2010 Project Abstract
Final Report

PROJECT TITLE: Assessing Septic System Discharge to Lakes
PROJECT MANAGER: Richard L. Kiesling
AFFILIATION: U.S. Geological Survey
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2010, Chp. 362, Sec. 2, Subd. 5e
M.L. 2013, Chapter 52, Section 2, Subdivision 17

APPROPRIATION AMOUNT: $594,000

Overall Project Outcome and Results

The current study (1) sampled 20 Minnesota lakes that receive groundwater under the potential influence of septic systems to determine the occurrence of pharmaceutically active compounds (PhACs) and endocrine active compounds (EACs), (2) assessed watershed and groundwater characteristics that may contribute to the frequency of PhAC and EAC detections, (3) assessed the histo-pathology of actively spawning bluegill sunfish for biomarkers of EAC exposure to compounds in the near-shore zone of four target lakes, and (4) enhanced EAC analytical capabilities at the Minnesota Department of Health (MDH) through the purchase of new analytical equipment. Study lakes were chosen based on depth to water table, septic system density regardless of functionality, bluegill nesting habitat, and groundwater temperature surveys in the near-shore zone. Lake water or lake-sediment pore water (water stored between sediment particles,contained within the lake-bed sediment) samples were collected and analyzed for a broad suite of 179 PhACs, EACs, and other waste compounds. All surface water samples and over three quarters of pore water samples had at least one compound detected. Overall, 43 of 69 (62%) waste compounds and 5 of 110 (4%) pharmaceuticals were detected in all samples. Twelve known or suspected endocrine active compounds were detected in at least one lake. On average, the prevalence of detections normalized to the number of compounds tested was three to four times higher in near-shore lake water than in near-shore pore water. Actively spawning male bluegill sunfish were collected from reference and groundwater discharge sites in four lakes. Pathologies were more common in fish collected at near-shore sites when compared to fish collected across entire lakes in the 2008 statewide study. The greater abundance of indicators of adverse biological impact suggests that a lake-wide sampling of fish will underestimate the impact of contaminant exposure to fish during reproductively important life stages

Project Results Use and Dissemination

Information from this project has been disseminated to scientific audiences via presentations at Minnesota Water Resources and Midwest Groundwater Conferences.

Environment and Natural Resources Trust Fund (ENRTF)
2010 Work Program Final Report

Date of Report: 8/15/2014
Date of Next Progress Report: **Final Report**
Date of Work Program Approval:
Project Completion Date: 6/30/2014

I. PROJECT TITLE: Assessing Septic System Discharge to Lakes

Project Manager: Richard L. Kiesling
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Location: Statewide project

Total ENRTF Project Budget:	**ENRTF Appropriation**	**$ 594,000**
Amount Spent as of 6/30/14:		**$ 594,000**
Equal Balance as of 12/31/12:		**$ 0**

Legal Citation: M.L. 2010, Chp. 362, Sec. 2, Subd. 5e
M.L. 2013, Chapter 52, Section 2, Subdivision 17

Appropriation Language:
The availability of the appropriations for the following projects are extended to June 30, 2014: (8) Laws 2010, chapter 362, section 2, subdivision 5, paragraph (e), Assessing Septic System Discharge to Lakes; $594,000 is from the trust fund to the commissioner of health for department activities and for an agreement with the United States Geologic Survey in cooperation with St. Cloud State University to develop quantitative data on septic system discharge of estrogenic and pharmaceutical compounds and assess septic and watershed influences on levels of contamination and biological responses in Minnesota lakes. The United States Geologic Survey is not subject to the requirements in Minnesota Statutes, section 116P.10. This appropriation is available until June 30, 2013, by which time the project must be completed and final products delivered.

II. FINAL PROJECT SUMMARY AND RESULTS:

Overall Project Outcome and Results

The current study (1) sampled 20 Minnesota lakes that receive groundwater under the potential influence of septic systems to determine the occurrence of pharmaceutically active compounds (PhACs) and endocrine active compounds (EACs), (2) assessed watershed and groundwater characteristics that may contribute to the frequency of PhAC and EAC detections, (3) assessed the histo-pathology of actively spawning bluegill sunfish for biomarkers of EAC exposure to compounds in the near-shore zone of four target lakes, and (4) enhanced EAC analytical capabilities at the Minnesota Department of Health (MDH) through the purchase of new analytical equipment. Study lakes were chosen based on depth to water table, septic system density regardless of functionality, bluegill nesting habitat, and groundwater temperature surveys in the near-shore zone. Lake water or lake-sediment pore water (water stored between sediment particles,contained within the lake-bed sediment) samples were collected and analyzed for a broad suite of 179 PhACs, EACs, and other waste compounds. All surface water samples and over three quarters of pore water samples had at least one compound detected. Overall, 43 of 69 (62%) waste compounds and 5 of 110 (4%) pharmaceuticals were detected in all samples. Twelve known or suspected endocrine active compounds were detected in at least one lake. On average, the prevalence of detections normalized to the number of compounds tested was three to four times higher in near-shore lake water than in near-shore pore water. Actively spawning male bluegill sunfish were collected from reference and groundwater discharge sites in four lakes. Pathologies were more common in fish collected at near-shore sites when compared to fish collected across entire lakes in the 2008 statewide study. The greater abundance of indicators of adverse biological impact suggests that a lake-wide sampling of fish will underestimate the impact of contaminant exposure to fish during reproductively important life stages

Project Results Use and Dissemination

Information from this project has been disseminated to scientific audiences via presentations at Minnesota Water Resources and Midwest Groundwater Conferences.

III. PROGRESS SUMMARY REPORTS

Amendment Request (03/07/2013)

We are requesting a no-cost, six-month extension to collect additional data through the summer of 2013 and to compare and contrast our results with a comparable data set collected by the State of Minnesota during the summer of 2012. An extension would provide the following project enhancements:

- we could complete additional biological sampling under Result 3.
    - The extension would provide the opportunity to continue to collect data on a stable lake population of exposed bluegill sunfish through September of

2013. These data would provide a link between our 2009 LCCMR project and the 010-A2 project.

- we would be able to compare our chemistry data collected under Result 1 in 2012 and 2013 with the Minnesota Pollution Control Agency (MPCA) lake survey data collected from approximately 50 Minnesota lakes in 2012. These data were analyzed using comparable methods.
- we would be able to use USGS FY 2014 financial resources to complete the project.

Amendment Approved: 05/09/2013

PROGRESS SUMMARY AS OF June 30, 2011:

A Joint Funding Agreement (JFA) with the Minnesota Department of Health was executed in October of 2010. During the fall of 2010, five candidate lakes in four regions of the state were sampled for evidence of groundwater influence. Samples of shallow groundwater were collected from the littoral zone of the lakes and compared to surface water grab samples. A hand-driven piezometer was used to sample groundwater at a number of locations around each lake. Groundwater samples were compared to surface water samples using standard stable isotopes of oxygen and hydrogen.

Additional efforts were initiated to collect the information necessary to complete site selection for the project. These efforts are ongoing. Geo-spatial information on the groundwater setting of additional candidate lakes is being collated and entered into a geo-spatial database. This information will be combined with data on the distribution of septic systems as well as with existing GIS data on watershed characteristics. Together, these sources of information were used to select candidate lakes scheduled to be sampled under Result 1.

Additional ongoing sampling continues to build the lakes database. A synoptic study of sites in Spirit Lake, Pokegama Bay, St. Louis Bay, and Superior Harbor was conducted with additional funding from State and Federal partners. Water and sediment samples were submitted for analysis from 30 sites from the synoptic study. Further analysis of results is pending return of data from the lab.

Dollars spent to date reflect budget estimates as of September 30, 2011.

PROGRESS SUMMARY AS OF December 31, 2011:

Efforts to collect additional information necessary to complete site selection for the project continue using information from the county well log file and the county groundwater atlas. Geo-spatial information on the groundwater setting of additional candidate lakes is being collated and entered into a geo-spatial database. This information will be combined with data on the distribution of septic systems as well as with existing GIS data on watershed characteristics. Together, these sources of information were used to characterize candidate lakes scheduled to be sampled under Result 1.

Additional ongoing sampling continues to build the lakes database. A synoptic study of sites in Spirit Lake, Pokegama Bay, St. Louis Bay, and Superior Harbor was conducted with additional funding from State and Federal partners. Water and sediment samples were submitted for analysis from 30 sites from the synoptic study. Further analysis of results is pending return of data from the lab.

PROGRESS SUMMARY AS OF June 30, 2012:

Sampling was completed on a large synoptic study of sites in Spirit Lake, Pokegama Bay, St. Louis Bay, and Superior Harbor conducted with additional funding from State and Federal partners. Water and sediment samples were submitted for analysis from

30 sites from the synoptic study. Analyses of these data have been completed and the results are being used to select locations for sampling fish populations under the influence of septic systems.

From April through June, eight additional lake sites were sampled for water and sediment. Two sites in each lake were sampled, bringing the total sampling effort to 46 sites. Bluegill spawning surveys were completed for four lakes, and two lakes were sampled for spawning bluegill sunfish. Two sites were sampled in each lake: one site in the vicinity of septic collection and treatment systems and one reference site.

PROGRESS SUMMARY AS OF December 31, 2012:

Chemical data has been received and reviewed for water and sediment samples collected during last reporting period. Preliminary analysis of that data has been completed. Preliminary results were presented at the Midwest Groundwater and MN Water Resources Conferences in October.

Histopathological analysis of fish tissues is in progress at St. Cloud State University. Completion of this analysis is projected to be completed in early 2013.

PROGRESS SUMMARY AS OF June 30, 2013

Water from two lakes was collected using a modified method from the previous year. The sampling methods were modified to increase the likelihood of obtaining a representative sample of groundwater seeping into lakes. Additionally, improved analytical capabilities at the USGS National Water Quality Laboratory provided an opportunity for more detailed chemical profiles using an expanded pharmaceutical list.

Biological data from fish collected during 2012 were received from St. Cloud State University and preliminary analyses completed. Preliminary results do not show any trends in biological response between septic and non-septic sites within lakes. Male bluegills were collected from one lake between ice-off and June 30, 2013. Egg sampling methods were modified to obtain a more representative sample using a peristaltic pump. Eggs were preserved for quantification and analysis of viability.

PROGRESS SUMMARY AS OF December 31, 2013

Ten more lakes were sampled, completing the goal of sampling 20 lakes across the state. Eighty percent of the chemical data has been received from the USGS National Water Quality Laboratory. Data review is in progress.

Fish were collected from one additional lake during July 2013. Histopathological analyses are complete and data have been received from St. Cloud State University. Data analysis is in progress.

IV. OUTLINE OF PROJECT RESULTS*:*

RESULT/ACTIVITY 1: Quantify the occurrence of estrogenic or pharmaceutical compounds in 20 Minnesota lakes that receive groundwater inputs from septic systems.

Description:

Pharmaceuticals and EACs will be measured in water and sediment from 20 Minnesota lakes. Lakes chosen will have significant numbers of septic systems (>35% shoreline development) and will be distributed among the State's hydrologic and ecological regions. Sampling will follow the general protocol from the recently completed Statewide Survey with MPCA, with site selection and sample distribution designed to follow gradients in groundwater and surface water hydrology within and between lakes. Results from the proposed survey will be combined with the results from the recently completed Statewide Survey to develop an EAC contaminant database for Minnesota lakes. The database will be used to analyze patterns in contaminant occurrence relative to watershed characteristics.

Summary Budget Information for Result/Activity 1:	**ENRTF Budget:**	**$247,000**
	Amount Spent:	**$247,000**
	Balance:	**$0**

Deliverable/Outcome	Completion Date	Budget
1.Survey of 10 lakes	9/30/2011	$96,000
2.Survey of 10 additional lakes	9/30/2013	$96,000
3. Data report on lakes	6/30/2014	$55,000

Result Completion Date: 11/30/2014

Result Status as of: 12/31/2010

Site selection criteria were developed and sample distribution design elements were defined. Gradients in groundwater and surface water hydrology within and between candidate lakes are being documented, reviewed, and accepted or rejected on a lake by lake basis. Sampling protocols from the recently published Statewide Survey with MPCA are under review and are being revised to meet project objectives. Data from published studies including the Minnesota county geologic atlases for the lake regions are under review.

Result Status as of: 6/30/2011

Intensive site surveys were completed for fifteen lake sites. Five additional lakes sites are under further study. A synoptic study of sites in Spirit Lake, Pokegama Bay, St. Louis Bay, and Superior Harbor was conducted with additional funding from State and Federal partners. Water and sediment samples were submitted for analysis from a total of 35 sites.

Result Status as of: **12/31/2011**

Results from a synoptic study of sites 35 sites distributed in Spirit Lake, Pokegama Bay, St. Louis Bay, and Superior Harbor were received and analyzed. Results identify a number of sites with significant detections of endocrine active compounds. Results

from a series of lakes sampled as part of a previous state-wide study were combined with the results from the synoptic survey into a single geospatial database for analysis of the distribution of individual constituents.

Result Status as of: **6/30/2012**

Sixteen lake sites under the influence of groundwater inputs were sampled between March and June of 2012. Four of these sixteen sites had active bluegill spawning in the vicinity of the groundwater inflows. Laboratory analysis should be complete by October of 2012.

Result Status as of: **12/31/2012**

Chemical data from the sixteen lake sites sampled between March and June of 2012 has been received and reviewed. Four of these sixteen sites had active bluegill spawning in the vicinity of the groundwater inflows. Paired sites within these four lakes did not show significant trends between septic and non-septic zones. Chemical results will be compared to biological data when preliminary analysis of fish tissues has been completed.

Result Status as of: 6/30/2013

Two lakes were sampled between ice out and June 2013. Sample methods were modified from the previous year to collect interstitial water (pore water from below the sediment surface) to obtain a better representation of groundwater seeping into the lake. A new, expanded pharmaceutical schedule available from the USGS National Water Quality Laboratory was included in analyses to provide a greater detailed characterization of water samples.

Result Status as of: 12/31/2013

Ten lakes were sampled between July 2013 and November 2013. These ten lakes completed the goal of sampling 20 lakes, as indicated in the work plan. Eighty percent of the chemical data has been received. We are awaiting results for the rest of the lakes, at which time the data will be reviewed and analyzed.

Final Report Summary: 6/30/2014

The presence of emerging contaminants, including EACs, and pharmaceuticals was determined in 20 lakes distributed throughout the state (Figure 1). Lake selection was based on: (1) the presence and density of septic systems around the lake (2) gradients in groundwater and surface water hydrology and (3) the presence of suitable bluegill spawning habitat near potential groundwater inflows. Septic system design and functionality was not taken into consideration when selecting study lakes. Lake water or lake-sediment pore water (water stored between sediment particles, contained within the lake-bed sediment) samples were collected from near-shore sites with suspected groundwater inputs. The USGS National Water Quality Laboratory analyzed all water samples for 69 waste compounds, such as plasticizers, surfactants, polycyclic aromatic hydrocarbons, alkylphenols, fragrances, and fire retardants, some of which are EACs. Initial sampling methods included sampling two sites per lake: a septic influenced site and a non-septic influenced site. The sampling strategy was modified during the second year to collect only one sample per lake because of the lack of differences between intra-lake sites. Additionally, use of a new pharmaceutical analytical method from the National Water Quality Laboratory provided greater in-depth analysis of 110 pharmaceuticals for the 12 lakes sampled during the second year of the study.

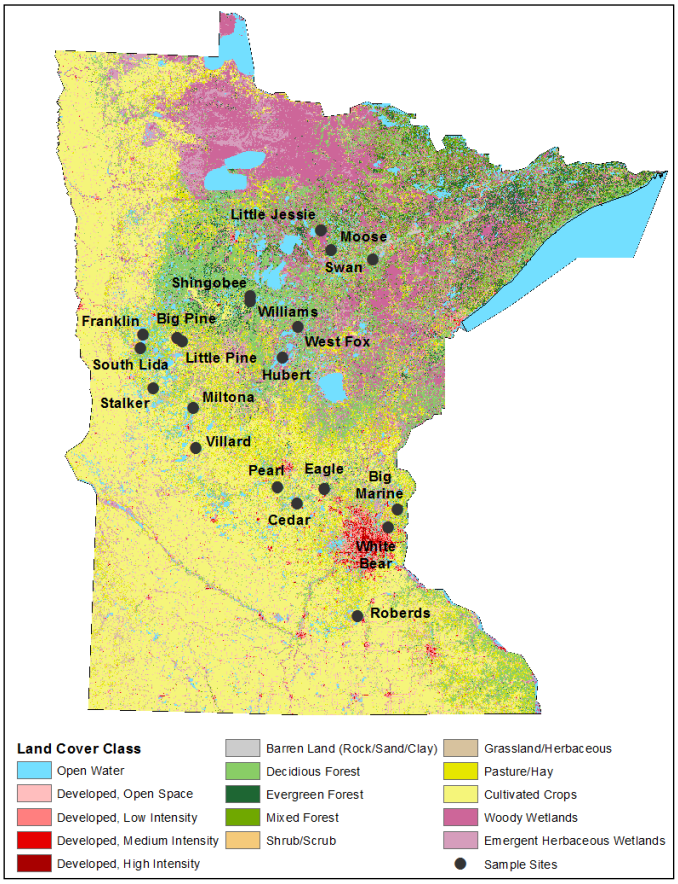

Figure 1. Map of 20 sampled lakes during 2012 and 2013.

Relatively few compounds were detected in each of the 20 lakes sampled, and in general, fewer compounds were detected in lake-sediment pore water samples compared to surface-water samples (Figure 2). At least two waste compounds were detected in every surface-water sample; three pore-water samples had no detections. Overall, 43 (62%) of the waste compounds were detected in at least one lake, representing compounds from a variety of compound uses, and including several known endocrine disruptors (Figure 3). For example, at least one alkylphenol (known endocrine disruptor) was detected in 25 and 8% of surface-water and pore-water samples, respectively. Atrazine, *beta*-Sitosterol, *beta*-Stigmastanol, cholesterol, DEET, isophorone, and nicotine were the most frequently detected compounds (Figure 4); these compounds were detected in at least 30% of samples. Waste compound concentrations ranged from 0.004 ug/L (carbazole) to 49 ug/L [bis(2-

ethylhexyl)phthalate] (Table 1). With the exceptions of cholesterol, *beta*-Sitosterol, 4-nonylphenol, and 4-nonylphenol diethoxylate, most concentrations were less than 1 ug/L.

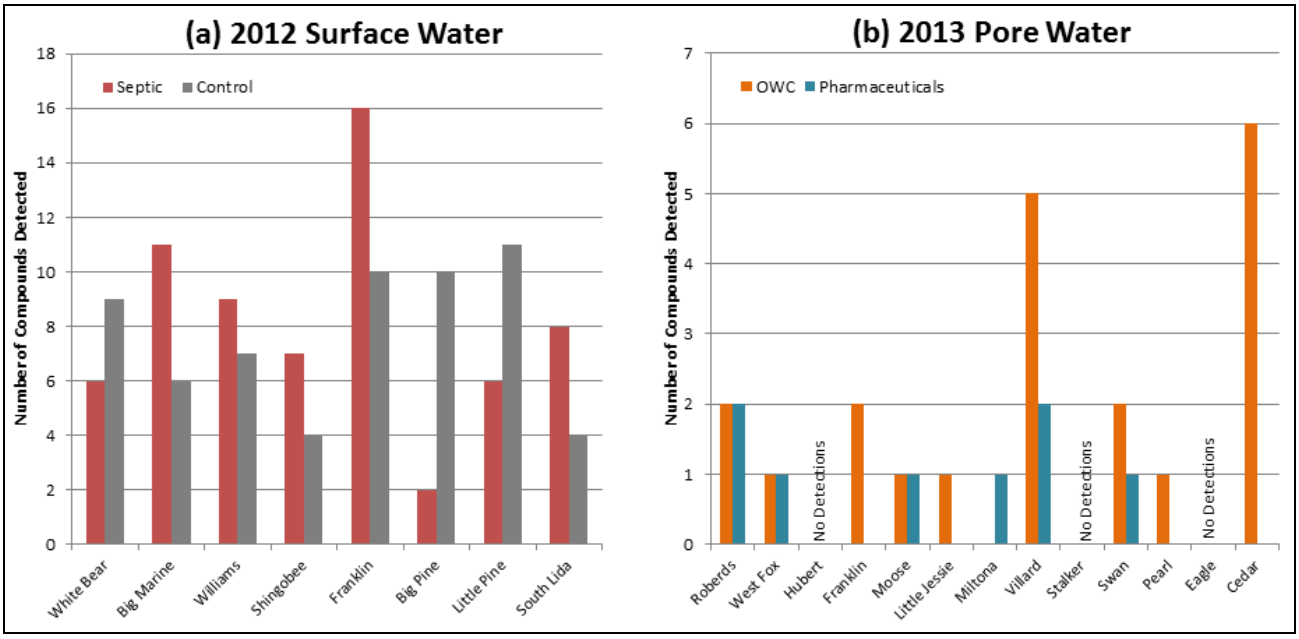

Figure 2. Number of compounds detected in (a) surface water collected during 2012 and (b) Pore water in 2013.

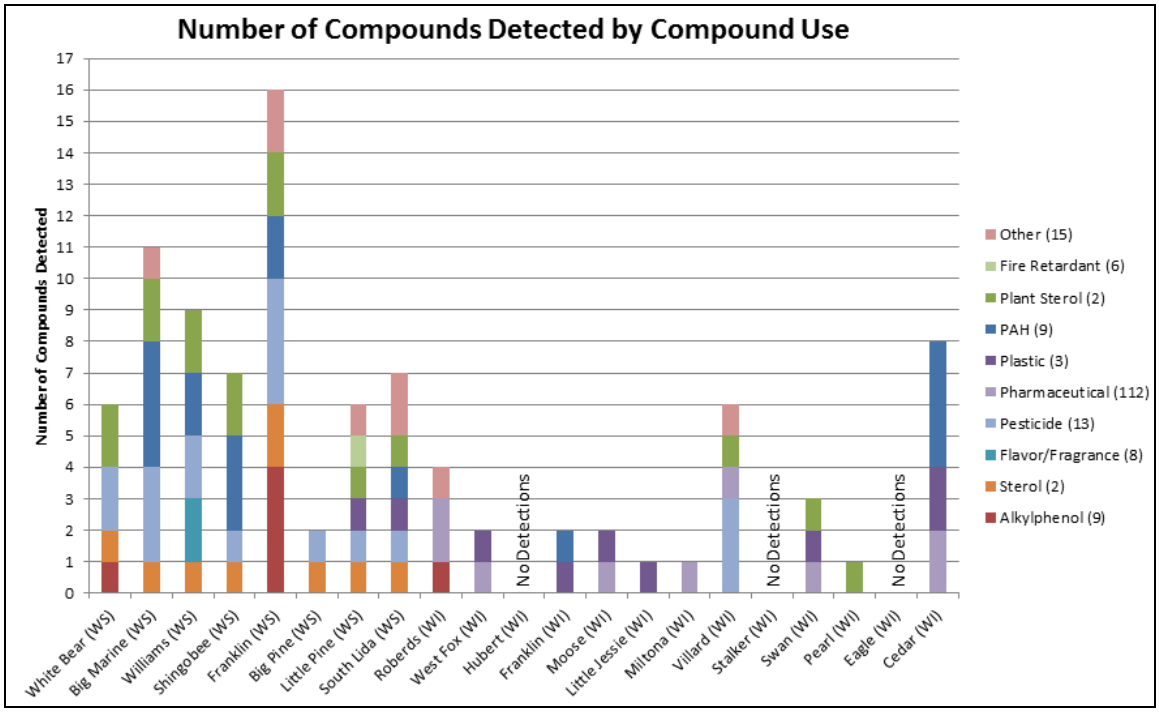

Figure 3. Number of compounds detected within each compound use category. WS, surface water; WI, pore water

Table 1. Waste compound concentrations detected in surface-water and pore-water samples during 2012 and 2013.

Compound (Number of detections)	Average Concentration, ug/L (standard deviation)	Compound Use
Bis(2-ethylhexyl)phthalate (4)	13.7 (23.5)	Plastic
beta-Sitosterol (8)	2.51 (2.61)	Plant sterol
Cholesterol (8)	2.37 (1.84)	Sterol
4-Nonylphenol (1)	1.8	Alkylphenol
Diethyl phthalate (3)	1.47 (1.29)	Plastic
4-Nonylphenol diethoxylate (2)	1.05 (0.919)	Alkylphenol
3-*beta*-Coprostanol (1)	0.900	Sterol
beta-Stigmastanol (7)	0.829 (0.736)	Plant sterol
4-Nonylphenol monoethoxylate (1)	0.48	Alkylphenol
d-Limonene (1)	0.28	Flavor/Fragrance
4-*tert*-Octylphenol diethoxylate (1)	0.2	Alkylphenol
Phenanthrene (1)	0.12	Polycyclic aromatic hydrocarbon
Triclosan (1)	0.11	Antimicrobial
Pentachlorophenol (1)	0.10	Other/Multiple Uses
4-*tert*-Octylphenol monoethoyxlate (1)	0.10	Alkylphenol
Camphor (1)	0.07	Flavor/Fragrance
Atrazine (6)	0.058 (0.019)	Pesticide
DEET (8)	0.057 (0.029)	Pesticide
2-Methylnaphthalene (3)	0.047 (0.063)	Polycyclic aromatic hydrocarbon
Bisphenol A (2)	0.04 (0.014)	Plastic
Caffeine (1)	0.04	Pharmaceutical
Naphthalene (2)	0.035 (0.021)	Polycyclic aromatic hydrocarbon
Triphenyl phosphate (1)	0.03	Fire Retardant
1-Methylnaphthalene (3)	0.027 (0.029)	Polycyclic aromatic hydrocarbon
Metolachlor (3)	0.027 (0.021)	Pesticide
2,6-Dimethylnaphthalene (4)	0.025 (0.017)	Polycyclic aromatic hydrocarbon
Benzophenone (1)	0.02	Other/Multiple Uses
Fluoranthene (1)	0.02	Polycyclic aromatic hydrocarbon
Isopropylbenzene (1)	0.02	Other/Multiple Uses
Isophorone (5)	0.019 (0.010)	Other/Multiple Uses
Pyrene (2)	0.01 (0)	Polycyclic aromatic hydrocarbon
Anthracene (1)	0.01	Polycyclic aromatic hydrocarbon
Carbazole (1)	0.004	Pesticide

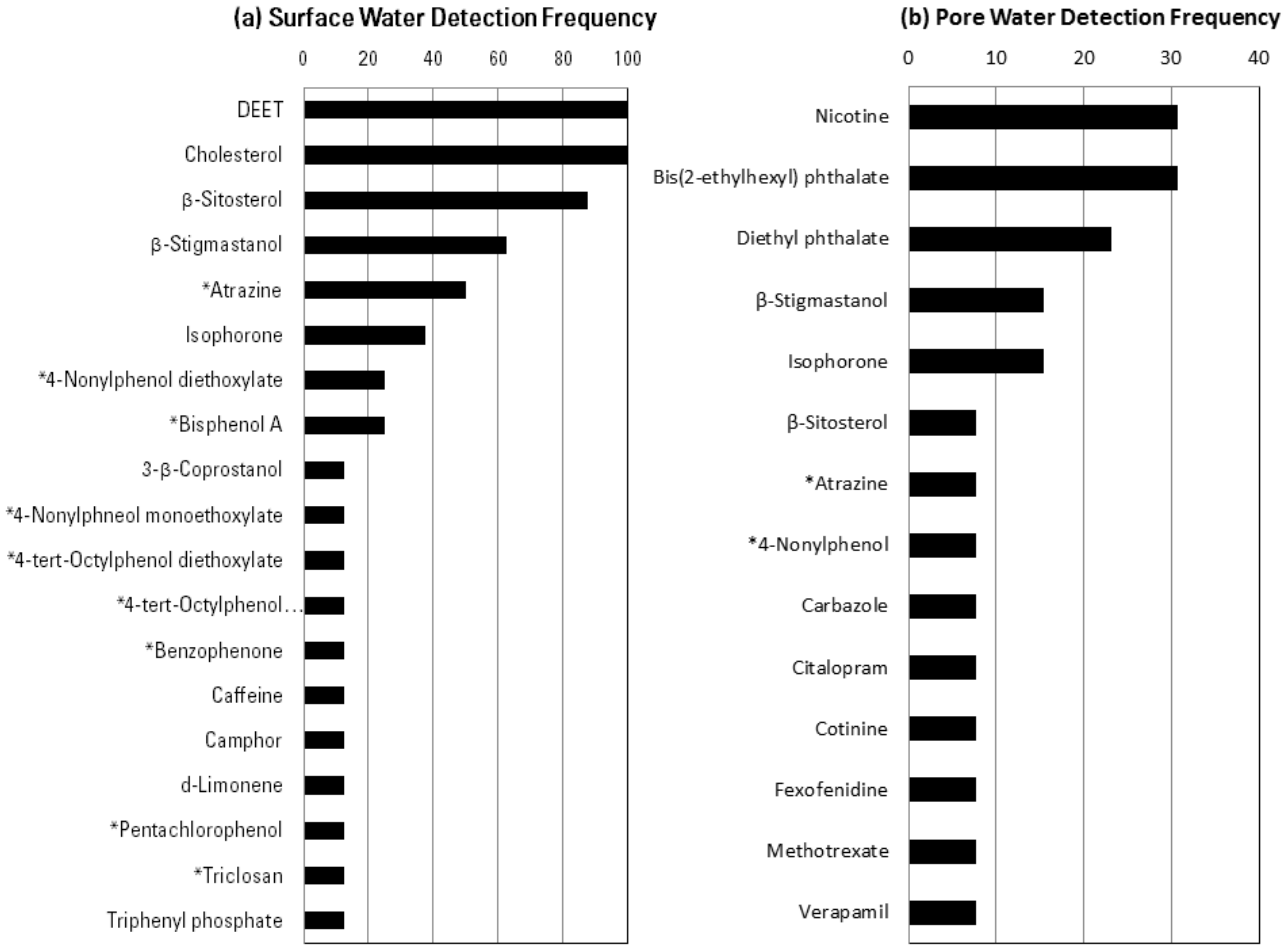

Figure 4. Most frequently detected compounds in (a) surface water and (b) pore water. Polycyclic aromatic hydrocarbons are not included in these graphs because industrial activities are major sources and they are not necessarily indicative of septic waste. *, known or suspected endocrine disruptor

At least one pharmaceutical was detected in 58% of pore-water samples (not analyzed in surface water). Two pharmaceuticals were detected in one lake, the maximum number detected at one lake. Overall, seven different pharmaceuticals were detected in pore water (Table 2) representing a variety of drug classes including a(n) antidepressant, stimulant, antihistamine, antimetabolite, and calcium channel blocker. Concentrations of detected pharmaceuticals ranged from 0.16 to 47 ng/L. Nicotine was the most frequently detected pharmaceutical (detections in one-third of pore-water samples) and had the highest concentrations of detected pharmaceuticals (42 and 47 ng/L in Roberds and West Fox Lakes, respectively). Concentrations detected in this study were generally slightly lower but on the same order of magnitude as average concentrations for nicotine, caffeine, and cotinine reported in a study conducted by Godfrey, et al. (2007).

Table 2. Pharmaceutical concentrations detected in pore-water samples collected from 12 lakes during 2013.

Compound (Number of times detected)	Concentration Range, ng/L
Nicotine (4)	16 – 47
Caffeine (1)	40
Methotrexate (1)	19
Fexofenedine (1)	9.2
Cotinine (1)	5.0
Verapamil (1)	0.74
Citalopram (1)	0.16

To date, three studies have now been completed which investigate the occurrence of CECs in Minnesota lakes: (1) 2008 Statewide Survey (USGS, Writer and others 2010), (2) 2012 National Lake Assessment (Ferrey 2013), and (3) 2012/13 Lake Septic (this study; USGS). Results from all three studies suggest that CECs are generally being detected more frequently in lakes located in Central and South Central Minnesota (Figure 5). In general, this area is characterized by higher road densities, more complete development of shorelines, and more intensive agricultural land use than north-central and north-east parts of the state sampled by the three studies.

The three studies mentioned above had different objectives, different sampling methods, and analyzed slightly different suites of chemicals. However, for general comparison with the current study, the number of detections in each lake was normalized to the number of compounds analyzed for each study. Normalized detection frequencies indicate that, on average, more compounds are detected in near-shore surface water grab samples compared to near-shore pore-water samples or to mid-lake grab samples. Comparisons between results from all three studies suggest that sampling in the near shore zone and using research analytical methods coupled with passive water quality samplers provides the highest rates of compound detection.

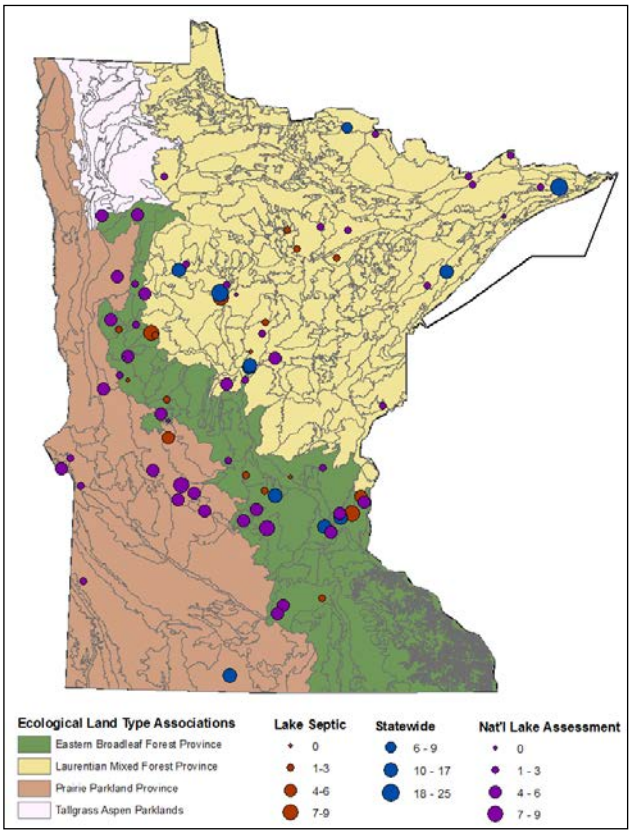

Figure 5. Map showing detection frequency of contaminants of emerging concern in Minnesota lakes sampled during three different studies throughout the period of 2008-2013. The number of compounds detected in each lake was normalized to the number of compounds analyzed for inter-study comparison.

Results from the current study suggest that the detection of CECs is dependent on the type of environmental media being sampled. Generally, compounds detected in pore-water samples were more indicative of waste compounds and therefore consistent with septic system influence. Compounds detected in surface water can be associated with a broader range of contaminant sources, including septic systems. Pharmaceutical concentrations were similar to those reported by others; however literature regarding the effects on aquatic biota is somewhat limited and tends to focus on specific drug classes (e.g. antidepressants). In lakes receiving shallow groundwater, analysis of both near-shore surface water and pore water would provide more information regarding the presence and sources of emerging contaminants.

Godfrey, E., Woessner, W.W., and Benotti, M.J.,2007, Pharmaceuticals in on-site sewage effluent and ground water, Western Montana, *Groundwater,* 45, 3, 263-71.

Ferrey, M. 2013. Pharmaceuticals and Endocrine Active Chemicals in Minnesota Lakes. Minnesota Pollution Control Agency Document number: tdr-g1-16

Writer, J. H., Brown, G. K., Taylor, H. E., Schoenfuss, H. L., Jahns, N. D., Bartell, S. E., Kiesling, R.L., Ferrey, M.L., and Barber, L.B. 2010. Anthropogenic Tracers, Endocrine Active Chemicals, and Endocrine Disruption in Minnesota Lakes not Impacted by Point-Source Discharges. Science of the Total Environment 409:100-111. (doi.org/10.1016/j.scitotenv.2010.07.018)

RESULT/ACTIVITY 2: Assess the hydrology and ecology of the watershed contributing to water or sediment concentrations of estrogenic/pharmaceutical compounds in the surveyed lakes.

Description:
Each lake sampled under Objective 1 will be classified by geologic, hydrologic, and ecological characteristics. Groundwater hydrology, including depth to groundwater, and groundwater contribution to lake water balance (determined using $\delta^{18}O$ and $\delta^{2}H$ isotopes) will be coupled with surface water hydrology (contributing area, morphometry) and watershed characteristics (land use, land cover; fragmentation) to develop categorical classifications for each lake. Groundwater level data will be collected for each lake in the vicinity of sampling sites, and seasonal groundwater isotope sampling in each lake will provide an estimate of groundwater contribution to water balance.

Summary Budget Information for Result/Activity 2:	**ENRTF Budget:**	**$120,000**
	Amount Spent:	**$120,000**
	Balance:	**$0**

Deliverable/Outcome	Completion Date	Budget
1.Survey of 10 lakes	11/30/2010	$48,000
2.Survey of 10 additional lakes	6/30/2013	$48,000
3. Data report on groundwater characteristics	12/31/2013	$24,000

Result Completion Date: 12/31/2013

Result Status as of: 12/31/2010

Each sampled lake is being evaluated for groundwater setting using published reports and available GIS data sets. Data from published studies including the Minnesota county geologic atlases for the lake regions are being compiled and entered into a geospatial database for lake water balance evaluation. Groundwater well database records in the vicinity of sampled lakes or candidate lakes are being collated and entered into a geo-spatial database.

Result Status as of: 12/31/2011

Five lakes were sampled for groundwater characteristics including depth to groundwater and groundwater contribution to lake water balance using $\delta^{18}O$ and $\delta^{2}H$ isotopes. Data from published studies including the Minnesota county geologic atlases for the lake regions were compiled from published sources and entered into a geospatial database for lake water balance evaluation. Groundwater well database records in the vicinity of sampled lakes or candidate lakes are being collated and entered into a geo-spatial database.

Result Status as of: 6/30/2012

Thirty-five lakes have been evaluated for groundwater characteristics including depth to groundwater and groundwater contribution to lake water balance using $\delta^{18}O$ and $\delta^{2}H$ isotopes where available from the literature. Data from published studies including the Minnesota county geologic atlases for the lake regions were used to make the assessments. Groundwater well database records in the vicinity of sampled lakes were analyzed to estimate groundwater gradients around candidate lakes.

Result Status as of: **12/31/2012**

Data from published studies including the Minnesota county geologic atlases for the lake regions were used to develop a draft standard assessment protocol for specific lake regions. The standard assessment protocol includes the use of the groundwater well database records in the vicinity of candidate lake regions lakes where groundwater is known to contribute to the water budget of ponded surface water features like lakes and wetlands.

Result Status as of: 6/30/2013

Groundwater contributions to samples were evaluated at each sampling location using mini-piezometer sampling of interstitial water (IW) from lake sediments in the near-shore zone. IW samples were pumped from mini-piezometers and screened for groundwater (GW) influence using temperature and specific conductance field data. IW samples that met screening criteria were analyzed for major ions to confirm GW influence.

Result Status as of: 12/31/2013

Field screening of candidate lakes continued through November of 2013. Of the 35 candidate lakes identified using published sources, a total of 28 lakes were screened in the field using the mini-piezometer sampling methods. Of these 28 lakes, 20 were sampled for chemical evaluation. Data sources for watershed characteristics were reviewed and information compiled into a single database for analysis by lake region.

Final Report Summary: 6/30/2014

Each sampled lake was evaluated for groundwater setting using published reports and available GIS data sets. Minnesota County Geologic Atlases and U.S. Geological Survey Hydrologic Atlases provided various data such as water table elevations around lakes, depth to groundwater, general direction of groundwater movement, and stable isotope data providing evidence of groundwater/surface water interaction. The County Well Index was used to obtain static groundwater levels to estimate groundwater gradients around select lakes. Using these parameters to select sampling locations proved to be more than seventy-percent successful. Groundwater influence was evaluated at each sampling location using mini-piezometer sampling of interstitial water from lake sediments in the near-shore zone. Interstitial water was pumped up through the mini-piezometer and screened for groundwater influence using temperature, dissolved oxygen and specific conductance field data. Upon confirmation of qualities indicative of groundwater, interstitial water samples were collected and analyzed for major ions to confirm groundwater influence.

Lake and watershed characteristics were obtained from Minnesota Department of Natural Resources (Table 3) and used to categorize sampled lakes to investigate patterns in CEC detections. Overall, the highest frequency of detections was in near-shore surface water, and the most important factor contributing to the pattern of detections was the influence of a shallow water table (Table 4).

Table 4. Results of correlations between the proportion of compounds that were not detected in the study and a number of watershed and lake characteristics.

Correlation Coeffiencts [r] between Non-detections of CECs and Watershed Characteristics					
	Littoral Area	Direct Contributing Area:Lake Area	Watershed Area:Lake Area	Depth to Water Table	Area
Frequency of Pore-water Non-detections	0.04	0.36	-0.48	0.42	0.02
Frequency of Near-shore Surface Water Non-detections	0.54	0.03	0.38	0.60	0.59

Table 3. Lake and watershed characteristics of 20 sampled lakes during 2012 and 2013. NCHF, North Central Hardwood Forests; NLF, Northern Lakes and Forests

Lake Name	Ecoregion	Area, acres	Littoral Area, acres	Direct Contributing Area:Lake Area	Watershed Area:Lake Area	Depth to Water Table*, feet	Topography	Moraine Type	Sedimentary Association
Big Marine	NCHF	1799	1152	4	4	6	Undifferentiated	Outwash	Outwash
Big Pine	NCHF	4726	2386	3	76	31	Rolling	Outwash	Outwash
Cedar	NCHF	783	315	5	11	13	Rolling	End Moraine	Outwash
Eagle	NCHF	463	330	11	11	14	Level	Outwash	Outwash
Franklin	NCHF	1088	749	6	6	5	Steep	Stagnation moraine	Supraglacial drift complex
Hubert	NLF	1288	465	2	3	16	Steep	Outwash	Lacustrine
Little Jessie	NLF	626	206	3	3	8	Hummocky	Outwash	Supraglacial drift complex
Little Pine	NCHF	2080	698	2	120	15	Level	Outwash	Outwash
Miltona	NCHF	5724	2802	2	8	14	Rolling	Stagnation moraine	Outwash
Moose	NLF	1274	345	2	9	18	Steep	Ground moraine	Outwash
Pearl	NCHF	753	511	8	22	11	Level	Outwash	Outwash
Roberds	NCHF	632	393	8	15	--	Hummocky	Ground moraine	Supraglacial drift complex
Shingobee	NLF	160	43	61	61	12	Rolling	Outwash	Outwash
South Lida	NCHF	775	356	2	4	--	Steep	Stagnation moraine	Supraglacial drift complex
Stalker	NCHF	1357	575	4	27	23	Steep	Stagnation moraine	Ice contact
Swan	NLF	2467	507	4	26	21	Rolling	End moraine	Till plain
Villard	NCHF	544	541	2	45	2	Rolling	Outwash	Supraglacial drift complex
West Fox	NLF	450	138	3	17	14	Steep	End moraine	Ice contact
White Bear	NCHF	2428	1314	3	3	13	Undifferentiated	End moraine	Undifferentiated
Williams	NLF	102	43	61	61	25	Steep	Outwash	Ice contact

* Depth to water table was calculated by averaging values provided in County Well Index for wells surrounding the immediate sample area.

RESULT/ACTIVITY 3: Assess biological exposure and response to known estrogenic and pharmaceutical compound contamination in Minnesota lakes.

Description:

A subset of the 24 lakes sampled under Objective 1 will be chosen for detailed biological analysis based on the severity of contamination. Adult bluegill sunfish will be sampled during the spring reproductive period from active nesting areas in each lake. Nesting areas will be associated with developed and undeveloped shorelines. Adult male and female fish will be evaluated using a variety of biomarkers, including condition factors, blood-chemistry (e.g., plasma vitellogenin) and histo-pathological indices of abnormal gonad development (e.g., intersex). Nesting sites will be evaluated for selected Phac and EAC residues in food-web components at the time of active reproduction. Samples from major trophic levels including adult bluegill will also be collected for stable isotope analysis to determine bluegill food web structure during the nesting period.

Summary Budget Information for Result/Activity 3:	**ENRTF Budget:**	**$117,000**
	Amount Spent:	**$117,000**
	Balance:	**$0**

Deliverable/Outcome	Completion Date	Budget
1.Tag adult fish in spawn areas	9/30/2013	$24,000
2.Sample fish in target lakes	9/30/2013	$66,000
3. Collect samples for food-web analysis	6/30/2013	$10,000
3. Data report on lakes	12/31/2013	$17,000

Result Completion Date: 6/30/2014

Result Status as of: 12/31/2010

No activity under task 3.

Result Status as of: 6/30/2011

No activity under task 3.

Result Status as of: 12/31/2011

A list of candidate lakes has been developed and preliminary surveys of bluegill spawning sites in five candidate lakes were completed. Bluegill spawning assessment methods were tested in controlled conditions at the USGS fish culture facility in Onalaska, WI, as part of the partner project funded by the ENRTF. Methods for total egg counts and fry counts as measures of reproductive success were evaluated as part of this ongoing research at the facility.

Result Status as of: 6/30/2012

Four candidate lakes were assessed during the bluegill spawning period of May through mid-June. Surveys of bluegill spawning sites found appropriate sampling locations in all four lakes, but active spawning fish were sampled in only two of the lakes. Male bluegills were sampled off of the nests they were guarding, and eggs were immediately sampled from the nests. Problems were encountered with quantitative sampling of eggs from the nests given uneven substrate size and composition. Additional sampling methods are under development for sampling in the spring of 2013. All fish are being analyzed at the laboratory of Dr. Heiko Schoenfuss, Saint Cloud State University.

Result Status as of: 12/31/2012

Histopathological analysis of fish tissue is in progress. Analysis is projected to be completed in early 2013. Biological results will be compared to chemical data when analysis of fish tissues has been completed. Chemical data from the four active bluegill spawning sites did not show significant trends between septic and non-septic zones. Additional sampling is planned for April and May of 2013.

Result Status as of: 6/30/2013

Fourteen male bluegills were sampled from Sullivan Lake. Fish were collected directly off the nests they were guarding and processed for histopathological analysis at St. Cloud State University. A new method was employed for collection of eggs off the nest using a peristaltic pump. The method worked well; fry were observed swimming in several of the samples. Egg samples were preserved for quantification and analysis of viability.

Result Status as of: 12/31/2013

Nine male bluegills were sampled from Lake Franklin. Fish and eggs were collected with identical methods identified above. Histopathological analysis of all fish collected during 2013 was completed by St. Cloud State University. Biological data will be compared to chemical data from the respective lakes.

Final Report Summary: 6/30/2014

Analysis of male bluegill sunfish captured while guarding nest sites in near-shore sites with suspected groundwater inputs exhibited a range of biological responses consistent with exposure to emerging contaminants. In two lakes, male sunfish exhibited plasma vitellogenin (a precursor protein for egg yolks typically present in females that is also an indicator of recent exposure to estrogenic compounds) concentrations 10-15 times greater than in sunfish collected from the other two lakes (Figure 6) and approaching concentrations more commonly measured in female sunfish.

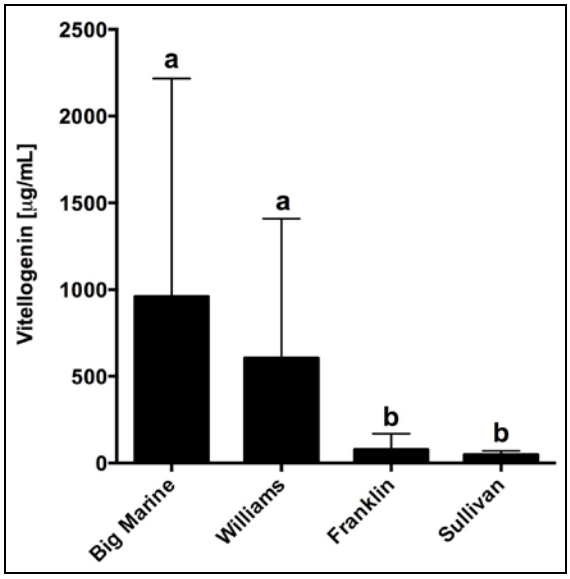

Figure 6. Plasma vitellogenin concentrations (mean and standard error) in male sunfish captured while guarding nest sites in near-shore sites with suspected groundwater inputs. Letters indicate significant differences between lakes ($p<0.05$; ANOVA with Tukey's multiple comparison test).

Interestingly, elevated plasma vitellogenin concentrations were not predictive of reproductive abnormalities such as intersex, a condition in which male fish become hermaphroditic as a consequence of exposure to estrogenic compounds. Intersex males were only found in Sullivan Lake where fish did not exhibit elevated plasma vitellogenin concentrations. The disconnect between plasma vitellogenin concentrations and presence of intersex suggests adverse impacts of non-estrogenic emerging contaminants on fish in Sullivan Lake. When the totality of pathologies (macroscopic and microscopic pathologies, parasite presence) are considered, sunfish from Sullivan Lake were found to have not only the highest rate of intersex, but also the highest rate of pathological occurrences (Figure 7B). Sullivan and Williams Lakes also contained male sunfish with lower body condition factors (Figure 7A), indicating that the fish were generally less healthy compared to the fish collected from Big Marine and Franklin lakes. Additionally, in the case of Sullivan Lake, greater relative testis size (Figure 7E) and a greater abundance of mature sperm (Figure 7F) were observed.

When comparing data generated for the current study with a previous assessment of fish health in Minnesota Lakes (MN Statewide Study 2008), the importance of sampling fish in habitats influenced by groundwater inputs likely containing emerging contaminants becomes apparent. Indicators of adverse health effects were generally much higher in fish collected in these near-shore habitats in the current study then in the 2008 study where fish were collected across the entire lake (Figure 7). General pathologies (Figure 7B) were more common in mature male sunfish collected in near-shore environments likely impacted by groundwater containing emerging contaminants than in fish collected across entire lakes in 2008. Similarly, hepatocyte vacuolization, an indicator of contaminant stress experienced by the liver was higher in near-shore male sunfish when compared to 2008 lake-wide collected fish (Figure 7D). The greater abundance of indicators of adverse biological impact suggests that a lake-wide sampling of fish will underestimate the impact of contaminant exposure to fish during reproductively important life stages. Only adverse effects observed in urban runoff lakes

in 2008 came close to adverse effects observed during the current study. As urban runoff provides an untreated inflow of many contaminants of emerging concern into lakes, it may not be surprising that its' effects rival those observed in some near-shore environments in the current study. It remains to be seen whether the observed, pronounced effects in mature male sunfish guarding nest sites translate into adverse health effects in developing embryos and larvae in direct contact with sediment and pore water assumed to carry septic discharge with groundwater.

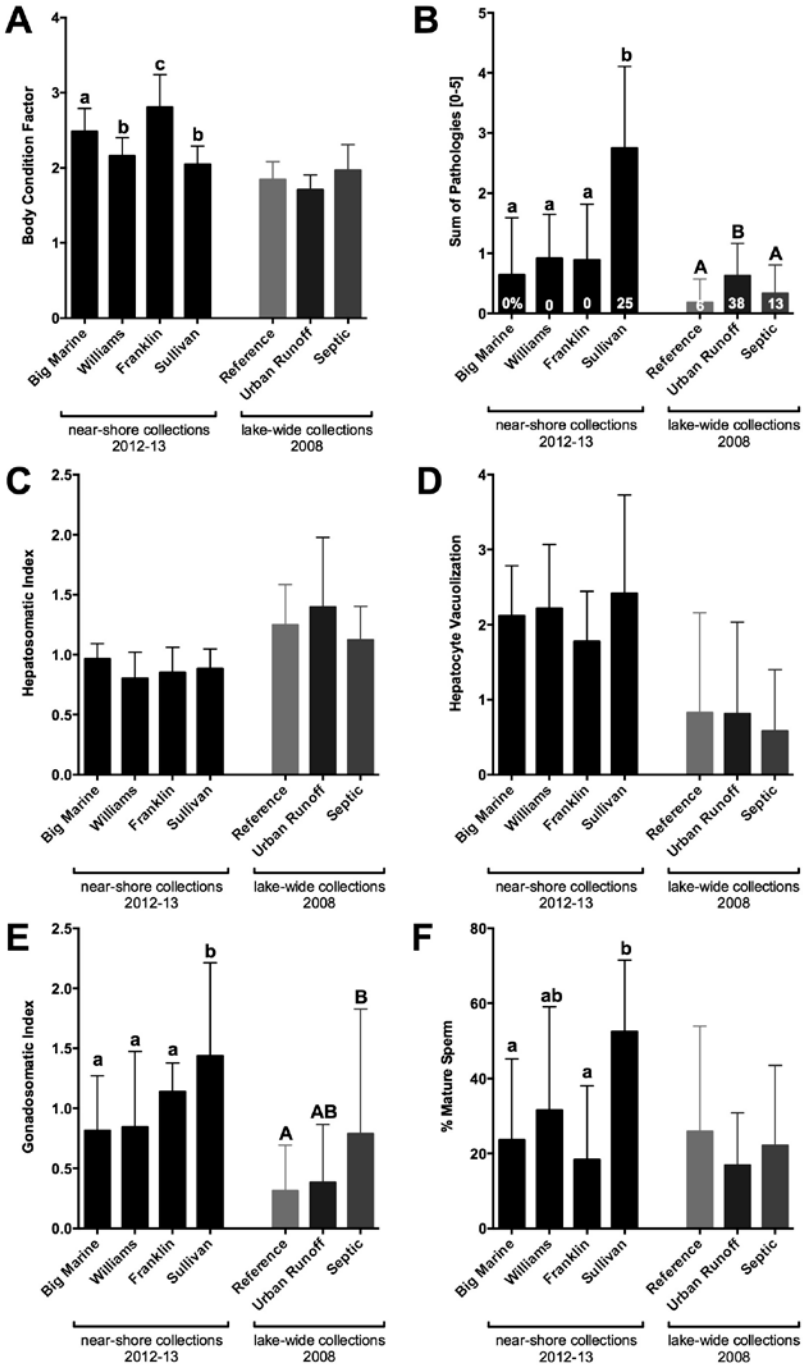

Figure 7. Biological indicators in sunfish collected for the current study in four Minnesota Lakes and comparison with a previous study (2008). For the 2008 study, lakes were grouped based on their primary input of contaminanats of emerging concern as either reference lakes (Elk), urban runoff lakes (Cedar,

Owasso, Budd), or septic lakes (Shingobee, Sullivan, White Sand).Letters indicate significant differences between lakes (P<0.05; ANOVA with Tukey's multiple comparison test).

RESULT/ACTIVITY 4: Enhance EAC analytical capabilities at the Minnesota Department of Health (MDH).

Description:

MDH currently has the capability to quantify a number of Phacs and organic compounds including the EACs bisphenol A, nonyl phenol (NP), and octyl phenol (OP). As part of this project, MDH will enhance its existing Phacs and EAC methods while implementing advanced laboratory techniques to quantify NP precursors. All MDH Phac and EAC analytical capabilities will be used to analyze water samples from the 20 Minnesota lakes in this study.

Summary Budget Information for Result/Activity 4:	**ENRTF Budget:**	**$110,000**
	Amount Spent:	**$110,000**
	Balance:	**$0**

Deliverable/Outcome	**Completion Date**	**Budget**
1.Purchase of triple quadrupole mass spectrometer	9/30/2010	$110,000

Result Completion Date: 12/31/2011

Result Status as of: 12/31/2010

MDH completed the product review process and selected an Agilent 7000 Quadrupole MS/MS with an Agilent 7890 Gas Chromatograph as the appropriate system. The order for the machine will be placed in early January. The instrument should be in-house within the next quarter and should be available to analyze samples collected during the upcoming field season.

Result Status as of: 6/30/2011

MDH has received the Agilent 7000 Quadrupole MS/MS with an Agilent 7890 Gas Chromatograph and placed it in service in their research lab. Methods transfer is ongoing. The machine will be ready for sample analysis by December of 2011.

Result Status as of: 12/30/2011

MDH has run diagnostics on the Agilent 7000 Quadrupole MS/MS with an Agilent 7890 Gas Chromatograph and placed it in service in their research lab. Methods transfer is complete. The machine is ready for sample analysis by January 2012.

Result Status as of: 6/30/2012

MDH has methods running on the Agilent 7000 Quadrupole MS/MS with an Agilent 7890 Gas Chromatograph The machine has analyzed samples since January 2012.

Result Status as of: 12/31/2012

MDH continues to run methods on the Agilent 7000 Quadrupole MS/MS with an Agilent 7890 Gas Chromatograph The machine has analyzed samples since January 2012.

Result Status as of: 6/30/2013

MDH has methods running on the Agilent 7000 Quadrupole MS/MS with an Agilent 7890 Gas Chromatograph The machine has analyzed samples since January 2012.

Result Status as of: 12/31/2013

MDH continues to run methods on the Agilent 7000 Quadrupole MS/MS with an Agilent 7890 Gas Chromatograph The machine has analyzed samples since January 2012.

Final Report Summary: 6/30/2014

MDH purchased an Agilent 7000 Quadrupole MS/MS with an Agilent 7890 Gas Chromatograph for their research laboratory. Diagnostics were run on the machine and methods transfers were completed. MDH has analyzed samples with the new machine since January 2012.

V. TOTAL ENRTF PROJECT BUDGET: $594,000

Personnel: $ 235,400; $198,400 to USGS and $40,000 to Saint Cloud State University

Capital Equipment: $110,000 to MDH to aid in the purchase of a Triple quadrupole mass spectrometer for the Organic Research Public Health Laboratory

Supplies: $ 26,000

Travel: $ 24,600

Additional Budget Items: $ 198,000 (laboratory analytical costs at USGS analytical labs)

TOTAL ENRTF PROJECT BUDGET: $594,000

Explanation of Capital Expenditures Greater Than $3,500:

Triple quadrupole mass spectrometer for MDH research lab to use in method application to analyze for the compounds of interest in this study - $110,000.

This piece of equipment will be will remain in service at MDH and will continue to analyze environmental samples for endocrine disruptors and pharmaceuticals after the end of the project.

VI. PROJECT STRATEGY:

A. Project Partners: This project is a continuing partnership between the United States Geological Survey (USGS) and the State of Minnesota to document the occurrence and effects of emerging contaminants in lakes. Team members from the USGS include Dr. Richard Kiesling (project Leader), Sarah Elliott (hydrologist), Kathy Lee (project co-leader; USGS Biologist), and Dr. Mindy Erickson (USGS Groundwater Specialist). USGS will manage project planning, sampling, and sample analysis. Dr. Heiko L. Schoenfuss, Professor and Director of the Aquatic Toxicology Laboratory, Department of Biological Sciences, St. Cloud State University, will participate in this project by performing histo-pathology analysis of fish tissues and data analysis and interpretation. Minnesota Department of Health laboratory team members include Paul Swedenborg, (MDH organics lab supervisor) and Dr. Carin Husset (MDH organics lab research scientist). All team members will participate in writing the final report and communicating results to state user groups.

B. Project Impact and Long-term Strategy: In Minnesota rivers, endocrine disruption has been observed in short- and long-lived fish species including vitellogenin induction in male fathead minnows, male carp, and walleye (Folmar and others, 1996, 2001; Lee and others, 2000; 2004). Vitellogenin in male carp was also observed at numerous sites downstream of WWTP discharges throughout central Minnesota (Lee and others, 2000). Two ongoing studies in Minnesota have recently identified additional fish species affected by EACs in tributaries of the Mississippi and the St. Croix Rivers (Jahns and others in prep; Lee and others in review) as well as urban lakes (Schoenfuss and others – unpublished data). Taken as a whole, these results indicate that Minnesota fish communities are vulnerable to reproductive impacts from EACs. The proposed study helps answer how vulnerable a common fish species is to EAC exposure during spawning in lakes across Minnesota. The study takes advantage of the specific life-history characteristics of bluegill sunfish to investigate the impact of EAC exposure on their spawning activity and reproductive output. The anticipated outcomes of the study will provide the following benefits:

1. Most EACs are found at very low concentrations in water but reach higher concentrations in sediment. Despite these low concentrations, research has identified developmental and reproductive effects on fish species at environmentally relevant concentrations. The proposed work will determine if lakes under the influence of septic systems are at risk for significant contamination from Phacs and EACs while providing details on what pharmaceutical and estrogenic compounds are present in lakes with high numbers of SSTS systems.

2. In Minnesota, pharmaceuticals and EACs have been observed in a range of lake types from a diverse set of background conditions. This study will provide a comprehensive analysis of the frequency and magnitude of contamination relative to

important factors including hydrology and geology, groundwater hydrology and watershed characteristics.

3. Three recent studies in Minnesota indicate river and lake fish communities are vulnerable to reproductive impacts from EACs. The proposed project uses a representative fish (bluegill) to determine how vulnerable adult fish are to pharmaceutical and estrogenic compounds exposure during spawning.

C. Other Funds Proposed to be spent during the Project Period:

Additional funds to be spent on the project include $215,000 of USGS Cooperative Research funding and $110,000 in equipment funding match for purchase of equipment by the MDH. MDH will also provide $153,900 of in-kind staff services during the course of the three year project.

D. Spending HIstory:

See Attachment A fiscal report.

VII. DISSEMINATION: Details of results will be available as a final project report to LCCMR in the form of a USGS scientific investigations series report and a scientific journal article manuscript. Results will be communicated to local groups, state agencies and national peer groups through presentations at regional and national meetings including state resource management meetings.

VIII. REPORTING REQUIREMENTS: Periodic work program progress reports will be submitted every six months by Result according to the schedules provided in Section IV above. A final work program report and associated products will be submitted between June 30 and August 1, 2014 as requested by the LCCMR.

IX. RESEARCH PROJECTS: see research addendum.

Attachment A 31 Final Report for 2010 Project 10-A2														
Attachment A: Budget Detail for 2010 Project 10-A2														
Project Title:	**Estrogenic and Pharmaceutical Septic System Discharge to Lakes**													
Project Manager Name:	**Richard Kiesling**													
Trust Fund Appropriation for Pro	**$594,000**													
2010 Trust Fund Budget	**Result 1 Budget:**	**Amount Spent (6/30/2014)**	**Balance (6/30/2014)**	**Result 2 Budget:**	**Amount Spent (6/30/2014)**	**Balance (6/30/2014)**	**Result 3 Budget:**	**Amount Spent (6/30/2014)**	**Balance (6/30/2014)**	**Result 4 Budget:**	**Amount Spent (6/30/2014)**	**Balance (6/30/2014)**	**TOTAL BUDGET**	**TOTAL BALANCE**
	Quantify the occurrence of estrogenic/pharmaceutical compounds in 30 Minnesota lakes that receive groundwater inputs from septic systems			*Assess surface water/groundwater hydrology/watershed characteristics contributing to water/sediment concentrations of estrogenic/pharmaceutical compounds in lakes*			*Assess biological exposure and response to known estrogenic/pharmaceutical compound contamination in Minnesota lakes*			*Enhance EAC analytical capabilities at the Minnesota Department of Health (MDH).*				
BUDGET ITEM														
PERSONNEL: wages and benefits *(List individual names, amount budgeted and %FTE; add rows as needed)*														
USGS Hydrologist: 0.15 FTE per year for three years; USGS Biologist: 0.12 FTE per year for three years; USGS Hydrology Tech: 0.35 FTE per year for three years; USGS Biology Tech: 0.35 FTE per year for two years	$85,400	$85,400	$0	$55,000	$55,000	$0	$55,000	$55,000	$0				$195,400	$0
Saint Cloud State University (SCSU) GRA: 0.5 FTE per year for one year	$10,000	$10,000	$0				$12,000	$12,000	$0				$22,000	$0
SCSU Full Professor eligible for summer salary: 3 months summer salary for one year	$9,000	$9,000	$0				$9,000	$9,000	$0				$18,000	$0
Additional Budget Items: USGS analytical costs for water, biota, and sediment samples	$118,000	$118,000	$0	$50,000	$50,000	$0	$30,000	$30,000	$0				$198,000	$0
Capital equipment over $3,500 *(list specific items)*														
MN Department of Health - Triple quadrupole mass spectrometer for the Public Health Laboratory, Minnesota Department of Health										$110,000	$110,000	$0	$110,000	$0
Supplies *(list specific categories)*														
Supplies for sample collection, storage, and	$10,000	$10,000	$0	$10,000	$10,000	$0	$6,000	$6,000	$0				$26,000	$0
									$0					
Travel expenses in Minnesota	$14,600	$14,600	$0	$5,000	$5,000	$0	$5,000	$5,000	$0				$24,600	$0
Travel outside Minnesota														
COLUMN TOTAL	**$247,000**	**$247,000**	**$0**	**$120,000**	**$120,000**	**$0**	**$117,000**	**$117,000**	**$0**	**$110,000**	**$110,000**	**$0**	**$594,000**	**$0**