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Final Report to the Wisconsin Department of Natural Resources

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Assessment of Virus Presence and Potential Virus Pathways in Deep Municipal Wells

Abstract

Among the many waterborne pathogens of humans, enteric viruses have the greatest potential to move deeply through the subsurface environment, penetrate aquitards, and reach confined aquifers. Previous research revealed the presence of viruses in water from two of three deep bedrock wells sampled in Madison, WI. Virus presence in these wells was particularly surprising because the wells were cased through a regional aquitard thought to provide protection for the wells. This present study is a follow-up to the previous work and is intended to (1) obtain a time series of virus, isotopic, and geochemical data from several municipal wells completed in a deep bedrock aquifer, (2) use these data sets to evaluate virus presence and, if present, the potential sources of the viruses and pathways to the wells, and (3) evaluate the possibility that virus transport occurs through the well casing, grout or annular space.

During 2007 and 2008 we sampled six deep municipal wells for viruses on an approximately monthly basis. Three of these wells had shallow casings, and three were cased through a regional aquitard. We also collected virus samples from local lakes and from untreated sewage and sampled groundwater and lake water for major inorganic ions and isotopes of hydrogen and oxygen.

Viruses were detected at least twice in every one of the six wells, but no well was virus-positive in every sampling round. Overall, 43 percent of the samples were virus-positive, and virus concentrations ranged from 0.00 to 6.15 genomic copies per liter (gc/l), with a mean of 0.47 gc/l. Samples from three wells were positive for virus infectivity. Lake samples were positive 78 percent of the time, and ranged from 0.00 to 27.6 gc/l, with a mean of 5.8 gc/l. Not surprisingly, Madison sewage was extremely high in viruses, with all samples positive, and concentrations ranging from about 50,000 to over two million gc/l, with a mean of 581,000 gc/l. Virus results varied significantly with time, and there is apparent correlation between virus levels in sewage, lakes, and groundwater.

Several different species (serotypes) of viruses were identified in wells, sewage, and lake water during this study, and in many cases wells and sewage contained identical virus serotypes. Detected viruses include Enteroviruses echovirus 3, echovirus 6, echovirus 11, Coxsackie A16 and B4, Adenoviruses 2, 6, 7, 41, as well as G1 norovirus and Rotovirus. The apparent correlation between viral serotypes found in sewage, lakes, and groundwater suggests very rapid transport from the sources to wells. Viral serotypes vary seasonally and annually, and so correlation between surface and subsurface serotypes would be unexpected if transport times from the surface to groundwater exceed many months. The Madison Lakes are probably not the main source of the viruses found in the wells as lake water contained some but not all of the serotypes found in the wells, and wells without lake-derived water had viruses present. Furthermore, the $^{18}O/^{2}H$ signature of water produced by these wells is not consistent with a significant lake water

component of recharge to most of the wells sampled. Virus levels in surface water were much lower than in sewage, thus significant volumes of lake water would be required to produce the virus levels measured in the wells.

The most likely source of the viruses in the wells is the leakage of untreated sewage from the Madison sewer system. Given the high concentrations (millions of genomic copies per liter) of viruses in sewage, it would take very little sewage to produce the virus concentrations observed in the wells.

Human enteric viruses might be excellent tracers of recently recharged groundwater in urban settings if virus sources exist. They have the desirable tracer characteristics of detectability over several orders of magnitude, high mobility, and are time-specific due to constantly changing serotypes. Although the presence of detectable tritium in a well is almost always an indicator of recent recharge to the well, the absence of tritium (at a detection limit of 0.8 TU) does not necessarily indicate that the well will be virus-free. In fact detection of viruses many be a far more sensitive indicator than tritium of a proportion of "young" groundwater in a well.

Table of Contents

Introduction

Virus contamination of groundwater

Among the many waterborne pathogens of humans, enteric viruses have the greatest potential to move deeply through the subsurface environment, penetrate aquitards, and reach confined aquifers. Enteric viruses are extremely small (27-75 nm), readily passing through sediment pores that would trap much larger pathogenic bacteria and protozoa. Viruses have been found in groundwater at depths of 67 m (Keswick and Gerba 1980; Robertson and Edberg 1997) and 52 m (Borchardt et al 2003) and lateral transport has been reported as far as 408 m in glacial till and 1600 m in fractured limestone (Keswick and Gerba 1980). Several recent studies have demonstrated widespread occurrence of viruses in domestic and municipal wells in the United States (Abbaszadegan et al 2003; Borchardt et al 2003; Fout et al 2003; Borchardt et al 2004), and approximately half of waterborne disease outbreaks attributable to groundwater consumption in the United States have a viral etiology (National Primary Drinking Water Regulations, 2006) . The US Environmental Protection Agency has listed several viruses on its drinking water Contaminant Candidate List, emphasizing that waterborne viruses are a research priority (http://www.epa.gov/safewater/ccl/index.html). Although the vulnerability of groundwater to virus contamination is now recognized, the occurrence of viruses in confined aquifers has rarely been explicitly investigated. In the most comprehensive groundwater-virus study to date, Abbaszadegan et al (2003) sampled 448 groundwater sites in 35 states and found 141 sites (31.5%) were positive for at least one virus type.

Previous virus sampling in the Madison area

During 2005 and 2006 we undertook initial virus sampling of three deep bedrock wells serving the city of Madison, Wisconsin (Borchardt et al. 2007a). Each of these high-capacity wells is over 700 feet deep and cased to at least 220 feet below the surface. The vertical hydraulic gradient is downward due to a major cone of depression beneath Madison. Two of the wells (wells 7 and 24) are cased through the Eau Claire shale, a regional aquitard described by Bradbury and others (1999) and thought to provide excellent protection to the underlying sandstone aquifer. A third well (well 5, now abandoned) was open both above and below the shale. Conventional wisdom suggested that viruses would not be detected in any of the three wells due to the probable long travel times from the surface to the wells, the depths of the wells, and the assumed short (six months to two years) lifetime of the viruses. The surprising result of the study was that viruses were repeatedly detected in the two wells thought to have greatest protection due to their deep casings (wells 7 and 24). Viruses were detected in 4 of 10 samples from well 7 and 3 of 10 samples from well 24 (Borchardt et al. 2007a). Moreover, five of the seven positive samples tested positive for infectivity, suggesting relatively rapid transport from the virus source to the wells. Replicate sampling and careful laboratory procedures have ruled out laboratory contamination as a source for the viruses. The human enteric viruses detected include serogroups coxsackieviruses and echoviruses as wells as poliovirus vaccine strain Sabin 1. The Madison, Wisconsin wells are typical of

wells now in use in many cities throughout Wisconsin and the United States. These high-capacity wells range in age from less than five to over 50 years and were constructed according to accepted well drilling practices, which include grouted well casing to depth. The wells produce water from one or both of two aquifers. The shallow bedrock aquifer is composed of sandstone and dolomite. The deeper bedrock aquifer is composed of sandstone. A regional aquitard, the Eau Claire aquitard, is composed of shale and siltstone, and separates the two aquifers, but may contain fractures or be absent beneath the nearby Madison lakes. Although the water utility samples the wells regularly for a long list of organic and inorganic contaminants, including bacteria, the wells are not tested for viruses, presumably because viruses have not been thought to be present in the subsurface. Our previous work in Madison shows that this assumption is false.

Understanding how the viruses moved from a near-surface source (humans) to the deep bedrock wells is critical to assessing the magnitude of the virus problem, the human health risks, and to developing remedial actions. However, based on the limited sampling to date it was difficult to elucidate a pathway or mechanism to deliver the viruses to the wells. Given that the viruses originated near the land surface there are four conceptual models of virus transport to the confined aquifer: (1) transport through the aquitard by porous-media flow; (2) transport by porous-media flow around the edge of the aquitard or through nearby "windows" or breaches in the aquitard, including local lakes; (3) transport by rapid flow through fractures in the aquitard or through cross-connecting nearby wells; and (4) transport by rapid flow along the well annulus through damaged, deteriorated, or poorly installed grout or breaches in the well casing.

Knowledge about the local hydrogeologic system and virus survival time makes some of these conceptual models more probable than others. The only environmental source of human enteric viruses is human fecal waste, and within the city limits of Madison human fecal waste is presumably only present in sanitary sewers. From this presumed point of entry, viruses must travel viruses downward over 200 feet though the upper sandstone aquifer, an additional 10 to 30 feet downward through the Eau Claire aquitard to reach the top of the Mount Simon aquifer. Once in the Mt Simon aquifer the viruses must move laterally some unknown distance to the production wells. Based on such a travel path, pathway 1 seems very unlikely because travel times would likely be far longer than the six months to two years these viruses can survive in the environment (Yates et al 1985, John and Rose 2005, Schijven et al 2006). Transport pathways 2 and 3, through breaches in the aquitard or through fracture pathways, are more probable, but one must still account for the long travel distance through the upper sandstone aquifer above the aquitard. Pathway 4, transport down the annulus of the well itself through deteriorated or poorly installed grout or through breaches in the well casing, seems the most likely mechanism for virus transport. This pathway could produce rapid downward movement of water with delivery directly to the well bore. Although the three wells tested in the previous study were drilled, cased, and grouted according to accepted practice it is impossible to confirm that the grout has remained intact over the entire length of the casing in wells that are now 27 years (Well 24) and 41 years old (Well 7).

During the previous virus study in Madison (Borchardt and others, 2007a) we collected limited samples for analysis of environmental isotopes. Tritium, deuterium, and oxygen-18 have long been used in hydrogeologic studies to help distinguish groundwater age and source areas (Clark and Fritz, 1997). Previous tritium data suggested that Madison wells 5 and 24 produce relatively "old" groundwater (little or no tritium content), while well 7 produces "younger" water (tritium near the levels in modern precipitation). We hoped that oxygen-18/deuterium data would be useful in confirming or discarding flow paths that include surface water contributions from the nearby Madison lakes. However, the oxygen-18/deuterium data were not definitive, possibly due to subsurface mixing and or seasonal variations in the ^{18}O concentrations in precipitation. Hunt and others (2005) showed that a time series of ^{18}O/deuterium rations is necessary to unambiguously distinguish surface-water inputs from terrestrial recharge; the previous study obtained only single isotope samples from each well.

In a population, like that of Madison, various viruses have a temporal signature, arriving and disappearing from the population over the course of a year. For example, late summer and autumn is the time of year for enterovirus infections in Wisconsin. Infected people in Madison shed enteroviruses, which are flushed through the sanitary sewers to the sewage treatment plant. There are 64 serotypes of enteroviruses and only a couple of serotypes are present in the population at any given time. One enterovirus strain will be dominant in Madison in August and a different strain dominant in October, which will differ from the strains present the following year. These temporal patterns and changes in the relative abundance of viruses and virus serotypes have been documented in wastewater for enteroviruses and adenoviruses (Sedmak et al. 2003; Sedmak et al. 2005; Carducci et al. 2006). Add in all the other human enteric viruses that can be detected and sequenced, and the viruses in the wastewater shed by the population become a "virus signature" for that point in time. The signatures can be used as a tracer of virus movement from source(s) (presumably leaking sanitary sewers or lake water) to the study wells. Using deuterium and O-18 as an isotope signature, Hunt et al. (2005) used a similar conceptual approach for estimating the time of travel of river water through the riverbank to adjacent wells.

The virus signature has several information components: (1) the general type of virus (e.g., norovirus or enterovirus), which gives information on the size, charge, and "lifespan" of the virus particle; (2) the quantity of virus (e.g. genomic copies/liter), which provides a time-varying signal whose amplitude may be observed along the suspected transport route and well; and (3) the virus serotype or nucleic acid molecular fingerprint, which can be tracked over time in wastewater and well water and, in conjunction with virus quantity, gives information on transport time. For example, the presence of echovirus 18 in wastewater in October followed by its detection in a well in December might suggest a 2 month time of travel from the source(s) to the well, but could also suggest a 14-month travel time if echovirus 18 had been present the previous October. This is why obtaining a measure of virus variation in the source water is critical. Of course, one would want to base time estimates on multiple virus detections and samples. Working with these virus signature components as separate lines of evidence, or perhaps combining them using multivariate techniques such as cluster analysis or

multidimensional scaling, and corroborated with isotope and chloride data, we believe will allow powerful inferences about virus transport routes to the drinking water wells.

One limitation of this approach is that for reasons not well understood among environmental virologists, there is substantial spatial and temporal variability in virus occurrence in groundwater. One approach to compensate for spatial variability is to take large sample volumes (~ 1000 liters) as commonly practiced. An approach to compensate for temporal variability is to increase sampling frequency, which is now affordable. The benefit of collecting numerous large sample volumes is that, spatial and temporal variability notwithstanding, the underlying biological and hydrogeologic patterns begin to emerge. A similar approach was recently reported by Borchardt et al (2007b) where several hundred water samples for viruses allowed the study team to quantify virus intrusions into municipal drinking water distribution systems.

Project objectives and scope

The objectives of this project are (1) to obtain a time series of virus, isotopic, and geochemical data from several municipal wells completed in a deep bedrock aquifer, (2) to use these data sets to evaluate virus presence and, if present, the potential sources of the viruses and pathways to the wells, and (3) to evaluate the possibility that virus transport occurs through the well casing, grout or annular space. This one-year project was entirely conducted in Madison WI, using wells owned and operated by the Madison Water Utility.

Acknowledgments

This project was funded by the Wisconsin Department of Natural Resources through the State of Wisconsin Groundwater Joint Solicitation Program. The Madison Water Utility participated in the project by providing information about wells and access to wells for sampling. WGNHS Geotechnician Peter Chase spent many hours in sample collection. Susan Spencer and Phil Bertz of the Marshfield Clinic performed the virus analyses. The Madison Metropolitan Sewerage District provided samples of sewage influent.

Procedures and Methods

Selection of wells for sampling

The Madison Water Utility currently operates 27 deep high-capacity wells completed in bedrock aquifers. The wells draw from a Cambrian-age sandstone aquifer underlying the city (Bradbury and others, 1999). This aquifer lies beneath 30 to 100 feet of glacially-deposited sand and gravel and lake sediment. Most of these high-capacity wells are over 700 feet deep and cased to about 200 feet below the surface. Water enters the wells through open boreholes in the rock below the casing. Although the well casings are supposed to be sealed to the surrounding geologic materials using cement grout, the integrity of these grout seals is often suspect and nearly impossible to test. About one-third of the wells are cased through the Eau Claire shale, a regional aquitard described by Bradbury and others (1999) and thought to provide excellent protection to the underlying sandstone aquifer. The other two-thirds of the wells, most of which are the older wells, are "cross-connecting"; open both above and below the shale or drilled in places where the shale is thin or absent. These wells are more vulnerable to contamination than the deeply cased wells.

The funding level for this project prohibited sampling of all 27 Madison wells. In order to understand the scope of the virus problem we decided initially to sample 11 wells and then sample fewer wells in subsequent rounds. Our rationale was to insure that we were working with some virus-positive wells and that we had a variety of well construction and well locations. We chose six wells reported to be multi-aquifer wells (open both above and below the Eau Claire aquitard) and five wells reported to be cased through the aquitard. We sampled surface water from Lakes Mendota, Monona, and Wingra as well as clarified sewage influent at the Madison Metropolitan Sewage District. Samples were also collected for inorganic chemistry and isotope analyses. Following the initial sampling rounds we chose six wells for repeated monthly sampling.

Figure 1 shows the spatial distribution of wells, and figures 2 and 3 show the construction of the sampled wells. Figure 2 also shows the typical conceptualization of subsurface hydrostratigraphy in Madison. The complex geologic stratigraphy is simplified to consist of upper glacial materials (till, sand and gravel, or lake sediment) covering a shallow bedrock aquifer composed of sandstone and dolomite. Shale of the Eau Claire Formation forms a regional aquitard and separates the upper bedrock aquifer from a deep bedrock aquifer composed of sandstone. Crystalline PreCambrian rock bounds the bottom of the system. Vertical hydraulic gradients in groundwater beneath the city are downward due to a regional cone of depression beneath the Madison metropolitan area (Bradbury and others, 1999). Figure 2 shows this diagrammatically – the potentiometric surface of the deep sandstone aquifer is lower than the water table in the shallow aquifer. In this situation water and any contaminants in the upper aquifer have the hydraulic potential to move vertically downward and reach the underlying deep aquifer. Wells are typically cased and grouted through the upper geologic units and consist of open holes below the casing.

Construction diagrams of individual wells (figures 2 and 3) show the variation in well construction, thickness of layers, and presence and thickness of the aquitard. Wells 11, 12, 13, 16, and 17 are termed "cross-connected" wells because either the aquitard is missing completely (wells 11, 13) or the well casings do not extend through the aquitard (wells 12, 16, 17) and the open hole provides a vertical conduit between the upper and lower aquifers. These cross-connecting wells are much more susceptible to contamination than "confined" wells (wells, 7, 8, 19, 24, 28, and 30), in which the casing extends through the aquitard.

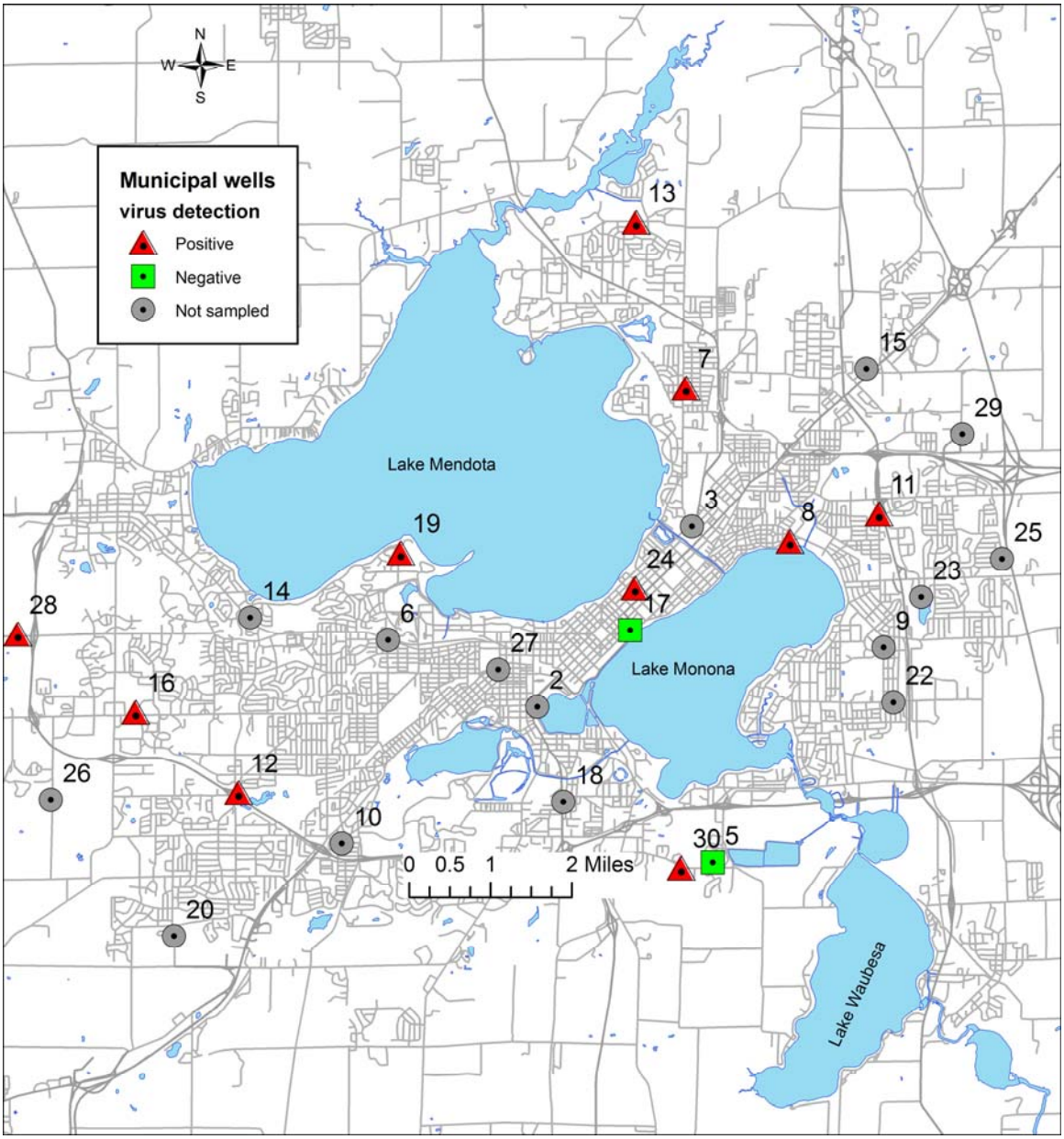

Figure 1. Location of sampled wells and virus detections. "Positive" denotes a well testing positive for viruses on at least one date. Numbers refer to Madison Water Utility well numbers.

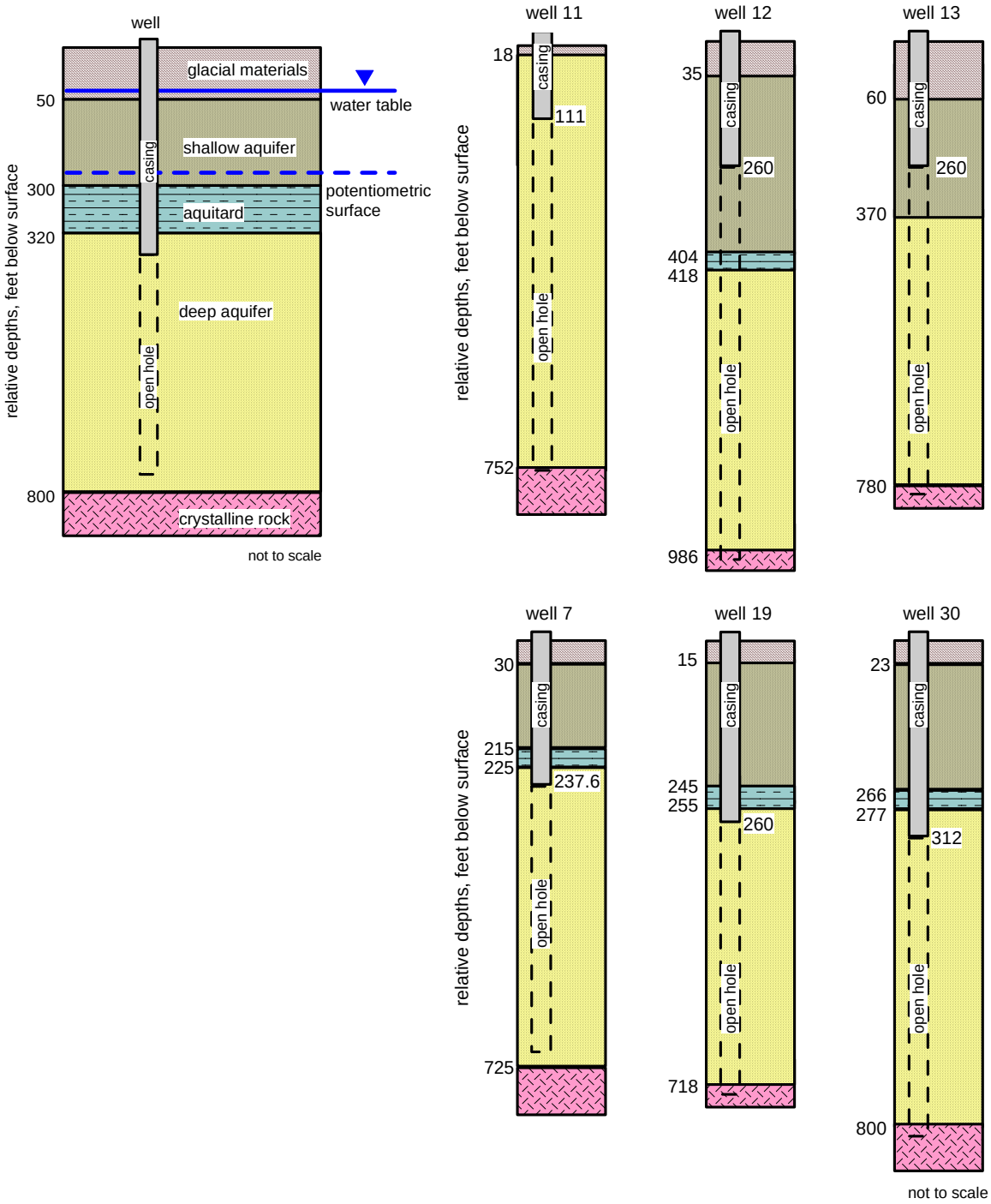

Figure 2. Construction details of the municipal wells sampled throughout the project. Diagram at upper left shows typical hydrostratigraphy and well construction for the Madison area.

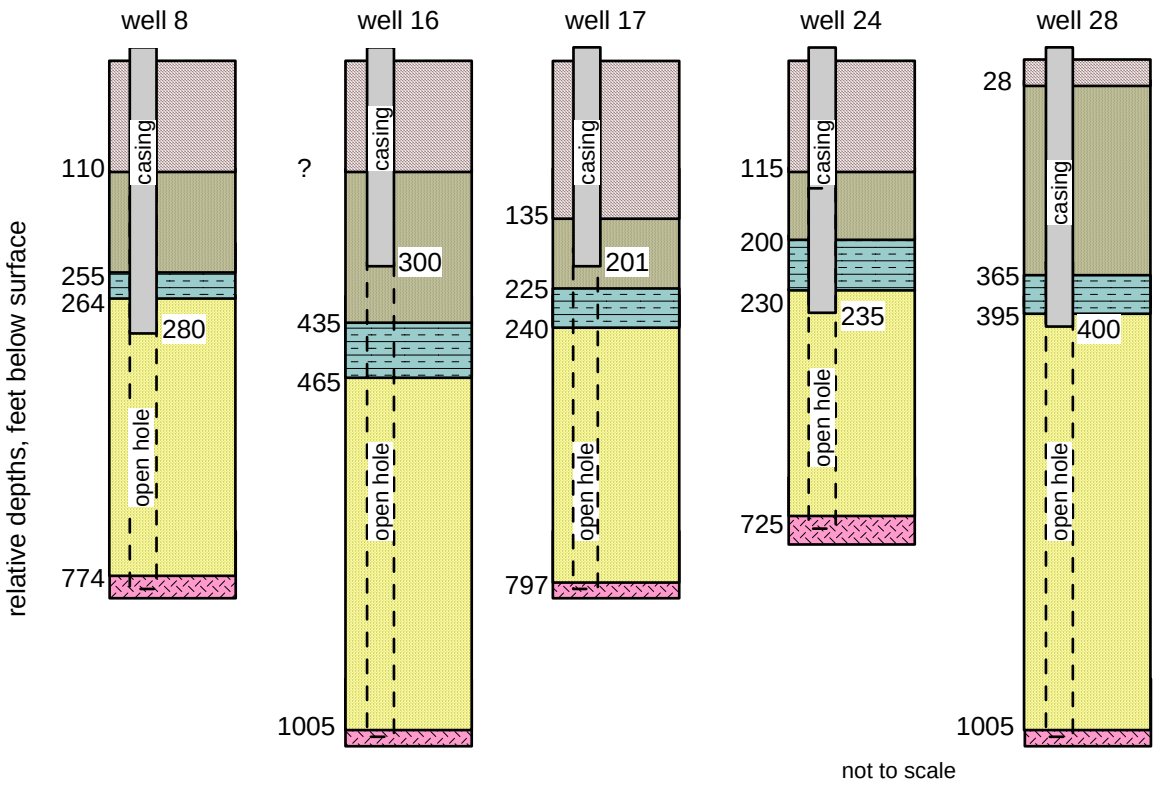

Figure 3 . Construction details of the additional five wells sampled during the initial phase of the project.

Sampling procedure at municipal wells

All well samples were collected at the wellhead while the well pumps were running. Viruses were concentrated using glass wool filters, a method that has been fully validated (Lambertini et al. 2008). Samples were obtained from a sampling tap on the well discharge line prior to discharge to the well reservoir. At wells where the pH exceeded 7.5, the pH was adjusted to between 6.5 and 7.0 using an acid injection ahead of the filter. The Madison wells are plumbed so that there is zero back pressure between the reservoir and the well discharge line; this lack of pressure required the use of a booster pump to force the sample through the glass wool filter. We used a portable heavy-duty peristaltic pump and food-grade tubing for this purpose; the pump and tubing were sterilized with a chlorine solution between each sample. Sampling each well required several hours of pumping; between 700 and 1000 liters of water were passed through the filter and the filtered volume was measured using a flow accumulator. A field blank was collected by pumping nineteen liters of reverse-osmosis water through a glass wool filter, using decontaminated field equipment. The filters were stored, transported and analyzed as described below.

Sampling procedure at lakes

The procedure for sampling lakes was similar to that for sampling the wells. A decontaminated pump and tubing were submerged in the lake, approximately 10 feet offshore. The water was pumped through a pre-filter to remove particulate matter. The sample stream was then acidified to a pH between 6.5 and 7.0, because the lake water was typically above pH 7.5. The acidified influent was split between two glass wool filters used in parallel. Filter effluent was directed onto the lake shore. Lake water was pumped at a rate of approximately 4 liters/minute until a total sample volume of about 1000 liters was passed through the filters. The pre-filter and two glass wool filters were transported on ice to Marshfield for analysis. The field equipment was decontaminated according to Marshfield standard procedures prior to re-use.

Sewage influent sampling

Clarified and settled sewage influent was collected and provided by the staff of the Madison Metropolitan Sewerage District at the Nine Springs sewage treatment plant. The influent was transferred to four-liter containers and shipped to Marshfield for analysis.

Virus analyses and sequencing

Pre-filters and glass wool filters were transported to the laboratory on ice and processed the next day after sampling. Filters were eluted with beef extract/glycine and the eluate flocculated and concentrated with polyethylene glycol following the methods described in Borchardt et al (2004) and Lambertini et al (2008).

Samples were analyzed for six virus groups: enteroviruses, adenoviruses, rotavirus, hepatitis A virus (HAV), and norovirus genogroups 1 and 2. Viruses were detected by real-time quantitative reverse-transcription polymerase chain reaction (RT-PCR) and TaqMan probe using the LightCycler (Roche Inc.) platform. The procedures, primers, and probes are described in Borchardt et al (2003, 2004) and Lambertini et al (2008). Standard curves were established by treating stocks of each virus type with Benzonase (Novagen, Madison, WI) for 30 min at 37°C, followed by incubation for 2 days at 4°C, leaving only the nucleic acid contained within intact capsid-protected virions, and removing extraneous viral nucleic acid that would have inflated the estimate of genomic copy number. Viral RNA or DNA mass was measured fluorometrically using RiboGreen (Molecular Probes, Eugene, OR) or PicoGreen (Molecular Probes) and a CytoFluor Series 4000 fluorimeter (Applied Biosystems, Framingham, MA), then converted to genomic copies based on the nucleic acid molecular weight of that virus. Intact viruses were serially diluted, and each dilution was seeded into separate 0.14 ml volumes of negative final concentrated sample volume (FCSV) and extracted using the QIAamp DNA Blood Mini Extraction Kit (Qiagen). Therefore, the standard curves represent the entire quantitation process and include any matrix effects from the elution and flocculation procedures. Crossing points were calculated automatically by the

LightCycler with the second derivative maximum method, and plotted against the decimal logarithm of viral RNA or DNA concentration.

RT-PCR controls for each batch of reactions included an extraction negative control (unseeded FCSV), negative controls for the RT and PCR cocktails, and a positive control of known low viral concentration seeded into an FCSV matrix. This positive control also served as the LightCycler reference control, validating the use of the standard curves. qRT-PCR inhibition was evaluated by seeding 800 copies of hepatitis G virus (HGV) Armored RNA® (Asuragen Inc., Austin, TX) into the RT reaction of every sample. qRT-PCR was performed using HGV primers provided by the manufacturer and a laboratory-designed probe. Inhibition was considered absent when the crossing point of the HGV seeded samples was less than one cycle higher than the inhibition reference control (crossing point = 32).

Samples that were qRT-PCR-positive for enteroviruses were further evaluated for virus infectivity by cell culture using three cell lines (BGMK, RD, and Caco-2). Infectivity was gauged by two outcome measures: 1) observation of cytopathic effect (CPE) in cultures held six weeks; 2) a ≥ 10-fold increase in virus genomic copies in cell lysates from 2 week or 6 week cultures compared to the initial virus quantity in the FCSV cell culture inoculum.

All enterovirus and adenovirus positive samples were identified to serotype by sequencing using the ABI Prism 3100 Genetic Analyzer and previously described methods (Borchardt et al 2004 and 2007).

Isotopic and geochemical sampling and analysis

Samples for major ions and isotopes were collected at the municipal wells from the sampling tap while the wells were running. Field collection followed standard procedures for collection of field parameters (pH, temperature, dissolved oxygen), filtration, and acidification of metals (e.g. Karklins, 1996). Surface water samples were collected from open water along the shoreline during periods when the lakes were fully mixed. Samples were analyzed for the following parameters: Ca, Mg, Na, K, Fe, Mn, HCO_3, SO_4, NO_3, Cl. Analyses were conduced at the Wisconsin State Laboratory of Hygiene, a certified water analysis laboratory. Isotope samples were analyzed at the University of Waterloo (Ontario) Environmental Isotope Laboratory or at the US Geological Survey Isotope Laboratory. Deuterium was determined by manganese reduction. Oxygen-18 was determined by mass spectrometry on CO_2 gas. Tritium was determined by liquid scintillation counting on enriched samples.

Results

Precipitation, climate, and water levels during the study period

The Madison area received unusually high precipitation during the study period. Figure 4 shows the distribution of precipitation and air temperature between July, 2007 and September, 2008. Intense rainfall during August, 2007 caused minor flooding during that Fall. Record snowfall (over 100 inches) occurred during the winter of 2007-2008. Finally, June, 2008 was the second wettest month on record, with a rainfall of 10.9 inches in the Madison area (MMSD, 2008). Very intense rainfall between June 9 and 12, 2008 cause major flooding across southern Wisconsin.

Surface-water and groundwater levels and storm sewer flows responded to the precipitation events. Figure 5 summarizes storm sewer flows, the elevation of Lake Mendota, and groundwater levels in two local monitoring wells. Rapid increases in groundwater levels show that rapid recharge occurred after storm events. The Spring Harbor storm sewer drains street runoff from west Madison and discharges into Lake Mendota. It is one of several such storm sewers in the Madison area. Maximum storm flows occurred after the heavy rains in August 2007 and June 2008. A significant flow event also occurred during early January, 2008 following an unusually warm "January thaw".

The June, 2008 precipitation event is also important because it resulted in extremely high flows in the Madison sanitary sewers (MMSD, 2008). Sewage flows often increase during precipitation evens due to stormwater infiltration through leaky sewers and basements. The average flow to the Nine Springs Wastewater Treatment Plant is about 41 million gallons per day (MGD). During the fist significant rains on June 8, flows increased to 122 MGD, and then declined to about 80 MGD for several days. Several discharges of sewage diluted with rainwater in the system occurred during this rain event, on June 9. The largest discharge was into the Cherokee Marsh and the Yahara River upstream of the Highway 113 bridge (1,080,000 gallons). There was a smaller discharge into the Cherokee Marsh on the south side on Golf Road (17,200 gallons). There were also two discharges that would have entered Starkweather Creek (245,000 gallons on the east side of the Dane County Regional Airport and 48,000 gallons near Milwaukee Street), a small discharge into Lake Mendota at Carroll Street, and two small discharges into Squaw Bay on Lake Monona; one on the south shore (50,000 gallons) and one on the east shore (4,000 gallons) (Jon Schellpfeffer, MMSD, written communication).

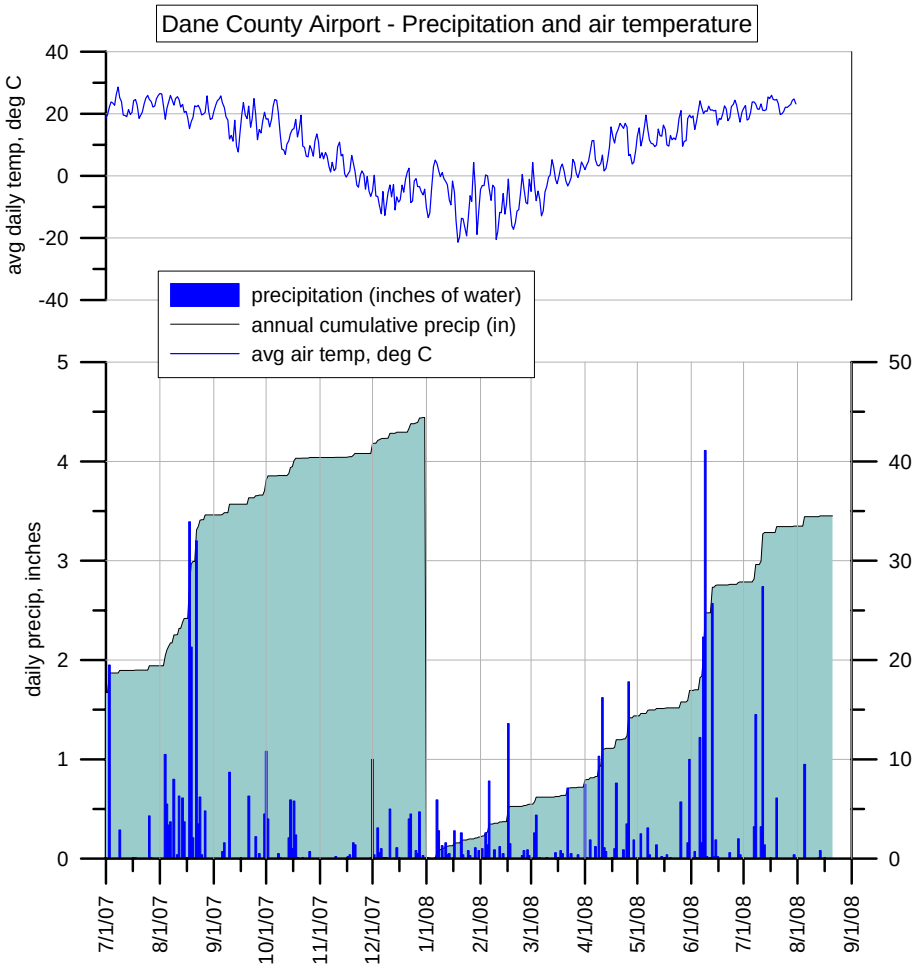

Figure 4. Precipitation and air temperature in the Madison area

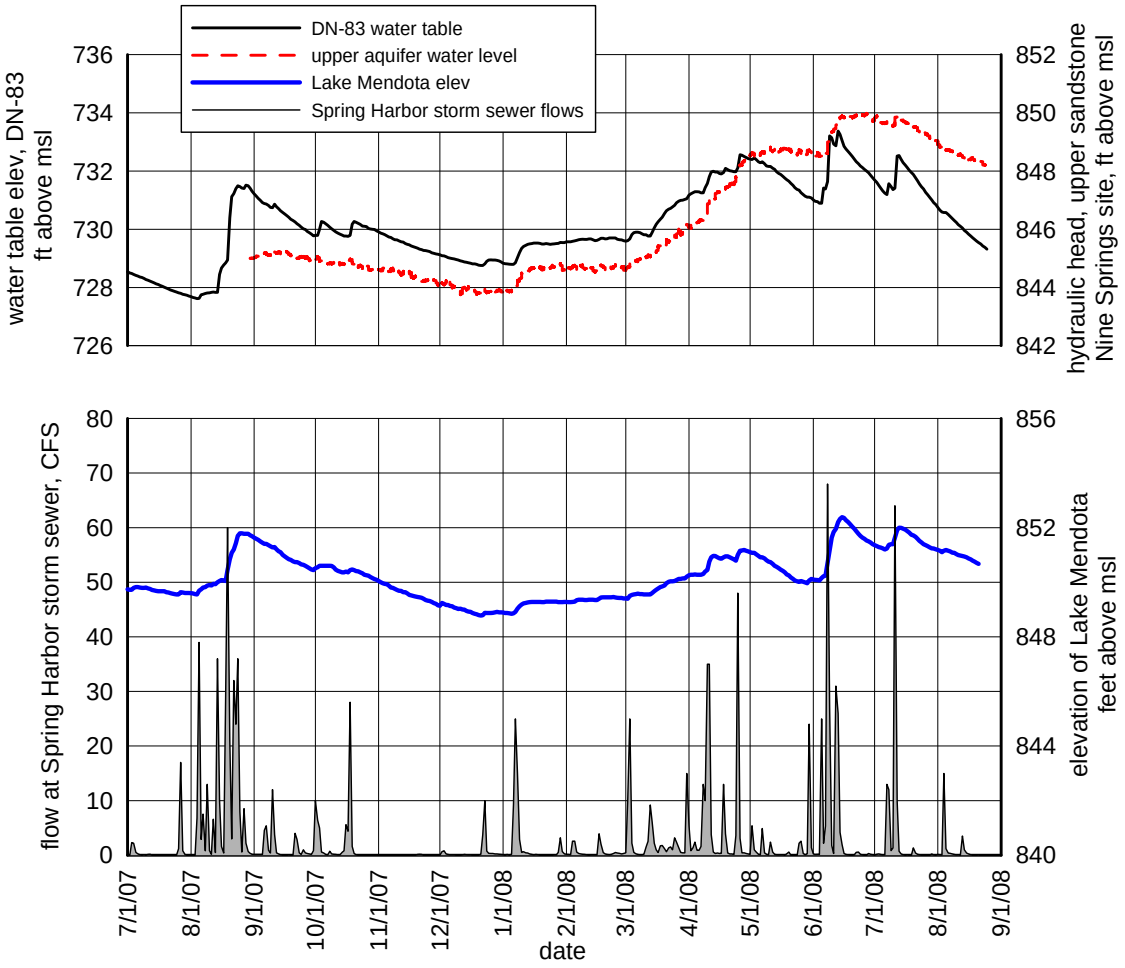

Figure 5. Stormwater flows, lake levels, and groundwater levels during the study period. Wastewater flows are from the Spring Harbor Storm Sewer (USGS site ID 05427965). Groundwater levels are from observations well DN-83 (USGS) and DN-1464 (unpublished data).

Viruses

The overall virus sampling consisted of 95 samples from wells, lakes, and sewage influent (Appendix A). Well samples included 76 samples from 11 different wells. The three Madison lakes (Mendota, Monona, and Wingra) were each sampled three times. Sewage influent was sampled at ten different dates. The initial sampling rounds (September and October, 2007) consisted of eleven wells (wells 7, 8, 11, 12, 13, 16, 17, 19, 24, 28, and 30). Following the October round we selected six wells (7, 11, 12, 13, 19, and 30) for regular monthly sampling. Our selection was based on initial virus detection, well construction, and some wells being off-line during the winter months.

Table 1 summarizes the overall virus results by sample source. Overall, water samples from wells were positive for viruses in 43 percent of the samples, and virus concentrations ranged from 0.00 to 6.15 gc/l (genomic copies per liter), with a mean of 0.47 gc/l. Lake samples were positive 78 percent of the time, and ranged from 0.00 to 27.6 gc/l, with a mean of 5.8 gc/l. Not surprisingly, Madison sewage was extremely high in viruses, with all samples positive, and concentrations ranging from about 50,000 to over two million gc/l, with a mean of 581,000 gc/l.

Virus results varied significantly with time, and there is apparent correlation between virus levels in sewage, lakes, and groundwater. Figure 6 shows the percentage of virus detections in wells along with virus concentrations in sewage and lake water. During the fall and winter of 2007, the wells were about 50 percent virus-positive. The positive percentage declined to about 20 percent in early 2008, and to zero in late May, 2008 before jumping to over 80 percent in July, 2008. Virus concentrations in sewage, while always in the thousands of gc/l, peaked in November, 2007, declined through May, 2008, and then rose in July, 2008. Although the lakes were only sampled three times, these samples are consistent with the apparent temporal trend. All three lakes contained viruses in September, 2007. Only lake Mendota contained detectable viruses in May, 2008, but all three lakes were positive in July, 2008. It is interesting to note that the July increases in virus detections followed the extreme rainfall events in June, 2008.

Table 1. Summary of virus detections by water source

	Virus detection (gc/l)			
Water source	Percent positive	min	max	mean
Wells	43.4	0.00	6.15	0.47
Lakes	77.8	0.00	27.6	5.80
Sewage	100.0	48,600	2,078,000	581,000

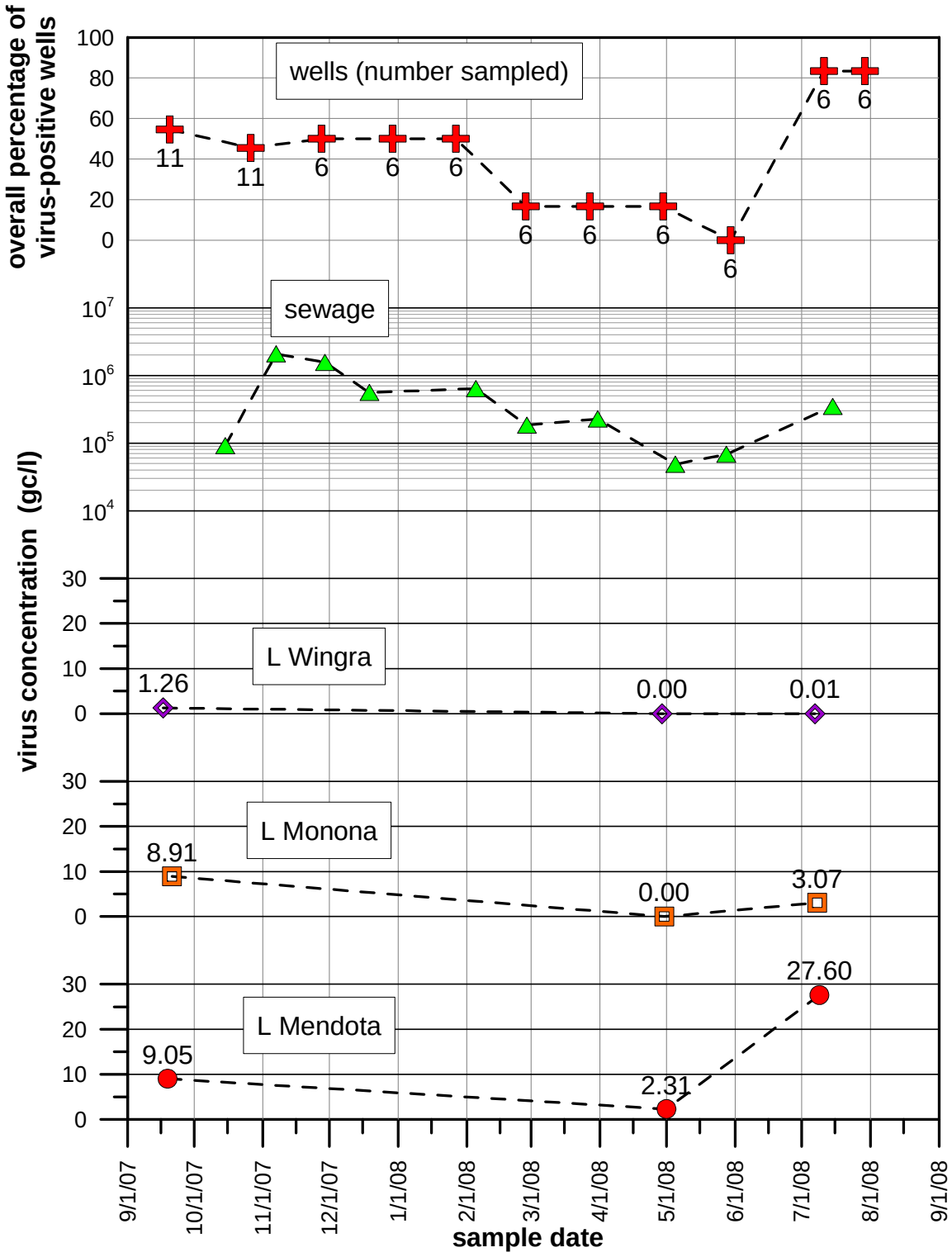

Figure 6. Overall virus detections in wells and concentrations in lakes and sewage.

Well-by-well virus results

Viruses were detected at least twice in every one of the six wells repeatedly sampled for this study, but no well was virus-positive in every sampling round. Figure 7 shows virus concentrations through time for each well, along with the overall percentage of detections in each well. Note that each well had a spike in virus concentrations in June and July 2008.

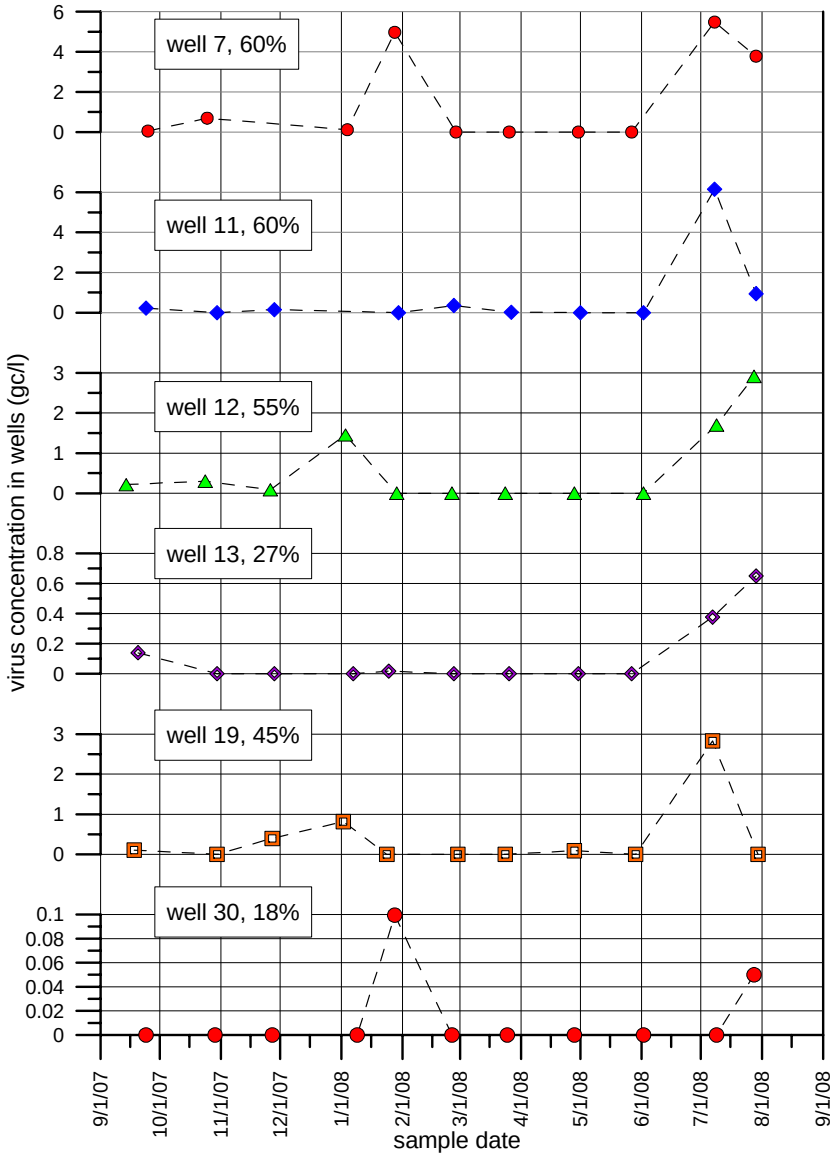

Figure 7. Virus concentrations through time for each of the six long-term wells. Percentages next to well labels show percent virus-positive samples.

Virus speciation and Infectivity

Several different species (serotypes) of viruses were identified in wells, sewage, and lake water during this study, and in many cases wells and sewage contained identical virus serotypes (table 2). Detected viruses include Enteroviruses echovirus 3, echovirus 6, echovirus 11, Coxsackie A16 and B4, Adenoviruses 2, 6, 7, 41, as well as G1 norovirus and rotovirus. The apparent correlation between viral serotypes found in sewage, lakes, and groundwater is important because it suggests very rapid transport from the surface to groundwater. Viral serotypes vary seasonally and annually, and so correlation between surface and subsurface serotypes would be unexpected if transport times from the surface to groundwater exceed many months. Although some viruses (A41, A2, echovirus 3, echovirus 11) were found in both lakes and wells, other viruses found in wells (A7, echovirus 6, CoxA16) were never found in lakes, suggesting that the lakes are not a source for these viruses in groundwater. With the exception of A7, all viruses found in wells were also detected in Madison sewage.

Infectious enteroviruses were found in wells 7, 11, and 19 in some, but not all, samples tested from these wells (infectivity testing on all samples was not completed in time for this report).

Table 2. Speciation of viruses detected. Numbers and letters refer to virus serotypes; E6 (echovirus 6) enterovirus, Adneovirus 41, etc.

	sample period		Sep-07	Oct-07	Nov-07	Dec-07	Jan-08	Feb-08	Mar-08	Apr-08	May-08	Jun-08	Jul-08
long-term wells	well 7	Enterovirus		E3		E6	E6					CoxA16	CoxB3
		Adenovirus	7	***41***			7					7	7
	well 11	Enterovirus			E11							E3	
		Adenovirus	***41***						***41***			***41***	
	well 12	Enterovirus		E11								E3	E3
		Adenovirus	***41***	***41***	***41***	***41***							
	well 13	Enterovirus										CoxA16	CoxA16
		Adenovirus	***41***				***41***						***41***
	well19	Enterovirus								E11		E3	
		Adenovirus	***41***		**2**	**2**							***41***
	well 30	Enterovirus											
		Adenovirus					**2**						***41***
initial wells	well 8	Adenovirus		E11, 41									
	well 16	Adenovirus	***41***										
	well 28	Adenovirus		***41***									
	well 24	Adenovirus											
lakes	L Mendota	Enterovirus										E3, E30	
		Adenovirus	***41***							**2**		***41***	
	L Monona	Enterovirus										E30	
		Adenovirus	***41***									**2**	
	L Wingra	Enterovirus											
		Adenovirus	***41***										
sewage	Sewage	Enterovirus	E11	E11	E11			E11		E11		E3	
				E3	CoxB4			E6				CoxA16	
		Adenovirus	***41***	6	***41***	***41***		***41***					
				2		**2**	**2**		**2**				
		other	G1		G1			G1	G1, R	G1, R		G1	

Groundwater and lake water chemistry

Samples were collected twice from the six study wells and once from the three lakes for analysis of major ions and tritium (Appendix B). Measurements of pH, specific conductance and dissolved oxygen were collected during each sampling event (Appendix C). Results are available from six sets of well samples and one round of lake samples for the stable isotopes of water, O^{18} and deuterium.

Groundwater from all of the six wells has similar composition, and all six are higher in calcium and lower in chloride than lake water. As shown by a Piper diagram of major ion concentrations (Figure 8), lake water and groundwater are bicarbonate type. Nitrate and chloride are naturally occurring constituents of groundwater, however elevated concentrations of these constituents may be attributed to contamination from septic systems or fertilizer, and road salt. Background nitrate levels in Wisconsin aquifers are generally less than 2 mg/L, and average chloride concentrations in Dane County wells are about 8 mg/L (Kammerer 1981). Chloride and nitrate concentrations in the six study wells are compiled in Table 4. Well 11 has elevated nitrate and chloride, indicating that it receives a relatively large amount of shallow, or recently recharged, groundwater. This conclusion is consistent with its shallow casing depth (111 ft.) and elevated tritium level (Table 3). Well 7 contains elevated chloride and tritium, and although cased through the Eau Claire aquitard, well 7 apparently receives a significant proportion of recent recharge and is vulnerable to contamination from the ground surface. Although Well 13 has a shallow casing depth (128 ft), it has less tritium and lower nitrate and chloride than Well 11, suggesting that it receives a smaller proportion of shallow or recently recharged groundwater.

Wells 30 and 19 are cased through the Eau Claire aquitard and are low in nitrate and chloride, indicating little vulnerability to shallow contaminants. However, the appreciable tritium measured in well 19 samples suggests that the well receives a significant proportion of recent recharge. Well 12 is low in nitrate and chloride, and similar to well 30, has no detectable tritium. The apparently small volume of young groundwater that reaches well 12 is surprising it is open to 120 feet of the upper aquifer (figure 2).

Environmental Isotopes in virus study wells

Tritium (^3H) contents and the deuterium (^2H) and oxygen-18 (^{18}O) contents of water help discriminate wells and show which wells are most vulnerable to surface-water recharge. Wells 19 and 7 are reportedly cased through the Eau Claire aquitard (Table 4). These wells are located close to lakes (Fig. 2), and oxygen isotope ratios plot to the right of other samples (Fig. 9), shown with a local meteoric water line (LMWL) from Dane County (Swanson and others, 2006). This lighter water suggests some contribution of lake water to these wells. As discussed above, both wells have tritium levels that indicate a significant volume of recent recharge reaches these wells. In contrast, the third confined aquifer well in the study, well 30, has tritium at less than detection (<0.8 TUs) and a $\delta^{18}O$

composition lower than reported for modern groundwater by Bradbury and others (1999), Hunt and Steuer (2000), and Kurtz et al. (2007). Lower compositions are indicative of cooler climates; thus, it is likely that well 30 pumps appreciable amounts of glacial melt water from the Pleistocene – amounts not seen in the other study wells.

The multi aquifer wells in the study are located further from the Madison lakes, however well 12 is near a retention basin (at Odana Hills) and well 13 is close to Cherokee Marsh. Their oxygen isotope signatures plot to the left of wells 19 and 7, indicating little to no contribution of fractionated surface water at these wells.

The variability in a well's isotopic composition can also help identify wells with surface water contributions (Hunt et al. 2005). The median and standard deviation of $\delta^{18}O$ collected in wells over the study period form a direct relation because the isotopic composition of terrestrially derived groundwater should reflect little to no surface evaporation and is expected to be less variable than surface water (Hunt et al. 2005). Well 30 has little variability in contrast to well 19 (Fig. 10), supporting the conclusion that terrestrially derived water dominates flow to well 30 (separated from modern water by the aquitard, away from lakes) whereas well 19 has some contribution from lake water. Well 12 also has a greater degree of variability than might be expected given its distance from the lakes and well 13 (which has a similar median isotopic composition). This could result from surface water contributions from the near-by retention basin. Alternatively, Hunt et al. (2005) identify changes to pumping schedule – both in the well of interest as well as nearby wells – as being a mechanism that can affect the variability in water isotope composition.

Table 3. Tritium results and well characteristics

well or lake	aquifer	year constructed	total depth (ft)	casing depth (ft)	distance to surface water (ft)	Tritium (TUs) Sept., 2007	± 1σ	Tritium (TUs) April, 2008	± 1σ	Tritium
7	confined	1939	736	238	3411.2	4.6	0.5	5.40	0.6	8.9[1], 9.9[1], 19.6[2]
30	confined	2003	800	312	4526.4	<0.8	0.3	<0.8	0.4	
19	confined	1970	710	260	836.4	4.4	0.5	3.70	0.5	
12	multi-aquifer	1957	529	260	1,115*	<0.8	0.6	<0.8	0.4	
11	multi-aquifer	1959	752	111	4739.6	6.3	0.9	5.40	0.6	
13	multi-aquifer	1959	780	128	2,510**	2.5	0.7	1.30	0.4	
Monona						8.7	0.7			15.1[2]
Wingra						9.2	0.8			13.7[2]
Mendota						8.5	0.7			11.4[2]

* Well 12 distance reported is to storm water retention pond; well is about two miles from Lake Wingra

** Well 13 distance reported is to Cherokee Marsh

[1]Tritium reported in Borchardt et al. 2007;samples collected in June, 2003 & May, 2004

[2]Tritium reported in Bradbury et al.1999;samples collected in June, 1995

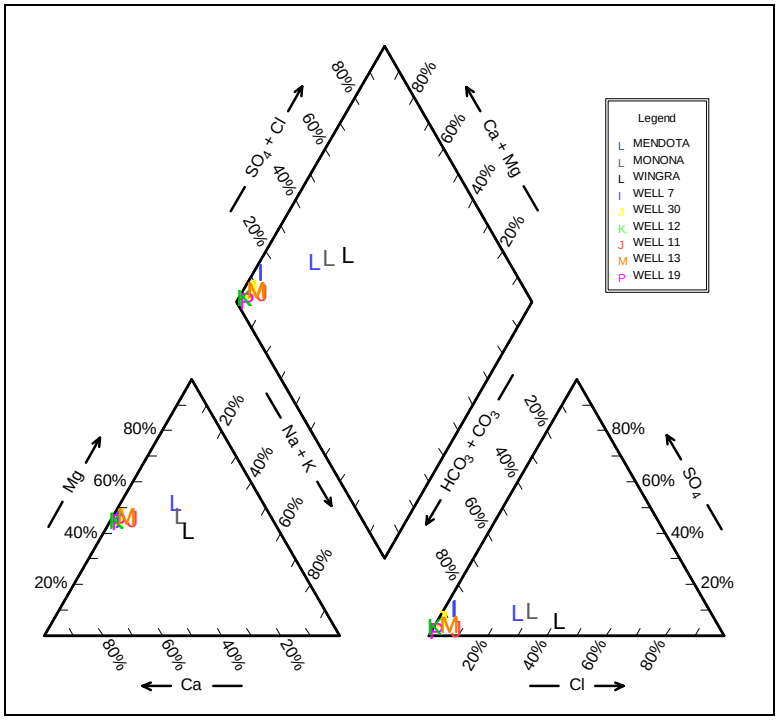

Figure 8. Piper diagram of well and lake water samples.

Table 4. Dissolved chloride and nitrate in the study wells.

Well	Chloride (mg/L)				Nitrate (mg/L)			
	Jun-07[1]	Sep-07	Jan-08	Jun-08[1]	Jun-07[1]	Sep-07	Jan-08	Jun-08[1]
7	12.49	10.40	10.50	5.91	ND	0.02	0.02	ND
11	45.86	17.30	NS	45.19	2.66	1.14	NS	2.58
12	2.49	1.07	1.08	2.62	0.72	0.77	0.73	0.78
13	8.36	7.74	8.06	8.50	1.71	1.76	1.74	1.73
19	5.72	3.60	3.70	5.89	ND	ND	ND	ND
30	4.11	3.75	2.58	4.39	ND	0.04	ND	ND

[1] Data from June 2007 and June 2008 provided by Dane County Health Department.

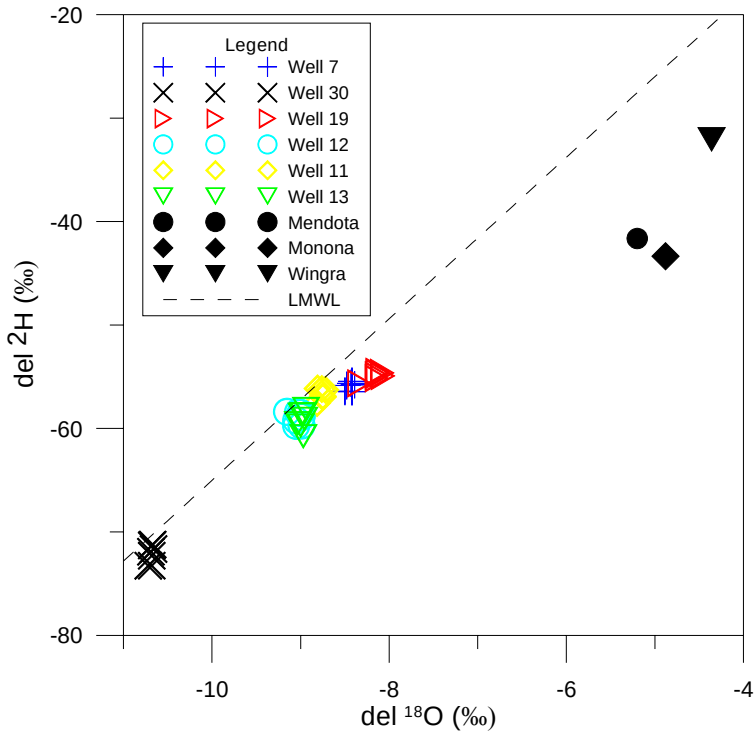

Figure 9. Deuterium/oxygen-18 results. N = 6 or 7 samples from wells, n = 1 from lakes (September only).

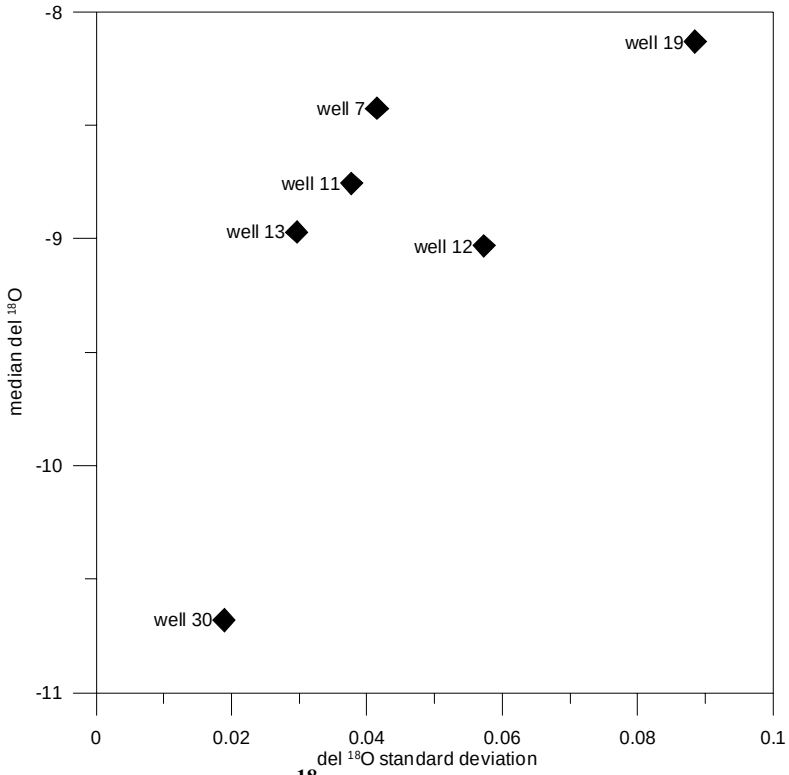

Figure 10. Median $\delta^{18}O$ compared to standard deviation, n = 6 or 7.

Discussion

Significance of virus detections

Viruses were detected in at least one sample from all but one of the municipal wells sampled for this project and in at least two samples from each of the six wells chosen for long-term sampling. These findings are consistent with our previous work (Borchardt and others, 2007a) and show that even deeply cased municipal wells in confined aquifer settings can be susceptible to pathogen contamination.

Potential virus pathways to wells

As stated in the introduction to this report, the four conceptual models of virus transport to the confined aquifer include (1) transport through the aquitard by porous-media flow; (2) transport by porous-media flow around the edge of the aquitard or through nearby "windows" or breaches in the aquitard, including local lakes; (3) transport by rapid flow through fractures in the aquitard or through cross-connecting nearby wells; and (4) transport by rapid flow along the well annulus through damaged, deteriorated, or poorly installed grout or breaches in the well casing. This current project has not been able to confirm or discount any of these potential flow paths. We had hoped to undertake in-well borehole sampling during this project in order to evaluate pathway 4 above, however logistical considerations prohibited this work during the past year. We intend to carry out the in-well sampling as part of a follow-up project during 2008-2009.

Lakes as a source of viruses

Although at first glance infiltrating lake water seems a plausible source for the viruses found in the municipal wells, several lines of evidence show that the lakes are probably not the primary virus source. First, the deuterium/oxygen-18 relationships (figure 9) suggest that only two wells (7 and 19) receive a significant proportion of lake-derived water, while all wells contained viruses. Second, with the exception of the July 2008 levels in Lake Mendota, virus concentrations in the lakes are generally as low as or lower than virus concentrations in the wells. Assuming significant mixing and dilution with virus-free water in the aquifer, the lake virus contents are likely too low to account for the virus levels in the wells. Third, the lakes contained only four of the six virus species detected in the wells.

Sanitary sewers as a source of groundwater contamination

Sanitary sewers are a major part of civic infrastructure in urban settings and represent a significant potential source of groundwater contamination. Sewer exfiltration, or outward leakage of sewage wastes, represents a potential source of pathogens, toxic chemicals, pharmaceutical compounds and other materials to the subsurface environment (Bishop et al. 1998). There have been two schools of thought on the significance of sewer exfiltration (Rutsch et al. 2008). Some investigators argue that the overall impact of

sewer exfiltration is insignificant due to the small volumes of leakage and to biodegradation and sorption of contaminants in the soil zone. Others (e.g. Leif Wolf 2004; Osenbrück et al. 2007) believe that exfiltration can be a major source of groundwater contamination. Most studies conclude that the impact of sewage exfiltration on groundwater is quite variable in time and space and there is currently a lack of knowledge about both the quantity of leakage and its consequences for the environment (Rutsch et al. 2008).

Relationships between sewer leakage and the hydrogeologic setting

Engineers commonly acknowledge that sanitary sewer systems leak. Most urban sewer systems consist of tens to hundreds of miles of buried pipes of a variety of ages, materials, and construction. Leaks can occur due to deteriorated materials, failed joints and junctions, damage from shifting soil or construction practices, tree roots, faulty construction, and many other natural and/or man-made sources. Historically, the overriding concern for sewage and wastewater management and treatment has been sewer infiltration – or groundwater leaking into sewers. Infiltration increases the volumes of sewage to be handled, treated, and disposed of, and can represent a major expense for communities. Sewer utilities usually inspect their lines for damage and infiltration leaks using remotely-operated television cameras, and it is not uncommon to see streams of water entering the sewers through joints or breaks in the pipes (B. Borelli, MMSD, personal communication, 2008). Exfiltration, on the other hand, is much more difficult to quantify. Outward-leaking sewage presents no obvious visual signal in televised pipe inspections, and mass-balance approaches to quantifying exfiltration are difficult because the rates of exfiltration may be below the uncertainty of flow measurements in the sewer system**.** Moreover, exfiltration is often thought not to pose a risk to the environment because it is expected to operate similar to a septic field whereby subsurface filtering and attenuation mitigates any adverse impact.

The relationships between sewers and the local hydrogeologic setting controls the potential for sewer infiltration and exfiltration. Figure 11 shows, in cross section, the four possibilities for sewer construction relative to the water table. In the figure, H_1 represents the hydraulic head inside the sewer, and H_2 represents the hydraulic head in the adjacent aquifer. There are two types of sewers. Gravity-drain sewers operate along an elevation gradient, and are only occasionally completely full of liquid. More commonly these gravity-drain sewers are only one-third to one-half full (A and B on figure 11). Gravity-drain sewers can temporarily fill under conditions of heavy sewer discharge, or permanently fill at low points in the system. Pressurized or force main sewers (C and D on figure 11) are permanently full of liquid and are connected to booster pumps that maintain positive pressure in the lines. Where leaks exist, the relationship between H_1 and H_2 controls the flow direction between the sewer and the environment. From figure 11, the only situation where infiltration can occur ($H_1 < H_2$) is A, where a gravity-drain sewer lies below the water table. In each of the other three possibilities (B, C, D) the head in the sewer can be higher than the head in the aquifer, and exfiltration can occur. In the cases of pressurized force mains (C, D) the potential for outflow can be very large due to large head differentials ($H_1 >> H_2$).

Wastewater for the entire Madison Metropolitan area is collected through laterals to individual homes and businesses and moved by gravity and force mains to treatment at the Nine Springs sewage treatment plant operated by the Madison Metropolitan Sewerage District (MMSD). Although the treatment plant itself is quite modern many of the wastewater mains and laterals are up to 50 years old, and some older lines are up to 90 years old. The sewer lines are constructed of a variety of materials, including (ranging in general from older to newer construction) vitrified clay, cast iron, ductile iron, reinforced

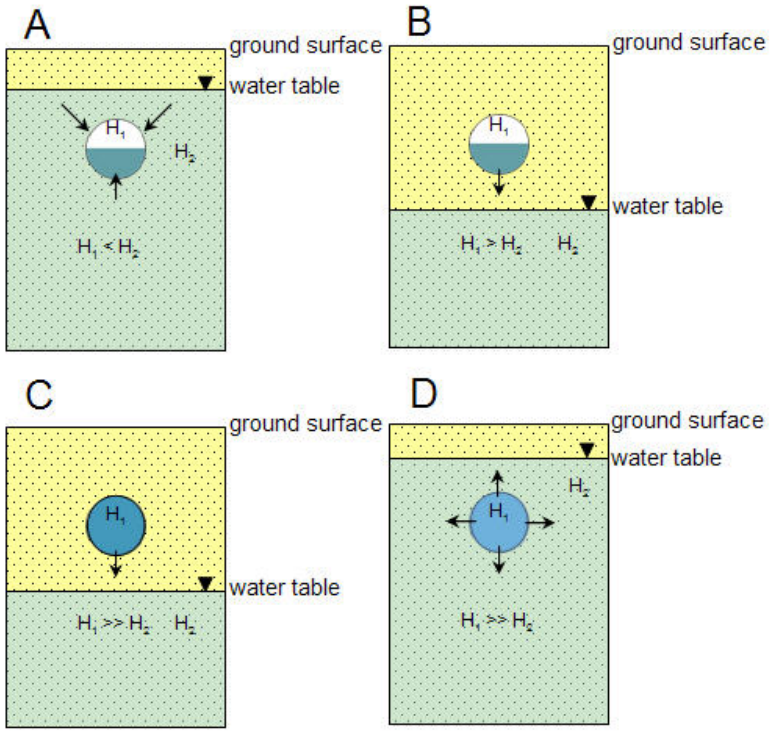

Figure 11. Cross sections showing the possible locations of a sewer relative to the water table. A: gravity-drain sewer below water table; B: gravity-drain sewer above water table; C: force main above water table; D: force main below water table. H_1 and H_2 represent hydraulic head inside and outside the sewer. Arrows show directions of potential sewer leakage.

concrete, asbestos cement, and PVC plastic. The sewers are generally placed in trenches 10 to 20 feet deep on top of a gravel bed backfilled with native material. The City of Madison manages nearly 800 miles of sewer lines that extend along each city street. The Madison Metropolitan Sewage District (MMSD) manages larger collector and connector sewers that extend from various locations in the city to the MMSD regional sewage treatment plant. The MMSD sewers include about 93 miles of gravity-flow lines and 30 miles of force mains.

Calculated source volumes of viral contaminants

Calculation of potential mixing between groundwater and sanitary sewer leakage suggests that the sewer leakage is a likely source of viral contamination of groundwater. Very little sewage is needed to produce the concentrations seen in the wells, as would be

expected when a gram of feces from an infected person can contain over one trillion infectious viruses. A simple calculation using some results from the study is provided below to illustrate this point. This calculation is based on the amount of water a well pumps during one virus sampling event (around 4 hours of pumping). During a typical sampling event a well produces over one million liters of water (1500 gal/min x 4 hr pumping x 60 min/hr x 3.78 l/gal). We assume that the viral concentration of the pumped water is constant during the four-hour sampling period, and that the viral filter collects a representative sub-sample of this water. Also assuming complete mixing in the aquifer and well bore and that "background" groundwater contains no viruses, we can calculate the volume of sewage needed to produce the observed concentrations in the wells. Table 5 summarizes viral and tritium concentrations observed in this study.

Table 5. Summary of virus and tritium observations

Water source	Virus concentration, gc/l	Tritium content, TU
Madison lakes	0 – 27	8.5-9.2
Madison sewage	49,000 – 2,100,000	0-6 (assumed)
Madison wells	0 – 6.2	0-10

Assuming that all viruses originate in the source water, the basic conservation of mass equation is:

$V_s \times C_s = V_{gw} \times C_{gw}$

Where

V_s = volume of sewer leakage,

C_s = concentration of viruses in sewer leakage,

V_{gw} = volume of groundwater, and

C_{gw} = virus concentration in groundwater.

If the source volume is the only unknown, the equation becomes:

$V_s = V_{gw} \times C_{gw} / C_s$

For the minimum sewage concentration (49,000 gc/l) and maximum well concentration (6.2 gc/l):

$V_s = 1.36 \times 10^6$ l x 6.2 gc/l / 49,000 gc/l = 172 l

For the maximum sewage concentration (2,100,000 gc/l) we have :

$V_s = 1.36 \times 10^6$ l x 6.2 gc/l / 2,100,000 gc/l = 4 l

Accordingly, about 4 to 170 liters (1 to 44 gallons) of sewage leakage into the recharge area could produce the maximum virus concentration observed in well water during the 4-hour long sampling event when the well was pumping. This analysis is only meant to be illustrative does not include the true contaminant transport processes. This disclaimer notwithstanding, such a minor sewer leak does not seem unreasonable given length of sewer pipe in a typical Madison well capture zone,

The small amount of virus-contaminated leakage required to produce the viral concentrations seen in the well samples explains why a well (such as wells 12 and 30) can be virus positive but not contain detectable tritium. Ambient tritium concentrations in surface water are on the order of 10 TU. Sewage, which is mostly derived from locally-pumped groundwater, is assumed to have tritium concentrations in the same range as the wells (0-6 TU). Mixing these small amounts of tritiated water with "old" groundwater (assumed to contain less than 0.5 TU) would not raise the tritium content above the laboratory detection limit of 0.8 TU. For the example above, mixing 172 liters of virus-laden sewage with one million liters of uncontaminated water produces detectable virus concentrations in the water. However, mixing the same 172 liters of water having a tritium content of 10 TU with one million liters of tritium-free water would produce a mixed concentration of about 0.002 TU, far below the laboratory detection limit.

The dilution calculations above demonstrate that human viruses have the potential to be used as very sensitive groundwater tracers. They possess several characteristics necessary for good tracer performance. First, when present, they are detectable over several orders of magnitude, from 1 gc/l to millions of gc/l. Second, they are extremely mobile. Third, virus speciation allows correlation of specific viral serotypes which vary through time, giving a temporal measure to tracer experiments. Finally, there has been much progress in reducing the time and cost of analyses, bringing such a tracer into the reach of more studies. However, they can only be used as tracers where there is a virus source, which limits their use to urban areas or areas affected by sewage treatment systems. Additional investigation of the use of viral tracers in groundwater study should be the focus of future research.

Conclusions and Recommendations

Conclusions

Human enteric viruses are a common contaminant in water produced by municipal wells in Madison, Wisconsin. Viruses were found in all wells sampled monthly, though not in every sample from every well. The percentage of virus-positive samples ranged from 60% in wells know to have multi-aquifer construction or shallow casings to 18 % in well 30, a new, deep well deeply cased across a regional aquitard. The presence of viruses in wells cased and grouted 200 to 300 feet below a regional aquitard raises disturbing questions about aquifer vulnerability in confined-aquifer settings usually thought to be well-protected from surface contaminants.

Although we are unable at this time to elucidate the transport pathway for viruses from the surface to the wells, several lines of evidence suggest that transport is rapid – on the order of months or weeks rather than years. Because they require a human host, these viruses must originate at or just below the land surface. Identical viral serotypes were found in sewage and groundwater, and the mix of viral species varied with time through the project. Moreover, virus detections in wells, and virus concentrations in lakes and sewage varied together through time. This temporal correlation is consistent with relatively rapid transport.

The Madison Lakes are probably not the main source of the viruses found in the Madison municipal wells. Lake water contained some but not all of the serotypes found in the wells, and virus levels in lake water are generally low. Furthermore, the $^{18}O/^{2}H$ signature of water produced by most Madison wells is not consistent with a significant lake water component of recharge.

The most likely source of the viruses in the wells is the leakage of untreated sewage from the Madison sewer system. Untreated sewage sampled at the Madison sewage treatment plant contains virus concentrations several orders of magnitude higher than concentrations observed in wells or lakes. Review of sewer construction and location data, the shear total length of city sewers (hundreds of miles), and the evidence that sewers are not completely water-tight suggests that leakage of sewage to the subsurface environment probably occurs in at least some parts of Madison. Given the high concentrations (millions of genomic copies per liter) of viruses in sewage, it would take very little sewage to produce the virus concentrations observed in the wells.

Human enteric viruses might be excellent tracers of recent groundwater. They have the desirable tracer characteristics of detectability over several orders of magnitude, high mobility, short analytic times and relatively reasonable cost, and are time-specific due to constantly changing serotypes. Although the presence of detectable tritium in a well is

almost always and indicator or recent recharge to the well, the absence of tritium (at a detection limit of 0.8 TU) does not necessarily indicate that the well will be virus-free. In fact detection of viruses many be a far more sensitive indicator than tritium of a proportion of "young" groundwater in a well if the well captures a virus source.

Recommendations

This study shows that human viruses can be commonly present in groundwater in deep bedrock wells. To protect human health, communities in Wisconsin and elsewhere that use groundwater for a drinking water source should consider using chlorination or other water treatment techniques to deactivate viruses, and work to ensure that these systems are operating correctly.

Sampling for viruses requires a time series approach because virus concentrations, and virus species, vary with time in individual wells.

Untreated sewage contains very high concentrations of viruses and should be considered a source of groundwater contamination. Wisconsin communities should evaluate sewer infrastructure to determine the potential for leakage of untreated sewage to the subsurface. For example, communities might wish to prioritize sewer repair or replacement within the contributing areas of municipal wells. Research on the impacts of sewers on groundwater quality should be encouraged.

Human enteric viruses represent a potentially powerful new tracing tool for hydrogeologic studies. Both fundamental (theoretical and column studies) and applied (field evaluations) research on the use and effectiveness of viruses as tracers should be undertaken.

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Appendices

Appendix A: Virus results.

Key: MC sample ID = Marshfield Clinic sample ID; Sample ID = field sample ID; type= well, lake, or wastewater; well ID = local number of well or lake name; Total virus concentration = virus concentration in genomic copies per liter; collection date = date of collection; filtration volume = volume of water filtered.

MC Sample ID	Sample ID	type	well ID	Total Virus Conc (gc/L)	Collection Date	Filtration volume (l)
32117	7-1	well	7	0.05	9/25/2007	1139.4
32427	7-2	well	7	0.69	10/25/2007	817.6
32587	7-4	well	7	0.11	1/4/2008	556.5
32605	7-5	well	7	4.97	1/28/2008	919.9
32621	7-6	well	7	0.00	2/28/2008	813.9
32637	7-7	well	7	0.00	3/26/2008	829.0
32838	7-8	well	7	0.00	4/30/2008	829.0
32865	7-9	well	7	0.00	5/27/2008	916.0
32944	7-10	well	7	5.48	7/8/2008	950.1
32970	7-11	well	7	3.78	7/29/2008	817.6
32118	8-1	well	8	0.00	9/25/2007	817.6
32540	8-2	well	8	0.30	11/2/2007	832.8
32115	11-1	well	11	0.22	9/24/2007	836.6
32457	11-2	well	11	0.00	10/30/2007	1237.8
32567	11-3	well	11	0.15	11/28/2007	844.1
32608	11-5	well	11	0.00	1/30/2008	851.7
32622	11-6	well	11	0.35	2/27/2008	768.4
32639	11-7	well	11	0.02	3/27/2008	829.0
32842	11-8	well	11	0.00	5/1/2008	942.6
32876	11-9	well	11	0.00	6/2/2008	859.3
32945	11-10	well	11	6.15	7/8/2008	806.3
32971	11-11	well	11	0.94	7/29/2008	855.5
32002	12-1	well	12	0.21	9/14/2007	1018.3
32425	12-2	well	12	0.30	10/24/2007	806.3
32568	12-3	well	12	0.09	11/26/2007	1294.6
32588	12-4	well	12	1.45	1/3/2008	681.4
32607	12-5	well	12	0.00	1/29/2008	836.6
32618	12-6	well	12	0.00	2/26/2008	829.0
32631	12-7	well	12	0.00	3/24/2008	1449.8
32831	12-8	well	12	0.00	4/28/2008	806.3
32878	12-9	well	12	0.00	6/2/2008	810.1
32948	12-10	well	12	1.70	7/9/2008	878.2
32969	12-11	well	12	2.91	7/28/2008	817.6
32072	13-1	well	13	0.14	9/20/2007	863.1
32937	13-10	well	13	0.00	10/30/2007	874.4
32972	13-11	well	13	0.00	11/28/2007	810.1

MC Sample ID	Sample ID	type	well ID	Total Virus Conc (gc/L)	Collection Date	Filtration volume (l)
32458	13-2	well	13	0.00	1/7/2008	829.0
32572	13-3	well	13	0.02	1/25/2008	806.3
32589	13-4	well	13	0.00	2/27/2008	908.5
32604	13-5	well	13	0.00	3/26/2008	280.1
32623	13-6	well	13	0.00	4/30/2008	1059.9
32638	13-7	well	13	0.00	5/27/2008	794.9
32839	13-8	well	13	0.38	7/7/2008	806.3
32866	13-9	well	13	0.65	7/29/2008	855.5
32003	16-1	well	16	0.07	9/14/2007	787.4
32460	16-2	well	16	0.00	10/31/2007	802.5
32569	16-3	well	16	0.00	11/26/2007	870.6
32584	16-4	well	16	0.00	12/19/2007	813.9
32148	17-1	well	17	0.00	9/26/2007	1362.7
32455	17-2	well	17	0.00	10/29/2007	802.5
32034	19-1	well	19	0.11	9/18/2007	840.4
32938	19-10	well	19	0.00	10/30/2007	810.1
32967	19-11	well	19	0.40	11/27/2007	972.8
32459	19-2	well	19	0.82	1/2/2008	813.9
32570	19-3	well	19	0.00	1/24/2008	863.1
32586	19-4	well	19	0.00	2/29/2008	878.2
32603	19-5	well	19	0.00	3/24/2008	969.1
32624	19-6	well	19	0.09	4/28/2008	806.3
32635	19-7	well	19	0.00	5/29/2008	806.3
32833	19-8	well	19	2.83	7/7/2008	851.7
32867	19-9	well	19	0.00	7/30/2008	855.5
32428	24-2	well	24	0.06	10/25/2007	836.6
32044	27-1	well	27	0.00	9/19/2007	931.2
32035	28-1	well	28	0.00	9/17/2007	829.0
32426	28-2	well	28	0.09	10/24/2007	836.6
32116	30-1	well	30	0.00	9/24/2007	844.1
32949	30-10	well	30	0.00	10/29/2007	893.4
32968	30-11	well	30	0.00	11/27/2007	1188.6
32456	30-2	well	30	0.00	1/9/2008	810.1
32571	30-3	well	30	0.10	1/28/2008	1052.3
32590	30-4	well	30	0.00	2/26/2008	1067.5
32606	30-5	well	30	0.00	3/25/2008	832.8
32619	30-6	well	30	0.00	4/28/2008	798.7
32636	30-7	well	30	0.00	6/2/2008	904.7
32832	30-8	well	30	0.00	7/9/2008	1048.6
32877	30-9	well	30	0.05	7/28/2008	923.6
32033	Wingra-1-GW	SW	Wingra	1.26	9/17/2007	832.8
32043	Mendota-1-GW	SW	Mendota	9.05	9/19/2007	889.6

MC Sample ID	Sample ID	type	well ID	Total Virus Conc (gc/L)	Collection Date	Filtration volume (l)
32074	Monona-1-GW	SW	Monona	8.91	9/21/2007	821.4
32834	Wingra-8	SW	Wingra	0.00	4/29/2008	847.9
32840	Monona-8	SW	Monona	0.00	4/30/2008	1150.8
32843	Mendota-8	SW	Mendota	2.31	5/1/2008	859.3
32933	Wingra-10-P	SW	Wingra	0.01	7/7/2008	984.2
32935	Monona-10-P	SW	Monona	3.07	7/8/2008	984.2
32946	Mendota-10	SW	Mendota	27.60	7/9/2008	984.2
32334	MMSW-1-a	WW		91569.00	10/15/2007	3.0
32543	MMSW-2	WW		2077558.00	11/7/2007	4.0
32573	MMSW-3	WW		1561945.00	11/29/2007	8.0
32585	MMSW-4	WW		558965.77	12/19/2007	4.0
32609	MMSW-5	WW		640625.00	2/5/2008	4.0
32620	MMSW-6	WW		184734.00	2/28/2008	4.0
32640	MMSW-7	WW		227578.00	3/31/2008	3.8
32845	MMSW-8	WW		48623.00	5/5/2008	4.0
32864	MMSW-9	WW		68482.14	5/28/2008	4.0
32950	MMSD-10	WW		348944.00	7/15/2008	1.0

Appendix B: Geochemical results.

Key: Field ID = field sample ID; well or lake = local number of well or lake name; LOD = laboratory limit of detection.

Date Collected	Field ID	Well or Lake	Parameter	Result	Units	LOD
9/24/2007	7-1	WELL 7	ALKALINITY TOTAL CACO3	311	MG/L	2.5
9/24/2007	7-1	WELL 7	CALCIUM DISS	74.3	MG/L	0.1
9/24/2007	7-1	WELL 7	CHLORIDE DISS	10.4	MG/L	0.026
9/24/2007	7-1	WELL 7	IRON DISS	0.4	MG/L	0.1
9/24/2007	7-1	WELL 7	MAGNESIUM DISS	42.4	MG/L	0.1
9/24/2007	7-1	WELL 7	MANGANESE DISS	29	UG/L	0.5
9/24/2007	7-1	WELL 7	NITROGEN NO3-N DISS	0.02	MG/L	0.006
9/24/2007	7-1	WELL 7	PH LAB	7.42	SU	
9/24/2007	7-1	WELL 7	POTASSIUM DISS	1.4	MG/L	0.1
9/24/2007	7-1	WELL 7	SODIUM DISS	6.2	MG/L	0.1
9/24/2007	7-1	WELL 7	SULFATE DISS	33.9	MG/L	0.02
9/24/2007	7-1	WELL 7	TURBIDITY	<1.0	NTU	
1/4/2008	7-4	WELL 7	ALKALINITY TOTAL CACO3	309	MG/L	2.5
1/4/2008	7-4	WELL 7	CALCIUM DISS	75	MG/L	0.1
1/4/2008	7-4	WELL 7	CHLORIDE DISS	10.5	MG/L	0.026
1/4/2008	7-4	WELL 7	CONDUCTIVITY AT 25C	669	US/CM	
1/4/2008	7-4	WELL 7	IRON DISS	0.4	MG/L	0.1
1/4/2008	7-4	WELL 7	MAGNESIUM DISS	43.9	MG/L	0.1
1/4/2008	7-4	WELL 7	MANGANESE DISS	28	UG/L	0.5
1/4/2008	7-4	WELL 7	NITROGEN NO3-N DISS	0.021	MG/L	0.006
1/4/2008	7-4	WELL 7	PH LAB	7.78	SU	
1/4/2008	7-4	WELL 7	POTASSIUM DISS	1.5	MG/L	0.1
1/4/2008	7-4	WELL 7	SODIUM DISS	6.5	MG/L	0.1
1/4/2008	7-4	WELL 7	SULFATE DISS	34.2	MG/L	0.02
1/4/2008	7-4	WELL 7	TURBIDITY	<1.0	NTU	
9/24/2007	8-1	WELL 8	ALKALINITY TOTAL CACO3	301	MG/L	2.5
9/24/2007	8-1	WELL 8	CALCIUM DISS	67.9	MG/L	0.1
9/24/2007	8-1	WELL 8	CHLORIDE DISS	14	MG/L	0.026
9/24/2007	8-1	WELL 8	IRON DISS	0.5	MG/L	0.1
9/24/2007	8-1	WELL 8	MAGNESIUM DISS	41.1	MG/L	0.1
9/24/2007	8-1	WELL 8	MANGANESE DISS	54	UG/L	0.5
9/24/2007	8-1	WELL 8	NITROGEN NO3-N DISS	ND	MG/L	0.006
9/24/2007	8-1	WELL 8	PH LAB	7.63	SU	
9/24/2007	8-1	WELL 8	POTASSIUM DISS	1.4	MG/L	0.1
9/24/2007	8-1	WELL 8	SODIUM DISS	8.6	MG/L	0.1
9/24/2007	8-1	WELL 8	SULFATE DISS	16.6	MG/L	0.02
9/24/2007	8-1	WELL 8	TURBIDITY	<1.0	NTU	
9/24/2007	11-1	WELL 11	ALKALINITY TOTAL CACO3	317	MG/L	2.5

Date Collected	Field ID	Well or Lake	Parameter	Result	Units	LOD
9/24/2007	11-1	WELL 11	CALCIUM DISS	77.5	MG/L	0.1
9/24/2007	11-1	WELL 11	CHLORIDE DISS	17.3	MG/L	0.026
9/24/2007	11-1	WELL 11	IRON DISS	ND	MG/L	0.1
9/24/2007	11-1	WELL 11	MAGNESIUM DISS	48.9	MG/L	0.1
9/24/2007	11-1	WELL 11	MANGANESE DISS	9	UG/L	0.5
9/24/2007	11-1	WELL 11	NITROGEN NO3-N DISS	1.14	MG/L	0.006
9/24/2007	11-1	WELL 11	PH LAB	7.44	SU	
9/24/2007	11-1	WELL 11	POTASSIUM DISS	1.3	MG/L	0.1
9/24/2007	11-1	WELL 11	SODIUM DISS	15.2	MG/L	0.1
9/24/2007	11-1	WELL 11	SULFATE DISS	11.4	MG/L	0.02
9/24/2007	11-1	WELL 11	TURBIDITY	<1.0	NTU	
9/14/2007	12-1	WELL 12	ALKALINITY TOTAL CACO3	264	MG/L	2.5
9/14/2007	12-1	WELL 12	CALCIUM DISS	57.3	MG/L	0.1
9/14/2007	12-1	WELL 12	CHLORIDE DISS	1.07	MG/L	0.026
9/14/2007	12-1	WELL 12	IRON DISS	ND	MG/L	0.1
9/14/2007	12-1	WELL 12	MAGNESIUM DISS	31.8	MG/L	0.1
9/14/2007	12-1	WELL 12	MANGANESE DISS	1	UG/L	0.5
9/14/2007	12-1	WELL 12	NITROGEN NO3-N DISS	0.774	MG/L	0.006
9/14/2007	12-1	WELL 12	PH LAB	7.5	SU	
9/14/2007	12-1	WELL 12	POTASSIUM DISS	1.2	MG/L	0.1
9/14/2007	12-1	WELL 12	SODIUM DISS	2.3	MG/L	0.1
9/14/2007	12-1	WELL 12	SULFATE DISS	9.96	MG/L	0.02
9/14/2007	12-1	WELL 12	TURBIDITY	<1.0	NTU	
1/3/2008	12-4	WELL 12	ALKALINITY TOTAL CACO3	263	MG/L	2.5
1/3/2008	12-4	WELL 12	CALCIUM DISS	60.2	MG/L	0.1
1/3/2008	12-4	WELL 12	CHLORIDE DISS	1.08	MG/L	0.026
1/3/2008	12-4	WELL 12	CONDUCTIVITY AT 25C	518	US/CM	
1/3/2008	12-4	WELL 12	IRON DISS	ND	MG/L	0.1
1/3/2008	12-4	WELL 12	MAGNESIUM DISS	32.7	MG/L	0.1
1/3/2008	12-4	WELL 12	MANGANESE DISS	ND	UG/L	0.5
1/3/2008	12-4	WELL 12	NITROGEN NO3-N DISS	0.734	MG/L	0.006
1/3/2008	12-4	WELL 12	PH LAB	7.78	SU	
1/3/2008	12-4	WELL 12	POTASSIUM DISS	1.2	MG/L	0.1
1/3/2008	12-4	WELL 12	SODIUM DISS	2.4	MG/L	0.1
1/3/2008	12-4	WELL 12	SULFATE DISS	9.98	MG/L	0.02
1/3/2008	12-4	WELL 12	TURBIDITY	<1.0	NTU	
9/20/2007	13-1	WELL 13	ALKALINITY TOTAL CACO3	284	MG/L	2.5
9/20/2007	13-1	WELL 13	CALCIUM DISS	63.4	MG/L	0.1
9/20/2007	13-1	WELL 13	CHLORIDE DISS	7.74	MG/L	0.026
9/20/2007	13-1	WELL 13	IRON DISS	ND	MG/L	0.1
9/20/2007	13-1	WELL 13	MAGNESIUM DISS	38.2	MG/L	0.1
9/20/2007	13-1	WELL 13	MANGANESE DISS	14	UG/L	0.5
9/20/2007	13-1	WELL 13	NITROGEN NO3-N DISS	1.76	MG/L	0.006
9/20/2007	13-1	WELL 13	PH LAB	7.55	SU	
9/20/2007	13-1	WELL 13	POTASSIUM DISS	2	MG/L	0.1

Date Collected	Field ID	Well or Lake	Parameter	Result	Units	LOD
9/20/2007	13-1	WELL 13	SODIUM DISS	5.5	MG/L	0.1
9/20/2007	13-1	WELL 13	SULFATE DISS	14.1	MG/L	0.02
9/20/2007	13-1	WELL 13	TURBIDITY	<1.0	NTU	
1/8/2008	13-4	WELL 13	ALKALINITY TOTAL CACO3	280	MG/L	2.5
1/8/2008	13-4	WELL 13	CALCIUM DISS	61.4	MG/L	0.1
1/8/2008	13-4	WELL 13	CHLORIDE DISS	8.06	MG/L	0.026
1/8/2008	13-4	WELL 13	CONDUCTIVITY AT 25C	580	US/CM	
1/8/2008	13-4	WELL 13	IRON DISS	ND	MG/L	0.1
1/8/2008	13-4	WELL 13	MAGNESIUM DISS	37.9	MG/L	0.1
1/8/2008	13-4	WELL 13	MANGANESE DISS	12	UG/L	0.5
1/8/2008	13-4	WELL 13	NITROGEN NO3-N DISS	1.74	MG/L	0.006
1/8/2008	13-4	WELL 13	PH LAB	7.74	SU	
1/8/2008	13-4	WELL 13	POTASSIUM DISS	1.7	MG/L	0.1
1/8/2008	13-4	WELL 13	SODIUM DISS	5	MG/L	0.1
1/8/2008	13-4	WELL 13	SULFATE DISS	13.5	MG/L	0.02
1/8/2008	13-4	WELL 13	TURBIDITY	<1.0	NTU	
9/14/2007	16-1	WELL 16	ALKALINITY TOTAL CACO3	270	MG/L	2.5
9/14/2007	16-1	WELL 16	CALCIUM DISS	63.2	MG/L	0.1
9/14/2007	16-1	WELL 16	CHLORIDE DISS	35.8	MG/L	0.026
9/14/2007	16-1	WELL 16	IRON DISS	ND	MG/L	0.1
9/14/2007	16-1	WELL 16	MAGNESIUM DISS	38.2	MG/L	0.1
9/14/2007	16-1	WELL 16	MANGANESE DISS	ND	UG/L	0.5
9/14/2007	16-1	WELL 16	NITROGEN NO3-N DISS	3.15	MG/L	0.006
9/14/2007	16-1	WELL 16	PH LAB	7.48	SU	
9/14/2007	16-1	WELL 16	POTASSIUM DISS	1.1	MG/L	0.1
9/14/2007	16-1	WELL 16	SODIUM DISS	13.7	MG/L	0.1
9/14/2007	16-1	WELL 16	SULFATE DISS	9.9	MG/L	0.02
9/14/2007	16-1	WELL 16	TURBIDITY	<1.0	NTU	
12/19/2007	16-4	WELL 16	ALKALINITY TOTAL CACO3	269	MG/L	2.5
12/19/2007	16-4	WELL 16	CALCIUM DISS	67.6	MG/L	0.1
12/19/2007	16-4	WELL 16	CHLORIDE DISS	40.2	MG/L	0.026
12/19/2007	16-4	WELL 16	CONDUCTIVITY AT 25C	664	US/CM	
12/19/2007	16-4	WELL 16	IRON DISS	ND	MG/L	0.1
12/19/2007	16-4	WELL 16	MAGNESIUM DISS	38.8	MG/L	0.1
12/19/2007	16-4	WELL 16	MANGANESE DISS	ND	UG/L	0.5
12/19/2007	16-4	WELL 16	NITROGEN NO3-N DISS	2.7	MG/L	0.006
12/19/2007	16-4	WELL 16	PH LAB	7.77	SU	
12/19/2007	16-4	WELL 16	POTASSIUM DISS	1.1	MG/L	0.1
12/19/2007	16-4	WELL 16	SODIUM DISS	13.7	MG/L	0.1
12/19/2007	16-4	WELL 16	SULFATE DISS	18.6	MG/L	0.02
12/19/2007	16-4	WELL 16	TURBIDITY	<1.0	NTU	
9/26/2007	17-1	WELL 17	ALKALINITY TOTAL CACO3	274	MG/L	2.5
9/26/2007	17-1	WELL 17	CALCIUM DISS	64.8	MG/L	0.1
9/26/2007	17-1	WELL 17	CHLORIDE DISS	33.4	MG/L	0.026

Date Collected	Field ID	Well or Lake	Parameter	Result	Units	LOD
9/26/2007	17-1	WELL 17	IRON DISS	ND	MG/L	0.1
9/26/2007	17-1	WELL 17	MAGNESIUM DISS	41.8	MG/L	0.1
9/26/2007	17-1	WELL 17	MANGANESE DISS	31	UG/L	0.5
9/26/2007	17-1	WELL 17	NITROGEN NO3-N DISS	0.014	MG/L	0.006
9/26/2007	17-1	WELL 17	PH LAB	7.49	SU	
9/26/2007	17-1	WELL 17	POTASSIUM DISS	1.4	MG/L	0.1
9/26/2007	17-1	WELL 17	SODIUM DISS	15.2	MG/L	0.1
9/26/2007	17-1	WELL 17	SULFATE DISS	43.3	MG/L	0.02
9/26/2007	17-1	WELL 17	TURBIDITY	<1.0	NTU	
9/18/2007	19-1	WELL 19	ALKALINITY TOTAL CACO3	276	MG/L	2.5
9/18/2007	19-1	WELL 19	CALCIUM DISS	59.6	MG/L	0.1
9/18/2007	19-1	WELL 19	CHLORIDE DISS	3.6	MG/L	0.026
9/18/2007	19-1	WELL 19	IRON DISS	0.2	MG/L	0.1
9/18/2007	19-1	WELL 19	MAGNESIUM DISS	31.9	MG/L	0.1
9/18/2007	19-1	WELL 19	MANGANESE DISS	50	UG/L	0.5
9/18/2007	19-1	WELL 19	NITROGEN NO3-N DISS	ND	MG/L	0.006
9/18/2007	19-1	WELL 19	PH LAB	7.58	SU	
9/18/2007	19-1	WELL 19	POTASSIUM DISS	1.8	MG/L	0.1
9/18/2007	19-1	WELL 19	SODIUM DISS	3.9	MG/L	0.1
9/18/2007	19-1	WELL 19	SULFATE DISS	6.89	MG/L	0.02
9/18/2007	19-1	WELL 19	TURBIDITY	<1.0	NTU	
1/2/2008	19-4	WELL 19	ALKALINITY TOTAL CACO3	274	MG/L	2.5
1/2/2008	19-4	WELL 19	CALCIUM DISS	62.2	MG/L	0.1
1/2/2008	19-4	WELL 19	CHLORIDE DISS	3.7	MG/L	0.026
1/2/2008	19-4	WELL 19	CONDUCTIVITY AT 25C	534	US/CM	
1/2/2008	19-4	WELL 19	IRON DISS	0.2	MG/L	0.1
1/2/2008	19-4	WELL 19	MAGNESIUM DISS	33.9	MG/L	0.1
1/2/2008	19-4	WELL 19	MANGANESE DISS	52	UG/L	0.5
1/2/2008	19-4	WELL 19	NITROGEN NO3-N DISS	ND	MG/L	0.006
1/2/2008	19-4	WELL 19	PH LAB	7.84	SU	
1/2/2008	19-4	WELL 19	POTASSIUM DISS	1.8	MG/L	0.1
1/2/2008	19-4	WELL 19	SODIUM DISS	3.9	MG/L	0.1
1/2/2008	19-4	WELL 19	SULFATE DISS	6.8	MG/L	0.02
1/2/2008	19-4	WELL 19	TURBIDITY	<1.0	NTU	
9/19/2007	27-1	WELL 27	ALKALINITY TOTAL CACO3	300	MG/L	2.5
9/19/2007	27-1	WELL 27	CALCIUM DISS	77.6	MG/L	0.1
9/19/2007	27-1	WELL 27	CHLORIDE DISS	39	MG/L	0.026
9/19/2007	27-1	WELL 27	IRON DISS	0.1	MG/L	0.1
9/19/2007	27-1	WELL 27	MAGNESIUM DISS	42.3	MG/L	0.1
9/19/2007	27-1	WELL 27	MANGANESE DISS	35	UG/L	0.5
9/19/2007	27-1	WELL 27	NITROGEN NO3-N DISS	0.397	MG/L	0.006
9/19/2007	27-1	WELL 27	PH LAB	7.34	SU	
9/19/2007	27-1	WELL 27	POTASSIUM DISS	1.6	MG/L	0.1
9/19/2007	27-1	WELL 27	SODIUM DISS	17.5	MG/L	0.1
9/19/2007	27-1	WELL 27	SULFATE DISS	41.5	MG/L	0.02

Date Collected	Field ID	Well or Lake	Parameter	Result	Units	LOD
9/19/2007	27-1	WELL 27	TURBIDITY	<1.0	NTU	
9/17/2007	28-1	WELL 28	ALKALINITY TOTAL CACO3	266	MG/L	2.5
9/17/2007	28-1	WELL 28	CALCIUM DISS	59.9	MG/L	0.1
9/17/2007	28-1	WELL 28	CHLORIDE DISS	0.887	MG/L	0.026
9/17/2007	28-1	WELL 28	IRON DISS	0.2	MG/L	0.1
9/17/2007	28-1	WELL 28	MAGNESIUM DISS	32.8	MG/L	0.1
9/17/2007	28-1	WELL 28	MANGANESE DISS	24	UG/L	0.5
9/17/2007	28-1	WELL 28	NITROGEN NO3-N DISS	0.022	MG/L	0.006
9/17/2007	28-1	WELL 28	PH LAB	7.42	SU	
9/17/2007	28-1	WELL 28	POTASSIUM DISS	1	MG/L	0.1
9/17/2007	28-1	WELL 28	SODIUM DISS	2.4	MG/L	0.1
9/17/2007	28-1	WELL 28	SULFATE DISS	17.8	MG/L	0.02
9/17/2007	28-1	WELL 28	TURBIDITY	<1.0	NTU	
9/24/2007	30-1	WELL 30	ALKALINITY TOTAL CACO3	249	MG/L	2.5
9/24/2007	30-1	WELL 30	CALCIUM DISS	58.7	MG/L	0.1
9/24/2007	30-1	WELL 30	CHLORIDE DISS	3.75	MG/L	0.026
9/24/2007	30-1	WELL 30	IRON DISS	ND	MG/L	0.1
9/24/2007	30-1	WELL 30	MAGNESIUM DISS	34.2	MG/L	0.1
9/24/2007	30-1	WELL 30	MANGANESE DISS	3	UG/L	0.5
9/24/2007	30-1	WELL 30	NITROGEN NO3-N DISS	0.04	MG/L	0.006
9/24/2007	30-1	WELL 30	PH LAB	7.55	SU	
9/24/2007	30-1	WELL 30	POTASSIUM DISS	1.7	MG/L	0.1
9/24/2007	30-1	WELL 30	SODIUM DISS	3.9	MG/L	0.1
9/24/2007	30-1	WELL 30	SULFATE DISS	16.8	MG/L	0.02
9/24/2007	30-1	WELL 30	TURBIDITY	<1.0	NTU	
1/9/2008	30-4	WELL 30	ALKALINITY TOTAL CACO3	252	MG/L	2.5
1/9/2008	30-4	WELL 30	CALCIUM DISS	54.4	MG/L	0.1
1/9/2008	30-4	WELL 30	CHLORIDE DISS	2.58	MG/L	0.026
1/9/2008	30-4	WELL 30	CONDUCTIVITY AT 25C	509	US/CM	
1/9/2008	30-4	WELL 30	IRON DISS	0.2	MG/L	0.1
1/9/2008	30-4	WELL 30	MAGNESIUM DISS	32.3	MG/L	0.1
1/9/2008	30-4	WELL 30	MANGANESE DISS	13	UG/L	0.5
1/9/2008	30-4	WELL 30	NITROGEN NO3-N DISS	ND	MG/L	0.006
1/9/2008	30-4	WELL 30	PH LAB	7.81	SU	
1/9/2008	30-4	WELL 30	POTASSIUM DISS	1.6	MG/L	0.1
1/9/2008	30-4	WELL 30	SODIUM DISS	3.6	MG/L	0.1
1/9/2008	30-4	WELL 30	SULFATE DISS	16.8	MG/L	0.02
1/9/2008	30-4	WELL 30	TURBIDITY	<1.0	NTU	
9/19/2007	MENDOTA-1	MENDOTA	ALKALINITY TOTAL CACO3	155	MG/L	2.5
9/19/2007	MENDOTA-1	MENDOTA	CALCIUM DISS	26.1	MG/L	0.1
9/19/2007	MENDOTA-1	MENDOTA	CHLORIDE DISS	38	MG/L	0.026
9/19/2007	MENDOTA-1	MENDOTA	IRON DISS	ND	MG/L	0.1
9/19/2007	MENDOTA-1	MENDOTA	MAGNESIUM DISS	30.4	MG/L	0.1
9/19/2007	MENDOTA-1	MENDOTA	MANGANESE DISS	3	UG/L	0.5
9/19/2007	MENDOTA-1	MENDOTA	NITROGEN NO3-N DISS	0.023	MG/L	0.006

Date Collected	Field ID	Well or Lake	Parameter	Result	Units	LOD
9/19/2007	MENDOTA-1	MENDOTA	PH LAB	8.55	SU	
9/19/2007	MENDOTA-1	MENDOTA	POTASSIUM DISS	3.3	MG/L	0.1
9/19/2007	MENDOTA-1	MENDOTA	SODIUM DISS	18.9	MG/L	0.1
9/19/2007	MENDOTA-1	MENDOTA	SULFATE DISS	20.1	MG/L	0.02
9/19/2007	MENDOTA-1	MENDOTA	TURBIDITY	<1.0	NTU	
9/21/2007	MONONA-1	MONONA	ALKALINITY TOTAL CACO3	152	MG/L	2.5
9/21/2007	MONONA-1	MONONA	CALCIUM DISS	26.6	MG/L	0.1
9/21/2007	MONONA-1	MONONA	CHLORIDE DISS	48.5	MG/L	0.026
9/21/2007	MONONA-1	MONONA	IRON DISS	ND	MG/L	0.1
9/21/2007	MONONA-1	MONONA	MAGNESIUM DISS	27.7	MG/L	0.1
9/21/2007	MONONA-1	MONONA	MANGANESE DISS	1	UG/L	0.5
9/21/2007	MONONA-1	MONONA	NITROGEN NO3-N DISS	ND	MG/L	0.006
9/21/2007	MONONA-1	MONONA	PH LAB	8.88	SU	
9/21/2007	MONONA-1	MONONA	POTASSIUM DISS	3	MG/L	0.1
9/21/2007	MONONA-1	MONONA	SODIUM DISS	23.7	MG/L	0.1
9/21/2007	MONONA-1	MONONA	SULFATE DISS	24.5	MG/L	0.02
9/21/2007	MONONA-1	MONONA	TURBIDITY	<1.0	NTU	
9/17/2007	WINGRA-1	WINGRA	ALKALINITY TOTAL CACO3	142	MG/L	2.5
9/17/2007	WINGRA-1	WINGRA	CALCIUM DISS	30.4	MG/L	0.1
9/17/2007	WINGRA-1	WINGRA	CHLORIDE DISS	69.1	MG/L	0.026
9/17/2007	WINGRA-1	WINGRA	IRON DISS	ND	MG/L	0.1
9/17/2007	WINGRA-1	WINGRA	MAGNESIUM DISS	27.3	MG/L	0.1
9/17/2007	WINGRA-1	WINGRA	MANGANESE DISS	4	UG/L	0.5
9/17/2007	WINGRA-1	WINGRA	NITROGEN NO3-N DISS	0.016	MG/L	0.006
9/17/2007	WINGRA-1	WINGRA	PH LAB	8.61	SU	
9/17/2007	WINGRA-1	WINGRA	POTASSIUM DISS	2.1	MG/L	0.1
9/17/2007	WINGRA-1	WINGRA	SODIUM DISS	34.1	MG/L	0.1
9/17/2007	WINGRA-1	WINGRA	SULFATE DISS	15.8	MG/L	0.02
9/17/2007	WINGRA-1	WINGRA	TURBIDITY	<1.0	NTU	

Appendix C: Field measurements.

Key: well id = local number of well or lake name; Sample id = field sample id; collection date and Marshfield Clinic sample ID provided in Appendix A.

well id	sample id	pH	Dissolved oxygen (mg/L)	Specific conductance (µhos/cm)
7	7-1	7.03	0.2	791
7	7-2	7.34	0.8	741
7	7-4	7.09	0.4	700
7	7-5	7.37	0.4	712
7	7-6	7.26	0.6	854
7	7-7	7.39	0.05	715
7	7-8	7.26	0.5	697
7	7-9	7.04	0.1	741
7	7-10	7.45	NA	731
7	7-11	7.26	NA	682
8	8-1	7.47	0.5	710
8	8-2	7.28	0.6	662
11	11-1	6.79	3.5	850
11	11-2	7.31	0.4	826
11	11-3	7.27	3	811
11	11-5	7.34	2	805
11	11-6	7.29	2	833
11	11-7	7.19	3	788
11	11-8	7.4	2	821
11	11-9	7.09	3.5	839
11	11-10	7.4	3.5	840
11	11-11	7.26	3	815
12	12-1	6.78	3.5	612
12	12-2	7.1	3	560
12	12-3	7.27	3	516
12	12-4	7.09	4	590
12	12-5	7.42	2	571
12	12-6	7.31	5	545
12	12-7	7.11	2	591
12	12-8	6.99	2	588
12	12-9	7.16	1.5	597
12	12-10	7.47	3.5	537
12	12-11	7.3	3.5	632
13	13-1	6.99	1.5	621
13	13-10	7.45	2.5	624
13	13-11	7.36	2	606
13	13-2	7.4	2	630
13	13-3	7.3	2.5	670

well id	sample id	pH	Dissolved oxygen (mg/L)	Specific conductance (µhos/cm)
13	13-4	6.97	1.5	704
13	13-5	7.39	2	725
13	13-6	7	2	583
13	13-7	7.36	1	595
13	13-8	7.37	3	624
13	13-9	7.43	NA	649
16	16-1	7.2	6.6	708
16	16-2	7.26	5	701
16	16-3	7.36	4	740
16	16-4	7.2	3.5	732
17	17-1	7.5	0.8	670
17	17-2	7.76	0.05	837
19	19-1	6.84	1	589
19	19-10	7.3	0.5	555
19	19-11	7.19	0.15	NA
19	19-2	7.35	1.5	583
19	19-3	7.38	0	538
19	19-4	7.42	0.2	547
19	19-5	7.32	0.2	660
19	19-6	7.28	0.05	538
19	19-7	7.44	1	549
19	19-8	7.32	0	570
19	19-9	7.01	0.5	596
24	24-2	7.52	0.6	554
27	27-1	6.52	1	778
28	28-1	6.47	3	NA
28	28-2	7.14	0.6	600
30	30-1	6.7	0.1	653
30	30-10	7.46	0.05	541
30	30-11	7.29	0.05	603
30	30-2	7.44	0.1	578
30	30-3	7.38	0	526
30	30-4	7.07	0.05	523
30	30-5	7.44	0.8	546
30	30-6	7.48	0.05	536
30	30-7	7.45	0.1	532
30	30-8	7.49	0	560
30	30-9	7.06	0.1	729
lake	Mendota 10	8.43	NA	NA
lake	Mendota- 8	8.18	9	546
lake	Mendota-1	8.25	6	472
lake	Monona- 8	7.82	9	715
lake	Monona-10	8.43	NA	NA

well id	sample id	pH	Dissolved oxygen (mg/L)	Specific conductance (µhos/cm)
lake	Monona-1	8.77	8	574
lake	Wingra- 8	8.24	9	741
lake	Wingra-10	8.1	NA	NA
lake	Wingra-1	8.23	6	552