Endocrine Active Compound Monitoring in Minnesota Lakes, 2009-2011: Lake Habitat and Land Use

Minnesota Pollution Control Agency

April 2012

Legislative Charge

This study was authorized in the FY 2010-2011 biennial budget by the Minnesota Legislature and the Governor, enacted in 2009 Session Laws Chapter 172, Article 2 Clean Water Fund, Section 4(k): "$7,500,000 the first year and $7,500,000 the second year are for completion of 20 percent of the needed statewide assessments of surface water quality and trends. Of this amount, $175,000 the first year and $200,000 the second year are for monitoring and analyzing endocrine disruptors in surface waters."

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Minnesota Pollution Control Agency

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Abbreviations

APE	Alkylphenol ethoxylate – detergents that degrade to endocrine active alkylphenols such as nonylphenol or octylphenol
BCF	Body condition factor – the ratio of body length to body weight
EAC	Endocrine active chemical
GSI	Gonadosomatic index – the ratio of the weight of gonads to body weight
HSI	Hepatosomatic index – the ratio of the weight of the liver to body weight
LAS	Linear alkylbenzene sulfonate – detergents that are not endocrine active
PPCPs	Pharmaceuticals and personal care products
VTG	Vitellogenin – a protein normally produced in female fish associated with egg development
VNP	Voyageur's National Park

Units of Concentration

ppm, mg/L	Parts per million
ppb, µg/L	Parts per billion
ppt, ng/L	Parts per trillion

Summary

Several recent studies of Minnesota lakes and rivers have shown that endocrine active chemicals (EACs) and pharmaceuticals are surprisingly widespread in the state's surface water and sediment. Fish that were analyzed in these studies showed evidence of exposure to EACs.

This study, following the Statewide Endocrine Disrupting Compound Monitoring Study of Minnesota Lakes in 2008, was done to understand how endocrine disruption in fish might vary spatially within a single lake, and how lake "microhabitats" are influenced by localized sources of EACs to the surface water. In addition to studying fish from these habitats, surface water samples and samples of groundwater flowing into the lake were collected for chemical analysis from each microhabitat.

The results of this intensive investigation of a single lake suggest that fish undergo morphological (such as body size and weight), physiological (such as metabolic or enzyme functions), and genetic changes when they are exposed to habitats influenced by septic fields, urban runoff, and agricultural sources of contaminants. Analysis of groundwater entering the lake and lake surface water revealed the presence of a variety of endocrine active alkylphenols, hormones, and other chemicals.

Water samples were also taken from lakes in very remote locations. Results of this sampling revealed that although remote lakes in Voyageur's National Park (VNP) and Itasca State Park did contain some contaminants, they appear to contain fewer chemicals than were discovered in lakes in more developed watersheds.

Introduction and Rationale for Study

In 2008, the Minnesota Pollution Control Agency (MPCA) collaborated with the U.S. Geological Survey (USGS) and St. Cloud State University (SCSU) to study pharmaceuticals and personal care products (PPCPs) and other contaminants of emerging concern in 12 Minnesota lakes and 4 streams (Figure 1). The study included the analysis of endocrine disruption in fish in those waters (Ferrey et al. 2008; Writer et al. 2010). That study, together with several previous investigations (Lee et al. 2010), demonstrated that several PPCPs and EACs are widespread in Minnesota's lakes and rivers, sometimes occurring in areas without obvious sources of contamination. It also showed that fish from these lakes and streams – some considered relatively pristine – were exhibiting signs of endocrine disruption.

While limited sampling of lakes and rivers has shown that these contaminants are present in the aquatic environment, several questions remain unanswered. First, it is unclear if EACs and PPCPs are present in lakes that are not exposed to obvious sources of contamination (such as septic fields, wastewater treatment plants, or agricultural areas). Second, the variability in contaminant concentration across a lake is not known. It is unclear if fish populations within a lake are exposed to contaminants uniformly or are more heavily exposed to contaminants in close proximity to identifiable sources, such as residential septic systems. Finally, it is not known if fish populations exhibit a variation in endocrine disrupting effects within a given lake depending on location.

In this study, four microhabitats in one lake (Sullivan Lake in Wright County) were investigated for:

a) The presence and concentration of PPCPs and EACs in the surface water.

b) The presence of detergents in the groundwater entering the lake at these four locations.

c) Temporal variability in PPCP and EAC concentrations in lake surface water.

d) Evidence of endocrine disruption in native fish at these locations.

e) Evidence of endocrine disruption in fish that were caged for a limited time at each microhabitat.

In addition, four remote lakes in northern Minnesota – Ryan and Beast Lakes in Voyageur's National Park, and Bohall and Elk Lakes in Itasca State Park (Figure 1) – were sampled and analyzed for EACs and other chemicals of emerging concern to determine what contaminants are present in pristine and undeveloped lakes that lack nearby sources of these chemicals.

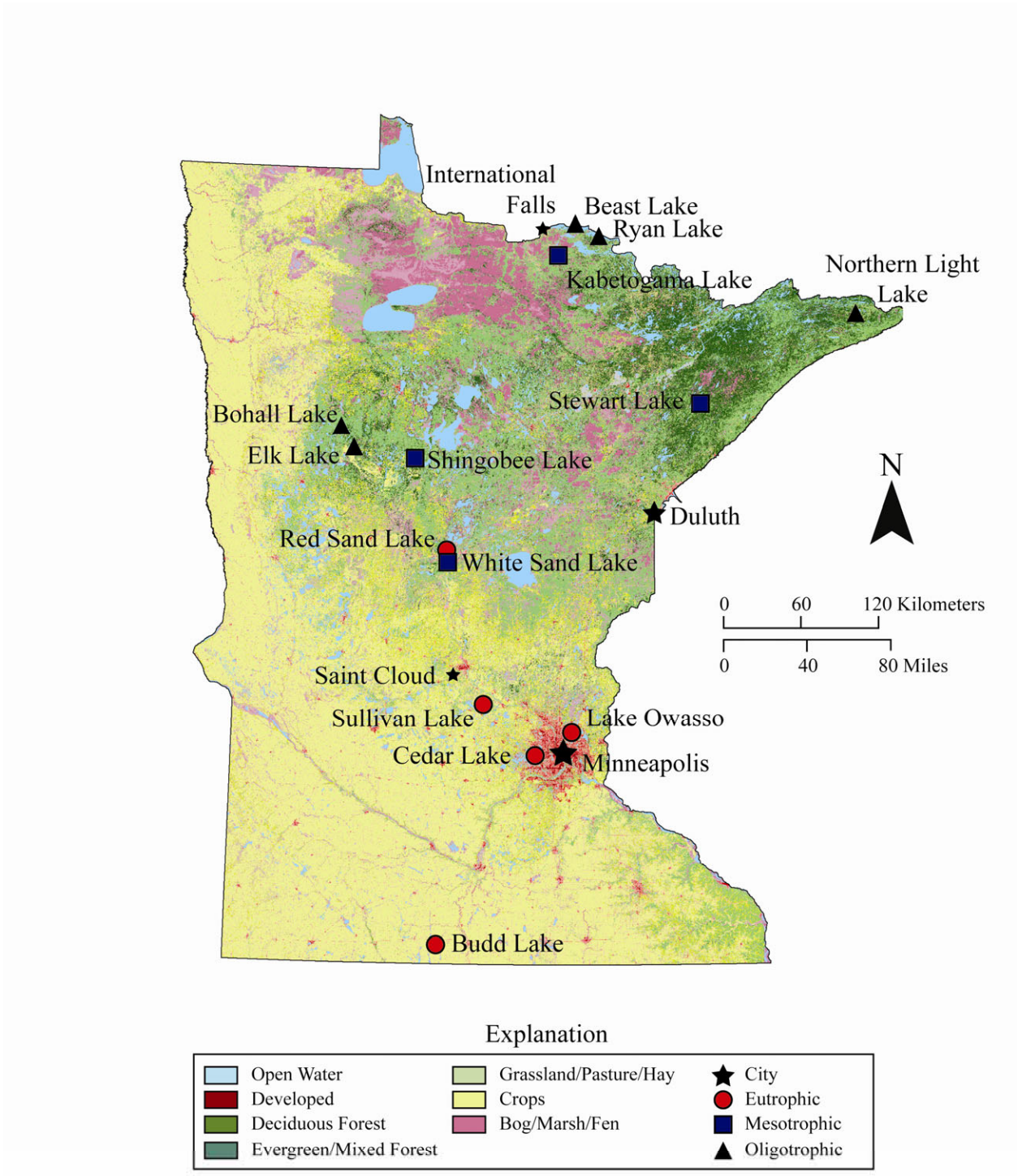

Figure 1. Lakes sampled for the 2008 Statewide Endocrine Disrupting Compound Study and this study.

Approach

Focused lake study. Sullivan Lake in Wright County, Minnesota, was selected for the focused lake study because the lake is influenced by a variety of land use: residential septic systems, runoff from a boat landing (road runoff), and agricultural activity (Figure 2). Aquatic microhabitats near these potential sources of contamination were studied to determine how particular land use affects nearby aquatic environments.

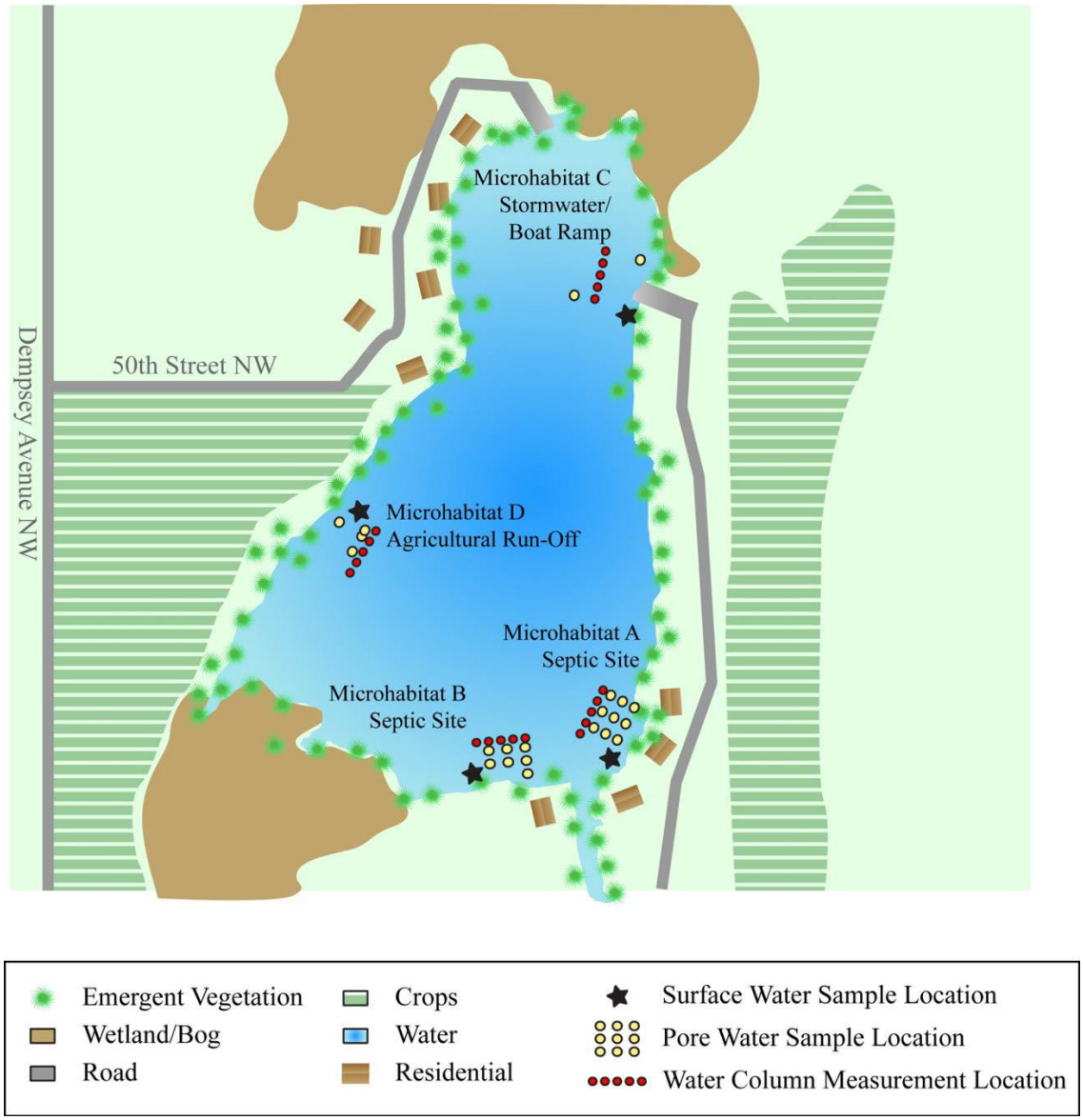

Figure 2. Locations selected for sampling on Sullivan Lake corresponding to land use.

Sullivan Lake surface water and groundwater sampling. Locations of groundwater discharge to Sullivan Lake were identified using temperature gradients and inorganic chemical measurements (e.g., nitrate) that indicate where groundwater flows upward through near-shore lake sediment and into the lake. Two areas of groundwater discharge to the lake near residences were chosen as locations where residential septic systems may be impacting the lake habitat (Figure 2). Groundwater was sampled as it passed upward through the lake sediment (Figure 3) and was analyzed for linear alkylbenzene sulfonates (LAS) and alkylphenol ethoxylates (APEs).

Surface water was sampled at the four lake locations shown in Figure 2 – areas influenced by road runoff, agricultural activities, and two locations influenced by residential septic drainfields – and analyzed for a broad suite of contaminants. For a complete list of analytes, see Appendix A.

Fish studies. Twenty native sunfish from each microhabitat location were collected during the spawning season and studied for evidence of endocrine disruption, including vitellogenin (VTG) production in male fish, morphological/body condition changes, and tissue analysis of gonads and liver. These analyses were also done on laboratory-raised sunfish that were caged at each microhabitat for 21 days. Results were compared to baseline or control fish that were maintained in the laboratory during the course of the experiment. Fathead minnow larvae in the laboratory were exposed to sediment pore-water that was collected from the two locations of groundwater upwelling into the lake near residential septic drainfields. After a 21-day exposure to this groundwater, the larvae were tested for behavioral changes using a predator escape performance assay in the laboratory.

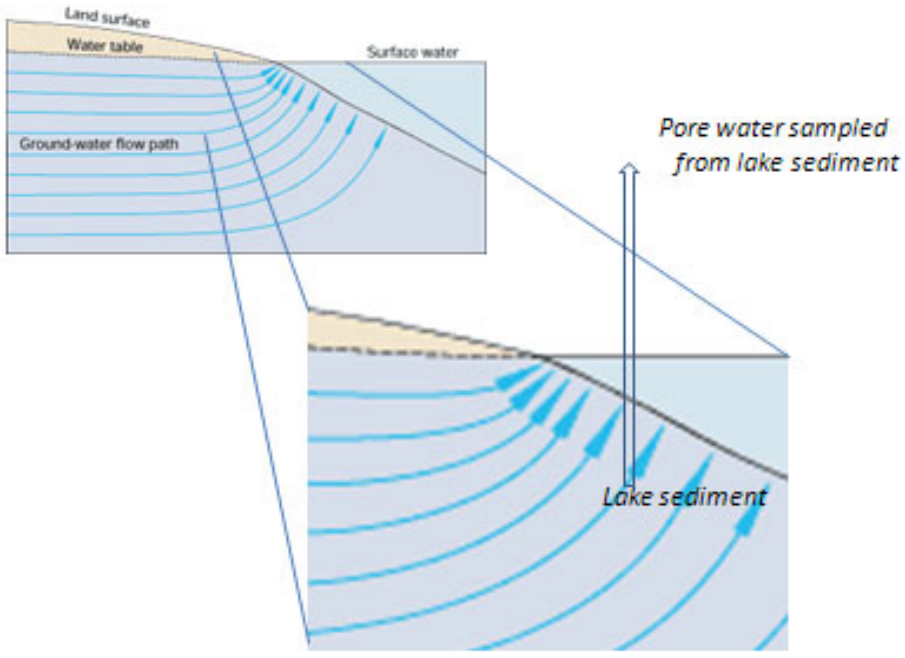

Figure 3. Diagram of groundwater flow to surface water. Groundwater samples can be taken as it flows upward through the lake sediment by inserting a narrow, hollow tube into the lake sediment and withdrawing water from the tube with a syringe *(Diagram modified from USGS*).

After exposure to the sediment pore water from the two septic-influenced sites (Sites A and B), the road runoff location (Site C), and the agriculture influenced location (Site D), fathead minnow larvae were analyzed for alterations in the expression of two genes – the estrogen receptor (ER) gene and the steroidogenic acute regulatory protein (StAR) gene – to measure the genetic effects of exposure to contaminants.

Remote lake sampling. Two remote lakes in Voyageur's National Park (VNP) – Ryan Lake and Beast Lake – and two lakes in Itasca State Park – Bohall Lake and Elk Lake (Figure 1) – were sampled in October 2010. The surface water was analyzed for a variety of hormones and industrial chemicals, several of which are EACs (for a complete list of the analytes, see Appendix A). Ryan, Beast, and Bohall Lakes are remote and receive very little human impact. Elk Lake, situated near Lake Itasca, is undeveloped, but is located on an access road and is exposed to light recreational traffic. Elk Lake was included in the previous sampling of 12 lakes in the Statewide Endocrine Disrupting Compound Study of 2008.

Results

Effect of land use on water quality

Sullivan Lake surface water analysis

Summer 2010

- EDTA (a metal binding agent and food preservative), nitrilotriacetic acid, and nonylphenolmonoethoxycarboxylic acid (a detergent) were detected at every microhabitat site.
- All sites had detections of bisphenol A (the chemical used to make polycarbonate plastic and a known EAC), di-butyl benzoquinone (a breakdown product of BHT), butylated hydroxytoluene (BHT), DEET (insect repellent), methylphenol, and nonylphenol (a breakdown product of APE detergents and a known EAC).
- Caffeine was detected at microhabitat A (septic influenced), which also had the highest levels of DEET and nonylphenol.
- Cholesterol, coprostanol (a metabolite of cholesterol), and estrone (a steroid hormone) were detected at all microhabitat sites. The hormone androstenedione was detected at all sites except microhabitat A.

Fall 2010

- Bisphenol A, butylated hydroxytoluene, di-butyl benzoquinone, DEET, nonylphenol, and octylphenol (a breakdown product of APE detergents and a known EAC), cholesterol, and coprostanol were detected at all sites. Estrone was detected at the agricultural microhabitat site.
- Caffeine was detected at both septic-influenced sites A and B, while butylated hydroxytoluene was found at site A and methylphenol was detected at site B.

Sullivan Lake microhabitat sediment pore water (ground water) analysis

Microhabitat A (septic –influenced location)

- LAS concentrations in pore water samples ranged from <0.02 to 0.92 mg/L.
- There was one detection of APE (0.14 mg/L).

Microhabitat B (septic-influenced location)

- LAS concentrations ranged from 0.02 to 0.12 mg/L.
- APEs were detected up to 0.11 mg/L.

Microhabitat C (road-runoff location)

- LAS was detected at 0.03 mg/L.
- APE was detected at 0.11 mg/L.

Microhabitat D (agricultural-influenced location)

- LAS was detected at concentrations ranging from 0.02 to 0.11 mg/L.

Remote lake surface water (Elk, Beast, Bohall, and Ryan Lakes)

- EDTA was detected in Elk Lake (0.53 µg/L) and in Ryan Lake (0.40 µg/L).
- Di-butyl benzoquinone was detected in Elk Lake (5 ng/L), Beast Lake (4 ng/L), and Bohall Lake (2 ng/L).
- Methylphenol was found (1 ng/L) in Beast and Ryan Lakes.
- Cholesterol was detected in all of the remote lakes sampled. Coprostanol (a biological metabolite of cholesterol) was detected in every lake except for Ryan Lake. These chemicals can occur due to the presence of either humans or wildlife.
- The hormone estrone was detected in Elk Lake (0.1 ng/L), Beast Lake (0.1 ng/L), and Ryan Lake (0.8 ng/L). Estrone is a metabolite of 17β-estradiol, which can be due to the presence of either wildlife or humans.

Sullivan Lake microhabitat fish studies

- Caged male sunfish at one septic-influenced location produced more VTG than those caged at the urban/road runoff site. (VTG production in male fish is evidence of endocrine disruption, since it is a protein required for egg development in female fish and is not normally found in males.)
- No differences were found in the hepatosomatic index (HSI) between caged and native fish at any of the locations. (HSI is a ratio of liver to body weight and is an indicator of physiological stress.) However, the livers from sunfish that were caged at one of the septic-influenced locations showed more signs of stress (vacuoles in the liver tissue) than the livers from native fish.
- Native sunfish had a significantly different gonadosomatic index (GSI) between the two septic locations. Change in GSI is an indication of endocrine disruption.
- Reproductive organs from wild male and female fish from one of the septic locations showed decreased maturity compared to those of the baseline/control fish.
- There were no measurable effects on predator escape responses in fathead minnow larvae that were exposed to sediment pore water that was collected from any of the locations.
- Minnow larvae that were exposed to sediment pore water from the septic-influenced locations and the road-runoff locations showed increases in the activity of ER and StAR genes.

Discussion

In 2008, the Minnesota Legislature funded the Statewide Endocrine Disrupting Compound Study of Minnesota lakes (Writer et al. 2010), which revealed the widespread presence of PPCPs and EACs in lakes that often lacked obvious sources of contaminants. That study (of 12 lakes and four rivers) also showed that endocrine disruption in fish is a common phenomenon in Minnesota's lakes and streams.

The results of this study indicate that land use is important in determining the type and concentration of contaminant in the surface water and incoming groundwater. In Sullivan Lake, surface water from every location was found to contain nonylphenol ethoxylate, nonylphenol, EDTA, DEET, di-butyl benzoquinone, cholesterol, and estrone. The highest concentrations of DEET in surface water were found at one of the septic-influenced locations.

Groundwater entering the lake from the residential/septic influenced areas contained LAS up to 1 mg/L as well as nonylphenol and octylphenol, indicating that groundwater associated with a particular land use can be a significant source of EACs to lake surface water. By contrast, lakes without surrounding development or human impact appear to contain very few of the contaminants that are present in lakes in a more developed watershed. Of the chemicals that are solely attributable to human impact, the remote Ryan Lake in VNP contained only the food preservative EDTA, while Beast, Bohall, and Elk Lakes contained di-butyl benzoquinone. Although these lakes also contained cholesterol, coprostanol, and estrone, the presence of these chemicals may be attributable to wildlife.

It has been established that several of the chemicals detected in this study are known to elicit hormone-like effects in fish. Nonylphenol, for example, is an established EAC that is weakly estrogenic with effects on the behavior of fish at concentrations in the parts per trillion range(Schoenfuss et al. 2008). Surface water standards have not been established for endocrine active compounds. Regardless, the chemicals detected in the surface water in this study are similar in concentration to what has been found in previous investigations. The results reported here are consistent with earlier work that suggests EACs and other chemicals of emerging concern are widespread in our aquatic environment.

This is the first study of the influence of land use on "microhabitats" in an individual lake. The results suggest that endocrine disruption in fish varies spatially within a lake, and is associated with the proximity to sources of contaminants. Subtle but clear differences in exposure effects between caged and resident sunfish, the variation in estrogenic responses in fish, and differences in body condition factors all suggest that fish are exposed to different stressors that can be location specific. For example, decreased BCF was the only adverse effect that indicated exposure to EACs in the habitat associated with agricultural land use, while no adverse effects were found on sunfish in the habitat exposed to road runoff. Fish from both septic influenced sites, however, showed more evidence of exposure to estrogenic chemicals, and changes in reproductive organs were observed in fish from these habitats. In addition to a decreased BCF, male sunfish caged at septic site B had higher levels of VTG, while fish caged at septic site A were found to have an increase in HSI and decreased sexual maturity.

Fathead minnow larvae exposed to groundwater that contained estrogenic contaminants did not exhibit behavioral changes in the predator avoidance tests that have been previously observed following exposure to estrogenic compounds. However, alterations of these fish on a genetic level (alterations in the expression of the ER and StAR genes) indicate that chemicals were exerting estrogen-like effects at the locations where this groundwater was entering the lake.

Studies in Minnesota and elsewhere have demonstrated that exposure to EACs in surface water can elicit changes in patterns of gene expression (Lee et al. 2011), the unnatural production of VTG in male fish of several species (Writer et al. 2010), and the collapse of fish populations and disruption of aquatic ecosystems (Kidd et al. 2007).This study demonstrates that effects of EACs apparently extend across different life stages (larvae and adult fish) as well as across species (sunfish and fathead minnows). Moreover, the study shows that the physiology of individual organisms can also be affected, including alterations of:

- gene expression (such as the ER and StAR gene expression changes)
- protein expression (such as vitellogenin production in male fish)
- tissues (the observed changes in liver structure)
- reproductive condition (observed changes in gonad tissues in wild fish)

These results are consistent with those of previous studies in suggesting that EACs released into our aquatic environments may be having adverse effects on fish and wildlife, and heighten concern over how fish populations – and other wildlife – may be affected over the long term.

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Appendix A

Chemicals analyzed in this study

Hormone	Description
11-ketotestosterone	The oxidized form of testosterone, the primary male sex hormone in fish
17α-estradiol	A form of estradiol that has no estrogenic properties used for the treatment of hair loss
17β-estradiol	One of three naturally occurring estrogens; a female hormone
Androstenedione	Precursor to testosterone and estrogen, the male and female sex hormones
Androsterone	Steroidal hormone with weak androgenic activity
Equilenin	An estrogen used in hormone replacement therapy
Equilin	An estrogen used in hormone replacement therapy
Estriol	One of three naturally occurring estrogens; a female hormone
Estrone	One of three naturally occurring estrogens; a female hormone
Ethinylestradiol	Synthetic oral contraceptive
Mestranol	An estrogen used in oral contraceptives, converted to ethinylestradiol
Progesterone	A female steroid hormone
Testosterone	A male sex hormone and anabolic steroid

Organic Wastewater Compound	Application/description
1,3-dichlorobenzene	Pesticide; deodorizer
1,4-dichlorobenzene	Pesticide; deodorizer
2,6-di-tert-butyl-1,4-benzoquinone (di-butyl benzoquinone)	Metabolite of 2,6-di-tert-butylphenol and BHT (an antioxidant food additive)
2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene)	BHT. Antioxidant food additive, also used in fuels, cosmetics, pharmaceuticals, and embalming fluid.
2,6-di-tert-butylphenol	A UV stabilizer and an antioxidant for hydrocarbon-based products. Prevents gumming in aviation fuel.
3β-coprostanol	Metabolite of cholesterol formed in the gut of animals; indicator of fecal matter in the environment
4-ethylphenol	A naturally occurring degradation product of lignocellulose
4-methylphenol (cresol)	Wood preservative; can also be a naturally occurring chemical in the environment
4-*tert*-octylphenol	Nonionic surfactant; an alkylphenol with endocrine active properties
4-n-octylphenol	An alkylphenol with endocrine active properties; a breakdown product of octylphenol ethoxylate

-continued-

Organic Wastewater Compound (continued)	Application/description
4-*tert*-octylphenol monoethoxylate; 4-OP1EO	Nonionic detergent
4-*tert*-octylphenol diethoxylate; 4-OP2EO	Nonionic detergent
4-*tert*-octylphenol tetraethoxylate; 4-OP4EO	Nonionic detergent
4-*tert*-octylphenol pentaethoxylate; 4-OP5EO	Nonionic detergent
4-*tert*-pentylphenol	An alkylphenol with estrogenic properties
4-nonylphenol	An alkylphenol with estrogenic properties; breakdown product of nonylphenol ethoxylate detergent
4-nonylphenol monoethoxylate; 4-NP1EO	Nonionic detergent
4-nonylphenol diethoxylate; 4-NP2EO	Nonionic detergent
4-propylphenol	An alkylphenol used in flavor and fragrance chemistry
4-tert-butylphenol	An alkylphenol precursor to other chemicals such as antioxidants and UV protection chemicals.
4-tert-pentylphenol	An alkylphenol used in the formation of antioxidants and UV protection chemicals
5-methyl-1H-benzotriazole	Anticorrosive; rust inhibitor
AHTN (Acetylhexamethyltetrahydronaphthalene)	Fragrance chemical
APECs (Alkylphenol ethoxycarboxylates)	Synthetic detergents that can break down to endocrine active alkylphenols
Bisphenol A	Monomer used to synthesize polycarbonate plastic
DEET (N,N-diethyl-*meta*-toluamide)	Insect repellant
EDTA (Ethylenediaminetetraacetic acid)	Chemical that binds metal ions in solution; widespread industrial and commercial application
HHCB (Hexahydrohexamethylcyclopentabenzopyran)	Fragrance chemical
NTA (Nitrilotriacetic acid)	Chemical that binds metal ions in solution; widespread industrial and commercial application
Triclosan	A widely used antimicrobial chemical

Pharmaceutical	Description
Caffeine	Stimulant; an indicator of wastewater