THE INFLUENCE OF DAM RELEASES ON MICROBIAL AND

PHYSIOCHEMICAL PARAMETERS IN THE ALLUVIAL AQUIFER OF A

REGULATED RIVER

A Thesis

by

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ABSTRACT

Elevated fecal indicator bacteria in river systems is a continuing concern in Texas and throughout the United States. It has been discovered that bacterial concentrations in surface waters can increase drastically during elevated flow events. These events can be induced by natural, or anthropologic causes. An example of the latter is releases of large amounts of water from dams. These large releases of water to a river system may also influence the state of the surrounding alluvial groundwater. The aim of this study was to better understand changes in bacterial concentrations in the saturated alluvium approximately 19.3 kilometers downstream of the Tom Miller Dam, following regular, dam releases every 24 hours. A transect of 4 groundwater wells, at distances 1.5 to 6 meters from the river, was installed perpendicular to the bank of the Colorado River near Austin, TX. The wells were sampled every 4 hours over a 24-hour period. Field measurements were taken for temperature, electrical conductivity, pH and dissolved oxygen (DO). Laboratory tests were used to determine concentrations of total coliforms, fecal coliforms, E. coli, PO₄-P, NO₃-N, dissolved organic carbon (DOC), and total nitrogen (TN). Results indicated that the dam release had little impact on bacterial concentrations of the surrounding alluvium with increases detected only in the well closest to the river at peak water levels. During the time in which these samples were collected, the river system was under relatively low-flow conditions with the regular dam release only raising the water level about 0.06 m (0.2 ft) during sampling. Therefore, following sampling, the wells were fitted with pressure transducers to

quantify lateral hydraulic gradients as a function of river level. Lateral hydraulic gradients confirmed the small dam releases, currently occurring upstream of the site, were not large enough to reverse the natural groundwater flow direction away from the river, consistent with the microbial and chemical results.

DEDICATION

I dedicate this thesis to my grandparents, Mr. and Mrs. Arnoldo Barrera Sr. and Mr. and Mrs. Carlos Tamayo. You all always taught me to appreciate everything that I have, to pursue my dreams and, no matter what, to work hard in order to reach my fullest potential. I love you all more than I could ever hope to express.

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1. INTRODUCTION

In many locations throughout the world, clean drinking water is not accessible to communities that are in desperate need of it. In such cases, shallow drinking water wells are installed in close proximity to surface water in the hopes that these groundwater wells are less contaminated than the nearby surface water. Natural filtration processes that occur in the adjacent saturated alluvium tend to purify the surface water of many contaminants (Tufenkji et al., 2002). Natural exchange between surface and groundwater fluctuates in direction and magnitude depending on the hydraulic gradient of the area. In an area where water table and river levels are similar, low flow river conditions may cause the river to receive groundwater. This is known as a gaining stream. During high river stage, however, the hydraulic gradient may be reversed and water would be forced into the surrounding alluvium.

The river associated with this project is the Colorado River. This river is the longest to be completely within the borders of the state of Texas. Its headwaters begin around Lamesa, TX, and the river travels approximately 1,387.25 km (862 miles) until it empties into Matagorda Bay near Houston, TX. There are two man-made lakes along the river that affect this project. The first is Austin Lake, created due to the construction of Tom Miller Dam, and the second is Lady Bird Lake, created due to the construction of Longhorn Dam (Figure 3.3). Tom Miller Dam was constructed primarily for flood control and hydroelectricity production purposes. This facility provides electricity to the city of Austin, especially during high use summer months. The dam is also used to

supplement the natural downstream flow in the river, provide water to the residents living along the river and supply the coastal estuaries with freshwater. Water is released from Austin Lake once every 24 hours causing a pulse in river flow that travels downstream until it reaches Lady Bird Lake. If the lake levels at Lady Bird lake are low, this water refills the lake. However, if the water levels at Lady Bird Lake are normal, the additional water passes through the spillway gates at Longhorn Dam and continues downstream.

This pulse in river flow and its effects on the breadth of the hyporheic zone of the river was studied by Sawyer et al. (2002). They found that the river stage, at the time, increased up to 2.8 m (9.2 ft) over a 24 hour period. Additionally, the river pulses were reversing the hydraulic gradient of the area up to 30 m (98.4 ft) into the river bank. In addition to the findings from that project, in 2010, the U.S. Environmental Protection Agency classified 8 km (5 mi.) of river, 5.8 km (3.6 mi.) upstream of Hornsby Bend (Figure 3.3.), as impaired due to bacterial contamination (USEPA, 2015). These two veracities could have a profound impact on the alluvial groundwater of the Colorado river system if the change in hydraulic gradient meant that impaired surface water was interacting with the alluvial groundwater.

The main objective of this research was to measure the effect of a rising and falling river stage along the Colorado River in Texas, over 24 hour periods, in order to determine if concentrations of microbial fecal indicators and pathogens in the river affected the microbial concentrations of the alluvial groundwater.

2. LITERTURE REVIEW

Water is an undeniably important resource for continuation of life on this planet. The state of water quality all over the world is of growing concern. All topics addressed in this review are key components to the purpose of this thesis. Without these components, an accurate understanding of the influence of dam releases on the state of alluvial groundwater would be impossible. The topics covered include the impact of hydroelectric dam releases on the down-stream hydrology, stream and groundwater exchange through riverbank filtration, utilization of physiochemical water parameters as water tracers, and the measurement of microbial water quality indicators. Despite the array in which these topics are presented, this review focus' on their application toward understanding the impacts that dam releases may have on the microbial and physiochemical parameters in the alluvial aquifer of a regulated river.

2.1. Dam Releases

All water, whether in liquid, solid, or gaseous form, make up the hydrosphere of the earth (Shiklomanov, 2000), which totals $1.386 \times 10^6 \text{ km}^3$. Of this hydrosphere only 2.5% is freshwater, and of this freshwater, 68.7% is frozen, 29.9% is classified as groundwater and only about 0.26% is available as surface water in lakes, rivers and streams (Carpenter et al., 2011). Currently, the upper limit of available fresh water has not been exceeded by human water usage, however, that is based on the global average. There are approximately 2.4 billion people living in areas that are considered highly water stressed (Oki and Kanae, 2006), and water management is crucial in these places.

The human solution to this problem is to install dams within large river systems to impound water in places that are in short supply during most of the year. Of the major river systems on the earth, dams affect over half (Nilsson et al., 2005). These effects are seen in the both the upstream and downstream portions of the river, and range from inundation of the floodplain upstream to temperature and water chemistry alterations downstream (Nilsson et al., 2005; World Commission on Dams, 2000).

Downstream effects are especially predominant below hydroelectric dams due to the large scheduled releases of water that power the turbines. These large releases cause a rise in the river stage that has the potential to force water into the surrounding riverbank and change the hydraulic gradient of the area. Then, if normal conditions favor a gaining stream, as the water recedes, the gradient changes back so that groundwater flows toward the river (Curry et al., 1994; Singh, 2004). This changes drainage water chemistry at specific locations along the river. Smart et al. (2001) found evidence that the geochemical characteristics of the riparian area had a significant influence on the chemical composition of the receding water. River stage increases from anthropogenic influences are often quite different from river stage changes brought about by natural events; such as storms, and so may cause changes not seen during natural occurrences. Temperature is a physical water characteristic that is often measured to be different upstream and downstream of a dam. Cooler waters, associated with the hypolimnion of the water body upstream of the dam, are released and alter the temperatures and oxygen concentrations of downstream environments (Hancook, 2002). Hill et al. (2000) found

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that microbial respiration, of the sediment near a stream containing cooler water, decreased, due to the negative impact on mesophilic microorganisms.

2.2. Stream and groundwater exchange

Natural river and stream systems are not consistent in their discharge and, instead, change from season to season and even from day to day (Cardenas, 2009). Under normal flow conditions, a stream system is considered to be either a gaining or a losing stream. When the groundwater table is higher than the surface of the stream, water will flow toward the stream, replenishing the river with what is known as base flow. Conversely, if the groundwater table is lower than the surface of the stream then water will flow toward the groundwater table (Sawyer et al., 2009). Surrounding these stream systems is an area of surface water and groundwater exchange known as the hyporheic zone, which literally means "under flow" (Boulton, 2000) and is named so since it is the area in which surface water infiltrates the alluvial aquifers and underlying groundwater (Figure 2.1).

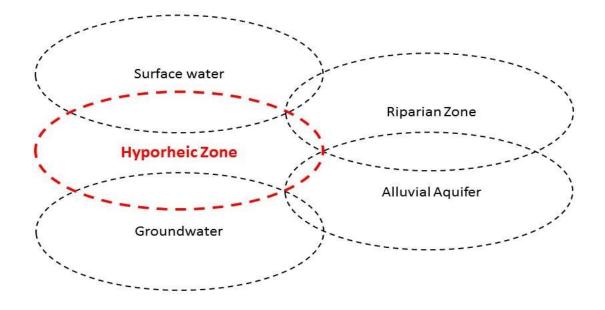


Figure 2.1. The hyporheic zone allows for water exchange from the surface stream into underlying groundwater and adjacent alluvial aquifers.

Hyporheic zone exchange is influenced by pressure and hydraulic gradients of the area (Cardenas, 2009). For rivers regulated by dams, changes in water height are consistent, as opposed to natural systems that are subject to seasonal flooding. In cases of rivers that are regulated by hydroelectric dams, water stage changes in the river follow an undulating pattern as water is released regularly for the production of electricity. During peak flow, under release schedules, it is possible that the hydraulic gradient between the surface water and the groundwater reverses from its natural state. Another way to explain this occurrence is through the Gill-Lung Model (Sawyer et al., 2009). The model illustrates that during base-flow the hyporheic zone exchange of water is shallow relative to the river, however, during regulated flow, such as under dam release conditions, the water exchange in the hyporheic zone is much deeper and dynamic.

Due to the influence of surface water, pressure gradients and directional flow changes, there are substantial amounts of water mixing occurring within the hyporheic zone and extending, sometimes, into groundwater. This mixing of these separate bodies of water establishes an environment that is suitable for high levels of biogeochemical cycling and bacterial growth. This dynamic nature of the hyporheic zone and its relative inaccessibility which makes physical mapping and special characterization of the aforementioned processes difficult. However, in one study, Cardenas et al. (2011) utilized electrical resistivity imaging (ERI) to map the extent and dynamics of water mixing and flow paths within the alluvial material along the Colorado River located in Austin, Texas. This study found that the hyporheic zone of the area extends several meters deep and across the entire channel bed. This size and extent would not be possible if the Colorado River was not a regulated river. Rivers that remain at low flow status for extended periods tend to have a shallow hyporheic zone, where surface water is not forced into and out of the hyporheic zone but merely flows through it. Thus, it can be hypothesized that the dam releases that result in an undulating river stage may also affect the amount of bacteria found in the surrounding groundwater due to water being forced downward.

2.3. Filtration properties of the riverbank

The fate of microbial organisms in soils likely depends on both biotic and abiotic controlling factors (van Keen et al., 1997). Biotic controls include competition and the

production of inhibitory substances by other organisms that may affect the survival of soil organisms. Due to this, increases in the survival rates of bacteria, such as *Escherichia coli*, occur when the microbial soil community is diminished in its complexity (van Elsas et al., 2007). Abiotic factors include mechanisms such as colloid straining. Escherichia coli was found to become retained through straining in finertextured soils at a greater rate than mechanisms like physico-chemical attachment (Bradford and Bettahar, 2005). As water moves within soils, deposition profiles for a range of microorganisms, like bacteria, viruses and protozoa, suggest that with increasing distance from the origin of contamination there is a decreasing rate of deposition (Albinger et al., 1994; DeFlaun et al., 1997; Baygents et al., 1998; Simoni et al., 1998; Bolster et al., 2000; Redman et al., 2001; Zhang et al., 2001; Bradford et al., 2002; Li et al., 2004; Bradford and Bettahar, 2005). In combination with straining, physico-chemical filtration aid in the removal of colloidal particles from water that moves through the soil matrix. The surface charge of soil particles, coupled with the charges on colloids enhance physico-chemical filtration. (Li et al., 2004).

2.4. Escherichia coli

When scientists look for the existence of pathogens in water samples, they do not test every water sample for the presence of every known pathogen, due to the high cost associated with such a method. In order to test many samples at a relatively low cost they test for the presence of indicator organisms. An important characteristic of an indicator organism is that it is able to persist in the same environment as the organism(s) of concern. However, because of many factors, including a variation in rates of survival, the lifespan of specific bacteria or viruses cannot be correlated with the presence or absence of indicator organisms (Dorner et al., 2004; Ferguson et al., 2012). The indicator organisms primarily used are total coliforms, fecal coliforms and *E. coli. Escherichia coli* is itself a fecal coliform but is the most widely used as an indicator organism because of its association with fecal contamination (CDC, 2009). These indicator organisms are of bacterial origin and are easily cultured in a laboratory setting on agar plates or through the use of Most Probable Number methods.

2.5. *Cryptosporidium* and Enterovirus

Cryptosporidium parvum was recognized first as an animal pathogen in 1955, and not until 1976 was it identified as a human pathogen. *C. parvum* is a protozoan, obligate parasite that multiplies within its host. It causes infections and self-limiting diarrhea in healthy adults, but in infants, the elderly, or anyone that is immunocompromised, it may cause fatal diarrhea. The oocysts of this species of *Cryptosporidium* are very hardy and environmentally resistant, which allows them to persist outside of their host once they are shed from the body through the fecal matter (Szewzyk et al., 2000). The Enterovirus group encompasses a few different virus types which include Polioviruses 1-3, Coxsackievirus A, Coxsackievirus B, Echoviruses, and Enterovirus 68-71. All of these virus types inhabit the human alimentary tract, which means that they are transmitted via the fecal-oral route. The spectrum of Enterovirus disease is vast, from permanent paralysis to subclinical infection. The latter is much more common than the occurrence of a clinically manifested disease (Melnick, 1982).

2.6. Water chemistry

There is a substantial link between water shortage and water quality. Water contamination reduces the amount of usable water available and also drives up the price of water treatment. Nonpoint pollution is considered to be the most significant source for nitrogen, phosphorus and carbon in natural water systems (Carpenter et al., 1998). Nitrogen is an abundant element in the environment. Natural sources to watersheds include rock weathering, lightning and wet precipitation, N-fixation to vegetation, fecal matter from wildlife and death and decay of vegetation and animals. Anthropogenic sources include mineral fertilizers applied to agricultural crops and homeowner landscapes in urban and suburban watersheds, rain out of volatilized ammonia and fecal matter and death and decay of domestic animals. Of the nitrogen species, nitrate is an extremely conservative molecule and is easily leached through soil toward groundwater or washed out of the soil during rainstorms and runoff events. This will cause the nitrogen to become unusable to plants, because it will be out of the reach of their root systems. When that occurs the end point for this nitrogen species is usually groundwater pools or rivers and streams (Bai et al., 2012).

Phosphorus is used as an additive to agricultural farms and private lawn and garden soils as a fertilizer. Although, anthropogenic sources of phosphorus have almost tripled the global mobilization of this nutrient, a small portion is made mobile due to natural sources such as decay of dead biomass, and solubilization of soil phosphates by microorganisms (Smil, 2000). Phosphorus is unique in its cycling because phosphorus rarely enters a gaseous form, which confines it to the soil and water of the environment. Phosphorus is the limiting nutrient in freshwater aquatic systems and any increase, no matter how small, could cause unwanted effects. These could include increased algal growth and decreased oxygen levels that could in turn negatively influence the population of aquatic life. There are two forms of phosphorus is the aquatic environment, organic and inorganic. Organic phosphorus is incorporated in plant and animal tissues, while inorganic phosphorus, the form available for plant uptake, is not. Both forms have the potential to be dissolved or suspended in the water column. The concentration of phosphate in the water column, largely depends on the adsorption and desorption properties to the surrounding sediment, as equilibrium is controlled by this exchange of phosphate into and off of particle surfaces (Kerr et al., 2011). The same may also be true of organic carbon within shallow groundwater.

Dissolved organic carbon (DOC) in shallow groundwater, especially groundwater that is used as a drinking water source, has unwanted aesthetic considerations as well as an influence on microbial growth (Wassenaar et al, 1996). Most DOC concentrations in natural groundwater sources are between the concentrations of < 1 mg/L to 100 mg/L (Chomycia et al 2008; Wassenaar et al, 1991 and Wassenaar et al, 1996), however, the concentrations all depend on the differences in organic matter origin and materials that makeup the overlying aquifer soils (Chomycia et al, 2008). High humic soils and seasonal changes in the amount of vegetative growth in the area all contribute to a change in carbon concentrations. Groundwater with high DOC concentrations provide significant sources of carbon for microbes (Findlay et al, 1993), such as *E. coli*. Studies performed by Bolster et al. (2005) and Surbeck et al. (2010) showed results that *E. coli* thrive and even show signs of population recovery in waters that contain high DOC and other nutrients.

Surface water and groundwater has very distinct chemical characteristics, these characteristics allow them to be useful tools in monitoring the movement of water between streams and alluvial aquifers.

3. MATERIALS AND METHODS

3.1 Monitoring wells

A transect of four wells was installed along the Colorado River in Austin, Texas (TCEQ Station ID #21411). The well site (N30°13'56.8", W97°39'12.6") is located at Hornsby Bend and the Center for Environmental Research. All wells were installed using a Geoprobe and were installed to a depth of approximately 3 m (10 ft). They were all screened across the entire water table and are each located 1.5 m (5 ft.) from the previous, with the first well being installed 1.5 m from the edge of the river at the time of installation (Figure 3.1 and 3.2).

Throughout the duration of the project, all river height data was obtained from a USGS gauge that is located under highway 183, approximately 11.6 km (7.2 mi) upstream of the research site (USGS 08158000 Colorado River at Austin, TX) (Figure 3.3). The elevation measurements were adjusted by applying a constant, which was calculated by taking the difference in datum elevations (meters above sea level) from each site and then applying that difference to all subsequent water elevation measurements.

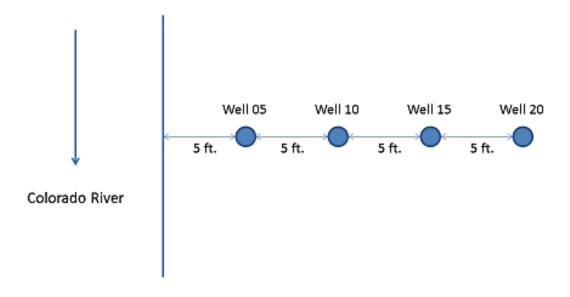


Figure 3.1. Well transect located along the Colorado River at Hornsby Bend in Austin, TX. TCEQ Station ID #21411



Figure 3.2. Hornsby Bend well transect showing the Colorado River in the background and groundwater wells 05, 10, 15, and 20.

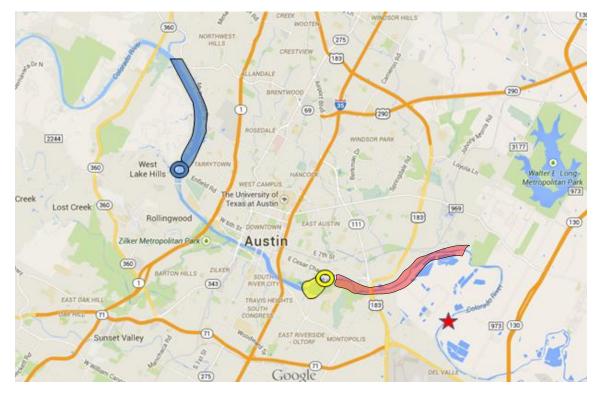


Figure 3.3. Map of Austin area, showing the locations of Austin Lake and Tom Miller Dam (blue), Lady Bird Lake and Longhorn Dam (yellow) and Hornsby Bend research well transect (red star, N30°13'56.8", W97°39'12.6"). In 2010, the section of the river highlighted in red was classified as impaired due to bacterial contamination by recreational standards.

3.2 Pumps

During the first round of sampling (Table 3.1), a Masterflex E/S portable peristaltic pump (Cole-Parmer, Vernon Hills, IL) was utilized. Sampling from the groundwater wells, during the second and third rounds of sampling, was done using four Poseidon 12V submersible water pumps (Groundwater Essentials, Bradenton, FL); one individual pump was used for each of the four wells in order to avoid crosscontamination between wells. The tubing setup remained the same for all three rounds, with only minor alterations in order to accommodate the pumps. The tubing used was standard 6.35 mm (1/4 in) ID polypropylene tubing with a backflow regulator attached to the end to prevent contamination of the well from outside sources. Before each sampling event, the pumps and tubing system were disinfected using a 200 mg/l chlorine solution, which was run through the pumps and tubing until 19 liters was used. The pump and tubing was then rinsed using 40 liters of reverse osmosis water to ensure that all residual chlorine was removed. A HACH DR 2800 spectrophotometer and TNT 867 chlorine detection kit (Hach Company, Loveland, Colorado) was used to confirm that chlorine removal was successful. Before each sample was collected from the groundwater wells, two entire well volumes were purged to ensure that water from the alluvium was being sampled and not stagnant water in the well column. Samples were collected from the river, adjacent to the well transect, using a telescoping swing sampler (Nasco, Ft. Atkinson, WI). These surface water samples were collected from an area 3 m from the river bank.

3.3 Sampling

There were a total of four sampling dates throughout the duration of this project (Table 3.1). Of the four events, three were over a period of 24 hours, with one exception, while only one was used for the purposes of *Cryptosporidium* and Enterovirus collection.

Approximately a week prior to the October event, there was a significant storm event that passed through the Austin area and caused a 1.8 m (6 ft) increase in river stage. This sizable increase in river stage caused the Hornsby Bend well field to become inundated with river water (Figure 3.4). Another storm, prior to the June 16-17 event, caused an increase in the river stage, however, it was not significant enough to flood the well field.

Event Date	Label*	Period	Parameters Measured
October 2-3, 2013	А	24 hours	Temperature Electrical Conductivity
			Dissolved Oxygen Microbial Chemical
June 8-9, 2014	В	20 hours	Temperature Electrical Conductivity Dissolved Oxygen Microbial Chemical
June 11, 2014	М	-	Cryptosporidium Enterovirus
June 16-17, 2014	С	24 hours	Temperature Electrical Conductivity Dissolved Oxygen Microbial Chemical

Table 3.1. Shows sampling event dates and samples collected during each event. *All labels correspond to their assigned sampling date throughout this thesis.



Figure 3.4. Colorado River flowing at approximately 10,000 cfs following a storm event in October 2013.

3.3.1 Bacteria and chemical collection

All three of the 24-hour sampling rounds were scheduled to capture normal flow conditions of the river, and were not performed unless there had been, at least, three days between the end of the last rain event and the sampling event. Each round consisted of a complete 24-hours of sampling, with the exception of round 2 (June 8-9) for which the last sample was not collected due to the onset of rain in the area. Samples were collected every 4 hours relative to the first sample collected. For every groundwater and river

sample, two sample bottles of water were collected. One 500 mL bottle was used for bacterial samples that were transferred to the Lower Colorado River Authority (LCRA) Environmental Services Laboratory, and one 125 mL, acid washed, collection bottle was used for the collection of chemical samples which were returned to Texas A&M University and analyzed in Dr. Aitkenhead-Peterson's laboratory.

3.3.2 Cryptosporidium and Enterovirus collection

Sampling for *Cryptosporidium* and Enterovirus was performed on June 11, 2014, which was separate from the normal sampling dates. Water samples were collected as described for sampling events 2 and 3 above. Once well purging was completed, a total of 10 liters from each site was pumped through an Envirochek[®] HV sampling capsules (Pall, Port Washington, NY) for *Cryptosporidium* and a NanoCeram[®] cartridge filter (Argonide, Sanford, FL) for Enterovirus collection. All filters were placed on ice in a cooler and transported back to Texas A&M University for further analysis.

3.3.3. Field parameters

Field parameters, including temperature, specific conductivity, pH and dissolved oxygen was measured at the same time that samples were taken. During the first sampling event a YSI Professional Plus Multiparameter Handheld instrument with Quatro Cable and respective detection probes was used (YSI Incorporated, Yellow Springs, Ohio). However, due to equipment malfunction, pH was unable to be taken with the YSI probe during the second sampling event. The LCRA Environmental Services lab conducted pH measurements on all the water samples during that event. During the final sampling event, a second YSI instrument was utilized, a YSI 556 MPS. All instruments were calibrated during the morning of the scheduled sampling event, again halfway through the sampling event, and then a final time upon the conclusion of the event to make certain that they remained calibrated over the entire 24 hours.

3.3.4 Pressure transducer data collection

Two pressure transducers (Model 3001 Levelogger Edge, Solinst Canada Ltd., Georgetown, Ontario, Canada) and two TLC probes (Model 3001 LTC Junior, Solinst Canada Ltd., Georgetown, Ontario, Canada) were installed in the shallow wells. All probes measure temperature and pressure, whereas the TLC probes also measures specific conductance of the water. The two TLC probes were installed closest to the river since this zone experiences the greatest fluctuations in specific conductance due to dilution of groundwater with river water during the high river stage (Sawyer et al., 2009). The probes recorded temperature and pressure every 10 minutes. Pressures were converted to water level elevation in two steps. The first step was subtracting atmospheric pressure, which was recorded by a barologger on site (Barologger Edge, Solinst, Georgetown, Canada). This step assumes a hydraulic efficiency of one which is valid for shallow, unconfined aquifers (Spane, 1999; Gonthier, 2007). The second step was to reference the transducer pressure readings to a manual water level below the top of the well casing using a water level tape (Model 101, P7 Water Level Meter, Solinst Canada Ltd., Georgetown, Ontario, Canada). To ensure that the calibration worked correctly, each logger was calibrated twice; once at deployment and once after removal. Calibrations were performed using a measured water depth, from the top of the well casing, and compared to the measured pressure readings at the time of logger

deployment. The "actual water table" depth was calculated by subtracting the constant that was measured immediately before deployment. The relative elevations of the tops of the wells was measured using a Total Station (GTS-226, Topcon Positioning Systems, Inc. Livermore, CA). This allowed water levels within each well to be compared across the aquifer in meters above an arbitrary datum.

3.4 Laboratory analysis

3.4.1. Bacterial analysis

To test for the presence and number of total and fecal coliforms in the samples provided to them, the LCRA Environmental Services Laboratory used Standard Method 9222 membrane filtration (MF). For the detection of *Escherichia coli* the laboratory used Standard Method 9223 Colilert Quanti Trays by IDEXX (IDEXX Laboratories Incorporated, Westbrook, Maine). Error bars associated with most probable number (MPN) method were calculated using 95% confidence intervals (Hurley and Roscoe, 1983).

3.4.2. Chemical analysis

3.4.2.1. Ammonium nitrogen

Ammonium nitrogen content was determined using the United States Environmental Protection Agency (USEPA) Method 350.1 (NH₄-N USEPA Method 350.1). The method using phenate hypochlorite was further enhanced using nitroprusside (Aitkenhead-Peterson et al., 2009).

3.4.2.2. Nitrate nitrogen

Nitrate nitrogen was determined using USEPA Method 353.2 (NO₃-N USEPA Method 353.2). Using this method, all samples underwent cadmium-copper reduction (Aitkenhead-Peterson et al., 2009). Nitrate concentrations were determined by subtracting the nitrate concentrations determined by SmartChem 200 Method 375-100E-1 (Rev: A-03-0906) from the total organic nitrogen (NO_x)⁻ determined by this same method.

3.4.2.3. Phosphate phosphorus

Total orthophosphate phosphorus was found using USEPA Method 365.1 (PO₄-P USEPA Method 365.1). Ammonium molybdate and antimony potassium tartrate reacted in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex was reduced to an intensely blue-colored complex by ascorbic acid. The color, measured at 880 or 660 nm, is proportional to the phosphorus concentration present within the sample.

3.4.2.4. Dissolved organic carbon and total nitrogen

Determination of non-purgeable organic carbon (NPOC) and total nitrogen (TN) was done using a Shimadzu TOC-VCSH with Dual TN Module (Shimadzu Scientific Corp. Koyoto, Japan) for NPOC/TN. NPOC, specifically, was found using 720°C combustion catalytic oxidation/chemiluminescence method and TN was determined using a combustion tube and oxidation catalyst which is shared with TOC analysis, and chemiluminescence method.

3.4.3. *Cryptosporidium* and Enterovirus analysis

Prior to laboratory analysis, all sample cartridges were transported from the field on ice, back to Texas A&M University, within 24 hours of the samples being collected. All laboratory analysis, then, was executed within a 72 hours of that collection event. In order to elute Cryptosporidium off of the Envirochek HV sampling capsules, USEPA Method 1623.1 was followed. The filters were submerged in sodium hexametaphosphate (NaHMP) solution and then agitated for five minutes at 900 rpm on a laboratory shaker. NaHMP was removed and the filters were rinsed with reagent grade water. Elution buffer was then added to the filter capsules and agitated for another five minutes at 900 rpm. The elution was collected in a 250 mL conical tube, and the elution process was repeated another time to ensure all oocysts were removed from the filter. To elute Enterovirus from NanoCeram[®] cartridge filters, USEPA Method 1615 was followed. Five hundred milliliters of 1.5% beef extract was allowed to sit for one minute. The extract was then pushed through the filter using a peristaltic pump and collected. An additional round of beef extract was pumped through the filter housing, allowed to sit for fifteen minutes, and then collected in the same beaker. The pH of the collected beef extract was adjusted to 3.5 so that the viruses would adhere to the flocculent that formed. The samples were transferred to centrifuge tubes, and centrifuged at 2,500 g and 4°C for 15 minutes to the collect the pellet. The pellet was dissolved in 30 mL of 0.15 M sodium phosphate (pH 9.0). The resuspended pellet was centrifuged at 4,000 x g for 10 minutes at 4^oC and the supernatant was retained. The sample pH was adjusted to 7, and the

supernatant was sterilized by using a $0.22 \ \mu m$ sterilizing filter. The final sample was divided into three storage portions for later use.

The concentration methods continued with Method 1623.1 for the concentration of *Cryptosporidum* oocysts by centrifuging the 250 mL conical tubes at 1,500 g for 15 minutes or until a pellet formed. All supernatant was removed until 5 mL above the pellet so as not to lose any oocysts. The samples were transferred to microcentrifuge tubes until the DNA extraction step; Method 1615 for Enterovirus continued with Vivaspin-20 centrifugal concentrators. Samples were centrifuged at 3,000 g until all samples were concentrated to 50 μ l.

The final step, before analysis in a thermocycler, was to extract the nucleic acid from all concentrated oocysts. Cryptosporidum sample extraction was performed according to the DNeasy Blood & Tissue Kit. Initially, 200 μ l of PBS and 20 μ l of proteinase K was added to each sample eluent. Following completion of that step, 200 μ l of buffer AL, without ethanol, was added to each of the samples. The samples were then incubated at 56°C for 10 minutes, after which, 200 μ l of ethanol was placed into each of the sample tubes. All samples were transferred to DNeasy Mini spin columns and centrifuged at 6000 g for 1 minute. The flow through was discarded and 500 μ l of Buffer AW1 was added to the spin column. The samples underwent another centrifugation session at 6000 g for 1 minute. After the flow through was discarded again, 500 μ l of Buffer AW2 was added to the spin column and the column was centrifuged at 20,000 g for 3 minutes. The flow through was discarded and the removable column was transferred to a new 1.5 microfuge tube. For the final washing, 200 μ l AW2 was added to the DNeasy membrane and allowed to incubate for 1 minute. The final centrifugation was done at 6,500 g for 1 minute. All samples were collected, quantified via Nanodrop and stored at -20°C until qPCR was performed.

Extraction of Enterovirus viral RNA was achieved using a QIAamp Viral RNA Spin Kit. Initially, 310 µl of buffer AVE was added to the tube containing carrier RNA. Next, 67.2 µl of carrier RNA was mixed with 67.2 µl of AVL Buffer. For each of the previously concentrated Enterovirus cell samples, 200 µl of RNA was transferred to a 1.5 mL microcentrifuge tube. Subsequently, 200 µl of the RNA carrier-AVL mixture was also transferred to the 1.5 mL microcentrifuge tube. The tubes were vortexed for 15 seconds each to mix the solution before incubation at 56°C for 10 minutes. Following incubation, the samples were centrifuged at 5,500 g for 5 seconds and then 200 µl of ethanol was added to the tubes. The samples were vortexed and centrifuged again for 5 seconds. All samples were then transferred to the QIAamp Mini Spin column and centrifuged at 6,000 g for 1 minute. The removable column was transferred to a new, clean collection tube and 500 µl of Buffer AW1 was added. The mixture was centrifuged again at 6,000 g for 1 minute and, after, the column was transferred to a new collection tube. Another 500 µl of Buffer AW2 was added carefully to the column and the mixture was centrifuged at 20,000 g for 3 minute. Column transfer to a new collection tube occurred again and the whole tube was centrifuged at 20,000 g for 1 minute. Before the final centrifugation step, the column was transferred to a clean RNA-free and DNA-free collection tube, 50 µl of Buffer AE was added to the column and it was all allowed to incubate at room temperature for 1 minute. This was followed by centrifugation at 6,000

g for 1 minute. The final samples were quantified by Nanodrop and stored in a -80°C freezer until reverse transcriptase was performed.

The kit used to assay for *Cryptosporidium* DNA was a PCR *Cryptosporidium* kit (Ceeram Tools, France). For each of the 40 samples, 20 µl of CRYPT/IPC Master Mix was transferred to a 96 well optical plate. In the case of the control samples, 5 µl of positive control and 5 µl of negative control was added to 20 µl of Master Mix each and pipetted into individual wells. Five microliters of each of the regular samples were added into individual wells, along with 20 µl of Master Mix. The solution in each well was thoroughly mixed using a pipette and the plate was centrifuged at 3000 g for 3 minutes. An optical cover was used to cover the plate and the plate was installed into a qPCR thermocycler using these conditions: 50°C for 2 minutes, 95°C for 10 minutes, a cycling step of 95°C for 15 seconds and 60°C for 1 minute for a total of 40 cycles, followed by a 4°C holding temperature.

A RT-PCR Enterovirus Test kit (Ceeram Tools, France) was used to obtain data from the viral RNA. For all twelve samples, a master mix containing RT-PCR enzymes was created using 19 µl of ENTERO/IPC Master Mix and 1 µl RT-PCR Enzyme Mix. Five microliters of positive, negative, and sample aliquots were transferred to a 96 well plate. Twenty microliters of Master Mix was then added to each well containing liquid sample. Each of the samples were mixed thoroughly using a pipette and the plate was centrifuged at 3,000 g for 3 minutes. An optical cover was applied to the plate and the plate was inserted into a RT-PCR Thermocycler using these conditions: 45°C for 10

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minutes, 95°C for 10 minutes, a cycling step of 95°C for 15 seconds and 60°C for 45 seconds for 40 cycles with a 4°C holding temperature.

3.5 Statistical analysis

Statistical analysis software (SPSS) with backwards stepwise regression modeling was used to model the potential predictors of *E.coli* in water samples collected during all three sampling events.

4. RESULTS AND DISCUSSION

4.1. Water levels and hydraulic gradients

The pressure transducers installed in the wells showed the change in water gradient within the subsurface of the river bank. The hydraulic gradient of the area seemed to change with very large surges in the river, which were only brought on by storm events. Figures 4.1 and 4.2 show the rainfall and river height changes prior to, during and after all scheduled sampling events.

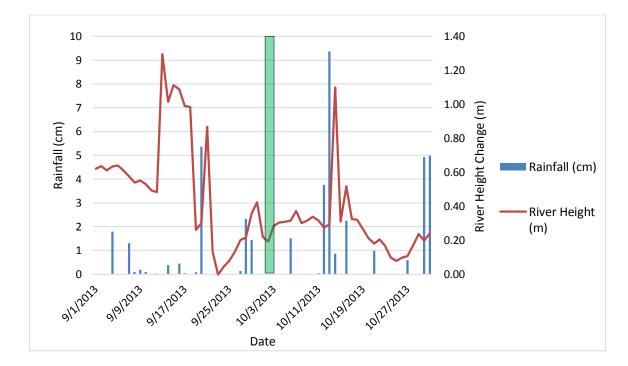


Figure 4.1. Graph shows the average rainfall in Austin, Texas and the change in the height of the Colorado River at Hwy 183, prior to, during, and after the October 2-3 sampling event, highlighted in green.

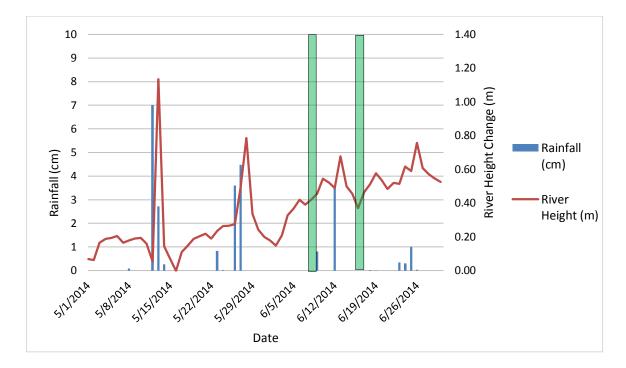


Figure 4.2. Graph shows the average rainfall in Austin, Texas and the change in river height at Hwy 183 prior to, during, and after the June 8-9 and 16-17 sampling events, highlighted in green.

Figure 4.3 is a profile plot of the Hornsby Bend well transect. The plot begins at the water's edge (00) and reaches to Well 20. This presents the increase in water level from the rivers lowest point, at 6:00 PM on October the 8th, 2013 until the river reaches peak flow at 3:00 AM on October 9th, 2013. The information suggests that the small positive pulse in river level has little effect on the groundwater table during the same time span.

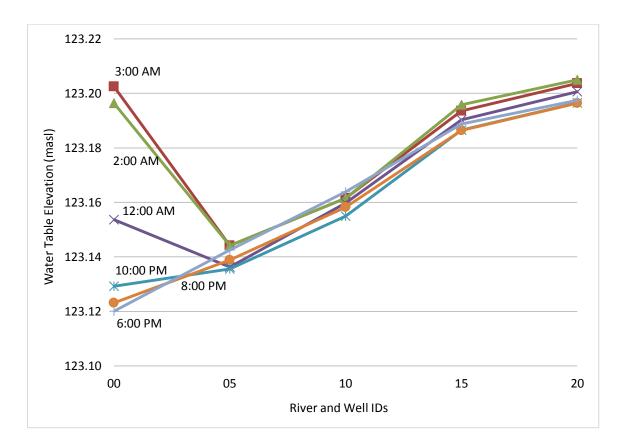


Figure 4.3. Profile plot of the water table beginning at the river (00) and reaching to Well 20 at Hornsby Bend over a 9 hour period, when the river pulses from its lowest to its highest point, on October 8-9, 2013.

The river heights shown in Fig. 4.3 were calculated by subtracting the difference between two synoptic river level measurements at the USGS gauge site 11.6 km upstream (Highway 183, Austin, TX) and at the well transect site to account for a sloping river surface. As well as assuming a sloping river surface, this method is based on the assumption that the magnitude of river pulses remain constant as they travel downstream to Hornsby Bend. This assumption is not valid because the magnitude of the river pulses decrease with distance downstream of a dam. Therefore the range in surface water heights shown in Fig. 4.3 are larger than those that actually occurred on site. This gives the impression of larger hydraulic head differences between the river and the aquifer. It is therefore more ideal that river stage and elevation be measured adjacent to the well transect to accurately know river stage and hydraulic forcing conditions in the riverbank aquifer.

Figure 4.4 shows the corresponding changes in hydraulic gradient during two separate storm events following the first round of sampling in October of 2013. Any hydraulic flow away from the river is shown in the upper graph where the line falls below zero on the y axis. This shows that the hydraulic gradient of the area only reverses under very large increases in the water table of the area. Suggesting that under a normal dam release schedule, there is not enough of a shift in hydraulic gradient to force river water into the alluvial aquifer.

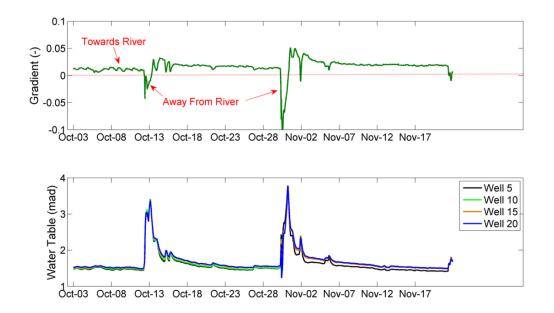


Figure 4.4. Hydraulic gradient change graphs that correspond to increases in the water table.

Flow velocities of the groundwater also change during times of very high flow in the river; Figure 4.5 illustrates this change. The further the graph lines deviate from zero on the y axis, the higher the velocity. Any movement in the positive direction shows groundwater flow toward the river, and movement in the negative direction shows water flow away from the river. This is in agreement with information reported in Sawyer et al (2009), where on average, the river at this section is a gaining stream.

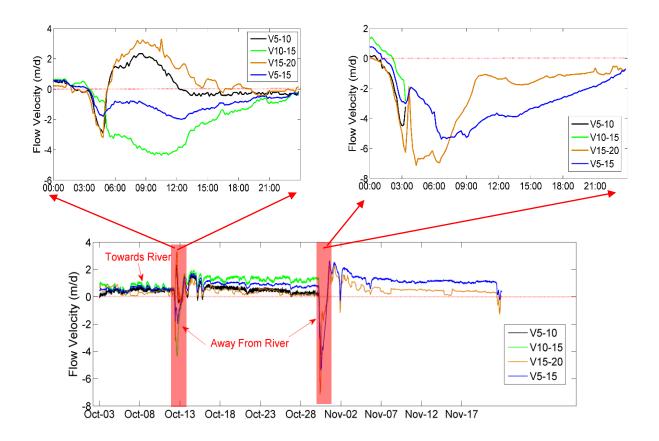


Figure 4.5. Flow velocity graphs of the groundwater in the alluvial aquifer adjacent to the Colorado River.

During the storm event that occurred on October 31st, 2013, the pressure transducer that was taking readings in Well 10 was forcibly removed from the well by storm debris and so data following that period of time was lost. However, what can be concluded is that the flow of groundwater within the river bank is not uniform throughout the extent of the well transect. The data shows that the groundwater has a tendency to flow away from Well 10 and toward Well 15. Suggesting that either Well 10 is situated in a lower permeable area of the soil, or that Well 15 acted as a water sink.

4.2. Field measurements

Field measurements were collected during the time of sampling and included water temperature, pH, conductivity, and dissolved oxygen of all samples. Figure 4.6. show the temperature of the river and all groundwater well samples during all three of the sampling events associated with this project. Among the three sampling events, the highest peaks in the river height occurred between 1:00 AM and 2:00 AM. The overall trends in river temperature fluctuation depended most heavily on the time of day, and not on any fluctuation in the height of the river (Sawyer et al., 2009). During each of the sampling events, the lowest water temperatures in the surface water were observed between 9:00 PM and 9:00 AM of the 24-hr sampling period. At approximately 9:00 AM, the temperatures of the river samples began to rise gradually. The trends in groundwater sample temperatures also followed this pattern, however, the range over which the temperature changed was dampened when compared to river water measurements.

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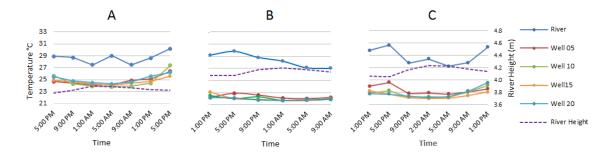


Figure 4.6. Water temperature of samples collected during October 2-3 (A), June 8-9 (B), and June 16-17 (C) sampling events (TCEQ Station ID #21411).

With the exception of the June 8-9 sampling event (Figure 4.7), the pH increased in the river water with increased river stage over a 24-hr period. The pH pulses in the wells were not as drastic in their temporal variability, although they did exhibit slight changes. During the June events, spatial variability between wells was lower than the variability in October. This is likely due to the degree of water mixing that occurred prior to the October sampling event. Figure 4.1. shows an elevated river stage that was maintained for approximately a week in the later part of September 2013. This prolonged, elevated river stage, likely reversed the natural hydraulic gradient of the river bank enough to allow for a higher amount of stream water to enter the shallow aquifer.

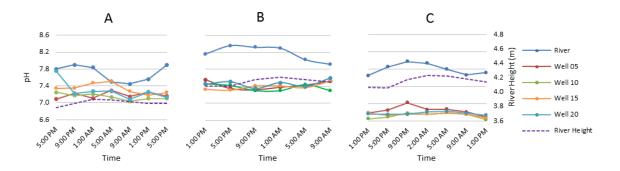


Figure 4.7. pH of water samples collected during October 2-3 (A), June 8-9 (B), and June 16-17 (C) sampling events (TCEQ Station ID #21411).

A much more accurate way to trace water using field measurements is by examining the conductivity of the water. This is because the conductivity of surface water is naturally much lower than the conductivity of groundwater in the area. This is illustrated in Figure 4.8, where the value of the river water samples lay at an average reading far below that of any groundwater sample. In comparison, the conductivity values of the groundwater samples were recorded at levels at or above 1000 µs/cm, with Well 15 as the exception during the October 2-3 sampling event. The samples pulled form this well had an average reading of 774 µs/cm. The field parameter, pH, also show evidence to the level of water mixing that occurred prior to the sampling events. The October pH readings between the river and well water samples show a much smaller difference. However, when compared to the June events, there was a much greater distinction between the pH values of the river and those of the well water samples. This alludes to a much higher level of water mixing in the subsurface prior to the month of October, in comparison to the month of June when it appeared that there was a much more distinct separation between river and groundwater.

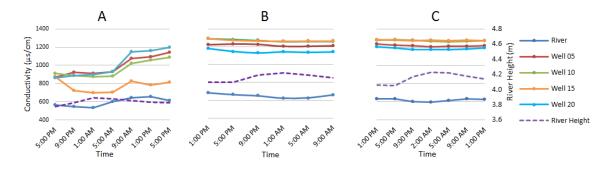


Figure 4.8. Electrical conductivity of water samples collected during October 2-3 (A), June 8-9 (B), and June 16-17 sampling events (TCEQ Station ID #21411).

Dissolved oxygen measurements in water are used as tracers and as indicators of water quality. Any decline in oxygen concentration in the well water samples following the initial collection was likely due to the collection itself. After the initial collection, DO concentration remained relatively constant. Dissolved oxygen changes in the river mimic the changes in water temperature closely, during the October sampling event. As the temperature of the river water spiked slightly, fell and then increased gradually in the early morning hours (Figure 4.6), the same trend was seen in the DO concentrations of the river during the same sampling event (Figure 4.9). The relationship between water temperature and dissolved oxygen concentration was not demonstrated as strongly during the June sampling events. While there were small increases in the steady undulating cycle of water temperature that occurred during the June sampling events at 1:00 AM and 2:00 AM, respectively. However, there were no observable changes in dissolved oxygen concentration at these sampling times.

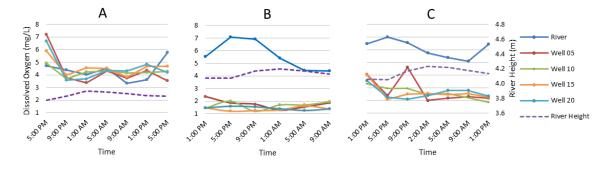


Figure 4.9. Dissolved oxygen concentrations in water samples collected during October 2-3 (A), June 8-9 (B), and June 16-17 (C) sampling events (TCEQ Station ID #21411).

4.3. Water chemistry

The chemical makeup of environmental waters change over time and under different conditions, this change can be followed and recorded in order to better understand the cycling of chemicals within the boundaries of a surface water-groundwater interaction. Nitrate in the surface water during the month of October averaged 6.5 mg/L, which was higher than the nitrate measured in the river during the month of June, which was between 3.5 and 3.8 mg/L (Figure 4.10). United States EPA (2015) has set the maximum contaminant level (MCL) for nitrate-nitrogen at 10 mg/L, following this standard, the nitrate measured in the river during all sampling events is within the regulatory standards. However, concentrations measured in unpolluted water bodies are usually <4.0 mg/L. Higher nitrate levels in October were consistent with the fact that there were larger, more intense rainstorms that occurred prior to that month, followed by reduced base flow levels. Since nitrate is a highly water-soluble molecule, more of it would have been washed into the river during these rainstorms and then there

was not enough water to dilute the dissolved molecules. This same reasoning can be used to explain the higher levels of nitrate in the groundwater in October, also.

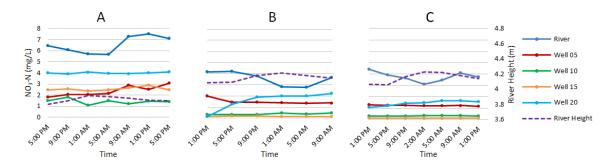


Figure 4.10. Nitrate-nitrogen concentrations for samples collected during the October 2-3 (A), June 8-9 (B), and June 16-17 (C) sampling events (TCEQ Station ID #21411).

Total nitrogen concentrations (Figure 4.11) were not significantly different from nitrate-nitrogen readings because of the low levels of other detectible forms of nitrogen found in the natural environment. Water chemistry analysis of the ammonium-nitrogen levels of the samples collected showed very low levels of this type of nitrogen (Figure 4.12), and thus supports the fact that nitrate-nitrogen was the most abundant form of nitrogen in the water samples at the time of sampling.

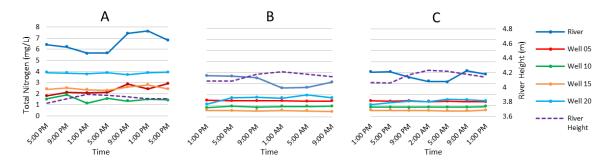


Figure 4.11. Total nitrogen concentrations from samples collected during the October 2-3 (A), June 8-9 (B), and June 16-17 (C) sampling events (TCEQ Station ID #21411).

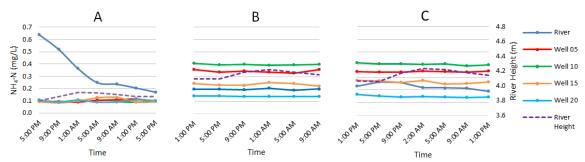


Figure 4.12. Ammonium-nitrogen concentrations from the samples collected during the October 2-3 (A), June 8-9 (B), and June 16-17 (C) sampling events (TCEQ Station ID #21411).

Phosphate-phosphorus concentrations averaged around 0.1 mg/L in the groundwater samples during all of the sampling events (Figures 4.13). The concentrations in the river, like the river level, pulsed over a 24-hr period. However, phosphate-phosphorus concentrations appeared to become reduced during times of heightened flow in the river, and increased as the river level began to decline. This occurred during all three of the sampling events.

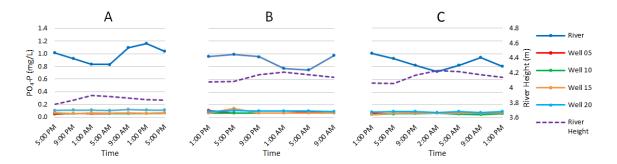


Figure 4.13. Phosphate-phosphorus concentrations from samples collected during the October 2-3 (A), June 8-9 (B), and June 16-17 (C) sampling events (TCEQ Station ID #21411).

Of all the chemical components of an environmental water system, dissolved organic carbon (DOC) can give the best indication as to where the run-off of the area is originating from, especially if the land use of the surrounding area is known (Gron et al, 1996). The height of the river, in relation to the DOC concentration in the river, did not appear to have an appreciable effect. According to Wassenaar et al. (1991), common DOC concentrations are on the order of 5 - 100 mg/L. The month of October results showed levels that are expected in the alluvial groundwater of a river system, however, the June sampling events showed concentrations that were much higher (Figure 4.14). Since there was not as much water movement in and out of the alluvial transect during the month of June, in comparison to the level predicted in October, the DOC content of the groundwater was uninfluenced for an extended amount of time. If runoff from the area transported large quantities of carbon into the subsurface from the surrounding area, without continued runoff events the levels of DOC measured in the samples are not so surprising.

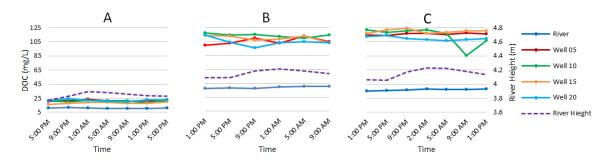


Figure 4.14. Dissolved organic carbon concentrations in samples collected during the October 2-3 (A), June 8-9 (B), and June 16-17 (C) sampling events (TCEQ Station ID #21411).

4.4 Bacterial concentrations

As mentioned in the methods section of this thesis, the LCRA Environmental Laboratory used the Colilert method for bacterial detection. The Colilert method is an enzyme substrate method. The method is able to detect a single, viable organism in a sample and it suppresses noncoliform bacteria that also may be present within the sample. It is also capable of specifically identifying *E. coli* cells that are present.

During all sampling rounds, *E. coli* concentrations in the river mirrored the rise and fall of the river height. Peak concentrations of the bacteria occurred shortly after the peak river height and then decreased over time (Figure 4.15). Overall, *E. coli* concentrations were generally low, with the exception of two sampling periods during the first round of sampling. During the 5:00 PM and 9:00 PM collection on October 3rd, *E. coli* concentrations rose to 416 MPN/100mL and 260 MPN/100mL, respectively. During sampling rounds 2 and 3, *E. coli* levels never rose above 67 MPN/100mL. *E. coli* concentrations in the groundwater remained lower than concentrations measured in the river all three sampling trips.

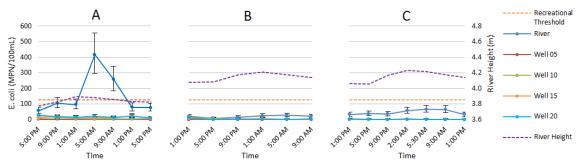


Figure 4.15. *Escherichia coli* concentrations in water samples collected during the October 2-3 (A), June 8-9 (B), and June 16-17 sampling events (TCEQ Station ID #21411). Error bars represent the upper and lower 95th percentile limits of uncertainty for the data collected by IDEXX Collect Quanti Trays.

Fecal coliform concentrations, like *E. coli* levels, remained lower in the groundwater than concentrations found in the river, except for a single collection point during round 2 where Well 10 had a slightly higher concentration (16 CFU/100 mL) than the concentration measured in the river (10 CFU/100mL) (Figure 4.16). Fecal coliform concentrations in the river peaked slightly after the peak in river height during all three of the sampling events. When comparing sampling event results to one another, the third round of sampling yielded concentrations in the groundwater that were consistently near 1 CFU/100mL, which is lower than concentrations measured during the previous two events.

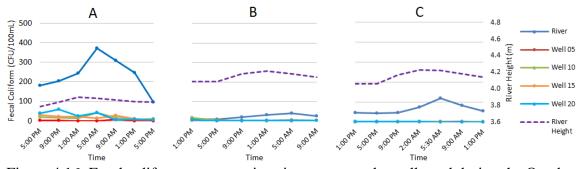


Figure 4.16. Fecal coliform concentrations in water samples collected during the October 2-3 (A), June 8-9 (B), and June 16-17 (C) sampling events (TCEQ Station ID #21411).

Concentrations of total coliforms in river samples, like *E. coli* and fecal coliform concentrations, increased as the height of the river increased, during all three sampling events (Figures 4.17). While concentrations of fecal coliforms, generally, remained lower than concentrations measured in river samples, there was an exception in Well 05 during the second sampling event. Well 05 concentrations followed the increasing trend seen in river samples, and only showed signs of decreasing during the 5:00 PM collection, however, immediately increased again. Noticeably lower concentrations (<15 CFU/100 mL) during rounds 2 and 3, were measured in the groundwater when compared to concentrations measured during round 1. Overall, there were no consistent trends in change for total coliforms in the groundwater samples.

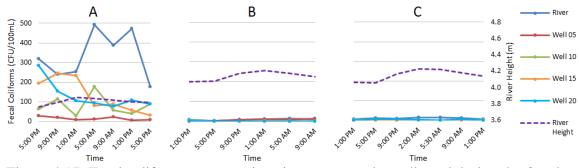


Figure 4.17. Total coliform concentrations in water samples collected during the October 2-3 (A), June 8-9 (B), and June 16-17 (C) sampling events (TCEQ Station ID #21411).

4.5 Statistical analysis

River water samples were the only samples that were analyzed with any confidence. Two separate models were run, with the only difference being the inclusion of the parameter river stage. Despite the difference, both models reported the same Standard Error of the Estimate. Results showed that water chemistry was the only predictor of *E. coli* concentrations in the water samples (Figure 4.18).

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.916 ^a	.839	.759	48.00358
2	.916 ^b	.839	.778	46.12044
3	.916°	.839	.793	44.48596
	Constant), pH, N Constant), pH, F			
c. Predictors: (Constant), pH, [OOC, EC, NO3		

Model Summary B						
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate		
1	.928 ^a	.860	.749	49.0116		
2	.927 ^b	.860	.770	46.8775		
3	.923°	.851	.777	46.1863		
4	.920 ^d	.846	.787	45.1758		
5	.916 ^e	.839	.793	44.4859		
	Constant), Stag Constant), Stag					
c. Predictors: (Constant), Stag	e, Temp, EC, p	H, NO3, DOC			
d. Predictors: (Constant), Stag	e, EC, pH, NO3	B, DOC			
e. Predictors: (Constant), EC, pH, NO3, DOC						

Figure 4.18. Statistical results for the *E. coli* predictors in river water samples.

These results were surprising considering that the measured *E. coli* concentrations obtained from IDEXX plate counts indicate that concentrations were affected by river pulses over 24 hours. An explanation for this could lie in the fact that while bacterial concentrations did increase with river stage increases, they were delayed approximately 4 to 5 hours. This lag in bacterial increase, which was not considered for in the model, most likely affected the results.

4.6 *Cryptosporidium* and Enterovirus

None of the samples collected and analyzed tested positive for the presence of either *Cryptosporidium* or Enterovirus. These results were most likely due to the extremely low concentration of these pathogens in the water system as evidenced by low levels of *E. coli* and other fecal indicator microorganisms.

5. CONCLUSIONS

In contrast to the river samples, where most chemical and microbial parameters fluctuated with river stage, there were no congruent fluctuations in those parameters in groundwater samples. This is likely due to the low magnitude river stage fluctuations caused by the low intensity of dam releases. Sawyer et al. (2009) found daily changes in the river level, at an adjacent site on the river, impacted water-table fluctuations 30 m into the surrounding aquifer and resulted in a hyporheic zone that extended approximately 1-5 m into the riparian aquifer. However, daily dam releases to the river were much larger during their study, producing changes in river levels of 2.5 m as compared to 0.15 m during this study.

With the exception of two samples, river *E. coli* levels were below regulatory limits (126 MPN/100 ml). This is consistent with data collected from February 2006 until February 2014, at a nearby site (TCEQ Station 12469) for which only 4 out of 48 samples exceeded 126 MPN/100 ml. Interestingly, *E. coli* levels rose with river height and peaked shortly after the river level began to fall. An explanation for this is that rising river levels collected additional *E. coli* from the surrounding riparian area and washed them into the river as the water receded. The results from the *Cryptosporidium* and Enterovirus tests found no evidence of these pathogens in the water system.

All the field parameters measured were found to be in agreement with the results previously found by Sawyer et al. (2009). Temperature cycles are congruent with normal diurnal river water temperature patterns. Conductivity was found to be lower in the river water samples and increase in samples with increasing distance from the river. Dissolved oxygen concentrations and pH of samples both showed strong evidence to support the difference in the level of water mixing that occurred between sampling months. The similarity between DO and pH values in in surface water and groundwater in October suggests mixing of surface and groundwater occurred at a much higher rate in October in comparison to the month of June. The results for these two field measurements in June show a much more prominent separation between river water and groundwater measurements.

Other water chemistry parameters also suggested evidence of higher degrees of water mixing during the month of October, especially the organic carbon concentrations. Whereas dissolved organic carbon concentrations were low in groundwater in October and similar to river water, during June they were abnormally high (between 105 - 125 mg/L) (Strohmeier et al., 2013). This was likely due to an accumulation of organic carbon from run-off events and no influx of river water to dilute the added carbon. The low flow events in the river during the month of June also, may have, contributed to the higher concentrations, since there was less water to dilute the added organic carbon.

Overall, the low levels of pathogens and bacterial indicator organisms coupled with weak evidence of the mixing of river and surface water when compared to levels seen historically, suggest that it is unlikely that the alluvial groundwater was adversely impacted by surface water contaminants under the current dam release conditions. Nevertheless, there is a possibility that if the dam release schedule was ever to return to

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historical levels, the risk of pathogen infiltration into the alluvial aquifer would possibly increase.

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