Pederson Hill Creek
Water Quality Monitoring

May 2014 – June 2014

This Final Report was prepared by the Alaska Department of Environmental Conservation as continuation to the Alaska Clean Water Actions Grant # ACWA 13-07 (FY13).

Contents

1. Summary

Pederson Hill Creek (also known as Casa Del Sol Creek) in the Mendenhall Valley of Juneau, Alaska, is an anadromous stream (ADFG Catalog #111-50-10490-2013) and bordered by Glacier Highway and Engineers Cutoff Road. The creek drains from uplands and forested wetlands, through residential and commercial development into the Mendenhall Wildlife Refuge.

Pederson Hill Creek was first included on the State's 303(d) list of impaired waterbodies in 1990, based on non-attainment of the Alaska Water Quality Standards (WQS) for fecal coliform bacteria. Failing septic tanks were identified as the probable pollution source. An assessment was conducted in 2006 and a Total Maximum Daily Load (TMDL) was approved by the U.S. Environmental Protection Agency (USEPA) in 2008. The impaired stream length consists of 3.56 miles.

In 2010 and 2011, the City and Borough of Juneau (CBJ) extended the municipal sewage treatment system into the watershed. All properties on Glacier Highway that may have added to fecal coliform bacteria contamination in the watershed have since connected to the CBJ sewage treatment system and properly decommissioned their on-site septic tanks. However, the municipal sewer line was not extended onto Engineers Cutoff Road, and according to CBJ[1] there are no plans for an extension. The Engineers Cutoff Road area is serviced by individual septic systems and continues to negatively impact the west fork of Pederson Hill Creek.

This project continued the 2013 evaluation of current hydrologic and selected chemical and biological conditions of Pederson Hill Creek. Water quality sampling for fecal coliform bacteria and other water quality parameters took place from May 2014 through June 2014 during high and low flow events.

The Alaska Department of Environmental Conservation (ADEC)-approved ***Pederson Hill Creek Tier 2 Quality Assurance Project Plan for Water Quality Monitoring – Sampling and Analysis Activities*** (dated March 2013) was applied for these 2014 sampling activities. Samples were collected at five separate sample locations within the Pederson Hill Creek watershed over a period of 30 days from May 20 to June 16, 2014.

Based on 2013 and 2014 analytical tests, the contributors of fecal pollution in the northern portion of the watershed (monitoring station PHC-3) appear to be contributed by non-human sources (wildlife and upgradient horse farm/pastures), and in the central portion of the watershed (monitoring station PHC-5) by human sources from upgradient septic systems on Engineers Cutoff Road. A more comprehensive monitoring program throughout the watershed, including microbial source tracking, will verify this initial assessment.

[1] Personal communication with John Bohan, P.E., CBJ Chief CIP Engineer on October 21, 2013; reconfirmed with staff Autumn Sapp on August 7, 2015.

2. About Pederson Hill Creek

Pederson Hill Creek, located in the Mendenhall Valley, is approximately two miles long and contains roughly one mile of tidally influenced estuarine channels. The creek originates on the north side of Glacier Highway from springs at the base of a bedrock outcrop and discharges into the Mendenhall River. Pederson Hill Creek is an anadromous system that supports populations of coho, pink, and chum salmon, cutthroat trout, and Dolly Varden char. The creek runs through forested wetlands, hemlock forest, and developed areas. This watershed provides habitat for a variety of waterfowl, raptors, songbirds, as well as small and large mammals.

Pederson Hill Creek was first included on the State's 303(d) list of impaired waterbodies in 1990, based on non-attainment of the Alaska WQS for fecal coliform bacteria. Failing septic tanks were identified as the likely pollution source. At that time, the residential and commercial buildings within the Pederson Hill Creek watershed were not connected to the CBJ sewer system and relied on individual on-site septic systems. The Pederson Hill Creek watershed had a documented history of problems associated with failing septic systems. An assessment was conducted in 2006 and a TMDL was approved by the EPA in 2008 in order to help the waterbody meet Alaska WQS. The presence of fecal coliform bacteria can indicate an increased risk of pathogen contamination. The consumption of, or contact with pathogen contaminated water presents a health risk.

In 2010 and 2011, the CBJ extended the municipal sewage treatment system into the watershed. According to CBJ in August 2015, all but one property within 200 feet of the sewage treatment line have connected and properly decommissioned their on-site septic tanks. The property to date has not connected to the municipal line and still has an on-site septic tank; CBJ has issued a violation. The property is located on Glacier Highway north of Wilma Avenue. Based on the property location, topography, and analytical sample results (PHC-2), it is unlikely that fecal coliform bacteria from this property had/will have an environmental impact on Pederson Hill Creek. Sample site PHC-2 is situated downstream of the property. The analytical results show only one elevated level of fecal coliform bacteria in PHC-2; this sample was collected following a heavy rain event. The municipal sewer line was not extended onto Engineers Cutoff Road, which is located up- and cross-gradient of the west fork of Pederson Hill Creek. According to CBJ there are no plans in the foreseeable future for an extension.

The TMDL recommends educating area homeowners of the importance of the proper operation and maintenance (O&M) of their onsite septic systems as a primary best management practice (BMP). Site conditions in this area, specifically on Engineers Cutoff Road, are less than ideal for onsite wastewater treatment and disposal. Steep slopes, wetlands, shallow bedrock and high levels of precipitation are not suited to traditional septic systems. Most of the systems installed in this area are secondary treatment systems (i.e., aerobic treatment units) and rely on mechanical parts that require periodic O&M. Failure to follow the maintenance requirements often results in system failure and the release of improperly treated wastewater. On March 16, 2016, Alaska Department of Fish and Game (ADFG) reported a suspected household waste and sewage discharge into the west fork of Pederson Hill Creek (aka Casa Del Sol Creek).

The March 2016 ADFG memorandum is attached as Appendix A. Upon inspection, an outfall coming from a residence was creating a blueish-gray organic deposit smelling of fecal matter into the creek. ADEC compliance program conducted an inspection on March 29, and contacted the responsible party. Similar complaints and violations have occurred on Engineers Cutoff Road throughout the years, and have resulted in inspections and required compliance measures. In addition, outreach to educate area homeowners of the importance of proper storage and BMPs for animal manure has been undertaken by the Natural Resources Conservation Service (NRCS).

3. Pederson Hill Creek Monitoring Locations

Pederson Hill Creek is a lightly forested watershed comprised of mixed use areas including homes, industrial/construction development areas, temporary asphalt batch plant, horse farms and office complexes. The soil and vegetation are primarily forested wetland. Glacier Highway is perpendicular to the northern part of the Pederson Hill Creek watershed and contributes road run-off to the creek. Monitoring sites PHC-1, PHC-2 and PHC-3 are located near a residential and industrial development area while PHC-4 and PHC-5 are downstream of urbanized development and open wetland areas. Table 1 provides a site description for each monitoring location. The site location selection is based on the representativeness of the site as well as previous use for TMDL assessment, development, and implementation. Table 2 describes the rationale for developing each of the sampling/monitoring locations and the criteria for site selection.

Table 1. Monitoring Locations and Site Descriptions

Site ID	Latitude	Longitude	Site Description
PHC-A	58° 22' 36.44"N	134° 37' 14.74"W	Upstream of the background sample PHC-1.
PHC-1	58° 22' 33.96"N	134° 37' 13.80"W	Above development at the end of Hamilton Drive. The area has dense brush and many areas of stagnant flow.
PHC-2	58° 22' 25.32"N	134° 37' 20.6"W	Southeast side of Engineers Cut-off Road. The site is downstream from the confluence of two stems of Pederson Hill Creek in the wooded forest. Receives runoff from Glacier Hwy, Engineers Cutoff Road and a culvert that runs under Engineers Cutoff Road.
PHC-3	58° 22' 23.52"N	134° 37' 6.56"W	Outlet of a culvert located on the southwest side of Glacier Hwy. This culvert receives water runoff collected from both sides of Glacier Hwy and receives direct contribution from horse farms and pastures upgradient.

PHC-4	58° 22' 17.15"N	134° 37' 2.32"W	Culvert intersection adjacent to the northwest side of Sherwood Lane. Receives contribution from ditches along Sherwood Lane and parking lot and buildings located on Sherwood Lane.
PHC-5	58° 22' 12.72"N	134° 37' 7.75"W	Downstream of all the parking lots and building structures on Sherwood Lane, including a decommissioned septic field that serviced several office buildings in this area. Site is located underneath a bridge that crosses the stream, just upstream of a mitigation pond (Millers Pond).

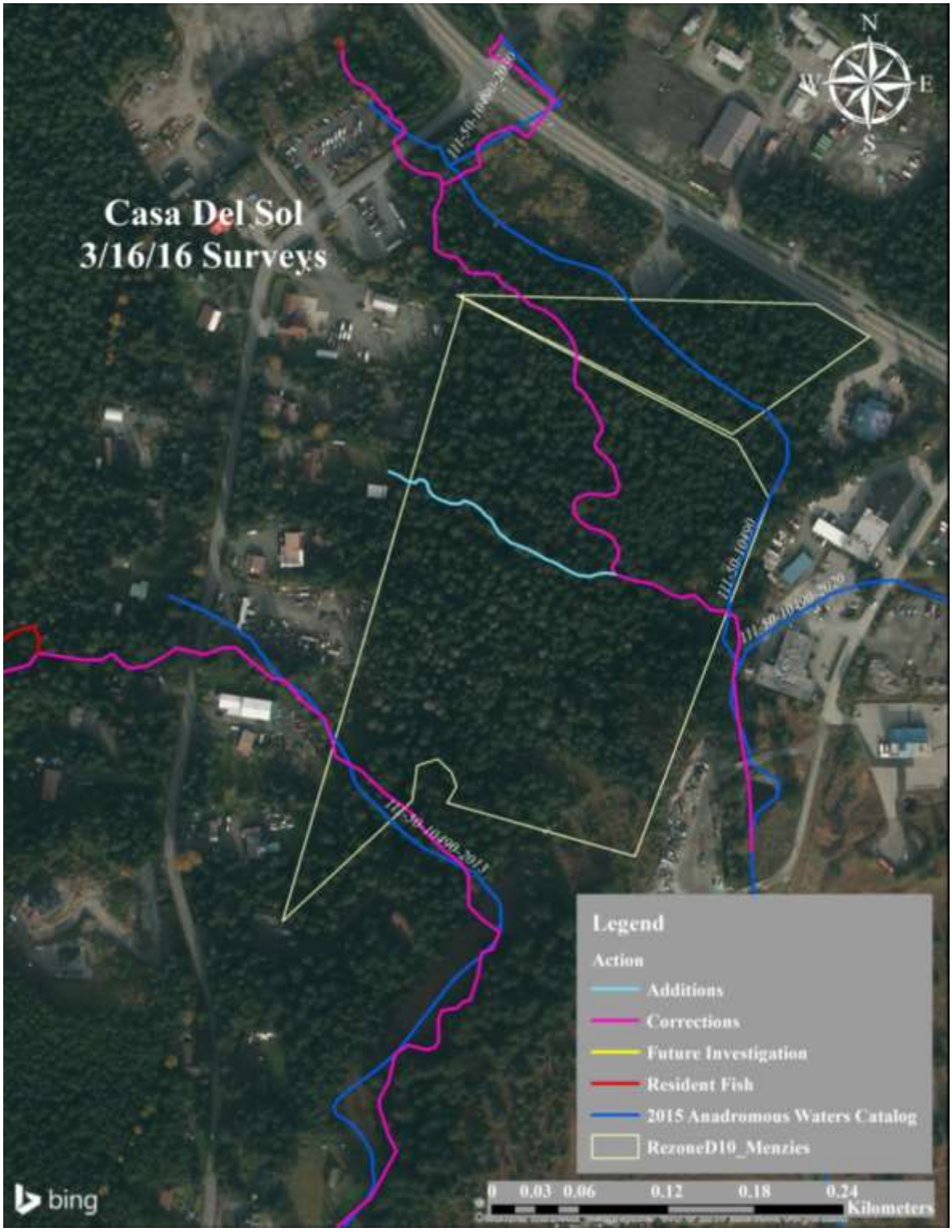
Table 2. Monitoring Site Rationale

Site ID	Monitoring Purpose	Measurements
PHC-A	Comparison with Alaska WQS and quality control	Background
PHC-1	Comparison with Alaska WQS	Background
PHC-2	Comparison with Alaska WQS	Nonpoint source and drainage ditch contribution
PHC-3	Comparison with Alaska WQS	Residential, drainage ditch and horse stable/farm contribution
PHC-4	Comparison with Alaska WQS	Residential, industrial, commercial and drainage ditch contribution
PHC-5	Comparison with Alaska WQS	Total contributions of PHC-1 – PHC-4

Figure 1 shows the aerial view of the Pederson Hill Creek watershed and the location of each monitoring site. Potential sources of pollution and runoff are also labeled on the map, including the location of a decommissioned septic tank, urbanized development and animal pastures. Figure 2 shows the updated stream information provided by ADFG during a March 2016 trip report resulting from a rezoning request application. The March 2016 ADFG memorandum is attached as Appendix A.


PHC-A
PHC-1
Figure 1
Pederson Hill Creek
2014 Monitoring Samples
Gravel Construction Site Entrance (left)
Engineers Cut-Off
PHC-2
Glacier Hwy
PHC-3
Sherwood Lane
Glacier Hwy
PHC-4
DMV
PHC-5
Legend
2014 monitoring sites
Pederson Hill Creek
Decommissioned Septic Tank Area
Horse Farm and Animal Pastures
0 15 30 60 90 120
Meters
N
Cartography by the Juneau Watershed Partnership 2013

Figure 2. Updated stream information and approximate parcel boundaries[2]



[2] State of Alaska Department of Fish and Game, Division of Habitat Memorandum (dated March 21, 2016).

4. Methods

Samples were collected for the Pederson Hill Creek project at five different sample locations (see Figure 1) within the Pederson Hill Creek watershed over a 30-day period. An additional one-time sample (PHC-A) was collected upstream from the PHC-1 background sample to provide laboratory quality control. All field data sheets and laboratory analytical reports are attached as Appendix B.

The first sample was collected on May 20, 2014 and the final sample was collected on June 16, 2014. The sampling period was chosen to meet the requirements of the TMDL for fecal coliform bacteria in the waters of Pederson Hill Creek, which was established for the achievement of water quality standards when a waterbody is water quality limited. The TMDL represents the amount of pollutant the waterbody can assimilate while maintaining compliance with applicable water quality standards. Applicable water quality standards for fecal coliform bacteria in Pederson Hill Creek establish water quality criteria for the protection of designated uses for water supply, water recreation, and growth and propagation of fish, shellfish, other aquatic life, and wildlife. The TMDL for Pederson Hill Creek was developed for the most stringent of guidelines - the fecal coliform bacteria criteria for drinking, culinary, and food processing water supply that state that "in a 30-day period, the geometric mean may not exceed 20 FC/100 mL, and not more than 10 percent of the samples may exceed 40 FC/100 mL (18 AAC 70 (1)(A)(i))."

Table 3. Alaska fresh water quality criteria for fecal coliform (18 AAC 70.020)

Designated use	**Description of criteria**
(2) Fecal coliform bacteria (FC), for fresh water uses (not applicable to groundwater)	
(A) Water supply	
(i) Drinking, culinary, and food processing	In a 30-day period, the geometric mean may not exceed 20 FC/100 ml, and not more than 10% of the samples may exceed 40 FC/100 ml.
(ii) Agriculture, including irrigation and stock watering	The geometric mean of samples taken in a 30-day period may not exceed 200 FC/100 ml, and not more than 10% of the samples may exceed 400 FC/100 ml. For products not normally cooked and for dairy sanitation of unpasteurized products, the criteria for drinking water supply, (2)(A)(i), apply.
(iii) Aquaculture	For products normally cooked, the geometric mean of samples taken in a 30-day period may not exceed 200 FC/100 ml, and not more than 10% of the samples may exceed 400 FC/100 ml. For products not normally cooked, the criteria for drinking water supply, (2)(A)(i), apply.

(iv) Industrial	Where worker contact is present, the geometric mean of samples taken in a 30-day period may not exceed 200 FC/100 ml, and not more than 10% of the samples may exceed 400 FC/100 ml.
(B) Water recreation	
(i) Contact recreation	In a 30-day period, the geometric mean of samples may not exceed 100 FC/100 ml, and not more than one sample, or more than 10% of the samples if there are more than 10 samples, may exceed 200 FC/100 ml.
(ii) Secondary recreation	In a 30-day period, the geometric mean of samples may not exceed 200 FC/100 ml, and not more than 10% of the total samples may exceed 400 FC/100 ml.
(C) Growth and propagation of fish, shellfish, other aquatic life, and wildlife	Not applicable.

Source: 18 AAC 70.020 (ADEC 2016)

Samples were collected to evaluate fecal coliform bacteria concentrations and to measure field parameters at the five sites on Pederson Hill Creek. Three samples were also collected for Microbial Source Tracking (MST)[3] analysis; these samples were selected based on the positive fecal coliform bacteria results. Each fecal coliform bacteria sample was collected using the grab method with a 120 ml bottle preserved with sodium thiosulfate, and each MST sample was collected into a 500 ml sterile polycarbonate Corning bottle, unpreserved. Only laboratory-supplied bottles were used for sample collection. Field parameter samples were collected separately in a 1000 ml high-density polyethylene (HDPE) sample bottle and were measured in-situ immediately after sample collection using a Hach 40d rugged portable multi-parameter meter. A field replicate was collected from one site per sampling period on a rotating schedule so that replicates were collected from each sampling site. Temperature blanks accompanied all coolers to ensure that samples remained within acceptable limits.

ADEC staff collected all samples by following Standard Operating Procedures as described in the *Pederson Hill Creek Tier II Quality Assurance Project Plan (QAPP) for Water Quality Monitoring, Sampling and Analysis Activities*. The water samples were collected with sterile sampling gloves and changed between each sampling site. Water samples were collected at approximately 1 inch (in shallow background sample locations) to 3-5 inches below the surface of the water to avoid collecting surface scum. During sampling at each location, notes were taken on observations of wildlife, weather, and potential sources of pollution.

Two laboratories were used for microbiological sample analysis for the Pederson Hill Creek project; Admiralty Environmental and Source Molecular. Admiralty Environmental, an ADEC-approved water quality laboratory in Juneau, performed analyses of fecal coliform bacteria colonies present in the samples.

[3] MST is a set of methods used to determine the host (different animals or Human) that contributes fecal pollution to a variety of waterbodies.

Admiralty Environmental provided all sampling bottles, materials, Chain of Custody (COC) forms, and coolers. After collection, samples were stored in a cooler between 1 and 10 degrees Celsius (°C) and were returned to the laboratory within six hours of collection. Sample analysis was started within eight hours of collection time, the standard holding period required by the ADEC's Division of Water. Laboratory staff at Admiralty Environmental checked each temperature blank upon receipt to ensure that samples were delivered less than 10 °C. All sample temperatures were within acceptable limits.

Source Molecular, an environmental laboratory in Miami, Florida that provides molecular and genetic microbial source tracking services for determination of sources of fecal pollution, provided sampling kits for each MST sampling event, which included sample bottles, cooler, ice packs, COC forms and shipping instructions. MST samples were packed in the cooler with gel ice and accompanying COC and temperature blank, and were shipped Fed Ex standard overnight to Source Molecular immediately after the project sample collection. The samples were stored at less than 6 °C until ADEC provided verbal instruction as to which samples to perform MST on.

Admiralty Environmental provided verbal fecal coliform bacteria results to ADEC immediately after the final fecal coliform bacteria result was determined at the laboratory. Based on these results, ADEC contacted Source Molecular and outlined which samples required MST testing, which target hosts to analyze for, and which samples needed to be discarded. Three samples were budgeted for MST analyses. Therefore, several sampling events had all of the MST samples discarded and none were analyzed for MST.

Field Forms and COC were scanned and placed in the project file. All field data was entered into the Alaska Water Quality Monitoring System (AWQMS) data template. Data was then reviewed for quality control and assurance by ADEC staff and uploaded to the AWQMS and EPA's STOrage and RETrieve Data Warehouse (STORET) databases.

5. Results

Five of the Pederson Hill Creek sampling sites were sampled five times during the month of May and June 2014 (see Table 3). An additional one-time sample (PHC-A) was collected upstream from the background sample (PHC-1) to provide laboratory quality control. Graphs for measured field parameters and laboratory analytical reports are listed in Appendix B.

Fecal Coliform

Presence of fecal coliform bacteria colonies ranged from non-detect (<2 FC/100 ml) to >6,000 FC/100 ml at the Pederson Hill Creek sampling sites, some of these samples failed to meet the Alaska water quality standards for fecal coliform bacteria. According to 18 AAC 70.040 and the final TMDL developed for Pederson Hill Creek in 2008, the most stringent water quality criteria applies to Pederson Hill Creek, which are drinking, culinary, and food processing water supply. The geometric mean and exceedance limits that the fecal coliform bacteria samples must meet for each sampling site are summarized in Table 4 below. Of the

five sites monitored, all of the sites exceeded the fecal coliform bacteria limits of the State of Alaska, stating that "not more than 10 percent of the samples may exceed 40 FC/100 mL (18 AAC 70 (1)(A)(i))." And four of the five sites monitored exceeded the geometric mean limit of 20 FC/100 ml, with the exception of Site PHC-2 which had a geometric mean result of 9.48 FC/100 ml. Table 3 shows the summary results.

Table 4. Summary of Fecal Coliform Bacteria results

Sampling location	Fecal Coliform Bacteria Results (FC/100 mL)			
	Total # of samples	Geomean [4]	# of samples > 40	% of samples > 40 [5]
PHC-A	1	NA	1	100%
PHC-1	5	**183.38**	5	100%
PHC-2	5	9.48	1	20%
PHC-3	5	**357.09**	4	80%
PHC-4	5	**22.02**	2	40%
PHC-5	5	**103.51**	3	60%

NA – not applicable

Southeast Alaska had cool and wet weather in May and June, with moderate to heavy precipitation on May 19, and June 7-9. The heavier the precipitation, the more elevated the fecal coliform bacteria was found in the samples. Specifically, May 19 with 0.45 inches of rain (>600 and 3,600 FC/100ml), and June 7, 8 and 9 with 0.34, 1.54 and 0.50 inches of rain (>600, 4,000, and >6,000 FC/100ml), respectively. Samples collected during periods of drier weather yielded fecal coliform bacteria results magnitudes lower (low to middle 100s FC/100ml). Specifically, 0.00 inches of rain on May 25-27 (range <2 to 450 FC/100ml), 0.18 inches in June 1-3 (range 6 to 480 FC/100ml), and 0.13 inches in June 14-16 (range 3.3 to 120 FC/100ml).

The final value for three site samples were reported as Too Numerous to Count (TNTC) or ">" by the analytical laboratory. Admiralty Environmental, the analytical laboratory, did not run a low enough dilution during the analysis method to produce a countable range of fecal coliform bacteria colonies, therefore producing the final result of TNTC or ">" value. Unofficially, the lab was able to estimate that the final count would be at least greater than a specific value, such as >6000 FC/100 ml. Sampling site PHC-3 yielded the highest overall sampling results for fecal coliform bacteria on May 20 and June 9, along with PHC-5 on June 9; see Table 5 for analytical results. These high fecal counts will be discussed below in the MST analysis discussion.

[4] Geomean may not exceed 20 FC/100ml

[5] Not > 10% of samples may exceed 40 FC/100ml

The background sample, PHC-1, had elevated levels of fecal coliform bacteria in all five of the samples, the entire sampling period. These levels ranged from 82 to >600 FC/100ml. To determine if there was a quality control issue with the analytical laboratory, an additional sample (PHC-A) was collected further upstream from the PHC-1 background sample. PHC-A was collected on June 9, 2014, and the results were similar to the background sample concluding the analytical laboratory procedures were adequate. The elevated background levels could be attributable to the limited water available for sample collection, and introduction of organic particles, or influenced by wildlife in the forested area.

Table 5. Fecal Coliform Bacteria and MST Bacteroidetes Results

Sample Locations	May 20	May 27	June 3	June 9	June 16
PHC-A	- [1]	-	-	**82** [2, 3]	-
PHC-1	**>600**	**450**	**100**	**64**	**120**
PHC-2	<2	4	10	**580**	3.3
PHC-3	**3,600** (no human, no horse) [4]	10	**480**	**>6,000** (no human)	**56**
PHC-4	20	<2	8.3	**>600**	**52**
PHC-5	**110** (human important contributor 9.69^{+03}/100ml water, no horse)	10	**27**	**4,000**	**100**

[1] **-** Denotes no sample was collected.
[2] Numeric values are fecal coliform bacteria in FC/100ml.
[3] Bolded samples indicate exceedence of Alaska WQS.
[4] Narrative description denotes the 3 samples selected and tested for MST analysis.

MST

Based on the positive fecal coliform bacteria results found in the samples, three samples were chosen for human and horse bacteroidetes ID analyses. Table 5 below depicts which samples had MST analyses requested, the host bacteria that were targeted, and the final results of the analyses. Of the three samples analyzed for a source bacteria present, only site PHC-5 (sampled May 20, 2014) was positive for human bacteroidetes ID, with a quantification count of (9.69 +03) 9,690 copy numbers per 100 mL of water.

The sample collected from site PHC-5 on May 20, 2014 was reported with 110 FC/100ml. The MST analyses was present for human bacteroidetes ID (noted as "important contributor") with a quantification

count of (9.69 +03) 9,690 copy numbers per 100 mL of water, and absent for horse bacteroidetes ID. Site PHC-5 is located downstream of the total watershed area sampled, with contributions from PHC-1 – PHC-4 (see Figure 2 stream addition in blue) and downstream from Engineers Cutoff Road which has no municipal sewer line and numerous on-site septic systems. There are also several polyvinyl chloride (PVC) and plastic pipes that have direct discharge into the creek. Several of these pipes run underground from the Department of Motor Vehicles (DMV) parking lot and the actual point of discharge from the pipe is located midway up the stream bank.

The other two samples selected for MST analyses were collected from site PHC-3. The May 20, 2014 sample reported fecal coliform bacteria at 3,600 FC/100ml. The MST analyses included human and horse bacteroidetes, but was absent for both bacteroidetes. The June 9, 2014 sample reported an estimated value of >6,000 FC/100ml. The MST analysis included human bacteroidetes, and the results were absent for human bacteroidetes. Site PHC-3 is located at a culvert receiving water runoff from both sides of Glacier Highway and direct contribution from an upgradient horse farm and pasture. Additional MST analyses were not performed due to budgetary reasons.

Field measurements

Field parameters were measured at each site in junction with the bacteria sample collection. In general, these field parameters confirmed typical, natural values. The graphs for each of these field parameters are available in Appendix C. Photographs of the sample sites during each of the five sampling events are shown in Appendix D.

Dissolved Oxygen (DO) values for sites PHC-1, PHC-2, PHC-4 and PHC-5 were all within the >7 mg/L water quality standard recommended for anadromous fish habitat. However, during four of the five sampling events, Site PHC-3 values fell below the >5 mg/L water quality standard required for healthy non-anadromous fish populations.

The pH values for all the sampled sites PHC-A, and PHC-1 through PHC-5 ranged between 6.5 and 8.5, the water quality standard set by the State of Alaska to support aquatic life.

Conductivity maintained a level trend during the May 20 through June 16, 2014 sampling program. An overall lower conductivity is seen on June 9th due to the heavy rain. The addition of freshwater (rain) with its inherently lower conductivity and the dilution of mineral concentrations from the heavier rains, results in lower creek water conductivity. The conductivity values ranged from 16.01 uS/cm to 816 uS/cm, all values which support aquatic life in freshwater streams.

Southeast Alaska had cooler weather in May and June (48° – 54° F), with an increase in temperature in mid-June (70° F). Moderate to heavy precipitation occurred in mid-May (0.45 inches of rain) and early-June (0.34, 1.54 and 0.50 inches of rain). Water temperatures reflected the precipitation events by remaining cooler with moderate to heavy rain, and warmer during light to no rain. This trend is also shown in the conductivity values collected for this project. All of the stream temperatures were less than 20° C, which is the Alaska Water Quality standard for waters available to support aquatic life.

Turbidity levels generally stayed steady throughout the sampling project, with the exception of two sampling events. During the sample collection on June 3, 2014 the water at Site PHC-1 was slightly elevated at 14.8 Nephelometric Turbidity Unite (NTU), and on June 16, 2014 the water at Site PHC-3 was slightly elevated at 12.4 NTU.

2013-2014 Summary

Based on 2013 and 2014 analytical tests, the contributors of fecal pollution at PHC-3 appear to be contributed by non-human sources (wildlife and upgradient horse farm/pastures), and at PHC-5 by human sources from upgradient septic systems on Engineers Cutoff Road. Table 6 displays 2013-2014 fecal coliform data, and Table 7 summarizes 2013-2014 both fecal coliform and MST data results. A more comprehensive monitoring program throughout the watershed, including microbial source tracking, will verify this initial assessment.

Please note that the PHC-3 sample collected on June 5, 2013 showed a fecal coliform result of >4,000 FC/100ml, and was selected for MST. This sample is significant because it exhibited a signal indicating the presence of the horse-associated Bacteroidetes gene, but the signal was below the laboratory's limit of detection. The sample was therefore classified as negative and required further sampling to draw definitive conclusions about the contributor(s) of fecal pollution. All field data sheets and laboratory analytical reports are attached as Appendix B.

Table 6. Fecal Coliform Bacteria Results Comparison 2013 and 2014

	2013 data					2014 data				
Sample Locations	June 5	June 12	June 19	June 24	June 26	May 20	May 27	June 3	June 9	June 16
PHC-A	- [1]	-	-	-	-	-	-	-	**82** [2, 3]	-
PHC-1	2	4	<2	<2	ND	**>600**	**450**	**100**	**64**	**120**
PHC-2	**50**	8.3	20	15	5	<2	4	10	**580**	3.3
PHC-3	**>4,000**	**220**	**146**	18	**150**	**3,600**	10	**480**	**>6,000**	**56**
PHC-4	**66**	**30**	18	5	18	20	<2	8.3	**>600**	**52**
PHC-5	**120**	**46**	**35**	**46**	**50**	**110**	10	**27**	**4,000**	**100**

[1] - Denotes no sample was collected.
[2] numeric values are fecal coliform bacteria in FC/100ml.
[3] Bolded samples indicate exceedence of Alaska WQS.

Table 7. Fecal Coliform Bacteria and MST Bacteroidetes Results

	2013/2014 MST Bacteroidetes Requests and Results for PHC-3 and PHC-5					
	PHC-3				**PHC-5**	
	6/5/13	6/12/13	5/20/14	6/9/14	6/5/13	5/20/14
Tests performed	Human Horse Dog Pig Bird Goose Chicken	Human Horse	Human Horse	Human	Human Horse	Human Horse
MST results	absent	absent	absent	absent	**Human (2.98^{+03}/100ml water)**	**Human (9.69^{+03}/100ml water)**
Fecal Coliform Bacteria results FC/100ml	>4,000	220	3,600	>6,000	120	110

[1] **Bolded** Host Species tested present (important contributor) for Bacteroidetes.
[2] Sample exhibited a signal indicating presence of the horse-associated Bacteroidetes gene, but signal was below laboratory's limit of detection.

6. Conclusions

Based on previous data collection, and the current, targeted 2013 and 2014 data, fecal coliform bacteria in Pederson Hill Creek waters continue to exceed water quality standards. Even with the installation of the municipal sewer system in 2010 and 2011, concentrations do not show a significant downward trend. The bacteria source(s) in the northern portion of the watershed (monitoring station PHC-3) appears to be associated with non-human sources (wildlife and upgradient horse farm/pastures), and in the central portion of the watershed (monitoring station PHC-5) contributions by human sources from upgradient septic systems on Engineers Cutoff Road. A more comprehensive monitoring program throughout the watershed, including microbial source tracking, will verify this assessment.

Efforts by ADEC and NRCS will continue to educate area homeowners on proper on-site septic system operation, and proper storage and BMPs for animal manure maintenance to reduce the potential migration of fecal bacteria contamination into adjacent wetlands and Pederson Hill Creek waters. ADEC will continue to respond to complaints about malfunctioning systems and will conduct compliance investigations when needed.

7. References

Alaska Department of Environmental Conservation. 2016. 18 AAC 70, Water Quality Standards. Amended as of February 19, 2016.

Ebert, J. 2013. Pederson Hill Creek Water Quality Monitoring. July 2, 2012 – June 30, 2013. Final Report for the Alaska Department of Environmental Conservation. ACWA 13-07. Juneau Watershed Partnership. Juneau, AK.

City and Borough of Juneau, Engineering Department. 2008. Juneau Sewer System, West Mendenhall Valley Sewer, Phase II – LID 98.

City and Borough of Juneau, Engineering Department. 2009. Juneau Sewer System, West Mendenhall Valley Sewer, Phase III – LID 130.

City and Borough of Juneau, Engineering Department. 2014. LID- 98 Phase II Permit records.

City and Borough of Juneau, Engineering Department. 2014. LID- 130 Phase III Permit records.

State of Alaska Department of Fish and Game, Division of Habitat Memorandum (dated March 21, 2016).

Appendix A – ADFG March 30, 2016 Memorandum

MEMORANDUM

State of Alaska

Department of Fish and Game
Division of Habitat

TO: Jackie Timothy
Southeast Regional Supervisor

DATE: March 30, 2016

FILE NO: 58.3710 N, 134.6256 W

SUBJECT: 111-50-10490-2013
Outfall

FROM: Greg Albrecht
Habitat Biologist

PHONE NO: (907) 465-6384

On March 16, 2016, Habitat Biologist Johnny Zutz and I visited the West Fork of Casa Del Sol Creek (Stream No. 111-50-10490-2013; cataloged for coho salmon and Dolly Varden char) to survey its upper extent for anadromous fish. In the fall, Johnny submitted a nomination to the Anadromous Waters Catalog for spawning chum salmon observed upstream of the Engineer's Cutoff Road.

About 40 feet above the Engineer's Cutoff Road culvert, we observed an outfall coming from a residence that was creating a blueish-grey deposit in the stream that smelled of fecal matter (Figures 1–3). The surrounding habitat was suitable for rearing and spawning fish. I reported the discharge to the Department of Environmental Conservation, who conducted a site visit on March 29 and made contact with the responsible party.

Figure 1.–Outfall observed in the West Fork of Casa Del Sol Creek.

Figure 2.–View towards origin of outfall.

Figure 3.–View culvert inlet under Engineer's Cutoff Road.

Email cc:

Al Ott, ADF&G Habitat, Fairbanks
Staff, ADF&G Habitat, Juneau
Dan Teske, ADF&G-SF, Juneau
Dave Harris, ADF&G-CF, Juneau
Stephanie Sell, ADF&G-WC, Juneau
Kevin Hanley, ADEC, Juneau
Kristi Asplund, ADEC, Juneau
Teri Camery, CBJ, Juneau
Ron King, CBJ, Juneau
Linda Speerstra, USACE, Sitka
Cindy Hartmann Moore, NMFS, Juneau
Steve Brockmann, USFWS, Juneau

Appendix B – Field Data Sheets and Laboratory Analytical Reports

May 20, 2014

Pederson Hill Creek Water Quality Field Data Sheet

Date: 5-20-14

Personnel: Gretchen Pikul

Weather: cloudy, light rain changed to mostly cloudy/partly sunny + no rain at 9:00 am.

Air temperature: 8.2°C 46.7°F

Sample ID	PHC-1	PHC-1
Date/Time	~~5-20-14~~ 7:18	5-20-14 12:43
Water temperature °C	8.3	11.2 10.8 avg 11.0
pH	7.22	7.10
Conductivity (µS/cm)	97.0	76.4
Dissolved Oxygen (mg/L)	11.12	9.92
Dissolved Oxygen (%)	94.9	90.9
Turbidity (NTU)	8.22	2.06

Sample ID	PHC-3
Date/Time	5-20-14 8:45
Water temperature °C	8.8
pH	6.61
Conductivity (µS/cm)	618
Dissolved Oxygen (mg/L)	3.47
Dissolved Oxygen (%)	29.9
Turbidity (NTU)	5.06

Sample ID	PHC-2
Date/Time	5-20-14 8:18
Water temperature °C	9.0 8.6 avg 8.8
pH	6.94
Conductivity (µS/cm)	186.4
Dissolved Oxygen (mg/L)	10.84
Dissolved Oxygen (%)	94.0
Turbidity (NTU)	4.24

Sample ID	PHC-4
Date/Time	5-20-14 9:02
Water temperature °C	9.0
pH	6.94
Conductivity (µS/cm)	~~210~~ 212
Dissolved Oxygen (mg/L)	8.97
Dissolved Oxygen (%)	77.8
Turbidity (NTU)	2.87

Sample ID	PHC-5 and Rep
Date/Time	5-20-14 9:35
Water temperature °C	9.8 9.6 avg 9.7
pH	7.10
Conductivity (µS/cm)	205.2
Dissolved Oxygen (mg/L)	10.69
Dissolved Oxygen (%)	94.3
Turbidity (NTU)	2.58

Sample ID	
Date/Time	
Water temperature °C	
pH	
Conductivity (µS/cm)	
Dissolved Oxygen (mg/L)	
Dissolved Oxygen (%)	
Turbidity (NTU)	

Calibrated multi-meter and turbidimeter on May 6, 2014. prior to any sampling.

Notes:

PHC-1 background sample location on Hamilton St.
* Resampled at 12:43 due to lack of sample size (<100 mL). Shallow water depth, difficult to fill sample bottle, low flow.

PHC-2 downstream of PHC confluence off intersection of Engineers Cutoff. good flow, sufficient water depth

PHC-3 downstream of culvert running under Glacier Hwy, downstream of Swampy Acres and runoff from hill. low flow, sufficient water depth.

PHC-4 intersection pool of culverts midway down Sherwood Lane, between 2 main bldgs along N edge of Hwy. medium flow, sufficient water depth.

PHC-5 upstream of bridge + mitigation pond, downstream of all bldgs, culverts, + all tributaries merge together. good flow, sufficient water depth.

Admiralty ENVIRONMENTAL 641 W. Willoughby Ave., Suite 301 Juneau, AK 99801 (907) 463-4415 (480) 247-4476 (f)

Gretchen Pikul
ADEC, Division of Water
410 Willoughby Ave., Ste. 303
Juneau, AK 99801

June 5, 2014

ADEC Division of Water Pedersen Hill Project

Date of Collection: May 20, 2014
Sampling Location: Juneau, AK

Summary

Samples from Pedersen Hill Creek were collected as grab samples between 08:18 and 09:35 hours on May 20, 2014 for the Pedersen Hill Creek Project. The samples were collected by Ms. Gretchen Pikul of ADEC's Division of Water.

Samples were each collected into a 120 ml sterile bottle preserved with sodium thiosulfate for the tests for fecal coliforms. All sampling and sample handling procedures were conducted in accordance with standard EPA guidelines for environmental sampling. The samples were hand-delivered to Admiralty Environmental, Juneau. All regulatory holding times were met for all tests. One sample was rejected as there was insufficient water in the bottle; that sample was re-collected by the client and the results will be sent in a separate report.

A complete report of the final lab results is enclosed. The official laboratory report follows this letter and includes the analytical results, case narrative, chain of custody form, and cooler receipt form.

Kindest Regards,

David Wetzel
Admiralty Environmental

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(907) 463 - 4415 Fax (480) 247 - 4476
www.admiraltyenvironmental.com

ADEC Division of Water

Pedersen Hill Creek

Analytical Report

May 20, 2014

Admiralty Environmental EPA ID AK 00976

Juneau, AK

AE 9872

Sample Location	Date & Time Sampled	Fecal Coliform (FC/100 ml)
PHC_2_052014	5/20/14, 08:18	< 2
PHC_3_052014	5/20/14, 08:45	3600
PHC_4_052014	5/20/14, 09:02	20
PHC_5_052014	5/20/14, 09:35	110
PHC_5Rep_052014	5/20/14, 09:35	82

Quality Control:

Analysis	MB	LCS	LCS Duplicate	RPD	Date/Time Commenced	Holding Time Met
FC	< 2	---	---	---	5/20/2014; 12:30	yes

Analysis Description:

Analysis	Method	MDL	PQL	Unit
FC	SM 9222D	1.0	2.0	FC/100ml

Case Narrative:

All sample collection and sample analysis QA/QC parameters were met for this event.

Key:

Enter.	Enterococci
FC	Fecal Coliform
LCS	Laboratory Control Standard
MB	Method Blank
MDL	Method Detection Limit
mg/L	Milligrams Per Liter
ND	Not Detected
PQL	Practical Quantitation Limit
RPD	Relative Percent Difference

David Wetzel
President, Admiralty Environmental
dwetzel@admiraltyenv.com

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CHAIN OF CUSTODY/TRANSMITTAL RECORD
PAGE 1 OF 1

CLIENT: State of Alaska DEC Division of Water

PROJECT: Pederson Hill Creek

AE 9872

REPORT TO: Gretchen Pikul

PHONE#: (907) 465-5023

ADDRESS: 410 Willoughby Ave Suite 303 Juneau, AK 99801

SAMPLED BY: Gretchen Pikul

EMAIL: gretchen.pikul@alaska.gov.

SAMPLES TAKEN IN THE PRESENCE OF: Please call email Gretchen at DEC with preliminary results after Final Readout

DATE	TIME	SITE DESCRIPTION /IDENTIFIER	MATRIX	# OF BOTTLES	Fecal Coliforms	pH	Temp	D.O.	
* ~~5-20-14~~	~~7:18~~	~~PHC-1_052014~~	~~H_2O~~	~~1~~	~~1~~	7.22	8.3	11.12	mg/L
5-2014	8:18	PHC-2_052014	H_2O	1	1	6.94	8.6	10.84	
5-20-14	8:45	PHC-3_052014	H_2O	1	1	6.61	8.8	3.47	
5-20-14	9:02	PHC-4_052014	H_2O	1	1	6.94	9.0	8.97	
5-20-14	9:35	PHC-5_052014	H_2O	1	1	7.10	9.8	10.69	
5-20-14	9:35	PHC-5Rep_052014	H_2O	1	1	7.10	9.8	10.69	↓

RELINQUISHED BY:	RECEIVED BY:	RELINQUISHED BY:	RECEIVED BY:
Signature: Gretchen Pikul	Signature: Jessica Wood	Signature	Signature
Printed Name: Gretchen Pikul	Printed Name: Jessica Wood	Printed Name	Printed Name
DATE: 5-20-14	DATE: 5/20/14	DATE	DATE
TIME: 10:47	TIME: 10:47	TIME	TIME

Temp/Loc: 5.07°C
Thermo ID#: SR#2
Condition of Custody Seals: ✓
Initialed By: JW
Shipped Via: —

Please *sample rejected. Filled below 100ml line. Client will resample today. [illegible]

- Results Reported verbally 5/21/14 @ 1210 [illegible]

Admiralty Environmental Cooler Receipt Form

Client: **Admiralty Environmental, LLC**
Project: **ADEC Water Division** AE# 9872

Date Opened: **5/20/2014** Opened by: **J. Wood**

A. External Cooler Conditions

• Local Sampling Event

1. Project ID: **Peterson Hill Creek**

2. COC Attached? **yes** Properly Completed? **yes** Signed by AE employee? **yes**

Small Temp. Blank **5.07** (temp in Celsius)
Large Temp. Blank: **N/A** (temp in Celsius)

• Air-Transported Sampling Event

1. Project ID: **N/A**

2. COC Attached? **N/A** Properly Completed? **N/A** Signed by AE employee? **N/A**
3. Airbill attached? **N/A** Airbill #: **N/A**
4. Custody Seals? **N/A**
5. Seals intact? **N/A**

Temp. Blank: N/A (temp in Celsius)

COMMENTS:

B. Sample Conditions

Number of Samples Received: **6** Packing type: **N/A**
Number of Bottles Received: **6**
1. Samples in proper bags? **yes**
2. Bottles intact? **yes**
3. Sufficient sample volume? **yes**
4. Labels agree with COC? **yes**
5. Samples delivered within holding time? **yes**

Problems encountered: **N/A**

Was the project manager called? **N/A**

COMMENTS:

Signature: Jessica Wood Date and time: 5/20/14 1107

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Gretchen Pikul
ADEC, Division of Water
410 Willoughby Ave., Ste. 303
Juneau, AK 99801

June 5, 2014

ADEC Division of Water Pedersen Hill Project

Date of Collection: May 20, 2014
Sampling Location: Juneau, AK

Summary

A sample from Pedersen Hill Creek was collected as grab sample at 12:43 hours on May 20, 2014 for the Pedersen Hill Creek Project. The sample was collected by Ms. Gretchen Pikul of ADEC's Division of Water.

The sample was collected into a 120 ml sterile bottle preserved with sodium thiosulfate for the tests for fecal coliforms. All sampling and sample handling procedures were conducted in accordance with standard EPA guidelines for environmental sampling. The sample was hand-delivered to Admiralty Environmental, Juneau. All regulatory holding times were met for all tests.

A complete report of the final lab results is enclosed. The official laboratory report follows this letter and includes the analytical results, case narrative, chain of custody form, and cooler receipt form.

Kindest Regards,

David Wetzel
Admiralty Environmental

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ADEC Division of Water

Pedersen Hill Creek

Analytical Report

May 20, 2014

Admiralty Environmental EPA ID AK 00976

Juneau, AK

AE 9873

Sample Location	Date & Time Sampled	Fecal Coliform (FC/100 ml)
PHC_1_052014	5/20/14, 12:43	> 600 (TNTC)

Quality Control:

Analysis	MB	LCS	LCS Duplicate	RPD	Date/Time Commenced	Holding Time Met
FC	< 2	---	---	---	5/20/2014; 13:45	yes

Analysis Description:

Analysis	Method	MDL	PQL	Unit
FC	SM 9222D	1.0	2.0	FC/100ml

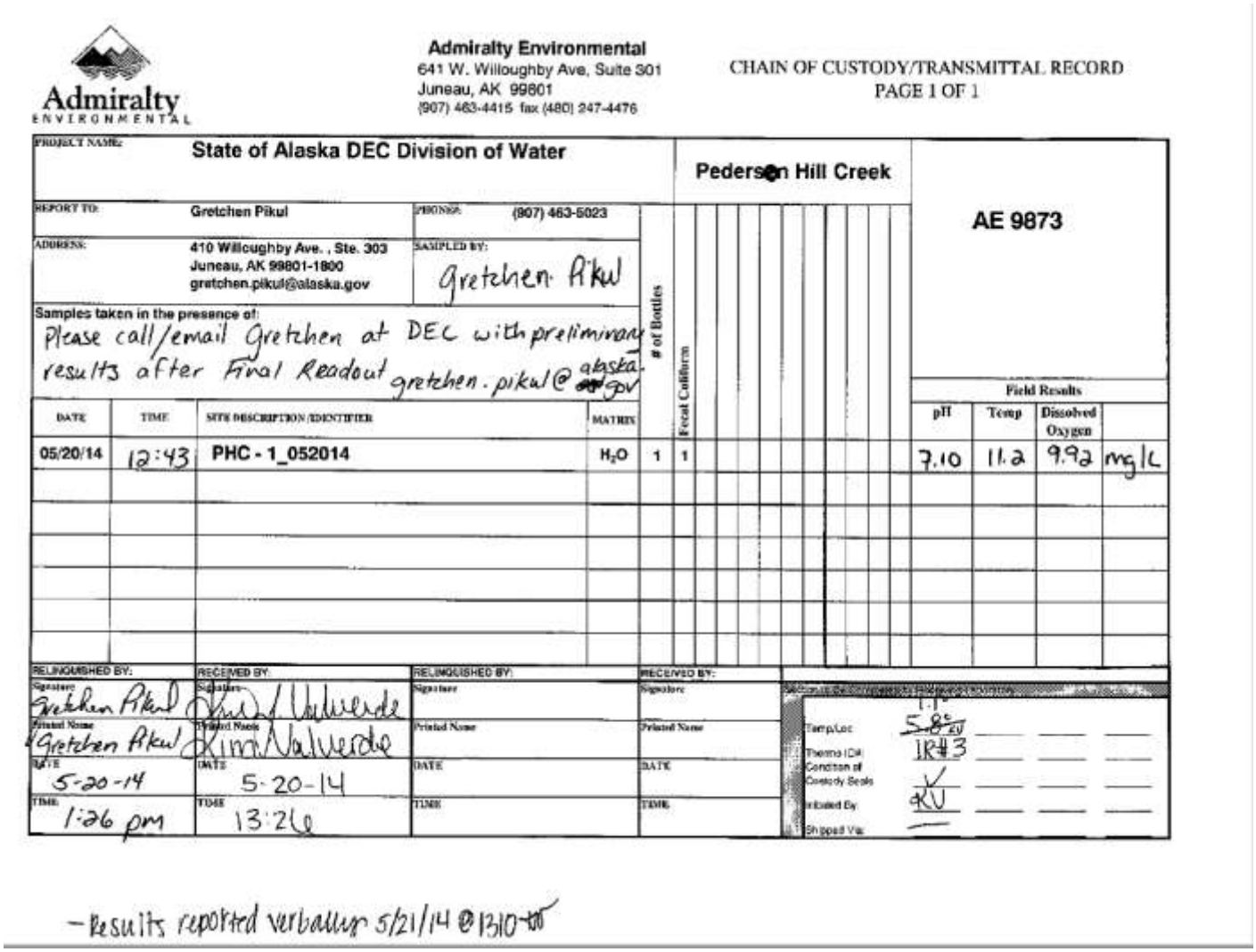
Case Narrative:

All sample collection and sample analysis QA/QC parameters were met for this event.

Key:

Enter.	Enterococci
FC	Fecal Coliform
LCS	Laboratory Control Standard
MB	Method Blank
MDL	Method Detection Limit
mg/L	Milligrams Per Liter
ND	Not Detected
PQL	Practical Quantitation Limit
RPD	Relative Percent Difference
TNTC	Too Numerous to Count

David Wetzel
President, Admiralty Environmental
dwetzel@admiraltyenv.com



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CHAIN OF CUSTODY/TRANSMITTAL RECORD
PAGE 1 OF 1

PROJECT NAME: **State of Alaska DEC Division of Water** — **Pederson Hill Creek** — **AE 9873**

REPORT TO: Gretchen Pikul — PHONE: (907) 463-5023

ADDRESS: 410 Willoughby Ave., Ste. 303, Juneau, AK 99801-1800, gretchen.pikul@alaska.gov — SAMPLED BY: Gretchen Pikul

Samples taken in the presence of:
Please call/email Gretchen at DEC with preliminary results after Final Readout gretchen.pikul@alaska.gov

DATE	TIME	SITE DESCRIPTION/IDENTIFIER	MATRIX	# of Bottles	Fecal Coliform	Field Results: pH	Temp	Dissolved Oxygen	
05/20/14	12:43	PHC - 1_052014	H_2O	1	1	7.10	11.2	9.92	mg/L

RELINQUISHED BY:	RECEIVED BY:	RELINQUISHED BY:	RECEIVED BY:
Signature: Gretchen Pikul	Signature: Kim Valverde	Signature	Signature
Printed Name: Gretchen Pikul	Printed Name: Kim Valverde	Printed Name	Printed Name
DATE: 5-20-14	DATE: 5-20-14	DATE	DATE
TIME: 1:26 pm	TIME: 13:26	TIME	TIME

Temp/Loc: 5.8°C IR#3
Thermo ID#:
Condition of Custody Seals: ✓
Initialed By: KV
Shipped Via:

- Results reported verbally 5/21/14 @ 1310 KV

Admiralty Environmental Cooler Receipt Form

Client: **Admiralty Environmental, LLC**
Project: **ADEC-Division of Water** AE# 9873

Date Opened: **5/20/2014** Opened by: **K.Valverde**

A. External Cooler Conditions

• Local Sampling Event

1. Project ID: **PHC-1**

2. COC Attached? **yes** Properly Completed? **yes** Signed by AE employee? **yes**

Small Temp. Blank **1.10** (temp in Celsius)
Large Temp. Blank: **N/A** (temp in Celsius)

• Air-Transported Sampling Event

1. Project ID: **N/A**

2. COC Attached? **N/A** Properly Completed? **N/A** Signed by AE employee? **N/A**
3. Airbill attached? **N/A** Airbill #: **N/A**
4. Custody Seals? **N/A**
5. Seals intact? **N/A**

Temp. Blank: N/A (temp in Celsius)

COMMENTS:

B. Sample Conditions

Number of Samples Received: **1** Packing type: **N/A**
Number of Bottles Received: **1**
1. Samples in proper bags? **yes**
2. Bottles intact? **yes**
3. Sufficient sample volume? **yes**
4. Labels agree with COC? **yes**
5. Samples delivered within holding time? **yes**

Problems encountered: **N/A**

Was the project manager called? **N/A**

COMMENTS:

Signature: K.J. Valverde Date and time: 5/20/14 13:24

Source Molecular
Leader in Microbial Source Tracking
4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-220-0379, Fax: (1) 786-513-2733, Email: info@sourcemolecular.com

Horse Fecal Toolbox ID™

Detection of the fecal Horse gene biomarker for Horse fecal contamination by quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: State of Alaska Department of Environmental Conservation
Date Received: May 21, 2014
Date Reported: June 4, 2014

SM #	Client #	Analysis Requested	DNA Analytical Results
SM-4E21046	PHC-3-052014	Horse Bacteroidetes ID	Absent
SM-4E21048	PHC-5-052014	Horse Bacteroidetes ID	Absent

Laboratory Comments

Negative Results
The results for all sample(s) were below the detection limits of the horse-associated Bacteroidetes gene real-time qPCR assay. The sample(s) were therefore classified as negative. It is important to note that a negative result does not mean that the sample does not definitely have horse fecal contamination. Only repeated sampling (both during wet and dry sampling events) will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Horse Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the horse-associated fecal genetic marker in the geographic region of interest. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **sourcemolecular.com/tests**.

DNA Analytical Method Explanation

Each submitted water sample was filtered through 0.45 micron membrane filters. Each filter was placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample was homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul containing sample extract, forward primer, reverse primer, probe and an optimized buffer. The following thermal cycling parameters were used: 95°C for 10 min and 40 cycles of 95°C for 15 s and 60°C for 1 min. All assays were run in duplicate.

For quality control purposes, a positive control consisting of horse fecal DNA and a negative control consisting of PCR-grade water, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives. The accumulation of PCR product is detected and graphed in an amplification plot. If the fecal indicator organism is absent in the sample, this accumulation is not detected and the sample is considered negative. If accumulation of PCR product is detected, the sample is considered positive.

Horse Bacteroidetes Theory Explanation

The phylum *Bacteroidetes* is composed of three large groups of bacteria with the best-known category being *Bacteroidaceae*. This family of gram-negative bacteria is found primarily in the intestinal tracts and mucous membranes of warm-blooded animals and is sometimes considered pathogenic.

Comprising *Bacteroidaceae* are the genus *Bacteroides* and *Prevotella*. The latter genus was originally classified within the former (i.e. *Bacteroides*), but since the 1990's it has been classified in a separate genus because of new chemical and biochemical findings. *Bacteroides* and *Prevotella* are gram-negative, anaerobic, rod-shaped bacteria that inhabitant of the oral, respiratory, intestinal, and urogenital cavities of humans, animals, and insects. They are sometimes pathogenic.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.[1] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*. Furthermore, these latter two organisms are facultative anaerobes and as such they can be problematic for monitoring purposes since it has been shown that they are able to proliferate in soil, sand and sediments.

The Horse Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[2,3,4,5,6] Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately detected in horses. Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found in horses.[2,6] As such, these bacterial strains can be used as indicators of horse fecal contamination.

One of the advantages of the Horse Bacteroidetes ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve. The absence of an amplification curve would indicate that the horse *Bacteroidetes* gene biomarker is not present.

References

[1] Scott, Troy M., Rose, Joan B., Jenkins, Tracie M., Farrah, Samuel R., Lukasik, Jerzy **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. (2002) 68: 5796-5803.

[2] Bernhard, A.E., and K.G. Field (2000a). **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Applied and Environmental Microbiology, 66: 1,587-1,594.

[3] Bernhard, A.E., and K.G. Field (2000b). **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Applied and Environmental Microbiology, 66: 4,571-4,574.

[4] Kreader, C.A. (1995). **Design and evaluation of Bacteroides DNA probes for the specific detection of human fecal pollution.** Applied and Environmental Microbiology, 61: 1,171-1,179.

[5] Fogarty, Lisa R., Voytek, Mary **A.Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species** Appl. Environ. Microbiol. 2005 71: 5999-6007.

[6] Dick, Linda K., Bernhard, Anne E., Brodeur, Timothy J., Santo Domingo, Jorge W., Simpson, Joyce M., Walters, Sarah P., Field, Katharine G.

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Preliminary Interpretation of Human Fecal Pollution ID™ Results

Detection and quantification of the fecal Human gene biomarker for Human fecal contamination by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: State of Alaska Department of Environmental Conservation
Date Received: May 21, 2014
Date Reported: June 4, 2014

SM #	Client #	Approximate Contribution of Human Fecal Pollution in Water Sample	Comment
SM-4E21041	PHC-3-052014	Negative	Negative for human fecal biomarker
SM-4E21043	PHC-5-052014	Important Contributor	Presence of human fecal biomarker

Source Molecular
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Human Fecal Pollution ID™ Quantification

Detection and quantification of the fecal Human gene biomarker for Human fecal contamination by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: State of Alaska Department of Environmental Conservation
Date Received: May 21, 2014
Date Reported: June 4, 2014

SM #	Client #	Analysis Requested	Species	Human Specific Marker Quantified*	DNA Analytical Results
SM-4E21041	PHC-3-052014	Human Bacteroidetes ID	Dorei	**<LOD	Absent
SM-4E21043	PHC-5-052014	Human Bacteroidetes ID	Dorei	9.69E+03	Present

*Numbers reported as copy numbers per 100 mL of water
**Below limit of detection (10 copy numbers per reaction)

Laboratory Comments

Submitter: State of Alaska Department of Env
Report Date: June 4, 2014

Negative Results
In sample(s) PHC-3-052014, the biomarker was either not detected or detected in quantities below the human-associated *Bacteroidetes* real-time qPCR assay analytical limit of detection. The sample(s) (were) therefore classified as negative. It is important to note that a negative result does not mean that the sample does not definitely have human fecal contamination. Only repeated sampling (both during wet and dry sampling events) will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.
In order to strengthen the result, a negative sample should be analyzed further for human fecal contamination with other DNA analytical tests. A list of human fecal ID tests can be found at **www.sourcemolecular.com/human**.

Positive Results
Sample(s) PHC-5-052014 tested positive for the human-associated Bacteroidetes gene biomarker(s) suggesting that human fecal contamination is present in the water sample(s). The biomarker(s) serve as an indicator of the targeted fecal pollution, but the absence of the biomarker does not signify conclusively the absence of that form of fecal pollution. Only repeated sampling (both during wet and dry sampling events) will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Human Fecal Reference Samples
The client is encouraged to submit samples from the surrounding wastewater facilities and/or septic systems in order to gain a better understanding of the concentration of the human-associated fecal Bacteroidetes genetic marker as well as the concentration of the general fecal Bacteroidetes genetic marker in the geographic region of interest. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
Quantitative results are reported along with interpretations. Interpretations are given as "negative", "trace", "minor contributor", "important contributor", or "major contributor" based on the concentration and proportion of the genetic markers found in the water samples.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **www.sourcemolecular.com/tests**

DNA Analytical Method Explanation

All reagents, chemicals and apparatuses were verified and inspected beforehand to ensure that no false negatives or positives could be generated. In that regard, positive and negative controls were run to attest the integrity of the analysis. All inspections and controls tested negative for possible extraneous contaminates, including PCR inhibitors.

Each submitted water sample was filtered through 0.45 micron membrane filters. Each filter was placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample was homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol.

Amplifications were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul containing sample extract, forward primer, reverse primer, probe and an optimized buffer. The following thermal cycling parameters were used: 50°C for 2 min, 95°C for 10 min and 40 cycles of 95°C for 15 s and 60°C for 1 min. All assays were run in duplicate. Absolute quantification was achieved by extrapolating genome copy numbers from standard curves generated from serial dilutions of Human specific and generic genomic DNA.

For quality control purposes, a positive control consisting of appropriate genomic DNA and a negative control consisting of PCR-grade water were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Human Bacteroidetes ID™ Species*: B. dorei*

The **Human Bacteroidetes ID™ Species*: B. dorei*** service targets the species *Bacteroides dorei*. *B. dorei* is an anaerobe that is frequently shed from the gastrointestinal tract and isolated from human feces worldwide. It is a newly discovered species that is widely distributed in the USA.[1,2] The human-associated marker DNA sequence is located on the 16S rRNA gene of *B. dorei*.[3] The marker is the microbial source tracking (MST) marker of choice for detecting human fecal pollution due to its exceptional sensitivity and specificity. Internal validations have been conducted on hundreds of sewage, septage, human and animal host fecal samples collected from throughout the U.S and archived in the Source Molecular fecal bank. The marker has also been evaluated in both inland and coastal waters. A recent, comprehensive, multi-laboratory MST method evaluation study, exploring the performance of current MST methods, concluded the *B. dorei* qPCR assay to be the top performing human-associated assay amongst those tested. The success and consistency of this marker in numerous studies around the world[1,3,4] makes the **Human Bacteroidetes ID™ Species*: B. dorei*** service the primary service for identifying human fecal pollution at Source Molecular.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.[5] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*.

The Human Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[3,5,6,7,8] Furthermore, certain strains of *Bacteroidetes* have been found to be associated with humans.[3,6] As such, these bacterial strains can be used as indicators of human fecal contamination.

Accuracy of the results is possible because the method amplifies DNA into a large number of small copies of the gene biomarker of interest. This is accomplished with small pieces of DNA called primers that are complementary and specific to the unique *B. dorei* DNA sequence. Through a heating process called thermal cycling, the double stranded DNA is denatured, hybridized to the complementary primers and amplified to create many copies of the DNA fragment desired. If the primers are successful in finding a site on the DNA fragment that is specific to the *B. dorei* DNA sequence, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve by the qPCR software. The absence of an amplification curve indicates that the *B. dorei* gene biomarker is not detected in the water sample because it is either not present or present at concentrations below the analytical detection limit.

To strengthen the validity of the results, additional tests targeting other high-ranking, human-associated *Bacteroidetes* species should be performed, such as
Human Bacteroidetes ID™ Species*: B. stercoris*,
Human Bacteroidetes ID™ Species*: B. fragilis*, and
Human Bacteroidetes ID™ Species*: B. thetaiotaomicron*.

[1]Boehm, A., Fuhrman, J., Mrse, R., Grant, S. **Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California**. Environ Sci Technol. 2003 37: 673–680.
[2]Bakir, M., Sakamoto, M., Kitahara, M., Matsumoto, M., Benno, Y. **Bacteroides dorei sp. nov., isolated from human faeces**. Int. J. Syst. Evol. Microbiol. 2006 56: 1639–1641.
[3] Bernhard, A., Field, K. **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA**. Appl. Environ. Microbiol. 2000b 66: 4571-4574.
[4]Ahmed, w., Masters, N., Toze, S. **Consistency in the host specificity and host sensitivity of the Bacteroides HF183 marker for sewage pollution tracking. Lett. Appl. Microbiol. 2012 55: 283-289.**
[5] Scott, T., Rose, J., Jenkins, T., Farrah, S., Lukasik, J. **Microbial Source Tracking: Current Methodology and Future Directions**. Appl. Environ. Microbiol. 2002 68: 5796-5803.
[6] Bernhard, A., Field, K. **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes**. Appl. Environ. Microbiol. 2000a 66: 1587-1594.
[7] Fogarty, L., Voytek, M. **A Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species**. Appl. Environ. Microbiol. 2005 71: 5999-6007.
[8] Dick, L., Bernhard, A., Brodeur, T., Santo Domingo, J., *et al.* **Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic**

Chain Of Custody Record

Source Molecular Corporation
Leaders in Genetic Microbial Source Tracking

SHIPPING ADDRESS:
4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

Company Name:	State of Alaska DEC Div. of Water
Contact Name(s):	Gretchen Pikul
E-mail Address(es):	gretchen.pikul@alaska.gov
Phone:	(907) 465-5023
Address:	410 Willoughby Ave. Suite 301
City/State/Zip:	Juneau, AK 99811
Country:	USA

Sample ID	Media	Sample Date	Sample Time	# of Containers	Analysis Requested (see pg. 2) Mark boxes with "X": MST (Human + Horse Bacteroides)	qPCR	ID	Quantification (Yes, No, If Positive)	Comments
PHC-1-052014	H_2O	05-20-14	7:18	1	X			Yes, if positive	Please HOLD samples until verbal confirmation of sample results for fecal coliforms.
PHC-2-052014	↓	↓	8:18	1	X			↓	
PHC-3-052014			8:45	1	X				Gretchen will call around 13:00 AKST on 5-21-14
PHC-4-052014			9:02	1	X				
PHC-5-052014	↓	↓	9:35	1	X				Do not filter and freeze until samples are chosen.

Completed by Client:

SAMPLES DELIVERED BY	Fed Ex Standard Overnight
SAMPLER NAME	Gretchen Pikul
SAMPLE SITE	Pederson Hill Creek
WITNESS	
SIGNATURE	

Relinquished 5-20-14 10:13 GPikul

Completed by Source Molecular:

SAMPLES RECEIVED BY	
RECEIVED DATE/TIME	
TEMPERATURE	
CONDITION	
SIGNATURE	

May 27, 2014

Pederson Hill Creek Water Quality Field Data Sheet

Date: 5-27-14

Personnel: Gretchen Pikul

Weather: cloudy, overcast, slight breeze

Air temperature: 11.9 °C 53.8°F

Sample ID	PHC-1
Date/Time	5-27-14 10:56
Water temperature °C	14.1 14.5 11.5 avg 13.4
pH	7.36
Conductivity (µS/cm)	62.1
Dissolved Oxygen (mg/L)	8.92
Dissolved Oxygen (%)	85.9
Turbidity (NTU)	6.81

Sample ID	PHC-3
Date/Time	5-27-14 11:52
Water temperature °C	10.6 11.5 12.5 avg 11.5
pH	6.51
Conductivity (µS/cm)	816
Dissolved Oxygen (mg/L)	2.02
Dissolved Oxygen (%)	17.9
Turbidity (NTU)	3.27

Sample ID	PHC-2
Date/Time	5-27-14 11:24
Water temperature °C	9.7 10.7 11.1 avg 10.5
pH	6.98
Conductivity (µS/cm)	206
Dissolved Oxygen (mg/L)	11.00
Dissolved Oxygen (%)	95.7
Turbidity (NTU)	4.79

Sample ID	PHC-4 and Rep
Date/Time	5-27-14 12:13
Water temperature °C	10.9 10.6 11.7 avg 11.1
pH	6.87
Conductivity (µS/cm)	286
Dissolved Oxygen (mg/L)	8.50
Dissolved Oxygen (%)	75.3
Turbidity (NTU)	2.79

Page 1 of 2

Sample ID	PHC-5
Date/Time	5-27-14 12:32
Water temperature °C	11.5 11.9 avg 12.6 14.3
pH	6.97
Conductivity (µS/cm)	214.2
Dissolved Oxygen (mg/L)	10.41
Dissolved Oxygen (%)	94.3
Turbidity (NTU)	1.64

Sample ID	
Date/Time	
Water temperature °C	
pH	
Conductivity (µS/cm)	
Dissolved Oxygen (mg/L)	
Dissolved Oxygen (%)	
Turbidity (NTU)	

Notes:

PHC-1 shallow water depth, low flow

PHC-2 good flow, sufficient water depth

PHC-3 low flow, sufficient water depth

PHC-4 medium flow, sufficient water depth

PHC-5 good flow, sufficient water depth

Calibrated multi-meter and turbidimeter prior to sampling.

previous day 5-26-14 - rain

Page 2 of 2

Admiralty ENVIRONMENTAL 641 W. Willoughby Ave., Suite 301 Juneau, AK 99801 (907) 463-4415 (480) 247-4476 (f)

Gretchen Pikul
ADEC, Division of Water
410 Willoughby Ave., Ste. 303
Juneau, AK 99801

June 6, 2014

ADEC Division of Water Pederson Hill Project

Date of Collection: May 27, 2014
Sampling Location: Juneau, AK

Summary

Samples from Pederson Hill Creek were collected as grab samples between 10:56 and 12:32 hours on May 27, 2014 for the Pederson Hill Creek Project. The samples were collected by Ms. Gretchen Pikul of ADEC's Division of Water.

Samples were each collected into a 120 ml sterile bottle preserved with sodium thiosulfate for the tests for fecal coliforms. All sampling and sample handling procedures were conducted in accordance with standard EPA guidelines for environmental sampling. The samples were hand-delivered to Admiralty Environmental, Juneau. All regulatory holding times were met for all tests.

A complete report of the final lab results is enclosed. The official laboratory report follows this letter and includes the analytical results, case narrative, chain of custody form, and cooler receipt form.

Kindest Regards,

David Wetzel
Admiralty Environmental

Admiralty ENVIRONMENTAL
641 W Willoughy Ave., Suite 301 Juneau, AK 99801
(907) 463 - 4415 Fax (480) 247 - 4476
www.admiraltyenvironmental.com

ADEC Division of Water

Pederson Hill Creek

Analytical Report

May 27, 2014

Admiralty Environmental EPA ID AK 00976

Juneau, AK

AE 9905

Sample Location	Date & Time Sampled	Fecal Coliform (FC/100 ml)
PHC-1_052714	5/27/2014; 10:56	450
PHC-2_052714	5/27/2014; 11:24	4
PHC-3_052714	5/27/2014; 11:52	10
PHC-4_052714	5/27/2014; 12:13	< 2
PHC-4Rep_052714	5/27/2014; 12:13	< 2
PHC-5_052714	5/27/2014; 12:32	10

Quality Control:

Analysis	MB	LCS	LCS Duplicate	RPD	Date/Time Commenced	Holding Time Met
FC	< 2	---	---	---	5/27/2014; 14:20	yes

Analysis Description:

Analysis	Method	MDL	PQL	Unit
FC	SM 9222D	1.0	2.0	FC/100ml

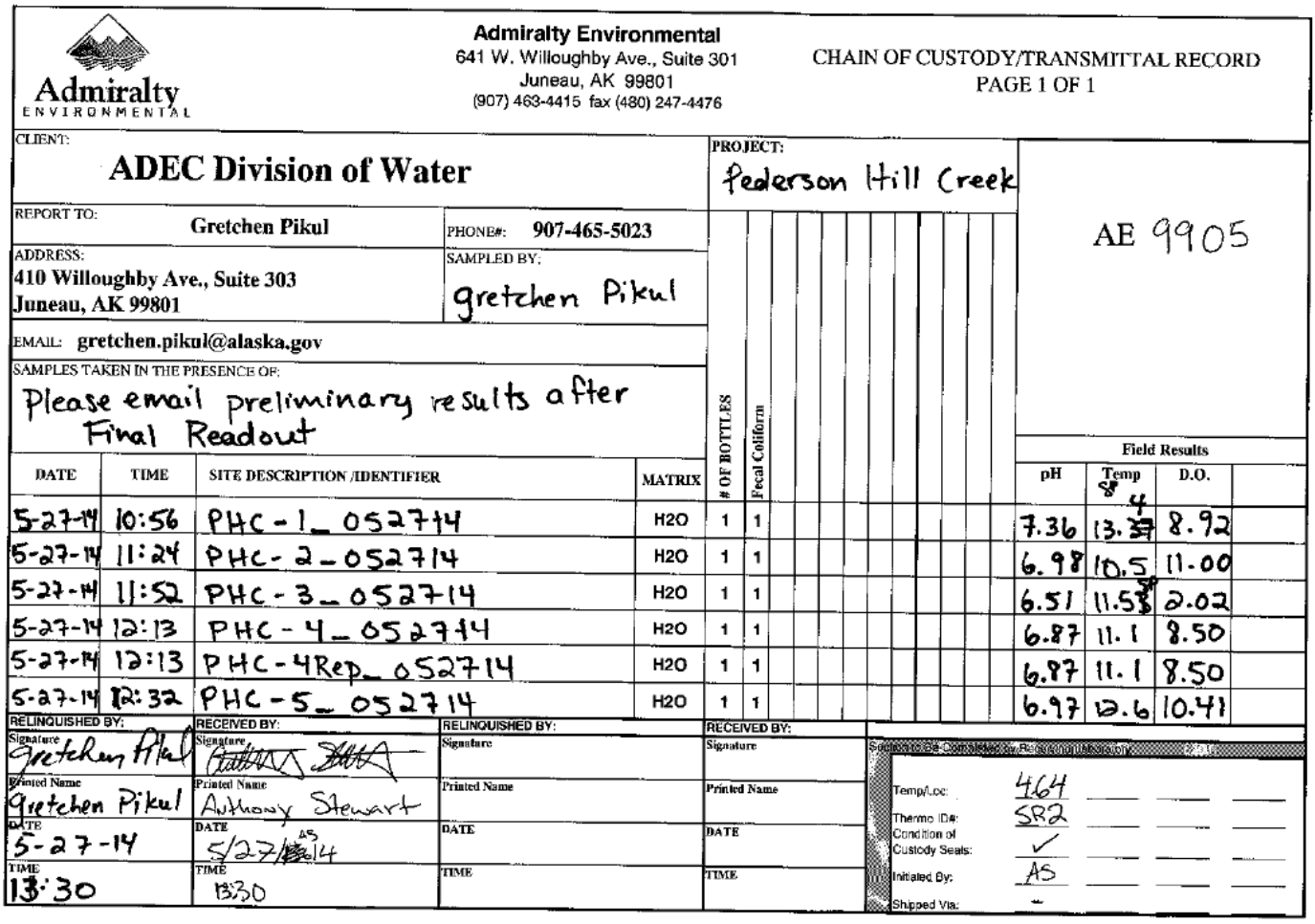
Case Narrative:

All sample collection and sample analysis QA/QC parameters were met for this event.

Key:

Enter.	Enterococci
FC	Fecal Coliform
LCS	Laboratory Control Standard
MB	Method Blank
MDL	Method Detection Limit
mg/L	Milligrams Per Liter
ND	Not Detected
PQL	Practical Quantitation Limit
RPD	Relative Percent Difference

David Wetzel
President, Admiralty Environmental
dwetzel@admiraltyenv.com



Admiralty ENVIRONMENTAL

Admiralty Environmental
641 W. Willoughby Ave., Suite 301
Juneau, AK 99801
(907) 463-4415 fax (480) 247-4476

CHAIN OF CUSTODY/TRANSMITTAL RECORD
PAGE 1 OF 1

CLIENT: **ADEC Division of Water**

PROJECT: Pederson Hill Creek

AE 9905

REPORT TO: Gretchen Pikul | PHONE#: 907-465-5023

ADDRESS: 410 Willoughby Ave., Suite 303, Juneau, AK 99801 | SAMPLED BY: Gretchen Pikul

EMAIL: gretchen.pikul@alaska.gov

SAMPLES TAKEN IN THE PRESENCE OF:
Please email preliminary results after Final Readout

DATE	TIME	SITE DESCRIPTION /IDENTIFIER	MATRIX	# OF BOTTLES	Fecal Coliform	Field Results pH	Temp °F	D.O.
5-27-14	10:56	PHC-1_052714	H2O	1	1	7.36	13.3 4	8.92
5-27-14	11:24	PHC-2_052714	H2O	1	1	6.98	10.5	11.00
5-27-14	11:52	PHC-3_052714	H2O	1	1	6.51	11.5	2.02
5-27-14	12:13	PHC-4_052714	H2O	1	1	6.87	11.1	8.50
5-27-14	12:13	PHC-4Rep_052714	H2O	1	1	6.87	11.1	8.50
5-27-14	12:32	PHC-5_052714	H2O	1	1	6.97	12.6	10.41

RELINQUISHED BY:	RECEIVED BY:	RELINQUISHED BY:	RECEIVED BY:
Signature: Gretchen Pikul	Signature: Anthony Stewart	Signature:	Signature:
Printed Name: Gretchen Pikul	Printed Name: Anthony Stewart	Printed Name:	Printed Name:
DATE: 5-27-14	DATE: 5/27/14 AS	DATE:	DATE:
TIME: 13:30	TIME: 1330	TIME:	TIME:

Temp/Loc: 464
Thermo ID#: SR2
Condition of Custody Seals: ✓
Initialed By: AS
Shipped Via: —

Admiralty Environmental Cooler Receipt Form

Client: **Admiralty Environmental, LLC**
Project: **ADEC Division** AE# 9905

Date Opened: **5/27/2014** Opened by: **A Stewart**

A. External Cooler Conditions

• Local Sampling Event

1. Project ID: **Pederson Hill Creek**

2. COC Attached? **yes** Properly Completed? **yes** Signed by AE employee? **yes**

Small Temp. Blank **4.64** (temp in Celsius)
Large Temp. Blank: **0.00** (temp in Celsius)

• Air-Transported Sampling Event

1. Project ID: **N/A**

2. COC Attached? **N/A** Properly Completed? **N/A** Signed by AE employee? **N/A**
3. Airbill attached? **N/A** Airbill #: **N/A**
4. Custody Seals? **N/A**
5. Seals intact? **N/A**

Temp. Blank: N/A (temp in Celsius)

COMMENTS:

B. Sample Conditions

Number of Samples Received: **6**
Number of Bottles Received: **6**
Packing type: **N/A**

1. Samples in proper bags? **yes**
2. Bottles intact? **yes**
3. Sufficient sample volume? **yes**
4. Labels agree with COC? **yes**
5. Samples delivered within holding time? **yes**

Preliminary email sent: 5/28, 16:30.

Problems encountered: **N/A**

Was the project manager called? **N/A**

COMMENTS:

DC

Signature: _____ Date and time: 5/27/1330

June 3, 2014

Pederson Hill Creek Water Quality Field Data Sheet

Date: 6-3-14

Personnel: Gretchen Pikul

Weather: rain, no wind

Air temperature: 11.3°C 52.2°F

Sample ID	PHC-1
Date/Time	6-3-14 11:14
Water temperature °C	13.4°C, 14.1°C, ~~10.0°C~~ gp, 11.9°C
pH	7.60
Conductivity (µS/cm)	61.7
Dissolved Oxygen (mg/L)	9.78
Dissolved Oxygen (%)	93.1
Turbidity (NTU)	14.8

13.1

Sample ID	PHC-3
Date/Time	6-3-14 11:59
Water temperature °C	10.1°C, 10.7°C, 10.8°C
pH	6.71
Conductivity (µS/cm)	735
Dissolved Oxygen (mg/L)	3.87
Dissolved Oxygen (%)	34.1
Turbidity (NTU)	2.91

10.5

Sample ID	PHC-2
Date/Time	6-3-14 11:41
Water temperature °C	10.1°C, 10.0°C, 11.3°C
pH	7.32
Conductivity (µS/cm)	205.7
Dissolved Oxygen (mg/L)	10.98
Dissolved Oxygen (%)	96.9
Turbidity (NTU)	4.20

10.5

Sample ID	PHC-4+Rep
Date/Time	6-3-14 12:16
Water temperature °C	10.3°C, 10.6°C, 10.7°C
pH	7.25
Conductivity (µS/cm)	219
Dissolved Oxygen (mg/L)	9.29
Dissolved Oxygen (%)	82.2
Turbidity (NTU)	2.9

10.5

10.8

Sample ID	PHC-5
Date/Time	6-3-14 12:34
Water temperature °C	11.0°C, 10.7°C, 10.7°C
pH	7.35
Conductivity (µS/cm)	194.3
Dissolved Oxygen (mg/L)	10.59
Dissolved Oxygen (%)	95.1
Turbidity (NTU)	2.37

Sample ID	
Date/Time	
Water temperature °C	
pH	
Conductivity (µS/cm)	
Dissolved Oxygen (mg/L)	
Dissolved Oxygen (%)	
Turbidity (NTU)	

Notes:

PHC-1 shallow water depth, low flow

PHC-2 sufficient water depth, good flow

PHC-3 sufficient water depth, low flow

PHC-4 sufficient water depth, medium flow

PHC-5 sufficient water depth, good flow

Calibrated multi-meter and turbidimeter prior to sampling.

previous day 6-2-14 - rain

Admiralty ENVIRONMENTAL 641 W. Willoughby Ave., Suite 301 Juneau, AK 99801 (907) 463-4415 (480) 247-4476 (f)

Gretchen Pikul
ADEC, Division of Water
410 Willoughby Ave., Ste. 303
Juneau, AK 99801

June 16, 2014

ADEC Division of Water Pederson Hill Project

Date of Collection: June 3, 2014
Sampling Location: Juneau, AK

Summary

Samples from Pederson Hill Creek were collected as grab samples between 11:14 and 12:34 hours on June 3, 2014 for the Pederson Hill Creek Project. The samples were collected by Ms. Gretchen Pikul of ADEC's Division of Water.

Samples were each collected into a 120 ml sterile bottle preserved with sodium thiosulfate for the tests for fecal coliforms. All sampling and sample handling procedures were conducted in accordance with standard EPA guidelines for environmental sampling. The samples were hand-delivered to Admiralty Environmental, Juneau. All regulatory holding times were met for all tests.

A complete report of the final lab results is enclosed. The official laboratory report follows this letter and includes the analytical results, case narrative, chain of custody form, and cooler receipt form.

Kindest Regards,

David Wetzel
Admiralty Environmental

Admiralty ENVIRONMENTAL
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www.admiraltyenvironmental.com

ADEC Division of Water

Pederson Hill Creek

Analytical Report

June 3, 2014

Admiralty Environmental EPA ID AK 00976

Juneau, AK

AE 9953

Sample Location	Date & Time Sampled	Fecal Coliform (FC/100 ml)
PHC-1_060314	6/3/2014; 11:14	100
PHC-2_060314	6/3/2014; 11:41	10
PHC-3_060314	6/3/2014; 11:59	480
PHC-4_060314	6/3/2014; 12:16	8.3
PHC-4Rep_060314	6/3/2014; 12:16	6
PHC-5_060314	6/3/2014; 12:34	27

Quality Control:

Analysis	MB	LCS	LCS Duplicate	RPD	Date/Time Commenced	Holding Time Met
FC	< 2	---	---	---	6/3/2014; 15:45	yes

Analysis Description:

Analysis	Method	MDL	PQL	Unit
FC	SM 9222D	1.0	2.0	FC/100ml

Case Narrative:

All sample collection and sample analysis QA/QC parameters were met for this event.

Key:

Enter.	Enterococci
FC	Fecal Coliform
LCS	Laboratory Control Standard
MB	Method Blank
MDL	Method Detection Limit
mg/L	Milligrams Per Liter
ND	Not Detected
PQL	Practical Quantitation Limit
RPD	Relative Percent Difference

David Wetzel
President, Admiralty Environmental
dwetzel@admiraltyenv.com

Admiralty ENVIRONMENTAL

Admiralty Environmental
641 W. Willoughby Ave., Suite 301
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(907) 463-4415 fax (480) 247-4476

CHAIN OF CUSTODY/TRANSMITTAL RECORD
PAGE 1 OF 1

CLIENT: **ADEC Division of Water**

PROJECT: Pederson Hill Creek

AE 9953

REPORT TO: Gretchen Pikul | PHONE#: 907-465-5023

ADDRESS: 410 Willoughby Ave., Suite 303, Juneau, AK 99801

SAMPLED BY: Gretchen Pikul

EMAIL: gretchen.pikul@alaska.gov

SAMPLES TAKEN IN THE PRESENCE OF:
Please email Gretchen with preliminary results after final read-out

DATE	TIME	SITE DESCRIPTION /IDENTIFIER	MATRIX	# OF BOTTLES	Fecal Coliform	Field Results pH	Temp °C	D.O.	
6-3-14	11:14	PHC-1_060314	H2O	1	1	7.60	13.1	9.78	mg/L
	11:41	PHC-2_060314	H2O	1	1	7.32	10.5	10.98	
	11:59	PHC-3_060314	H2O	1	1	6.71	10.5	3.87	
	12:16	PHC-4_060314	H2O	1	1	7.25	10.5	9.29	
	12:16	PHC-4 Rep_060314	H2O	1	1	7.25	10.5	9.29	
	12:34	PHC-5_060314	H2O	1	1	7.35	10.8	10.59	

RELINQUISHED BY:	RECEIVED BY:	RELINQUISHED BY:	RECEIVED BY:
Signature: Gretchen Pikul	Signature:	Signature:	Signature:
Printed Name: Gretchen Pikul	Printed Name: Anthony Stewart	Printed Name:	Printed Name:
DATE: 6-3-14	DATE: 6/3/14	DATE:	DATE:
TIME: 1:51	TIME: 13:51	TIME:	TIME:

Sample Receipt Verified by Shipping Laboratory

Temp/Loc:	5.8°C			
Thermo ID#:	SR2			
Condition of Custody Seals:	✓			
Initialed By:	AS			
Shipped Via:	–			

Admiralty Environmental Cooler Receipt Form

Client: **Admiralty Environmental, LLC**
Project: **ADEC Division of Water** AE# 9953

Date Opened: **6/3/2014** Opened by: **A. Stewart**

A. External Cooler Conditions

• Local Sampling Event

1. Project ID: **Peterson Hill Creek**

2. COC Attached? **Yes** Properly Completed? **Yes** Signed by AE employee? **Yes**

Small Temp. Blank **N/A** (temp in Celsius)
Large Temp. Blank: **5.89** (temp in Celsius)

• Air-Transported Sampling Event

1. Project ID: **N/A**

2. COC Attached? **N/A** Properly Completed? **N/A** Signed by AE employee? **N/A**
3. Airbill attached? **N/A** Airbill #: **N/A**
4. Custody Seals? **N/A**
5. Seals intact? **N/A**

Temp. Blank: N/A **(temp in Celsius)**

COMMENTS:

B. Sample Conditions

Number of Samples Received: 6 Packing type: **N/A**
Number of Bottles Received: 6
1. Samples in proper bags? **yes**
2. Bottles intact? **yes**
3. Sufficient sample volume? **yes**
4. Labels agree with COC? **yes**
5. Samples delivered within holding time? **yes**

Problems encountered: **N/A**

Was the project manager called? **N/A**

COMMENTS:

Signature: _____ Date and time: 6/3/14 13:51

June 9, 2014

Pederson Hill Creek Water Quality Field Data Sheet

Date: 6-9-14

Personnel: Gretchen Pikul

Weather: rain

Air temperature: 12.6°C, 54.2°F

Sample ID	PHC-A
Date/Time	6-9-14 8:03
Water temperature °C	9.0°C, 9.0°C,
pH	7.24
Conductivity (µS/cm)	16.01
Dissolved Oxygen (mg/L)	11.32
Dissolved Oxygen (%)	101.0 *
Turbidity (NTU)	0.68

Sample ID	PHC2 + Rep
Date/Time	6-9-14 9:03
Water temperature °C	9.5°C 12.6°C, 9.6°C, 10.6°C 9.9
pH	~~8.00~~ 7.17
Conductivity (µS/cm)	86.9
Dissolved Oxygen (mg/L)	11.55
Dissolved Oxygen (%)	100.9
Turbidity (NTU)	5.49

Sample ID	PHC-1
Date/Time	6-9-14 8:39
Water temperature °C	10.2°C, 10.6°C, 9.7°C 10.2
pH	7.29
Conductivity (µS/cm)	50.6
Dissolved Oxygen (mg/L)	11.05
Dissolved Oxygen (%)	98.0
Turbidity (NTU)	2.59

Sample ID	PHC-3
Date/Time	6-9-14 9:27 11.3
Water temperature °C	11.1°C, 11.1°C, 11.6°C
pH	7.00
Conductivity (µS/cm)	316
Dissolved Oxygen (mg/L)	9.32
Dissolved Oxygen (%)	84.4
Turbidity (NTU)	~~5.85~~ 3.85

Multi-meter and turbidimeter calibrated prior to sampling.

* DO probe calibration showed L-? even after calibration with result of 100.0% and meter display "calibration passed."

Sample ID	PHC-4
Date/Time	6-9-14 9:45
Water temperature °C	10.2°C, 9.7°C, 10.2°C
pH	7.26
Conductivity (µS/cm)	55.5
Dissolved Oxygen (mg/L)	9.90
Dissolved Oxygen (%)	87.6
Turbidity (NTU)	2.02

10.0

Sample ID	PHC-5
Date/Time	6-9-14 10:07
Water temperature °C	10.6°C, 10.5°C, 9.8°C, ~~10.0~~ 9P
pH	7.14
Conductivity (µS/cm)	63.4
Dissolved Oxygen (mg/L)	10,51
Dissolved Oxygen (%)	93.8
Turbidity (NTU)	3.94

10.1

Prior 48 hours heavy rain; many areas with high water.

Notes:

PHC-A upstream of background sample PHC-1. good flow, sufficient water sample depth.

PHC-1 good flow, sufficient water sample depth.

PHC-2 heavy flow, sufficient water sample depth. strong high water

PHC-3 moderate flow, sufficient water sample depth.

PHC-4 good-strong flow, high water, sufficient water sample depth.

PHC-5 good-strong flow, high water level, sufficient water sample depth.

pH probe took several minutes longer to read on PHC-A, PHC-1; quick on rest of samples.

Admiralty ENVIRONMENTAL 641 W. Willoughby Ave., Suite 301 Juneau, AK 99801 (907) 463-4415 (480) 247-4476 (f)

Gretchen Pikul
ADEC, Division of Water
410 Willoughby Ave., Ste. 303
Juneau, AK 99801

June 27, 2014

ADEC Division of Water Pederson Hill Project

Date of Collection: June 9, 2014
Sampling Location: Juneau, AK

Summary

Samples from Pederson Hill Creek were collected as grab samples between 08:03 and 10:07 hours on June 9, 2014 for the Pederson Hill Creek Project. The samples were collected by Ms. Gretchen Pikul of ADEC's Division of Water.

Samples were each collected into a 120 ml sterile bottle preserved with sodium thiosulfate for the tests for fecal coliforms. All sampling and sample handling procedures were conducted in accordance with standard EPA guidelines for environmental sampling. The samples were hand-delivered to Admiralty Environmental, Juneau. All regulatory holding times were met for all tests.

A complete report of the final lab results is enclosed. The official laboratory report follows this letter and includes the analytical results, case narrative, chain of custody form, and cooler receipt form.

Kindest Regards,

David Wetzel
Admiralty Environmental

Admiralty ENVIRONMENTAL
641 W Willoughy Ave., Suite 301 Juneau, AK 99801
(907) 463 - 4415 Fax (480) 247 - 4476
www.admiraltyenvironmental.com

ADEC Division of Water

Pederson Hill Creek

Analytical Report

June 9, 2014

Admiralty Environmental EPA ID AK 00976

Juneau, AK

AE 9988

Sample Location	Date & Time Sampled	Fecal Coliform (FC/100 ml)
PHC-A_060914	6/9/2014; 08:03	82
PHC-1_060914	6/9/2014; 08:39	64
PHC-2_060914	6/9/2014; 09:03	580
PHC-2Rep_060914	6/9/2014; 09:03	450
PHC-3_060914	6/9/2014; 09:27	>6,000
PHC-4_060914	6/9/2014; 09:45	>600
PHC-5_060915	6/9/2014; 10:07	4,000

Quality Control:

Analysis	MB	LCS	LCS Duplicate	RPD	Date/Time Commenced	Holding Time Met
FC	< 2	---	---	---	6/9/2014; 12:30	yes

Analysis Description:

Analysis	Method	MDL	PQL	Unit
FC	SM 9222D	1.0	2.0	FC/100ml

Case Narrative:

All sample collection and sample analysis QA/QC parameters were met for this event.

Key:

Enter.	Enterococci
FC	Fecal Coliform
LCS	Laboratory Control Standard
MB	Method Blank
MDL	Method Detection Limit
mg/L	Milligrams Per Liter
ND	Not Detected
PQL	Practical Quantitation Limit
RPD	Relative Percent Difference

David Wetzel
President, Admiralty Environmental
dwetzel@admiraltyenv.com

Admiralty ENVIRONMENTAL

Admiralty Environmental
641 W. Willoughby Ave., Suite 301
Juneau, AK 99801
(907) 463-4415 fax (480) 247-4476

CHAIN OF CUSTODY/TRANSMITTAL RECORD
PAGE 1 OF 1

CLIENT: **ADEC Division of Water**

PROJECT: Pederson Hill Creek

AE 9988

REPORT TO: Gretchen Pikul | PHONE#: 907-465-5023

ADDRESS: 410 Willoughby Ave., Suite 303, Juneau, AK 99801 | SAMPLED BY: gretchen Pikul

EMAIL: gretchen.pikul@alaska.gov

SAMPLES TAKEN IN THE PRESENCE OF:
* Please call/email Gretchen at DEC with
* preliminary results after Final Readout.
*

DATE	TIME	SITE DESCRIPTION /IDENTIFIER	MATRIX	# OF BOTTLES	Fecal Coliform	Field Results pH	Temp	D.O.	
6-9-14	8:03	PHC-A_060914	H2O	1	1	7.24	9.0	11.32	mg/L
	8:39	PHC-1_060914	H2O	1	1	7.29	10.2	11.05	
	9:03	PHC-2_060914	H2O	1	1	7.17	9.9	11.55	
	9:03	PHC-2Rep_060914	H2O	1	1	7.00	11.3	9.32	
	9:27	PHC-3_060914	H2O	1	1	7.08	11.3	9.32	
	9:45	PHC-4_060914	H2O	1	1	7.26	10.0	9.90	
	10:07	PHC-5_060914	H2O	1	1	7.14	10.1	10.51	

RELINQUISHED BY:	RECEIVED BY:	RELINQUISHED BY:	RECEIVED BY:
Signature: Gretchen Pikul	Signature: Jessica Wood	Signature:	Signature:
Printed Name: Gretchen Pikul	Printed Name: Jessica Wood	Printed Name:	Printed Name:
DATE: 06-09-14	DATE: 6/9/14	DATE:	DATE:
TIME: 11:10	TIME: 11:13	TIME:	TIME:

Relinquished by Courier to Receiving Laboratory

Temp/Loc: 4.34°C
Thermo ID#: #2
Condition of Custody Seals: V
Initialed By: JW
Shipped Via: —

Admiralty Environmental Cooler Receipt Form

Client: **Admiralty Environmental, LLC**
Project: **ADEC Division of Water** AE# 9988

Date Opened: **6/9/2014** Opened by: **J. Wood**

A. External Cooler Conditions

• Local Sampling Event

1. Project ID: **Pederson Hill Creek**

2. COC Attached? **yes** Properly Completed? **yes** Signed by AE employee? **yes**

Small Temp. Blank **4.34** (temp in Celsius)
Large Temp. Blank: **N/A** (temp in Celsius)

• Air-Transported Sampling Event

1. Project ID: **N/A**

2. COC Attached? **N/A** Properly Completed? **N/A** Signed by AE employee? **N/A**
3. Airbill attached? **N/A** Airbill #: **N/A**
4. Custody Seals? **N/A**
5. Seals intact? **N/A**

Temp. Blank: N/A (temp in Celsius)

COMMENTS:

B. Sample Conditions

Number of Samples Received: **7** Packing type: **N/A**
Number of Bottles Received: **7**
1. Samples in proper bags? **yes**
2. Bottles intact? **yes**
3. Sufficient sample volume? **yes**
4. Labels agree with COC? **yes**
5. Samples delivered within holding time? **yes**

Problems encountered: **N/A**

Was the project manager called? **N/A**

COMMENTS:

Signature: Jessica L Wood Date and time: 6/9/14 1113

Source Molecular
Leader in Microbial Source Tracking

4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-220-0379, Fax: (1) 786-513-2733, Email: info@sourcemolecular.com

Human Fecal Toolbox ID™

Detection of the fecal Human gene biomarker for Human fecal contamination by quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: State of Alaska Department of Environmental Conservation
Date Received: June 10, 2014
Date Reported: June 27, 2014

SM #	Client #	Analysis Requested	Species	DNA Analytical Results
SM-4F10106	PHC-3-060914	Human Bacteroidetes ID	Dorei	Absent

Laboratory Comments

Negative Results
In sample(s) classified as negative, the human-associated Bacteroidetes gene biomarker was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis. It is important to note that a negative result does not mean that the sample does not definitely have human fecal contamination. Only repeated sampling (both during wet and dry sampling events) will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.
In order to strengthen the result, a negative sample should be analyzed further for human fecal contamination with other DNA analytical tests. A list of human fecal ID tests can be found at www.sourcemolecular.com/human.

Positive Results
In sample(s) classified as positive, the human-associated Bacteroidetes gene biomarker(s) was detected in both test replicates suggesting that human fecal contamination is present in the water sample(s). All detected concentration levels are classified as "Present", including trace levels. For more insight on the concentration of the biomarker, quantification is required. The biomarker(s) serve as an indicator of the targeted fecal pollution, but the absence of the biomarker does not signify conclusively the absence of that form of fecal pollution. Only repeated sampling (both during wet and dry sampling events) will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Human Fecal Reference Samples
The client is encouraged to submit samples from the surrounding wastewater facilities and/or septic systems in order to gain a better understanding of the concentration of the human-associated fecal Bacteroidetes genetic marker as well as the concentration of the general fecal Bacteroidetes genetic marker in the geographic region of interest. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at www.sourcemolecular.com/tests

DNA Analytical Method Explanation

Each submitted water sample was filtered through 0.45 micron membrane filters. Each filter was placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample was homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol.

Amplifications were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul containing the sample extract, forward primer, reverse primer, probe and an optimized buffer. The following thermal cycling parameters were used: 50°C for 2 min, 95°C for 10 min and 40 cycles of 95°C for 15 s and 60°C for 1 min. '
All assays were run in duplicate.

For quality control purposes, a positive control consisting of appropriate genomic DNA and a negative control consisting of PCR-grade water were run alongside the sample(s) to ensure a properly functioning reaction and to reveal any false negatives or false positives. The accumulation of PCR product is detected and graphed in an amplification plot. If the fecal indicator organism is absent in the sample, this accumulation is not detected and the sample is considered negative. If accumulation of PCR product is detected, the sample is considered positive.

Human Bacteroidetes ID™ Species*: B. dorei*

The **Human Bacteroidetes ID™ Species*: B. dorei*** service targets the species *Bacteroides dorei*. *B. dorei* is an anaerobe that is frequently shed from the gastrointestinal tract and isolated from human feces worldwide. It is a newly discovered species that is widely distributed in the USA.[1,2] The human-associated marker DNA sequence is located on the 16S rRNA gene of *B. dorei*.[3] The marker is the microbial source tracking (MST) marker of choice for detecting human fecal pollution due to its exceptional sensitivity and specificity. Internal validations have been conducted on hundreds of sewage, septage, human and animal host fecal samples collected from throughout the U.S and archived in the Source Molecular fecal bank. The marker has also been evaluated in both inland and coastal waters. A recent, comprehensive, multi-laboratory MST method evaluation study, exploring the performance of current MST methods, concluded the *B. dorei* qPCR assay to be the top performing human-associated assay amongst those tested. The success and consistency of this marker in numerous studies around the world[1,3,4] makes the **Human Bacteroidetes ID™ Species*: B. dorei*** service the primary service for identifying human fecal pollution at Source Molecular.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.[5] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*.

The Human Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[3,5,6,7,8] Furthermore, certain strains of *Bacteroidetes* have been found to be associated with humans.[3,6] As such, these bacterial strains can be used as indicators of human fecal contamination.

Accuracy of the results is possible because the method amplifies DNA into a large number of small copies of the gene biomarker of interest. This is accomplished with small pieces of DNA called primers that are complementary and specific to the unique *B. dorei* DNA sequence. Through a heating process called thermal cycling, the double stranded DNA is denatured, hybridized to the complementary primers and amplified to create many copies of the DNA fragment desired. If the primers are successful in finding a site on the DNA fragment that is specific to the *B. dorei* DNA sequence, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve by the qPCR software. The absence of an amplification curve indicates that the *B. dorei* gene biomarker is not detected in the water sample because it is either not present or present at concentrations below the analytical detection limit.

To strengthen the validity of the results, additional tests targeting other high-ranking, human-associated *Bacteroidetes* species should be performed, such as
Human Bacteroidetes ID™ Species*: B. stercoris*,
Human Bacteroidetes ID™ Species*: B. fragilis*, and
Human Bacteroidetes ID™ Species*: B. thetaiotaomicron*.

[1]Boehm, A., Fuhrman, J., Mrse, R., Grant, S. **Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California**. Environ Sci Technol. 2003 37: 673–680.
[2]Bakir, M., Sakamoto, M., Kitahara, M., Matsumoto, M., Benno, Y. **Bacteroides dorei sp. nov., isolated from human faeces**. Int. J. Syst. Evol. Microbiol. 2006 56: 1639–1641.
[3] Bernhard, A., Field, K. **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA**. Appl. Environ. Microbiol. 2000b 66: 4571-4574.
[4]Ahmed, w., Masters, N., Toze, S. **Consistency in the host specificity and host sensitivity of the Bacteroides HF183 marker for sewage pollution tracking. Lett. Appl. Microbiol. 2012 55: 283-289.**
[5] Scott, T., Rose, J., Jenkins, T., Farrah, S., Lukasik, J. **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. 2002 68: 5796-5803.
[6] Bernhard, A., Field, K. **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Appl. Environ. Microbiol. 2000a 66: 1587-1594.
[7] Fogarty, L., Voytek, M. **A Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species.** Appl. Environ. Microbiol. 2005 71: 5999-6007.
[8] Dick, L., Bernhard, A., Brodeur, T., Santo Domingo, J., *et al.* **Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic**

Chain Of Custody Record

Source Molecular Corporation

SHIPPING ADDRESS:
4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

Company Name:	State of Alaska DEC Div. of Water
Contact Name(s):	Gretchen Pikul
E-mail Address(es):	gretchen.pikul@alaska.gov
Phone:	(907) 465-5003
Address:	410 Willoughby Ave Suite 303
City/State/Zip:	Juneau, AK 99811
Country:	USA

Sample ID	Media	Sample Date	Sample Time	# of Containers	MST (Human Bacteroides)	Quantification (Yes, No, If Positive)	Comments
PHC-1_060914	H_2O	6-9-14	~~8:03~~ 8:03	1	x	yes, if positive	Please HOLD samples until verbal confirmation of fecal sample results
PHC-2_060914	↓	↓	8:39	↓	x		
PHC-3_060914			9:03		x		Gretchen will call around 13:00 AKST on 6-10-14
PHC-4_060914			9:27		x		
PHC-5_060914			9:45		x		Do not filter and freeze until samples are chosen.
			10:07				

Completed by Client:

SAMPLES DELIVERED BY: Fed Ex Standard Overnight
SAMPLER NAME: Gretchen Pikul
SAMPLE SITE: Pederson Hill Creek
WITNESS:
SIGNATURE:

Relinquished 6-9-14 10:24 GPik

Completed by Source Molecular:

SAMPLES RECEIVED BY:
RECEIVED DATE/TIME:
TEMPERATURE:
CONDITION:
SIGNATURE:

June 16, 2014

Pederson Hill Creek Water Quality Field Data Sheet

Date: 6-16-14

Personnel: Gretchen Pikul

Weather: sunny, slight wind from south

Air temperature: 70.6°F 21.0°C

Sample ID	PHC-1
Date/Time	6-16-14 10:14
Water temperature °C	14.9°C, 13.9°C, 12.9°C
pH	7.84
Conductivity (µS/cm)	64.3
Dissolved Oxygen (mg/L)	9.06
Dissolved Oxygen (%)	89.5
Turbidity (NTU)	1.49

13.9

Sample ID	PHC-3
Date/Time	6-16-14 10:51
Water temperature °C	11.6°C, 12.7°C, ~~16.0~~ 13.4°C, 12.2
pH	6.90
Conductivity (µS/cm)	612
Dissolved Oxygen (mg/L)	~~2.83~~ 3.17
Dissolved Oxygen (%)	~~26.0~~ 29.6
Turbidity (NTU)	12.4

Sample ID	PHC-2
Date/Time	6-16-14 10:35
Water temperature °C	11.7°C, 12.9°C
pH	7.45
Conductivity (µS/cm)	223
Dissolved Oxygen (mg/L)	10.16
Dissolved Oxygen (%)	93.3
Turbidity (NTU)	4.73

12.3

Sample ID	PHC-4
Date/Time	6-16-14 11:11
Water temperature °C	12.5°C, 12.9°C, 12.3°C 12.6
pH	7.26
Conductivity (µS/cm)	222
Dissolved Oxygen (mg/L)	8.14
Dissolved Oxygen (%)	76.2
Turbidity (NTU)	3.05

Multi-meter and turbidimeter calibrated prior to sampling.

Sample ID	PHC-5 + Rep
Date/Time	6-16-14 11:25
Water temperature °C	13.9°C, 13.5°C, 14.5°C
pH	7.40
Conductivity (µS/cm)	194.8
Dissolved Oxygen (mg/L)	9.38
Dissolved Oxygen (%)	90.6%
Turbidity (NTU)	1.84

14.0

Sample ID	
Date/Time	
Water temperature °C	
pH	
Conductivity (µS/cm)	
Dissolved Oxygen (mg/L)	
Dissolved Oxygen (%)	
Turbidity (NTU)	

Notes:

PHC-1 low flow, shallow water depth
PHC-2 good flow, sufficient water depth
PHC-3 low flow, sufficient water depth
PHC-4 medium flow, sufficient water depth
PHC-5 good flow, sufficient water depth

previous day weather 6-15-14 - sun
6-14-14 - some rain

Confirmed with Hach, DO probe is calibrated properly but in need of the yearly sensor cap. DEC ordered one on 6-11-14.

Page 2 of 2

Admiralty ENVIRONMENTAL 641 W. Willoughby Ave., Suite 301 Juneau, AK 99801 (907) 463-4415 (480) 247-4476 (f)

Gretchen Pikul
ADEC, Division of Water
410 Willoughby Ave., Ste. 303
Juneau, AK 99801

June 27, 2014

ADEC Division of Water Pederson Hill Project

Date of Collection: June 16, 2014
Sampling Location: Juneau, AK

Summary

Samples from Pederson Hill Creek were collected as grab samples between 10:14 and 11:25 hours on June 16, 2014 for the Pederson Hill Creek Project. The samples were collected by Ms. Gretchen Pikul of ADEC's Division of Water.

Samples were each collected into a 120 ml sterile bottle preserved with sodium thiosulfate for the tests for fecal coliforms. All sampling and sample handling procedures were conducted in accordance with standard EPA guidelines for environmental sampling. The samples were hand-delivered to Admiralty Environmental, Juneau. All regulatory holding times were met for all tests.

A complete report of the final lab results is enclosed. The official laboratory report follows this letter and includes the analytical results, case narrative, chain of custody form, and cooler receipt form.

Kindest Regards,

David Wetzel
Admiralty Environmental

Admiralty ENVIRONMENTAL
641 W Willoughy Ave., Suite 301 Juneau, AK 99801
(907) 463 - 4415 Fax (480) 247 - 4476
www.admiraltyenvironmental.com

ADEC Division of Water

Pederson Hill Creek

Analytical Report

June 16, 2014

Admiralty Environmental EPA ID AK 00976

Juneau, AK

AE 10047

Sample Location	Date & Time Sampled	Fecal Coliform (FC/100 ml)
PHC-1_061614	6/16/2014; 10:14	120
PHC-2_061614	6/16/2014; 10:35	3.3
PHC-3_061614	6/16/2014; 10:51	56
PHC-4_061614	6/16/2014; 11:11	52
PHC-5_061614	6/16/2014; 11:25	100
PHC-5Rep_061614	6/16/2014; 11:25	108

Quality Control:

Analysis	MB	LCS	LCS Duplicate	RPD	Date/Time Commenced	Holding Time Met
FC	< 2	---	---	---	6/16/2014; 14:45	yes

Analysis Description:

Analysis	Method	MDL	PQL	Unit
FC	SM 9222D	1.0	2.0	FC/100ml

Case Narrative:

All sample collection and sample analysis QA/QC parameters were met for this event.

Key:

Enter.	Enterococci
FC	Fecal Coliform
LCS	Laboratory Control Standard
MB	Method Blank
MDL	Method Detection Limit
mg/L	Milligrams Per Liter
ND	Not Detected
PQL	Practical Quantitation Limit
RPD	Relative Percent Difference

David Wetzel
President, Admiralty Environmental
dwetzel@admiraltyenv.com

Admiralty ENVIRONMENTAL

Admiralty Environmental
641 W. Willoughby Ave., Suite 301
Juneau, AK 99801
(907) 463-4415 fax (480) 247-4476

CHAIN OF CUSTODY/TRANSMITTAL RECORD
PAGE 1 OF 1

CLIENT: **ADEC Division of Water**

PROJECT: Pederson Hill Creek

AE 10047

REPORT TO: Gretchen Pikul | PHONE#: 907-465-5023

ADDRESS: 410 Willoughby Ave., Suite 303, Juneau, AK 99801

SAMPLED BY: Gretchen Pikul

EMAIL: gretchen.pikul@alaska.gov

SAMPLES TAKEN IN THE PRESENCE OF:

DATE	TIME	SITE DESCRIPTION /IDENTIFIER		MATRIX	# OF BOTTLES	Fecal Coliform	Field Results pH	Temp	D.O.	
6-16-14	10:14	PHC-1_061614	A	H2O	1	1	7.84	13.9	9.06	mg/L
	10:35	PHC-2_061614	B	H2O	1	1	7.45	12.3	10.16	
	10:51	PHC-3_061614	C	H2O	1	1	6.90	12.2	3.17	
	11:11	PHC-4_061614	D	H2O	1	1	7.26	12.6	8.14	
	11:25	PHC-5_061614	E	H2O	1	1	7.40	14.0	9.38	
	11:25	PHC-5Rep_061614	F	H2O	1	1	7.40	14.0	9.38	
				H2O	1	1				

RELINQUISHED BY:	RECEIVED BY:	RELINQUISHED BY:	RECEIVED BY:
Signature: Gretchen Pikul	Signature: Halstead	Signature:	Signature:
Printed Name: Gretchen Pikul	Printed Name: HALSTEAD	Printed Name:	Printed Name:
DATE: 6-16-14	DATE: 6/16/14 IF	DATE:	DATE:
TIME: 12:49	TIME: 1249	TIME:	TIME:

Section to Be Completed by Receiving Laboratory

Temp/Loc:	4.09			
Thermo ID#:	SR#2			
Condition of Custody Seals:	✓			
Initialed By:	IF			
Shipped Via:	—			

Admiralty Environmental Cooler Receipt Form

Client: **Admiralty Environmental, LLC**
Project: **ADEC division of Water** AE# 10047

Date Opened: **6/16/2014** Opened by: **I Felstead**

A. External Cooler Conditions

• Local Sampling Event

1. Project ID: **Pederson Hill Creek**

2. COC Attached? **yes** Properly Completed? **yes** Signed by AE employee? **yes**

Small Temp. Blank **4.09** (temp in Celsius)
Large Temp. Blank: **N/A** (temp in Celsius)

• Air-Transported Sampling Event

1. Project ID: **N/A**

2. COC Attached? **N/A** Properly Completed? **N/A** Signed by AE employee? **N/A**
3. Airbill attached? **N/A** Airbill #: **N/A**
4. Custody Seals? **N/A**
5. Seals intact? **N/A**

Temp. Blank: N/A (temp in Celsius)

COMMENTS:

B. Sample Conditions

Number of Samples Received: **1** Packing type: **N/A**
Number of Bottles Received: **6**
1. Samples in proper bags? **yes**
2. Bottles intact? **yes**
3. Sufficient sample volume? **yes**
4. Labels agree with COC? **yes**
5. Samples delivered within holding time? **yes**

Problems encountered: **N/A**

Was the project manager called? **N/A**

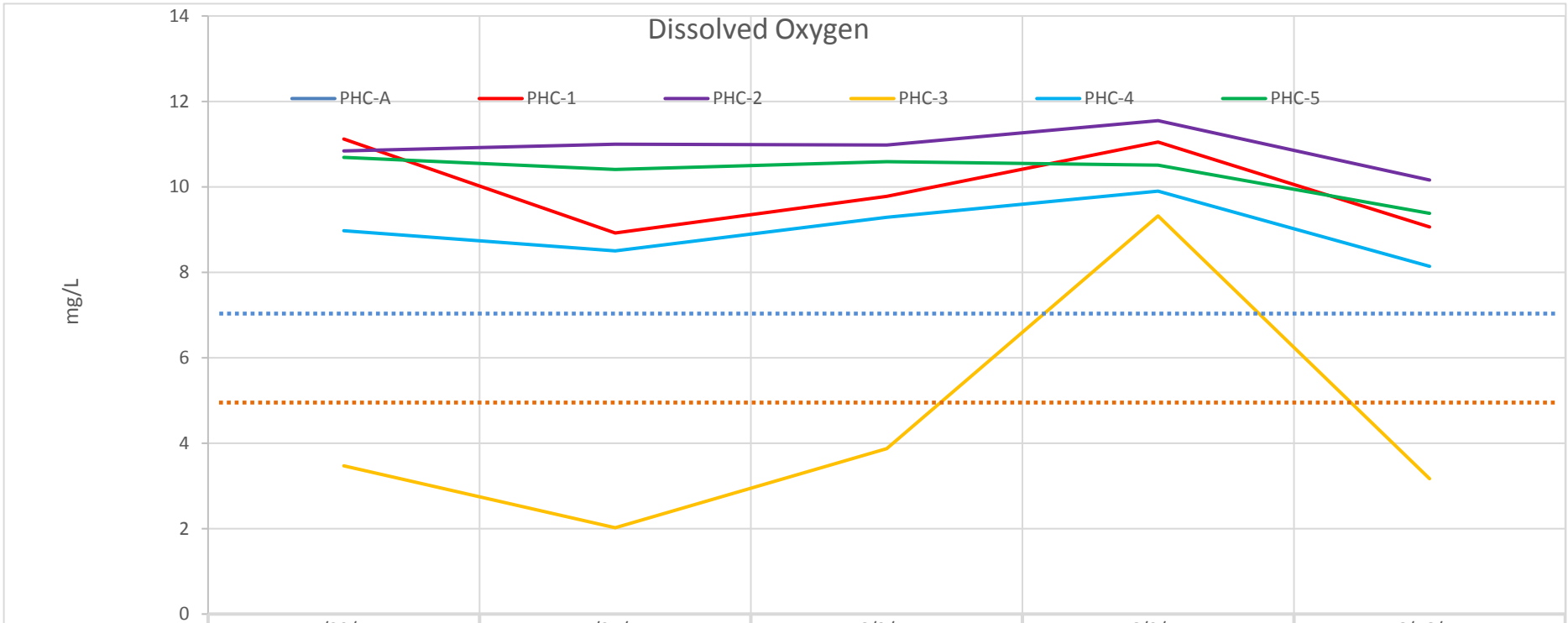
COMMENTS:

Signature: IFelstead Date and time: 6/16/14 12:49

This page intentionally left blank.

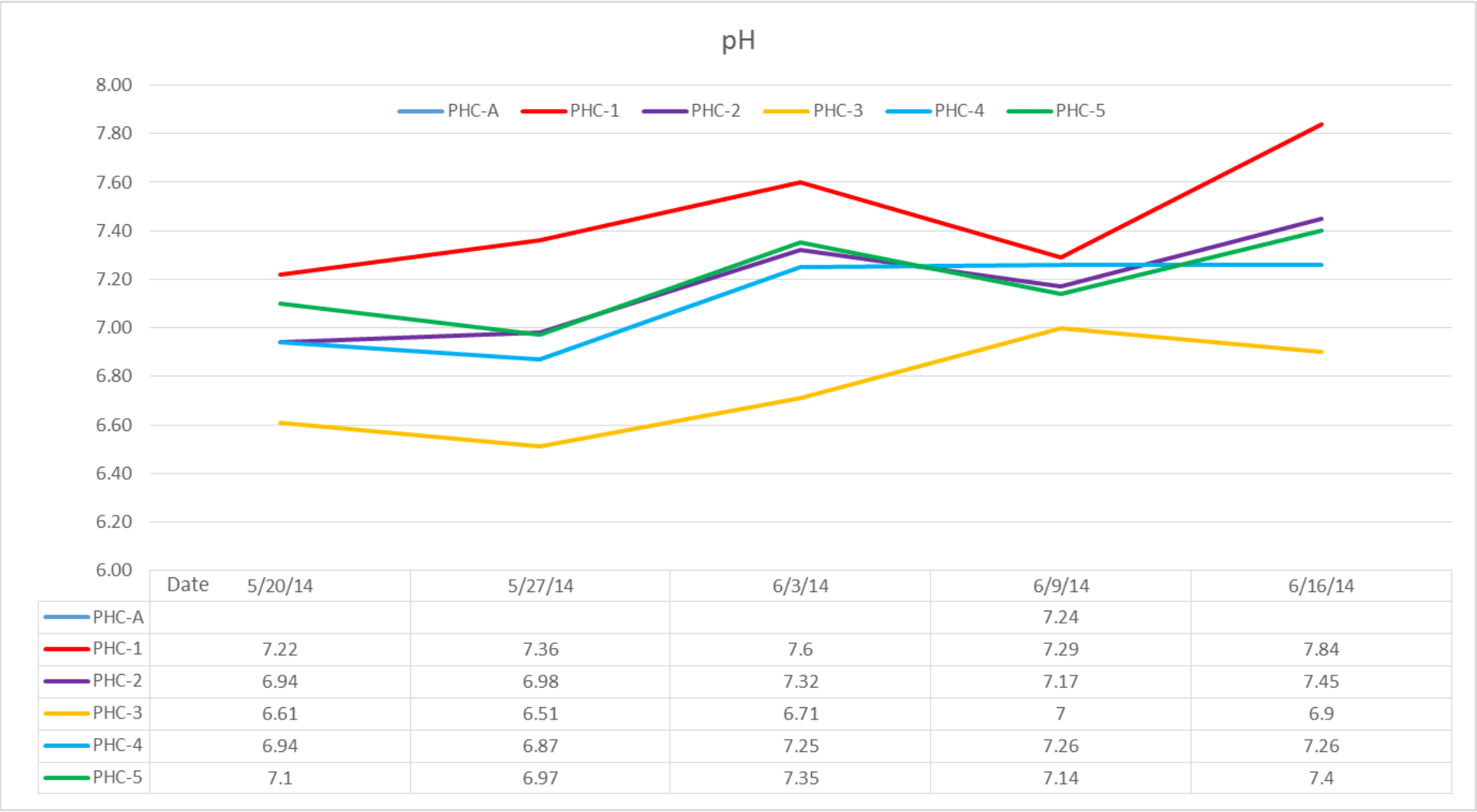
Appendix C – Data Graphs

Figure 1. Dissolved Oxygen (DO) values from Pederson Hill Creek water quality monitoring during May-June 2014. Alaska WQS requires > 7 mg/l for anadromous fish; > 5 mg/l for non-anadromous fish; < 17 mg/l for aquatic life (the dotted lines correlate to DO concentrations required for healthy fish populations).



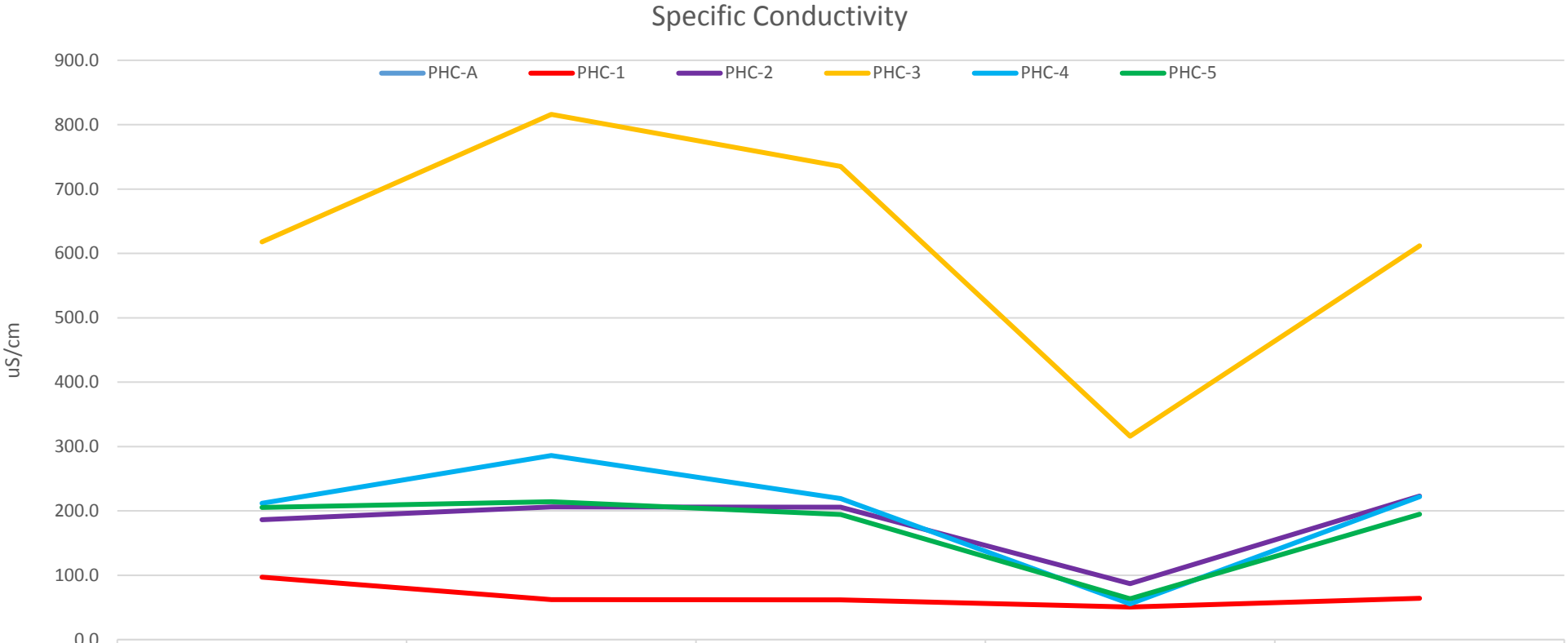
Date	5/20/14	5/27/14	6/3/14	6/9/14	6/16/14
PHC-A				11.32	
PHC-1	11.12	8.92	9.78	11.05	9.06
PHC-2	10.84	11	10.98	11.55	10.16
PHC-3	3.47	2.02	3.87	9.32	3.17
PHC-4	8.97	8.5	9.29	9.9	8.14
PHC-5	10.69	10.41	10.59	10.51	9.38

Figure 2. pH values from Pederson Hill Creek water quality monitoring during May-June 2014. Alaska WQS requires pH values ranging from 6.5 to 8.5 to support aquatic life; the dotted line correlates to pH values required for healthy fish populations.



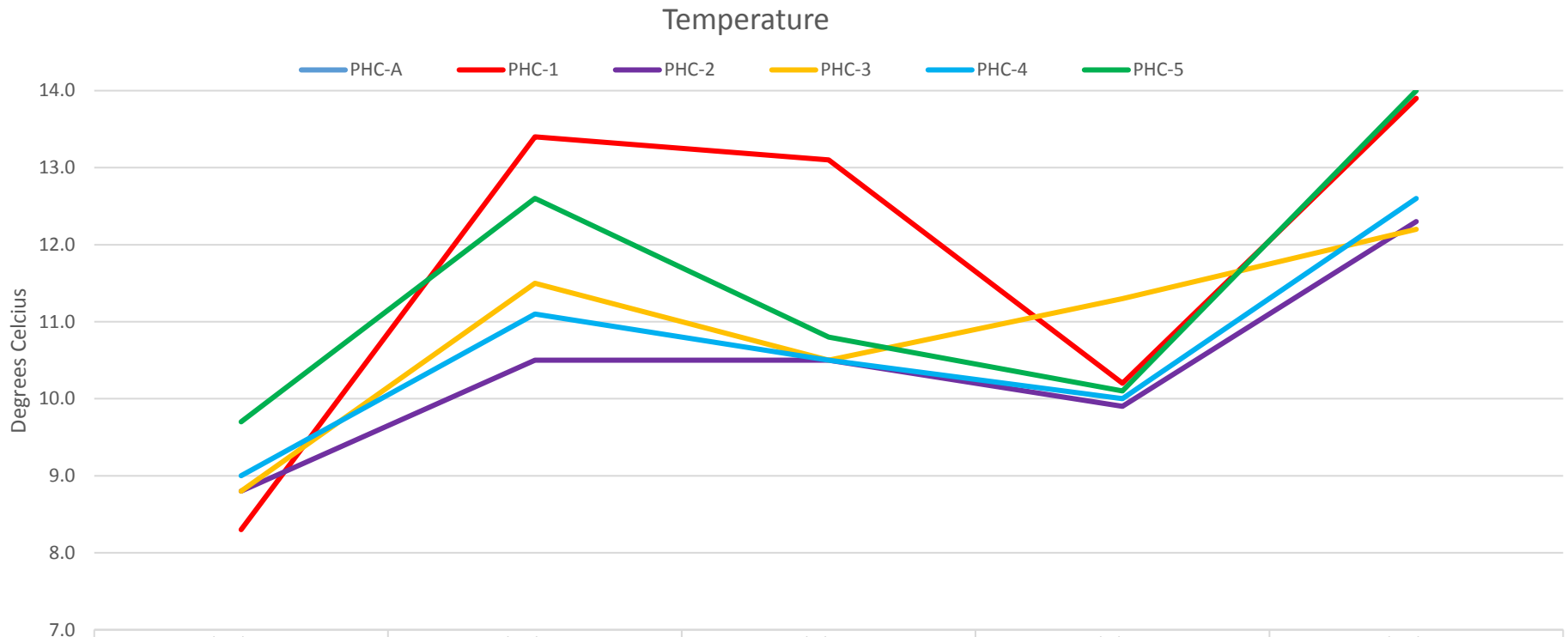
Date	5/20/14	5/27/14	6/3/14	6/9/14	6/16/14
PHC-A				7.24	
PHC-1	7.22	7.36	7.6	7.29	7.84
PHC-2	6.94	6.98	7.32	7.17	7.45
PHC-3	6.61	6.51	6.71	7	6.9
PHC-4	6.94	6.87	7.25	7.26	7.26
PHC-5	7.1	6.97	7.35	7.14	7.4

Figure 3. Conductivity values from Pederson Hill Creek water quality monitoring during May-June 2014.



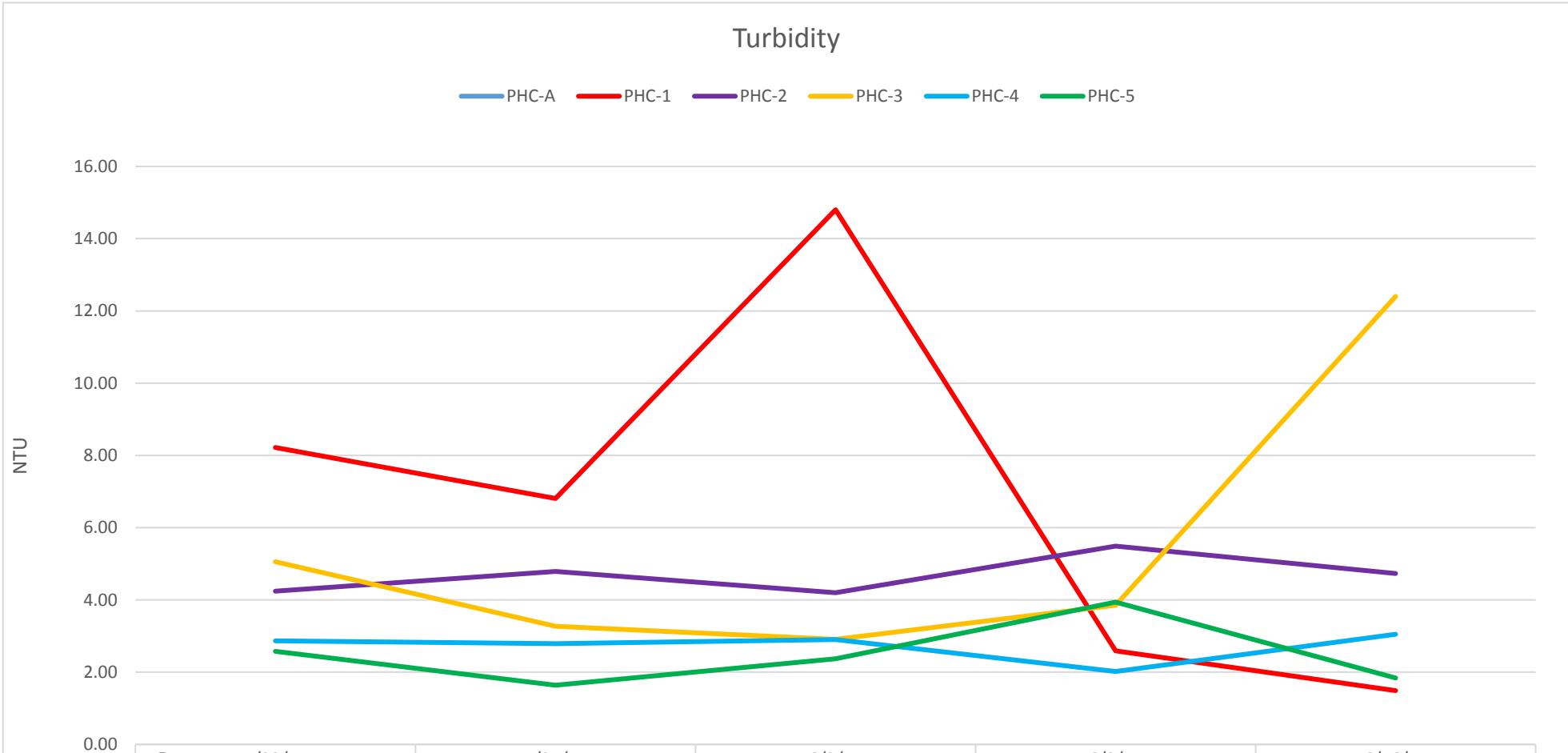
Date	5/20/14	5/27/14	6/3/14	6/9/14	6/16/14
PHC-A				16.01	
PHC-1	97	62.1	61.7	50.6	64.3
PHC-2	186.4	206	205.7	86.9	223
PHC-3	618	816	735	316	612
PHC-4	212	286	219	55.5	222
PHC-5	205.2	214.2	194.3	63.4	194.8

Figure 4. Temperature values from Pederson Hill Creek water quality monitoring during May-June 2014. Alaska WQS call for temperatures < 20 °C to support aquatic life.



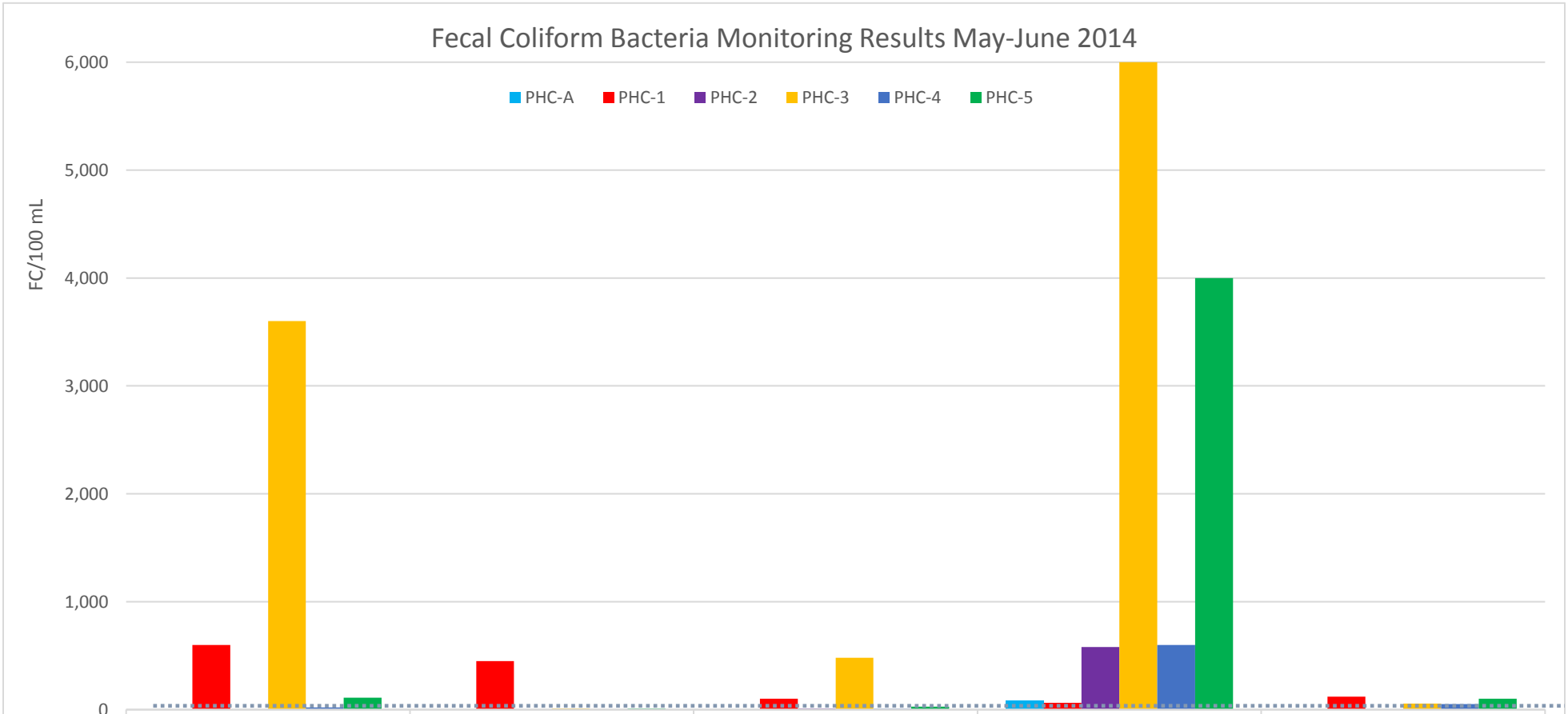
Date	5/20/2014	5/27/2014	6/3/2014	6/9/2014	6/16/2014
PHC-A				9	
PHC-1	8.3	13.4	13.1	10.2	13.9
PHC-2	8.8	10.5	10.5	9.9	12.3
PHC-3	8.8	11.5	10.5	11.3	12.2
PHC-4	9	11.1	10.5	10	12.6
PHC-5	9.7	12.6	10.8	10.1	14

Figure 5. Turbidity values from Pederson Hill Creek water quality monitoring during May-June 2014.



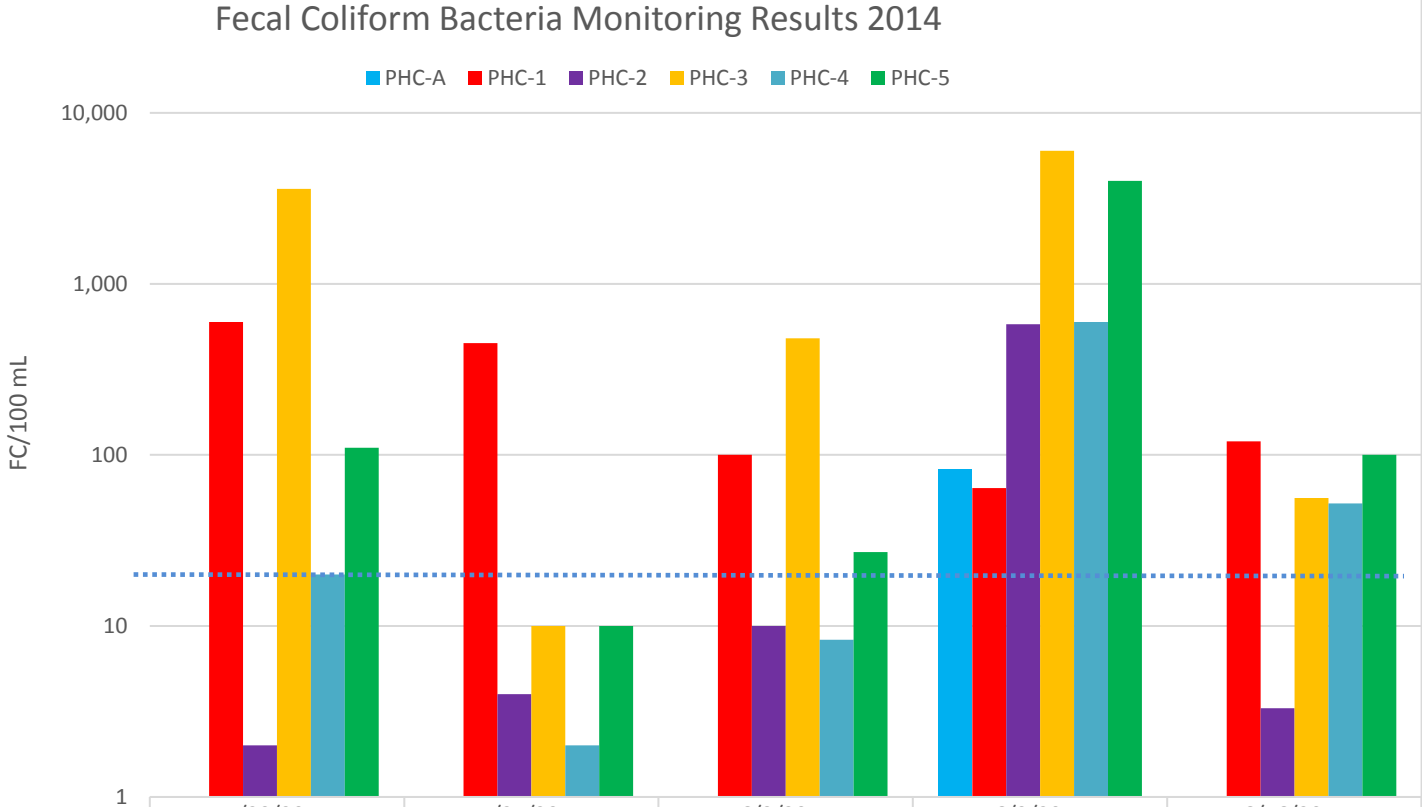
Date	5/20/14	5/27/14	6/3/14	6/9/14	6/16/14
PHC-A				0.68	
PHC-1	8.22	6.81	14.8	2.59	1.49
PHC-2	4.24	4.79	4.2	5.49	4.73
PHC-3	5.06	3.27	2.91	3.85	12.4
PHC-4	2.87	2.79	2.9	2.02	3.05
PHC-5	2.58	1.64	2.37	3.94	1.84

Figure 6. Fecal Coliform Bacteria Results from Pederson Hill Creek water quality monitoring during May-June 2014. The blue line represents the Alaska WQ criteria in a 30-day period, the geometric mean may not exceed 20 FC/100 ml, and not more than 10% of the samples may exceed 40 FC/100 ml. All five Pederson Hill sampling sites exceed the water quality limits as set by the State of Alaska.



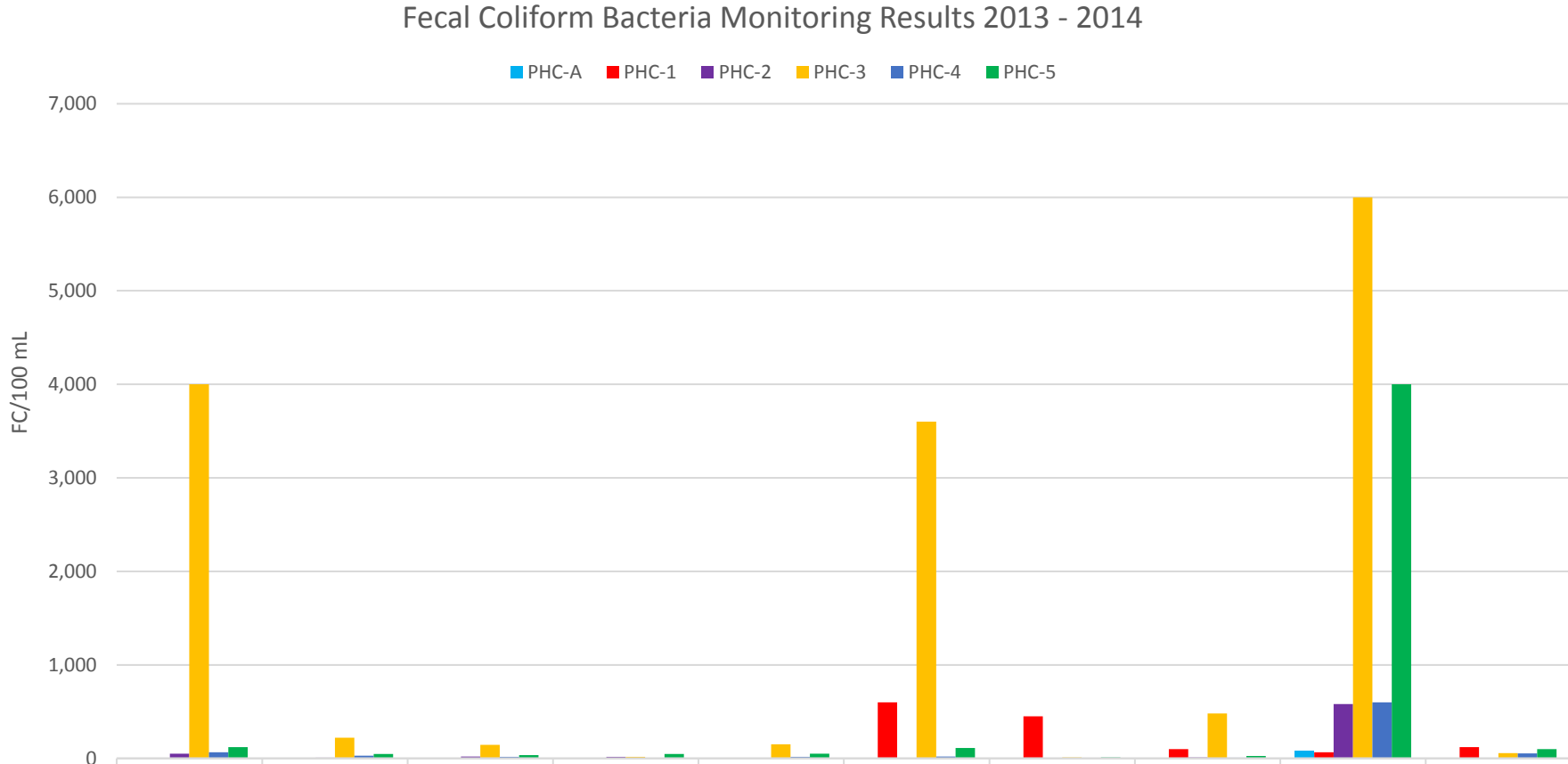
Date	5/20/2014	5/27/2014	6/3/2014	6/9/2014	6/16/2014
PHC-A				82	
PHC-1	600	450	100	64	120
PHC-2	2	4	10	580	3.3
PHC-3	3600	10	480	6,000	56
PHC-4	20	2	8.3	600	52
PHC-5	110	10	27	4,000	100

Figure 7. Fecal Coliform Bacteria Results from Pederson Hill Creek water quality monitoring during May-June 2014 – **using logarithmic scale.** The blue line represents the Alaska WQ criteria in a 30-day period, the geometric mean may not exceed 20 FC/100 ml, and not more than 10% of the samples may exceed 40 FC/100 ml.



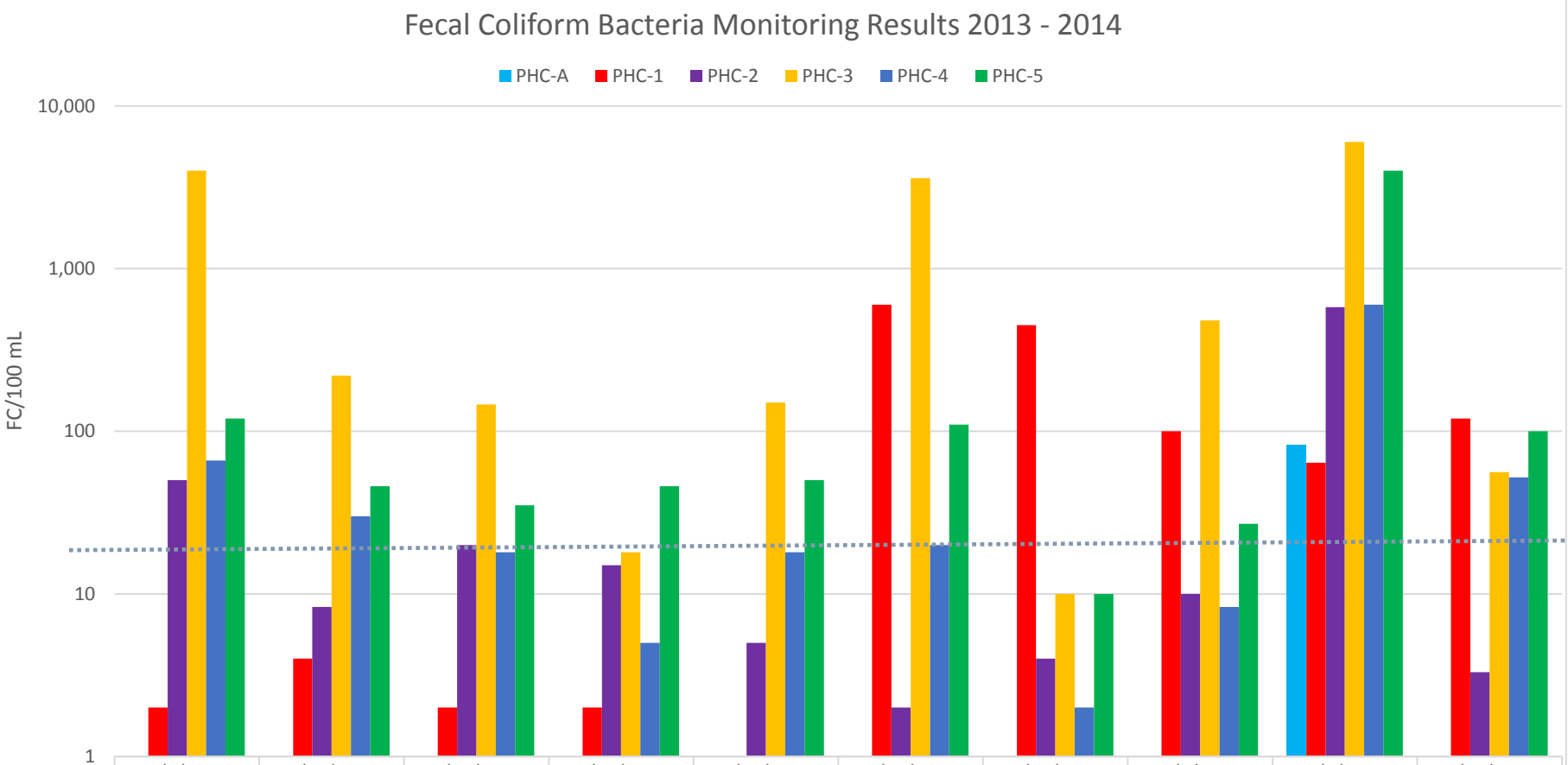
Date	5/20/2014	5/27/2014	6/3/2014	6/9/2014	6/16/2014
PHC-A				82	
PHC-1	600	450	100	64	120
PHC-2	2	4	10	580	3.3
PHC-3	3600	10	480	6,000	56
PHC-4	20	2	8.3	600	52
PHC-5	110	10	27	4,000	100

Figure 8. Fecal Coliform Bacteria Results from Pederson Hill Creek water quality monitoring during 2013 - 2014.



Date	6/5/2013	6/12/2013	6/19/2013	6/24/2013	6/26/2013	5/20/2014	5/27/2014	6/3/2014	6/9/2014	6/16/2014
PHC-A									82	
PHC-1	2	4	2	2	0	600	450	100	64	120
PHC-2	50	8.3	20	15	5	2	4	10	580	3.3
PHC-3	4000	220	146	18	150	3600	10	480	6,000	56
PHC-4	66	30	18	5	18	20	2	8.3	600	52
PHC-5	120	46	35	46	50	110	10	27	4,000	100

Figure 9. Fecal Coliform Bacteria Results from Pederson Hill Creek water quality monitoring during May-June 2014 – **using logarithmic scale**. The blue line represents the Alaska WQ criteria in a 30-day period, the geometric mean may not exceed 20 FC/100 ml, and not more than 10% of the samples may exceed 40 FC/100 ml.



Date	6/5/2013	6/12/2013	6/19/2013	6/24/2013	6/26/2013	5/20/2014	5/27/2014	6/3/2014	6/9/2014	6/16/2014
PHC-A									82	
PHC-1	2	4	2	2	0	600	450	100	64	120
PHC-2	50	8.3	20	15	5	2	4	10	580	3.3
PHC-3	4000	220	146	18	150	3600	10	480	6,000	56
PHC-4	66	30	18	5	18	20	2	8.3	600	52
PHC-5	120	46	35	46	50	110	10	27	4,000	100

Appendix D – Site Photos

May 20, 2014 sampling site photos

PHC-1 sample site

PHC-2 sample site

PHC-3 sample site
(Natural biological sheen shown in photo)

PHC-4 sample site

PHC-5 sample site

Downstream of PHC-5 sample site

May 27, 2014 sampling site photos

PHC-1 sample site

PHC-2 sample site

PHC-3 sample site
(Natural biological sheen shown in photo)

PHC-4 sample site

PHC-5 sample site

Downstream of PHC-5 sample site

June 3, 2014 sampling site photos

PHC-1 sample site

PHC-2 sample site

PHC-3 sample site
(Natural biological sheen shown in photo)

PHC-4 sample site

PHC-5 sample site

June 9, 2014 sampling site photos

PHC-1 sample site

PHC-2 sample site

PHC-3 sample site

PHC-4 sample site

PHC-5 sample site

Downstream of PHC-5 sample site

June 16, 2014 sampling site photos

PHC-1 sample site

PHC-2 sample site

PHC-3 sample site
(Natural biological sheen shown in photo)

PHC-4 sample site

PHC-5 sample site

Downstream of PHC-5 sample site

Mitigation pond downstream of PHC-5 sample site

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2014 Pederson Hill Creek Water Quality Monitoring