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ORIGINAL ARTICLE

High-frequency and long-term observations of eDNA from imperiled salmonids in a coastal stream: Temporal dynamics, relationships with environmental factors, and comparisons with conventional observations

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Abstract

A greater understanding of eDNA behavior in the environment is needed before it can be employed for ecosystem monitoring applications. The objectives of this study were to use autonomous sampling to conduct long-term, high-frequency monitoring of the eDNA of native salmonid species in a Californian coastal stream, describe temporal variation of eDNA on multiple scales and identify environmental factors that drive this variation, and evaluate the ability of the eDNA datasets to detect rare species and represent organismal abundance. Using high-throughput autonomous environmental sample processors (ESPs) and qPCR, we enumerated eDNA concentrations from 674 water samples collected at subdaily intervals over 360 days at a single site. We detected eDNA from two imperiled salmonids (coho salmon *Oncorhynchus kisutch* and steelhead/rainbow trout *O. mykiss*) in most samples; *O. kisutch* eDNA was generally in lower concentration and more variable than *O. mykiss* eDNA. High-frequency (i.e., subdaily and daily) variability in salmonid eDNA concentrations showed occasional patchiness (i.e., large differences between consecutive samples), while seasonal differences were observed consistent with the ecology of the species at this site. Salmonid eDNA concentrations were significantly associated with creek discharge, photoperiod, and whether the creek mouth was open or closed by a seasonal sandbar. The release of hatchery-origin *O. kisutch* parr into the stream was associated with a significant increase in eDNA concentration for the remainder of the study. We compared eDNA signals with fish abundance data collected from traps located at the site. Fish were detected more often by eDNA than from trapping. Significant positive associations between fish abundance and eDNA concentrations were observed for *O. mykiss*; however, no such associations were observed for *O. kisutch*. This study adds to our knowledge on the occurrence and behavior of fish eDNA in lotic systems and informs future biomonitoring efforts using automated sampling technology.

KEYWORDS
abundance, autonomous sampling, environmental DNA, fish ecology

1 | INTRODUCTION

In riverine ecosystems, traditional fisheries monitoring methods such as trapping, snorkel surveys, or electrofishing involve the direct collection or observation of organisms; however, such methods can be cumbersome and costly to implement (Levi et al., 2019), require taxonomic expertise, and disrupt or harm the organisms being monitored (Thomsen & Willerslev, 2015). Environmental DNA (eDNA) methods, which involve analyzing water samples for genetic material liberated by a target species without direct handling or observation of the organisms (Kelly et al., 2014; Lodge et al., 2012), have shown promise in alleviating some of these issues. Monitoring eDNA has been used to detect invasive species (Dejean et al., 2012; Sepulveda et al., 2020), monitor species of conservation concern (Deiner et al., 2021; Mizumoto et al., 2020), identify the presence of biological pollutants (Smith et al., 2015; Yamahara et al., 2015), and characterize the biodiversity of various marine and freshwater ecosystems (Ruppert et al., 2019).

Because eDNA monitoring does not provide direct observations of organisms, it is essential to calibrate eDNA measurements to the presence, abundance, and location of organisms in the environment. There have been many attempts to relate eDNA concentrations with organism abundance (Rourke et al., 2021), but the results of those studies are equivocal. While many studies have shown empirically that eDNA concentrations in water samples correlate positively with more traditional measures of organism abundance (Doi et al., 2017; Pilliod et al., 2013; Shelton et al., 2019; Takahara et al., 2012; Tillotson et al., 2018), some indicate no correlation (Capo et al., 2019; Hinlo et al., 2018; Hongo et al., 2021; Perez et al., 2017). The discrepant results between different studies could be attributable to variable source strength (i.e., abundance or biomass) of the target organism, as well as differences in the fate and transport of eDNA in different systems. This is partially supported by the finding that abiotic and biotic environmental factors can modify the association between measured eDNA and organism abundance (Harrison et al., 2019; Lacoursière-Roussel et al., 2016).

To realize the full potential of eDNA for ecosystem monitoring, there is a need to better understand eDNA ecology, or how eDNA concentration is controlled in space and time by environmental processes (Barnes & Turner, 2016). While studies have addressed questions related to the discrete shedding, transport, and fate mechanisms that affect eDNA concentrations in the environment (Allan et al., 2021; Sassoubre et al., 2016; Shogren et al., 2017; Stewart, 2019), these are typically done using mesocosm studies or modeling approaches. Observations of eDNA behavior in the field in the presence of varying organism abundances and behavior and changing environmental conditions (which can occur over multiple temporal and spatial scales) are needed to better understand the relationship between organisms and the eDNA they shed.

Robotic sampling instrumentation presents an opportunity to overcome some logistical limitations for ecosystem monitoring over time. The environmental sample processor (ESP) is a robotic instrument designed to automate water sample filtration and filter preservation, and (if desired) process samples for in situ analyses (Scholin et al., 2017). Sampling with ESPs can yield eDNA datasets much longer and with higher temporal resolution than traditional manual sampling could produce. ESPs can be programmed to sample at distinct times without a human operator, and collection times and target volumes can be altered at-will via wireless communication. Thus, eDNA data can be acquired at times that are logistically or physically challenging for a human to do so, such as during weekends and holidays, odd hours of the day, or during poor weather or high-stream discharge regimes. Recent work has examined the efficacy of using ESPs to sample eDNA at high frequencies for studying fish ecology. Hansen et al. (2020) used the instrument in a large mesocosm to accumulate a daily fish eDNA time series over the course of 51 days. Sepulveda et al. (2020) found that eDNA sampling performed by an ESP every 3 hours provided stronger evidence than weekly, manual grab sampling for pathogen and fish detection in a mountain stream. However, ESPs have yet to be used to explore eDNA dynamics in the field over multiple temporal scales.

The objective of the present study was to use autonomous sampling methods afforded by the ESP to assess the dynamics of eDNA shed from anadromous fish in the field. Since anadromous fish exhibit behaviors that are both episodic and seasonal in nature (e.g., spawning and migration), their study warrants both the high-frequency and long-term observation enabled by autonomous methods. There is precedent for using high-frequency eDNA data to inform migratory fish ecology (Levi et al., 2019; Thalinger et al., 2019; Yatsuyanagi et al., 2019). For example, Levi et al. (2019) determined that at-least daily eDNA sampling was required to capture the ephemeral migration and spawning of anadromous salmonids in an Alaskan stream. Long-term (i.e., on the order of seasons to years) observations of fish assemblage patterns conducted using traditional methods are commonly used to inform conservation efforts and determine how species respond to chronic threats such as climate change (Adams et al., 2011; Quinn, 2018), yet long-term eDNA studies are few and those that exist are often conducted at the expense of sampling interval (Doi et al., 2017; Hongo et al., 2021; Pilliod et al., 2019).

Here, we used ESPs to perform high-frequency (on the order of subdaily to daily) water sampling over an entire year in a coastal California stream and subsequently quantified eDNA of native, imperiled salmonids. We assessed temporal variation in the eDNA signals over multiple time scales, examined the association between eDNA and specific environmental factors, and evaluated the utility of eDNA for detecting rare species and representing organismal

abundance by comparing the signals with fish count data collected from traps collocated at the study site.

2 | MATERIALS AND METHODS

2.1 | Field site

The study was conducted in Scott Creek (37°03′03.6″ N, 122°13′37.2″ W), a coastal stream located near Santa Cruz, California, USA (Figure 1). The region is characterized by a Mediterranean climate with a distinct wet period between November and April, followed by a protracted dry period with little to no precipitation. Mean annual rainfall in the Scott Creek watershed is ~100 cm/year (Bond et al., 2019). The catchment has a drainage area of 78 km^2 and contains approximately 23 km of streams that are accessible to anadromous fish. Typical of most small coastal California watersheds, a seasonal sandbar (barrier beach) forms across the mouth of Scott Creek during the dry season when stream discharge is low, precluding movement of fish between marine and freshwater environments. During our study, the creek mouth closed on September 5, 2019, and the sandbar persisted until breached by high-stream flows on December 4, 2019 (90 days).

The Scott Creek watershed supports populations of coho salmon (*Oncorhynchus kisutch*) and steelhead/rainbow trout (*O. mykiss*), which are listed under the US Endangered Species Act (ESA) as endangered and threatened species, respectively. The seasonal lagoon provides feeding habitat and refuge from predators for the oversummer rearing of salmonids (Hayes et al., 2008). When water quality degrades in the lagoon, coho and steelhead have been reported to retreat back upstream to the lower reaches of Scott Creek (Osterback et al., 2018).

The National Oceanic and Atmospheric Administration (NOAA) and University of California Santa Cruz operate a salmonid life cycle monitoring station (Adams et al., 2011) in lower mainstem Scott Creek (~1.5 km upstream from the Pacific Ocean), which includes a weir trap to intercept and count upstream migrating adults and a smolt trap (100 m upstream from the weir) to count downstream-migrating juveniles. A small conservation hatchery located ~3 km upstream from the weir on one of the Scott Creek tributaries (Figure 1) periodically releases juvenile *O. kisutch* into the watershed. Nevertheless, *O. mykiss* is the dominant salmonid species in the watershed (Osterback et al., 2018), in terms of both abundance and biomass.

2.2 | ESP sampling and processing

Between March 25, 2019, and April 4, 2020 (377 days), ESPs were used to autonomously collect and preserve eDNA water samples (Scholin et al., 2017). Instruments were located adjacent to the weir trap and were programmed to collect water samples at a frequency of one to three times per day. ESP sample collection and preservation methods followed those described by Yamahara et al. (2015) and Sepulveda et al. (2020). Briefly, creek water was pumped to an external sampling module from which eDNA water samples were drawn. The stream water sampler consisted of a submersible pump (WSP-12V-4 Waterra USA Inc., Bellingham, WA, USA) installed ~2 m downstream of the weir trap at 0.15 m above the creek bottom, pressurizing the self-draining 1.1-L sampling module tank to 10 psi. Prior to the collection of ESP water samples, the pump and sampling module were flushed for 15 min with creek water to reduce the potential for carry-over contamination. To reduce the potential for contamination within the ESP, the ESP sampling path was filled with 10% bleach after the completion of sampling. Bleach was flushed from the sampling path using a solution of 0.1% tween-20 prior to the initiation of each sample collection event. ESP eDNA samples were collected on 25 mm, 0.22-μm pore-size, mixed cellulose ester filters (GSWP02500, MilliporeSigma, Burlington, MA, USA). Sampling would cease after 2 L passed through the filter or when the flow rate with a 22 PSI pressure gradient across it reduced to below 0.05 ml/second; the volume of water filtered was recorded by the instrument. After filtration, eDNA filters were preserved within the ESP using RNAlater (Invitrogen, Carlsbad, CA, USA) (Yamahara et al., 2015, 2019).

Prior to and after each instrument deployment, 4L of MilliQ water was connected to the ESP intake via a Flexboy 2D Bag

FIGURE 1 Map of study site. Images show the ESP housing (top) and intake collocated at the weir trap (bottom)

(Sartorius, Bohemia, NY, USA), and three 1-L MilliQ water samples were collected as negative instrument controls (field blanks). At the end of each deployment, filters were aseptically removed from the ESP, transferred to 2-ml screw-cap microcentrifuge tubes containing DNA extraction beads, and stored at −80°C until DNA extractions were performed (within 6 months). ESP metadata included unique sample ID, date and time of sampling start and finish, sampling duration, and total sample volume (Table S1). Due to sampling capacity (126 field samples per instrument), ESP instruments were routinely exchanged serially throughout the duration of the experiment; three unique ESPs were deployed. There were 18 days during the duration of the study when samples were not collected due to ESP malfunction or maintenance. On one of those days (February 11, 2020), a 10-L sample was collected manually and triplicate 1-L subsamples were processed in the laboratory with vacuum filtration. A total of 13 samples were omitted from the dataset due to low (<25 ml) total sample volume. A total of 674 field samples (including 673 ESP filters and 1 manually collected filter) and 48 negative field blanks were processed.

2.3 | Molecular analyses

To reduce the possibility of contamination, all DNA extractions were conducted in a separate purpose-designated laboratory. Total DNA was extracted from filters using a modified Dneasy Blood and Tissue (Qiagen, Germantown, MD, USA) protocol (Thomsen et al., 2012). DNA was eluted in two 50-µl fractions, for a total of 100 µl. DNA extracts were aliquoted into three ~33-µl volumes and stored at −80°C until used as template in qPCR reactions (within 4 months). On each day an extraction was performed, a negative extraction blank control utilizing a sterile filter was carried out through the entire extraction process ($N = 32$). DNA concentrations were measured in the extracts using NanoDrop One Spectrophotometer (ThermoScientific, Waltham, MA, USA) and ranged from 0.63 to 104.9 ng/µl (average = 21.9 ± 1.1 ng/µl (95% CI)).

PCR reactions were prepared in a designated DNA-free PCR hood (UVP, Upland, CA, USA), and subsequent amplifications were performed in a separate isolated laboratory room. DNA from coho salmon (*O. kisutch*) and rainbow trout (*O. mykiss*) were quantified using previously described assays that target mtDNA (Table 1). Reactions consisted of 1X Taqman Environmental Mastermix 2.0 (Life Technologies, Foster City, CA, USA), forward and reverse primers and probe, and 2 µl of template DNA (over the entire extraction process, this is equivalent to 1.5% of the filtered volume) in a final reaction volume of 20 µl. Triplicate reactions were run on a StepOnePlus real-time PCR system (Life Technologies, Foster City, CA, USA) under the following thermal cycling conditions: 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, and 1 min at the appropriate annealing temperature.

PCR inhibition of environmental samples was assessed using dilutions. Both the undiluted and (1:5) diluted DNA extracts were

TABLE 1 Assay specifications and standard curve parameters

Target	Primer/Probe ID	Sequences (5′–3′)	Annealing Temp. (°C)	Conc. (uM)	Slope (Eff. %)	Intercept	R^2	LOD (copies/rxn)	LOQ (copies/rxn)	Threshold	Refs.
O. kisutch (CYTB)	Ok_CYTB_F	CCT TGG TGG CGG ATA TAC TTA TCT TA	60	0.4	−3.46 (94.5%)	40.7	0.98	12	12	0.2	Pilliod and Laramie (2016)
	Ok_CYTB_R	GAA CTAG GAA GAT GGC GAA GTA GAT C		0.4							
	Ok_CYTB_P	6FAM-TGG AAC ACC CAT TCA T-MGBNFQ		0.25							
O. mykiss (NADH)	Omy_NAHD_F	5′-AGTCTCTCCCTG-TATATCGTC–3′	60	0.3	−3.49 (93.3%)	39.8	0.98	21	21	0.2	Wilcox et al. (2015)
	Omy_NAHD_R	5′-GATTTAGTTCATGAAG TTGCGTGAGTA–3′		0.6							
	Omy_NAHD_P	6FAM–5′-CCAACAACTCT-TTAACCATC–3′-MGBNFQ		0.25							

Note: Target organisms (genes) and primer probe identifiers are provided in the first two columns. Assay specific sequences and corresponding PCR chemistries for assay annealing temperature (Annealing Temp., °C) and primer/probe concentrations (Conc., µM), and standard curve results are reported for Slope and Efficiency (Slope – Eff., %), y-intercept (Intercept), R-squared (R^2), limit of detection (LOD), limit of quantification (LOQ), cycle threshold (Threshold) and corresponding assay references are shown in the subsequent columns.

amplified in triplicate. Inhibition was assessed by calculating the ΔC_T between the triplicate mean C_T values of the dilution series of each sample ($\Delta C_T = C_{T(\text{undiluted})} - C_{T(\text{diluted})}$). The theoretical ΔC_T between an undiluted sample and a 1:5 diluted sample is −2.3 cycles. To allow for experimental variability, we declared samples with ΔC_T greater than −1.8 inhibited. We were unable to assess inhibition for samples where one or both of the sample dilutions did not amplify any target species. If inhibition was detected, we used the 1:5 dilution as the representative sample; otherwise, we used the undiluted samples as the representative sample. Inhibition was more common for *O. kisutch* than for *O. mykiss* samples and occurred more frequently in samples collected in the between the months of June and October (Figures S1–S2).

Concentrations of targets in the sample reactions were determined using standard curves (Figure S3). Synthetic DNA gBlock (IDT, Skokie, IL, USA) standard concentrations were measured using a NanoDrop One Spectrophotometer (ThermoScientific, Waltham, MA, USA) at 100-ng/μl concentration before diluting to a working solution of 10^9 copies/μl and stored at −80°C until used. On each plate, a triplicate standard curve was run using a tenfold dilution series from 10^6 to 10^1 copies/reaction as well as 5 copies/reaction. At the onset and end of the qPCR laboratory analyses, a full plate of standards (12 replicates) was also run to establish a master standard curve discussed below. On each plate, triplicate no template control (NTC) reactions were analyzed using PCR grade water as template.

Master standard curves for both targets were calculated using the standards data from all plates in aggregate regardless of plate-specific amplification efficiency. Standard curves were fit using ordinary least-squares regression between C_T values and $\log_{10}$-transformed standard reaction concentration (units of copies/reaction). Level of detection (LOD) and level of quantification (LOQ) were determined using the curve fitting methods outlined by Klymus et al. (2020). See Table 1 for the assay specifications (probes, standard curves, and LOD and LOQ values) for both targets. Information regarding standard curve performance and sample inhibition can be found in Figures S1–S2.

All negative controls including no template controls, negative extraction controls, and negative field controls used throughout the project were negative. Additional details to satisfy EMMI guidelines for reporting (Borchardt et al., 2021) are also provided in the Supporting Information.

Data from replicate wells were combined as follows to calculate eDNA concentrations in water samples. The result from a specific replicate was considered an outlier if its concentration was greater than 10,000 copies/reaction, and the replicate was the only of the sample triplicates to amplify (which occurred in three *O. kisutch* samples). Outliers, unamplified replicates (i.e., replicates where no quantitative amplification occurred within 40 PCR cycles), and amplified replicates with concentrations below the LOQ, were flagged and their concentrations were substituted with a quantity of 0 copies/reaction (Wilcox et al., 2016). The dilution factor was then applied to replicate reaction concentrations when appropriate, and mean sample reaction concentrations (copies/reaction) were calculated by averaging the triplicate concentrations. If none of the sample triplicates amplified, a sample was considered a nondetect (ND) for the target (Wilcox et al., 2016). Samples with mean reaction concentrations below the LOQ (BLOQ) were flagged and replaced with a quantity of 0 copies/reaction. This replacement value was chosen to standardize across samples because different sample volumes were collected throughout the study period. eDNA concentrations (units of copies/ml sampled) were then calculated using dimensional analysis using the sample reaction concentrations, the reaction volumes, and the total sampled volumes.

2.4 | Fish trap, hatchery, and environmental data

Count and biometric data of salmonid individuals were collected regularly at weir and smolt traps co-located with the ESP. Trapping was conducted throughout the adult (December–May) and juvenile/smolt (March–May) migration periods. However, there were periods during the study when traps were offline due to unsafe stream flows or for logistical reasons. In total, fish abundance data were available for 180 out of 377 days of the study period. Trap counts typically began around 10:00 h local time. For each counted fish, mass was either measured directly or estimated using a regression that considered the fish's length, species, and life stage (Figure S4). When a fish's length was not measured, the mean mass of the fish's species and life stage were assigned. Daily fish abundance was calculated by aggregating the day's total fish counts and biomass. The capture and handling of ESA-listed salmonids was authorized by the National Marine Fisheries Service under Section 10(a)(1)(A) permit No. 17292-2A. All fish handling procedures were carried out in accordance with approved protocols from the Institutional Animal Care and Use Committee at University of California Santa Cruz (Protocol No. KIERJ1604_A1).

Hatchery-origin juvenile *O. kisutch* was occasionally released into Scott Creek at various locations in the watershed during the study period (Table S2). While a majority of *O. kisutch* smolt releases occurred primarily downstream of the sampling site, a fraction was released upstream. Over 10,000 *O. kisutch* parr were released over the course of one day (November 21, 2019) in various locations throughout the Scott Creek watershed; unlike smolts, these fish were not expected to immediately migrate downstream (Quinn, 2018). A pilot study (data not shown) suggested that the eDNA signal from the hatchery itself (which is hydraulically connected to Scott Creek) is significantly attenuated before reaching the weir (20-fold reduction, from ~200 copies/ml at the hatchery outflow to ~10 copies/ml at the weir), and a transport study in a nearby watershed suggest similar attenuation of caged *O. kisutch* eDNA at distance of 1 km (Spence et al., 2020).

Environmental parameters were collated from different sources (Figure S5). Water temperature was collected at the weir at 15-min resolution using a Hobo temperature logger (Onset, Bourne, MA, USA) and was averaged by day (between 00:00 and 23:59 Pacific Standard Time (PST)) to obtain a daily mean temperature. The mean

water temperature during the entire experiment was 12.7°C and ranged from 6.8 to 17.6°C. Daily mean air temperature, cumulative precipitation, and mean stream discharge were collected and provided by the Cal Poly Swanton Pacific Ranch. A day was considered a 'wet' day if the total precipitation over the previous 3 days were greater than 10 mm; it was considered a 'dry' day otherwise. Missing days of streamflow data were imputed using a regression on USGS flow gages located in nearby streams in the region. The mean discharge during the experiment was 1.57 m^3/s (range 0.30–15.0 m^3/s). Creek discharge was classified into the following regimes: low (discharge <0.65 m^3/s), medium (0.65 m^3/s < discharge <1.47 m^3/s), and high (≥1.47 m^3/s); categorizations were based on historical discharge measured between 2010 and 2020. We used daily photoperiod (in fraction of a day) to represent the length of each day and as a proxy for season and total daily UV irradiance. Finally, the status of the mouth of Scott Creek (i.e., whether open to the Pacific Ocean or closed by the seasonal sandbar) was recorded each day by NOAA.

2.5 | Data analysis—eDNA signals

Data analysis was performed using the Python programming language. Differences in eDNA concentration by sample groupings were assessed using the Mann–Whitney U (MW-U) and Kruskal–Wallis (KW) tests for unpaired data and the Friedman chi-squared and Wilcoxon rank-sum tests for paired data. Unless otherwise noted, statistical significance was considered when $p < 0.05$.

The temporal dynamics of the eDNA data were examined on multiple time scales. We first assessed whether eDNA concentrations were significantly different by time of day for days in which samples were collected each in the morning (earlier than 1100 PST), midday (1100 to 1700 PST), and evening (later than 1700 PST) periods. High-frequency variation was assessed by examining subdaily and daily variability in eDNA detection frequency and concentration. Subdaily (i.e., within-day) variability was assessed between samples collected less than 12 h apart. The number of samples in which the detection status (i.e., above or below the LOD) differed between two subsequent samples was determined. Subdaily variability in eDNA concentration was determined by calculating the relative difference in concentration between the two samples (defined as $\delta_i = |c_{i+1} - c_i| / c_i$, where c_i and c_{i+1} are the eDNA concentrations of samples i and $i + 1$). Similarly, daily variation was assessed by calculating the relative difference in mean eDNA concentration between consecutive days (defined as $\Delta_i = |C_{i+1} - C_i|/C_i$, where C_i and C_{i+1} are the mean eDNA concentrations of samples collected on days i and $i + 1$). Long-term trends were assessed by categorizing samples by season of the year. Seasons were defined according to the month a sample was collected: spring (March–May), summer (June–August), autumn (September–November), and winter (December–February).

To examine the relationship between eDNA and environmental factors, we first assessed differences in eDNA concentration by grouping samples by stream discharge regime (low, medium, and high) and creek mouth status (open or closed). We also assessed the importance of hatchery-origin juvenile releases on the *O. kisutch* eDNA signal by conducting similar analyses on samples grouped by if they were collected after the November 21, 2019, parr release and if they were collected within 3 days of a smolt release. We chose to distinguish samples within 3 days of a smolt release because, while *O. kisutch* smolts are expected to immediately migrate downstream after release, it has been observed that they can reside in place temporarily.

We then used multivariate linear regression to determine the level of association between eDNA concentrations and environmental parameters. eDNA concentrations were averaged over each day and $\log_{10}$-transformed before use as the models' dependent variables. Environmental parameters (i.e., the independent variables) included daily mean water temperature, photoperiod (length of the day), $\log_{10}$-transformed daily mean stream discharge, whether the sample was collected on a wet or dry day (to account for spikes in discharge), and the creek mouth status. All environmental parameters have been shown in previous works to modulate eDNA concentrations or affect fish behavior in coastal streams (Lusardi et al., 2020; Quinn, 2018; Sassoubre et al., 2016; Takahara et al., 2012). To control for hatchery-origin juvenile releases in the *O. kisutch* model, we also included variables that indicated if the sample was collected after the November 21, 2019, parr release and if smolt releases had occurred within the previous 3 days of sample collection (see above). *O. kisutch* and *O. mykiss* were modeled independently, and eDNA of one species was not considered in the models of the other.

To fit models, we used generalized least-squares regression with a second-order autoregressive covariance term to control for serial correlation that was found to be present in the eDNA data. This was accomplished using the 'glsar' function in the 'statsmodels' package in Python. Durbin-Watson statistic values for all models were approximately 2 indicating that serial correlation was not present in model residuals. Multicollinearity between independent variables was assessed using variance inflation factors (VIFs); all VIFs were less than 5, revealing that multicollinearity was not of concern in the models.

Models were assessed by first examining the model F-statistic. A significant F-statistic suggests that independent variables (environmental parameters) in a model can explain the variability of the dependent variable (eDNA concentrations) significantly better than the null model (intercept only). We also calculated adjusted R^2 values to determine quality of fit and assessed model coefficients and their p-values to determine which independent variables were important in explaining eDNA concentration variation after accounting for others.

2.6 | Data analysis—comparing eDNA with fish trap data

To assess congruity between eDNA and traditional monitoring methods, we compared salmonid eDNA concentrations and trap

abundance data for days in which both sampling methods were performed (N = 180 days). The McNemar's test was used to test the null hypothesis that daily "fish detection rate," defined as the fraction of monitoring days where fish were detected by a given sampling method, is the same between eDNA and fish trapping methods. On days in which multiple water samples were collected, eDNA was considered detected on that day if any of the samples were detected. We assessed the relationship between fish abundance and eDNA concentrations by performing univariate linear regression to determine whether there was a linear relationship between the two parameters. In this analysis, models were fit such that fish abundance and eDNA concentrations were the dependent and independent variables, respectively. We used both daily fish counts (i.e., total individuals counted in the traps on a given day) and total daily biomass as abundance metrics. Models were fit and assessed using the regression methodology and metrics described above.

3 | RESULTS

3.1 | High-throughput ESP sampling and qPCR

We analyzed a total of 674 water samples, which were collected on 360 days during the 377-day study period. There were 95 (26%) sampling days where water samples were collected three times per day, 124 (34%) days where they were collected twice per day, and 141 (39%) days where they were collected once per day. The mean ESP deployment duration was 52 days, and the longest deployment was 100 days. 52%, 16%, and 32% of samples were collected in the morning (before 6:00 PST), midday (between 6:00 and 17:00 PST), and the evening (after 17:00 PST), respectively. The mean sample volume was 729 ml (range 28–1825 ml) (Figure S6), and the mean sampling duration was 45 min.

3.2 | eDNA signal—overview

For both salmonid species, eDNA was consistently detected over the course of the study (Figure 2): 7.4% and 1.0% of water samples were ND (i.e., when no replicates amplified) for *O. kisutch* and *O. mykiss* eDNA, respectively, while 59.3% and 91.7% of samples had quantifiable (i.e., detected and above the LOQ) eDNA (Table 2). Concentrations of *O. kisutch* eDNA were significantly lower (MW-U, $p < 0.01$) than *O. mykiss* (Figure 2). The median and maximum eDNA concentrations for *O. kisutch* (N = 674) were 2 and 636 copies/ml, respectively, while the median and maximum eDNA concentrations for *O. mykiss* were 35 and 6976 copies/ml, respectively (Table 2). The Spearman rank correlation coefficient between the *O. kisutch* and *O. mykiss* eDNA concentrations was 0.48 ($p < 0.01$).

3.3 | eDNA signal—temporal dynamics and associations with abiotic factors

3.3.1 | Temporal variation

Of the 92 days in which samples were collected each during the morning, midday, and evening periods, there was a significant difference by time of day in *O. kisutch* eDNA concentrations (Wilcoxon Signed Rank, $p = 0.02$); concentrations were generally higher in samples collected in the middle of the day and lower in samples collected in the evening. No difference by time of day was found in *O. mykiss* eDNA concentrations (Friedman Chi-squared, $p > 0.05$).

Over high frequencies (i.e., subdaily to daily), eDNA concentrations showed temporal patchiness and this variability was generally higher for *O. kisutch* than for *O. mykiss*. There were 367 samples in which a consecutive sample was collected less than 12 hours later. Of these, there were 208 *O. kisutch* eDNA samples and 63 *O. mykiss* eDNA samples in which the detection status above or below the LOD alternated in the subsequent sample. The median relative difference in eDNA concentration between subdaily samples (δ) was 61% (range 0–4474%) for *O. kisutch* and 54% (range 0–18,044%) for *O. mykiss*. There were 350 days when samples were collected on a consecutive day. The median relative difference in daily mean eDNA concentration (Δ) was 44% (range: 0–4630%) for *O. kisutch* and 37% (range: 0–18,821%) for *O. mykiss*.

eDNA concentrations for both salmonid species were significantly different between seasons (MW-U, $p < 0.05$) (Figure S7). *O. kisutch* eDNA concentrations were generally highest in the winter (12 copies/ml median), lowest in autumn (0 copies/ml), and similar between the spring and summer seasons (2 copies/ml). *O. mykiss* eDNA concentrations were generally highest in the summer season (median of 61 copies/ml), yet similar in magnitude to the remaining seasons (33, 27, and 30 copies/ml in spring, autumn, and winter, respectively).

3.3.2 | Association between eDNA concentrations and environmental factors

eDNA in Scott Creek varied by environmental condition (Figure S8). Concentrations of both salmonid species' eDNA were significantly different by creek flow regime (MW-U, $p < 0.01$). The highest concentrations were measured during periods of medium discharge, while the lowest concentrations were measured during periods of low discharge. Concentrations for both species were also significantly higher (MW-U, $p < 0.01$) when the creek mouth was open compared with when it was closed. No significant difference in *O. kisutch* eDNA was observed in samples collected within 3 days of a hatchery-origin smolt release; however, concentrations were significantly higher (MW-U, $p < 0.01$) after the November 21, 2021, release of approximately 10,000 hatchery-origin parr into the watershed (median of 1.5 and 7.5 copies/ml before and after, respectively) (Figure S9).

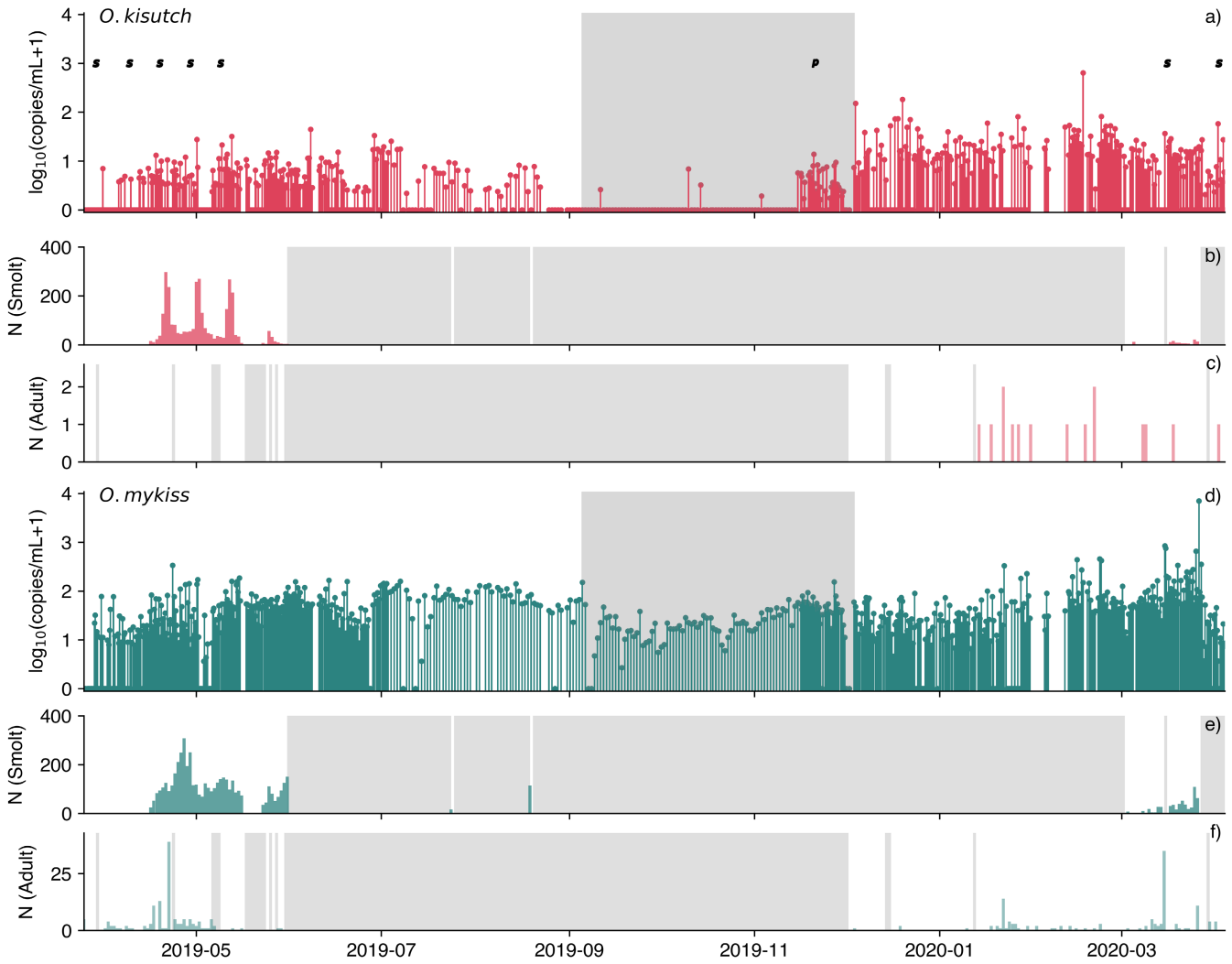

FIGURE 2 Time series of salmonid eDNA concentrations and trap counts. Concentrations of *O. kisutch* and *O. mykiss* eDNA are plotted in (a) and (e), respectively. Samples measured below the LOQ (i.e., both ND and detected but below the LOQ) are plotted as zeros. Gray shading in these plots indicates the dates when the mouth of Scott Creek was closed due to sandbar formation (September 5, 2019) and when the creek mouth reopened due to elevated streamflow (December 4, 2019). Days when releases of hatchery-origin *O. kisutch* smolts occurred are indicated by the 's' symbols; the date when hatchery-origin *O. kisutch* parr were released (21 November 2019) is indicated by the 'p' symbol. Trap counts of *O. kisutch* smolts and adults are plotted in (b) and (c), respectively, and counts of *O. mykiss* smolts and adults are plotted in (e) and (f), respectively. Gray shading in these plots indicates dates when the adult and smolt traps were not operational

We performed multivariate regressions between salmonid eDNA concentrations and environmental factors (Table 3). For both targets, the variability in eDNA concentrations explained by environmental factors was significant (*F*-statistic, $p < 0.01$) but low (adjusted R^2 values of 0.16 and 0.05 for *O. kisutch* and *O. mykiss*, respectively). After controlling for the effects from the release of hatchery-origin parr ($p < 0.01$) and smolts ($p > 0.05$), *O. kisutch* eDNA was significantly associated (in the positive direction) with the status of the creek mouth ($p < 0.05$) and whether the release of hatchery-origin parr had occurred ($p < 0.01$); *O. mykiss* eDNA was significantly associated with creek discharge ($p = 0.01$, in the negative direction) and creek mouth status ($p < 0.01$, in the positive direction). Photoperiod was associated with *O. mykiss* eDNA concentrations in the positive direction at $p < 0.01$. Water temperature and whether the sample was collected on a wet day (i.e., occurrence of a spike in discharge) were not significant factors in our models.

3.4 | eDNA signal—comparison with fish trap data

3.4.1 | Comparing fish detection rate

There were 180 days in which both eDNA was sampled and the weir and smolt traps were operational and sampled for fish (Figure 2). *O. kisutch* were present in the traps on 60 days, and a total of 3119 individuals were counted during this time; *O. mykiss* were present on 96 days, and a total of 5506 individuals were counted. The median and maximum daily biomass of fish in the traps were 0 and 7.6 kg,

TABLE 2 Summary statistics describing concentrations of salmonid eDNA in Scott Creek during the study

Target	*O. kisutch*	*O. mykiss*
	N Samples	
Total	674	
ND	50 (7.4%)	7 (1.0%)
Detected but BLOQ	224 (33.2%)	49 (7.3%)
Above LOQ	400 (59.3%)	618 (91.7%)
	Concentration (copies/ml)	
Mean	8.2	62.2
Std. Deviation	28.2	276.2
Minimum	0.0	0.0
25% Quartile	0.0	14.8
Median	2.3	35.2
75% Quartile	9.1	63.8
Maximum	636.2	6975.7

Abbreviations: BLOQ, below the level of quantification; ND, nondetect.

TABLE 3 Multivariate regressions of eDNA concentrations for *O. kisutch* and *O. mykiss*

Target	*O. kisutch*	*O. mykiss*
Parameter	Coefficient (*p*-value)	
Intercept	−0.02 (0.96)	1.44 (<0.01)
Water temperature	−0.01 (0.65)	−0.02 (0.41)
$\log_{10}$ discharge	−0.03 (0.81)	−0.49 (0.01)
Photoperiod	0.64 (0.51)	1.64 (0.09)
Wet day	−0.08 (0.29)	−0.03 (0.77)
Creek mouth	0.31 (0.02)	0.35 (<0.01)
Smolt release	−0.01 (0.96)	
Parr release	0.51 (<0.01)	–
Adjusted R^2	0.16	0.05
F-statistic (*p*-value)	11.00 (<0.01)	3.62 (<0.01)
Durbin-Watson	2.00	2.05

Note: The dependent variable of the models was log10-transformed daily mean eDNA concentration. The independent variables (i.e., environmental and hatchery parameters) included were daily mean water temperature, log10-transformed stream discharge, photoperiod, whether the sample was collected on a wet day, whether the creek mouth was open or closed, whether hatchery-origin smolts were released in the previous three days (for the *O. kisutch* models only), and whether the November 21, 2019, hatchery-origin parr release had occurred (for the *O. kisutch* models only). All variables had VIFs less than 5.

respectively, for *O. kisutch* and 0.5 and 104 kg, respectively, for *O. mykiss* (Table S3).

eDNA sampling detected salmonids significantly more frequently than fish traps (McNemar's, $p < 0.01$). *O. kisutch* and *O. mykiss* were detected in eDNA samples on 99% and 100% of days, respectively, while these species were present in the traps on 33% and 53% of days, respectively. There were no days in which fish were present in the traps and eDNA was not detected; however, there were 118 and 84 days, respectively, in which eDNA detected *O. kisutch* and *O. mykiss* while these species were absent from the traps (Table S4).

TABLE 4 Simple linear regressions of fish abundance

	√Count	√Biomass
Parameter	Coefficient (*p*-value)	
O. kisutch		
Intercept	2.03 (0.04)	0.43 (<0.01)
$\log_{10}$ eDNA	−0.16 (0.56)	−0.09 (0.34)
R^2	0.002	0.005
F-statistic (*p*-value)	0.35 (0.56)	0.92 (0.34)
Durbin-Watson	2.00	2.02
O. mykiss		
Intercept	1.79 (0.36)	0.08 (0.85)
$\log_{10}$ eDNA	0.94 (<0.01)	0.72 (<0.01)
R^2	0.04	0.05
F-statistic (*p*-value)	8.12 (<0.01)	8.82 (<0.01)
Durbin-Watson	1.98	2.14

Note: The dependent variable of the models was either daily fish count (left column) or total daily biomass (right column) measured in the weir and smolt traps. Both dependent variables were square-root transformed. The independent variable of the models was log10-transformed daily mean eDNA concentration.

3.4.2 | Association between fish abundance and eDNA concentration

No significant relationship between *O. kisutch* abundance (count and biomass) and eDNA was found (Table 4, Figure S10). A significant ($p < 0.01$), positive association was found between both *O. mykiss* abundance metrics and eDNA (Table 4); however, the amount of variability in fish abundance explained by eDNA was low (model R^2 values of 0.04 and 0.05 for fish count and biomass, respectively).

4 | DISCUSSION

Coupling eDNA analyses with automated water samplers (such as the ESP) provides high temporal resolution biological data for watershed monitoring and surveillance. Previous studies have utilized the ESP for the investigation of invasive species (Sepulveda et al., 2020), marine fish (Hansen et al., 2020; Yamahara et al., 2019), and numerous marine microbes and phytoplankton (Scholin et al., 2017). Here, we utilize the ESP for continuous long-term biomonitoring of salmonids in a California coastal stream.

The results provide insight into the temporal dynamics of anadromous fish eDNA in lotic environments. Over longer time scales, we observed distinct seasonal patterns in eDNA concentrations

that may correspond to the ecology and life histories of salmonids in Scott Creek. The highest concentrations of *O. kisutch* eDNA were measured during the winter. This may be due to the upstream migration, spawning, and subsequent decomposition of adult fish that typically occurs in this time (Osterback et al., 2018; Quinn, 2018), though it may also be due to the release of hatchery-origin parr into the watershed at the end of November (see below). Conversely, the lowest *O. kisutch* eDNA concentrations were observed during autumn, the season when *O. kisutch* eDNA shedding may be lowest as individuals tend to seek refuge in pools during periods of low discharge until it is time to migrate (Lusardi et al., 2020). *O. mykiss* eDNA concentrations were generally higher and less variable throughout the year than *O. kisutch* eDNA, aligning with knowledge that *O. mykiss* is in larger abundance in Scott Creek and has populations that reside there year-round (represented by multiple age classes). Such congruence with salmonid life history patterns provides confidence in the quality of the eDNA signals. It also points to the potential of eDNA being an effective tool for biomonitoring purposes.

On shorter time scales (i.e., subdaily and day-to-day), eDNA concentrations exhibited patchy behavior. There were instances in which eDNA concentrations varied multiple orders of magnitude between consecutive samples, and in which eDNA was detected in one sample but not in the consecutive sample. Patchy behavior in the eDNA signals may be due to low abundance of fish in the system, the episodic nature of fish migration (Quinn, 2018), or diurnal variation in fish metabolism (which could also explain the differences by time of day observed in the *O. kisutch* eDNA signal). An additional reason for these observations could be the inability of a single sampling location to capture the spatially heterogeneous nature of eDNA in aquatic systems. Finally, it is a possibility that the activities of the life cycle monitoring station (i.e., fish accumulating in traps over 24 hours and handling of fish during processing) could also contribute to subdaily variability in eDNA concentrations. Regardless of the cause, the high-frequency variability observed in eDNA signals in this system should serve as a caution to researchers to not rely on a single measurement for eDNA monitoring as it may not represent the true state of the system and may give rise to high false-positive or false-negative detection rates (Darling et al., 2021).

We observed significant associations between eDNA and a number of environmental factors. Status of the creek mouth and photoperiod are two of these; both vary at low frequencies and likely represent the seasonality observed in the eDNA data. A closed creek mouth represents a barrier to migration for salmonids into the stream (Osterback et al., 2018), which corresponds to less eDNA shed into a system. This may be one reason why *O. kisutch* eDNA was detected less often and concentrations were lowest in autumn; however, data collected near the creek mouth and over multiple years of varying migrant population sizes would be needed to verify this. Photoperiod (or the length of the day) has been also shown to drive fish behavior (Quinn, 2018), but the mechanism by which it affects eDNA in Scott Creek is unclear. For example, we found that the photoperiod variable had a positive effect on *O. mykiss* eDNA, but no effect on *O. kisutch* eDNA. Rather than representing solar irradiance (which could drive eDNA decay (Allan et al., 2021)), photoperiod could be a correlated proxy of other seasonal effects in Scott Creek, which drive fish activity and subsequent increased eDNA shedding (e.g., water temperature or prey availability (Lusardi et al., 2020)). Targeted, controlled studies could lend insight into this by focusing on determining the importance of photodegradation as an eDNA decay mechanism in the field.

Creek discharge was also an important factor in explaining eDNA concentration in the study. Discharge—like sandbar status and photoperiod—tends to vary in Scott Creek primarily on a seasonal basis; however, flow in the creek can spike diurnally owing to episodic precipitation events. In our study, the highest eDNA concentrations were measured during periods of medium discharge and not during flow extremes. One explanation for this pattern could be that eDNA is more efficiently dispersed and thus measured by ESPs in medium flow regimes, whereas high flow may dilute eDNA to concentrations below the detection limit of the assays or increase eDNA patchiness. Indeed, this notion was confirmed by Thalinger et al. (2020) when they observed attenuation in caged fish eDNA signals with increasing stream discharge. Alternatively, low discharge may insufficiently transport eDNA to sampling locations before decay and settling mechanisms remove it from the system (Shogren et al., 2016; Spence et al., 2020).

The release of over 10,000 hatchery-origin *O. kisutch* parr into the watershed at the end of November 2019 was associated with an elevated eDNA signal for the remainder of the study. Because *O. kisutch* parr are not able to migrate to the ocean, it is expected that these fish redistributed throughout the Scott Creek watershed. Thus, the sudden increase in *O. kisutch* biomass likely shifted the background eDNA measured at the study site. The contribution of eDNA from these parr into Scott Creek could have masked the eDNA shed from migrants caught in the adult and smolt traps at the study site, and thus be a reason for the poor association between *O. kisutch* eDNA and abundance.

eDNA and fish trap measurements were not congruous. We detected salmonid eDNA more frequently than trapping did: eDNA indicated salmonid presence on all days in which they were observed in traps, but eDNA was also measured on days in which fish were absent from traps. This highlights that eDNA and trapping methods provide fundamentally different information about a system. In lotic environments, the presence of eDNA indicates that organisms were likely present upstream, but eDNA does not necessarily co-occur with the organism. eDNA measurements alone cannot delineate the exact location of organisms in the system (e.g., nearby the sampling site, farther upstream, or residual hatchery DNA), when they were in the system, their specific activity (e.g., migrating, spawning, or in refuge), or their life stage or status—information that traps can provide. Conversely, fish traps provide direct observations of fish, but only represent those fish captured and retained by the trap during the period it is deployed and when fish are migrating. In Scott Creek, fish trap data are not inclusive of

parr (natural- or hatchery-origin), nor are they a continuous time series, as there are periods in which the traps are not functional or are not efficiently trapping all salmonids (e.g., during high flow regimes that crest the weir). As such, eDNA concentrations and trapping data should be considered complementary. In order to maximize the information gained about a system, eDNA sampling should continue to be paired with traditional observation methods in long-term biomonitoring studies.

A significant, positive association between eDNA concentrations and fish count and biomass was observed for *O. mykiss*, yet no such relationship existed for *O. kisutch*. In addition to the influence of hatchery-origin juvenile releases and methodological differences between eDNA and trap observations discussed above, we speculate that this contrast in model results may be attributed to the relative density of the two salmonid species in Scott Creek. *O. mykiss* is the more abundant salmonid species and often has a more protracted freshwater residency (Hayes et al., 2008; Osterback et al., 2018) with correspondingly higher and less variable eDNA concentrations compared with *O. kisutch*. It could be that eDNA shed by fish in high enough densities persists long enough to be measured before it becomes undetectable. This is supported by other studies which found significant associations between eDNA and abundance for migratory fish in systems in which fish were in much higher abundance than in our study (Levi et al., 2019; Thalinger et al., 2019).

The disparities between eDNA and traditional observational data not only reveal the need to account for methodological differences but also to resolve eDNA fate and transport when modeling organism abundance. We attempted to do this by including life-stage information and environmental factors into our statistical models to control for differential shedding by juveniles and adults and for stream discharge, water temperature, photoperiod, and creek mouth status (results not shown). While these parameters improved model fits per adjusted R^2 values, they did not yield more accurate abundance predictions. This is likely because statistical models alone do not fully account for or represent all of the important environmental processes that drive the relationship between organisms and the eDNA they shed into a system (Harrison et al., 2019). Pilot studies that elucidate the important drivers of eDNA concentrations in specific systems (such as that conducted by Thalinger et al. (2020) where spatial variation of eDNA by level of discharge was examined) as well as a process-based modeling approach (such as the use of hybrid modeling (Wang et al., 2016)) may be needed in order to establish the relationship between eDNA and fish abundance in individual waterways.

To our knowledge, the data presented herein represent one of the longest continuous eDNA collections at a single site. Automated sampling (like that performed by ESPs) can enable collection of such datasets from which temporally variable information about ecosystems can be extracted. Measurement of eDNA occurred during times when fish traps were not operational, something that is especially beneficial for monitoring salmonids as these fish can exhibit activity outside of typical trapping seasons (Bennett et al., 2015; Hayes et al., 2008; Quinn, 2018). Additionally, high-frequency sampling by ESPs allowed for the accumulation of sequential observations that give higher confidence to determinations of species presence or absence (Sepulveda et al., 2020). While we have shown that automated sampling technologies alleviate the human resource challenges of biomonitoring for extended time periods, the full potential of automated sampling technologies will be realized with the incorporation of in situ analyses. Further work should consider autonomous samplers for investigating spatiotemporal eDNA signals to elucidate eDNA fate and transport mechanisms, build robust occupancy models to elucidate detection probabilities and inform optimal sampling frequencies based on different management use cases (McKelvey et al., 2016; Sepulveda et al., 2020; Wilcox et al., 2016), and improve organism abundance estimation (Levi et al., 2019; Thalinger et al., 2019).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

JMB, CS, KSV, and KMY conceptualized the idea and acquired funding for the project. KMY, CMP, SJ, BR, and JMB assisted in development and deployment of the ESP at the study site. KMY, CW, CMP, and JDK contributed to sample collection and laboratory analyses. RTS contributed to data analysis and interpretation and wrote the first draft of the manuscript. ABB contributed data interpretation and edited early drafts of the manuscript. All authors edited and provided feedback on later drafts of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at the Stanford Digital Repository: https://purl.stanford.edu/xn861qv4853.

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REFERENCES

Adams, P., Boydstun, L. B., Gallagher, S., Lacy, M., Mcdonald, T. & Shaffer, K. (2011). California Coastal Salmonid Population Monitoring: Strategy, Design, and Methods. Department of Fish and Game (Fish Bulletin 180). https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=30284

Allan, E. A., Zhang, W. G., Lavery, A. C., & Govindarajan, A. F. (2021). Environmental DNA shedding and decay rates from diverse animal forms and thermal regimes. *Environmental DNA*, *3*(2), 492–514. https://doi.org/10.1002/edn3.141

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, *17*(1), 1–17. https://doi.org/10.1007/s10592-015-0775-4

Bennett, T. R., Roni, P., Denton, K., McHenry, M., & Moses, R. (2015). Nomads no more: Early juvenile coho salmon migrants contribute to the adult return. *Ecology of Freshwater Fish*, *24*(2), 264–275. https://doi.org/10.1111/eff.12144

Bond, R. M., Nicol, C. L., Kiernan, J. D., & Spence, B. C. (2019). Occurrence, fate, and confounding influence of ghost passive integrated transponder tags in an intensively monitored watershed. *Canadian Journal of Fisheries and Aquatic Sciences*, *76*(2), 286–298. https://doi.org/10.1139/cjfas-2017-0409

Borchardt, M. A., Boehm, A. B., Salit, M., Spencer, S. K., Wigginton, K. R., & Noble, R. T. (2021). The Environmental Microbiology Minimum Information (EMMI) guidelines: QPCR and dPCR quality and reporting for environmental microbiology. *Environmental Science & Technology*, *55*(15), 10210–10223. https://doi.org/10.1021/acs.est.1c01767

Capo, E., Spong, G., Norman, S., Königsson, H., Bartels, P., & Byström, P. (2019). Droplet digital PCR assays for the quantification of brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*) from environmental DNA collected in the water of mountain lakes. *PLoS One*, *14*(12), e0226638. https://doi.org/10.1371/journal.pone.0226638

Darling, J. A., Jerde, C. L., & Sepulveda, A. J. (2021). What do you mean by false positive? *Environmental DNA*, *3*(5), 879–883. https://doi.org/10.1002/edn3.194

Deiner, K., Yamanaka, H., & Bernatchez, L. (2021). The future of biodiversity monitoring and conservation utilizing environmental DNA. *Environmental DNA*, *3*(1), 3–7. https://doi.org/10.1002/edn3.178

Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E., & Miaud, C. (2012). Improved detection of an alien invasive species through environmental DNA barcoding: The example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology*, *49*(4), 953–959. https://doi.org/10.1111/j.1365-2664.2012.02171.x

Doi, H., Inui, R., Akamatsu, Y., Kanno, K., Yamanaka, H., Takahara, T., & Minamoto, T. (2017). Environmental DNA analysis for estimating the abundance and biomass of stream fish. *Freshwater Biology*, *62*(1), 30–39. https://doi.org/10.1111/fwb.12846

Hansen, B. K., Jacobsen, M. W., Middelboe, A. L., Preston, C. M., Marin, R., Bekkevold, D., Knudsen, S. W., Møller, P. R., & Nielsen, E. E. (2020). Remote, autonomous real-time monitoring of environmental DNA from commercial fish. *Scientific Reports*, *10*(1), 13272. https://doi.org/10.1038/s41598-020-70206-8

Harrison, J. B., Sunday, J. M., & Rogers, S. M. (2019). Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1915), https://doi.org/10.1098/rspb.2019.1409

Hayes, S. A., Bond, M. H., Hanson, C. V., Freund, E. V., Smith, J. J., Anderson, E. C., Ammann, A. J., & MacFarlane, R. B. (2008). Steelhead growth in a small central California watershed: Upstream and estuarine rearing patterns. *Transactions of the American Fisheries Society*, *137*(1), 114–128. https://doi.org/10.1577/T07-043.1

Hinlo, R., Lintermans, M., Gleeson, D., Broadhurst, B., & Furlan, E. (2018). Performance of eDNA assays to detect and quantify an elusive benthic fish in upland streams. *Biological Invasions*, *20*(11), 3079–3093. https://doi.org/10.1007/s10530-018-1760-x

Hongo, Y., Nishijima, S., Kanamori, Y., Sawayama, S., Yokouchi, K., Kanda, N., Oori, S., Uto, Y., Ishii, M., Mita, H., Akimoto, S., Kusano, A., Okabe, K., Yamazaki, T., Fukuda, N., Onitsuka, T., Minagawa, M., Okamura, H., Niwa, K., … Kurogi, H. (2021). Fish environmental DNA in Tokyo Bay: A feasibility study on the availability of environmental DNA for fisheries. *Regional Studies in Marine Science*, *47*, 101950, https://doi.org/10.1016/j.rsma.2021.101950

Kelly, R. P., Port, J. A., Yamahara, K. M., Martone, R. G., Lowell, N., Thomsen, P. F., Mach, M. E., Bennett, M., Prahler, E., Caldwell, M. R., & Crowder, L. B. (2014). Harnessing DNA to improve environmental management. *Science*, *344*(6191), 1455–1456. https://doi.org/10.1126/science.1251156

Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., Jackson, C. A., Lance, R. F., Mangan, A. M., Monroe, E. M., Piaggio, A. J., Stokdyk, J. P., Wilson, C. C., & Richter, C. A. (2020). Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA*, *2*(3), 271–282. https://doi.org/10.1002/edn3.29

Lacoursière-Roussel, A., Rosabal, M., & Bernatchez, L. (2016). Estimating fish abundance and biomass from eDNA concentrations: Variability among capture methods and environmental conditions. *Molecular Ecology Resources*, *16*(6), 1401–1414. https://doi.org/10.1111/1755-0998.12522

Levi, T., Allen, J. M., Bell, D., Joyce, J., Russell, J. R., Tallmon, D. A., Vulstek, S. C., Yang, C., & Yu, D. W. (2019). Environmental DNA for the enumeration and management of Pacific salmon. *Molecular Ecology Resources*, *19*(3), 597–608. https://doi.org/10.1111/1755-0998.12987

Lodge, D. M., Turner, C. R., Jerde, C. L., Barnes, M. A., Chadderton, L., Egan, S. P., Feder, J. L., Mahon, A. R., & Pfrender, M. E. (2012). Conservation in a cup of water: Estimating biodiversity and population abundance from environmental DNA. *Molecular Ecology*, *21*(11), 2555–2558. https://doi.org/10.1111/j.1365-294X.2012.05600.x

Lusardi, R. A., Hammock, B. G., Jeffres, C. A., Dahlgren, R. A., & Kiernan, J. D. (2020). Oversummer growth and survival of juvenile coho salmon (*Oncorhynchus kisutch*) across a natural gradient of stream water temperature and prey availability: An in situ enclosure experiment. *Canadian Journal of Fisheries and Aquatic Sciences*, *77*(2), 413–424. https://doi.org/10.1139/cjfas-2018-0484

McKelvey, K. S., Young, M. K., Knotek, W. L., Carim, K. J., Wilcox, T. M., Padgett-Stewart, T. M., & Schwartz, M. K. (2016). Sampling large geographic areas for rare species using environmental DNA: A study of bull trout *Salvelinus confluentus* occupancy in western Montana. *Journal of Fish Biology*, *88*(3), 1215–1222. https://doi.org/10.1111/jfb.12863

Mizumoto, H., Mitsuzuka, T., & Araki, H. (2020). An environmental DNA survey on distribution of an endangered salmonid species, *Parahucho perryi*, in Hokkaido, Japan. *Frontiers in Ecology and Evolution*, *8*, 383. https://doi.org/10.3389/fevo.2020.569425

Osterback, A.-M.- K., Kern, C. H., Kanawi, E. A., Perez, J. M., & Kiernan, J. D. (2018). The effects of early sandbar formation on the abundance and ecology of coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Oncorhynchus mykiss*) in a central California coastal lagoon. *Canadian Journal of Fisheries and Aquatic Sciences*, *75*(12), 2184–2197. https://doi.org/10.1139/cjfas-2017-0455

Perez, C. R., Bonar, S. A., Amberg, J. J., Ladell, B., Rees, C., Stewart, W. T., Gill, C. J., Cantrell, C., & Robinson, A. T. (2017). Comparison of American Fisheries Society (AFS) standard fish sampling techniques and environmental DNA for characterizing fish communities in a large reservoir. *North American Journal of Fisheries*

Management, *37*(5), 1010–1027. https://doi.org/10.1080/02755947.2017.1342721

Pilliod, D. S., Goldberg, C. S., Arkle, R. S., & Waits, L. P. (2013). Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*(8), 1123–1130. https://doi.org/10.1139/cjfas-2013-0047

Pilliod, D. S., & Laramie, M. B. (2016). Salmon Redd Identification Using Environmental DNA (eDNA). U.S. Geological Survey Open-File Report, 1091. http://dx.doi.org/https://doi.org/10.3133/ofr20161091

Pilliod, D. S., Laramie, M. B., MacCoy, D., & Maclean, S. (2019). *Integration of eDNA-Based Biological Monitoring within the U.S. Geological Survey's National Streamgage Network*. https://onlinelibrary.wiley.com/doi/abs/https://doi.org/10.1111/1752-1688.12800

Quinn, T. P. (2018). *The behavior and ecology of pacific salmon and trout*. University of Washington Press, http://ebookcentral.proquest.com/lib/stanford-ebooks/detail.action?docID=5667611

Rourke, M. L., Fowler, A. M., Hughes, J. M., Broadhurst, M. K., DiBattista, J. D., Fielder, S., Walburn, J. W., & Furlan, E. M. (2021). Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. *Environmental DNA*, *4*(1), 9–33. https://doi.org/10.1002/edn3.185

Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, *17*, e00547. https://doi.org/10.1016/j.gecco.2019.e00547

Sassoubre, L. M., Yamahara, K. M., Gardner, L. D., Block, B. A., & Boehm, A. B. (2016). Quantification of environmental DNA (eDNA) shedding and decay rates for three marine fish. *Environmental Science & Technology*, *50*(19), 10456–10464. https://doi.org/10.1021/acs.est.6b03114

Scholin, C., Birch, J., Jensen, S., Marin, R. III, Massion, E., Pargett, D., Preston, C., Roman, B., & Ussler, W. I. (2017). The quest to develop ecogenomic sensors: A 25-year history of the environmental sample processor (ESP) as a case study. *Oceanography*, *30*, https://doi.org/10.5670/oceanog.2017.427

Sepulveda, A. J., Birch, J. M., Barnhart, E. P., Merkes, C. M., Yamahara, K. M., Marin, R., Kinsey, S. M., Wright, P. R., & Schmidt, C. (2020). Robotic environmental DNA bio-surveillance of freshwater health. *Scientific Reports*, *10*(1), 14389. https://doi.org/10.1038/s41598-020-71304-3

Shelton, A. O., Kelly, R. P., O'Donnell, J. L., Park, L., Schwenke, P., Greene, C., Henderson, R. A., & Beamer, E. M. (2019). Environmental DNA provides quantitative estimates of a threatened salmon species. *Biological Conservation*, *237*, 383–391. https://doi.org/10.1016/j.biocon.2019.07.003

Shogren, A. J., Tank, J. L., Andruszkiewicz, E. A., Olds, B., Jerde, C., & Bolster, D. (2016). Modelling the transport of environmental DNA through a porous substrate using continuous flow-through column experiments. *Journal of the Royal Society Interface*, *13*(119), 20160290. https://doi.org/10.1098/rsif.2016.0290

Shogren, A. J., Tank, J. L., Andruszkiewicz, E., Olds, B., Mahon, A. R., Jerde, C. L., & Bolster, D. (2017). Controls on eDNA movement in streams: Transport, retention, and resuspension. *Scientific Reports*, *7*(1), 1–11. https://doi.org/10.1038/s41598-017-05223-1

Smith, M. B., Rocha, A. M., Smillie, C. S., Olesen, S. W., Paradis, C., Wu, L., Campbell, J. H., Fortney, J. L., Mehlhorn, T. L., Lowe, K. A., Earles, J. E., Phillips, J., Techtmann, S. M., Joyner, D. C., Elias, D. A., Bailey, K. L., Hurt, R. A., Preheim, S. P., Sanders, M. C., … Hazen, T. C. (2015). Natural bacterial communities serve as quantitative geochemical biosensors. *MBio*, *6*(3), https://doi.org/10.1128/mBio.00326-15

Spence, B. C., Rundio, D. E., Demetras, N. J., & Sedoryk, M. (2020). Efficacy of environmental DNA sampling to detect the occurrence of endangered coho salmon (*Oncorhynchus kisutch*) in Mediterranean-climate streams of California's central coast. *Environmental DNA*, *3*(4), https://doi.org/10.1002/edn3.175

Stewart, K. A. (2019). Understanding the effects of biotic and abiotic factors on sources of aquatic environmental DNA. *Biodiversity and Conservation*, *28*(5), 983–1001. https://doi.org/10.1007/s10531-019-01709-8

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., & Kawabata, Z. (2012). Estimation of fish biomass using environmental DNA. *PLoS One*, *7*(4), e35868. https://doi.org/10.1371/journal.pone.0035868

Thalinger, B., Kirschner, D., Pütz, Y., Moritz, C., Schwarzenberger, R., Wanzenböck, J., & Traugott, M. (2020). Lateral and longitudinal fish environmental DNA distribution in dynamic riverine habitats. *Environmental DNA*, *3*(1), 305–318. https://doi.org/10.1002/edn3.171

Thalinger, B., Wolf, E., Traugott, M., & Wanzenböck, J. (2019). Monitoring spawning migrations of potamodromous fish species via eDNA. *Scientific Reports*, *9*(1), 15388. https://doi.org/10.1038/s41598-019-51398-0

Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., Orlando, L., & Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, *21*(11), 2565–2573. https://doi.org/10.1111/j.1365-294X.2011.05418.x

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, *183*, 4–18. https://doi.org/10.1016/j.biocon.2014.11.019

Tillotson, M. D., Kelly, R. P., Duda, J. J., Hoy, M., Kralj, J., & Quinn, T. P. (2018). Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. *Biological Conservation*, *220*, 1–11. https://doi.org/10.1016/j.biocon.2018.01.030

Wang, X., Zhang, J., & Babovic, V. (2016). Improving real-time forecasting of water quality indicators with combination of process-based models and data assimilation technique. *Ecological Indicators*, *66*, 428–439. https://doi.org/10.1016/j.ecolind.2016.02.016

Wilcox, T. M., Carim, K. J., McKelvey, K. S., Young, M. K., & 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*(11), e0142008. https://doi.org/10.1371/journal.pone.0142008

Wilcox, T. M., McKelvey, K. S., Young, M. K., Sepulveda, A. J., Shepard, B. B., Jane, S. F., Whiteley, A. R., Lowe, W. H., & Schwartz, M. K. (2016). Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*. *Biological Conservation*, *194*, 209–216. https://doi.org/10.1016/j.biocon.2015.12.023

Yamahara, K. M., Demir-Hilton, E., Preston, C. M., Marin, R., Pargett, D., Roman, B., Jensen, S., Birch, J. M., Boehm, A. B., & Scholin, C. A. (2015). Simultaneous monitoring of faecal indicators and harmful algae using an in-situ autonomous sensor. *Letters in Applied Microbiology*, *61*(2), 130–138. https://doi.org/10.1111/lam.12432

Yamahara, K. M., Preston, C. M., Birch, J., Walz, K., Marin, R. I., Jensen, S., Pargett, D., Roman, B., Ussler, W. I., Zhang, Y., Ryan, J., Hobson, B., Kieft, B., Raanan, B., Goodwin, K. D., Chavez, F. P., & Scholin, C. (2019). In situ autonomous acquisition and preservation of marine environmental DNA using an autonomous underwater

vehicle. *Frontiers in Marine Science*, *6*(373), https://doi.org/10.3389/fmars.2019.00373.

Yatsuyanagi, T., Ishida, R., Sakata, M. K., Kanbe, T., Mizumoto, H., Kobayashi, Y., Kamada, S., Namba, S., Nii, H., Minamoto, T., & Araki, H. (2019). Environmental DNA monitoring for short-term reproductive migration of endemic anadromous species, Shishamo smelt (*Spirinchus lanceolatus*). *Environmental DNA*, *2*(2), 130–139. https://doi.org/10.1002/edn3.50

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