Total Maximum Daily Load
for
Johnson Lake

Parameter of Concern: Fecal coliform Bacteria

Nebraska Department of Environmental Quality
Planning Unit, Water Quality Division

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Executive Summary

Johnson Lake was included on the 2004 Surface Water Quality Integrated Report as a Category 5 waterbody needing a total maximum daily load (TMDL) (NDEQ 2004) due to impairment by excessive fecal coliform. As such, a total maximum daily load must be developed for fecal coliform in accordance with the Clean Water Act. By definition, a TMDL is the sum of individual wasteload allocations for point sources and load allocations for nonpoint sources and natural background with a margin of safety. This document presents the TMDL for fecal coliform that is designed to allow Johnson Lake to fully support the primary contact recreation beneficial use. The information contained herein should be considered one (1) TMDL.

This TMDL has been prepared to comply with the current (1992) regulations found at 40 CFR Part 130.7.

1. **Name and geographic location of the impaired waterbody for which the TMDL are being developed.**
   Johnson Lake; Gosper and Dawson Counties, Nebraska; Lake Identification Number: MP2-L0520

2. **Identification of the pollutant and applicable water quality standard**
   The pollutant causing the impairment of the water quality standard and designated beneficial use is fecal coliform bacteria. Designated uses assigned to the above-identified waterbody include: primary contact recreation, aquatic life Warmwater class A, agriculture water supply class A, industrial water supply and aesthetics (NDEQ 2002b). Excessive fecal coliform have been determined to be impairing the primary contact recreation beneficial use. The applicable water quality standards are a seasonal geometric mean of 200/100 ml with <10% of the samples being greater that 400/100ml.

3. **Quantification of the pollutant load that may be present in the waterbody and still allows attainment and maintenance of the water quality standards.**
   The allowable pollutant load is based upon the available lake volume expressed as elevation or E (feet). That is, loading capacities are developed for each flow by multiplying the water quality standard (WQS) by the selected elevation with the equation being:

   $$\text{Loading capacity} = \text{WQS} * \text{E}$$

4. **Quantification of the amount or degree by which the current pollutant load in the waterbody, including upstream sources that is being accounted for as background loading deviates from the pollutant load needed to attain and maintain water quality standards.**
   From the monitoring conducted in 2001, 2002 and 2003 the number of samples exceeding 400/100 ml are 37 of 284 which is 13%.

5. **Identification of the pollutant source categories.**
   No National Pollutant Discharge Elimination System (NPDES) permits have been issued for the discharge to Johnson Lake or the Tri-County Canal upstream of Johnson Lake. Therefore, nonpoint sources (including natural sources) have been identified to be contributing to the pollutant loads being delivered Johnson Lake. Nonpoint sources have been determined to originate from human and animal sources.

6. **Wasteload allocations (WLAs) for pollutants from point sources.**
   Because no point sources discharge to Johnson Lake, the wasteload allocations for point source discharges will zero (0).

7. **Load allocations (LAs) for pollutants from nonpoint sources.**
   The load allocations assigned to this TMDL will be based upon the lake volume defined by the elevation and will be calculated by:

$$LA_i = E_i*400/100 \text{ ml}$$

Where:
LA_i = load allocations at the i^{th} elevation
E_i = lake volume/elevation at the i^{th} elevation
400/100 ml = applicable/target water quality criteria for fecal coliform from Title 117

8 A margin of safety.
This TMDL contains an implicit margin of safety (MOS). Specifically, the targeted reductions will result in seasonal geometric means and the percent of samples exceeding 400/100 ml well below the applicable water quality criteria.

9. Consideration for seasonal variation.
The water quality criteria are only applicable during the Title 117 defined recreation season that starts May 1 and ends September 30. Because of this, the water quality and lake elevation data was limited to this time period.

10. Allowances for reasonably foreseeable increases in pollutant loads.
There was no allowance for future growth included in this TMDL.

11. Implementation Plan
Implementation of the reductions for fecal coliform will be carried out through a combination of regulatory and non-regulatory activities. In the future and if necessary, point sources will be regulated under the auspice of the National Pollutant Discharge Elimination System and the Rules and Regulations Pertaining to Livestock Waste Control. On-site wastewater treatment systems will be regulated under the Rules and Regulations for the Design, Operation and Maintenance of On-Site Wastewater Treatment Systems. Nonpoint source pollution will be addressed using available programs, technical advice, information and education and financial incentives such as cost share.

The TMDL included in the following text can be considered a “phased TMDL”. As such, is an iterative approach to managing water quality based on the feedback mechanism of implementing a required monitoring plan that will determine the adequacy of load reductions to meet water quality standards and revision of the TMDL in the future if necessary. A description of the future monitoring (Section 4.0) that is planned has been included.

Monitoring is essential to all TMDLs in order to:

- Assess the future beneficial use status;
- Determine if the water quality is improving, degrading or remaining status quo;
- Evaluate the effectiveness of implemented best management practices.

The additional data collected should be used to determine if the implemented TMDL has been or is effective in addressing the identified water quality impairments. As well, the data and information can be used to determine if the TMDL has accurately identified the required components (i.e. loading/assimilative capacity, load allocations, in lake response to pollutant loads, etc.) and if revisions are appropriate.

1.0 Introduction

Johnson Lake was listed on Part 5 of the 2002 Nebraska Section 303(d) list of impaired waters (NDEQ 2002a) as a water quality concern with the pollutant of concern being fecal coliform bacteria using data collected during 2001. Additional data collected during the 2002 and 2003 recreation season (May 1 – September 30) indicated the waterbody was not supporting the primary contact recreation beneficial use and the waterbody has been included as a Category 5 waterbody on the 2004 Surface Water Quality Integrated Report as needing a TMDL.

Therefore, based on the above and as required by Section 303(d) of the Clean Water Act and 40 CFR Part 130.7, a TMDL for fecal coliform bacteria has been developed and contained herein to address the impairment.

1.1 Background Information

Johnson Lake is located in Gosper and Dawson Counties, Nebraska (Figure 1.1), and was constructed by the Central Nebraska Public Power and Irrigation District (CNPPID). The waterbody was constructed primarily as a regulating structure with completion of the earthen dam and the initial fill occurring in 1940 (Olmstead 2002). The CNPPID was organized in 1933 to bring irrigation to south-central Nebraska and generate electricity for the region. The waterbody also supports recreation (primary contact, fishing, etc.) as a secondary use. A description of the physical information is provided in Table 1.1. The Nebraska Game and Parks Commission (NGPC) manages the fishery along with two areas that are operated as state recreation areas. Water provided to the lake is delivered by a minimal watershed immediately surrounding the lake and the Tri-County Canal that originates near North Platte, NE where water from the North and South Platte River basins are diverted. Water flows out of Johnson Lake through two smaller impoundments and two hydroelectric plants then is either returned to the Middle Platte River or diverted to irrigation delivery canals.

Recreation did not become popular at Johnson Lake until the late 1940s and early 1950s. Since that time many houses and cabins have been built around the lake and it has become one of the most popular recreational lakes in central Nebraska (Olmstead, 2002).

1.1.1 Waterbody Description

1.1.1.1 Waterbody Name: Johnson Lake

Lake Identification Number: MP2-L0520 (Title 117 – Nebraska Surface Water Quality Standards)

1.1.1.2 Major River Basin: Missouri River

1.1.1.3 Minor River Basin: Middle Platte

1.1.1.4 Hydrologic Unit Code 10200101

1.1.1.5 Assigned Beneficial Uses: Primary contact recreation, Aquatic Life Warmwater Class A, Agricultural Water Supply Class A, Industrial Water Supply and Aesthetics (Title 117 – Nebraska Surface Water Quality Standards)

1.1.1.6 Major Tributary: Tri-County Supply Canal: Segment identification MP2-20500

1.1.2 Watershed Characterization

1.1.2.1 Physical Features: Johnson Lake is located in the Central Great Plains (Level III) ecoregion as defined by Chapman, et al. (2001). The reservoir was completed in 1940 by the CNPPID who retains ownership. However, the lake's fishery is managed by the NGPC along with a portion of the surrounding area that is managed as a State Recreation Area. The lake is a regulating reservoir that is used to regulate water volume through the CNPPID downstream hydroelectric and irrigation facilities.

CNPPID retains ownership of all of the shoreline and adjacent property around the lake that is designated by the Federal Energy Regulatory Commission boundary. The shoreline is a mix of private residential homes, seasonal cabins, public use and commercial areas the majority of which have obtained leases from CNPPID.

Figure 1.1 Location of Johnson Lake in Dawson and Gosper Counties, Nebraska

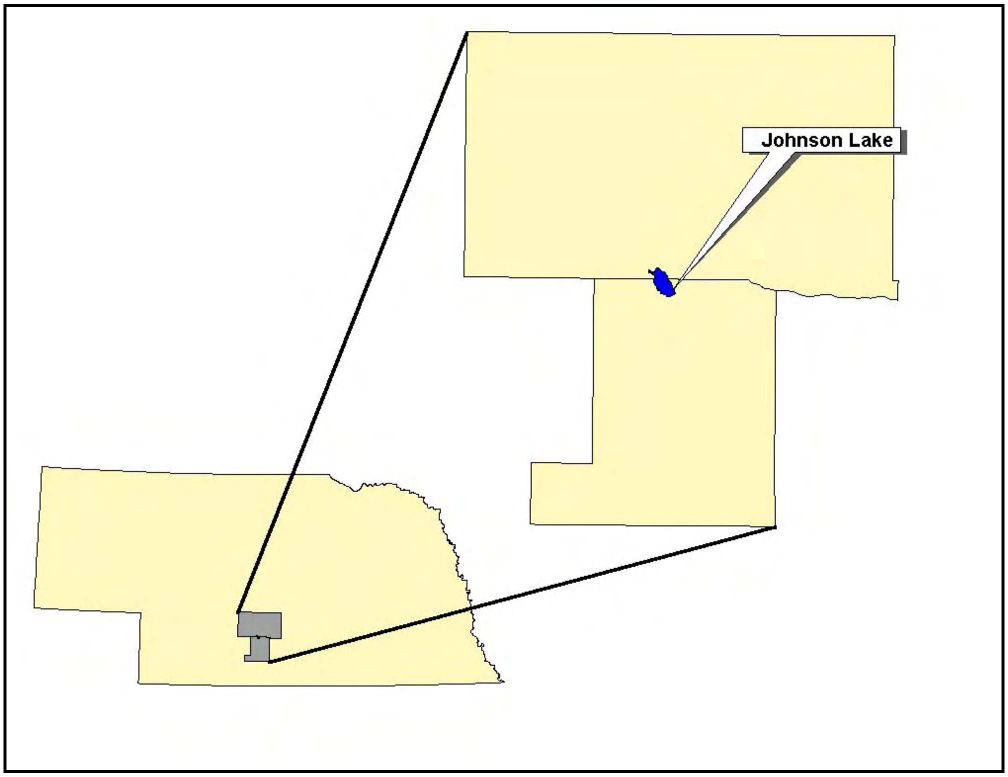


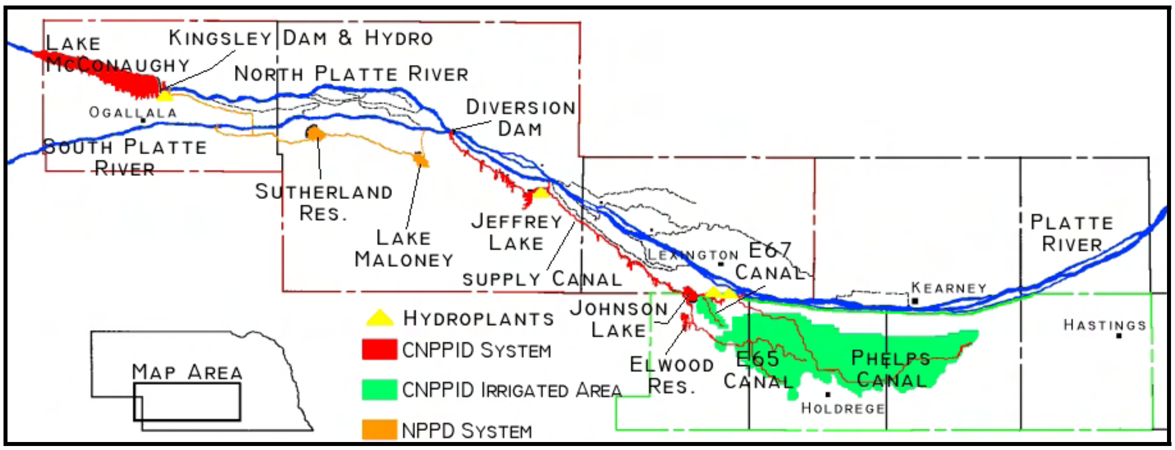
Table 1.1 Physical Description of Johnson Lake

Parameter	Holmes Lake
State	Nebraska
County	Dawson and Gosper
Latitude (center of dam)	40° 40' 34.2"
Longitude (center of dam)	99° 50' 15.4"
Legal Locations (dam)	Section 8, Township 8 North, Range 22 West
Surface Area (Elevation 2617 feet)	2,189.2 acres
Shoreline Length	10.9 miles (approximately)
Mean Depth – 2003 (Elevation 2617 feet)	19.4 feet (5.9 meters)
Volume – 2003 (Elevation 2617 feet)	42,465 acre/feet
Number of Major Inlets	1

The watershed contributing to the lake is limited to areas immediately adjacent to the waterbody. This is often defined by the paved access road that encircles the lake. With the exception of this small watershed that immediately surrounds the lake, the majority of water in the lake is provided by the Tri-County Supply Canal. Water in the supply canal ultimately originates from the upper North and South Platte basins and is diverted at North Platte, NE. A map of the CNPPID project can be found in figure 1.1.2.1.

1.1.2.2 Climate: Precipitation in the immediate area of Johnson Lake averages approximately 22 inches per year. Typically, a majority of the precipitation occurs as rainfall during the growing season and the distribution may not always favor crop production. Temperatures in the vicinity of the lake range from an average high in the upper 80s during the summer to average lows in the 10s during the winter (NRC Databank 2004).

Figure 1.1.2.1 CNPPID Project Map (Source: CNPPID Internet Site)



1.1.2.2 Demographics: Johnson Lake lies approximately 7 miles southwest of Lexington, NE (population 10,011). There are approximately 900 houses and cabins surrounding the lake with approximately 1/3 occupied by year-round residents, resulting in a permanent population of 600 persons. During the summer recreation season, the population can increase to 3,400 with peak weekend and holiday numbers exceeding 5,200 people. Additionally, the NGPC estimates an average of 1,300 can be present during the summertime (Olmstead, 2002).

1.1.2.3 Land Use: Land use in the immediate watershed consists of residential dwelling, commercial facilities, recreational areas and crop ground. As stated above approximately 900 houses and cabins occupy the lake's 10.9 miles of shoreline with several others on adjacent areas (Figure 1.1.2.3). All the businesses, residences and cabins have individual, common or community water supplies with ground water as the sole source of the supply water and the majority of the facilities are individually served by on-site septic tanks and drain fields.

2.0 Pathogen (Fecal coliform) TMDL

2.1 Problem Identification

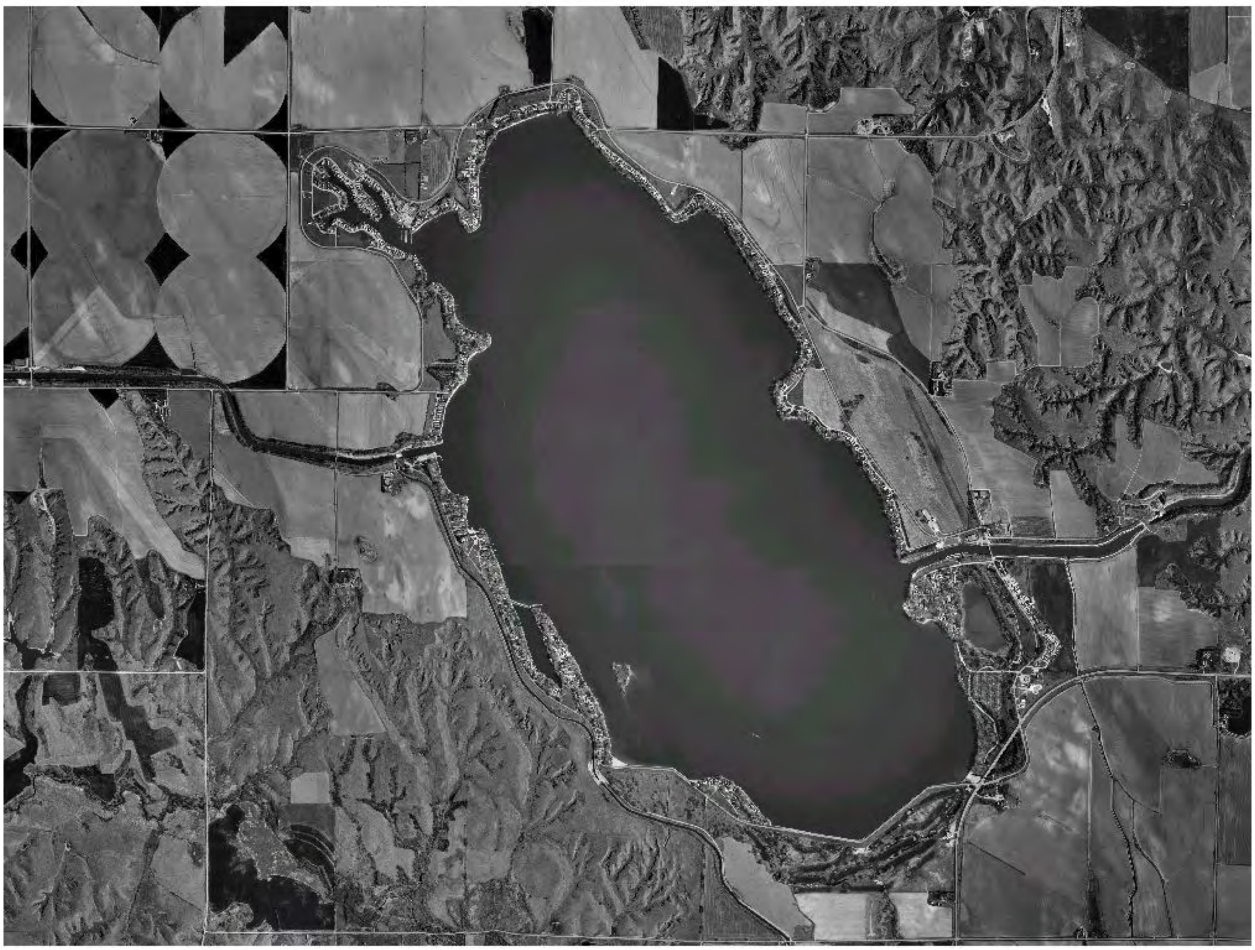
Johnson Lake was included on the 2004 Nebraska Surface Water Quality Integrated Report as having an impaired primary contact recreation beneficial use with the parameter of concern being fecal coliform.

This section deals with the extent and nature of water quality impairments caused by excessive bacteria in Johnson Lake.

2.1.1 Water Quality Criteria Violated and/or Beneficial Uses Impaired

The *Primary Contact Recreation* beneficial use has been deemed impaired within Johnson Lake. The *Primary Contact Recreation* beneficial use applies to surface waters which are used or have the potential to be used for primary contact recreation that includes activities where the body may come into prolonged or intimate contact with the water such that water may be accidentally ingested or sensitive body organs (e.g. eyes, ears, nose) may be exposed (NDEQ 2002b).

Figure 1.1.2.3 Aerial Photograph of Johnson Lake



2.1.2 Data Sources

The Nebraska Department of Environmental Quality (NDEQ) monitors surface waters based upon a rotating basin scheme, whereby monitoring is limited to 2 or 3 river basins each year with all 13 basins being (partially) examined in a 5 year period. Under the auspice of the rotating basin plan, data was initially collected from southeast swimming beach from Johnson Lake in 2001. Additional data was collected from several locations in 2002 and 2003. These additional locations were located around the perimeter of the lake and are depicted in Figure 2.1.2. It should be noted, the 2003 sampling did not include a sample of the outlet canal whereas the 2002 sampling did. As well, the 2002 sampling did not include a sample site located at the inlet to Plum Creek Reservoir.

2.1.3 Water Quality Assessment

Water quality data assessments were based upon the beneficial use assessment procedures used to identify impaired waters for the 2003 Section 303(d) list/305(b) Report update and as well as the 2004 Nebraska Surface Water Quality Integrated Report. The procedures are based on the application of the "binomial distribution" method that applies a confidence interval to the exceedance rate in an effort to determine the true exceedance of the waterbody versus that of the data set. A complete description of the water quality data assessment procedures can be found in the *Methodologies for Waterbody Assessments and Development the 2004 Integrated Report for Nebraska*, October 2003 (NDEQ 2003a). The process used in assessing data to determine the use support of the *Primary Contact Recreation* beneficial use can be found in table 2.1.3.

Table 2.1.3: Assessment of the Primary Contact Recreation Beneficial Use Using Fecal Coliform and *E. coli* Bacteria Data.

Parameter	Season Geometric Mean	Single Sample Maximum	Supported	Impaired
Fecal coliform	≤200/100 ml	No more than 10% of Samples >400/100 ml	Season geometric mean ≤200/100 ml or ≤10% of samples exceed 400/100ml	Season geometric mean >200/100 ml and/or >10% of samples exceed 400/100ml
E. coli	≤126/100 ml	235-576/100 ml depending upon frequency of use	Season geometric mean ≤126/100 ml or ≤10% of samples exceed applicable maximum	Season geometric mean >126/100 ml and/or >10% exceed applicable maximum

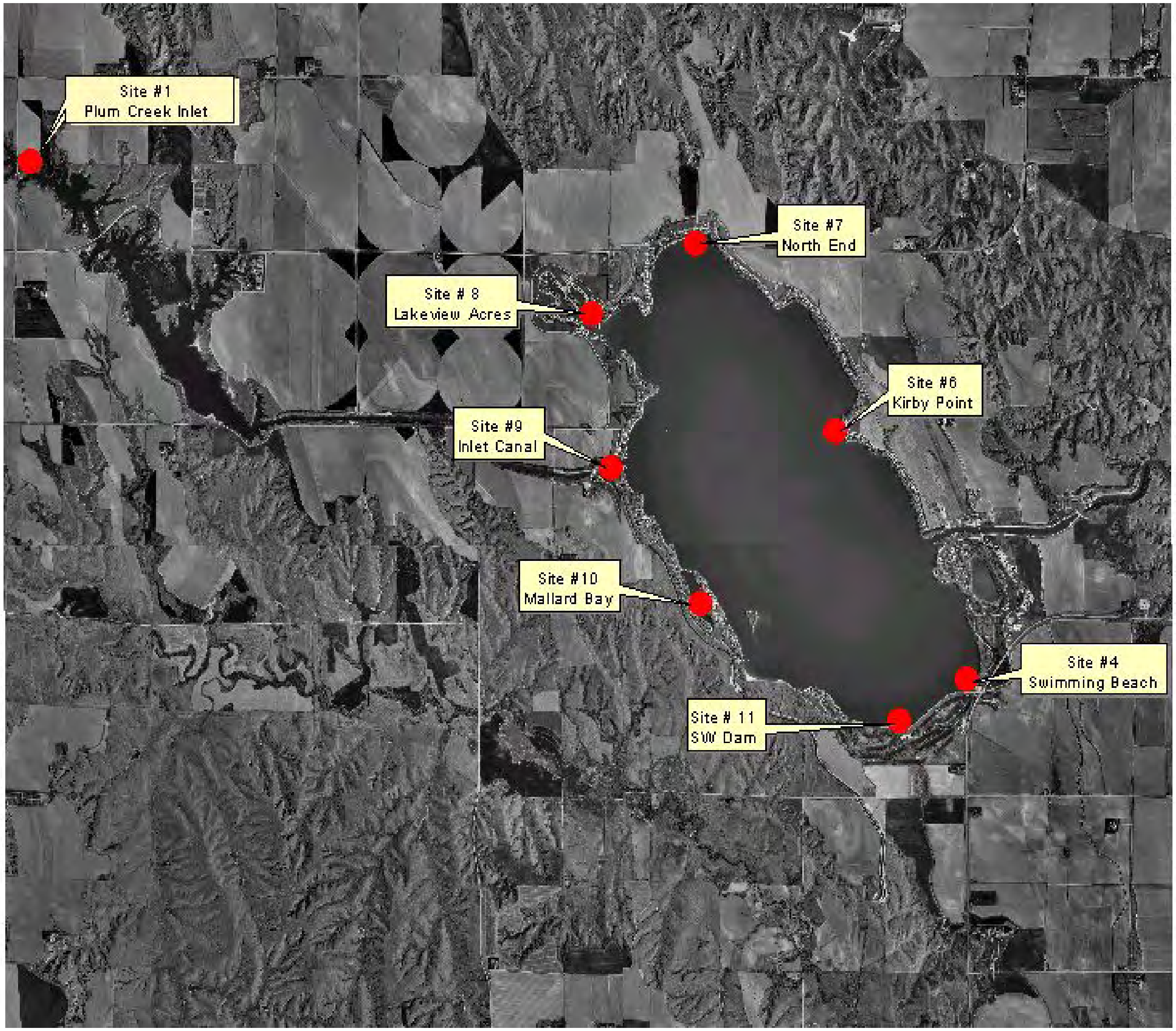
2.1.4 Water Quality Conditions

Fecal coliform data collected during the 2001-2003 recreation seasons (May through September) was assessed to determine the beneficial use support for primary contact recreation. Table 2.1.4 and figure 2.1.4 present the fecal coliform results.

Table 2.1.4 Johnson Lake – 2001-03 Fecal coliform Data and Assessments

Site Number	Site Name/Location	Number of Samples	Maximum Observed Season Geometric Mean (#/100 ml) (year)	# Samples >400/100 ml	% Samples >400/100 ml
1	Plum Creek Inlet	22	12 (2003)	1	5%
4	SE Swimming Beach	65	155 (2002)	10	15%
5	Outlet Canal	23	102 (2002)	2	9%
6	Kirby Point	45	85 (2002)	3	7%
7	North End	45	153 (2002)	7	16%
8	Lakeview Acres	45	105 (2002)	4	9%
9	Inlet Canal	45	129 (2002)	5	11%
10	Mallard Bay	39	162 (2002)	8	21%
11	SW Corner of Dam	45	110(2002)	5	11%
4,6,7,8,10 & 11	Lake Samples-Canal excluded	284		37	13%

Figure 2.1.2 Johnson Lake Sampling Locations

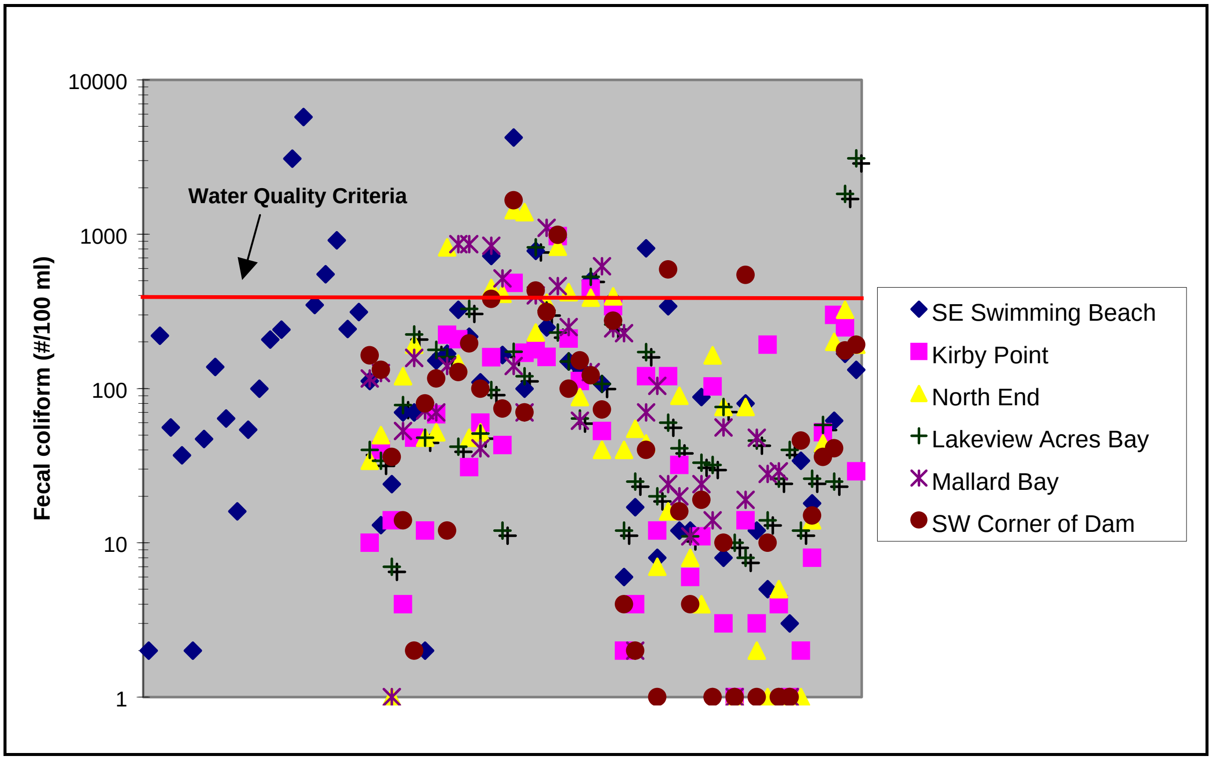


2.1.5 Potential Pollutant Sources

2.1.5.1 Point Sources: Title 117 - Nebraska Water Quality Standards (Title 117), Chapter 6 Lakes and Impounded Waters, sections 004 and 004.01 state:

"No discharge of wastewater from domestic, municipal, or industrial sources shall be allowed directly into these waters except: wastewater from sources authorized by NPDES permits to discharge to these waters prior to May 10, 1982, which have operated under active NPDES permits since then."

Figure 2.1.4 Johnson Lake Fecal coliform Measurements by Sampling Location



The NDEQ has not permitted any facilities, nor are there facilities operating under NPDES permits to discharge to the Tri-County Canal or Johnson Lake. Point sources do however discharge to the North and South Platte River upstream of the Tri-County Supply Canal diversion.

Illicit connections, discharges, combined sewer overflows; sanitary sewer overflows, direct discharges from failing septic tanks or other on-site wastewater systems can also be point sources of fecal coliform bacteria.

Animal feeding operations that have been issued State of Nebraska permits, required for construction and operation of livestock waste control facilities (LWCF) if the operation has discharged, or have the potential to discharge, livestock waste to waters of the State are also considered potential sources. Facilities issued state operating or NPDES permits are designed to contain any run-off that is generated by storm events that are less in intensity than the 25 year, 24-hour rainfall. There are 12 animal feeding operations upstream of Johnson Lake and in the vicinity of the Tri-County canals and adjacent drainages.

2.1.5.2 Nonpoint Sources: Nonpoint sources have the potential to deliver fecal coliform to Johnson Lake. These sources include: indirect discharges from failing septic tanks or other on-site wastewater systems, run-off from livestock pastures, improper or over-application of biosolids (wastewater treatment facility sludge, septage or manure) and urban stormwater runoff not regulated by an NPDES permit.

2.1.5.3 Natural Sources: The primary natural source of fecal coliform is wildlife. A variety of wildlife is native to or have adapted to the area surrounding and upstream of Johnson Lake. Big game, upland game, furbearers, waterfowl and non-game species have been observed in the area.

2.2 TMDL Endpoint

The endpoint for this TMDL will be based on the numeric criteria associated with the *Primary Contact Recreation* beneficial use.

2.2.1 Numeric Water Quality Criteria

Water quality criteria established for the protection of the *Primary Contact Recreation* beneficial use can be found in Title 117, Chapter 4 and are as follows:

Fecal Coliform
Bacteria of the Fecal coliform group shall not exceed a geometric mean of 200/100 ml, nor equal or exceed 400/100 ml, in more than 10% of the samples. These criteria are based upon a minimum of 5 samples taken within a 30-day period. This does not preclude fecal coliform limitations based on effluent guidelines.

These criteria apply during the recreational period of May 1 through September 30.

E. coli
E. coli bacteria shall not exceed a geometric mean of 126/100 ml. For increased confidence of the criteria, the geometric mean should be based on a minimum of five samples taken within a 30-day period. This does not preclude fecal coliform limitations based on effluent guidelines. Single sample minimum allowable densities shall not exceed the following criteria.

235/100 ml at designated bathing beaches
298/100 ml at moderately used recreational waters
406/100 ml at lightly used recreational waters
576/100 ml at infrequently used recreational waters

2.2.2 Selection of Critical Environmental Conditions

The water quality criteria associated with the *Primary Contact Recreation* beneficial use only applies from May 1 through September 30. Therefore, the critical conditions for these TMDL will be those occurring from May 1 through September 30.

2.2.3 Waterbody Pollutant Loading Capacity

By regulation, a TMDL requires a loading capacity value for the pollutant of concern. In the case of fecal coliform, a "load" (flow rate/volume x concentration x time) could be calculated, but may not be the best way to approach this non-conservative parameter in a lake environment. Therefore, for the purposes of this TMDL, a loading capacity will not be "calculated" but by default, will be the water quality standard. The TMDL endpoint (Title 117 criteria) will be met through a targeted reduction from the 2001-2003 sampling concentrations.

2.3 Pollutant Source Assessment

For this pathogen TMDL the existing pollutant conditions will be described as fecal coliform densities rather than daily or annual loads.

2.3.1 Existing Pollutant Conditions

The existing pollutant conditions are shown in the elevation fecal coliform target curve (Figure 2.3.1) prepared for Johnson Lake. The points plotted above the line indicate a deviation from the water quality criteria. The chart was prepared solely as a graphical representation of the fecal coliform data in comparison to the water quality target. The lake elevation measurements were provided by CNPPID in October 2003.

The target curve was developed by the following equation:

Elevation x 400/100 ml

Where:
Elevation = Observed (0-100th percentile) lake elevations from 2001-03
400/100 ml = Fecal coliform water quality criteria

The data point values were calculated by multiplying the fecal coliform measurement by the observed daily elevation.

The comparison of fecal coliform density to elevation is used to determine if there are trends in the water quality such an increased fecal coliform density associated with a high or low lake elevation, with elevation being a representation of volume. From this evaluation and assessment of figure 2.3.1, there does not appear to be a significant trend observed and the water quality exceedances are observed across the range of observed elevations, thus fecal coliform densities are independent of elevation. Lacking a trend based on water elevation/volume and the variable nature of water volume management at the lake, alternative source identification techniques must be employed. These will be described in section 2.3.3 below.

2.3.2 Deviation from Acceptable Pollutant Loading Capacity

Table 2.3.2 describes the deviation from the acceptable water quality criteria based upon the in-lake samples (Plum Creek Inlet, Inlet Canal and Outlet Canal excluded) 2001-03 fecal coliform monitoring information.

Table 2.3.2 Johnson Lake Sample Deviation From the Acceptable Water Quality Criteria

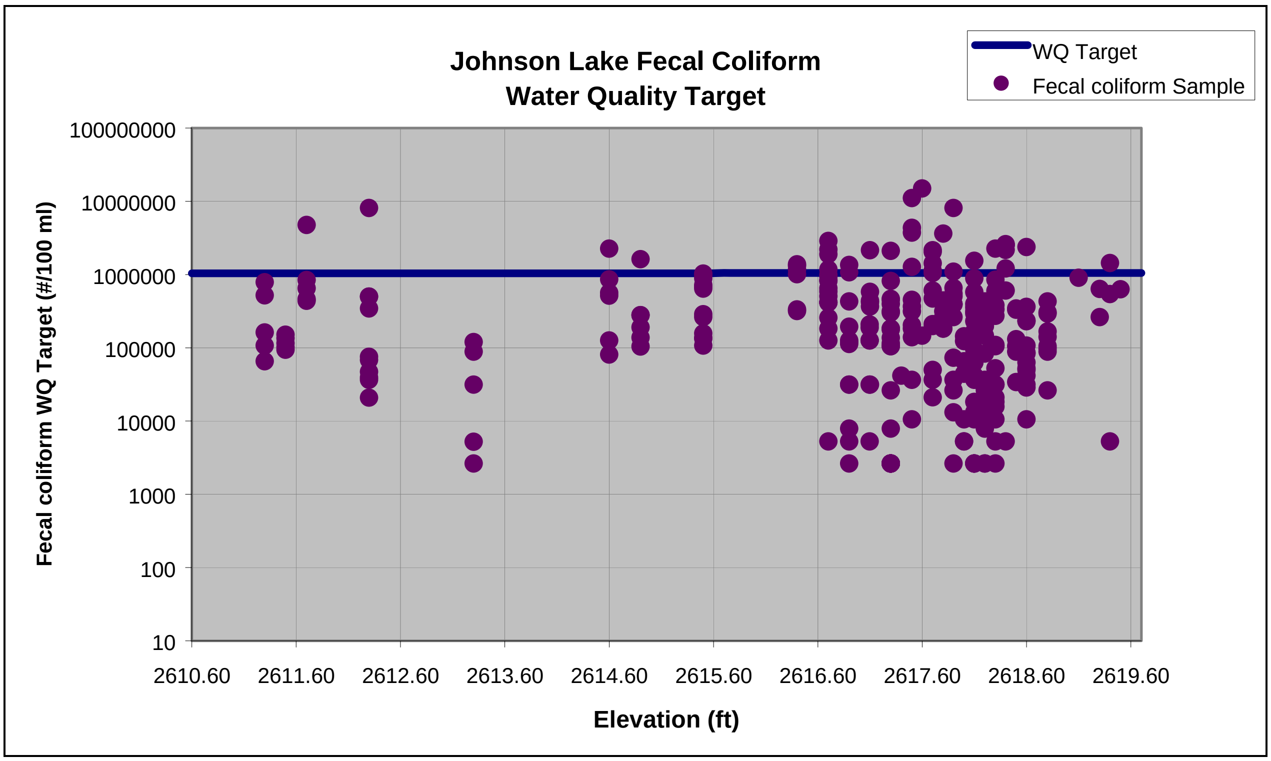
Site Number	Site/Location	Total Samples	# Samples >400 /100 ml	# Of Samples Greater than allowed to meet WQS
4	SE Swimming Beach	65	10	4
6	Kirby Point	45	3	0
7	North End	45	7	3
8	Lakeview Acres	45	4	0
10	Mallard Bay	39	8	5
11	SW Corner of Dam	45	5	1
	All Samples	284	37	9

2.3.3 Identification of Pollutant Sources

In the identification of pathogen sources, consideration must be given for the diffuse nature of fecal coliform sources, the land use in the area of the lake and the water quantity/volume management. As a result of these several factors that potentially influence fecal coliform densities, multiple techniques were employed to assist in the identification of specific sources or source categories. These techniques includes traditional water quality sampling and analysis of Johnson Lake and the source water provided by the Tri-County Canal along with microbial source tracking and specific wastewater compound sampling conducted by the United States Geologic Survey (USGS).

The details of the data analysis will be separated into two categories, external sources and internal sources. Specifically, external will refer to the information obtained from the inlet canal(s) to Plum Creek and Johnson Lake and internal refers to the data and information collected from the sites located around Johnson Lake proper.

Figure 2.3.1 Johnson Lake Fecal coliform Target Curve

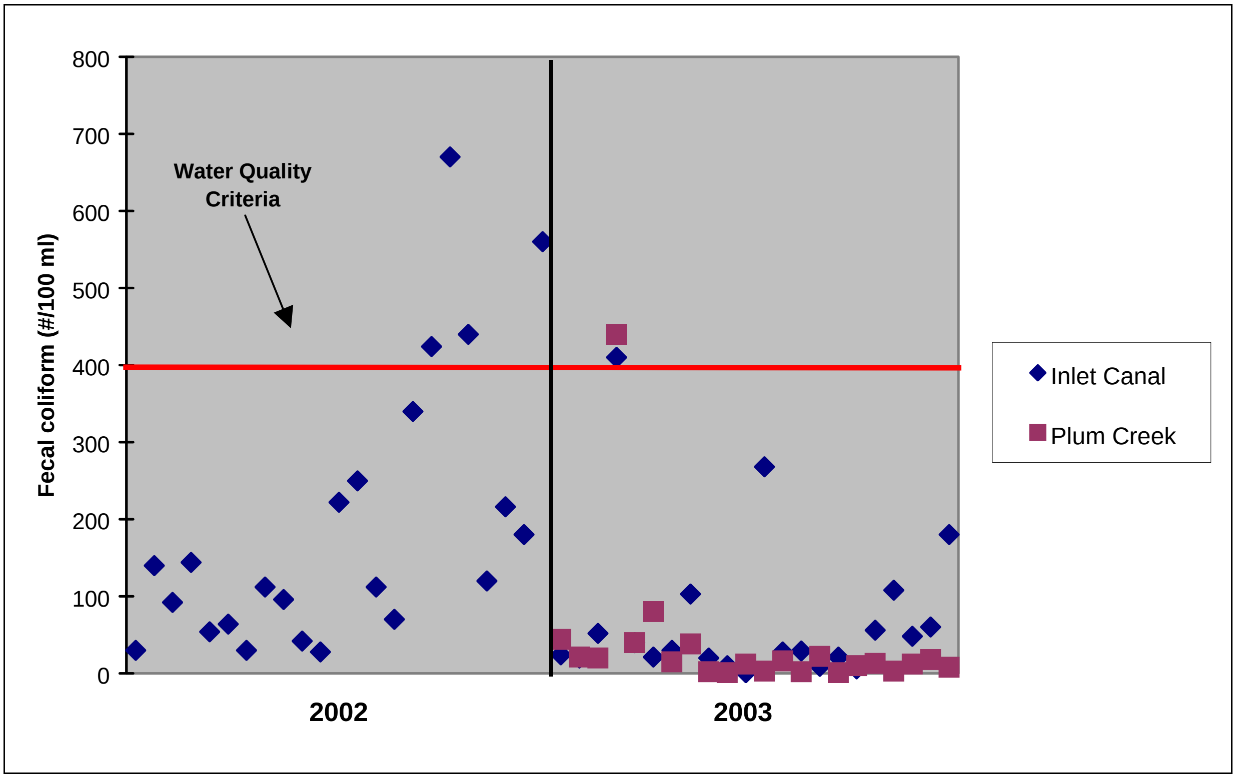


2.3.3.1 Internal vs. External Contributions of Fecal coliform: The term “external source” refers to those sources that may contribute to the Tri-County Canal, upstream of Johnson Lake. In contrast, the term “internal” refers to those sources that may contribute to the lake directly and are located in close proximity (i.e. storm drains). As shown in figure 2.1.2 above, fecal coliform samples were obtained from the Inlet Canal during 2002 and 2003 and from the inlet to Plum Creek Lake during 2003. A summary of the data is presented in table 2.3.3.1 with the individual data points being shown in Figure 2.3.3.1.

Table 2.3.3.1 Fecal coliform Information from the Johnson Lake and Plum Creek Inlets

Site Name/Location	Number of Samples	Maximum Observed Season Geometric Mean (#/100 ml)	# Samples >400/100 ml	% Samples >400/100 ml	Maximum Observed Value
Johnson Lake Inlet	45	129 (2002)	5	11%	670/100 ml
Plum Creek Inlet	22	12 (2003)	1	4.5%	440/100 ml

Figure 2.3.3.1 Johnson Lake and Plum Creek Lake Inlet Canal Fecal coliform Data



In order to assess the potential impacts of the inflow concentration, simple dilution calculation was utilized considering the inflow volume, lake volume, Inlet Canal maximum concentration, and a default “background” concentration for the purpose of determining the potential influence of the inflow on the water quality of Johnson Lake.

$$FC_{Final} = \{(FC_i * Q_i) + (FC_L * V_L)\}/(Q_i + V_L)$$

Where:
FC_{Final} = Lake Fecal coliform (#/100 ml)
FC_i = In-flow Fecal coliform (#/100 ml)
Q_i = In-flow Volume/flow (acre/feet)
FC_L = Initial Lake Fecal coliform (#/100 ml)
V_L = Lake Volume (acre/feet)

To populate this equation the maximum observed fecal coliform of 670/100 ml measured at the inlet canal, the average recreation season inflow of 1605 ac-ft, a (default) lake fecal coliform of 10/100 ml, and the lake volume of 42,465 ac-ft (at elevation 2617 feet) are used with the result being:

$$FC_{Final} = \frac{(670/100ml*1605)+(10/100ml*42,465)}{1605+42,465} = 34/100ml$$

Based on the maximum inflow concentration, the Johnson Lake fecal coliform would be 34/100 ml. The question then arises as to cumulative or long-term impacts based on the maximum observed fecal coliform density. Using the above equation and using the calculated fecal coliform density (FC_{Fianal}) for the initial fecal coliform for day +1 the below table was generated.

Day	**Cumulative Fecal coliform (#/100 ml)**
1	34
2	57
3	80
4	101
5	122
6	142
7	161
8	179
9	197
10	215
11	231
12	247
13	262
14	277
15	292
16	305
17	319
18	331
19	344
20	356
21	367
22	378
23	389
24	**399**
25	**409**
26	418

Actual "modeling" would involve the inclusion of decay/die off coefficients and the determination of the portion or percentage of the lake that is actually impacted. The values presented in the table were derived assuming no decay/die-off of fecal coliform is occurring and the lake completely mixes immediately. These assumptions were made to simplify the analysis. Using these predicted fecal coliform densities in the table, it would take approximately 24 days of continuous loading at 670/100 ml for the water quality of Johnson Lake to exceed the applicable criteria. A review of the data does not reflect that the maximum observed fecal coliform density persisted for the necessary 24 days.

One conclusion reached by this data analysis is that the Inlet Canal is having an impact on the water quality of Johnson Lake. However, the data does not support the inlet canal being the sole source causing the fecal coliform impairment. From this conclusion, fecal coliform reductions must be targeted to both internal and external sources.

2.3.3.2 **Source Tracking:** Recent advances in microbiology have included the use of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in the identification of bacteria sources. These techniques are commonly referred to as "microbial source tracking". In simplistic terms, the DNA or RNA of bacteria is converted to a "band pattern" and the obtained pattern is then compared to a library of known samples. The process is comparable to a fingerprint analysis.

A method of source tracking analysis using a E. coli (microbial) as the target parameter and commonly referred to as "Ribotyping" was utilized in August 2003. The source tracking methods employed and the laboratory report(s) are in Appendix A with a summary of the analysis being provided in Table 2.3.3.2.

The sampling results indicate the coliform sampled at the North End, Inlet Canal, SW Dam and Plum Creek Reservoir Inlet were likely from animal sources. The sample from Lakeview Acres Bay indicated a combination of animal and human sources.

Table 2.3.3.2 Ribotyping Analytical Results

Monitoring Location	Fecal coliform Result (mpn/100 ml)	*E. coli* Isolate #	Probable source
SE Swimming Beach	<3	Cancelled	Unable to isolate *E. coli*
Kirby Point	<3	Cancelled	Unable to isolate *E. coli*
North End	4	1	**Animal**
		2	**Animal**
		3	**Animal**
		4	**Animal**
		5	**Animal**
Lakeview Acres Bay	9	1	**Animal**
		2	**Animal**
		3	**Animal**
		4	***Human***
		5	***Human***
Inlet Canal	4	1	**Animal**
		2	**Animal**
		3	**Animal**
		4	**Animal**
		5	**Animal**
Mallard Bay	<3	Cancelled	Unable to isolate *E. coli*
SW Dam	4	1	**Animal**
		2	**Animal**
		3	**Animal**
		4	**Animal**
		5	**Animal**
Plum Creek Reservoir Inlet	4	1	**Animal**
		2	**Animal**
		3	**Animal**
		4	**Animal**
		5	**Animal**

2.3.3.3 Wastewater Compound Analysis To determine sources of bacteria to Johnson Lake, the NDEQ contracted with the United States Geologic Survey to collect samples that would be analyzed using the "*Method of Analysis by the U.S. Geological Survey National Water Quality Laboratory-Determination of Wastewater Compounds by Polystryrene-Divinylbenzene Solid Phase Extraction and Capillary-Column Gas Chromatography/Mass Spectrum*" also referred to as **Schedule 1433**. Schedule 1433 is a method for the determination of 67 compounds that have been found in domestic and industrial wastewaters. The compounds include food additives, fragrances, antioxidants, flame-retardants, plasticizers, industrial solvents, disinfectants, fecal sterols, polycyclicaromatic hydrocarbons and high use domestic pesticides (USGS 2002).

Samples were taken and analyzed on two different occasions, July 7 and August 18, 2003. The result of those events is summarized in Table 2.3.3.3. A copy of the report submittal and summary letter has been included as Appendix B of this TMDL.

Table 2.3.3.3 Results of Schedule 1433 Analysis by USGS

Compound	Site Detected	Date Detected	General Origin/Use
Para-nonylphenol	Kirby Point	8/19/03	Nonionic detergent metabolite
Caffeine	Mallard Bay and Lakeview Acres	7/7/03	Coffee, tea, etc.
Beta-stigmastanol	Plum Creek	8/19/03	Plant sterol
Cholesterol	Plum Creek	8/19/03	Originates from plant and animal sources
Phenol	SE Swimming Beach, Lakeview Acres Bay, North End, Inlet Canal,	7/7/03 and 8/19/03	General disinfectant
N, N-Diethyl-Meta-Toluamide (DEET)	All Sites	7/7/03 and 8/19/03	Insect repellant
Metolachlor	Mallard Bay, Southwest Dam, Inlet Canal, North End, Lakeview Acres, and SE Swimming Beach	7/7/03 and 8/19/03	Herbicide
Benzophenone	North End and Inlet Canal	8/19/03	Fixative for heavy perfumes, antihistamines, hypnotics and insecticides
1,4-Dichlorobenzene	Mallard Bay	7/7/03	Moth fumigant and deodorant
1-Methlynaphthalene and 2-methylnaphthalene	Mallard Bay	8/19/03	Components of gasoline and diesel fuel
Acetophenone	Mallard Bay	8/19/03	Flavoring agent and fragrance ingredient

Metolachlor is pre-emergent herbicide used on many crops including corn, soybeans and milo and can be persistent in the soils and water for as long as 200 days. The presence of metolachlor at several locations in the lake and the Inlet Canal indicates some agriculture drainage to the waterbody has occurred in either the form of nonpoint source runoff due to precipitation or irrigation return flow.

Cholesterol can originate from plant and animal sources while the source of *beta*-stigmastanol is likely plant materials. Both the cholesterol and *beta*-stigmastanol indicate a nonpoint or external contributions.

The presence of 1-Methlynaphthalene and 2-methylnaphthalene may be attributed to the large amount of boat traffic on Johnson Lake or other source such as direct contribution or storm water discharges (spill, illicit discharge, etc.).

Caffeine was detected at the Lakeview Acres Bay and Mallard Bay monitoring locations. Caffeine is a potential indicator of domestic wastewater because it is clearly of anthropogenic origin and often has been detected in wastewater (Selier, et. al., 1999).

Given the general origin and use of the remaining compounds measured in Johnson Lake (*para*-nonylphenol, phenol, DEET, benzophenone, 1,4-dichlorobenzene, and acetephenone), the Inlet Canal and Plum Creek Inlet, human sources of wastewater are entering Johnson Lake.

2.3.3.4 Source Determination: The data and information collected and analyzed/considered includes: weekly fecal coliform and *E. coli* samples, source tracking/ribotyping and wastewater compound analysis. Each category or series of data provides evidence as to the sources of fecal coliform and *E. coli* that have caused impairment of Johnson Lake. Unfortunately, the data and information is not such that specific sources can be identified; rather, the segregation will be into categories of sources.

A summary of the data and information considered is as follows:

- ➢ The maximum observed fecal coliform density at the Inlet Canal location was 670/100 ml
- ➢ High levels of fecal coliform did not persist for the 24 days required to elevate the levels of all the Johnson Lake sites that exceeded the water quality criteria.
- ➢ Source tracking methods indicate animals source contributions to the bacteria loading at the North End, Inlet Canal, SW Dam and Plum Creek monitoring locations
- ➢ Source tracking methods indicated animals and human source contributions to the bacteria loading at the Lakeview Acres Bay monitoring location
- ➢ The USGS Schedule 1433 Wastewater Compound Analysis detection of Metolachlor, *beta*-stigmastanol and cholesterol indicate nonpoint sources of bacteria are likely from agricultural drainage.
- ➢ The USGS Schedule 1433 Wastewater Compound Analysis detection of *Para*-nonylphenol, caffeine, phenol, N, N-Diethyl-Meta-Toluamide (DEET), Benzophenone, 1,4-Dichlorobenzene, and Acetophenone indicate human oriented wastewater is entering the waterbody.
- ➢ The NDEQ has not issued an NPDES permit for the discharge of domestic or compatible wastewater to the Tri-County Canal from the point of diversion from the Platte River to Johnson Lake

Based on this information, source identification conclusions are:

1. Fecal coliform bacteria is contributed to Johnson Lake through the Inlet Canal
2. Fecal coliform bacteria is contributed by internal sources
3. Fecal coliform bacteria is contributed by animal sources
4. Fecal coliform bacteria is contributed by human sources
5. Nonpoint and natural sources are the principal sources of fecal coliform bacteria in Johnson Lake

It should be noted; the order of listing does not represent a ranking or a level of importance assigned to the conclusions.

2.4 Pollutant Allocation

A TMDL is defined as:

TMDL = Loading Capacity = Wasteload Allocation + Load Allocation + Background + Margin of Safety

As stated above, the loading capacity is based upon elevation/volume in the hydrograph and is defined by:

Load Capacity = Elevation x 400/100 ml

Where:

Elevation = Recreation (May-September) season lake elevation
400/100 ml = applicable/target water quality criteria for fecal coliform from Title 117

By regulation, a TMDL requires a loading capacity value for the pollutant of concern. In the case of fecal coliform, a "load" (volume x concentration) could be calculated, but the approach may not be appropriate for expressing this non-conservative parameter. Therefore, for the purposes of this TMDL, a loading capacity will not be "calculated" but will be expressed as the water quality standard. Because the water quality criteria is expressed as a concentration, the LC will not equal the wasteload allocation + the load allocation.

To achieve the desired loading capacities requires the following allocations:

2.4.1 Wasteload Allocations (WLAs)

As stated above, there are no permitted facilities that discharge to Johnson Lake or the Tri-County Canal upstream of Johnson Lake. Based upon this the WLA for this TMDL will be zero (0).

2.4.2 Load Allocations (LAs)

Due to the diverse nature, distribution and delivery method, nonpoint and natural sources will not be separated. Given the difficulty in expressing loads using fecal coliform information, the below formula utilizes the lake elevation and the water quality criteria is used to define the acceptable water quality condition at a specific elevation and allows for illustration or plotting of the measurements. The process has been modified but is similar in principal to the procedure to that utilized for developing TMDLs for streams and described in the document *Nebraska's Approach for Developing TMDLs for Streams Using the Load Duration Curve Methodology.* (NDEQ 2002c) Therefore:

$$LA_i = E_i * 400/100 \text{ ml}$$

Where:
LA_i = load allocations at the i^{th} elevation
E_i = Elevation (ft) at the i^{th} elevation
400/100 ml = applicable/target water quality criteria for fecal coliform from Title 117

2.4.2.1 Reduction in Nonpoint Source and Natural Background Loads to Meet Water Quality Criteria

It is important to report the reductions necessary to meet the water quality criteria. The necessary reductions were determined based upon the 2001-03 data, which is considered representative information. The targeted reduction has been determined to be 55% with the results of the reduced loads being described in Table 2.4.2.1. This procedure provides water quality managers with a quantitative endpoint by which implementation planning can be carried out. The noted reductions, if achieved, should result in the waterbody fully supporting the primary contact recreation beneficial use.

Table 2.4.2.1 Targeted Nonpoint Source and Natural Background Reductions

Location/Monitoring Site	Targeted Reduction	Expected Geometric Mean	Expected Percentage of Samples >400/100 ml
SE Swimming Beach	55%	32/100 ml	8%
Kirby Point		16/100 ml	2%
North End		24/100 ml	4%
Lakeview Acres		30/100 ml	4%
Mallard Bay		30/100 ml	3%
SW Corner of Dam		17/100 ml	4%
All In-Lake Samples		24/100 ml	5%

2.4.3 Margin of Safety (MOS)

A margin of safety must be incorporated into TMDLs in an attempt to account for uncertainty in the data, analysis or targeted allocations. The MOS can either be explicit or implicit and for this TMDL is as follows:

- In order to achieve the applicable water quality criteria, the load allocation reduction must focus on the extreme measurements. In doing this, the expected seasonal geometric means for the specific locations and the lake as a whole are 84-91% less than the required seasonal geometric mean.
- Achieving the 55% reduction of fecal coliform will result in values that exceed 400/100 ml that is 50% less than necessary for the composite of all lake samples. In regards to the specific sites, the range is 20-80%.

2.4.4 Pathogen TMDL Summary

TMDL/Waterbody Loading Capacity = 0/100 ml (WLA) + E_i*400/100 ml (LA & Natural Background) + Implicit Margin of Safety

3.0 Implementation Plan

The implementation of controls to manage fecal coliform being delivered to Johnson Lake includes but is not limited to:

3.1 Animal Feeding Operations

Sources of fecal coliform bacteria to Johnson Lake include humans and animals as a result of nonpoint source. While no animal feeding operations exist in the direct vicinity of Johnson Lake, several are adjacent or near the Tri-County Canal upstream of the lake as shown in Figure 3.1. For these facilities, Title 130 – Rules and Regulations Pertaining to Livestock Waste Control (NDEQ 2001) states:

> *001 A livestock waste control facility shall be required for an existing or proposed livestock operation of three hundred animal units or larger, when livestock wastes:*

<u>001.01</u> *Violate or threaten to violate Title 117 (Neb. Administrative Code (NAC)), Nebraska Surface Water Quality Standards;*
<u>001.02</u> *Violate or threaten to violate Title 118 (NAC), Ground Water Quality Standards and Use Classification;*
<u>001.03</u> *Discharge into waters of the State; or*
<u>001.04</u> *Violate The Nebraska Environmental Protection Act.*

<u>002</u> *Any livestock operation less than three hundred animal units is exempt from the permitting process, including the requirement to request an inspection, unless there has been a confirmed discharge into waters of the State, or the Department has determined that because of conditions at the livestock operation there is a high potential for discharge into waters of the State in which case the Department shall notify the owner of the livestock operation by certified mail that the owner is subject to the Livestock Waste Management Act.*

When a livestock waste control facility is required, the owner/operator must also be issued a construction and/or a state-operating permit. State operating permits require facilities be properly operated and maintained to prevent water pollution and to protect the environment of the State.

By regulation, livestock waste control facilities for open lots must be designed and constructed to contain all waste generated under conditions less than a 25 year/24 hour precipitation event. Confined animal feeding operations are required to maintain 180 days of storage or a lagoon to treat the waste products. Meeting these permit requirements should equate to "zero" discharge during under conditions less than a 25 year 24 hour precipitation event, or a chronic wet period.

Wastewater and biosolids (manure) produced by animal feeding operations are most often land applied for beneficial reuse. Permitted facilities are required to follow best management practices (BMPs) for the land application as defined in Title 130, Chapter 11. Those BMPs include:

1. Utilize application areas which are under proper conservation treatment to prevent run-off into waters of the State
2. Not apply waste within 30 feet of any stream, lake or impounded waters identified in Chapter 6 and Chapter 7 of Title 117, unless in accordance with an approved comprehensive nutrient management plan
3. When waste is applied within 100 feet of any streams, lakes an impounded waters identified in Chapter 6 and 7 of Title 117, the Department may also require additional buffer and/or vegetative buffers, and that the livestock waste be applied in a manner which reduces potential for run-off of nutrients or pathogens by incorporation, injection of waste or other approved practices.

Based upon the above, it shall be recommended that the NDEQ's Agriculture Section stipulate in the state operating or other permits, for facilities located near the Tri-County Canal and upstream of Johnson Lake, that the application of livestock waste occurring during or 10 days prior to the Recreation Season (May 1 – September 30) be consistent with BMPs #1 and #2 above and the application setback distance be the minimum of 30 feet regardless of the status of the comprehensive nutrient management plan. In those areas where land slope or drainage is such where the application has a greater potential to run-off, or where biosolids application has been observed to have run-off, the recommendation will be consistent with the requirements of BMP #3 with the minimum setback being 100 feet.

3.2 Exempt Facilities/Other Agricultural Sources

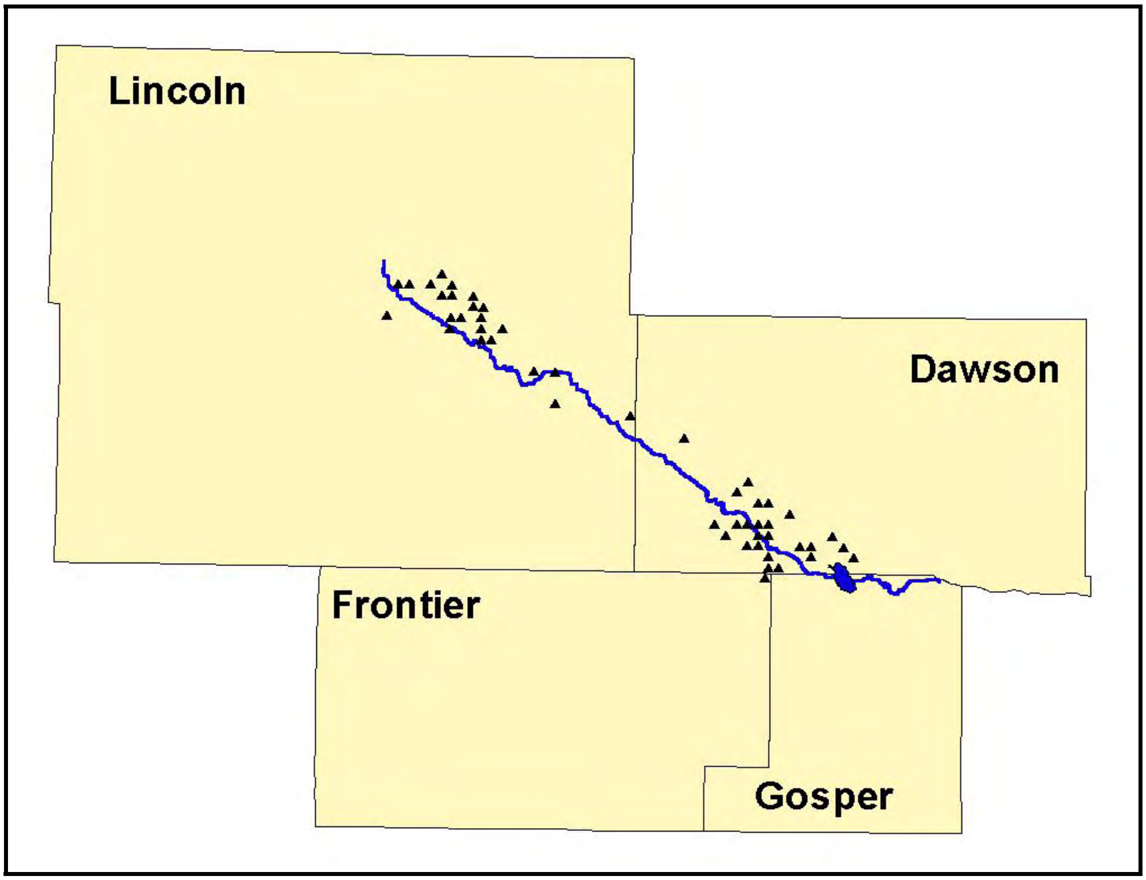
Animal feeding operations are exempt from the regulations set forth in Title 130 if:

- The operation is less than 300 animal units,
- There has not been a confirmed discharge to waters of the State, or
- The Department has determined that because of conditions at the livestock operation there is **not** a high potential for discharge into waters of the State.

Periodically, the NDEQ will receive a complaint on or a request for an inspection of a facility operating with <300 animal units. Should deficiencies be noted during the on-site visit, the owners/operator will often be given an opportunity to make corrections prior to enforcement or permit action being taken. In the event the efforts at voluntary compliance fail, civil enforcement or the issuance of a permit will be pursued to bring about the necessary corrective measures.

Because these facilities are "non-regulated", it is difficult to assess the impacts to the environment. As well, pastures or other temporary feeding practices may contribute to the fecal coliform impairments if conditions are such that run-off from the site occurs. In lieu of regulatory requirements, the NDEQ will first look to the USDA-Natural Resource Conservation Service (NRCS) for assistance utilizing programs under the control of the Service such as the Conservation Reserve Program, Environmental Quality Incentives Program, Conservation Farm Option, Conservation of Private Grazing Land Initiative, the Wetlands Reserve Program and others that aid in the maintenance and improvement of water quality.

Figure 3.1 Animal Feeding Operations Near the Tri-County Canal Issued or Requesting a State Construction or Operating Permit or Requesting an Inspection.



3.3 Dry Weather Discharges

Title 119 – Rules and Regulations Pertaining to the Issuance of Permits Under the National Pollutant Discharge Elimination System, Chapter 2 states:

> *"All persons discharging pollutants from a point source into any waters of the State are required to apply for and have a permit to discharge."*

Discharges not permitted should be required to obtain the proper authorization to discharge. All discharges are then subject to the appropriate limitations consistent with the WLAs established by this TMDL. Elimination of the discharge should be undertaken in the event permitting and control is not feasible.

3.4 Storm Water Discharges

The population of the Johnson Lake Area ranges from a wintertime low of approximately 600 persons and peaks near 5,200 people during the summer recreation period. In comparison (2004 Nebraska Directory of Municipal Officials) the peak population is similar to that of Schuyler or Ogallala, Nebraska. In order to gain a perspective of the potential stormwater impacts, the following is offered. In 1995, the NDEQ undertook sampling of the watershed contributing inflow to Holmes Lake in Lincoln, NE. One of monitoring locations captured the run-off from a residential area that included 1,045 homes (compared to approximately 900 in the direct vicinity of Johnson Lake). The maximum fecal coliform results, measured during run-off were 60,000 colonies/100 ml. From this, it is reasonable to expect stormwater impacts from the area surrounding Johnson Lake.

Due to the potential for the area to contribute significant amounts of fecal coliform during run-off events, actions should be taken to minimize the loadings. Activities include but are not limited to:

- Information and education program
- Identify and eliminate illicit discharges
- Pet waste management program or activities
- Stormwater pollution prevention program

3.5 On-Site Wastewater Treatment Systems

All of the residences that surround Johnson Lake are served by individual or shared septic tanks and drain fields. Since a majority of the lots are small and the area requirements of septic tanks and drain fields are relatively large, there is only nominal space available on each lot for the individual systems. As a result of tight spacing, ongoing building improvements and expansions, and rigid setback requirements, conflicts have arisen, resulting in the compromising of health and sanitary conditions. As old systems cease to function or become overloaded, many lots do not have adequate space for replacement systems. Also, the increasing trend of year round or extended seasonal use is leading to an increase in wastewater volume at Johnson Lake. Although the soil around the lake is generally acceptable for drain field purposes, it is being forced to handle more and more sewage, leading to saturation in some areas (Olmstead 2002).

Along with the spacing issues, there are no permanent records that detail individual treatment systems installed during the initial development. However, field observations during the replacement of the failed or undersized systems have noted various conditions and material (steel, concrete block, etc.) used for the early systems. The systems being removed are likely an accurate representation of those that remain in use.

Title 124 – Rules and Regulations for the Design, Operation and Maintenance of On-Site Wastewater Treatment Systems (NDEQ 2003b) includes the regulation for the installation of new facilities or maintenance of existing facilities. Maintenance activities and requirements for existing systems are found in Chapter 15 and include:

> *001 The owner of any septic tank or his agent shall regularly inspect and arrange for the removal and sanitary disposal of septage from the tank whenever the top of the sludge layer is less than 12 inches below the bottom of the outlet baffles or whenever the bottom of the scum layer is less than three inches above the bottom of the outlet baffle.*
>
> *002 Disposal of septage shall be in accordance with Federal, State and local rules and regulations.*

Failed systems, as defined in Title 124, Chapter 1, must be addressed in accordance to the regulations, including setback and spacing requirements.

Desired implementation activities for this TMDL for on-site wastewater treatment systems include:

- Information and education program on proper usage, maintenance, inspections, etc.
- Preventative maintenance of existing systems to increase efficiency
- Regular inspection and removal of septage in accordance with Title 124, Chapter 15
- Identification of failed systems with an emphasis on those with a direct conduit to Johnson Lake.
- Replacement of failed systems in accordance with Title 124
- Proper closure of abandoned systems
- Identification of saturated areas and the development and implementation of a water conservation plan for these areas

3.6 Section 319 – Nonpoint Source Management Program

The United States Environmental Protection Agency supplies grant funds to states to aid in managing nonpoint source pollution. When grant applications are submitted for review, an effort should be made to include the control of fecal coliform and surface run-off for the proposed projects in the area of Johnson Lake. As well, an effort will be made to redirect applicants to develop proposals consistent with the goals of this TMDL. Preference may be given to those projects that will have a direct reduction in the fecal coliform contributions of nonpoint source discharges.

3.7 Reasonable Assurances

The NDEQ is responsible for the issuance of NPDES or state operating permits for industrial and municipal wastewater discharges, regulated stormwater discharges and livestock operations (open lot or confined). Issued permits must be consistent with or more stringent than the wasteload allocations set forth by this TMDL. Compliance with the permit may require construction or modification of a facility and the issued permits may account for this through the inclusion of a compliance schedule or administrative order.

NDEQ is also responsible for ensuring adherence with the rules and regulations set forth in Title -124 Rules and Regulations for the Design, Operation and Maintenance of On-Site Wastewater Treatment Systems.

Effective management of nonpoint source pollution in Nebraska necessarily requires a cooperative and coordinated effort by agencies and organizations, both public and private. Several organizations (e.g. The Groundwater Foundation) are uniquely equipped to deliver specific services and assistance to the citizens of Nebraska to help reduce the effects of nonpoint source pollution on the State’s water resources. Agencies and organizations should be included when responsibilities include program oversight or fund allocation that may be useful in addressing and reducing fecal coliform contributions to Johnson Lake. Participation will depend on the agency/organization's program capabilities.

4.0 Future Monitoring

Future monitoring will be conducted to evaluate the beneficial use status of Johnson Lake. Monitoring locations will be specified annually depending upon the level of data necessary to determine the use status and activities that have occurred that may have an impact on the fecal coliform loadings (i.e. installation of best management practices). At a minimum, the NDEQ intends to monitor the SE swimming beach and one other representative location on an annual basis.

The utility of future specialized monitoring and analysis will be determined at a later date and as necessary to identify sources, health concerns, etc.

5.0 Public Participation

The availability of the TMDL in draft form was published in the Kearney Daily Hub and Lexington Clipper Herald with the public comment period running from approximately July 9, 2004 to August 16, 2004. This TMDL was also made available to the public on the NDEQ's Internet site and announcement letters were mailed to interested stakeholders. No comments were received in response to the public notice.

6.0 References

Chapman, Shannen, S. Omernik, J.M., Freeouf, J.A., Huggins, D.G., McCauley, J.R., Freeman, C.C., Steiner, G.A., Robert, T., Schlepp, R.L., 2001. Ecoregions of Nebraska and Kansas (color poster with map, descriptive text, summary tables and photographs): Reston, Virginia, U.S. Geological Survey

NDEQ 2001. Title 130 – Rules and Regulations Pertaining to Livestock Waste Control. Nebraska Department of Environmental Quality. Lincoln, NE.

NDEQ 2002a. 2002 Section 303(d) List of Impaired Waters. Nebraska Department of Environmental Quality. Lincoln, NE.

NDEQ 2002b. Title 117 – Nebraska Surface Water Quality Standards. Nebraska Department of Environmental Quality. Lincoln, NE.

NDEQ 2002c. Nebraska's Approach for Developing TMDLs for Streams Using the Load Duration Curve Methodology. Nebraska Department of Environmental Quality. Lincoln, NE.

NDEQ 2003a. Methodologies for Waterbody Assessment and Developing the 2004 Integrated Report for Nebraska. Nebraska Department of Environmental Quality. Lincoln, NE.

NDEQ 2003b. Title 124 – Rules and Regulations for the Design, Operation and Maintenance of On-Site Wastewater Treatment Systems. Nebraska Department of Environmental Quality. Lincoln, NE.

NDEQ 2004. 2004 Surface Water Quality Integrated Report. Nebraska Department of Environmental Quality Lincoln, NE.

NDNR. 2004. Nebraska Department of Natural Resources Databank, NDNR Internet Site, Nebraska Department of Natural Resources. Lincoln, NE.

Olmstead & Perry Consulting Engineers Inc. 2002. Wastewater and Water Supply Feasibility Study. Olmstead & Perry Consulting Engineers Inc. Omaha, NE.

Sieler, R. L., Zaugg, S.D., Thomas, J. M., and Howcroft, D.L., 1999, Caffeine and pharmaceuticals as indicators of wastewater contamination in wells: Ground Water, v. 37 no. 3, p 405-410.

Appendix A – E. coli ID – DNA Fingerprinting of *E. coli* Report by Source Molecular Corporation

Laboratory Identification Number	Monitoring Site
LMP2JOHNSN04	SE Swimming Beach
LMP2JOHNSN06	Kirby Point
LMP2JOHNSN07	North End
LMP2JOHNSN08	Lakeview Acres Bay
LMP2JOHNSN09	Inlet Canal
LMP2JOHNSN10	Mallard Bay
LMP2JOHNSN011	SW Dam
LMP2PLUMCR12	Plum Creek Reservoir Inlet

SOURCE MOLECULAR CORPORATION
4989 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-268-8363, Fax: (1) 786-513-2733, Email: info@sourcemolecular.com

E. coli ID™ – DNA Fingerprinting of *E. coli*

(Discriminant Analysis of Ribotype Profiles of *E. coli*)

Submitter: Nebraska Dept. of Environmental Quality
Source Molecular #: SM 4924
Submitter #: LMP2JOHNSN04
Sample Received: August 19, 2003
Date Reported: September 11, 2003

Fecal Coliform[4] mpn*/100 ml	*E. coli* Isolate # (0 colonies of cultured *E. coli* were analyzed)	Probable Source
< 3		CANCELLED

* mpn = most probable number of fecal coliforms in 100mL of sample after 20 hrs of cultivation at 44.5°C.

Laboratory Comments

We were unable to isolate any *E. coli* for DNA fingerprinting therefore the sample was cancelled.

Method and theory explanation page is included on a separate page in this report & on the website at www.sourcemolecular.com

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(Discriminant Analysis of Ribotype Profiles of *E. coli*)

Submitter: Nebraska Dept. of Environmental Quality
Source Molecular #: SM 4925
Submitter #: LMP2JOHNSN06
Sample Received: August 19, 2003
Date Reported: September 11, 2003

Fecal Coliform[4] mpn*/100 ml	*E. coli* Isolate # (0 colonies of cultured *E. coli* were analyzed)	Probable Source
< 3		CANCELLED

* mpn = most probable number of fecal coliforms in 100mL of sample after 20 hrs of cultivation at 44.5°C.

Laboratory Comments

We were unable to isolate any *E. coli* for DNA fingerprinting therefore the sample was cancelled.

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(Discriminant Analysis of Ribotype Profiles of *E. coli*)

Submitter: Nebraska Dept. of Environmental Quality
Source Molecular #: SM 4926
Submitter #: LMP2JOHNSN07
Sample Received: August 19, 2003
Date Reported: September 11, 2003

Fecal Coliform[4] mpn*/100 ml	*E. coli* Isolate # (5 colonies of cultured *E. coli* were analyzed)	Probable Source
= 4	1	Animal
	2	Animal
	3	Animal
	4	Animal
	5	Animal

* mpn = most probable number of fecal coliforms in 100mL of sample after 20 hrs of cultivation at 44.5°C.

Laboratory Comments

The DNA fingerprints of 5 colonies of *E. coli* cultured from the water sample statistically matched animal sources recorded in a database of known source DNA fingerprints. The results do not represent that animal *E. coli* is the only *E. coli* in the system under investigation. Further analysis of multiple samples from multiple locations, in repetition would add further confidence. Pinpointing the source of animal *E. coli* is possible by collecting fecal matter from the predicted sources along with further contaminated water samples, and then looking for a direct DNA match.

Method and theory explanation page is included on a separate page in this report & on the website at www.sourcemolecular.com

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(Discriminant Analysis of Ribotype Profiles of *E. coli*)

Submitter: Nebraska Dept. of Environmental Quality
Source Molecular #: SM 4927
Submitter #: LMP2JOHNSN08
Sample Received: August 19, 2003
Date Reported: September 11, 2003

Fecal Coliform[4] mpn*/100 ml	*E. coli* Isolate # (5 colonies of cultured *E. coli* were analyzed)	Probable Source
= 9	1	Animal
	2	Animal
	3	Animal
	4	Human
	5	Human

* mpn = most probable number of fecal coliforms in 100mL of sample after 20 hrs of cultivation at 44.5°C.

Laboratory Comments

The DNA fingerprints of 5 colonies of *E. coli* cultured from the water sample statistically matched both human and animal sources when compared to a database of known source DNA fingerprints. Pinpointing the source of animal *E. coli* is possible by collecting fecal matter from the predicted sources along with further contaminated water samples, and then looking for a direct DNA match.

Method and theory explanation page is included on a separate page in this report & on the website at www.sourcemolecular.com

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E. coli ID™ – DNA Fingerprinting of *E. coli*

(Discriminant Analysis of Ribotype Profiles of *E. coli*)

Submitter: Nebraska Dept. of Environmental Quality
Source Molecular #: SM 4928
Submitter #: LMP2JOHNSN09
Sample Received: August 19, 2003
Date Reported: September 11, 2003

Fecal Coliform[4] mpn*/100 ml	*E. coli* Isolate # (5 colonies of cultured *E. coli* were analyzed)	Probable Source
= 4	1	Animal
	2	Animal
	3	Animal
	4	Animal
	5	Animal

* mpn = most probable number of fecal coliforms in 100mL of sample after 20 hrs of cultivation at 44.5°C.

Laboratory Comments

The DNA fingerprints of 5 colonies of *E. coli* cultured from the water sample statistically matched animal sources recorded in a database of known source DNA fingerprints. The results do not represent that animal *E. coli* is the only *E. coli* in the system under investigation. Further analysis of multiple samples from multiple locations, in repetition would add further confidence. Pinpointing the source of animal *E. coli* is possible by collecting fecal matter from the predicted sources along with further contaminated water samples, and then looking for a direct DNA match.

Method and theory explanation page is included on a separate page in this report & on the website at www.sourcemolecular.com

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(Discriminant Analysis of Ribotype Profiles of *E. coli*)

Submitter: Nebraska Dept. of Environmental Quality
Source Molecular #: SM 4929
Submitter #: LMP2JOHNSN10
Sample Received: August 19, 2003
Date Reported: September 11, 2003

Fecal Coliform[4] mpn*/100 ml	*E. coli* Isolate # (0 colonies of cultured *E. coli* were analyzed)	Probable Source
< 3		CANCELLED

* mpn = most probable number of fecal coliforms in 100mL of sample after 20 hrs of cultivation at 44.5°C.

Laboratory Comments

We were unable to isolate any *E. coli* for DNA fingerprinting therefore the sample was cancelled.

Method and theory explanation page is included on a separate page in this report & on the website at www.sourcemolecular.com

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(Discriminant Analysis of Ribotype Profiles of *E. coli*)

Submitter: Nebraska Dept. of Environmental Quality
Source Molecular #: SM 4930
Submitter #: LMP2JOHNSN11
Sample Received: August 19, 2003
Date Reported: September 11, 2003

Fecal Coliform[4] mpn*/100 ml	*E. coli* Isolate # (5 colonies of cultured *E. coli* were analyzed)	Probable Source
= 4	1	Animal
	2	Animal
	3	Animal
	4	Animal
	5	Animal

* mpn = most probable number of fecal coliforms in 100mL of sample after 20 hrs of cultivation at 44.5°C.

Laboratory Comments

The DNA fingerprints of 5 colonies of *E. coli* cultured from the water sample statistically matched animal sources recorded in a database of known source DNA fingerprints. The results do not represent that animal *E. coli* is the only *E. coli* in the system under investigation. Further analysis of multiple samples from multiple locations, in repetition would add further confidence. Pinpointing the source of animal *E. coli* is possible by collecting fecal matter from the predicted sources along with further contaminated water samples, and then looking for a direct DNA match.

Method and theory explanation page is included on a separate page in this report & on the website at www.sourcemolecular.com

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E. coli ID™ – DNA Fingerprinting of *E. coli*

(Discriminant Analysis of Ribotype Profiles of *E. coli*)

Submitter: Nebraska Dept. of Environmental Quality
Source Molecular #: SM 4931
Submitter #: LMP2PLUMCR12
Sample Received: August 19, 2003
Date Reported: September 11, 2003

Fecal Coliform[4] mpn*/100 ml	*E. coli* Isolate # (5 colonies of cultured *E. coli* were analyzed)	Probable Source
= 4	1	Animal
	2	Animal
	3	Animal
	4	Animal
	5	Animal

* mpn = most probable number of fecal coliforms in 100mL of sample after 20 hrs of cultivation at 44.5°C.

Laboratory Comments

The DNA fingerprints of 5 colonies of *E. coli* cultured from the water sample statistically matched animal sources recorded in a database of known source DNA fingerprints. The results do not represent that animal *E. coli* is the only *E. coli* in the system under investigation. Further analysis of multiple samples from multiple locations, in repetition would add further confidence. Pinpointing the source of animal *E. coli* is possible by collecting fecal matter from the predicted sources along with further contaminated water samples, and then looking for a direct DNA match.

Method and theory explanation page is included on a separate page in this report & on the website at www.sourcemolecular.com

DNA Fingerprinting Method Explanation

E. coli were enumerated by taking plates that are positive for fecal coliforms, transferring the membrane filter to EC with MUG media (Difco), and incubating for an additional 24 hours at 37°C. Colonies that fluoresced under UV light were counted as *E. coli* and isolated for ribotyping.

Ribotyping of *E. coli* isolates was accomplished by the method of Parveen *et al* (1999)[1]. Chromosomal DNA was extracted from *E. coli* isolates and digested with *Hind*/III. Fragments were separated by agarose electrophoresis. The DNA was then transferred and fixed to a Zeta-probe membrane. A cDNA probe complementary to the *E. coli* 16S and 23S rDNA was labeled with digoxigenin-dUTP and was used to probe the membranes. The resulting genetic fingerprint was translated to a binary code based on the presence and absence of predetermined bands. The resulting binary code was then analyzed by discriminant analysis using SAS (registered) software against a library of known source isolates - similar to the method elaborated in Scott *et al* (2003)[3].

DNA Fingerprinting Theory Explanation

After cultivating *E. coli* from the submitted sample, one or more *E. coli* isolates are selected. Isolates are clusters of *E. coli* colonies on an agar plate. A DNA fingerprinting analysis called ribotyping is performed on each *E. coli* isolate selected. This genetic fingerprint comes from genes that code for ribosomal ribonucleic acids (rRNA) of *E. coli*. Ribosomal RNA together with various proteins makes up the cell structure called a ribosome.

The ribosome is the cell structure where proteins are manufactured. In order to produce proteins, the messenger RNA and the amino acids are transferred to the ribosome. As the ribosome moves down the messenger RNA, it places the correct amino acid in the growing protein. It has been shown that looking at small differences in the DNA that codes for these 16S and 23S rRNA's help identify different strains of *E. coli*.

Ribosomal genes are also known to be highly conserved in microbes, meaning that the genetic information coding for rRNA will vary much less within bacteria of the same strain than it will between bacterial strains. This characteristic allows for a greater ability to distinguish between different bacterial strains.

In ribotyping, restriction enzymes are used to cut the genes coding for rRNA into pieces, and electrophoresis separates the pieces by size through a gel. Genetic probes then visualize locations of different-size fragments of DNA in the gel, which appear as bands. The banding pattern of DNA fragments corresponding to the relevant rRNA is known as the ribotype. The banding patterns are compared to a database of other *E. coli* strains and matched for each determined strain. If the client submits fecal samples, then direct banding pattern matches are also investigated between the fecal samples and blind samples submitted.[2]

[1] Parveen, Salina, Portier, Kenneth M., Robinson, Kevin, Edmiston, Lee, Tamplin, Mark L. **Discriminant Analysis of Ribotype Profiles of Escherichia coli for Differentiating Human and Nonhuman Sources of Fecal Pollution** Appl. Environ. Microbiol. (1999) 65: 3142-3147

[2] Carson, C. Andrew, Shear, Brian L., Ellersieck, Mark R., Asfaw, Amha **Identification of Fecal Escherichia coli from Humans and Animals by Ribotyping** Appl. Environ. Microbiol. (2001) 67: 1503-1507

[3] Scott, Troy M., Parveen, Salina, Portier, Kenneth M., Rose, Joan B., Tamplin, Mark L., Farrah, Samuel R., Koo, Andrew, Lukasik, Jerzy **Geographical Variation in Ribotype Profiles of Escherichia coli Isolates from Humans, Swine, Poultry, Beef, and Dairy Cattle in Florida** Appl. Environ. Microbiol. (2003) 69: 1089-1092

[4] Standard methods for the Examination of Water and Wastewater Method 9223 A1 (APAHA, 1998)

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Appendix B – Transmittal Letter of USGS Schedule 1433 Monitoring

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
U.S. Geological Survey
Water Resources Division
100 Centennial Mall North
Federal Building 406
Lincoln, NE 68508

October 27, 2003

Pat O'Brien, Total Daily Maximum Load (TMDL) Coordinator
Nebraska Department of Environmental Quality
Planning Unit-Water Quality Division
1200 N Street, Suite 400
Lincoln, NE 68509-8922

Subject: Water-quality results for Johnson Lake Bacteria Source Assessment Project, Johnson Lake, Dawson and Gosper Counties, Nebraska

Dear Pat:

The U.S. Geological Survey (USGS) has completed the water-quality analysis for the Johnson Lake Bacteria Source Assessment Project, Johnson Lake, Dawson and Gosper Counties, Nebraska. The water-quality parameters analyzed for included USGS-National Water Quality Laboratory (NWQL) Schedule 2711-National Water Quality Assessment Program (NAWQA), surface water, nutrients, and Schedule 1433-Wastewater compounds, filtered, solid-phase extraction (SPE), gas chromatography/mass spectrometry (GC/MS), and field parameters, which included water temperature, conductivity, pH, and dissolved oxygen. The samples were collected during two different events, the first occurred July 7, 2003, and the second occurred August 18, 2003. The samples were obtained at a depth of approximately 3 feet at eight locations, which included Johnson Lake Swimming Beach, Southwest Dam, Mallard Bay, Inlet Canal, Lakeview Acres, North End, and Kirby Point. A quality-assurance sample was collected during each event.

The site map, quality-assurance monitoring project plan, analyte lists for wastewater compounds and nutrients, a copy of Water-Resources Investigations Report 01-4186, Methods of analysis by the USGS NWQL--determination of wastewater compounds by polystyrene-divinylbenzene solid-phase extraction and capillary-column GC/MS, 2002, and the Johnson Lake water-quality and field-parameter results are presented in table format and in charts.

The wastewater compounds method analyzes for 67 compounds typically found in domestic and industrial wastewater. The method was developed in response to increasing concern over the impact of endocrine-disrupting chemicals in wastewater on aquatic organisms. The method focuses on the determination of compounds that are an indicator of wastewater or that have been chosen on the basis of their endocrine-disrupting potential or toxicity. These compounds include the alkylphenol ethoxylate nonionic surfactants and their degradates, food additives, fragrances, antioxidants, flame retardants, plasticizers, industrial solvents, disinfectants, fecal sterols, polycyclicaromatic hydrocarbons, and high-use domestic pesticides.

The alkylphenol *(*AP*)* compound *para*-nonylphenol, a nonionic detergent metabolite, was detected at Kirby Point at a concentration of 1.3 µg/L. Various investigators have reported concentrations of APs ranging from 10 to 100 mg/kg in sediment samples near sewage-treatment plants. This compound is known for its estrogenic activity (hormonal disruption) in aquatic organisms at concentrations greater than 20 µg/L.

Caffeine was detected at Mallard Bay and Lakeview Acres in concentrations of 0.033 and 0.022 µg/L, respectively. Detection of caffeine can be an important indicator of wastewater contamination in surface-water samples; however, caffeine is not persistent in the environment because of rapid degradation by bacteria.

The compounds of *beta*-stigmastanol and cholesterol were detected at Plum Creek in concentrations of 0.95 and 1.0 µg/L, respectively. B*eta*-stigmastanol is a plant sterol. Cholesterol can originate from plant and animal sources and often is an indicator of fecal contamination, it is used in pharmaceutical and dermal preparations as an emulsifying agent. Cholesterol is not classifiable as to its carcinogenicity to humans.

Phenol was detected at all sites. As a general disinfectant, either in solo or mixed with slaked lime for toilets, stables, cesspools, floors, drains, etc. Phenol is also used in the manufacturing of colorless or light-colored artificial resins, many medical and industrial organic compounds and dyes; and as a reagent in chemical analysis. Phenol is not classifiable as to its carcinogenicity to humans.

N, N-Diethyl-Meta-Toluamide (DEET) was detected at all sites. DEET is a commonly used insect and acarid repellent. Because it was detected at all sites, it is possible that members of the sampling team had applied DEET prior to or during the sampling event.

Metolachlor was detected at Mallard Bay, Southwest Dam, Inlet Canal, North End, Lakeview Acres, and Johnson Lake Beach. It is a preemergence and preplant incorporated weed control herbicide, used to control of annual grasses and some broad-leaved weeds. Metolachlor is often used in combination with broad-leaved herbicides, in order to extend the spectrum of activity. It is a possible human carcinogen.

Benzophenone was detected at the North End and the Inlet Canal in small concentrations. It is a fixative for heavy perfumes, such as geranium new-mown hay, especially when used in soaps and it is used in the manufacture of antihistamines, hypnotics and insecticides. Benzophenone is a suspected endocrine-disrupting compound (EDC).

1,4-Dichlorobenzene was detected in small concentrations at Mallard Bay. It is a moth repellent fumigant, and it is used in deodorants. There is inadequate evidence in humans for the carcinogenicity of dichlorobenzene

The compounds 1-Methylnaphthalene and 2-Methylnaphthalene were detected at Mallard Bay in low concentrations. They are components of gasoline and diesel fuel.

Acetophenone was detected at Mallard Bay in low concentrations. It is used as a flavoring agent in non-alcoholic beverages, ice cream, candy, baked goods, gelatins and puddings, chewing gum and a fragrance ingredient in soaps, detergents, creams, lotions, and perfumes. It is not classifiable as to human carcinogenicity

The NAWQA nutrient method analyzes for six compounds typically found in runoff from inorganic fertilizers applied to cultivated farmland, from manure in feedlots, and from domestic wastewater. Total nitrogen (ammonia, nitrate, nitrite, organic) was detected at Johnson Lake Beach and Mallard Bay in concentrations of 0.75 and 1.3 mg/L, respectively. Phosphorus was detected at Lakeview Acres, Kirby Point, North End, Inlet Canal, Southwest Dam, and the Plum Creek Inlet in concentrations that ranged from 0.8 to 1.0 mg/L. Johnson Lake Beach and Mallard Bay had the lowest concentrations of dissolved oxygen, which could be attributed to the nitrification process, which uses oxygen.

If you have any questions or would like to discuss the results further please call me at 437-5115.

Sincerely,

Rick Wilson, P.E.
Chief, Hydrologic Studies Section

Attachments:

1. Site map of Johnson Lake sampling locations
2. Quality Assurance Monitoring Project Plan for the Johnson Lake Bacteria Source Assessment Project # SWS-03-001
3. USGS-National Water Quality Laboratory, Analyte List for Schedule 2711, National Water Quality Assessment Program (NAWQA), Surface Water, Nutrients
4. USGS-National Water Quality Laboratory, Analyte List for Schedule 1433, Waste Water Compounds, Filtered, Solid-Phase Extraction (SPE), Gas Chromatography/Mass Spectrometry (GCMS)
5. Water Resources Investigation Report 01-4186, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory---Determination of wastewater compounds by polystyrene-divinylbenzene solid-phase extraction and capillary-column gas chromatography/mass spectrometry, 2002
6. Wastewater compound parameter (Schedule 1433) results, nutrient parameter (Schedule 2711) results, field parameter (water temperature, conductivity, pH, dissolved oxygen) results