

Fathead Minnow and Pearl Dace Pilot at Canadian Experimental Lakes Area

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The Story

From 1999 through 2004 the Canadian Department of Fisheries and Oceans Freshwater Research Institute conducted a whole-lake ecosystem study to evaluate organism through population effects of exposure to a model endocrine disrupting compound, the synthetic estrogen ethynyl estradiol. This study at the Experimental Lakes Area site in Western Ontario was visited by EPA scientists for field sampling of exposed indigenous fish and deployment of unexposed fish in cages, in both curtained enclosures (limnocorrals) and the lake. Lake water was also shipped for laboratory exposures of cultured fish at the NERL-Cincinnati laboratory. EPA diagnostic molecular indicator measurements showed increased levels of estrogenic activity across time, in both the water column and in sediments, consistent with chemical data that were associated with dramatic changes in fish populations. Invaluable experiences and data were also gained that provided ORD with the knowledge of its capabilities in monitoring in very remote locations, with indigenous and laboratory-cultured fish across extensive periods of time.

Background Information

- Increased concern about impacts of endocrine disruptors on aquatic organisms
- Potent synthetic estrogens used in contraceptives, such as ethynylestradiol (EE2), are found in waterways downstream of wastewater treatment plants
- Studies have found EE2 in surface waters at concentrations up to 60 ng/L but more commonly at low ng/L levels
- Fisheries and Oceans Canada conducted a whole-lake experiment at the Experimental Lakes Area (ELA) in northwestern Ontario
- For three summers beginning in 2001, EE2 was added to surface waters to achieve 5-6 ng/L concentrations in one lake
- Population-, organism-, biochemical- and cellular-level effects were examined in fathead minnow and pearl dace exposed to environmentally-relevant concentrations of this synthetic estrogen

U.S. EPA NERL Objectives

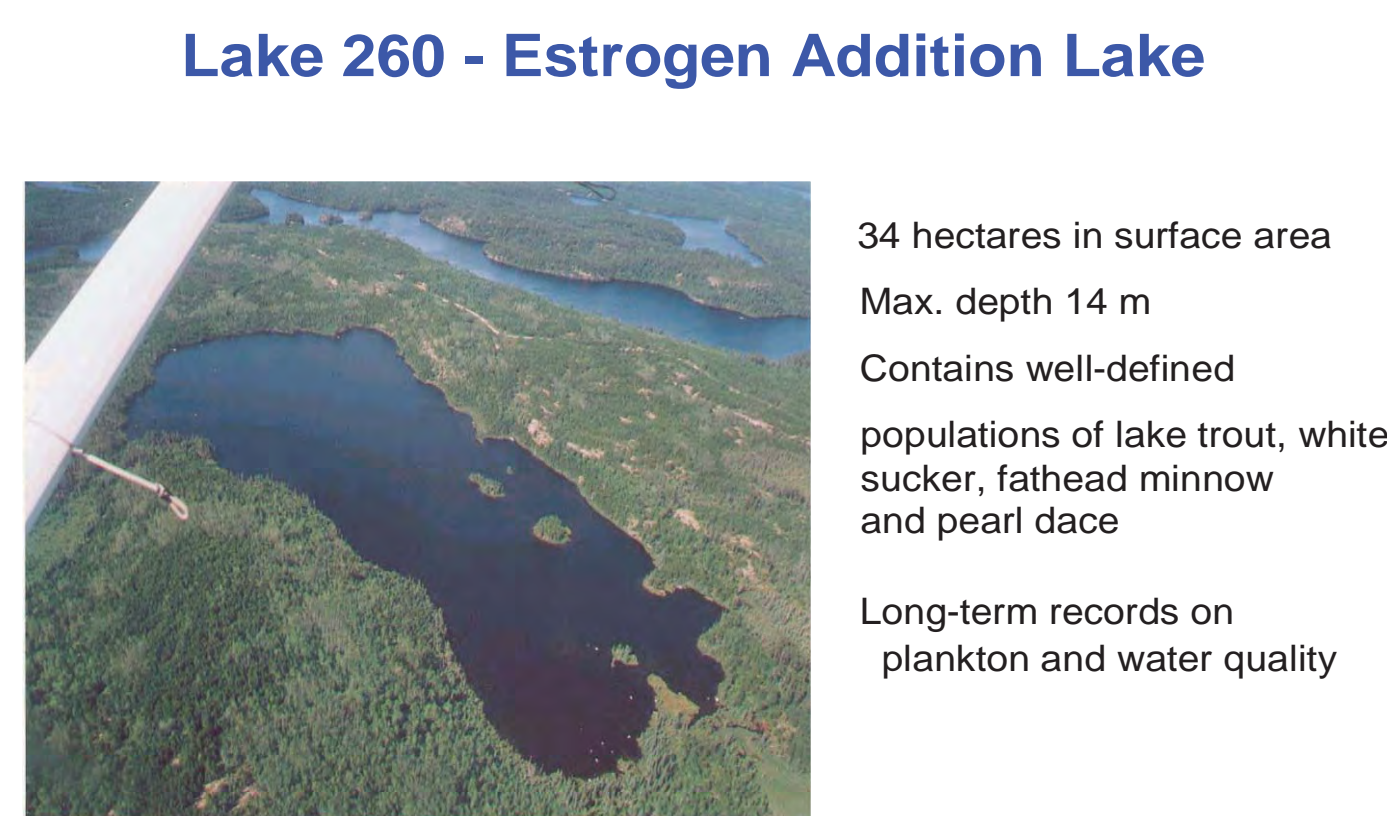
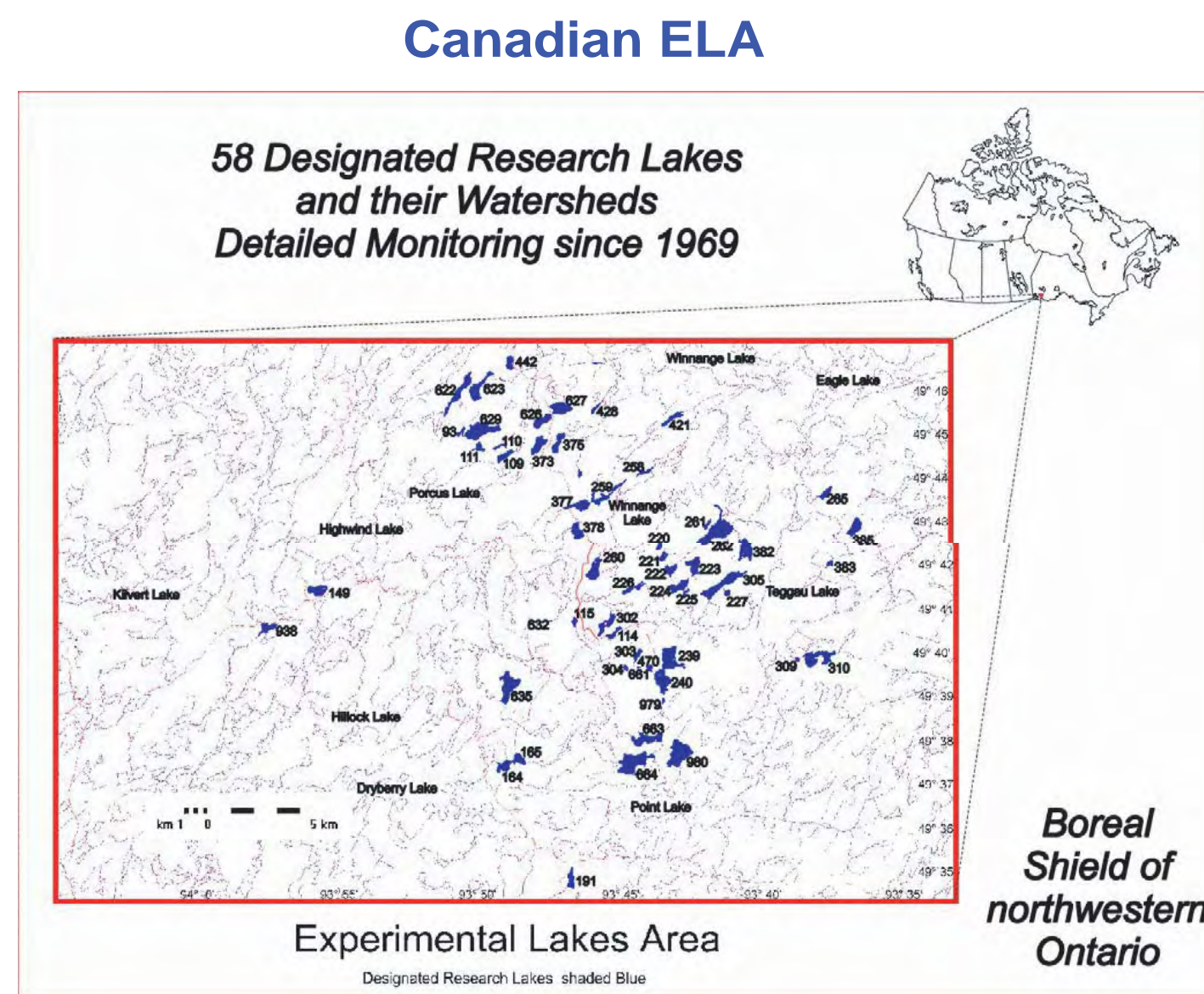
- To evaluate the performance of U.S. EPA's vitellogenin gene expression PCR methods (Gel based and QPCR) on indigenous male fathead minnows exposed to ethynylestradiol.
- To develop relationships of gene expression with protein expression and histopathological effects
- To apply the fathead minnow gene expression method to other cyprinids, i.e. pearl dace

DFO - Study Objectives

- To assess biochemical through population level effects of a potent estrogen
- To determine the timing and magnitude of impacts of an estrogen on aquatic populations
- To link effects across levels of biological organization

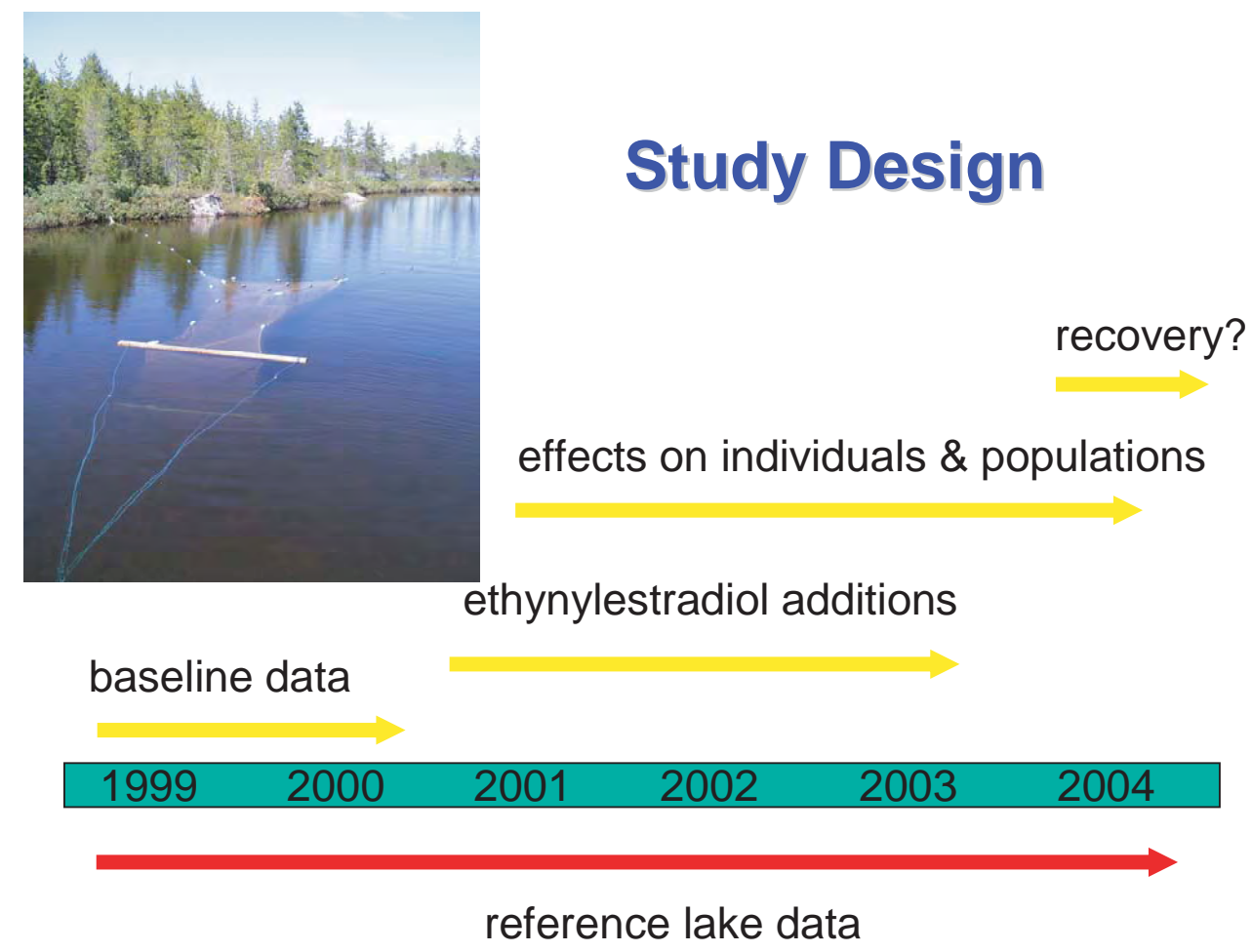
Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy

Methods/Approach



Field and Lab Methods

- Male fathead minnows were collected from dosed Lake 260 and reference lake 114 after 7 weeks, 9 weeks and 12 weeks of dosing. (2001-2003) – minnow traps
- Male fathead minnows from reference lake 114 placed into dosed lake 260 for 13-days. (2001 only)
- Water and sediment samples from Lakes 260 and 114 collected and shipped to Cincinnati and tested with lab reared fatheads.
- Livers were collected shipped to Cincinnati for RNA Isolation and vitellogenin gene expression quantification relative to 18s



Conclusions

U.S. EPA Results

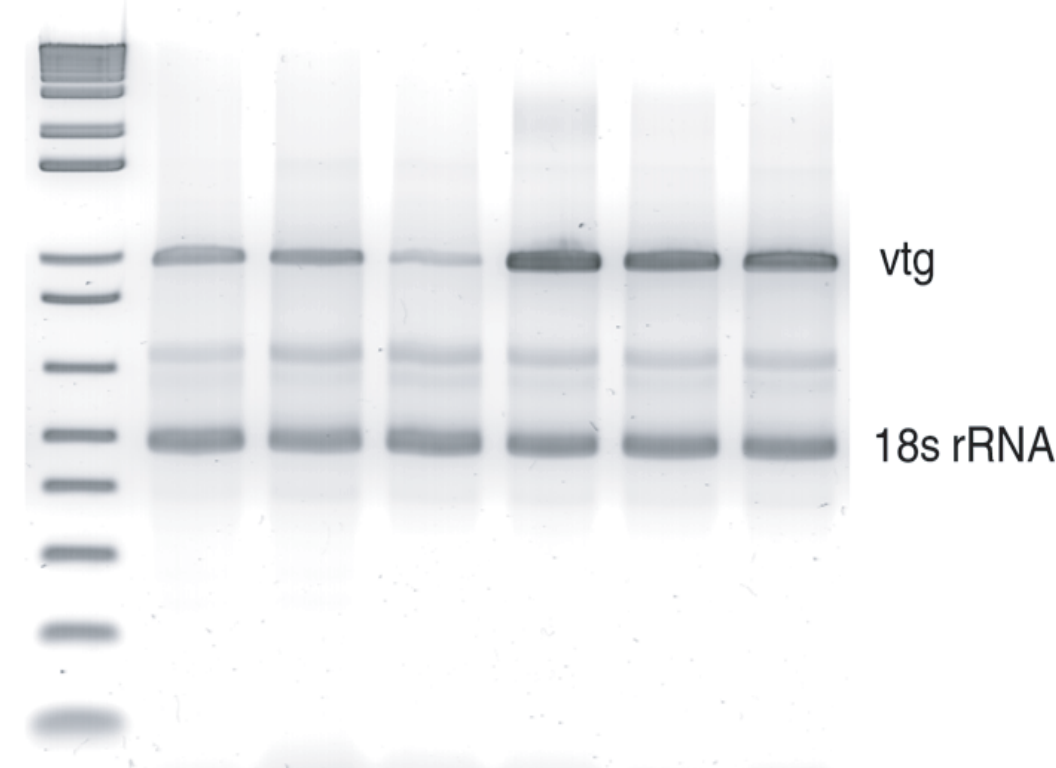
- Gene expression in the 2001 fathead minnow deployment study was detected within 24-hrs after deployment of control fish into the treated lake and stayed elevated for the entire 13-day study.
- Indigenous male fathead minnows and pearl dace collected at all time intervals from the dosed lake showed a constant level of elevation in vitellogenin gene expression in both males and females and beyond normal breeding season.
- Methods developed to measure vitellogenin gene expression in fathead minnows were validated for use in another cyprinid, pearl dace.
- Fathead minnow primers were applied to field collected pearl dace and fathead minnows. This indicates the potential for assessing exposure to EDCs in other field collected cyprinids.
- Gene expression was elevated in male adult fathead minnows exposed to sediment elutriates from sediments collected in 2004.

Whole Lake Experiment – Main Findings

- Male and female fathead minnow and pearl dace showed elevated whole body concentrations of vitellogenin within 7 weeks of EE2 additions to Lake 260
- Egg development was delayed in fathead minnow and pearl dace exposed to EE2; testes development was severely impaired and testes-ova were observed in males of these species
- Reproductive failure was observed in both of these minnow species during the second year of additions

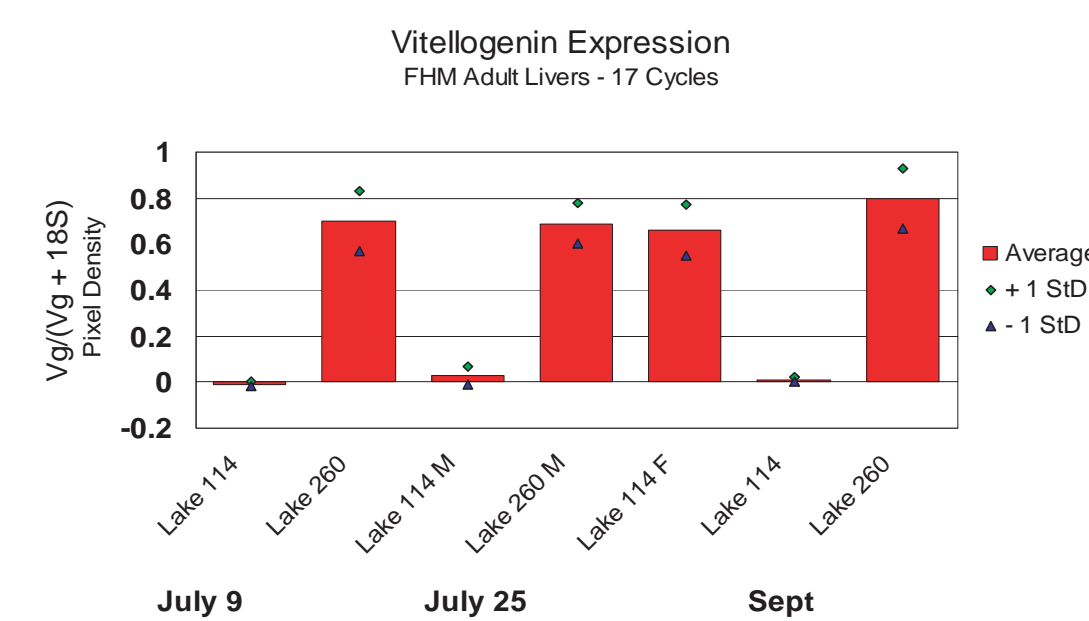
Results

2001 Semi-quantitative PCR Gel Based

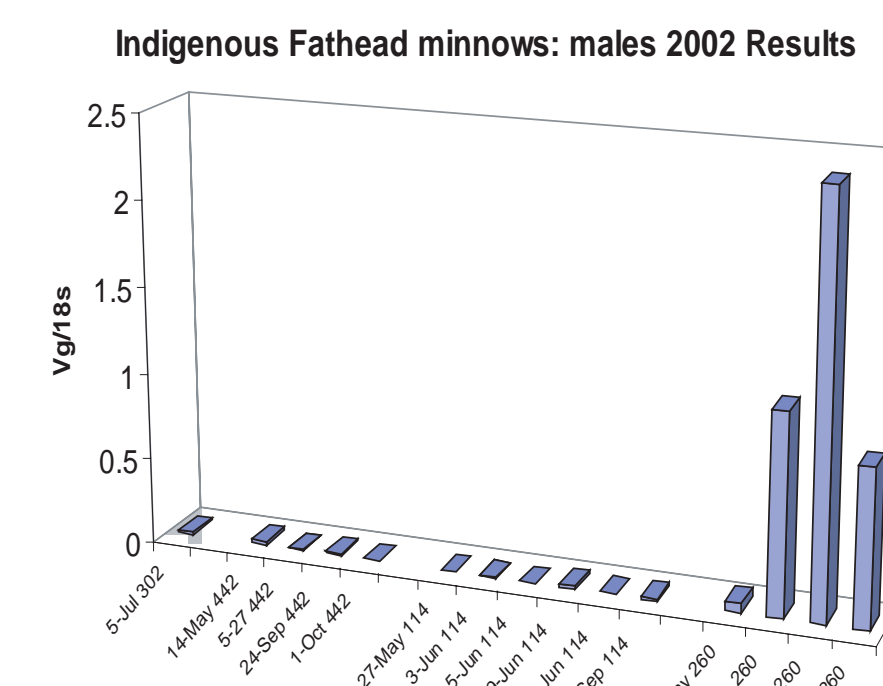


Gel-based measurement of Vg gene expression. Each column is a separate sample. Size standards are at left. Vitellogenin PCR products are separated by charge (vertical axis) and measured relative to an internal cell standard (18S rRNA).

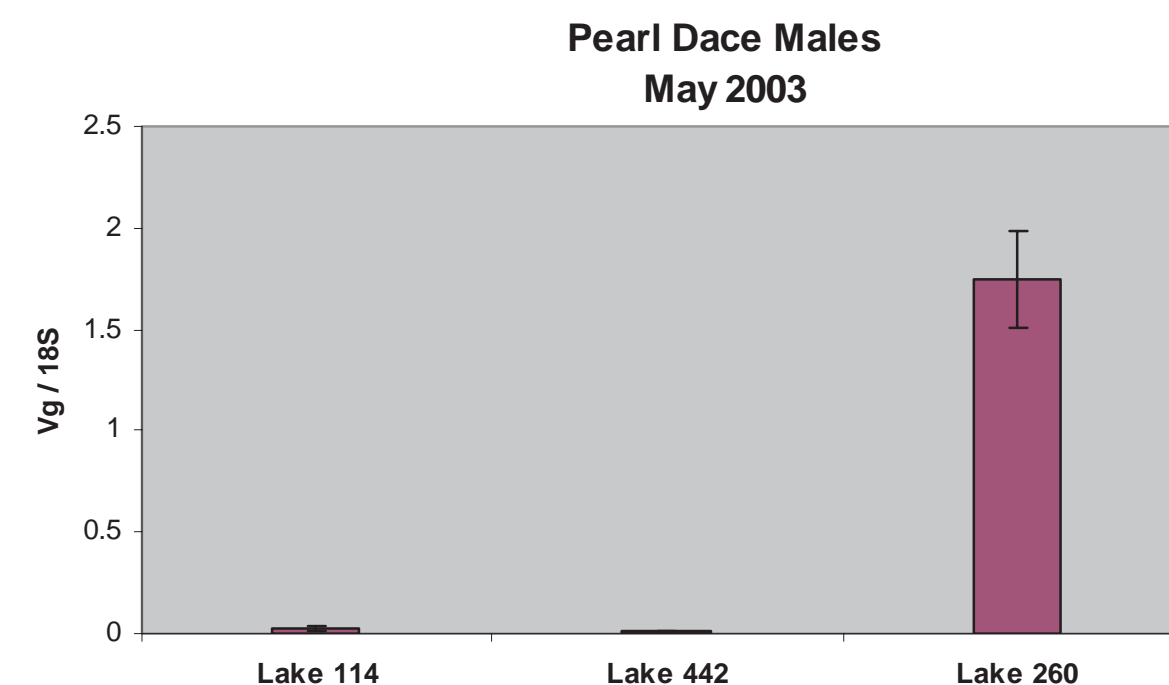
2001 Results for Indigenous Male Fathead Minnows



2002 Results for Indigenous Male Fathead Minnows



2003 Results for Indigenous Male Pearl Dace



Impact and Outcomes

- RT-PCR analyses of total RNA can be used to provide a rapid and timely estimate of exposure to estrogenic substances to indigenous organisms and in laboratory animals exposed to water and sediment samples.
- Gene expression results in combination with biochemical, histological and chemical information show that indigenous fathead minnows and pearl dace continuously exposed to 5 ng/L of EE2 are adversely affected.

Future Directions

- An EDC Multiyear Plan "Annual Performance Measure" report has been prepared and will be distributed to Office of Water and Regional Offices to support the development of environmental policy and water quality criteria and standards development.
- A manuscript will also be prepared and submitted.
- Overall, this pilot research activity extended the capabilities of EPA/ORD EDC monitoring efforts to a successful collaboration on an international whole-lake ecosystem study at an extremely remote location.

References

- Lattier, D.L., Reddy, T.V., Gordon, D.A., Lazorchak, J.M., Smith, M.E., Williams, D.E., Weichman, B., Flick, R.W., Miracle, A.L. and Toth, G.P., 2002; 17 d - ethynylestradiol - induced vitellogenin gene transcription quantified in livers of adult males, larvae, and gills of fathead minnows (*Pimephales promelas*). Environ. Toxicol. Chem 21 (11): 2385-2393.



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