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12 Spatial and Temporal Surveys of Salmon Environmental DNA (eDNA) in a Seattle Urban 13 Creek

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22 **Abstract**

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

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43 **Introduction**

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

55 Monitoring is an important element of urban stream management and fundamental to
56 adaptive management (Alberti et al. 2007, O'Neal et al. 2016, Rubin et al. 2017). In the city of
57 Seattle, WA, Seattle Public Utilities (SPU) has spent several decades monitoring and studying
58 fish to track salmonid abundance, distribution, and movements, and to evaluate fish migration
59 barriers. This information has helped to minimize environmental impacts associated with
60 operations, services, and capital investments in the City's urban watersheds. Seattle Public
61 Utilities uses information from fish surveys to acquire permits for operation and replacement of
62 infrastructure located in urban watersheds, acquire funding, track regulatory and contractual
63 obligations, and to plan urban watershed focused programs and projects. In addition, SPU has
64 completed several urban creek restoration projects to remove barriers to fish passage, expand
65 flood storage capacity, and improve aquatic and riparian habitat, with the goal of gathering

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66 information on project performance through post-project monitoring (Peter et al. 2019, Bakke et
67 al. 2020, Morley et al. 2021). A key performance measure of these creek restoration projects is
68 whether fish access and use newly restored habitats, which can be assessed through monitoring
69 and fish surveys.

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

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88 The advancement of environmental DNA (eDNA) for detecting and monitoring aquatic
89 species has expanded the toolbox for resource managers (Rees et al. 2014, Thomsen and
90 Willerslev 2015). Aquatic organisms shed DNA in the form of cellular and extra-cellular genetic
91 material into their environment through skin cells, mucous, feces, gametes, and other tissues,
92 enabling target species residing in aquatic habitats to be surveyed through eDNA. These surveys
93 collect water samples and aim to associate the presence of DNA from a target species in the
94 water sample with their physical presence in the environment. Consequently, eDNA surveys
95 have broad application to resource managers including monitoring for invasive (Erickson et al.
96 2017, Carim et al. 2019) and imperiled species (Bylemans et al. 2017), monitoring spawning
97 migrations (Thalinger et al. 2019, Duda et al. 2021), assessing species re-introductions (Riaz et
98 al. 2020), providing information on spatial distributions (Schmelzle and Kinziger 2016, Ostberg
99 et al. 2018), identifying migration barriers (Yamanaka and Minamoto 2016, Halvorsen et al.
100 2020), and evaluating recolonization following barrier removal (Duda et al. 2021). Sampling and
101 analysis of eDNA is efficient and cost-effective, and several studies have demonstrated that
102 eDNA surveys perform as well or better than traditional field sampling methods in detecting
103 target species (Jerde et al. 2011, Dejean et al. 2012, Pilliod et al. 2013, Schmelzle and Kinziger
104 2016, Hinlo et al. 2017, Ostberg et al. 2019). With consistent monitoring over time, eDNA
105 surveys can provide information on spatial and temporal changes in species distributions
106 (Gingera et al. 2016, Bracken et al. 2019, Duda et al. 2021).

107 The purpose of this study was to use eDNA surveys to assess occupancy and distribution
108 of Chinook Salmon, Coho Salmon, and Coastal Cutthroat Trout (*O. clarki clarki*) in Thornton
109 Creek, a recently restored urban stream in Seattle. We used eDNA detection as a proxy for
110 species presence. The study objectives were to assess the temporal and spatial distribution of

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111 adult salmon in fall and juvenile salmon in spring, compare eDNA detection between transient
112 species with low densities (Chinook Salmon and Coho Salmon) and a common species with
113 higher densities (Coastal Cutthroat Trout), and fit multiscale hierarchical occupancy models to
114 evaluate the effects of distance upstream, year, and day on the probabilities of occupancy,
115 occurrence, and detection of eDNA from adult Chinook Salmon and Coho Salmon in Thornton
116 Creek during the adult return time. While this study is specific to SPU and Thornton Creek, it
117 has broader implications for natural resource managers as a case study for surveying fish eDNA
118 in watersheds where traditional survey methods, like spawner surveys and smolt trapping, can be
119 challenging when fish occur in low densities and streams exhibit flashy flows following the onset
120 of precipitation.

121 **Methods**

122 Study area

123 Thornton Creek is the largest watershed within Seattle, covering approximately 2,942 ha (Figure
124 1). The creek is about 32 km in length, consisting of two main branches (North Branch and
125 South Branch) and 20 smaller tributaries. The creek flows generally from northwest to southeast
126 and drains into Lake Washington. Much of the mainstem is a low gradient channel (median
127 0.5%–0.9%). The North Branch is also low gradient (median around 1%) whereas the South
128 Branch is steeper gradient (median 1%–2%) (City of Seattle 2007). The watershed hosts at least
129 16 different fish species of which 12 are native species, including Chinook Salmon, Coho
130 Salmon, Sockeye Salmon (*O. nerka*), Coastal Cutthroat Trout, Rainbow Trout (*O. mykiss*),
131 Peamouth (*Mylocheilus caurinus*), Largescale Sucker (*Catostomus macrocheilus*), Longnose
132 Dace (*Rhinichthys cataractae*), Lamprey spp. (Petromyzontidae), Prickly Sculpin (*Cottus asper*),
133 Coastrange Sculpin (*Cottus aleuticus*), and Threespine Stickleback (*Gasterosteus aculeatus*).

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134 The four non-native species include Largemouth Bass (*Micropterus salmoides*), Rock Bass
135 (*Ambloplites rupestris*), Pumpkinseed (*Lepomis gibbosus*), and Pond Loach (*Misgurnus*
136 *anguillicaudatus*). Coastal Cutthroat Trout are the most abundant fish species in Thornton Creek
137 (Prokop et al. 2009, Tabor et al. 2010).

138 Coho Salmon adults typically spawn in low numbers in the Thornton Creek mainstem,
139 which is about 2.2 km long, but also travel further upstream on the South and North branches.
140 Chinook Salmon adults also spawn in the mainstem and two branches but typically at lower
141 numbers than Coho Salmon. Past salmon spawning surveys conducted between 1999 and 2008
142 documented between 8 and 135 Coho Salmon adult observations (both live and dead) and
143 between 2 and 12 Chinook Salmon adults per year (Wild Fish Conservancy 2008). More
144 recently, SPU conducted salmon spawning surveys between 2016–2018. The surveys were
145 mostly restricted to the mainstem of Thornton Creek. Between one and five Coho Salmon
146 observations (both live and dead) were documented in 2016 and 2017 and none in 2018,
147 although three redds were documented in 2018. Chinook Salmon were not observed during the
148 2016–2018 spawning surveys; however, on October 17, 2018, during an SPU educational site
149 tour an adult female hatchery Chinook Salmon was observed by one of the authors (C. Pier,
150 Seattle Public Utilities) and a few days later, a male hatchery Chinook Salmon was observed
151 spawning with the female. Juvenile Coho Salmon and Chinook Salmon have been captured in
152 smolt traps deployed in the lower mainstem between 2001 and 2008 (roughly 350 m upstream of
153 site M1). A single juvenile Coho Salmon was also collected during electrofishing surveys as
154 recently as summer of 2019.

155 Environmental DNA sampling and analysis

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156 Sampling was carried out in fall (October–December), with the goal of detecting eDNA from
157 adults returning to spawn, and in spring (May), with the goal of detecting eDNA from juveniles
158 hatched in Thornton Creek and/or occupying habitats within the creek during their outmigration
159 from other locations in the Lake Washington basin (e.g., Cedar River, Bear Creek, Issaquah
160 Creek, and Issaquah Creek Hatchery). Sampling was also carried out in early September to
161 provide information on background eDNA levels prior to adult returns. We assumed that young-
162 of-the year Coho Salmon could be present in September because their juveniles typically
163 outmigrate during spring of their second year (Sandercock 1991, Weitkamp et al. 1995) and
164 juvenile Chinook Salmon would not be present because they typically outmigrate in spring as
165 young-of-the-year (Tabor and Moore 2020). Most of the mainstem sample sites represented
166 hotspots for salmon spawning activity based on past surveys. Conversely, the most upstream
167 sites on the South and North branches (S4 and N4) were selected as sites where salmon were not
168 expected to be detected due to downstream partial fish barriers and absence of historical salmon
169 sightings.

170 We surveyed eDNA in Thornton Creek by collecting water samples on 24 sampling days
171 across multiple locations between October 17, 2018, and December 17, 2020 (Figure 1). A total
172 of nine sites were sampled in Thornton Creek during fall 2018. In 2019, three sites (M1.5, N4,
173 S4) were added based on the 2018 results. At the beginning of the salmon run in October, the
174 objective was to focus the sampling effort on sites in the mainstem and lower South and North
175 branches. Later in the season, the focus shifted to include sampling of upstream sites on the two
176 branches with the assumption that salmon would be more widely distributed throughout the
177 watershed.

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178 To survey eDNA at a site on a specific date, two 1-L sub-surface water sample replicates
179 were collected using pre-sterilized Nalgene plastic bottles. Water samples were placed on ice in a
180 cooler until they were filtered in the laboratory, which typically occurred within 6 hours after
181 collection. Each water sample was filtered through a pre-sterilized, 47 mm diameter filter funnel
182 with a 1 µm pore size cellulose nitrate sterile filter membrane (Thermo Fisher Scientific,
183 Waltham, Maine) to capture genetic material onto filters. A 1-L negative control sample
184 composed of deionized water (negative field control) was filtered in the laboratory, alongside
185 field collected water samples, at the end of each day that water samples were collected. After
186 filtration, filters were removed from the funnel by using sterile forceps and placed into sterile 5
187 mL tubes containing 95% ethanol and stored at -20 °C until DNA extraction. All Nalgene
188 bottles, filter funnels and forceps were sterilized prior to their use by soaking in 10% bleach for
189 at least 15 minutes followed by rinsing in tap water.

190 All laboratory procedures were designed to avoid cross contamination (Goldberg et al.
191 2016). The eDNA workflow and sample preparation was separated into designated work rooms
192 including a clean room where DNA was extracted (no amplified PCR products or highly
193 concentrated target DNA sequences allowed), a second room where PCR reagents were prepared
194 and loaded, a third room where DNA standards were diluted and loaded, and a fourth room
195 dedicated to PCR amplification. Sample preparation was performed in UV hoods using
196 equipment dedicated to processing eDNA samples at each workstation. Workstations were
197 decontaminated with UV and/or 10% bleach before and after each use.

198 The DNA collected onto filters was extracted following the protocol described in Duda et
199 al. (2021), using one half of each filter for extraction and archiving the other half at -20 ^{o}C.
200 Negative DNA extraction controls (extraction buffers only) were included during the DNA

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201 extraction process to identify any contamination of equipment and reagents during this
202 procedure.
203 All DNA extracts were tested for the presence of PCR inhibitors prior to testing for target
204 species, by performing an internal positive control (IPC) assay using TaqMan Exogenous
205 Internal Positive Control Reagents (EXO-IPC) (Applied Biosystems, Foster City, CA) and
206 quantitative PCR (qPCR). The IPC assay was performed in duplicate on each DNA sample in 10
207 µl volumes consisting of 5 µl of Gene Expression Master Mix (Thermo Fisher Scientific), 1 µl
208 EXO-IPC mix, 0.2 µl EXO-IPC DNA, 0.8 µl Nanopure sterile water and 3 µl DNA template or
209 sterile water for the non-template control. Samples were run on a ViiA 7 real-time PCR system
210 (Applied Biosystems) and cycling conditions for the IPC consisted of 10 min initial heat
211 activation at 95 ^{o}C, followed by 40 cycles of denaturing at 95 ^{o}C for 15 s and
212 annealing/extension at 60 ^{o}C for 1 min. Results were analyzed using ViiA 7 RUO 1.2.4 software
213 (Applied Biosystems). A DNA sample was considered inhibited when it had > 1 cycle threshold
214 (C_t) shift relative to the mean non-template control. Samples that were inhibited were treated
215 with OneStep PCR Inhibitor Removal kit (Zymo Research Corporation, Irvine, California) and
216 re-tested with the IPC assay to confirm that PCR inhibition was alleviated.
217 Target species assays included Coho Salmon (COCytb_980–1093), Chinook Salmon
218 (CKCO3_464–534), and Coastal Cutthroat Trout (CCCytb_572–685) (Duda et al. 2021), but not
219 all DNA samples were assayed for each species (Supplemental Tables 1–3). Assays were
220 performed in triplicate (i.e., three PCR technical replicates) on each sample in 10 µl reaction
221 volumes consisting of 3 µl DNA template, 1x Gene Expression Mastermix (Thermo Fisher
222 Scientific) and 1X custom TaqMan primer and probe mix consisting of a final concentration of
223 450 nM for each forward and reverse primers and 125 nM probe. All target species PCR assays

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224 were run on a ViiA 7 real-time PCR system (Applied Biosystems) with cycling parameters
225 consisting of initial steps of 2 min at 50 °C then 10 min at 95 °C, followed by 45 cycles of
226 denaturing at 95 °C for 15 s and annealing/extension at 60 °C for 1 min, and results were
227 analyzed using ViiA 7 RUO 1.2.4 software (Applied Biosystems). Each qPCR run consisted of a
228 five-point serial dilution of a DNA standard composed of a gBlock double-stranded DNA
229 fragment (Integrated DNA Technologies, Coralville, Iowa) specific to the target species, negative
230 field controls, negative DNA extraction controls, and no-template controls (sterile water in place
231 of DNA), all of which were run in triplicate.

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

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

245 To analyze detection-nondetection data from eDNA surveys, we fitted multiscale
246 occupancy models using the R package eDNAoccupancy (Dorazio & Erickson, 2018). The

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247 multiscale eDNA occupancy model design consisted of three nested, hierarchical levels: (i) the
248 site occupancy probability (Ψ_i, occupancy), defined as the probability of occurrence of eDNA at
249 site *i*; (ii) the occurrence probability (θ_{ij}, occurrence), defined as the conditional probability of
250 eDNA occurrence in water sample *j* given occupancy of eDNA at site *i*; and (iii) the detection
251 probability (p_{ijk}, detection), defined as the conditional probability of eDNA detection in PCR
252 technical replicate *k* given that it occurs in water sample *j* and site *i*.

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

259 $$\text{logit}(\Psi) = \beta_0 + \beta_1 \cdot Distance$$

260 where logit(x) is the logit link function [log(x) / (1+log(x)], β_0 is the intercept, and β_1 is the
261 slope measuring the effect of distance on Ψ. We hypothesized that downstream sites would have
262 higher occupancy probabilities. Both occurrence (θ) and detection (p) probability were each
263 modeled as a function of sample year (2018, 2019, and 2020) and sample day, where October 1
264 represented day 1:

265 $$\text{logit}(\theta) = \alpha_0 + \alpha_1 \cdot I_{(2019)} + \alpha_2 \cdot I_{(2020)} + \alpha_3 \cdot Day + \alpha_4 \cdot Day^2$$

266 $$\text{logit}(p) = \delta_0 + \delta_1 \cdot I_{(2019)} + \delta_2 \cdot I_{(2020)} + \delta_3 \cdot Day + \delta_4 \cdot Day^2.$$

267 Here, α and δ are the coefficients associated with covariate effects on θ and p. Year was
268 modeled as a factor where the intercepts (α_0 and δ_0) represent 2018 as the reference group,

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269 $I_{(year)}$ is an indicator function resolving to 1 for the specified year and zero otherwise, and
270 coefficients estimate each year's difference from 2018. Day was modeled with both linear
271 (Day) and quadratic (Day^2) terms because we hypothesized that occurrence and detection
272 probability might first increase and then decrease over time, following the timing of the salmon
273 spawning. All continuous covariates were standardized to zero mean and unit standard deviation.
274 Models were run using 500,000 Markov chain iterations with 250,000 burn-in steps and
275 graphically checked for convergence and stationarity. Model runs generated estimates of
276 posterior means for covariate parameter coefficients and covariates with significant effect were
277 identified as coefficients with 95% credible intervals (CI) that did not overlap zero.

278 **Results**

279 Coho Salmon

280 We tested for Coho Salmon eDNA on all 24 survey days (Supplemental Table 1). Positive
281 detections varied spatially and temporally (Figure 2). The spatial distribution of Coho Salmon
282 eDNA was most widespread in fall 2018, with the highest detection rates occurring in November
283 and positive detections occurring in multiple replicate water samples and across all sampling
284 days. In fall 2018, detection rates ranged from 33.3%–55.6% across mainstem sites, 12.5%–
285 54.2% across North Branch sites, and 4.2%– 58.3% across South Branch sites, and positive
286 detections occurred as far upstream as N3 and S3. In fall 2019, a single PCR amplified from
287 October 3 (M3) and the next positive detection occurred over one month later on November 14 at
288 the uppermost site on the South Branch (S4) where all three PCR technical replicates amplified
289 in one of two water samples. One week later (November 21), a single PCR amplified at the same
290 site, but no other sites registered a positive detection. In fall 2020, detection rates ranged between
291 24% and 43% across mainstem sites, with the highest detection rates occurring mid-late October,

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292 and sampling days where the only positive result was a single PCR amplification occurred on
293 September 10 at N1 and on December 17 at M3. Five eDNA surveys were performed across
294 spring 2019 and 2020, yielding only a single PCR amplifying for Coho Salmon, occurring at M1
295 on May 20, 2020 (Figure 2).

296 Chinook Salmon

297 We tested for Chinook Salmon eDNA on 23 survey days (Supplemental Table 2). Positive
298 detections were found primarily in the mainstem (Figure 3). In fall 2018, each replicate water
299 sample collected at each site on the mainstem was positive on October 17 coinciding with a
300 visual sighting of a female Chinook Salmon on that day. Samples collected in both November
301 and December of the same year yielded positive detections across multiple sites and primarily in
302 the mainstem, though these detections typically represented single PCR amplifications. In fall
303 2019, detections occurred at low levels (mostly single PCR amplifications), few sites (M1.5, M3,
304 N1, and N4), and on few sampling days (October 3, October 15, and November 5). In fall 2020,
305 Chinook Salmon eDNA was detected on October 15 at all four sites sampled in the mainstem
306 and the only other detections occurred as single PCR amplifications at M2 on November 10 and
307 at M1 on December 4. During the spring surveys, Chinook Salmon eDNA was detected on May
308 7, 2019, in the mainstem and North and South branches and in 2020 at N1 as single PCR
309 amplification (Figure 3).

310 Coastal Cutthroat Trout

311 We tested for Coastal Cutthroat Trout eDNA on 6 sampling occasions between October 2018
312 and May 2019 (Supplemental Table 3). Coastal Cutthroat Trout eDNA was widespread and
313 prevalent, being detected at all sample sites and on all sampling occasions (Figure 4). Detection

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314 rates ranged between 75% and 100% across sample sites and between 86% and 100% across
315 sampling days.
316 Occupancy modeling
317 The mean site occupancy probability across survey years was higher for Coho Salmon (0.801)
318 than Chinook Salmon (0.657) (Table 1). As hypothesized, downstream sites tended to have
319 higher occupancy probabilities than upstream sites for both species, indicated by mean β_1
320 coefficient estimates that were negative for Coho Salmon and Chinook Salmon, although the
321 effect of distance was stronger for Chinook Salmon (Figure 5). However, the 95% CI for β
322 coefficient estimates for both species overlapped zero (Table 1), indicating uncertainty in the
323 posterior estimates for the effect of distance on site occupancy.
324 The mean occurrence probability of Coho Salmon eDNA was considerably lower in 2019
325 (0.13) than in either 2018 (0.49) or 2020 (0.49) and 95% CIs did not overlap, suggesting that
326 survey year had a significant effect (Table 1, Figure 6). The occurrence probability of Chinook
327 Salmon eDNA was not different among years, noted by overlapping 95% CIs, although the mean
328 probability was considerably lower in 2019 (0.29) compared to 2018 (0.51) and 2020 (0.44),
329 possibly indicating that Chinook Salmon eDNA was not sampled as effectively at occupied sites
330 in 2019 (Figure 6). Day and Day^2 did not have a significant effect on the occurrence of eDNA in
331 water samples for either species (Table 1).
332 The mean detection probability of Chinook Salmon eDNA was substantially higher in
333 2018 (0.50) than either 2019 (0.15) or 2020 (0.24) and 95% Cis did not overlap, suggesting that
334 survey year had a significant effect (Table 1, Figure 6). Survey year, however, did not have an
335 apparent effect on the detection of Coho Salmon eDNA as mean probabilities were similar
336 among years (2018, 0.55; 2019, 0.48; 2020, 0.46) and 95% CIs overlapped. Both Day and Day^2

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337 had a significant effect on the detection of Coho Salmon eDNA, indicating detection
338 probabilities first increased and then decreased over time with mean detection probabilities
339 peaking between approximately 0.40 and 0.50 among years between mid-October and mid-
340 November (Figure 6). In contrast, Day and Day^2 did not have a significant effect on detection of
341 Chinook Salmon eDNA, although the negative value and magnitude of the δ coefficient for Day
342 (Table 1) and distribution of detection probabilities (Figure 6) suggest that detection decreased
343 during the adult survey period.

344 **Discussion**

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

357 Both Coho Salmon and Chinook salmon eDNA was detected further upstream than
358 expected on the North and South branches during fall surveys. The Coho Salmon detections at
359 S3 and N3 in 2018 and at S4 in 2019 were unexpected due to lack of historical sightings near

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360 these sites. The detections at S3 and N3 in 2018 coincided with positive detections at nearly all
361 other downstream sites sampled on the same day, providing confidence in these results, and
362 suggesting that adults migrated above partial barriers that are present in both branches. The Coho
363 Salmon eDNA detection at S4 in 2019 is idiosyncratic because although this detection
364 represented amplification across all three PCR technical replicates from one water sample, Coho
365 Salmon eDNA was not detected below this site at any time during the 2019 fall survey, with the
366 exception of a single PCR amplification at M3 on October 3, suggesting the detection at S4 be
367 interpreted with caution in the larger context of the Coho Salmon distribution. While Chinook
368 Salmon eDNA was detected above their expected distribution during the fall surveys (i.e., a
369 single PCR amplifying at N4 in 2019), Chinook Salmon eDNA was only sparsely detected and at
370 low levels during the fall 2019 survey, suggesting the detection at N4 could be a false-positive,
371 possibly resulting from contamination, allochthonous DNA, or non-specific amplification. False-
372 positive errors can produce biased estimates of occupancy, occurrence, and detection
373 probabilities, but removing samples that register only a single PCR amplification can also bias
374 these estimates (Lahoz-Monfort et al. 2016). Study designs often incorporate replication across
375 water samples, PCRs, and spatial and temporal levels because repeatable results improve the
376 strength of evidence, which in turn provides greater confidence in the results. The cases where
377 only a single PCR amplified across replicate water samples provide lower strength of evidence,
378 particularly when the single amplification was the only case of detection on a sampling day.

379 Detection of Chinook Salmon DNA in water samples collected downstream of an adult
380 female demonstrates that eDNA is effective for detecting adult salmon at extremely low densities
381 in small urban streams. The single female Chinook Salmon observed on October 17, 2018, was
382 the first confirmed sighting of a Chinook Salmon in Thornton Creek since 2010. Coincidently,

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

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

402 Spring surveys for Coho Salmon eDNA produced only a single detection at the
403 lowermost site (M1), suggesting few or no juvenile Coho Salmon were present at the time of
404 spring surveys. Evidence for few juvenile Coho Salmon inhabiting Thornton Creek, at least in
405 2019, is corroborated by an electrofishing survey performed across approximately 168 meters of

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406 continuous stream habitat on July 1–2, 2019, where a single individual young-of-the-year was
407 captured on successive days (C. Pier, Seattle Public Utilities, unpublished data). Several reasons
408 could explain why Coho Salmon eDNA was not detected during the 2019 spring eDNA survey,
409 although one or more juveniles was likely present. First, studies suggest juveniles shed less total
410 eDNA than adults (Maruyama et al. 2014, Takeuchi et al. 2019); therefore, we may expect few
411 juveniles to have lower detectability than few adults. Second, eDNA concentrations tend to be
412 positively correlated with fish abundance (Bracken et al. 2019, Levi et al. 2019, Sepulveda et al.
413 2021), suggesting eDNA may be sparse in habitats with few juveniles. Third, the amount of
414 eDNA that can be sampled from the water column is a function of the amount shed into the water
415 column and the amount lost through degradation and deposition. Consequently, detectability
416 decreases with increasing distance between eDNA source and sample collection sites (Jane et al.
417 2015, Balasingham et al. 2017, Spence et al. 2021). Further studies in small urban streams
418 coupling eDNA sampling with traditional field methods that yield biomass estimates could
419 provide greater understanding on the efficacy of eDNA for detecting juvenile salmon at low
420 densities.

421 We found a substantial difference in eDNA detections between salmon and Coastal
422 Cutthroat Trout, both spatially and temporally, reinforcing the importance of considering the
423 ecology and life history of target species when designing eDNA monitoring surveys (Erickson et
424 al. 2017, Ostberg et al. 2018, Duda et al. 2021). Our survey was designed to collect water
425 samples around the time when salmon were historically present in Thornton Creek. The inclusion
426 of temporally stratified sampling and sample replication into the sample design improved the
427 probability of detecting salmon because their occupancy can be short lived in small urban
428 streams like Thornton Creek, particularly for Chinook Salmon. In contrast to migratory species

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429 such as salmon, sample timing and sample replication may be less important for common and
430 widespread resident species, such as Coastal Cutthroat Trout, which were consistently detected at
431 high frequency across temporal and spatial gradients.

432 Occupancy models fitted to eDNA survey data collected across the adult salmon return
433 time suggested that distance, year, and day influenced site occupancy, occurrence, and detection
434 probabilities. In our model, the site occupancy parameter estimated the probability that a site was
435 occupied by eDNA at some point over the course of the eDNA survey. The occupancy
436 probability for Coho Salmon eDNA was higher than Chinook Salmon eDNA, which is consistent
437 with historically greater number of Coho Salmon adults returning to spawn in Thornton Creek
438 (Wild Fish Conservancy 2008). Year-to-year variation in numbers of returning adults and their
439 distribution within the stream can affect occupancy probability estimates. We evaluated the
440 effect of distance upstream on site occupancy, and while the effect of distance was not
441 significant, our results suggests higher occupancy probabilities for Chinook Salmon eDNA in the
442 mainstem Thornton Creek compared to sites upstream. According to past surveys, most
443 spawning activity for Chinook Salmon has occurred in the upper mainstem and in the lower
444 North Branch (Prokop et al. 2009). The effect of sample site distance was nominal in Coho
445 Salmon, supporting their broader spawning distribution. Historically, Coho Salmon spawning
446 activity has focused on the mainstem, but they are more widely distributed in the watershed with
447 documented sightings further upstream than Chinook Salmon in the North and South branches
448 (Prokop et al. 2009).

449 The sample collection year had a notable effect on the occurrence and detection
450 probabilities of Coho and Chinook salmon eDNA. Specifically, mean occurrence probabilities
451 were 3.5 times and 1.5–1.8 times lower for Coho Salmon and Chinook Salmon eDNA,

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452 respectively, in 2019, compared to 2018 and 2020. Moreover, mean detection probabilities for
453 Chinook Salmon eDNA in 2019 and 2020 were more than 2 times lower compared to 2018 while
454 mean detection probabilities for Coho Salmon eDNA were roughly similar across years.
455 Variability in occurrence and detection probabilities is apparent among years and between the
456 species, suggesting that future eDNA survey designs could benefit by incorporating flexibility in
457 sampling effort to attain desired probability thresholds. Thornton Creek spawning surveys
458 indicate year-to-year variability in adult returns (Wild Fish Conservancy 2008, Prokop et al.
459 2009), which could explain differences in eDNA occurrence and detection among years. Fish
460 abundance has been shown to have a positive association with eDNA concentrations in the water
461 (Rourke et al. 2021, Sepulveda et al. 2021) and a positive effect on eDNA occurrence and
462 detection probabilities (Strickland and Roberts 2018, Spence et al. 2020). Thus, at low densities,
463 there is less eDNA available in the water column to be sampled, which, in turn, can affect the
464 likelihood of detecting eDNA in a PCR replicate. The significantly higher detection probability
465 of Chinook Salmon eDNA in 2018 is noteworthy and was likely influenced by the female that
466 was observed digging a redd on the day when samples were collected.

467 Regarding an effect of sample collection day, we hypothesized that occurrence and
468 detection probabilities might follow a run timing curve by first increasing and then decreasing
469 across the spawning run. There was no evidence for a day effect on eDNA occurrence, but an
470 effect on eDNA detection was evident for Coho Salmon and highly suggestive for Chinook
471 Salmon. A day effect on eDNA detection may be expected when eDNA concentrations track
472 adult salmon returns (Tillotson et al. 2018, Levi et al. 2019). Spawn timing for Coho Salmon in
473 Thornton Creek occurs between October and mid-December (Prokop et al. 2009), and eDNA
474 detection probabilities captured a run timing curve for Coho Salmon in Thornton Creek across

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475 survey days, with peak detection probabilities occurring from roughly mid-October through late
476 November. Although sampling effort was similar across days, detection was variable across the
477 run timing curve, with probabilities peaking between approximately 0.40 and 0.50 and dipping to
478 approximately 0.10 and 0.15 at the tails of the curve among years. Spawn timing for Chinook
479 Salmon in Thornton Creek is earlier than Coho Salmon (Prokop et al. 2009), as indicated by high
480 detection probabilities at the beginning of the survey with a near linear reduction in detection
481 probability shortly thereafter. Like Coho Salmon, Chinook eDNA detection probabilities were
482 variable across each survey season and dropped by more than 3-fold from the beginning to the
483 end of the survey. It is apparent that our eDNA survey did not fully cover the timeframe for
484 returning adult Chinook Salmon and inclusion of earlier sampling dates into the survey design
485 would have likely provided the data to create a run timing curve.

486 Surveys of eDNA are not necessarily a replacement for traditional fish surveys, and both
487 have distinct advantages and can be complimentary (Beng and Corlett 2020, Carim et al. 2020,
488 Keller et al. 2022). Traditional survey methods are advantageous because fish can be captured
489 for species identification, collection of biometric data, diet analysis, tissue sampling for genetic
490 analysis, and abundance estimates (Bonar et al. 2009, Radinger et al. 2019). However, traditional
491 fish survey methods can be time-consuming, intensive, typically require multiple personnel, and
492 can be invasive (Moser et al. 2007, Bonar et al. 2009, Radinger et al. 2019). Private property
493 ownership, which is common on urban streams, can restrict stream access for deploying
494 traditional survey methods. Surveys of eDNA have great capacity as a monitoring tool because
495 sampling is simple, noninvasive, and can be completed by a single person. Also, many sites can
496 be sampled in a short period of time; samples can be collected at public access points; and eDNA
497 methods have high sensitivity for detecting target species (Rees et al. 2014, Beng and Corlett

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498 2020). In this study, eDNA was particularly effective at tracking adult salmon presence when
499 sparse in numbers. The effectiveness for tracking juveniles in urban streams when juveniles are
500 sparse in number is less clear, suggesting further studies are warranted.

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

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692 **Tables**

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

Species	Parameter	Logit scale Mean (95% CI)	Probability scale Mean (95% CI)
Coho	Site (Ψ)		
	Intercept (β_0)	1.479 (0.505 – 2.664)	0.801 (0.624 – 0.935)
	Distance (β_1)	-0.268 (-1.292 – 0.823)	
	Water Sample (θ)		
	Intercept (α_0)	-0.059 (-0.417 – 0.339)	0.485 (0.397 – 0.584)
	Year_2019 (α_1)	**-1.848** (-2.436 – -1.274)	0.132 (0.080 – 0.202)
	Year_2020 (α_2)	-0.003 (-0.484 – 0.498)	0.485 (0.384 – 0.609)
	Day (α_3)	0.250 (-0.622 – 1.112)	
	Day^2 (α_4)	-0.411 (-1.449 – 0.717)	
	PCR detection (p)		
	Intercept (δ_0)	0.190 (-0.145 – 0.527)	0.547 (0.464 – 0.629)
	Year_2019 (δ_1)	-0.292 (-1.329 – 0.657)	0.476 (0.249 – 0.693)
	Year_2020 (δ_2)	-0.364 (-0.811 – 0.089)	0.457 (0.369 – 0.550)
	Day (δ_3)	**1.479** (0.640 – 2.326)	
	Day^2 (δ_4)	**-2.111** (-3.053 – -1.145)	
Chinook	Site (Ψ)		
	Intercept (β_0)	0.692 (-0.256 – 1.779)	0.657 (0.436 – 0.856)
	Distance (β_1)	-0.561 (-1.648 – 0.543)	
	Water Sample (θ)		
	Intercept (α_0)	-0.052 (-0.614 – 0.909)	0.512 (0.351 – 0.713)
	Year_2019 (α_1)	-1.021 (-2.071 – 0.528)	0.292 (0.109 – 0.678)
	Year_2020 (α_2)	-0.284 (-1.286 – 1.107)	0.443 (0.217 – 0.814)
	Day (α_3)	-0.514 (-1.732 – 0.726)	
	Day^2 (α_4)	0.425 (-0.931 – 1.798)	
	PCR detection (p)		
	Intercept (δ_0)	-0.003 (-0.549 – 0.551)	0.499 (0.366 – 0.634)
	Year_2019 (δ_1)	**-1.804** (-2.827 – -0.827)	0.152 (0.058 – 0.312)
	Year_2020 (δ_2)	**-1.202** (-1.973 – -0.374)	0.238 (0.128 – 0.412)
	Day (δ_3)	-1.115 (-2.303 – 0.122)	
	Day^2 (δ_4)	0.341 (-0.975 – 1.608)	

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

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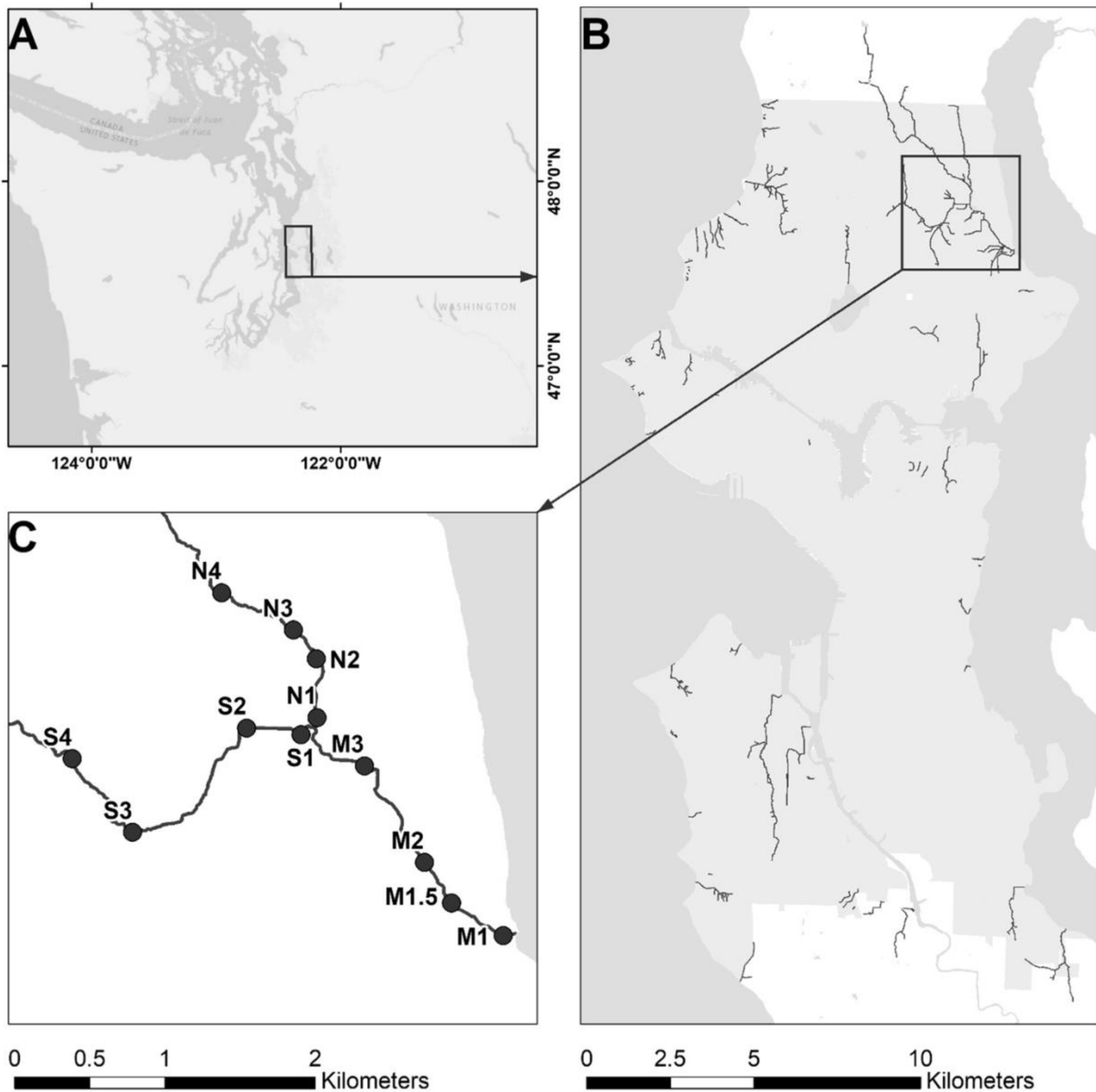
702 **Figure**



703

704 Figure 1. (A) Puget Sound region, Washington, USA. (B) City of Seattle (lighter gray

705 shaded area) displaying Thornton Creek (boxed area) in northeast Seattle. (C) eDNA sampling

706 locations (black points) on Thornton Creek with mainstem sites (M), South Branch sites (S), and

707 North Branch sites (N).

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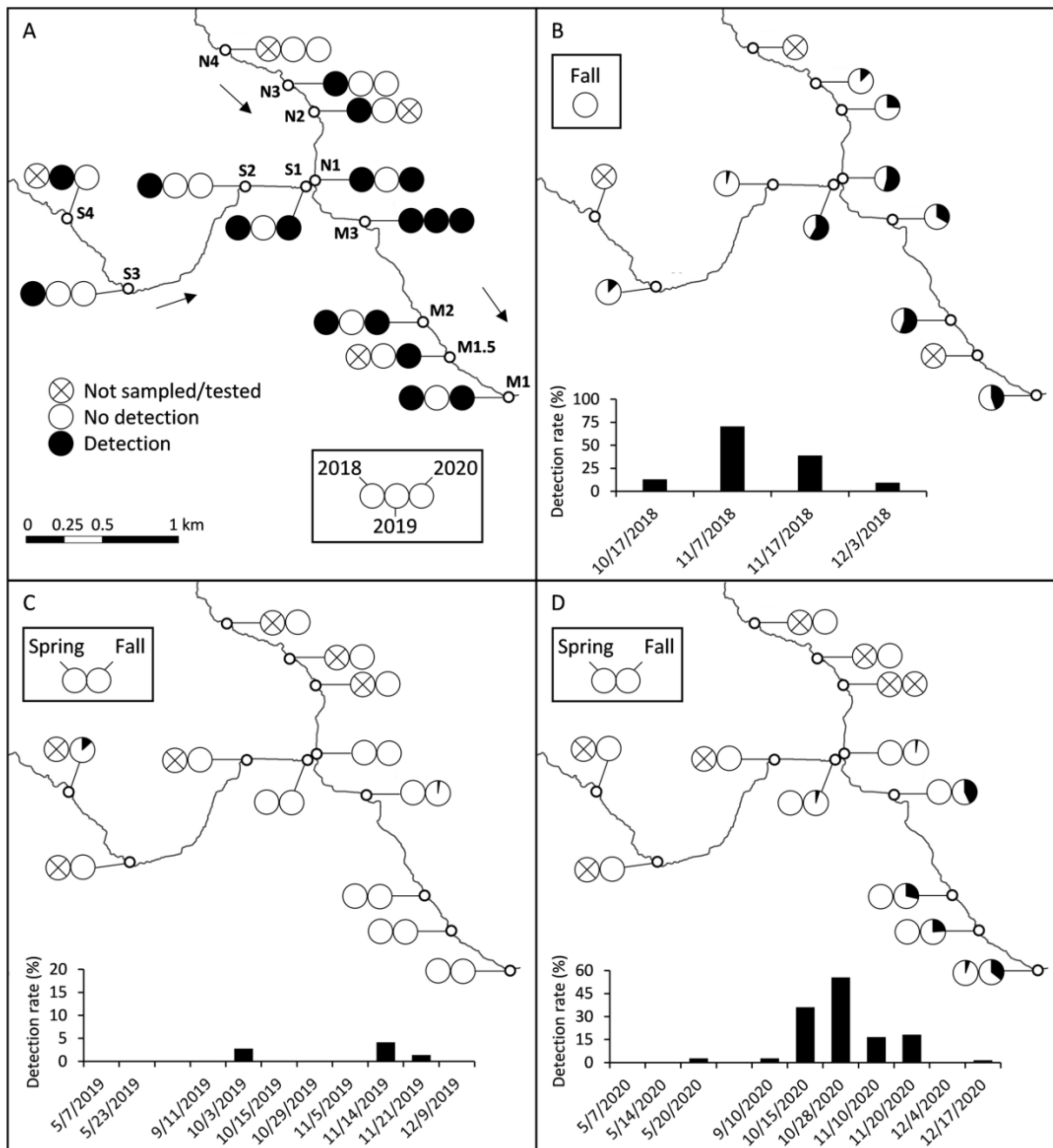

708

709 Figure 2. (A) Summary of Thornton Creek Coho Salmon eDNA survey results for each site

710 sampled in 2018, 2019 and 2020. Arrows indicate direction of stream flow. B–D: eDNA

711 detection rates across sites (black color in pie graphs) and across sites on each sample day (bar

712 graphs) for samples collected in fall 2018 (B), spring (May) and fall (September–December)

713 2019 (C), and spring (May) and fall (September–December) 2020 (D). Vertical axes on bar

714 graphs are on different scales. See Supplemental Table 1 for comprehensive results.

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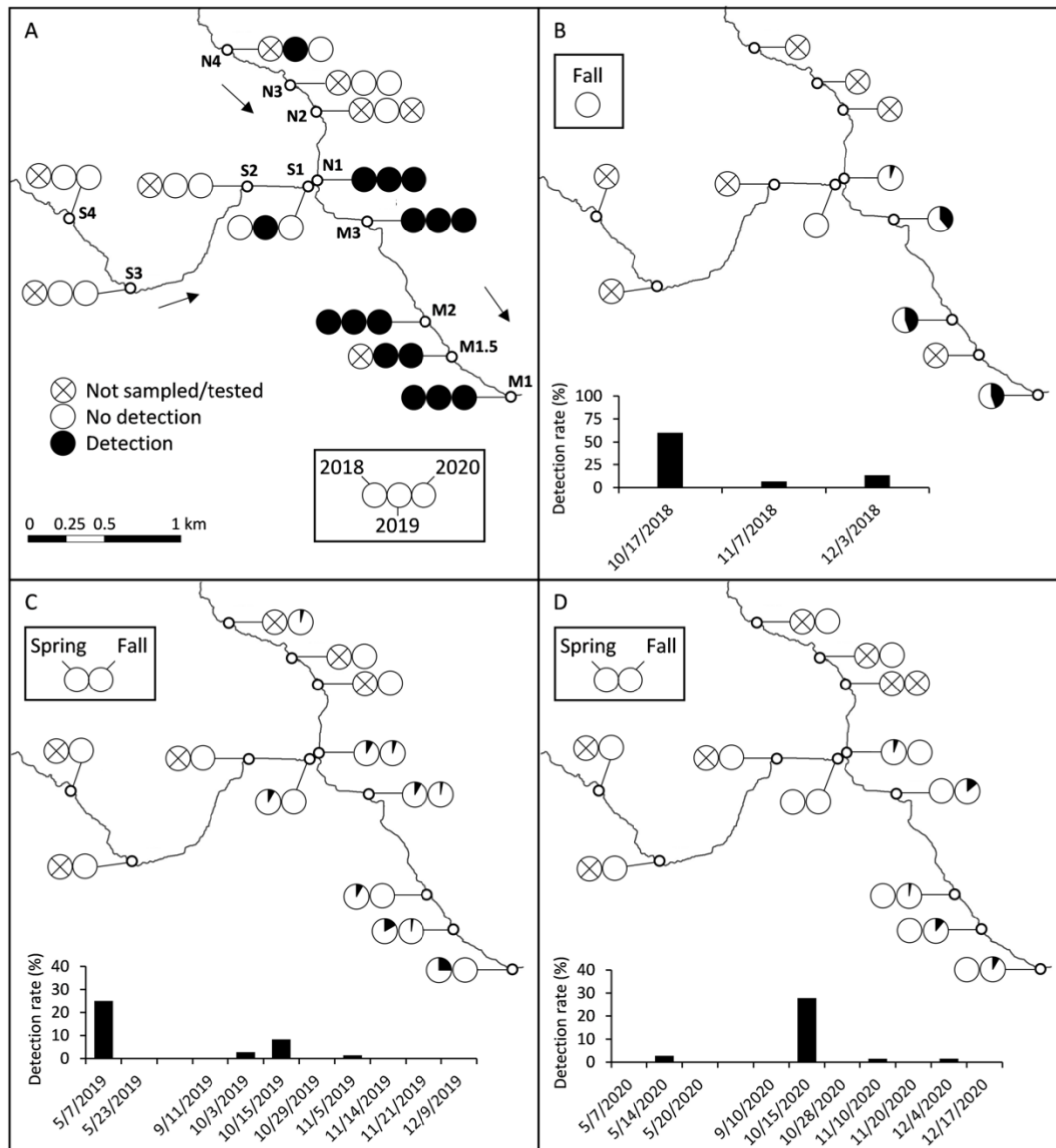

715

716 Figure 3. (A) Summary of Thornton Creek Chinook Salmon eDNA survey results for each
717 site sampled in 2018, 2019 and 2020. Arrows indicate direction of stream flow. B–D: eDNA
718 detection rates across sites (black color in pie graphs) and across sites on each sample day (bar
719 graphs) for samples collected in fall 2018 (B), spring (May) and fall (September–December)
720 2019 (C), and spring (May) and fall (September–December) 2020 (D). Vertical axes on bar
721 graphs are on different scales. See Supplemental Table 2 for comprehensive results.

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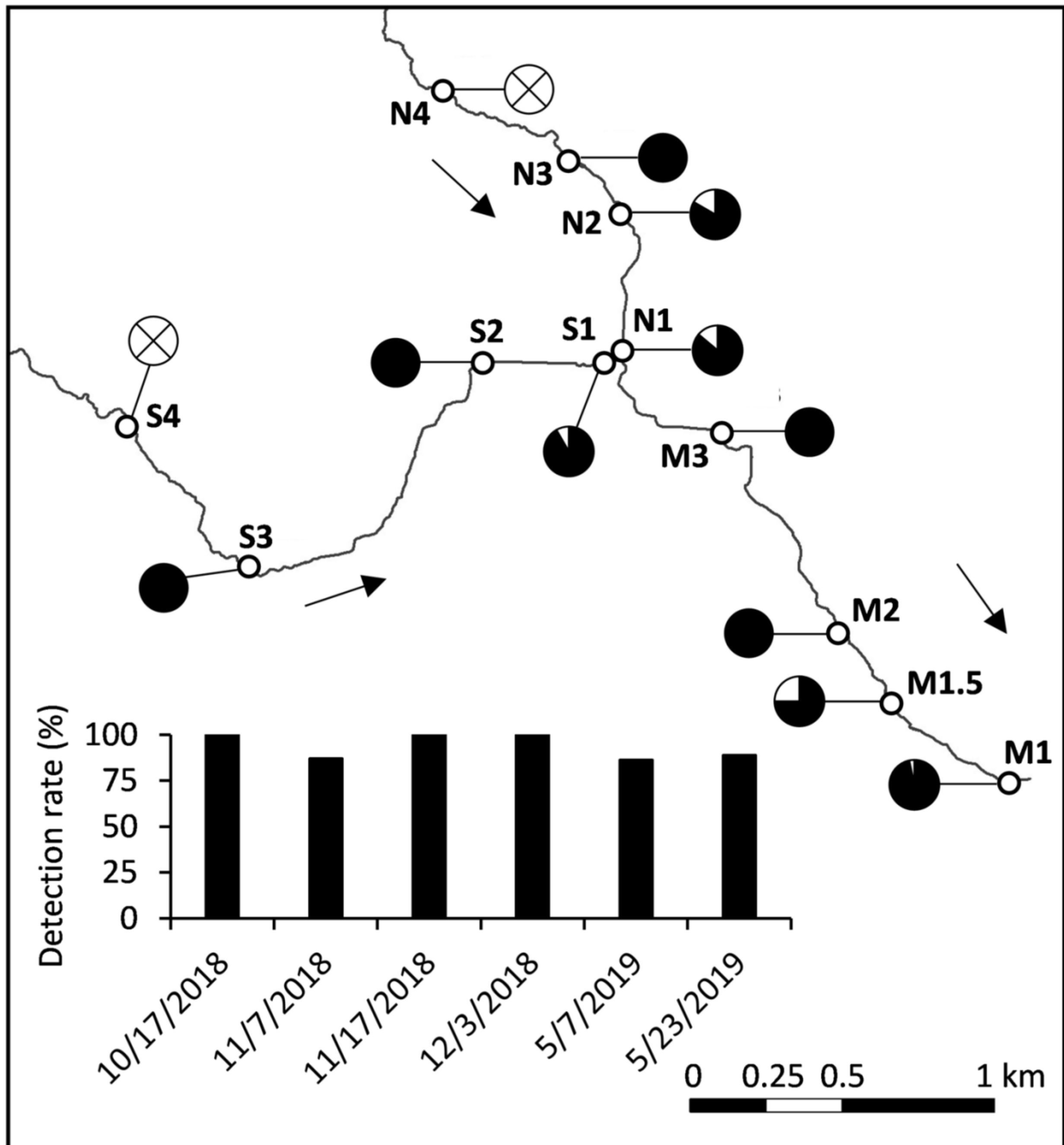

722

723 Figure 4. Summary of Thornton Creek Coastal Cutthroat Trout eDNA detection rates

724 between October 2018 and May 2019 across sites (black color in pie graphs) and across sites on

725 each sample day (bar graphs). Sites S4 and N4 were not sampled. Arrows indicate direction of

726 stream flow. See Supplemental Table 3 for comprehensive results.

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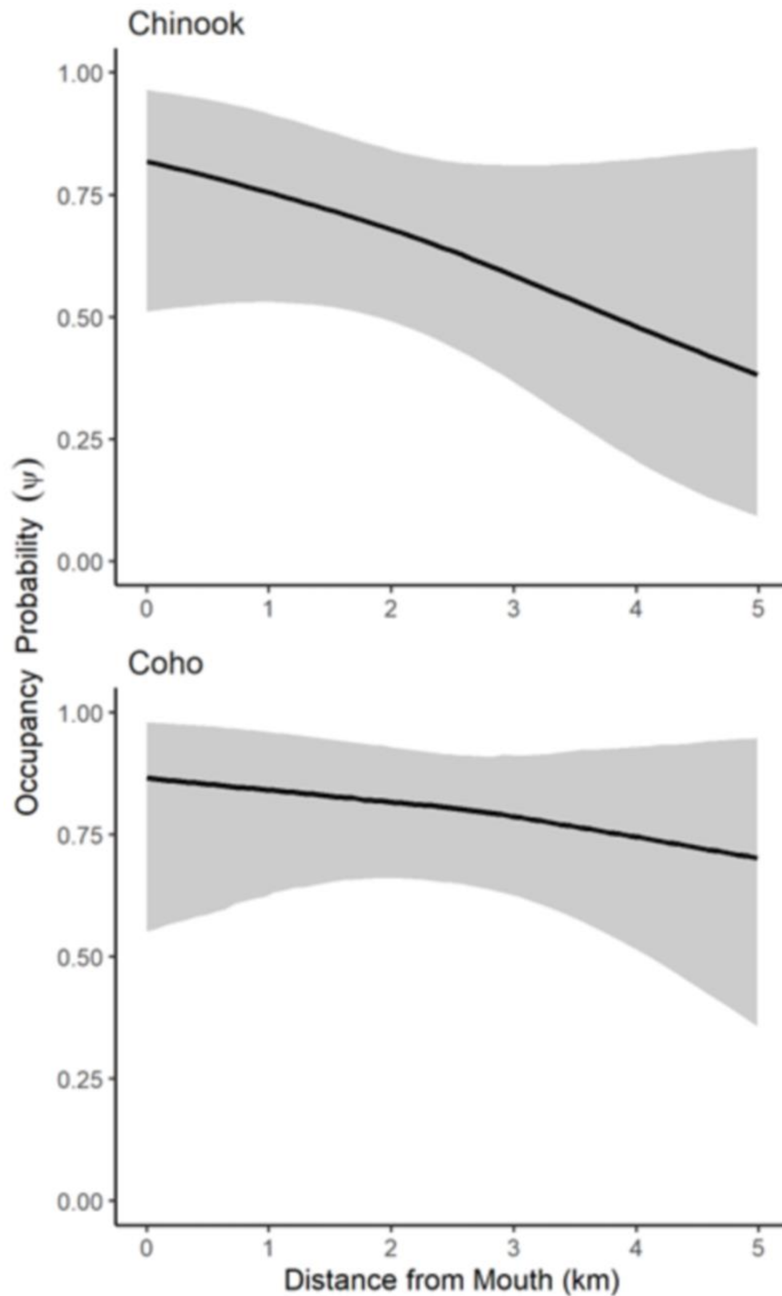

727

728 Figure 5. Occupancy probability estimates (with shaded areas indicating 95% credible

729 intervals) for Chinook Salmon and Coho Salmon eDNA as a function of distance (km) from

730 Lake Washington during the adult return time frame (October–December) across 2018, 2019,

731 and 2020.

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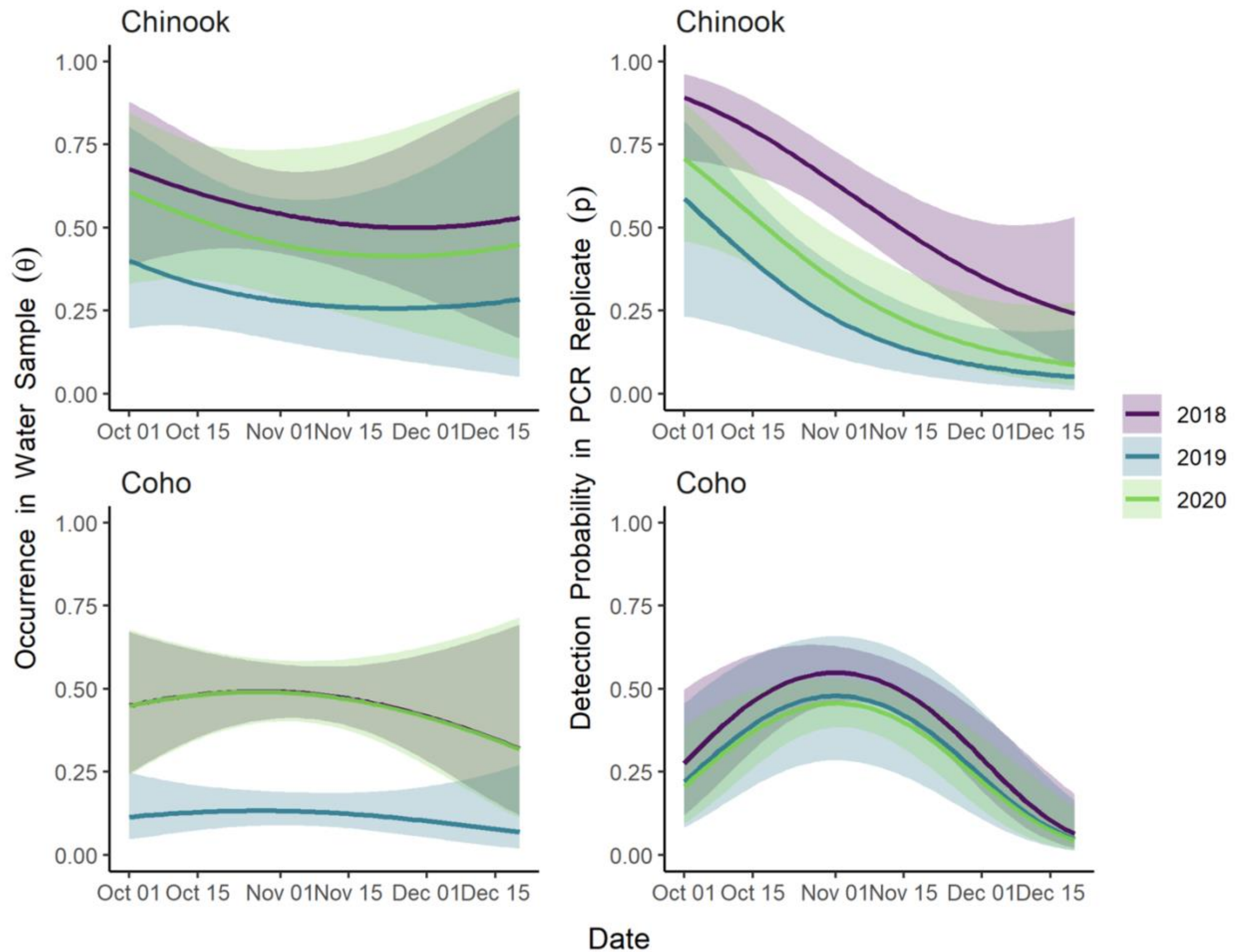

732

733 Figure 6. Results from occupancy modeling showing the occurrence probability of eDNA

734 in a water sample (θ) and the detection probability of eDNA in a PCR technical replicate (p)

735 during the adult return time frame (October–December) for each year (2018, 2019, and 2020) as

736 a function of days from the start of the study. Day 1 for each year is October 1. Shaded areas

737 indicate 95% credible intervals. In the panel for the occurrence probability of Coho Salmon

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Ostberg CO, Pier C, Chase DM. 2024. Spatial and temporal surveys of salmon environmental DNA (eDNA) in a Seattle urban creek. Northwest Science 97(3): *in press*.

Supplemental Tables

SUPPLEMENTAL TABLE 1. Coho salmon eDNA survey results for Thornton Creek (2018–2020). Two replicate water samples were collected at each site and three PCR technical replicates were performed on each water sample. The number of PCR technical replicates amplifying (0, 1, 2, or 3) in each of the two water samples collected at each site (separated by a slash, "/") is indicated. Sites that were not sampled on a given day are indicated by "ns" (not sampled).

Date	M1	M1.5	M2	M3	N1	N2	N3	N4	S1	S2	S3	S4
10/17/18	0/**2**	ns	**1/2**	0/0	0/0	0/0	0/0	ns	0/**2**	0/0	0/0	ns
11/7/18	**3/3**	ns	**3/3**	**3/3**	**3/3**	**1/3**	0/**2**	ns	**3/2**	0/0	**3**/0	ns
11/17/18	ns	ns	ns	ns	**3/3**	**1/1**	0/0	ns	**3/2**	0/**1**	0/0	ns
12/3/18	0/0	ns	**1**/0	0/0	0/**1**	0/0	0/**1**	ns	**1/1**	0/0	0/0	ns
5/7/19	0/0	0/0	0/0	0/0	0/0	ns	ns	ns	0/0	ns	ns	ns
5/23/19	0/0	0/0	0/0	0/0	0/0	ns	ns	ns	0/0	ns	ns	ns
9/11/19	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
10/3/19	0/0	0/0	0/0	**1**/0	0/0	ns	ns	ns	0/0	ns	ns	ns
10/15/19	0/0	0/0	0/0	0/0	0/0	ns	ns	ns	0/0	ns	ns	ns
10/29/19	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
11/5/2019	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
11/14/2019	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	**3**/0
11/21/2019	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/**1**
12/9/2019	0/0	0/0	ns	ns	0/0	0/0	0/0	ns	0/0	0/0	ns	ns
5/7/2020	0/0	0/0	0/0	0/0	0/0	ns	ns	ns	0/0	ns	ns	ns
5/14/2020	0/0	0/0	0/0	0/0	0/0	ns	ns	ns	0/0	ns	ns	ns
5/20/2020	**1**/0	0/0	0/0	0/0	0/0	ns	ns	ns	0/0	ns	ns	ns
9/10/2020	0/0	0/0	0/0	0/0	**1**/0	ns	ns	ns	0/0	ns	ns	ns
10/15/2020	**2/1**	0/**1**	**3/1**	**1/3**	0/0	ns	ns	ns	**1**/0	ns	ns	ns
10/28/2020	**2/3**	**2/2**	**1/3**	**3/3**	0/0	ns	ns	ns	0/**1**	ns	ns	ns
11/10/2020	**2/1**	**1/1**	**2**/0	**3/1**	0/0	ns	0/0	0/0	0/0	0/0	0/0	0/0
11/20/2020	**2/2**	**2/1**	**1/1**	**1/2**	0/0	ns	0/0	0/0	0/0	0/0	0/0	0/0
12/4/2020	0/0	0/0	0/0	0/0	0/0	ns	0/0	0/0	0/0	0/0	0/0	0/0

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12/17/2020	0/0	0/0	0/0	0/**1**	0/0	ns	0/0	0/0	0/0	0/0	0/0	0/0

Ostberg, C. O., and D. M. Chase. 2022. Spatial and temporal surveys of salmon eDNA in Seattle urban creeks, Washington, 2018 - 2020. U.S. Geological Survey data release. https://doi.org/10.5066/P9JY06SS.

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SUPPLEMENTAL TABLE 2. Chinook salmon eDNA survey results for Thornton Creek (2018–2020). Two replicate water samples were collected at each site and three PCR technical replicates were performed on each water sample. The number of PCR technical replicates amplifying (0, 1, 2, or 3) in each of the two water samples collected at each site (separated by a slash, "/") is indicated. Sites that were not sampled on a given day are indicated by "ns" (not sampled). Sites that were sampled but not tested are indicated by "nt" (not tested).

Date	M1	M1.5	M2	M3	N1	N2	N3	N4	S1	S2	S3	S4
10/17/18	**3/3**	ns	**3/3**	**3/3**	0/0	nt	nt	ns	0/0	nt	nt	ns
11/7/18	0/**1**	ns	0/0	0/**1**	0/0	nt	nt	ns	0/0	nt	nt	ns
12/3/18	0/**1**	ns	0/**2**	0/0	0/**1**	nt	nt	ns	0/0	nt	nt	ns
5/7/19	**3**/0	**1/1**	**1**/0	**1**/0	0/**1**	ns	ns	ns	**1**/0	ns	ns	ns
5/23/19	0/0	0/0	0/0	0/0	0/0	ns	ns	ns	0/0	ns	ns	ns
9/11/19	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
10/3/19	0/0	0/0	0/0	0/**1**	0/0	ns	ns	ns	0/0	ns	ns	ns
10/15/19	0/0	0/**1**	0/0	0/0	**2**/0	ns	ns	ns	0/0	ns	ns	ns
10/29/19	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
11/5/2019	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/**1**	0/0	0/0	0/0	0/0
11/14/2019	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
11/21/2019	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
12/9/2019	0/0	0/0	ns	ns	0/0	0/0	0/0	ns	0/0	0/0	ns	ns
5/7/2020	0/0	0/0	0/0	0/0	0/0	ns	ns	ns	0/0	ns	ns	ns
5/14/2020	0/0	0/0	0/0	0/0	**1**/0	ns	ns	ns	0/0	ns	ns	ns
5/20/2020	0/0	0/0	0/0	0/0	0/0	ns	ns	ns	0/0	ns	ns	ns
9/10/2020	0/0	0/0	0/0	0/0	0/0	ns	ns	ns	0/0	ns	ns	ns
10/15/2020	**2**/0	**1/2**	0/**1**	**2/3**	0/0	ns	ns	ns	0/0	ns	ns	ns
10/28/2020	0/0	0/0	0/0	0/0	0/0	ns	ns	ns	0/0	ns	ns	ns
11/10/2020	0/0	0/0	**1**/0	0/0	0/0	ns	0/0	0/0	0/0	0/0	0/0	0/0
11/20/2020	0/0	0/0	0/0	0/0	0/0	ns	0/0	0/0	0/0	0/0	0/0	0/0
12/4/2020	0/**1**	0/0	0/0	0/0	0/0	ns	0/0	0/0	0/0	0/0	0/0	0/ns
12/17/2020	0/0	0/0	0/0	0/0	0/0	ns	0/0	0/0	0/0	0/0	0/0	0/0

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SUPPLEMENTAL TABLE 3. Coastal cutthroat trout eDNA survey results for Thornton Creek (2018–2019). Two replicate water samples were collected at each site and three PCR technical replicates were performed on each water sample. The number of PCR technical replicates amplifying (0, 1, 2, or 3) in each of the two water samples collected at each site (separated by a slash, "/") is indicated. Sites that were not sampled on a given day are indicated by "ns" (not sampled).

Date	M1	M1.5	M2	M3	N1	N2	N3	N4	S1	S2	S3	S4
10/17/18	**3/3**	ns	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	ns	**3/3**	**3/3**	**3/3**	ns
11/7/18	**3/3**	ns	**3/3**	**3/3**	**3**/0	**2**/0	**3/3**	ns	**3/3**	**3/3**	**3/3**	ns
11/17/18	ns	ns	ns	ns	**3/3**	**3/3**	**3/3**	ns	**3/3**	**3/3**	**3/3**	ns
12/3/18	**3/3**	ns	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	ns	**3/3**	**3/3**	**3/3**	ns
5/7/19	**2/3**	**3/2**	**3/3**	**3/3**	**3/3**	ns	ns	ns	**2/1**	ns	ns	ns
5/23/19	**3/3**	**3/1**	**3/3**	**3/3**	**2/2**	ns	ns	ns	**3/3**	ns	ns	ns

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