SUMMARY OF MAJOR RESEARCH PROJECTS AT THE EXPERIMENTAL LAKES AREA DURING 2003

9 December 2003

Research activity on site at the ELA during 2003, while somewhat reduced from the record levels of the previous two years, remained well above the historic average. More than 200 different researchers, representing more than 30 different universities, government agencies and private companies, worked on site during the field season.

The 2003 activities covered a broad spectrum of research. The METAALICUS and EDC (estrogen) studies were both in their third year of ecosystem-scale, experimental manipulation, while the FLUDEX study, at a reduced activity level, was in its 5th, and final, experimental year. The experimental phase of the Cage Aquaculture study got underway in June, and this project squeezed past METAALICUS to become largest single project in terms of on-site activity. The long-term, ecological research (LTER) program continued with support from core funding. Research on recovery of lake ecosystems from acidification continued, as did a number of smaller studies, both new and continuing.

The following is an attempt to summarize the status of major research projects by providing some information about their purposes and designs. It should be noted, however, that data analyses are ongoing and results are not provided here. These projects are grouped under several broad category headings.

Note:

Using information provided by research project leaders and other ELA staff, John Shearer compiled this summary. Where appropriate, the names of principal investigators, graduate students, and their affiliations are noted. However, DFO Experimental Lakes Area staff members and seasonal employees, many of whom have not been specifically mentioned, are conducting many aspects of most major projects. The summary is intended as an overview of research activities at the ELA during 2003. In most cases, the results have been omitted, so as not to preclude future, peer-reviewed publication. For more detailed information, particularly concerning results, the reader should contact those researchers responsible for each study, or refer to published literature.

TABLE OF CONTENTS

HABITAT ALTERATION AND ECOSYSTEM 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.

FERTILIZATION OF LAKE 227

Rationale:

Eutrophication remains one of the most common water quality problems in much of the world. As most ELA lakes are naturally oligotrophic, it has proved advantageous to maintain at least one study lake in which the primary productivity is elevated. This enables researchers to compare physical, chemical and food web characteristics in other ELA lakes with those in a more productive system, more typical of those in many areas of Canada, and elsewhere.

Research Activities:

Lake 227 was fertilized with phosphorus for the 35th consecutive year in 2003. This original ELA ecosystem-scale 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 2003, 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.

We continued to monitor water chemistry, phytoplankton, and zooplankton in Lake 227 during 2003. The following paper was recently published:

- Paterson, M.J., D. Findlay, A.G. Salki, L.L. Hendzel, and R.H. Hesslein. 2002. The effects of *Daphnia* on nutrient stoichiometry and filamentous cyanobacteria: a mesocosm experiment in a eutrophied lake. *Freshwater Biology* **47**: 1217-1233.

EFFECTS OF MACROPHYTE REMOVAL ON PIKE POPULATIONS

Principal Investigator:

Dr. Ken Mills, DFO Freshwater Institute

Rationale and Design:

Many property owners along lake shorelines remove rooted and floating plants (macrophytes) from the nearshore areas to facilitate water access for boating and swimming. However, these plants may provide important habitat for fish and other aquatic species. What are the impacts of mechanical removal of macrophytes from a small boreal lake?

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 July 1996. A mechanical harvester removed fifty percent of the macrophytes present in the lake. The harvesting continued in 1997 and 1998. No macrophyte harvesting has occurred in the years following 1998. We have now completed 5 years, 1999–2003, of recovery monitoring of the macrophyte community and organisms in the lake.

EMPIRICAL MODELS FOR EVALUATING THE PRODUCTIVE CAPACITY OF FISH HABITAT - LINKING FISH BIOMASS AT SPECIFIC HABITATS TO TOTAL POPULATION PRODUCTION

Project Goal:

The goal of this research is to assess the productive capacity of littoral habitats for fish in lakes.

Principal Investigators:

This project is part of a national collaborative study, with Dr. R. Randall (DFO, Burlington, ON) as project manager. Dr. P. Blanchfield, with biologist Lori Flavelle, is responsible for the ELA component of this study.

Work Completed in 2003:

In 2003 we shifted our study of habitat-specific cyprinid abundance and biomass estimates from Lake 442 to Lake 223. This move was done to find a lake with habitat types more similar to the collaborative research being done at the Turkey Lakes field site.

Five types of habitat were identified by visual underwater observation; open, rocky, woody, beaver lodges and vegetated. We dye-marked individuals of all cyprinid species captured (minnow traps and fyke nets) according to habitat. Three subsequent recapture periods allowed us to determine whether marked fish remained in the original habitat type where they were marked, or utilised several of the habitat types.

Additionally, distance-sampling methodology was employed as a means of assessing the abundance of different cyprinid species in the five habitat types. This method involves an observer swimming a pre-placed transect, and noting the presence of individuals, as well as recording each individual's distance from the main transect line. These observations will be used to assess fish densities in the various habitat types.

Plan for 2004:

No further research is planned.

RESERVOIR IMPACTS

In Canada, 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.

Over the past decade, ELA researchers have been investigating the ecological effects of flooding caused by reservoir creation and operation. In most cases, this has involved experimental alteration of water levels, as a simulation of what typically occurs during the creation and operation of reservoirs. The focus of these studies has been the production and fate of methylmercury and various greenhouse gases. One mesocosm-scale study is investigating the effects of burning vegetation prior to flooding as a means of mitigating mercury effects in reservoirs.

EXPERIMENTAL LAKES AREA RESERVOIR PROJECT (ELARP)

Objective:

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 (CO2) and methane (CH4) to the atmosphere.

Design and Results:

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 by-products of the decomposition of the flooded vegetation in the peatland are CO2 and CH4. Mobilization of MeHg within the flooded ecosystem and release to the atmosphere of CO2 and CH4 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.

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. During the open water period of 2001, the system was flooded once again and a regular monitoring program was carried out.

Flooding was repeated in 2002 and in 2003, but only minimal general monitoring was conducted. However, researchers from the University of Waterloo conducted two special studies, as described below.

Fluxes and Isotopic Composition of Greenhouse Gases in a Flooded Wetland - Ten Years Post Flood.

Project Goal:

To determine if the flooding and drawdown of a boreal wetland over ten years has changed the production and flux of greenhouse gases using concentration data, stable carbon isotopes (12C and 13C) and vegetation coverage.

Principal Investigators:

Dr. Sherry Schiff, Michelle Saquet (MSc. candidate), and Jason Venkiteswaran (PhD candidate)

(Department of Earth Science, University of Waterloo, Waterloo, ON, N2L 3G1)

Work completed in 2003:

In summer 2003, L979 centre buoy, L979 east inflow, and L240 outflow samples were collected for DIC, CH_4, and O_2 concentration, as well as stable carbon and oxygen isotopes. Four vertical profiles at the L979 centre buoy were collected for for DIC, CH_4, and O_2 concentration, as well as stable carbon and oxygen isotopes.

Changes in Vegetation and Carbon Pool in the Aboveground Biomass after the Peatland Flooding Experiment.

Principal Investigators:

Dr. Taro Asada (Post-doctorial fellow) and Dr. Barry G. Warner

(Department of Geography and Wetlands Research Centre, University of Waterloo, Waterloo, Ontario, N2L 3G1)

Objectives:

The purpose is to clarify the effect of flooding on the change in peatland vegetation and total aboveground carbon pool. More specifically, the goal is to describe and map the current vegetation communities on the floating peat mat as of 2002, and examine the change in vegetation caused by the flooding. To quantify the total aboveground biomass and aboveground carbon pool, and examine their change that is attributable to the flooding.

Summary of Work carried out during 2003:

No field work was carried out on this project during 2003. Analyses of photographs from before and after the flooding have continued.

One publication based on these analyses is now in press; namely,

- Asado,T., B.G. Warner, and S.L. Schiff. 2003. Vegetation response to experimental flooding in a boreal basin peatland. *Ecosystems* (in press).

Continuation of Work for 2003:

Continue analyses on the vegetation and biomass using data from both before and after the flooding.

FLOODED UPLAND DYNAMICS EXPERIMENT (FLUDEX)

Purpose and Approach:

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 summers of 1999 to 2003, inclusive, to create experimental hydroelectric reservoirs. Greenhouse gases fluxes before and after flooding were measured at all three sites. Carbon dioxide, methane and nitrous oxide were monitored. Fluxes are being 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 were measured in the experimental reservoirs. Mitigation strategies that will have direct planning application are being investigated. The fifth year of flooding (2003) was the last for the experimental reservoirs. Studies on the effectiveness of selenium additions to reduce mercury in food chains began in 2003 with a survey of mercury and selenium levels in reservoirs, wetlands and natural lakes at the ELA and in northern Quebec.

Principal Investigators:

- 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 and Andrew Majewski (Freshwater Institute)
  - David Findlay (Freshwater Institute)
  - Mariah Mailman (University of Manitoba)
- Greenhouse gases and carbon decomposition:
  - Elizabeth Joyce, Cory Matthews and Vincent St.Louis (University of Alberta)
  - Natalie Boudreau, Jason Venkiteswaran and Sherry Schiff (University of Waterloo)
  - Len Hendzel (Freshwater Institute)

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 the use of controlled burns to mitigate mercury problems in boreal reservoirs.

2002-03: Year 4 of flooding, study of GHG emissions and mercury dynamics; completion of studies on controlled burning.

2003-04: Year 5 (last year) of flooding, study of GHG emissions and mercury dynamics; removal of reservoir dikes and rehabilitation of sites; initiation of studies on the possible use of selenium additions to reservoirs to mitigate mercury problems.

The three areas flooded are: a High Carbon moist forest (Site 1), a Medium Carbon dry forest (Site 2), and a Low Carbon 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 (10^4 m^3)	Dike length (m)
Site 1	0.69	1.0	0.69	190
Site 2	0.50	0.8	0.40	130
Site 3	0.66	1.2	0.79	350

Reservoirs were filled with water pumped from Roddy Lake using a diesel-powered unit. Water from all sites drained back to Roddy Lake. Water renewal times of approximately 10 days were maintained during the open water season. Maximum dike height and reservoir depths are approximately 2 m.

Site Rehabilitation:

Upon completion of final sampling in October 2003, all water pipes were removed. Removal of the reservoir dikes and site rehabilitation was contracted for November and December 2003. The rehabilitation is being conducted according to guidelines prescribed by the Ontario Ministry of Natural resources and includes bulldozing and on-site burning of the dead jack pine trees in the former reservoirs. The diesel pump, fuel tank, and storage trailer will also be removed as part of this rehabilitation process.

Funding Support:

The FLUDEX project has been funded from a variety of sources, including Manitoba Hydro, Hydro-Québec, the Department of Fisheries and Oceans, the Natural Sciences and Engineering Research Council (Canada), the United States Geological Survey, and the Centre for Research in Earth and Space Technology (Ontario).

Primary Publications:

- Hall, B.D, V.L. St.Louis, K.R. Rolfhus, R.A. Bodaly, K.G. Beaty and M.J. Paterson. In press. The impact of reservoir creation on the biogeochemical cycling of methyl and total mercury in boreal upland forests. *Ecosystems*.
- Matthews, C.J.D., E.M. Joyce, V.L. St.Louis, S.L. Schiff, R.A. Bodaly, J.J. Venkiteswaran, K.G. Beaty, and B.D. Hall. In press. Carbon dioxide (CO_2) and methane (CH_4) production in small reservoirs flooding upland boreal forest. *Ecosystems*.
- Hall, B.D., V.L. St. Louis, and R.A. Bodaly. in press. The stimulation of methylmercury production by decomposition of flooded birch leaves and jack pine needles. *Biogeochemistry*.

Conference Presentations:

- 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. 1999. 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.
- Matthews, C.J.D. V.L. St.Louis, and R.A. Bodaly. 2001. The impact of reservoir creation on greenhouse gas fluxes from three forested upland subcatchments. International Society of Limnology, Melbourne, Australia.
- Paterson, M.J., C. Podemski, R.A. Bodaly, and K.A. Peech. 2001. Bioaccumulation of methyl mercury by invertebrates from four reservoirs and three natural lakes at the Experimental Lakes Area, Ontario, Canada. Sixth International Conference on Mercury as a Global Pollutant, Minamata, Japan.
- Rolfhus, K.R., J.P. Hurley, B.D. Hall, and D.P. Krabbenhoft. 2001. The response of soil/water mercury fluxes to periodic inundation of upland boreal forest reservoirs. Sixth International Conference on Mercury as a Global Pollutant, Minamata, Japan.
- Hall, B.D., V.L. St.Louis, R.A. Bodaly, and K.G. Beaty. 2001. Impacts of reservoir creation on the biogeochemical cycling of methylmercury in boreal forest uplands. Sixth International Conference on Mercury as a Global Pollutant, Minamata, Japan.
- Bodaly, R.A., A.R. Majewski, W.A. Jansen, R.J.P. Fudge, and M.J. Paterson. 2001. Uptake of mercury by fish in three experimental reservoirs that flooded forested boreal uplands. Sixth International Conference on Mercury as a Global Pollutant, Minamata, Japan.
- Hall, B.D., V.L. St.Louis and R.A. Bodaly. 2001. Methylmercury production in flooded birch leaves and jack pine needles. Sixth International Conference on Mercury as a Global Pollutant, Minamata, Japan.
- Peech, K.A., M.J. Paterson, R.A. Bodaly and L. Wesson. 2001. Methylmercury n zooplankton in reservoirs that flooded boreal forest catchments: The Flooded Uplands Dynamics Experiment. Sixth International Conference on Mercury as a Global Pollutant, Minamata, Japan.

- Hall, B.D., Bodaly, R.A., and 8 co-authors. 2002. Mercury cycling and greenhouse gas fluxes from flooded boreal forest uplands: the FLUDEX (FLooded Uplands Dynamics EXperiment) Project at the Experimental Lakes Area. American Society of Limnology and Oceanography, Victoria. (invited)

Theses Completed:

- Boudreau, Natalie M. 2000. Soil carbon, carbon dioxide, and methane in three experimentally flooded upland boreal forest reservoirs: a δ13C inventory of sources and processes. M.Sc. thesis, University of Waterloo, Department of Earth Sciences, 180 p.
- Hall, B.D. 2003. Impacts of reservoir creation on the biogeochemical cycling of methylmercury in boreal forest uplands. Ph.D. thesis. Department of Biological Sciences, University of Alberta, 231 p.
- Joyce, E. M. 2001. The impact of experimental reservoir creation on greenhouse gas fluxes from forested uplands. M.Sc. thesis, Department of Biological Sciences, University of Alberta, 70 p.
- Matthews, C.J.D. 2002. Greenhouse gas production in experimental reservoirs flooding upland boreal forest. M.Sc. thesis, Department of Biological Sciences, University of Alberta, 101 p.
- Peech Cherewyk, K.A. 2002. Methylmercury bioaccumulation in zooplankton: an assessment of exposure routes and accumulation in newly flooded reservoirs. M.Sc. thesis, Department of Entomology, University of Manitoba. 89 p.

Nitrous Oxide Gas Fluxes Related to Reservoir Creation (FLUDEX) and Aquaculture Activities

Purpose:

There were two studies at the ELA during 2003 that examined nitrous oxide fluxes. One as a consequence of upland reservoir creation (FLUDEX Project) and the other as a consequence of rearing of fish enclosures (Aquaculture Project).

The FLUDEX-N_2O study was initiated during the summer of 1998 to look at the pre-flood fluxes of nitrous oxide from soils within three upland sites that were primarily distinguishable from each other by moisture content and nutrient composition, two factors which interact to control N_2O fluxes from soils. It was hypothesized that these factors would control the emission of N_2O from these upland soils. Once flooding started in 1999 it was hypothesized that nitrification and denitrification within the reservoirs would be limited by severe anoxia and low pH. Furthermore it was hypothesized that based on carbon availability N_2O concentrations would either continue to decline or remain relatively unchanged in subsequent years. N_2O fluxes were tracked for one complete year from the pre-flood upland reservoir sites and for five years following flooding. Also for comparison, lake 979, a peat-based reservoir with much more stored carbon than the FLUDEX reservoirs, was again monitored for N_2O during summer flooding.

The lake 375 Aquaculture project was an opportunity to study what the impacts of aquaculture waste production (i.e. waste food, metabolism products) had on nitrification-denitrification at the sediment surface. It was hypothesized that N_2O emissions would increase if there were any substantial accumulation of uneaten food or waste products of fish metabolism accumulating on the sediments in the area beneath the fish pen. In order to determine this, water column N_2O

concentrations at two stations in lake 375 (375 N & S) and in one control lake (373) have been monitored before and during the first season of operation of an experimental fish enclosure.

Principal Investigators:

Len Hendzel (DFO, Freshwater Institute)

Work carried out during 2003:

Surface waters from the FLUDEX reservoirs and epi-, meta- and hypolimnetic water in lakes 375 N & S and 373 were routinely sampled for dissolved N_2O every 2-4 weeks through the field season. In addition, lake 979 (ELARP) surface water N_2O concentrations, and fluxes over floating and stationary peat vegetation were measured during the summer of 2003. In total about 150 samples were collected. Analysis of all samples will be completed by the new year and tabulation of the data will follow.

Publications or Spin-off Research resulting from the Study:

The 2002 data were presented at the Aquaculture Data Workshop held at the Freshwater Institute during March. There were no workshops in support of the FLUDEX project during 2002-03.

A manuscript summarizing this work has been prepared and submitted to *Ecosystems*, where it is currently under review.

Investigating Carbon Sources for Greenhouse Gas Flux using Stable Carbon Isotopes

Principal Investigators:

Dr. Sherry Schiff, Jason Venkiteswaran (PhD candidate), Dr. Maren Oelbermann (Post-Doctoral Fellow)

(Department of Earth Sciences, University of Waterloo, Waterloo, Ontario, N2L 3G1)

Objectives:

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

Summary of Work during 2003:

Post-flood Greenhouse Gas Source, Greenhouse Gas Process and Heterotrophy-Autotrophy

In summer 2003, we collected reservoir water samples for dissolved inorganic carbon (DIC), methane (CH_4) and oxygen (O_2) concentration and isotopic ratio analyses. Continued monitoring of the DIC and CH_4 concentrations will be used to construct carbon mass budgets for the reservoirs to determine net greenhouse gas production. Isotopic ratio work on DIC will separate two competing DIC processes: decomposition of organic stores and primary production. Isotopic ratio work on CH_4 will allow us to discriminate between CH_4 production from decomposition of organic stores and CH_4 consumption by oxidation. Isotopic ratio work on O_2 will allow us to determine the heterotrophy-autotrophy relationship of each reservoir and how it changes with time.

The following samples were taken from each reservoir on an intensive schedule throughout the flooded season: inflows, outflows, samples throughout the water column from sippers, and bubbles from bubble traps. CH_4, DIC, and O_2 samples were taken during each sampling event. Concentration and isotopic ratio samples were collected at the same time so that they could be used together to construct and isotopic ratio mass budget for each reservoir.

Continuation of Study / Spin-off Research:

Because 2003 was the final year for this experiment, three detailed studies were performed:

1. Soil Collection:
To study the isotope dynamics of soil decomposition under flooded conditions, soil was collected from FLUDEX reference sites and from the FLUDEX reservoirs after flooding ended in 2003. From the reference sites, soil representing the different soil and vegetation communities was collected. From the reservoirs, twenty-five soil cores from each reservoir were collected. Soil samples were divided into soil horizons, elemental and isotopic abundances of C and N in each layer will be determined, and the soil will be incubated to monitor decomposition.

2. Mid-season Diurnal Experiments:
To determine the importance of primary production and to test new isotope hypotheses, a two day diurnal experiment was conducted on each FLUDEX reservoir. DIC and CO_2 concentration samples were collected four times per day. Continuous O_2 concentration and temperature measurements were collected by a Hydrolab Minisonde. Continuous CO_2 concentrations measurements were collected by equipment supplied by Patricia Ramlal (FWI). Isotope samples were collected hourly. The diurnal change in concentration and isotopes will be used to determine the rates of photosynthesis and community respiration in each reservoir.

3. Gas Exchange Technique Comparison Experiment:
To determine whether ELA-style floating chambers over- or under-estimate gas exchange, a comparison experiment was conducted. Sulphur hexafluoride (SF_6) was added to each reservoir and allowed to mix completely. The SF_6 concentration decline rate is used to determine the whole reservoir gas exchange coefficient for SF_6 (k_{SF6}). k_{SF6} can be related to other gases such as CO_2, CH_4, and O_2. Seven floating chambers were deployed for one hour on two consecutive days and the increase in SF_6 concentration in the chamber is used to determine k_{SF6}. Since the whole reservoir SF_6 addition is believed to be a more accurate method for determining k_{SF6}, these two methods of determining k_{SF6} will be compared to asses the ability of ELA-style floating chambers to determine k_{SF6} and thus gas exchange rates of CO_2, CH_4, and O_2.

Previous, spin-off research includes a series of sealed chamber experiments and CH_4 oxidation microcosms. Sealed chamber experiments were designed to test the theories behind and precision of process-based research. Results from these experiments are currently being drafted into a manuscript for submission to a peer-reviewed journal. CH_4 oxidation microcosm experiments were continued to determine the carbon isotopic fractionation factor associated with CH_4 oxidation. Since the fractionation factor varies widely (cf. Whiticar 1999) it is imperative to determine it for the FLUDEX reservoirs and in ELARP (L979) at environmentally-relevant temperatures. Results from these experiments have been submitted to *Applied Geochemistry*, a peer-reviewed journal.

Related Publications:

- Boudreau NM. 2000. Soil carbon, carbon dioxide, and methane in three experimentally flooded upland boreal forest reservoirs: a $d^{13}C$ inventory of sources and processes. *MSc thesis*. University of Waterloo.

- Baril M. 2001. Dissolved inorganic carbon from the decomposition of vegetation and soils of flooded reservoirs: stable carbon isotope ratios. *BSc thesis*. University of Waterloo.
- Soil Classification Working Group. 1998. *The Canadian System of Soil Classification*. 3rd edition. Agriculture and Agri-Food Canada Publication **1646** (Revised). 187 pp.
- Whiticar MJ. 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology* **161**(1-3): 291-314.

MITIGATION STRATEGIES FOR MERCURY CONTAMINATION OF RESERVOIRS

Now that the issue of methyl mercury contamination in reservoirs has been well documented and the processes are becoming better understood, the question arises as to what strategies might be effective in mitigating this contamination. Current studies at the ELA are attempting to address this issue.

Effects of Burning before Flooding on Methyl Mercury and Greenhouse Gas Production in Boreal Reservoirs

Principal Investigators:

Mariah Mailman (M.Sc. Candidate, U of Manitoba) and Dr. Drew Bodaly (Freshwater Institute)

Purpose:

Flooding terrestrial plants and soil causes decomposition of organic matter, which stimulates microbial methyl mercury (MeHg) production. Burning before flooding was tested as a method to ameliorate MeHg contamination in reservoirs.

Research Activities:

In a controlled, replicated field experiment conducted at the Experimental Lakes Area in 2002, vegetation and soil were burned in a controlled manner using propane torches. Combinations of fresh and burned vegetation and soil were added to limnocorrals to simulate flooded ecosystems. Vegetation and soil were added to limnocorrals in the following combinations: fresh vegetation and soil (Fresh treatments), burned vegetation and fresh soil (Partial Burn treatments), and burned vegetation and burned soil (Complete Burn treatments). Controls had no added vegetation or soil. Subsequent analysis of the experiment during 2003 produced the following a number of results. These will be published in the coming months.

Are Selenium Concentrations Depressed in Reservoirs?

Principal Investigators:

Mariah Mailman (Ph.D. Candidate, U. of Manitoba) and Dr. Drew Bodaly (DFO, Freshwater Institute)

Objective:

The objective for the 2003 field season was to gain information on the geochemical cycling of selenium (Se) to understand whether selenium is depressed in reservoirs and whether depressed selenium concentrations in reservoirs correlate with higher methyl mercury (MeHg) concentrations in fish tissue. This work is preliminary to a mesocosm-scale study planned for the ELA in 2004.

Activities in 2003:

Reservoirs and reference lakes were sampled for MeHg and Se in water, zooplankton, insect larvae, and fish, as well as flooded and sub-aerial soil from the reservoirs. The specific reservoirs sampled were at the ELA and at two hydroelectric complexes in Québec.

At the ELA, samples were collected from the three experimental reservoirs located in an upland forest catchment of the FLooded Uplands Dynamics EXperiment (FLUDEX), from the experimentally flooded wetland of the ELA Reservoir Project (ELARP), from two reference ponds (L632 and L115), and from two reference lakes (L468 is the FLUDEX inflow and L240 is the ELARP inflow).

In northern Québec, two reservoirs were sampled in the eastern sector of the 20 year old La Grande Complex, Laforge-1 and Caniapiscau Reservoir, as well as one reference lake, lac Serigny. In north-eastern Québec, Robertson Reservoir (eight years old) was sampled and one reference lake, lac Ivry. Due to natural, regional variations in selenium concentrations, comparisons will be made on a geographical basis.

Samples of water for THg and MeHg were collected using the clean trace metal techniques. Water samples for Se and water chemistry were collected at the same time. Zooplankton were collected from the large water bodies using a 160 µm tow net and from the small water bodies using the same mesh size sweep net. At the ELA, insects were collected as they emerged in floating traps. In Québec, benthic invertebrates were collected using D-ring nets with 1 mm mesh, sieved, and insects were separated to the family level. At the ELA, Finescale dace were collected from experimental reservoirs, reference ponds, and L468. Yellow perch were collected from L468 and L240, and White Suckers were collected from only L240. In Québec, fishes were collected by contractors hired from Genivar by Hydro-Québec. The fish species sampled from the La Grande Complex are Lake Whitefish, Longnose Sucker, Pike, and Lake Trout. Fish species to be sampled in Robertson Reservoir and reference lakes are Brook Trout, Arctic Char, dwarf Whitefish, and Rainbow Smelt. The fish muscle tissue was sub-sampled and given to me for Se analysis. Samples were frozen and are currently being prepared for analysis. I expect to have all data by mid December.

Data Analyses:

Se in water is currently being analyzed by Fei Wang at the University of Manitoba Chemistry Department using inductively coupled plasma mass spectrometry (ICPMS). Se in biota is being analyzed by Vince Palace at the Freshwater Institute in Winnipeg. THg and MeHg in water and MeHg in zooplankton and insects are being analyzed by Flett Research in Winnipeg by cold vapour atomic fluorescence spectrometry (CVAFS). MeHg in the ELA fish will be analyzed by Laurie Wesson at the Freshwater Institute by cold vapour atomic absorption spectrometry (CVAAS). MeHg data for fishes from Québec will be provided by the PSC laboratory in Anjou, Québec, which will also be analyzed by CVAAS. Some cross laboratory checks will be performed to affirm the quality and comparability of the data. The results will be presented at a workshop in Montréal in January.

NATURAL VARIABILITY AND CLIMATIC FACTORS

In order to assess objectively 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.

LONG-TERM ECOLOGICAL RESEARCH (LTER) AND DATA MANAGEMENT

Principal Investigators:

- S. Kasian, LTER Coordinator and ELA Data Manager
- K. Beaty, P. Blanchfield, D. Findlay, D. Guss, L. Hendzel, R. Hesslein, M. Lyng, K. Mills, S. Page, M. Paterson, J. Shearer, M. Stainton, M. Turner.

All principal investigators are ELA/DFO staff.

Project Description 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.

Activities in 2003:

Long-term records of meteorology and hydrology of the Lake 239 basin were maintained. Meteorological variables (air temperature, precipitation, wind speed and direction, bright sunshine and evaporation) were monitored daily. The 3 inflowing streams and outflow of Lake 239 were monitored for volume of flow (continuous record) and chemical composition (weekly). The five core lakes (114, 224, 239, 373, and 442) were again monitored, where possible, for all major disciplines which include: hydrology, water temperature, secchi depth and light extinction profiles, water chemistry, primary production, epilithon, phytoplankton populations and nutrient status, zooplankton, and fish. Discipline specific monitoring continued in other lakes to maintain long-term records.

Measurements of limnological variables and samples for chemistry, phytoplankton and zooplankton analyses were taken every 2 weeks through the open water season and twice over winter (shortly after ice-on and before ice-off). Epi-, meta- and hypolimnetic water in Lake 373 were routinely sampled for dissolved N_2O every 2-4 weeks through the field season to provide reference data for the aquaculture experiment in Lake 375. Phytoplankton nutrient status (alkaline phosphatase activity and nutrient debt) was measured approximately every 2-4 weeks during the open water season in both epi- and meta-limnetic waters. Epilithon were sampled during midsummer in all LTER lakes and analyses included metabolism, chemical composition (stoichiometry) and algal and bacterial composition. Phytoplankton and zooplankton analyses included identification to species and biomass estimates. *Chaoborus* and *Mysis* in lakes 373 and 240 were sampled for comparisons to specific experimental lakes. Mark-recapture work to estimate fish populations occurred in spring and/or fall, depending on the species. Movements of lake trout and white suckers in Lake 373 were studied with acoustic telemetry for the purpose of comparison to those in the Aquaculture experimental lake. Cyprinid population data collection continued in the spring and fall in Lake 442 to provide specific reference information for the EDC experiment in Lake 260.

Progress continued on archiving data in the ELA Database, a secure and managed database. Further improvements were made to the functionality of the Retrieval application. Most core data sets are up to date to 2002 and 2003 data will be added over the winter of 2003/4 after analyses and QA/QC are complete. Content summaries were added to the Retriever, which give users concise overviews of what data is available for which lakes and years. Documentation was improved as work began on developing information sheets (meta-data) about each data set. Also, more processed data functions were incorporated which provide monthly and annual summaries of meteorological and hydrological data. Major improvements were made to the hydrology and field services projects data management applications. The data sets containing light transparency profiles (PAR) and light attenuation coefficients were given major attention, brought up to date and added to the Database. Work began on compiling digital bathymetric maps of major ELA lakes for the purpose of having the most up-to-date version included in the ELA Database.

New Monitoring Initiatives:

- A new temperature logger chain was deployed in Lake 239, at the centre buoy, to continuously monitor temperature at 0, 5, 10, 15, 20, 25 and 29 metres.
- A reconditioned "Wet Fall Precipitation" collector was deployed at the Met Site in August, 2003.
- A ground-based UV meter was installed at the Met Site in October, 2003.
- The weir at the NW inflow of Lake 239 was rebuilt in the fall of 2003.

Presentations:

Dr. Ken Mills gave the following presentations:

- Growth, recruitment, and age-at-maturity of lake whitefish after pulse exploitation in a small Ontario lake. (Oral presentation at AFS annual meeting)
- Effects of macrophyte removal on yellow perch and other fish species in a small boreal lake (Poster at Percis III international symposium).

- Effects of macrophyte removal and recovery on the fish populations of Lake 191 (Poster at AFS 2003 annual meeting).

The following were presented at the SCL Annual Meeting, 2-5 January 2003, Ottawa, ON:

- Baulch, H. M., M. A. Turner D. L. Findlay, R. Vinebrooke, and L. L. Hendzel. Is Chla often misused as a measure of biomass and productivity in benthic algal studies?
- Mark D. Graham, Michael A. Turner, and Rolf D. Vinebrooke. Empirical evidence of the importance of pollen rain to algal communities in boreal lakes.
- M. A. Turner, D. L Findlay, E. M. Watkins, H. Baulch, S. E. M. Kasian and D. McNicol. Resilience of benthic algal associations in a boreal forest lake recovering from acidification.

Grant Proposals (ELA LTER data used as leverage) and Collaborative Projects with External Researchers:

- Michael Turner to Environment Canada ($20 K in 2003/4): Memorandum of understanding for a joint project to determine the ability of aquatic biota in boreal forest lakes to recover from acidification
- Michael Turner to Environment Canada ($10 K in 2003/4): Memorandum of understanding for a joint project to determine the ability of boreal forest lakes to recover physically and chemically from acidification
- Michael Turner with Rolf Vinebrooke: EJLB Foundation proposal, which should be providing $5 K/yr for the next two years should also be noted. Rolf's proposal is titled "Identification of Ecological Bottlenecks to Recovery of Biodiversity in Acidified Canadian Shield Lakes".
- Michael Turner with Rolf Vinebrooke.: DFO University Science Subvention Fund successful proposal should also be noted (3 yr @ $15 K/yr beginning in 2003). It is titled: Impacts of biological delay responses and species re-introductions on the productive capacity of recovering boreal lakes. Mark Graham is a Ph.D. student.
- Michael Turner: Letter of intent to the Climate Change Action Fund was submitted Nov 2002 for research beginning in 2003. Title: the climatic variables of increased temperature and diminished dissolved organic matter as modifiers of the productivity and biological diversity of boreal forest lakes. (unsuccessful)
- Michael Turner: Letter of intent to the Ontario Great Lakes Area Fish Habitat Management group. The proposal was titled: Can food quality (stoichiometry) refine our understanding of the factors controlling productive capacity and be useful as a tool for measuring adverse effects on benthic fish habitats? (unsuccessful)

Publications, Reports and Theses (using LTER data):

Published or In Press:

- Baulch, H. M., M. A. Turner, D. L. Findlay, R. Vinebrooke, W. Donahue, and L. Hendzel. 2004. Is chlorophyll appropriate as a measure of biomass in benthic algal studies? *J. North Amer. Benthol. Soc*. **23** (Submitted as Perspectives Article 9 September 2003)
- Baulch, H. M., D. W. Schindler, M. A. Turner, D. L. Findlay, P. Leavitt and M. P. Paterson. 2004. The effects of experimental warming on epilithic composition in a boreal lake. *Limnol. Oceanogr*. (in revision)

Summary of ELA Research for 2003

- Baulch, H. M., M. A. Turner and D. W. Schindler. 2004. Effects of increased temperature on epilithic metabolism and potential implications of climatic change. *Limnol. Oceanogr.* (in revision)
- Donahue, W. F., M. A. Turner, D. L. Findlay, and P. R. Leavitt. 2003. The role of solar radiation in structuring of shallow benthic communities of boreal lakes. *Limnol. Oceanogr.* **48**(1): 31-47.
- Findlay, D.L. 2003. Response of phytoplankton communities to acidification and recovery in Killarney Park and the Experimental Lakes Area (Ontario). *Ambio* **32**: 190-195.
- Findlay, D.L., S.E.M. Kasian, M.J. Vanni, M. Paterson, A Salki, and K. Mills. 2004. Dynamics of a boreal lake ecosystem during a long-term manipulation of top predators. *Ecosystems* (Submitted).
- Frost, P. C., S. E. Tank, M. A. Turner, and J. J. Elser. 2003. Elemental composition of littoral invertebrates from oligotrophic and eutrophic Canadian lakes. *J. North Amer. Benthol. Soc.* **22**: 51-62.
- Huebert, D., M. A. Turner, D. L. Findlay, L. L. Hendzel, R. A. Bodaly, and W. A. Jansen. 2004. The divergent impacts of experimental lake-level drawdown on planktonic and benthic plant communities in a boreal forest lake. *Can. J. Fish. Aquat. Sci.* (in revision)
- Jeffries, D. R., T. A. Clair, S. Couture, P. J. Dillon, J. Dupont, W. Keller, D. K. McNicol, M. A. Turner, R. Vet, and R. Weeber. 2003. Assessing the recovery of lakes in southeastern Canada from the effects of acidic deposition. *Ambio* **32**: 176-182.
- Mills, K.H., S.M. Chalanchuk, D.J. Allan, K. L. Howland, and R.F. Tallman. 2003. The fin-ray method of aging lake trout. *Proceedings of the Lake Trout Workshop*, Whitehorse 2002. (in press).
- Mills, K.H., S.M. Chalanchuk, and D.J. Allan. 2003. Lake trout spawning locations and spawning site fidelity in small Ontario lakes. *Proceedings of the Lake Trout Workshop*, Whitehorse 2002. (in press).
- Mills, K.H., S.M. Chalanchuk, and D.J. Allan. 2003. A long-term study of the dynamics of lake trout populations in small lakes. *Proceedings of the Lake Trout Workshop*, Whitehorse 2002. (in press).
- Mills, K.H., S.M. Chalanchuk, and D.J. Allan. 2003. The population dynamics of unexploited lake whitefish (*Coregonus clupeaformis*) populations and their responses to stresses. *Proceedings of the whitefish-Diporea Great Lakes Fishery Commission Workshop* – Ann Arbor, 2002. (in press)
- Mills, K.H., S.M. Chalanchuk, and D.J. Allan. 2003. The fin-ray method of aging lake whitefish. *Ann. Zool. Fennici*. (in press).
- Mills, K.H., E.C. Gyselman, S.M. Chalanchuk, and D.J. Allan. 2003. Growth, annual survival,age and length frequencies for unexploited lake whitefish populations. *Ann. Zool. Fennici.* (in press).
- Turner M. A., H. M. Baulch, S. E. M. Kasian, D. L. Findlay, R. D. Vinebrooke, and D. K. McNicol. 2004. Resilience and hysteresis in benthic algal associations of a boreal forest lake recovering from acidification. *Limnol. Oceanog*. (Submitted 10 November 2003).
- Valeo, C., K. Beaty and R. Hesslien. 2003. Influence of forest fires on climate change studies in the central boreal forest of Canada. *J. of Hydrology* **280**: 91-104.

- Vinebrooke, R., M.D. Graham, D.L. Findlay, and M.A. Turner. 2003. Compositional stability of epilithic algal assemblages in atmospherically and experimentally acidified boreal lakes. *Ambio* **32**: 196-202.
- Vinebrooke, R. D., D. W. Schindler, D. L. Findlay, M. A. Turner, M. Paterson, and K. H. Mills. 2003. Trophic dependence of ecosystem resistance and species compensation in experimentally acidified Lake 302S (Canada). *Ecosystems* **6**: 101-113.
- Weidman, P., M. A. Turner, and G. G. Goldsborough. 2004. The depth distribution of UV effects in the shallow littoral zone. *J. North Amer. Benthol. Soc*. (in revision)

In Preparation:

- Baulch, H. M, M. A. Turner, D. L. Findlay, R. D. Vinebrooke, W. F. Donahue, and L. L. Hendzel. 2003. Is chlorophyll misused as a measure of biomass in benthic algal studies? (In prep)
- Graham, M., R. Vinebrooke, M. A. Turner et al. 2004. Empirical evidence of the importance of pollen rain to algal communities in boreal lakes. Limnol. Oceanogr. (In preparation as note as of 2003 Jan.)
- Jeffries, D. R., T. Clair, S. Couture, P. J. Dillon, C. Gagnon, S. E. M. Kasian, W. Keller, D. McNicol, M. A. Turner and R. Weeber. 2004/5. Effects of climate on the coherence of the responses of boreal lakes in central and eastern Canada to reductions in sulphur deposition. (In preparation as of summer 2003)
- Turner, M. A., H. M. Baulch, S. M. Chalanchuk, I. J. Davies, D. L. Findlay, B. J. Hann, L. Hendzel, R. H. Hesslein, S. E. M. Kasian, D. K. McNicol, K. H. Mills, M. J. Paterson, C. G. Trick, and R. D. Vinebrooke. 2004. Biological disorder in a boreal forest lake recovering from acidification. Ecosystems (In revision after first co-author review; delayed by co-author data unavailability)

Long-term Data Sets Requested for Research Projects:

- Bristow, Corben. R. Hesslein's graduate student from University of Ottawa. Stream Discharge, water level, bathymetry, chemistry, water temperature and wind speed for a range of lakes, to be used in the development of predictive models.
- Classen, Jessie. University of British Columbia. Physical Limnology from M. Lyng
- Crusius, John. Woods Hole. Precipitation data.
- Jeffries, Dean. Environment Canada. Water chemistry from M. Turner.
- Laird, Kathleen, and Brian Cumming. Queen's University. Bathymetric and other physical lake data for sediment coring sites from J. Shearer.
- Minns, Ken. Fisheries and Oceans Canada. Physical limnology data.
- Molot, Lewis. York University. From M. Turner.
- Parks, John. Ontario Ministry of the Environment. Water chemistry data from M. Turner.
- Radomske, Erin. Okanagan University College. PAR data.
- Regoli, Lidia. International Zinc Association - Europe. Water chemistry.
- Saquet, Michelle. University of Waterloo. Physical Limnology from M. Lyng.
- Valeo, Cathy. University of Calgary. Evaporation Pan data.

- Venkiteswaren, Jason. University of Waterloo. Physical Limnology from M. Lyng.
- Vinebrooke, Rolf. University of Alberta. Phytoplankton population data from D. Findlay.
- Weeber, Russ and Don McNicol. Environment Canada. Trace metal data. From M. Turner.

Plans for 2004:

The overall plan is that the LTER and Data Management project will continue in 2004 with little modification. Given a similar budget and availability of external funding support, most field sampling and data collection will continue in order to maintain long-term records. As well, data management initiatives, both for the centralized ELA Database and for applications at the project level, will continue to be supported.

Some more specific plans for 2004 are:

Len Hendzel reports that sampling for nutrient status work will not continue in 2004. Sufficient data has been collected over the past 10+ years, and efforts will now be directed towards publications.

Mark Lyng states that some site cleanups around LTER lakes are necessary. Further, a new data management initiative is required to handle the data generated from loggers monitoring temperature at lake surfaces and at depth profiles in L239.

Depending upon funding, Michael Turner will pursue an exploration of the role of stoichiometry in littoral productive capacity. Further, he will likely participate in P. Weidman's Ph.D. research into understanding physico-chemical impacts of climate change and reduced DOC on aquatic biota.

Ken Beaty recommends that several weirs require attention to maintain the hydrological network. The weir at the L224 outflow should be re-established and one at the L442 outflow should be rebuilt. Further, the weirs at L239 NEIF and EIF require repairs.

Susan Kasian will continue the exploration of climate regimes at the ELA, and their impact on physical and chemical limnological variables and biota. The goal is a publication.

Many other publications, not yet identified and which rely on LTER data records, will be pursued by project researchers.

Specific Ancillary Studies:

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, Ian Delorme, and others, has primary responsibility for this facility and data are contributed to the AES national climate database. With more than 34 years of continuous monitoring, this site is an official long-term station in the AES Climate network. Meteorological variables (air temperature, precipitation, wind speed and direction, bright sunshine and evaporation) were monitored daily again in 2003.

<u>Canadian Air and Precipitation Monitoring Network (CAPMoN)</u>

ELA personnel, under the direction of K. Beaty, continued to operate a CAPMoN station at the ELA met site in 2003. 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_2 and HNO_3 in the atmosphere, Cl, SO_4, NO_3, Na, NH_4, 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.

Canadian Network Isotopes in Precipitation (CNIP)

The ELA is a node in a Canadian network monitoring isotopes (^{18}O, Deuterium) in precipitation. This network (*http://sciborg.uwaterloo.ca/~twdedwar/cnip/ela.html*), coordinated from the University of Waterloo, comprises 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.

Impacts of Natural Disturbances on the Lake 239 Watershed

Long-term hydrological, meteorological, and chemical monitoring in the calibrated catchments of this watershed continued during 2003. Portions of the watershed have been perturbed by a major forest blow-down in 1973, by a less extensive wind event in August 2001, 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 34 years. Long-term records of meteorology and hydrology of the Lake 239 basin were maintained in 2002. The 3 inflowing streams and outflow of Lake 239 were monitored for volume of flow (continuous record) and chemical composition (weekly). No chemical additions are made.

In June, 2003, researchers from the United States Forest Service in Minnesota, the Ontario Ministry of Natural Resources, and the Canadian Forestry Service toured sites in the eastern portion of this watershed to evaluate forest re-growth following the sequential fires in 1974 and 1980. The U.S. researchers hope to use the information to assess the feasibility and value of using prescribed burns to rehabilitate large areas of forest blowdown in the Superior National Forest.

Limnological Monitoring of Core LTER Lakes

The five core lakes (114, 224, 239, 373, and 442) were again monitored, where possible, for all major disciplines. Sampling includes hydrology, water temperature, Secchi depth and light extinction profiles, water chemistry, primary production, epilithon, phytoplankton populations and nutrient status, zooplankton, and fish. Discipline specific monitoring continued in other lakes to maintain long-term records. Measurements of limnological variables and samples for chemistry and biological analyses were taken every 2 weeks through the open water season and twice over winter (shortly after ice-on and before ice-off).

Concentrations of N_2O were measured bi-weekly through the open water season in the epi-, meta- and hypolimnion of LTER lakes. Phytoplankton nutrient status was measured approximately every 2-4 weeks. Epilithon monitoring varied in intensity by lake and included metabolic parameters, stoichiometric information and taxonomy. Phytoplankton and zooplankton analyses included identification to species and biomass estimates. *Chaoborus* and *Mysis* in Lakes 373 and 239 were sampled for comparisons to specific experimental lakes. Mark-recapture work to estimate fish populations occurred in spring and/or fall, depending on the species. Movements of lake trout and white suckers in Lake 373 were studied with acoustic telemetry for the purpose of comparison to those in the Aquaculture experimental lake. Benthic invertebrate monitoring is still unfunded.

Phytoplankton Nutrient Status in ELA Lakes

Purpose or Goals:

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 it's 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 lakes during 2003 in support of a number of ELA projects. These included:

- LTER – long term reference database (114, 224, 239, 373, 442),
- Aquaculture – measuring the impacts of aquaculture waste production on water column primary productivity, nutrient levels and secondary production (375 N & S, 373),
- Acid Recovery – studying the recovery of an acidified system (302S) ,and
- Food Chain Studies – artificial eutrophication study (227).

Principal Investigator:

- Len Hendzel (DFO, Freshwater Institute)

Work carried out during 2003:

2002 was to have been the last year for nutrient status monitoring of the LTER lakes, as well as Lake 302S (Acid Recovery) and Lake 227 (Food Chain Studies); however work on these lakes was continued through 2003. Phytoplankton nutrient status (alkaline phosphatase activity and nitrogen debt) was measured on epilimnetic and metalimnetic water samples from LTER lakes 114 (epilimnion only) 224, 239, 373, 442 and other ELA lakes (227, 302S 375 North and South) approximately every 2-4 weeks between early May and early November. In addition, Lake 227 nitrogen fixation also was measured on several occasions during the summer.

Publications or Spin-off Research:

A major analysis of the ELA nutrient status data was planned for the summer of 2003, but was not initiated because of time limitations. A complete analysis of the data is planned for 2004. This will then be followed by publishing the data in a report or primary publication.

Plans for 2004:

Sampling of the LTER lakes, as well as L227 and L302S, for nutrient status will not be continued in 2004. As noted, sufficient data have been collected over the past 10+ years and they now need to be analyzed and published. It is uncertain at this time whether nutrient status measurements for L375 North and South will be continued next year.

OXYGEN DYNAMICS IN LAKES, RIVERS AND GROUNDWATER: A NEW ISOTOPIC APPROACH

Principal Investigators:

Dr. Sherry Schiff and Jason Venkiteswaran (Ph.D. candidate)

(Department of Earth Sciences, University of Waterloo, Waterloo, Ontario, N2L 3G1)

Objectives:

Continue to develop and deploy an oxygen stable isotope (^{16}O and ^{18}O) method of determining the balance of heterotrophy, autotrophy and atmospheric influence in lakes, rivers and groundwater in Canada.

Summary of Work carried out during 2003:

Six ELA lakes (L224, L227, L239, L373, L442, L979) were sampled monthly by the ELA Lake Sampling group for O_2 concentration and isotopic ratio in profile (various depths from the centre buoy). These lakes comprise some of the lakes routinely sampled for primary productivity. In addition, FLUDEX reservoirs were also routinely sampled. All concentration analyses were performed at the ELA. Analyses for isotopic ratio will begin shortly and continue into the new year. Results will be available in the spring.

Continuation of Study / Spin-off Research:

Samples will be taken for concentration and isotopic ratio during the next few years. This data set is part of a larger survey involving several researchers at the University of Waterloo.

RECOVERY OF BOREAL LAKES FROM ACIDIFICATION

ELA researchers and colleagues have focused on characterizing the natural recovery potential of several lake ecosystems that had previously been experimentally acidified. Beginning in 1974, ELA researchers had embarked on a major program of 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. This program provided scientific evidence that was instrumental in the development of the 1991 Canada-US Clean Air Act, which reduced sulphur emissions, partly in an effort to protect the integrity of acid-sensitive aquatic ecosystems. ELA recovery research is similarly proving useful for management of fisheries and for adjusting legislation designed to protect Canadian aquatic ecosystems and their biota, and finds its rationale in DFO's 1986 Policy for the Management of Fish Habitat.

Our study of the natural potential of boreal forest lakes to recover from acidification remains particularly relevant. Despite substantial reductions in the release of acidifying emissions, there are a large numbers of lakes in southeastern Canada and northeastern United States that have not yet recovered from anthropogenic acidification. Acid deposition continues to exceed critical loads across large portions of eastern Canada, causing acidification in these areas to continue, and threatening the biodiversity of these aquatic ecosystems. For example it has been estimated that even with full implementation of the Canada – USA Air Quality Agreement by 2010, about 76,000 lakes or about 10^6 hectares will remain chemically damaged (i.e., their pH will remain below 6 even though they could be expected to have pHs higher than 6). Similarly, Department of Environment chemical models predict that outside of the Sudbury area about one quarter of the lakes in eastern Canada will still be acidified after 2010, and integrated assessment models have predicted that about 160,000 Canadian fish populations will remain at risk.

The unexpected delays in recovery of many lakes is caused by several factors. These include:

- nitrogen oxide emissions have not been reduced as much as sulphur emissions;
- some of the buffering components in precipitation have declined;
- watersheds are still releasing acidifying substances;
- some of the buffering potential in the watersheds has been exhausted by previous acidification.

Also, importantly, from our research we have come to recognize that some recovering systems are more vulnerable to acid inputs than was previously recognized, and that some of our expectations for the recovery of aquatic ecosystems have been incorrect.

RECOVERY OF LAKES 302 AND 223 FROM EXPERIMENTAL ACIDIFICATION

Purpose or Goal of the Research:

The general goal of our studies 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. To achieve this goal we are currently studying the ability of two experimental lakes to recover from acidification. We have been studying the recovery of the physical, chemical and biological properties of these aquatic ecosystems as their target pH has been relaxed following years of experimental acidification. Although the principal experimental system recently under study has been Lake 302S, we have continued to spend a small amount of effort studying Lake 223 and L302N.

In Lake 302S, we have found that several indirect and complex effects of acidification have complicated our ability to predict the rate and form of recovery. Sometimes a lake's internal ability to buffer acid can be impaired. As a result, we have been studying chemical and biological impediments to recovery. Previously we have conducted studies to discover possible remedies where such blockages exist, and to ascertain the possible influences of climate warming upon rates of recovery from acidification.

The policy implications of our studies include indirect testing of the suitability of current acidifying emissions standards, and importantly, better defining the nature of recovery of boreal forest lakes from acidification. These goals support the Federal, Provincial and Territorial governments' commitment to the recent Canada-Wide Acid Rain Strategy for Post-2000. Our research is also proving to be an important component of the upcoming Canadian Acid Rain Assessment in 2004. The research also supports DFO's 1986 Policy for the Management of Fish Habitat by enhancing Canadians' ability to mitigate acid-related threats to the productive capacity of fish habitats.

Participating Agencies and Principal Investigators:

There were primarily two funding agencies supporting our research in 2003. (a) Environment Canada (EC, specifically Mr. Don McNicol of the Canadian Wildlife Service and Dr. Dean Jeffries of National Water Research Institute) provided operating funds that allowed us to continue the Lake 302S experiment and to conduct limited sampling in Lake 223. (b) The

Department of Fisheries and Oceans (DFO) provided salary support for its researchers, and provided support for the ELA platform from which these experiments has been conducted.

DFO members of the research group in 2003 (arranged alphabetically) were H. Baulch, K. Beaty, S. Chalanchuk, D. Findlay, Dr. R. Hesslein, L. Hendzel, S. Kasian, M. Lyng, Dr. K. Mills, S. Page, Dr. M. Paterson, and Dr. M. Turner. Colleagues from a variety of agencies who were also involved either directly in the experiments or in the interpretation of the associated experimental results during 2003 included: Dr. B. Hann (U. of Manitoba), Dr. D. Jeffries (EC), D. McNicol (EC), Dr. L. Molot (York U.) and Dr. R. Vinebrooke (U. of Alberta). Dr. R. Vinebrooke's involvement was supported by DFO's University Subvention Fund and by the EJLB Foundation. Dr. Michael Turner (DFO) is the scientist in charge of the ELA study.

Description of Work during 2003:

pH was unregulated in Lake 302S during 2003 for the third year. 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 in the littoral zone (littoral microcrustacea and epilithon). We also conducted follow-up sampling to continue to monitor the success of the lake whitefish addition that we made to Lake 302S in the fall of 1999.

We continued to sample Lake 223 to evaluate the recovery of lake trout and the permanence of changes in the white sucker population, and Lake 302N to monitor the recovery of the lake whitefish population from the dual stressors of acidification and eutrophication. There was only restricted sampling of water chemistry and phytoplankton in Lake 223 and L302N.

Listing of New Publications and those in Preparation:

- Donahue, W. F., M. A. Turner, D. L. Findlay, and P. R. Leavitt. 2003. The role of solar radiation in structuring of shallow benthic communities of boreal lakes. *Limnol. Oceanogr*. **48**(1): 31-47.
- Findlay, D.L. 2003. Response of phytoplankton communities to acidification and recovery in Killarney Park and the Experimental Lakes Area (Ontario). *Ambio* **32**: 190-195.
- Jeffries, D. R., T. A. Clair, S. Couture, P. J. Dillon, J. Dupont, W. Keller, D. K. McNicol M. A. Turner, R. Vet, and R. Weeber. 2003. Assessing the recovery of lakes in southeastern Canada from the effects of acidic deposition. *Ambio* **32**: 176-182.
- Jeffries, D. R., M. A. Turner, R. Hesslein, S. E. M. Kasian, and R. Hecky. 2004. Hysteresis in the physicochemical properties of a boreal forest lake recovering from acidification. *Ecosystems* (In preparation).
- Mills, K.H., S.M. Chalanchuk, D.L. Findlay, D.J. Allan, and B.R. McCulloch. 2002. Condition, recruitment, and abundance of lake whitefish (*Coregonus clupeaformis*) in a fertilized acid lake. *Arch. Hydrobiol. Spec. Issues Advanc. Limnol*. **57**: 423-433.
- Mills, K.H., S.M. Chalanchuk, and D.J. Allan. 2002. Abundance, annual survival, and recruitment of unexploited and exploited lake charr, *Salvelinus namaycush*, populations at the Experimental Lakes Area, northwestern Ontario. *Environmental Biology of Fishes* **64**: 281-292.
- Mills, K.H., S.M. Chalanchuk, and D.J. Allan. 2002. Biomass and production of lake charr during the acidification and pH recovery of a small Ontario lake. *Environmental Biology of Fishes* **64**: 293-301.

- Molot, L. A., and P. J. Dillon. 2003. Variation in iron, aluminum and dissolved organic carbon mass transfer coefficients in lakes. *Water Research* **37**: 1759-1768.
- Paterson, M. et al. 2005. Consequences of the patterns of zooplankton recovery from acidification. *Freshwater Biology* (In preparation)
- Turner, M. A., H. M. Baulch, S. M. Chalanchuk, I. J. Davies, D. L. Findlay, B. J. Hann, L. Hendzel, R. H. Hesslein, S. E. M. Kasian, D. K. McNicol, K. H. Mills, M. J. Paterson, C. G. Trick, and R. D. Vinebrooke. 2004. Biological disorder in a boreal forest lake recovering from acidification. *Ecosystems* (In preparation).
- Turner, M. A., H. M. Baulch, S. E. M. Kasian, D. L. Findlay, R. D. Vinebrooke, and D. McNicol. 2004. Resilience and hysteresis in benthic algal associations of a boreal forest lake recovering from acidification. *Limnology and Oceanography* (Submitted).
- Vinebrooke, R. D., D. W. Schindler, D. L. Findlay, M. A. Turner, M. Paterson, and K. H. Mills. 2003. Trophic dependence of ecosystem resistance and species compensation in experimentally acidified Lake 302S (Canada). *Ecosystems* **6**: 101-113.
- Vinebrooke, R. D., M. Graham, D. L. Findlay and M. A. Turner. 2003. Resilience of epilithic algal assemblages in atmospherically and experimentally acidified boreal lakes. *Ambio* **32**: 196-202.

Plans for 2004:

2004 will be the fourth consecutive year during which no acid will be added to Lake 302S, i.e. there will be no set target pH and the lake will be allowed to achieve whatever pH level that it can. Dr. Vinebrooke (University of Alberta) and his students will conduct process-oriented research into factors controlling the rate of biological recovery in conjunction with parallel investigations in lakes of southeastern Ontario that are recovering from regional acidification. His research will be supported by DFO's University Subvention Fund (year 2 of 3) and by the EJLB Foundation (year 2 of 2). If funding continues to be available from Environment Canada during 2004, we will monitor the lake's physical, chemical & biological properties, including further assessing the success of the new whitefish cohort. As in recent years, we anticipate being able to conduct only a limited amount of sampling in Lakes 223 and 302N.

MERCURY LOADING AND BIOACCUMULATION

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.

Current studies at the ELA, both on a whole watershed scale, and in various mesocosms, are helping to answer the questions about mercury contamination in aquatic biota, particularly fish, and delineate the linkages between mercury in fish and the mercury that is deposited from the atmosphere.

MERCURY EXPERIMENT TO ASSESS ATMOSPHERIC LOADING IN CANADA AND THE UNITED STATES (METAALICUS)

Background and Rationale:

The presence of methylmercury (MeHg) in fish is a natural occurrence, but fish MeHg concentrations in remote lakes in eastern Canada, including Ontario, are often above the 0.5 ppm limit for commercial sale. This is the case even in the absence of direct anthropogenic discharges of mercury to the lakes. Methylmercury is produced from inorganic mercury by bacteria, and is accumulated preferentially by fish. Fish with elevated mercury concentrations are a health concern due to the toxicity of MeHg and exposure via fish consumption. There is a general consensus that elevated MeHg concentrations of fish in remote lakes are influenced by inputs of atmospheric inorganic mercury directly to lakes and indirectly via their watersheds. Furthermore, anthropogenic mercury emissions are likely contributing to mercury loading rates to lakes. Anthropogenic inputs originate as emissions from coal combustion, waste incineration, and as emissions from other industrial and mining processes.

Much research has been undertaken to better understand the impacts of mercury emissions on fish mercury concentrations, and the potential effectiveness of emissions controls. In North America alone, emission controls for electric utilities have been estimated to cost billions of dollars per year. Despite this research activity, a fundamental question remains unanswered: What will happen to fish mercury concentrations if atmospheric mercury deposition is reduced? Uncertainty remains regarding both the magnitude and timing of the response.

The METAALICUS experiment (Mercury Experiment To Assess Atmospheric Loading in Canada and the US) is currently underway at the Experimental Lakes Area (ELA) to examine this issue. METAALICUS involves the addition of stable, non-radioactive, mercury isotopes to a whole ecosystem to see if there is a response in mercury concentrations fish. In 2001, 2002 and 2003, mercury was added to Lake 658 and its watershed at an annual rate approximately equal to 3 times the annual wet deposition of mercury at the ELA.

Some of the added mercury is being measured in fish, but it is very likely that the mercury concentrations in fish have not yet stabilized in response to the changing mercury load. We cannot yet say what the eventual response of fish mercury concentrations will be following a change in mercury loading. Permission is currently being requested therefore to continue mercury additions at the same levels as in the first three years (up to 30 $\mu g/m^2/yr$ of additions), for up to 3 more years (2004 through 2006 inclusive).

Experimental Objectives:

METAALICUS is designed with the following overall objectives:

- To determine the relationship between the atmospheric deposition of mercury to a lake ecosystem 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 (Hg(II)).
- To establish the relative importance of mercury deposited on uplands, wetlands, or onto the lake surface as sources of MeHg to fish.

Summary of ELA Research for 2003

Participants:

Principle Investigators:

R.A. Bodaly, DFO Winnipeg, and R. Harris, Tetra Tech Inc., Toronto

Other DFO Winnipeg Investigators:

M. Paterson, P. Blanchfield, C. Podemski.

Other Investigators:

J. Rudd, R & K Research Inc., Saltspring Is., BC
C. Kelly, R & K Research Inc., Saltspring Is., BC
H. Hintelmann, Trent University, Peterborough, ON
C. Gilmour, Academy of Natural Sciences, Estuarine Research Center, St. Leonard, MD
J. Hurley, U. of Wisconsin, Madison WI
C. Babiarz, U. of Wisconsin, Madison, WI
D. Krabbenhoft, USGS, Madison WI
V. St.Louis, U. of Alberta, Edmonton, AB
S. Lindberg, Oakridge National Laboratory, Oakridge, TN
M. Amyot, Université de Montréal, Montréal, QC
B. Branfireun, U. of Toronto, Toronto, ON

International Advisory Panel:

A. Iverfeldt, 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, Winnipeg.
J. Wiener, University of Wisconsin, Lacrosse

Study Description:

As described above, METAALICUS is a whole-ecosystem experiment in which mercury loading to a headwater lake and its watershed is being altered experimentally. Lake 658 at ELA was selected for the study. It is a small (8.3 ha), low productivity, headwater lake on the Canadian Shield and is one of the lakes reserved for research at the ELA. Background studies documenting site conditions prior to the experiment were carried out in 1999-2000 and are discussed in the 2001 *Summary of Major Research Projects at the ELA*.

Mercury is being added in the form of stable, non-radioactive isotopes of inorganic mercury (Hg(II)). The power of using isotopes lies in the ability to follow the newly deposited mercury separately from background mercury. Furthermore, different mercury stable isotopes are being added to the upland, wetland and lake surface (^{200}Hg, ^{198}Hg and ^{202}Hg respectively) to determine the relative contributions of these sources to fish mercury levels.

ELA is a low deposition area for mercury, with approximately 7 ug/m2/yr of wet mercury deposition. The low mercury deposition rate at ELA means that adding the equivalent of about 1/6th of a teaspoon of mercury per year (approximately 12.5 g) increases wet Hg deposition to the 52 ha Lake 658 ecosystem (lake and watershed) by 3-5 fold. This action results in a mercury wet deposition rate to the experimental system that is comparable to rates currently observed in some parts of the US Northeast and Florida.

Mercury concentrations are being tracked in all major compartments in the lake, watershed, and atmosphere. Detailed process studies are also being carried out to follow the movement and transformations of mercury through the watershed and lake, as well as air/surface exchange of mercury. This process-based approach will allow us not only to document what happens, but also to understand why. This is essential if we are to use the results of the study to make predictions for other locations. The approach is providing critical information for an existing model that predicts fish mercury concentrations in lakes and the effects of remedial actions such as reductions in mercury loading.

The experiment is being carried out in two phases. Phase I involved pilot and baseline studies in 1999-2000, to prepare for Phase II. The ELA Management Board approved Phase I studies at the February 1999 and February 2000 meetings. Final approval of the full-scale experiment for 2001 through 2003 was obtained in March 2001. The full-scale experiment began in 2001. Mercury additions have been carried out in 2001, 2002 and 2003. Permission is currently being sought to continue additions for 1 to 3 additional years (2004 - 2006).

Milestones:

- (1999-2000) Two years of pilot scale experiments; pre-addition background monitoring of both candidate lakes.
- (2001-2003) Initial three years of whole-ecosystem isotope additions to upland and wetland areas of the watershed and to the lake surface, and continued pilot studies.
- (2004 and beyond) Permission being requested for 1 to 3 more years of whole-ecosystem isotope additions. The loading phase of the experiment followed by monitoring of the food chain and fish mercury concentrations until conditions return to pre-addition levels.

Pilot Scales Studies:

Pilot-scale studies from 1999-2002 were described in the *Summaries of Major Research Projects at the ELA* for 2000, 2001, and 2002, based on results available at the time. These included:

- Isotopic Hg(II) additions to small upland plots;
- Isotopic Hg(II) additions to a wetland plot; and
- Additions of isotopic mercury to lake enclosures

In 2003, some of the pilot-scale studies done from 1999-2002 were continued or expanded to gain additional knowledge. Updates to the pilot-scale studies follow:

Pilot Isotopic Hg(II) Additions to Upland and Wetland Catchments

Hg additions to pilot-scale upland plots at ELA were carried out from 1999-2002. These experiments broadened the understanding of mercury behaviour in upland systems, and provided important information when designing a method to apply mercury to the terrestrial system. During simulations of dry deposition or light rain (the conditions under which mercury is being applied for the full scale experiment), little of the mercury applied to upland plots has been exported in runoff since the pilot studies began (up to four years of data for some test areas).

Pilot scale studies from 1999-2002 at a 500 m^2 wetland plot bordering Lake 115 at ELA suggested that the vertical mobility of mercury added to the wetland plot was greater than expected, and confirmed wetlands as active methylation sites.

Additions of Isotopic Mercury to Lake Enclosures

During 2000-2001, a study of isotopic additions of $^{200}Hg(II)$ to four enclosures was carried out at Lake 239 at the ELA. The enclosure experiments in 2000-2001 confirmed for the first time that the new isotopic analytical methods being used could indeed follow added mercury isotopes through the water, sediments and food web, including fish.

In 2002, 11 corrals were set up in Lake 240, ELA, and dosed with varying amounts of mercury, up to 15X current annual wet deposition at ELA. In 2003, the corrals in Lake 240 were dosed a second time but with a different isotope than in 2002. This effort is described separately in this current report (see MESOSIM section, below).

Whole-Ecosystem Mercury Additions at Lake 658

Public Consultation:

During 2000, public information meetings were conducted 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 positive. It should be noted that there has been no public opposition or negative media coverage in connection with METAALICUS since permission was originally granted and the project began. Scientific, public, and governmental feedback has been very positive.

Research Activities:

After two years of pilot and baseline studies in 1999-2000, METAALICUS went "full scale" in June 2001. Stable non-radioactive Hg(II) isotopes are being applied to the upland, wetland, and directly to the lake surface in the Lake 658 watershed at ELA. The upland, wetland and lake are receiving ^{200}Hg, ^{198}Hg, and ^{202}Hg respectively. In each case the total mercury application rate is approximately 25 ug m^{-2} yr^{-1}. Isotopes have been applied during the 2001-2003 field seasons. The upland and wetland areas are being sprayed once per year by an airplane flying low over the canopy. Isotopes are being applied directly to the lake by mixing from a boat at a depth of 0.7m, over a series of 9 applications during the ice-free season. The mercury loading rate directly to the lake surface is approximately 22 ug/m2/yr.

Impact on Downstream Lakes:

Based on pilot-scale studies and our knowledge of the behaviour of mercury in ELA lakes, most of the added mercury will bound in the upland soils, wetland peat and lake sediments in the long term. Mercury in the Lake 658 outflow enters a very large downstream lake (Winnange Lake). We expect 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 Lake 658 and the very large volume of Winnange Lake relative to Lake 658 (approximately 1000x larger). Monitoring is being carried out in Winnange Lake to verify that the Winnange Lake food web is not impacted by the experiment. The concentrations of the three different mercury isotopes added to the Lake 658 watershed were examined in young-of-the-year yellow perch and northern pike prior to the addition of isotopes (June 2001) and two years after mercury additions began (May 2003). None of the mercury isotopes added as part of the METAALICUS study is presently detectable in Winnange Lake fish or sediments. This is not surprising considering that the transport of mercury isotopes out of Lake 658 has been measured and determined to be very small.

2004 Field Season:

The 2004 field season will be the fourth field season during which whole ecosystem mercury additions are planned. Permission is being requested to continue with 1-3 more years of full-scale mercury isotope additions to the Lake 658 lake and watershed. Detailed monitoring of site conditions, mercury concentrations, and the fate and transport of mercury will be undertaken. Pilot-scale studies will also continue.

Lake Restoration:

Prior to the beginning of METAALICUS, it was anticipated that MeHg concentrations in the food web following the mercury additions would be within the range presently observed in remote Canadian lakes that do not receive local anthropogenic mercury sources. This has been the case to date. If fish mercury concentrations do increase significantly in Lake 658, as a result of METAALICUS, it is expected that concentrations will return to background levels after mercury additions are stopped.

After the experiment has been completed, the study lake will be monitored 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*. During this recovery period, concentrations of mercury in fish and sediments in Winnange lake will also be monitored every second year.

Publications Completed or In Progress:

- Amyot, M., G. Southworth, S.E. Lindberg, H. Hintelmann, J.D. Lalonde, N. Ogrinc, A.J. Poulain, and K.A. Sandilands. Evolution of dissolved gaseous mercury in large enclosures amended with $^{200}HgCl_2$. (submitted to *Can. J. Fish. Aquat. Sci*.)
- Babiarz, C.L, J.P. Hurley, D.P. Krabbenhoft, C.C. Gilmour, and B.A. Branfireun. 2003. Application of ultrafiltration and stable isotope amendments to the partitioning of mercury in lake water and over land runoff. *Science of the Total Environment* **304**: 295-303.
- Branfireun, B.A., D.P. Krabbenhoft, H. Hintelmann, R.J. Hunt, J.P. Hurley, and J.W.M. Rudd. The transport and speciation of atmospheric mercury in a Boreal forest wetland: A stable mercury isotope approach. (submitted, October 2003)
- Hintelmann, H., R. Harris, A. Heyes, J. Hurley, C. Kelly, D. Krabbenhoft, S. Lindberg, J.W.M. Rudd, K. Scott and V. St. Louis. 2002. Reactivity and mobility of new and old mercury deposition in a boreal forest ecosystem during the first year of the METAALICUS study. *Env. Sci. Technol*. **36**: 5034-5040.
- Kelly, C.A., J.W.M. Rudd, and M.H. Holoka. 2003. The effect of pH on mercury uptake by an aquatic bacterium - implications for Hg cycling. *Environ. Sci. & Technol*. **37**:2941-2946.
- Lalonde, J.D., M. Amyot, M-R. Doyon, and J-C. Auclair. 2003. Photo-induced Hg(II) reduction in snow from the remote and temperate Experimental Lakes Area (Ontario, Canada). *J. Geophys. Res.* **108**, No. D6, 4200, doi: 10.1029/2001JD001534.
- Ogrinc, N., H. Hintelmann, C. Eckley, and S. Lojen. 2003. Biogeochemical influence on carbon isotope signature in boreal lake sediments. *Hydrobiologia* **494**: 207-213.
- St. Louis, V.L., J.W.M. Rudd and C.A. Kelly, B.D. Hall, K.R. Rolfhus, K.J. Scott, S.E. Lindberg and W. Dong. 2001. Importance of the rorest canopy to fluxes of methyl mercury and total mercury to boreal ecosystems. *Environ. Sci. Technol.* **35**: 3089-3098.

MESOCOSM SIMULATIONS OF ATMOSPHERIC MERCURY DEPOSITION TO LAKES (MESOSIM)

Rationale:

Waste products emitted from industrial activities, such as fossil fuel burning, have increased mercury levels in the atmosphere. Atmospheric mercury, primarily in the form of Hg(II), is dispersed by global air circulation and deposited to freshwater systems, where it is converted by bacteria to methyl mercury (MeHg). Methyl mercury is a toxic compound that is known to bioaccumulate in aquatic food webs, leading to elevated levels of mercury in fish. Wildlife and humans who consume these contaminated fish may be at serious risk of mercury exposure. The control regulations for mercury emissions to the atmosphere are being re-evaluated, for which a greater understanding of the relationship between atmospheric mercury deposition and methyl mercury concentrations in fish is required.

Goals:

The primary research objectives of the MESOSIM Project are:

1. To quantify the relationship between the rate of deposition of inorganic mercury from the atmosphere to aquatic systems and methyl mercury in fish.
2. To examine and model the biogeochemical cycling of atmospheric mercury in aquatic systems and the transfer of mercury through the food web, across a global range of wet deposition rates.

The outcomes anticipated from the MESOSIM Project are: to facilitate the extrapolation of results from METAALICUS to other systems, to lead to improvements in the predictive capabilities of mercury cycling models, and to contribute to policy reforms on industrial mercury emissions.

Principle Investigators:

Dr. Marc Amyot	*Dissolved Gaseous Mercury Production* Département des sciences biologiques, Université de Montréal, Montréal, QC m.amyot@umontreal.ca
Dr. Paul Blanchfield	*Experimental Design, Fish* Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, MB BlanchfieldP@dfo-mpo.gc.ca
Dr. Drew Bodaly	*Diane Orihel's Academic Supervisor* Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, MB BodalyD@dfo-mpo.gc.ca
Dr. Cynthia Gilmour	*Sediments and Periphyton* Estuarine Research Center, Academy of Natural Science, St. Leonard, MD, United States gilmour@acnatsci.org
Mr. Reed Harris	*Modeling* Tetra Tech Inc., Oakville, ON rharris6@cogeco.ca
Dr. Holger Hintelmann	*Hg Analyses* Environment and Resource Studies, Trent University, Peterborough, ON HHintelmann@trentu.ca
Ms. Diane Orihel	*M.N.R.M Thesis Project* Natural Resources Institute, University of Manitoba, Winnipeg, MB OrihelD@dfo-mpo.gc.ca
Dr. Michael Paterson	*Project Director, Experimental Design, Zooplankton* Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, MB PatersonM@dfo-mpo.gc.ca
Mr. Alex Poulain	*Dissolved Gaseous Mercury Production* Université de Montréal, Montréal, QC alexandre.poulain@umontreal.ca
Dr. John Rudd	*Experimental Design* Rudd and Kelly Research Inc., Salt Spring Island, BC r_kresearch@saltspring.com

Mr. George Southworth	*Evasion - Flux Chamber* Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, United States. southworthgr@ornl.gov

Work completed in 2003:

In June 2002, eleven 10m-diameter mesocosms were installed over the littoral sediments of Lake 240 at the Experimental Lakes Area, Ontario. In 2002 and 2003, the mesocosms were loaded with mercury at rates representing 0-15 times the natural annual rate of wet deposition of mercury to the Experimental Lakes Area (0-107 µgTHg/m^2). Mercury loading in the mesocosms was manipulated by adding eight weekly doses of a mercury stable isotope ($^{202}HgCl_2$ in 2002; $^{200}HgCl_2$ in 2003). At the beginning of the experiment, the mesocosms were stocked with 1+ year-old yellow perch (*Perca flavescens*) collected from L240, in densities comparable to natural systems. Over the course of the 10-week experiment, mercury levels in biota and their habitat were monitored. In the food web, periphyton, zooplankton, zoobenthos, and fish were analyzed for mercury concentrations. In the surrounding environment, samples of air, water, sediments were collected for mercury analyses. In September, 2003, the enclosures and all associated equipment were removed form Lake 240.

Plans for 2004:

No further field work is planned for this project in 2004. Analysis of samples collected in 2002 and 2003 will be completed and the results written up.

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 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?

EFFECTS OF A POTENT ESTROGEN MIMIC ON AQUATIC POPULATIONS - A WHOLE-LAKE ADDITION EXPERIMENT

Project Team:

Freshwater Institute:

Karen Kidd, Principal Investigator
Paul Blanchfield
Bob Evans
Dave Findlay
Ken Mills
Vince Palace
Michael Paterson
Cheryl Podemski
Alex Salki

Other Agencies:

Scott Brown, Environment Canada, Burlington, Ontario
David Graham, Department of Civil and Environmental Engineering, University of Kansas
Karsten Liber, Toxicology Centre, University of Saskatchewan
Mark McMaster, Environment Canada, Burlington, Ontario
Glen Van Der Kraak, Department of Biological Sciences, University of Guelph
Greg Toth, Mark Bagley, and Jim Lazorchak, US EPA, Cincinnati

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 through the development of egg proteins and eggs. One of the most sensitive and common tools used to assess exposure to endocrine disrupting chemicals (EDCs) is the presence of vitellogenin (VTG), an egg yolk protein precursor, in the plasma of male fish. Recent studies have shown elevated plasma VTG in male fish downstream of sewage treatment plants.

Natural and synthetic estrogens such as estriol and 17α-ethynylestradiol (EE2), two of the main active components of birth control pills, are present at ng/L 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 populations. Laboratory studies have confirmed that these compounds are causing the feminization and elevated VTG levels observed in male fish and developmental problems in females 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, such as VTG production, observed in fish exposed to EDCs are indicative of changes in population viability. Though significant progress has been made in characterizing the effects of hormone mimics on individuals, population-level approaches to identify and quantify effects are lacking.

Purpose of Experiment:

This whole ecosystem study has been developed to determine whether aquatic populations are being adversely impacted by EDCs, and to determine the relationship between organism- and population-level responses to these compounds. This six-year study (1999-2004) will determine the impacts of the synthetic estrogen, EE2, on well-defined fish and invertebrate populations at the Experimental Lakes Area (ELA). EE2 was chosen for this experiment because it is a 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 assess the 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 and Experimental Design:

Lake 260 was chosen for this whole-lake addition experiment and it 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 extensively in laboratory EDC assays) and pearl dace (*Semotilus margarita*). Nearby Lakes 114, 224, 442, 373, and Roddy (468) are being used as reference systems throughout this study because most are similar in physical and chemical characteristics, have the same fish species and long-term data on their populations, and some historical data on lower-trophic-level biota.

Two years of baseline research (1999 & 2000) and three years of EE2 additions (2001-2003) have now been completed. It is our intention to continue to monitor impacts and/or recovery of the vertebrate and invertebrate populations in 2004.

EE2 Additions and Water Column Concentrations

Addition and Quantification of EE2 in Lake 260:

In April of 2003 the collaborators met to discuss the previous season's results. Fish population data suggested some reproductive failures for the fathead minnows in Lake 260 after the second year of EE2 additions (see below). The group felt that a third season of EE2 additions was needed to completely ascertain the population-level effects. We decided to target the same surface water concentrations (5-6 ng EE2/L), as in 2001 and 2002. The lake stratifies over the summer, thermally isolating different depths of the water column and making it difficult to add the EE2 to the meta- and hypolimnion (middle and lower waters respectively) while ensuring that the estrogen is thoroughly mixed. In contrast, the epilimnion (upper) waters are mixed continuously by wind and previous studies at ELA have demonstrated rapid vertical and horizontal distribution of chemicals added to the upper water column.

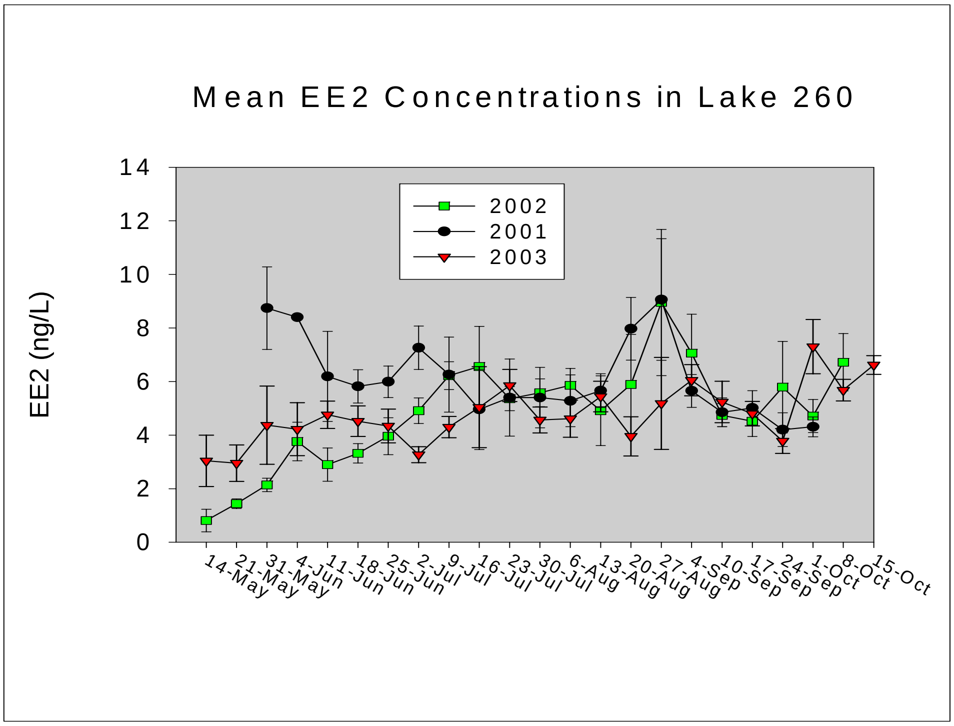

Figure E-1. Mean (+/- SD) concentrations of EE2 (ng/L) in the epilimnion of Lake 260, 2001, 2002 and 2003.

EE2 additions to Lake 260 were started on 20 May 2003, after the epilimnion of the lake had been established. The estrogen was added three times weekly in a 100% methanol solution into the propeller wash of the boat as it was driven around the lake. Between 100 and 450 mg of EE2 was added per day (4.5% loss per day) to maintain target concentrations, and a total of 38.7 g was added between 20 May and 6 October of 2003 (44 g was added in 2002). Weekly epilimnion water samples (replicates) were collected from 4 sites, filtered, processed on solid phase extraction columns, and quantified for EE2 using radioimmunoassay. Testosterone was added to each sample as an internal standard and blanks were run each week (mean of 0.6 ng/L). Prior to any additions of EE2 to the lake, water concentrations of EE2 were assessed. Concentrations of EE2 were between 2 and 3 ng/L in May just prior to the beginning of the additions for 2003. Over the addition period, mean EE2 concentrations (blank corrected) were 4.8 ± 1.0 ng/L between 26 May to 8 October (Figure E-1) and similar to the two previous years. Samples of the meta-, hypolimnion and outflow were also collected on a monthly basis and averaged 3.1 ± 1.1, 2.7 ± 0.7, and 1.6 ± 1.3 ng/L (outflow collected in May and June only due to low water year).

Effects of EE2 on Fish

Fish Population Studies:

We sampled the fish populations in Lakes 260, 442 and 224 during spring and fall of 2003 in a similar fashion to previous years. 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 all fish populations in Lake 260. Lake trout were caught and sampled using trap nets and short sets of gill nets in the fall while white sucker were caught and sampled in trap nets in the spring. Other species – fathead minnow, pearl dace, lake chub, and slimy sculpin - were caught in trap nets and sampled in spring and fall in each lake. Most of the spring and fall data from 2003 are still being analysed

Population sizes of fathead minnow and pearl dace were evaluated using a mark-and-recapture program on Lakes 260 and 442 in 1999-2003. The data are based on a fall marking period of 10 days and a spring recapture period of 10 days. Each time a total of 30 baited minnow traps are used. In the fall, fish are released during the marking period, whereas in the spring, the fish are caged over the recapture period.

Because effects in longer-lived populations will take time to manifest, netting will be continued in 2004 (funded by DFO) to determine the impacts of an estrogen mimic on fish sustainability and to assess the ecological relevance of biomarkers used to determine estrogen exposure in fish. It is our hope to find support in 2004 to continue the fish monitoring for additional years in order to adequately assess the population-level impacts of EE2.

Fathead Minnow Reproductive Behaviour:

Laboratory studies have shown that estrogen exposure affects the reproductive behaviour of fathead minnows. It is not known whether such impacts would occur in fish under natural conditions. Underwater video was used to record spawning behaviour of male fathead minnows in Lake 260 and in reference lakes.

Fish Fertilization Success:

Laboratory studies have shown decreased survival and skewed sex ratios of fish larvae exposed to estrogen mimics. In this study, fertilizations of lake trout eggs have been done during 3 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 is 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. In 2001, 5 females from lake 260, 5 from Roddy Lake (Lake 468) and 5 from Lake 442 were spawned. In 2002, 5 females from Lake 260 and 6 females from Lake 442 were spawned. In 2003, 5 females from each of lakes 260, 442 and Roddy were spawned. Individual egg diameters and total egg volumes were recorded to calculate fecundities for each female.

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 were reared at 8C with sub samples 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.

Molecular and Cellular Responses in Fish:

The purpose of this component of the project is to assess the biochemical and tissue-level effects of EE2 exposure using both baseline data collected from fish populations in Lake 260 and three years of reference lake data. Several parameters are being examined and include vitellogenin (VTG; egg protein precursor) and its mRNA production, sex steroids, thyroid hormones, vitamins and gonadal development.

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, and Roddy Lake (Lake 468) (and Lake 114 for minnows) in 2003 in conjunction with the spring and fall netting programs for the population-level research. The following three paragraphs detail the number of fish collected for these analyses.

Spring Collections for 2003: Fathead minnow histological samples taken include: Lake 260, 8 males, 13 females; Lake 442, 5 males, 4 females; Lake 114, 0 males, 0 females. Vitellogenin samples. Lake 260 0 males, 0 females. Lake 442 8 males and 7 females. Lake 114, 7 males, 7 females. Pearl dace histological samples include Lake 260, 6 males, 8 females; Lake 442, 10 males, 12 females; Lake 114, 13 females, 12 males. Vitellogenin and vitamin samples. Lake 260 7 males, 7 females, Lake 114 8 males, 8 females, Lake 442 8 males, 8 females. Lake trout blood sampling in spring for Lake 260 = 21, Roddy lake = 18 and Lake 442 = 15 (sex not evident for spring collection of lake trout). White suckers for blood in spring. Lake 260 = 6 males, 7 females, Roddy Lake = 7 males, 8 females, Lake 442 = 8 males, 11 females.

Mid-Summer Collections for 2003: Vitellogenin samples. Lake 260 10 females, 4 males, Lake 442 0 males, 0 females. Lake 114 8 males, 5 females. Pearl dace histological samples taken include: Lake 260, 2 males, 12 females; Lake 442, 0 males, 0 females; Lake 114 8 males, 5 females. Pearl dace vitellogenin samples. Lake 260, 7 males, 7 females, Lake 114, 7 males, 7 females, Lake 442, 7 males, 7 females.

Fall Collections for 2003: Fathead minnow histological samples taken include: Lake 260, 15 males, 15 females; Lake 442, 15 males, 15 females; Lake 114, 10 males, 10 females. Fathead minnow for VTG and vitamins from Lake 260, 7 males, 7 females; Lake 114, 6 males, 7 females; Lake 442 7 males, 7 females. Pearl Dace histological samples include Lake 260, 10 males, 16 females; Lake 114, 15 males, 14 females; Lake 442, 16 males, 16 females. Pearl dace for VTG and vitamins include Lake 260; 8 males, 8 females, Lake 114; 4 males, 7 females and Lake 442; 7 males, 7 females. Lake trout (blood, histology, and tissues) Lake 260 = 8 females, 6 males; Roddy Lake = 10 males, 7 females; Lake 442 6 males, 8 females. White sucker for blood, histology and tissues. Lake 260 = 7 males, 7 females; Roddy Lake = 8 males, 7 females; Lake 442 = 6 males, 5 females.

Vitellogenin:

Plasma samples were collected each fall from male and female lake trout to assess the production of the egg protein precursor vitellogenin (VTG). Fathead minnows and pearl dace were collected for VTG analyses in the spring and fall of 1999-2002.

Circulating Sex Steroids and Steroid Production:

Alterations in circulating levels of the major biologically active reproductive steroid hormones are a common response in fish following exposure to endocrine disrupting substances. Reductions in steroid levels have been correlated to reductions in gonadal development, reduced expression of secondary sexual characteristics, increased age to maturation and altered fecundity in fish populations. Detailed mechanistic studies examining the pituitary-gonadal axis identified reduced gonadal steroid productive capacity as one of the major contributors to these reduced circulating steroid levels (Van Der Kraak et al., 1992; McMaster et al., 1996). As part of this ELA study, we have collected plasma from white sucker and lake trout from three lakes prior to EE2 additions and in the fall of 2001-2003 following exposure. We measure the two biologically active steroids in both sexes, testosterone and 17ß-estradiol in females and testosterone and 11-ketotestosterone in males, and compare levels between sites as well as to the other reproductive endpoints being measured. In the fall of 2002 and 2003 we also conducted *in vitro* gonadal incubations on female white sucker ovarian tissue from all three of the lakes to determine the steroid biosynthetic capacity of the ovarian tissue and to determine whether EE2 addition altered this reproductive endpoint. Blood samples of white sucker from 1999-2001 have also been analysed

In 2002 *in vitro* gonadal incubations were completed on female white sucker in order to evaluate the impact of EE2 addition on steroid biosynthetic capacity. Incubations were conducted under both basal incubation conditions as well as following stimulation with human Chorionic Gonadotropin (hCG). Incubations were repeated in the fall of 2003 to determine whether any EE2-related impacts occurred during the third season of additions.

In the small forage fish species, pearl dace, we used an *in vitro* gonadal incubation procedure to compare the ability of gonadal tissue to produce steroid hormones following EE2 exposures. This procedure has been used previously in fish too small to obtain sufficient amounts of blood.

Vitamin Concentrations:

In mammals, estrogenic compounds are known to alter lipid metabolism and the whole body distribution of lipid soluble vitamins A and E (Demacker et al. 1991, Mooij et al. 1991). We have also confirmed altered concentrations of these vitamins in fish exposed to estrogenic compounds in the laboratory (Palace et al. 2001a, 2001b). Plasma samples from the 1999-2001 collections of large fish from Lake 260 and reference lakes have been analyzed for lipid soluble vitamins. Collections for 2002 and 2003 are still to be analyzed.

Impacts on Gonad, Kidney and Liver Tissues:

Tissue sections from fathead minnow were examined in fish collected from the study and reference lakes in 1999-2001.

Gonadal development in fish was assessed using histological sections to determine whether EE2 exposure affects the timing and maturation of testes and ovaries in white sucker, pearl dace and fathead minnow. Medial sections of ovaries were collected and were examined for state of maturation, presence of atretic follicles, frequency distribution of oocyte stages, lesions and the presence of intersex (development of both male and female tissues). Testes were 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).

Female fathead minnow were best examined in the spring of each year because this was the time of year when their gonads were most developed and least subjected to effects of asynchronous spawning.

Effects of EE2 on Lower Trophic-level Biota

Effects on Phytoplankton and Bacteria:

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 carbon and nitrogen). Bacteria samples were also taken from Lake 260 and reference Lakes 373 and 239 over the same time period.

Impacts of EE2 on Zooplankton:

Vertical water column tows were collected bi-weekly from ice-off to ice-on in 2003, as was done in 1999-2002 on Lake 260 and the reference lakes. All zooplankton samples from 1999-2002 have been identified and counted to determine abundances, community composition, and sex and egg ratios (used to estimate birth and mortality rates).

Leeches:

Baited leech traps were set overnight in Lakes 260, 442 and 373 once each month over the summers of 1999-2002 (but not 2003) to assess the size and catch-per-unit effort of different species in these lakes. Leeches from these traps were preserved and these samples were examined for species composition and the dry gonad/somatic body weight ratios.

Clay pots were placed in the nearshore area of Lake 260 and the reference lakes, and every second week leech cocoons were collected from this artificial substrate.

Larval and Adult Insects:

Artificial substrates (rock baskets) were used to assess the effects of EE2 exposure on larval benthic invertebrates. In September of 2000 and 2001, the rock baskets were placed at five sites with different substrate types in Lake 260 and reference Lake 442. These baskets were allowed to colonize over the winter and were removed from the lake in May, July and August of each of the following years. Larval invertebrates were preserved, identified and counted. Results from this study indicated no effects of EE2 on species abundances or community composition of these invertebrates (data not shown).

Duplicate emergence traps were set at 5 sites in Lakes 260 and 442 and the adult insects were collected weekly over the summers of 1999-2002.

Effects on Mink and Green Frog Tadpoles:

Hormone mimics are suspected of contributing to the recent and rapid declines and extinction of amphibian populations all over the world (Carey and Bryant 1995). Laboratory studies have demonstrated that exogenous hormones can have numerous impacts on amphibians, including inhibited tail resorption (incomplete metamorphosis), and increased time to metamorphosis. Little is known about the impacts of estrogen mimics on wild populations.

The main objectives of this component of the experiment was to determine whether EE2 exposure 1) alters the timing of tadpole growth and development, 2) causes changes in tissue concentrations of thyroid hormones that control metamorphosis, and 3) effects changes in gonad differentiation resulting in intersex or skewed sex ratios in these tadpoles. Wild tadpoles were netted in Lake 260, 114, 442 and 224 in the spring and fall of 1999-2003. These individuals were weighed, staged and preserved for gonadal development analysis using histological methods. Field caging studies were also conducted in 2001 and 2002 using both green and mink frog egg masses collected from Lake 442. These egg masses were divided and placed into cages on Lakes 114, 224 and 260 to examine effects of EE2 on hatching success, and survival, growth, metamorphosis, sex ratios, and gonadal development of the tadpoles. Green frog tadpoles (1 year olds) were also collected in 2001 from Lake 114 and caged in Lakes 260, 114 and 224 to look at impacts of EE2 exposure on thyroid hormone concentrations (control metamorphosis) in body tissues.

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 resident tadpoles. He completed his thesis in August of 2003. J. Werner is a Ph.D. student at the University of Manitoba that is conducting studies on the fertilization success and biochemical responses of fish in Lake 260 exposed to EE2.

Future Research Schedule:

The collaborators on this project will be meeting in March of 2004 to review results from the past field seasons. One additional year of funding for this experiment has been received from DFO and it is our intention to use this funding to monitor the continued impacts and/or recovery of the fish populations after three seasons of EE2 additions. In 2004, residual concentrations of EE2 in the lake and its outflow will be monitored on a monthly basis to determine whether any estrogen remains in the water column.

We will continue the monitoring of biochemical-, tissue-, organism- and population-level responses in the fish from Lake 260 and our reference systems in 2004 using the same sampling strategies as in previous years. Some lower-trophic-level monitoring will likely be done but it is not clear at this stage what will be repeated. No funding is currently available for continued monitoring of invertebrates, algae or bacteria.

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.

Funding Sources for 2003/04:

- Environmental Sciences Strategic Research Fund, DFO
- American Chemistry Council
- Schering Pharmaceutical, Germany

Funding Total – 156K
In-Kind Contributions – 90K
Total Annual Budget in 2003/04 – 246K

ENVIRONMENTAL IMPACTS OF AQUACULTURE

As wild fish populations come under increased pressures from human exploitation, commercial aquaculture or "fish farming" has become increasingly important as a source of fish protein for humans. While most Canadian aquaculture has focused on marine systems, there is increasing interest in freshwater aquaculture, in the Great Lakes and potentially in smaller inland systems. Until now, little research has focused on the environmental impacts of such activities in freshwater lakes.

IMPACTS OF CAGE AQUACULTURE ON LAKE ECOSYSTEMS

Project Team:

DFO Freshwater Institute:

Cheryl Podemski, Project Coordinator
Paul Blanchfield
Sandy Chalanchuk
Dave Findlay
Lori Flavelle
Len Hendzel
Ray Hesslein
Karen Kidd
Ken Mills
Michael Paterson
Michael Turner
Patrick Buat

Other Agencies:

Mike Meeker, Meeker Aquaculture
Murray Charlton, Environment Canada, CCIW
Sherri Schiff, University of Waterloo
Dominique Bureau, University of Guelph, Fish Nutrition Laboratory
Scott Findlay, University of Ottawa
Corben Bristow, University of Ottawa (MSc student under S. Findlay and Hesslein)
Rebekah Rooney, University of Manitoba (MSc student under Podemski)

Background:

The environmental impacts of cage aquaculture are complex but largely related to two issues: the potential impacts of escapees on native fish populations and the production and release of waste materials. The impacts of escapees on native fish stocks can result directly through competition, predation, and genetic mixing, or indirectly through the introduction of parasites and diseases, or as a result of environmental degradation by the introduced species (Arthington and Bluhdorn 1996, Welcomme 1996). There is currently insufficient information in the scientific literature to predict the impacts of escapees on Canadian freshwater stocks. Nutrient-induced stimulation of local algal blooms (eutrophication) and the creation of anoxic waters and sediments underlying the netpens are the primary environmental concerns associated with waste generation. Between 4 – 26 kg of phosphorus (P) and 50 – 125 kg of nitrogen are released to the environment for every tonne of fish that is produced (Enell and Lof 1983; Ackefors and Enell 1994; Cho et al. 1994). These wastes are in the form of fish faeces and uneaten feed (Beveridge 1984). Dissolved carbon, nitrogen, and phosphorus are released to surface waters by solubilization from feed and faeces, and through the gill and urinary excretions of the cultured fish and these nutrients can significantly stimulate primary production. Solid wastes settle to the lake bottom where they may serve as a food source to wild fish and invertebrates. Remaining wastes decompose, and this decomposition may result in the creation of anoxic conditions in sediments and overlying waters.

Ontario's aquaculture industry is the largest producer of freshwater finfish in Canada, and cage aquaculture of rainbow trout dominates the Ontario industry (77% of total production in 2000, Moccia and Bevan 2000). This segment of the industry occurs almost exclusively in Northern Ontario. While marine aquaculture is a rapidly expanding industry in Canada (OCAD, 2001), the freshwater industry has lagged behind, showing little increase in total production over the last three years. Public perception that the industry is not environmentally sustainable and an uncertain regulatory environment are major factors limiting further development of this industry. These pressures are due in part to the lack of scientific data. Little research has been undertaken in Canadian or North American freshwater ecosystems, and much of the published literature is out-dated and of limited relevance due to changing industry practices. There is a need for scientific evaluation of the impacts of current aquacultural practices in Canadian freshwater environments, and the development of cost-effective methods to predict and monitor these impacts.

Assessment of the impacts of cage-culture is difficult at existing commercial sites because:

1. most operations have insufficient or no pre-operational data;
2. confounding influences such as other aquacultural operations, industry, or human habitation make it difficult to unequivocally assign responsibility for environmental impacts; and
3. many impacts such as effects on wild fish populations cannot be readily quantified in the large, open systems typical of many operations.

An experimental approach in a controlled ecosystem where a mass balance approach can be taken to track the movement and fate of materials will be the strongest method to objectively evaluating the impacts of freshwater aquaculture. The Experimental Lakes Area Aquaculture Project is conducting a whole lake experiment to address these issues.

Purpose of the Experiment:

This whole ecosystem study has been developed to assess the environmental and ecological impacts of cage aquaculture under current industry practices. The three-year study will determine the impacts of aquaculture on water quality, primary production, and native invertebrate and fish communities. A mass balance approach, the measurement of stable sulphur, carbon and nitrogen isotopes, and the incorporation of inert tracers into feed will be used to trace the movement of aquaculture-related waste materials in the ecosystem.

Study Site and Experimental Design:

Lake 375 was chosen as the study lake for this experiment and Lake 373 serves as the reference. Lake 375 is a double basin lake with a surface area of 19.7 hectares and a maximum depth of 26 meters. In the shallower, north basin, maximum water depth is 17 m. The lake has been considered in the past for whole lake experiments and has been used for a small limnocorral experiment. As a result, many years of zooplankton, phytoplankton and water chemistry data exist. Fish populations in the lake have been monitored annually since 1988 through spring and fall trap netting, short-set gill netting, and mark-recapture. The lake supports a stable population of approximately 305 Lake trout (*Salvelinus namaycush*) and approximately

65 white sucker (*Catostomus commersoni*). Smaller fish species present include the fathead minnow (*Pimephales promelas*), pearl dace (*Semotilus margarita*), northern redbelly dace (*Chrosomus eos*), finescale dace (*Chrosomus neogaeus*) and slimy sculpin (*Cottus cognatus*).

We have installed a cage-culture facility to rear approximately 10 tonnes (total mass at harvest) of rainbow trout in Lake 375 per production cycle. Fish will be triploid or all-female to reduce any potential long-term consequences of escapees. The farm consists of a single, 30’x30’, steel cage anchored in the north basin of the study lake over approximately 17m of water. A commercial fish farmer (Mike Meeker, Meeker Aquaculture) will act as an off-site manager of the facility to ensure that all practices at the experimental facility conform to commercial practices. This will include administration of chemotherapeutants and antibiotics, if deemed necessary. Installation occurred in the spring of 2003 and trout will be raised through two, 1-year production cycles. During this time comprehensive monitoring of the ecosystem will occur.

The following is a summary of what was accomplished in 2003, the first year of fish production, collection, by the various collaborators on this experiment.

Farm Operation:

On June 5, 2003, a team of 23 people moved 10,000 female rainbow trout into the net pen in Lake 375. This process took approximately 5 hours, with close to one hundred ATV trips down the 375 trail, and ran very smoothly. We had fewer than 150 mortalities in the two weeks following fish introduction, a very low mortality rate. Total mortality rate over the summer was approximately 5%. Fish were harvested on November 19, 2003. Fish remained healthy throughout and no antibiotic use was required. Total feed usage over the production cycle was 8713.8 kg.

Water Quality:

On a monthly basis, samples for water quality analysis were collected along depth profiles in both the north and south basin of the lake. Also on a monthly basis, but on alternate weeks (resulting in biweekly sampling), depth-integrated samples were collected from the epi, meta, and hypolimnion at the same stations. During spring and fall turn-over, additional depth integrated samples were collected and analyzed for all forms of phosphorus and nitrogen. Sediment traps were collected weekly in both basins to determine sedimentation rates of carbon, nitrogen, and phosphorus. Water quality samples are still being analyzed.

On a biweekly basis, a YSI multi-parameter probe was used to construct meter by meter depth profiles for temperature, oxygen, pH, conductivity, turbidity, and fluorescence at 12 stations placed along the north-south axis of the lake. Secchi depths were also determined at each station.

Phytoplankton and Bacteria:

Phytoplankton and bacteria were sampled bi-weekly from the deep stations in the north and south basins throughout the ice-free season in 2003. Samples have been analysed and data in the process of being worked up. The phytoplankton community in both basins of Lake 375 is typical for oligotrophic shield lakes and in 2003 appears to have been unimpacted by the introduction of the rainbow trout. Chrysophytes dominated seasonally with high abundances of cyanobacteria (*Chroococcus limnetica)*. A similar increase in other reference systems has been observed but not at the same magnitude. Total biomass for 2003 was within the confidence limits of the suite of reference lakes.

Benthic Algae:

We designed a benthic algal sampling program to evaluate the hypothesis that if phosphorus (and nitrogen) is mobilized from the aquaculture operation, we can expect the productive capacity of the littoral zone to increase.

If phosphorus (and nitrogen) availability increases, then from a metabolic perspective we could expect to observe increased photosynthetic potential. This differs somewhat from other ELA lakes because of the high dissolved inorganic carbon in L 375 relative to other ELA lakes, in which we would expect little if any metabolic enhancement because of carbon limitation of epilithic biofilms. From a food-quality perspective, we also expect that if the availability of phosphorus (and nitrogen) are increased, stoichiometry of littoral biofilms (especially C:P) will be enhanced. Note though that timelines associated with nutrient dynamics will likely vary with the nutrient and in terms of sensitivity to detection by a mid-summer synoptic sampling program. Although N might become available during the open-water season if ammonia from fish wastes is soluble and exceeds pelagic demand, P release from the fish waste will likely depend upon cycling through the anoxic hypolimnion and require entrainment into the epilimnion during spring and fall overturns.

In 2003, because of severe funding constraints, we were able only to conduct metabolic sampling along the northern shore, with the objective of continuing long-term epilithic observations at north station in the first year of the aquaculture operation. Given that little immediate effect was anticipated, we expected that this sampling would better establishing the baseline conditions, and preliminary data analysis suggests that this was the case. We also conducted compositional sampling of biofilms along L 375's west shore with the objective of further establishing the stoichiometric baseline for epilithon in a rocky region of the littoral zone near the aquaculture operation. In cooperation with Dave Findlay's biofilm research we also collected tiles at each of his previously established western and northern sampling locations.

Zooplankton:

On a biweekly basis, vertical net hauls were used to collect epilimnetic and hypolimnetic zooplankton samples from stations in the north and south basins of L375 and from a single station in L373. The stations were identical to those used for water quality sampling. These samples are currently being analyzed and will be used to determine species composition, biomass, and secondary production. On a monthly basis, vertical tows were collected at each of 6 stations in L375 at least 1 hour after sundown to monitor Mysis, Chaoborus and Leptodora. Also on a monthly basis, collections were made to look for diurnal changes in the vertical distribution of zooplankton in the south basin.

Zoobenthos and Sediment:

To examine the impacts of the farm on the lake benthos, on a biweekly basis sediment cores were collected by a KB gravity corer along depth transects (11, 13, 15, 17, 19, 21 m) in both basins of L 375 and in the reference lake (L 373). Samples were collected at 11m and deeper because experience has shown that hard (rock) substrates in shallower waters of these two lakes prevent operation of the corer. Samples were sieved through a 250 µm sieve and were preserved in 10% formalin for later analysis.

In order to examine the near-field impacts of the farm on sediments and benthic invertebrates, core samples were obtained on a monthly basis from seven sites located 0 m, 5 m, 6 m, 8 m, 10 m, 20 m, and 50 m from the centre of the cage. Five replicate cores were washed using a 250 µm sieve and were preserved in 10% formalin for analysis of the benthic invertebrate community. Three replicate cores were used to generate vertical profiles of dissolved oxygen and redox potential using micro-electrodes at 2 mm depth increments. Three replicate cores were sub-sectioned into slices representing 0-2 cm depth and 2-4 cm depth, and the pore water was removed by filtration. Both slicing and filtration were carried out in a glove box under nitrogen atmosphere to prevent oxidation of sulphide. The concentration of ammonia and sulphide in each 2 cm slice was then determined using ion-selective electrodes. Three replicate cores from each site were taken and sub-sectioned into 0-2 cm, 2-4 cm, and 4-6 cm depth samples. These sub-samples were frozen, and will be analysed for carbon, nitrogen, and phosphorus content.

A series of eight floating emergence traps were placed above a water depth of 15 m in L 375 and L 373, and were collected weekly during the stratified period. Traps were placed along a distance transect from the proposed fish farm location. These traps will supplement our knowledge about changes in community composition with distance from the cage.

Energy Transfer to the Native Food Web:

One possible impact of freshwater aquaculture practices on native fish communities is related to the discharge of waste feed and faeces, which can represent a novel food source for these populations. Native salmonids that switch to waste food can attain significantly larger size than fish feeding on natural food at reference sites (Gabrielsen 1999)(Phillips et al. 1985). European studies have shown that wild fish can use up to 100% of waste materials (Borum et al. 1995); however, no similar measures from Canadian ecosystems exist.

Marine proteins in fish feed used in the cage culture contain a stable sulfur isotope signature that is distinct from those in freshwater lakes (Hesslein et al. 1988). This signature in the diet is preserved when proteins are incorporated into tissues of the consumer (Hesslein 1993), and can be used, along with carbon isotope ratios, to assess the importance of different energy sources to the organism (Peterson and Fry, 1989). Nitrogen isotope ratios are used to determine an organism's trophic level because the heavier isotope of nitrogen (^{15}N) is preferentially retained over the lighter isotope (^{14}N) resulting in an increase in the ratio from prey to predator. Measurements of these tissue stable isotopes are commonly used in freshwater and marine studies to examine the structure of the food web, to assess changes in dietary habits, and to characterize the importance of different energy sources to primary through tertiary consumers. Advantages to using these measurements over traditional gut content analyses include longer-term information on dietary habits (weeks to months rather than days), assessments of what is used by an organism for growth, information when guts are empty, and an ability to calculate the proportion of energy coming from food differing in isotopic composition. In this experiment, we will measure sulfur, carbon, and nitrogen isotope signatures in invertebrates and resident fishes to determine whether the aquaculture feed is used directly (through consumption of the food) or indirectly (by organisms that fed upon the feed). These data will be important in assessing the timing and magnitude of the impacts of aquaculture feed on the pre-existing biota in lakes.

In May, June, July and August of 2002 and 2003, nearshore invertebrates and macrophytes were collected from several sites in Lakes 375 and reference Lake 373. The invertebrates were sorted into major taxa in the lab and frozen for further identifications and processing. Vertical zooplankton tows and deep-water benthic invertebrates were also collected from each lake once a month, sorted when necessary, and frozen for further analyses. Several minnow species (pearl dace, northern redbelly dace, finescale dace and fathead minnow) were collected from Lakes 375 and 373 in the spring and fall of 2002 and 2003. Tissues from lake trout and white sucker were also obtained as part of the index netting program. Fish tissues and invertebrates are being dried, ground and processed for stable carbon, nitrogen and sulphur isotope analyses. Samples of water, sediment, and fish faeces are being analyzed by Dr. S. Schiff's lab at the Earth Isotope Laboratory, University of Waterloo, to look at stable isotope signatures of organic and inorganic sulfur.

Summary of ELA Research for 2003

Fish Populations:

Lake trout, white sucker, fathead minnow, pearl dace, finescale dace, and slimy scuplin were captured in trap nets and gill nets (non-destructive) in Lake 375 in spring and fall of 2003. Lake trout (fall only) and whitesucker (spring and fall) were weighed, measured, fin-clipped (for aging) and marked by fin-scarring before release back to Lake 375. Each species is involved in long-term mark-recapture population studies. The other species were counted and measured before release back to Lake 375.

Acoustic and Video Tracking of Fish Behaviour:

Our first activities on Lake 375 involved the construction of an extensive fish barrier (~25 m long) across a portion of the outflow to Manomin Lake to prevent the downstream movement of any potential escaped rainbow trout. This fence, which also included upstream and downstream traps to monitor cyprinid movement and abundance, was inspected over the course of the field season.

We installed and maintained three VRAP (VEMCO Radio-Linked Acoustic Positioning) systems that continually monitor the movements of acoustically-tagged lake trout and white suckers in the study lake (375; two systems) and the reference lake (373; one system). This set-up allows for total coverage of fish movements in Lake 375 and partial coverage in Lake 373. We augmented these data with occasional manual tracking and the use of multiple passive receivers. We were faced with several mortalities of tagged fish, some of which we were able to retrieve and use in different fish (lake trout n=4, white sucker n=1). In addition, prior to the cage harvest, ten farmed rainbow trout were fitted with acoustic transmitters and were released as experimental “escapees” in order to examine behaviour of escaped farm fish in natural systems.

We examined cyprinid abundance and schooling behaviour through underwater visual observation and video recording. Minnow trapping was conducted at the cage site, at littoral sites around Lake 375, and at similar sites in Lake 373. Length and weight data were recorded in order to examine size distribution of minnow species, and samples were preserved for stable isotope analysis.

Training:

Two graduate students (Corben Bristow, University of Ottawa, and Rebekah Rooney, University of Manitoba) are receiving training as part of this project. Corbin is responsible for constructing the mass balance model for C, N and P in the study lake. Rebekah is examining the effects of organic waste loading on benthic invertebrate community composition and sediment chemistry (nitrogen, carbon and phosphorus content, pore water ammonia, and oxygen/redox profiles).

Future Research Schedule:

We plan to install the fish cage in late winter and start fish production in early summer 2003. We plan to continue the same level of sampling for two years, with the addition of underwater video to document any accumulations of material under the net pen. The experiment has funding for a further 2 years.

Funding Sources for 2002:

The funding source for this experiment is DFO’s Aquaculture Cooperative Research and Development Program (ACRDP). This fund requires joint industry and government participation. Our industry partners are the Northern Ontario Aquaculture Association and Meeker Aquaculture. Martin Mills provided the NOAA with the fingerlings and also a discounted price on feed used in this experiment.

Literature Cited:

- Ackefors, H. and M. Enell. 1994. The release of nutrients and organic matter from aquaculture systems in Nordic countries. *Journal of Applied Ichthyology* **10**(4): 225-241.
- Arthington, A. H. and D. R. Bluhdorn. 1996. The effects of species interactions resulting from aquaculture operations. Pp 114-139 in*: Aquaculture and Water Resource Management*. D. J. Baird, M. C. M. Beveridge, L.a. Kelly and J.F. Muir. Blackwell Science.
- Beveridge, M. C. M., L.G. Ross and L.A. Kelly. 1994. Aquaculture and biodiversity. *Ambio* **23**(8): 497-502.
- Borum, K., et al. 1995. The importance of wild fish to the distribution of phosphorus from fish farms. *Vatten/Water* **51**: 125-134.
- Cho, C. Y., K. Hynes, K.R.Wood, and H.K. Yoshida. 1994. Development of high-nutrient-dense, low pollution diets and prediction of aquaculture wastes using biological approaches. *Aquaculture* **124**: 293-305.
- Enell, M. and J. Lof 1983. Environmental impacts of aquaculture sedimentation and nutrient loadings from fish cage culture farming. *Vatten/Water* **39**(4): 364-375.
- Gabrielsen, S.-E. 1999. Effects of fish-farm activity on the limnetic community structure of brown trout, Salmo trutta, and Arctic charr, Salvelinus alpinus. *Environmental Biology of Fishes* **55**: 321-332.
- Hesslein, R. H., et al. 1988. Sulfur isotopes in sulfate in the inputs and outputs of a Canadian Shield watershed remote from sources of anthropogenic sulfate. *Biogeochem*. **5**: 263-273.
- Hesslein, R. H., K.A. Hallard and P. Ramlal 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (Coregonus nasus) in response to a change in diet traced by 34S, 13C, and 15N. *Can. J. Fish. Aquat. Sci*. **50**: 2071-2076.
- Moccia, R. D. and D. J. Bevan 2000. *Aquaculture legislation in Ontario*, University of Guelph: **8**.
- OCAD, 2001. *Legislative and Regulatory Review of Aquaculture in Canada.* 77pp.
- Peterson B.J. and Fry B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18**:293-320.
- Phillips, M. J., et al. (1985). Waste output and environmental effects of rainbow trout cage culture., *ICES Report*: **17**.
- Welcomme, R. L. 1996. International measures for the control of introductions of aquatic organisms. *Fisheries* **11**(2): 4-9.