Water and Environmental Research Center
Annual Technical Report
FY 2005

Introduction

The demand for water-related research in Alaska is undergoing a change. Resource development and growth are the main drivers. Parts of Alaska that have never been developed before are being considered for resource extraction (metals, oil and gas, etc.) such as ANWR (Arctic National Wildlife Refuge) and NPRA (National Petroleum Reserve Alaska). Management of these water resources is difficult because related data networks in the state are sparse, poorly distributed and cover short durations. Minimal hydrologic data was collected in the state prior to World War II, and most stations are located at low elevations along the coast and major rivers. There is also the issue of collecting hydrologic data in an extreme environment. At the same time, parts of the state where there has been sustained growth are encountering conflicts for water resources.

Although Alaska is a very large state with considerable water resources, the environment (long winters and low temperatures) and the distribution, spatially and temporally, result in insufficient water resources at times during the year. A good example is the North Slope of Alaska, where long, cold winters and low annual precipitation make it a challenge to ensure water for domestic and industrial uses during the winter months. A trip to this area, particularly right after snowmelt, would give the impression that this region is water-rich with abundant lakes and rivers. However this resource is only a thin veneer over the deep, cold permafrost that prevails over the entire region. Even the numerous lakes that are present are deceiving as they are quite shallow (~2 m), and many completely freeze to the bottom during the winter months. Precipitation over the remainder of the state varies considerably, from a maritime climate for a thin strip of land along the southerly coast (extending from southeast Alaska out into the Aleutian Islands) to a dry continental climate over the remainder of the state.

Phase change (ablation, sublimation, soil freezing and thawing, ice growth and decay, evaporation, transpiration, etc.) is an important component of the hydrology of this region. This, coupled with the ongoing climatic warming makes for some interesting problems. The development of thermokarsts (thawing of ice-rich permafrost) is impacting terrain, sediment transport and the drainage of water (drained lakes).

In the past year there has been substantial growth in the research activities of the Water and Environmental Research Center (WERC) at the University of Alaska. We are obtaining considerably more state funding than we have in the past. This is mainly in response to development on state land where significant water management issues exist. For example, on the North Slope of Alaska, obtaining water for many uses in the winter months is a significant management issue.

An expanded picture of faculty, staff and student activities can be followed on the Water and Environmental Research Centers website: http://www.uaf.edu/water/

Research Program

During the research year 2005-2006 (March 1, 2005 to February 28, 2006), three research projects were funded through the U.S. Geological Survey 104b program. We have over the years used this small amount of grant money exclusively for graduate student support (stipend, tuition, and project supplies) to help young faculty get started with their research programs. This program (along with some outside funding) allows us to fund about three to four graduate students per year at about $31,000/year for a M.S. student. During the past year we funded the following three projects: Monitoring Thermokarst Evolution at Caribou-Poker Creeks Research Watershed, PI: Horacio Toniolo; Investigation of the Mechanism of Arsenic Biosorption by Modified Crab Shells, PI: Silke Schiewer; Antibiotic Resistance Analysis of Enterococci in Chester Creek, PI: Bill Schnabel.

Monitoring Thermokarst Evolution at Caribou-Poker Creeks Research Watershed (Year 2)

Basic Information

Title:	Monitoring Thermokarst Evolution at Caribou-Poker Creeks Research Watershed (Year 2)
Project Number:	2004AK25B
Start Date:	3/1/2005
End Date:	2/28/2006
Funding Source:	104B
Congressional District:	AK
Research Category:	Ground-water Flow and Transport
Focus Category:	Hydrology, None, None
Descriptors:	
Principal Investigators:	Horacio Toniolo

Publication

1. Kodial, P. (2005). Thermokarst evolution and sediment transport study in the Caribou-Poker Creeks Research Watershed, Alaska. MS Thesis, University of Alaska Fairbanks, 102 pp.

Monitoring Thermokarst Evolution at
Caribou-Poker Creeks Research Watershed

Introduction

Research focused on the evolution of a thermokarst located in the Caribou-Poker Creeks started in 2004 (Project Number 2004AK25B). Initial results reported last year indicated enormous changes in the study area. These landscape changes were mainly related to lateral and upward bank erosion.

This report summarizes the field and laboratory work conducted during 2005. The region evolved, in a two-year period, from a relatively stable landscape with no perturbations to a well-defined water channel that was preserved during the last winter (2005-2006). Detailed information on the study site and an extensive up-to-date review of existing literature on thermokarsts can be found in Kodial (2005).

Methods

Our initial report speculated that this landscape change could be due to a cryogenic process (piping). A survey using Ground Penetrating Radar (GPR), using instrumentation available through the Water and Environmental Research Center, University of Alaska Fairbanks, was conducted in early March 2005 in the area where piping was hypothesized.

Discharge measurements were performed during breakup. Water samples were collected and analyzed for suspended sediment concentration. Analyses were conducted in WERC labs.

Soil temperature on both left and right banks was measured on 7 August 20005 and 13 October 2005. A topographical field survey was carried out in October to assess the thermokarst's growth from the previous year. Abundant digital photographs were taken during field visits to document morphologic changes in the study area.

Results

GPR survey

The survey was conducted in area of approximately 10 m^2. Results from this survey did not provide conclusive evidence for the pipe hypothesized during the summer of 2004. According to Dr. Yoshikawa, research scientist at WERC, the instrument's performance was inhibited due to the higher electrical conductivity of the silty soil present in the area. However, an unfrozen water pocket was found at approximately 1 m below the ground surface. The presence of the unfrozen water indicated that the active layer did not completely freeze during the cold winter. Its presence could indicate permafrost degradation (deepening of permafrost table). The GPR image can be found in Kodial (2005).

Cryogenic piping

As mentioned before, the existence of cryogenic pipes in the study area was hypothesized at the beginning of the study in 2004, when high sediment flows were noticed in the right margin of the thermokarst. Piping was documented during the summer of 2005. Specifically, piping

phenomenon was observed at the upstream site and in the intermediate section between the upstream site and downstream site. The cryo-pipe in the study area was exposed when rapid erosion progressed upward from the upstream site. At the peak of its development, the outer pipe diameter was approximately 50 cm, while an inner pipe had a diameter of around 10 cm (see Figure 1). These pipes can significantly improve the transport of fine sediment from the soil matrix to downstream sites (Holden 2005). The temporal development (from initial exposure to collapse) occurred in a 20-day period (Figure 1). Additional pictures showing ice-rich- sediment degradation in the pipe were presented by Kodial (2005).

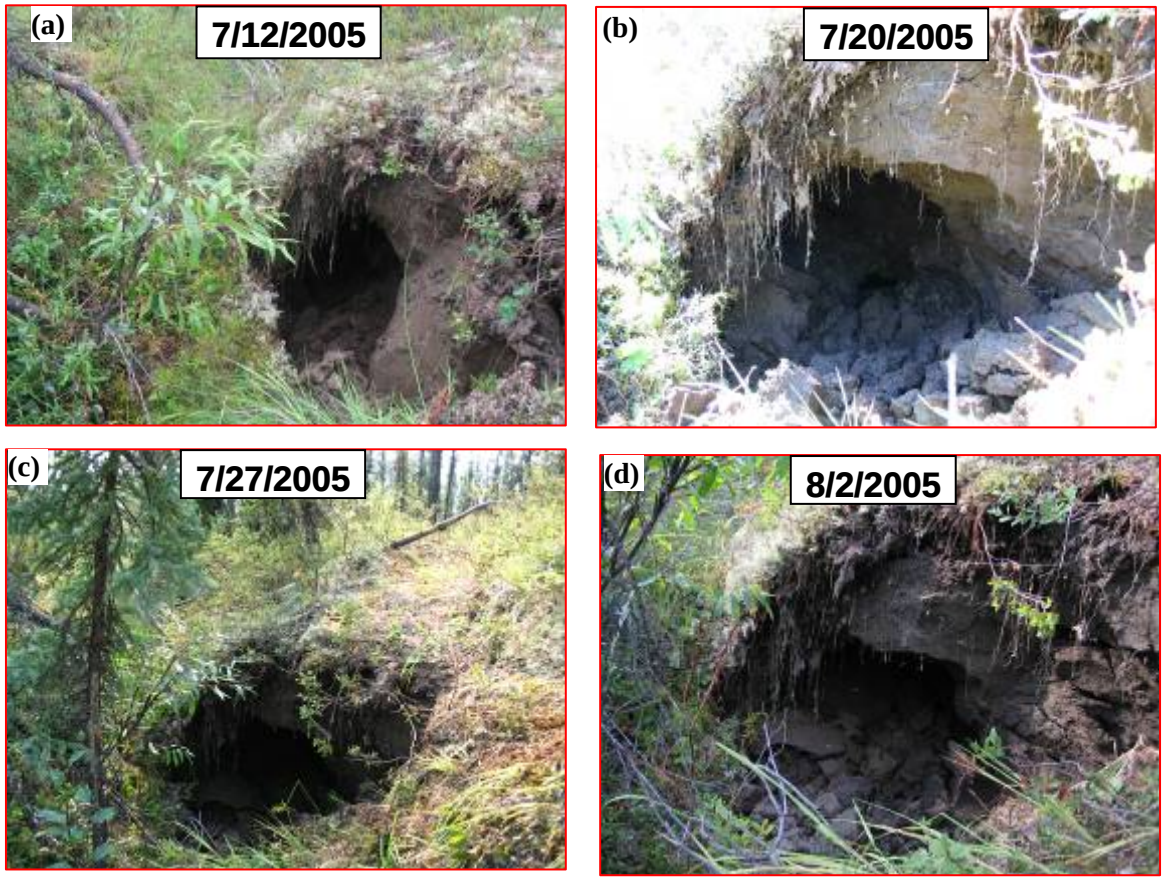

Figure 1. *Cryogenic pipe evolution. (a) initial pipe exposure; (b) pipe enlargement and start of erosion; (c) advanced stage of pipe subsidence; (d) final state of the collapsed pipe with eroded material covering the pipe entrance. Ten-centimeter inner pipe is the dark area in the middle of the picture (from Kodial, 2005).*

Discharge and sediment concentration

Discharge measurements at the study site were only possible during breakup because most of the water moved as subsurface flow during 2005. Water samples were collected and analyzed for suspended sediment concentration in a manner similar to the 2004 field season.

Two locations were selected along the thermokarst for discharge measurements and water sampling. One of them was located at the thermokarst water input, namely, “upstream”. The other one was located in the central portion of the thermokarst, here defined as “downstream”.

The discharge was measured using the volume-by-time method. Water samples were collected in 1000 ml plastic bottles for suspended sediment concentration.

The collected water samples were analyzed in laboratories at the Water and Environmental Research Center (WERC), UAF to determine the suspended sediment concentration.

A key difference between measured discharges in 2004 and 2005 is the magnitude. Measured peak flows were 2.05 l/s and 5.13 l/s for 2004 and 2005 respectively. All the water flowing into the thermokarst during the 2005 breakup appeared attributable to snowmelt due to the fact that the area was precipitation-free during the period of discharge measurement. Figure 2 shows the discharge variation in two sites (upstream and downstream) as well as the precipitation (secondary axis).

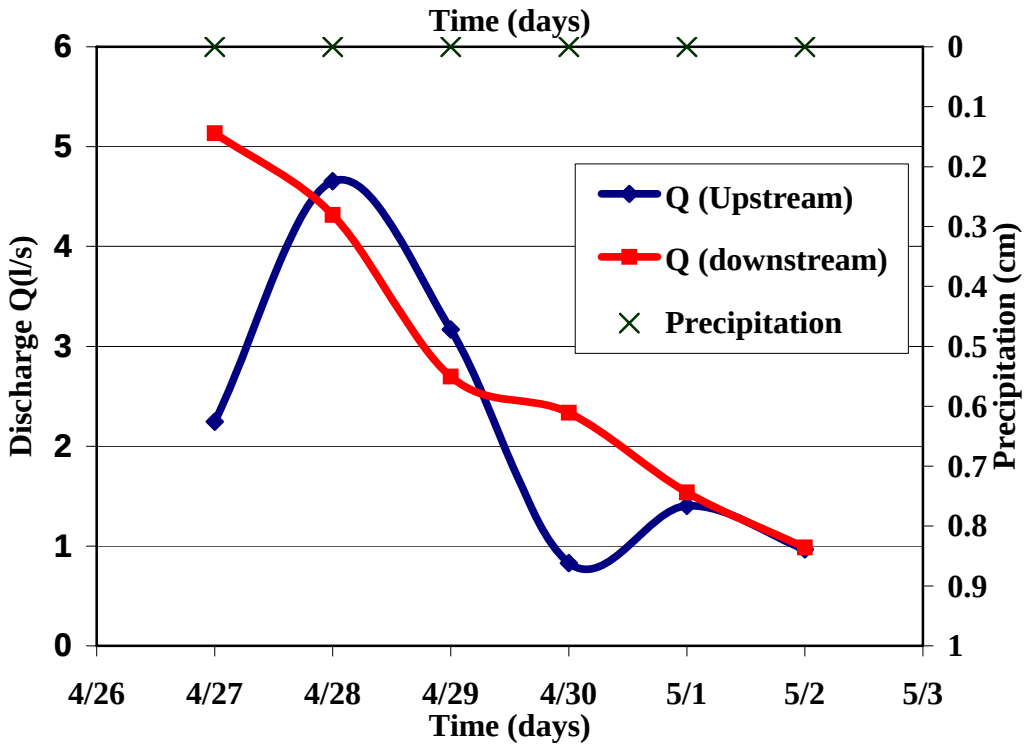

Figure 2. *Discharge plot for 2005 at the upstream and downstream sites. No precipitation occurred during the days discharge was measured (from Kodial 2005).*

Peak sediment concentration was 44.81 mg/g. A major difference in the data observed in 2004 and 2005 was that the first high sediment concentration calculated in 2005 was almost a month earlier than that in 2004. Additional details can be found in Kodial (2005).

Soil temperature profiles

Soil temperatures were measured on both right and left banks in two different locations in the study site. One of these locations was a disturbed area (i.e., erosive processes, unstable banks); the other place was an undisturbed spot (i.e., natural soil conditions, stable bank). Temperature profiles were measured on August and October 2005. Figures 3 and 4 show the temperature profiles for the banks in the disturbed and undisturbed sites respectively. Steep temperature gradients were detected in the disturbed site during August. More uniform temperature profiles were measured at the beginning of Alaska's winter (October) on the same site. Contrary to this shift, both temperature measurements in the undisturbed site indicated only small temperature change along the vertical profile.

A possible explanation for the steep temperature gradient at the disturbed site is the drying of the eroded bank material, which can impact the latent and sensible heat flux movements (Yoshikawa and Hinzman 2003). A secondary explanation could be the difference in thermal conductivities of the organic layer and the underlying soil.

Topographical survey

A topographical survey was conducted on 13 October 2005 and compared with previous surveys carried out during 2004. A comparison between the initial (24 May 2004) and final surveys indicated a 7 m displacement in the upstream site. Figure 5 shows a 3D plot of the study area. A series of small pool-riffle-like features can be observed in the graph.

Morphological changes

A well-defined, small fluvial channel was established in the study site by the end of the 2005 summer. New sediment deposits were noticeable along the stream. Sediment was eroded from the soil matrix and transported through the cryogenic pipes. A view of the channel is shown in Figure 6. Additional photographs are available in Kodial (2005).

Conclusions

The study site has substantially changed in two consecutive summers. Major changes are related to lateral and upstream bank erosion. A continuous channel was established by the end of the 2005 field season. High sediment concentrations, on the order of 40 mg/g, were calculated in both summers. In 2005, cryogenic piping was the key sediment supplier to the area. The thermokarst growth rate was on the order of 3.5 m/year.

Future research work should be focused on cryogenic processes because they influence several components in the system (i.e., soil subsidence, sediment characteristics, sediment supply).

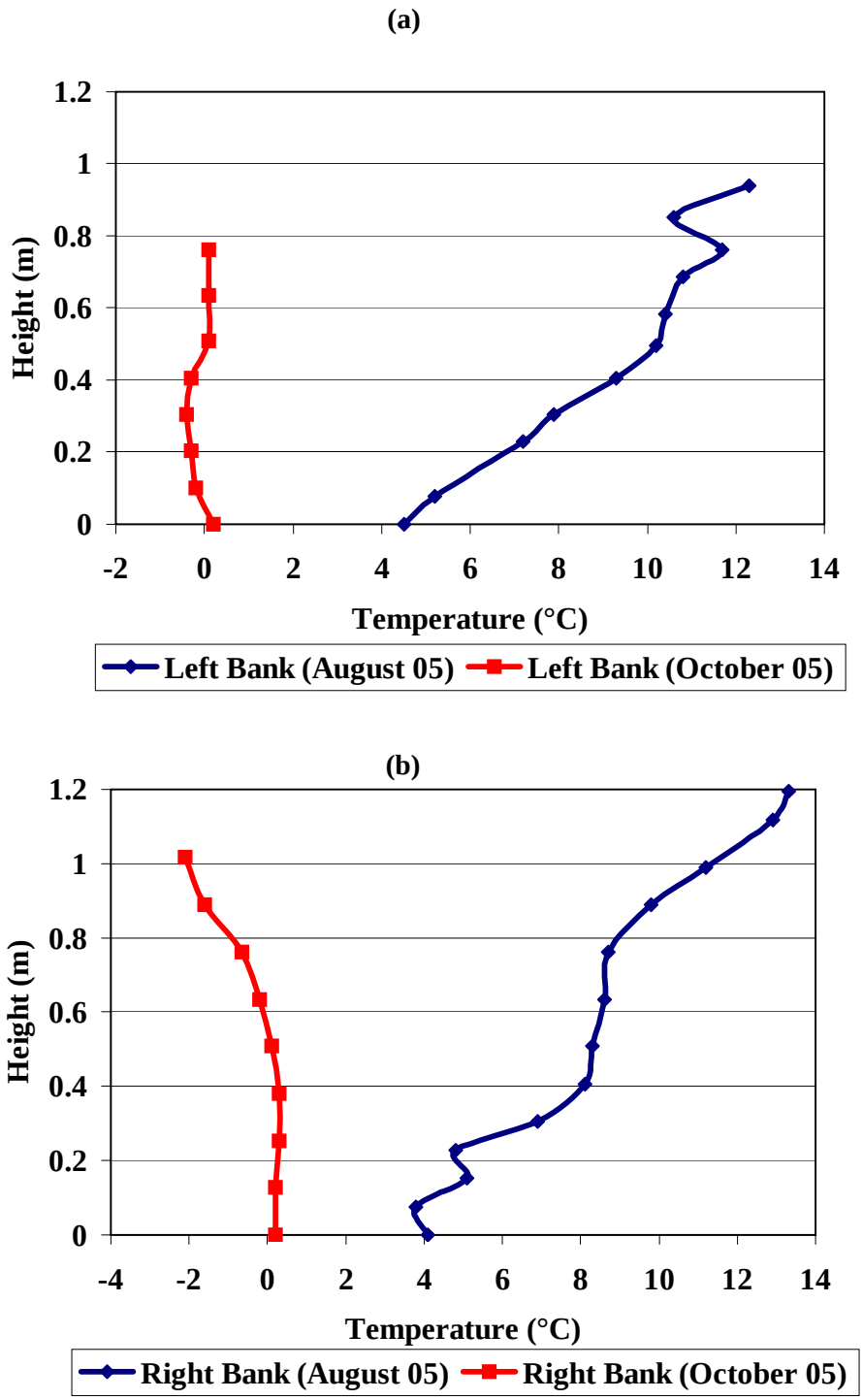

Figure 3. *Temperature profiles at the disturbed site measured on 8/7/2005 and 10/13/2005. (a) left bank temperature profile; (b) right bank temperature profile.*

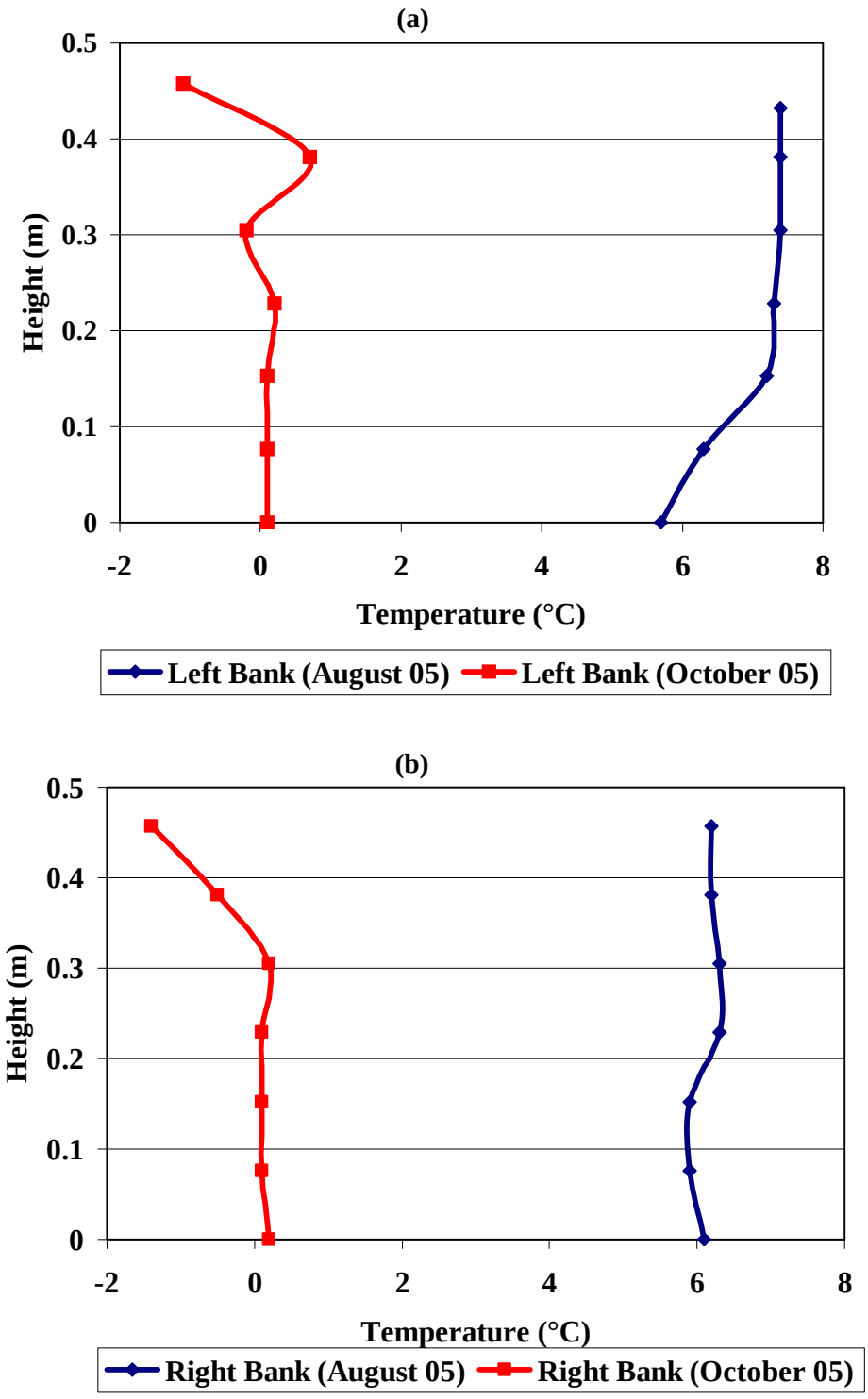

Figure 4. *Temperature profiles at the undisturbed site measured on 8/7/2005 and 10/13/2005. (a) left bank temperature profile; (b) right bank temperature profile.*

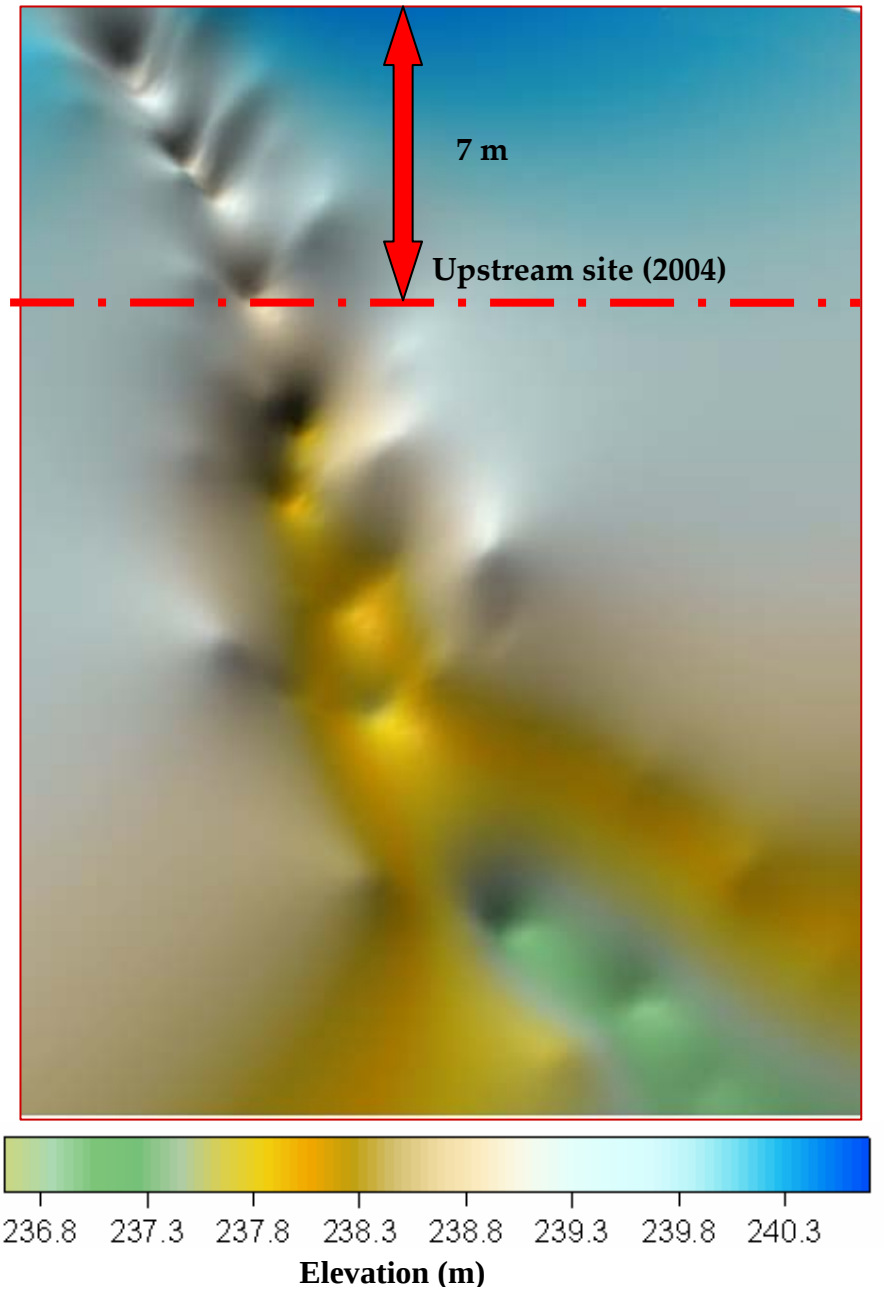

Figure 5. *3-D plot of topographical survey conducted on October 2005. Flow direction is from top to bottom. Reference coordinate in the color scale is 240 m, an arbitrary datum.*

Figure 6. *Channel formed in the study area. Flow direction is from bottom to top (foreground to background). Photograph taken on 17 September 2005.*

References

Holden, J. (2005). Piping and woody plants in peatlands: Cause or effect? *Water Resources Research*, 41, 10 pp.

Kodial, P. (2005). Thermokarst evolution and sediment transport study in the Caribou-Poker Creeks Research Watershed, Alaska. *MS Thesis,* University of Alaska Fairbanks, 102 pp.

Yoshikawa, K. and Hinzman, L. D. (2003). Shrinking thermokarst ponds and groundwater dynamics in discontinuous permafrost near Council, Alaska. *Permafrost and Periglacial Processes* 14, 151-160.

Investigation of the mechanism of arsenic biosorption by modified crab shells

Basic Information

Title:	Investigation of the mechanism of arsenic biosorption by modified crab shells
Project Number:	2005AK43B
Start Date:	3/1/2005
End Date:	2/28/2006
Funding Source:	104B
Congressional District:	AK
Research Category:	Water Quality
Focus Category:	Waste Water, None, None
Descriptors:	
Principal Investigators:	Silke Schiewer

Publication

1. Zhang, H. and Schiewer, S. "Influence of chitosan physicochemical properties on its adsorption of arsenate". Presentation given at the 231st American Chemical Society National Meeting, Atlanta, GA, March 26-30 2006.

Investigation of the Mechanism of Arsenic Biosorption by Modified Crab Shells

Problem and research objectives

Problem: potential pollution of surface waters by mining activities

Alaska's economy is largely resource-based with mining being one of the major economic activities in the interior. Water resources may be impacted by mine tailings and leachate. Toxic heavy metals present in these waste streams may bio-accumulate in the food chain if released into the environment. Arsenic is a toxic metalloid that can be associated with gold mining tailings. Therefore it is necessary to properly treat any effluent and waste from mining operations. Especially for industries like mining, where large quantities of waste streams are generated, it is important to develop cost-efficient clean-up technologies. In this respect, biosorption is a promising avenue, since cheap raw materials from other industries can be used as biosorbents. Biosorption combines the advantages of being, on the one hand, highly efficient at metal removal and, on the other hand, much more cost-effective than comparable techniques such as ion exchange (Volesky 1990).

Results from prior research

The first phase of this research project (see last year's report) investigated the production of biosorbents from crab shells. Crab shells are a waste product of Alaska's fisheries industry, which is an important economic factor in the coastal regions. Our goal for the first phase of the research was to produce a material rich in chitosan, which carries amine groups that can bind arsenate.

Our past research showed that by increasing the concentration of NaOH up to 40%, chitin could be efficiently converted to chitosan. The efficiency of this conversion was measured by FTIR as the degree of deacetylation (DDA). Increased DDA lead, to some extent, to increased metal binding. The optimal binding of arsenate was obtained at pH 5, where arsenate is negatively charged and chitosan should be positively charged. This preliminary research indicated that electrostatic interactions between negatively charged arsenate complexes and positively charged amine groups of chitosan are likely an important binding mechanism.

However, the relationships between deacetylation, availability of amine groups, surface charge, and arsenate binding including the binding mechanism were not investigated in our past work. Therefore the goal of the second phase of our research, as reported in this document, addressed this issue.

Hypothesis and objectives

The main ***hypothesis*** of the present research was that amine groups of chitosan play an important role in As binding by modified crab shells.

Objectives of the research were to investigate the effect of pH and electrostatic properties (sorbent charge), characterize the sorbent, and investigate the binding mechanism, particularly with respect to chitosan amine groups.

Materials and Methods

All experiments used double deionized (DDI) water and ACS reagent grade chemicals. Arsenic stock solution with concentration of 10 mM at pH 5 ± 0.2 was prepared by dissolving $Na_2HAsO_4 \cdot 7\ H_2O$ (J.T. Baker) in DDI water.

Preparation of chitin and chitosan. The freeze-dried Alaska king crab shells were crushed manually. The particle size fraction between 1-2 mm was sieved out for further processing. The decalcification of chitin was carried out according to the method suggested by Muzzarelli (17), by soaking the crushed crab shells in 50 g/L hydrochloric acid (HCl) at room temperature for 24 hours. After that, 5% NaOH solutions were used for a three-fold extraction for about 40 minutes to remove the proteins. The chitin produced was washed to neutral pH after those reactions. Chitosan samples with different DDA were obtained by hydrolyzing chitin in concentrated NaOH solutions (10% to 40% (w/w)) at a temperature of 90-12° C for 5-120 minutes. A sample with DDA ~100% was obtained by successive alkali treatments, the samples were hydrolyzed twice with 40% NaOH at 120° C for 120 minutes each, and washed with distilled water till neutral pH before the 2nd treatment.

Determination of degree of deacetylation (DDA). The DDA for chitosan samples obtained by different processing methods was determined with ^{1}H-nuclear magnetic resonance (NMR) spectroscopy. To obtain the ^{1}H-NMR spectra of low DDA chitosan, 10 mg of sample were dissolved in 2 mL 20% (wt./wt.) deuterium chloride (DCl), while for high DDA chitosan the same amount of sample was dissolved into 1.96 mL of D_2O and 0.04 mL of 20% DCl. The spectra were recorded on a 300 MHz ^{1}H-NMR (Varian-Mercury-300BB) spectrometer. The software (VNMR) was employed for data analysis. Dividing the peak area for the acetyl group HAc, which possesses three hydrogen atoms, by three and the signal area H26 of the ring structure, which possesses six hydrogen atoms, by six, allows calculating the fraction of acetylated groups as (HAc/3)/(H26/6).

Determination of crystalline size and crystallinity**.** Powder x-ray diffraction (XRD) was applied to study the crystallographic properties of chitosan after fine grinding. The x-ray diffraction patterns were obtained on a wide-angle x-ray diffractometer (for $Ka_{1,Cu}$, radiation λ is 1.5405Å). The voltage was 40 kV and the intensity was 40 mA. The 2θ angle was scanned between 3^o to 35^o, and the counting time was 2 s at each step (0.01^o).

Adsorption study. Sorption isotherm and kinetic studies were carried out by using chitosan with 1 g/L concentration and As(V) solution with concentration ranging from 0.75 ppm to 75 ppm. The pH was controlled at 5.0 throughout the adsorption process by adding 0.1N HNO_3 and 0.1N NaOH using an Methohm 719s autotitrator. Continuous mixing was applied by magnetic stirring. Preliminary kinetic studies (data not shown) suggested that 20 minutes were sufficient for equilibration. After 20 minutes, the samples where filtered out through a macrofilter (0.45 μm pore size). The initial and equilibrium concentrations of As(V) were determined by graphite furnace atomic adsorption spectrometry (PerkinElmer AAnalyst 300). The uptake amount q (mg/g) was calculated from the mass balance for arsenic.

Principal findings and significance

Determination of DDA by NMR

Representative ^{1}H-NMR spectra of some chitosan samples with high and low DDA, respectively, are presented in Figure 1. Generally, higher temperature, higher base concentration and longer reaction time will lead to higher DDA. However, to achieve 100% deacetylated chitosan, successive alkali treatment must to be applied.

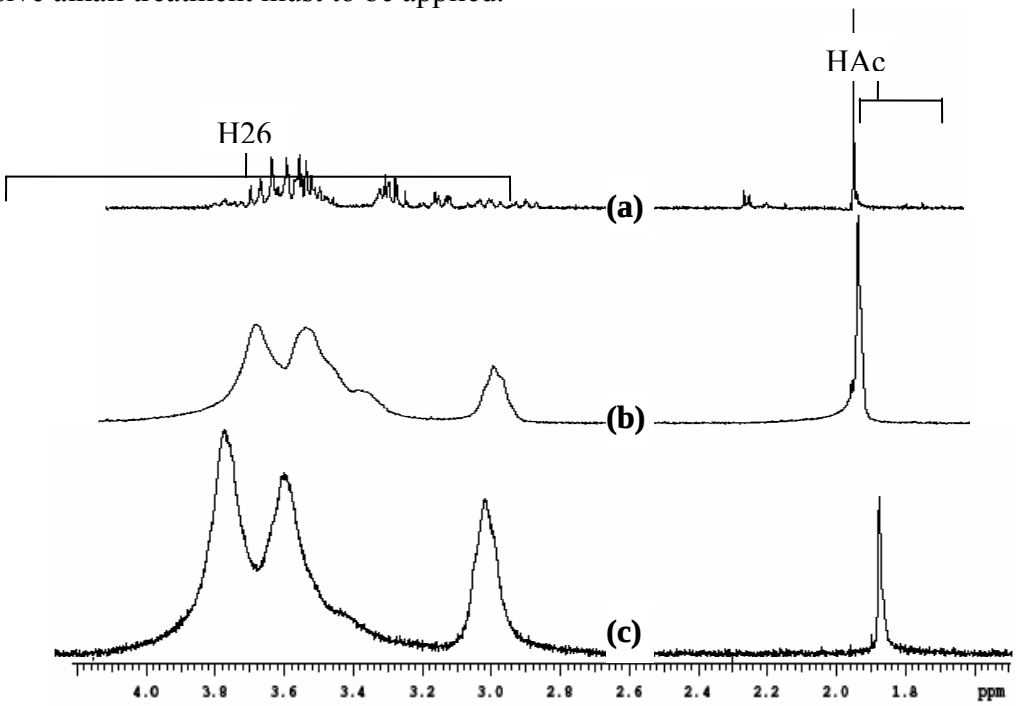

Figure 1. ^{1}H-NMR spectrum for chitosan with (a)DDA~43 (with 20%DCl solution), (b) DDA~71 and (c) DDA~91.

Comparison of FTIR and NMR for determining the DDA

In our past research (see prior report) we used the DDA as determined by FTIR as the measure to quantify the degree to which chitin was converted to chitosan. However, while the FTIR method can reliably be used to compare the efficiency of conversion in qualitative terms, it is not necessarily a quantitatively reliable method. Depending on which peak ratios are calculated based on the obtained FTIR spectra, different DDA values can be calculated from the same samples. Therefore our current research also employed the more complex method of nuclear magnetic resonance (NMR) for determining the DDA. NMR can quantify the numbers of hydrogen atoms in different locations of the chitosan structure and can therefore provide a quantitative measure. The NMR DDA results were used to calibrate the FTIR DDA values. As shown in Figure 2, a very good correlation exists between both methods of measurement. The correlation has a slope of almost one, which is ideal. The offset by a DDA of 0.72 is negligible in the measured DDA range. This indicates that for further research it is possible to utilize either technique. The simpler FTIR method can also be used reliably for DDA determination.

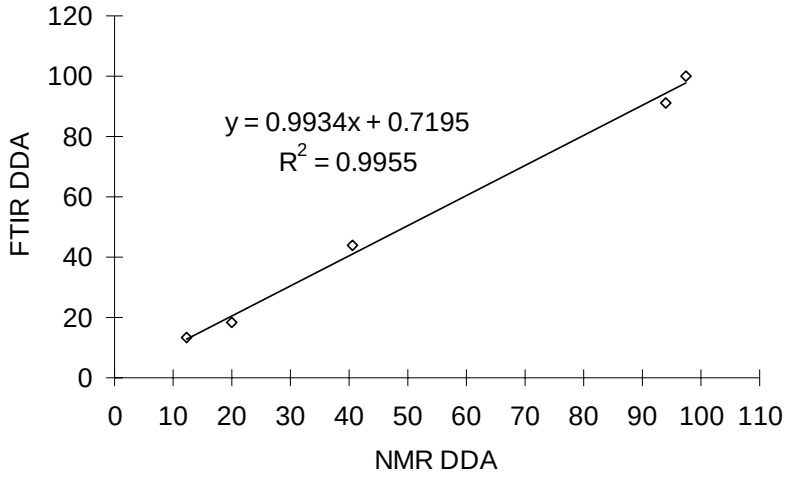

Figure 2. Correlation between DDA values determined by FTIR and NMR, respectively.

Characterization of surface charge by measuring the zeta potential
The zeta potential indicates the surface charge of a particle at a certain distance from the surface (shear plane). For materials that undergo acid base reactions, the surface charge depends on pH. Since chitosan has amine groups, which are positively charged at lower pH values, it is expected that the charge would be positive at low pH and then decrease when the pKa of the surface functional groups is reached.

This is confirmed by our experiment data. As shown in Figure 3, for all chitin/chitosan materials, the surface charge is positive up to ~pH 8. The magnitude of charge increases somewhat with increasing DDA. However, the charges are not directly proportional to the DDA, as one might have expected. At pH 5, for example, where most As binding experiments were performed, only the samples with DDA 13 show a much lower zeta potential than the samples with DDA 42-DDA95, which all have a similar potential.

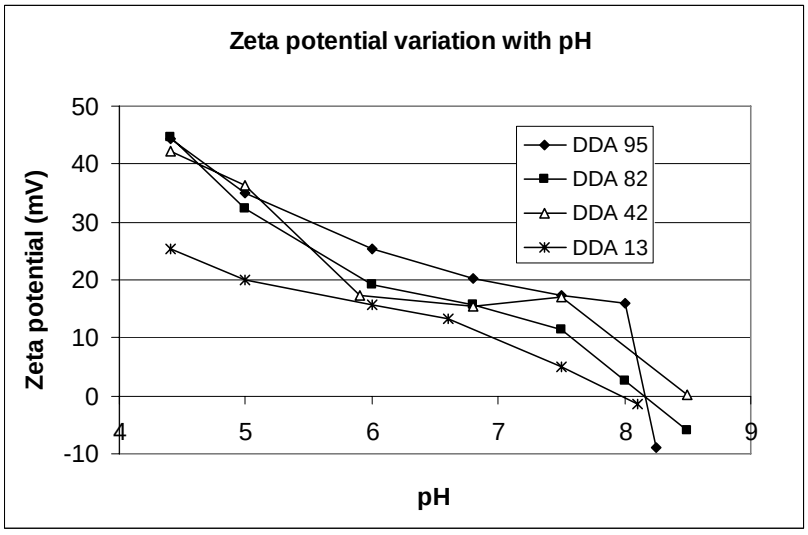

Figure 3. Electrical surface potential of chitin/chitosan with different DDA as a function of pH.

Arsenate uptake

The observation that there are no large differences among samples with DDA > 40 matches the findings with respect to arsenate binding by chitin/chitosan samples with different DDA. Beyond a DDA of 40, no significant improvement of arsenate binding was achieved for increasing DDA, as shown in Figure 4.

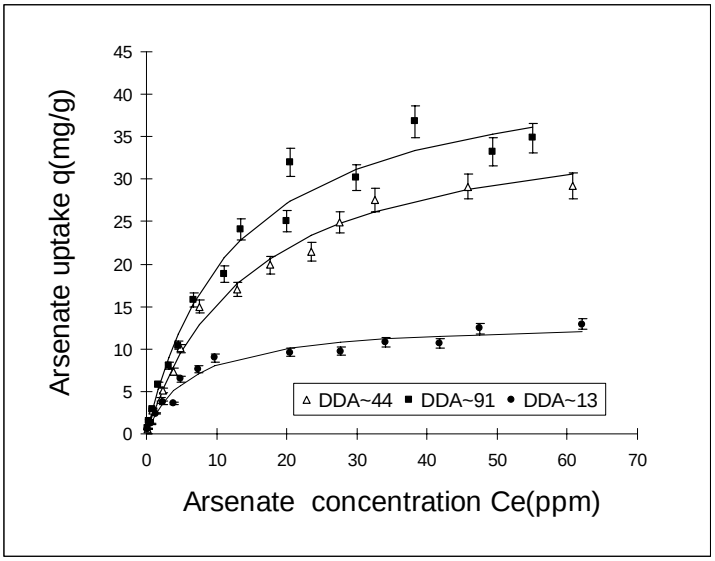

Figure 4. Arsenate sorption isotherms at pH 5 for chitin/chitosan with different DDA.

A potential reason for no increase in arsenate binding beyond DDA 40 may be that not all deacetylated amine groups are available for arsenate binding. Those in crystalline regions may be less accessible than those in amorphous regions. Therefore the crystallinity and crystallite size were measured using XRD. For DDA lower than 50%, both the CrI and the crystallite size did not vary significantly with increasing DDA, whereas at high DDA (≥ 90 %), the CrI reduced to approximately 60% (data not shown).

References

Muzzarelli, R.A.A. 1973. *Chitin*, Pergamon Press, Oxford, p 17.

Volesky, B. (1990) Removal and recovery of heavy metals by biosorption. In: Volesky, B. (Ed.), *Biosorption of Heavy Metals*, CRC Press, Boca Raton, FL, 7-43.

Characterizing sources and growth potential of indicator bacteria in cold region streams

Basic Information

Title:	Characterizing sources and growth potential of indicator bacteria in cold region streams
Project Number:	2005AK45B
Start Date:	3/1/2005
End Date:	2/28/2006
Funding Source:	104B
Congressional District:	Alaska
Research Category:	Water Quality
Focus Category:	Water Quality, None, None
Descriptors:	Enterococci, Antibiotic Resistence Analysis
Principal Investigators:	William Schnabel

Publication

1. Stahnke, G., Schnabel, W., Duddleston, K. and Wilson, T.; Antibiotic resistance analysis of Enterococci in Chester Creek. 2005. In: Proceedings of ASCE EWRI World Water & Environmental Resources Congress, Anchorage, AK, May 15-19.

Antibiotic Resistance Analysis of *Enterococci* in Chester Creek

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Abstract

Antibiotic Resistance Analysis (ARA) is a technique that can be employed to identify the source of fecal indicator bacteria in rural and urban watersheds. In this ongoing study, ARA is being utilized to investigate the sources of *Enterococcus* bacteria in Chester Creek, Anchorage, AK. Possible sources of fecal bacteria in the Chester Creek Watershed include waterfowl, moose, bear, beaver, domestic animals, and sewer/septic inputs. Thus far, 170 isolates have been collected and used for ARA. Results to date indicate that the antibiotic resistance of unknown isolates increases with downstream distance. Isolates originating from moose have shown to be resistant to only five of the eleven antibiotics tested, and indicate that antibiotic resistance in moose may depend on the age of the animal. Canine isolates have shown to be primarily resistant to four of the eleven antibiotics tested including CEP, GEN, KAN, and STR. Further isolate testing using ARA is ongoing and more complete results will be presented at the conference.

Introduction

Fecal contamination is a problem currently being faced in many urban and rural watersheds. Fecal pollution can lead to disease outbreaks and regulatory closure of surface water bodies to recreation and other activities. Efforts to detect fecal indicator organisms are easily achieved, but tracking fecal indicator sources has proved to be much more difficult. Microbial Source Tracking (MST), also known as Bacterial Source Tracking (BST), refers to a group of analytical techniques that can be used to trace the origins of fecal indicator bacteria such as *Eschericia coli* (Scott et al., 2002; Simpson et al., 2002). Antibiotic Resistance Analysis (ARA) is a phenotypic MST technique that can provide reliable results regarding the origination of microbial pollutants. It has been studied and applied in numerous locations such as Virginia, Florida, and California (Graves et al., 2002; Jiang, 2003). Presently, ARA has not been investigated for use in extremely cold climates such as Alaska. The ARA technique is based upon the premise that fecal bacteria in humans and animals differ in their antibiotic sensitivity due to different levels of exposure encountered throughout an animal's life. This difference allows for a library style classification scheme using multi-variate statistical techniques such as discriminant analysis.

Background

Anchorage area streams experience a considerable fecal load from wildlife, domestic animals, and human sources. Twelve of the Municipality of Anchorage's bodies of water are contaminated with fecal coliforms (ADEC, 2003). In addition, Chester Creek is listed on the EPA Clean Water Act under Section 303(d) for contamination by fecal coliforms (Rice et al., 2003). The creek has been studied for years without yielding a decrease in fecal coliform levels, and efforts to study and characterize the fecal coliform problem are ongoing through numerous local, state, and federal agencies.

Potential sources of fecal pollution in Anchorage area streams include waterfowl, moose, bear, beaver, domestic animals, and sewer/septic inputs. Efforts to track the source of this pollution using either phenotypic or genotypic methods of MST have not previously been undertaken.

This study seeks to employ ARA to identify the primary contributors to the fecal coliform load in Chester Creek. This information will allow more informed decisions to be made regarding Best Management Practices (BMPs) for Chester Creek and other Anchorage watersheds contaminated with fecal coliforms.

Project goals are being pursued through the collection and analysis of water samples from five locations in Chester Creek. The first sampling location on Fort Richardson Army Base is relatively pristine with no development upstream of this site. The second and third sites are located at the inlet and outlet to University Lake, a popular recreation area located in a more urbanized area of the stream. University Lake also serves as a no-leash dog park, and fecal contributions from canines are being thoroughly explored at these two locations. There is also considerable channelization between the Fort Richardson site and University Lake, which could impact fecal coliform concentration and survival. The fourth sampling location is at the University of Alaska Anchorage, downstream of two hospitals as well as University Lake. The final sampling location is near Arctic Boulevard, where channelization and development are extensive.

Fecal coliforms as a group are not amenable to ARA due to the wide variety of bacteria that are encompassed within the classification. Instead, ARA is being performed on a fecal coliform subgroup from the genus *Enterococcus* due to their ease of acquisition, culture, and the availability of supporting literature (Wiggins et al., 2003).

Materials and Methods

Samples for Enterococci enumeration are obtained at least bimonthly and processed for use in ARA. A complete schematic of the ARA procedure can be seen in Figure 1. Enterococci samples are collected in sterile 125 ml whirl-pak bags, and immediately transported to the laboratory on gel ice for analysis. Samples are filtered in 20, 50, and 100 mL aliquots through sterile filter funnels (Pall MicroFunnel), with 0.45-µm Gelman GN-6 filters. Filters are then transferred to 50 mm Petri dishes containing mEnteroccocus agar (Difco) and incubated at 37°C for 48 hours. Individual isolates of *Enterococci* appear as red dots and are then transferred using sterile toothpicks into sterile 96-well micro-titer plates (NUNC) filled with 0.2 ml of Enterococcosel broth (BBL). The 96-well plates are incubated for an additional 48 hours at 37°C. A dark brown color in the well indicates a positive response to the esculin catalase test, and these samples are employed for ARA analysis. Isolates that do not hydrolyze esculin (i.e., produce a dark brown color) are not considered to be enterococci and are discarded.

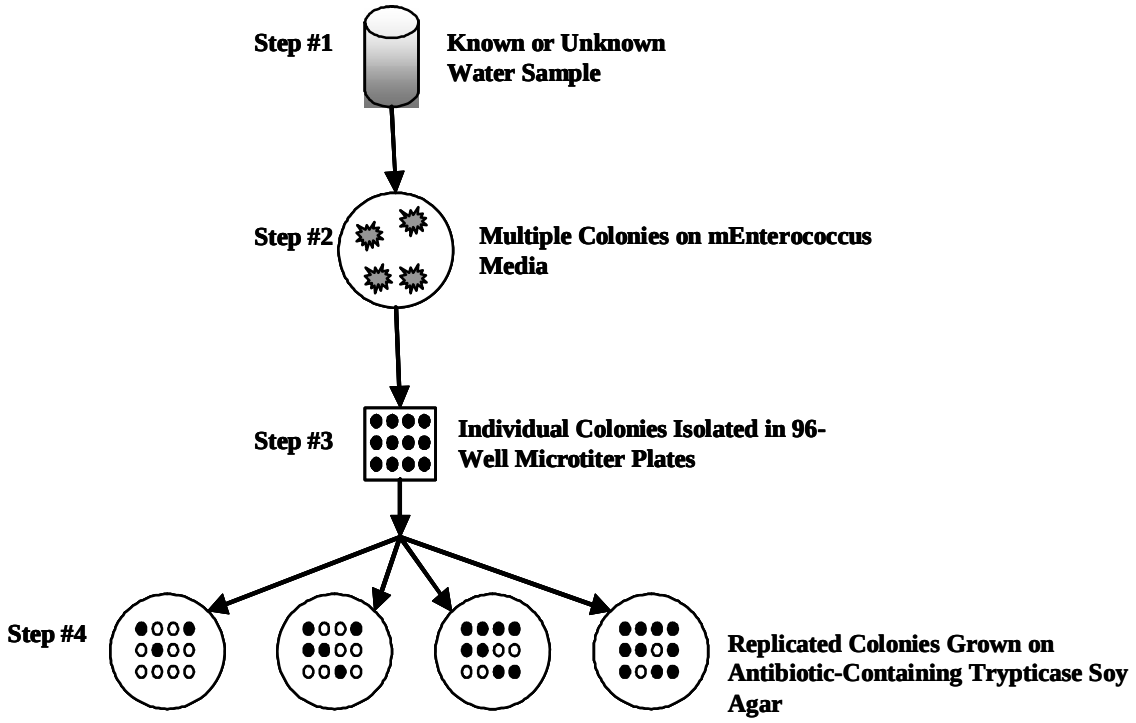

Figure 1. Schematic of ARA procedure.

Based on previous work by Wiggins, eleven antibiotics are used to test the isolates. These include bacitracin (BAC, Sigma), cephalothin (CEP, Sigma), chlortetracycline hydrochloride (CTC, Sigma), erythromycin (ERY, Sigma), gentamicin (GEN, Sigma), kanamycin monosulfate (KAN, Sigma), neomycin sulfate (NEO, Sigma), oxytetracycline hydrochloride (OTC, Sigma), streptomycin sulfate (STR, Sigma), tetracycline (TET, Sigma), and vancomycin (VAN, Sigma). Antibiotic plates are prepared in Trypticase Soy Agar (BBL) in the following concentrations: 10, 25, 50, and 100 µg/ml BAC; 10, 15, and 50 µg/ml CEP; 20, 60, and 80 µg/ml CTC; 10, 30, and 50 µg/ml ERY and TET; 5, 10, and 20 µg/ml GEN; 10, 15, 30, and 50 KAN and NEO; 20, 40, and 80 µg/ml OTC; 20, 40, 60, and 80 µg/ml STR; 5, 10, and 30 µg/ml VAN (Wiggins, 2003).

Following a positive response to the esculin catalase test, bacterial isolates are transferred to 100 mm sterile Petri dishes containing the various antibiotic concentrations in TSA using a 48-prong replica-plater (Sigma). For each test, there are a total of 37 plates containing TSA with antibiotics and two blank plates containing TSA with no antibiotics. To test for the possibility of antibiotic cross contamination, one blank is replica-plated before and after the replica-plating of the antibiotics.

Resistance to antibiotics is determined by comparison with the isolates grown on the plates containing no antibiotics. Isolates that show decreased growth are considered to be sensitive to that concentration of antibiotic. A spreadsheet showing various isolates and their resistances can be seen in Table 1.

Library generation is performed by collecting fresh fecal samples from within the Chester Creek Watershed boundaries as defined by the USGS and the Municipality of Anchorage (Rice et al., 2003). The fecal material is mixed with a sterile saline buffer in amounts varying

from 0.1 – 1.0 g. The samples are filtered as previously described for water samples in aliquots of 20, 50, and 100 ml. When possible, fecal samples are collected within two hours of deposition. Human samples will be collected from the Municipality's water treatment plant as well as from septic pump trucks. ARA is performed as specified above.

Discriminant analysis will be performed using the SAS statistical software. As different combinations of antibiotics are expected to result in different Average Rate of Correct Classification (ARCC), multiple combinations of antibiotics will be analyzed to determine the most appropriate discriminant variable (Wiggins, 1996).

Classification using discriminant analysis will be performed with respect to three different classification schemes. The first is the human vs. non-human classification scheme. This is expected to quantify the relative input from any septic or sewer sources. The second classification scheme, termed "management level classification scheme," sorts isolates into human, domestic animals, or wildlife categories. This is the most useful classification scheme of the three as it is anticipated to provide enough information to allow better BMPs to be established. The majority of the statistical analysis performed will be aimed at establishing a good management level classification with a high ARCC. The third classification scheme is a species level classification and can provide information into what particular species are polluting the creek (i.e. differentiation between ducks, geese, moose, dogs, etc.). As ARCCs for species level classification have historically been low using ARA (50-70%), results are not anticipated to provide conclusive species level discrimination (Wiggins et al., 1999).

In addition to the three different classification schemes, discriminant analysis will be performed for specific sites, at different times of the month or year, and at all locations and all sampling times. This will allow for the determination of source changes related to time or downstream distance.

Results and Discussion

This study is currently ongoing. Thus far, 170 isolates have been cultured under the antibiotic regimen described above. A total of 1100 additional isolates are planned for development of the library and determination of the discriminant variables.

Preliminary results from unknown samples have shown that in general, antibiotic resistance of *Enterococci* bacterial isolates increases with downstream distance, with the highest antibiotic resistances observed near the University Lake and University of Alaska Anchorage, as well as downstream at the Arctic site.

Isolates analyzed from a female adult moose were observed to have some resistance to KAN, NEO, and BAC, but very little resistance to other antibiotics.
Isolates obtained from a calf, however, displayed little resistance to antibiotics other than KAN, NEO, and STR. It is possible then, that the age of the animal influences the ARA profile of the indicator bacteria, due to different exposures throughout the animal's life.
Isolates analyzed from canine fecal samples have been observed to have some antibiotic resistance to CEP, GEN, KAN, and STR but little resistance to other antibiotics. Table 1 shows six isolates from three known sources and their resistances to the various antibiotics.

	Maximum antibiotic concentration showing growth (µg/ml)											
Source	BAC	CEP	CTC	ERY	GEN	KAN	NEO	OTC	STR	TET	VAN	BLANK
Female Moose	10	NG	NG	NG	NG	10	15	NG	NG	NG	NG	G
Female Moose	NG	NG	NG	NG	NG	10	15	NG	NG	NG	NG	G
Female Moose	10	NG	NG	NG	NG	50	15	NG	20	NG	NG	G
Female Moose	10	NG	NG	NG	NG	30	15	NG	NG	NG	NG	G
Female Moose	10	NG	NG	NG	NG	10	15	NG	NG	NG	5	G
Female Moose	10	NG	NG	NG	NG	30	15	NG	NG	NG	NG	G
Calf (Moose)	NG	NG	NG	NG	NG	30	NG	NG	NG	NG	NG	G
Calf (Moose)	NG	NG	NG	NG	NG	30	NG	NG	NG	NG	NG	G
Calf (Moose)	NG	NG	NG	NG	NG	50	15	NG	20	NG	NG	G
Calf (Moose)	NG	NG	NG	NG	NG	50	15	NG	20	NG	NG	G
Calf (Moose)	NG	NG	NG	NG	NG	50	15	NG	20	NG	NG	G
Calf (Moose)	NG	NG	NG	NG	NG	30	15	NG	NG	NG	NG	G
Dog	NG	NG	NG	NG	NG	30	NG	NG	20	NG	NG	G
Dog	NG	15	NG	NG	5	50	NG	NG	20	NG	NG	G
Dog	NG	15	NG	NG	5	50	NG	NG	20	NG	NG	G
Dog	NG	15	NG	NG	5	50	NG	NG	20	NG	NG	G
Dog	NG	15	NG	NG	5	50	NG	NG	20	NG	NG	G
Dog	NG	15	NG	NG	5	50	NG	NG	20	NG	NG	G

NG- No Growth G- Growth

Table 1. Antibiotic resistance of various *Enterococcus* isolates.

These general antibiotic resistance profiles will be used in conjunction with many profiles yet to be obtained to identify which antibiotics will be used as discriminant variables in the discriminant analysis. This will allow more informed decisions to be made regarding the management of Chester Creek and other Anchorage bodies of water contaminated with fecal coliforms.

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Information Transfer Program

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	0	0	0	0	0
Masters	2	0	0	0	2
Ph.D.	1	0	0	0	1
Post-Doc.	0	0	0	0	0
Total	3	0	0	0	3

Notable Awards and Achievements

Publications from Prior Projects

1. 2004AK26B ("Development of Crab Shell Based Biosorbents for Removing Anionic Metal Complexes From Contaminated Water") - Articles in Refereed Scientific Journals - Psoch, C.; Schiewer, S.: Long-term study of an intermittent air sparged MBR for synthetic wastewater treatment. Journal of Membrane Science 260 (2005), 56-65.
2. 2004AK26B ("Development of Crab Shell Based Biosorbents for Removing Anionic Metal Complexes From Contaminated Water") - Articles in Refereed Scientific Journals - Psoch, C.; Schiewer, S.: Critical Flux aspects of air sparging and backflushing in membrane bioreactors. Desalination 175 (2005), No. 1, 61-71.