

## Minnesota Pollution Control Agency

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# Statewide Endocrine Disrupting Compound Monitoring Study, 2007 - 2008



Minnesota Pollution Control Agency

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## Acknowledgements

This report contains the results of a state-wide study (Study) on the presence and effects of endocrine disrupting compounds, pharmaceuticals, pesticides, and other contaminants normally associated with wastewater in Minnesota's lakes and rivers. The study was completed through a cooperative effort between the Minnesota Pollution Control Agency (MPCA), St. Cloud State University, and the U.S. Geological Survey.

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## Glossary of Terms and Abbreviations

EDC	Endocrine Disrupting Compound
Estrogenic	A chemical that binds to estrogen receptors and elicits a response similar to natural estrogen hormones.
Reference Lake	A lake without surrounding development to which other lakes in this study was compared.
ISTS	individual sewage treatment system or septic system.
OWC	Organic wastewater compounds. Chemicals normally associated with wastewater.
SSRI	Selective serotonin reuptake inhibitor; antidepressant drugs
VTG	Vitellogenin; the protein normally found in female fish associated with egg development.
WWTP	Wastewater Treatment Plant

### Units

One part per million:

ppm  
mg/Kg  
mg/L  
µg/g

One part per billion:

ppb  
µg/Kg  
ng/g  
µg/L

One part per trillion:

ppt  
ng/Kg  
ng/L

# Executive Summary

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It is well established that some chemicals can mimic the effects of hormones in animals and cause adverse physiologic effects such as changes to the reproductive system or to the growth and development of an organism. These *endocrine disrupting compounds*, or EDCs, do not usually exhibit acute toxicity at the levels normally found in the environment, but instead can alter the normal functioning and growth of the exposed organism at very low concentrations.

Previous investigations of the Mississippi River and its tributaries have shown that EDCs are widespread at low concentrations in rivers. The studies also demonstrated that fish in these waters show signs of endocrine disruption, such as the feminization of male fish. Most of these investigations focused on locations near wastewater treatment plants (WWTPs). Much less is known, however, about the presence of EDCs and their effects in Minnesota's lakes.

In this study, commissioned by Minnesota lawmakers in the 2007 Legislative Session, twelve lakes and four rivers in Minnesota were sampled for the presence and concentrations of a diverse group of EDCs and other chemicals in surface water and sediment. In addition, caged and wild-caught fish from these bodies of water were examined for evidence of endocrine disruption and environmental stress. The lakes and rivers were selected to represent a wide range of land use and development. Two lakes without development were selected as reference lakes. Three rivers in south-central Minnesota and one in western Minnesota were also included in the study.

Water and sediment samples from each lake or river were analyzed for 110 chemicals, including many known or suspected EDCs including pharmaceuticals, hormones, pesticides, personal care products, and compounds commonly associated with wastewater contamination. Several species of wild fish collected from each location were assessed for the induction of the protein vitellogenin (VTG) in male fish (VTG is the protein associated with reproduction in female fish and an indicator of feminization in male fish). These fish also were assessed for changes in body, liver, and reproductive organ weight, and histological abnormalities (changes in the abundance of cell types in the liver and reproductive organs). Caged minnows that were deployed at each location for three weeks were also analyzed with the same suite of endpoints for evidence of endocrine disruption.

Several contaminants, including hormones and pharmaceuticals, were detected in many of the lakes and rivers sampled for this study. The most frequently detected compounds in water were:

- The hormones androstenedione (64 percent of sampled lakes, 50 percent of sampled rivers), estrone (55 percent of the lakes, 75 percent of the rivers), and 17 $\beta$ -estradiol (55 percent of the lakes, 38 percent of the rivers). These may be of human origin, naturally occurring, or both.
- Bisphenol-A (the chemical used to manufacture polycarbonate) (45 percent of the lakes, 38 percent of the rivers).

For sediment, the most frequently detected compounds were:

- Bisphenol-A (82 percent of the lakes, 57 percent of the rivers)
- Acetaminophen (a common pain medication) (50 percent of all samples)
- 4-octylphenol diethoxylate (an ingredient in some detergents) (36 percent of the lakes, 71 percent of the rivers)
- Carbamazepine (a medication used to treat ADHD) (36 percent of all samples)
- 4-(tert)octylphenol (an ingredient in detergents) (45 percent of the lakes, 14 percent of the rivers)
- Triclosan (a household antibacterial agent) (18 percent of the lakes, 57 percent of the rivers)



In reference lakes (“pristine” lakes without any surrounding development to which other lakes in this study were compared), N-N, diethyl-m-toluamide (commonly known as the insecticide DEET), nonylphenol and nonylphenolethoxylates, octylphenolethoxylates (ingredients in detergents), bisphenol-A, and the hormones 17- $\beta$ -estradiol, and estrone were all detected in the water. Reference lake sediment contained 4-(tert) octylphenol, bisphenol-A, acetaminophen, and carbamazepine. The detection of several of these compounds in these waters was not anticipated given the remote locations of the lakes.

Concentrations of contaminants in sediments appear to be much higher than the lake water at the same locations. This suggests that these chemicals are accumulating over time in the lake sediment. More study is needed to determine how persistent these chemicals are in sediment and to understand the impact of this accumulation to aquatic ecosystems.

Vitellogenin was found in male fish collected from several of the lakes and rivers, including fish collected from the two reference lakes. This indicates likely exposure of the fish to estrogenic compounds in their environment. No correlation between land use or detected chemicals and VTG in wild male fish emerged from these data. Caged fathead minnows from urban lakes had higher liver fat-cell counts and reduced liver size (indications of contaminant stress and not necessarily indications of endocrine disruption). Caged fathead minnows exhibited elevated VTG levels, which is an indication of exposure to endocrine disrupting compounds. Urban lakes were associated with the highest occurrence of intersex (evidence of testicular feminization) in wild fish.

This study shows that endocrine disrupting chemicals that include pharmaceuticals and other contaminants typically associated with wastewater are present in the surface water and sediment of Minnesota lakes not receiving effluent from wastewater treatment plants. It also shows that fish in lakes and rivers are being exposed to estrogenic chemicals in their environment, although it is unclear which chemicals caused these effects in wild fish collected in this investigation. The results of this investigation are consistent with previous studies done on the Mississippi River and its tributaries, where many of the same contaminants were found in the surface water and sediment and fish were found to exhibit similar forms of endocrine disruption.

It is not known how long these contaminants persist in the aquatic environment or how rapidly they break down. Although the results of this study suggest that they are accumulating in lake sediment, further study is needed to understand the overall fate of these chemicals in lakes and rivers.

## Introduction

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In the last decade, national and statewide studies have revealed that many pharmaceuticals, personal care products, chemicals associated with wastewater effluent, and a variety of industrial compounds with known or suggested endocrine disrupting potential are found in the aquatic environment [1, 2]. Apart from the disquieting realization that wastewater chemicals and drugs are detectable in much of our surface water, there is a growing concern that even at low concentrations, chemicals, or mixtures of them, may adversely affect fish, wildlife, ecosystems, and possibly human health. Our collective understanding of the extent and impact of these chemicals on our aquatic environment is still quite limited.

## What is an endocrine disrupting chemical?

Endocrine disrupting chemicals (EDCs) do not usually exhibit acute toxicity at concentrations typically found in the environment. Instead, EDCs are hormonally active at very low, non-toxic concentrations, and may alter normal physiological functions in the exposed organism.

Scientists collaborating and advising on this study developed the following working definition of EDCs:

“An EDC is an anthropogenic chemical (human-made compound or natural compounds at unnatural concentrations due to human activity) that may have an adverse effect on reproduction or development, mediated directly through the endocrine system of fish, wildlife, and humans.”

EDCs can include pharmaceuticals, personal care products, general anthropogenic (man-made) compounds, pesticides, biogenic (naturally occurring) compounds, or inorganics. An unknown number of the more than 87,000 chemicals that are manufactured worldwide may possess endocrine disrupting properties; the UK Institute for Environment and Health lists 966 known and potential EDCs. (For a more detailed review on EDCs, see [3]).

## Barriers to understanding and regulating EDCs

Because EDCs exert adverse physiologic effects far below concentrations that are considered toxic, conventional approaches to understanding and addressing their sources are not sufficient. Some of the challenges in defining the problem, setting environmental standards and limiting discharges include:

- A lack of scientific data on EDC effects and toxicology of suspected EDCs.
- EDC effects may be so subtle that they are not consistently measurable or identifiable.
- Natural variations in the environment such as water flow and temperature add more variables to characterizing the effects of EDCs in surface waters.
- The timing of exposure to EDCs (e.g. infancy vs. adulthood) may be a critical factor.
- Mixtures of pollutants create synergistic, antagonistic and additive effects, adding difficulty to developing environmental standards for EDCs.
- Compliance determination methods for dischargers have not been developed.
- Treatment technology effectiveness, efficiency and costs are largely unknown.

Though several effects of exposure to EDCs have been documented in laboratory studies, it is difficult to link observed effects in nature to the presence of a chemical in the environment. Nonetheless, evidence is accumulating that EDCs can dramatically reduce reproductive success and survival, alter sex ratios and cause intersex in fish and other organisms, and cause developmental abnormalities. Specific studies on EDCs include those that demonstrate that:

- Nonylphenol or octylphenol cause the feminization of male fish [4, 5]
- The synthetic hormone in birth control pills, ethinylestradiol, can cause dramatic population changes in fish at five parts per trillion in surface water [6] and has been observed to cause vitellogenin induction at less than one part per trillion [7]

The antidepressant compound fluvoxamine can induce spawning in freshwater mussels at a concentration of 30 parts per trillion [8].

These and other EDCs originate from a variety of sources ranging from personal care products to industrial chemicals and are therefore present in the effluent of wastewater treatment plants (WWTPs)[9, 10], septic systems[11], stormwater and agricultural runoff, and natural sources.

## Purpose of the Minnesota statewide EDC study

Previous studies of EDCs in Minnesota have focused primarily on Minnesota's rivers that often included obvious sources such as WWTPs ([12], [13],[14]). These studies showed a widespread occurrence of EDCs in surface water and sediment and evidence that endocrine disruption in fish is occurring where these compounds are detected.

Little is known about the presence, concentration, or effect of EDCs in Minnesota lakes. Like rivers, lakes receive contaminants from a wide variety of sources including septic systems (individual sewage treatment systems, or ISTSs), lawns, confined animal feedlot operations, cropland, atmospheric deposition, and storm water from paved and other impervious surfaces. These sources contribute pesticides, surfactants, pharmaceuticals, and other compounds to lakes that have unknown effects on wildlife and human health.

In 2007, the Minnesota Legislature and Governor Pawlenty approved funding for a state-wide EDC study with the following goals:

1. to measure known and suspected EDCs in representative lakes and rivers
2. to measure EDCs and pharmaceutical compounds in sediment
3. to measure the degree of fish abnormalities due to endocrine disruption at the same locations

The MPCA collaborated with the U.S. Geological Survey (USGS) and St. Cloud State University (SCSU) to plan and execute this investigation at twelve lakes and three rivers across the state. The USGS collected and analyzed water and sediment samples for EDCs, pharmaceutical compounds, and other organic wastewater contaminants (OWCs). SCSU caged fish and sampled wild-caught fish at each location and performed morphological and histological examinations to detect endocrine disruption in fish. The results of these investigations are presented in this report.

## Methods

### Site selection

The 12 lakes and four rivers included in the study were selected to represent the geographic and ecological diversity of aquatic habitats across Minnesota (Table 1, Figure 1). Lakes were selected to represent different trophic levels (eutrophic, mesotrophic, or oligotrophic) that differ in development density and treatment of wastewater (sewered vs. septic) and land use (urban, rural, forested).



**Figure 1. Map of sampling locations**

Budd Lake, Lake Owasso, and Cedar Lake are mesotrophic urban lakes. Development surrounding these lakes is sewerage, and storm water runoff is assumed to be the primary source of contaminants to these lakes. Sullivan Lake, White Sand Lake, and Red Sand Lake reflect mesotrophic lakes influenced by non-sewered residences with ISTSs. Stewart Lake, Shingobee Lake, and Lake Kabetogama represent northern, oligotrophic lakes with non-sewered residences with ISTSs. Northern Light Lake and Elk Lake (oligotrophic and mesotrophic, respectively) were selected as reference lakes due to their remote locations, lack of development, and absence of any clearly identifiable source of contaminants. Two Harbors on Lake Superior was selected as typical of the municipalities located on its shore. Finally, four rivers and streams were also included in the study: Seven Mile Creek, the LeSueur River, the Redwood River, and the Little Cobb River.

## Sampling procedures

Lake and bed-sediment samples were collected according to established USGS protocols (USGS, 2003). Samples were collected from the top ten centimeters of bed sediment at four to six depositional areas at each sampling location with stainless-steel sampling equipment. The composite bed-sediment sample was transferred to a series of baked-glass containers and placed on ice for transport. Samples were frozen and held until shipment to USGS National Research Program laboratories for analyses of selected chemicals using research methods or to the USGS National Water Quality Lab for analysis of wastewater indicator compounds using standard methods.

Fish samples for this study were collected through two mechanisms. Laboratory-reared mature male fathead minnows were obtained from a laboratory fish supplier and were caged in each lake or stream for 21 days. In addition, an attempt was made to collect 20 male and 20 female fish from at least two of four species of fish identified for this study (minnow, shiner, sunfish, and perch). Wild fish were collected using shallow-water seines and back-pack and boat electro-shocking techniques. Regardless of collection technique or source (caged or wild-caught), all fish were maintained alive and moved to the Aquatic Toxicology Laboratory at St. Cloud State University within six hours of collection. Laboratory processing of the fish followed established USGS guidelines [15].

Briefly, fish were measured for weight and length, sampled for blood, and dissected to extirpate livers and reproductive organs (testis, ovaries) for histopathological analysis. Blood samples were analyzed for concentrations of vitellogenin (a female reproductive protein found to be produced by male fish exposed to estrogenic EDCs) using a published enzyme-linked immunosorbent assay (ELISA) technique. Livers and reproductive organs were prepared for microscopic viewing using standard techniques and then evaluated for the abundance of particular cell types using a technique validated previously by the U.S. EPA[16].

## Results

### Contaminant analysis

#### Surface Water

Results for organic wastewater compounds and EDCs are presented in Tables 2-4, which are condensed to show only the compounds that were detected in at least one surface water sample.

The endocrine disrupting compounds 4-nonylphenol, 4-octylphenol, nonylphenol ethoxylates, octylphenol ethoxylates, and bisphenol-A was detected in one or more of the lakes and rivers included in this study (Table 2). Nonylphenol was detected in Seven Mile Creek and the Redwood River (upstream of the Marshall wastewater treatment plant) at concentrations ranging from 0.542 to 0.245 µg/L (parts per billion), respectively. However, the highest concentrations of these and several other EDCs and organic wastewater chemicals were measured in the Two Harbors WWTP effluent as it enters Lake Superior, where 4-nonylphenol and 4-nonylphenol-2-ethoxylate were both detected in excess of 1.46 µg/L. In the reference lake Northern Light Lake, 4-nonylphenol was detected at 0.213 µg/L, similar to the concentration of 4-nonylphenol (0.215 µg/L) found below the Redwood River WWTP. Other detections in Northern Light Lake included nonylphenol ethoxylates, octylphenol ethoxylates, and bisphenol-A.

The antibacterial chemical triclosan was found in the Little Cobb River (0.015 µg/L), the Two Harbors WWTP effluent (0.572 µg/L), the Redwood River (0.010 µg/L), and the sample at the site of the Redwood River WWTP (0.112 µg/L).

N,N, diethyl-m-toluamide (DEET, the common ingredient in insect repellent) was detected in all surface water, with the highest concentration of 1.855 µg/L found in the Two Harbors effluent, followed by 0.579 µg/L in Shingobee Lake. DEET is commonly detected in surface water investigations. Caffeine, a tracer for anthropogenic impacts to surface water, was detected in 36 percent of the lake and 88 percent of the river samples.

Several hormones were analyzed in each of the water samples (Table 3). The hormone 17β-estradiol was found in 55 percent of the lake samples and 38 percent of the river samples. Northern Light Lake and Elk Lake contained the highest concentrations of any of the lakes tested at 0.45 and 0.41 ng/L (parts per trillion). The highest concentration of 17β-estradiol detected overall was 1.71 ng/L in the Two Harbors WWTP effluent. Detections of estrone (82 percent lakes, 75 percent rivers) and androstenedione (91 percent lakes, 50 percent rivers) followed a similar pattern. The highest concentrations of these compounds were again found in the Two Harbors WWTP effluent, while the highest concentrations in the other rivers or lakes were found in Seven Mile Creek (for estrone) and White Sand Lake (for androstenedione). The synthetic hormone ethinylestradiol was not detected in any of the samples.

The largest number of hormone detections was in the Two Harbors WWTP effluent with nine detections, followed by White Sand Lake and the Redwood River WWTP effluent with five detections each. The fewest hormone detections were in the reference lake Northern Light Lake.<sup>1</sup>

Water samples were analyzed for 37 pesticides and pesticide degradates. While atrazine and de-ethylatrazine were present in several lakes (Table 4), all were below the analytical reporting limit. No other pesticides were detected in any of the lakes or streams included in this study.

Polar organic chemical integrative samplers (POCIS)

Table 5 contains the results from the POCIS water samples. Using this sampling method, DEET was detected in 91 percent of the lakes; nonylphenol ethoxylates were detected in 27 percent of the lakes; and bisphenol-A and octylphenol ethoxylate were each detected in one lake. (Note: POCIS sample results are the total amount of a chemical sequestered onto an absorption medium over the time the sampler is deployed in the lake.) Results in Table 5 do not show actual surface water concentrations of a chemical, because the volume of water the sampler is exposed to over time is not known. Sullivan Lake and the urban lakes Cedar Lake and Lake Owasso each tested positive for six chemicals, while only one chemical was detected in Budd Lake. POCIS sampling in the reference lakes Elk Lake and Northern Light Lake showed the presence of five and four compounds, respectively.

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<sup>1</sup>P Androstenedione and estrone were detected in all urban lakes but were not quantifiable due to method reporting limits.

P<sup>1</sup>P POCIS analysis is not directly comparable to water sample concentration data. POCIS membranes absorb certain chemicals more than others, the detection limits for water samples and POCIS samples are different, and the POCIS samples reflect a continuous integration of the contaminants in water over an extended period of time. Water “grab samples”, on the other hand, represent a “snapshot” of water quality at a given time. POCIS sampling offers an alternative analytical method that, used in conjunction with water, fish, and sediment sampling, assists in assessing impacts to a water body. It is not intended as a substitute for water samples.

## Sediment

Organic wastewater compounds found in sediment are shown in Table 6. Bisphenol-A was present in most lake sediment samples, including the reference lakes Northern Light Lake and Elk Lake. The highest concentration was detected in White Sand Lake at 35.4 ng/g (parts per billion). Bisphenol-A was not detected in sediment from Cedar Lake, Stewart Lake, or Seven Mile Creek. The EDC 4-nonylphenol was found in sediment from Seven Mile Creek and the Redwood River downstream of the wastewater treatment plant. The presence of 4-nonylphenol in Shingobee Lake sediment at 224 ng/g seems to reflect the presence of this chemical found in the surface water for this lake. However, no nonylphenol was detected in the sediments of Northern Light Lake, even though it was detected in the lake water at this location. Octylphenol was detected in sediments from Lake Owasso, White Sand Lake, Sullivan Lake, Shingobee Lake, and Elk Lake, as well as the Little Cobb River sediments; nonylphenol ethoxylates and octylphenol ethoxylates were detected in sediment from several lakes. However, nonylphenol, octylphenol, nonylphenol ethoxylates, and octylphenol ethoxylates were not detected in the sediment from the urban lakes Cedar Lake and Budd Lake.

## Pharmaceutical and personal care compounds

The antibacterial compound triclosan was present in Cedar Lake and Lake Owasso sediment, the Redwood River below the WWTP, and in the upper reaches of Seven Mile Creek, but this compound was absent in the sediment samples from most of the lakes. Acetaminophen was often present in the sediment, including the reference lake Northern Light Lake (Table 7). Acetaminophen was also present in Seven Mile Creek and the Little Cobb River. Interestingly, this compound was absent in the sediment from the Two Harbors and Redwood River locations near the WWTPs, perhaps reflecting the degradation of this compound in the WWTP plants. Carbamazepine (an anticonvulsive used in the treatment of attention-deficit hyperactivity disorder, or ADHD) was found in the sediment from the Little Cobb River, Red Sand Lake, Sullivan Lake, and Stewart Lake; it was also detected in the sediment of Northern Light Lake. The antidepressant fluoxetine was only found in the sediment from the Two Harbors location, which contained a total of nine pharmaceuticals (Table 7, Table 8). No pharmaceuticals were detected in samples from Lake Kabetogama, Shingobee Lake, Elk Lake, or White Sand Lake.

## Endocrine disrupting effects in fish

Tables 9 through 28 show the results of the histological and morphological studies conducted on caged and wild-caught fish collected at each location.

VTG concentrations (Table 30) in male fish varied depending on the sampling location and the species of fish. Concentrations were generally greater in fathead minnows and common shiners than sunfish and perch. The increased concentration of VTG in fish from Lake Owasso, White Sand Lake, and Lake Kabetogama suggests that these fish were exposed to estrogenic compounds in the aquatic environment. Differences in VTG in female fish may be due to variations in reproductive timing among lakes across the state.

The number of liver-fat cells was greater in male fish from Budd Lake, Sullivan Lake, and Lake Kabetogama, and in Budd Lake for female fish. This indicates a loss of liver function and stress from greater exposure to pollution in these waters than in the reference lakes. The highest rates of intersex (evidence of testicular feminization in male fish) were found in urban lakes, suggesting that these fish have been exposed to estrogenic compounds in their environment.

No clear trend emerged in gametogenesis (the development of sperm in males and eggs in females) or the hepatosomatic index (the ratio of the weight of the liver to the weight of the fish). Observations of overall body condition (length and weight) were similar for fish of the same species in different lakes, with a slight increase in Sullivan Lake, Stewart Lake, and Shingobee Lake.

Elevated concentrations of VTG compared to control fish were observed in male fathead minnows that were caged in urban lakes and in Lake Kabetogama. This suggests that these fish were exposed to estrogenic compounds over the three weeks of exposure in the lakes.

Measurements of the caged fathead minnows indicated that the body condition in these fish did not vary between lake types or between “test” and reference lakes. However, the minnows caged in lakes categorized as septic and mesotrophic had larger sex organs in relation to body size (gonadosomatic index) when compared to the caged minnows in reference lake Elk Lake. (This may be due to greater nutrient availability. Similar observations of higher gonadosomatic indices downstream of WWTPs have been made in previous studies.)

Caged minnows deployed in urban lakes had smaller livers than minnows that were caged in the reference lakes.

Abundance of fat cells in the liver was greater in caged minnows deployed in urban lakes, indicating a loss of liver function in these minnows after three weeks exposure to the urban lake waters. While immature sperm abundance in caged minnows decreased in both urban and septic/mesotrophic lakes, mature sperm abundance increased in caged minnows in urban lakes. This shift in the ratio of immature to mature sperm in urban lake-deployed minnows indicates a possible reduction in sperm production by these fish during the time they were deployed in the lakes.

Finally, caged fathead minnows exhibited a low but persistent rate of intersex. This may be the result of a normally occurring base-rate of intersex in this fish species. However, the relatively high rate of intersex observed at the downstream Seven Mile Creek location probably indicates an exposure to EDCs.

## Discussion

This is the first state-wide investigation of EDCs that focuses largely on surface water and sediment from Minnesota lakes. Similar to previous studies of Minnesota rivers ([12-14]), the results from this study show that low concentrations of EDCs and other contaminants are present in Minnesota lakes regardless of region or land use. Although this analysis was done on relatively few surface water and sediment samples from a limited number of lakes, several of the results from this survey are particularly noteworthy.

The detection of 17 $\beta$ -estradiol and estrone in many of the lake water samples likely reflects that these hormones are excreted by wildlife as well as humans. However, the presence of the endocrine disrupting compounds 4-nonylphenol, bisphenol-A, 4-octylphenol, nonylphenol ethoxylate, octylphenol ethoxylate, as well as the pharmaceuticals carbamazepine and acetaminophen in the reference lakes Elk Lake and Northern Light Lake, was unexpected. These undeveloped lakes were included in this study to provide a baseline of undeveloped or “pristine” lakes to which the other “test” lakes in the study could be compared. The source(s) of these compounds to these lakes is not known. However, the detection of these compounds in these lakes shows that EDCs in surface water do not emanate solely from wastewater treatment plants and individual septic systems, since these lakes are clearly not influenced by these sources.

No apparent correlation is evident between observed effects in fish and the presence of particular chemicals analyzed in this investigation. Nonylphenols, octylphenols, nonylphenol ethoxylates, and octylphenol ethoxylates were not detected in Budd Lake or Lake Owasso, which are both urban lakes, while they were clearly present in the northern, oligotrophic Shingobee Lake (with an estimated three septic systems) and Northern Light Lake (with no homes). VTG induction in male wild fish was relatively low in the urban lakes Cedar Lake and Budd Lake. The high VTG ratios (VTG concentrations in male fish divided by VTG concentrations in female fish) in some species of wild fish from Northern Light Lake and Elk Lake suggest that these fish populations were exposed to estrogenic compounds in their environment (Table 30). An upward trend in VTG concentrations in male fish was observed from upstream to downstream in Seven Mile Creek and in the Redwood River (Table 29), suggesting that there may be an increase in exposure to estrogenic compounds in the lower reaches of these streams.

The analytical results also show that several EDCs and organic wastewater compounds are entering Lake Superior directly through wastewater treatment plant effluent. While this is not unexpected based on the results of studies of other WWTPs, it does show that Lake Superior is receiving a continuous stream of EDCs and wastewater-associated contaminants from WWTPs along its shores with unknown consequences for the lake.

Table 30 ranks the study lakes by relative concentration of frequently detected contaminants in the water, sediment and POCIS samples. Lake Owasso (an urban lake) consistently had elevated relative concentrations of contaminants in the water, sediment and POCIS samples.

Results were more variable between sampled media for the other lakes. For example, the urban Cedar Lake, as well as the reference lakes Elk Lake and Northern Light Lake, contained higher relative concentrations of contaminants in the water column than the majority of lakes surveyed. However, Northern Light Lake and Cedar Lake had lower relative concentrations of contaminants in the sediments. Lake Kabetogama (which has



hundreds of septic systems on the south shore) had fewer elevated contaminant concentrations in the water column but had relatively high concentrations of contaminants in the sediments.

When ranked by POCIS results, the urban lake Budd Lake and the reference lake Northern Light Lake contained the fewest elevated contaminant concentrations, while the samples from Cedar Lake were found to have the highest elevated contaminant concentrations.

Water samples reflect more transitory conditions, whereas sediment and POCIS samples integrate contaminant presence over time. Therefore, differences between the sampled mediums are not surprising and indicate the likelihood of multiple contaminant sources.

Table 31 compares the maximum concentrations of organic wastewater contaminants reported in this study with those found in the tributary study ([13]), the Mississippi River longitudinal study ([12]), and the 2000-2002 USGS reconnaissance study ([14]). All of these studies differ in scope, purpose, and locations (locations sampled in the prior three studies, for example, included locations downgradient of WWTPs). While the maximum concentrations are generally greater in the 2002 USGS reconnaissance study and the Mississippi River longitudinal study, these data demonstrate that EDCs and other contaminants are widespread in lakes, rivers, and streams, regardless of whether the locations are downgradient of WWTPs, associated with urban development, or in rural settings.

Table 33 shows maximum contaminant concentration for surface water that was categorized as urban (sewered), ISTS, or undeveloped lakes. All the lake types contained bisphenol-A, DEET, nonylphenol-1-ethoxylate (NP1EO), and octylphenol-2-ethoxylate (OP2EO) in the water. The urban lakes in this study, however, contained these compounds at lower concentration than either the lakes with septic systems or the undeveloped reference lakes. Three EDCs were detected in urban lakes, while eight EDCs were found in lakes with septic systems. Five EDCs were detected in the reference lakes. Aside from concentration, there were also fewer compounds detected in the urban lakes than in the lakes influenced by septic systems (Table 35). These results are consistent with the study by Conn *et al.* [17] who sampled 30 residential septic tanks. They reported that several alkylphenols were consistently detected in effluent from ISTSs, including 4-nonylphenol and 4-nonylphenol ethoxylates that were detected in 63 percent and 70 percent, respectively, of the septic systems in that study.

A similar pattern was observed for contaminants in sediment (Table 34, 36). More alkylphenol and alkylphenol ethoxylate chemicals were detected in the sediment of lakes under the influence of septic systems than in urban lakes where the development is sewerred. In this study, the three urban lakes selected for sampling were similar to the reference lakes with two contaminants detected, while a total of seven contaminants were detected in those lakes influenced by ISTSs.

Concentrations of EDCs in sediment were much higher than in water. Nonylphenol concentrations in the water, for example, are 0.2 to 0.8 ppb, while the maximum concentration for sediment was 234 ppb, representing a 1000-fold increase in concentration. Other chemicals in Table 31 show the same increase in concentration for sediment (Table 32), indicating that sediments may be a long-term sink for some chemicals released into the aquatic environment[18]. The exception seems to be DEET, which is found frequently in surface water but not detected in sediment in this study. Without a mechanism for degradation of these compounds in the sediment, they may continue to accumulate in freshwater sediment with unknown consequences to benthic organisms and aquatic ecosystems.

## Summary

This study had a two-fold purpose: 1) to assess the presence of EDCs, pharmaceuticals, and other contaminants in lakes and streams in Minnesota and 2) to determine whether fish from these locations showed signs of endocrine disruption.

The results show that known and suspected EDCs and other contaminants are widespread in low concentrations in Minnesota lakes. Although the scope of this study is not wide enough to draw statistically significant conclusions, the data indicates that these compounds are present in both lakes that lack obvious sources of contamination and in lakes with substantial residential development. The analytical results of this study suggest that lakes surrounded by sewerred development had lower concentrations of alkylphenols in

surface water and sediment than lakes that have homes with ISTSs. The results also show that municipal WWTP effluent is contributing EDCs to Lake Superior. Contaminants were also detected in the three rivers sampled as part of this study, results that are consistent with earlier studies on tributary streams and rivers in Minnesota[12]. Sediment concentrations of compounds analyzed in this study are much higher than the corresponding water concentrations, suggesting that these chemicals may be accumulating in sediment over time.

Examination of fish shows that they are probably being affected by estrogenic chemicals in many of the lakes and rivers selected for this study. Vitellogenin induction in male fish – evidence of feminization of these fish - was observed in urban lakes, in rural lakes with septic systems, in the undeveloped reference lakes, and in three of the four rivers sampled. No clear correlation could be drawn between evidence of endocrine disruption and a particular set of chemicals in the water. However, fish exposed to urban lake water show evidence of physiologic stress through increased liver fat-cell numbers.

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Table 36: Number of organic contaminants detected in sediment by lake category

**Table 1. Lakes and rivers selected for the statewide study**

**(a) Lakes**

<b>Name</b>	<b>Location</b>	<b>Type</b>	<b>Category</b>
Cedar Lake	Minneapolis	Eutrophic	Sewered
Lake Owasso	St. Paul	Eutrophic	Sewered
Budd Lake	Fairmont	Eutrophic	Sewered
White Sand Lake	Baxter	Mesotrophic	Septic
Red Sand Lake	Baxter	Mesotrophic	Septic
Sullivan Lake	Buffalo	Mesotrophic	Septic
Elk Lake	Park Rapids	Mesotrophic	Reference
Stewart Lake	North of Two Harbors	Oligotrophic	Septic
Shingobee Lake	Akeley	Oligotrophic	Septic
Lake Kabetogama	Voyageurs National Park	Oligotrophic	Septic
Northern Light Lake	Arrowhead Region	Oligotrophic	Reference
Lake Superior	Two Harbors	Oligotrophic	WWTP

**(b) Rivers**

<b>Name</b>	<b>Location</b>	<b>Category</b>	
Little Cobb River	South-central Minnesota, near Mankato	Agriculture	
LeSueur River	South-central Minnesota, near Mankato	Agriculture	
7 Mile Creek, Upstream	South-central Minnesota, near Mankato	Agriculture	
7 Mile Creek, Midstream	South-central Minnesota, near Mankato	Agriculture	
7 Mile Creek, Downstream	South-central Minnesota, near Mankato	Agriculture	
Redwood River, Upstream of WWTP	Southwest Minnesota, near Marshall	Agriculture	
Redwood River, Downstream 1	Southwest Minnesota, near Marshall	WWTP Influenced	
Redwood River, Downstream 2	Southwest Minnesota, near Marshall	WWTP Influenced	

Table 2. Organic wastewater compounds in surface water (ng/L).

Location	1,4-dichlorobenzene	4-methylphenol	4-ethylphenol	4-propylphenol	4-tert-butylphenol	4-tert-pentylphenol	2,6-di-tert-butylphenol	2,6-di-tert-butyl-1,4-benzoquinone	2,6-di-tert-butyl-4-methylphenol	5-methyl-1H-Benzotriazole	N,N-diethyl-m-tolamide	4-(tert-octyl)phenol	4-nonylphenol (Avg. Spec.)	4-n-octylphenol	caffeine	Diphenhydramine HCl	4-NP1EO (Avg. Spec.)	triclosan	4-OP2EO	bisphenol A	4-NP2EO (Ave. Spec.)	4-OP3EO	4-NP3EO (Avg. Spec.)	4-NP4EO (Avg. Spec.)	3-b-coprosterol	cholesterol
Cedar Lake	<10	19.9	<10	<10	<10	<10	<10	141.6	64.9	38.3	48.1	<10	<100	<10	87.4	<10	<50	<10	<10	<10	<50	<10	<50	<10	77.7	
Lake Owasso	<10	28.6	<10	<10	<10	<10	<10	<10	65.5	<10	90.0	<10	<100	<10	129.4	<10	<50	<10	<10	25.6	<50	<10	<50	<10	258.3	
Budd Lake	<10	<10	<10	<10	<10	<10	180.0	<10	68.7	13.6	25.3	<10	<100	<10	17.7	<10	59.1	<10	25.8	37.9	<50	<10	<50	<50	<10	40.4
White Sand Lake	<10	23.0	<10	<10	<10	<10	<10	87.8	15.5	<10	33.4	<10	<100	<10	<10	<10	<50	<10	<10	<10	<50	<10	<50	<50	24.0	367.2
Red Sand Lake - Average	<10	15.0	<10	<10	<10	<10	<10	38.0	17.0	<10	11.5	<10	<100	<10	<10	14.0	<50	<10	<10	<10	<50	<10	<50	<50	22.3	206.8
Sullivan Lake	<10	19.5	<10	<10	<10	<10	<10	82.6	<10	<10	30.5	<10	<100	<10	<10	<10	<50	<10	<10	<10	<50	<10	<50	<50	<10	507.0
Elk Lake - Average	<10	14.4	<10	<10	<10	<10	<10	76.5	18.4	<10	217.8	<10	<100	<10	10.7	<10	<50	<10	<10	<10	<50	<10	<50	<50	<10	658.1
Stewart Lake	<10	16.9	<10	<10	<10	<10	<10	<10	<10	<10	51.2	10.2	<100	<10	13.8	<10	<50	<10	<10	20.0	<50	<10	<50	<50	<10	63.5
Shingobee Lake	<10	13.8	<10	<10	<10	<10	<10	<10	<10	<10	579.4	10.2	110.8	<10	12.8	35.7	86.3	<10	33.7	19.9	170.5	13.8	123.0	<50	16.8	164.4
Lake Kabetogma - Average	<10	18.6	<10	<10	<10	<10	<10	<10	<10	<10	17.6	<10	<100	<10	<10	<10	<50	<10	<10	<10	<50	<10	<50	<50	<10	217.2
Northern Light Lake - Average	<10	41.4	<10	<10	<10	<10	<10	<10	12.0	<10	50.7	<10	213.8	<10	19.3	<10	106.0	<10	42.7	12.4	65.8	<10	<50	<50	145.6	71.3
Little Cobb R. - Average	<10	30.7	15.5	<10	<10	<10	<10	<10	<10	<10	177.9	<10	<100	<10	52.4	<10	<50	15.1	<10	<10	<50	<10	<50	<50	<10	102.2
Le Suer R.	<10	20.8	<10	<10	<10	<10	<10	207.7	<10	<10	73.9	<10	<100	<10	83.8	<10	<50	<10	<10	<10	<50	<10	<50	<50	<10	89.5
7 mile upstream	<10	25.5	<10	<10	<10	<10	<10	<10	<10	<10	157.7	<10	**	<10	<10	<10	<50	<10	<10	<10	<50	<10	<50	<50	<10	69.3
7 mile midstream - Average	<10	24.8	<10	<10	<10	<10	<10	<10	<10	<10	36.6	<10	541.7	<10	21.3	<10	<50	<10	<10	10.1	<50	<10	<50	<50	21.1	45.9
7 mile downstream - Average	<10	25.5	<10	<10	<10	<10	<10	37.2	12.9	<10	178.7	<10	358.4	<10	18.8	29.8	<50	<10	<10	12.1	<50	<10	<50	<50	<10	86.5
Redwood R Upstream	<10	26.1	<10	<10	<10	<10	<10	241.2	<10	72.9	31.7	17.6	245.2	<10	41.7	<10	<50	<10	49.7	53.8	<50	<10	<50	<50	<10	118.6
Redwood R DS1 - Average	<10	33.4	<10	<10	14.5	<10	<10	188.2	17.0	62.7	554.3	<10	858.4	13.7	59.4	<10	<50	10.5	76.0	<10	<50	<10	112.3	57.0	91.6	347.7
Redwood R DS2 - Average	<10	21.9	<10	<10	<10	<10	<10	165.0	<10	52.6	294.3	<10	622.4	<10	78.4	<10	<50	<10	<10	<10	<50	<10	<50	<50	34.5	323.3
Two Harbors - Average	184.0	177.5	<10	55.3	116.7	57.0	<10	1387.6	26.1	264.9	1855.2	129.0	1456.3	<10	13815.1	<10	867.0	572.5	<10	45.6	1484.0	<10	519.6	60.5	8458.0	5375.5
Redwood R WW eff - Average	54.5	44.3	<10	12.2	91.2	<10	83.7	365.9	21.3	583.7	244.8	26.1	215.1	<10	117.7	195.7	161.9	112.5	30.9	<10	<50	<10	134.0	<50	476.5	559.5

Table 3. Hormones in surface water (ng/L).

Location	cis-androsterone	17-alpha-estradiol	androstenedione	estrone	17-beta-estradiol	testosterone	equilin	11-ketotestosterone	mestranol	equilenin	ethinyl estradiol	estriol	progesterone	coprostanol	cholesterol
Cedar Lake	ND	ND	QA	QA	0.38	ND	ND	ND	ND	ND	ND	ND	ND	27.89	7842.55
Lake Owasso	ND	ND	QA	QA	0.10	ND	ND	ND	0.17	ND	ND	ND	ND	ND	8982.17
Budd Lake	ND	ND	QA	QA	0.04	ND	ND	ND	ND	ND	ND	ND	ND	39.07	2536.52
White Sand Lake	ND	ND	1.00	0.64	0.12	0.23	0.16	ND	ND	ND	ND	ND	ND	552.46	7520.07
Red Sand Lake - Average	ND	ND	0.22	0.63	0.03	ND	ND	ND	ND	ND	ND	ND	ND	200.75	5688.40
Sullivan Lake	ND	ND	0.72	0.70	0.08	ND	ND	ND	ND	ND	ND	ND	ND	50.28	7133.56
Elk Lake - Average	ND	ND	0.81	1.10	0.41	ND	ND	ND	ND	ND	ND	ND	ND	50.99	8279.33
Stewart Lake	ND	ND	0.33	1.45	ND	ND	ND	ND	ND	ND	ND	ND	ND	161.64	1363.99
Shingobee Lake	ND	ND	0.54	1.08	ND	ND	ND	ND	ND	ND	ND	ND	ND	418.53	1369.67
Lake Kabetogma - Average	ND	ND	0.50	0.02	ND	ND	ND	ND	ND	ND	ND	ND	ND	25.30	1975.58
Northern Lights Lake - Average	ND	ND	ND	ND	0.45	ND	ND	ND	ND	ND	ND	ND	ND	14.80	931.08
Maximum	0.0	0.0	1.0	1.4	0.4	0.2	0.2	0.0	0.2	0.0	0.0	0.0	0.0	552.5	8982.2
% Detection	0.0	0.0	63.6	63.6	72.7	9.1	9.1	0.0	9.1	0.0	0.0	0.0	0.0	90.9	100.0
Little Cobb R. - Average	ND	ND	ND	0.14	ND	ND	ND	ND	ND	ND	ND	ND	ND	55.12	1225.54
Le Suer R.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	70.10	767.62
7 mile upstream	ND	ND	0.32	ND	0.10	ND	ND	ND	ND	ND	ND	ND	ND	62.40	1473.68
7 mile midstream - Average	ND	0.18	0.25	1.56	ND	ND	ND	ND	ND	ND	ND	ND	ND	171.61	1127.86
7 mile downstream - Average	ND	ND	0.43	2.20	ND	ND	ND	ND	ND	ND	ND	ND	ND	116.10	970.75
Redwood R Upstream	ND	ND	0.09	0.24	0.02	ND	ND	ND	ND	ND	ND	ND	ND	21.90	752.21
Redwood R DS1 - Average	ND	ND	0.04	1.16	0.96	ND	ND	ND	ND	ND	ND	ND	ND	272.61	1147.90
Redwood R DS2 - Average	ND	ND	0.04	0.66	0.15	ND	ND	ND	ND	ND	ND	ND	ND	144.80	1283.68
Maximum	0.0	0.2	0.4	2.2	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	272.6	1473.7
% Detection	0.0	12.5	75.0	75.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0
Two Harbors - Average	10.95	0.47	17.99	20.91	1.71	0.27	ND	0.55	ND	ND	ND	2.67	1.32	#####	35948.84
Redwood R WW eff - Average	ND	ND	1.04	1.38	0.09	ND	ND	ND	0.08	0.10	ND	ND	ND	2222.09	2968.52
Maximum	10.9	0.5	18.0	20.9	1.7	0.3	0.0	0.6	0.1	0.1	0.0	2.7	1.3	21885.9	35948.8
% Detection	50.0	0.0	50.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0

Table 4. Pesticides in surface water (ug/L).

Location	Atrazine (ug/L)	De-ethyl- atrazine (ug/L)
Cedar Lake	P	ND
Lake Owasso	P	ND
Budd Lake	P*	P
White Sand Lake	P	P
Red Sand Lake	P	P
Sullivan Lake	P	P
Elk Lake	P	ND
Stewart Lake	ND	ND
Shingobee Lake	ND	ND
Kabetogama Lake	P	P
Northern Light Lake	ND	ND
Two Harbors (Lake Superior)	ND	P

\* P (present) is equal to 1/2 the MRL, or 0.025 micrograms/L.



**Table 5. POCIS results (ng/sample membrane).**

	4-methylphenol	4-ethylphenol	2,6-di-tert-butyl-1,4-benzoquinone	2,6-di-tert-butyl-4-methylphenol	5-methyl-1H-Benzotriazole	N,N-diethyl-m-tolamide	4-nonylphenol (Avg. Spec.)	caffeine	Diphenhydramine HCl	4-OP2EO	bisphenol A	4-NP2EO (Ave. Spec.)	4-NP3EO (Avg. Spec.)
Cedar Lake	27.3	37.3	<10	<10	64.0	86.7	<10	104.7	18.7	<10	<10	<10	<10
Owassa Lake	57.3	16.0	<10	351.3	<10	115.3	<10	17.3	26.0	<10	<10	<10	<10
Budd Lake	14.7	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
White Sand Lake	11.7	<10	16.5	62.3	<10	85.5	<10	<10	<10	<10	<10	<10	86.5
Red Sand Lake	41.8	<10	37.7	167.3	<10	29.8	116.2	<10	<10	<10	<10	122.8	<10
Sullivan Lake - Day 7	10.2	<10	70.0	223.8	<10	14.7	<10	<10	<10	21.8	<10	<10	<10
Sullivan Lake - Day 14	12.3	<10	74.7	239.3	<10	18.8	<10	<10	<10	<10	<10	<10	<10
Sullivan Lake - Day 21	17.7	10.2	54.2	<10	<10	38.5	<10	<10	<10	<10	<10	<10	<10
Sullivan Lake	59.8	<10	53.7	223.5	<10	78.2	<10	<10	<10	<10	14.0	<10	61.3
Elk Lake	28.5	30.7	63.7	179.3	<10	29.0	<10	<10	<10	<10	<10	<10	<10
Stewart Lake	55.8	13.2	211.2	12.3	<10	58.0	<10	<10	<10	<10	<10	<10	<10
Shingobee Lake	56.0	14.2	293.5	16.3	<10	13.5	<10	<10	<10	<10	<10	<10	<10
Lake Kabetogama	255.2	10.7	181.0	158.0	<10	20.8	<10	<10	<10	<10	<10	<10	<10
Northern Lights Lake	42.7	<10	77.7	183.2	<10	38.0	<10	<10	<10	<10	<10	<10	<10
Maximum concentration	255.2	37.3	293.5	351.3	64.0	115.3	116.2	104.7	26.0	21.8	14.0	122.8	86.5

**Table 6. Organic wastewater compounds in sediment (ng/g)**

Location	4-methylphenol	4-ethylphenol	4-tert-butylphenol	4-tert-pentylphenol	2,6-di-tert-butyl-4-methylphenol	4-(tert-octyl)phenol	4-nonylphenol (Avg. Spec.)	caffeine	4-OP1EO	4-NP1EO (Avg. Spec.)	triclosan	4-OP2EO	bisphenol A	4-NP2EO (Ave. Spec.)	4-NP3EO (Avg. Spec.)	3-b-coprostenol	cholesterol
Detection Limit (ng/g)	3.00	3.00	1.00	3.00	3.00	3.00	100.00	1.00	1.00	20.00	1.00	1.00	3.00	1.00	1.00	10.00	120.00
Cedar Lake (avg)	8.18	5.50	ND	ND	ND	ND	ND	71.21	ND	ND	14.58	ND	ND	ND	ND	21.10	157.50
Lake Owasso	595.44	58.69	ND	88.03	ND	98.15	ND	ND	ND	ND	33.19	ND	19.52	ND	ND	732.19	2306.13
Budd Lake	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	16.76	ND	ND	59.34	395.85
White Sand Lake	185.94	22.81	ND	74.18	ND	33.14	ND	ND	ND	ND	ND	73.89	35.44	ND	ND	ND	377.40
Red Sand Lake (avg)	355.38	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	10.58	16.59	ND	ND	389.05	779.42
Sullivan Lake	131.43	21.30	5.65	22.61	ND	8.09	ND	ND	ND	ND	ND	ND	6.74	ND	61.83	969.35	1560.83
Elk Lake	33.56	17.75	ND	ND	ND	4.92	ND	ND	ND	ND	ND	ND	7.67	ND	ND	853.68	3397.24
Stewart Lake	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.42	ND	ND	ND	47.82	183.27
Shingobee Lake (avg)	160.30	95.63	ND	ND	ND	11.08	223.88	ND	3.23	ND	ND	ND	19.11	29.31	ND	409.98	686.54
Lake Kabetogama	1099.69	607.45	ND	ND	ND	ND	ND	ND	ND	ND	ND	45.34	15.53	ND	ND	346.74	1652.64
Northern Lights Lake	128.39	33.94	ND	ND	3.97	ND	ND	ND	ND	ND	ND	ND	3.11	ND	ND	ND	ND
Little Cobb River	ND	3.86	ND	7.50	ND	3.79	ND	ND	ND	ND	ND	2.64	4.04	ND	ND	77.96	557.46
Le Sueur River	86.19	3.16	ND	4.87	3.32	ND	ND	ND	ND	ND	2.27	3.22	3.55	ND	ND	78.24	693.85
7 Mile Ck - Upstream	106.69	19.18	ND	ND	ND	ND	101.51	ND	ND	ND	2.92	4.61	ND	ND	ND	100.53	614.08
7 Mile Ck - Midstream	30.90	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	49.85	ND	ND	425.45
7 Mile Ck - Downstream	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.60	ND	ND	ND	10.47	315.01
Redwood R. DS1	78.75	29.12	ND	ND	ND	ND	104.65	ND	ND	ND	5.18	ND	4.58	ND	ND	191.90	532.04
Redwood R. DS2	1782.09	122.68	ND	ND	ND	ND	136.98	ND	ND	61.47	35.70	48.58	9.28	ND	ND	541.62	1478.35
Two Harbors	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.40	ND	ND	ND	132.63
Redwood R. WWTP	28.82	13.19	1.56	ND	ND	ND	ND	ND	1.22	ND	3.07	5.79	ND	ND	ND	59.64	170.89

Table 7. Pharmaceuticals in sediment. (ug/Kg)

Location	Acetaminophen	Cimetidine	Caffeine	Trimethoprim	Carbamazepine	Diphenhydramine	Fluoxetine	Miconazole	Venlafaxine	Citalopram	Fluoxetine 1	Sertraline	# Detections
Cedar Lake	225.99	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1
Lake Owasso	279.28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1
Budd Lake	232.97	13.20	ND	ND	ND	ND	ND	12.92	ND	ND	ND	ND	3
White Sand Lake	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
Red Sands Lake	ND	ND	ND	ND	12.39	ND	ND	14.18	ND	ND	ND	ND	2
Sullivan Lake	231.70	ND	ND	ND	11.54	11.09	ND	ND	ND	ND	ND	ND	3
Elk Lake	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
Stewart Lake	544.21	ND	ND	ND	15.20	ND	ND	ND	ND	ND	ND	ND	2
Shingobee Lake	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
Lake Kabetogama	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
Northern Lights Lake	131.35	ND	ND	ND	8.46	ND	ND	ND	ND	ND	ND	ND	2
Lake Superior, Two Harbors	ND	ND	26.21	11.59	ND	45.36	16.29	13.58	4.37	2.64	1.00	0.38	9
Little Cobb River	106.00	ND	ND	ND	8.01	7.99	ND	9.02	ND	ND	ND	ND	4
LeSueur River													
7 Mile Cr Upstream	144.31	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1
7 Mile Cr Downstream	119.92	ND	21.15	ND	ND	12.03	ND	ND	ND	ND	ND	ND	3
Redwood River	ND	ND	ND	ND	ND	21.14	ND	ND	ND	0.34	ND	ND	2

**Table 8. Number of pharmaceutical detections by lake type.**

<b>Number of pharmaceutical detections for MN lake sites – 2008</b>					
<b>Trophic Category</b>	<b>Development Category</b>				
	<b>Effluent</b>	<b>Reference</b>	<b>Septic</b>	<b>Urban Runoff*</b>	<b>Total</b>
<b>Eutrophic</b>	No data	No data	No data	4	4
<b>Mesotrophic</b>	No data	0	5	1	6
<b>Oligotrophic</b>	9	2	2	No data	13
<b>Total</b>	5	2	7	5	23

\*Missing results for two urban runoff lakes.

Table 9.

**Lake Owasso, (02LOW)**

## Fish Collection Information:

- 1) GPS Coordinates: Lat 45.03133 Long -93.13004; iFINDER® Expedition™ GPS
- 2) County: Ramsey
- 3) Type of Water Body: Eutrophic, Urban Runoff Lake
- 4) Collection Gear: Seine
- 5) Other Samples: Passive Samplers, Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	23	51.4	143.7	114.8	0.56±0.2	1±0.04	1.7±0.04
	F	18	46	141.4	114.6	3.2±0.8	1.6±0.2	1.6±0.03
Minnow	M	17	2.6	67.1	56.8	1±0.3	1.7±0.1	0.8±0.02
	F	16	2.1	62.9	53.5	6.2±0.9	1.8±0.4	0.8±0.02
Perch	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Shiner	M	18	4.4	82	65	2.4±0.5	1.2±0.3	0.8±0.01
	F	19	5.2	85.9	69.4	11±2	1.4±0.1	0.8±0.3
Caged Minnow	M	18	2.5	61.1	50.4	0.99±0.1	1.1±0.08	1.1±0.03

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	22	3.6±1.5	0	11	1%
	F	18	400±137	n/a	n/a	n/a
Minnow	M	12	955±406	0	1	47.5%
	F	8	2,013±742	n/a	n/a	n/a
Perch	M	0	-	-	-	-
	F	0	-	-	-	-
Shiner	M	18	643±185	3	3	28.3%
	F	18	2,272±679	n/a	n/a	-
Caged Minnow <sup>1</sup>	M	17	20±7	0	0	1%

<sup>1</sup> To calculate caged FHM VTG ratio, caged male VTG was multiplied by 100/ Female-wild-FHM VTG

**Table 10.**

Cedar Lake (01LCE)

Fish Collection Information:

- 1) GPS Coordinates: Lat 44.95616 Long -93.3211; NAD83
- 2) County: Hennepin
- 3) Type of Water Body: Eutrophic, Urban Runoff Lake
- 4) Collection Gear: Seine, Backpack Electro-shocker, Boat Electro-shocker
- 5) Other Samples: Passive Samplers, Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	31	32	121	100	0.3±0.03	2±0.1	2±0.04
	F	9	23.2	106	88	1±0.1	2±0.1	2±0.08
Minnow	M	28	2.7	68	58	0.7±0.08	1±0.07	0.8±0.01
	F	18	2	61	52	10±1	2±0.2	0.8±0.03
Perch	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Shiner	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Caged Minnow	M	18	2.2	59.6	49.2	0.98±0.09	1.1±0.1	0.98±0.04

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	31	0.7±0.4	3	8	0.6%
	F	9	118±94	n/a	n/a	n/a
Minnow	M	20	559±225	0	0	14%
	F	5	3,973±1,606	n/a	n/a	n/a
Perch	M	0	-	-	-	
	F	0	-	-	-	
Shiner	M	0	-	-	-	
	F	0	-	-	-	
Caged Minnow <sup>1</sup>	M	16	25±6	1	2	0.6%

<sup>1</sup> To calculate caged FHM VTG ratio, caged male VTG was multiplied by 100/ Female-wild-FHM VTG

**Table 11.**

White Sand Lake (04LWS)

Fish Collection Information:

- 1) GPS Coordinates: Lat 46.35991 Long -94.28740; iFINDER® Expedition™ GPS
- 2) County: Crow Wing
- 3) Type of Water Body: Meso-Eutrophic, Septic Input Lake
- 4) Collection Gear: Seine, Boat Electro-shocker
- 5) Other Samples: Passive Samplers, Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	26	23.5	114.2	94.6	0.2±0.07	1.3±0.05	1.5±0.04
	F	17	22.9	111.6	92.9	1.1±0.1	1.5±0.1	1.6±0.06
Minnow	M	0	-	-	-			
	F	0	-	-	-			
Perch	M	0	-	-	-			
	F	0	-	-	-			
Shiner	M	33	4.6	83.2	67.8	1.6±0.07	1±0.09	0.8±0.007
	F	6	4	76.8	63.2	8.5±3	2±0.4	0.9±0.03
Caged Minnow	M	27	2.4	61.8	51.3	1.3±0.1	1.1±0.1	1±0.03

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	24	7.4±0.8	0	0	105.7%
	F	17	7±1.1	n/a	n/a	n/a
Minnow	M	0	-	-	-	
	F	0	-	-	-	
Perch	M	0	-	-	-	
	F	0	-	-	-	
Shiner	M	31	972±223	0	0	44.7%
	F	4	2,174±854	n/a	n/a	n/a
Caged Minnow	M	26	8.2±2	0	2	n/a

**Table 12.**

Budd Lake (03LBU)

Fish Collection Information:

- 1) GPS Coordinates: Lat 43.63965 Long -94.47441; iFINDER® Expedition™ GPS
- 2) County: Martin
- 3) Type of Water Body: Eutrophic, Urban Runoff Lake
- 4) Collection Gear: Seine, Boat Electro-shocker
- 5) Other Samples: Passive Samplers, Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	28	42.7	128.2	105.3	0.3±0.06	1.2±0.04	1.8±0.03
	F	19	59.6	141.5	117.6	1.5±0.2	1.5±0.08	1.8±0.02
Minnow	M	21	2.1	61.6	51.3	1±0.1	1.9±0.2	0.9±0.03
	F	21	1.6	56.4	47.2	6.5±0.4	1.7±0.1	0.9±0.03
Perch	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Shiner	M	16	2.1	63.3	52.6	1.4±0.3	1.3±0.1	0.8±0.02
	F	26	2.3	65.8	54.5	7.1±1	1.7±0.2	0.8±0.02
Caged Minnow	M	29	2.5	61.2	50.7	1.3±0.1	1.1±0.08	1.1±0.03

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	28	0	10	0	0%
	F	44	149±43	n/a	n/a	n/a
Minnow	M	18	30±8	0	0	1.3%
	F	9	2,255±669	n/a	n/a	n/a
Perch	M	0	-	-	-	
	F	0	-	-	-	
Shiner	M	13	57±24	1	1	8%
	F	20	707±140	n/a	n/a	n/a
Caged Minnow <sup>1</sup>	M	27	37±8	2	2	1.6%

<sup>1</sup> To calculate caged FHM VTG ratio, caged male VTG was multiplied by 100/ Female-wild-FHM VTG



**Table 13.**

Sullivan Lake (06LSU)

Fish Collection Information:

- 1) GPS Coordinates: Lat 45.22004 Long -93.94226; iFINDER® Expedition™ GPS
- 2) County: Wright
- 3) Type of Water Body: Meso-Eutrophic, Septic Input Lake
- 4) Collection Gear: Seine, Boat Electro-shocker
- 5) Other Samples: Passive Samplers, Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	31	64.	144.2	119	1.4±0.2	1±0.04	2.1±0.04
	F	10	46.8	135	112	2.1±0.5	1.1±0.1	1.8±0.05
Minnow	M	0	-	-	-			
	F	0	-	-	-			
Perch	M	0	-	-	-			
	F	0	-	-	-			
Shiner	M	0	-	-	-			
	F	0	-	-	-			
Caged Minnow	M	29	2.4	60.4	49.8	1.2±0.1	1.5±0.08	1.1±0.02

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	31	3.3±1.5	1	0	0.1%
	F	10	3,310±888	n/a	n/a	n/a
Minnow	M	0	-	-	-	
	F	0	-	-	-	
Perch	M	0	-	-	-	
	F	0	-	-	-	
Shiner	M	0	-	-	-	
	F	0	-	-	-	
Caged Minnow	M	24	20±5	1	0	n/a

**Table 14.**

Red Sand Lake (05LRS)

Fish Collection Information:

- 1) GPS Coordinates: Lat 46.38103 Long -94.28610; iFINDER® Expedition™ GPS
- 2) County: Crow Wing
- 3) Type of Water Body: Meso-Eutrophic, Septic Input Lake
- 4) Collection Gear: Seine, Boat Electro-shocker
- 5) Other Samples: Passive Samplers, Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	0	Lake iced out Winter 2007-08					
	F	0						
Minnow	M	0						
	F	0						
Perch	M	0						
	F	0						
Shiner	M	0						
	F	0						
Caged Minnow	M	10	2.2	59	48.9	1.2±0.2	1.4±0.2	1.1±0.05

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	0	-	-	-	
	F	0	-	-	-	
Minnow	M	0	-	-	-	
	F	0	-	-	-	
Perch	M	0	-	-	-	
	F	0	-	-	-	
Shiner	M	0	-	-	-	
	F	0	-	-	-	
Caged Minnow	M	10	9.4±2	0	0	n/a

**Table 15.**

Stewart Lake (08LST)

Fish Collection Information:

- 1) GPS Coordinates: Lat 47.18246 Long -91.75473; iFINDER® Expedition™ GPS
- 2) County: Crow Wing
- 3) Type of Water Body: Oligotrophic, Septic Input Lake
- 4) Collection Gear: Seine, Boat Electro-shocker
- 5) Other Samples: Passive Samplers, Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	19	88.3	151.6	124	1.1±0.4	1.2±0.1	2.3±0.04
	F	13	90.8	154.6	126	6±0.9	1.8±0.1	2.2±0.05
Minnow	M	0	-	-	-			
	F	0	-	-	-			
Perch	M	16	69.5	172.9	142	0.3±0.1	1.1±0.06	1.3±0.04
	F	28	85.2	183	155	0.9±0.2	1.2±0.09	1±0.03
Shiner	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Caged Minnow	M	0	-	-	-	-	-	-

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	19	155±147	2	3	9.1%
	F	14	1,708±268	n/a	n/a	n/a
Minnow	M	0	-	-	-	
	F	0	-	-	-	
Perch	M	17	7.3±4.7	1	3	3.8%
	F	28	192±133	n/a	n/a	n/a
Shiner	M	0	-	-	-	
	F	0	-	-	-	
Caged Minnow	M	0	-	-	-	n/a

**Table 16.**

Elk Lake (07LEL)

Fish Collection Information:

- 1) GPS Coordinates: Lat 47.19646 Long -95.22157; iFINDER® Expedition™ GPS
- 2) County: Clearwater
- 3) Type of Water Body: Meso-Eutrophic, Reference Lake
- 4) Collection Gear: Seine, Boat Electro-shocker
- 5) Other Samples: Passive Samplers, Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	21	49.6	125.7	107	0.4±0.1	1.2±0.07	1.8±0.05
	F	25	75.6	154.5	125	1.6±0.1	1.4±0.06	1.8±0.04
Minnow	M	14	3	67.64	56.6	1.1±0.2	1.4±0.1	0.9±0.05
	F	22	2.2	61.73	51.2	13±2	1.9±0.1	0.9±0.03
Perch	M	2	14.4	111	95.5	0.4±0.2	1.2±0.2	1±0.02
	F	24	84.2	176.5	147	0.7±0.06	1.2±0.04	1.1±0.02
Shiner	M	13	3.2	68.23	57.1	2.2±0.3	1±0.1	0.8±0.03
	F	26	2.1	62.77	51.7	6±0.6	1±0.1	0.8±0.02
Caged Minnow	M	14	2.6	61.9	50.6	1±0.09	1.4±0.1	1.1±0.3

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	20	529±201	0	11	59%
	F	25	897±236	n/a	n/a	n/a
Minnow	M	15	837±624	0	0	20.1%
	F	17	4,168±1,150	n/a	n/a	n/a
Perch	M	2	3.7±3.7	0	0	2.1%
	F	23	179±73	n/a	n/a	n/a
Shiner	M	9	69±20	0	0	5.6%
	F	21	1,243±421	n/a	n/a	n/a
Caged Minnow <sup>1</sup>	M	13	8.1±3	1	0	0.2%

<sup>1</sup> To calculate caged FHM VTG ratio, caged male VTG was multiplied by 100/ Female-wild-FHM VTG

**Table 17.**

Kabetogama Lake (10LKA)

Fish Collection Information:

- 1) GPS Coordinates: Lat 48.44423 Long -93.02840; iFINDER® Expedition™ GPS
- 2) County: St. Louis
- 3) Type of Water Body: Oligotrophic, Septic Input Lake
- 4) Collection Gear: Seine
- 5) Other Samples: Passive Samplers, Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Minnow	M	15	3.2	67	57.3	1.9±0.1	1±0.02	1±0.02
	F	32	2.8	65.75	55.4	16±0.6	4.4±1.1	1±0.01
Perch	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Shiner	M	3	1.9	61.67	51.7	1.2±0.3	0.8±0.05	0.8±0.05
	F	0	-	-	-	-	-	-
Caged Minnow	M	7	2.6	64.1	53.1	0.95±0.2	1.1±0.08	1±0.05

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	0	-	-	-	
	F	0	-	-	-	
Minnow	M	15	893±272	0	0	11.7%
	F	30	7,661±577	n/a	n/a	n/a
Perch	M	0	-	-	-	
	F	0	-	-	-	
Shiner	M	3	295±177	0	1	
	F	0	-	-	-	
Caged Minnow <sup>1</sup>	M	6	40±21	0	2	0.5%

<sup>1</sup> To calculate caged FHM VTG ratio, caged male VTG was multiplied by 100/ Female-wild-FHM VTG

**Table 18.**

Shingobee Lake (09LSH)

Fish Collection Information:

- 1) GPS Coordinates: Lat 46.99936 Long -94.68737; iFINDER® Expedition™ GPS
- 2) County: Hubbard
- 3) Type of Water Body: Oligotrophic, Septic Input Lake
- 4) Collection Gear: Boat Electro-shocker
- 5) Other Samples: Passive Samplers, Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	16	96.6	146.5	121	0.5±0.1	0.9±0.07	4.7±2.6
	F	6	101.7	160.7	137	5±1.2	1.3±0.2	2.2±0.1
Minnow	M	6	2.1	64.17	54.2	1.3±0.2	0.8±0.2	0.8±0.04
	F	5	2	61.8	51.6	8.3±1	1.5±0.2	0.9±0.07
Perch	M	13	12.1	106.2	91.5	0.7±0.1	1±0.05	1±0.03
	F	27	24.8	130.2	111	0.7±0.03	1±0.03	1±0.02
Shiner	M	4	4.6	81.5	66.5	0.6±0.1	1±0.09	0.8±0.04
	F	5	2.1	61.8	51.4	10±1.4	1.3±0.2	0.9±0.02
Caged Minnow	M	17	1.9	57.4	47.4	0.8±0.08	0.94±0.1	0.97±0.03

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	16	58±50	4	1	31.2%
	F	6	183±179	n/a	n/a	n/a
Minnow	M	6	170±67	0	0	10.9%
	F	5	1,562±837	n/a	n/a	n/a
Perch	M	12	2.9±1	0	0	39.7%
	F	26	7.3±1.1	n/a	n/a	n/a
Shiner	M	4	82±35	0	0	0.8%
	F	5	10,156±9,673	n/a	n/a	n/a
Caged Minnow <sup>1</sup>	M	15	6.2±2	1	0	0.4%

<sup>1</sup> To calculate caged FHM VTG ratio, caged male VTG was multiplied by 100/ Female-wild-FHM VTG

## Table 19.

Lake Superior – Two Harbors Site (12LTH)

### Fish Collection Information:

- 1) GPS Coordinates: Lat 47.0174 Long -91.66425; iFINDER® Expedition™ GPS
- 2) County:
- 3) Type of Water Body: Oligotrophic, Septic Input Lake
- 4) Collection Gear: NA
- 5) Other Samples: Water Samples, Caged Fathead Minnows

*Note:* The mobile exposure laboratory was set up in July 2008 at the Wastewater Treatment Plant in Two Harbors, MN. Unfortunately, a pulse of effluent produced increased levels of ammonia in the effluent of the wastewater treatment plant that resulted in unacceptably higher mortality among the exposed fathead minnows, forcing us to end the experiment and to exclude biological data from this site.

**Table 20.**

Northern Light Lake (11LNL)

Fish Collection Information:

- 1) GPS Coordinates: Lat 47.90807 Long -90.25210; iFINDER® Expedition™ GPS
- 2) County: Cook
- 3) Type of Water Body: Oligotrophic, Reference Lake
- 4) Collection Gear: Boat Electro-shocker
- 5) Other Samples: Passive Samplers, Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Minnow	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Perch	M	7	33.8	131.3	112	0.4±0.2	1±0.08	1.2±0.03
	F	3	46.7	151	129	0.4±0.2	0.8±0.03	1.1±0.06
Shiner	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Caged Minnow	M	16	2	58.8	47.9	0.9±0.08	1.1±0.07	0.98±0.04

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	0	-	-	-	
	F	0	-	-	-	
Minnow	M	0	-	-	-	
	F	0	-	-	-	
Perch	M	7	3.5±1.3	0	0	66%
	F	3	5.3±2.7	n/a	n/a	n/a
Shiner	M	0	-	-	-	
	F	0	-	-	-	
Caged Minnow	M	14	7.3±4	0	2	n/a



**Table 21.**

Le Sueur River (14RLS)

Fish Collection Information:

- 1) GPS Coordinates: Lat 44.01466 Long -93.52647; iFINDER® Expedition™ GPS
- 2) County: Blue Earth
- 3) Type of Water Body: Agriculturally Dominated River, Downstream Site
- 4) Collection Gear: Backpack Electro-shocker
- 5) Other Samples: Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	1	18.001	101	86	0.3	1.2	1.7
	F	0	-	-	-	-	-	-
Minnow	M	21	2.27181	61.05	50.8	1.6±0.4	2.7±0.1	1±0.02
	F	15	1.45993	52.53	43.7	5.9±0.7	2.2±0.2	1±0.04
Perch	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Shiner	M	15	2.1544	63	52.4	1±0.2	1.2±0.1	0.9±0.02
	F	11	1.99527	61.09	50.1	11±1.6	2.3±0.2	0.9±0.05
Caged Minnow	M	24	2.1	59	48.8	0.86±0.08	1±0.06	1±0.02

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	1	0	0	0	n/a
	F	0	-	-	-	n/a
Minnow	M	18	265±192	4	0	4.3%
	F	13	6110±1983	n/a	n/a	n/a
Perch	M	0				
	F	0				
Shiner	M	13	532±91	0	0	43.4%
	F	10	1226±243	n/a	n/a	n/a
Caged Minnow <sup>1</sup>	M	22	33±6	1	1	0.5%

<sup>1</sup> To calculate caged FHM VTG ratio, caged male VTG was multiplied by 100/ Female-wild-FHM VTG

**Table 22.**

Little Cobb River (13RLC)

Fish Collection Information:

- 1) GPS Coordinates: Lat 43.999738 Long -93.828832; iFINDER® Expedition™ GPS
- 2) County: Blue Earth
- 3) Type of Water Body: Agriculturally Dominated River, Downstream Site
- 4) Collection Gear: Seine, Backpack Electro-shocker
- 5) Other Samples: Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	4	12.6573	84	71.5	1.3±0.4	1.4±0.3	1.8±0.06
	F	2	4.745	71.5	63	13±4.1	3.3±1.3	1.3±0.3
Minnow	M	12	2.44683	59.33	50.1	2.2±0.3	2.2±0.3	1.1±0.05
	F	10	1.724	53.6	45.1	8.6±0.9	3.1±1	1.1±0.07
Perch	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Shiner	M	12	2.72617	63.67	52.8	1.1±0.08	1.1±0.08	0.9±0.03
	F	11	2.04982	59.18	49.5	8.4±1	1.6±0.1	1±0.03
Caged Minnow	M	26	2.3	59.8	49.6	1.1±0.1	1.2±0.1	1.1±0.02

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	4	3.8±1.3	0	0	0%
	F	2	728±622	n/a	n/a	n/a
Minnow	M	10	4987±1650	0	0	560% !!!
	F	7	890±454	n/a	n/a	n/a
Perch	M	0	-	-	-	
	F	0	-	-	-	
Shiner	M	12	297±153	0	0	37.2%
	F	8	798±176	n/a	n/a	n/a
Caged Minnow <sup>1</sup>	M	11	7.5±1.6	1	0	0.8%

<sup>1</sup> To calculate caged FHM VTG ratio, caged male VTG was multiplied by 100/ Female-wild-FHM VTG

**Table 23.**

7 Mile River – Upstream Site (15R7A)

Fish Collection Information:

- 1) GPS Coordinates: Lat 44.29110 Long -94.07558; iFINDER® Expedition™ GPS
- 2) County: Nicollet
- 3) Type of Water Body: Agriculturally Dominated River, Upstream Site
- 4) Collection Gear: Backpack Electro-shocker
- 5) Other Samples: Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Minnow	M	19	2.55211	62.37	51.7	1.6±0.2	1.6±0.1	1±0.02
	F	3	2.13067	61.33	50	3.7±0.8	2.1±0.4	0.9±0.08
Perch	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Shiner	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Caged Minnow	M	22	2.1	57.9	48.1	0.7±0.08	1.2±0.1	1.1±0.02

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	0	-	-	-	
	F	0	-	-	-	
Minnow	M	19	2106±748	0	0	22.2%
	F	3	9475±4565	n/a	n/a	n/a
Perch	M	0	-	-	-	
	F	0	-	-	-	
Shiner	M	0	-	-	-	
	F	0	-	-	-	
Caged Minnow <sup>1</sup>	M	19	4.9±0.6	2	3	0%

<sup>1</sup> To calculate caged FHM VTG ratio, caged male VTG was multiplied by 100/ Female-wild-FHM VTG

**Table 24.**

7 Mile River – Midstream Site (16R7B)

Fish Collection Information:

- 1) GPS Coordinates: Lat 44.26807 Long -94.04393; iFINDER® Expedition™ GPS
- 2) County: Nicollet
- 3) Type of Water Body: Agriculturally Dominated River, Midstream Site
- 4) Collection Gear: Backpack Electro-shocker
- 5) Other Samples: Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Minnow	M	3	3.76233	72.67	62.3	0.7±0.1	1.2±0.2	1±0.07
	F	4	2.0525	60.75	51.3	9.3±1.5	2.1±0.5	0.9±0.05
Perch	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Shiner	M	16	2.12194	63.06	52.9	1.6±0.3	1±0.1	0.8±0.01
	F	5	2.0652	64	53	7.2±1.1	1.6±0.2	0.8±0.009
Caged Minnow	M	26	2.3	60.8	50.9	1±0.1	1.1±0.08	1±0.02

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	0	-	-	-	
	F	0	-	-	-	
Minnow	M	3	3987±2,072	1	0	65%
	F	4	6132±4341	n/a	n/a	n/a
Perch	M	0	-	-	-	
	F	0	-	-	-	
Shiner	M	15	776±195	0	5	118.5%
	F	5	655±346	n/a	n/a	n/a
Caged Minnow <sup>1</sup>	M	11	3.2±0.2	3	0	0%

<sup>1</sup> To calculate caged FHM VTG ratio, caged male VTG was multiplied by 100/ Female-wild-FHM VTG

**Table 25.**

7 Mile River – Downstream Site (17R7C)

Fish Collection Information:

- 1) GPS Coordinates: Lat 44.29111 Long -94.07518; iFINDER® Expedition™ GPS
- 2) County: Nicollet
- 3) Type of Water Body: Agriculturally Dominated River, Downstream Site
- 4) Collection Gear: Backpack Electro-shocker
- 5) Other Samples: Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Minnow	M	17	2.54924	63.12	53.9	0.7±0.1	1.6±0.07	1±0.02
	F	7	1.90157	57.57	49	12±1.3	1.4±0.1	1±0.03
Perch	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Shiner	M	12	1.91208	59.25	49.3	0.8±0.09	1±0.07	0.9±0.02
	F	8	1.80813	57.88	48.4	5.1±0.5	1.3±0.2	0.9±0.03
Caged Minnow	M	27	2.1	59.1	48.9	1.1±0.09	0.9±0.07	1±0.02

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	0	-	-	-	
	F	0	-	-	-	
Minnow	M	16	1,329±275	1	0	226.4%
	F	6	587±544	n/a	n/a	n/a
Perch	M	0	-	-	-	
	F	0	-	-	-	
Shiner	M	12	36±12	0	1	14.4%
	F	8	251±76	n/a	n/a	n/a
Caged Minnow <sup>1</sup>	M	23	3.9±0.3	7	2	0.7%

<sup>1</sup> To calculate caged FHM VTG ratio, caged male VTG was multiplied by 100/ Female-wild-FHM VTG

**Table 26.**

Redwood River – Upstream Site (18RRA)

Fish Collection Information:

- 1) GPS Coordinates: Lat 44.4775 Long -95.776389; Google Earth
- 2) County: Lyon
- 3) Type of Water Body: WWTP Influenced River, Upstream Site
- 4) Collection Gear: Backpack Electro-shocker
- 5) Other Samples: Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Minnow	M	7	2±0.2	58±1.4	-	-	-	1±0.05
	F	0	-	-	-	-	-	-
Perch	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Shiner	M	15	19±3.3	115±8.7	-	-	-	1±0.02
	F	9	5.9±0.8	84±4.7	-	-	-	0.9±0.04

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	0	-	-	-	
	F	0	-	-	-	
Minnow	M	7	0	0	0	
	F	1	0	n/a	n/a	
Perch	M	0	-	-	-	
	F	0	-	-	-	
Shiner	M	11	3±1.6	0	0	0.1%
	F	5	3,259±1,882	n/a	n/a	n/a

**Table 27.**

Redwood River – Downstream 1 Site (19RRB)

Fish Collection Information:

- 1) GPS Coordinates: Lat 44.486806 Long -95.766111; Google Earth
- 2) County: Lyon
- 3) Type of Water Body: WWTP Influenced River, Downstream 1 Site
- 4) Collection Gear: Backpack Electro-shocker
- 5) Other Samples: Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Minnow	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Perch	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Shiner	M	19	16±2.2	110±4.3	-	-	-	1.1±0.04
	F	9	8.3±0.8	92±2.9	n/a	n/a	n/a	1±0.02

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	0	-	-	-	
	F	0	-	-	-	
Minnow	M	0	-	-	-	
	F	0	-	-	-	
Perch	M	0	-	-	-	
	F	0	-	-	-	
Shiner	M	18	1.3±0.7	0	0	0.6%
	F	4	232±11	n/a	n/a	n/a

**Table 28.**

Redwood River – Downstream 2 Site (20RRC)

Fish Collection Information:

- 1) GPS Coordinates: Lat 44.486806 Long -95.766111; Google Earth
- 2) County: Lyon
- 3) Type of Water Body: WWTP Influenced River, Downstream 2 Site
- 4) Collection Gear: Backpack Electro-shocker
- 5) Other Samples: Water Samples, Caged Fathead Minnows

Field Data								
Fish Species	Sex	n	Average Weight (g)	Average Total Length (mm)	Average Standard Length (mm)	Gonadosomatic Index	Hepatosomatic Index	Body Condition Index
Sunfish	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Minnow	M	2	2.1±0.9	57±8.5	-	-	-	1.1
	F	0	-	-	-	-	-	-
Perch	M	0	-	-	-	-	-	-
	F	0	-	-	-	-	-	-
Shiner	M	14	21±2.1	123±4.3	-	-	-	1.1±0.03
	F	7	15±4.4	106±11	-	-	-	1.1±0.04

Lab Data						
Fish Species	Sex	n	Vtg (µg/mL)	Histological Data (Abundance)		
				# of Intersex	# of Testis Feminization	VTG Male/Female Ratio (100%/Female VTG)*Male VTG
Sunfish	M	0	-	-	-	
	F	0	-	-	-	
Minnow	M	2	0	0	0	
	F	0	-	-	-	
Perch	M	0	-	-	-	
	F	0	-	-	-	
Shiner	M	14	0.4±0.2	0	0	1.1%
	F	5	36.6±34.9	n/a	n/a	n/a



Table 29. Normalized plasma vitellogenin (VTG) levels in fish.

	VTG Male/Female Ratio (100%/Female VTG) * Male VTG				
Location	Sunfish	Minnow	Perch	Shiner	Caged fathead minnow
01 Cedar	0.6	14			0.6
02 Owassa	1	47.5		28.3	1
03 Budd	0	1.3		8	1.6
04 White Sand	105.7			44.7	
05 Red Sand					
06 Sullivan	0.1				
08 Stewart	9.1		3.8		
09 Shingobee	31.2	10.9	39.7	0.8	0.4
10 Kabetogama		11.7			0.5
11 Northern Light			66		
07 Elk	59	20.1	2.1	5.6	0.2
12 Superior					
13 Little Cobb	0	560		37.2	0.8
14 Le Sueur		4.3		46.4	0.5
15 7 Mile Up		22.2			0
16 7 Mile Mid		65		118.5	0
17 7 Mile Down		226.4		14.4	0.7
18 Redwood Up				0.1	
19 Redwood Down 1				0.6	
20 Redwood Down 2				1.1	

Table 30. Relative ranking of lakes by matrix sampled

	<b>Rank (water)</b>		<b>Rank (sediment)</b>		<b>Rank (POCIS)</b>
Lake Owasso	6.2	Lake Owasso	1.8	Cedar Lake	4.1
Elk Lake	6.5	Shingobee Lake	2.5	Sullivan Lake	4.3
Northern Light Lake	6.8	Lake Kabetogama	2.8	Stewart Lake	4.8
Cedar Lake (avg)	7	White Sand Lake	3.9	Lake Owasso	5
White Sand Lake	7.1	Red Sand Lake	4.3	Red Sand Lake	5.3
Shingobee Lake	7.2	Elk Lake	4.3	Elk Lake	5.6
Sullivan Lake	7.7	Sullivan Lake	4.5	White Sand Lake	6.3
Budd Lake	8.4	Northern Light Lake	5.6	Shingobee Lake	6.4
Stewart Lake	8.5	Stewart Lake	6.1	Lake Kabetogama	6.5
Red Sand Lake	9.3	Budd Lake	6.5	Budd Lake	6.8
Lake Kabetogama	9.3	Cedar Lake	6.9	Northern Light Lake	7.1

(Lower value indicates higher relative concentrations were measured, individual compounds aggregated )

Table 31. Maximum concentrations of organic wastewater compounds in surface water

	Statewide EDC Study	Tributary Study**	Mississippi River Study	2002 Minnesota reconnaissance study	
Maximum concentrations detected (ppb)					
	Lakes	Rivers*			
Bisphenol A	0.0379	0.054	0.137	2.76	1.5
DEET	0.5794	0.554	0.7	0.224	0.37
4-nonylphenol	0.2138	0.858	0.88	ND	2.6
Nonylphenoltetraethoxylate (NP4EO)	ND	0.057	0.15		
Nonylphenoltriethoxylate (NP3EO)	0.123	0.112	0.87		
Nonylphenoldiethoxylate (NP2EO)	0.1705	ND	0.37	ND	34
Nonylphenolmonoethoxylate (NP1EO)	0.106	ND	0.66	ND	
Octylphenoltetraethoxylate (OP4EO)	ND	ND	0.0153		
Octylphenoltriethoxylate (OPEO3)	0.0138	ND	0.11		
Octylphenoldiethoxylate (OPEO2)	0.0427	0.076	0.13	0.43	ND
Octylphenolmonoethoxylate (OPEO1)	ND	ND	0.028	0.208	ND
4-octylphenol	ND	0.0137	0.01459	ND	1.6
tert-octylphenol	0.0102	0.0176	0.11		ND
Triclosan	ND	0.0151	0.15	ND	0.31
Cholesterol	0.658	0.348	2.2	1.69	6
Caffeine	0.129	0.838	3	ND	0.52

\* Little Cobb, Seven Mile Creek, LeSueur, and Redwood Rivers.

\*\* Crow, South Fork, Grindstone, and Redwood Rivers.

Table 32. Maximum concentrations of organic wastewater compounds in sediment.

	Statewide EDCStudy	Tributary Study**	Mississippi River Study	
Maximum concentrations detected (ppb)				
	Lakes	Rivers*		
Bisphenol A	35.4	9.3	ND	18.2
DEET	ND	ND	ND	ND
4-nonylphenol	223.9	137	260	2024
Nonylphenoltetraethoxylate (NP4EO)	ND	ND	ND	
Nonylphenoltriethoxylate (NP3EO)	61.8	ND	ND	
Nonylphenoldiethoxylate (NP2EO)	29.3	49.9	ND	1140
Nonylphenolmonoethoxylate (NP1EO)	ND	61.5	17	1389
Octylphenoltetraethoxylate (OP4EO)	ND	ND	ND	
Octylphenoltriethoxylate (OPEO3)	ND	ND	ND	
Octylphenoldiethoxylate (OPEO2)	73.9	48.6	ND	ND
Octylphenolmonoethoxylate (OPEO1)	3.2	ND	ND	54
4-octylphenol	ND	ND	ND	5.08
tert-octylphenol	98.15	3.8	ND	57
Triclosan	33.19	35.7	ND	78.3
Cholesterol	3397	1478	820	14600
Caffeine	71.2	ND		

\* Little Cobb, Seven Mile Creek, LeSueur, and Redwood Rivers.

\*\* Crow, South Fork, Grindstone, and Redwood Rivers.

**Table 33. Maximum concentrations of organic wastewater compounds in surface water by lake category**

<b>Statewide EDC Study</b>				
<b>Maximum concentrations detected (ppb)</b>				
	<b>Urban</b>	<b>Septic</b>	<b>Reference</b>	<b>Two Harbors effluent</b>
<b>Bisphenol A</b>	<b>0.0379</b>	<b>0.02</b>	<b>0.0124</b>	<b>0.0456</b>
<b>DEET</b>	<b>90</b>	<b>0.5794</b>	<b>0.2178</b>	1.855
<b>4-nonylphenol</b>	ND	<b>0.1108</b>	<b>0.2138</b>	1.456
<b>Nonylphenoltetraethoxylate (NP4EO)</b>	ND	ND	ND	0.0605
<b>Nonylphenoltriethoxylate (NP3EO)</b>	ND	<b>0.123</b>	ND	0.5196
<b>Nonylphenoldiethoxylate (NP2EO)</b>	ND	<b>0.1705</b>	<b>0.0658</b>	1.484
<b>Nonylphenolmonoethoxylate (NP1EO)</b>	<b>0.0591</b>	<b>0.0863</b>	<b>0.106</b>	<b>0.867</b>
<b>Octylphenoltetraethoxylate (OP4EO)</b>	ND	ND	ND	ND
<b>Octylphenoltriethoxylate (OPEO3)</b>	ND	<b>0.0138</b>	ND	ND
<b>Octylphenoldiethoxylate (OPEO2)</b>	<b>0.0258</b>	<b>0.0337</b>	<b>0.0427</b>	ND
<b>Octylphenolmonoethoxylate (OPEO1)</b>	ND	<b>ND</b>	ND	ND
<b>4-octylphenol</b>	ND	ND	ND	ND
<b>tert-octylphenol</b>	ND	<b>0.0102</b>	ND	0.129
<b>Triclosan</b>	ND	ND	ND	0.573
<b>Caffeine</b>	<b>129.4</b>	<b>0.0138</b>	<b>0.0193</b>	<b>13.815</b>

Table 34. Maximum concentrations of organic wastewater compounds in sediment by lake category.

	Statewide EDCStudy		
	Maximum concentrations detected (ppb)		
	Urban	Septic	Reference
<b>Bisphenol A</b>	<b>19.52</b>	<b>35.44</b>	<b>7.67</b>
<b>DEET</b>	ND	ND	ND
<b>4-nonylphenol</b>	ND	<b>223.88</b>	ND
<b>Nonylphenoltetraethoxylate (NP4EO)</b>	ND	ND	ND
<b>Nonylphenoltriethoxylate (NP3EO)</b>	ND	<b>61.83</b>	ND
<b>Nonylphenoldiethoxylate (NP2EO)</b>	ND	<b>29.31</b>	ND
<b>Nonylphenolmonoethoxylate (NP1EO)</b>	ND	ND	ND
<b>Octylphenoltetraethoxylate (OP4EO)</b>	ND	ND	ND
<b>Octylphenoltriethoxylate (OPEO3)</b>	ND	ND	ND
<b>Octylphenoldiethoxylate (OPEO2)</b>	ND	<b>73.89</b>	ND
<b>Octylphenolmonoethoxylate (OPEO1)</b>	ND	<b>3.23</b>	ND
<b>4-octylphenol</b>	ND	ND	ND
<b>tert-octylphenol</b>	<b>98.15</b>	<b>33.14</b>	<b>4.92</b>
<b>Triclosan</b>	<b>33.19</b>	ND	ND
<b>Caffeine</b>	<b>71.21</b>	ND	ND

**Table 35. Number of organic contaminants detected in surface water by lake category.**

<b>Surrounding development</b>	<b>Number OWC compounds detected</b>	<b>Number hormones detected</b>	<b>Total</b>
<b>Urban</b>	<b>10</b>	<b>2</b>	<b>12</b>
<b>Septic</b>	<b>15</b>	<b>5</b>	<b>20</b>
<b>Reference</b>	<b>11</b>	<b>3</b>	<b>14</b>

**Table 36. Number of organic contaminants detected in sediment by lake category.**

<b>Surrounding development</b>	<b>Number OWC compounds</b>	<b>Number of pharmaceuticals</b>	<b>Total</b>
<b>Urban</b>	<b>8</b>	<b>3</b>	<b>11</b>
<b>Septic</b>	<b>12</b>	<b>4</b>	<b>16</b>
<b>Reference</b>	<b>6</b>	<b>2</b>	<b>8</b>

## Appendix A

### List of compounds, sources, and their uses

<b>Organic Wastewater Compound</b>	<b>Application/description</b>
<b>1,4-dichlorobenzene</b>	Moth repellent, deodorant
<b>2,6-di-tert-butyl-1,4-benzoquinone</b>	
<b>2,6-di-tert-butyl-4-methylphenol</b>	BHT. Antioxidant food additive, also used in fuels, cosmetics, pharmaceuticals.
<b>2,6-di-tert-butylphenol</b>	One of many alkylphenols. Alkylphenols are used to synthesize detergents, fragrances, polymers, and other compounds
<b>3-b-coprostenol</b>	Breakdown product of cholesterol; indicator of fecal matter
<b>4-(tert-octyl)phenol</b>	Nonionic detergent or breakdown product of detergent
<b>4-ethylphenol</b>	One of many alkylphenols. Alkylphenols are used to synthesize detergents, fragrances, polymers, and other compounds
<b>4-methylphenol (cresol)</b>	Wood preservative; can also be naturally occurring.
<b>4-n-octylphenol</b>	Nonionic detergent or breakdown product of detergent
<b>4-nonylphenol</b>	Nonionic detergent or breakdown product of detergent
<b>4-NP1EO</b>	Nonionic detergent
<b>4-NP2EO</b>	Nonionic detergent
<b>4-NP3EO</b>	Nonionic detergent
<b>4-NP4EO</b>	Nonionic detergent
<b>4-OP2EO</b>	Nonionic detergent
<b>4-OP3EO</b>	Nonionic detergent
<b>4-propylphenol</b>	One of many alkylphenols. Alkylphenols are used to synthesize detergents, fragrances, polymers, and other compounds
<b>4-tert-butylphenol</b>	One of many alkylphenols. Alkylphenols are used to synthesize detergents, fragrances, polymers, and other compounds
<b>4-tert-pentylphenol</b>	One of many alkylphenols. Alkylphenols are used to synthesize detergents, fragrances, polymers, and other compounds



<b>5-methyl-1H-benzotriazole</b>	A common additive in de-icing fluids
<b>Bisphenol A</b>	Monomer used to synthesize polycarbonate plastic
<b>Caffeine</b>	Beverages; tracer for waste compounds; biodegradable
<b>Cholesterol</b>	Fecal indicator; also a plant sterol
<b>N,N-diethyl-m-toluamide</b>	Insect repellent (DEET)
<b>Triclosan</b>	Antimicrobial disinfectant

<b>Hormone</b>	<b>Description</b>
<b>Androsterone</b>	Steroid hormone with weak androgenic activity
<b>17<math>\alpha</math>-estradiol</b>	A female estrogenic hormone
<b>Androstenedione</b>	Precursor to testosterone and estrogen, the male and female sex hormones
<b>Estrone</b>	One of three naturally occurring estrogens; a female hormone
<b>17<math>\beta</math>-estradiol</b>	One of three naturally occurring estrogens; a female hormone
<b>Testosterone</b>	A male sex hormone and anabolic steroid
<b>Equilin</b>	An estrogen used in hormone replacement therapy
<b>11-ketotestosterone</b>	A male sex hormone
<b>Mestranol</b>	An estrogen used in oral contraceptives, converted to ethinylestradiol
<b>Equilenin</b>	An estrogen used in hormone replacement therapy
<b>Ethinyl estradiol</b>	Synthetic oral contraceptive in birth control prescriptions
<b>Estriol</b>	One of three naturally occurring estrogens; a female hormone
<b>Progesterone</b>	A female steroid hormone

<b>Pharmaceutical</b>	<b>Description</b>
<b>Acetaminophen</b>	Analgesic (active ingredient in Tylenol <sup>®</sup> )
<b>Cimetidine</b>	Antacid
<b>Trimethoprim</b>	Antibiotic
<b>Carbamazepine</b>	Anticonvulsive used to treat epilepsy and attention-deficit hyperactivity disorder (ADHD)
<b>Diphenhydramine</b>	Antihistamine
<b>Fluoxetine</b>	Antidepressant drug
<b>Miconazole</b>	Topical antifungal medication
<b>Venlafaxine</b>	Antidepressant drug
<b>Citalopram</b>	Antidepressant drug
<b>Sertraline</b>	Antidepressant drug

## Appendix B

List of compounds analyzed but not detected in any of the samples:

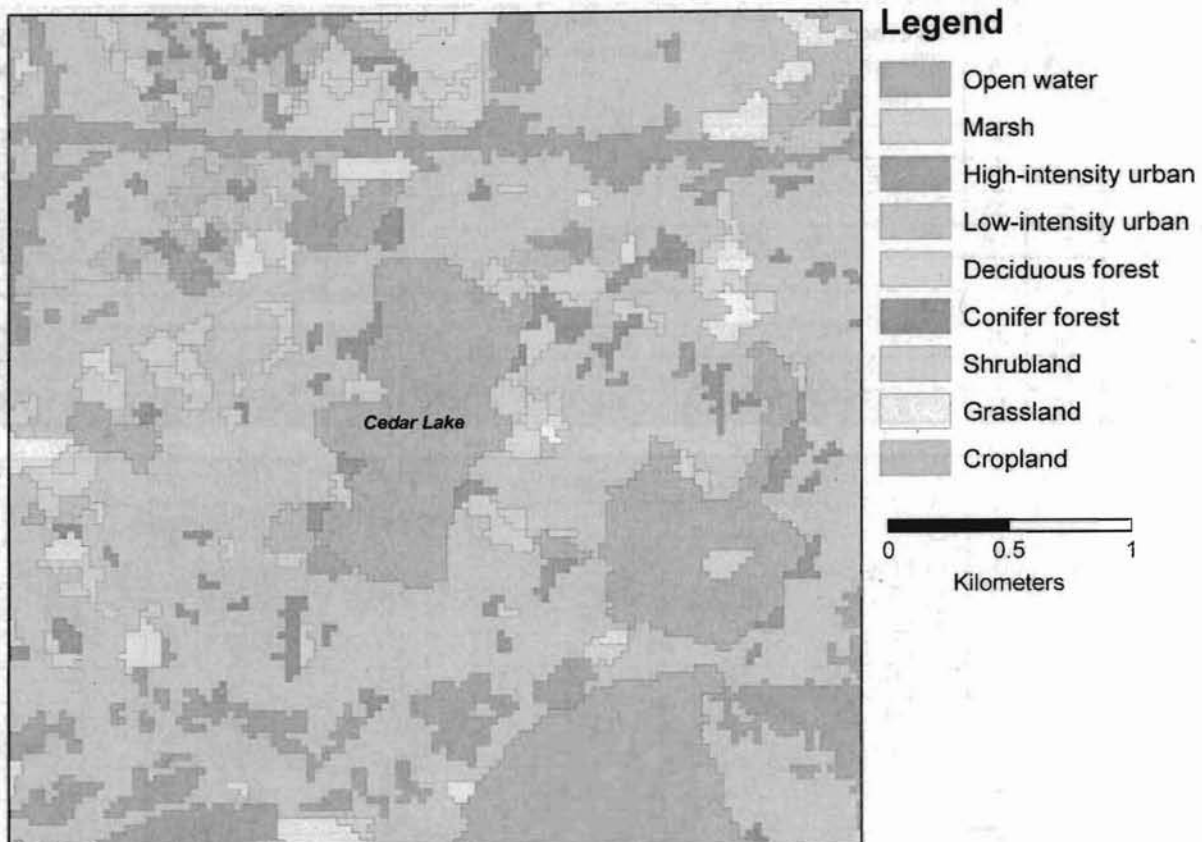
### **Water analysis:**

Epitestosterone  
Dihydrotestosterone  
Norethindrone  
Ethinylestradiol  
1,3-dichlorobenzene  
1,2-dichlorobenzene  
2[3]-tert-butyl-4-methylphenol  
4-octylphenoltetraethoxylate  
4-nonylphenolpentaethoxylate  
Cotinine

### **Sediment analysis:**

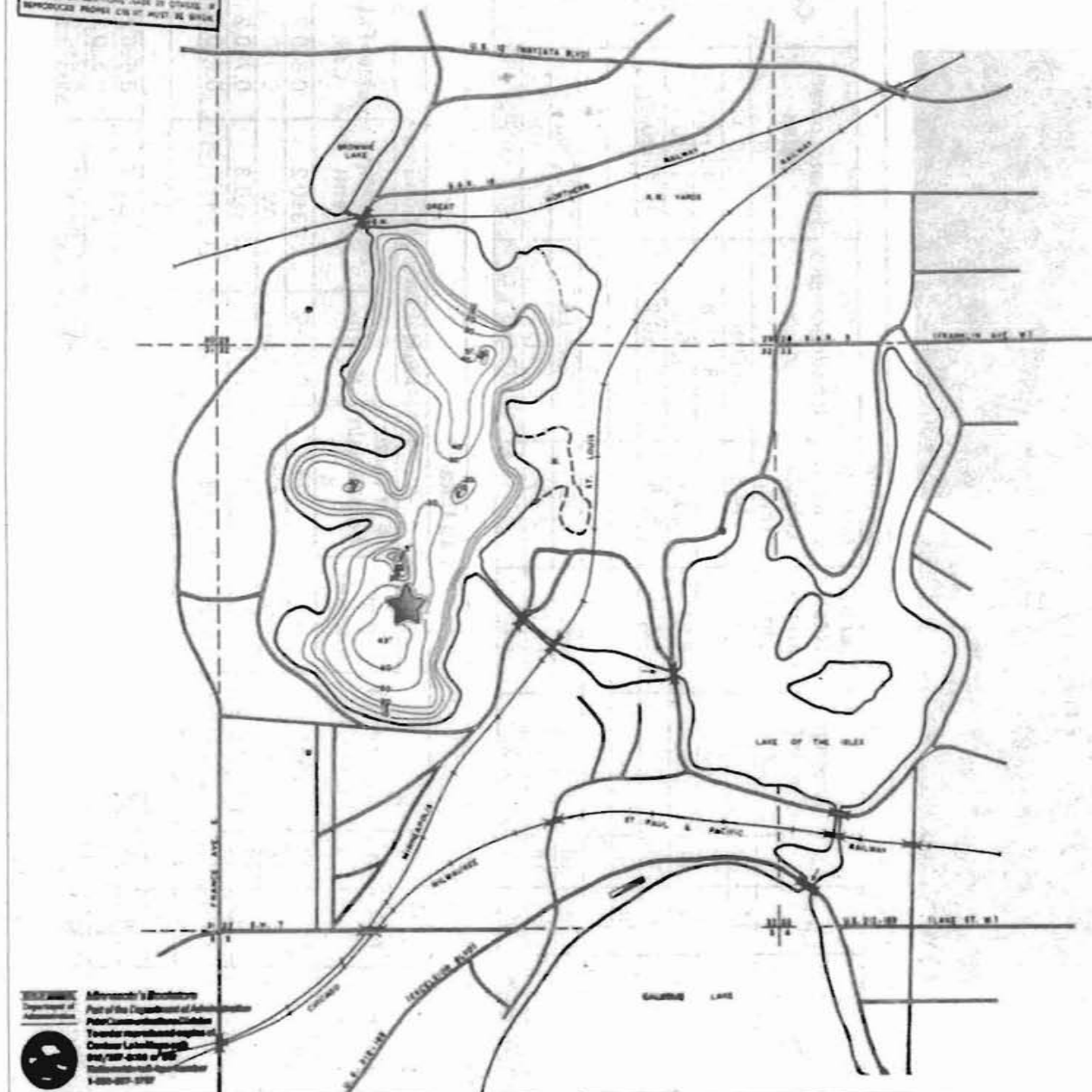
1,3-dichlorobenzene  
1,2-dichlorobenzene  
4-propylphenol  
2,6-di-tert-butyl-1,4-benzoquinone  
5-methyl-1H-benzotriazole  
N,N-dimethyl-m-toluamide  
Cotinine  
4-n-octylphenol  
4-octylphenoltriethoxylate  
4-octylphenoltetraethoxylate  
4-nonylphenoltetraethoxylate  
4-nonylphenolpentaethoxylate  
Albuterol  
1,7-dimethylxanthine  
Ranitidine  
Codeine  
Thiabendazole  
Sulfamethoxazole  
Azithromycin  
Diltiazem  
Erythromycin  
Dehydronifedipine  
Warfarin

## Land Cover Types Surrounding Cedar Lake Hennepin County, MN



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W.E. 1/4 S.W. 1/4 S.E. 1/4.  
AREA 186.5 ACRES (PLANIMETERED)  
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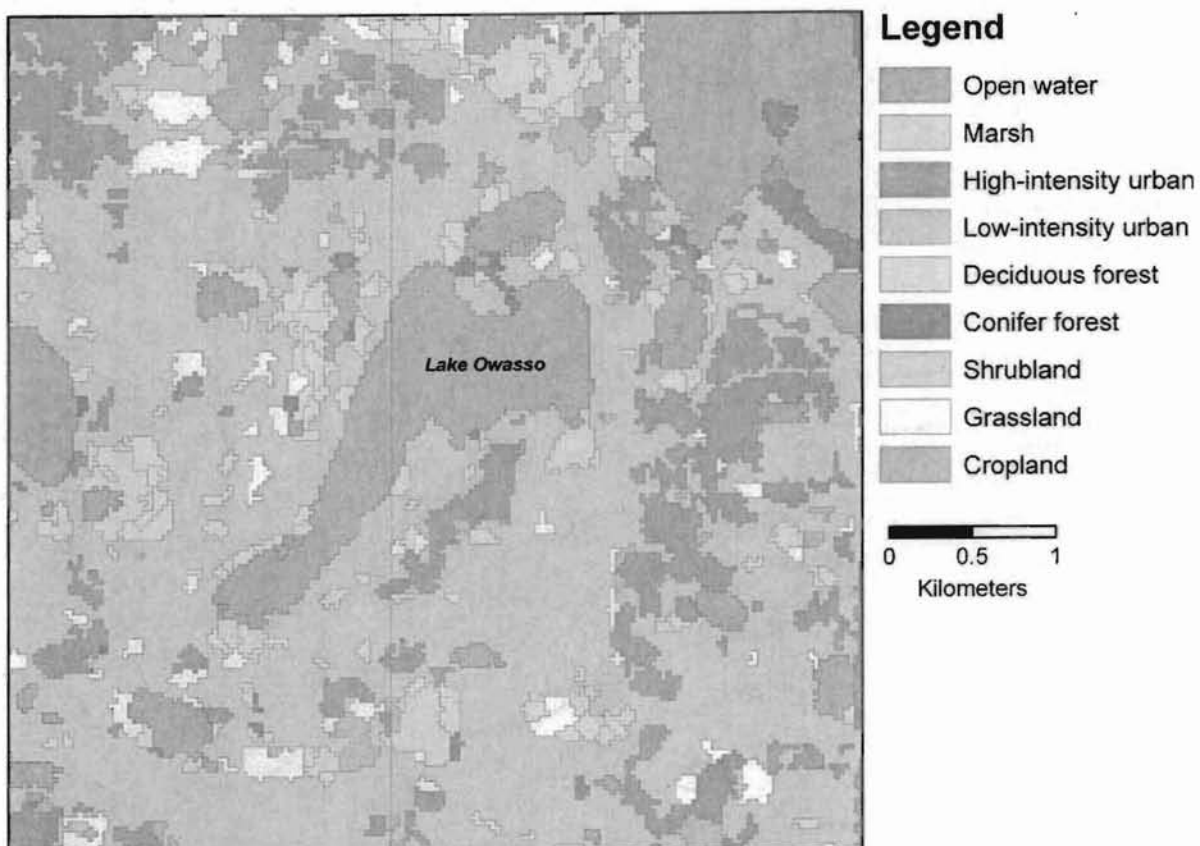
DIVISION OF GAME AND FISH  
RESEARCH AND PLANNING SECTION

CEDAR LAKE 27-39  
HENNEPIN COUNTY

TWP. 28 N.	R. 24 W.	SEC. 29 E.
RELINQUISH S.W. 1/4 S.E. 1/4	SECTION OF S.W. 1/4	PLAT. 10000 100
DATE 5-27-86	DATE 12-2-86	FILE 1-2

★ indicates position of samples.

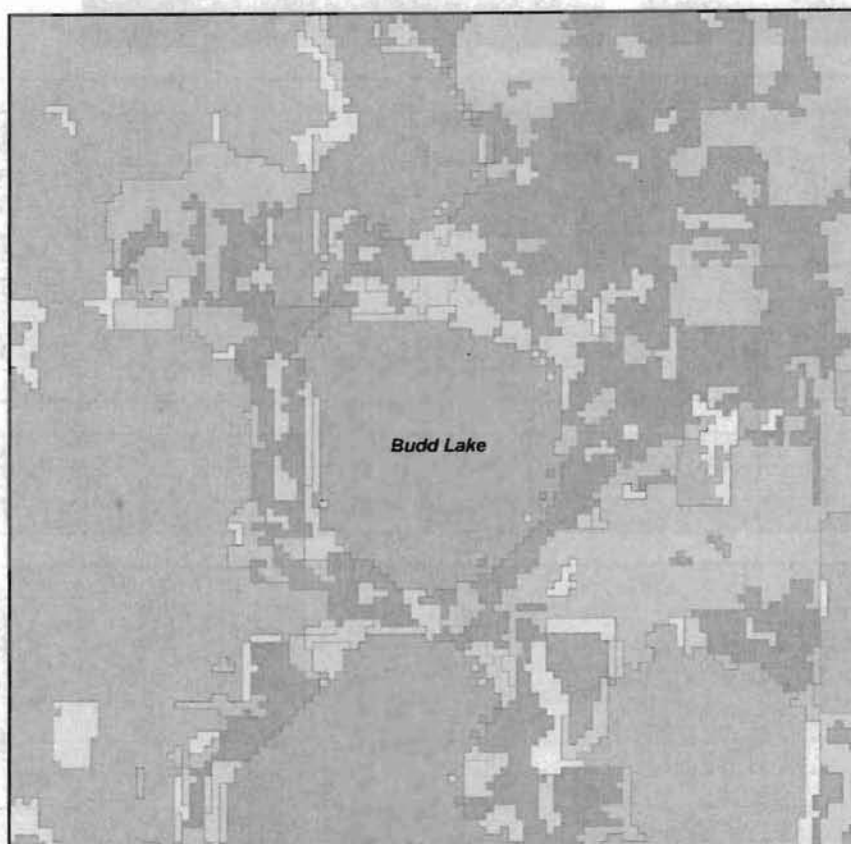
## Land Cover Types Surrounding Lake Owasso Ramsey County, MN



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## Land Cover Types Surrounding Budd Lake Martin County, MN



### Legend

- Open water
- Marsh
- High-intensity urban
- Low-intensity urban
- Deciduous forest
- Grassland
- Cropland

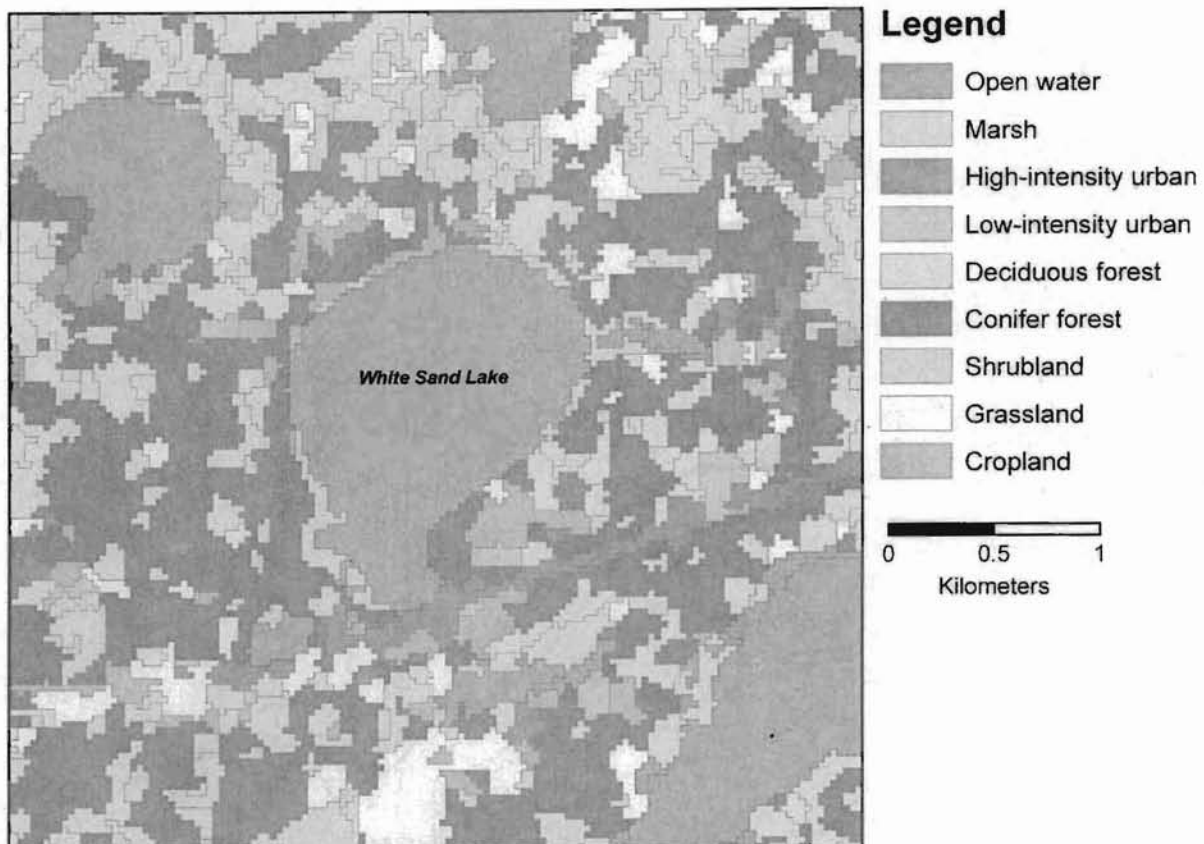
0 0.5 1  
Kilometers

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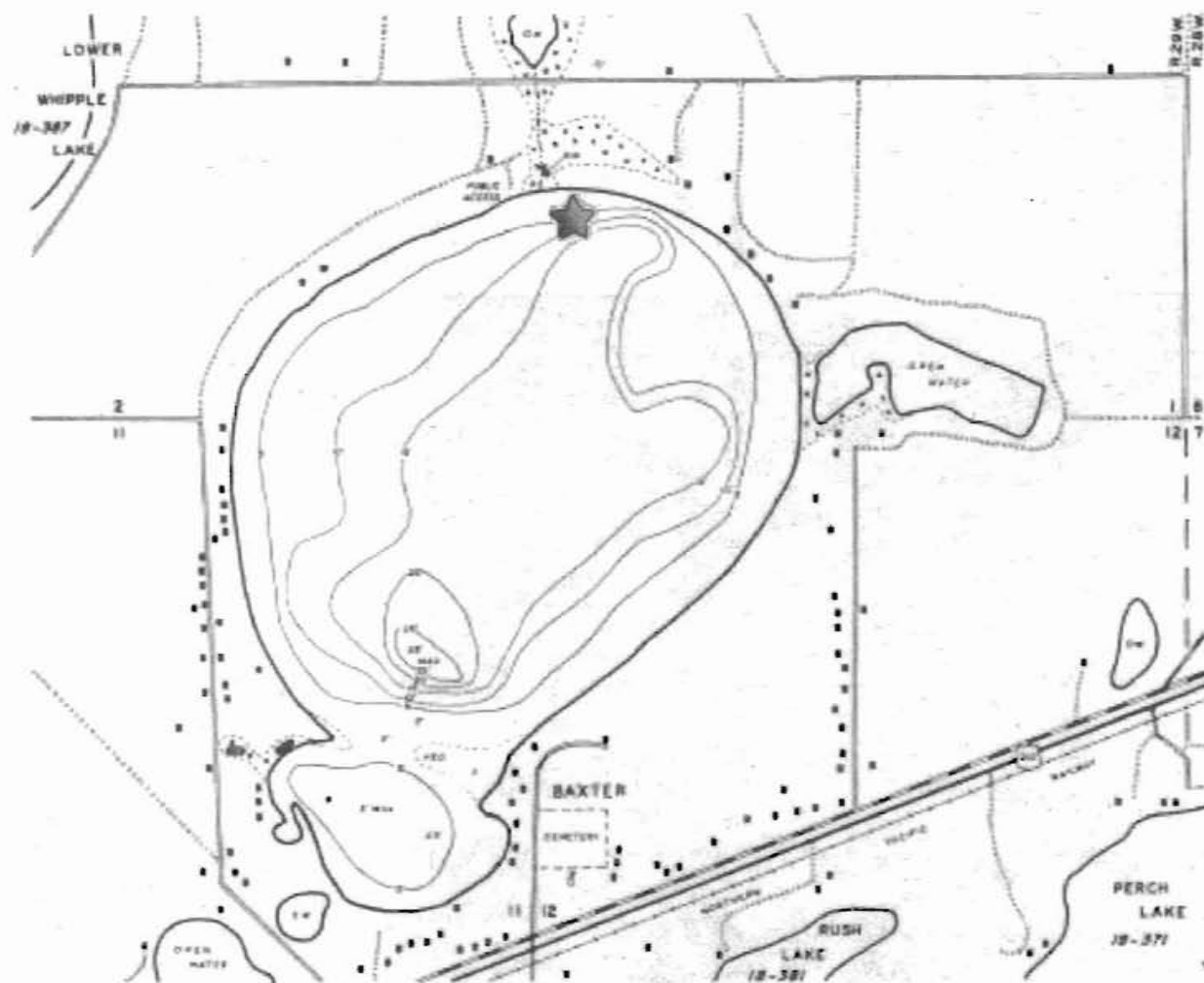




## Land Cover Types Surrounding White Sand Lake Crow Wing County, MN



Land cover types were derived by computer classification of combined two-season pairs of early-1990s Landsat 4/5 Thematic Mapper (TM) satellite imagery, as part of the Upper Midwest Gap Analysis Program of the U.S. Geological Survey.



# LEGEND

- Water (shaded)
- Public Road
- Unimproved Road
- Unimproved Road
- Intermittent Stream
- Water
- Shoreline

PLANNED AREA 1300 ACRES  
 ACTUAL AREA 1250 ACRES  
 LENGTH OF SHORELINE 12.3 MILES

S.W. QUARTER OF SOUTHEAST CORNER OF 36th  
 STRUCTURE AND EAST OF ACCESS SW  
 300' FROM SHORELINE, 100' FROM NORTH  
 CENTRAL SHORE OF LAKE  
 N.E. ELEVATION OF BUILDING 54'

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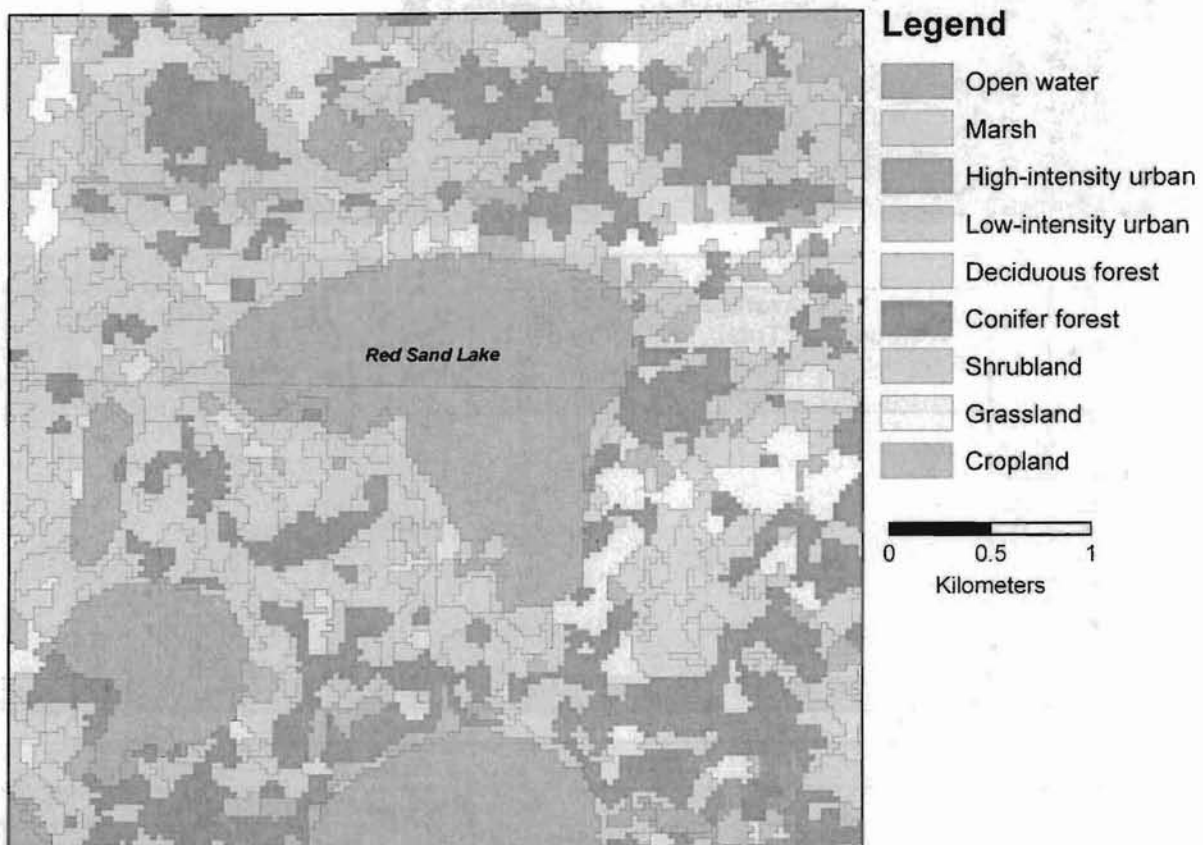


*[Signature]*

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WHITE SAND LAKE 18-379 CROW WING COUNTY		
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DATE 1-1-84	DATE 1-1-84	FW-10-12

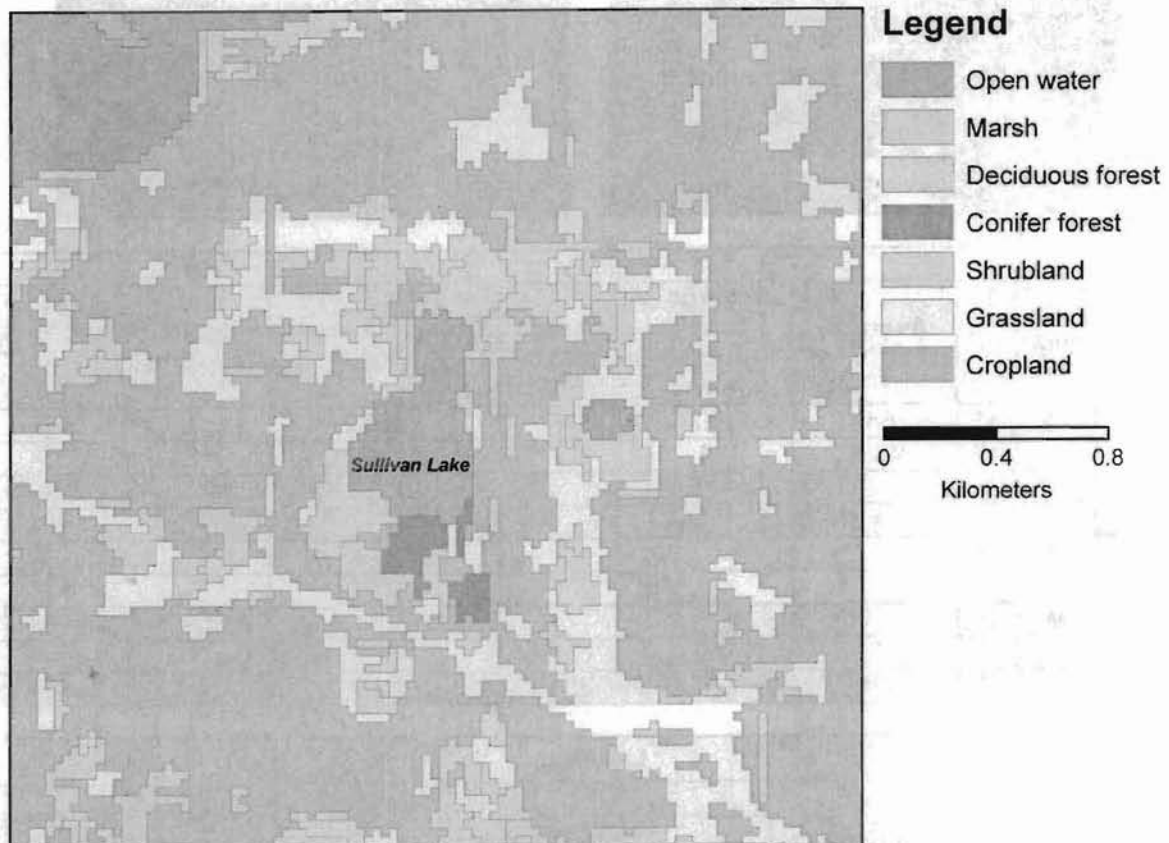
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## Land Cover Types Surrounding Red Sand Lake Crow Wing County, MN



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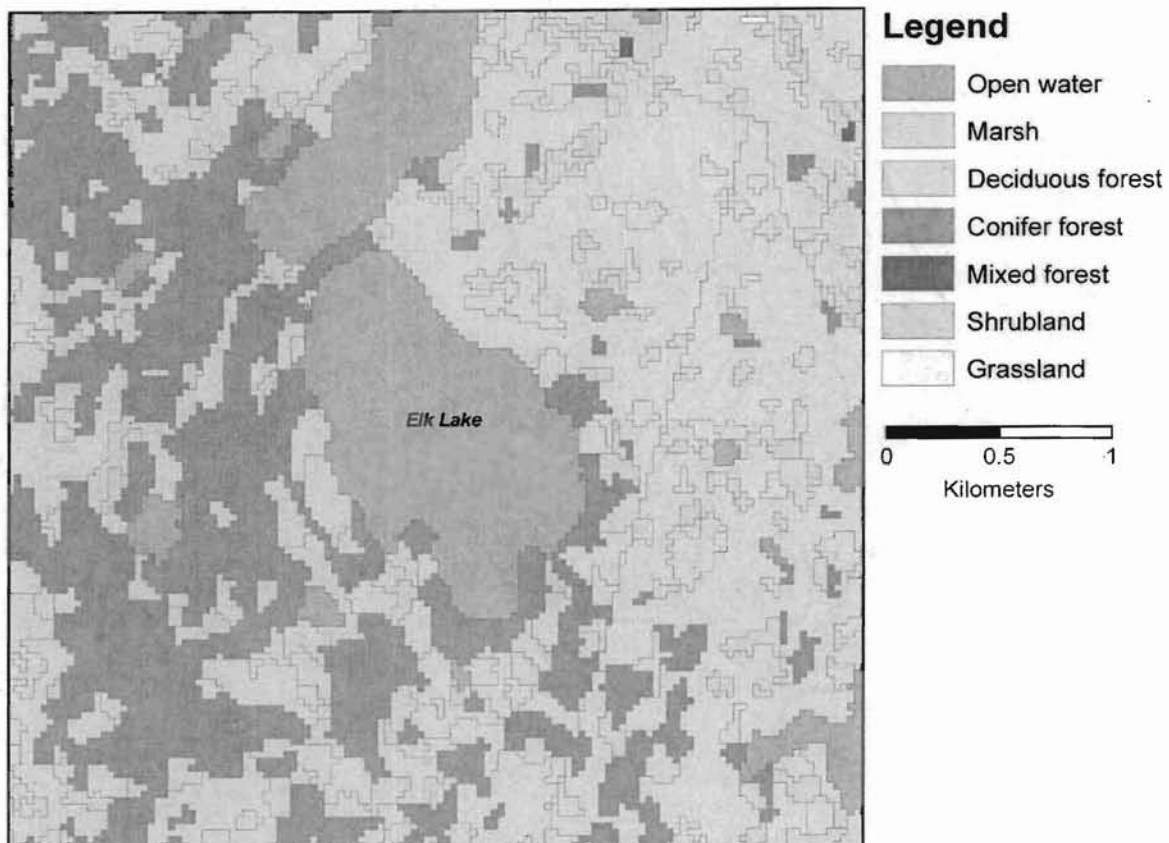
## Land Cover Types Surrounding Sullivan Lake Wright County, MN



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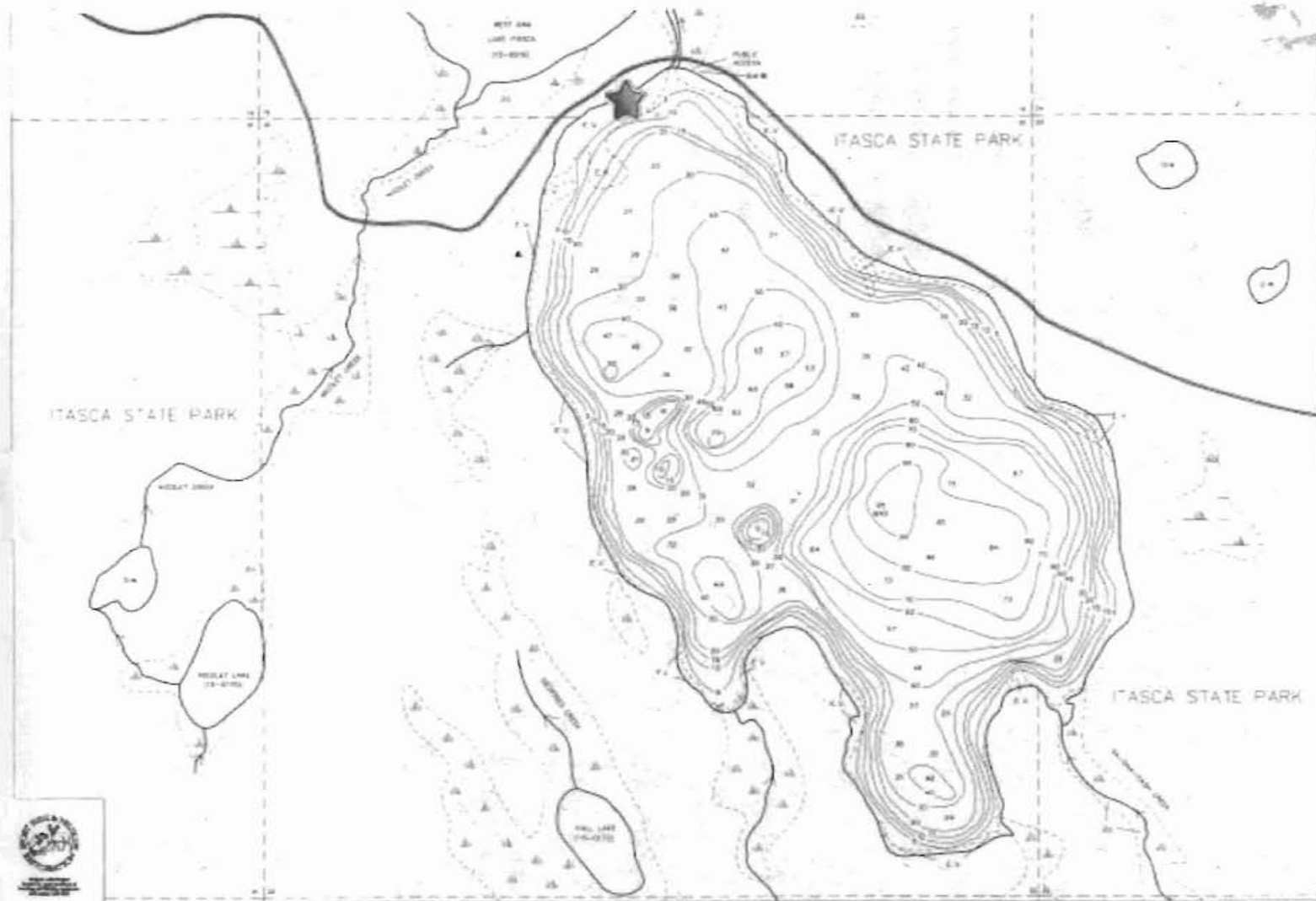


## Land Cover Types Surrounding Elk Lake Clearwater County, MN



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#### LEGEND

- BOUNDARY 1/4" = 1/4"
- WATER 1/4" = 1/4"
- OPEN WATER 1/4" = 1/4"
- FORESTED VEGETATION 1/4" = 1/4"
- FLUENT LEAF VEGETATION 1/4" = 1/4"
- STATE PARK ROAD 1/4" = 1/4"
- STREAM 1/4" = 1/4"
- CAMPFIRE 1/4" = 1/4"
- POLE STAKE 1/4" = 1/4"
- SECTION LINE 1/4" = 1/4"
- SECTION NUMBER 1/4" = 1/4"

ELK LAKE & WEST LAKE, MINN.  
 Map - LAKE LOCATED AT PUBLIC WATER ACCESS  
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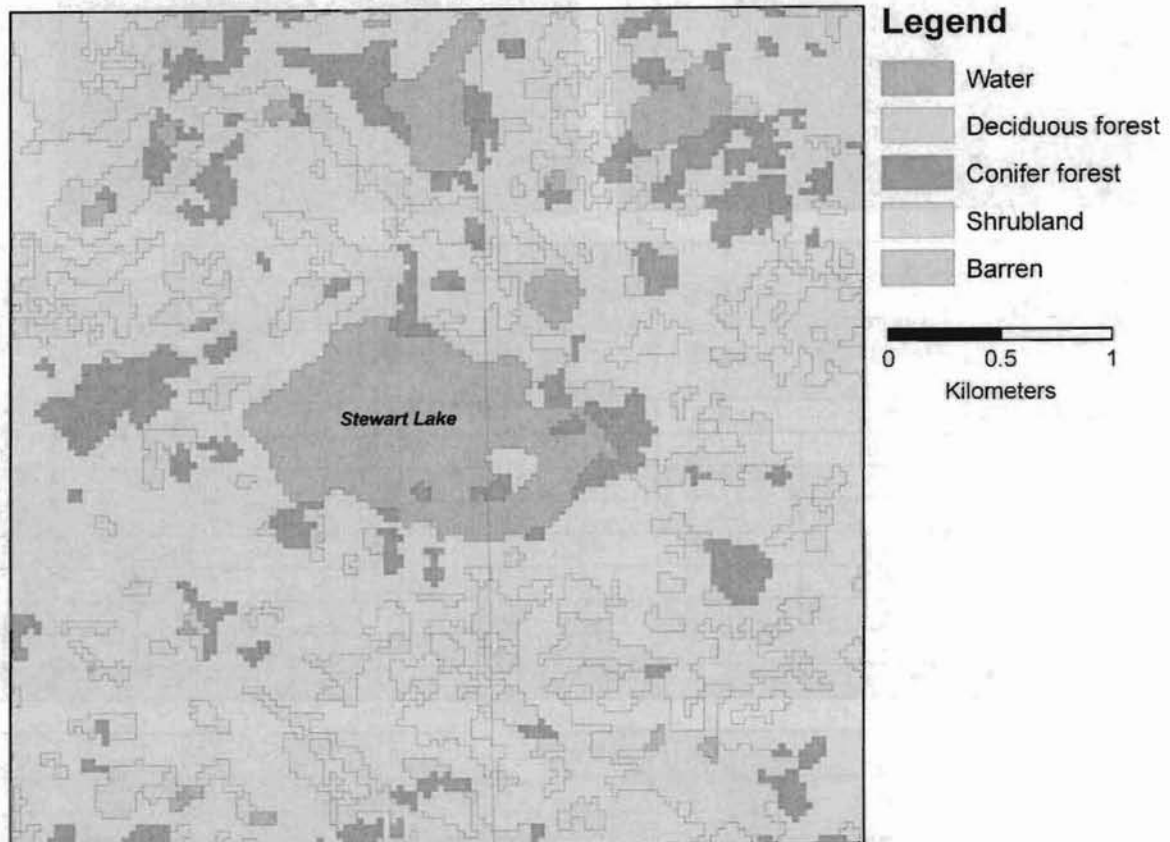
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STATE NUMBER	15-000	LAKE AREA	2000 ACRES
TOWNSHIP	15 N	LAKE AREA	2000 ACRES
RANGE	15 E	LAKE AREA	2000 ACRES
SECTION	15.00.00	LAKE AREA	2000 ACRES

## ELK LAKE

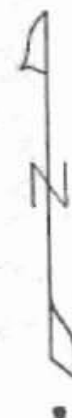
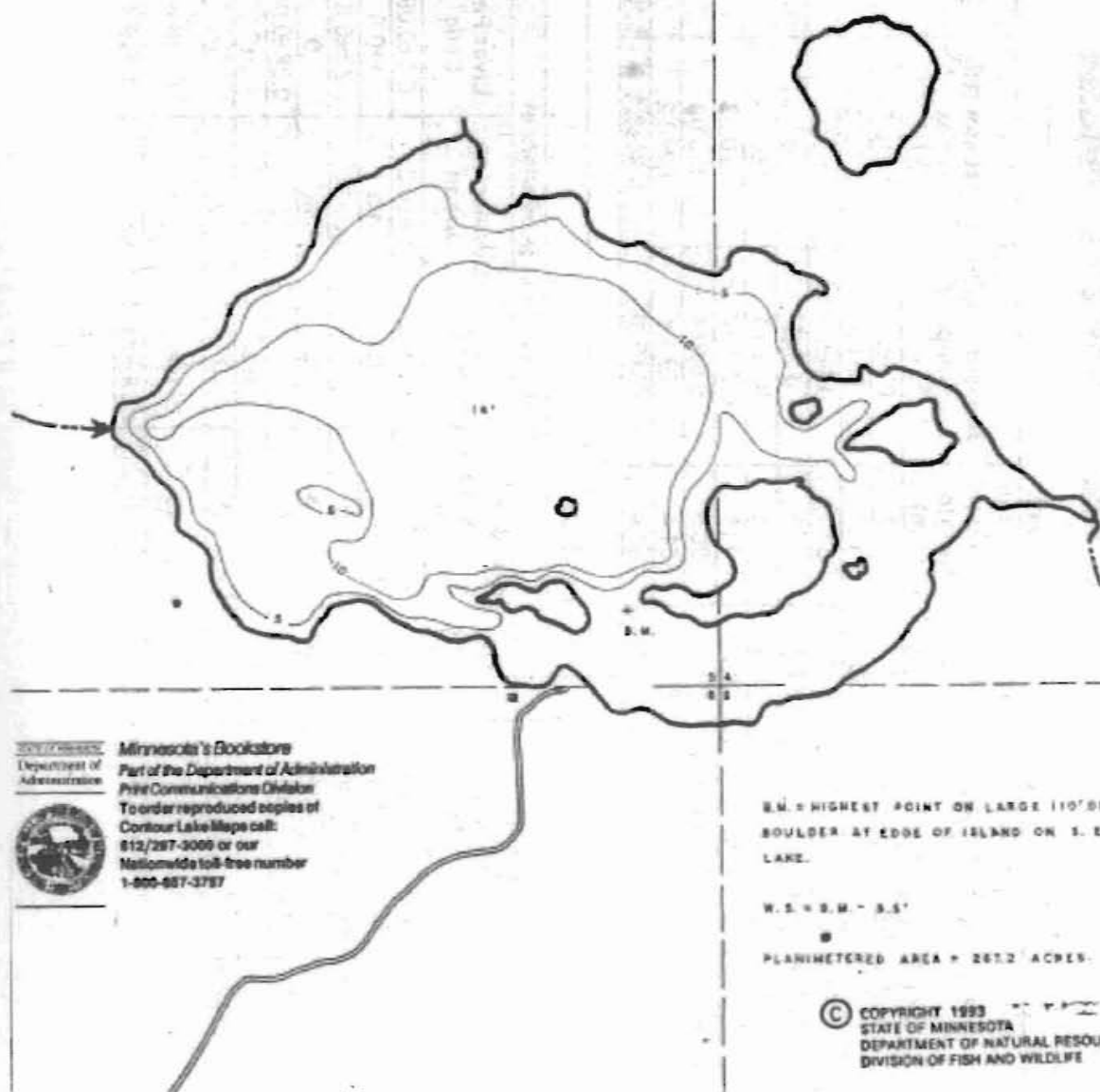
LAKE AREA	2000 ACRES
LAKE AREA	2000 ACRES
LAKE AREA	2000 ACRES
LAKE AREA	2000 ACRES



## Land Cover Types Surrounding Stewart Lake Crow Wing County, MN



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LAKE.

W.S. = S.M. - 3.5'

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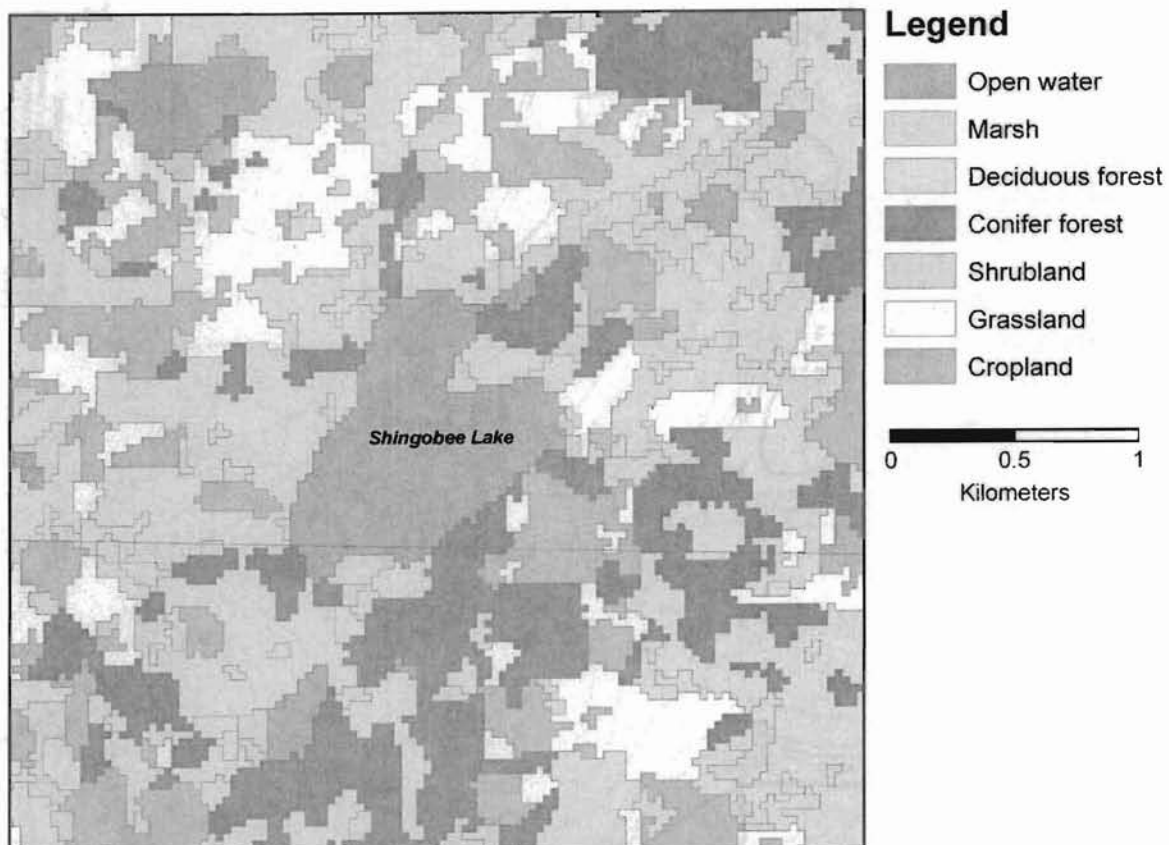
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DATE 10-18-88	DATE 5-17-87	D.J.-F.W.-T.N.

## Land Cover Types Surrounding Shingobee Lake Hubbard County, MN



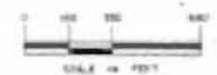
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LEGEND

- COUNTY ROAD
- SHADEL ROAD
- UNIMPROVED ROAD
- STREAM
- CULVERT
- NATURAL AREA
- EMERGENCY RESTRICTION
- RESTRICTED RESTRICTION
- PLANNED AREA
- LITTORAL AREA
- LENGTH OF SHORELINE

SHINGOBEE LAKE - 29-43  
 ON SOUTH SIDE OF SHINGOBEE LAKE  
 AT FROM WATER'S EDGE ON THE  
 SOUTHWEST END OF LAKE AT  
 PROXIMITY RESIDENCE  
 N.E. 1/4 - 1.5 MILES S.W. 1/4  
 N.W. 1/4 - 1.5 MILES S.W. 1/4  
 N.E. 1/4 - 1.5 MILES S.W. 1/4  
 N.W. 1/4 - 1.5 MILES S.W. 1/4

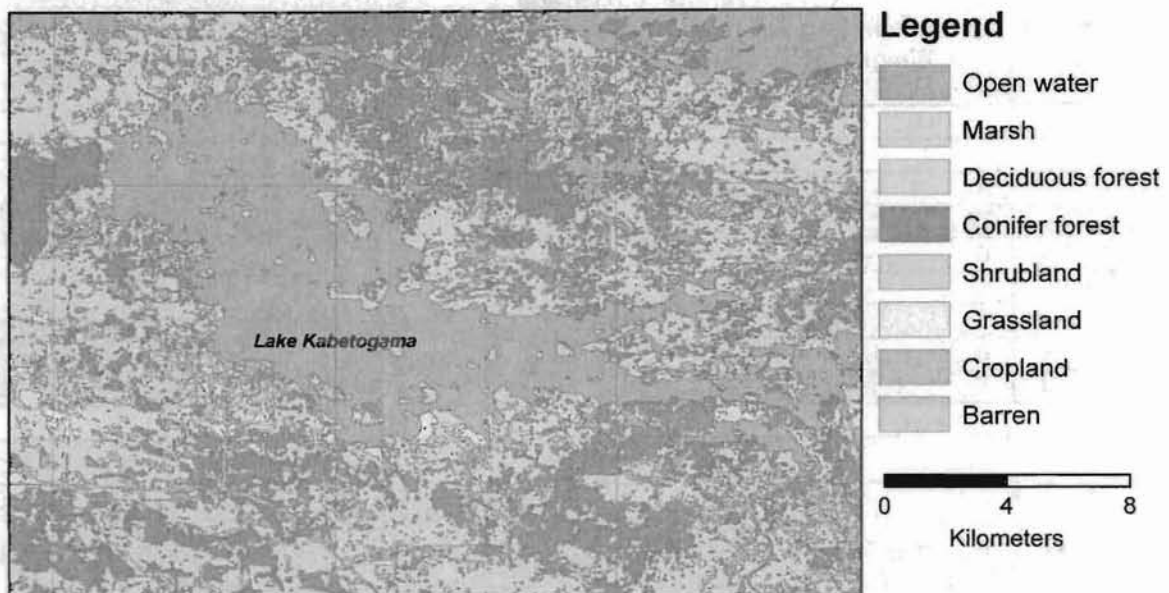


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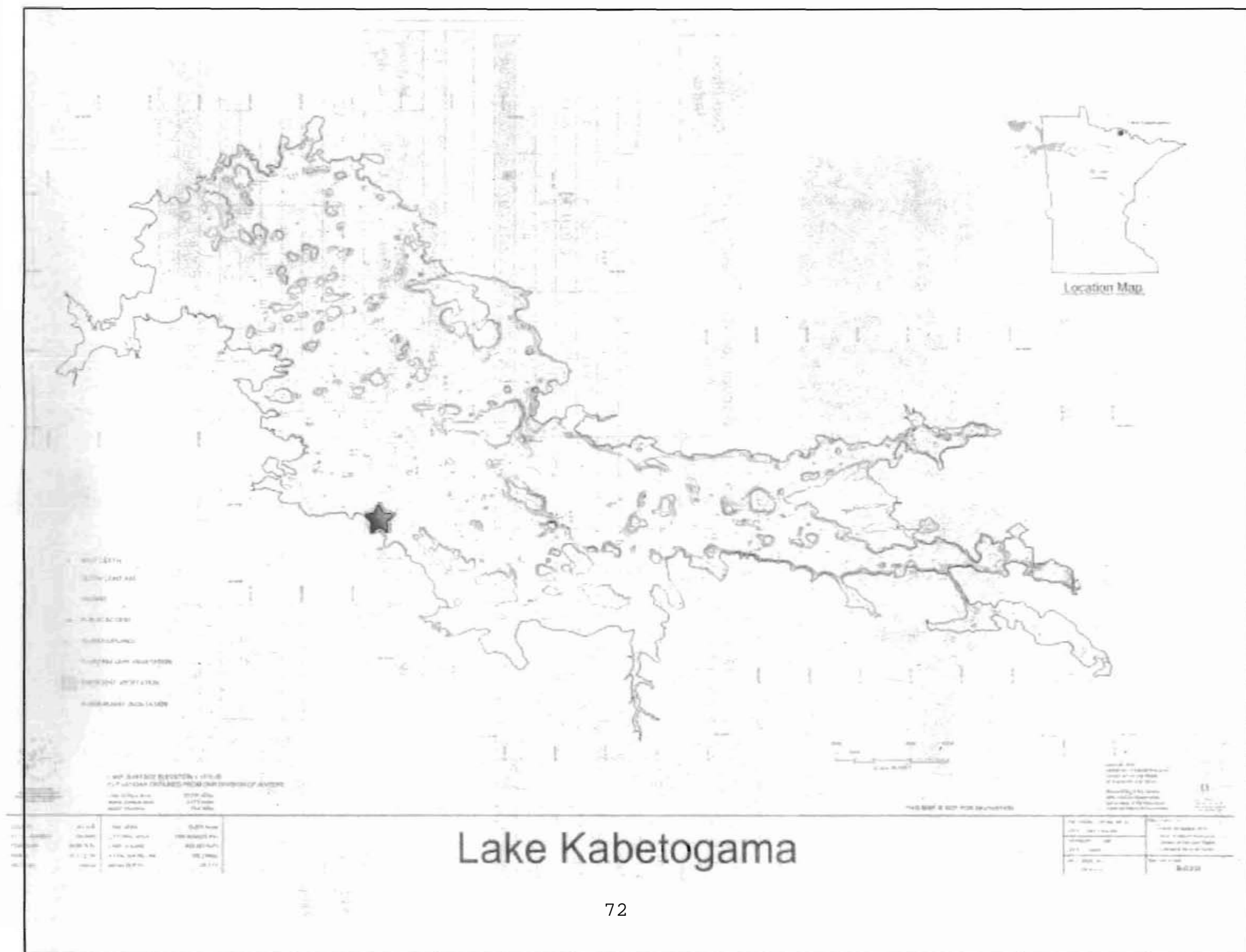
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SHINGOBEE LAKE 29-43		
HUBBARD COUNTY		
T-10-H	S-10-M	S-25, 34, 35, 40, 22
SECTION T-10-H	SECTION S-10-M	SECTION S-25, 34, 35, 40, 22
DATE 7-1-75	DATE 10-25-75	DATE 10-18-75

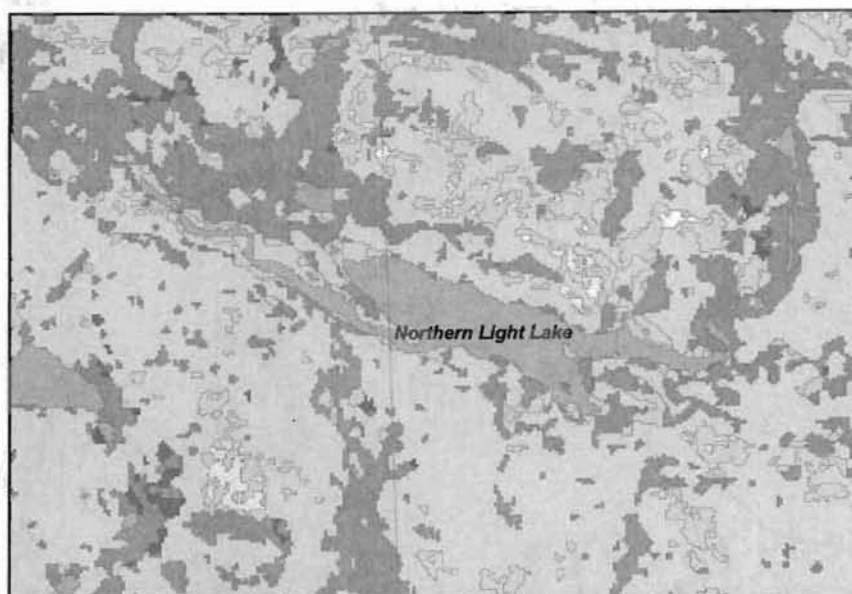
## Land Cover Types Surrounding Lake Kabetogama Koochiching and St. Louis Counties, MN



Land cover types were derived by computer classification of combined two-season pairs of early-1990s Landsat 4/5 Thematic Mapper (TM) satellite imagery, as part of the Upper Midwest Gap Analysis Program of the U.S. Geological Survey.



## Land Cover Types Surrounding Northern Light Lake Cook County, MN



### Legend

- Water
- Marsh
- Deciduous forest
- Conifer forest
- Mixed forest
- Shrubland
- Grassland

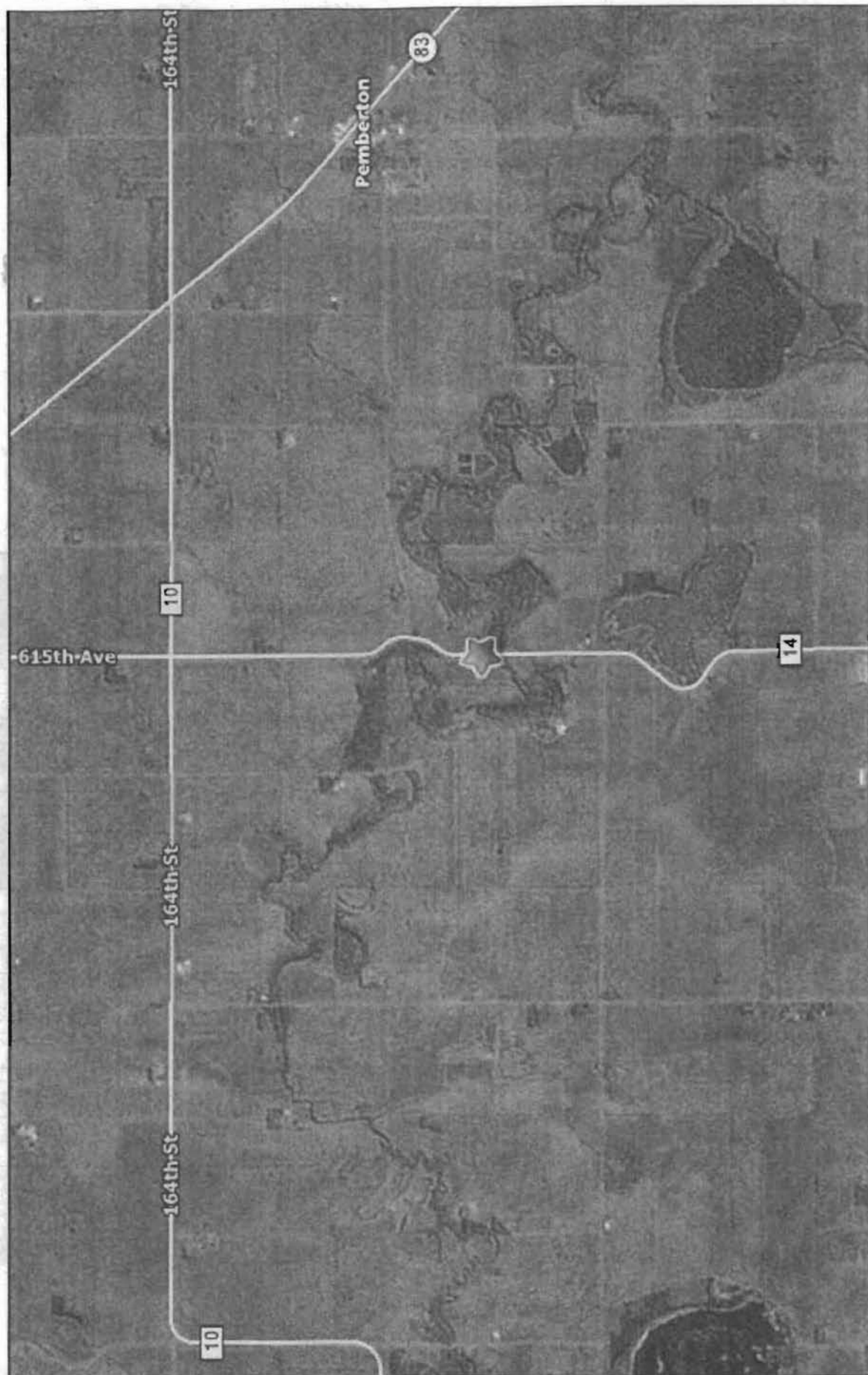
0 1 2  
Kilometers

Land cover types were derived by computer classification of combined two-season pairs of early-1990s Landsat 4/5 Thematic Mapper (TM) satellite imagery, as part of the Upper Midwest Gap Analysis Program of the U.S. Geological Survey.





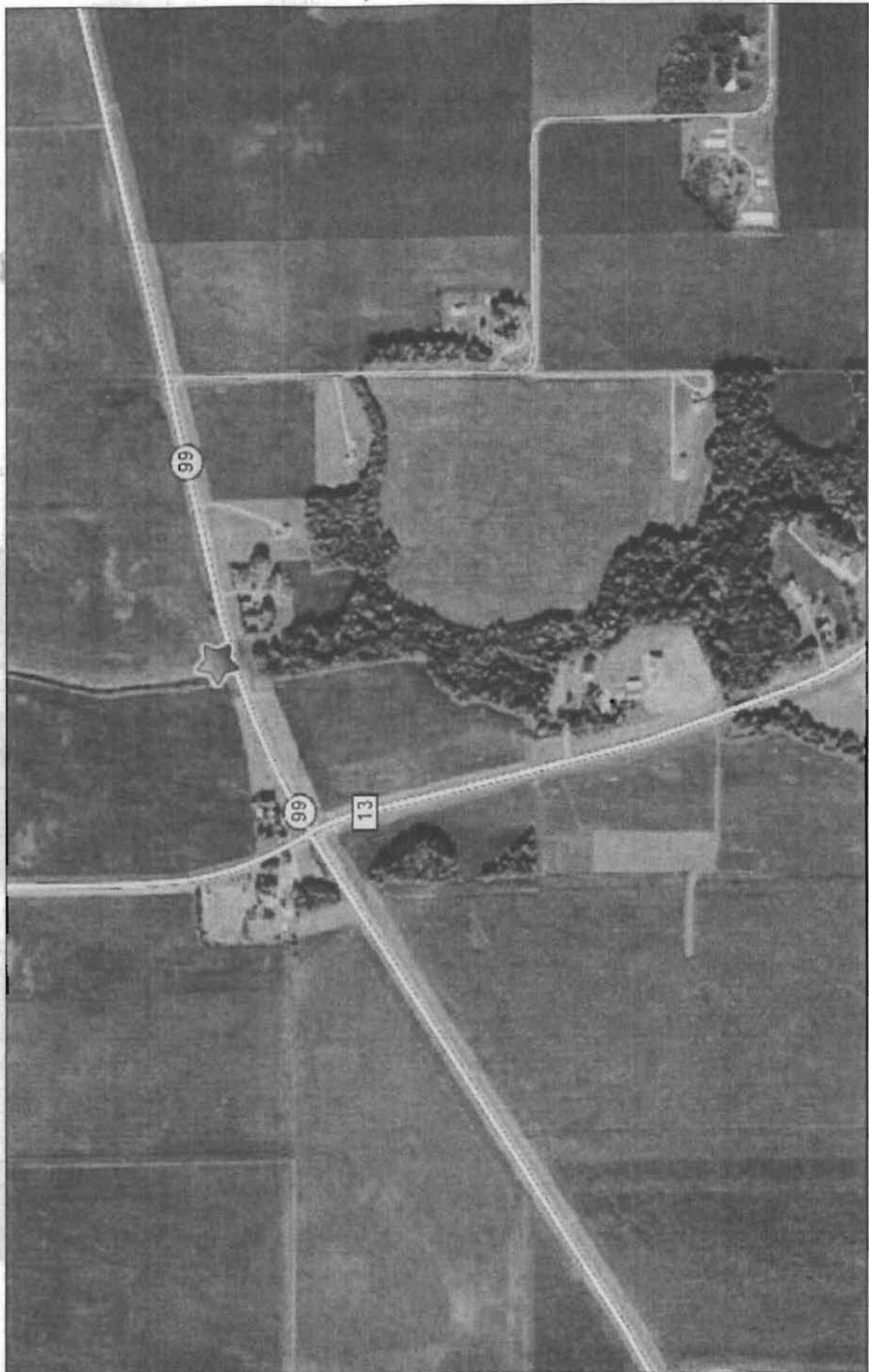
Little Cobb River



*Le Sueur River*



7 mile Creek - UPSTREAM



7 MILE CREEK - MID-STREAM





# 7 MILE CREEK - DOWNSTREAM

(Aerial view of the creek)



REDWOOD RIVER - UPSTREAM



REDWOOD RIVER - DOWNSTREAM #1



REDWOOD RIVER - DOWNSTREAM #2





## Appendix D Quality assurance information

The quality assurance program consisted of field blanks, field duplicates, duplicate matrix spikes, and distilled water matrix spikes. Target compounds were spiked into distilled water and natural water matrices and processed through the entire procedure in the same manner as environmental samples to evaluate method recoveries. Recoveries for individual samples were evaluated using the surrogate standards. No standard reference materials were available for any of the organic compounds evaluated in this study. For the CLLE GC/MS analyses, surrogate standards ( $d_{21}$  2,6-di-*tert*-butyl-4-methylphenol,  $d_6$  bisphenol A, 4-*normal*-nonylphenol, 4-*normal*-nonylphenolmonoethoxylate, and 4-*normal*-nonylphenoldiethoxylate obtained from CIL and Aldrich Chemical) were added to the samples prior to isolation to evaluate whole method recovery. Deuterated polynuclear aromatic hydrocarbon internal standards (Supelco, Bellefonte, PA) were added to the extracts prior to GC/MS analysis. The surrogate standards for the EVAP-GC/MS method were  $d_{12}$  ethylenediaminetetraacetic acid, 4-bromophenylacetic acid, and 4-*normal*-nonylphenoldiethoxycarboxylic acid and the internal standard was 1-phenylnonane (CIL and Aldrich Chemicals). The C18-SPE GC/MS/MS analysis used 12 deuterated surrogate standards ( $d_7$  androstenedione,  $d_7$  cholesterol,  $d_3$  diethylstilbestrol,  $d_4$  dihydrotestosterone,  $d_4$  17 $\beta$ -estradiol,  $d_3$  estriol,  $d_4$  estrone,  $d_4$  17 $\alpha$ -ethynylestradiol,  $d_4$  mestranol,  $d_6$  norethindrone,  $d_9$  progesterone, and  $d_5$  testosterone obtained from CIL. Target compound quantitation for the CLLE GC/MS, and EVAP GC/MS analysis was based on internal standards and a multi-point calibration curve. Quantitation for the C18-SPE GC/MS/MS method was based on multi-point calibration curves constructed using isotope dilution quantitation protocols and exact deuterated analogs (12 compounds) or deuterated analogs of structurally similar compounds (7 compounds).

For the sediment samples, surrogate standards ( $d_{21}$  2,6-di-*tert*-butyl-4-methylphenol,  $d_6$  bisphenol A, 4-*normal*-nonylphenol, 4-*normal*-nonylphenolmonoethoxylate, 4-*normal*-nonylphenoldiethoxylate,  $d_7$  androstenedione,  $d_7$  cholesterol,  $d_3$  diethylstilbestrol,  $d_4$  dihydrotestosterone,  $d_4$  17 $\beta$ -estradiol,  $d_3$  estriol,  $d_4$  estrone,  $d_4$  17 $\alpha$ -ethynylestradiol,  $d_4$  mestranol,  $d_6$  norethindrone,  $d_9$  progesterone, and  $d_5$  testosterone) were added to the samples prior to ACE extraction to evaluate whole method recovery. Deuterated polynuclear aromatic hydrocarbon internal standards (Supelco, Bellefonte, PA) were added to the extracts prior to GC/MS analysis for organic wastewater analysis. After GC/MS analysis extracts were derivitized (MSTFA II), and triphenylene was added as an internal standard prior to analysis by GC/MS/MS.

For the POCIS samples, surrogate standards ( $d_{21}$  2,6-di-*tert*-butyl-4-methylphenol,  $d_6$  bisphenol A, 4-*normal*-nonylphenol, 4-*normal*-nonylphenolmonoethoxylate, 4-*normal*-nonylphenoldiethoxylate,  $d_7$  androstenedione,  $d_7$  cholesterol,  $d_3$  diethylstilbestrol,  $d_4$  dihydrotestosterone,  $d_4$  17 $\beta$ -estradiol,  $d_3$  estriol,  $d_4$  estrone,  $d_4$  17 $\alpha$ -ethynylestradiol,  $d_4$  mestranol,  $d_6$  norethindrone,  $d_9$  progesterone, and  $d_5$  testosterone) were added to the samples prior to fluorocil column cleanup. Deuterated polynuclear aromatic hydrocarbon internal standards (Supelco, Bellefonte, PA) were added to the extracts prior to GC/MS analysis for organic wastewater analysis. After GC/MS analysis extracts were derivitized (MSTFA II), and triphenylene was added as an internal standard prior to analysis by GC/MS/MS.

Reporting limits for water samples were defined by analytical method as the concentration equivalent to three times the mean detection value in method blanks or five times the baseline, whichever was greater. For the sediments, reporting limits were determined to be three times the mean detection value in method blanks, corrected for sediment mass extracted. For the POCIS samples, reporting limits were set at three times the instrument detection values, corrected for number of POCIS extracted. Average relative percent difference (RPD) values are reported for duplicate samples in which the compound was detected in both samples. Spike and recovery studies were not done for the POCIS samplers.