

Effects of a Synthetic Estrogen on Aquatic Populations: A Whole Ecosystem Study

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Despite the overt physiological evidence that fish are being affected by hormone mimics in municipal wastewater effluents, it remains unclear whether these compounds are affecting the ability of aquatic organisms to reproduce and sustain their population sizes. This study has been designed to determine whether hormone mimics have adverse effects on aquatic populations, and to assess the relationships between organism- and population-level responses. We conducted a multi-year whole-ecosystem study at the Experimental Lakes Area (ELA) in northwestern Ontario that will expose well-defined fish and lower-trophic-level populations to environmentally-relevant concentrations of the synthetic estrogen ethynylestradiol (EE2). EE2 was chosen for this experiment because it is present in municipal wastewater effluents at concentrations known to impact the endocrine system and reproductive development of fish; for example, male fish exposed to low ng/L concentrations of EE2 produce vitellogenin, an egg protein precursor normally produced only in females. In 1999 and 2000, baseline data were collected for this study on the aquatic populations in the study and reference lakes. We examined vitellogenin, sex steroids, thyroid hormones, vitamin stores, gonadal and kidney tissues, gonad and liver size, and reproductive behavior (fathead minnow only) in four species of fish. The population-level parameters monitored for fish included fertilization success, sex ratios, abundance, age-to-maturity, size distributions, and growth rates. In the summers of 2001 through 2003, EE2 was added continuously to the study lake (Lake 260) to maintain a constant and environmentally-relevant concentration of this hormone mimic during the open water season. Mean concentrations of EE2 in 2001-2003 were 6.0 +/- 2.9, 5.0 +/- 1.8 and 4.8 +/- 1.0 (SD) ng/L in the surface waters, respectively, of Lake 260 over the summer seasons. No EE2 was added in 2004 or 2005 but we continued to monitor plankton and fish populations for continued effects and/or recovery. Analyses of fathead minnow and lake trout tissues and plasma indicate that males and females from Lake 260 had vitellogenin concentrations that are significantly elevated in 2002 through 2004 when compared to pre-addition and reference lake data. Histological analyses have revealed delayed ovarian development in female pearl dace (a minnow), ova-testes in 30% of male pearl dace, and delayed development and fibrotic tissues in male fathead minnow testes. Fish population data collected in the fall of 2002 through 2005 and spring of 2003 through 2005 suggested year class failures for the shortest-lived species the fathead minnow; the other minnow species, the pearl dace, showed an increase in median size and a progressive decline in the smallest size classes. Continued monitoring is required to assess these longer-term parameters for large fish species. Species abundance and community composition have been studied for benthic invertebrates, zooplankton, microbes, and algae. To date, EE2 has not affected the abundance and production of microorganisms, and the biomass and community composition of algae in Lake 260 (2001 through 2005). Zooplankton have also not shown any direct or indirect effects of EE2 additions to date. Samples of emergent adult and juvenile invertebrates were examined only in the two baseline years and in the first year of additions; there were no effects of EE2 on species abundance and community composition for this part of the food web. Leech catches or gonad sizes have not been impacted by estrogen exposure but accelerated reproduction was observed for a species that lays cocoons on artificial substrates. For the native frog species, there were no effects of EE2 on survival, growth, rates of tadpole metamorphosis, and levels of thyroid hormones in caged animals, and on the sex ratios of wild and caged individuals. However, significantly reduced hatching success was observed in green frog eggs exposed to EE2, and intersex in up to 28% of the individuals was observed in wild and caged mink frog

tadpoles from the treated lake in the three years of additions. Results from this study will be used to understand the ecological relevance of the screening tools, such as vitellogenin production, that are used to assess exposure to and impacts of hormone mimics on aquatic organisms. In addition, these data will improve our understanding of the ecosystem-level effects of estrogen mimics on aquatic biota and the risks these compounds pose to wildlife. We are currently in the seventh year of the project but hope to continue monitoring the fish populations beyond what is presently funded.

Start and end date: April 1999 - March 2006

Presentation(s):

Lazorchak, J., Flick, R., Kidd, K., Palace, V., and Evans, R. (2004). Fathead minnow and pearl dace pilot at Canadian experimental lakes area. Presentation at Program Review of the Environmental Protection Agency's Endocrine Disruptors Research. Research Triangle Park, NC, December 13-15: 2004.

Lazorchak, J., Palace, V., Evans, R., Wautier, K., Flick, R., Wiechman, B., Braam, A, Kidd, K., Lattier, D. (2004). Molecular, protein and histological indicators in fathead minnow and pearl dace exposed to EE2 in a whole lake dosing experiment. Presentation at SETAC Meeting, Portland, Oregon, November 14-18, 2004.

Kidd, K., Palace, V., Blanchfield, P., Mills, K., Wautier, K., Lazorchak, J., Lattier, D. (2004). Differences in the biochemical- through population-level responses of two minnow species exposed to a potent estrogen mimic. Presentation at SETAC Meeting, Portland, Oregon. November 14-18, 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. Presentation at SETAC Meeting, Portland, Oregon, November 14-18, 2004.

Kidd, K.A., Paterson, M., Salki, A., Mills, K., Blanchfield, K. (2004). Effects of a potent estrogen mimic on aquatic populations. Presentation at Aquatic Toxicity Workshop, October 25-27, 2004.

Palace, V., Kidd, K., Blanchfield, P., Mills, K., Evans, R.E., Baron, C.L., and Wautier, K. (2003). Vitellogenin induction and histopathological effects in pearl dace (*Semotilus margarita*) captured from a lake experimentally treated with the synthetic estrogen ethynylestradiol. Presentation at SETAC 24th Annual Meeting, Austin, TX, November 10-13, 2003.

Lazorchak, J.M., Flick, R., Lattier, D., Toth, G., Kidd, K., Palace, V., Evans, B., Mills, K., and Hodge, T. (2003). Vitellogenin gene expression in fathead minnows exposed to EE2 in a whole lake dosing experiment. Presentation at SETAC 24th Annual Meeting, Austin, TX, November 10-13, 2003.

Kidd, K., Podemski, C., Salki, A., Paterson, M., Findlay, D., Liber, K., Lazorchak, J. and Watson, C. (2003). Impacts of whole-lake synthetic estrogen additions on lower-trophic-level biota. Presentation at SETAC 24th Annual Meeting, Austin, TX, November 10-13, 2003.

Park, B. and K. Kidd. (2003). Effects of 17 α -ethynylestradiol on development and gonad differentiation of larval ranids: a lake exposure. SETAC 24th Annual Meeting, Austin, TX, November 10-13, 2003.

Kidd, K.A. (2003). Effects of estrogenic substances on a freshwater ecosystem. Mid-Atlantic Society of Toxicology Meeting on "Pharmaceuticals in the Environment: Risks and Realities" Invited plenary talk, October 7, 2003. East Windsor, New Jersey. .

Kidd, K.A., Podemski, C.L., Paterson, M.J., Salki, A.G., Findlay, D.L., Palace, V.P., Blanchfield, P.J., Mills, K.H., Liber, K., McMaster, M.E., Evans, R.E., and Park, B.J. (2003). Responses of a freshwater food web to synthetic estrogen additions. Presentation at Aquatic Toxicity Workshop, Ottawa ON, September, 28 – October 1, 2003.

Kidd, K.A., Palace, V.P., Blanchfield, P.J., Mills, K.H., Evans, R.E., Wautier, K., Vandenbyllaardt, L., Majewski, A. McMaster, M. (2003). Reproductive and population-level effects in fish from a lake experimentally treated with a potent estrogen mimic. American Fisheries Society Meeting, Quebec City, QC, August 10-13, 2003.

Blanchfield, P., Majewski, A., Palace, V., and Kidd, K. (2003). The effect of a synthetic estrogen on the reproductive success of male fathead minnows: A whole-lake addition experiment. Canadian Council of Fisheries Research Meeting, Ottawa, ON, January 5-7, 2003.

Lazorchak, J.M., Flick, R., Kidd, K., Palace, V., and Hodge, T. (2002). Vitellogenin Gene Expression in Fathead Minnows Exposed to EE2 in a Whole Lake Dosing Experiment. Society of Environmental Toxicology and Chemistry (SETAC) Meeting, Salt Lake City, UT, November 16-20, 2002. .

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Kidd, K., Palace, V., Evans, B., Mills, K., Salki, A., Blanchfield, P., Mills, K., Paterson, M., Podemski, C., and McMaster, M. (2002). Effects of experimental additions of a synthetic estrogen on freshwater food webs. Aquatic Toxicity Workshop, Whistler, BC, October 20-23, 2002.

Park, B., Kidd, K., and Eales, G. (2002). Effects of a synthetic estrogen on tadpole development: a lake exposure study. Aquatic Toxicity Workshop, Whistler, BC, October 20-23, 2002.

Kidd, K. Effects of a potent estrogen mimic on freshwater populations. (2002). Invited Presentation at the Great Lakes WATER Institute, Milwaukee, WI, March 7, 2002.

Kidd, K., Palace, V., Evans, B., Mills, K., Blanchfield, P., Vandenbyllaardt, L., Wautier, K., Lazorchak, J., Lattier, D., and Flick, R. (2002). Biochemical and physiological effects in wild fathead minnows from a lake experimentally treated with the synthetic estrogen, ethynylestradiol. Canadian Symposium on Water Quality, Burlington, ON, February 4-5, 2002.

Kidd, K., Palace, V., Evans, B., Mills, K., Salki, A., Blanchfield, P., Liber, K., Mills, K., Paterson, M., Podemski, C., and McMaster, M. (2002). A whole-lake experiment to examine the effects of a synthetic estrogen on aquatic populations. Canadian Society of Limnologists Meeting, Vancouver, BC, January 3-5, 2002.

Kidd, K., Palace, V., Evans, B., Mills, K., Blanchfield, P., Brown, S., McMaster, M., Van Der Kraak, G., Lattier, D., and Lazorchak, J. (2001). Organism- and population-level responses to a synthetic estrogen: preliminary results from a whole-lake experiment. Presentation at the Society of Environmental Toxicology and Chemistry (SETAC) Meeting, Baltimore, MD, November 20-23, 2001.

Kidd, K., Podemski, C., Paterson, M., Findlay, D., Salki, A., Palace, V., Evans, B., Blanchfield, P., Mills, K., Park, B., Werner, J., Brown, S., McMaster, M., Van Der Kraak, G., Lazorchak, J., Toth, G., Lattier, D., Bagley, M., Liber, K., Graham, D., and Londry, K. A. (2001). Whole-lake experiment to examine the effects of a synthetic estrogen on aquatic populations. Presentation at the Toxic Substances Research Initiative Regional Conference, Hamilton, ON, November 9-10, 2001.

Kidd, K., Palace, V., Blanchfield, P., Mills, K., Evans, B., McMaster, M., Brown, S., Van Der Kraak, G., Lattier, D., and Lazorchak, J. (2001). A whole-lake experiment to examine the effects of a synthetic estrogen on fish populations. Presentation at the Aquatic Toxicity Workshop, Winnipeg, MB, September 30 – October 3, 2001.

Palace, V., Kidd, K., Wautier, K., Evans, R., Dick, R., Werner, J., and Baron, C. (2001). Freshwater fish exposed to environmental estrogens have altered lipid soluble vitamin status. Presentation at the Aquatic Toxicity Workshop, Winnipeg, MB, September 30 – October 3, 2001.

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Kidd, K. (2000). Effects of a potent estrogen mimic on aquatic populations: a whole-lake addition experiment. Presentation at the Endocrine Issues Science Forum, Heidelberg, Germany, November 29, 2000.

Kidd, K., Vandenbyllaardt, L., Weidman, P., and Hesslein, R. (2000). The fate of ethynylestradiol in aquatic enclosures. Poster presented at the 21st Annual Society of Environmental Toxicology and Chemistry (SETAC) Meeting, Nashville, TN, November 12-16, 2000.

Kidd, K.A., Findlay, D., Paterson, M., Podemski, C., Salki, A., Vandenbyllaardt, L., and Liber, K. (2000). Effects of the synthetic estrogen ethynylestradiol on invertebrate, algal and microbial communities. Poster presented at the 21st Annual SETAC Meeting, Nashville, TN, November 12-16, 2000.

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Kidd, K.A. (2000). Effects of a potent estrogen mimic on aquatic populations: a whole-lake addition experiment. Presentation at the Aquatic Toxicity Workshop, St. John's, Newfoundland, October 2-4, 2000.

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Majewski A. R., Blanchfield P., Palace V., and Wautier K. (2002). Waterborne 17 α -ethynylestradiol affects aggressive behaviour of male fathead minnows (*Pimephales promelas*) under artificial spawning conditions. *Can. J. Water Qual.* 37: 697-710.

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Palace, V. P., Dick, T. A., Evans, R. E., Wautier, K., Baron, C. L., Werner, J., Klaverkamp, J. F., and Kidd, K. A. (2001). Altered distribution of lipid-soluble antioxidant vitamins in juvenile sturgeon exposed to waterborne ethynylestradiol. *Environ. Toxicol. Chem.* 20(10): 2370-2376.

Palace, V. P., Wautier, K., Evans, R. E., Baron, C. L., Werner, J., Ranson, C. R., Klaverkamp, J. F., and Kidd, K. (2000). Effects of 17 β -estradiol exposure on metallothionein and fat soluble antioxidant vitamins in juvenile lake trout (*Salvelinus namaycush*). *Bull. Environ. Contam. Toxicol.* 66: 591-596.

Palace, V.P., Wautier, K.G., Evans, R.E., Blanchfield, P., Mills, K., Chalanchuk, S., Godard, D., McMaster, M., Tetrault, G., Peters, L.E., Vandenbyllaardt, L. and K.A. Kidd. 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 review.

Park, B.J. and K.A. Kidd. Effects of the synthetic estrogen ethynylestradiol on early life states of mink frogs and green frogs in the wild and *in situ*. *Environ. Toxicol. Chem.* In review.

Other publication(s):

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Werner, J. Effects on environmental estrogens and metal exposure on metallothionein expression in tissues of wild lake trout and white sucker. University of Manitoba, Ph.D. Thesis. (In preparation).

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