Environment Canada
Water Science and Technology Directorate

Direction générale des sciences et de la technologie, eau
Environnement Canada

Biogeochemical Study of an Avian Botulism Outbreak

By:
T. Murphy, A. Lawson, C. Nalewajko, H. Murkin

NWRI Contribution # 99-263

99-263

Biogeochemical Study of an Avian Botulism Outbreak

T.P. Murphy, A. Lawson, C. Nalewajko, H. Murkin, L. Ross, and T. McIntyre

Manuscript Perspective

This presentation was a review of our research on algal toxins. The weight of evidence is tightening the linkage between the presence of microcystins and the initiation of avian botulism. However, the specific pathway is uncertain. For example, algal toxins might kill small animals that become the substrate for Clostridium which then kills birds. Our ongoing studies indicate that if the algal toxin kills birds directly, it must be initially protected from digestive juices perhaps by bioaccumulation into duck food. The other focus of this study was to resolve why certain areas of the lake were much more toxic. Ongoing studies indicate that hotspots are set up by wind and appear to involve release of an essential nutrient, not phosphorus from sediments.

Abstract

Sediment release appeared responsible for the mean total phosphorus concentrations in Whitewater Lake increasing from about 73 µg/L on May 23 to a seasonal maximum of about 470 µg/L on June 17. Spatially the sediment anoxia varied greatly with the south-east corner being more reduced. The redox of the sediments appeared to decrease after calcium carbonate precipitation in the water column. Sulphate concentrations in the sediments were influenced by groundwater and probably macrophytes so that sulphate was never completely reduced to sulphide. The rich nutrients resulted in intense blooms of *Microcystis* and *Aphanizomenon* which produced the toxins microcystin-LR microcystin-RR and anatoxin-A.. Northern parts of the lake had more algal biomass and associated algal toxins. Wind appeared to produce these algal hotspots.

Étude biogéochimique d'une flambée de botulisme aviaire

SOMMAIRE À L'INTENTION DE LA DIRECTION

Cette présentation était une synthèse de notre recherche sur les toxines algales. De bonnes preuves tendent à confirmer le lien qui existe entre la présence de microcystines et l'apparition du botulisme aviaire, mais il reste à en déterminer le mécanisme exact. Par exemple, les toxines algales peuvent tuer de petits animaux qui deviennent un substrat pour *Clostridium*, laquelle, à son tour, tue des oiseaux. Nos études en cours démontrent que si des oiseaux sont tués directement par une toxine algale, cette dernière doit être initialement protégée des sucs digestifs, peut-être par bioaccumulation dans les aliments des canards. L'autre objectif de cette recherche était de comprendre pourquoi certaines régions du lac étaient beaucoup plus toxiques. Les études en cours indiquent que des concentrations algales sont créées par le vent et sont associées à la libération d'un nutriant essentiel autre que le phosphore des sédiments.

Mots-clés : toxines algales, botulisme aviaire, microcystines

Résumé

Le phosphore libéré par les sédiments s'est avéré responsable des concentrations moyennes de phosphore total du lac Whitewater, lesquelles sont passées d'environ 73 µg/L, le 23 mai, à un maximum saisonnier de 470 µg/L, le 17 juin. L'anoxie des sédiments variait grandement dans l'espace, le degré de réduction étant le plus élevé dans le coin sud-est. Le potentiel d'oxydoréduction des sédiments a diminué après la précipitation de carbonate de calcium dans la colonne d'eau. Les concentrations de sulfate dans les sédiments étaient tributaires des eaux souterraines et probablement des macrophytes, de sorte que le sulfate n'était jamais complètement réduit en sulfure. Les riches nutriants ont entraîné une forte prolifération de *Microcystis* et d'*Aphanizomenon* qui produisent les toxines microcystine-LR, microcystine-RR et anatoxine-A. Certains secteurs du nord du lac renfermaient une biomasse algale plus élevée et aussi une quantité de toxines algales plus élevée. Le vent serait responsable de la production de ces concentrations algales.

Algal Toxins - Initiators of Avian Botulism?

Tom Murphy and Annette Lawson,
National Water Research Institute, 1-905-336-4602, fax 1-905-336-8901, tom.murphy@cciw.ca
867 Lakeshore Road, Burlington, Ontario, L7R 4A6

Czesia Nalewajko
University of Toronto, 1265 Military Trail, Westhill, Ontario M1C 1A4

Henry Murkin and Lisette Ross
Institute for Wetland and Waterfowl Research, Stonewall, P.O. Box. 1160, Oak Hammock Marsh, Manitoba, R0C 2Z0.

Keiji Oguma, Department of Bacteriology, Medical School, Okayama University, 2-5-1, Shikata-cho, Okayama 700, Japan

Terry McIntyre, Environment Canada, 351 St. Joseph Blvd, Hull, Quebec. K1A 0H3

Abstract

An outbreak of avian botulism in Whitewater Lake, Manitoba was associated with reducing sediments. But any linkage between sediments and botulism was only indirect; *Clostridium botulinum* was not observed in the sediments. The source of the *Clostridium botulinum* was unclear but carcasses that overwintered appeared to perpetuate the outbreak. The algal toxins anatoxin-a and microcystin-LR were present (17 ≤μg/L) when many birds were moulting and unable to fly, likely making them more sensitive to botulism. The sediment anoxia released phosphorus into lakewater so that concentrations increased from about 73 μg/L to 470 μg/L and enhanced growth of *Microcystis* and *Aphanizomenon*. Wind resuspension of sediments resulted in areas with more algal biomass and associated algal toxins.

Key Words; algal toxins, microcystins, avian botulism, Clostridium, biogeochemistry

Introduction

Avian botulism is a wildlife disease caused by *Clostridium botulinum*. In this decade, the frequency of disease outbreaks in the prairies appears to have increased and in 1998 avian botulism spread north into the boreal region. As many as 10^6 waterfowl died in a major avian botulism outbreak (Ball et al. 1998). Furthermore, species that are sensitive to avian botulism are declining to alarmingly small populations. Wobeser (1997) hypothesized that a normal low frequency mortality is "cleaned up" by scavengers, but larger die-offs can trigger avian botulism outbreaks. The management of avian botulism relies on the assumption that removal of dead birds can control the outbreak. This belief has lead to extensive programs to pick up dead ducks

in the USA. This is also done in Alberta and Manitoba but there are serious reservations whether this strategy is appropriate for Canada's large wetlands. The efficiency of carcass retrieval is less than 25% (Bollinger, T. presentation to interagency botulism workshop, January 15, 1999, Saskatoon). It might be more effective to control the initiation of the botulism outbreak but first the mechanisms associated with the initiation of the outbreaks must be better understood.

Although the precise conditions responsible for the outbreak of avian botulism are not understood, the general conditions are well known. *Clostridium botulinum* is an obligate anaerobe thus, organic rich environments are typically where botulism occurs. The discharge of sewage and garbage can encourage conditions favouring botulism (Locke and Friend 1989, Ortiz and Smith 1994). Nutrient enrichment from farms has also been identified as a cause of botulism in wetlands (Crowder and Bristow 1988).

Storms have often been associated with outbreaks of avian botulism (Ball et al. 1998). Subtle physical changes such as wind driven (seiche) flooding of soils could kill invertebrates, but lightning and high winds could be as important. Storm events also often appear to trigger the collapse of algal blooms in prairie lakes (Papst et al. 1980). Recent studies indicated that algal toxins might initiate outbreaks of avian botulism. Ducks were observed dying in Finland and Thailand when *Microcystis* blooms and microcystins were present, respectively (Ericksson and Lindholm 1988, Mahakhant et al. 1999). Lysis of toxic algal cells during bloom collapse would release algal toxins into solution but any relationship between these toxicity aspects of storm events is speculative.

Study Site

Whitewater Lake is in an agricultural (grain and cattle) area of southwestern Manitoba (100° 20'W, 49° 18'N, Figure 1). When full, the lake's maximum length, width and depth are about 14 km, 6 km and 1 m, respectively. The lake is a terminal basin and it has been dry at least a few times (Ransom and Hochbaum 1972, Ransom 1972). Emergent vegetation like whitetop grass (*Scolochloa festucacea*), cattail (*Typha*), and bulrush (*Scripus acutus*) can be found along the shore and throughout the lake. The habitat attracts many waterfowl including ducks, pelicans, coots and shorebirds. Whitewater Lake is the most important staging area in Manitoba for migratory birds. The large expanse of shallow water rich in plants offers the appearance of safe habitat. Many ducks pick this site to moult. In 1996, over 116,463 dead birds were collected from this lake. By some estimates over 200,000 birds died of avian botulism (Pratt 1996). In 1997, 48,683 dead birds were collected (Ball et al. 1998).

Like many prairie lakes (Barica 1974, 1980), Whitewater Lake is very eutrophic. Although agricultural discharges of nutrients might be a big part of the eutrophication problem, the natural geochemistry of these lakes enhances the availability of phosphorus. The high concentration of sulphate increases the potential for sediment release of phosphorus (Caraco et al. 1989, Murphy et al. 1997). Bacterial metabolism in the sediments converts the high concentration of sulphate to high concentrations of sulphide that inactivates iron that in turn enhances mobility of phosphorus and increases eutrophication (Manning et al. 1994). The lake is also alkaline and in

August 1996, precipitation of calcite and dolomite was a major event settling as much as 35 mg/l of inorganic precipitants (Murphy et al. 1997). The sudden settling of algae with calcium carbonate to the bottom of the lake should enhance consumption of oxygen and produce conditions favourable for the growth of *Clostridium botulinum*.

The objectives of this study were as follows;
1) assess effect of redox on outbreaks of avian botulism,
2) measure algal biomass, species and toxin production,
3) determine spatial and temporal changes in sediment chemistry.

Methods

Sample Collection

A differential Global Positioning System (GPS) was used for site positioning of six main sampling stations. Nine sampling trips were conducted from May 23, 1997 to October 5, 1997. The dissolved oxygen, conductivity, redox, pH, temperature and depth of the water column were measured with a H20 Model Hydrolab on two trips (June 7 and August 23).

Sediment Samples

Sediment cores were collected with a benthos corer in triplicate from six sites in the lake June 7 and August 23, and sectioned at 0-5 cm and 5-10 cm depths. The redox of each section was measured with a Fisher Scientific Accumet 1003 pH/Eh meter equipped with an Orion combination redox electrode. The samples were placed in plastic bags and the air was removed by displacement.

Within 24 hours of sample collection, the sediment core sections were homogenized and the acid volatile sulphide (AVS) was measured by ion selective electrodes using a diffusion method (Brouwer and Murphy 1994). Freeze-dried sediment was weighed before and after ashing in a muffle furnace at 490°C for two hours to determine loss on ignition.

Sediment Interstitial Water Samplers (Peepers)

Diffusion chambers (peepers) were installed in triplicate May 24 and August 9 and retrieved on June 7 and August 23 from six sites in the lake. Peepers were designed to sample water 0-5 cm above the sediments and the interstitial porewater from 0-5 cm and 6-11 cm below the sediment surface. The peepers were made of two Plexiglas components; a bottom reservoir with 6 cells of approximately 12 ml capacity each and a cover, which were secured together by screws to hold polysulfone dialysis paper (Gelman HT 450) firmly in place over the reservoir. The cover had holes corresponding to the cells to allow diffusion of chemicals from the sediment, across the dialysis membrane, and into the reservoirs containing de-ionized distilled water. Peepers were prepared to avoid contamination with oxygen. For example, the peepers were de-oxygenated by purging with nitrogen gas for several hours.

Once assembled, the peepers were checked for trapped gas bubbles and bubbled with nitrogen for at least 48 hours prior to deployment (Hesslein 1976, Rosa and Azcue 1993). A surrounding baffle was attached then the peepers were inserted into the sediment with one row of cells above the sediment and water interface. The baffle acted as a stabilizer to minimize movement of the peeper. Two weeks later the peepers were removed and the pore water was extracted from the cells with syringes and acidified immediately with 0.02% nitric acid.

Chemical Analyses

For metal analysis, filtered water samples (0.45 µm cellulose acetate) were brought to a pH of 2 with concentrated nitric acid. The metals and major ions were analysed by automated inductively coupled argon plasma emission spectroscopy with ultrasonic nebulisation and direct aspiration (NLET, Method 02-2051). Filtered water samples (0.45 µm cellulose acetate) were preserved with 0.3% sulphuric acid then analysed for total phosphorus by automated colorimetric technique (NLET Method 01-1190). Total and dissolved inorganic carbon (TIC, DIC), total and dissolved organic carbon (TOC, DOC), and total Kjeldahl nitrogen (TKN) were also measured by NLET methods (1994). Water samples for ammonia analysis were filtered near the lake, preserved with a 4% phenol solution in the field, and analyzed by the colorimetric phenate method 4500-NH_3 D (APHA 1989). Absorbance was measured on a LKB Biochrom UV spectrophotometer at 640 nm. Anions in pore water were analysed on a Dionex model 2010i ion chromatograph.

Algal Analysis

Samples for chlorophyll a analysis were filtered through GF/F filters and frozen until analysis by DMSO extraction (Burnison 1980).

Samples for algal analysis were preserved with Lugol's iodine and shipped to the University of Toronto for identification of blue-green algae with the Utermöhl technique. Phytoplankton enumerations were carried out using a Wild inverted microscope. Large algae were counted at x 200 magnification, smaller ones at x 400 or x 1000. In all instances, at least 400 and usually 800 individuals were examined.

Algal samples for toxin analysis were isolated by filtering a known volume through a 30 µm nitex mesh. The residue was rinsed off the mesh with lakewater into a bottle for freeze-drying. Algal toxins were extracted from these algal concentrates by bath sonication (5 minutes) in Milli-Q water followed by a second extraction (5 minutes) in a 75:20:5 water/methanol/butanol mixture (Meriluoto and Erickson 1988). Samples were centrifuged and filtered (0.45 µm) after each extraction then pooled and evaporated to 1 ml. The HPLC system consisted of dual Waters 510 pumps, WISP autoinjector, and a Kratos UV detector set at 238 nm. The column was a 300 mm x 3.9 mm I.D. µBondapak C18 (10 µm, 125 Å, Waters) connected to a µBondapak guard insert. The mobile phase consisted of a 10 mM ammonium acetate:acetonitrile (76:24) mixture operating at 1 ml/min isocratic for 40 minutes. Injection volumes were 100 µL per standard and

sample. Target compounds (microcystin-LR, microcystin-RR, anatoxin-A) were identified by retention time comparison of standards and sample spiking. Concentrations were determined by comparing peak areas against a calibration curve.

For polymerize chain reaction (PCR) analysis, bottles were filled 1/3 full with sediment and the remainder with surface water. The bottle was capped and shaken vigorously for two minutes to resuspend the sediment. The bottle was set aside to allow sediment to settle; the water was decanted and frozen immediately. PCR analysis followed the procedure of Takeshi et al. 1996, using an applied Biosystems DNA Synthesizer Model 380A. Attempts were made to grow *Clostridium* from sediments in cooked meat media under an anaerobic atmosphere.

Meteorological Station

A meteorological station was installed May 24 west of Sexton's Island near site 4 on a floating raft. On June 8 a storm damaged the equipment. After repairs, the meteorological station was installed July 2 east of Sexton's Island near site 1 (Figure 1). The following variables were recorded every ten minutes; air and water temperature, relative humidity, wind speed, wind direction, solar radiation and water oxygen concentration.

Results

Sediment Chemistry

The sediments were firm with a low water content (57% 0-5 cm, 45% 5-10 cm, n=36) and moderate organic content (Table 1, TOC 4.25%, TON 0.38%; n=36). In general, sediment composition was highly variable, both within triplicate samples collected at each site and among sites. Since most of the sediments were sand and the relationship between loss on ignition (LOI) and organic content was excellent (r^2=0.85, 95% confidence level), much of the variability was probably associated with erratic deposition of sand, clay, shells (TIC 1.96%, n = 36) and organic matter. In August, the LOI of surface sediments increased at site 3 on the eastern portion of the lake. This observation is important in that it might represent an external source of organic carbon entering from Cherry Creek. Furthermore, this hypothesis is substantiated by the sediment redox values and water chemistry data.

Table 1 Sediment Organic Content

	Site 3, May	Site 3, Aug	Lake Mean May	Lake Mean Aug.
TOC	4.93 ± 1.0	ND	4.25 ± 1.44	ND
LOI	12.2 ± 2.3	17.9 ± 2.0	11.0 ± 2.7	9.9 ± 3.83

ND - no data,

Redox readings indicated sediments were reduced in May (-53 to -593 mV) and in August (-136 to -398 mV). Acid volatile sulphide concentrations in the surface sediments were in the range of

0.12 to 0.55 mg S/g in May and had increased threefold in August (0.42 to 0.88 mg S/g) (Figure 2). This large increase followed precipitation of calcium carbonate. AVS concentrations varied significantly between sites and there was some local variability. Site 3 in the southeast corner near Cherry Creek had the lowest redox readings (-371 ± 18 mV) and highest sediment AVS concentrations (0.57 ± 0.13 mg/g). The reduced sediments could produce conditions favourable for the growth of *Clostridium botulinum*.

Concentrations of sediment sulphide and pore water sulphate varied significantly between sites and there were no strong spatial trends (Figure 3). Also, we could not establish a correlation between sulphide and sulphate concentrations. In general, sediment variability was large and at least triplicate analysis is required for sediment studies. Phosphorus concentrations in sediment porewater were high in all measurements. The sediments were a large potential source of nutrients.

Table 2 Total Phosphorus mg/L

Depth	June 7	August 23
surface water	0.30 ± 0.12	0.22 ± 0.05
bottom water	1.09 ± 0.85	0.60 ± 0.42
0-5 cm porewater	1.80 ± 1.20	1.59 ± 0.62
5-10 cm porewater	2.23 ± 1.82	2.55 ± 0.30

June mean of duplicates from 6 sites: August mean of triplicates from 6 sites

Porewater movement into the lake can be seen indirectly from the sodium data. The surface porewater was rich in sodium which increased both with depth and as the summer advanced (Table 3). The seasonal increase in sodium may indicate a groundwater supply of saline water, which would could lead to flushing of phosphorus rich sediment porewater into the lake. An alternative hypothesis would be a slow diffusion of the surface porewater between two larger compartments, the deeper porewater and the lakewater. The sodium concentration of the lakewater was significantly correlated to water depth, albeit there was enough variability for other factors like groundwater to still have importance ($r = -0.577$, $n = 54$). By comparison the correlation of calcium and depth was much weaker, presumably because of precipitation of calcium carbonate ($r = -0.325$, $n = 54$).

Table 3 Sodium (mg/L)

Depth	June 7	August 23
surface water	242 ± 25	315 ± 28
bottom water	239 ± 29	357 ± 28
0-5 cm porewater	345 ± 153	496 ± 151
5-10 cm porewater	796 ± 577	1060 ± 673

June mean of duplicates from 6 sites: August mean of triplicates from 6 sites

Water Chemistry

The sodium concentrations were moderately high and increased in summer as the lake level decreased (May 23, 186 mg/L; October 15, 577 mg/L). The decrease in water depth of 40 cm from as deep as 80 cm, could easily explain the increase in sodium by evaporative concentration. However, the large increase in phosphorus from May to June was associated with only a 2 cm decrease in water level. Sediment release appeared responsible for the increase in the mean total phosphorus concentrations in the lake from about 73 µg/L on May 23 to a seasonal maximum of about 470 µg/L on June 17 (Figure 4). Average total phosphorus concentrations at all sites excluding site 5 were 0.26 to 0.328 mg/L, which is typical of extreme eutrophic conditions. Phosphorus in the bottom water as measured by peepers was much higher than surface samples (571 µg/L vs. 301 µg/L in May/June; 657 µg/L vs. 223 µg/L in August). This difference was much bigger than with the more conservative sodium and was due to precipitation of phosphorus from the water column and sediment release. The variability of the water quality within a few metres was relatively small and the standard deviation on all plotted water column measurements was less than 2%.

Unlike in August 1996 when we observed an anoxic water column near Sexton's Island, in 1997, the water column was always oxic. Dissolved oxygen and redox measurements in May and August indicated an aerobic water column. The oxygen sensor on the meteorological station provided a more complete record. Although oxygen concentrations decreased by as much as 4 mg/L at night, anoxic water was never detected.

Dissolved organic carbon concentrations in the water column ranged from 16.2 to 57.4 mg/L. Highest concentrations were measured at sites 3 and 6, nearest to the inflow from Cherry Creek, which is suspected to be contaminated with sewage. Water overlying the sediments in the southeast corner of the lake (site 6) consistently had high concentrations of iron or manganese, which indicates extreme anoxia, nutrient regeneration from sediments into the water column and potential for growth of toxic algae and *Clostridium botulinum*. The concentration of iron was variable and not correlated to either phosphorus or chlorophyll a concentrations. This does not necessarily mean that iron phosphorus precipitation did not take place or that iron was not an essential trace metal to algae. It probably means that other factors such as calcium carbonate precipitation or wind mediated sediment resuspension were more important.

Calcium Carbonate Precipitation

Calcium concentrations decreased at most stations in mid to late summer. The marked decrease in calcium concentration at Site 5 from July 2 to July 22 continued until after September 11 (Figure 5). By October 5, the calcium concentrations in the water had increased as the water became colder and calcite partially dissolved. The dense algal biomass produced a pH >9.0 which must have resulted in precipitation of calcium carbonate (calcite) and perhaps dolomite.

The decrease of about 16 mg Ca/L indicated a precipitation of 40 to 60 mg of calcite per liter. The higher estimates results were obtained by adjusting for an estimated 50% loss in water by evaporation. The concentrations of calcium in the sediment porewater were particularly high (June 7, 0-5 cm, 81 mg/L; 5-10 cm, 154 mg/L: August 23, 0-5 cm, 90 mg/L; 5-10 cm, 177 mg/L); presumably salts and organic matter suppressed calcium carbonate precipitation.

Algal Blooms

Dense algal blooms in Whitewater Lake were first measured on July 22. The bloom was found mainly on the northern part of the lake. Phytoplankton analysis confirmed visual observations that the bloom of blue-green alga *Microcystis aeruginosa* was largest at site 4 (biomass of 107 mg/L) (Figure 5). *Microcystis aeruginosa* was also the dominant blue-green algal species at site 1 but the concentrations were much lower (biomass of 27.5 mg/L). Maximum peaks of *Microcystis aeruginosa* at site 1 were measured on August 8 and September 9 (biomasses of 130 and 79.8 mg/L, respectively). At site 5, the dominant alga on July 22 was *Aphanizomenon flos-aquae* (biomass of 34.2 mg/L, Figure 17) with some *Microcystis aeruginosa* present (biomass of 1.43 mg/L). By August 8, however, *Microcystis aeruginosa* also dominated site 5 (biomass of 92.2 mg/L) and continued to dominate on August 22 and September 9 (biomasses of 1802 and 111 mg/L, respectively). Chlorophyll a concentrations on July 22 were extremely high at site 5 (283 μg/L) (Figure 5) and this site usually had the highest chlorophyll a concentration.

The biomass of blue-green algae varied greatly among sites. Prior to July 22, winds predominantly blew from the southwest, perhaps concentrating nutrients in the northeastern portion of the lake (Figure 5). Wind direction shifted after the blooms, originating from both the east and southeast directions (Figure 5). This appeared to concentrate algal blooms into the east central part of the lake, as observed on August 23. Storm breakage, movement and concentration of reed beds also could have had a major effect on nutrients and toxic algal blooms. The algal toxins microcystin-LR and anatoxin-A were the major algal toxins associated with the algal blooms. This wind driven concentration of algal blooms is not supported by other aspects of water chemistry and microbiology. For example, conservative ions like chloride and sodium varied spatially albeit not as much as phosphorus (Figure 4) and water analysis did not indicate wind mixing. Perhaps only the very surface layers with algal blooms moved or perhaps wave action enhanced release of a critical nutrient from sediments. This last idea fits well with the increase in sulphate associated with sites with more algae.

Blue-green algae are characteristically found in waters receiving high nutrient inputs.. Total phosphorus concentrations were >2 times lower at site 5, where the densest algal blooms occurred. In the hotspots of algal biomass, water conditions in the lake were ideal for blue-green algal growth and apparently not phosphorus limited. Also from June to September, ammonia was never less than 0.4 mg N/L and nitrogen limitation was unlikely. No obvious trends were observed between blue-green algal biomass and alkalinity, total phosphorus, TKN, iron concentrations and other ions. Nutrient limitation could potentially have a major impact on blue-green algal growth outside of the hotspots, but our data does not indicate any obvious limitation.

Total iron is not a good measurement of bioavailable iron so no conclusions can be made from our iron data.

The lack of blue-green algal blooms throughout the lake is surprising. The density of macrophytes was not enough to produce light limitation to phytoplankton but macrophytes might have influenced the blooms in other ways such as via their roots. There was an interesting relationship between sediment porewater sulphate and biomass of blue-green algae. In late summer, the algal biomass was higher where the sediment porewater sulphate concentration increased or remained constant. If macrophyte oxidation of the sediments produced this effect, then whatever stimulated macrophytes stimulated the blue-green algae. The influence of wind on the algal blooms via physical enhancement of sediment mixing is also an important hypothesis that could be mediated via nitrogen or iron availability, or physical resuspension of cells resting on the sediments.

Efforts to grow *Clostridium botulinum* from these sediments were unsuccessful.

Discussion

Geochemistry

Laboratory studies on sediments indicated the complete reduction of sulphate to sulphide and suggested that this critical switch in redox reactions might be significant in the development of *Clostridium* (Murphy et al. 1997). This hypothesis might be valid in microniches, but in general, it is not supported by 1997 studies in the lake. The geochemistry was atypical of most lakes. In 1997, AVS was not correlated to sulphate concentrations in porewater. This observation is counterintuitive for a lake, but Whitewater Lake is a wetland not a typical lake. As a dimictic lake stratifies in summer, sediments become more reduced and sulphate is converted to sulphide. In Whitewater Lake, macrophyte roots could have oxidized reduced sulphur to sulphate. Wind mixing of sediments, and resulting inflow of saline groundwater could be replenishing sediment porewater sulphate faster than it is reduced to sulphide. This flushing of sediment porewater phosphorus into the lake by groundwater or waves would show both short term and year-to-year variation associated with changes in weather. These changes should have an impact on many variables influencing avian botulism. Sodium may be a useful tracer to measure these seasonal and year-to-year changes.

The large spatial differences in algal biomass were reflected in large changes in carbonate precipitation and were also observed in 1996. Furthermore, the carbonate precipitation was confirmed by geochemical analysis of the sediments. The water chemistry of the lake varied spatially indicating that the water was not circulating freely. Presumably the rooted macrophytes suppressed most water movement.

Attempts to grow *Clostridium botulinum* or to use PCR in NWRI and Okayama University to measure *Clostridium botulinum* in sediments of Whitewater Lake were unsuccessful. The

Okayama University Laboratory is very experienced handling *Clostridium botulinum* so our negative results could indicate either a lack of *Clostridium botulinum* in the sediments, or an interfering substrate. We suspect that humic acids interfered with PCR measurements. *Clostridium botulinum* survived the winter in carcasses that alone may have perpetuated the botulism outbreak the following year.

Algal Toxins

Many of the sediment geochemical processes influencing growth of *Clostridium* also influence the growth of toxic blue-green algae. Typically large celled blue-green algae spend part of their life cycle on sediments (Trimbee and Harris 1984) and sediment chemistry can influence whether toxic algae can grow from sediments (Nalewajko and Murphy 1998).

Although we detected hotspots of algal blooms, the concentration of algal toxins was less than has been reported in some studies (Figure 6). Our measurements are higher than those found in some Finnish lakes and most years in Lake Suwa, Japan. Without laboratory bioassays, these results are inconclusive. The pathway for assimilation of microcystins can be more complex than represented by algal analysis. Birds that are moulting may be forced by hunger to eat food that ducks capable of flying would reject by taste. Limited work on bioaccumulation of algal toxins has demonstrated detectable microcystin-LR in pulmonate snails (Zurawell and Prepas 1995). Similar work by Eriksson et al. (1989) observed a 50% bioaccumulation of a microcystin like compound by freshwater mussels. The concentrations of microcystin-LR in Whitewater Lake were higher than those known to cause chronic toxicity in fish (0.5 μg/L, Oberemm et al. 1997) and only moderately less than those known to induce acute toxicity response in fish. The potential for interactions between the presence of microcystins and botulism is high and should be resolved by laboratory study. Many birds come to large wetlands like Whitewater Lake to moult; thus, they are unable to avoid long-term contact with algal toxins. It is highly likely that microcystins or anatoxins at least stress the birds and enhance the probability of an avian botulism outbreak.

Acute and chronic studies of microcystins on ducks are required. Almost all toxic studies have been done on mice or rats. One laboratory study of quail produced very different results than obtained from mice or rat studies (Takahashi and Kaya 1993). Quail spleens were enlarged two fold by microcystin-RR, but the livers did not change. Postmortem analysis of birds dying from algal toxins may require different analysis than that used for mice. Usually the quails died between 14 and 18 hr after the injection. The LC_{50} value obtained was 256 μg/kg quail. By comparison, microcystin-LR has an LD_{50} of 50 μg/kg, by intraperitoneal injection in mice.

If microcystins are demonstrated to be an initiator of avian botulism, then many aspects of disease control become clearer, but not simple. In culture, high nitrate concentrations have been shown to enhance the production of microcystins (Sivonen 1990) and nitrogen fluxes are complex and large in prairie lakes (Murphy and Brownlee 1981a, Murphy and Brownlee 1981b, Brownlee and Murphy 1983). *Microcystis* does not have heterocysts and does not fix nitrogen. Nitrogen limitation is a valid possibility that makes nitrogen sources like sewage and duck feces

important. A study of the nutrient inputs into the lake is recommended. Cherry Creek should be sampled for nutrients and coliforms during the sampling season especially during storm events. Also, the significant number of birds nesting on the lake gives rise to another possible flux of nutrients into the lake. A census of bird nesting should be part of a nutrient budget. If source control of nutrients cannot be effectively implemented, the frequency of any direct treatment of the lake must be estimated before any engineering efforts such as in situ sediment treatment. If the algal blooms are confined to the same small areas of the lake, treatment of these hotspots is more amenable. However if these hotspots move randomly with the wind, in situ treatment would be more difficult.

Acknowledgements

Ducks Unlimited supplied funding. The Canadian Biotechnology Strategy Renewal Fund also financially supported the project. Several individuals within Manitoba Department of the Environment, Ducks Unlimited and NWRI's Technical Support Division assisted in the collection of samples. The engineering department of NWRI calibrated and repaired the weather station. The Water Technology International Corporation allowed the use of their HPLC equipment for measurement of algal toxins.

References

American Public Health Association. Standard Methods for the Examination of Water and Wastewater. 17th edn. Washington D.C., American Public Health Association, 1989.

Ball, G., Bollinger, T.; Conly, M.; Kadlec, J.; Kehoe, P.; MacFarlane, B.; Murkin, H.; Murphy, T.P; Pybus, M; Rocke, T; Samuel, M.; Sharp, D.; and Wobeser, G. 1998. Report to the Prairie Habitat Joint Venture by the Working Group on Avian Botulism.

Barica, J. Arch. Hydrobiol. 1974, 73,334-360.

Barica, J. In: D.T. Waite (ed.). Prairie surface waters: problems and solutions. Canadian Plains Proceedings No. 7., 1980

Brownlee, B.G.; Murphy, T.P. Can. J. Fish. Aq. Sci. 1983, 40,1853-1860.

Brouwer, H.; Murphy, T.P. Envir. Chem. Toxicol. 1994, 13,1273-1275.

Burnison, B. K. Can. J. Fish. Aquat. Sci. 1980, 37, 729-733.

Crowder A.A.; Bristow, J.M. J. Great Lakes Res. 1988,14(1),115-127.

Eriksson, J.E., Meriluoto, J.A.O.; Lindholm, T., Hydrobiologia, 1989,183,211-216.

Eriksson, J.E.;Lindholm, T. Lahti. 1988, 18(5),19-35.

Hesslein, R.H. Limnol. Oceanogr. 1976, 21, 912-914.

Locke, L.N.; Friend, M. U.S. Fish and Wildlife Leaflet 1989,13.2.4.

Mahakhant, A., Klungsugya, P.; Arunpairojana, A.; Sano, T.; Watanabe, M.; Kaya, K.; Atthasampunna, P. Toxicity of cyanobacterial blooms in Thailand. Thailand Institute of Scientific and Technological Research. Research Project 1999, No 39-02.

Manning, P.G.; Murphy, T.P.; Prepas, E.E. Canadian Mineralogist 1994,32,459-468.

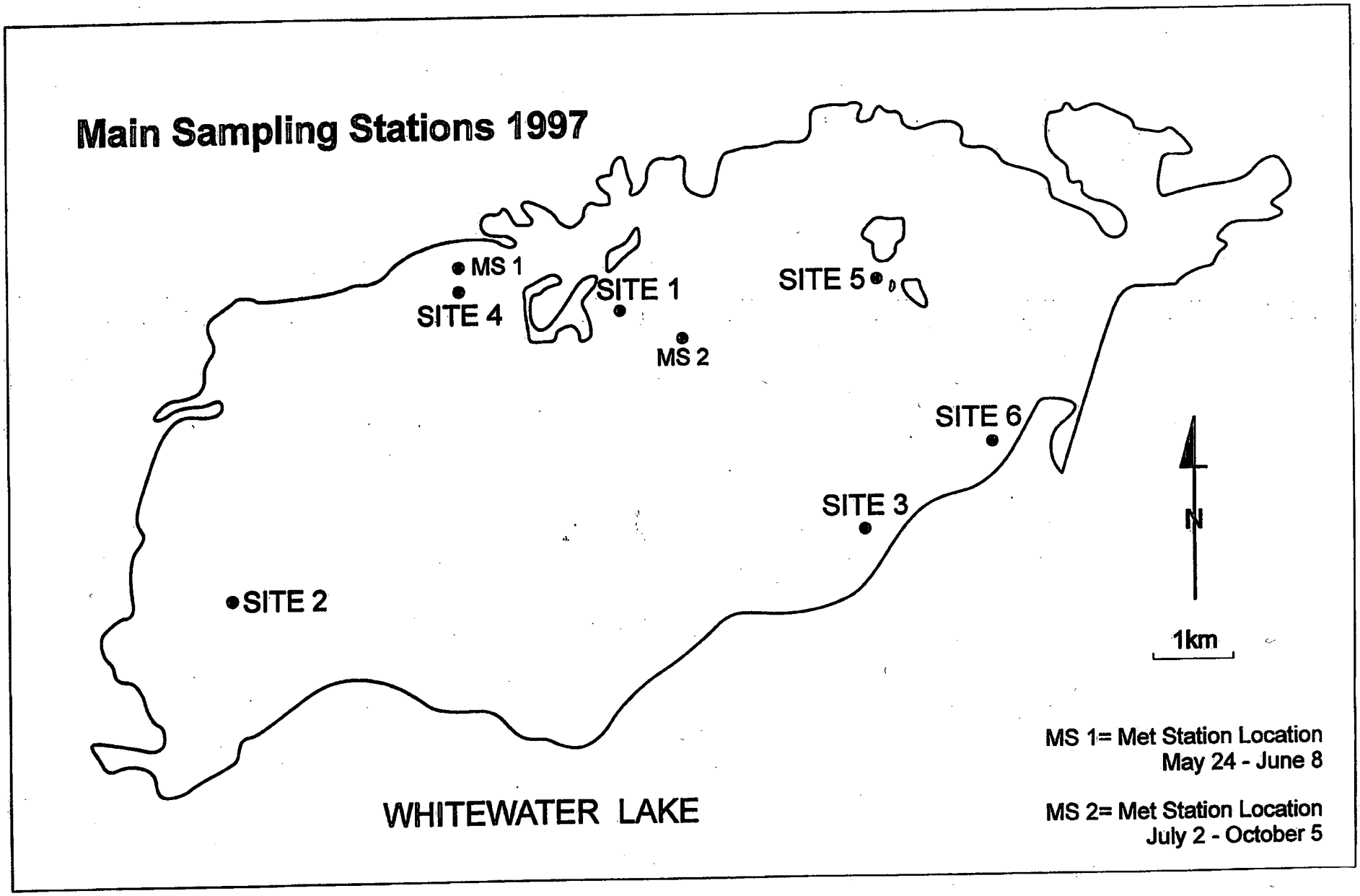
Manual of Analytical Methods. 1994. Volume I and II, National Laboratory for Environmental Testing, Environment Canada.

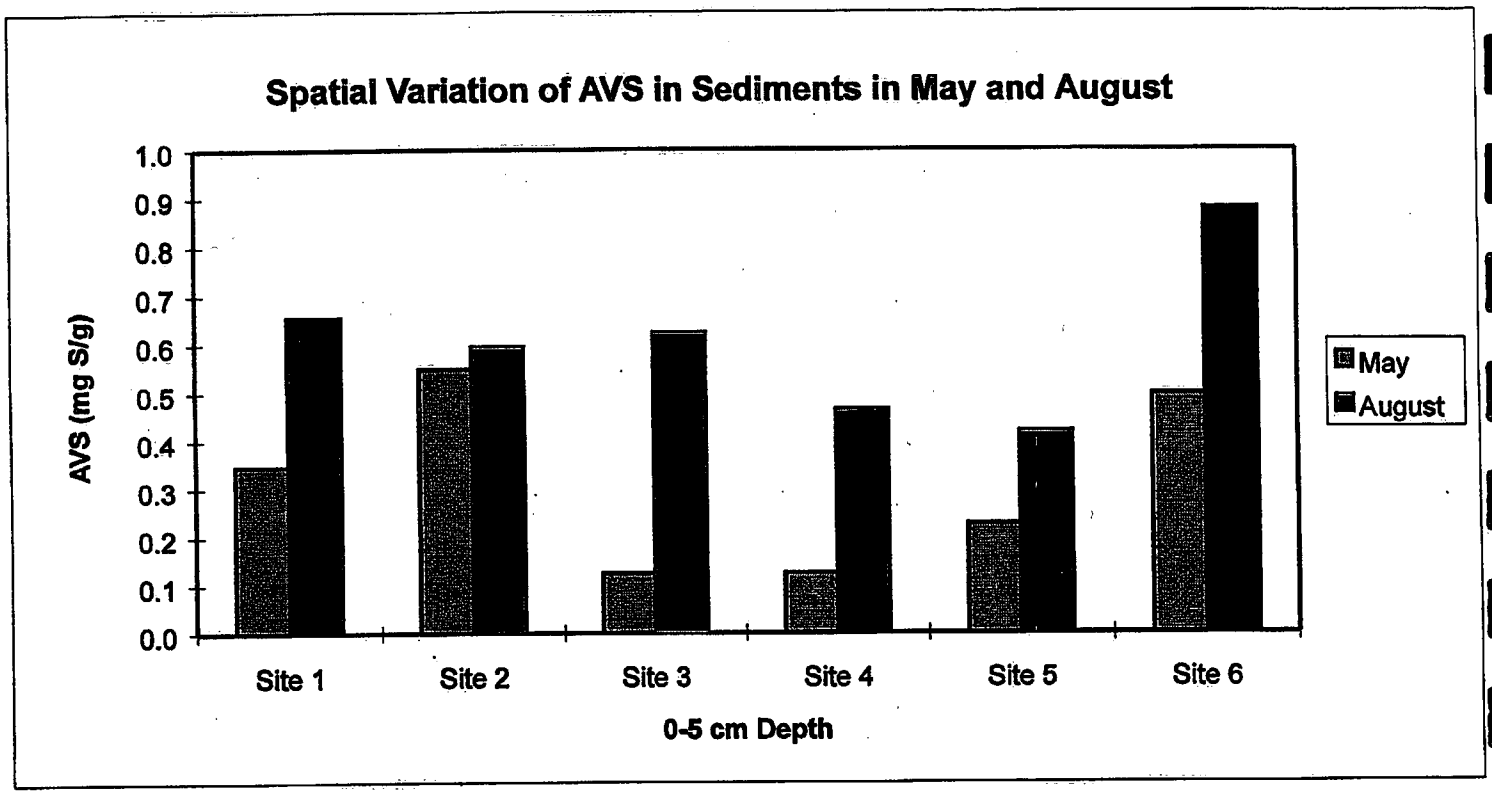
Meriluoto, J.A.O. and J.E. Eriksson. J. Chromotogr.1988, 438,93-99.
Murphy, T.P.; Brownlee, B.G. Can. J. Fish. Aquat. Sci. 1981a, 38,1035-1039.
Murphy, T.P.; Brownlee, B.G. Can. J. Fish. Aquat. Sci. 1981b,38,1040-1044.
Murphy, T.P.; Lawson, A.; Corsini, J.; Gray, I.; Rancourt, D.G. Whitewater Lake, Biogeochemical Study of 1996 Botulism Outbreak, Report to Ducks Unlimited. National Water Research Institute Contribution 1997, No. 97-211.
Nalewajko, C.; Murphy, T.P. J. Appl. Phycol. 1998,10,341-348.
Oberemm, A.; Fastern, J.; Steinberg, C.E.W. Wat. Res. 1997,31,2918-2921.
Ortiz, N.E.,; Smith, G.R. Epidemiol. Infect. 1994,112(2),385-391.
Papst, M.H.; Mathias, J.A.; Barica, J. Can. J. Fish. Aquat. Sci. 1980,37,1433-1438.
Pratt, A. Whitewater Botulism Data Summary Report. Ducks Unlimited Canada, Brandon, Manitoba, 1996, 53 p.
Ransom, A.B. Resource management alternatives in the Whitewater Lake area. Manitoba Department of Mines, Resources and Environmental Management. 1972.
Ransom, A.B.; Hochbaum, G. Wildlife Management Problems and Potentials at Whitewater Lake. Manitoba Department of Mines, Resources and Environmental Management. 1972.
Rosa, F.; Azcue, J.M.; Peeper Methodology - A detailed procedure from field experience. National Water Research Institute contribution, 1993, 93-33.
Sivonen, K. Appl. Environ. Microbiol. 1990, 56,2658-2666.
Takahashi, S.; Kaya, K. Nat. Toxins. 1993,1(5),283-285.
Takeshi, K.; Fujinaga, Y.; Inoue, K; Nakajima, H.; Oguma, K.; Ueno, T.; Sunagawa, H.; Ohyama, T. Microbiol. Immunol. 1996, 40(1),5-11.
Trimbee, A.M.; Harris, G.P. J. Plankton Res. 1984, 6(5),897-918.
Zurawell, R.; Prepas, E.E.. 1995. Lake Reserv. Manage. 1995,11(2),206.

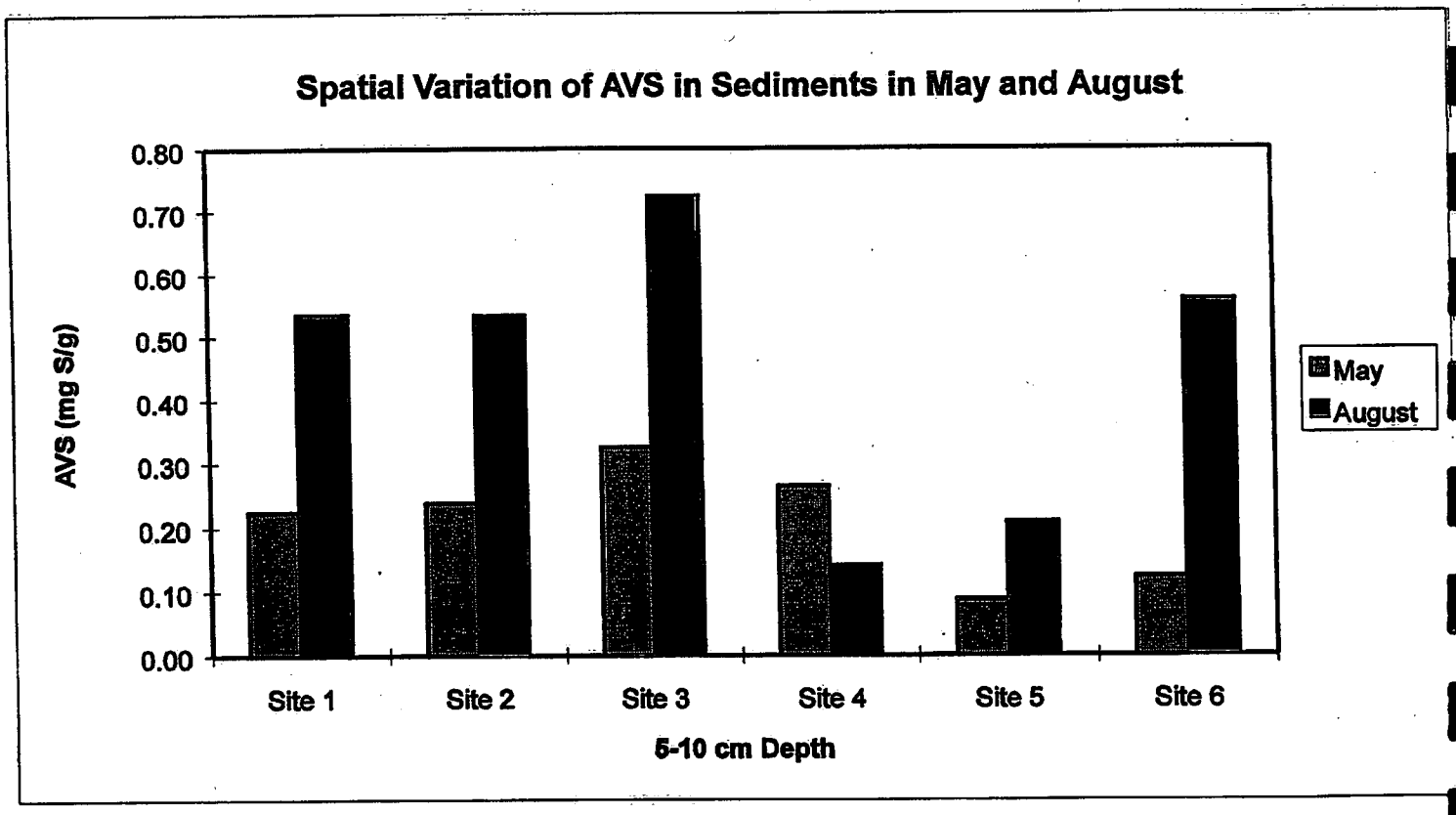
List of Figures

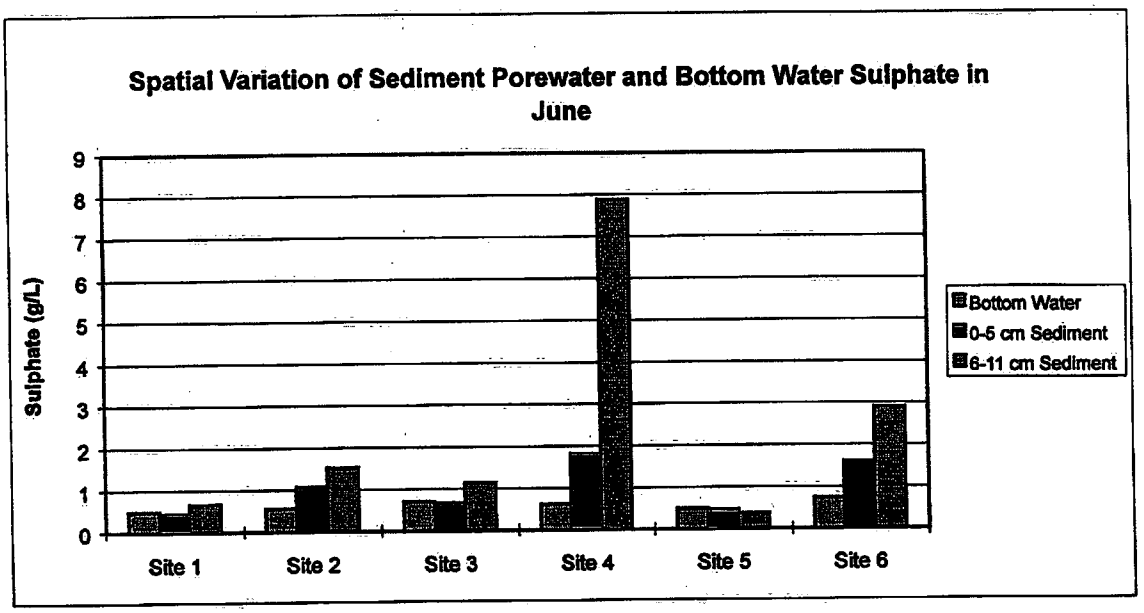
f) Wind Direction and Speed after Algal Blooms

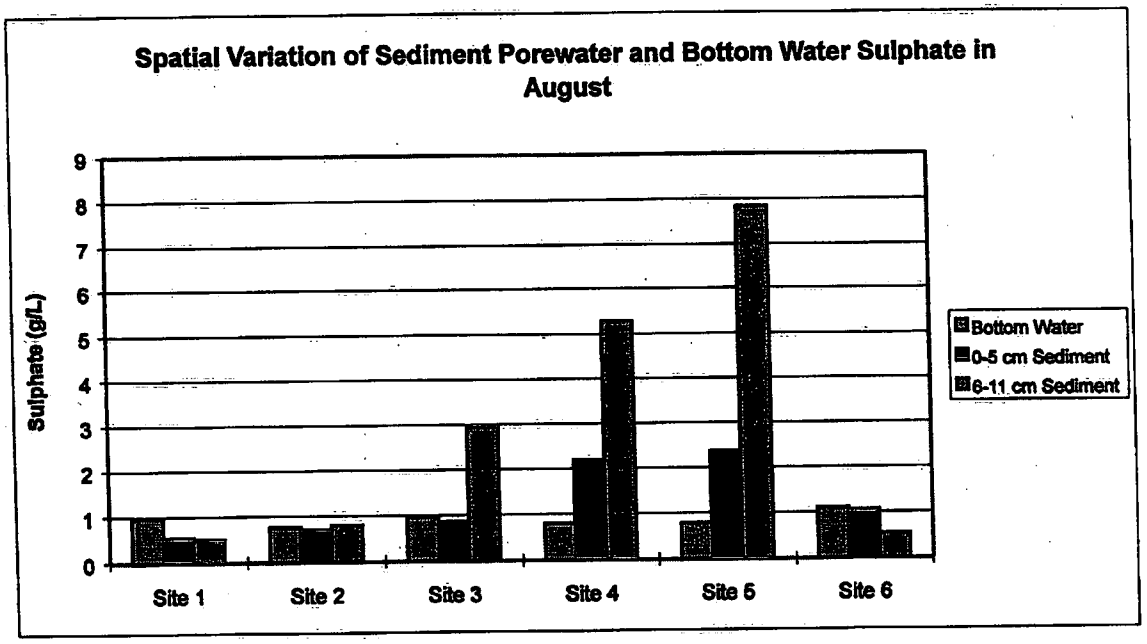
Fig. 6 Microcystin-LR and Anatoxin-A in Whitewater Lake

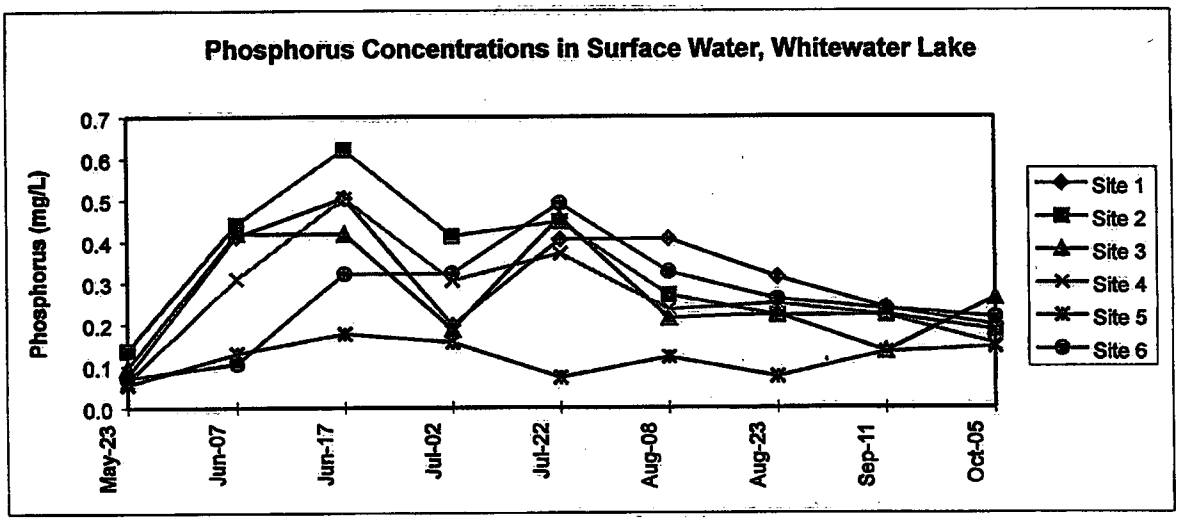

Main Sampling Stations 1997
MS 1
SITE 4
SITE 1
MS 2
SITE 5
SITE 6
SITE 3
SITE 2
N
1km
WHITEWATER LAKE
MS 1= Met Station Location
May 24 - June 8
MS 2= Met Station Location
July 2 - October 5

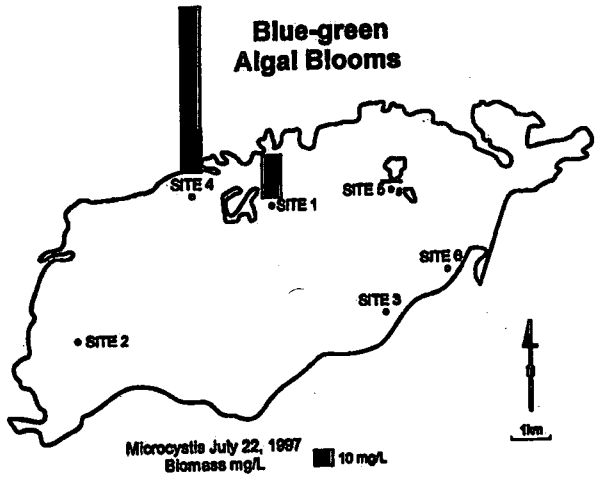

Spatial Variation of AVS in Sediments in May and August
AVS (mg S/g)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
0-5 cm Depth
May
August

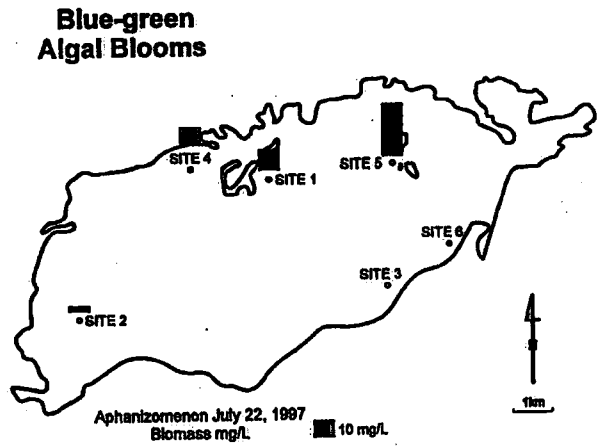

Spatial Variation of AVS in Sediments in May and August
AVS (mg S/g)
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
5-10 cm Depth
May
August

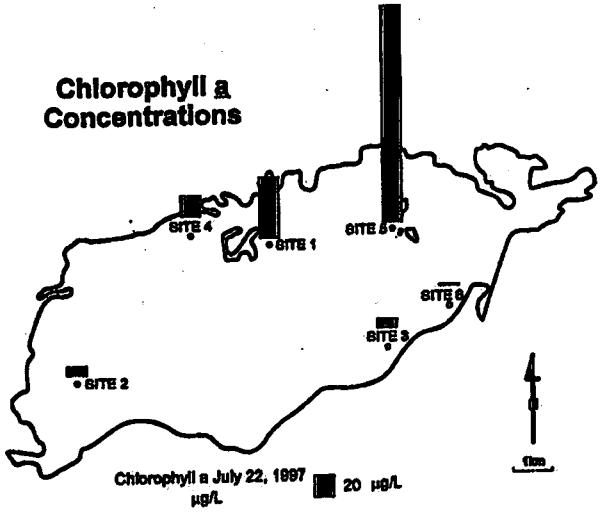

Spatial Variation of Sediment Porewater and Bottom Water Sulphate in June
Sulphate (g/L)
0
1
2
3
4
5
6
7
8
9
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
Bottom Water
0-5 cm Sediment
6-11 cm Sediment

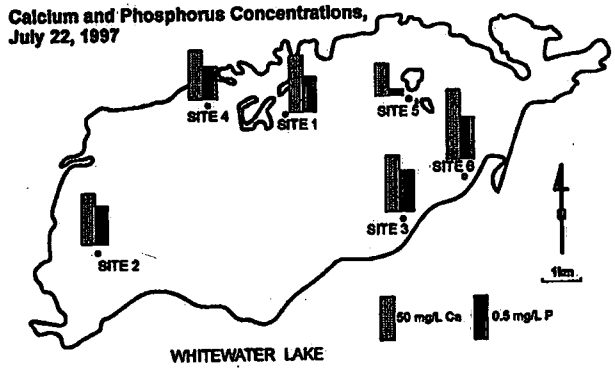

Spatial Variation of Sediment Porewater and Bottom Water Sulphate in August
Sulphate (g/L)
0
1
2
3
4
5
6
7
8
9
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
Bottom Water
0-5 cm Sediment
6-11 cm Sediment

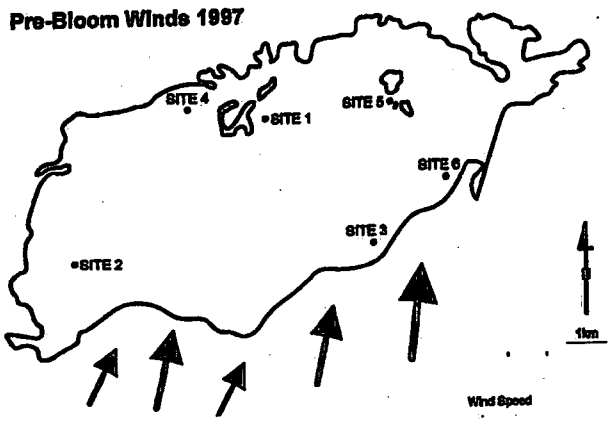

Phosphorus Concentrations in Surface Water, Whitewater Lake
Phosphorus (mg/L)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
May-23
Jun-07
Jun-17
Jul-02
Jul-22
Aug-08
Aug-23
Sep-11
Oct-05
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6

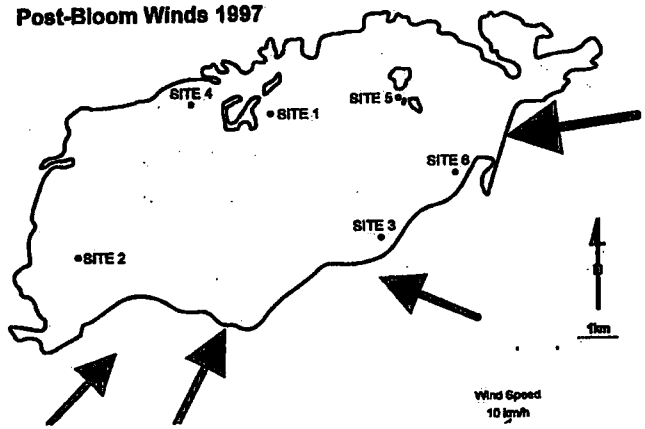

Blue-green
Algal Blooms
SITE 4
SITE 1
SITE 5
SITE 6
SITE 3
SITE 2
1km
Microcystis July 22, 1997
Biomass mg/L
10 mg/L

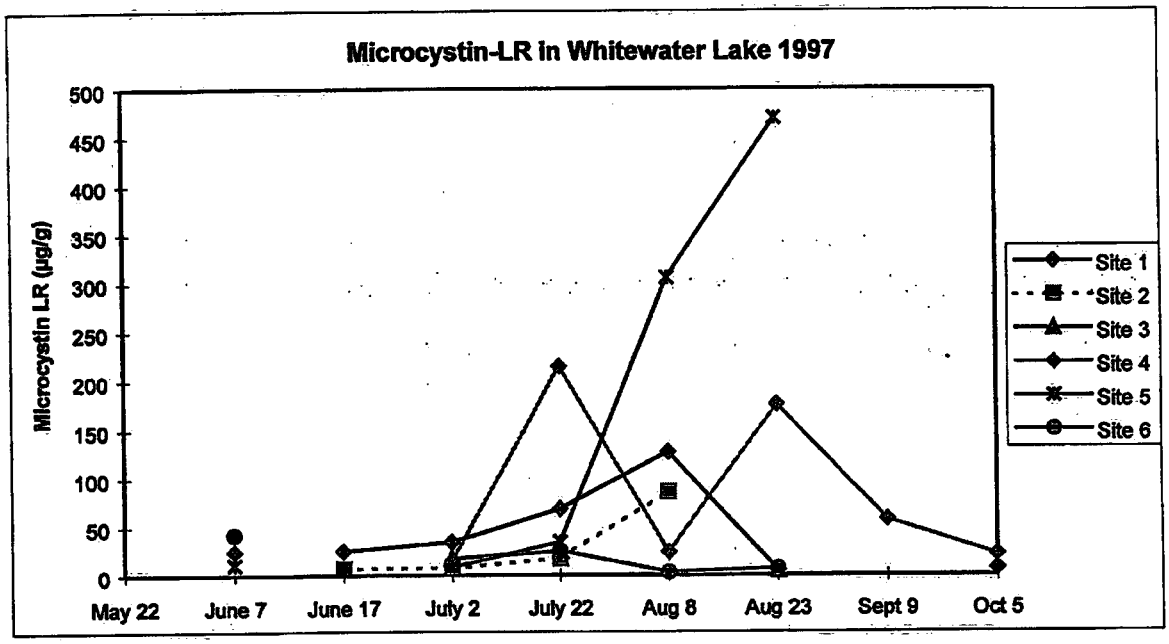

Blue-green
Algal Blooms
SITE 4
SITE 1
SITE 5
SITE 6
SITE 3
SITE 2
1km
Aphanizomenon July 22, 1997
Biomass mg/L
10 mg/L

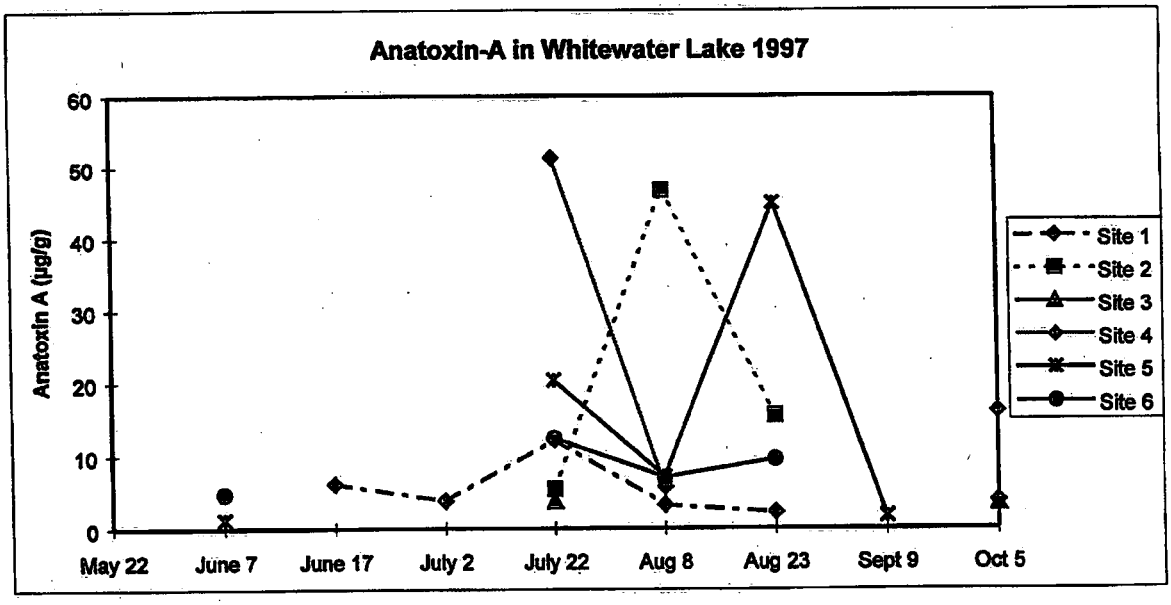

Chlorophyll a
Concentrations
SITE 4
SITE 1
SITE 5
SITE 6
SITE 3
SITE 2
1km
Chlorophyll a July 22, 1997
µg/L
20 µg/L


Calcium and Phosphorus Concentrations,
July 22, 1997
SITE 4
SITE 1
SITE 5
SITE 6
SITE 3
SITE 2
1km
50 mg/L Ca
0.5 mg/L P
WHITEWATER LAKE


Pre-Bloom Winds 1997
SITE 4
SITE 1
SITE 5
SITE 6
SITE 3
SITE 2
1km
Wind Speed


Post-Bloom Winds 1997
SITE 4
SITE 1
SITE 5
SITE 6
SITE 3
SITE 2
1km
Wind Speed
10 km/h


Microcystin-LR in Whitewater Lake 1997
Microcystin LR (µg/g)
500
450
400
350
300
250
200
150
100
50
0
May 22
June 7
June 17
July 2
July 22
Aug 8
Aug 23
Sept 9
Oct 5
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6


Anatoxin-A in Whitewater Lake 1997
Anatoxin A (µg/g)
60
50
40
30
20
10
0
May 22
June 7
June 17
July 2
July 22
Aug 8
Aug 23
Sept 9
Oct 5
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6

ENVIRONMENTAL TOXICOLOGY

Date: 9June 2000

EDITOR

Ian R. Falconer
Editorial Office
44, Mirning Crescent,
Aranda, ACT 2614
Australia
Fax 61-2-6251-7621
e-mail ifalconer@medicine.adelaide.edu.au

Dr.Murphy
National Water Research Institute

Dear Dr Murphy

We are pleased to tell you that your paper entitled "Algal Toxin- Initiators of Avian Botulism" has been accepted for publication in the Special Issue of Environmental Toxicology to be published as issue 5 of 2000. It is scheduled to be published on 20th November.
With best wishes,
Yours sincerely,

Em.Professor Ian Falconer

Environment Canada | Environnement Canada

Canada

Canada Centre for Inland Waters
P.O. Box 5050
867 Lakeshore Road
Burlington, Ontario
L7R 4A6 Canada

Centre canadien des eaux intérieures
Casé postale 5050
867, chemin Lakeshore
Burlington (Ontario)
L7R 4A6 Canada

National Hydrology Research Centre
11 Innovation Boulevard
Saskatoon, Saskatchewan
S7N 3H5 Canada

Centre national de recherche en hydrologie
11, boul. Innovation
Saskatoon (Saskatchewan)
S7N 3H5 Canada

St. Lawrence Centre
105 McGill Street
Montreal, Quebec
H2Y 2E7 Canada

Centre Saint-Laurent
105, rue McGill
Montréal (Québec)
H2Y 2E7 Canada

Place Vincent Massey
351 St. Joseph Boulevard
Gatineau, Quebec
K1A 0H3 Canada

Place Vincent-Massey
351 boul. St-Joseph
Gatineau (Québec)
K1A 0H3 Canada