IOWA'S WATER
Ambient Monitoring Program

Bacteria Source Tracking
in Lake Darling Watershed

Monitoring of Iowa's surface waters during the past five years has demonstrated the regular occurrence of fecal bacteria in surface water resources. While the presence of fecal bacteria in water indicates the input of a relatively fresh source of human and/or animal waste, it does not identify the source(s). A recent economic study by Iowa State University found that Iowans value water quality above all other factors when choosing a lake for recreation. Specifically, Iowans are concerned

Lake Darling in Washington County.

about safety from bacterial contamination and health advisories while swimming[1]. In 2003, the Iowa DNR initiated a study of Lake Darling and its watershed (Washington County) in an attempt to identify the source(s) of fecal bacteria in the lake through the use of several source tracking tools. Numerous source tracking methodologies have been developed to determine the sources of fecal bacteria from environmental samples (e.g., from human, livestock, or wildlife origins).

Lake Darling Beach has periodically experienced high bacteria levels since beach monitoring began in 2000. The lake area is less than 0.5 mi^2 and drains a watershed that is nearly 20 mi^2, about 55% of which is in agricultural production. Water samples for source tracking were taken during nine sampling events under varying flow conditions (Table 1). Ten sites on the lake and throughout the watershed were monitored (Figure 1). Source tracking tools used in this project included DNA ribotyping, antibiotic resistance analysis (ARA), sterols/caffeine/cotinine analysis, and pathogens analysis.

Methods

DNA ribotyping involves comparing DNA profiles or "fingerprints" of *E.coli* bacteria from impacted waters to DNA profiles of *E.coli* from known sources of fecal material in the watershed. Fecal samples were collected from geese, humans, deer, cattle, and swine within the watershed and the isolated *E.coli* were ribotyped to create a library of DNA patterns for known *E.coli* sources in this watershed. *E.coli* isolates from lake, beach, and stream water samples that were taken throughout the watershed were compared to the DNA library of known sources to identify contributing sources within the Lake Darling watershed.

Table 1. *Sampling events conducted for Lake Darling Source Tracking Project.*

Sampling Date	Flow Regime*	Rainfall previous 24 hours
6/26/03	Mid-flow	1.4
7/9/03	Mid-flow	0.9
8/6/03	Low-flow	0.0
8/18/03	Low-flow	0.0
9/2/03	Low-flow	0.0
9/15/03	Low-flow	0.3
3/5/04	High-flow	1.6
3/26/04	High-flow	1.2
6/11/04	High-flow	0.6

*Flow Regime based on discharge, not rainfall in past 24 hours.

Antibiotic resistance analysis (ARA). The source(s) contributing fecal pollution to a water body can often be determined by analyzing the antibiotic resistance profiles of *E.coli* bacteria isolated from both known sources and surface water. *E.coli* isolates used for ARA were taken from the same groupings as those used by DNA ribotyping. These isolates were exposed to 26 separate antibiotics at multiple concentrations and their ability to grow in the presence of these antibiotics was noted. The growth characteristics of bacteria from known sou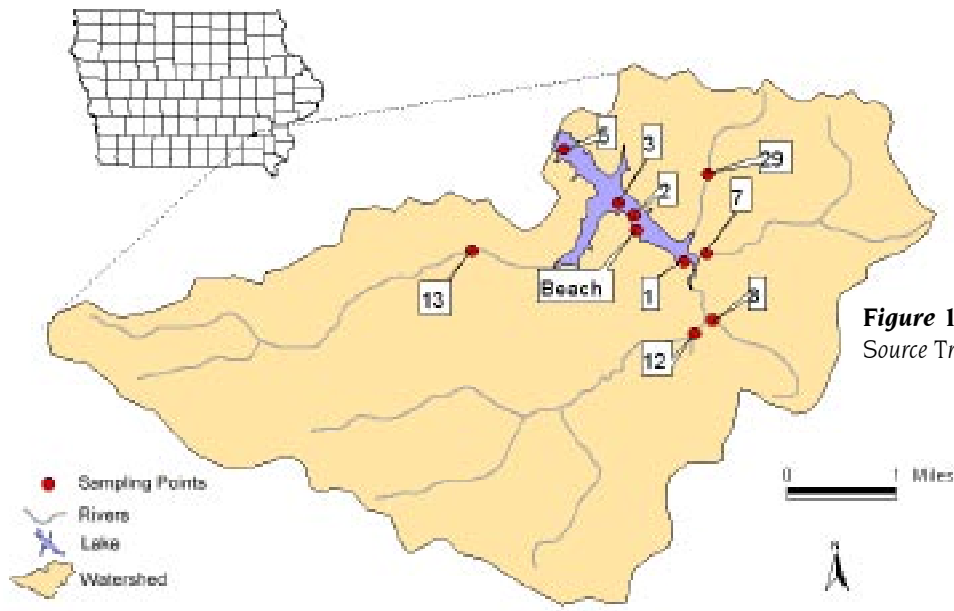rces were compared to growth characteristics of bacteria from lake, beach, and stream water samples to determine contributing sources within the Lake Darling watershed.

Sterols/caffeine/cotinine. Past research has shown sterols (chemical markers found in the fatty acids of cell walls and membranes) to be effective in identifying sewage contamination of sediment, however their use in water samples is experimental. Sterols analysis focused on a coprostanol ratio (coprostanol to cholesterol+dihydrocholesterol). Research conducted on this ratio has revealed that a high coprostanol ratio in sediment could signify a sewage source of bacteria[2]. However, the validity of this relationship has not been established for sterols collected from water samples.

Caffeine and cotinine (nicotine by-product) in water samples can indicate human sources of bacteria, although caution must be used when interpreting caffeine results, as this compound has become quite common in the environment. A small portion of the water samples (10%) were tested for sterols, caffeine, and cotinine.

Pathogens are disease-causing microorganisms. All of the water samples collected in this project were analyzed for multiple pathogens: *E.coli* O157:H7 and several species of *Salmonella*. While some strains of these two pathogens are common in both animals and humans, others are specific to certain hosts. *E.coli* O157:H7 is not a strain of *E.coli* typically used as an indicator, but can itself cause illness if ingested.

Results

DNA ribotyping indicated that multiple sources of *E.coli* bacteria in the watershed were influencing water quality in Lake Darling. As this type of source tracking study is new in the state of Iowa, the size of known DNA libraries needed for comparison in Iowa waters was unknown. Despite this drawback, analysis was still able to determine that 25% of the *E.coli* isolates observed in the water samples originated from wild animal sources (geese and deer). Only one *E.coli* isolate from the Lake Darling water samples was consistent with DNA profiles exhibited by known human sources. Because of the small library size, not enough known isolates were collected from the groups of domesticated animals (cattle and swine). This resulted in domesticated animal isolates falling into a group of "unknown"

Figure 1. *Sampling sites for Lake Darling Source Tracking Project.*

sources. Consequently, 75% of the *E.coli* isolates from water were classified to be from this "unknown" group, meaning many "unknown" isolates could originate from domesticated animals.

The result of more "unknown" source classifications than "known" sources (human and wild animal) suggests that the *E.coli* isolate library was either not large enough or was not representative of all of the sources in the watershed. The abundance of "unknown" identifications from the ribotyping analysis most likely reflects the small isolate library for cattle and swine sources. When all animal isolates were grouped together and compared to human isolates, many of the previously "unknown" samples were identified as animal, suggesting these isolates may be from cattle or swine sources.

Antibiotic resistance analysis results were similar to DNA ribotyping, while having similar drawbacks with limited library size. A high percentage of water samples (39%) were identified as originating from geese and deer, while 12% were classified as originating from livestock (cattle or swine). With ARA, 10% of the samples had similar profiles to known human isolates, while a relatively large percentage (39%) were of "unknown" origin.

Sterols/caffeine/cotinine analysis showed a high coprostanol ratio only at Site 7, located on a tributary entering Lake Darling from the east side of the watershed. If the coprostanol ratio used for sediment is applied to water, this is the only site where a sewage source would be expected.

Analysis of water samples for caffeine and cotinine did not indicate a human source. One site had a small amount of caffeine (site 5, near the dam), while no cotinine was found in any of the water samples.

Pathogen analysis identified *Salmonella* species at all sites, except site 5 near the dam, during different times. The *Salmonella* species found could not be identified as belonging to any particular source group. No *E.coli* O157:H7 was found at any of the sites.

Discussion

As a whole, data from this project indicated that the sources of bacteria at the beach and in the lake were from animals throughout the watershed. In order to more fully understand the sources of bacterial contamination in the Lake Darling watershed, additional fecal samples are being collected from domesticated animal sources. This should increase the size of the DNA library significantly and allow these groups to be looked at individually in determining sources of fecal contamination in the water.

All of the different source tracking tools used have their advantages and limitations. A tool box approach using numerous source tracking tools, coupled with bacteria monitoring, investigation of sources in the watershed, and animal inventories, is recommended for source tracking in Iowa. All these tools generate several lines of evidence to more accurately identify the contributing source(s) of fecal bacteria in a watershed.

A final project report is available on the Iowa DNR Water Monitoring Section web site in the publications section at wqm.igsb.uiowa.edu.

References

1. Azevedo, C.D., K.J. Egan, J.A. Herriges, and C.L. Kling, 2003. The Iowa Lakes Valuation Project: Summary and Findings from Year One. Ames, IA: Iowa State University, 34 p.

2. Writer, J.H., J.A. Leenheer, L.B. Barber, G.L. Amy, and S.C. Chapra, 1995. Sewage Contamination in the Upper Mississippi River as Measured by the Fecal Sterol, Coprostanol. Water Resources 29(6):1427-1436.

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