THE CHEMICAL AND MICROBIOLOGICAL

LIMNOLOGY OF

WOOD LAKE, B.C., 1984

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1. INTRODUCTION

a) Objective

A limnological study of Wood Lake, B.C. was conducted during 1984. Previous studies have catagorized the lake as being "mildly-eutrophic" where algal growth is enhanced by nutrient input from the surrounding water shed. A 1980 government study (Jasper and Gray, Environment Canada) has suggested that nitrate leaching from Hiram Walker exfiltration beds into the groundwater system has contributed to the changing nutrient balance of Wood Lake. It has also been suggested that this potential leaching and subsequent changes in the lake's nutrient balance may not necessarily be detrimental to water quality (ie: water clarity). The 1980 report indicates that summer blue-green (cyanophyte) blooms, which frequented the lake during the early 1970's, seem to have subsided. One possible reason for this is that a spring bloom of diatoms which do not reduce water clarity to the same extent that blue-greens do, stimulated by increased nitrogen levels utilize available phosphorus thus limited its availability for a summer blue-green bloom.

The objective of the present study is to attempt to determine whether the 1980 study is an indication that a trend to improved water quality in Wood Lake is occurring or whether 1980 was an anomolous year in terms of a reduction in the number and duration of summer blue-green blooms.

b) Acknowledgements

I would like to thank R. Liboiron, D. Materi, B. Stagg, T. Thorburn, K. Zangbell, K. Kupser and D. Newman for their invaluable assistance with the field work. And a special thanks to Bonnie Fresu for the typing of this report.

2. SAMPLE COLLECTION

Samples were collected for biological analysis between March 15, 1984 and October 15, 1984. Samples for chemical analysis were collected on March 15, June 11 and October 15, 1984. Measurements of temperature, dissolved oxygen and light extinction were gathered between March 15 and October 15, 1984. All above samples were collected at a point located at the center of the lake. In addition, temperature, dissolved oxygen and light extinction measurements were made at a north and south point in the lake from March 15 to October 15, 1984. (See Appendix)

Water samples were collected using a 1.5 L Van Doren bottle from discrete depths down through the water column (5 for biology and 7 for chemistry). Glass B.O.D. bottles were filled for dissolved oxygen analysis. Water samples were then placed in 1 L plastic bottles for biological analysis (chlorophylls). Sample preparation (filtration, preservation, etc.) and most biological analyses were performed by Eco-Tech Labs of Kamloops.

3. ANALYTICAL METHODS

a) Physical

Temperature measurements were made at 1 M intervals down to 30 M. Dissolved oxygen measurements were taken at 5 M intervals down to 30 M using an Oxygen electrode up until July and, after July, samples were analyzed using the azide-modified Winkler titration method usually within 4 hours of sample collection. Water clarity measurements were made with the aid of a Secchi disc.

b) Chemistry

Samples for nitrogen, phosphorus and silica analysis were filtered through prewashed (distilled water and sample), Sartorius cellulose acetate membrane filters with a 0.45 micron nominal pore size. Analysis for total phosphorus was performed on unfiltered samples. Chemical analyses were by methods described in the Water Quality Branch Analytical Methods Manual (IWDM, 1981). All samples were processed and sent for analysis within 24 hours.

c) Biology

Investigations centered around distribution and composition of phytoplankton.

(i) Two independent measures of biomass were obtained; chlorophyll a and total cell volumes.

For chlorophyll a determinations, varying aliquots of lake water were filtered through Whatman GF/C filters and, unless pigments were extracted immediately, filters were stored frozen, in the dark, with dessicant. Chlorophyll samples were done in triplicate and results averaged. When filtering, vacuum pressure did not exceed 10" of mercury. Chlorophyll analysis followed Stainton et al. (1977, see appendix) with pigments extracted in 90% acetone for 16 hours in a refrigerator, ground, centrifuged and read on a Turner Designs III fluorometer. Calculation of chlorophyll a and phaeopigments used the formulae of Strickland and Parsons (1972, see appendix).

Total cell volumes were calculated from measurements of cell volumes and abundance of individual species (see Appendix).

(ii) Phytoplankton species composition and abundance.

Samples (normally 250 mls) were preserved with Lugol's solution in the field. They were shaken and settled in 1 ml settling chambers for approximately one hour. They were then checked under low power for random distribution of cells and enumerated at 125X. For almost all samples over 200 cells were identified and counted. For most samples three 1 ml aliquots were counted and the results averaged.

For each species the dimensions of several cells (filaments or colonies) were determined and volumes calculated assuming standard geometric shapes (eg. for ovoid with a circle in cross-section: $4/3\ \pi r^2 \cdot l$).

4. PHYSICAL MEASUREMENTS

a) Temperature

Temperature data are given in Appendix 1. Spring overturn and stratification preceded the first sampling date. Surface temperatures continued to rise through the summer until early August reaching a maximum of 22°C. At this time a strong thermocline had been established between 6 and 20 meters. Past studies indicate that fall overturn and isothermal conditions probably commence around the end of November.

b) Water Clarity

Secchi depth readings are given in Appendix I. An average Secchi depth of 19.5 feet was recorded over the period of the study. There appears to be a slight correlation between water clarity and algal biomass levels. The relatively small (as compared to 1980) blue-green bloom in early June saw the Secchi disc reading fall to its lowest value (14') though such low readings were not recorded during the fall bloom where chlorophyll values were three times those of June (Table 1). As well, algal biomass variations throughout the year do not seem to be reflected with any consistency with respect to Secchi depths.

5. CHEMICAL MEASUREMENTS

A list of chemical measurements is given in Appendix II.

a) Dissolved Oxygen

Dissolved oxygen (D.O.) decreased with depth in the hypolimnion during the stratified period, almost disappearing (<1.0 mg O_2/ L) near the sediment-water interface, beginning in the first week of August, presumably due to phytoplankton respiration. Elevated D.O. concentrations (>9.0 mg O_2/ L) were recorded above the hypolimnion in March, May and June (no D.O. recordings were taken during April). These elevated D.O. values probably reflect photosynthetic activity although unsettled weather during much of April-May could also have been a contributing factor in increasing D.O. values in the upper regions of the water column.

b) Phosphorus

Total phosphorus (TP) in the water column remained fairly constant from spring to summer but had showed a decrease by the time of fall sampling (see Appendix II). A similar pattern was observed with dissolved phosphorus (DP), however, orthophosphate dipped to below the limit of detection during June in the upper 5 M but recovered by the fall. For all chemical species mentioned above the highest concentrations were found in the hypolimnion perhaps indicating exchange with the sediments.

c) Nitrogen

Total dissolved nitrogen (TDN) remained fairly constant from spring to summer but doubled in the top 15 M in October (see Appendix II).

Nitrate (NO_3) was highest in the spring and was reduced to below the detection limit throughout the rest of the year although there was an increase in the hypolimnion in June. Nitrite (NO_2) was almost always below the limit of detection during the year.

Ammonia nitrogen (AN) was almost always below the detection limit in the spring and summer but increased in the fall and was fairly evenly distributed throughout the water column indicating that there had been no significant sedimenting of phytoplankton by early October.

d) Silica

Silica (SiO_2) concentrations were fairly uniform throughout the water column during spring and summer (see Appendix II). October 1st saw a decrease in the top 15 M to below the limit of detection. The expected depletion of silica in the spring, due to diatom growth, was not seen indicating that the March 15 sampling date was too early.

6. PHYTOPLANKTON BIOMASS

In terms of phytoplankton, the period of study can be divided into four stages though each stage is not always discrete (See Figure I).

From mid-March through early May there was a diatom bloom composed mainly of Fragilaria sp. (80μ in width) which peaked on April 17. Green algae (chlorophytes) had a minor peak on this same date. However, this was mainly composed of plankton species (denoted "large eugleniod", see pictures 6 and 7) that was not identified with any certainty and this peak should be viewed with some skepticism.

As diatoms began a precipitous decline in early May a green algal bloom (Sphaerocystis sp.) occurred which peaked at the end of May (chlorophyll a 13.3 $mg \cdot m^{-3}$ in epilimnion). Concomitant with the rise in green algae, blue-greens (mainly Anabaena sp.) also peaked though not to the same extent as greens did. As greens declined into August so did blue-greens.

The third stage spans July to Mid-August where total algal biomass was relatively low (see Table 1). During this period diatoms completely disappeared.

The final stage, which began in the latter part of August and continued to the end of the study, saw a blue-green bloom (mainly Aphanizomenon sp. and Lynbya sp. chlorophyll a: 15.8 $mg \cdot m^{-3}$ in epilimnion). Diatoms (mainly Melosira sp. and Fragilaria sp.[110μ width]) also reappeared at this time while greens (mainly Oocystis sp.) began to decline after a slight rise in early September. The October rise in diatoms seems to be reflected in the depletion of silica (Appendix II).

Figure 1: Algal Volumes from 0-20m

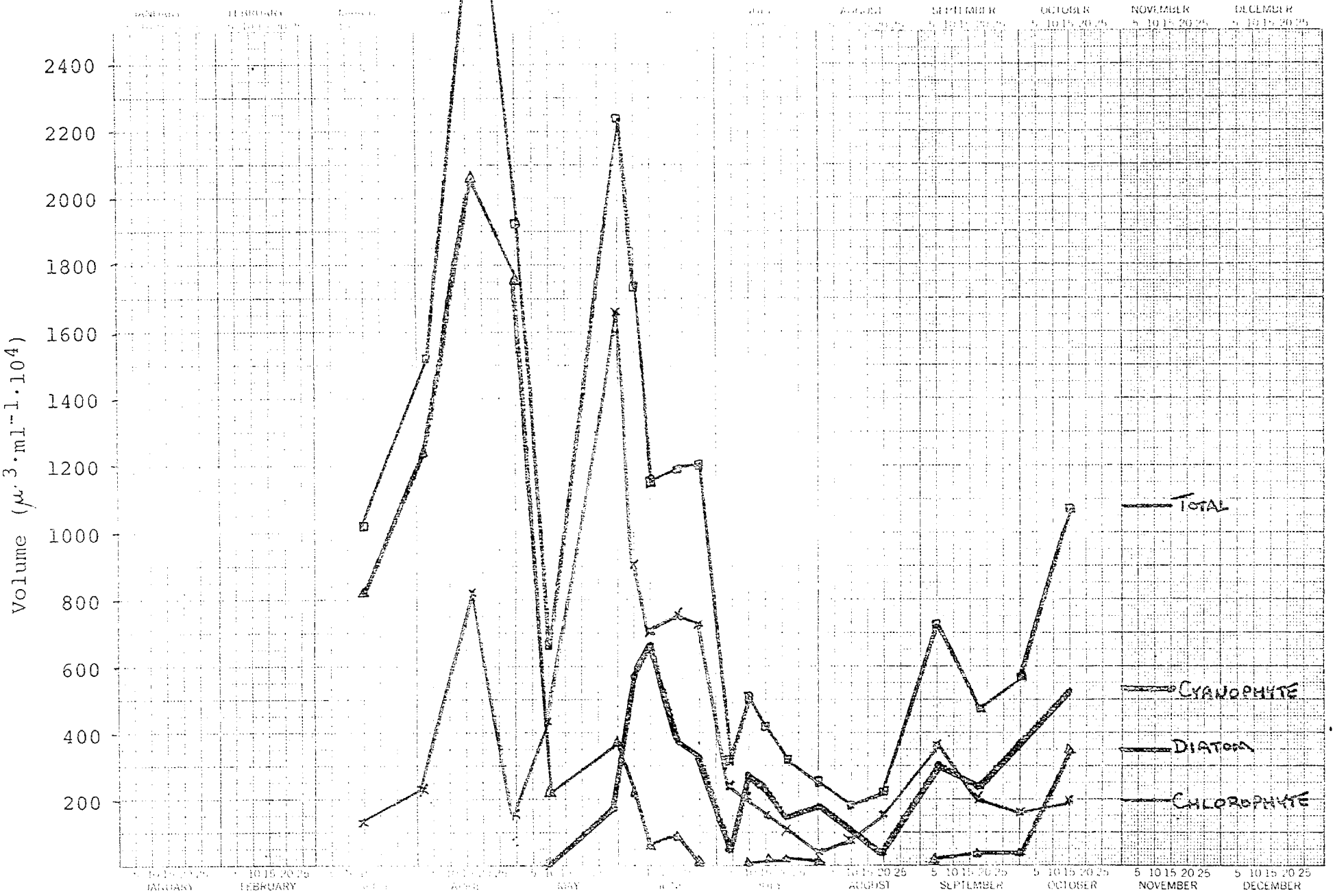

TABLE I: WOOD LAKE PHYTOPLANKTON BIOMASS MEASUREMENTS AT SPECIFIC DEPTHS

Date	Depth	Chlorophyll a ($mg \cdot m^{-3}$)	a Phaeopigments ($mg \cdot m^{-3}$)	Total Volume ($\mu^3 \cdot ml^{-1} \times 10^4$)	Dominant Phytoplankton (by volume)
84/03/15	0.5	-	-	99.67	Diatom (*Fragilaria* sp.)
	5	-	-	179.11	"
	10	-	-	238.45	"
	15	-	-	304.0	"
	20	-	-	212.1	"
84/04/02	0.5	-	-	318.99	Diatom (*Fragilaria* sp.)
	5	-	-	346.86	"
	10	-	-	365.90	"
	15	-	-	251.77	"
	20	-	-	-	
84/04/17	0.5	-	-	233.16	Diatom (*Fragilaria* sp.)
	5	-	-	622.69	"
	10	-	-	964.80	"
	15	-	-	613.16	"
	20	-	-	516.10	"
84/05/01	0.5	-	-	44.07	Diatom (*Fragilaria* sp.)
	5	5.88	0.67	178.2	"
	10	-	-	503.3	"
	15	-	-	623.0	"
	20	0.91	0.77	571.0	(*Stephanodiscus* sp.)
84/05/09	0.5	1.71	0.35	175.9	Chlorophyte?
	5	1.90	0.28	113.4	"
	10	1.64	0.44	156.6	"
	15	1.72	0.61	124.4	Diatom (*Fragilaria* sp.)
	20	1.42	0.78	95.7	"
84/05/14	0.5	1.18	0.37	-	
	5	0.91	0.27	-	
	10	0.61	0.25	-	
	15	0.85	0.48	-	
	20	1.23	0.41	-	
84/05/22	0.5	2.41	0.79	-	
	5	2.21	1.08	-	
	10	1.45	0.36	-	
	15	0.64	0.24	-	
	20	0.83	0.24	-	
84/05/28	0.5	1.85	0.46	503.9	Chlorophyte (*Sphaerocystis* sp.)
	5	2.21	1.39	738.7	"
	10	1.68	0.75	485.1	"
	15	-	-	293.9	"
	20	-	-	221.4	"
84/06/84	0.5	6.36	0.44	724.2	Cyanophyte (*Anabaena* sp.)
	5	5.44	0.83	620.3	Cyanophyte/Chlorophyte
	10	1.55	0.88	258.9	Chlorophyte (*Sphaerocystis* sp.
	15	0.77	0.51	285.0	"
	20	0.49	0.47	-	

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84/06/11	0.5	2.71	1.00	392.17	Cyanophyte (Anabaena sp.)
	5	2.40	0.62	276.97	"
	10	2.09	0.43	258.47	"
	15	1.46	0.48	161.17	"
	20	0.63	0.52	63.52	Chlorophyte (Sphaerocystis sp.)
84/06/18	0.5	1.89	0.38	460.93	Cyanophyte (Anabaena sp.)
	5	2.98	0.74	309.35	Chlorophyte (Sphaerocystis sp.)
	10	2.39	0.60	211.26	Chlorophyte
	15	2.28	0.63	122.64	"
	20	1.25	0.48	90.44	"
84/06/25	0.5	3.46	0.40	305.88	Cyanophyte (Anabaena sp.)/ Chlorophyte (Sphaerocystis sp.)
	5	4.24	0.96	339.90	"
	10	3.74	0.75	223.10	"
	15	2.02	0.86	107.90	"
	20	2.17	0.70	227.80	"
84/07/03	0.5	3.19	0.20	141.90	Chlorophyte (Sphaerocystis sp.)
	5	3.55	0.38	70.05	"
	10	3.44	0.83	93.06	"
	15	0.70	0.20	16.90	"
	20	0.42	0.21	14.05	"
84/07/09	0.5	2.30	0.17	169.48	Cyanophyte (Anabaena sp.)
	5	3.46	0.75	240.02	"
	10	3.73	1.03	95.58	Chlorophyte (Sphaerocystis sp.)
	15	2.16	0.54	5.52	"
	20	0.74	0.22	0.35	"
84/07/16	0.5	1.78	0.43	52.70	Cyanophyte/Chlorophyte
	5	3.54	0.43	272.30	Cyanophyte (Anabaena sp.)
	10	3.65	0.81	61.73	Chlorophyte (Spaerocystis sp.)
	15	2.00	0.31	26.39	"
	20	0.88	0.16	5.00	"
84/07/23	0.5	1.42	0.22	42.07	Cyanophyte/Chlorophyte
	5	1.87	0.43	147.50	"
	10	1.98	0.30	81.16	"
	15	-	-	33.69	"
	20	1.42	0.22	8.39	"
84/07/31	0.5	1.85	0.27	18.33	Chlorophyte (Oocystis sp.)
	5	1.72	0.19	153.73	Cyanophyte (Anabaena sp.)
	10	1.72	0.41	68.03	"
	15	1.07	0.45	10.25	"
	20	1.77	0.45	-	-
84/08/08	0.5	1.69	0.31	23.82	Cyanophyte (Anabaena sp.)
	5	1.60	0.19	57.99	(Aphanizomenon sp.)
	10	1.50	0.44	56.76	Chlorophyte (Oocystis sp.)
	15	1.08	0.15	19.97	"
	20	0.47	0.38	14.17	"

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Date	Depth	Chlorophyll a ($mg \cdot m^{-3}$)	a Phaeopigments ($mg \cdot m^{-3}$)	Total Volume ($\mu^3 \cdot ml^{-1} x 10^4$)	Dominant Phytoplankton (by volume)
84/08/20	0.5	1.70	0.67	51.94	Chlorophyte (*Oocystis* sp.)
	5	1.31	0.55	81.20	"
	10	1.25	0.48	34.40	"
	15	0.59	0.42	18.50	"
	20	0.62	0.57	3.97	"
84/09/05	0.5	1.56	0.24	135.84	Cyanophyte (*Lynbya* sp. +
	5	2.07	0.42	247.28	*Aphanizomenon* sp.)
	10	1.62	0.34	147.45	Chlorophyte (*Oocystis* sp.)
	15	1.48	0.27	88.53	"
	20	1.25	0.26	98.96	"
84/09/18	0.5	3.14	1.18	127.28	Cyanophyte (*Aphanizomenon*/*Lyngb*
	5	5.43	1.41	182.57	Chlorophyte (*Oocystis* sp.)
	10	5.60	2.24	115.34	"
	15	1.65	0.80	33.55	"
	20	0.63	0.55	18.02	"
84/10/01	0.5	4.63	0.90	156.39	Cyanophyte (*Aphanizomenon* sp. +
	5	5.04	2.02	182.92	*Lynbya* sp.)
	10	6.09	1.67	107.81	"
	15	1.61	0.69	117.20	"
	20	0.49	0.97	--	
84/10/15	0.5	10.8	2.75	266.42	Cyanophyte (*Aphanizomenon* sp.)
	5	14.07	0.78	307.00	"
	10	9.90	2.77	313.20	"
	15	3.47	1.33	94.40	Diatom (*Melosira* sp.)
	20	0.81	0.59	86.88	"

7. DISCUSSION AND CONCLUSIONS

Wood Lake, during 1984, became strongly stratified with a well-defined epilimnion and thermocline. Secchi disc levels were consistent with the characteristics of a meso-trophic lake although there did not seem to be any correlation with these levels and chlorophyll a measurements. Dissolved oxygen concentrations throughout the year decreased in the hypolimnion at rates typical of a mesotrophic lake with low levels measured at the sediment-water interface during the fall.

Dissolved phosphorus levels did not fall in the summer as would be expected from algal growth but did decrease by approximately 1/3 by the fall paralleling the October blue-green bloom.

Total dissolved nitrogen remained constant during spring and summer with an increase in October. Since no heterocysts were seen in blue-greens in the summer it can be postulated that the lake was not under nitrogen-stress which would have encouraged the production of the nitrogen-fixing cell.

Dissolved silica concentrations in the epilimnion were reduced to low levels in the fall which was accompanied by a small diatom bloom. It is probable that spring sampling was too early to detect silica depletion during the April diatom bloom.

Phytoplankton biomass levels in the euphotic zone, measured by chlorophyll a and cell volumes, indicate that the quality of the lake water has improved since 1980. This statement is based primarily on the single, relatively small blue-green bloom in June. Because summer blue-greens did not display heterocyst development it may be postulated that the lake was under P-limition and not under N-limition which may explain the shift in the algal community structure from that of 1980.

It is possible that greens (particularily Sphaerocystis sp.) were better able to exploit available phosphorus and suppressed blue-green growth. Continued lake studies must be under-taken, however, in order to determine if this apparent improvement in water quality can be documented from one year to the next or whether it is sporadic in nature.

Any conclusions regarding the specific influence that Hiram Walker may be having on the ecology of Wood Lake is beyond the scope of the present study.

APPENDIX 1

WOOD LAKE TEMPERATURE DATA (°C) FROM MID-STATION A

DEPTH (Metres)	84/03/15	84/05/01	84/05/09	84/05/14	84/05/22	84/06/06	84/06/11
0	3	8	8.5	10	10.5	13	14
1	3	8	8.5	10	10.5	13	14
2	3	8	8.5	10	10	12.5	13
3	3	8	8	10	10	12	13
4	3	8	8	10	10	12	12
5	4	8	8	9.5	10	12	12
6	4	8	8	9	10	11	11.5
7	4	8	8	9	9.5	11	11
8	4	8	8	9	9.5	11	11
9	4	7	8	9	9	10.5	10.5
10	4	7	8	9	9	10	10
11	4	7	8	9	8.5	9.5	9.5
12	4	6	8	8	8.5	9	9
13	4	6	8	8	8	7.5	9
14	4	6	8	7	7.5	6.5	8
15	4	6	7.5	6.5	7	6.5	7.5
16	4	6	8	6	6.5	6	7
17	4	6	8	6	6.5	6	6
18	4	5	8	5.5	6	5.5	6
19	4	5	7	5.5	6	5.5	5.5
20	3	5	7	5	6	5	5.5
21	3	5	7	5	5.5	5	5
22	3	5	7	5	5	4.5	5
23	3	5	7	5	5	4.5	5
24	3	5	7	5	5	4.5	4.5
25	3	5	6.5	5	5	4	4
26	3	5	6.5	5	4.5	4	4
27	3	5	6.5	5	4.5	4	4
28	3	4	6	5	4.5	4	4
29	3	4	6	5	4.5	4	4
30	3	4	6	5	4.5	4	4
Secchi Disc Reading		21'	20'	33'	31'	14'	20'

Note: Depth not taken for 84/05/28 - Secchi Disc reading 23'

WOOD LAKE TEMPERATURE DATA (^{o}C) FROM MID-STATION A

DEPTH (Metres)	84/06/18	84/06/25	84/07/03	84/07/09	84/07/16	84/07/23	84/07/31
0	16.5	17.5	16.5	18	21	20	21
1	16.5	17	16	17.5	20.5	20	21
2	16.5	17	16	17	20.5	19.5	20
3	16.5	16.5	16	17	20	19.5	19.5
4	15	16.5	15.5	17	19	19.5	19
5	14.5	16	15.5	16.5	18.5	19.5	18
6	14	15.5	15	16.5	17	19.5	17.5
7	13	15.5	15	16	16.5	17	15.5
8	11.5	15	15	15.5	16	15.5	14.5
9	10.5	13.5	15	14.5	15	13.5	13.5
10	10	12	14	13	13.5	12.5	12
11	10	10.5	12	12	12.5	12	11.5
12	9.5	10	10	10.5	11	11	10.5
13	9.5	9	9	9	10	10	10
14	9	8.5	8.5	8	9	9	9
15	9	8	7.5	8	8	8	7.5
16	8	7.5	7	7.5	7.5	7.5	7
17	7.5	7	6.5	7	7	7	6.5
18	7	6	6	7	6.5	6.5	6
19	6	6	5.5	6.5	6	6	5.5
20	6	6	5	6	5.5	5.5	5
21	6	5.5	5	5.5	5	5	5
22	5.5	5.5	5	5	5	5	5
23	5	5.5	5	5	5	5	4
24	5	5	4.5	5	5	5	4
25	5	5	4.5	4.5	4.5	4.5	4
26	4.5	4.5	4.5	4	4.5	4.5	4
27	4.5	4.5	4	4	4.5	4.5	4
28	4	4	4	4	4	4	3.5
29	4	4	4	4	4	4	3.5
30	4	4	4	4	4	4	3.5
Secchi Disc Reading	16'	16'	15'	16'	17'	18'	17'

WOOD LAKE TEMPERATURE DATA (^{O}C) FROM MID-STATION A

DEPTH (Metres)	84/08/08	84/08/20	84/09/05	84/09/18	84/10/01	84/10/15	84/10/24
0	22	21.5	18	16.5	13.5	11	9.5
1	22	21.5	18	16.5	13.5	11	9.5
2	22	21.5	18	16	13.5	11	9.5
3	22	21.5	18	16	13.5	11	9.5
4	22	21.5	18	16	13.5	11	9.5
5	22	21.5	18	16	13.5	11	9.5
6	22	21.5	18	16	13.5	11	9.5
7	20.5	21	18	16	13.5	11	9.5
8	18.5	20	18	16	13.5	11	9.5
9	16	16	18	16	13.5	11	9.5
10	14.5	13	17.5	16	13.5	11	9.5
11	12.5	12	15.5	15	13.5	11	9.5
12	12	11	13	13	13.5	11	9.5
13	11	9	11	10.5	13.5	11	9.5
14	10	8.5	10	9.5	13.5	11	9.5
15	9	8	9.5	9	11	11	9.5
16	8.5	7.5	8.5	8	8.5	10.5	9.5
17	8	7	8	7	7.5	7.5	9.5
18	7	6.5	7.5	6.5	7	6.5	9.5
19	6.5	6	7	6	6.5	6	7
20	6	6	7	6	6.5	5.5	6
21	6	5.5	6	6	6	5	6
22	5.5	5	6	5.5	5.5	5	5.5
23	5	5	6	5.5	5.5	5	5.5
24	5	5	5.5	5	5	5	5
25	5	4.5	5	5	5	5	5
26	5	4.5	5	5	5	5	5
27	5	4.5	5	5	5	4.5	5
28	5	4.5	5	4.5	5	4.5	5
29	5	4.5	5	4.5	5	4.5	4.5
30	5	4.5	4.5	4.5	5	4.5	4.5
Secchi Disc Reading	20'	16'	17'	19'	21'	23'	20'

Appendix 2

WOOD LAKE CHEMICAL DATA FROM MID-STATION

PARAMETER	SYMBOL	UNITS
Depth	Z	Meters
Temperature	^{o}C	Degrees Centigrade
pH	pH	pH
Total Nitrogen	TN	Milligrams/Liter N
Total Dissolved Nitrogen	TDN	Milligrams/Liter N
Nitrate Nitrogen	NO_3	Milligrams/Liter N
Nitrite Nitrogen	NO_2	Milligrams/Liter N
Ammonium Nitrogen	AN	Milligrams/Liter N
Total Phosphorus	TP	Milligrams/Liter P
Dissolved Phosphorus	DP	Milligrams/Liter P
Phosphorus Ortho	OP	Milligrams/Liter P
Reactive Silica	SiO_2	Milligrams/Liter SiO_2
Dissolved Oxygen	DO	Milligrams/Liter O
(Field Measurement)	(F)	Hach Kit
(Lab Measurement)	(L)	Winkler Azide-Modified Method

Appendix 2 (con't)

Monitor Date: March 16, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
	10.6	10.6			0.77	0.032	L.005	L0.05	0.061	0.055	0.030	1.1
5	10.8	10.8			1.01	0.042	L.005	L0.05	0.057	0.042	0.036	0.9
10	10.0	10.0			0.68	0.033	L.005	L0.05	0.045	0.029	0.029	0.9
15	6.9	6.9			0.69	0.028	L.005	L0.05	0.036	0.032	0.027	1.0
20	6.2	6.2			0.59	0.031	L.005	L0.05	0.030	0.029	0.024	1.1
25	6.3	6.3			0.56	0.029	0.005	L0.05	0.059	0.036	0.020	1.0
30*	6.2	6.2			14.5	0.089	L.005	4.24	0.218	0.117	0.017	2.3

Monitor Date: May 1, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	8	11.6										
5	8	12.1										
10	7	11.4										
15	6	10.8										
20	5	10.4										
25	5	10.7										
30	4	11.2										

Monitor Date: May 9, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	8.5	10.4		7.5								
5	8.0	-		7.5								
10	8.0	10.2		8.1								
15	7.5	-		8.0								
20	7.0	10.2		8.0								
25	6.5	-										
30	6.0	10.4										

Monitor Date: May 14, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	10.0	9.2		8.4								
5	9.5	-		8.4								
10	9.5	9.4		8.4								
15	6.5	-		8.15								
20	5.0	9.0		8.15								
25	5.0	-										
30	5.0	9.4										

Appendix 2 (con't)

Monitor Date: May 22, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	10.5	10.7		8.5								
5	10.0	-		8.45								
10	9.0	10.6		8.35								
15	7.0	-		8.25								
20	6.0	10.0		8.20								
25	5.0	9.2										
30	4.5	9.5										

Monitor Date: May 28, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	14	12.0		8.35								
5	-	-		8.35								
10	-	-		8.25								
15	-	-		8.25								
20	-	11.0		8.15								
25	-	-										
30	-	10.0										

Monitor Date: June 6, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	13	10.8		8.1								
5	12	-		8.1								
10	10	8.9		7.95								
15	6.5	-		7.80								
20	5	8.5		7.60								
25	4	-										
30	4	7.4										

Monitor Date: June 11, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	14	10.0		8.00	0.61	L.005	L.005	L0.05	0.042	0.039	L.010	1.0
5	12	-		8.00	0.41	L.005	L.005	L0.05	0.059	0.058	L.010	0.8
10	10	9.4		7.95	0.35	0.007	L.005	L0.05	0.060	0.050	0.013	0.9
1	7.5	-		7.60	0.55	0.018	L.005	0.13	0.068	0.047	0.029	0.9
[illegible]	5.5	8.3		7.35	0.65	0.019	L.005	L0.05	0.078	0.035	0.035	1.0
[illegible]	4	-		7.20	0.67	0.038	L.005	L0.05	0.098	0.065	0.065	0.9
30	4	6.6		7.05	0.76	0.066	L.005	0.23	0.131	0.120	0.111	1.3

Monitor Date: June 18, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	16.5	9.7		8.15								
5	14.5	-		8.0								
10	10	9.1		7.90								
15	9	-		7.85								
20	6	7.9		7.55								
25	5	-										
30	4	6.6										

Monitor Date: June 25, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	17.5	11.0		8.15								
5	16	-		8.10								
10	12,0	9.6		8.00								
15	8	-		7.75								
20	6	8.2		7.80								
25	5	-										
30	4	5.7										

Monitor Date: July 3, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	16.5	10.0		7.20								
5	15.5	-		7.15								
10	14	8.9		7.00								
15	7.5	-		6.45								
20	5	8.1		6.30								
25	4.5	-										
30	4	5.5										

Monitor Date: July 9, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	18	8.8										
5	16.5	-										
10	13	7.5										
15	8	-										
20	6	6.0										
25	4.5	-										
30	4	4.0										

Monitor Date: June 18, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	16.5	9.7		8.15								
5	14.5	-		8.0								
10	10	9.1		7.90								
15	9	-		7.85								
20	6	7.9		7.55								
25	5	-										
30	4	6.6										

Monitor Date: June 25, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	17.5	11.0		8.15								
5	16	-		8.10								
10	12,0	9.6		8.00								
15	8	-		7.75								
20	6	8.2		7.80								
25	5	-										
30	4	5.7										

Monitor Date: July 3, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	16.5	10.0		7.20								
5	15.5	-		7.15								
10	14	8.9		7.00								
15	7.5	-		6.45								
20	5	8.1		6.30								
25	4.5	-										
30	4	5.5										

Monitor Date: July 9, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	18	8.8										
5	16.5	-										
10	13	7.5										
15	8	-										
20	6	6.0										
25	4.5	-										
30	4	4.0										

Appendix 2 (con't)

Monitor Date: July 16, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	21	7.2										
5	18.5	-										
10	13.5	5.7										
15	8	-										
20	5.5	4.6										
25	4.5	-										
30	4	5.0										

Monitor Date: July 23, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	20	11.6		8.10								
5	19.5	-		8.10								
10	12.5	7.8		7.95								
15	8	-		7.80								
20	5.5	5.9		7.60								
25	4.5	-										
30	4	2.5										

Monitor Date: July 31, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	21		8.8	8.15								
5	18		9.4	8.20								
10	12		8.1	7.80								
15	7.5		7.3	7.45								
20	5		-	7.25								
25	4		-									
30	3.5		0.9									

Monitor Date: August 8, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	22		8.8	8.15								
5	22		9.0	8.10								
10	14.5		8.0	7.65								
[illegible]	9		7.9	7.50								
[illegible]	6		4.8	7.45								
25	5		-									
30	5		-									

Appendix 2 (con't)

Monitor Date: Aug 20, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	21.5		9.1	8.10								
5	21.5		9.3	8.05								
10	13		6.8	7.50								
15	8		6.0	7.25								
20	6		3.4	7.20								
25	4.5		-									
30	4.5		0.3									

Monitor Date: Sept 5, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	18		9.0	8.20								
5	18		7.5	8.35								
10	17		5.1	8.35								
15	9.5		4.4	8.00								
20	7		-	8.00								
25	5		-									
30	4.5		0.6									

Monitor Date: Sept 18, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	16.5		9.35	8.35								
5	16		9.2	8.35								
10	16		8.5	8.30								
15	9		4.3	7.65								
20	6		2.1	7.60								
25	5		0.3									
30	4.5		0.4									

Monitor Date: Oct 1, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	13.5		L9.0		1.84	0.008	L.005	0.88	0.022	0.022	0.022	L0.5
5	13.5		9.2		1.81	0.019	L.005	1.01	0.018	0.017	0.017	L0.5
10	13.5		8.9		1.85	0.032	L.005	0.96	0.017	0.016	0.011	L0.5
15	11		4.1		1.22	0.038	0.007	0.30	0.057	0.057	0.057	L0.5
20	6.5		1.4		0.72	0.181	0.009	0.37	0.104	0.100	0.098	1.0
25	5		0.2		0.87	0.385	0.146	0.44	0.174	0.155	0.150	0.7
30	5		0.3		0.98	0.413	0.146	0.68	0.216	0.190	0.185	1.9

Appendix 2 (con't)

Monitor Date: Oct 15, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
	11		8.7	7.90								
5	11		8.9	7.95								
10	11		8.8	7.90								
15	11		7.9	7.80								
20	5.5		0.5	7.30								
25	5		0.4									
30	4.5		0.2									

Monitor Date: Oct 24, 1984

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2
0	9.5											
5	9.5											
10	9.5											
15	9.5											
20	6											
25	5											
30	4.5											

Monitor Date:

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2

Monitor Date:

Z	°C(F)	D.O.(F)	D.O.(L)	pH	TDN	NO_3	NO_2	AN	TP	DP	OP	SiO_2

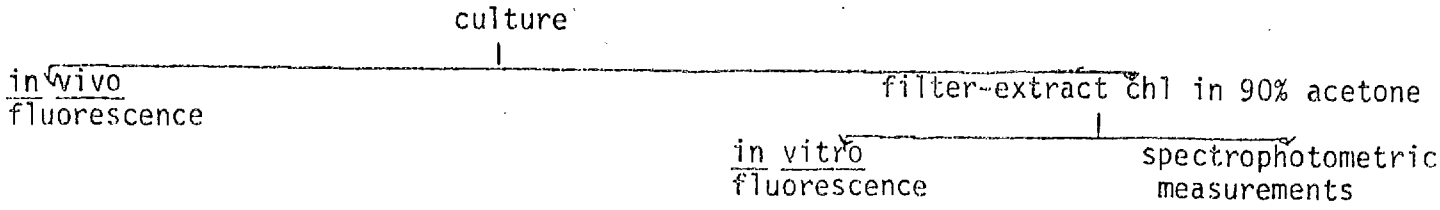
Biomass Measurements

Experimental Objectives

1) To determine chlorophyll *a* and phaeopigment concentrations in a phytoplankton culture during (a) exponential growth phase (b) scensecent phase. The pigment concentrations will be determined by (a) Spectrophotometric methods (b) Fluorescence - *in vivo* and *in vitro*

2) To examine qualitatively the efficiency of extracting some marine macrophytes with the solvents, acetone and dimethyl sulphoxide

Note: Proceed with objective (1) according to the following flow diagram



I. Determination of Chlorophyll Spectrophotometrically

A. Chlorophyll extraction

(1) Set up filter apparatus. Use a glass fiber filter.

(2) Squirt about 1 ml of a saturated solution of $MgCO_3$ (shake immediately before use) onto a glass fiber filter (to make pore size of filter more uniform)

(3) The volume of the culture required, will depend on how dense the culture is. If you cannot see a green-brown color on the filter when you are finished filtering, then filter more. Try 25 ml at first. Pour culture sample into filter funnel and start vacuum pump ($\sim \frac{1}{2}$ atm setting)

(4) Remove the filter as soon as filtration is finished, fold it in half and emerse it in 5 ml of pre-chilled 90% acetone in a screw-top test tube or calibrated centrifuge tube. Let the chlorophyll extraction proceed in a refrigerator, in the dark (light-tight box) for about 20 hrs. For this lab we will extract for only 2 hrs (room temperature). (In some diatoms the enzyme chlorophyllase is still not completely inactivated in 90% acetone and therefore chl *a* degrades to chlorophyllide *a* with time. While you are waiting during the extraction period measure *in vivo* fluorescence (II. A-P.3) and extract macrophyte pigments (III-P.3).

B. Sample analysis - done preferably in dim light.

(1) Empty the filter and acetone into a tissue grinder and grind of ~1 min at 500 rpm. Keep grinder tube in ice bath during grinding. Transfer the homogenized sample to the original tube and rinse (~3 ml) the tissue grinder twice with acetone, adding the rinses to the rest of the sample. It is important to keep the sample in the dark (light-tight box) as much as possible while carrying out the above operation. The final volume (original sample + 2 rinses) should be 11 ml.

(2) Shake the tube and centrifuge for 5 to 10 min. at 3000 to 5000 g.

(3) Carefully pipette off the supernatant into a small graduated cylinder and measure the volume. An easier way if a graduated centrifuge tube is used, is to read off the volume occupied by the ground-up filter and subtract it from the total volume of the solution.

(4) Fill a cuvette with the extract and read the extinction at 750,664 , 647, 630, and 430 nm against a 90% acetone blank.

(5) Add 2 drops of 10% HCl to sample in the cuvette and mix (tap tube with finger). After 2 min read at 750 and 664 nm. (Make sure acid is completely washed out of the cuvette afterwards).

B. Calculations

(a) The chl a concentration (really chlorophyll a + chlorophyllide a) for a diatom culture can be calculated according to the following equation (Jeffrey and Humphrey, 1975),

$$\frac{\mu g \text{ chl } a}{\text{litre}} = \frac{S\,(11.47\,E_{664} - 0.40\,E_{630})}{V}$$

where S = vol. of acetone (ml)
V = vol. of sample filtered (liter)
E_{664} - and other extinction measurements must be corrected for turbidity by subtracting measurement at E_{750}.

The equation assumes a 1 cm cell or cuvette is used.

(b) Physiological index,

$$E_{430}/E_{664} = \frac{E_{430} - E_{750}}{E_{664} - E_{750}}$$

(c) Phaeopigments - really phaeophytin a + phaeophorbide a

$$\text{Phaeopigment } a = \frac{26.7\,[1.7\,(E_{664_a}) - E_{664_o}] \times S}{V}$$

Symbols are as defined above in (a). Subscript 'a' = with acid addition and 'o' = without acid. (See Lorenzen 1967)

II. Determination of Chlorophyll by Fluorescence

The readings on the fluorometer are in relative units (dial goes from 0 to 100). Therefore, the fluorometer must be calibrated in order to convert these readings to chl a concentrations, and it must be recalibrated each time any changes or repair work is done on the instrument. The fluorometer has been calibrated for you. These factors were obtained by taking some of the 90% acetone extract of chlorophyll that had been read on the spectrophotometer and obtaining a reading on the different scales of the fluorometer (i.e. factor = $\frac{\text{chl conc'n } (\mu g/l)}{\text{fluor. reading}}$

A. In vivo fluorescence

1) Turn on fluorometer and lamp for a 15 minute warm-up period.

2) Zero the fluorometer with a cuvette filled with filtered seawater. A solid black plug may be inserted into the culture holder also.

3) Fill the cuvette with the culture sample and read on the appropriate scale. Repeat, taking duplicate readings.

B. In vitro fluorescence

1) Fill cuvette with some of the extracted chlorophyll sample obtained from first part of lab and read on an appropriate scale. It may be necessary to dilute the sample. Which ever scale is chosen zero first with reagent blank (90% acetone).

C. Calculations

1) If no phaeopigments are present, the in vivo or in vitro fluorometer reading can be multiplied by appropriate factor to get µg chla/l.

2) If phaeopigments are thought to be present:

$$\underset{(\mu g/l)}{\text{chl a}} = \text{factor } (F_o - F_a)$$

$$\underset{(\mu g/l)}{\text{Phaeo}} = \text{factor } (1.8\, F_a - F_o$$

- where F_a and F_o are the fluorometer readings with and without acid added.

III. Efficiency of Different Solvents in Extracting Macrophyte Pigments

Procedure:

1) Wash algal blade with tap water to remove adhering salt (prevents turbidity during extraction).

2) Place blade in a beaker and treat blade with a volume of dimethyl sulphoxide (DMSO) = 4 x blade wt. (Caution: Wear plastic gloves when handling DMSO).

3) After about 5 min. drain off yellow-brown extract into another beaker. What pigments are contained in this extract?

4) Treat the blade with a similar volume of acetone.

5) After 5 min drain off the extract into another beaker. What pigments are contained in this extract?

6) Describe the appearance of the blade, noting areas where pigments remain.

Lab Write-up

1) Write up results of objective (1) and discuss results. Note the conditions that can cause variation in the results. The following references from your reference list should be helpful: Jeffrey, 1974; Kiefer 1973

2) Write up observations of objective (2), answering questions in the procedure and referring to the paper by Seeley et al. 1972.

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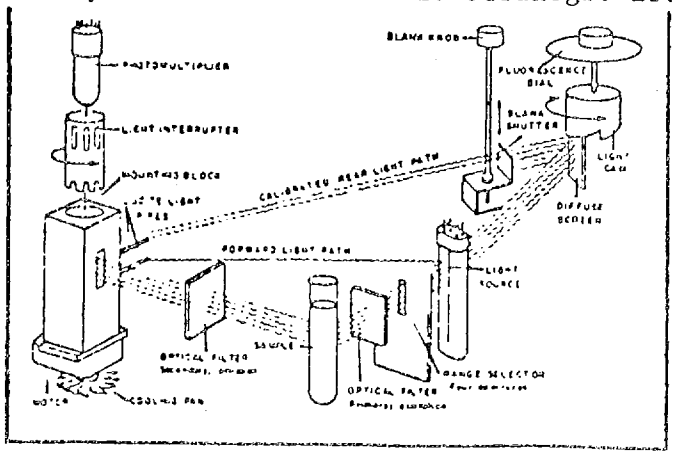

Diagram of Turner Double Beam Filter Fluorometer

References (continued)

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Biomass Measurements

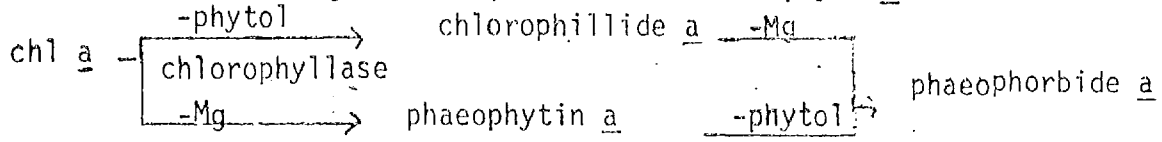
1) Cell Counting (a) Microscope- can obtain numbers, species and volume. The latter is determined by measuring certain dimensions of the cell (e.g. length and width) and assuming the cell approximates a certain geometric shape (e.g. cylinder, where Volume = $\pi r^2 h$) Cell Volume can be converted into carbon using an equation by Strathman (1967).
Disadvantages are 1) time consuming 2) human errors 3) preservation problems

(b) Particle counter

- counts particles and therefore chains of cells.
- counts detritus (dead particles).
- gives good estimate of total volume since number of particles analyzed is large.

2. Chemical Methods

(a) Pigments (i) Spectrophotometric method
using trichromatic equation (SCOR-UNESCO, 1966) to measure chlorophyll a, b, c and carotenoids.
- can get losses if store samples or expose to light.
- degradation products of chlorophyll a are as follows:



Pigment diversity index or physiological index

$\frac{E_{430}}{E_{664}}$ - a ratio above 3 (i.e. getting more carotenoids formed) indicates senescent algal population.

methods measuring- phaeophytin a (phaeopigments) are really measuring both phaeophytin a + phaeophorbide a (Jeffrey, 1974)

Phaeopigments - measured by adding 2 drops of 10% HCl to chlorophyll extrac
- if chl b present will exaggerate phaeophytin value since chl b increases fluorescence when acid added. Acid ratio = Eo/Ea = 1.7 (when no breakdown present)

chl c - new equations (Jeffrey and Humphrey, 1975) primarily affect determination of chl c. Old equations (UNESCO, 1966) give chl c values 2 times too high because old extinction coefficient determined with chl c which polymerized when purified, giving a lower extinction coefficient.

note: - Spectrophotometric method does not distinguish between:
(1) chl a and chlorophyllide a
(2) phaeophytin a, phaeophorbide a or chl b

The new equations (Jeffrey and Humphrey, 1975) are:

1. Higher plants and green algae containing chlorophylls *a* and *b* (solvent 90% acetone)
   Chlorophyll *a* $= 11.93\ E_{664} - 1.93\ E_{647}$
   Chlorophyll *b* $= 20.36\ E_{647} - 5.50\ E_{664}$
2. Diatoms, chrysomonads and brown algae containing chlorophylls *a*, and c_1 and c_2 in equal proportions (solvent 90% acetone)
   Chlorophyll *a* $= 11.47\ E_{664} - 0.40\ E_{630}$
   Chlorophylls $c_1 + c_2$ $= 24.36\ E_{630} - 3.73\ E_{664}$
3. Dinoflagellates and cryptomonads containing chlorophylls *a* and c_2 (solvent 100% acetone)
   Chlorophyll *a* $= 11.43\ E_{663} - 0.64\ E_{630}$
   Chlorophyll c_2 $= 27.09\ E_{630} - 3.63\ E_{663}$
4. Mixed phytoplankton populations containing chlorophylls *a* and *b* and equal amounts of chlorophylls c_1 and c_2 (solvent 90% acetone)
   Chlorophyll *a* $= 11.85\ E_{664} - 1.54\ E_{647} - 0.08\ E_{630}$
   Chlorophyll *b* $= -5.43\ E_{664} + 21.03\ E_{647} - 2.66\ E_{630}$
   Chlorophylls $c_1 + c_2$ $= -1.67\ E_{664} - 7.60\ E_{647} + 24.52\ E_{630}$

(ii) Fluorescence method

- a fluorometer is used to measure chlorophyll fluorescence
- blue light passes through primary filter (430 nm) - causes chl a to fluoresce (gives off red light). This light passes through a secondary filter (676 nm) which helps decrease the interference due to fluorescence of chl b and c. (660 and 642 nm, respectively).

- in vitro - Yentsch & Menzel (1963)
  - extract chl into acetone - read in fluorometer
    - easy - insensitive to turbidity
    - however - not as sensitive as spectrophotometric (trichromatic) method
      - loss of detail (no values can be determined for chl b, c or carotenoids)
      - interference between chlorophylls and phycobilin fluorescence.

- in vivo - Lorenzen (1966), also Loftus and Carpenter (1971)
  - read fluorescence on live culture
  - can continually read fluorescence of experimental cultures in tubes (culture not used up)
  - however - ~ 1/10 as sensitve as in vitro method
    - affected by temperature (fluor. ↑ as temp. ↑)

(iii) Thin layer chromatography (Jeffrey 1974)
- necessary for accurate determinations of degradation products of chl a
- extract - concentrate pigment from solvent - TLC-elute-spectrophotometry.

(b) ATP - method of Holm-Hansen & Booth (1966)
- ATP is very labile, therefore method estimates total living biomass
- the determination of ATP is by bioluminescence where 1 photon of light is emitted for each molecule of ATP hydrolyzed according to the following equation.

$$\underset{\text{(reduced)}}{\text{Luciferin} + \text{ATP} + O_2} \xrightarrow[\text{Luciferase}]{Mg^{++}} \underset{\text{(oxidized)}}{\text{Luciferin} + \text{PPi} + \text{AMP} + H_2O} + 1 \text{ photon}$$

(c) Protein - can now be quickly measured using a fluorescent compound, fluorescamine (Packard and Dortch, 1975)

(d) Total Carbon - high temperature combustion of particulate organic carbon into CO_2 (Sharp, 1973)

Conversion from one biomass estimate to another

1) Fluorescence ⟶ chl a - ratio varies by over a factor of 2, due to:
   a) Light intensity - as Lt intensity ↑ fluor.↓ in 15 min. due to chloroplast movement (Kiefer, 1973)
   b) Nutrient deficiency - ratio ↑ as cells become stationar

2) chl a ⟶ C - ratio varies between 10 & 90 (avg. ∿ 30 to 40)

Note: To convert from fluorescence to C is a 2 step process. If one assumes a certain ratio for each conversion, then final estimate could be high or low by up to a factor of ∿ 4.

3) ATP ⟶ C - C/ATP ratio varies from 125 to 250.

IV.3.IV. FLUOROMETRIC DETERMINATION OF CHLOROPHYLLS

INTRODUCTION

The method described here is based on the use of the Turner fluorometer as suggested by C.S. Yentsch and D.W. Menzel (*Deep-Sea Res.*, 10: 221, 1963) and subsequently investigated by Holm-Hansen et al. (*J. Conseil, Conseil Perm. Intern. Exploration Mer*, 30: 3, 1965). The method is not so accurate as the spectrophotometric approach but has the convenience of speed and the requirement of much smaller sample volumes for a given sensitivity. Only chlorophyll *a* is determined.

METHOD

A. CAPABILITIES

The limit of detection will depend upon the volume of water filtered and the sensitivity of the fluorometer. With a 2-liter sample about 0.01 mg chlorophyll a/m^3 should be detectable with surety. The precision is very much dependent on the amount of pigment being determined but P (*see* NOTE ON STATISTICAL LIMITS) is better than 8% of any value of chlorophyll *a* exceeding 0.5 mg/m^3.

B. OUTLINE OF METHOD

Extracts obtained as described in IV.3.I are measured fluorometrically with the Turner fluorometer.

C. SPECIAL APPARATUS AND EQUIPMENT

See IV.3.I,C but only glass filters should be used. The Turner fluorometer is fitted with the "high sensitivity" door, F.4T4-BL lamp, Wratten 47B or Corning CS.5-60 filter for the excitation light and Corning CS.2-64 filter for the emitted light.

D. SAMPLING PROCEDURE AND SAMPLE STORAGE

See IV.3.I,D.

E. SPECIAL REAGENTS

See IV.3.I,E.

F. EXPERIMENTAL

The extracts from 0.25–2 liters, obtained exactly as described in IV.3.I,F.1–5, but using *only 10.0 ml* of 90% acetone, are measured in a Turner fluorometer with the scale "zeroed" for each door opening against a tube of 90% acetone. Provided that phaeo-pigments are absent:

$$\text{mg chlorophyll } a/m^3 = F_D \times R$$

where R is the reading of the fluorometer and F_D is a factor for each door (*see* Section H *below*). Do not use solutions which necessitate the use of door 1 or which give readings much greater than 50 on door 3. With such solutions the concentrations of chlorophyll are too great for there to be linearity between fluorescence and concentration. If solutions are too concentrated dilute 3 ml (pipette) of extract with 3 ml of 90% acetone in a second clean, dry tube.

G. Determination of Blank

As the conditions used in the fluorometer are specific for chlorophyll and this is not introduced as a contaminant in reagents we have never found a blank, *per se*, with this method. The output (probably from scatter) of a tube of 90% acetone is not negligible on door 10 and the instrument should be zeroed against a tube of 90% acetone with all doors immediately prior to use.

H. Calibration

This must be done on extracts from marine phytoplankton as pure chlorophyll *a* is difficult to obtain. With the conditions recommended in this method the instrument responds almost exclusively to chlorophyll *a* but there is a slight and variable response to other chlorophylls. For this reason factors vary a little from species to species. We recommend that a healthy culture of *Skeletonema costatum* or, even more, a mixture of about equal amounts (by pigment) of *Skeletonema costatum*, *Coccolithus huxleyii*, and *Peridinium trochoidium* be used as a source of chlorophyll. If such cultures are not available natural populations can be used but there is then always uncertainty as to the presence of phaeo-pigments. Take samples from near the surface in eutrophic waters under early "bloom" conditions.

Extract sufficient culture or natural population to give 50 ml of extract having a reading of about 50 on door 3 of the fluorometer (R_3). Determine the amount, C_a, as described in IV.3.I,F, having ensured that the wavelength alignment of the spectrophotometer is carefully adjusted. Determine F_3 for door 3 from the formula:

$$F_3 = \frac{C_a}{R_3}$$

Dilute a known volume of the extract with a known volume of 90% acetone so that readings greater than 50 are obtained for doors 10 and 30 with known new values of C_a. Calculate F_{10} and F_{30} from expressions analogous to the above.

Note: Generally it is so little extra trouble to determine both chlorophyll and phaeo-pigments together that the approach given in the following addendum is recommended for work with samples other than phytoplankton cultures.

ADDENDUM TO IV.3.IV. FLUOROMETRIC DETERMINATION OF PHAEO-PIGMENTS

METHOD

This procedure is taken from Holm-Hansen, et al. (*J. Conseil, Conseil Perm. Intern. Exploration Mer*, 30: 3, 1965) and is essentially the same as IV.3.IV except that after the first reading, R_B, is taken on the fluorometer, the tube is removed, and 2 drops of 5% v/v hydrochloric acid are added. The contents of the tube are mixed by inverting once or twice, and a second reading, R_A, is taken between 30 and 60 sec later, after a stable value is reached.

Note: The tube must be washed out well with 90% acetone between determinations to make sure that no acid remains.

The precision varies from sample to sample and is not so high as the precision obtainable when only measurements on unacidified solutions are made. Quantities of phaeo-pigments less than 10% of the total pigment should be interpreted with caution.

$$\text{mg chlorophyll } a/\text{m}^3 = F_D \frac{r}{r-1}(R_B - R_A) \times \frac{\text{Volume extracted in (ml)}}{\text{volume filtered (ml)}}$$

$$\text{mg phaeo-pigment/m}^3 = F_D \frac{r}{r-1}(rR_A - R_B)$$

Where F_D is the door factor and r is a ratio obtained as described below. There is no blank *per se* with this method.

CALIBRATION

The door factors, F_D, are identical with those obtained in IV.3.IV,H and should be obtained in the same way, ideally from mixed cultures. The ratio, r, is the ratio $\frac{R_B}{R_A}$ obtained on any extract free from phaeo-pigments but, preferably, an extract used for standardization. This ratio, which should be near to 2.2 with the equipment specified here, must be obtained by making all measurements on one door, the R_B value being as near to 100 as practicable. The value of r is not quite constant, especially if a great deal of chlorophyll *c* is present in some extracts, so slightly negative values for phaeo-pigments may sometimes be obtained. A single formula is good enough, however, for most field work and will estimate the fraction of phaeo-pigments present in samples sufficiently well for most ecological studies. For work of the highest precision use the method given in the Addendum to IV.3.I.

PICTURE KEY

1. Fragilaria sp. (110 microns in width, 125X)
2. Sphaerocystis sp. (100 microns in diameter, 125X)
3. Sphaerocystis sp. (500X)
4. ? (denoted on count sheets as "Large Euglenoid", 50-25 microns 1000X)
5. Anabaena sp. + "large euglenoid"
6. Anabaena sp. clump (125X)
7. Staurastrum sp. (400X)
8. Ceratium sp. (1000X)
9. Asterionella sp. (125X)
10. Oocystis sp. (mother cell, 1000X)
11. Stephanodiscus sp. (90μ in diameter, 500X)
12. Stephanodiscus sp. (1000X)
13. Aphanizomenon sp. (+ akinete, 125X)
14. Melosira sp. (500X)
15. Melosira sp. (1000X)
16. ? (denoted 8 on count sheets, 7 microns wide, 125X)
17. ? (denoted 8 on count sheets, 500X)

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ENVIRONMENTAL TESTING
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ASSAYING

783 Notre Dame Drive, Kamloops, B.C. V2C 5N8 - Telephone (604) 372-9700
Telex: 048-8393

March 22, 1984

ANALYTICAL RESULTS

CLIENT: Hiram Walker & Sons Ltd.
Okanagan Distillery
P. O. Box 250
WINFIELD, B. C.
V0H 2C0

ATTENTION: Mr. Len Russell, Quality Control Supervisor

SAMPLE IDENTIFICATION: 7 lake study water samples received 0800 hrs. March 16, 1984 collected March 15,1984

PARAMETER:

	SITE NO. 1	2	3	4	5	6	7*
Nitrogen - Total Kjeldahl (as N)	0.77	1.01	0.68	0.69	0.59	0.56	14.5
Nitrogen - Dissolved Nitrate (as N)	0.032	0.042	0.033	0.028	0.031	0.029	0.089
Nitrogen - Dissolved Nitrite (as N)	<0.005	<0.005	<0.005	<0.005	<0.005	0.005	<0.005
Nitrogen - Ammonia (as N)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	4.24
Phosphorus - Total (as P)	0.061	0.057	0.045	0.036	0.030	0.059	0.218
Phosphorus - Total Dissolved (as P)	0.055	0.042	0.029	0.032	0.029	0.036	0.117
Phosphorus - Ortho (as P)	0.030	0.036	0.029	0.027	0.024	0.020	0.017
Silica - Reactive (as SiO_2)	1.1	0.9	0.9	1.0	1.1	1.0	2.3

NOTES: All results expressed in mg per litre.
< = less than
* Sample taken at lake bottom - some sediment in sample.

ECO-TECH LABORATORIES LTD.
Sandra M. Taylor, M.Sc.
Chief Chemist

SMT/mil

Over Page

Wood Lake Analyses 84/06/21

RESULTS FOR LAKE SAMPLES COLLECTED 12/04 AS FOLLOWS

NO 1: 0 M JUNE 11/84
2: 5 M "
3: 10 M "
4: 15 M
5: 20 M
6: 25 M JUNE 11/84
7: 30 M "

PARAMETER	NO 1	NO 2	NO 3	NO 4	NO 5	NO 6	NO 7
TOT ORTHOPHOS							
NITROGEN	0.61	0.41	0.25	0.55	0.05	0.67	0.76
NITRATE, DISS	L0.005	L0.005	L0.007	0.018	0.019	0.026	0.066
NITRITE, DISS	L0.005	L0.005	L0.005	L0.005	L0.005	L0.005	L0.005
AMMONIA	L0.05	L0.05	L0.05	0.13	L0.05	L0.05	0.23
PHOSPHORUS, TOTAL	0.042	0.059	0.060	0.063	0.078	0.055	0.151
PHOSPHORUS, DISS	0.029	0.055	0.050	0.047	0.025	0.005	0.129
PHOSPHATE, DISS	L0.010	L0.010	0.013	0.009	0.0133 0.035	0.065	0.111
CHLOROPHYLL A, ACETONE	1.0	0.8	0.4	0.5	1.0	0.2	1.3

* = RESULTS ARRIVED TOO LATE FOR INCLUSION IN REPORT
L = LESS THAN
+ = TESTS PERFORMED ON FILTERED SAMPLES

LB Russell

DM Booth

cc: C. MacDougall

ENVIRONMENTAL TESTING
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ASSAYING

ECO-TECH LABORATORIES LTD.

10041 E. Trans Canada Hwy., R.R. #2, Kamloops, B.C. V2C 2J3 Phone (604) 573-5700
Telex: 048-8393

October 16, 1984

ANALYTICAL RESULTS

CLIENT: Hiram Walker & Sons Ltd.
Okanagan Distillery
P. O. Box 250
WINFIELD, B. C.
V0H 2C0

ATTENTION: Mr. Len Russell, Quality Control Supervisor

SAMPLE IDENTIFICATION: 16 lake study water samples received 0930 hrs. October 4, 1984 labelled as follows:

No. 1: Wood Lake - 0m
2: " " - 5m
3: " " - 10m
4: " " - 15m
5: " " - 20m
6: " " - 25m
7: " " - 30m
8: Kal Lake - 0m
9: " " - 5m
10: " " - 10m
11: " " - 15m
12: " " - 20m
13: " " - 25m
14: " " - 30m
15: Duck #1
16: Duck #2

PARAMETER:	SITE NO. 1	2	3	4	5	6
Nitrogen - Total Kjeldahl (as N)	1.84	1.81	1.85	1.22	0.72	0.87
Nitrogen - Dissolved Nitrite (as N)	<0.005	<0.005	<0.005	0.007	0.009	0.146
Nitrogen - Dissolved Nitrate (as N)	0.008	0.019	0.032	0.038	0.181	0.385
Nitrogen - Ammonia (as N)	0.88	1.01	0.96	0.30	0.37	0.44
Phosphorus - Total (as P)	0.022	0.018	0.017	0.057	0.104	0.174
Phosphorus - Total Dissolved (as P)	0.022	0.017	0.016	0.057	0.100	0.155
Phosphorus - Ortho (as P)	0.022	0.017	0.011	0.057	0.098	0.150
Silica (as SiO_2)	<0.5	<0.5	<0.5	<0.5	1.0	0.7

.../2

SOUTH STATION. (BLUE WATERS HOUSE).

Hiram Walker & Sons Ltd. - 2 - October 16, 1984

depth meters.

PARAMETER:	WQC 0 30	KAL 0	KAL 5	KAL 10	KAL 15	KAL 20
	SITE NO. 7	8	9	10	11	12
Nitrogen - Total Kjeldahl (as N)	0.98	0.31	0.12	1.29	1.48	1.22
Nitrogen - Dissolved Nitrite (as N)	0.146	<0.005	<0.005	<0.005	<0.005	<0.005
Nitrogen - Dissolved Nitrate (as N)	0.413	0.014	0.009	<0.005	<0.005	0.010
Nitrogen - Ammonia (as N)	0.68	<0.05	<0.05	<0.05	<0.05	<0.05
Phosphorus - Total (as P)	0.216	0.027	0.035	0.018	<0.010	<0.010
Phosphorus - Total Dissolved (as P)	0.190	0.021	0.020	0.018	<0.010	<0.010
Phosphorus - Ortho (as P)	0.185	0.018	<0.010	<0.010	<0.010	<0.010
Silica (as SiO_2)	1.9	<0.5	<0.5	<0.5	<0.5	<0.5

PARAMETER:	KAL 25	KAL 30	Duck
	SITE NO. 13	14	15	16
Nitrogen - Total Kjeldahl (as N)	0.11	0.13	0.43	1.49
Nitrogen - Dissolved Nitrite (as N)	0.014	0.005	<0.005	<0.005
Nitrogen - Dissolved Nitrate (as N)	0.025	0.022	0.037	0.033
Nitrogen - Ammonia (as N)	<0.05	0.15	0.22	0.20
Phosphorus - Total (as P)	<0.010	<0.010	0.029	0.022
Phosphorus - Total Dissolved (as P)	<0.010	<0.010	<0.010	<0.010
Phosphorus - Ortho (as P)	<0.010	<0.010	<0.010	<0.010
Silica (as SiO_2)	<0.5	<0.5	0.5	1.0

NOTES: All results expressed in mg per litre.
< = less than

Sandra M. Taylor

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xc: Mike Booth