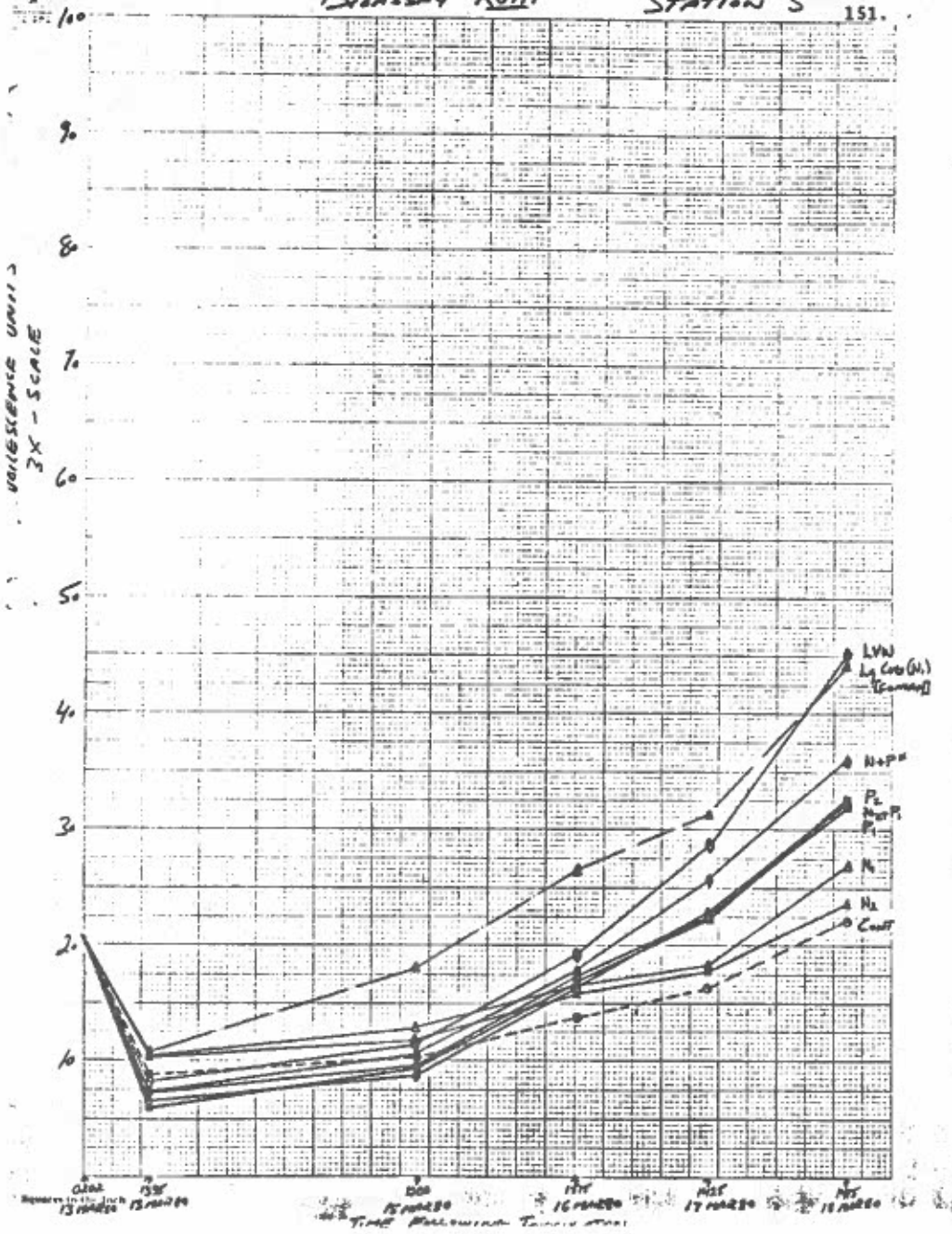
SPECIAL BIOASSAY XVII 10% UNTREATED : 90% ALUM TREATED STA



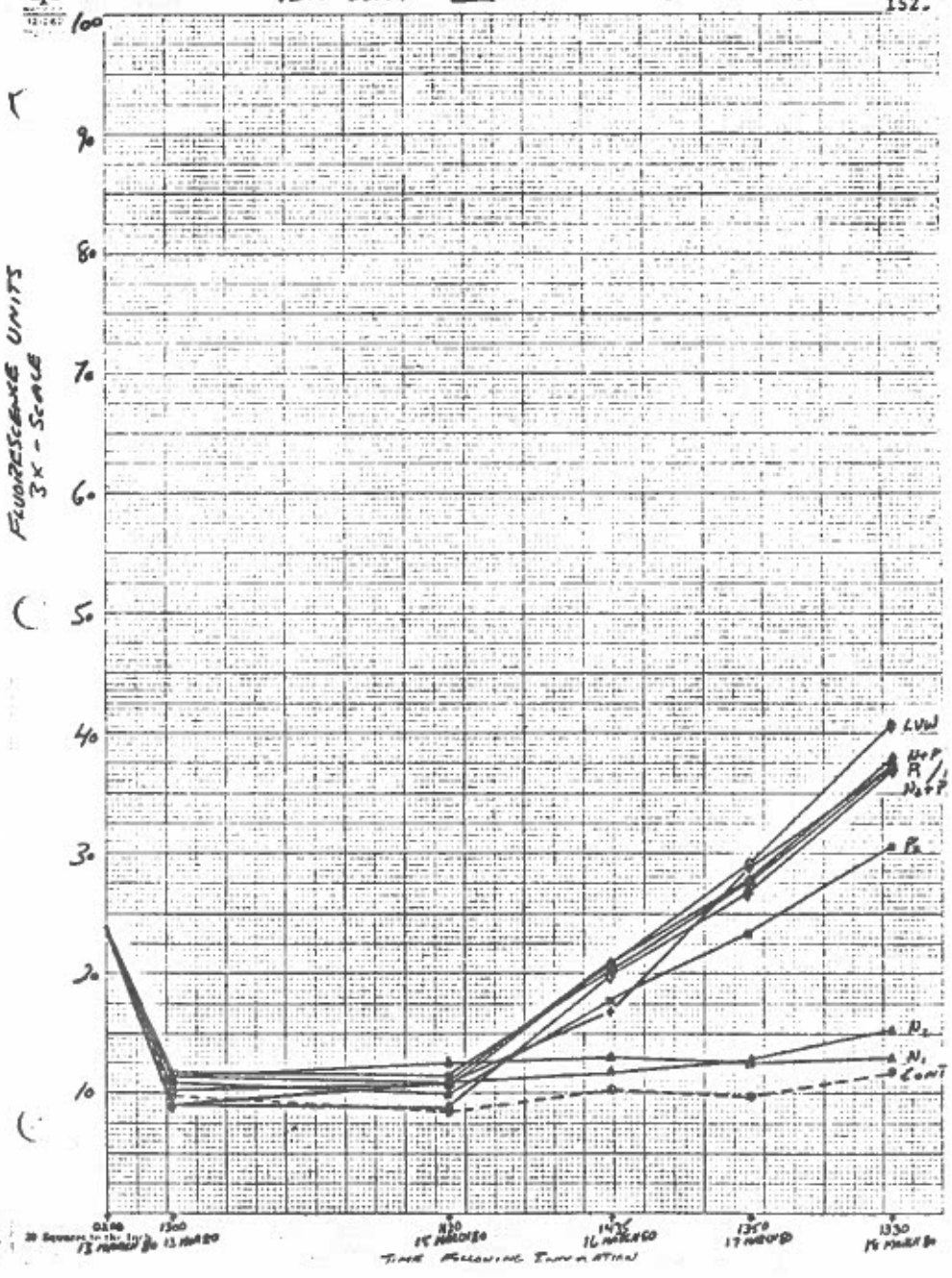

BIOASSAY XVIII STATION 2
149.-
FLUORESCENCE UNITS
1X - SCALE
LVN
N+P
N1
Cont

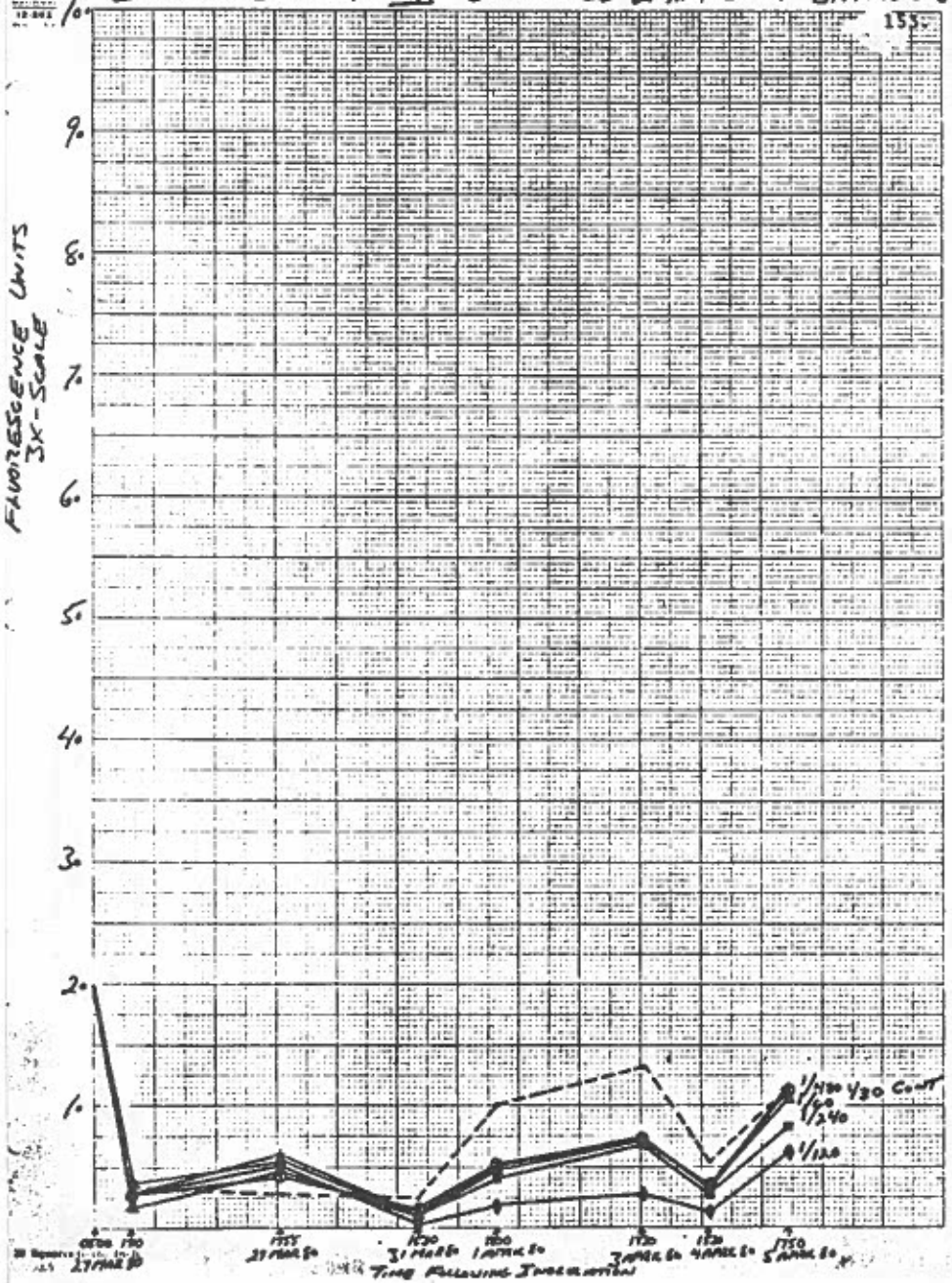

BIOASSAY XVIII STATION 4
150.
FLUORESCENCE UNITS
1X - SCALE
LVN
N+P
N1
Cont

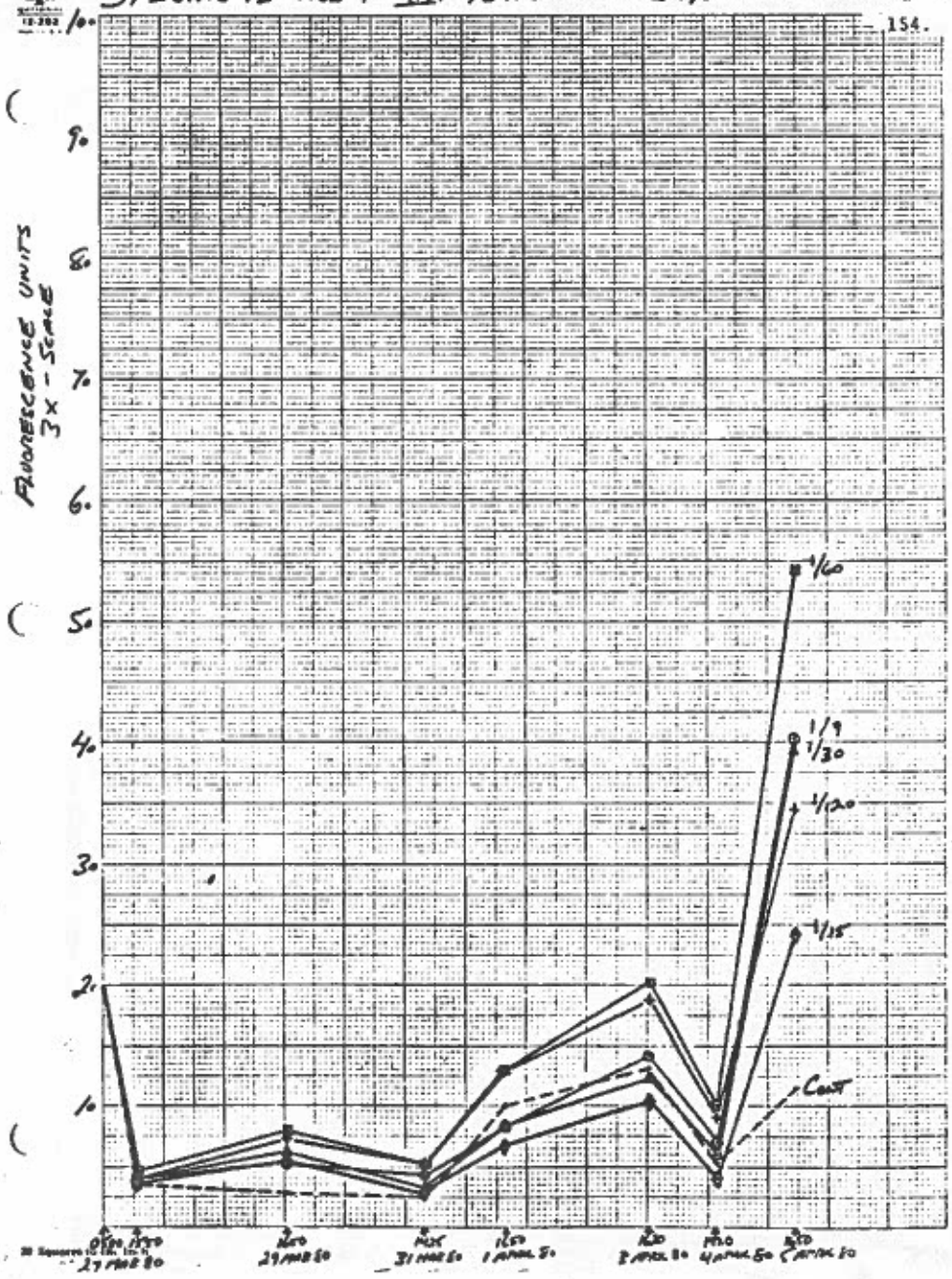
BIOMASSAY XVIII STATION 5

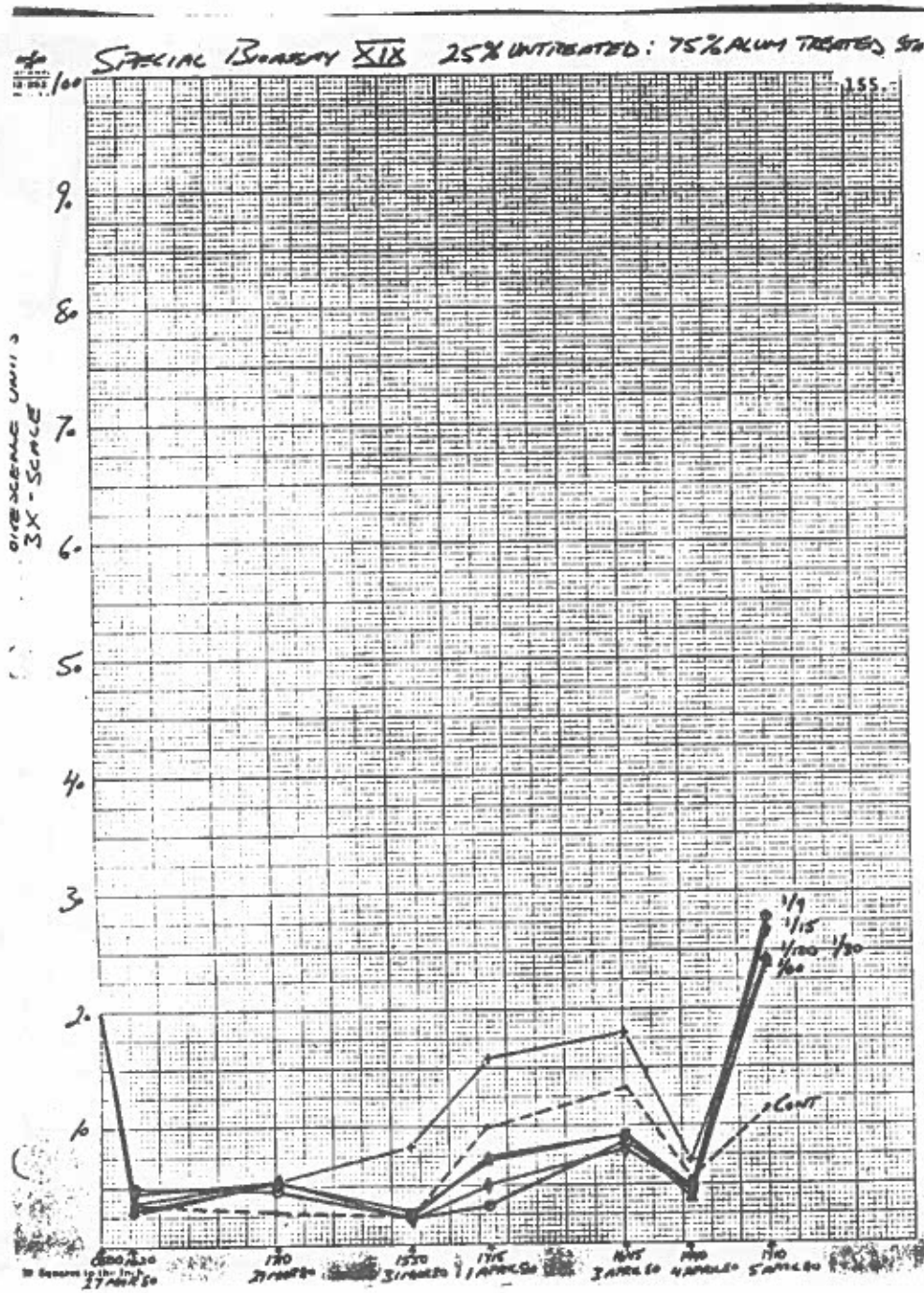


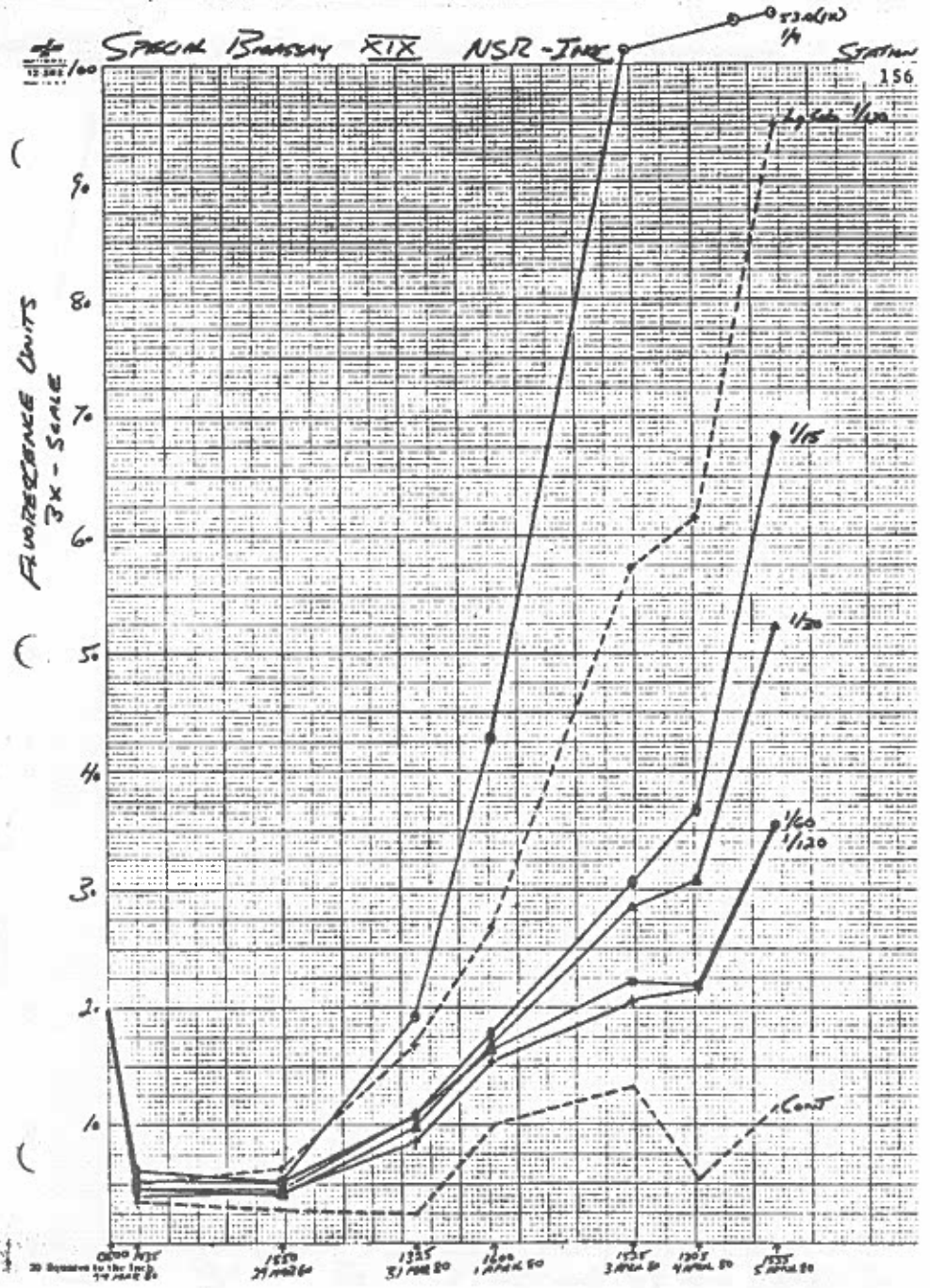
BIOMASSAY XVIII STATION 8




SPECIAL BIOASSAY XIX UNTREATED 2ND ARY EFF. STATION 5
155.
FLUORESCENCE UNITS 3X-SCALE
1/430 1/30 CONT
1/60
1/240
1/120
TIME FOLLOWING INOCULATION

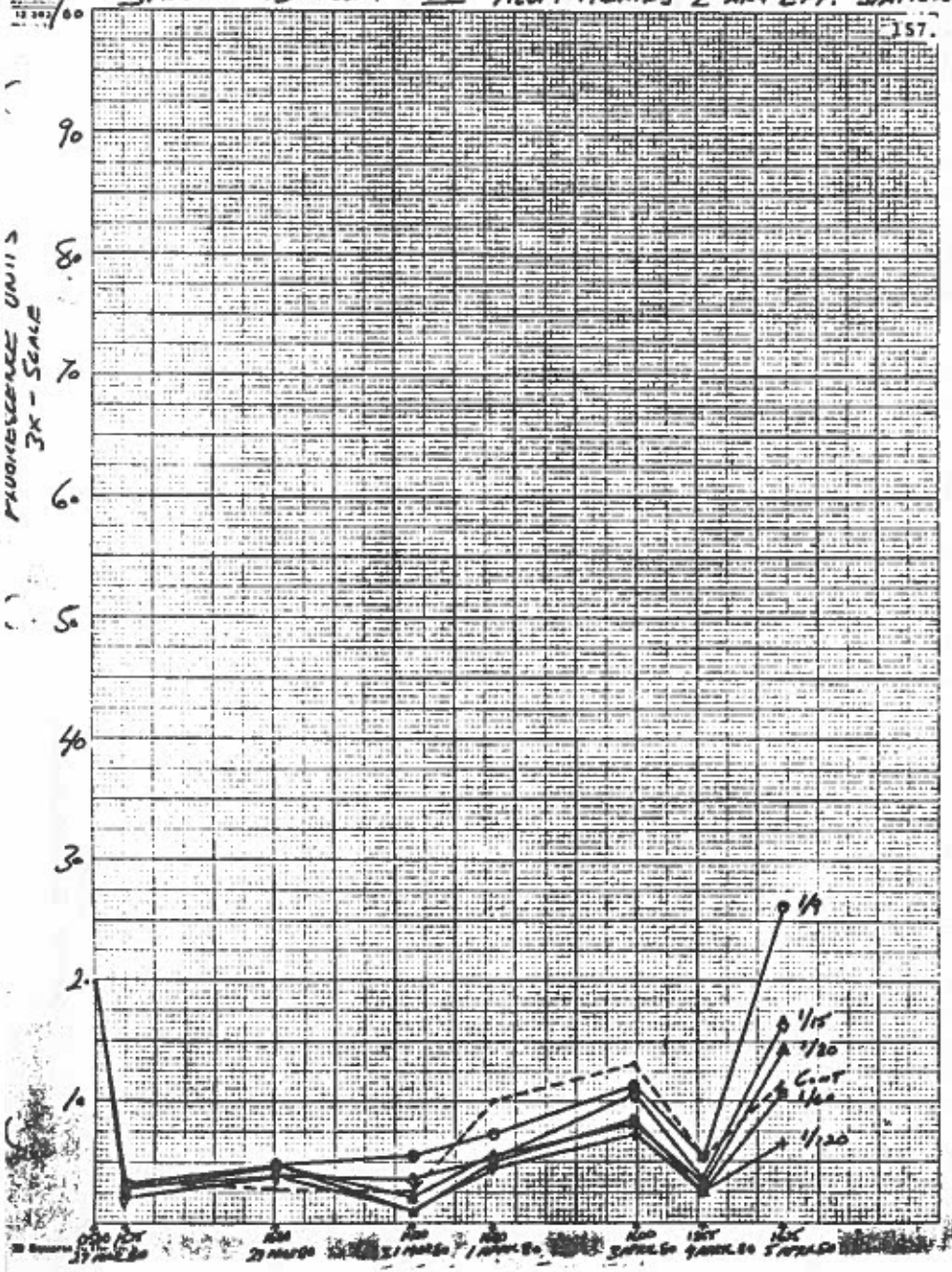

SPECIAL BIOASSAY XIX 10% UNTREATED : 90% ALUM TREATED STA
154.
FLUORESCENCE UNITS 3X-SCALE
1/60
1/9
1/30
1/120
1/15
CONT


SPECIAL BIOASSAY XVIII 25% UNTREATED : 75% ALUM TREATED Sta
155
FLUORESCENCE UNITS 3X - SCALE
9
8
7
6
5
4
3
2
1
1/9
1/15
1/30
1/60
1/120
CONT


SPECIAL BIOASSAY XIX NSR - JUNE
Sta(N)
1/4
STATION
156
FLUORESCENCE UNITS 3X - SCALE
9
8
7
6
5
4
3
2
1
1/15
1/30
1/60
1/120
CONT

157.

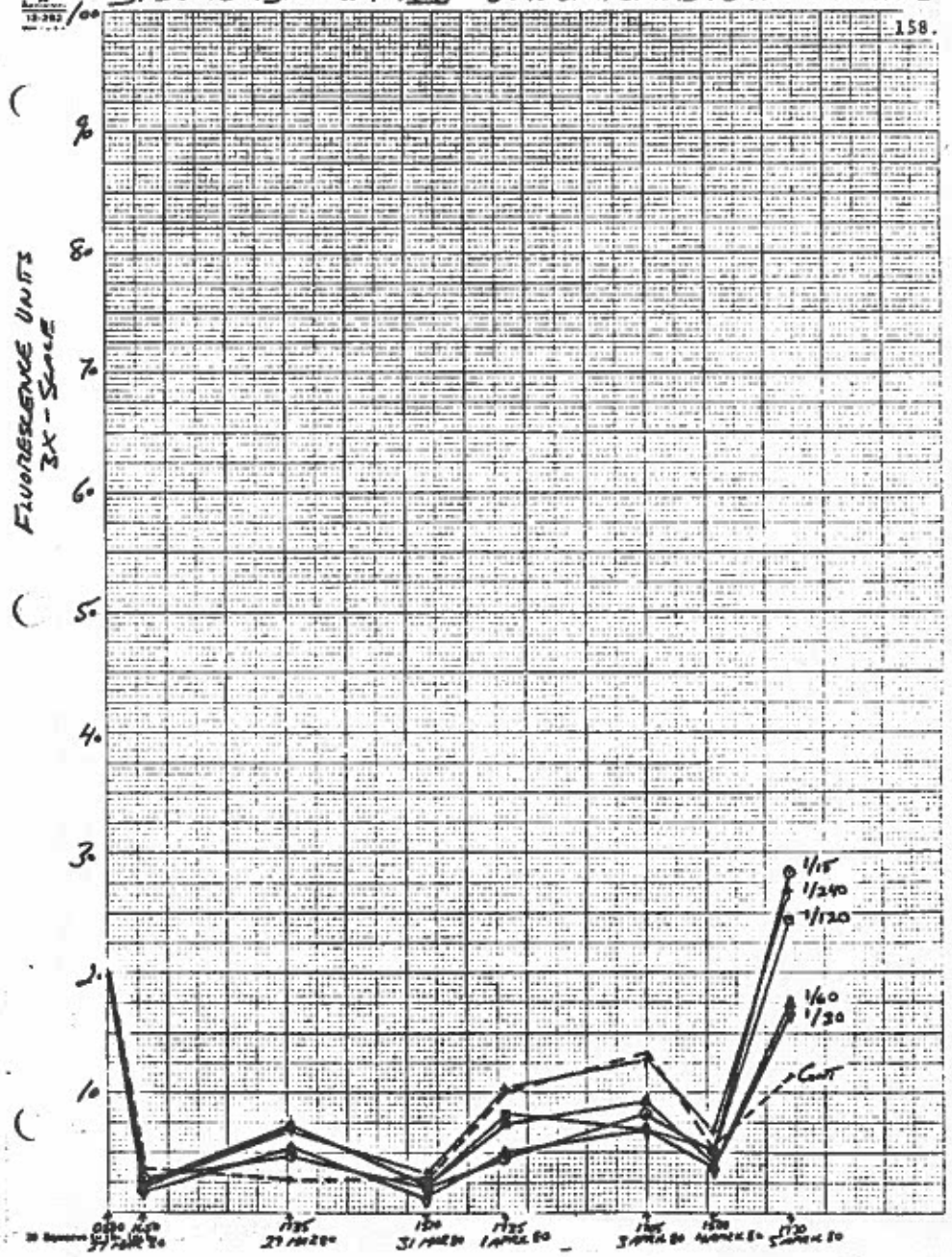
SPECIAL BIOASSAY XIX ALUM TREATED 2ndARY EFF. STATION 5

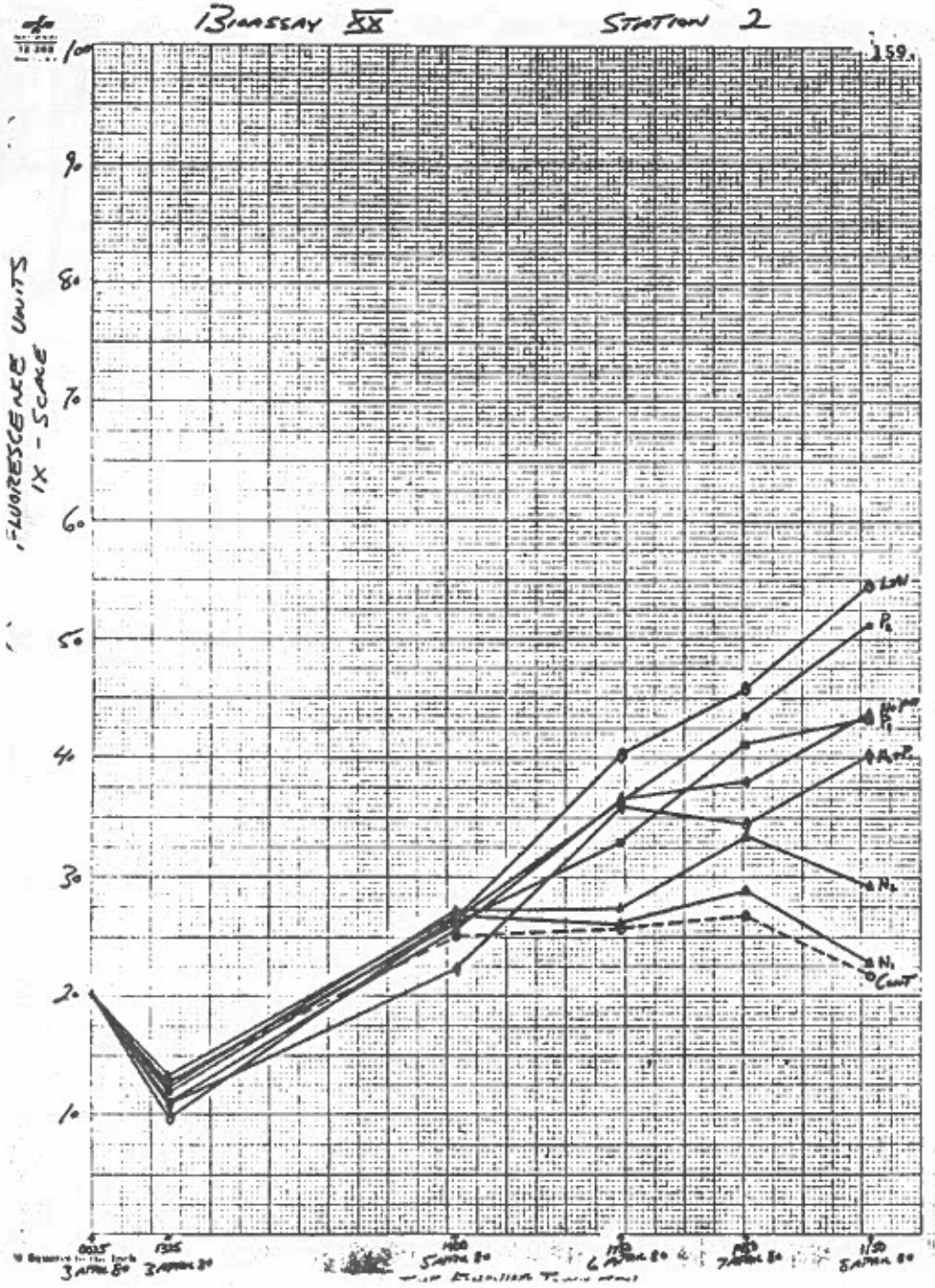


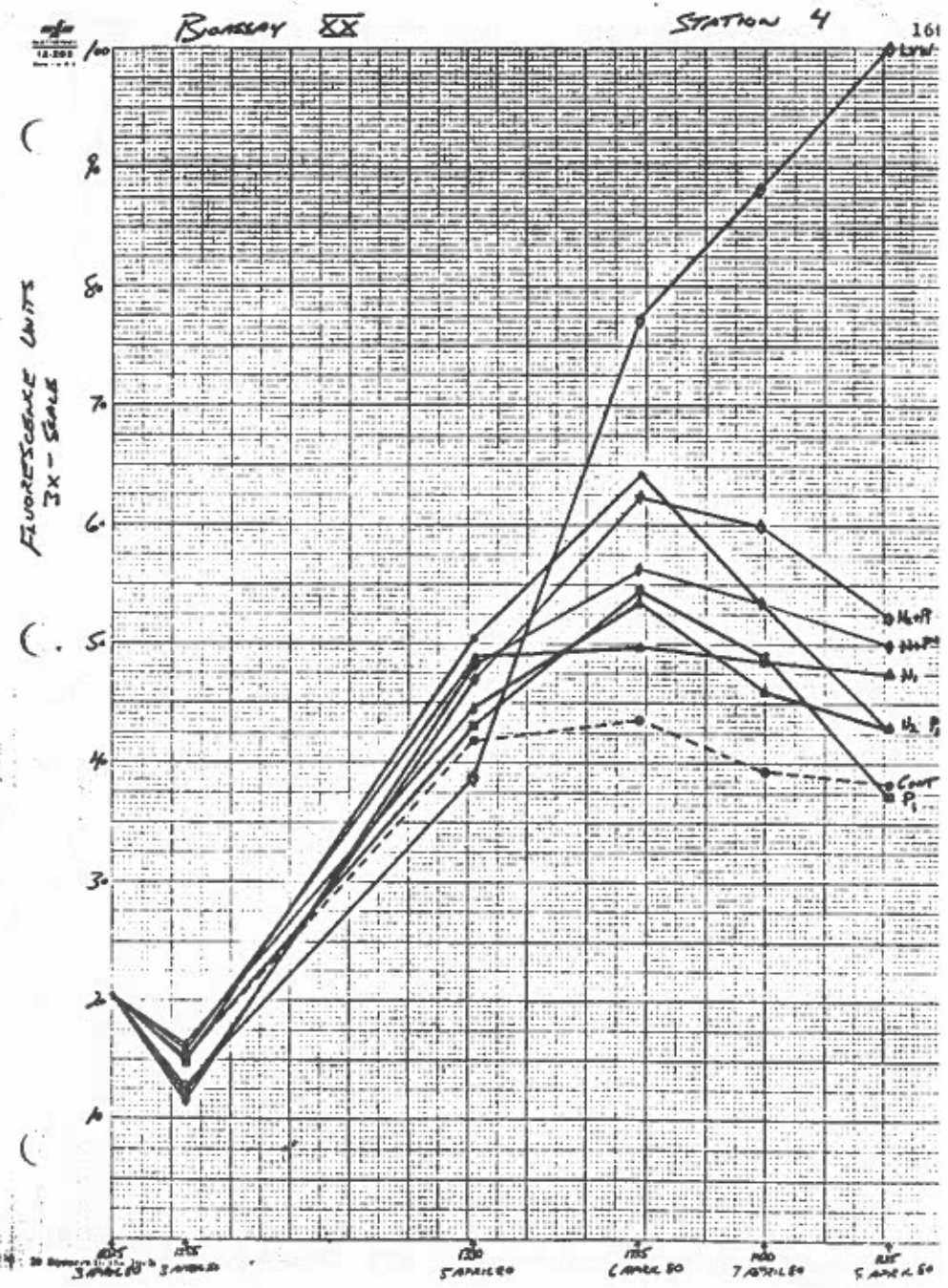
158.

STATION 5

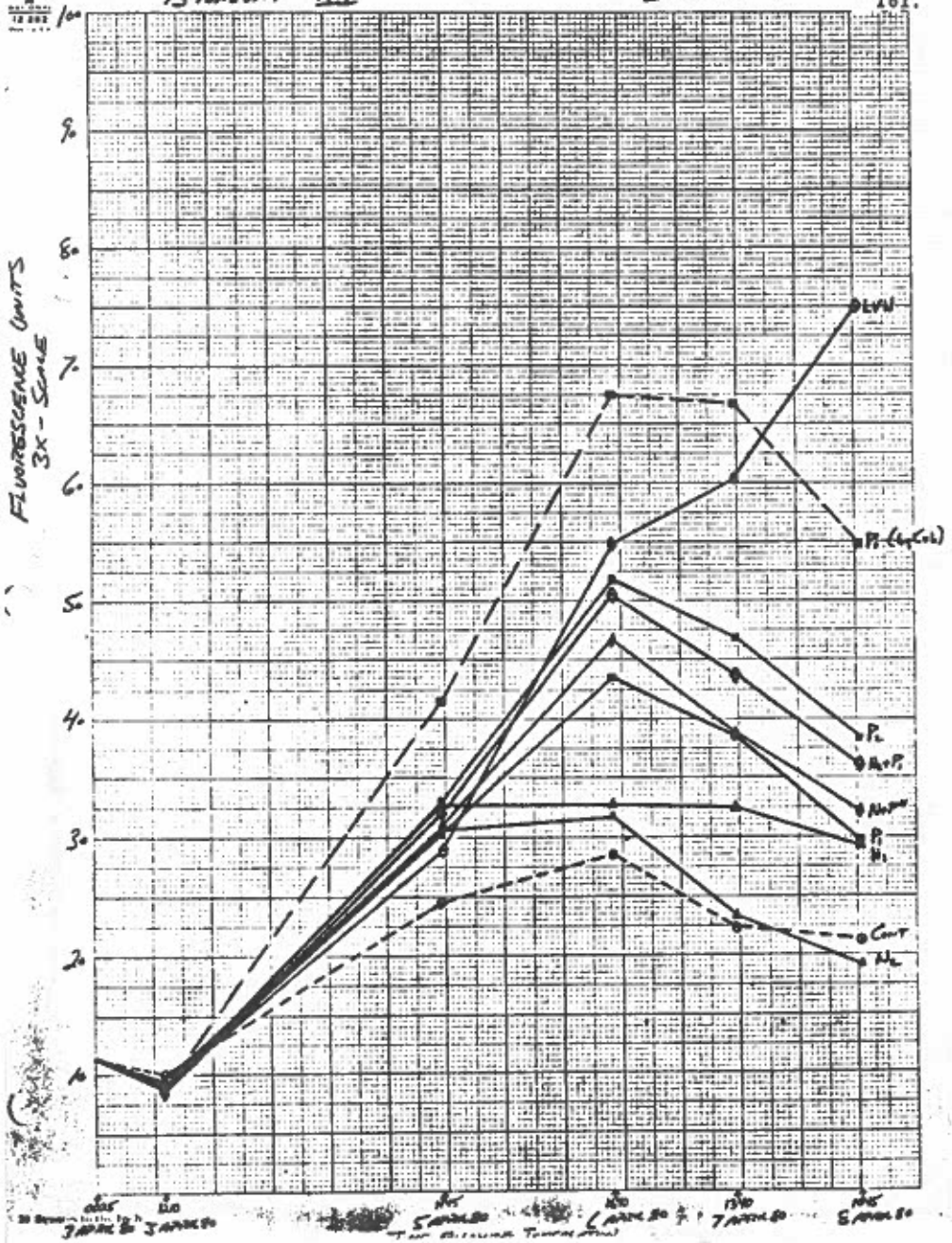
SPECIAL BIOASSAY XIX 50% UNTREATED : 50% ALUM TREATED



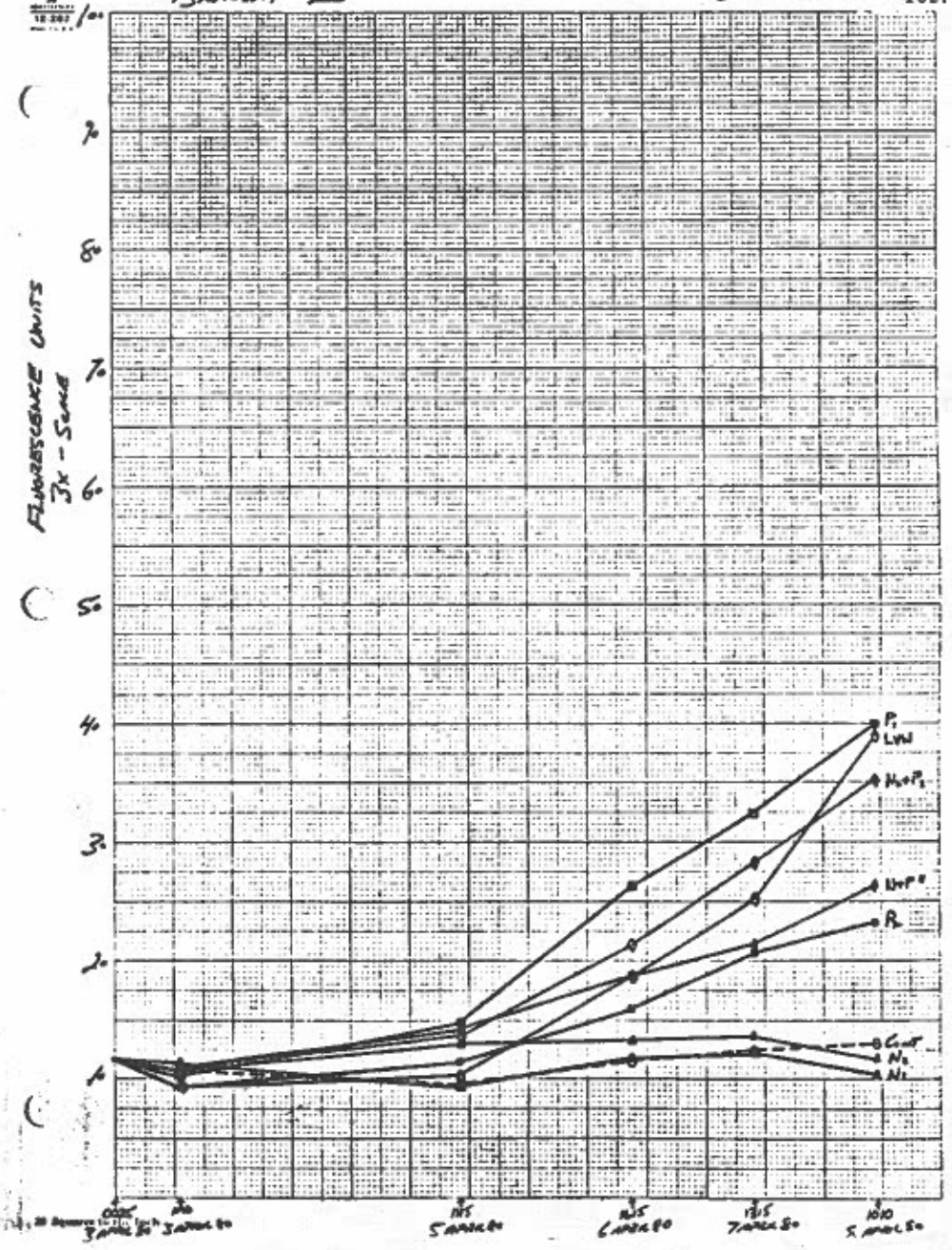

BIOASSAY XX
STATION 2
159
FLUORESCENCE UNITS
1X - SCALE
100
90
80
70
60
50
40
30
20
10
LVW
P2
N2
N1
CONT
0025
1325
1400
1150
3 APRIL 80
3 APRIL 80
5 APRIL 80
6 APRIL 80
7 APRIL 80
8 APRIL 80

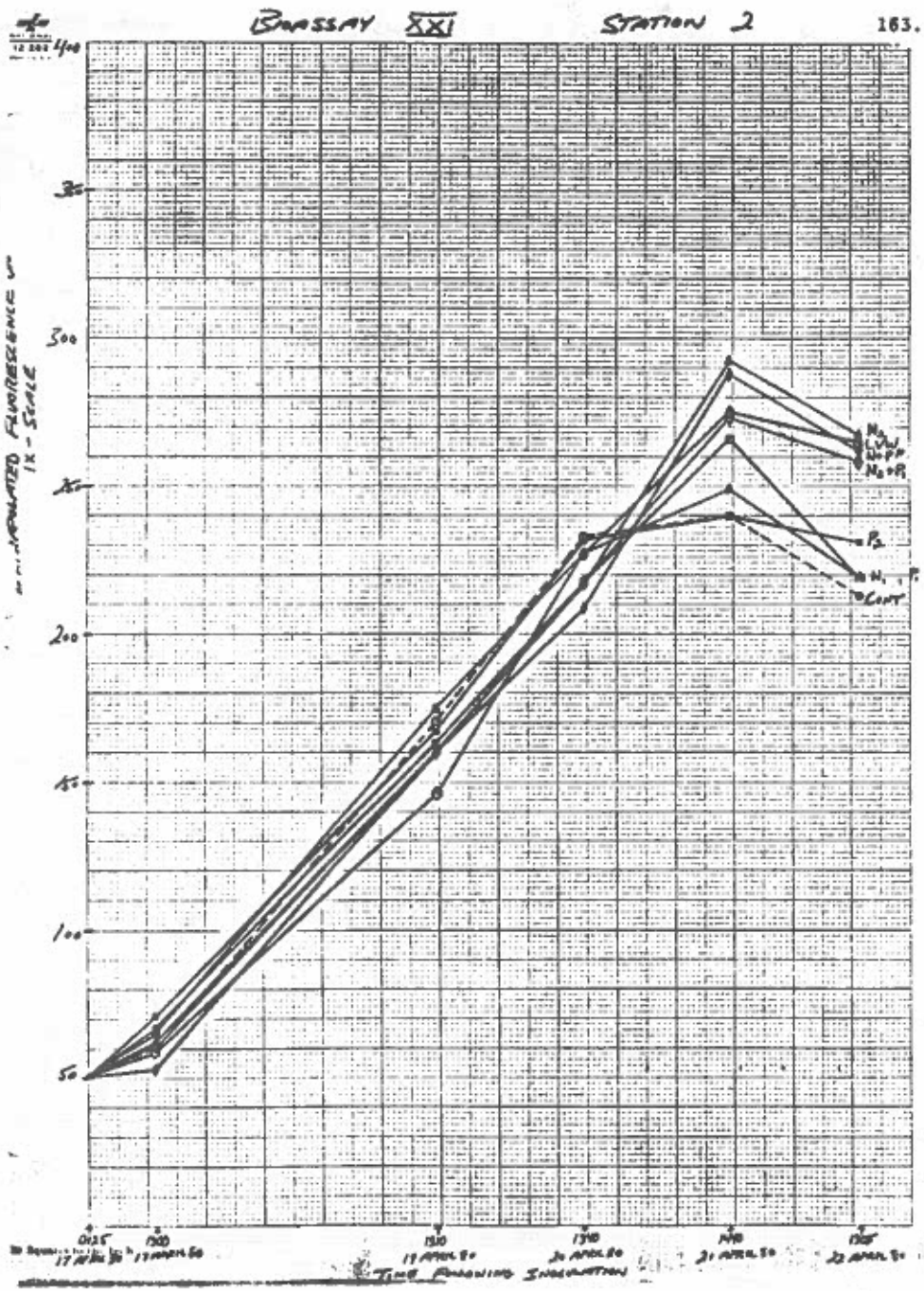

BIOASSAY XX
STATION 4
161
FLUORESCENCE UNITS
3X - SCALE
100
90
80
70
60
50
40
30
20
10
LVW
N2+P1
N1
N2 P1
CONT
P1
1330
1325
1400
3 APRIL 80
3 APRIL 80
5 APRIL 80
6 APRIL 80
7 APRIL 80
8 APRIL 80

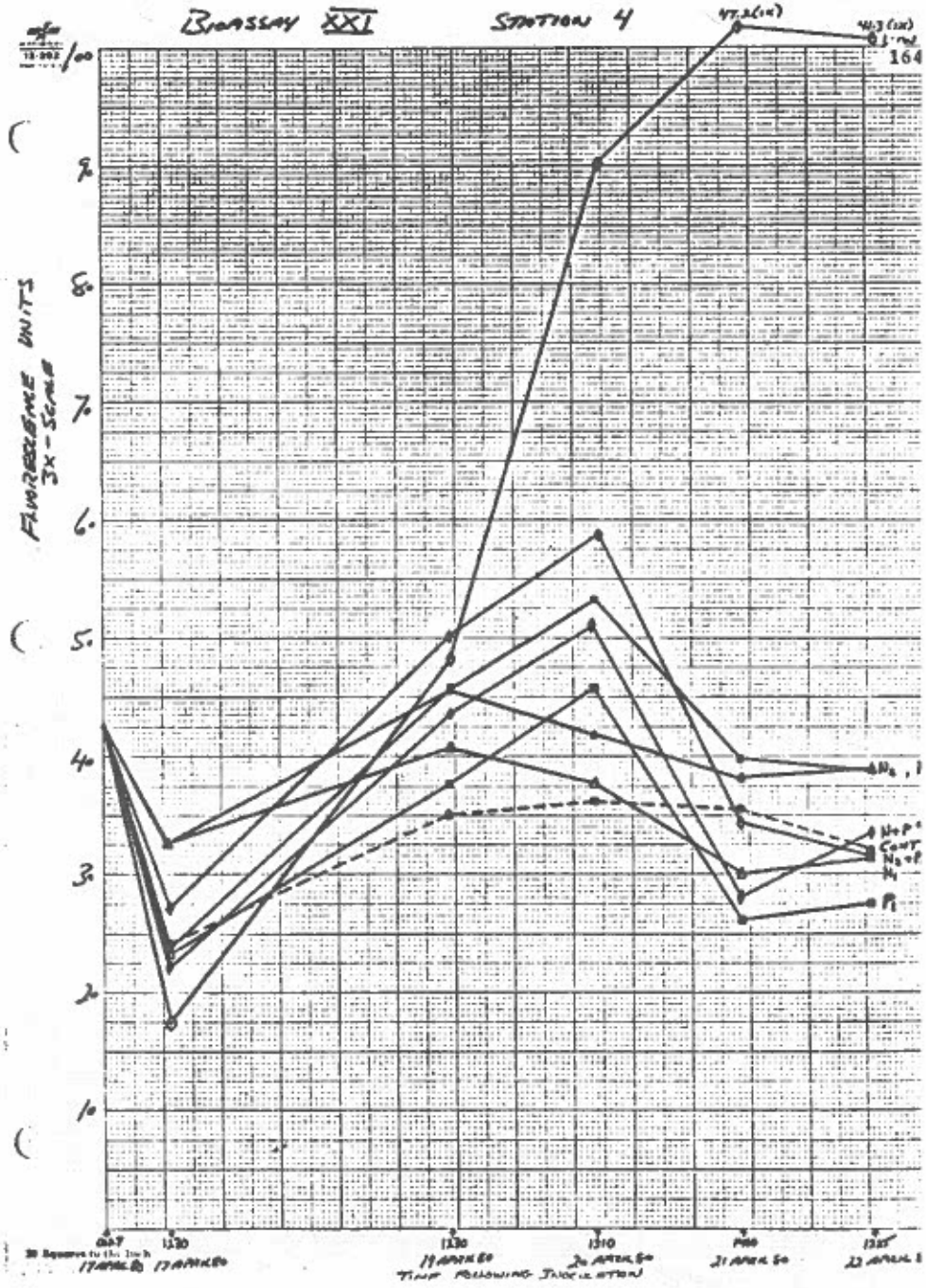
BIOASSAY XX — STATION 5

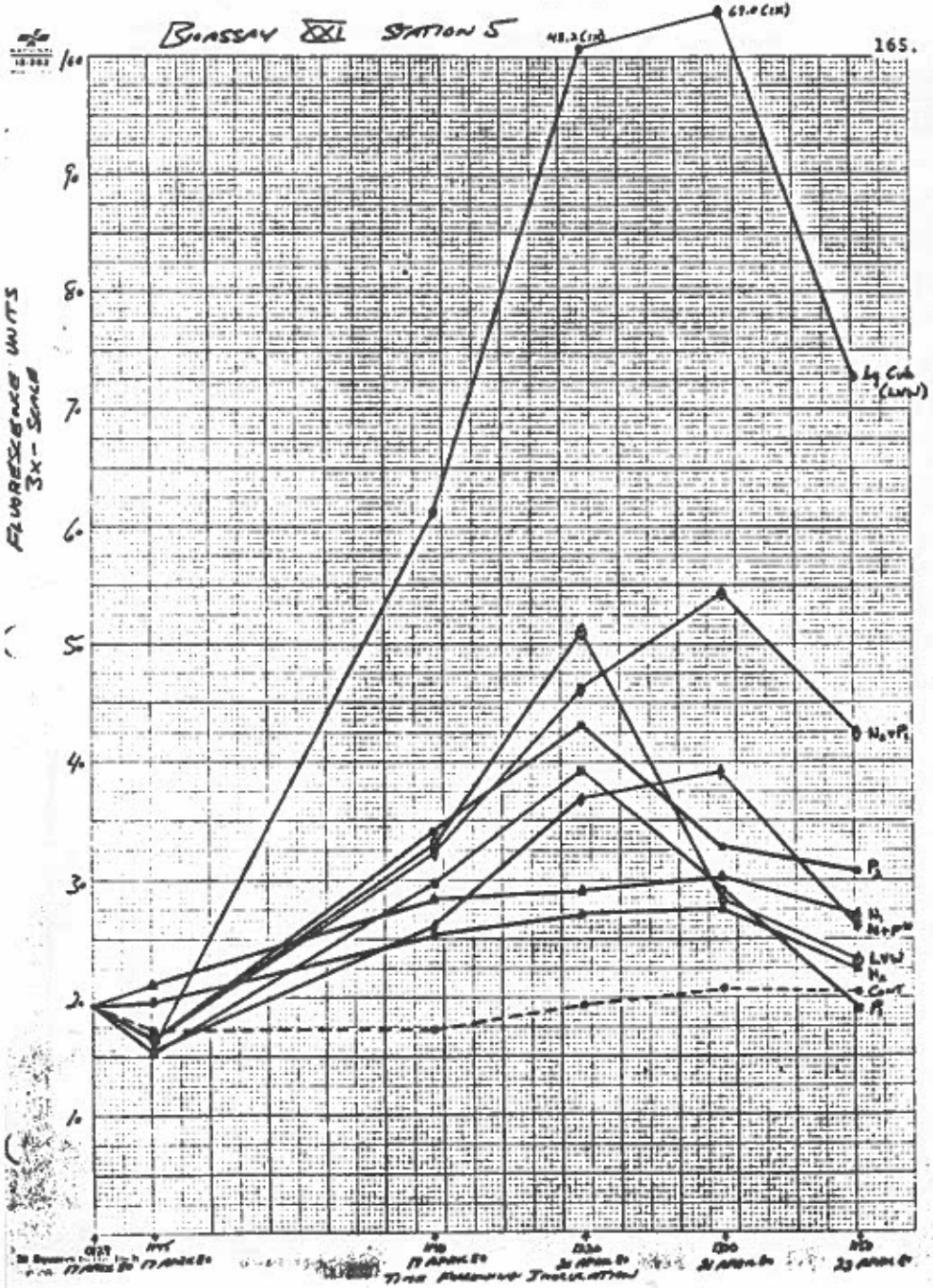


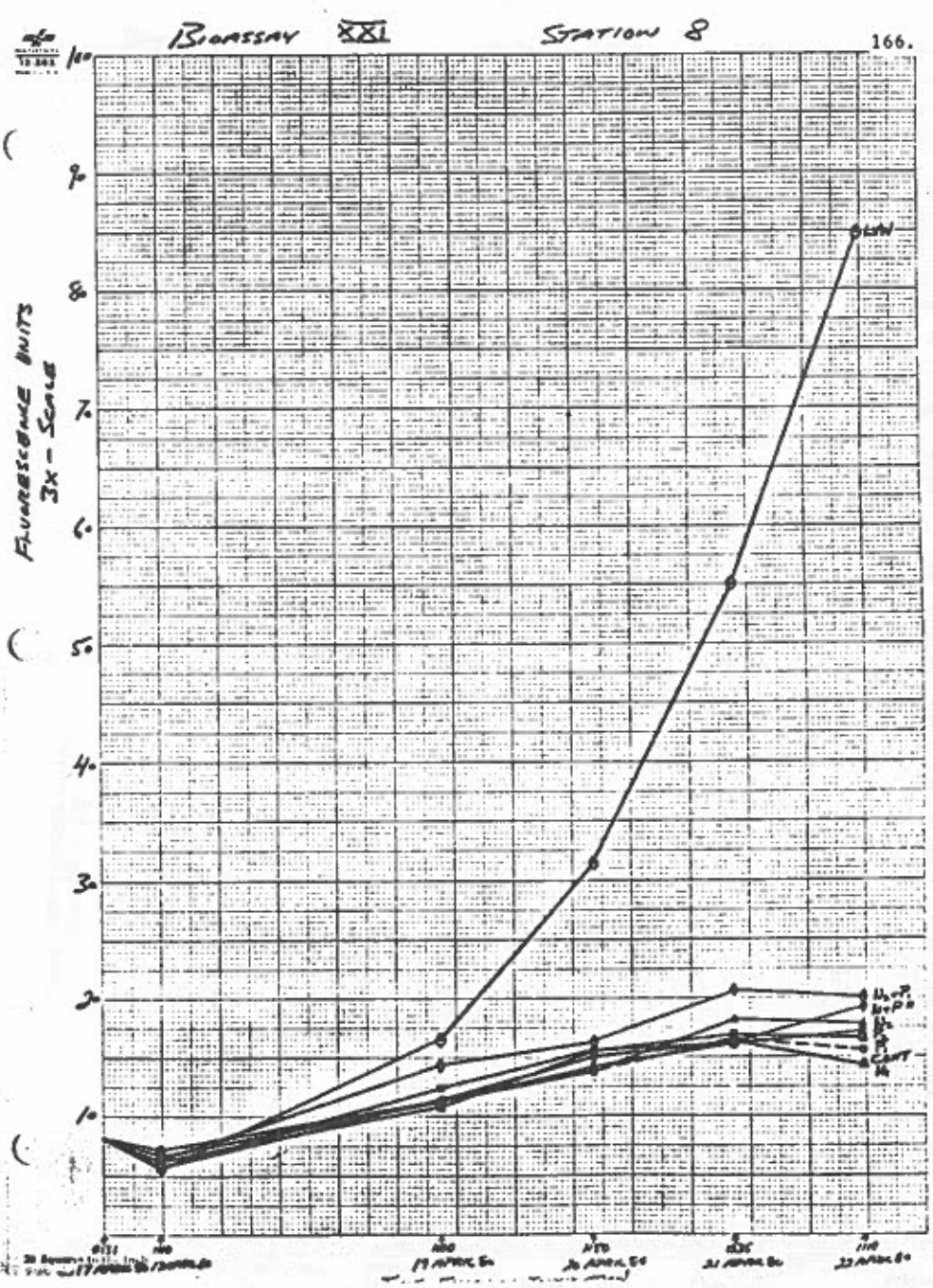
BIOASSAY XX — STATION 8

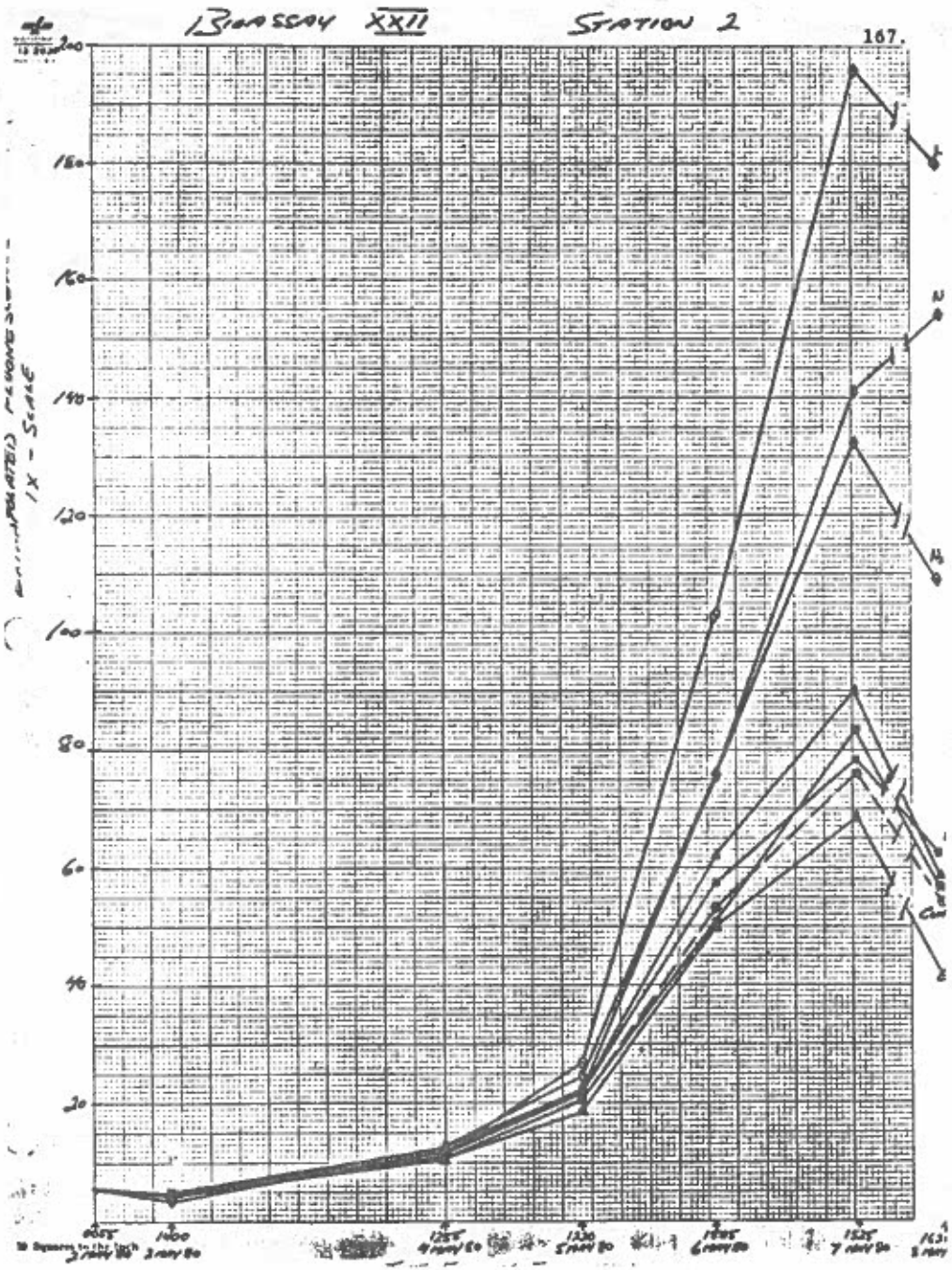


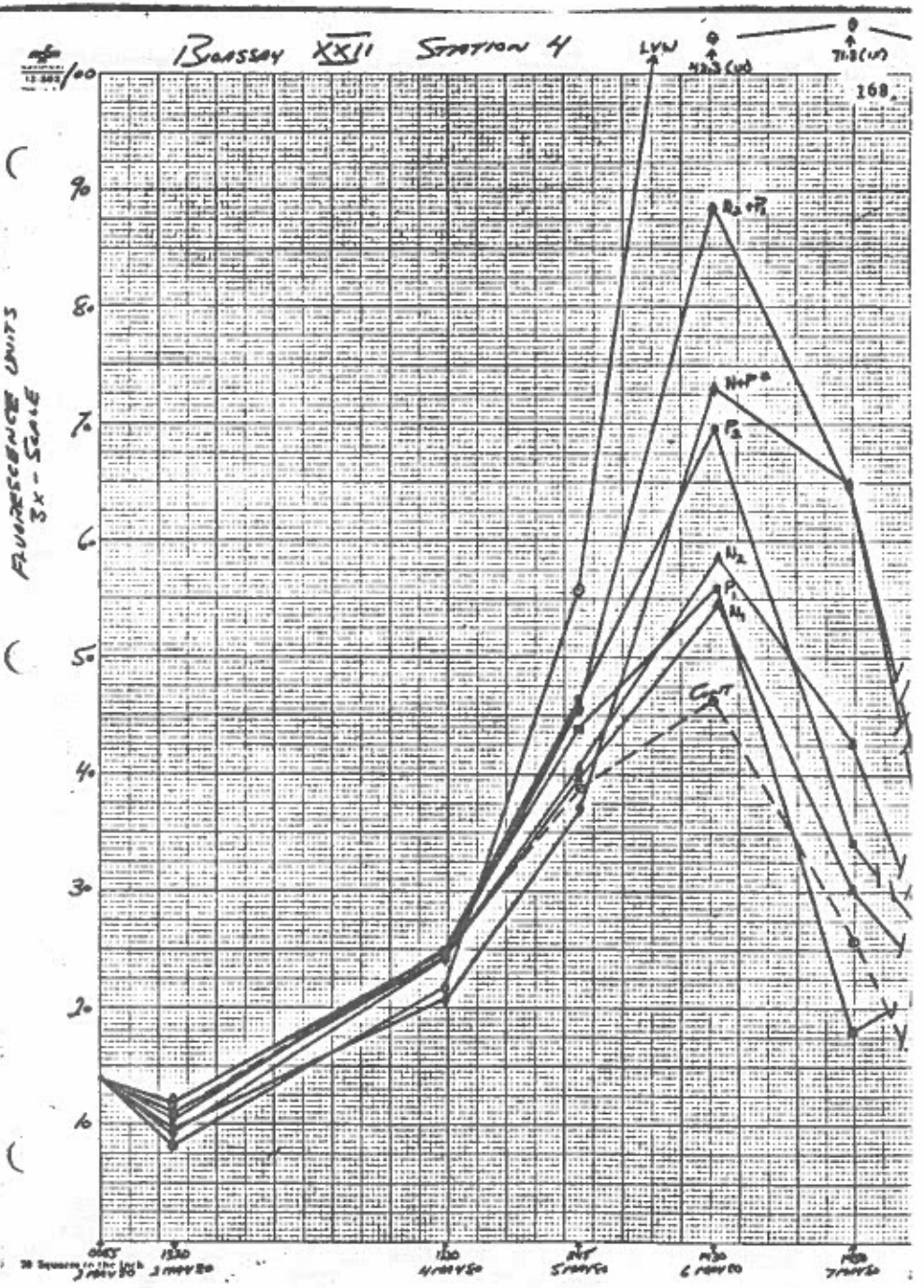

BIOASSAY XXI
STATION 2
163.
FLUORESCENCE UNITS
1X - SCALE
400
300
200
100
50
N2
LVW
N+P
N2+P1
P2
N1, P1
CONT
0125
1300
1310
1340
1440
1505
17 APRIL 80
19 APRIL 80
20 APRIL 80
21 APRIL 80
22 APRIL 80
TIME FOLLOWING INOCULATION

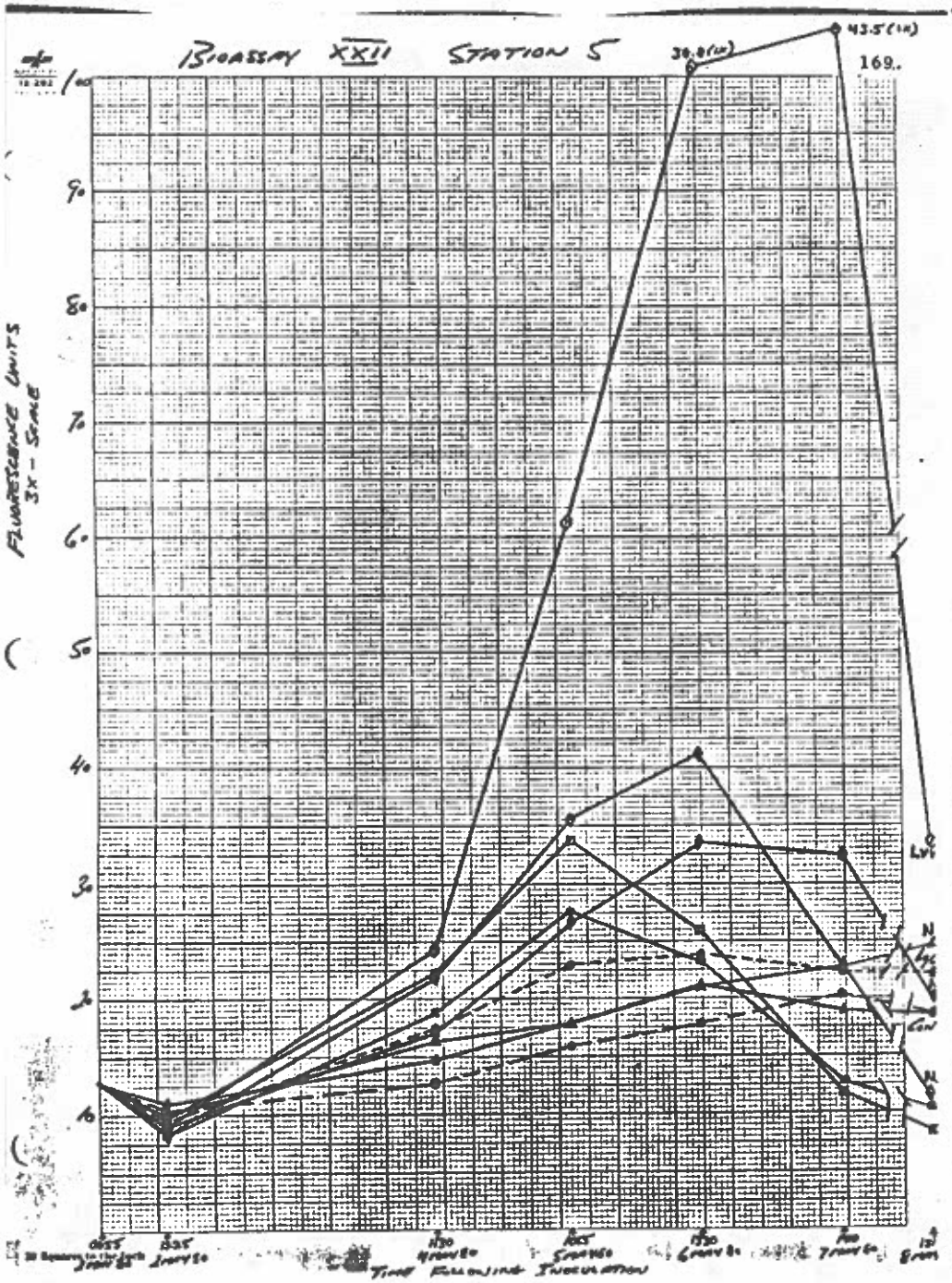

BIOASSAY XXI
STATION 4
164
FLUORESCENCE UNITS
3X - SCALE
100
90
80
70
60
50
40
30
20
10
N2, P
N+P
CONT
N2+P
N1
P1
0127
1230
1330
1310
1440
1505
17 APRIL 80
19 APRIL 80
20 APRIL 80
21 APRIL 80
22 APRIL 80
TIME FOLLOWING INOCULATION

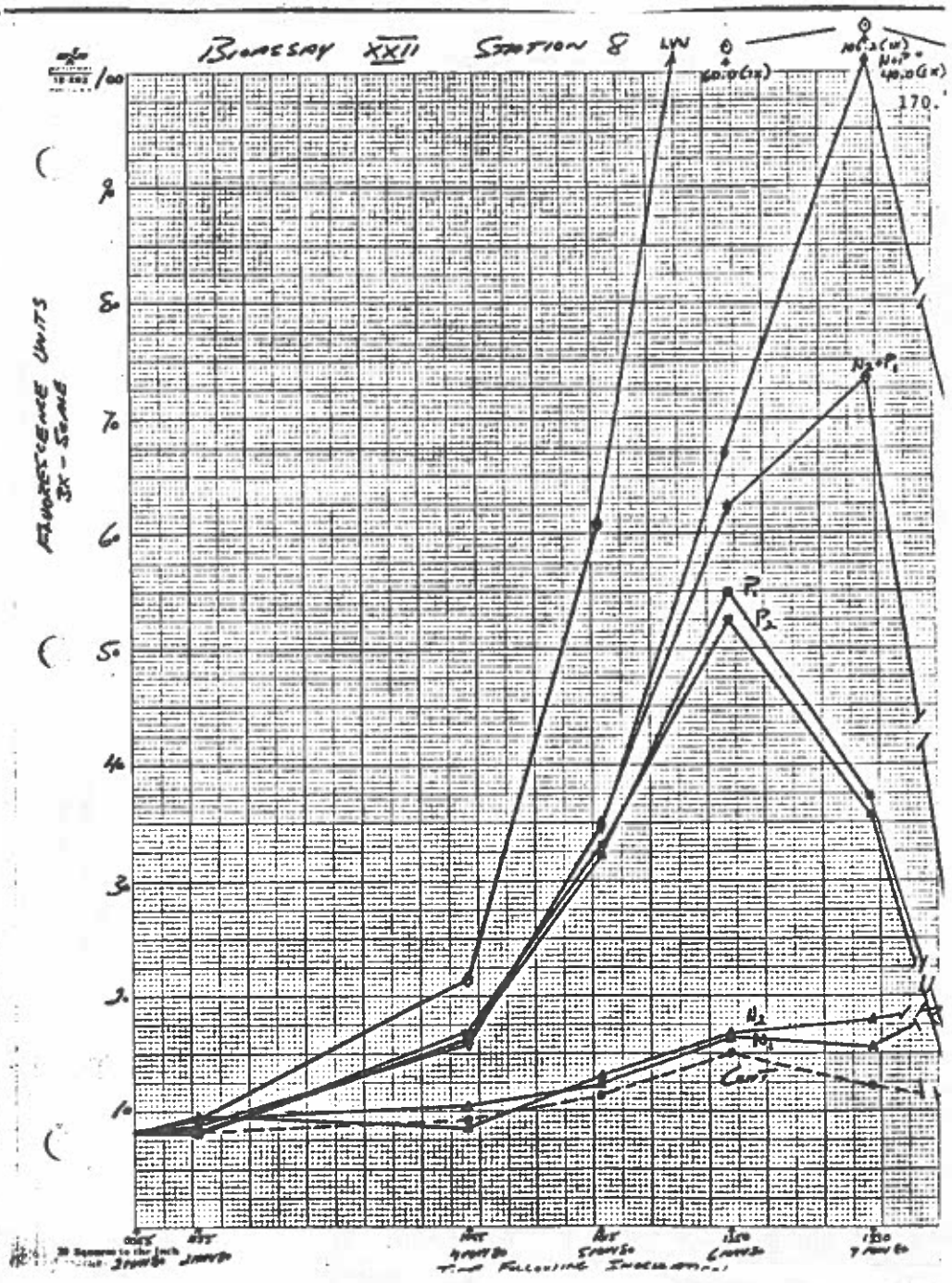

BIOASSAY XXI STATION 5
165.
FLUORESCENCE UNITS
3X - SCALE
100
90
80
70
60
50
40
30
20
10
48.2 (1X)
69.0 (1X)
Lg Cub (LVW)
N2+P1
P2
N1
N+P
LVW
N2
CONT
P1
TIME FOLLOWING INOCULATION

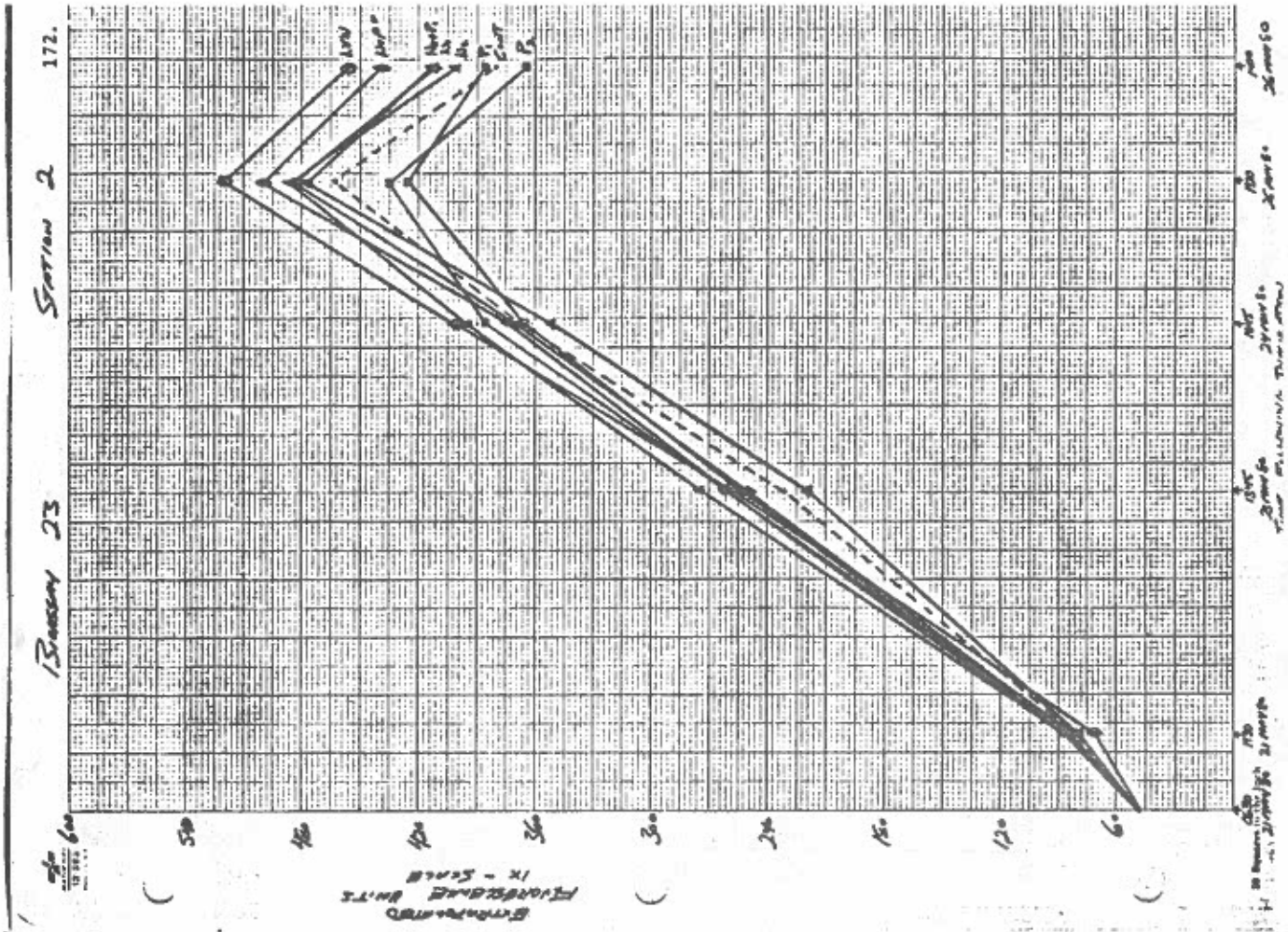

BIOASSAY XXI STATION 8
166.
FLUORESCENCE UNITS
3X - SCALE
100
90
80
70
60
50
40
30
20
10
LVW
N2+P1
N+P
N2
P2
P1
CONT
N1


BIOASSAY XXII
STATION 2
167.
FLUORESCENCE UNITS
1X - SCALE

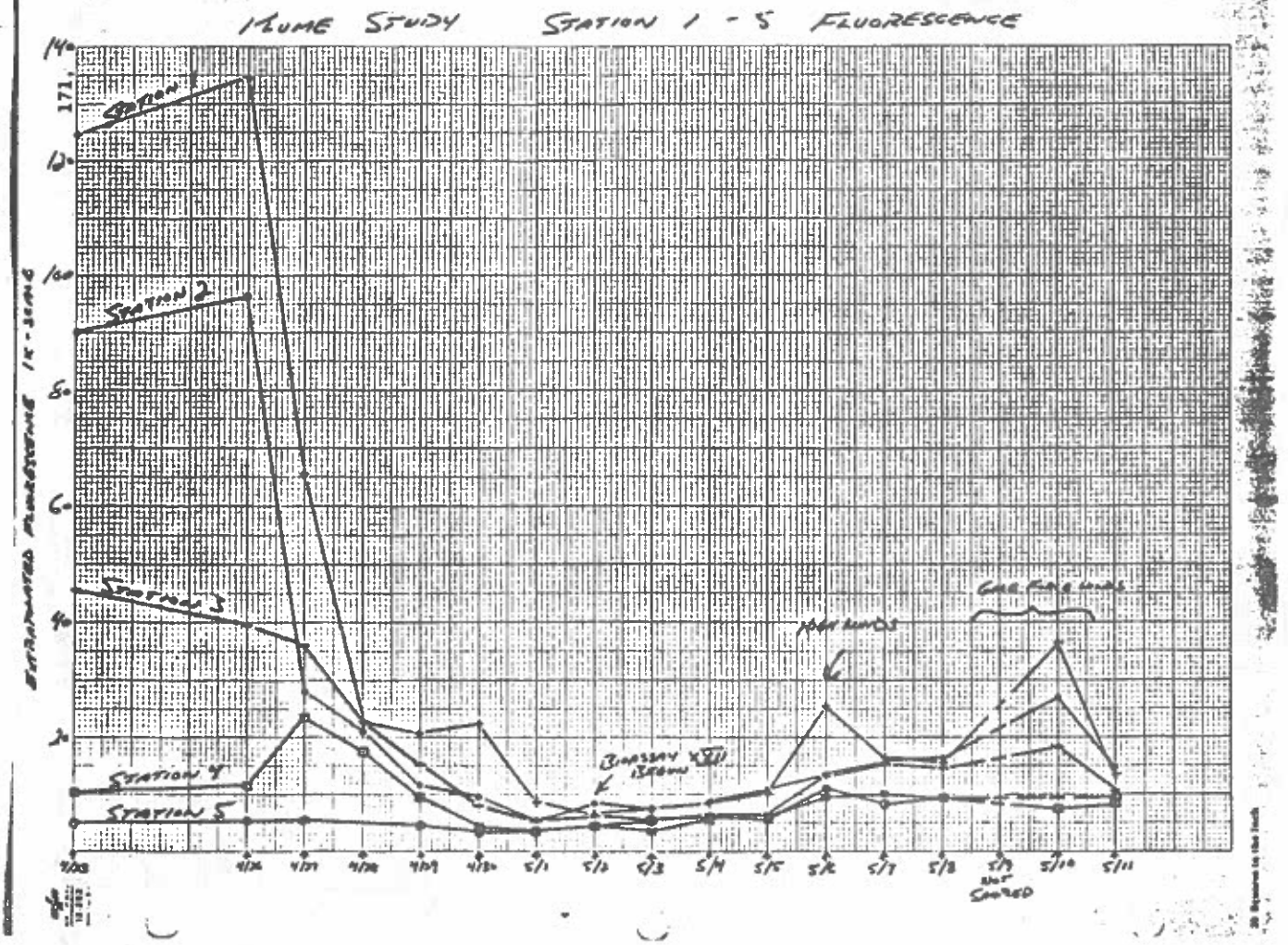

BIOASSAY XXII
STATION 4
168.
FLUORESCENCE UNITS
3X - SCALE


BIOASSAY XXII STATION 5
169.
FLUORESCENCE UNITS 3X - SCALE
TIME FOLLOWING INOCULATION
LVN
N
CON

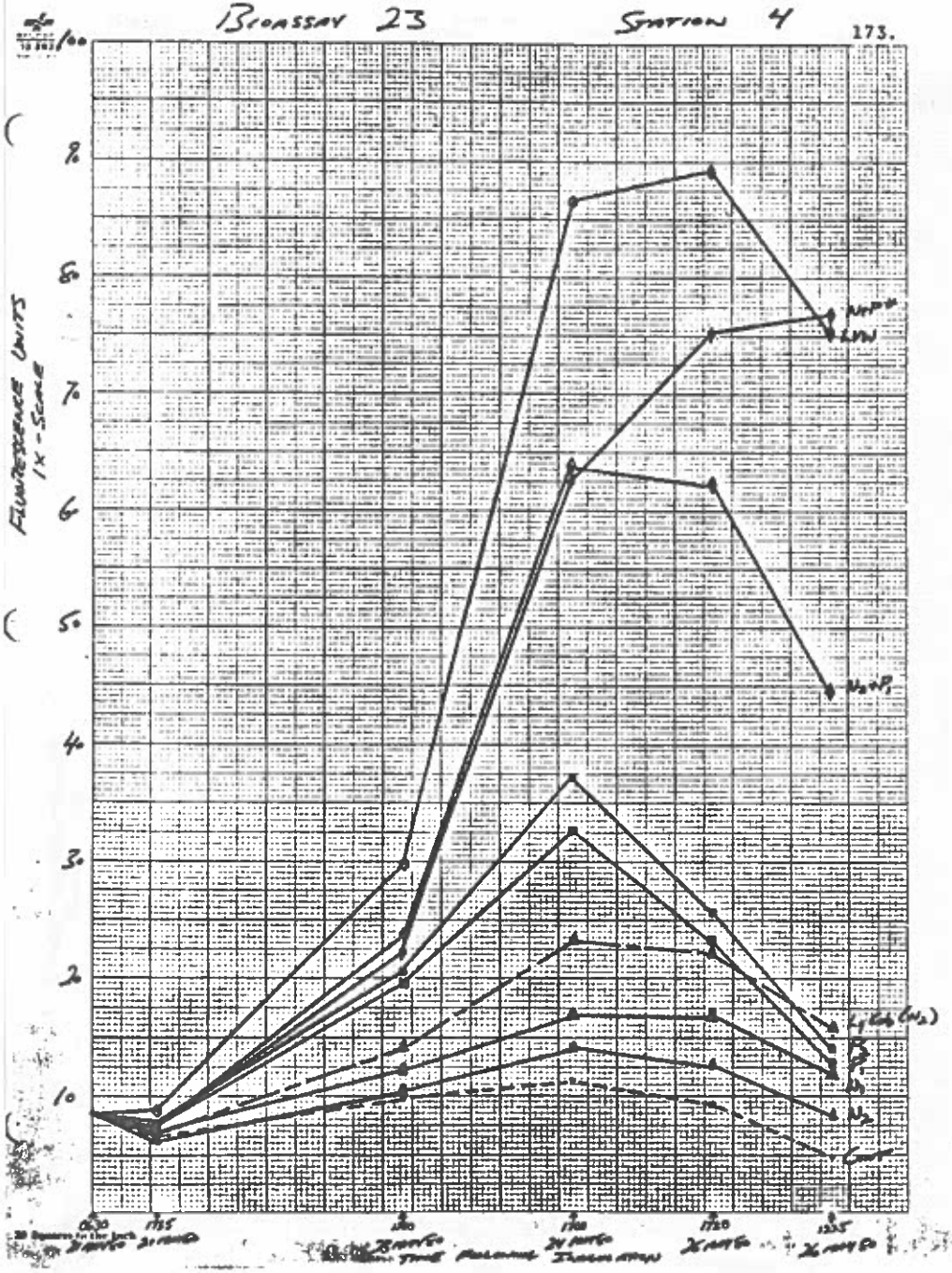

BIOASSAY XXII STATION 8
170.
FLUORESCENCE UNITS 3X - SCALE
TIME FOLLOWING INOCULATION
LVN
N2+P1
P1
P2
N2
N1
CONT



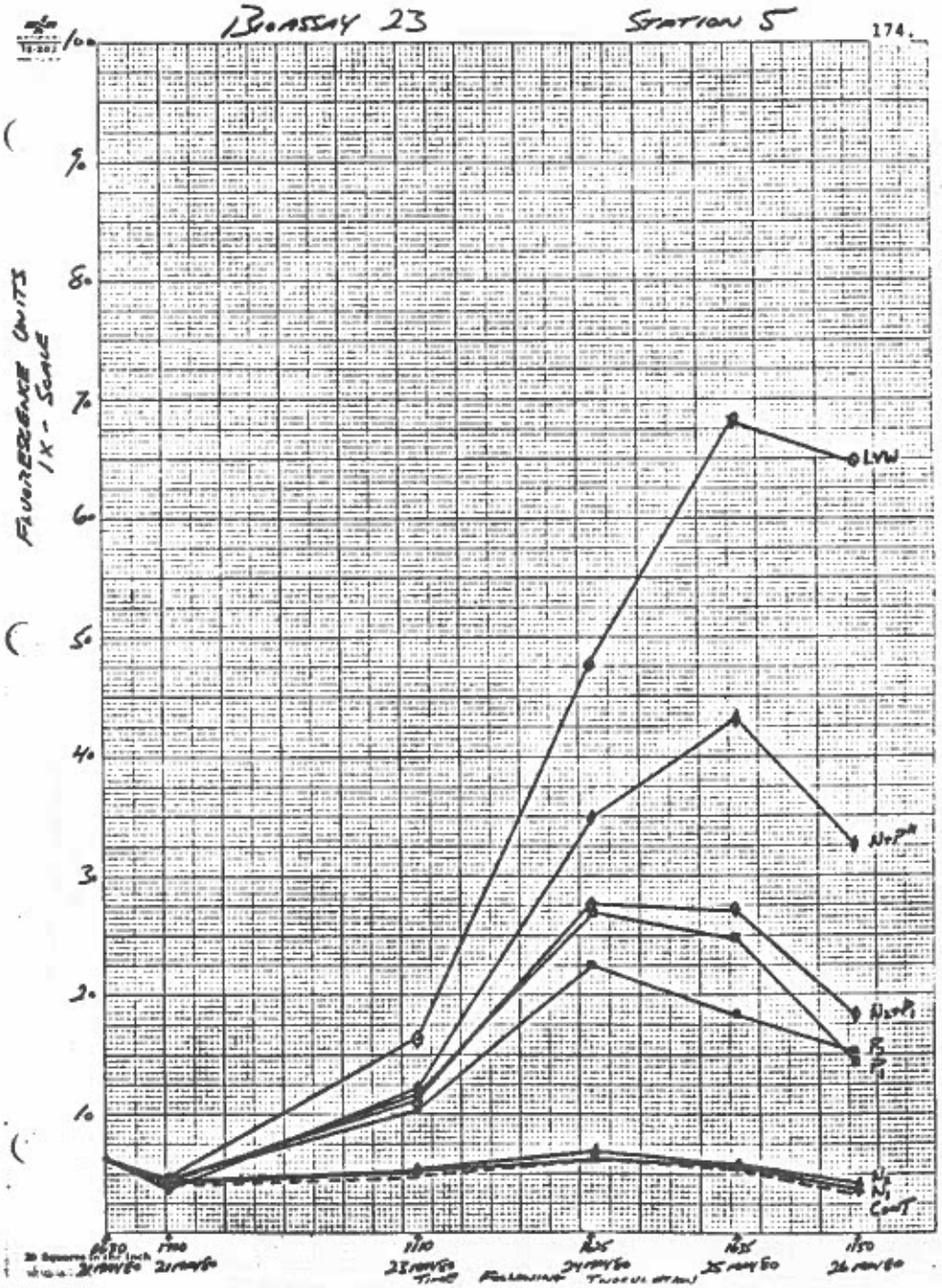
Plume Study Station 1 - 5 Fluorescence



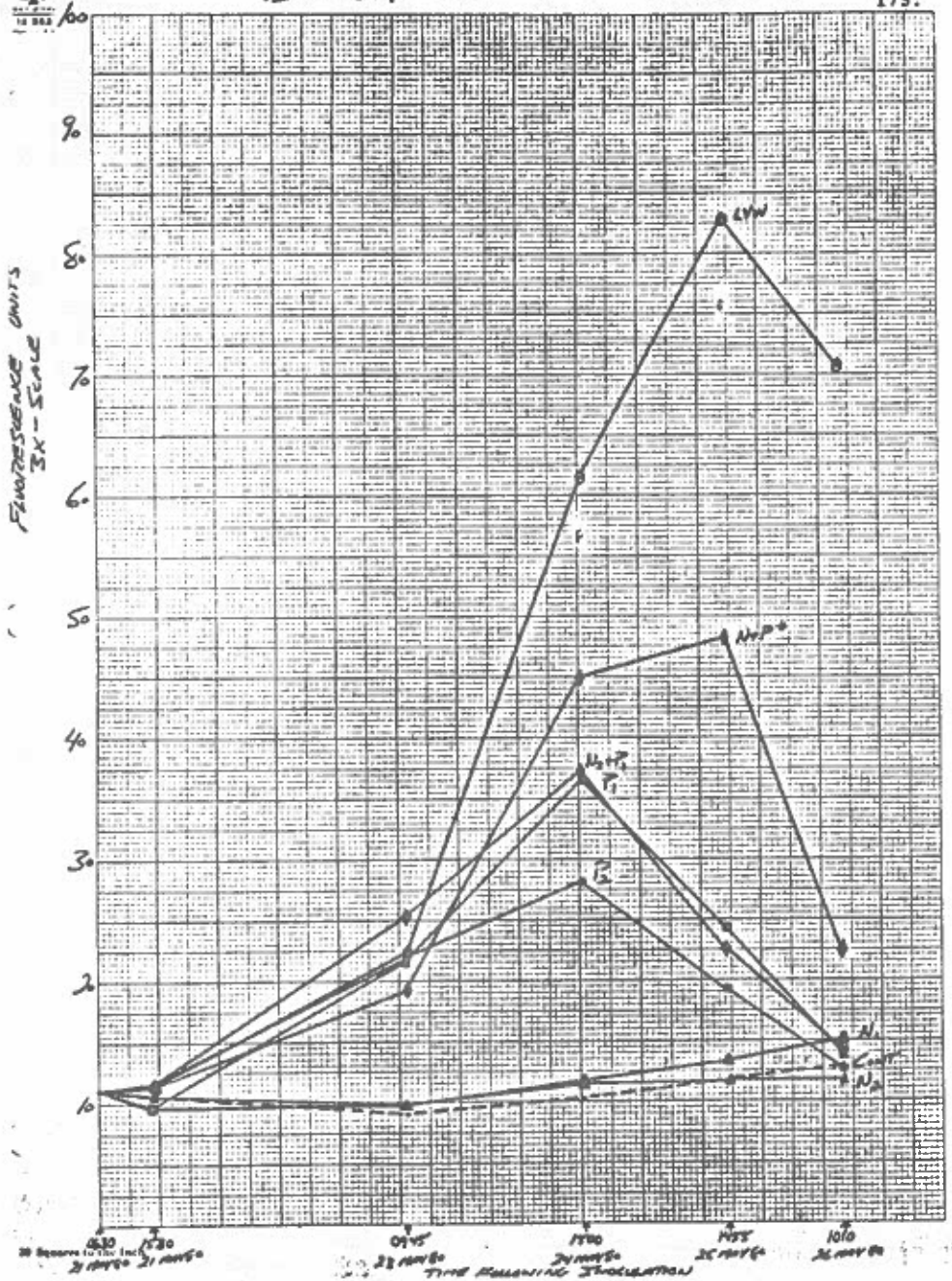
BIOASSAY 23 STATION 4



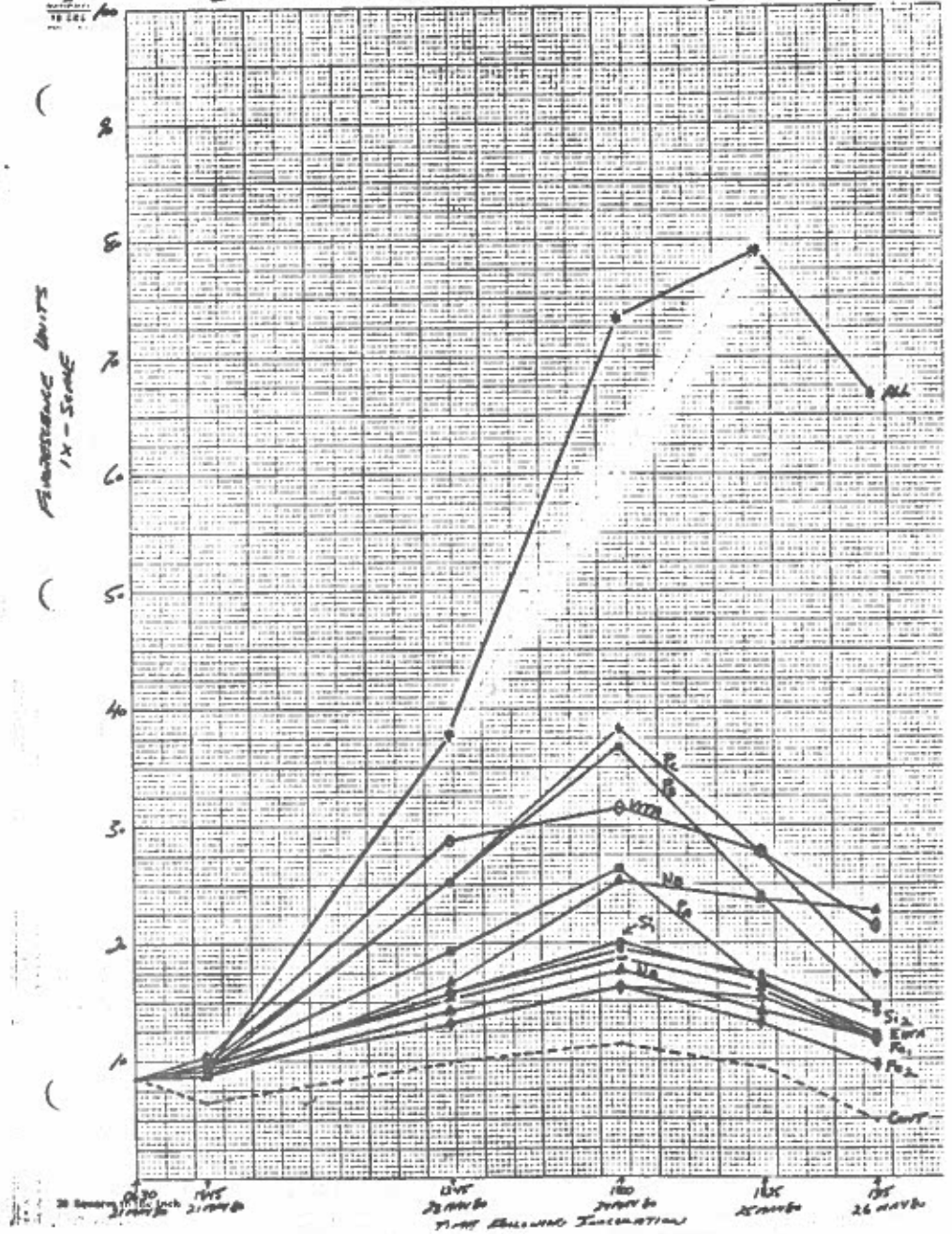
BIOASSAY 23 STATION 5

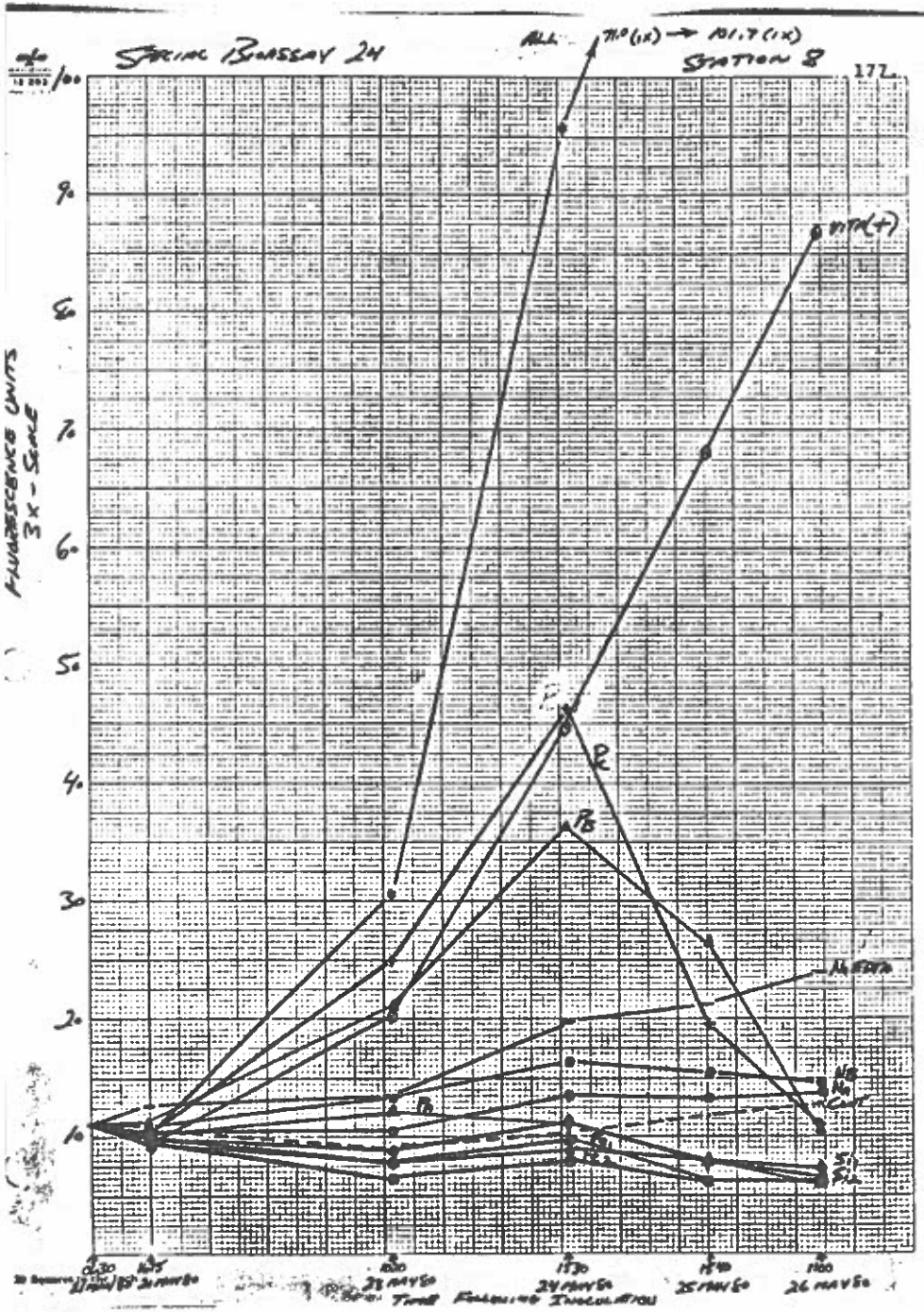


BIOASSAY 23 STATION 8

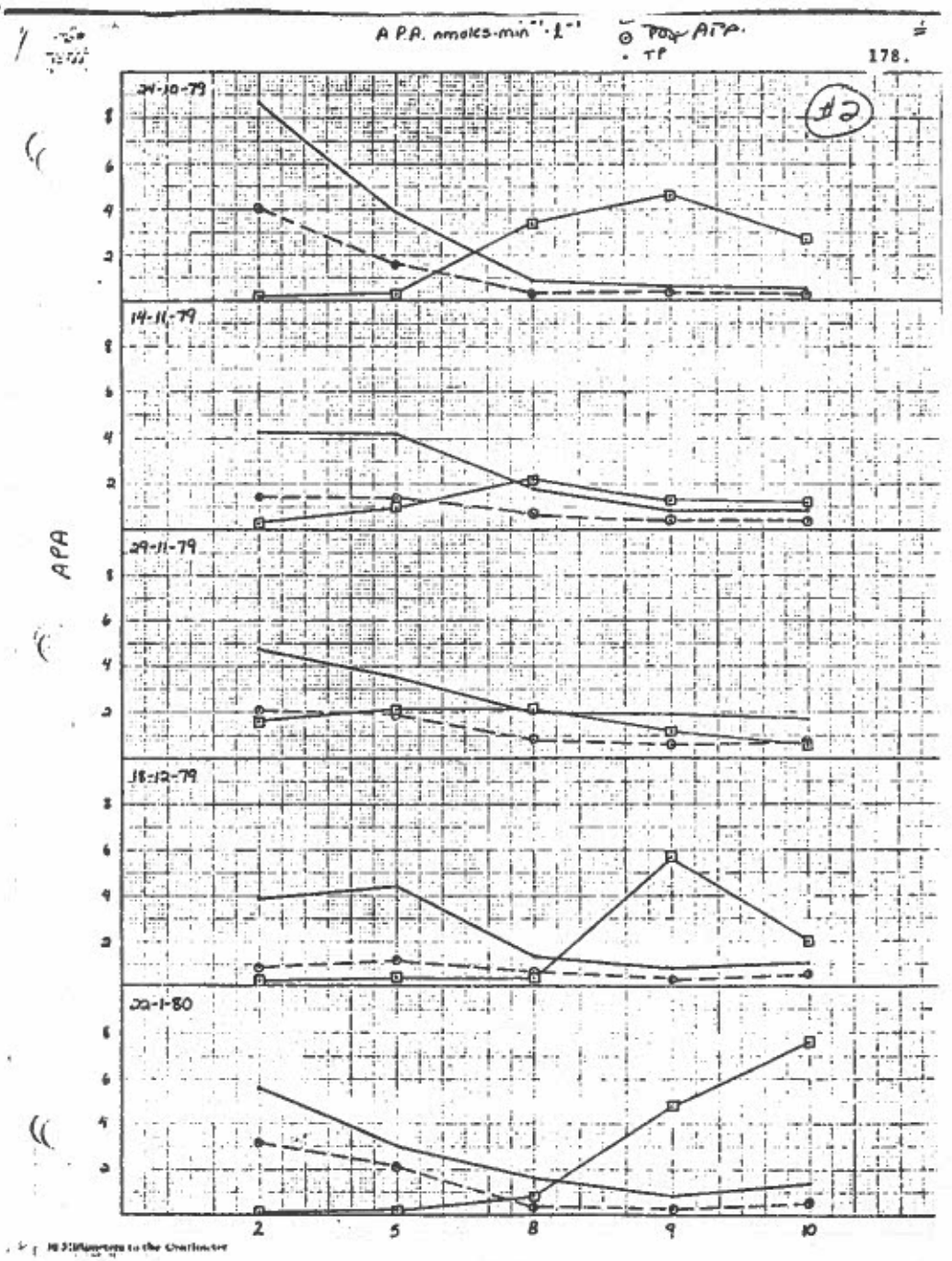


SPECIAL BIOASSAY 24 STATION 4

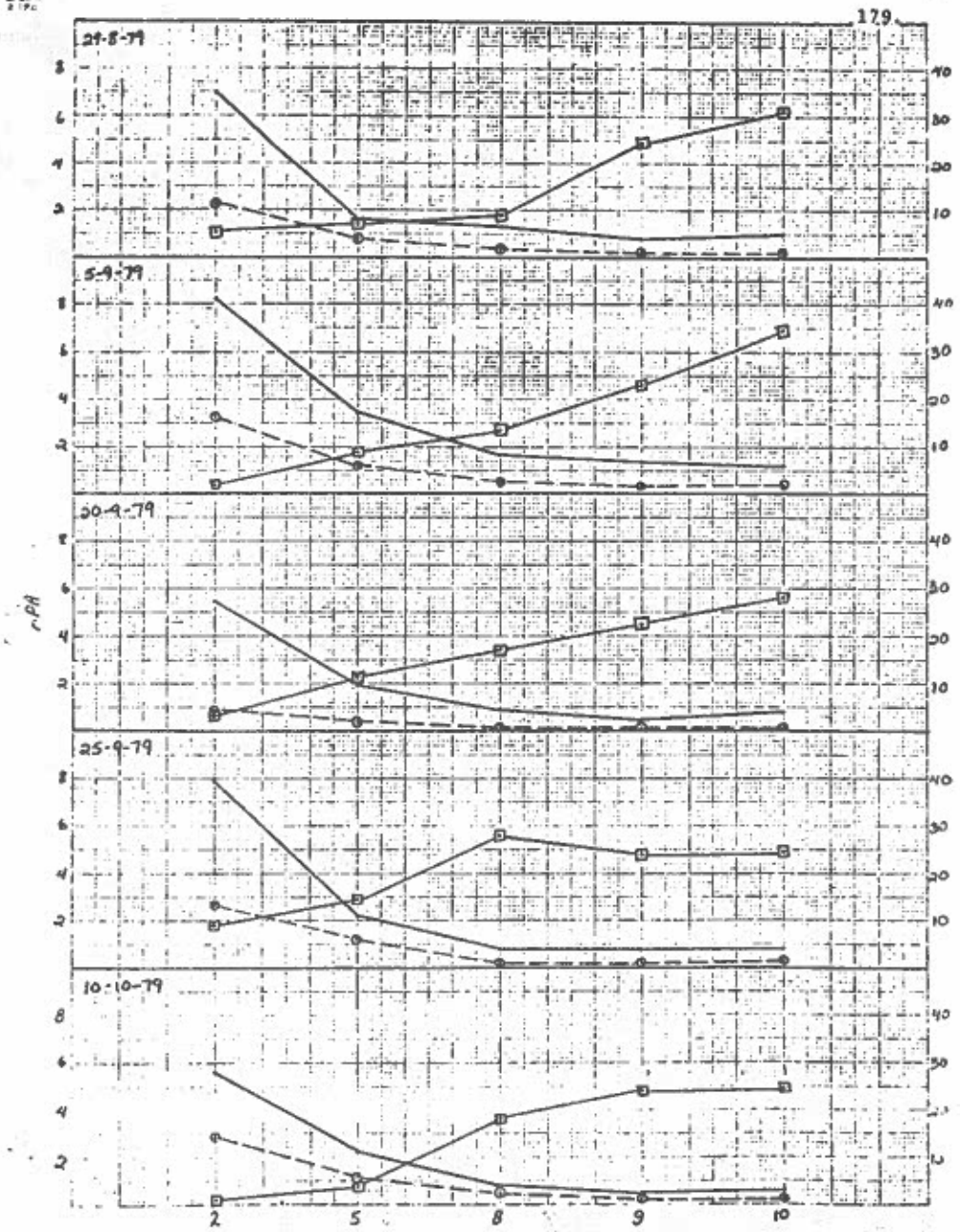


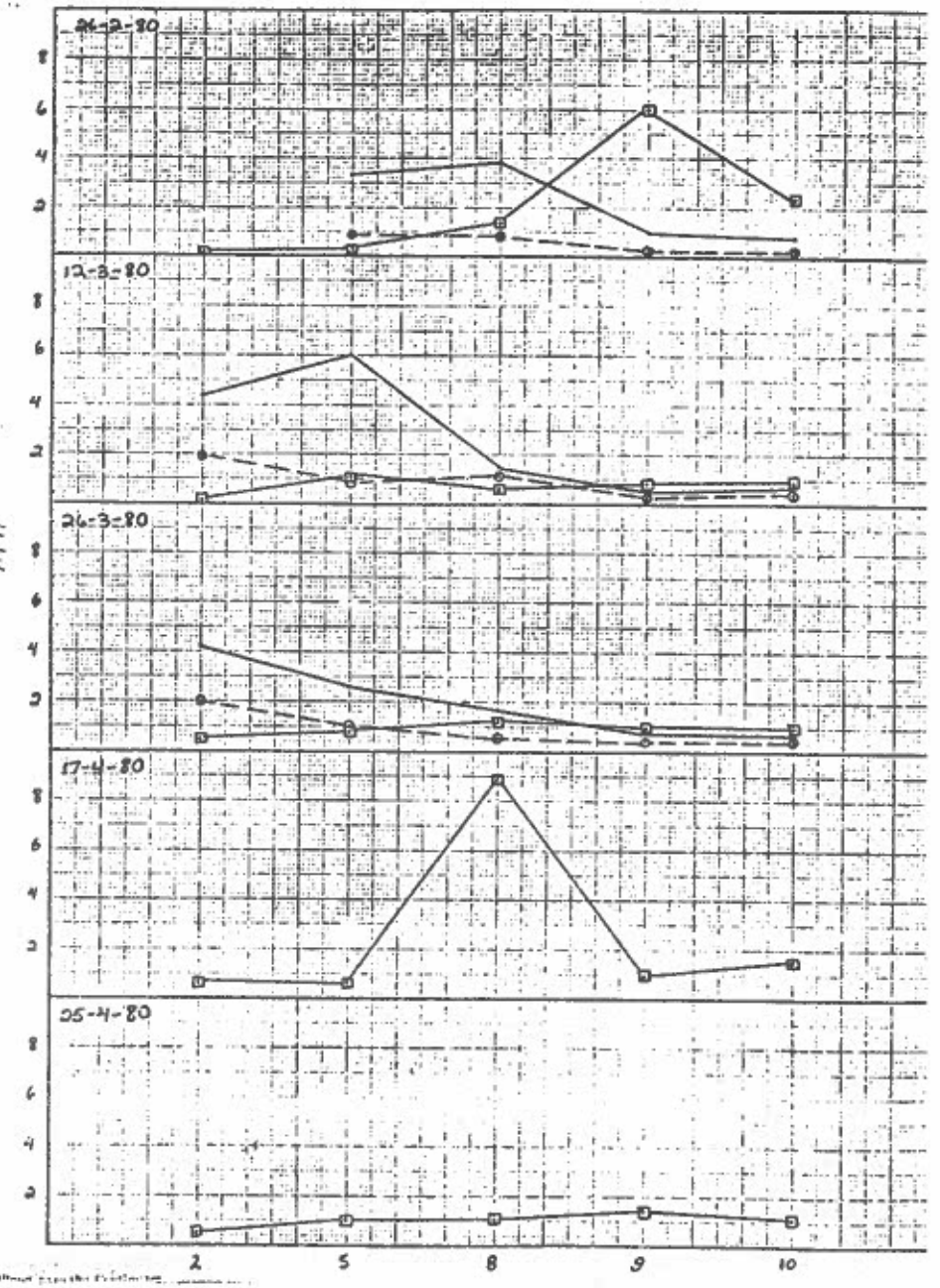


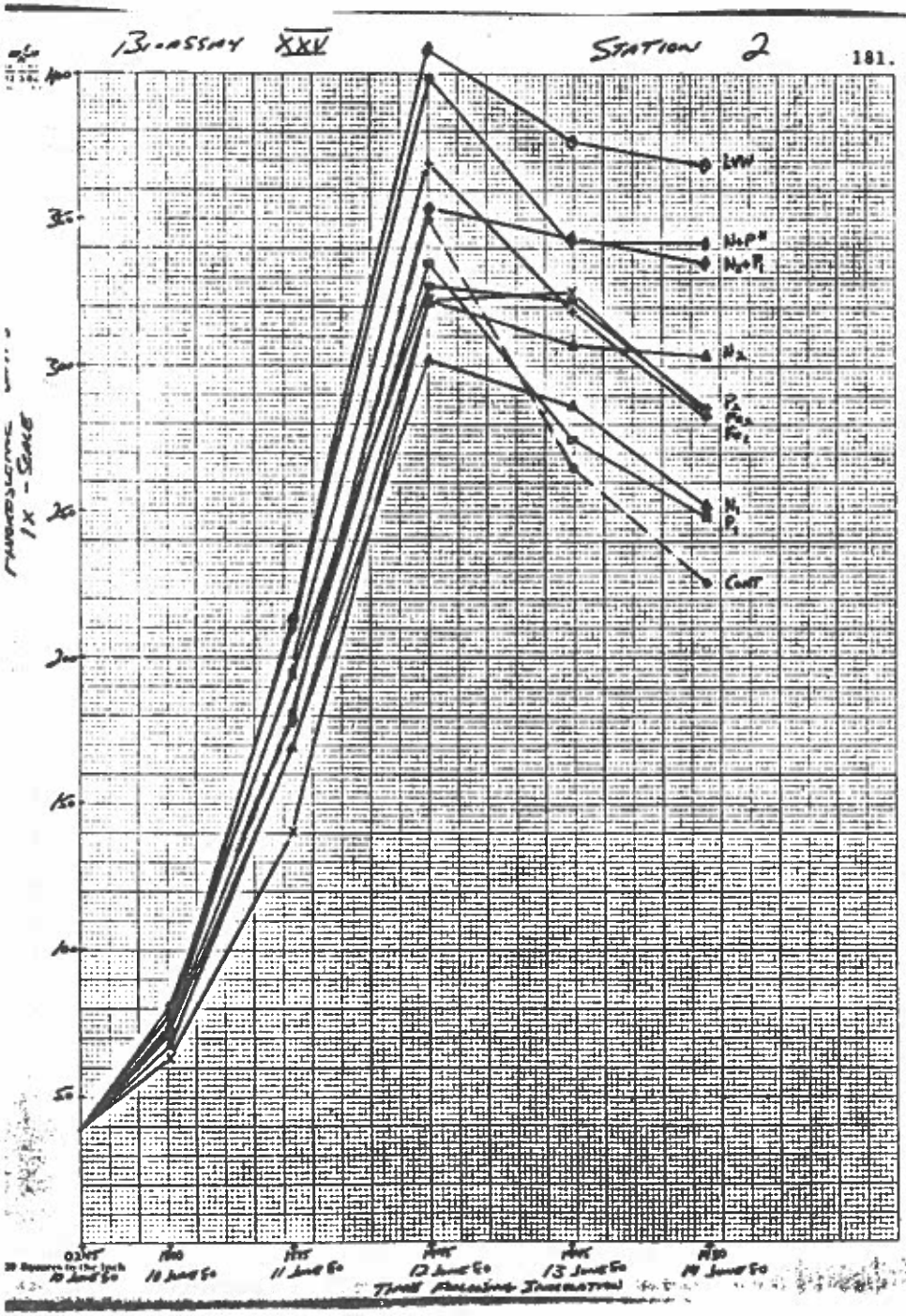
Special Bioassay 24
Station 8
177.
Fluorescence Units 3x - Scale
Time Following Inoculation

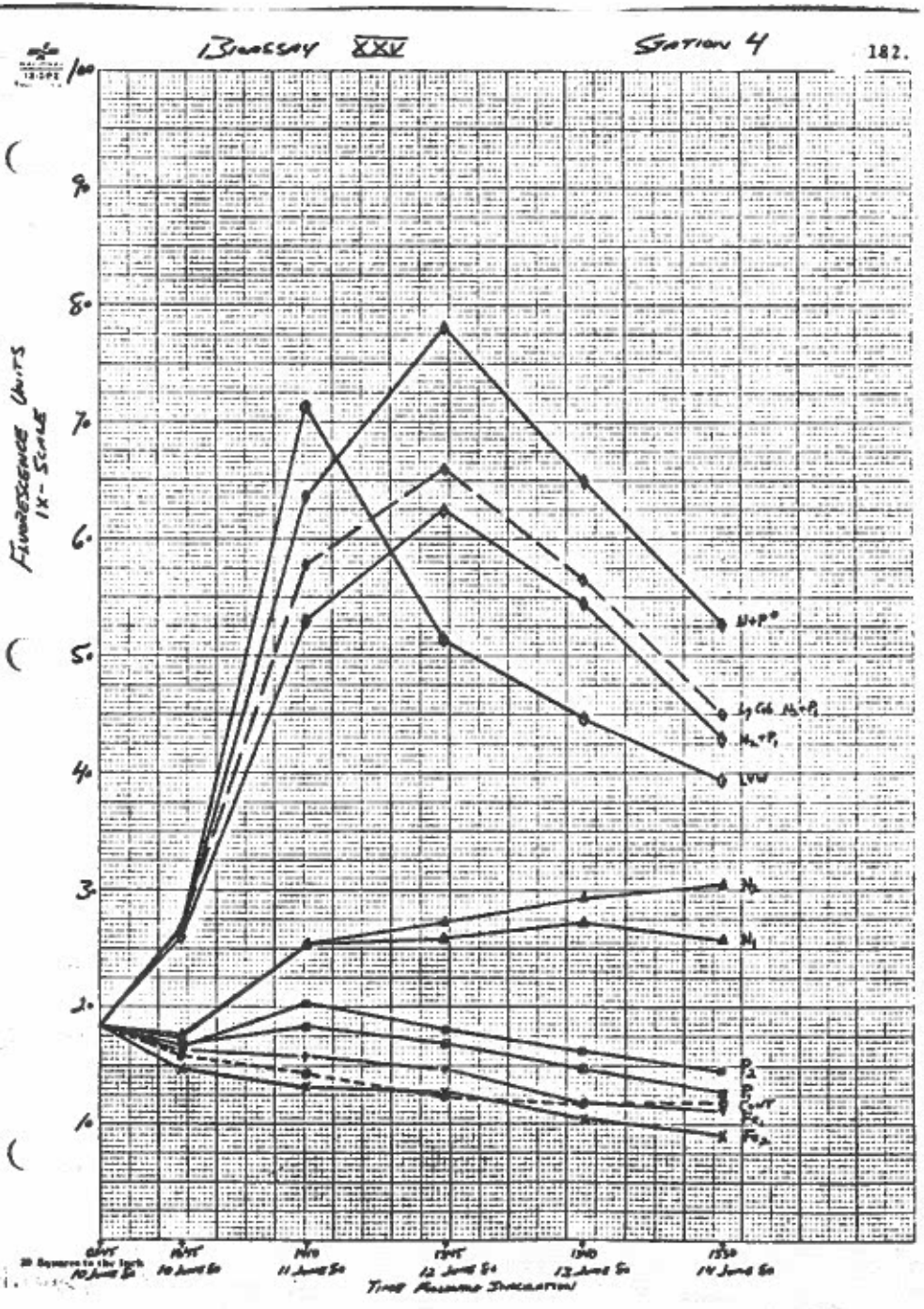


A.P.A. nmoles·min⁻¹·l⁻¹
178.
24-10-79
14-11-79
29-11-79
18-12-79
22-1-80
APA

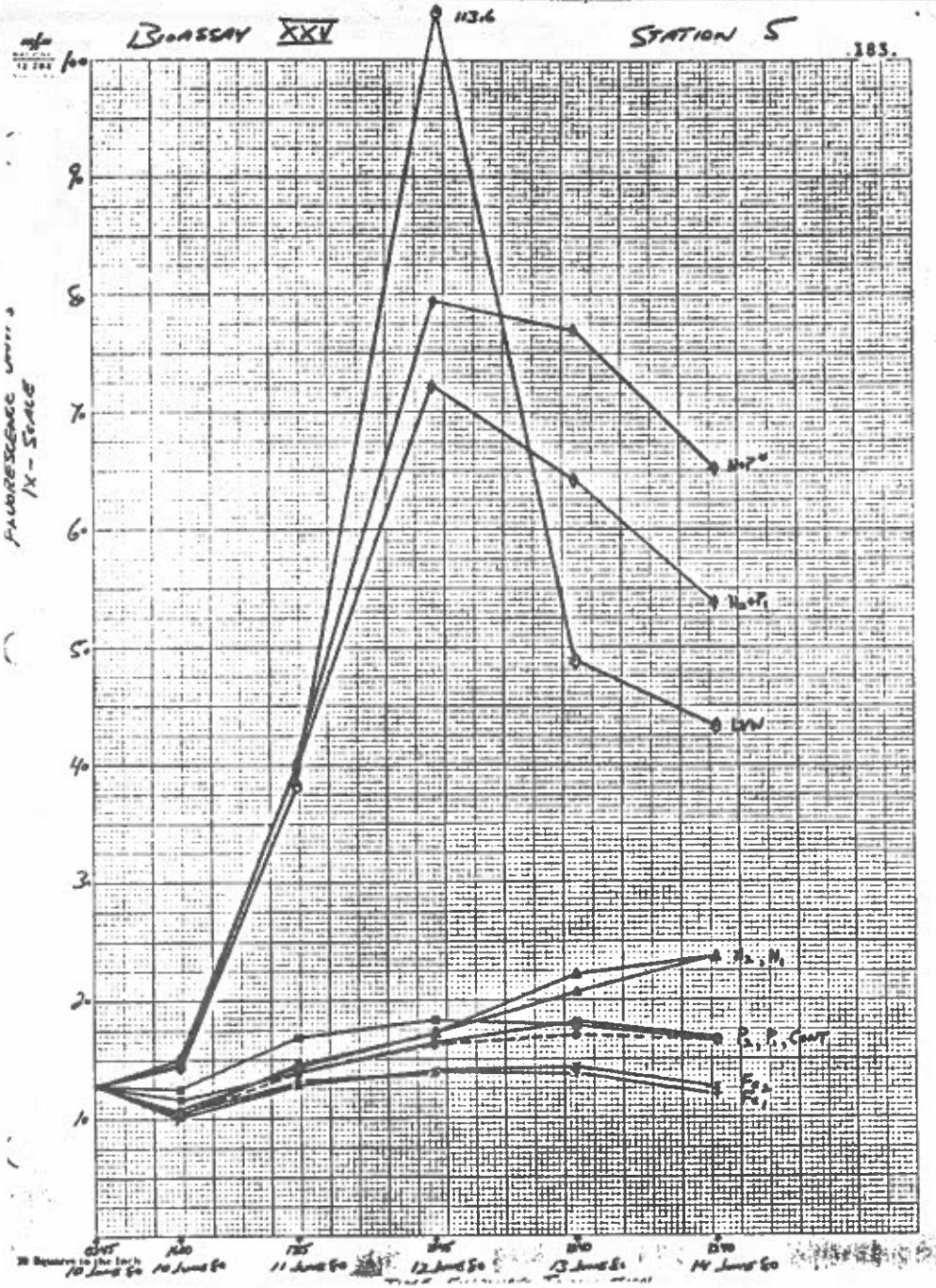

24-8-79
5-9-79
20-9-79
25-9-79
10-10-79
APA
2
5
8
9
10


24-2-80
12-3-80
26-3-80
17-4-80
25-4-80
APA
2
5
8
9
10

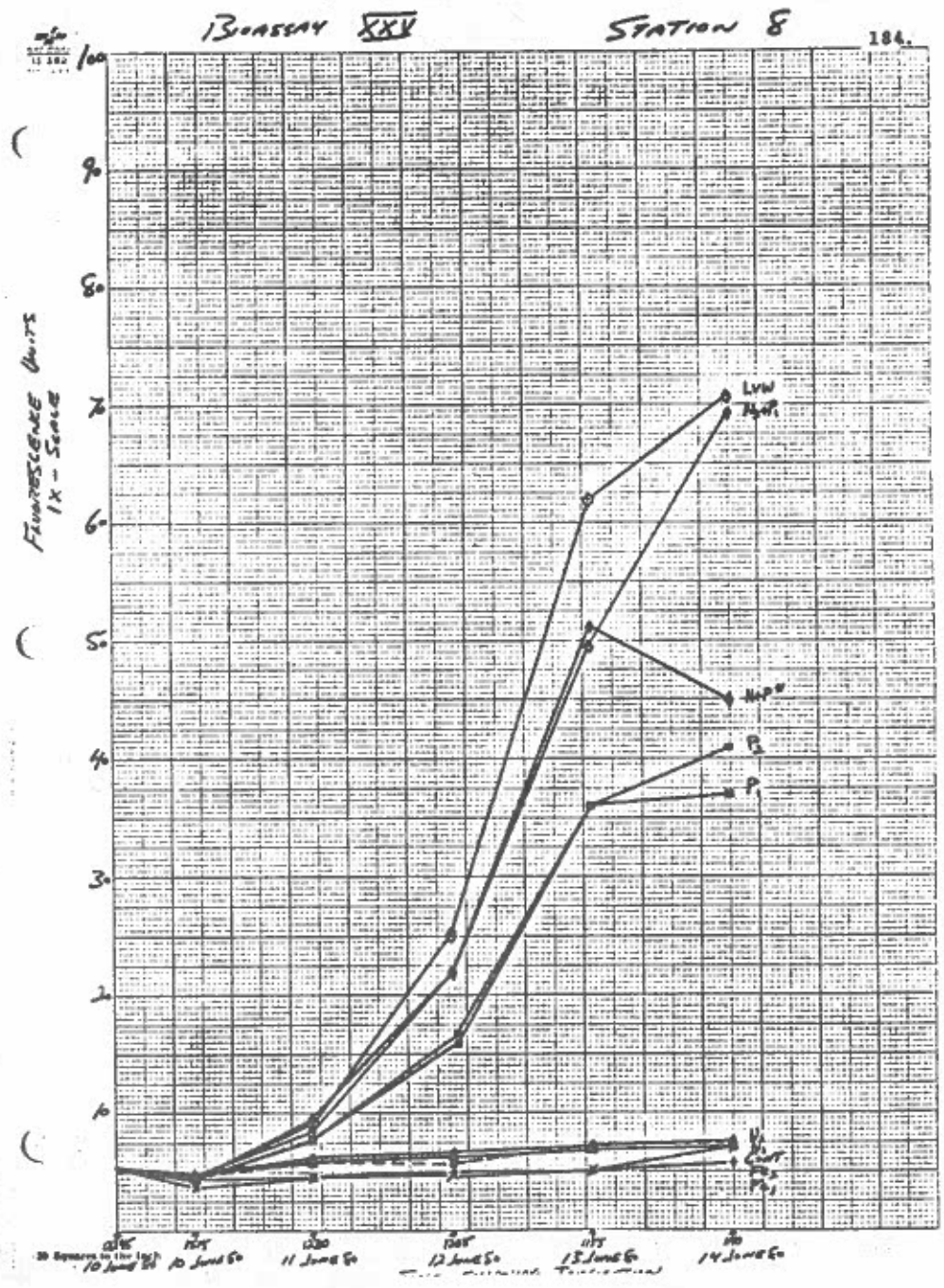

BIOASSAY XXV
STATION 2
181.
FLUORESCENCE UNITS 1X - SCALE
LVW
N+P*
N2+P1
N2
P2
Fe2
Fe1
N1
P1
CONT
TIME FOLLOWING INOCULATION


BIOASSAY XXV
STATION 4
182.
FLUORESCENCE UNITS 1X - SCALE
N+P*
Lg Cob N2+P1
N2+P1
LVW
N2
N1
P2
P1
CONT
Fe1
Fe2
TIME FOLLOWING INOCULATION

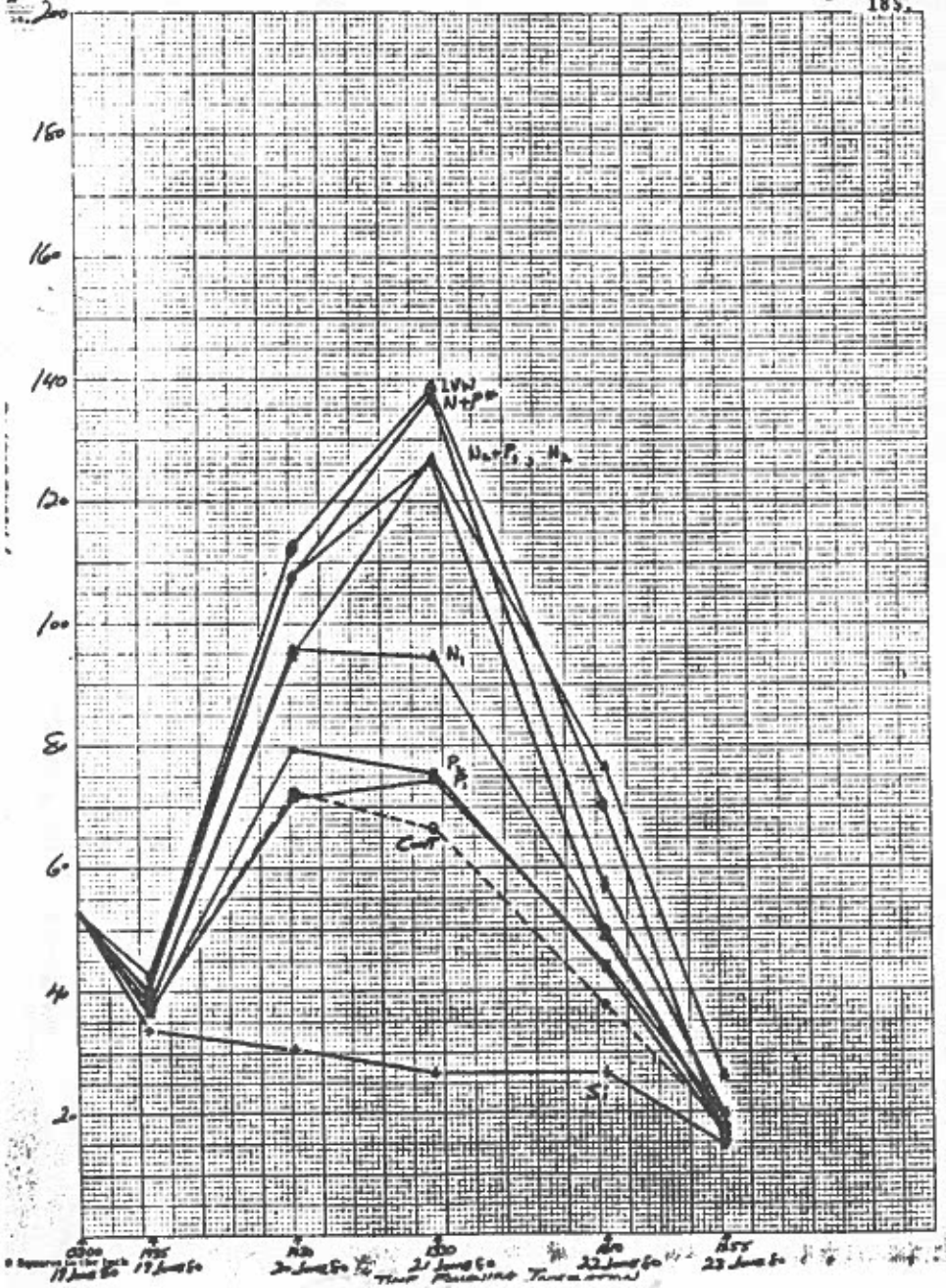
BIOASSAY XXV — STATION 5



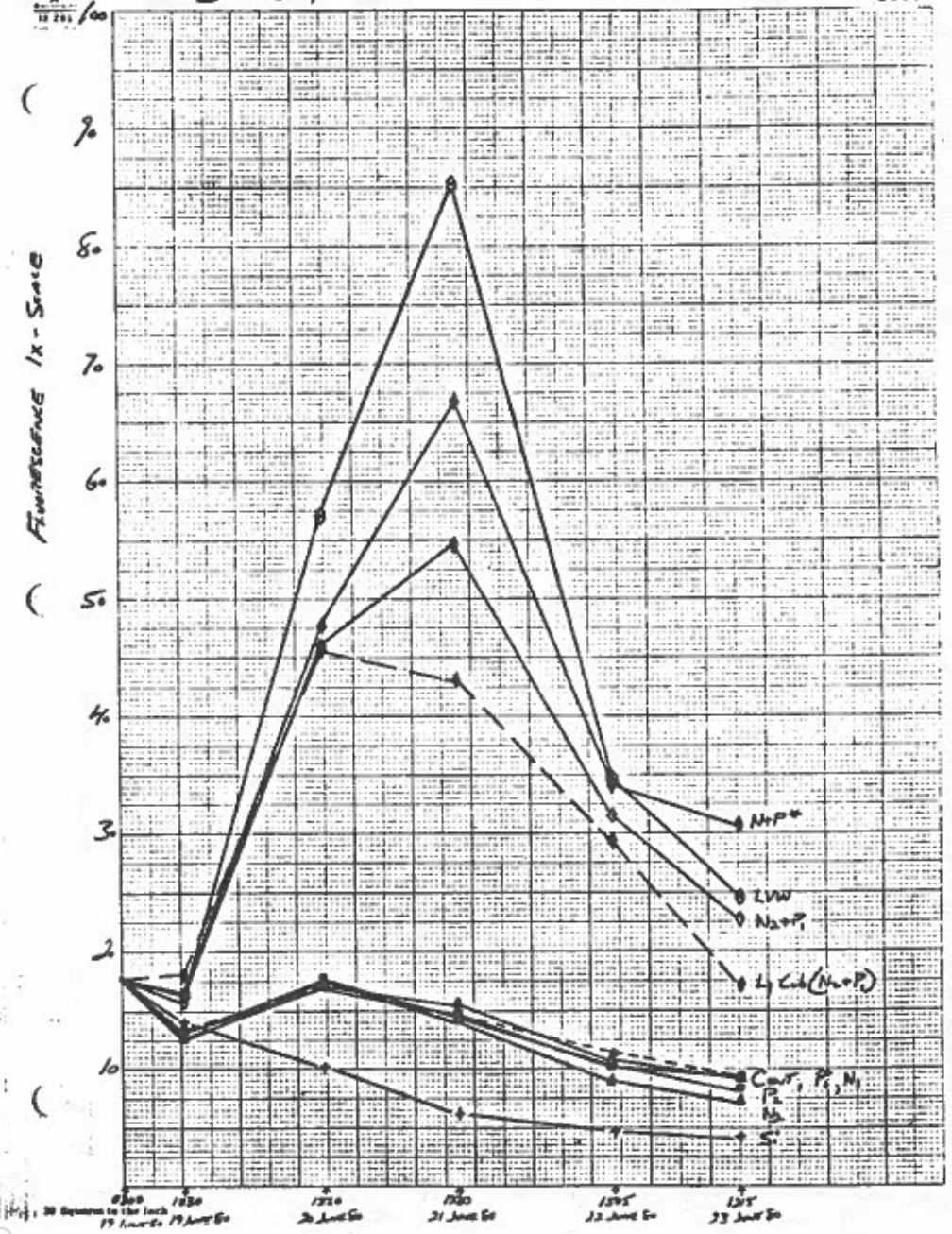
BIOASSAY XXV — STATION 8



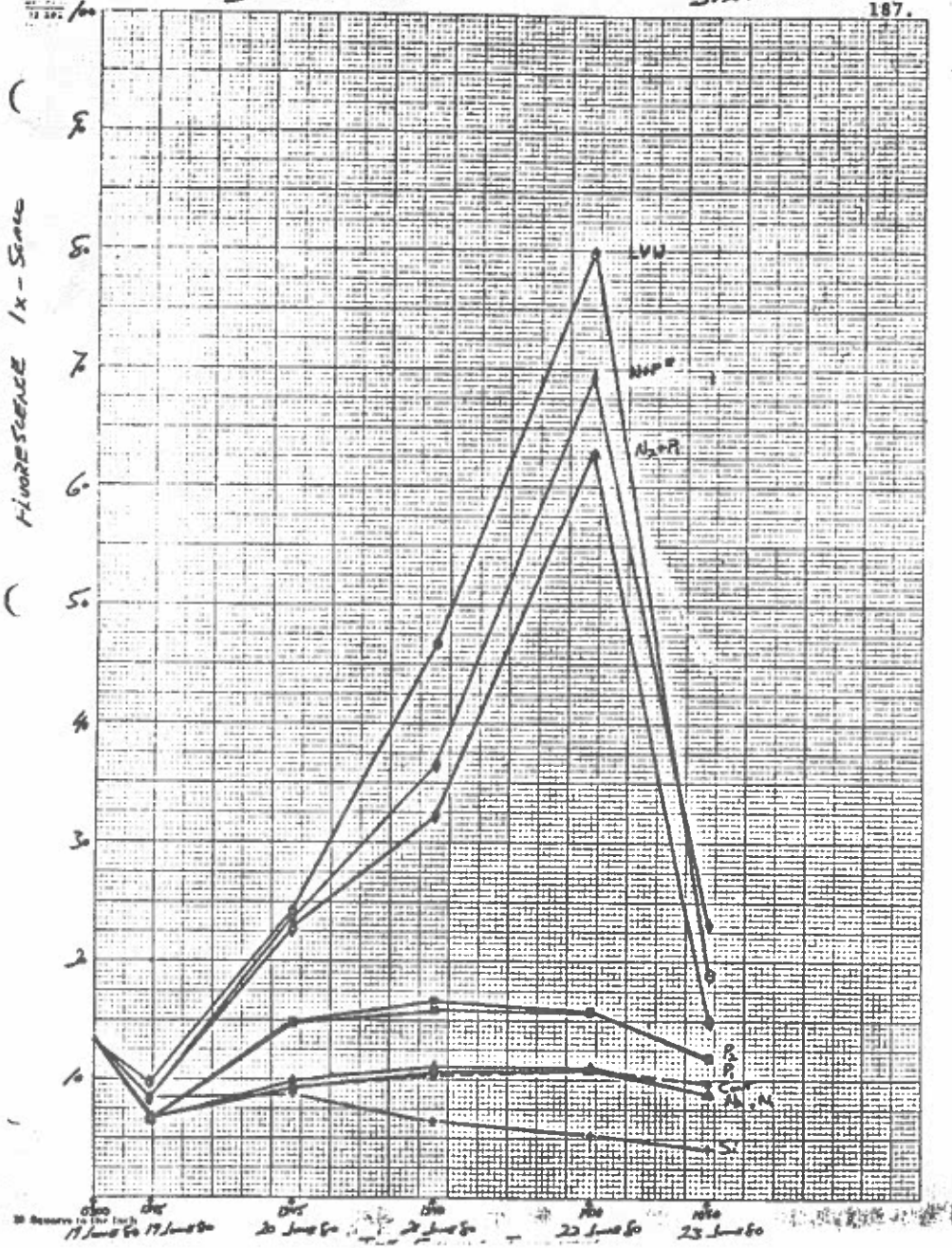
Bombay 26 — Station 2



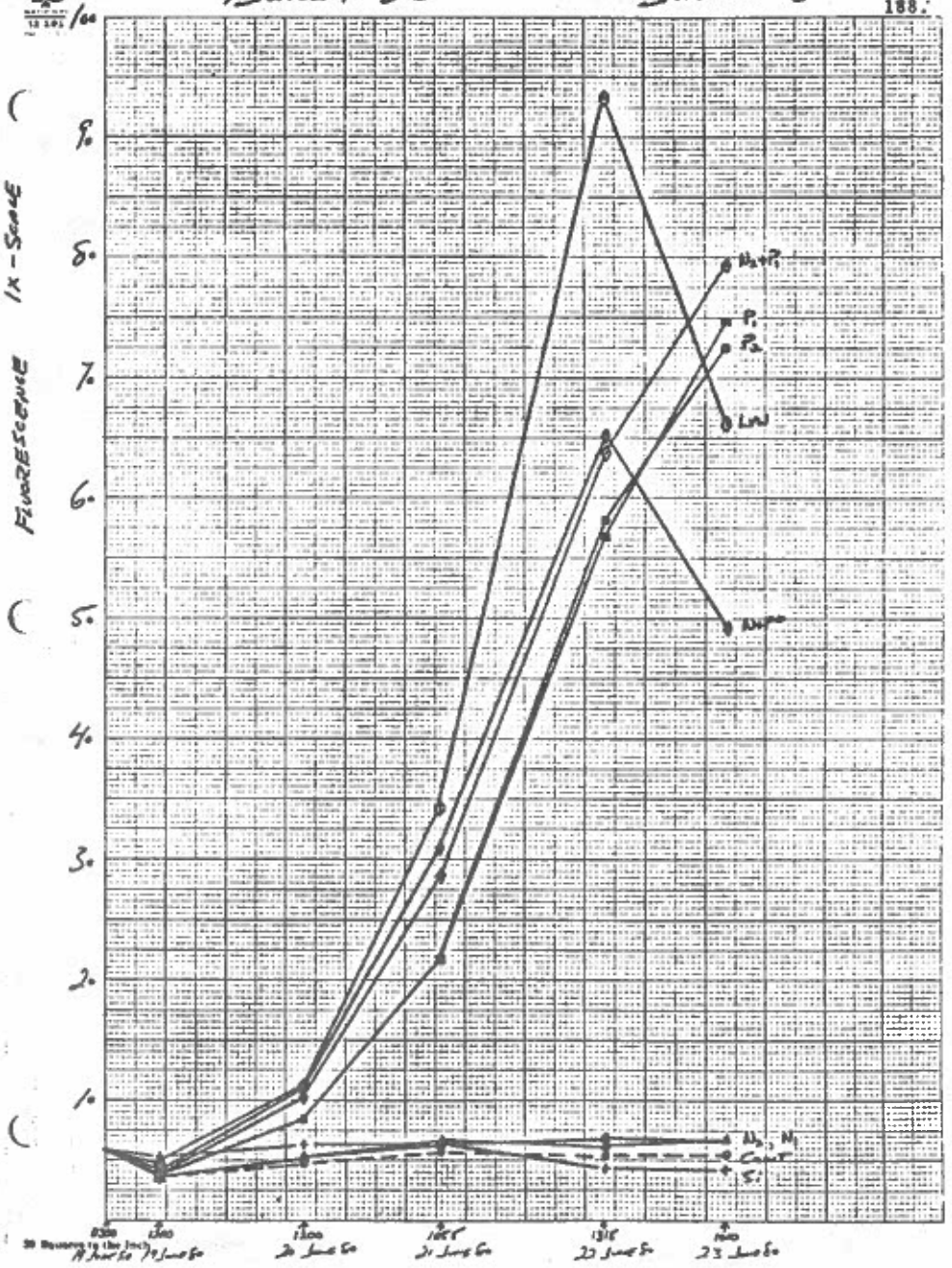
Bombay 26 — Station 4

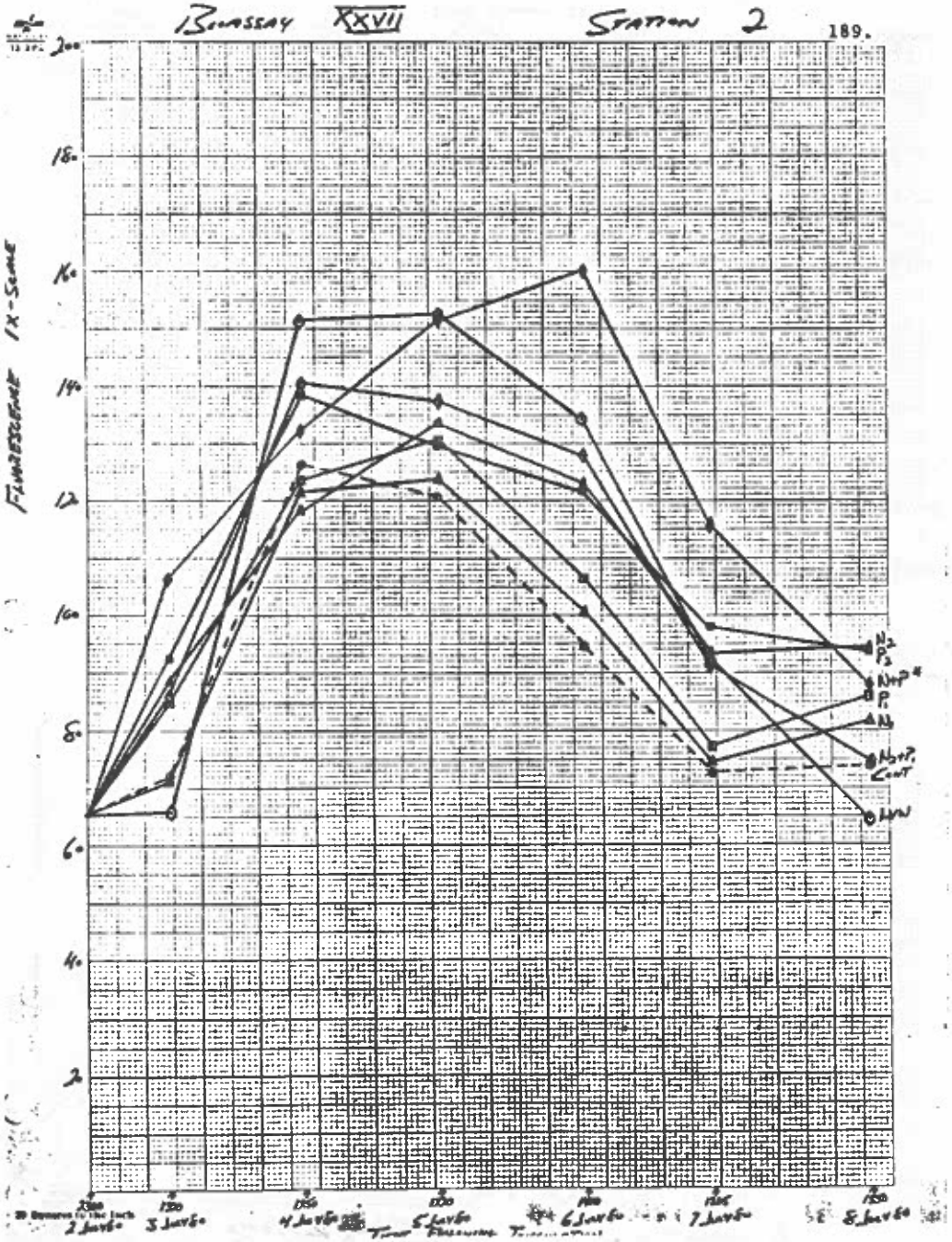


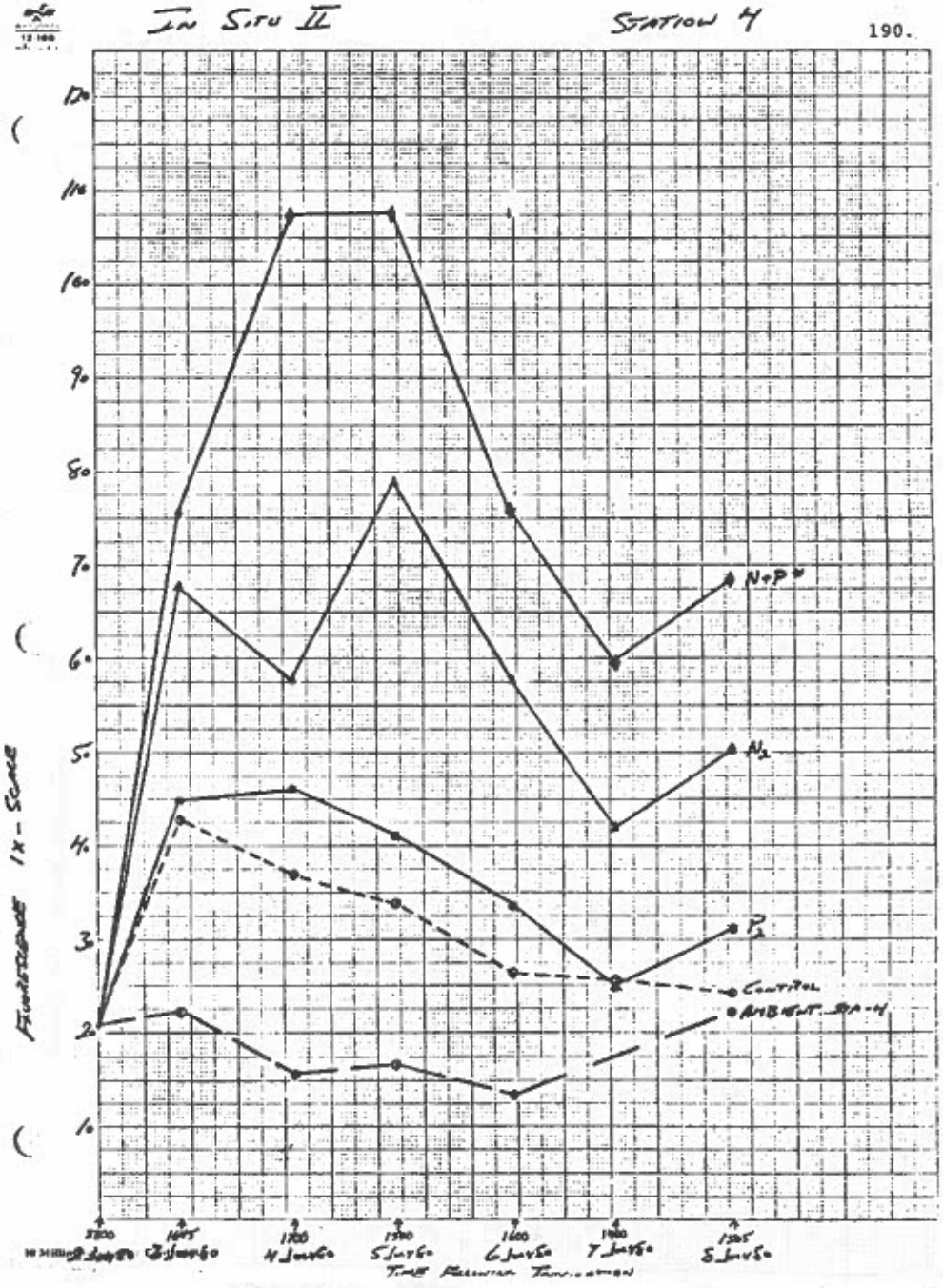
BIOASSAY 26 STATION 5

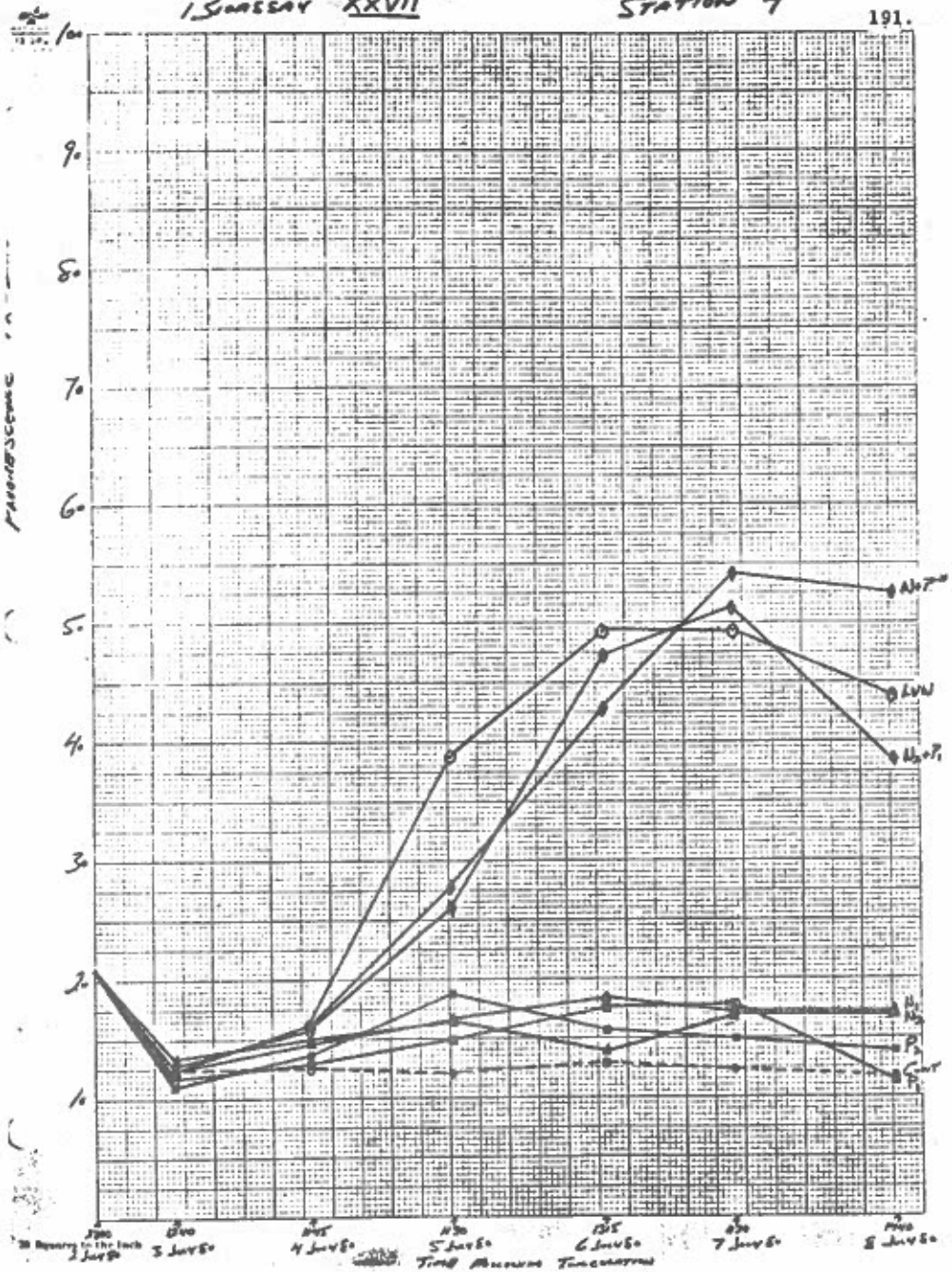


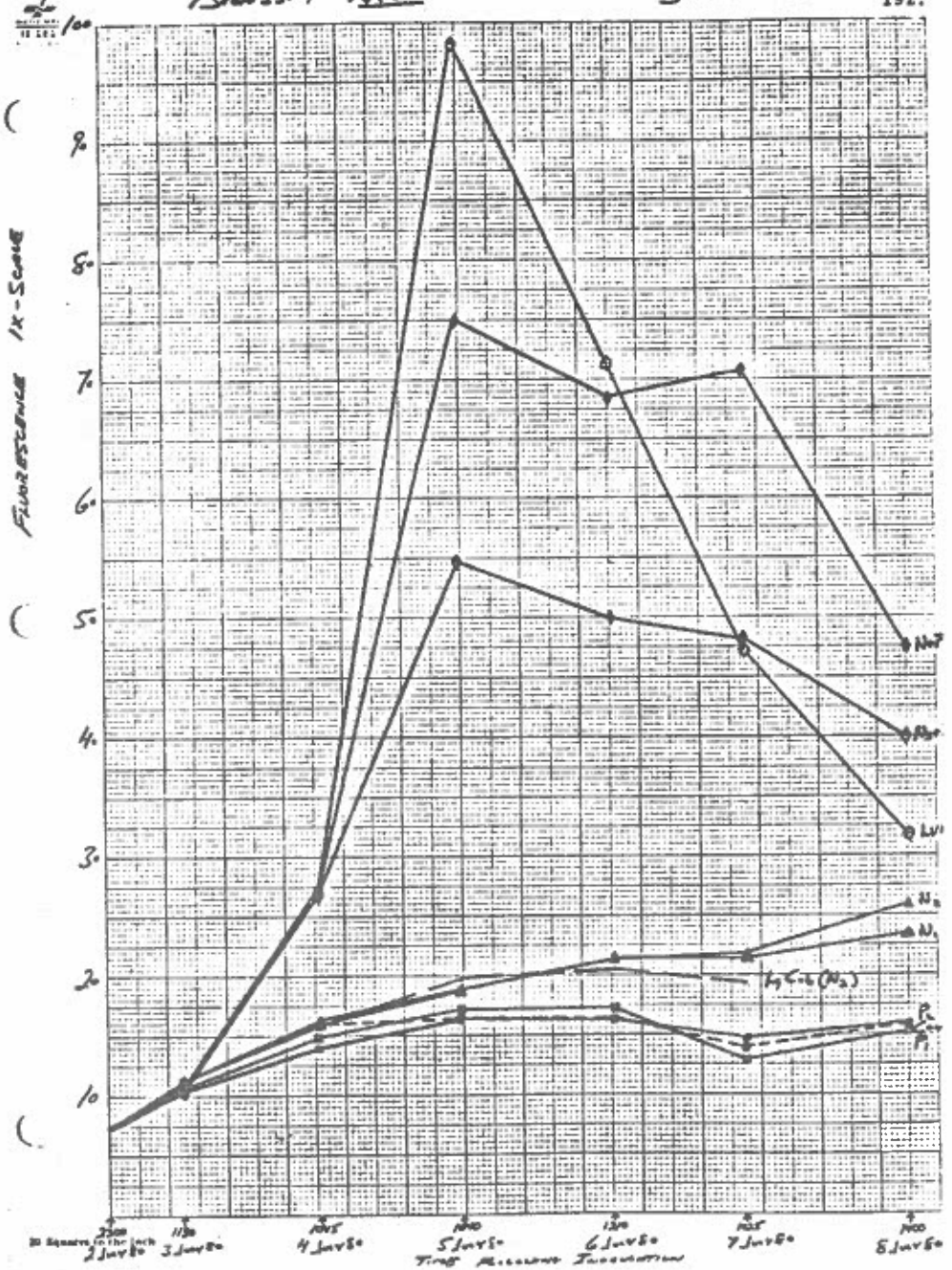
BIOASSAY 26 STATION 8



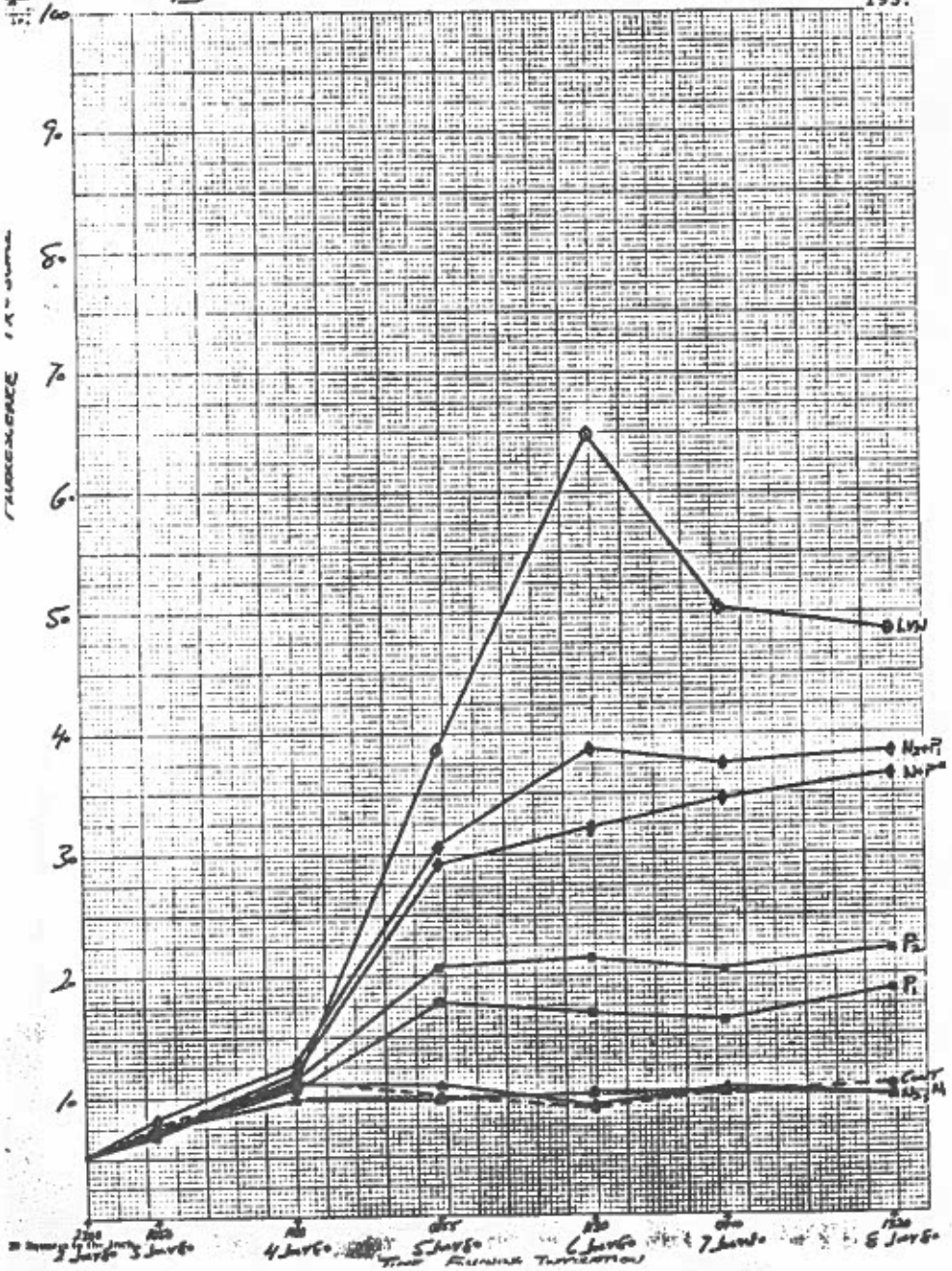

Bioassay XXVII
Station 2
189.
Fluorescence 1x-Scale
N2
P2
N+P*
P1
N1
N2+P1
Cont
WW
2 June 80
3 June 80
4 June 80
5 June 80
6 June 80
7 June 80
8 June 80


In Situ II
Station 4
190.
Fluorescence 1x-Scale
N+P*
N2
P2
Control
Ambient Sta-4
2 June 80
3 June 80
4 June 80
5 June 80
6 June 80
7 June 80
8 June 80

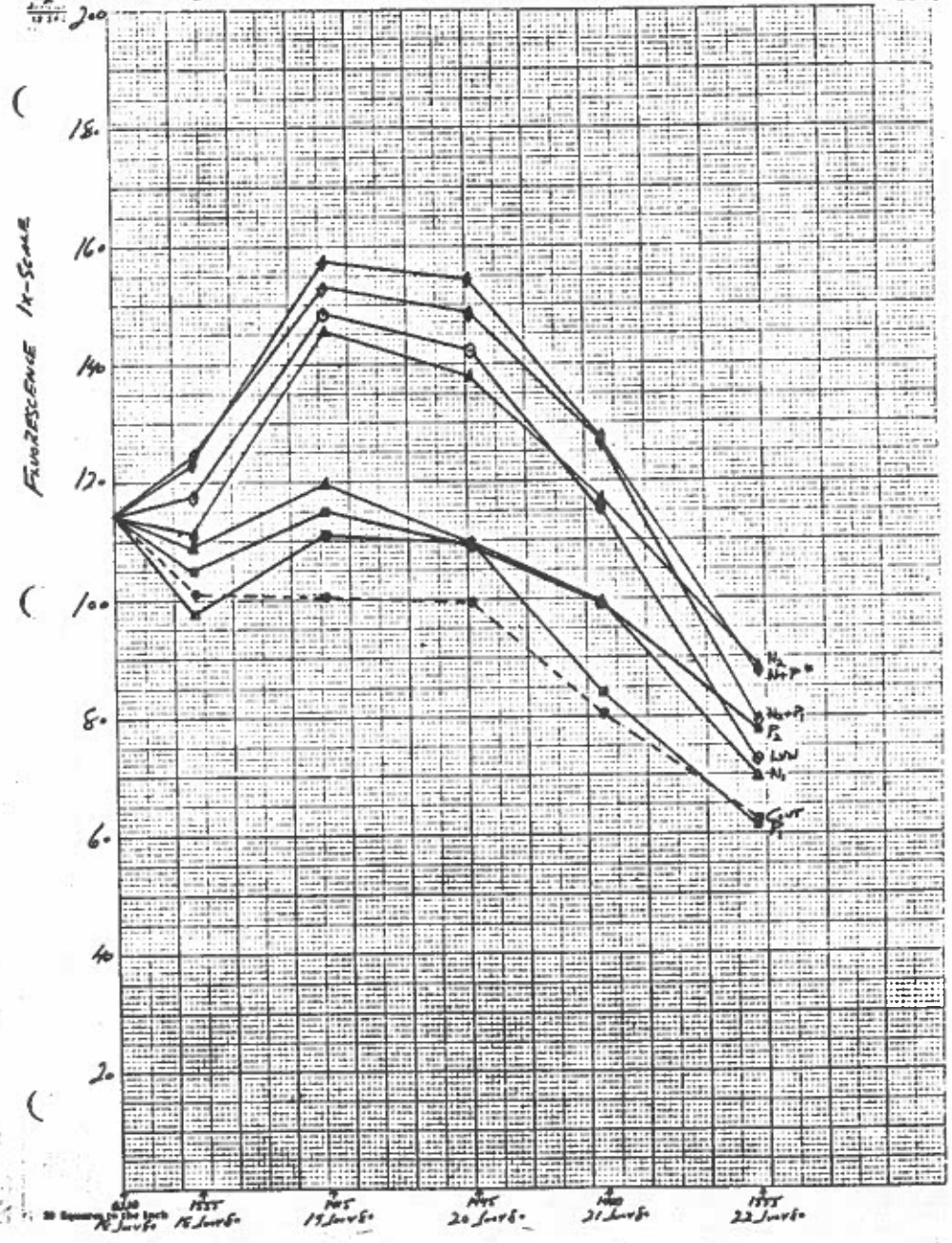

Bioassay XXVII
Station 4
Fluorescence
100
90
80
70
60
50
40
30
20
10
LVN
Time Following Inoculation


Bioassay XXVII
Station 5
Fluorescence 1x-Scale
100
90
80
70
60
50
40
30
20
10
Time Following Inoculation

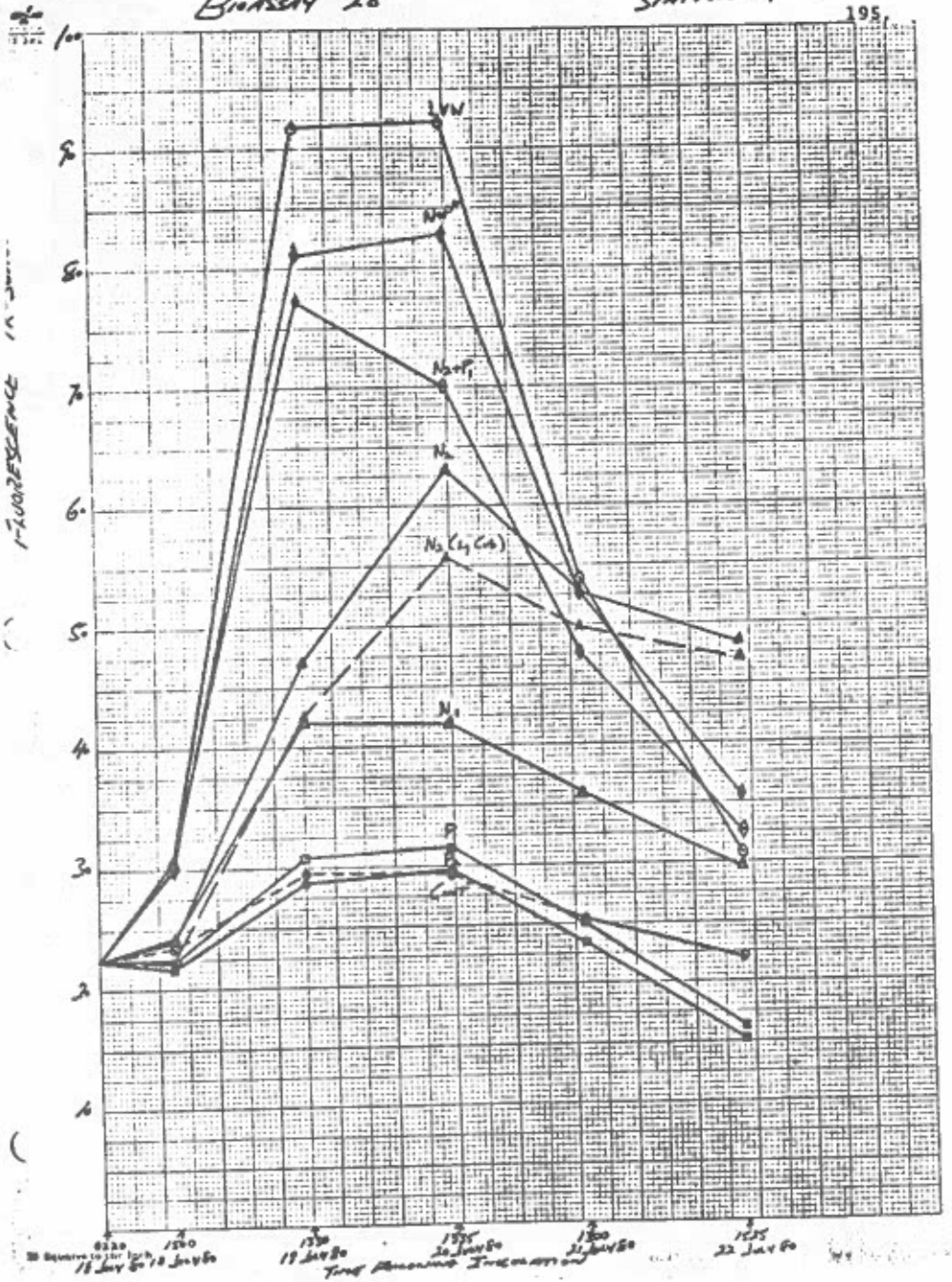
BIOASSAY XXVII — STATION 8



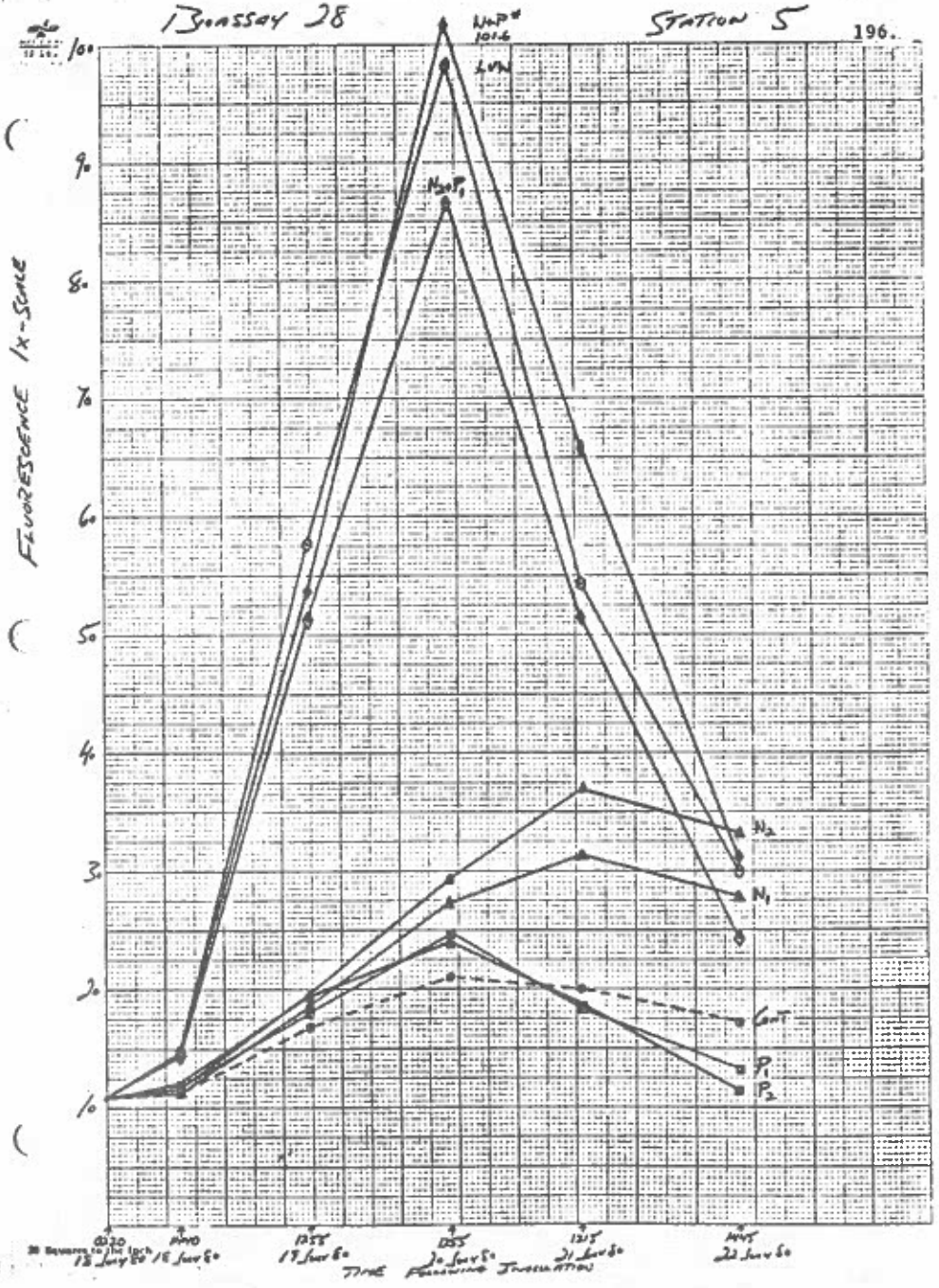
BIOASSAY 28 — STATION 2

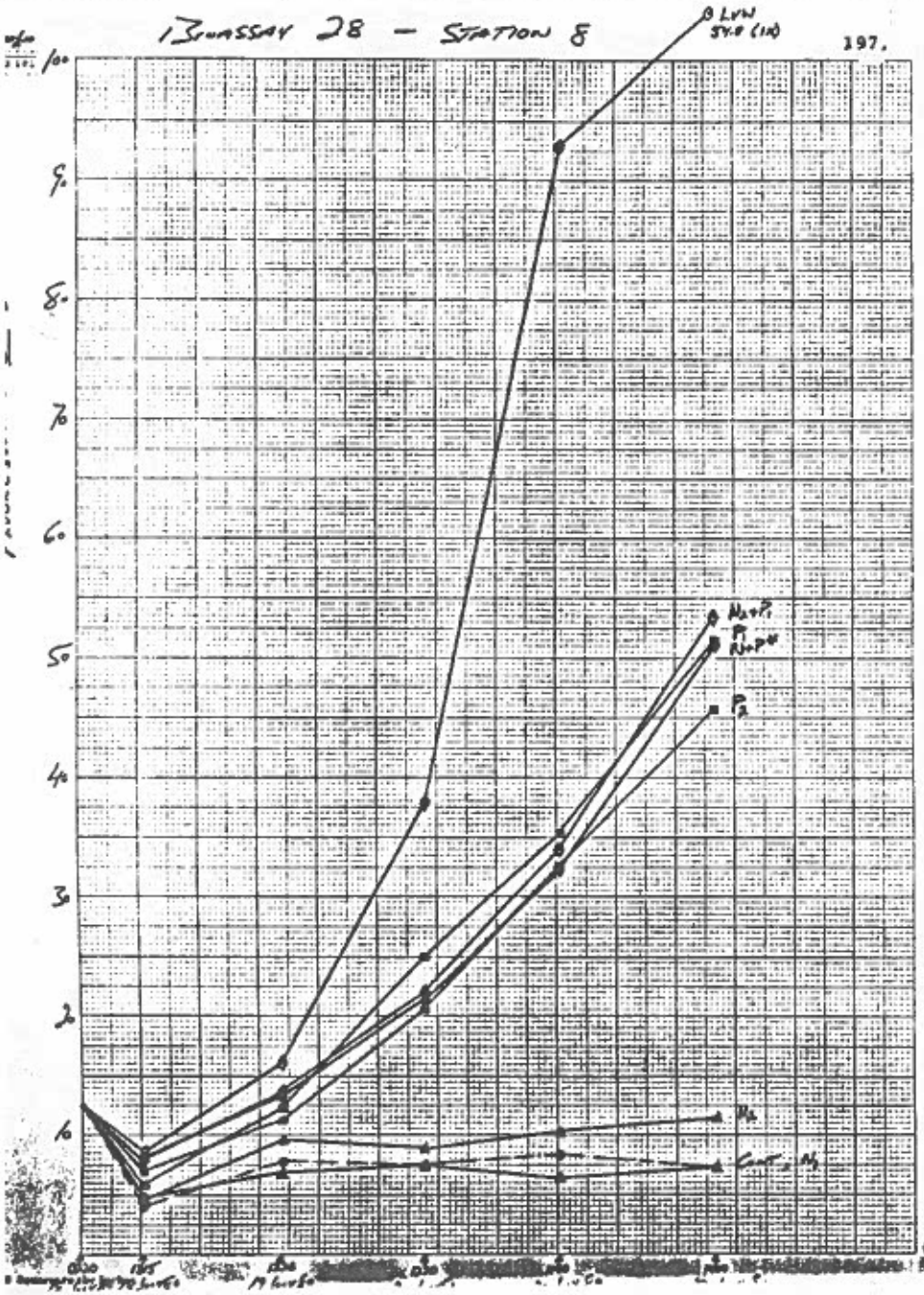


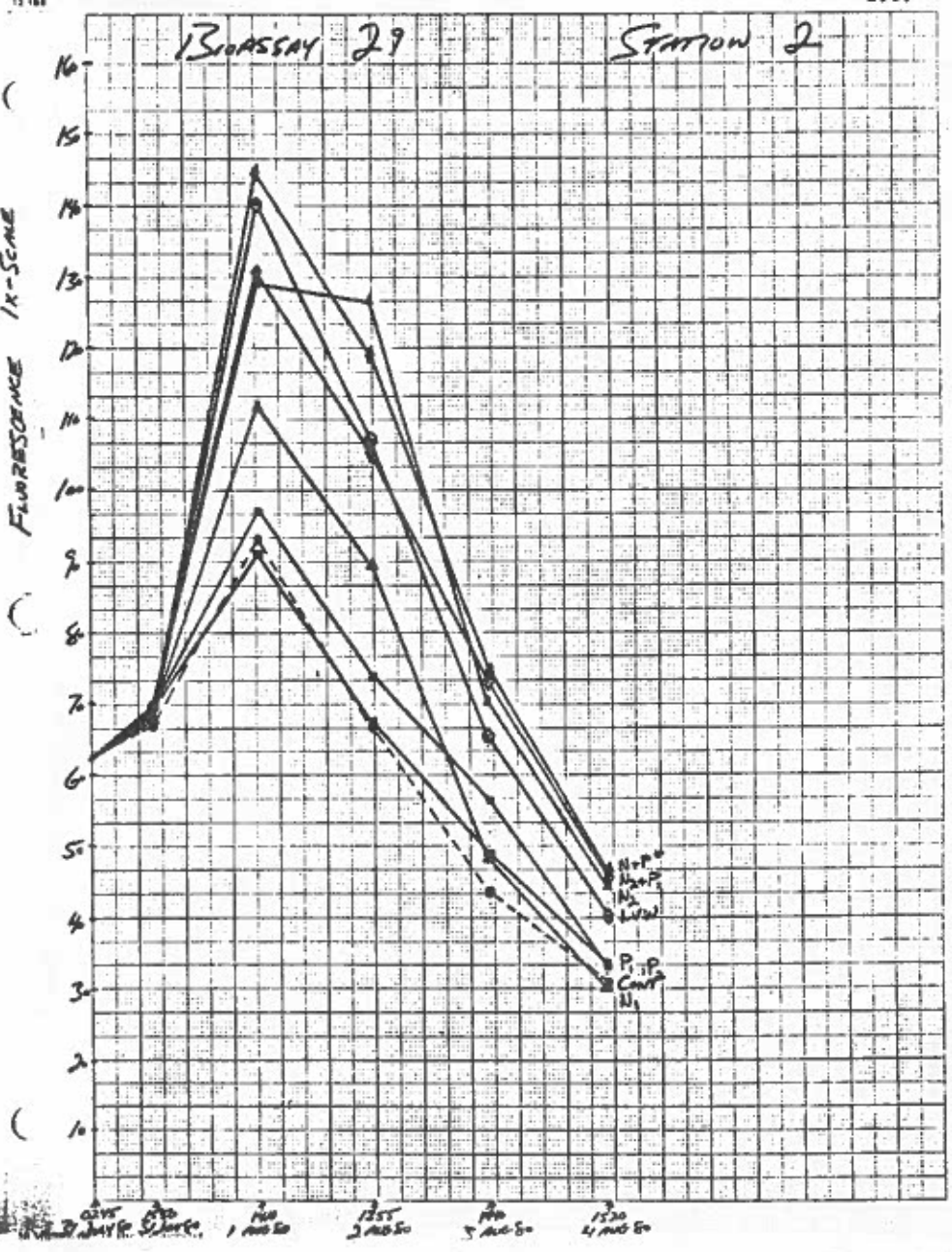
Biomass 28 — Station 4

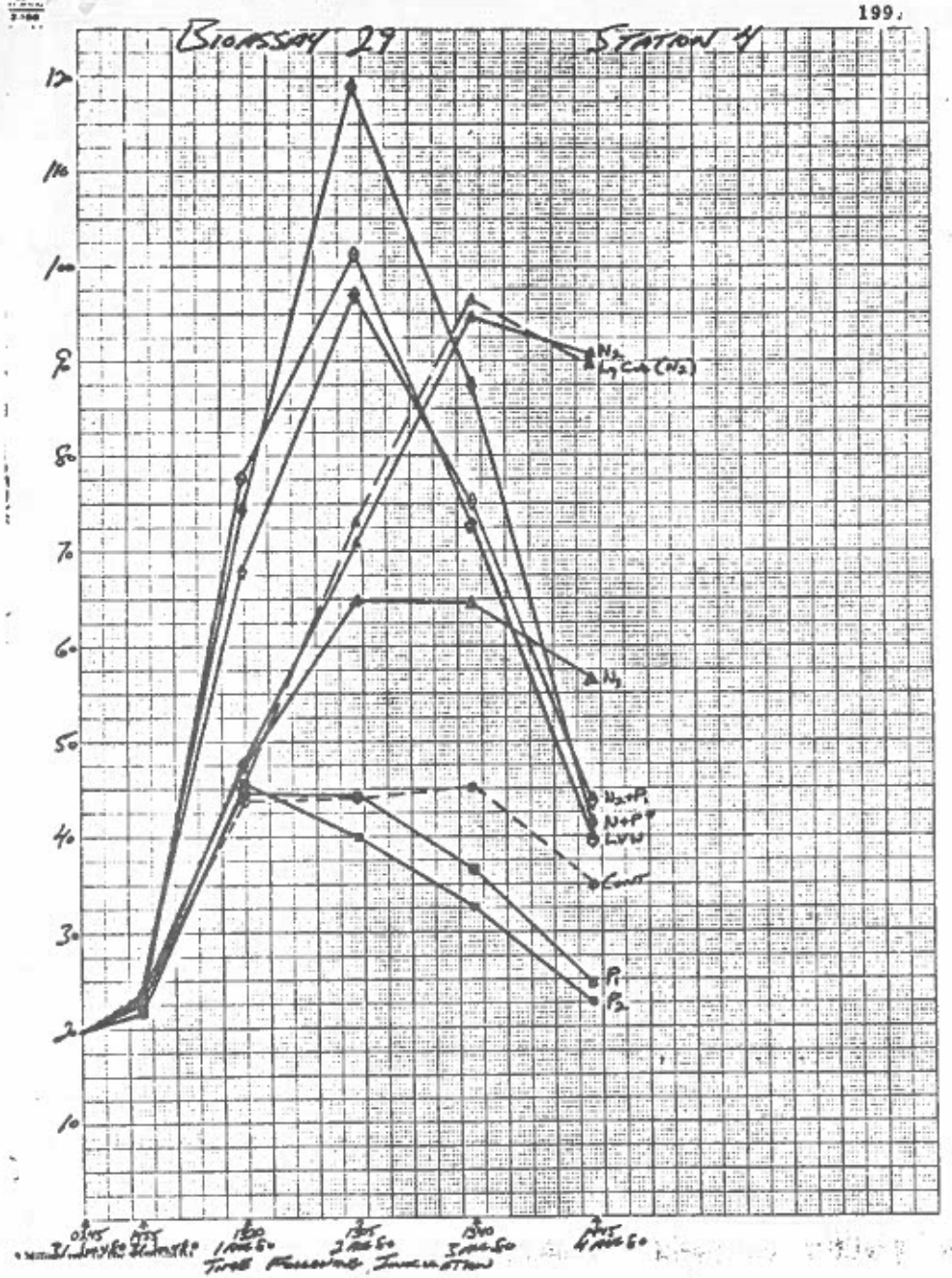


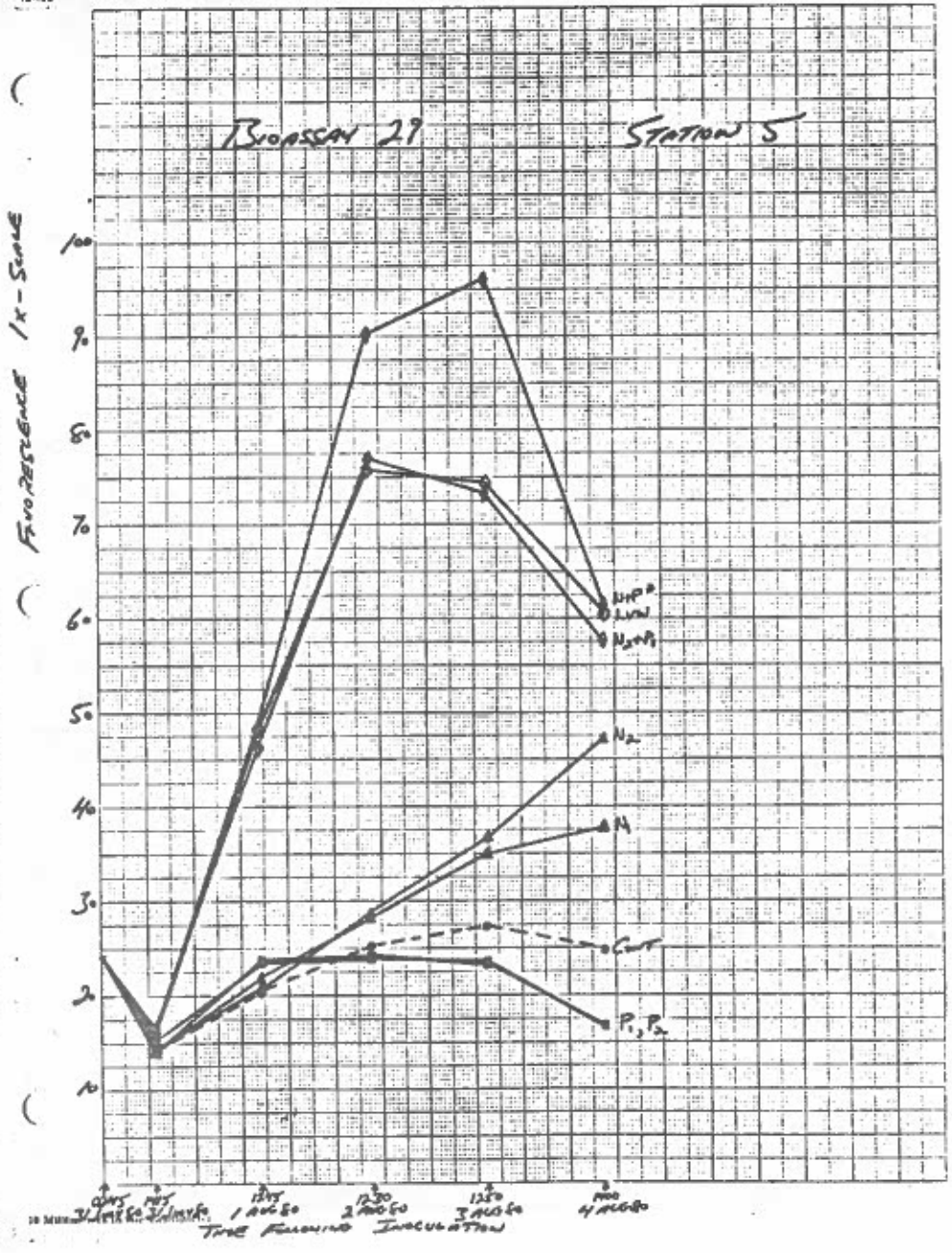
Biomass 28 — Station 5

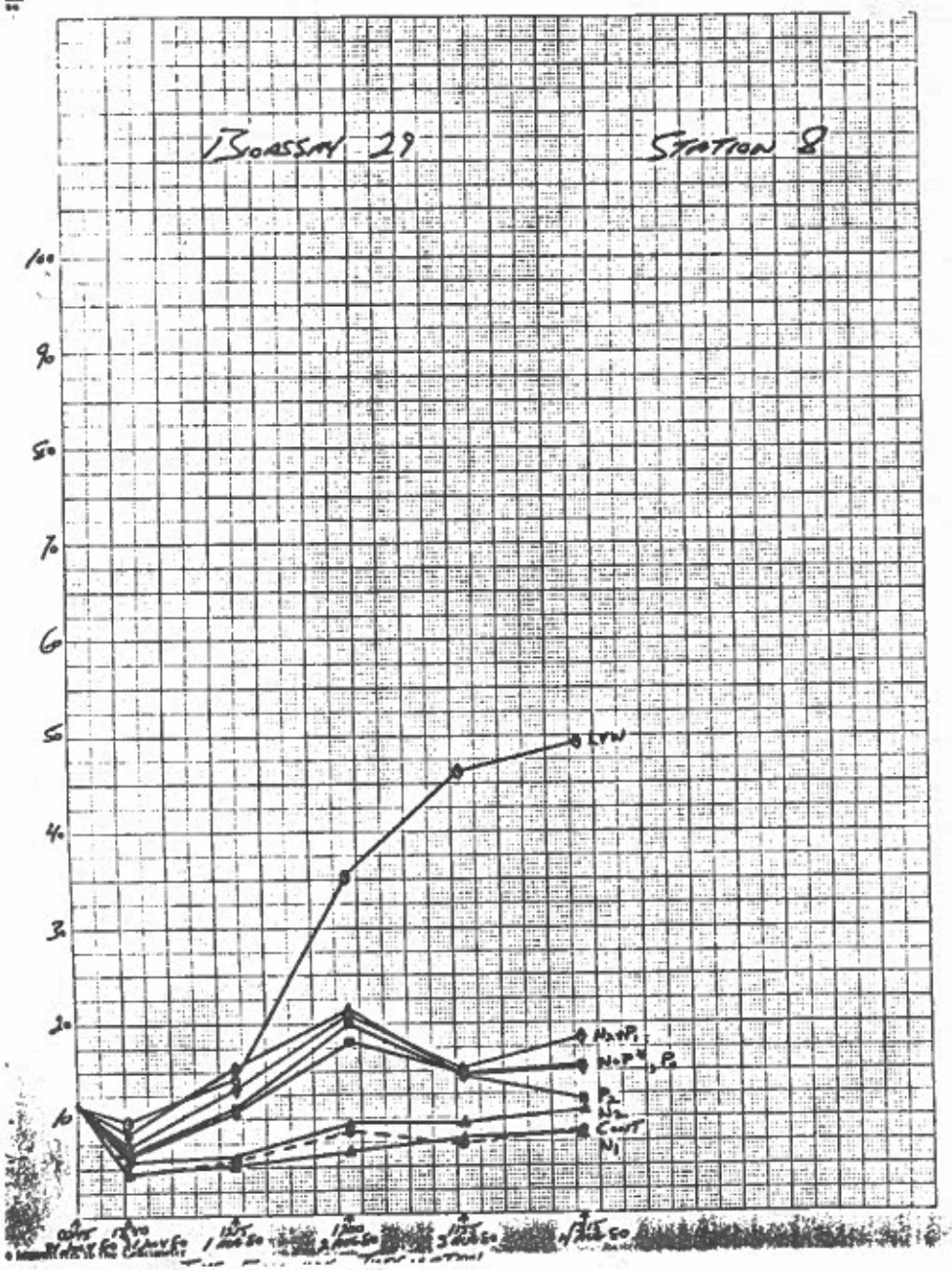


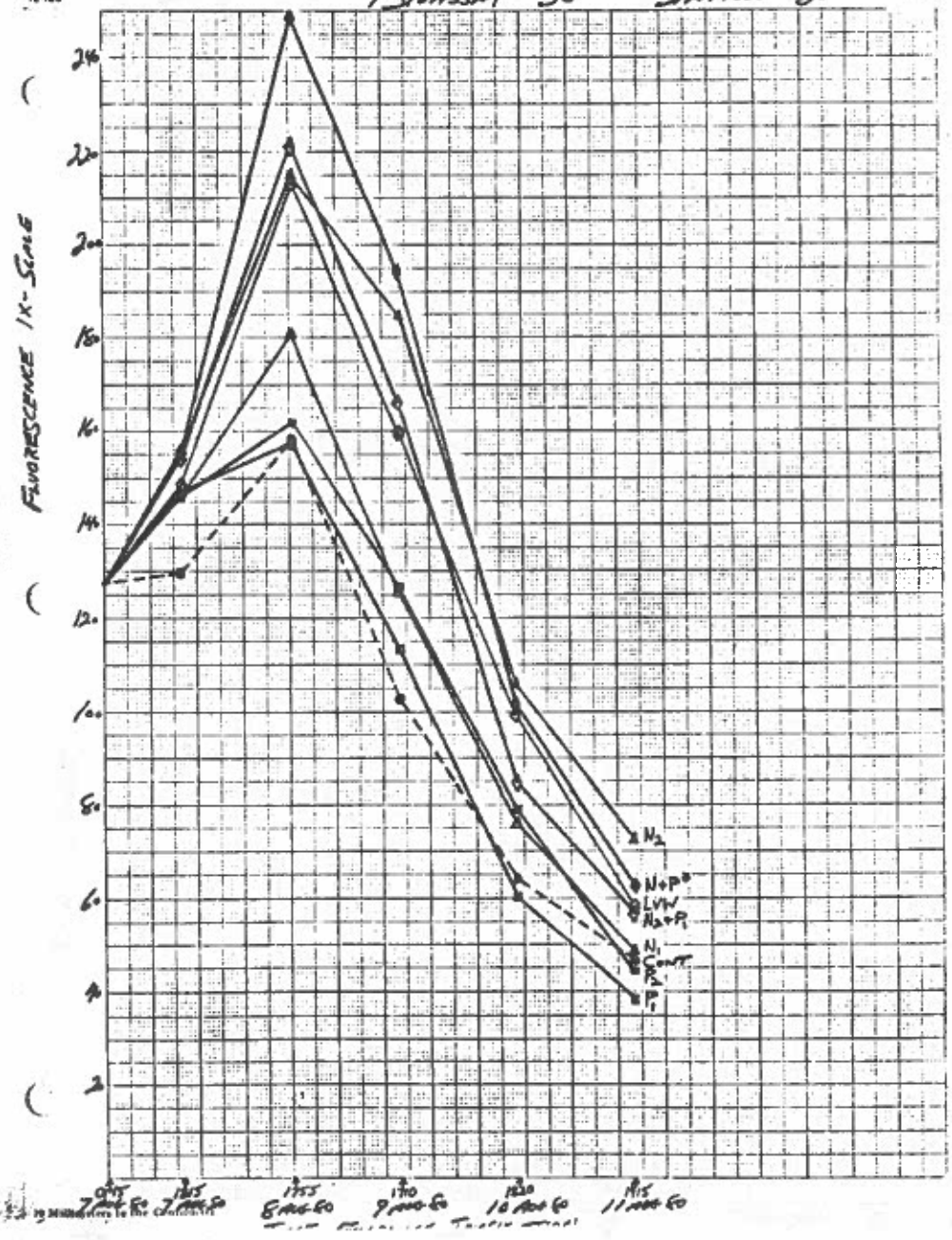

BIOASSAY 28 – STATION 8
LVW
N2+P1
P1
N+P*
P2
N2
CONT, N1

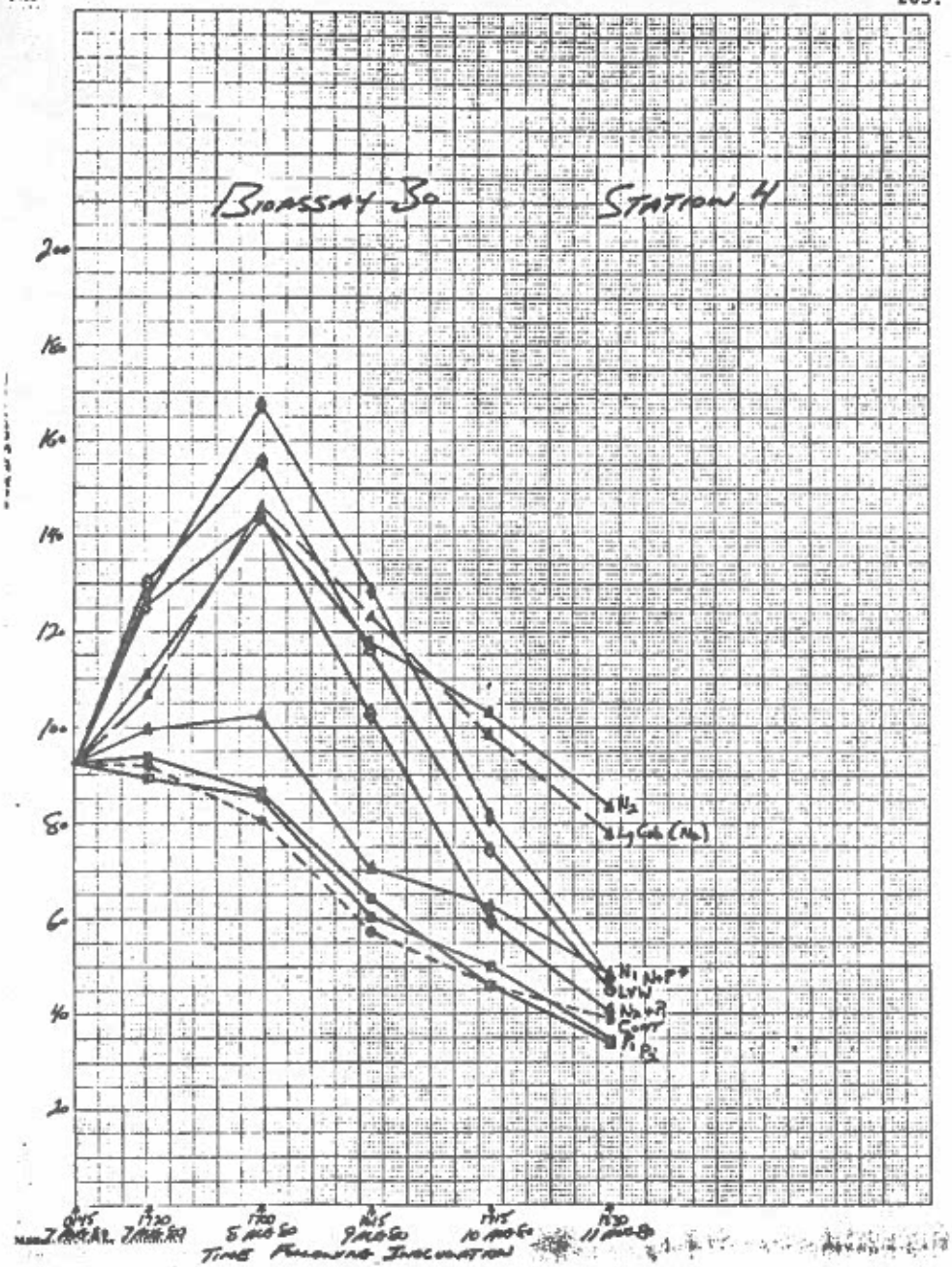

BIOASSAY 29
STATION 2
FLUORESCENCE 1x-SCALE
N+P*
N2+P1
N2
LVW
P1, P2
CONT
N1

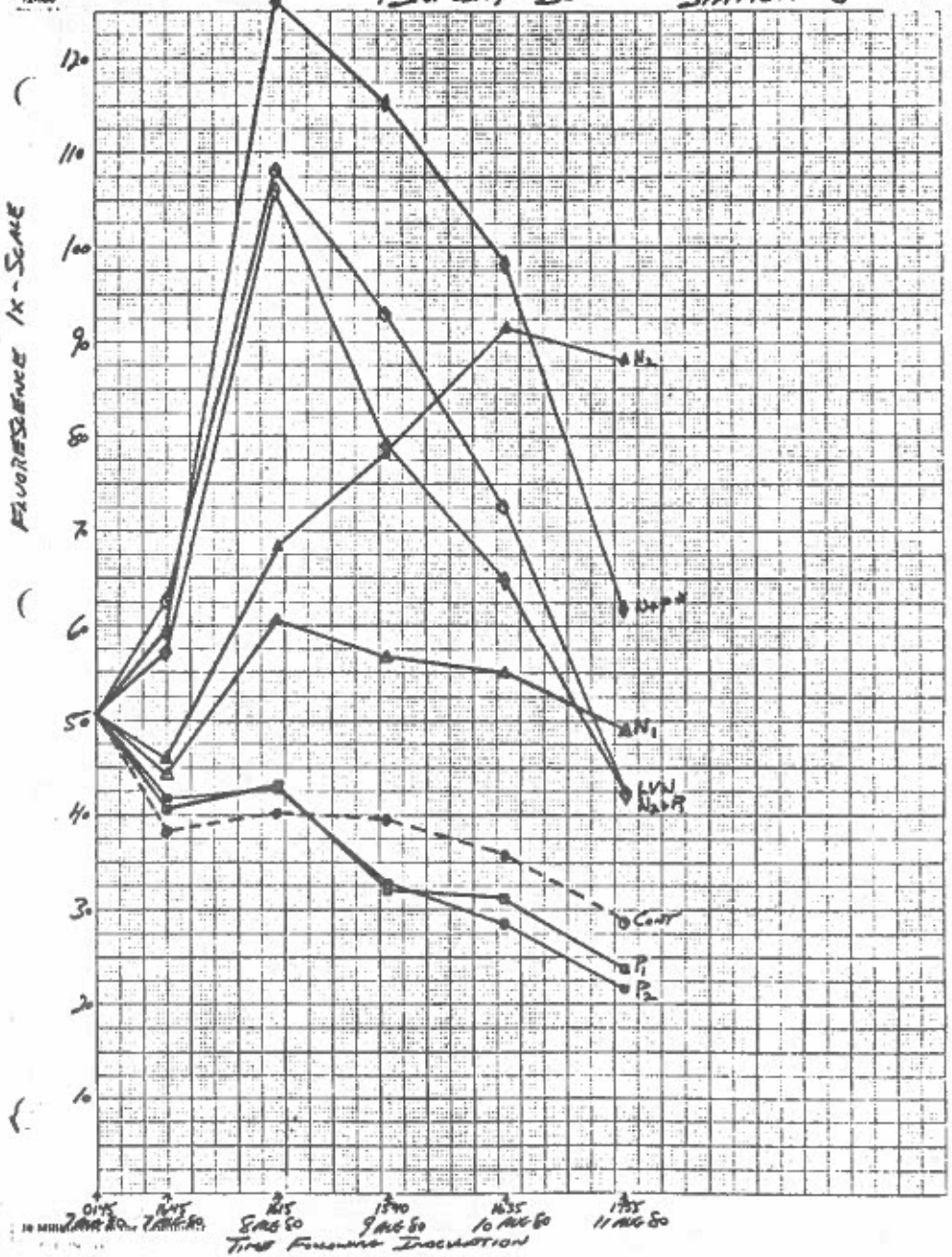

BIOASSAY 29
STATION 4
120
110
100
90
80
70
60
50
40
30
20
10
N2
N1
N2+P1
N+P*
LVW
CONT
P1
P2
0245
1455
1320
1305
1340
1445
1 AUG 80
2 AUG 80
3 AUG 80
4 AUG 80
TIME FOLLOWING INOCULATION

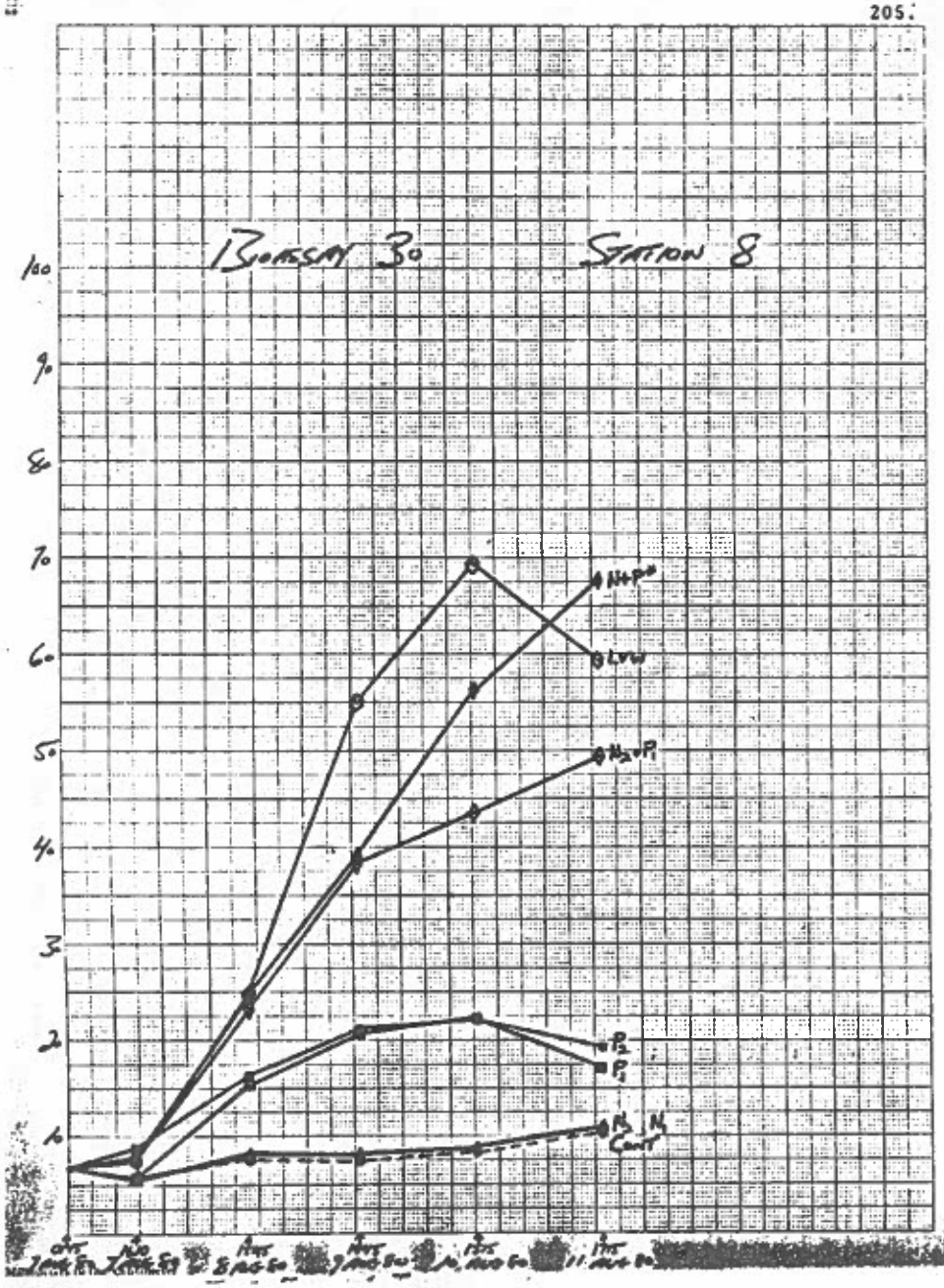

BIOASSAY 29
STATION 5
FLUORESCENCE 1x-SCALE
100
90
80
70
60
50
40
30
20
10
N+P*
LVW
N2+P1
N2
N1
CONT
P1, P2
0245
1445
1245
1230
1250
1400
1 AUG 80
2 AUG 80
3 AUG 80
4 AUG 80
TIME FOLLOWING INOCULATION

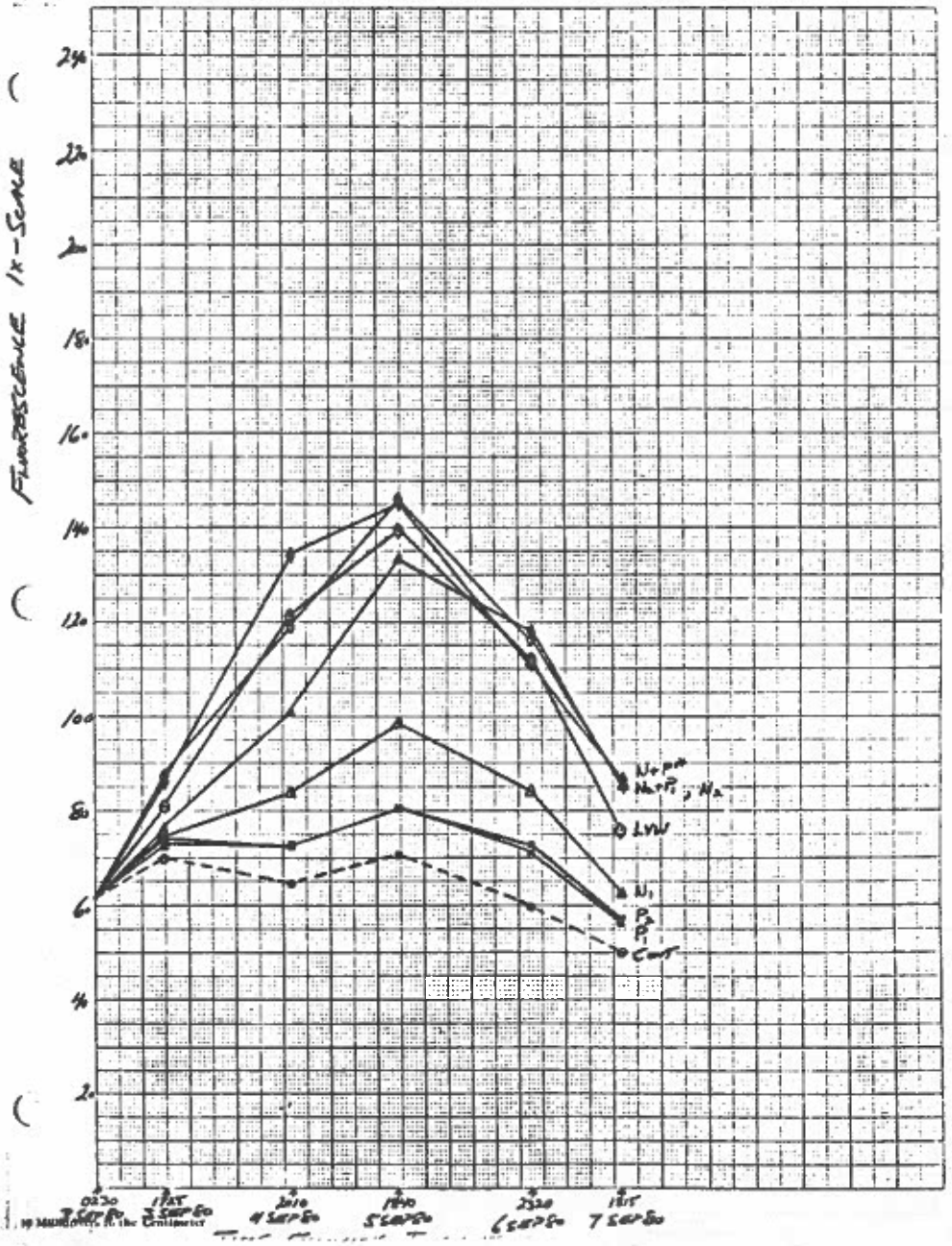

BIOASSAY 29
STATION 8
100
90
80
70
60
50
40
30
20
10
LVN
N+P1
N+P2
P2
LVN
CONT
N1

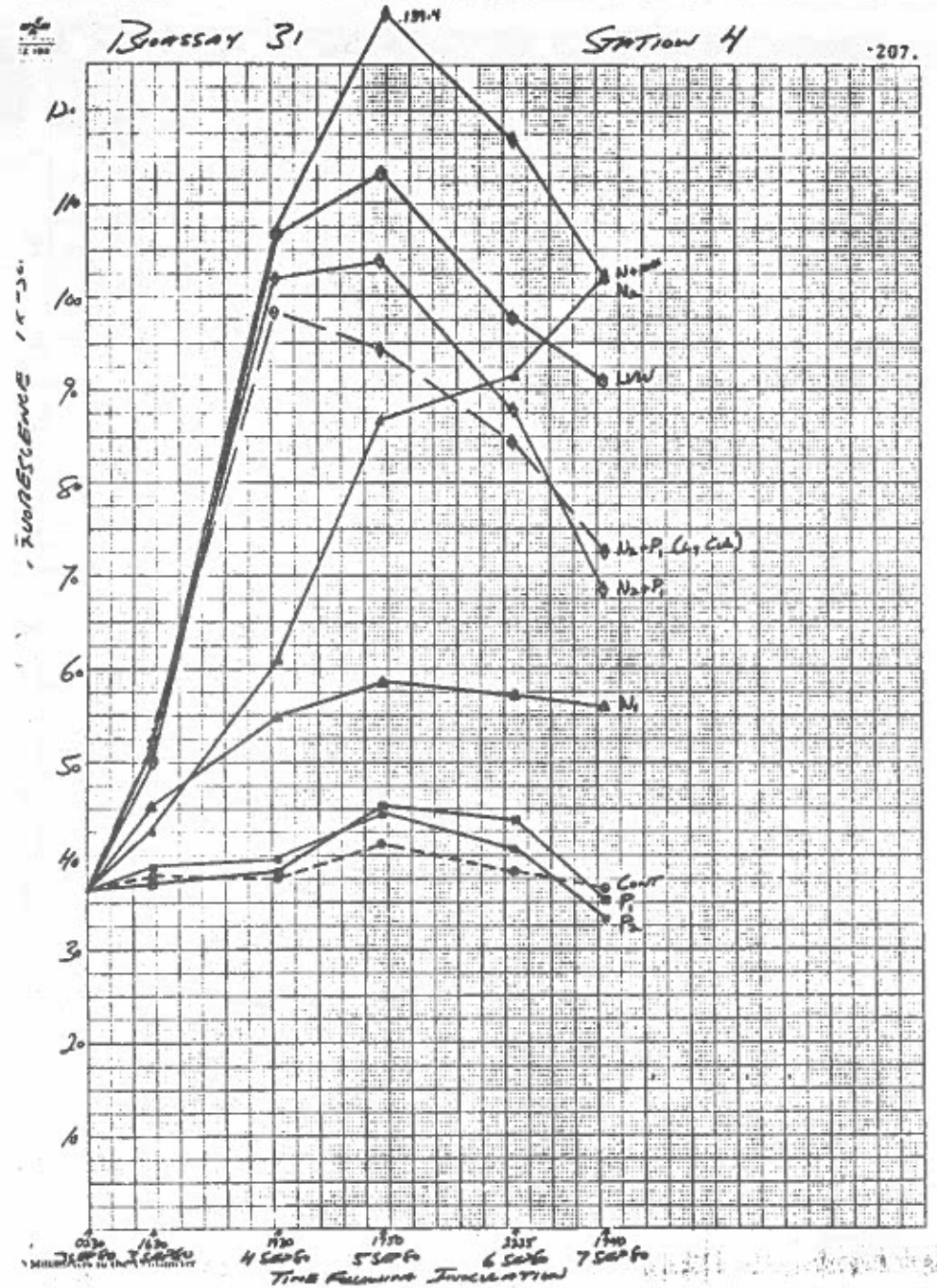

BIOASSAY 30
STATION 2
FLUORESCENCE 1X-SCALE
24
22
20
18
16
14
12
10
8
6
4
2
N2
N+P2
LVN
N2+P1
N1
CONT
P2
P1
7 MIN-GO
8 MIN-GO
9 MIN-GO
10 MIN-GO
11 MIN-GO


BIOASSAY 30
STATION 4
200
180
160
140
120
100
80
60
40
20
N2
Lg Cob (N2)
N1 N+P*
LVW
N2+P1
CONT
P1 P2
0145 7 AUG 80
1720 7 AUG 80
1700 8 AUG 80
1615 9 AUG 80
1715 10 AUG 80
1630 11 AUG 80
TIME FOLLOWING INOCULATION


BIOASSAY 30
STATION 5
FLUORESCENCE 1X-SCALE
120
110
100
90
80
70
60
50
40
30
20
10
N2
N+P*
N1
LVW
N2+P1
CONT
P1
P2
0145 7 AUG 80
1645 7 AUG 80
1615 8 AUG 80
1540 9 AUG 80
1635 10 AUG 80
1755 11 AUG 80
TIME FOLLOWING INOCULATION


BIOASSAY 30
STATION 8
100
90
80
70
60
50
40
30
20
10
N+P*
LVW
N2+P1
P2
P1
N2, N1
CONT


BIOASSAY 31
STATION 2
FLUORESCENCE 1x-SCALE
240
220
200
180
160
140
120
100
80
60
40
20
N+P*
N2+P1, N2
LVW
N1
P2
P1
CONT
0230
3 SEP 80
1725
3 SEP 80
2010
4 SEP 80
1840
5 SEP 80
2320
6 SEP 80
1815
7 SEP 80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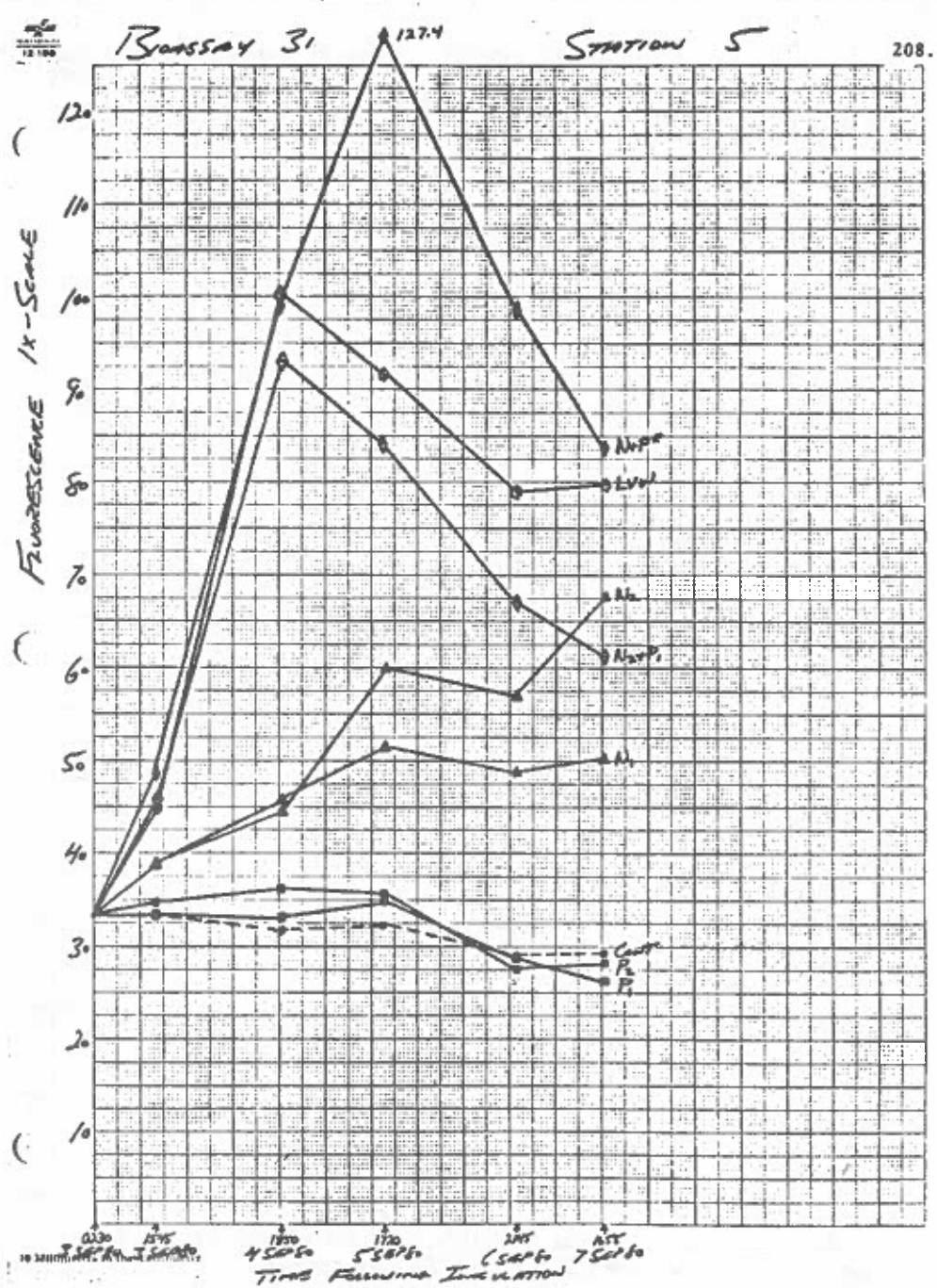
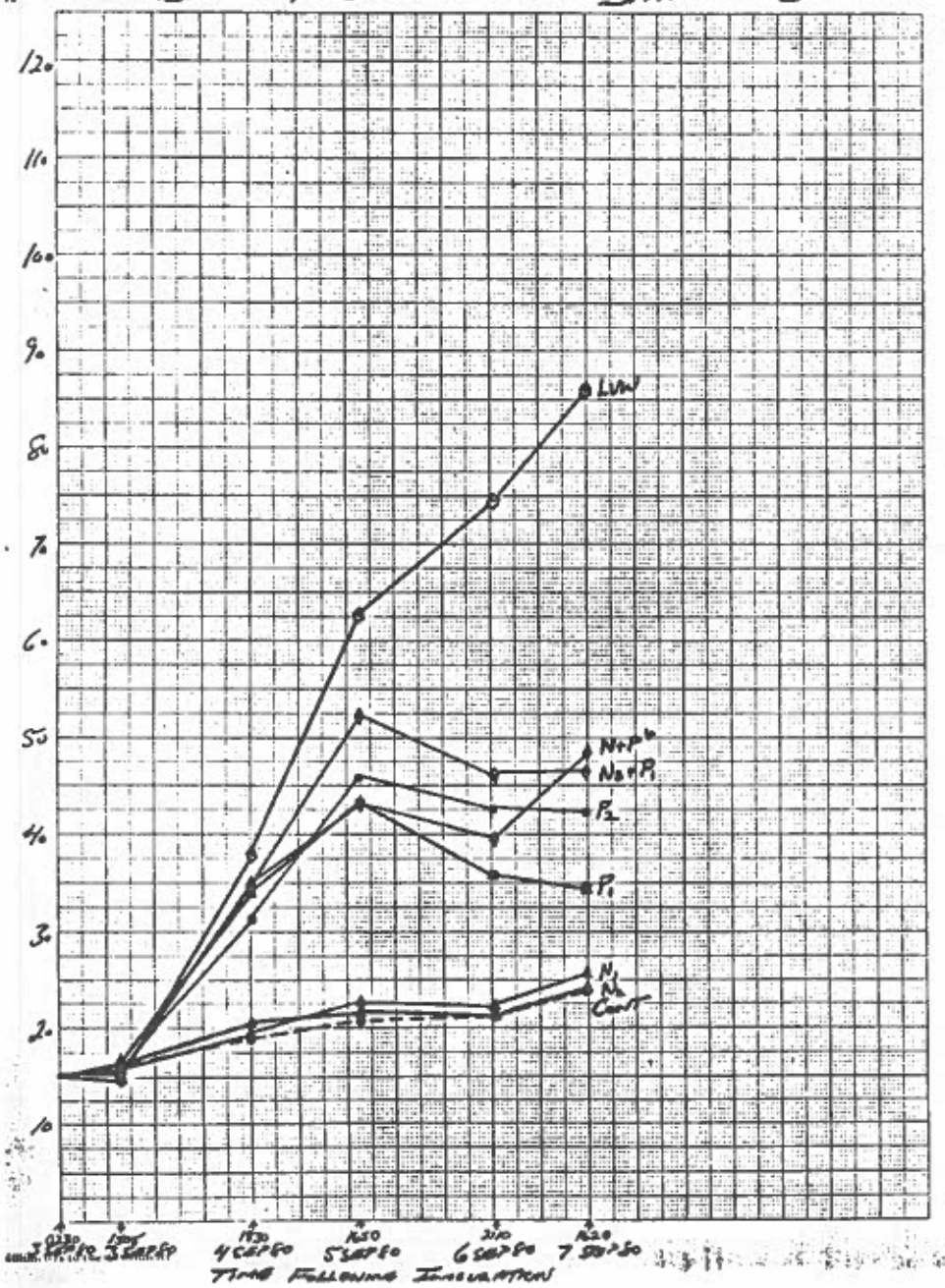
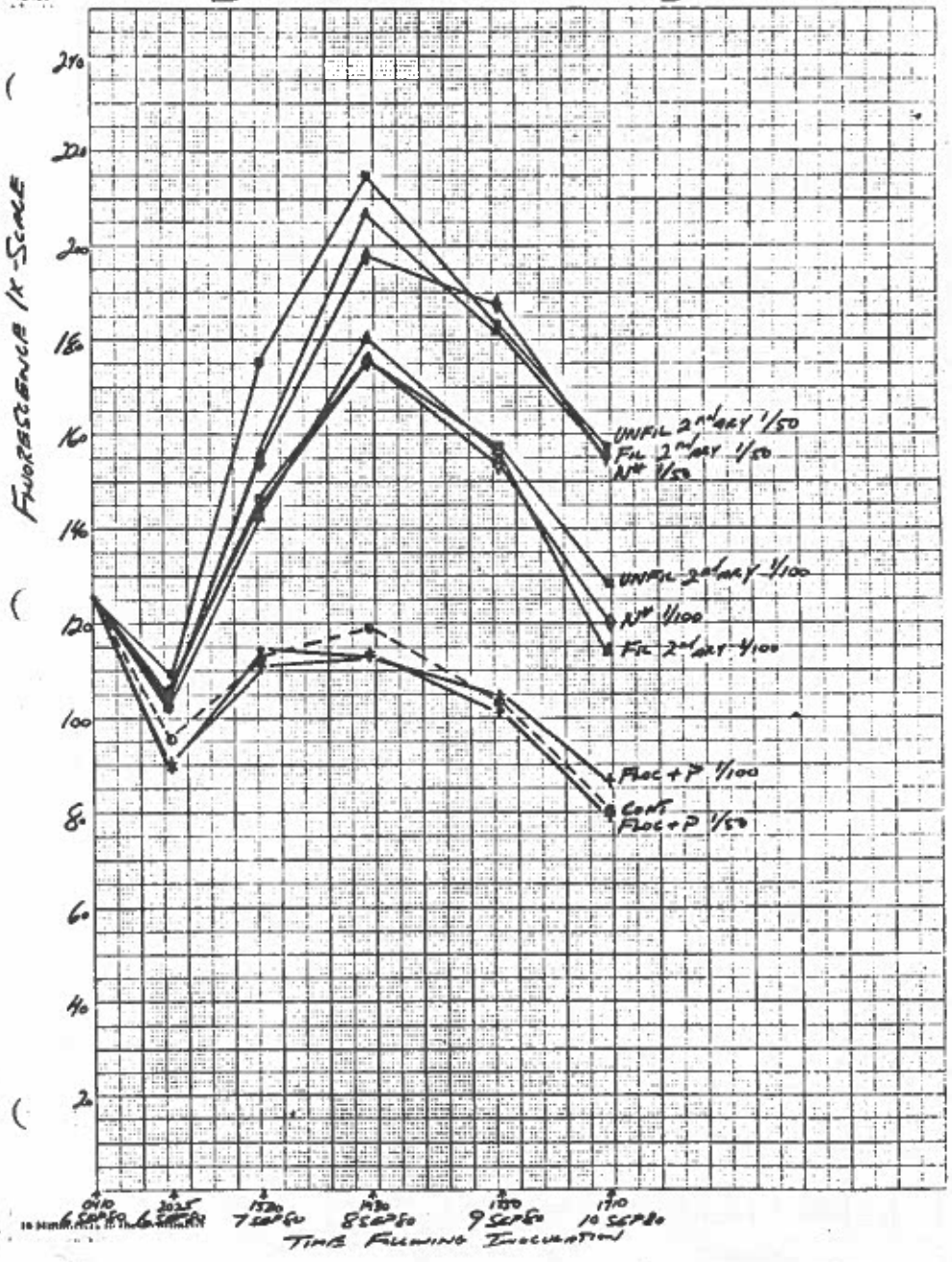
Station 4
139.4
N+P*
N2
LVW
N2+P1 (Lg Cul)
N2+P1
N1
Cont
P1
P2
Fluorescence 1x-Scale
Time Following Inoculation
0230
1630
1930
1950
2225
1940
3 Sep 80
4 Sep 80
5 Sep 80
6 Sep 80
7 Sep 80


Bioassay 31
Station 5
127.4
N+P*
LVW
N2
N2+P1
N1
Cont
P2
P1
Fluorescence 1x-Scale
Time Following Inoculation
0230
1545
1850
1720
2145
1655
3 Sep 80
4 Sep 80
5 Sep 80
6 Sep 80
7 Sep 80

Buzzard Bay 31 Station 8



Special Buzzard Bay 32 Station 2



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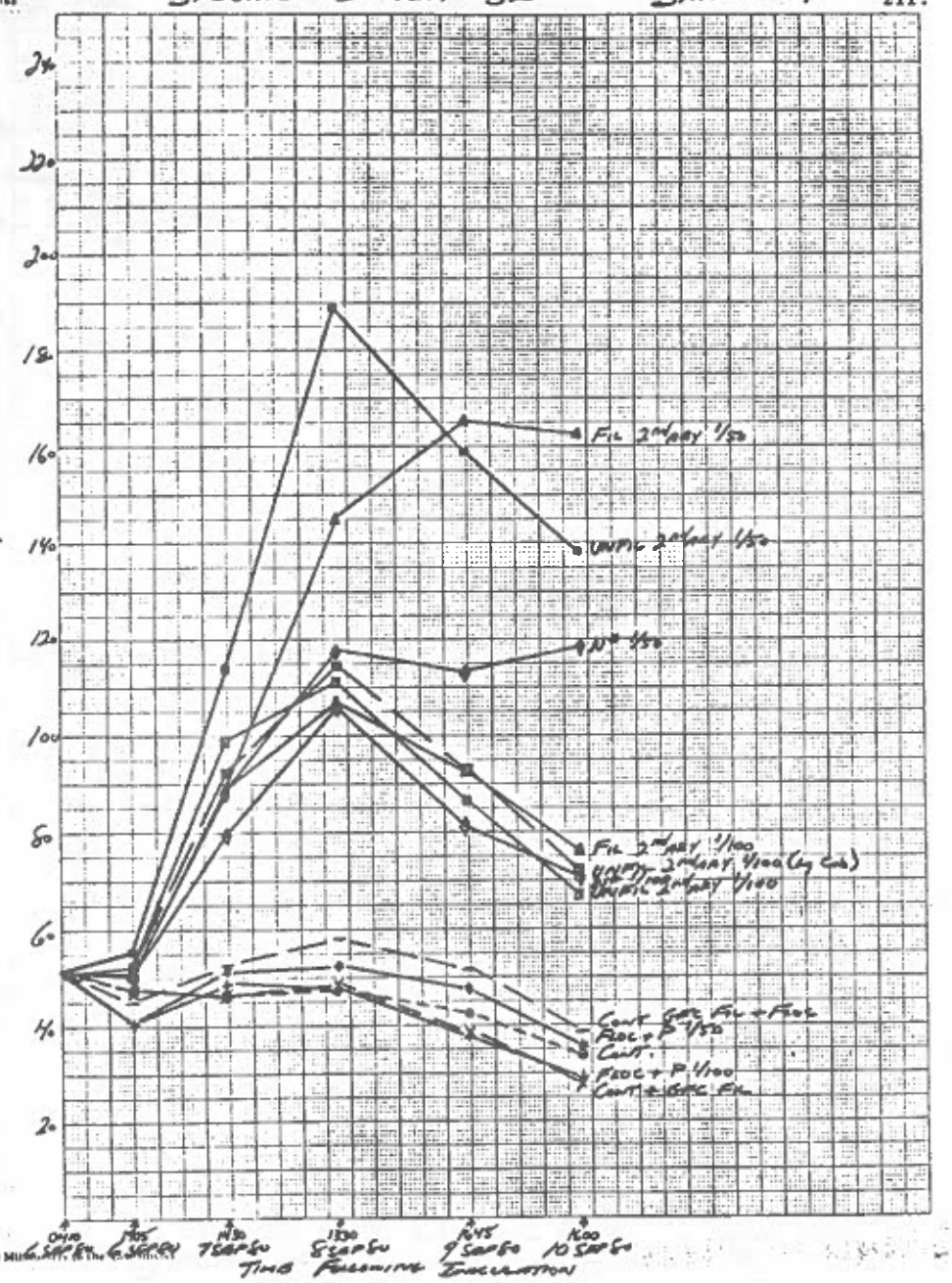
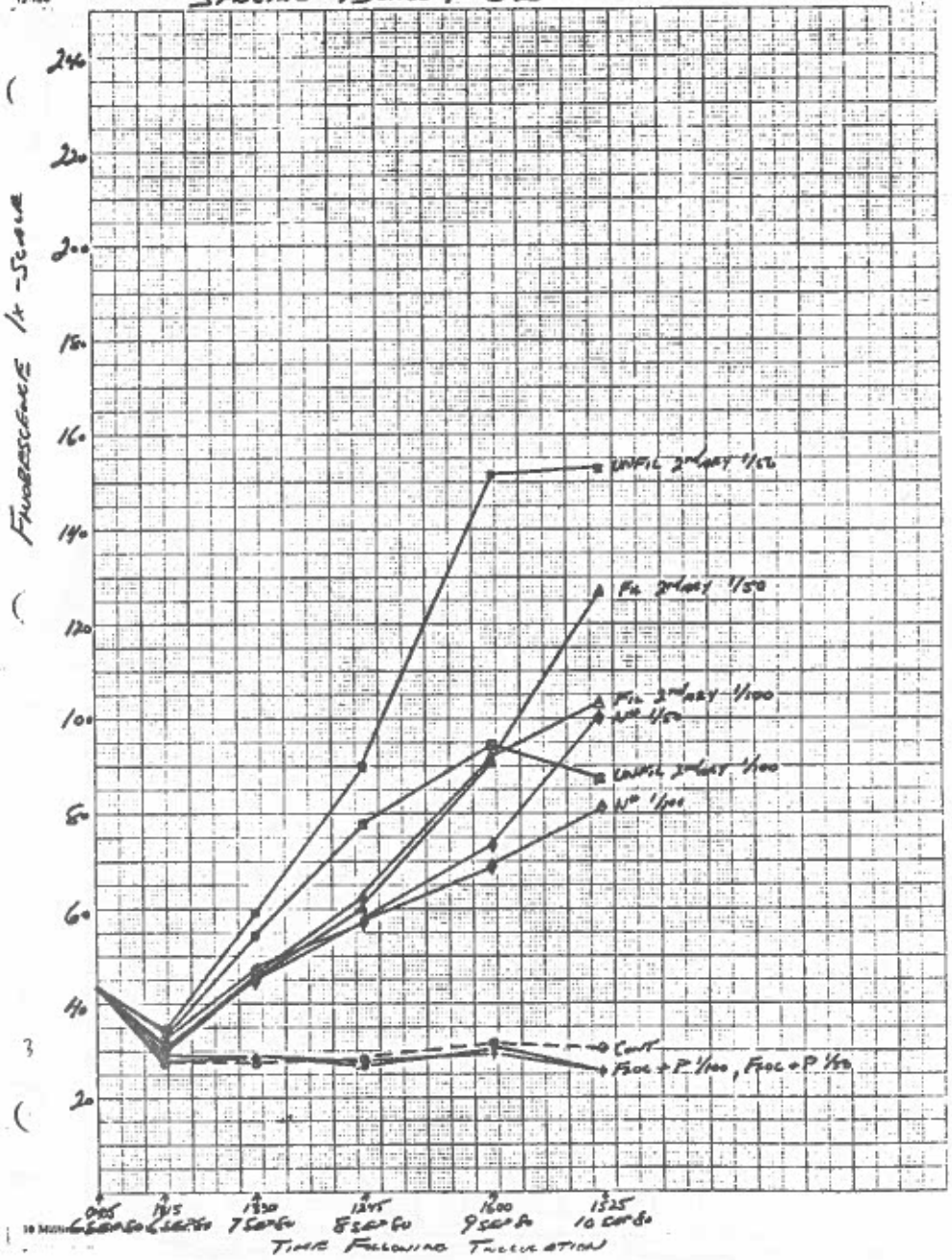
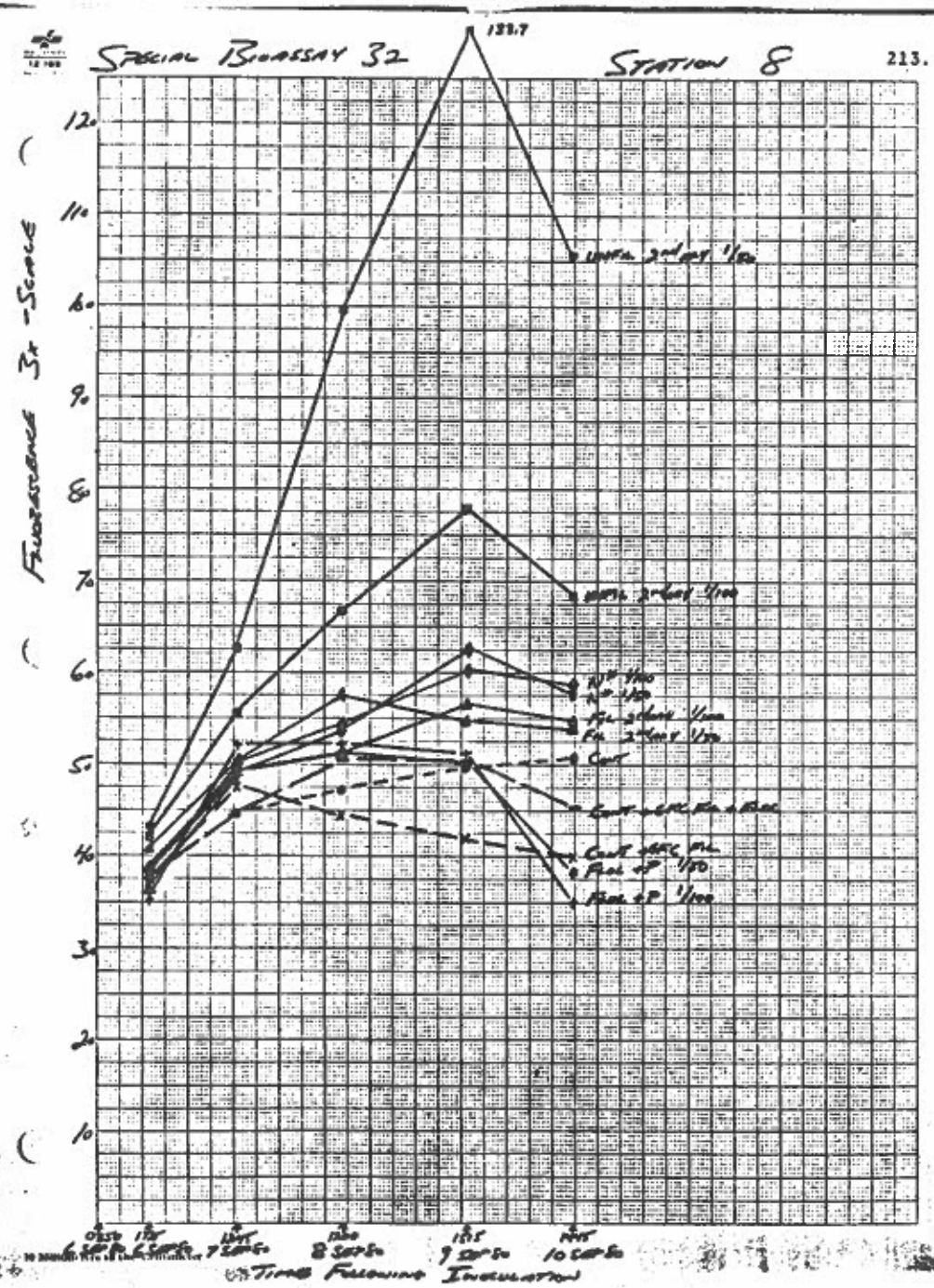


SPECIAL BOMBAY 32 STATION 5




SPECIAL BIOASSAY 32
STATION 8
FLUORESCENCE 3x -SCALE
133.7
12
11
10
9
8
7
6
5
4
3
2
1
TIMES FOLLOWING INOCULATION

APPENDIX C

Maximum Response as % Control and Statistical Analysis

(Regular Bioassay)

Maximum Response As % Control

Bioassay No.	Treatment	Station 2	Station 4	Station 5	Station 8
1	N_1	107	83	102	102
	N_2	116	103	119	98
	P_1	89	82	119	150
	P_2	113	70	125	106
	N + P	116	118	539	108
	LVW 1/100	137	436	864	131
2	N_1	150	182	86	144
	N_2	173	139	121	144
	P_1	95	121	97	106
	P_2	102	132	93	117
	$N_2 + P_2$	188	363	334	167
	LVW 1/50	206	568	562	439
4	N_1	109	103	172	78
	N_2	119	199	164	141
	P_1	93	110	108	71
	P_2	89	96	125	73
	$N_2 + P_1$	119	191	348	120
	LVW 1/50	123	250	438	134
5	N_1		115		112
	N_2		127		115
	P_1		97		103
	P_2		104		97
	$N_2 + P_1$		277		147
	LVW 1/50		471		394

Bioassay No.	Treatment	Station 2	Station 4	Station 5	Station 8
6	N_1	160	182	162	133
	N_2	223	219	155	171
	P_1	115	96	98	113
	P_2	119	101	89	111
	$N_2 + P_1$	317	395	374	209
	N + P*	476	857	528	244
	LVW 1/50	305	538	372	538
8	N_1	129	93	114	200
	N_2	178	107	137	215
	P_1	88	65	84	92
	P_2	94	62	94	82
	$N_2 + P_1$	218	237	167	185
	N + P*	234	185	273	177
	LVW 1/50	177	166	200	223
9	N_1	129	158	193	121
	N_2	209	217	228	158
	P_1	98	102	108	122
	P_2	97	110	106	123
	$N_2 + P_1$	172	241	330	177
	N + P*	186	281	391	147
	LVW 1/50	201	340	401	245
10	N_1	132	122	124	104
	N_2	127	126	116	140
	P_1	103	110	124	138
	P_2	107	126	117	117
	$N_2 + P_1$	114	122	105	97
	N + P*	92	118	105	103
	LVW 1/50	103	121	88	107

Bioassay No.	Treatment	Station 2	Station 4	Station 5	Station 8
11	N_1	95	113	95	108
	N_2	101	115	102	107
	P_1	113	149	153	116
	P_2	118	160	159	120
	$N_2 + P_1$	148	158	180	127
	N + P*	146	171	150	117
	LVW	152	192	232	123
12	N_1	98	114	129	112
	N_2	95	118	102	106
	P_1	99	130	98	131
	P_2	101	130	118	120
	$N_2 + P_1$	109	130	121	107
	N + P*	102	141	128	130
	LVW 1/50	114	137	143	140
13	N_2	105	105		
	P_2	127	118		
	$N_2 + P_1$	117	131		
14	N_1	103	92	119	121
	N_2	103	106	110	120
	P_1	100	244	224	192
	P_2	101	234	259	184
	$N_2 + P_1$	97	224	361	183
	N + P*	97	288	390	181
	LVW 1/50	99	342	559	455

Bioassay No.	Treatment	Station 2	Station 4	Station 5	Station 8
16	N_1	120	142	115	98
	N_2	125	151	151	100
	P_1	106	190	191	261
	P_2	103	207	201	266
	$N_2 + P_1$	72	159	102	165
	N + P*	83	122	129	155
	LVW 1/50	102	211	207	246
18	N_1	111	120	121	110
	N_2	115	109	106	130
	P_1	114	302	143	315
	P_2	115	298	147	258
	$N_2 + P_1$	111	288	146	314
	N + P*	115	310	162	322
	LVW 1/50	141	410	202	345
20	N_1	108	114	115	94
	N_2	125	123	112	106
	P_1	162	125	153	308
	P_2	192	148	181	178
	$N_2 + P_1$	151	144	177	271
	N + P*	164	129	164	202
	LVW 1/50	205	230	263	300
21	N_1	104	112	146	98
	N_2	122	126	133	109
	P_1	111	126	190	102
	P_2	100	147	208	104
	$N_2 + P_1$	114	162	262	125
	N + P*	120	142	189	116
	LVW 1/50	115	391	246	515

Bioassay No.	Treatment	Station 2	Station 4	Station 5	Station 8
22	N_1	90	117	121	132
	N_2	118	127	104	129
	P_1	110	121	167	367
	P_2	103	151	136	350
	$N_2 + P_1$	174	192	203	490
	N + P*	203	159	167	800
	LVW 1/50	271	466	643	2124
23	N_1	103	149	101	117
	N_2	106	124	112	91
	P_1	92	289	428	285
	P_2	94	329	361	220
	$N_2 + P_1$	105	562	443	291
	N + P*	108	679	690	444
	LVW 1/50	113	789	1093	647
25	N_1	86	174	139	96
	N_2	92	193	139	101
	P_1	96	117	108	493
	P_2	93	129	107	544
	$N_2 + P_1$	101	396	425	924
	N + P*	114	497	466	683
	LVW 1/50	117	453	668	943
26	N_1	133	100	102	121
	N_2	176	103	103	119
	P_1	103	98	148	1334
	P_2	110	103	155	1295
	$N_2 + P_1$	175	320	585	1416
	N + P*	190	392	646	1164
	LVW 1/50	192	499	743	1668

Bioassay No.	Treatment	Station 2	Station 4	Station 5	Station 8
27	N_1	98	144	144	89
	N_2	106	133	159	95
	P_1	103	138	106	165
	P_2	110	148	100	195
	$N_2 + P_1$	112	401	337	342
	N + P*	127	425	461	324
	LVW 1/50	121	386	604	572
28	N_1	118	143	149	88
	N_2	144	215	176	139
	P_1	110	108	117	620
	P_2	114	101	113	551
	$N_2 + P_1$	151	264	412	645
	N + P*	156	283	484	617
	LVW	147	315	468	3961
29	N_1	120	143	139	97
	N_2	139	209	173	122
	P_1	98	98	89	208
	P_2	104	101	88	227
	$N_2 + P_1$	141	215	282	239
	N + P*	156	263	353	244
	LVW 1/50	151	224	278	560
30	N_1	114	111	151	106
	N_2	136	156	228	110
	P_1	99	97	107	222
	P_2	103	102	106	219
	$N_2 + P_1$	140	157	264	488
	N + P*	157	183	313	670
	LVW 1/50	135	169	269	687

Bioassay No.	Treatment	Station 2	Station 4	Station 5	Station 8
31	N_1	140	142	155	108
	N_2	188	247	204	102
	P_1	114	110	105	182
	P_2	114	107	109	192
	$N_2 + P_1$	206	252	279	220
	N + P*	205	333	383	205
	LVW 1/50	197	274	302	362

Statistical Analysis: student's two-tailed t-test, 95% level

Bioassay No.	Treatment	Station 2	Station 4	Station 5	Station 8
1	N_1	NS	NS	NS	NS
	N_2	NS	NS	NS	NS
	P_1	NS	NS	NS	NS
	P_2	NS	NS	NS	NS
2	N_1	.025	.010	NS	.025
	N_2	.001	NS	NS	NS
	P_1	NS	NS	NS	NS
	P_2	NS	NS	NS	NS
4	N_1	NS	NS	.050	NS
	N_2	NS	.001	NS	NS
	P_1	NS	NS	NS	NS
	P_2	NS	NS	NS	NS
5	N_1		NS		NS
	N_2		.050		NS
	P_1		NS		NS
	P_2		NS		NS
6	N_1	.001	.005	.001	.005
	N_2	.001	.005	.001	.025
	P_1	NS	NS	NS	NS
	P_2	NS	NS	NS	NS
8	N_1	.005	NS	NS	.001
	N_2	.010	NS	.025	.025
	P_1	.025	.001	NS	NS
	P_2	NS	.001	NS	NS
9	N_1	.001	.001	.001	NS
	N_2	.001	.001	.001	.010
	P_1	NS	NS	.050	NS
	P_2	NS	NS	.025	.050

Bioassay No.	Treatment	Station 2	Station 4	Station 5	Station 8
10	N_1	.005	.050	NS	NS
	N_2	.050	NS	.050	.005
	P_1	NS	NS	.010	NS
	P_2	NS	NS	.025	NS
11	N_1	.050(-)	.025	.010(-)	NS
	N_2	NS	.025	NS	NS
	P_1	NS	.010	.001	.025
	P_2	.005	.001	.001	NS
12	N_1	NS	NS	.010	NS
	N_2	NS	NS	NS	NS
	P_1	NS	.010	NS	.050
	P_2	NS	.025	NS	NS
13	N_2	NS	NS		
	P_2	.005	.025		
14	N_1	NS	NS	.050	NS
	N_2	NS	NS	NS	NS
	P_1	NS	.001	.001	.001
16	N_1	NS	NS	NS	NS
	N_2	.050	NS	NS	NS
	P_1	NS	.025	.050	.001
	P_2	NS	.010	.050	.001
18	N_1	NS	.050	NS	NS
	N_2	.050	NS	NS	NS
	P_1	.050	.001	NS	.005
	P_2	NS	.001	NS	.050

Bioassay No.	Treatment	Station 2	Station 4	Station 5	Station 8
20	N_1	NS	NS	NS	NS
	N_2	.005	NS	NS	NS
	P_1	.001	.050	.050	.005
	P_2	.005	.005	.005	NS
21	N_1	NS	NS	.005	NS
	N_2	.010	NS	.005	NS
	P_1	.025	NS	.001	NS
	P_2	NS	.005	.001	NS
22	N_1	NS	NS	NS	NS
	N_2	NS	NS	NS	NS
	P_1	NS	NS	.001	.001
	P_2	NS	.025	.010	.001
23	N_1	NS	.010	NS	NS
	N_2	NS	NS	NS	NS
	P_1	NS	.001	.001	.005
	P_2	.050(-)	.001	.005	NS
24	N_a		.025		NS
	N_b		.001		.025
	P_a		.050		NS
	P_b		.001		.010
	P_c		.001		.001
	Fe_1		.050		.025(-)
	Fe_2		.010		.025(-)
	Si_1		.001		NS
	Si_2		.001		.050(-)
	NaEDTA		.005		.025

Bioassay No.	Treatment	Station 2	Station 4	Station 5	Station 8
25	N_1	NS	.001	.010	NS
	N_2	NS	.001	.005	NS
	P_1	NS	.050	NS	.001
	P_2	NS	.010	NS	.005
	Fe_1	NS	NS	NS	.025(-)
	Fe_2	NS	NS	NS	NS
26	N_1	.005	NS	NS	.050
	N_2	.001	NS	NS	NS
	P_1	NS	NS	.001	.001
	P_2	.050	NS	.001	.001
	Si	.001(-)	.005(-)	.025(-)	NS
27	N_1	NS	.005	.050	NS
	N_2	NS	.025	.005	NS
	P_1	NS	.025	NS	.005
	P_2	.025	.050	NS	.001
28	N_1	.050	.001	.001	NS
	N_2	.001	.001	.001	NS
	P_1	NS	NS	.010	.001
	P_2	NS	NS	.010	.005
29	N_1	.005	.025	.005	NS
	N_2	.001	.001	.005	NS
	P_1	NS	NS	NS	.001
	P_2	NS	NS	NS	.001
30	N_1	.001	.025	.001	NS
	N_2	.001	.001	.001	NS
	P_1	NS	NS	.010	.001
	P_2	NS	NS	NS	.001

Bioassay No.	Treatment	Station 2	Station 4	Station 5	Station 8
31	N_1	.001	.001	.001	.005
	N_2	.001	.001	.001	NS
	P_1	.010	.025	NS	.001
	P_2	.025	.010	NS	.005

APPENDIX D

Regular Bioassays

(Detailed Protocol)

Regular Bioassay 1 (July 23 - July 28, 1979)

1. Lakewater samples collected at 2030 - 2130 on the evening of July 23, 1979 from stations 2,4,5, and 8.

2. Enrichments were different for this, the first bioassay, than for all of the other bioassays which follow the standard enrichment schedule. In this bioassay N_1 and P_1 were 22µg/l N and P enrichments and N_2 and P_2 were 55µg/l N and P enrichments, respectively. The $N_2 + P_1$ treatment was 55µg/lN + 22µg/lP. There was no N + P* treatment.

3. Additional special treatments included a 22µg/l Fe (as $FeCl_3$) enrichment, and two levels of enrichment by filtered LVW water, a 1/50 and a 1/100 dilution.

4. The bioassay was incubated for only four days, with in vivo fluorescence measured daily on a Turner Designs fluorometer. All other bioassays utilized the Turner 111 fluorometer. The bioassay was terminated on July 28.

Regular Bioassay 2 (July 30 - August 4, 1979)

1. Lakewater samples collected at 2000-2130 on July 30, 1979 from stations 2, 4, 5, and 8.

2. Enrichments were initiated at 2230 on July 30, as per the standard enrichment schedule, except there was no N+P* treatment.

3. The 3 replicate large cubitainers (N_2 + P_1 enrichment) showed an algal growth response very similar to that of the regular size cubitainers.

4. The bioassays were incubated for five days and *in vivo* fluorescence was measured daily. The bioassays were terminated on August 4, 1979.

Regular Bioassay 4 (August 21 - August 25, 1979)

1. Lakewater samples were collected from stations 2, 4, 5, and 8 at 1930 - 2130 August 20.

2. Enrichments were initiated at 0030 on August 21 as per standard enrichment schedule. There was no N+P* treatment.

3. The 3 replicate large cubitainers (LVW enrichment) showed a mean maximum algal growth response approximately 50% higher than for the regular size cubitainers. Both treatments showed responses significantly above the control.

4. The bioassays were incubated for five days with *in vivo* fluorescence measured daily. The bioassays were terminated on August 25.

Regular Bioassay 5 (August 30 - September 4, 1979)

1. Lakewater samples were collected from Station 2, 4, 5, and 8 at 2000-2100 on August 30.

2. Enrichments were initiated at 2300 as per standard enrichment schedule. There was no N+P* treatment.

3. The 3 replicate large cubitainers (N_2 + P_1 enrichment) at station 5 showed a similar algal growth response to that of the regular size cubitainers.

4. The bioassays were incubated for five days with in vivo fluorescence measured daily. This bioassay was the first to be shaded by parachute netting draped over the outdoor pools. All subsequent bioassays were similarly shaded. The bioassay was terminated on September 4.

Regular Bioassay 6 (September 6 to September 11, 1979)

1. LVW samples (in duplicate) were obtained at Northshore Rd. at 0700 on September 6.

2. Chemical analysis of LVW samples was performed at the CCSD(Clark County Sanitation District) Laboratory:

	#1	#2	#3
NO_3	9.1 mg/1	9.1 mg/1	9.1 mg/1
NO_2	2.0 "	1.9 "	1.9 "
NH_3	5.2 "	5.1 "	5.1 "
org-N	0.17"	0.11"	0.14"
total-P	1.66"	1.66"	1.66"
ortho-P	1.35"	1.33"	1.34"

TDN (Total Dissolved Nitrogen) - 16.2 mg/1 N

Therefore, to prepare the N+P* treatment we add 292 ugN and 24 ugP to yield final concentrations of 324 ug/1N and 27 ug/1P in the cubitainers.

3. Lakewater was collected at 1930-2100 at stations 2,4,5, and 8.

4. Enrichments were initiated at 2300 as per standard enrichment schedule

5. Additional special treatments included a comparison of the response due to enrichment by 166 ug/1 of N and $NaNO_3$ to the response elicited by enrichment with 83 ug/1 of N as NH_4Cl and 83 ug of N as $NaNO_3$. At both stations 2 and 4 the results showed the two treatments were not significantly different.

Bioassay 6 (continued)

6. The maximum growth response of the 3 replicate large cubitainers (N+P* treatment) was very similar in value to that of the regular size cubitainers. Both were significantly elevated above the control levels.

7. The bioassay was incubated for five days with *in vivo* fluorescence measured daily. The bioassay was terminated on September 11.

Regular Bioassay 8 (September 24 - September 29, 1979)

1. LVW samples were collected at 0700 on September 24, 1979.

2. The N+P* treatment was prepared by adding 184µgN + 23 gP to yield final concentrations of 204µg/1N and 25µg/1P in the cubitainers.

3. Lakewater samples were collected at 1930-2100 on September 24.

4. Enrichments were initiated at 2300 as per standard enrichment schedule.

5. The maximum response for the 3 replicate large cubitainers (N_2+P_1) was significantly higher than for the control and for the regular size cubitainers.

6. The bioassay was incubated for five days with in vivo fluorescence measured daily. The bioassay was terminated on September 29, 1979.

Regular Bioassay 9 (October 10 - October 15, 1979)

1. LVW sample was collected at Northshore Rd at 0700 on October 10. Duplicate samples were collected (#1 and #2).

2. Chemical analysis of OVW samples was performed at CCSD Laboratory:

	#1	#2	Mean
NO_3	8.4 mg/l	9.0 mg/l	8.7 mg/l
NO_2	1.6 "	1.7 "	1.65"
NH_4	6.2	6.0 "	6.1 "
Total-P	2.32"	2.32"	2.32"
Ortho-P	1.16"	1.10"	1.13"

TDN = 16.45 mg/lN

Therefore, to prepare the N+P* treatment we added 296µgN + 20µgP to yield a final concentration of 329µg/lN + 23µg/lP.

3. Lakewater was collected from station 2, 4, 5, and 8 at 2-0-2200.

4. Enrichments were initiated at 2230 as per standard enrichment schedule.

5. Special treatments in addition to the regular protocol consisted of a set of dark controls from stations 2, 4, and 5. This was designed to test whether light shock was causing initial fluorescence declines observed in several bioassays. The results showed that the dark controls evidenced the same decline as unshaded samples, indicating light shock was probably not the cause of the initial decline.

Rioassay 9 (continued)

6. Bioassays were incubated for 5 days in the outdoor pools, with fluorescence monitored daily. The bioassay was terminated on October 15, 1979.

7. The mean maximal algal growth response for the 3 replicate larbe cubitainers (LVW enrichment) at station 4 was only about 70% of that for the regular size cubitainers, but both were significantly above control.

Regular Bioassay 10 (October 25 - November 1, 1979)

1. LVW sample was taken at Northshore Road at 0700 on October 24.

2. Chemical analysis of LVW sample was performed at CCSD Laboratory:

NO_3 - 10.2 mg/1
NO_2 = 1.25 mg/1
NH_3 = 7.4 mg/1
ortho-P= 1.8 mg/1
TDN = 18.85 mg/1N

Therefore, to prepare the N+P* treatment we added 339µgN + 32µgP to yield a final concentration of 377µg/1N + 36µg/1P.

3. Lakewater was collected from station 2, 5 and 8 at 1900-2100 on October 25.

4. Enrichments were initiated at 2100 as per standard enrichment schedule.

5. Lakewater from station 4 was collected for use in both the in situ plastic enclosure bioassay and the regular bioassay between 1300-1400 on October 26, 1979. The water was sampled using a gas-powered water pump. An integrated 0-5 m sample was obtained by raising and lowering the intake hose through the water column.

6. The station 4 lakewater was returned to the UNLV Lab at 1800 on October 26 and enrichments were initiated at 2000-3000 as per the regular enrichment schedule.

Bioassay 10 (continued)

7. Assays for stations 2, 5, and 8 were incubated for seven days, station 4 bioassays were incubated for six days. The bioassay was terminated on November 1, 1979.

8. The mean maximum algal growth response for the 3 replicate large cubitainers (N+P* enrichment) at station 4 was higher than that of the regular cubitaners (1.7 fold higher), however, both showed significant stimulation as compared to the control.

Regular Bioassay 11 (November 15-November 21, 1979)

1. LVW sample was collected at Northshore Road at 0700 on November 15. Two samples were collected (#1 and #2).

2. Chemical analysis of LVW samples was performed at CCSD laboratory:

	#1	#2	Mean
NO_3	13.8mg/l	14.8mg/l	14.30mg/l
NO_2	1.20"	1.00"	1.10"
NH_3	7.73"	7.90"	7.82"
Total-P	2.58"	2.75"	2.67"
Ortho-P	1.04"	1.32"	1.18"

TDN = 23.22 mg/lN

Therefore to prepare the N+P* treatment, we added 417.6µgN + 21.6µgP to yield a final concentration of 464µg/lN + 24µg/lP.

3. Lakewater was collected from stations 2, 4,5 and 8 at 1900=2100.

4. Enrichments were initiated at 2000 as per the standard enrichment schedule.

5. In addition to standard enrichments, a set of dark controls was prepared to compare their response to unshaded controls. Enrichment was also made to Station 2,4,5, and 8 samples with unfiltered LVW water (1:30 dilution) in order to evaluate the stimulatory response to the particulate fraction

6. In this bioassay the set of foil covered (dark) controls at station 5 did not decline in the initial 24-36 hours, as did the unshaded controls. This indicated light shock

Bioassay 11 (continued)

in some way was implicated, although this result has not been substantiated by similar tests in other bioassays.

7. The unfiltered LVW treatments (1:50 dilution) paralleled the growth response shown by the filtered LVW enrichments (1:50 dilution). This implies that the particulate fraction (suspended silt load) of the LV Wash was not by itself stimulatory to algal growth.

8. Bioassays were incubated for six days in outdoor pools with in vivo fluorescence measured daily. The bioassay was terminated on November 21, 1979.

9. The growth response of the 3 replicate large cubitainers (N+P*) at station 5 was perfectly parallel to that demonstrated in the regular size cubitainers, both treatments significantly above controls.

Regular Bioassay 12 (December 6 - December 13, 1979)

1. Duplicate LVW samples (#1 & #2) were collected at Northshore Rd at 0700 on Dec. 6, 1979.

2. Chemical analysis of LVW samples was performed at CCSD Laboratory:

	#1	#2	Mean
NO_3	6.0 mg/l	5.0 mg/l	5.5 mg/l
NO_2	0.8 "	0.7 "	0.75"
NH_4	9.07"	9.13"	9.10"
Total P	3.96"	4.30"	4.13"
Ortho P	1.23"	1.27"	1.25"

TDN = 15.35 mg/1N

∴ To prepare N+P* treatment we added 276µgN + 22.5 gP to yield a final concentration of 307µg/1N + 25µg/1P.

3. Lakewater was collected from station 2,4,5 and 8 at 2000 - 2200.

4. Enrichments were initiated at 0045 on Dec. 7 as per standard enrichment schedule.

5. Samples were incubated for seven days with <u>in vivo</u> fluorescence monitored daily. The bioassay was terminated on December 13, 1979.

6. The mean maximal algal growth response shown by the 3 replicate large cubitainers (LVW) was only slightly different than the regular size cubitainers (LVW enrichment), within 13%. However, the maximum was attained nearly two days earlier in the larger cubitainers.

Regular Bioassay 14 (January 15 - January 21, 1980)

1. LVW sample was collected at Northshore Rd. at 0630 on January 15.

2. Chemical analysis of LVW samples was performed at CCSD Laboratory:

NO_3 - 3.1 mg/1
NO_2 - 0.7 mg/1
NH_4 = 8.5 mg/1
Total P = 3.24 mg/1
Ortho P = 0.70 mg/1

TDN - 12.3 mg/1N

Therefore to prepare the N+P* treatment we added 221µgN + 12.6µgP to yield a final concentration of 246νg/1N and 14µg/1P.

3. Lakewater was collected from station 2,4,5 and 8 at 1700-1830.

4. Enrichments were initiated at 1930 as per standard enrichment schedule.

5. In addition to the standard enrichments, a set of 3 replicate treatments for stations 2,4,5, and 8 were enriched with several additions of P such that the five daily additions, each contributing 33.2µgP/1, would in sum equal the P_2 treatment (166µg/1P). This procedure would allow a comparison between serial and batch enrichments. In fact, it was observed that the serial additions made no significant difference at Station 2, and little difference at Sta. 4,

Bioassay 14 (continued)

however at stations 5 and 8 the maximum growth response attained with the serial P addition was nearly two-fold that of the regular P_2 enrichment. One explanation for this phenomenon is that when the algae are exposed to large surplus amounts of P, they tend to accumulate polyphosphate storage product, the formation of which not only takes energy (ATP) but also delays the onset of subsequent utilization. Organisms exposed to serial additions of lower amounts of P would utilize the inorganic P directly and not expend a fraction of ATP for polyP formation. This would confer a growth advantage and would also explain why at Sta. 2, where there is already a surplus of P, no difference was seen to exist between serial P additions and batch additions (166µg/l).

6. The mean maximal algal growth response for the 3 replicate large cubitainers (P_2 enrichment) was somewhat higher than for the regular cubitainer P_2 enrichments (30% higher); however, both types of container indicated the same result, namely that the P_2 treatment was significantly higher than the controls.

Regular Bioassay 16 (February 15 - February 21, 1980)

1. LVW sample was collected at Northshore Rd. at 0630 on February 13.

2. Chemical analysis of LVW samples was performed at CCSD Laboratory:

NO_3 - 0.88 mg/l
NO_2 - 1.00 "
NH_4 - 5.26 "
Ortho P - 1.04 "
Total P - 1.32 "

TDN = 7.14 mg/lN

Therefore, to prepare the N+P* treatment, we added 129µgN + 19µgP to yield a final concentration of 143µg/lN + 21µg/lP.

3. Lakewater was collected from station 2,4,5, and 8 at 1700-1830 on February 14. Note that Station 8 was sampled during a heavy thunderstorm and wind mixing might have led to increased nutrient levels.

4. In addition to the regular treatments, a set of special nitrogen enrichments were prepared (Sta. 5) using very high levels of both NH_4 and NO_3. Results indicated that even very high enrichments of NO_3 were not significantly different than the controls, although corresponding high levels of NH_4 (1.98 mg/l) did in fact show a nearly two-fold increase above control levels.

5. Since a heavy storm increased LV Wash flow on the day before the initiation of this bioassay, a special comparison of LVW treatments was set up, using LVW samples taken before the

Bioassay 16 (continued)

storm (2.14). This test should determine the effect of high storm flows on the algal growth response in LVW enrichments. It is most interesting to note that at Station 5, the LVW* enrichments (filtered) taken after the storm showed a 75% higher positive response when compared to LVW enrichments using water sampled before the storm. At Station 8 the difference was even greater, the post-storm LVW samples yielding nearly twofold higher responses than the pre-storm samples. Apparently the high flows somehow change the nutrient status of the LVW samples and are more stimulatory, especially at outer Bay and Boulder Basin.

6. Bioassays were incubated for seven days. In vivo fluorescence was measured daily (except Feb. 17). The bioassay was terminated on February 21.

7. The profile of algal growth response for the large cubitainers (P_2 treatment) was nearly identical to that demonstrated by the P_2 enrichments in the regular size cubitainers, both significantly above control.

Regular Bioassay 18 (March 13 - March 18,1980)

1. LVW sample was collected at Northshore Rd. at 0630 on March 12. Duplicate samples were collected.

2. Chemical analysis of LVW samples was performed at CCSD Laboratory:

	#1	#2	Mean
NO_3	1.30 mg/l	1.30 mg/l	1.30 mg/l
NO_2	0.29 "	0.29 "	0.29 "
NH_4	8.28 "	8.29 "	8.29 "
org-N	0.34 "	9.34 "	9.34 "
Total-P	3.4 "	3.3 "	3.35 "
Ortho-P	2.1 "	2.1 "	2.1 "

TDN = 9.88 mg/lN

Therefore to prepare the N+P* treatment we added 178.2µgN and 37.8µgP to yield a final concentration in the cubitainer of 198µg/lN and 42µg/lP.

3. Lakewater was collected at 2100 on March 12 from Stations 2,4,5, and 8.

4. Enrichments were initiated at 2300 on March 12 as per standard enrichment schedule.

5. Special treatments, in addition to the regular protocol, included a comparison of the response elicited by KH_2PO_4 as opposed to the NaH_2PO_4, the latter used normally in the regular bioassay. At both stations 4 and 8 little difference was evident.

6. The large cubitainer treatments on this bioassay were contaminated and do not reflect the true response.

7. The bioassay was incubated for five days, with in vivo fluorescence measurements taken daily (except March 18). The bioassay was terminated on March 18.

Regular Bioassay 20 (April 3 - April 8, 1980)

1. LVW sample was collected at Northshore Rd. at 0645 on April 2.

2. Chemical analysis of LVW sample was performed at CCSD laboratory:

NO_3 - 1.92 mg/1
NO_2 - 0.36 mg/1
NH_3 - 10.5 mg/1
org-N - 0.17 mg/1
Total P - 2.3 mg/1
Ortho P - 1.6 mg/1

TDN = 12.78 mg/1N

Therefore to prepare the N+P* treatment, we added 230µgN and 29µgP to yield a final concentration in the cubitainer of 256µg/1N and 32µg/1P.

3. Lakewater was collected from stations 2,4,5, and 8 at 1800-2000.

4. Enrichments were initiated at 2200 as per the standard enrichment schedule.

5. The mean maximal algal growth response for the 3 replicate large cubitainers (P_1 enrichment) was significantly higher than the equivalent regular size cubitainer P_1 enrichments (~50% higher) at Station 5; however, as in almost all cases thus far, both size cubitainers yielded the same result, in this case P_1 treatments were significantly above control.

Bioassay 20 (continued)

6. The bioassays were incubated for 5 days with *in vivo* fluorescense measured daily (except April 4). The bioassay was terminated on April 8.

Regular Bioassay 21 (April 17 - April 22, 1980)

1. LVW sample was collected at Northshore Rd. at 0630 on April 16.

2. Chemical analysis of LVW sample was performed at CCSD Laboratory:

NO_3 - 1.95 mg/l
NO_2 - 0.65 mg/l
NH_4 - 11.70 mg/l
org-N - 0.34 mg/l
Total P - 3.3 mg/l
Ortho P - 2.2 mg/l

TDN = 14.3 mg/lN

Therefore to prepare the N+P* treatment we added 258µgN and 40µgP to yield a final concentration in the cubitainer of 286µg/lN + 44µg/lP.

3. Lakewater was collected from stations 2,4,5 and 8 at 1800-2000.

4. Enrichments were initiated at 2200 as per standard enrichment schedule.

5. The mean maximal growth response for the 3 replicate large cubitainers (LVW enrichment) at Station 5 was over twofold that of the regular size cubitainers; however, both treatments were significantly above the control levels.

6. Bioassays were incubated for six days and in vivo fluorescence was measured daily (except April 18). The bioassay was terminated on April 22, 1980.

Regular Bioassay 22 (May 2 - May 8, 1980)

1. LVW sample was collected at Northshore Rd at 0630 on May 1.

2. Chemical analysis of LVW sample was performed at CCSD Laboratory:

NO_3 - 1.88 mg/1
NO_2 - 0.62 mg/1
NH_4 - 11.40 mg/1
org-N - 0.28 mg/1
Total P - 3.13 mg/1
Ortho P - 1.85 mg/1

TDN = 13.9 mg/1

Therefore, to prepare the N+P* treatment, we added 250µgN + 33µgP to yield a final concentration of 278µg/1N and 37µg/1P in the cubitainer.

3. Lakewater was collected from Stations 2,4,5 and 8 at 1800-2000.

4. Enrichments were initiated at 2200 as per standard enrichment schedule.

5. The algal growth response in the 3 replicate large cubitainers (controls) generally followed that response demonstrated by the regular size control cubitainers, however, the large cubitainers attained a mean maximal value about 20% higher.

6. Bioassays were incubated for seven days with <u>in vivo</u> fluorescence measured daily (except May 3). The bioassay was terminated on May 8, 1980.

Regular Bioassay 23 (May 20-May 26, 1980)

1. LVW sample was collected at Northshore Rd. at 0630 on May 20.

2. Chemical analysis of LVW sample was performed at CCSD Laboratory:

NO_3 - 2.8 mg/1
NO_2 - 0.9 mg/1
NH_4 - 10.1 mg/1
org N - 0.22 mg/1
Total P - 2.39 mg/1
Ortho P - 1.75 mg/1

TDN = 13.8 mg/1N

Therefore, to prepare the N+P* treatment, we added 248µgN and 32µgP to yield a final concentration of 276µg/1N and 35µg/1 P in the cubitainers.

3. Lakewater was collected at Stations 2,4, 5, and 8 at 2200. A dense algal bloom evident at Station 2.

4. Enrichments were prepared as per the standard enrichment schedule at 2400 - 0530, May 21.

5. The mean maximum growth response for the 3 replicate large cubitainers (N_2 enrichment) was 23.5, this compared to a value of 14 for the regular size cubitainers. In this case there appears to be a difference in the response of the two treatments.

6. The bioassays were incubated for 5 days with <u>in vivo</u> fluorescence measured daily (except May 22). The bioassays were terminated on May 26, 1980.

Regular Bioassay 25 (June 10 - June 14, 1980)

1. LVW sample was collected at Northshore Rd. at 0630 on June 9.

2. Chemical analysis of LVW sample was performed at CCSD laboratory:

NO_3 - 1.38 mg/l
NO_2 - 0.42 mg/l
NH_4 - 9.24 mg/l
org-N - 0.22 mg/l
Total-P - 1.98 mg/l
Ortho-P - 1.67 mg/l

TDN - 11.24 mg/lN

Therefore, to prepare the N+P* treatment we added 203µgN + 30µgP to yield a final concentration of 225µg/lN + 33µg/lP in the cubitainers.

3. Lakewater was collected from stations 2,4,5 and 8 at 2000-2200.

4. Enrichments were initiated at 2300 as per standard enrichment schedule.

5. Special treatments, in addition to the regular protocol, consisted of two levels of Fe (iron) enrichment at levels of 10µg/l and 50µg/l Fe (as $FeCl_3$). At none of the stations was there any evidence of significant stimulation due to Fe enrichment.

6. The algal growth response for the 3 replicate large cubitainers (N_2 + P_1 enrichment) at Sta 4 was very similar to that for the regular size cubitainers, both significantly elevated above control levels.

Bioassay 25 (continued)

7. Bioassays were incubated for 5 days in the outdoor pools, with *in vivo* fluorescence measured daily. The bioassay was terminated on June 14, 1980.

Regular Bioassay 26 (June 19 - June 23, 1980)

1. LVW sample was collected at Northshore Rd. at 0630 on June 16.

2. Chemical analysis of LVW sample was performed at CCSD Laboratory:

NO_3 - 2.05 mg/1
NO_2 - 1.20 mg/1
NH_4 - 8.40 mg/1
org-N - 0.22 mg/1
Total-P - 1.88 mg/1
Ortho-P - 1.48 mg/1

TDN = 11.75 mg/1 N

Therefore, to prepare the N+P* treatment, we added 212µgN and 27µgP to yield a final concentration of 235µg/1N and 30µg/1P in the cubitainers.

3. Lakewater was collected from Station 2,4,5 and 8 at 19-2100 on 18 June.

4. Enrichments were initiated at 2310 as per standard enrichment schedule.

5. Special treatments, in addition to the regular protocol, consisted of a silica enrichment of 20 mg/1 ($Na_2SiO_3 \cdot 9H_2O$). Results showed that in no case did silica enrichment stimulate algal growth. In fact, at Station 2, silica showed a considerable suppression of algal growth, even as compared to the control. This pattern was the same at Stations 4 and 5.

Bioassay 26 (continued)

6. The algal growth response for the 3 replicate large cubitainers (N_2 + P_1) enrichment was very similar to that for the regular size cubitainers, although the latter was about 20% higher. Both sizes showed significant responses as compared to the controls.

7. Bioassays were incubated for 5 days, with in vivo fluorescence measured daily. The bioassay was terminated on June 23.

Regular Bioassay 27

1. LVW sample was collected at Northshore Rd. at 0630 on July 1.

2. Chemical analysis of LVW samples was performed at CCSD Laboratory:

NO_3 - 3.40 mg/1
NO_2 - 1.25 mg/1
NH_4 - 9.10 mg/1
org-N - 0.28 mg/1
Total-P - 2.24 mg/1
Ortho-P - 1.68 mg/1

TDN = 13.75 mg/1N

Therefore, to prepare the N+P* treatment, we added 248µgN and 31µgP to yield a final concentration of 275µg/1N and 34µg/1P in the cubitainers.

3. Lakewater was collected on 2 July 1980, the delay due to rain and severe thunderstorm activity. Station 4 water was collected at 1130, the sample for the regular bioassay taken at the same time as the sample for the in situ assay in plastic enclosures. Sampling at Station 4 was accomplished using a gas powered impeller pump, the integrated sample obtained by continuously raising and lowering the intake hose through the water column (0-5m). Lakewater at Station 2,5, and 8 was collected at 2000-2200.

4. Enrichments were initiated at 2300 as per the standard enrichment schedule.

Bioassay 27 (continued)

5. The growth response for the 3 replicate large cubitainers (N_2 treatment) at Station 5 closely paralleled the response for the regular size cubitainers.

6. Bioassays were incubated for 6 days in the outdoor pools with *in vivo* fluorescence measured daily. The bioassay was terminated on July 8, 1980.

Regular Bioassay 28 (July 18 - July 22, 1980)

1. LVW sample was collected at Northshore Rd. at 0620 on October 17.

2. Chemical analysis of LVW samples was performed at CCSD Laboratory:

NO_3 - 1.9 mg/1
NO_2 - 1.07 mg/1
NH_4 - 7.50 mg/1
org-N - 0.28 mg/1
Total-P - 1.68 mg/1
Ortho-P - 1.50 mg/1
TDN = 10.47 mg/1N

Therefore to prepare the N+P* treatment, we added 188µgN + 27µgP to yield a final concentration of 209µg/1N and 30µg/1P in the cubitainers.

3. Lakewater was collected from Stations 2,4,5 and 8 at 2000-2130.

4. Enrichments were initiated at 2300 as per the standard enrichment schedule.

5. The algal response profile for the 3 replicate large cubitainers (N_2 enrichment) was very similar to that for the regular size cubitainers, with the maximum response of the latter about 10% greater.

6. Bioassays were incubated for 5 days in the outdoor pools with <u>in vivo</u> fluorescence measured daily. The bioassay was terminated on 22 July 1980.

Regular Bioassay 29 (July 31 - August 4, 1980)

1. LVW sample was collected at Northshore Rd. at 0630 on March 12.

2. Chemical analysis of LVW samples was performed at CCSD Laboratory.

NO_3 - 2.50 mg/l
NO_2 - 1.34 mg/l
NH_4 - 7.10 mg/l
org-N - 1.57 mg/l
Total-P - 2.63 mg/l
Ortho-P - 1.53 mg/l

TDN = 10.94 mg/lN

Therefore, to prepare the N+P* treatment, we added 197µg/N + 28µgP to yield a final concentration of 219µg/lN and 31µg/lP in the cubitainers.

3. Lakewater was collected from Station 2,4,5 and 8 at 2130-2330.

4. Enrichments were initiated at 2400 as per standard enrichment schedule.

5. The algal growth response for the 3 replicate large cubitainers (N_2 enrichment) was almost exactly the same as that response shown for the regular size cubitainers.

6. The bioassays were incubated for 3 days in outdoor pools with <u>in vivo</u> fluorescence measured daily. The bioassay was terminated on August 4, 1980.

Regular Bioassay 30 (August 7 - August 11, 1980)

1. LVW sample was collected at Northshore Rd. at 0620 on August 6.

2. Chemical analysis of LVW samples was performed at CCSD Laboratory; however, due to a malfunction of the CCSD spectrophotometer, data for total P and ortho-P were not forthcoming. Also, a substitute chemist performed the N analyses on this date, and the values reported were considered to be anomalous:

NO_3 - 3.50 mg/1
NO_2 - 1.35 mg/1
org-N - 8.01 mg/1

Therefore to determine the amount of N+P to be used to prepare the N+P* enrichment, we used the mean TDN levels and ortho-P concentrations for Bioassays 26-29, which were 11.32mg/1N and 1.55mg/1P. We added 203µgN and 28µgP to yield a final concentration of 226µg/1 N and 31µg/1P in the cubitainers.

3. Lakewater was collected from stations 2,4,5 and 8 at 2000-2200.

4. Enrichments were initiated at 2300 as per the standard enrichment schedule.

5. The algal growth response curve for the 3 replicate large cutitainers (N_2 enrichment) was greatly similar to that for the regular size cubitainer. Both treatments were significantly higher than the control.

6. Bioassays were incubated for 5 days, in the outdoor pools, with <u>in vivo</u> fluorescence measured daily. The bioassay was terminated on August 11, 1980.

Regular Bioassay 31 (September 3 - September 7, 1980)

1. LVW sample was collected at Northshore Rd. at 0630 on September 2.

2. Chemical analysis of the LVW sample was performed at the CCSD Laboratory.

NO_3 - 3.1 mg/l
NO_2 - 1.5 mg/l
NH_4 - 7.2 mg/l
org-N - 0.28 mg/l
Total-P - 1.82 mg/l
Ortho-P - 1.18 mg/l

TDN = 11.8 mg/lN

Therefore to prepare the N+P* treatment, we added 212µgN and 21µgP to yield a final concentration of 236µg/lN and 24µg/lP in the cubitainers.

3. Lakewater was collected from station 2,4,5 and 8 at 2000-2200.

4. Enrichments were initiated at 2300 as per the standard enrichment schedule.

5. The algal growth response for the 3 replicate large cubitainers (N_2+P_1 enrichment) was nearly the same as that for the standard size cubitainers. Both treatments were significantly above the control level.

6. Bioassays were incubated for 5 days in the outdoor pools with in vivo fluorescence measured daily. The bioassay was terminated on September 7, 1980.

APPENDIX E

Special Bioassays

(Regular Protocol)

SPECIAL BIOASSAY 3 (Aug. 8 - Aug. 13, 1979)

Objective

The purpose of this special bioassay was to measure the growth response of the indigenous algal populations at stations 2, 4, 5, and 8 to enrichments of lakewater by untreated secondary effluent, alum treated effluent, and filtered water from the Las Vegas Wash density plume (sampled at 8 m at station 2).

In addition, a regular bioassay enrichment scheme, that is $N_{1,2}$, $P_{1,2}$, and $N_2 + P_1$ treatments, was used in order to delineate whether the pattern of limitation in Virgin Basin was similar to that in Boulder Basin and in L.V. Bay.

Methods

At 1930 on 8 August 1979, water samples were taken at Virgin Basin (station 9) and then between 2030 and 2130 at stations 2, 4, 5, and 8. Untreated secondary effluent was obtained from the city of Las Vegan Treatment Plant. Alum treated effluent was also obtained from the city's plant. The treatment scheme was as follows:

1) untreated secondary effluent ($\frac{1}{50}$ dilution) -18 ml of GF/C filtered effluent per 900 ml of lakewater.

2) alum treated effluent ($\frac{1}{50}$ dilution) -18 ml of GF/C filtered effluent per 900 ml of lakewater.

3) plume enrichment ($\frac{1}{50}$ dilution) -18 ml of water obtained at station 2 at an 8 meter depth.

4) water from Virgin Basin received the following enrichment.

N_1 - 55 µg/ℓ N as $NaNO_2$

N_2 - 166 µg/ℓ N as $NaNO_3$

P_1 - 55 µg/ℓ P as KH_2PO_4

P_2 - 166 µg/ℓ P as KH_2PO_4

N_2+P_1 - 166 µg/ℓ N and 55 µg/ℓ P.

Results

At the time this special bioassay was conducted in early August, the regular bioassays indicated that N was the major limiting nutrient at all of the Las Vegas Bay stations (2, 4, 5) and at Boulder Basin (station 8). Indeed dissolved N: ortho P ratios were 7:1, 3.2:1, 3.2:1 at station 2,4, and 5, respectively, on August 8. At station 8 the ratio was somewhat higher (≃ 8.5:1) and therefore the conditions should have been more in favor of N and P colimitation. At station 8, the untreated addition reached a maximum level nearly 6-fold the control, while the alum treated addition was the same as the control. At this time in the summer, alum treatment and its associated phosphorus removal, had its largest effect at station 8 where P was closest to limitation. At the L.V. Bay stations, both treated and untreated additions showed responses significantly higher than the controls. Presumably the effect of nitrogen (unreduced by alum treatment) is evidenced here. In fact at station 2 (where ortho P levels of 58 µg/ℓ were measured on August 7) the treated addition yielded a maximum response higher than the untreated enrichment. The addition of plume water was only significantly higher than the controls at station 5. Enrichment of Virgin Basin lakewater showed a pattern most similar

to that at Boulder Basin, that is, the highest response by N_2+P_1 additions, and slight stimulation by N alone (N_2), while both P_1 and P_2 treatments yielded no significant response. This is surprising since N:P ratios of >30 persist for most of the summer and ortho P levels are usually less than 3 µg/ℓ.

SPECIAL BIOASSAY 7 (SEPT. 18 - SEPT. 24, 1979)

Objective

This bioassay was aimed at identifying the effect of alum treatment of wastewater effluent on the growth of indigenous algal populations from station 2, 4, 5 and 8. Treatments included additions to lakewater of untreated secondary effluent, alum treated effluent, and also of treated effluent with dissolved ortho P added back, so that the final P concentration was equivalent to that in untreated effluent samples. This latter treatment was designed to isolate the enrichment response to that of phosphorus alone, since alum treatment may change the concentration of other constituents besides P in wastewater. Additionally, treated effluent enrichments were prepared without filtration in order to evaluate the contribution of particulate matter to the overall algal growth response.

Methods

On September 7, 1979, untreated secondary effluent was obtained from the Clark County Sanitation District treatment Plant just downstream of the trickling filter. Treated effluent was also obtained, after alum and polymer addition, from the surface of the clarification tanks. Samples of effluent were returned to UNLV laboratory, filtered, and refrigerated for use. On the afternoon of September 17, Sam Shannon (CCSD chemist) communicated the results of chemical analysis of effluent samples. They were as follows:

	Untreated	Treated
Total P	19.0 mg/1	2.87 mg/1
Diss. Ortho P	1.8 mg/1	0.7 mg/1

The treatments for special bioassay 7 include (3 replicates each)

1. Control - no addition to cubitainer
2. untreated - untreated effluent, filtered (GF/C) and diluted 18 ml to make 900 ml (1/50 dil).
3. treated - treated effluent, filtered (GF/C) and diluted 18 ml to make 900 ml (1/50 dil).
4. treated + PO_4-P - treated effluent with P added as KH_2PO_4 to bring the level of dissolved ortho P in treated samples to the same concentration as in untreated samples. This entailed the addition of 19.8 mg to the cubitainers with treated effluent, to yield a final concentration of 36 ppb in the containers.
5. treated, unfiltered - treated effluent, with no filtration (1/50 dil.)

Due to misjudgement by UNLV sampling crew and a dark moonless night, they were not able to find the station buoys and the bioassay had to be delayed one night. All wastewater samples were kept refrigerated until use on September 18, 1979.

Water samples for special bioassay 7 were taken on the evening of September 18, 1979 between 1930-2100. Samples were epilimnetic composites (1,3,5m) taken by Van Dorn using a 80μ screen to remove zpoplankton. Stations sampled included 2, 4, 5, and 8. Initial fluorescence readings were made on the evening of Sept. 18.

Each day, the large cubitainers were subsampled and 300 ml was filtered through 42.4 mm GF/C filters for subsequent chlorophyll extraction and analysis. Final phytoplankton samples were taken as composites of all three replicates for each treatment and preserved with lugols. Initial water samples from stations 2, 4, 5, and 8 were frozen for subsequent chemical analysis.

Results

This special bioassay was conducted in late September when the regular bioassays indicated primarily N limitation at all stations 2, 4, 5, and 8. On September 20, 1979 ratios of dissolved N:ortho P were 1.5:1, 1.5:1, 1:1, 2:1, at all four of these stations, respectively, and total inorganic N levels were in all cases less than 6 µg/l.

At station 2, where ambient nutrient concentrations were highest, it was not surprising that the maximal algal response was not significantly different when alum treated enrichments were compared to untreated enrichments. Obviously, whether treated or untreated, addition of effluent contained enough N and other factors to show enhanced response over that of the control.

At stations 4 and 5, the highest response to enrichments was demonstrated by unfiltered additions, even those these unfiltered samples were from alum treated effluents. The particulate matter residing in treated waters must contain enough bound nutrients to stimulate algal growth over and above that of the dissolved fraction. At stations 4 and 5 the removal of phosphorus by alum treatment did show up in the significantly higher algal growth response evidenced by untreated enrichments as compared to treated

enrichments. Paradoxically, this effect is demonstrated at a time during the year when N is the primarly limiting nutrient, mostly because effluent enrichments really involve both N and P additions and it is only at these elevated levels of N that P becomes a limiting factor. It is important to note that the treatments using treated effluent with ortho P added back, yield the same high response as that shown by untreated enrichments, thus indicating that the reduction in algal growth response evidenced by alum treatment is solely due to P removal.

At station 8 there are only slight differences between all of the treatments, although the higher responses are shown by untreated and treated, unfiltered enrichments as compared to filtered effluent enrichments.

SPECIAL BIOASSAY 13 (DEC. 18 - DEC 23, 1979).

Objective

The objective of this bioassay was twofold. First, to measure the growth response of the indigenous algal community to enrichments of lakewater by secondary effluent as compared to similar enrichments using treated (alum flocculation) effluent with much of the phosphorus removed (≈ 88% reduction of dissolved P). In some of the treatments, dissolved ortho P was added back to the alum treated enrichments to examine whether the dissolved P fraction was indeed primarily responsible for differential algal growth response. A second aim of this special bioassay was to ascertain whether "light shock" or time of sampling (night vs. day) was the cause of the initial (24-36 hr) decline in fluorescence (and chlorophyll) commonly observed in some of the bioassays performed.

Methods

Untreated and treated secondary effluent was obtained from the Clark County Sanitation District Facility before and after alum flocculation and clarification, respectively. These samples were collected at 0700 on 17 December 1979, and chemical analyses were performed immediately. Results were as follows:

	Untreated mg/l	Treated mg/l
Total P	12.32	0.04
Diss. Ortho P	1.00	0.0
Total Diss. P	3.34	0.40

The available phosphorus removed through alum treatment was considered to be that fraction of the dissolved ortho phosphorus which was reduced. Knowing this concentration, it was then possible

to design an enrichment scheme involving the re-addition of ortho P to the treated enrichments so that the growth response solely attributable to phosphorus removal could be determined. The enrichment scheme is summarized as follows:

Treatment	Station	Enrichment
control	2,4,5,8	-none
untreated	2,4,5,8	-18 ml filtered secondary effluent
treated	2,4,5,8	-18 ml filtered alum treated effluent
treated +P	5	-18 ml filtered alum treated effluent + 1.8 ml of 10 mg/ℓ P-stock solution.
Large cubitainer	5	-72 mg filtered alum treated effluent

Effluent samples used for enrichment were obtained at the same time as those samples used for nutrient analysis. Samples were returned to UNLV Laboratory on ice. Volumes of 1000 ml and 1200 ml for treated and untreated effluent respectively, were filtered through 4.25 cm GF/C filters with 500 ml of each filtrate frozen. The remaining portions of the filtrate were placed in refrigeration for subsequent use as enrichments. Lake water was collected at about 1900-2100 December 18 from stations 2, 4, 5, and 8 using a Van D rn Water Sampler. Standard 1-3-5 m composites were made with zooplankton removed by passing through 80 µ mesh netting, Lake samples were returned immediately to the lab and enrichments were as per the above scheme 0300-0500 December 19, 1979.

In order to determine whether the time of sampling was implicated in the initial decline in fluorescence observed in several of the bioassays, samples of lakewater (station 2 and 4) were collected

both at mid-day and at night. Between 1300-1500 on December 18, 1979, epilimnetic composites were filtered through 80 µ mesh netting into 25 L carboys. The carboys were then enclosed in plastic semiopaque bags to reduce light exposure. Samples were immediately returned to the lab and stored in cool shade for later enrichment. That night at about 1900-2100, lakewater was collected from stations 2 and 4 (the standard 1-3-5 m composite) and filtered through 80µ mesh. The water in a 25L carboy was returned to the lab and enrichments were made to both daylight/night collected samples at 0200 December 19, 1979 as per the following schedule:

Treatment	Station 2&4 Daylight/night collected	Rep	Enrichment
Control	↓	3	none
N_2	↓	3	166 µg/ℓ N
P_2	↓	3	166 µg/ℓ
N_2+P_1		3	166 µg/ℓ N+55 µg/ℓ P

Upon completion of all enrichments as above, the remaining inoculum was reagitated and subsampled as follows: 500 ml transferred to an acid rinsed poly bottle and frozen; 125ml transferred to poly bottles and preserved with 1 ml Lugol's reagent; 300 ml of "night-collected" station 2,4,5, and 8 water treated with $MgCO_3$ filtered through a 4.25 cm GF/C filte and the filter frozen for later pigment extraction; triplicate 8 ml samples of each used for determination of initial in vivo floures-cence.

In vivo fluorescence of non-enriched lake water, collected both during the day and night, at 1, 3 and 5 m discrete depths was monitored through five days of incubation to determine relative growth potentials. At 1330, December 18, 1979, a 3L Van Dorn water sampler was used to collect station 4 water from 1, 3 and 5 m depths respectively. From each haul, approximately 900 ml was passed through 80µ mesh netting directly into each of two small 1 quart containers. These were capped, labeled with time and depth sampled, placed in an ice chest and returned to UNLV at 1900, December 18, 1979. A similar set of "night-collected" samples were taken at station 4 and returned to the lab. Initial in vivo fluorescenses for both sets of duplicates were then recorded and samples placed in the incubation pools. Samples were incubated for five days with fluorescence monitored daily.

In order to determine whether light inhibition may have been the sources of initial population declines, three cubitainers were filled with 900 ml non-enriched station 8 water and completely covered by aluminum foil so as to exclude all light. These were placed in the pools at the same time as normal station 8 controls. Fluorescence of covered cubitainers was monitored.

Bioassays were terminated on December 23, 1970. Final fluorescence measurements were made and 100 ml of each replicate/treatment of the following were composited to give 300 ml, treated with $MgCO_3$, filtered through GF/C filters, and filters frozen for subsent chlorophyll extraction:

Station 2 "treated"

Station 2 "untreated"

Station 2 "untreated + P"

300 ml subsamples from each "large cubitainer" were similarly treated with $MgCO_3$, GC/C filtered and filters frozen for subsequent pigment analysis. Ten ml of each replicate per treatment were composited to 30 ml, preserved with Lugol's reagent and stored for subsequent phytoplankton enumeration.

Results

1) Alum treatment did indeed lower the algal growth response after five days of incubation. This would be expected, as the time of this special bioassay corresponded to a period during which the regular bioassays showed P stimulation at stations 4, 5, and 8. The untreated enrichments generally showed a higher maximum response than the treated +P enrichments, indicating that there are other nutrients or growth factors besides phosphorus which are removed by alum treatment and which may stimulate algal growth.

2) Light shock is probably not the cause of the initial decline in fluorescence. In this special bioassay, both covered and uncovered controls declined initially and the declines were of about equal magnitude. The time of sampling (night vs. day) was also not seen to be a major determinant in the initial decline.

SPECIAL BIOASSAY 15 (JAN. 30 - FEB. 5, 1980)

Objective

In this bioassay, enrichments to lakewater from station 5 were made with varying dilutions of both untreated secondary effluent and alum treated effluent, or selected blends of both together. The objective was to prepare enriched lake water samples with a range of dissolved N: Ortho P ratios, in order to examine how the algal growth response varied as this ratio changed from a range representing N limitation (<10:1) to that of P limitation (>10:1). Nitrogen was added back to treated effluent enrichments to yield high (15:1) N:P ratios. Additionally the direct effect of alum treatment (75% reduction of total dissolved P and >96% reduction of dissolved ortho P) on the algal growth response was evaluated by comparing untreated vs. treated enrichments. Untreated effluent was obtained from the secondary clarifier of the City of Las Vegas Treatment Plant and treated effluent was obtained from the Clark County Sanitation District Plant subsequent to alum flocculation and clarification.

Methods

At 0700-0730 on January 30, 1980, two 4 liter effluent samples were taken, one from the secondary clarifier at the city of Las Vegas Treatment Plant and the other from the clarifier, after alum flocculation at the C.C.S.D. Treatment Plant. One liter of each sample was given to the C.C.S.D. Lab for analysis and the remainder placed on ice and returned to UNLV. Approximately 1.5

liters of each were immediately filtered through 4.25 cm GF/C filters, with 500 ml of the filtrate transferred to an acid rinsed poly bottle and frozen; the remaining filtrate was placed in refrigeration for use as enrichment that same night. Analytical results for the above were reported at 1500 by C.C.S.D.:

	C.L.V.T.P.	C.C.S.D.
NO_3	1.1 mg/l	0.38 mg/l
NO_2	0.18	0.02
NH_4	13.5	18.3
Organic -N	0.84	1.12
Total -P	7.6	3.5
Total Diss. P	8.4	2.18
Diss. Ortho P	5.66	0.20
Fil. Diss. Acid Hyd. P.	7.04	1.20

From these data, N:P ratios were calculated for enrichments by each effluent or blend. Available nitrogen was calculated as the sum of the nitrogen fractions (NO_3+NO_2+NH_4+Org N) whereas available phosphorus was considered to be equal to the total dissolved phosphorus fraction. The C.C.S.D. sample had a ratio of 19.82 mg/l N:2.18 mg/l P or (9.09:1), and the C.L.V.T.P. sample had a ratio or 15.62 mg/l N:8.4 mg/l P or (1.86:1). From these data additions of effluent were developed which alone or blended could yield a broad range of N:P ratios; and for each ratio, a series of dilutions were made giving a broad range of final nutrient concentrations. These constituted the enrichments for the bioassay and are summarized in the following table:

Rep.	Enrichment	ml Sta. 5 Water	Final Dilution	µg N/Enrich	µgP/Enrich.	N:P ratio
3	72 ml filCSSD	828	1/12.5	1427	157	9.09
3	36	864	1/25	713.5	78.5	9.09
3	18	882	1/50	356.8	39.3	9.09
3	9	891	1/100	178.4	19.7	9.09
3	4.5	895.5	1/100	89.2	9.9	9.09
3	18 ml CLVTP	882	1/50	281	151	1.86
3	9	891	1/100	140.5	75.5	1.86
3	4.5	895.5	1/200	70.25	37.75	1.86
3	2.5	898	1/400	35.13	18.88	1.86
3	1.13	899	1/800	17.56	9.44	1.86
3	30 ml 50/50	870	1/30	532	159	3.35
3	15	885	1/60	266	79.5	3.35
3	7.5	892.5	1/120	133	39.8	3.35
3	3.75	897	1/240	66.5	19.8	3.35
3	1.88	898	1/480	33.25	9.95	3.35
3	45 ml 25/75	855	1/20	845	168	5.03
3	22.5	877.5	1/40	422.5	84	5.03
3	11.25	888.75	1/80	211.25	42	5.03
3	5.63	894.5	1/160	105.63	21	5.03
3	2.82	897	1/320	52.82	10.5	5.03
3	72ml filCCSD+9.28 ml 100mg/l-N	819	1/12.5	2355	157	15
3	36ml filCCSD+4.64 ml	859.5	1/25	1177.5	78.5	15
3	18ml filCCSD+2.32 ml	880	1/50	588.75	39.25	15
3	9ml filCCSD+1.16 ml	890	1/100	294.38	19.63	15
3	4.5ml filCCSD+0.58 ml	895	1/200	147.19	9.82	15
3	Control	3564				
3	Lg cub 36 ml CLVTP					

Rep.	Enrichment	ml Sta.5 Inoc.	Final Dilution	µg N/Enrich	µg P/Enrich	N:P Ratio
3	100 ml fil CCSD	800	1/9	1673	15	111.5
	60	840	1/15	1004	9	111.5
	30	870	1/30	502	4.5	111.5
	15	885	1/60	251	2.25	111.5
	7.5	892.5	1/120	125.5	1.125	111.5
3	100 ml fil CLVTP	870	1/30	406	164	2.48
	15	885	1/60	203	82	2.48
	7.5	892.5	1/120	101.5	41	2.48
	3.75	896	1/240	50.75	20.5	2.48
	1.88	898	1/480	25.38	10.25	2.48
3	100 ml 10/90	800	1/9	1641	69	23.8
	60	840	1/15	984	41	23.8
	30	870	1/30	492	20.5	23.8
	15	885	1/60	246	10.25	23.8
	7.5	892.5	1/120	123	5.125	23.8
3	90 ml 25/75	810	1/10	1433	133	10.8
	60	840	1/15	956	95	10.8
	30	870	1/30	478	44.5	10.8
	15	885	1/60	239	22.25	10.8
	7.5	892.5	1/120	119.5	11.125	10.8
3	60 ml 50/50	840	1/15	908	168.5	5.4
	30	870	1/30	454	84.25	5.4
	15	885	1/60	227	42.125	5.4
	7.5	892.5	1/120	113.5	21.06	5.4
	3.75	896	1/240	56.75	10.53	5.4
3	Control					
3	Lg Cub Control					

To prepare the 10/90 solution (10% C.L.V.T.P./90% C.C.S.D.) 67 ml of filtered C.L.V.T.P. effluent were combined with 603 ml of filtered C.S.S.D. effluent and mixed thoroughly. Preparation of the 25/75 solution (25% C.L.V.T.P./75% C.C.S.D.) consisted of the combination of 167.5 ml of filtered C.L.V.T.P. effluent with 502.5 ml of filtered C.C.S.D. effluent. A 50/50 solution was prepared combining 335 ml of both effluent filtrates.

Lake water was collected at 1900-2100 at stations using a Van Dorn Water Sampler. 120 L of a 1-3-5 M composite sample were taken with zooplankton removed by passing through 80 μ mesh netting. Small volume composite samples were also collected from stations 2, 4 and 8. 500 ml of each were placed in acid rinsed poly bottles and later frozen. 125 ml of each were preserved with 1 ml lugols reagent. Lake samples were returned immediately to the lab and enrichments made, as per the enrichment table at 2400 February 26, 1980 - 0430 February 27, 1980. Station 5 water was subsampled for analysis as follows: 500 ml transferred to an acid rinsed poly bottle and frozen; 125 ml transferred to a screw capped poly bottle and preserved with 1 ml lugols reagent; 300 ml treated with $MgCO_3$, filtered through a 4.25 Dia GF/c filter, and the filter frozen for later chlorophyll extraction; triplicate 8 ml samples used for determination of initial in vivo fluorescence.

Samples were incubated for twelve days in the outdoor pools with in vivo fluorescence measured daily excepting two days. 300 ml subsamples were taken daily (except 2 days) from the large cubitainers, treated with $MgCO_3$, GF/C filtered and frozen for later chlorophyll extraction. The bioassay was terminated on

March 9, 1980 after observing fluorescence peaks the previous day. Final fluorescence measurements were made and the following 300 ml composites treated with $MgCO_3$ and GF/c filtered for chlorophyll extraction in addition to the large cubitainters:

10/90 enrichment	1/30 dilution
10/90 enrichment	1/120 dilution
10/90 enrichment	1/9 dilution
10/90 enrichment	1/60 dilution
10/90 enrichment	1/15 dilution

10 ml of each replicate/treatment were composited and preserved with 0.5 ml Lugols reagent. Finally, 80 ml of each replicate for C.C.S.D., C.L.V.T.P., 25/75 enrichments as well as the controls were composited to give 240 ml and frozen.

Results

At the time this special bioassay was conducted, the regular bioassays showed that at station 5 there was a significant response of the indigenous algal population to stimulation by both P and N, although the trends were not totally consistent. In regular bioassay 18 in early March, both P_1 and P_2 additions to station 5 water showed no significant increase above control level, while the N_1 treatment (in large cubitainers only) did stimulate significantly. Earlier, at the end of February P (singly) did yield a significant and high response (in Bioassay 16), while the regular N treatments did not.

This data makes it difficult to interpret the results of this special bioassay, which in fact demonstrated that equivalent dilutions

of untreated versus treated effluent enrichments showed little difference with regard to indigenous algal growth response. For example, in the case of the 1/30 dilution of each, the treated addition gives approximately a 10% higher response. This may be due to the fact that the treated samples had a 20% higher concentration of dissolved N than the untreated samples and that at the time of this special bioassay, the algal population responded positively to the high N:P ratios generated by enrichment with treated effluent (112:1). Interestingly enough, the highest response was attained in the 25:75 (untreated:treated) blend addition where the ratio of 10.8:1 was the most balanced from the point of view of availability of both N and P.

SPECIAL BIOASSAY 19 (MARCH 26-APRIL 5, 1980)

Objective

The objective of this bioassay was very similar to that of the preceeding special bioassay 17, that is to compare the indigenous algal growth response (station 5 water) to enrichments of untreated secondary effluent versus enrichments of alum treated effluent. The dissolved N:P ratio of the treated effluent was high (54:1) while the same ratio for untreated effluent was low (3.5:1). Enrichment was also made with Las Vegas Wash water (Northshore Road) which yielded a relatively low ratio (5.7:1) Treated and untreated effluent was blended to yield intermediate enrichment ratios in order that we might ascertain the value for the N:P ratio where the transition from N to P stimulation occurs.

Methods

At 0600 on March 26, 1980 a 4 liter Las Vegas wash sample was obtained at the North Shore Road sampling station and placed on ice. At 0630-0645 two additional four liter effluent samples were taken, one from the secondary clarifier at the city of Las Vegas Treatment Plant and the other from a point downstream of the alum flocculation tank at the C.C.S.D. Treatment Plant. Samples were placed on ice and transported to the C.C.S.D. lab, where one 1 of each were used for analysis. The remaining samples were returned to UNLV where approximately 1.5 liter of the secondary effluent samples and 1.3 liter of the NSR sample were immediately filtered through 4.25 dia. GF/C filters. 500 ml of each filtrate was transferred to an acid rinsed poly bottle and frozen; the remaining

To prepare the 50/50 solution, 90 ml of filtered CCSD effluent were combined with 90 ml of filtered CLVTP effluent in an acid rinsed 500 ml beaker and mixed thoroughly. Preparation of the 25/75 solution was made similarly with 70 ml of CLVTP fil. effluent, added to 210 ml CCSD effluent. To prepare an enrichment with a 15:1 N:P ratio, CCSD fil. effluent was enriched with NH_4NO_3 stock reagent. For example, a 1/12.5 enrichment of C.C.S.D. effluent + N yielding an N:P ratio of 15 was made by considering the fact that .072 ℓ of C.C.S.D. effluent has 1.427 mg N and 0.57 mg P. Multiplying 0.157 mg P by 15 gives the final nitrogen enrichment desired (2.355). Therefore 0.928 mg N was added to the C.C.S.D. effluent to give the final desired enrichment ratio.

Lake water (1-3-5 m composite) was collected at 1800-2000 at station 5 using a Van Dorn Water Sampler. Also collected were 1-3-5 m composite samples from stations 2 and 4. 500 ml of each were placed in acid rinsed poly bottles and frozen, 125 ml of each were preserved with 1 ml Lugol's reagent. Lake samples were returned immediately to the lab and enrichments initiated at 2200, as per the the enrichment schedule.

Enrichments were completed and all samples were placed in the incubation pools by 0100 on January 31, 1980. The remaining station 5 water was thoroughly remixed and subsample as follows: 500 ml transferred to an acid rinsed poly bottle and frozen; 125 ml transferred to a screw cap poly bottle and preserved with 1 ml Lugol's reagent; 300 ml treated with $MgCO_3$, filtered through a GF/c filter and the filter frozen for later pigment extraction; triplicate 8 ml samples used for determination of initial <u>in vivo</u> fluorescence.

Samples were incubated for six days in the outdoor pools with *in vivo* fluorescence measured daily. 300 ml subsamples were taken daily from the large cubitainers, treated with $MgCO_3$, GF/c filtered and frozen for later chlorophyll extraction. The bioassay was terminated on February 5, 1980. Final fluorescence measurements were made and the following 300 ml composites treated with $MgCO_3$ GF/C filtered for pigment analysis in addition to the large cubitainers.

25/75 enrichment	1/40 dilution
50/50 enrichment	1/30 dilution
50/50 enrichment	1/240 dilution
CCSD + N enrichment	1/200 dilution
CLVTP enrichment	1/100 dilution
Control -	- -

10 ml of each replicate/treatment were composited and preserved with 0.5 ml Lugol's reagent. Finally, selected 240 ml composites were frozen for later analysis.

Results

When this special bioassay was performed, the regular bioassays encompassing this period indicated that at station 5, phosphorus was the primary nutrient that stimulated algal growth. It was therefore an ideal time to test the effect of alum treatment and the concomitant reduction of dissolved P, on the indigenous algal growth response. Indeed, this special bioassay did show that enrichments of treated effluent to lake water (station 5) yielded a mean growth response only 63% of that response shown by similar

dilutions of untreated effluent in lake water.

Data also showed that the indigenous algal growth response in N-enriched treatments with ratios of N:P of 15:1 did not greatly differ from enrichments where ratios were about 9:1. This points to the fact that N was not the primary limiting nutrient at this time and also that above ratios of about 9:1, P exerts the main stimulatory effect on algal growth.

SPECIAL BIOASSAY 17 (FEB. 26 - MARCH 9, 1980)

Objective

In this bioassay, enrichments were made to station 5 lakewater with both untreated secondary effluent and treated (alum flocculation) effluent. The effluents were also blended and added in different dilutions so as to yield a range of final nutrient concentrations. The objective of this bioassay was to prepare additions to station 5 lakewater that yielded a range of dissolved N:P ratios in order to evaluate the algal growth response as a function of the availability of both N and P; a ratio of <10:1 indicated N limitation and a ratio >10:1 indicated P limitation. In this bioassay the direct effect of alum treatment upon the indigenious algal growth response was examined by comparing similar enrichment dilutions of treated versus untreated effluent.

Methods

At 0630-0700 on February 23, 1980, two 4 liter effluent samples were taken, one from the secondary clarifier at the City of Las Vegas Treatment Plant and the other from the flocculation tank at the C.C.S.D. Treatment Plant. One liter of each sample were given to the C.C.S.D. Lab for analysis and the remainder placed on ice and returned to UNLV. Approximately 1.5 liter of each were immediately filtered through 4.25 Dia. GF/C filters, with 500 ml of each filtrate transferred to an acid rinsed poly bottle and frozen; the remaining filtrate placed in refrigeration for use as enrichment that night. Analytical results for the above were

reported at 1500 by C.C.S.D.:

	C.L.V.T.P.	C.C.S.D.
NO_3	0.21	0.04
NO_2	0.09	0.03
NH_4	12.5	15.6
ORG-N	0.73	1.06
Tot-P	6.32	2.95
Tot Dis-P	5.96	0.27
Ortho-P	5.46	0.15

From these data, respective N:P ratios were calculated for each effluent sample. Available nitrogen was calculated as the sum of nitrogen fractions reported (NO_3 + NO_2 + NH_4 + ORG-N) whereas available phosphorus was considered the total dissolved phosphorus fraction. The C.C.S.D. sample had a ratio of 16.73 mg/l N:0.15 mg/l P or 111.5:1 and the C.L.V.T.P. sample had a ratio of 13.53 mg/l N:5.45 mg/l P or 2.48:1. From these data additions of effluent were calculated which alone or in combination gave a broad range of N:P ratios, and for each ratio a series of dilutions was prepared yielding a broad range of concentrations. The enrichments are summarized in the following table:

filtrate was placed in refrigeration for use as enrichment that same night. Analytical results for the above were reported at 1500 by C.C.S.D.:

	LVW	LVCTP	CCSD
NH_4	8.57	12.2	16.4
NO_3	1.61	0.9	0.42
NO_2	0.03	0.20	0.023
ORG-N	0.06	0.62	0.73
Tot-P	5.95	7.25	3.32
Tot Dis-P	3.65	-	1.35
Ortho-P	1.80	3.9	0.325

From these data, respective N:P ratios were calculated for each sample. Available nitrogen was calculated as the sum of nitrogen fractions reported (NO_3 + NO_2 + NH_4 + ORG-N) whereas available phosphorus was considered as equal to the concentration of Ortho-P (not tot Dis. phos, due to lack of data for L.V.C.T.P.). The LVW sample had a ratio of 10.27 mg/l N: 1.80 mg/l P (5.7). The L.V.C.T.P. samples had 13.53 mg N: 3.19 mg P(3.47), and the C.C.S.D. sample 17.57 mg: 0.325 mg P (54.1). From these data additions of LVW, CLVTP and CCSD effluent were calculated which alone or in combination (C.L.V.T.P. + C.C.S.D) yielded a broad range of N:P ratios. For each ratio, a series of dilutions was prepared to give a broad range of concentrations. These consituted the enrichments and are summarized in the following table:

Rep	Enrichment	ml Sta.5 Inoc	Final Dilution	µg/N Enrich.	µg P Enrich.	N:P Ratio
3	100 ml fil. LVW	800	1/9	1027	180	5.7
	60	840	1/15	616	108	5.7
	30	870	1/30	308	54	5.7
	15	885	1/60	154	27	5.7
	7.5	892.5	1/120	77	13.5	5.7
3	100 ml fil. CCSD	800	1/9	1757	32.5	54.1
	60	840	1/15	1054	18.5	54.1
	30	870	1/30	527	9.75	54.1
	15	885	1/60	263.5	4.88	54.1
	7.5	892.5	1/120	131.75	2.44	54.1
3	30 ml Fil. CLVTP	870	1/30	406	117	3.47
	15	885	1/60	203	58.5	3.47
	7.5	892.5	1/120	101.5	29.25	3.47
	3.75	896	1/240	50.75	14.6	3.47
	1.88	898	1/480	25.4	7.3	3.47
3	100 ml 10/90	800	1/9	1716	68	25
	60	840	1/15	1030	41	25
	30	870	1/30	515	20.5	25
	15	885	1/60	257.5	10.25	25
	7.5	892.5	1/120	128.75	5.3	25
3	100 ml 25/75	800	1/9	1656	122	13.6
	60	840	1/15	994	74	13.6
	30	870	1/30	497	37	13.6
	15	885	1/60	248.5	18.5	13.6
	7.5	892.5	1/120	128.75	5.3	13.6
3	60 ml 50/50	840	1/15	933	127	7.3
	30	870	1/30	466.5	63.5	7.3
	15	885	1/60	233.25	31.75	7.3
	7.5	892.5	1/120	116.6	15.9	7.3
	3.75	896	1/240	58.3	7.94	7.3
3	Control					
3	1g Cub LVW 30 ml	3570				

To prepare the 10/90 solution (10% CLVTP/90%CCSD) 67 ml of filtered CLVTP effluent were combined with 603 ml of filtered CCSD effuent and mixed thoroughly. Preparation of the 25/75 solution (25% CLVTP/75% CCSD) consisted of the combination of 167.5 ml of filtered CLVTP effluent with 502.5 ml of filtered CCSD effluent. A 50/50 solution was prepared combining 335 ml of both effluent filtrates.

Lake water was collected at 1800-2000 at station 5 using a Van Dorn Water sampler. 120 L of 1-3-5 M composited sample were taken with zooplankton removed by passing through 80 μ mesh netting. Lake samples were returned immediately to the lab and enrichments made, as per the enrichment table, 2400 March 28, 1980 to 0200 March 29, 1980. Station 5 water was subsampled for analysis as follows: 500 ml transferred to an acid rinsed poly bottle and frozen; 125 ml transferred to a screw capped poly bottle and preserved with 1 ml lugols reagent; 300 ml treated with $MgCO_3$, filtered through a 4.25 cm GF/c filter and the filter frozen for later chlorophyll extraction; triplicate 8 ml samples used for determination of initial in vivo fluorescence.

Samples were incubated for ten days in the outdoor pools with in vivo fluorescence measured on seven of ten days. For days on which fluorescence was measured, 300 ml subsamples were taken from the large cubitainters, treated with $MgCO_3$, GF/c filtered and frozen for later chlorophyll extraction. The bioassay was terminated on April 5, 1980. Final fluorescence measurements were made and the following 300 ml subsamples treated with $MgCO_3$ and GF/c filtered for chlorophyll extraction in addition to the large cubitainers:

LVW enrichment	1/19 dilution
LVW enrichment	1/60 dilution
LVW enrichment	1/60 dilution
LVW enrichment	1/60 dilution

10 ml of each replicate/treatment for low, intermediate and high dilutions of all enrichments were composited to give 30 ml photoplankton samples and preserved with 0.5 ml lugols reagent. 240 ml water chemistry composites were made for selected enrichment replicates.

Results

The pattern of algal response to enrichment in regular bioassay 20, initiated on April 3, 1980 showed that only phosphorus (alone) could significantly stimulate algal growth. However, the results of this special bioassay were surprising in that the alum treated enrichments did not show a reduced algal growth enhancement as compared to the untreated enrichment. In fact the 100% treated enrichment was somewhat more stimulating to algal growth at almost equivalent dilution factors and the 10:90 (treated:untreated) treatment showed a significant and large response as compared to the control. The greatest response of any of the treatments was evidenced by the LVW (Northshore Road) addition, which unlike all of the other treatments, responded positively after the period (48 hrs) of initial decline. The decline in all treatments with effluent (treated or untreated) continued for 4 days. The presence of inhibitory substances (e.g., trace metals) in the effluent of both treatment plants, but not present in LVW waters, might explain this effect.

SPECIAL BIOASSAY 24 (MAY 20 - MAY 26, 1980

Objective

This special bioassay was designed to test the algal response to enrichment by the following factors known to be required for algal growth, including Iron ($FeCl_3$), Silica (SiO_2) EDTA (a chelator) a combination of vitamins (B_{12}, biotin, thiamin) and a combination of nutrients and the vitamin blend (NH_4NO_3, $NaHPO_4$, vitamins $FeCl_3$, SiO_2).

A second objective of this special bioassay was aimed at examining the algal response to enrichment by a broader range of concentrations of both N and P (each added singly) than is normally used in the regular bioassays. In this instance the treatments (station 4 and 8 water) were designed to yield 10, 55, 166 and 500 µg/l N additions and 5, 20, 55, 166 and 500 µg/l P additions

Methods

At 0630 on May 20, 1980 a 4 liter Las Vegas Wash sample was obtained at the Northshore Road sampling station and placed on ice. One liter of the sample was given to the CCSD Lab for chemical analysis and the remainder returned to UNLV. Approximately 750 ml were immediately filtered through a 4.25 cm Dia. GF/C filter, with 500 ml of filtrate transferred to an acid rinsed poly bottle and frozen; the remaining filtrate was placed in refrigeration for use as enrichment that night. Analytical results for the above were reported at 1500 by CCSD:

NO_3	2.8mg/1
NO_2	0.9 "
NH_4	10.1 "
ORG-N	0.22"
Total-P	2.39"
Ortho-P	1.75"

Available nitrogen was calculated as the sum of (NO_3+NO_2 +NH_4) or 13.8 mg/1 nitrogen, whereas available phosphorus was considered to be ortho-phosphorus (1.75 mg/1). These values were used to calculate an N+P* enrichment corresponding to the weight of available N and P contained in 18 ml of filtered Las Vegas Wash enrichment. Multiplying available N and P fractions by 0.018, enrichment with 248 mg nitrogen and 31.5 mg phosphorus were indicated. The iron enrichment was calculated to provide 1x and 5x ambient Lake Mead mean concentrations, which was assumed to be 10 mg/1. Vitamin enrichments employed were identical to those used by Smayda (1974) and consisted of Bi tin, B_{12} and thiamine-HCl, vitamins known to limit various groups of phytoplankton. The Na_2 EDTA enrichment was calculated to provide EDTA chelator in a concentration slightly less than half of that used in Smayda (1974) in a marine system. All enrichments are summarized in the following table:

Treatment	Station	Rep	Enrichment
Control	2,4,5,8	3	none
N_1	2,4,5,8	3	55 µg/l -N
N_2	2,4,5,8	3	166 µg/l -N
P_1	2,4,5,8	3	55 µg/l -P
P_2	2,4,5,8	3	166 µg/l -P
N_2+P	2,4,5,8	3	166 µg/l-N+55mg/l-P
N+P*	2,4,5,8	3	276 µg/l-N+35mg/l-P
LVW	2,4,5,8	3	.018 liter filtered LVW
Na	4,8	3	11.1 µg/l-N
Nb	4,8	3	556 µg/l -N
Pa	4,8	3	5.6 µg/l -P
Pb	4,8	3	22.2 µg/l -P
Pc	4,8	3	556 µg/l -P
Fe_1	4,8	3	10 µg/l -Fe
Fe_2	4,8	3	50 µg/l -Fe
Si_1	4,8	3	10 µg/l -Si
Si_2	4,8	3	50 µg/l -Si
Vit	4,8	3	1 µg/l B_{12} + 1 µg/l Biotine + 200 µg/l Thiamine·HCl
All	4,8	3	276 µg/l -N + 35 µg/l -P+ 50µg/l Fe + 50 µg/l -Si + 1 µg/l B_{12} + 1 µg/l Biotin + 200 µg/l thiamine·HCl
Na_2EDTA	4,8	3	11.1 µg/l Na_2EDTA
Lg Cub (N_2)	4	3	166 µg/l -N

Nutrient stock solutions required for the above (other than nitrogen, phosphorus and SiO_2) were prepared just prior to use. These included 5 mg/1 $FeCl_3$, 1 g/1 Na_2 EDTA, 0.5 mg/1 B_{12} + Biotin and 100 mg/1 thiamine HCI stock. B_{12}+ Biotin stock solution were prepared by dissolving a crushed 100 microgram pharmaceutical tablet of each vitamin into 0.2 liter of DDW over low heat. Thiamine·HCI stock was prepared dissolving a crushed 100 mg thiamin HCi tablet into 1 liter DDW. Note that reagent grade vitamins were not used and these pharmaceutical preparations contain a matrix, presumably yeast extract, which may provide a source of nutrients in addition to the pure vitamin. Without knowing the nutritional contribution of the vitamin matrix, interpretation of the response to these enrichments is unfortunately limited.

Lake water was collected at 1800-2000 at stations 2, 4, 5, and 8 using a Van Dorn water sampler. 75 liters of 1-3-5 M composited sample were taken from each of stations 4 and 8, with 25 liters of composite obtained from both stations 2 and 5. Zooplankton were removed by passing lake water through 80 μ mesh netting. Lake samples were returned immediately to the lab and enrichment made as per the enrichment table at 2400 May 30 - 0530 May 21, 1980. Remaining portions of station 2, 4, 5, and 8 lake water were thoroughly reagitated and subsampled as follows: 500 ml of each transferred to an acid rinsed poly bottle and frozen; 125 ml each transferred to a screw capped poly bottle and preserved with 1 ml lugols reagent; 500 ml each treated with $MgCO_3$, filtered through a 4.25 cm. dia. GF/c filter and the filters frozen for later chlorophyll extraction; triplicate 8 ml samples of each used for

determination of initial in vivo fluorescence.

Samples were incubated for six days in the outdoor pools with in vivo fluorescence measured daily excepting May 22. 500 ml subsamples were taken daily from the large cubitainers, treated with $MgCO_3$, GF/c filtered and filters frozen for later chlorophyll extraction. The bioassay was terminated on May 26, 1980. Final fluorescence measurements were made and the following 500 ml subsamples treated with $MgCO_3$ and GF/c filtered for pigment analysis.

Large cubitainers I, II, III

Stations 8 (all) w/fluor 104.5 (1x)

Stations 5 (N+P*) w/fluor 93.0 (3x)

Stations 4 (N_2+P_1) w/fluor 40.0 (1x)

Ten ml of each replicate/treatment were composited and preserved with 0.5 ml lugols reagent. Finally, selected 240 ml composites were frozen for later analysis.

Literature Cited

Samayda (1974) "Bioassay of the Growth Potential of the Surface Water of Lower Narragansett Bay over an Annual Cycle Using the Diatom *Thalassiosira pseudonana* (oceanic clone, 13-1)," Limnology and Oceanography, (19), 889-901.

Results

The results of this special bioassay indicated that at station 4, at this time in late May, Fe was slightly stimulatory to algal growth, while at station 8, Fe did not enhance algal growth at all. These results are consistent with findings that N (by itself) is stimulatory at station 4 but not at station 8. It is known that

iron is a cofactor of nitrate reductase and is therefore needed for assimilation of nitrate by algae.

Additions of NaEDTA showed a small positive response at station 4 and a larger significant stimulation at station 8. Perhaps the addition of this chelating agent increased metal cofactor availability The vitamin combination was highly stimulatory to algal growth both at station 4 and station 8, as was the nutrient combination. In fact at station 8 the nutrient and vitamin mixture yielded a maximum response nearly 4 times that of LVW (1/50 dilution) treatment and over 6-fold that of the N+P* treatment, indicating the fact that at Boulder Basin (station 8) there are perhaps several other factors besides N and P which may limit algal growth. This makes sense, since station 8 is far removed from the influence of Las Vegas Wash, whose waters may well supply some of these necessary growth factors.

The algal response to the broad range of concentrations of N+P (individually) showed that at phosphorus limited station 8, there was little difference in the growth yield between the 556 and 22 µg/ℓ P treatments. This must indicate that in enrichments with high concentrations of P, some other factor (particularly N) becomes limiting very quickly as the growth rate increases in response to P enrichment. At station 4, where N+P were more colimiting, high enrichments of N (556 µg/ℓ) yielded only a slightly higher response than additions of N one order of magnitude less. Likewise, the treatments of P addition at 556 µg/ℓ and 22 µg/ℓ, differ only so slightly and clearly show again that when both nutrients are co-limiting, additions of one (even in great excess) do not show

marked effects unless the other is also added. Indeed, the results of regular bioassay 23 conducted at the same time as this special bioassay, show that at station 4 the N_2+P_1 treatment is 5.5-fold the control, compared to 3-fold for P alone.

SPECIAL BIOASSAY 32 (SEPT. 5 - SEPT. 10, 1980)

Objective

This bioassay was designed to test whether phosphorus, bound to an artificially prepared alum "floc," still had the potential to stimulate algal growth. The "floc" was prepared by combining a 7mg/l P stock solution with 150 mg/l alum ($Al_2(SO_4) \cdot 18H_2O$) and 1 mg/l Dow Polymer Purifloc^r. This artifically prepared floc was similar to the formulation used in the tertiary treatment process of the Clark County Sanitation District Treatment Facility.

Additionally, lake water from station 2, 4, 5, and 8 was enriched with filtered and unfiltered alum treated effluent to determine if particulate matter that remains subsequent to alum flocculation, has the capacity to stimulate growth in indigenous algal bioassay cultures. Treatments were also prepared by enrichment with NH_4NO_3, to yield N concentrations equivalent to those exhibited by enrichment with alum treated effluent. Such treatments would more specifically identify an enhanced algal response as being derived from P (alone) stimulation.

Methods

At 0700 on Sept. 5, 1980 an eight liter effluent sample was obtained from the C.C.S.D. plant and placed on ice. One liter of the sample was given to the C.C.S.D. lab for chemical analyses and the remainder returned to UNLV. Approximately one liter was immediately filtered through 4.25 cm diameter GF/c filters, with 500 ml of the filtrate transferred to an acid rinsed poly bottle

and frozen. The remaining filtrate was placed in refrigeration for use as an enrichment that night. 500 ml of non-filtered sample were similarly transferred to an acid rinsed poly bottle and frozen, with the remaining returned to refrigeration for later use. Analytical results for the above were reported at 1500 by C.C.S.D.:

NH_4	11.1 mg/l
NO_3	1.68
NO_2	0.45
ORG-N	0.39
Tot-P	0.44
Ortho-P	0.00

Total dissolved nitrogen (TDN) was calculated as the sum of NH_4 + NO_3 + NO_2 or 13.23 mg/l nitrogen; no dissolved phosphorus was detected. The total dissolved nitrogen value was used to calculate N* enrichments corresponding to the weight of TDN in 9 and 18 ml of GF/c filtered, treated effluent. Enrichments of 119 and 238 micrograms N were indicated. Additional enrichments were made with 9 and 18 ml of GF/c filtered, treated effluent. In order to determine the growth response resulting from the particulate post alum flocculation fraction, enrichment was also made with 9 and 18 ml of non-filtered, treated effluent. Finally, in order to estimate the growth response resulting from particulate, flocculant bound phosphorus, enrichment was made with an artificial floc, similar in composition to the C.C.S.D floc, but free of bound contaminating nutrients.

In preparation of the artificial floc, 7 mg/l phosphorus was assumed to be the flocculant bound fraction. Alum, $Al_2(SO_4)_3 \cdot 12H_2O$, is added by C.C.S.D. in concentrations of 150 mg/l assuming

aluminum complexes with phosphate-P in a molar ratio of 2:1, the above is slightly more $Al_2(SO_4)_3 \cdot 12H_2O$ than necessary to fully remove 7 mg/l P from solution:

$$\frac{.007 \text{ g P}}{31 \text{ g P mole}^{-1}} = .0002258 \text{ mole P in 7 mg/l P to remove}$$

need Al:P = 2:1 therefore need .4516 M mole Al

or .4516 M mole x 27 g mole^{-1} Al

= 0.122g Al to remove 7 mg/l P

$$\frac{54\text{g Al}}{558\text{g Al2}(SO_4)_3 \cdot 12H_2O} = \frac{.0122\text{g Al}}{.1261 \text{ g } Al_2(SO_4)_3 \cdot 12H_2O}$$

only 126.1 mg/l Alum are required to fully complex 7mg/l P.

1 mg/l of Dow Polymer Purifloc^r flucculant is added by C.C.S.D. to facilitate flocculation, and we used this same concentration. In summary, C.C.S.D. floc was assumed to consist of 7 mg/l P, 0.01222 g/l Al as alum and 1 mg/l Dow Polymer Purifloc^r. The artificial floc was prepared having equivalent concentrations of the above: 150.4 mg $Al_2(SO_4)_3 \cdot 18H_2O$* was added to one liter of DDW to give .0122g Al liter^{-1},

$$\frac{54 \text{ g Al}}{666 \text{ g } Al_2(SO_4)_3 \cdot 18H_2O} = \frac{.0122 \text{ g Al}}{.1504 \text{ g } Al_2(SO_4)_3 \cdot 18H_2O}$$

* $Al_2(SO_4)_3 \cdot 12H_2O$ not available.

To this solution was added 0.0312 g $NaH_2PO_4 \cdot H_2O$ to yield 7 mg/l P_1. Finally 1 mg of the Dow Polymer Purifloc^r was added and formation of an ultra fine white floc was observed.

To isolate the floc for enrich ment it was necessary to pass the floc solution through 4.25 cm dia. GF/c filters, thus concentrating quantities of the floc on the filters. (Isolation by

centrifugation and washing was not feasible due to the small quantities involved). 9 and 18 ml volumes of thoroughly agitated floc solution were drawn through separate filters, each volume containing an equivalent amount of floc as that contained in 9 and 18 ml respectively of non-filtered, alum treated effluent. Twelve filtrations of 9 ml and twelve filtrations of 18 ml were made to give 24 total floc containing GF/C filters. Following filtration, filters were placed in individual aluminum tins, covered and stored in refrigeration for later enrichment. The filtrate from all filtrations was composited, transferred to an acid rinsed poly bottle and frozen for later analysis. Enrichment consisted of direct addition of floc containing filters to the cubitainters.

All enrichments are summarized in the following:

Treatment	Station	Rep	Enrichment
Control	2,4,5,8	3	none
N* 1/100	2,4,5,8	3	1.19 ml 100mg/l NH_4NO_3-N
N* 1/50	2,4,5,8	3	2.39 ml 100mg/l NH_4NO_3-N
GF/C fil 1/100	2,4,5,8	3	9ml GF/c fil. treated eff.
GF/c fil 1/50	2,4,5,8	3	18 ml GF/c fil. treated ef
Non-fil 1/100	2,4,5,8	3	9 ml non-fil. treated eff.
Non-fil 1/50	2,4,5,8	3	18 ml non-fil, treated eff.
Floc +P 1/100	2,4,5,8	3	GF/c filter w/floc from 9 ml
Floc +P 1/50	2,4,5,8	3	GF/c filter w/floc from 18 ml artificial eff.

(Cont.)

Treatment	Station	Rep	Enrichment
GF/C control	4,8	3	GF/C filter
Floc + GF/C control	4,8	3	GF/C filter w/floc from 18 ml of polymer + $Al_2(SO_4)_3 \cdot 18H_2O$*

* Same solution as floc containing solution minus 7 mg/l - P.

At 2000-2200 on September 5, 1980, lake water was sampled at stations 2, 4, 5, and 8 using a Van Dorn water sampler. 25 liters of 1-3-5 meter composited sample were collected for each of stations 2,5, and 8 with 45 liters taken at station 4. Zooplankton were removed by passing the sample through 80 μ mesh netting and composited in 25 liter carboys. Samples were returned immediately to the lab and enrichments initiated at 2300 as per the above. Upon completing all enrichments at 0300 September 6, 1980, remaining water was reagitated and subsampled as follows: 500 ml of each transferred to acid rinsed poly bottles and frozen for later analysis; 125 ml of each preserved with 1 ml lugols reagent and stored for later phytoplankton enumeration; 500 ml each treated with $MgCO_3$, GF/c filtered and filters frozen for later chlorophyll extraction; 8 ml triplicates of each used to measure initial in vivo fluorescence.

Samples were incubated for five days in the outdoor pools with in vivo fluorescence measured daily. 500 ml subsamples were taken daily from the large cubitainers, treated with $MgCO_3$, GF/c filtered and filters frozen for chlorophyll extraction. The bioassay was terminated on September 10, 1980. Final fluorescence measurements

were made and the following 500 ml subsamples treated with $MgCO_3$ and GF/c filtered for chlorophyll extraction:

Lg Cubitainters I, II, III

Station 8 Control w/fluor 50.5 (3x)

Station 4 Control w/fluor 33.5 (1x)

Station 4 Fil 1/100 w/fluor 100.0 (1x)

10 ml of each replicate/treatment were composited and preserved with0.5 ml lugols reagent.

Results

The results of this special bioassay indicate that the floc bound phosphorus fraction (artifically prepared) in no case enhances the algal growth as compared to control. Interpretation of the significance of these findings is clouded somewhat by the fact that at the time this bioassay was conducted the algal communities at station 2, 4, and 5 were definitely N-limited, and consequently could not be expected to respond to P addition (whether dissolved or floc-bound). Dissolved N:P ratios at these three stations were 5.8:1, 2.4:1 and 4.8:1, respectively on September 11, 1980. Indeed, the N-enrichments performed in this special bioassay show significant nitrogen stimulation (as compared to the controls) at all three stations although it also can be seen that equivalent dilutions of effluent (either filtered or unfiltered) with equivalent N-concentrations, yield significantly a higher response.

Only station 8 may have been truly P limited when this bioassay was conducted. In fact P limitation exists for most of the summer of 1980 at station 8, but by regular bioassay 31 the P response

was beginning to diminish. Nitrogen enrichments yield slight positive responses in this special bioassay at station 8, however, the dissolved N:P ratio of 15:1 on September 11, points to a continuing response to P (alone) enrichment, and therefore the results of the special bioassay with regard to the effect of enrichment by artificially prepared floc-bound P, indicate that this phosphorus fraction is not available for algal assimilation.

At stations 4, 5, and 8 dilutions of unfiltered alum treated effluent yield higher algal growth responses than equivalent dilutions of filtered alum treated effluent. Since floc-bound P is probably not available, then other nutrients, in particular bound NH_4^+ might serve to stimulate algal response over and above that shown by dissolved nutrients in filtered, treated enrichments.

SECTION 2

EPA Bioassay Report

ALGAL GROWTH POTENTIAL RESULTS OF TESTS PERFORMED ON LAKE MEAD, NEVADA WATERS COLLECTED OCTOBER 1979 AND JULY 1980

RECEIVED
MAR 0 2 1981
BROWN AND CALDWELL

Completion Report

by

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INTRODUCTION

A vigorous legal, technical, and political dispute over advanced waste treatment (AWT) has been underway in Las Vegas, Nevada. The cities of Las Vegas and North Las Vegas sued Clark County, the State of Nevada, and the U.S. Environmental Protection Agency (EPA) in an attempt to halt construction of a one-quarter complete 3.6 m^3/d (90 MGD) AWT plant designed to remove phosphorus from the area's secondary effluent. Las Vegas charged that the Nevada water quality standards, on which the need for an AWT facility was based, had not been set in accord with requirements of the Clean Water Act.

In early 1979, all parties signed a consent decree which, in part, mandates a comprehensive water quality study of Lake Mead. At the request of the EPA Regional Office in San Francisco, California the EPA Algal Assay Team at the Corvallis Environmental Research Laboratory, Oregon performed tests on selected Lake Mead waters.

Brown and Caldwell (Sacramento, California) were the program management consultants to the Las Vegas Valley Water Quality Program. Jerry E. Ongerth of Brown and Caldwell was liaison between the Las Vegas Program and EPA Corvallis.

Dr. Ongerth selected four Lake Mead stations for algal bioassay. The stations are identified as numbers 2, 4, 5 and 8. Their locations can be determined from Figure I.

Algal assays were performed to determine the primary and secondary limiting nutrients of the Lake Mead test stations as well as their trophic

SAMPLING STATION LOCATIONS

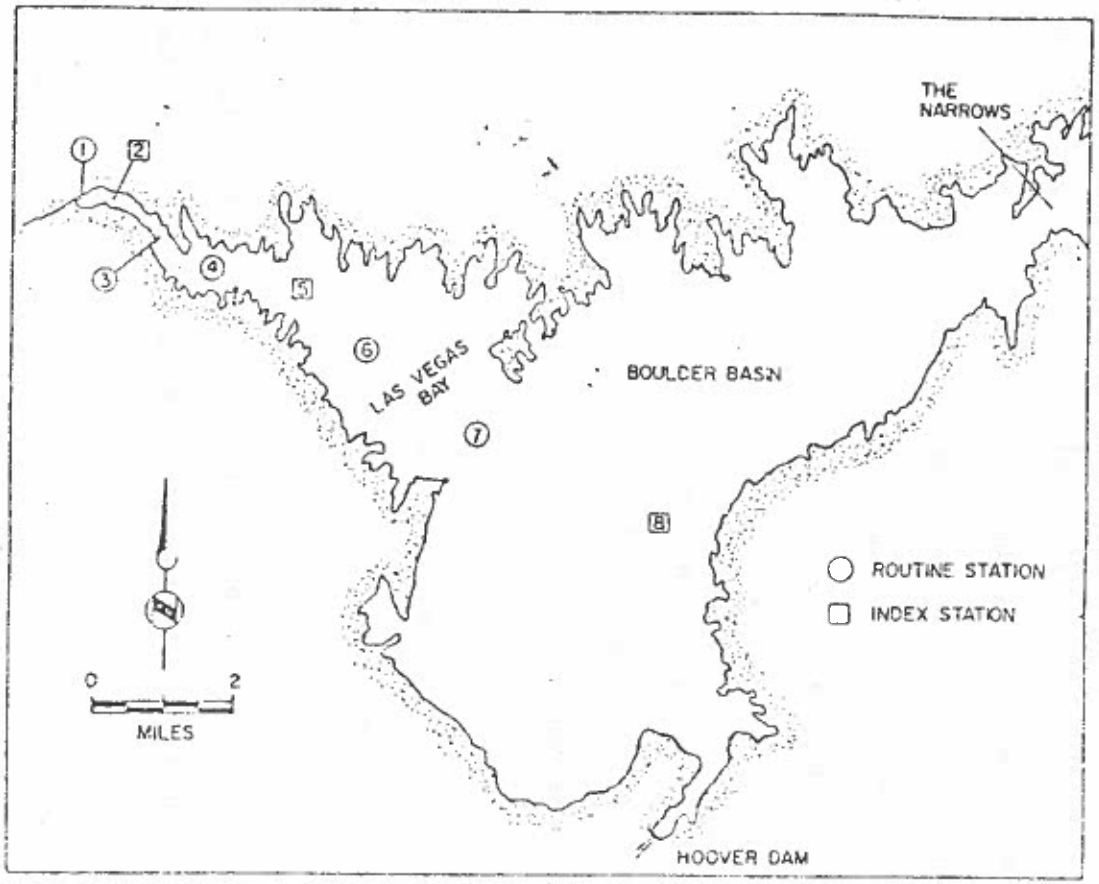

Fig. 1 Limnological Monitoring Program Sampling Stations - Las Vegas Bay and Boulder Basin

STATION NO.	LAT	LONG
1	36° 07' 39"	114° 52' 55"
2	36° 07' 56"	114° 52' 24"
3	36° 07' 41"	114° 51' 53"
4	36° 07' 09"	114° 51' 29"
5	36° 06' 59"	114° 49' 57"
6	36° 06' 20"	114° 48' 54"
7	36° 05' 34"	114° 47' 18"
8	36° 04' 02"	114° 44' 08"
9	36° 03' 17"	114° 25' 54"
10	36° 04' 41"	114° 26' 47"
11	36° 02' 10"	114° 28' 47"

classification. Background information indicated that metal concentrations might be high enough to inhibit the growth of S. capricornutum. As a precaution, a set of nutrient spikes with the chelator EDTA were included in the test design. A comprehensive analysis of heavy metals was also performed.

Jerry Ongerth requested that the algal assays be performed in filtered water. I counseled Dr. Ongerth against that pretreatment because large amounts of nutrients can be removed with the filterable organisms. The result might be an error in the primary limiting nutrient as well as the trophic classification. The recommended standard method of pretreatment is autoclaving followed by filtration. I decided to perform the assay using both pretreatments to achieve the most complete data base.

METHODOLOGY

Water samples were collected by employees of Brown and Caldwell for shipment to Corvallis.

The first set of Lake Mead samples, collected October 24-26, 1979, were received approximately 12 days later (November 5-6, 1979). Bioassay began on November 9.

The second set of water samples were collected July 2, 1980. They were shipped by air to Corvallis and arrived on July 3 at 1740 hours. Bioassay began on July 11, 1980.

Selenastrum capricornutum Printz was used to assess the algal growth response to water samples taken from Lake Mead, Nevada. The procedure outlined in the Algal Assay Bottle Test (Miller, Greene and Shiroyama, 1978) was followed. Each sample was pretreated in two parts prior to assay. One part was filtered through a 0.45 µm membrane filter (FO) prior to shipment. The second was shipped raw, autoclaved upon arrival, cooled to room temperature, re-equilibrated to the original pH with 1.0% CO_2 in air and filtered through a 0.45 µm membrane filter (AF).

Fifty milliliters of sample was dispensed into 125 ml Erlenmeyer flasks. Triplicate samples were spiked with 1.00 mg N L^{-1}, 0.05 mg P L^{-1}, N+P, 1.00 mg EDTA L^{-1} (Disodium Ethylenedinitrilotetraacetate), N+EDTA, P+EDTA, and N+P+EDTA. Samples without the addition of nutrients or a chelator were used as controls.

The test flasks were inoculated to a final concentration of 1,000 cells S. capricornutum ml^{-1} and cultured for 14 days under constant light (4,304 lumens) and temperature (24°C).

Aliquots were taken from the raw, filtered and autoclaved-filtered samples for nutrient and metals analysis. Analyses for Ortho-P, Total-P, NH_3, NO_2+NO_3, and Kjeldahl-N were performed on a three-channel Technicon Auto-Analyzer II. An initial screen for metals was performed on a Quantrometric (Plasma Emission) Analyzer. Metals that appeared in high concentration in the plasma emission scan were further analyzed for greater accuracy on a Perkin-Elmer Model 305B atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Bioassay of the nutrient enrichment problem in Las Vegas Bay and Lake Mead was complicated by inhibitory concentrations of heavy metals in all of the water samples collected on October 24-26, 1980. A comprehensive "Plasma Emission" screening analysis of 20 metals was performed on these waters (Table I). Nickel, zinc, copper and lead concentrations appeared high in the screening test and were selected for more accurate analysis by atomic absorption spectrophotometry (Table II). Nickel concentrations in all samples were consistently high. They ranged from 0.045 to 0.067 mg nickel L^{-1}.

The study of heavy metal interaction is complicated by an uncertainty of form or biological reactive state of the metals and lack of information on antagonism or synergism between the metals. Algal assay response is an integration of the combined effects of metal solubility and concentration, contact time, hardness, and ionic strength of the test water. Miller, Greene, Shiroyama and Merwin (1973) showed that the addition of 1.0 mg EDTA L^{-1} to waters containing heavy metals allowed the algae to grow to the level predicted from analysis of the primary limiting nutrient. More comprehensive studies established that the EDTA-addition technique was a viable tool for testing the limiting nutrient potential in metal-laden waters (Miller, Greene and Shiroyama, 1976; Greene et al., 1976a; Greene et al., 1976b; Greene et al., 1978).

Inhibition of growth in a culture containing laboratory algal test species indicates that non-adapted algal species will display some degree of

TABLE I

HEAVY METALS SCREEN (mg L^{-1}) OF LAKE MEAD, NEVADA WATERS COLLECTED OCTOBER 24-26, 1979 (PLASMA EMISSION).

Metals Analyzed	Station 2			Station 4			Station 5			Station 8		
	Raw[1]	AF[2]	FO[3]	Raw	AF	FO	Raw	AF	FO	Raw	AF	FO
Zn	0.036	0.076	0.024	0.026	0.050	0.022	0.028	0.078	0.025	0.033	0.040	0.027
Cu	0.022	0.028	0.020	0.028	0.028	0.020	0.028	0.028	0.022	0.028	0.022	0.022
Cd	---	---	---	0.003	---	---	---	---	---	---	---	---
Ni	0.090	0.090	0.090	0.100	0.080	0.080	0.090	0.090	0.090	0.080	0.080	0.080
Cr	0.009	0.008	0.009	0.012	0.008	0.011	0.011	0.008	0.008	0.011	0.011	0.008
Co	0.210	0.170	0.190	0.210	0.170	0.170	0.210	0.170	0.170	0.170	0.130	0.130
Pb	0.350	0.350	0.290	0.320	0.290	0.290	0.350	0.230	0.290	0.230	0.290	0.230
Al	0.290	0.160	0.210	0.290	0.170	0.230	0.210	0.160	0.190	0.230	0.160	0.190
As	0.180	0.180	0.180	0.210	0.160	0.160	0.210	0.180	0.180	0.140	0.140	0.120
V	0.003	0.003	0.003	0.009	---	---	0.003	---	0.003	0.003	---	---
B	0.175	0.165	0.169	0.162	0.165	0.162	0.158	0.160	0.155	0.140	0.141	0.140
Mo	---	---	---	0.080	---	---	0.040	---	---	---	---	---
Zr	---	---	---	0.002	---	---	0.002	---	---	---	---	---
Sr	1.429	1.383	1.370	1.373	1.378	1.363	1.358	1.383	1.315	1.230	1.169	1.229
Se	0.101	---	---	0.030	---	---	0.010	---	0.030	0.030	0.030	---
Mn	0.012	0.008	0.001	0.006	0.006	0.001	0.003	0.004	0.001	0.002	0.001	---
Ca	85.900	77.600	81.600	82.600	79.400	81.500	83.300	82.020	79.000	76.500	64.200	76.400
Mg	34.100	33.300	32.300	32.900	32.000	32.800	32.600	33.200	31.500	30.100	29.900	30.000
Fe	0.028	0.001	0.001	0.018	0.001	0.003	0.011	0.001	0.005	0.008	0.001	0.001

[1] Well mixed untreated sample.
[2] Autoclaved followed by filtration through 0.45 µm membrane filter.
[3] Filtered through 0.45 µm membrane filter.

TABLE II

HEAVY METALS ANALYSIS (mg L^{-1}) OF LAKE MEAD, NEVADA WATERS COLLECTED OCTOBER 24-26, 1979 (ATOMIC ABSORPTION).

Metals Analyzed	Station 2			Station 4			Station 5			Station 8		
	Raw[1]	AF[2]	FO[3]	Raw	AF	FO	Raw	AF	FO	Raw	AF	FO
Zn	0.016	0.049	0.007	0.008	0.029	0.004	0.010	0.005	0.007	0.024	0.021	0.008
Cu	0.006	0.017	0.008	0.009	0.013	0.014	0.009	0.014	0.016	0.016	0.016	0.014
Ni	0.051	0.067	0.054	0.059	0.065	0.055	0.056	0.051	0.058	0.047	0.045	0.056
Pb	<0.001	0.002	<0.001	<0.001	<0.001	0.004	0.002	01001	<0.001	<0.001	<0.001	0.003

[1] Well mixed untreated sample.
[2] Autoclaved followed by filtration through 0.45 µm membrane filter.
[3] Filtered through 0.45 µm membrane filter.

reduced growth. These results should not be construed to mean that algae indigenous to Lake Mead will have reduced growth. It is quite likely that indigenous algal growth is controlled by nutrient limitations rather than heavy metal inhibition because of their adaption to the metals.

Shiroyama, Miller and Greene (1973) developed coefficients for predicting the maximum yield of S. capricornutum. If phosphorus is limiting growth, the analyzed ortho-P is multiplied by 430. The result is the predicted mg dry weight of S. capricornutum L^{-1} ± 20 percent. A similar result for nitrogen is achieved by multiplying the sum of NO_2+NO_3+NH_3 (= total soluble inorganic nitrogen = TSIN) by 38. Actual and predicted maximum yields of S. capricornutum cultured in Lake Mead water collected October 24-26, 1979 are reported in Table III. There was little growth in the water samples without EDTA addition.

Chemical analysis for ortho-P in the autoclaved-filtered water sample collected at Station 2 (Table IV) suggested a very high growth potential based on assumed phosphorus limitation. The predicted yield in the EDTA spiked sample is 8.60 mg dry weight L^{-1} (430 x 0.020 mg P L^{-1}). With a range of ± 20 percent, one could expect the sample to yield from 6.88 to 10.32 mg dry weight L^{-1}. The actual bioassay yield was 10.30 mg dry weight L^{-1}. The study of all P-limited predicted yields and their associated algal assay yield revealed that the ortho-P analysis was verified by the bioassay results.

The accuracy of chemical analysis of nitrogen is seriously questioned. It is physiological impossible for S. capricornutum to produce biomass unless the essential nutrients are present in the test solution. However, when the test alga was relieved of metal inhibition and phosphorus growth limitation (PE spiked samples), all stations regardless of pretreatment produced biomass from 61.2 to 75.1-percent greater than that which could be supported through utilization of all the analyzed TSIN. For example, the 0.175 mg TSIN L^{-1}

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TABLE III

Actual and predicted yields of Selenastrum capricornutum (mg dry weight L^{-1}) cultured in Lake Mead, Nevada waters collected October 24-26, 1979.

	Control	1.0 N[3]	0.05 P	NP	1.0 EDTA	NE	PE	NPE	Limiting Nutrient	% Yield > TSIN Potential[4]	Percent[5] Inhibition
Station 2 (AF)[1]	0.07	0.13	0.23	0.18	10.30	10.42	17.40	26.30	P/N	262	99.3
Predicted Yield					8.60	8.60	6.65	30.10			
Station 2 (FO)[2]	0.06	0.07	0.14	3.96	0.17	0.13	2.57	30.73	P/N	378	94.5*
Predicted Yield					<1.00	<1.00	0.68	21.50			
Station 4 (AF)	0.07	0.13	0.26	1.03	2.36	5.55	14.10	35.55	P/N	320	97.0
Predicted Yield					4.73	4.73	4.41	26.23			
Station 4 (FO)	0.66	0.37	6.14	20.59	1.90	1.10	9.03	34.01	P/N	396	65.3
Predicted Yield					2.58	2.58	2.28	23.40			
Station 5 (AF)	0.18	0.24	0.33	0.29	8.97	11.25	9.60	24.98	N/P	402	98.0
Predicted Yield					10.75	10.75	2.39	32.25			
Station 5 (FO)	0.08	0.10	1.01	0.19	0.07	0.08	5.68	21.68	P/N	364	82.2*
Predicted Yield					<1.00	<1.00	1.56	21.50			
Station 8 (AF)	0.06	0.06	1.42	0.30	0.10	0.12	11.22	20.06	P/N	295	87.3*
Predicted Yield					<1.00	<1.00	3.80	21.50			
Station 8 (FO)	0.06	0.12	0.81	0.80	0.11	0.07	10.62	22.26	P/N	345	92.4
Predicted Yield					<1.00	<1.00	3.08	21.50			

[1] Autoclaved, followed by 0.45 µm filtration.
[2] 0.45 µm filtration.
[3] All spikes mg l^{-1}.
[4] Percent of the total nitrogen limited yield achieved from an organic nitrogen source (assuming TSIN analysis is correct). Estimated from the difference in growth between the predicted and actual yields under the PE column.
[5] Difference between the yields produced in the control and EDTA spiked cultures.
* Due to nutrient limitation, percent inhibition was calculated from the yields produced in P and PE spiked cultures.

TABLE IV

Chemical analysis of nutrients (mg L^{-1}) in Lake Mead, Nevada waters collected October 24-26, 1979.

	Station 2			Station 4			Station 5			Station 8		
Analysis	Raw	AF[1]	FO[2]	Raw	AF	FO	Raw	AF	FO	Raw	AF	FO
Total-P	----	<0.010	0.019	----	<0.010	<0.010	----	0.025	<0.010	----	0.010	<0.010
Ortho-P	----	0.020	0.006	----	0.011	0.006	----	0.025	<0.005	----	0.010	0.005
$NO_2 + NO_3$	----	0.101	0.018	----	0.026	0.022	----	0.063	0.026	----	0.056	0.026
NH_3	----	0.074	<0.005	----	0.090	0.038	----	<0.005	0.015	----	0.044	0.055
TSIN[3]	----	0.175	0.018	----	0.116	0.060	----	0.063	0.041	----	0.100	0.081
Organic N	----	Neg	0.283	----	Neg	Neg	----	<0.050	Neg	----	Neg	Neg
pH	8.05	7.80	7.78	8.25	8.23	8.50	7.88	7.79	8.14	8.02	8.02	8.15
Hardness	358	333	339	345	335	338	346	344	330	318	285	317

[1] Autoclaved, followed by 0.45 μm filtration.
[2] 0.45 μm filtration.
[3] Total Soluble Inorganic Nitrogen ($NO_2 + NO_3 + NH_3$).

analyzed in the Station 2 sample was expected to yield approximately 6.65 mg dry weight of S. capricornutum L^{-1}. The actual yield was 17.40 mg dry weight L^{-1}; 61.2 percent more biomass than could be supported by the analyzed TSIN (Table III). Selenastrum can utilize some species of organic nitrogen, but chemical analysis indicated that little if any was present. Results of nitrogen analysis in the October Lake Mead water samples are obviously unreliable.

Lake Mead water is very hard, ranging from 285 to 358 mg $CaCO_3$ L^{-1} (Table IV). It has been argued that the AF pretreatment is undesirable because phosphorus precipitated with the calcium ions during autoclaving. Autoclaved-filtered phosphorus limited Lake Mead waters produced yields that were larger than the same stations FO pretreated sample (Table III). These data indicate that phosphorus was removed with the filterable organisms, but not lost during autoclaving.

Filtration of a water sample, when a large algal population is present, will produce an erroneous trophic classification. Miller, Maloney and Greene (1974) published a trophic index of waters measured by algal assay. The productivity groups were defined as: low productivity (0.00 to 0.10 mg dry weight L^{-1}); moderate productivity (0.11 to 0.80 mg dry weight L^{-1}); moderately high productivity (0.81 to 6.00 mg dry weight L^{-1}); and high productivity (> 6.00 mg dry weight L^{-1}). The error FO pretreatment makes in properly classifying the productivity classification of a lake can be seen in the bioassay results of Station 2 (Table III). Growth in the EDTA spiked FO sample is 0.17 mg dry weight L^{-1} and fits into the low productivity classification. The same sample that had AF pretreatment yielded 10.30 mg dry weight L^{-1} and is classified as highly productive.

Productivity classification of the October Lake Mead sample, based on the yields in the EDTA spiked cultures, is high productivity, moderately high productivity, high productivity and low productivity for stations 2, 4, 5 and 8, respectively.

The Lake Mead waters were primarily phosphorus-limited and secondarily nitrogen-limited in every case except Station 5 AF, which was the reverse (Table III).

The Lake Mead samples collected July 2, 1980 were also screened for their heavy metals content (Table V). Eight of the metals were re-analyzed by the more accurate method of atomic absorption spectrophotometry (Table VI). Nickel, once again, was consistently high in all samples regardless of pre-treatment. It ranged from 0.040 to 0.073 mg nickel L^{-1}. Heavy metals affected growth of the test alga in all of the July samples except Station 2 AF (Table VII). There was reduced growth in the control and 1.0 mg N L^{-1} spiked cultures relative to their associated EDTA spiked flasks. However, all of the other Station 4 AF cultures were without growth inhibition.

Nutrient analysis of the 1980 Lake Mead water samples (Table VIII) showed very high levels of orthophosphorus in raw and pretreated Station 2 samples. Orthophosphorus concentrations in water collected at Station 4, 5 and 8 were below the level of detection. These analyses are consistent with the algal assay results. The Station 2 samples produced high yields and were primarily nitrogen-limited or too close to call (N+P). All other samples were primarily phosphorus limited.

As was found in the October 1979 Lake Mead samples, the July 1980 samples consistently produced more algal biomass in nitrogen-limited samples than could be supported by the analyzed TSIN. These data strongly suggest that a substantial portion of this growth was supported by organic nitrogen.

TABLE V

HEAVY METALS SCREEN (mg L^{-1}) OF LAKE MEAD, NEVADA WATERS COLLECTED JULY 2, 1980 (PLASMA EMISSION).

Metals Analyzed	Station 2			Station 4			Station 5			Station 8		
	Raw[1]	AF[2]	FO[3]	Raw	AF	FO	Raw	AF	FO	Raw	AF	FO
Zn	0.012	0.021	0.021	0.021	0.013	0.023	0.012	0.012	0.019	0.012	0.009	0.017
Cu	0.027	0.006	0.031	0.019	<0.005	0.027	0.023	<0.005	0.031	0.023	<0.005	0.028
Ni	0.070	0.030	0.070	0.060	0.020	0.060	0.050	0.010	0.070	0.060	0.010	0.060
Cr	0.003	---	0.003	---	---	0.006	0.002	---	0.006	0.003	---	0.007
Co	0.300	---	0.350	0.220	---	0.300	0.240	---	0.330	0.220	---	0.330
Pb	0.300	0.210	0.340	0.260	0.100	0.280	0.280	0.100	0.280	0.260	0.130	0.280
Al	0.220	0.090	0.230	0.200	0.030	0.200	0.190	0.030	0.200	0.190	0.070	0.200
As	0.220	0.070	0.220	0.160	0.020	0.160	0.130	0.010	0.160	0.140	0.020	0.170
V	---	---	---	---	---	---	---	---	0.003	---	---	---
B	0.241	0.205	0.213	0.173	0.141	0.160	0.153	0.134	0.146	0.141	0.125	0.137
Mo	---	---	---	---	---	0.040	---	---	0.080	---	---	0.040
Zr	---	---	---	---	---	---	---	---	0.002	---	---	0.002
Sr	1.652	1.827	1.625	1.403	1.343	1.315	1.337	1.249	1.291	1.236	1.208	1.206
Mn	0.018	0.010	0.008	0.003	---	0.001	---	---	0.004	---	---	0.001
Ca	98.900	105.200	96.500	79.600	72.800	74.900	76.900	60.500	74.000	74.000	66.300	71.900
Mg	37.000	38.600	36.500	32.300	31.500	31.300	31.100	29.800	30.400	30.400	29.400	29.900
Fe	0.024	---	0.001	0.287	---	0.010	0.001	---	0.004	0.004	---	0.004

[1] Well mixed untreated sample.
[2] Autoclaved followed by filtration through 0.45 µm membrane filter.
[3] Filtered through 0.45 µm membrane filter.

TABLE VI

HEAVY METALS ANALYSIS (mg L^{-1}) OF LAKE MEAD, NEVADA WATERS COLLECTED JULY 2, 1980 (ATOMIC ABSORPTION).

Metals Analyzed	Station 2			Station 4			Station 5			Station 8		
	Raw[1]	AF[2]	FO[3]	Raw	AF	FO	Raw	AF	FO	Raw	AF	FO
Zn	<0.001	0.004	0.003	0.009	0.009	0.008	0.002	0.003	0.002	<0.001	0.002	0.002
Cu	0.013	0.009	0.008	0.004	0.007	0.007	0.003	0.005	0.007	0.006	0.006	0.008
Ni	0.073	0.040	0.057	0.059	0.055	0.047	0.042	0.039	0.042	0.043	0.042	0.059
Co	0.013	0.011	0.016	0.021	0.010	0.016	0.020	0.012	0.024	0.016	0.006	0.009
Pb	0.004	0.002	0.003	0.005	0.005	0.003	0.003	0.004	0.006	0.006	0.006	0.004
Al*	0.154	0.016	0.046	0.035	0.019	0.036	0.033	0.024	0.041	0.032	0.030	0.027
As	0.087	0.044	0.092	0.105	0.058	0.112	0.108	0.079	0.085	0.104	0.083	0.100
Mo	0.108	0.086	0.128	0.120	0.076	0.117	0.141	0.092	0.092	0.157	0.146	0.144

[1] Well mixed untreated sample.
[2] Autoclaved followed by filtration through 0.45 µm membrane filter.
[3] Filtered through 0.45 µm membrane filter.
* Aluminum analysis ±0.020 mg/l.

TABLE VII

Actual and predicted yields of *Selenastrum capricornutum* (mg dry weight L^{-1}) cultured in Lake Mead, Nevada waters collected July 2, 1980.

	Control	†1.0 N	0.05 P	NP	1.0 EDTA	NE	PE	NPE	Limiting Nutrient	% Organic N Bioavailable	Percent Inhibition
Station 2 (AF)[1]	16.36	15.71	17.80	36.11	20.12	23.66	23.01	38.72	N+P	29.6	18.7
Predicted Yield					20.21	20.21	16.19	41.71			
Station 2 (FO)[2]	0.90	3.70	0.66	5.01	14.05	17.90	14.24	40.23	N/P	3.4	93.6
Predicted Yield					11.63	13.76	11.63	35.26			
Station 4 (AF)	0.63	1.12	8.43	24.23	2.68	1.74	7.33	24.44	P/N	72.7	76.5
Predicted Yield					2.15	2.15	1.98	23.65			
Station 4 (FO)	0.11	0.17	1.89*	9.53	0.11	0.09	4.01	29.29	P/N	77.3	52.9
Predicted Yield					<1.00	<1.00	0.91	21.50			
Station 5 (AF)	0.06	0.08	3.96	12.89	0.14	0.12	4.67	17.66	P/N	89.5	15.2
Predicted Yield					<1.00	<1.00	0.49	21.50			
Station 5 (FO)	0.08	0.07	4.93	21.54	0.08	0.06	4.71	28.47	P/N	75.0	0
Predicted Yield					<1.00	<1.00	1.18	21.50			
Station 8 (AF)	0.05	0.07	5.18	6.37	0.05	0.05	6.21	21.53	P/N	81.6	16.6
Predicted Yield					<1.00	<1.00	1.14	21.50			
Station 8 (FO)	0.05	0.06	5.92	18.96	0.11	<0.05	5.74	21.71	P/N	68.3	0
Predicted Yield					<1.00	<1.00	1.82	21.50			

† All spikes mg L^{-1}.
[1] Autoclaved, followed by 0.45 µm filtration.
[2] 0.45 µm filtration.
* Slow growth in replicate flasks.

TABLE VIII

Chemical analysis of nutrients (mg L^{-1}) in Lake Mead, Nevada waters collected July 2, 1980.

	Station 2			Station 4			Station 5			Station 8		
Analysis	Raw	AF[1]	FO[2]	Raw	AF	FO	Raw	AF	FO	Raw	AF	FO
Total-P	0.097	0.086	0.045	0.024	0.015	<0.010	0.019	<0.010	<0.010	<0.010	<0.010	<0.010
Ortho-P	0.041	0.047	0.032	<0.005	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
NO_2+NO_3	0.124	0.129	0.141	<0.010	0.010	0.015	<0.010	<0.010	0.015	0.014	0.017	0.026
NH_3	0.297	0.297	0.165	0.022	0.042	0.009	<0.005	0.013	0.016	<0.005	0.013	0.022
TSIN[3]	0.421	0.426	0.306	0.022	0.052	0.024	<0.010	0.013	0.031	0.014	0.030	0.048
Organic N	0.585	0.487	0.409	0.315	0.439	0.417	0.268	0.328	0.308	0.259	0.432	0.326
pH	8.20	8.15	8.45	8.45	8.30	8.40	8.40	8.40	8.40	8.35	8.00	8.40
Hardness	402	424	394	335	313	318	323	275	312	312	288	305

[1] Autoclaved, followed by 0.45 µm filtration.
[2] 0.45 µm filtration.
[3] Total Soluble Inorganic Nitrogen (NO_2 + NO_3 + NH_3).

The July 1980 samples were classified as having high productivity, moderately high productivity, moderate productivity and low productivity for Stations 2, 4, 5 and 8, respectively. These results are consistent with nutrient loading in Las Vegas Bay (Station 2) and maximum dilution of nutrients (if they reach that area) in Lake Mead (Station 8).

REFERENCES

Greene, J. C., W. E. Miller, T. Shiroyama, R. A. Soltero, and K. Putnam. 1976a. Use of algal assays to assess the effects of municipal and smelter wastes upon phytoplankton production. Proc. Symp. Terr. Aquat. Ecol. Studies of NW, EWSC Press, Cheney, Wash. 1976:326-336.

Greene, J. C., R. A. Soltero, W. E. Miller, A. F. Gasperino, and T. Shiroyama. 1976b. The relationship of laboratory algal assays to measurements of indigenous phytoplankton in Long Lake, Washington. p. 93-126 In E. J. Middlebrooks, D. A. Falkenborg, and T. E. Maloney [eds.], Biostimulation and Nutrient Assessment. Ann Arbor Sci.

Greene, J. C., W. E. Miller, T. Shiroyama, R. A. Soltero, and K. Putnam. 1978. Use of laboratory cultures of Selenastrum, Anabaena, and the indigenous isolate Sphaerocystis to predict effects of nutrient and zinc interactions upon phytoplankton growth in Long Lake, Washington. Mitt. Int. Ver. Limnol. 21:372-384.

Miller, W. E., T. E. Maloney, and J. C. Greene. 1974. Algal productivity in 49 lake assays as determined by algal assays. Water. Res. 8:667-679.

Miller, W. E., J. C. Greene, T. Shiroyama, and E. Merwin. 1975. The use of algal assays to determine effects of waste discharge in the Spokane River System. p. 113-131. U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-600/3-75-034.

Miller, W. E., J. C. Greene, and T. Shiroyama. 1976. Application of algal assays to define the effects of wastewater effluents upon algal growth in multiple use river systems. p. 77-92 In E. J. Middlebrooks, D. A.

Falkenborg, and T. E. Maloney [eds.], Biostimulation and Nutrient Assessment. Ann Arbor Sci.

Miller, W. E., J. C. Greene, and T. Shiroyama. 1978. The Selenastrum capricornutum Printz Algal Assay Bottle Test: Experimental Design, Application, and Data Interpretation Protocol. U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-600/9-78-018. 126 p.

Shiroyama, T., W. E. Miller, and J. C. Greene. 1975. Effects of nitrogen and phosphorus on the growth of Selenastrum capricornutum Printz. p. 132-142 In Proc. Biostm. Nutr. Assess. Workshop, 16-17 October 1973, U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-660/3-75-034.

TABLE III

Actual and predicted yields of *Selenastrum capricornutum* (mg dry weight L^{-1}) cultured in Lake Mead, Nevada waters collected October 24-26, 1979.

	Control	1.0 N[3]	0.05 P	NP	1.0 EDTA	NE	PE	NPE	Limiting Nutrient	% Yield > TSIN Potential[4]	Percent[5] Inhibition
Station 2 (AF)[1]	0.07	0.13	0.23	0.18	10.30	10.42	17.40	26.30	P/N	61.2	99.3
Predicted Yield					8.60	8.60	6.65	30.10			
Station 2 (FO)[2]	0.06	0.07	0.14	3.96	0.17	0.13	2.57	30.73	P/N	73.5	94.5*
Predicted Yield					<1.00	<1.00	0.68	21.50			
Station 4 (AF)	0.07	0.13	0.26	1.03	2.36	5.55	14.10	35.55	P/N	68.7	97.0
Predicted Yield					4.73	4.73	4.41	26.23			
Station 4 (FO)	0.66	0.37	6.14	20.59	1.90	1.10	9.03	34.01	P/N	74.8	65.3
Predicted Yield					2.58	2.58	2.28	23.40			
Station 5 (AF)	0.18	0.24	0.33	0.29	8.97	11.25	9.60	24.98	N/P	75.1	98.0
Predicted Yield					10.75	10.75	2.39	32.25			
Station 5 (FO)	0.08	0.10	1.01	0.19	0.07	0.08	5.68	21.68	P/N	72.5	82.2*
Predicted Yield					<1.00	<1.00	1.56	21.50			
Station 8 (AF)	0.06	0.06	1.42	0.30	0.10	0.12	11.22	20.06	P/N	66.1	87.3*
Predicted Yield					<1.00	<1.00	3.80	21.50			
Station 8 (FO)	0.06	0.12	0.81	0.80	0.11	0.07	10.62	22.26	P/N	71.0	92.4
Predicted Yield					<1.00	<1.00	3.08	21.50			

[1] Autoclaved, followed by 0.45 µm filtration.
[2] 0.45 µm filtration.
[3] All spikes mg l^{-1}.
[4] Percent of the total nitrogen limited yield achieved from an organic nitrogen source (assuming TSIN analysis is correct). Estimated from the difference in growth between the predicted and actual yields under the PE column.
[5] Difference between the yields produced in the control and EDTA spiked cultures.
* Due to nutrient limitation, percent inhibition was calculated from the yields produced in P and PE spiked cultures.

TABLE III

Actual and predicted yields of *Selenastrum capricornutum* (mg dry weight L^{-1}) cultured in Lake Mead, Nevada waters collected October 24-26, 1979.

	Control	1.0 N[3]	0.05 P	NP	1.0 EDTA	NE	PE	NPE	Limiting Nutrient	% Yield > TSIN Potential[4]	Percent[5] Inhibition
Station 2 (AF)[1]	0.07	0.13	0.23	0.18	10.30	10.42	17.40	26.30	P/N	61.2	99.3
Predicted Yield					8.60	8.60	6.65	30.10			
Station 2 (FO)[2]	0.06	0.07	0.14	3.96	0.17	0.13	2.57	30.73	P/N	73.5	94.5*
Predicted Yield					<1.00	<1.00	0.68	21.50			
Station 4 (AF)	0.07	0.13	0.26	1.03	2.36	5.55	14.10	35.55	P/N	68.7	97.0
Predicted Yield					4.73	4.73	4.41	26.23			
Station 4 (FO)	0.66	0.37	6.14	20.59	1.90	1.10	9.03	34.01	P/N	74.8	65.3
Predicted Yield					2.58	2.58	2.28	23.40			
Station 5 (AF)	0.18	0.24	0.33	0.29	8.97	11.25	9.60	24.98	N/P	75.1	98.0
Predicted Yield					10.75	10.75	2.39	32.25			
Station 5 (FO)	0.08	0.10	1.01	0.19	0.07	0.08	5.68	21.68	P/N	72.5	82.2*
Predicted Yield					<1.00	<1.00	1.56	21.50			
Station 8 (AF)	0.06	0.06	1.42	0.30	0.10	0.12	11.22	20.06	P/N	66.1	87.3*
Predicted Yield					<1.00	<1.00	3.80	21.50			
Station 8 (FO)	0.06	0.12	0.81	0.80	0.11	0.07	10.62	22.26	P/N	71.0	92.4
Predicted Yield					<1.00	<1.00	3.08	21.50			

[1] Autoclaved, followed by 0.45 µm filtration.
[2] 0.45 µm filtration.
[3] All spikes mg l^{-1}.
[4] Percent of the total nitrogen limited yield achieved from an organic nitrogen source (assuming TSIN analysis is correct). Estimated from the difference in growth between the predicted and actual yields under the PE column.
[5] Difference between the yields produced in the control and EDTA spiked cultures.
* Due to nutrient limitation, percent inhibition was calculated from the yields produced in P and PE spiked cultures.