Central Alaska Network

Stream Monitoring Program

2019 Annual Report



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On the cover: An unnamed tributary to the Ruth Glacier in Denali National Park and Preserve. The Central Alaska Network Stream Monitoring Program sampled this location in 2018 as part of an effort to characterize the biodiversity of small clearwater streams that terminate at large glaciers.

Summary
During the 2019 field season, the Central Alaska Network (CAKN) Stream Monitoring Program made 66 sampling visits to 36 unique stream sites across the network, with each site being sampled between one and three times from early May through late September (Figure 1, Table 1). Extensive fire activity prevented sampling in YUCH, where use of a helicopter is required to visit most sites, and also cut short sampling at remote sites in WRST. Accordingly, sampling during the 2019 field season was focused on road-accessible sites. The program has now sampled 206 unique sites since 2006 (Figure 4). The data collected included instantaneous water chemistry, stream flow, water chemistry samples, macroinvertebrates, stable isotope samples, habitat data and environmental DNA (eDNA) samples. Continuous year-round temperature monitoring at 23 sites across the network is ongoing, and is now in its 11th year at some sites. We successfully used eDNA to detect the presence of multiple fish species at several sites in the network. Recently we have begun applying the same methodology to the detection of multiple aquatic invasive species, including the aquatic plant *Elodea spp*. We recently completed an analysis of replicate samples collected at 58 sites in 3 park units, including Wrangell-St. Elias (WRST); all samples tested negative for *Elodea*. Three site visits were made to the stream gage on the Nabesna River just outside of WRST to perform station maintenance and collect discharge and water chemistry data. This gage has been operated as a cooperative effort between CAKN, The US Fish and Wildlife Service and the NOAA River Forecast Center since 2012. 13 years of continuous discharge and temperature data have now been collected. All of the published CAKN Stream Monitoring Program Annual Reports (2006-2018) are available in IRMA at the Stream Monitoring Project page – (https://irma.nps.gov/DataStore/Reference/Profile/2215908. Beginning in 2012, we stopped issuing extensive annual reports that were published as Natural Resource Technical Reports, and replaced them with field season or abbreviated annual reports. More extensive synthesis reports including analyses of most data collected by the program to date will be published over the next several years. The Stream Monitoring Protocol, including a Protocol Narrative and 18 Standard Operating Procedures, was approved and published in 2017 and is also available in IRMA at the same location.

Background

This study is part of the National Park Service Vital Signs (Inventory and Monitoring) Program for the Central Alaska Network. Climate change and other anthropogenic impacts can be expected to have a dramatic effect on freshwater ecosystems in Alaskan National Parks. The streams monitoring portion of the CAKN Inventory and Monitoring program has been designed to detect trends in the status of important components of lotic ecosystems, whether resulting from climate change or the effects of other natural or anthropogenic drivers. These components include hydrologic regime, geomorphology (habitat structure), temperature regime, water quality and the distribution and abundance of freshwater species. The overall goal is to develop a logistically feasible, repeatable and scientifically robust monitoring program that will detect change in any or all of these ecosystem indicators. To that end, several types of data are collected across the network, including continuous temperature data at multiple sites, continuous stage and discharge data at a single site, and a large suite of discrete physical, chemical and biological data collected during field visits.

Field data collection

Collection of field data during repeated visits to selected sites across the network is a critical component of the CAKN Stream Monitoring Program. Three types of sites are sampled – sentinel sites, GRTS sites, and judgment sites. Sentinel sites, all of which are currently located along the Denali park road and the Nabesna and McCarthy roads in WRST, are sampled approximately annually to maximize our ability to detect changes. Some of these sites are also sampled multiple times per year as a way to help quantify seasonal variability in ecological metrics. Many of the sentinel sites in DENA were originally part of the Long Term Ecological Monitoring (LTEM) program. Although at this point there are no sentinel sites in YUCH, because there are no road-accessible areas, we plan to identify at least one in the near future. GRTS sites are selected using a probabilistic algorithm (known as "Generalized Random Tessellation Stratified", or "GRTS"). Sampling based on a probabilistic survey design allows us to use data collected at these sites to infer conditions at sites that have not been sampled. Although some of these GRTS sites are also sentinel sites (due to a coincident location with an existing sentinel site), most are in the backcountry and will be sampled approximately once every 10 years due to the high cost of accessing them. Judgment sites are selected on an *ad hoc* basis, and for a variety of reasons. For example, the so-called "Travertine stream", located along the DENA park road near Stony Creek, has been sampled occasionally due to its unusual water chemistry and macroinvertebrate fauna. Lake Creek was selected to make sure that a lake outlet stream was included in the set of DENA sentinel sites, since their biogeochemistry and ecology are often quite different from other stream types. Similarly, in 2018 we sampled a tributary to the Ruth Glacier in DENA because it was a small clearwater stream that is disconnected from downstream habitats by the glacier. Streams like this are common in both DENA and WRST, and due to their isolation may harbor unique flora and fauna. They may also be sensitive to climate-induced changes in glacial extent. In all of these cases we are attempting to broaden the ecological scope of streams that have been sampled. This is a critical aspect of the overall sampling approach of the Stream Monitoring Program, because probabilistic sampling schemes do not necessarily capture the full range of ecological conditions across a landscape, something that is important for ecological modeling.

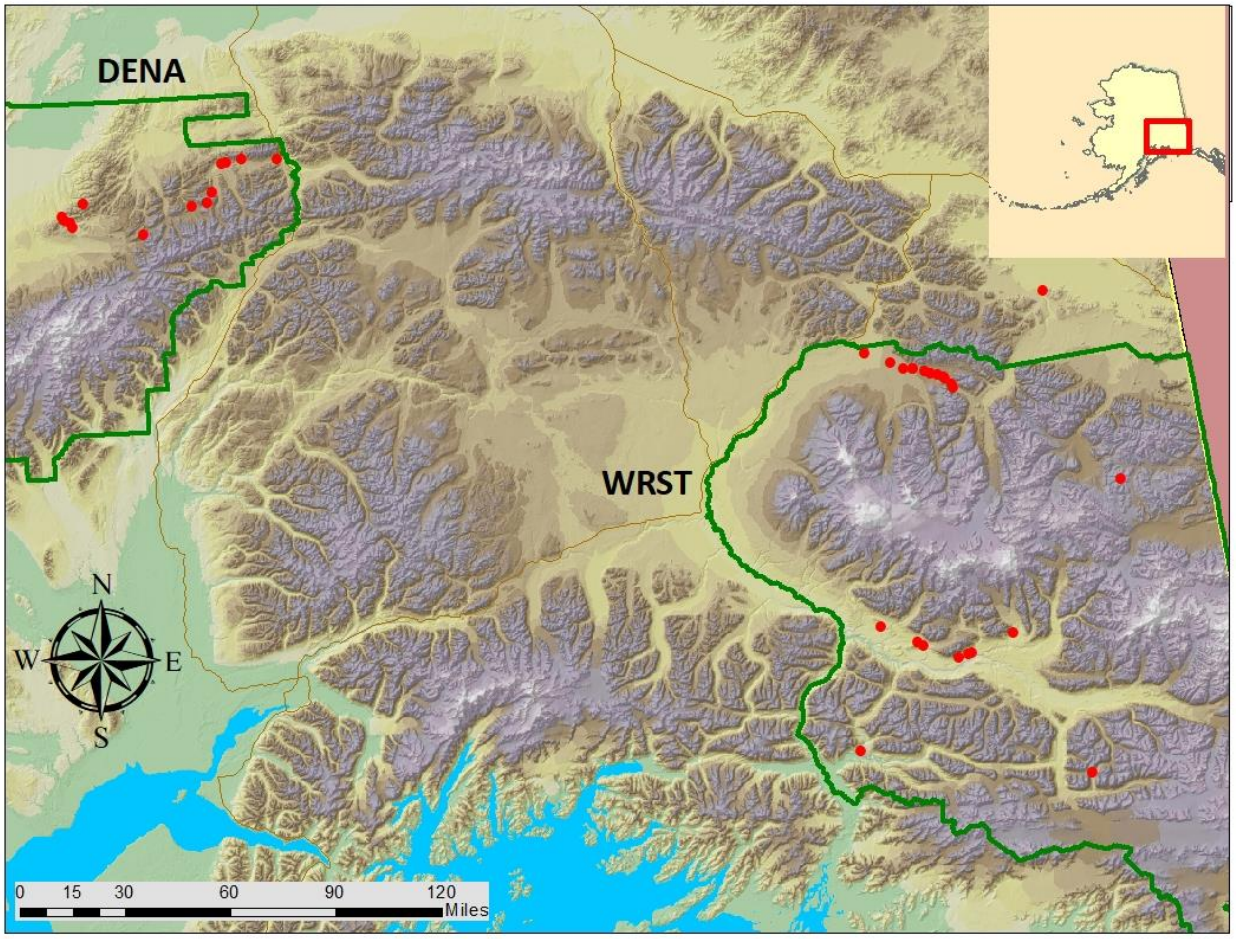


Figure 1. Locations of the 36 stream sites that were sampled by the CAKN Stream Monitoring Program in 2018.

Overall in 2019, 66 sampling visits were made to 36 unique sites in the network (Figure 1, Table 1). Data collected during these sampling visits included instantaneous water chemistry (temperature, conductivity, dissolved oxygen, and pH), habitat characteristics and stream flow, as well as water chemistry samples, stable isotope samples, benthic invertebrate samples and environmental DNA (eDNA) samples. Not all data were collected during every sampling visit, but at a minimum instantaneous water chemistry parameter values were recorded. In most cases, a qualitative assessment of stream flow, rather than an actual flow measurement, was recorded, due to time and personnel constraints.

Table 2 shows the chemical constituents and parameters that are collected by the CAKN Stream Monitoring Program, along with a brief description of what each can tell us about stream ecosystems. Several of these constituents are important determinants of water quality; therefore, any observed exceedances of Alaska state water quality standards will be reported to the Alaska Department of Environmental Conservation. Through 2019, instantaneous water chemistry data have been collected during 883 site visits, and 392 water chemistry samples have been collected and analyzed. Note that laboratory analyses for the 2019 water chemistry samples have not yet

been completed. We are currently in the process of analyzing all of the instantaneous and laboratory results. There are several objectives for these analyses. First, we are using these data

Table 1. List of sites sampled by the CAKN Stream Monitoring Program in 2019

Site Code	Site Name	Park Unit	Notes
DENA-001	Rock Creek	DENA	Sampled annually, first LTEM site*
DENA-008	Little Stony Creek	DENA	Sampled annually, LTEM site
DENA-017	Tattler Creek	DENA	Sampled annually, LTEM site
DENA-021	Hogan Creek	DENA	Sampled annually, LTEM site
DENA-029	Lake Creek	DENA	Sampled most years
DENA-035	Eureka Creek	DENA	Legacy mining impacts
DENA-036	Friday Creek	DENA	Legacy mining impacts
DENA-GRTS-010	Moose Creek	DENA	Sampled annually, LTEM site**
DENA-GRTS-014	E.F. Toklat tributary	DENA	Sampled annually, LTEM site, [Coal Cr.]
DENA-GRTS-027	E.F. Toklat River	DENA	Sampled annually, LTEM site
DENA-GRTS-060	Savage River	DENA	Sampled annually
DENA-GRTS-084	Igloo Creek	DENA	Sampled annually, LTEM site
DENA-GRTS-104	Sanctuary River	DENA	Sampled annually, LTEM site
NABA2	Nabesna River	Tetlin NWR	Site of the cooperative gaging station
WRST-004	Chalk Creek	WRST	Sampled annually
WRST-006	Rock Creek	WRST	Sampled annually
WRST-011	Rock Creek tributary	WRST	Sampled annually
WRST-013	Gilahina River	WRST	Sampled annually
WRST-015	Little Jack Creek	WRST	Sampled annually
WRST-036	Rufus Creek	WRST	Sampled most years
WRST-039	Caribou Creek	WRST	Sampled periodically
WRST-040	Chokosna River	WRST	Sampled most years
WRST-043	Lakina River	WRST	Sampled periodically
WRST-045	Kennicott River	WRST	Sampled periodically
WRST-051	Long Lake Creek	WRST	Sampled most years
WRST-060	Paco Creek	WRST	Sampled annually
WRST-069	Trail Creek	WRST	Sampled annually
WRST-070	Lost Creek	WRST	Sampled annually
WRST-071	Boyden Creek	WRST	Sampled annually
WRST-072	Crystal Creek	WRST	Sampled most years
WRST-082	Stream nr. Salmon Cr.	WRST	Judgment site
WRST-098	Twelvemile Creek	WRST	Judgment site
WRST-105	Willow Creek	WRST	Judgment site
WRST-GRTS-021	Caribou Creek	WRST	Sampled annually
WRST-GRTS-030	Strelna Creek	WRST	Sampled most years
WRST-GRTS-130	Jack Creek	WRST	Sampled annually

*LTEM sites are sites that were part of the Denali Long Term Ecological Monitoring program, which preceded the Inventory and Monitoring program. **Sites with “GRTS” as part of the Site Code are sites that were selected using an algorithm that generates a probabilistic, spatially-balanced list of potential sampling locations to maximize inference to conditions at unsampled sites. Some of these sites are sampled annually, but most are sampled on a long-term rotation, approximately once every 10 years.

to look for spatial patterns in stream water chemistry, as well as to develop stream classifications that may reveal non-geographic patterns, and how those patterns may relate to other aspects of stream ecosystems, such as species distribution and diversity. These analyses have shown that

there are statistically significant differences among the network parks for a number of chemical parameters, including nutrients (total nitrogen and phosphorous, nitrate and phosphate, dissolved organic carbon), alkalinity, conductivity and sulfate. Secondly, we are using data collected repeatedly at sentinel sites to search for any trends in the value of water chemistry parameters, either at individual sites, or at a regional scale. A statistical power analysis based on data from 12 sentinel sites with at least 5 years of data indicated that the high interannual variability observed for many important water chemistry parameters, nutrients in particular, would require over a decade of data to detect a relatively modest trend (3% per year). Nevertheless, preliminary nonparametric trend analyses indicate potentially interesting changes at several sentinel sites. The results of these analyses will be published in the near future.

Table 2. List of chemical parameters measured by the CAKN Stream Monitoring Program along with a brief rationale for each.

Water Chemistry Parameter	Rationale for Sampling
Instantaneous temperature*	Required WRD core parameter
Continuous temperature	Affects habitat suitability, life history for aquatic organisms
Dissolved oxygen (DO)*	Required WRD core parameter, primary productivity and distribution of biota
pH*	Required WRD core parameter, affects habitat suitability for aquatic organisms
Specific conductivity*	Required WRD core parameter. Indicator of geology, affects distribution of biota, often correlated with mean annual temperature and presence of pollutants
Total nitrogen	Trophic state, nutrient dynamics
Total nitrate/nitrite nitrogen	Measure of biologically available nitrogen, trophic state, biogeochemistry, nutrient dynamics
Ammonia-nitrogen	Biogeochemistry, nutrient limitation
Dissolved organic nitrogen	Biogeochemistry, nutrient dynamics
Total dissolved nitrogen	Biogeochemistry, nutrient dynamics
Total phosphorus	Trophic state, biogeochemistry, nutrient dynamics
Total dissolved phosphorous	Trophic state, biogeochemistry, nutrient dynamics
Orthophosphate	Measure of biologically available phosphorus, trophic state, biogeochemistry, nutrient dynamics
Apparent color	Light availability, carbon metabolism
Silica	Required for diatom growth
Alkalinity	Buffering capacity of water – changes have been related to permafrost degradation
Calcium and magnesium	Measure of water hardness
Base cations/anions	Measure of weathering rates and indication of retrogressive thaw slumps
Dissolved organic carbon (DOC)	Measure of organic matter contributions from terrestrial ecosystems, indication of permafrost degradation, correlated with bioavailable Hg

*represent core water quality parameters required by the NPS Water Resources Division (WRD)

Since 2010, the CAKN Stream Monitoring Program has also been collecting samples for analysis of stable isotopes of oxygen and hydrogen. Through 2019, we have collected and analyzed 194 stable isotope samples. Ratios for the concentration of deuterium (^{2}H) relative to ^{1}H, and for ^{18}O relative to ^{16}O in a water sample can be used together to estimate the relative contributions of different water sources (*e.g.*, glacial, snowmelt, rainfall, groundwater) to stream runoff. We expect climate warming to alter all of these source waters, and to alter their impact, relative or absolute, on the hydrology of network streams and rivers, with subsequent effects on stream and river ecosystems. We are currently working with the Alaska Stable Isotope Facility to analyze these isotope data.

Since 2006, 252 benthic macroinvertebrate samples have been collected by the CAKN Stream Monitoring Program, resulting in the identification of 219 unique taxa. Not counting specimens collected in 2019, we have identified a large number of new state species records for Alaska (28), two new species records for North America, and at least five previously undescribed species, in the collection. Because it is often challenging to identify larval invertebrates to species, many of the taxa in the collection are genus-level identifications, suggesting the true species diversity in our collection is much higher than 219, and that many more state and continental species records, as well as other previously undescribed species are likely in the collection. To address this challenge, the program has adopted a two-pronged approach – collecting adult specimens, and using DNA barcoding. For many aquatic macroinvertebrate species, only adult specimens display sufficiently distinct morphological characteristics to allow a confident assignment of species identity. Accordingly, starting in 2013, the CAKN Stream Monitoring Program began efforts to collect adult macroinvertebrates during routine sampling visits. However, this effort has so far yielded only modest returns, due to the synchronized emergence of adults that characterizes the life histories of most aquatic insect species. Such synchrony means the presence of adults at a given location is dependent on the timing of the sampling visit. The second approach to obtain species-level information relies on DNA barcoding, which refers to the sequencing of a standard portion (called the DNA barcode) of the mitochondrial cytochrome oxidase gene of a larval specimen, and comparing that sequence to a reference library of identified sequences. This reference library, call the Barcode of Life Database (BOLD), is maintained by the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph. Since 2012, the CAKN Stream Monitoring Program has been collaborating with the CCDB to develop a barcode library of Alaskan aquatic invertebrate species.

To date, we have sequenced 490 specimens from the CAKN macroinvertebrate collection. This has resulted in the identification of a substantial number of additional species in our collection. However, the effort is complicated by the incomplete nature of the BOLD library, in that a majority of aquatic invertebrate species are not yet represented in the library. This means that a significant proportion of the DNA barcodes from the CAKN collection return “no match” from BOLD, or that the closest match occurs at a % identity well below the threshold for species separation. Ongoing efforts to expand the representation of aquatic insects in the database should alleviate this challenge in the future. A report summarizing the results of morphology-based taxonomic analysis and DNA barcoding through the 2018 samples is in draft form and will be published soon. In addition, a manuscript describing the new state and continental species records is in preparation.

New taxa added to the dataset based on 2016 collections and barcoding in 2017 include the genus *Deuterophlebia* (the “mountain midges”), collected at two sites in YUCH, and the stonefly species *Mesocapnia variabilis*, collected at Tattler Creek in DENA. In addition, two new potential species of the stonefly genus *Zapada*, and two new potential species of the stonefly genus *Nemoura*, were identified via DNA barcoding (they are separate species, but do not match any known DNA sequence), and corresponding sets of larval morphological characteristics. Based on the latest round of DNA barcoding, completed very recently, 9 additional species were added to the dataset, several of which appear to be new state records. The most recent barcoding

effort, conducted in 2019, involved specimens collected through 2018, and the results are still under review.

Benthic invertebrate and basin descriptor data (derived from delineations of the upstream basin of each sampling location combined with data from a large number of GIS layers) have been used to construct several empirical predictive biological assessment indices (O/E indices) for the Central Alaska Network. These indices allow us to quantify the ecological condition of stream ecosystems, and hence their water quality, based on the degree to which the observed macroinvertebrate taxon composition at a site conforms to the expected composition (as predicted by the model). The precision of the latest iteration of the O/E index we have developed (as quantified by the standard deviation of derived reference site scores [0.17]) is comparable to other large-scale indices developed in the lower 48 states. These constitute the first set of O/E biological assessment indices ever developed for Alaska. In 2018 the CAKN received funding to expand and refine this bioassessment tool and apply it to quantifying the ecological condition of historically mined sites across the network. This project will take advantage of data collected by multiple agencies, both inside and outside of network parks, and will include the parallel development of a predictive Multi-Metric Index (MMI), a complementary method for quantifying stream ecological condition. The project is being conducted in collaboration with the BLM Assessment, Inventory and Monitoring program and with the Western Center for Monitoring and Assessment of Freshwater Ecosystems at Utah State University. We expect the results of this effort to be published in 2020.

Continuous temperature data collection

Beginning in 2008, continuously recording temperature loggers have been deployed in streams along the Denali Park Road in DENA, and along the Nabesna and McCarthy Roads in WRST. There are also currently 2 dataloggers in YUCH collecting conductivity data in addition to temperature data. At present, there are temperature loggers deployed at 23 sites across the network (Table 3). In 2008 and 2009, the dataloggers were deployed in late spring and removed in the fall, but they are now left in place throughout the year. Currently the dataloggers are changed out approximately every 2 years, although because temperature data are recorded hourly, there is enough memory to leave them deployed for 5 years in cases where they cannot be retrieved after 2 years. In 2016, all deployed recorders were changed out, as they were nearing the end of their battery life, and duplicate units ones were deployed at 10 sites. Duplicate loggers were added to another 11 sites in 2017. As funding allows, all temperature loggers will eventually be replaced by loggers that record conductivity as well as temperature. Continuous temperature data will allow us to detect changes in the temperature regimes of park streams over time. In 2017-2018, the CAKN worked with staff at the National Center for Ecological Synthesis and Analysis to get all current CAKN stream temperature data cleaned up and entered into a publicly accessible database, the Knowledge Network for Biocomplexity, an international data repository (National Park Service, Central Alaska Network. 2017. In-situ stream temperature monitoring, central Alaska, 2008-2017. Knowledge Network for Biocomplexity. urn:uuid:6ccfbb82-1b69-4638-b3f7-99d0bbaae6e6), as part of an effort to begin large-scale analyses of stream temperature trends across Alaska (Jones, L. J. Clark, S. Mauger and M. Geist 2018. Status of stream temperature monitoring and synthesis of a statewide database for Alaska. Western Division of the American Fisheries Society meeting, May 21-25, 2018, Anchorage, Alaska.).

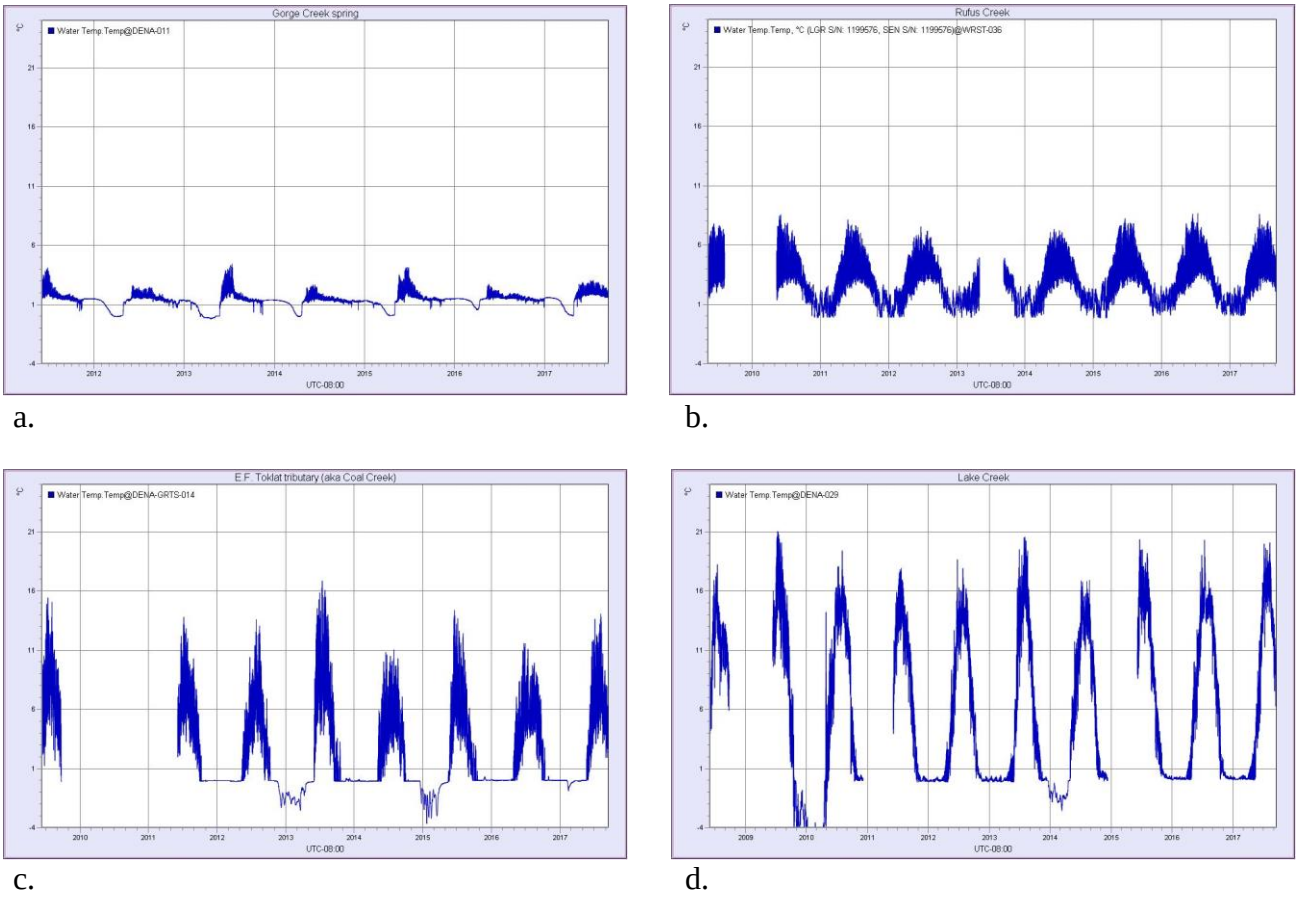
As Figure 2 shows, there is a great deal of variability in the temperature regimes we have observed. Four representative temperature regimes are depicted. Spring-fed creeks (Figure 2a) exhibit very little variation in temperature within or between years, with the temperature essentially remaining between 2 and 4 degrees Celsius year round. Groundwater-dominated streams like Rufus Creek (Figure 2b) exhibit increased, though still modest, variability through the year. Rufus Creek varies from ~3 to 8 degrees Celsius in the summer, and between 0 and 2 degrees in the winter. Streams like this often do not freeze in the winter, or freeze only partly. Runoff dominated streams like Coal Creek (Figure 2c, a tributary to the East Fork of the Toklat River) show more variability in temperature, both through the year and on a daily basis in the summer, and are in general much warmer in the summer than springfed or groundwater

Table 3. Locations of currently-deployed continuous temperature dataloggers in CAKN parks. The dataloggers at Coal Creek and the Amy Lake outlet stream also record conductivity.

Site Code	Site Name	Park Unit	Length of data record
DENA-001	Rock Creek	DENA	Since 2009
DENA-008	Little Stony Creek	DENA	Since 2008
DENA-017	Tattler Creek	DENA	Since 2009
DENA-021	Hogan Creek	DENA	Since 2008
DENA-029	Lake Creek	DENA	Since 2008
DENA-033	Gorge Creek spring	DENA	Since 2009
DENA-034	Wonder Lake inflow	DENA	Since 2012
DENA-GRTS-010	Moose Creek	DENA	Since 2008
DENA-GRTS-014	E.F. Toklat tributary	DENA	Since 2009
DENA-GRTS-060	Savage River	DENA	Since 2008
DENA-GRTS-084	Igloo Creek	DENA	Since 2008
DENA-GRTS-104	Sanctuary River	DENA	Since 2008
WRST-004	Chalk Creek	WRST	Since 2008
WRST-006	Rock Creek	WRST	Since 2008
WRST-013	Gilahina River	WRST	Since 2008
WRST-015	Little Jack Creek	WRST	Since 2008
WRST-036	Rufus Creek	WRST	Since 2009
WRST-051	Long Lake Creek	WRST	Since 2008
WRST-060	Paco Creek	WRST	Since 2011
WRST-GRTS-032	Crystal Creek	WRST	Since 2010
WRST-GRTS-130	Jack Creek	WRST	Since 2008
YUCH-GRTS-003	Coal Creek	YUCH	Since 2015
YUCH-FIRE-001	Amy Lake outlet	YUCH	Since 2018

dominated streams. Streams like this warm up faster in the spring, cool down faster in the fall, and usually freeze over completely during the winter. Coal Creek varies between ~4 to ~15 degrees Celsius in the summer and stays at or below 0 degrees throughout the winter. Lake outlet streams like Lake Creek (Figure 2d) are similar to runoff dominated streams in terms of intra-annual variability, but tend to be much warmer in the summer as their source tends to be the epilimnion of the lake. In addition, lake outlet streams show less diurnal variability than runoff dominated streams. Lake Creek varies between 12 and 20 degrees Celsius during the summer, and stays at or near 0 degrees throughout the winter.

Exploratory analysis of the data collected to date has revealed a variety of temperature regimes among network streams, and substantial variation among years at some sites (Figure 2), but as of yet have not revealed any temporal trends. Interannual variability in summer temperatures



a. b.

c. d.

Figure 2. Representative continuous temperature data. All data are shown on the same vertical scale. 2a (upper left) shows data from a spring-fed creek near the Eielson Visitor Center in DENA. 2b (upper right) shows data from Rufus Creek in WRST. 2c (lower left) shows data from a tributary of the East Fork of the Toklat River in DENA (sometimes called Coal Creek). 2d (lower right) shows data from Lake Creek in DENA, the outlet stream for Wonder Lake.

appears to be driven at least in part by summer air temperature and precipitation, as expected. For example, summer temperatures in Denali streams were substantially higher in 2013, which was abnormally hot and dry, than in 2014, which was abnormally cool and wet (Figure 2a, c, d). As expected, the largest interannual difference occurs in the runoff dominated stream (Figure 2c). Formal analyses of the temperature data collected to date are in progress.

Nabesna River cooperative gaging station

2019 marked the eighth field season for the collaborative operation of the Nabesna River gaging station. This is currently the only active gaging station located within or downstream of a network park unit. This station, located a few miles downstream of WRST in Tetlin National Wildlife Refuge (NWR), has been operated by the USFWS Water Resources Branch since 2007,

and was scheduled to be removed in 2012. However, the CAKN Stream Monitoring Program has entered into a collaboration with the USFWS Water Rights Branch, Tetlin NWR and the National Weather Service Alaska River Forecast Center (NWS) to maintain the station indefinitely. Under this agreement, the CAKN Stream Monitoring Program is responsible for field data collection and station maintenance, Tetlin NWR provides staff time and logistical support for field visits, and the Water Rights Branch and NWS are responsible for technical assistance, data management and analysis, and publication of the data. In 2012 the CAKN Stream Monitoring Program installed a new datalogger and GOES satellite transmitter to provide real-time river stage/discharge and air and water temperature data. Real-time stage/discharge and temperature data are available at the NOAA Hydrometeorological Automated Data System (HADS) website (http://www.nws.noaa.gov/oh/hads/). The NWSLI Identifier for the Nabesna River station is NABA2. The NESDIS Identifier is FED0E484.

In 2019, we made three visits to the gaging station. The March visit, when an under-ice discharge measurement is normally conducted, was cancelled when the scheduled helicopter was unable to meet the crew. Additional visits were made in late May, early August, and mid-September for discharge measurements, monument surveys, and routine station maintenance.

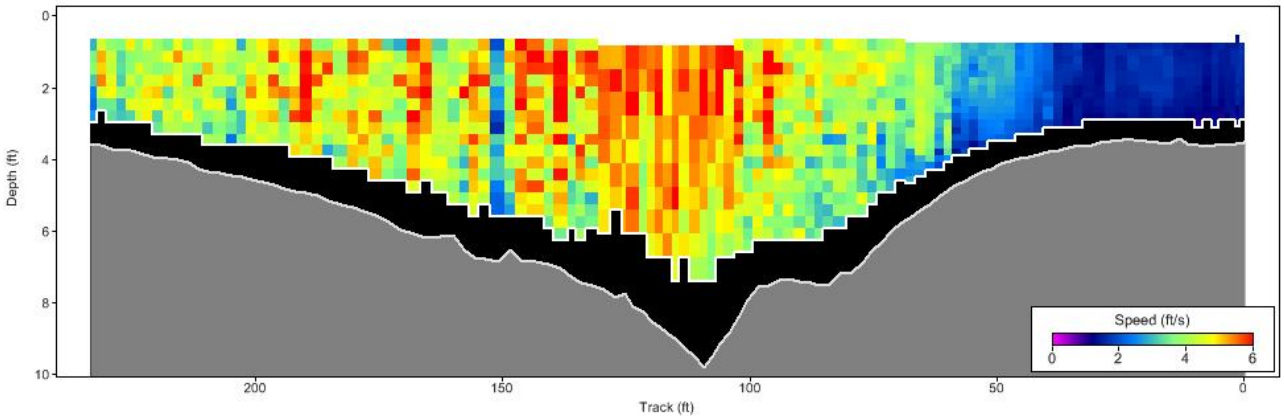


Figure 3. Example of discharge data obtained using an Acoustic Doppler Current Profiler at the Nabesna River. The data include a bathymetric profile of the river bed, a 2-dimensional profile of water velocity, and an integrated discharge value derived from these.

Discharge data from this station, the only currently operating gage within or downstream of a network park, will be used to assess hydrologic conditions upstream in WRST. As of 2019, we now have 13 years of discharge data, which is approaching the data record necessary to achieve the requisite statistical power to detect long-term changes in flow. Preliminary analyses of the data collected to date have so far revealed no such trends. A formal analysis and data summary for the current period of record, being conducted in collaboration with USFWS hydrologists, is in progress and should be published soon. Since 2013 we have also been collecting water chemistry and eDNA samples during each site visit. Detection of coho salmon in eDNA samples at the Nabesna station was used to help guide the sampling design for an ongoing Alaska Department of Fish and Game project to map salmon distribution in the upper Tanana basin. In 2020 we will be replacing the datalogger and satellite transmitter with an updated model as required to meet new standards for GOES satellite data transmission that go into effect in 2022.

Assessing fish distribution using environmental DNA

The CAKN Stream Monitoring Program has been working with the USGS Molecular Ecology Lab since 2012 to develop methods to detect the presence of multiple fish species and other aquatic organisms in streams and lakes by analyzing DNA present in the environment (eDNA). Currently we use a portable peristaltic pump that allows us to filter large volumes of water in a relatively short time. Shed skin cells, feces and other biological material containing DNA are retained on the filter, which is then preserved in a detergent buffer. The DNA is then isolated and purified in the lab. We have developed a set of mitochondrial DNA markers that in combination allow us to distinguish 42 species of freshwater fish native to Alaska. We use the polymerase chain reaction (PCR) to specifically amplify these segments of DNA and then sequence them individually using next-generation sequencing technology to identify the species present in the sample. The methods were worked out using water samples from an aquarium, so that species composition was known. Importantly, we successfully detected all 6 species present in the aquarium, and there were no false positives (i.e. we did not "detect" any species that were not present).

Our results indicate that the method works well, and that we can accurately assess the fish species composition of a water body based on a moderately sized water sample (0.25 – 2 liters), although there are still challenges in accurately distinguishing among the closely related species of the genera *Cottus* and *Salvelinus*. We now have specifically designed additional primers targeted to those genera that are able to distinguish the 3 species of *Cottus* found in Alaska from one another, as well as the 3 species of *Salvelinus* found in Alaska.

The method was developed primarily using samples collected as part of fisheries surveys in a set of lakes on the North Slope, as having generally contemporaneous species composition data allowed us to refine the method. However we also included samples collected at the Gilahina River in WRST, and from Igloo Creek and Wonder Lake in DENA, where historical fisheries data were available, in the initial development phase. Analysis of the Gilahina River samples revealed the presence of eDNA from most of species that have been documented to occur there (Dolly Varden, rainbow trout, coho salmon, and slimy sculpin). Similarly, analysis of samples from Igloo Creek revealed the presence of eDNA from Arctic grayling and slimy sculpin, the only two species documented in that system. In the Wonder Lake sample, we detected lake trout, burbot and slimy sculpin, representing all of the species documented in that system as well. We are now in the process of analyzing a large number of samples collected from streams and lakes in both the Central Alaska and Arctic Networks. A manuscript documenting the results of this portion of the project has recently been published (Menning, D, T. Simmons and S. Talbot 2020. Using redundant primer sets to detect multiple native Alaskan fish species. Conservation Genetics Resources 12:109-123).

The latest effort to arise out of this collaboration has been to design similar eDNA-based methods for the detection of multiple aquatic invasive species (AIS), as well as species that are cryptic or otherwise difficult to monitor. The first phase of this project has resulted in the development of multiple nuclear and chloroplast markers for the detection of both species of the invasive aquarium plant genus *Elodea* (*canadensis and nuttallii*, as well as a purported hybrid), which is already known to be present and spreading in Alaska. The use of multiple markers increases the specificity of the assay and greatly reduces the likelihood of false positive detections. We have successfully tested this assay using eDNA samples taken from 2 locations

with known Elodea infestations. In 2016, 2017 and 2018, field crews collected eDNA samples from multiple lakes in WRST, as well as in Kenai Fjords, Lake Clark, Katmai, and Yukon Flats NWR in concert with standard "rake" physical surveys for the plant. All of the samples collected in 2016 and 2017 have been tested using this assay (59 total) and all tested negative. A manuscript describing these results should be published later in 2020.

We are currently in the process of expanding this eDNA-based detection methodology to include an additional 10 species with the potential to invade Alaskan aquatic ecosystems in the near future (Table 4). If successful, this effort will lead to the ability to assay simultaneously for the presence of multiple AIS in a single water sample. The long-term goal is to facilitate the development of a multi-agency landscape-scale "early warning" system based on eDNA technology to allow detection of AIS before they are fully established. Such early detection is critical if eradication efforts are to have a chance at success.

Table 4. List of potentially invasive aquatic species we are developing DNA-based assays for. Underlined species are those that we can already detect (the invasive fish species listed can be detected using our existing fish species assay). Priority was determined following consultations with state and federal land management agencies.

High priority

American waterweed – *Elodea canadensis (present and spreading)*
Nuttall's waterweed – *Elodea nuttallii (present and spreading)*
Northern pike – *Esox lucius* [S of the Alaska Range] *(present, possibly spreading)*
Red swamp crayfish – *Procambarus clarkia (present, reproduction documented)*
Atlantic salmon – *Salmo salar (occasionally detected, reproduction not documented)*
New Zealand mud snail – *Potamopyrgus antipodarum (not yet detected)*

Secondary priority

Zebra mussel – *Dreissena polymorpha (not yet detected)*
Signal crayfish – *Pacifasticus leniusculus (not yet detected)*
Northern red-legged frog – *Rana aurora (present, reproduction documented)*
Whirling disease parasite – *Myxobolus cerebralis (not yet detected)*
Chinese mittencrab – *Eriocheir sinensis (not yet detected)*
Spiny waterflea – *Bythotrephes longimanus (not yet detected)*
Northern Pacific tree frog – *Pseudacris regilla (present, reproduction documented)*
Quagga mussel – *Dreissena bugensis (not yet detected)*
American shad – *Alosa sapidissima (rarely detected, reproduction not documented)*

The next planned phase of the project is to include the ability to detect a number of native species that are either cryptic or otherwise difficult to survey for or that may be species of concern. We are currently in the early stages of developing markers for the detection of multiple species of aquatic and semi-aquatic mammals (beaver, mink, muskrat, river otter), amphibians (wood frog, western toad, rough-skinned newt) and birds (American dipper, harlequin duck, 3

species of eider, common merganser, 3 species of grebe, 2 species of swans, and 5 species of loons). Note that we have already developed and successfully tested an assay that allows for the eDNA-based detection of all 5 species of loons.

Cumulative stream sampling and additional data sources

Since 2006, the CAKN Stream Monitoring Program has made 885 sampling visits to 206 unique sites across the network. Figure 4 shows the locations of all 206 sites. Individual sites have been sampled from one to 47 times over that period. Not all data types were collected at each visit, although the 4 core water chemistry parameters (temperature, specific conductivity, pH and

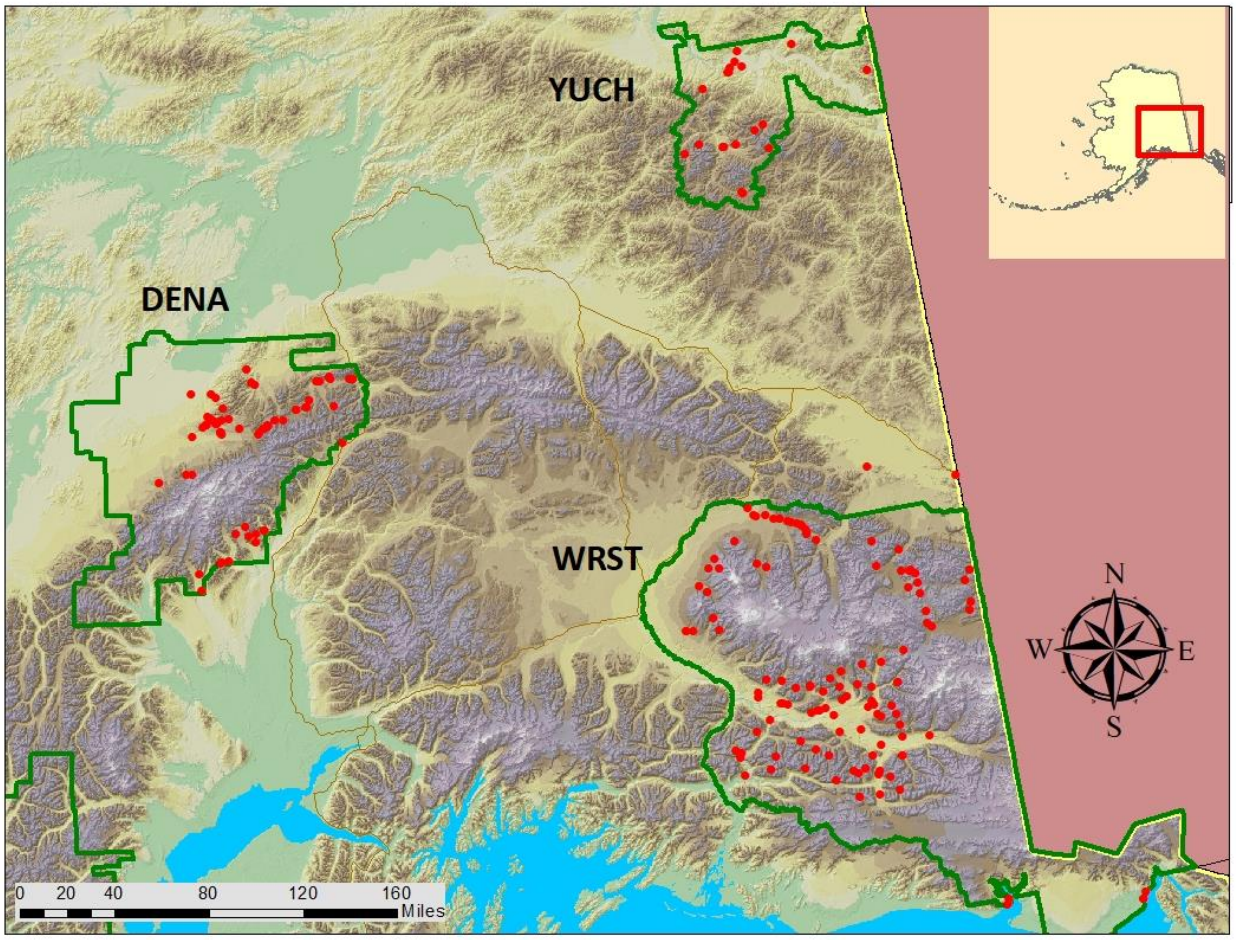


Figure 4. Locations of all 206 sites sampled by the CAKN Stream Monitoring Program since 2006. The majority have been sampled once, but some sites have been sampled as many as 47 times.

dissolved oxygen) were collected every time unless the stream was waterless at the time of the site visit. Examination of Figure 4 reveals that some areas of the network are underrepresented, most notably in YUCH. This is partly due to the phased roll-out of the monitoring program, which initially focused on WRST, and then expanded gradually to include DENA and then YUCH. Other areas that remain underrepresented include the western third of DENA, as well as the far northeastern, southeastern and coastal areas of WRST. This is primarily due to the difficulty and high cost of accessing these areas by helicopter. Not shown in Figure 4 are 12 sites

that were sampled in Tetlin National Wildlife Refuge in 2014 as part of a new cooperative effort with the US Fish and Wildlife Service. These sites in Tetlin NWR were sampled for eDNA to identify fish species, and included both lakes and streams.

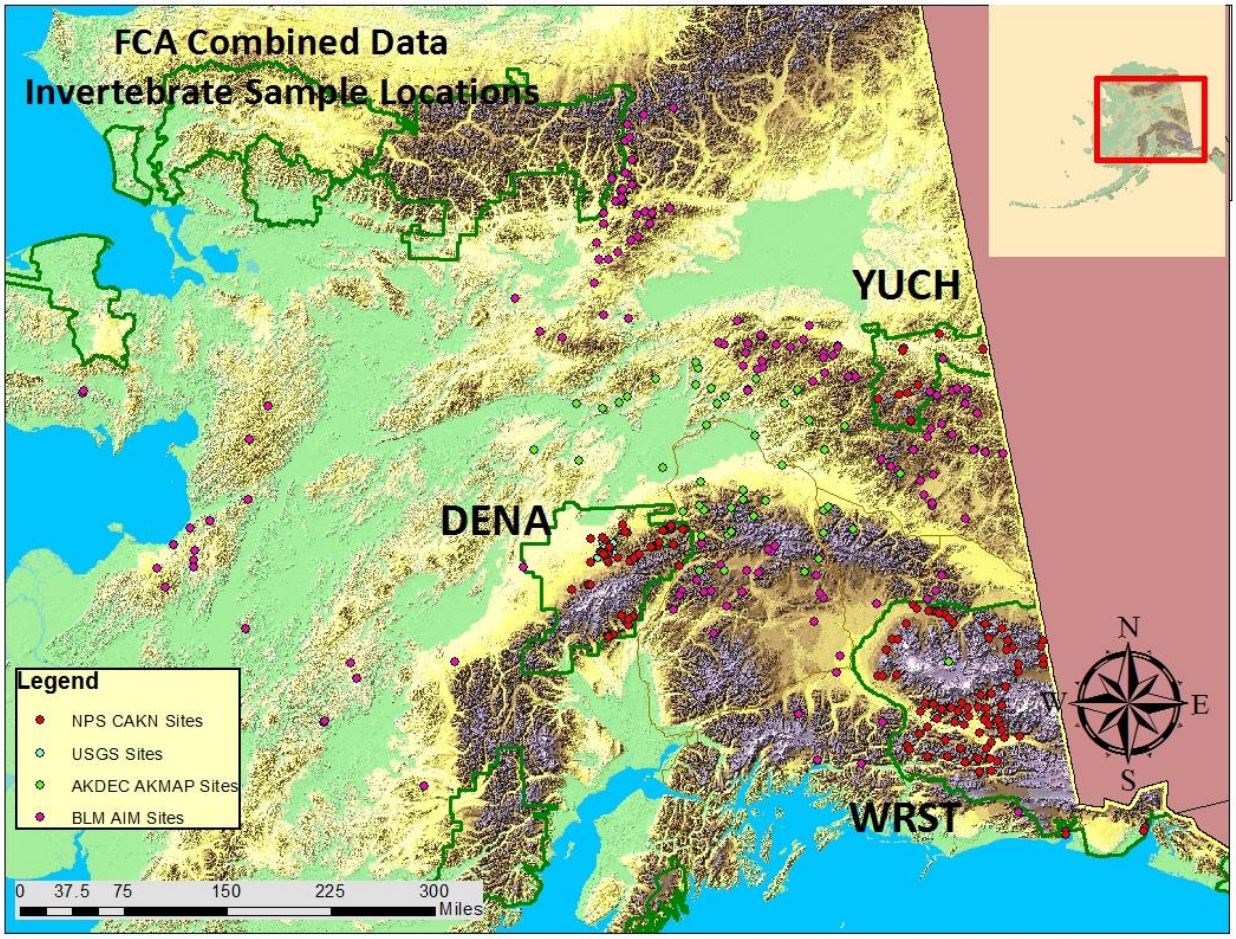


Figure 5. Locations of data sources being incorporated into the development of invertebrate-based bioassessment indices for the CAKN and surrounding areas. Sites shown in red are locations where the CAKN has collected data since 2006 (142 sites). Sites shown in blue are locations where data were collected by the USGS along the McCarthy Road and in the Kantishna Hills from 2007-2009 (21 Sites). Sites shown in green are locations where data were collected as part of an EPA-funded pilot project (AKMAP) by the Alaska Department of Environmental Conservation in 2004-2005 (46 sites). Sites shown in purple are locations where the BLM Assessment, Inventory and Monitoring Program collected data in 2016-2017 (156 sites).

As part of an ongoing effort to improve the performance and geographic scope of the O/E biological water quality assessment indices that the program is developing, as well as to improve understanding of spatial patterns in and determinants of water chemistry regimes and invertebrate communities across the network, we have been actively engaged in locating and incorporating stream data collected by other agencies and programs from sites both inside and outside of the network parks.

In 2004-2005, the Environmental Protection Agency (EPA) provided funds to the Alaska Department of Environmental Conservation to collect biological, chemical and habitat data from wadeable streams in the Tanana Basin. The field protocols used in this effort, known as the Alaska Monitoring and Assessment Program (AKMAP), were very similar to those that the CAKN Stream Monitoring Program uses (which are largely based on EPA methods). AKMAP data were collected at 46 sites across the Tanana Basin (Figure 5). We have also obtained biological, physical and chemical data collected by the US Geological Survey (USGS) at 21 stream sites in DENA and WRST (Figure 5). These data were collected from 2007-2009 using protocols developed for the USGS National Water-Quality Assessment Program (NAWQA).

Although NAWQA field protocols differ in some respects from protocols used by the EPA and CAKN, the resulting data are fundamentally compatible. Finally, as discussed above, we recently obtained funding to develop two related bioassessment indices in collaboration with the BLM Assessment, Inventory and Monitoring Program (AIM) and the Western Center for Monitoring and Assessment of Freshwater Ecosystems at Utah State University. A significant focus of this project is to combine data from multiple agencies to maximize the precision and sensitivity of the indices. This has resulted in the inclusion of data from an additional 156 sites sampled by the AIM program in recent years (Figure 5). The combined dataset now includes data from 449 invertebrate samples collected at 362 unique sites, mostly from interior Alaska, and a majority from in and around the CAKN network parks. We expect initial index development and evaluation to be completed in 2020. One immediate use of these indices will be to evaluate the ecological condition of historically mined streams in DENA and YUCH for which we already have the required data on hand. These indices will also be incorporated into the regionalized Stream Quantification Tool (SQT) currently under development for Interior Alaska. This effort, funded primarily by the BLM with participation from multiple local, state and federal agencies, is designed to provide a framework for assessing and prioritizing degraded streams for restoration and for designing and assessing stream restoration projects. The SQT regionalization is expected to be completed by the fall of 2020.