Using Areas of Concentrated *E. coli* Bacteria to Identify Species Specific Sources in Urbanized Sections of the Concho River, Tom Green County, Texas

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**Abstract**

Seasonality has been shown to play an extremely responsible role in the fluctuation of *Escherichia coli* (*E. coli*) loading on the Concho River System in San Angelo, Texas. However even with temperature change and other physicochemical parameters varying with seasonal change, several sites exceed EPA’s “safe versus unsafe for contact” surface water standard by three to ten times the respected < 320 colony forming units per 100 mL standard threshold value (at 3%). The objective of this project is to quantify areas of *E. coli* loading to further understand local sources of bacteria pollution. Ten sites located along highly urbanized sections of the Concho River will be sampled for *E. coli* and physiochemical properties including temperature, dissolved oxygen, specific conductance, conductance, total dissolved solids, and pH. The data set will encompass twenty-six sampling periods spread out over a year. The *E. coli* data was used to isolate areas where Bacteroides identification DNA markers which was sampled for birds, humans, and dogs. This project gives way to putting a numerical and biological answer to pollution of an urbanized surface water system with located within the area of interest.

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**Introduction**

Urbanization and human influenced alteration may negatively impact environmental quality and sustainability of surface water systems throughout the world. Continuous research is discovering new detection methods and means of expressing how impactful human and/or animal influences truly are on surface water systems. With the elevated risk of humans contracting pathogen-illness from surface water systems, a serious health related concern becomes evident when a surface water system becomes 303(d) impaired (TCEQ 2013). The Concho River in Concho and Tom Green Counties in west central Texas has been listed as a 303(d) impaired surface water system for two parameters (dissolved oxygen and bacteria) since 2008. Following the 2008 Texas Commission on Environmental Quality integrated report, the Concho River has been researched by local and regional based water science entities to understand where, what, and how the system is being altered and consequently impaired. *E. coli* bacteria has served as a water quality indicator for decades (Edberg 1988). Along with technological advancements, the accuracy and precision at
which *E. coli* bacteria is quantified is becoming better and better every year. Bacteroi-
des bacteria are the most ideal fecal source tracking target for this experiment for sev-
eral key reasons. Although Bacteroides and *E. coli* are quite similar, they don’t express
the same intestinal activities in a warm blooded animal. For that reason, Bacteroides
function as bacterium that aid in nutrient absorption in the host and whatever the host
species’ food consumption is, allows for different gene profiles separating certain spe-
cies from one another from a microbial standpoint (Coakley 2011, Hooper et al. 2001).
However, only in recent years has a DNA based approach become regularly utilized
to analyze specie specific hosts of contamination. With an extremely wide variety of
physicochemical parameters, seasonality flux, and both point and non-point sources
pollution impacting the Concho River, the focus of this research project is locating
areas where *E. coli* bacteria is the most concentrated and using those sampling sites to
conduct DNA based source host analysis. The objective of this project is to determine
host specific data in order to transition a possible non-point source of contamination
to a point source and then develop best management practices to low the possible con-
taminant sources to a “safe for contact” standard.

**Project Statement:** Previous research and analysis has determined the Concho River
is subject to high levels of fecal coliform bacteria and specifically *E. coli* year
around. Traditional analysis of *E. coli* bacteria shows a direct relation will
temperature change. However, at certain localities of the Concho River within the
city limits of San Angelo, Texas *E. coli* bacteria remains at a very high level regardless
of temperature change, an excess of 2419.6 cfu/100 mL. Thus, the idea of possibly
one or several year around contamination sources are impacting the system and
leading to excessively high *E. coli* bacteria results. Therefore, the project aims
to document the possible source of contamination and restore the Concho River
to a non-impaired surface water statue. The Concho River is a valuable resource
for eleven counties within the river’s watershed and a large sub-basin of the
Colorado River basin that stretches through the heart of the state of Texas.

**Hypothesis:** Fecal coliform and *E. coli* concentrated areas have one or several direct
DNA based Bacteroides hosts contributing to the high levels of contamination
found at two specific sites of interest in the research area.

**Objective #1:** Quantify locations of *E. coli* loading at ten different sampling sites
  **Task #1:** Collect biweekly *E. coli* samples to isolate areas of high contamination
load.
  **Task #2:** Collect and document physiochemical data at each site per *E. coli* sample
  **Task #3:** Document human, bird, and animal activity at each site during sampling

**Objective #2:** Conduct DNA based sampling at highly contaminated sites
  **Task #1:** Collect samples using Source Molecular Corporation’s procedures and
protocols for Bacteroides analysis for human, bird, and dog specific DNA markers

Task #2: Aid in creating best management practices to lower negative environmental impact on the Concho River upon results from Source Molecular Corporation Laboratory

The Concho River: Location, Geology, and Impact

The Concho River is regionally broken into three major watershed contributories; North, Middle, and South Concho Rivers. All three major contributories meet near the Bell Street Dam located within the city limits of San Angelo, Texas (Figure 1). The Concho River Basin as a whole encompasses eleven Texas counties and runs into Lake O.H. Ivie (Smith 2015, LCRA 2008) all while supplying essential water sources to families, farmers, and ranch near the river system.

The North Concho River – is a highly variable watershed of the Concho River that extends from northcentral Glasscock County to O.C. Fisher Reservoir. Although the watershed extends through a large portion of the Concho River basin, the North Concho River remains dry though most of the year.

The Middle Concho River – is a west to east contributory to the Concho River basin that extends as far west as central Upton County. Much like the North Concho River, and with the exception of isolated spring contributions, the watershed remains dry throughout most of the year. The watershed extends to the north pool of Twin Buttes Reservoir.

The South Concho River – is a south to north contributory that offers continuous spring fed influence to the Concho River basin. Although many dams are located throughout the river system, it is the only branch of the Concho River that offers water flow year around. Flow continues through much of southern Tom Green County and extends to the south pool of Twin Buttes Reservoir.

All three watersheds are controlled by manmade reservoir structures that control the overall flow of the three watersheds prior to all three watersheds meeting at the Bell Street Dam (Henry 1986). From the Bell Street Dam, flow continues eastward to O.H. Ivie where the Concho River meets the Colorado River. Within the Concho River watershed, shallow, Permian aged aquifers contribute upwelling sources of groundwater in isolated locations (Dutton et al. 1989).

The lithological influences on the Concho River Basin mostly consist of Cretaceous-age carbonates with a small area of Permian aged sandstones and conglomerates near the convergence of the three watersheds (UCRA, 2011). The Cretaceous-age carbonates found at the surface throughout the Concho River Basin are subject to many secondary structures such as joints and karstic type conduits and vugs (Henderson 1928, Orndorff et al. 2001, Craig 1988).

Many rural communities rely on irrigation and other agricultural activities to be supplemented from water use out of the Concho River. Large cotton, milo, and other cash crops are grown within the Concho River Basin and in Tom Green County alone.
956,852 acres exist in farm production (USDA 2012). Consequently, the more negatively impacted the Concho River becomes upstream, the larger the possibility of adverse effects becoming evident downstream. Controlling human impact and developing large scale management practices will help control the contamination within urbanized sections of the Concho River in San Angelo, Texas and consequently downstream.

**Methodology and Experimentation**

The twenty-six biweekly sampling period as directed in the Quality Assurance Project Plan (QAPP) began on May 8, 2014 with the first round of *E. coli* sampling. The collection period for *E. coli* extended through April 29, 2015. All physiochemical parameters were taken using a pre and post calibrated Professional Series Yellow Spring Instrument Multi-Parameter Aquatic Sampling Soude and calibrated under United States Geological Survey calibration protocols (Wagner et al. 2006). These physiochemical parameters included; water temperature and pressure, dissolved oxygen levels (% and mL), specific conductance, conductance, total dissolved solids, and pH. *E. coli* collections were done using the IDEXX Laboratories’ Colilert Procedures (2013, Eaton et al. 2005). Upon collection of parameters and *E. coli* samples, the visible environmental conditions were documented and described in a field notebook. This information included: bird and other animal activity, human activity, water color and odor, trash and debris, and prior weekly precipitation. No sample collection period was conducted prior to a thirty-six hour buffer time period after a local rain event. The IDEXX Colilert method is a process at which a 100 mL of sampling media is collected from a site of interest to generate a most probable number of colony forming units per 100 mL to represent the *E. coli* level at the site (cfu/100 mL). Similar experimentations have been conducted on other surface water systems in a regional proximity such as the Rio Grande River (Mendoza et al. 2004), along the Kentucky River (Black et al. 2007), and as far away as Sweden (Novel, Novel 1976) making the Colilert method a local, regional, and internationally recognized method used for quantifying *E. coli*. As directed in the QAPP, no dilution or duplications were conducted because the main objective was to locate the areas with the most consistently high *E. coli* measurements in order to collect the sites for future DNA based Bacteroides analysis.

**Results**

After completion of the final *E. coli* sampling period on April 29, 2015, a twenty-six sample baseline was created with averages of all ten research sampling sites. The ten sampling sites and the associated *E. coli* (cfu/100 mL) values are as follows: Site 1 – 151.7, Site 2 – 190.0, Site 3 – 64.4, Site 4 – 776.3, Site 5 – 399.8, Site 6 – 181.6, Site 7 – 58.2, Site 8 – 1525.9, Site 9 – 364.3, Site 10 (was only sampled three times, no water present). In order to understand the severity of *E. coli* contamination, the data was compared to the Recommendation 2 criteria element for the statistical threshold value
for *E. coli* in fresh water, 320 cfu/100 mL. Recommendation 2 was developed by the Environmental Protection Agency in 2012 when the federal entity released the 2012 Recreational Water Quality Criteria (RWQC) recommendations (EPA 2012). The standard expresses an estimated illness rate of 32/1,000 (3.2%) which approximates the 90th percentile of water quality distribution and is intended to be a value that should not be exceeded by more than 10% of samples analyzed. When coupling this analysis with data from the Concho River sampling baseline, two sites, Site 4 and Site 8, exceed the 320 cfu/100 mL correlation valve by 2.426 times and 4.768 times, respectively.

After deliberation, research stakeholders concluded that sampling Site 4 and Site 8 for Human, Dog, and Bird Fecal detection would be the most conducive means of isolating species specific sources of contamination. Physical presents of all three hosts were documented consistently at these localities. At Site 4 and Site 8, respectively, an average bird population of ≈25 and ≈35 birds were counted and documented during the sampling period. Numerous samplings at Sites 4 and 8 were also conducted with heavy human traffic and an occasional dog(s) present. On June 11, 2015, the first round of DNA based sampling was conducted by Mr. Chuck Brown of the Upper Colorado River Authority and analyzed by Source Molecular Corporation in Miami, Florida on June 22, 2015. All three host analysis came back negative. Site 4 and Site 8 were collected and analyzed for two Human Bacteroidetes ID™ Species; Bacteroides dorei. B and EPA Developed Assay biomarkers, one Bird Fecal ID™; genus Helicobacter (classified as genus Campylobacter prior to 1989), and one Dog Fecal ID™; Dog Bacteroidetes.

**Discussion**

The results of the Bacteroides sampling when compared to the *E. coli* baseline averages offers an interpretation and possible an environmental factor as well. Between the last sampling period for *E. coli* and the first Bacteroides sampling, according to the National Weather Service, Tom Green County alone received 9.12 inches of rainfall. In one month, Tom Green County almost received half of the county’s annual rainfall total (Henderson 2010). Thus a possible interpretation of the negative Bacteroids bacteria could be a result of a natural “flushing” of the surface water system through massive storm water run-off. Comparatively to *E. coli*, Bacteroids bacteria are the most numerous intestinal bacteria and encompass as my as 1,011 cells per gram of dry fecal matter (Coakley 2011, Finegold et al. 1983). The baseline data set of *E. coli* sampling reflects a large number of *E. coli* cfu/100 mL regardless of temperature fluctuation. Therefore, if year around *E. coli* bacteria are documented at a site, Bacteroids bacteria should be within that same area and be environmentally impacting a water system as well.

The *E. coli* data also revealed some interesting information in regards to sites with low *E. coli* bacteria counts. Site 3 and Site 7 are located in two very different places on the Concho River and both exhibit low *E. coli* averages (64.4 and 58.2 cfu/100
mL) consistently throughout the project timeline. Site 3 was sampled at the middle of the Bell Street Dam structure and was the only site that had a continuous year around discharge from a drain pipe located in the middle of the structure and controlled by the City of San Angelo. Site 7 is the furthest upstream site located on the newly re-mediated Concho River Trail in downtown San Angelo. Both sites are located directly downstream from the two most polluted sites along the river and show very different correlations to their polluted counterparts: Site 3 vs. Site 4 (64.4 - 776.3 cfu/100 mL) and Site 7 vs. Site 8 (58.2 - 1525.9 cfu/100 mL).

### Conclusion

The high points of *E. coli* sampling show a strong relation in variation when comparing them to the low points of *E. coli* sampling in a relatively short distance. Could the dam structures in between site locations be serving as impediments to the surface water system’s nature flow and consequently variation in bacteria populations? How long can the bacteria polluting the river survive under nature conditions? Even though there were two different bacteria of interest in this research, the project gives way to areas that have been documented with high *E. coli* values but still have an unknown DNA based analysis of the pollution as to where the pollution is coming from. Although the Bacteroides sampling was not conducive of any species specific data for the contamination, it did give rise to how the Concho River can exhibit a return to a low bacteria count river system after a strong influence of rain. This could have made the system reflect a more “nature” flow pattern even with dam structures throughout the river. In order to apply some of these ideas to the research, future work in creating a larger baseline of *E. coli* data at each site and further sampling of Bacteroides primers/markers at the most contaminated sites could offer the proper explanation to exactly what is contaminating the Concho River and the most effective means of remediation to control the contamination.
Table 1: *E. coli* sampling results (cfu/100 mL)

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<th>Site #2</th>
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<th>Site #5</th>
<th>Site #6</th>
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Table 2: Bacteroides Analysis Results for Site 4 and Site 8

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<th>Date Reported</th>
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<td>22-Jun-15</td>
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<td>22-Jun-15</td>
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Figure 1: Sampling Site Locations 1-10 located in downtown San Angelo, Texas

Figure 2: Physiochemical Sampling at Site 5 (photo by Monique Ching, 2014)
References


13. IDEXX Laboratories Inc., 2013, Colilert, 06-12999-06.


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