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1 # Environmental DNA for the enumeration and management of

2 # Pacific salmon

3 **short title**: Counting salmon with eDNA

4

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Abstract

18 Pacific salmon are a keystone resource in Alaska, with an economic impact of well over
19 ~US$500 million/yr. Due to their anadromous life history, adult spawners distribute amongst
20 thousands of streams, posing a huge management challenge. Currently, spawners are enumerated
21 at just a few streams because of reliance on human counters and, rarely, sonar. The ability to
22 detect organisms by shed tissue (environmental DNA, eDNA) promises a more efficient
23 counting method. However, although eDNA correlates generally with local fish abundances, we
24 do not know if eDNA can accurately enumerate salmon. Here we show that daily, and near-daily,
25 flow-corrected eDNA rate closely tracks daily numbers of immigrant sockeye and coho spawners
26 and emigrant sockeye smolts. eDNA promises accuracy and efficiency, but to deliver the most
27 robust numbers will need higher-resolution stream-flow data, at-least-daily sampling, and to
28 focus on species with simple life histories, since shedding rate varies amongst jacks, juveniles,
29 and adults.

30

31 **Keywords:** environmental DNA, qPCR, Southeast Alaska, fisheries management,
32 *Oncorhynchus*, ecosystem services, ecosystem functions

33

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Introduction

34 Pacific salmon (*Oncorhynchus* spp.) support a $449 million/yr commercial fishery and play a
35 significant role in the $470 million/yr sport fishery (National Marine Fisheries Service 2017) in
36 Alaska alone and remain a key cultural and subsistence resource for humans. Salmon are also a
37 major source of marine nutrients and energy, which subsidize terrestrial and aquatic food webs
38 (Gende *et al.* 2002a; Gende *et al.* 2002b; Gende *et al.* 2004; Schindler *et al.* 2003), including
39 large numbers of bears and eagles, which are themselves important to the regional ecotourism
40 industry. Due to their anadromous life history, salmon fisheries are often managed by setting
41 escapement goals, where escapement refers to the number of fish that escape the mostly ocean-
42 based fishery and are thus available for spawning in fresh water. For example, from April to
43 October each year, the Alaska Department of Fish and Game (ADFG) continuously estimates
44 salmon breeding population sizes in some western Alaskan streams and issues temporary fishery
45 closure notices to ensure that these escapements exceed minimum target sizes per species.
46 *Current salmon monitoring*. – Of course, it is very costly to count fish. A typical salmon weir
47 consists of a series of closely spaced bars across an entire stream to prevent the passage of
48 salmon, except through a single, narrow gate over which a human observer tallies and identifies
49 to species salmon as they file through (alternatively, Didson sonar can be used to count and size
50 salmon individuals as they pass with species inferred from body size and run timing). The annual
51 running cost of a weir is approximately $80,000, not including installation or major maintenance
52 (Fox 2018), and even this setup might be prone to undercounting (Eggers *et al.* 2009).

53 More than 6,000 streams are used by Pacific salmon in Southeast Alaska alone, and more
54 than 1000 of those streams have been documented as hosting spawning populations (Johnson &
55 Blossom 2018; Fig. S1). Not surprisingly, almost all these salmon runs are left unmonitored or

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56 are monitored only every few years with crude indices, such as visual transects conducted on
57 foot or from the air. Detailed sampling effort varies depending upon budgets, but only a few
58 streams are enumerated and are given escapement targets in any given year. For example, coho
59 salmon (*O. kisutch*) are managed in Southeast Alaska by monitoring escapements and
60 commercial fishery take from four to nine full indicator stock streams (Shaul *et al.* 2005). Full
61 indicator stock streams are systems in which juveniles (usually outmigrating smolt) are tagged
62 with coded wire tags and marked with an adipose fin clip. The proportion of marked fish
63 sampled upon return, along with fishery and escapement sampling, are used to estimate smolt
64 production, fishery interception rate, and escapement. Additional coho streams near urban
65 centers are surveyed by air or on foot, and in some cases escapement goals are established, but
66 there is no guarantee that these intermittent surveys overlap with the peak abundances of runs.
67 Similarly, sockeye salmon (*O. nerka*) escapements are at least partially enumerated at 14 streams
68 in Southeast Alaska (Munro & Volk 2016). Nearly all pink (*O. gorbuscha*) and chum (*O. keta*)
69 salmon runs are left un-enumerated by weirs or sonar, despite these species making up the
70 majority of salmon biomass, harvest, and economic value in this region. Instead, several larger
71 chum and pink streams are surveyed by air or on foot several times each year (Munro & Volk
72 2016), but even this is complicated by the difficulty of distinguishing pink and chum because
73 their migration timing and habitat use often overlap. Finally, enumeration is naturally focused on
74 the largest, most economically valuable streams, leaving large numbers of subdominant runs for
75 most salmon species unmonitored most years.

76 Fry and smolt production resulting from spawning salmon is monitored even less, which
77 limits inference of future expected recruitment and harvest. Poor understanding of fry and smolt
78 production also limits inference regarding the degree to which salmon productivity is limited by

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79 spawning habitat for adults or by rearing habitat for juveniles, and whether changes in marine or
80 freshwater productivity are responsible for changes in salmon recruitment and abundance. Such
81 information is critical for informed management and for judging the potential efficacy of stock
82 enhancement programs.

83 More generally, the under-monitoring of Pacific salmon stocks hinders the construction
84 of reliable spawner-recruit models, which are used to determine escapement goals for maximum
85 sustainable yield, increases uncertainty about whether, and where, there are sufficient spawners
86 to maximize salmon recruitment, and, finally, increases the risk of long-term decline or loss,
87 especially of the small, subdominant components of salmon runs. These smaller salmon runs
88 have value in that they could be increasing the resilience of salmon stocks through portfolio
89 effects (Schindler *et al.* 2010) or by restocking of a dominant component that has suffered a
90 negative shock, and they provide key resources for wildlife by extending the spatial range and
91 phenology of salmon availability to terrestrial and aquatic food webs (Gende *et al.* 2002b;
92 Schindler *et al.* 2013). As fisheries increasingly transition towards ecosystem-based fisheries
93 management, identifying, monitoring, and maintaining such spatially and temporally distributed
94 salmon resources becomes increasingly important for conservation and management.

95

96 *Environmental DNA*. – The advent of environmental DNA (eDNA) methods that detect DNA
97 shed by organisms (Bohmann *et al.* 2014; Goldberg *et al.* 2016) provides a promising tool for
98 monitoring salmon escapements and juvenile production, which could increase management-
99 relevant information at low cost. However, while the efficacy of using eDNA for species
100 *detection* is now widely recognized (Goldberg *et al.* 2016; Rees *et al.* 2014) and while several
101 studies have demonstrated that eDNA is generally correlated with fish abundance in mesocosm

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102 experiments, lakes, and streams (Doi *et al.* 2015; Handley *et al.* 2018; Lacoursière-Roussel *et al.*
103 2016; Takahara *et al.* 2013; Tillotson *et al.* 2018; Wilcox *et al.* 2016), we do not yet know
104 whether eDNA contains sufficient information to allow robustly accurate estimates of fish
105 abundance, particularly for anadromous fish as they enter and leave a watershed. By robust, we
106 mean accuracy that is not greatly affected by variation among *inter alia* years, species, stream,
107 and/or details of the sampling protocol.

108 Anadromous fish such as salmon provide a potentially straightforward scenario for
109 testing whether eDNA can be used to count fish, because large numbers of salmon release their
110 DNA as they pass a fixed sampling point, either as they swim up a river or stream as
111 immigrating adults or swim downstream as emigrating juveniles. If eDNA degrades or settles
112 quickly (as suggested by Jane *et al.* 2015; Jerde *et al.* 2016; Sassoubre *et al.* 2016; Shogren *et al.*
113 2016; Turner *et al.* 2015), then eDNA concentrations should primarily detect fish that are locally
114 present in space and time. Thus, rather than simply accumulating as fish enter a watershed,
115 eDNA concentrations might spike up and down as a pulse of fish swims past a sampling point,
116 with the size of the spike correlated with fish number and/or biomass. Because the concentration
117 of eDNA in streamwater results from both the amount of DNA shed by organisms and the flow
118 of water, the product of eDNA concentration and streamflow (measured in units of water volume
119 per time) can be used to calculate absolute quantities of eDNA per unit time. Such ‘flow-
120 corrected eDNA rates’ measured at regular intervals (e.g. daily) could then be substituted for or
121 complement gold-standard count data from weirs.

122 In the most comprehensive and relevant study to date, Tillotson *et al.* (2018) have
123 demonstrated that local counts of sockeyes in a spawning creek, particularly dead sockeye,
124 indeed predict local eDNA concentrations. As Tillotson *et al.* (2018) put it, the next step is

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125 "reversing the model to predict abundance from eDNA." Here we take advantage of a daily
126 census of sockeye and coho salmon carried out at the Auke Creek research weir in Juneau,
127 Alaska to test whether eDNA concentrations and stream-flow measurements together produce
128 quantitative and management-relevant indices of salmon escapement and juvenile outmigration.
129 To explore the general ecology of eDNA, we also quantify the relative influences of salmon
130 counts on the same day of water sampling, salmon that entered the watershed one day prior, and
131 salmon that entered two days prior to an eDNA measurement, and we assess the eDNA signal
132 produced by salmon of different life stages and body sizes. The purpose of these latter analyses
133 is to test for two possible sources of error (long-distance transport of eDNA and differential
134 shedding rates by body size and type) when using eDNA to enumerate salmon.

Methods

Weir operation

135 The Auke Creek research weir is located 19.2 km north of Juneau, Alaska, 400 m downstream
136 from the outlet of Auke Lake above the high tide line at the mouth of Auke Creek. The ~1072.5
137 ha watershed includes five tributaries that feed into Auke Lake, which is 1.6 km long and 1.2 km
138 wide, with a surface area of 67 ha. The weir is cooperatively operated by the National Marine
139 Fisheries Service, in collaboration with the University of Alaska, and the Alaska Department of
140 Fish and Game, with the objective of capturing all emigrant and immigrant fish at Auke Creek.
141 All emigrant fish (from upstream) are enumerated from the beginning of March to the middle of
142 June and released below the weir, after which the weir is converted to capture immigrating adult
143 salmonids (from downstream), which are released above the weir. During monitoring of adult
144 salmonids, fish are classified by species and life stage. The Auke Creek dataset represents
145 probably the highest-temporal-resolution and most accurate salmon census data in Alaska, if not

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146 the world. Life stages for coho salmon include a 'juvenile' life-history strategy in which young
147 coho rearing in the estuary and ocean return upstream, early maturing and small-bodied 'jack'
148 males, and typical adult male and female fish. Sockeye salmon can also produce jacks, but
149 infrequently. Complete methods for weir operation can be found in Vulstek *et al.* (2018) (Weir
150 photos in Supporting information S2). River height is recorded daily and converted to
151 streamflow (cubic feet per second) using an established rating curve (Bell *et al.* 2017).

Environmental DNA quantitation

152 We collected water samples for three years, from 2014-2016, after each day's salmon
153 enumeration. In a 2014 pilot study, we collected three 1L water samples weekly from 28 May to
154 11 December. Based on promising results, and to minimize reduce costs, in 2015, we sampled
155 weekly when few fish were entering the river and then increased sampling frequency up to daily
156 during periods in which many salmon were entering the river. Because salmon eDNA
157 disappeared entirely after October in 2014, we sampled from 12 May to 3 November in 2015.
158 Based on further promising results from 2015, we increased sampling frequency to daily in 2016
159 from 10 May to 20 October. Because previous technical replicates had yielded consistent results,
160 and because of the high frequency of water collection, we collected only two 1L water samples
161 daily in 2015 and 2016. All water samples were collected using 1L disposable sterile Whirlpak
162 bags and filtered through a 0.45 micron cellulose nitrate filter. Filters were then folded and
163 stored in 100% ethanol at 4C until laboratory processing.

164 We maintained strict protocol to prevent contamination of filters and reagents. We
165 performed DNA extraction and PCR setup inside of separate heap-filtered and UV-irradiated
166 PCR cabinet (Air Science LLC, Fort Meyers, FL) within a separate lab where PCR product is
167 prohibited. Filters were first removed from ethanol and air-dried overnight in sterile, disposable

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168 weigh boats. A modified protocol for the Qiagen DNeasy Blood and Tissue kit was used to
169 isolate DNA. This included the addition of 1.0 millimeter zirconia/silica beads to the initial lysis
170 buffer and then a 15 minute vortex step to loosen the DNA from the filters. Incubation in lysis
171 buffer was increased to 48 hours. After incubation, 300ul of the lysed product was transferred to
172 a new 1.7ml microcentrifuge tube. Thereafter, we followed the manufacturers protocol for
173 isolation of tissue. DNA was eluted in a total volume of 100ul.

174 Using species-specific primers and TaqMan minor groove binder (MGB) probes
175 (ThermoFisher Scientific, Waltham, MA), developed by Rasmussen Hellberg *et al.* (2010)
176 (Table 1), we targeted a fragment of the cytochrome c oxidase subunit 1 (COI) gene. For each
177 species, each sample was run in triplicate PCR reactions. Each 20ul qPCR reaction contained 6ul
178 of DNA template, 10ul Environmental Master Mix 2.0 (ThermoFisher Scientific, Waltham,
179 MA), 0.2 uM of both forward and reverse primers, 0.2um of the TaqMan MGB probe, and sterile
180 water. Additionally, each plate contained a four-point standard curve using DNA obtained from
181 salmon tissue from each species. Extracted tissue was quantified using a Qubit Fluorometer
182 (ThermoFisher Scientific, Waltham, MA) and diluted 10-fold from 10^{-1} to 10^{-4} ng/ul. PCR
183 cycling conditions involved an initial denaturation step of 10 min at 95C to activate the HotStart
184 Taq DNA polymerase, followed by 50 cycles of 95C for 15 s and 60C for 60 s. All reaction
185 plates contained a negative control (water) as well as extraction blanks. PCR was performed on
186 an ABI PRISM 7500 FAST Sequence Detection System (Applied Biosystems, Foster City, CA),
187 and analyzed on 7500 Software v2.0.6 (Applied Biosystems, Foster City, CA). Cycle values
188 were converted to target-DNA concentration using on the standard curve derived from the tissue
189 samples, and each day's eDNA concentration was taken as the mean across the two extractions
190 and the three qPCR replicates from that day for that species.

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Data analysis

191 To calculate the flow-corrected eDNA rate, we multiplied each day's qPCR-estimated target-
192 DNA concentration ($\frac{ng}{\mu l}$) against that day's streamflow ($\frac{cubic\ feet}{sec}$). There is no need to harmonize
193 units because the product is now an estimate of DNA biomass rate (ng/sec) multiplied by a
194 dimensionless constant (volume/volume): $\frac{ng}{sec} \cdot \frac{cubic\ feet}{\mu l}$, and the fitted model parameters
195 incorporate the conversion factor. Streamflow was usually taken at 8 AM each day, near the time
196 that eDNA was sampled. However, this measure is only for one time point and might not be fully
197 representative of streamflow during the whole day. We return to this point in the Discussion.
198 We predicted salmon counts from flow-corrected eDNA rate using a quasipoisson regression in
199 order to account for overdispersion. The quasipoisson model produces the same coefficients as
200 standard Poisson generalized linear models for count data, but it is more inferentially
201 conservative (i.e. lower Type I error rates due to wider confidence intervals). We fit separate
202 models in 2015 and 2016 for immigrating adult sockeye salmon, immigrating total coho salmon,
203 and outmigrating sockeye smolts. In our analysis, we included data for adult sockeye salmon
204 from 18 June - 1 August, adult coho salmon from 15 August – 30 October, and outmigrating
205 sockeye smolts from 15 April – 10 June. This time period captured the full runs of each species
206 and life stage, but did not include a time period after the adult sockeye salmon run when DNA
207 was transported downstream as salmon died in the lake. We used total coho, not just adult coho,
208 because the coho run includes a varying mixture of juveniles, jacks, and adults, which are
209 different sizes. We return to this point also in the Discussion.

210 To determine whether the relationship between flow-corrected eDNA and salmon counts
211 was consistent between the two years, we combined data for the two years (the adult datasets
212 only) and fitted a model with an additional interaction term between year and flow-corrected

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213 eDNA. A significant interaction effect would indicate a different relationship between count and
214 eDNA between years, which would indicate a lack of model transferability.
215 *Ecology of eDNA*. – We also used the dataset to explore the 'ecology of eDNA,' using salmon
216 counts from the same and previous days to predict that day's flow-corrected eDNA rate. The
217 purpose is to test for the possibility that long-distance, albeit attenuated, transport of eDNA from
218 far-upstream salmon degrades the real-time quantitative accuracy of eDNA. We also test for the
219 possibility that body size and/or life-history affects per-fish shedding rates.
220 To directly estimate the timescale over which eDNA was detected in Auke Creek, we
221 used a series of three linear regression models to relate daily counts of sockeye salmon in 2016
222 (the year with daily sampling) to flow-corrected eDNA concentration. We first modeled flow-
223 corrected eDNA as a function of salmon counts from the same day. We then used the residuals
224 from that model in a second regression that instead included salmon counts from the previous
225 day as a predictor. Finally, we used the residuals from the second model in a regression using
226 salmon counts from two days prior as a dependent variable. We interpreted significant lag
227 variables from salmon counts in the second or third models as evidence that salmon entering the
228 river one or two days ago influence the measured flow-corrected eDNA concentration. In order
229 to explore the eDNA production by coho salmon of different life stages, we additionally used
230 multiple linear regression with counts of adults, jacks, and juveniles in 2015 and 2016 as
231 predictors of flow-corrected eDNA measured that same day.
232

Results

233 Neither the concentration of eDNA nor flow-corrected eDNA rate increased monotonically as
234 salmon accumulated in the Auke Creek watershed. Instead, flow-corrected eDNA rates reflected

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235 a highly local signal of salmon abundance in space and time, effectively tracking salmon that had
236 passed near the water sampling site over the previous day (Figs. 2-4). This was true for both
237 adult salmon and smolts.
238
239 *Tracking of salmon phenology and abundances with eDNA*. – The product of stream flow (cubic
240 feet per second, cfs) and eDNA concentration (ng/µl), which we refer to as flow-corrected eDNA
241 rate, was highly predictive of the counts of immigrating adult sockeye and coho salmon, as well
242 as of outmigrating sockeye salmon smolts in both 2015 and 2016 (Fig. 5; Adult sockeye 2015: β
243 $= 3.83 \pm 1.05$, $p < 0.003$; Adult sockeye 2016: $\beta = 4.34 \pm 0.76$, $p < 10^{-5}$; Total coho 2015: $\beta =$
244 3.61 ± 0.49, $p < 10^{-8}$; Total coho 2016: $\beta = 8.87 \pm 2.32$, $p < 0.004$; Sockeye smolts 2015: $\beta =$
245 570.24 ± 128.09, $p < 0.004$; Sockeye smolts 2016: $\beta = 681.99 \pm 148.83$, $p < 0.002$).
246 The combined models for 2015 and 2016 produced unambiguously non-significant
247 interaction effect between year and flow-corrected eDNA rate for adult sockeye salmon ($p =$
248 0.71) or sockeye salmon smolts ($p = 0.60$), indicating that eDNA had a consistent relationship
249 with sockeye adult counts across years. However, the interaction term was significant for coho,
250 indicating that the same value of flow-corrected eDNA rate was associated with a higher count
251 of total coho in 2016 ($\beta = 5.26 \pm 2.20$, $p < 0.02$), which is likely due to a higher abundance of
252 smaller-bodied life stages of coho salmon in 2016 (many more juveniles and jacks).
253 With the exception of coho salmon in 2016, which were composed of a diverse mix of
254 life histories and body sizes, the quasipoisson regression models using flow-corrected eDNA rate
255 as a single predictor produced visually representative predictions of counts through time that
256 captured the phenology, temporal dynamics, and relative abundance of each run (Fig. 6).
257 However, while the quasipoisson model correctly predicted the timing and size of the peaks in

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258 salmon counts, the models also consistently predicted small but nonzero counts of salmon even
259 when no salmon where counted (Fig. 6). This occurred because the model estimated a positive
260 intercept term despite near zero eDNA concentrations in the absence of salmon (Fig. 2-4). When
261 we used this biological information to fix the intercept to zero at zero flow-corrected eDNA rate,
262 the model fit was worsened in periods when salmon were abundant. Inspection of the residuals
263 gave us no reason to infer zero-inflation in the data-generating mechanism. We thus retained the
264 original models with non-zero intercepts. Similarly, we tried models with water temperature as a
265 second predictor, but saw no consistently significant effects and we were concerned about
266 spurious correlations caused by the temporal trend in temperature data. This result is not
267 surprising since visual inspection of the temperature timelines (Figs. 2, 3, 4) reveals no
268 covariance between temperature and fish count peaks.
269
270 *Ecology of eDNA*. – Sockeye salmon counts from the current day in 2016 significantly predicted
271 flow-corrected eDNA rate ($\beta = 0.0011 \pm 0.00016$, $p < 10^{-7}$), but salmon from one day prior was
272 only marginally related to any residual variation from the first model ($\beta = 0.00026 \pm 0.00015$, $p <$
273 0.09), and salmon from two days prior was completely unrelated to residual variation not
274 accounted for by salmon counts from the same day and one day prior ($p = 0.99$).
275 Of the three coho salmon life-history categories (adults, jacks, and juveniles), adults
276 produced the strongest flow-corrected eDNA signal ($\beta = 0.0060 \pm 0.00045$, $p < 10^{-15}$), which was
277 an effect 3.5 times higher than that produced by each juvenile fish ($\beta = 0.0017 \pm 0.00058$, $p <$
278 0.005). When accounting for the eDNA signal produced by adults and juveniles, counts of jacks
279 were negatively associated with flow-corrected eDNA ($\beta = -0.0020 \pm 0.00080$, $p < 0.02$), but a
280 jack-only model (no adults) yielded no relationship ($\beta = 0.002 \pm 0.0012$, $p = 0.14$). Statistically

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281 speaking, jacks are invisible on the eDNA radar, which is intriguingly consistent with their
282 reproductive strategy of gaining access to females via 'sneaking.'

Discussion

283 Since the efficacy of eDNA was first demonstrated for the detection of invasive bullfrogs
284 (Ficetola *et al.* 2008), a rapidly growing body of literature has highlighted the efficacy of eDNA
285 for rare species detection (Rees *et al.* 2014; Wilcox *et al.* 2016), has explored the technical
286 aspects of eDNA (Goldberg *et al.* 2016), and has suggested that eDNA holds promise for
287 quantifying the abundance of species (Doi *et al.* 2015; Lacoursière-Roussel *et al.* 2016; Takahara
288 *et al.* 2013; Tillotson *et al.* 2018). The next, and most transformative, technical step for
289 mobilizing the use of eDNA for environmental management is to determine whether, and under
290 what conditions, eDNA can be used to *precisely enumerate* organisms. The possibility of
291 enumerating Pacific salmon as they immigrate and emigrate represents a particularly promising
292 application, with large economic and risk-management implications for a multibillion dollar
293 fishery and keystone wildlife resource.

294 To test the efficacy of eDNA for salmon enumeration, we coupled a complete census of
295 immigrating and emigrating anadromous salmon with daily quantitation of environmental DNA.
296 We demonstrate that flow-corrected eDNA rate
297 (1) predicts same-day, daily counts of two species of adult salmon immigrating into the
298 watershed (Figs. 2, 3) and of one species of emigrating salmon smolt (Fig. 4),
299 (2) does not simply accumulate over time, which would have otherwise reflected the total
300 number of salmon that have entered the watershed this season,
301 (3) is highly accurate at delimiting the phenologies of immigrating adult and emigrating juvenile
302 salmon, and

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303 (4) appears to be affected by differential DNA-shedding rates across different life-history
304 strategies.
305 However, we have also identified several remaining obstacles to straightforward implementation
306 of eDNA for the enumeration of salmon. Most importantly, accurate measures of streamflow are
307 crucial. This is particularly true because pulses of adult salmon immigration sometimes coincide
308 with high streamflow events (Figs. 2-4), and the error in estimating streamflow is exacerbated
309 because the ratings curves that relate river height (the measure that is actually recorded daily) to
310 flow contain more error at extreme values, since extreme-flow estimates are either based on few
311 calibration points or on none at all and just represent extrapolations.
312 The 2015 and 2016 adult sockeye runs are excellent examples of the importance of
313 obtaining accurate streamflow data (Fig. 2). In 2015, non-flow-corrected sockeye eDNA
314 concentration ('DNA' timeline) was highest around 1 July and declined monotonically through
315 the month despite few adult immigrating sockeye in early July. However, early July was also a
316 period of low stream flow. Only after accounting for stream flow ('Flow X DNA' timeline),
317 which included a flood event around 15 July, did eDNA correctly predict the observed sockeye
318 immigration peak on 15 July ('Counts' timeline).
319 In 2016, there were three non-flow-corrected eDNA peaks ('DNA' timeline), the timings
320 of which very closely matched the three count peaks. However, the first two non-flow-corrected
321 eDNA peaks, in early July, were taller than the third peak, which is the opposite to that seen in
322 the count data. The reason is that the third eDNA concentration peak, in late July, occurred just
323 as streamflow also rose, diluting the eDNA ('Flow (cfs)' timeline). The third eDNA peak's shape
324 and size more closely matched the count data after flow correction ('Flow X DNA' timeline),
325 although the third eDNA peak is still too small, relative to the sizes of the first two peaks. We

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326 hypothesize that the streamflow value that we used to multiply the first day of the third eDNA
327 concentration peak was too low, probably recorded before most of that day's flow increase had
328 occurred, causing us to under-correct and thus under-predict. We have informally substituted in
329 the next day's much higher streamflow value (flow during the third sockeye peak rapidly more
330 than tripled from 6.6 to 23.1 cfs between 23 and 24 July), and the third flow-corrected eDNA
331 peak matches the count data more closely (data not shown).

332 A second critical consideration for quantifying anadromous fish counts with eDNA is the
333 time scale of inference being made by an eDNA measurement. As adult salmon move upstream,
334 the signal produced by their shedding of DNA attenuates and is eventually not detectable.
335 Therefore, effective monitoring of anadromous fish with highly variable daily counts requires
336 eDNA to be sampled at least daily. Even with daily sampling, we can imagine that the eDNA
337 signal produced by a medium-sized pulse of fish could be the same strength as the signal
338 produced by a large pulse of fish that passed by only hours ago. This potential timescale
339 mismatch produces errors that set an upper limit on the accuracy of eDNA for quantifying
340 anadromous fish abundance.

341 How much the above two *within*-stream sources of error reduce reliability in decision-
342 making depends in part on the level of variation *across* streams. If a single stream, regardless of
343 how expensively and accurately it is censused, does not reflect regional escapement sizes, due to
344 temporal variation in salmon abundance across streams, it might be more robust to collect data
345 from multiple streams (presumably only feasible with eDNA), even at a cost of reduced accuracy
346 per stream. Currently, the Alaska salmon fishery does not have enough data to judge this
347 possibility.

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348 A third consideration is that some salmon runs contain a mix of individuals with different
349 life histories. This was particularly the case for coho salmon in 2016, for which jacks were
350 numerically dominant early in the run and a juvenile coho life history strategy was dominant late
351 in the run. Both juveniles and jacks were rare in 2015. The many arriving jacks and juveniles did
352 not produce levels of DNA concordant with the production by adult salmon (Fig. 3), which
353 introduced substantial error into the relationship between flow-corrected eDNA and salmon
354 counts (Figs. 5-6). In contrast, flow-corrected eDNA was a much better index of coho salmon
355 counts in 2015 when the coho salmon run was dominated by adult fish (i.e. not jacks and
356 juveniles) despite fewer data points collected in that year (Figs. 3, 5, 6).

357 Pacific salmon are a valuable resource, but their distributed spawning and rearing habitat,
358 due to their anadromous life history, makes monitoring their distribution and abundance a
359 formidable challenge, which consequently injects an unknown but probably non-trivial amount
360 of inefficiency and risk into management. Our results suggest that using eDNA quantitation to
361 estimate fish abundance has serious potential for reducing inefficiency and risk, but will require
362 (1) accurate and ideally time-averaged streamflow measures and (2) frequent (at-least-daily)
363 eDNA sampling due to the ephemeral nature of the eDNA signal. On the other hand, this very
364 ephemerality is what makes eDNA such a sensitive correlate of salmon abundance.

365 Given the strong observed correlations between daily eDNA samples and fish counts
366 (Figs. 5-6), investment in technology to allow frequent or even near real-time eDNA
367 quantitation, and stream flow measurement, could provide a more accurate and cost-effective
368 means of enumerating anadromous fish and thus informing management. This would be
369 especially true if daily eDNA samples from many streams turn out to provide a more accurate
370 estimate of regional escapement sizes than do intensive measurements at just a few streams.

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371 Even with the same budget, it should be possible for a technician who would otherwise be paid
372 to count fish in a single stream to instead collect water samples from many spawning streams
373 across a watershed. In addition, water sampling could be extended to quantify smolt runs, which
374 are currently only estimated in Southeast Alaska at a limited number of index systems.
375 Moreover, because post-sampling filters can be stored in a refrigerator or freezer for many days
376 after sampling, it should be feasible to train and pay a network of citizen scientists to carry out
377 sampling across multiple watersheds. Note also that although our analysis focused on sockeye
378 and coho salmon, the same eDNA sample can be used to monitor any number of aquatic species
379 with the development of appropriate assays. Against these potential gains in sampling efficiency
380 and information must be balanced the additional cost of the qPCR assays to be carried out in a
381 dedicated eDNA lab.

382 Our study is of a single stream in Southeast Alaska. However, it provides strong
383 justification for an expanded effort to sample salmon eDNA over more streams, more species,
384 and more days, both in the streams that currently have weirs, so that a robustly transferable
385 model can be parameterized and validated, and in some of the many streams that are not
386 currently monitored, to test for the possibility that multiple streams sampled daily with eDNA
387 provide more useful information than a few streams counted intensively. Given the huge size of
388 the Alaska salmon fishery, even a small improvement in management effectiveness and/or a
389 small decline in the risk of population decline or establishment by alien salmonids would more
390 than justify this investment.

391

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402 Marine Fisheries Service. Any use of trade, firm, or product names is for descriptive purposes
403 only and does not imply endorsement by the U.S. Government.

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484

485

486 Data Accessibility

487 The R scripts and data for analyses are at github.com/dougwyu/2014_2015_2016_Auke_qPCR

488 Author Contributions

489 TL, DWY, DAT, and CYY conceived the research and designed the experiments. DB, JJ, SCV,

490 and JRR collected field samples. JA and CYY performed laboratory analyses. TL and DY

491 performed the statistical analysis. TL, DWY, and DAT wrote the manuscript, with comments

492 from all other authors.

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493 Table 1. Species-specific primers and probes used in this study (Rasmussen Hellberg *et al.* 2010)

494

Target species	Forward Primer (5'-3')	Reverse Primer (5'-3')	Probe (5'-3')
Sockeye (*Oncorhynchus nerka*)	GGAAACCTTGCCCACGCG	AAAAGTGGGGTCTGGTACTGAG	FAM-CTCTGTTGACTTAACCATC-MGB
Coho (*Oncorhynchus kisutch*)	CGCTCTTCTAGGGGATGATC	CTCCGATCATAATCGGCATG	FAM-ATTTACAACGTAATCGTC-MGB

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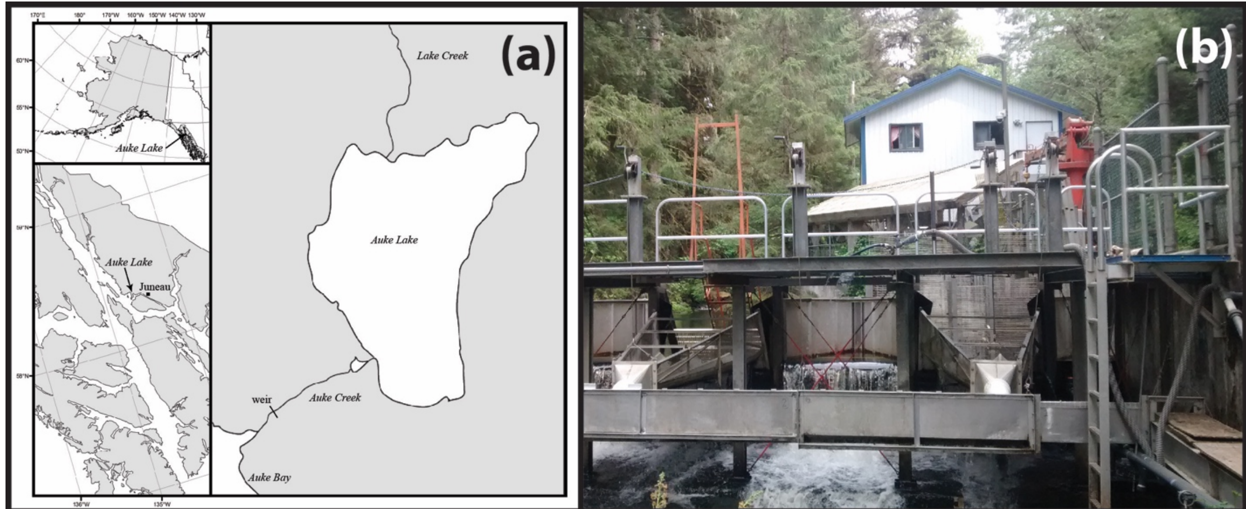
496 **Figure 1.** The Auke Creek research weir is (A) located in Juneau, Alaska at the outflow of Auke
497 Lake. (B) The weir is a permanent structure used to sort and enumerate immigrating juvenile
498 salmon and emigrating adult salmon.
499



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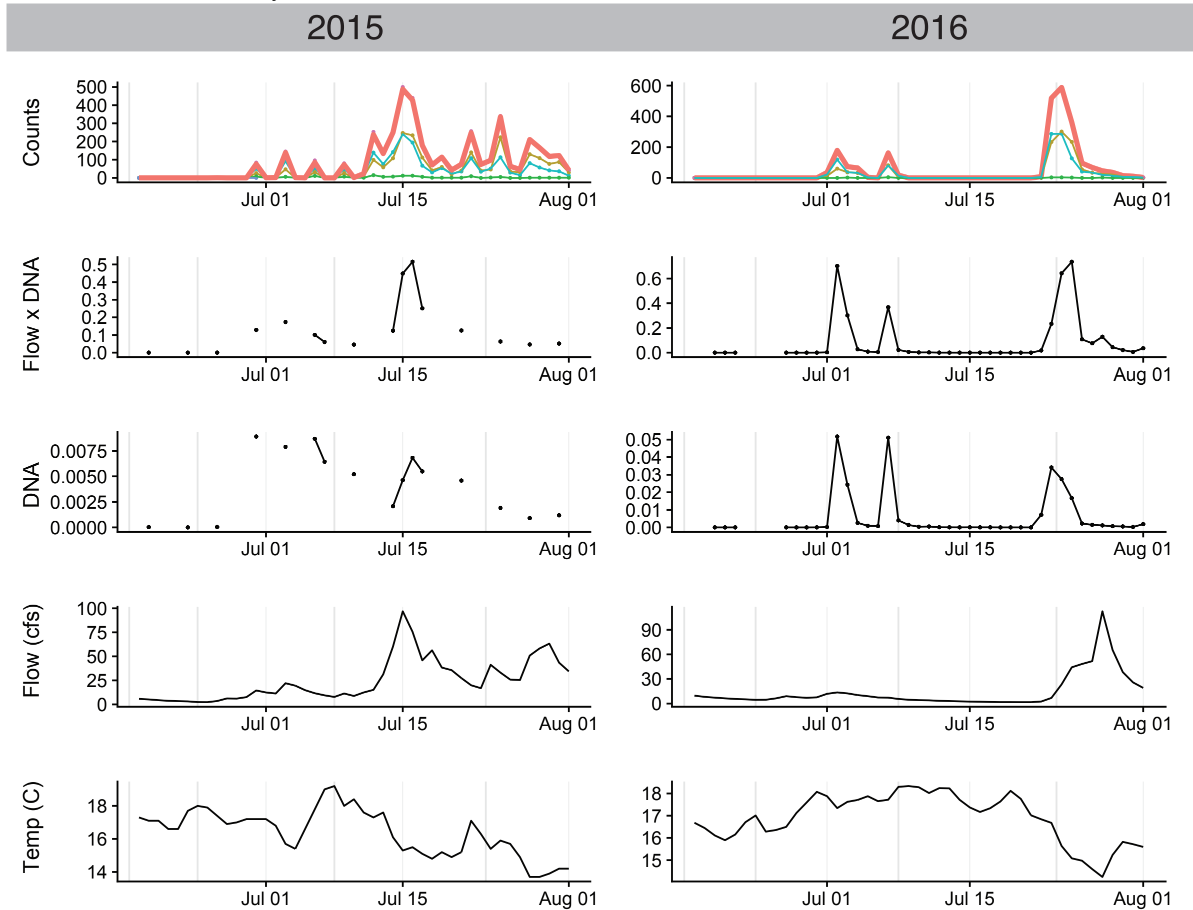
503 **Figure 2.** Timeline from June 18 to August 1 of adult sockeye salmon counts, flow-corrected
504 eDNA concentration (ng/µl*cfs), uncorrected eDNA concentration (ng/µl), stream flow (cfs,
505 cubic-feet/sec), and stream temperature (C) in 2015 and 2016. Environmental DNA results from
506 consecutive days are connected by lines. Male and female salmon are denoted by yellow-brown
507 and blue lines respectively, and jacks are denoted by green lines. Total adult sockeye salmon
508 counts are denoted by thick red lines.



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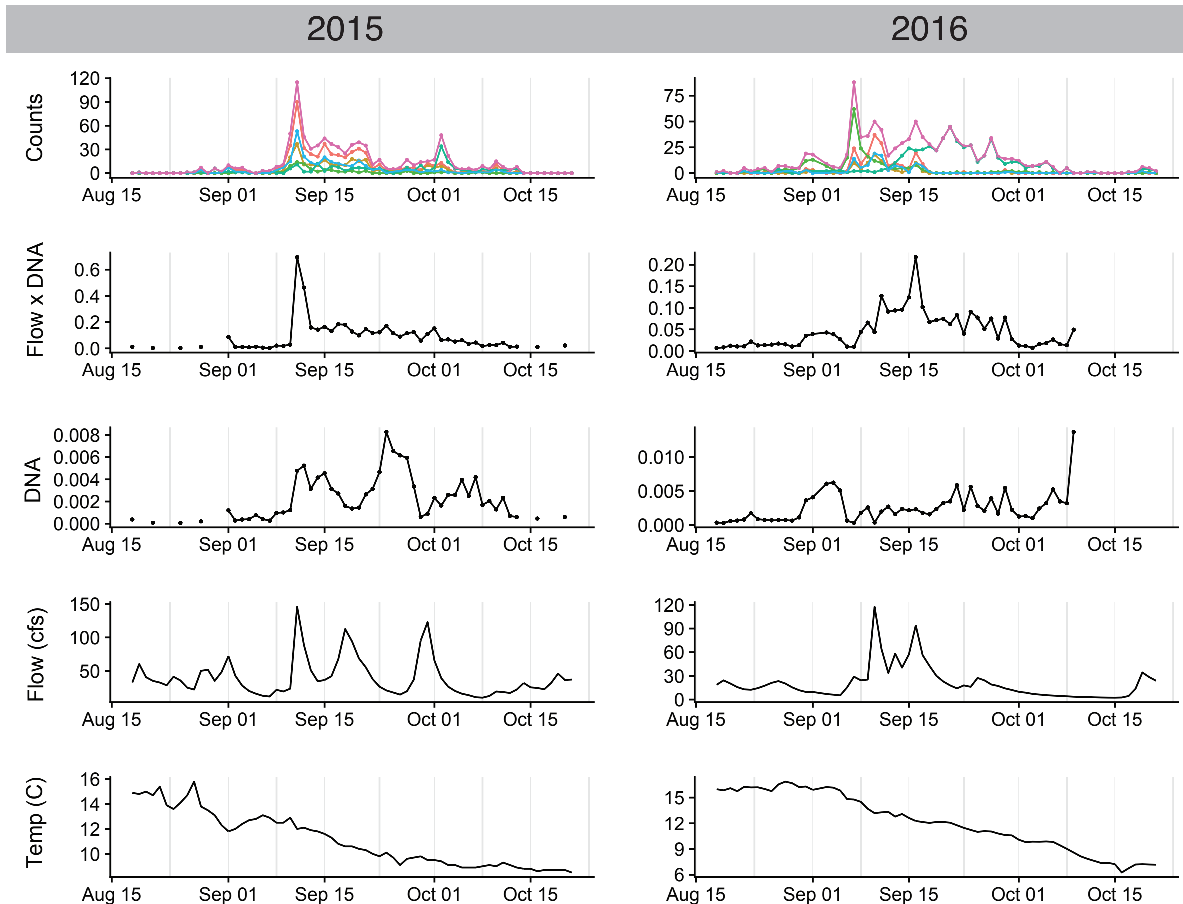
512 **Figure 3.** Timeline from August 15 to October 30 of coho salmon counts, flow-corrected eDNA
513 concentration (ng/µl*cfs), uncorrected eDNA concentration (ng/µl), stream flow (cfs), and
514 stream temperature (C) in 2015 and 2016. Environmental DNA results from consecutive days are
515 connected by lines. Male and female coho salmon are denoted by yellow-brown and blue lines
516 respectively, jacks are denoted by green lines, counts of a juvenile nomadic life history strategy
517 are denoted by teal lines, total adult (male + female) coho salmon counts are denoted by red
518 lines. Total coho salmon counts including jacks and juveniles are denoted by pink lines. Note
519 that the adult male and female coho salmon were the dominant component of the run in 2015
520 while the jack and juvenile life history strategy was a major component of the run in 2016.
521



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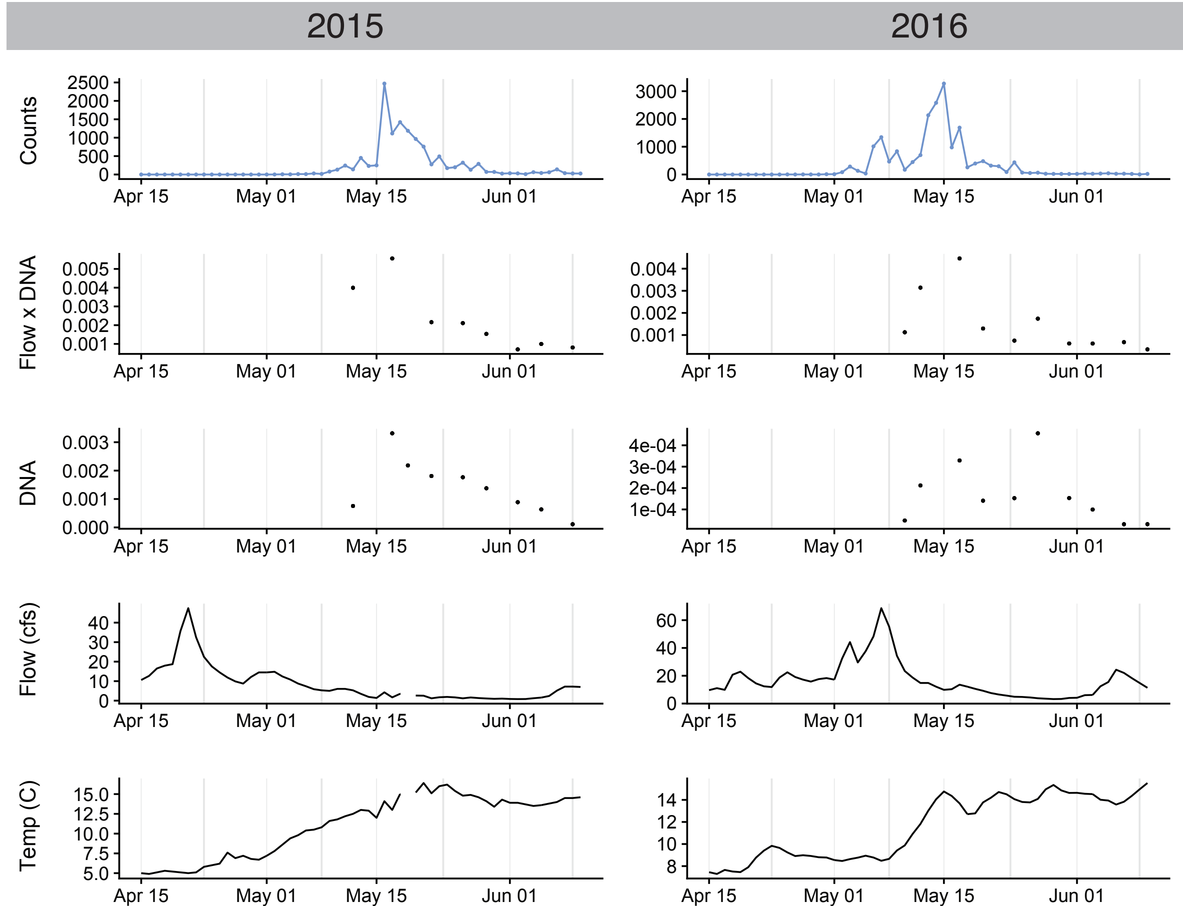
523 **Figure 4.** Timeline from April 15 to June 10 of outmigrating sockeye salmon smolt counts, flow-
524 corrected eDNA concentration (ng/µl*cfs), uncorrected eDNA concentration (ng/µl), stream
525 flow (cfs), and stream temperature (C) in 2015 and 2016. Environmental DNA results from
526 consecutive days are connected by lines.
527



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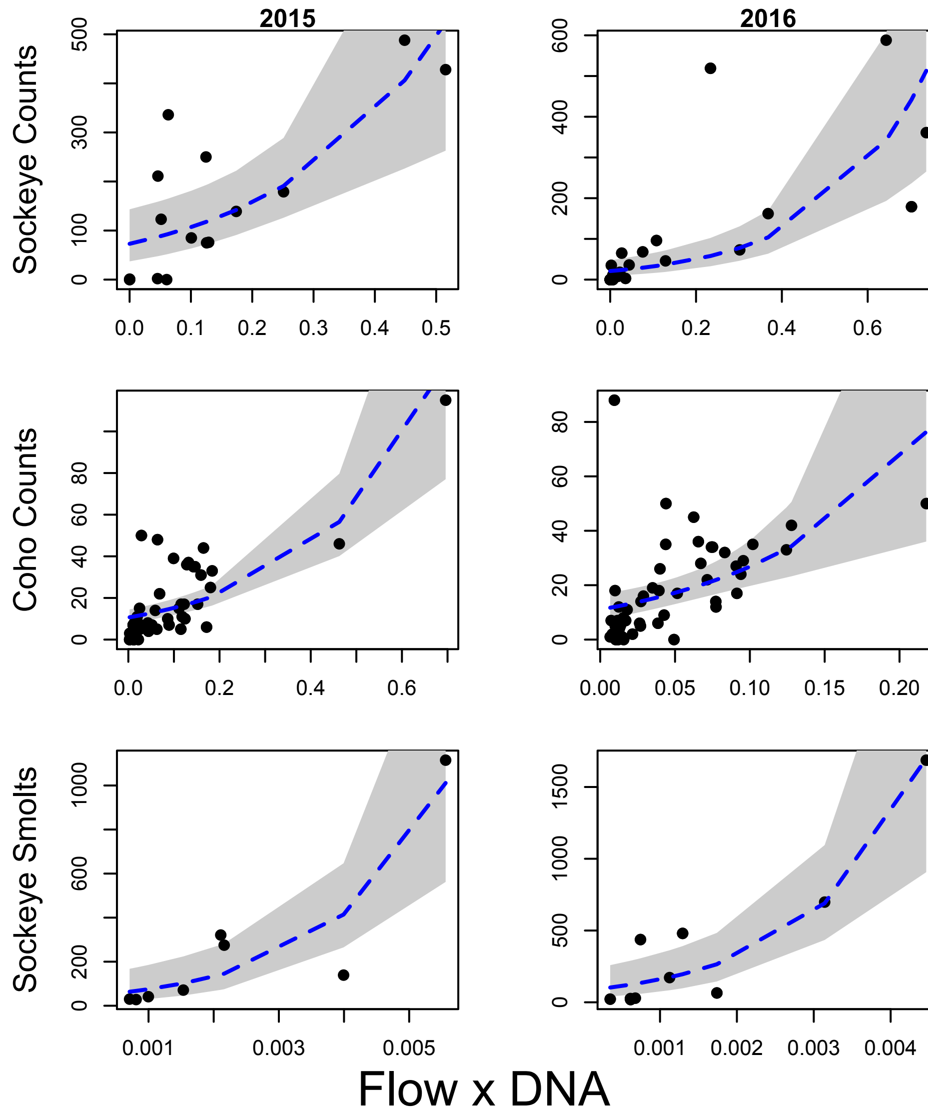
530 **Figure 5.** Results of quasipoisson regression models relating flow-corrected eDNA
531 concentration to adult sockeye salmon counts (2015: $p<0.003$, 2016: $p<2e-6$), total coho salmon
532 counts (2015: $p<2e-9$, 2016: $p<0.004$), and counts of sockeye salmon smolts (2015: $p<0.005$,
533 2016: $p<0.002$). Gray shading denotes the 95% confidence interval.



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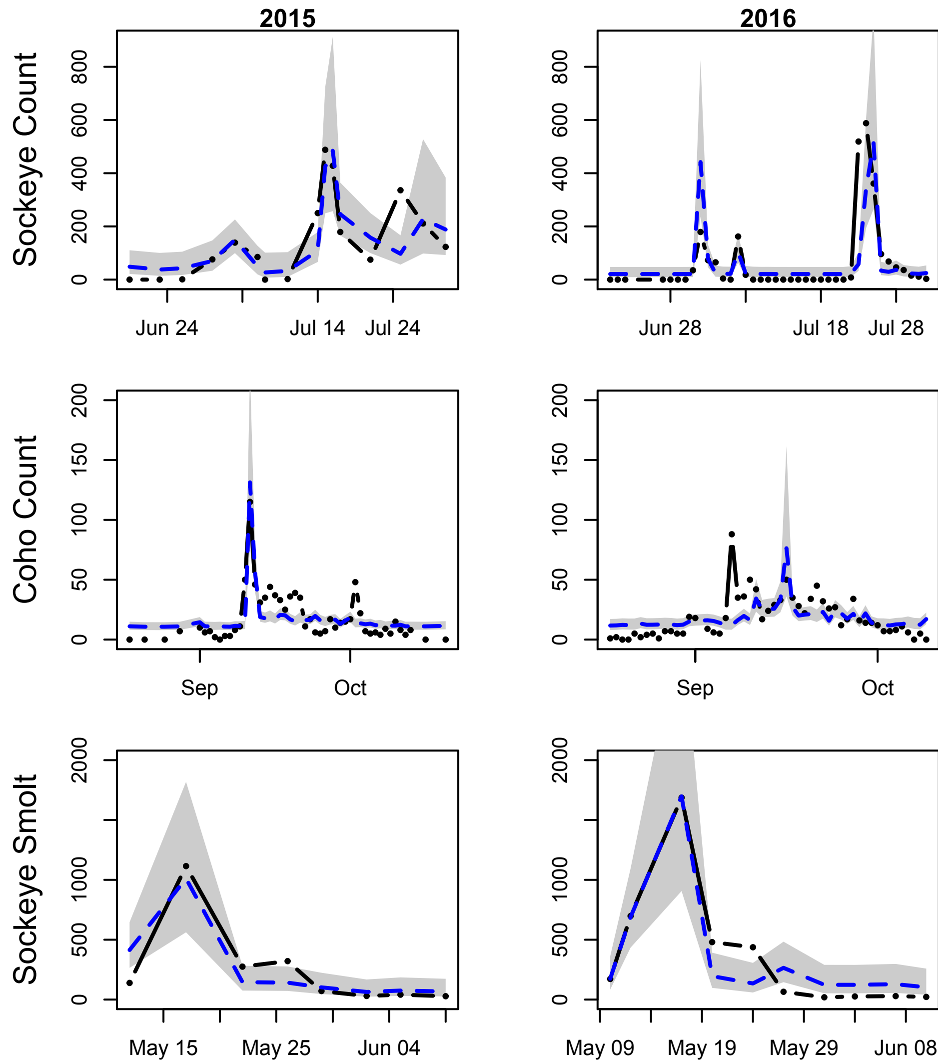
536 **Figure 6.** Counts of adult sockeye salmon, total coho salmon (including all life history
537 strategies), and sockeye salmon smolts (black dots) and the predicted number of counts based on
538 the flow-corrected eDNA concentration predictor in the quasipoisson regression model (blue
539 dashed lines). Gray shading denotes the 95% confidence interval.



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Supplemental Information for:

Environmental DNA for the enumeration and management of Pacific salmon

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Table of Contents:

S1. Salmon spawning streams in Southeast Alaska	Page 1
S2. Photos of weir structure	Page 2

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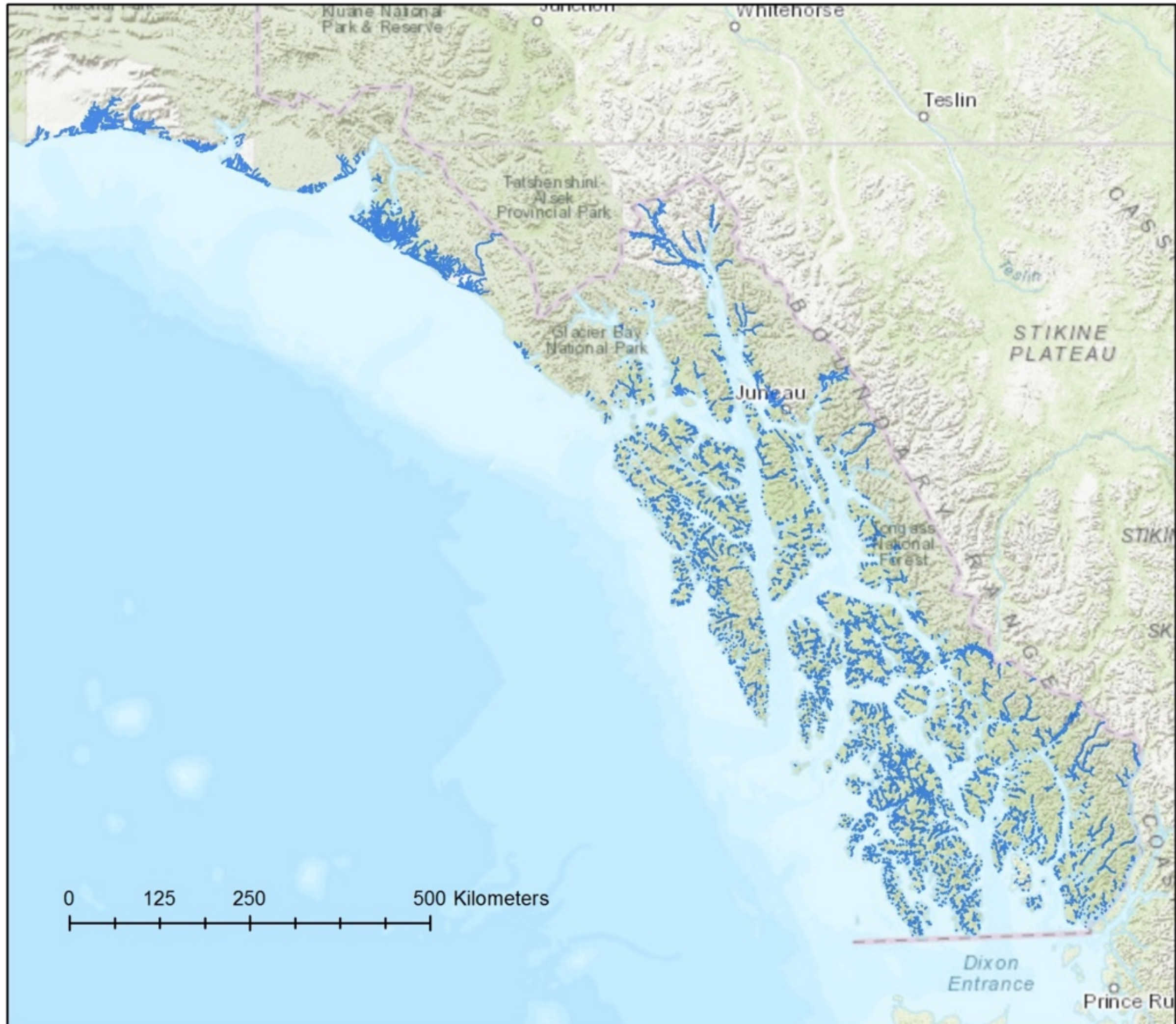
Supporting Information

S1. Salmon spawning streams in Southeast Alaska



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S2. Photos of weir structure

Smolt Capture Pen

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Upstream side of weir

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Spring downstream weir

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Fall upstream weir