

# SUMMARY OF MAJOR RESEARCH PROJECTS AT THE EXPERIMENTAL LAKES AREA DURING 2000

## *Note:*

*This summary was compiled by John Shearer, using information provided in November 2000 by research project leaders and other ELA staff. Where appropriate, the names of principal external investigators, graduate students, and their affiliations are noted. However, many aspects of major projects are being conducted by DFO Experimental Lakes Area staff, many of whom have not been specifically mentioned. The summary is intended as an overview of research activities at the ELA during 2000. In most cases, results have been excluded to facilitate peer-reviewed publication at later times. Those provided are preliminary and subject to revision. For more detailed information, the reader should contact those researchers responsible for each study, or refer to published literature.*

7 December 2000

Research activity at the ELA during 2000 focused on one major experimental study (FLUDEX), and on background studies and pilot experiments for two ecosystem-scale experiments (METAALICUS, EDC) scheduled to begin in 2001. In addition, research on lake ecosystem recovery from acidification continued, and a long-term stoichiometric study came to an end. The long-term, ecological research program (LTER) was again supported by core funding, in recognition of the inherent value of long-term data records on unperturbed systems. Several “climate change” projects were utilizing the long-term ELA data sets to answer questions pertinent to the effects of climatic change on Boreal Shield lake ecosystems and their water budgets

Total ELA site use remained similar to 1999, with activity levels in both September and October being at, or near, record levels for those months. In October, the ELA was again the site for a two-week field course on ecological monitoring presented to students from the Centre for Indigenous Ecological Resources (CIER).

The following is an attempt to summarize the status of major projects by providing some information about their purpose, design and, where possible, significant results. It should be noted, however, that data analyses are ongoing and most of the results provided here are preliminary. These projects are grouped under several broad category headings.

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## 1 BIOMANIPULATION AND SYSTEM PRODUCTIVITY

As humans have perturbed and manipulated aquatic ecosystems for various purposes, unexpected impacts have frequently occurred. Often these impacts have been manifested in major population shifts and alterations of energy flow within the food web. If we can better understand the factors which control system productivity and structure, and the food chain linkages affected by these perturbations, we will be better able to develop effective management and regulatory strategies for minimizing the adverse effects on aquatic ecosystems of many human perturbations. The following projects are intended to improve our knowledge of these linkages.

### 1.1 Biomanipulation and Fertilization of Lake 227, and Biomanipulation of Lake 110

Lake 227 was fertilized with phosphorus for the 32th consecutive year in 2000. The original experiment was initiated in 1969 to demonstrate that atmospheric carbon dioxide could provide the carbon necessary for algal blooms in eutrophic lakes. Prior to 1990, all additions included various combinations of nitrogen and phosphorus. The ratio of phosphorus to nitrogen was changed during these previous stages of the experiment to test whether this would influence the dominant algal groups. Since 1990, only phosphorus has been added. During 2000, phosphorus, as phosphoric acid, was again added to Lake 227 surface waters for twenty consecutive weeks (2.5 litres per week) during the ice-free season. The acid was diluted with lake water in a plastic barrel and dribbled via *Tygon* tubing into the near-shore water. The required acid was carried to the lake weekly. Sodium bicarbonate, to be used as a neutralizing agent in case of an acid spill, is stored on site.

In May of 1993, 54 male northern pike (*Esox lucius*), a piscivorous fish, were added to Lake 227. These fish were transferred from Lakes 222 and 663 at spawning time, when individuals could easily be sexed. An identical transfer of pike to Lake 110 was also completed in the spring of 1993. In May, 1994, another 130 pike were added to Lake 227 and 126 pike were added to Lake 110. These transfers of pike were undertaken after obtaining approval from the Ontario Ministry of Natural Resources. Major ions, nutrient chemistry, nitrogen fixation, primary production, zooplankton, benthos, and fish populations were examined in both lakes and in reference Lake 240 throughout the study. Changes in background levels of mercury and organochlorines (PCB's) in fishes and other biota were also monitored during the experiment.

In the fall of 1999, as in each year since 1995, Lake 227 was fished to ensure removal of all adult pike. No fish have been caught for several years and we assume that all pike have been removed from the lake. There has been no evidence of pike reproduction.

No fish studies were carried out in Lake 110 during 1999. We believe that some adult pike remain in the lake, but no evidence of pike reproduction has been observed.

### 1.2 Stoichiometry and Food Web Dynamics

For the past number of years, a joint research undertaking (The Stoichiometry Project), involving DFO and researchers from Arizona State University (ASU)(Dr. James Elser), the University of Texas at Arlington (Dr. Thomas Chrzanowski, Dr. James Grover), Kyoto University (Dr. Jotaro Urabe) and the University of Minnesota (Dr. Robert Sterner), has investigated how changes in zooplankton community structure alter the relative availability of N and P supporting phytoplankton and bacterial production.

Originally, this research centred on changes in the stoichiometry of N and P in two lakes, artificially-eutrophic Lake 227 and oligotrophic Lake 110, following introduction of piscivores [see 1.1., above]. Recent studies have also included mesocosm experiments in Lake 239.

In 2000, another series of research projects at the ELA provided follow-up to the earlier studies and concluded the project. These studies, largely funded by the United States National Science Foundation (NSF), were headed by Dr. Chrzanowski and Dr. Grover.

### **2.3.2 *Microbial Indicators of Biological Integrity and Nutrient Stress***

**Principle investigators:** J.P. Grover and T.H. Chrzanowski (Univ. of Texas – Arlington)

**Project Dates:** 1 September 97 – 31 August 00

**Goals of the Project:** The researchers are examining several chemical and biological variables that may provide not only a broadly applicable approaches to understanding the biological consequences of nutrient loading in aquatic systems, but may also provide a means of predicting the resulting community structure. The indicators examined are seston C:N:P ratio, species level responses of algae to nutrient bioassays, community level responses of bacteria to nutrient bioassays community structure of algae, community structure of bacteria, and the estimated ratio of algal to bacterial specific growth rates.

**Activity during 2000:** Technical staff (Kory Pennebaker and Brenda Smith) were on site from the middle of May through end of August. The principle investigators were on site part of this time. During the period from 15 May to 28 August lakes 227 and 239 were each sampled weekly. Data on physical limnology (light, temperature, mixing depth, attenuation coefficients, and dissolved oxygen profiles) were collected for each sampling interval. Epilimnetic water samples were collected synoptically with data on physical limnology. Water samples will be analyzed to determine dissolved nutrient loads (C, N and P species). Particulate fractions of the water were analyzed to establish seston C:N:P ratios.

To test the idea that bacterial substrate utilization is related to lake trophic status, the researchers performed a wider spatial survey during this field season. They selected twelve lakes of differing size, morphometry, trophic status, and experimental history to be surveyed five times over the growing season. In practice, each survey period covered fewer lakes due to logistical problems. For each of these lakes, algal and microbial abundance, nutrient concentrations, seston composition, and Biolog assays of substrate utilization were measured in triplicate. For a subset of eight lakes, dilution bioassay experiments were conducted to determine  $\mu_0$  and  $\mu_{\max}$  for algae and bacteria, but factorial nutrient additions to further decompose nutrient effects were omitted.

Data for this field season have not yet been analysed.

No new experimental studies or manipulations are currently planned.

### **2.3.3 *Benthic Stoichiometry Stable Isotope Study***

**Purpose of research:**

This study examines the extent of elemental differences between benthic consumers and their food sources in boreal lakes across a trophic gradient.

**Participating agencies and/or principal investigators:**

Paul Frost, Arizona State University, M.A. Turner (FWI), J.J. Elser (ASU), Suzanne Tank (Univ. of Alberta)

**Year 2000 activities:**

During the summer of 2000, Paul Frost collected benthic invertebrates and their food sources from eight lakes that encompassed a wide trophic gradient across western Canada. Littoral zones of ELA lakes (L239 and L373) were sampled in late-July for insect larvae and other invertebrates, periphyton, macrophytes, phytoplankton, and zooplankton. Additional lakes were sampled on the boreal plain near Edmonton, AB and in the Rocky Mountains at Jasper National Park. All samples were dried and/or preserved for elemental analysis and identification.

**Future research plans:**

Current research plans are focused on finishing analysis of samples obtained during the summer of 2000. No other research activities are currently planned for this project.

**1.2. Biomanipulation of Lake 221**

Lake 221 is the site of a biomanipulation experiment that started in 1987 when 123 northern pike were transferred to this lake from nearby Lake 222. Water chemistry, primary productivity, phytoplankton populations, zooplankton, zoobenthos, and fish populations were studied in Lake 221 before and after pike addition. The Recovery phase of the experiment started in 1994, when approximately 80% of the northern pike were removed from this lake. Netting continued in 1995, 1996 and 1997. The presence of several younger fish in the 1997 catch indicated that the pike are reproducing in this lake. Water chemistry, phytoplankton, and zooplankton populations were monitored again in 1999, and some fishing was done in the fall.

The Lake 221 ecosystem underwent significant changes during the initial 5 years following pike introduction. Yellow perch populations, pearl dace populations, total zooplankton biomass, and phytoplankton biomass were significantly reduced. Two populations (Chaoborus and bacteria) benefited from the introduction of pike. Overall, Lake 221 shifted from having a plankton-supported food web to having one supported by bacteria.

Limited monitoring of the lake has continued through 2000 to address the key question whether such a lake ecosystem can reach a new equilibrium and, more importantly for management, to verify whether this new equilibrium can be sustained. Lake 221 results, to date, suggest that this is possible. In Lake 221, the zooplankton, phytoplankton, and bacteria have been stable since 1994, and there has been no significant change in major nutrients. Some sampling of fish, zooplankton and phytoplankton was carried out again in 2000, but there is nothing new to report.

**1.3. Experimental Cropping of Lakes**

The lake whitefish populations of Lakes 258 and 305 were experimentally fished in 1981 and 1982 to simulate a pulse commercial fishery. Recovery of these populations has been monitored by over-night sets of gill nets one to two nights per year periodically since the initial cropping. Reference Lakes 259 and 468 are also monitored using similar netting techniques. In 2000, only Lakes 259 and 468 were

fished. Data from these reference lakes are used to interpret lake whitefish data from other ELA experiments.

#### **1.4. Effects of Macrophyte Removal on Pike Populations**

Lake 191 at the Experimental Lakes Area has been the site of a macrophyte removal experiment to determine the change in northern pike production when 50% of the macrophytes present in the littoral zone were removed. Changes in water chemistry and all trophic levels in the lake have been monitored during the study.

After two years of background study, macrophyte harvesting began in 1996. By August 1996, a mechanical harvester removed fifty percent of the macrophytes present in the lake, and the cut areas were maintained in 1997 and 1998. Macrophyte harvesting was terminated after 1998 and the recovery phase of the experiment was initiated. We have now completed 2 years, 1999 and 2000, of recovery monitoring.

Few changes have occurred during the two years of recovery. Recovery of macrophytes in areas cut from 1996 – 1998 has been minimal, although some re-colonization occurred in 2000. Although it has been two years since macrophytes were harvested, there has been less than 15% recovery of plant coverage and biomass in cut areas.

A master's thesis in Natural Resource Management was completed by Rob Rabasco, based on studies of trophic effects on the fish populations of Lake 191:

Rabasco, R.M. 2000. Trophic effects of macrophyte removal on fish populations in a boreal lake. *M.N.R.M thesis*. Univ. of Manitoba, Winnipeg, viii + 113 p.

## **2 PHYSICAL PERTURBATIONS**

This section includes experimental studies in which some physical aspect of the ecosystem has been manipulated.

In most cases, this has involved experimental alteration of the water level, as a simulation of what typically occurs during the creation and operation of reservoirs. In Canada, these reservoirs are generally created primarily for generation of hydroelectricity. Many cause flooding over large areas of northern wetland and forest land. The water levels in these reservoirs tend to be drawn down during the winter periods when electrical demand is high and water flows are low.

Another study, carried out in mesocosms, is investigating the effects of warmer water on the community inhabiting nearshore rock surfaces.

### **2.1. Experimental Lakes Area Reservoir Project (ELARP)**

The Experimental Lakes Area Reservoir Project (ELARP) is a whole-ecosystem flooding experiment designed to examine the production and mobilization of methylmercury (MeHg) in response to flooding,

and to determine if reservoirs are significant sources of the GHG's carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) to the atmosphere.

In June, 1993, following two years of background studies, the outflow of a ELA Lake 979 and its surrounding wetland was dammed, and the water level raised 1.4 meters to flood 14 hectares of peatland. Direct byproducts of the decomposition of the flooded vegetation in the peatland are CO<sub>2</sub> and CH<sub>4</sub>. Mobilization of MeHg within the flooded ecosystem and release to the atmosphere of CO<sub>2</sub> and CH<sub>4</sub> in response to the flooding were monitored intensively. A non-flooded wetland system (ELA Lake 632), was monitored as a reference. Following winter drawdown, flooding of Wetland 979 was repeated in summer and fall of 1994 and 1995, as detailed studies continued in both wetland systems. In all three years, dramatic increases in MeHg and in release of the GHG were observed in response to flooding.

During the open-water periods of 1996 through 1998, the 979 wetland was experimentally flooded, but the system was studied less intensively. GHG emissions and MeHg mass-balance budgets were monitored.

In 1999 and again in 2000, the system was flooded, but no ecosystem monitoring was conducted. The next program of widespread monitoring on the system is scheduled for the year 2001.

#### **2.3.4 "Bioreporter" Project**

In a project related to ELARP, the use of a genetically engineered "bioreporter" to measure the bioavailability of inorganic mercury in lake water is being tested. Inorganic mercury is turned into MeHg, which is more toxic, by bacteria that carry out methylation. The inorganic mercury must enter the bacterial cells to become methylated, but a lot of mercury in aquatic systems is bound to particles and DOC, and so is not bioavailable for methylation. The bioreporter system, which is a strain of bacteria that produces light when mercury enters its cells, allows scientists to quantify bioavailability. The ELA part of this work has been done by Karen Scott, who is a Ph.D candidate in Microbiology at the University of Manitoba. During 2000, she has continued to measure bioavailability of mercury through testing and refining the use of the Bioreporter system in natural waters at the ELA.

### **2.1. Lake 226 Drawdown Study**

The purpose of this Lake 226 experiment was to study the impacts of winter, water level drawdown, simulating the water level fluctuations in a hydroelectric reservoir. The lake was studied for one year prior to drawdown (1994), for three years after drawdown (1995, 1996, and 1997), and for two years after recovery from drawdown (1998 and 1999). In the winter of 1994-95, the water level was reduced by 2 m below natural levels. Approximately 30% of the lake volume was removed, resulting in a decrease of about 11% in lake surface area. The lake rose during the spring and early summer by about 0.5 m, but, as runoff into the lake was approximately balanced by evaporation, the level stayed relatively constant during the summer and fall at about 1.5 m below natural levels. In the winters of 1995-96 and 1996-97, the water level was reduced by 3 m below natural levels. The lake rose during the spring and summer of 1996 and 1997 due to snow melt and summer rains. Lake elevation at freeze-up in 1996 and 1997 was approximately 0.8 m below natural levels. The lake was not drawn down in the winter of 1997-98 and natural lake elevations were achieved early in the summer of 1998.

The water level of Lake 226 was allowed to fluctuate at natural levels throughout 1999 and 2000. With no funds available to continue this study, only limited monitoring of fish populations was carried out. Dr.

Ken Mills reports that the abundance of lake whitefish in Lake 226 appears to be increasing rapidly under the natural water level conditions.

**2.2. Flooded Upland Dynamics Experiment (FLUDEX)**

The purpose of the Upland Flooding Experiment (FLUDEX; Flooded Upland Dynamics Experiment) is to study the greenhouse gas and mercury impacts of flooding forested upland areas. Three forested uplands, a moist forest and two dry forested areas, located in the watershed of Roddy Lake were flooded in the summer of 1999 and again in summer 2000 to create experimental hydroelectric reservoirs. The three areas were flooded by pumping water from a nearby lake low in mercury and dissolved organic carbon. Flushing rates were equalized among the reservoirs by maintaining pumping rates proportional to estimated reservoir volumes. Greenhouse gases fluxes before and after flooding were measured at all three sites. Carbon dioxide, methane and nitrous oxide were monitored. Fluxes have been compared to the previously flooded boreal wetland (ELARP project) and to existing hydroelectric reservoirs to determine the potential greenhouse gas contribution of global freshwater reservoirs. The production of methyl mercury from flooded soils and the bioaccumulation of methyl mercury through the food chain are being measured in the experimental reservoirs. Mitigation strategies that will have direct planning application will be developed.

**Study schedule and plan:**

1998-99: dam construction, background data collection including site characterization of vegetation, mercury inventories in soils and vegetation, carbon inventories, and set up of pump and piping water supply system hydrological network completed

1999-00: year 1 of flooding, measurement of greenhouse gas (GHG) emissions and mercury dynamics

2000-01: year 2 of flooding, study of GHG emissions and mercury dynamics

2001-02: year 3 of flooding, study of GHG emissions and mercury dynamics; beginning of studies on mercury mitigation

The three areas flooded are: a moist forest (Site 1), a dry forest (Site 2), and a very dry forest with areas of exposed bedrock (Site 3). The amount of organic carbon stored on the sites before flooding was found to be highest in Site 1, intermediate in Site 2, and lowest in Site 3. The approximate sizes of each of these impoundments are as follows:

Site	Area (ha)	Mean Depth (m)	Volume (10 <sup>4</sup> m <sup>3</sup> )	Dike Length (m)
1	0.69	1.0	0.69	190
2	0.50	0.8	0.40	130
3	0.66	1.2	0.79	350

Reservoirs were filled by pumping water from Roddy Lake. Water from all sites drained back to Roddy Lake. Water was pumped using a diesel unit.

Water renewal times of approximately 10 days were maintained during the open water season. Dikes were constructed of gravel, sealed to bedrock with plastic (dikes 1 m in height or less) and with wood construction sealed to bedrock with cement and plastic (dikes greater than 1 m in height). Maximum dike height and reservoir depths are approximately 2 m.

The following groups and individuals are involved in the experiment:

Project coordination: Drew Bodaly, Freshwater Institute

Hydrology and project design: Ken Beaty and Mark Lyng (Freshwater Institute)

Mercury dynamics: Kristofer Rolfhus, James Hurley and David Krabbenhoft (University of Wisconsin and USGS)  
 Britt Hall and Vincent St.Louis (University of Alberta)  
 Katharine Peech and Michael Paterson (University of Manitoba and Freshwater Institute)  
 Drew Bodaly (Freshwater Institute)  
 David Findlay (Freshwater Institute)

Greenhouse gases and carbon decomposition:

Cory Matthews and Vincent St.Louis (University of Alberta)  
 Jason Venkiteswaran and Sherry Schiff (University of Waterloo)  
 Len Hendzel (Freshwater Institute)

Conference presentations resulting from the project:

Bodaly, R.A., K.G. Beaty, R.J.P. Fudge and D. Huebert. 1999. Introduction to the upland flooding experiment. *Air and Waste Management Association Conference on Mercury in the Environment*, Minneapolis.

Hall, B.D., V.L. St.Louis, and R.A. Bodaly. 1999. Impact of reservoir creation on the biogeochemical cycling of methylmercury in boreal forest uplands. *Air and Waste Management Association Conference on Mercury in the Environment*, Minneapolis.

Rolfhus, K.R., J. Hurley, and D.P. Krabbenhoft. 1999. The burden and mobilization of total and methyl mercury from upland soils at the Experimental Lakes Area, Ontario, Canada. *Air and Waste Management Association Conference on Mercury in the Environment*, Minneapolis.

Hall, B.D., V.L. St.Louis and R.A. Bodaly. 1999. Production of methylmercury in three experimental boreal upland reservoirs. *Canadian Society of Limnologists*, Annual Meeting, Edmonton.

Joyce, E. and V.L. St.Louis. Concentrations of carbon dioxide and methane in three experimental boreal upland reservoirs. *Canadian Society of Limnologists*, Annual Meeting, Edmonton.

Hall, B.D., V.L. St.Louis, R.A. Bodaly and K.G. Beaty. 2000. Methylmercury production in flooded forest uplands. *Society of Environmental Toxicology and Chemistry*, Nashville.

Rolfhus, K.R., J.P. Hurley, and K.P. Krabbenhoft. 2000. The dynamics of mercury species fluxes from inundated upland boreal forest soils. *Society of Environmental Toxicology and Chemistry*, Nashville.

Bodaly,R.A., R.J.P. Fudge, M.J. Paterson, K.A. Peech, and L. Wesson. 2000. Methyl mercury bioaccumulation in the food chains of three experimental reservoirs that flooded boreal forest uplands. *Society of Environmental Toxicology and Chemistry*, Nashville.

Thesis completed:

Boudreau, Natalie M. 2000. Soil carbon, carbon dioxide, and methane in three experimentally flooded upland boreal forest reservoirs: a  $\delta^{13}\text{C}$  inventory of sources and processes. *M.Sc. thesis*, University of Waterloo, Department of Earth Sciences, 180 p.

### 2.3.5 2.3.1 *Mercury Methylation Rates and Methyl Mercury Concentrations in Fish*

Principal Investigator: Britt Hall, University of Alberta (Ph.D. candidate)

#### **Purpose or Goal:**

Ms. Hall's specific research objective is to determine, using a whole ecosystem, mass balance approach, if Hg methylation rates increase, and therefore lead to increased MeHg concentrations in fish. Inputs (precipitation, throughfall, and inputs from the source lake) and storage (vegetation and soil) of MeHg and inorganic Hg are measured and subtracted from the amount of MeHg and inorganic Hg leaving the system (via surface run off, groundwater run off, outflows from reservoirs, and photodegradation).

#### **Preliminary Results:**

In 1998 and 1999, data were collected to quantify the MeHg and inorganic Hg inputs to and outputs from the three sites. We measured MeHg and total Hg (THg) concentrations in precipitation (collected at the ELA Met site), throughfall (collected in each of the three sites and at the ELA Met site), and run off. Litterfall samplers were also set to determine an annual flux of MeHg and THg from the litter.

The summer of 2001 is the last scheduled FLUDEX flooded field season. Ms. Hall plans to continue mass balance budget calculations. However, she is planning on spending the majority of her efforts next season assessing the methylation of Hg in periphyton.

### 2.3.6 *Impact of Reservoir Creation on Greenhouse Gas Fluxes from Forested Uplands.*

Cory Matthews, M.Sc. candidate, University of Alberta  
Dr. Vince St. Louis, Supervisor

#### **Research goal:**

Mr. Mathews is carrying out research on the flooded uplands dynamics experiment (FLUDEX) at the ELA to study the levels of  $\text{CO}_2$  and  $\text{CH}_4$  fluxing from reservoirs created in three forested upland catchment sites that vary in carbon stores. He will examine the biogeochemical processes controlling rates of decomposition of flooded organic matter and greenhouse gas (GHG) production in the three sites and determine if the amount and type of flooded organic carbon is related to the levels of GHG fluxing from the reservoir. Results of this research will be incorporated into mathematical models currently being developed to help hydroelectric utilities and government agencies make informed decisions about the sites for future reservoirs, estimate rates of GHG production in these and existing reservoirs, and include reservoirs in domestic and international GHG inventories.

#### **Principle investigations:**

The research in the summer of 2000 focused on tracking the movement of carbon within the reservoirs, especially focusing on the amount of carbon being released at the water surface and sediment. To determine the amount of carbon fluxing from the water to the atmosphere (as  $\text{CO}_2$  and  $\text{CH}_4$ ), a gas tracer technique was used. Briefly, sulfur hexafluoride ( $\text{SF}_6$ ), a chemically and biologically inert gas in aqueous

environments, was injected into the reservoirs. Over time, it gases off to the atmosphere; the decline in concentration was monitored to determine a gas transfer value ( $k$ ). Once  $k$  for the gas tracer is known, that of  $\text{CO}_2$  and  $\text{CH}_4$  can be determined. The  $k$  value, along with the surface concentrations of  $\text{CO}_2$  and  $\text{CH}_4$ , are used to determine gas flux from the water surface. This data are currently being worked up.

To determine fluxes at the sediment water interface, submerged chambers were placed on the sediment. The chambers were allowed to sit for about four to five hours, during which time water samples were collected (initial, intermediate, and final). The samples were then analyzed for dissolved inorganic carbon (DIC) and methane concentration to determine increases or decreases over time. Gas concentrations are presently being used to determine production/consumption rates. These data, along with the surface flux data, will be used to determine factors affecting rates of decomposition and to determine if gas fluxes from the surface reflect gas production in the flooded soils and ground vegetation.

Research was also carried out on the amount of gas leaving the reservoir in the form of bubbles arising from the sediments to the water surface. Dissolved gas leaving the reservoir via the outflow was also monitored.

Findings from summer 2000 will be presented at the Societas Internationalis Limnologiae (SIL) conference in February, 2001, in Melbourne, Australia at a session titled "Reservoirs and Greenhouse Gases".

#### **Research, 2001:**

Plans are to sample for another field season, lasting from May to October, 2001. Generally, the same procedures will be performed to monitor GHG production in the reservoirs during the third season of flooding.

#### ***2.3.7 Pre- and Post-flood Nitrous Oxide Gas Fluxes from Forested Upland Soils***

Principal Investigator: Len Hendzel (Freshwater Institute)

A study was initiated during the summer of 1998 to look at the pre-flood fluxes of nitrous oxide from within three upland sites that were primarily distinguishable from each other by soil moisture content and soil nutrient composition, two factors which interact to control  $\text{N}_2\text{O}$  fluxes in soils. It was hypothesized that these factors would control the emission of  $\text{N}_2\text{O}$  from the upland soils. During the later part of 1999, profile water samples collected earlier from the three reservoirs were analyzed for  $\text{N}_2\text{O}$  concentrations. In 2000, during the second year of flooding of the three upland sites, it was hypothesized that nitrification and denitrification within most of the flooded basins would be limited by severe anoxia and low pH, and any  $\text{N}_2\text{O}$  emissions should only occur along the shallow edges where expected oxygen concentrations will be sufficient to support nitrification and denitrification. Total anoxia and/or a  $\text{pH} < 5.5$  will inhibit both microbial processes.

With the help of graduate students Cory Matthews and Jason Venkiteswaran, over 500  $\text{N}_2\text{O}$  samples were collected from the three reservoirs. Each reservoir was extensively sampled for surface  $\text{N}_2\text{O}$  fluxes on two occasions during May prior to flooding, followed by bi-weekly sampling of the flooded reservoirs between June and September. Sampling of the flooded reservoirs included extensive spatial and temporal sampling for surface and profile  $\text{N}_2\text{O}$  concentrations in addition to measurement of sediment  $\text{N}_2\text{O}$  fluxes and the collection of  $\text{N}_2\text{O}$  emissions in the form of bubbles. Greater than 90% of all the  $\text{N}_2\text{O}$  samples have been analyzed to date. Tabulation of this data will be initiated in the near future. As well, synoptic

measurements of phytoplankton nutrient status were undertaken on two occasions during the summer on surface water samples from reservoirs 1,2 and 3.

Analysis of this year's data is still incomplete. This study is planned to continue in 2001.

Preliminary results were presented at the 1998-1999 data at the FLUDEX Workshop in Jasper, AB, April 7-9, 2000. Speakers' notes and figures have been published in a workshop report.

### ***2.3.8 Investigating Carbon Sources for Greenhouse Gas Flux using Stable Carbon Isotopes***

#### **Principal Investigators:**

Dr. Sherry Schiff

Jason Venkiteswaran (M.Sc. Student)

Dr. Ramon Aravena

Department of Earth Sciences, University of Waterloo, Waterloo, Ontario

#### **Objectives:**

Identify sources and sinks of greenhouse gases carbon dioxide and methane using stable carbon isotopes  $^{12}\text{C}$  and  $^{13}\text{C}$ . Utilise the previous carbon inventory (Boudreau 2000, MSc thesis) to identify processes affecting greenhouse gases (GHGs) using stable carbon isotopes.

#### **Summary of work carried out during 2000:**

##### Post-flood carbon inventory work:

Post-flood field work involved identification and collection of possible carbon sources for greenhouse gas production. The researchers conducted soil coring and sectioning, collected vegetation samples, and identified remaining surface vegetation in each reservoir. Post-flood lab work will involve running soil and vegetation samples on an elemental analyzer to determine the percent carbon and carbon to nitrogen ratio remaining in each soil horizon and vegetation type. From this data they can calculate the actual amount of carbon contributed by specific soil horizons and construct spatial maps of soil organic carbon in comparison to the samples collected prior to flooding, in 1999.

##### Post-flood GHG source/process work:

In summer 2000 they collected reservoir water samples for dissolved inorganic carbon (DIC) and methane ( $\text{CH}_4$ ) isotopic analysis. Isotope work on DIC will identify the vegetation type or soil horizon source of the gases and improve flux calculations. Isotope work on  $\text{CH}_4$  will allow them to discriminate between  $\text{CH}_4$  production from microbial sources and consumption by oxidation.

The following samples were taken from each reservoir on an intensive schedule throughout the flooded season: inflows, outflows, seeps, samples at the sediment-water interface from clear and shaded submerged chambers, samples throughout the water column from sippers, and bubbles from bubble traps. Methane and DIC samples were taken during each sampling event. Sampling was coordinated with Cory Matthews (Univ. of Alberta) so that isotope and concentration data could be used in conjunction to improve flux calculations.

Additionally, microcosms to identify the fractionation factor associated with microbial CH<sub>4</sub> oxidation were undertaken. When methane is oxidised, <sup>12</sup>CH<sub>4</sub> is preferentially oxidised over <sup>13</sup>CH<sub>4</sub>. The fractionation factor is the measure of this preference. This information will allow us to determine the importance of CH<sub>4</sub> oxidation in the overall carbon cycle in each reservoir.

### **Findings:**

#### Post-flood C isotope work:

Water samples are currently being run for DIC and CH<sub>4</sub> isotopic signature. Soil and vegetation signatures are known from pre-flood work (Boudreau 2000, MSc thesis). Inflow waters have very little CH<sub>4</sub> and a DIC isotope signature of ranging from -12 to -5 permil. The measured DIC signature of outflow waters will be a combination of the inflow and the source signature. The measured CH<sub>4</sub> signature of water column samples will indicate whether CH<sub>4</sub> is being produced (depleted signature) or consumed (enriched signature). Isotopic analysis of reservoir waters will continue into the winter and results will be available in the next few months.

### **Continuation of Study/ Spin-off Research**

Samples will be taken for isotopic analysis in other post-flood years. This information will tell the researchers if the source of the GHGs is changing over time and how processes affecting the gases are changing over time.

Spin-off research includes an undergraduate thesis by Melissa Baril, currently underway. Melissa will incubate a variety of plant and sediment material to determine the isotopic signature and fractionation associated with the DIC produced from decomposition. This information will help in the interpretation of sources since DIC produced from decomposition does not have an identical isotopic signature to the source carbon.

## **2.4 Temperature Influences on the Epilithic Community**

Helen Baulch (University of Alberta)  
Supervisor: D.W. Schindler

Despite a long history of human alteration of natural thermal conditions and concern over global climate change, few *in situ* studies have addressed the effects of temperature on the littoral subecosystem, and very few have addressed this issue using controlled studies.

Helen Baulch is the principal investigator, and both D. W. Schindler (U. of Alberta) and M. A. Turner (DFO) have participated in all components of the study. D. L. Findlay (DFO), R. D. Vinebrooke (U. of Regina), and M. Paterson (DFO) collaborated in the community composition study, and D.L Findlay and R.D. Vinebrooke contributed to the biomass portion of the study.

Funding for this research was derived from NSERC (scholarship to HB and grant to DWS), NRCAN (Climate Change Action Fund grant to MAT), and DFO (support of the ELA).

In August – September 2000, eight littoral mesocosms in L239 were manipulated to assess the effects of experimental warming on the epilithon. Experimental enclosures were warmed to 4.5°C above control enclosures using a heat exchange system. Four major warming responses were monitored:

#### Community metabolism

It has been suggested that algal photosynthetic rates may or may not be temperature dependent under different light and nutrient conditions. However, optimum temperatures for photosynthesis vary among species, suggesting that photosynthetic rates in natural communities may vary if temperature change induces a taxonomic shift in the community. The temperature dependence of respiration rates is well documented. Rates of net photosynthesis and dark respiration were measured in this study.

#### Epilithic biomass

Increased temperature has been associated with increased biomass in stream, lake and pond periphyton. However, effects may depend on the magnitude of the change in temperature, the long-term mean temperature of the environment or the length of colonization of the epilithon. Changes in biomass were monitored during the eight-week experiment on pre-colonized and newly colonized substrates using HPLC-based measurements of total areal chlorophyll and carotenoids, and estimates of algal biovolume.

#### Community composition

The shift of algal communities from diatoms at low temperatures to flagellates, and then to greens and blue greens as temperature increases is well documented in studies of thermal pollution, and in artificial stream studies. Warming-induced changes in the epilithon will be assessed by direct algal counts and HPLC pigment analyses. Effects on algal richness and diversity, bacterial density and the benthic invertebrate community will also be assessed.

#### Benthic stoichiometry

Temperature may affect nutrient uptake rates and the minimum cellular nutrient content required for algal growth. It may also affect the nutrient ratios of algal tissues. Research addressing the effect of temperature on nutrient ratios and cellular nutrient concentration has focussed on cultured marine algae. To date no studies have addressed the effects of temperature on nutrient content of epilithon. Areal concentrations of carbon, nitrogen and phosphorus were measured during the experiment, and will be used to assess the effects of the warming treatment on ratios of carbon: nitrogen, and carbon: phosphorus.

#### ***Future plans:***

In addition to continuing sample and data analysis from the above studies, a long-term data-analysis will be performed using epilithic data collected by M. A. Turner. Community variables will be correlated with air and water temperature data to determine whether past temperature variability is associated with changes in epilithic metabolism, biomass, taxonomy or stoichiometry.

### **3 NATURAL VARIABILITY AND CLIMATIC FACTORS**

In order to objectively assess the effects of anthropogenic perturbations on aquatic ecosystems, it is essential to systematically monitor non-perturbed systems over long time periods. Only thus can we hope to evaluate the effects of naturally-occurring events (weather, cyclic climatic oscillations) on these ecosystems and factor these effects into our interpretations of impacts resulting from human activities. Of course, natural perturbations also can have significant effects on processes within these small lake ecosystems.

Over more than three decades, researchers at the E.L.A. have been collecting data on natural lake ecosystems in support of, and as references for, the experimental studies. Increasingly, these data sets are becoming invaluable in their own right because of the unusual scope and length of the records.

### 3.1 Long-Term Ecological Research and Data Management

#### Principal Investigators:

K. Beaty, P. Blanchfield, D. Findlay, L. Hendzel, R. Hesslein, S. Kasian, M. Lyng, K. Mills, M. Paterson, C. Podemski, J. Shearer, M. Stainton, M. Turner

#### Project Summary and Goals:

In 1998 the Long-Term Ecological Research (LTER) project was established to co-ordinate the hydrological, chemical, and biological monitoring of long-term reference lakes at the ELA. Responsibilities for collection of meteorological data and management of the ELA multidisciplinary database were added to the project in 1999.

There are three objectives for the project:

1. To provide an envelope of expected natural variability against which experimental results can be assessed.
2. To provide a long-term record for the detection of change due to the effects of region-wide perturbances resulting from global stressors (e.g. climate change, atmospheric contaminant loading and stratospheric ozone depletion), for the assessment of variance and for the interpretation of ecological relationships.
3. To provide a secure and accessible database of ecological data collected at the ELA, which serves the information needs of ELA researchers.

The project will monitor two groups of lakes. The first is a core set of reference lakes that will be protected against experimental manipulation for the long-term. The core lakes will have good historical records, be typical of the ELA, and be monitored by as many disciplines as possible for full ecosystem coverage. The second set of lakes contributes to discipline-specific reference data sets, answering particular questions or expanding the range of type of lake or biological community.

**2000:** Five core lakes (114, 224, 239, 373, and 442) received, where possible, full discipline coverage. Data collected included hydrology, secchi depth and water temperature, water chemistry, primary production, periphyton, phytoplankton, zooplankton, and fish. Discipline specific monitoring continued in other non-manipulated lakes.

Benthic invertebrate and minnow studies were added to the LTER discipline coverage. Preliminary studies were conducted to determine appropriate methods for long-term monitoring. Rock baskets were placed in L442 and colonising invertebrates are being identified. A mark-recapture study of minnows in Lakes 442 and 115 was done in the fall 1999 and spring 2000.

The year 2000 was unusual in many hydro-meteorological respects. It was the earliest ice-out and longest ice-free season in 32 years. June was the wettest and August the third wettest month on record, resulting in the wettest open water season on record. Preliminary results indicate that lakes are flushing faster than in any other 10 consecutive year period.

The following publications and presentations have resulted from the project this year.

**Publications:**

D.L. Findlay, S.E.M. Kasian, M.P. Stainton, K. Beaty, M. Lyng. 2000. Climatic influences on algal populations of boreal forest lakes in the Experimental Lakes Area. (Submitted to *Limnology and Oceanography*)

**Conference Presentations:**

Findlay D.L., S.E.M. Kasian. 2001. Response of dinoflagellates to acidification and climate induced DOC decrease. (American Society of Limnology and Oceanography)

Kasian, S.E.M., M.P. Stainton, R.H. Hesslein. 2001. Three decades of variation in chemistry of 17 pristine boreal shield lakes: implications for detecting climate change. (American Society of Limnology and Oceanography)

Mills, K. 2000. Annual variability of abundance, annual survival, and recruitment of lake trout at the ELA. (Char Conference, Midwest Fish and Wildlife Conference)

**Theses:**

Simmons, J.R. (in progress). Runoff response to land cover and climatic changes in the Lake of the Woods Watershed. University of Manitoba. Funded by Manitoba Hydro.

The following *Canadian Climate Action Fund* proposals, which rely on LTER data, were funded in 2000:

Hesslein, R.H. Changes in water quantity and quality as a result of climate change.

Hesslein, R.H. The impact of climate on the thermal structure of boreal forest lakes and its potential impact on important fish communities.

Turner, M.A., R.H. Hesslein, S.E.M. Kasian, M.P. Stainton. Testing the reversibility of climate change impacts on in-lake metabolism of DOC and its aftermath for boreal forest lakes.

**3.3.2 Meteorological Monitoring**

The ELA is the site of long-term monitoring of meteorological variables via a meteorological station (met site) that uses equipment provided by the Meteorological Service of Canada (MSC) of Environment Canada and is operated by ELA staff. Ken Beaty, with assistance from Mark Lyng, Neil Fisher, and others, has primary responsibility for this facility and data are contributed to the AES national climate database. Data on air temperature, precipitation (rain and snow), wind speed and direction, bright sunshine, and evaporation are collected on a daily basis at the site. In June of 1999, this site reached the milestone of 30 years of continuous monitoring record, making it an official long-term station in the AES Climate network. In recognition of this achievement, the ELA was presented with the Morley Thomas Award by MSC officials in May, 2000.

**3.3.3 Impact of Natural Disturbances on the Lake 239 Watershed**

Long-term hydrological, meteorological, and chemical monitoring in the calibrated catchments of this watershed continued during 2000. Portions of the watershed were perturbed by a major forest blow-down in 1973, and by forest wildfires in 1974 and 1980. This and other ELA watersheds have been subjected to extremes of precipitation over the monitoring period. The monitoring is intended to evaluate long-term effects of these and other natural perturbations on the lake ecosystems, and to calibrate other hydrological studies at the ELA. The watershed has been continuously monitored for 32 years. No chemical additions are made.

### **3.3.4 Canadian Air and Precipitation Monitoring Network (CAPMoN)**

ELA personnel, under the direction of K. Beaty, continued to operate a CAPMoN station at the ELA met site in 2000. The CAPMoN program, which monitors both atmospheric and precipitation chemistry at a network of sites across southern Canada, is funded and coordinated by the Meteorological Service of Canada. The ELA site monitors ground-level ozone, SO<sub>2</sub> and HNO<sub>3</sub> in the atmosphere, Cl, SO<sub>4</sub>, NO<sub>3</sub>, Na, NH<sub>4</sub>, Ca, K, Mg, pH, and mercury in precipitation. The ELA site, which has been operating since the 1980's, is frequently used as a baseline reference for sites in eastern Canada.

### **3.3.5 Canadian Network Isotopes in Precipitation (CNIP)**

The ELA is a node in a Canadian network monitoring isotopes (<sup>18</sup>O, Deuterium) in precipitation. This network ([www.fes.uwaterloo.ca/u/jgibson/gibson\\_files/cnip/ela.html](http://www.fes.uwaterloo.ca/u/jgibson/gibson_files/cnip/ela.html)), coordinated from the University of Waterloo, comprises 17 sites distributed broadly across Canada, including the high arctic. Its current goal is "to discern fundamental linkages between the isotopic composition of precipitation and synoptic climate and to aid in designing and optimizing a more permanent future network". Ken Beaty is the ELA researcher responsible for the ELA site.

### **3.3.6 Phytoplankton Nutrient Status in ELA Lakes**

Principal Investigator: Len Hendzel (Freshwater Institute)

Phytoplankton nutrient status measurements which include the use of composition ratios and physiological measurements (alkaline phosphatase, nitrogen debt, and nitrogen fixation activity) deal with the basic view that algae interacting with its environment provide direct and relevant answers regarding algal interactions within the aquatic food chain. Algal physiology and phytoplankton nutrient status explores the roles of essential nutrients (C, N, P) and physical factors in controlling algal species composition, succession and blooms, and chemical composition (lipids/carbohydrates, proteins, composition ratios, cell quotas) and determine to what extent laboratory studies can be applied to field situations. The species composition and biochemical composition of algae, together with other phytoplankton and zooplankton data can determine the efficiency of food chains, effect of perturbation, the production and consequences of harmful phycotoxins, and also the bio-availability of environmental toxic substances and their rates of removal from surface waters. As part of a continuing dataset, synoptic measurements of phytoplankton nutrient status were made on a selected number of ELA lakes during 2000.

Phytoplankton nutrient status (alkaline phosphatase activity and nitrogen debt) was measured on epilimnetic and metalimnetic water samples from lakes 227, 239, 302S, and 373 approximately every three weeks between May and September. In addition, nitrogen fixation also was measured routinely for lake 227.

Analysis of this year's data is still incomplete but previous data have consistently indicated that ELA lakes primarily are strongly phosphorus limited with some seasonal indications of secondary nitrogen deficiency. Also metalimnetic populations are less nutrient deficient than epilimnetic phytoplankton. These observations are consistent with other north temperate freshwater lakes in Canada and the world. Perturbations such as eutrophication or acidification usually impact phytoplankton nutrient status in ways that tend to either increase phosphorus deficiency or increase the seasonal variability of phosphorus and nitrogen deficiency indicators.

Synoptic surveys will continue if funding through the LTER and/or Food Chain Studies program continues to be provided.

Nutrient status measurements have been used to support food chain experiments conducted over the past years at ELA (L227 in 1998, and L239, 260, 302S, 304 in 1999) with M. Paterson and D. Findlay. One publication describing the 1998 L227 phytoplankton-zooplankton feeding experiment is in the manuscript stage.

### ***3.3.7 Comparison of Methods to Measure Bacterial Productivity in ELA lakes.***

Principal Investigators: Len Hendzel and Dave Findlay (Freshwater Institute)

The investigators wanted to test and compare two methods for measuring bacterial productivity in the water column. Both methods have been used to measure bacterial productivity for some time now but each have had several drawbacks that apparently are related to how the methods are specifically applied. They compared the more complex  $^3\text{H}$ -thymidine method with the more direct frequency of dividing cells (FDC) method in order to determine whether FDC could be incorporated into normal phytoplankton identification and enumeration protocols. Temporal bacterial productivity estimates, have to our knowledge, never been done at ELA and being able to estimate bacterial productivity would provide additional information about the movement of carbon through the food chain as well as providing a direct estimate of secondary productivity.

Frequency of dividing cells and  $^3\text{H}$ -thymidine incorporation were determined for epilimnetic water samples from lakes 227, 239, 373, and 260 last summer. Further laboratory work with ELA lake water samples was planned in order to define the conversion factor used to calculate the number of cells produced per mole of  $^3\text{H}$ -thymidine incorporated. However this work had already been done for a number of ELA lakes by Thomas Chrzanowski (Univ. of Texas) and he has agreed to share this data with Mr. Hendzel. Therefore no further experimental work was done during 2000.

No further thymidine measurements are planned, but David Findlay will continue to do FDC analysis on LTER lakes.

A manuscript detailing this work will be prepared this winter.

## **3 RECOVERY OF BOREAL LAKES FROM ACIDIFICATION**

Beginning in 1974, researchers at the ELA embarked on a major program investigating the effects of acidification on lakes of the Boreal Shield. Several lakes (223, 114, 302) and a wetland (239 Fen) were

acidified using various experimental designs. Subsequent to these acidification studies, the researchers have focused on monitoring and documenting the natural recovery of these systems. This work remains particularly relevant given the large numbers of lakes in eastern Canada, which, despite reductions in the emissions of acid precursors, have not yet recovered from anthropogenic acidification.

#### **4.1 Recovery of Lakes 302 and 223 from Experimental Acidification**

##### **Purpose(s) or Goal(s) of the Research:**

Our general goal in this continuing study has been to evaluate the ability of boreal forest lakes to recover from acidification without deliberate intervention in the recovery process to raise lake pH. This goal is in support of the recent Canada-Wide Acid Rain Strategy for Post-2000 that was committed to by the Federal, Provincial and Territorial governments.

To achieve this goal we have been studying the recovery of the physical, chemical and biological properties of aquatic ecosystems as their target pH has been relaxed following years of experimental acidification. Although the principal experimental system studied has been Lake 302S, a small amount of effort was spent on Lakes 302N and 223.

##### **Participating Agencies and/or Principal Investigators:**

There were three funding agencies supporting our research in 2000. (a) The Department of Environment (specifically Dr. Dean Jeffries of National Water Research Institute and Mr. Don McNicol of the Canadian Wildlife Service) provided operating funds that allowed us to continue the Lake 302S experiment and to conduct limited sampling in lakes 223 and 302N. (b) The DFO provided salary support for its researchers, and provided support for the ELA platform from which this experiment has been conducted. (c) The Climate Change Action Fund (Natural Resources Canada) supported preliminary research into the possible interaction between acidification recovery and climate change in terms of alkalinity generation and DOC metabolism.

DFO members of the principal research study in 2000 were M. Lyng, D. Findlay, L. Hendzel, K. Mills, S. Chalanchuk, P. Blanchfield, M. Paterson, L. Wesson, K. Beaty, and M. Stainton (All DFO, Freshwater Institute). R. Vinebrooke (U. Regina), B. Hann (U. Manitoba), and D. Schindler (U. Alberta) continue to be involved in the interpretation of earlier results from the experiment. As well, we are hopeful that D. Jeffries, D. McNicol and C. Podemski will become involved in future data analysis and interpretation. Participants in the acid-climate interaction study were P. Weidman (U. Manitoba), R. Hesslein (DFO), W. Donahue (U. Alberta), and J. Curtis (Okanagan University College). The principal investigator is Michael Turner (DFO).

##### **Brief Description of Work done during 2000:**

2000 was the third year for which Lake 302S had a target pH of 6.2 during the pH recovery phase of this experiment. We continued to monitor many of the physical (hydrology, temperature, and transparency), chemical (nutrient and ionic chemistry) and biological (phytoplankton, zooplankton and Chaoborus) properties of the pelagic zone. We carried out synoptic sampling of a limited number of corresponding properties (epilithon and metaphyton); I. Davies (CanSun) conducted a zoobenthic survey paralleling earlier LRTAP biomonitoring. We also conducted the first stage of sampling to follow-up on the lake whitefish addition that we made to Lake 302S in the fall of 1999.

There was restricted sampling of water chemistry and phytoplankton in Lakes 223 and 302N. We also sampled Lake 223 to determine the recovery of lake trout and permanence of changes in the white sucker population, and Lake 302N to monitor the recovery of the lake whitefish population from acidification and fertilization.

As part of our climate – acidification recovery interaction study, we performed a mesocosm test in Lake 302S of the potential role of DOC in internal alkalinity generation in recovering lakes. A companion study this winter, to be led by R. Hesslein, will evaluate the potential interaction between acidification recovery and climate change and variability on the in-lake metabolism of DOC.

#### **Listing of New Publications:**

- Donahue, W.F. 2000. The direct and indirect effects of solar ultraviolet radiation in boreal lakes of the Experimental Lakes Area, northwestern Ontario. *Ph.D. thesis*, Univ. Alberta, Edmonton, AB, 177 p.
- Frost, P., M. A. Turner, and J. J. Elser. 2001. Variable epilithon C:N:P stoichiometry and its causes in boreal lakes. *Can. J. Fish. Aquat. Sci.* (In revision).
- Hann, B. J., and M. A. Turner. 2000. Littoral microcrustacea in Lake 302S in the Experimental Lakes Area of Canada: acidification and recovery. *Freshwater Biol.* 43:133-146.
- Mills, K.H., S.M. Chalanchuk, and D.J. Allan. 2000. Recovery of fish populations in Lake 223 from experimental acidification. *Can. J. Fish. Aquat. Sci.* 57: 192-204.
- Mills, K.H., S.M. Chalanchuk, and D.J. Allan. 2001. Biomass and production of lake charr during the acidification and pH recovery of a small Ontario lake. *Environ. Biol. Fish.* (Submitted).
- Mills, K.H., S.M. Chalanchuk, D.L. Findlay, D.J.Allan, and B.R. McCulloch. 2001. Condition, recruitment, and abundance of lake whitefish (*Coregonus clupeaformis*) in a fertilized acid lake. *Arch. Hydrobiol. Spec. Issues Advanc. Limnol.* (accepted).
- Vinebrooke, R. D., D. W. Schindler, D. L. Findlay, M. A. Turner, M. Paterson, and K. H. Mills. 2001. Changing biodiversity and ecosystem function of acid-sensitive boreal lakes. *Ecosystems.* (Will be submitted mid Dec.).
- Vinebrooke, R., M. A. Turner, K. Kidd, B. J. Hann, and D. W. Schindler. 2001. Truncated food-web effects of omnivorous minnows in a recovering acidified lake. *J. North American Benthol. Soc.* (Submitted).

#### **Plans for 2001 and Beyond:**

We intend that 2001 will be the first of three study years in which no acid will be added to Lake 302S, *i.e.*, there will be no set target pH. These three years are intended to be the endgame of the experiment. During this period we will continue to monitor its physical, chemical & biological properties, including further assessing the success of the 1999 whitefish addition. As occurred this year, we anticipate conducting only a limited amount of sampling in lakes 223 and 302N, which have not received acid additions for several years.

Although, as in the past, we will once be again be seeking financial support, we are hopeful that the DOE will continue to provide us with sufficient assistance to conduct our monitoring program.

## 4 PERSISTENT TOXIC SUBSTANCES

Certain substances, when released into natural ecosystems, may persist for years in a toxic form, and may bioaccumulate within the food chain to create health problems for higher organisms, including humans, particularly when exposures are chronic. While such persistent toxicants are often experimentally studied under laboratory conditions, only studies conducted in real ecosystems can effectively examine the complexity of ecosystemic pathways and compartments in which these substances move and accumulate. Some controlled experimentation in real ecosystems is required to validate existing and proposed regulatory standards for these substances.

In addition, these experimental studies with persistent toxicants provide an opportunity to determine the physiological bases of ecosystem effects, thereby identifying indicators of stress at lower (physiological, histological) levels of biological organization. Once identified, these indicators can be extremely useful for the assessment and remediation of environmental problems.

### 5.1 Mercury Experiment to Assess Atmospheric Loading in Canada and the United States (METAALICUS)

#### Whole-Ecosystem Mercury Loading Experiment (proposed)

This proposed experiment addresses a need for research that will be useful to the Canadian Council of Ministers of the Environment (CCME) for setting regulations on emission levels of mercury to the atmosphere by electrical utilities. This is a unique experiment of international scope that can only be carried out at the Experimental Lakes Area, where whole-ecosystems can be manipulated to give understanding of environmental stressors at the ecosystem level.

#### I. BACKGROUND AND RATIONALE

Methyl mercury (MeHg) is the most common contaminant of fish in Canada, with unacceptable levels occurring in all regions and Territories. For example, in Ontario, where survey information is most complete, the guide to Ontario Sport Fisheries (MOE, 1999) reports that 99% of lakes and rivers tested now have consumption advisories due to MeHg. The primary concern is the human consumption of fish. Recently, Health Canada has reduced the daily allowable intake of MeHg for pregnant women and children under 12 from 0.47 to 0.2 ug/Kg/day. There is also increasing concern for loons and fish-eating mammals. Most recently, fish reproduction has been found to be sensitive at environmentally relevant concentrations of MeHg.

Bacteria that are active in lake sediments and peatlands produce MeHg from inorganic mercury (HgII). Most of the mercury in fish is MeHg ( $\text{CH}_3\text{Hg}^+$ ), which is a much more toxic form of mercury than is HgII.

There is a broad consensus among mercury experts that the most widespread cause of high fish MeHg concentrations is elevated atmospheric deposition of mercury to lakes and their watersheds. Emissions from the US Midwest and southeastern Canada are transported and deposited in both countries in a pattern similar to the deposition of sulfuric and nitric acids. In addition, there is increased global circulation of Hg in the atmosphere and increased deposition even in remote locations. One of the major sources of anthropogenic mercury is mercury emitted from coal-fired utilities, and controls on these emissions have recently been proposed. On a North American basis, the estimated cost of these controls would be several *billion* dollars per year (EPA, 1998). Despite these moves towards controls, mercury

experts are in agreement that the relationship between atmospheric mercury deposition and MeHg concentrations in fish is unknown. Although emission reductions of 50% have been proposed (Conference of New England State Governors and Maritime Province Premiers), there is no understanding of whether this reduction would be inadequate or more than necessary, and this could result in the waste of billions of dollars on control technologies. The uncertainty comes from the fact that the amount of stored Hg in ecosystems is many times greater than annual deposition. If the newly deposited Hg is much more bioavailable than the stored Hg, ecosystems will respond quickly (1-2 yr.) to changes in atmospheric deposition, but if all the Hg is equally bioavailable, response time will be very slow.

We propose to address these fundamental questions by adding trace amounts of inorganic mercury (HgII) to a lake and its watershed at the Experimental Lakes Area (ELA), northwestern Ontario. The ELA is situated in a region of low atmospheric deposition of mercury, and we propose that the Hg loading to an ELA lake be experimentally increased by a factor of 4 to simulate the atmospheric loading of mercury to lakes in Eastern Canada (Southern Ontario and the Maritime Provinces). This will be a whole-ecosystem experiment, which, in addition to following changes in MeHg concentration of fish, will study other key biological and chemical processes that determine the rate of MeHg production and its bioaccumulation into fish.

We hypothesize that additions of mercury to the lake basin will proportionately increase the MeHg bioaccumulation by fish. We further hypothesize that inorganic mercury added directly to the lake surface will be more bioavailable to mercury methylating bacteria than mercury entering the lake from the watershed, where it has been bound to dissolved organic carbon.

## II. EXPERIMENTAL OBJECTIVES

- To determine the relationship between the atmospheric loading of mercury to lakes and the MeHg concentration of fish.
- To determine the response time of MeHg in a whole ecosystem, including fish, to changes in rate of atmospheric deposition of mercury (HgII).

## III. PARTICIPANTS

Principle Investigators: John Rudd, DFO, and R. Harris, Tetra Tech Inc.

DFO participants: D. Bodaly, K. Kidd, M. Paterson, C. Kelly, P. Blanchfield, C. Podemski.

Other participants: H. Hintelmann, Trent University, Peterborough, ON  
 C. Gilmour, Academy of Natural Sciences, Benedict  
 Estuarine Research Center, Benedict, MD  
 J. Hurley, U. of Wisconsin & Wisconsin DNR, Madison WI  
 D. Krabbenhoft, USGS, Madison WI  
 V. St.Louis, U. of Alberta, Edmonton, AB  
 S. Lindberg, Oakridge National Laboratory, Oakridge, TN  
 M. Amyot, INRS-Eau Université du Québec  
 B. Branfireun, U. of Toronto, Toronto, ON

International Advisory Panel:

A. Iverfledt, Swedish Environmental Research Institute (IVL).

J. Munthe, Swedish Environmental Research Institute (IVL).  
E. Swain, Minnesota Pollution Control Agency  
R. Hesslein, Department of Fisheries and Oceans.

#### IV. DESCRIPTION OF THE STUDY, AND 2000 RESEARCH RESULTS

The experiment is designed to be carried out in two phases. The ELA Management Board approved the phase one study (pilot studies) at the Feb. 1999 and Feb. 2000 meetings. We have made final application for approval for phase two of the study (whole-ecosystem mercury addition). This approval is needed by late January of 2001.

At the time of writing of this report, samples from year 2000 pilot studies are still being analyzed. These results will be presented to the team and discussed at a project workshop to be held in Chicago in February of 2001. A summary of these results will be discussed at the ELA Management Board Meeting.

##### Phase One: Pilot Studies (1999, 2000)

##### *3.3.8 Isotopic Hg(II) Additions to a Small Upland Catchment (1100m<sup>2</sup>)*

In 2000, at monthly intervals, a stable mercury isotope (<sup>200</sup>Hg) was applied to a tiny, treed upland subcatchment at the ELA. All of the added mercury was isotopic mercury. It was sprayed onto the understory of the forest at a height of 1 meter. In 1999, <sup>202</sup>Hg was added to the same subcatchment. During the 2000 field season, loss of both isotopes to the atmosphere, to stream flow as well as storage in soils was followed. This dual isotope approach gives us a short and longer term understanding of mercury movement and storage in upland an catchment.

The objective was to determine the extent and time course of loss of the isotope by volatilization to the atmosphere and by runoff. Data for 2000 are still being analysed.

##### *3.3.9 Additions of <sup>202</sup>Hg and <sup>200</sup>Hg To A Wetland Plot*

In 1999, a pulse (one-time) addition of mercury isotope (<sup>202</sup>Hg) was applied to a 500 m<sup>2</sup> wetland area bordering Lake 115, at the ELA.

In 2000, at monthly intervals, mercury isotope (<sup>200</sup>Hg) was applied to the same 500 m<sup>2</sup> wetland area bordering Lake 115. The same sampling procedure was used in 2000 to determine the movement of both isotopes in the peat, loss to the atmosphere, and production of methyl mercury. These samples are still undergoing analysis.

### 3.3.10 5.1.3 Additions of Isotopic Mercury to Lake Enclosures

Pilot scale mercury additions were carried out in four 10 m diameter lake enclosures installed in Lake 239. The objectives of this experiment were 1) to test and refine our isotopic methods in the aquatic environment in preparation for the whole-ecosystem experiment, and 2) to study the relationship between HgII loading and production and bioaccumulation of MeHg.

Two experiments were conducted. In the first experiment, duplicate enclosures were spiked with  $^{200}\text{Hg}$  ( $20\mu\text{g}/\text{m}^2$ ) only at the beginning of the experiment. This was a pool labeling experiment designed to determine the length of time required for the added isotopic inorganic mercury to enter the bottom sediments, be methylated, and then be bioaccumulated as methyl mercury by fish and food chain organisms.

A second set of duplicate enclosures received the same amount of mercury, but in five equal aliquots between June and mid August. The purpose of this experiment was to simulate the periodic addition of labeled mercury that will be applied to L. 658 in 2001. This experiment will give us a preview of expected concentrations of isotope in sediment, water and the food chain in the study lake after the first year of isotope addition.

Samples from these experiments are presently being analyzed.

### **Phase Two: Whole-Ecosystem Mercury Addition (2001, 2002, 2003)**

We intend that Hg loading to an ELA lake and its water shed be increased by adding 3 non-radioactive (stable) isotopes of mercury - one to the lake surface, a second to wetland areas in the lake watershed, and a third to upland areas of the watershed. This experimental design will enable us to answer several crucial questions:

- Is there a direct relationship between HgII deposition to a lake and its watershed and the MeHg concentration of fish?
- What is the lag time between the deposition of HgII and MeHg bioaccumulation by fish, and does this lag vary for uplands, wetlands, and lake inputs?
- What is the relative importance of mercury deposited on uplands, wetlands or onto the lake surface as sources of MeHg to fish?

We propose to experimentally increase the deposition of mercury to an ELA lake and its watershed by a factor of four, which is the difference in deposition rate between ELA (a relatively clean site) and eastern North America, where deposition is much more polluted. The most recent estimate of total mercury deposition rate at the ELA is approximately  $14\ \mu\text{g}/\text{m}^2/\text{yr}$ . We therefore propose to add mercury ( $\text{HgCl}_2$ ) to an ELA lake and its watershed at a rate of  $56\ \mu\text{g}/\text{m}^2/\text{yr}$ .

### **Background studies on the Lake 658 Basin, the site of the proposed whole-ecosystem isotope addition beginning May 2001:**

We did extensive background data collections on L. 658 in preparation for addition of labeled mercury in 2001. At regular intervals samples for water chemistry, mercury species, concentrations of mercury in fish and foodchain organisms were taken. Sedimentation of particle bound mercury to the sediment-water interface and return of dissolved mercury to the water-column was studied. In addition, instrumentation was installed to measure water flow and take water samples from streams exiting wetland and upland

catchments. Precipitation samplers were installed and regular sampling for mercury in precipitation commenced. Pre-addition soil, peat and vegetation samples were taken and are being analyzed.

### Public Consultation

During 2000, we conducted public information meetings in Dryden and Kenora to discuss the project with the public. In addition a presentation was made to three NGO's at a meeting in Toronto. Feedback from these presentations was consistently positive.

### V. MILESTONES

- (2000) Second year of pilot scale experiments, pre-addition background monitoring of both candidate lakes.
- (2001) First year of whole-ecosystem isotope additions to upland and wetland areas of the watershed and to the lake surface, and a 3<sup>rd</sup> year of pilot studies.
- (2002) Second year of whole-ecosystem isotope additions.
- (2003) Third year of whole-ecosystem isotope additions.
- (2003 and beyond) Monitoring foodchain and fish mercury concentrations until they return to return to pre-addition levels.

### VI. PROPOSED ACTIVITIES FOR THE 2001 FIELD SEASON

Continuation of Pilot Scale Experiments, and background studies.

A third stable isotope of mercury will be added to the upland and subcatchment and to the wetland plot so that mobility of the isotopes and production of MeHg can be studied over a three-year period.

Snow study: Marc Amyot of the University of Ottawa proposes to begin a study of the fate of <sup>199</sup>HgII in snow. He will study the production and volatilization of Hg<sup>0</sup>, and he will also study the fate of <sup>199</sup>HgII at the time of snowmelt. He proposes to do these studies in 100 m<sup>2</sup> plots on the frozen surface of L. 114 and in the terrestrial catchment of L. 114.

In May of 2001, a dike will be constructed across the outflow of L. 658. This structure will be used to measure water flow out of the lake and into Winnange Lake. This will enable us to construct a water budget for the lake, and determine whole lake mass-balance budget for mercury and other chemical species. The dike, will be constructed of gravel and wood, and will be removed at the end of the experiment. Detailed plans for the dike are now being finalised, and appropriate permits will be obtained before construction begins.

Our present plan is to begin whole-ecosystem isotope addition to the Lake 658 basin in May of 2001. We have obtained sufficient funding for the purchase of isotope for the first two years of experiment. It has been ordered, and is being produced in Russia, with delivery beginning next May. We have made arrangements with the Canadian Forestry Service, who will supply, at cost, an aircraft and experienced pilot for the aerial application of the mercury isotopes. The isotopes will be applied by aerial spraying to the upland and wetland areas on five or six occasions between May and October of 2001. On the same schedule, the third mercury isotope will be added to the surface of the lake by injection into the propeller wash of a small boat.

After two years of preparation and pilot study we are now ready, contingent upon receiving final approval from Ontario, to begin addition of isotope to the Lake 658 basin in the spring of 2001.

## VII. IMPACT ON DOWNSTREAM LAKES

Based on our first year of upland and wetland pilot studies, and on our knowledge of the behavior of mercury in ELA lakes, we can estimate with confidence that less than 10% of the added mercury will leave the lake basin by the outflow on the long term. A somewhat larger percentage (perhaps 20%) will be emitted to the atmosphere. Most of the added mercury will bound in the upland soils, wetland peat and lake sediments on the long term.

Mercury in the Lake 658 outflow will enter a very large downstream lake (Winnange Lake). Based on our first year of pilot studies, and our knowledge of mercury in ELA aquatic ecosystems, we are confident that the added mercury isotope will not be detected in the Winnange Lake food chain due to the small amount that will be discharged from L. 658 and the very large volume of Winnange Lake relative to L. 658 (approx. 1000 x).

To verify that the Winnange Lake foodchain is not impacted by the experiment, we propose to study Winnange L. near the inflow from L. 658. Prior to addition of isotope, a sediment core will be taken in the vicinity of the L. 658 inflow. It will be sliced at 2 cm intervals to a depth of 10 cm and the isotopic composition of the mercury in the core slices will be assayed to determine if there is detectable isotopic mercury immediately downstream of L. 658. In addition 20 specimens of a piscivorous species will be caught in the vicinity of the L. 658 inflow. The flesh of these fish will be analyzed for mercury concentration and for isotopic mercury composition. During the period of experimental addition (2001, 2002, 2003), in the fall of each year, the sediments and fish will be re-sampled to determine if there has been any change in mercury concentrations or mercury isotopic composition.

## VIII. LAKE RESTORATION

If the mercury additions cause increased MeHg concentrations in food chain organisms, it is anticipated that these increases will be within the range presently observed in remote Canadian lakes that do not have industrial mercury sources. Because the piscivorous fish are longer lived, and they bioaccumulate MeHg over many years, their mercury concentrations would be less influenced over the three year addition period than shorter lived invertebrate organisms and prey fish species. The possible increased mercury concentrations would not be toxic to any of these organisms.

Even though the ELA is located in a region of low atmospheric mercury deposition, concentrations of MeHg in large, piscivorous fish in ELA lakes approach, and sometimes exceed, the 0.5 ppm commercial limit. Thus, it is possible that the large, piscivorous fish in our study lake will exceed the 0.5 ppm limit at the end of the experiment. However, if we do see an increase in mercury concentrations in fish during the 3 years of elevated mercury additions, we will have demonstrated that the ecosystem is very responsive to changes in mercury loading. We would therefore anticipate that, after we stop adding mercury to the lake, the fish will return to their pre-addition concentrations within a few years. After the experiment has been completed, the study lake will be monitored every second year until fish mercury concentrations return to pre-addition levels and the lake returns to conditions specified in Section VII. 3. of the ELA Memorandum of Agreement. We expect that natural recovery will take less than ten years.

During this recovery period, concentrations of mercury in fish and sediments in Winnange lake will be monitored every second year as described above.

## 5 ENDOCRINE DISRUPTING CHEMICALS (EDC'S)

Humans are producing and releasing to the environment a number of chemicals which are structurally similar to naturally occurring endocrine substances or hormones. There is considerable evidence to suggest that some of these manufactured chemicals may imitate the natural hormones and, under certain conditions, disrupt normal endocrine functioning in a number of species. Can these chemicals, when present in lakes and streams, disrupt the endocrine functioning of fishes? If so, what are the potential consequences?

### 5.1 Effects of a Potent Estrogen Mimic on Aquatic Populations

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#### Background:

Considerable evidence exists that aquatic organisms are being exposed to, and impacted by, a wide range of compounds that mimic hormones. Fish exposed to these compounds often exhibit an array of responses including depressed circulating sex steroid levels, reduced gonad size and fecundity, and males have become feminized. One of the most sensitive and common tools used to assess exposure to endocrine disrupting chemicals (EDCs) with estrogenic activity is the presence of vitellogenin (VTG), an egg yolk protein precursor, in the plasma of male fish. Recent have shown elevated plasma VTG in male fish downstream of sewage treatment plants.

Natural, and synthetic estrogens such as estriol and  $17\alpha$ -ethynylestradiol (EE2), two of the main active components of birth control pills, are present at  $\text{ng}\cdot\text{L}^{-1}$  concentrations in sewage effluents. Though other estrogenic compounds are present in these effluents, the natural and synthetic estrogens are believed to be posing the greatest threat to the endocrine systems of the resident fish. Laboratory studies have

confirmed that these compounds are causing the intersex and elevated VTG levels observed in fish downstream of sewage plants.

Despite the overt physiological evidence that fish are being adversely impacted by EDCs, it remains unclear whether these compounds are impacting a population's sustainability. It has been recognized nationally and internationally that there is a need to determine whether the molecular and cellular effects observed in fish exposed to estrogen mimics are indicative of changes in population viabilities. Though significant progress has been made in characterizing the effects of estrogenic contaminants in individuals, population level approaches to identify and quantify effects are lacking.

### **Purpose of the Experiment:**

This whole ecosystem study has been developed to determine whether aquatic populations are being adversely impacted by EDCs, and to calibrate and validate the relationship between organism- and population-level responses to these compounds. This four-year study will determine the impacts of the synthetic estrogen, EE2, on well-defined fish and invertebrate populations at the ELA. EE2 was chosen for this experiment because it is potent estrogen mimic that is known to affect the endocrine system of fish and other vertebrates. EE2 will act directly and effectively upon the endocrine system of organisms, and, therefore, research results will be broadly applicable to field and laboratory studies of other estrogen-like compounds.

The main objective of this study is to determine the ecological relevance of molecular, cellular and organism-level screening tools currently used to determine exposure of freshwater organisms to EDCs. In addition, this experiment will determine 1) the magnitude, mechanisms, and timeframe of EDC impacts on fish populations, 2) the impacts of an EDC on lower-trophic-level organisms, and 3) the most sensitive species and life history stages of freshwater biota exposed to an EDC. Results will be critical in determining whether EDCs are impacting the viability of freshwater populations, in interpreting the ecological relevance of assessment data from studies by DFO and other Departments (*e.g.* Environment Canada studies on fish downstream of sewage treatment plants), in identifying sentinel species for field studies, and in developing the science used by regulators and industries for ecological risk assessments, mitigation strategies and release regulations for EDCs.

### **Study Site:**

Lake 260 was chosen for this whole-lake addition experiment and has a surface area of 34 hectares and a maximum depth of 14.4 m. This lake has been part of a long term monitoring program at the ELA and considerable data exist on its limnological, physical, and biological characteristics. It supports well-defined lake trout (*Salvelinus namaycush*) and white sucker (*Catostomus commersoni*) populations of approximately 300 and 500 individuals, respectively, as well as the small fish species fathead minnow (*Pimphales promelas*; used in laboratory EDC assays) and pearl dace (*Semotilus margarita*). Nearby lakes, Lakes 442, 224 and 373 are being used as reference systems throughout this study as they are similar in physical and chemical characteristics, have the same fish species with similar population sizes to Lake 260, have long-term data on fish populations, and historical data on lower-trophic-level biota.

### **Baseline and Background Studies in 2000:**

The first two years of the study, 1999 and 2000, consisted of data collections and enclosure experiments in preparation for the whole-lake additions of EE2 in 2001 and 2002. As described below, population data were collected for fish and invertebrates, and background samples were collected to obtain biochemical, histological and reproductive information for fishes from these lakes. These data, and those

obtained from the reference lakes throughout the study will be critical in assessing the natural variability of these systems and in interpreting effects of the EE2 whole-lake additions.

### ***3.3.11 Enclosure Pilot Experiments***

One enclosure experiment was conducted in 2000 to examine the addition rates of EE2 in preparation for the whole-lake addition in 2001. Nine enclosures (2 m in diameter and 2 m deep) were set up in the littoral area of Lake 260. EE2 was added to 6 of the enclosures at an initial rate of 22% per day for two weeks to ramp up the water concentrations. After two weeks, daily addition rates were reduced to 5% per day to maintain water column concentrations at 15 and 30 ng EE2/L. The water concentrations were maintained at these target levels for 4 weeks and then the additions were discontinued. No results are available to date on the concentrations of EE2 in sediments from these and previous enclosure experiments. However, laboratory experiments are currently examining the microbial degradation of EE2 in these sediments and results will be available early in 2001. Results from this and other studies will be modelled and used to determine EE2 addition rates for the whole-lake experiment to be conducted in 2001 and 2002.

### ***3.3.12 Fish Population Studies***

Spring and fall trap net and short gill net sets were used to continue the mark-and-recapture program that evaluates the populations of lake trout and white sucker in Lakes 260, 442 and 224. Sampling was done in a manner that minimizes mortality, and the data will be used to assess age and size distributions, sex ratios, age to maturity, condition factors, abundance, growth rates, and annual survival and recruitment for lake trout and white sucker. These data and those collected from the reference systems during this study will be used to assess annual variability in unmanipulated populations, and to determine the effects of EE2 on fish populations in Lake 260.

Population sizes of fathead minnow and pearl dace were evaluated using a fall mark-and-recapture program on Lake 260 and 442 in 1999 and 2000. Catch-per-unit effort data for these species was also obtained from trap nets using volumetric methods, and a subsample of the total catch was weighed and measured.

### ***3.3.13 Molecular and Cellular Responses in Fish***

The purpose of this component of the project is to obtain background data on the gonadal development and the endocrine system function of fish populations. These data will be used to assess the effects of EE2 on fish during the whole-lake EE2 additions.

To avoid confounding interpretations of population-level effects, a small percentage (less than the annual natural mortality) of the lake trout, white sucker, fathead minnow and pearl dace populations were sacrificed to obtain gonad weights, fecundities, and gonad sections for histology and steroidogenesis. Fish were sampled from Lakes 260, 442, 304 and Roddy Lake (and Lake 114 for minnows) in 1999 and 2000 in conjunction with the spring and fall netting programs for the population-level research.

*Spring collections for 1999:* Fathead minnow histological samples taken include: Lake 260, 10 males, 14 females; Lake 442, 12 males, 10 females; Lake 114, 15 males, no females. Vitellogenin and vitamin samples. Lake 260 20 males, 18 females. Lake 442 13 males and 14 females. Lake 114 20 males, no

females. Pearl Dace include Lake 260, 13 males, 15 females; Lake 442, 9 males, 2 females. Vitellogenin and vitamin samples. Lake 260 14 males and 14 females, Lake 114 11 males and 5 females, Lake 442 33 males and 7 females. Lake trout blood sampling in spring for Lake 260=6, Lake 375 = 7 and Lake 442 =5 (sex not evident for spring collection of lake trout) White suckers for blood in spring. Lake 260 = 1 male and 4 females, Lake 375 = 5 males and 7 females, Lake 442 = 3 males and 13 females.

*Fall collections for 1999.* Fathead minnow from Lake 260, 13 males, 2 females; Lake 114, 13 males, 2 females; no fish from Lake 442. Pearl dace include 12 males and 12 females from each of Lakes 260, 114 and 442. Lake Trout (blood, histology, and tissues) Lake 260 = 4 females and 5 males, lake 375 = 9 males and 4 females, Lake 442 4 males and 5 females. White sucker for blood, histology and tissues. Lake 260 = 9 males and 6 females, Lake 375 = 1 male and 1 female, Lake 442 = 5 males and 7 females.

*Spring Collections for 2000:* Fathead minnow histological samples taken include: Lake 260, 8 males, 5 females; Lake 442, 13 males, 12 females; Lake 114, 10 males, 8 females. Vitellogenin and vitamin samples. Lake 260 10 males, 8 females. Lake 442 13 males and 9 females. Lake 114 10 males, 8 females. Pearl Dace histological samples include Lake 260, 8 males, 10 females; Lake 442, 13 males, 13 females. Vitellogenin and vitamin samples. Lake 260 24 males and 21 females, Lake 114 6 males and 7 females, Lake 442 22 males and 21 females. Lake trout blood sampling in spring for Lake 260=18, Lake 305 = 13 and Lake 442 = 14 (sex not evident for spring collection of lake trout) White suckers for blood in spring. Lake 260 = 4 male and 10 females, Lake 305 = 8 males and 8 females, Lake 442 = 6 males and 7 females.

*Mid-Summer Collections for 2000:* Fathead minnow histological samples taken include: Lake 260, 12 males, 4 females; Lake 442, 0 males, 0 females; Lake 114, 12 males, 11 females. Vitellogenin and vitamin samples. Lake 260 12 males, 4 females. Lake 442 10 males and 7 females. Lake 114 12 males, 10 females.

*Fall collections for 2000.* Fathead minnow from Lake 260, 6 males, 2 females; Lake 114, 0 males, 0 females; Lake 442 12 males and 4 females. Pearl Dace histological samples include Lake 260, 7 males, 14 females; Lake 114, 14 males, 14 females; Lake 442, 8 males, 9 females. Pearl dace for VTG and vitamins include 12 males and 12 females from each of Lakes 260, 114 and 442. Lake Trout (blood, histology, and tissues) Lake 260 = 6 females and 6 males, Roddy Lake = 5 males and 4 females, Lake 442 5 males and 6 females. White sucker for blood, histology and tissues. Lake 260 = 9 males and 6 females, Roddy Lake = 3 males and 3 females, Lake 442 = 5 males and 7 females.

Plasma samples were collected in the fall from male and female lake trout and sucker to assess circulating reproductive hormone, egg-protein (VTG), and sodium, potassium and calcium levels. Hormone samples are currently being analysed for the steroids estradiol, testosterone, 11-ketotestosterone, and  $17\alpha, 20\beta$ dihydroxy-4-pregnen-3-one by M. McMaster (EC, Burlington) and GtH II (LH-like gonadotropin) content by G. Van Der Kraak (University of Guelph). Whole body homogenates of fathead minnows and pearl dace were collected for VTG analyses in the spring and fall and are currently being analysed by V. Palace (DFO, Winnipeg).

Background information on gonadal development in fish was assessed using several techniques. Mature gonadal tissues from sucker and fathead minnows were collected in the fall to assess sex steroid production in females. Medial sections of ovaries were collected and are being examined by B. Evans for state of maturation, presence of atretic follicles, frequency distribution of oocyte stages, lesions and the presence of intersex. Testes will be examined for delayed testicular maturation, inhibited

spermatogenesis, asynchronous cyst maturation, seminiferous lobule deformities, replacement of generative tissue with connective tissue and other lesions (intersex/testis/ova). In addition, sections of gonadal tissues from these species are being assessed for estrogen receptors and apoptosis (programmed cell death) by G. Van Der Kraak because of increased incidences in fish exposed to contaminants.

Another consequence of EE2 exposure in fish may be disruption of thyroid function. Background information on thyroid function in fish from Lake 260 is being examined by S. Brown (Environment Canada, Burlington) using thyroid histological appearance, thyroid hormone levels in whole-body homogenates and in plasma and muscle.

Laboratory studies have shown decreased survival and skewed sex ratios of fish larvae exposed to EDCs. In this study, fertilizations of lake trout eggs have been done during 2 successive fall spawning seasons in the study and reference lakes to evaluate % fertilization, survival, growth and hatching success (V. Palace), and for developmental abnormalities. Fry survival and development are being assessed in the laboratory at the Freshwater Institute. Lake trout were captured using trap nets in Lakes 260 and 442, and in Roddy Lake during fall 2000. Eggs were collected from 10 females in Lake 260 and 5 females in Lake 442 during 1999. In 2000, 7 females in Lake 260, 5 in Lake 442 and 2 from Roddy Lake were spawned. Individual egg diameters and total egg volumes were recorded to calculate fecundities for each female and eggs. Unfertilized eggs were transported in sterile plastic bags back to the Freshwater Institute in Winnipeg where they were fertilized with a composite of milt (>3 males) obtained from male lake trout from the same lake. Eggs are being reared at 8C with subsamples being collected and preserved for measures of gross embryonic development and thyroid hormone and vitamin analysis. Methodologies to evaluate reproduction in white sucker in a manner similar to that described for lake trout were not previously available. However, over the first two seasons of this study, several methods were evaluated and a successful technique has been established.

Physiological and tissue-level responses and population-level effects in fish will be examined and correlated following each field season. Comparisons of effects at different levels of biological organization will be made at project workshops at the Freshwater Institute following each field season, and results will be examined and modelled through a collaborative effort by this multi-disciplinary team.

### ***3.3.14 Background Studies on Lower-trophic-levels***

Little is known about the impacts of hormone mimics on lower-trophic-level organisms and this experiment provides an excellent opportunity to advance the understanding of the effects of estrogenic compounds on such organisms. Baseline information on the tadpole, invertebrate, algal and microbial communities in the study and reference lakes has been collected in 1999 and 2000.

Tadpoles from Lake 114 were caged for 3 months in Lake 260, 224 and 114 to monitor their timing to complete metamorphosis and sizes at different stages of metamorphosis (n=60 to 75/lake). Tadpoles were fed every second day and weighed and measured weekly. Some of the tadpoles have been preserved to examine gonadal development and to analyse tissues for the thyroid hormones that control metamorphosis. In addition, individuals from the wild populations were collected and measured using trap nets for comparison to the caged animals. These studies in the pre-addition year will be repeated in 2001 to determine whether EE2 impacts the timing of development of the resident tadpoles. This research is part of a thesis project being done by B. Park at the University of Manitoba.

Emergence traps were used along the shoreline (~ 1 m depth) to sample emerging benthic insects from five different sites on Lakes 260 and 442. Two emergence traps were used per site and the subsamples were collected weekly from early June until the end of September.

Artificial substrates (0.01m<sup>3</sup> wire baskets containing local rocks) were used to monitor benthic invertebrate populations at the same stations established for monitoring insect emergence on Lakes 260 and 442. Rock baskets provide standardized samples from rocky lake substrates that are not easily sampled by more conventional means such as dredges. Fifteen rock baskets from each lake were collected and processed three times during the summer of 2000. These samples are currently being analysed to determine the abundance of taxa and community composition on these substrates.

Baited leech traps were set overnight in Lakes 260, 442 and 373 once each month over the summer of 2000 to assess the size and catch-per-unit effort of different species in these lakes. Leeches from these traps were preserved and these samples will be examined for species composition and gonad/somatic body weight ratios.

Vertical water column net tows were collected bi-weekly from ice-off to ice-on in 2000 as was done in 1999 on Lake 260 and the reference lakes. Nightly water column tows were done once a month to collect the invertebrate predators *Chaoborus* spp. Zooplankton samples from 1999 have been identified and counted to determine abundances, community composition, and sex and egg ratios (used to estimate birth and mortality rates).

Bi-weekly water samples were collected from these lakes in conjunction with zooplankton samples for phytoplankton and chemical analyses (phosphorous and nitrogen, chlorophyll *a*, suspended C and N). Bacteria samples were also taken from Lake 260 and reference Lakes 373 and 239. Phytoplankton samples from 1999 and 2000 have been processed and analyzed. The results indicate that the phytoplankton assemblage in Lake 260 is highly variable, averaging 950 mg.m<sup>-3</sup> in 1999 and 725 mg.m<sup>-3</sup> in 2000.

### **Training:**

Two graduate students are conducting research as part of this experiment. B. Park is a M.Sc. student at the University of Manitoba who is looking at the effects of EE2 on tadpole metamorphosis. J. Werner is a Ph.D. student at the University of Manitoba who is conducting studies on the fertilization success and biochemical responses of fish in Lake 260 in response to EE2 exposure.

### **Future Research Schedule:**

The whole-lake addition of EE2 will begin at ice-off in 2001 and will be continuous throughout the open-water seasons of 2001 and 2002. EE2 addition rates will be determined from the models of the enclosure studies done in 1999 and 2000, and will maintain water concentrations at a target dose (likely 10 to 20 ng/L, similar to estrogen concentrations downstream of some sewage treatment plants) that will be determined at a collaborators' workshop to be held early in 2001. EE2 will be added continuously from several locations on the lake by hand and using automated pumps. In the spring and fall of each year, EE2 applied to surface waters will mix vertically in the water column during lake turnover. Water, particulates, and sediments will be collected and analysed bi-weekly during the open-water season for EE2 using analytical methods developed at the Freshwater Institute for the enclosure studies. It is estimated that up to 750 g of EE2 will be needed each year to maintain water column concentrations at target levels.

The aquatic population research conducted in 1999 and 2000 will be repeated over the two years of EE2 additions to assess the impacts of this hormone mimic on the microbial, algal, invertebrate and vertebrate populations in Lake 260.

**Conclusion:**

By exposing well-defined aquatic populations to a known and potent EDC, we will determine whether estrogen mimics affect the reproductive success of organisms under wild conditions where EE2 is the only stressor. This information is critical for determining the ecological relevance of the screening tools currently used to assess effects of EDCs on aquatic biota in both laboratory and field studies. Linking organism-level responses to impacts on populations will also improve our ability to assess the risks that EDCs pose to wildlife.

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