Final Report
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Assessing Groundwater Quality in Kewaunee County, Wisconsin and Characterizing the Timing and Variability of Enteric Pathogen Contamination within the Dolomite Aquifer in Northeastern Wisconsin

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ABSTRACT

The Silurian dolomite aquifer is an important water source in northeastern Wisconsin. Previous studies have shown that the aquifer is vulnerable to contamination because the dolomite is extensively fractured and many regions have thin soils that do not attenuate contaminants moving from the land surface to the water table. Wastewater from septic systems and livestock manure from farming operations are possible contaminant sources, while environmental conditions and geology (e.g., precipitation, groundwater recharge, and depth to bedrock) can affect the timing and extent of private well contamination. We address these aspects of groundwater contamination in six main objectives that produced nine key findings. Private domestic wells completed in the Silurian dolomite aquifer and located in Kewaunee County, Wisconsin were sampled and tested in three ways: 1) Wells were randomly selected based on depth to bedrock and tested for coliform bacteria and nitrate during two synoptic events, the fall of 2015 and summer of 2016. 2) Samples were collected on a seasonal basis during five sampling events and analyzed by quantitative polymerase chain reaction (qPCR) for microbes that originate in fecal material, including those specific to human fecal wastes and manure from ruminant animals and cattle. 3) Automated sampling devices collected time-series samples and continuously monitored water-quality parameters. Overall, we collected 980 samples from 624 wells. Depth-to-bedrock data were abstracted from well construction reports, and sentinel wells monitored groundwater levels and a variety of water-quality parameters that were assessed for their utility as indicators of recharge. Key findings are as follows: 1) The Kewaunee county-wide contamination rate for wells positive for coliform bacteria or with nitrate-N concentrations greater than 10 mg/L was 26% in the fall of 2015 and 28% in the summer of 2016. 2) In regions with shallow depths to bedrock the contamination rate can be much higher than the county-wide average. 3) Both human wastewater and cattle manure were identified as sources of fecal contamination among those wells positive for coliforms or with nitrate-N concentrations greater than 10 mg/L; 60% of tested wells had evidence of fecal contamination. 4) Pathogens that cause illness in people were detected in private wells using qPCR. 5) Analysis of existing nitrate data for the Silurian dolomite aquifer in northeast Wisconsin suggests increased nitrate-N concentrations correlate with nutrient application rather than periods of groundwater recharge. 6) Water-quality data collected at non-pumping sentinel wells suggest that sharp inflections in specific conductance and dissolved organic matter are good recharge indicators (in addition to

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rising water levels). 7) Water-quality data collected by automated samplers placed in households indicate that private well water quality is highly variable over time. 8) Microbial data collected by the automated samplers indicate the presence of microbes in private wells depends on both recharge events and the strength of the contamination source. 9) Forecasts of runoff predicted from the Runoff Risk Advisory Tool appear to be associated with bovine contamination of private wells. Other research activities related to this report but not funded by the Wisconsin DNR include multivariable statistical models of risk factors related to private well contamination, DNA sequencing confirmation of well water samples positive for human *Bacteroides* and ruminant *Bacteroides*, genetic subtyping analyses of samples positive for rotavirus group A to determine the fecal source of the virus, and estimates of illness risk in Kewaunee County caused by private well contamination. These additional findings are presented in the scientific papers resulting from this work. Considered *in toto*, findings from the present study and from previous hydrogeological and water-quality investigations of the Silurian dolomite aquifer provide many opportunities for ensuring clean groundwater for the residents of northeastern Wisconsin.

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INTRODUCTION

Project Purpose and Objectives

Areas underlain by the Silurian dolomite aquifer in northeastern Wisconsin are extremely vulnerable to groundwater contamination from land-use activities, especially the disposal of human wastewater and dairy manure. These areas have had long-standing water-quality problems with elevated nitrate-N concentrations, bacteria levels, and instances of "brown water" impacting domestic wells. The well contamination event near Luxemburg in October 2016 (http://www.wbay.com/content/news/DNR-tests-tainted-water-in-Kewaunee-County-415745143.html) and the ongoing water-quality concerns in Kewaunee County (https://www.wiscontext.org/fecal-microbes-found-kewaunee-county-wells-raise-concerns-about-dairy-manure-septic-waste) have refocused public attention on the aquifer's susceptibility to contamination. While these events have received recent media attention, they are not isolated incidents.

Historically, "brown-water" events have been noted in several other counties underlain by the Silurian aquifer, specifically in Brown, Calumet, Door, Kewaunee, and Manitowoc Counties. Previous compilations of water-quality data by the Northeast Wisconsin Karst Task Force revealed extensive contamination by nitrate and coliform bacteria more than a decade ago (Erb and Steiglitz, 2007). Sporadic instances of water-borne disease in the area have focused attention on the presence of enteric pathogens in the aquifer. The brown-water events and cases of illness prompt questions about the extent and causes of contamination. Prior to this project, there was no consensus as to which source of wastewater, livestock or septic systems, generated the greater impact to the aquifer. In addition, we did not have a good understanding of how pathogen transport related to recharge rates and associated transport processes.

This report summarizes the findings of two companion projects funded in part by the Wisconsin DNR: 1) Assessing the Groundwater Quality in Kewaunee County, Wisconsin; and 2) Characterizing the Timing and Variability of Enteric Pathogen Contamination within the Dolomite Aquifer in Northeastern WI. The overall purpose of these projects was to quantify the extent of groundwater contamination in Kewaunee County by nitrate, coliform bacteria, and enteric pathogens. Some specific objectives of the two projects overlapped and can be summarized as follows:

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1) Estimate the county-wide contamination rate for indicator bacteria and nitrate as related to depth of bedrock;
2) Characterize seasonal variation in recharge and groundwater quality;
3) Determine sources of fecal contamination in private wells using viruses and fecal markers capable of distinguishing septic system versus bovine fecal material;
4) Install an automated sampling system on one or more wells to determine the timing of peak transport for pathogens and indicator bacteria and identify those time periods/recharge conditions that lead to the highest pathogen loads;
5) Compare water-quality data from samples collected during this study to existing water-quality data for Kewaunee County;
6) Compare private well contamination rate of bovine manure-specific microbes with the risk predictions for surface water runoff from the Runoff Risk Advisory Tool.

Two decisions made by the DNR and investigators during the course of the projects altered the objectives and, consequently, the deliverables. First, the DNR and investigators jointly agreed there was already sufficient data on the seasonal variation of nitrate in groundwater for northeast Wisconsin (Objective 2) and these existing data could be summarized by the investigators in lieu of additional sampling. Funds for Objective 2 were re-directed to Objective 3 that addressed the source of fecal contamination. Secondly, the DNR decided not to fund statistical analyses relating land use practices, rainfall, groundwater recharge, depth-to-bedrock, and well construction characteristics to the risk of private well contamination. Instead the USDA-Agricultural Research Service (ARS) funded the statistical work, and the models are reported in a scientific manuscript being prepared by Borchardt et al.

Additional related work funded by USDA-ARS that is not reported here are DNA sequencing analyses of samples positive for HF183 human *Bacteroides* and ruminant *Bacteroides* and genetic subtyping analyses of samples positive for Rotavirus group A to determine the fecal source of the virus. These data are reported in the scientific manuscript.

Background

Hydrogeologic Setting. Kewaunee County, located in northeastern Wisconsin, is underlain by dolomite of Silurian age which forms an important regional aquifer (Figure 1). The Silurian

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strata dip gently into the Michigan basin to the east-southeast at approximately 0.5 degrees or 40-50 feet per mile and are underlain by Ordovician-aged shale, which forms a regionally extensive aquitard (Sherrill 1978). The dolomite forms a prominent escarpment that runs along the western shore of the Door Peninsula and continues southward along the eastern side of the Lower Fox River valley in Brown County and the eastern shore of Lake Winnebago and Horicon Marsh before being obscured by thick deposits of glacial sediment. The dolomite is densely fractured and secondary dissolution has enlarged both fracture apertures and primary porosity. Groundwater flow is characterized by recharge through vertical fractures and rapid lateral movement along horizontal high-permeability zones (Sherrill 1978, Bradbury and Muldoon 1992) that appear to be laterally continuous on the scale of kilometers (Muldoon et al. 2002).

The dolomite is the primary aquifer for Kewaunee County. The area receives approximately 30.7 inches per year of precipitation, including both rain and snow (http://www.usclimatedata.com/ climate/kewaunee/wisconsin/united-states/uswi0363). Groundwater recharge occurs rapidly, on the order of 24 to 48 hours after a rain or melt event even in areas with up to 18 feet of surficial sediment (Muldoon and Bradbury, 2010). Groundwater recharge does not occur uniformly throughout the year; the primary recharge period is during spring snowmelt. Additional recharge usually occurs in the fall of the year when vegetation has gone quiescent and during winter melt events (Rayne et al. 2001, Muldoon and Bradbury, 2010). There is a regional groundwater divide that roughly coincides with the surface water divide that separates streams that flow to the Fox River and Green Bay from those that flow to Lake Michigan (Figure 2). Groundwater discharges to local streams as well as to Green Bay and Lake Michigan.

Previous hydrogeologic and contamination studies. The general geology and stratigraphy of the Silurian dolomite was first described by T.C. Chamberlain in his *Geology of Wisconsin* (1877). While Chamberlain noted the occurrence of artesian conditions in some portions of the aquifer, he focused more on the potential mineral resources of the unit rather than the groundwater characteristics. Weideman and Schultz (1915) noted that the dolomite was "fairly well intersected by transverse and bedding joints, which greatly increase its permeability" thus making the aquifer the main source of water supply for wells along Lake Michigan. Sherrill (1975, 1978) also notes that groundwater flow is primarily through vertical fractures and along horizontal bedding-plane partings and he correlates eight, regionally important "water-bearing"

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zones in Door County based on borehole geophysics. Sherrill also noted chronic groundwater contamination concerns within the Silurian aquifer of Door County.

Previous work has demonstrated that recharge to the aquifer can be exceedingly rapid (e.g., Bradbury et al., 2002; Muldoon and Bradbury, 2010). Figure 3 (from Muldoon and Bradbury, 2010) illustrates variation in fluid temperature, electrical conductivity, and water levels for a shallow monitoring well in Kewaunee County. Recharge events, indicated by sharp rises in water-level, are both rapid and episodic throughout the year. Changes in fluid conductivity in response to recharge indicate the complexities of the recharge process. The lower graph indicates a rain event in early December that led to no groundwater recharge as the ground was frozen. But then a second rain event in late December, when the temperature was above freezing for several days, produced a sharp rise in conductivity (as vadose water drained) followed by a drop in conductivity as low-conductivity recharge water entered the aquifer. While these data illuminate the timing and rapidity of recharge events to the dolomite aquifer, prior to this project we had very limited understanding on the delivery and transport of enteric pathogens within this aquifer. If we can understand pathogen breakthrough, in relation to other indicators of groundwater recharge, we will be able to design better sampling protocols.

Early work that documented the degraded water quality of the dolomite aquifer and the extensive contamination by nitrate and coliform bacteria focused on Door County. Sherrill (1978) collected fifteen samples for total and fecal coliform bacteria from five wells and two springs for the period March 1972 to October 1973. He noted that the highest concentrations of total coliform bacteria occur during recharge periods (times of rising groundwater levels) and that the lowest concentrations occur during periods of falling water levels (non-recharge periods). Bradbury and Muldoon (1992) collected over 2000 samples from domestic wells during the interval 1986 to 1990 as part of the northern Door priority watershed study. Parameters studied included nitrate, chloride, fluid conductivity, turbidity, and total coliform bacteria. They noted that coliform bacteria were the most commonly detected contaminant in northern Door County and that a typical well contained bacteria 35% of the time. They also provide data on seasonal variation in nitrate that will be discussed in a later section.

The Northeast Wisconsin Task Force (Erb and Steiglitz, 2007) was the first effort to look at contamination of the aquifer more regionally. That group compiled existing water-quality data from five northeast counties (Door, Brown, Kewaunee, Calumet and Manitowoc) and noted that

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in areas with thin soils, there was extensive contamination by nitrate and coliform bacteria as well as the occasional brown-water event during groundwater recharge.

Kewaunee County has conducted an annual groundwater sampling program, coordinated by the Land and Water Conservation Department and the UW-Stevens Point Environmental Analysis Lab, since August 2004. Results from more than 550 different private rural wells in Kewaunee County indicate that 29.7% of the wells sampled were not safe for human consumption due to the presence of coliform bacteria and/or nitrate-N above the human health standard of 10 mg/L. Figure 4 summarizes the nitrate and bacteria data for Kewaunee County that have been reported to the UW-Stevens Point Center for Watershed Science. Exceedances of groundwater standards are not randomly distributed but rather appear to correlate with areas where thin soils overlie the fractured dolomite aquifer.

Enteric pathogens and nitrate in groundwater. Contamination of North American groundwater by human enteric pathogens is well established in the scientific literature (Hynds et al. 2014) resulting in notable disease outbreaks (Wallender et al. 2014) and less obvious levels of illness that are sporadic but, nonetheless, measurable (Borchardt et al. 2012). Wallender et al. (2014) recently reported that of the 248 documented outbreaks during 1971 to 2008 related to untreated groundwater, 26% happened in hydrogeological settings similar to northeastern Wisconsin. In Door County, Wisconsin in 2007, 229 people became severely ill after drinking norovirus-contaminated well water at a new restaurant. Dye trace tests showed rapid transport to the restaurant's well from the septic tanks, where a broken connecting pipe was later discovered, and from the septic leach field located up-gradient from the well (Borchardt et al. 2011).

Nitrogen is a widespread and vexing contaminant to ground and surface water, affecting the drinking water resources and the biotic integrity of many of the nation's water bodies (USEPA, 2009; USGS 2010). Background or natural levels of nitrate in groundwater are generally less than 1 mg/L. Concentrations above 1 mg/L indicate influence by one or more of the following sources: nitrogen fertilizers, manure or other bio-solids (both application to land-surface or leakage from storage), or septic system drainfields. Nitrate-N concentrations above the drinking water standard of 10 mg/L should not be consumed by infants or women who are pregnant or expecting to become pregnant; all other persons are encourage to avoid long-term consumption of water containing greater than 10 mg/L nitrate-N (WI DNR, 2014).

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Sources of contamination. In Kewaunee County, dairy farming and associated crop production comprise the primary land use, and manure is commonly applied to crop land prior to spring planting and again in fall after crops have been harvested. In addition, crop land receives commercial fertilizers, septic wastes, as well as industrial and municipal wastes. Housing is the second leading land use in Kewaunee County, and residences in the rural environs dispose their household wastewater through septic systems. All of these land-released materials are long-term, diffuse sources that can contribute to nitrate contamination. Fecal wastes from humans and dairy cattle can be diffuse, long-term sources of enteric pathogens. Prior to this project there was no consensus as to whether the main source of fecal contamination in the dolomite aquifer was human wastewater or bovine manure.

OBJECTIVE 1: County-wide Contamination Rate for Indicator Bacteria and Nitrate

Procedures and Methods

Selection of sampling sites. Wells were selected using a stratified random approach to provide a more precise and representative estimate of the county-wide well contamination rate. Stratification was by depth to bedrock to ensure equal representation of wells with different bedrock depths.

The total number of wells in Kewaunee County is estimated to be approximately 4900 based on county tax parcel and septic system data (D. Bonness, personal communication). The sampling frame was obtained from ArcMap coverage of Kewaunee County of all of the property parcels in the county that were valued at greater than $30,000 so as to include parcels that likely contained buildings and therefore wells. We excluded parcels within the areas that are served by a municipal water-supply system (those within the villages of Algoma, Kewaunee and Luxemburg).

Depth to bedrock was categorized based on the existing map (Sherrill, 1979). While Sherrill's map contains five categories (less than 5 feet, 5 to 20 feet, 20 to 50 feet, 50-100 feet, and >100 feet), we used only three categories (less than 5 feet, 5 to 20 feet, and greater than 20 feet to rock) as these were the ArcMap data readily available from Kewaunee County. By intersecting the two coverages in ArcMap, tax parcel and depth to bedrock, we were able to randomly choose parcels within specific depth-to-bedrock categories.

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Selection of sampling dates. The sampling dates were chosen so that one round of samples was collected during a recharge period (November 2015) and one round was collected during a non-recharge period (July 2016). In addition, the November 2015 recharge sampling event followed a period of manure application. Previous work (Muldoon and Bradbury, 2010) suggests that recharge events tend to dilute concentrations of conservative contaminants (such as nitrate and chloride) and increase detections for bacteria. Spatially the proposed sampling design was random, but temporally the design was weighted towards the times when groundwater is most vulnerable to contamination by the various parameters of interest. In other words, we designed the random sampling program to provide information on the proportion of contaminated wells and not to determine the proportion of contaminated samples. Even with weighting the sampling times to when groundwater is most vulnerable, the proportion of wells that are contaminated will likely be underestimated because of the rapid, transient nature of contamination events in this aquifer.

The study designed called for synoptic sampling events during which all samples were to be collected over a period of a few days and dropped off at one of several collection sites over a single weekend. Because environmental conditions that affect groundwater contamination, like rainfall, are similar for wells within a county, a synoptic "snapshot" approach was used. Interpretation of snapshot data is less confounded by temporal changes and does not have to account for the variation in conditions that exists for wells sampled over a long period. The study captured two "snapshots" of contamination to represent water quality under different conditions (fall and summer).

Recruitment of homeowners. We mailed recruitment letters to 800 and 1001 well owners for the November 2015 and July 2016 sampling events, respectively. Included with the letter was a pre-addressed postcard for the well owner to return to the Kewaunee Land and Water Conservation Department. That office compiled the positive responses and provided the addresses to the UW-Oshkosh Environmental Research and Innovation Center (ERIC).

Sample collection and analysis. Sample collection and analysis was conducted by staff of the Environmental Research and Innovation Center (ERIC lab) at UW-Oshkosh. Sample bottles and detailed instructions on proper sample collection techniques were mailed to the well owners. Well owners returned samples within 24 hours of collection to designated sample drop-off sites in Kewaunee County. We received 323 well owner-collected samples for the November 2015

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event and 401 samples for the July 2016 event. Coliforms and *E. coli* were analyzed by Colilert Quanti-Trays (IDEXX, Westbrook, ME) within 48 hours of sample collection. Nitrate was measured by cadmium reduction on an AQ1 Discrete Analyzer (SEAL Analytical).

Notifying well owners of results. The ERIC lab followed its regular procedures in terms of providing well owners with their water-quality results. If a sample exceeded the drinking water standard for either nitrate or bacteria, the well owner received a phone call from the lab as soon as the analysis had been completed. All well owners, including those who had been called, received a standard letter from the lab which noted their results. If the results indicated contamination, the lab included in the mailing the appropriate DNR literature on methods for dealing with nitrate and bacteria.

Data analysis and statistical approach. Location information for the sampled wells included street address and Kewaunee County parcel number. We used the State Cartographer's Public Land Survey System (PLSS) finder program (http://maps.sco.wisc.edu/PLSSFinder/#) to determine the Township, Range and section of each sample location because well construction reports (WCRs) are located by the PLSS. We attempted to match each sample location to a WCR by comparing the owner and location information to the following databases: 1) Kewaunee County's GIS database of WCRs, 2) DNR's online database of WCRs, (https://prodoasext.dnr.wi.gov/inter1/watr$.startup) and 3) the WGNHS Hydro Data Viewer. The first two databases contain WCRs for wells constructed after 1988 and these wells have been assigned a Wisconsin Unique Well Number (WUWN). Wells constructed prior to 1988 are included in the WGNHS Hydro Data Viewer and these wells have a unique image identifier assigned by WGNHS.

If the sampled well could confidently be matched to a specific WCR in the above databases, the depth to rock was recorded as an exact value. A WCR was considered a good match to a sample location if the well owner's name on the WCR matched the owner of the parcel. If the names did not match, which is common with older wells, a WCR might still be considered a good match if the WCR location information was consistent with the sample's parcel location and if the geology was consistent with other wells in the area. If we could not match a sample location to a specific WCR, we used the surrounding WCRs and an understanding of the geologic setting to estimate depth to rock. When estimating depth to rock, we did not use pre-assigned categories but rather estimated a range based on the surrounding WCRs.

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Subsequent to our bedrock determinations, Kewaunee County contracted the WGNHS to develop a more complete WCR database for the county. For each well in WDNR's database and the WGNHS Hydro Data Viewer, WGNHS staff assigned the well a location based on historic ownership information contained within published plat books and noted a location confidence for each well. Once the database was complete, Steve Mauel, WGNHS GIS specialist, performed a spatial join between the well database and the Kewaunee County Parcel data and he then attached the Parcel ID from each parcel to every well that had a location confidence of 500 feet or better. He then matched our sampling data to specific wells using the Parcel ID as the primary key.

Our statistical approach used the stratified random sampling design to generate estimated contamination rates at the county level and within the sampling strata defined by depth-to-bedrock (DTB) categories (<5 feet, 5-20 feet, >20 feet). Smaller DTB strata were oversampled relative to a simple random sample. This approach, in conjunction with the use of corresponding analytic weights and finite population correction factors in the analyses, resulted in more precise estimates for the smaller DTB strata without sacrificing the ability to estimate a county-wide contamination rate. The analytic weight was defined as the product of the inverse of the sampling probability and the inverse of the response rate within the appropriate DTB stratum. Statistical computations accounted for the complex sampling design. Rao-Scott likelihood ratio chi-square tests were used to test associations between contamination rates and DTB, as well as compare Fall 2015 and Summer 2016 estimated contamination rates, both overall and within DTB strata The latter comparisons entailed the assumption that the data from the two sampling periods were independent which may not strictly hold since some wells appeared in both samples. SAS was used to conduct the analyses (SAS Institute Inc., Cary, NC).

Results and Discussion

Estimated number of wells in depth-to-bedrock strata. While well selection for sampling and analytical weighting was by depth-to-bedrock strata identified from the map created by Sherrill (1979), the county-wide contamination rates were calculated using depth-to-bedrock values derived from well construction reports (How depth-to-bedrock values were interpolated for wells with missing construction reports was described above). "Ground-truthing" using well construction reports resulted in some wells having different depth-to-bedrock values than

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originally determined from the Sherrill map. Assuming the change we observed in the number of study wells among the depth-to-bedrock strata applies to all wells in the county, we can estimate the number of wells in each depth-to-bedrock stratum for the county (Table 1).

There are fewer wells with depth to bedrock < 5 feet in Kewaunee County than the number estimated from the Sherrill map; the map indicated 318 wells at depth-to-bedrock < 5 feet (6.5% of wells in the county), whereas the number estimated from data collected for the Fall 2015 and Summer 2016 sampling events is 76 and 104 wells, respectively (about 2% of wells in the county) (Table 1). In contrast, there are more wells with depth to bedrock > 20 feet (4,156, 84.9% of the county wells) than originally estimated from the map (4,021, 82.1% of county wells). While wells were removed and added to the 5 to 20 feet depth-to-bedrock stratum, the net change in the percentage of wells in that stratum was small (original estimate 11.4%, updated estimated about 11.8%). We estimate 1% to 2% of wells in the county have an indeterminate depth-to-bedrock value.

While our approach provides an estimate and 95% confidence intervals for the number of wells in each depth-to-bedrock stratum, it cannot indicate the spatial distribution of wells with respect to depth-to-bedrock. For that, it would be necessary to update the Sherrill (1979) map.

Estimated coliforms, E. coli, and nitrate contamination rates. During the November 2015 and July 2016 synoptic sampling campaigns for coliform bacteria, *E. coli*, and nitrate sampling kits were returned by 323 and 401 well owners, respectively; by random chance 103 wells were selected for sampling in both events.

Some samples were excluded from data analysis. In the fall of 2015, five samples (one nitrate and four coliforms) did not meet laboratory compliance standards. In addition, depth-to-bedrock values could not be determined for four wells. In the summer of 2016 there were 4 non-compliant samples (zero nitrate and four coliforms) and depth-to-bedrock values were missing for two wells.

The county-wide private well contamination rate for any of the three contaminants (coliforms, *E. coli*, or nitrate-N > 10 mg/L) was 26% in the fall (recharge) and 28% in the summer (no recharge) (Table 2).

The Wisconsin statewide contamination rates for the same three contaminants were reported in two previous studies (US GAO 1997; Knobeloch 2013). Aggregated across the county, contamination rates of coliforms, *E. coli*, and nitrate in Kewaunee County did not differ greatly

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from their contamination rates across Wisconsin (Table 2). (Note the previous studies did not report the "any contaminant" category, precluding a comparison with the present study.) However, for wells with shallow bedrock depths (bedrock depth strata < 5 feet and 5 to 20 feet) the contamination rates in Kewaunee County were generally higher than rates statewide (Table 2). The highest contamination was during groundwater recharge in the fall when 50% and 42% of wells in the two shallowest bedrock depth strata (< 5 feet and 5 to 20 feet, respectively) were positive for coliforms or nitrate-N > 10 mg/L. (Groundwater recharge measurements from monitoring wells are described below in Objective 2.)

When comparing contamination rates of groundwater contaminants like coliforms and nitrate between regions, for example between counties, it is important to account for the factors that can result in localized higher or lower contamination. In Kewaunee County that factor is depth to bedrock. Aggregating contaminant data at large geographic scales could potentially ignore contamination levels that are important at smaller scales (e.g. county versus township).

Contamination rates were statistically different among depth-to-bedrock strata. In the fall, coliform detections and detection of any of the three contaminants differed by depth to bedrock (chi-square test, $p = 0.047$ and 0.019, respectively) with contamination rates decreasing as depth to bedrock increased. Nitrate contamination was highest in the 5 to 20 feet stratum; 20% of wells had nitrate-N > 10 mg/L ($p = 0.113$). In the summer, coliform detections did not differ by depth-to-bedrock strata, but nitrate contamination, again, was highest in wells with depths to bedrock between 5 and 20 feet ($p = 0.026$). The highest nitrate contamination in the mid-range depth-to-bedrock stratum may reflect less agricultural activity and lower nitrogen inputs in shallower bedrock areas. Values from wells with deeper bedrock depths likely reflect the deeper soil column providing greater attenuation of nitrate before it reaches private wells.

E. coli contamination rates did not differ statistically among depth-to-bedrock strata, although the small number of positive wells likely resulted in low statistical power to evaluate this association.

In addition to depth to bedrock, well contamination also likely depends on surrounding land uses (e.g., residential and agriculture). Inventory of land use near sampled wells was not within the scope of the present study, but its role in well contamination was part of the statistical models that will be reported in the scientific paper stemming from this work.

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Table 1. Estimated number of private wells in depth-to-bedrock categories in Kewaunee County. Estimates were derived from well construction report data in combination with the statistical analyses for determining county-wide contamination rates from two stratified random sampling events.

Source of Data	Number of Wells (95% Confidence Interval) (Percent of Total)				
	Depth-to-Bedrock (feet)				Total
	Indeterminate	< 5	5-20	> 20	
Original estimate from map (Sherrill 1979)		318 6.5%	557 11.4%	4021 82.1%	4,896
Fall 2015 sampling event	89 (0 – 204) 1.8%	76 (53 – 99) 1.6%	575 (425 – 724) 11.7%	4156 (3970 – 4341) 84.9%	4,896
Summer 2016 sampling event	52 (0 – 123) 1.1%	104 (47 – 160) 2.1%	583 (445 – 722) 11.9%	4157 (3996 – 4318) 84.9%	4,896

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Table 2. Percentage of private wells positive for total coliform bacteria, *E. coli*, or nitrate-N > 10 mg/L.

			Percent Positive Wells (95% confidence interval)			
Season and groundwater recharge condition	Region or depth-to-bedrock category	Number of wells sampled	Total coliforms	*E. coli*	nitrate-N > 10 mg/L	Total coliform or nitrate-N > 10 mg/L
Fall, 2015 Recharge	< 5 ft to bedrock	26	46 (30 – 63)	4 (0 – 9)	7 (0 – 15)	50 (34 – 66)
	5 – 20 ft to bedrock	120	28 (18 – 37)	1 (0 – 2)	20 (7 – 33)	42 (28 – 55)
	> 20 ft to bedrock	167	19 (11 – 26)	0.3 (0 – 0.6)	6 (1 – 10)	23 (15 – 31)
	Kewaunee County	313[1,2] 316[2,3]	21 (14 – 27)	0.4 (0.1 – 0.7)	7 (3 – 11)	26 (19 – 34)
Summer, 2016 No recharge	< 5 ft to bedrock	24	23 (6 – 39)	7 (0 – 15)	10 (0 – 20)	33 (12 – 53)
	5 – 20 ft to bedrock	122	29 (16 – 41)	1 (0 – 3)	19 (9 – 28)	40 (28 – 53)
	> 20 ft to bedrock	252	21 (15 – 27)	1 (0 – 2)	5 (2 – 8)	26 (19 – 32)
	Kewaunee County	396[1,2] 400[2,3]	22 (17 – 28)	1 (0.1 – 2)	7 (4 – 10)	28 (22 – 33)
Not applicable	Wisconsin 1997[4]	534	23	3	7	–
Not applicable	Wisconsin 2013[5]	3,838	18	–	10	–

[1] n for coliforms and *E. coli*.
[2] In addition to samples excluded from the data analysis as described in the text, n's reflect wells with missing DTB values based on the Sherrill 1979 map (6 for Fall 2015 and 1 for Summer 2016) for which analytic weights could not be generated.
[3] n for nitrate
[4] Data for private wells; US General Accounting Office 1997.
[5] Knobeloch et al. 2013.

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Private well contamination rates for coliforms, *E. coli*, or nitrate-N > 10 mg/L, whether reported by depth-to-bedrock stratum or county-wide, did not differ statistically between fall (recharge) and summer (no recharge) (Table 2). There was one exception; wells with bedrock depths < 5 feet had higher coliform detection rates in the fall than in the summer (chi-square test, $p = 0.046$).

The coliform, *E. coli*, and nitrate contamination rates reported here are unique in that the randomized well selection and large sample sizes yield a representative picture of well contamination in the county. Moreover, the stratified sampling design improved contamination rate precision for the depth-to-bedrock strata with small numbers of wells as these strata were oversampled.

Concentrations of coliforms, E. coli, and nitrate-N. Descriptive statistics for coliform bacteria, *E. coli*, and nitrate-N concentrations measured in the study wells during the fall and summer sampling campaigns are reported in Table 3. The statistics do not include samples that did not have measureable concentrations of the contaminants (reported as non-detects in Table 3). Notably, of the wells with measureable nitrate (n = 203, fall sampling; n = 205, summer sampling) 25% had nitrate-N concentrations of 9 mg/L or higher. Similarly, of the coliform-positive wells, 25% had coliform concentration greater than 17 MPN/100 ml (fall) and 55 MPN/100 ml (summer). Several wells exceeded the upper limit of quantification for coliform bacteria.

All sample-level data on total coliforms, *E. coli*, and nitrate are reported in Appendix 2. Data have been de-identified to maintain well owner confidentiality.

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Table 3. Descriptive statistics of coliform bacteria, *E. coli*, and nitrate-N concentrations for positive samples.

Season and groundwater recharge condition	Measurement	N	Number of non-detects	Concentration of positive samples[1]					
				Mean	Median	Minimum	25th percentile	75th percentile	Maximum
Fall, 2015 Recharge	Coliforms	87	232	73.2	5.2	1.0	2.0	17.3	> 2419.6
	E. coli	5	314	5.0	2.0	1.0	2.0	4.1	16.1
	Nitrate-N	203	119	6.3	4.7	0.2	1.6	9.0	29.7
Summer, 2016 No recharge	Coliforms	87	310	116.8	6.2	1.0	2.0	55.4	> 2419.6
	E. coli	10	387	105.0	3.1	1.0	1.3	8.8	1011.2
	Nitrate-N	205	196	6.5	5.2	0.2	2.1	9.1	33.3

[1]Coliforms and *E. coli*, MPN/100 ml; nitrate-N, mg/L

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OBJECTIVE 2: Seasonal Variation in Recharge and Groundwater Quality

Procedures and Methods

One of the objectives of this project was to assess seasonal variation in groundwater quality by 1) sampling a subset of wells from the randomized sampling frame on an every other week basis and 2) collecting water-level and water-quality data at two non-pumping sentinel wells to characterize the timing and magnitude of recharge events. Locations of the non-pumping sentinel wells, the Casco weather station, and the automated sampler sites are shown in Figure 5.

Meteorological data. Meteorological data were not recorded as part of the current study. Instead, we used climatic data that were available from a weather station in Casco, WI which is part of Michigan State University's Enviroweather network and from the National Weather Service (NWS) station in Green Bay. We gathered meteorological data for the period October 22, 2015 to May 29, 2017. The MSU network does not record snow precipitation. The NWS station in Green Bay was the closest weather station that records the water-equivalence of winter snowfall. We obtained the precipitation data from both stations so as to have a complete record of precipitation for the study period.

Data at the Casco/Luxemburg station are recorded hourly. The hourly parameters that we accessed at the MSU web site (https://enviroweather.msu.edu/weather.php?stn=lux) included air temperature, leaf wetness, precipitation, relative humidity, soil temperature at 5-cm depth, solar flux, and wind direction and speed. We also downloaded the following daily data: maximum and minimum air temperature, precipitation, maximum and minimum soil temperature at 5-cm depth, and total solar radiation.

Preliminary monthly climate data for Green Bay were accessed at the NWS web site http://www.weather.gov/climate/index.php?wfo=grb and include the following daily data: temperature (maximum, minimum, and average), 24-hr precipitation totals (including the water equivalent of any snow fall), snow depth, as well as information concerning heating/cooling degree days and wind conditions.

Figure 6 compares the daily precipitation records from both weather stations for the periods Oct 22, 2015 to May 31, 2016 (top) and June 1, 2016 to May 29, 2017 (bottom). We plotted the data over these two time periods so that the details of the precipitation records were clearer. The pattern of precipitation at both sites is generally similar except that the Green Bay station records more precipitation in the winter since the water-equivalence of snowfall is recorded for that

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station, but not at the Casco station. Over the time period from Oct 22, 2015 to May 31, 2017, the Green Bay station recorded 21.37 inches of total precipitation while the Casco station recorded only 15.72 inches. Over the time period June 1, 2016 to May 29, 2017, the Green Bay station recorded 34.93 inches of total precipitation whereas the Casco station only recorded 32.26 inches.

Modified study objective. As the project progressed, this objective was modified in consultation with the DNR because several existing data sets addressed the seasonal variation in nitrate-N concentrations and that the funds for this objective would be better spent investigating the source of the pathogen contamination (see next objective). Three previous studies, summarized below, provide an overview of seasonal variation in nitrate.

Upper Door Priority Watershed Study (1986-1990). This was a collaborative project by the Door County Soil and Water Conservation Department and the Wisconsin Geological and Natural History Survey (WGNHS). The results are presented in Bradbury and Muldoon (1992). The well-sampling program concentrated on the central portion of northern Door County and was initiated in February 1986. The initial sampling effort, conducted by the Door County Soil and Water Conservation Department, collected samples every two weeks from five springs and 45 homeowners and it continued through June of 1986 (Blanchard, 1988). The WGNHS became involved in the project in July, 1986 and after reviewing the data, the sampling program was changed in order to focus on a smaller geographic area and to add additional wells for which well construction reports were available. After a year (June 1987), sampling was discontinued for all wells except those in a 15 mi^2 subarea where sampling of 14 wells continued every two weeks through August 1988 and monthly through July 1990. For three of these wells, monthly sampling continued through June of 1992.

Northeast Wisconsin Recharge Study (2008-2009). Results of this study are summarized in a final report to the WDNR (Muldoon and Bradbury, 2010). The goal of this project was to gain an understanding of seasonal variations in recharge, the timing of recharge events, and the resulting water-quality variations in the Silurian dolomite aquifer in areas with 10 feet or more of surficial sediment. Shallow bedrock wells were installed in four counties (Brown, Calumet, Kewaunee, and Manitowoc) where the Silurian aquifer was the uppermost bedrock aquifer and soil was greater than 10 feet thick. All wells were located at the edge of agricultural fields where manure or sewage sludge was being applied. Wells were sited to avoid interference from septic systems.

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Geophysical logs were used to identify high-permeability bedding-plane fractures. Water levels and water temperature were recorded every 30 minutes using Solinst Leveloggers™. Each of the wells was also instrumented with a downhole temperature/conductivity probe placed adjacent to a major horizontal fracture. Probes were connected to a surface datalogger that was programmed to record hourly average values. All wells were sampled approximately monthly for nitrate, chloride, and dissolved phosphorus during the period September 2007 to August 2008. Samples were collected by lowering a submersible Grundfos sampling pump into the well to a point opposite the major bedding-plane-parallel fracture penetrated by that well.

Town of Lincoln Study of the Intra-Annual Variability of Well Water Quality. Bonness and Masarik (2014) sampled ten wells in the Town of Lincoln, Kewaunee County, on a monthly basis for one year to investigate seasonal variability in water quality.

Monitoring recharge at non-pumping sentinel wells. Monitoring well KW183 was installed as part of Muldoon and Bradbury's project "Assessing Seasonal Variations in Recharge and Water Quality in the Silurian Aquifer in Areas with Thicker Soil Cover" and that report contains details on its construction as well as geophysical logs. The well is now part of the USGS Climate Response Network (https://nwis.waterdata.usgs.gov/nwis/uv?site_no=443535087345401). The Fish Hatchery well was installed as part of this project and site selection criteria included the following: 1) located on public land, 2) soils were less than 15 ft in thickness, and 3) the surrounding land use was primarily agricultural. The six-inch diameter well was installed using air-rotary methods on August 29, 2016. A tri-cone bit was used to advance the borehole through the unconsolidated sediment. The casing was seated into firm rock and the borehole was advanced into the bedrock by air-rotary drilling. Portland cement was pumped through a tremie line in order to seal the annular space and grout the casing in to the rock. Samples were collected and described approximately every 5 feet. Upon completion of drilling, the well was air-lifted for several minutes to clear the borehole of any drill cuttings. The well was logged on Sept 8, 2016 by Pete Chase of the Wisconsin Geological and Natural History Survey (WGNHS) using the Mt Sopris digital borehole logger. All geophysical data were recorded relative to depth from the top of the casing. Logs completed included caliper, which measures borehole diameter, and can help identify fracture zones; natural gamma, which measures natural radiation and can be used for stratigraphic correlation; and single-point resistivity and spontaneous potential which are also useful for stratigraphic correlation. Logs used to help locate high-permeability bedding plane

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fractures include fluid temperature and resistivity. Figure 7 illustrates well location, land use, selected borehole logs, and details of lithology and well construction for the Fish Hatchery well.

Water levels in both sentinel wells were recorded hourly using vented pressure transducers. KW183 water levels were monitored beginning October 22, 2015 and monitoring continues to the present. Water levels at the Fish Hatchery well were monitored for the period October 21, 2016 to June 13, 2017. Water-quality sensors, enclosed within a Yellow Springs Instrument EXO sonde, were placed near a high-permeability fracture in each well such that there was continuous water flow across the sensors. Water-quality data collected at both sentinel wells included the following: fluid temperature, specific conductance, pH, dissolved organic matter (DOM) and turbidity. Nitrate data were collected at the Fish Hatchery well and chloride data were collected at KW183. Water-quality data were collected hourly at KW183 for the period October 21, 2016 to June 13, 2017. At the Fish Hatchery well, water-quality data were recorded at 5-minute intervals for the period December 1, 2016 to May 10, 2017.

Results and Discussion

Seasonal variation in nitrate (from previous studies). The three studies described above provide an overview of seasonal variation in nitrate in the fractured Silurian dolomite of northeast Wisconsin. Two of the studies used a monthly sampling schedule while the Door County study sampled every two weeks for part of the study period and monthly for the later part of the study. While it is beyond the scope of this section to present all of the nitrate data from these previous studies, we will highlight some of the trends that can be identified from these projects.

The Town of Lincoln study (Bonness and Masarik, 2014) noted that nitrate-N concentrations were stable in one-half of the sampled wells (standard deviation < 1.0 mg/L) while one-half were determined to have significant intra-annual variability (standard deviation >1.0 mg/L). Figure 8, which is figure 4 in the original report, presents the monthly sample results for nitrate along with fluid conductivity, total coliform bacteria, alkalinity, and chloride. Based on data presented in Figure 9 (Figure 5 in the original report) the authors note that overall water-quality appears to be most stable during periods of frozen ground and that variability increases in spring, presumably due to snowmelt recharge and then variability decreases during summer months as recharge is

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less common. These trends are more apparent for total coliform and chloride and less obvious for the nitrate data.

The northeast Wisconsin recharge study (Muldoon and Bradbury, 2010) also used a monthly sampling schedule, but this study adds additional insight into nitrate variations as water levels were also monitored in the four sampled wells. Five discrete recharge periods were identified during the study period. Figure 10 presents the nitrate results as well as the water-level variations in two of the wells, and precipitation data. Nitrate-N concentrations varied over time in all wells and most wells varied by 9 to 10 mg/L; the Calumet well varied by over 28 mg/L. Three of the wells (all but MN-544) show drops in nitrate-N concentrations in January and April of 2008. Both of these sampling events followed a large recharge event. These results suggest that recharge is a significant component of the temporal variability in nitrate-N concentrations and that it has a dilution effect in terms of nitrate.

For the Northern Door Study, we focused our compilation efforts on wells that had four or more years of record. Figure 11 is a modification of figure 3-4 from the original report. It shows variations in nitrate-N concentrations in four domestic wells oriented along a north-south line about one mile east of the village of Carlsville. The figure also shows hydraulic head from well MW1 at the Jarmen Road research site and precipitation at the Sturgeon Bay Experimental Farm. The distance between well 66 (the northernmost well) and well 64 (the southernmost well) is three miles. For each well in Figure 11, we note average concentration (which is also shown as a gray horizontal line), median concentration, and the number of samples.

These data suggest a more complex relationship between recharge and nitrate-N concentrations than was seen in the northeast Wisconsin recharge study. Time periods shaded red on the graph are times when nitrate-N concentrations vary in similar ways in all four wells, suggesting that the source of the contamination is diffuse and ubiquitous across the area rather than coming from discrete point sources. For the first period, October to December 1987, all wells had below average nitrate-N concentrations for much of fall, followed by a sharp spike in concentration in December. The hydrograph for well MW1, based on periodic measurements, suggests that water levels were rising for this entire time. After spring snowmelt in 1988, water levels peaked in early April. For three of the wells, excluding well 54, there was also a peak in nitrate-N concentrations in early April. A second spike in nitrate-N was observed in well 66 in mid-May, during a period of falling water levels. In November and December of 1988, we see

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rising nitrate-N concentrations again during a period of rising water levels. In 1990, there were rising water levels for the period of mid-February through March. During this time, three of the four wells also exhibited rising nitrate-N levels. Finally, there was an increase in nitrate-N in all four wells in July 1990. This increase seems correlated with an unusual summer recharge pulse as noted by the rising water level in well MW1.

In summary, the seasonal variation in nitrate-N concentrations is not a simple function of recharge. We can note that nitrate-N concentrations appear most stable during periods of falling water levels. Typically water-levels decline during the frozen ground-period of the winter, but also in the summer. Increases in nitrate-N concentrations seem to occur primarily in late fall and in spring with occasional increases in summer months. In order to explain the seasonal variation in nitrate-N concentrations, it is useful to consider the variations in the seasonal applications of nutrients at the land surface. In a typical diary cropping systems, manure is applied in fall, after crops are harvested, and again in spring prior to the growing season. If inorganic nitrogen is applied, it is typically applied early in the growing season. Based on the northern Door study, periods of increased nitrate-N concentration seem to correlate better with periods of nutrient application rather than with periods of recharge. Periods of variable nitrate-N concentration seem to correlate with recharge periods, whereas periods of stable nitrate-N concentration correlate with non-recharge periods.

Water-level fluctuations and recharge events. Figure 12 compares precipitation data to water levels in both non-pumping monitoring wells. Since only the Green Bay weather station recorded the water-equivalence of snow during winter, those data were used in this figure. Both wells exhibited similar water-level trends over the period when both were being monitored other than excursions due to pumping at the Fish Hatchery. Water-level fluctuations at KW183 were on the order 8 feet while the Fish Hatchery well exhibited about 10 feet of water-level fluctuation. The Fish Hatchery well's water levels were clearly impacted by the presence of two nearby high-capacity wells used to occasionally supply water to the hatchery. Pumping of those wells began on October 7 and 19; one well was shut-off on November 2 and both wells were turned off on December 7. The sharp rebounds in water-levels are highlighted by red arrows in Figure 12.

We define three broad recharge periods based on generally rising water levels at one or both of the non-pumping sentinel wells. These are shown as gray, shaded areas in Figure 12. The first major recharge period occurred in Fall 2015, from the start of monitoring in late October through

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mid-December. From mid-December 2015 to mid-February 2016 there was a period of generally declining water levels. The major snowmelt recharge event in 2016 extended from approximately February 18 to April 1, 2016. Then water levels generally fell from April through early September 2016. A wet fall in 2016 and a warm winter, led to a prolonged recharge period from approximately September 7, 2016 to May 2, 2017. During this time interval, water-levels show an overall rising trend punctuated by sharp, brief increases in water levels and short periods of declining water-levels. After May 2, 2017 water levels generally declined to the end of the study period.

*Seasonal variation in groundwater quality data (from YSI Sondes installed in sentinel wells).*One of the goals of the water-quality measurements at the sentinel wells was to identify those parameters that were most useful in identifying periods of groundwater recharge. The idea being that those parameters could also be monitored at the automated sampler sites where pumping due to homeowner water use would complicate the water-level record such that it would be useless for identifying recharge periods. Water-quality data recorded at the automated sampler sites are presented under Objective 4. The water-quality data from automated sampler Site 2 were published to ScienceBase (Owens et al., 2019a) as supplemental data to a Technical Note published in the journal Groundwater (Owens et al., 2019b). We are planning to create a ScienceBase site that will include the water-quality data from the sentinel wells and automated sampler Sites 1 and 3. Alternatively, the water-quality data can be obtained by containing the authors of this report.

Water-quality monitoring results from KW183 and the Fish Hatchery well are presented in Figures 13 and 14 respectively. Specifically, the plots show variation in specific conductance, dissolved organic matter, pH, and turbidity at each well. Figure 14 includes the nitrate data for the Fish Hatchery well. The chloride probe installed in KW183 experienced significant problems over the course of the study and so those data are not included in Figure 13. All of the water-quality probes produced highly variable data records and so the plotted lines in Figures 13 and 14 are running averages of the water-quality data with a window size of 11 data points. In addition to the water-quality data, both figures include the daily precipitation data from the Casco station, the cumulative precipitation from the Green Bay station (so as to account for snow precipitation) and water-level data from both wells. Comparison of both figures indicate that the Fish Hatchery well showed little chemical variation over time. The Fish Hatchery well is located near a regional

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discharge point, the Kewaunee River, whereas KW183 is located much closer to the regional water-table divide. It is likely that the different responses between the two wells reflect this difference in flow system position. The more upgradient well, KW183, exhibits greater variability in water quality than the more downgradient well, the Fish Hatchery well.

The data from KW183 suggest that specific conductance and colored dissolved organic matter (CDOM) are useful indicators of groundwater recharge in that variations in both of these parameters generally tend to mimic the variations in water levels. While Figure 13 shows these parameters for the entire period of record (October 2016 to March 2017), Figures 15 and 16 are plots of these parameters for shorter time intervals so that the details of the temporal record are clearer. Figure 15 illustrates the period October to December 2016 and Figure 16 illustrates the period January to March, 2017. In both plots, dashed blue vertical lines highlight discrete recharge events marked by sharp rises in water level. Examining the details of specific recharge events reveals notable trends. In Figure 15, the recharge events on October 26 and November were due to large rain events, whereas the event on December 27 was due to a combination of rain and snowmelt. For both rain events, there is an initial drop in specific conductance and a spike in CDOM at the start of the event. Recharging precipitation has lower specific conductance than the groundwater and the spike in CDOM is likely due to the recharging water moving dissolved organic matter from the soil zone into the aquifer. After those initial inflections, the water-quality parameters then tend to mimic the water levels until the next recharge event. This pattern is less clear in the December event which doesn't exhibit a sharp drop in specific conductance and the spike in CDOM is a bit delayed. Figure 16 also illustrates a prolonged period of recharge during which water-levels show an overall rising trend punctuated by sharp, brief increases in water levels and short periods of declining water-levels. The water-quality parameters mimic this overall trend and the discrete recharge events in mid-January, mid-February, late March and late April do not show the initial inflections in water-quality parameters that were observed in the Fall events. Water-quality parameters peak with the end of the snowmelt season in mid-March while water levels peaked in early May.

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OBJECTIVE 3: Determining Sources of Fecal Contamination

Procedures and Methods

Selection of sampling sites and dates. Wells positive for coliforms or with nitrate-N > 10 mg/L were eligible for additional sampling for assessing sources of fecal contamination and the occurrence of enteric pathogens. From this group, wells were selected for five sampling events: April 18-22, August 1-3, and October 31-November 2 of 2016 and January 23-24 and March 27-29 of 2017. Selection was randomized and stratified by the three depth-to-bedrock categories. We sampled 22 to 30 wells during each event, resulting in 138 samples from 131 wells; seven wells were sampled in two events.

Sample collection and processing. Sampling was conducted by USDA and USGS laboratory staff using dead-end ultrafiltration (Smith and Hill, 2009) with Hemodialyzer Rexeed-25s filters (Asahi Kasei Medical MT Corp., Oita, Japan). Water taps were flame-sterilized before filter attachment; all filter tubing and fittings were new for each sample. Well water was collected prior to softening or other treatment systems. Mean sample volume was 839 L (range 522 – 1517 L, n = 138). Filters were bagged, placed on ice, and back-flushed in the laboratory within 72 hours.

Filter back-flushing followed the method of Smith and Hill (2009) and the eluate was further concentrated by polyethylene glycol (PEG) flocculation (Lambertini et al., 2008). Nucleic acids were extracted with the QIAamp® DNA blood mini kit and buffer AVL using a QIAcube® (Qiagen, Valencia, CA). Virus RNA was reverse-transcribed as described in Borchardt et al. 2012.

Laboratory analyses. Samples were analyzed by real-time quantitative polymerase chain reaction (qPCR) for 33 gene targets specific to 29 microbial taxa or groups (see Appendix 1). The microbes tested were all fecal-borne and, based on the biology of the microbe or validation studies reported in the scientific literature, placed in one of three host-specificity categories: human-specific, bovine- or ruminant-specific, and no host specificity.

qPCR was performed with a LightCycler® 480 instrument (Roche Diagnostics, Mannheim, Germany) using the LightCycler 480 Probes Master kit. Fluorescence for all qPCR assays was by hydrolysis probes (IDT, Coralville, IA). Each qPCR was performed in duplicate. Primers (IDT) and hydrolysis probes are reported in Appendix I.

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To ensure laboratory contamination was absent (i.e., no false-positives), we performed no-template controls of every target for the extraction, reverse transcription, and qPCR steps of each qPCR batch; and we tested for every target in each batch of filter backflush solution. All tests had to be negative (i.e. no cycle of quantification (Cq) value) for sample data to be accepted. All quality control data for the qPCR assays are reported in Appendix 2.

Extraction positive controls were bovine herpes virus vaccine for DNA and bovine respiratory syncytial virus vaccine for RNA (both vaccines from Zoetis Inc., Kalamazoo, MI), the latter serving also as the reverse transcription positive control. qPCR positive controls were gBlocks® or Ultramers® (IDT) of each target, with sequences modified to distinguish from wildtype while still maintaining the same GC-content.

Inhibition was evaluated following the approach of Gibson et al. (2012), using as controls Hepatitis G virus RNA oligonucleotide (IDT) and G-lambda DNA for reverse transcription and qPCR inhibition, respectively. Twelve of 138 samples were qPCR-inhibited, requiring dilution with AE buffer.

Standard curves were generated for each gene target by serially dilutions of the positive controls. Cq values were calculated using the second derivative maximum method and regressed against the decimal logarithm of target concentration using the non-linear function provided by the LightCycler 480 software.

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Table 4. Microbes and gene targets detected in private household wells (n = 131) and samples (n = 138).

Host specificity	Microbe[1]	Gene target	Positive wells (n)	Positive samples (n)	Concentration of positive samples (gene copies/L)	
					Median	Range
Human-specific	*Bacteroidales*-like Hum M2	Glycosyl hydrolase family 92	7	8	4	<1 – 1,050
	HF183 *Bacteroides*	16s rRNA	27	28	<1	<1 – 34
	Cryptosporidium hominis	18s rRNA	1	1	<1	<1
	Adenovirus A	hexon	1	1	1	1
	Rotavirus group A, G1 P[8]	*NSP3*	7	7	<1	<1 – 3
		VP7	3	3	1	<1 – 22
	Any human microbe		33	34	<1	<1 – 1,050
Bovine- or ruminant-specific	*Bacteroidales*-like Cow M2	DHIG domain protein	2	2	472	29 – 915
	Bacteroidales-like Cow M3	HD super family hydrolase	4	4	174	3 – 49,818
	Ruminant *Bacteroides*	16s rRNA	36	36	1	<1 – 42,398
	Bovine polyomavirus	*VP1*	8	8	4	<1 – 451
	Bovine enterovirus	5' non-coding region	1	1	2	2
	Rotavirus group A, G10 P[11]	*NSP3*	12	12	12	2 – 4,481
		VP7	5	5	23	<1 – 732
	Any bovine or ruminant microbe		44	44	3	<1 – 49,818
No host specificity	Pepper mild mottle virus	replication-associated protein	13	14	14	2 – 3,811
	Cryptosporidium spp.	18s rRNA	2	2	<1	<1 – 1
	Cryptosporidium parvum	18s rRNA	13	13	<1	<1 - 14
	Giardia lamblia	*β-giardin*	2	2	<1	<1
	Campylobacter jejuni	*mapA*	1	1	<1	<1
	Salmonella spp.	*invA*	3	3	6	<1 – 13
		ttr	5	5	10	5 – 59
	E. coli (pathogenic)	*eae*	1	1	4	4
		stx1	1	1	16	16
		stx2	1	1	1	1
	Rotavirus group C	*VP6*	3	3	50	45 – 1,301
	Any non-specific microbe		37	46	5	<1 – 3,811
All	Any microbe		79	82	2	<1 – 49,818

[1] Microbial targets analyzed but not detected: human adenovirus groups B, C, D, and F, human enterovirus, human norovirus genogroups I and II, human polyomavirus, *Cryptosporidium bovis*, bovine adenovirus, bovine coronavirus, and bovine viral diarrhea virus types 1 and 2

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Results and Discussion

Microbial detections and concentrations. Eighteen microbe taxa were detected in the wells. The pathogens detected included *Cryptosporidium parvum, Giardia lamblia, Salmonella* spp., pathogenic *E. coli, Campylobacter jejuni*, and rotavirus group C (Table 4). *Bacteroidales*-like Hum M2, HF183 *Bacteroides, Bacteroidales*-like Cow M2, *Bacteroidales*-like Cow M3, Ruminant *Bacteroides*, and pepper mild mottle virus are non-pathogenic.

Thirteen microbe taxa were analyzed but not detected in the wells: human adenovirus groups B, C, D, and F, human enterovirus, human norovirus genogroups I and II, human polyomavirus, *Cryptosporidium bovis,* bovine adenovirus, bovine coronavirus, and bovine viral diarrhea virus types 1 and 2. These negative results could stem from specific characteristics of a microbe such as low survival in groundwater or low transport potential through soil. Another possibility is the non-detected microbes may not have been circulating in the human or cattle populations in the county at the time of the study.

Microbe concentrations in the wells were low except for ruminant *Bacteroides* and *Bacterioidales*-like CowM2 (Table 4). Among pathogens detected, rotavirus group C and bovine-related rotavirus group A had the highest concentrations.

Extent of fecal contamination. Fecal contamination, as evidenced by the presence of fecal-borne microbes, occurred in 82 samples from 79 wells (59% of samples, 60% of wells) (Table 4). Two or more fecal-borne microbe types were detected in 70 wells.

While these wells were selected randomly for sampling, selection was from the group of wells that had shown previously to be positive for coliforms or had nitrate-N > 10 mg/L. We took this approach to enhance the likelihood of achieving this particular study objective, that is, determine sources of fecal contamination. However, these wells may not be necessarily representative of all wells in Kewaunee County and the 60% prevalence value for fecal contamination could be overestimated. On the other hand, 60% prevalence could be an underestimate because 95% of the wells were sampled only once, and the probability of detecting a contaminant increases the more frequently a well is sampled (Atherholt et al. 2015).

Sources of fecal contamination. Microbes from both human wastewater and bovine manure were present in the study wells. Thirty-three wells had evidence of human wastewater primarily by the detection of HF183 *Bacteroides* and *Bacteroidales*-like Hum M2 (Table 4). Forty-four

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wells had evidence of bovine manure, indicated primarily by the positive detection of ruminant *Bacteroides*. Both fecal sources, human and bovine, were detected in nine wells.

Whether the prevalence of fecal contamination from bovine manure (44 wells) is greater than that contributed by human wastewater (33 wells) cannot be determined from these data for three reasons: First, the probability of detecting a host-specific microbe was not equally weighted between hosts, 13 human microbes were analyzed versus 11 bovine microbes; Second, the prevalence of host-specific pathogens used to determine fecal source could have differed between the human and bovine populations in the county during the study. For example, if fewer people in the county were infected with gastrointestinal pathogens then fewer wells would be positive for human wastewater; Third, the analytical sensitivity of the detection method (qPCR) can vary by microbe type leading some microbes to be more likely detected than others.

Forty-six samples from 37 wells were positive for pathogenic and non-pathogenic microbes that are not related to a specific host and therefore the source of fecal contamination cannot be determined.

All sample-level data on microbial targets measured by qPCR are reported in Appendix 2. Data have been de-identified to maintain well owner confidentiality.

OBJECTIVE 4: Automated Sampling for Timing of Transport of Pathogens and Indicator Bacteria

Procedures and Methods

Overview of approach. The flow characteristics of the dolomite aquifer --rapid transport from soil surface to the saturated zone and high groundwater velocities within the aquifer -- can result in changes to groundwater quality that are rapid, intermittent, and short-lived. Prior to this project, there were no data that addressed the temporal variability of pathogen loading nor any data that illustrated how pathogen transport related to recharge events. As noted above, we monitored water levels and water-quality parameters at non-pumping sentinel wells throughout this project. During specific recharge events, we collected detailed time-series of the concentrations of pathogens and microbial markers using specialized automated samplers that were installed on three domestic wells.

Automated samplers site selection. The automated samplers were designed to collect a time-series of samples of drinking water from private domestic wells during recharge events. The site

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instrumentation required a continual flow of water past the water-quality sensors both before and during sampling of recharge events. During automated sampling, a study team member visited the sampler every few days. One of our primary considerations for site selection of the automated sampler was having cooperative homeowners who were interested in the research that we were conducting and who were likely to be home during daytime hours. The questions that we hoped to address were: 1) what is the temporal variability of pathogen loading? and 2) how does pathogen transport related to recharge events? As such, we wanted to sample wells with known water-quality problems. The ideal well would have a history of bacteria detections, nitrate-N over 5 mg/L, and shallow soil cover, ideally < 20 ft. If the well had been sampled for pathogens, we were interested that both human and bovine indicator microbes had been detected. Since Kewaunee County has restrictions on manure application until mid-April, and the spring snowmelt recharge event often occurs in March, we thought it would be good to deploy one of the samplers in Door County. A final consideration was to include wells of differing age and construction. The locations of the automated samplers are shown on Figure 5 and a summary of well location and characteristics is presented in Table 5.

Sampler design and construction. The initial design for the automated sampler was similar to that used by Corsi et al. (2014) to sample streams. A primary goal of the design was to develop a compact sampler that would be easy to transport and able to be quickly deployed on domestic wells. Each sampler contained the following components: dead-end hemodialysis ultrafiltration filters for collecting samples, a flow-through chamber containing a multi-probe water-quality sonde, several inline rotary flow meters, a datalogger for running the system and recording the resulting flow and water-quality data, and a remote telemetry communication system that allowed the researchers operators to monitor current conditions, adjust operational parameters, and control the start and end of the sampling periods. The sampler used at Site 1 was capable of holding four dead-end hemodialysis ultrafiltration filters that were housed in an unrefrigerated case. The design of the second sampler was modified such that it was capable of holding 8 filters and was enclosed within a small refrigerator thus extending the hold time for the filters. The design of the second sampler is described by Owens et al. (2019b). Figure 17 includes photos of both automated sampler designs.

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Table 5. Automated sampler sites

Site	Location	Operation time	Well depth (feet)	Casing depth (feet)	Date constructed
Site 1	SW1/4, SE1/4 sec 23 T25N, R23E	Oct 2016 to April 2017	303	200	August 2013
Site 2	NW1/4, SE 1/4, sec 27, T26N, R23E	Jan to April 2017	43	29	Unknown
Site 3	NE1/4, NW1/4, sec 23, T24N, R23E	May 2017	120	40	July 1975

The samplers were installed in the basements of Sites 1 and 2. For each installation the household water supply was tapped at the water pressure tank and fed through the sampler. For Site 3, the sampler was deployed outside the house and the outside spigot was tapped for sampling. A flow rate was set using a valve on the automated sampler intake. Water moving through the sampler was monitored using a multi-probe water-quality sonde and a flow-through chamber. Parameters measured include fluid temperature, specific conductance, pH, nitrate, chloride, fluorescent dissolved organic matter (FDOM), and turbidity. Water-quality measurements were made every minute and stored on the sampler's datalogger. Water-quality data collected were available real-time using the two-way telemetry of the sampler. The purpose of this monitoring was to characterize the dynamics and conditions of the household system during time periods before and during the time pathogen samples were taken. During initial deployment, degassing of the water in the flow-through chamber caused bubble artifacts in results from the water-quality sensors; orientating the sonde more vertically reduced, but did not eliminate, bubble formation. Water was continually flushed through the flow-through chamber at a rate of approximately 0.6 L/min. This resulted in a constant relatively low-flow rate discharge that required continual disposal using a sump pump (Site 1) or gravity drainage into a floor drain (Site 2) or away from the house (Site 3). Because the sampler was installed in household basements, it included an emergency shutdown capability triggered by the occurrence of water in the bottom of the sampler. A pressure transducer was also used to measure backpressure in the

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filter system, an indication of filter clogging and/or system failure. When a threshold pressure of 345 kPa (50 psi) was met the system would be shut down by closing the main valve into the sampler. However, no clogging was observed at any site during our study.

When a target recharge event was identified, the smartphone app was used to open the valve to the first filter. The sampler program then monitored the flow rate until the user-specified volume passed through the filter, at which point the valve to the first filter was shut and the valve to the second filter was opened. This continued until all filters were used or the user canceled the sampling event. Target volumes could be changed for the current or future filters during the sampling event. This allowed the researchers to optimize the event sample coverage as the event progressed. A minimum of 600 liters of water was run through a filter and a maximum of 1600 liters of water was run through a single filter.

Filters were collected every 1 to 3 days to minimize holding times and maintain continual coverage over the sampling period. During collection, the sampler was paused, filters were replaced, used filters placed in a plastic bag, sealed, and shipped to the analytical laboratory. Protective latex gloves were worn throughout filter collection to prevent contamination. Samples were analyzed for the four microbial targets that had been most frequently detected during the sampling conducted to determining the source of pathogen contamination (described above). This included HF183 *Bacteroides*, ruminant *Bacteroides*, pepper mild mottle virus, and rotavirus group A. We also analyzed for total coliform and *E. coli* bacteria concentrations.

Results and Discussion

Groundwater quality data. Geochemical parameters were recorded at minute time steps for the entire time an automated sampler was deployed at a given monitoring site. Those data can be used to characterize the variability of water quality at each of the domestic wells that were sampled.

Parameters measured at Site 1 include fluid temperature, pH, nitrate, chloride, turbidity, fluorescent dissolved organic matter (FDOM), and specific conductance and all of these (excluding fluid temperature) are presented in Figure 18. Bubble formation, discussed in Owens et al. (2019b), affected those parameters that rely on optical measures (FDOM and turbidity) in the early part of the monitoring period. Adjusting the orientation of the EXO sonde in late November reduced, but did not eliminate the problem. Several parameters, specifically FDOM,

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chloride and nitrate all tend to vary in a similar fashion and also tend to mimic the variation in water levels seen at the sentinel wells. This suggests that these parameters could serve as good indicators of recharge at domestic wells where the water-level record is confounded by homeowner pumping. Breaks and jumps in the record are due to periodic calibration of the water-quality sensors.

Sampler 2, which was deployed at Sites 2 and 3, did not include probes for measurements of pH and turbidity. The geochemical results for Site 2, plotted in Figure 19, also show significant variations in nitrate, chloride, specific conductance, and FDOM on time frames ranging from days to weeks. Unlike what was seen at Site 1, these parameters do not vary in a similar fashion to each other, nor do they track with water levels at the sentinel wells. This may be due the following factors: greater depth of unconsolidated sediment at this site and flow system position further from a regional recharge area and the sentinel well.

Site 3 was monitored during a period of generally falling water levels, but even in the absence of significant recharge events, chloride, specific conductance and FDOM all show significant variation on a timescale of a day or two (Figure 20). Nitrate-N concentrations climbed for a few days after installation until reaching a value on the order of ~42 mg/L. The nitrate-N concentrations then climbed ~10 mg/L over the sampling period. The probes were not calibrated during this sampling event and so some of the variation in these data may be due to probe drift.

Results from all three domestic wells serve to illustrate the time-variance in the quality of water produced by domestic wells completed in the dolomite aquifer. All three wells show significant variation in monitored parameters on time scales ranging from hours to weeks. Some of the variation is clearly correlated to specific recharge events. The correlation of water-quality variations and recharge events is perhaps most clearly seen at Site 1 which is also the well that is located closest to the regional recharge area.

Microbial samples. The automated samplers were used to collect microbial samples during five distinct recharge events and one period of declining water levels. Figure 21 illustrates the sampling periods in relation to the water-level data from the two non-pumping sentinel wells. The sampler at Site 1 was used to sample recharge events in October 2016 and December 2016. The sampler was installed at Site 2 and was used to sample a January 2017 recharge event. Both samplers were used to sample a short-duration recharge event in late February and a long-duration recharge event from late March to early April. Sampler 2 was also used to sample Site 3

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during a period of generally declining water levels, however, there were some small recharge pulses during the sampling period and the site was adjacent to a field where manure had been recently applied. The results of the six sampling events are summarized in Figures 22 to 27. The hydrologic conditions and microbial sampling results of each event are discussed below.

October event. The rain event of October 26, 2016 led to a brown water event on Rockledge Road (https://www.wbay.com/content/news/DNR-tests-tainted-water-in-Kewaunee-County-415745143.html). Figure 22 shows the hydrologic conditions over the duration of this sampling event. The figure includes hourly precipitation data from the Casco weather station along with the resulting changes in water level in sentinel well KW183. The rain began at 8:00 AM and the water level at the sentinel well began to increase approximately 5 hours later. Sampling was initiated prior to the start of the rain event and continued until approximately midday on November 2. Microbial targets, other than total coliform, were detected only sporadically and were best characterized by presence/absence in filters that were collected sequentially. In addition to the microbial targets shown in the top graph, *E. coli* bacteria were also not detected during this event. In contrast, total coliform bacteria, were detected in every sample and exhibited concentrations that varied over two orders of magnitude across the recharge event (note log axis in Figure 22). The peak concentration of total coliform occurred approximately 24 hours after the initiation of the rain event and preceded the peak water level observed in sentinel well KW183.

December event. A brief recharge event in late December appears to have been triggered by small rain events on December 24 and 25, 2016 and a short period with air temperature above freezing on Christmas day. Figure 23 shows the hydrologic conditions over the duration of this sampling event. The water level began to rise in KW183 at approximately 11 PM on Christmas day. We initiated sampling approximately mid-morning on December 26 and collected only four samples. No microbial targets, other than total coliform were detected in any sample. Total coliform bacteria were again detected in every sample and the peak concentration was observed in the last sample collected. Given the short nature of this sampling event, it is difficult to relate microbial concentrations with well dynamics.

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Table 6. Summary of sampling events using the automated samplers

Initiation of recharge event	Sampling locations	Sampling duration	Number samples; average hours/filter
October 26, 2016	Site 1	10/26/16 5:30 to 11/2/16 11:30	16 samples; 12.5 hours/filter
December 26, 2016	Site 1	12/26/16 10:25 to 12/28/16 10:42	4 samples; 12.1 hours/filter
January 18, 2017	Site 2	1/18/17 15:32 to 1/23/17 18:41	7 samples; 17.6 hours/filter
February 27, 2017	Site 1	2/27/17 18:40 to 3/2/14 4:45	4 samples; 14.5 hours/filter
	Site 2	2/27/17 18:41 to 3/4/17 0:19	8 samples; 12.7 hours/filter
March 20, 2017	Site 1	3/20/17 10:54 to 4/9/17 10:00	29 samples; 14.85 hours/filter
	Site 2	3/21/17 11:44 to 4/9/17 11:52	26 samples; 17.5 hours/filter
Water-levels begin declining May 1	Site 3	5/11/17 17:30 to 5/29/17 12:32	24 samples; 17 hours/filter

January event. Sampler 2, deployed at Site 2, was used to sample across a January, 2017 recharge event that occurred due to both snowmelt and winter rainfall (Figure 24). The NWS Green Bay station reported 5 inches of snow depth on January 16, 2017 that began to melt that day as air temperatures rose above freezing for the majority of the next ten days. The water level in KW183 rose steadily until January 26, at which times it began to decline, signifying the end of this recharge event. We collected seven samples between January 18 and January 23. Total coliform bacteria were detected in every sample and *E. coli* bacteria were detected in all but one sample. Pepper mild mottled virus and Rotavirus group A were each detected in one sample; while neither human nor bovine *Bacteroides* were detected in any sample. Unfortunately, the last sample again contained the peak bacterial concentrations meaning the breakthrough curve is not well defined Thus, we cannot determine the relationship between peak concentrations and recharge dynamics.

Late February event. A period of mixed rain and snow on February 28 and March 1 led to

a short-term rise in the water level at sentinel well KW183 (Figure 25). Sampling at both Sites 1 and 2 was initiated the evening of February 27. At Site 1, we collected 4 samples and ceased sampling at approximately 5 AM on March 2. We continued sampling at Site 2 until a few minutes after midnight on March 4 and collected eight samples from that site. Site 1 (top graph) had one detection of bovine *Bacteroides* and one detection for total coliform bacteria. At Site 2 (middle graph), total coliform bacteria were detected in every sample, pepper mild mottle virus was detected in four samples, and human *Bacteroides* was detected in one sample. Total coliform concentrations appear to track similarly as the water level in KW183, with both rising during February 28 and peaking on March 1.

Late March to early April event. The water level in KW183 declined until March 19 and then began to increase (Figure 26). The steepest rise in water levels occurred March 24 to 28 when air temperatures were generally above freezing and there were several days with rain events. Water levels began to decline on April 6. We initiated sample at Site 1 on March 20 and at Site 2 on March 21. Sampling at both sites continued until April 9. A total of 29 samples were collected at Site 1 and 26 at Site 2. Total coliform bacteria were again the most commonly detected microbe. All samples from Site 2, excluding the first sample, contained detectable concentrations of total coliform. For Site 1, the first seven samples did not contain detectable concentrations of total coliform, but all subsequent samples did. Sporadic rain events between March 30 and April 5 seemed to result in spikes in total coliform at both sites. Other microbes were again only detected occasionally with human *Bacteroides* and pepper mild mottle virus detected at both sites and bovine *Bacteroides* and Rotovirus A detected at neither site.

May sampling event. One of the primary goals of the automated sampler work was to gain an understanding of pathogen transport in relation to recharge. We were particularly interested in the transport of enteric pathogens such as human and bovine *Bacteroides*. As noted above, these pathogens were detected only sporadically during the five recharge events that we sampled. We believe the infrequent detection of these pathogens was due to the relatively sparse distribution of septic systems and the lack of manure applications in the vicinity of the automated samplers.

Manure application was delayed due to wet weather in spring 2018. In early May, we identified a well that was located next to a field that was expected to receive manure yet that spring. We chose to move sampler 2 to this location (Site 3) and continue sampling even though

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the peak spring recharge period had passed. Sampling was initiated the evening of May 11 and continued to mid-day on May 29. Manure applications occurred on May 10 and May 18. While water levels generally declined over the sampling period, periodic rain events did result in small recharge events. Figure 27 shows the hydrogeologic conditions for mid to late May as well as the results of our sampling. Similar to the other sampling events, total coliform bacteria were detected in every filter that we collected and *E. coli* bacteria were detected in 7 of 15 samples. A significant rain event that began late on May 23 and continued into early May 24 appears to lead to spikes in both total coliform and *E. coli* concentrations. Site 3 also had significantly more detections of other microbial targets. Bovine *Bacteroides* and Rotavirus A were detected in every sample and these results are plotted as symbols connected by solid lines in Figure 27. Human *Bacteroides* and pepper mild mottle virus were detected only sporadically and these are show as isolated symbols in Figure 27. The variations in the microbial targets, other than total coliform and *E. coli*, are not clearly correlated with precipitation or water-level variations and may represent the baseline contaminant load for this well.

OBJECTIVE 5: Comparison with Existing Well Water Quality Data for Kewaunee County

Procedures and Methods

Existing data on private well contamination in Kewaunee County is available from the Well Water Quality Viewer managed by the Center for Watershed Science and Education, University of Wisconsin – Stevens Point (https://www.uwsp.edu/cnr-ap/watershed/Pages/WellWaterViewer.aspx). These data are from private well water samples submitted voluntarily by well owners over the past 25 years. Data are aggregated (e.g., percent positive samples) as a "running" statistic, meaning new data are added to the aggregate measure as new samples are analyzed. We compared our estimates for private well contamination obtained by the two synoptic stratified random sampling events with data in the Well Water Viewer accessed online on June 6, 2019. Comparisons were made at the geographic scale of county. To be consistent with our study design we assumed each sample in the Viewer represents one well (i.e., no multiple samples per well).

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Results and Discussion

The Well Water Quality Viewer reports 11% of 1,350 samples analyzed for nitrate-N in Kewaunee County exceeded the health standard of 10 mg/L. This percentage falls within the 95% confidence interval for the nitrate contamination prevalence we determined in Fall 2015 (3% - 11%), but it is slightly higher than the prevalence determined in Summer 2016 (Table 2). The coliform contamination prevalence reported in the Viewer (19% of 1,206 samples) lies within the 95% confidence intervals of both our estimates (Table 2), suggesting our coliform data and the Viewer data are similar. In contrast, *E. coli* prevalence reported in the Viewer (2.2% of 1206 samples) is higher than the 95% confidence intervals for our two estimates, suggesting our data and the Viewer data for *E. coli* are not similar.

Overall, it appears the two data collection methods, voluntary well water samples submitted by well owners and the synoptic stratified random sampling conducted for this study, give similar results, at least when the data are aggregated at the large geographic scale of county. We cannot determine whether the Viewer data set is unbiased or both data sets are biased in similar directions. Furthermore, we did not assess how sensitive the comparison between data collection methods is to the number of samples in each data set.

OBJECTIVE 6: Comparison with Runoff Risk Advisory Tool (RRAT)

Procedures and Methods

The Runoff Risk Advisory Tool (RRAT) combines National Oceanic and Atmospheric Administration (NOAA) weather forecasting, precipitation, snowmelt, and flood prediction models with soils data and ground temperature/weather station data to predict when runoff to surface waters will occur. The RRAT has focused on surface water since its inception, and does not have a groundwater component. However, the basic predictor variables are also some of the key variables that contribute to water infiltration and recharge of the shallow fractured dolomite aquifer – soil type, precipitation runoff, snowmelt, and presence of frozen soil conditions. Given the similarities, we explored the potential of RRAT's relevance for groundwater by analyzing the concordance of predicted runoff risk with observed groundwater contamination.

Runoff risk maps for the state of Wisconsin for the study period (years 2016 and 2017) were obtained from the Department of Soil Science, University of Wisconsin – Madison. Unlike the current 2019 version of the Runoff Risk Advisory Forecast (RRAF) that has a spatial resolution

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of 2 km x 2 km grids, the resolution of the previous RRAT in operation during the study period was at the watershed scale. We examined the runoff risk levels for three watersheds: Ahnapee River, East Twin River, and Kewaunee River. These three watersheds comprise 86% of the Kewaunee County land area (Kewaunee County Land and Water Management Plan 2019).

The RRAT risk output was compared with the prevalence of bovine-specific microbes (i.e., bovine manure) in private wells sampled throughout the county. To match spatial scales between well contamination prevalence (county) and RRAT output (watershed) we scaled up the latter by characterizing the county runoff risk as the risk level possessed by at least two of the three watersheds in our analysis. All three watersheds had the same risk level in 65 of the 80 time periods examined; 15 time periods had two of the three watersheds with the same risk level and at no time did each watershed have a different runoff risk. Runoff risk was characterized for five time periods corresponding to the specific dates wells were sampled: risk on the same day as sampling, risk on days one, two, and three previous to sampling, and the highest risk level forecast within seven days previous to the sampling date. Runoff risk predictions from RRAT are forecast by NOAA three times per day; we selected the forecast with the highest risk for the time period examined. Runoff risk levels were recorded "as is" without consideration of forecast outlook (10 days if the ground is frozen or snow-covered, 3 days for all other conditions). Comparisons between RRAT runoff risk and well contamination were by chi-square using SigmaPlot, version 13.

Results and Discussion

The Runoff Risk Advisory Tool outputs five risk levels of which four levels occurred during the five time windows relative to sample date for which risk was characterized (defined in previous paragraph). The risk levels and the number of samples associated with each were as follows: frozen/snow high risk (22 samples); unfrozen ground, high risk (59 samples); unfrozen, moderate risk (28 samples); unfrozen, low risk (29 samples). No samples were collected when the runoff risk was categorized as frozen/snow, no runoff.

The percentage of samples contaminated with bovine-specific microbes was associated with runoff risk category (Table 7). The strongest association between well contamination and runoff risk was when risk was categorized as the highest level within 7 days previous to the sampling date ($P = 0.018$). Excluding the frozen/snow category to focus on the time of year when the

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ground was unfrozen did not improve the associations. When the runoff risk category was "frozen/snow high risk" the percentage of samples positive for bovine manure was high (50%) and consistent irrespective of the time period in which risk was characterized. However, only when the previous 7-day period was used to characterize risk was there a plausible trend with the percentage of contaminated samples decreasing with risk level (Table 7). As the trend was significant (P = 0.018) we conducted post-hoc pairwise chi square tests. The percentage of contaminated samples in the frozen/snow high risk category was significantly greater than the moderate and low risk categories (P = 0.035 and 0.028, respectively), but not higher than the high risk category (P = 0.522). The percentage of contaminated samples in the unfrozen high risk category was marginally significantly greater than the moderate and low risk categories (P = 0.085 and 0.070, respectively).

Rapid surface water infiltration into the fractured Silurian dolomite aquifer in northeastern Wisconsin is well documented. Thus, it is plausible that the output of a tool developed to predict surface runoff is, at least for the Silurian dolomite aquifer, correlated with groundwater contamination. Indeed, the associations observed here strongly suggests RRAT has potential to predict when well owners should be concerned about the quality of their well water. Perhaps the new tool version, the Runoff Risk Advisory Forecast (RRAF), provides greater predictive power for well water quality and at finer spatial resolutions. As the coliform, *E. coli*, and nitrate samples for the present study were collected synoptically, in effect holding weather and runoff constant during the sampling timeframe, it was not possible to evaluate whether coliform and nitrate contamination rates were associated with RRAT-predicted runoff risk. Besides bovine manure-specific microbes, future studies evaluating RRAF for predicting well water quality could use other outcomes such as coliforms, nitrate, enterococci, and coliphages.

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Table 7. Comparison of runoff risk forecasts from the Runoff Risk Analysis Tool and private well contamination with microbes specific to bovine manure.

Risk period relative to sampling date	Chi-square statistic	P-value	Percent samples positive for bovine-specific microbes by risk level			
			Frozen-high	High	Moderate	Low
Same day	9.23	0.026	50	19	50	27
1 day previous	5.62	0.132	50	20	37	30
2 days previous	7.51	0.051	50	20	50	28
3 days previous[1]	5.22	0.073	50	20	–	32
Highest risk level within 7 days previous	10.09	0.018	50	39	18	17
Analysis excluding frozen ground category						
Same day	5.63	0.060	–	19	50	27
1 day previous	1.77	0.412	–	20	37	30
2 days previous	3.79	0.150	–	20	50	28
3 days previous[1]	1.05	0.306	–	20	–	32
Highest risk level within 7 days previous	6.55	0.038	–	39	18	17

[1]Medium risk was excluded from the chi-square test because there were only 2 wells.

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SUMMARY POINTS

Contamination rates of coliforms, E. coli, and nitrate.

- In two synoptic randomized sampling events the county-wide percentage of private wells positive for coliform bacteria, *E. coli*, or nitrate-N > 10 mg/L was 26% (Fall 2015, 95% confidence interval 19% - 34%) and 28% (Summer 2016, 95% confidence interval 22% - 33%). These contamination rate estimates are unique for their accuracy and precision as sampling was by a stratified design, accounting for the number of wells located at different depths to bedrock.

Depth to bedrock and contamination

- Depth to bedrock is one of the most important factors related to private well contamination in Kewaunee County. Wells located in the two shallowest depth-to-bedrock categories used in the present study (< 5 feet and 5 to 20 feet) had the highest contamination rates of coliform bacteria, *E. coli*, or nitrate-N > 10 mg/L. Statistical modeling reported elsewhere (Borchardt et al. in preparation) suggests the depth to bedrock must be greater than 50 feet for the risk of well contamination to be similar to the Wisconsin statewide averages for coliforms, *E. coli*, and nitrate.
- About 2% of private wells in Kewaunee County are located where the depth to bedrock is less than 5 feet. This statistical estimate is substantially less than the visual estimate (6.5% of wells) derived from the depth-to-bedrock map created by Sherrill in 1979. While it might be encouraging there are fewer wells considered highly vulnerable to contamination, the data show contamination vulnerability extends to much deeper depths to bedrock than originally thought (e.g., wells with 5 to 20 feet depth to bedrock are more likely to be contaminated than wells with bedrock depths greater than 20 feet).

Fecal contamination

- Wells were randomly selected for fecal source tracking from the group of wells positive for coliforms or with nitrate-N concentrations greater than 10 mg/L. Of the wells sampled (131), 60% had evidence of microbes associated with fecal wastes.
- The fecal wastes in private wells in Kewaunee County stemmed from both human and bovine sources. Septic systems and cattle manure are the two largest fecal sources on the

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Kewaunee County rural landscape. Statistical modeling (Borchardt et al. in preparation) shows significant quantitative relationships between septic system density and well contamination with human fecal microbes, and similarly, quantitative relationships between agricultural activities and well contamination with bovine manure microbes.

- Genes specific to fecal-borne pathogens of significant concern were detected in the study wells: *Salmonella* species, enterohemorrhagic *E. coli*, *Cryptosporidium parvum* and *C. hominis*, *Giardia lamblia*, rotavirus, and *Campylobacter jejuni*. Studies have shown measurements of pathogen genes in water can be linked to health risk; however, the health risk of pathogen genes in Kewaunee County wells is currently unknown. Nonetheless, scientists would agree that the health risk from drinking water positive for pathogen genes is greater than drinking the same water absent pathogen genes.

Other groundwater quality data for Kewaunee County

- The Well Water Quality Viewer managed by the Center for Watershed Science and Education, University of Wisconsin – Stevens Point reports groundwater quality data for thousands of private well samples collected by homeowners. The Viewer's coliform and nitrate contamination rates for Kewaunee County are similar to the rates reported in the present study when the data are aggregated at the level of county. We did not evaluate other geographic scales such as township or watershed. Having accurate Viewer data does not obviate sample collection for future studies as the research questions addressed may have specific sampling requirements to maximize statistical power, minimize bias, and avoid confounding variables.

Runoff Risk Advisory Tool for predicting private well contamination

- The Runoff Risk Advisory Tool managed by the University of Wisconsin – Madison Department of Soil Science predicts when runoff to surface waters will occur. The proportion of private well samples positive for bovine manure was associated with the runoff risk level predicted by the tool, particularly when risk was characterized for the 7 day period prior to well water sample collection. In hindsight, while the study design effectively addressed several important research objectives, it was not optimal for evaluating the runoff tool. Especially now that the new Runoff Risk Forecast Advisory is

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available, it would be worthwhile to further evaluate the tool's predictions with the occurrence of private well contamination.

Recharge and Water Quality

- Seasonal variation in nitrate-N concentrations is not a simple function of recharge. In general, periods of variable nitrate-N concentration seem to correlate with recharge periods, whereas periods of stable nitrate-N concentration correlate with non-recharge periods. Periods of elevated nitrate-N concentration seem to correlate with periods of nutrient application rather than with periods of recharge.
- Water-level variations at both non-pumping monitoring wells tended to track together suggesting that both wells respond similarly to precipitation and melt events. The water-level rise at both sentinel wells typically occurs within 4 to 5 hours of a large rain event. This suggests that flow-system position does not exert a strong control on water-level response. However, flow-system position does seem to affect water-quality parameter response. This was noted at both the non-pumping sentinel wells and the domestic wells where automated samplers were installed. Wells located nearest to the regional water-table divide, which also serves as the regional recharge area, had the clearest recharge signal in terms of water-quality parameters.
- Water-quality data collected at the non-pumping sentinel wells suggest that sharp inflections in specific conductance and Colored Dissolved Organic Matter (CDOM) are good indicators of groundwater recharge (in addition to rising water levels). Water-quality monitoring at automated sampler sites suggest that several parameters, specifically FDOM, chloride, and nitrate all tend to vary in a similar fashion and also tend to mimic the variation in water levels seen at the sentinel wells.

Automated Samplers and Pathogen Transport

- Water-quality data collected using the automated samplers indicate that household water quality is highly variable over timescales ranging from hours to days. Automated samplers provide an efficient method of characterize this chemical variability and the variability of pathogen load over time.

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- The microbial sampling results indicate that microbe arrival at a domestic well is dependent upon both recharge events and source term. Total coliform was the most commonly detected microbe. Since these bacteria are ubiquitous, frequent detection associated with recharge events is not a surprising result. The fact that the concentrations of total coliform bacteria over time looked similar to traditional breakthrough curves was surprising. Host-specific microbes (human and bovine *Bacteroides)* and non-specific Rotavirus group A were sporadically detected due to sparser distribution of septic systems and episodic nature of manure application. During periods of manure application, the bovine enteric pathogens were persistent.

ACKNOWLEDGEMENTS

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In addition, we thank the laboratory staff of the Environmental Research and Innovation Center at University of Wisconsin – Oshkosh for their extraordinary efforts to analyze hundreds of samples that arrived nearly simultaneously as a result of the synoptic sampling design.

Finally, we thank the well owners and citizens of Kewaunee County for their support and participation, which without the study would not have been completed.

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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REFERENCES

Atherholt TB, Korn LR, Louis JB, Procopio NA. 2015. Repeat sampling and coliform bacteria detection rates in New Jersey domestic wells. Groundwater Monitoring and Remediation. 35: 70-80.

Blanchard MC. 1988. Investigation of the shallow fractured dolomite aquifer in Door County, Wisconsin. Unpublished M,S. thesis, University of Wisconsin-Madison, 186 p.

Bonness D. and Masarik K. 2014. Investigating intra-annual variability of well water in Lincoln Township. Final Report to Town of Lincoln, WI. http://www.uwsp.edu/cnr-ap/watershed/Documents/Lincoln_FinalReport.pdf

Borchardt MA, Bradbury KR, Alexander EC, Archer J, Braatz L, Forest B, Kolberg R, Olson C, Spencer SK. 2011. Norovirus outbreak caused by a new septic system in a dolomite aquifer. Groundwater, 49:85-97.

Borchardt MA, Spencer SK, Kieke BA Jr., Lambertini E, Loge FJ. 2012. Viruses in non-disinfected drinking water from municipal wells and community incidence of acute gastrointestinal illness. Environmental Health Perspectives, 120:1272-1279.

Bradbury K R and Muldoon MA. 1992. Hydrogeology and groundwater monitoring of fractured dolomite in the Upper Door Priority Watershed, Door County, Wisconsin. Wisconsin Geological and Natural History Survey Open File Report, WOFR 92-2, 84 p.

Chamberlin TC. 1877. Geology Of Wisconsin, Volume 2, Survey of 1873-1877. Wisconsin Geological and Natural History Survey, Madison, Wisconsin. p. 335-389.

Corsi SR, Borchardt MA, Spencer SK, Hughes PE, Baldwin AK. 2014. Human and bovine viruses in the Milwaukee River watershed: hydrologically relevant representation and relations with environmental variables. Science of the Total Environment, 490: 849-860.

Erb K. and Steiglitz R. (editors), 2007. Final Report of the Northeast Wisconsin Karst Task Force, 46 p. http://learningstore.uwex.edu/Final-Report-of-the-Northeast-Wisconsin-Karst-Task-Force-P1234C120.aspx

Gibson KE, Schwab KJ, Spencer SK, Borchardt MA. 2012. Measuring and mitigating inhibition during quantitative real time PCR analysis of viral nucleic acid extracts from large-volume environmental water samples. Water Research, 46:4281-4291.

Hynds PD, Thomas MK, and Pintar KDM. 2014. Contamination of groundwater systems in the US and Canada by enteric pathogens, 1990-2013: A review and pooled-analysis. PLoS ONE, 9(5):e93301.

Kewaunee County Land and Water Management Plan 2019. http://cherrylan.kewauneeco.org/FTP/landwater/LWRMaster.pdf [accessed July 29, 2019].

ED_004892A_00000077-00055

Knobeloch L, Gorski P, Christenson M, Anderson H. 2013. Private drinking water quality in rural Wisconsin. Journal of Environmental Health, 75:16-20.

Lambertini E, Spencer SK, Bertz PD, Loge FJ, Kieke BA, Borchardt MA. 2008. Concentration of enteroviruses, adenoviruses, and noroviruses from drinking water by use of glass wool filters. Applied and Environmental Microbiology, 74:2990-2996.

Muldoon MA, Simo JA, Bradbury KR. 2002. Correlation of high-permeability zones with stratigraphy in a fractured-dolomite aquifer, Door County, Wisconsin. Hydrogeology Journal, 9:570-583.

Muldoon MA. and Bradbury KR. 2010. Assessing seasonal variations in recharge and water quality in the Silurian aquifer in areas with thicker soil cover: Final Report to the Wisconsin Department of Natural Resources, 45 p.

Owens DW, Hunt RJ, Firnstahl AD, Muldoon MA, Borchardt MA. 2019a. Water-quality data collected for Kewaunee County, Wisconsin: U.S. Geological Survey data release, https://doi.org/10.5066/P9VO1KF9.

Owens DW, Hunt RJ, Firnstahl AD, Muldoon MA, and Borchardt MA. 2019b. Automated time series measurement of microbial concentrations in groundwater-derived water supplies. Groundwater 57:329-336.

Rayne TW, Bradbury KR, Muldoon MA, Roffers PD. 2001. Delineation of capture zones for municipal wells in complex fractured carbonate rock. Hydrogeology Journal, 9:432-450.

Shaver RH, Ault CH, Ausich WI, Droste JB, Horowitz AS, James WC, Okla SM, Rexroad CB, Suchomel DM, Welch JR. 1978. The search for a Silurian reef model, Great Lakes area: Indiana Department of Natural Resources Geological Survey Special Report 15, 36 p.

Sherrill MG. 1975. Ground-water contamination in the Silurian dolomite of Door County, Wisconsin. Groundwater, 13:209-213.

Sherrill MG. 1978. Geology and ground water in Door County, Wisconsin, with emphasis on contamination potential in the Silurian dolomite. U.S. Geological Survey Water-Supply Paper 2047, 38 p.

Sherrill MG. 1979. Contamination potential in the Silurian Dolomite aquifer, eastern Wisconsin. USGS Water-Resources Investigations Report 78-108, 2 plates.

Skinner EL. Borman RG. 1973. U.S. Geological Survey Hydrologic Atlas 432, Water Resources of Wisconsin--Lake Michigan basin, 4 plates.

Smith CM and Hill VR. 2009. Dead-end hollow-fiber ultrafiltration for recovery of diverse microbes from water. Applied and Environmental Microbiology, 75:5284-5289.

ED_004892A_00000077-00056

USEPA. 2009. National Water Quality Inventory: Report to Congress, 2004, Reporting Cycle United States Environmental Protection Agency publication 841-R-08-001.

US GAO. Information on the quality of water found at community water systems and private wells. United States General Accounting Office/RCED-97-123, June 1997.

USGS. 2010. The quality of our Nation's waters - Nutrients in the Nation's streams and groundwater, 1992-2004: US Geological Survey Circular 1350. Washington, DC: US Department of the Interior.

Wallender EK, Ailes EC, Yoder JS, Roberts VA, Brunkard JM. 2014. Contributing factors to disease outbreaks associated with untreated groundwater. Groundwater 52:886-897.

Weideman S. and Schultz AR. 1915. The underground and surface water supplies of Wisconsin. Wisconsin Geological and Natural History Survey Bulletin 35, 664 pages.

WI DNR. 2014. Nitrate in Drinking Water. Wisconsin Department of Natural Resources, Bureau of Drinking Water and Groundwater. PUB-DG-001 2014.

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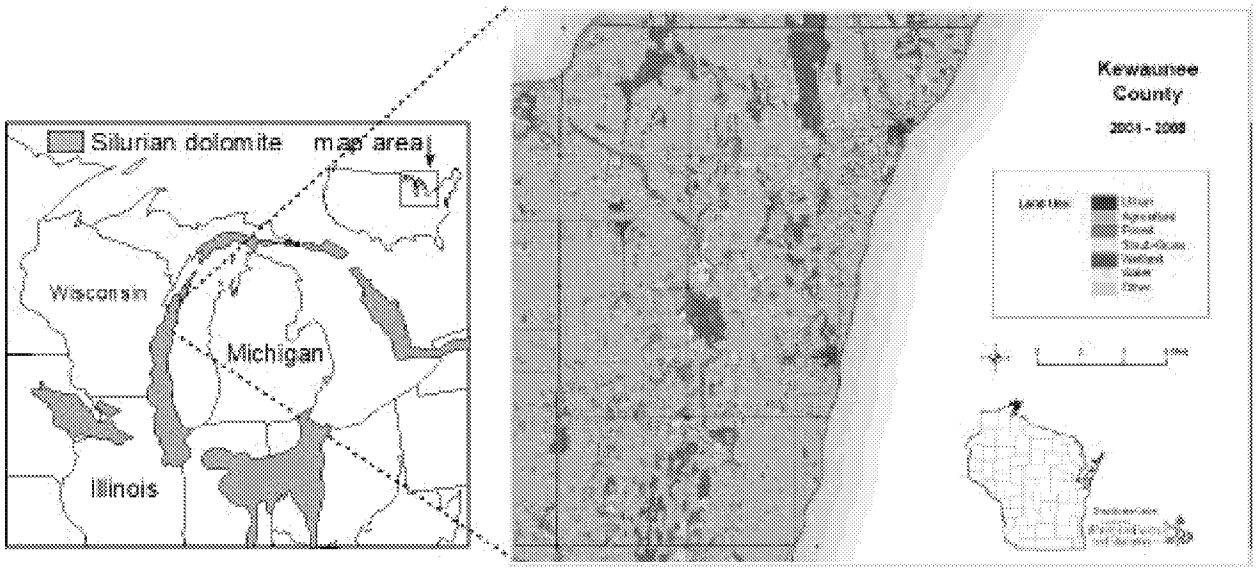
FIGURES



(A) (B)

Figure 1. Location of Kewaunee County including A) map of generalized Silurian subcrop shown as shaded area (modified from Shaver et al., 1978), and B) a map of land use within the county.

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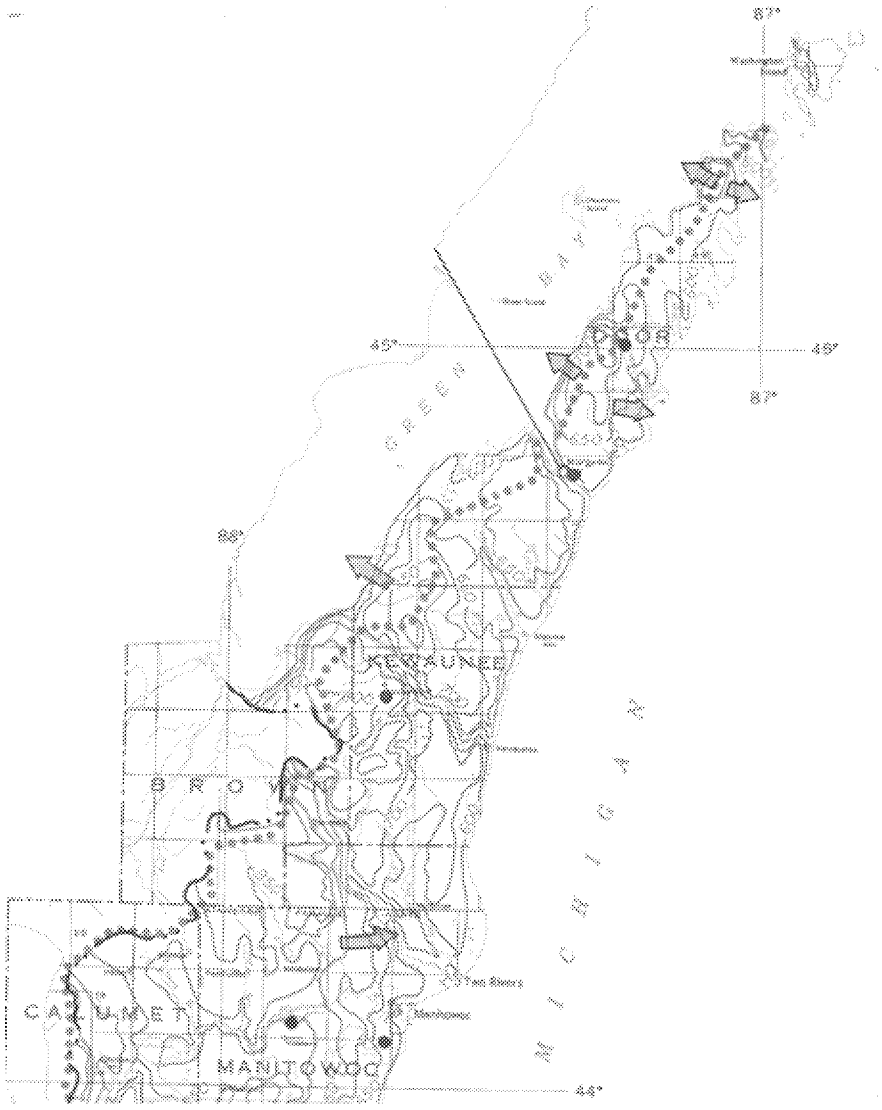

Figure 2. Portion of the regional water-table map taken from USGS HA432, Water Resources of the Lake Michigan Basin (Skinner and Borman, 1973) that includes Kewaunee County.

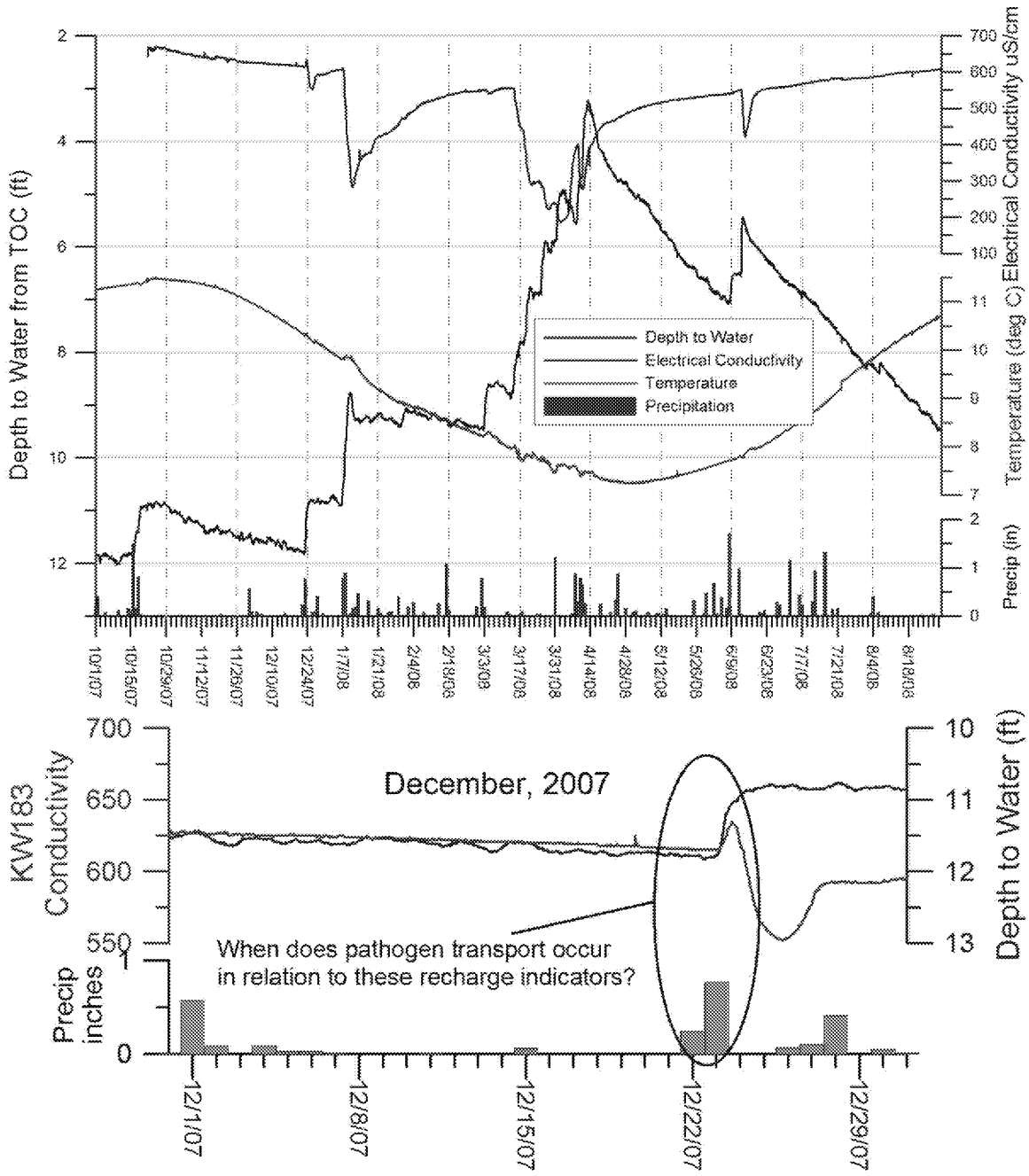

Figure 3. Variation in water level, fluid temperature and electrical conductivity as indicators of recharge in well KW183 along with the precipitation record (from Muldoon and Bradbury, 2010). The top graph indicates that there were five recharge events during the study period. The lower graph illustrates the details of the recharge event in December 2007.

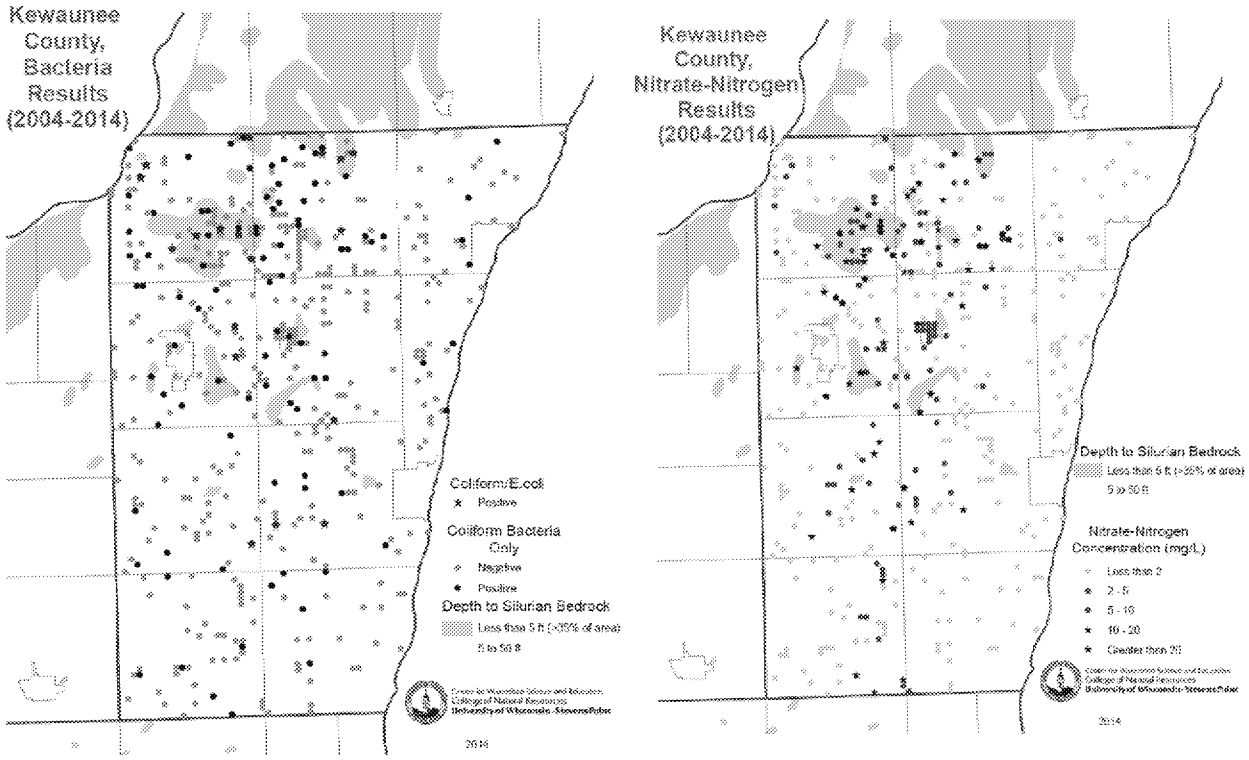

Figure 4. Maps of the bacteria (left) and nitrate-N (right) data for Kewaunee County that have been reported to the UW-Stevens Point Center for Watershed Science. Depths to bedrock in the underlying base map are based on Sherrill's map (1979).

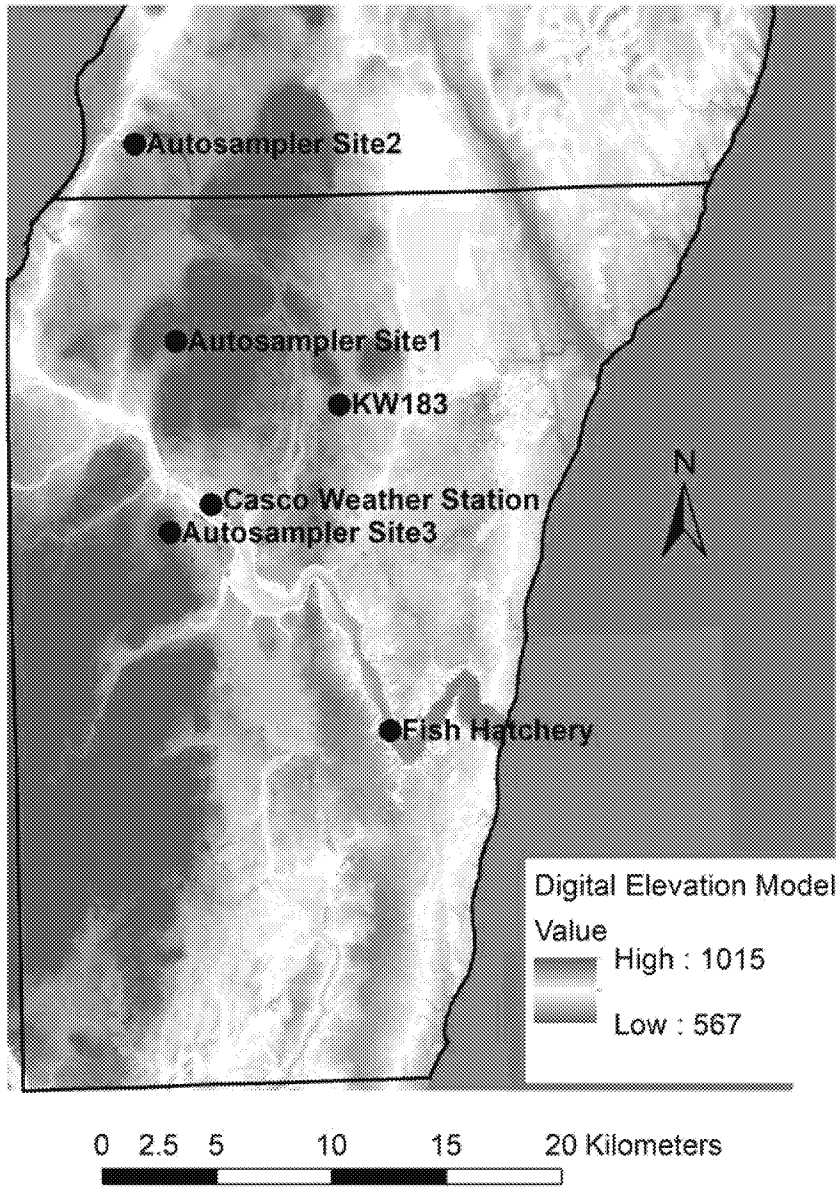

Figure 5. Map showing location of the non-pumping monitoring wells (KW183 and Fish Hatchery), automated samplers, and the weather station. The underlying map is a digital elevation model (DEM) of the study area showing land surface elevation in feet above Mean Sea Level.

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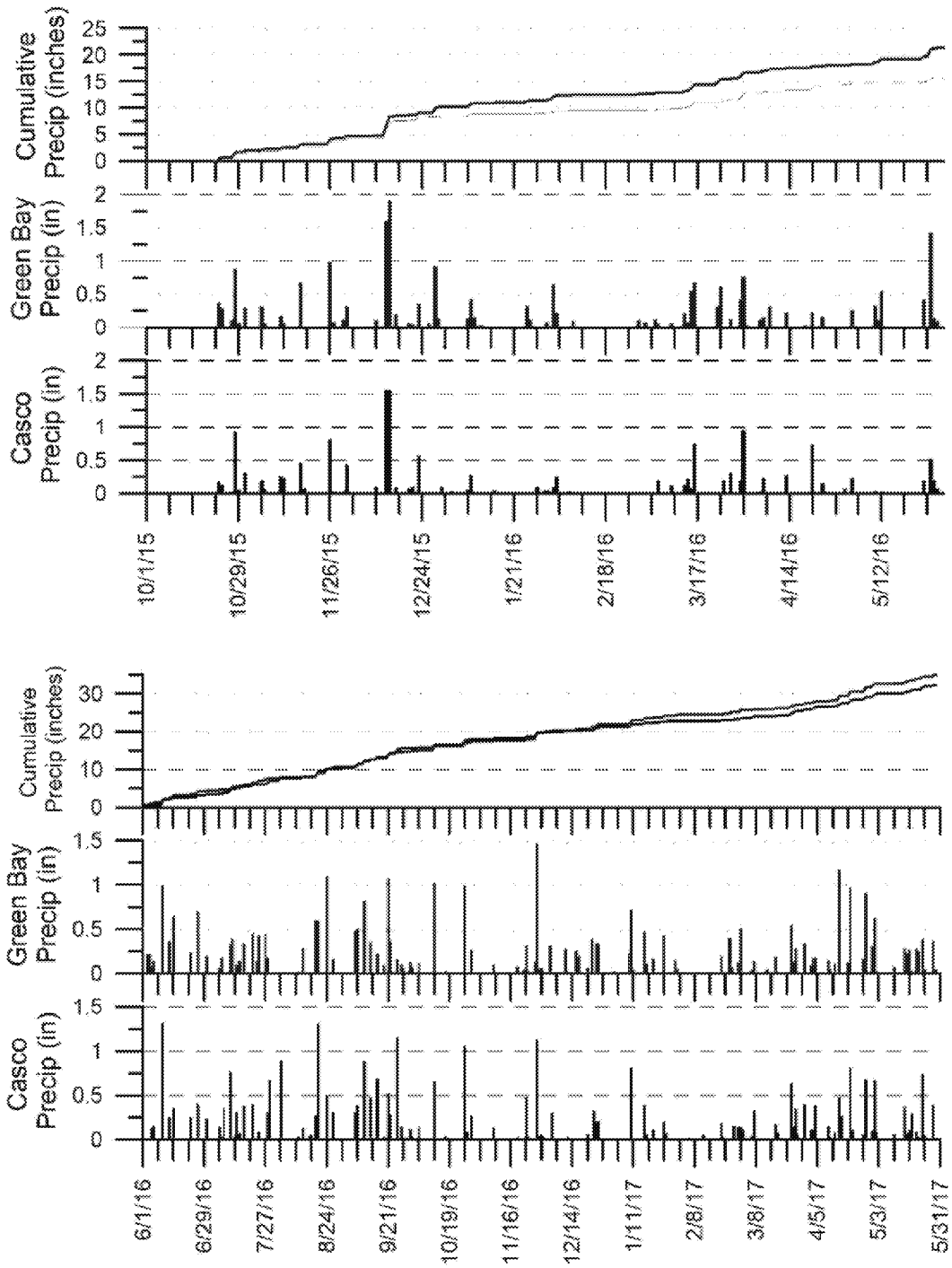

Figure 6. Comparison of the daily precipitation records from MSU's weather station in Casco, WI (black) and from the National Weather Service station in Green Bay (blue) for the periods Oct 22, 2015 to May 31, 2016 (top) and June 1, 2016 to May 29, 2017 (bottom). For both time periods, the Green Bay station records more total precipitation as the water-equivalence of winter snowfall is included in the record whereas the Casco station does not record snow precipitation.

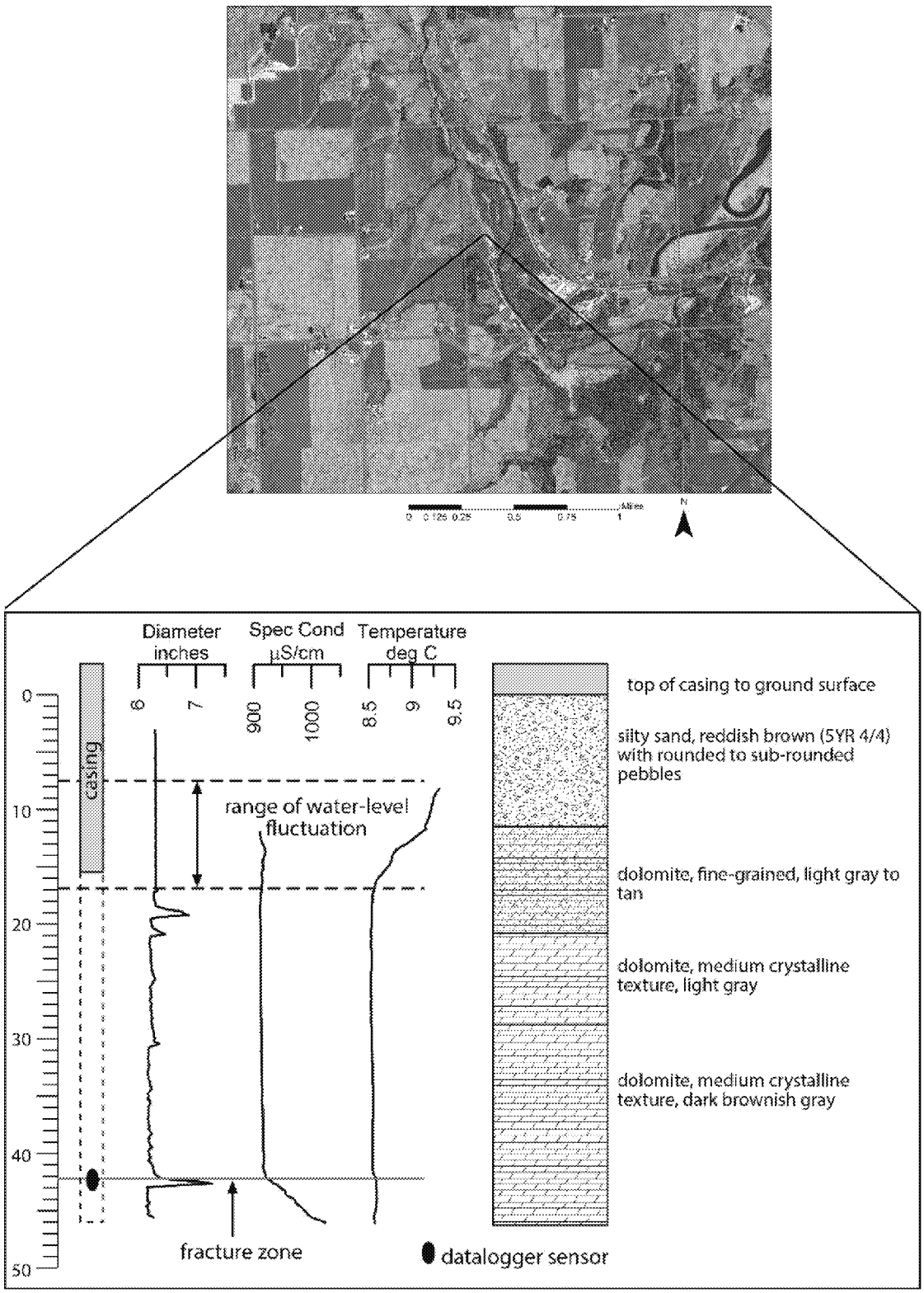

Figure 7 Air photo showing location of the Fish Hatchery well and surrounding land use. Lower diagram illustrates well construction, borehole logs, range of water-level fluctuation, sonde placement, and lithology.

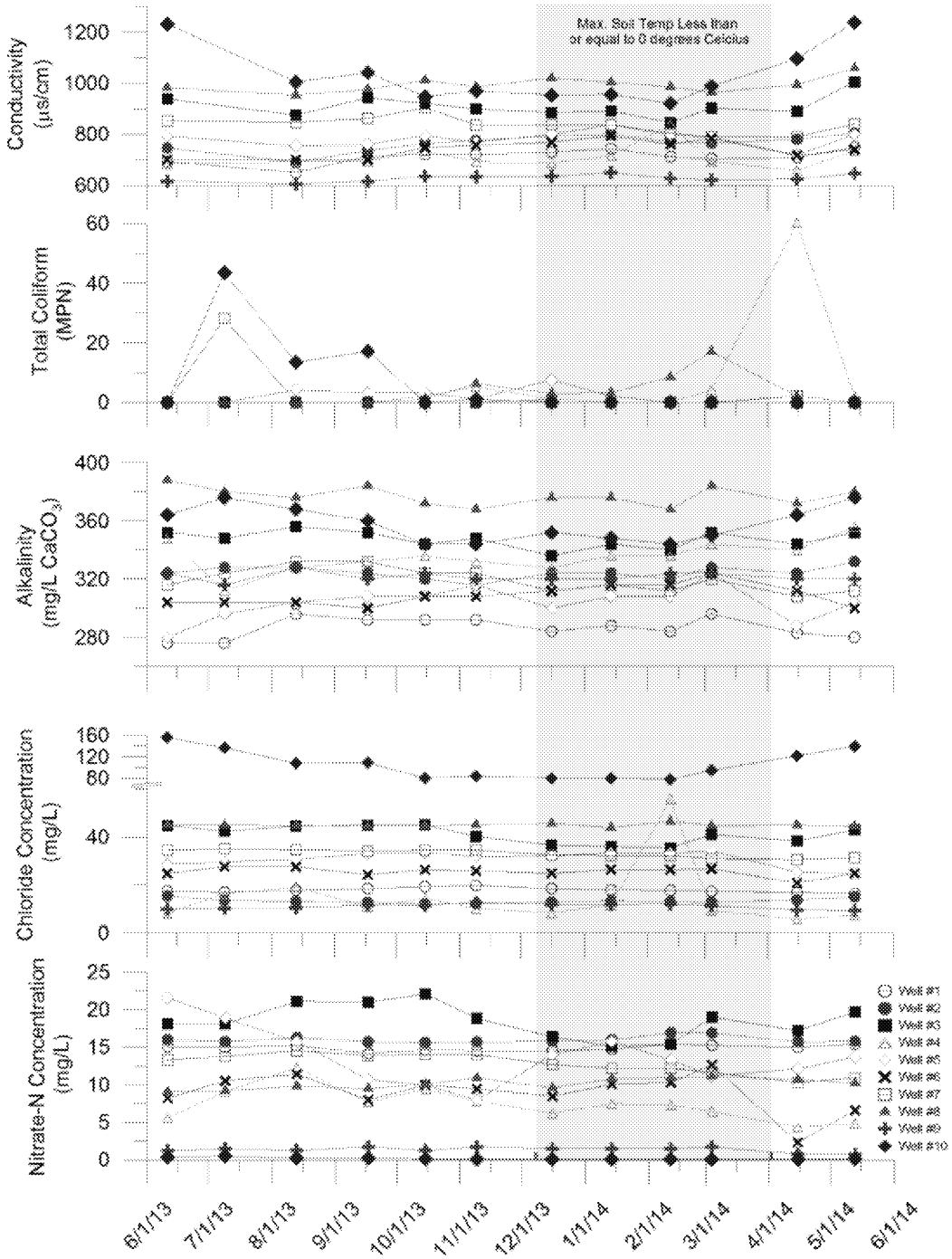

Figure 8. Monthly water-quality results from ten domestic wells sampled for the Town of Lincoln study (Bonness and Masarik, 2014). Nitrate-N results are shown in the bottom graph.

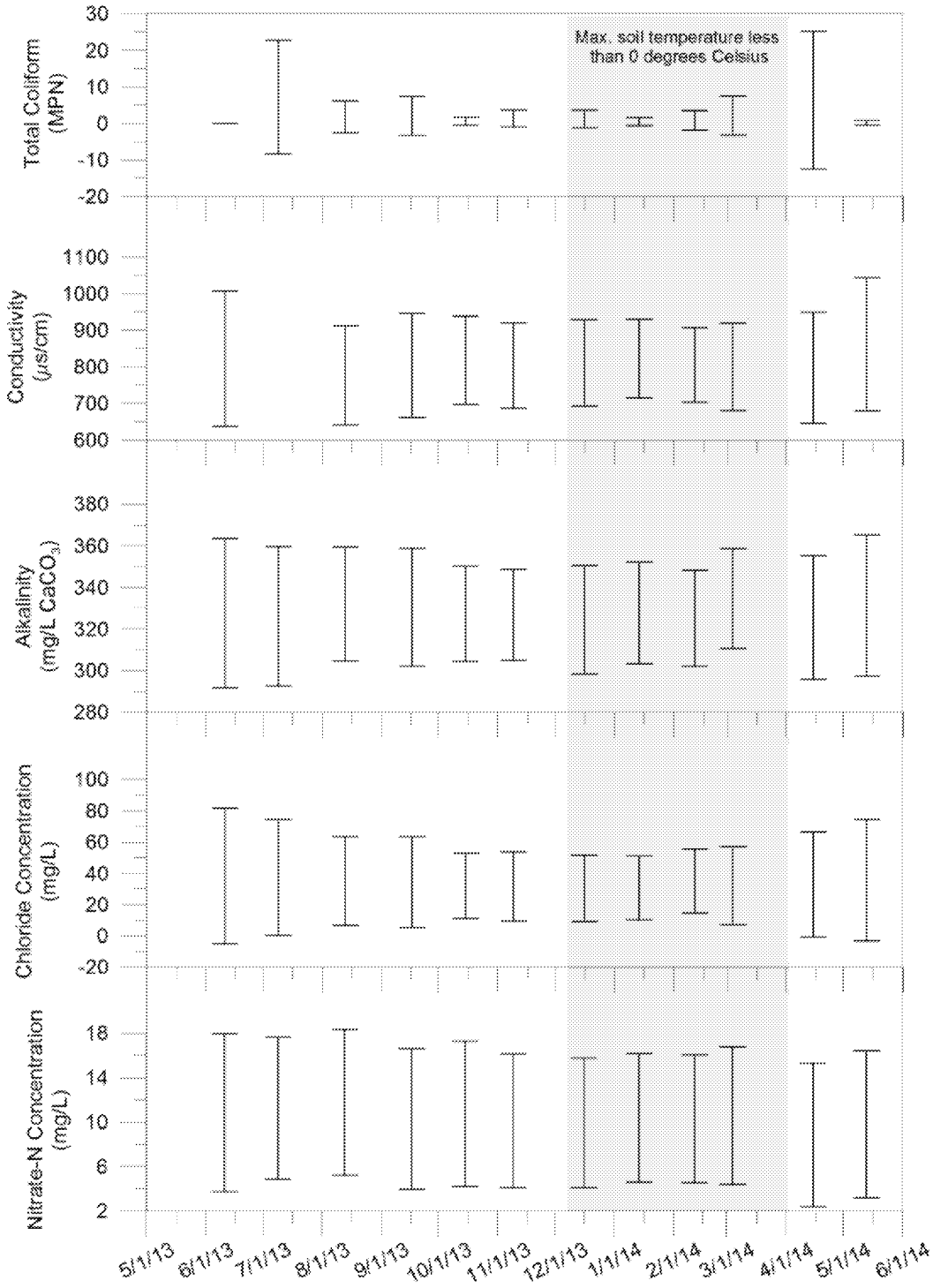

Figure 9. Graph illustrating variability of water-quality results from the Town of Lincoln study (Bonness and Masarik, 2014). The graphs illustrate the standard deviation from the average of all ten wells for each month sampled. Nitrate-N results have a high standard deviation throughout the year whereas the variability decreases during the frozen-ground months (shaded gray) for some of the other water-quality parameters.

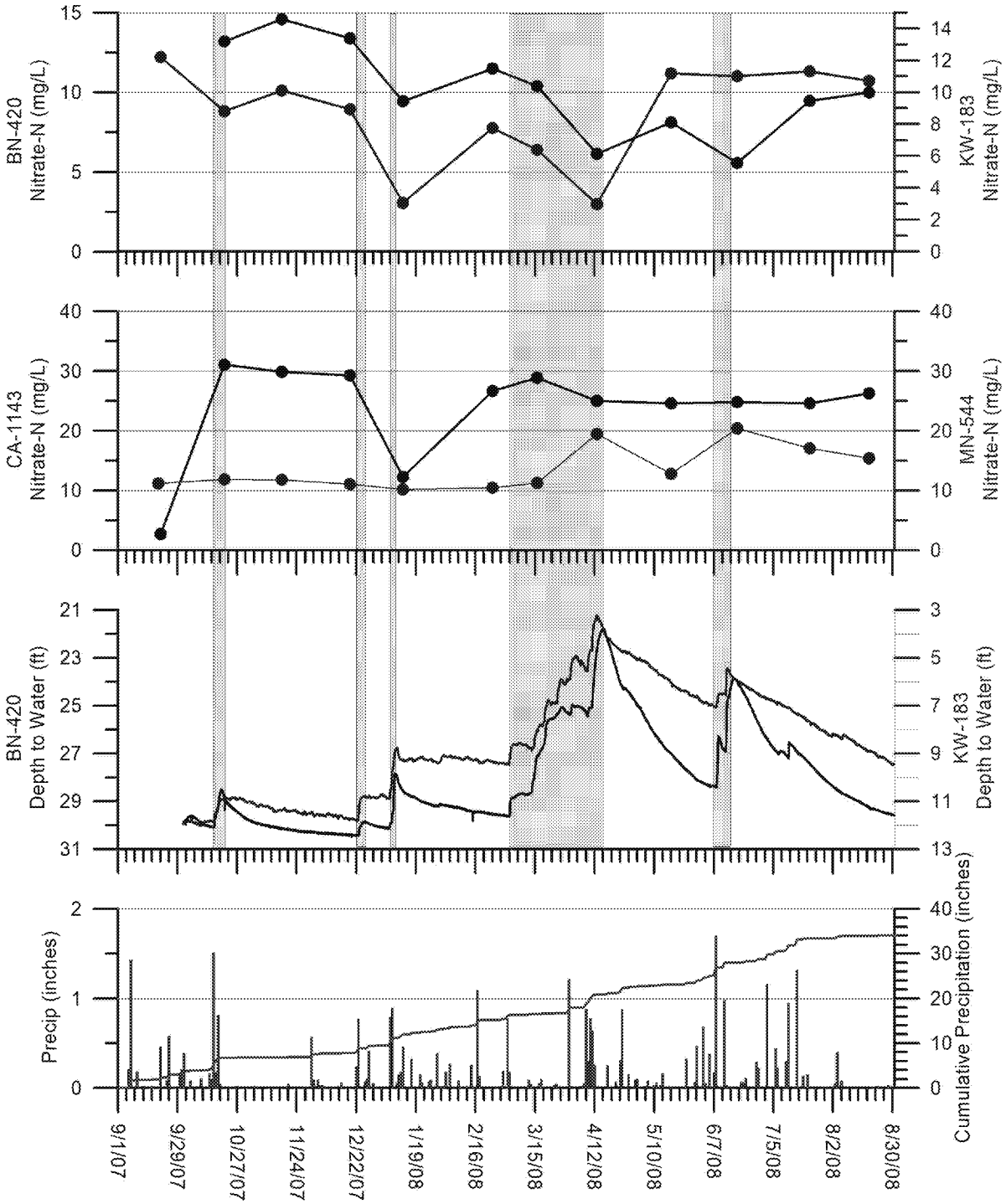

Figure 10. Nitrate data from the northeast Wisconsin recharge study (Muldoon and Bradbury, 2010). The top two graphs show monthly variation in nitrate-N concentrations. Bottom two graphs: water-level variations in the Brown and Kewaunee County monitoring wells and precipitation and cumulative precipitation from the NWS Green Bay weather station. The gray, shaded areas are periods of rising water levels during which recharge is occurring.

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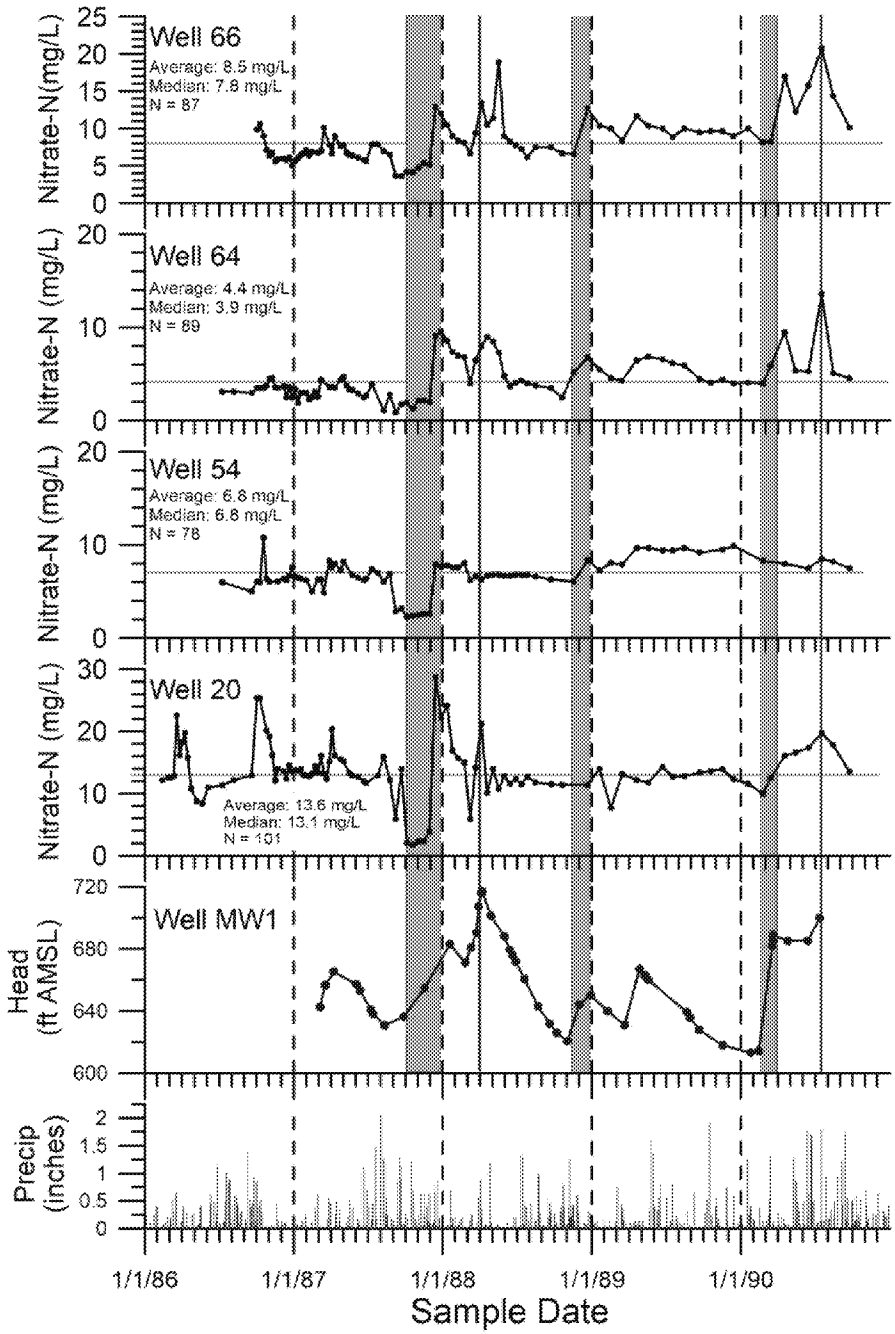

Figure 11. Sample nitrate data from the Northern Door Priority Watershed project (Bradbury and Muldoon, 1992). The top 4 graphs show variations in nitrate-N concentrations for four domestic wells over a 4-year time period. For each well the average nitrate-N concentration is also shown as a gray horizontal line. The bottom two graphs show hydraulic head from well MW1 at the Jarmen Road research site and precipitation at the Sturgeon Bay Experimental Farm. The red shaded areas highlight time periods where the nitrate-N concentrations in these wells varied in a similar way.

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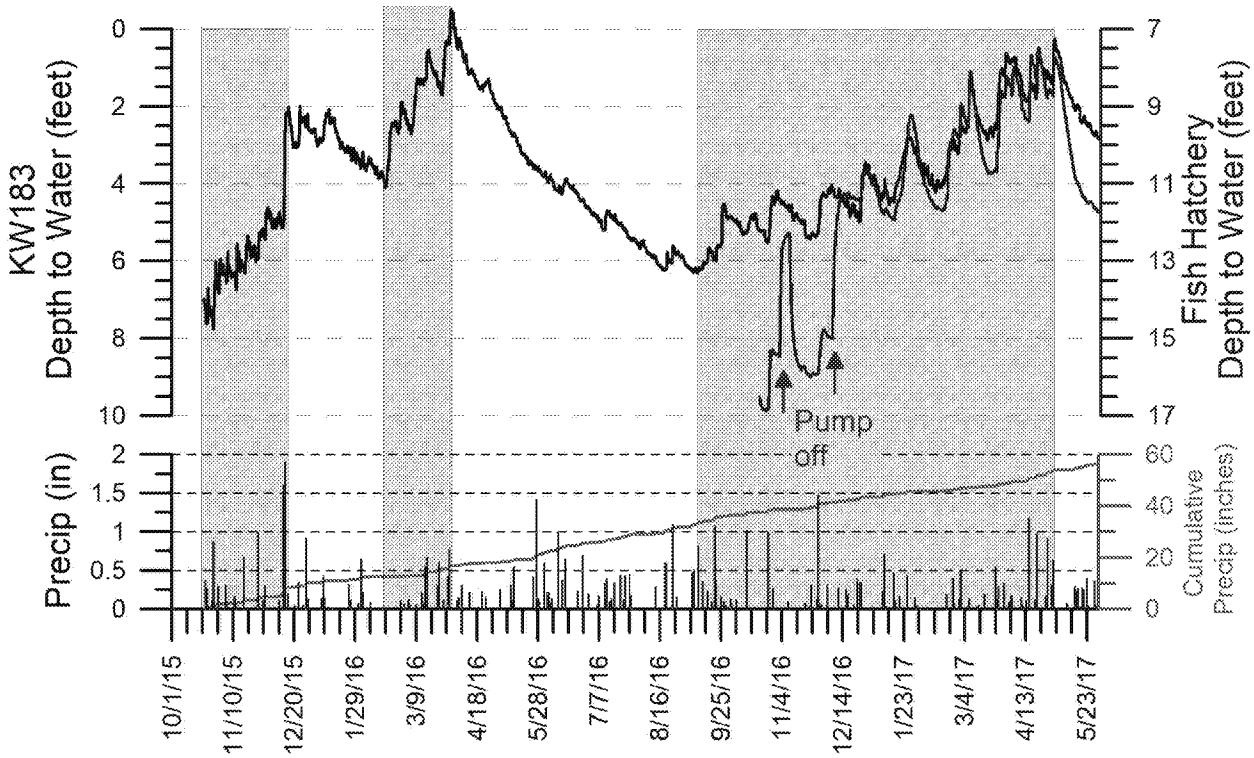

Figure 12. The top graph shows water-levels from both non-pumping sentinel wells from October 22, 2015 to May 29, 2017. The gray, shaded areas highlight recharge periods. The bottom graph illustrates precipitation data recorded at the NWS Green Bay weather station.

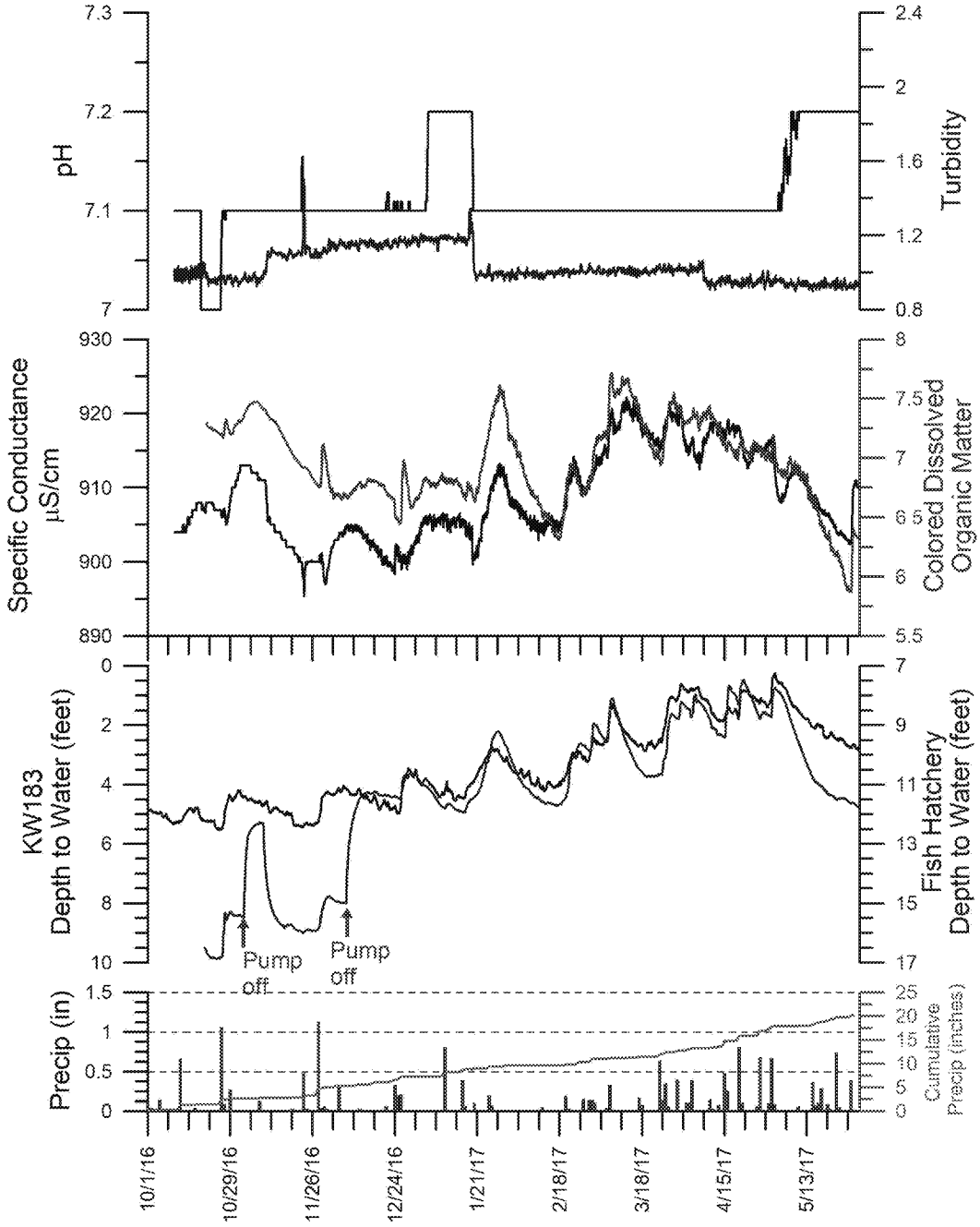

Figure 13. Water-quality data from the sonde installed in sentinel well KW183 (top two graphs) for the entire monitoring period. Water levels from both sentinel wells and precipitation data are shown in the bottom two graphs. While neither turbidity nor pH exhibited much variation over the monitoring period, comparison of water-level and water-quality data suggests that Specific Conductance and Colored Dissolved Organic matter (CDOM) are good indicators of groundwater recharge at this well.

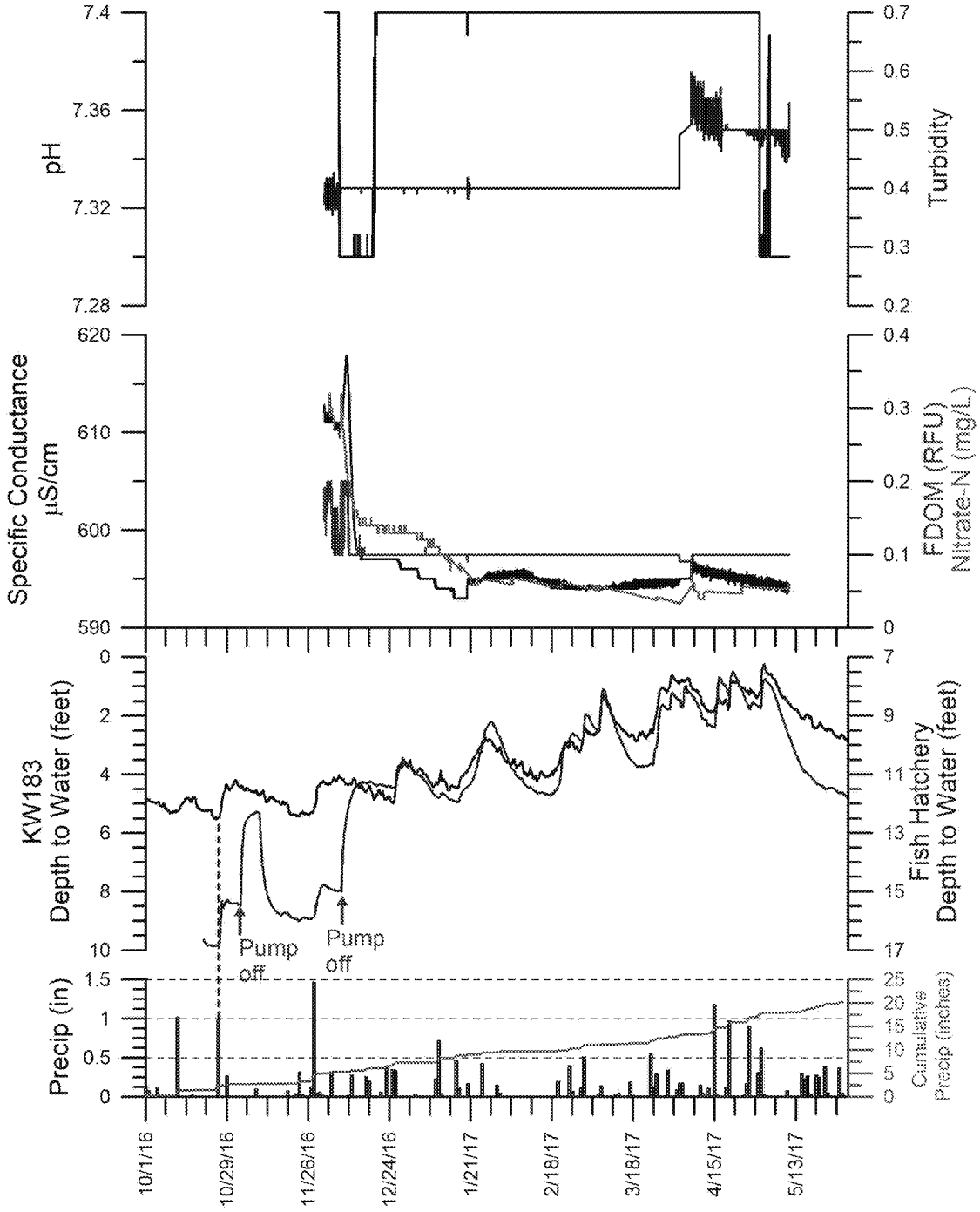

Figure 14. Water-quality data from the sonde installed in sentinel well at the Fish Hatchery (top two graphs) for the entire monitoring period. Water levels from both sentinel wells and precipitation data are shown in the bottom two graphs. None of the water-quality parameters exhibited much variation over the monitoring period.

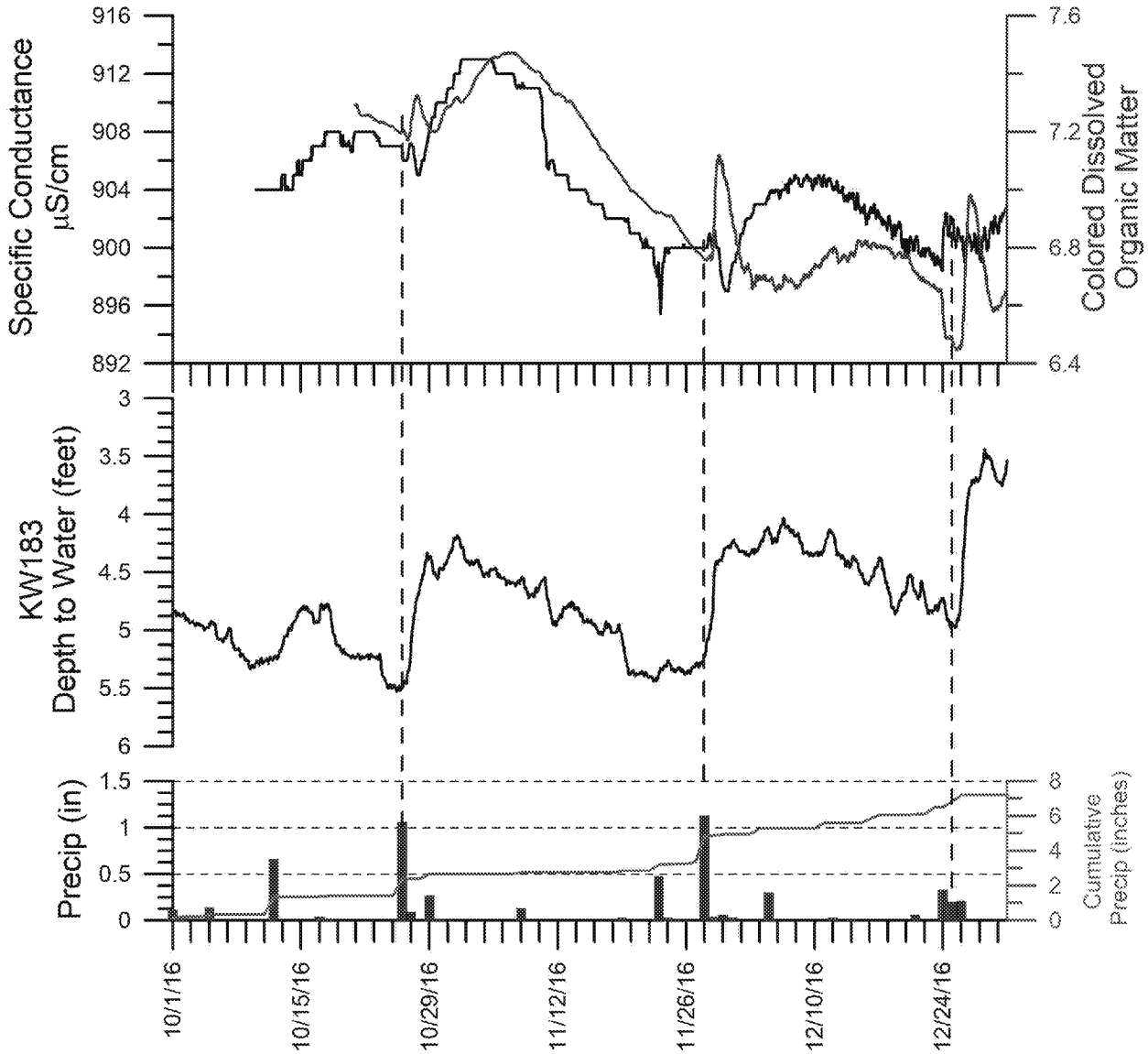

Figure 15. Detailed water-quality and recharge data for KW183 for the period October 10 to December 31, 2016. Note that recharge events (shown by dashed blue lines) in October and November are marked by sharp, but opposite, inflections in both specific conductance and CDOM. The December recharge event also produces a sharp inflection in CDOM.

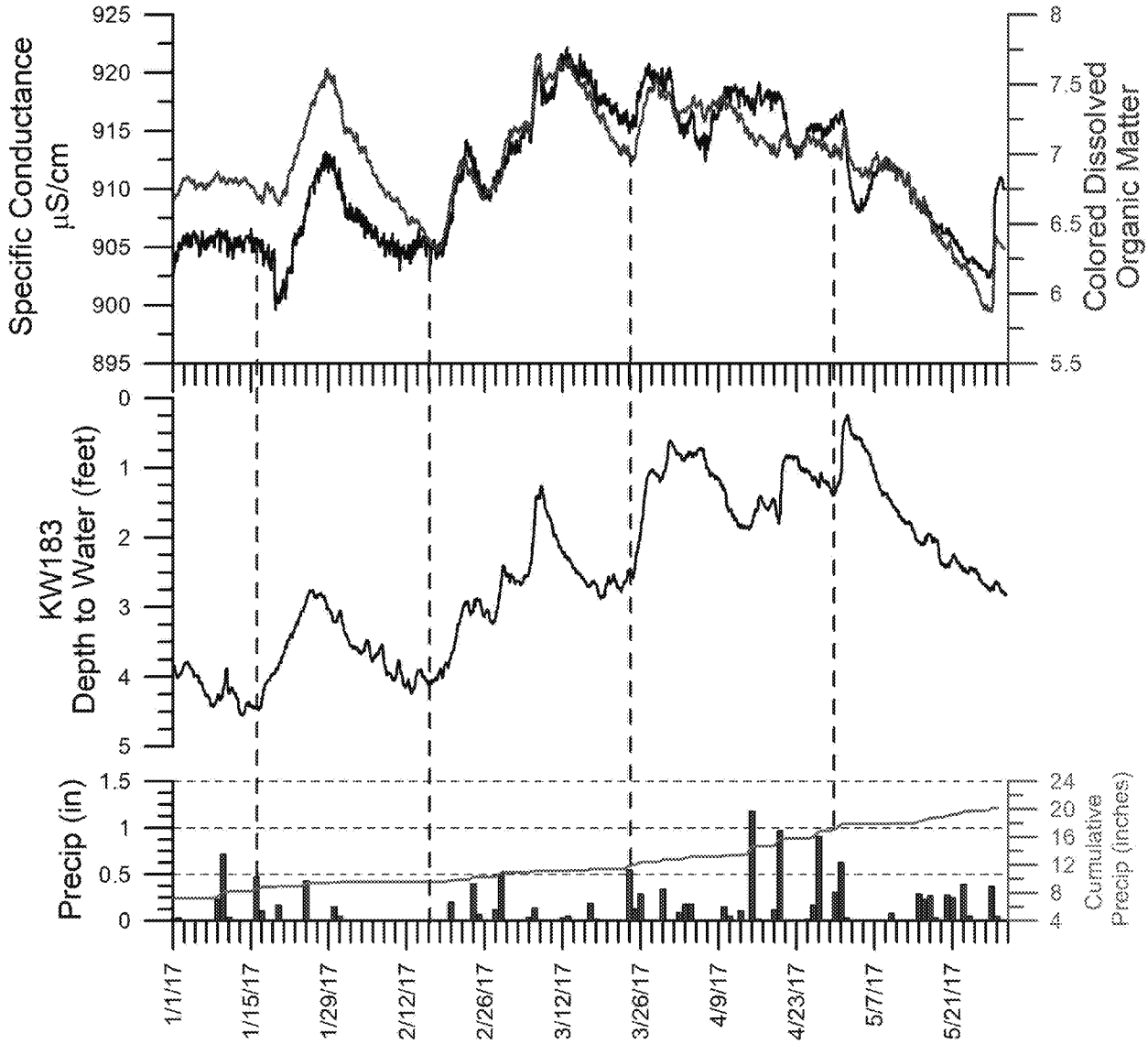

Figure 16. Detailed water-quality and recharge data for KW183 for the period January 1 to May 30, 2017. The recharge events through March are due to a mix of rain and snowmelt, while the April and May events are driven by rainfall. For all of the recharge events in this time period, there does not appear to be sharp and opposite inflections in specific conductance and CDOM. Rather these parameters seem to generally mimic the water-level variations.

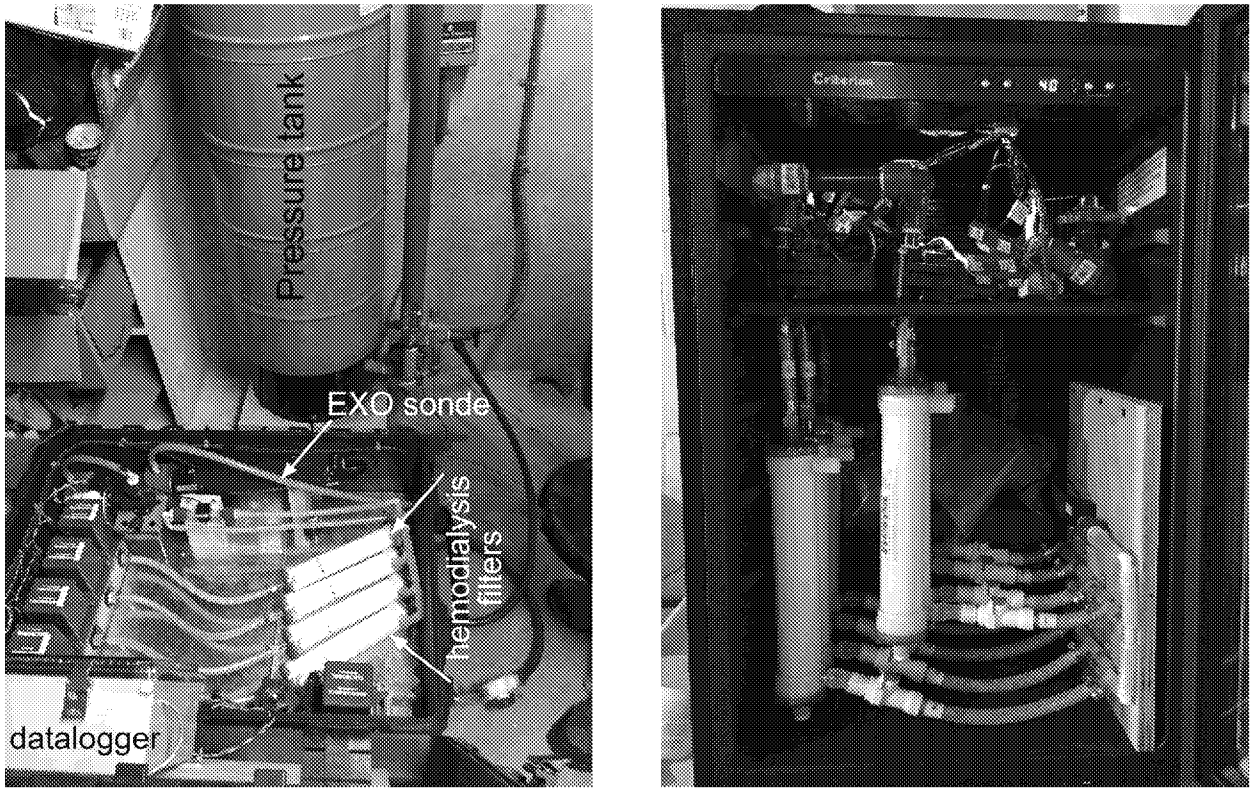

Figure 17. Photos of both automated sampler designs. Sampler 1 (on left) is labeled to show the location of the YSI EXO sonde containing the water-quality sensors, the datalogger and the four hemodialysis filters. Sampler 2 (on right) holds eight filters and is contained within a small refrigerator. The datalogger (not in the picture) sits on top of the refrigerator.

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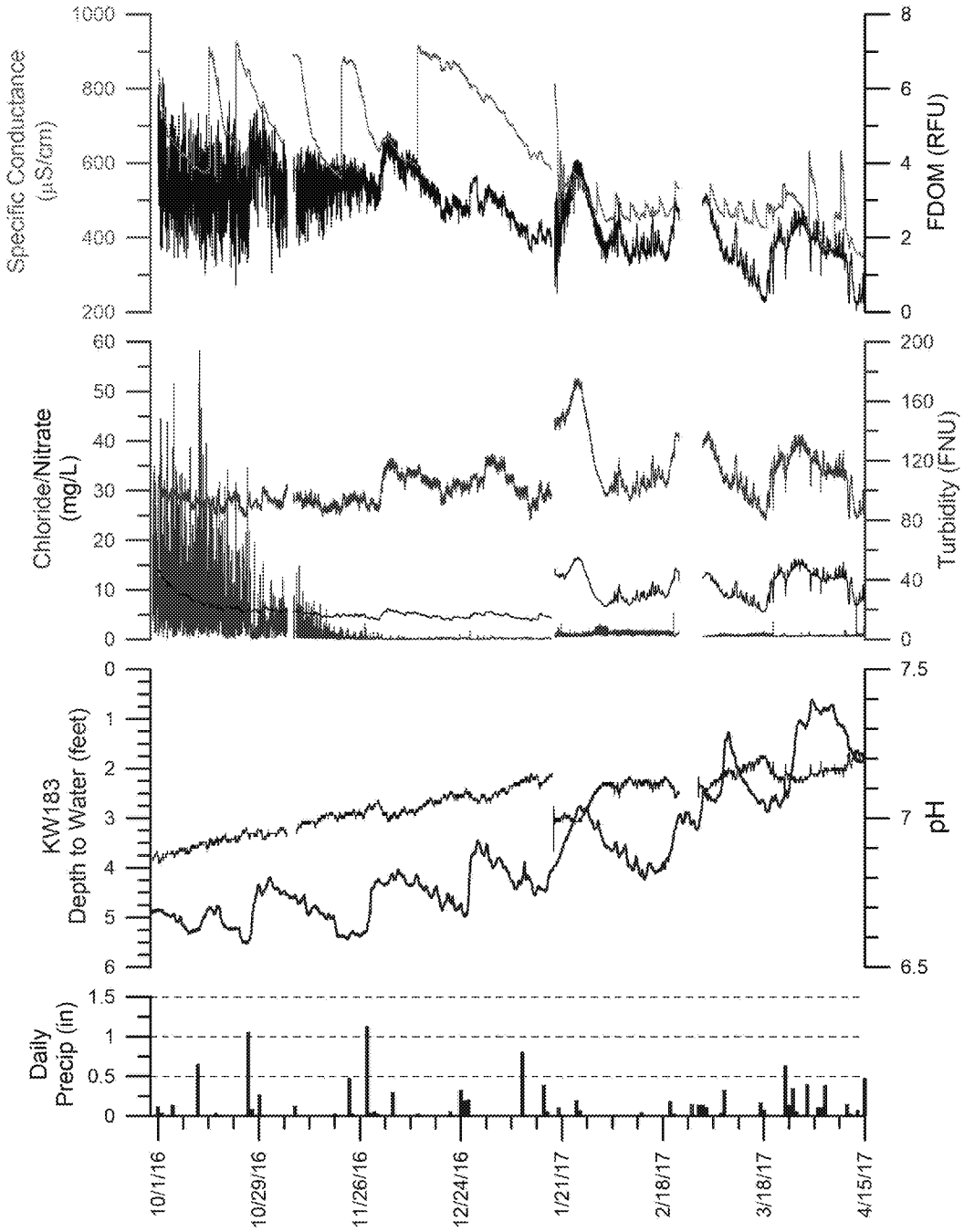

Figure 18. Variation in water-quality parameters at automated sampler Site 1 for the period September 29, 2016 to April 15, 2017.

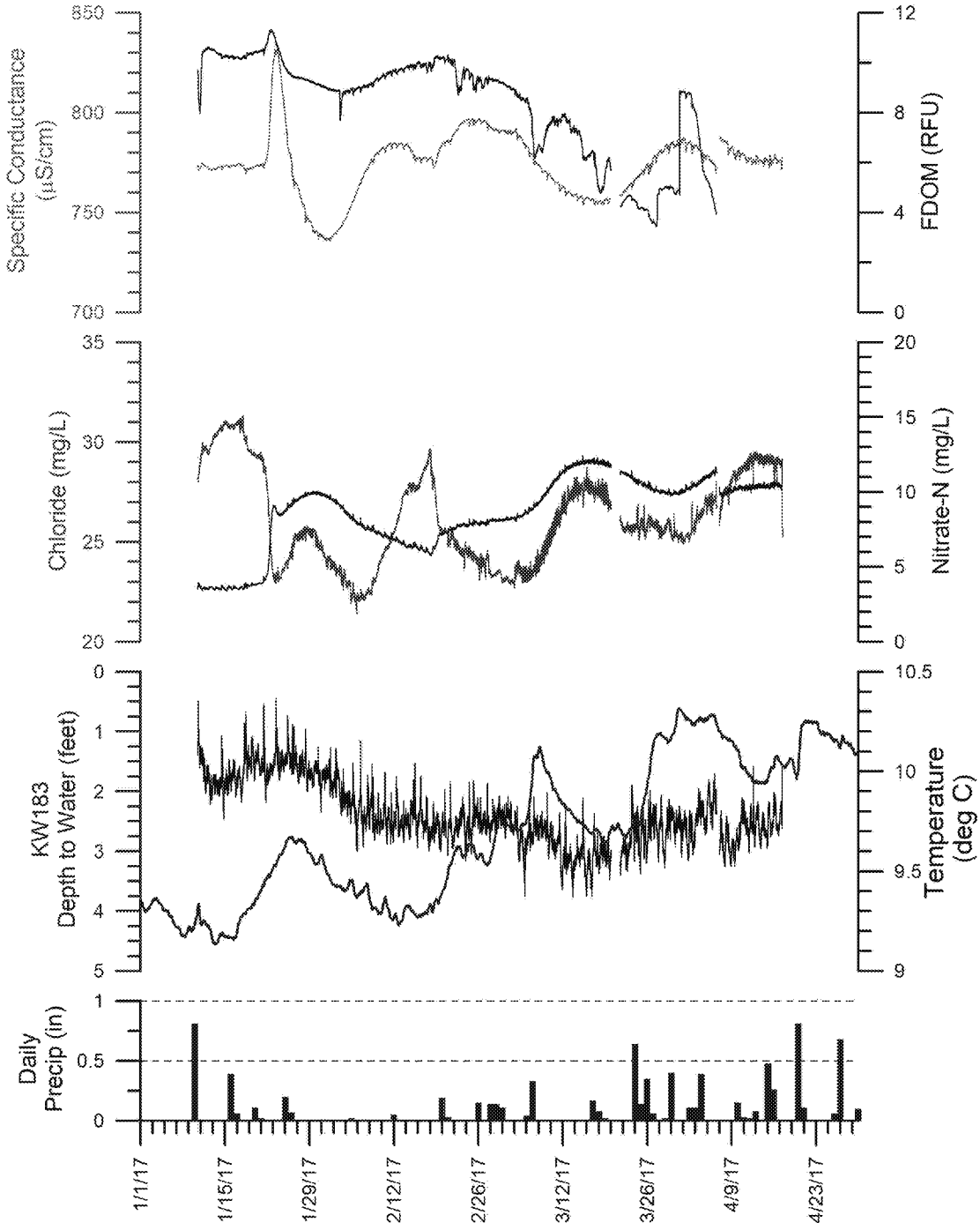

Figure 19. Variation in water-quality parameters at automated sampler Site 2 for the entire monitoring period (January 11 to April 17, 2017)

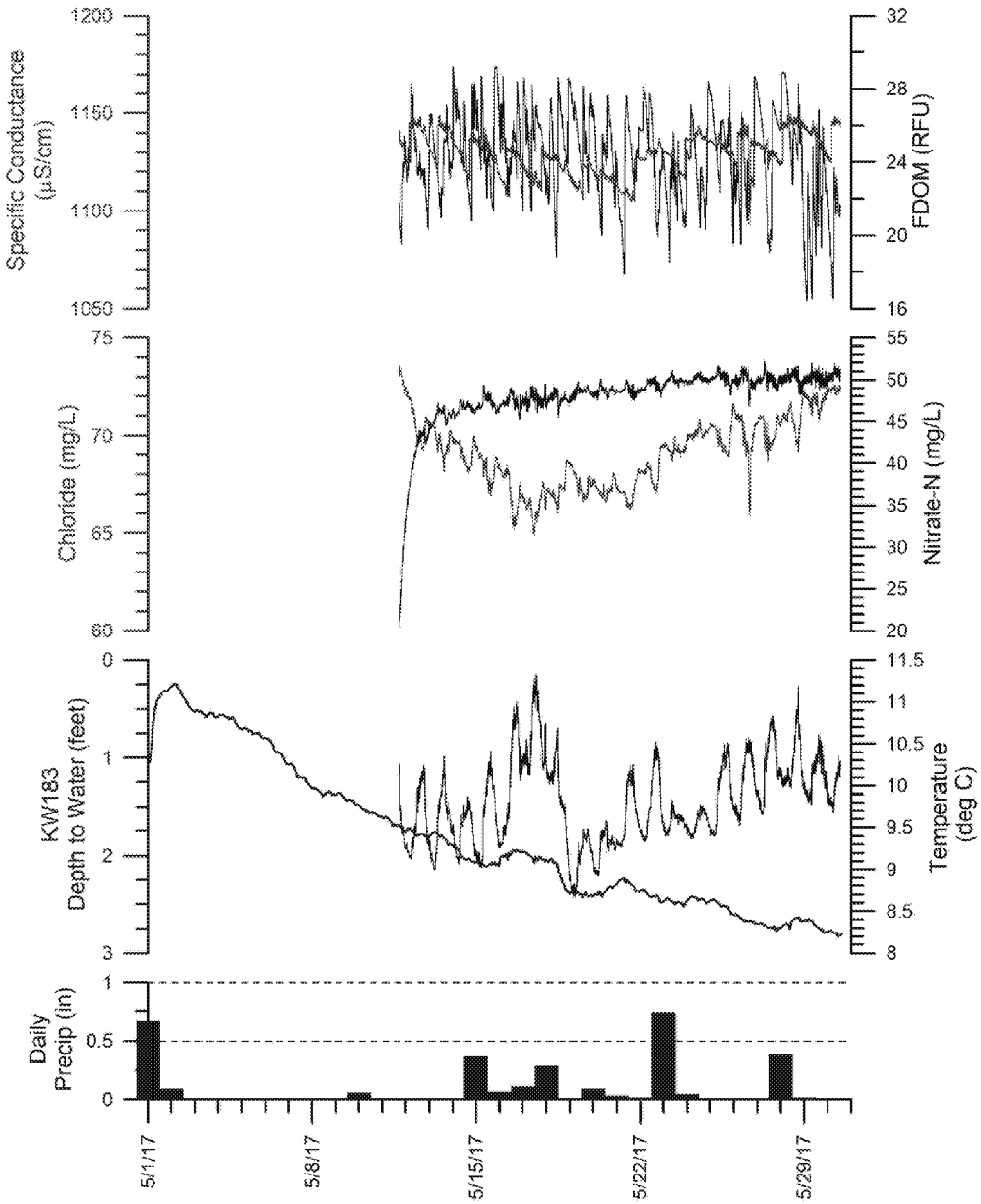

Figure 20. Variation in water-quality parameters at automated sampler Site 3 for the entire monitoring period (May 11 to 30, 2017)

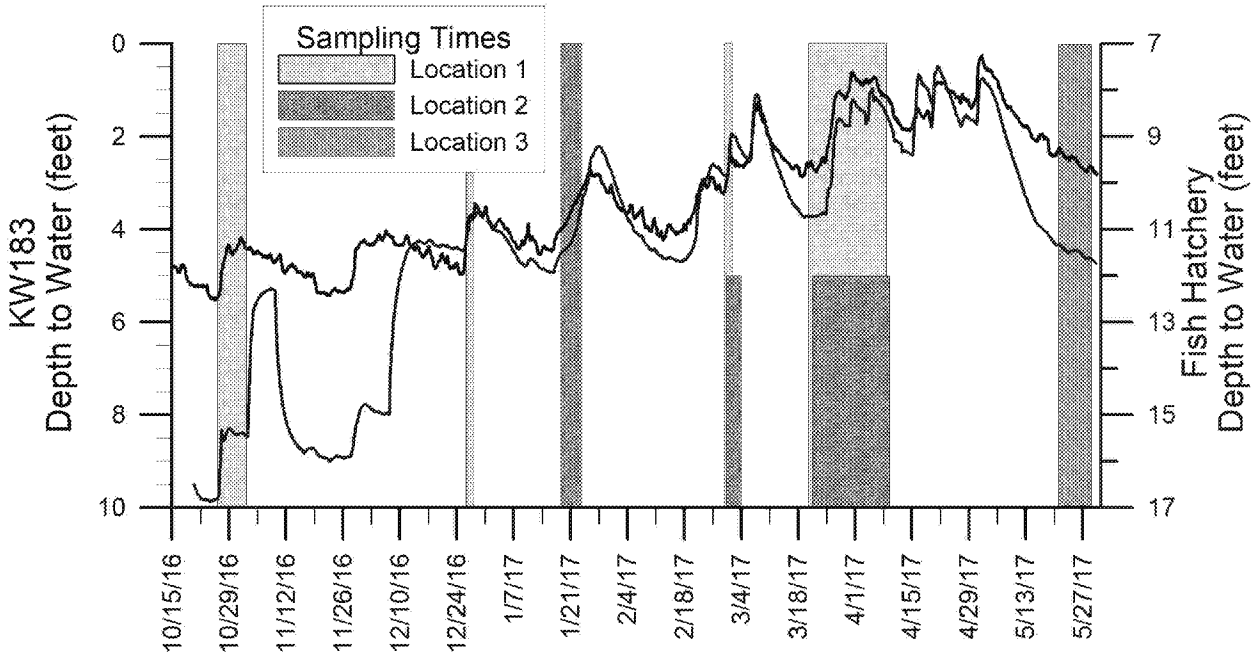

Figure 21. Graph showing sampling events for the automated samplers in relation to the water-level variations at the non-pumping sentinel wells.

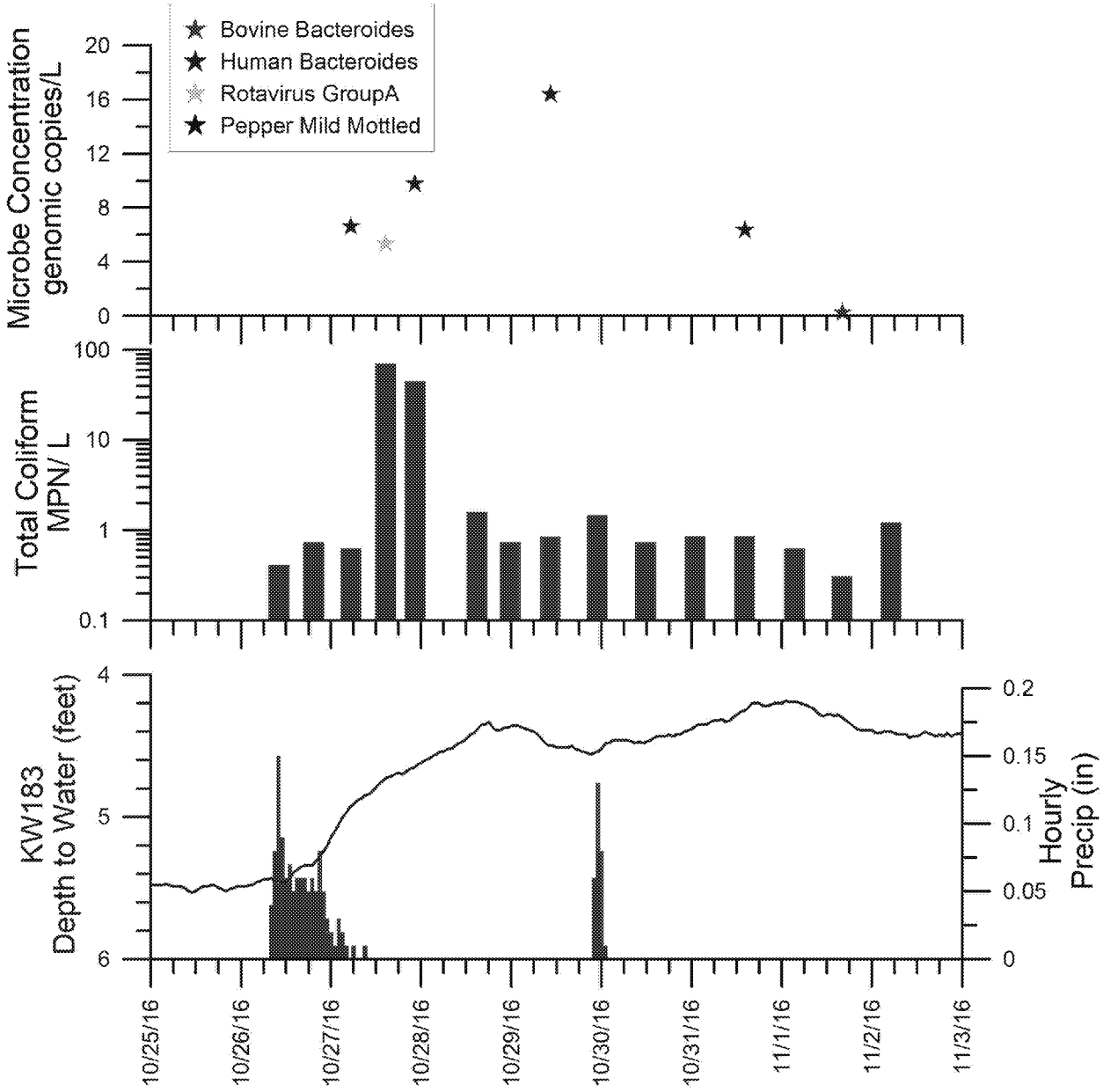

Figure 22. Results of microbial sampling at automated sampler Site 1 for the October 2016 recharge event. The bottom graph illustrates the hydrologic conditions: the water-level in sentinel KW183 and hourly precipitation from the Casco weather station. Total coliform concentrations are shown in the middle graph. Total coliform bacteria were detected in every sample collected; no *E. coli* bacteria were detected during this sampling event. The top graph illustrates detections of other microbes.

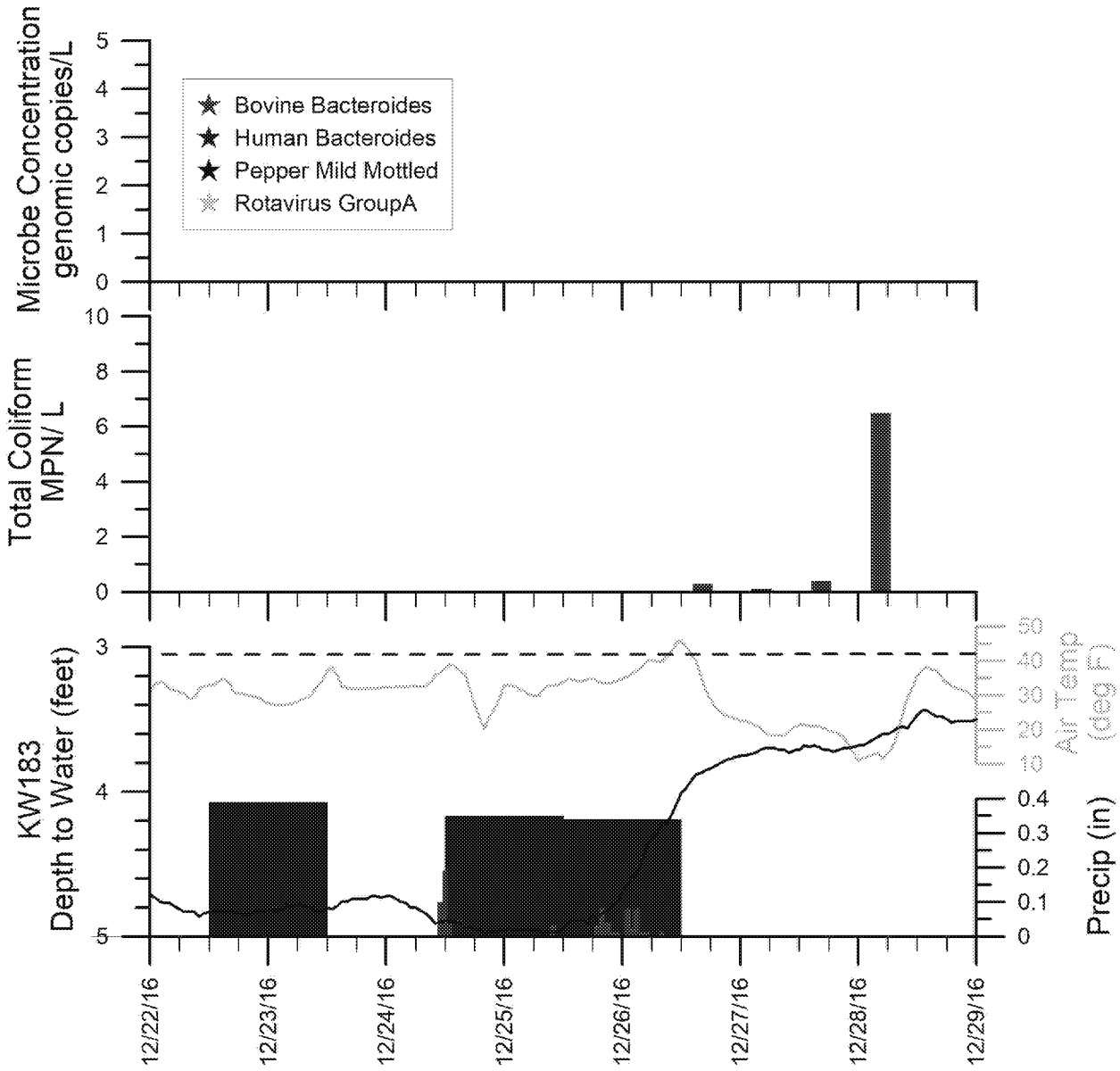

Figure 23. Results of microbial sampling at automated sampler Site 1 for the December 2016 recharge event. The bottom graph illustrates the hydrologic conditions: the water-level in sentinel KW183 (black line), daily precipitation from the NWS Green Bay station which includes the water equivalence of snowfall (blue bars), hourly rainfall (red bars) and air temperature (green line) from the Casco weather station. Total coliform concentrations are shown in the middle graph. Total coliform bacteria were detected in every sample collected; no *E. coli* bacteria or other microbes were detected during this event.

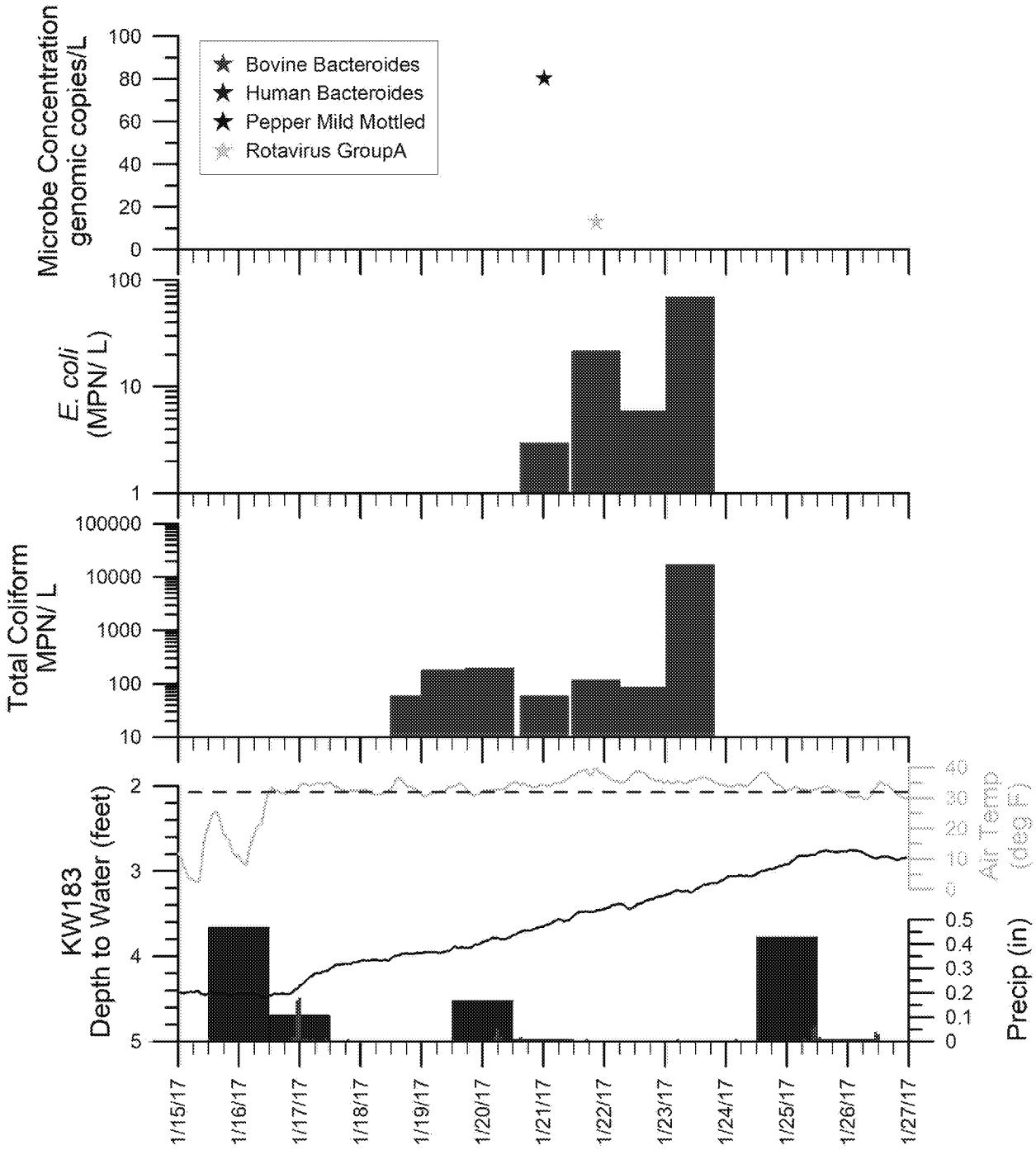

Figure 24. Results of microbial sampling at automated sampler Site 2 for the January 2017 recharge event. The bottom graph illustrates the hydrologic conditions: the water-level in sentinel KW183 (black line), daily precipitation from the NWS Green Bay station which includes the water equivalence of snowfall (blue bars), hourly rainfall (red bars) and air temperature (green line) from the Casco weather station. Total coliform and *E. coli* concentrations are shown in the middle two graphs. The top graph illustrates detections of other microbes.

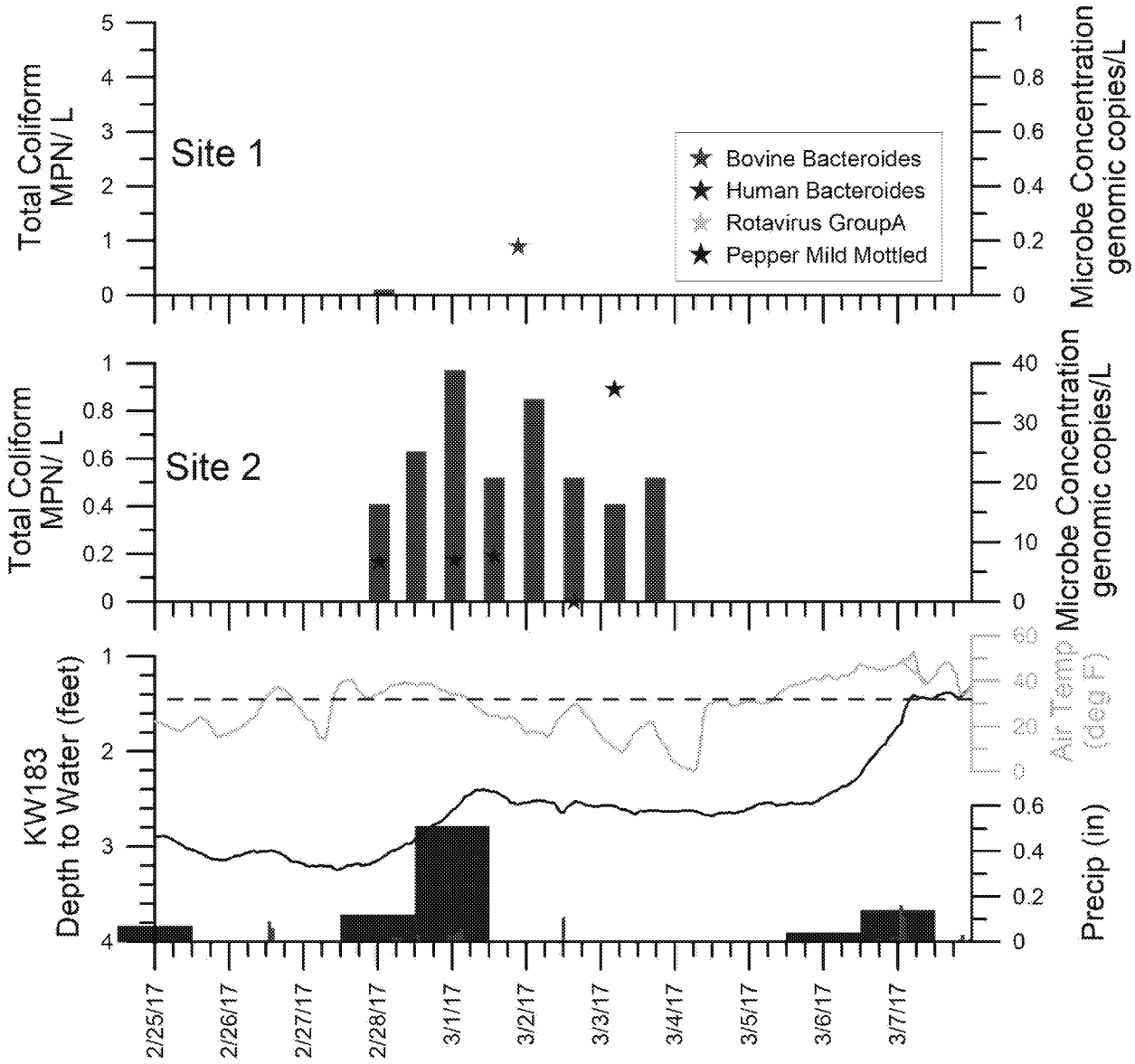

Figure 25. Results of microbial sampling at automated sampler Sites 1 and 2 for the late February-early March 2017 recharge event. The bottom graph illustrates the hydrologic conditions: the water-level in sentinel KW183, daily precipitation from the NWS Green Bay station which includes the water equivalence of snowfall (blue bars), hourly rainfall (red bars) and air temperature (green line) from the Casco weather station. The top graph shows both total coliform and other microbe detections for Site 1 while the middle graph shows both total coliform and other microbe detections for Site 2.

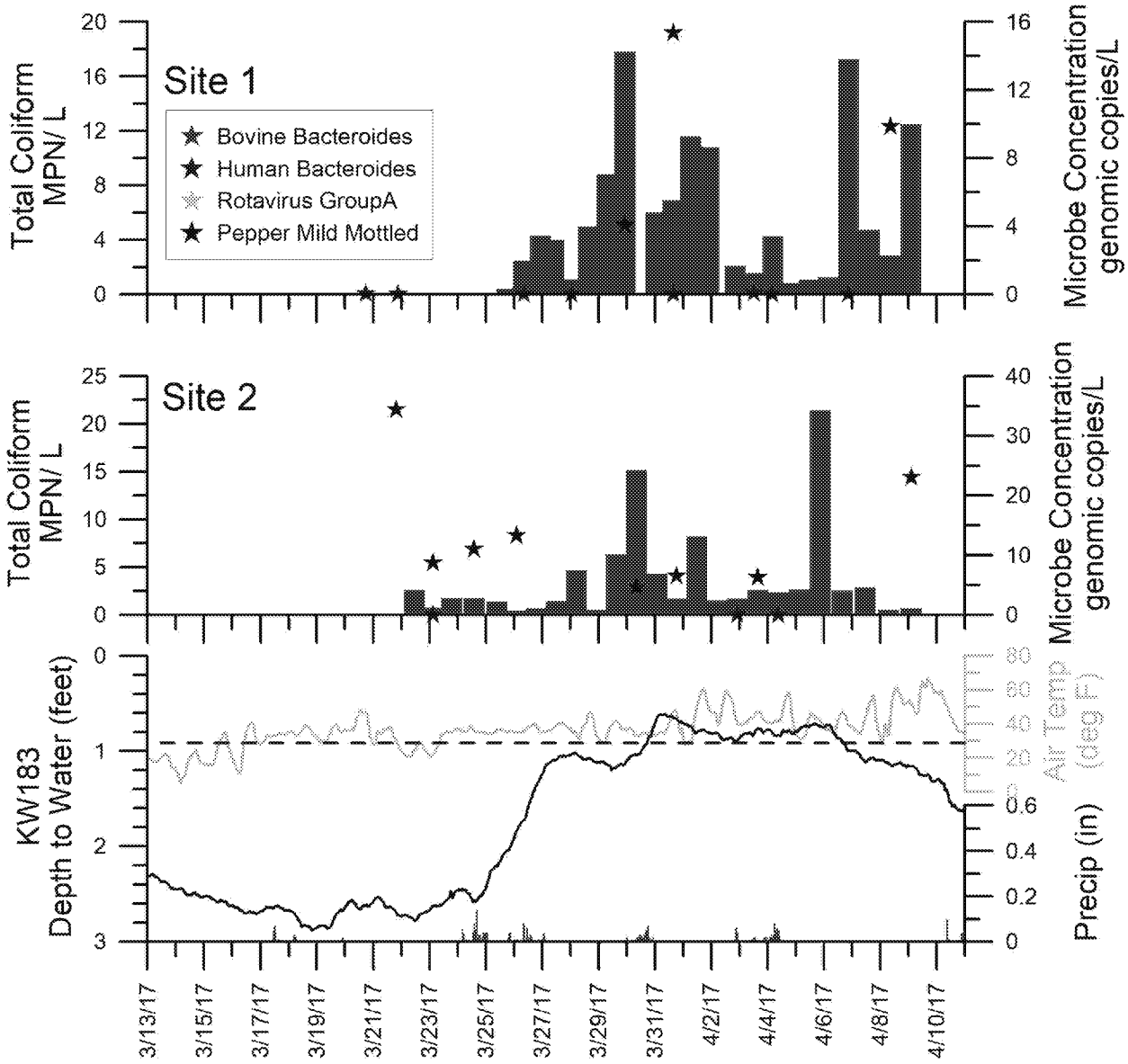

Figure 26. Results of microbial sampling at automated sampler Sites 1 and 2 for the major spring 2017 snowmelt recharge event. The bottom graph illustrates the hydrologic conditions: the water-level in sentinel KW183, daily precipitation from the NWS Green Bay station which includes the water equivalence of snowfall (blue bars), hourly rainfall (red bars) and air temperature (green line) from the Casco weather station. The top graph shows both total coliform and other microbe detections for Site 1 while the middle graph shows both total coliform and other microbe detections for Site 2.

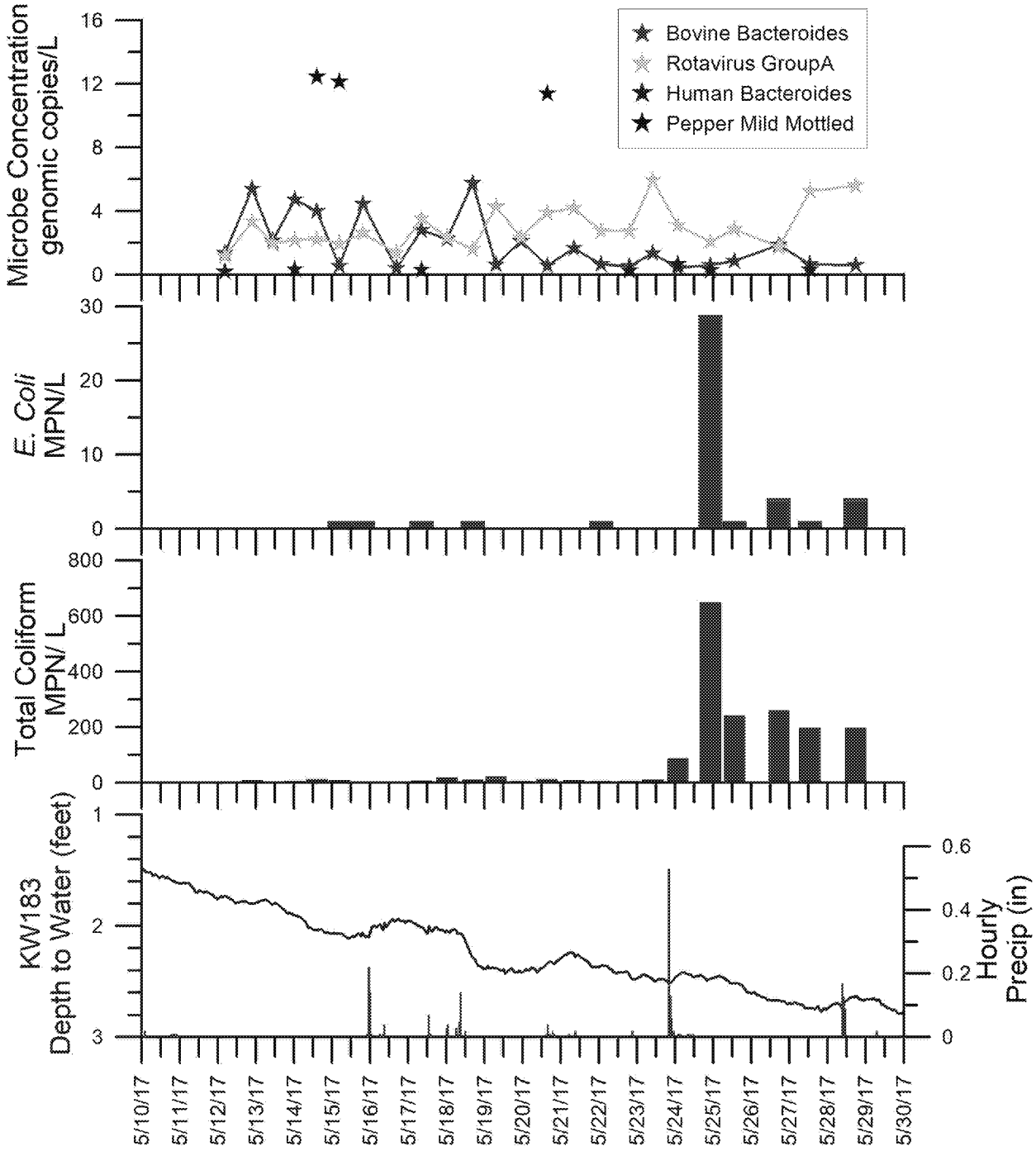

Figure 27. Results of microbial sampling at automated sampler Site 3 for a period in May 2017 with declining water levels. The bottom graph illustrates the hydrologic conditions: the water-level in sentinel KW183, hourly rainfall (red bars) from the Casco weather station. Total coliform and *E. coli* concentrations are shown in the middle two graphs. The top graph illustrates other microbe detections.

APPENDICES

Appendix I. Primer and probe sequences, concentrations, and references for qPCR assays.

Microorganism (gene target)	Primer sequences	Primer conc. (nM)	Probe sequence	Probe conc. (nM)	Primer/probe reference
Adenovirus group A (hexon)	GGACGCCTCGGAGTACCTGA	300	TGCGTTTTGTGCCCGTGGAT	50	Kuo et al. 2009
	GCTTRAAACTGGGACCRCG	300			
Adenovirus group B (hexon)	GGACGCCTCGGAGTACCTGA	500	ACCCACGATGTGACCACCGA	50	Kuo et al. 2009
	ATGTCAAAGAAHGTGCTGGCC	500			
Adenovirus groups C,D,F (hexon)	GGACGCCTCGGAGTACCTGA	500	CACCGATACGTACTTCAGCCTGGGT	50	Kuo et al. 2009
	CGCCGCGGATGTCAAAGTA	500			
Bacteroidales-like Cow M2 (DHIG domain protein)	CGGCCAAATACTCCTGATCGT	500	AGGCACCTATGTCCTTTACCTCATCAACTACAGACA	50	Shanks et al. 2008
	GCTTGTTGCGTTCCTTGAGATAAT	500			
Bacteroidales-like Cow M3 (HD super family hydrolase)	CCTCTAATGGAAAATGGATGGTATCT	500	TTATGCATTGAGCATCGAGGCC	50	Shanks et al. 2008
	CCATACTTCGCCTGCTAATACCTT	500			
Bacteroidales-like Hum M2 (Glycosyl hydrolase family 92)	CGTCAGGTTTGTTTCGGTATTG	500	TATCGAAAATCTCACGGATTAACTCTTGTGTACGC	50	Shanks et al. 2009
	TCATCACGTAACTTATTTATATGCATTAGC	500			
Bovine adenovirus (hexon)	CRAGGGAATAYYTGTCTGAAAATC	500	TTCATCWCTGCCACWCAAAGCTTTTTT	50	Wong and Xagoraraki 2010
	AAGGATCTCTAAATTTYTCTCCAAGA	500			
Ruminant *Bacteroides* (16SrRNA)	ACAGCCCGCGATTGATACTGGTAA	500	ATGAGGTGGATGGAATTCGTGGTGT	50	Mieszkin et al. 2010
	CAATCGGAGTTCTTCGTGAT	500			
Bovine enterovirus (5' non-coding region)	GCCGTGAATGCTGCTAATCC	500	CGCACAATCCAGTGTTGCTACGTCGTAAC	50	Gibson and Schwab 2011
	GTAGTCTGTTCCGCCTCCACCT	500			
Bovine herpes virus-extraction positive (glycoprotein B)	TGTGGACCTAAACCTCACGGT	500	AGGACCGCGAGTTCTTGCCAC	100	Wang et al. 2007
	GTAGTCGAGCAGACCCGTGTC	500			

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Bovine polyomavirus (VP1)	TGGCTTTCTGACTCAGCCAAA	500	ACCAACAGCAATTTAGAGGCCTTCCCAG	50	Wong and Xagoraraki 2011
	TCTCTTCCTGAGAGTCACAGACATG	500			
Bovine respiratory syncytial virus-extraction positive (β-actin)	GCAATGCTGCAGGACTAGGTATAAT	500	ACCAAGACTTGTATGATGCTGCCAAAGCA	100	Boxus et al. 2005
	ACACTGTAATTGATGACCCCATTCT	500			
Bovine viral diarrhea virus type 1 (5' non-coding region)	TAGCCATGCCCTTAGTAGGAC	200	CAGTGGTGAGTTCGTTGGATGGCT	50	Brooks et al. 2007
	GACGACTACCCTGTCCTCAGG	200			
Bovine viral diarrhea virus type 2 (5' non-coding region)	TAGCCATGCCCTTAGTAGGAC	200	AGGGGACTAGCGGTAGCAGTGAGTTC	50	Brooks et al. 2007
	GACGACTCCCCTGTACTCAGG	200			
Campylobacter jejuni (*mapA*)	CTGGTGGTTTTGAAGCAAAGATT	400	TTGAATTCCAACATCGCTAATGTATAAAAGCCCTTT	50	Best et al. 2003
	CAATACCAGTGTCTAAAGTGCGTTTAT	400			
Coronavirus (M protein)	ATTAGAACTGGAAGTTGGTGGA	500	ACAATAATACGTGGTCATCTTTACATGCAAG	50	Our lab
	TCACATAAGCTGGCAAATCT	500			
Cryptosporidium spp. (18S rRNA)	CATGGATAACCGTGGTAAT	300	CTAGAGCTAATACATGCGAAAAAA	50	Mary et al. 2013
	TACCCTACCGTCTAAAGCTG	300			
Cryptosporidium bovis (18S rRNA)	CATGGATAACCGTGGTAAT	300	CCCGACTTCTTGGAA	50	Mary et al. 2013
	TACCCTACCGTCTAAAGCTG	300			
Cryptosporidium hominis (18S rRNA)	CATGGATAACCGTGGTAAT	300	ATCACAATTAATGT	50	Mary et al. 2013
	TACCCTACCGTCTAAAGCTG	300			
Cryptosporidium parvum (18S rRNA)	CATGGATAACCGTGGTAAT	300	ATCACATTAAATGT	50	Mary et al. 2013
	TACCCTACCGTCTAAAGCTG	300			
Enterohemorrhagic *E. coli* (*eae*)	GTAAGTTACACTATAAAAGCACCGTCG	500	AAATGGACATAGCATCAGCATAATAGGCTTGCT	50	Ibekwe et al. 2004
	TCTGTGTGGATGGTAATAAATTTTTG	500			
Enterohemorrhagic *E. coli* (*stx 1*)	ACATTGTCTGGTGACAGTAGC	500	ATCAGTCGTACGGGGATGCAGATAAAT	50	Derzelle et al. 2011
	CGACATTAAATCCAGATAAGAAGTAGT	500			
Enterohemorrhagic *E. coli* (*stx 2*)	ATGACAACGGACAGCAGTTAT	500	ATGCAAATCAGTCGTCACTCACTGG	50	Derzelle et al. 2011
	CTGAACTCCATTAACGCCAGATA	500			

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Human enterovirus (5' non-coding region)	CCTCCGGCCCCTGAATG	300	CGGAACCGACTACTTTGGGTGTCCGT	50	De Leon et al. 1989 Monpoeho et al. 2000
	ACCGGATGGCCAATCCAA	300			
Giardia lamblia group B (*β-giardin*)	GGCCCTCAAGAGCCTGAAC	500	CTCGAGACAGGCATC	100	Baque et al. 2011
	GGGCGATCGTCTCCTTCTC	500			
Hepatitis G – RT inhibition control (5' non-coding region)	CGGCCAAAAGGTGGTGGATG	500	AGGTCCCTCTGGCGCTTGTGGCGAG	100	Schlueter et al. 1996
	CGACGAGCCTGACGTCGGG	500			
Human *Bacteroides* (16S rRNA)	ATCATGAGTTCACATGTCCG	500	CTAATGGAACGCATCCC	50	Green et al. 2014
	CTTCCTCTCAGAACCCCTATCC	500			
Lambda gDNA - PCR inhibition control (genomic DNA: chromosome I)	AGACGAATGCCAGGTCATCTGAAACAG	500	CGTCAACGGCATCCACGAAGGCGACAGA	100	Rutledge et al. 2010
	CTTTTGCTCTGCGATGCTGATACCG	500			
Norovirus genogroup I (ORF1-ORP2 junction)	GCCATGTTCCGITGGATG	400	TGTGGACAGGAGATCGCAATCTC	50	Jothikumar et al. 2005
	TCCTTAGACGCCATCATCAT	400			
Norovirus genogroup II (ORF1-ORP2 junction)	CARGARBCNATGTTYAGRTGGATGAG	400	TGGGAGGGCGATCGCAATCT	50	Kageyama et al. 2003
	TCGACGCCATCTTCATTCACA	400			
Pepper mild mottle virus (replication-associated gene)	GAGTGGTTTGACCTTAACGTTTGA	500	CCTACCGAAGCAAATG + MGB	50	Rosario et al. 2009
	TTGTCGGTTGCAATGCAAGT	500			
Human polyomavirus (T-antigen region)	AGTCTTTAGGGTCTTCTACCTTT	250	TCATCACTGGCAAACAT + MGB	50	McQuaig et al. 2009
	GGTGCCAACCTATGGAACAG	250			
Rotavirus group A (*NSP3*)	TTGCCACCAATTCAGAATAC	300	ACAGTATAAGAGAGCACAAGTTAATGAAGACA	50	Zeng et al. 2008
	ATTTCGGACCATTTATAACC	300			
Rotavirus group A (*VP7*)	TGCCACACTGTTGTCAATATTA	300	GGTAAGCCGCTAGAAGCAGATTTGACAGTG	50	Chang et al. 1999
	TCCTCTGCTGTTGGGAAAAGTT	300			
Rotavirus group C (*VP6*)	GAAGCTGTATGTGATGATGA	300	CAACGTTAATCGCATTAGCTTCA	50	Chang et al. 1999
	AGAATATATGAATTTCTATATTCAAA	300			
Salmonella sp. (*invA*)	TCGTCATTCCATTACCTACC	500	TCTGGTTGATTTCCTGATCGCA	50	Hoorfar et al. 2000
	AAACGTTGAAAAACTGAGGA	500			
Salmonella sp. (*ttr*)	CTCACCAGGAGATTACAACATGG	500	AAAGTCGGTCTCGCCGTCGGTG	50	Malorny et al. 2004
	AGCTCAGACCAAAAGTGACCATC	500			

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References for Appendix I

Baque RH, Gilliam AO, Robles LD, Jakubowski W, Slifko TR. 2011. A real-time RT-PCR method to detect viable Giardia lamblia cysts in environmental waters. Water Res 45:3175-84.

Best E, Powell EJ, Swift C, Grant KA, Frost JA. 2003. Applicability of a rapid duplex real-time PCR assay for speciation of Campylobacter jejuni and Campylobacter coli directly from culture plates. FEMS Microbiol Lett. 229:237-41.

Boxus M, Letellier C, Kerkhofs P. 2005. Real Time RT-PCR for the detection and quantitation of bovine respiratory syncytial virus. J Virol Methods. 125:125-30.

Chang KO, Nielsen PR, Ward LA, Saif LJ. 1999. Dual infection of gnotobiotic calves with bovine strains of group A and porcine-like group C rotaviruses influences pathogenesis of the group C rotavirus. 1999. J of Virol. 73(11): 9284-93.

Brooks JW, Key DW, Hattel AL, Hovingh EP, Peterson R, Shaw DP, Fisher JS. 2007. Failure to detect bovine viral diarrhea virus in necropsied farm-raised white-tailed deer (Odocoileus virginianus) in Pennsylvania. J Vet Diagn Invest. 19:298-300.

De Leon R, Shieh C, Baric RS, Sobsey MD. 1990. Detection of enteroviruses and hepatitis A virus in environmental samples by gene probes and polymerase chain reaction, p 833-853. *In* Proceedings of the 1990 Water Quality Technology Conference. American Water Works Association, Denver, CO

Derzelle S, Grine A, Madic J, de Garam CP, Vingadassalon N, Dilasser F, Jamet E, Auvray F. 2011. A quantitative PCR assay for the detection and quantification of Shiga toxin-producing Escherichia coli (STEC) in minced beef and dairy products. Int J Food Microbiol. 151:44-51.

Green HC, Haugland RA, Varma M, Millen HT, Borchardt MA, Field KG, Walters WA, Knight R, Sivaganesan M, Kelty CA, Shanks OC. 2014. Improved HF183 quantitative real-time PCR assay for characterization of human fecal pollution in ambient surface water samples. Appl Environ Microbiol. 80:3086-94.

Hoorfar J, Ahrens P, Rådström P. 2000. Automated 5' nuclease PCR assay for identification of Salmonella enterica. J Clin Microbiol. 38:3429-35.

ED_004892A_00000077-00088

Ibekwe AM, Watt PM, Shouse PJ, Grieve CM. 2004. Fate of Escherichia coli O157:H7 in irrigation water on soils and plants as validated by culture method and real-time PCR. Can J Microbiol. 50:1007-14.

Jothikumar N, Lowther JA, Henshilwood K, Lees DN, Hill VR, Vinjé J. 2005. Rapid and sensitive detection of noroviruses by using TaqMan-based one-step reverse transcription-PCR assays and application to naturally contaminated shellfish samples. Appl. Environ. Microbiol. 71:1870-75.
Kageyama, T. Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, Takeda N, Katayama K. 2003. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J. Clin. Micro. 41:1548-57.

Kuo DH, Simmons F, Xagoraraki I. 2009. A new set of PCR assays for the identification of multiple human adenovirus species in environmental samples. J Appl Microbiol. 107:1219-29.

Malorny B, Paccassoni E, Fach P, Bunge C, Martin A, Helmuth R. 2004. Diagnostic real-time PCR for detection of Salmonella in food. Appl Environ Microbiol. 70:7046-52.

Mary C, Chapey E, Dutoit E, Guyot K, Hasseine L, Jeddi F, Menotti J, Paraud C, Pomares C, Rabodonirina M, Rieux A, Derouin F. 2013. ANOFEL Cryptosporidium National Network. Multicentric evaluation of a new real-time PCR assay for quantification of Cryptosporidium spp. and identification of Cryptosporidium parvum and Cryptosporidium hominis. J Clin Microbiol. 51:2556-63.

McQuaig SM, Scott TM, Lukasik JO, Paul JH, Harwood VJ. 2009. Quantification of human polyomaviruses JC Virus and BK Virus by TaqMan quantitative PCR and comparison to other water quality indicators in water and fecal samples. Appl Environ Microbiol. 75:3379-88.

Mieszkin S, Yala JF, Joubrel R, Gourmelon M. 2010. Phylogenetic analysis of Bacteroidales 16S rRNA gene sequences from human and animal effluents and assessment of ruminant faecal pollution by real-time PCR. J Appl Microbiol. 108:974-84.

Monpoeho S, Dehée A, Mignotte B, Schwartzbrod L, Marechal V, Nicolas JC, Billaudel S, Férré V. 2000. Quantification of enterovirus RNA in sludge samples using single tube real-time RT-PCR. Biotechniques. 29:88-93.

Rosario K, Symonds EM, Sinigalliano C, Stewart J, Breitbart M. 2009. Pepper mild mottle virus as an indicator of fecal pollution. Appl Environ Microbiol. 75:7261-7.

ED_004892A_00000077-00089

Rutledge RG, Stewart D. 2010. Assessing the performance capabilities of LRE-based assays for absolute quantitative real-time PCR. PLoS One. 5:e9731.

Schlueter V, Schmolke S, Stark K, Hess G, Ofenloch G, Haehnle B, Engel AM. 1996. Reverse transcription PCR detection of hepatitis G virus. J. Clin. Microbiol. 34:26602664.

Shanks OC, Atikovic E, Blackwood AD, Lu J, Noble RT, Domingo JS, Seifring S, Sivaganesan M, Haugland RA. 2008. Quantitative PCR for detection and enumeration of genetic markers of bovine fecal pollution. Appl Environ Microbiol. 74:745-52.

Shanks OC, Kelty CA, Sivaganesan M, Varma M, Haugland RA. 2009. Quantitative PCR for genetic markers of human fecal pollution. Appl Environ Microbiol. 75: 5507–13.

Wang J, O'Keefe J, Orr D, Loth L, Banks M, Wakeley P, West D, Card R, Ibata G, Van MK, Thoren P, Isaksson M, Kerkhofs P. 2007. Validation of a real-time PCR assay for the detection of bovine herpesvirus 1 in bovine semen. J Virol Methods. 144:103–108.

Wong K, Xagoraraki I. 2010. Quantitative PCR assays to survey the bovine adenovirus levels in environmental samples. J Appl Microbiol. 109:605-12.

Wong K, Xagoraraki I. 2011. Evaluating the prevalence and genetic diversity of adenovirus and polyomavirus in bovine waste for microbial source tracking. Appl Microbiol Biotechnol 90:1521-26.

Zeng SQ, Halkosalo A, Salminen M, Szakal ED, Puustinen L, Vesikari T. 2008. One-step quantitative RT-PCR for the detection of rotavirus in acute gastroenteritis. J Virol Methods. 153:238-40.

ED_004892A_00000077-00090

Appendix 2. Private well data on total coliforms, *E. coli*, nitrate,and microbial targets measured by qPCR

I. Synoptic sampling: Total coliform, *E. coli*, and nitrate
- A. November 2015
- B. July 2016

II. qPCR analyses for microbial targets
- A. Human-specific microorganims
- B. Bovine-specific microorganisms
- C. Not host-specific microorganisms--Part 1
- D. Not host-specific microorganisms--Part 2

III. Quality assurance/quality control for qPCR analysis
- A. Standard curve performance parameters
- B. 95% limits of detection (LOD) for qPCR assays
- C. Negative controls--Part 1
- D. Negative controls--Part 2

Kewaunee County Groundwater Study

Synopotic sampling event November 2015

Total coliform, *E. coli* , and nitrate results for well samples. MPN, most probable number; NA, not analzyed; ND, not detected; Unknown, well construction reports not available.

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
VC100	11/14/2015	63	<1	<1	ND
VC101	11/14/2015	65	<1	<1	ND
VC102	11/21/2015	48	<1	<1	ND
VC103	11/14/2015	58	<1	<1	6.9
VC104	11/14/2015	42	<1	<1	ND
VC108	11/14/2015	3	<1	<1	13.4
VC109	11/14/2015	2	<1	<1	8.1
VC11	11/14/2015	40	8.4	<1	15
VC112	11/21/2015	43	<1	<1	0.17
VC113	11/14/2015	153	2	<1	ND
VC115	11/14/2015	52	<1	<1	ND
VC116	11/20/2015	50	<1	<1	ND
VC117	11/21/2015	45	<1	<1	ND
VC118	11/14/2015	4	11	<1	4
VC119	11/14/2015	5	<1	<1	ND
VC12	11/14/2015	28	<1	<1	ND
VC120	11/14/2015	80	1203.3	<1	ND
VC121	11/14/2015	30	<1	<1	ND
VC124	11/14/2015	4	<1	<1	ND
VC125	11/14/2015	11	1.0	<1	2.3
VC126	11/14/2015	22	<1	<1	0.74
VC127	11/21/2015	73	<1	<1	13.5
VC13	11/21/2015	5	<1	<1	0.53
VC130	11/20/2015	59	2	<1	9.7
VC132	11/21/2015	54	<1	<1	9.1
VC133	11/14/2015	63	<1	<1	8.9
VC134	11/14/2015	80	4.1	<1	ND
VC135	11/14/2015	6	7.2	<1	8
VC139	11/14/2015	35	117.8	<1	0.9
VC140	11/20/2015	80	<1	<1	ND
VC141	11/14/2015	154	6.3	<1	ND
VC142	11/14/2015	30	<1	<1	ND
VC143	11/21/2015	43	57.1	<1	2.6
VC144	11/21/2015	96	<1	<1	4.9
VC145	11/20/2015	72	<1	<1	3.7
VC146	11/14/2015	5	<1	<1	1.2
VC147	11/20/2015	Unknown	<1	<1	9.9
VC148	11/14/2015	66	<1	<1	0.18

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
VC149	11/14/2015	18	<1	<1	ND
VC15	11/14/2015	70	<1	<1	3.4
VC150	11/14/2015	35	<1	<1	7
VC151	11/14/2015	13	<1	<1	7
VC152	11/14/2015	54	<1	<1	ND
VC153	Not recorded	52	<1	<1	0.36
VC154	11/21/2015	3	14.8	2	4.9
VC155	11/14/2015	5	<1	<1	14.1
VC157	11/21/2015	52	<1	<1	9.6
VC158	11/14/2015	14	<1	<1	ND
VC159	11/14/2015	10	<1	<1	1.7
VC16	11/14/2015	120	1	<1	ND
VC161	11/21/2015	5	<1	<1	1.8
VC162	11/14/2015	7	3.1	<1	29.7
VC163	11/14/2015	45	<1	<1	10.5
VC164	11/21/2015	3	<1	<1	1.6
VC165	11/20/2015	4	18.3	<1	ND
VC166	11/21/2015	46	<1	<1	ND
VC167	11/21/2015	5	<1	<1	7.1
VC168	11/21/2015	6	<1	<1	0.95
VC169	11/14/2015	21	1.0	<1	4.5
VC17	11/13/2015	152	<1	<1	ND
VC170	11/14/2015	14	<1	<1	2.5
VC171	11/14/2015	2	8.6	<1	8.1
VC172	11/14/2015	18	14.8	<1	19.7
VC173	11/14/2015	65	<1	<1	8.5
VC174	11/14/2015	99	<1	<1	0.15
VC178	11/14/2015	20	<1	<1	6.6
VC18	11/20/2015	160	<1	<1	ND
VC180	11/21/2015	80	<1	<1	ND
VC182	11/21/2015	140	<1	<1	ND
VC183	11/21/2015	63	NA	NA	9
VC184	11/20/2015	91	<1	<1	1.2
VC185	11/21/2015	25	<1	<1	ND
VC187	11/21/2015	64	<1	<1	ND
VC189	11/21/2015	Unknown	<1	<1	ND
VC190	11/14/2015	3	<1	<1	12.5
VC194	11/14/2015	21	<1	<1	0.28
VC197	11/20/2015	78	<1	<1	ND
VC199	11/14/2015	11	69.1	<1	6.2
VC20	11/20/2015	23	<1	<1	ND
VC200	11/21/2015	8	<1	<1	2.8
VC201	11/21/2015	6	17.3	<1	9.7
VC202	11/21/2015	120	<1	<1	ND

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
VC204	11/14/2015	48	<1	<1	1.1
VC205	11/21/2015	18	<1	<1	ND
VC206	11/14/2015	45	<1	<1	ND
VC207	11/20/2015	51	<1	<1	ND
VC208	11/14/2015	4	2.0	<1	1.5
VC209	11/14/2015	55	<1	<1	6.1
VC21	11/14/2015	8	<1	<1	3.5
VC212	11/21/2015	44	<1	<1	3.8
VC214	11/21/2015	20	6.3	<1	3.3
VC215	11/21/2015	58	1	<1	5.8
VC216	11/14/2015	32	<1	<1	ND
VC218	11/14/2015	5	8.5	<1	2.4
VC219	11/14/2015	45	<1	<1	ND
VC22	11/14/2015	5	2	1	3.2
VC223	11/14/2015	10	16	<1	29
VC224	11/14/2015	46	<1	<1	6.4
VC225	11/14/2015	14	<1	<1	ND
VC226	11/21/2015	10	NA	NA	3.2
VC228	11/14/2015	20	<1	<1	ND
VC230	11/14/2015	65	133.3	<1	6.8
VC232	11/14/2015	5	<1	<1	9.2
VC233	11/21/2015	16	<1	<1	0.38
VC234	11/20/2015	53	<1	<1	3
VC235	11/20/2015	30	<1	<1	ND
VC238	11/14/2015	120	NA	NA	3.4
VC239	11/21/2015	3	64	<1	4.7
VC24	11/21/2015	5	29.2	<1	13.3
VC240	11/14/2015	10	1.0	<1	ND
VC241	11/21/2015	64	<1	<1	ND
VC243	11/20/2015	87	<1	<1	ND
VC244	11/20/2015	103	<1	<1	1.2
VC245	11/21/2015	10	9.7	<1	18.9
VC247	11/14/2015	18	<1	<1	0.17
VC249	11/21/2015	6	<1	<1	13.4
VC25	11/14/2015	50	<1	<1	3.5
VC250	11/21/2015	5	<1	<1	ND
VC251	11/21/2015	1	<1	<1	1.5
VC253	11/21/2015	31	<1	<1	ND
VC254	11/14/2015	42	<1	<1	6.2
VC257	11/21/2015	20	3	<1	18
VC259	11/20/2015	76	<1	<1	0.66
VC261	11/21/2015	100	<1	<1	ND
VC262	11/14/2015	17	<1	<1	9.3
VC263	11/14/2015	4	1.0	<1	5.6

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
VC265	11/21/2015	63	<1	<1	ND
VC267	11/21/2015	65	<1	<1	2.1
VC268	11/14/2015	5	<1	<1	10.1
VC269	11/21/2015	3	<1	<1	ND
VC271	11/21/2015	83	<1	<1	0.17
VC272	11/21/2015	167	<1	<1	ND
VC274	11/21/2015	43	<1	<1	ND
VC277	11/20/2015	40	<1	<1	6.1
VC278	11/21/2015	10	<1	<1	8.1
VC280	11/20/2015	166	<1	<1	ND
VC283	11/20/2015	180	<1	<1	ND
VC285	11/20/2015	57	2	<1	12.8
VC286	11/14/2015	13	<1	<1	ND
VC287	11/14/2015	17	<1	<1	ND
VC288	11/14/2015	18	<1	<1	ND
VC289	11/20/2015	8	<1	<1	16.2
VC29	11/14/2015	84	<1	<1	ND
VC290	11/21/2015	3	<1	<1	1.9
VC291	11/14/2015	16	<1	<1	11.3
VC292	11/21/2015	12	1	<1	10.1
VC293	11/21/2015	20	<1	<1	7.5
VC294	11/21/2015	7	<1	<1	ND
VC295	11/20/2015	15	<1	<1	7.1
VC296	11/20/2015	8	1	<1	3.9
VC297	11/20/2015	45	387.3	4.1	2.7
VC298	11/20/2015	5	<1	<1	7.9
VC3	11/14/2015	7	155.3	16.1	2.9
VC30	11/14/2015	68	<1	<1	ND
VC300	11/20/2015	10	<1	<1	18.5
VC301	11/21/2015	55	<1	<1	ND
VC302	11/14/2015	146	<1	<1	ND
VC304	11/14/2015	Unknown	<1	<1	23.4
VC305	11/21/2015	10	<1	<1	ND
VC307	11/14/2015	15	1.0	<1	10.2
VC309	11/14/2015	14	2.0	<1	8.6
VC31	11/21/2015	15	<1	<1	10
VC310	11/14/2015	3	2.0	<1	12.1
VC311	11/21/2015	41	<1	<1	16.3
VC313	11/14/2015	143	4.1	<1	ND
VC314	11/14/2015	165	<1	<1	ND
VC315	11/20/2015	3	7.5	<1	0.44
VC316	11/20/2015	15	<1	<1	2.2
VC317	11/21/2015	30	<1	<1	8.2
VC318	11/14/2015	122	<1	<1	ND

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
VC319	11/21/2015	170	<1	<1	ND
VC32	11/14/2015	12	4.1	<1	1.6
VC320	11/21/2015	17	27.9	<1	5.2
VC321	11/21/2015	22	12.2	<1	4.4
VC322	11/21/2015	116	<1	<1	ND
VC323	11/21/2015	65	<1	<1	4.7
VC324	11/20/2015	70	<1	<1	4.1
VC325	11/14/2015	10	<1	<1	20
VC326	11/14/2015	10	<1	<1	12
VC327	11/20/2015	7	4.1	<1	7.5
VC329	11/21/2015	7	<1	<1	26
VC33	11/14/2015	2	2	<1	1.4
VC330	11/14/2015	12	<1	<1	6.8
VC331	11/21/2015	50	<1	<1	0.52
VC332	11/14/2015	35	<1	<1	ND
VC333	11/14/2015	47	<1	<1	3.2
VC336	11/14/2015	105	<1	<1	ND
VC337	11/14/2015	10	<1	<1	0.77
VC338	11/14/2015	9	9.7	<1	4.8
VC339	11/14/2015	45	<1	<1	ND
VC34	11/14/2015	94	<1	<1	ND
VC341	11/14/2015	40	<1	<1	13.5
VC343	11/20/2015	47	<1	<1	0.36
VC347	11/14/2015	150	<1	<1	ND
VC349	11/21/2015	35	<1	<1	0.95
VC350	11/21/2015	9	4.1	<1	3.6
VC351	11/21/2015	10	<1	<1	4.4
VC352	11/14/2015	0	9.7	<1	ND
VC353	11/14/2015	10	<1	<1	0.3
VC354	11/14/2015	58	31.7	<1	1.7
VC355	11/14/2015	30	<1	<1	18.2
VC357	11/20/2015	7	<1	<1	ND
VC358	11/20/2015	11	2	<1	ND
VC359	11/14/2015	15	1.0	<1	9.65
VC360	11/20/2015	30	<1	<1	7.7
VC361	11/20/2015	40	<1	<1	ND
VC362	11/20/2015	45	<1	<1	9
VC363	11/24/2015	48	<1	<1	7.3
VC365	11/20/2015	20	<1	<1	6.7
VC367	11/21/2015	20	44.1	<1	4.3
VC368	11/20/2015	44	<1	<1	ND
VC369	11/21/2015	63	<1	<1	3.8
VC370	11/21/2015	20	<1	<1	ND
VC371	11/21/2015	0	<1	<1	1.2

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
VC372	11/21/2015	5	2	<1	2.5
VC374	11/14/2015	12	<1	<1	0.64
VC375	11/14/2015	58	<1	<1	ND
VC376	11/14/2015	3	<1	<1	ND
VC377	11/14/2015	14	<1	<1	ND
VC378	11/14/2015	20	<1	<1	ND
VC38	11/14/2015	6	4.1	<1	0.19
VC380	11/14/2015	70	<1	<1	6.2
VC383	11/14/2015	63	<1	<1	ND
VC384	11/14/2015	42	4.1	<1	ND
VC386	11/14/2015	21	<1	<1	ND
VC387	11/14/2015	40	4.1	<1	9
VC388	11/14/2015	63	<1	<1	3.8
VC389	11/14/2015	138	<1	<1	ND
VC39	11/20/2015	5	<1	<1	ND
VC390	11/14/2015	70	1.0	<1	ND
VC391	11/21/2015	45	<1	<1	ND
VC392	11/14/2015	40	9.7	<1	NA
VC394	11/14/2015	25	<1	<1	3.5
VC395	11/14/2015	137	<1	<1	0.25
VC396	11/14/2015	31	<1	<1	16.2
VC397	11/14/2015	167	18.7	<1	ND
VC398	11/14/2015	56	<1	<1	ND
VC399	11/14/2015	7	<1	<1	6.8
VC4	11/14/2015	3	<1	<1	ND
VC40	11/14/2015	24	7.3	2	5.4
VC400	11/14/2015	40	<1	<1	7.4
VC402	11/14/2015	39	10.7	<1	ND
VC403	11/14/2015	3	66.3	<1	5.1
VC404	11/14/2015	46	<1	<1	1.4
VC405	11/14/2015	145	<1	<1	ND
VC406	11/14/2015	30	<1	<1	3.3
VC407	11/14/2015	55	3.1	<1	ND
VC408	11/14/2015	2	<1	<1	9.4
VC409	11/14/2015	0	3.0	<1	5.8
VC41	11/14/2015	0	<1	<1	1.9
VC410	11/14/2015	15	<1	<1	6.7
VC411	11/14/2015	20	<1	<1	ND
VC412	11/14/2015	15	3.1	<1	0.64
VC414	11/14/2015	115	<1	<1	ND
VC415	11/14/2015	10	<1	<1	ND
VC416	11/14/2015	8	<1	<1	0.18
VC418	11/14/2015	58	<1	<1	2.8
VC419	11/14/2015	5	15.8	<1	16.3

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
VC42	11/14/2015	10	172.3	<1	0.48
VC420	11/14/2015	8	<1	<1	7.4
VC421	11/14/2015	5	5.2	<1	4
VC424	11/14/2015	25	<1	<1	11.2
VC43	11/14/2015	100	5.2	<1	ND
VC436	11/14/2015	15	9.7	<1	2.7
VC437	11/14/2015	30	1	**<1**	**15**
VC44	11/21/2015	10	25	<1	7.7
VC443	11/14/2015	102	<1	<1	ND
VC45	11/14/2015	20	<1	<1	8.9
VC46	11/21/2015	11	<1	<1	12.4
VC460	11/14/2015	10	<1	<1	18.2
VC462	11/14/2015	2	29.9	<1	9.1
VC466	11/14/2015	100	<1	<1	ND
VC468	11/20/2015	25	<1	<1	3.7
VC47	11/20/2015	8	<1	<1	ND
VC471	11/13/2015	3	<1	<1	ND
VC474	11/20/2015	85	<1	<1	ND
VC476	11/14/2015	7	868.4	<1	0.22
VC48	11/14/2015	44	<1	<1	0.51
VC480	11/14/2015	51	<1	<1	0.81
VC49	11/14/2015	145	<1	<1	ND
VC52	11/23/2015	16	<1	<1	7.3
VC53	11/21/2015	42	<1	<1	ND
VC54	11/20/2015	6	2	<1	0.9
VC55	11/14/2015	8	1	<1	4.5
VC56	11/14/2015	10	<1	<1	11.9
VC57	11/21/2015	10	<1	<1	0.32
VC58	11/14/2015	17	<1	<1	0.154
VC6	11/14/2015	20	<1	<1	0.18
VC60	11/14/2015	14	101.7	<1	0.23
VC62	11/14/2015	43	1.0	<1	0.21
VC63	11/14/2015	25	<1	<1	1.04
VC64	11/20/2015	45	<1	<1	26.2
VC65	11/14/2015	Unknown	1.0	<1	9.1
VC66	11/14/2015	69	<1	<1	6.5
VC68	11/14/2015	63	<1	<1	4.3
VC69	11/20/2015	16	<1	<1	ND
VC70	11/13/2015	43	<1	<1	0.6
VC71	11/14/2015	185	>2419.6	<1	0.154
VC72	11/14/2015	55	<1	<1	ND
VC73	11/21/2015	30	<1	<1	9.44
VC74	11/21/2015	104	<1	<1	ND
VC75	11/21/2015	181	<1	<1	ND

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
VC76	11/21/2015	5	3.1	<1	2.5
VC77	11/21/2015	9	<1	<1	1.7
VC78	11/21/2015	8	<1	<1	ND
VC79	11/14/2015	21	<1	<1	ND
VC8	11/14/2015	15	4.1	<1	ND
VC81	11/21/2015	62	<1	<1	3.4
VC82	11/21/2015	11	<1	<1	ND
VC83	11/21/2015	9	<1	<1	ND
VC84	11/21/2015	13	<1	<1	0.34
VC85	11/14/2015	40	<1	<1	0.18
VC87	11/20/2015	15	<1	<1	7.6
VC88	11/21/2015	43	<1	<1	1
VC89	11/14/2015	77	<1	<1	8.1
VC91	11/14/2015	25	<1	<1	ND
VC93	11/21/2015	11	<1	<1	ND
VC94	11/21/2015	5	<1	<1	4
VC95	11/14/2015	3	<1	<1	ND
VC96	11/14/2015	5	1.0	<1	7.5
VC97	11/14/2015	27	<1	<1	ND
VC98	11/14/2015	69	<1	<1	3.5
VC99	11/14/2015	40	NA	NA	8.8

Kewaunee County Groundwater Study

Synopotic sampling event July 2016

Total coliform, *E. coli* , and nitrate results for well samples. MPN, most probable number; NA, not analzyed; ND, not detected; Unknown, well construction reports not available; Absent indicates qualitative result.

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
K001	7/29/2016	32	<1	<1	0.28
K002	7/30/2016	10	<1	<1	ND
K003	7/30/2016	Unknown	10.9	<1	4.5
K004	7/29/2016	115	<1	<1	ND
K005	7/30/2016	77	<1	<1	ND
K006	7/29/2016	167	<1	<1	ND
K007	7/30/2016	99	<1	<1	ND
K008	7/29/2016	13	<1	<1	9.1
K009	7/29/2016	10	<1	<1	3.0
K010	7/29/2016	85	816.4	<1	ND
K011	7/30/2016	16	<1	<1	ND
K012	7/30/2016	161	2.0	<1	ND
K014	7/30/2016	6	<1	<1	0.63
K018	7/30/2016	3	<1	<1	1.0
K019	7/30/2016	55	<1	<1	ND
K020	7/29/2016	100	67.0	<1	ND
K021	7/30/2016	5	<1	<1	1.2
K022	7/30/2016	10	<1	<1	4.4
K023	7/30/2016	13	<1	<1	0.23
K024	7/29/2016	101	<1	<1	ND
K025	7/29/2016	69	<1	<1	1.6
K026	7/29/2016	125	<1	<1	ND
K028	7/30/2016	43	<1	<1	ND
K030	7/29/2016	4	83.6	<1	2.1
K031	7/29/2016	5	<1	<1	5.8
K033	7/29/2016	5	<1	<1	23.5
K035	7/30/2016	48	<1	<1	ND
K036	7/30/2016	42	<1	<1	ND
K037	7/30/2016	42	<1	<1	4.9
K038	7/30/2016	200	<1	<1	ND
K039	7/30/2016	30	<1	<1	13.8
K042	7/29/2016	97	<1	<1	ND
K043	7/29/2016	154	<1	<1	ND
K044	7/30/2016	134	<1	<1	ND
K045	7/29/2016	50	<1	<1	6.3
K046	7/30/2016	143	<1	<1	ND
K048	7/30/2016	52	8.6	<1	ND

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
K049	7/29/2016	25	<1	<1	3.8
K050	7/29/2016	18	<1	<1	ND
K051	7/29/2016	30	<1	<1	5.2
K052	7/30/2016	51	17.3	<1	ND
K053	7/29/2016	40	<1	<1	6.7
K054	7/29/2016	15	<1	<1	6.6
K055	7/30/2016	40	<1	<1	ND
K056	7/29/2016	3	<1	<1	11.5
K057	7/29/2016	9	<1	<1	24.4
K059	7/30/2016	52	<1	<1	ND
K060	7/29/2016	26	45.5	<1	ND
K061	7/30/2016	63	<1	<1	8.4
K062	7/29/2016	170	<1	<1	ND
K063	7/29/2016	137.5	<1	<1	ND
K064	7/29/2016	65	<1	<1	ND
K065	7/30/2016	115	<1	<1	ND
K066	7/29/2016	7	6.3	1	1.0
K068	7/30/2016	9	4.1	<1	11.6
K069	7/30/2016	7	2	<1	33.3
K070	7/30/2016	5	<1	<1	7.2
K072	7/29/2016	88	<1	<1	0.63
K074	7/30/2016	113	<1	<1	ND
K077	7/29/2016	105	<1	<1	ND
K080	7/29/2016	120	<1	<1	ND
K083	7/30/2016	129	<1	<1	ND
K084	7/30/2016	42	1	<1	ND
K085	7/29/2016	130	<1	<1	ND
K086	7/30/2016	101	<1	<1	ND
K088	7/30/2016	168	<1	<1	ND
K089	7/28/2016	50	<1	<1	ND
K091	7/29/2016	75	<1	<1	2.3
K092	7/30/2016	54	<1	<1	ND
K093	7/30/2016	28	59.4	<1	9.6
K094	7/30/2016	77	<1	<1	ND
K095	7/29/2016	12	<1	<1	3.0
K099	7/30/2016	200	2	<1	ND
K100	7/30/2016	121	<1	<1	ND
K102	7/29/2016	2	2.0	<1	6.1
K103	7/29/2016	66	<1	<1	ND
K104	7/29/2016	10	4.1	<1	ND
K106	7/29/2016	68	<1	<1	11.2
K107	7/29/2016	45	<1	<1	6.8
K110	7/29/2016	23	1.0	<1	9.0
K111	7/29/2016	62	<1	<1	0.28

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
K112	7/29/2016	7	<1	<1	8.7
K114	7/30/2016	102	7.5	<1	ND
K115	7/30/2016	10	<1	<1	11.5
K116	7/29/2016	147	<1	<1	ND
K119	7/30/2016	46	<1	<1	ND
K120	7/29/2016	13	5.2	<1	ND
K121	7/29/2016	58	<1	<1	3.7
K122	7/29/2016	33	<1	<1	5.1
K123	7/30/2016	20	17.1	<1	11.6
K124	7/30/2016	25	2	<1	5.9
K125	7/30/2016	41	<1	<1	14.6
K126	7/29/2016	Unknown	<1	<1	11.4
K127	7/29/2016	68	<1	<1	ND
K129	7/30/2016	12	<1	<1	ND
K130	7/30/2016	5	2	<1	1.7
K132	7/30/2016	146	<1	<1	ND
K134	7/29/2016	35	23.1	4.1	ND
K135	7/29/2016	165	Broken Bottle	Broken Bottle	ND
K136	7/30/2016	67	<1	<1	4.2
K138	7/29/2016	15	<1	<1	2.7
K139	7/29/2016	12	<1	<1	ND
K140	7/30/2016	20	<1	<1	8.0
K142	7/29/2016	115	<1	<1	ND
K143	7/29/2016	129	<1	<1	ND
K144	7/30/2016	80	1.0	<1	2.3
K145	7/30/2016	129	1.0	<1	ND
K147	7/29/2016	10	<1	<1	0.24
K148	7/30/2016	7	<1	<1	8.0
K149	7/30/2016	53	<1	<1	27.1
K150	7/30/2016	4	<1	<1	ND
K151	7/29/2016	56	<1	<1	ND
K152	7/29/2016	50	<1	<1	1.9
K153	7/29/2016	71	<1	<1	5.3
K156	7/30/2016	4	<1	<1	6.3
K157	7/30/2016	45	<1	<1	13.8
K158	7/30/2016	51	3.1	<1	5.7
K160	7/29/2016	22	<1	<1	6.7
K163	7/29/2016	15	<1	<1	10.9
K164	7/29/2016	7	<1	<1	ND
K165	7/30/2016	63	<1	<1	ND
K166	7/29/2016	3	<1	<1	ND
K167	7/30/2016	10	3.1	<1	1.9
K168	7/29/2016	4.5	5.2	<1	3.5
K169	7/29/2016	8	<1	<1	3.9

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
K170	7/29/2016	>50	<1	<1	ND
K172	7/29/2016	93	<1	<1	ND
K173	7/29/2016	9	11.0	<1	6.9
K174	7/30/2016	110	<1	<1	ND
K176	7/30/2016	83	6.3	<1	0.28
K177	7/29/2016	12	<1	<1	6.1
K179	7/30/2016	15	1.0	<1	0.23
K181	7/30/2016	4	13.4	1	3.4
K182	7/30/2016	35	2	<1	ND
K183	7/30/2016	67	<1	<1	4.7
K184	7/30/2016	165	2419.6	<1	ND
K185	8/5/2016	15	Absent	Absent	ND
K186	7/30/2016	0	<1	<1	0.95
K188	7/29/2016	20	<1	<1	26.4
K190	7/30/2016	10	261.3	<1	0.83
K191	7/30/2016	7	<1	<1	4.4
K192	7/30/2016	133	<1	<1	ND
K194	7/29/2016	>105	<1	<1	ND
K195	7/30/2016	143	<1	<1	ND
K197	7/29/2016	4	<1	<1	7.2
K199	7/29/2016	57	<1	<1	ND
K200	7/29/2016	5	<1	<1	ND
K201	7/29/2016	47	<1	<1	12.3
K202	7/30/2016	65	<1	<1	ND
K203	7/29/2016	178	<1	<1	ND
K206	7/29/2016	41	<1	<1	ND
K207	7/30/2016	83	3.1	<1	ND
K208	7/30/2016	78	<1	<1	ND
K209	7/29/2016	95	<1	<1	ND
K210	7/29/2016	40	<1	<1	ND
K212	7/29/2016	42	<1	<1	ND
K215	7/30/2016	41	<1	<1	2.1
K216	7/30/2016	31	<1	<1	4.8
K217	7/29/2016	50	<1	<1	9.4
K219	7/29/2016	40	<1	<1	ND
K220	7/29/2016	30	<1	<1	ND
K221	7/30/2016	10	56.5	<1	5.8
K222	7/29/2016	95	114.5	<1	ND
K224	7/29/2016	154	<1	<1	ND
K225	7/30/2016	67	1.0	<1	7.1
K226	7/30/2016	50	<1	<1	11.3
K227	7/30/2016	10	112.2	<1	1.5
K228	7/29/2016	27	<1	<1	ND
K229	7/30/2016	63	<1	<1	ND

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
K230	7/30/2016	5	<1	<1	16.3
K231	7/30/2016	17	48.0	<1	2.3
K234	7/29/2016	17	<1	<1	11.7
K236	7/30/2016	45	980.6	<1	8.6
K237	7/29/2016	8	<1	<1	ND
K238	7/29/2016	42	<1	<1	ND
K239	7/29/2016	1	<1	<1	ND
K240	7/30/2016	58	<1	<1	ND
K241	7/29/2016	77	<1	<1	ND
K244	7/29/2016	44	<1	<1	ND
K245	7/30/2016	33	<1	<1	8.9
K247	7/29/2016	98	<1	<1	ND
K249	7/30/2016	147	3.0	<1	ND
K251	7/29/2016	128	<1	<1	ND
K252	7/29/2016	22	<1	<1	9.9
K253	7/29/2016	73	<1	<1	1.0
K254	7/29/2016	80	<1	<1	0.17
K255	7/30/2016	50	<1	<1	ND
K257	7/30/2016	64	<1	<1	ND
K258	7/30/2016	63	1.0	<1	ND
K260	7/30/2016	69	<1	<1	ND
K262	not recorded	12.5	<1	<1	8.2
K265	7/29/2016	26	<1	<1	ND
K267	7/29/2016	11	<1	<1	ND
K268	7/29/2016	90	<1	<1	ND
K270	7/30/2016	60	2.0	<1	ND
K271	7/29/2016	18	<1	<1	ND
K272	7/30/2016	12	<1	<1	5.7
K273	7/29/2016	50	<1	<1	2
K275	7/29/2016	12.5	<1	<1	ND
K276	7/29/2016	5	13.4	<1	0.71
K277	7/29/2016	28	2.0	<1	ND
K278	7/29/2016	8	<1	<1	16.3
K280	7/30/2016	3	<1	<1	2.2
K281	7/29/2016	63	<1	<1	1.9
K282	7/29/2016	10	140.1	<1	14.5
K285	7/30/2016	48	<1	<1	0.84
K286	7/29/2016	13	<1	<1	7.9
K287	7/30/2016	4	<1	<1	ND
K289	7/30/2016	4	<1	<1	ND
K290	7/29/2016	12	<1	<1	10.2
K291	7/29/2016	4	12.1	<1	2.3
K292	7/29/2016	25	<1	<1	ND
K293	7/29/2016	8	1.0	<1	ND

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
K294	7/29/2016	8	<1	<1	ND
K295	7/29/2016	25	<1	<1	2.5
K297	7/30/2016	40	<1	<1	ND
K301	7/30/2016	54	<1	<1	6.5
K302	7/30/2016	55	<1	<1	6.2
K303	7/29/2016	30	>2419.6	9.7	ND
K304	7/30/2016	30	<1	<1	ND
K305	7/30/2016	111	<1	<1	ND
K306	7/30/2016	69	<1	<1	ND
K307	7/30/2016	138	<1	<1	ND
K308	7/29/2016	85	<1	<1	0.76
K309	7/30/2016	127	<1	<1	ND
K310	7/29/2016	59	< 1	< 1	7.7
K311	7/29/2016	4	<1	<1	ND
K312	7/29/2016	30	<1	<1	10.6
K314	7/29/2016	77	<1	<1	6.7
K315	7/29/2016	9	<1	<1	3.4
K316	7/29/2016	15	<1	<1	5.4
K317	7/30/2016	50	<1	<1	ND
K318	7/30/2016	145	110.6	<1	ND
K319	7/29/2016	5	1011.2	1011.2	7.3
K320	Not Recorded	115	<1	<1	ND
K321	7/29/2016	54	<1	<1	ND
K323	7/30/2016	8	<1	<1	ND
K324	7/30/2016	65	<1	<1	ND
K325	7/29/2016	41	<1	<1	3.1
K326	7/29/2016	12.5	<1	<1	5.7
K327	7/29/2016	10	2.0	<1	4.5
K328	7/30/2016	21	<1	<1	5.0
K329	7/29/2016	1	727	<1	0.9
K330	7/29/2016	12	<1	<1	9.9
K331	7/29/2016	90	<1	<1	ND
K332	7/30/2016	14	<1	<1	ND
K333	7/30/2016	45	<1	<1	0.51
K334	7/29/2016	95	<1	<1	3.6
K335	7/29/2016	10	<1	<1	6.7
K336	7/29/2016	9	6.3	<1	ND
K338	7/29/2016	21	<1	<1	ND
K339	7/29/2016	8	<1	<1	13.4
K341	7/29/2016	5	<1	<1	ND
K342	7/29/2016	20	1.0	<1	ND
K343	7/29/2016	3	<1	<1	ND
K344	7/30/2016	77	<1	<1	4.9
K345	7/29/2016	3	<1	<1	ND

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
K347	7/29/2016	10	1.0	<1	17.5
K348	7/29/2016	17	<1	<1	10.1
K349	7/30/2016	5	<1	<1	ND
K350	7/29/2016	21	<1	<1	ND
K352	7/30/2016	20	<1	<1	15.1
K353	7/29/2016	7	<1	<1	ND
K354	7/29/2016	7	<1	<1	28.0
K355	7/30/2016	43	2.0	<1	0.32
K356	7/29/2016	40	<1	<1	ND
K357	7/29/2016	40	<1	<1	9.6
K358	7/30/2016	13	<1	<1	ND
K359	7/30/2016	10	<1	<1	2.1
K361	7/29/2016	51	<1	<1	4.0
K362	7/29/2016	5	<1	<1	13.3
K363	7/29/2016	43	26.5	<1	ND
K365	7/29/2016	52	6.2	6.2	1.2
K366	7/30/2016	8	135.4	<1	ND
K367	7/30/2016	8	<1	<1	ND
K368	7/29/2016	6	<1	<1	1.5
K369	7/29/2016	15	19.9	<1	9.1
K370	7/30/2016	7	<1	<1	ND
K373	7/29/2016	80	<1	<1	ND
K375	7/30/2016	30	<1	<1	ND
K376	7/29/2016	5	<1	<1	12.1
K377	7/30/2016	90	<1	<1	ND
K378	7/29/2016	107	<1	<1	ND
K380	7/29/2016	12.5	<1	<1	2.7
K381	7/29/2016	5	<1	<1	3.5
K382	7/30/2016	42	1.0	<1	14.3
K384	7/30/2016	136	<1	<1	ND
K385	7/29/2016	41	313.0	<1	ND
K386	7/29/2016	76	<1	<1	ND
K387	7/30/2016	65	<1	<1	ND
K388	7/30/2016	180	<1	<1	ND
K389	7/29/2016	30	<1	<1	10.3
K391	7/30/2016	86	<1	<1	ND
K394	7/30/2016	92	<1	<1	ND
K395	7/30/2016	87	<1	<1	ND
K396	7/30/2016	123	<1	<1	0.47
K397	7/29/2016	46	<1	<1	1.3
K398	7/30/2016	57	<1	<1	11.7
K399	7/29/2016	140	<1	<1	ND
K400	7/30/2016	100	<1	<1	ND
K401	7/30/2016	105	<1	<1	ND

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
K403	7/30/2016	50	<1	<1	10.0
K404	7/29/2016	64	71.2	<1	ND
K405	7/29/2016	45	<1	<1	ND
K406	7/29/2016	35	1.0	<1	ND
K408	7/30/2016	16	2.0	<1	ND
K412	7/30/2016	56	<1	<1	0.19
K413	7/30/2016	18	<1	<1	ND
K414	7/29/2016	49	<1	<1	7.2
K415	Not Recorded	63	< 1	< 1	6.5
K416	7/30/2016	67	<1	<1	7.1
K417	7/30/2016	53	<1	<1	0.29
K418	7/30/2016	7	<1	<1	7.0
K419	7/29/2016	8	<1	<1	0.74
K420	7/30/2016	10	60.2	<1	ND
K421	7/29/2016	8	<1	<1	4.6
K422	7/29/2016	5	<1	<1	8.7
K423	7/29/2016	20	<1	<1	ND
K424	7/30/2016	45	<1	<1	ND
K426	7/30/2016	40	<1	<1	14.4
K427	7/30/2016	32	1.0	<1	8.7
K429	7/29/2016	51	1.0	<1	4.0
K430	7/30/2016	94	4.1	<1	2.1
K431	7/29/2016	84	<1	<1	ND
K433	7/29/2016	87	<1	<1	ND
K435	7/29/2016	37	<1	<1	ND
K436	7/30/2016	49	20.3	<1	8.3
K438	7/30/2016	94	<1	<1	2.2
K439	7/29/2016	58	>2419.6	12.2	ND
K440	7/29/2016	58	23.1	<1	5.5
K442	7/30/2016	45	<1	<1	ND
K444	7/29/2016	15	<1	<1	ND
K445	7/29/2016	111	<1	<1	ND
K446	7/30/2016	2	<1	<1	0.17
K448	7/29/2016	150	<1	<1	ND
K452	7/29/2016	40	2.0	<1	3.2
K453	7/29/2016	30	<1	<1	13.7
K454	7/29/2016	12.5	<1	<1	2.9
K455	7/29/2016	24	<1	<1	3.3
K456	7/29/2016	13	1.0	<1	6.4
K458	7/29/2016	1	<1	<1	0.94
K459	7/29/2016	12	<1	<1	19.6
K460	7/30/2016	34	<1	<1	6.9
K461	7/29/2016	20	<1	<1	ND
K462	7/29/2016	47	<1	<1	7.9

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
K463	7/30/2016	28	<1	<1	3.7
K464	7/29/2016	22	<1	<1	3.1
K465	7/29/2016	26	<1	<1	0.66
K466	8/5/2016	57	Absent	Absent	0.33
K468	7/29/2016	190	<1	<1	ND
K469	7/30/2016	190	<1	<1	ND
K470	7/29/2016	162	<1	<1	2.6
K471	7/30/2016	41	<1	<1	ND
K472	7/30/2016	10	<1	<1	15.6
K473	7/29/2016	69	<1	<1	8.2
K474	7/29/2016	40	<1	<1	ND
K475	7/30/2016	16	<1	<1	ND
K476	7/29/2016	33	6.3	2.0	ND
K477	7/29/2016	15	<1	<1	2.0
K478	7/29/2016	33	3.0	<1	ND
K479	7/29/2016	40	<1	<1	11.5
K480	7/29/2016	6	<1	<1	ND
K481	7/29/2016	65	<1	<1	ND
K482	7/29/2016	8	<1	<1	25.7
K484	7/29/2016	40	<1	<1	2.3
K485	7/30/2016	14	<1	<1	4.9
K486	7/30/2016	22	<1	<1	2.2
K487	7/29/2016	41	<1	<1	11.4
K488	7/30/2016	43	<1	<1	0.19
K489	7/30/2016	13	<1	<1	0.74
K492	7/29/2016	74	<1	<1	3.6
K493	7/29/2016	15	<1	<1	2.9
K494	7/29/2016	20	<1	<1	ND
K496	7/30/2016	41	3.1	<1	5.2
K498	7/29/2016	17	<1	<1	ND
K500	7/29/2016	43	71.2	2.0	7.3
K501	7/29/2016	73	<1	<1	6.1
K502	7/29/2016	42	1	<1	1.5
K503	7/30/2016	11	<1	<1	ND
K504	7/29/2016	30	<1	<1	3.7
K505	7/29/2016	60	<1	<1	ND
K508	7/30/2016	30	<1	<1	ND
K509	7/29/2016	5	<1	<1	14.4
K510	7/29/2016	63	1.0	<1	ND
K512	7/29/2016	16	<1	<1	ND
K513	7/29/2016	20	<1	<1	0.89
K515	7/30/2016	35	<1	<1	8.4
K516	7/29/2016	67	<1	<1	4.3
K517	7/29/2016	5	<1	<1	ND

ID	Date	Depth to bedrock (feet)	Total coliform (MPN 100 mL^{-1})	*E. coli* (MPN 100 mL^{-1})	Nitrate (mg L^{-1})
K519	7/29/2016	63	<1	<1	ND
K521	7/29/2016	4	55.4	1.0	3.5
K524	7/30/2016	15	<1	<1	17.8
K525	7/29/2016	8	<1	<1	0.63
K526	7/30/2016	8	<1	<1	0.31
K527	7/29/2016	7	3.0	<1	0.86
K531	7/30/2016	45	1299.7	<1	0.16
K532	7/30/2016	4	524.7	<1	11.2
K533	7/30/2016	7	<1	<1	ND
K534	7/30/2016	27	<1	<1	0.16
K535	7/30/2016	2	<1	<1	14.8
K536	8/5/2016	147	Absent	Absent	ND

Kewaunee County Groundwater Study

Wells for microbial analysis were selected based on the presence of total coliform, *E. coli*, or high nitrate ($N\text{-}NO_3^-$ > 10 ppm) during the November 2015 or July 2016 sampling events.

Mircrobial targets. Analyses by quantitative polymerase chain reaction (qPCR) except rotavirus genotyping

Human-specific	***Not host-specific***
Adenovirus group A	*Campylobacter jejuni*
Adenovirus group B	Enterohemorrhagic *E. coli* (*eae* gene)
Adenovirus groups C,D,F	Enterohemorrhagic *E. coli* (*stx1* gene)
Human enterovirus	Enterohemorrhagic *E. coli* (*stx2* gene)
Norovirus genogroup I	Rotavirus group A (*NSP3* gene)
Norovirus genogroup II	Rotavirus group A (*VP7* gene)
Human polyomavirus	Rotavirus group C
Human *Bacteroides*	*Salmonella* (*invA* gene)
Bacteroidales-like Hum M2	*Salmonella* (*ttr* gene)
Cryptosporidium hominis (qualitative test)[a]	Pepper mild mottle virus
	Cryptosporidium parvum (qualitative test)[a]
Bovine-specific	*Cryptosporidium* spp.
Bovine adenovirus	*Giardia lamblia* group B
Ruminant *Bacteroides*	
Bovine viral diarrhea virus type 1	***Rotavirus genotyping by sequencing***[b]
Bovine viral diarrhea virus type 2	G genotyping (*VP7* gene) (qualitative test)
Bovine enterovirus	P genotyping (*VP4* gene) (qualitative test)
Bovine polyomavirus	
Coronavirus	
Bacteroidales-like cow M2	
Bacteroidales-like cow M3	

[a]Only samples positive for the general *Cryptosporidium* spp. assay were tested using the species-specific tests

[b]Only samples positive for the general rotavirus group A assays were genotyped; G1P[8] and G10P[11] are considered human- and bovine-specific genotypes, respectively.

Analyses by Laboratory for Infectious Disease and the Environment (USDA-ARS/USGS)

THE FOLLOWING TABLES EXCLUDE MICROBIAL TARGETS NOT DETECTED IN ANY SAMPLE

Human-specific microbial targets

Results for microbial analysis of well samples (genomic copies L^{-1} except for qualitative tests). Human-specific microbial targets not shown were not detected in any samples. NA, not analyzed; Unknown, well construction reports not available.

LIMS ID	Depth to bedrock (feet)	Date	Adenovirus group A	*Bacteroidales* -like Hum M2	Human *Bacteroides*	*Cryptosporidium hominis* [a]	Human rotavirus group A genotype[b]
103327	70	18-Apr-16	0	0	0	NA	NA
103328	143	18-Apr-16	0	0	0	NA	NA
103329	100	18-Apr-16	0	0	0	NA	NA
103330	3	19-Apr-16	0	0	0.37	NA	NA
103331	11	19-Apr-16	0	0	0	Negative	NA
103332	80	19-Apr-16	0	0	0	NA	NA
103333	10	19-Apr-16	0	0	0.55	Negative	NA
103334	14	19-Apr-16	0	0	0.40	NA	NA
103335	10	19-Apr-16	0	0	0.75	NA	Positive
103336	8	20-Apr-16	0	0	0	Negative	NA
103337	7	20-Apr-16	0	0.47	2.0	Negative	NA
103338	11	19-Apr-16	0	0	0	NA	NA
103339	11	19-Apr-16	0	0	0	NA	Positive
103340	45	19-Apr-16	0	0.52	0.38	NA	NA
103341	12	19-Apr-16	0	0	0	NA	NA
103342	11	18-Apr-16	0	0	0	NA	NA
103343	2	18-Apr-16	0	0	0	NA	NA
103344	120	18-Apr-16	0	0	0.31	NA	NA
103345	15	20-Apr-16	0	0	0.24	NA	NA
103346	15	20-Apr-16	0	0	0	NA	NA
103347	14	20-Apr-16	0	0	0	NA	Positive
103348	25	20-Apr-16	0	0	0	NA	NA
103349	73	20-Apr-16	0	0	0	NA	Positive
103350	35	21-Apr-16	0	0	0	NA	NA
103351	58	21-Apr-16	0	0	2.3	Positive	NA
103352	30	21-Apr-16	0	0	0	NA	NA

LIMS ID	Depth to bedrock (feet)	Date	Adenovirus group A	*Bacteroidales* -like Hum M2	Human *Bacteroides*	*Cryptosporidium hominis* [a]	Human rotavirus group A genotype[b]
103353	15	21-Apr-16	0	0	0.51	NA	Positive
103354	6	21-Apr-16	0	0.49	0	NA	Positive
103355	21	22-Apr-16	0	0	0	NA	Positive
103356	55	22-Apr-16	0	0	0	NA	NA
103651	70	1-Aug-16	0	0	0	NA	NA
103652	153	1-Aug-16	0	0	0	NA	NA
103653	13	1-Aug-16	0	0	0	Negative	NA
103654	42	1-Aug-16	0	0	0	NA	NA
103655	41	1-Aug-16	0	0	0	NA	Negative
103656	12	1-Aug-16	0	0	0	NA	NA
103657	7	1-Aug-16	0	0	0	NA	NA
103658	57	1-Aug-16	0	0	0	NA	NA
103659	20	1-Aug-16	0	0	1.46	NA	NA
103660	45	1-Aug-16	0	0	0	NA	NA
103661	5	2-Aug-16	0	0	0	Negative	Negative
103662	5	2-Aug-16	0	0	0	NA	NA
103663	5	2-Aug-16	0	0	0	NA	NA
103664	5	2-Aug-16	0	0	0	NA	NA
103665	3	2-Aug-16	0	21.77	13.89	NA	NA
103666	5	2-Aug-16	0	0	0.65	NA	NA
103667	59	2-Aug-16	0	0	0	NA	NA
103668	65	2-Aug-16	0.99	0	0.50	NA	NA
103669	30	2-Aug-16	0	0	0	NA	NA
103670	15	2-Aug-16	0	0	0	NA	NA
103671	31	2-Aug-16	0	0	0	NA	NA
103672	8	3-Aug-16	0	0	0	NA	NA
103673	24	3-Aug-16	0	0	0	NA	NA
103674	4	3-Aug-16	0	0	2.64	NA	NA
103675	58	3-Aug-16	0	0	0	NA	NA
103676	80	3-Aug-16	0	0	0	NA	NA
103677	80	3-Aug-16	0	0	0.90	NA	NA
103678	6	3-Aug-16	0	0	0	NA	NA

LIMS ID	Depth to bedrock (feet)	Date	Adenovirus group A	*Bacteroidales* -like Hum M2	Human *Bacteroides*	*Cryptosporidium hominis* [a]	Human rotavirus group A genotype[b]
103679	63	3-Aug-16	0	0	0	NA	NA
104109	10	31-Oct-16	0	0	0	NA	NA
104110	10	31-Oct-16	0	0	0	NA	Negative
104111	42	31-Oct-16	0	0	0	NA	NA
104112	30	31-Oct-16	0	0	0	NA	NA
104113	42	31-Oct-16	0	0	0	NA	NA
104114	10	31-Oct-16	0	0	0	NA	NA
104115	51	31-Oct-16	0	0	0	NA	NA
104116	5	31-Oct-16	0	0	0	NA	NA
104117	9	1-Nov-16	0	0	0	NA	NA
104118	7	1-Nov-16	0	0	0	NA	NA
104119	21	1-Nov-16	0	0	0	NA	NA
104120	52	1-Nov-16	0	0	0	NA	Negative
104121	2	1-Nov-16	0	0	0	NA	NA
104122	41	1-Nov-16	0	0	0	NA	NA
104123	12	1-Nov-16	0	0	0	NA	NA
104124	4	1-Nov-16	0	0	0	NA	Negative
104125	3	1-Nov-16	0	0	0	NA	NA
104126	8	1-Nov-16	0	0	0.46	NA	NA
104127	43	1-Nov-16	0	0	0	NA	NA
104128	63	1-Nov-16	0	0	0	NA	NA
104129	5	2-Nov-16	0	0	0	NA	NA
104130	25	2-Nov-16	0	0	0	NA	NA
104131	Unknown	2-Nov-16	0	0	0	NA	NA
104132	45	2-Nov-16	0	0	0	NA	Negative
104133	10	2-Nov-16	0	0	0	NA	NA
104134	73	2-Nov-16	0	0	0	NA	NA
104135	94	2-Nov-16	0	0	0	NA	NA
104340	10	23-Jan-17	0	2.56	0	NA	NA
104341	5	23-Jan-17	0	0	0	Negative	NA
104342	8	23-Jan-17	0	0	0	NA	NA
104343	7	23-Jan-17	0	0	0	NA	NA

LIMS ID	Depth to bedrock (feet)	Date	Adenovirus group A	*Bacteroidales* -like Hum M2	Human *Bacteroides*	*Cryptosporidium hominis* [a]	Human rotavirus group A genotype[b]
104344	8	23-Jan-17	0	0	0	NA	NA
104345	85	23-Jan-17	0	0	0	Negative	NA
104346	30	23-Jan-17	0	0	0	NA	NA
104347	53	23-Jan-17	0	0	0	NA	NA
104348	45	23-Jan-17	0	0	0	NA	NA
104349	57	23-Jan-17	0	0	0	Negative	NA
104350	35	23-Jan-17	0	0	0	Negative	NA
104351	20	23-Jan-17	0	6.31	0.68	NA	NA
104352	83	23-Jan-17	0	0	0	NA	NA
104353	43	24-Jan-17	0	0	0	NA	NA
104354	20	24-Jan-17	0	0	0	Negative	NA
104355	35	24-Jan-17	0	0	0	NA	NA
104356	83	24-Jan-17	0	0	0	NA	NA
104357	67	24-Jan-17	0	0	0	NA	NA
104358	3	24-Jan-17	0	23.80	1.47	NA	NA
104359	40	24-Jan-17	0	0	0	Negative	NA
104360	68	24-Jan-17	0	0	0	NA	NA
104361	30	24-Jan-17	0	0	0	Negative	NA
104380	9	27-Mar-17	0	0	0.08	NA	NA
104381	17	27-Mar-17	0	0	0.03	NA	NA
104382	8	27-Mar-17	0	0	0	NA	NA
104383	10	27-Mar-17	0	0	0	NA	NA
104384	16	27-Mar-17	0	0	0	NA	NA
104385	7	28-Mar-17	0	0	0.04	NA	NA
104386	5	28-Mar-17	0	0	0	NA	NA
104387	5	28-Mar-17	0	0	0	NA	NA
104388	2	28-Mar-17	0	0	0	NA	NA
104389	1	28-Mar-17	0	0	0.04	NA	NA
104390	10	28-Mar-17	0	0	0	NA	NA
104391	20	29-Mar-17	0	0	0	NA	NA
104392	50	29-Mar-17	0	0	0	NA	NA
104393	32	29-Mar-17	0	0	0	NA	NA

LIMS ID	Depth to bedrock (feet)	Date	Adenovirus group A	*Bacteroidales* -like Hum M2	Human *Bacteroides*	*Cryptosporidium hominis* [a]	Human rotavirus group A genotype[b]
104394	40	29-Mar-17	0	0	0	NA	NA
104395	15	27-Mar-17	0	0	0.03	NA	Negative
104396	5	27-Mar-17	0	0	0	NA	NA
104397	50	27-Mar-17	0	0	0.03	NA	NA
104398	45	27-Mar-17	0	0	0	NA	NA
104399	17.5	27-Mar-17	0	0	0.03	NA	NA
104400	33	28-Mar-17	0	0	0	Negative	NA
104401	3	28-Mar-17	0	1049.85	33.58	NA	Negative
104402	64	28-Mar-17	0	0	0	NA	NA
104403	17	28-Mar-17	0	0	0.69	NA	NA
104404	47	28-Mar-17	0	0	0	NA	NA
104405	10	28-Mar-17	0	0	0	NA	Negative
104406	95	29-Mar-17	0	0	0	NA	NA
104407	41	29-Mar-17	0	0	0	NA	NA
104408	55	29-Mar-17	0	0	0	Negative	Negative
104409	53	29-Mar-17	0	0	0	NA	NA

[a]Only samples positive for the general *Cryptosporidium* spp. assay were tested using the species-specific tests. Qualitative test.

[b]Only samples positive for the general rotavirus group A assays were genotyped. G1P[8] and G10P[11] are considered human- and bovine-specific genotypes, respectively. Qualitative test.

Bovine-specific microbial targets

Results for microbial analysis of well samples (genomic copies L^{-1} except for qualitative tests). Bovine-specific microbial targets not shown were not detected in any samples. NA, not analyzed; Unknown, well construction reports not available.

LIMS ID	Depth to bedrock (feet)	Date	*Bacteroidales* - like Cow M2	*Bacteroidales* - like Cow M3	Ruminant *Bacteroides*	Bovine enterovirus	Bovine polyomavirus	Bovine rotavirus group A genotype[b]
103327	70	18-Apr-16	0	0	0	0	0	NA
103328	143	18-Apr-16	0	0	0	0	0	NA
103329	100	18-Apr-16	0	0	0	0	0	NA
103330	3	19-Apr-16	0	0	0	0	0	NA
103331	11	19-Apr-16	0	0	0	0	0	NA
103332	80	19-Apr-16	0	0	0	0	0	NA
103333	10	19-Apr-16	0	0	0	0	0	NA
103334	14	19-Apr-16	0	0	0	0	0	NA
103335	10	19-Apr-16	0	0	0	0	0	Negative
103336	8	20-Apr-16	0	0	0	0	0	NA
103337	7	20-Apr-16	0	0	0	0	0	NA
103338	11	19-Apr-16	0	0	0	0	0	NA
103339	11	19-Apr-16	0	0	1.31	0	3.42	Negative
103340	45	19-Apr-16	0	0	1.65	0	0	NA
103341	12	19-Apr-16	0	0	4.20	0	0	NA
103342	11	18-Apr-16	0	0	0	0	0	NA
103343	2	18-Apr-16	0	0	0	0	0	NA
103344	120	18-Apr-16	0	0	0	0	0	NA
103345	15	20-Apr-16	0	0	0	0	0	NA
103346	15	20-Apr-16	0	0	0	0	0	NA
103347	14	20-Apr-16	0	0	0	0	0	Negative
103348	25	20-Apr-16	0	0	0	0	0	NA
103349	73	20-Apr-16	0	0	0	0	0	Negative
103350	35	21-Apr-16	0	0	0	0	0	NA
103351	58	21-Apr-16	0	0	0	0	0	NA
103352	30	21-Apr-16	0	0	0	0	0	NA

LIMS ID	Depth to bedrock (feet)	Date	*Bacteroidales* - like Cow M2	*Bacteroidales* - like Cow M3	Ruminant *Bacteroides*	Bovine enterovirus	Bovine polyomavirus	Bovine rotavirus group A genotype[b]
103353	15	21-Apr-16	0	0	0	1.52	4.06	Positive
103354	6	21-Apr-16	0	0	0	0	1.15	Positive
103355	21	22-Apr-16	0	0	0	0	0	Negative
103356	55	22-Apr-16	0	0	0	0	0	NA
103651	70	1-Aug-16	0	0	0	0	0	NA
103652	153	1-Aug-16	0	0	0	0	0	NA
103653	13	1-Aug-16	0	0	0	0	0	NA
103654	42	1-Aug-16	0	0	0	0	0	NA
103655	41	1-Aug-16	0	0	0	0	5.57	Positive
103656	12	1-Aug-16	0	0	0	0	0	NA
103657	7	1-Aug-16	0	0	0	0	0	NA
103658	57	1-Aug-16	0	0	0	0	0	NA
103659	20	1-Aug-16	0	0	0	0	0	NA
103660	45	1-Aug-16	0	0	0	0	0	NA
103661	5	2-Aug-16	914.59	49817.63	42397.98	0	451.24	Positive
103662	5	2-Aug-16	0	0	0.56	0	0	NA
103663	5	2-Aug-16	0	0	0	0	0	NA
103664	5	2-Aug-16	0	3.23	0.77	0	0	NA
103665	3	2-Aug-16	0	0	0	0	0	NA
103666	5	2-Aug-16	0	0	0	0	0	NA
103667	59	2-Aug-16	0	0	0	0	0	NA
103668	65	2-Aug-16	0	0	0	0	0	NA
103669	30	2-Aug-16	0	0	0	0	0	NA
103670	15	2-Aug-16	0	0	0	0	0	NA
103671	31	2-Aug-16	0	2.54	0	0	0	NA
103672	8	3-Aug-16	0	0	0	0	0	NA
103673	24	3-Aug-16	0	0	0	0	0	NA
103674	4	3-Aug-16	0	0	0	0	0	NA
103675	58	3-Aug-16	0	0	0	0	0	NA
103676	80	3-Aug-16	0	0	0	0	0	NA
103677	80	3-Aug-16	0	0	0	0	0	NA
103678	6	3-Aug-16	0	0	0	0	0	NA

LIMS ID	Depth to bedrock (feet)	Date	*Bacteroidales* - like Cow M2	*Bacteroidales* - like Cow M3	Ruminant *Bacteroides*	Bovine enterovirus	Bovine polyomavirus	Bovine rotavirus group A genotype[b]
103679	63	3-Aug-16	0	0	0	0	0	NA
104109	10	31-Oct-16	0	0	0.53	0	0	NA
104110	10	31-Oct-16	28.52	343.79	1056.24	0	37.45	Positive
104111	42	31-Oct-16	0	0	0	0	0	NA
104112	30	31-Oct-16	0	0	0.61	0	0.44	NA
104113	42	31-Oct-16	0	0	0.62	0	0	NA
104114	10	31-Oct-16	0	0	0	0	0	NA
104115	51	31-Oct-16	0	0	0.36	0	0	NA
104116	5	31-Oct-16	0	0	0.55	0	0.41	NA
104117	9	1-Nov-16	0	0	0	0	0	NA
104118	7	1-Nov-16	0	0	0.65	0	0	NA
104119	21	1-Nov-16	0	0	0	0	0	NA
104120	52	1-Nov-16	0	0	0	0	0	Positive
104121	2	1-Nov-16	0	0	0.59	0	0	NA
104122	41	1-Nov-16	0	0	0.69	0	0	NA
104123	12	1-Nov-16	0	0	0	0	0	NA
104124	4	1-Nov-16	0	0	4.67	0	0	Positive
104125	3	1-Nov-16	0	0	0	0	0	NA
104126	8	1-Nov-16	0	0	0	0	0	NA
104127	43	1-Nov-16	0	0	3.13	0	0	NA
104128	63	1-Nov-16	0	0	0.53	0	0	NA
104129	5	2-Nov-16	0	0	1.80	0	0	NA
104130	25	2-Nov-16	0	0	0	0	0	NA
104131	Unknown	2-Nov-16	0	0	3.33	0	0	NA
104132	45	2-Nov-16	0	0	2.30	0	0	Positive
104133	10	2-Nov-16	0	0	0	0	0	NA
104134	73	2-Nov-16	0	0	2.44	0	0	NA
104135	94	2-Nov-16	0	0	0	0	0	NA
104340	10	23-Jan-17	0	0	22.34	0	0	NA
104341	5	23-Jan-17	0	0	4.87	0	0	NA
104342	8	23-Jan-17	0	0	0	0	0	NA
104343	7	23-Jan-17	0	0	4.84	0	0	NA

LIMS ID	Depth to bedrock (feet)	Date	*Bacteroidales* - like Cow M2	*Bacteroidales* - like Cow M3	Ruminant *Bacteroides*	Bovine enterovirus	Bovine polyomavirus	Bovine rotavirus group A genotype[b]
104344	8	23-Jan-17	0	0	0	0	0	NA
104345	85	23-Jan-17	0	0	0	0	0	NA
104346	30	23-Jan-17	0	0	0	0	0	NA
104347	53	23-Jan-17	0	0	4.59	0	0	NA
104348	45	23-Jan-17	0	0	0	0	0	NA
104349	57	23-Jan-17	0	0	0.92	0	0	NA
104350	35	23-Jan-17	0	0	1.07	0	0	NA
104351	20	23-Jan-17	0	0	0	0	0	NA
104352	83	23-Jan-17	0	0	0	0	0	NA
104353	43	24-Jan-17	0	0	0.83	0	0	NA
104354	20	24-Jan-17	0	0	0.74	0	0	NA
104355	35	24-Jan-17	0	0	0	0	0	NA
104356	83	24-Jan-17	0	0	0	0	0	NA
104357	67	24-Jan-17	0	0	0	0	0	NA
104358	3	24-Jan-17	0	0	4.21	0	0	NA
104359	40	24-Jan-17	0	0	0.76	0	0	NA
104360	68	24-Jan-17	0	0	0	0	0	NA
104361	30	24-Jan-17	0	0	1.12	0	0	NA
104380	9	27-Mar-17	0	0	0	0	0	NA
104381	17	27-Mar-17	0	0	0.55	0	0	NA
104382	8	27-Mar-17	0	0	0	0	0	NA
104383	10	27-Mar-17	0	0	0	0	0	NA
104384	16	27-Mar-17	0	0	0	0	0	NA
104385	7	28-Mar-17	0	0	0	0	0	NA
104386	5	28-Mar-17	0	0	0	0	0	NA
104387	5	28-Mar-17	0	0	0	0	0	NA
104388	2	28-Mar-17	0	0	0	0	0	NA
104389	1	28-Mar-17	0	0	0	0	0	NA
104390	10	28-Mar-17	0	0	0	0	0	NA
104391	20	29-Mar-17	0	0	0	0	0	NA
104392	50	29-Mar-17	0	0	0	0	0	NA
104393	32	29-Mar-17	0	0	0	0	0	NA

LIMS ID	Depth to bedrock (feet)	Date	*Bacteroidales* - like Cow M2	*Bacteroidales* - like Cow M3	Ruminant *Bacteroides*	Bovine enterovirus	Bovine polyomavirus	Bovine rotavirus group A genotype[b]
104394	40	29-Mar-17	0	0	0	0	0	NA
104395	15	27-Mar-17	0	0	3.47	0	0	Positive
104396	5	27-Mar-17	0	0	0	0	0	NA
104397	50	27-Mar-17	0	0	0	0	0	NA
104398	45	27-Mar-17	0	0	0	0	0	NA
104399	17.5	27-Mar-17	0	0	0	0	0	NA
104400	33	28-Mar-17	0	0	0	0	0	NA
104401	3	28-Mar-17	0	0	0	0	0	Positive
104402	64	28-Mar-17	0	0	0	0	0	NA
104403	17	28-Mar-17	0	0	0	0	0	NA
104404	47	28-Mar-17	0	0	0	0	0	NA
104405	10	28-Mar-17	0	0	0	0	0	Positive
104406	95	29-Mar-17	0	0	0.37	0	0	NA
104407	41	29-Mar-17	0	0	0	0	0	NA
104408	55	29-Mar-17	0	0	0	0	0	Positive
104409	53	29-Mar-17	0	0	0	0	0	NA

[a]Only samples positive for the general *Cryptosporidium* spp. assay were tested using the species-specific tests. Qualitative test.

[b]Only samples positive for the general rotavirus group A assays were genotyped. G1P[8] and G10P[11] are considered human- and bovine-specific genotypes, respectively. Qualitative test.

Not host-specific microbial targets--Part 1

Results for microbial analysis of well samples (genomic copies L^{-1} except for qualitative tests). Non-specific microbial targets not shown were not detected in any samples. EHEC, enterohemorrhagic *E. coli* ; NA, not analyzed; Unknown, well construction reports not available.

LIMS ID	Depth to bedrock (ft)	Date	*Campylobacter jejuni*	*Cryptosporidium* spp.	EHEC (*eae* gene)	EHEC (*stx1* gene)	EHEC (*stx2* gene)	*Giardia lamblia* group B	Pepper mild mottle virus
103327	70	18-Apr-16	0	0	0	0	0	0	0
103328	143	18-Apr-16	0	0	0	0	0	0	0
103329	100	18-Apr-16	0	0	0	0	0	0	0
103330	3	19-Apr-16	0	0	0	0	0	0	0
103331	11	19-Apr-16	0	0.01	0	0	0	0	0
103332	80	19-Apr-16	0	0	0	0	0	0	0
103333	10	19-Apr-16	0	0.17	0	0	0	0	0
103334	14	19-Apr-16	0	0	0	0	0	0	0
103335	10	19-Apr-16	0	0	0	0	0	0	0
103336	8	20-Apr-16	0	0.02	0	0	0	0	0
103337	7	20-Apr-16	0	0.02	0	0	0	0	0
103338	11	19-Apr-16	0	0	0	0	0	0	2.32
103339	11	19-Apr-16	0	0	0	0	0	0	44.17
103340	45	19-Apr-16	0	0	0	0	0	0	0
103341	12	19-Apr-16	0	0	0	0	0	0	0
103342	11	18-Apr-16	0	0	0	0	0	0	0
103343	2	18-Apr-16	0	0	0	0	0	0	0
103344	120	18-Apr-16	0	0	0	0	0	0	0
103345	15	20-Apr-16	0	0	0	0	0	0	0
103346	15	20-Apr-16	0	0	0	0	0	0	2.09
103347	14	20-Apr-16	0	0	0	0	0	0	0
103348	25	20-Apr-16	0	0	0	0	0	0	0
103349	73	20-Apr-16	0	0	0	0	0	0	0
103350	35	21-Apr-16	0	0	0	0	0	0	0
103351	58	21-Apr-16	0	0.02	0	0	0	0	0
103352	30	21-Apr-16	0	0	0	0	0	0	0

LIMS ID	Depth to bedrock (ft)	Date	*Campylobacter jejuni*	*Cryptosporidium* spp.	EHEC (*eae* gene)	EHEC (*stx1* gene)	EHEC (*stx2* gene)	*Giardia lamblia* group B	Pepper mild mottle virus
103353	15	21-Apr-16	0	0	0	0	0	0	3.11
103354	6	21-Apr-16	0	0	0	0	0	0	2.55
103355	21	22-Apr-16	0	0	0	0	0	0	0
103356	55	22-Apr-16	0	0	0	0	0	0	0
103651	70	1-Aug-16	0	0	0	0	0	0	0
103652	153	1-Aug-16	0	0	0	0	0	0	0
103653	13	1-Aug-16	0	0.03	0	0	0	0	0
103654	42	1-Aug-16	0	0	0	0	0	0	0
103655	41	1-Aug-16	0	0	0	0	0	0	21.64
103656	12	1-Aug-16	0	0	0	0	0	0	0
103657	7	1-Aug-16	0	0	0	0	0	0	0
103658	57	1-Aug-16	0	0	0	0	0	0	0
103659	20	1-Aug-16	0	0	0	0	0	0	0
103660	45	1-Aug-16	0	0	0	0	0	0	0
103661	5	2-Aug-16	0	1.95	0	0	0	0	0
103662	5	2-Aug-16	0	0	0	0	0	0	0
103663	5	2-Aug-16	0	0	0	0	0	0	16.53
103664	5	2-Aug-16	0	0	0	0	0	0	21.61
103665	3	2-Aug-16	0	0	0	0	0	0	1704.50
103666	5	2-Aug-16	0	0	0	0	0	0	0
103667	59	2-Aug-16	0	0	0	0	0	0	0
103668	65	2-Aug-16	0	0	0	0	0	0	0
103669	30	2-Aug-16	0	0	0	0	0	0	0
103670	15	2-Aug-16	0	0	0	0	0	0	0
103671	31	2-Aug-16	0	0	0	0	0	0	0
103672	8	3-Aug-16	0	0	0	0	0	0	0
103673	24	3-Aug-16	0	0	0	0	0	0	0
103674	4	3-Aug-16	0	0	0	0	0	0	14.77
103675	58	3-Aug-16	0	0	4.43	0	0	0	0
103676	80	3-Aug-16	0	0	0	0	0	0	0
103677	80	3-Aug-16	0	0	0	0	0	0	0
103678	6	3-Aug-16	0	0	0	0	0	0	0

LIMS ID	Depth to bedrock (ft)	Date	*Campylobacter jejuni*	*Cryptosporidium* spp.	EHEC (*eae* gene)	EHEC (*stx1* gene)	EHEC (*stx2* gene)	*Giardia lamblia* group B	Pepper mild mottle virus
103679	63	3-Aug-16	0	0	0	0	0	0	0
104109	10	31-Oct-16	0	0	0	0	0	0	0
104110	10	31-Oct-16	0	0	0	16.47	1.38	0	0
104111	42	31-Oct-16	0	0	0	0	0	0	0
104112	30	31-Oct-16	0	0	0	0	0	0	0
104113	42	31-Oct-16	0	0	0	0	0	0	0
104114	10	31-Oct-16	0	0	0	0	0	0	0
104115	51	31-Oct-16	0	0	0	0	0	0	0
104116	5	31-Oct-16	0	0	0	0	0	0	0
104117	9	1-Nov-16	0	0	0	0	0	0	0
104118	7	1-Nov-16	0	0	0	0	0	0	11.37
104119	21	1-Nov-16	0	0	0	0	0	0	0
104120	52	1-Nov-16	0	0	0	0	0	0	0
104121	2	1-Nov-16	0	0	0	0	0	0	0
104122	41	1-Nov-16	0	0	0	0	0	0	0
104123	12	1-Nov-16	0	0	0	0	0	0	0
104124	4	1-Nov-16	0	0	0	0	0	0	0
104125	3	1-Nov-16	0	0	0	0	0	0	0
104126	8	1-Nov-16	0	0	0	0	0	0	0
104127	43	1-Nov-16	0	0	0	0	0	0	0
104128	63	1-Nov-16	0	0	0	0	0	0	0
104129	5	2-Nov-16	0	0	0	0	0	0	0
104130	25	2-Nov-16	0	0	0	0	0	0	0
104131	Unknown	2-Nov-16	0	0	0	0	0	0	0
104132	45	2-Nov-16	0	0	0	0	0	0	0
104133	10	2-Nov-16	0	0	0	0	0	0	0
104134	73	2-Nov-16	0	0	0	0	0	0	0
104135	94	2-Nov-16	0	0	0	0	0	0	0
104340	10	23-Jan-17	0	0	0	0	0	0	0
104341	5	23-Jan-17	0	0.05	0	0	0	0	0
104342	8	23-Jan-17	0	0	0	0	0	0	0
104343	7	23-Jan-17	0	0	0	0	0	0	0

LIMS ID	Depth to bedrock (ft)	Date	*Campylobacter jejuni*	*Cryptosporidium* spp.	EHEC (*eae* gene)	EHEC (*stx1* gene)	EHEC (*stx2* gene)	*Giardia lamblia* group B	Pepper mild mottle virus
104344	8	23-Jan-17	0	0	0	0	0	0	0
104345	85	23-Jan-17	0	0.05	0	0	0	0	0
104346	30	23-Jan-17	0	0	0	0	0	0	0
104347	53	23-Jan-17	0	0	0	0	0	0	0
104348	45	23-Jan-17	0	0	0	0	0	0	0
104349	57	23-Jan-17	0	0.27	0	0	0	0	0
104350	35	23-Jan-17	0	0.43	0	0	0	0	0
104351	20	23-Jan-17	0	0	0	0	0	0	0
104352	83	23-Jan-17	0	0	0	0	0	0	0
104353	43	24-Jan-17	0	0	0	0	0	0	0
104354	20	24-Jan-17	0	2.03	0	0	0	0	0
104355	35	24-Jan-17	0	0	0	0	0	0	0
104356	83	24-Jan-17	0	0	0	0	0	0	0
104357	67	24-Jan-17	0	0	0	0	0	0	0
104358	3	24-Jan-17	0.78	0	0	0	0	0	0
104359	40	24-Jan-17	0	3.17	0	0	0	0	0
104360	68	24-Jan-17	0	0	0	0	0	0	0
104361	30	24-Jan-17	0	1.24	0	0	0	0	0
104380	9	27-Mar-17	0	0	0	0	0	0	0
104381	17	27-Mar-17	0	0	0	0	0	0	0
104382	8	27-Mar-17	0	0	0	0	0	0	0
104383	10	27-Mar-17	0	0	0	0	0	0.39	0
104384	16	27-Mar-17	0	0	0	0	0	0	0
104385	7	28-Mar-17	0	0	0	0	0	0	0
104386	5	28-Mar-17	0	0	0	0	0	0	13.33
104387	5	28-Mar-17	0	0	0	0	0	0	0
104388	2	28-Mar-17	0	0	0	0	0	0	0
104389	1	28-Mar-17	0	0	0	0	0	0	0
104390	10	28-Mar-17	0	0	0	0	0	0	0
104391	20	29-Mar-17	0	0	0	0	0	0	0
104392	50	29-Mar-17	0	0	0	0	0	0	0
104393	32	29-Mar-17	0	0	0	0	0	0	0

LIMS ID	Depth to bedrock (ft)	Date	*Campylobacter jejuni*	*Cryptosporidium* spp.	EHEC (*eae* gene)	EHEC (*stx1* gene)	EHEC (*stx2* gene)	*Giardia lamblia* group B	Pepper mild mottle virus
104394	40	29-Mar-17	0	0	0	0	0	0	0
104395	15	27-Mar-17	0	0	0	0	0	0	0
104396	5	27-Mar-17	0	0	0	0	0	0	0
104397	50	27-Mar-17	0	0	0	0	0	0	0
104398	45	27-Mar-17	0	0	0	0	0	0.36	0
104399	17.5	27-Mar-17	0	0	0	0	0	0	0
104400	33	28-Mar-17	0	14.09	0	0	0	0	0
104401	3	28-Mar-17	0	0	0	0	0	0	3810.90
104402	64	28-Mar-17	0	0	0	0	0	0	0
104403	17	28-Mar-17	0	0	0	0	0	0	0
104404	47	28-Mar-17	0	0	0	0	0	0	0
104405	10	28-Mar-17	0	0	0	0	0	0	13.78
104406	95	29-Mar-17	0	0	0	0	0	0	0
104407	41	29-Mar-17	0	0	0	0	0	0	0
104408	55	29-Mar-17	0	6.07	0	0	0	0	0
104409	53	29-Mar-17	0	0	0	0	0	0	0

Not host-specific microbial targets--Part 2

Results for microbial analysis of well samples (genomic copies L^{-1} except for qualitative tests). Non-specific microbial targets not shown were not detected in any samples. NA, not analyzed; Unknown, well construction reports not available.

LIMS ID	Depth to bedrock (ft)	Date	Rotavirus group A (*VP7* gene)	Rotavirus group A (*NSP3* gene)	Rotavirus group C	*Salmonella* (*invA* gene)	*Salmonella* (*ttr* gene)	*Cryptosporidium parvum* [a]
103327	70	18-Apr-16	0	0	0	0	0	NA
103328	143	18-Apr-16	0	0	0	0	0	NA
103329	100	18-Apr-16	0	0	0	0	4.70	NA
103330	3	19-Apr-16	0	0	0	0	0	NA
103331	11	19-Apr-16	0	0	0	0	0	Positive
103332	80	19-Apr-16	0	0	0	0	0	NA
103333	10	19-Apr-16	0	0	0	5.92	10.12	Positive
103334	14	19-Apr-16	0	0	0	0	0	NA
103335	10	19-Apr-16	0	< 0.01	0	12.88	58.86	NA
103336	8	20-Apr-16	0	0	0	0	0	Positive
103337	7	20-Apr-16	0	0	0	0	0	Positive
103338	11	19-Apr-16	0	0	0	0	0	NA
103339	11	19-Apr-16	1.00	1.11	0	0	0	NA
103340	45	19-Apr-16	0	0	0	0	0	NA
103341	12	19-Apr-16	0	0	0	0.03	31.40	NA
103342	11	18-Apr-16	0	0	0	0	0	NA
103343	2	18-Apr-16	0	0	0	0	0	NA
103344	120	18-Apr-16	0	0	0	0	0	NA
103345	15	20-Apr-16	0	0	0	0	8.19	NA
103346	15	20-Apr-16	0	0	0	0	0	NA
103347	14	20-Apr-16	0	< 0.01	0	0	0	NA
103348	25	20-Apr-16	0	0	0	0	0	NA
103349	73	20-Apr-16	0	< 0.01	0	0	0	NA
103350	35	21-Apr-16	0	0	0	0	0	NA
103351	58	21-Apr-16	0	0	0	0	0	Negative
103352	30	21-Apr-16	0	0	0	0	0	NA

LIMS ID	Depth to bedrock (ft)	Date	Rotavirus group A (*VP7* gene)	Rotavirus group A (*NSP3* gene)	Rotavirus group C	*Salmonella* (*invA* gene)	*Salmonella* (*ttr* gene)	*Cryptosporidium parvum* [a]
103353	15	21-Apr-16	22.82	1.97	0	0	0	NA
103354	6	21-Apr-16	0	3.20	0	0	0	NA
103355	21	22-Apr-16	< 0.01	0.63	0	0	0	NA
103356	55	22-Apr-16	0	0	0	0	0	NA
103651	70	1-Aug-16	0	0	0	0	0	NA
103652	153	1-Aug-16	0	0	0	0	0	NA
103653	13	1-Aug-16	0	0	0	0	0	Positive
103654	42	1-Aug-16	0	0	0	0	0	NA
103655	41	1-Aug-16	0	8.97	0	0	0	NA
103656	12	1-Aug-16	0	0	0	0	0	NA
103657	7	1-Aug-16	0	0	0	0	0	NA
103658	57	1-Aug-16	0	0	0	0	0	NA
103659	20	1-Aug-16	0	0	0	0	0	NA
103660	45	1-Aug-16	0	0	0	0	0	NA
103661	5	2-Aug-16	< 0.01	4481.02	1301.17	0	0	Positive
103662	5	2-Aug-16	0	0	0	0	0	NA
103663	5	2-Aug-16	0	0	0	0	0	NA
103664	5	2-Aug-16	0	0	0	0	0	NA
103665	3	2-Aug-16	0	0	50.24	0	0	NA
103666	5	2-Aug-16	0	0	0	0	0	NA
103667	59	2-Aug-16	0	0	0	0	0	NA
103668	65	2-Aug-16	0	0	0	0	0	NA
103669	30	2-Aug-16	0	0	0	0	0	NA
103670	15	2-Aug-16	0	0	0	0	0	NA
103671	31	2-Aug-16	0	0	0	0	0	NA
103672	8	3-Aug-16	0	0	0	0	0	NA
103673	24	3-Aug-16	0	0	0	0	0	NA
103674	4	3-Aug-16	0	0	0	0	0	NA
103675	58	3-Aug-16	0	0	0	0	0	NA
103676	80	3-Aug-16	0	0	0	0	0	NA
103677	80	3-Aug-16	0	0	0	0	0	NA
103678	6	3-Aug-16	0	0	0	0	0	NA

LIMS ID	Depth to bedrock (ft)	Date	Rotavirus group A (*VP7* gene)	Rotavirus group A (*NSP3* gene)	Rotavirus group C	*Salmonella* (*invA* gene)	*Salmonella* (*ttr* gene)	*Cryptosporidium parvum* [a]
103679	63	3-Aug-16	0	0	0	0	0	NA
104109	10	31-Oct-16	0	0	0	0	0	NA
104110	10	31-Oct-16	732.44	352.41	44.84	0	0	NA
104111	42	31-Oct-16	0	0	0	0	0	NA
104112	30	31-Oct-16	0	0	0	0	0	NA
104113	42	31-Oct-16	0	0	0	0	0	NA
104114	10	31-Oct-16	0	0	0	0	0	NA
104115	51	31-Oct-16	0	0	0	0	0	NA
104116	5	31-Oct-16	0	0	0	0	0	NA
104117	9	1-Nov-16	0	0	0	0	0	NA
104118	7	1-Nov-16	0	0	0	0	0	NA
104119	21	1-Nov-16	0	0	0	0	0	NA
104120	52	1-Nov-16	0	2.09	0	0	0	NA
104121	2	1-Nov-16	0	0	0	0	0	NA
104122	41	1-Nov-16	0	0	0	0	0	NA
104123	12	1-Nov-16	0	0	0	0	0	NA
104124	4	1-Nov-16	271.50	91.03	0	0	0	NA
104125	3	1-Nov-16	0	0	0	0	0	NA
104126	8	1-Nov-16	0	0	0	0	0	NA
104127	43	1-Nov-16	0	0	0	0	0	NA
104128	63	1-Nov-16	0	0	0	0	0	NA
104129	5	2-Nov-16	0	0	0	0	0	NA
104130	25	2-Nov-16	0	0	0	0	0	NA
104131	Unknown	2-Nov-16	0	0	0	0	0	NA
104132	45	2-Nov-16	3.62	3.24	0	0	0	NA
104133	10	2-Nov-16	0	0	0	0	0	NA
104134	73	2-Nov-16	0	0	0	0	0	NA
104135	94	2-Nov-16	0	0	0	0	0	NA
104340	10	23-Jan-17	0	0	0	0	0	NA
104341	5	23-Jan-17	0	0	0	0	0	Positive
104342	8	23-Jan-17	0	0	0	0	0	NA
104343	7	23-Jan-17	0	0	0	0	0	NA

LIMS ID	Depth to bedrock (ft)	Date	Rotavirus group A (*VP7* gene)	Rotavirus group A (*NSP3* gene)	Rotavirus group C	*Salmonella* (*invA* gene)	*Salmonella* (*ttr* gene)	*Cryptosporidium parvum* [a]
104344	8	23-Jan-17	0	0	0	0	0	NA
104345	85	23-Jan-17	0	0	0	0	0	Positive
104346	30	23-Jan-17	0	0	0	0	0	NA
104347	53	23-Jan-17	0	0	0	0	0	NA
104348	45	23-Jan-17	0	0	0	0	0	NA
104349	57	23-Jan-17	0	0	0	0	0	Positive
104350	35	23-Jan-17	0	0	0	0	0	Negative
104351	20	23-Jan-17	0	0	0	0	0	NA
104352	83	23-Jan-17	0	0	0	0	0	NA
104353	43	24-Jan-17	0	0	0	0	0	NA
104354	20	24-Jan-17	0	0	0	0	0	Positive
104355	35	24-Jan-17	0	0	0	0	0	NA
104356	83	24-Jan-17	0	0	0	0	0	NA
104357	67	24-Jan-17	0	0	0	0	0	NA
104358	3	24-Jan-17	0	0	0	0	0	NA
104359	40	24-Jan-17	0	0	0	0	0	Positive
104360	68	24-Jan-17	0	0	0	0	0	NA
104361	30	24-Jan-17	0	0	0	0	0	Negative
104380	9	27-Mar-17	0	0	0	0	0	NA
104381	17	27-Mar-17	0	0	0	0	0	NA
104382	8	27-Mar-17	0	0	0	0	0	NA
104383	10	27-Mar-17	0	0	0	0	0	NA
104384	16	27-Mar-17	0	0	0	0	0	NA
104385	7	28-Mar-17	0	0	0	0	0	NA
104386	5	28-Mar-17	0	0	0	0	0	NA
104387	5	28-Mar-17	0	0	0	0	0	NA
104388	2	28-Mar-17	0	0	0	0	0	NA
104389	1	28-Mar-17	0	0	0	0	0	NA
104390	10	28-Mar-17	0	0	0	0	0	NA
104391	20	29-Mar-17	0	0	0	0	0	NA
104392	50	29-Mar-17	0	0	0	0	0	NA
104393	32	29-Mar-17	0	0	0	0	0	NA

LIMS ID	Depth to bedrock (ft)	Date	Rotavirus group A (*VP7* gene)	Rotavirus group A (*NSP3* gene)	Rotavirus group C	*Salmonella* (*invA* gene)	*Salmonella* (*ttr* gene)	*Cryptosporidium parvum* [a]
104394	40	29-Mar-17	0	0	0	0	0	NA
104395	15	27-Mar-17	0	38.26	0	0	0	NA
104396	5	27-Mar-17	0	0	0	0	0	NA
104397	50	27-Mar-17	0	0	0	0	0	NA
104398	45	27-Mar-17	0	0	0	0	0	NA
104399	17.5	27-Mar-17	0	0	0	0	0	NA
104400	33	28-Mar-17	0	0	0	0	0	Positive
104401	3	28-Mar-17	0	5.03	0	0	0	NA
104402	64	28-Mar-17	0	0	0	0	0	NA
104403	17	28-Mar-17	0	0	0	0	0	NA
104404	47	28-Mar-17	0	0	0	0	0	NA
104405	10	28-Mar-17	0	15.92	0	0	0	NA
104406	95	29-Mar-17	0	0	0	0	0	NA
104407	41	29-Mar-17	0	0	0	0	0	NA
104408	55	29-Mar-17	0	14.27	0	0	0	Positive
104409	53	29-Mar-17	0	0	0	0	0	NA

[a]Only samples positive for the general *Cryptosporidium* spp. assay were tested using the species-specific tests. Qualitative test.

< 0.01 indicates that the microorganism was detected below 0.01 genomic copies L^{-1} and was rounded to 0.01 for presentation.

Kewaunee County Groundwater Study

Quality control/quality assurance for qPCR analysis

A. Standard curve performance parameters
B. 95% limits of detection (LOD) for qPCR assays determined by the probit approach
C. Negative controls--Part 1
    1. Extraction
    2. Reverse transcription
D. Negative controls--Part 2
    1. qPCR
    2. Secondary concentration

Kewaunee County Groundwater Study

Standard curve performance parameters. N/A, not applicable; assays used qualitatively

Assay	Curve #	Efficiency[a]	Mean square error	r^{2b}	Highest C_q measured
Adenovirus group A	1	0.931	0.0166	0.9834	38.93
	2	1.007	0.0386	0.9614	40
	3	0.975	0.019	0.981	40
Adenovirus group B	1	0.922	0.117	0.883	40
	2	0.897	0.101	0.899	40
	3	0.911	0.101	0.899	40
Adenovirus groups C,D,F	1	0.864	0.0446	0.9554	40
	2	0.949	0.0158	0.9842	40
	3	0.892	0.0291	0.9709	40
Bacteroidales -like Cow M2	1	0.982	0.0814	0.9186	40
	2	0.994	0.0096	0.9904	39.5
Bacteroidales -like Cow M3	1	0.964	0.0107	0.9893	37.18
	2	0.977	0.105	0.895	38.46
	3	0.997	0.0402	0.9598	37.67
Bacteroidales -like Hum M2	1	1.014	0.0516	0.9484	38.22
	2	0.994	0.0096	0.9904	38.74
Bovine adenovirus	1	1.022	0.0535	0.9465	40
	2	0.971	0.118	0.882	40
Ruminant *Bacteroides*	1	0.886	0.0269	0.9731	40
	2	0.938	0.00611	0.99389	40
	3	0.897	0.00191	0.99809	40
Bovine enterovirus	1	0.982	0.00769	0.99231	40
	2	0.985	0.0122	0.9878	40
Bovine polyomavirus	1	0.925	0.00663	0.99337	39.22
	2	0.895	0.00852	0.99148	40
	3	0.913	0.0034	0.9966	40
Bovine viral diarrhea virus type 1	1	1.003	0.12	0.88	37.24
	2	0.959	0.0764	0.9236	39.38
Bovine viral diarrhea virus type 2	1	1.003	0.0139	0.9861	36.98
	2	0.988	0.0874	0.9126	39.31
Campylobacter jejuni	1	1.023	0.0246	0.9754	40
	2	0.956	0.0361	0.9639	40
	3	0.897	0.0046	0.9954	40
Coronavirus	1	0.98	0.0523	0.9477	40
	2	0.963	0.0124	0.9876	40
Cryptosporidium spp.	1	0.973	0.128	0.872	38.4
	2	0.918	0.144	0.856	40
Cryptosporidium hominis	1	N/A	N/A	N/A	N/A
	2	N/A	N/A	N/A	N/A
	3	N/A	N/A	N/A	N/A
Cryptosporidium parvum	1	N/A	N/A	N/A	N/A
	2	N/A	N/A	N/A	N/A

Assay	Curve #	Efficiency[a]	Mean square error	r^{2b}	Highest C_q measured
	3	N/A	N/A	N/A	N/A
Enterrohemorrhagic *E. coli* (*eae* gene)	1	0.939	0.00362	0.99638	39.16
	2	0.952	0.0101	0.9899	40
	3	0.95	0.0079	0.9921	40
Enterrohemorrhagic *E. coli* (*stx 1* gene)	1	0.913	0.0136	0.9864	40
	2	0.958	0.00473	0.99527	40
	3	0.938	0.0082	0.9918	40
Enterrohemorrhagic *E. coli* (*stx 2* gene)	1	0.999	0.0315	0.9685	36.82
	2	0.945	0.0162	0.9838	38.81
	3	0.971	0.011	0.989	40
Human enterovirus	1	0.943	0.0332	0.9668	39.01
	2	0.931	0.0221	0.9779	40
	3	0.946	0.0085	0.9915	37.66
Giardia lamblia group B	1	0.960	0.004	0.996	40
Human *Bacteroides*	1	1.023	0.0232	0.9768	39.41
	2	0.899	0.0112	0.9888	39.61
Norovirus genogroup I	1	0.98	0.133	0.867	39.52
	2	0.949	0.0387	0.9613	40
	3	0.981	0.0206	0.9794	40
Norovirus genogroup II	1	0.983	0.00825	0.99175	38.94
	2	0.918	0.145	0.855	40
	3	0.913	0.118	0.882	40
Pepper mild mottle virus	1	0.969	0.198	0.802	38.23
	2	1.024	0.101	0.899	40
	3	0.952	0.0712	0.9288	40
Human polyomavirus	1	0.961	0.00581	0.99419	40
	2	0.955	0.0321	0.9679	40
	3	0.933	0.0036	0.9964	39.17
Rotavirus group A (*NSP3* gene)	1	1.069	0.00909	0.99091	37.1
	2	1.008	0.0299	0.9701	40
	3	0.972	0.0171	0.9829	40
Rotavirus group A (*VP7* gene)	1	1.066	0.0283	0.9717	38.22
	2	0.948	0.0292	0.9708	39.39
Rotavirus group C	1	0.98	0.0467	0.9533	40
	2	0.884	0.026	0.974	40
Salmonella (*invA* gene)	1	1.008	0.00973	0.99027	38.1
	2	0.985	0.00525	0.99475	38.75
	3	0.948	0.0143	0.9857	40
Salmonella (*ttr* gene)	1	0.988	0.0245	0.9755	40
	2	1.027	0.015	0.985	40
	3	0.939	0.0065	0.9935	38.89

[a]Efficiency = $10^{(-1/slope)}-1$

[b]The r^2 value was calculated as one minus the mean square error determined by the LightCycler software (Roche technical support, personal communication)

95% limits of detection (LOD) for qPCR assays determined by the probit approach (CLSI 2012; Stokdyk et al. 2016). gc, genomic copies

DNA assay	Assay 95% LOD (gc reaction^{-1})	RNA assay	Assay 95% LOD (gc reaction^{-1})
Adenovirus group A	1.9	Bovine enterovirus	3.7
Adenovirus group B	1.4	Bovine viral diarrhea virus type 1	3.3
Adenovirus groups C,D,F	5.7	Bovine viral diarrhea virus type 2	5.5
Bacteroidales -like Cow M2	4.5	Coronavirus	15.1
Bacteroidales -like Cow M3	11.9	Human enterovirus	12.0
Bacteroidales -like Hum M2	3.5	Norovirus genogroup I	5.8
Bovine adenovirus	5.3	Norovirus genogroup II	3.5
Ruminant *Bacteroides*	6.1	Pepper mild mottle virus	13.1
Bovine polyomavirus	3.0	Rotavirus group A (*NSP3* gene)	3.7
Campylobacter jejuni	5.6	Rotavirus group A (*VP7* gene)	19.3
Cryptosporidium spp.	27.8	Rotavirus group C	4.5
Enterrohemorrhagic *E. coli* (*eae* gene)	3.1		
Enterrohemorrhagic *E. coli* (*stx 1* gene)	2.0		
Enterrohemorrhagic *E. coli* (*stx 2* gene)	3.8		
Giardia lamblia group B	3.8		
Human *Bacteroides*	1.7		
Human polyomavirus	2.3		
Salmonella (*invA* gene)	7.4		
Salmonella (*ttr* gene)	5.4		

CLSI (Clinical and Laboratory Standards Institute). 2012. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline, second ed. CLSI document EP17eA2.

Stokdyk J, Firnstahl AD, Spencer SK, Burch TR, Borchardt MA. 2016. Determining the 95% limit of detection for waterborne pathogen analyses from primary concentration to qPCR. Water Research 96:105-113.

Negative controls--Part 1. All controls are analyzed in duplicate.

	Extraction negative					Reverse transcription negative				
Microbial target	Apr 2016	Aug 2016	Oct 2016	Jan 2017	Mar 2017	Apr 2016	Aug 2016	Oct 2016	Jan 2017	Mar 2017
Adenovirus group A	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Adenovirus group B	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Adenovirus groups C,D,F	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Bacteroidales -like Cow M2	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Bacteroidales -like Cow M3	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Bacteroidales -like Hum M2	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Bovine adenovirus	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Ruminant *Bacteroides*	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Bovine enterovirus	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Bovine polyomavirus	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Bovine viral diarrhea virus type 1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Bovine viral diarrhea virus type 2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Campylobacter jejuni	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Coronavirus	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Cryptosporidium spp.	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Cryptosporidium hominis	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Cryptosporidium parvum	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Enterrohemorrhagic *E. coli* (*eae* gene)	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Enterrohemorrhagic *E. coli* (*stx 1* gene)	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Enterrohemorrhagic *E. coli* (*stx 2* gene)	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Human enterovirus	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Giardia lambia group B	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Human *Bacteroides*	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Norovirus genogroup I	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Norovirus genogroup II	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Pepper mild mottle virus	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Human polyomavirus	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Rotavirus group A (*NSP3* gene)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Rotavirus group A (*VP7* gene)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Rotavirus group C	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Salmonella (*invA* gene)	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA
Salmonella (*ttr* gene)	NEG	NEG	NEG	NEG	NEG	NA	NA	NA	NA	NA

NA, not applicable; reverse transcription is only performed for organisms with an RNA genome.

Negative controls--Part 2. All controls are analyzed in duplicate.

	qPCR negative					Secondary concentration negative				
Microbial target	Apr 2016	Aug 2016	Oct 2016	Jan 2017	Mar 2017	Apr 2016	Aug 2016	Oct 2016	Jan 2017	Mar 2017
Adenovirus group A	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Adenovirus group B	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Adenovirus groups C,D,F	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Bacteroidales -like Cow M2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Bacteroidales -like Cow M3	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Bacteroidales -like Hum M2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Bovine adenovirus	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Ruminant *Bacteroides*	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Bovine enterovirus	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Bovine polyomavirus	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Bovine viral diarrhea virus type 1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Bovine viral diarrhea virus type 2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Campylobacter jejuni	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Coronavirus	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Cryptosporidium spp.	NEG	NEG	NEG	NEG	NEG	NEG	NEG			
Cryptosporidium hominis	NEG	NEG	NEG	NEG	NEG					
Cryptosporidium parvum	NEG	NEG	NEG	NEG	NEG					
Enterrohemorrhagic *E. coli* (*eae* gene)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Enterrohemorrhagic *E. coli* (*stx 1* gene)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Enterrohemorrhagic *E. coli* (*stx 2* gene)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Human enterovirus	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Giardia lambia group B	NEG	NEG	NEG	NEG	NEG		NEG			
Human *Bacteroides*	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		
Norovirus genogroup I	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Norovirus genogroup II	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Pepper mild mottle virus	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Human polyomavirus	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Rotavirus group A (*NSP3* gene)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Rotavirus group A (*VP7* gene)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Rotavirus group C	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG
Salmonella (*invA* gene)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Salmonella (*ttr* gene)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		NEG