

SUMMARY OF MAJOR RESEARCH PROJECTS AT THE EXPERIMENTAL LAKES AREA DURING 2005

5 January 2006

The level of research activity on site at the ELA during 2005 was considerably less than during the previous several years, but remained at a level close to the historic average. Approximately 120 different researchers, representing more than a dozen different universities, government agencies and private companies, worked on site during the field season.

The experimental phase of the Cage Aquaculture study was in its third year, although the level of on-site activity was somewhat reduced from previous years. The METAALICUS study was in its fifth year of ecosystem-scale, experimental manipulation, at a level of on-site sampling activity slightly reduced from 2004. An ancillary experiment investigated the movement and fate of stable isotopes of mercury in a small stream segment. The estrogen (EDC) field study was effectively completed, except for a small amount of follow-up monitoring on Lake 260. Two new enclosure experiments were conducted. One of these investigated selenium and mercury interactions, while the other examined the roles of biologically-available iron and ultraviolet radiation in the control of noxious cyanobacteria. Several other graduate student studies were ongoing. The long-term, ecological research (LTER) program continued, with very limited support from core funding. On-site meteorological monitoring, with support from Environment Canada, moved into its 37th consecutive year.

The following is an attempt to summarize the status of most major research projects by providing some information about their purposes, designs and, where possible, significant results. It should be noted, however, that data analyses and interpretations are ongoing, and most of the recent results have been omitted unless they have been previously published in peer-reviewed journals. These projects are grouped under several broad category headings.

Note:

Using information provided by research project leaders and other ELA staff, John Shearer compiled this summary. Where appropriate, the names of principal investigators, graduate students, and their affiliations are noted. However, DFO Experimental Lakes Area staff members and seasonal employees, many of whom have not been specifically mentioned, are conducting many aspects of most major projects. The summary is intended as an overview of research activities at the ELA during 2005. In most cases, experimental results have been omitted unless previously published. For more detailed information, the reader should contact those researchers responsible for each study, or refer to published literature.

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LONG-TERM MONITORING AND CLIMATIC FACTORS

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

Over almost four decades, researchers at the E.L.A. have been collecting data on natural lake ecosystems in support of, and as references for, the experimental studies. Increasingly, these data sets have become invaluable in their own right because of the unusual scope and length of the records, and we have established a formal long-term monitoring program at the ELA. In recent years, various external groups have also conducted various monitoring research, particularly in relation to climatic change.

LONG-TERM ECOLOGICAL RESEARCH (LTER) AND DATA MANAGEMENT

Principal Investigators:

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

All principal investigators are Fisheries and Oceans Canada, Experimental Lakes Area staff.

Project Description and Goals:

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

There are three objectives for the project:

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

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Activities in 2005:

Long-term records of meteorology and hydrology of the Lake 239 basin were maintained. Meteorological variables (air temperature, precipitation, wind speed and direction, bright sunshine and evaporation) were monitored daily. The 3 inflowing streams and outflow of Lake 239 were monitored for volume of flow (continuous record) and chemical composition (weekly). The five core lakes (114, 224, 239, 373, and 442) were again monitored, where possible, for all major disciplines which include: hydrology, water temperature, secchi depth and light extinction profiles, water chemistry, primary production, epilithon, phytoplankton populations and nutrient status, zooplankton, and fish. Primary production and epilithon monitoring programs were severely reduced. Some discipline specific monitoring continued in other lakes to maintain long-term records. Fertilization and basic monitoring of Lake 227 continued.

Measurements of limnological variables and samples for chemistry, phytoplankton and zooplankton analyses were taken every 2 weeks through the open water season and twice over winter (shortly after ice-on and before ice-off). Surface water temperature was measured continually in all LTER lakes. Phytoplankton nutrient status (alkaline phosphatase activity and nutrient debt) was measured approximately every 2-4 weeks from May to early September in both epi- and meta-limnetic waters. A few strategic primary production measurements were done in mid-summer. L373 and L239 epilithon was sampled synoptically for particulate chemical composition and algal taxonomy. Phytoplankton and zooplankton analyses included identification to species and biomass estimates. *Chaoborus* and *Mysis* in lakes 373 and 240 were sampled for comparisons to specific experimental lakes. Mark-recapture work to estimate fish populations occurred in spring and/or fall, depending on the species. Movements of lake trout and white suckers in Lake 373 were studied with acoustic telemetry for the purpose of comparison to those in the Aquaculture experimental lake. Cyprinid population data collection continued in the spring and fall in Lake 442 to provide specific reference information for the EDC experiment in Lake 260.

Progress continued with archiving data in the ELA Database, improving the functionality of the Retrieval application and developing day-to-day data management applications for researchers. Most core data sets were brought up to date with 2004 data. A stand-alone PC version of the database was developed primarily for use by staff when they are at the ELA or other location where there is no, or poor, ability to connect to the Freshwater Institute's network. Significant enhancements were made to the Retriever application, mostly focused on value-added analyses or reporting of data. Users can now obtain monthly and annual means, long-term means or medians and percentiles along with respective plots for several data sets. Further, an analysis of long-term chemical yields from the Lake 239 basin was incorporated. Finally progress was made on developing information sheets about various data sets that record useful historical information about methods, cautionary notes, site locations, etc.

Major Findings or Conclusions:

Analyses of this year's data are still largely incomplete. See the list of presentations, publications, etc. for research pursuits and use of past LTER data.

A new pursuit is the examination of fish behaviour with respect to winter snow depth, ice on/off dates and sunlight.

LTER researchers joined with those from Dorset, Ontario and Trout Lake, Wisconsin to form a Regional Limnology Network for the purpose of facilitating collaborative research at a regional scale. We expect to examine the temporal and spatial variation in physical and chemical lake characteristics in the Upper Great Lakes region and relate this variation to fluctuations in climate and lake biology. Five papers have been proposed.

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Publications and Presentations with a Significant LTER Component:

Published:

- Aherne, J., T.A. Clair, I.F. Dennis, M. Gilliss, S. Couture, D. McNicol, R. Weeber, P.J.Dillon, W.B. Keller, D.S. Jeffries, S. Page, K. Timoffee, J. Cosby. Dynamic Modelling of Lakes in Eastern Canada. Chapter 6. *In: a submission to the next CCE status report (ICP M&M under UNECE LRTAP Convention).*
- Baulch, H.M., D.W. Schindler, M. A. Turner, D.L. Findlay, M.P. Paterson, and R.D. Vinebrooke. 2005. Effects of temperature on benthic communities in a boreal lake: Implications of climate warming. *Limnol. Oceanogr.* **50(5)**: 1377-1392.
- Mills, K.H., E.C. Gyselman, S.M. Chalanchuk, and D.J. Allan. 2005. The population dynamics of unexploited lake whitefish (*Coregonus clupeaformis*) populations, p 247-259. In (ed. LC Mohr and TF Nalepa) Proceedings of a workshop on the dynamics of lake whitefish (*Coregonus clupeaformis*) and the amphipod *Diporeia* spp. In the Great Lakes. *Great Lakes Fish. Com. Tech Rep.* **66**.
- Turner, M.A., D.B. Huebert, D.L. Findlay, L.L. Hendzel, R.A. Bodaly, W.A. Jansen, L.M. Armstrong, and S.E.M. Kasian. 2005. Divergent impacts of experimental lake-level drawdown on planktonic and benthic plant communities in a boreal forest lake. *Can. J. Fish. Aquat. Sci.* **62**: 991-1003.
- Watmough, S.A., J. Aherne, C. Alewell, P. Arp, S. Bailey, T. Clair, P. Dillon, L. Duchesne, C. Eimers, I. Fernandez, N. Foster, T. Larssen, E. Miller, M. Mitchell, and S. Page. 2005. Sulphate, nitrogen and base cation budgets at 21 forested catchments in Canada, the United States and Europe. *Environmental Monitoring and Assessment* **109**: 1-36.
- Weidman, P., M.A. Turner, and G.G. Goldsborough. 2005. The depth distribution of UV effects in the shallow littoral zone. *J. North American Benthol. Soc.* **25(4)**: 820-831.

In Press:

- Graham, M., Vinebrooke, R. D., and M. A. Turner. 2006. Coupling of boreal forests and lakes: Effects of conifer pollen on littoral communities. *Limnol. Oceanogr.* (In revision 22 July 2005; accepted provisionally).

In Preparation:

- Clair, T.A., J. Aherne, I.F. Dennis¹, M. Gilliss, S. Couture, D. McNicol, R. Weeber, P. J. Dillon, W.B. Keller, D.S. Jeffries, S. Page, K. Timoffee, and B.J. Cosby. Past and future changes to acidified eastern Canadian lakes: a geochemical modeling approach.

Presentations:

- Clair, T.A., J. Aherne, I. Dennis, M. Gilliss, S. Couture, D. McNicol, R. Weeber, P. Dillon, W. Keller, D.S. Jeffries, S. Page, K. Timoffee, B. J. Cosby. Past and future changes to acidified eastern Canadian lakes: a geochemical modeling approach. (talk in Prague).
- Mills, K.H., S.M. Chalanchuk, P.J. Blanchfield, C.L. Podemski, and D.J. Allan. Enhanced growth and condition of lake trout in a small Ontario lake during cage aquaculture of rainbow trout. Poster Presentations at the 2nd North American Lake Trout Symposium.

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- Mills, K.H., S.M. Chalanchuk, and D.J. Allan. The fin-ray method of aging lake trout. Poster Presentations at the 2nd North American Lake Trout Symposium.
- Mills, K.H., S.M. Chalanchuk, and D.J. Allan. The long-term recovery of a lake trout population from lake acidification. Poster Presentations at the 2nd North American Lake Trout Symposium.
- Mills, K.H., S.M. Chalanchuk, and D.J. Allan. Spawning site fidelity of lake trout in small Ontario lakes. Poster Presentations at the 2nd North American Lake Trout Symposium.
- Mills, K.H., S.M. Chalanchuk, and D.J. Allan. Long-term impacts of acidification and nutrient additions on a lake whitefish population. Oral presentation at the 9th International Symposium on the Biology and Management of Coregonid Fishes (Olsztyn, Poland); (Manuscript submitted for proceedings of conference in Arch. Hydrobiol. Spec. Issues Advanc. Limnol.)
- Mills, K.H., S.M. Chalanchuk, E.C. Gyselman, and D.J. Allan. Growth, recruitment, and age-at-maturity of lake whitefish after pulse exploitation in small Ontario lakes. Poster presentation at the 9th International Symposium on the Biology and Management of Coregonid Fishes (Olsztyn, Poland).
- Turner, M.A., D.L. Findlay, R.H. Hesslein, L.L. Hendzel, L.M. Armstrong, D. McNicol. Differing responses of benthic and planktonic algal assemblages to reduced stress. Society of Canadian Limnologists. Windsor. January 2005.
- Turner, M.A., M.J. Paterson, R.H. Hesslein, D.L. Findlay, D. McNicol and L.L. Hendzel. Lessons and surprises from a study of limits to ecosystem resilience in a boreal forest lake recovering from experimental acidification. Ecological Society of America. Montreal. August 2005.

Long-term Data Sets Requested by External Researchers:

Three copies of the stand-alone version of the ELA Database were given to external researchers who are collaborating with staff on investigations of long-term behaviour of boreal shield lakes. Those researchers are: David W. Schindler, University of Alberta; Jim Rusak, University of Wisconsin, Madison, and Jeff Hudson, University of Saskatchewan, Saskatoon.

To date, there have been twenty responses to formal requests by external researchers for long-term data during 2005. ELA staff and graduate students have logged 300 requests for data from the ELA Database.

Plans for 2006:

The intention is to continue the LTER monitoring so that long-term records of natural variation in boreal shield lakes can be maintained. Funding is currently not secure.

LTER researchers expect to continue involvement with the Regional Limnology Network to conduct collaborative limnological research at a regional scale. Long-term data collected at the ELA, northwestern Ontario, Dorset, south-central Ontario, and Trout Lake, Wisconsin, will facilitate this work.

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Specific Ancillary Studies:

Meteorological Monitoring

The ELA is the site of long-term monitoring of meteorological variables via a meteorological station (met site) that uses equipment provided by the Meteorological Service of Canada (MSC) of Environment Canada and is operated by ELA staff. Ken Beaty, with assistance from Mark Lyng, Frank McCann, and others, has primary responsibility for this facility and data are contributed to the MSC national climate database. Established in June of 1969, this site is now in its 37th year of continuous monitoring. Meteorological variables (air temperature, precipitation, wind speed and direction, bright sunshine and evaporation) were monitored daily again in 2005.

These climatic data are essential for our understanding of interactions between climatic variables and the lake ecosystems we study. Increasingly, they provide a basis for understanding many of the long-term patterns observed in our ELA data sets.

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 2005. The CAPMoN program (http://www.msc.ec.gc.ca/capmon/index_e.cfm), which monitors both atmospheric and precipitation chemistry at a network of sites across Canada, is funded and coordinated by Environment 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, has frequently been used as a baseline reference for sites in eastern Canada.

Canadian Network Isotopes in Precipitation (CNIP)

The ELA is a node in a Canadian network monitoring isotopes (¹⁸O, Deuterium) in precipitation. This network (<http://sciborg.uwaterloo.ca/~twdedwar/cnip/cniphome.html>), coordinated from the University of Waterloo, comprises sites distributed broadly across Canada, including the high Arctic. Its current goal is "to discern fundamental linkages between the isotopic composition of precipitation and synoptic climate and to aid in designing and optimizing a more permanent future network". Ken Beaty is the ELA researcher responsible for the ELA site.

LONG-TERM CLIMATIC RECORDS IN THE WINNIPEG RIVER DRAINAGE BASIN

Three external research groups from Queen's University, the Bedford Institute, and the Geological Survey of Canada conducted sampling surveys within the Experimental Lakes Area during 2004 as part of a larger project to examine long-term climatic trends within the drainage basin of the Winnipeg River. The ELA sits astride a height of land which divides two major sub-basins of Winnipeg River drainage. This, coupled with the ELA's existing long-term data sets, attracted these groups to the ELA for these surveys. The primary purpose of these studies is to assess the variability in climatic conditions in northwestern Ontario over the last 2 thousand years. Brief descriptions of the sampling surveys are provided in the *Summary of Major Research Projects at the Experimental Lakes Area during 2004*.

No further sampling was conducted in 2005 and we await the results of sample and data analyses, which could take several years.

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SEISMIC MONITORING STATION

Natural Resources Canada, Seismology & Electromagnetism Section, installed an automated seismic monitoring station at the ELA in June of 2004. The station is located atop a bedrock ridge between Lake 239 and Roddy Lake (468), in the clearing created in 2003 by the removal of the FLUDEX site 1 reservoir. Fully automated with a satellite data uplink, this is part of a small network of stations installed in northwestern Ontario and is expected to remain in place for 2 to 5 years.

HABITAT ALTERATION AND ECOSYSTEM PRODUCTIVITY

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

FERTILIZATION OF LAKE 227

Rationale:

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

Research Activities:

Lake 227 was fertilized with phosphorus for the 37th consecutive year in 2005. This original ELA ecosystem-scale experiment was initiated in 1969 to demonstrate that atmospheric carbon dioxide could provide the carbon necessary for algal blooms in eutrophic lakes. Prior to 1990, all additions included various combinations of nitrogen and phosphorus. The ratio of phosphorus to nitrogen was changed during these previous stages of the experiment to test whether this would influence the dominant algal groups. Since 1990, only phosphorus has been added. During 2005, phosphorus, as phosphoric acid, was again added to Lake 227 surface waters for twenty consecutive weeks (2.5 litres per week) during the ice-free season. The acid was diluted with lake water in a plastic barrel and dribbled via *Tygon* tubing into the near-shore water. The required acid was carried to the lake weekly. Sodium bicarbonate, to be used as a neutralizing agent in case of an acid spill, is stored on site.

We continued to monitor water chemistry, phytoplankton, and zooplankton in Lake 227 during 2005. Also, Dr. Lewis Molot, of York University, and colleagues conducted enclosure studies of noxious cyanobacteria in this lake, utilizing the enhanced productivity of this system and the 37 years of historic data record during which the lake has been experimentally fertilized.

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CONTROL OF NOXIOUS CYANOBACTERIA BY BIOLOGICALLY AVAILABLE IRON AND ULTRAVIOLET RADIATION

Principal Investigators:

- Lewis Molot, Faculty of Environmental Studies, York University, Toronto ON, M3J 1P3
- Guiyou Li, M.Sc. Candidate, York University, Toronto
- David Findlay, Fisheries and Oceans Canada, Winnipeg.

Project Purpose:

In spite of many years of research, the specific mechanisms leading to cyanobacterial dominance in eutrophic waters are not well understood. We tested several hypotheses:

- Reduction of iron in the euphotic zone will reduce phytoplankton biomass and also cause a shift in species composition from cyanobacteria to eukaryotic
- UVA and UVB are essential to maintaining high iron levels in eutrophic waters
- Cyanobacterial toxin levels are affected by UV and iron levels

Description of Work Undertaken During 2005:

Twelve 2 x 3 m enclosures were installed in fertilized Lake 227 using four treatments in triplicate. Treatments included:

1. Control
2. UVA & UVB removal with lexan covers
3. Nitrate addition to oxidize sediments and reduce Fe flux from sediments,
4. Deferroxamine addition to chelate iron and reduce biological availability

Installation was completed in mid-May and enclosures were sampled twice weekly until the end of June for chlorophyll *a*, ferrous iron, dissolved and particulate iron, phytoplankton, nitrate, TP, pH, ammonia, TKN, and cyanobacterial toxins. Surface waters were also sampled every two weeks at the main stations in Lakes 239 and 227.

Publications Resulting from this Research:

A manuscript entitled "Regulation of small and large cyanobacterial relative abundance by iron and phytoplankton productivity in oligotrophic and eutrophic waters" by L.A. Molot, S.A. Miller, D.L. Findlay, N. Kelton, P.J. Dillon, S.B. Watson and G. Li is undergoing final revisions and will be submitted later this month. The paper includes only the ferrous data from the main stations of Lakes 227 and 239.

Plans for Continuation of this Study in Future Years:

In the second year of this study, variations in the iron removal methods will be tried in an attempt to lower iron levels to those in Lake 239. Treatments will include:

1. a higher dose of nitrate
2. nitrate + oxine (a cheaper and stronger chelator)
3. identical N dose but as ammonia
4. control

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Partial funding is available for a third year, but Year 3 will depend on securing additional funds.

RESERVOIR IMPACTS AND POSSIBLE MITIGATION

In Canada, reservoirs are generally created primarily for generation of hydroelectricity. Many cause flooding over large areas of northern wetland and forest land. The water levels in these reservoirs tend to be drawn down during the winter periods when electrical demand is high and water flows are low.

Since the early 1990s, ELA researchers have been investigating the ecological effects of flooding caused by reservoir creation and operation. In most cases, this has involved experimental alteration of water levels, as a simulation of what typically occurs during the creation and operation of reservoirs. The focus of these studies has been the production and fate of methylmercury and various greenhouse gases.

This work is now winding down, but data analyses are ongoing and a mesocosm study of possible mercury mitigation using low concentrations of selenium was conducted in 2005.

EXPERIMENTAL LAKES AREA RESERVOIR PROJECT (ELARP)

Objective:

The Experimental Lakes Area Reservoir Project (ELARP) is a whole-ecosystem flooding experiment designed to examine the production and mobilization of methylmercury (MeHg) in response to flooding, and to determine if reservoirs are significant sources of the greenhouse gases (GHG) carbon dioxide (CO₂) and methane (CH₄) to the atmosphere.

Design and Results:

In June, 1993, following two years of background studies, the outflow of ELA Lake 979 and its surrounding wetland was dammed, and the water level raised 1.4 meters to flood 14 hectares of peatland. Direct by-products of the decomposition of the flooded vegetation in the peatland are CO₂ and CH₄. Mobilization of MeHg within the flooded ecosystem and release to the atmosphere of CO₂ and CH₄ in response to the flooding were monitored intensively. A non-flooded wetland system (ELA Lake 632), was monitored as a reference. Following winter drawdown, flooding of Wetland 979 was repeated in summer and fall of 1994 and 1995, as detailed studies continued in both wetland systems. In all three years, dramatic increases in MeHg and in release of the GHG were observed in response to flooding.

During the open-water periods of 1996 through 1998, the 979 wetland was experimentally flooded, but the system was studied less intensively. GHG emissions and MeHg mass-balance budgets were monitored. In 1999 and again in 2000, the system was flooded, but no ecosystem monitoring was conducted. During the open water period of 2001, the system was flooded once again and a regular monitoring program was carried out.

Flooding was repeated in 2002, 2003, 2004, and 2005, but only minimal general monitoring was conducted. The wooden components of the dam are showing their age, but researchers hope to flood again in 2006 and conduct regular monitoring for mercury, similar to that done in 2001.

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FLOODED UPLAND DYNAMICS EXPERIMENT (FLUDEX)

Purpose and Approach:

The purpose of the Upland Flooding Experiment (FLUDEX; Flooded Upland Dynamics Experiment) was to study the greenhouse gas and mercury impacts of flooding forested upland areas. Three forested uplands, a moist forest and two dry forested areas, located in the watershed of Roddy Lake were flooded in the summers of 1999 to 2003, inclusive, to create experimental hydroelectric reservoirs. Greenhouse gases fluxes before and after flooding were measured at all three sites. Carbon dioxide, methane and nitrous oxide were monitored. Fluxes are being compared to the previously flooded boreal wetland (ELARP project) and to existing hydroelectric reservoirs to determine the potential greenhouse gas contribution of global freshwater reservoirs. The production of methyl mercury from flooded soils and the bioaccumulation of methyl mercury through the food chain were measured in the experimental reservoirs. Mitigation strategies that will have direct planning application are being investigated. The fifth year of flooding (2003) was the last for the experimental reservoirs. Studies on the effectiveness of selenium additions to reduce mercury in food chains began in 2003 with a survey of mercury and selenium levels in reservoirs, wetlands and natural lakes at the ELA and in northern Quebec.

Principal Investigators:

- Project coordination:
 - Drew Bodaly (Freshwater Institute)
- Hydrology and project design:
 - Ken Beaty and Mark Lyng (Freshwater Institute)
- Mercury dynamics:
 - Kristofer Rolfhus, James Hurley and David Krabbenhoft (University of Wisconsin and USGS)
 - Britt Hall and Vincent St.Louis (University of Alberta)
 - Katharine Peech and Michael Paterson (University of Manitoba and Freshwater Institute)
 - Drew Bodaly and Andrew Majewski (Freshwater Institute)
 - David Findlay (Freshwater Institute)
 - Mariah Mailman (University of Manitoba)
 - Greenhouse gases and carbon decomposition:
 - Elizabeth Joyce, Cory Matthews and Vincent St.Louis (University of Alberta)
 - Natalie Boudreau, Jason Venkiteswaran and Sherry Schiff (University of Waterloo)
 - Len Hendzel (Freshwater Institute)

Funding Support:

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

Activity in 2005:

All experimental activity was concluded in 2003 and rehabilitation of the sites was completed in 2004. Analysis of data and publication of results have continued in 2005, but results are

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essentially as reported in the 2004 project summary. The main conclusions are listed below, as are a summary of primary publications to date, and a listing of these produced during this study. Aerial photographs of the reservoirs during the flooding phases have been used for cover photos by two international research journals; namely, *Environmental Science and Technology* in September, 2004, and *Ecosystems* in April, 2005.

Conclusions:

Four main conclusions can be drawn from the results of the FLUDEX experiment to date:

1. Rates of carbon decomposition, oxygen depletion, dissolved nutrients, greenhouse gas fluxes, production of methyl mercury and uptake of methyl mercury by the food chain were not directly related to the amount of carbon stored on the three flooded sites, at least during the first one or two years of flooding, although rates came into line with estimates of organic C storage on the sites in later years.
2. In contrast to the ELARP experimental reservoir that flooded a wetland, the FLUDEX reservoirs showed definite indications of decreases in the rates of carbon decomposition, greenhouse gas fluxes, and MeHg production over the five years of flooding.
3. MeHg bioaccumulation in the food chain was not generally closely linked to the rates of production of MeHg in the reservoirs. Although concentrations of MeHg in the water of the FLUDEX reservoirs were lower than those in the ELARP reservoir in the first three years after flooding, concentrations in zooplankton were as high and concentrations in fish were higher in the FLUDEX reservoirs. DOC concentrations may be important in modifying the uptake of MeHg from the water to the food chain.
4. Although the FLUDEX reservoirs have been significant sources of the greenhouse gases CO₂ and methane, surprisingly they have been determined to be sinks for N₂O.

Primary Publications:

- Bodaly, R.A., K.G. Beaty, L.L. Hendzel, A.R. Majewski, M.J. Paterson, K.R. Rolffhus, A.F. Penn, V.L. St.Louis, B.D. Hall, C.J.D. Matthews, K.A. Cherewyk, M. Mailman, J.P. Hurley, S.L. Schiff and J.J. Venkiteswaran. 2004. Experimenting with hydroelectric reservoirs. *Environmental Science and Technology* **38**: 347A-352A.
- Hall, B.D., V.L. St. Louis, and R.A. Bodaly. 2004. The stimulation of methylmercury production by decomposition of flooded birch leaves and jack pine needles. *Biogeochemistry* **68**: 107-109.
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MERCURY AND SELENIUM INTERACTIONS IN LAKES AND RESERVOIRS

Principal Investigators:

- Mariah Mailman, Ph.D. Candidate
- Drew Bodaly, Research Scientist and Adjunct Professor
 - Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, and Natural Resources Institute, University of Manitoba, Winnipeg

Purpose of the Experiment:

In new reservoirs, decomposition of flooded organic matter releases stored inorganic mercury (Hg) and nutrients that enhance microbial conversion of Hg to methyl mercury (MeHg). MeHg is highly toxic and biomagnifies. Contaminated fish are the main source of MeHg to humans and the main cause of fish consumption advisories, but MeHg mitigation approaches remain elusive. The most efficient mitigation approach may be minute additions of selenium (Se) to aquatic ecosystems, which has been shown to lower Hg concentrations in fish. Minimum Se concentrations required to affect MeHg uptake have not been established and the biogeochemical processes are unclear. Although Se is an essential element at low concentrations, it can cause toxic effects at slightly elevated concentrations. There might be a range of Se concentrations that will lower MeHg in fish without causing toxicity, but before this could be accepted, a significant amount of research will be required.

Before applying Se as a method to ameliorate MeHg bioaccumulation, it would be desirable to establish if Se additions can lower MeHg uptake in organisms without causing toxicity, and to understand the mechanisms by which this occurs. Additions of Se could potentially be applied to both new and existing reservoirs and natural lakes to protect the integrity of the ecosystem and the health of subsistence fishers and fisheries.

In 2005 we conducted a limnocorral experiment to determine the lowest Se concentrations that will decrease MeHg bioaccumulation in aquatic ecosystems. We applied a range of Se concentrations to limnocorrals in a linear regression design, from background levels up to 1.6 $\mu\text{g.L}^{-1}$. The limnocorrals were three metres deep by ten metres in diameter. These impermeable plastic tubes were sealed to the bottom sediment and open to the atmosphere. They were installed in a natural lake (Lake 239) at the Experimental Lakes Area. Samples of water, sediment, algae, zooplankton, benthic invertebrates, and small fish were collected to assess the effects of Se on MeHg bioaccumulation and the risk of Se toxicity. Samples were also taken to assess Se induced changes in community composition.

The upper limit concentration of Se additions in the experiment was 1.6 $\mu\text{g.L}^{-1}$. This compares to the Canadian standard to protect aquatic life of 1.0 $\mu\text{g.L}^{-1}$ and background concentrations in natural freshwaters in North America of up to approximately 1.0 $\mu\text{g.L}^{-1}$. Natural concentrations in surface waters at the ELA reach 0.15 $\mu\text{g.L}^{-1}$. Small amounts of a mercury stable isotope were also be added to the limnocorrals to act as a tracer for the experiment. Addition rates were approximately 15 $\mu\text{g.m}^{-2}$ or approximately 100 μg per limnocorral. This equates to

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concentrations of about 7 ng.L^{-1} in the limnocorrals, which are within the natural range for total mercury in pristine waters.

Study Site:

The study site for the 2005 limnocorral experiment was Lake 239. A shallow bay that has a consistent depth and soft substrate was chosen for the experiment. Lake 239 is a designated ELA research lake. It offered ready access from the ELA field station and has been used for limnocorral experiments in the past. ELA researchers are the only regular users of this lake. The amount of Se that was released into the lake at the end of the experiment was very small compared to amount naturally present in the lake.

Specific Experimental Procedures:

Six limnocorrals measuring ten meters in diameter were installed along the three meter isobath. Installation took place in early June. Limnocorral walls were sealed to the sediment with sandbags installed by SCUBA divers and the collars were anchored. Water inside the limnocorrals was in contact with the substrate.

Se was added to the limnocorrals as sodium selenite at concentrations of 0, 0.1, 0.2, 0.4, 0.8, and $1.6 \mu\text{g.L}^{-1}$. Applying Se at one time was representative of how Se could be applied for mitigating Hg.

Yellow perch were added to the limnocorrals. All fish handling protocols were approved by the Canadian Council on Animal Care. Yellow perch are native to Lake 239. The fish for the limnocorrals were collected from the host lake. Added fish were individually measured, weighed, and tagged.

Effects of Se on Hg cycling will be determined by measuring concentrations of mercury in sediments, water and biota, including fish. Total and methyl mercury are being analyzed in the water and sediment. MeHg is being analyzed in algae, zooplankton, and benthic invertebrates. Only THg will be analyzed in the fish because THg is a good estimate of MeHg in fish. Total Se and species of Se will be analyzed in water, sediment, and algae, zooplankton, benthic invertebrates and in fish. Measuring the species of Se is essential for understanding how Se cycles through the food web. Analyses of samples should be complete by January 2006.

Various components of fish will be analyzed for total Se and Hg and species of Se and MeHg. We will analyze fish muscle, liver, brain, gonads, and gills. Se in fish gonads is currently the best way to assess the risk of Se toxicity. Because gonads were so small, only Se will be analyzed in gonads.

The experiment was decommissioned in late August. All materials were removed from the bay where the limnocorrals had been installed and from the shore of Lake 239.

Anticipated Impacts on the Lake Ecosystem:

This study increased boat traffic in Lake 239 by one to two trips per day on average. Installing and decommissioning the limnocorrals disturbed the bottom sediment in a localized area. Se added to limnocorrals mixed with the lake water when the limnocorrals were removed. The total amount of Se added to limnocorrals (less than 1 gram) was also sequestered by biota and sedimentation. Any Se released when the limnocorrals were decommissioned would have been diluted by the total volume of water in the lake. The amount of Se that was released into the lake at the end of the experiment would be very small compared to amount naturally present in the lake. This is because concentrations in the limnocorrals would only be slightly higher than

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those in the lake and the volume of water in the limnocorrals (about 170 m³) is much smaller than the estimated volume of the lake.

Mitigation Measures for Lake Rehabilitation after the Study Ends:

Decommissioning included removal of all foreign materials (limnocorral collars and walls, sand bags, anchors, floating docks). A small amount of residual Se and Hg would have remained in the sediments at the site of the limnocorrals. Se bioaccumulation and toxicity is more strongly linked with Se taken up from water rather than sediment (reviewed in Hamilton and Lemly 1999). A mass balance of Se added in the study will allow us to estimate the concentrations of Se in the sediment. In the limnocorral receiving the greatest dose of Se, the sediment concentrations could reach 0.07 µg.g⁻¹ of Se, if all of the added Se went to the sediment. This concentration is not significant compared to naturally present concentrations.

Reference:

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MERCURY LOADING AND BIOACCUMULATION

Certain substances, when released into natural ecosystems, may persist for years in a toxic form, and may bioaccumulate within the food chain to create health problems for higher organisms, including humans, particularly when exposures are chronic.

While such persistent toxicants are often experimentally studied under laboratory conditions, only studies conducted in real ecosystems can effectively examine the complexity of ecosystemic pathways and compartments in which these substances move and accumulate. Some controlled experimentation in real ecosystems is required to validate existing and proposed regulatory standards for these substances.

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

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

Background and Rationale:

The relationship between atmospheric mercury deposition and fish mercury concentrations has not been established, but is central to assessing the benefits of emissions controls being considered or implemented in North America and internationally. Efforts to examine this relationship with field datasets are confounded by many factors that can affect mercury cycling and bioaccumulation in the environment. Changes in sulphur deposition, lake acidity, land use, fish growth rates, hydrology and climate, for example, all have the potential to complicate attempts to isolate the effects of mercury loading on fish mercury concentrations.

As a result of the above complications for interpreting field data, an experiment was designed to use stable isotopes to examine the effect of mercury loading on MeHg production and

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concentrations in biota. METAALICUS involves the addition of stable, non-radioactive, mercury isotopes to a whole ecosystem to see if there is a response in fish mercury concentrations. Pilot scale studies began in 1999 and the full-scale experiment began at Lake 658 in 2001. Mercury has been added to the Lake 658 ecosystem each year since 2001, and permission has been obtained to continue the loading phase through 2006. Multiple years of mercury additions are needed because the ecosystem has not yet fully responded.

Experimental Objectives:

METAALICUS is designed with the following overall objectives:

- To determine the relationship between the atmospheric deposition of mercury to a lake ecosystem and the MeHg concentration of fish.
- To determine the response time of MeHg in a whole ecosystem, including fish, to changes in rate of atmospheric deposition of mercury (Hg(II)).
- To establish the relative importance of mercury deposited on uplands, wetlands, or onto the lake surface as sources of MeHg to fish.

Participants:

Principle Investigators:

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

Other DFO Winnipeg Investigators:

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J. Hurley, U. of Wisconsin, Madison WI
C. Kelly, R & K Research Inc., Saltspring Is., BC
D. Krabbenhoft, USGS, Madison WI
S. Lindberg, Oakridge National Laboratory, Oakridge, TN
J. Rudd, R & K Research Inc., Saltspring Is., BC
V. St.Louis, U. of Alberta, Edmonton, AB
M. Tate, USGS, Madison WI

International Advisory Panel:

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

Study Description:

As described above, METAALICUS is a whole-ecosystem experiment in which mercury loading to a headwater lake and its watershed is being altered experimentally. Lake 658 at ELA was selected for the study. It is a small (8.4 ha), low productivity, headwater lake on the Canadian

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Shield and is one of the lakes reserved for research at the ELA. Background studies documenting site conditions prior to the experiment were carried out in 1999-2000 and are discussed in the 2001 *Summary of Major Research Projects at the ELA*.

Mercury additions with different isotopic signatures are being applied to the lake, upland and wetland (^{202}Hg , ^{200}Hg and ^{198}Hg respectively) at a rate of $22 \mu\text{g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$. The power of using isotopes lies in the ability to follow the newly deposited mercury separately from background mercury. Applying mercury with different isotopic signatures to the upland, wetland and lake also allows us to determine the relative contributions of these sources to fish mercury levels.

The ELA is a low deposition area for mercury, with approximately 2 to $7 \mu\text{g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ of wet mercury deposition (2000-2003). The low mercury deposition rate at ELA means that adding the equivalent of about 1/6th of a teaspoon (approximately 12.5 g) of mercury per year increases wet Hg deposition to the 52 ha Lake 658 ecosystem (lake and watershed) by approximately 6 fold. This addition results in a mercury wet deposition rate to the experimental system that is comparable to rates currently observed in some parts of the US Northeast and Florida.

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

The experiment is being carried out in two phases. Phase I involved pilot and baseline studies in 1999-2000, to prepare for Phase II. The ELA Management Board approved Phase I studies at the February 1999 and February 2000 meetings. Final approval of the full-scale experiment for 2001 through 2003 was obtained in March 2001. Permission to continue adding mercury to the ecosystem for the 2004 to 2006 period was granted in February 2004.

Milestones:

- (1999-2000) Two years of pilot scale experiments; pre-addition background monitoring of candidate lakes. The Lake 658 ecosystem was selected for study.
- (2001-2005) Five years of whole-ecosystem isotope additions to the lake surface. Due to difficulties obtaining the services of a pilot in 2005, only the shoreline areas of the upland and wetland had mercury applied in 2005.
- (2006) The remaining 2005 doses will be applied to the main areas of the upland and wetland in 2006. If funding is obtained, a 6th annual load of mercury will also be applied to the ecosystem.
- (2007 and beyond) Monitoring of food web methylmercury concentrations until conditions return to pre-addition levels.

Public Consultation:

During 2000, public information meetings were conducted in Dryden and Kenora to discuss the project with the public. In addition a presentation was made to three NGO's at a meeting in Toronto. Feedback from these presentations was positive. It should be noted that there has been no public opposition or negative media coverage in connection with METAALICUS since permission was originally granted and the project began. Scientific, public, and governmental feedback has been very positive.

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Pilot Scales Studies:

Pilot-scale studies from 1999-2004 were described in the *Summaries of Major Research Projects at the ELA* for the years 2000-2004, based on results available at the time. These included:

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

In 2005, some of the pilot-scale studies done from 1999-2004 were continued or expanded to gain additional knowledge. Updates to the pilot-scale studies follow:

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

Results from studies in a small upland plot (600 m²) initiated in 1999 and continued to the present show a steady migration into the soil profile of four years of successive spike additions (different isotope each year). These results suggest that more recent mercury deposition to terrestrial soils can eventually be expected to be incorporated into runoff and transported down gradient. Continued monitoring at this site, and experiments specifically designed to examine small-scale, soil-zone migration of the spike, should help to estimate transfer rates and aid predictions of whole-ecosystem response for the Lake 658 study.

Another plot-scale study at the Lake 115 wetland involved the the addition of ²⁰²Hg to the surface of the wetland to test whether we could detect the spike above background concentrations and variability, and as a tool to examine fate and transport processes. The added Hg migrated both vertically and/or horizontally in peat and pore waters, and rapid methylation of the spike was observed within one day of application. Results of this study were published by Branfireun et al. (2005)

Additions of Isotopic Mercury to Lake Enclosures

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

In 2002, eleven mesocosms were installed in Lake 240 at the ELA. Over two years, mesocosms received different amounts of inorganic mercury [Hg(II)] to simulate atmospheric deposition rates between 1x and 15x the annual rate of wet deposition at the ELA. Mercury added to the mesocosms in 2002 and 2003 was enriched in ²⁰²Hg and ²⁰⁰Hg, respectively. Results of this 'MESOSIM' experiment are discussed in *Summary of Major Research Projects at the ELA* for 2002 and for 2003.

Whole-Ecosystem Mercury Additions at Lake 658

Lake 658 Research Activities:

After two years of pilot and baseline studies in 1999-2000, METAALICUS went "full scale" in June 2001. Stable non-radioactive Hg(II) isotopes are being applied to the upland, wetland, and directly to the lake surface in the Lake 658 watershed at ELA. The upland, wetland and lake are receiving mercury additions enriched with ²⁰⁰Hg, ¹⁹⁸Hg, and ²⁰²Hg respectively. In each case the annual application rate in terms of the total amount of mercury being added is approximately

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$22 \mu\text{g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$. Isotopes are being applied directly to the lake by mixing from a boat at a depth of 0.7 m, over a series of 9 applications during the ice-free season. Upland and wetland areas are being sprayed once per year by an airplane flying low over the canopy. Isotopes have been applied during the 2001-2005 field seasons. In 2005, mercury was added to the lake surface and shoreline areas of the upland and wetland, but not to the main parts of the upland and wetland that have mercury applied by airplane. The pilot scheduled to apply mercury to the upland and wetland by airplane was unable to participate in 2005. These areas will receive the intended 2005 applications in 2006. If funds are obtained to purchase a 6th year of mercury additions, these isotopes will also be applied to the ecosystem in 2006.

In 2005, terrestrial METAALICUS researchers also:

- Continued to collect precipitation in the open and under the forest canopy (throughfall), as well as canopy and ground vegetation, to examine the long-term cycling of both the ambient and added Hg through the various catchment compartments. Automated *Tekran* air monitoring equipment was also installed at the ELA meteorological site to continuously measure concentrations of gaseous elemental Hg, reactive gaseous Hg and particulate Hg in the atmosphere. Data acquired from this instrumentation will help to understand the importance of dry deposition, which may be much larger than the wet deposition contribution, to the loading of Hg to watersheds.
- Installed shallow soil water samplers and groundwater wells in the hillslope of the Lake 658 catchment to determine if aerially applied Hg can be measured in runoff along either or both of these flowpaths.
- Carried out a “LIDAR” vegetation and biomass survey to a) better quantify the component of the annual Hg mass balance that is in the vegetation canopy, and b) to more accurately measure the topography of the L658 watershed (and other ELA watersheds) to develop more accurate soil and hydrologic mercury models.
- Sampled two peatlands at ELA (Lake 658 and Lake 239) and two peatlands in Minnesota at the Marcell Experimental Forest, in a grid pattern (~30 sites per peatland) three times over the ice free season (June; August; late Sept) for pore water and solid phase MeHg and total mercury. The objective is to assess the transferability of our understanding of the amount and distribution of total mercury and MeHg in peatlands. Analyses are ongoing
- Continued studies of Hg methylation and demethylation in upland and wetland components of the Lake 658 watershed.

Impact on Downstream Lakes:

Based on pilot-scale studies and our knowledge of the behaviour of mercury in ELA lakes, most of the added mercury will be bound to particles (soils, peat, sediments) in the Lake 658 ecosystem or returned to the atmosphere in the long term. Mercury in the Lake 658 outflow enters a very large downstream lake (Winnange Lake). We expect that the added mercury isotope will not be detected in the Winnange Lake food chain due to the small amount that will be discharged from Lake 658 and the very large volume of Winnange Lake relative to Lake 658 (approximately 1000x larger). Monitoring is being carried out in Winnange Lake to verify that the Winnange Lake food web is not impacted by the experiment. The concentrations of the three different mercury isotopes added to the Lake 658 watershed were examined in young-of-the-year yellow perch and northern pike prior to the addition of isotopes (June 2001) and at two year intervals after mercury additions began (spring 2003, spring 2005). None of the mercury isotopes added as part of the METAALICUS study were detectable in Winnange Lake fish or

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sediments as of 2003. This is not surprising considering that the transport of mercury isotopes out of Lake 658 has been measured and determined to be very small. Northern pike and yellow perch collected in the spring of 2005, and sediment samples from Winnange Lake in September 2005, have not yet been analyzed.

2006 Field Season:

The 2006 field season will be the sixth field season during which mercury additions are planned. Detailed monitoring of site conditions, mercury concentrations, and the fate and transport of mercury will be undertaken. Pilot-scale studies will also continue.

Lake Restoration:

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

After the experiment has been completed, the study lake will be monitored until fish mercury concentrations return to pre-addition levels and the lake returns to conditions specified in Section VII. 3. of the *ELA Memorandum of Agreement*. During this recovery period, concentrations of mercury in fish and sediments in Winnange lake will also be monitored every second year.

Publications Completed or In Progress:

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IN-STREAM CYCLING AND TRANSPORT OF MERCURY FROM THE LAKE 222 OUTFLOW

Participants:

Principle Investigators:

D. Krabbenhoft, USGS, Middleton, WI
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Other Participants:

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C. Babiarz, University of Wisconsin, Madison, WI
S. Chadwick, University of Wisconsin, Madison, WI
J. Hurley, University of Wisconsin, Madison, WI
M. Olson, USGS, Middleton, WI

Project Purpose:

The purpose of this experiment was to gain a better understanding of mercury cycling and transport in small streams. The results from the stream experiment will be complimentary to the METAALICUS project, and will aid in the overall interpretation and transferability of that project.

Experimental Objectives:

- To determine how natural humic acid complexes control mercury transport downstream
- To better understand mercury partitioning when complexed with different ligands in the stream environment (e.g., natural dissolved organic carbon (DOC), chloride)

Study Description:

The stream injection experiment was carried out at the outflow of Lake 222 during the week of August 28th, 2005. The average discharge of the study stream reach during the week was very low ($0.001 \text{ m}^3 \cdot \text{s}^{-1}$), and the tracer injection rate was $40 \text{ ml} \cdot \text{s}^{-1}$, which had no significant impact on stream discharge.

Over the course of the experiment, four different injections were executed. The first, a potassium bromide injection, was done on the day before the three mercury injections to establish the transport characteristics for a conservative tracer (bromide). The information from this injection is critical for establishing the precise sampling locations and times during the mercury tracer injections. For each mercury tracer injection, a different single mercury isotope was used for each test, with the exception of the first injection, which contained two mercury isotope tracers: inorganic mercury (^{201}Hg) and methylated mercury (Me^{199}Hg) that were diluted into a concentrated solution of DOC from a Lake 302 upland stream. On the following two days, successive injections of ^{202}Hg in a concentrate of Everglades DOC, and ^{200}Hg diluted into a HCl matrix were made.

Each mercury tracer injection lasted approximately 8 minutes. Samples were collected simultaneously at four stations downstream from the injection point (10 m, 17 m, 37 m, 51 m). Samples were collected up to 120 minutes after the injection started. Temperature, conductivity, dissolved oxygen, and pH were continuously monitored at the closest sampling site to the injection point. At the end of the experiment, a limited number of small fish (about 12) were collected from the stream.

Summary of ELA Research for 2005

Future Plans:

Currently, there are no further plans to continue this study during the 2006 field season. Once the samples have been analyzed and the data interpreted, another experiment focusing on an aspect of this experiment in more detail may be proposed.

MERCURY DEPURATION FROM YELLOW PERCH AND NORTHERN PIKE

Principal Investigators:

- Jillian Van Walleggem, M.Sc. Candidate, University of Manitoba
- Paul Blanchfield, Research Scientist and Adjunct Professor, Fisheries and Oceans Canada, and University of Manitoba, Winnipeg

Project Purpose:

If the levels of mercury in lakes can be reduced, how quickly can we expect fish living in these lakes to show a reduction of the mercury in their tissues?

This field study is examining the rate of loss (deposition) of stable isotopes of mercury from yellow perch and northern pike tissues. It is also studying the effects of mercury exposure on the loss of mercury stable isotopes.

Work undertaken during 2005:

Fish in ELA Lake 658, site of the METAALICUS mercury experiment (see above), have been exposed to experimentally elevated concentrations of special isotopic mercury for several years. They now have detectable levels of this stable isotope in their tissues. Other lakes in the ELA do not have detectable levels of this mercury isotope.

Yellow perch were transported live from Lake 658 and placed in enclosures in ELA Lakes 240 and 979. Samples of tissue from fish in these enclosures were collected periodically over time and analysed for mercury content.

Four northern pike were also transported live from Lake 658 to Lake 240 and surgically implanted with acoustic telemetry tags. These joined 8 northern pike previously transported in 2004, bringing the total number of former Lake 658 pike now in Lake 240 to twelve. Muscle biopsies were taken from 5 of these pike in the spring and fall for mercury analysis. Hour-long activity tracks, which give an estimate of swimming speed, were done on the pike during the summer months.

Future Research Plans:

The northern pike will be collected for a final time in the spring of 2006 and muscle biopsies will be conducted to determine mercury concentrations.

No publications on this research have been produced to date.

ENDOCRINE DISRUPTING CHEMICALS (EDCs)

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 that some of these manufactured chemicals, intentionally or otherwise,

Summary of ELA Research for 2005

imitate 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, including reproduction, of fishes? If so, what are the potential consequences? A whole-lake experiment at the ELA from 2001 through 2003 examined the ecosystemic impacts of one potent endocrine mimic.

EFFECTS OF A POTENT ESTROGEN MIMIC ON AQUATIC POPULATIONS

Project Team:

Freshwater Institute:

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Paul Blanchfield
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Other Agencies:

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

Background:

Considerable evidence exists that aquatic organisms are being exposed to and impacted by a wide range of compounds that mimic hormones. Fish exposed to these compounds often exhibit an array of responses including depressed circulating sex steroid levels, reduced gonad size and fecundity, and males have become feminized through the development of egg proteins and eggs. One of the most sensitive and common tools used to assess exposure to endocrine disrupting chemicals (EDCs) is the presence of vitellogenin (VTG), an egg yolk protein precursor, in the plasma of male fish. Recent studies have shown elevated plasma VTG in male fish downstream of sewage treatment plants (Harries et al. 1997; Jobling et al. 2002).

Natural and synthetic estrogens such as estriol and 17 α -ethynylestradiol (EE2), two of the main active components of birth control pills, are present at ng/L concentrations in sewage effluents (Kolpin et al. 2002; Kirk et al. 2002; Tilton et al. 2002). Though other estrogenic compounds are present in these effluents, the natural and synthetic estrogens are believed to be posing the greatest threat to the endocrine systems of the resident fish populations. Laboratory studies have confirmed that these compounds are causing the feminization and elevated VTG levels observed in male fish and developmental problems in females downstream of sewage plants

Summary of ELA Research for 2005

(Kramer et al. 1998; Lange et al. 2001; Miles-Richardson et al. 1998; Parrott et al. in press; Schultz et al. 2003; Jobling et al. 1996).

Despite the overt physiological evidence that fish are being adversely impacted by EDCs, it remains unclear whether these compounds are impacting a population's sustainability. It has been recognized nationally and internationally that there is a need to determine whether the molecular and cellular effects, such as VTG production, observed in fish exposed to EDCs are indicative of changes in population viability (Campbell and Hutchinson 1998; Arcand-Hoy and Benson 1998). Though significant progress has been made in characterizing the effects of hormone mimics on individuals, population-level approaches to identify and quantify effects are lacking.

Purpose of Experiment:

This whole ecosystem study has been developed to determine whether aquatic populations are being adversely impacted by EDCs, and to determine the relationship between organism- and population-level responses to these compounds. This seven-year study (1999-2005) will determine the impacts of the synthetic estrogen, EE2, on well-defined fish and invertebrate populations at the Experimental Lakes Area (ELA). EE2 was chosen for this experiment because it is a potent estrogen mimic that is known to affect the endocrine system of fish and other vertebrates. EE2 will act directly and effectively upon the endocrine system of organisms, and, therefore, research results will be broadly applicable to field and laboratory studies of other estrogen-like compounds.

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

Study Site and Experimental Design:

Lake 260 was chosen for this whole-lake addition experiment; it has a surface area of 34 hectares and a maximum depth of 14.4 m. This lake has been part of a long term monitoring program at the ELA and considerable data exist on its limnological, physical, and biological characteristics. It supports well-defined lake trout (*Salvelinus namaycush*) and white sucker (*Catostomus commersoni*) populations of approximately 300 and 500 individuals, respectively, as well as the small fish species fathead minnow (*Pimphales promelas*; used extensively in laboratory EDC assays) and pearl dace (*Semotilus margarita*). Nearby lakes, Lakes 442, 224, 114, Roddy (468) and 373 are being used as reference systems throughout this study because most are similar in physical and chemical characteristics, have the same fish species and long-term data on their populations, and some historical data on lower-trophic-level biota.

Two years of baseline research (1999 & 2000) and three years of EE2 additions (2001-2003) have now been completed. In 2004 and 2005, we did not add EE2 to the lake in order to monitor continued impacts and/or recovery of the lake and its biota. The following is a summary of what has been accomplished to date in 2005 by the various collaborators on this experiment.

Summary of ELA Research for 2005

EE2 Additions & Water Column Concentrations:

In the summers of 2001-2003, EE2 was added three times weekly to the surface waters of Lake 260 to maintain low, but environmentally-relevant, concentrations of this potent estrogen in the surface waters. Methods of additions and quantitation have been described in previous reports to the ELA Management Board. The mean concentrations of EE2 in the epilimnion of the Lake were 6.0 ± 2.9 , 5.1 ± 1.8 , and 4.8 ± 1.0 ng/L in 2001-2003, respectively. Monthly surface water samples were collected from May until October in 2004 and in September of 2005 to determine whether there are residual levels of EE2 in the water. These samples have been extracted and quantified using radioimmunoassay techniques but we are still awaiting confirmation of the numbers using gas chromatography and mass spectrometry over the winter of 2005/06.

Effects of EE2 on Fish

Fish Population Studies:

We sampled the fish populations in Lakes 260, 442 and 224 during spring and fall of 2005 in a similar fashion to previous years. Sampling was done in a manner that minimizes mortality, and the data are being 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. Lake trout were caught and sampled using trap nets and short sets of gill nets in the fall while white sucker were caught and sampled in trap nets in the spring. Other species - fathead minnow, pearl dace, lake chub, and slimy sculpin - were caught in trap nets and sampled in spring and fall in each lake. Most of the spring and fall data from 2005 are still being analysed.

Population sizes of fathead minnow and pearl dace were evaluated using a mark-and-recapture program on Lakes 260 and 442 in 1999-2005. The data are based on a fall marking period of 10 days and a spring recapture period of 10 days. Each time 30 baited minnow traps are used daily. In the fall, fish are released during the marking period, whereas in the spring, the fish are caged over the recapture period.

Because effects in longer-lived populations will take time to manifest, we hope to continue netting in 2006-07 to determine the impacts of an estrogen mimic on fish sustainability and to assess the ecological relevance of biomarkers used to determine estrogen exposure in fish. At present, there are no funds to continue with this research; it is our hope to find support prior to the 2006 field season in order to adequately assess the population-level impacts of EE2.

Fathead Minnow Reproductive Behaviour:

Laboratory studies have shown that estrogen exposure affects the reproductive behaviour of fathead minnows (Majewski et al. 2002). It is not known whether such impacts would occur in fish under natural conditions. Underwater video was used to record spawning behaviour of male fathead minnows in Lake 260 and in reference lakes.

Fish Fertilization Success:

Laboratory studies have shown decreased survival and skewed sex ratios of fish larvae exposed to estrogen mimics (Parrott et al. in press). In this study, fertilizations of lake trout eggs have been done during 5 successive fall spawning seasons in the study and reference lakes to evaluate % fertilization, survival, growth and hatching success, and for developmental abnormalities. Fry survival and development is being assessed in the laboratory at the Freshwater Institute. Lake trout were captured using trap nets in Lakes 260 and 442 and in

Summary of ELA Research for 2005

Roddy Lake during fall 1999-2003. Eggs were collected from 10 females in Lake 260 and 5 females in Lake 442 during 1999. In 2000, 5 females in Lake 260, 5 in Lake 442 and 1 from Roddy Lake were spawned. In 2001, 5 females from Lake 260, 5 from Roddy Lake (Lake 468) and 6 from Lake 442 were spawned. In 2002, 5 females from Lake 260 and 6 females from Lake 442 were spawned. In 2003, 6 females from Lake 260 and 7 females from lake 442 were spawned. Individual egg diameters and total egg volumes were recorded to calculate fecundities for each female. Unfertilized eggs were transported in sterile plastic bags back to the Freshwater Institute in Winnipeg where they were fertilized with a composite of milt (>3 males) obtained from male lake trout from the same lake. Eggs were reared at 8°C with subsamples collected and preserved for measures of gross embryonic development and thyroid hormone and vitamin analysis.

Molecular and Cellular Responses in Fish:

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

To avoid confounding interpretations of population-level effects, a small percentage (less than the annual natural mortality) of the lake trout, white sucker, fathead minnow and pearl dace populations were sacrificed to obtain gonad weights, fecundities, and gonad sections for histology and steroidogenesis. Fish were sampled from Lakes 260, 442, and Roddy Lake (Lake 468) (and Lake 114 for minnows) in 2005 in conjunction with the spring and fall netting programs for the population-level research.

Circulating Sex Steroids and Steroid Production:

Alterations in circulating levels of the major biologically active reproductive steroid hormones are a common response in fish following exposure to endocrine disrupting substances. Reductions in steroid levels have been correlated to reductions in gonadal development, reduced expression of secondary sexual characteristics, increased age to maturation and altered fecundity in fish populations (McMaster et al., 1991). Detailed mechanistic studies examining the pituitary-gonadal axis identified reduced gonadal steroid productive capacity as one of the major contributors to these reduced circulating steroid levels (Van Der Kraak et al., 1992; McMaster et al., 1996).

As part of this study, we have collected plasma from white sucker and lake trout from three lakes prior to EE2 additions, in the fall of 2001-2003 following exposure and in 2004 for recovery. We measure the two biologically active steroids in both sexes, testosterone and 17 β -estradiol in females and testosterone and 11-ketotestosterone in males, and compared levels between sites as well as to the other reproductive endpoints being measured.

In the fall of 2002, 2003 and 2004 (but not 2005) we also conducted *in vitro* gonadal incubations on female white sucker ovarian tissue from all three of the lakes to determine the steroid biosynthetic capacity of the ovarian tissue and to determine whether EE2 addition altered this reproductive endpoint. Incubations were conducted under both basal incubation conditions as well as following stimulation with human chorionic gonadotropin (hCG).

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

Summary of ELA Research for 2005

Impacts on Gonad, Kidney and Liver Tissues:

Tissue sections from fathead minnow were examined in fish collected from the study and reference lakes in 1999-2005. Liver cells in the EE2-exposed individuals were enlarged when compared to baseline data from Lake 260 and reference lake fish in the fall of 2001. Hepatocyte volume index (HVI) is determined by counting the number of nuclei within a specific microscopic field of view and is a measure of the relative size of liver cells (Leatherland and Sonstegard 1984).

Gonadal development in fish was assessed using histological sections to determine whether EE2 exposure affects the timing and maturation of testes and ovaries in white sucker, pearl dace and fathead minnow. Medial sections of ovaries were collected and were examined for state of maturation, presence of atretic follicles, frequency distribution of oocyte stages, lesions and the presence of intersex (development of both male and female tissues). Testes were examined for delayed testicular maturation, inhibited spermatogenesis, asynchronous cyst maturation, seminiferous lobule deformities, replacement of generative tissue with connective tissue and other lesions (intersex/testis/ova).

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

Effects of EE2 on Lower Trophic Level Biota

Effects on Phytoplankton and Bacteria:

Bi-weekly water samples were collected from these lakes in conjunction with zooplankton samples for phytoplankton and chemical analyses (phosphorous and nitrogen, chlorophyll *a*, suspended carbon and nitrogen). Bacteria samples were also taken from Lake 260 and reference Lakes 373 and 239 over the same time period.

Impacts of EE2 on Zooplankton:

Vertical water column tows were collected bi-weekly from ice-off to ice-on in 2005 as was done in 1999-2004 on Lake 260 and on the reference lakes. All zooplankton samples from 1999-2003 and most from 2004 have been identified and counted to determine abundances, community composition, and sex and egg ratios (used to estimate birth and mortality rates). Samples from 2005 are still being processed.

Other Lower Trophic Level Biota:

Monthly samples of the zooplankton predator *Chaoborus* spp. have been taken to determine possible changes in abundance.

In previous years we examined the effects of EE2 on leeches (1999-2003), and larval and adult aquatic insects (1999-2001) using traps and artificial substrates. No research was done in 2004 or 2005 on these organisms. Data from earlier years is being written up for publication and has been summarized in last year's report to the ELA Management Board.

We have also conducted studies on the effects of EE2 on mink and green frog larvae and tadpoles. No research was done on these organisms in 2004. All results for this component of the study have been summarized in the previous year's report. A publication on this research is currently in review (see below).

Summary of ELA Research for 2005

Training

Two graduate students have been trained as part of this experiment. B. Park was a M.Sc. student at the University of Manitoba who was looking at the effects of EE2 on resident tadpoles. He completed his thesis in August of 2003. J. Werner is a Ph.D. student at the University of Manitoba who is conducting studies on the fertilization success and biochemical responses of fish in Lake 260 exposed to EE2.

Future Research Schedule

At present there are no funds to continue research on this experiment. We are seeking funds to continue with the fish population monitoring (at minimum) and some basic plankton monitoring for another 2 years. Continued research on the lake will allow us to determine whether the fathead minnow and pearl dace populations are recovering from EE2 exposure or whether there are ongoing impacts on these species. In addition, we would continue to look for any delayed effects of EE2 on the longer-lived white sucker and lake trout.

Summary of Research Completed to Date

- Continuous EE2 additions have been completed for three summer seasons. Mean concentrations of EE2 in Lake 260 were 6.0, 5.1 and 4.8 ng/L over the 5 months of additions in 2001-03, respectively.
- The surface waters of Lake 260 were sampled in September of 2005 to look for residual concentrations of EE2. These samples are still being processed.
- Spring and fall netting of fish indicate a recruitment failure of fathead minnow in Lake 260 in 2002 - 2005.
- Male and female fathead minnow, pearl dace and lake trout from Lake 260 are being exposed to effective concentrations of EE2. These fish are producing concentrations of the egg protein precursor, vitellogenin, that are up to 9000 and 2000 times higher, respectively, than the males and females from the reference lakes.
- Gonadal sex steroid production in pearl dace has been adversely affected by EE2 exposure.
- EE2 additions are delaying gonadal development in both species of minnows and in suckers. Testes-ova were observed in male pearl dace and fathead minnow in years 1 and 3 of the EE2 additions, respectively. In addition, liver cells and kidney tissues from fish in Lake 260 are showing signs of protein accumulation likely due to the high production of egg protein precursors in these individuals.
- No changes in the microbial or algal population sizes have been observed in Lake 260 in 2001-2005. Diversity of the algal community was lower in Lake 260 in both 2001 and 2002 when compared to reference lake systems, but this was not observed in 2003-2005.
- Zooplankton densities in 2004 were comparable to data collected in 1999-2002 and do not suggest an effect of EE2 on these biota.
- The zooplankton predator *Chaoborus* spp. increased in abundance by 2 times in Lake 260 in 2002-2004. This may be linked to top-down effects in the food web of the lake.

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.

Summary of ELA Research for 2005

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Funding for 2005/06

- American Chemistry Council

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Manuscripts Published, In Review or In Preparation

- Werner, J., K. Wautier, K. Mills, S. Chalanchuk, K. Kidd and V. Palace. Reproductive fitness of lake trout (*Salvelinus namaycush*) exposed to environmentally relevant concentrations of the potent estrogen ethynylestradiol (EE2) in a whole lake exposure experiment. Proceedings of the 26th Annual Larval Fish Conference. In review.
- Blanchfield, P., Kidd, K.A., Palace, V.P., Mills, K., Lazorchak, J. and Lattier, D. Population-level effects of a potent estrogen: results from a whole lake experiment. In preparation.
- Podemski, C., Kidd, K.A., K. Dszyz and M. Dobrin. Effects of a potent estrogen on leeches. In preparation.
- Palace, V.P., Wautier, K.G., Evans, R.E, Blanchfield, P., Mills, K., Chalanchuk, S., Godard, D., McMaster, M., Tetrault, G., Peters, L.E., Vandenbyllardt, L. and K.A. Kidd. 2005. Biochemical and histopathological effects of ethynylestradiol in pearl dace (*Semotilus margarita*) exposed to the synthetic estrogen in a whole lake experiment. Environ. Toxicol. Chem. In press.
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Recent Conference Presentations

- Kidd, K.A., D. Findlay, A. Salki, M. Paterson, P. Blanchfield and K. Mills. Food web effects of a potent estrogen mimic. Society of Environmental Toxicology and Chemistry 26th Annual Meeting, Baltimore, Maryland 13-17th November 2005.
- Werner, J., K. Wautier, K. Mills, S. Chalanchuk, K. Kidd and V. Palace. Reproductive fitness of lake trout (*Salvelinus namaycush*) exposed to environmentally relevant concentrations of the potent estrogen ethynylestradiol (EE2) in a whole lake exposure experiment. Proceedings of the 26th Annual Larval Fish Conference, Barcelona Spain July 11-14, 2005
- Lazorchak, J., Palace, V., Evans, R., Wautier, K., Flick, R., Wiechman, B., Braam, A, Kidd, K., Lattier, D., Molecular, Protein and Histological Indicators in Fathead Minnow and Pearl Dace Exposed to EE2 in a Whole Lake Dosing Experiment . SETAC 25th Annual Meeting, Portland, Oregon 14-18 November 2004.
- Kidd, K., Palace, V., Blanchfield, P., Mills, K., Wautier, K., Lazorchak, J., Lattier, D. Differences in the biochemical- through population-level responses of two minnow species exposed to a potent estrogen mimic. SETAC 25th Annual Meeting, Portland, Oregon 14-18 November 2004.
- Kidd K., Paterson, M., Salki, A., Findlay, D., Blanchfield, P, Mills, K. Responses of a freshwater food web to whole-lake additions of a potent estrogen. SETAC 25th Annual Meeting, Portland, Oregon 14-18 November 2004.
- Kidd, K.A., Paterson, M., Salki, A., Mills, K., Blanchfield, K. Effects of a potent estrogen mimic on aquatic populations. Aquatic Toxicity Workshop 25-27 October 2004.
- Palace, V., K. Kidd, P. Blanchfield, K. Mills, R.E. Evans, C.L. Baron, and K. Wautier. Vitellogenin induction and histopathological effects in pearl dace (*Semotilus margarita*) captured from a lake experimentally treated with the synthetic estrogen ethynylestradiol. SETAC 24th Annual Meeting, Austin, TX 10-13 November 2003.
- Lazorchak, J.M., Flick, R., Lattier, D., Toth, G., Kidd, K., Palace, V., Evans, B., Mills, K., and T. Hodge. Vitellogenin gene expression in fathead minnows exposed to EE2 in a whole lake dosing experiment. SETAC 24th Annual Meeting, Austin, TX 10-13 November 2003.
- Park, B. and K. Kidd. Effects of 17 α -ethynylestradiol on development and gonad differentiation of larval ranids: a lake exposure. SETAC 24th Annual Meeting, Austin, TX 10-13 November 2003.
- Kidd, K., C. Podemski, A. Salki, M. Paterson, D. Findlay, K. Liber, J. Lazorchak and C. Watson. Impacts of whole-lake synthetic estrogen additions on lower-trophic-level biota. SETAC 24th Annual Meeting, Austin, TX 10-13 November 2003.

ENVIRONMENTAL IMPACTS OF AQUACULTURE

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

Summary of ELA Research for 2005

IMPACTS OF CAGE AQUACULTURE ON LAKE ECOSYSTEMS

Purpose of the Study:

This whole ecosystem study has been developed to assess the environmental and ecological impacts of cage aquaculture under current industry practices. The study will determine the impacts of aquaculture on water quality, primary production, sediments and native invertebrate and fish communities. A mass balance approach and the measurement of stable sulphur, carbon and nitrogen isotopes will be used to trace the movement of aquaculture-related waste materials in the ecosystem. Originally planned for three years, this year the project received approval for an additional four years of funding from the Aquaculture Co-operative Research and Development Program. We have just completed the first of an additional two years of fish production and then will proceed to study ecosystem recovery for two years.

Major Participants/Contributors:

Participants:

- Cheryl L. Podemski, Freshwater Institute, Fisheries & Oceans
- Ken Mills, Freshwater Institute, Fisheries & Oceans
- Michael Paterson, Freshwater Institute, Fisheries & Oceans
- Michael Turner, Freshwater Institute, Fisheries & Oceans
- Paul Blanchfield, Freshwater Institute, Fisheries & Oceans
- Ray Hesslein, Freshwater Institute, Fisheries & Oceans
- Lori Tate, Freshwater Institute, Fisheries & Oceans
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- Rebekah Rooney, MSc candidate, University of Manitoba
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Contributors:

- Aquaculture Co-operative Research and Development Program
- Northern Ontario Aquaculture Association
- MTM Aquaculture
- Martin Mills
- NSERC

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2005 Study Activities and Preliminary Results:

Farm Operation:

On May 30, 2004, a team of over 30 ELA staff and students moved approximately 10,440 female rainbow trout into the net pen in Lake 375. The fingerlings were obtained from Lyndon Farms, a certified disease-free hatchery. Total mortality over the summer was 613 fish. Fish were harvested on October 30, 2005. Fish remained healthy throughout and no antibiotic use was required. Total feed usage over the production cycle was approximately 10 tonnes. This is the highest annual feed use thus far and was due to two factors: a larger initial size (200g versus 90g) and to the maintenance of moderate surface water temperatures over the majority of the summer. Estimated total production of trout was 10120 kg.

Temperature and dissolved oxygen were measured and recorded within the cage daily. Fish were fed two times per day and total feed usage was carefully monitored and recorded. Mortalities were removed daily by netting and weekly by SCUBA diving; all morts were enumerated and individually weighed. Inventories to determine average weight of the fish were conducted monthly. Dr. Paula Azevedo and Dr. Bureau are modeling the farm's waste production using feed inputs, fish size, water temperature, and digestibility coefficients measured from the same batch of feed. This year, on a weekly basis, waste feed was estimated by suspending eight sediment traps (diameter approximately 10 cm) at the bottom of the cage during feeding and then retrieving the traps and counting pellets captured by each trap. In general, waste feed appears to be minimal; the majority of traps were consistently empty.

Lake Ecosystem Studies:

Water Quality

On a monthly basis, samples for water quality analysis were collected along depth profiles in both the north and south basin of the lake. Also on a monthly basis, but on alternate weeks (resulting in biweekly sampling), depth-integrated samples were collected from the epi, meta, and hypolimnion at the same stations. During spring and fall turn-over, additional depth-integrated samples were collected over the depth of the cage (1-10m) and analyzed for all forms of phosphorus and nitrogen. Sediment traps were collected weekly in both basins to determine sedimentation rates of carbon, nitrogen, and phosphorus. Many of the water chemistry samples remain to be analyzed. On a biweekly basis, a YSI multi-parameter probe was used to construct meter-by-meter depth profiles for temperature, oxygen, pH, conductivity, turbidity, and fluorescence at 12 stations placed along the north-south axis of the lake. Secchi depths were also determined at each station.

Phytoplankton and Bacteria

Phytoplankton and bacteria were sampled bi-weekly from the deep stations in the north and south basins throughout the ice-free season in 2005 using an integrated sampler. Samples have been analyzed and data are in the process of being worked up.

Algal physiology and phytoplankton nutrient status measurements, which include the use of composition ratios and physiological measurements (alkaline phosphatase, nitrogen debt, and nitrogen fixation activity), 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). The species composition and biochemical composition of algae, together with other phytoplankton and zooplankton data, can

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determine the efficiency of food chains, the effects of perturbation, the production and consequences of harmful phycotoxins, and also the bio-availability of environmentally toxic substances and their rates of removal from surface waters. Unfortunately, these measures had to be discontinued in L375 and L373 in 2005 due to the impending retirement of the individual responsible for this work.

Bacterial biomass and turn-over rates in Lake 375 for 2004 have been analysed.

Littoral Periphyton

Our benthic algal sampling program was designed to evaluate the hypothesis that if phosphorus (and nitrogen) is mobilized from the aquaculture operation, we can expect the productive capacity of the littoral zone to increase. From a metabolic perspective we would expect to observe increased photosynthetic potential because of the high dissolved inorganic carbon in L375, which alleviates carbon limitation typical of epilithic biofilms in ELA lakes. From a food-quality perspective, we also expect that if the availability of phosphorus (and nitrogen) are increased, stoichiometry of littoral biofilms (especially C: P) will be enhanced. Note though that timelines associated with nutrient dynamics will likely vary with the nutrient and in terms of sensitivity to detection by a mid-summer synoptic sampling program. Although N might become available during the open-water season, if ammonia from fish wastes exceeds pelagic demand, P release from the fish waste will likely depend upon cycling through the anoxic hypolimnion and require entrainment into the epilimnion during spring and fall overturns.

In 2005, L375 and L373 were sampled synoptically once each for metabolic information and for taxonomy + chemical composition. A failed equipment redesign resulted in the loss of all metabolic information for 2005.

In order to simulate littoral shorelines in closer proximity to cage operations, six Plexiglas trays holding 8 ceramic tiles ($2 \times 2 \text{ cm}^2$) were placed in Lake 375 and 1 tray placed in reference Lake 373 in May 2004. In addition, 1 tray was placed directly inside the cage. The trays were positioned in a transect 50, 100, and 150 m north and south of the fish cage at a depth of 1 m. In 2004 and 2005, an additional tray was placed directly inside the cage. The trays were sampled monthly with 2 tiles per tray being removed. The September sample represented accumulated seasonal growth of periphyton. All samples have been analysed.

Zooplankton

In 2005, we continued to collect samples to estimate the abundance, biomass, and species composition of zooplankton and invertebrate predators (primarily *Mysis relicta*) in Lake 375 and reference Lake 373. These organisms are important food for fish and also act as indicators of changes in water quality. Zooplankton samples were collected at the deep station in both the north and south basins of L375 on a biweekly basis. Samples were collected in L375 from the epilimnion and the hypolimnion at each station using a double-barreled net in L375. A tube sampler was used to collect samples from six locations located around L373. *Mysis* were collected monthly in L373 and L375 at least one hour after sunset using a 0.75 m diameter net. Samples were collected along a transect down the long axis of each lake; there was a total of 11 stations in L375 and 8 stations in L373. Samples from 2005 have not yet been processed.

Sediments and Associated Biota

To examine the impacts of the farm on productivity of lake benthos, on a biweekly basis sediment cores were collected by a KB gravity corer along depth transects (11, 13, 15, 17, 19, 21 m) in both basins of Lake 375 and in the reference lake (373). Samples were collected at 11 m and deeper because experience has shown that hard (rock) substrates in shallower waters of

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these two lakes prevent operation of the corer. Samples were sieved through a 250 µm sieve and were preserved in 10% formalin. These samples are being archived until funds for their analyses are obtained.

In order to examine the near-field impacts of the farm on sediments and benthic invertebrates we have been sampling sediments along a distance transect from the farm. In 2005, the frequency of sampling along this transect was reduced to two times a year: once in May prior to the introduction of fish to the cage, and once after fish harvest. The transect ran along the 15 m isobath and had sites located directly beneath the cage, at the cage edge, and at 1 m, 3 m, 5 m, 10 m, 15 m, 20 m, and 45 m from the centre.

Core samples were collected by a Kajak-Brinkhurst corer. The water on top of each core was removed by siphoning and a pH probe was used to measure the pH of the sediments at 1 cm, 2 cm, 3 cm and 4 cm of depth within the core (September only). The top 0-1, 1-2 and 2-4 cm of sediment were extruded from four replicate cores from each station in September; in May, sediment cores were sectioned into the top 0-2 and 2-4 cm for this analysis. Pore-water was collected via filtration and ammonia concentration was measured using an ion selective electrode. An additional 4 cores from each station were similarly sectioned and the sediment frozen for later analysis of carbon, nitrogen, phosphorus and metals content. From each station 10 replicate cores were collected and preserved for enumeration of benthic invertebrates.

Energy Transfer to the Native Food Web

Little is known about the effects of cage culture on the native food web. The main objective of this component of the study is to assess whether the aquaculture fish feed or trout faeces are being used as a novel energy source by the Lake 375 biota. To achieve this, we are examining the carbon, nitrogen, and sulphur stable isotope signatures of invertebrates and fish collected before and after the introduction of cage aquaculture into L375. Adding to the 2002 - 2004 collections, aquatic invertebrates, macrophytes and periphyton were collected in August 2005 from L375 and the upstream reference lake, L373. All invertebrates were sorted into families and frozen. Zooplankton and *Mysis* were collected monthly from May through October. Samples of fish were collected in the spring and fall of 2004. Samples will be processed in winter 2005/2006.

Wild Fish Populations

We continued our yearly sampling of fish populations in Lake 375 and two reference lakes, as in previous years. White sucker, slimy sculpin, and cyprinid populations were sampled in the spring and fall with trap nets. Lake trout were sampled in the fall with trap nets and short sets of small-mesh gillnets. Almost all fish were returned live to each lake. Each white sucker and lake trout was anaesthetized, weighed, measured, and marked before release. A few fin-rays were removed for age determinations from a representative sample of each species. Each cyprinid or slimy sculpin was anaesthetized and measured. Data collected in the spring and fall are still being analyzed, but trends that first emerged in 2004 continued in 2005. We captured two untagged rainbow trout escapees from the culture pen in fall 2005 (1.8 kg and 2.3 kg, respectively). These individuals were caught in the fall during lake trout spawning, but captured distant from the lake trout spawning area in the lake.

We again examined cyprinid abundance and size-distributions through monthly minnow trapping at the cage site, at littoral sites around Lake 375, and at similar sites in Lake 373. Samples of minnow species from each site were preserved for stable isotope analysis and other measures.

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Fish Movements

The barrier fence on the outflow of Lake 375 was inspected over the course of the field season to ensure that no escaped rainbow trout (experimental or accidental) could move downstream to Manomin Lake. As a result of very high water levels in the spring, the fence was extended to ensure that escapees had no wetted channel through which to migrate into the outflow.

We continued to maintain three radio-linked acoustic fish positioning systems (VRAP, *Vemco*, Nova Scotia) that continually monitor the movements of acoustically-tagged fish in the study lake (375; two systems) and the reference lake (373; one system). We augmented the data collected using the automated positioning systems with the use of multiple passive receivers which record date and time, depth, and unique fish identifier number.

Most of the transmitters implanted in the initial year of the study (2002) were no longer functioning due to loss of battery power. New transmitters were implanted into wild fish to continue monitoring distribution and movements of native fish species. In the spring of 2005, transmitters were implanted into 8 and 7 lake trout in the study and reference lakes, respectively. We implanted transmitters into 5 white suckers in each of the study and reference lakes in the fall. There has been some mortality of these newly-tagged fish. We also released an additional 10 rainbow trout with active transmitters into the experimental lake prior to the harvest of caged fish. Two of the rainbow trout “escapees” we released into Lake 375 in the fall of 2004 have survived and are still being monitored.

Presentations and Publications:

There are no publications as yet from this project.

This year two members of the ELA project (Podemski and Kidd) organized a special session at the annual Aquatic Toxicity Workshop, focusing on environmental impacts of cage aquaculture. Four presentations from the ELA project were presented and two of our graduate students, Marilynn Kullman and Rebekah Rooney were awarded the second and third prizes respectively for student presentations. Ken Mills presented a poster entitled “Enhanced growth and condition of lake trout in a small Ontario lake during cage aquaculture of rainbow trout” at the 2nd North American Lake Trout Symposium.

Corben Bristow has submitted his MSc thesis for defense at the University of Ottawa. Rebekah Rooney (University of Manitoba) and Marilynn Kullman (University of New Brunswick) have both completed their fieldwork on this project. Rebekah Rooney is currently writing her thesis and expects to defend in the spring of 2006. Marilynn is still completing her laboratory processing, and has a draft of an as yet untitled manuscript describing a sediment bioassay almost complete. She expects to defend in the Autumn of 2006.

Plans for 2006:

As our research proposal to ACRDP last year was successful, the project will continue with another year of fish production next year. Impending retirements and lack of staff renewal will pose challenges for us to continue to produce the quantity of data of previous years, particularly in the areas of water and sediment chemistry, primary production and algal physiology. Dr. Paula Azevedo will be starting a 1-year NSERC Visiting Fellowship with the Freshwater Institute in Mid-November. Dr. Azevedo will be continuing to model the waste outputs from the farm with Dr. Bureau from the University of Guelph, but will additionally focus some of her efforts on production of the mass balance for nitrogen and phosphorus and on determining nutrient release rates and oxygen consumption from the sediment under the farm.

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ECOSYSTEM INTERACTIONS

If we are to fully understand how human activities impact lake ecosystems and fish habitat, we must gain a better understanding of how the populations that make up lake food webs interact with each other, and how processes on the land can impact the lakes. At the ELA, these questions are often addressed by graduate students as part of their theses requirements. During 2005, two such studies were underway.

IMPACT OF INVERTEBRATE PREDATORS ON CRUSTACEAN ZOOPLANKTON

Investigators:

- Dalila Seckar, M.Sc. Candidate, Department of Entomology, University of Manitoba
- Dr. Michael Paterson, Research Scientist, Department of Fisheries and Oceans, Freshwater Institute

Study Goals:

Zooplankters have an important role in the food webs of freshwater lakes; they consume phytoplankton and are eaten in turn by invertebrate predators and fish. The goal of this project is to examine the interactions among three common invertebrate predators and to determine their impact on the zooplankton community using experimental mesocosms. Eighteen mesocosms were installed that contained different combinations of the invertebrate predators *Mysis relicta*, *Chaoborus* spp., and *Leptodora kindtii*.

Study Activities:

The goal of the summer 2005 activities was to replicate the previous summer's research with some adjustments. Enclosures 1.58m in diameter and 11 to 12m deep open to the atmosphere and with closed bottoms were anchored to the bottom of lake 239. The enclosures were pumped full of water from the lake and stocked with zooplankton from L239. Two invertebrate predators were added to the sixteen enclosures in various combinations. *Mysis relicta* was collected from Lake 239 and *Chaoborus* spp. were collected from Lake 227 for these additions. Both predators occur naturally in Lake 239. Target densities in the enclosures were within the natural range of these predators in ELA lakes. Zooplankton were collected from the enclosures weekly and water for chemistry analyses was collected at the beginning, middle, and end of the experiment. Sampling lasted for five weeks and in the final sample week, collections for the invertebrate predators were conducted.

Future Plans:

No further manipulations in deep mesocosms are expected.

This research could be enhanced in the future by surveying predators and zooplankton communities in a wide range of ELA lakes, analysing predators' crop contents, and using predator data from ELA lakes in bioenergetics models.

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MODELLING CARBON STOCKS AND NPP IN ONTARIO'S BOREAL FOREST ECOSYSTEM

Principal researcher and affiliation:

- Ms. Jessica Galarza-Macias, PhD candidate, Trent University Watershed Ecosystems Graduate Program
- Supervisor: Dr. Shaun Watmough, Trent University

Project goal:

The principal objective of this research is to evaluate uncertainty in carbon budget model predictions for the boreal region in Ontario, employing the following procedures:

- Incorporate fires and fire severity into the CBM-CFS model.
- Evaluate the impact of including nitrogen deposition (processes) in carbon budget models.
- Apply an integrated modelling approach that incorporates carbon sequestration in upland forests, wetlands and fluxes to lakes at the landscape level at the ELA.
- Compare simulated NPP and carbon stocks outputs from three carbon budget models currently used in Ontario.

Description of work undertaken during 2005:

A detailed field survey at ELA was conducted in the summer of 2005 to collect forest and soil data, with a follow up visit planned for 2006. The field survey consisted of 25 sampling plots (24 m x 24 m) around the catchment of Lakes 239 and 240. These plots were selected using a stratified random sampling method. The stratification of the area was done using the Ontario Land Cover map and a false colour composite image (using Landsat TM images). On each sample plot site descriptive characteristics were recorded: site number, position, elevation, slope, aspect, vegetation type, percentage coverage, topography, succession status, ecological zone, and disturbance. Allometric tree measurements for all the trees with a DBH \geq 3 cm were acquired, and litter and woody biomass measured. In addition, one soil sample was collected for each of the 25 plots to measure nitrogen and carbon content. Soil type characterization (profile description and laboratory analyses) will be performed at Trent University. These analyses will enable carbon and nitrogen content in soil and biomass at each plot to be determined.

Plans for continuation of this study in future years:

Another field season is planned for the summer of 2006, when the same plots will be re-measured and new plots from wetland areas will be added to obtain vegetation and soil information and characteristics.

RECOVERY OF BOREAL LAKES FROM ACIDIFICATION

Research at the Experimental Lakes Area was instrumental in demonstrating that lake ecosystems and their food webs could be seriously damaged at pH levels above 5.0. This was an important factor in convincing the United States and Canada to implement measures to partially control the release of acid precursors to the atmosphere. Later, ELA researchers were able to demonstrate that lakes contained natural bacterial populations that were capable of

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natural buffering of acidic inputs, thereby permitting lake ecosystems to recover from acidification if acidic inputs were sufficiently reduced. These are just two of the important results of ELA acidification research over the past three decades.

RECOVERY OF LAKES 223 AND 302 FROM EXPERIMENTAL ACIDIFICATION

Investigations on the impacts of lake acidification began in Lake 223 at the ELA in 1974. Our active investigations of recovery from acidification wrapped up in Lake 302 South in 2004. These two study lakes, in which most of the ELA acidification research was focused, are now largely recovered from the experimental studies. With no dedicated funding for continuation of this research, no further experimental work in this area was conducted during 2005.

While chemical recovery was relatively rapid in both acidified lakes, biological recovery and re-colonization of species extirpated during experimental acidification has taken longer. Monitoring during 2005 in L223 demonstrated that slimy sculpin (*Cottus cognatus*) and crayfish (*Orconectes virilis*) have re-colonized the lake and are present in good numbers. The lake trout population now seems comparable to its pre-acidification size. We do not know yet whether *Mysis relicta* has returned to L223.

In Lake 302, the curtains separating the two basins are still in place. The crayfish, *O. virilis*, extirpated from the south basin during the experimental acidification phase, has apparently not re-established in that basin. As part of a larger study focusing on Invasive Species, Michael Turner (Fisheries and Oceans), Rolf Vinebrooke (Univ. of Alberta), and colleagues are proposing to study the experimental re-introduction and restoration of this crayfish in Lake 302S, perhaps beginning in 2006. This will be done in conjunction with other studies of how the rusty crayfish, *O. rusticus*, is invading and displacing *O. virilis* in the Lake of the Woods, and how *O. virilis* is apparently invading other lakes in western Canada.

We will continue to do periodic surveys to confirm whether *Mysis* and other species are able to naturally re-colonize these formerly acidified lakes. Also, at some time in the near future, researchers may wish to use one, or both, of these lakes for new experimental studies.