



OPEN ACCESS

EDITED BY

Arne Ludwig,
Leibniz Institute for Zoo and Wildlife Research
(LG), Germany

REVIEWED BY

Zuogang Peng,
Southwest University, China
Leonardo Congiu,
University of Padua, Italy

*CORRESPONDENCE

Samuel J. Brenkman
✉ samuelbrenkman@spherosenv.com

†PRESENT ADDRESSES

Samuel J. Brenkman,
Four Peaks Environmental Science and Data
Solutions, Wenatchee, WA, United States
Katie E. Kierczynski,
Washington Department of Fish and Wildlife,
Olympia, WA, United States
Trevor J. Kumec,
Resource Environmental Solutions, LLC.,
Sacramento, CA, United States

RECEIVED 03 September 2025

REVISED 26 November 2025

ACCEPTED 08 December 2025

PUBLISHED 15 January 2026

CITATION

Brenkman SJ, Duda JJ, McCaffery RM,
Kierczynski KE, Hoy MS, Kumec TJ, Baccus W,
Goldberg CS, Ostberg CO and Fradkin SC
(2026) Unveiling a legacy of fish introductions
to mountain lakes using historical records and
eDNA surveys in a National Park.
Front. Conserv. Sci. 6:1698619.
doi: 10.3389/fcsc.2025.1698619

COPYRIGHT

© 2026 Brenkman, Duda, McCaffery,
Kierczynski, Hoy, Kumec, Baccus, Goldberg,
Ostberg and Fradkin. This is an open-access
article distributed under the terms of the
[Creative Commons Attribution License \(CC BY\)](#).
The use, distribution or reproduction in other
forums is permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original publication in
this journal is cited, in accordance with
accepted academic practice. No use,
distribution or reproduction is permitted
which does not comply with these terms.

Unveiling a legacy of fish introductions to mountain lakes using historical records and eDNA surveys in a National Park

Samuel J. Brenkman^{1*†}, Jeffrey J. Duda²,
Rebecca M. McCaffery³, Katie E. Kierczynski^{1†}, Marshal S. Hoy²,
Trevor J. Kumec^{1†}, William Baccus¹, Caren S. Goldberg⁴,
Carl O. Ostberg² and Steven C. Fradkin¹

¹National Park Service, Olympic National Park, Port Angeles, WA, United States, ²U.S. Geological Survey, Western Fisheries Research Center, Seattle, WA, United States, ³U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Port Angeles, WA, United States, ⁴U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Pacific Northwest Environmental DNA Laboratory, Boise, ID, United States

Across the western United States, introductions of non-native fish into historically fishless mountain lakes have impacted native biota. Understanding the impacts of fish introductions is essential for conservation in Olympic National Park, a Biosphere Reserve. We reconstructed fish plantings using records dating back to 1930, followed by environmental DNA (eDNA) surveys to estimate the current distribution of fish and amphibians in 117 remote mountain lakes. We used Bayesian multiscale occupancy models to determine how lake attributes and planting history related to fish and amphibian occupancy. The most frequently detected species were Brook Trout, Rainbow Trout, Cascades Frog, and Northwestern Salamander. eDNA sampling revealed 52 lakes with amphibians only, 45 with fish and amphibians, 14 with fish only, and 6 unoccupied. Of the 53 lakes with planting records, 38 had fish eDNA detected. Fish eDNA was also detected in 21 lakes lacking planting records, which could reflect incomplete records, unauthorized plantings, and false positive detections. Of the three species planted, Cutthroat Trout had the highest failure rate and did not become established in 23 of 28 historically planted lakes. In a subset of 9 lakes sampled for up to 7 years, those with known fish and amphibian presence showed consistent eDNA detections over time. The number of times a lake was stocked was the best predictor of occupancy for Brook and Rainbow trout, while higher occupancy for Brook Trout was also associated with lower elevations, lower solar radiation, and larger lake area. We did not observe widespread negative associations between amphibian occupancy and fish presence, although there was a negative relationship between fish presence and Rough-skinned Newt and Long-toed Salamander occupancy. Cascades Frog occupancy showed no relationship to fish presence or lake traits. Our results suggest mechanisms of fish persistence over time and highlight areas where native amphibians are impacted by introduced fish. These results can guide

management options like targeted fish removals that benefit native fauna while still supporting recreational fishing. More broadly, our work demonstrates the value of combining historical records with contemporary surveys and the utility of eDNA for broad-scale surveys of species distribution in remote wilderness areas.

KEYWORDS

amphibians, Brook Trout, multiscale occupancy model, non-native trout, protected area, Rainbow Trout

1 Introduction

Global introductions of salmonid fishes have occurred in 65% of United Nations countries (Crawford and Muir, 2008). In the western United States, introductions of non-native salmonids into historically fishless lakes began in the late 1800's, with initial efforts by cattle ranchers, miners, and anglers, followed by government efforts to provide recreational angling opportunities (Pister, 2001). In a broad survey of fish in 16,000 high mountain lakes in 11 western states, 60% of all lakes and 95% of large and deep (> 2 ha and 3 m in depth) lakes contained introduced trout, including many that lie within the boundaries of national parks and congressionally designated wilderness areas (Bahls, 1992). The three most prevalent fish species introduced into those lakes were Rainbow Trout (*Oncorhynchus mykiss*), Cutthroat Trout (*Oncorhynchus clarkii*), and Brook Trout (*Salvelinus fontinalis*).

Non-native trout are now among the most widespread introduced species in aquatic habitats and threaten receiving ecosystems and native fauna (Fuller et al., 1999; Lowe et al., 2000; Kats and Ferrer, 2003; Courtenay and Fuller, 2004; Dunham et al., 2004). The history, extent, controversy, and ecological consequences of these introductions have been well documented (Moyle, 1986; Pister, 2001; Dunham et al., 2004; Crawford and Muir, 2008). Introducing non-native fish into historically fishless lakes can dramatically alter ecosystem structure and trophic interactions, resulting in the alteration of vertebrate and invertebrate community structure, reduction of native species abundance, extirpation of native species, and changes in lake productivity (Stoddard, 1987; Liss and Larson, 1991; Leavitt et al., 1994; Carlisle and Hawkins, 1998; Schindler et al., 2001; Larson and Hoffman, 2002). Fish in headwater lakes may also disperse into downstream habitats occupied by native, rare, or threatened fish populations (Adams et al., 2001). Additionally, non-native fish influence the terrestrial environment by reducing aquatic prey for birds (Epanchin et al., 2010), small mammals, and snakes (Eby et al., 2006).

Non-native fishes have specifically been linked to extirpations and population declines of amphibians worldwide (Knapp and Matthews, 2000; Adams et al., 2001; Kats and Ferrer, 2003; Pilliod et al., 2010). At least eight amphibian species have negative associations (presence and/or abundance) with non-native trout

in mountain lakes in the western United States (Dunham et al., 2004), and high predation pressure by fish has led to the loss of amphibians in many permanent mountain ponds and lakes (Knapp et al., 2001; Pilliod et al., 2010).

The history of intentional introductions (hereafter fish planting) in mountain lakes is often unknown or incomplete. Where records are available, they can provide a valuable context for the legacy of fish introductions. This legacy includes the frequency and magnitude of planting events, the temporal persistence of planted fish, the differential success of different planted species, and the resultant impacts on amphibian community structure. Few studies have examined a reconstructed history of non-native fish planting in conjunction with contemporary fish and amphibian sampling (Metcalf et al., 2012; Miño and Ventura, 2013; Love Stowell et al., 2015). Here, we examined extensive historical records of fish planting alongside contemporary lake surveys for fish and amphibians in Olympic National Park (OLYM), located in western Washington, United States.

Since the establishment of Yellowstone National Park in 1872, sport fishing has been a popular recreational activity in many U.S. national parks and is one of the few consumptive uses allowed (Panek, 1994). Early National Park Service (NPS) fisheries management efforts, in an effort to gain public support and increase tourism, prescribed systematic introductions of both native and non-native fish species to supplement fishing opportunities (Panek, 1994; Kulp and Moore, 2005; Sellars, 2009; Brenkman et al., 2014; Love Stowell et al., 2015). Concerns about non-native species and their disruption of ecosystem processes in parks were expressed by the scientific community as early as the 1920's (Houston and Schreiner, 1995), and the NPS highlighted concerns regarding the effects of introduced trout on native faunas (Wallis, 1960). In western National Parks, the presence of non-native fish conflicts with management objectives to preserve and protect natural ecological processes and native fauna (National Park Service, 2006). Surveys to assess contemporary distributions of fish and amphibians alongside historical reconstructions of planting histories are thus critical to effective management going forward.

Quantifying nonnative fish and native amphibian distribution on a park-wide scale requires tools suitable for remote, wilderness

settings across large landscapes. The widespread use of environmental DNA (eDNA) for inventory, monitoring, and conservation of species, communities, and ecosystems has rapidly increased over the past two decades (Rees et al., 2014; Beng and Corlett, 2020). The use of eDNA offers benefits over traditional sampling approaches, including lower costs, non-invasive sampling, higher detection probabilities, and less intensive field logistics (Kelly et al., 2024). For remote areas, eDNA is a particularly useful tool because samples can be collected with modest gear and personnel when compared to more intensive survey methods involving rafts and gill-netting (Hering et al., 2018; Duda et al., 2021). However, there are limitations to the efficacy and power of eDNA (Roussel et al., 2015), including: inference limited to species presence or non-detection; difficulties in quantifying biomass and abundance of target organisms; resolution and persistence of the eDNA signal; contamination and false positives; and variability and difficulty in quantifying the impacts of environmental conditions on the fate, transport, and persistence of eDNA signals (Mathieu et al., 2020). Standardization of approaches to minimize detection errors and established reporting requirements have led to more widespread use and adoption of eDNA in several fields (Klymus et al., 2020; Shu et al., 2020; Kelly et al., 2024).

Our overall objectives were to reconstruct and summarize the historical fish planting of mountain lakes in OLYM and determine

the current distribution and co-occurrence patterns of introduced fish and amphibian species in these lakes using eDNA sampling. From our eDNA surveys, we aimed to 1) determine the efficacy of eDNA to assess the presence and interannual variability of fish and amphibian species; 2) test the hypothesis that introduced fish impact amphibian occupancy in naturally fishless mountain lakes; and 3) determine which factors influence occupancy of fish and amphibians. Our results can inform management options to meet NPS preservation and recreation mandates.

2 Methods

2.1 Study area

This study occurred primarily within the boundaries of OLYM, a largely roadless protected area that is 95% congressionally designated wilderness, a UNESCO International Biosphere Reserve and World Heritage Site. Three additional lakes were sampled in adjacent Olympic National Forest (ONF) lands. The Olympic Mountains are a 9,300 km² mountain range and central feature of Washington's Olympic Peninsula (Figure 1). The core of the range is protected in OLYM (3,734 km²) and bounded on the west, south and east by 2,604 km² of National Forest lands. Rising

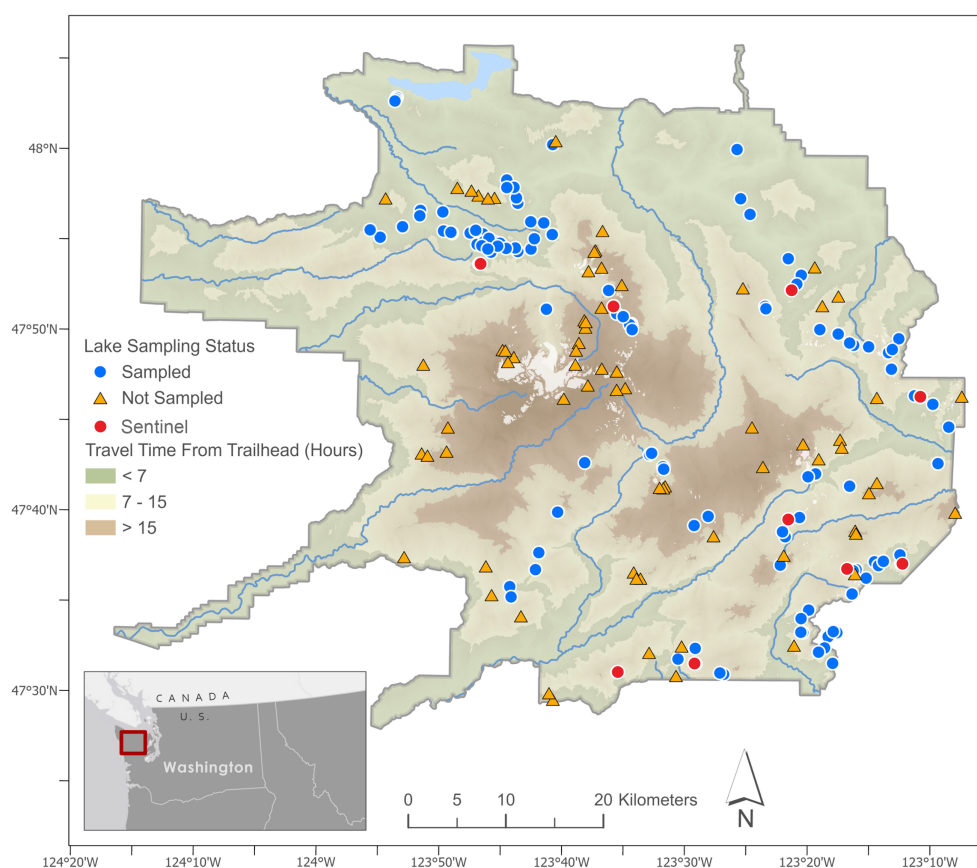


FIGURE 1

Locator map denoting eDNA collections from 117 mountain lakes (blue circles) of the 178 lakes that met study selection criteria across 13 watersheds in Olympic National Park, Washington. One-way hiking times denoted by colored shading.

rapidly from sea level to an elevation of 2,430 m in only 35 km, the Olympic Mountains form a major barrier that intercepts moisture-laden storms from the Pacific Ocean, resulting in extreme precipitation gradients (90 cm to 500 cm annually; Davey et al., 2006).

The park contains over 800 mountain lakes, ponds, and tarns which are typically ice free from early July to November. They were historically fishless due to steep gradients, waterfalls, and lack of connectivity that prevented fish from moving into lakes after glaciation. Nearly all of these mountain lakes occur within glacially carved cirques; lowland lakes are rare in the park. Although characteristics vary according to elevation, bedrock geology, aspect, subbasin size and vegetation, all lakes are generally cold, oligotrophic, and relatively free of anthropogenic impacts. Despite having over 1,000 km of maintained and primitive trails in the park, only some of the study lakes were accessible by trail, while the rest were only accessible via off-trail hiking in rugged terrain.

We focused on the following ten species that occur in or have been introduced into high elevation mountain lakes in OLYM: Rainbow Trout, Brook Trout, Coastal Cutthroat Trout (*O. clarkii clarkii*), Yellowstone Cutthroat Trout (*O. virginalis bouvieri*), Westslope Cutthroat Trout (*O. clarkii lewisi*), Cascades Frog (*Rana cascadae*), Western Toad (*Anaxyrus boreas*), Rough-Skinned Newt (*Taricha granulosa*), Northwestern Salamander (*Ambystoma gracile*), and Long-toed Salamander (*Ambystoma macrodactylum*).

2.2 Planting history of mountain lakes in OLYM

Over the last century, non-native fish were systematically and casually introduced into mountain lakes in the Olympic Mountains via backpack, stock animals, and aircraft (Figure 2a) (Garlick, 1950). Records from OLYM archives and Washington State Trail Blazers (a club dedicated to alpine fishing) included information on fish release locations, date and year of planting, species and subspecies stocked, annual number of releases, and hatchery name of origin (Brenkman et al., 2025). We summarized all existing data to the best of our ability, but recognize that some records were incomplete or did not include the number of fish released; thus, estimates herein were considered the minimum number of years stocked and numbers released.

2.3 Study design for lake sampling

Our goal was to sample as many potentially fish-bearing mountain lakes on foot as possible within a three-year study period given the constraints of accessibility and field-crew safety. We used a Geographic Information System (GIS) to select lakes within the montane and subalpine zones (914 m to 1,828 m in elevation) with surface areas greater than 0.3 ha, resulting in an initial study target population of 178 lakes. Smaller tarns and alpine lakes freeze completely over winter precluding fish survival.

We then used a GIS resistance model incorporating travel times, non-trail travel, slope steepness, and river crossings to consider site accessibility and personnel safety. This reduced the sample frame to 98 accessible and 41 possibly accessible target lakes. Of those, we sampled a total of 117 lakes (114 in OLYM; three in ONF) from 2019 to 2021, including three opportunistically sampled lakes that were lower than the original 914 m elevation or smaller than the 0.3 ha surface area criteria (Figure 1).

The lakes sampled included nine lakes (hereafter “sentinel lakes”; Figure 1) that have been monitored annually over a ~20 yr period for biological, physical, and chemical attributes as part of the NPS long-term monitoring program (Glesne et al., 2012), including annual visual surveys of amphibians and visual/angling/gillnetting surveys of fish (hereafter referred to as visual detections). Amphibian surveys consisted of searching the whole perimeter of each lake in a 2 m band, 1 m from the water’s edge to 1 m into the lake. eDNA samples were collected annually from the nine sentinel lakes, three lakes from 2015 to 2021 and the other six lakes from 2016 to 2021 (at the same time of year as this study). From 2015 through 2018, 3 replicate 1-L water samples were collected from the outlet of each sentinel lake. Beginning in 2019, the 3 1-L samples were collected around the perimeter of each lake consistent with the sampling design of the broader eDNA program described below. The sentinel lake samples were analyzed to evaluate interannual variation of eDNA results within each lake across seven years (2015 to 2021) of repeated sampling (See section 2.7).

2.4 Physical habitat attributes of mountain lakes

We used GIS to estimate the physical characteristics of each mountain lake including elevation (m), lake area (ha), and a measure of solar radiation (hr yr^{-1}). The Total Duration of Direct Solar Radiation (hereafter, solar radiation), representing the duration of direct incoming solar radiation for lake centroids, was determined in ArcGIS Pro 3.2 using the Spatial Analyst Area Solar Radiation tool. Default tool values were used except for the sky size resolution, diffuse model type, diffuse proportion and transmittivity parameters, which were adjusted to better reflect conditions on the Olympic Peninsula.

2.5 Field collection of eDNA samples and visual surveys

We adapted methods from Laramie et al. (2015) to collect and filter water samples for eDNA, taking care to avoid contamination between samples and among sites with use of nitrile gloves and single use water collection and filter apparatus. We used aerial photographs and visual estimation to divide each lake into thirds, and three water samples were collected at each lake (one in each section). Samples were collected equidistant from one another, with necessary adjustments made to accommodate site-specific access challenges that included steep cliffs, thick vegetation, or odd-shaped

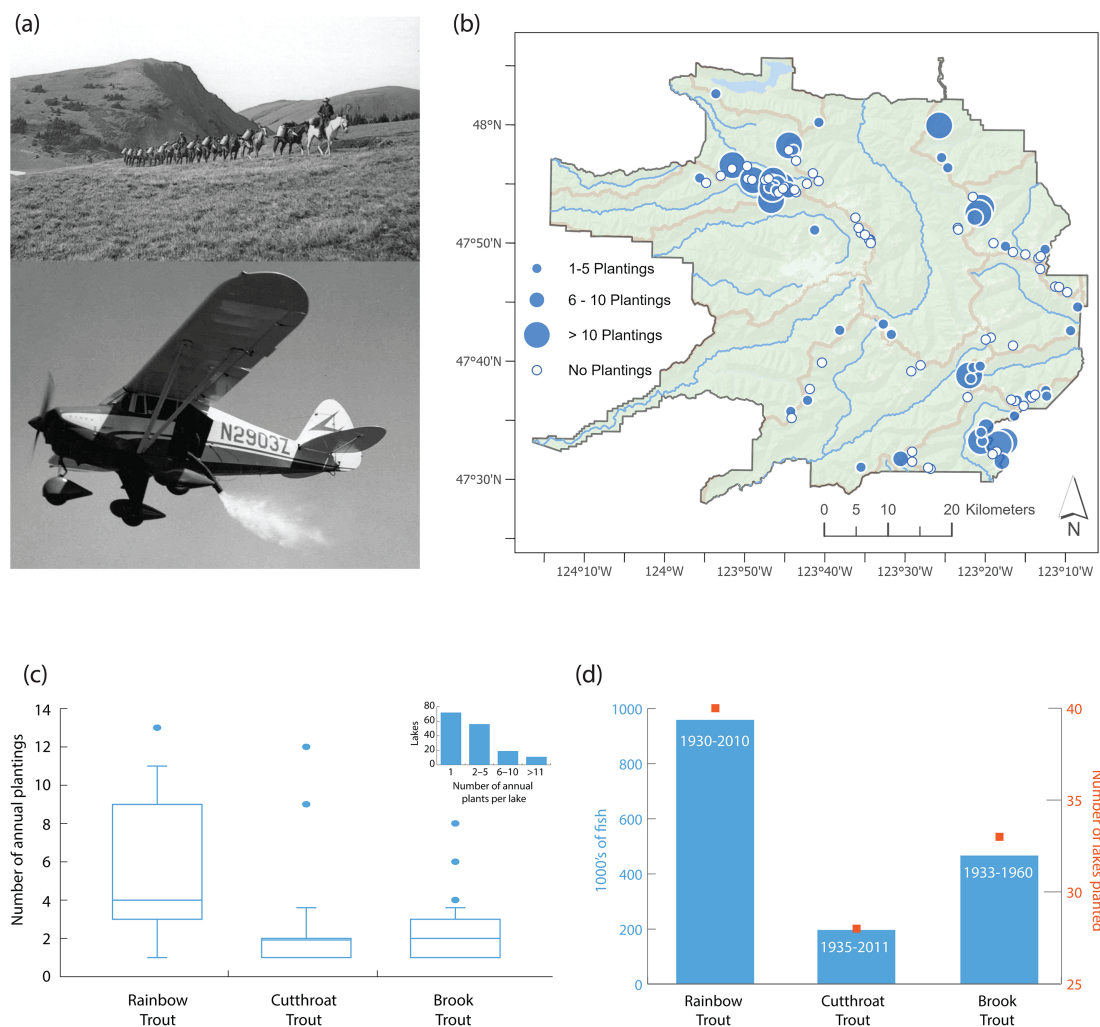


FIGURE 2

Summary of non-native trout plantings into 53 previously fishless mountain lakes in Olympic National Park: **(a)** historical pictures of fish plantings via aircraft and stock animals; **(b)** map of fish plantings in lakes, with the size of the point scaled to the number of annual plantings; **(c)** box plot (bar is median, box is interquartile range, whiskers are 95% confidence intervals) of annual plants by species. Inset is a histogram of the number of annual plants per lake; and **(d)** period of fish planting records, thousands of fish, and number of lakes with plantings of Rainbow Trout, Brook Trout, and Cutthroat Trout. At least 1,625,292 trout were introduced over the period of record.

lakes. For lakes with outlets, one sample was collected near the outlet flow.

At each sample location, we collected water from shore using a 1-L Whirl-Pak bag, typically without wading into the lake. Each sample was filtered through a 250 ml Nalgene filter funnel loaded with a 5 µm cellulose nitrate filter using a hand pump, with filtered water volume recorded using a graduated cylinder. Filters were carefully folded using sterile forceps and an applicator stick and stored in 5 ml vials containing 95% ethanol. Vials were stored in sealed plastic bags in a -80 °C freezer after returning from the field. Upon return to the office, a 1-L negative control sample was taken using deionized water to test for equipment contamination.

After collecting eDNA samples, we conducted angling surveys at 54 lakes. Surveyors did not touch angling equipment prior to the collection of eDNA samples to avoid cross-contamination. Fish that were caught during angling were identified, recorded, and released near their point of capture. Visual surveys were conducted for the

presence or non-detection of amphibians (including adult, juvenile, and egg masses) and fish along the perimeter of each lake. Additional visual surveys were conducted for fish in any lake where angling did not occur due to time constraints.

2.6 eDNA laboratory analyses

All laboratory protocols and analyses were designed to avoid cross-contamination (Goldberg et al., 2016). The eDNA workflow and sample preparation were separated into designated work rooms including a clean room where DNA was extracted (i.e., no amplified PCR products or highly concentrated target DNA sequences allowed), a second room where PCR reagents were prepared and loaded, a third room where DNA standards were diluted and loaded, and a fourth room dedicated to PCR amplification. Sample preparation was performed in UV hoods using equipment

dedicated to processing eDNA samples at each workstation. Workstations were decontaminated with UV and/or 10% bleach before and after each use.

Each whole filter was cut into eight 2-mm-wide strips with decontaminated scissors prior to DNA extraction and strips were incubated together in lysis buffer for 1 hour at 55 °C. DNA was extracted from filters using Qiagen DNeasy Blood & Tissue extraction kits with the following modifications: 360 µl ATL buffer and 40 µl of Proteinase K were used for cell lysis and the volume of AL buffer and 95% ethanol was adjusted to 400 µl post-lysis. DNA was eluted into 200 µl of AE buffer and stored at –20 °C until qPCR analysis. Negative DNA extraction controls (extraction reagents only) were included during the DNA extraction process to identify any contamination of equipment and reagents that may have occurred during this procedure. We found no evidence of contamination in these samples.

All DNA extracted from water samples was first tested for the presence of PCR inhibitors with an internal positive control (IPC) assay using TaqMan Exogenous Internal Positive Control Reagents (EXO-IPC) (Applied Biosystems). Each DNA sample was run in duplicate, and each IPC assay was performed in 12 µl volumes consisting of 5 µl of Gene Expression Master Mix, 1 µl EXO-IPC mix, 0.2 µl EXO-IPC DNA, 2.8 µl Nanopure sterile H₂O, and 3 µl DNA template or sterile water for the non-template control (NTC). Internal positive control samples were run on a ViiA 7 real-time PCR system (Applied Biosystems) and cycling conditions for the IPC consisted of 10 min initial heat activation at 95 °C, followed by 40 cycles of denaturing at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. Results were analyzed using Vii7 RUO 1.2.4 software (Applied Biosystems). Environmental samples were considered inhibited when samples displayed a > 2 cycle threshold (Ct) shift relative to the mean NTC. Samples that were inhibited (25 of 171) were treated with OneStep PCR Inhibitor Removal Kit (Zymo Research Corporation) and retested with the IPC assay to confirm that PCR inhibition was alleviated. If the treatment did not resolve the inhibition, samples (10 of 25) were diluted (1:10) with AE buffer and retested with IPC to confirm PCR inhibition was alleviated.

We tested the DNA extracted from water samples using the qPCR assays for Rainbow Trout, Brook Trout, Westslope Cutthroat Trout, Yellowstone Cutthroat Trout (Wilcox et al., 2015), Coastal Cutthroat Trout (Duda et al., 2021), Western Toad (Franklin et al., 2018), Cascades Frog (Pope et al., 2020), and Northwest Salamander (Hoy and Ostberg, 2023). We developed assays for Rough-skinned Newt and Long-toed Salamander (see supplemental information for description and [Supplementary Table S1](#) for forward primer, reverse primer, and probe sequence information). Assays were validated for species specificity by testing fish assays against fish tissue and amphibian assays against amphibian tissues. All assays were performed in triplicate on each DNA extract in 12 µl reaction volumes consisting of 3 µl DNA template and 5 µl Gene Expression Mastermix (Thermo Fisher Scientific). Primer and probe concentrations for each assay were based on the methods reported in the respective publications or as described in the supplemental information.

PCRs were run on the ViiA 7 Real-Time PCR system (Applied Biosystems) with cycling parameters consisting of an initial step of 2

min at 50 °C, then 10 min at 95 °C, followed by 45 cycles of denaturing at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. Results were analyzed using ViiA 7 RUO 1.2.4 software (Applied Biosystems). We included a five-point dilution (10,000, 2,000, 400, 80 and 16 copies per reaction) of a gBlock double-stranded DNA fragment (Integrated DNA Technologies) representing the amplicons and run in quadruplicate. Negative controls consisting of field filtered sterile water controls, DNA extraction controls, and no-template PCR controls were each run in triplicate. We considered a positive detection as any sample amplifying at less than 40 Ct with a uniform curve morphology, as suggested by Klymus et al. (2020).

To summarize fish and amphibian detection at each lake, we differentiated “trace” detections as those cases where only 1 of 9 PCR replicates were positive from cases where ≥ 2 were positive, following Duda et al. (2021). But in occupancy modeling, we retained these as detections to avoid inflating bias in an attempt to remove false positives (Lahoz-Monfort et al., 2016).

2.7 Occupancy modeling

We conducted two different sets of occupancy models for the datasets containing multiple years of data versus those sampled only once. For the nine sentinel lakes sampled multiple years, we conducted a longitudinal occupancy analysis to estimate species occupancy (Ψ) and detection probabilities (p) across multiple years using the unmarked package (Fiske and Chandler, 2011) in R (ver. 4.5.1). Each species was modeled with year as a categorical covariate, allowing for year-specific estimates of occupancy and detection probabilities. We also used visual surveys (i.e., angling, gill netting, and visual identification) as a covariate in occupancy models to determine the change in detection probability when an organism was confirmed by visual surveys.

For the survey of 117 lakes, we implemented Bayesian multiscale occupancy models to determine which physical and planting history factors might be influencing presence of fish and amphibians (Stratton et al., 2020). We fit separate, single-season occupancy models for each species. For each lake, we used one year of data in the analysis and for the 9 sentinel lakes we used eDNA data from 2021. For six lakes that were sampled in 2019 and resampled in 2021, we used eDNA results from 2021 due to limited sampling (i.e., filter tears or equipment failure) in 2019. All other lakes were sampled a single time in either 2019, 2020, or 2021.

For the fish models, we focused on Brook Trout and Rainbow Trout, as these were the only species detected at a high enough frequency for an occupancy analysis. For the amphibian models, we examined Cascades Frog, Northwestern Salamander, Long-toed Salamander, and Rough-skinned Newt. Western Toad detections were too infrequent to estimate occupancy.

Predictors of eDNA detection probability for all species included lake area and whether the sample was taken at the lake outlet. We hypothesized that the probability of eDNA detection would be higher in lakes that had smaller areas and in samples taken

at the lake outlet (if there was one). Predictors of occupancy for both fish and amphibians included lake area, lake elevation, and solar radiation. For Brook Trout and Rainbow Trout, we also included the number of years the lake was stocked with each species according to the historical planting record. Number of years stocked was correlated with years since last planting (for Rainbow Trout, $r^2 = 0.584$, $p < 0.0001$; for Brook Trout, $r^2 = 0.675$, $p < 0.0001$), so we only included the total number of years stocked in our model. We hypothesized that the probability of occupancy would be higher in lakes that had been stocked for more years. For all amphibian species, we included a covariate indicating whether any fish species was detected with eDNA in each lake. We hypothesized that lakes with fish eDNA would have lower probabilities of occupancy by amphibians than fish-free lakes. Thus, each species had a model with two covariates related to detection probability and four covariates related to occupancy probability. We ran models for 50,000 iterations for each species using the package *msocc* (Stratton et al., 2020) in R (ver. 4.5.0).

3 Results

3.1 Legacy of fish stocking

We summarized 355 fish planting records throughout the period of record (1930 to 2011) from the 117 OLYM mountain lakes where eDNA sampling occurred (Brenkman et al., 2025). Records indicated that trout were planted in 53 historically fishless mountain lakes for recreational fishing purposes (Figure 2a). Most lakes were planted a single time but ranged from 1 to 18 years (Figures 2b, c). From 1930 to 2011, at least 1,625,292 trout were planted including 958,971 Rainbow Trout (in 40 lakes), 466,813 Brook Trout (33 lakes), and 196,508 Cutthroat Trout of three subspecies (28 lakes). For Cutthroat subspecies, 107,440 Yellowstone, 24,357 Westslope, 2,397 Coastal, and 62,314 unspecified cutthroat trout were planted (Figure 2d). First and last years of plantings were 1930 and 2010 for Rainbow Trout, 1933 and 1960 for Brook Trout, and 1935 and 2011 for Cutthroat Trout (Figure 2d).

The introduced trout species originated from hatcheries in California, Iowa, Idaho, Colorado, New Mexico, Missouri, Utah, Washington, and Wyoming. Eggs were shipped from those states to the U.S. Fish and Wildlife Service Quilcene and Quinalt Fish hatcheries located immediately adjacent to the park. When reported in records, the stock origins included Mount Whitney Rainbow Trout, Kamloops Rainbow Trout, steelhead (the anadromous life history of Rainbow Trout), Coastal Cutthroat Trout (Tokul Creek), Westslope Cutthroat Trout, (Twin Lakes, ID), and Yellowstone Cutthroat Trout (referred to in records as Montana Blackspotted Trout).

3.2 Lake attributes and eDNA detection of fish and amphibians

To sample the 117 lakes, field crews conducted sampling trips ranging from 1 to 6 days. Mountain lakes averaged 2.55 ha in area

(0.1 ha to 17.1 ha) across an elevation range from 774 m to 1,791 m (Figure 3). Our sampling design resulted in 346 water samples and 10,329 technical PCR replicates being conducted across the five fish and five amphibian species-specific qPCR assays. The month of sampling did not significantly affect the eDNA detections of fish or amphibians (Supplementary Materials [Supplementary Figure S1](#)). No laboratory or field blank controls amplified eDNA or showed evidence of contamination.

Of the 117 lakes sampled, eDNA sampling revealed 52 lakes with amphibians only, 45 lakes with fish and amphibians, 14 lakes with fish only, and 6 with no fish or amphibians detected (Figure 3). We detected at least 1 introduced fish species in 59 lakes, while the other 58 lakes had no eDNA detections of fish. For the lakes that had fish eDNA detections, 44 had 1 fish species, 13 had 2 fish species, and 2 lakes had 3 fish species. Brook Trout were the most frequently detected fish species, found in 40 lakes (including 7 lakes with trace detections i.e., 1 of 9 PCR replicates positive) and Rainbow Trout were the next most common fish species, detected in 29 (6 trace) lakes (Figure 4). Westslope Cutthroat Trout were detected in 5 lakes (2 trace), Yellowstone Cutthroat Trout in 2 lakes (1 trace), and Coastal Cutthroat Trout were not detected in any samples. Interestingly, the 2 lakes where Yellowstone Cutthroat Trout were detected also had detections of Westslope Cutthroat Trout. We detected at least 1 amphibian species in 97 lakes, while the other 20 lakes had no detections of amphibians. Thirty-seven lakes had 1 amphibian species, 32 lakes had two amphibian species, 24 lakes had three amphibian species, 4 lakes had four amphibian species, and no lakes had all 5 target amphibian species detected. Cascades Frog, Northwestern Salamander, Long-toed Salamander, Rough Skinned Newt, and Western Toad were detected in 62 (17 trace), 54 (2 trace), 35 (4 trace), 33 (6 trace), and 2 lakes, respectively (Figure 4).

We also compared current eDNA fish detections to records of documented historical fish plantings (Table 1). Of the 53 lakes with planting records, 38 had fish detections from eDNA sampling. Brook Trout had the highest level of contemporary presence based on eDNA, persisting in 70% of planted lakes, followed by Rainbow Trout (48%; 19 of 40 lakes) and Cutthroat Trout (all 3 species combined) persisting in only 7% (2 of 28) of planted lakes. We compared the average years since planting and the average number of plantings for eDNA positive (+) and eDNA negative (-) groups of lakes by species. Excluding Cutthroat Trout, the only significant difference between lakes with and without fish was a 10-year difference in the average years since last planting for Brook Trout (72 versus 82 years). We also detected fish eDNA in 21 of 64 (33%) lakes with no planting records; with Rainbow Trout, Brook Trout, and Cutthroat Trout being detected in 6, 15, and 4 lakes, respectively.

Lake aspect and the terrain of the surrounding watershed determine the amount of solar radiation. Lakes with different combinations of fish and amphibian presence differed by elevation and lake area, but not by solar radiation (ANOVA, $P = 0.246$). Lakes with both fish and amphibians occurred at significantly lower elevations than lakes with fish only, amphibians only, or no vertebrate taxa (ANOVA, $P < 0.001$). Lakes with only fish detected were significantly larger than lakes

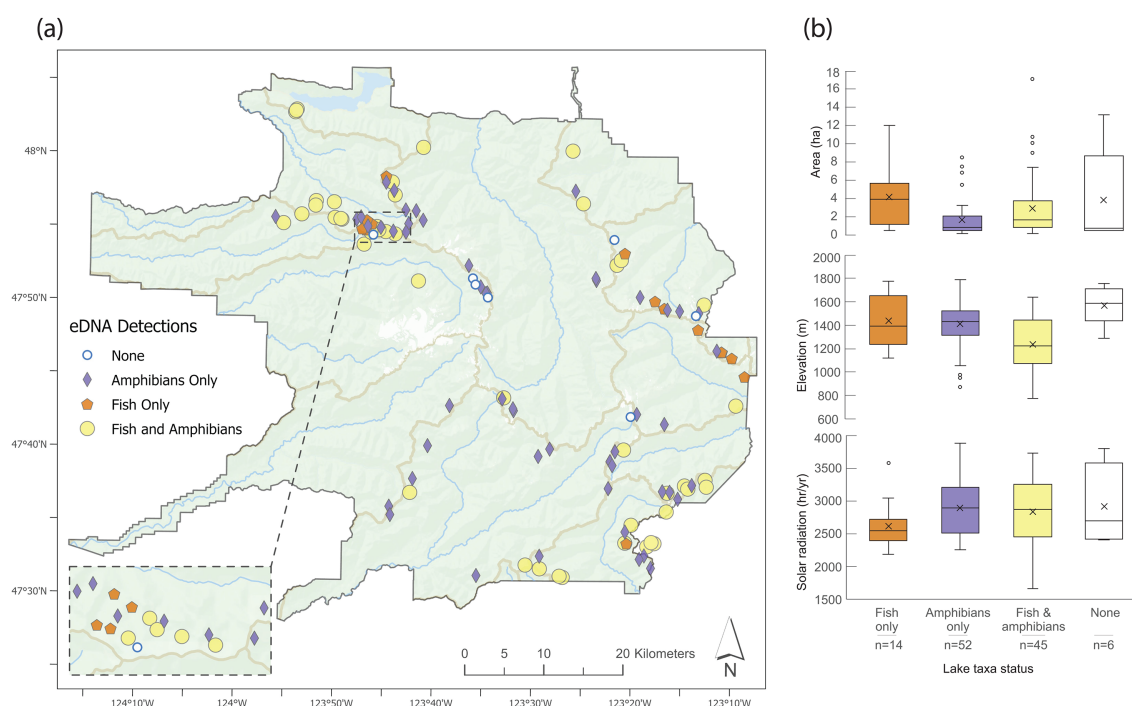


FIGURE 3

Map of mountain lakes in Olympic National Park (a) with eDNA detections showing lakes with fish only, amphibian only, fish and amphibians, or no species detected and (b) box plot summary (bar is median, x is mean, box is interquartile range, whiskers are 95% confidence intervals) of the physical characteristics of each lake.

with only amphibians detected (ANOVA, $P = 0.003$). Finally, we found that lakes with only Long-toed Salamanders occurred at higher elevations relative to lakes with only Northwestern Salamanders (Figure 5; $P = 0.008$). Lakes containing both species occurred mainly at mid elevations between the mean elevations of single species only lakes.

3.3 Sentinel lakes – interannual and between method variability

Based on the repeated annual sampling of sentinel lakes, we were able to assess interannual patterns of eDNA detections and levels of agreement with concurrent visual surveys. Generally, there was agreement between the two methods (Figure 6), but with some important differences noted below.

Overall, fish were never observed in a lake without also being detected via eDNA (Figure 6). Three of the nine sentinel lakes were known to contain fish from repeated angling and gillnet surveys. Gladys and Hoh Lakes contained Brook Trout that were detected both visually and via eDNA in every year sampled. Lake Sunup contained Cutthroat Trout that were detected via eDNA in 5 of the 6 years sampled. For the other six sentinel lakes which were putatively fishless, at least one fish species was detected in the lakes during at least one year ($n = 14$ of 114, or 12% of all possible species-lake-year combinations).

Amphibians in sentinel lakes were consistently detected in lakes across time (Figure 6). There was agreement (i.e., eDNA+/Visual+

or eDNA-/Visual-) in 84% of all 220 possible species-lake-year combinations. For newts and salamanders, almost all the cases of disagreement were of eDNA detecting presence that was not confirmed by visual identification. The opposite was true for Cascades Frog, which were visually observed but not detected with eDNA in 24% of 55 lake-year cases for this species.

For Brook Trout, Northwestern Salamander, Long-toed Salamander, and Cascades Frog, we determined if visual detection of the species was related to detection probability of that species via eDNA. For the other species, all eDNA detections matched confirmed visual detections. We found that eDNA detection probability increased when visual observation was included as a covariate for Brook Trout (from $p = 0.68$ to $p = 0.96$), Long-toed Salamander (from $p = 0.60$ to $p = 0.77$), and Northwestern Salamander (from $p = 0.74$ to $p = 0.99$). Visually detecting Cascades Frog, on the other hand, decreased detection probability from 0.50 to 0.32.

3.4 Estimated detection and occupancy probabilities of fish and amphibians

Estimated mean eDNA detection probability was 0.69 (95% credible interval [CRI] 0.58 – 0.79) for Rainbow Trout and 0.88 (95% CRI 0.82 – 0.93) for Brook Trout. Brook Trout detection was somewhat higher in smaller lakes than in larger lakes ($b = -0.27$; 95% CRI -0.58 – -0.02), but not for Rainbow Trout (Table 2). There was no effect of sampling location (outlet v. not at outlet) for either

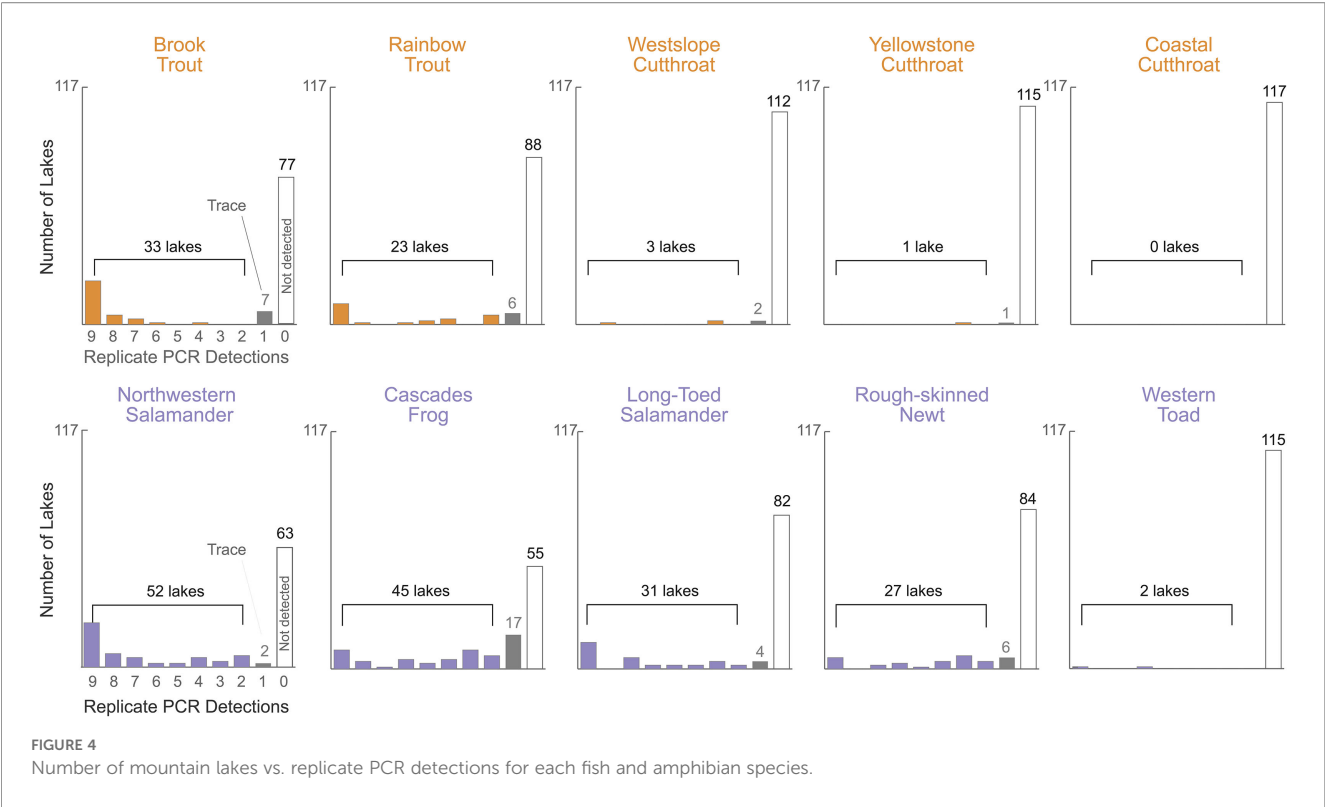


TABLE 1 Fate of fish plantings based on contemporary eDNA sampling, with average number of plants and average years since last planting for lakes that had detections (eDNA+) or failure to detect (eDNA-) three species of introduced fish.

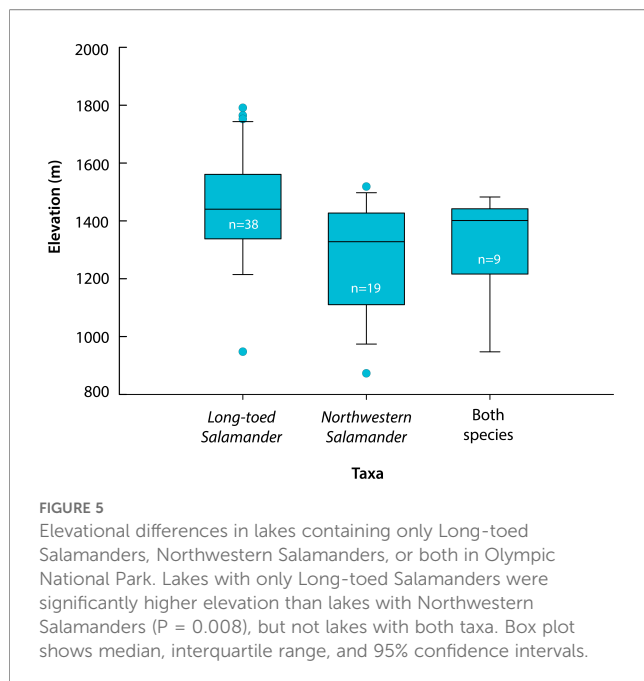
Factor	eDNA +	eDNA -	t-test
Rainbow Trout			
Lakes with fish planting record	19	21	
Average years since last planting	53.6	57.7	$t_{(38)} = 0.798$, $p=0.43$
Average number of plantings	6.0	5.1	$t_{(38)} = 0.772$, $p=0.44$
Brook Trout			
Lakes with fish planting record	23	10	
Average years since last planting	72.0	81.9	$t_{(31)} = 2.58$, $p=0.02$
Average number of plantings	2.4	1.5	$t_{(31)} = 1.46$, $p=0.15$
Cutthroat Trout*			
Lakes with fish planting record	2	26	
Average years since last planting	37	67.5	$t_{(26)} = 1.89$, $p=0.07$
Average number of plantings	1	2.2	$t_{(26)} = -1.19$, $p=0.05$

*Combination of Yellowstone Cutthroat Trout, Coastal Cutthroat Trout, and Westslope Cutthroat Trout.

fish species. Estimated mean eDNA detection probability was 0.56 (95% CRI 0.48 – 0.65) for Cascades Frogs, 0.88 (95% CRI 0.83 – 0.93) for Northwestern Salamanders, 0.84 (95% CRI 0.76 – 0.91) for Long-toed Salamanders, and 0.66 (95% CRI 0.54 – 0.77) for Rough-skinned Newts. Lake area and sampling location had no effect on detection probability for any amphibian species (Table 2).

Mean Rainbow Trout eDNA occupancy was 0.26 (95% CRI 0.19 – 0.35) and mean Brook Trout occupancy was 0.35 (95% CRI 0.26 – 0.43). Rainbow Trout occupancy was best predicted by the number of years stocked, where occupancy was higher in lakes that had been stocked for more total years ($b = 0.71$, 95% CRI 0.29 – 1.18; Table 2). No other physical characteristics of lakes were important to Rainbow Trout occupancy. Brook Trout occupancy was higher in lakes with higher number of years stocked ($b = 1.19$, 95% CRI 0.59 – 1.85), larger areas ($b = 0.90$, 95% CRI 0.07 – 2.30), at lower elevations ($b = -0.53$, 95% CRI -0.99 – -0.08), and with lower solar radiation ($b = -0.49$, 95% CRI -0.96 – -0.04).

For amphibians, Cascades Frogs had the highest mean eDNA occupancy (0.59, 95% CRI 0.49 – 0.70), followed by Northwestern Salamanders (0.46, 95% CRI 0.38 – 0.55), Long-toed Salamanders (0.31; 95% CRI 0.23 – 0.39), and Rough-skinned Newts (0.30; 95% CRI 0.22 – 0.40). Each amphibian species had a different combination of variables best predicting occupancy (Table 2). Cascades Frog occupancy was higher in areas with increased solar radiation, though the credible interval crossed zero ($b = 0.40$, 95% CRI -0.04 – 0.90). Frog occupancy was not associated with area, elevation, or presence of fish eDNA. Long-toed Salamander occupancy had a strong negative relationship with fish presence ($b = -1.58$; 95% CRI -2.48 – -0.72), higher occupancy at higher elevations ($b = 0.90$; 95% CRI 0.35 – 1.45), and a suggestion of higher occupancy with higher solar radiation ($b =$



0.46; 95% CRI -0.01 – 0.95), though this variable was not statistically significant. Similarly, Rough-skinned Newts had a strong negative relationship with fish presence ($b = -1.02$; 95% CRI -1.20 – -0.10), but in contrast showed higher occupancy at lower elevations ($b = -1.46$; 95% CRI -2.18 – -0.85). Finally, Northwestern Salamanders showed higher occupancy at lower elevations ($b = -1.23$; 95% CRI -1.79 – -0.73), but the relationship with fish presence was not statistically significant. None of the salamander species' occupancy probabilities were associated with lake area.

4 Discussion

We documented the extent and frequency of fish planting in naturally fishless mountain lakes in Olympic National Park and used eDNA to estimate contemporary occupancy patterns of fish and native amphibians in 117 lakes. The legacy of fish plantings, a practice dating back to at least 1930 that officially ended nearly 45 years ago in OLYM waters, was evident in the 59 sampled lakes that had fish. Additionally, historical records and contemporary detections of fish in 21 lakes that lacked planting records suggested that illicit plantings sporadically occurred. Contrary to other studies (e.g., Kats and Ferrer, 2003; Knapp and Matthews, 2000; Pilliod and Peterson, 2001), we did not observe widespread negative associations between amphibian occupancy and fish presence. In fact, there were 3.2 times more lakes that had both fish and amphibians detected versus those with only fish, but results differed by amphibian species. Rough-Skinned Newt and Long-Toed Salamander occupancy was negatively associated with fish presence, while Cascades Frogs and Northwestern Salamanders did not have a negative association. Geographically, fish eDNA was typically detected in lower elevation lakes near the park boundary and in areas popular with backpackers. These patterns of non-

native fish in historically fishless lakes provides a current benchmark upon which future management actions can be based. More broadly, our results showcase the strengths and limitations of historical records for assessing contemporary nonnative fish occupancy, contribute to our understanding of co-occurrence of native amphibians and nonnative fish in the U.S. mountain west, and highlight the value of eDNA as a tool for assessing species occupancy in remote aquatic environments. Our results can inform study design and management actions in the U.S. Pacific Northwest and beyond as land managers balance competing interests for recreation opportunity and biodiversity conservation.

4.1 Legacy of fish planting and contemporary patterns

Our work showed the persistence of legacy fish introductions in OLYM while highlighting challenges to building an accurate historical record of plantings. Stocking of lakes was mostly discontinued by the NPS in the late 1960s and early 1970s as the result of shifting priorities, policies, and conservation mandates (Pister, 2001; Bahls, 1992), with the latest planting record from 1986. Olympic National Forest policies continued to allow fish planting as late as 2011). Although we reconstructed the planting history from all available historical records, questions about the accuracy of these records remain. For example, the extent to what was planned (i.e., documented in the records) deviated from what occurred (i.e., where the fish were actually planted) is unknown. Additionally, extensive aerial planting of mountain lakes occurred in U.S. Forest Service lands immediately adjacent to the park boundary, with evidence that some air plants of fish missed entering the target lake. Finally, illicit fish introductions into public waters has been a well-known problem in the western United States (Johnson et al., 2009; Rahel, 2004) and it is apparent that an unknown level of planting occurred outside of official records. We identified several lakes where Brook Trout eDNA was detected but we have no records of fish plantings. This occurred in both single sampled lakes and in some sentinel lakes that were sampled up to 7 years in a row. Thus, known planting history alone is insufficient to determine the contemporary status of fish in historically fishless mountain lakes.

Our eDNA surveys revealed that 58% of the historically planted lakes had fish eDNA detected, suggesting that historical planting was not a guarantee of long-term success. There are several reasons why fish may not have persisted in a particular lake, including: the number of annual plantings; physical or habitat characteristics of lakes; a mismatch between the species niche requirements and mountain lake habitat; or because fish were present but not detected with eDNA. For Rainbow Trout, we found that the number of years lakes were stocked was the best predictor of contemporary occupancy. This suggests that planting frequency (and, thus, the cumulative number of fish planted) increased the likelihood of persistence for that species. Because the most recent date of planting was correlated with planting frequency, this factor could also be related to occupancy.

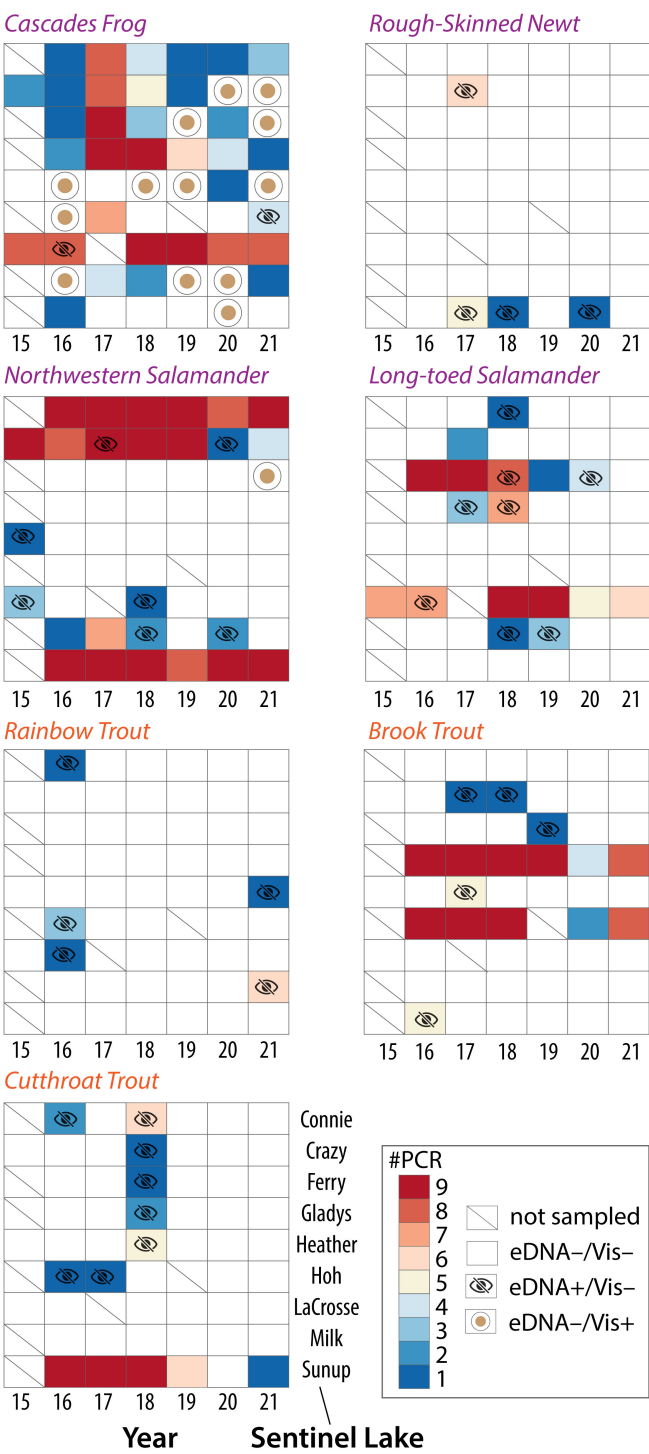


FIGURE 6 Results of annual replicate eDNA detections and visual surveys (see methods) of amphibians and fish in 9 sentinel lakes repeatedly sampled from 2015–2021. The PCR color ramp depicts the number of positive PCR replicates out of 9 possible (3 water samples, each with 3 technical replicates) for each cell that depicts a lake–year–species sampling event. Cells with symbol overlays depict different combinations of pairwise eDNA/Visual results as indicated, whereas cells with just the color ramp code indicate eDNA+/Vis+ result.

Furthermore, no other physical features of lakes were important to Rainbow Trout occupancy, consistent with their ability to survive and persist at a variety of elevations and temperature profiles as evidenced by their history as a major species for introduction globally (Crawford and Muir, 2008). In contrast, Brook Trout

occupancy was best predicted by planting frequency, larger lake area, lower elevation, and lower solar radiation. Despite Rainbow Trout having a larger number of lakes planted, total number of annual plants, and total numbers of fish, Brook Trout had higher detection probability and occupancy. A potential explanation for

TABLE 2 Parameter estimates for covariates hypothesized to affect detection and species occupancy from eDNA data collected at 117 lakes in Olympic National Park, Washington, for two nonnative fish and four amphibian species, with bold indicating statistically significant covariates.

Species	Parameter	Covariate	Mean	2.5 CRI	97.5 CRI
Rainbow Trout	Detection	Lake Area	-0.115	-1.946	1.984
		Sampled at outlet	0.509	-0.476	1.584
	Occupancy	Lake Area	-0.145	-1.890	1.897
		Elevation	-0.244	-0.689	0.239
		Solar radiation	-0.190	-0.654	0.266
		Number years stocked	0.712	0.290	1.185
Brook Trout	Detection	Lake Area	-0.272	-0.577	-0.023
		Sampled at outlet	0.536	-0.613	1.789
	Occupancy	Lake Area	0.904	0.066	2.302
		Elevation	-0.526	-0.993	-0.081
		Solar radiation	-0.491	-0.964	-0.040
		Number years stocked	1.187	0.587	1.848
Cascades Frog	Detection	Lake Area	-0.372	-2.207	1.653
		Sampled at outlet	0.552	-0.119	1.266
	Occupancy	Lake Area	-0.386	-2.178	1.600
		Elevation	-0.198	-0.654	0.241
		Solar radiation	0.402	-0.041	0.901
		Fish eDNA present	-0.232	-1.070	0.604
Northwestern Salamander	Detection	Lake Area	-0.372	-2.182	1.682
		Sampled at outlet	0.179	-0.812	1.272
	Occupancy	Lake Area	-0.252	-2.007	1.707
		Elevation	-1.233	-1.790	-0.727
		Solar radiation	0.216	-0.192	0.631
		Fish eDNA present	-0.586	-1.406	0.213
Long-toed Salamander	Detection	Lake Area	-0.376	-2.218	1.693
		Sampled at outlet	0.405	-1.685	1.628
	Occupancy	Lake Area	-1.377	-2.171	1.614
		Elevation	0.902	0.354	1.500
		Solar radiation	0.462	-0.013	0.952
		Fish eDNA present	-1.579	-2.480	-0.718
Rough-skinned Newt	Detection	Lake Area	-0.371	-2.215	1.638
		Sampled at outlet	0.302	-0.596	1.260
	Occupancy	Lake Area	-0.134	-1.925	1.819
		Elevation	-1.455	-2.177	-0.847
		Solar radiation	0.142	-1.347	0.632
		Fish eDNA present	-1.024	-1.997	-0.095

CRI, Credible Interval, derived from the posterior distribution of each parameter.

this discrepancy could be that, as described above, Brook Trout were more often stocked illicitly. However, number of years planted was a strong predictor of occupancy for both species, suggesting that despite the reality that some illicit planting data were not represented in our dataset, the number of years of planting was associated with higher contemporary occupancy overall. Cutthroat Trout fared the worst of the three fish species planted, with the three subspecies failing to persist in 82% of the planted lakes, although planting frequency was also considerably less for this species (Table 1).

Elements of fish life history may explain patterns of introduced fish persistence in OLYM, notably spawn timing and location. Spawn timing may influence the persistence of fish species planted in mountain lakes where winterkill can be common and pervasive in shallow and ice-covered lakes (Mathias and Barica, 1980; Davis et al., 2019). Brook Trout, which had the highest occupancy across the sampled lakes, spawn in the fall. Because larger lakes at lower elevations freeze later, winterkill could have been lower when compared to lakes at higher elevations. In contrast to Brook Trout, Cutthroat Trout persisted in only 5 of 28 lakes where they were originally planted. The lack of Cutthroat Trout detections may be due to the low number of fish planted and the fewest average number of annual plantings. This could be coupled with the fact that the subspecies of Cutthroat Trout planted primarily spawn in lake inlet or outlet tributaries (Gresswell, 1995; McIntyre and Rieman, 1995) and many of the mountain lakes in OLYM were isolated, lacking these tributaries. The timing of reproduction influences population success, and all three subspecies of Cutthroat Trout typically spawn during winter and spring months (Trotter, 1989; Behnke, 2002), a period when many mountain lakes are still frozen. Notably, Hagen (1961) reported that despite many plants of Cutthroat Trout in OLYM, there was no evidence of their presence in sampled lakes. Furthermore, it is possible that where Cutthroat Trout were introduced alongside Brook Trout or Rainbow Trout, they were outcompeted by the latter species.

4.2 Impacts of fish presence and lake characteristics on amphibian distribution

Lakes containing only amphibian species were the most common in our study, followed by those with both amphibians and fish (Figure 3), demonstrating the broad distribution and persistence of amphibians both in lakes with and without fish. There were only 14 lakes where fish were exclusively detected. However, individual amphibian species varied in their distribution and association with fish presence. Cascades Frogs had the highest estimated occupancy and did not appear to be influenced by trout occupancy. They were found throughout OLYM mountain lakes across a wide range of the physical habitat features that we examined. The lack of a negative association with fish may not be surprising as this species is more likely to breed in ephemeral wetlands and ponds adjacent to lakes than in the lakes themselves, thus creating a refuge where breeding occurs in areas isolated from

the effects of fish (Vredenburg, 2004). Cascades Frogs can also modify their habitat preferences and behavior in the presence of fish to facilitate co-occurrence (Hartman et al., 2014). Cascades Frog occupancy did appear to be higher in areas with higher solar radiation, though the credible interval slightly crossed zero, indicating the effect may not be significant. Since frogs are likely using larger lakes for basking and foraging in the post-breeding season, occupancy could be higher in those lakes receiving more solar radiation. Finally, of all the amphibians assessed with eDNA, Cascades Frogs had the highest levels of mismatch between visual (+) and eDNA (-) occupancy. This may be due to the shift from aquatic (tadpole) to more terrestrial (adult) habitats that occur over the course of the summer, in contrast to other species with a multi-year aquatic life stage. Our visual survey methods captured observations of both aquatic and riparian presence without discriminating between the two. Thus, this pattern may be a function of the sampling timing, as Cascades Frog could have shifted from lake water to riparian terrestrial habitats by the time sampling crews obtained water samples in mid-summer, allowing time for degradative processes to eliminate the eDNA signal within lake water (Barnes et al., 2014). Additionally, breeding may have occurred in smaller ephemeral waterbodies that we did not sample, meaning that the more detectable larval stage was not available for detection in the lake eDNA samples.

Both Rough-skinned Newts and Long-toed Salamanders had lower predicted occupancy in the presence of trout. The negative effect of introduced trout on Long-toed Salamanders has been well-documented in previous studies and is consistent with what we found in OLYM (Tyler et al., 1998; Pilliod and Peterson, 2001; Pearson and Goater, 2009). In addition to direct predation on Long-toed Salamanders, trout presence can incur non-lethal effects such as smaller size, lower weight, slower growth, and delayed metamorphosis (Kenison et al., 2016). The evidence for nonnative fish affecting Rough-skinned Newts is more equivocal. Some studies have found lower densities or presence of newts in the presence of nonnative fish, but others have found no effect (Welsh et al., 2006). Rough-skinned Newts produce a coevolutionary anti-predator tetrodotoxin, although toxicity varies widely between populations (Hague et al., 2016). The toxicity of OLYM newts is unknown, but a low toxicity would be consistent with lower occupancy in the presence of trout.

Salamander species showed different occupancy patterns by elevation. Long-toed Salamander occupancy was greater in higher elevation lakes and potentially in lakes with increased solar radiation. Rough-skinned Newts and Northwestern Salamanders showed higher occupancy at lower elevations. The elevational segregation between Northwestern and Long-toed Salamanders is consistent with previous research which suggested an elevational transition zone between the two species (Figure 5; Hoffman et al., 2003; Tyler et al., 1998).

The detection of Western Toads in only two lakes was insufficient for occupancy analysis. Most sampling occurred later in the summer after toads would have metamorphosed from breeding ponds and entered the terrestrial environment. Regardless, previous work has shown that toad presence can be unaffected in the presence of introduced trout because they are

unpalatable to fish and thus may be less affected by fish presence (Kats et al., 1988).

One limitation of our study was the lack of data describing meso- and micro-habitat characteristics at our sampled lakes, specifically the presence of aquatic vegetation and other structural complexity that can promote coexistence of fish and amphibians. For example, Long-toed Salamanders can use more highly vegetated areas in lakes containing non-native trout than in lakes without trout (Kenison et al., 2016) and in areas with more emergent vegetation generally (Hoffman et al., 2003). Cascades Frog occupancy can also be higher in areas with increased emergent vegetation (Briggs, 1987; Garwood and Welsh, 2007), and DNA transport can be blocked by emergent vegetation, preventing detection (Mayne et al., 2024).

The implications of our results for amphibian conservation are somewhat limited by our focus on larger, permanent lakes. Cascades Frog breeding habitat is typically free from fish because they often breed in smaller waterbodies that melt earlier and dry each summer, providing a breeding refugia free from fish predation. However, these breeding ponds are vulnerable to increased temperature and drought, which is expected to shorten hydroperiods and make some of this habitat unsuitable in the future (Ryan et al., 2014). In that case, lakes and permanent ponds will become even more important to amphibian persistence in these environments, and the negative impact of fish could be heightened. Further work using eDNA to sample amphibians in wetlands and ponds surrounding these lakes would provide a deeper understanding of factors contributing to fish and amphibian coexistence. It would also enhance our understanding of how amphibians use different waterbodies in the landscape during different parts of their life histories.

4.3 Lessons from sentinel lakes

Repeated annual visits to the sentinel lakes allowed us to assess the temporal consistency and reliability of eDNA sampling. By repeatedly visiting the same lakes annually we had confidence from visual methods (including angling and gillnetting) that 3 lakes had stable fish populations and 6 lakes lacked fish. eDNA sampling at locations with fish were consistent with expectations from visual sampling (Figure 6). We also had fish eDNA detections at the sentinel lakes that did not meet our expectations based on visual data. Of a total of 165 lake-year-fish taxa combinations (i.e. all 9 sentinel lakes), we found 19 cases of eDNA detections that did not match the visual occurrence of fish taxa. When this 12% error rate is applied to the overall study population, it is possible that 7 of 59 lakes with eDNA detections could be false positives. We tried to assess the source of error in several ways. First, we examined the field and laboratory control samples, yet none showed evidence of contamination. Next, we examined the patterns of fish detections in putatively fishless sentinel lakes relative to when eDNA sample batches were prepped for PCR (12 dates) and when PCR samples were amplified (5 dates). No patterns consistent with laboratory contamination were evident. Additionally, a subset of 11 PCR samples were rerun to verify

previous results of fish presence and these showed consistent results. Finally, we examined field visitation schedules and did not find any patterns to suggest interlake contamination.

Although we found no direct evidence of either field or laboratory contamination to explain the detection of fish in a subset of putatively fishless sentinel lakes, there are other mechanisms that could result in eDNA detection of fish in these systems (Darling et al., 2021). Fish eDNA may have been transported to these lakes by other organisms, including wildlife like piscivorous birds, ducks, and mammals, especially if occupied lakes were nearby. Similarly, park visitors that visit and recreate within multiple mountain lakes over a short time period may act as a vector. Finally, there were multiple instances where we had trace eDNA detections with low eDNA concentration. These waterbodies merit repeat sampling to confirm fish presence.

4.4 Management implications

The extent and effects of non-native fish in OLYM has received very little attention since the park's inception in 1938, particularly considering the long history and widespread implementation of fish plantings. The first comprehensive inventories of introduced fish in mountain lakes started in the late 1950s (Hagen, 1961), leading park biologists to raise ecological concerns in the 1990's. Olson and Meyer (1994) conducted a limited inventory of non-native trout in 20 mountain lakes, with continued concerns from resource managers at OLYM about threats of introduced fish on lake amphibians and native fish downstream, including those that are federally threatened and rare. Reconstruction of legacy fish introductions and high-resolution information on existing trout distributions were necessary first steps to effective conservation of unique mountain lake ecosystems and can help establish eradication priorities (Adams et al., 2001; Miró and Ventura, 2013), particularly in locations where downstream dispersal could have additional ecological impacts to lotic ecosystems.

Fishing is a popular recreational activity in national parks even while conflicting with other park goals related to preserving native species biodiversity and ecosystem function. Our surveys clarify the current extent of non-native fish and amphibian distributions in OLYM mountain lakes where fish were not historically present. To develop management strategies to minimize the impacts of introduced fish while maintaining recreational opportunity, next steps could include classifying sampled lakes into categories based on relative risks to native fauna (e.g., lakes with and without outlets) and visitor usage (e.g., relative fishing popularity). Given adequate adjacent source populations, amphibian communities in high mountain lakes can rapidly recover following removal of nonnative fish (Vredenburg, 2004; Knapp et al., 2007), so our work can help with prioritizing fish removal in targeted areas that address simultaneous goals of restoring native amphibian communities while still providing fishing opportunity in lakes that remain. Other national parks in the U.S. and abroad have taken similar approaches to the issue of non-native fish in alpine lakes (Chiapella et al., 2018; Tiberti et al., 2021).

Our study showed the advantages of using eDNA as a tool for surveying remote wilderness lakes, revealing current distribution patterns of non-native fish and native amphibians. The non-invasive water sampling, highly sensitive detection, logistically efficient and relatively safe field work compared to traditional methods allowed us to conduct a broad survey across a large national park, much of it in remote and rugged wilderness terrain (Rees et al., 2014; Thompson and Willerslev, 2015). We were able to survey many more lakes than would have been possible using traditional techniques with similar levels of funding and personnel, allowing us to meet our goal of understanding the implications of past management actions in the face of modern conservation needs.

The results of this study have implications far beyond the park's borders. Our combination of historical records with contemporary studies provides a blueprint for other land managers grappling with the legacy of past fish introductions relative to current distributions of nonnative fish. Our demonstration of the utility of eDNA sampling in remote environments can be replicated in other areas of interest in the mountains of the western United States and beyond, both to assess current species' distributions and to quantify the success of management actions such as targeted fish removal (Kamoroff and Goldberg, 2018). Our eDNA results add empirical information about where and how fish and amphibians persist in these landscapes, providing a deeper understanding of putative drivers of occupancy while highlighting opportunities for management and further investigation, such as integrating elements of microhabitat and landscape context to these results. Together, our work informs ongoing efforts to address the legacy of fish introductions while working towards common stewardship goals.

Data availability statement

The datasets presented in this study can be found online at Brenkman et al. (2025).

Ethics statement

Ethical approval was not required for the studies involving animals in accordance with the local legislation and institutional requirements. The research involved Environmental DNA sampling of water.

Author contributions

SB: Conceptualization, Supervision, Investigation, Writing – review & editing, Data curation, Writing – original draft, Project administration, Funding acquisition. JD: Writing – original draft, Formal analysis, Project administration, Visualization, Methodology, Writing – review & editing, Data curation, Investigation, Conceptualization. RM: Investigation, Writing –

original draft, Writing – review & editing, Formal analysis. KK: Writing – original draft, Writing – review & editing, Investigation, Data curation. MH: Writing – original draft, Investigation, Data curation, Writing – review & editing. TK: Investigation, Writing – original draft, Writing – review & editing. WB: Investigation, Writing – review & editing, Writing – original draft. CG: Writing – review & editing, Investigation, Resources, Validation. CO: Writing – review & editing, Investigation, Conceptualization, Supervision. SF: Writing – review & editing, Conceptualization, Supervision, Investigation, Formal analysis, Funding acquisition, Methodology, Visualization, Writing – original draft.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This study was supported by Natural Resource Preservation Program and Inventory and Monitoring Program; Washington's National Park Fund; and U.S. Geological Survey, Ecosystems Mission Area.

Acknowledgments

We thank the following field crew members for their dedication to sampling remote, mountain lakes: Kurt Anderson, Amanda Bacon, Kate Baustain, Abraham Brenkman, Sam Carter, Mary Commins, Heidi Connor, Pat Crain, Mike Danisiewicz, Keelan Dann, Josh Geffre, Eric Guzman, Emma Froehlich, Gay Hunter, Miren Jayo, Dylan Keel, Crystal Kersey, Philip Kennedy, Martin Knowles, Mallory Mintz, Elizabeth Rieger, Paul Seyler, Carter Urnes, and Rebecca Wanagel. Special thanks to Brian Curtis from Washington Trail Blazers for sharing fish planting records and to Gordon Malarkey (deceased) for compiling Olympic National Park (OLYM) archived fish planting records. Thanks to Roger Hoffman and Natasha Antonova for their assistance with GIS and to John Meyer and Rich Olson (deceased) for curating planting records during their tenure at OLYM. We appreciate internal and journal reviewers for their comments on an earlier version of this manuscript.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Adams, S. B., Frissell, C. A., and Rieman, B. E. (2001). Geography of invasion in mountain streams: Consequences of headwater lake fish introductions. *Ecosystems* 4, 296–307. doi: 10.1007/s10021-001-0012-5
- Bahls, P. (1992). The status of fish populations and management of high mountain lakes in the western United States. *Northwest Sci.* 66, 183–193.
- Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., and Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environ. Sci. Technol.* 48, 1819–1827. doi: 10.1021/es404734p
- Behnke, R. J. (2002). *Trout and Salmon of North America* (New York, NY: The Free Press, Simon and Schuster, Inc.).
- Beng, K. C., and Corlett, R. T. (2020). Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. *Biodivers. Conserv.* 29, 2089–2121. doi: 10.1007/s10531-020-01980-0
- Brenkman, S. J., Duda, J. J., Fradkin, S. C., McCaffery, R., Kierczynski, K., Hoy, M. S., et al. (2025). Environmental DNA survey dataset for fish and amphibians in mountain lakes of Olympic National Park. U.S. Geological Survey Data Release. doi: 10.5066/P13X8MSW
- Brenkman, S. J., Duda, J. J., Kennedy, P. R., and Baker, B. M. (2014). A legacy of divergent fishery management regimes and the resilience of rainbow and cutthroat trout populations in Lake Crescent, Olympic National Park, Washington. *Northwest Sci.* 88, 280–304. doi: 10.3955/046.088.0404
- Briggs, J. L. (1987). Breeding biology of the cascade frog, *Rana cascadae*, with comparisons to *R. aurora* and *R. pretiosa*. *Copeia* 1987, 241. doi: 10.2307/1446066
- Carlisle, D. M., and Hawkins, C. P. (1998). Relationships between invertebrate assemblage structure, 2 Trout Species, and habitat structure in Utah mountain lakes. *J. N. Am. Benthol. Soc.* 17, 286–300. doi: 10.2307/1468332
- Chiapella, A. M., Nielsen-Pincus, M., and Strecker, A. L. (2018). Public perceptions of mountain lake fisheries management in national parks. *J. Environ. Manage.* 226, 169–179. doi: 10.1016/j.jenvman.2018.08.040
- Courtenay, W. R., and Fuller, P. F. (2004). Under water and out of sight: Invasive fishes in the United States, implications for National Parks. *Park Sci.* 22, 42–45.
- Crawford, S. S., and Muir, A. M. (2008). Global introductions of salmon and trout in the genus *Oncorhynchus*: 1870–2007. *Rev. Fish Biol. Fisheries* 18, 313–344. doi: 10.1007/s11160-007-9079-1
- Darling, J. A., Jerde, C. L., and Sepulveda, A. J. (2021). What do you mean by false positive? *Environ. DNA* 3, 879–883. doi: 10.1002/edn3.194
- Davey, C. A., Redmond, K. T., and Simeral, D. B. (2006). *Weather and climate inventory, National Park Service, North Coast and Cascades Network. Natural resource technical report NPS/NCCN/NRTR-2006/010, Fort Collins, CO: national park service.* Available online at: <https://www.npshistory.com/publications/climate-change/nrtr-2006-010.pdf> (Accessed September 2, 2025).
- Davis, M. N., McMahon, T. E., Webb, M. A. H., Ilgen, J. E., Hitch, A. T., Jaeger, M. E., et al. (2019). Winter survival, habitat use, and hypoxia tolerance of Montana Arctic grayling in a winterkill-prone lake. *Trans. Am. Fish. Soc.* 148, 843–856. doi: 10.1002/tafs.10176
- Duda, J. J., Hoy, M. S., Chase, D. M., Pess, G. R., Brenkman, S. J., McHenry, M. M., et al. (2021). Environmental DNA is an effective tool to track recolonizing migratory fish following large-scale dam removal. *Environ. DNA* 3, 121–141. doi: 10.1002/edn3.134
- Dunham, J. B., Pilliod, D. S., and Young, M. K. (2004). Assessing the consequences of nonnative trout in headwater ecosystems in western North America. *Fisheries* 29, 18–26. doi: 10.1577/1548-8446(2004)29%5B18:ATCONT%5D2.0.CO;2
- Eby, L., Roach, W., Crowder, L., and Stanford, J. (2006). Effects of stocking-up freshwater food webs. *Trends Ecol. Evol.* 21, 576–584. doi: 10.1016/j.tree.2006.06.016
- Epanchin, P. N., Knapp, R. A., and Lawler, S. P. (2010). Nonnative trout impact an alpine-nesting bird by altering aquatic-insect subsidies. *Ecology* 91, 2406–2415. doi: 10.1890/09-1974.1
- Fiske, I., and Chandler, R. (2011). unmarked: An R package for fitting hierarchical models of wildlife occurrence and abundance. *J. Stat. Soft.* 43, 1–23. doi: 10.18637/jss.v043.i10
- Franklin, T. W., Dysthe, J. C., Golden, M., McKelvey, K. S., Hossack, B. R., Carim, K. J., et al. (2018). Inferring presence of the western toad (*Anaxyrus boreas*) species complex using environmental DNA. *Global Ecol. Cons* 15, e00438. doi: 10.1016/j.gecco.2018.e00438
- Fuller, P. L., Nico, L. G., and Williams, J. D. (1999). *Nonindigenous fishes introduced into inland waters of the United States*. (Bethesda, Md: American Fisheries Society).
- Garlick, L. R. (1950). The helicopter in fish-planting operations in Olympic National Park. *Progressive Fish-Culturist* 12, 72–76. doi: 10.1577/1548-8640(1950)12%5B72:THIFOI%5D2.0.CO;2
- Garwood, J. M., and Welsh, H. H. (2007). *Ecology of the Cascades frog (Rana cascadae) and interactions with garter snakes and nonnative trout in the Trinity Alps Wilderness* (California. Arcata, CA: USDA Forest Service). Available online at: <https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=3823&inline> (Accessed September 2, 2025).
- Glesne, R. S., Fradkin, S. C., Samora, B. A., Boetsch, J. R., Holmes, R. E., and Christoe, C. (2012). "Protocol for long-term monitoring of mountain lakes in the North Coast and Cascades Network: Version July 9, 2012," (Fort Collins, CO: National Park Service). Available online at: <https://www.govinfo.gov/content/pkg/GOVPUB-I29-PURL-gpo138380/pdf/GOVPUB-I29-PURL-gpo138380.pdf> (Accessed September 2, 2025).
- Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., et al. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods Ecol. Evol.* 7, 1299–1307. doi: 10.1111/2041-210X.12595
- Gresswell, R. E. (1995). "Yellowstone cutthroat trout," in *Conservation assessment for inland cutthroat trout*, vol. 61, 36–54. (U.S. Department of Agriculture, Fort Collins, CO).
- Hagen, H. K. (1961). *An inceptive study of the distribution and relative condition of the endemic and exotic fishes of several selected areas in Olympic National Park* (Port Angeles, WA: National Park Service).
- Hague, M. T. J., Avila, L. A., Hanifin, C. T., Snedden, W. A., Stokes, A. N., Brodie, E. D., et al. (2016). Toxicity and population structure of the Rough-Skinned Newt (*Taricha granulosa*) outside the range of an arms race with resistant predators. *Ecol. Evol.* 6, 2714–2724. doi: 10.1002/ece3.2068
- Hartman, R., Pope, K., and Lawler, S. (2014). Factors mediating co-occurrence of an economically valuable introduced fish and its native frog prey. *Conserv. Biol.* 28, 763–772. doi: 10.1111/cobi.12218
- Hering, D., Borja, A., Jones, J. I., Pont, D., Boets, P., Bouchez, A., et al. (2018). Implementation options for DNA-based identification into ecological status assessment under the European Water Framework Directive. *Water Res.* 138, 192–205. doi: 10.1016/j.watres.2018.03.003

Author disclaimer

This article has been peer reviewed and approved for publication consistent with USGS Fundamental Science Practices (<https://pubs.usgs.gov/circ/1367/>). Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcsc.2025.1698619/full#supplementary-material>

- Hoffman, R. L., Larson, G. L., and Brookes, B. J. (2003). Habitat segregation of *Ambystoma gracile* and *Ambystoma macrodactylum* in mountain ponds and lakes, Mount Rainier National Park, Washington, USA. *J. Herpetol.* 37, 24–34. doi: 10.1670/0022-1511(2003)037%5B0024:HSOAGA%5D2.0.CO;2
- Houston, D. B., and Schreiner, E. G. (1995). Alien species in National Parks: Drawing lines in space and time. *Conserv. Biol.* 9, 204–209. doi: 10.1046/j.1523-1739.1995.09010204.x
- Hoy, M. S., and Ostberg, C. O. (2023). Development of a quantitative PCR assay for detecting northwest salamander (*Ambystoma gracile*) in environmental DNA samples. *Conserv. Genet. Resour.* 15, 109–112. doi: 10.1007/s12686-023-01308-4
- Johnson, B. M., Arlinghaus, R., and Martinez, P. J. (2009). Are we doing all we can to stem the tide of illegal fish stocking? *Fisheries* 34, 389–394. doi: 10.1577/1548-8446-34.8.389
- Kamoroff, C., and Goldberg, C. S. (2018). An issue of life or death: using eDNA to detect viable individuals in wilderness restoration. *Freshw. Sci.* 37, 685–696. doi: 10.1086/699203
- Kats, L. B., and Ferrer, R. P. (2003). Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Divers. Distrib.* 9, 99–110. doi: 10.1046/j.1472-4642.2003.00013.x
- Kats, L. B., Petranka, J. W., and Sih, A. (1988). Antipredator defenses and the persistence of amphibian larvae with fishes. *Ecology* 69, 1865–1870. doi: 10.2307/1941163
- Kelly, R. P., Lodge, D. M., Lee, K. N., Theroux, S., Sepulveda, A. J., Scholin, C. A., et al. (2024). Toward a national eDNA strategy for the United States. *Environ. DNA* 6, e432. doi: 10.1002/edn3.432
- Kenison, E. K., Litt, A. R., Pilliod, D. S., and McMahon, T. E. (2016). Larval long-toed salamanders incur nonconsumptive effects in the presence of nonnative trout. *Ecosphere* 7, e01258. doi: 10.1002/ecs2.1258
- Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., et al. (2020). Reporting the limits of detection and quantification for environmental DNA assays. *Environ. DNA* 2, 271–282. doi: 10.1002/edn3.29
- Knapp, R. A., Boiano, D. M., and Vredenburg, V. T. (2007). Removal of nonnative fish results in population expansion of a declining amphibian (mountain yellow-legged frog, *Rana muscosa*). *Biol. Conserv.* 135, 11–20. doi: 10.1016/j.biocon.2006.09.013
- Knapp, R. A., Corn, P. S., and Schindler, D. E. (2001). The introduction of nonnative fish into wilderness lakes: Good intentions, conflicting mandates, and unintended consequences. *Ecosystems* 4, 275–278. doi: 10.1007/s10021-001-0009-0
- Knapp, R. A., and Matthews, K. R. (2000). Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. *Conserv. Biol.* 14, 428–438. doi: 10.1046/j.1523-1739.2000.99099.x
- Kulp, M. A., and Moore, S. E. (2005). A case history in fishing regulations in Great Smoky Mountains National Park: 1934–2004. *N. Am. J. Fish. Manage.* 25, 510–524. doi: 10.1577/M03-254.1
- Lahoz-Monfort, J. J., Guillera-Arroita, G., and Tingley, R. (2016). Statistical approaches to account for false-positive errors in environmental DNA samples. *Mol. Ecol. Resour.* 16, 673–685. doi: 10.1111/1755-0998.12486
- Laramie, M. B., Pilliod, D. S., and Goldberg, C. S. (2015). Characterizing the distribution of an endangered salmonid using environmental DNA analysis. *Biol. Conserv.* 183, 29–37. doi: 10.1016/j.biocon.2014.11.025
- Larson, G. L., and Hoffman, R. L. (2002). Abundances of northwestern salamander larvae in Montane lakes with and without fish, Mount Rainier National Park, Washington. *Northwest Sci.* 76, 35–40.
- Leavitt, P. R., Schindler, D. E., Paul, A. J., Hardie, A. K., and Schindler, D. W. (1994). Fossil pigment records of phytoplankton in trout-stocked alpine lakes. *Can. J. Fish. Aquat. Sci.* 51, 2411–2423. doi: 10.1139/f94-241
- Liss, W. J., and Larson, G. L. (1991). Ecological effects of stocked trout on North Cascades naturally fishless lakes. *Park Sci.* 11, 22–23.
- Love Stowell, S. M., Kennedy, C. M., Beals, S. C., Metcalf, J. L., and Martin, A. P. (2015). The genetic legacy of more than a century of stocking trout: a case study in Rocky Mountain National Park, Colorado, USA. *Can. J. Fish. Aquat. Sci.* 72, 1565–1574. doi: 10.1139/cjfas-2015-0009
- Lowe, S., Browne, M., Boudjelas, S., and De Poorter, M. (2000). 100 of the world's worst invasive alien species: a selection from the global invasive species database. (Auckland, New Zealand: ISSG). 12pp
- Mathias, J. A., and Barica, J. (1980). Factors controlling oxygen depletion in ice-covered lakes. *Can. J. Fish. Aquat. Sci.* 37, 185–194. doi: 10.1139/f80-024
- Mathieu, C., Hermans, S. M., Lear, G., Buckley, T. R., Lee, K. C., and Buckley, H. L. (2020). A systematic review of sources of variability and uncertainty in eDNA data for environmental monitoring. *Front. Ecol. Evol.* 8, doi: 10.3389/fevo.2020.00135
- Mayne, S. R., Manning, J. A., Henderson, S. M., Parsley, M. B., Strickler, K. M., Nielson, J. R., et al. (2024). Hydrodynamics and aquatic vegetation drive spatial patterns of environmental DNA in ponds. *Environ. DNA* 6, e70036. doi: 10.1002/edn3.70036
- McIntyre, J. D., and Rieman, B. E. (1995). "Westslope cutthroat trout." in *Conservation Assessment for Inland Cutthroat Trout*, M. K., Young (ed), (General Technical Report RM-256. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. p. 1–15.), 61.
- Metcalf, J. L., Love Stowell, S., Kennedy, C. M., Rogers, K. B., McDonald, D., Epp, J., et al. (2012). Historical stocking data and 19th century DNA reveal human-induced changes to native diversity and distribution of cutthroat trout. *Mol. Ecol.* 21, 5194–5207. doi: 10.1111/mec.12028
- Miró, A., and Ventura, M. (2013). Historical use, fishing management and lake characteristics explain the presence of non-native trout in Pyrenean lakes: Implications for conservation. *Biol. Conserv.* 167, 17–24. doi: 10.1016/j.biocon.2013.07.016
- Moyle, P. B. (1986). The Frankenstein effect: impact of introduced fishes on native fishes in North America. *Fish. Culture Fisheries Manage.* 1986, 415–426.
- National Park Service (2006). *Management policies* 2006. (Washington, D.C.: U.S. Government Printing Office).
- Olson, R. W., and Meyer, J. H. (1994). *Survey of non-native fish in selected interior lakes of olympic national park* 1992–1994. (Port Angeles, WA: National Park Service).
- Panek, F. M. (1994). Recreational fishing in the national parks: Why is there a question? *Fisheries* 19, 6–7.
- Pearson, K. J., and Goater, C. P. (2009). Effects of predaceous and nonpredaceous introduced fish on the survival, growth, and antipredation behaviours of long-toed salamanders. *Can. J. Zool.* 87, 948–955. doi: 10.1139/Z09-084
- Pilliod, D. S., Hossack, B. R., Bahl, P. F., Bull, E. L., Corn, P. S., Hokit, G., et al. (2010). Non-native salmonids affect amphibian occupancy at multiple spatial scales. *Divers. Distrib.* 16, 959–974. doi: 10.1111/j.1472-4642.2010.00699.x
- Pilliod, D. S., and Peterson, C. R. (2001). Local and landscape effects of introduced trout on amphibians in historically fishless watersheds. *Ecosystems* 4, 322–333. doi: 10.1007/s10021-001-0014-3
- Pister, E. P. (2001). Wilderness fish stocking: History and perspective. *Ecosystems* 4, 279–286. doi: 10.1007/s10021-001-0010-7
- Pope, K. L., Goldberg, C. S., Nelson, N. L., Cummings, A. K., Seaborn, T., and Piovato-Scott, J. (2020). Designing environmental DNA surveys in complex aquatic systems: Backpack sampling for rare amphibians in Sierra Nevada meadows. *Aquat. Conserv.* 30, 1975–1987. doi: 10.1002/aqc.3444
- Rahel, F. J. (2004). Unauthorized fish introductions: Fisheries management of the people, for the people, or by the people? *Am. Fisheries Soc. Symposium* 44, 431–443.
- Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., and Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA – a review of eDNA as a survey tool in ecology. *J. Appl. Ecol.* 51, 1450–1459. doi: 10.1111/1365-2664.12306
- Roussel, J.-M., Paillisson, J.-M., Tréguier, A., and Petit, E. (2015). The downside of eDNA as a survey tool in water bodies. *J. Appl. Ecol.* 52, 823–826. Available online at: <https://www.jstor.org/stable/43869253> (Accessed July 24, 2025).
- Ryan, M. E., Palen, W. J., Adams, M. J., and Rochefort, R. M. (2014). Amphibians in the climate vise: Loss and restoration of resilience of montane wetland ecosystems in the western US. *Front. Ecol. Environ.* 12, 232–240. doi: 10.1890/130145
- Schindler, D. E., Knapp, R. A., and Leavitt, P. R. (2001). Alteration of nutrient cycles and algal production resulting from fish introductions into mountain lakes. *Ecosystems* 4, 308–321. doi: 10.1007/s10021-001-0013-4
- Sellers, R. W. (2009). *Preserving nature in the national parks: A history* (New Haven, CT: Yale University Press).
- Shu, L., Ludwig, A., and Peng, Z. (2020). Standards for methods utilizing environmental DNA for detection of fish species. *Genes* 11, 296. doi: 10.3390/genes11030296
- Stoddard, J. L. (1987). Microcrustacean communities of high-elevation lakes in the Sierra Nevada, California. *J. Plankton Res.* 9, 631–650. doi: 10.1093/plankt/9.4.631
- Stratton, C., Sepulveda, A. J., and Hoegh, A. (2020). msocc: Fit and analyse computationally efficient multi-scale occupancy models in R. *Methods Ecol. Evol.* 11, 1113–1120. doi: 10.1111/2041-210X.13442
- Thompson, P. F., and Willerslev, E. (2015). Environmental DNA - An emerging tool in conservation for monitoring past and present biodiversity. *Biol. Conserv.* 183, 4–18.
- Tiberti, R., Buchaca, T., Boiano, D., Knapp, R. A., Pou Rovira, Q., Tavecchia, G., et al. (2021). Alien fish eradication from high mountain lakes by multiple removal methods: Estimating residual abundance and eradication probability in open populations. *J. Appl. Ecol.* 58, 1055–1068. doi: 10.1111/1365-2664.13857
- Trotter, P. C. (1989). Coastal cutthroat trout: a life-history compendium. *Transactions of the American Fisheries Society*. 118, 463–473. doi: 10.1577/1548-8659(1989)118%3C0463:CCTALH%3E2.3.CO;2
- Tyler, T., Liss, W. J., Ganio, L. M., Larson, G. L., Hoffman, R., Deimling, E., et al. (1998). Interaction between introduced trout and larval Salamanders (*Ambystoma macrodactylum*) in high-elevation lakes. *Conserv. Biol.* 12, 94–105. doi: 10.1111/j.1523-1739.1998.96274.x
- Vredenburg, V. T. (2004). Reversing introduced species effects: Experimental removal of introduced fish leads to rapid recovery of a declining frog. *Proc. Natl. Acad. Sci. U.S.A.* 101, 7646–7650. doi: 10.1073/pnas.0402321101
- Wallis, O. L. (1960). Management of sport fishing in national parks. *Trans. Am. Fish. Soc.* 89, 234–238. doi: 10.1577/1548-8659(1960)89%5B234:MOSFIN%5D2.0.CO;2
- Welsh, H. H., Pope, K. L., and Boiano, D. (2006). Sub-alpine amphibian distributions related to species palatability to non-native salmonids in the Klamath mountains of northern California. *Divers. Distrib.* 12, 298–309. doi: 10.1111/j.1366-9516.2006.00254.x
- Wilcox, T. M., Carim, K. J., McKelvey, K. S., Young, M. K., and Schwartz, M. K. (2015). The dual challenges of generality and specificity when developing environmental DNA markers for species and subspecies of *Oncorhynchus*. *PloS One* 10, e0142008. doi: 10.1371/journal.pone.0142008