Research Technical Completion Report

DEVELOPMENT OF TOXIC BLUE-GREEN ALGAL BLOOMS IN BLACK LAKE, KOOTENAI COUNTY, IDAHO

by

Jacob Kann
C. Michael Falter

Department of Fish and Wildlife Resources

Idaho Water Resources Research Institute
University of Idaho
Moscow, Idaho 83843

October, 1987

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

Research Technical Completion Report

14-08-0001-G1014-05

DEVELOPMENT OF TOXIC BLUE-GREEN ALGAL BLOOMS IN BLACK LAKE, KOOTENAI COUNTY, IDAHO

by

Jacob Kann
C. Michael Falter

Department of Fish and Wildlife Resources

Submitted to
U.S. Geological Survey
United States Department of the Interior
Washington, D.C. 20242

Idaho Water Resources Research Institute
University of Idaho
Moscow, Idaho 83843

October, 1987

ACKNOWLEDGEMENTS

The authors would like to acknowledge the initial toxicological assistance provided by Bob Krieger and Doug Stevens of the University of Idaho Veterinary Toxicology Laboratory. We are particularly grateful to Doug Stevens who refined and performed the GC-MS analyses for anatoxin-a. This research was supported by the Idaho Water Resources Research Institute and McIntire-Stennis funding.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Increasing occurrences of blue-green algal blooms in lakes throughout the western United States have been linked to recreational use, sewage inputs, and nonpoint runoff from agricultural and grazing sources. In certain instances these blooms produce toxins that can be lethal to fish, aquatic invertebrates, mammals and humans. Black Lake in northern Idaho has experienced late summer and fall growths of a toxic alga, Anabaena flos-aquae. Demonstrated fatal toxicity to cattle and small mammals occurred in 1981, 1982, 1983, and 1985. The eutrophication and subsequent toxic blooms in Black Lake can be related to its large nonpoint nutrient input from the surrounding watershed. The presence or absence of cattle on adjacent meadows may be a major determining factor in bloom formation. Despite a high proportion of sediments (55%) exposed to anaerobic conditions during summer stratification, it appears that internal loading alone does not play a significant role in the triggering of a toxic bloom in Black Lake. Development of a toxic A. flos-aquae bloom in Black Lake is dependent on a series of interdependent environmental controls. The most important conditions in Black Lake appear to be high spring nutrient load (compounded by the presence of cattle), high fall water temperature, and stable water column conditions in the fall. The A. flos-aquae strain present in Black Lake

formed surface concentrations and produced anatoxin-a, despite comprising only 1-2% of the total algal biovolume in the water column. Anatoxin-a is produced at times other than when massive surface scums are formed, indicating toxic strains are more widespread in occurrence than previously perceived.

INTRODUCTION

Certain species of freshwater blue-green algae (Cyanophyta) produce toxins detrimental to man and other animals. Since the first documented case reported by Francis (1878), when mass killings of sheep and cattle were observed along the shores of Lake Alexandrina, South Australia, sporadic incidents of toxicity have occurred in all parts of the world. Well-documented cases have been reported in North America, Europe, South Africa, Australia and Asia (Prescott, 1948; Olson, 1960; Gorham, 1962; Gentile, 1971; Moore, 1977; Elleman et al. 1978; Watanabe et al. 1980; Henning and Kohl, 1981; Leeuwangh et al. 1983; Persson at al. 1984; Skulberg at al. 1984; Carmichael et al. 1985). Some of the more common bloom-forming species capable of toxin production are Microcystis aeruginosa, Aphanizomenon flos-aquae, Anabaena flos-aquae, and Oscillatoria agardhii. These species produce a variety of endotoxins, each with varying pharmacological action (Carmichael et al. 1985). Toxin production and release usually occurs during the terminal stages of the algae bloom, implying that toxicity is affected by different environmental conditions prior to or at the time of bloom development (Carmichael and Gorham, 1977).

Ecological factors which play a major role in development of these blooms are nutrients (especially phosphorus

and nitrogen), temperature, pH, CO_2, light intensity, oxygen, summer hypolimnetic oxygen depletion, and water column stability (Vollenweider, 1968; Fogg et al. 1973; Shapiro, 1983; Reynolds, 1984). When ecological conditions are optimal, the species responsible for toxin production exhibit explosive growth. Gas vacuolate forms, through buoyancy regulation, can then optimize their position in the water column in response to changing environmental conditions. Light intensity, nutrients, oxygen, and CO_2 can affect buoyancy either positively or negatively depending on their respective intensity or concentrations (Booker and Walsby, 1981; Klemer et al. 1982; Paerl and Ustach, 1982; Klemer, 1983; Oliver and Walsby, 1984; Klemer et al. 1985; Spencer and King, 1985). Under a combination of positive buoyancy and calm surface waters, cells can float to the surface, forming thick green surface scums on the leeward shores. At such times, usually in the late summer or fall, these concentrations can result in livestock, pet, and possible human poisoning.

Although no known human deaths have occurred, there is evidence that liver, gastrointestinal, and dermatological disorders have been caused by direct contact with the toxins or by ingesting water from a blue-green infected drinking water supply (Ingram and Prescott, 1954; Schwimmer and Schwimmer, 1964; Billings, 1981; Sykora and Keleti, 1981; Bourke et al. 1983, Falconer et al. 1983).

In addition to toxic effects on terrestrial animals, these algal toxins can affect a variety of lake organisms, including zooplankton, protozoa, fish, and waterfowl (Mills and Wyatt, 1974; Carmichael and Gorham, 1977; Ransom et al. 1978; Snell, 1980; Lampert, 1981; Phillips et al. 1985).

Due to the serious implications of algal toxicity, water managers are faced with a situation of severe water use limitations for a variety of water users, including municipalities, lake homeowners, fishermen, recreational boaters, and ranchers. Unfortunately, the timing, intensity, or even the occurrence of toxic blooms cannot be predicted at this time.

Description of The Study Area

Black Lake is a shallow, eutrophic lake located in the flood plain of the Coeur d'Alene River (RM 135) in northern Idaho. Over the last fourteen years it has experienced periodic fall blooms of a toxic strain of Anabaena flos-aquae. The first documented case of animal poisoning at Black Lake was reported by the Idaho Department of Fish and Game in 1972 (Beckwith, 1985). The next reported case of animal poisoning occurred in October 1981, when wildlife, adult cattle and domestic dogs died shortly after drinking from the lake where algae had become concentrated in an embayment. Based upon the speed of death and symptoms of neural toxicity occurring just

prior to death (paralysis, respiratory distress, and convulsions), anatoxin-a (a powerful neuromuscular blocking agent) was judged to be the agent of toxicity (Robert Krieger pers comm, 1983). This was further substantiated by mouse bioassays on the lake water (Kann and Falter, 1985).

Massive blooms and subsequent animal poisoning again occurred in 1982 and 1983. In 1984 (the first year of this study) no fall bloom of A. flos-aquae occurred, but small toxic blooms did occur in September 1985 and October 1986 (although no animal poisoning was reported). Toxicity was shown only from the 1985 bloom using mouse bioassays, but recent analyses using a gas chromatograph-mass spectrophotometer (GC-MS) method of anatoxin-a detection have shown the presence of anatoxin-a in samples from the mild October 1986 bloom.

Because of the sporadic nature of toxic blue-green blooms (as evidenced by the yearly variability of toxic blooms in Black Lake) we initiated this study to better understand the development and timing of these toxic blooms in Black Lake. The specific objectives were to document the biological elements and timing of the toxic blooms, and to relate these blooms to the physical-chemical environment before and during the bloom occurrence.

METHODS

Phytoplankton and Environmental Indices

We established a lake sampling plan to monitor Black Lake phytoplankton development. We concurrently measured salient physical and chemical variables in the water column (Table 1), selected for their known influence on algal growth. Water samples were collected with either a 1 or 3 liter Kemmerer bottle. The lake was monitored monthly from June through August, and biweekly or weekly during the September-October bloom period for all years (1984-1986). In 1984 and 1985, eight monitoring sites were selected to take into account both bay and open water sites. In 1986, two representative sites were monitored (one bay and one open-lake). Deep open-lake sites were sampled at the surface, 1.0 m, 3.0 m and 5.0 m. Shallow bay sites were sampled at surface, mid-, and off-bottom points in the water column. A bathymetric map was constructed from soundings taken along transects in July 1984. Weather variables (rainfall and daily total minutes of sunlight) were obtained from NOAA climatological records for Idaho.

Correlation and Multiple Regression Analysis

Correlation coefficients were calculated to determine the relationship between A. flos-aquae biovolume and the 14 environmental variables listed in Table 2 (starting

Table 1. Environmental and phytoplankton indices monitored in Black Lake

Parameter	Unit	Methodology
Environmental Indices		
Water temperature	^{0}C	YSI Model 33 S-C-T Meter
Dissolved oxygen	mgl^{-1}	Membrane electrode method (APHA 1980), YSI Model 57 Dissolved Oxygen Meter
Electrical Conductivity	mmbo cm^{-1}	Platinum electrode method (APHA 1980), YSI Model 33 S-C-T Meter
pH	---	Corning Model 7 pH meter
Turbidity	NTU	Nephelometric method. (APHA 1980) Hach Model 2100A Turbidimeter
Nitrogen availability (NO_3,NH_3,total N)	mgl^{-1}	Ultraviolet spectrophotometric screening method, macro-Kjeldahl method (APHA 1980)
Total phosphorus	mgl^{-1}	Stannous chloride method (APHA 1980)
Inorganic carbon (CO_2 and HCO_3)	mgl^{-1}	Titrimetric methods (APHA 1980)
Zinc and Cadmium	mgl^{-1}	AA spectrophotometry (EPA 1979)
Phytoplankton Indices		
Algal biovolume	mm^3l^{-1}	Cell volume method. (APHA 1980)
Algal Chlorophyll content	mgm^{-3}	Spectrophotometric determination of chlorophyll "a" - trichromatic method (APHA 1980)
Algal composition	mm^3l^{-1}	Inverted microscope method (Lund et al 1958)
Density of toxin producers	mm^3l^{-1}	Inverted microscope method (Lund et al 1958)
Water transparency	m	Secchi disk (Tyler 1968)
Algal toxin		Mouse bioassay (Carmichael 1986) GC-MS analysis (Doug Stevens - Univ. of Idaho)

Table 2. Summer, fall, and yearly volume weighted means of selected limnological variables in Black Lake, 1984-86.

Variable	1984			1985			1986		
	Summer	Fall	1984	Summer	Fall	1985	Summer	Fall	1986
Chlorophyll a (mg/m^3)	14.0	10.9	11.8	7.3	18.4	15.6	11.6	13.4	12.6
Total Biovolume (mm^3/m^3)	2105.0	3428.0	3050.0	2275.0	5461.7	4665.0	4823.3	2715.2	3417.9
A. flos-aquae Biovolume (mm^3/m^3)	165.5	6.0	51.6	782.5	1359.6	1215.3	131.9	13.7	53.1
Total Phosphorus (mg/l)	0.029	0.042	0.038	0.027	0.032	0.030	0.028	0.037	0.032
Total Nitrogen (mg/l)	0.499	0.520	0.514	0.549	0.587	0.578	0.393	0.450	0.422
TN:TP Ratio	18.0	12.8	14.3	24.0	18.2	19.6	14.0	12.0	13.0
Temperature (C)	21.3	13.0	15.4	19.3	14.5	15.7	19.5	12.1	14.6
Dissolved Oxygen (mg/l)	9.8	11.8	11.2	6.2	8.3	7.8	6.6	8.7	8.0
Conductivity (umhos)	88.7	84.1	85.4	76.8	70.6	72.2	71.6	61.5	64.8
Carbon Dioxide (mg/l)	5.9	6.2	6.1	7.1	1.7	3.0	3.4	4.1	3.8
Total Alkalinity (mg/l)	33.7	33.2	33.3	26.0	30.5	29.4	26.3	27.9	27.3
pH	7.93	7.45	7.58	7.21	7.57	7.48	7.76	7.52	7.61
Turbidity (NTU)	3.84	4.95	4.63	1.98	3.95	3.45	2.13	3.47	2.96
Secchi (m)	2.70	1.29	1.69	3.55	1.45	1.98	3.22	2.20	2.54
Stability ($gm-cm/m^2$)	28.10	0.60	18.10	29.40	1.10	19.10	26.20	3.80	18.10
Total Rainfall (in.)	3.84	2.91	6.75	1.66	6.61	8.27	2.81	NA	NA
% of Tot. Poss. Sunlight	80.70	61.00	72.80	81.70	51.00	69.40	82.70	69.50	77.40

with total phosphorus). A two week mean prior to each sampling date was used for stability, total rainfall, and sunlight values in the correlation analysis. Values of all remaining variables were simultaneously measured at the time of algal collection. A. flos-aquae biovolume was correlated with the environmental variables using all sampling dates over the three years (n=24), all summer dates (n=7), and all fall dates (n=17). The above analysis was run on both mean water column values (volume weighted) and mean epilimnial values. An additional analysis was performed with biovolume of all other algal species as the dependent variable. A stepwise multiple regression analysis (SAS, 1981) was used to determine the model of best fit for those variables which showed significant correlations with A. flos-aquae biomass and with biomass of all other algal species.

Toxicity Testing

Toxicity testing was conducted at times when A. flos-aquae trichomes became concentrated at or near the lake surface. At such times, cells were carefully skimmed from the lake surface and brought to the University of Idaho Veterinary Toxicology lab in polyethylene containers. Concentrations of cells were lysed either by freezing and thawing or running the container under hot tap water. Suspensions of lysed cells were then injected intraperitoneally into mice. Time until death and

symptoms of toxicity were noted (Astrachan and Archer, 1981; Carmichael, 1986). Development in 1986 of an extraction-derivitization process (developed by D. Stevens and R. Krieger at the University of Idaho Veterinary Toxicology Laboratory) coupled with GC-MS confirmation, enabled detection of anatoxin-a at levels 1000X lower than those of the above mouse bioassay. This method was utilized on the October 1986 samples.

Nutrient Loading

Estimates of nitrogen and phosphorus loading were made in 1985. Discharge and nutrient concentrations were monitored at all tributaries and inflows during selected periods of the hydrograph, chosen to take into account varying high and low flow regimes. Estimates of internal loading for 1985 were made using the difference between observed phosphorus retention and predicted retention for an oxic lake (Nrnberg, 1984). Precipitation and dryfall inputs were estimated from analysis of collected rainwater and dryfall during the year. An acid-washed polyethylene container (d=40 cm) was set out from April through November. The container was left standing between rain events to collect dryfall, and samples were collected and frozen after each significant rain event (a rewashed container was replaced after each sample collection). Inputs from septic systems were estimated from a shoreline survey of the lake (Panhandle Health District, 1977).

RESULTS

Limnological Characteristics

Summer, fall, and yearly means of selected limnological parameters are presented in Table 2. Black Lake morphometric and hydrologic characteristics are presented in Table 3. Typical of shallow north temperate lakes, Black Lake experiences weak stratification from June through August. The lower meter of the water column becomes anaerobic during this time. Some mixing of deeper water occurs during windy periods throughout summer stratification. Black Lake has steep sides and uniform depth across much of the basin (Z_{max} = 7.3 m, $\bar{Z}$ = 4.3 m). The low ratio of $Z_{max}/\bar{Z}$ translates to a high proportion (55 %) of lake sediments deeper than 5m, the depth below which anaerobosis occurs. Both flushing rate and hydraulic residence time are high (1.4 yr^{-1} and 0.55 yrs respectively) because of the the high watershed area to lake surface area ratio (28:1).

Phytoplankton and Toxicity Trends

Cell biovolume data (mm^3/m^3) of Anabaena flos-aquae in 1984, 1985, and 1986 are presented in Figure 1. In 1984 A. flos-aquae biovolume peaked in early August at only 350 mm^3/m^3 (20% of the total phytoplankton biovolume), and then remained below 2 mm^3/m^3 (less than 1% of the total phytoplankton biovolume) through the fall. No surface scums formed in 1984. High biovolume values of A. flos-

Table 3. Black Lake morphometric and hydrologic descriptors.

ELEVATION		647.1 m
SURFACE AREA	SA	151.9 ha
VOLUME	V	6.5×10^6 m^3
MEAN DEPTH	Z	4.3 m
MAXIMUM DEPTH	Z_m	7.3 m
MAXIMUM LENGTH	l	2.65 km
MAXIMUM WIDTH	b	1.04 km
MEAN WIDTH	b	0.57 km
SHORELINE LENGTH	SL	8.77 km
SHORELINE DEVELOPMENT	D_L	2.01
TOTAL SEDIMENT AREA		1.5×10^6 m^2
MAXIMUM SEDIMENT AREA BELOW ANAEROBIC WATER AT SUMMER STRATIFICATION		8.4×10^5 m^2
WATERSHED AREA TO LAKE SURFACE AREA RATIO		28:1
FLUSHING RATE	p	1.4 yr^{-1}
HYDRAULIC RESIDENCE TIME	T_w	0.55 yrs
AREAL WATER LOADING	q_s	7.85 m yr^{-1}

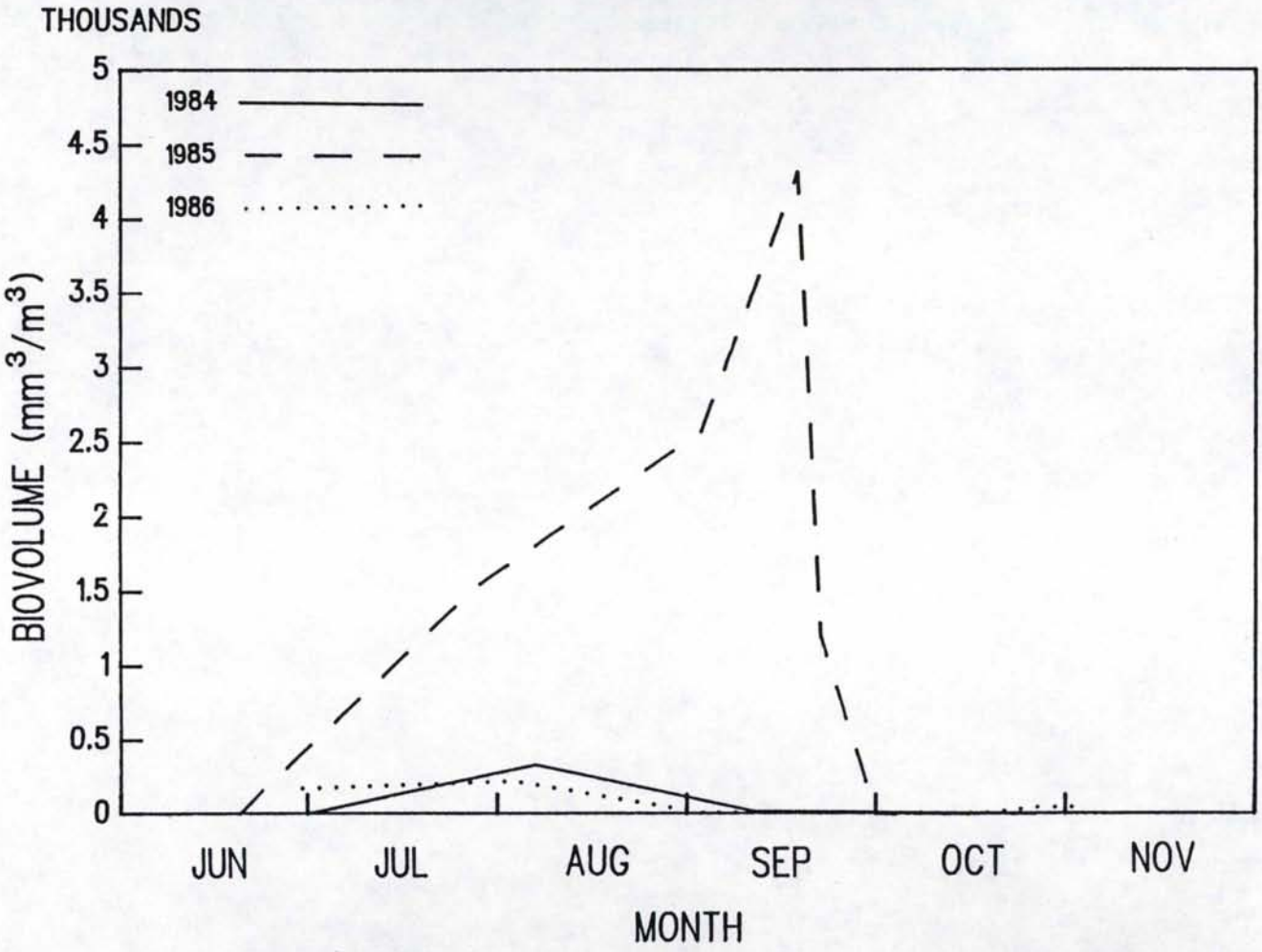

Figure 1. Water column mean biovolume of *Anabaena flos*-aquae in Black Lake, 1984-1986.

aquae during the summer and fall months occurred only in 1985 when biovolume peaked in early September at 4,300 mm^3/m^3 or 65% of the total phytoplankton biovolume (95% by cell number). Not only was A. flos-aquae biovolume higher in August 1985 (5 to 8 times higher than that of August 1984), but biovolume remained high well into September when small surface scums formed along the shoreline. Cell biovolume of A. flos-aquae was lowest in 1986, peaking at 220 mm^3/m^3 (5% of the total phytoplankton biovolume) in early August. Despite accounting for only 1-2% of the biovolume composition ($40mm^3/m^3$) in mid-October 1986, A. flos-aquae formed isolated surface sheens at this time.

Biovolume of the dominant phyla are presented in Figure 2. The Chrysophyta component (comprised mainly of the genera Melosira, Fragilaria, and Asterionella) dominated throughout most of the three years. Cyanophyta (composed almost exclusively of A. flos-aquae) was dominant only in early August 1984 and late July through mid-September 1985. Euglenophyta (comprised mainly of the genera Trachelomonas, Phacus, and Euglena) increased slightly during the fall months of all years. Values in 1986, however, were somewhat higher than those of 1984 and 1985. Other Phyla (Chlorophyta, Pyrrophyta, and Cryptophyta) were minor components of the total phytoplankton in all years.

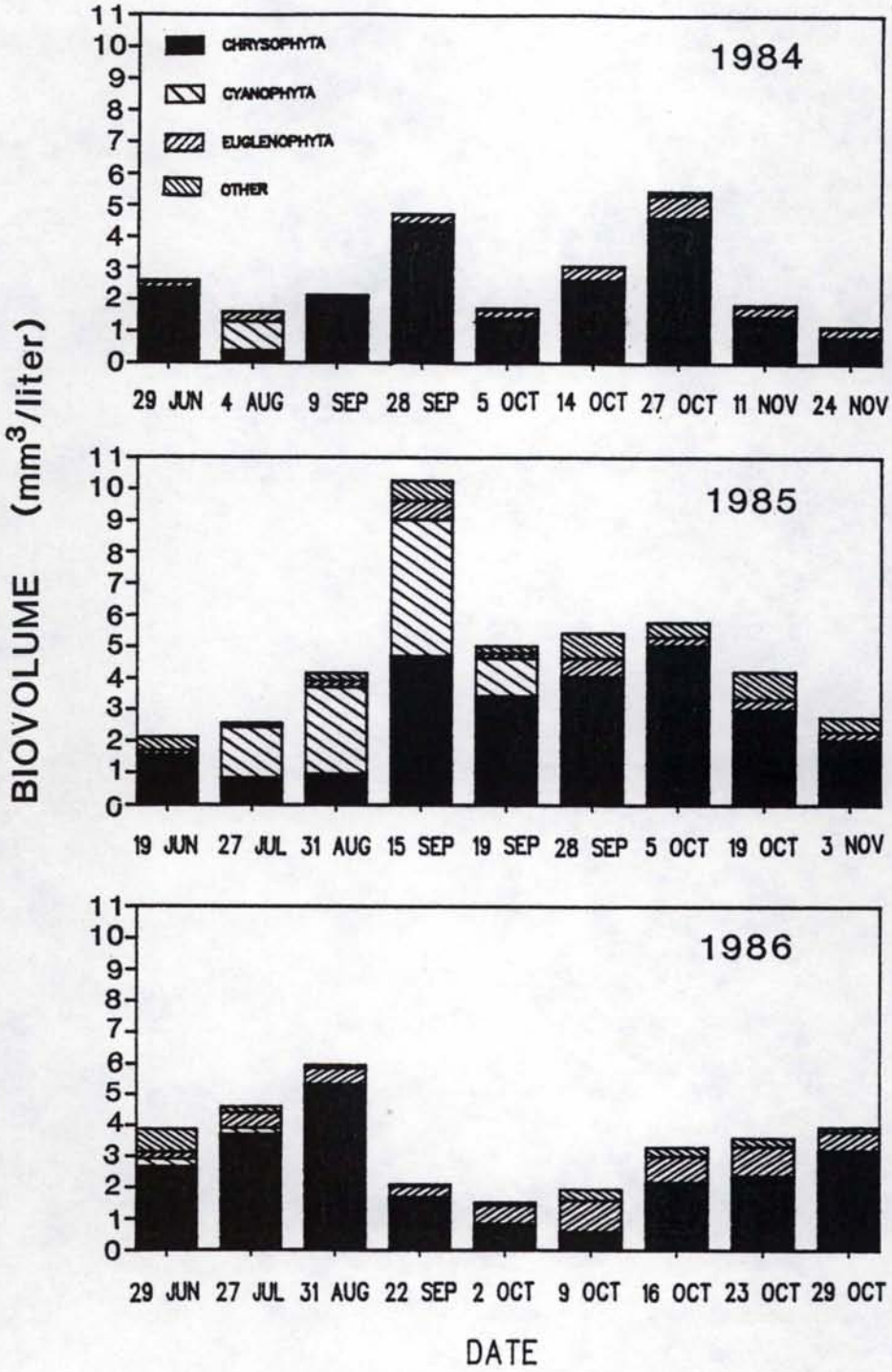

Figure 2. Water column mean biovolume of major phytoplankton phyla in Black Lake, 1984-1986.

Differences in fall phytoplankton biomass between the three years can also be expressed by chlorophyll a data (Figure 3). Peak water column mean chlorophyll a values reached 31 mg/m^3 in mid-September 1985. In 1984, values peaked at only 17 mg/m^3 in late September, with diatoms accounting for most of the chlorophyll a present. Trends in 1986 were similar to 1984, with values peaking at 19 mg/m^3 in late september and early October. Again, diatoms accounted for most of the chlorophyll a present.

Toxicity as determined by mouse bioassays was shown only during the mid-September 1985 bloom, and then only from cells collected on the 19th of September. Surface cell collections taken one week earlier showed no toxicity using the mouse bioassay. No surface scums even formed in 1984, so no toxicity was shown. The 1986 slight October surface accumulation showed no toxicity using mouse bioassays, however, anatoxin-a was shown to be present (~0.05 µg anatoxin per mg of algae) using GC-MS analysis. These anatoxin-a levels were ca. 20X higher (per mg dry weight of algae) than levels from the massive 1981 bloom.

Correlation and Multiple Regression Analysis

Results of the correlation analyses are presented in Table 4. Only those coefficients with significance levels of $p \leq 0.05$ are shown. Secchi disk and turbidity are also not included as they are direct functions of algal biomass in Black Lake. Water column mean A. flos-aquae biovolume

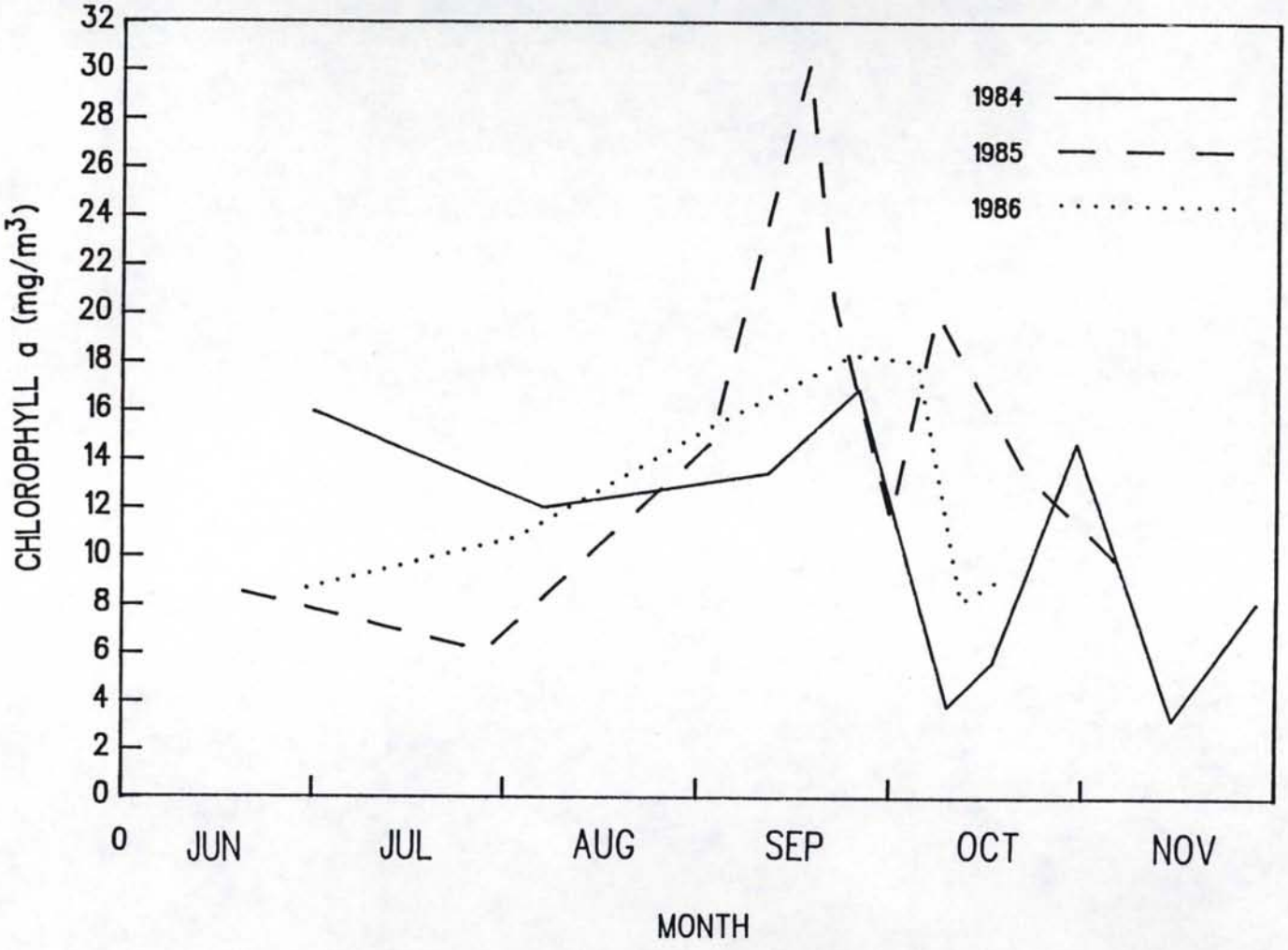

Figure 3. Water column mean chlorophyll *a* in Black Lake, 1984-1986.

Table 4. Correlation coefficients for *A. flos-aquae* biovolume (mm^3/m^3) and biovolume of other algal species with physical-chemical variables over all dates combined, summer dates, and fall dates. Data is presented for water column means and epilimnial means. Only those variables with significant correlation coefficients ($p \leq 0.05$) are shown (*=$P \leq 0.01$).

	Water Column Mean			Epilimnion Mean		
	All Dates n=24	Summer n=7	Fall n=17	All Dates n=24	Summer n=7	Fall n=17
	Biomass of *Anabaena flos-aquae*					
Variable						
TP	-0.46	-0.80	---	---	---	---
TN	---	---	0.59	---	---	0.81*
TN:TP	0.64*	0.69	0.63*	0.55*	---	0.55
Temperature	0.40	---	0.52	0.42	---	0.50
Dissolved Oxygen	-0.50*	---	---	-0.46	---	---
Carbon Dioxide	---	---	---	-0.42	---	---
pH	-0.41	---	---	---	---	---
	Biomass of Other Algal Species					
Variable						
TN:TP	-0.44	-0.84	---	---	---	---
Temperature	---	---	---	-0.43	---	---
pH	0.50	---	0.52	---	---	---
Stability	-0.44	---	-0.60*	-0.52*	---	-0.43

was negatively correlated with TP, dissolved oxygen and pH over all dates combined, and was positively correlated with the TN:TP ratio and temperature. Epilimnial results were comparable, but showed no correlation with TP and a negative correlation with carbon dioxide. The only significant summer correlation was negative between water column mean A. flos-aquae and TP. Fall values were positively correlated with TN, TN:TP, and temperature for both water column and epilimnial means.

Water column mean biovolume of all other algal species was negatively correlated with TN:TP and stability, and positively correlated with pH over all dates. Epilimnial means over all dates were negatively correlated with temperature and stability. Water column means were negatively correlated with TN:TP for summer dates. Water column and epilimnial means were both negatively correlated with stability for fall dates.

Results of the stepwise multiple regression analysis yielded no significant two-or-more-variable models. Due to the interrelationship of TP, TN, and the TN:TP ratio with algal biomass, these variables were not included in the multiple regression analysis. Significant single variable models show that temperature accounts for 21% ($p \leq 0.05$) of the variability in water column mean A. flos-aquae biovolume over all dates, and 28% ($p \leq 0.05$) of the variability over fall dates. Likewise, temperature accounted for 25% ($p \leq 0.05$) of the variability in

epilimnial mean A. flos-aquae over fall dates. Stability accounted for 34% ($p \leq 0.01$) of the variability in the water column mean biovolume of all other algal species over fall dates, and 33% ($p \leq 0.01$) of the variability for epilimnial means over all dates.

Thermal Stability

Stability (gm-cm/cm^2) (an expression of the work required to mix the lake to homothermous conditions) was calculated according to Cole (1979). Mean June stability values were 35% higher in 1985 than for the same period in 1984 and 1986 (Figure 4). However, the overall mean summer stability was similar in all years (Table 2). Stability values in 1984 were lower than the other years through October (Figure 4). The overall fall means for 1985 and 1986 were 92% and 300% higher, respectively, than 1984 (the non-bloom year).

External Nutrient Loading

External nutrient loading to Black Lake comes from a variety of sources including two major tributaries (Black Creek and Lamb Creek), two smaller un-named tributaries, reverse flow through the outlet channel, pumped effluent from adjacent grazed or cultivated meadows, precipitation, and septic drainage. Reverse flow through the outlet channel occurs during an influx of Coeur d' Alene River water into the lake during peak flows in spring. On both

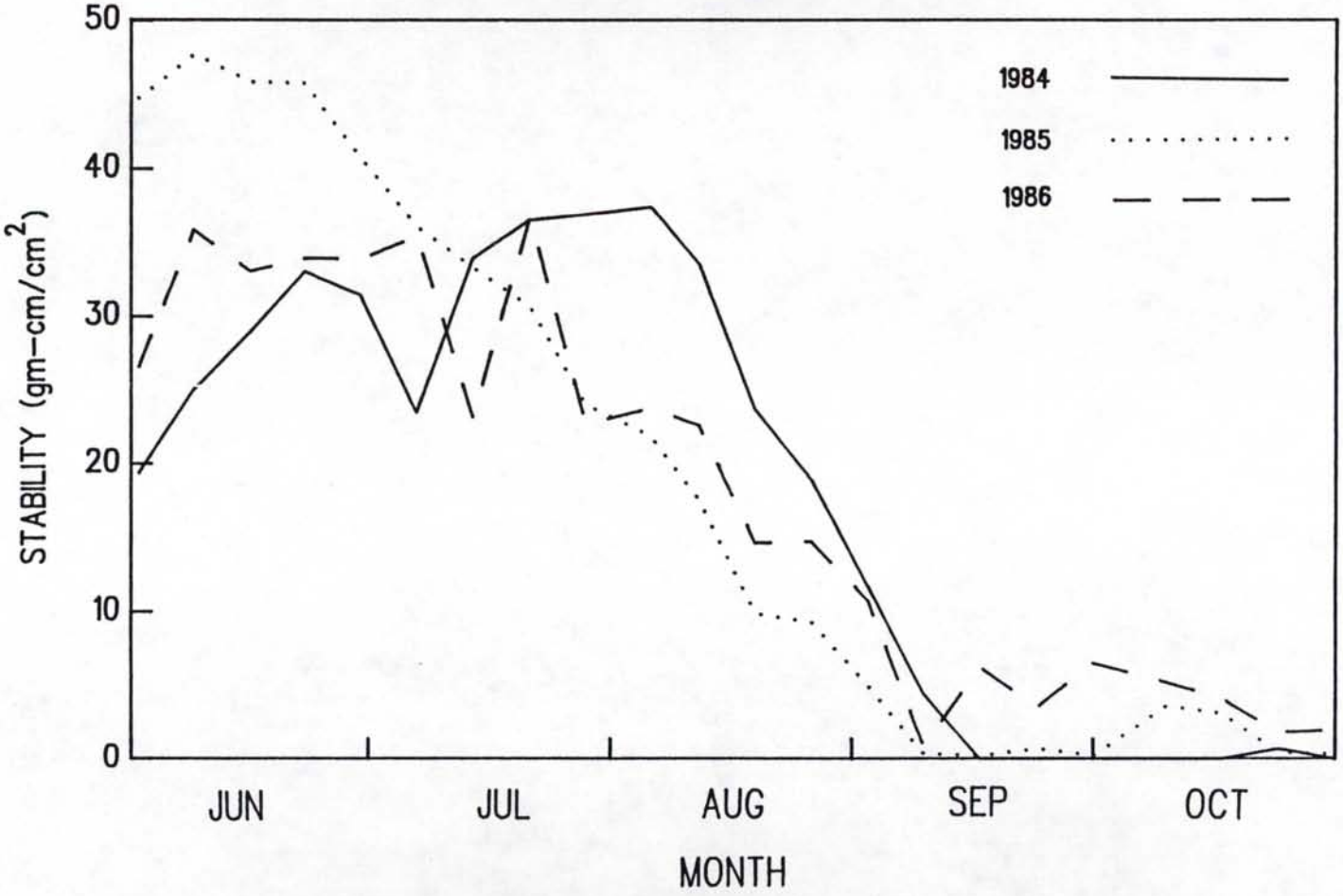

Figure 4. Summer-fall stability values calculated for Black Lake, 1984-1986.

the east and west sides of the lake are two large meadows which fill with 1-2 m of water during early spring snowmelt. Over a several month period this water is pumped over a dike into Black Lake.

Absolute and relative contribution of TN and TP from each source during 1985 are presented in Table 5. Black Creek and Lamb Creek supply the largest tributary inputs of phosphorus, contributing 33.5% of the total load. Effluent from the pumped meadows contributes 26.8% of the total load. The remaining 39.7% comes from the other lesser sources, each contributing less than 10% of the total load. Due to high concentrations of TP in the meadow effluent (120-260 ug/l), TP input is high from that source (26.8%) relative to its hydrologic input (15.7%). The 1985 areal TP loading rate to Black Lake was 0.84 $g/m^2/yr$.

Internal Phosphorus Loading

Adequate hydrologic data in 1985 enabled internal loading estimates to be made using a phosphorus retention model developed by Nurnberg (1984):

$$L_{int} = -L_{ext} \times (R_{obs} \; D \; R_{pred})$$

where L_{int} is internal P load in $mg/m^2/yr$, L_{ext} is external P load in $mg/m^2/yr$, R_{obs} is retention measured as 1-(P outflow/P inflow), and R_{pred} is predicted retention ($R=15/(18+q_s)$, where q_s is the areal water loading rate in m yr^{-1}).

Table 5. Total annual flows and total phosphorus and nitrogen loads to Black Lake, 1985.

Source	Annual Water Flow ($M^3 x 10^4$)	Percent of Annual Water Inflow (%)	Annual TP Loading (kg)	Percent TP Contributed by each Source	Annual N Loading (kg)	Percent N Contributed by each Source
Black Creek	452.3	37.9	218.1	17.2	2902.8	20.6
Un-named Creek #1	80.9	6.8	91.4	7.2	1333.1	9.5
Lamb Creek	236.2	19.8	206.8	16.3	4993.4	35.4
Un-named Creek #2	101.0	8.5	118.8	9.4	1576.7	11.2
Outlet Channel (reverse flow)	46.3	3.9	50.9	4.0	226.9	1.6
East Pump	82.4	6.9	214.1	16.8	1014.6	7.2
West Pump	105.9	8.9	127.1	10.0	754.9	5.4
Precipitation	88.8	7.4	71.0	5.6	1056.7	7.5
Septic Drainage			54.0	4.3	234.0	1.7
Internal Loading			117.9	9.3		
TOTAL INFLOWS	1193.6	100.0	1270.26	100.0	14093.1	100.0
Areal Loading Rate	7.85m/yr	--	0.84g/m^2/yr	--	9.27g/m^2/yr	--

With this model we obtained an internal phosphorus loading estimate of 78 mg/m^2/yr (9.3 percent of the total TP loading). This translates to an average sediment release rate of 1.75 mg TP per m^2 of sediment per day during the stratified period.

DISCUSSION

The pattern of toxic A. flos-aquae blooms in Black Lake exhibits extreme year to year variability. This is clearly shown by a comparison of A. flos-aquae biovolume, Cyanophyta density, and chlorophyll a between 1984, 1985, and 1986. Not only was the density (by biovolume) of A. flos-aquae greatest in August of 1985, but it continued to increase well into September, when a toxic surface scum formed. A. flos-aquae was present in 1984, but its density did not increase through the fall, and no surface bloom (toxic or non-toxic) occurred. The scenario of no bloom in 1984, and mild blooms in 1985 and 1986, suggests a combination of certain environmental controls required to initiate bloom development.

Because Black Lake becomes anaerobic during summer stratification, and because a high proportion of sediments underlies anaerobic water (55%), we initially surmised that phosphorus import from the sediments might have played a role in bloom development. Despite higher fall TP values in 1984 ($42mg/m^3$) than in either 1985 ($32mg/m^3$) or 1986 ($37mg/m^3$), the highly available nature of internally released phosphorus (Nurnberg and Shaw, 1986) could still theoretically trigger a fall bloom upon overturn. If stratification had been weaker in 1984 (a non-bloom year), we would expect less anaerobosis, and therefore less available phosphorus to be released from

the sediments. There would then be less phosphorus available for a blue-green algae bloom upon fall mixing. During the summer stratification period, water stability values in 1984 were similar to other bloom years indicating that summer internal loading was probably similar to that calculated for 1985 (78mg/m^2/yr).

If one were to extend this analysis (using the sediment release rate calculated in 1985) and consider an additional 30 days of anaerobic conditions (an unlikely event in shallow Black Lake) the internal loading rate would only increase to 93 mg/m^2/yr, or 11 percent of the TP loading in the 1985 water year. Because the variability of internal phosphorus loading does not appear to be great between bloom and non-bloom years, we have concluded that summer internal phosphorus loading alone could not explain annual bloom variations in Black Lake.

Annual loading of TP from external sources is excessive, with 26.9% derived from pumping of adjacent flooded meadows in 1985. Reduction of external loading by exclusion of meadow effluent would decrease TP loading nearly 30% in the 1985 water year. It is then likely that the eutrophication rate would be slowed considerably, with a concomitant decrease in blue-green algal blooms. At the time of the massive toxic blooms during the early 1980's, over 700 head of cattle were overwintered on the adjacent meadows. From 1984 to 1986 fewer cattle were overwintered, but some cultivation (mainly oats) took

place. If we use the per cow nitrogen and phosphorus export figures obtained from Viets (1971), and assume 25% of this reaches the lake during spring pumping (because all water from the flooded meadows enters the lake, we feel 25% is very conservative), the potential annual loading due to the cattle is 462 kg phosphorus (a 27% increase over 1985 loading) and 2,856 kg nitrogen (a 17% increase over 1985 loading). Plans call for high numbers of cattle to again be overwintered starting in fall 1987. It will be interesting to note whether the massive toxic blooms of the early 1980's reappear. Black Lake's high external loading ($0.76 g/m^2/yr$) is certainly a basic cause for its high productivity, but with only one year of nutrient loading data (1985) we could not make the connection between high annual loading alone and the subsequent occurrence of a toxic algae bloom in a given year. However, that is not to say a connection does not exist. It is clear, however, from the sporadic nature, timing, and intensity of these blooms that other factors also play a role in week to week development of these blooms.

Correlation coefficient and multiple regression analyses were performed to determine which factors might have a dominant regulatory role in bloom development. Given the fact that all variable measurements were taken at the same time as the algal biovolume measurements, only variables which are not directly affected by the algae

themselves provide useful information. For example, a positive relationship was obtained between A. flos-aquae biovolume and the TN:TP ratio, and a negative relationship obtained with TP. In most lakes, low TN:TP ratios favor nitrogen fixing Cyanobacteria such as A. flos-aquae (Schindler, 1977; Flett et al. 1980; Smith, 1983). The Black Lake anomalous relationship may be explained by the the simultaneous collection of TP, TN and algae samples. Because nitrogen-fixing Cyanobacteria can actively fix atmospheric nitrogen when nitrogen is limiting, nitrogen content in the algal cell remains non-limiting, allowing phosphorus deficiency in the cell to develop (Healey, 1982). Since the measurement of TP and TN includes phosphorus and nitrogen contained in the algal cells, water samples collected during high A. flos-aquae densities could conceivably show higher TN:TP ratios. The negative relationship with TP can be explained similarly. At peak A. flos-aquae densities, TP has actually become deficient in the algal cells. Given the above scenario, all variables (eg., nutrients, CO_2, pH, dissolved oxygen, etc...) which at a specific point in time may be more a result of algal biomass than actual controlling factors of the biomass, should not be included in predictive analyses.

More accurate predictive models could be obtained by regressing mean values of variables over certain time periods prior to algal cell growth (eg. one, two, etc...

weeks prior to increasing algal growth) with algal biomass. In addition, measurement of nutrient forms more available to algae would more accurately reflect upcoming events. For example, the measurement of dissolved phosphorus and nitrogen at fall overturn (thereby excluding N and P contained in the algal cells) may better reflect a particular water body's ability to support fall Cyanobacterial blooms. Regularly spaced and more frequent sampling through the summer and fall is necessary to provide the data base for these time lag analyses.

Because temperature and stability are not affected by algal biomass, more useful predictive relationships can be obtained for these variables. The positive correlation (r^2=0.25) of A. flos-aquae biovolume with fall temperature, indicates some relationship of fall temperature to bloom development. Likewise, a negative correlation (r^2=0.34) of all other algae with fall stability indicates the strong effect of stability.

Stability values were lower (92-300%) during September and October 1984 than other bloom years, indicating well-circulating waters following fall overturn. Woods (1981) reported that phytoplankton can be circulated out of the photic zone when weak thermal structure exists, thereby causing a trophic state lower than that expected based on external loading. It is possible that fall circulation may prevent A. flos-aquae from maintaining its optimal water column position. In addition, the negative

correlation between stability and non-buoyant algal species in Black Lake, indicates that stable conditions inhibit production of non-buoyant species. Viner (1985) also states that high stability promotes Cyanobacterial growth by allowing them to obtain optimal light intensity, and at the same time inhibits resuspension of non-buoyant species. Turbulent waters, therefore, tend to negate the competitive advantage of buoyancy regulating species (Reynolds, 1975; Paerl and Ustach, 1982; Viner, 1985; Cooke et al. 1986).

It appears that development of a toxic A. flos-aquae bloom in Black Lake is dependent on a series of inter-dependent environmental controls, each of which forms part of a complex picture of bloom development. Although conditions must be optimal to cause some Cyanobacterial growth (eg. nutrients, temperature, light intensity, circulation pattern, etc...), conditions of CO_2, dissolved oxygen, light intensity, nutrient concentration and stability, must also be optimal for buoyancy and surface scum formation to take place. For example, even though A. flos-aquae biovolume was only 1-2% of the total algal biovolume; calm, warm conditions in October 1986 enabled it to form surface concentrations of algae containing anatoxin-a. High fall stability and warmer fall temperatures, along with high spring nutrient loading (compounded by the presence of cattle) may be the most important factors regulating A. flos-aquae blooms in Black

Lake. The detection of low levels of toxin with GC-MS methodology in October 1986 indicates that toxic blooms are much more widespread than previously perceived. They generally go undetected unless animal deaths occur. The October 1986 bloom was both localized and small, indicating that toxicity is produced at times other than during massive scum formation. This supports Carmichael and Gorham's (1981) premise that sub-lethal pulses of toxicity recur seasonally even when poisoning episodes do not. Factors regulating selection of toxic strains over non-toxic ones are, as yet, unknown.

CONCLUSIONS

1. Black Lake in northern Idaho has developed toxic Anabaena flos-aquae blooms in the fall during four years since 1980.

2. Over our limnological study of the last three years, toxicity developed in the falls of 1985 and 1986.

3. Annual loading from external sources is excessive, with 30% derived from pumping of adjacent flooded meadows. The presence of cattle on these adjacent meadows may play a large role in bloom formation. Prevention of meadow effluent from reaching the lake would likely cause a decrease in A. flos-aquae blooms.

4. Despite a high proportion of sediments exposed to anaerobic conditions during summer stratification, it appears that internal loading alone does not play a significant role in the triggering of a toxic algae bloom in Black Lake.

5. GC-MS analysis enabled detection of anatoxin-a when concentrations were to low to be detected with the mouse bioassay, indicating that toxic strains of A. flos-aquae are more common than previously perceived.

6. The finding of anatoxin-a in slight surface scums when A. flos-aquae comprised only 1-2% of the total algae biovolume in the water column, suggests that toxin production may be a routine occurrence in those eutrophic

lakes which have demonstrated the presence of a toxic strain.

7. Development of a toxic A. flos-aquae bloom in Black lake is dependent on a series of interdependent environmental controls. The most important conditions in Black Lake appear to be high spring nutrient load (compounded by the presence of cattle), high fall water temperature, and stable water column conditions in the fall.

REFERENCES

American Public Health Association. 1980. Standard methods for the examination of water and wastewater, 15th ed. New York. 1134p.

Astrachan, N. B. and B. C. Archer. 1981. Simplified monitoring of Anatoxin-A in rats. Pages 437-446 in W. W. Carmichael, ed. The water environment-algal toxins and health. Plenum Press, New York.

Beckwith, M. A. 1985. Water quality assessment, Black Lake, Kootenai County. Idaho Dept. Health and Welfare, Division of Environment, Water Quality Bureau, Statehouse, Boise, Idaho 83702. 65p.

Billings, W. H. 1981. Water-associated human illness in northeast Pennsylvania and its suspected association with blue-green alga blooms. Pages 243-255 in W. W. Carmichael, ed. The water environment-algal toxins and health. Plenum Press, New York.

Booker, M. J. and A. E. Walsby. 1981. Bloom formation and stratification by a planktonic blue-green alga in an experimental water column. Br. Phycol. J. 16:411-421

Bourke, A.T.C., R.B. Hawes, A. Neilson, and N. D. Stallman. 1983. An outbreak of hepato-enteritis (the Palm Island mystery disease) possibly caused by algal intoxication. Toxicon, Suppl. 3 (Abst.):45.

Carmichael, W.W. 1986. Isolation, culture and toxicity testing of toxic freshwater cyanobacteria (blue-green algae). In V. Shilov, ed. Fundamental research in homogeneous catalysis, Vol. 3 1284p.

Carmichael, W. W. and P.R. Gorham. 1977. Factors influencing the toxicity and animal susceptibility of Anabaena flos-aquae blooms. Journal of Phycology 13:97-101.

Carmichael, W.W., and P.R. Gorham. 1981. The mosaic nature of toxic blooms of cyanobacteria. Pages 161-172 in W. W. Carmichael, ed. The water environment-algal toxins and health. Plenum Press, New York.

Carmichael, W.W., C.L.A. Jones, N.A. Mahmood, and W.C. Thiess. 1985. Algal toxins and water-based diseases. CRC Critical Reviews in Environmental Control 15(3):275-313.

Cole, G. A. 1979. Textbook of limnology. The C. V. Mosby Company, St. Louis. 426p.

Cooke, G.D., E.B. Welch, S.A. Peterson, and P.R. Newroth. 1986. Lake and Reservoir Restoration. Butterworth Publishers, Stoneham, Mass.

Elleman, T.C., I.R. Falconer, A.R.B. Jackson, and M.T. Runnegar. 1978. Isolation, characterization and pathology of the toxin from a Microcystis aeruginosa (Anacystis cyana bloom). Aust. J. Biol. Sci. 31:209-218.

Falconer, I.R., A M. Beresford, and M. T.C. Runnegar. 1983. Evidence of liver damage by toxin from a bloom of the blue-green algae, Microcystis aeruginosa. Med. J. Aust. 1(11):511-514.

Flett, R.J., D.W. Schindler, R.D. Hamilton, and N.E. R. Campbell. 1980. Nitrogen fixation in Canadian Precambrian Shield Lakes. Can. J. Fish. Aquat. Sci. 37:494-505.

Fogg, G.E., W. D.P. Stewart, P. Fay, and A.E. Walsby. 1973. The blue-green algae. Academic Press, New York. 459p.

Francis, C. 1878. Poisonous Australian lake. Nature 18:11-12.

Gentile, J.H. 1971. Blue-green and green algal toxins. Pages 27-66 in S. Kadis, A. Ciegler, S.J. Ajl, (eds.), Microbial toxins, vol. 7. Academic Press, New York.

Gorham, P.R. 1962. Laboratory studies on the toxins produced by waterblooms of blue-green algae. American Journal of Public Health 52(12):2100-2105.

Healy, F.P. 1982. Chapter 5: Phosphate. Pages 105-124 in Carr, N.G., and B. A. Whitton, (eds.), The biology of cyanobacteria. University of California Press, Los Angeles.

Henning, M. and J.G. Kohl. 1981. Toxic blue-green algae water blooms found in some lakes in the German Democratic Republic. Revue ges. Hydrobiol. 66(4):553-561.

Ingram, W.M. and G.W. Prescott. 1954. Toxic fresh-water algae. Am. Midland Nat. 52(1):75-87.

Kann, J. and C.M. Falter. 1985. Blue-green algae toxicity in Black Lake, Kootenai County, Idaho. Idaho Water Resources Research Institute. Research Technical Completion Report G903-02. NTIS PB86 157385/AS.

Klemer, A.R. 1983. Cyanobacterial buoyancy regulation and blooms. pages 234-236 in Lake Restoration, Protection and Management. Proc. 2nd Annu. Conf. N. Am. Lake Manage. Soc., Vancouver, Can. EPA 440/5-83-001. U.S. Environ. Prot. Agency, Washington DC.

Klemer, A.R., J. Feuillade, and M. Feuillade. 1982. Cyanobacterial blooms: carbon and nitrogen limitation have opposite effects on the buoyancy of Oscillatoria. Science, 215:1629-31.

Klemer, A.R., D.C. Pierson, and M.C. Whiteside. 1985. Blue-green algal (cyanobacterial) nutrition, buoyancy and bloom formation. Verh. Internat. Verein. Limnol. 22:2791-98.

Krieger, R. 1983. Personal communication. Department of Veterinary Medicine, University of Idaho, Moscow, Id. 83843.

Lampert, W. 1981. Inhibitory and toxic effects of blue-green algae on Daphnia. Revue ges. Hydrobiol. 66(3):285-298.

Leeuwangh, P., F.I. Kappers, M. Dekker, and W. Koerselman. 1983. Toxicity of cyanobacteria in Dutch lakes and reservoirs. Aquatic Toxicology 4:63-72.

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

Mills, D.H. and J.T. Wyatt. 1974. Ostracod reactions to non-toxic and toxic algae. Oecologia 17:171-177.

Moore, R.E. 1977. Toxins form blue-green algae. Bioscience 27(12):797-802.

Nurnberg, G.K. 1984. The prediction of internal phosphorus load in lakes with anoxic hypolimnia. Limnol. Oceanogr. 29(1):111-124.

Nurnberg, G.K., and M. Shaw, 1986. Internal phosphorus load in an oligotrophic precambrian shield lake with an anoxic hypolimnion. Can. J. Fish. Aquat. Sci., 43:574-580.

Oliver, R.L., and A.E. Walsby. 1984. Direct evidence for the light-mediated gas vesicle collapse in the buoyancy regulation of Anabaena flos-aquae (cyanobacteria). Limnol. Oceanogr. 29(4):879-886.

Olson, T.A. 1960. Water poisoning-a study in poisonous algal blooms in Minnesota. American Journal of Public Health 50:883-884.

Paerl, W.P., and J.F. Ustach. Blue-green algal scums: an explanation for their occurrence during freshwater blooms. Limnol. Oceanogr. 27(2):212-217.

Panhandle Health District I. 1977. Kootenai and Shoshone Counties shoreline survey report. 1309 Best Ave., Coeur d'Alene, Idaho. 17p.

Persson, P.E., K. Sivonen, J. Keto, K. Kononen, M. Niemi, and H. Viljama. 1984. Potentially toxic blue-green algae (cyanobacteria) in Finnish natural waters. Aqua Fennica 14(2):147-154.

Phillips, M.J., R.J. Roberts, J.A. Stewart, and G.A. Codd. 1985. The toxicity of the cyanobacterium Microcystis aeruginosa to rainbow trout, Salmo gairdneri Richardson. Journal of Fish Diseases 8:339-344.

Prescott, G.W. 1948. Objectionable algae with reference to killing fish and other animals. Hydrobiologia 1:1-13.

Ransom, R.E., T.A. Nerad, and P.G. Meier. 1978. Acute toxicity of some blue-green algae to the protozoan Paramecium caudatum. Journal of Phycology 14:114-116.

Reynolds, C.S. 1984. Phytoplankton periodicity: the interactions of form, function and environmental variability. Freshwater Biology 14:111-142.

SAS Users Guide. Statistical Analysis Systems Inc., 1981. P.O. Box 10066, Raleigh, North Carolina 17605.

Schindler, D.W. 1977. Evolution of phosphorus limitation in lakes. Science 195:260-262.

Schwimmer, D. and M. Schwimmer. 1964. Algae and medicine. Pages 368-412 in D.F. Jackson, ed. Algae and man. Plenum Press, New York.

Shapiro, J. 1983. Blue-green dominance in lakes: the role and management significance of pH and CO_2. Pages 219-227 in Lake Restoration, Protection and Management. Proc. 2nd Annu. Conf. N. Am. Lake Manage. Soc., Vancouver, Can. EPA 440/5-83-001. U.S. Environ. Prot. Agency, Washington DC.

Skulberg, O.M., G.A. Codd, and W. W. Carmichael. 1984. Blue-green algal (cyanobacteria) toxins: water quality and health problems in Europe. Ambio 13:244-247.

Smith, V.H. 1983. The nitrogen and phosphorus dependence of blue-green algal dominance in lakes. pages 237-241 in Lake Restoration, Protection and Management. Proc. 2nd Annu. Conf. N. Am. Lake Manage. Soc., Vancouver, Can. EPA 440/5-83-001. U.S. Environ. Prot. Agency, Washington DC.

Snell, T.W. 1980. Blue-green algae and selection in rotifer populations. Oecologia 46:343-346.

Spencer, C.N., and D.L. King. 1984. Interactions between light, NH_4^+, and CO_2 in buoyancy regulation of Anabaena flos-aquae (Cyanophyceae)

Sykora, J.L. and G. Keleti. 1981. Cyanobacteria and endotoxins in drinking water supplies. Pages 285-301 in W. W. Carmichael, ed. The water environment-algal toxins and health. Plenum Press, New York.

Viner, A.B. 1985. Thermal stability and phytoplankton distribution. Hydrobiologia 125:47-69.

Vollenweider, R.A. 1968. Scientific fundamentals of the eutrophication of lakes and flowing water, with particular reference to nitrogen and phosphorus as factors in eutrophication. O.E.C.D. Technical Report DAS/CSI 68.27.

Watanabe, M.F. and S. Oishi. 1980. Toxicities of Microcystis aeruginosa collected from some lakes, reservoirs, ponds and a moat in Tokyo and adjacent regions. Jpn. J. Limnol. 41:5-9.

Woods, P.F. Physical limnological factors suppressing primary productivity in Lake Koocanusa, Montana. Pages 224-233 in H.G. Stefan, ed. Proceedings of a symposium on surface water impoundments, Vol. 2. American Society of Civil Engineers, New York.