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Molecular Ecology Resources in press

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, generating annual revenues 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 returning sockeye and coho spawners
26 and outmigrating sockeye smolts. eDNA thus promises accurate and efficient enumeration, but to
27 deliver the most robust numbers will need higher-resolution stream-flow data, at-least-daily
28 sampling, and a focus on species with simple life histories, since shedding rate varies amongst
29 jacks, juveniles, and adults.

30

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

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## Introduction

34 Pacific salmon (*Oncorhynchus* spp.) support a $449 million/yr commercial fishery, 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 nutrient and energy subsidies to terrestrial and aquatic food webs, in
38 large part by being important seasonal prey resources for bears, eagles, and other culturally,
39 biologically, and economically important consumers (Gende *et al.* 2002; Gende *et al.* 2004;
40 Schindler *et al.* 2003; Shakeri *et al.* 2018; Wheat *et al.* 2017). Due to their anadromous life
41 history, salmon fisheries are often managed by setting escapement goals, where escapement
42 refers to the number of fish that escape the mostly ocean-based fishery and are thus available for
43 spawning in fresh water. For example, from April to October each year, the Alaska Department
44 of Fish and Game (ADFG) continuously estimates salmon breeding population sizes in some
45 Alaskan streams and issues temporary fishery closure notices to ensure that these escapements
46 exceed minimum target sizes per species.

47 Of course, it is very costly to count fish. A typical salmon weir consists of a series of
48 closely spaced bars across an entire stream to prevent the passage of salmon, except through a
49 single, narrow gate over which a human observer tallies and identifies to species salmon as they
50 file through (alternatively, Didson sonar can be used to count and size salmon individuals as they
51 pass with species identity inferred from body size and run timing). The annual operating cost of a
52 weir is approximately $80,000, not including installation or major maintenance (Fox 2018), and
53 even this setup might be prone to undercounting (Eggers *et al.* 2009).

54 More than 6,000 streams are used by various combinations of the five species of Pacific
55 salmon in Southeast Alaska alone, and more than 1000 of those streams have been documented
56 as hosting spawning populations (Johnson & Blossom 2018; Fig. S1). Not surprisingly, almost

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

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

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

85 More generally, the under-monitoring of Pacific salmon stocks hinders the construction
86 of reliable spawner-recruit models, which are used to determine escapement goals for maximum
87 sustainable yield. The lack of such models increases uncertainty about whether, and where, there
88 are sufficient spawners to maximize recruitment and increases the risk of long-term decline or
89 loss, especially of the small, subdominant components of salmon runs. These smaller salmon
90 runs increase the resilience of salmon stocks through portfolio effects (Schindler *et al.* 2010), can
91 restock a dominant component that has suffered a negative shock, and provide key resources for
92 wildlife by extending the spatial range and phenology of salmon availability to terrestrial and
93 aquatic food webs (Gende *et al.* 2002; Levi *et al.* 2015; Schindler *et al.* 2013). As fisheries
94 increasingly transition towards ecosystem-based fisheries management (Levi *et al.* 2012),
95 identifying, monitoring, and maintaining such spatially and temporally distributed salmon
96 resources becomes increasingly important for conservation and management.

97 The advent of environmental DNA (eDNA) methods that detect DNA shed by organisms
98 (Bohmann *et al.* 2014; Goldberg *et al.* 2016) provides a promising tool for monitoring salmon
99 escapements and juvenile production because it could increase management-relevant information
100 at low cost. However, while the efficacy of using eDNA for species *detection* is now widely
101 recognized (Goldberg *et al.* 2016; Rees *et al.* 2014) and while several studies have demonstrated
102 that eDNA is generally correlated with fish abundance in mesocosm experiments, lakes, and
103 streams (Doi *et al.* 2015; Handley *et al.* 2018; Lacoursière-Roussel *et al.* 2016; Takahara *et al.*
104 2013; Tillotson *et al.* 2018; Wilcox *et al.* 2016), we do not yet know whether eDNA contains

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105 sufficient information to robustly and accurately estimate fish abundance, particularly for
106 anadromous fish as they enter and leave a watershed. By robust, we mean accuracy that is not
107 greatly affected by variation among years, species, stream, and/or details of the sampling
108 protocol.

109 Anadromous fish such as salmon provide a straightforward scenario for testing whether
110 eDNA can be used to count fish, because potentially large numbers of salmon release their DNA
111 as they pass a fixed sampling point, either as they swim upstream as returning adults or swim
112 downstream as outmigrating juveniles. If eDNA degrades or settles quickly (as suggested by
113 Jane *et al.* 2015; Jerde *et al.* 2016; Sassoubre *et al.* 2016; Shogren *et al.* 2016; Turner *et al.*
114 2015), then eDNA concentrations should primarily detect fish that are locally present in space
115 and time. Thus, rather than simply accumulating as fish enter a watershed, eDNA concentrations
116 might spike up and down as a pulse of fish swims past a sampling point, with the size of the
117 spike correlated with fish number and/or biomass. Because the concentration of eDNA in
118 streamwater results from both the amount of DNA shed by organisms and the flow of water, the
119 product of eDNA concentration and streamflow (measured in units of water volume per time)
120 can be used to calculate absolute quantities of eDNA per unit time. Such 'flow-corrected eDNA
121 rates' measured at regular intervals (e.g. daily) could then be substituted for, or complement,
122 gold-standard count data from weirs. For sockeye, coho, and chinook salmon, which produce
123 juveniles that typically rear in freshwater prior to outmigrating to the ocean as smolts, whether
124 this is plausible depends on the strength of eDNA signal produced by adults relative to what is
125 produced by juveniles residing upstream. If, for instance, juveniles rear sufficiently far upstream,
126 the signal of their eDNA should be weak or undetectable, eliminating a source of noise that
127 would prevent the robust enumeration of adult salmon entering lower stream reaches with
128 eDNA.

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129 In the most comprehensive and relevant study to date, Tillotson *et al.* (2018)
130 demonstrated that local counts of sockeye salmon in a spawning creek, particularly dead
131 sockeye, indeed predict local eDNA concentrations. As Tillotson *et al.* (2018) put it, the next
132 step is "reversing the model to predict abundance from eDNA." We accomplish this by taking
133 advantage of a daily census of sockeye and coho salmon carried out at the Auke Creek research
134 weir in Juneau, Alaska to test whether eDNA concentrations and stream-flow measurements
135 together produce quantitative and management-relevant indices of salmon escapement and
136 juvenile outmigration. To explore the general ecology of eDNA, we also quantify the relative
137 influences of salmon counts on the same day of water sampling, salmon that entered the
138 watershed one day prior, and salmon that entered two days prior to an eDNA measurement, and
139 we assess the eDNA signal produced by salmon of different life stages and body sizes. The
140 purpose of these latter analyses is to test for two possible sources of error (long-distance
141 transport of eDNA and differential shedding rates by body size and type) when using eDNA to
142 enumerate salmon.

## Methods

### *Weir operation*

143 The Auke Creek research weir is located 19.2 km north of Juneau, Alaska, 400 m downstream
144 from the outlet of Auke Lake above the high tide line at the mouth of Auke Creek (Fig. 1). The
145 ~1072.5 ha watershed includes five tributaries that feed into Auke Lake, which is 1.6 km long
146 and 1.2 km wide, with a surface area of 67 ha. The weir is cooperatively operated by the
147 National Marine Fisheries Service, in collaboration with the University of Alaska, and the
148 Alaska Department of Fish and Game, with the objective of capturing all outmigrants and
149 returning spawners at Auke Creek. All outmigrants (from upstream) are enumerated from the
150 beginning of March to the middle of June and released below the weir, after which the weir is

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151 converted to capture returning adult salmonids (from downstream), which are counted and then
152 released above the weir. During monitoring of adult salmonids, fish are classified by species and
153 life stage. The Auke Creek dataset represents probably the highest-temporal-resolution and most
154 accurate wild Pacific salmon census data in Alaska, if not the world. Life stages for coho salmon
155 include typical adult male and female fish along with smaller early maturing and small-bodied
156 'jack' males, and a unique 'nomadic' juvenile life-history strategy in which coho fry rearing in
157 the estuary and ocean return upstream (Koski 2009). Coho 'nomads' are similar to ocean-type
158 chinook and sockeye salmon that outmigrate as fry rather than rearing in freshwater, with the
159 exception that coho 'nomads' rear in the estuary within their salt tolerance and return to
160 freshwater in the fall where they overwinter as juveniles before outmigrating to the ocean as
161 smolts the following year (see Koski 2009 for details). Sockeye salmon can also produce jacks,
162 but infrequently. Complete methods for weir operation can be found in Vulstek *et al.* (2018)
163 (Weir photos in Supplemental information S2). River height is recorded daily and converted to
164 streamflow (cubic feet per second) using an established rating curve (Bell *et al.* 2017).

*Environmental DNA quantitation*

165 We collected water samples from just upstream of the weir (location photograph in Supplemental
166 Information S2) for three years, from 2014-2016, after each day's salmon enumeration. In a 2014
167 pilot study, we collected three 1L water samples weekly from 28 May to 11 December. Based on
168 promising results, and to reduce costs, in 2015, we sampled weekly when few fish were entering
169 the river and then increased sampling frequency up to daily during periods in which many
170 salmon were entering the river. Because salmon eDNA disappeared entirely after October in
171 2014, we sampled from 12 May to 3 November in 2015. Based on further promising results from
172 2015, we increased sampling frequency to daily in 2016 from 10 May to 20 October. Because
173 previous technical replicates had yielded consistent results, and because of the high frequency of

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174 water collection, we collected only two 1L water samples daily in 2015 and 2016. All water
175 samples were collected using 1L disposable sterile Whirlpak bags and filtered through a 0.45
176 micron cellulose nitrate filter. Filters were then folded and stored in 100% ethanol at 4C until
177 laboratory processing.

178 We maintained strict protocol to prevent contamination of filters and reagents. We
179 performed DNA extraction and PCR setup inside of separate HEPA-filtered and UV-irradiated
180 PCR cabinets (Air Science LLC, Fort Meyers, FL) within a separate lab where PCR product is
181 prohibited. Filters were first removed from ethanol and air-dried overnight in sterile, disposable
182 weigh boats. A modified protocol for the Qiagen DNeasy Blood and Tissue kit was used to
183 isolate DNA. This included the addition of 1.0 mm zirconia/silica beads to the initial lysis buffer
184 and then a 15 minute vortex step to loosen the DNA from the filters. Incubation in lysis buffer
185 was increased to 48 hours. After incubation, 300 ul of the lysed product was transferred to a new
186 1.7 ml microcentrifuge tube. Thereafter, we followed the manufacturer's protocol. DNA was
187 eluted in a total volume of 100 ul.

188 Using species-specific primers and TaqMan minor groove binder (MGB) probes
189 (ThermoFisher Scientific, Waltham, MA), developed by Rasmussen Hellberg *et al.* (2010)
190 (Table 1), we targeted a fragment of the cytochrome c oxidase subunit 1 (COI) gene. For each
191 species, each sample was run in triplicate PCRs. Each 20 ul qPCR contained 6 ul of DNA
192 template, 10 ul Environmental Master Mix 2.0 (ThermoFisher Scientific, Waltham, MA), 0.2 uM
193 of both forward and reverse primers, 0.2 um of the TaqMan MGB probe, and sterile water.
194 Additionally, each plate contained a four-point standard curve using DNA obtained from salmon
195 tissue from each species. Extracted tissue was quantified using a Qubit Fluorometer
196 (ThermoFisher Scientific, Waltham, MA) and diluted 10-fold from $10^{-1}$ to $10^{-4}$ ng/ul. PCR
197 cycling conditions involved an initial denaturation step of 10 min at 95 °C to activate the

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198 HotStart Taq DNA polymerase, followed by 50 cycles of 95 °C for 15 s and 60 °C for 60 s. All
199 reaction plates contained a negative control (water) as well as extraction blanks. PCR was
200 performed on an ABI PRISM 7500 FAST Sequence Detection System (Applied Biosystems,
201 Foster City, CA) and analyzed on 7500 Software v2.0.6 (Applied Biosystems, Foster City, CA).
202 Cycle values were converted to target-DNA concentration using the standard curve derived from
203 the tissue samples, and each day's eDNA concentration was taken as the mean across the two
204 extractions and the three qPCR replicates from that day for that species.

*Data analysis*

205 To calculate the flow-corrected eDNA rate, we multiplied each day's qPCR-estimated target-
206 DNA concentration ($\frac{ng}{\mu l}$) against that day's streamflow ($\frac{cubic\ feet}{sec}$). There is no need to harmonize
207 units because the product is now an estimate of DNA biomass rate (ng/sec) multiplied by a
208 dimensionless constant (volume/volume): $\frac{ng}{sec} \cdot \frac{cubic\ feet}{\mu l}$, and the fitted model parameters
209 incorporate the conversion factor. Streamflow was usually taken at 8 AM each day, near the time
210 that eDNA was sampled. Note that this measure is only for one time point per day and might not
211 be fully representative of streamflow over the whole day.

212 We predicted salmon counts from the natural log of flow-corrected eDNA rate using a
213 quasipoisson regression with a log-link function in order to account for overdispersed count data.
214 The quasipoisson model produces the same coefficients as standard Poisson generalized linear
215 models for count data, but it is more inferentially conservative (i.e. lower Type I error rates due
216 to wider confidence intervals). Log transformation of flow-corrected eDNA rate (1) allowed for
217 the fit of zero salmon counts in the Poisson model, which would otherwise only be achievable if
218 the eDNA rate approached negative infinity due to the log-link, and (2) fit a flexible power law
219 (a linear model fit in log-log space). We fit separate models in 2015 and 2016 for returning adult

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220 sockeye salmon, returning total coho salmon, and outmigrating sockeye smolts. In our analysis,
221 we included data for adult sockeye salmon from 18 June - 1 August, adult coho salmon from 15
222 August – 30 October, and outmigrating sockeye smolts from 15 April – 10 June. This time
223 period captured the full runs of each species and life stage, but did not include a time period after
224 the adult sockeye salmon run when DNA was transported downstream as salmon died in the
225 lake. We used total coho, not just adult coho, because the coho run includes a varying mixture of
226 nomadic juveniles, jacks, and adults, which are different sizes but with unknown relative
227 contributions to DNA that we found to not scale predictably with biomass (see *Ecology of*
228 *eDNA*). This is complicated in part by the fact that much larger-bodied adult salmon do not eat or
229 defecate unlike juvenile nomads, potentially unlinking the rate of eDNA shedding to biomass or
230 surface area. We detected a single high leverage outlier for coho salmon in 2016 in which a day
231 with a large pulse of jacks retained a low concentration of eDNA. To avoid poor model
232 predictions due to this outlier, we removed this data point from the results in the main text and
233 include this outlier in the models in Supplemental Information S3.

234 To determine whether the relationship between flow-corrected eDNA and salmon counts
235 was consistent between the two years, we combined the data from the two years and fit a model
236 with an additional interaction term between year and flow-corrected eDNA. A significant
237 interaction effect would indicate a different relationship between count and eDNA between
238 years, which would indicate a lack of model transferability.

239 We collected daily water temperature data, but we observed a strong negative correlation
240 of temperature and flow ($r$ = -0.75 for Sockeye adult dataset, $r$ = -0.53 for Sockeye smolt dataset,
241 $r$ = -0.10 for coho total dataset), and we were concerned about spurious correlations caused by
242 the temporal trend in temperature. Nevertheless, we explored models with stream temperature
243 and observed no consistent results in the magnitude, sign, or significance of the temperature

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244 effect across years or species, which suggested to us that our concern about spurious temperature
245 effects were warranted and could lead to overfitting that exaggerated the precision of our
246 predicted number of counts. That is, temperature effects were not transferable among years
247 within the same salmon type or among salmon types.
248

249 *Ecology of eDNA*. – We also used the dataset to explore the 'ecology of eDNA,' using salmon
250 counts from the same and previous days to predict that day's flow-corrected eDNA rate. The
251 purpose is to test for the possibility that long-distance, albeit attenuated, transport of eDNA from
252 far-upstream salmon degrades the real-time quantitative accuracy of eDNA. We also test for the
253 possibility that body size and/or life-history affects per-fish shedding rates.

254 To directly estimate the timescale over which eDNA was detected in Auke Creek, we
255 used a series of three linear regression models to relate daily counts of sockeye salmon in 2016
256 (the year with daily sampling) to flow-corrected eDNA concentration. We first modeled flow-
257 corrected eDNA as a function of salmon counts from the same day. We then used the residuals
258 from that model in a second regression that instead included salmon counts from the previous
259 day as a predictor. Finally, we used the residuals from the second model in a regression using
260 salmon counts from two days prior as a dependent variable. We interpreted significant lag
261 variables from salmon counts in the second or third models as evidence that salmon entering the
262 river one or two days ago influence the measured flow-corrected eDNA concentration. In order
263 to explore the eDNA production by coho salmon of different life stages, we additionally used
264 multiple linear regression with counts of adults, jacks, and nomad juveniles in 2015 and 2016 as
265 predictors of flow-corrected eDNA measured that same day.
266

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## Results

267 Neither the concentration of eDNA nor flow-corrected eDNA rate increased monotonically as
268 salmon accumulated in the Auke Creek watershed. Instead, flow-corrected eDNA rates reflected
269 a highly local signal of salmon abundance in space and time, effectively tracking salmon that had
270 passed near the water sampling site over the previous day (Figs. 2-4). This was true for both
271 adult salmon and smolts.

272

273 *Tracking of salmon phenology and abundances with eDNA*. – The natural logarithm of the
274 product of stream flow (cubic feet per second, cfs) and eDNA concentration (ng/μl), which we
275 refer to as flow-corrected eDNA rate, was highly predictive of the counts of returning adult
276 sockeye and coho salmon, as well as of outmigrating sockeye salmon smolts in both 2015 and
277 2016 (Fig. 5; Adult sockeye 2015: $\beta = 0.63 \pm 0.20$, $p = 0.008$; Adult sockeye 2016: $\beta = 0.79 \pm 0.11$,
278 $p < 2e\text{-}8$; Adult sockeye both years: $\beta = 0.71 \pm 0.09$, $p < 2e\text{-}10$; Total coho 2015: $\beta = 0.70 \pm 0.10$, $p$
279 $< 2e\text{-}8$; Total coho 2016: $\beta = 0.78 \pm 0.10$, $p < 3e\text{-}10$; Total coho both years: $\beta = 0.66 \pm 0.06$, $p <$
280 $2e\text{-}16$; Sockeye smolts 2015: $\beta = 1.64 \pm 0.37$, $p = 0.004$; Sockeye smolts 2016: $\beta = 1.42 \pm 0.35$, $p$
281 $= 0.003$); Sockeye smolts both years: $\beta = 1.33 \pm 0.30$, $p = 0.0005$).

282 The combined models for 2015 and 2016 unambiguously failed to identify an interaction
283 effect between year and flow-corrected eDNA rate for adult sockeye salmon ($p = 0.43$), total
284 coho salmon ($p = 0.59$), and for sockeye salmon smolts ($p = 0.71$), indicating that eDNA had a
285 consistent relationship with salmon counts across years.

286 In all models, the quasipoisson regression models using flow-corrected eDNA rate as a
287 single predictor produced visually representative predictions of counts through time that captured
288 the phenology, temporal dynamics, and relative abundance of each run (Fig. 6). Similarly, we
289 tried models with water temperature as an additional predictor but saw no consistently significant

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290 effects, and we were concerned about spurious correlations caused by the temporal trend in
291 temperature data and its strong anti-correlation with flow ($r$ = -0.75 for Sockeye adult dataset, $r$
292 = -0.53 for Sockeye smolt dataset, $r$ = -0.10 for coho total dataset). This result is not surprising
293 since visual inspection of the temperature timelines (Figs. 2-4) reveals no covariance with fish
294 counts.
295
296 *Ecology of eDNA*. – As expected, sockeye salmon counts from the current day in 2016
297 significantly predicted flow-corrected eDNA rate ($\beta = 0.0011 \pm 0.00016$, $p < 10^{-7}$), but salmon
298 countsfrom one day prior were only marginally related to any residual variation from the first
299 model ($\beta = 0.00026 \pm 0.00015$, $p < 0.09$), and salmon counts from two days prior were completely
300 unrelated to residual variation not accounted for by salmon counts from the same day and one
301 day prior ($p = 0.99$).
302 When pooling 2015 and 2016 data, of the three coho salmon life-history categories
303 (adults, jacks, and nomadic juveniles), adults produced the strongest flow-corrected eDNA signal
304 ($\beta = 0.0059 \pm 0.00048$, $p < 10^{-15}$), which was 3.5 times higher than that produced by each juvenile
305 fish class ($\beta = 0.0017 \pm 0.00058$, $p < 0.004$). When accounting for the eDNA signal produced by
306 adults and juveniles, counts of jacks were uncorrelated with flow-corrected eDNA ($\beta$ = -
307 $0.0006 \pm 0.0014$, $p = 0.69$).

## Discussion

308 Since the efficacy of eDNA was first demonstrated for the detection of invasive bullfrogs
309 (Ficetola *et al.* 2008), a rapidly growing body of literature has highlighted the efficacy of eDNA
310 for rare species detection (Rees *et al.* 2014; Wilcox *et al.* 2016), has explored the technical
311 aspects of eDNA (Goldberg *et al.* 2016), and has suggested that eDNA holds promise for

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312 quantifying the abundance of species (Doi *et al.* 2015; Lacoursière-Roussel *et al.* 2016; Takahara
313 *et al.* 2013; Tillotson *et al.* 2018). The next, and most transformative, technical step for
314 mobilizing the use of eDNA for resource managers is to determine whether, and under what
315 conditions, eDNA can be used to *enumerate* organisms. The possibility of enumerating Pacific
316 salmon as they outmigrate or return to spawn represents a particularly promising application,
317 with large economic and risk-management implications for a multibillion dollar fishery and
318 keystone wildlife resource.

319 To test the efficacy of eDNA for salmon enumeration, we coupled a complete census of
320 returning and outmigrating anadromous salmon with daily quantitation of environmental DNA.
321 We have demonstrated that flow-corrected eDNA rate:
322 (1) predicts same-day, daily counts of two species of adult salmon returning into the watershed
323 (Figs. 2, 3) and of one species of outmigrating salmon smolt (Fig. 4),
324 (2) does not simply accumulate over time, which would have otherwise reflected the total
325 number of salmon that have entered the watershed this season (Figs. 2, 3),
326 (3) is minimally affected by upstream-rearing juveniles (*Ecology of eDNA*), given that the eDNA
327 from the coho and sockeye fry rearing in Auke Lake appears to settle and/or attenuate prior to
328 reaching lower stream reaches,
329 (4) is highly accurate at delimiting the phenologies of returning adult and outmigrating juvenile
330 salmon (Figs 2, 3, 4), and
331 (5) is affected by differential DNA-shedding rates across different life-history strategies and
332 body sizes (*Ecology of eDNA*).
333 We have also identified several remaining obstacles to straightforward implementation of eDNA
334 for the enumeration of salmon. Most importantly, accurate measures of streamflow are crucial.
335 This is particularly true because pulses of adult salmon immigration sometimes coincide with

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336 high streamflow events (Figs. 2-4), and the error in estimating streamflow is exacerbated because
337 the ratings curves that relate river height (the measure that is actually recorded daily) to flow
338 contain more error at extreme values, since extreme-flow estimates are either based on few
339 calibration points or on none at all and just represent extrapolations.

340 The adult sockeye runs are excellent examples of the importance of obtaining accurate
341 streamflow data (Fig. 2). In 2015, non-flow-corrected sockeye eDNA concentration ('DNA'
342 timeline) was highest around 1 July and declined monotonically through the month despite few
343 adult returning sockeye in early July. However, early July was also a period of low stream flow.
344 Only after accounting for stream flow ('Flow X DNA' timeline), which included a flood event
345 around 15 July, did eDNA correctly predict the observed sockeye immigration peak on 15 July
346 ('Counts' timeline). In 2016, there were three non-flow-corrected eDNA peaks ('DNA'
347 timeline), the timings of which very closely matched the three count peaks. However, the first
348 two non-flow-corrected eDNA peaks, in early July, were taller than the third peak, which is the
349 opposite to that seen in the count data (Fig. 2). This occurred because the third eDNA
350 concentration peak, in late July, occurred just as streamflow also rose, diluting the eDNA ('Flow
351 (cfs)' timeline). The third eDNA peak's shape and size more closely matched the count data after
352 flow correction ('Flow X DNA' timeline), although the third eDNA peak is still smaller than
353 expected based on the size of the first peak. We hypothesize that the streamflow value that we
354 used to multiply the first day of the third eDNA concentration peak was too low, potentially
355 because it was recorded before most of that day's flow increase had occurred, causing us to
356 under-correct and thus under-predict. We have informally substituted in the next day's much
357 higher streamflow value (flow during the third sockeye peak rapidly more than tripled from 6.6
358 to 23.1 cfs between 23 and 24 July), and the third flow-corrected eDNA peak matches the count
359 data more closely (data not shown).

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360 A second critical consideration for quantifying anadromous fish counts with eDNA is the
361 temporal resolution of an eDNA measurement. As adult salmon move upstream, the signal
362 produced by their shedding of DNA attenuates and is eventually not detectable. Therefore,
363 effective monitoring of anadromous fish with highly variable daily counts requires eDNA to be
364 sampled at least daily. Even with daily sampling, we can imagine that the eDNA signal produced
365 by a medium-sized pulse of fish now could be the same strength as the signal produced by a
366 large pulse of fish that passed by hours ago. This ambiguity sets an upper limit on the accuracy
367 of eDNA for quantifying anadromous fish abundance.

368 How much the above two *within*-stream sources of error reduce reliability in decision-
369 making depends in part on the level of variation *across* streams. If a single stream, regardless of
370 how accurately it is censused, does not reflect regional escapement sizes, due to variation in
371 salmon abundance across streams, it might be more robust to collect data from many streams
372 (probably only feasible with eDNA), even at a cost of reduced accuracy per stream. Currently,
373 the Alaska salmon fishery does not have enough data to judge this possibility.

374 A third consideration is that some salmon runs contain a mix of individuals with different
375 life histories. This was particularly the case for coho salmon in 2016, for which jacks were
376 numerically dominant early in the run and a nomadic juvenile coho life history strategy was
377 dominant late in the run. Both nomadic juveniles and jacks were rare in 2015. Jacks and
378 juveniles did not produce levels of DNA concordant with the production by adult salmon (Fig.
379 3), which introduced error into the relationship between flow-corrected eDNA and coho salmon
380 counts (Figs. 5-6). For unknown reasons, coho jacks produced no detectable eDNA when
381 controlling for adults and nomads.

382 A fourth consideration is the location of sample collection versus the locations of rearing
383 juvenile salmon and spawning adults. Given our results, salmon enumeration should occur in

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384 lower stream reaches, as far as possible from spawning areas that will shed large quantities of
385 eDNA from gametes and decaying fish and from large numbers of rearing salmon fry. It is
386 possible that the presence of the lake upstream of our sampling location facilitated settling or
387 degradation of eDNA, which may have increased the ratio of signal (current salmon moving past
388 the weir) to noise (eDNA from other sources upstream) in our measurements. Similarly, the
389 presence of the weir led to fish released upstream shortly before eDNA sampling.

390 Implementation in a stream

391 Finally, noise in enumeration with eDNA can be caused by a lack of primer specificity.
392 Our assays are much more sensitive to sockeye and coho salmon DNA than to non-target
393 salmonids, but there can be non-zero amplification of some non-target DNA. In particular,
394 chinook and coho cross-amplify at low levels (data not shown), which was not an issue in this
395 research because Auke Creek does not have a resident population of chinook salmon (although
396 strays do attempt to enter at the weir). Ensuring good primer specificity to the extant species will
397 help reduce noise in future efforts to enumerate anadromous fish with eDNA.

398 Pacific salmon are a valuable resource, but their distributed spawning and rearing habitat,
399 due to their anadromous life history, makes monitoring their distribution and abundance a
400 formidable challenge, which consequently injects an unknown but probably non-trivial amount
401 of inefficiency and risk into management. Given the strong observed correlations between daily
402 eDNA samples and fish counts (Figs. 5-6), investment in technology to allow frequent or even
403 near-real-time eDNA quantitation and stream-flow measurement could provide a more accurate
404 and cost-effective means of reducing this inefficiency and risk. This would be especially true if
405 daily eDNA samples from many streams turn out to provide a more accurate estimate of regional
406 escapement sizes than do intensive direct-count measurements at a few streams. However, our
407 results also suggest that using eDNA to estimate fish abundance will require (1) accurate and

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408 ideally time-averaged streamflow measures and (2) frequent (at-least-daily) eDNA sampling due
409 to the ephemeral nature of the eDNA signal. On the other hand, this very ephemerality is what
410 makes eDNA such a sensitive correlate of salmon abundance.

411 Even with a fixed budget constraint, it should be possible for a technician who would
412 otherwise be paid to count fish in a single stream to instead collect water samples from many
413 spawning streams across a watershed. In addition, water sampling could be extended to quantify
414 smolt runs, which are currently only estimated in Southeast Alaska at a small number of index
415 systems. Moreover, because post-sampling filters can be stored in a refrigerator or freezer for
416 many days after sampling, it should be feasible to train and pay a network of citizen scientists to
417 carry out sampling across multiple watersheds. Note also that although our analysis focused on
418 sockeye and coho salmon, the same eDNA sample can be used to detect and/or quantify any
419 number of aquatic species with the development of appropriate assays. Against these potential
420 gains in sampling efficiency and information must be balanced the additional cost of the qPCR
421 assays to be carried out in a dedicated eDNA lab.

422 Our study is of a single stream in Southeast Alaska. However, it provides strong
423 justification for an expanded effort to sample salmon eDNA over more streams, more species,
424 and more days, both in the streams that currently have weirs, so that a robustly transferable
425 model can be parameterized and validated, and in some of the many streams that are not
426 currently monitored, to test for the possibility that multiple streams sampled daily with eDNA
427 provide more useful information than a few streams counted intensively. The applicability of
428 eDNA to expand monitoring of anadromous salmon to currently unmonitored rivers will depend
429 on the transferability of flow-corrected eDNA rate among streams. It is possible that differences
430 in stream size, morphology, and hyporheic flow will be too idiosyncratic for results calibrated on
431 one weir to be transferable among rivers, thus requiring independent calibration on every river to

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432 be monitored. Alternatively, results might be transferable among systems with similar
433 morphology. For example, Auke Creek is a short river course below a lake, which may lead to
434 calibration results that are only transferable to systems with an upstream lake where eDNA
435 settles prior to downstream transport. Given the huge size of the Alaska salmon fishery, even a
436 small improvement in management effectiveness and/or a small decline in the risk of population
437 decline or establishment by alien salmonids could justify the investment in large-scale eDNA
438 calibration tests and an assessment of the efficacy of deploying eDNA to expand the portfolio of
439 streams that can be effectively monitored.
440

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452 or product names is for descriptive purposes only and does not imply endorsement by the U.S.
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454

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541

542

## 543 Data Accessibility

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

545 and on Dryad doi: 10.5061/dryad.94d37g3

## 546 Author Contributions

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

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

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

550 from all other authors.

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

552

| 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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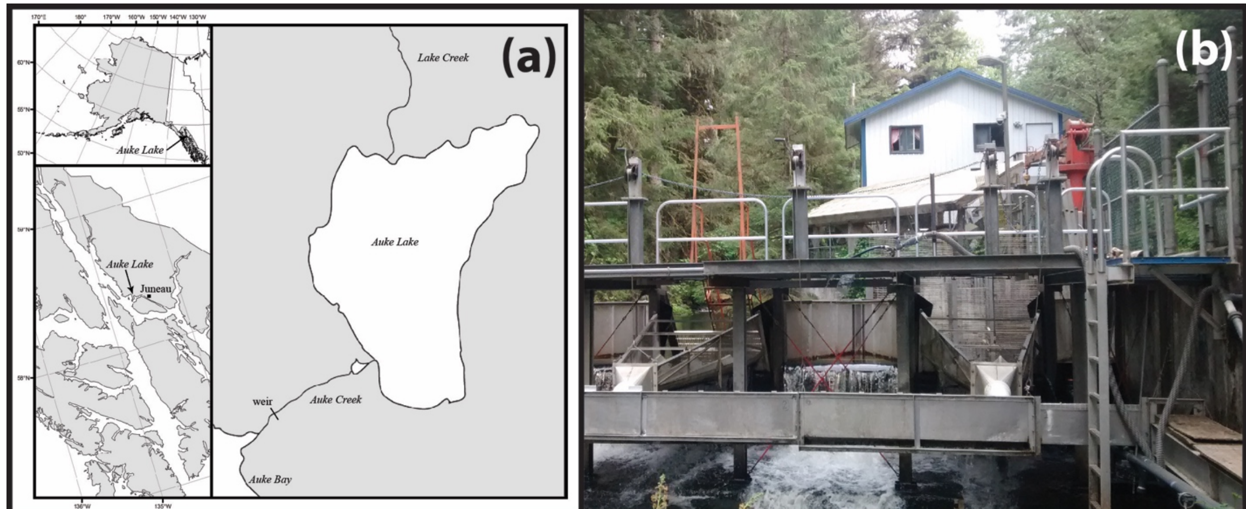
**Figure 1.** The Auke Creek research weir is (A) located in Juneau, Alaska at the outflow of Auke Lake. (B) The weir is a permanent structure used to sort and enumerate outmigrating juvenile salmon and returning adult salmon.



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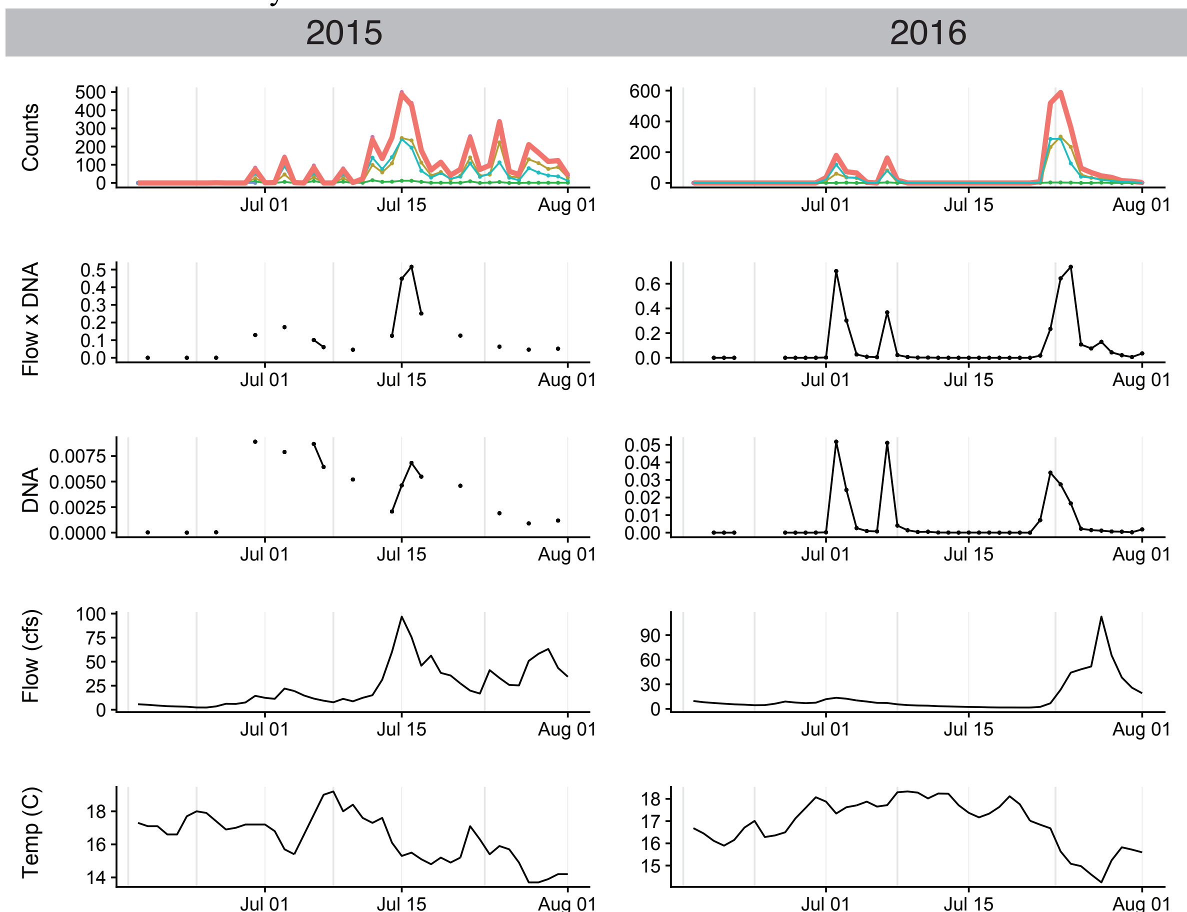
561 **Figure 2.** Timeline from June 18 to August 1 of adult sockeye salmon counts, flow-corrected
562 eDNA concentration (ng/µl*cfs), uncorrected eDNA concentration (ng/µl), stream flow (cfs,
563 cubic-feet/sec), and stream temperature (°C) in 2015 and 2016. Environmental DNA results from
564 consecutive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

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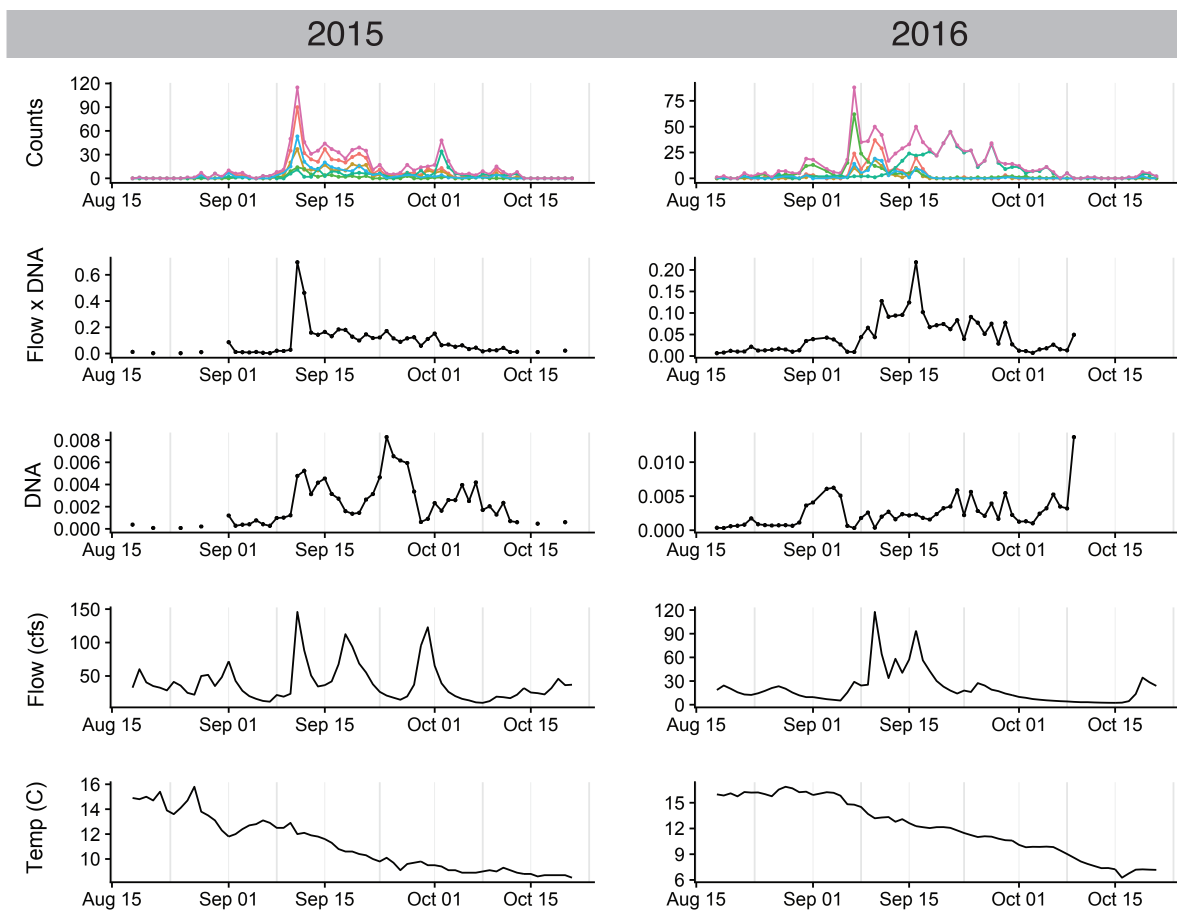
570 **Figure 3.** Timeline from August 15 to October 30 of coho salmon counts, flow-corrected eDNA
571 concentration (ng/µl*cfs), uncorrected eDNA concentration (ng/µl), stream flow (cfs), and
572 stream temperature (C) in 2015 and 2016. Environmental DNA results from consecutive days are
573 connected by lines. Male and female coho salmon are denoted by yellow-brown and blue lines
574 respectively, jacks are denoted by green lines, counts of a nomadic juvenile life history strategy
575 in which young coho rear in the estuary and ocean and return upstream are denoted by teal lines,
576 total adult (male + female) coho salmon counts are denoted by red lines. Total coho salmon
577 counts including jacks and juveniles are denoted by pink lines. Note that the adult male and
578 female coho salmon were the dominant component of the run in 2015 while the jack and juvenile
579 life history strategy was a major component of the run in 2016. A pulse of 62 coho jacks was
580 recorded on Sep 7, 2016, but no concomitant eDNA signal was recorded.
581

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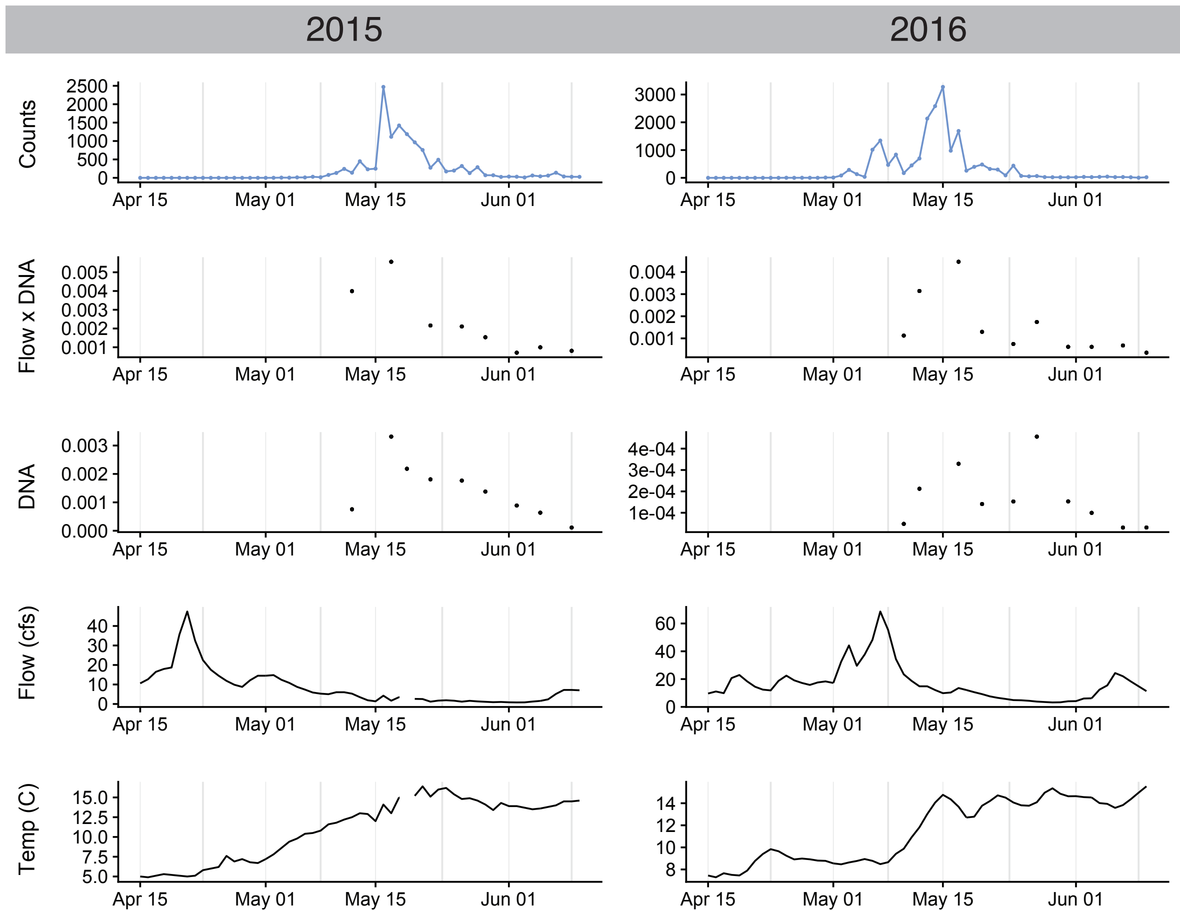
583 **Figure 4.** Timeline from April 15 to June 10 of outmigrating sockeye salmon smolt counts, flow-
584 corrected eDNA concentration (ng/μl*cfs), uncorrected eDNA concentration (ng/μl), stream
585 flow (cfs), and stream temperature (C) in 2015 and 2016. Environmental DNA results from
586 consecutive days are connected by lines.
587

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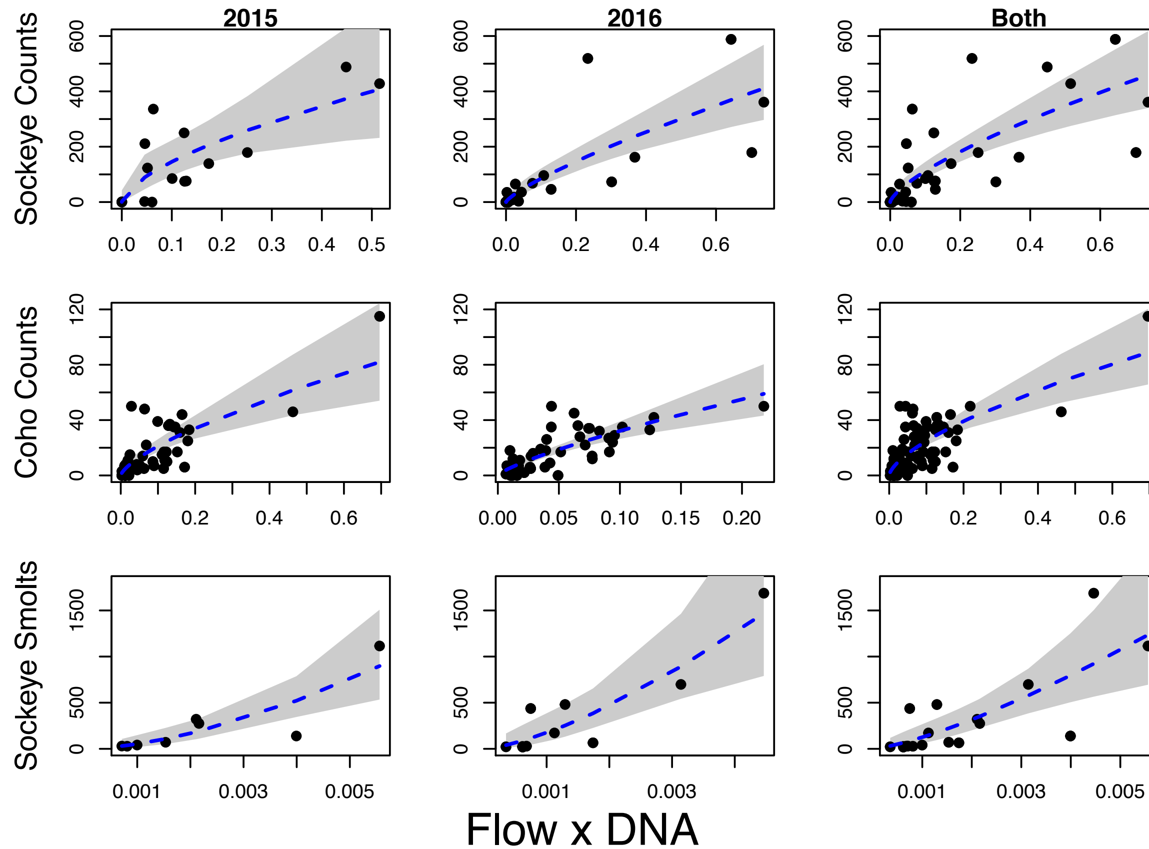
590 **Figure 5.** Results of quasipoisson regression models relating flow-corrected eDNA
591 concentration to adult sockeye salmon counts (2015: p=0.008, 2016: p<2e-8, both years: p<1e-
592 10), total coho salmon counts (2015: p<2e-8, 2016: p<3e-8, both years: p<2e-16), and counts of
593 sockeye salmon smolts (2015: p=0.004, 2016: p=0.003, both years: p<0.005). Gray shading
594 denotes the 95% confidence interval.
595

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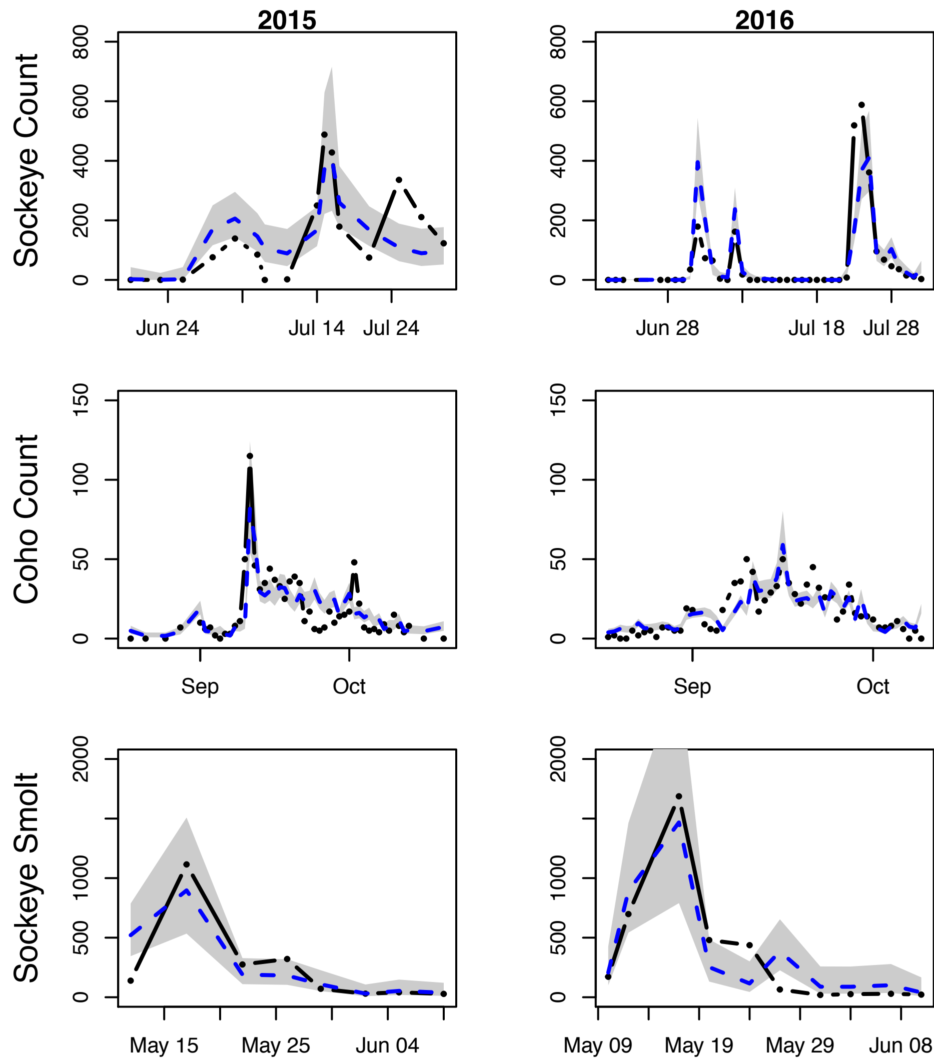
597 **Figure 6.** Counts of adult sockeye salmon, total coho salmon (including all life history
598 strategies), and sockeye salmon smolts (black dots) and the predicted number of counts based on
599 the flow-corrected eDNA concentration predictor in the quasipoisson regression model (blue
600 dashed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

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MOLECULAR ECOLOGY RESOURCES

**Supplemental Information for:**

# Environmental DNA for the enumeration and management of Pacific salmon

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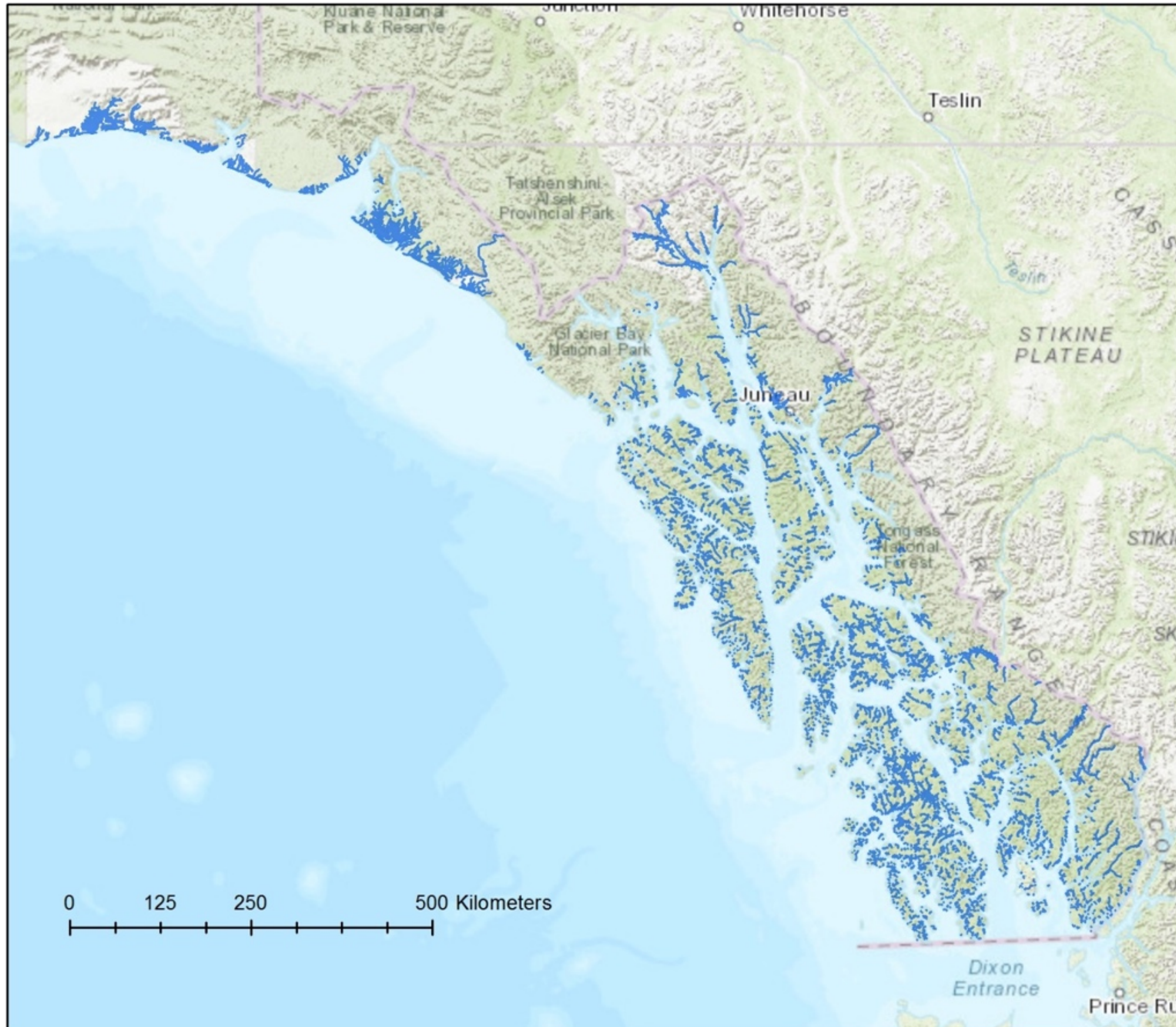
MOLECULAR ECOLOGY RESOURCES

## S1. Salmon spawning streams in Southeast Alaska indicated by blue shading.



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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

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## Sampling location upstream from weir

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## S3. Results including outlier day with pulse of jacks

**Figure S3.1.** Results of quasi-Poisson regression models relating the natural logarithm of flow-corrected eDNA concentration to adult sockeye salmon counts (2015: $p=0.008$, 2016: $p<2e-8$), total coho salmon counts (2015: $p<2e-8$, 2016: $p=0.002$), and counts of sockeye salmon smolts (2015: $p=0.004$, 2016: $p=0.003$). Gray shading denotes the 95% confidence interval.

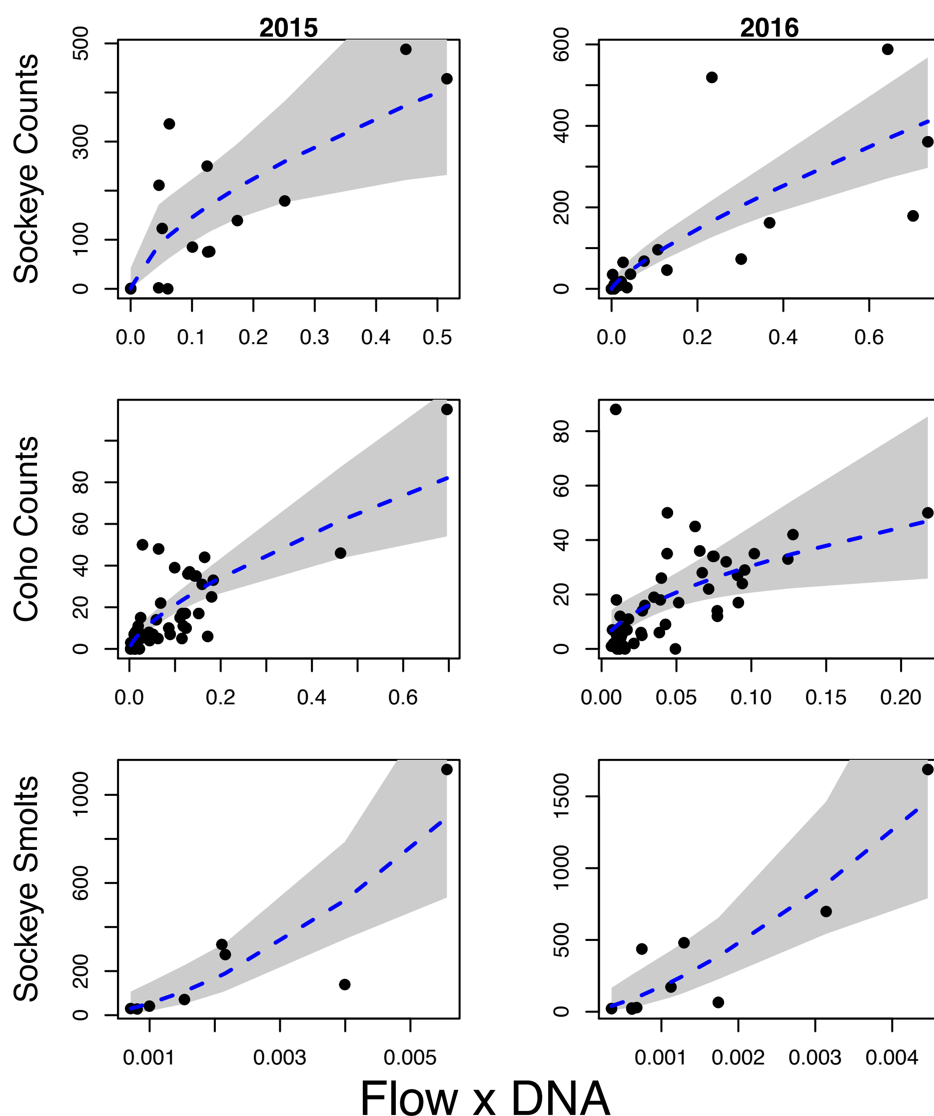

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**Figure S3.2.** Counts of adult sockeye salmon, total coho salmon (including all life history strategies and the single outlier in 2016), and sockeye salmon smolts (black dots) and the predicted number of counts based on the flow-corrected eDNA concentration predictor in the quasi-Poisson regression model (blue dashed lines). Gray shading denotes the 95% confidence interval.