Briefing Paper

Klamath River Renewal Project Molecular Library: using environmental DNA and RNA to establish a genetic time capsule prior to unprecedented dam removal

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Additional Project Information

Klamath River Restoration
https://res.us/home/restoring-at-scale/klamath-river-restoration/

Genidaqs | Fisheries Science, eDNA & Population Genetics
https://genidaqs.com/

Klamath River Renewal (klamathrenewal.org)

Executive Summary

The removal of the four dams along the Klamath River represents an opportunity to evaluate restoration effectiveness at scale and environmental DNA (eDNA) has been shown to an effective tool for monitoring the distribution of aquatic species following large-scale dam removals. Prior to dam removal, Resource Environmental Solutions (RES) and Genidaqs (GIQ) staff collected, filtered, and preserved the genetic material within over 400 water samples from 45 mainstem and tributary monitoring locations, across 114 river kilometers in California and Oregon. These monitoring locations were selected across the Klamath River Renewal Project (KRRP; also known as the Lower Klamath Project) to include sites with a range of characteristics as well as control sites both in and out of the impacted watersheds for a Before-After-Control-Impact (BACI) monitoring design. All samples were stabilized following protocols for the long term storage and viability of both eDNA and environmental RNA (eRNA), were collected with sufficient replicates to run 200 individual analyses per site and are suitable for analyses including: detection of rare or cryptic species, distribution and relative abundance of aquatic organisms, community composition of aquatic organisms through eDNA metabarcoding, and subsequent population demography inference through eRNA analysis. The spatial and temporal configuration, as well as the relative long-term stability of this baseline data makes this first year of the KRRP Molecular Library an invaluable genetic time capsule and resource for future studies. This dataset was collected independent of the restoration effectiveness monitoring responsibilities defined for the project and the completion of the capture and preservation of the irreplaceable baseline data is awaiting future opportunities and partnerships for analysis.

Introduction

The Klamath River Renewal Project (KRRP; also known as the Lower Klamath Project) represents an effort to remove four hydroelectric dams along the Klamath River in California and Oregon. The removal of the four dams and subsequent restoration of the former reservoir footprints to a riverine condition is intended to reconnect over 640 kilometers of habitat for migratory fishes, restore native vegetation across nearly 1,000 hectares of previously drowned land, and improve water quality in the Klamath River Basin (US DOI 2013).

The KRRP objectives include restoration actions that will benefit the distribution and abundance of economically, recreationally, and culturally valuable organisms following a return to riverine conditions and an ecosystem shift towards native biodiversity across a vast landscape. Environmental DNA (eDNA) has been shown to be a cost effective (Evans et al. 2017), non-invasive (Goldberg et al. 2016), and effective tool for monitoring the distribution of aquatic species at broad geographic scales (Schmelzle and Kinziger 2016, Sutter and Kinziger 2018, Miya et al. 2022), and specifically after large-scale dam removals (Duda et al. 2021, Muha et al. 2021, Huang et al. 2023). The inclusion of pre-restoration data is an important

element in quantifying change over time (England et al. 2021) and the long-term stability of the samples collected in this effort are an opportunity to do so for decades to come.

The purpose of this document is to briefly describe the methods, spatial extent, and analytical potential of a pre-restoration data collection effort completed in July 2023 prior to major dam removal along the Klamath River. Herein, we demonstrate that the Before-After-Control-Impact (BACI) sampling design and the molecular techniques employed to isolate and preserve genetic material indefinitely, make the KRRP Molecular Library a valuable genetic time capsule and tool with which to conduct, support, and facilitate future research and monitoring efforts in the Klamath Basin.

Methods

Field Sampling and Preservation

A total of 405 samples were collected from 45 mainstem and tributary monitoring locations by RES and Genidaqs (GIQ) staff between 17 and 20 July 2023. The summer season from June through August was identified as the preferred time to complete this sampling annually due to lower stream discharge that minimizes the dilution of eDNA in streams (Curtis et al. 2021), to maximize the probability of detecting key indicator species such as including bacteria, algae, macroinvertebrates, amphibians, fishes, and pathogens, and to collect genetic material from only the juvenile life-stage of Pacific salmon (*Oncorhynchus tshawytscha* and *Oncorhynchus kisutch*) (Thompson et al. 2020, Hamilton et al. 2011, Sutton & Soto 2012).

At each site, three liters of stream water were collected from both left and right banks and the center of the channel, where accessible, for a total of nine liters. The nine liters of water were combined into a single vessel and nine replicate samples were collected from the composite. Each replicate was filtered in the field through 0.45 µm pore-size Sterivex filters and preserved with 1.6-2.0 mL of RNAprotect Tissue Reagent following Miyata et al. (2022) to maximize the probability of stabilizing genetic material. Samples were stored at ambient temperature before being transferred to a non-frost-free freezer at -20°C. Field crews followed protocols to minimize and assess the risk of contamination including flushing gear with sample water for one minute before filtering, changing clean nitrile gloves frequently, and collecting field controls as in Figure 1.

Figure 1: RES staff filter genetic material from water samples using peristaultic pumps at a boat access monitoring site along the Klamath River.

Site Selection

Monitoring locations were selected systematically every 2 km along mainstem Klamath River and reservoir sites and every 1 km along priority tributaries, with additional sites included near likely long-term monitoring locations. Overall, monitoring locations covered approximately 114 km of mainstem and tributary habitat. Sites were selected along navigable streams and only at locations with a high probability of long-term continued accessibility. Sites along the Klamath River extend from approximately 3.5 km downstream of Iron Gate Dam, upstream to where the Klamath River enters J. C. Boyle Reservoir, and tributary sites extended upstream either to barriers to fish migration or as far as was feasible (Maps 1 & 2). Best practices for implementation of a BACI sampling design include multiple controls, or sites that will not receive the impact/treatment (England et al. 2021). We selected six control sites outside of the impacted watershed in the nearby Scott River Watershed that consisted of both mainstem and tributary locations (Map 3), and two in-watershed controls located upstream of barriers to migratory fish in Fall and Jenny Creeks (Map 1).

Representative Analysis and Future Directions

Species Distribution and Relative Abundance

Reconnected habitat with volitional passage for native species above dams and reservoirs is a core goal of the KRRP. Environmental DNA analyzed with quantitative polymerase chain reactions (qPCR) has been shown to be an effective tool to monitor the reestablishment of populations of migratory fishes of the families Salmonidae, Petromyzontidae, Anguillidae, & Clupeidae following large-scale dam removals (Duda et al. 2021, Muha et al. 2021, Huang et al. 2023). Additionally, eDNA analyzed with qPCR has shown to have equal or greater detection probability than many traditional methods of surveying for aquatic species (Pilliod et al. 2013, Smart et al. 2015, Schmelzle & Kinziger 2016, Wilcox et al. 2016). The nine field replicates collected in this pre-restoration dataset will create sufficient extracted genetic material for approximately 200 individual analyses per site, making this dataset ideal for using many technical qPCR replicates to increase the detection probability of rare species.

While the methodology for using eDNA in water samples to estimate abundance/biomass is less established than analysis of presence/absence, most studies resolve a positive relationship between eDNA concentration and abundance/biomass (Rourke et al. 2022, Takahara et al. 2012, Tillotson et al. 2018, Levi et al. 2019, Pilliod et al. 2019, Shelton et al. 2019, Pochardt et al. 2020, Capo et al. 2020, Shelton et al. 2022). Additionally, qPCR is already the preferred method of monitoring the abundance of the Salmonid parasite *Ceratonova shasta* throughout the Klamath River Basin (Hallet & Bartholomew 2006). This dataset presents the opportunity to integrate with existing qPCR based monitoring in the Klamath River Basin, and this baseline and subsequent years of data could expand the capacity of managers and researchers to evaluate changes in relative abundance of species before and after dam removal.

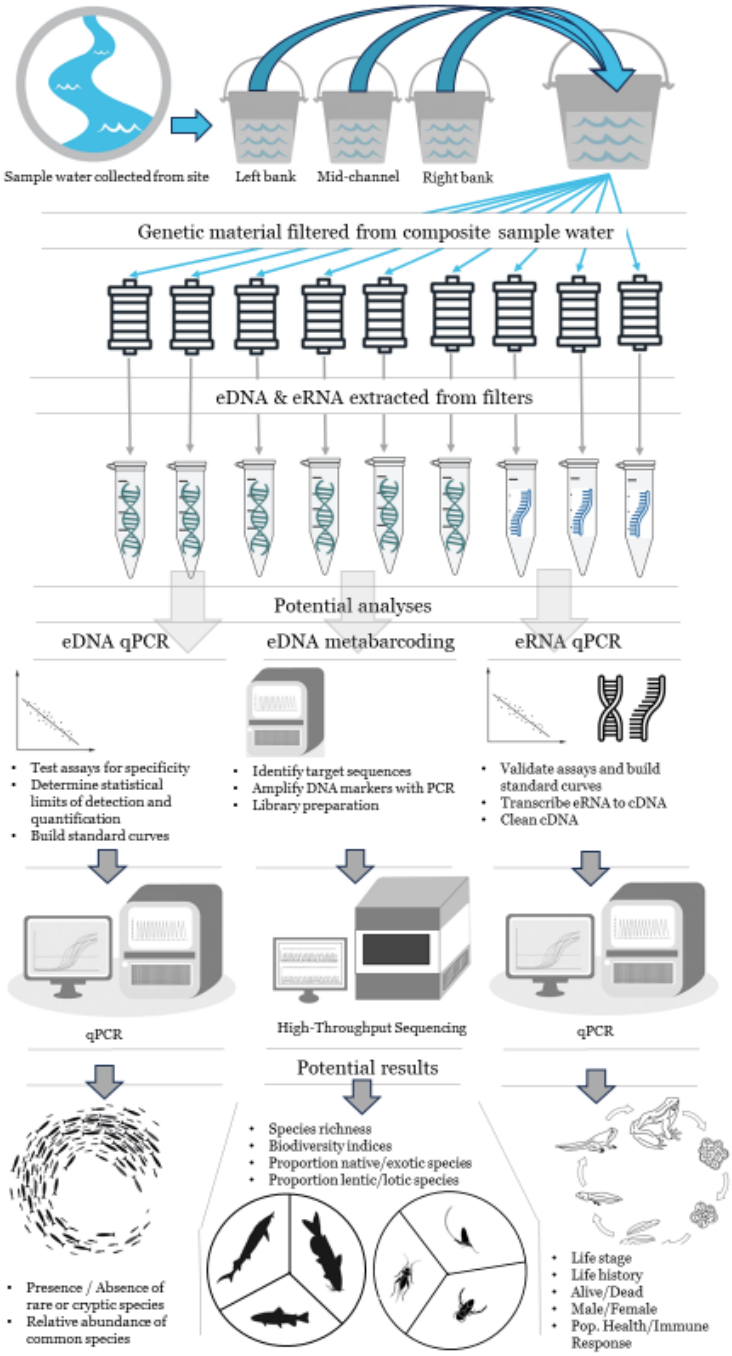

Figure 2: workflow of isolation of genetic material from environmental samples to create nine field replicates per site per year, potential analyses, and potential results. Baseline samples collected in 2023 have been filtered, preserved in RNATissueProtect, and are awaiting extraction and analysis which could include any or all of the following: eDNA qPCR, eDNA metabarcoding, and eRNA qPCR.

Species Composition

In addition to the restoration of volitional passage of aquatic organisms through the KRRP, reestablishment of native riverine species could be an important indicator of restoration effectiveness. Although traditional biodiversity assessments utilize a myriad of methodologies, they are often marred by similar limitations in that they tend to be dependent on the skill of the surveyor, are prone to observational biases, are often expensive and time consuming, may require many permits, or may be invasive to the species and habitats themselves (Goldberg et al. 2016). A metabarcoding approach of eDNA offers a parsimonious method to evaluate species richness, biodiversity indices, and drivers of community composition (i.e. restoration actions), which is relatively cost effective (Evans et al. 2017), non-invasive (Goldberg et al. 2016), and highly repeatable through time regardless of surveyor skill or

experience. This dataset is highly suitable for eDNA metabarcoding analyses and provides a unique opportunity to evaluate changes in community composition of a variety of taxonomic groups through time following large-scale dam removal.

Population Demography

Although transcriptomic analysis (eRNA qPCR) has only recently been introduced as a method of evaluating river restoration effectiveness, the approach holds the potential to revolutionize biological monitoring (England et al. 2021, Fediajevaite et al. 2021). Currently, eRNA is being used to detect bacterial and fungal genes and transcripts with known ecological functions such as organic matter breakdown and cellulose degradation to characterize the rate of ecosystem recovery following river restoration (Clark et al. 2018). Additionally, a variety of management and conservation applications of eRNA have been identified based on the complex life history patterns of fish and amphibians that involve physiological changes related to differential gene expression (Stevens and Parsley 2022). These include: distinguishing living organisms, quantifying living communities, determination of population age structure, determination of sex ratios, assessment of population health, and assessment of population stress. Many of these eRNA assays are currently under development around the world, some are being implemented in resource management applications, and significant advances in the technology are expected on the timeline of the KRRP (Clark et al. 2018). This baseline data captured and preserved eRNA for posterity as well as for currently available eRNA applications related to determining fish life-history that have been developed by GIQ. At the time of writing, the KRRP Molecular Library baseline samples represent the largest collection of eRNA samples related to a river restoration project known to the authors and represent a completely unique opportunity to evaluate changes in population demography at scale following dam removal.

Data Timeline

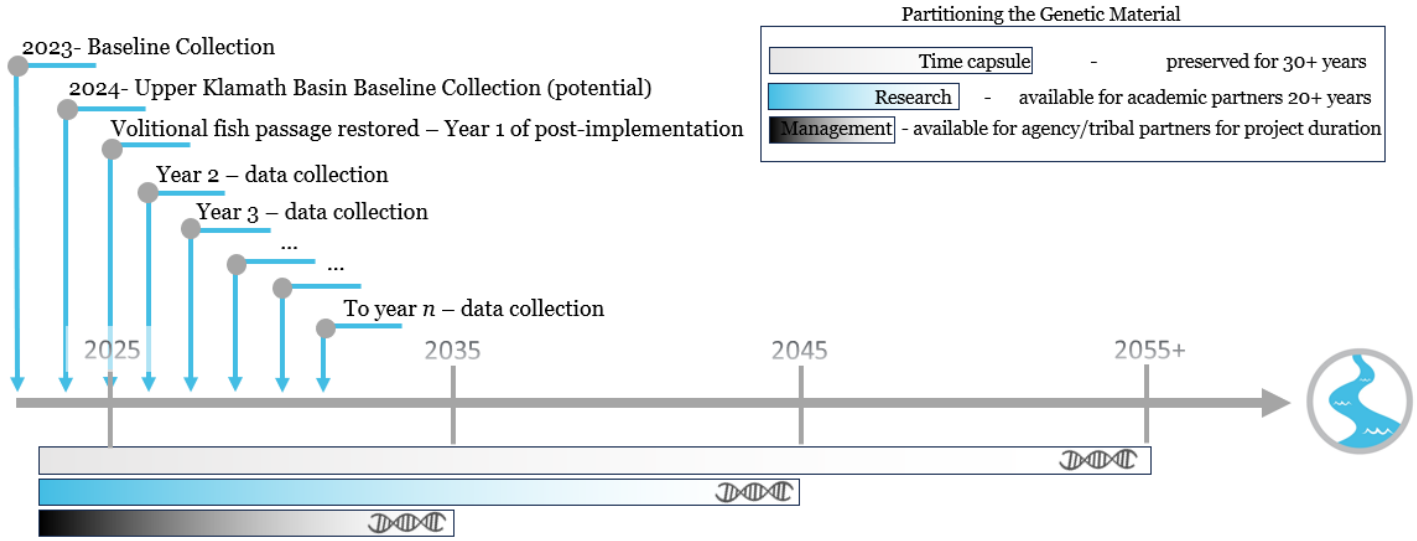

Figure 3: Timeline of the KRRP Molecular Library. Baseline data collection and preservation of genetic material was completed for the hydroelectric reach in 2023, there is the potential for using the KRRP Molecular Library sampling design to collect an equivalent baseline in the Upper Klamath Basin in 2024, 2025 will be the first full year with volitional fish passage, and replicate sampling could be completed for n years for research, managmenet, and posterity. Preserved genetic material will be saved for management needs, for research, and for long-term preservation with the intention of informing future restoration projects and for use with yet undeveloped eDNA/eRNA technologies.

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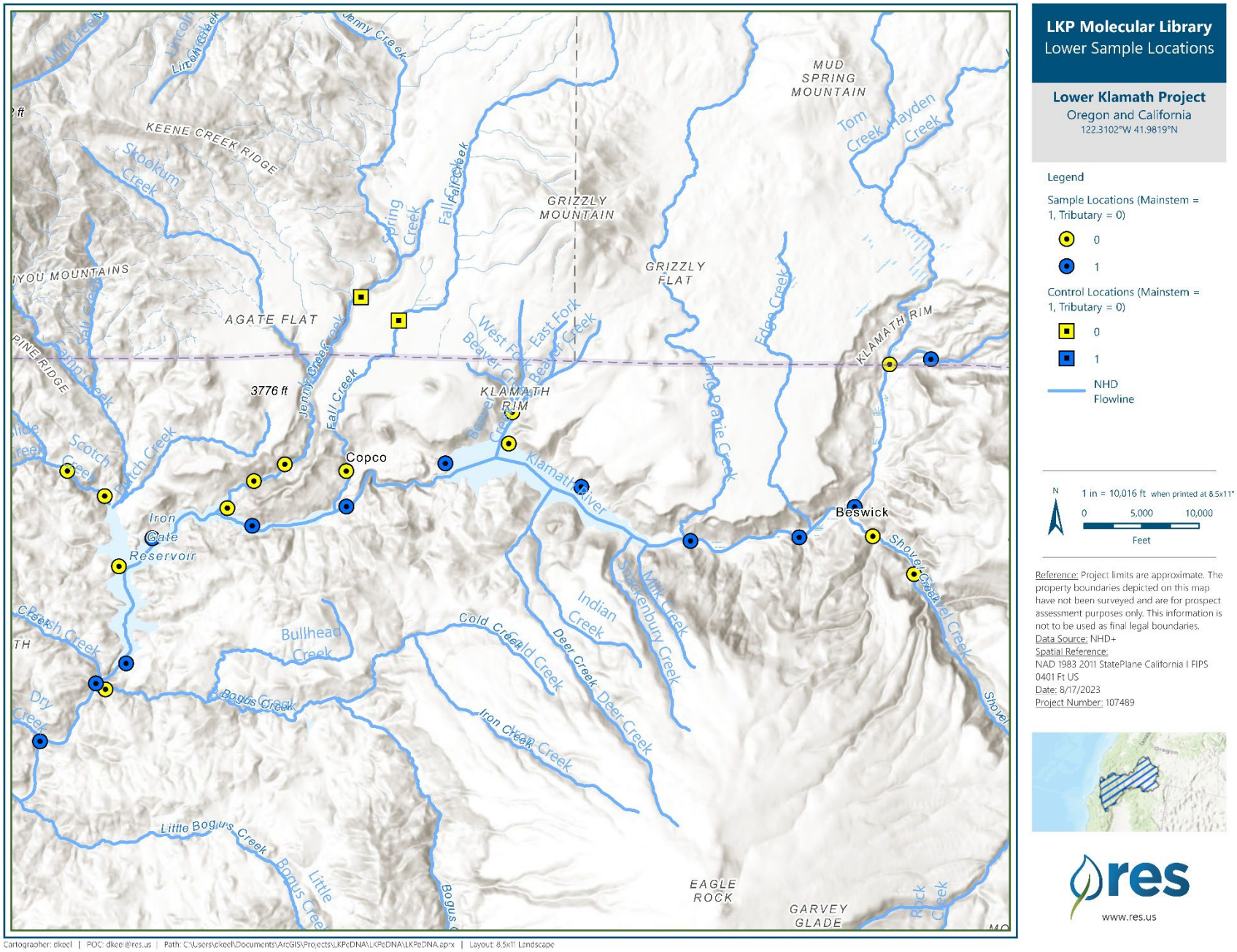
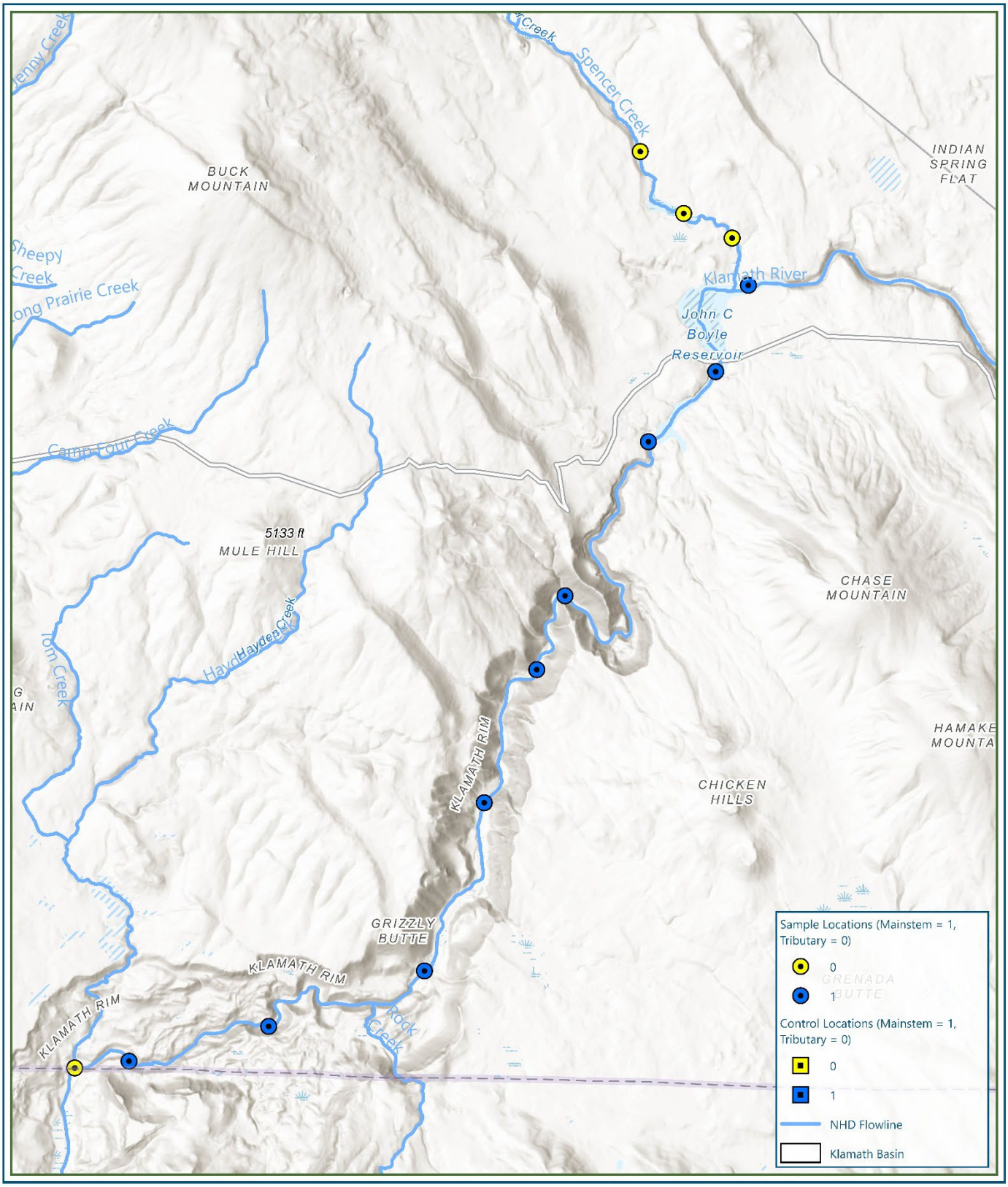
Maps

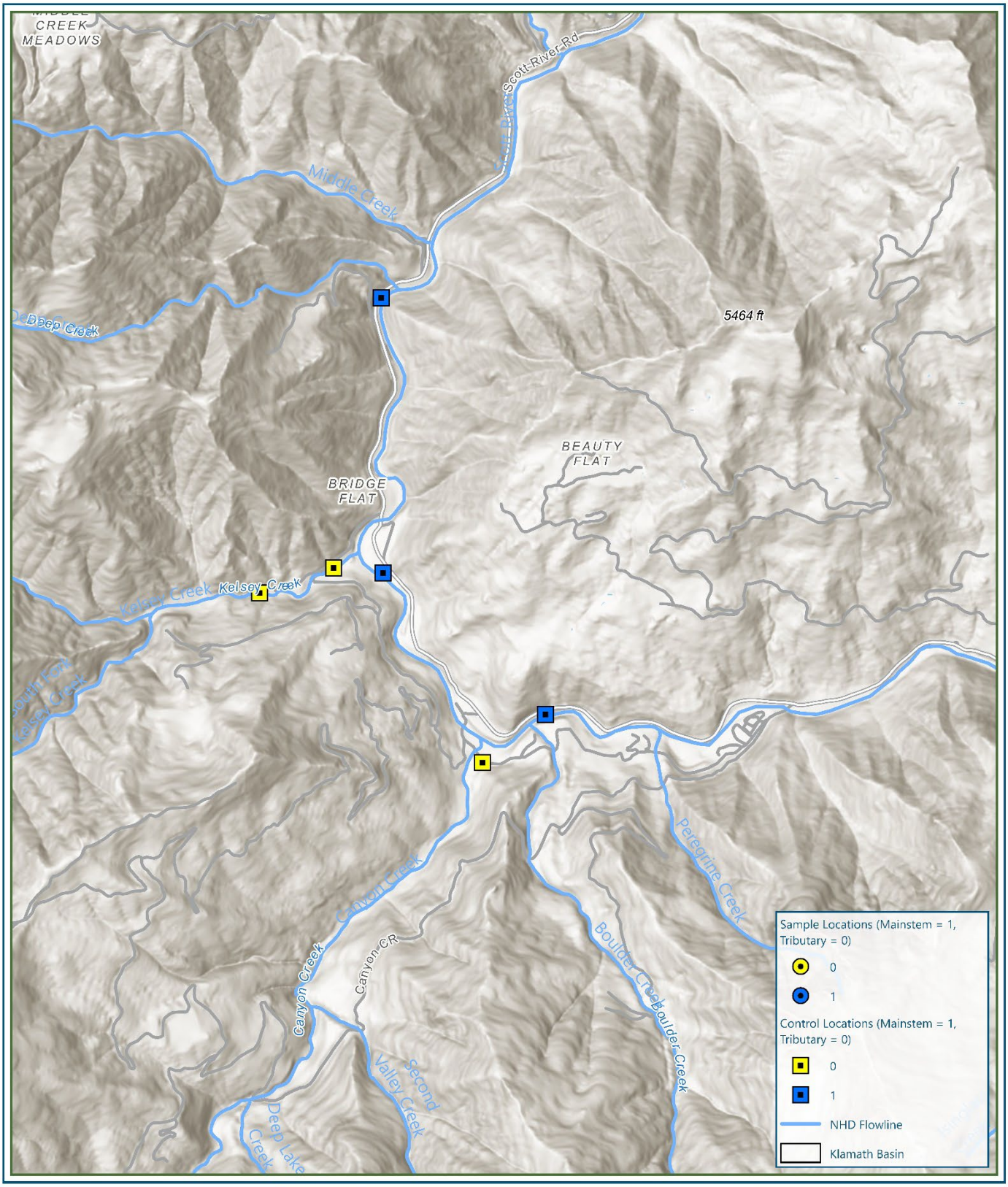


Cartographer: dkeel | POC: dkee@res.us | Path: C:\Users\dkeel\Documents\ArcGIS\Projects\LKPeDNA\LKPeDNA.aprx | Layout: 8.5x11 Landscape

Map 1: Klamath River Renewal Project Molecular Library downstream sampling locations



Map 2: Klamath River Renewal Project Molecular Library upstream sampling locations



Map 3: Klamath River Renewal Project Molecular Library Scott River Control locations.