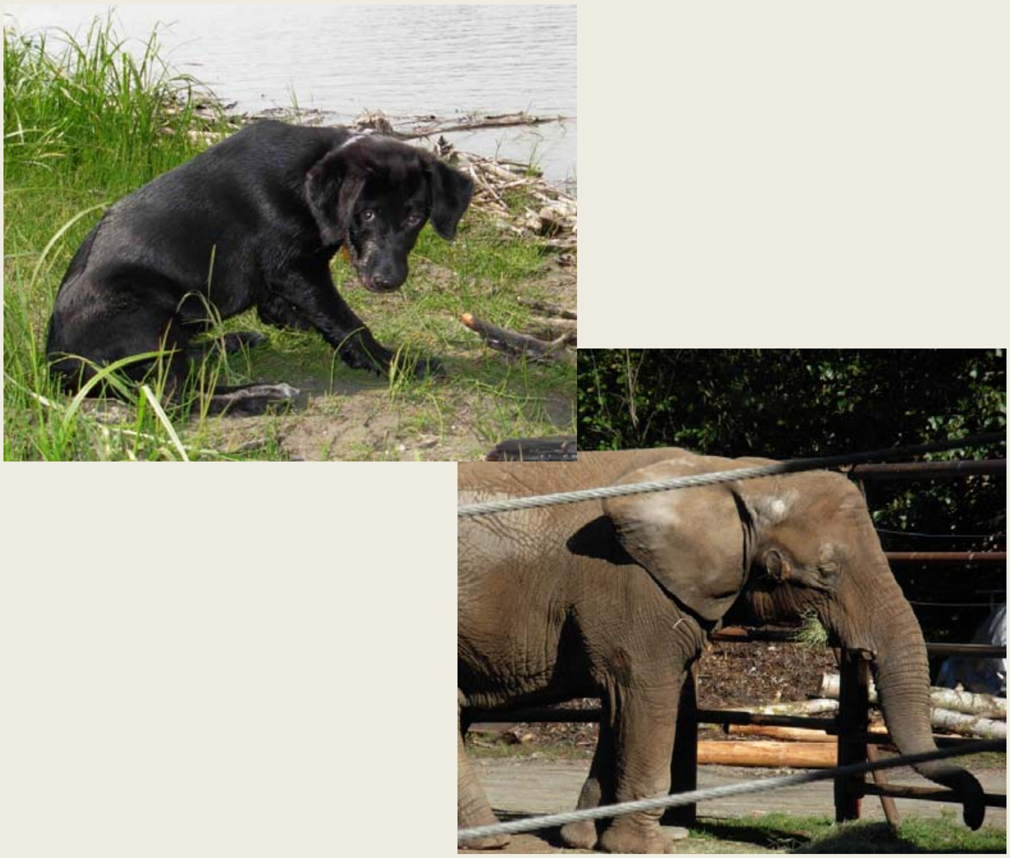
Fecal Coliform Bacteria Source Assessment in the waters of Cottonwood Creek, Wasilla, and Little Campbell Creek, Anchorage

Final Report



Aquatic Restoration & Research Institute

Jeffrey C. Davis, Gay A. Davis
P.O. Box 923, Talkeetna AK, 99676
arri@mtaonline.net 907.733.5432

ALASKA Department of Environmental Conservation

Timothy Stevens, Division of Water

November 2010

This project was funded by the Alaska Department of Environmental Conservation, contract no. 18-2011-21-7.

Table of Contents

Table of Figures

Executive Summary

Previous sampling has documented concentrations of fecal coliform bacteria in Cottonwood Creek (Wasilla) and Little Campbell Creek (Anchorage) above state water quality standards. The source of these bacteria, however, is unknown. Potential sources include humans, dogs, horses, waterfowl, and other wildlife. The sources of fecal bacteria in streams can be determined through the differences in the populations of the bacteria residing within the digestive system of warm-blooded animals. These isolated groups of bacteria evolve genetically allowing for differentiation based upon DNA analyses.

Water samples were collected at three locations on Cottonwood Creek, at two locations on the North Fork Little Campbell Creek, and at three sites on the South Fork Little Campbell Creek. Sampling locations were selected to bracket potential sources and to replicate previous studies. Samples were collected during spring runoff, summer base flow, and following storm events. Water samples from each site on each sampling date were analyzed for total fecal coliform bacteria and genetic markers for fecal *Bacteroides* and *Bacteroides* DNA segments specific to humans, dogs, horses, and waterfowl. Stream water turbidity, temperature, and discharge were measured to account for environmental factors that could influence bacterial concentrations. Specific conductivity and pH were measured to differentiate between stream flows dominated by ground water or surface runoff. Stream surveys were conducted in order to identify locations where potential fecal sources could be originating.

Human-specific *Bacteroides* markers were found in samples collected during storm flows from two of the three Cottonwood Creek sampling locations. Horse-specific markers were detected concurrent with human markers at these two sites. Therefore, bacteria from humans and human activities are contributing to the contamination of water quality in Cottonwood Creek. Their presence following storms indicates that fecal material is being transported in surface or shallow subsurface flows. Since human markers were not present during base flow conditions, septic systems are not likely to be the source of contamination. Markers from dogs also were detected at one location during base flow conditions.

No human, dog, horse, or waterfowl markers were found at the North Fork Little Campbell Creek sampling site in Far North Bicentennial Park. Human-specific markers and waterfowl markers were found in samples collected from the North Fork Little Campbell Creek downstream from the Old Seward Highway following a fall rain storm. Therefore, bacteria from humans are contributing to the contamination of the North Fork Little Campbell Creek. Bacteria are being transported in surface flows following storms making septic sources unlikely.

Human-specific markers were found in samples collected from all three South Fork Little Campbell Creek sites during base flow and at the downstream site following a storm event. Therefore, human sources are contributing to the fecal contamination of the South Fork Little Campbell Creek and are likely coming from ground water and surface water

sources. Their infrequent occurrence, and absence during high flow conditions, suggests low abundance of bacteria from humans. Markers specific to dogs were found at the site located below Abbott Loop. Markers from waterfowl were present but no horse-specific markers were detected in South Fork Little Campbell Creek.

Bacteroides markers were more likely to be found when fecal coliforms were more abundant. *Bacteroides* markers were absent from spring samples which may be due to their inability to survive more than 4 to 5 days in aerobic environments outside of their host species.

Introduction

Human and animal fecal contamination can spread many types of diseases and can cause significant economic loss due to the closure of recreational areas and fisheries (EPA 2005). Roughly 13% of the nation's surface waters do not meet water quality standards for fecal coliform bacteria. Contamination through point sources has largely been controlled, leaving non-point sources as the primary cause of fecal contamination (EPA 2005). Animal and human waste can enter streams and rivers through leaking, overloaded, or failed sewage systems; carried by surface runoff; or deposited directly. While human sources from sewage systems are suspected to enter through the groundwater and dominate during base flow conditions, some studies have documented increasing human fecal contamination during storm events (Shehane et al. 2005 as cited by Stedtfeld et al. 2006). Investigations of surface water fecal contamination have documented a strong correlation with stormwater flow (summarized in Stedtfeld et al. 2006). Fecal bacteria attach to sediment and, therefore, water column concentrations are positively correlated with turbidity (Fries et al. 2007). In addition, some fecal bacteria can persist or even reproduce in anaerobic benthic sediments (Evanson and Ambrose 2005) and can increase in water column concentrations with sediment resuspension.

Documentation of fecal contamination is accomplished through the presence of indicator species including total coliform bacteria or E. coli. Investigations of water quality in Cottonwood Creek and Little Campbell Creek have detected the presence of indicator species that suggest the presence of fecal waste. The abundance of fecal coliform bacteria in these streams exceeds state water quality standards (18 AAC 70). The decision to list or delist these waters for non-attainment of water quality standards, however, depends on whether the fecal bacteria are from a human, or a human-caused source. In addition, development of Total Maximum Daily Load (TMDL) calculations and treatment options requires an understanding of the sources of fecal bacteria.

Cottonwood Creek is located near Wasilla, Alaska. Cottonwood Creek arises from springs located between the Little Susitna River and Wasilla Creek drainages (Figure 1). The Creek is composed of two first order tributaries: Cottonwood Creek which flows into Cornelius Lake to the east and Dry Creek which flows out of Anderson Lake to the west. Both streams flow into Neklason Lake. The total length of all stream segments is 16.6 miles. There are 10 lakes within the drainage with a total of over 1,000 acres and 22 miles of shoreline (Davis and Davis 2005). An estimated 20.69% of the Cottonwood Creek drainage is developed (Mauger et al. 2010). Development upstream of Wasilla Lake is primarily residential. From Wasilla Lake to the Old Matanuska Road development is mixed commercial and residential. The lower 8 miles of Cottonwood Creek, from the Old Matanuska Road to Cook Inlet, is primarily residential development. Elevation at the Old Matanuska Road is near 300 feet and stream slope through this reach is 0.7%.

Previous Cottonwood Creek water sample collection for fecal coliform bacteria was conducted monthly from April through September in 2004 from sites distributed throughout the drainage. This initial screening documented fecal coliform bacteria in abundances above state water quality standards (18 AAC 70). Geometric means from monthly samples above Wasilla Lake were less than 20 cfu/100 ml; however, at the 4 downstream sampling locations geometric means were from 60 to 100 cfu/100 ml with maximum values of up to 250 cfu/100 ml at Surrey Road, the site located farthest downstream. This initial sampling was followed up with 4 samples collected within a 30 day period at one of the upstream sites near Bogard Road, and two downstream sites at Old Matanuska Road and Surrey Road. Consistent with initial screening, the geometric mean of these samples was near 5 at the upstream Bogard Road site and near 100 cfu/100 ml at Old Matanuska Road and Surrey Road, with the maximum value of 197 cfu/100 ml at the Old Matanuska Road site. Additional samples were collected in the fall of 2007 (4 samples in August and 2 in September following storm events) and spring of 2008 at sites located at the inlet and outlet of Wasilla Lake, the Parks Highway, Old Matanuska Road, Edlund Road and Surrey Road. The abundance of fecal coliform bacteria from the Parks Highway upstream were below 20 cfu/100 ml, with the exception of a geometric mean of 62 cfu/100 ml from 2 samples collected following storms at the Parks Highway. All of the sites from the Old Matanuska Road downstream had geometric mean fecal coliform counts over 20 cfu/100 ml for August samples and following September storms; however, not during spring runoff. The highest geometric means were found at Surrey Road, 175 to 200 cfu/100 ml, which also had the highest individual value of 690 cfu/100 ml.

The abundance of fecal coliform bacteria in Cottonwood Creek increase downstream from Wasilla Lake to Surrey Road. Waterfowl congregating at the outlet of Wasilla Lake could explain bacterial abundance within this reach, however, human sources from wastewater systems cannot be ruled out. Alternatively, poor septic systems and dense development downstream from Marble Way to Fairview Loop provide a possible human source, but the presence of stables and private duck ponds also could be contributing factors. Concentrations of fecal coliforms were high during the summer of 2004; however, this could be misleading due to the extreme high temperatures and low flows that occurred that year. Concentrations also increased during fall storm events, but total fecal coliform abundance can't differentiate between bacteria within stormwater, and bacteria that are already present and are resuspended into the water column.

Little Campbell Creek is made up of two main branches, the North Fork and the South Fork. Approximately 20,000 residences are located in the watershed which contains 40 to 65% impervious surface (Little Campbell Creek Watershed Plan 2007). The North Fork is spring fed originating at approximately 400 feet elevation within the Far North Bicentennial Park (Figure 2). The North Fork flows approximately 5.5 miles to its confluence with the South Fork at approximately 100 feet in elevation. The North Fork has a small tributary stream referred to as the North Branch of the North Fork that originates near Elmore Road and flows into the North Fork near Lake Otis Parkway. The upper 3 miles of the North Fork is within Bureau of Land Management (BLM) and municipal parks. The lower 2.5 miles flow through mixed commercial and residential development with the portion of commercial property increasing downstream.

The South Fork of Little Campbell Creek originates within Chugach State Park at an elevation of approximately 2,000 feet and flows for approximately 8 miles to the confluence. The South Fork receives spring flow tributary input from Craig Creek, located to the north and flowing through the Spring Creek subdivision. From Hillside Drive to below O'Malley Road, the South Fork flows through low density residential development. From O'Malley Road to Abbott Loop, the stream flows through the Alaska Zoo and Ruth Arcand Park. Residential development is predominant from Abbott Loop to Lake Otis Parkway, downstream of which commercial and industrial development increases.

Previous water sample collection for fecal coliform analyses has been conducted at 5 sampling locations in Little Campbell Creek in the fall (October and November) of 2003 weekly through breakup (March and April), May and June of 2004. Samples were collected at two locations in the North Fork, at the Coyote Trail Crossing and at 68th Street near Lake Otis Parkway; two locations in the South Fork, above Hillside Drive and near Abbott Loop; and one location below the confluence of the North and South Forks. At the upstream North Fork site, geometric means were above 20 cfu/100 ml for fall (October and November), May, and June intervals. The maximum geometric mean of 291 occurred in May. The lowest mean was 5.6 for the spring (March and April) sampling interval. The highest individual value was 7200 cfu/100 ml in May of 2004. At the North Fork crossing of 68th Street, geometric means were greater than 20 cfu/100 ml for all 4 sampling periods with the highest geometric mean of 551 occurring in June and the highest individual value of 2300 cfu/100 ml in early June.

At the upper sampling station above most development within the South Fork Little Campbell Creek drainage, geometric means from fall samples were above 20 cfu/100 ml, but below this value during breakup, May, and June of 2004. The highest individual value at this site was 310 cfu/100 ml in June. Downstream at Abbott Road, which is below developed reaches, the Alaska Zoo, and Ruth Arcand Park, geometric mean fecal coliform counts exceeded 20 cfu/100 ml through all 4 sampling periods. The maximum geometric mean of 408 occurred in June of 2004 with the maximum individual value of 1400 cfu/100 ml. The geometric mean of fecal coliform counts below the confluence of the North and South Forks also exceeded 20 cfu/100 ml through all sampling periods. The highest geometric mean of 475 occurred in June of 2004 and also the highest individual sample count of 1700 cfu/100ml.

The peak in fecal coliforms during spring snow melt in the North Fork Little Campbell Creek likely are due to bacteria that had accumulated over the winter (presumably surviving within dog feces). However, at the remaining North Fork locations, the South Fork site near Abbott Road, and below the confluence of these two tributaries, bacteria abundance increased from late May through June of 2004. Given the extreme draught conditions in 2004, this suggests inputs from groundwater (presumably human) sources. Other potential sources within the North Fork Little Campbell Creek include horse waste from stables (Equestrian Center on Abbott Road) and trails; and waste from animals held in the Alaska Zoo.

Fecal indicators can document contamination of surface waters, but they are unable to distinguish among multiple sources. Microbial source tracking (MST) and sample collection during spring runoff, base flow, and storm events allow for determination of causes, the delivery routes of bacteria, and the information necessary to develop effective treatment options. Microbial source tracking can identify sources of fecal contamination through the identification of bacteria that are species specific. *Bacteroides* is an obligate anaerobe residing within the digestive system of warm-blooded animals. Therefore, *Bacteroides* can be used as an indicator of fecal contamination of water (Field et al. 2003). The presence of *Bacteroides* in water is accomplished through the isolation, replication, and identification of gene sequences specific to this bacterium. The number of gene sequences identified within a sample, however, is not a measure of the number of organisms present and can't be related directly to fecal coliform counts. As fecal contamination increases the number of *Bacteroides* markers identified will increase along with the number of fecal coliform bacteria. The ratio of fecal coliform bacteria to *Bacteroides* is not consistent within or among species. In addition, a portion of the gene sequences found within the population of *Bacteroides* are specific to the host organism. Therefore, the identification of species-specific *Bacteroides* markers can be used to identify sources of microbial pollution. The portion of *Bacteroides* markers that are species-specific varies among hosts (Silkie and Nelson 2009). Approximately 80% of the *Bacteroides* in human are species-specific; however, in dogs, only approximately 6% are species specific. This means that the number of markers found in a sample is not comparable between species and that markers for some species are more likely to be found within a sample if present at the same concentration.

The project objective developed by the Alaska Department of Environmental Conservation (ADEC) is to: "Collect water quality samples in Cottonwood Creek and Little Campbell Creek under a ADEC approved QAPP and analyze samples using a laboratory capable of determining bacteria sources using MST Bacteroides markers analyses. Source determination will confirm if the fecal coliform bacteria pollution found are from human sources, naturally occurring (wildlife), or caused by human related activities (pets and horses). Knowing the source(s) of fecal coliform bacteria pollution will help the city of Wasilla, Matanuska-Susitna Borough and the Municipality of Anchorage to determine which best management practices to use to reduce the amount of fecal coliform bacteria reaching the streams, and will help the State determine if these waters can be delisted from the State's list of impaired waters."

Methods

Assessing the source of fecal contamination of Cottonwood and Little Campbell Creeks has been designed to isolate areas of potential sources and differentiate between times when groundwater or surface waters dominate stream flows. Detailed descriptions of the project design, sample collection and handling, sample processing, and reporting are provided in the Quality Assurance Project Plan (QAPP) found in Appendix D.

Sampling Locations

Sampling locations on Cottonwood Creek were selected based upon the distribution of fecal coliform bacteria from previous studies (Davis and Davis 2005 and 2008, Davis et al. 2006). Cottonwood Creek sampling was conducted at the Old Matanuska Road Bridge, the Marble Way Road crossing, and at the Surrey Road crossing (Figure 1). Fecal coliform counts increase downstream from Wasilla Lake. The Old Matanuska Road sampling location (CWOMR) evaluates potential sources including waterfowl concentrated at Wasilla Lake outlet, runoff from the Parks Highway and shopping areas, and potential discharge from wastewater pipes that cross under Cottonwood Creek. Differences between road runoff and wastewater should be elucidated by comparing bacterial sources and concentrations between stormwater and base flows. Sampling sites at Marble Way (CWMW) and Surrey Road (CWSR) bracket a reach of Cottonwood Creek that has dense residential development, private duck ponds, and the few remaining horse stables (see Figure 1).

Two sampling locations were selected along the North Fork Little Campbell Creek (Figure 2). The North Fork sampling site at the Coyote Trail crossing within Far North Bicentennial Park (LCCNF01) isolates potential animal sources upstream, from potential downstream human sources to the Old Seward Highway (LCCNF02).

Three sampling locations were selected on the South Fork Little Campbell Creek. The farthest upstream sampling site was located below O'Malley Road to isolate potential sources from residential development. This site was located at the Our Road stream crossing (LCCSF01). Sampling at the Abbott Road crossing (LCCSF02) targeted potential sources from the equestrian park and the Alaska Zoo. Samples collected downstream of the Old Seward Highway upstream of the confluence with the North Fork (LCCSF03), tracks sources from older residential and commercial development.

Field Data Collection and Sample Handling

Sampling was conducted on April 12, May 3, May 17, June 22, June 24, August 11, August 30, and September 30, 2010. Latitude, longitude and altitude of sampling locations were recorded using a hand-held GPS receiver. Photographs of the stream were taken showing the stream channel and riparian vegetation both upstream and downstream. Measures of turbidity, water temperature, and discharge were collected in the field. Measures of stream water pH and specific conductivity were included beginning on May 17.

Water samples were collected from a well-mixed area at each sampling site. Water-column integrated samples for fecal coliform bacteria were collected by drawing water into a 60 ml sterile syringe while moving the syringe up from near the stream bottom to near the water surface and discharging into the sample container twice. The water within the syringes was discharged into pre-labeled sample bottles for a final sample volume of 100 to 120 ml. A new syringe was used for each sample location. The sampler wore nitrile exam gloves and aseptic technique was maintained.

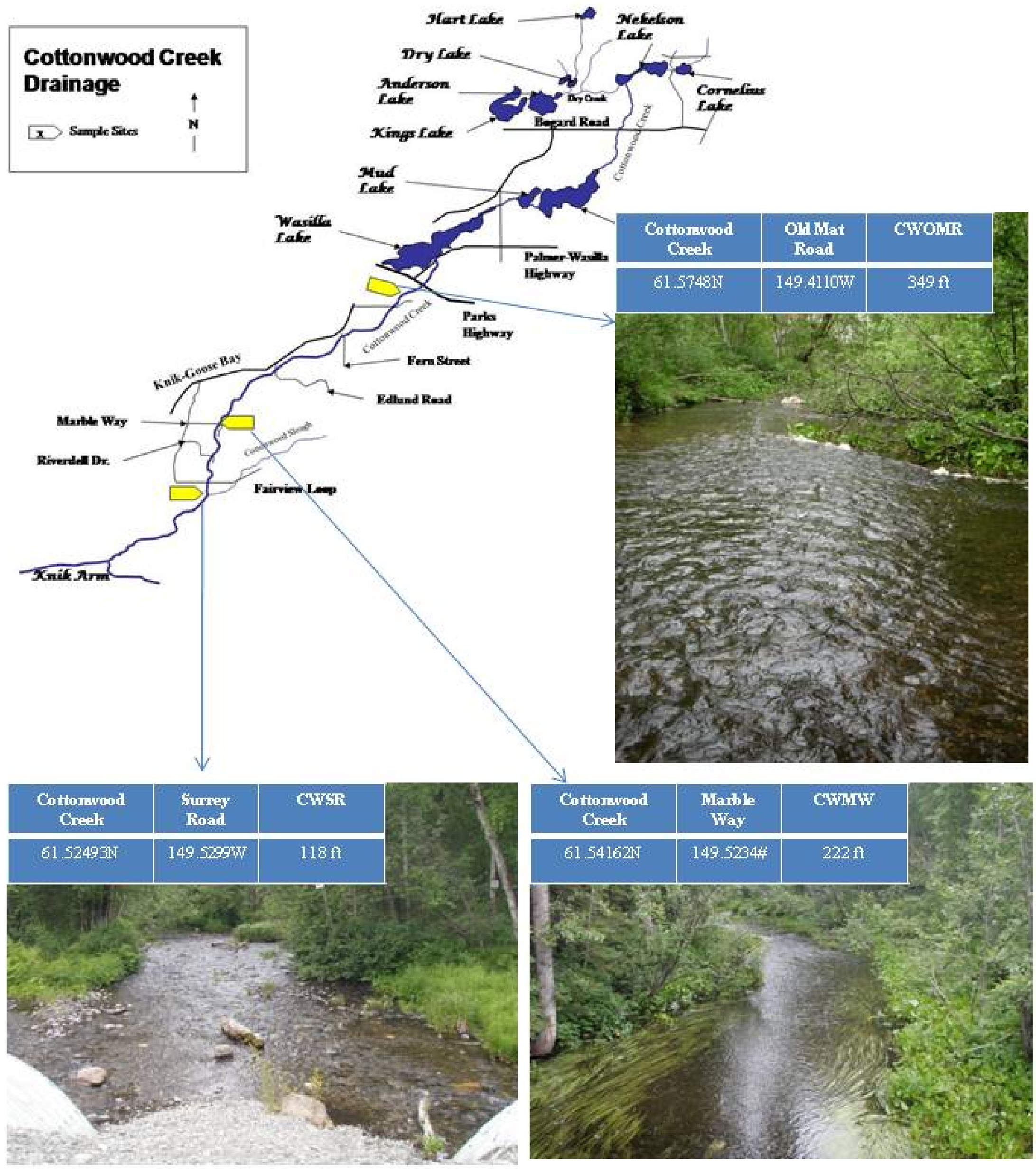


Figure 1. Cottonwood Creek sampling locations with site latitude, longitude, and elevation.

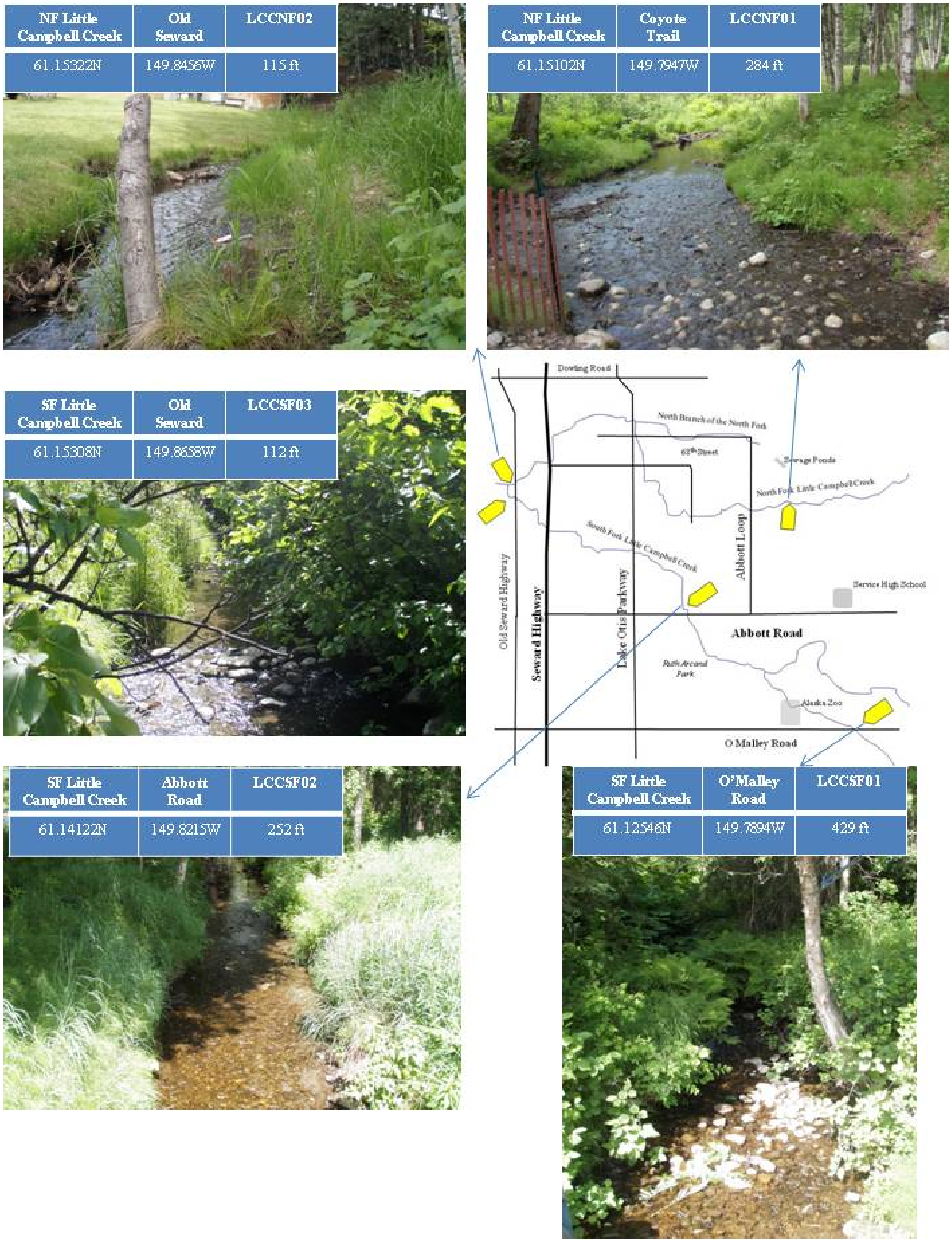


Figure 2. North and South Fork Little Campbell Creek sampling location latitude, longitude, and elevation.

Sample bottles were placed in a cooler with ice to maintain water temperatures of less than 6°C. Samples were transported to SGS – North America, in Anchorage, within 4 hours of collection for total fecal coliform analyses by membrane filtration (SM9222D).

Depth-integrated water samples were collected in pre-labeled 500 ml nalgene bottles for microbial source tracking analysis. The water samples along with a temperature blank were shipped (FedEx overnight) within coolers with frozen gel-paks to the Oregon State University microbiology laboratory. Immediately upon receipt, 100 ml of each sample was filtered (0.2 micron glass-fiber filter), preserved (in 700 µL GITC lysis buffer), and stored at -80ºC. Five replicate samples were filtered and analyzed for samples collected on May 17 (spring runoff), June 24 (summer baseflow), and August 30 (fall storm) at the Surrey Road Crossing of Cottonwood Creek and at the North and South Fork Little Campbell Creek sites just upstream from their confluence. The portion of replicates that contained markers was used to quantify the relative contribution of sources to contamination. That is, a marker present in all five replicates was considered to be a larger contributor to pollution than a marker that appeared in one of five replicates. Samples were analyzed for bacterial sources using polymerase chain reaction amplification of *Bacteroides* 16S rDNA and 16S rRNA (Field et al. 2003).

Turbidity was measured *in situ* on all sampling dates and sampling locations using a LaMotte TC-3000e turbidimeter. Water turbidity was calculated from the average of 3 samples collected at each site. Stream water pH and specific conductivity were measured using aYSI meter and probe. Stream water temperature was measured using a hand-held thermometer or the YSI meter. Stream water temperature was recorded hourly at the Old Matanuska and Surrey Road crossings of Cottonwood Creek (March 4 through September 28) and in Little Campbell Creek upstream of the Campbell Creek trail (March 18 through September 28) with HOBO Water temperature ProV2 data loggers.

Discharge was measured on each sampling date at the Old Matanuska Road Crossing of Cottonwood Creek and on Little Campbell Creek downstream of the North and South Fork confluence. Discharge was measured using the methods of Rantz et al. (1982). A meter tape was suspended across the stream. Water velocity was measured at multiple intervals across the stream using a Swofffer 3000 velocity meter. A top-setting wading rod was used to ensure velocity is measured at 0.6 depth. Water stage in Cottonwood Creek was measured using a staff gauge secured to the Old Matanuska Road Bridge and in Little Campbell Creek with a HOBO water level logger.

Daily weather data summaries (air temperatures and precipitation) were downloaded from the National Oceanic and Atmospheric Administration (NOAA) web site (http://www.ncdc.noaa.gov/oa/ncdc.html) for the Palmer and Anchorage Municipal Airports. The Wasilla Airport, while closer to Cottonwood Creek, does not record precipitation. Weather during sampling was based upon direct observations.

Stream surveys (See Appendix C) were conducted on Cottonwood Creek and Little Campbell Creek to identify land use and potential sources of fecal contamination. Field crews walked Cottonwood Creek from the outlet of Wasilla Lake to Surrey Road. Surveys of North and South Forks of Little Campbell Creek were conducted from the upstream sampling site to the confluence. Observations were made at each road crossing and through foot surveys at locations without road access. Field Technicians documented the types of development and riparian modification. Surveys were used to identify any potential sources of fecal contamination including presence of waterfowl, horse use, dog waste, and grey water outfalls. All potential sources were photographed and latitude and longitude recorded.

Results

Weather and Stream Flow

Sampling was conducted on April 12, May 3, May 17, June 22, June 24, August 11, August 30, and September 30, 2010. April and May sampling dates were selected to capture initial snow melt and high spring flow events. Timing of spring sampling was based upon visual observations of snow melt runoff along streets and gutters and through discussions with the ADEC project manager. June sampling was conducted during base flow conditions when stream discharge was largely due to groundwater inputs. Sampling in August and September was timed to follow storm events.

Cottonwood and Little Campbell Creek discharge on sampling dates is shown in Figure 3. Flows in both streams were high in April and May due to melting snow and ice. In April, many sites were ice covered, and samples were collected from openings. By May 3, ice was absent from Cottonwood Creek but remained upstream of the sampling location on the North Fork Little Campbell Creek at Coyote Trail and at the two upstream locations on the South Fork Little Campbell Creek. Stream flow in both streams was the highest on May 3. Riparian vegetation was still dormant. By May 17, stream flow had reached base flow conditions in Cottonwood Creek and was declining in Little Campbell Creek. There were no signs of ice and leaf-out had occurred. Low flow conditions were present in both streams by the June sampling dates. Stream discharge increased over summer base flow in both streams in August. Discharge was low at both sites on September 30; however, there was very little precipitation in either watershed throughout the month of September.

Precipitation recorded at the Palmer and Anchorage Airports is shown in Figure 4. There was 0.005 inches of rain recorded at the Palmer Airport prior to the June 22 sampling date and no rain recorded between June 22 and the next sampling date of June 24. The August 11 sampling occurred following 0.16 inches of rain the previous 3 days in Palmer. However, 0.44 inches of rain had been recorded from August 1 to August 8. The August 30 sampling followed a three day accumulation of 1.13 inches of rain in Palmer. September was extremely dry with less than 0.03 cumulative inches of rain falling between September 8 and September 27. Roads in Wasilla were wet and there was light rain on September 30 during sampling; however, no rain had been recorded at the Palmer Airport over the previous 3 days.

In Anchorage, 0.001 inches of rain was recorded prior to the June 22 sampling date, with recorded precipitation from June 22 to June 24. The August 11 sampling occurred following 0.25 inches of rain in Anchorage over the previous 3 days. The August 11 sampling occurred following 0.25 inches of rain over the previous 3 days; however, 1.25 inches of rain had been recorded in Anchorage from August 1 to August 8, with a large storm of 0.82 inches of rain the

24 hours prior to August 8. The August 30 sampling followed a three day accumulation of 1.33 inches of rain in Anchorage. This was followed by a dry spell where a cumulative 0.02 inches of rain fell between September 8 and September 27. Sampling on September 30 followed 0.27 inches of rain in Anchorage.

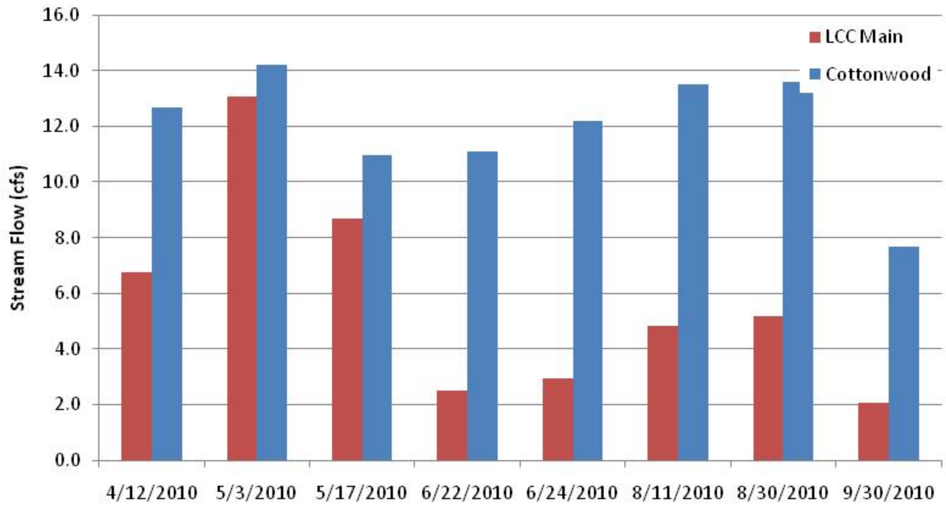


Figure 3. Stream discharge on each sampling date showing low flow conditions during sampling in June and high flows during spring runoff and August storm events.

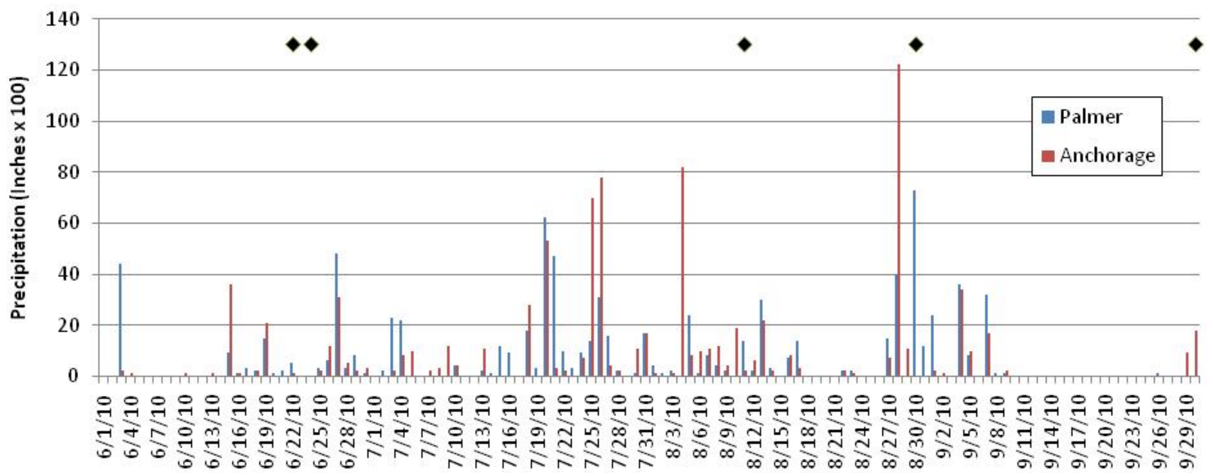


Figure 4. Precipitation recorded in the 24 hours previous at the Anchorage and Palmer airports for June through September. Black diamonds represent sampling dates.

Cottonwood Creek

Temperature, Turbidity, pH, Specific Conductivity

Water temperature and turbidity for all sampling dates at the Cottonwood Creek sampling sites are shown in Figures 5 and 6. Stream water temperatures are consistently higher at the Old Matanuska Road site, approximately 1 km below the outlet of Wasilla Lake, and decrease downstream. Temperatures increased through April and May and were consistent from June through July, decreasing again in August.

Turbidity was below 5 NTU on all sampling dates and at all sampling locations. There were only minor differences among sites. Turbidity in Cottonwood Creek did not increase during spring breakup or following precipitation events in August. However, turbidity was slightly higher during low flow samples in June at the Marble Way and Surrey Road sites. Turbidity was not correlated with changes in discharge or precipitation.

Stream water pH and specific conductivity in Cottonwood Creek for each sampling date is shown in Figures 7 and 8. Stream water pH tends to decrease during storm events, while specific conductivity was highest during spring runoff but did not increase following August storms. Stream water pH was positively correlated with discharge and specific conductivity was negatively correlated with discharge.

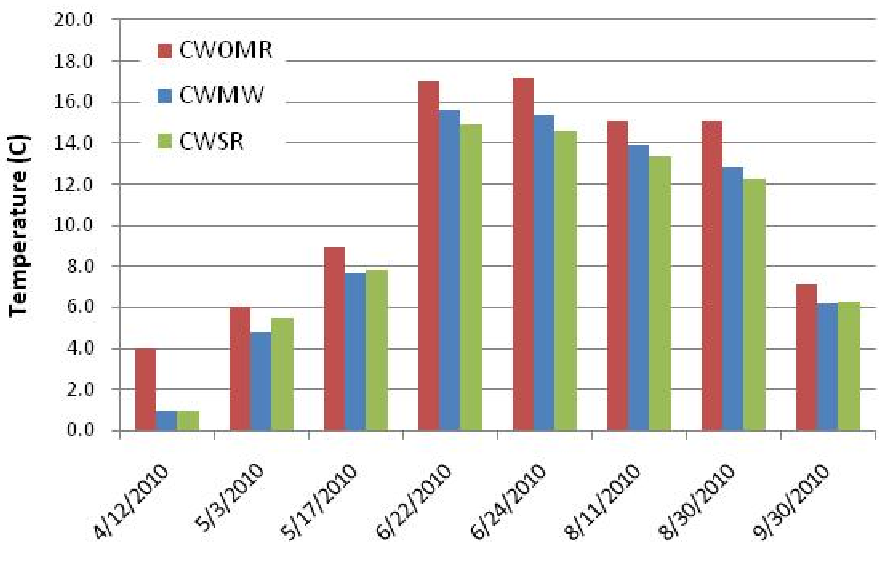


Figure 5. Cottonwood Creek water temperature for all sampling dates and locations.

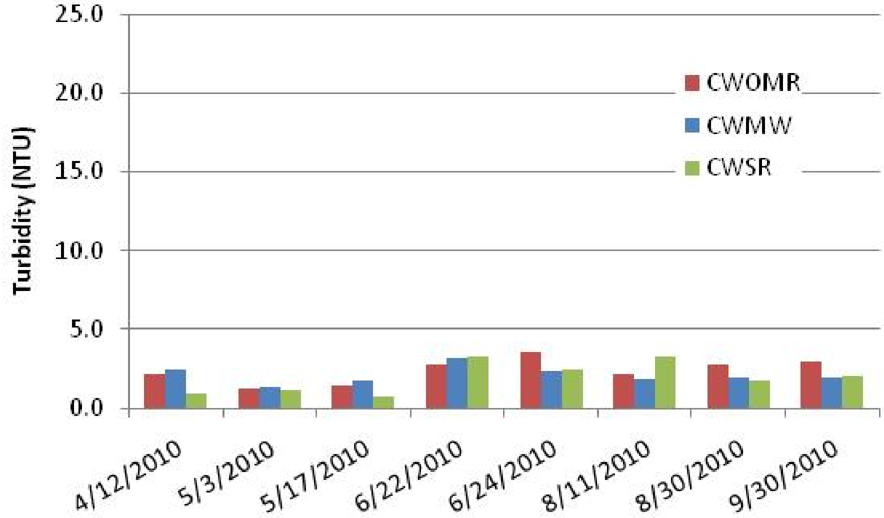


Figure 6. Turbidity of Cottonwood Creek for all sampling dates and locations showing overall low turbidity with lowest values during spring runoff.

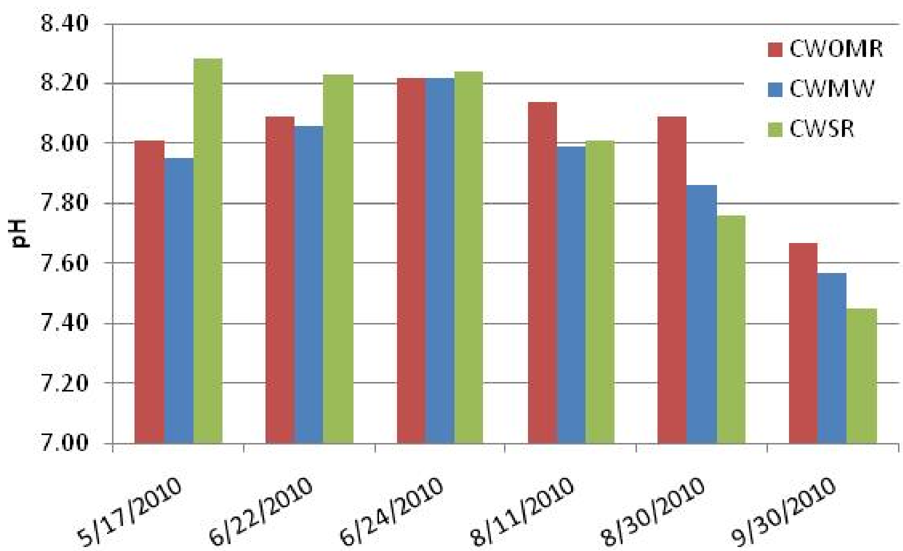


Figure 7. Cottonwood Creek pH for all sampling dates except April 12 and May 3, at all sampling locations showing highest pH during base flow conditions, and lower pH during spring runoff and fall storms.

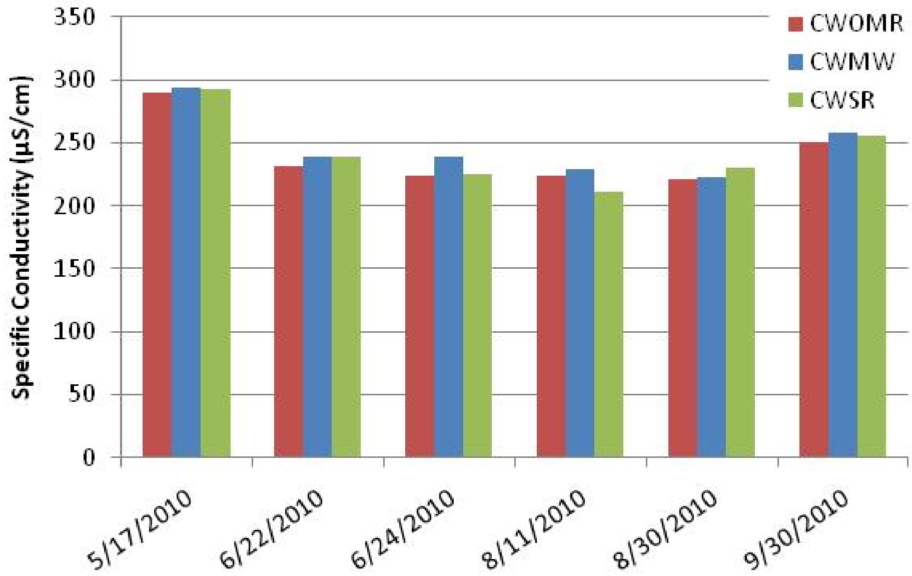


Figure 8. Cottonwood Creek specific conductivity showing higher values following spring runoff.

Stream Survey Cottonwood Creek

Site-specific information and photographs from surveys are provided in Appendix C. Land use is primarily commercial between Wasilla Lake and the Old Matanuska Road. The riparian vegetation has been modified just downstream of the Palmer/Wasilla Highway, ducks are often present between the Palmer/Wasilla and Parks Highways, and duck feces are present on rocks and other resting areas. Stormwater from the Parks Highway and shopping centers on both sides of Cottonwood Creek discharge just upstream from the highway culvert. From the Parks Highway to the Old Matanuska Road, stream slopes are low, the substrate is composed of fine sediment and macrophytes proliferate. Land use is primarily residential downstream of the Old Matanuska Road to Surrey road, with the highest development occurring between Marble Way and Fairview Loop Road.

Fecal Coliform Bacteria and *Bacteroides* Markers

A total of 27 water samples were collected from Cottonwood Creek and analyzed for fecal coliform bacteria. Of these, 15 (55%) of the samples were greater than 20 cfu/100 ml, and 8 (30%) of the samples were greater than 100 cfu/100ml. Concentrations of fecal coliform bacteria were low at the Old Matanuska Road sampling site on all sampling dates. Concentrations increased slightly during the sampling events following August storms to a maximum of 20 cfu/100 ml on August 30 (Figure 9). Fecal coliforms abundance at Old Matanuska Road were within the range observed in 2007 and 2008 but less than recorded previously in 2004 and 2005. The concentrations of fecal coliforms at Marble Way were high during early spring runoff in April, then declined to less than 5 with decreasing flows by May 17. Concentrations of fecal coliforms were highest on the June 22 sampling date and decreased until September. The abundance of fecal coliforms at Marble Way were higher at over 200 cfu/100 ml, than previous sampling in 2007 and 2008 where maximum counts of 59 cfu/100 ml were recorded. At Surrey Road, located further downstream, concentrations of fecal coliforms peaked during low flows in June and then gradually declined through the season. Maximum recorded counts of over 200 were within the range of counts obtained in previous sampling, but less than the previous maximum count of 690 cfu/100 ml recorded following a storm event in September of 2007.

The abundance of total fecal coliforms at Old Matanuska Road were strongly correlated with precipitation (correlation coefficient 0.93), and negatively correlated with specific conductivity (correlation coefficient -0.55). The concentration of fecal coliforms at Marble Way and Surrey Road were not correlated with precipitation or discharge, but were positively correlated with turbidity (correlation coefficients 0.7 and 0.9) and negatively correlated with specific conductivity (correlation coefficients -0.7 and -0.8). Total fecal coliforms were positively correlated with water temperature, with a strong positive correlation at Surrey Road (correlation coefficient 0.85).

The presence of species-specific *Bacteroides* markers at Cottonwood Creek sampling sites is shown in Figures 10 through 12. Specific markers were detected in 5 of 15 samples (33%) with greater than 20 cfu/100 ml fecal coliform counts, and 5 of 8 samples (63%) with over 100 cfu/100 ml fecal coliform counts. Markers were found at all sites on August 30, following stormwater runoff. Human-specific markers were detected at the two downstream locations following precipitation prior to August 30. Markers also were found at two locations during base-flow conditions.

Markers for horses and waterfowl were detected at the Old Matanuska Road site (Figure 10). The horse marker was found during base flow conditions in June. On this date, five replicate samples were collected at this location, and the horse marker was found in one of the five samples. Therefore, there was a 20% chance of this marker occurring in a sample. This suggests that there was a low abundance of horse markers which is supported by the low fecal coliform counts. Total fecal coliform counts of 3 cfu/100 ml were measured at this site on this date. The marker for waterfowl was present on August 30, coinciding with higher discharge and following precipitation. The total fecal coliform count was 20 cfu/100 ml on this same date, which was the highest recorded value at the Old Matanuska Road site.

Markers for dogs, humans, horses, and waterfowl were detected at the Marble Way sampling location (Figure 11). The marker for dogs was found on June 22 when discharge was low. This coincided with the highest fecal coliform bacteria count for this location. Markers for humans, horses, and waterfowl were detected on August 30 following a rain storm. Five replicate samples were collected at this site for this date. Markers for dogs and humans were detected in 20% of the samples and markers for waterfowl were detected in 60% of the samples. Fecal coliform counts were 121 cfu/100 ml at this location on this date.

At the furthest downstream site, Surrey Road, markers for humans and horses were detected on August 30 (Figure 12). Concentrations of total fecal coliforms were 76 cfu/100 ml on this date. These were the 4th highest counts of the 8 sampling events. Replicate samples were collected at this site on this sampling date and markers for both humans and horses were detected in one of the two samples.

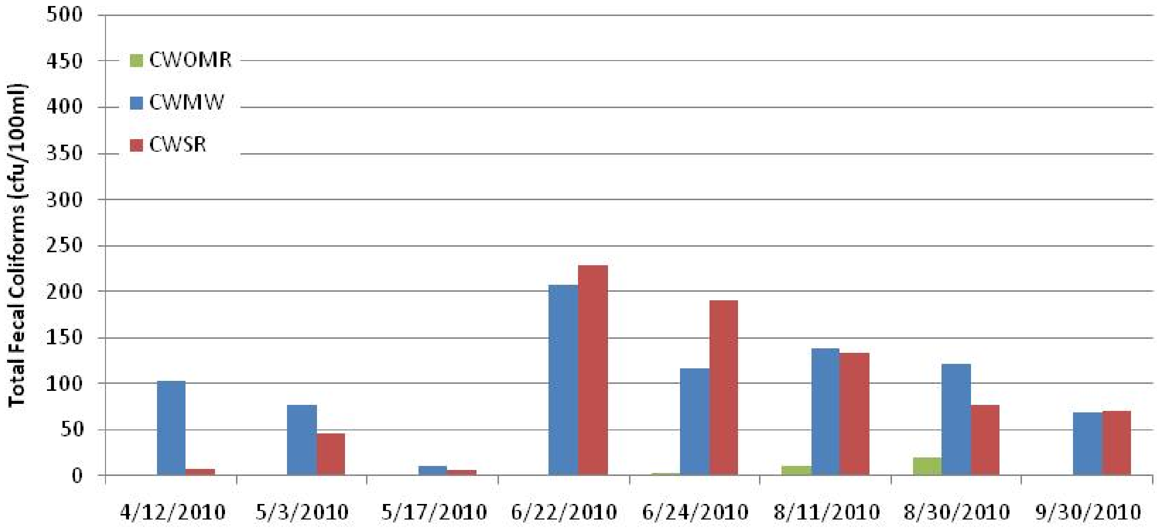


Figure 9. Total fecal coliform bacteria at the three Cottonwood Creek sampling stations. Concentrations are lowest at Old Matanuska Road and similar at Marble Way and Surrey Roads.

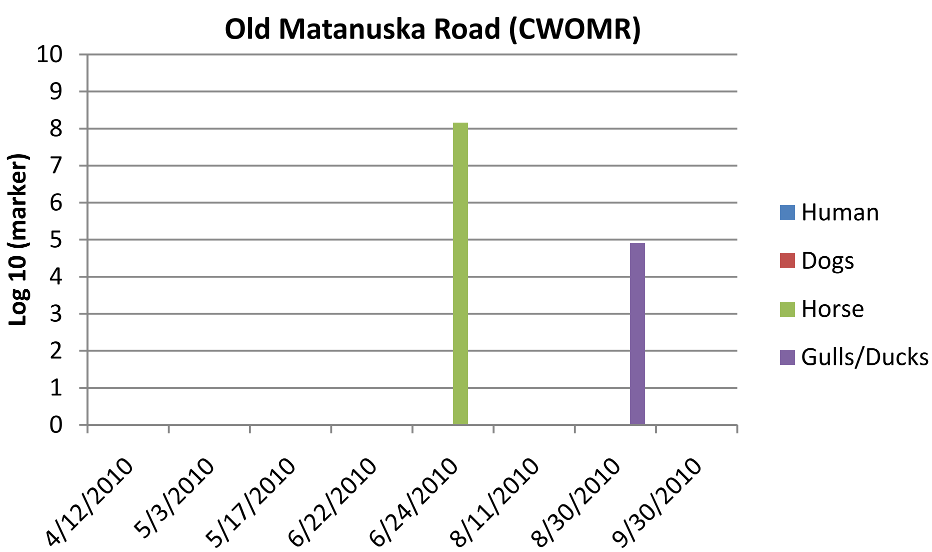


Figure 10. Species-specific *Bacteroides* markers for each sampling date showing presence of markers for horses on one date during low flow conditions and waterfowl following precipitation.

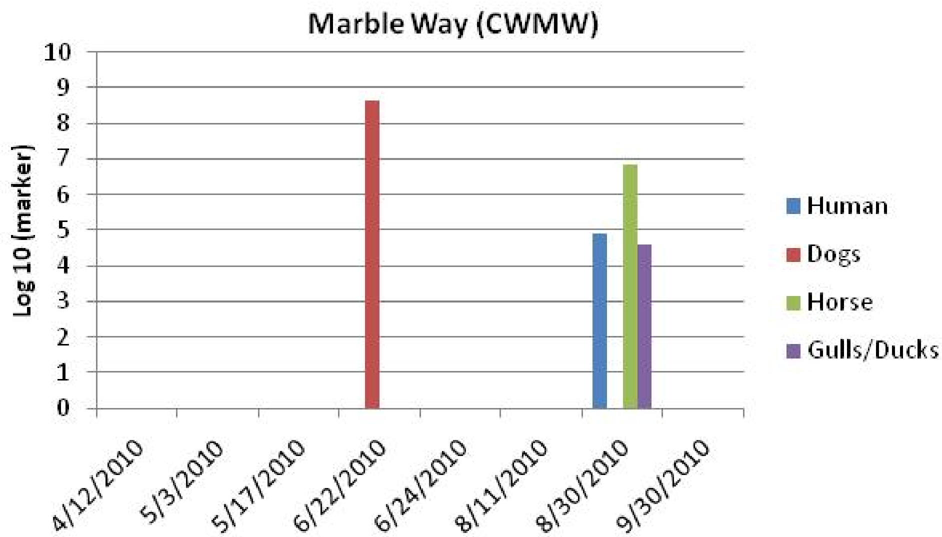


Figure 11. Species-specific *Bacteroides* markers for each sampling date, showing the presence of markers for dogs during low-flow conditions, and humans, horse, and waterfowl following precipitation.

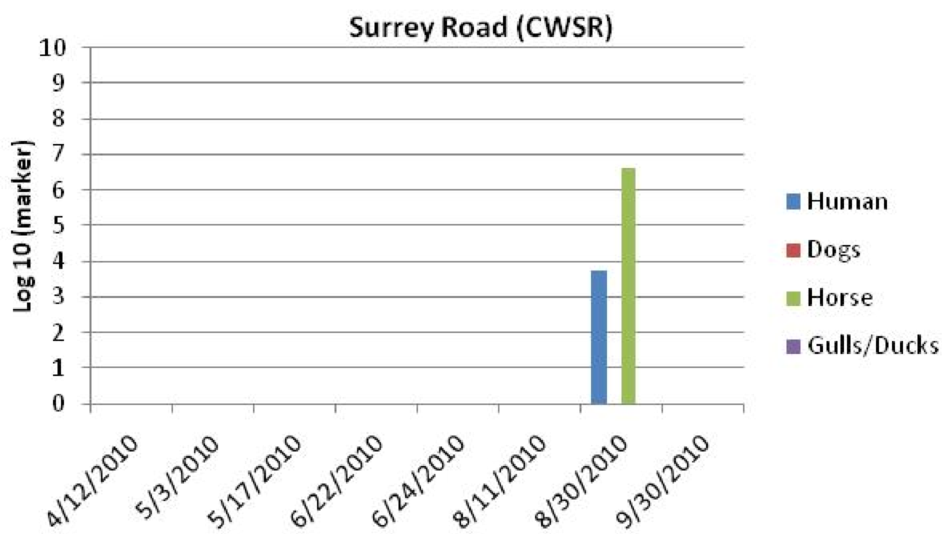


Figure 12. Species-specific *Bacteroides* markers for each sampling date, showing the presence of markers for humans and horses following precipitation.

North Fork Little Campbell Creek

<u>Temperature, Turbidity, pH, Specific Conductivity</u>

Stream water temperature at the two North Fork Little Campbell Creek sites is shown in Figure 13. Water temperatures remained cool at the upstream sampling location within Bicentennial Park. Maximum water temperature was near 7°C. Water temperature was higher downstream, increasing up to 7°C above upstream values, with maximum summer temperatures near 14°C.

Turbidity was low, generally below 3 NTU at the upstream location (LCCNF01) (Figure 14). There was a slight increase in turbidity at this site to 5.7 NTU on June 22 during low flow conditions, but following a 0.01 inch rain event. Turbidity was not strongly correlated with flow or precipitation but was negatively correlated with specific conductivity and specific conductivity was positively correlated with precipitation at LCCNF01. Turbidity at the downstream sampling location just upstream from the confluence with the South Fork was higher during spring runoff in April and early May at 13.9 and 7.0 NTU, respectively. During base flow, turbidity was near 5 NTU but then increased on June 22 and following precipitation events prior to August 11 and August 30. Turbidity downstream was 5 or more NTU higher than the upstream sampling site on April 12, May 3, and August 30. Turbidity at LCCNF02 was inversely related to specific conductivity; however, conductivity at this downstream sampling location increased following precipitation.

Stream water pH at the upper and lower North Fork Little Campbell Creek sites ranged from 7.7 to 8.0 (Figure 15). The stream water was more acidic during spring runoff and following precipitation events. Specific conductivity was stable at the upstream location, and increased slightly with precipitation events (Figure 16). Specific conductivity downstream was variable and negatively correlated with precipitation and pH.

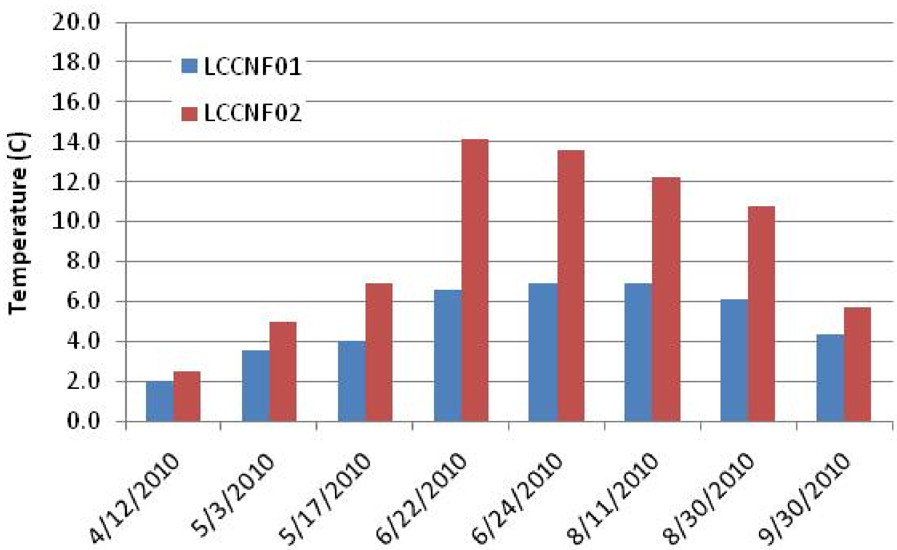


Figure 13. Water temperature during sampling at the two North Fork Little Campbell Creek sites.

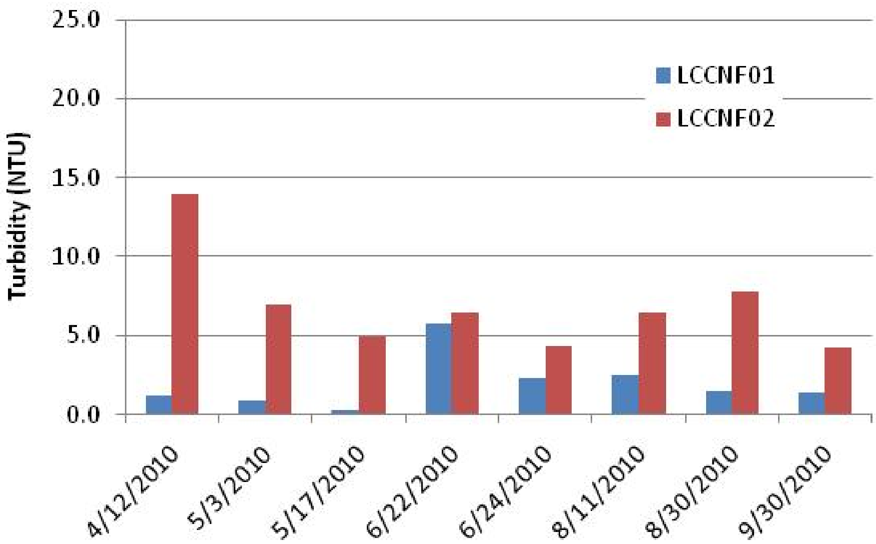


Figure 14. Stream water turbidity on all sampling dates at the two North Fork Little Campbell Creek sampling sites.

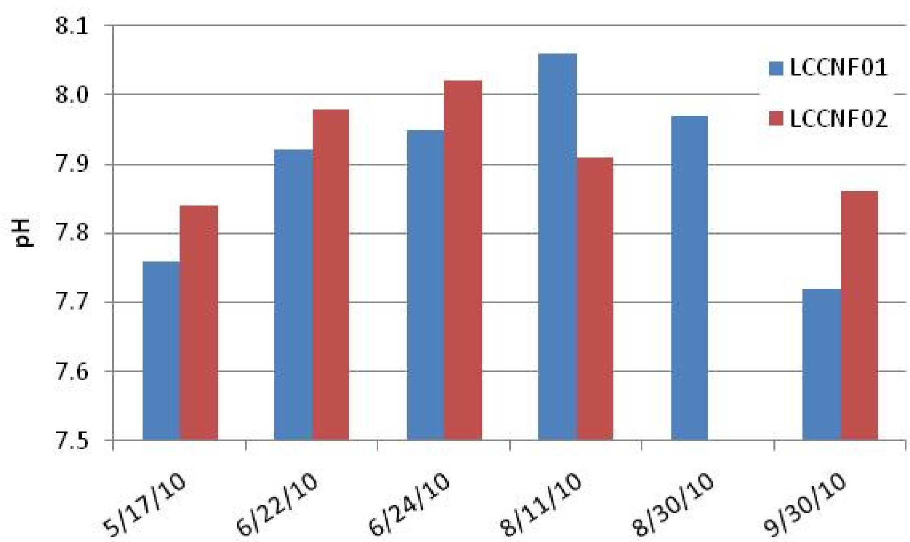


Figure 15. Stream water pH in the North Fork Little Campbell Creek for most sampling dates.

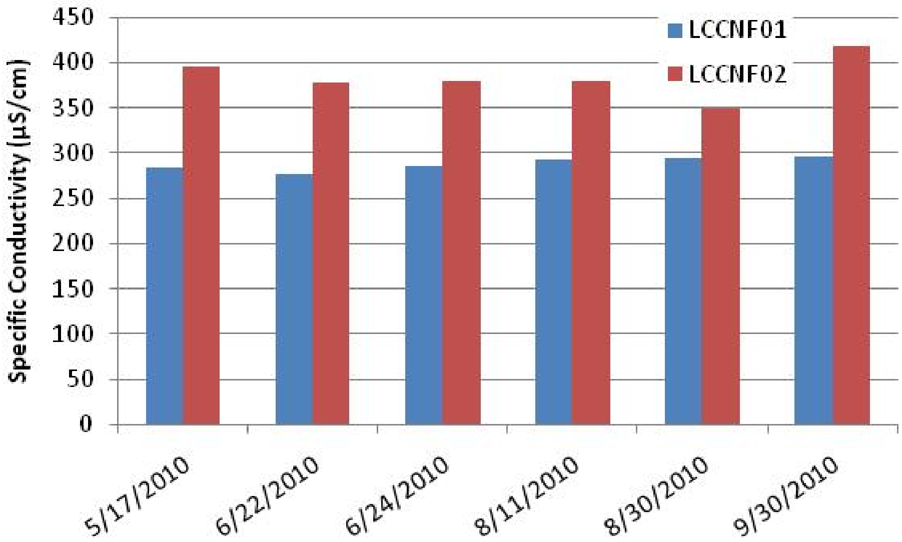


Figure 16. North Fork Little Campbell Creek specific conductivity.

Stream Survey North Fork Little Campbell Creek

Site-specific information and photographs from surveys are provided in Appendix C. Land use surrounding the North Fork Little Campbell Creek from the Old Seward Highway to Lake Otis Parkway is a mix of residential, commercial and industrial. Riparian vegetation improves from downstream to upstream. Waterfowl and private residences were the main possible source of microbial input. Along the North Branch North Fork Little Campbell Creek from Lake Otis Parkway to BLM land, land use is residential to undeveloped from downstream to upstream and riparian vegetation improves along the same gradient. Dogs are the main source of possible microbial input. The old sewage lagoons on BLM land have been capped and sample wells have not found any fecal contamination of surrounding groundwater (personal communication BLM staff). The North Fork Little Campbell Creek upstream of Lake Otis is largely residential to Elmore Road. Possible contamination sources include dogs and septic systems. There is a large stable at Elmore Road that could be contributing bacteria to the North Fork. Upstream of Elmore Road, the North Fork Little Campbell Creek flows through the Far North Bicentennial Park. The stream is crossed by recreational trails where canine, horse, and wildlife feces could enter the stream. Dog tracks and feces have been observed at the Coyote Trail Bridge.

Fecal Coliform Bacteria and *Bacteroides* Markers

Total fecal coliform bacteria results for the two North Fork Little Campbell Creek sites are shown in Figure 17. A total of 16 samples were collected and analyzed for total fecal coliform bacteria. Of these samples, 12 (75%) were over 20 cfu/100 ml, and 4 (25%) were greater than 100 cfu/100 ml. Markers were found in two of the North Fork samples. Source markers were detected in 1 (12.5%) of the samples where fecal coliform counts exceeded 20 cfu/100 ml and 2 (50%) of the samples where fecal coliform counts exceeded 100 cfu/100 ml.

Concentrations of fecal coliform bacteria at the upstream site located within Bicentennial Park were over 20 cfu/100 ml on 5 of the 8 sampling dates. Maximum counts near 200 cfu/100 ml occurred on August 30. Fecal coliform counts at this site were strongly correlated with cumulative precipitation prior to sampling (correlation coefficient 0.93). No *Bacteroides* markers were detected at this sampling location for humans, horses, dogs, or waterfowl; therefore, these sources are not likely responsible for the high fecal coliform counts.

Fecal coliform bacteria exceeded 20 cfu/100 ml on 6 of the 8 sampling dates at the downstream sampling site (LCCNF02). High counts, over 300 cfu/100 ml, were obtained during June base flow. The maximum seasonal value of 600 cfu/100 ml was on August 30. Total fecal coliform counts were positively correlated with cumulative precipitation prior to sampling (correlation coefficient 0.68) and pH (correlation coefficient 0.87).

Bacteroides markers were found for waterfowl on June 24 and for humans and waterfowl on August 30 (Figure 18). Five replicate samples were collected on August 30, and waterfowl markers were present in all samples. Human markers were present in 3 of the 5, or 60%, of the replicates.

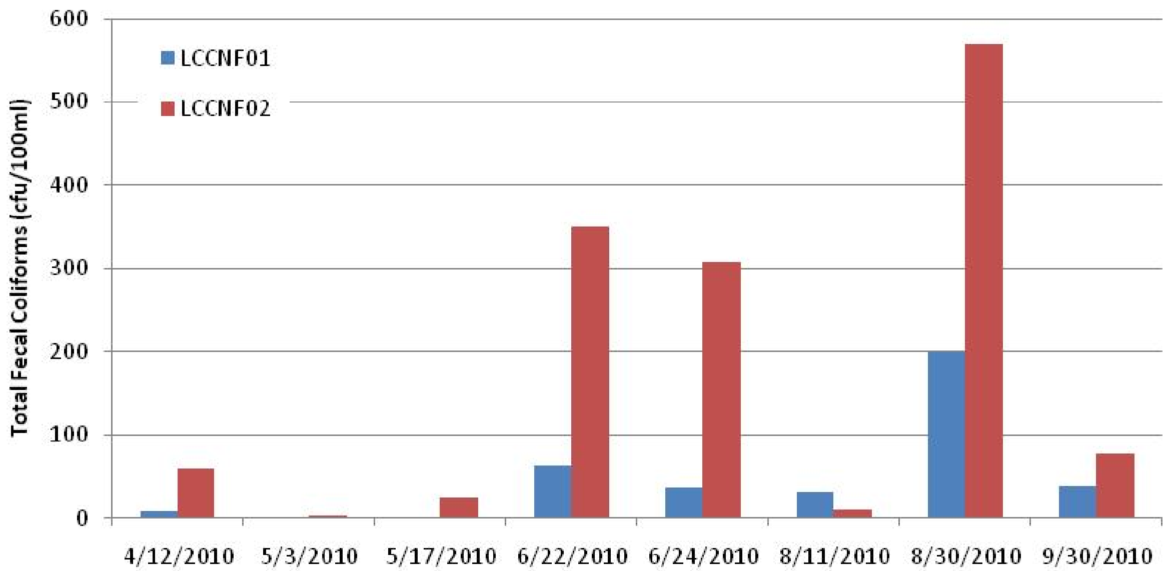


Figure 17. Total fecal coliform bacteria for the North Fork Little Campbell Creek sampling sites.

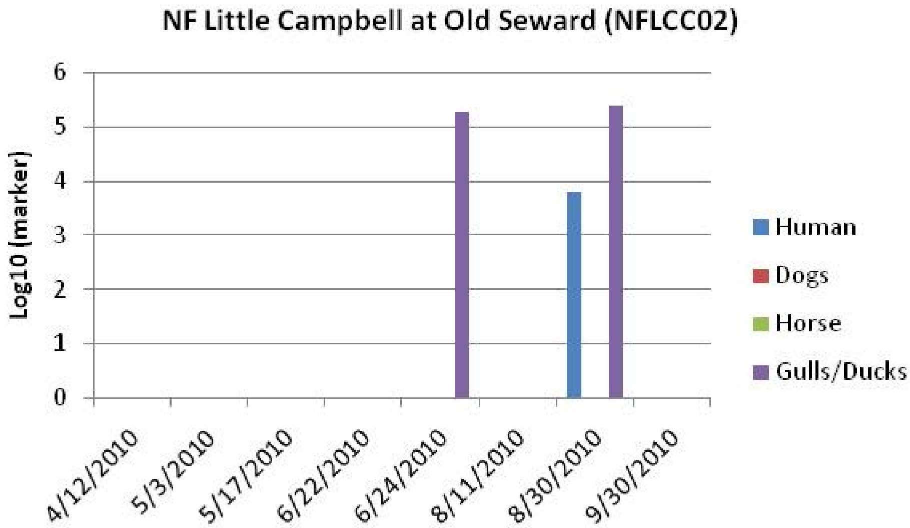


Figure 18. Species-specific *Bacteroides* markers showing the presence of bacteria from waterfowl during low flow and following storms, and human sources following storm runoff.

South Fork Little Campbell Creek

Temperature, Turbidity, pH, Specific Conductivity

Stream water temperature at the three South Fork Little Campbell Creek sites during sampling is shown in Figure 19. Water temperature decreased between the upstream site (LCCSF01) located near O'Malley Road, and the next downstream site (LCCSF02) located near Abbott Loop, due to tributary input from Craig Creek. Craig Creek enters the South Fork just downstream of the Alaska Zoo. On August 30, water temperature in Craig Creek was 6.2°C compared to 10.8°C at LCCSF01. Water temperature increased from Abbott Loop to the site below the Old Seward Highway (LCCSF03).

Turbidity at the two upstream sampling locations on the South Fork was generally below 5 NTU (Figure 20), with the exception of a slight increase on June 22, which was also observed at the upstream site on the North Fork. Turbidity at the downstream site was high during spring runoff and decreased gradually. Turbidity at this site also increased relative to the upstream sites following precipitation prior to August 30. Turbidity at LCCSF03 was greater than 5 NTU above values at LCCSF01 on April 12 and May 5 during spring runoff and on August 30 following precipitation.

Stream water pH in the South Fork Little Campbell Creek ranged from 7.8 to 8.3 (Figure 21). As within the other streams, values were more acidic during spring runoff and following precipitation in August. Specific conductivity was lowest at the upstream site near O'Malley Road and highest downstream near the Old Seward Highway. Specific conductivity increased due to inputs from Craig Creek. Specific conductivity in Craig Creek was 450 µS/cm on August 30 and September 30, compared with values close to 250 µS/cm at LCCSF01. Specific conductivity increased at the upstream site with precipitation (Figure 22) suggesting that the conductivity of surface runoff was greater than stream values. At the sites below Craig Creek, specific conductivity had a strong negative correlation with discharge (correlation coefficients of -0.8 to -0.9) suggesting that the conductivity of surface runoff was less than stream values.

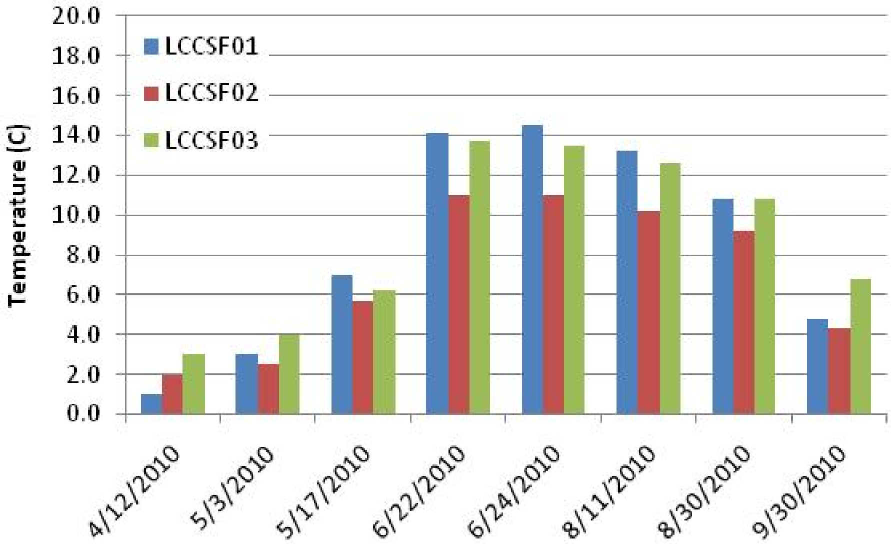


Figure 19. Stream water temperature during South Fork Little Campbell Creek sampling events showing a decrease from LCCSF01 to LCCSF02.

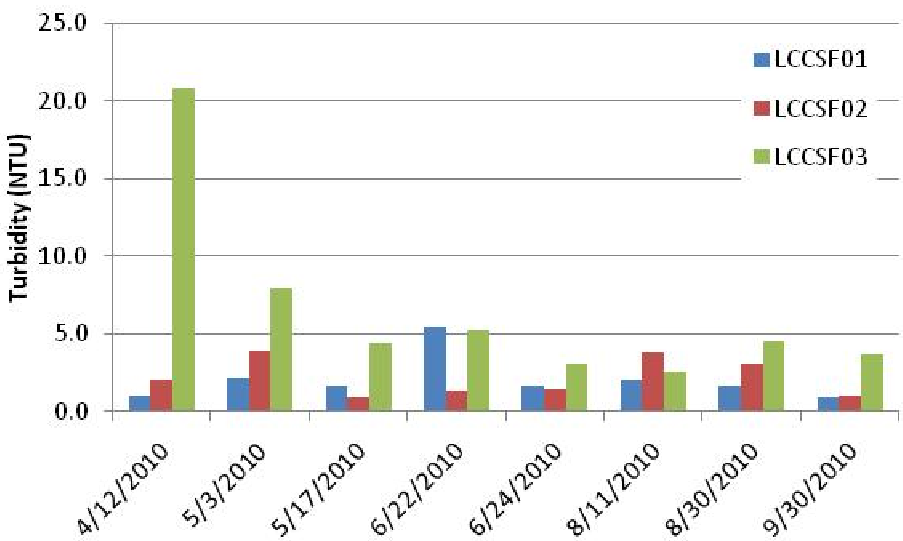


Figure 20. Turbidity in the South Fork Little Campbell Creek during sampling events showing high turbidity at the Old Seward Highway site (LCCSF03) during spring runoff.

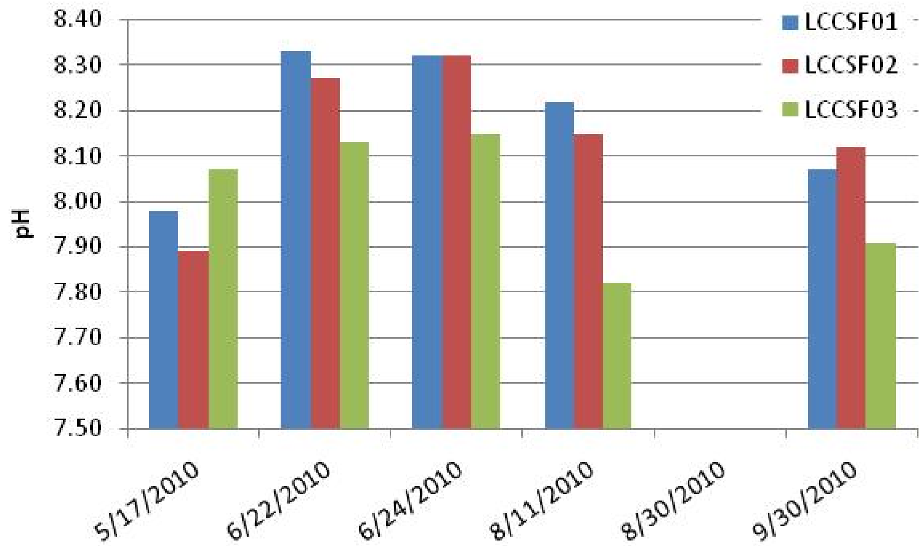


Figure 21. South Fork Little Campbell Creek pH during sampling events showing more acidic water following spring runoff and precipitation.

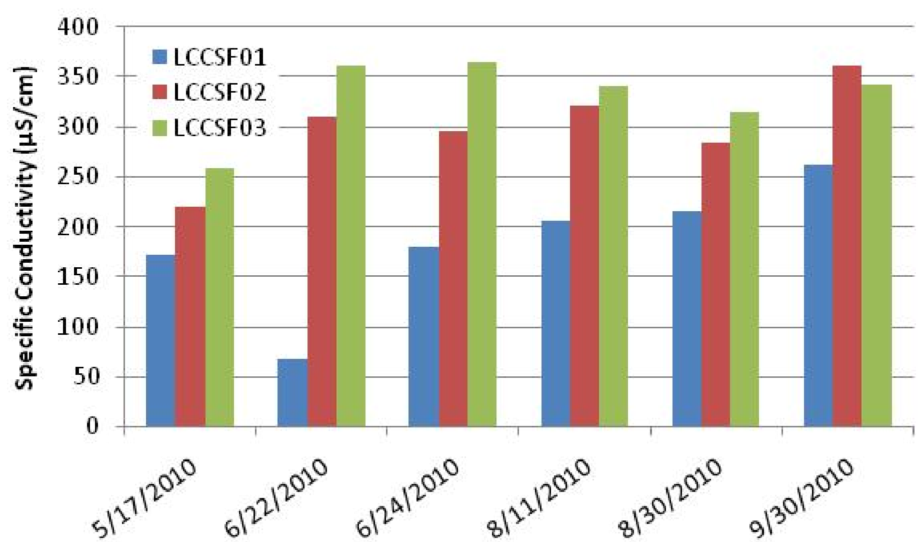


Figure 22. Specific conductivity at the three South Fork Little Campbell Creek sampling locations.

Stream Survey South Fork Little Campbell Creek
Site specific information and photographs from surveys are provided in Appendix C. Land use along the South Fork Little Campbell Creek from the confluence with Little Campbell Creek to Lake Otis Parkway is predominately developed, mixed commercial and industrial. The riparian vegetation is altered to the streambank in more than half of the sites observed. Possible sources of microbial contamination include dogs, moose, garbage facilities, and birds. South Fork Little Campbell Creek land use from Lake Otis Parkway to Abbott Road is 100% residential. Some alteration to the riparian vegetation was observed. Possible microbial contamination is predominately from dogs, but also from moose and birds. From Abbott Road to Elmore Road, land is predominately undeveloped. The major change to the riparian vegetation is due to the power line right-of-way along the undeveloped section of Elmore Road. Horseback riding is prevalent in the area and is likely to be a source of microbial contamination in combination with dogs. The South Fork Little Campbell Creek upstream from Elmore Road is composed of the Alaska Zoo and residential development. Riparian vegetation is largely intact. Possible sources of microbial contamination are zoo animals, horses, and birds. Craig Creek is a north tributary to South Fork Little Campbell Creek below Our Road. Land use is 100% residential in this area. Some riparian alteration has occurred from homeowners mowing to the stream edge. Bird feces were the only observed source of microbial contamination.

South Fork Little Campbell Creek Fecal Coliform Bacteria and *Bacteroides* Markers
Total fecal coliform results are shown in Figure 23. A total of 24 samples were collected in the South Fork Little Campbell Creek and analyzed for total fecal coliform bacteria. Of those, 16 samples (67%) had values over 20 cfu/100 ml and 11 (45%) had counts over 100 cfu/100 ml. Markers for *Bacteroides* were found in 7 of the samples.

Fecal coliform counts were low at the upstream site (LCCSF01) during spring break up and following precipitation events in August. Counts during base flow conditions, June 22 and June 24 were greater than 100 cfu/100 ml. High counts also were obtained on September 30, following a small storm, but when discharge was still low. Fecal coliform counts at this upstream site had a strong negative correlation with discharge (correlation coefficient -0.72). Markers for human *Bacteroides* were found at this site (LCCSF01) on June 22 coinciding with low flows and high fecal coliform counts (Figure 24). Markers for waterfowl were found on August 30, following precipitation, higher discharge, and lower total fecal coliform counts.

Total fecal coliforms at the site located downstream, below Abbott Loop (LCCSF02) were low during spring runoff but increased during base flow and following precipitation. Fecal coliform bacteria counts had a weak negative relationship with discharge, and a week positive relationship with precipitation. Markers were found for humans, dogs, and waterfowl on June 24, during low flows, and for humans and dogs on August 11 (Figure 25) following a small precipitation event.

At the downstream South Fork Little Campbell Creek site near the Old Seward Highway (LCCSF03), fecal coliform bacteria counts were high during spring runoff, decreased during base flow, and then increased again following the August 30 rain event. Total fecal coliform bacteria counts were higher than at most other sites, with values over 100 cfu/100 ml on 4 of the 8 sampling dates, and greater than 20 cfu/100 ml on 7 of the 8 sampling dates. Total fecal coliforms had a strong negative correlation with specific conductivity (correlation coefficient -

0.87), which decreased with increasing flows. Total fecal coliform counts were greater than 500 cfu/100 ml on May 17; however, no *Bacteroides* markers were present on this date (Figure 26). Markers for humans were found during low flow conditions on June 24, and following precipitation on August 11 and August 30. Markers for waterfowl also were present on the two sampling dates in August. Replicate samples were collected on this date at LCCSF03. Human markers occurred in 2 of 5 samples, or 40%, and waterfowl occurred in 3 of 5 samples, or 60% of the replicates.

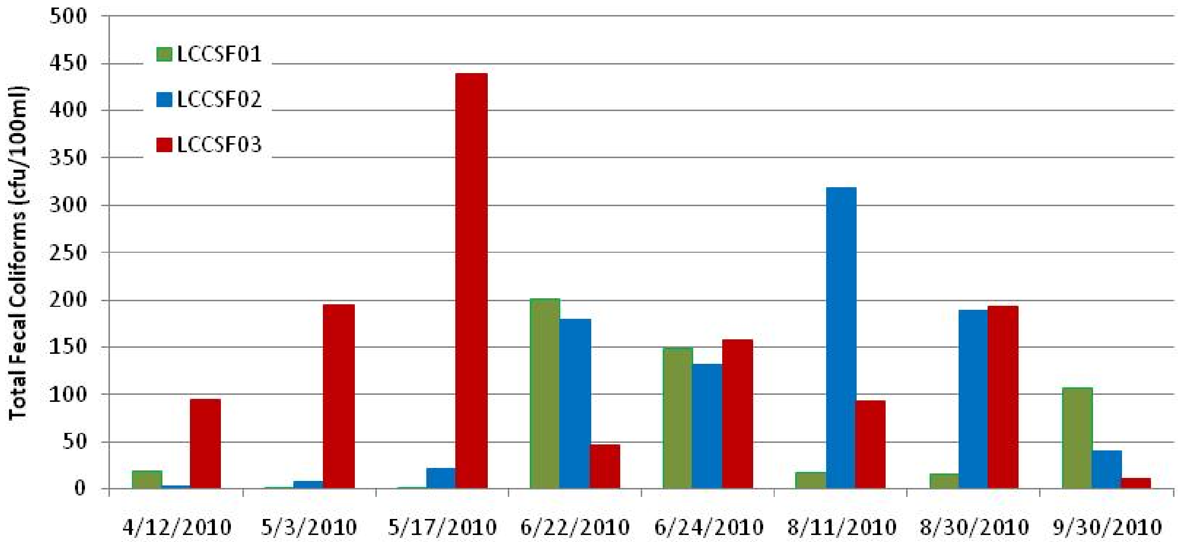


Figure 23. Fecal coliform bacteria at the three South Fork Little Campbell Creek sampling locations showing the divergent patterns among locations.

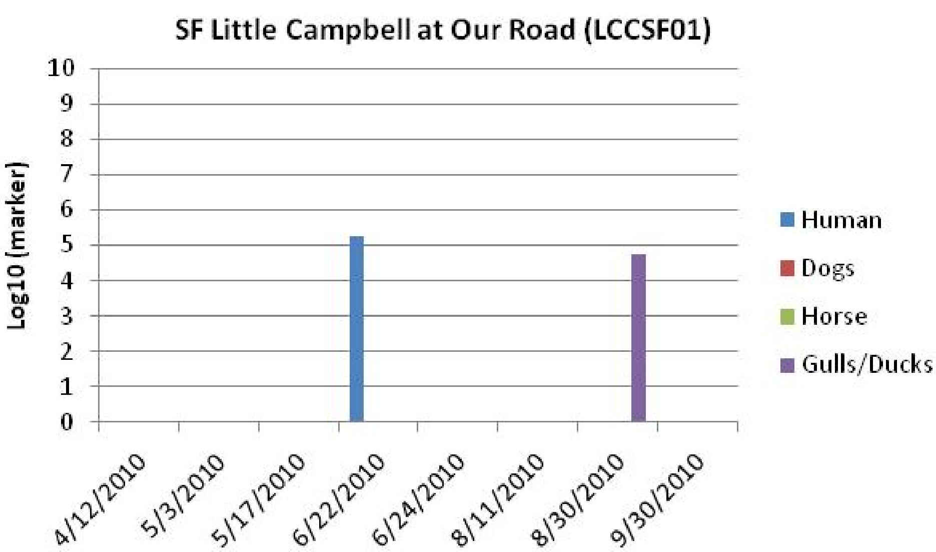


Figure 24. Species-specific markers at the upstream sampling location showing human sources during base flows and waterfowl following storm events.

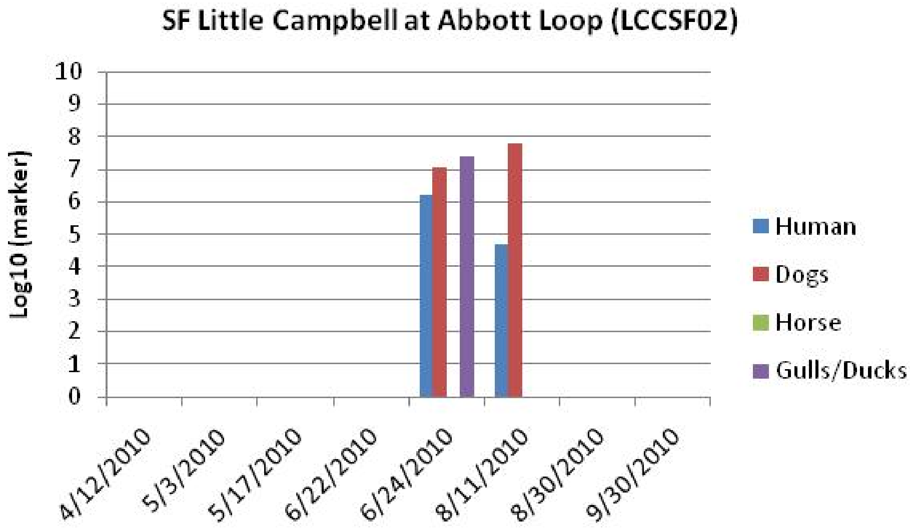


Figure 25. Species-specific markers showing presence of humans, dogs and waterfowl during base flow conditions and a small precipitation event.

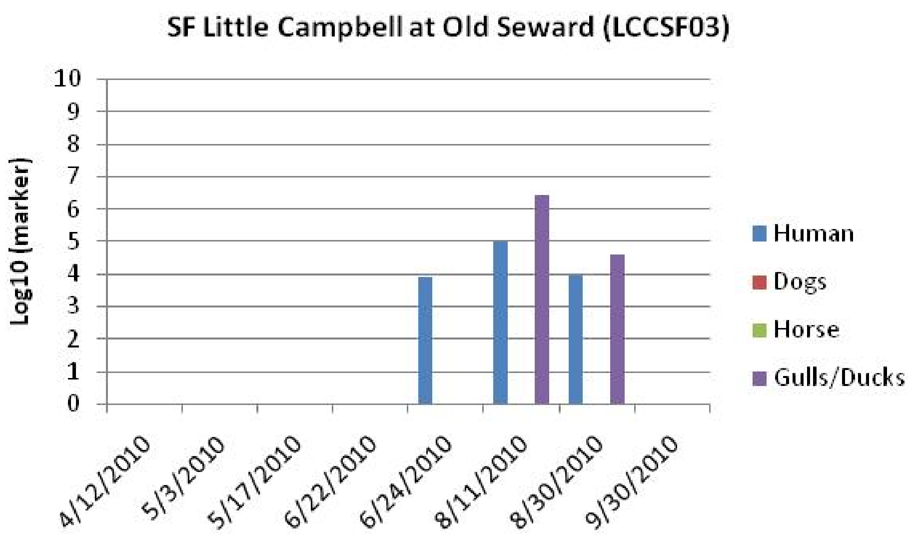


Figure 26. Species-specific markers at the farthest downstream South Fork Little Campbell Creek location showing the presence of human and waterfowl markers during base flow and following rain storms.

Discussion

Human Sources

The primary objective of this project was to determine whether fecal bacteria in Cottonwood Creek and Little Campbell Creek were directly or indirectly human caused. Results of this project support the conclusion that fecal bacteria from humans are present in Cottonwood Creek, North Fork Little Campbell Creek, and the lower South Fork Little Campbell Creek site following precipitation. Human-specific markers also were detected during base flow at the two upper South Fork Little Campbell Creek sites.

Human markers were found in all three streams; however, in Cottonwood Creek and the North Fork Little Campbell Creek, human markers were only found at downstream sites following precipitation, and no human markers were detected on spring sampling dates with high total fecal coliform counts. This supports the conclusion that human sources are not contributing to the bacterial load during spring runoff and are not likely due to septic or sewer system failure. It is still possible that septic systems could be a contributing factor in Cottonwood Creek, if subsurface flows during storm events are saturating and flushing bacteria from leaking tanks or drain fields. In Cottonwood Creek, high fecal coliform counts (>100 cfu/100 ml) were obtained during spring runoff at Marble Way, and during both June and the first August sampling date, at Marble Way and Surrey Road. On June 22 total fecal coliform counts were greater than 200 cfu/100 ml at these two sites; however no human markers were detected. Human-specific *Bacteroides* make up about 82% of the *Bacteroides* community in humans (Silkie and Nelson 2009). If *Bacteroides* from humans were present it is likely they would be detected in analyses.

In the North Fork Little Campbell Creek no human markers were found on most sampling dates even though total fecal coliform counts were high. Human markers were absent in samples collected from the downstream North Fork site (LCCNF02 near Old Seward Highway) in June, with total fecal coliform counts over 300 cfu/100 ml. Human markers were not found following precipitation prior to August 30 sampling at the upstream North Fork site even though total fecal coliform bacteria counts were 199 cfu/100 ml.

Human markers in Cottonwood Creek and the North Fork Little Campbell Creek were found only on the August 30 sampling date, which followed cumulative precipitation of more than 1.0 inch in the previous 3 days. This suggests that human feces are entering these streams in surface or shallow subsurface flow. The transportation of fecal coliform bacteria and human markers in surface flow is consistent with the relationships between total fecal coliform bacteria and environmental variables. Total fecal coliforms in Cottonwood Creek at Old Matanuska Road (the farthest upstream site) and North Fork Little Campbell Creek in Bicentennial Park (the farthest upstream site) were strongly correlated with precipitation (correlation coefficients near 0.9). This means that fecal coliforms at these sites were not present during spring runoff or base flows, but increased with precipitation in August. Total fecal coliform bacteria at the downstream sampling locations in these two streams (Marble Way and Surrey Road in Cottonwood Creek, and near the Old Seward Highway in the North Fork Little Campbell Creek) were not well correlated with precipitation because they also received inputs during spring runoff. Total fecal coliforms were, however, correlated with changes in specific conductivity, which decreased with increasing discharge or precipitation.

The surface transport of human feces into Cottonwood Creek and the North Fork Little Campbell Creek is unpleasant to imagine. Development adjacent to these streams is entirely residential (Cottonwood Creek) or a mix of residential and industrial (North Fork Little Campbell Creek) (see Surveys in Appendix C). However, one possible source was identified through stream surveys on Cottonwood Creek, where a camp was set up on an island within the stream complete with a “Honey Bucket” and toilet paper. Another possible source could be stored garbage containing diapers or other fecal material.

Human markers were found in the South Fork Little Campbell Creek at all sampling locations during base flow conditions and at the lower two sites, following the August rain storms. These results support the conclusion that fecal contamination is occurring through ground water sources, although at low concentrations, and from surface runoff. The most likely source of groundwater contamination is from septic systems which are still in use in the older subdivision within the upper drainage.

The conclusion that human contamination is occurring at low concentrations is based upon intermittent appearance in samples, and presence only during low discharge. Human markers were found at the upstream South Fork Little Campbell Creek sampling site (LCCSF01 near O’Malley Road) on one date that coincided with the lowest discharge and the highest total fecal coliform counts. This could be explained by consistent low numbers of bacteria that are diluted when discharge increases, and only appear intermittently in samples even when stream flow is low and concentrations are relatively high. This is supported by the strong negative correlation between total fecal coliform bacteria and discharge at this upstream South Fork Little Campbell Creek sampling location.

The evidence at the South Fork Little Campbell Creek site near Abbott Loop (LCCSF02 at Our Road) is less clear but still supports human contamination through groundwater sources. Human markers were found on June 24 and August 11. High total fecal coliform counts (>100 cfu/100 ml) were present on all June and August sampling dates. Similar to the discussion for LCCSF01, the results are best explained by low concentrations of human markers that did not appear during high flows due to dilution, did not appear on June 22 even though concentrations were increasing, due to their low abundance and probability of being collected in a 500 ml sample, but were present on June 24 and August 11 because of their persistence and high relative concentrations due to low discharge. The August 11 sampling date followed 0.25 inches of cumulative rainfall, which caused only a small increase in discharge. The small increase in discharge would not have diluted concentrations by very much, and fecal bacteria from humans at low concentration could still have shown up in samples. However, with larger increases in discharge following 1 inch of precipitation, further dilution without surface water inputs could explain the absence human markers from samples.

The downstream South Fork Little Campbell Creek site (LCCSF03) shows human markers during both base flow (June 24) and following initial, and then larger precipitation events. Similar to the discussion for LCCSF02, low concentrations of bacteria from human sources are present during base flows, entering between these two sites, or being transported from upstream sites. Concentrations of human markers are absent during spring runoff due to dilution by high flows. Unlike the other two sampling locations on this stream, human markers in August 30 samples, following over 1 inch of cumulative precipitation and increasing discharge suggests possible surface transport. However, it is possible, given the presence of human markers during previous low flows and the minor increase in flow following this precipitation event, that groundwater remained the predominate source, and that the change in flow did not result in dilution to the point that bacteria were not picked up in samples. While human markers were detected, relatively low abundance of human bacteria is supported by their occurrence in only 2 of the 5 replicate filters from the August 30 sample.

<u>Other Sources</u>

Species-specific markers for horses, dogs, and waterfowl were found in Cottonwood Creek; however, their presence is infrequent and cannot account for high total fecal coliform counts on other sampling dates. Horse markers were found on June 24 at the Old Matanuska Road site in one of five replicate filters from that sample. Total fecal coliform bacteria abundance was low on this date at 3 cfu/100 ml. We have observed tracks at the ATV crossing below the sampling site, which could be from stables located near Glenwood Road. A more probable source would be the use of horse manure as a fertilizer on lawns or gardens upstream. Dog markers were found in Marble Way samples collected on June 22. Dogs are common throughout the residential areas upstream and the presence of dog markers was expected. However, markers from dogs were not seen in Cottonwood Creek following rain storms.

Markers were detected at all three Cottonwood Creek sites on August 30, following precipitation. The surface transportation of fecal contaminants in storm flows to Cottonwood Creek is clearly occurring. Horse markers were present at Marble Way and Surrey Road on August 30. Stables and gardens are more common in this lower section of the creek and horse fecal material transported in surface flow is reasonable. Therefore, both humans and human related activities are contributing to the fecal contamination of Cottonwood Creek from Marble Way to Surrey Road due to surface transport of contaminants following storm events. The increase in fecal coliform bacteria due to storm flow in urban environments is well documented (Mallin et al. 2000, Mallin 2009). Feces from waterfowl were observed on substrate within or adjacent to the channel on surveys along the entire length of Cottonwood Creek. While ducks may congregate at the outlet of Wasilla Lake or be present on private ponds adjacent to Cottonwood Creek, it is impossible to differentiate human supported ducks from the natural waterfowl community.

Besides humans, dog and waterfowl markers were the only other sources of bacteria in the North and South Forks of Little Campbell Creek. The lack of horse markers in these two streams was surprising given the abundance of stables and trails. This may be due to the low percent of horse-specific *Bacteroides* markers in the *Bacteroides* community of horse feces, which is about 2% (Silkie and Nelson 2009). That is, only 2% of the *Bacteroides* found in horse feces are specific to horses. Dog markers were found only at the Abbott Loop site during low flow conditions. This is also surprising given the number of dogs we observed, particularly at the North Fork Little Campbell Creek site in Bicentennial Park. Species-specific *Bacteroides* make up about 6% of the *Bacteroides* community within dog feces (Silkie and Nelson 2009); therefore, species-specific markers for dogs could be missed even when dog feces are present. The lack of dog markers also may be due to the effectiveness of education programs (scoop the poop) implemented throughout the Anchorage area.

Relationship between Fecal Coliform Bacteria and *Bacteroides* Markers

Species-specific *Bacteroides* markers were not found during spring runoff sampling and on many other dates when total fecal coliforms were present and often abundant. This may be because for some species, *Bacteroides* may be less abundant than fecal coliforms and/or because fecal coliform bacteria are able to survive in the environment. *Bacteroides* markers were more likely to appear when fecal coliform bacteria abundance was high. Markers were detected in 8% of the samples collected when total fecal coliform counts were less than 20 cfu/100 ml. Detection increased to 21% when fecal coliform counts were between 20 and 100 cfu/100 ml, 34% when greater than 100 cfu/100 ml, and 63% of the time when counts were greater than 200 cfu/100 ml.

Bacteroides make up one third of the community within humans and outnumber coliforms (Holdeman *et al.*, 1976; Moore and Holdeman, 1974; Salyers, 1984 per Field et al. et al. 2003); however this may not be the case when species-specific *Bacteroides* are only 2 to 6% of the *Bacteroides* community (i.e. dogs and horses). In this case, it would require greater numbers of total fecal coliforms (based on the ratio of coliforms to host-specific *Bacteroides*) before species-specific markers could be detected. Alternatively, fecal coliform bacteria are able to live and possibly reproduce within the environment. Therefore, measures of total fecal coliform bacteria may exaggerate fecal contamination or indicate fecal contamination when it is not occurring. In this case, *Bacteroides* may be a more accurate indicator of fecal contamination.

The ability for fecal coliform bacteria to survive in the environment may explain the lack of markers in spring runoff samples. If fecal coliforms are surviving through the winter within fecal material under the snow they would appear in samples collected during spring runoff. *Bacteroides* are unable to survive in an aerobic environment for more than a few days, and cannot easily be grown under laboratory conditions (EPA 2005). Therefore, *Bacteroides* would unlikely survive in feces throughout the winter and would not be detected in spring samples. If this is the case, it should be assumed that sources detected in storm runoff samples are also present during spring runoff.

The sampling design and use of source tracking also helped focus treatment efforts toward surface or groundwater sources. Sampling effectively captured spring runoff and base flow events, but had limited success sampling during storm events due to the lack of intense fall storms that usually occur in September. However, there were distinct differences among sampling events in the presence of species specific markers. This allowed for differentiation between surface and subsurface routes of fecal contamination. For example in the North Fork Little Campbell Creek, the presence of human markers at the lower sampling location following precipitation should direct efforts toward the transport of fecal material in surface flows. This allows for limited resources to be effectively applied to the specific locations and routes of delivery to stream systems.

The objective of this project was to use source tracking to determine if fecal contamination is due to humans or human related activities, and this project objective was achieved. For example, the use of source tracking identified bacteria from humans and horses in Cottonwood Creek at

Marble Way and Surrey Road. This coincided with total fecal coliform counts of 76 to 121 cfu/100 ml. Therefore, humans and human related activities were contributing to the bacterial load on days when abundances exceeded water quality standards. However, source tracking cannot tell us the portion of the total load these sources are contributing. In addition, source tracking cannot tell us what is causing high fecal coliform counts when markers are absent. It may be due to other sources (i.e. wildlife) or, it could be that species-specific markers were present but not detected. Regardless of the limitations, source tracking has identified sources and locations within these streams where treatments should be directed.

Literature Cited

Davis, J. C., and G. A. Davis. 2005. Cottonwood Creek TMDL Development—Residue. Aquatic Restoration and Research Institute. Final Report for the Alaska Department of Environmental Conservation, ACWA 05-02. Talkeetna, Alaska.

Davis, J.C., and G.A. Davis. 2008. Cottonwood Creek fecal coliform and temperature evaluation. Final Report for the Alaska Department of Environmental Conservation. ACWA 08-01. Aquatic Restoration and Research Institute. Talkeetna, Alaska.

Davis, J.C., G.A. Davis, and L. Eldred. 2006. Cottonwood Creek Ecological Assessment. Aquatic Restoration and Research Institute. Final Report for the Alaska Department of Environmental Conservation. ACWA 06-02. Talkeetna, Alaska.

Doyle, JD, B Tunnicliff, R Kramer, R Kuehl, SK Brickler. 1992. Instability of fecal-coliform populations in waters and bottom sediments at recreational beaches in Arizona. Water Research 26 (7): 979-988.

Evanson, M., and R.F. Ambrose. 2006. Sources and growth dynamics of fecal indicator bacteria in a coastal wetland system and potential impacts to adjacent waters. Water Research 40: 475-486.

Field, K.G., A.E. Bernhard, and T.J. Brodeur. 2003. Molecular approaches to microbiological monitoring fecal source detection. Environmental Monitoring and Assessment 81:313-326.

Fries, J.S., G.W. Characklis, and R.T. Noble. 2006. Attachment of fecal indicator bacteria to particles in the Neuse river Estuary, N.C. Journal of Environmental Engineering:1338-1346.

Fries, J.S., G.W. Characklis, R.T. Noble. 2008. Sediment-water exchange of Vibrio sp. And fecal indicator bacteria: Implications for persistence and transport in the Neuse River Estuary, North Carolina, USA. Water Research 42: 941-950.

Holdeman, V., Cato, E. P., and Moore, W. E. C.: 1976, 'Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress', Appl. Environ. Microbiol. 31, 359–375.

Kuntz, R.L., P.G. hartel, D.G. Godfrey, J.L. McDonald, K.W. Gates, and W.I Segars. 2003. Targeted sampling protocol as prelude to bacterial source tracking with Enterococcus faecalis. Journal of Environmental Quality 32: 2311-2318.

Little Campbell Creek Watershed Management Plan. 2007. Downloaded on December 4 from http://wms.geonorth.com/library/.

Mallin, M.A. 2009. Comparative impacts of stormwater runoff on water quality of an urban, a suburban, and a rural stream. Environmental Monitoring and Assessment 159: 475.

Mallin, M.A., K.E. Williams, E.C. Esham, and R.P. Lowe (2000) Effect of Human Development on Bacteriological Water Quality in Coastal Watersheds. *Ecological Applications* 10(4): 1047-1056.

Mallin, MA, LB Cahoon, BR Toothman, DC Parsons, MR McIver, ML Ortwine, RN Harrington. 2007. Impacts of a raw sewage spill on water and sediment quality in an urbanized estuary. Marine Pollution Bulletin 54(1): 81-88.

Mauger, S, J.Davis, G. Davis, R. Ruffner, and M. Geist. 2010. Stream temperature monitoring network for Cook Inlet Salmon Streams. Cook Inletkeeper, Homer, Alaska. 36p.

Moore, W. E. C., and Holdeman, L. V.: 1974, 'Human fecal flora: the normal flora of 20 Japanese-Hawaiians', Appl. Microbiol. 27, 552–555.

Rantz, S. E., and others. 1982. Measurement and computation of streamflow--Volume 1. Measurement of stage and discharge. U.S. Geological Survey Water-Supply Paper 2175, 284p.

Salyers, A. A.: 1984, '*Bacteroides* of the human lower intestinal tract', Ann. Rev. Microbiol. 38, 293–313.

Scott, T.M., J.B. Rose, T.M. Jenkins, S.R. Farrah, and J. Lukasik. 2002. Microbial source tracking: current methodology and future directions. Applied and Envirnmental Microbiology 68(12): 2796-4803.

Shehane, S. D.; Harwood, V. J.; Whitlock, J. E.; Rose, J. B. 2005. The Influence of Rainfall on the Incidence of Microbial Faecal Indicators and the Dominant Sources of Faecal Pollution in a Florida River. J. Appl. Microbiol., 98, 1127.

Stedtfeld, R.D., D. Yurtsever, M. Duran, E.W. Alm, and S.A. Hashsham. 2006. Detection ad occurrence of indicator organisms and pathogens. Water Enviromental Research 70(10):1054-1077.

United States Department of Environmental Protection Agency. 2005. Microbial source tracking guide document. National Risk Management Research Laboratory, Office of Research Development. EPA/600/R-05/064.

Appendix A. Completed Field Data Sheets

Appendix B. Laboratory Reports

Appendix C. Stream Surveys

Appendix D. Quality Assurance Project Plan