Technical Report No. 25-01

Baseline Aquatic Biomonitoring for the Lost River Prospect, 2024

By
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and

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February 2025

Alaska Department of Fish and Game **Habitat Section**

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Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat. or long.
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Measures (fisheries)

fork length	FL
mideye-to-fork	MEF
mideye-to-tail-fork	METF
standard length	SL
total length	TL

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	e
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	E
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

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Cover: Curve Creek, tributary to Lost River, July 2024. Photograph by Audra Brase.

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INTRODUCTION

In 2024 the Alaska Department of Fish and Game – Habitat Section (ADF&G) performed the second year of a multi-year baseline aquatic monitoring program in the Lost River drainage. The Lost River system is located on the Seward Peninsula approximately 137 kilometers northwest of Nome, between Brevig Mission and Wales. The objectives of 2024 baseline work were to collect a range of biological data from the aquatic ecosystem at each of the eight baseline sites identified in 2023 and document any additional fish use of the drainage. These data will be useful for preparing environmental documents and permit authorizations as mining exploration activities continue in the area.

Tin mining on the Seward Peninsula has occurred intermittently since the early 1900's. The Lost River mine was discovered in 1903, but production did not begin until Federal funding was provided under the Defense Production Act of 1950 (Lorain et al. 1958). Both placer and lode deposits were considered a strategic interest due to a lack of a domestic supply of tin. Lost River was the largest tin deposit of the United States and was a production mine from 1951 to 1955 (Aleksandrov 2010, Figure 1). Tin was widely used for many household and industry needs prior to World War II, but after World War II aluminum took the place of tin in most applications due to its lower cost and higher durability.

Figure 1.–Lost River Mine, looking towards Cassiterite Creek, 1961. *Photo from Don Grybeck slides, University of Alaska Anchorage, Archives and Special Collections, Consortium Library.*

The Lost River drainage originates at an elevation of 425 meters in the York Mountains and flows south 15 kilometers to the Bering Sea. The river is a clear braided system that flows through a wide unvegetated gravel floodplain. The surrounding landscape is made up of rocky low hills with minimal vegetation, small flowering plants and occasional willows that are no more than 30 centimeters tall. The surrounding hills provide habitat for both caribou and introduced muskox[1]. There is debris from past mining operations scattered throughout the Lost River drainage, although it is primarily concentrated at the old mine site on Cassiterite Creek (Figure 2).

Lost River Mining, Inc. started exploration activities in 2022, and since 2023 has supported two camps for staff and contractors. The lower camp is located near the mouth of Lost River and the airstrip, primarily housing geologists, consultants and visiting employees. The upper camp is approximately 11 kilometers upstream on Lost River and houses the exploration crew and support staff, as it is closest to the drill rigs near the old mine site. Current exploration activities are for minerals associated with lithium including tin, tungsten and fluorspar (Gannon 2022).

Prior to the work performed by ADF&G in 2023, there was little documentation of the fish resources of Lost River. Steidtmann and Cathcart (1922) mention that "grayling and trout" were "fairly well stocked" in the drainages of the surrounding streams, but they made no mention of Pacific salmon species. This report summarizes the periphyton, aquatic invertebrate, and fish samples collected in July 2024, as well as the results of an aerial survey and environmental DNA collection in August 2024.

Figure 2.–Examples of historic mining debris located adjacent (left) and upstream (right) of the upper camp at Cassiterite Creek.

[1] Muskox were reintroduced to the Seward Peninsula from Nunivat in 1970, those Nunivat animals were descendants of muskox that were originally brought from Greenland in 1930 (Woodford 2021).

METHODS

SAMPLING OVERVIEW

The objectives of this second year of baseline aquatic monitoring at Lost River were to document the productivity of the aquatic instream community at the eight sample sites identified in 2023 and quantify adult salmon use of the system. In 2024 there were two sampling events of the Lost River drainage. Periphyton, benthic macroinvertebrates, and juvenile fish were sampled from July 22-25, and an aerial survey and environmental DNA (eDNA) collection occurred on August 22.

The eight sites identified for baseline sampling near the Lost River exploration site (Table 1 and Figure 3) were selected based on whether they could be accessed safely, the availability of appropriately sized rocks for periphyton collection, and whether there was deep enough water to fish minnow traps effectively. Three of the sampling locations are located upriver of the former mine site (Crystal and Esch creeks, and Upper Lost River), three are located downriver (Cassiterite Creek, Middle and Lower Lost River) and two are lower tributaries of Lost River (Curve Creek and Rapid River; Figures 4 – 11). In 2024 the Rapid River baseline site was moved upriver to avoid a section of river that intermittently goes dry/subsurface (Brase and Clawson 2024). The characteristics of the streams at most of the sample sites were very similar – relatively shallow (easily wadable), clear, riffle systems; swiftly flowing over clean rock and cobble. The Lower Lost River site was deeper, and tidally influenced since it was located approximately 500 meters from the river mouth.

At each of the eight baseline sample sites replicate samples of the aquatic community were collected, including benthic macroinvertebrates, periphyton, and fish (Table 1). Measurements of basic water quality parameters (temperature, dissolved oxygen, conductivity, pH and turbidity) were also taken at each site. In 2023, an additional fish sampling site was added in Cassiterite Creek due to fish being observed, but not caught at the first baseline site. This additional site was sampled again in 2024.

Table 1.–List of baseline sites in the Lost River drainage sampled for periphyton, benthic macroinvertebrates and fish, July 2024.

Sample Site	Latitude	Longitude	Invertebrates	Periphyton	Fish
Lower Lost River	65.3927	-167.1480	X	X	X
Middle Lost River	65.4532	-167.1766	X	X	X
Upper Lost River	65.4914	-167.1892	X	X	X
Rapid River[1]	65.4069	-167.1803	X	X	X
Curve Creek	65.4276	-167.1894	X	X	X
Cassiterite Creek	65.4662	-167.1695	X	X	X
Upper Cassiterite Creek	65.4742	-167.1627			X
Esch Creek	65.4751	-167.1816	X	X	X
Crystal Creek	65.4879	-167.1885	X	X	X

[1]In 2024 the Rapid River baseline site was moved upriver from the 2023 location to avoid the portion of the river that occasionally goes dry/ subsurface.

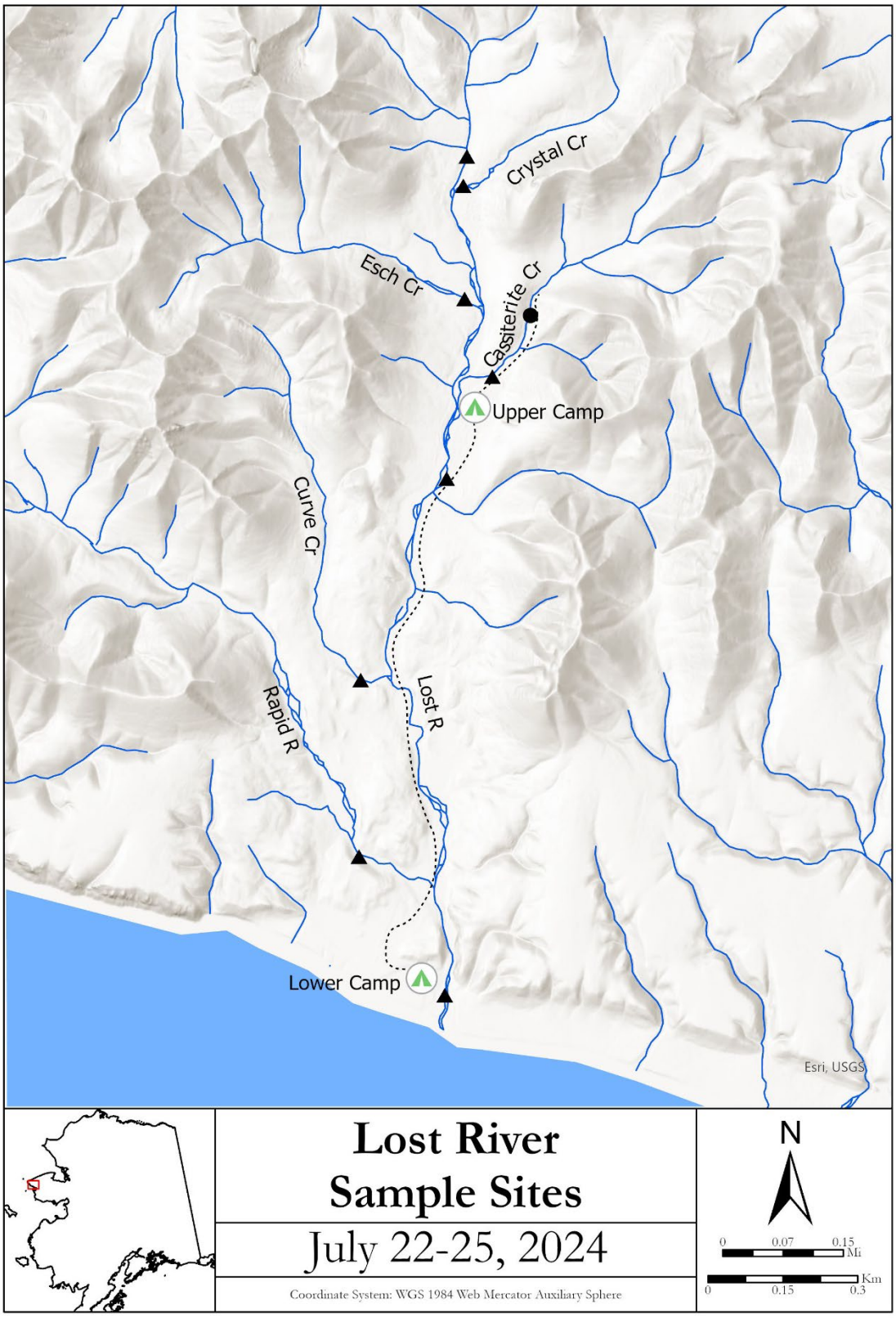

Figure 3.–All locations sampled in the vicinity of the Lost River prospect in July 2024. Triangles were sampled for periphyton, aquatic invertebrates, and fish presence. The circle was only sampled for fish presence. The dashed line is the primitive road that runs along Lost River and Cassiterite Creek.

Figure 4.–Crystal Creek baseline site, upstream (left) and downstream (right), July 2024.

Figure 5.–Esch Creek baseline site, upstream (left) and downstream (right), July 2024.

Figure 6.–Upper Lost River baseline site, upstream (left) and downstream (right), July 2024.

Figure 7.–Cassiterite Creek baseline site, upstream (left) and downstream (right), July 2023.

Figure 8.–Middle Lost River baseline site, upstream (left) and downstream (right), July 2024.

Figure 9.–Lower Lost River baseline site, upstream (left) and downstream (right), July 2023.

Figure 10.–Curve Creek baseline site, upstream (left) and downstream (right), July 2023.

Figure 11.–Rapid River baseline site, upstream (left) and downstream (right), July 2024.

WATER QUALITY

Water quality can be variable in the vicinity of highly mineralized geologic features, therefore point measurements of water quality were taken at each baseline sampling site. These samples were concurrent with aquatic invertebrate and periphyton sampling but were collected above any sampling disturbance. A handheld multiparameter YSI was used to measure water temperature (°C), dissolved oxygen (mg/L), specific conductance (µS/cm), conductivity (µS/cm), and pH. The probe was placed in flowing water, and measurements were allowed to equilibrate for 15 minutes before being recorded. An Orion AQUAfast Turbidity meter was used to measure turbidity (NTU). At each site, the sample vial was rinsed with sample water three times, then filled with flowing water. Three readings of the sample were taken, and the average value of those readings was recorded.

PERIPHYTON

Field Methods

Periphyton is composed of chlorophyll producing organisms, such as algae, attached to submerged surfaces in a waterbody. Algal density and community structure are influenced by water and sediment quality through physical chemical and biological factors that change throughout the year (Barbour et al. 1999). The concentration of chlorophyll-a pigments in periphyton samples provides an estimate of active algal biomass, and is often used in monitoring studies to detect changes in aquatic communities. Periphyton samples were collected at the eight baseline sample sites in the Lost River drainage (Table 1).

Ten smooth, flat, undisturbed and perennially wetted rocks, each at least 25 cm^2 were collected at each site. A 5 cm by 5 cm square of high-density flexible foam was placed on the rock. All the material around the foam was scrubbed off with a toothbrush and rinsed back into the stream. The toothbrush was also rinsed. The foam square was then removed from the rock, and that section of the rock was brushed and rinsed onto a 0.45 µm glass fiber filter receptacle attached to a hand vacuum pump. Material from the toothbrush was also rinsed onto the filter. The water was extracted from the periphyton covered filter using a hand vacuum pump. Just before all the water was pumped through the filter, one to two drops of magnesium carbonate ($MgCO_3$) were added to the water to prevent acidification and additional conversion of chlorophyll-a to phaeophytin.

Filters from each rock were folded in half, with the sample material on the inside, and placed in individual dry paper coffee filters. All ten coffee filters were placed in a zip-lock bag containing desiccant to absorb remaining moisture. The bags were then wrapped in aluminum foil to prevent light from reaching the samples, placed in a cooler with ice packs, then transferred to a freezer in camp. Samples were kept frozen until they were analyzed at the ADF&G laboratory in Fairbanks.

Laboratory Methods

In the lab, periphyton samples were removed from the freezer, the glass fiber filters were cut into small pieces and placed in individual 15 ml centrifuge tubes with 10 ml of 90% spectrophotometric grade acetone. Samples were secured in a vial rack covered with aluminum foil to reduce light exposure and stored in a dark refrigerator overnight. On the following day (~18-24 hours after preparation), samples were placed in a centrifuge and spun at 1,600 rpm for 20 minutes. Samples were then decanted individually into cuvettes and absorption values at 750 nm, 664 nm, 647 nm,

and 630 nm were recorded on a split beam spectrophotometer. Each sample was treated with 80 µL of 0.1N hydrochloric acid for 90 seconds to convert the chlorophyll to phaeophytin and then absorbance was measured at 750 nm and 665 nm.

Trichromatic equations were used to estimate chlorophyll a, -b, and -c concentrations. Phaeophytin was calculated to determine if a chlorophyll-a conversion had occurred, and to correct chlorophyll-a concentrations for the presence of phaeophytin. Additional details regarding periphyton sampling and analysis methods can be found in ADF&G Technical Report No. 17-09 (Bradley 2017).

BENTHIC MACROINVERTEBRATES

Field Methods

At each of the eight sample sites, five samples of the benthic macroinvertebrate community were collected using a Hess sampler (Table 1). The Hess stream bottom sampler has a 0.086 m^2 sample area and material is captured in a 200 mL cod end constructed with 300 µm mesh net. Rocks within the sample area were scoured by hand, and gravel, sand, and silt were disturbed to about 10 cm depth to dislodge benthic macroinvertebrates into the net. The cod end contents were then removed and placed in individual pre-labeled Nalgene bottles with denatured ethyl alcohol to preserve the samples.

Laboratory Methods

Samples were sorted and invertebrates identified by a private aquatic invertebrate lab in Fairbanks. Insects of the orders Ephemeroptera, Plecoptera, Trichoptera were identified to genus. Insects of the order Diptera were identified to genus, except the nonbiting midges of the family Chironomidae. Oligochaeta, Platyhelminthes, Nematoda, and Nematomorpha were identified to class level. Because invertebrates belonging to the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) (EPT) are more sensitive to water quality, the total number of individual specimens of EPT was calculated and compared to groups of other invertebrates, which are less sensitive. Macroinvertebrate density was calculated for each sample by dividing the number of macroinvertebrates by 0.086 m^2, the Hess sampling area. Mean density was estimated for each site by calculating the mean density among the five samples. Taxa richness is reported as the number of taxonomic groups identified to the lowest practical level. Terrestrial organisms were excluded from all calculations.

FISH

Minnow Trapping

During the July sampling trip, ten minnow traps baited with cured salmon eggs were placed upstream and downstream of each of the periphyton and aquatic invertebrate sampling locations. Where possible, traps were placed in a variety of habitats, including cut banks and pools. Typically, minnow traps are placed near large woody debris that provide habitat and shelter for juvenile fish, but there is virtually no large woody debris in the Lost River drainage. In areas with high streamflow, rocks were added to the bottom of each trap for weight and to provide refuge for captured fish. Traps were soaked overnight and checked about 24 hours later. All captured fish were measured for fork or total length, depending on species.

Aerial Survey

The aerial survey was conducted by two observers in a helicopter flying slowly and low enough to accurately count fish but minimize fish disturbance (~30 m above the river). The survey began at the mouth of Lost River and proceeded up the main and side channels, and the tributaries. Because there was no helicopter based at Lost River in 2024, a helicopter was chartered from Nome (Bering Air). The aerial survey crew departed Nome at 10:30am on August 22, and after arriving at Lost River, performed the helicopter survey from approximately 12 – 1pm. A handheld GPS was used to mark large schools of fish, spawning redds and the upper extent of fish presence. Tributaries were flown until no additional fish were seen for approximately 15 minutes.

Environmental DNA

Environmental DNA (eDNA) samples were collected in 2024 in an attempt to positively identify the red colored salmon observed during the aerial surveys of Lost River in 2023 and 2024; and confirm fish use of Rapid River as no fish had been captured in that drainage, although salmon skeletons were found along the riverbank in 2023 (Brase and Clawson 2024). After completing the aerial survey on August 22, replicate eDNA samples were collected from lower Lost River, middle Lost River, and Rapid River (Figure 24). Methods were performed as described in Appendix B1 - Citizen Scientist eDNA sampler collection protocol.

RESULTS

GENERAL

The weather during the July sampling event was ideal, with sunshine, high clouds and light winds. Air temperatures ranged from 60-70°F and there was no precipitation during the four days of sampling. Lost River and its tributaries were all low and clear. Curve Creek, Rapid River, and Cassiterite Creek did not have surface connections to Lost River during the sampling event (Figure 12). Water temperatures were higher, and turbidity was lower than in 2023 when there were heavy rains throughout the July sampling period (Figures 13 and 14).

Figure 12.–Curve Creek 2023 (left) and 2024 (right), note dry/ subsurface connection to Lost River in 2024.

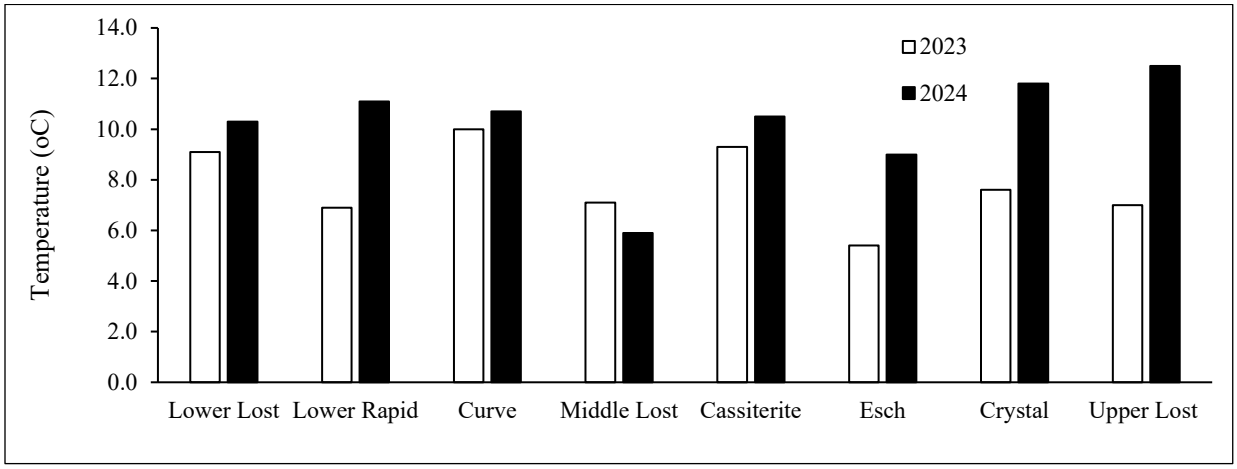

Figure 13.–Water temperature in the Lost River drainage, July 2023 and 2024.

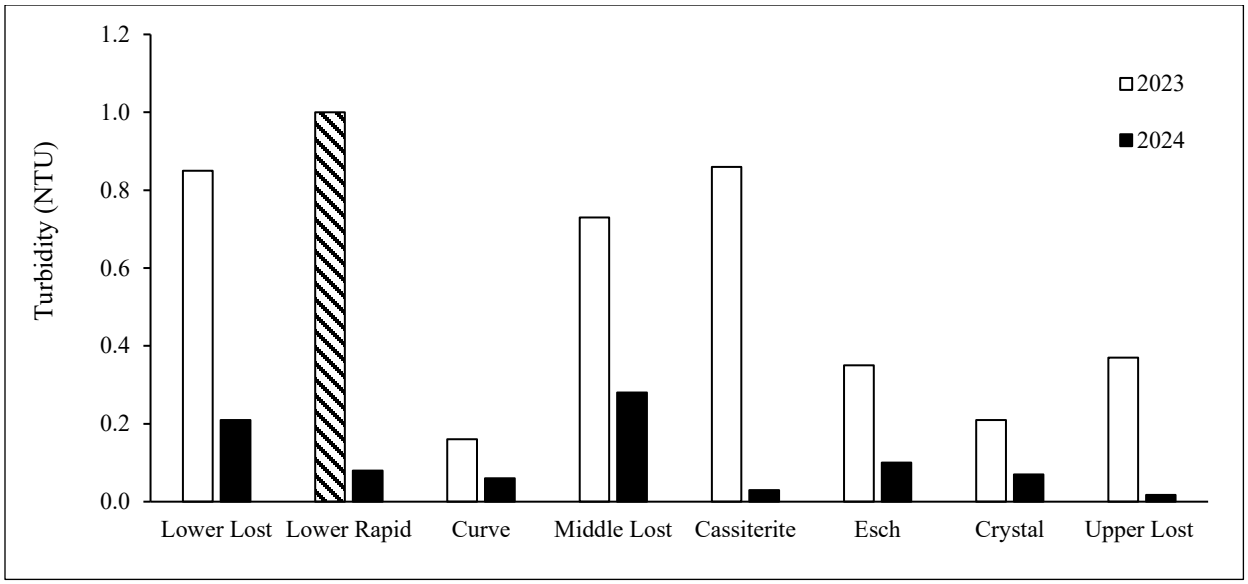

Figure 14.–Turbidity in the Lost River drainage, July 2023 and 2024. Note that the turbidity measurement taken at Lower Rapid River was 11.37 in 2023, however it has been truncated here for illustrative purposes.

WATER QUALITY

Although there was some variability, the water at all sites was cool, alkaline and well oxygenated. The EPA pH standard for aquatic life in freshwater is 6.5 – 9 (USEPA 1086). Point measurements for pH at all sites except for Lower Lost River and Curve Creek were within this range in 2024 (Figure 15 and Table 2). In 2023 the highest pH among all sites was 8.51 at Esch Creek. The water temperature point measurement at Middle Lost River was the lowest among all sites at 6°C in 2024 and was the only site where the temperature point measurement was colder in 2024 than 2023. In 2023, the lowest water temperature point measurement was 5.4°C at Esch Creek. The turbidity at all sample sites was considerably lower in 2024 compared to 2023 (Figure 14).

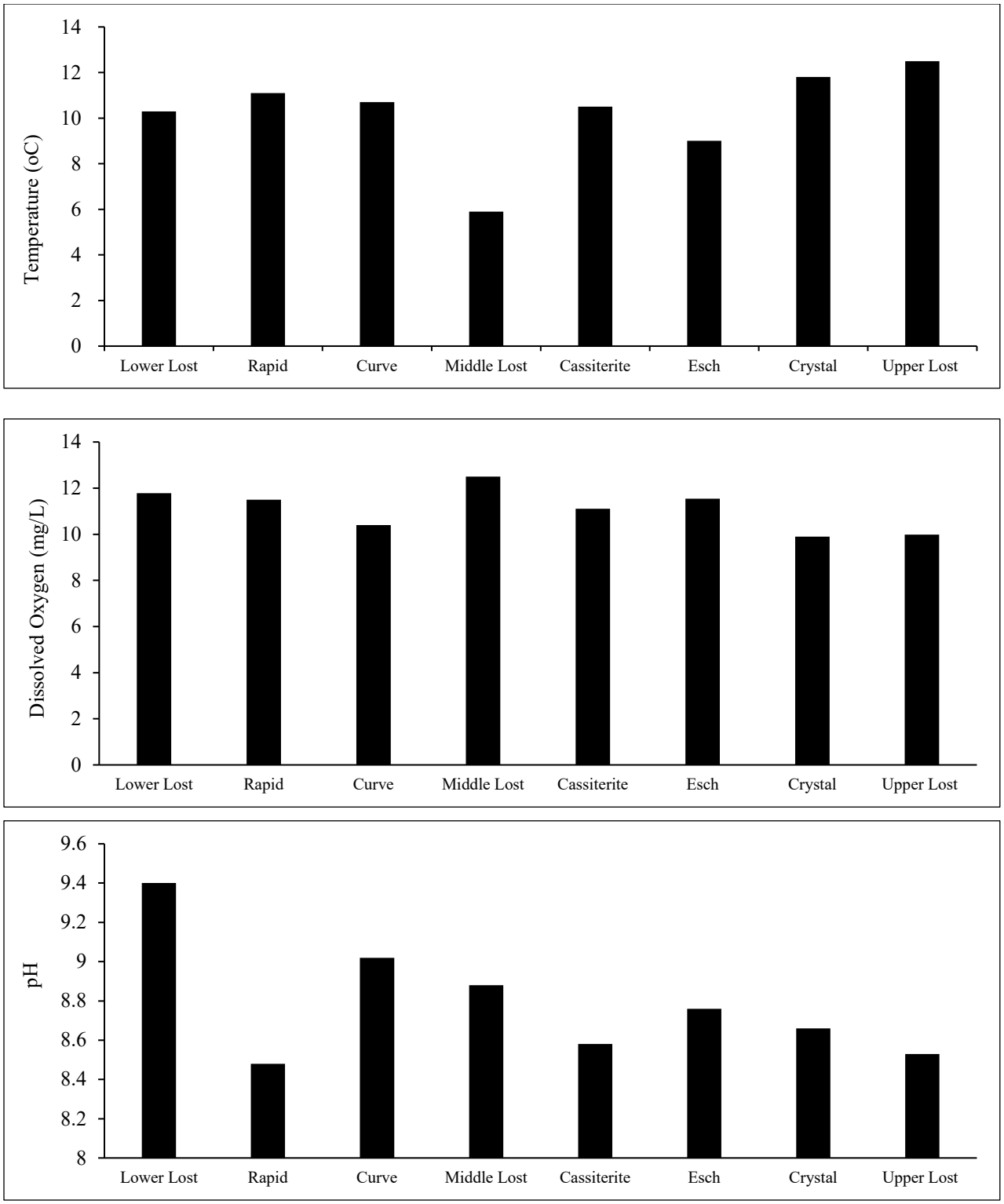

Figure 15.–Temperature (top), dissolved oxygen (middle), and pH (bottom) water quality parameters in the Lost River drainage, July 2024. Sample sites are listed from downriver to upriver - left to right on the x-axis.

Table 2.–Water quality parameters from Lost River drainage sample sites, July 2024.

Sample Site	Temp (ºC)	Dissolved O^2 (mg/L)	Specific Conductance (µS/cm)	Conductivity (µS/cm)	pH	Turbidity (NTU)
Lower Lost	10.30	11.78	258.4	185.6	9.40	0.21
Rapid	11.10	11.50	170.2	125.6	8.48	0.08
Curve	10.70	10.40	207.5	151.2	9.02	0.06
Middle Lost	5.90	12.50	208.2	133.5	8.88	0.28
Cassiterite	10.50	11.11	198.8	143.7	8.58	0.03
Esch	9.00	11.54	189.6	131.9	8.76	0.1
Crystal	11.80	9.90	158.5	118.6	8.66	0.07
Upper Lost	12.50	9.98	223.3	170.1	8.53	0.017

PERIPHYTON

Mean chlorophyll-a concentrations were lower at most sites in 2024 than in 2023 ranging from 0.18 mg/m^2 at Crystal Creek to 2.45 mg/m^2 at Lower Lost River (Figure 16; Appendix A1). These values are low but are within the range of chlorophyll-a concentrations found in other river systems monitored by ADF&G Habitat, such as those in the vicinity of Red Dog Mine and the Arctic-Bornite prospect (Clawson 2024a; Clawson 2024b; Edwards 2024).

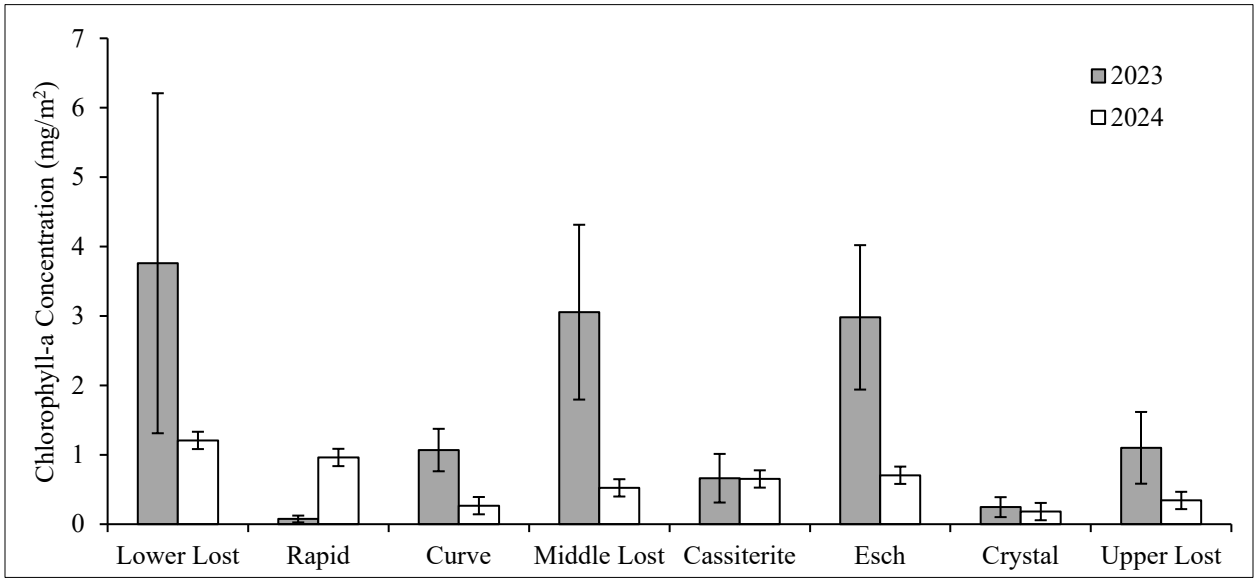

Figure 16.–Mean chlorophyll-a concentrations ± 1 SD for all sites at Lost River, July 2023 and 2024.

BENTHIC MACROINVERTEBRATES

Taxa richness of the benthic macroinvertebrates found in the Lost River system ranged from 6 to 13 taxa per sampling site in 2024, with an average of 10 taxa per site. Some sites had lower taxa richness when compared to 2023, when taxa richness ranged from 7 to 18 taxa, with an average of 11 taxa per site (Figure 17). The average density of BMI found in the Lost River system in 2024 was 613 BMI/m^2 (Figure 18). The Curve Creek site had the greatest density with an average of 1,170 BMI/m^2, and the Cassiterite Creek site had the lowest density with an average of 279 BMI/m^2 (Appendix 2). Benthic macroinvertebrate density varies widely among sample sites at other locations monitored by ADF&G Habitat. For example, BMI density ranged from 28 to 10,393 BMI/m^2 at sample sites near Red Dog Mine in 2023, and from 88 to 4,971 BMI/m^2 at sample sites near the Arctic-Bornite prospect in 2023 (Clawson 2024a and Clawson 2024b). While BMI densities at all sample sites from Lost River fall within the range seen at other monitoring locations throughout Northwestern Alaska, it is notable that the range of densities is much tighter than other sampled drainages, and there are no very high density sites.

The BMI species composition was highly variable among the sample sites. In general, there were more EPT species in the lower portion of the drainage. Samples from Lower Lost and Rapid rivers contained the highest percentages of Plecoptera. Crystal Creek had the highest percentage of Diptera among all sites (Figure 19). Only one Trichoptera was captured in 2024 across all Lost River samples. Trichoptera are typically very low to completely absent in samples from other areas in northern Alaska. In streams near the Arctic-Bornite prospect, Trichoptera were only captured at one out of the nine sample sites in 2023 (Clawson 2024a). Also of note were the numerous oligochaetes (segmented worms) and Platyhelminthes (flatworms) observed in several of the samples from Lost River (Appendix A2).

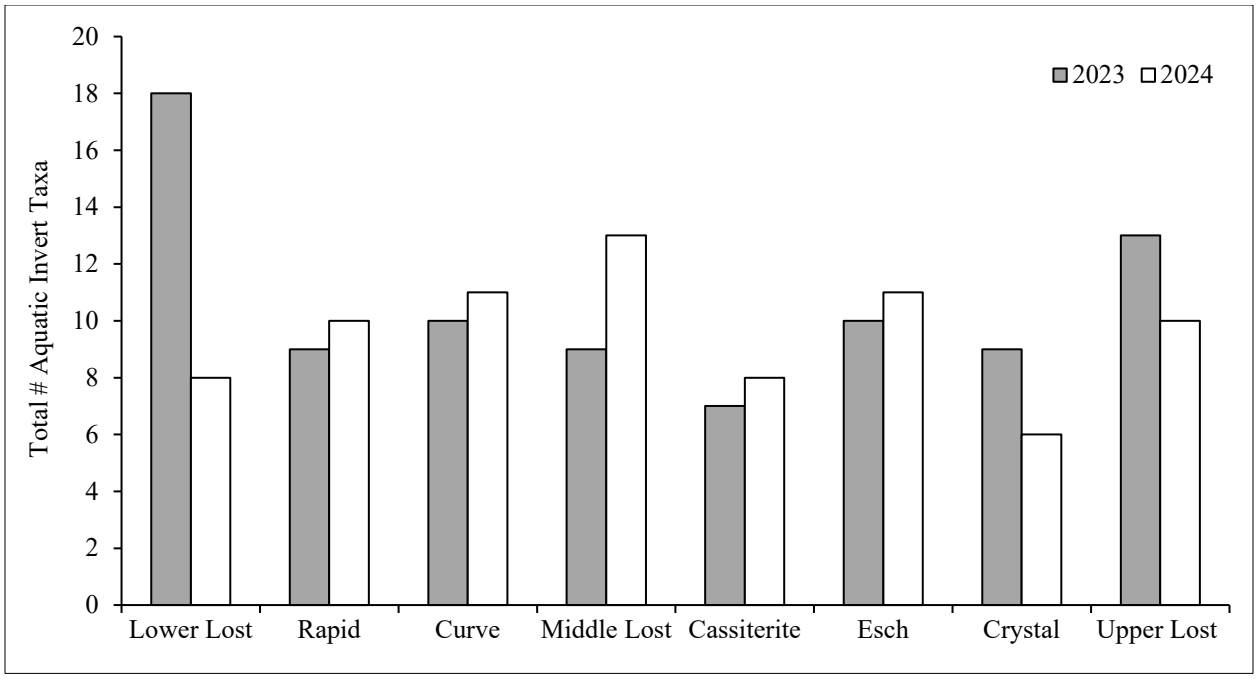

Figure 17.–Benthic macroinvertebrate taxa richness at Lost River drainage sample sites, 2023-2024. Sample sites are listed from downriver to upriver - left to right on the x-axis.

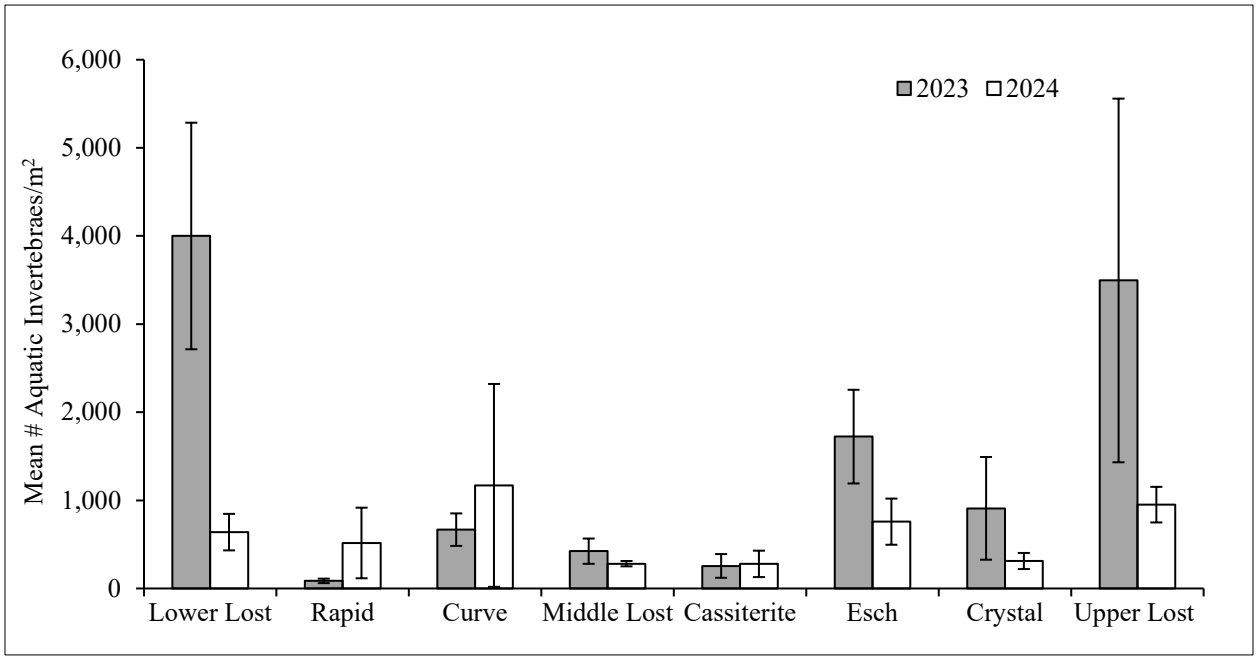

Figure 18.–Mean number of benthic macroinvertebrates/m^2 substrate at Lost River drainage sample sites, 2023-2024.

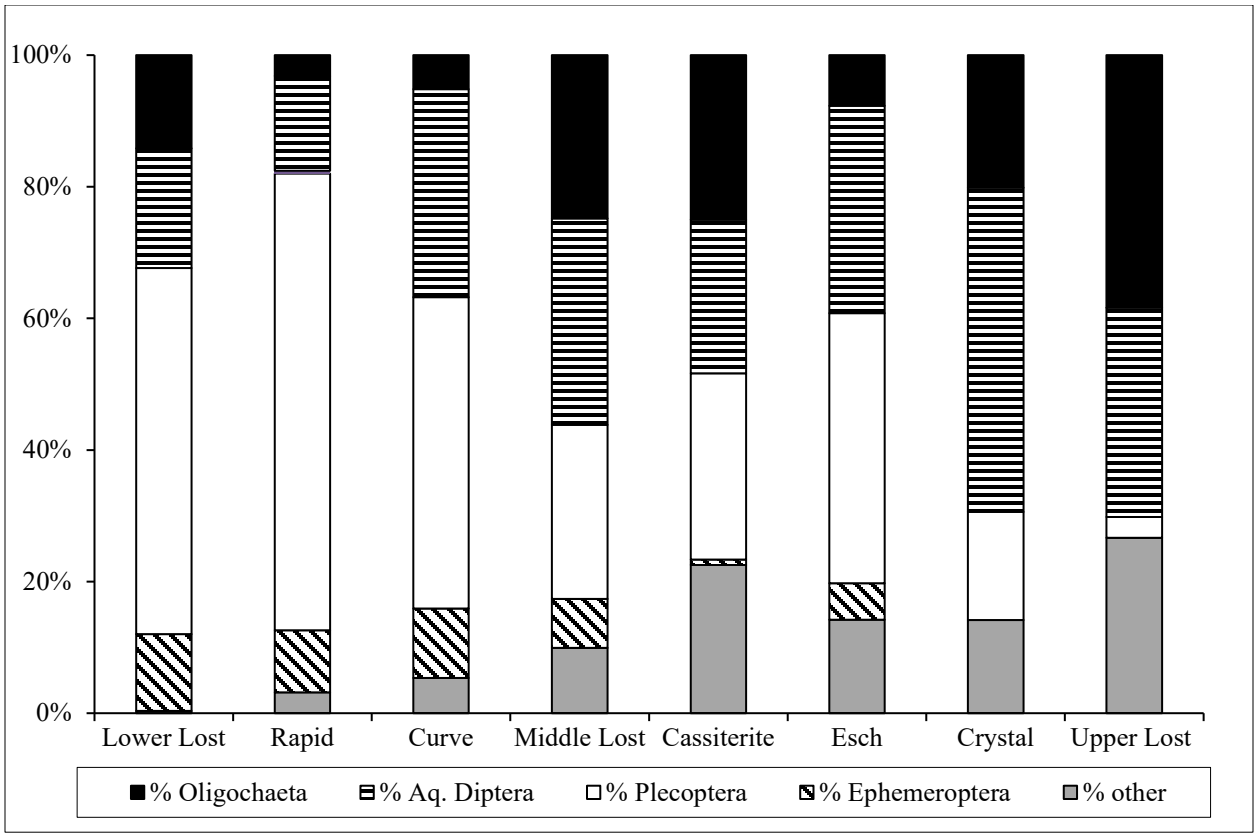

Figure 19.–Mean percent EPT, Oligochaeta, Diptera and other taxa in the Lost River benthic macroinvertebrate samples, July 2024.

FISH CAPTURES

During the July sampling event a total of 43 Dolly Varden were captured using minnow traps in the Lost River drainage, a substantial increase over the 6 fish captured in 2023. The Dolly Varden captured in 2024 were generally robust and ranged in size from 82 – 193 mm (Table 3, Figure 20). No other fish species were captured in minnow traps. However, two large schools of several hundred pink salmon were observed in lower Lost River during July (Figure 21).

Table 3.–Number, mean length, and length range of Dolly Varden captured in minnow traps, Lost River drainage, July 2024.

Sample Site	Number captured	Mean fork length (mm)	Length range (mm)
Lower Lost River	3	129	120-139
Middle Lost River	10	123	99-145
Upper Lost River	0	-	-
Rapid River	0	-	-
Curve Creek[1]	9	140	82-193
Cassiterite Creek[2]	5	142	119-179
Upper Cassiterite Creek[3]	13	151	126-189
Esch Creek	0	-	-
Crystal Creek	3	125	122-129

[1]Approximately 17 additional fish were observed in pools near minnow traps.
[2]Additional fish were observed near minnow traps.
[3]Only 8 traps set at this site.

Figure 20.–Dolly Varden captured at Curve Creek (left), and lower Cassiterite Creek (right), July 2024.

Figure 21.–Two large schools of pink salmon observed in lower Lost River, July 22, 2024.

AERIAL SURVEY

Survey conditions were good on August 22 with cloudy skies, a steady breeze gusting to 20 mph, and no precipitation. In 2024 all tributaries had surface connections to Lost River, in contrast to 2023, when Curve Creek and Rapid River had no surface connections (Figure 22). The total fish count was approximately 90 pink salmon and 3 fish tentatively identified as sockeye salmon (Figure 23). No salmon carcasses were observed and very few spawning redds were noted. This was a sharp contrast to the 2023 aerial survey where over 2,500 fish and many redds were counted.

Figure 22.–Rapid River flow differences between August 2023 (left) and August 2024 (right). The yellow arrow is pointing to an old culvert to provide a sense of location.

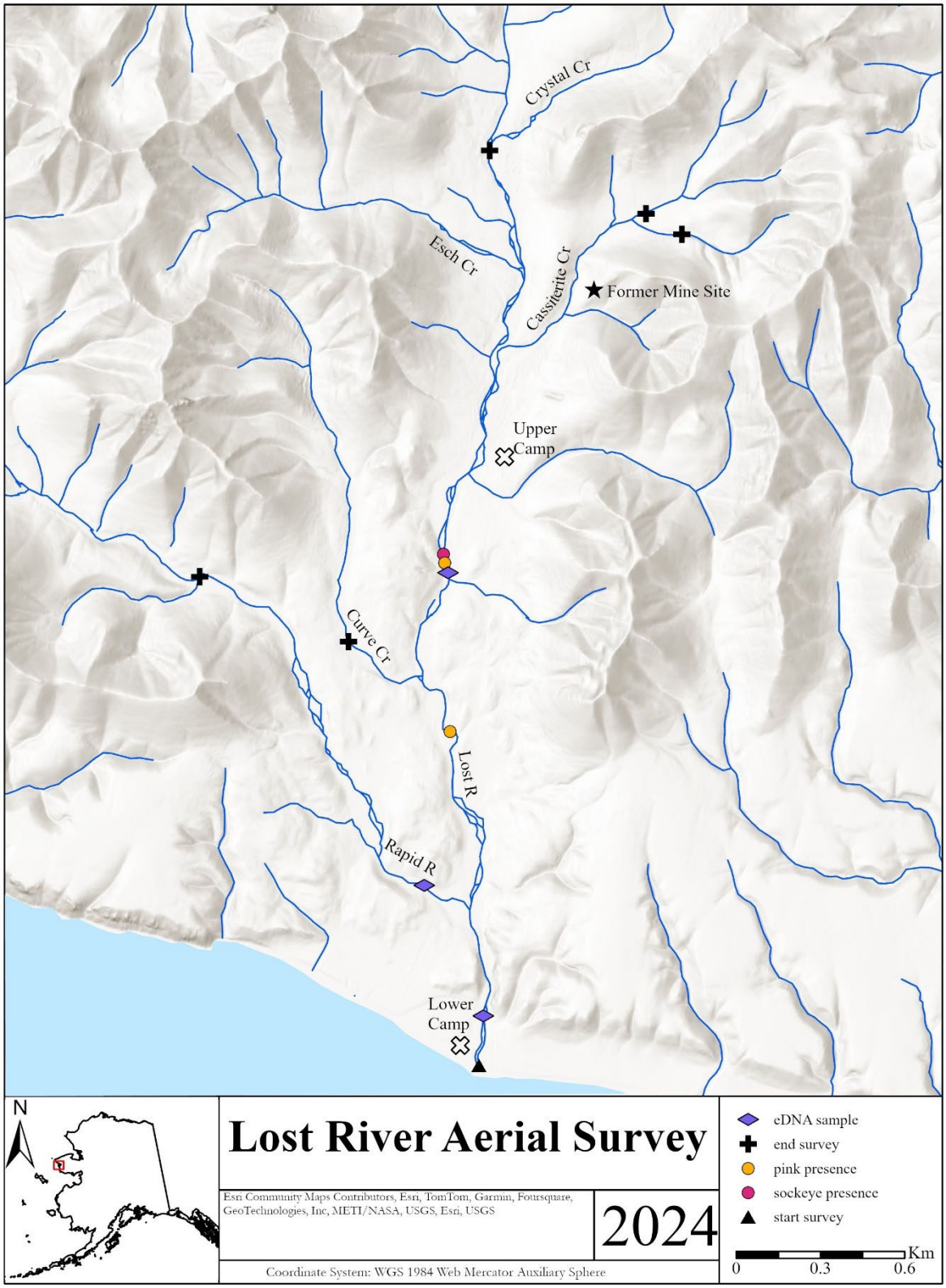

Figure 23. –Map of Lost River drainage showing salmon extent observed during the 2024 aerial survey and the collection locations for eDNA. Colored circles indicate fish species, and spawning reaches.

ENVIRONMENTAL DNA

After completing the aerial survey, eDNA samples were collected from lower Lost River, middle Lost River, and Rapid River (Figures 23 and 24). The samples were collected in an attempt to positively identify the red colored salmon observed in both 2023 and 2024, and to provide evidence as to whether Rapid River may support fish. The laboratory results detected pink and Chinook salmon eDNA at the Lower Lost River site, pink salmon and Dolly Varden eDNA at the middle Lost River site, and no fish eDNA at the Rapid River site.

Figure 24.–Collecting eDNA samples from Lower Lost River, August 22, 2024.

DISCUSSION

Overall, there was high variability between the 2023 and 2024 aquatic samples and observations. In July 2024 both the air and water temperatures were warmer, with sunny skies and low water in the Lost River drainage. The low water levels allowed for very effective use of minnow traps, with higher numbers of fish caught in several new locations (Lower & Middle Lost River, and Lower Cassiterite Creek), and more fish caught at Upper Cassiterite and Crystal creeks. These conditions were a stark contrast to July 2023 when there was consistent rainfall, high water throughout the drainage, and many minnow traps were not able to fish effectively due to high flows.

The water quality parameters, periphyton, and benthic macroinvertebrate results between 2023 and 2024 were also quite variable. Water temperatures were warmer, turbidity was lower, and water measured more alkaline in 2024 than in 2023. Chlorophyll-a concentrations were generally lower, as were the diversity and densities of aquatic invertebrates in 2024. These differences may not be unusual, but additional years of baseline studies will help quantify the variability and range within the Lost River aquatic ecosystem.

The weather for the aerial survey was similar between the 2023 and 2024 surveys, however the river conditions were quite different. In 2024 all tributaries had surface connections to Lost River, whereas in 2023 Curve Creek and Rapid River had no surface connections (Brase and Clawson 2024). In 2023 the total fish count during the aerial survey was approximately 2,500 pink salmon and 20 fish tentatively identified as sockeye salmon, and spawning redds were noted throughout the drainage. Conversely, in 2024 only 90 pink salmon were observed and 3 fish were tentatively identified as sockeye salmon. Very few spawning redds were noted in 2024.

The lack of fish noted during the 2024 aerial survey was unexpected since several hundred pink salmon were observed staging in lower Lost River during the July 2024 aquatic biomonitoring trip, and Norton Sound even year pink salmon return numbers are typically greater than odd years. During the aerial survey we flew further up Rapid River and Cassiterite Creek thinking that the pink salmon were spawning upriver, but this was not the case. It's possible the pink salmon observed in lower Lost River in July spawned prior to the aerial survey and their carcasses were washed out of the river during subsequent high rainfall events. In future years, we suggest planning more time in August on site and ensuring more fuel availability so a more comprehensive aerial survey can be performed within the large Rapid River tributary.

Although eDNA was collected and analyzed in 2024, it is difficult to determine how to best interpret the results. The laboratory detected pink salmon and Dolly Varden eDNA in one of the two middle Lost River samples. This location was directly downriver of at least two red salmonids observed during the August 22 aerial survey that were tentatively identified as sockeye salmon from the air. It is unlikely the red salmonids were adult Dolly Varden since Dolly Varden typically spawn a month later in mid to late September. Instead, the Dolly Varden eDNA signature was likely from the juvenile Dolly Varden that inhabit the drainage. However, no salmon species consistent with the field observation of red colored salmon were detected in the eDNA samples, despite the samples being collected just downriver of the fish. The laboratory detected pink and Chinook salmon eDNA in one of the two lower Lost River samples, which is a tidally influenced location where we may have detected some estuarine fish species. Neither adult nor juvenile Chinook salmon have been recorded in the Lost River drainage. The most significant limitation of eDNA is the chance of contamination leading to false positive results, as the polymerase chain reaction (PCR) amplification of gene fragments acts on extremely small quantities of DNA (Sepulveda et al 2020). The field blanks collected at Lost River were all negative for any eDNA, indicating that field contamination is a lower likelihood, and the closed nature of the Smith-Root Citizen Science Sampling filters packs also minimizes the risk of field contamination (Appendix B). But contamination can also occur in the laboratory setting, even with stringent protocols to minimize the likelihood. Due to these discrepancies, eDNA results will not be used as an indication of definitive fish presence.

As stated in the 2023 technical report (Brase and Clawson 2024) it is still unknown whether the juvenile Dolly Varden captured in the Lost River drainage are anadromous or resident fish. Lost River has a direct connection to the marine environment, so it is possible that the fish are anadromous. To make a definitive determination of anadromy lethal fish sampling would be required for analysis of their otoliths to assess strontium (Sr) concentrations in these structures.

ADF&G recommends that baseline aquatic studies continue at Lost River for at least another three years. The high variability in all the aquatic samples collected in 2023 and 2024, and the vastly different aerial survey results between 2023 and 2024 illustrate the importance of multi-year baseline programs. Typically, a minimum of three years of aquatic baseline data is recommended before evaluating potential impacts from development activities. However, in the case of Lost River, with the high variability observed in the first two years of baseline work, and unusual nature

of some of the tributaries periodically going subsurface, it is particularly important to collect several years of data. In 2024 exploration activities were increasing in the vicinity of upper Cassiterite Creek. Therefore we suggest adding an additional baseline sampling site somewhere in the upper Cassiterite Creek drainage, well above any potential mine influence.

It will also be important to continue the aerial survey component of the biomonitoring program to document the variability among years in the number of spawning pink salmon. Norton Sound even-year spawners are typically more numerous than odd-year spawners, but that was not the case observed at Lost River in 2024. Additionally, during the next aerial survey, if red colored salmon are again observed, an attempt to capture them with hook and line gear should be made to positively determine the species.

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APPENDIX A: PERIPHYTON AND MACROINVERTEBRATE DATA

Appendix A1. Periphyton standing crop detail, Lost River 2024.

2024 Chloro Results - Lost River

IDL = 0.14 mg/m^2	
EDL = 0.51 mg/m^2	Linear Check Maximum = 69.02 mg/m^2

			Phaeo Corrected				
	Date	Vial	**Chl a**	**Chl a**	**664/665**	Chl b	Chl c
Site	Analyzed	Chl a	**mg/m2**	**mg/m2**	**Ratio**	mg/m2	mg/m2
Lower Lost R	11/22/2024	0.28	1.13	0.96	1.56	0.07	0.18
Lower Lost R	11/22/2024	0.26	1.05	0.96	1.64	0.00	0.15
Lower Lost R	11/22/2024	0.27	1.09	1.07	1.71	0.00	0.22
Lower Lost R	11/22/2024	0.26	1.04	0.96	1.64	0.09	0.39
Lower Lost R	11/22/2024	0.41	1.64	1.50	1.64	0.00	0.24
Lower Lost R	11/22/2024	0.34	1.37	1.28	1.67	0.00	0.14
Lower Lost R	11/22/2024	0.29	1.14	1.07	1.67	0.00	0.11
Lower Lost R	11/22/2024	0.38	1.51	1.39	1.65	0.00	0.19
Lower Lost R	11/22/2024	0.17	0.68	0.64	1.67	0.04	0.24
Lower Lost R	11/22/2024	0.58	2.33	2.24	1.70	0.00	0.24
Rapid R	11/22/2024	0.39	1.55	1.39	1.62	0.00	0.19
Rapid R	11/22/2024	0.38	1.50	1.39	1.65	0.04	0.26
Rapid R	11/22/2024	0.32	1.27	1.17	1.65	0.06	0.32
Rapid R	11/22/2024	0.27	1.09	1.07	1.71	0.01	0.12
Rapid R	11/22/2024	0.24	0.95	0.85	1.62	0.07	0.24
Rapid R	11/22/2024	0.23	0.91	0.85	1.67	0.02	0.17
Rapid R	11/22/2024	0.25	1.00	0.96	1.69	0.05	0.23
Rapid R	11/22/2024	0.20	0.81	0.75	1.64	0.06	0.19
Rapid R duplicate	11/22/2024	0.19	0.77	0.75	1.70	0.08	0.19
Rapid R	11/22/2024	0.12	0.50	0.53	1.83	0.06	0.20
Rapid R	11/22/2024	0.26	1.05	0.85	1.53	0.03	0.22
Curve Cr	11/22/2024	0.13	0.50	0.43	1.57	0.00	0.03
Curve Cr	11/22/2024	0.06	0.22	0.21	1.67	0.04	0.10
Curve Cr	11/22/2024	0.06	0.22	0.21	1.67	0.04	0.10
Curve Cr	11/22/2024	0.05	0.18	0.21	2.00	0.00	0.04
Curve Cr	11/22/2024	0.03	0.13	0.21	3.00	0.08	0.12
Curve Cr	11/22/2024	0.08	0.31	0.21	1.40	0.07	0.16
Curve Cr	11/22/2024	0.07	0.27	0.32	2.00	0.02	0.10
Curve Cr	11/22/2024	0.07	0.27	0.32	2.00	0.10	0.06
Curve Cr	11/22/2024	0.08	0.31	0.32	1.75	0.07	0.16
Curve Cr	11/22/2024	0.08	0.32	0.21	1.40	0.00	0.09

Middle Lost R	11/22/2024	0.13	0.54	0.53	1.71	0.11	0.26
Middle Lost R	11/22/2024	0.09	0.36	0.43	2.00	0.05	0.15
Middle Lost R	11/22/2024	0.30	1.19	1.07	1.63	0.00	0.20
Middle Lost R	11/22/2024	0.11	0.45	0.43	1.67	0.01	0.04
Middle Lost R	11/22/2024	0.08	0.32	0.21	1.40	0.00	0.09
Middle Lost R	11/22/2024	0.10	0.41	0.43	1.80	0.02	0.14
Middle Lost R	11/22/2024	0.16	0.63	0.64	1.75	0.07	0.15
Middle Lost R	11/22/2024	0.24	0.96	0.85	1.62	0.00	0.17
Middle Lost R	11/22/2024	0.08	0.32	0.21	1.40	0.00	0.09
Middle Lost R	11/22/2024	0.10	0.41	0.43	1.80	0.02	0.14
Cassiterite Crk	11/21/2024	0.07	0.26	0.21	1.50	0.09	0.16
Cassiterite Crk	11/21/2024	0.19	0.76	0.75	1.70	0.16	0.26
Cassiterite Crk	11/21/2024	0.08	0.32	0.32	1.75	0.00	0.09
Cassiterite Crk	11/21/2024	0.10	0.41	0.43	1.80	0.02	0.14
Cassiterite Crk	11/21/2024	0.23	0.91	0.85	1.67	0.02	0.17
Cassiterite Crk	11/21/2024	0.21	0.82	0.75	1.64	0.00	0.12
Cassiterite Crk	11/21/2024	0.16	0.63	0.53	1.56	0.07	0.15
Cassiterite Crk	11/21/2024	0.09	0.36	0.43	2.00	0.05	0.15
Cassiterite Crk	11/21/2024	0.59	2.36	1.50	1.36	0.60	1.37
Cassiterite Crk	11/21/2024	0.23	0.91	0.75	1.54	0.02	0.17
Esch Crk	11/21/2024	0.15	0.59	0.53	1.63	0.02	0.09
Esch Crk	11/21/2024	0.26	1.05	0.96	1.64	0.03	0.22
Esch Crk	11/21/2024	0.22	0.86	0.85	1.73	0.05	0.08
Esch Crk	11/21/2024	0.24	0.96	0.85	1.62	0.00	0.17
Esch Crk	11/21/2024	0.14	0.54	0.53	1.71	0.04	0.09
Esch Crk	11/21/2024	0.09	0.36	0.21	1.33	0.06	0.05
Esch Crk	11/21/2024	0.25	1.00	0.96	1.69	0.06	0.13
Esch Crk	11/21/2024	0.17	0.68	0.53	1.50	0.05	0.14
Esch Crk	11/21/2024	0.22	0.86	0.75	1.58	0.04	0.18
Esch Crk	11/21/2024	0.22	0.86	0.85	1.73	0.04	0.18
Crystal Cr	11/22/2024	0.10	0.41	0.32	1.50	0.02	0.14
Crystal Cr	11/22/2024	0.06	0.22	0.11	1.25	0.03	0.20
Crystal Cr	11/22/2024	0.08	0.32	0.21	1.40	0.00	0.09
Crystal Cr	11/22/2024	0.07	0.27	0.21	1.50	0.02	0.10
Crystal Cr	11/22/2024	0.03	0.14	0.11	1.50	0.01	0.05
Crystal Cr	11/22/2024	0.03	0.14	0.11	1.50	0.01	0.05
Crystal Cr	11/22/2024	0.06	0.22	0.21	1.67	0.04	0.10
Crystal Cr	11/22/2024	0.07	0.27	0.21	1.50	0.02	0.10
Crystal Cr	11/22/2024	0.02	0.09	0.00	1.00	0.00	0.00
Crystal Cr	11/22/2024	0.03	0.14	0.32		0.01	0.05
Upper Lost R	11/22/2024	0.07	0.27	0.21	1.50	0.02	0.10
Upper Lost R	11/22/2024	0.13	0.50	0.53	1.83	0.00	0.13
Upper Lost R	11/22/2024	0.08	0.32	0.32	1.75	0.01	0.00
Upper Lost R	11/22/2024	0.08	0.32	0.32	1.75	0.00	0.09
Upper Lost R	11/22/2024	0.13	0.50	0.43	1.57	0.00	0.13
Upper Lost R	11/22/2024	0.10	0.41	0.43	1.80	0.04	0.04
Upper Lost R	11/22/2024	0.09	0.36	0.21	1.33	0.06	0.05
Upper Lost R	11/22/2024	0.07	0.27	0.21	1.50	0.02	0.10
Upper Lost R	11/22/2024	0.09	0.36	0.32	1.60	0.06	0.05
Upper Lost R	11/22/2024	0.11	0.45	0.43	1.67	0.00	0.14

Appendix A2. Benthic macroinvertebrate sample detail, summarized by site, Lost River 2024.

			Lower Lost	Rapid	Curve	Middle Lost	Cassiterite	Esch	Crystal	Upper Lost
Ephemeroptera	Baetidae	*Baetis*	4	10	23	1	1			
		not determined			3			1		
	Ephemerelidae	*not determined*								
	Heptageniidae	*Cinygmula*	25	9	25	6		14		
		not determined		1	1	2				
	Not Determined		3	1	1			3	1	
Plecoptera	Capniidae	*not determined*	2		1			6		2
	Chloroperlidae	*Suwallia*	2			8				
	Nemouridae	*Podmosta*	20	12	82	11	23	30	13	9
		not determined	124	141	151	5	11	82	3	2
	Perlodidae	*not determined*				2				
	Not Determined		5	1	4	6		17	5	
Trichoptera	Not Determined			1						
Diptera	Chironomidae	larvae	46	26	149	34	25	91	58	120
	Chironomidae	pupae	4	5	1		1		6	3
	Empididae	*Chelifera*				1				3
	Tabanidae				2			1		
	Tipulidae	*Hexatoma*							2	
		Tipula		1	4	2	2	9		
		not determined								4
	Simuliidae	*Simulium*				1				
		not determined			3			1		
Miscellaneous										
	Acari	Acarina		1		1	1	3		2
	Oligochaeta		33	7	26	30	30	25	27	157
	Copepoda	Harpacticoida		1	3		1			3
	Terrestrial Flies		2	1	1		7	3	7	
	Terrestrial Wasps								2	
	Nematoda					7				14
	Platyhelminthes		1	5	24	4	25	43	19	90

APPENDIX B: COLLECTION METHODS
ENVIRONMENTAL DNA

Field materials

- Citizen Scientist sampler with charged battery (Fig. 1)
- Filter packs (2 per day, or 3 when taking weekly field blank, in gallon ziplock)
- Gloves (2 pairs per day, or 3 when taking weekly field blank, in gallon ziplock)
- Clipboard with datasheet, pencil and sharpie
- *When taking weekly field blank:* 1 quart ziploc filled with ~300 mL distilled water (fill to red sharpie mark)

Lab/backup materials

- Backup sampler (drill pump, flask, and extra tubing)
- Season's worth of filter packs
- Extra datasheets
- Distilled water in 1-gal jugs
- Wet wipes containing 2% bleach

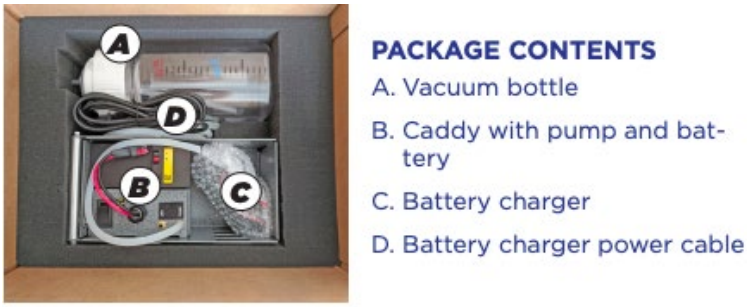

Figure 1. Citizen Scientist sampler components

Contamination precautions

- Wash your hands and put on a pair of clean, non-powdered, single-use gloves before gathering and setting up sampling gear. Change into a second pair before opening filter packs and collecting river water samples. When collecting a field blank, change into a third pair of gloves before opening that filter pack and collecting the blank.
- Do not let gloves contact contaminated surfaces, such as the stream, your waders, or any other equipment that may have fish DNA on it, prior to handling the filters
- If gloves or supplies become soiled or contaminated (e.g., drop filter, touch your slimy waders), replace with spare gloves or supplies rather than attempting to clean in the field. When in doubt err on the side of caution and replace gloves or supplies.
- If you think any eDNA sampling materials may have gotten contaminated (e.g., the tote containing the filter packs or ziploc holding the gloves gets splashed with river water), you can decontaminate them using the provided wipes containing a 2% bleach solution. (Alcohol is not effective for decontamination: it kills germs, but does not destroy DNA).

Methods

Choosing a sample site:

1. Determine and mark the sampling site

a. Select a location in the stream to collect the eDNA sample that has moderate flow (not a pool or eddy). eDNA sample collection should precede any activity in the stream or be positioned upstream of any other activity in-stream.
b. Find a level surface on the stream bank that allows you to stay downstream of your sampling site (where you will hold the tubing). Ensure that the tubing is long enough to reach into the stream.
c.

Each sampling day:

2. Prepare sampling gear indoors
    a. Wash hands and put on a pair of clean gloves.
    b. Label a gallon ziploc bag with the site and date in sharpie. Label 2 filter pack bags with sharpie: SITE-DATE-SUBSAMPLE:
        i. Site: Creek name, any other identifying information ie "Lower California"
        ii. Date in MMDDYY format
        iii. Subsample: "a" or "b"
        iv. e.g. LowerCalifornia062524a
    c. Write the same label name with pencil on the white sticker.
    d. Place labeled filter packs and a set of gloves inside a gallon ziploc and seal.
    e. If collecting a field blank:
        i. Label another gallon ziploc with the site, date, and "blank" with sharpie. Label 1 filter pack SITE-DATE-z (e.g. LowerCalifornia062524z).
        ii. Carefully open quart ziploc with red sharpie line on it, being careful not to touch the inside of the bag. Have person 1 fill the ziploc with distilled water to red sharpie line while person 2 holds the bag by both sides of the ziploc seal. Seal the ziploc bag.
        iii. Place filter pack and a second set of gloves inside gallon "blank" ziploc, and seal.
    f. Gather remaining field gear (see list above) and proceed to sampling site.

3. Go to sampling site and collect eDNA samples
    a. Switch to a new pair of gloves before opening filter pack
    b. Open a new filter pack. Grab the filter by the yellow barbed end to avoid touching the clear tube. Carefully attach clear tube to the white end of the filter housing while the clear tube is still in the bag (**Figure 3-3)**. Attach yellow barbed end of filter to pump's intake tube. **Avoid touching the clear tube before the sample is collected.**
    c. Turn pump on
    d. Holding the filter housing, place the clear tube in the water at arm's reach and slightly upstream from where you are kneeling on the bank **(Figure 3-6)**. Do not step into the water. Do not submerge the filter or get water on your gloves. **Be sure to sample from the same location each time.**
    e. While keeping the clear tube end steady and submerged, filter until the vacuum bottle reaches the red "invert filter" line just below the 2L mark **(Figure 3-7)**.
    f. **Keeping the pump ON, remove the clear tube from the water and quickly invert the filter and air dry for at least 30 sec (Figure 3-8)**.

i. If the filter is clogged (e.g. very turbid water) with standing water that will not drain inside the housing: pull gently on the tab located on the white half of the filter housing to carefully break the seal and allow filtering to continue. **Do not open fully**

ii. **(Figure 4-4)**

g. Turn pump off

h. Remove and discard the short clear tube piece from the filter housing,. Gently shake filter housing to remove excess water and seal only the white and yellow filter housing back in the original labeled ziplock. Place into the gallon ziploc.

i. **Do not put gloves, clear tube etc back into the original filter bag.**

i. **On the datasheet record** date, time, subsample letter, actual volume filtered, and any notes. Use a **separate** row for each filter.

j. Empty the vacuum bottle, and repeat for the second subsample. Collect 2 replicates (e.g. a, b) every time you sample.

4. Collect Field Blank sample (only once per week)

Field blanks are collected to make certain that we are not contaminating our normal river water samples. They are expected to contain no fish DNA.

a. Remove gloves used for river water samples.

b. Open the gallon ziploc labeled "blank" and put on the clean gloves inside. Once gloves are on, avoid touching anything that might contaminate the sample (e.g., river water, river water samples, waders, non-eDNA equipment).

c. Open the filter pack bag labeled "z", grab the filter by the yellow barbed end, and carefully attach the clear tube to the white end of the filter housing while the tube is still in the bag (Figure 3-3). **Avoid touching the clear tube before the sample is collected.**

d. Attach yellow barbed end of filter housing to intake tube of pump

e. Turn pump ON

f. Invert filter and carefully pour distilled water from ziploc into clear tube until bag is empty

g. **While pump remains ON, leave filter inverted and dry for at least 30 seconds** (Figure 3-8)

h. Turn pump off

i. Remove and discard the clear tube from the filter housing and seal only the filter back in the original filter bag. Place inside gallon ziploc and seal.

j. On the datasheet record date, time, subsample letter "z", actual volume filtered, and any notes. Use a separate row for the blank filter.

k. Empty the vacuum bottle

l. Note: always collect blank *after* first collecting your two river samples

5. Sample storage

a. Store all completed samples in the sealed filter bags they came in and store those inside the labeled gallon ziplocks. If a ziplock breaks, reseal it in a good ziplock.

b. Store all of the filters in a plastic tote indoors under relatively stable temps. If indoor temperatures exceed ~80˚F, move tote outside into the shade. Avoid allowing samples to freeze. Repeated freeze/thaw damages DNA.

c. eDNA filters are self preserving for about 6 months at room temperature

Figure 3. Visual steps for operation of the Smith-Root eDNA citizen science sampler

Figure 4. Visual steps for processing Smith-Root eDNA filter packs