Spatial and Temporal Surveys of Salmon Environmental DNA (eDNA) in a Seattle Urban Creek

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Abstract

Seattle Public Utilities (SPU) has a history of conducting traditional fish surveys in urban streams of Seattle, Washington. Limited staff resources have reduced SPU's capacity to monitor fish, and environmental DNA (eDNA) was recognized as an alternative survey method that could potentially improve the efficiency and capacity of SPU-sponsored fish surveys. We performed spatiotemporal surveys of eDNA to assess occupancy and distribution of Chinook Salmon (*Oncorhynchus tshawytscha*), Coho Salmon (*O. kisutch*), and Coastal Cutthroat Trout (*O. clarkii clarkii*) in Thornton Creek, Seattle, between October 2018 and December 2020. Peak Chinook and Coho eDNA detections occurred in October and October–November, respectively, coinciding with expected adult return time. Chinook and Coho eDNA was detected in May at the time when juveniles outmigrate through the Lake Washington basin. Coastal Cutthroat Trout eDNA was widespread and detected at high rates across seasons, reflecting their ubiquitous distribution. Results from multiscale occupancy modeling suggested that distance upstream affected site-level occupancy probabilities for adult Chinook, but not Coho. Model results also suggested that the probability of Coho and Chinook eDNA occurring in water samples was affected by survey year. Finally, model results suggested that the probability of detecting Chinook eDNA in PCR technical replicates was affected by survey year and collection day but detection of Coho eDNA was only affected by collection day. This study indicates eDNA surveys are effective for assessing distribution and occupancy of salmonids in Seattle's urban streams. Integrating eDNA surveys into urban stream monitoring programs can help alleviate the burden of limited assets facing many resource managers.

Keywords: Chinook Salmon, Coho Salmon, monitoring, occupancy, urban streams

Introduction

The Puget Sound region of western Washington has experienced extensive urban sprawl (Davis and Schaub 2005, Hepinstall-Cymerman et al. 2013), with substantial population growth expected to continue (Puget Sound Regional Council 2020). Urbanization has negatively affected stream ecosystems by altering stream hydrology and geomorphology, increasing nutrient and contaminant loads, and reducing biodiversity (Paul and Meyer 2001, Walsh et al. 2005). Seattle, Washington, has experienced significant urban development over the past 160 years that has degraded the ecological health of the city's watersheds. Years of deteriorating habitat conditions has led to dramatic declines in native fish populations and a change in relative abundance of these species. Despite these declines, Seattle's urban watersheds continue to harbor at least 15 different native fish species, including Coho Salmon (*Oncorhynchus kisutch*) and Endangered Species Act (ESA) listed Chinook Salmon (*O. tshawytscha*) (Prokop et al. 2009).

Monitoring is an important element of urban stream management and fundamental to adaptive management (Alberti et al. 2007, O'Neal et al. 2016, Rubin et al. 2017). In the city of Seattle,

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WA, Seattle Public Utilities (SPU) has spent several decades monitoring and studying fish to track salmonid abundance, distribution, and movements, and to evaluate fish migration barriers. This information has helped to minimize environmental impacts associated with operations, services, and capital investments in the city's urban watersheds. Seattle Public Utilities uses information from fish surveys to acquire permits for operation and replacement of infrastructure located in urban watersheds, acquire funding, track regulatory and contractual obligations, and to plan urban watershed focused programs and projects. In addition, SPU has completed several urban creek restoration projects to remove barriers to fish passage, expand flood storage capacity, and improve aquatic and riparian habitat, with the goal of gathering information on project performance through post-project monitoring (Peter et al. 2019, Bakke et al. 2020, Morley et al. 2021). A key performance measure of these creek restoration projects is whether fish access and use newly restored habitats, which can be assessed through monitoring and fish surveys.

Seattle Public Utilities has spent several years conducting salmon spawning surveys and smolt trapping surveys in the five major urban watersheds in Seattle city limits (Thornton Creek, Longfellow Creek, Piper's Creek, Taylor Creek, and Fauntleroy Creek). Typically, spawning surveys require at least two surveyors to walk in the stream channel once a week during the salmon spawning season, which occurs October–December. The surveys include counts of live fish, carcasses, and redds of each salmon species, and surveys can take most of the day depending on the length of stream that must be covered. In some areas, spawning surveys are incomplete because stream access is restricted by private property. Since 2009, annual salmon spawning surveys have been reduced mostly due to limited staff and resources; however, salmon spawning surveys have continued to be conducted in Longfellow Creek, Fauntleroy Creek, and Piper's Creek by local community groups. More recently (2016–2018), SPU conducted targeted salmon spawning surveys to document salmon use in recently restored reaches of Thornton Creek. From 2001–2009, smolt trap data were collected annually in Thornton Creek to assess outmigration of coho salmon smolts. The smolt trapping surveys were deployed for two to four weeks to coincide with the peak coho salmon smolt outmigration which typically occurs in May. The smolt traps have not been redeployed since 2009 largely due to the considerable staff time that is required for upkeep. Consequently, over the past decade, there have been large information gaps about the presence and distribution of fish in Seattle's urban watersheds.

The advancement of environmental DNA (eDNA) for detecting and monitoring aquatic species has expanded the toolbox for resource managers (Rees et al. 2014, Thomsen and Willerslev 2015). Aquatic organisms shed DNA in the form of cellular and extra-cellular genetic material into their environment through skin cells, mucous, feces, gametes, and other tissues, enabling target species residing in aquatic habitats to be surveyed through eDNA. These surveys collect water samples and aim to associate the presence of DNA from a target species in the water sample with their physical presence in the environment. Consequently, eDNA surveys have broad application to resource managers including monitoring for invasive (Erickson et al. 2017, Carim et al. 2019) and imperiled species (Bylemans et al. 2017), monitoring spawning migrations (Thalinger et al. 2019, Duda et al. 2021), assessing species re-introductions (Riaz et al. 2020), providing information on spatial distributions (Schmelzle and Kinziger 2016, Ostberg et al. 2018), identifying migration barriers (Yamanaka and Minamoto 2016, Halvorsen et al. 2020), and evaluating recolonization following barrier removal (Duda et al. 2021). Sampling and analysis of eDNA is efficient and cost-effective, and several studies have demonstrated that eDNA surveys perform as well or better than traditional field sampling methods in detecting target species (Jerde et al. 2011, Dejean et al. 2012, Pilliod et al. 2013, Schmelzle and Kinziger 2016, Hinlo et al. 2017, Ostberg et al. 2019). With consistent monitoring over time, eDNA surveys can provide information on spatial and temporal changes in species distributions (Gingera et al. 2016, Bracken

et al. 2019, Duda et al. 2021).

The purpose of this study was to use eDNA surveys to assess occupancy and distribution of Chinook Salmon, Coho Salmon, and Coastal Cutthroat Trout (*O. clarki clarki*) in lieu of traditional field sampling methods in Thornton Creek, a recently restored urban stream in Seattle. We used eDNA detection as a proxy for species presence. The study objectives were to assess the temporal and spatial distribution of adult salmon in fall and juvenile salmon in spring, compare eDNA detection between transient species with low densities (Chinook Salmon and Coho Salmon) and a common species with higher densities (Coastal Cutthroat Trout), and fit multiscale hierarchical occupancy models to evaluate the effects of distance upstream, year, and day on the probabilities of occupancy, occurrence, and detection of eDNA from adult Chinook Salmon and Coho Salmon in Thornton Creek during the adult return time. While this study is specific to SPU and Thornton Creek, it has broader implications for natural resource managers as a case study for surveying fish eDNA in watersheds where traditional survey methods, like spawner surveys and smolt trapping, can be challenging when fish occur in low densities and streams exhibit flashy flows following the onset of precipitation.

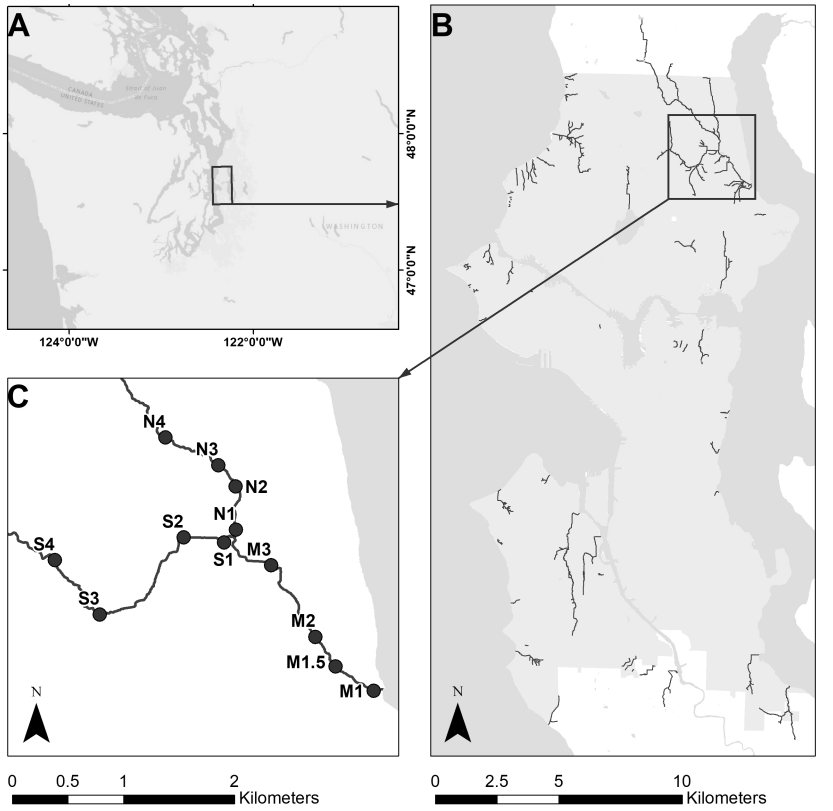

Figure 1. (A) Puget Sound region, Washington, USA. (B) City of Seattle (lighter gray shaded area) displaying Thornton Creek (boxed area) in northeast Seattle. (C) eDNA sampling locations (black points) on Thornton Creek with mainstem sites (M), South Branch sites (S), and North Branch sites (N).

Methods

Study Area

Thornton Creek is the largest watershed within Seattle, covering approximately 2,942 ha (Figure 1). The creek is about 32 km in length, consisting of two main branches (North Branch and South Branch) and 20 smaller tributaries. The creek flows generally from northwest to southeast and drains into Lake Washington. Much of the mainstem is a low gradient channel (median 0.5%–0.9%). The North Branch is also low gradient (median around 1%) whereas the South Branch is steeper gradient (median 1%–2%) (City of Seattle 2007). The watershed hosts at least 16 different fish species, of which 12 are

native species, including Chinook Salmon, Coho Salmon, Sockeye Salmon (*O. nerka*), Coastal Cutthroat Trout, Rainbow Trout (*O. mykiss*), Peamouth (*Mylocheilus caurinus*), Largescale Sucker (*Catostomus macrocheilus*), Longnose Dace (*Rhinichthys cataractae*), Lamprey spp. (Petromyzontidae), Prickly Sculpin (*Cottus asper*), Coastrange Sculpin (*Cottus aleuticus*), and Threespine Stickleback (*Gasterosteus aculeatus*). The four non-native species include Largemouth Bass (*Micropterus salmoides*), Rock Bass (*Ambloplites rupestris*), Pumpkinseed (*Lepomis gibbosus*), and Pond Loach (*Misgurnus anguillicaudatus*). Coastal Cutthroat Trout are the most abundant fish species in Thornton Creek (Prokop et al. 2009, Tabor et al. 2010).

Coho Salmon adults typically spawn in low numbers in the Thornton Creek mainstem, which is about 2.2 km long, but also travel further upstream on the South and North branches. Chinook Salmon adults also spawn in the mainstem and two branches but typically at lower numbers than Coho Salmon. Past salmon spawning surveys conducted between 1999 and 2008 documented between 8 and 135 Coho Salmon adult observations (both live and dead) and between 2 and 12 Chinook Salmon adults per year (Wild Fish Conservancy 2008). More recently, SPU conducted salmon spawning surveys between 2016–2018. The surveys were mostly restricted to the mainstem of Thornton Creek. Between one and five Coho Salmon observations (both live and dead) were documented in 2016 and 2017 and none in 2018, although three redds were documented in 2018. Chinook Salmon were not observed during the 2016–2018 spawning surveys; however, on October 17, 2018, during an SPU educational site tour an adult female hatchery Chinook Salmon was observed by one of the authors (C. Pier, Seattle Public Utilities) and a few days later, a male hatchery Chinook Salmon was observed spawning with the female. Juvenile Coho Salmon and Chinook Salmon have been captured in smolt traps deployed in the lower mainstem between 2001 and 2008 (roughly 350 m upstream of site M1). A single juvenile Coho Salmon was also collected during electrofishing surveys as recently as summer of 2019.

Environmental DNA Sampling and Analysis

Sampling was carried out in fall (October–December), with the goal of detecting eDNA from adults returning to spawn, and in spring (May), with the goal of detecting eDNA from juveniles hatched in Thornton Creek and/or occupying habitats within the creek during their outmigration from other locations in the Lake Washington basin (e.g., Cedar River, Bear Creek, Issaquah Creek, and Issaquah Creek Hatchery). Sampling was also carried out in early September to provide information on background eDNA levels prior to adult returns. We assumed that young-of-the year Coho Salmon could be present in September because their juveniles typically outmigrate during spring of their second year (Sandercock 1991, Weitkamp et al. 1995) and juvenile Chinook Salmon would not be present because they typically outmigrate in spring as young-of-the-year (Tabor and Moore 2020).

We surveyed eDNA in Thornton Creek by collecting water samples on 24 sampling days across multiple locations between 17 October 2018 and 17 December 2020 (Figure 1). A total of nine sites were sampled in Thornton Creek during fall 2018. In 2019, three sites (M1.5, N4, S4) were added based on the 2018 results. At the beginning of the salmon run in October, the objective was to focus the sampling effort on sites in the mainstem and lower South and North branches. Later in the season, the focus shifted to include sampling of upstream sites on the two branches with the assumption that salmon would be more widely distributed throughout the watershed. Most of the mainstem sample sites represented hotspots for salmon spawning activity based on past surveys. Conversely, the most upstream sites on the South and North branches (S4 and N4) were selected as sites where salmon were not expected to be detected due to downstream partial fish barriers and absence of historical salmon sightings.

To survey eDNA at a site on a specific date, two 1-L sub-surface water sample replicates were collected using pre-sterilized Nalgene plastic bottles. Water samples were placed on ice in a cooler until they were filtered in the laboratory, which typically occurred within six hours after

collection. Each water sample was filtered through a pre-sterilized, 47 mm diameter filter funnel with a 1 µm pore size cellulose nitrate sterile filter membrane (Thermo Fisher Scientific, Waltham, Maine) to capture genetic material onto filters. A 1 L negative control sample composed of deionized water (negative field control) was filtered in the laboratory, alongside field collected water samples, at the end of each day that water samples were collected. After filtration, filters were removed from the funnel by using sterile forceps and placed into sterile 5 mL tubes containing 95% ethanol and stored at -20 °C until DNA extraction. All Nalgene bottles, filter funnels and forceps were sterilized prior to their use by soaking in 10% bleach for at least 15 minutes followed by rinsing in tap water.

All laboratory procedures were designed to avoid cross contamination (Goldberg et al. 2016). The eDNA workflow and sample preparation was separated into designated work rooms including a clean room where DNA was extracted (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.

The DNA collected onto filters was extracted following the protocol described in Duda et al. (2021), using one half of each filter for extraction and archiving the other half at -20 °C. Negative DNA extraction controls (extraction buffers only) were included during the DNA extraction process to identify any contamination of equipment and reagents during this procedure.

All DNA extracts were tested for the presence of PCR inhibitors prior to testing for target species by performing an internal positive control (IPC) assay using TaqMan Exogenous Internal Positive Control Reagents (EXO-IPC) (Applied Biosystems, Foster City, CA) and quantitative PCR (qPCR). The IPC assay was performed in duplicate on each DNA sample in 10 µl volumes consisting of 5 µl of Gene Expression Master Mix (Thermo Fisher Scientific), 1 µl EXO-IPC mix, 0.2 µl EXO-IPC DNA, 0.8 µl Nanopure sterile water, and 3 µl DNA template or sterile water for the non-template 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 ViiA 7 RUO 1.2.4 software (Applied Biosystems). A DNA sample was considered inhibited when it had > 1 cycle threshold (C_t) shift relative to the mean non-template control. Samples that were inhibited were treated with OneStep PCR Inhibitor Removal kit (Zymo Research Corporation, Irvine, CA) and re-tested with the IPC assay to confirm that PCR inhibition was alleviated.

Target species assays included Coho Salmon (COCytb_980–1093), Chinook Salmon (CKCO3_464–534), and Coastal Cutthroat Trout (CCCytb_572–685) (Duda et al. 2021), but not all DNA samples were assayed for each species (Supplemental Tables S1–3, available online only). Assays were performed in triplicate (i.e., three PCR technical replicates) on each sample in 10 µl reaction volumes consisting of 3 µl DNA template, 1x Gene Expression Mastermix (Thermo Fisher Scientific), and 1X custom TaqMan primer and probe mix consisting of a final concentration of 450 nM for each forward and reverse primer and 125 nM probe. All target species PCR assays were run on a ViiA 7 real-time PCR system (Applied Biosystems) with cycling parameters consisting of initial steps 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, and results were analyzed using ViiA 7 RUO 1.2.4 software (Applied Biosystems). Each qPCR run consisted of a five-point serial dilution of a DNA standard composed of a gBlock double-stranded DNA fragment (Integrated DNA Technologies, Coralville, IA) specific to the target species, negative field controls, negative DNA extraction controls, and no-template controls

(sterile water in place of DNA), all of which were run in triplicate.

A positive detection was inferred for any sample amplifying at less than 40 cycles with a uniform curve morphology. The negative field controls, negative DNA extraction controls, and no-template controls yielded no positive detections, indicating a very low likelihood of false-positive results in the survey samples. All metadata for this study are publicly available (Ostberg and Chase 2022b).

To characterize general spatial and temporal eDNA patterns, we calculated observed eDNA detection rates for each target species as the proportion of PCR technical replicates that amplified. For Coho Salmon and Chinook Salmon, eDNA detection rates were calculated at each site for the expected adult return in fall (September–December) and for the juvenile outmigration in spring (May) for each sample year. For Coastal Cutthroat Trout, eDNA detection rates were calculated at each site between fall 2018 and spring 2019. Spatial patterns were evaluated by pooling samples across each sampling period at a given sampling site, and temporal patterns were assessed by pooling across sites for a given sampling day.

To analyze detection–non-detection data from eDNA surveys, we fitted multiscale occupancy models using the R package eDNAoccupancy (Dorazio and Erickson 2018). The multiscale eDNA occupancy model design consisted of three nested, hierarchical levels: (i) the site occupancy probability (Ψ_i, occupancy), defined as the probability of occurrence of eDNA at site *i*; (ii) the occurrence probability (θ_{ij}, occurrence), defined as the conditional probability of eDNA occurrence in water sample *j* given occupancy of eDNA at site *i*; and (iii) the detection probability (p_{ijk}, detection), defined as the conditional probability of eDNA detection in PCR technical replicate *k* given that it occurs in water sample *j* and site *i*.

We fitted models to Coho Salmon and Chinook Salmon eDNA data for the adult salmon return (October–December eDNA surveys) to evaluate the effects of covariates representing year, distance, and sampling day on occupancy, occurrence, and detection of eDNA. A single model was fitted for each species using covariates for each nested hierarchical level. Occupancy probability (Ψ) was modeled as a function of distance (km) of the sampling site from Lake Washington:

$$\Phi(\Psi)=\beta_0+\beta_1 \cdot Distance \quad (1)$$

where $\Phi(x)$ is the probit link function (i.e., the standard normal cumulative distribution function), β_0 is the intercept, and β_1 is the slope measuring the effect of distance on Ψ. We hypothesized that downstream sites would have higher occupancy probabilities. Both occurrence (θ) and detection (p) probability were each modeled as a function of sample year (2018, 2019, and 2020) and sample day, where 1 October represented day 1:

$$\Phi(\theta)=\alpha_0+\alpha_1 \cdot I_{(2019)}+\alpha_2 \cdot I_{(2020)}+\alpha_3 \cdot Day+\alpha_4 \cdot Day^2 \quad (2)$$

$$\Phi(p)=\delta_0+\delta_1 \cdot I_{(2019)}+\delta_2 \cdot I_{(2020)}+\delta_3 \cdot \mathrm{Day}+\delta_4 \cdot Day^2 \quad (3)$$

Here, α and δ are the coefficients associated with covariate effects on θ and p. Year was modeled as a factor where the intercepts (α_0 and δ_0) represent 2018 as the reference group, $I_{(year)}$ is an indicator function resolving to one for the specified year and zero otherwise, and coefficients estimate each year's difference from 2018. Day was modeled with both linear (Day) and quadratic (Day^2) terms because we hypothesized that occurrence and detection probability might first increase and then decrease over time, following the timing of the salmon spawning. All continuous covariates were standardized to zero mean and unit standard deviation. Models were run using 500,000 Markov chain iterations with 250,000 burn-in steps and graphically checked for convergence and stationarity. Model runs generated estimates of posterior means for covariate parameter coefficients and covariates with significant effect were identified as coefficients with 95% credible intervals (CI) that did not overlap zero.

Results

Coho Salmon

We tested for Coho Salmon eDNA on all 24 survey days (Supplemental Table S1). Positive detections varied spatially and temporally (Figure 2). The spatial distribution of Coho Salmon eDNA was most widespread in fall 2018, with the highest

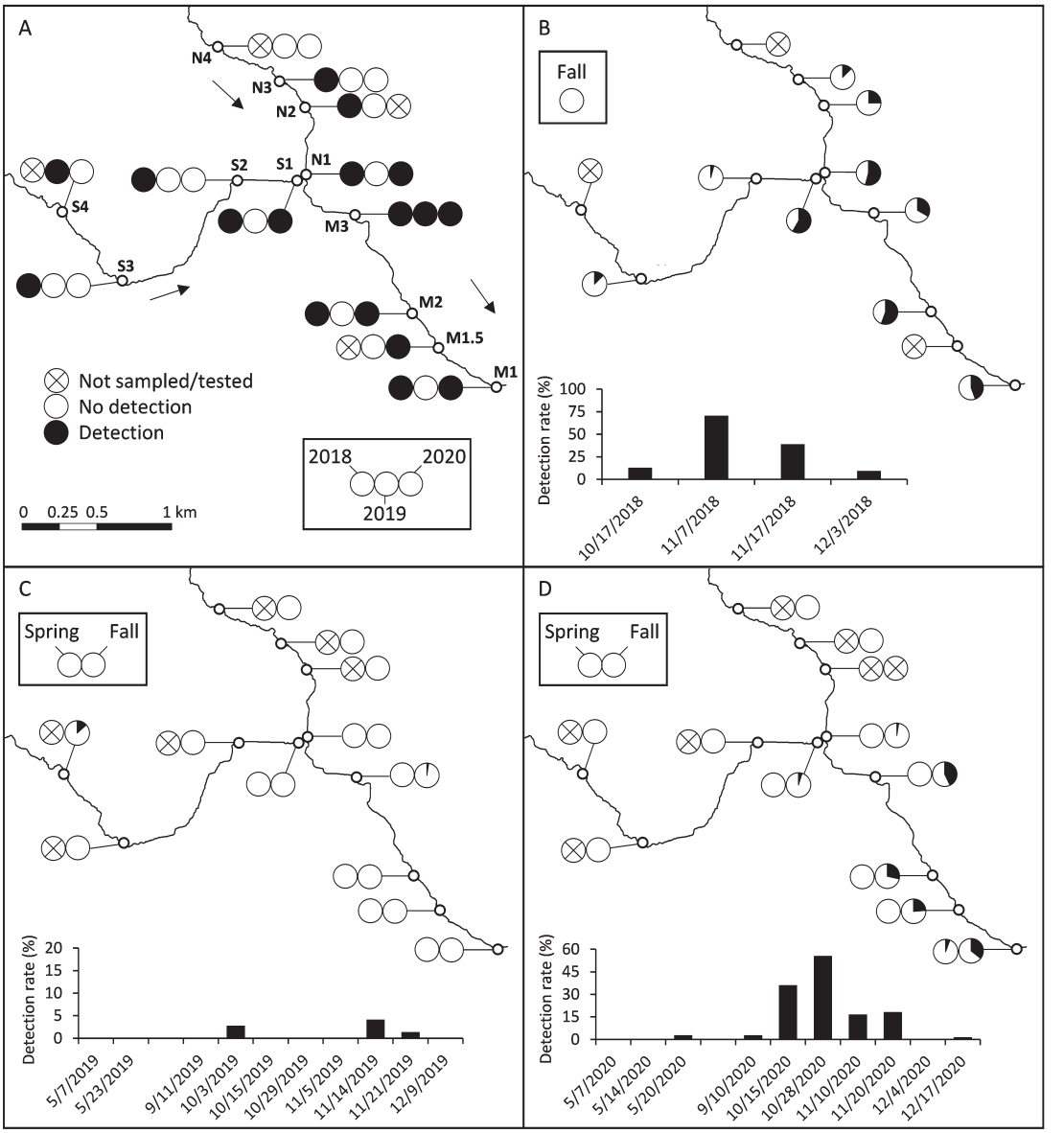

Figure 2. (A) Summary of Thornton Creek Coho Salmon eDNA survey results for each site sampled in 2018, 2019 and 2020. Arrows indicate direction of stream flow. B–D: eDNA detection rates across sites (black color in pie graphs) and across sites on each sample day (bar graphs) for samples collected in fall 2018 (B), spring (May) and fall (September–December) 2019 (C), and spring (May) and fall (September–December) 2020 (D). Vertical axes on bar graphs are on different scales. See Supplemental Table S1 for comprehensive results.

detection rates occurring in November and positive detections occurring in multiple replicate water samples and across all sampling days. In fall 2018, detection rates ranged from 33.3%–55.6% across mainstem sites, 12.5%–54.2% across North Branch sites, and 4.2%–58.3% across South Branch sites, and positive detections occurred as far upstream as N3 and S3. In fall 2019, a single PCR amplified from 3 October (M3) and the next positive detection occurred over one month later on

14 November at the uppermost site on the South Branch (S4) where all three PCR technical replicates amplified in one of two water samples. One week later (21 November), a single PCR amplified at the same site, but no other sites registered a positive detection. In fall 2020, detection rates ranged between 24% and 43% across mainstem sites, with the highest detection rates occurring in mid–late October, and sampling days where the only positive result was a single PCR amplification occurred on 10 September at N1 and on 17 December at M3. Five eDNA surveys were performed across spring 2019 and 2020, yielding only a single PCR amplifying for Coho Salmon, occurring at M1 on 20 May 2020 (Figure 2).

Chinook Salmon

We tested for Chinook Salmon eDNA on 23 survey days (Supplemental Table S2). Positive detections were found primarily in the mainstem (Figure 3). In fall 2018, each replicate water sample collected at each site on the mainstem was positive on 17 October coinciding with a visual sighting of a female Chinook Salmon on that day. Samples collected in both November and December of the same year yielded positive detections across multiple sites and primarily in the mainstem, though these detections typically represented single PCR amplifications. In fall 2019, detections occurred at low levels (mostly single PCR amplifications), few sites (M1.5, M3, N1, and N4), and on few sampling days (3 October, 15 October, and 5 November). In fall 2020, Chinook Salmon eDNA was detected on 15 October at all four sites sampled in the mainstem and the only other detections occurred as single PCR amplifications at M2 on 10 November and at M1 on 4 December. During the spring surveys, Chinook Salmon eDNA was detected on 7 May 2019 in the mainstem and North and South branches and in 2020 at N1 as single PCR amplification (Figure 3).

Coastal Cutthroat Trout

We tested for Coastal Cutthroat Trout eDNA on six sampling occasions between October 2018 and May 2019 (Supplemental Table S3). Coastal Cutthroat Trout eDNA was widespread and prevalent, being detected at all sample sites and on all sampling occasions (Figure 4). Detection rates ranged between 75% and 100% across sample sites and between 86% and 100% across sampling days.

Occupancy Modeling

The mean site occupancy probability across survey years was higher for Coho Salmon (0.90) than Chinook Salmon (0.73) (Table 1). As hypothesized, downstream sites tended to have higher occupancy probabilities than upstream sites for both species, indicated by mean β_1 coefficient estimates that were negative for Coho Salmon and Chinook Salmon, although the effect of distance was stronger for Chinook Salmon (Figure 5). However, the 95% CI for β coefficient estimates for both species overlapped zero (Table 1), indicating uncertainty in the posterior estimates for the effect of distance on site occupancy.

The mean occurrence probability of Coho Salmon eDNA was considerably lower in 2019 (0.03) than in either 2018 (0.48) or 2020 (0.48) and 95% CIs did not overlap, suggesting that survey year had a significant effect (Table 1, Figure 6). The occurrence probability of Chinook Salmon eDNA was not different among years, noted by overlapping 95% CIs, although the mean probability was considerably lower in 2019 (0.21) compared to 2018 (0.52) and 2020 (0.41), possibly indicating that Chinook Salmon eDNA was not sampled as effectively at occupied sites in 2019 (Figure 6). Day and Day^2 did not have a significant effect on the occurrence of eDNA in water samples for either species (Table 1).

The mean detection probability of Chinook Salmon eDNA was substantially higher in 2018 (0.50) than either 2019 (0.05) or 2020 (0.13) and 95% CIs did not overlap, suggesting that survey year had a significant effect (Table 1, Figure 6). Survey year, however, did not have an apparent effect on the detection of Coho Salmon eDNA as mean probabilities were similar among years (2018, 0.57; 2019, 0.46; 2020, 0.43) and 95% CIs overlapped. Both Day and Day^2 had a significant effect on the detection of Coho Salmon eDNA, indicating detection probabilities first increased

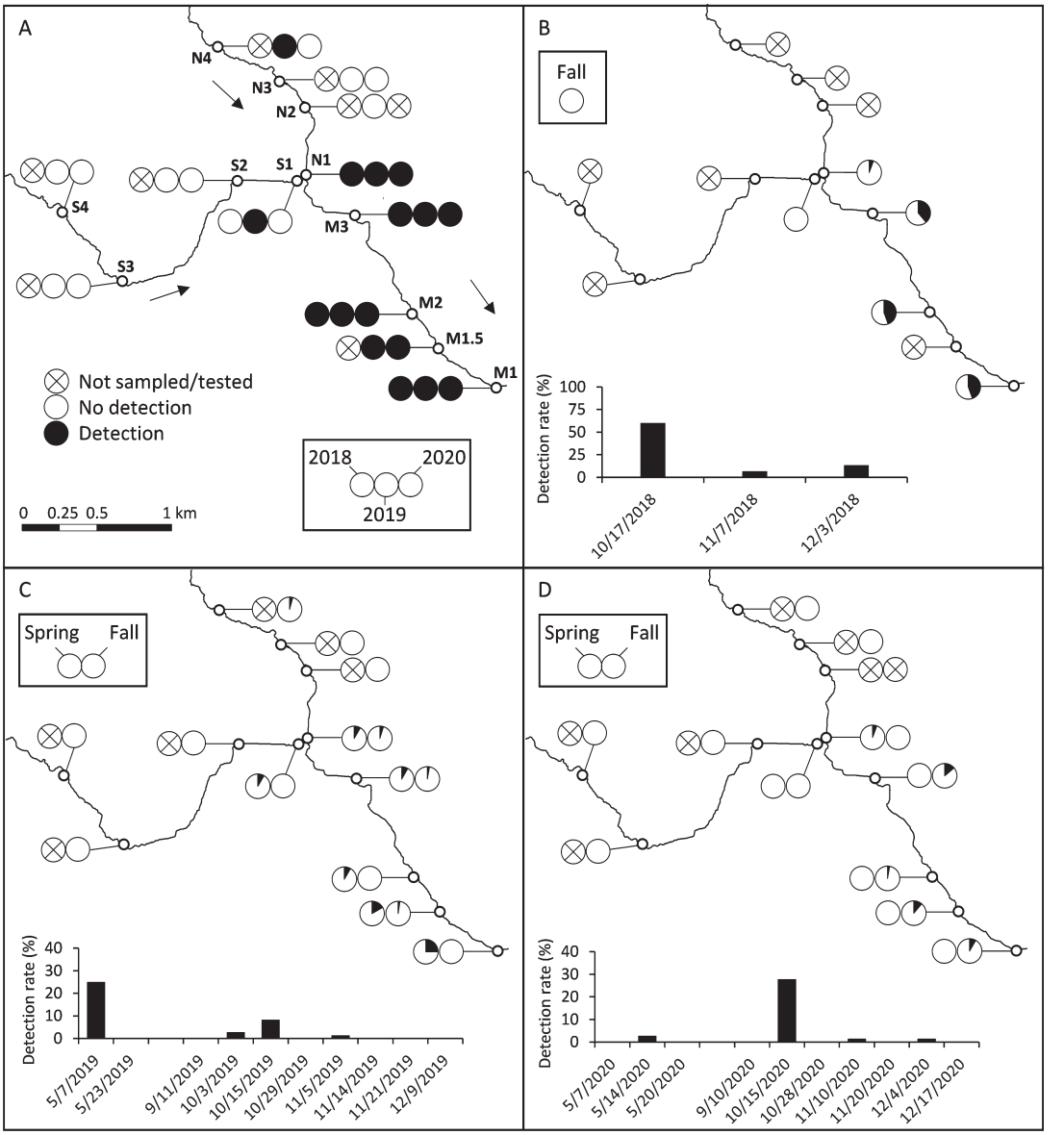

Figure 3. (A) Summary of Thornton Creek Chinook Salmon eDNA survey results for each site sampled in 2018, 2019 and 2020. Arrows indicate direction of stream flow. B–D: eDNA detection rates across sites (black color in pie graphs) and across sites on each sample day (bar graphs) for samples collected in fall 2018 (B), spring (May) and fall (September–December) 2019 (C), and spring (May) and fall (September–December) 2020 (D). Vertical axes on bar graphs are on different scales. See Supplemental Table S2 for comprehensive results.

and then decreased over time with mean detection probabilities peaking between 0.43 and 0.58 among years between mid-October and mid-November (Figure 6). In contrast, Day and Day^2 did not have a significant effect on detection of Chinook Salmon eDNA, although the negative value and magnitude of the δ coefficient for Day (Table 1) and distribution of detection probabilities (Figure 6) suggest that detection decreased during the adult survey period.

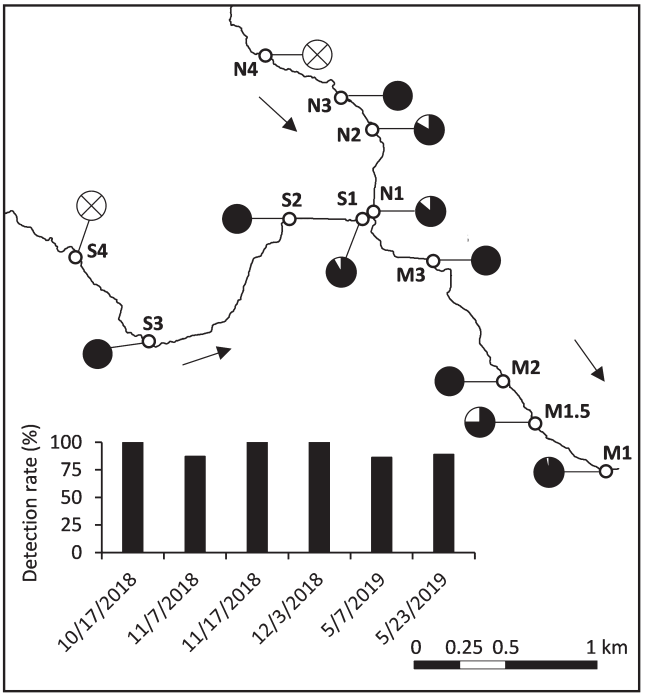

Figure 4. Summary of Thornton Creek Coastal Cutthroat Trout eDNA detection rates between October 2018 and May 2019 across sites (black color in pie graphs) and across sites on each sample day (bar graphs). Sites S4 and N4 were not sampled. Arrows indicate direction of stream flow. See Supplemental Table S3 for comprehensive results.

Discussion

Spatial and temporal surveys of Coho Salmon and Chinook Salmon eDNA in Thornton Creek revealed patterns of eDNA detection that were consistent with historical surveys of adults. During the fall, Coho Salmon eDNA was detected at multiple locations in the mainstem and both branches, and Chinook Salmon eDNA was typically detected in the mainstem. Positive and reproducible eDNA detection results across multiple years (2018 and 2020) and across sites sampled on the same day for Chinook Salmon in October and for Coho Salmon between October and November provides strength of evidence for the presence of adults and coincided with the time when adults would be expected to access spawning streams in the Lake Washington basin (Wild Fish Conservancy 2008, Prokop et al. 2009). Our findings support a growing body of studies demonstrating the effectiveness of eDNA as a tool for monitoring life history events associated with reproduction (Bylemans et al. 2017, Tillotson et al. 2018, Bracken et al. 2019, Takeuchi et al. 2019, Thalinger et al. 2019).

Both Coho Salmon and Chinook salmon eDNA was detected further upstream than expected on the North and South branches during fall surveys. The Coho Salmon detections at S3 and N3 in 2018 and at S4 in 2019 were unexpected due to lack of historical sightings near these sites. The detections at S3 and N3 in 2018 coincided with positive detections at nearly all other downstream sites sampled on the same day, providing confidence in these results, and suggesting that adults migrated above partial barriers that are present in both branches. The Coho Salmon eDNA detection at S4 in 2019 is idiosyncratic because although this detection represented amplification across all three PCR technical replicates from one water sample, Coho Salmon eDNA was not detected below this site at any time during the 2019 fall survey, with the exception of a single PCR amplification at M3 on 3 October, suggesting the detection at S4 be interpreted with caution in the larger context of the Coho Salmon distribution. While Chinook Salmon eDNA was detected above their expected distribution during the fall surveys (i.e., a single PCR amplifying at N4 in 2019), Chinook Salmon eDNA was only sparsely detected and at low levels during the fall 2019 survey, suggesting the detection at N4 could be a false positive, possibly resulting from contamination, allochthonous DNA, or non-specific amplification. False-positive errors can produce biased estimates of occupancy, occurrence, and detection probabilities, but removing samples that register only a single PCR amplification can also bias these estimates (Lahoz-Monfort et al. 2016). Study designs often incorporate replication across water samples, PCRs, and spatial and temporal levels

Table 1. Estimates of posterior means and 95% credible intervals (CI) for the occupancy models fitted to eDNA data from the Coho Salmon and Chinook Salmon adult return time (October–December) in 2018, 2019, and 2020. Estimates are shown on the probit scale for covariate coefficients (β, α, and δ) and on the probability scale at the mean of continuous covariates. Year was included as a factor for α and δ with 2018 as the reference year, indicated as Intercept (α_0 or δ_0), with the slope set to zero.

Species	Parameter	Probit scale Mean (95% CI)[1]	Probability scale Mean (95% CI)
Coho	Site (Ψ)		
	Intercept (β_0)	1.479 (0.505 to 2.664)	0.904 (0.693 to 0.996)
	Distance (β_1)	-0.268 (-1.292 to 0.823)	
	Water Sample (θ)		
	Intercept (α_0)	-0.059 (-0.417 to 0.339)	0.477 (0.338 to 0.633)
	Year_2019 (α_1)	**-1.848** (-2.436 to -1.274)	0.033 (0.007 to 0.085)
	Year_2020 (α_2)	-0.003 (-0.484 to 0.498)	0.476 (0.318 to 0.670)
	Day (α_3)	0.250 (-0.622 to 1.112)	
	Day^2 (α_4)	-0.411 (-1.449 to 0.717)	
	PCR detection (*p*)		
	Intercept (δ_0)	0.190 (-0.145 to 0.527)	0.574 (0.442 to 0.701)
	Year_2019 (δ_1)	-0.292 (-1.329 to 0.657)	0.464 (0.135 to 0.792)
	Year_2020 (δ_2)	-0.364 (-0.811 to 0.089)	0.432 (0.295 to 0.580)
	Day (δ_3)	**1.479** (0.640 to 2.326)	
	Day^2 (δ_4)	**-2.111** (-3.053 to -1.145)	
Chinook	Site (Ψ)		
	Intercept (β_0)	0.692 (-0.256 to 1.779)	0.730 (0.399 to 0.962)
	Distance (β_1)	-0.561 (-1.648 to 0.543)	
	Water Sample (θ)		
	Intercept (α_0)	-0.052 (-0.614 to 0.909)	0.518 (0.270 to 0.818)
	Year_2019 (α_1)	-1.021 (-2.071 to 0.528)	0.209 (0.018 to 0.772)
	Year_2020 (α_2)	-0.284 (-1.286 to 1.107)	0.412 (0.099 to 0.930)
	Day (α_3)	-0.514 (-1.732 to 0.726)	
	Day^2 (α_4)	0.425 (-0.931 to 1.798)	
	PCR detection (*p*)		
	Intercept (δ_0)	-0.003 (-0.549 to 0.551)	0.499 (0.292 to 0.709)
	Year_2019 (δ_1)	**-1.804** (-2.827 to -0.827)	0.054 (0.003 to 0.215)
	Year_2020 (δ_2)	**-1.202** (-1.973 to -0.374)	0.132 (0.028 to 0.361)
	Day (δ_3)	-1.115 (-2.303 to 0.122)	
	Day^2 (δ_4)	0.341 (-0.975 to 1.608)	

[1]Coefficient estimates with 95% credible intervals that do not overlap zero have less than 5% chance of obtaining the posterior mean estimate by chance and are shown in boldface type.

because repeatable results improve the strength of evidence, which in turn provides greater confidence in the results. The cases where only a single PCR amplified across replicate water samples provide lower strength of evidence, particularly when the single amplification was the only case of detection on a sampling day.

Detection of Chinook Salmon DNA in water samples collected downstream of an adult female demonstrates that eDNA is effective for detecting adult salmon at extremely low densities in small urban streams. The single female Chinook Salmon observed on 17 October 2018 was the first confirmed sighting of a Chinook Salmon in Thornton Creek since 2010. Coincidently, we had planned to begin the eDNA survey on 17 October. On this day, the female was observed digging a redd just below the confluence of the North and

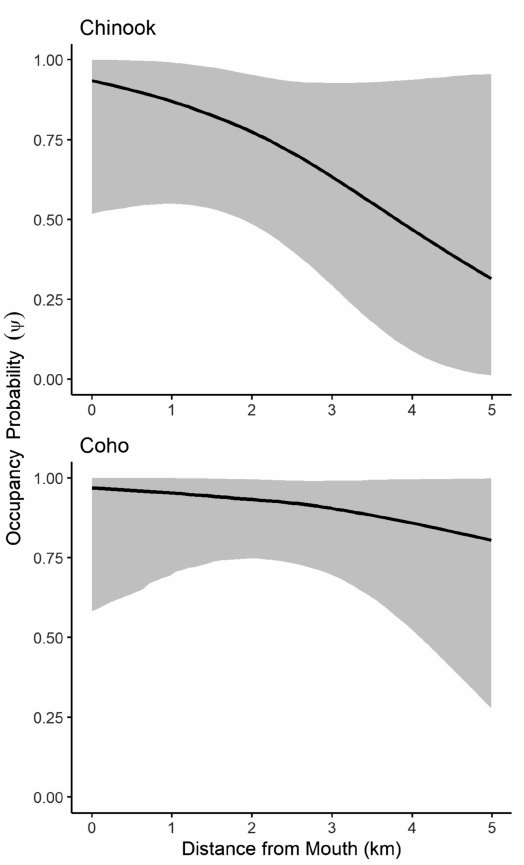

Figure 5. Occupancy probability estimates (with shaded areas indicating 95% credible intervals) for Chinook Salmon and Coho Salmon eDNA as a function of distance (km) from Lake Washington during the adult return time frame (October–December) across 2018, 2019, and 2020.

South branches. Three sites were sampled at approximately 0.4 km (M3), 1.3 km (M2), and 2.1 km (M1) downstream of the female and all PCR technical replicates amplified Chinook Salmon DNA across all water samples. The high detection rate was probably boosted by the abrasion of skin cells into the water column during redd construction. The female was accompanied by a male a few days later, although it is unknown whether the male was present, or any other Chinook Salmon for that matter, when water samples were collected on 17 October. Approximately two weeks after the initial sighting, the pair were no longer observed and subsequent eDNA sampling yielded low-level amplifications, possibly corresponding to eDNA shed from carcasses (Merkes et al. 2014) or eggs (Ostberg and Chase 2022a) derived from the adult Chinook Salmon that were previously observed.

During spring surveys, detection of Chinook Salmon eDNA was primarily limited to a single day (7 May 2019) with lower sections (sites M1 and M1.5) producing robust detections relative to sites upstream. The eDNA source could have been offspring from the pair observed spawning in October 2018 and/or juveniles that moved into Thornton Creek during their outmigration from the Lake Washington basin. During their spring outmigration, juvenile Chinook Salmon find refuge in lower sections of non-natal streams like Thornton Creek (Tabor et al. 2011, Tabor and Moore 2020).

Spring surveys for Coho Salmon eDNA produced only a single detection at the lowermost site (M1), suggesting few or no juvenile Coho Salmon were present at the time of spring surveys. Evidence for few juvenile Coho Salmon inhabiting Thornton Creek, at least in 2019, is corroborated by an electrofishing survey performed across approximately 168 m of continuous stream habitat on 1–2 July 2019 where a single individual young-of-the-year was captured on successive days (C. Pier, Seattle Public Utilities, unpublished data). Several reasons could explain why Coho Salmon eDNA was not detected during the 2019 spring eDNA survey, although one or more juveniles was likely present. First, studies suggest juveniles shed less total eDNA than adults (Maruyama et al. 2014, Takeuchi et al. 2019); therefore, we may expect few juveniles to have lower detectability than few adults. Second, eDNA concentrations tend to be positively correlated with fish abundance (Bracken et al. 2019, Levi et al. 2019, Sepulveda et al. 2021), suggesting eDNA may be sparse in habitats with few juveniles. Third, the amount of eDNA that can be sampled from the water column is a function of the amount shed into the water column and the amount lost through degradation and deposition. Consequently, detectability decreases with increasing distance between eDNA source and sample collection sites (Jane et al. 2015, Balasingham et al. 2017, Spence et al. 2021). Further studies in

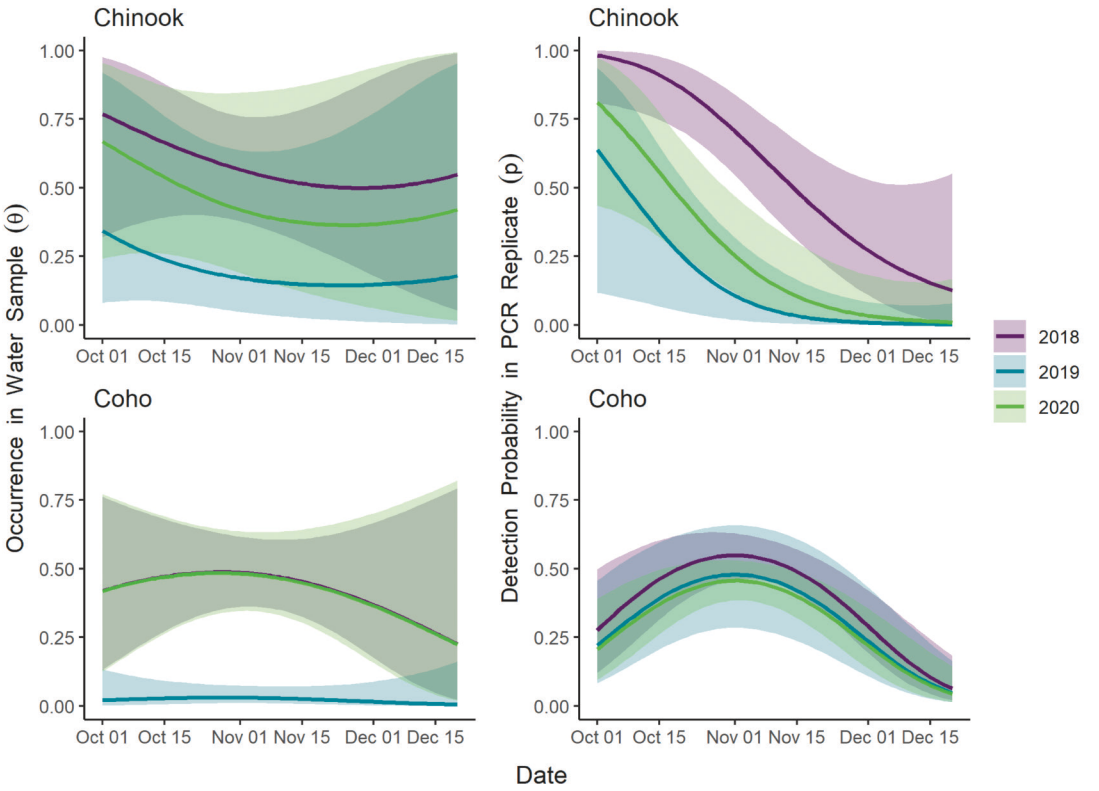

Figure 6. Results from occupancy modeling showing the occurrence probability of eDNA in a water sample (θ) and the detection probability of eDNA in a PCR technical replicate (*p*) during the adult return time frame (October–December) for each year (2018, 2019, and 2020). Day 1 for each year is October 1. Shaded areas indicate 95% credible intervals. In the panel for the occurrence probability of Coho Salmon eDNA, the mean and 95% credible intervals for 2018 and 2020 fall on top of each other.

small urban streams coupling eDNA sampling with traditional field methods that yield biomass estimates could provide greater understanding on the efficacy of eDNA for detecting juvenile salmon at low densities.

We found a substantial difference in eDNA detections between salmon and Coastal Cutthroat Trout, both spatially and temporally, reinforcing the importance of considering the ecology and life history of target species when designing eDNA monitoring surveys (Erickson et al. 2017, Ostberg et al. 2018, Duda et al. 2021). Our survey was designed to collect water samples around the time when salmon were historically present in Thornton Creek. The inclusion of temporally stratified sampling and sample replication into the sample design improved the probability of detecting salmon because their occupancy can be short-lived in small urban streams like Thornton Creek, particularly for Chinook Salmon. In contrast to migratory species such as salmon, sample timing and sample replication may be less important for common and widespread resident species, such as Coastal Cutthroat Trout, which were consistently detected at high frequency across temporal and spatial gradients.

Occupancy models fitted to eDNA survey data collected across the adult salmon return time suggested that distance, year, and day influenced site occupancy, occurrence, and detection probabilities. In our model, the site occupancy parameter estimated the probability that a site was occupied by eDNA at some point over the course of the eDNA survey. The occupancy prob-

ability for Coho Salmon eDNA was higher than Chinook Salmon eDNA, which is consistent with historically greater number of Coho Salmon adults returning to spawn in Thornton Creek (Wild Fish Conservancy 2008). Year-to-year variation in numbers of returning adults and their distribution within the stream can affect occupancy probability estimates. We evaluated the effect of distance upstream on site occupancy, and while the effect of distance was not significant, our results suggest higher occupancy probabilities for Chinook Salmon eDNA in the mainstem Thornton Creek compared to sites upstream. According to past surveys, most spawning activity for Chinook Salmon has occurred in the upper mainstem and in the lower North Branch (Prokop et al. 2009). The effect of sample site distance was nominal in Coho Salmon, supporting their broader spawning distribution. Historically, Coho Salmon spawning activity has focused on the mainstem, but they are more widely distributed in the watershed with documented sightings further upstream than Chinook Salmon in the North and South branches (Prokop et al. 2009).

The sample collection year had a notable effect on the occurrence and detection probabilities of Coho and Chinook salmon eDNA. Specifically, mean occurrence probabilities were 14 times and 2.0–2.5 times lower for Coho Salmon and Chinook Salmon eDNA, respectively, in 2019, compared to 2018 and 2020. Moreover, mean detection probabilities for Chinook Salmon eDNA in 2019 and 2020 were more than 3.5 times lower compared to 2018 while mean detection probabilities for Coho Salmon eDNA were roughly similar across years. Variability in occurrence and detection probabilities is apparent among years and between the species, suggesting that future eDNA survey designs could benefit by incorporating flexibility in sampling effort to attain desired probability thresholds. Thornton Creek spawning surveys indicate year-to-year variability in adult returns (Wild Fish Conservancy 2008, Prokop et al. 2009), which could explain differences in eDNA occurrence and detection among years. Fish abundance has been shown to have a positive association with eDNA concentrations in the water (Sepulveda et al. 2021, Rourke et al. 2022) and a positive effect on eDNA occurrence and detection probabilities (Strickland and Roberts 2018, Spence et al. 2021). Thus, at low densities, there is less eDNA available in the water column to be sampled, which, in turn, can affect the likelihood of detecting eDNA in a PCR replicate. The significantly higher detection probability of Chinook Salmon eDNA in 2018 is noteworthy and was likely influenced by the female that was observed digging a redd on the day when samples were collected.

Regarding an effect of sample collection day, we hypothesized that occurrence and detection probabilities might follow a run timing curve by first increasing and then decreasing across the spawning run. There was no evidence for a day effect on eDNA occurrence, but an effect on eDNA detection was evident for Coho Salmon and highly suggestive for Chinook Salmon. A day effect on eDNA detection may be expected when eDNA concentrations track adult salmon returns (Tillotson et al. 2018, Levi et al. 2019). Spawn timing for Coho Salmon in Thornton Creek occurs between October and mid-December (Prokop et al. 2009), and eDNA detection probabilities captured a run timing curve for Coho Salmon in Thornton Creek across survey days, with peak detection probabilities occurring from roughly mid-October through late November. Although sampling effort was similar across days, detection was variable across the run timing curve, with probabilities peaking between 0.43 and 0.58 and dipping to less than 0.15 at the tail of the curve among years. Spawn timing for Chinook Salmon in Thornton Creek is earlier than Coho Salmon (Prokop et al. 2009), as indicated by high detection probabilities at the beginning of the survey with a near linear reduction in detection probability shortly thereafter. Like Coho Salmon, Chinook eDNA detection probabilities were variable across each survey season and dropped by more than 3-fold from the beginning to the end of the survey. It is apparent that our eDNA survey did not fully cover the time frame for returning adult Chinook Salmon and inclusion of earlier sampling dates into the survey design would have likely provided the data to create a run timing curve.

Surveys of eDNA are not necessarily a replacement for traditional fish surveys, and both have distinct advantages and can be complementary (Beng and Corlett 2020, Carim et al. 2020, Keller et al. 2022). Traditional survey methods are advantageous because fish can be captured for species identification, collection of biometric data, diet analysis, tissue sampling for genetic analysis, and abundance estimates (Bonar et al. 2009, Radinger et al. 2019). However, traditional fish survey methods can be time consuming, intensive, typically require multiple personnel, and can be invasive (Moser et al. 2007, Bonar et al. 2009, Radinger et al. 2019). Private property ownership, which is common on urban streams, can restrict stream access for deploying traditional survey methods. Surveys of eDNA have great capacity as a monitoring tool because sampling is simple, noninvasive, and can be completed by a single person. Also, many sites can be sampled in a short period of time; samples can be collected at public access points; and eDNA methods have high sensitivity for detecting target species (Rees et al. 2014, Beng and Corlett 2020). In this study, eDNA was particularly effective at tracking adult salmon presence when sparse in numbers. The effectiveness for tracking juveniles in urban streams when juveniles are sparse in number is less clear, suggesting further studies are warranted.

Seattle Public Utilities has three primary fisheries-related information needs associated with urban streams: 1) identifying species and life stages present in each watershed; 2) identifying species distributions and upstream extent in watersheds; and 3) identifying hotspots of spawning and rearing activities. This information is important for evaluating projects focused on removing fish passage barriers, restoring aquatic and riparian habitat, improving water quality, and for obtaining salmon recovery focused grants. Environmental DNA-based methods can become part of the toolbox that helps address these information needs. While eDNA cannot differentiate life stages, it can be used to infer presence of different life stages, such as adults and juveniles, for species that have life stages with discrete seasonal differences in occupancy, like Chinook Salmon. Spatially stratified eDNA surveys in watersheds can be used to identify fish distributions and their upstream extent. Finally, eDNA surveys can be used as an initial survey method to efficiently identify habitats where traditional sampling methods might be employed to provide quantifiable fish abundance data for revealing hotspots of spawning and rearing activity.

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