

SUMMARY OF MAJOR RESEARCH PROJECTS AT THE EXPERIMENTAL LAKES AREA DURING 1999

1 January 2000

Research activity during 1999 continued to increase as new experimental studies began and background studies for proposed experiments were underway. The first phase of a new laboratory complex was constructed during the winter, and was used during the 1999 field season for water chemistry and other activities. Three new research scientist positions were staffed during the spring and these new personnel were all active on site during the field season.

The long-term, ecological research program continued to be supported by core funding, in recognition of the inherent value of long-term data records on unperturbed systems. Monitoring of lakes recovering from acidification experiments continued, albeit at a reduced level of activity. The investigation of stoichiometric linkages in lakes of different trophic status continued at an active pace, and several other projects investigated the impacts of UV radiation. Although the ELA Reservoir Project and the Lake 226 drawdown study were effectively dormant during 1999, much of the research activity again focused on the effects of reservoirs, as flooding of new upland reservoirs (FLUDEX project) began in June and continued until October. Mesocosm experiments and baseline measurements were carried out in preparation for two proposed ecosystem-scale experiments; namely, a whole-lake addition of a synthetic estrogen, and a small watershed addition of stable isotopic tracers of mercury (METAALICUS Project).

Total ELA site use increased significantly over 1998, with activity levels in both July and September being at, or near, record levels for those months. In September, the ELA hosted more than 30 international experts on algal taxonomy for a 6-day workshop, and was the site for a two-week field course on ecological monitoring presented to students from the Centre for Indigenous Ecological Resources (CIER).

The following is an attempt to summarize the status of major projects by providing some information about their purpose and design. Results, which are largely preliminary at this time, are not presented. Anyone wishing more information about specific studies should contact the researcher(s) directly involved. These projects are grouped under several broad category headings.

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1. BIOMANIPULATION AND SYSTEM PRODUCTIVITY

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

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

Lake 227 was fertilized with phosphorus for the 31th consecutive year in 1999. The original experiment was initiated in 1969 to demonstrate that atmospheric carbon dioxide could provide the carbon necessary for algal blooms in eutrophic lakes. Prior to 1990, all additions included various combinations of nitrogen and phosphorus. The ratio of phosphorus to nitrogen was changed during these previous stages of the experiment to test whether this would influence the dominant algal groups. Since 1990, only phosphorus has been added. During 1999, phosphorus, as phosphoric acid, was again added to Lake 227 surface waters for twenty consecutive weeks (2.5 litres per week) during the ice-free season. The acid was diluted with lake water in a plastic barrel and dribbled via *Tygon* tubing into the near-shore water. The required acid was carried to the lake weekly. Sodium bicarbonate, to be used as a neutralizing agent in case of an acid spill, is stored on site.

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

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

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

1.2. Stoichiometry and Food Web Dynamics

For the past number of years, a joint research undertaking (The Stoichiometry Project), involving DFO and researchers from Arizona State University (ASU)(Dr. James Elser), the University of Texas at Arlington (Dr. Thomas Chrzanowski, Dr. James Grover), Kyoto University (Dr. Jotaro Urabe) and the University of Minnesota (Dr. Robert Sterner), have investigated how changes in zooplankton community structure alter the relative availability of N and P supporting phytoplankton and bacterial production. Originally, this research centred on changes in the stoichiometry of N and P in two lakes, artificially-eutrophic Lake 227 and oligotrophic Lake 110, following introduction of piscivores [see **1.1.**, above]. Recent studies have also included mesocosm experiments in Lake 239.

In 1999, another series of research projects at the ELA provided follow-up to the earlier studies. These studies, largely funded by the United States National Science Foundation (NSF), are headed by Dr. Elser, Dr. Urabe, Dr. Chrzanowski and Dr. Grover.

1.2.1. Microbial Indicators of Biological Integrity and Nutrient Stress

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

Project Dates: 1 September 97 – 31 August 00

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

Activity during 1999: Technical staff (Kory Pennebaker and Brenda Smith) were on site from 16 May through 28 August. Principle investigators (2) were on site for approximately 25 days each. During the period from 15 May to 28 August, Lakes 227 and 239 were each sampled weekly. Data on physical limnology (light, temperature, mixing depth, attenuation coefficients, and dissolved oxygen profiles) were collected for each sampling interval. Epilimnetic water samples were collected synoptically with data on physical limnology. Water samples will be analyzed to determine dissolved nutrient loads (C, N and P species). Particulate fractions of the water were analyzed to establish seston C:N:P ratios. Fourteen nutrient bioassays were conducted (7 for L239, 7 for L227).

Activity planned for 2000: The University of Texas research team plans to replicate their sampling effort through the summer of 1999. No new experimental studies or manipulations are planned.

1.2.2. Light, Phosphorus, and Ecological Stoichiometry in Pelagic Communities

Dr Jim Elser, Department of Biology, Arizona State University

Collaborator: Dr. J. Urabe (Kyoto University)

1.2.2.1. Stoichiometric Responses to Food Chain Manipulation

Manipulated lakes (L110, L227) and a reference lake (L240) were again sampled in 1999. These are continued studies from our prior NSF project at ELA in which we added piscivorous pike to Lake 110 and L227 and monitored responses of the lower food web, with an emphasis on stoichiometric parameters, during the ensuing period. Both lakes continue to indicate interesting transient dynamics to the manipulations. A new manuscript for the Lake 110 experiment is planned for the period of 1996-1998; this is a follow-up to a published paper (Elser et al. 1998) reporting the potential existence of stoichiometric constraints on food web dynamics in L110 given its very high seston C:P ratios. Data analysis for the L227 experiment for the period of 1992-1996 is completed and a manuscript summarizing the spectacular re-organization of the L227 ecosystem during 1996's *Daphnia* bloom is in review at the journal *Ecosystems*.

1.2.2.2. Testing the Light:Nutrient Hypothesis

A large, mesocosm experiment was conducted in ELA Lake 239. This experiment involved the establishment of 18 enclosures (1-m diameter, 2-m depth) that were subjected to two separate manipulations. In the first, we manipulated the starting conditions in terms of the distribution of phosphorus among dissolved, seston, and zooplankton pools. In the second, we manipulated light:nutrient balance by a series of increasing nutrient (N, P) inputs under ambient and reduced light conditions. Both experiments ran about 5 weeks. These experiments are seeking to understand the factors that regulate algal nutrient limitation and seston C:P ratio, which we hypothesize to be major factors regulating pelagic community structure via stoichiometric mechanisms. A large suite of ancillary and core limnological parameters were measured in each enclosure on a 6-d sampling schedule. These included light extinction, inorganic nutrient dynamics, bacteria and protozoa abundance, phytoplankton and zooplankton abundance and species composition, seston C:N:P ratios, zooplankton C:N:P ratios, light vs. nutrient limitation of algae, DOC vs. nutrient limitation of bacteria, grazing and nutrient recycling rates by macrozooplankton, and C:P food quality of seston for *Daphnia* growth. Samples from this experiment are being processed and data should be available for analysis in spring 2000.

1.2.2.3. Student Projects

A variety of sampling efforts and experiments were performed by Paul Frost (ASU PhD student). These studies are a part of his PhD thesis studies investigating factors associated with the C:N:P stoichiometry of benthic communities in ELA lakes. An undergraduate research project was performed by Amy Waggener (Arizona State University) in which she attempted to modify laboratory approaches for direct testing of mineral P limitation of *Daphnia* growth for field conditions. An experiment was performed by postdoctoral researcher Wataru Makino (Kyoto University) investigating the possibility of intra-specific facilitation of growth of *Daphnia* via stoichiometric feedbacks; this is a follow-up to experiments run by undergraduate Craig Herbold during summer 1999. PhD student Takehito Yoshida (Kyoto University) completed a comparative sampling regime examining rates of microzooplankton grazing in relation to various environmental parameters (esp. lake trophic status) in a sampling of about 35 lakes at the ELA. Data from these projects are still being processed.

1.2.2.4. Ancillary studies

The ASU/Kyoto research group also performed some side projects of various kinds. For example, preliminary studies examining the effects of stoichiometric food quality on RNA levels in *Daphnia* were performed.

Upon completion of sample analyses, the researchers anticipate preparing several additional research papers reporting the results of summer 1998/1999 activities.

1.3. Biomanipulation of Lake 221

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

Limited monitoring of the lake has continued through 1999 to address the key question whether such a lake ecosystem can reach a new equilibrium and, more importantly for management, to verify whether this new equilibrium can be sustained. Two overnight gill net sets in late September, 1999, resulted in 37 pike and 56 perch being removed from the lake.

1.4. Experimental Cropping of Lakes

The lake whitefish populations of Lakes 258 and 305 were experimentally fished in 1981 and 1982 to simulate a pulse commercial fishery. Recovery of these populations has been monitored by over-night sets of gill nets one to two nights per year periodically since the initial cropping. Reference Lakes 259 and 468 are also monitored using similar netting techniques. In 1999 (fall), only Lakes 259 and 468 was fished. Data from these reference lakes are used to interpret lake whitefish data from other ELA experiments.

1.5. Effects of Macrophyte Removal on Pike Populations

Lake 191 at the Experimental Lakes Area is the site of a macrophyte (rooted aquatic plant) removal experiment to determine the change in northern pike production when half of the macrophytes present in the littoral zone are removed.

After two years of background study, we initiated macrophyte harvesting in 1996. A mechanical harvester removed fifty percent of the macrophytes present in the lake by August 1996 and the cut areas were maintained during 1997 and 1998. Changes in water chemistry and all trophic levels in the lake were monitored through 1998.

The experimental study was effectively unfunded during 1999 and no macrophyte cutting was done. Enough funding was available to permit a summer macrophyte inventory and to fish the lake for two weeks in September.

A graduate student, Rob Robasco, sampled stomach contents of pike, perch and pumpkinseed in Lake 191 during 1999, in support of his Masters thesis at the University of Manitoba, Natural Resources Institute.

The future of this study depends on whether additional funding can be obtained. If funding can be secured, macrophytes will be harvested in 2000 for the fourth year and impacts documented on the water chemistry, fish, and other biota of the lake. Otherwise, the study may have to be terminated prematurely.

1.6. Comparison of Methods to Measure Bacterial Productivity in ELA Lakes

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

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

Frequency of dividing cells was measured bi-weekly on epilimnetic water samples from lakes 227 and 239 between May and October 1999. ^3H -thymidine incorporation was measured on epilimnetic water samples from lakes 227, 239, 373, and 260 collected between June and October 1999. Fresh water samples were returned to Winnipeg, usually within 48 hours after collection, and ^3H -thymidine incorporation along with bacterial enumeration were conducted at the Freshwater Institute.

Further laboratory experiments are planned for next year in order to optimize both of these methods for ELA waters, reducing the amount of variability that was observed this past year.

2. PHYSICAL PERTURBATIONS (RESERVOIR STUDIES)

This section includes experimental studies in which some physical aspect of the ecosystem has been manipulated. In most cases, this has involved experimental alteration of the water level, as a simulation of what typically occurs during the creation and operation of reservoirs. In Canada, these reservoirs are generally created primarily for generation of hydroelectricity. Many cause flooding over large areas of northern wetland and forest land.

2.1. Experimental Lakes Area Reservoir Project (ELARP)

The Experimental Lakes Area Reservoir Project (ELARP) is a whole-ecosystem flooding experiment designed to examine the production and mobilization of methylmercury (MeHg) in response to flooding, and to determine if reservoirs are significant sources of the GHG's carbon dioxide (CO_2) and methane (CH_4) to the atmosphere.

In June, 1993, following two years of background studies, the outflow of a ELA Lake 979 and its surrounding wetland was dammed, and the water level raised 1.4 meters to flood 14 hectares of peatland. Direct byproducts of the decomposition of the flooded vegetation in the peatland are CO_2 and CH_4 . Mobilization of MeHg within the flooded ecosystem and release to the atmosphere of CO_2 and CH_4 in

response to the flooding were monitored intensively. A non-flooded wetland system (ELA Lake 632), was monitored as a reference. Following winter drawdown, flooding of Wetland 979 was repeated in summer and fall of 1994 and 1995, as detailed studies continued in both wetland systems. In all three years, dramatic increases in MeHg and in release of the GHG were observed in response to flooding.

During the open-water periods of 1996, 1997 and 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, the system was again flooded, but no ecosystem monitoring was conducted. The next program of widespread monitoring on the system is scheduled for the year 2001.

2.1.1. The Bioaccumulation of Hg in Birds nesting adjacent to a Flooded Boreal Wetland

Principal Investigators: P.M. Gerrard and V.L. St Louis, University of Alberta, Biological Science

Overview: A primary objective of the Experimental Lakes Area Reservoir Project was to improve our understanding of the biogeochemical cycling of MeHg in reservoirs. Following the experimental flooding of lake 979, concentrations of MeHg in the water of the reservoir increased up to 10 times those measured before flooding, resulting in a large increase in MeHg concentrations in food chain organisms. Despite extensive research on fish and invertebrates living in reservoirs, comparatively little is known about the impacts of increased Hg methylation within reservoirs on terrestrial species living around them. For example, many species of birds rely on invertebrates from aquatic ecosystems as a food resource during the breeding season. The purpose of the bird portion of the ELARP is to determine the extent to which increased concentrations of MeHg in the experimentally flooded reservoir impacted tree swallows (*Tachycineta bicolor*), an aerial, insectivorous bird nesting adjacent to it. This includes quantifying MeHg bioaccumulation in the birds and their invertebrate food, and determining the impact flooding has on their reproductive success.

1999: The spring of 1999 was the last field season for the bird portion of the ELARP. The investigators collected birds for Hg analysis from the experimental reservoir (Lake 979) as well as from three other reference lakes. Some reproductive parameters were measured, but on the whole it was a short field season and they focused on Hg analysis of 1998 and 1999 birds as well as working up the eight years of reproductive and Hg data.

Future work: The researchers are currently in the process of tying up loose ends and finishing this project. The winter will be spent working up the data and writing results, as Ms. Girard is hoping to finish her thesis for a defense sometime in early spring. Dr. St. Louis will probably continue monitoring birds on L979, although this work will be less intensive and will likely revert to an alternate year schedule.

Thanks to: Manitoba Hydro
Circumpolar/Boreal Alberta Research (C/BAR)
The Mary Louise Imrie Graduate Student Award (for travel assistance)

2.1.2. "Bioreporter" Project

In a project related to ELARP, the use of a genetically engineered "bioreporter" to measure the bioavailability of inorganic mercury in lake water is being tested. Inorganic mercury is turned into

MeHg, which is more toxic, by bacteria that carry out methylation. The inorganic mercury must enter the bacterial cells to become methylated, but a lot of mercury in aquatic systems is bound to particles and DOC, and so is not bioavailable for methylation. The bioreporter system, which is a strain of bacteria that produces light when mercury enters its cells, allows scientists to quantify bioavailability. The ELA part of this work has been done by Karen Scott, who is a graduate student in Microbiology at the University of Manitoba. During 1999, she has continued to measure bioavailability of mercury through testing and refining the use of the Bioreporter system in natural waters at the ELA.

2.2. Lake 226 Drawdown Study

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

The lake will not be drawn down over the winter of 1999-2000. Rather, to continue study of the lake during recovery from drawdown, the water level will be allowed to continue at natural levels over the spring and summer of 2000. Continued monitoring of the lake in the 2000 summer field season will depend on funds available.

The following publications and presentations have resulted thus far from the project:

Publications:

Begout Anras, M.L., R.A. Bodaly, and R. McNicol. 1998. Use of an acoustic beam actograph to assess the effects of external tagging procedure on lake whitefish swimming activity. *Transactions of the American Fisheries Society* **127**: 329-335.

Begout Anras, M.L., P. Cooley, R.A. Bodaly, and R.J.P. Fudge. Tracking lake whitefish during spawning with acoustic tags. *Transactions of the American Fisheries Society* (in press).

Cooley, P.M. 1999. A manual for analysis and display of lake habitat and fish position information using GIS: physical and hydraulic habitat, drawdown, and acoustic telemetry. *Canadian Technical Report of Fisheries and Aquatic Sciences* **2276**: ix + 46 p.

Conference Presentations:

Begout Anras, M.L., R.A. Bodaly, et al. 1996. Spawning movements of lake whitefish in a boreal lake. *International Conference on the Biology and Management of Coregonid Fishes*, Constance, Germany.

Begout Anras, M.L., R.A. Bodaly, and R. McNicol. 1996. Behavioural effects of an externally applied acoustic tag on lake whitefish. *International Conference on the Biology and Management of Coregonid Fishes*, Constance, Germany.

Bodaly, R.A. et al. 1998. The Lake 226 drawdown experiment: effects on productivity, habitat and fish populations. *Canadian Society of Limnologists*, Kingston, Ontario.

Mills, K.H., S.M. Chalanchuk, D.J. Allan, and R.A. Bodaly. 1999. Growth, condition and recruitment of lake whitefish as a function of lake drawdown. *International Conference on the Biology and Management of Coregonid Fishes*, Ann Arbor, Michigan.

2.3. Flooded Upland Dynamics Experiment (FLUDEX)

The purpose of the upland flooding 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 experimentally flooded, beginning in June 1999, to create experimental hydroelectric reservoirs. The three areas were flooded by pumping water from a nearby lake, low in mercury and dissolved organic carbon. Flushing rates were equalized among the reservoirs by maintaining pumping rates proportional to estimated reservoir volumes. Greenhouse gas fluxes before and during flooding were measured at all three sites. Carbon dioxide, methane and nitrous oxide were monitored. Fluxes will be compared to the previously flooded boreal wetland (ELARP, see 2.1, above) 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 with direct planning application will be developed.

With the approach of winter, water was drained from the reservoirs, beginning in early October. The reservoirs will be refilled in the spring of 2000.

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; mitigation mesocosm studies

2001-02: year 3 of flooding, study of GHG emissions and mercury dynamics; year 2 of mitigation mesocosm studies

The three areas flooded are: a moist forest (Site 1), a dry forest (Site 2), and a very dry forest with areas of exposed bedrock (Site 3). The approximate size of each of these impoundments is as follows:

Site	Area (ha)	Mean Depth (m)	Volume (10 ⁴ m ³)	Dike Length (m)
1	0.61	1.0	0.61	190
2	0.70	1.2	0.84	130
3	0.71	1.0	0.71	350

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

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

After filling, the experimental reservoirs were characterized by relatively warm temperatures, high nutrient levels (especially in deeper waters), and severe oxygen deficits. The production of methyl mercury in the reservoirs was measured by net difference of fluxes into and out of the water bodies. Food chain bioaccumulation was determined by sampling zooplankton, emerging insects, and by introducing and sampling small fish in each reservoir. Fluxes of methyl mercury from flooded soils were determined by close interval sampling in and near the soil/water interface.

The following groups and individuals are involved in the experiment:

- Project coordination: Drew Bodaly (Freshwater Institute)
- Hydrology and Project design: Ken Beaty and Mark Lyng (Freshwater Institute)
- Mercury dynamics: Kristofer Rolffhus, 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)
Robert Fudge (Freshwater Institute)
David Findlay (Freshwater Institute)
- Greenhouse gases and carbon decomposition:
Elizabeth Joyce and Vincent St.Louis (University of Alberta)
Natalie Boudreau and Sherry Schiff (University of Waterloo)
Len Hendzel (Freshwater Institute)

Conference presentations resulting from the project:

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

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

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

2.3.1. Impact of Reservoir Creation on Greenhouse Gas Fluxes from Forested Uplands.

Reservoirs created for hydroelectric power have recently been identified as sources of greenhouse gases (GHG), including methane (CH₄) and carbon dioxide (CO₂), to the atmosphere. Following flooding plants die and stop taking up atmospheric CO₂ via photosynthesis. In addition, bacteria mineralize carbon stored in plants and soils to CO₂ and CH₄, which then flux to the atmosphere. The long-term impact of reservoir creation on GHG emissions should be related to the amount of organic carbon stored in ecosystems prior to flooding. In the northern Boreal landscape, where many Canadian reservoirs are developed, carbon stores range from large in peatlands to small in pockets of ridge-top forests.

The three FLUDEX boreal upland catchments being experimentally flooded range in their degree of organic carbon stores. A study by Elizabeth Joyce, of the University of Alberta, is focusing on the decomposition of this flooded organic carbon with two major objectives:

- 1) to investigate biogeochemical processes controlling initial rates of decomposition and GHG production in the flooded catchments with varying stores of organic carbon, and
- 2) to compare these initial rates with those measured in a previously flooded experimental wetland with large stores of organic carbon in peat.

Pre-flood, gas flux data were collected until experimental flooding occurred in mid-June, 1999. Sampling continued in the reservoirs following flooding and the data are being analysed.

2.3.2. Investigating Carbon Sources for Greenhouse Gas Flux using Stable Carbon Isotopes

Principal Investigators (Department of Earth Sciences, University of Waterloo)

Dr. Sherry Schiff

Natalie Boudreau (M.Sc. Student)

Dr. Ramon Aravena

Objectives

1. Construct a soil organic carbon inventory for each experimental reservoir to determine amount of organic carbon available for decomposition, and where organic carbon is stored.
2. Identify sources of greenhouse gases carbon dioxide and methane using stable carbon isotopes ¹²C and ¹³C.
3. Identify processes affecting greenhouse gases using stable carbon isotopes.

Summary of work carried out during 1999

Pre-flood carbon inventory work:

Pre-flood field work involved identification and collection of possible carbon sources for greenhouse gas production. The researchers conducted detailed soil coring and sectioning, collected vegetation samples, and mapped soil depth and surface vegetation in each enclosure. Pre-flood lab work involved running soil and vegetation samples on an elemental analyzer to determine the percent carbon and carbon to nitrogen ratio of each soil horizon and vegetation type. From this data the investigators calculated the actual amount of carbon contributed by specific soil horizons and constructed spatial maps of soil organic carbon.

Post-flood GHG source/process work:

In summer 1999, the researchers collected reservoir water samples for dissolved inorganic carbon (DIC) and methane (CH₄) isotopic analysis. Isotope work on DIC will identify the vegetation type or soil horizon source of the gases and improve flux calculations. Isotope work on CH₄ will allow the research team to discriminate between CH₄ production from microbial sources and consumption by oxidation.

The following samples were taken from each reservoir on an intensive schedule throughout the flooded season: inflows, outflows, seeps, samples at depth from submerged chambers, samples throughout the water column from sippers, and bubbles from bubble traps. Methane and DIC samples were taken during each sampling event. Sampling was coordinated with Elizabeth Joyce (see 2.3.1., above) so that isotope and concentration data could be used in conjunction to improve flux calculations.

Post-flood C isotope work:

Water samples are currently being run for DIC and methane isotopic signature. Soil and vegetation signatures are known from pre-flood work. The measured DIC signature of outflow waters will be a combination of the inflow and the source signature. The measured CH₄ signature of water column samples will indicate whether CH₄ is being produced (depleted signature) or consumed (enriched signature). Isotopic analysis of reservoir waters will continue into the winter and results will be available in the next few months. One core per enclosure has been set aside for ¹⁴C analysis of carbon accumulation.

Continuation of Study/ Spin-off Research:

Samples will be taken for isotopic analysis in other post-flood years. This information will tell us if the source of the GHG's is changing over time and how processes affecting the gases are changing over time. Jason Venkiteswaran has recently begun his Masters program and will be doing field work on the FLUDEX project in 2000.

Spin-off research includes an undergraduate thesis by Grant Ferguson, currently underway. Grant will leach different soil horizons from each reservoir in an attempt to determine any differences in isotope signature between soil and the water leached from the soil. This information will help in our interpretation of carbon sources.

2.3.3. *Effects of Reservoir Creation on Mercury Methylation Rates*

The Upland Flooding Experiment has been designed to test the hypothesis that methylmercury (MeHg) and greenhouse gas production in reservoirs is related to the amount of carbon stored in the reservoir. The three sites have been chosen to represent three different types of upland forests; namely, a moist forest (Site 1: jack pine stands, with *Sphagnum* and *Ledum*), a dry forest (Site 2: thick jack pine stands

with some birch and alder), and a very dry forest (Site 3: jack pine stands with exposed bedrock outcrops). Each of these sites has different amounts of organic matter stored in the vegetation and soils.

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

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

During the summer of 1999, MeHg and THg concentrations in ten vegetation tissues were determined, and litterbags were placed in each reservoir to measure the change in MeHg and THg concentrations in vegetation over five years. Ms. Hall will continue to measure inputs and output and fluxes of MeHg and THg in years 2000 and 2001. Mass balance budgets will be constructed for each site and compared to one another, as well as to the Experimental Lakes Area Reservoir Project's seven-year-old reservoir (L979), created over a wetland that contained large stores of carbon as peat (see 2.1., above)

The results from this research, comparing MeHg cycles in different forest reservoirs, will be used to calibrate a mercury model to help predict the behaviour of MeHg in flooded ecosystems. Increased predictive abilities will in turn help to develop mitigation strategies for future reservoir creation.

3. NATURAL VARIABILITY, UV RADIATION, AND CLIMATIC FACTORS

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

Over more than three decades, researchers at the E.L.A. have been collecting data on natural lake ecosystems in support of, and as references for, the experimental studies. Increasingly, this data set is becoming important in its own right because of the unusual scope and length of the records.

3.1. Long-Term Ecological Research and Data Management

Principal Investigators (Freshwater Institute)

K. Beaty, D. Findlay, R. Hesslein, S. Kasian, M. Lyng, K. Mills, M. Paterson, J. Shearer, M. Stainton, M. Turner

Project Summary and Goals

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

There are three objectives for the project:

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

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

Work carried out in 1999

Five core lakes (114, 224, 239, 373, and 442) received, where possible, full discipline coverage. Data collected included hydrology, Secchi depth and water temperature, water chemistry, phytoplankton production, phytoplankton populations, periphyton, zooplankton, and fish. Discipline specific monitoring continued in other non-manipulated lakes. A multi-disciplinary workshop was held to examine aspects of spatial and temporal variability at the ELA, gleaned from existing long-term records, with the objective of setting criteria for the selection of lakes and sampling plans. The research presented will be used to establish the rationale for future sampling efforts. Three possible papers were identified: the relationship between precipitation and water yield and the effect on chemical transport from watersheds (master's thesis), within-lake degradation rates of DOC, and the relationship of within and between lake variability of chemical constituents to physiogeographic characteristics of lakes.

3.1.1. 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, and is operated by ELA staff. Ken Beaty, with assistance from Mark Lyng, Neil Fisher, and others, has primary responsibility for this facility and data are contributed to the MSC national climate database. Data on air temperature, precipitation (rain and snow), wind speed and direction, bright sunshine, and evaporation are collected on a daily basis at the site. In June of 1999, this site reached the milestone of 30 years of continuous monitoring record, making it an official long-term station in the MSC Climate network.

3.1.2. Impact of Natural Disturbances on the Lake 239 Watershed

Long-term hydrological, meteorological, and chemical monitoring in the calibrated catchments of this watershed continued during 1999. Portions of the watershed were perturbed by a major forest blow-down in 1973, and by forest wildfires in 1974 and 1980. This and other ELA watersheds have been subjected to extremes of precipitation over the monitoring period. The monitoring is intended to evaluate long-term effects of these and other natural perturbations on the lake ecosystems, and to calibrate other hydrological

studies at the ELA. The watershed has been continuously monitored for 31 years. No chemical additions are made.

3.1.3. Canadian Air and Precipitation Monitoring Network (CAPMoN)

ELA personnel, under the direction of K. Beaty, continued to operate a CAPMoN station at the ELA met site in 1999. 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, SO₂ and HNO₃ in the atmosphere, Cl, SO₄, NO₃, Na, NH₄, Ca, K, Mg, pH, and mercury in precipitation. The ELA site, which has been operating since the 1980's, is used as a baseline reference for sites in eastern Canada.

3.1.4. Canadian Network Isotopes in Precipitation (CNIP)

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

3.1.5. Phytoplankton Nutrient Status in ELA Lakes

Principal Investigator: Len Hendzel

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 their environment provide direct and relevant answers regarding algal interactions within the aquatic food chain. Algal physiology and phytoplankton nutrient status explore the roles of essential nutrients (C, N, P) and physical factors in controlling algal species composition, succession and blooms, and chemical composition (lipids/carbohydrates, proteins, composition ratios, cell quotas), and determine to what extent laboratory studies can be applied to field situations. The species composition and biochemical composition of algae, together with other phytoplankton and zooplankton data, can determine the efficiency of food chains, effect of perturbation, the production and consequences of harmful phycotoxins, and also the bio-availability of environmental toxic substances and their rates of removal from surface waters. As part of a continuing dataset, synoptic measurements of phytoplankton nutrient status were made on a selected number of ELA lakes during 1999.

Phytoplankton nutrient status (alkaline phosphatase activity and nitrogen debt) was measured on epilimnetic and metalimnetic water samples from lakes 227, 239, 302S, 373 and 260 on a bi-weekly schedule between May and June, then monthly until October.

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

3.2. Direct and Indirect Effects of Ultraviolet Radiation

Over the past several years, a research group from the University of Alberta, led by Dr. David Schindler, has carried out a variety of studies focusing on the effects on lakes and streams of ultraviolet-B (UV-B) radiation. These studies have examined the role of dissolved organic carbon (DOC) as a shield against UV-B in freshwater systems.

During 1999, aspects of this work were continued at the ELA by University of Alberta graduate students. They investigated the effects and interactions of UV radiation and DOC on algal populations in limnetic and littoral zones of lakes.

3.2.1. Importance of Near-surface Thermoclines in Combination with UV Radiation in Boreal Lakes

Marguerite A. Xenopoulos, Ph.D. candidate

Supervisor: Dr. D.W. Schindler

Dissertation Summary

Shallow, near-surface thermoclines form under sunny and near-windless conditions and can retain the plankton under photoinhibitory irradiances, including ultraviolet (UV) radiation, for prolonged periods of time. The objective of the following projects was to examine the combined effects of high UV penetration and shallow secondary thermoclines on phytoplankton. Particular attention was placed on the combination of very high incident UV and still weather, when planktonic organisms are held isolated in a shallow layer of high UV radiation. Such conditions have been found to be particularly detrimental to some phytoplankton species. Below is a brief description of projects conducted in 1999.

Brief Description of Projects conducted at ELA in 1999

3.2.1.1. Mixing Rates, Near-Surface Thermoclines and Phytoplankton Movement in Relation to Different Light Fields

(M.A. Xenopoulos, W. Edwards, D. A. Culver, and D.W. Schindler)

Fine-scale profiles of temperature and turbulence were taken using a microstructure profiler. Few ecological studies of phytoplankton have utilized realistic light variations experienced by cells in the water column due to vertical mixing. Many studies do not consider the potential for refuge from UVB in the lower portion of the well-mixed layer. However, data from microstructure profilers can be used to extrapolate realistic light variations with depth. From these data, the vertical extent of eddies and the time scales of vertical mixing within the water column can be determined. These data can be used to predict realistic cell trajectories, and, therefore, actual PAR and UV variation experienced by phytoplankton. This project was conducted in Lake 224 and Lake 302.

3.2.1.2. The Influence of Ultraviolet Radiation on Alkaline Phosphatase Activity in two Boreal Lakes

Suzanne E. Tank¹

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Although ultraviolet radiation (UVR) has been shown to influence primary production, little is known about the influence of UVR on alkaline phosphatase, an extracellular enzyme that cleaves inorganic phosphorus from dissolved organic matter. The impact of UV-A and -B radiation was assessed on alkaline phosphatase activity (APA) in two boreal lakes of differing light and chemical characteristics at the Experimental Lakes Area. Samples of surface water were incubated near the lake surface under three

different light regimes: ambient radiation, ambient radiation screened for UV-B, and ambient radiation screened for both UV-A and UV-B. Incubation occurred for 48 hours; treated samples and an additional lakewater sample were removed for analysis at the 24- and 48-hour mark. Both unfiltered water samples, and samples filtered through a 0.2 micron filter were measured for APA. Further analysis will be undertaken to correct samples for the level of biomass present in them, and assess the nutrient status of the organisms under differing radiation treatments. Decreases of APA in the presence of ultraviolet radiation could increase P-stress in low nutrient aquatic environments.

3.2.1.3. The influence of Ultraviolet Radiation on the Phytoplankton Populations of the Top and Bottom of the Mixed Layer

(M.A. Xenopoulos and D.W. Schindler)

Numerous studies have shown that there can be considerable variation in the thermal structure and mixing dynamics of a lake from one day to another. This variation can affect the average light intensity experienced by the phytoplankton. The objective of this study was to determine the effects of UVA, UVB, and PAR on near-surface phytoplankton populations and phytoplankton populations from the bottom of the mixed layer when trapped in near-surface waters for 4 hours, 24 hours and 48 hours. Variables measured were: ^{14}C incorporation, bacterial production, biomass (chlorophyll, carbon) and particulate phosphorus. The experiments were done in L224 and L302S.

3.2.1.4. The influence of UVR on phytoplankton growth and elemental composition in two boreal lakes

(M.A. Xenopoulos, P.C. Frost, D.W. Schindler and J.J. Elser)

The influence of UVR on *in situ* algal growth rates and elemental composition is still largely unknown. The objective of this study was to determine the influence of UVR on the growth rates and elemental composition of phytoplankton in near-surface waters. This project was conducted in L224 and L302S with the use of bioassays during three different periods: spring bloom (May), summer solstice (late June) and summer (July-August).

3.2.1.5. The Influence of Ambient UVR on Phytoplankton Pigments

(M.A. Xenopoulos, P.R. Leavitt and D.W. Schindler)

The objective of this study is to determine the influence of daily ambient UVR on the phytoplankton dynamics and pigmentation. Some studies have found a high concentration of certain carotenoids in phytoplankton under UV-stress. Carotenoids can protect the algae from intense UVB. This study was conducted in Lake 114, Lake 224, Lake 302S and Lake 239. Pigments were quantified with the use of HPLC.

3.2.1.6. The Influence of UVR on the Cyanobacteria of an Artificially Fertilized Boreal Lake

(R. Hazewinkle and M.A. Xenopoulos)

The influence of UVA and UVB was studied on the cyanobacteria of Lake 227. Lake 227 has been experimentally fertilized since 1969 (see 1.1, above) and the phytoplanktonic community is dominated by cyanobacteria during much of the open-water season. Data are still being processed.

Future Objectives (Preliminary for summer 2000)

This marks the end of planned field work at the Experimental Lakes Area. Future studies may use archived phytoplankton data from ELA for comparative surveys.

3.3. Effects of UV-B Radiation on a Pelagic Community

A research team from the University of Winnipeg, led by Drs. Rod Bollman, Judith Huebner, and Donna Young, and including two student research assistants, completed their first 6-week field season at the ELA, spanning three weeks before and three weeks after the 1999 summer solstice. The focus of this study is to try and estimate the impact of ultraviolet-B (UV-B) radiation on the pelagic community of boreal freshwater lakes. Two experiments were conducted during this time, the planning and development of which occurred in 1998 through two pilot studies at the ELA.

The first experiment, carried out in Lake 239, was designed to examine the effect of UV-B on total community structure. This involved placing six limnocorrals in a shallow, open bay. The 2 m wide and 1 m deep enclosures were divided into three replicates each of control (covered by UV-B-opaque Mylar polyester) and experimental (covered by polyethylene) treatments. While the original plan was to examine effects of UV-B on algae, *Daphnia* species, and minnows in these enclosures, the small numbers of the latter two organisms in Lake 239 precluded this. Instead, it was decided to focus on the effects of UV-B on algal community composition. The enclosures were maintained for six weeks and water samples were taken every three days for analysis of the changes in algal community structure. Light measurements (PAR, UV-B, and UV-A profiles), lake and enclosure temperature profiles, and dissolved oxygen content within and outside the corrals were also measured at three-day intervals. Chemical parameters in the lake and corrals, including total dissolved PO₄ and N, total particulate PO₄, NO₃, and C, and total chlorophyll were measured at six-day intervals. In order to examine benthic algal colonization rates under UV-B exposed and sheltered conditions, strips of triflex were suspended in the enclosures, and sampled intermittently over the six week run. At this time, the analysis of samples continues, and results will be reported at a later date.

A new experiment to investigate the effects of UV-B on *Daphnia sp.* survival, reproduction, and carotenoid pigment accumulation was developed and attempted during summer 1999. This involved the suspension of daphnids in small volume (1 L) enclosures at different depths in Lake 110. Groups of 25 UV-B shielded and exposed daphnids were counted every two days, and survival, number of offspring, and production of ephippial eggs were recorded. After ten days, carotenoid extractions would have been performed on ten daphnids from each depth and treatment; however, survival in the enclosures was too poor to allow for this. Problems were also encountered in terms of locating all the adult daphnids, as they tended to disappear altogether, perhaps due to rapid decomposition after death. Preliminary results suggested that re-design of the enclosures is probably necessary before next summer.

3.4. Metalimnetic and Hypolimnetic Chlorophyll Peaks

For the fourth consecutive summer, Dr. F.J. DeNoyelles, Jr. and Dr. David Graham of the University of Kansas conducted a survey of several ELA lakes to evaluate the magnitude and status of metalimnetic and hypolimnetic chlorophyll maxima attributable to concentrations of algae and photosynthetic bacteria at distinct depth strata. In 1999, Lake 260, the candidate lake for the estrogen addition, was a major focus of this work.

The work is a follow up to extensive studies of these peaks conducted at the ELA by Dr. DeNoyelles and other researchers during the 1970's and 1980's. Results from lakes at the ELA are being compared to those from artificial lakes in Kansas and other sites much further south. Similar work has been conducted for the Coldwater Lakes study area near Atikokan, Ontario.

4. POTENTIAL FOR RECOVERY OF BOREAL LAKES FROM ACIDIFICATION

Contributors:

Michael Turner, Brenda Hann (3), Dave Schindler (2), David Findlay, Ken Beaty, Kenneth Mills, Len Hendzel, Mark Lyng, Michael Paterson, Michael Stainton, Ray Hesslein, Rolf Vinebrooke (4), Sandy Chalanchuk, Susan Kasian, Vince St. Louis (4) and Willie Findlay (5).

Except as noted, researchers are members of (1) the ELA team of the Department of Fisheries and Oceans. Other affiliations are: (2) U. Alberta, (3) U. Manitoba, (4) U. Regina, and (5) Winnipeg, MB. Note also that the order of researchers after MT is based on the alphabetical order of their first names.

Our general goal has been to understand the potential of small boreal lakes to recover from acidification. Such research is germane to Canada and to other nations in both the northern and southern hemispheres. More specifically, we have been studying blockages (whether chemical or biological) to ecosystem recovery, and where they exist, to discover possible remedies. Additionally we have been preparing to investigate the possible linkages between recovery potential and climate change by examining the interaction of two master limnological variables, pH and DOC. Moreover, this research provides a fundamental opportunity to study the effects of long-term stress on aquatic ecosystems.

Despite the importance of this research, funding continues to limit the progress of this project. Specifically, achievement of our research goals was hampered during 1999 by the limited amount of funding available, and by further cuts to ELA's A-base funding. Although this should be one of the federal government's priorities for research support, for several years research into acidification recovery has been ignored as a priority.

Because of a one-time \$12K grant from the Department of Environment in 1999, some sampling of Lake 302S was continued in the pelagic zone, but we were forced to suspend almost all sampling of Lake 223. We maintained pH 6.2 as the target in Lake 302S for a second year. We studied fish populations, pelagic biology (zooplankton, algae and bacteria), water chemistry, and hydrology, although sampling effort in each was arguably sub optimal. Study of the littoral subecosystem was suspended in 1999, as was any process-oriented research. In the fall, we re-established the lake whitefish population in L302S, which will allow us to determine whether they can survive in both the new chemical soup of the recovering lake, and the new biological circumstances.

Pending sufficient funding, our plans for 2000 and beyond are to continue ecosystem monitoring in Lake 302S for about the final three years of step-wise pH recovery, and to make synoptic observations in Lake 223 now that its pH has returned to its pre-acidification level. We intend to conduct mesocosm and survey studies to further evaluate the chemical and biological blockages to ecosystem recovery that we have identified, and to explore possible remedies. We will pay close attention to the success of the lake whitefish reintroduction, and to the impacts of the fish on the ecosystem. Additional effort will be directed to evaluating the interactions of climate change (principally mediated through DOC) with the recovery from acidification.

We published, or made substantial progress in, the following journal publications during 1999:

Published or In Press

Findlay, D. L., S. E. M. Kasian, M. A. Turner, and M. P. Stainton. 1999. Responses of phytoplankton and epilithon during acidification and early recovery of a lake. *Freshwater Biol.* **42**: 157-175.

Hann, B. J., and M. A. Turner. 1999. Littoral microcrustacea in Lake 302S in the Experimental Lakes Area, northwestern Ontario, Canada: acidification and recovery. *Freshwater Biol.* (in press).

Hann, B. J., and M. A. Turner. 1999. Exploitation by microcrustacea of a new littoral habitat in an acidified lake. *Hydrobiol.* (in press).

Submitted or Under Review

Mills, K.H., S.M. Chalanchuk, and D.J. Allan. Recovery of fish populations in Lake 223 from experimental acidification. *Can. J. Fish. Aquat. Sci.*

Mills, K.H., S. M. Chalanchuk, and D. J. Allan. The loss of fish populations from acidified L302S due to recruitment failures and fish kills. *Freshwater Biology*

Vinebrooke, R. D., D. W. Schindler, D. L. Findlay, M. Paterson, E. U. Schindler and M. A. Turner. Loss of biodiversity and function in acidified boreal lakes. *Limnol. Oceanogr.*

In Preparation (note that the following authorship and titles are provisional)

Donahue, W. F., M. A. Turner, D. L. Findlay, P. R. Leavitt, D. W. Schindler, M. P. Stainton and S. J. Page. Analysis of physical, chemical, and biological interactions in the structuring of shallow epilithic communities in boreal lakes. *Ecol. Monographs* (Under external review)

Turner, M. A., M. P. Stainton, R. Hesslein, S. E. M. Kasian, and R. Hecky. Chemical features of the early recovery of a boreal forest lake from experimental acidification to pH 4.5. *Freshwater Biol.* (In prep.)

Vinebrooke, R., M. A. Turner, K. Kidd, B. J. Hann, and D. W. Schindler. 2000. Truncated food-web effects of omnivorous minnows in a recovering acidified lake. *Ecology* (In prep.).

5. PERSISTENT TOXIC SUBSTANCES

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

In addition, these experimental studies with persistent toxicants provide an opportunity to determine the physiological bases of ecosystem effects, thereby identifying indicators of stress at lower (physiological,

histological) levels of biological organization. Once identified, these indicators can be extremely useful for the assessment and remediation of environmental problems.

5.1. Organochlorines and Poly-Aromatic Hydrocarbon Study

The purpose of this study was to evaluate the effects of chlordane, toxaphene, and a dibenzofuran on lake trout and white sucker populations in Lake 260. It formed the basis for a Ph.D. thesis completed by Peter Delorme (University of Manitoba) in 1994.

In 1988 and 1989, low concentrations of each compound were injected into the bodies of adult lake trout and white sucker. These individuals were recaptured over a series of years to evaluate changes in spawning efficacy, through egg incubation experiments, and changes in the annual survival of the injected individuals. Non-injected individuals were monitored each year as reference fish for the injected fish. Most of the injected individuals were removed from the population for analysis of residue in their tissues.

In 1999, Lake 260 was studied intensively in preparation for a whole-lake addition of a synthetic estrogen, planned for 2000 (see 6.1., below). A fall fish survey revealed one individual that had been injected with the "control" substance, corn oil, during the experimental study, but no individuals injected with the toxic compounds. No individuals that had been injected with chlordane, toxaphene, or dibenzofuran have been recaptured since 1995. The fish community in Lake 260 will be monitored during the estrogen study, but it is unlikely that any of these contaminated fish remain in the lake.

5.2. Cadmium Addition to Lake 382

Between 1987 and 1992, trace amounts of cadmium were experimentally added to the surface waters of Lake 382. The purpose was to test the adequacy of existing guidelines for protecting against chronic cadmium contamination effects in real aquatic ecosystems. Concentrations of cadmium in Lake 382 were gradually raised each ice-free season so that approximately 200 nanograms per litre ($\text{ng}\cdot\text{L}^{-1}$) were present during the summers of 1991 and 1992. This is still almost 2 orders of magnitude below the Canadian drinking water standard, but at a level where biological impacts would probably occur if the treatment were chronically maintained. By the end of 1992, a total of 7 kg of cadmium had been added to Lake 382. Most of this has moved into the lake sediments. No cadmium has been added since 1992, but regular monitoring continued through 1996 to observe responses to the "zero discharge strategy".

Since 1996, no funding has been available to continue the detailed monitoring. Dr. Diane Malley, the study coordinator, has retired from DFO. Basic chemical parameters, and phytoplankton and zooplankton populations were monitored monthly in 1999. Fish sampling was also done in September 1999. Sediment samples for cadmium analysis will be taken from Lake 382 this winter. Current plan calls for periodic cadmium monitoring to continue, but not on an annual basis.

5.3. Mercury Experiment to Assess Atmospheric Loading in Canada and the United States (METAALICUS)

Why is METAALICUS Necessary?

This whole-ecosystem experiment describes research that will *directly answer for the first time* what happens to fish mercury concentrations when there is a change in atmospheric mercury deposition. Despite the massive amounts of scientific information published on mercury contamination of ecosystems, there remain very basic questions about the environmental behavior and effects of this element. A critical uncertainty is—whether a reduction in atmospheric mercury emissions will reduce mercury concentrations in fish. It is important to answer this question now because controls on industrial emissions of mercury have been proposed that will cost *billions* of dollars per year if implemented, yet the effectiveness of these controls is unknown.

Mercury is the most common contaminant in fish in the United States and Canada. Forty-two states have advisories against fish consumption due to high mercury levels, and unacceptable fish mercury concentrations exist in all Canadian provinces and the Northwest Territories, including remote “pristine” lakes. Ninety-seven percent of closures to fishing in Ontario are due to mercury contamination.

Mercury is a neurotoxin. There is a particular concern for prenatal life and infants, for whom safe levels of mercury exposure are lower than in adults. Concern also exists regarding the toxic effects of mercury on reproduction, behavior and survival in fish-consuming birds and marine mammals.

Experts agree that the relationship between atmospheric mercury deposition and the mercury in fish can not be understood by examining historical or regional data. This is mainly because empirical attempts to isolate the effects of mercury loading on fish mercury levels are confounded by other environmental factors which cause variations in fish mercury concentrations in lakes. These factors include a wide range of local site conditions and annual weather variability, often combined with poorly documented mercury loads. By contrast, this experiment provides the control necessary to examine the effects of one critical factor: mercury loading. Furthermore, this experiment will also allow us to distinguish newly deposited mercury from background mercury that has accumulated over hundreds of years.

What is METAALICUS?

METAALICUS is a whole-ecosystem experiment in which mercury inputs to a headwater lake (example Lake 658) and its watershed will be increased experimentally. An ecosystem approach will be used because the complex pathway of mercury from the atmosphere to fish cannot be simulated in laboratory experiments.

The mercury will be added as 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 the background mercury.

Different mercury isotopes will be added to the upland, wetland and the lake surface to determine if the route of entry affects how much is accumulated in the fish .

Comprehensive field studies will be carried out to establish site conditions and mercury concentrations in all compartments in the lake and watershed, and how they change with time. Detailed process studies will also be carried out. Movement of the mercury and transformations between mercury forms will be followed through the watershed and lake. Production of methylmercury (MeHg) will be studied in the lake sediments, upland and wetland, as will the bioaccumulation of MeHg into benthic organisms, plankton and fish. Methylmercury will be followed specifically because it is the dominant form in fish and the most toxic form of mercury in the environment.

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 will also provide 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 study will be carried out in two phases over a 5-year period. Phase 1 has started and involves a series of pilot studies, and background information on the study site. In Phase 2, the mercury loading to the whole ecosystem will be increased.

Study Area

The Experimental Lakes Area (ELA) is situated in Northwestern Ontario, where atmospheric deposition of mercury is relatively low. Uninhabited research watersheds have been set aside for ecosystem-scale, controlled studies of environmental stressors. ELA lakes and their drainage basins have been used over the past 30 years to provide leading limnological studies in freshwater lakes, including landmark whole-lake studies of the effects of nutrients, acidification and reservoir construction. Other mercury studies at ELA include the completed Experimental Lakes Area Reservoir Project (ELARP) (see **2.1.**, above) and the ongoing Flooded Uplands Dynamics Experiment (FLUDEX) (**2.3.**, above). These experiments are investigating mercury cycling in natural boreal ecosystems and in experimentally flooded wetlands and uplands.

How Much Hg will be Added?

Not much. Approval is being sought for experimental addition of mercury to an ELA lake and its watershed. ELA is naturally a low deposition area for mercury, with approximately 3 ug/m²/yr of wet mercury deposition. The low mercury deposition rate at ELA means that adding the equivalent of about half a teaspoon of mercury over the entire 5 year study is enough to increase the atmospheric load to a 50 ha system by 4 to 5 times. **This amount of mercury addition will result in a mercury deposition rate to the experimental system that is equivalent to rates currently observed in the Northeastern United States** (figure below).

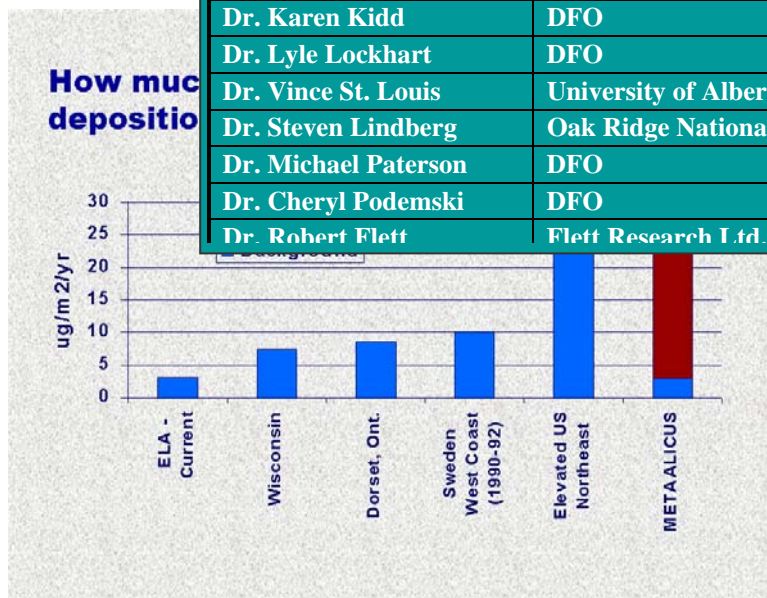
The Study Team

The project team (see table) includes leading mercury researchers from the United States and Canada. We also have an international advisory committee from Sweden, the USA and Canada. The team has extensive experience with whole ecosystem mercury experiments and a history of successful collaboration.

Current Activities

A general research plan was drafted at two workshops in Toronto, Ontario, in 1998 and in Washington, D.C., in 1999. It was decided that initial pilot scale studies are essential to refine the analytical methods and to ensure that the effects of the additions proposed will be measurable during the full-scale study.

METAALICUS ProjectTeam	
Principal Investigator	Organization
Mr. Reed Harris, P. Eng.	Tetra Tech Inc.
Dr. John Rudd	Canadian Department of Fisheries and Oceans (DFO)
Dr. Carol Kelly	DFO
Dr. Holger Hintelmann	Trent University, Ontario
Dr. Marc Amyot	University of Ottawa
Dr. R.A. (Drew) Bodaly	DFO
Mr. Ken Beaty	DFO
Dr. Paul Blanchfield	DFO
Dr. Brian Branfireun	University of Toronto
Mr. David Findlay	DFO
Dr. Cynthia Gilmour:	Academy of Natural Sciences, MD
Dr. Andrew Heyes	Academy of Natural Sciences, MD
Dr. James Hurley	University of Wisconsin/Wisconsin DNR
Dr. David Krabbenhoft	USGS
Dr. Karen Kidd	DFO
Dr. Lyle Lockhart	DFO
Dr. Vince St. Louis	University of Alberta
Dr. Steven Lindberg	Oak Ridge National Laboratory, TN
Dr. Michael Paterson	DFO
Dr. Cheryl Podemski	DFO
Dr. Robert Flett	Flett Research Ltd.



Pilot studies have been approved and undertaken during 1999. These include:

- Isotopic ²⁰²Hg(II) additions to a small upland catchment (1100m²), a wetland plot (300 m²), and a pine tree stand.
- Refinements to methods for measuring isotopic mercury concentrations in various media.
- Preliminary studies of the experimental lake and its watershed.

The pilot studies have confirmed that the analytical techniques are sufficiently sensitive to detect the small additions of added isotope, and that the general research design is viable.

International Advisory Panel	
Advisor	Organization
Dr. Åke Iverfeldt	Swedish Environmental Research Institute (IVL)
Dr. John Munthe	Swedish Environmental Research Institute (IVL)
Dr. Edward Swain	Minnesota Pollution Control Agency
Dr. Ray Hesslein	Canadian Department of Fisheries and Oceans

Future Activities and Schedule

In 2000 we will continue baseline studies and begin an aquatic mesocosm experiment with different Hg doses. The full-scale addition experiment is planned to start in 2001 and will run for three years. Mercury will be added at intervals to mimic natural seasonal deposition patterns. Initial full-scale results will emerge in 2001. 2004 will be dedicated to final reporting and publishing for the study. If appropriate, less intensive sampling in the longer term will be considered.

Communications

To ensure timely dissemination of research results we will employ different means of communications: a) annual workshops for government, industry and team members, b) publications in newsletters and primary research journals throughout the study, and c) general information distribution via the world-wide-web. The URL for the METAALICUS web site is:

<http://www.biology.ualberta.ca/metaalicus/metaalicus.htm>

METAALICUS Status and Funding

Funding for METAALICUS is about half complete. We presently have funding from the Canadian Department of Fisheries and Oceans, the Electric Power Research Institute, the U.S. EPA, the USGS, the Wisconsin DNR, the National Sciences Engineering and Research Council of Canada, and the U.S. Department of Energy. Approval is being sought for the whole-ecosystem additions.

6. ENDOCRINE DISRUPTING CHEMICALS (EDC'S)

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

6.1. Effects of a Synthetic Estrogen on Aquatic Populations

Project Team:

Freshwater Institute:

Karen Kidd, Principal Investigator
Paul Blanchfield
Bob Evans
Dave Findlay
Jack Klaverkamp
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

Background

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

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

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

Purpose of Experiment

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

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

Study Site

Lake 260 was chosen for this study and has a surface area of 34 hectares with a maximum depth of 14.4 m. This lake has been part of a long term monitoring program at the ELA and considerable data exist on its limnological, physical, and biological characteristics. It supports well-defined lake trout (*Salvelinus namaycush*) and white sucker (*Catostomus commersoni*) populations of approximately 300 and 500 individuals, respectively, as well as the small fish species fathead minnow (*Pimphales promelas*; used in laboratory EDC assays) and pearl dace (*Semotilus margarita*). Two nearby lakes, Lakes 442 and 375, will be used as reference systems throughout this study, as they are similar in physical and chemical characteristics, have the same fish species with similar population sizes to Lake 260, have long-term data on fish populations, and the former lake has historical data on lower-trophic-level biota.

Background Studies in 1999

This first year of the study, 1999, consisted of data collections and mesocosm studies in preparation for the whole-lake additions of EE2 in 2000 and 2001. As described below, population data were collected for fish and invertebrates, and background samples were collected to obtain biochemical, histological and reproductive information for fishes from these lakes. These data, and those obtained from the reference lakes throughout the study, will be critical in assessing the natural variability of these systems and in interpreting effects of the EE2 whole-lake additions.

Mesocosm Studies: Several mesocosm studies were conducted this year to determine the fate of EE2 in Lake 260. Four mesocosms were spiked with EE2, and water samples were collected to monitor the half-life of this chemical in lake water. Preliminary results suggest that this would not persist for long periods of time in the lake after experimental additions were discontinued. Results from this and other studies will be modelled and used to determine EE2 addition rates for the whole-lake experiment to be conducted in 2000 and 2001.

A second limnocorral study was conducted to determine whether continuous EE2 additions are likely to have direct impacts on lower-trophic-level biota. EE2 was added continuously to six mesocosms to maintain water concentrations at levels between 5 and 600 ng EE2/l (encompasses potential concentrations for the whole-lake study). Effects on zooplankton, phytoplankton, bacterial samples and benthic macroinvertebrate samples are currently being examined using samples collected throughout this five-week experiment.

Fish Studies: Spring and fall trap net and short gill net sets were used to continue the mark-and-recapture program that evaluates the populations of lake trout and white sucker in Lakes 260, 375 and 442. Sampling was done in a manner that minimizes mortality, and the data will be used to assess age and size distributions, sex ratios, age to maturity, condition factors, abundance, growth rates, and annual survival and recruitment for lake trout and white sucker. These data and those collected from the reference systems during this study will be used to assess annual variability in unmanipulated populations, and to determine the effects of EE2 on fish populations in Lake 260.

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

Molecular and Cellular Responses in Fish: (see 6.1.1., below)

Spring collections for 1999: Fathead minnow histological samples taken include: Lake 260, 10 males, 14 females; Lake 442, 12 males, 10 females; Lake 114, 15 males, no females. Vitellogenin and vitamin samples. Lake 260 20 males, 18 females. Lake 442 13 males and 14 females. Lake 114 20 males, no females. Pearl Dace include Lake 260, 13 males, 15 females; Lake 442, 9 males, 2 females. Vitellogenin and vitamin samples. Lake 260 14 males and 14 females, Lake 114 11males and 5 females, Lake 442 33 males and 7 females. Lake trout blood sampling in spring for Lake 260=6, Lake 375 = 7 and Lake 442 =5 (sex not evident for spring collection of lake trout) White suckers for blood in spring. Lake 260 = 1 male and 4 females, Lake 375 = 5 males and 7 females, Lake 442 = 3 males and 13 females.

Fall collections for 1999: Fathead minnow from Lake 260, 13 males, 2 females; Lake 114, 13 males, 2 females; no fish from Lake 442. Pearl dace include 12 males and 12 females from each of Lakes 260, 114 and 442. Lake Trout (blood, histology, and tissues) Lake 260 = 4 females and 5 males, lake 375 = 9 males and 4 females, Lake 442 4 males and 5 females. White sucker for blood, histology and tissues. Lake 260 = 9 males and 6 females, Lake 375 = 1 male and 1 female, Lake 442 = 5 males and 7 females. Plasma samples were collected in the fall from male and female lake trout and sucker to assess circulating reproductive hormone, egg-protein (VTG), and sodium, potassium and calcium levels. Hormone samples are currently being analysed for the steroids estradiol, testosterone, 11-ketotestosterone, and 17α , 20β dihydroxy-4-pregnen-3-one by M. McMaster (EC, Burlington) and GtH II (LH-like gonadotropin) content by G. Van Der Kraak (University of Guelph). Whole body homogenates of fathead minnows and pearl dace were collected for VTG analyses in the spring and fall and are currently being analysed by V. Palace (DFO, Winnipeg). Plasma potassium, sodium and calcium concentrations will also be measured (J. Klaverkamp) and linked to routine histological analyses of liver and kidney tissues (B. Evans).

Background information on gonadal development in fish was assessed using several techniques. Mature gonadal tissues from sucker and fathead minnows were collected in the fall to assess sex steroid production in females. Medial sections of ovaries were collected and will be examined by B. Evans for state of maturation, presence of atretic follicles, frequency distribution of oocyte stages, lesions and the presence of intersex. Testes will be examined for delayed testicular maturation, inhibited spermatogenesis, asynchronous cyst maturation, seminiferous lobule deformities, replacement of generative tissue with connective tissue and other lesions (intersex/testis/ova). In addition, sections of gonadal tissues from these species is being assessed for estrogen receptors and apoptosis (programmed cell death) by G. Van Der Kraak because of increased incidences in fish exposed to contaminants.

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

Laboratory studies have shown decreased survival and skewed sex ratios of fish larvae exposed to EDCs. In this study, fertilizations of lake trout eggs were done during the fall spawning season in the study and reference lakes to evaluate % fertilization, survival, growth and hatching success (V. Palace), and for developmental abnormalities using histological sections (B. Evans). Fry survival and development is being assessed in the laboratory at the Freshwater Institute. Lake trout were captured using short gill net sets (<20 min) from Lakes 260 and 442. Eggs were collected from 10 females in Lake 260 and 5 females in Lake 442. Individual egg diameters and total egg volumes were recorded to calculate fecundities for each female and eggs. Unfertilized eggs were transported in sterile plastic bags back to the Freshwater Institute in Winnipeg where they were fertilized with a composite of milt (>3 males) obtained from male lake trout from the same lake. Eggs are being reared at 6.7C with subsamples being collected and preserved for measures of gross embryonic development and thyroid hormone and vitamin analysis. Physiological and tissue-level responses and population-level effects in fish will be examined and correlated following each field season. Comparisons of effects at different levels of biological organization will be made at project workshops at the Freshwater Institute following each field season, and results will be examined and modelled through a collaborative effort by this multi-disciplinary team.

Background Studies on Lower-trophic-levels

Little is known about the impacts of hormone mimics on lower-trophic-level organisms and this experiment provides an excellent opportunity to advance the understanding of the effects of estrogenic compounds on such organisms. Studies were conducted this summer to obtain background information on the invertebrate, algal and microbial communities in the study and reference lakes.

Emergence traps were used along the shoreline (~ 1 m depth) to sample emerging benthic insects from five different sites on Lakes 260 and 442. Two emergence traps were used per site and the subsamples were collected weekly from early June until the middle of September. All samples are being sorted, and the invertebrates identified and enumerated to determine abundances and community composition.

Artificial substrates (0.01m³ wire baskets containing local rocks) were used to monitor benthic invertebrate populations at the same stations established for monitoring insect emergence on Lakes 260 and 442. Rock baskets provide standardized samples from rocky lake substrates that are not easily sampled by more conventional means such as dredges. Three baskets were sampled at each station twice over the course of the summer. After sampling, the baskets were returned to the lakes and will be sampled again in following years. Samples from the artificial substrates will be used to monitor community composition and abundance.

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

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

Bi-weekly integrated surface and deep water samples were collected from these lakes in conjunction with zooplankton samples for phytoplankton, bacteria, and chemical analyses (phosphorous and nitrogen, chlorophyll *a*, suspended C and N). These samples are currently being analysed at the Freshwater Institute.

Training

Five graduate students will be receiving training through this project starting in 2000. Three of these students will be attending the University of Manitoba, the fourth is through the University of Saskatchewan, and the fifth is attending the University of Guelph.

Future Research Schedule

The whole-lake addition of EE2 will begin at ice-off in 2000 and will be continuous throughout the open-water seasons of 2000 and 2001. EE2 concentrations and addition rates will be determined from the limnocorral studies done in 1999, and will maintain water concentrations at a target dose that will be determined at a collaborators' workshop to be held early in 2000. EE2 will be added continuously from several locations on the lake using the automated peristaltic pump apparatus used for the limnocorral studies. In the spring and fall of each year, EE2 applied to surface waters will mix vertically in the water column during lake turnover. Water, particulates, and sediments will be collected and analysed bi-weekly during the open-water season for EE2 using analytical methods developed at the Freshwater Institute for the limnocorral studies.

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

Conclusion

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

Funding Sources for 1999/2000

Toxic Chemicals Program, DFO
Toxic Substances Research Initiative
Chemical Manufacturer's Association

Canadian Chemical Producers Association (to K. Liber and G. Van Der Kraak)
Funding Total – 311K
In-Kind Contributions – 98K
Total Annual Budget – 409K

6.1.1. *Effects of a Synthetic Estrogen on Biochemical Parameters in Natural Populations of Fish*

Objectives

- to obtain background data on the gonadal development and the endocrine system function of fish populations.
- to establish natural variability in the methods used to detect endocrine disruption in fish. These data will be used to assess the effects of EE2 on fish during the whole-lake additions in 2000 and 2001.

Summary of 1999 Work

Lake trout, white sucker, fathead minnow and pearl dace were sacrificed to obtain gonad weights, fecundities, and gonad sections for histology and steroidogenesis. Fish were sampled from Lakes 260, 442 and 375 as well as in Lake 114 in 1999 in conjunction with the spring and fall netting programs for the population-level research. Plasma samples were collected in the fall from 9-15 lake trout and sucker to assess circulating reproductive hormone, egg-protein (VTG), and sodium, potassium and calcium levels. Hormone samples are currently being analysed for the steroids estradiol, testosterone, 11-ketotestosterone, and 17α , 20β dihydroxy-4-pregnen-3-one, and GtH II (LH-like gonadotropin) content. Whole body homogenates and plasma of fathead minnows and pearl dace have been collected and will be analyzed for VTG. These collections include spring and fall samples (11-30 males, 7-18 females). Analysis is currently ongoing.

Background information on gonadal development in fish was assessed using several techniques. Mature gonadal tissues from sucker and pearl dace were collected in the fall to assess sex steroid production in females. Medial sections of ovaries were collected and will be examined for their state of maturation, presence of atretic follicles, frequency distribution of oocyte stages, lesions and the presence of intersex. Testes will be examined for delayed testicular maturation, inhibited spermatogenesis, asynchronous cyst maturation, seminiferous lobule deformities, replacement of generative tissue with connective tissue and other lesions (intersex/testis/ova). In addition, sections of gonadal tissues from these species are being assessed for estrogen receptors and apoptosis (programmed cell death).

Laboratory studies have shown decreased survival and skewed sex ratios of fish larvae exposed to EDCs. In this study, fertilizations of lake trout eggs were done during the fall spawning season in the study and reference lakes to evaluate % fertilization, survival, growth and hatching success, and for developmental abnormalities using whole preserved and histological sections. Fry survival and development is being assessed in the laboratory at the Freshwater Institute.

Findings

In parallel laboratory-based studies, we have established that exposure to ethynylestradiol at concentrations similar to the anticipated addition rates are likely to have significant effects on fish. Specifically, impacts including enlarged liver, depletion of glycogen, histological evidence of liver cell hyperplasia and lipid soluble vitamin depletion in liver and plasma have all been observed. While the bulk

of analyses for the field collections will occur during the winter between the 1999 and 2000 field seasons, several key issues related to the feasibility of our planned studies have been resolved. These include the establishment of suitable reference populations for each of the 4 species to be examined and refinements in the methodology to be used for examining reproductive parameters.

Future Plans

Field collections and analyses similar to those performed during 1999 are planned for the 2000 and 2001 field seasons.

Productivity

Two manuscripts detailing the parallel laboratory studies are currently in preparation. In addition, the following presentations were made:

Palace, V.P., K. Wautier, C.L. Baron, R.E. Evans and J.F. Klaverkamp. 1999. Effects of Ethynylestradiol exposure on metallothionein and fat soluble vitamins in juvenile lake trout. *26th Annual Aquatic Toxicity Workshop* in Edmonton, October 2-6.

Palace, V.P., T.A. Dick, K. Wautier, C.L. Baron, R.E. Evans and J.F. Klaverkamp. 1999. Exposure to waterborne ethynylestradiol alters fat soluble vitamin and lipid metabolism in juvenile lake sturgeon. *26th Annual Aquatic Toxicity Workshop* in Edmonton, October 2-6.

Note:

This summary was compiled by John Shearer, using information provided by research project leaders and other ELA staff. Where appropriate, the names of principal external investigators, graduate students, and their affiliations are noted. However, many aspects of most major projects are being conducted by DFO Experimental Lakes Area staff, many of whom have not been specifically mentioned. The summary is intended as an overview of research activities at the ELA during 1999. For more detailed information, the reader should contact those researchers responsible for each study or refer to published literature.