



Lassen Volcanic National Park Cascades Frog and eDNA Inventory

Final Report

Natural Resource Report NPS/LAVO/NRR—2023/2567



ON THE COVER

A Cascades frog (*Rana cascadae*) surfaces from calm water and vegetation.
WSP USA Environment & Infrastructure Inc.

Lassen Volcanic National Park

Cascades Frog and eDNA Inventory

Final Report

Natural Resource Report NPS/LAVO/NRR—2023/2567

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Executive Summary

The Cascades frog (*Rana cascadae*) is one of two frog species known to occur historically in Lassen Volcanic National Park (LAVO) in Northern California. However, no Cascades frogs have been documented in LAVO for several decades, despite repeated surveys. Threats to the species include natural predators, introduction of fish into historically fishless habitats, diseases like fungal pathogens, airborne drift of pesticides from nearby agricultural areas, ultraviolet (UV-B) radiation, wildfires, and habitat loss from human development and climate change. Objectives of this project were to conduct surveys for Cascades frogs at LAVO using both visual encounter surveys (VES) and environmental DNA (eDNA) surveys at locations with potential for reintroduction, conduct VES and eDNA surveys for potential predators (rainbow trout, brook trout, brown trout, and leeches) to determine locations unsuitable for reintroduction, and to develop a suitability model to assess reintroduction potential within LAVO for Cascades frogs. Fifty locations around LAVO were sampled during this study. Suitability for Cascades frogs was ranked according to suitable vegetation availability, elevation, climatic factors, and perennial water availability. Projected climate data was used to derive future climate suitability for Cascades frogs within and around LAVO.

No Cascades frog egg masses, larvae, or adults were detected during VES or eDNA sampling within LAVO in 2021. Pacific tree frogs were observed at 18 sites, indicating suitable larval frog habitat at these sites. At 12 of these 18 sites, trout were not detected with either VES or eDNA sampling and leeches were only detected in two locations. The eDNA survey did not appear to be an effective way to detect leeches, but this method was effective for trout species and Cascades frogs.

Climate, vegetation, elevation, and perennial water availability were summarized and combined to identify suitable locations within and around LAVO. Climate data for known locations of Cascades frogs in California were used to define a range of suitable climate conditions for the species and then extrapolated across the Cascade frog's range in California. Within LAVO, occurrence data were not available and, thus, known habitat preferences were combined with spatial data to establish the location and density of suitable vegetation communities and the availability of perennial water. The most suitable areas within the park were identified to occur along the outer boundary and eastern side of the Park. These locations had optimal climate conditions, suitable vegetation communities and available perennial water sources. Many of the areas with high suitability in LAVO were impacted in recent burns (2021), which will reduce their current overall suitability until the vegetation recovers. Projected climate scenarios suggest that, in general, areas surrounding LAVO will decline in temperature suitability while areas within LAVO will increase.

Using all available information, sites (based on sub-watersheds) were determined to be of high, medium, or low reintroduction potential. Sites of high reproduction potential included the Cluster Lakes area; Feather Lake and nearby pond; Horseshoe Lake, Snag Lake and Grassy Creek, and vernal pool near Snag Lake trail; and Willow Lake. All the sites with high reproduction potential will require active management to prepare them for Cascades frogs and to maintain them as suitable habitat into the future.

List of Acronyms

CDFW	California Department of Fish and Wildlife
CNDDDB	California Natural Diversity Database
eDNA	environmental Deoxyribonucleic acid
GIS	Geographic Information System
HUC	Hydrologic Unit Code
IPC	Internal Positive Control
LAVO	Lassen Volcanic National Park
MIQE	Minimum Information for Quantitative Real-Time PCR Experiments
NAD	North American Datum
ND	Not Detected
NHD	National Hydrography Dataset
NPS	National Park Service
NWI	National Wetlands Inventory
PAC	Positive Amplification Controls
PC	Principal Component
PCA	Principal Component Analysis
PCT	Pacific Crest Trail
QAQC	Quality Assurance and Quality Control
qPCR	Quantitative Polymerase Chain Reaction
USFS	United States Forest Service
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
UV	Ultraviolet
VES	Visual Encounter Survey

Introduction

Park Setting and History

Lassen Volcanic National Park (LAVO or Park) encompasses 106,372 acres, spread across four counties in Northern California, and is almost completely surrounded by Lassen National Forest. The Cascades frog (*Rana cascadae*) is one of two frog species known to historically occur in LAVO, with records from at least 16 locations between 1920-1980 including Manzanita Lake, Emerald Lake, Crumbaugh Lake, Dersch Meadows, Kings Creek Meadows and several other pond, lake, and creek locations (Pope et al. 2014). Surveys in the 1990s suggested that Cascades frogs had become rare within the Park, with small numbers observed at only a few sites. Extensive surveys in 2004 (Stead et al. 2005) and resurveys in 2006 and 2007 (Fellers et al. 2007) found few remaining frogs at or near Juniper Lake. Subsequent surveys in 2008, 2009, and 2011 recorded no Cascades frogs, and there are no known locations of this species remaining in LAVO (Pope et al. 2014).

Threats to Cascades frogs include, but are not limited to, natural predators, introduction of fish into historically fishless habitats, disease (e.g., fungal pathogen *Batrachochytrium dendrobatidis*), airborne drift of pesticides from nearby agriculture areas, ultraviolet (UV-B) radiation, wildfires and fuel management, and habitat loss from human development and climate change (Plemons et al. 2015). Known predators of Cascades frogs within LAVO include:

- Rainbow trout (*Onocorhynchus mykiss*)
- Brook trout (*Salvelinus fontinalis*)
- Brown trout (*Salmo trutta*)
- Leech (*Haemopsis marmorata*)

The three trout species have been stocked throughout LAVO since the 1800's, with more focused stocking following the eruption of Mount Lassen in 1914 that decimated fish populations in LAVO (Plemons et al. 2015). Stocking from 1920 through to the 1970's included all three trout species into lakes and streams in LAVO, with the focus on enhancing brown and rainbow trout for recreational fishing. Policy changes in the 1970's resulted in discontinuation of artificial stocking in all but four lakes (Butte, Crystal, Manzanita and Summit) and two streams (Kings and Hot Springs creeks) by 1975. Stocking was discontinued completely in all LAVO waters in 1990 (Plemons et al. 2015).

Life History of Cascades Frogs

Cascades frogs breed soon after spring snowmelt. Elevation and seasonal differences affect timing of oviposition, which occurs between April and July. Egg masses are commonly found at the surface in shallow water with emergent vegetation. In the southern Cascades, larvae usually hatch in June and metamorphose in late August (Pope et al. 2014).

Recent Surveys

National Park Service (NPS) is considering reintroducing the Cascades frog to LAVO but needs to; 1) verify current species status in the Park; 2) document current condition of potential reintroduction sites; and, 3) identify potential reintroduction sites. As Cascades frog populations have declined and labor-intensive visual surveys have failed to detect frogs in LAVO within the last decade, new

methods such as environmental DNA (eDNA) sampling have been proposed. eDNA can be an effective monitoring tool to detect trace genetic evidence (such as shed skin, feces, urine, and mucus) for rare species of interest in aquatic environments (Pope et al. 2020).

Over the past decade, the use of eDNA to survey biological communities has grown tremendously (Beng and Corlett 2020). For example, quantitative polymerase chain reaction (qPCR) can selectively identify and quantify individual target species within an eDNA sample and are particularly useful for detecting the presence/absence of rare and invasive species (Evans et al. 2017, Beng and Corlett 2020). Single species eDNA approaches have robust Standard Operating Procedures (SOPs) (Abbott et al. 2021), are routinely used in legal and regulatory frameworks (Jerde 2021) and are particularly effective at detecting rare and invasive species in aquatic habitats (Beng and Corlett 2020, Gold et al. 2022).

The use of eDNA sampling does have limitations, such as variable production of eDNA across species and individuals (Stewart 2019), different rates of degradation in different habitats and conditions (Strickler et al. 2015, Goldberg et al. 2018), and that it does not travel far from its source in still water (Dunker et al. 2016, Goldberg et al. 2018). However, ongoing research to improve eDNA sampling and analytical approaches (Thomas et al. 2019, Goldberg et al. 2018, Kamoroff and Goldberg 2018) continues to improve sampling methods. Additionally, performing eDNA sampling, paired with visual surveys, has been proven to be an effective tool for detecting the presence of *R. cascadae* in the Lassen National Forest (Pope et al. 2020). Utilizing eDNA methods for fish and amphibian species presence within the Park has the potential to reduce time-consuming survey techniques that often disturb animals by netting and electrofishing and could result in reduced field time, cost, and risk to target species in order to better monitor these species and inform management efforts (Thomsen and Willerslev 2015, Pope et al. 2020).

Objectives

The objectives of this Project were:

- Conduct surveys for Cascades frogs using both visual encounter surveys (VES) and eDNA surveys at locations with the potential for reintroduction;
- Conduct surveys for potential predators (three trout species and leeches), using both VES and eDNA surveys to determine locations unsuitable for reintroduction; and
- Develop a site suitability model for both occupancy (if any) and restoration/reintroduction potential within LAVO for Cascades frogs.
- The results of the surveys as well as existing Cascades frogs observation data, habitat and abiotic environmental data were used to develop the site suitability model.

Methods

Site Selection

WSP USA Environment & Infrastructure, Inc. (WSP) compiled existing data on known Cascades frog locations, preferred habitats, and other relevant data from NPS, US Forest Service (USFS), US Geological Survey (USGS), US Fish and Wildlife Service (USFWS), and California Department of Fish and Wildlife (CDFW) via the California Natural Diversity Database (CNDDDB) including:

- Known (current and historic) Cascades Frog Locations (Pope and Larson 2013, Dinger 2016, 2019, CNDDDB 2020) – Appendix A, Figure A-1
- Leech Locations (Stead and Pope 2007, Pope 2008) – Appendix A, Figure A-1
- Trout Locations (NPS 2004) - Appendix A, Figure A-1
- Perennial waterbodies and streams from the National Hydrography Dataset (NHD) (USGS 2021a) – Appendix A, Figure A-2
- Wetlands from the National Wetland Inventory (NWI) (USFWS 2022) – Appendix A, Figure A-2
- National Elevation Data (USGS 2021b) – Appendix A, Figure A-3
- Vegetation types including wet herbaceous meadows, willow (*Salix* sp.) shrubland, and wet herbaceous vegetation (NPS 2016) – Appendix A, Figure A-4

Each of these datasets was evaluated for relevance in determining both survey locations and habitat suitability modelling. A workshop with NPS and CDFW was held on April 9, 2021, with additional follow-up conversations with USFS, to review the data and discuss potential survey locations; a spreadsheet was compiled of potential sites and their priority.

As the literature suggests that the Cascades frog occupies elevations between 3600 – 6100 feet above mean sea level (amsl), the first data used to refine locations was elevation. Elevation occurrence was based on existing Cascades frog points from CNDDDB (multiple decades), USFS (2013), and NPS (2013, 2016) amphibian surveys (Pope and Larson 2013, Dinger 2016, 2019, CNDDDB 2020). Perennial streams and water bodies (based on NHD, NWI, and other data) was a second filter. Known historic (pre-1980) locations of Cascades frogs and documented predator (primarily fish) locations were also incorporated. Additional input was gathered during the April 2021 meeting and from interviews with biologists with knowledge of LAVO. The result was 50 target sampling locations (Table B-1 in Appendix B). Vegetation density was collected at the sample locations and can be found in Table B-4 in Appendix B.

While surveying in 2021, some sites were unsafe to access or there was no evidence of surface water. If a potential site was unsafe to access or was dry upon arrival, a more suitable site was selected nearby. In a few cases, a site not originally identified as containing suitable Cascades frog habitat was readily available and was sampled instead. This resulted in a final set of 50 sites (Figure 1). One site, site 40-M, included composite samples (i.e., sampling water from multiple locations within one water resource into one eDNA filter) from two different habitat types and is displayed in Figure 1 as

40-M and 40-M-C2. Table B-1 in Appendix B summarizes the changes from the initial list of sites to the final set of sites (Figure 1), along with any composite sampling.

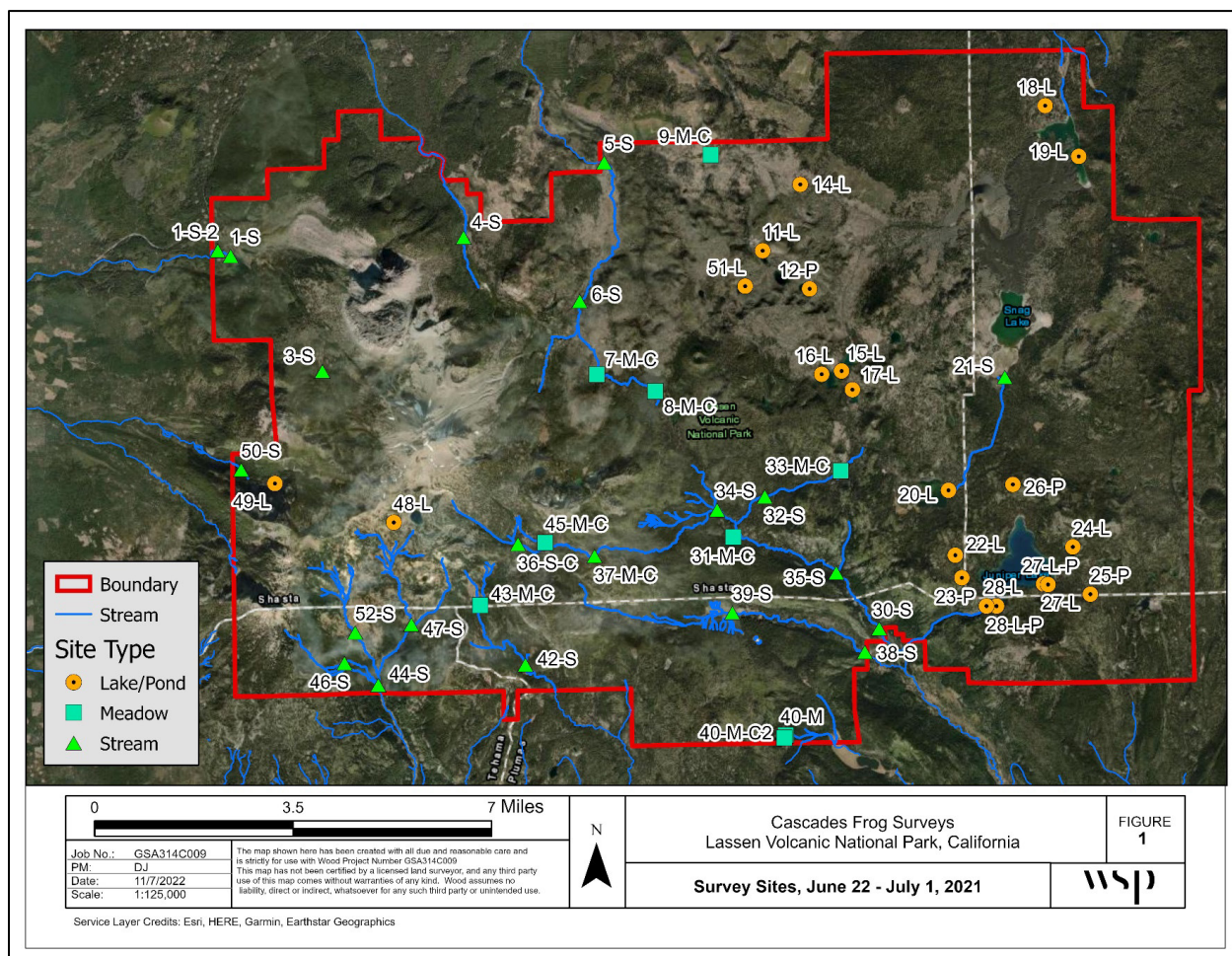


Figure 1. Survey sites for VES and eDNA sampling within LAVO, June 22 – July 1, 2021.

Survey Methods

Data attributes were collected at each survey site, including, but not limited to, site type (stream, marsh, etc.), weather conditions (air temperature, general conditions, wind), water condition (water temperature, pH), coordinates of each observation, any target species observations (frogs, leeches, or trout), and additional notes related to vegetation, aquatic substrate, and other incidental wildlife observations. Biologists recorded attributes and data for each site using Geographic Information Systems (GIS) in the ArcGIS Collector application (see Appendix B).

Global Positioning System points were collected with a Trimble® R1 Global Navigation Satellite System Receiver connected to ArcGIS Collector using tablets. Field data points were then overlaid with appropriate resource data in ArcGIS. Aerial imagery along with field data was used to modify resources such as vegetation communities, streams, and wetlands. Field data were collected using

North American Datum 1983 (NAD 1983), and field maps were generated using the NAD 1983 State Plane California VI FIPS 0406 projected coordinate system with linear units: feet.

Visual Encounter Surveys

The surveys conducted June 6-July 1, 2021 followed a methodology adapted from the CDFW protocol, A Standardized Protocol for Surveying Aquatic Amphibians (available at <https://irma.nps.gov/DataStore/Reference/Profile/2299761>) and adapted as applicable based on the experience of the lead herpetologist on the field team. Prior to VES within LAVO, WSP biologists visited reference sites south of LAVO with Cascades frog expert Dr. Karen Pope to identify and get a search image of tadpole, juvenile, and adult Cascades frog (and collect eDNA samples to be used as positive controls). VES was the preferred survey method as eggs, tadpoles, and adult frogs can be found this way, and maximum breeding counts were not the objective of the surveys (nor would they have been effective due to the unknown presence of the target species).

VES was conducted during daylight hours (mid-morning to late afternoon) and focused on Cascades frogs and their habitat. Surveys included streams, rivers, ponds, lakes and meadow streams which appeared suitable near the identified survey location. Unsuitable areas, such as dry stretches, were not included in the surveys. Upon arrival at each survey site, edges of habitat and banks were scanned for basking frogs and to make habitat observations. After scanning the habitat, biologists walked slowly, either at the edge of suitable habitat or through the water, scouting ahead for eggs, tadpoles, or frogs. Biologists carried at least one, but often two, dip nets for catching any observed tadpole or frog (Photo 1). Throughout the survey, biologists alternated between a stationary scan and a walking scan. In thicker vegetation, dip nets were used to sweep over the habitat to encourage movement and potential visual identification.

As all areas surveyed had clear water, dip net sweeps for tadpoles were not required. All encountered tadpoles were captured to verify identification. Tadpoles were caught and held in the water with the dip net. Adult frogs observed were also captured to confirm identification, collect biological data (if it was the target species), and photograph the animal. In cases where identification could be made from a distance, and the animal was not the target species, the animal was not captured.

Other species encountered were also documented and sometimes captured to confirm identification (e.g., predators such as leeches and trout). While the predators were not the target of the VES, they were observed visually and documented. Biological data to be collected upon finding Cascades frog included snout-to-vent length, weight, sex, general notes on apparent health, and swabs of the groin and throat. Since none were observed, this data was not collected.



Photo 1. Visual encounter survey in the Park using a dip net.

eDNA Sampling

Sampling was performed by filtering water through self-preserving eDNA filter packs using a hand pump and filtration flask as described below. Two replicate samples were collected at each site, as one filter was retained by the laboratory as an archive. Filter volumes and times were recorded for all samples.

Equipment

Water samples for eDNA were collected using a 47 mm self-preserving eDNA filter pack from Smith-Root (Thomas et al. 2019). The in-line filtration and preservation system (desiccant) reduces the chance of sample contamination from alternate sampling methods that require transfer of filters into preservative, and has been shown to yield more eDNA per liter of water than alternative methods using ethanol as a preservative (Thomas et al. 2019). Additionally, this method reduced the load on field crews by eliminating the need to carry ethanol into wilderness areas, with an added benefit that the filters are partially biodegradable to reduce plastic waste from the sampling design. The self-preserving filters have been shown to preserve eDNA for up to 6 months at room temperature before lab processing (Thomas et al. 2019).

Filter packs included sterile tubing used to draw a sample from the water body. Filtration was done using hand pumps attached via a filter flask and tubing to the outflow nozzle of the eDNA filter, allowing the sampler to measure the volume of water sampled (Photo 2). The use of manual hand pumps, as opposed to battery-operated peristaltic pumps that are commonly used for eDNA sampling (Pope et al. 2020), were a benefit to the field crew as this method reduced weight and did not require

carrying batteries into wilderness areas. Per technical specifications of the eDNA filter, the pressure of water passing through the eDNA did not exceed 10 psi (-20 inHg). Following collection, filters were placed back in the resealable bag, labeled, and stored until shipped for lab processing.



Photo 2. eDNA equipment set up to collect sample from the bank of a stream.

Filter Size

Water sampling for eDNA was done using 0.45 micron (μm) filters in streams and either 0.45 μm or 1.2 μm filters in lakes and wetland areas. In wetlands or lakes where the filter is more likely to clog due to suspended material or algae, crews used the slightly larger 1.2 μm filter to help obtain the target filter volumes. These filter pore sizes have been demonstrated to reliably detect fish and frogs (Kamoroff and Goldberg 2018, Bedwell and Goldberg 2020), and compared to larger sizes (5 or 10 μm) it is suggested that smaller filter pore sizes can increase the chances of detecting DNA, especially when low quantities of DNA may be present, or a small population of target organisms is present (Kamoroff and Goldberg 2018).

Filter Volume

Using the single-use tubing and filter pack, crews used the hand pump from the shore (or downstream of the tubing intake in moving water) to draw surface water (approximately 10 cm depth) through the filter. In the event a grab bottle was needed, a pre-cleaned bottle using decontamination procedures outlined below was used. The volume of filtered water was measured

using a pre-labeled filter flask. In streams and marsh channels, crews targeted 2L of filter volume (Pope et al. 2020), while in lakes and wetlands crews targeted 1L. The differing target volumes are consistent with results that show doubling the filter volume in moving water increased the likelihood of detecting rare frog species by 5x, while there was no effect of filter volume on detection in lakes in part due to filter clogging (Bedwell and Goldberg 2020). In areas where filter clogging reduces the ability to obtain the target filter volume, samples were filtered for 25 minutes (per replicate) at which time the total filtered volume was recorded, consistent with methods outlined in Bedwell and Goldberg (2020) for detection of Sierra Nevada yellow-legged frog (*Rana sierrae*) and the foothill yellow-legged frog (*Rana boylei*).

Sample Collection

When possible, samples collected at lakes targeted areas where the lake discharges into a stream in order to maximize the potential for water movement and mixing to increase the spatial resolution of eDNA samples. In lakes or wetland areas with no clear discharge point, sampling crews attempted to collect a sample from the downwind shoreline (preferably near potential frog habitat) to capture samples in areas where water movement was highest. Sampling from the shore (as opposed to wading into the water) is consistent with the littoral habitat preference of frogs and also reduces the chance for contamination by the sampler. Sampling in meadows and marsh channels targeted the downstream foot of the meadow as this has been shown to increase detection efficiency for *R. cascadae* (Pope et al. 2020).

Decontamination

Strict procedures were employed at every stage of DNA sampling to avoid contamination. Contamination can result from a variety of factors at every step in the sample collection process. Two common sources of contamination are 1) contamination that originates from equipment and 2) contamination that originates from the sampler.

Prior to the first day and after each day of field surveys, any equipment that was regularly handled by the sampling crew or came into contact with the water body being sampled, was decontaminated with a 10-30% bleach solution. During field sampling, if any equipment or boots of samplers came in contact with mud or water, they were decontaminated with a 10-30% bleach solution before departing for the next sampling site in areas where bleach could be safely contained and disposed of. Field crews also carried non-bleach decontamination solution (e.g., DNA-Away, Eliminas) if needed while in wilderness areas to eliminate the risk of spills. Equipment was wetted with the solution, wiped dry, and rinsed with deionized (DI) water. Any waste from these solutions was collected and disposed of properly.

Archive and Quality Assurance Samples

Of the two replicate samples collected at each sampling location, one was designated to be archived by the laboratory in case of discrepant results or for future analysis of additional species. In addition to the samples collected at target water bodies within the Park, two sets of positive control samples were taken for Cascades frogs at Carter Meadow which contains confirmed populations of this species. A total of two negative controls (one after the positive control samples were taken, prior to sample collection in the Park, and one approximately 75% through surveys in the Park) were also

collected using DNA-free DI water to ensure there was no eDNA signature resulting from the filtering equipment.

Assay Development

After collection, samples were shipped to Precision Biomonitoring Laboratory (Precision or Lab) in Guelph, Ontario, Canada for qPCR analysis. Existing assays were unavailable for two target species, leech and Cascades frog, requiring assay development for these species. A detailed description of the methodology used for assay development is provided in the Precision lab report (available at <https://irma.nps.gov/DataStore/Reference/Profile/2299758>). Precision already had assays developed for the three fish species and those are available in the report above. A brief summary is provided below for each species in which assay development was required.

Parasitic leech (Haemopsis marmorata)

The Triple Lock™ qPCR assay for *H. marmorata* was validated in accordance with parameters outlined in the Minimum Information for Quantitative Real-Time PCR Experiments (MIQE) Guidelines (Bustin et al. 2009). The specificity of the assay was then tested using genomic DNA extracts from the target species and on synthetic homologs of the target gene fragment in related species (e.g., *Haemopsis terrestris*, *Haemopsis elegans*). Results of this assay indicated the test was species-specific.

Cascades Frogs (Rana cascadae)

The Triple Lock™ qPCR assay for the Cascades frog qPCR assay was validated in accordance with parameters outlined in the MIQE Guidelines (Bustin et al. 2009). The specificity of the assay was then tested using genomic DNA extracted from specimens of sympatric species (such as *Rana boylei* or *Lithobates pipiens*). Results of the assay indicated the test was species-specific.

eDNA Extraction and qPCR Analysis

A detailed description of the methodology used for DNA extraction and analysis is included in the Precision Biomonitoring lab report (<https://irma.nps.gov/DataStore/Reference/Profile/2299758>). The methods are summarized briefly here.

eDNA Extraction from Filters

Individual filters from each sample envelope were subject to overnight digestion in lysis buffer ATL (Qiagen) and proteinase K before the lysate from each was pooled into single tubes representing each sample at the post-Qiashredder stage. eDNA extracts from any pooled filters were immediately stored at 4° C until further analysis.

Fluorometric Quantification of DNA Yield

DNA yields per sample were measured by fluorometric quantification on a Qubit Flex™ Spectrophotometer (ThermoFisher) to quantify total eDNA (including non-target DNA) captured on the filter papers and extracted into the final elution solution during DNA extraction. This quality control step is used to control against filter and extraction malfunctions to ensure that sufficient DNA was extracted for qPCR analyses.

qPCR for Target eDNA Detection

qPCR analysis was conducted using a CFX96 Real-Time Detection System (BioRad) thermocycler with optimized thermal cycling conditions. All qPCRs performed in triplicate per sample (N = 3). Positive amplification controls consisting of reactions containing the target species' DNA fragment were included in each qPCR run to verify qPCR assay performance. No-template controls were included in each qPCR run to detect the potential presence of sample or reagent contamination during analysis. Each qPCR reaction was 20 μ L, consisting of 15 μ L of customized master mix and 5 μ L of eDNA extract. Prior to target species analysis, each sample was tested for PCR inhibition by comparing the C_q value of each eDNA extract from the expectations of uninhibited samples. If the C_q value of the samples are delayed by at least one C_q value relative to the internal positive control, then the sample is diluted with ultrapure water and this diluted extract is subject to target species qPCR.

Using the results of the qPCR, analysis included the presence/absence of target species, the quantity of eDNA detected in each sample (as it relates to signal strength or relative population size), and the results of the positive and negative controls as part of the quality assurance and quality control (QAQC) process. Where only one or two qPCR replicates detected the target species, results have been flagged accordingly and should be regarded as positive detections on the edge of the detection limits of the assay.

Habitat Modeling

Recent occurrence data for Cascades frog within LAVO were limited, and thus a statistical model could not be properly developed and validated with the available data. Despite the lack of occurrence data, multiple high resolution GIS data sources were available for the Park and surrounding area and studies from the surrounding region provided occurrence data and some information on the habitat requirements for the species overall. These data sources and ecological information were used to quantify and rank the suitability of the landscape within LAVO according to three ecologically relevant criteria:

- Suitable Vegetation Availability,
- Elevation/Climatic Environment, and
- Perennial Water Availability

Suitable Vegetation Availability

Cascades frogs use a variety of aquatic habitats and can be found in lakes, ponds, wet meadows and streams (Pope et al. 2014). Within LAVO, these habitats were identified using detailed vegetation mapping (NPS Integrated Resource Management Application 2016) and the NWI data (USFWS 2022). Specifically, wet herbaceous meadows, willow (*Salix* species) shrubland, and wet herbaceous vegetation were extracted from the NPS 2016 data and persistent riverine and lacustrine wetland habitats were isolated from the NWI data. These data sources were combined and represented suitable vegetation.

The resulting vegetation maps were combined into a 50 x 50 m raster grid that represented the availability (0: no suitable vegetation, 1: suitable) of any of these habitats within the grid cell.

Isolated patches of habitat are likely not as valuable as multiple connected patches of habitat (Pope et al. 2014) and Cascades frogs can regularly travel more than 1 km between seasons (Garwood and Welsh Jr. 2007, Garwood 2009, Pope and Larson 2010 as cited in Pope et al. 2014). Thus, a moving window was used to transform each cell to represent the density of available habitat within a circular buffer with a 1.2 km radius (average minimum intra-basin dispersal distances, Garwood 2009). The resulting density was rescaled between 0 (lowest suitability) and 1 (highest suitability).

Elevation/Climate Suitability

Climatic variables (e.g., temperature, precipitation, etc.) can be an important factor in determining the distribution of amphibians and translocation success (Trumbo et al. 2012, Bellis et al. 2020).

Climate grid data were derived from ClimateNA (Wang et al. 2016), which extracts gridded climate data (4 x 4 km) from PRISM (Daly et al. 2008) and WorldClim (Hijmans et al. 2005) and combines it with elevational data to derive scale-free gridded climate data. This software and a digital elevation model (30 x 30 m resolution) were used to develop raster grids representing 30-year annual climate normals (1991 – 2020) for the following nine standard climate summaries:

- AHM: annual heat-moisture index $(MAT+10)/(MAP/1000)$
- DD5: degree-days above 5°C, growing degree-days
- FFP: frost-free period
- MAP: mean annual precipitation (mm)
- MAT: mean annual temperature (°C)
- MCMT: mean coldest month temperature (°C)
- MSP: May to September precipitation (mm)
- MWMT: mean warmest month temperature (°C)
- PAS: precipitation as snow (mm). For individual years, it covers the period between August in the previous year and July in the current year.

The selected grids are six of the nine (MAT, MWMT, MCMT, MAP, MSP and AHM) directly calculated annual variables in ClimateNA and represent temperature and precipitation variability across the range of Cascades frogs in California. DD5 and FFP were chosen to reflect the length of the active season, and PAS because it is correlated with temperature and spring flow events. The grids were generated across the entire range of the occurrence data for Cascades frogs in California and the average values for the region (1.2 km circular buffer) surrounding each occurrence location were calculated. As a comparison random ‘background locations’ were derived across the range of Cascades frogs in California and separately within the LAVO boundary. Comparing the climate at project locations to background locations gives an indication of where Cascades frogs are distributed in locations where climate conditions that deviate from what is randomly available. The number of climate parameters was reduced using a principal component analysis (PCA) including both occurrence and background locations. A PCA is a multivariate approach which transforms a group of variables into a smaller new set of uncorrelated variables (the principal components; PCs). The principal components are defined to be linear combinations of the original variables. Here this

approach was used to derive a meaningful, but reduced, set of climate components that could be used to derive climate suitability layers. Once completed, the PCs were used to generate PC raster maps and averaged within a 1.2 km moving window. PC layers were translated to suitability scores by using the percentiles of the PC scores for occurrence data that explained the greatest variability. In other words, we identified the range of PC scores where Cascades frogs are currently found in California. PC maps were ranked from lowest (0 –outside the range of occurrence data) to highest (1 –within the central percentiles) suitability. The range-wide PCs were resampled into a 50 x 50 m park-specific raster grid to match with the vegetation data and classified from low-to-high suitability using the percentiles.

Perennial Water Availability

The availability of perennial waterbodies and streams were established from the National Hydrography Dataset (USGS 2021a) and converted into a 50 x 50 m raster grid that represented the availability (0: no habitat, 1: habitat) of a perennial waterbody or stream within with each grid cell. Following the methods described for the vegetation availability, a moving window was used to transform each cell to represent the density of perennial water within a circular buffer (1.2 km radius) and the resulting density was rescaled between 0 (lowest suitability) and 1 (highest suitability).

Final Suitability

The final suitability model was derived by averaging across the layers for each of the three components and rescaling the result to values between 0 and 1. The resulting suitability was summarized at survey site locations (1.2 km buffer) and within sub-watershed US Geologic Survey hydrologic units code (HUC, specifically HUC12) and combined with information on predator occurrences and soil burn severity.

Climate Projections

Given the purpose of the project to identify suitable sites for reintroducing Cascades frogs into LAVO and climate suitability is known to influence reintroduction success (Bellis et al. 2020), it is relevant to evaluate climate projections to understand how climate change may influence suitability for the Park in the future. There is little purpose reintroducing Cascades frogs into an area that is suitable now, but which will likely be highly unsuitable in 30 years.

Projected climate grid data for the time periods of 2011 to 2040 and 2041 to 2070 were derived from ClimateNA for the 13 ensemble General Circulation Model projections. The projected climate data were used to derive future climate suitability using the current PCA and percentile ranges described above. This step was included to identify if the habitat suitability at LAVO would likely improve or decline in future decades.

Results

Visual Encounter Surveys

A total of 50 potential habitat sites within the Park were surveyed (Figure 1) including 28 streams and marsh channels and 22 lakes and ponds.

No Cascades frog egg masses, larvae, or adults was observed during VES within LAVO. Of the 50 sites surveyed within LAVO, Pacific tree frog (*Pseudacris* sp.) adults and tadpoles were observed at 18 sites (Figure 2, Photo 3), Western toad (*Anaxyrus boreas*) toadlets were observed at Soap Lake (14-L), leeches were observed at two sites, and trout were observed at 19 sites (Figure 3, Photo 4) (Table 1). The comprehensive VES results can be found in Table B-2, in Appendix B.

Table 1. Summary of VES results at 50 sites within LAVO between June 22 and July 1, 2021.

Category	Cascades frogs	Tree Frog Tadpoles and Adults	Trout Species	Leeches
Sites observed	0	18	19	2

Incidental wildlife observations were recorded as relevant and the incidental species results can be found in Table B-3 in Appendix B.

Cascades frog breed in still or slow-moving water where eggs are laid in shallow areas, often attached to vegetation (Slater 1939). These waterbodies that are considered breeding habitats are extremely important to amphibian biodiversity within a landscape and often represent the only breeding habitat for local populations. The best breeding areas are unpolluted, do not contain fish, and contain a variety of vegetation structures, both in and around the edge of the pond, for egg-laying and calling by frogs.

Cascades frog inhabits wet mountain areas such as small streams, meadow puddles, ponds, and lakes in open coniferous forests, rarely below 2,000 ft (Slater 1939). They are found in water or among grasses, ferns, and riparian vegetation (AmphibiaWeb 2021). The best adjacent habitats have dense undergrowth that maintains a damp environment.

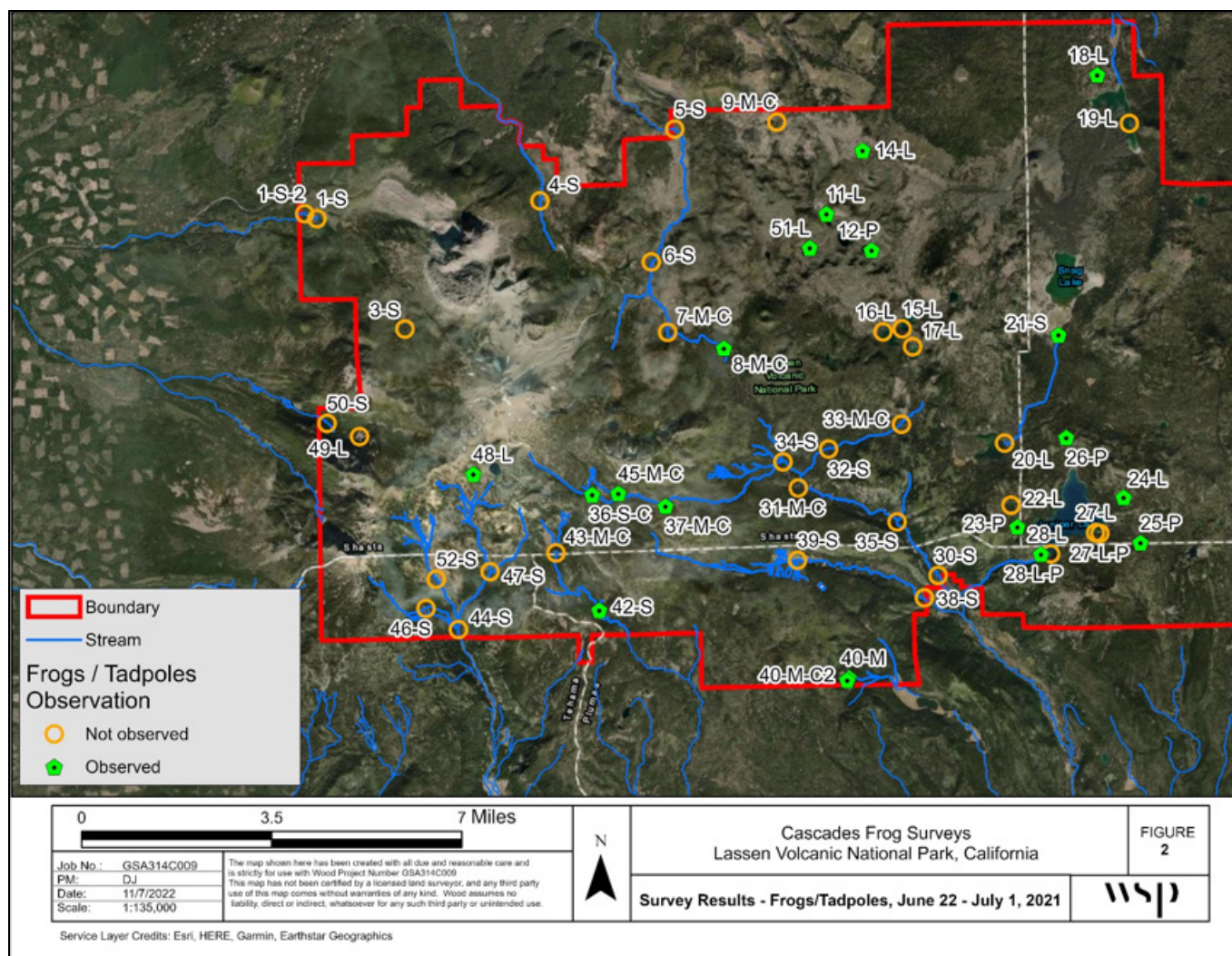


Figure 2. VES results for tree frog tadpoles and adults within LAVO, June 22 – July 1, 2021.



Photo 3. Pacific tree frog (*Pseudacris* sp., left), Pacific treefrog tadpole (right).

As discussed above, a desktop source review was completed to target sample sites which met habitat requirements for Cascades frog. However, many sites did not contain suitable habitat during fieldwork as a result of drought and the dry field conditions. A few sample sites had higher human activity than expected and previous fire areas had not recovered to the extent needed to support suitable vegetation. Drought dries up the ponds, streams, and pools, which serve as breeding habitats and the succession into thickets and forests similarly reduces breeding habitats (AmphibiaWeb 2021). Succession is often a result of common management practices that suppress natural fire regimes and the cessation of cattle grazing. Additionally, fire suppression leads to the buildup of vegetation biomass, and in fire-prone ecosystems, the buildup may produce more severe fires when they do burn. In 2012 the Reading Fire burned the north central portion of the Park, and in 2021 (after surveys), the Dixie fire burned the majority of the Park. All of these contributed a very limited amount of suitable habitat for Cascades frogs.

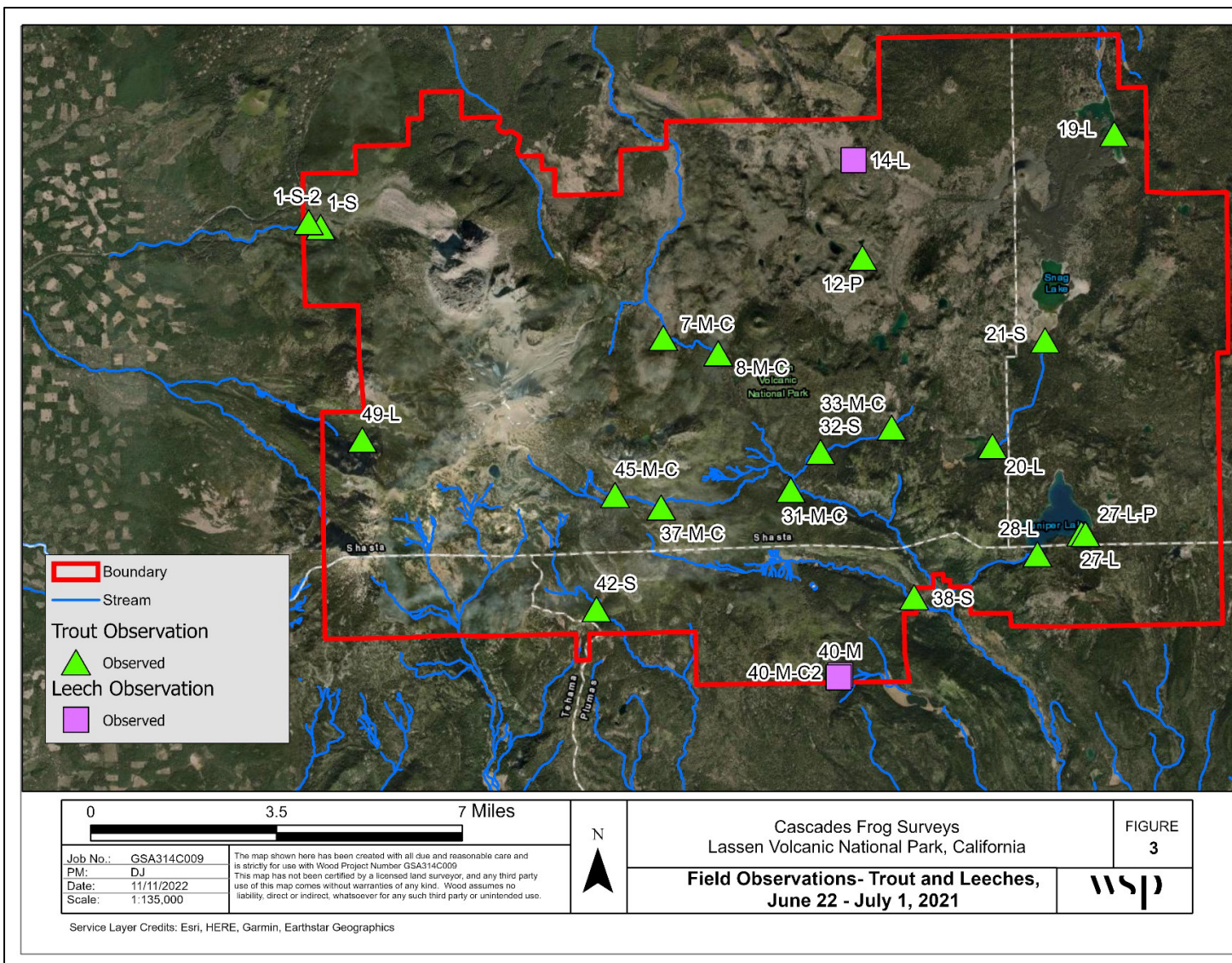


Figure 3. Field observations for trout (all species) and leeches within LAVO, June 22 - July 1, 2021.



Photo 4. Individual trout in a dip net.

Sample sites 5-S, 6-S, 7-M-C, 9-M-C, 11-L, 12-P, 51-L, and 14-L had previously been affected by fire, and suitable wet meadow sites and adjacent habitats were not present (Photo 5). Similarly, in July 2021, “Extreme to Exceptional” Drought occurred at LAVO (NIDIS 2021). Drought was noticeable at the majority of the sample sites by noticeably low water levels (Photo 6). Three sites had no water present and therefore were not surveyed. Other sites were moved to where potential habitat or water occurred. Lastly, the influence of human activity was apparent in some places, such as Emerald Lake, that could affect Cascades frogs (Plemons et al. 2015). At Emerald Lake, open tree cover to the water’s edge occurred, however, low riparian vegetation such as grasses and sedges were largely trampled, compacted, and otherwise impacted by human activity. Emerald Lake also had a green-yellow pond algal mat around the shore which was not seen in other sample locations. One adult male Pacific tree frog that was captured at Emerald Lake was lethargic and had distinct reddening of the groin and legs suggestive of chytrid fungus (*Batrachochytrium dendrobatidis*).

Sample sites which retained desirable features for Cascades frog, despite current environmental conditions, were also sites which contained fish. For example, site 45 contained a large wet meadow; however, the stream throughout contained an abundance of fish. When watercourses were separated from the main channels at site 45, Pacific tree frog tadpoles were documented. A site perched above a rock formation at site 37 contained a pond with Pacific tree frog tadpoles and old egg masses. Although water levels were very low at Snag Lake (site 21), wet meadow features and slow wooded creeks were still present, and numerous clusters of Pacific tree frog tadpoles were observed, as well as fish. The presence of Pacific tree frog tadpoles indicates that the area can at least sustain frog tadpoles, although it is unknown if Pacific tree frogs and Cascades frogs compete in any way.



Photo 5. Soap Lake (Site 14-L) displaying the burned stand from the 2012 Reading Fire.



Photo 6. Site 37, a dry location that previously had standing water.

Site 40, Willow Lake, contained suitable habitat and abundant Pacific tree frog juveniles. While no fish were documented at Site 40, leeches appeared abundant. Site 24-L, Crystal Lake, had Pacific tree frog juveniles along shallow edges with vegetation and no apparent sign of fish or leeches. However, Crystal Lake is a popular day hike location with access to swimming. Crystal Lake occurs east of Juniper Lake. Juniper Lake was sampled in four sites: 27-L, 27-L-P, 28-L, and 28-L-P. Juniper Lake sites 27-L and 27-L-P contained the invasive signal crayfish, and three of the four sites contained fish. Juniper Lake is also a campground, and vegetation trampling and compaction around shorelines and isolated ponds are common. While hiking to Snag Lake, a vernal pond was sampled and had dozens of Pacific tree frog sightings. This vernal pond became sites 26-P and had no signs of fish or leeches, and it contained fairy shrimp. Minimal encroachment from humans was found at site 26-P, and riparian vegetation did occur as well as an open coniferous canopy.

eDNA Survey

A total of 107 eDNA samples were collected, including two negative controls, four positive controls (at two sites outside LAVO), and a set of test and archive samples from 50 sites inside LAVO.

Total DNA Recovery

Of the total of 101 eDNA samples collected within LAVO, 57 were collected with a 0.45 μm filter and 44 were collected with a 1.2 μm filter. Filter volumes for 0.45 μm filters ranged from 170-2520 milliliters (mL) while 1.2 μm filter volumes ranged from 175-1995 mL. Total DNA recovery (including non-target species) for all samples ranged from 616-12,000 nanograms per milliliter (ng/mL) and provided enough material for all samples to be analyzed successfully.

Two negative control samples were analyzed and results were reported below the detection limit for DNA yield (<10 ng/mL), which confirms that appropriate precautions were taken to avoid equipment contamination during field sampling.

Target Species DNA Recovery

Of the 56 eDNA extracts analyzed, including positive and negative control samples, 14 showed no signs of PCR inhibition from material collected with the water sample and did not require further dilution. The remaining 42 samples were subject to both 1:10 and 1:20 dilutions in TE buffer, and the internal positive control (IPC) analysis was rerun. All samples exhibited IPC values akin to the baseline no-template controls IPC level with 1:20 dilutions, but only one sample did not with a 1:10 dilution (32-S). Thus, qPCR for the five target species were conducted on a 1:20 dilution of sample 32-S, but on a 1:10 dilution of all the other samples in which the undiluted samples showed initial PCR inhibition.

For all species, positive amplification controls (PACs) amplified 100% across replicates: Cascades frog (Mean $C_q = 19.19 \pm 0.88$); parasitic leech (Mean $C_q = 18.97 \pm 0.86$); brook trout (Mean $C_q = 21.83 \pm 0.66$); rainbow trout (Mean $C_q = 29.78 \pm 0.26$); brown trout (Mean $C_q = 24.56 \pm 0.64$). Successful PAC amplification renders any risk of false negative detection from degraded reagents or suboptimal or defective PCR cycling conditions as negligible.

Results of qPCR are shown as the copies of target eDNA per microliter (μl) of eDNA extract (micrograms per liter [$\mu\text{g/L}$]). For all target species, the number of copies of eDNA recovered ranged from 0.1-689.3 $\mu\text{g/L}$. For additional details about QAQC results, see Precision Biomonitoring lab report (<https://irma.nps.gov/DataStore/Reference/Profile/2299758>).

Cascades frog

There were no positive detections of Cascades frogs from within LAVO.

Positive control samples were taken from Carter Meadow outside the park where the presence of Cascades frogs was confirmed visually before eDNA samples were collected. At each of the two sampling locations in Carter Meadow, a sample was taken with a 0.45 μm and 1.2 μm filter, for a total of four positive control samples. Two of the four positive control samples were reported as not-detected (ND) for Cascades frog eDNA. These two samples (CAR-M-C1 and CAR-M-C2) were collected from the same location in Carter Meadow on US Forest Service lands (Carter Meadow 1; Table 2, Photo 7). Samples from this site were collected from a small marsh channel after one small individual Cascades frog had been identified approximately 5 meters upstream. However, prior to eDNA sampling, the frog was relocated downstream to avoid contamination due to handling of the animal. The relocation, along with other factors such as the small size of the individual and relatively low flow of the marsh channel, may have contributed to the absence of eDNA in the sample.

Table 2. Cascade frogs eDNA results from Carter Meadow, outside of LAVO.

Site Code	Site Name	Filter Size (μm)*	Average eDNA ($\mu\text{g/L}$)*
CAR-M-C1	Carter Meadow 1	0.45	ND
CAR-M-C2	Carter Meadow 1	1.20	ND
CAR-M-C3	Carter Meadow 2	0.45	106.2
CAR-M-C4	Carter Meadow 2	1.20	10.9

* μm = micron; $\mu\text{g/L}$ = microgram per liter, ND = not detected

Results from the remaining two positive control samples confirmed presence of Cascades frog within Carter Meadow Site 2. Samples from this site were collected from the foot of the meadow fed by multiple small channels, which drained a large portion of the meadow. Samples were taken approximately 10 meters downstream of the main convergence of multiple marsh channels, where at least four adult Cascades frogs were observed. For both 0.45 μm and 1.2 μm samples, positive eDNA signals for Cascades frogs were detected. Despite similar filter volumes (875 mL for 0.45 μm , 960 μmL for 1.2 μm), approximately 10x the target eDNA was detected in the 0.45 μm filter (Table 2).



Photo 7. eDNA collection at Carter Meadow 1.

Brook trout

Brook trout were detected at 18 sites in LAVO (Figure 4 and Table 3). All detections were made in streams and channeled marsh habitats, although detections at inflow/outflow points of Manzanita and Snag Lakes suggest that brook trout are likely present in those waterbodies. Within discrete watersheds such as Hat Creek, Kings Creek, and Hot Springs Creek detections were relatively consistent across all sites at similar magnitude, suggesting that populations of brook trout in these watersheds are well distributed.

In some cases, eDNA detection downstream is not necessarily additive and localized magnitudes may well reflect areas with more suitable habitat for this species or areas where eDNA persists for longer periods and may be more readily detected. For example, brook trout were detected at the outflow to Manzanita Lake (site 1-S, 24.9 $\mu\text{g/L}$); however, more eDNA was detected in an adjacent wetted area to Manzanita Lake (site 1-S-2, 137.7 $\mu\text{g/L}$). Within the Kings Creek watershed, brook trout were detected at similar magnitudes at most sites (5.05-81.9 $\mu\text{g/L}$). There were higher detections at Upper Grassy Swale Trail (site 33-M-C, 207.4 $\mu\text{g/L}$) and at a small wetland that empties into Kings Creek (site 31-M-C, 581.9 $\mu\text{g/L}$, Photo 8), which had the highest concentration of eDNA for brook trout detected among all sites. Of the sites with the highest concentrations of brook trout eDNA, most were characterized as marsh channels. The wetted area at these sites is relatively small with low flow and substantial grass and vegetation cover, which may have helped eDNA persist compared to stream sites with higher flow.

In the case of Hat Creek, eDNA magnitude for brook trout appeared to increase down the watershed, with upper watershed sites showing lower concentrations (sites 8-M-C and 7-M-C, 39.7 and 49.7 $\mu\text{g/L}$ respectively) than those downstream (sites 6-S and 5-S, 162.9 and 361.6 $\mu\text{g/L}$ respectively). This could be in part due to increased populations in these areas (and perhaps larger fish in the faster moving stream channel compared to small marsh channels) and may also be in part due to eDNA in this watershed persisting longer due to environmental conditions such as temperature or exposure to sunlight (Strickler et al. 2015).

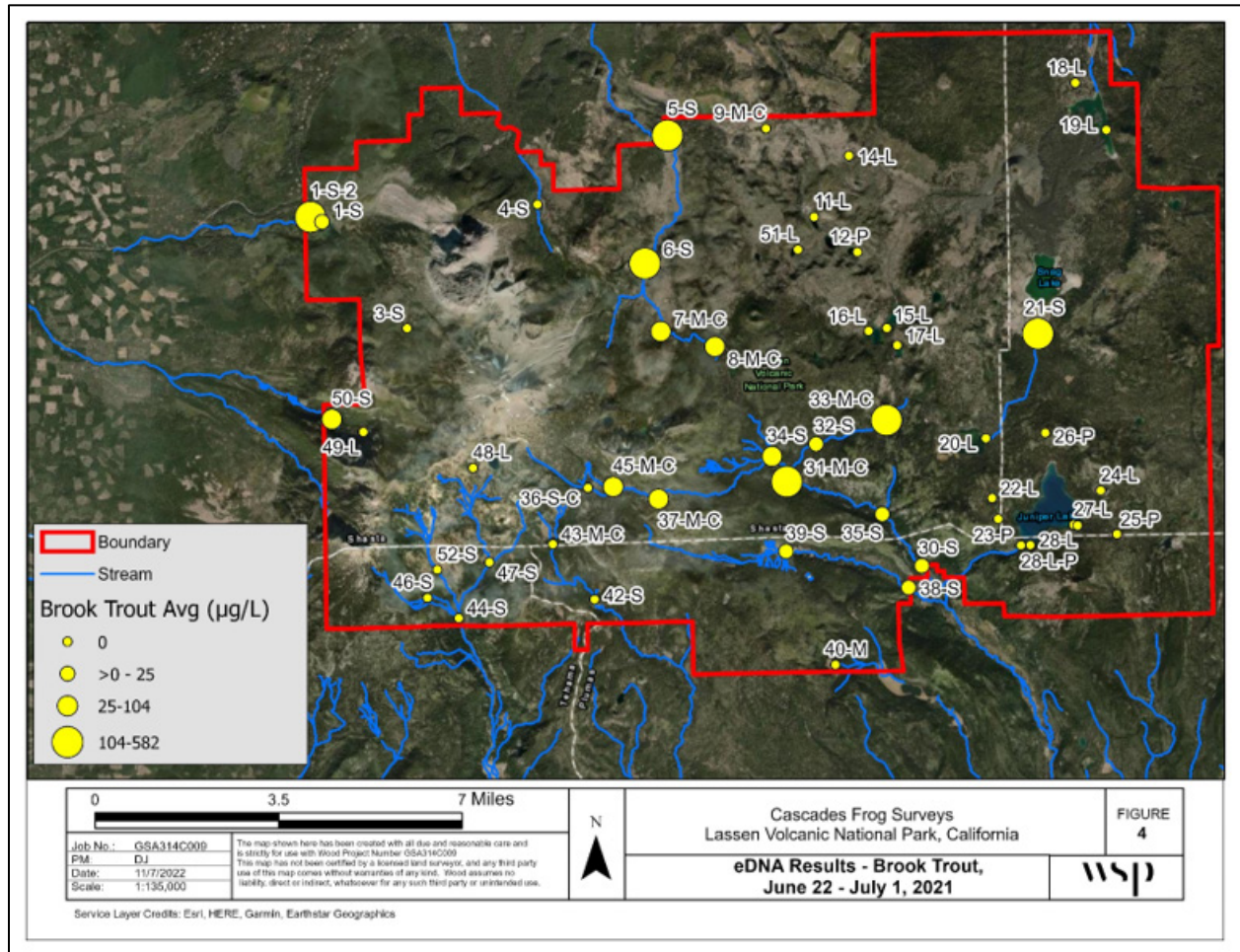


Figure 4. eDNA results for brook trout from samples collected in LAVO, June 22 - July 1, 2021.

Table 3. Brook trout eDNA sampling results within LAVO between June 22 and July 1, 2021 (PCT = Pacific Crest Trail).

Site Code	Site Name	Filter Size (µm)	Average eDNA (µg/L)
1-S	Manzanita Lake Outflow	0.45	24.9
1-S-2	Manzanita Wetted Area	0.45	137.7
5-S	Hat Creek - Downstream	0.45	361.6
6-S	Hat Creek Near Hat Creek Rd	0.45	162.9
7-M-C	East Fork Hat Creek	0.45	49.7
8-M-C	East Fork Hat Creek by Summit Lake Trailhead	0.45	39.7
21-S	Inflow to Snag Lake	0.45	227.4
30-S	Lower Kings Creek by Park Boundary	0.45	11.2
31-M-C	Wetland Near PCT and Kings Creek	0.45	581.9

^a eDNA detected in only 1 of 3 qPCR replicates

^b eDNA detected in only 2 of 3 qPCR replicates

Table 3 (continued). Brook trout eDNA sampling results within LAVO between June 22 and July 1, 2021 (PCT = Pacific Crest Trail).

Site Code	Site Name	Filter Size (µm)	Average eDNA (µg/L)
32-S	Lower Grassy Swale Trail	0.45	20.4 ^b
33-M-C	Upper Grassy Swale Trail	0.45	207.4
34-S	King and Summit Creek Junction	0.45	65.9
35-S	Lower Kings Creek at Kelly Camp Trail	0.45	5.1 ^b
37-M-C	Kings Creek Trail	0.45	81.9
38-S	Hot Springs Creek - Downstream near Park boundary	0.45	13.0
39-S	Hot Springs Creek in Drakesbad Meadow	1.20	8.0 ^a
45-M-C	Kings Creek Meadow	1.20	71.4
50-S	North Fork Bailey Creek	0.45	103.9

^a eDNA detected in only 1 of 3 qPCR replicates

^b eDNA detected in only 2 of 3 qPCR replicates



Photo 8. Trout visible in shallow marsh channel in King's Creek Meadow.

Rainbow trout

Rainbow trout were detected at 13 sites in LAVO (Figure 5 and Table 4) and at one site in Carter Meadow. Detections were primarily in stream and marsh channel habitats, but detections were also reported in samples collected from Blue Lake (site 49-L) and at the outflow/inflow to Manzanita and Snag Lakes. Consistent detections of rainbow trout were made in Hot Springs Creek watershed and within the Kings Creek watershed, primarily in the lower section of the watershed at and below the

Summit and Kings Creek Junction (site 34-S). The amount of eDNA detected in the lower end of these watersheds, before the creeks exit the park boundary, were higher than the amount detected upstream (the highest concentration of rainbow trout eDNA was 79.7 µg/L at site 30-S), suggesting increased populations downstream. Detections of rainbow trout were reported in samples collected from the furthest downstream points of Mill Creek (site 44-S) and Rice Creek (42-S) near the Park boundary, but no detections were reported upstream in those watersheds.

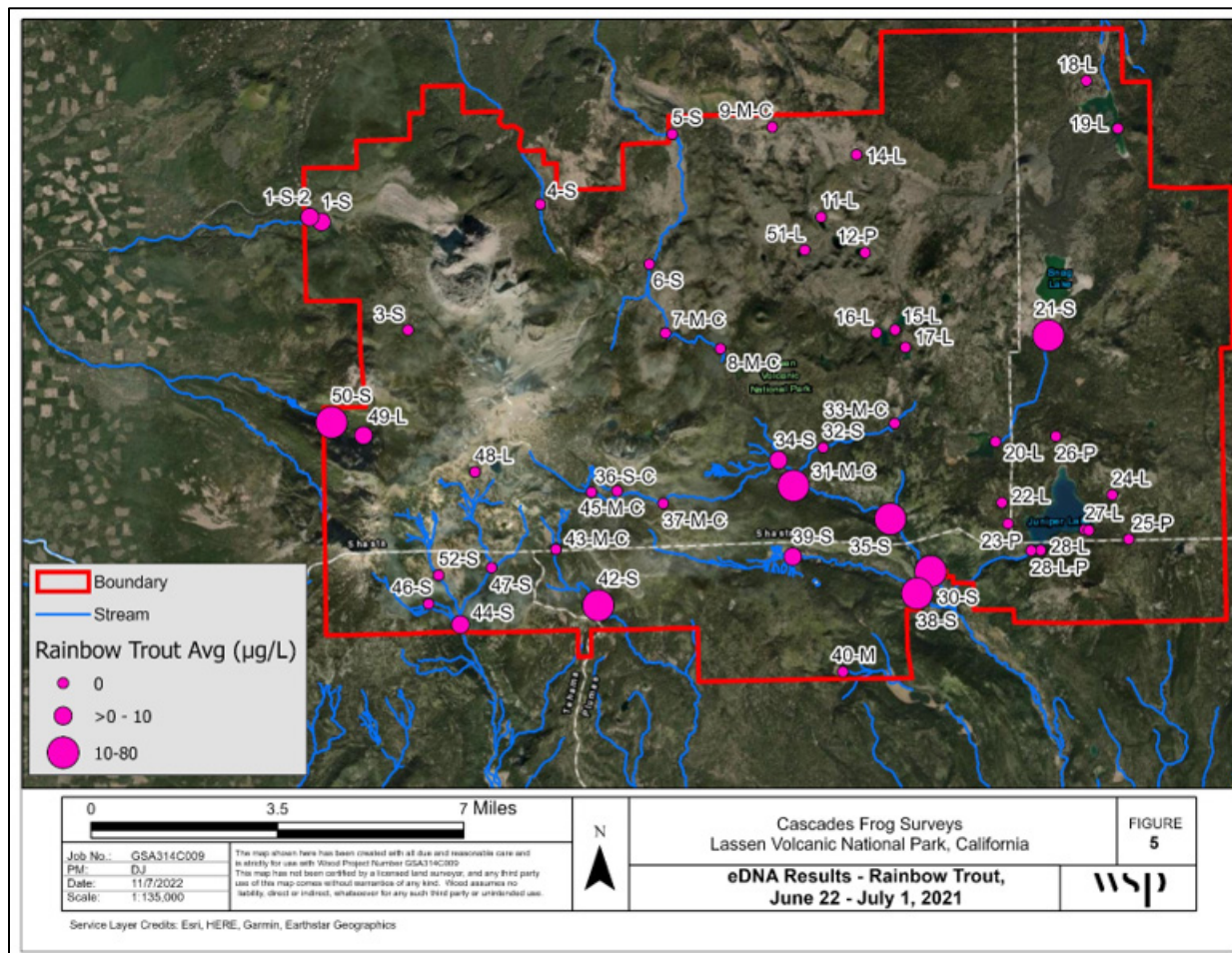


Table 4. Rainbow trout eDNA results within LAVO between June 22 and July 1, 2021.

Site Code	Site Name	Filter Size (µm)	Average eDNA (µg/L)
CAR-M-C3	Carter Meadow 2	0.45	3.1 ^a
1-S	Manzanita Lake Outflow	0.45	4.0
1-S-2	Manzanita Wetted Area	0.45	3.0 ^a
21-S	Inflow to Snag Lake	0.45	70.9 ^b

^aeDNA detected in only 1 of 3 qPCR replicates

^beDNA detected in only 2 of 3 qPCR replicates

Table 4 (continued). Rainbow trout eDNA results within LAVO between June 22 and July 1, 2021.

Site Code	Site Name	Filter Size (µm)	Average eDNA (µg/L)
30-S	Lower Kings Creek by Park Boundary	0.45	79.7
31-M-C	Wetland Near PCT and Kings Creek	0.45	12.5 ^a
34-S	King and Summit Creek Junction	0.45	9.8
35-S	Lower Kings Creek at Kelly Camp Trail	0.45	46.3
38-S	Hot Springs Creek - Downstream near Park boundary	0.45	51.6
39-S	Hot Springs Creek in Drakesbad Meadow	1.20	6.2
42-S	Rice Creek	0.45	36.3 ^a
44-S	Mill Creek	0.45	4.4 ^a
49-L	Blue Lake	1.20	5.9
50-S	North Fork Bailey Creek	0.45	16.3

^aeDNA detected in only 1 of 3 qPCR replicates

^beDNA detected in only 2 of 3 qPCR replicates

Brown trout

Brown trout were detected at 12 sites in LAVO (Figure 6 and Table 5). Detections were reported at nearly equal numbers of stream and lake (or lake inflow/outflow) sites. Brown trout were most prevalent in Kings Creek (at and below Kings/Summit Creek junction site 34-S) and Hot Springs Creek, with a single detection at the bottom of Rice Creek. Brown trout eDNA was detected in samples from Butte and Blue Lake as well as the inflow/outflow of Manzanita and Snag Lake. The magnitude of brown trout eDNA detected at most sites was low compared to other trout species (0.2-4.7 µg/L), with the exception of the two sites at the outflow of Manzanita Lake, in which concentrations were reported at 10.1 µg/L at the outflow point and 60.1 µg/L in the adjacent wetted area.

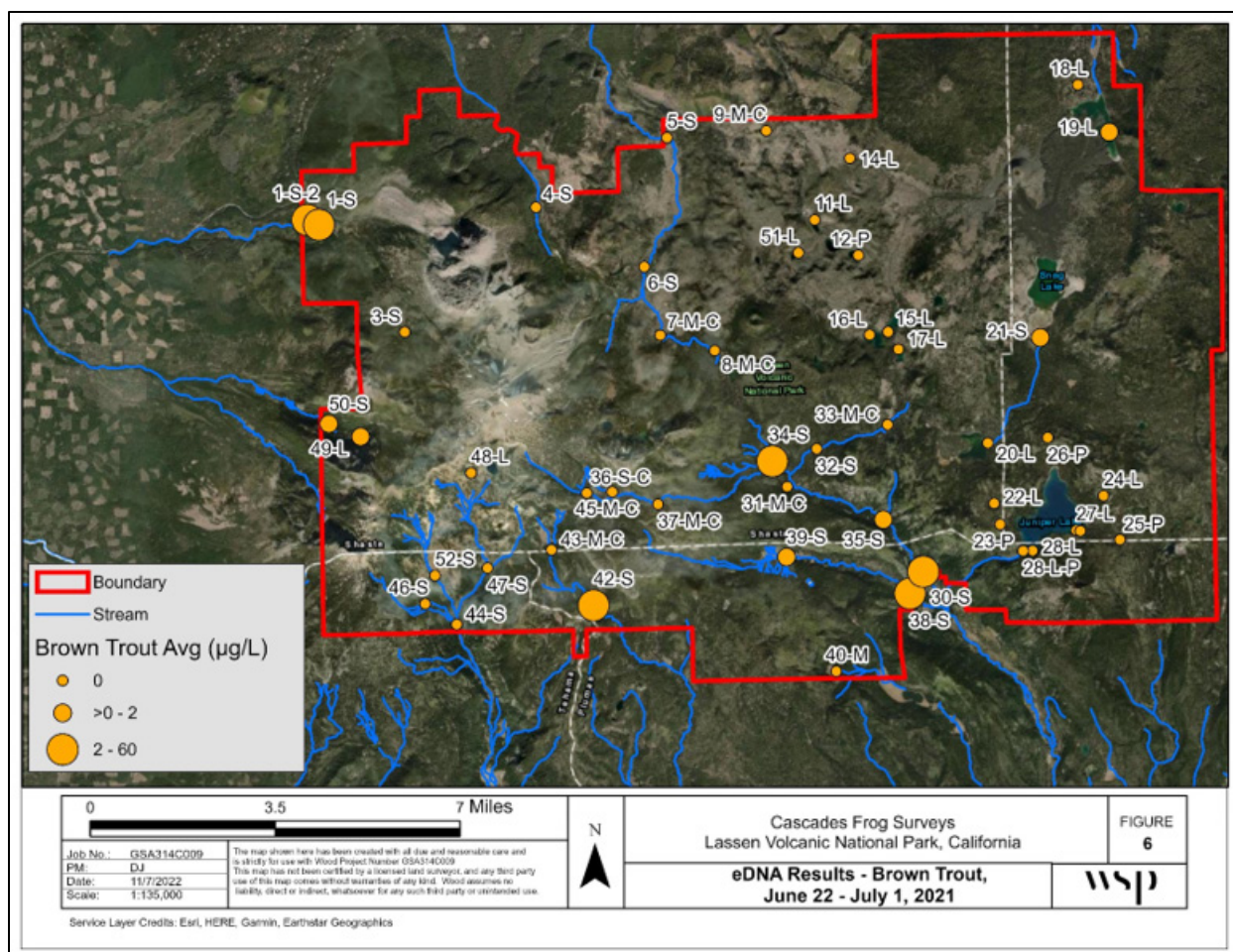


Table 5. Brown trout eDNA results within LAVO between June 22 and July 1, 2021.

Site Code	Site Name	Filter Size (µm)	Average eDNA (µg/L)
1-S	Manzanita Lake Outflow	0.45	10.1
1-S-2	Manzanita Wetted Area	0.45	60.1
19-L	Butte Lake - Eastern Shore	1.20	0.2 ^a
21-S	Inflow to Snag Lake	0.45	0.7
30-S	Lower Kings Creek by Park Boundary	0.45	2.6
34-S	King and Summit Creek Junction	0.45	2.6
35-S	Lower Kings Creek at Kelly Camp Trail	0.45	2.1
38-S	Hot Springs Creek - Downstream near Park boundary	0.45	4.7
39-S	Hot Springs Creek in Drakesbad Meadow	1.20	1.1

^aeDNA detected in only 1 of 3 qPCR replicates

^beDNA detected in only 2 of 3 qPCR replicates

Table 5 (continued). Brown trout eDNA results within LAVO between June 22 and July 1, 2021.

Site Code	Site Name	Filter Size (µm)	Average eDNA (µg/L)
42-S	Rice Creek	0.45	2.1
49-L	Blue Lake	1.20	0.7
50-S	North Fork Bailey Creek	0.45	1.2

^aeDNA detected in only 1 of 3 qPCR replicates

^beDNA detected in only 2 of 3 qPCR replicates

Leech

The parasitic leech *H. marmorata* was only detected through eDNA samples at one site (40-M, Little Willow Lake), with very low concentrations of eDNA, reported at 0.13 µg/L as an average of all three qPCR replicates. Invertebrates are generally known to shed less DNA compared to vertebrate species like fish (Gold et al. 2022), and their small size may make detection possible only in areas with high densities.

Comparison of VES and eDNA Results

Pairing VES with eDNA provides additional information when determining suitable reintroduction sites; however, comparing where the two results did not align is instructive for designing future surveys (Pope et al. 2020). Importantly, eDNA was not detected in negative control samples, which gives confidence that sampling efforts in the field were successful in preventing contamination. For the positive control samples collected at Carter Meadow for Cascades frogs, VES and eDNA sampling methods agreed on the presence of frogs within the larger stream system. Possible explanations for the disagreement between VES and eDNA of a juvenile Cascades frog at the small channel (CAR-M-C1 and CAR-M-C2) is the small size of the frog, handling and removal of the frog prior to sampling, and unknown shedding rate of eDNA between life stages. It is also consistent with previous eDNA surveys performed for Cascades frogs, where it was found that detection was less consistent at the head of a meadow compared to the foot where eDNA can accumulate from all habitats within the meadow (Pope et al. 2020).

Within the Park there were 13 sites where eDNA and VES results differed in the presence of trout, falling into two categories. The first category includes five sites where fish were observed visually but not detected by eDNA (Table 6), all of which were lake or pond sites. Both filter pore sizes (0.45 and 1.2 µm) were used for samples collected at these sites, indicating filter size was likely not a contributing factor to the observed discrepancies. Additionally, the only other two lakes where trout were observed (Butte Lake and Blue Lake, 19-L and 49-L respectively) had positive eDNA detections using 1.2 µm filters. The most likely explanations for the discrepancy between field observations and negative eDNA results are 1) the population of fish in these lakes was small or individual eDNA shedding rates were low (Stewart 2019); 2) water did not readily mix in the lake so if fish were not present in the immediate vicinity they may not have been detected (Dunker et al. 2016); and 3) these lakes were all relatively unvegetated along the shoreline and the combination of water temperature and UV exposure from sunlight rapidly degraded eDNA in the water (Strickler et

al. 2015). The second category where eDNA and VES results differed in the presence of trout includes eight sites where fish were not observed by field crews but were detected by eDNA (Table 6), all of which were stream sites. Notably, these sites were all part of some of the larger stream systems surveyed such as Hat Creek (5-S, 6-S), Kings Creek (30-S, 34-S, 35-S), Hot Springs Creek (39-S), Mill Creek (44-S), and Bailey Creek (50-S). It is most likely that fish were present in these systems but were either 1) too far upstream to be detected during VES or 2) well hidden in the stream and evaded detection. Compared to the historically available data for trout, these results indicate the trout are much more widely distributed throughout LAVO.

Table 6. Summary of target species detections for both VES and eDNA results.

Site ID	Site Description	Cascades Frog Detected	Trout Detected	Leech Detected
1-S	Manzanita Lake Outflow	No	VES, eDNA	No
1-S-2	Manzanita Wetted Area	No	VES, eDNA	No
3-S	Manzanita Creek	No	No	No
4-S	Lost Creek	No	No	No
5-S	Hat Creek - Downstream	No	eDNA	No
6-S	Hat Creek Near Hat Creek Rd	No	eDNA	No
7-M-C	East Fork Hat Creek	No	VES, eDNA	No
8-M-C	East Fork Hat Creek by Summit Lake Trailhead	No	VES, eDNA	No
9-M-C	Wetland Near PCT and Cluster Lake Trail	No	No	No
11-L	Cluster Lake	No	No	No
12-P	Pond Near Feather Lake	No	VES	No
14-L	Soap Lake	No	No	VES
15-L	Lower Twin Lake	No	No	No
16-L	Upper Twin Lake	No	No	No
17-L	Swan Lake	No	No	No
18-L	Bathtub Lake	No	No	No
19-L	Butte Lake - Eastern Shore	No	VES, eDNA	No
20-L	Horseshoe Lake - East	No	VES	No
21-S	Inflow to Snag Lake	No	VES, eDNA	No
22-L	Indian Lake	No	No	No
23-P	"Tadpole City" - Downstream of Indian Lakes	No	No	No
24-L	Crystal Lake	No	No	No
25-P	Juniper Pond - Downstream	No	No	No
26-P	Snag Lake Trail Pond	No	No	No
27-L	Juniper Lake - Southeast Corner	No	VES	No
27-L-P	Small pond adjacent to Juniper Lake	No	VES	No

Table 6 (continued). Summary of target species detections for both VES and eDNA results.

Site ID	Site Description	Cascades Frog Detected	Trout Detected	Leech Detected
28-L	Juniper Lake Outflow Area	No	VES	No
28-L-P	Juniper Lake Outflow Area 3	No	No	No
30-S	Lower Kings Creek by Park Boundary	No	eDNA	No
31-M-C	Wetland Near PCT and Kings Creek	No	VES, eDNA	No
32-S	Lower Grassy Swale Trail	No	VES, eDNA	No
33-M-C	Upper Grassy Swale Trail	No	VES, eDNA	No
34-S	King and Summit Creek Junction	No	eDNA	No
35-S	Lower Kings Creek at Kelly Camp Trail	No	eDNA	No
36-S-C	Kings Creek Picnic Area	No	No	No
37-M-C	Kings Creek Trail	No	VES, eDNA	No
38-S	Hot Springs Creek - Downstream near Park boundary	No	VES, eDNA	No
39-S	Hot Springs Creek in Drakesbad Meadow	No	eDNA	No
40-M	Little Willow Lake	No	No	VES, eDNA
42-S	Rice Creek	No	VES, eDNA	No
43-M-C	Crumbaugh Lake	No	No	No
44-S	Mill Creek	No	eDNA	No
45-M-C	Kings Creek Meadow	No	VES, eDNA	No
46-S	Creek Crossing at LAVO Park Hwy Near SW Entrance	No	No	No
47-S	East Sulphur Creek	No	No	No
48-L	Emerald Lake	No	No	No
49-L	Blue Lake	No	VES, eDNA	No
50-S	North Fork Bailey Creek	No	eDNA	No
51-L	Big Bear Lake	No	No	No
52-S	West Sulphur Creek Near Mill Creek Trail	No	No	No
CAR-M-C1	Carter Meadow 1	VES	No	No
CAR-M-C2	Carter Meadow 1	VES	No	No
CAR-M-C3	Carter Meadow 2	VES, eDNA	eDNA	No
CAR-M-C4	Carter Meadow 2	VES, eDNA	No	No
Neg Control 1	Negative Control 1	No	No	No
Neg Control2	Negative Control 2	No	No	No

Leeches were seen and detected genetically at Little Willow Lake (40-M) but were seen and not detected genetically at Soap Lake (14-L). Invertebrates are generally known to shed less DNA compared to vertebrate species (Stewart 2019, Gold et al. 2022), and their small size may make

detection possible only in areas where population size is large enough. Compared to historic data, leeches were less present and in different locations than in the past.

Table 6 summarizes the overlap between VES and eDNA results. See Table B-2 in Appendix B for more detail about survey results by site.

Habitat Modelling and Site Suitability Results

Using vegetation mapping, hydrology (perennial water), elevation, and climate data (current and projected), the habitat suitability for Cascades frogs was ranked within LAVO. Using the approach described in Methods for Habitat Modelling datasets for vegetation, perennial water, elevation, and climate parameters were compiled and used to delineate the landscape from low (shown in blue on the figures) to high suitability (shown in red on the figures) for Cascades frogs, both currently and under two climate projection scenarios.

Suitable Vegetation and Perennial Water Availability

Availability of suitable vegetation and perennial water varied throughout the Park, but were largely consistent with each other on the landscape (See Figure C-2 in Appendix C for raw density maps). Suitability scores ranging from 0 (lowest density) to 1 (highest density) were derived from the vegetation and perennial water density maps and the results are shown in Figure 7.

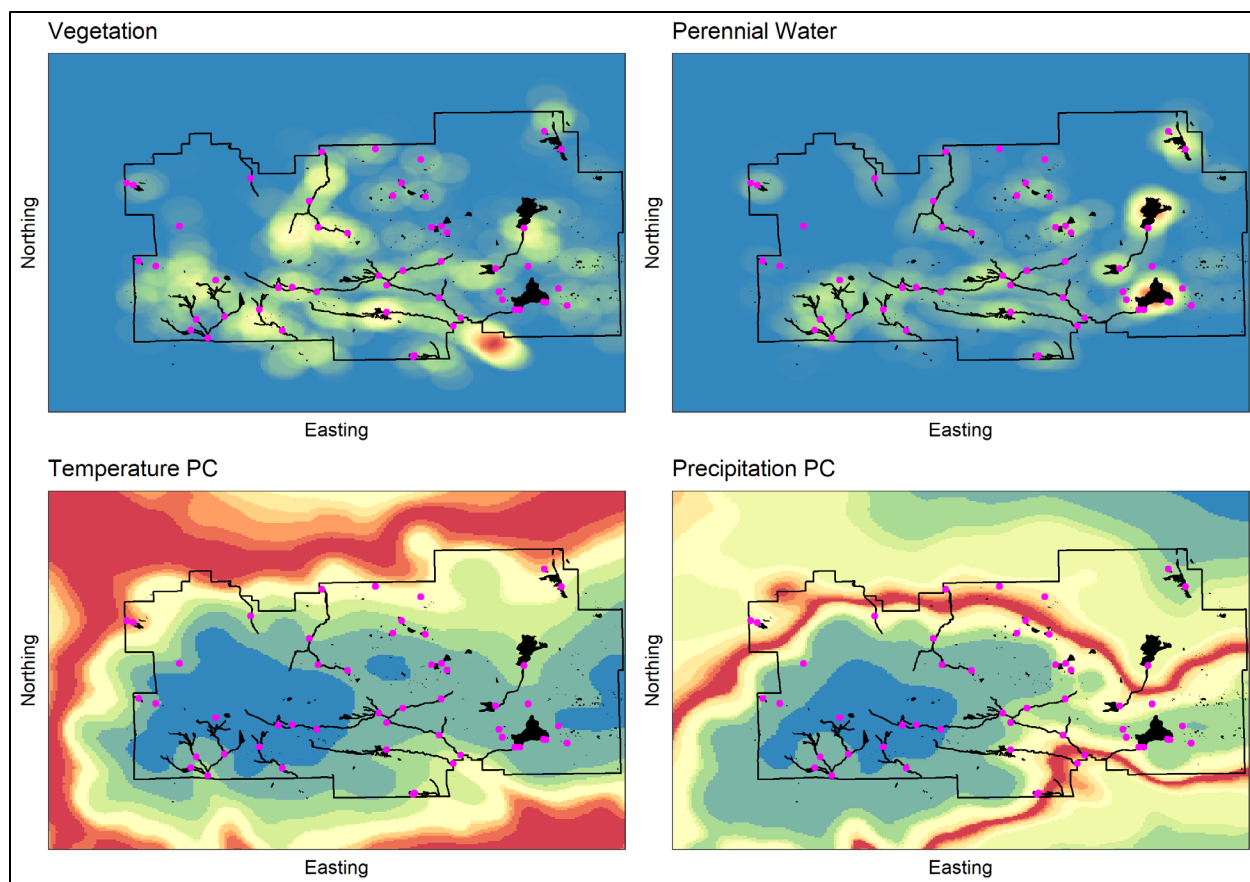


Figure 7. Survey locations (magenta circles) and suitability scores ranging from low (blue) to high (red) for Cascades frog in LAVO (black box) for vegetation, perennial water (shown in black), temperature, and precipitation.

Elevation and Climate Suitability

The first two components of the PCA explained 91 % of the variability (See Table C-1 in Appendix C). The first axis (62% explained variability) generally represented temperature variables, with lower scores having cooler temperatures. The west-central region of the Park associated with higher elevations had the lowest scores with the rest of the Park having moderate to high temperature scores (See Figure C-2 in Appendix C for density maps). The temperature variables having the greatest influence on the temperature PC were:

- DD5 - Degree-days above 5°C
- FFP - Frost-free period
- MAT - Mean annual temperature
- MCMT - Mean coldest month temperature
- MWMT - Mean warmest month temperature
- PAS - Precipitation as snow

The second PCA generally represented precipitation variables with lower scores having less precipitation and a lower heat-moisture index. Higher elevation areas within the park (central-southwest) had higher precipitation scores (See Figure C-2 in Appendix C). The north-east had lower precipitation scores (blue) with a zone on transition in between. The precipitation variables having the greatest influence on the precipitation PC were:

- MAP - Mean annual precipitation
- MSP - May to September precipitation
- AHM - Annual heat-moisture index

The raw suitability indices for temperature and precipitation are presented in Figure C-2 in Appendix C and climate suitability was delineated based on these two principal components (i.e., temperature PC and precipitation PC, respectively). When raw PC scores for occurrence locations were compared to scores for background locations generated randomly across the range and in LAVO, it was clear the occurrence data scores did not include the entire range (See Figure C-1 in Appendix C). In general, the temperature PC scores for background and survey locations in LAVO were lower than the occurrence data suggesting that, on average, locations in LAVO were cooler than locations with known Cascades frog occurrence. Scores for the precipitation PC in LAVO and at survey locations were more consistent with the occurrence data suggesting that the precipitation in LAVO is more consistent with known locations. Indeed, translating temperature PC scores to suitability scores suggested that the outer boundaries of LAVO were most suitable, with the interior generally being cooler than where Cascades frogs have been found across California (Figures 7 and 8). Precipitation suitability scores also suggested that the outer edges of LAVO in the transition zone between high and low precipitation were most suitable (Figures 7 and 8).

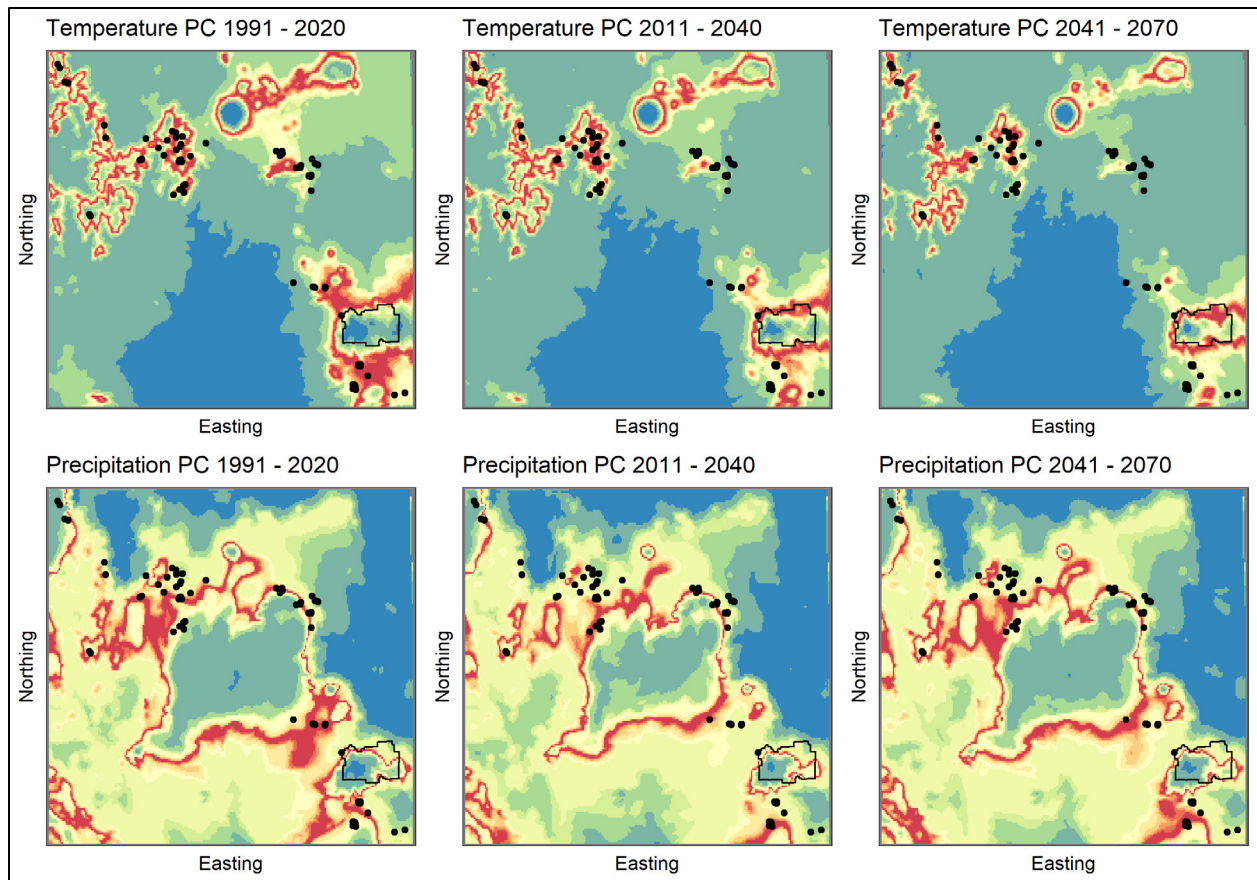


Figure 8. Cascades frog occurrence (black circles) in recently occupied areas in California in relation to current and projected climate suitability for temperature and precipitation (low [blue] to high [red] suitability). LAVO boundary delineated with a black box.

Climate Projections

Projected scenarios suggest that temperature suitability will shift across the current range of Cascades Frogs in California (Figure 8). In general, areas surrounding LAVO decline in temperature suitability while areas within the Park increased (Figure 8 and 9). All sub-watersheds (shown in Figure 10) are projected to see a net increase in mean temperature suitability with a mean net change of 0.33 from the current climate (1991-2020) to the last projected time-period (2041 – 2070). Snag Lake sub-watershed had the largest overall increase from a mean of 0.17 in 1991 – 2020 to a mean of 0.67 in the 2041 to 2070 projection. Box Canyon-Hat Creek watershed had a similar increase from 0.34 to a mean of 0.80.

Precipitation suitability appears to be much more stable over time for areas both within and outside of the Park (Figures 9 and 11). Sub-watersheds had small changes in both the positive and negative direction with a mean net change of -0.02 (Table C-2 in Appendix C).

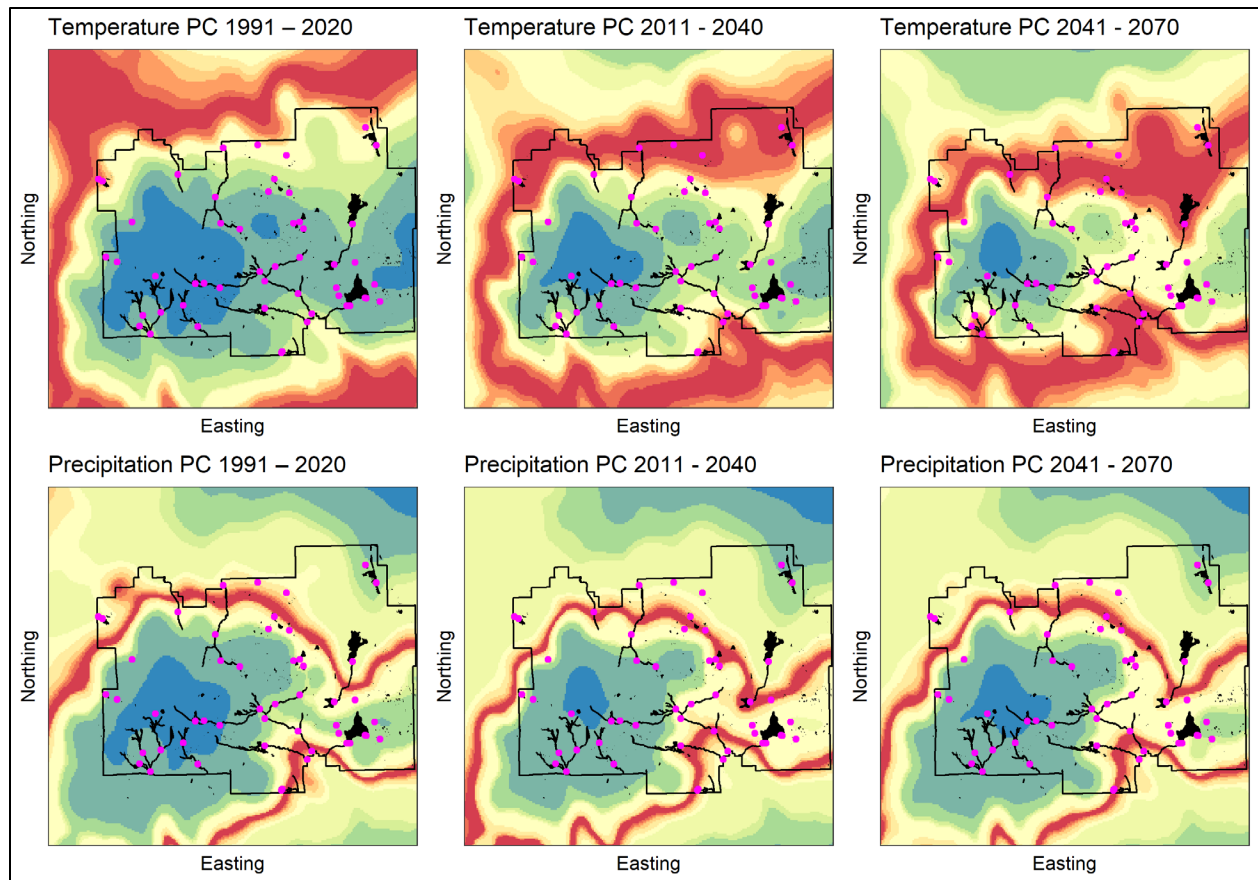


Figure 9. Survey locations (magenta circles) and suitability scores for temperature and precipitation for Cascades frog in LAVO for three time periods (1991-2020, 2011-2040, 2041-2070).

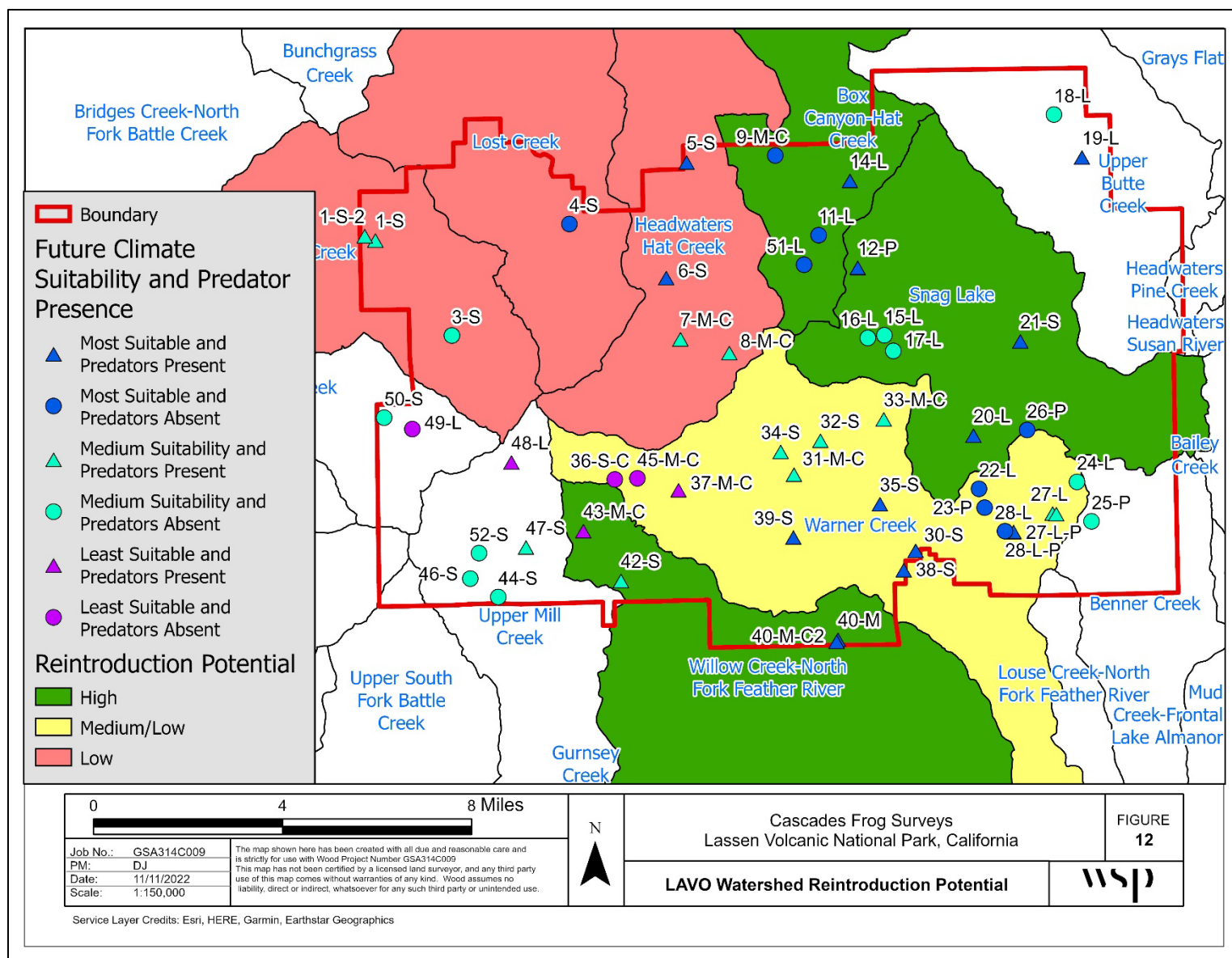


Figure 10. Watersheds and Cascades frog reintroduction potential within LAVO.

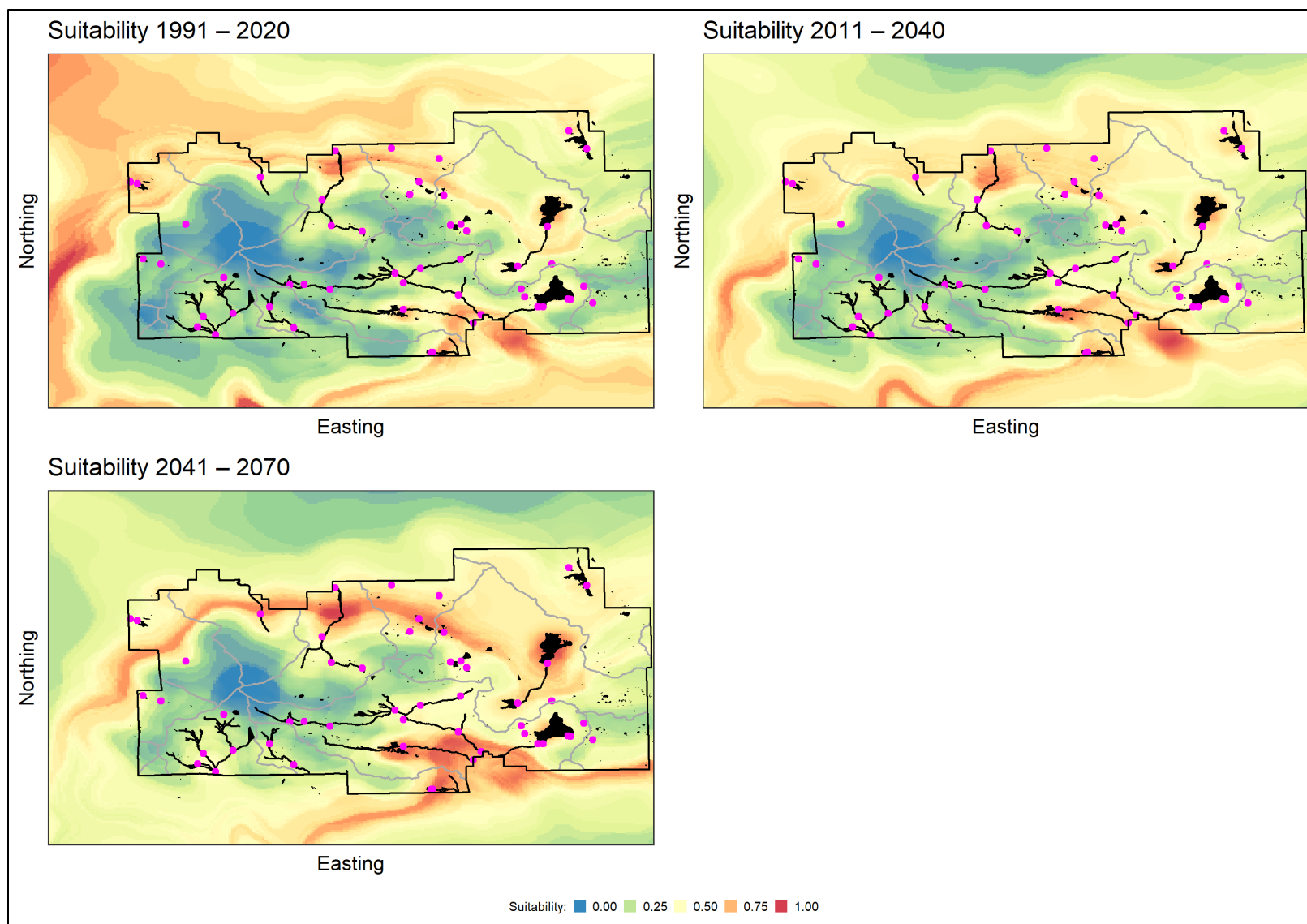


Figure 11. Overall mean suitability scores for Cascades frog in LAVO for three time periods (1991-2020, 2011-2040, 2041-2070). Sub-watersheds delineated in grey and perennial water in black.

Final Suitability

Averaging over the four components identified the most suitable areas of the Park were on the outer regions where climate was more suitable and suitable vegetation and perennial water was available (Figure 12). The northern boundary and eastern side of the Park were generally more suitable for Cascades frogs. The northern boundary has the added benefit of being adjacent to existing populations on USFS lands.

The sub-watersheds within the Park with the highest mean suitability were Box Canyon-Hat Creek, Headwaters Pine Creek, Upper Butte Creek, and Snag Lake, which all had a mean suitability ranking above 0.4 (Table 7). All other sub-watersheds had a mean ranking of 0.31 or less (Table 7). A significant portion of the Park suffered extensive damage from recent wildfires (Figure 12), which had some impact on eleven of the 15 watersheds and resulted in over 90 % of the total area burned for seven of the units (Table 7). Unfortunately, this included three (Headwaters Pine Creek, Upper Butte Creek, and Snag Lake) of the four units with the highest mean habitat suitability rankings. Box Canyon-Hat Creek, however, had the highest mean ranking and only 20 % of the area was burned in the recent forest fires. This area was burned in a 2012 fire, but the extent of that fire is not quantified here.

The three top sites according to their historical mean ranks were 38-S (Warner Creek), 5-S (Headwaters Hat Creek) and 1-S-2 (Deer Creek; Table 8). Consistent with the watershed rankings, the sites within Box Canyon-Hat Creek generally had high rankings with 3 of the 4 sites in this watershed having mean rankings above 0.5 and within the ten most suitable sites. Overall, many of the sites had an increase in mean suitability from the current climate (1991-2020) to the last projected time-period (2041 – 2070). In particular, 4-S (Lost Creek watershed) had the greatest overall increase (positive 0.29 change) and all of the sites in the Snag Lake and Warner Creek watersheds had increases in overall mean suitability. Projected 2011-2040 suitability top sites were 39-S (Warner Creek), 5-S (Headwaters Hat Creek), and 38-S/11-L (Warner Creek and Box Canyon-Hat Creek, respectively), while sites 30-S (Warner Creek), 38-S, and 21-S/11-L (Snag Lake and Box Canyon-Hat Creek, respectively) were identified as the top three sites for projected 2041-2070 suitability.

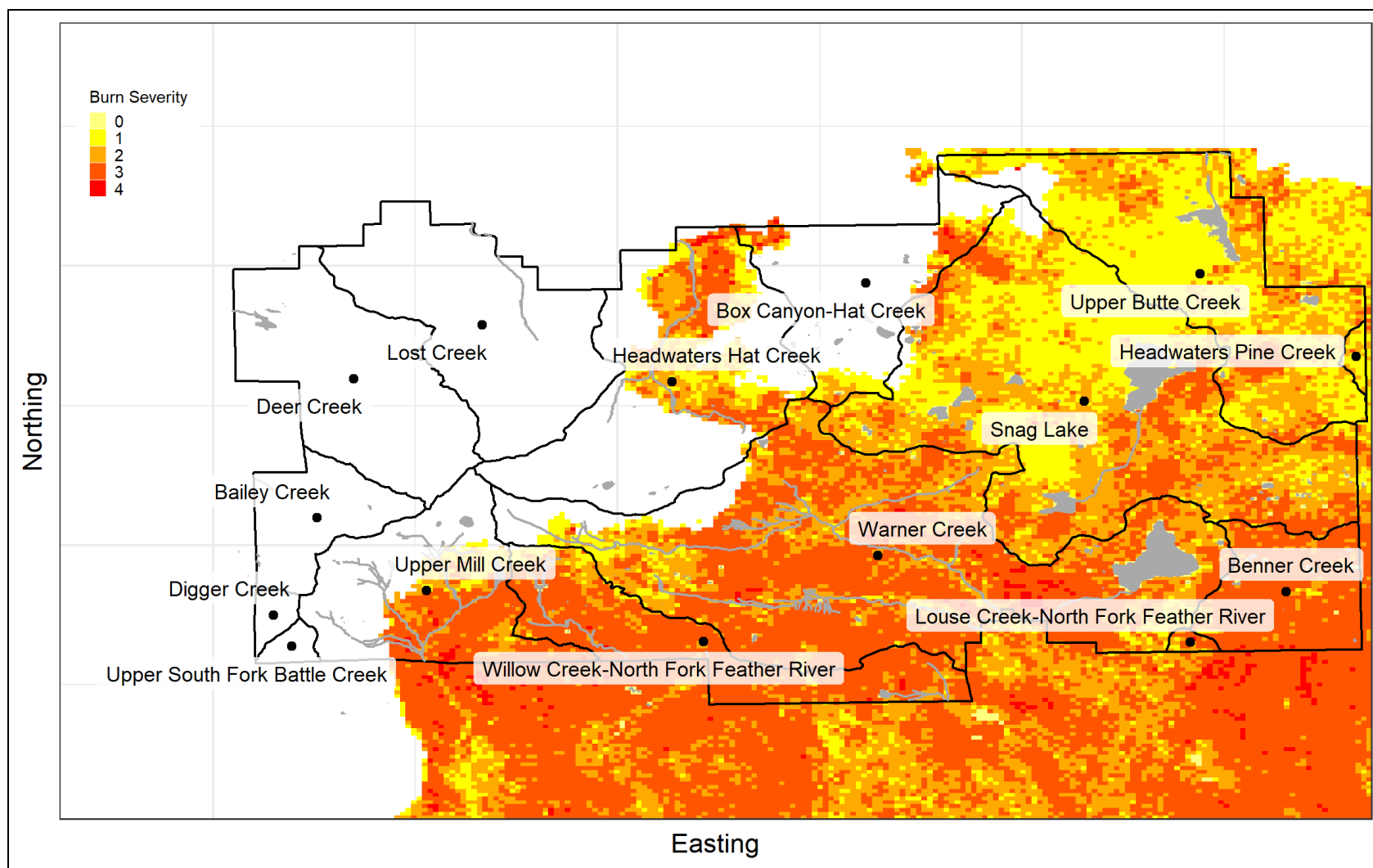


Figure 12. Sub-watersheds (labelled with black circle and associated label) and burn severity from the 2021 wildfire and watersheds on LAVO (black boundary). Perennial water delineated in grey

Table 7. Summary table for Cascades frog suitability by watershed, in descending order of historic climate suitability.

Watershed Name	Historic Climate 1991–2020 Overall Mean Suitability	Projected Climate 2011–2040 Overall Mean Suitability	Projected Climate 2041–2070 Overall Mean Suitability	2021 Burn Impact Proportion of Area	2021 Burn Damage Mean Severity	Trout Presence Proportion of Sites	Leech Presence Proportion of Sites
Box Canyon-Hat Creek	0.490	0.56	0.58	0.200000	1.9	0.00	0.25
Headwaters Pine Creek	0.420	0.30	0.41	1.000000	1.4	0.00	0.00
Upper Butte Creek	0.410	0.46	0.49	0.9400000	1.5	0.50	0.00
Snag Lake	0.400	0.47	0.55	0.9300000	1.8	0.43	0.00
Warner Creek	0.310	0.38	0.44	0.9000000	2.5	0.50	0.00
Deer Creek	0.310	0.35	0.35	0.000000	0.0	0.67	0.00
Lost Creek	0.310	0.37	0.40	0.000035	1.0	0.00	0.00
Headwaters Hat Creek	0.300	0.37	0.41	0.380000	2.1	0.50	0.00
Willow Creek-North Fork Feather River	0.250	0.31	0.38	0.990000	2.7	0.20	0.60
Benner Creek	0.250	0.37	0.37	0.990000	2.8	0.00	0.00
Louse Creek-North Fork Feather River	0.230	0.33	0.38	1.000000	2.6	0.00	0.00
Upper Mill Creek	0.190	0.21	0.27	0.470000	2.5	0.00	0.00
Bailey Creek	0.130	0.18	0.22	0.000000	0.0	0.50	0.00
Upper South Fork Battle Creek	0.089	0.13	0.20	0.000000	0.0	0.00	0.00
Digger Creek	0.045	0.11	0.15	0.000000	0.0	0.00	0.00

Table 8. Summary table for Cascades frog suitability, based on historic and projected climate, burn damage severity, and predator presence by station.

Station Name	Watershed	Historic Climate 1991– 2020 Mean Suitability	Projected Climate 2011–2040 Mean Suitability	Projected Climate 2041–2070 Mean Suitability	2021 Burn Damage Mean Severity	Trout Presence	Leech Presence
1-S	Deer Creek	0.69	0.64	0.48	0	VES, eDNA	No
1-S-2	Deer Creek	0.71	0.63	0.46	0	VES, eDNA	No
3-S	Deer Creek	0.15	0.30	0.34	0	No	No
4-S	Lost Creek	0.40	0.60	0.69	0	No	No
5-S	Headwaters Hat Creek	0.71	0.67	0.65	2.4	eDNA	No
6-S	Headwaters Hat Creek	0.32	0.49	0.56	1.8	eDNA	No
7-M-C	Headwaters Hat Creek	0.31	0.33	0.37	2.0	VES, eDNA	No
8-M-C	Headwaters Hat Creek	0.26	0.26	0.30	2.3	VES, eDNA	No
9-M-C	Box Canyon-Hat Creek	0.67	0.65	0.60	2.4	No	No
11-L	Box Canyon-Hat Creek	0.57	0.66	0.79	0	No	No
12-P	Snag Lake	0.49	0.56	0.70	1.8	VES	No
14-L	Box Canyon-Hat Creek	0.60	0.63	0.59	2.3	No	VES
15-L	Snag Lake	0.33	0.48	0.48	1.5	No	No
16-L	Snag Lake	0.27	0.38	0.40	1.5	No	No
17-L	Snag Lake	0.31	0.44	0.48	1.6	No	No
18-L	Upper Butte Creek	0.55	0.58	0.47	1.2	No	No
19-L	Upper Butte Creek	0.46	0.58	0.58	1.2	VES, eDNA	No
20-L	Snag Lake	0.48	0.63	0.61	1.8	VES	No
21-S	Snag Lake	0.62	0.62	0.79	1.9	VES, eDNA	No
22-L	Warner Creek	0.33	0.43	0.51	2.3	No	No
23-P	Warner Creek	0.35	0.46	0.53	2.3	No	No
24-L	Warner Creek	0.28	0.38	0.33	2.3	No	No
25-P	Benner Creek	0.25	0.36	0.33	2.8	No	No

Table 8 (continued). Summary table for Cascades frog suitability, based on historic and projected climate, burn damage severity, and predator presence by station.

Station Name	Watershed	Historic Climate 1991– 2020 Mean Suitability	Projected Climate 2011–2040 Mean Suitability	Projected Climate 2041–2070 Mean Suitability	2021 Burn Damage Mean Severity	Trout Presence	Leech Presence
26-P	Snag Lake	0.40	0.48	0.52	2.5	No	No
27-L	Warner Creek	0.42	0.51	0.48	1.7	VES	No
27-L-P	Warner Creek	0.40	0.50	0.45	1.8	VES	No
28-L	Warner Creek	0.41	0.51	0.55	1.9	VES	No
28-L-P	Warner Creek	0.42	0.52	0.58	2.2	No	No
30-S	Warner Creek	0.65	0.64	0.81	2.8	eDNA	No
31-M-C	Warner Creek	0.29	0.42	0.47	2.6	VES, eDNA	No
32-S	Warner Creek	0.27	0.41	0.46	2.6	VES, eDNA	No
33-M-C	Warner Creek	0.27	0.39	0.47	2.3	VES, eDNA	No
34-S	Warner Creek	0.29	0.39	0.43	2.5	eDNA	No
35-S	Warner Creek	0.45	0.59	0.68	2.7	eDNA	No
36-S-C	Warner Creek	0.16	0.19	0.18	1.6	No	No
37-M-C	Warner Creek	0.15	0.19	0.20	2.2	VES, eDNA	No
38-S	Warner Creek	0.73	0.66	0.80	2.7	VES, eDNA	No
39-S	Warner Creek	0.55	0.69	0.75	2.8	eDNA	No
40-M	Willow Creek-North Fork Feather River	0.52	0.64	0.75	2.8	No	VES, eDNA
42-S	Willow Creek-North Fork Feather River	0.24	0.24	0.30	3.0	eDNA	No
43-M-C	Willow Creek-North Fork Feather River	0.20	0.25	0.26	2.8	VES, eDNA	No
44-S	Upper Mill Creek	0.22	0.30	0.45	2.9	No	No
45-M-C	Warner Creek	0.17	0.21	0.20	1.8	No	No
46-S	Upper Mill Creek	0.23	0.28	0.41	2.7	No	No
47-S	Upper Mill Creek	0.20	0.23	0.31	2.7	VES, eDNA	No
48-L	Upper Mill Creek	0.16	0.17	0.17	1.4	eDNA	No

Table 8 (continued). Summary table for Cascades frog suitability, based on historic and projected climate, burn damage severity, and predator presence by station.

Station Name	Watershed	Historic Climate 1991– 2020 Mean Suitability	Projected Climate 2011–2040 Mean Suitability	Projected Climate 2041–2070 Mean Suitability	2021 Burn Damage Mean Severity	Trout Presence	Leech Presence
49-L	Bailey Creek	0.14	0.20	0.25	0	No	No
50-S	Bailey Creek	0.16	0.29	0.36	0	No	No
51-L	Box Canyon-Hat Creek	0.34	0.51	0.58	1.0	No	No
52-S	Upper Mill Creek	0.23	0.27	0.39	2.6	No	No

Future Uses of eDNA Sampling

The methods employed for this project using self-preserving filters and hand pumps maximized the ability to cover large distances and sample multiple sites per day, without hauling large mechanical pumps and batteries into protected wilderness areas. This method performed very well as a compliment to VES for detecting frogs (even without an integrated backpack sampler; see Pope et al. 2020), as well as trout and leeches. It is recommended that future eDNA sampling use 0.45 μm filters in streams and either 0.45 μm or 1.2 μm filters in lakes and wetland areas. In wetlands or lakes where the filter is more likely to clog due to suspended material or algae, it is recommended to use the slightly larger 1.2 μm to help obtain the target filter volumes (Kamoroff and Goldberg 2018, Bedwell and Goldberg 2020) and sample at the foot of meadow habitat (Pope et al. 2020).

The results of the eDNA survey yielded no detections of Cascades frogs in LAVO, which is consistent with recent VES efforts (Stead et al. 2005, Pope et al. 2014). At the 18 sites where tree frogs were observed by field crews, 12 of them did not have trout detected with VES or eDNA sampling. However, nearly all of the samples from these sites were collected from lakes or ponds, in which eDNA may be more susceptible to breakdown and/or more difficult to capture due to limited mixing. To effectively determine whether predators are absent from these sites, it is recommended to analyze samples for presence of tree frog eDNA. If results from this analysis corroborate visual observations for positive presence of tree frogs, this can help validate the lack of predator eDNA at these sites.

Additional suitable habitat for frogs could be determined by analyzing archived eDNA samples (currently frozen and viable for future analysis) for the tree frog to identify areas where they were present but not seen during VES. This effort could further inform decisions about the relative population size that trout would have to be controlled to in order to allow for viable frog populations in these areas. While the quantitative detection of target species eDNA is not perfectly correlated to the number of animals and should be used cautiously (Gold et al. 2022), eDNA is a field that is evolving rapidly and future efforts could benefit from targeted studies to help answer pertinent questions to the conservation of rare species. Examples include: 1) What is the amount and rate of eDNA shed by frogs and trout of various sizes? and 2) What factors in high altitude environments affect how long eDNA can be detected?

In addition to identifying areas for reintroduction, eDNA results for the three trout species shows promise for future use in monitoring trout populations throughout LAVO. Programs such as the management of trout in Manzanita Lake and nearby spawning areas under the California Fish and Game Commission Wild Trout Waters designation may be able to use eDNA to inform management (Plemons et al. 2015). Overall, the magnitude of brook trout eDNA was greater than any other species (of the positive detections, the average concentration was calculated at 120.8 $\mu\text{g/L}$, compared to 25.0 $\mu\text{g/L}$ and 7.3 $\mu\text{g/L}$ for rainbow and brown trout, respectively), suggesting a greater presence of this species at sample locations. Further investigation of eDNA shedding rates between these species could help distinguish relative population size between species.

Conclusions

Cascades frogs have not been documented in several years of surveys on LAVO and were not documented in this survey either, the extirpation of Cascades frogs from LAVO seems a reasonable conclusion. This leads to a consideration of whether reintroduction and/or habitat improvements are a viable strategy for the future of Cascades frogs in LAVO.

Given the current conditions, recent wildfires, and particularly incorporating climate projections and resulting suitability, the locations with some potential for reintroducing Cascades frogs to LAVO become fairly clear. Nearly all the locations will require some active management to prepare for and maintain any reintroduced Cascades frogs. Shifts in habitat suitability due to climate change may increase the possibility of Cascades frogs migrating into the Park during the next century, but active management to maintain those migration corridors would likely be necessary to ensure they do not result in high losses of migrating frogs.

Cascades Frog Reintroduction Suitability

The reintroduction potential for different areas was categorized (high, medium, low) based on the results of 2021 VES and eDNA surveys, final site suitability modeling results, and historical sightings of Cascades frogs within the Park. Each area and the factors contributing to the reintroduction potential are described below. See Figure 12 for a map of reintroduction potential by watershed.

High Reintroduction Potential

Cluster Lakes Area Including Cluster Lake (11-L), Big Bear Lake (51-L) and Soap Lake (14-L) in Box Canyon-Hat Creek Watershed. Feather Lake and the Nearby Pond (12-P) in Snag Lake Watershed

Suitability Summary: Climate suitability modeling gave Box-Canyon-Hat Creek watershed the highest mean suitability across all projections (0.49 for 1991-2020, 0.56 for 2011-2040 and 0.58 for 2041-2070). Note that site 12-P is right at border of Box Canyon-Hat Creek and Snag Lake watersheds but falls within similar suitability scores bands that span both watersheds. Site specific projections for these sites indicate that they are likely to become more suitable in the future (0.34-0.60 for 1991-2020, 0.51-0.66 for 2011-2040 and 0.58-0.79 for 2041-2070), with 11-L (Photo 9) ranked fourth among all sites (0.66) in 2011-2040 projections and third (0.79) in 2041-2070 projections.

These small lakes and ponds appear promising for future Cascades frog reintroduction efforts. There were tree frogs observed at all of them during VES and even at low water levels observed during the surveys there was plenty of riparian grassy habitat and cover (e.g., downed trees, overhanging vegetation). Predators were absent in eDNA samples at these sites, although this could be due to environmental factors (i.e., eDNA breakdown from UV, limited water movement as discussed above). Although there were some predators noted during VES such as leeches at Soap Lake and trout in the pond near Feather Lake. Low predator occurrence and density indicate that the predators could be controllable to levels that would allow for Cascades frog reintroduction. Additionally, these

sites are more difficult to access for most visitors and would be less prone to habitat trampling and disturbance.



Photo 9. Cluster Lake (11-L), a high reintroduction potential site.

A meadow that runs along the Pacific Coast Trail to the north (9-M-C) looked promising for frog habitat, but was mostly dry when surveyed except for a few spring fed pools that were relatively small but had toads and snakes present. Future predictions of climate and precipitation may indicate that these meadow habitats could become viable frog habitat and have some resilience to drought conditions if spring fed areas could be restored for frog reintroduction.

Limitations: This area is part of the ‘devastated area’ from the 2012 Reading fire and thus was largely spared from damage from the 2021 Dixie fire (only 20% of watershed burned), but it is still recovering from the 2012 Reading fire. VES in this area indicates that vegetation is already regenerating so vegetation remediation could be effective in promoting frog reintroduction.

Recommendations: The Box Canyon-Hat Creek area seems the best suited to reintroductions based on all the combined information, especially if any active management is undertaken to improve the area for Cascades frogs. Connectivity between reintroduction areas via small streams or meadows would increase the likelihood of frog dispersal, along with connectivity to the north to USFS lands.

Horseshoe Lake (20-L), Snag Lake and the Connecting Grassy Creek (21-S), and Vernal Pool Near Snag Lake Trail (26-P) in Snag Lake Watershed

Suitability Summary: Climate suitability modeling gave Snag Lake watershed the 4th highest mean suitability score (0.40) for 1991-2020 and the 2nd highest projected score (0.47) for 2011-2040 and (0.55) 2041-2070. Site specific projections for these sites indicate that they are likely to become more suitable in the future (0.40-0.62 for 1991-2020, 0.48-0.63 for 2011-2040 and 0.52-0.79 for 2041-2070), with 21-S (Photo 10) ranked third among all sites (0.79) in 2041-2070 projections.



Photo 10. Snag Lake (21-S), a high reintroduction potential site.

Lakes surveyed in this area had grassy riparian areas during VES which appeared suitable for frogs (VES confirmed tree frogs in Snag Lake), especially if water levels come back up. Trout were present in these areas during VES and in eDNA samples, especially in Grassy Creek, although predator control efforts could be considered here.

The vernal pond on the trail to Snag Lake had dozens of Pacific tree frog sightings, had no signs of fish or leeches, and it contained fairy shrimp (a food source and a sign of high quality habitat). The site appeared to have minimal encroachment from humans and riparian vegetation did occur as well as an open coniferous canopy.

Limitations: There appears to be potential for reintroduction into this watershed from suitability modeling, but this area also sustained substantial damage from the Dixie fire and would require additional vegetation remediation to restore riparian habitats.

Recommendations: The Snag Lake watershed is promising for reintroduction as there are several favorable habitat types that appear to support amphibian populations. An assessment of damage to riparian habitats following the Dixie fire would be required to determine if vegetation remediation would be required. Predator control in Snag and Horseshoe Lakes and the connecting Grassy Creek could increase viability for Cascades frog reintroduction.

Little Willow Lake (40-M) in Willow Creek-North Fork Feather River Watershed

Suitability Summary: Climate suitability modeling gave the Willow Creek-North Fork Feather River watershed a relatively low score (0.25, tied for 9th out of 15 watersheds) for 1991-2020 mean suitability. Projections for 2011-2040 (0.31) and 2041-2070 (0.38) were also relatively low compared to all watersheds but suggest better suitability in the future than currently exists. However, closer examination of Little Willow Lake (Photo 11) within the watershed shows that the site is located in the southeast corner of the watershed and scores much higher than the watershed as a whole for mean

suitability across all years (0.52 for 1991-2020, 0.64 for 2011-2040 and 0.75 for 2041-2070). Little Willow Creek scores in the top ten for projected suitability for both 2011-2040 and 2041-2070 but is not in the top ten for historical 1991-2020 suitability.



Photo 11. Little Willow Lake within the watershed.

During surveys, this area had extensive protected, marshy habitat with aquatic vegetation in the water. Tree frogs were observed throughout the lake which suggests this area already contains viable amphibian habitat. There were no trout observed during VES or detected in eDNA but leeches were both observed and detected with eDNA, although in low numbers. The lake is out of the way of most visitors, although there is a nearby road which would allow for easier access for future restoration work.

Limitations: This area of the Park was heavily impacted by the recent Dixie fire, with 99% of the watershed burned with the second highest average burn severity score for any watershed within the area of the Park affected by the fire.

Recommendations: Willow Lake is promising for reintroduction as there are large swaths of favorable habitat types across the lake that appear to support existing amphibian populations. An assessment of damage to riparian habitats following the Dixie fire would be required to determine if vegetation remediation would be required, and accessibility near the south end of the Park with nearby road access is a plus.

Medium Reintroduction Potential

Juniper Lake (25-P, 27-L, 27-L-P, 28-L, 28-L-P), Crystal Lake (24-L) and Indian Lake (22-L, 23-P) in Warner Creek Watershed

Suitability Summary: Climate suitability modeling gave the Warner Creek watershed a score of 0.31 for 1991-2020 historical mean suitability, which was tied for 5th out of 15 watersheds. Projections

for 2011-2040 and 2041-2070 showed increases in suitability to 0.38 and 0.44, respectively. Site specific projections for these sites indicate that they are likely to become slightly more suitable in the future (0.25-0.42 for 1991-2020, 0.36-0.52 for 2011-2040 and 0.33-0.58 for 2041-2070), although none of these sites ranked in the top ten for any historical or projected period.

Juniper Lake was one of the last areas within the Park that frogs were seen in surveys (albeit at low densities). In historical records, Crystal Lake was reported to have high densities of Cascades frogs present. These areas now appear to be heavily disturbed from human activity, with the surveys noting trampled riparian habitat around Crystal Lake and development evident around Juniper Lake. Tree frogs were seen at a few of the sampling locations, mostly in small, isolated ponds surrounding Juniper Lake. Trout were visually confirmed in many areas although eDNA results did not detect them, perhaps due to factors previously discussed such as not enough water movement, warm water, and exposure of the shallow areas near the shore to UV radiation that could result in more rapid breakdown of eDNA.

Limitations: This area of the Park was heavily impacted by the recent Dixie fire, with 90% of the watershed burned with the fourth highest average burn severity score for any watershed within the area of the Park affected by the fire.

Recommendations: While historical surveys suggest that frogs at one time were able to inhabit this area, it appears that many factors have changed such as increased human impact and recent disturbance from wildfires that may render these areas unsuitable for future introductions. Furthermore, suitability modeling predictions do not score the areas around Juniper Lake as highly as other areas of the watershed. The area is easily accessible but would likely require predator control, vegetation remediation, and restricted access to park visitors to consider reintroduction in this area.

Low Reintroduction Potential

Hat Creek (between 6-S and 5-S) in Headwaters Hat Creek Watershed

Suitability Summary: Climate suitability modeling ranked Headwaters Hat Creek watershed 8th (0.30) for historical mean suitability, tied for 5th (0.37) in 2011-2040 and 4th (0.44) in 2041-2070. Site specific suitability showed that 5-S (0.71; top three) was ranked higher than 6-S (0.32) for historical climate, but that by 2041-2070 the suitability scores become more similar (0.65 and 0.56, respectively) and 5-S drops out of the top ten.

Surveys did not identify viable riparian habitat for frogs in these areas as no tree frogs were observed although tree frogs were seen in the upper reach of Hat Creek in marshy areas (8-M-C).

Limitations: The creek is a relatively high flow habitat with high occurrence of trout throughout the watershed as indicated from eDNA and VES results. Historical trout stocking and active downstream stocking in Hat Creek may limit the ability to conduct predator control.

Recommendations: While climate modeling suggests this area has potential, there is little suitable riparian for reintroduction and predator control in the creek would require elimination of large

populations of trout throughout the reach. This area appears to have low potential for future introduction.

Manzanita Lake (Sites 1-S and 1-S-2) in Deer Creek Watershed

Suitability Summary: Climate suitability modeling ranked Deer Creek watershed tied for 5th (0.31) for historical mean suitability and a suitability of 0.35 in 2011-2040 and 2041-2070 which ranked 8th and 11th overall. Closer examination of the suitability projections show that the area around Manzanita Lake, and these sites specifically, decrease (0.69-0.71 for 1991-2020, 0.63-0.64 for 2011-2040 and 0.46-0.48 for 2041-2070) going from both sites ranked in the top 5 for 1991-2020 to outside of the top 20 by 2041-2070. Historical sightings of Cascades frogs have been made around Manzanita Lake, although not within the last 20 years.

The habitat around Manzanita Lake was variable and disturbed due to human impacts from the associated campground. A walking loop exists around the Lake, and various locations where footpaths encroach on the water's edge were noted. Locations of riparian habitat do occur, often comprised of emergents and shrubs along the northeast shore. A tributary to the northwest was also surveyed as grasses and wetland areas were observed.

Limitations: The lake is currently a managed trout fishery under the CDFW Wild Trout Waters designation (Plemons et al. 2015), thus predator control to promote frog populations would not be possible here. Additionally, the proximity to camping facilities increases the likelihood of human impacts on reintroduction efforts.

Recommendations: While historical sightings of Cascades frogs have been made in this area, the development of camping facilities, an active recreational trout fishery in the lake, and decreasing projected suitability due to climate change suggest that reintroduction efforts should be focused elsewhere.

Lower ends of Kings Creek (35-S, 30-S), Hot Springs Creek (39-S, 38-S) in Warner Creek Watershed

Suitability Summary: Climate suitability modeling gave the Warner Creek watershed a score of 0.31 for 1991-2020 historical mean suitability, which was tied for 5th out of 15 watersheds. Projections for 2011-2040 and 2041-2070 showed increases in suitability to 0.38 and 0.44, respectively. Closer examination of these sites within the watershed show that they locally score higher than the watershed as a whole, with all sites increasing for future projections (0.45-0.73 for 1991-2020, 0.64-0.69 for 2011-2040 and 0.68-0.81 for 2041-2070).

Limitations: These areas contain relatively high flow stream habitats that may not be suitable for amphibians as no tree frogs were observed throughout the watershed. Predator abundance appears to be high with trout observed throughout the watershed as indicated from eDNA and VES results, potentially established populations remaining from historical stocking efforts in these creeks (Plemons et al. 2015). The lower end of Kings Creek (30-S) has been channelized and berms constructed on the banks of the creek to protect nearby properties.

Recommendations: Despite this area of the creek scoring well in mean climate suitability as it exits the Park, it may not be feasible as private property, development, and high water flows may make it difficult to maintain suitable Cascades frog habitat in this area.

Lost Creek (4-S) in Lost Creek Watershed

Suitability Summary: Climate suitability modeling ranked Lost Creek watershed tied for 5th (0.31) for historical mean suitability, tied for 5th (0.37) in 2011-2041 and 7th (0.40) in 2041-2070. The site specific score also increased compared to the historical suitability score (0.40), to 0.60 in 2011-2040 and 0.69 in 2041-2070.

This small and entrenched stream had grassy undercut banks and no predators visible or detected from eDNA samples. The sample location was adjacent to a road and upstream of a dual concrete box culvert bridge with a weir.

Limitations: The high bridge may indicate that the stream could become much larger during significant storm events and/or snow melt. There were no tree frogs observed during VES in the area.

Recommendations: While the suitability modeling suggests this site may support frogs, it does not rank highly and there is no evidence of amphibians in the area to suggest that reintroduction efforts would be successful here.

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Appendix A. Maps of Cascades frogs and predators, water resources, elevation, and vegetation

This appendix contains a series of maps detailing key study attributes related to Cascades frogs and their predators (Figure A-1), perennial water resources and wetlands (Figure A-2), elevation (Figure A-3), and vegetation types (Figure A-4) within Lassen Volcanic National Park (LAVO).

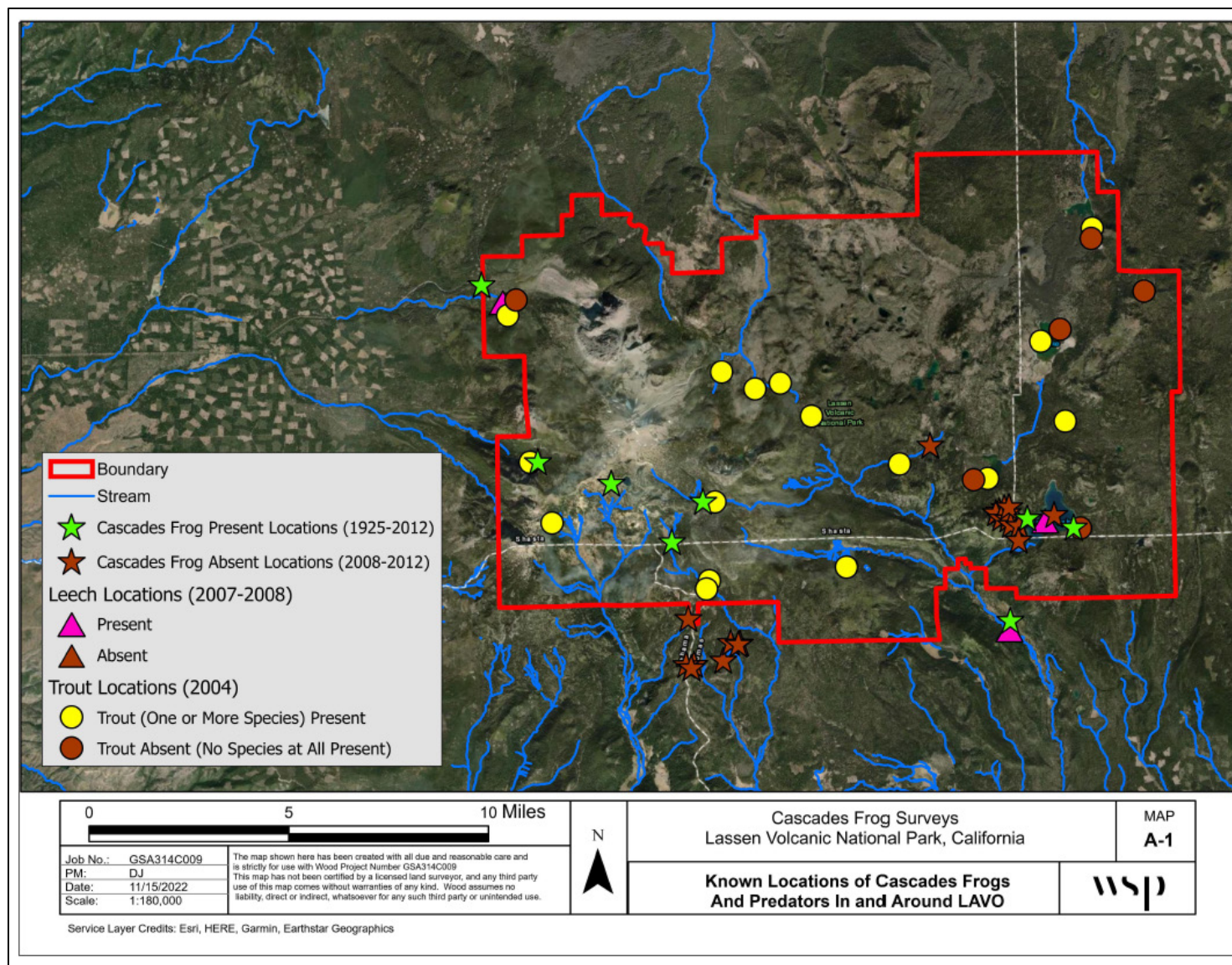


Figure A-1. Known (current and historic) locations of Cascades frogs and predators in and around LAVO.

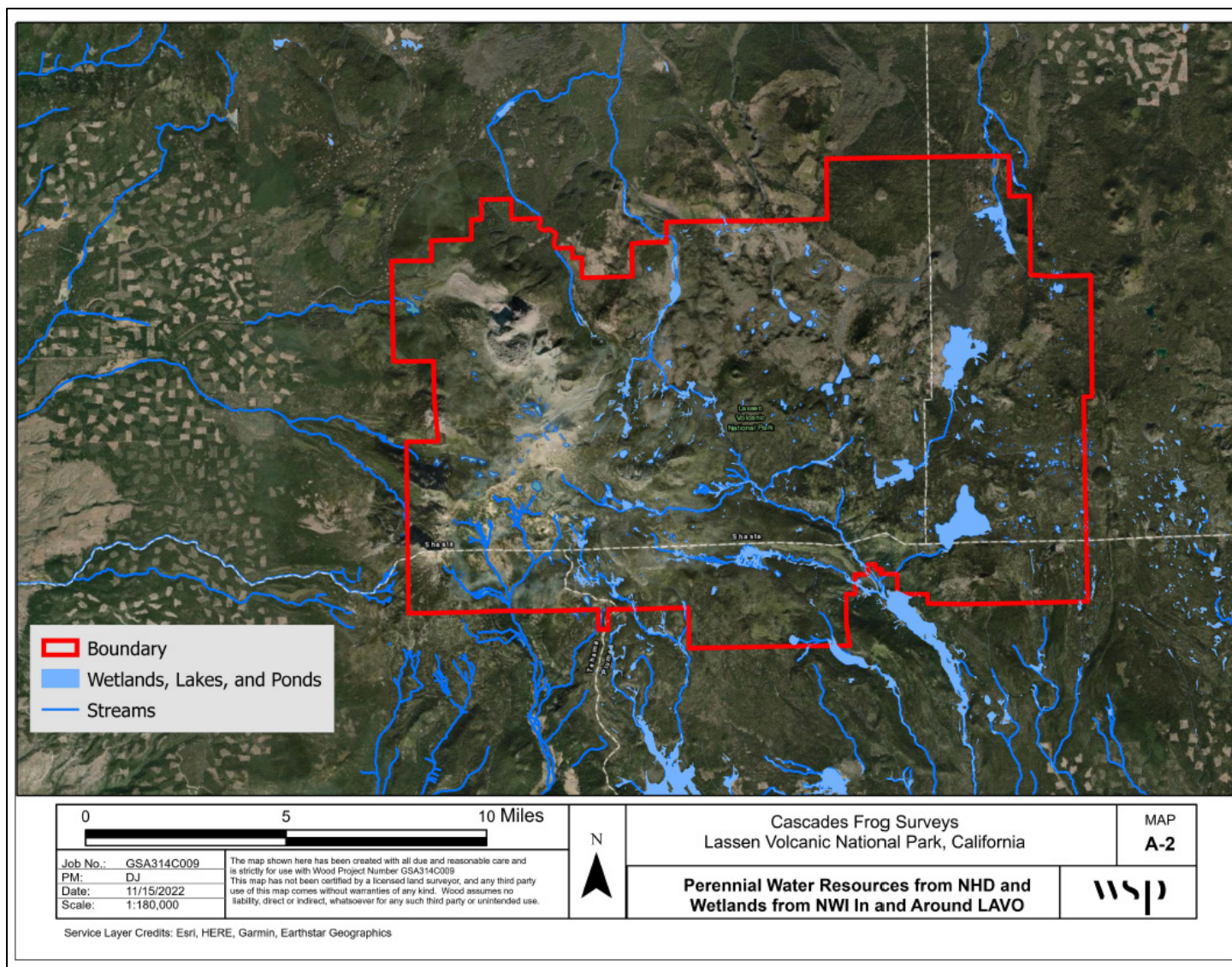


Figure A-2. Perennial water resources from NHD and wetlands from NWI in and around LAVO.

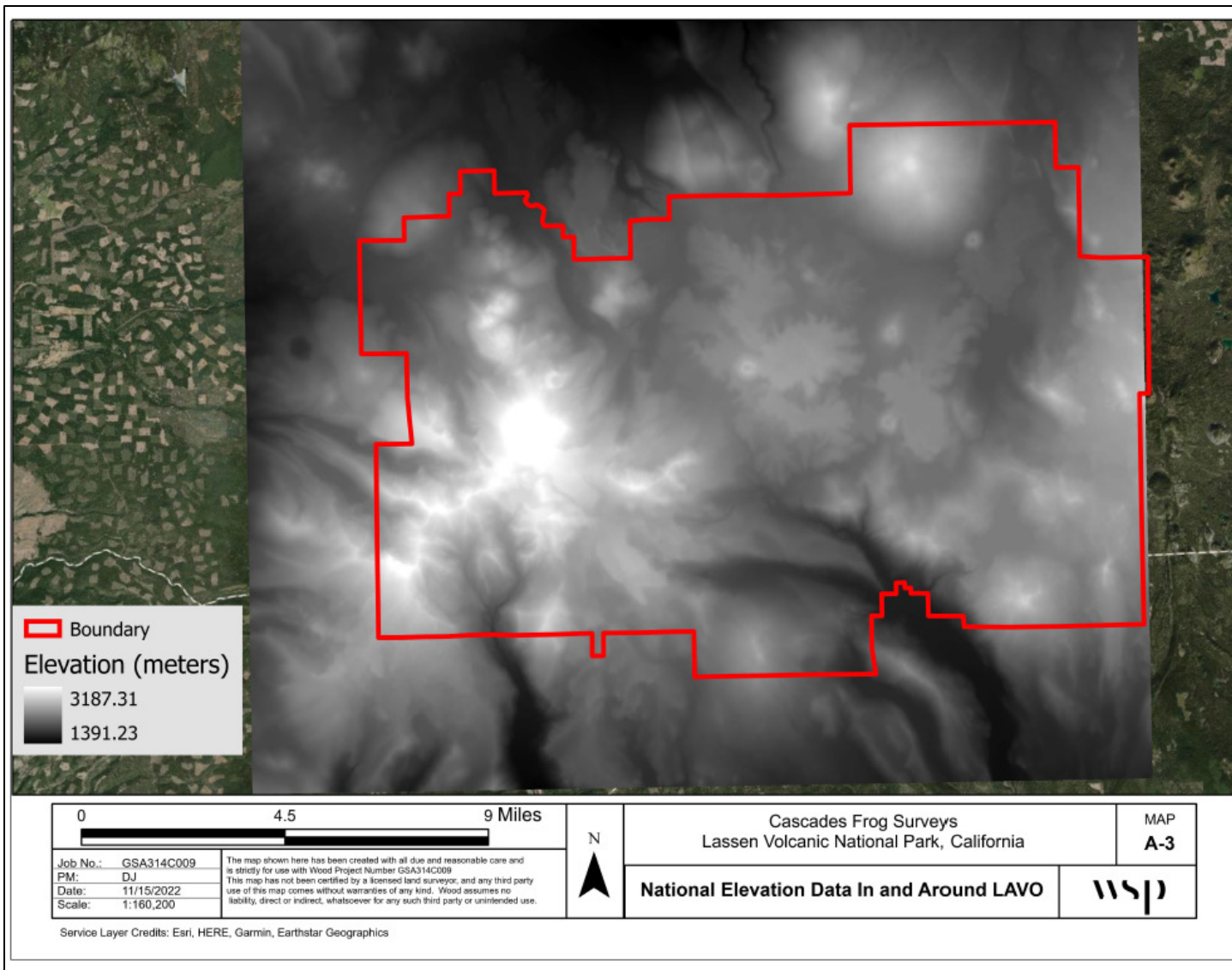


Figure A-3. National Elevation Data in and around LAVO.

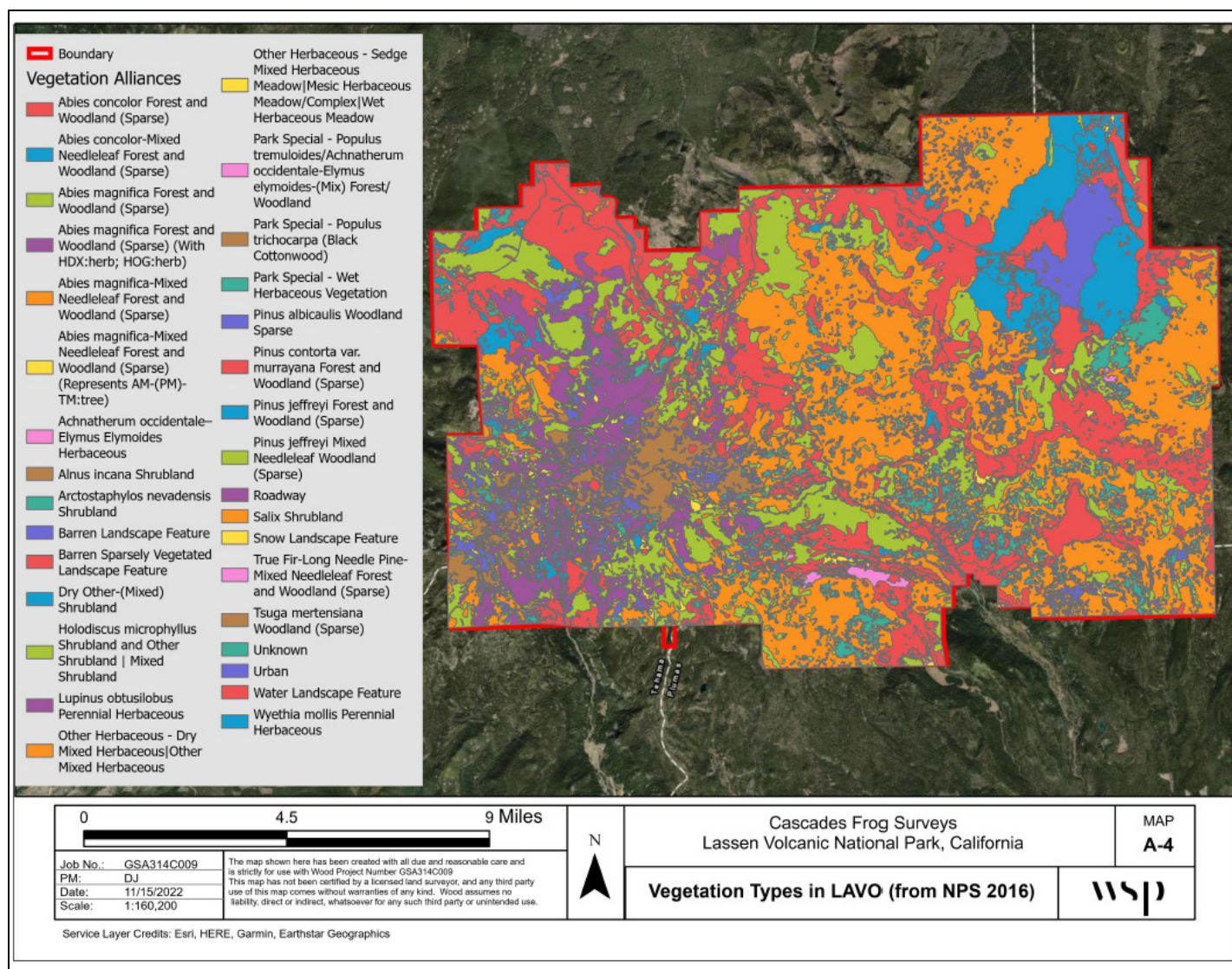


Figure A-4. Vegetation types in LAVO (from NPS 2016).

Appendix B. Data tables (survey sites, summary data, incidental species observations, and vegetation types)

This appendix includes a series of data tables detailing lists of survey sites (proposed versus final, Table B-1), final summary data from all sampling (VES and eDNA) during 2021 (Table B-2), lists of incidental species observations (Table B-3), and descriptions of vegetation density and community types (Table B-4).

Table B-1. Sampling Results: Proposed survey sites compared to final survey sites within LAVO, 2021.

Proposed Site	Priority	Final Site(s) Number	Comments
1	1	1-S-2	–
2	2	1-S	Not used, became 1-S on outflow of Manzanita Lake
3	4	3-S	Relocated to the south
4	3	4-S	Relocated to the south
5	4	5-S	–
6	2	6-S	–
7	2	7-M-C	Relocated to the south
8	2	8-M-C	–
9	4	9-M-C	Relocated to the northeast
10	3	–	Dry, unused
11	3	11-L	Relocated to the southeast
12	3	12-P	–
13	4	–	Dry, unused
14	3	14-L	Relocated to north of proposed site 13
15	3	15-L	Relocated to southwest
16	3	16-L	Relocated to northwest
17	3	17-L	Relocated to the north
18	4	18-L	Relocated to the southwest
19	3	19-L	Relocated to the south
20	3	20-L	Relocated to the edge of Horseshoe Lake (west)
21	3	21-S	Relocated slightly to the northeast
22	4	22-L	Relocated to the northwest
23	4	23-P	Relocated to the north
24	4	24-L	Relocated to Crystal lake to the northwest
25	4	25-P	Relocated to the northwest
26	4	26-P	Relocated to the northwest
27	1	27-L, 27-L-P	“Doubled”

*Three eDNA samples were taken from site 40-M from three different habitat types but only one eDNA sample was analyzed (from a composite of 40-M and 40-M-C2 as shown in Figure 1).

Table B-1 (continued). Sampling Results: Proposed survey sites compared to final survey sites within LAVO, 2021.

Proposed Site	Priority	Final Site(s) Number	Comments
28	2	28-L, 28-L-P	"Doubled"
29	2	–	Private property, not used
30	1	30-S	–
31	3	31-M-C	Relocated slightly to the west
32	4	32-S	Relocated slightly to the east
33	4	33-M-C	Relocated to the northeast
34	4	34-S	Relocated slightly to the east
35	4	35-S	Relocated to the south
36	2	36-S-C	–
37	2	37-M-C	–
38	2	38-S	–
39	3	39-S	Relocated slightly to the south
40	2	40-M*	Relocated to the west, "tripled"
41	2	–	Not used
42	2	42-S	Relocated to the northwest
43	2	43-M-C	–
44	2	44-S	Relocated slightly to the north
45	4	45-M-C	Relocated to the northeast
46	4	46-S	Dry, relocated slightly to the north
47	4	47-S	–
48	2	48-L	–
49	2	49-L	Relocated slightly to the west
50	4	50-S	Relocated slightly to the east
–	–	51-L	Added on Big Bear Lake
–	–	52-S	Added on West Sulphur Creek Near Mill Creek Trail

*Three eDNA samples were taken from site 40-M from three different habitat types but only one eDNA sample was analyzed (from a composite of 40-M and 40-M-C2 as shown in Figure 1).

Table B-2. Compiled summary table from all sampling (VES and eDNA).

Site Number	Site Name	Watershed	eDNA Filter Size (µm)	Filter Volume (mL)	Cascades Frogs Detected	Tree Frogs Detected	Brook Trout Detected	Rainbow Trout Detected	Brown Trout Detected	Leeches Detected
1-S	Manzanita Lake Outflow	Deer Creek	0.45	2025	No	No	eDNA, VES*	eDNA, VES*	eDNA, VES*	No
1-S-2	Manzanita Wetted Area	Deer Creek	0.45	1945	No	No	eDNA, VES*	eDNA, VES*	eDNA, VES*	No
3-S	Manzanita Creek	Deer Creek	0.45	1970	No	No				No
4-S	Lost Creek	Lost Creek	0.45	1025	No	No				No
5-S	Hat Creek - Downstream	Headwaters Hat Creek	0.45	2020	No	No	eDNA			No
6-S	Hat Creek Near Hat Creek Rd	Headwaters Hat Creek	0.45	2050	No	No	eDNA			No
7-M-C	East Fork Hat Creek	Headwaters Hat Creek	0.45	1590	No	No	eDNA, VES*	VES*	VES*	No
8-M-C	East Fork Hat Creek by Summit Lake Trailhead	Headwaters Hat Creek	0.45	2040	No	VES	eDNA, VES*	VES*	VES*	No
9-M-C	Wetland Near PCT and Cluster Lake Trail	Box Canyon-Hat Creek	1.20	1000	No	No				No
11-L	Cluster Lake	Box Canyon-Hat Creek	0.45	965	No	VES				No
12-P	Pond Near Feather Lake	Snag Lake	1.20	955	No	VES	VES*	VES*	VES*	No
14-L	Soap Lake	Box Canyon-Hat Creek	1.20	450	No	VES				VES
15-L	Lower Twin Lake	Snag Lake	1.20	1075	No	No				No
16-L	Upper Twin Lake	Snag Lake	1.20	1065	No	No				No
17-L	Swan Lake	Snag Lake	1.20	980	No	No				No
18-L	Bathtub Lake	Upper Butte Creek	1.20	1000	No	VES				No
19-L	Butte Lake - Eastern Shore	Upper Butte Creek	1.20	1050	No	No	VES*	VES*	eDNA, VES*	No
20-L	Horseshoe Lake - East	Snag Lake	0.45	810	No	No	VES*	VES*	VES*	No
21-S	Inflow to Snag Lake	Snag Lake	0.45	2140	No	VES	eDNA, VES*	eDNA, VES*	eDNA, VES*	No
22-L	Indian Lake	Warner Creek	1.20	1010	No	No				No
23-P	"Tadpole City" - Downstream of Indian Lakes	Warner Creek	1.2	1100	No	VES				No
24-L	Crystal Lake	Warner Creek	1.20	1310	No	VES				No
25-P	Juniper Pond - Downstream	Benner Creek	0.45	360	No	VES				No
26-P	Snag Lake Trail Pond	Snag Lake	1.20	660	No	VES				No
27-L	Juniper Lake - Southeast Corner	Warner Creek	1.20	405	No	No	VES*	VES*	VES*	No
27-L-P	Small pond adjacent to Juniper Lake	Warner Creek	1.20	875	No	No	VES*	VES*	VES*	No
28-L	Juniper Lake Outflow Area	Warner Creek	0.45	705	No	No	VES*	VES*	VES*	No
28-L-P	Juniper Lake Outflow Area 3	Warner Creek	1.20	875	No	VES				No
30-S	Lower Kings Creek by Park Boundary	Warner Creek	0.45	2070	No	No	eDNA	eDNA	eDNA	No
31-M-C	Wetland Near PCT and Kings Creek	Warner Creek	0.45	1970	No	No	eDNA, VES*	eDNA, VES*	VES*	No
32-S	Lower Grassy Swale Trail	Warner Creek	0.45	1775	No	No	eDNA, VES*	VES*	VES*	No
33-M-C	Upper Grassy Swale Trail	Warner Creek	0.45	1565	No	No	eDNA, VES*	VES*	VES*	No
34-S	King and Summit Creek Junction	Warner Creek	0.45	1905	No	No	eDNA	eDNA	eDNA	No

*VES for trout were not differentiated between species, and so were listed as detected under every trout species at a site. “-S”=stream/creek, “-L”=lake, “-P”=pond, “-M-C”=meadow creek.

Table B-2 (continued). Compiled summary table from all sampling (VES and eDNA).

Site Number	Site Name	Watershed	eDNA Filter Size (µm)	Filter Volume (mL)	Cascades Frogs Detected	Tree Frogs Detected	Brook Trout Detected	Rainbow Trout Detected	Brown Trout Detected	Leeches Detected
35-S	Lower Kings Creek at Kelly Camp Trail	Warner Creek	0.45	2040	No	No	eDNA	eDNA	eDNA	No
36-S-C	Kings Creek Picnic Area	Warner Creek	0.45	2520	No	VES	–	–	–	No
37-M-C	Kings Creek Trail	Warner Creek	0.45	1625	No	VES	eDNA, VES*	VES*	VES*	No
38-S	Hot Springs Creek - Downstream near Park boundary	Warner Creek	0.45	1350	No	No	eDNA, VES*	eDNA, VES*	eDNA, VES*	No
39-S	Hot Springs Creek in Drakesbad Meadow	Warner Creek	1.20	1910	No	No	eDNA	eDNA	eDNA	No
40-M	Little Willow Lake	Willow Creek- North Fork Feather River	1.20	300	No	VES	–	–	–	VES, eDNA
42-S	Rice Creek	Willow Creek- North Fork Feather River	0.45	1735	No	VES	VES*	eDNA, VES*	eDNA, VES*	No
43-M-C	Crumbaugh Lake	Willow Creek- North Fork Feather River	1.20	930	No	No	–	–	–	No
44-S	Mill Creek	Upper Mill Creek	0.45	1155	No	No	–	eDNA	–	No
45-M-C	Kings Creek Meadow	Warner Creek	1.20	1995	No	VES	eDNA, VES*	VES*	VES*	No
46-S	Creek Crossing at LAVO Park Hwy Near SW Entrance	Upper Mill Creek	0.45	2050	No	No	–	–	–	No
47-S	East Sulphur Creek	Upper Mill Creek	0.45	1720	No	No	–	–	–	No
48-L	Emerald Lake	Upper Mill Creek	1.20	1010	No	VES	–	–	–	No
49-L	Blue Lake	Bailey Creek	1.20	1020	No	No	VES*	eDNA, VES*	eDNA, VES*	No
50-S	North Fork Bailey Creek	Bailey Creek	0.45	2015	No	No	eDNA	eDNA	eDNA	No
51-L	Big Bear Lake	Box Canyon-Hat Creek	1.20	1005	No	VES	–	–	–	No
52-S	West Sulphur Creek Near Mill Creek Trail	Upper Mill Creek	0.45	1840	No	No	–	–	–	No
CAR-M-C1	Carter Meadow 1	Lost Creek-Deer Creek	0.45	1060	No	VES	–	–	–	No
CAR-M-C2	Carter Meadow 1	Lost Creek-Deer Creek	1.20	992	No	VES	–	–	–	No
CAR-M-C3	Carter Meadow 2	Lost Creek-Deer Creek	0.45	875	eDNA	VES, eDNA	–	eDNA	–	No
CAR-M-C4	Carter Meadow 2	Lost Creek-Deer Creek	1.20	960	eDNA	VES, eDNA	–	–	–	No
Neg Control 1	Negative Control 1	–	0.45	925	–	No	–	–	–	No
Neg Control2	Negative Control 2	–	0.45	950	–	No	–	–	–	No

*VES for trout were not differentiated between species, and so were listed as detected under every trout species at a site. “-S”=stream/creek, “-L”=lake, “-P”=pond, “-M-C”=meadow creek.

Table B-3. Species list from incidental observations.

Taxa category	Species Common Name	Species Scientific Name	Observation Location
Mammals	Mule deer	<i>Odocoileus hemionus</i>	Small herd at Emerald Lake and one individual at Manzanita Lake
	American black bear	<i>Ursus americanus</i>	Observed on the way to 44-S and one at Snag Lake. Scat observed at 37-M-C.
Reptiles and Amphibians	Common sagebrush lizard	<i>Sceloporus graciosus</i>	Multiple observed between Boiling Springs Lake and on the way to site 40-M
	Valley garter snake	<i>Thamnophis sirtalis</i> ssp. <i>fitchi</i>	11 sites. Notably, approximately 6 snakes seen at Juniper Lake site 27-L-P and approximately 6 more observed at 8-M-C. Two individuals documented at Soap Lake feeding on Western toads. A shed was found on north bank of Crystal Lake.
	Western toad	<i>Anaxyrus boreas</i>	Several dozen at Soap Lake in various tadpole development and toadlets. Several adult holes were observed between Boiling Springs Lake and on the way to site 40-M
	Tree frog	<i>Pseudacris</i> sp.	As reported in Results
Insects	Boisduval's blue	<i>Plebejus icarioides</i>	A small group mud-puddling at Juniper Lake.
	Pale swallowtail	<i>Papilio eurymedon</i>	Site 35-S
	Greater fritillary	<i>Speyeria</i> sp.	Site 14-L
	Woolly bee flies	<i>Systoechus</i> sp.	Site 4-S
	Bluet sp.	<i>Enallagma</i> sp.	Various locations. Notable site includes 18-L where all individuals were infested with red mites.
	Unknown dragonfly nymph	<i>Odonata</i>	Emerald Lake had many observable nymphs
Macroinvertebrates	Fairy shrimp	<i>Streptocephalus coloradensis</i>	Site 26-P
	Signal crayfish	<i>Pacifastacus leniusculus</i>	Juniper Lake

Table B-4. Vegetation density and community at each survey location in 2021.

Site Number	Emergent Vegetation ^a	Floating Vegetation ^b	Predominant Vegetation Community ^c
1-S	0–30	0	Variable: Mixed Needleleaf Forest and Woodland (Sparse), Other Mixed Herbaceous Meadow, Mesic-Wet Herbaceous Complex, Willow Shrubland
1-S-2	0	0	White and Red Fir Forest and Woodland (Sparse)
3-S	0	0	Mixed Needleleaf Forest and Woodland (Sparse), Mixed Shrubland, With Dry Mixed Herbaceous; Other Grasses
4-S	0 (undercut grassy banks)	0	Herbaceous; Other Grasses (Bordered by Mixed Needleleaf Forest and Woodland)
5-S	0	0	Manzanita Shrubland surrounded by old burn Needleleaf Forest and Woodland
6-S	100	0	Grasses, Dry Mixed Herbaceous, surrounded by old burn Needleleaf Forest and Woodland
7-M-C	0–90	0	Mesic-Wet Herbaceous Complex, Mixed Needleleaf Forest and Woodland (Sparse)
8-M-C	0–90	0	Mesic-Wet Herbaceous Complex, Mixed Needleleaf Forest and Woodland (Sparse)
9-M-C	0–10	0	Other Herbaceous - Dry Mixed Herbaceous Other Mixed Herbaceous, Grasses. Surrounded by Shrubland and Mixed Needleleaf Forest and Woodland
11-L	10	0	Burned Pine-Fir Forest and Woodland
12-P	0–25	0	Burned Pine-Fir Forest and Woodland
14-L	0–25	0	Burned Pine-Fir Forest and Woodland
15-L	0	0	Red Fir-Lodgepole Pine-Mixed Needleleaf Forest and Woodland
16-L	0–5	0	Red Fir-Lodgepole Pine-Mixed Needleleaf Forest and Woodland
17-L	0	0	Red Fir-Lodgepole Pine-Mixed Needleleaf Forest and Woodland, burned landscape
18-L	0	0	Mixed Needleleaf Forest and Woodland (Sparse), Barren Sparsely Vegetated Landscape Feature
19-L	0	0	Cinder Cone-Barren Landscape
20-L	0	0	Lodge pole Pine Forest and Woodland (Sparse)

^a This represents the percentage of water surface that is covered by vegetation that is rooted in the substrate. Such vegetation would include rushes, cattails, grasses, etc.

^b This is the percent cover for plants such as duckweed that float on the water.

^c This is the dominant plant community in the area being surveyed. Extracted from <https://www.nps.gov/im/vmi-lavo.htm> and field truthing.

Table B-4 (continued). Vegetation density and community at each survey location in 2021.

Site Number	Emergent Vegetation ^a	Floating Vegetation ^b	Predominant Vegetation Community ^c
21-S	0–100	0	Southern area of Snag Lake is Sedge Mixed Herbaceous Meadow/Complex, Wet Herbaceous Meadow
22-L	0–25	5	Mixed Needleleaf Forest and Woodland (Sparse)
23-P	50	0	Mixed Needleleaf Forest and Woodland (Sparse)
24-L	0–5	0	Mixed Needleleaf Forest and Woodland (Sparse) and Barren Sparsely Vegetated Landscape
25-P	0	0–25	Mixed Needleleaf Forest and Woodland (Sparse) and Other Herbaceous
26-P	25	0	Mixed Needleleaf Forest and Woodland (Sparse)
27-L	0	0	Mixed Needleleaf Forest and Woodland (Sparse), Rock Feature
27-L-P	10	0	Open Water surrounded by Mixed Needleleaf Forest, Woodland (Sparse) and Other Herbaceous
28-L	0	0–25	Mixed Needleleaf Forest and Woodland (Sparse) and Other Herbaceous
28-L-P	100	0	Mixed Needleleaf Forest and Woodland (Sparse) and Other Herbaceous
30-S	0	0	Mixed Needleleaf Forest and Woodland (Sparse) and Shrubland banks
31-M-C	50	0	Mixed Needleleaf Forest and Woodland (Sparse), Other Herbaceous - Wet Herbaceous Meadow
32-S	25	0	Other Herbaceous - Wet Herbaceous Meadow, Mixed Needleleaf Forest and Woodland (Sparse)
33-M-C	75	5	Mixed Needleleaf Forest and Woodland (Sparse), Other Herbaceous - Wet Herbaceous Meadow
34-S	0	0	Mixed Needleleaf Forest and Woodland (Sparse) and Shrubland banks
35-S	0	0	Mixed Needleleaf Forest and Woodland (Sparse) and Shrubland banks
36-S-C	10 (undercut banks)	0	Other Herbaceous - Sedge Mixed Herbaceous Meadow, Mesic Herbaceous Meadow Complex, Wet Herbaceous Meadow
37-M-C	0	0	Mixed Needleleaf Forest and Woodland (Sparse), Other Herbaceous - Wet Herbaceous Meadow, grasses
38-S	10	0	Mixed Needleleaf Forest and Woodland (Sparse), Shrubland banks

^a This represents the percentage of water surface that is covered by vegetation that is rooted in the substrate. Such vegetation would include rushes, cattails, grasses, etc.

^b This is the percent cover for plants such as duckweed that float on the water.

^c This is the dominant plant community in the area being surveyed. Extracted from <https://www.nps.gov/im/vmi-lavo.htm> and field truthing.

Table B-4 (continued). Vegetation density and community at each survey location in 2021.

Site Number	Emergent Vegetation ^a	Floating Vegetation ^b	Predominant Vegetation Community ^c
39-S	10	0	Mixed Needleleaf Forest and Woodland (Sparse) and Shrubland banks, Other Herbaceous - Wet Herbaceous Meadow
40-M	90	25	Wet Herbaceous Vegetation, Mixed Needleleaf Forest and Woodland (Sparse) and Shrubland
40-M-C2	90	25	Wet Herbaceous Vegetation, Mixed Needleleaf Forest and Woodland (Sparse) and Shrubland
42-S	25	0	Dry Mixed Herbaceous, Other Mixed Herbaceous
43-M-C	25 (low water)	0	Dry Mixed Herbaceous, Other Mixed Herbaceous
44-S	0	0	Mixed Needleleaf Forest and Woodland (Sparse), Barren Sparsely Vegetated Landscape Feature
45-M-C	75	0	Herbaceous Wet Meadow
46-S	0	0	Grey Alder Shrubland
47-S	0	0	Grey Alder and Manzanita Shrubland, Dry Other Mixed Shrubland on banks
48-L	10	0	Mountain Hemlock Woodland (Sparse), Mixed Woodland, Sparsely Vegetated Landscape Feature
49-L	0	0	Dry Mixed Herbaceous Other Mixed Herbaceous and Dry Other Mixed Shrubland
50-S	0	0	Mixed Needleleaf Forest and Woodland (Sparse), Shrubland and Other Mixed Herbaceous
51-L	25	0	Burned Pine-Fir Forest and Woodland
52-S	5	0	Grey Alder and Manzanita Shrubland, Perennial Herbaceous adjacent

^a This represents the percentage of water surface that is covered by vegetation that is rooted in the substrate. Such vegetation would include rushes, cattails, grasses, etc.

^b This is the percent cover for plants such as duckweed that float on the water.

^c This is the dominant plant community in the area being surveyed. Extracted from <https://www.nps.gov/im/vmi-lavo.htm> and field truthing.

Appendix C. Modeling results

This appendix details the results of modeling efforts related to Cascades frog occurrence relative to vegetation, elevation and climate, and perennial water availability.

Averages for nine climate summaries were calculated with 1.2 km buffers surrounding Cascades frog occurrence locations (R.c. Occ), random locations generated across the range of Cascades frogs in California (background locations - BG), random locations generated within LAVO and for the survey locations (Surv. Loc). Occurrence data had a smaller range of values for all raw climate variables (Figure C-1). The number of climate parameters was reduced using a principal component analysis (PCA) including both occurrence and background locations. A PCA is a multivariate approach which transforms a group of variables into a smaller new set of uncorrelated variables (the principal components; PCs). Table C-1 presents the explained variability and overall contribution of variables to the first two PCs, which combined explain 91 % of the variability.

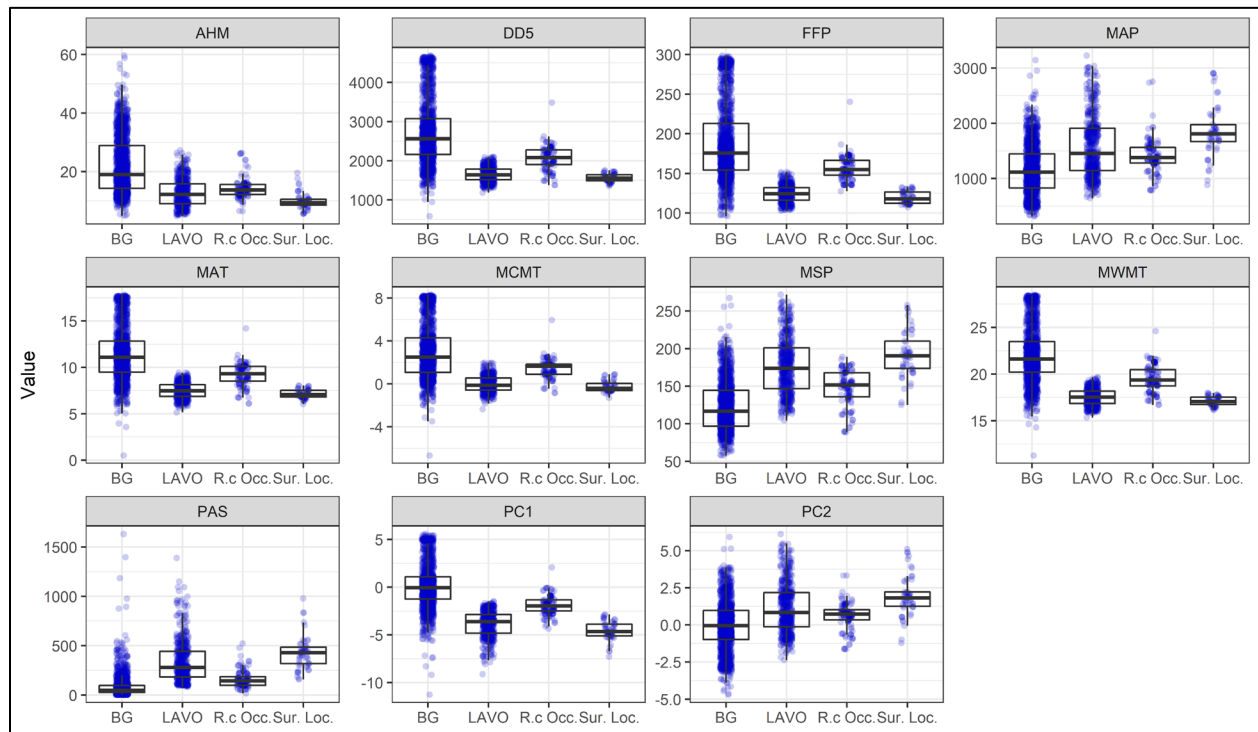


Figure C-1. Boxplots of climate and PCA scores for Cascades frog occurrence (R.c. Occ), random locations generated across the range of Cascades frogs in California (BG), random locations generated within the Park (LAVO) and for survey locations. Raw PC scores for occurrence locations do not extend the entire range of scores at background locations. Temperature PC scores for background and survey locations within the Park are lower than the occurrence data suggesting locations within the Park are generally cooler than locations with known Cascades frog occurrence. Scores for the precipitation PC within the Park and at survey locations are more consistent with the occurrence data.

Table C-1. Pearson correlations of climate variables and PCA axis scores. Noted values represent variables that contribute most in the positive (^a) and negative (^b) direction to the corresponding axis.

Variable	Description	PCA1 (62%)		PCA2 (29%)	
		P-value	rp	P-value	rp
AHM	Annual heat-moisture index	<0.001 ^D	0.438	<0.001 ^C	-0.826 ^b
DD5	Degree-days above 5°C	<0.001 ^D	0.973 ^a	<0.001 ^C	0.190
FFP	Frost-free period	<0.001 ^D	0.911 ^a	<0.001 ^C	0.345
MAP	Mean annual precipitation	<0.001 ^D	-0.261	<0.001 ^C	0.939 ^a
MAT	Mean annual temperature	<0.001 ^D	0.977 ^a	<0.001 ^C	0.198
MCMT	Mean coldest month temperature	<0.001 ^D	0.930 ^a	<0.001 ^C	0.336
MSP	May to September precipitation	<0.001 ^D	-0.437	<0.001 ^C	0.786 ^a
MWMT	Mean warmest month temperature	<0.001 ^D	0.981 ^a	<0.001 ^C	0.136
PAS	Precipitation as snow	<0.001 ^D	-0.756 ^b	<0.001 ^C	0.249

^a rp ≥ 0.6.

^b rp ≤ -0.6 .

^c AHM = annual heat-moisture index (MAT+10)/(MAP/1000)), DD5 = degree-days above 5°C, growing degree-days, FFP =frost-free period, MAP = mean annual precipitation (mm), MAT = mean annual temperature (°C), MCMT = mean coldest month temperature (°C), MSP: May = to September precipitation (mm), MWMT = mean warmest month temperature (°C), and PAS = precipitation as snow (mm). For individual years, it covers the period between August in the previous year and July in the current year.

^d Significant correlation (p-value < 0.05).

The first PC generally represented temperature variables (highlighted in blue or green in the table), with lower scores having cooler temperatures. The west-central region of the Park associated with higher elevations had the lowest scores with the rest of the Park having moderate to high temperature scores (Figure C-2). The second PC generally represented precipitation variables (highlighted in blue or green in the table), with lower scores having less precipitation and a lower heat-moisture index. Higher elevation areas within the Park (central-southwest) had higher precipitation scores (Figure C-2). The north-east had lower precipitation scores (blue) with a zone on transition in between. Figure C-2 also delineates the raw densities of suitable vegetation and perennial water within the Park, which are generally consistent with each other on the landscape.

The average climate and overall suitability with HUC12 watersheds in different time periods is presented in Table C-2. For context, the Table C-2 also summarizes predator observations (i.e., the proportion of survey locations with an observed predator) and recent (2021) burn severity (average value within the watershed and proportion of area burned [severity>0]).

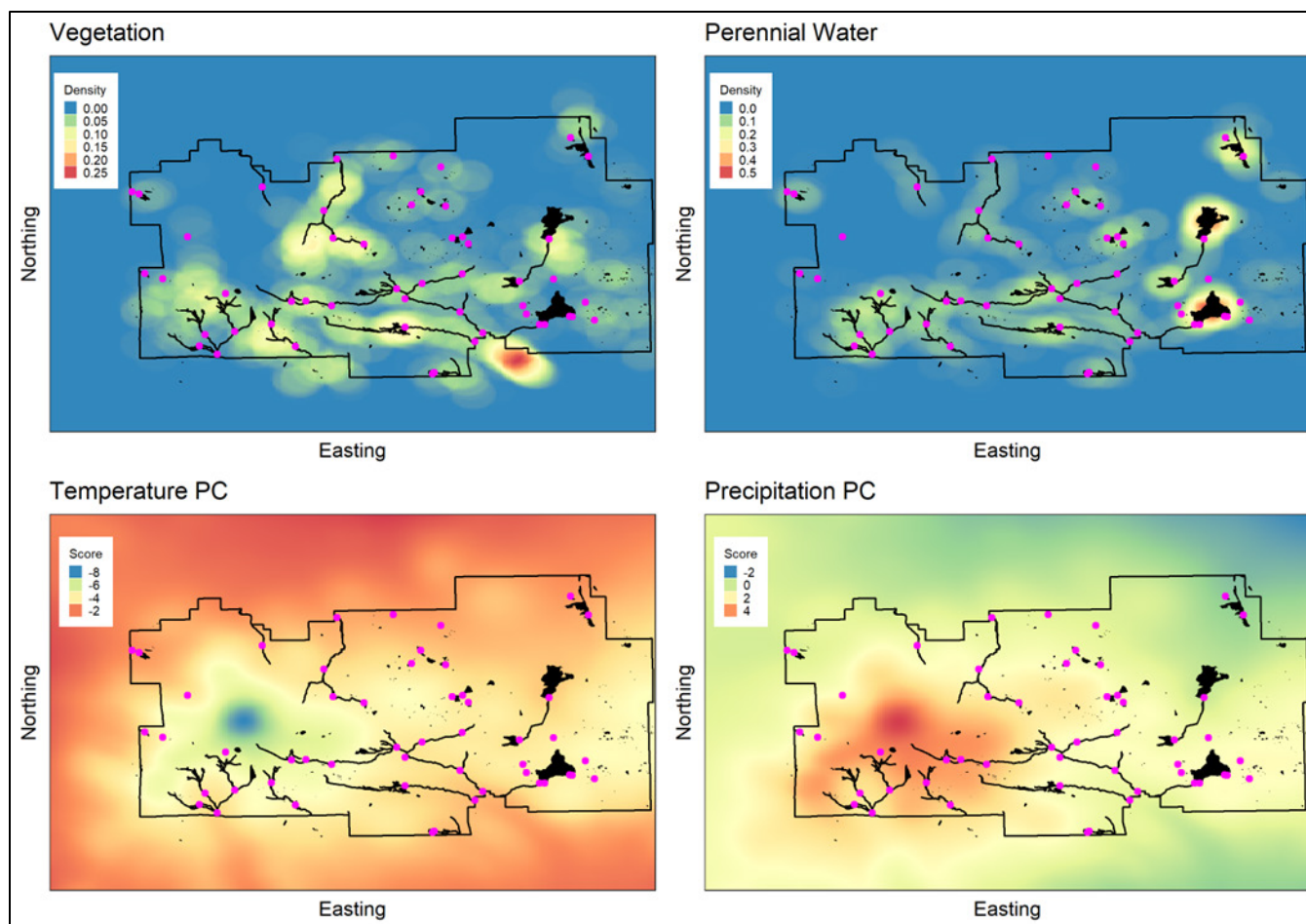


Figure C-2. Survey locations (magenta points) and raw density and PCA scores for Cascades frogs in LAVO for vegetation, perennial water (shown in black), temperature, and precipitation.

Table C-2. Watershed summary, including Cascades frog mean suitability, predator occurrence, and 2021 wildfire impacts.

Watershed Name (HUC12 ID)	Area in the Park (km2)	Number of Survey Locations	1991-2020 Mean Suitability					Projected Climate 2011-2040			Projected Climate 2041-2070			2021 Wildfire		Predator Proportion of Sites	
			Vegetation	Perennial Water	Temperature PC	Precipitation PC	Overall Mean	Temperature PC	Precipitation PC	Overall Mean	Temperature PC	Precipitation PC	Overall Mean	Proportion of Area	Mean Severity	Trout	Leech
Box Canyon-Hat Creek (180200030706)	24.00	4.0	0.056000	0.03000	0.340	0.550	0.49	0.74	0.510	0.56	0.80	0.550	0.56	0.20	1.9	0.00	0.25
Headwaters Pine Creek (180800030101)	1.00	1.0	0.006400	0.00200	0.096	0.730	0.42	0.18	0.530	0.30	0.34	0.660	0.30	1.00	1.4	0.00	0.00
Upper Butte Creek (180200030604)	45.00	2.0	0.023000	0.04700	0.340	0.400	0.41	0.67	0.360	0.46	0.73	0.400	0.46	0.94	1.5	0.50	0.00
Snag Lake (180200030601)	83.00	7.0	0.0740000	0.09100	0.170	0.470	0.40	0.41	0.550	0.47	0.67	0.520	0.47	0.93	1.8	0.43	0.00
Warner Creek (180201210101)	89.00	18	0.130000	0.13000	0.100	0.260	0.31	0.25	0.420	0.38	0.48	0.360	0.38	0.90	2.5	0.50	0.00
Deer Creek (180201530101)	31.00	3.0	0.030000	0.01400	0.230	0.340	0.31	0.46	0.330	0.35	0.49	0.330	0.35	0.00	0.0	0.67	0.00
Lost Creek (180200030702)	34.00	1.0	0.018000	0.01500	0.220	0.360	0.31	0.45	0.420	0.37	0.56	0.390	0.37	<0.01	1.0	0.00	0.00
Headwaters Hat Creek (180200030703)	45.00	4.0	0.170000	0.04100	0.130	0.260	0.30	0.32	0.340	0.37	0.49	0.310	0.37	0.38	2.1	0.50	0.00
Willow Creek-North Fork Feather River (180201210102)	23.00	5.0	0.140000	0.06500	0.099	0.200	0.25	0.28	0.270	0.31	0.49	0.240	0.31	0.99	2.7	0.20	0.60
Benner Creek (180201210401)	16.00	1.0	0.068000	0.02100	0.092	0.310	0.25	0.21	0.580	0.37	0.41	0.400	0.37	0.99	2.8	0.00	0.00
Louse Creek-North Fork Feather River 180201210103)	0.80	1.0	0.000086	0.00013	0.100	0.360	0.23	0.21	0.570	0.33	0.44	0.490	0.33	1.00	2.6	0.00	0.00
Upper Mill Creek (180201560301)	27.00	5.0	0.150000	0.12000	0.047	0.049	0.19	0.15	0.088	0.21	0.31	0.081	0.21	0.47	2.5	0.00	0.00
Bailey Creek (180201530104)	12.00	2.0	0.150000	0.01400	0.033	0.065	0.13	0.14	0.140	0.18	0.27	0.110	0.18	0.00	0.0	0.50	0.00
Upper South Fork Battle Creek (180201530202)	1.60	1.0	0.031000	0.01600	0.058	0.073	0.089	0.17	0.100	0.13	0.35	0.100	0.13	0.00	0.0	0.00	0.00
Digger Creek (180201530105)	1.80	1.0	0.032000	0.00880	0.016	0.033	0.045	0.12	0.100	0.11	0.22	0.100	0.11	0.00	0.0	0.00	0.00

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NPS 111/190224, September 2023

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