Environmental DNA for indigenous-led monitoring of a culturally important fish

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Abstract

Although the environmental DNA shed from an organism is now widely used for species detection in a wide variety of contexts, mobilizing environmental DNA for management requires estimation of population size and trends rather than simply assessing presence or absence. However, the efficacy of environmental-DNA-based indices of abundance for long-term population monitoring have not yet been assessed. Here we report on the relationship between five years of mark-recapture population estimates for eulachon (*Thaleichthys pacificus*) and 'eDNA rates,' which are calculated from the product of stream flow and DNA concentration. Eulachon are a culturally and biologically important anadromous fish that have significantly declined in the southern part of their range but were historically rendered into oil and traded between coastal and interior peoples on a network of 'grease trails'. Both the peak eDNA rate and the area under the curve of the daily eDNA rate were highly predictive of the mark-recapture population estimate, explaining 97.4% and 93.4% of the deviance respectively. In the absence of flow correction, the peak of the daily eDNA concentration explained only 41.9% while the area under the curve still explained 92.1% of the deviance. These results support the use of eDNA to monitor eulachon population trends and represent a >80% cost savings over mark-recapture, which could be further increased with automated water sampling, reduced replication, and focused temporal sampling. Due to its logistical ease and affordability, eDNA sampling can facilitate monitoring a larger number of rivers and in remote locations where mark-recapture is infeasible.

Introduction

Eulachon (*Thaleichthys pacificus*) are a lipid-rich, anadromous smelt of the family Osmeridae (Mecklenburg et al. 2002). Their historic range stretched from southern California to the Bering Sea in southwest Alaska (Hart, 1973). In Southeast Alaska, they are the first anadromous fish to return after the long winter, and as a result, are a key resource for indigenous people and for wildlife. For the Northwest Coast native people, eulachon are a culturally significant staple food source that is consumed fresh, dried, or smoked, and are frequently rendered into oil (Betts, 1994). Historically, eulachon oil was the most important trade item on a network of 'grease trails' between coastal and interior peoples, and it is still used and traded (Betts, 1994; Moody & Pitcher, 2010). Eulachon spawn just prior to the breeding season of many consumers, including marine mammals, thus providing a high-energy prey resource at an energetically demanding time (Sigler, Womble, & Vollenweider, 2004). The eulachon spawning aggregation draws enormous congregations of seabirds, bald eagles (*Haliaeetus leucocephalus*), Steller sea lions (*Eumetopias jubatus*), harbor seals (*Phoca vitulina*), and humpback whales (*Megaptera novaeangliae*) among many other smaller predators and scavengers.

The majority of eulachon populations have been declining since the 1990s (Hay & Mccarter, 2000). In 2010, the National Marine Fisheries Service (NMFS) listed the southern distinct population segment in Washington, Oregon, and California as Threatened under the Endangered Species Act (NOAA, 2010). Because there is no commercial eulachon fishery in northern Southeast Alaska, there is no harvest regulation or management, agency oversight, or monitoring of population trends. While some eulachon population declines have been well documented (Hay & Mccarter, 2000), most populations of eulachon are either unknown or anecdotal. In southeast Alaska, eulachon remain a key resource for indigenous people and

65 wildlife, but little is known about their physiology or spatiotemporal dynamics (Betts, 1994;
66 Olds, 2016).

67 A lack of eulachon population information and the cultural and subsistence value of the
68 species led to the development of an indigenous-led eulachon monitoring program in northern
69 Southeast Alaska. In 2010 the Chilkoot Indian Association and the Takshanuk Watershed
70 Council initiated a modified Lincoln-Petersen (Lincoln, 1930; Petersen, 1896) mark-recapture
71 population estimate on the Chilkoot River near Haines, Alaska at the northern end of southeast
72 Alaska (Fig. 1). This program was successful in gathering baseline eulachon population data
73 where none existed previously; however, monitoring is challenging and expensive (~$20,000
74 annually), limiting the feasibility of conducting long-term monitoring and limiting the possible
75 geographic scope of monitoring.

76 In an effort to develop a more cost-effective monitoring method, in 2014 we began
77 pairing the mark-recapture program with daily water sampling to evaluate the efficacy of
78 environmental DNA (eDNA) to produce an index of eulachon abundance. While the
79 environmental DNA shed from an organism is now widely used for species detection in a wide
80 variety of contexts (Barnes & Turner, 2016; Rees, Maddison, Middleditch, Patmore, & Gough,
81 2014; Jerde, Mahon, Chadderton, & Lodge, 2011; Laramie, Pilliod, & Goldberg, 2015; Mächler,
82 Deiner, Steinmann, & Altermatt, 2014; Rees et al., 2014; Takahara, Minamoto, Yamanaka, Doi,
83 & Kawabata, 2012), mobilizing environmental DNA for management requires estimation of
84 population size and trends rather than simply assessing presence or absence. Recent research
85 suggests that eDNA quantified with real-time quantitative polymerase chain reaction (PCR) or
86 digital PCR can provide a proxy for actual abundance in controlled experiments (Rees,
87 Maddison, Middleditch, Patmore, & Gough, 2014), in ponds (Lacoursière-Roussel, Côté,

Leclerc, & Bernatchez, 2016; Takahara et al., 2012) and in streams (Doi et al., 2015; Levi et al., 2019; Lodge et al., 2012; Tillotson et al., 2018; Wilcox et al., 2016). However, the efficacy of environmental DNA based indices of abundance have not yet been assessed in a management context for long-term population monitoring.

Here we compare five years (2014-2018) of mark-recapture eulachon abundance estimates with eulachon eDNA quantification to test whether long-term, affordable, indigenous-led monitoring of eulachon populations could be effectively achieved with environmental DNA. This method could facilitate intertribal cooperation for affordable monitoring of a culturally important subsistence and economic resource on a regional scale. Such regional monitoring is particularly important for eulachon, which exhibit low site-fidelity and thus regional broad-scale population structure (Flannery, Spangler, Norcross, Lewis, & Wenburg, 2013) such that a true population decline can only be verified by monitoring multiple river systems.

Methods

Study System

The Chilkoot River near Haines, Alaska has long been a culturally and ecologically important river. The lower Chilkoot River flows 1.5 km from Chilkoot Lake to the ocean at the terminus of a large fjord. The Chilkoot Tlingit village and fishcamp was historically located along the banks of the Chilkoot River, which is still utilized for eulachon fishing and processing today (Betts, 1994; Olds, 2016). Eulachon typically spawn in the lower reaches of the Chilkoot River (Hay & Mccarter, 2000) where mostly indigenous harvesters capture large quantities for smoking, frying, and rendering into oil in pits.

The Chilkoot Indian Association initiated a eulachon mark-recapture study to develop the first population baseline for the Chilkoot River and among the only population data from Southeast Alaska (Alaska Department of Fish and Game Aquatic Resources Permit: SF-2014-027, SF2015-066, SF2016-113, SF2017-062, SF2018-072). This effort was initiated because anecdotal observation suggested that the run size and timing on the Chilkoot River differed from traditional knowledge, and because the decline of the southern distinct population segment of eulachon was substantial enough to warrant threatened status under the Endangered Species Act. The Endangered Species Act listing of eulachon led to concern by Chilkoot Indian Association tribal members that a decline in northern Southeast Alaska, where a strong subsistence fishery remained, would go undocumented, and thus un-remediated, without quantification of the current run size (Olds, 2016).

Mark-Recapture

At the mouth of the Chilkoot River, eulachon were captured using a modified fyke net trap and dip nets. The initial captured eulachon (M group) were transferred in small groups to plastic dishpans where they could be easily handled to clip off the adipose fin using retina scissors and returned to the river. To avoid excessive increases in temperature and to reduce the possibility of disease transmission, the water in the dishpans was changed between each group. To allow time for the marked fish to mix with the unmarked fish, the recapture group was captured approximately 0.75 km upstream of the trap location (C and R group) (Fig 1). Eulachon in the second capture group were collected by field crews wading through the river with dip nets making sure to sample all portions of the river and with the help of subsistence harvesters. The captured fish were examined for a clipped adipose fin before releasing. To avoid repetitive sampling of the same fish, field crews started at a downstream point and worked their way

133 upstream. A modified Lincoln-Peterson estimator equation was used $N = \frac{(M+1)(C+1)}{R+1} - 1$ where
134 N = total population size, M = marked initially, C = total in second sample, and R = marked
135 recaptures. The standard error was calculated using the equation $SE =$
136 $\sqrt{[(M+1)(C+1)(M-R)(C-R)]/[(R+1)^2(R+2)]}$. The 95% confidence interval was
137 calculated as $CI = N \pm (1.96)(SE)$. Mark-recapture data were collected from 2010 through
138 2018, excluding 2013 where a lapse in funding prohibited collection.

139 *Environmental DNA*

140 We collected daily water samples for eulachon eDNA quantification at the mark-
141 recapture trap location near the mouth of the Chilkoot River (Fig. 1) from 2014 through 2018.
142 The samples were taken during low tide to avoid either DNA intrusion from the estuary and/or
143 dilution with an influx of tidal flow. Three 1 L water samples were collected in sterile Whirl Pak
144 bags starting in early to mid-April and continuing for at least one week beyond the end of the
145 mark-recapture study duration. Each sample was filtered through a 0.45 micron cellulose nitrate
146 filter using either a peristaltic pump or vacuum pump. Filters were stored in 100% ethanol in 2
147 mL cryovials. Filters were removed from ethanol and air-dried overnight in sterile, disposable
148 weight boats in a hepafiltered and UV-irradiated cabinet within a PCR-free laboratory to avoid
149 contamination. DNA was then extracted using the Qiagen DNeasy Blood and Tissue kit modified
150 to include a >48 hour soak in lysis buffer, which was found to produce higher and more
151 consistent yields. DNA was eluted in a total volume of 100 µl.

152 *DNA Quantification*

153 We developed a species-specific quantitative PCR assay for eulachon targeting a 187-bp
154 region of the *Cytochrome oxidase I* (COI) region of the mitochondrial genome while minimizing

155 overlap with other fishes in the family Osmeridae. The probe was labeled with a 5' FAM
156 fluorescent marker and a minor-groove-binding non-fluorescent quencher on the 3' end. Primer3
157 software (Untergasser et al. 2012) was used to select the following primers: Euc_COI_R (5'-
158 CTCCCTCCTTCCTTCTCCTT-3'), Euc_COI_R (5'- GGTCTGGTACTGGGAAATGG-3') and
159 the internal probe Euc_COI_I (5'-6FAM*AGCGGGAGCCGGGACTGGCT*MGBNFQ).
160 A Bio-Rad QX200 AutoDG Droplet Digital PCR system (Hercules, CA. USA) at the
161 Oregon State University Center for Genome Research and Biocomputing was used to quantify
162 DNA concentrations. A 22 µl reaction was carried out containing (final concentrations) 1 x
163 ddPCR Supermix for probes (no dUTP), 900 nM of both forward and reverse primers, 250 nM
164 internal probe and 4 µl of DNA extract. Droplets were generated using the QX200 AutoDG
165 system, which adds the ddPCR reaction mixture to the droplet oil. 40 µl is then dispensed into a
166 new 96-well plate. Cycling consisted of 95 °C for 10 mins, followed by 45 cycles of 94 °C for 30
167 secs, and 60 °C for 1 min, ending with 96 °C for 10 mins, allowing for a ramp rate of 2 °C/sec
168 between steps. After the reaction, the droplets were read on a Droplet Reader and analyzed with
169 QuantaSoft Analysis Pro software (version 1.0.596).
170 The concentration of eDNA is sensitive to both the amount of eDNA shed into the river
171 and dilution of eDNA due to increased stream flow. To calculate the flow-corrected eDNA rate,
172 we multiplied each day's ddPCR DNA concentration $\left(\frac{copies}{ul}\right)$ against the day's stream flow
173 $\frac{cubic\ feet}{sec}$. We refer to this as an eDNA rate because the volume units cancel, leading to a result
174 proportional to DNA copies/second (Levi et al. 2019). Stream flow measurements were taken
175 each day that an eDNA sample was collected immediately following the collection of the eDNA
176 sample. To measure streamflow, we used a rating curve developed by the Alaska Department of
177 Fish and Game for the Chilkoot River. To validate this rating curve, a stream flow measurement

was taken at the beginning of each field season on the Chilkoot following the USGS velocity-area method using a type AA current meter (Turnipseed & Sauer, 2010). Following the initial calibration of the rating curve, the daily river height was measured off of an established benchmark using surveying equipment, which was then transformed into a river discharge based on the rating curve.

Analysis

We evaluated the flow-corrected eDNA rate as an index of eulachon abundance based on two metrics. First, we use the maximum eDNA rate (i.e. size of peak). Second, we used area under the curve of the eDNA rate throughout the duration of the run. Particularly in cases of multimodal runs, the area under the curve is expected to provide a more accurate representation of the overall biomass. We computed the area under the curve with the AUC function in the *DescTools* package version 0.99.27 (Signorell, 2019) in RStudio version 1.1.383 (RStudio Team, 2015). We additionally assessed the need for flow correction by evaluating the relationship between uncorrected eDNA concentrations and mark-recapture population estimates. We used quasipoisson regression to model the mark-recapture population estimates as a function of the natural log of the two measures of eDNA rate and the uncorrected eDNA concentration.

Results

Mark-Recapture

The mark-recapture population estimate was initiated in 2010 and continued annually through 2018, excluding 2013. Eulachon exhibited substantial population fluctuations with a potential 5-6-year cyclic pattern for large returns (Fig. 2). The average eulachon population estimate for the mark-recapture method from 2010-2018 (excluding 2013) was 6.1 million, with

201 a maximum of 12.6 million in 2011 and 2017 (±521,961 and 1,113,520, respectively), and a
202 minimum of 319,568 in 2015 (±158,934) (Table 1).
203 Two notable anomalies occurred during the mark-recapture study period. In 2016 the run
204 consisted of multiple pulses with what appeared to be a definitive end of the run that was
205 followed by a final pulse of fish five days later. This final pulse of fish was not recorded as part
206 of the mark-recapture estimate but was captured in the eDNA data. Additionally, the 2015 run
207 was a failure, with the smallest number of fish during our study period.
208
209 *Environmental DNA*
210 The product of streamflow and eDNA concentration, which we refer to as 'flow-corrected eDNA
211 rate' (Fig. 3, see also Levi et al. 2019), was highly predictive of the eulachon population estimate
212 generated through the mark-recapture method. The natural log of the eDNA peak was
213 significantly related to, and explained 97.4% of the deviance in, the mark-recapture population
214 estimate (β=0.399, $p = 0.002$), despite a multimodal eulachon run in 2016 that contained three
215 distinct peaks. The area under the curve eDNA metric explained 93.4% of the deviance in the
216 mark-recapture population estimate (β=0.391, $p = 0.008$) (Fig. 4). The peak eDNA concentration
217 without flow correction explained only 41.9% of the deviance in, and was not significantly
218 correlated with, the mark-recapture population estimate (β=0.815, $p = 0.36$), but the area under
219 the curve even without flow correction still explained 92.1% of the deviance in the mark-
220 recapture population estimate (β=0.830, $p = 0.016$) (Fig. 4). The quasipoisson regression models
221 using either the peak or area under the curve of the flow-corrected eDNA rate as a single
222 predictor produced highly representative predictions of mark-recapture population estimates
223 (Fig. 5).

Discussion

The utility of eDNA for the detection of organisms has been widely documented (Ficetola, Miaud, Pompanon, & Taberlet, 2008; Rees et al., 2014; Wilcox et al., 2016). Recently the next generation of eDNA science has evaluated the efficacy of quantifying the abundance of species using eDNA (Doi et al., 2015; Levi et al., 2019; Takahara et al., 2012; Tillotson et al., 2018). However, the expansion of eDNA beyond academic settings and into species management and monitoring is just beginning. Otherwise neglected species and populations, such as eulachon, are a promising management application for eDNA methods. This is true even if eDNA provide less accurate or precise results than do traditional methods, because lower quality data from more streams could result in more robust management decision-making than higher quality data from just a few streams, particularly for a fish that exhibits low site fidelity. In addition, for many taxa, knowing whether a species is rare or common, and increasing or declining, is more important than precisely enumerating abundance. However, our results suggest that this tradeoff is largely moot; flow-corrected eDNA rate was highly predictive of the eulachon mark-recapture population estimates at a small fraction of the cost.

Unlike the mark-recapture method, which produced a single population estimate for the eulachon run, the eulachon eDNA rate captured within-run phenology as eulachon abundance varied in the Chilkoot River above the sampling location. eDNA was very effective at quantifying run timing and was particularly effective at demonstrating that the 2016 eulachon run was multimodal with three distinct pulses of eulachon that were separated by 4-5 days of inactivity (Fig. 3). The third pulse in 2016 was not represented in the mark-recapture estimate because field personnel had assumed that the run had terminated, but it was captured in the eDNA sampling continued due to the minimal labor required for eDNA sampling.

Given the results presented here, eDNA has great potential as an indigenous-led, efficient, and effective means of monitoring this culturally and ecologically important species. Because eulachon in many regions, like northern Southeast Alaska, are not a commercially harvested species, they do not receive agency funding for management. This leaves a financial gap in monitoring that is often filled through indigenous-led efforts. A primary benefit of using eDNA is the vastly reduced cost of monitoring under-funded species, such as eulachon. The mark-recapture study on the Chilkoot River costs approximately $20,000 per year; largely because two 5-person crews are needed to properly implement the mark-recapture method. The use of eDNA at the current Oregon State University rate of $42/sample for 3 samples/day for ~20 days is $2520. Further cost savings would be accrued by reducing sampling prior to and after the run, when DNA concentrations are low, and instead focusing measurement during the ~1 week peak of the run, because both the peak eDNA concentration and area under the curve eDNA metrics are invariant to sampling additional days with very low DNA concentrations. Such a reduced sampling window reduces the cost of sampling to below $900 annually, and further optimization such as taking only two replicate samples daily can reduce the cost to nearly $500. The economic viability of eDNA could be further increased with automated water samplers, currently in development, or the use of citizen scientists.

An additional benefit of eDNA methods is that mark-recapture estimation is not logistically feasible on all rivers. The Chilkoot poses a unique set of characteristics – single channel, road accessible, and with a relatively distinct upper limit to spawning activity. Many rivers in Southeast Alaska where eulachon spawn are glacially-fed, with wide, braided river mouths that are in remote, road-less areas. A mark-recapture method at these locations would be logistically challenging, in large part due to a large field-crew requirement. The appeal of eDNA

270 is the ability to simply take a water sample to derive an index of abundance, which can be done
271 by a small crew in only a few hours at most. The use of eDNA allows population data to be
272 gathered on rivers that otherwise would not be possible, which is vital in monitoring a population
273 that exhibits a regional genetic population structure where a decline in spawning biomass in any
274 one river system might not necessarily represent a decline in the regional eulachon population
275 (Flannery et al., 2013). The use of affordable and logistically feasible eDNA methods could
276 facilitate regional studies of eulachon population size, run timing, and synchrony among rivers,
277 which would allow for inference on regional population trends, environmental drivers of
278 population dynamics, and environmental drivers of spawning river selection (Bryant, 2009).
279 In addition to the affordability of eDNA, it may in fact present a *more* accurate estimate
280 of eulachon abundance than mark-recapture population estimation. While eDNA concentration is
281 a direct measurement, mark-recapture population estimates are derived from a statistical
282 estimator and often leads to very wide confidence intervals. In years with low returns, the mark-
283 recapture method might not reach the sample-size threshold for precisely determining a
284 population estimate. For example, the poor eulachon return during 2015 resulted in wide
285 confidence intervals around the population estimate (95% CI: 8,074 to 631,098). In contrast, the
286 eDNA signal represents a direct measurement of eulachon mitochondria in the water rather than
287 a statistical sampling process and is thus invariant to sample size. Further, the demographic-
288 closure assumptions of mark-recapture estimators are difficult to meet with an anadromous fish
289 that quickly enters and leaves the river (Pollock, 2018). The Chilkoot River mark-recapture study
290 lasts for the duration of the run (typically 4-8 days), beginning on the first day that fish are
291 observed in the river (typically late April) and ending once recapture sampling has exhausted all
292 new fish into the system (i.e. when recaptures are identifying double-marked fish). During this

time, new fish immigrate into the river while subsistence fishing activities actively remove fish, thus violating closure. However, population estimates can be robust to moderate violations of closure (Kendall, 1999). In this study, the closed-population assumption is thought to be reasonably met because (1) initial marking efforts remained relatively constant and continued until no new fish appear to be entering the system (i.e. approximately all individuals were potentially subject to marking), (2) there was an equal probability of capture of marked and unmarked fish by subsistence harvesters, and (3) we secured participation of subsistence harvesters to search their catch for marked fish.

We have demonstrated that eDNA provides reliable quantification of anadromous eulachon abundance. eDNA is thus a promising tool that can be mobilized by indigenous people to affordably monitor noncommercial species that are neglected by agencies but are culturally and/or economically important to native people. However, substantial uncertainty persists. In particular, it is unknown whether our model predicting eulachon run size with flow-corrected eDNA will be transferable to other rivers. This is unlikely to be the case when rivers have different morphologies, such as braided floodplains with pockets of eulachon spawning throughout. In such circumstances, a within-river index of abundance might be achieved by monitoring several braids where eulachon congregate, or perhaps even the estuary where mixing of water might homogenize the sample. Once sampling specifications for rivers of various morphologies are determined, the final step toward large-scale implementation is regional cooperation among Alaska natives, First Nations in British Columbia, and tribal governments in Washington and Oregon to facilitate monitoring of eulachon spawning rivers throughout the Pacific Northwest.

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Data Accessibility Statement

All data and code are made available on the Dryad repository.

Author Contributions

TH and MP organized the eulachon mark-recapture program. TL, DY, SM initiated the eDNA monitoring program. TL, MP, JA, collected eDNA samples that were processed in the lab by JA and MP. JA developed primers. MP and TL analyzed data and wrote the paper with input and edits from other authors.

Tables

Table 1. Annual eulachon population estimates for the Chilkoot River using a modified Lincoln-Peterson equation (excluding 2013). eDNA monitoring began in 2014.

Table 1. Eulachon population estimates for the Chilkoot River using a modified Lincoln-Peterson equation								
Measurement	**2010**	**2011**	**2012**	**2014**	**2015**	**2016**	**2017**	**2018**
M = Marked Initially-adipose clipped	8,017	49,814	27,525	24,084	306	9,384	33,681	30,542
C = Total in second sample captured above weir	20,210	143,444	48,376	19,886	3,122	8,865	47654	18,636
R = Marked recaptures above weir with clip	72	568	186	140	2	45	126	64
N1 = Population Estimate	2.2 Million	12.6 Million	7.1 Million	3.4 Million	319,586	1.8 Million	12.6 Million	8.7 Million
SE[2] = Standard Error	256,415	521,961	516,583	283,226	158,934	262,518	1,113,520	1,074,932
CI[3] = 95% Confidence Interval	1.7 to 2.7 Million	11.5 to 13.6 Million	6.1 to 8.1 Million	2.9 to 3.9 Million	8,074 to 631,098	2.3 to 1.3 Million	10.5 to 14.8 Million	6.6 to 10.9 Million

Figure 1. The Chilkoot River (A) is located in northern Southeast Alaska. The study area is located in the approximately 1.75 km. section of river between the outlet of Chilkoot Lake and where the river meets the estuary (B). At the initial capture location, the adipose fin of eulachon captured in the trap is clipped into a "shark fin" (C) for easy identification. The beginning of the recapture reach is located approximately 0.75 km. upstream of the initial capture location. Crews dip net or cast net to capture eulachon (D). (E) Depicts the Chilkoot River during a large eulachon run.

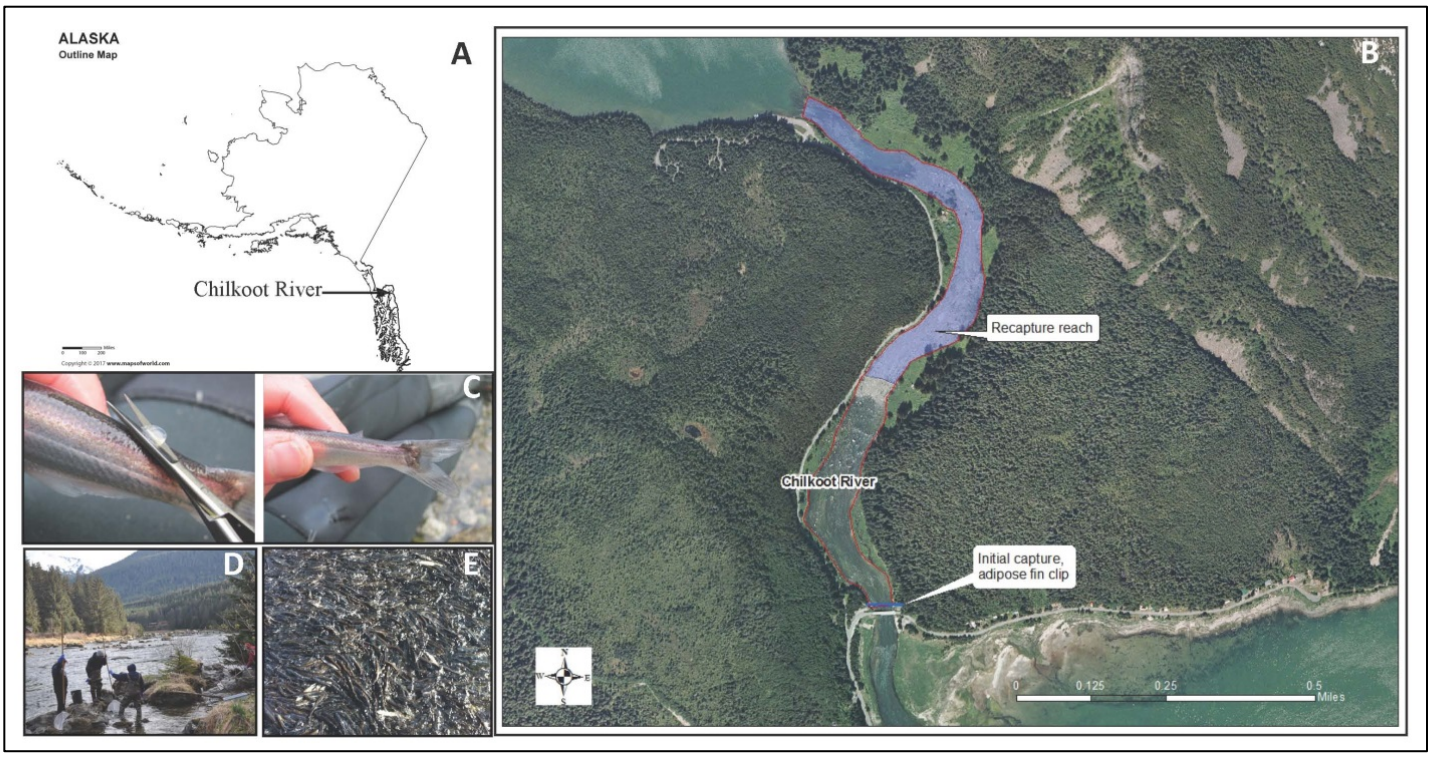

Figure 2 Results of mark-recapture population estimate for eulachon on the Chilkoot River using a modified Lincoln-Petersen method. Error bars represent one standard error.

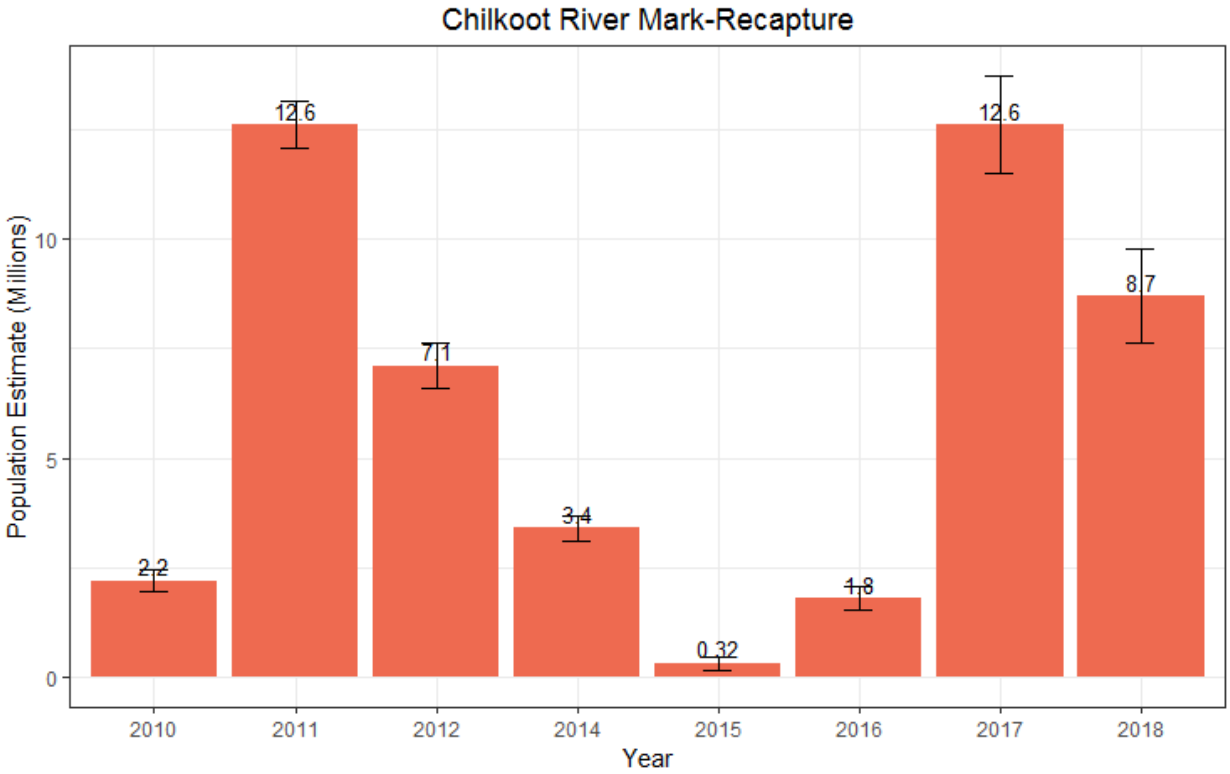

Figure 3. Annual results of the Chilkoot River flow-corrected eDNA concentration (copies/µl * discharge in cubic-feet/sec.). 2014-2016 (A), 2017-2018 (B). The boxplots illustrates the variability among the three daily samples.

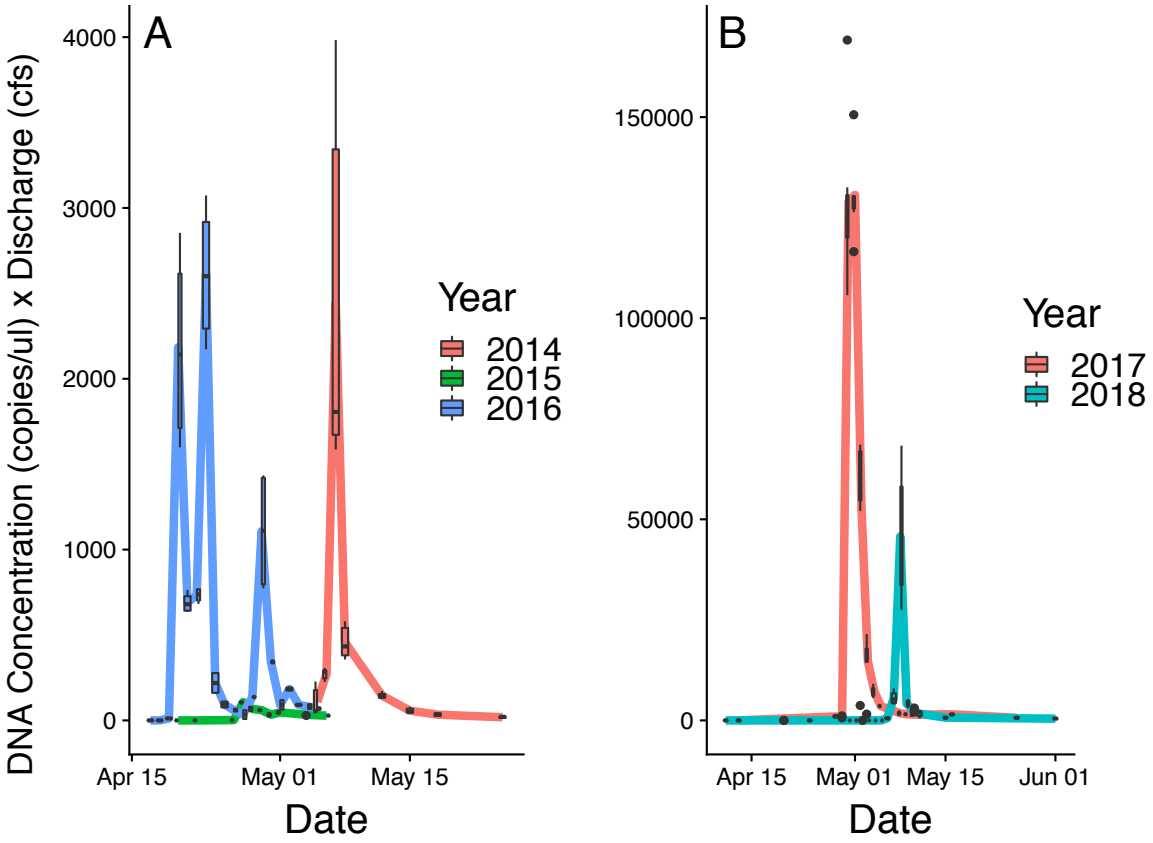

Figure 4. Results of quasipoisson regression models relating log-transformed mark-recapture population estimate to (A) the size of the peak flow-corrected eDNA rate ($p < 0.003$, 97.4% Deviance explained), (B) the area under the curve of the flow-corrected eDNA rate ($p < 0.01$, 93.4% Deviance explained), (C) the size of the peak uncorrected eDNA concentration ($p = 0.36$, 41.9% Deviance explained), and (D) the area under the curve of the uncorrected eDNA concentration ($p < 0.02$, 92.1% Deviance explained). Gray shading denotes 95% confidence interval.

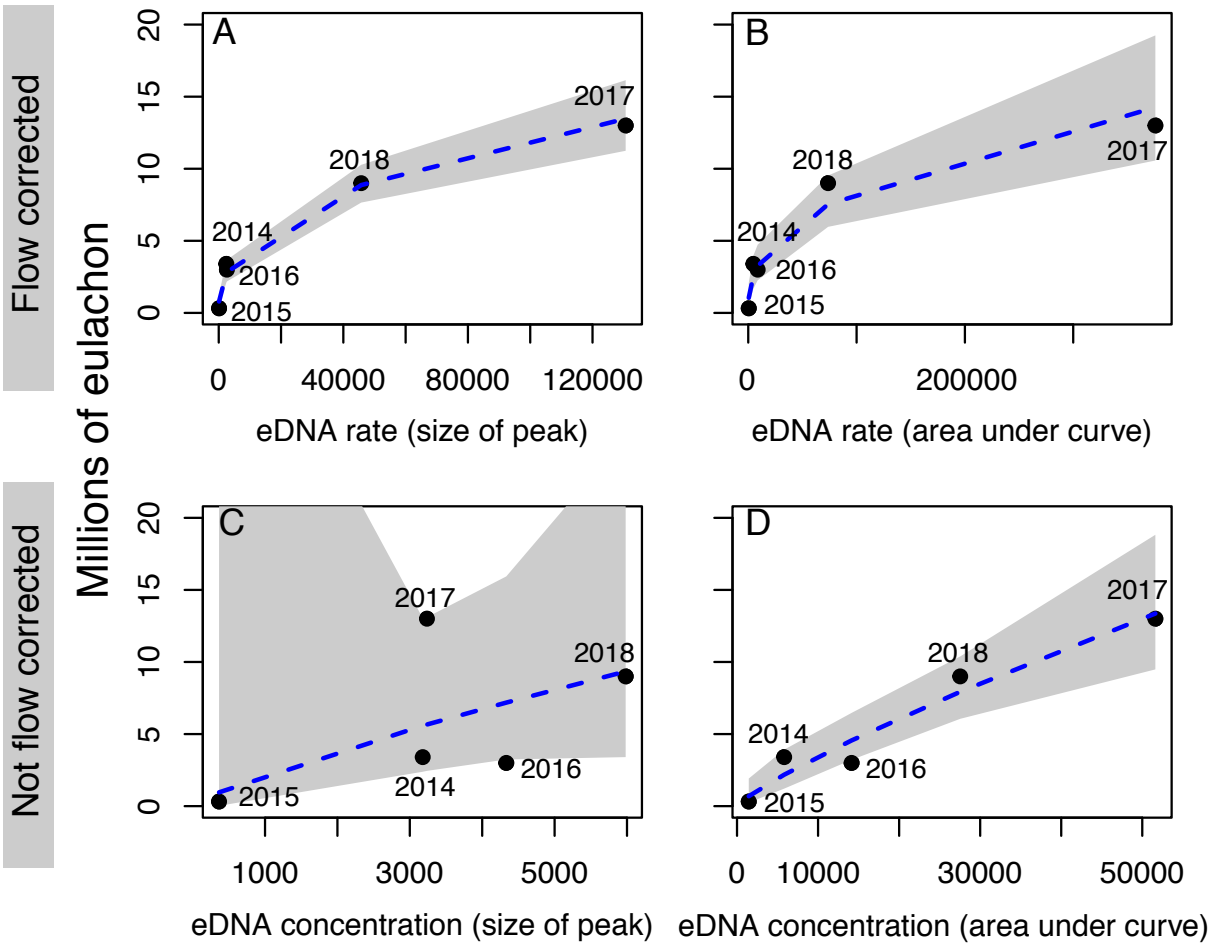
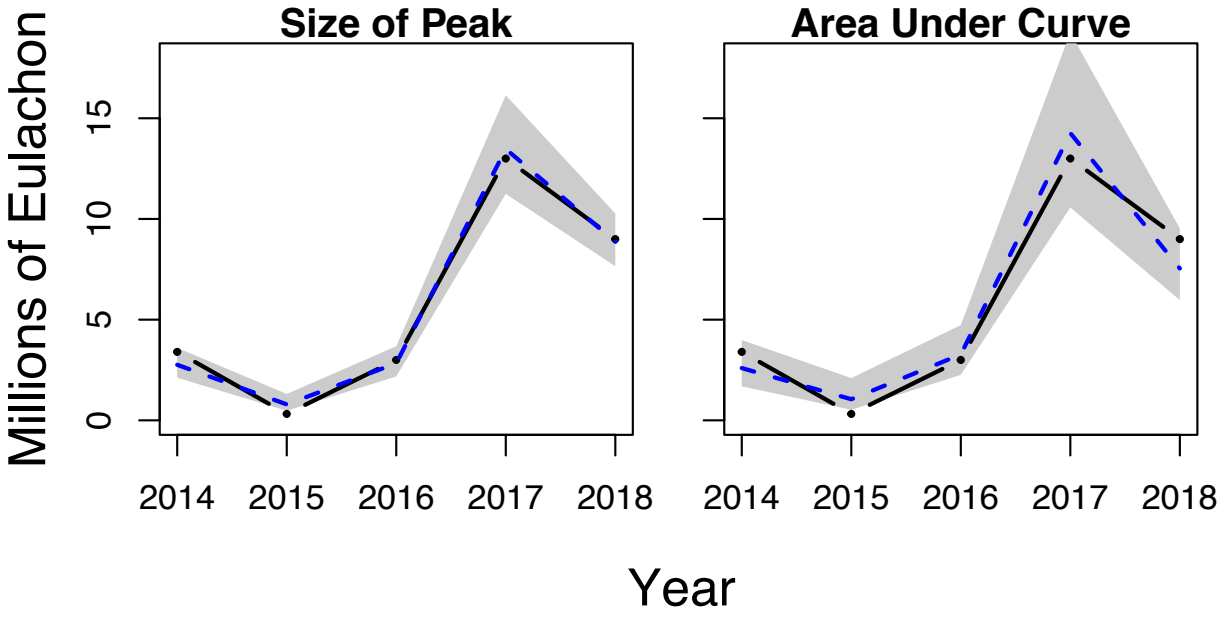
Figure 5. Mark recapture population estimate of eulachon runs (black dots) from 2014 to 2018 and the predicted number of eulachon based on the peak or area under the curve of the flow-corrected eDNA rate in the quasipoisson regression model (blue dashed lines). Gray shading denotes the 95% confidence interval.