Version of Record: https://www.sciencedirect.com/science/article/pii/S0006320718318469
Manuscript_4f6a095d789ac78bb47624d77499fd5f

1 # Environmental DNA provides quantitative estimates of
2 # a threatened salmon species

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15 *Keywords: Oncorhynchus, abundance estimator, Chinook salmon, eDNA, ESA, seine, qPCR*

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Abstract

Species of conservation interest are often rare or elusive, and often require labor-intensive population surveys for management. Sampling genetic traces of such species from environmental media such as water, air, or soil (environmental DNA; eDNA) can provide noninvasive and cost-effective means of monitoring. However, eDNA results may not align with traditional survey methods (e.g., visual, net) making it difficult to interpret eDNA results. We present the results of parallel beach seine and quantitative-PCR (qPCR) surveys of a threatened Chinook salmon (*Oncorhynchus tshawytscha*) from Skagit Bay, an estuary in Washington, USA. Our replicated design and hierarchical statistical model assesses the abundance, biomass, and DNA concentration at two spatial scales (site- and population-) over five months. We find both eDNA- and seine-derived abundance indices reflect the seasonal migration of salmon; at the population-scale, eDNA and seines provide virtually identical quantitative information. At the site scale, the methods are less correlated, suggesting the methods reveal different information about a patchily distributed species. Environmental DNA may act to smooth otherwise patchy biological signals in space and time. Reduced within-site variability for eDNA relative to seines suggests that eDNA may offer more precise population estimates. We partition sources of variability in space and time and compare eDNA and seine surveys – a first, to our knowledge – and so reveal the behavior of eDNA in the field. Our results underscore the value of using eDNA in conjunction with traditional surveys. Combining eDNA and seine estimates should improve the population data on which management of threatened species depends.

1. Introduction

Surveys of abundance and biodiversity enable ecological inquiry and quantitative management of species and ecosystems. But surveys are often complex, expensive, and labor-intenstive, particularly when target species are rare or elusive as is the case for many species of conservation concern. Furthermore, many survey types involve physical capture or disturbance of species, which can potentially harm sampled individuals and is particularly undesirable for species of conservation concern (Pimm et al., 2015). Developing new methods for censusing wild populations that are sensitive, non-invasive, and relatively inexpensive is an important component that can lead to improved management outcomes in a changing world (Snaddon et al., 2013; Burton et al., 2015; Marvin et al., 2016). Additionally, statistical methods that can integrate multiple data types into a unified framework for population assessment are increasingly important for management and conservation (Maunder & Punt, 2013; Robinson, Morrison, & Baillie, 2014; Ahrestani et al., 2017) and allow for disparate data types to be used in concert to address applied problems. Environmental DNA (eDNA) is an increasingly common tool for ecological sampling that examines minute traces of species (e.g., cells or waste) left behind in environmental media (e.g., water or soil) and can be used to detect and enumerate target species (Kelly et al., 2014; Laramie, Pilliod, & Goldberg, 2015; Spear et al., 2015; Thomsen & Willerslev, 2015). However, the use of eDNA in practice has been hindered by questions and concerns about the accuracy and interpretability of eDNA results.

Three key questions have repeatedly arisen about eDNA: 1) *how does the environmental DNA signal vary over space and time, such that we can understand what environmental DNA is revealing about the environment?*; 2) *how well do environmental DNA estimates of organismal abundance match the results from non-molecular survey techniques?*; 3) *how can we use eDNA-based survey results in practice?* As the answers to these questions come into focus, it will be increasingly possible to use genetic-based survey techniques to learn about the ecology of target organisms in a way that we could not otherwise, and to use eDNA results in a management context. However, answering these questions requires careful, quantitative investigations of eDNA under field conditions to uncover the empirical behavior of this new data source.

Surveying species of conservation concern is an attractive application for eDNA because the methods are sensitive and neither destructive nor invasive. Consequently, the past several years have seen the first such uses, across a wide range of spatial scales (from individual ponds to continental landscapes) and media (freshwater, saltwater, soil). Examples include eDNA studies of six endangered freshwater taxa across five European countries (Thomsen et al., 2012), the hellbender salamander in North Carolina, USA (Spear et al., 2015), Florida manatees (Hunter et al., 2018), salmonids in western rivers (Laramie et al., 2015), a

rediscovered frog in Israel (Renan et al., 2017), and mammals – including the orangutan and banteng – at a saltlick in Borneo (Ishige et al., 2017). To date, the majority of eDNA studies have focused on the occurrence of target species, not quantifying abundance or biomass (but see Thomsen et al., 2016; Knudsen et al., 2019). A critical step toward making eDNA methods useful for applied settings is determining the ability of eDNA to be used quantitatively.

Recent papers provide strong evidence that observed eDNA shed from multicellular organisms tends to reflect the organisms present in close proximity – in both space and time – to the sampled location. Most eDNA is thought to be contained in cells shed from a focal organism, including skin, feces, and mucus, not freely floating, extracellular DNA (Turner et al. 2014). In streams and nearshore estuarine habitats, in particular, the e ective genetic signal appears to degrade over the scale of 10s of meters away from its source (Jerde et al., 2016; O'Donnell et al., 2017; Tillotson et al., 2018), although sensitive assays may nevertheless detect target organisms from 100s of meters away or even further (Deiner & Altermatt, 2014; Wilcox et al., 2016; Pont et al., 2018). Similarly, studies of changes in DNA concentration with time show rapid decay of eDNA such that a majority of eDNA degrades within hours to a small number of days (Okabe and Shimazu 2007, Eichmiller et al. 2016, Tsuji et al. 2017, Collins et al. 2018). Available oceanographic information suggests that the residence time for salt water in the basin that includes Skagit Bay is on the order of 20 days (Sutherland et al. 2011) indicating that DNA within this area should reflect predominantly local sources (Sassoubre et al. 2017).

For studies examining quantitative estimates of species' abundances using environmental DNA, researchers often conflate the extent to which eDNA reflects the true abundance with how eDNA reflects the results from a di erent survey method. It is common for studies to assess the validity of eDNA methods by assuming a second survey method (e.g., visual or net; Wilcox et al., 2016; Tillotson et al., 2018) provides an unbiased and precise estimate of true abundance. Hence, eDNA surveys tend to be viewed as 'successful' only to the extent that their results mirror those of an alternative method (Thomsen et al., 2016; Yamamoto et al., 2016). We argue that this reasoning is likely to mislead investigators with regard to the value and efficacy of eDNA methods (Shelton et al., 2016; Kelly et al., 2017).

If well-designed, we expect di erent survey types to reflect the true – but unknown – abundance of organisms of interest. However, there are many reasons to expect the results of di erent surveys types to di er. Generally, survey methods have di erent suites of attributes – detection levels, precision, bias – and consequently we would not expect di erent survey types to yield identical results. Ultimately, we are interested in how surveys inform estimates of true abundance, not in correlations among di erent survey methods. Surveys are only related indirectly to one another via their relationship to the true abundance of the species in question.

When comparing eDNA to traditional sampling methods we need to acknowledge that each is a source of information that informs our estimates of abundance, but neither is itself truth, and under most situations neither is appropriate as a standard for judging the quality of the other. There is value in contrasting or combining population estimates derived from multiple methods – we perform some such analyses below – but treating one method as a benchmark against which to measure the success or failure of an alternative method is unwise. An additional complexity of many studies is that it is very difficult to sample all areas in which a species lives (e.g. due to sampling constraints in di erent habitat types) and thus in practice, surveys often provide indices of relative abundance, not an estimate of absolute abundance (Maunder & Punt 2013, Ahrestani et al. 2017).

To advance eDNA for conservation and management purposes, we need to work towards understanding the attributes of eDNA surveys under field conditions. A rigorous way of comparing eDNA to species abundance requires a workable model of the multi-step analytical process leading from a "true" value to observed samples (Shelton et al., 2016). We will focus on a single- species application of eDNA derived from the use of quantitative PCR (qPCR), but the conceptual framework for thinking about eDNA methods is also generally applicable to the more complicated multi-species surveys derived from high-throughput amplicon sequencing (Shelton et al., 2016; Thomsen et al., 2016), even if the specific statistical methods are somewhat distinct.

Here, we use qPCR alongside an intensive beach seine sampling e ort to quantify seasonal Chinook salmon (*Oncorhynchus tshawytscha*) abundance in Skagit Bay, an important estuarine habitat in Washington, USA. We develop quantitative models for each sampling method and examine estimates of Chinook salmon abundance from the perspective of beach seines and eDNA. Our analysis allows for the examination of the sources and scale of variability arising from each method. We examine the relationship between qPCR and beach seines at two spatial scales: the local, site-scale at the larger Skagit Bay-wide scale (the population scale).

Importantly for conservation and management purposes, Puget Sound Chinook salmon is a threatened species under the United States Endangered Species Act (ESA), triggering a suite of legal protections and management responsibilities for federal, state, and tribal entities. Our analyses answer the three key questions posed above – at least, for this particular habitat and management case – and provide data on which future endangered-species-monitoring e orts might be based. We find that eDNA sampling is comparable to traditional net sampling, and the two are especially similar at the population scale. Furthermore, we find higher precision among eDNA samples than among net-samples and thus conclude that eDNA surveys have great potential for revealing ecological patterns of management relevance.

2. Materials and Methods

We develop independent indices of abundance for Chinook salmon using two sampling methods (beach seine and qPCR) and compare the results from the surveys at two spatial scales (site- and population-) over multiple months. Here we outline the field sampling for each method, the laboratory processing for eDNA, and the statistical models used in analyzing each survey. Full laboratory and statistical details can be found in the supplemental materials.

We paired monthly eDNA sampling with beach-seine sampling in Skagit Bay, Washington, between February and June 2017, capturing the seasonal outmigration of juvenile salmonids from the Skagit River into saltwater (Fig. 1). Sampling dates and coordinates are provided in the supplementary information (Tables S2.1, S2.2). We took replicate water samples for eDNA analysis alongside beach seine sampling conducted by the Skagit River System Cooperative (SRSC), an organization supporting applied science for two Washington tribes. Tribal scientists are part of a larger federal-state coalition working toward recovery of listed Chinook salmon stocks, and therefore perform beach seine sampling as part of federal recovery guidelines. No permits were necessary for water sampling.

There are four discrete steps to our qPCR methodology: (1) environmental sample collection, (2) isolation of particulates from water via filtration, (3) isolation of DNA from filter membrane, and (4) amplification of target locus via PCR (see Supplement S1 for full details).

2.1. Field Sampling

2.1.1. Beach Seine

To monitor threatened salmon populations in the Skagit River watershed, SRSC conducts surveys for five salmon species at smolt and adult stages. Detailed protocols and reports of surveys can be found at http://skagitcoop.org/research (see also the Skagit Chinook Recovery Plan; Beamer et al., 2005).

We focus on sampling of shoreline-oriented outmigrants using beach seines, designed to count salmon smolts that have left the Skagit River and rear in Skagit Bay. Beach seines sample juvenile salmon biweekly between February and October to capture the entire salmon outmigration. For each sampling site, crews of two or three field biologists deploy a beach seine (37× 3.7m) of knotless nylon mesh (0.3 cm mesh size) by fixing one end on the beach and the other on a ski , setting the net across the current, and returning to the beach at a point upstream at a distance of approximately 60% of the net length (~22 m). The net is briefly held open against the longshore current to spread the net, and then the boat is returned to the shoreline edge and both

157 ends of the net are retrieved, yielding a catch in the bunt section. The average sample area is 0.486 ha for
158 beach seines.

159 Here, we include information from beach seine samples collected monthly between February and June at the
160 core eight beach seine locations ("index sites"; Fig. 1) where water samples were collected for eDNA analysis
161 alongside beach seines. Supplement S2 provides the sample dates and sites visited. Supplement S3 provides
162 information about abiotic variables (water temperature and salinity) measured in conjunction with beach
163 seines and water samples. In addition to counts of each species, lengths of individual fish are collected in
164 order to estimate biomass. For Chinook salmon, up to 25 fork lengths are sampled if fish are abundant, and
165 all lengths are recorded if catch is fewer than 25 fish.

166 2.2 Water collection and processing for qPCR

167 We summarize collection and laboratory procedures here and provide detailed protocols in Supplement S1.

168 We collected 5 replicate 1L water samples at the surface from eight index sites in sterilized bottles on each
169 sample occassion (Fig. 1, Table S2.1). Following preliminary analyses on a subset of bottles, we elected to
170 only analyze 4 of the water samples for most site-month combinations. We attempted to space collection
171 before seine deployment, after the deployment of the first seine and after the deployment of the second seine.
172 Samples consequently reflect water within approximately 10 minutes and 20m of one another. We assessed
173 potential cross-contamination by filling one bottle with deionized water before each sampling day, opened
174 and closed it in the field, and treated it identically to the samples for the remainder of the steps.

175 Each water sample was filtered in the lab on a sterile filter cup fitted with a 47 mm diameter cellulose acetate
176 membrane with 0.45 μm pores, preserved in Longmire bu er and stored at room temperature. To test for
177 the extent of contamination attributable to laboratory procedures, we filtered three replicate 1 L samples of
178 deionized water and processed them alongside field samples.

179 We purified DNA from the membrane using a phenol:chloroform:isoamyl alcohol protocol following Renshaw
180 et al. (2015). We used a novel qPCR primer and probe assay specific for chinook salmon targeting the
181 mitochondrial DNA Cytochrome oxidase III/NADH dehydrogenase 3 (COIII/ND3) gene. We tested for and
182 found no cross-amplification with this assay and the other Pacific salmon species (genus *Oncorhynchus*; see
183 Supplement S1 for information about cross-amplification assays).

184 Each purified eDNA template was amplified in triplicate, providing replicate measures of DNA concentration
185 for each water sample, resulting in a total of five separate qPCR plates. To assess comparability among

qPCR plates, we ran a total of 32 samples on multiple plates.

2.3. Statistical methods for qPCR and beach-seine sampling

We constructed a hierarchical statistical model to estimate all parameters in our analytical pathway – from regression parameters describing the DNA standard dilution series to the DNA concentration at each site and month. This modeling approach appropriately propagates uncertainty through the qPCR analyses from the regression analysis of the DNA dilution series to the estimates of DNA concentration in field samples. Standard analyses of qPCR data typically ignore uncertainty in the relationship between the DNA concentration in the dilution series standard and the PCR cycle at which amplification occurs, resulting in estimates DNA concentration of field samples that are potentially biased. Additionally, our model enables distinct processes to be modeled explicitly and compared, allowing us to identify processes that contribute to uncertainty. Analyses of eDNA involve many analytical steps and we believe that fully documented and transparent statistical approaches are important to complement the sophisticated laboratory procedures used with eDNA. We provide a full statistical description in Supplement S2.

We are primarily interested in the estimates of the DNA concentration at each site-month and combining these estimates to arrive at an estimate of overall abundance in Skagit Bay (see below). However, we are also interested in the parameters that reveal the causes of variation in DNA concentration. Two processes contribute to the variability in observed qPCR counts derived from a single water bottle: variance due to uncertainty in the standard curve ("PCR standards") and variance arising among PCR replicates from identical field samples ("PCR samples"). We expect these quantities to be small relative to variance among-bottles collected within a single site ("Bottles"). Our analyses assume that collected bottles are exchangeable samples of the eDNA at a particular site-location. As our samples are collected sequentially and coincident with the deployment of the beach seine, there is the possibility that the use of the beach seine a ected DNA concentrations. However, we found no evidence for bottle collection order on Chinook salmon DNA concentration (Fig. S2.6).

Together, the variability attributable to PCR standards, PCR replicates, and among-bottle replicates can be combined to describe the total within-site variability in DNA concentration at each site-month combination (denoted "PCR + Bottles"; see Supplement S2). This within-site variability is comparable to the within-site variation of beach seine sampling (see below). We also calculated two derived measures of variation to understand variation at the scale of Skagit Bay. We calculated the standard deviation in estimated DNA concentration among months at a each site ("Month" variation) and standard deviation in DNA concentration

216 among sites in each month (“Site” variation).

217 Similar to qPCR sampling, we created a hierarchical statistical model for all beach seine samples collected. 218 We modeled catches in beach seines using a Negative Binomial likelihood (Supplement S2). As with the 219 qPCR analysis, we are interested primarily in the estimated abundance at each site but we are also interested 220 in the components of variation. For beach seines we can calculate the variance of catches at a particular site 221 and month (“Seine” variation) and compare this values to the estimated variation among months at a each 222 site (“Month” variation) and variation among sites in each month (“Site” variation).

223 **2.3.1. Calculation of Biomass**

224 For Chinook salmon entering Skagit Bay during the spring and summer, not only are the number of Chinook 225 salmon changing over the period of sampling, but individual fish are growing rapidly as well. Thus the biomass 226 of Chinook salmon within Skagit Bay likely has a di erent spatio-temporal pattern than the abundance. There 227 are strong seasonal patterns with Chinook salmon length increasing over time (Fig. S2.4). To estimate biomass 228 at each site-month combination, we used lengths of Chinook salmon captured during beach seine surveys and 229 converted fork length (l; mm) to biomass (m; g) using an allometric equation $m = 4 \times 10^{-6} l^{3.2028}$ derived 230 from juvenile Chinook salmon sampled in Skagit Bay by beach seines between 1996 and 2010 ($N = 6028$, 231 $R^2 = 0.98$, E. Beamer unpublished data). We then calculate a monthly average mass in grams for the Chinook 232 salmon captured during each of our survey dates and multiplied this average mass by the estimated denstiy 233 to generate an estimated biomass for each site-month.

234 **2.3.2. Estimation**

235 We estimated both the statistical models for qPCR and beach seine in Stan, a Hamiltonian Markov Chain 236 Monte Carlo (MCMC) sampler for Bayesian statisical models (Gelman, Lee, & Guo, 2015; Carpenter et al., 237 2017) as implemented in the R environment (rstan, v.2.16.2; R Core Team, 2018, Stan Development Team 238 2018). For both beach seine and qPCR analyses we used 5 parallel chains with di use starting locations and 239 examined Gelman-Rubin diagnostics to ensure convergence and adequate mixing among chains. We used 240 di use prior distributions for all parameters (Table S2.3) and we provide all analytical code and data in the 241 supplement and online data repository (see Mendeley Data archive).

242 **2.3.3. Constructing Indices of Abundance**

243 Indices of abundance are widely used in management settings when it can be difficult to directly map survey

results to absolute measures of abundance. For example, many marine surveys are conducted using net sampling techniques, but most nets do not capture all of the fish present at a given location and time. Some fish may be too small or too elusive to be captured. Across many samples we expect a given net to have the same attributes, and therefore it is often reasonable to assume that the survey is proportional to true abundance or biomass, but that this constant of proportionality is not known *a priori*. This constant of proportionality – referred to as "catchability" in the fisheries literature (Arreguín-Sánchez, 1996; Fraser et al. 2007) – can be estimated using auxiliary information.

The objective of beach-seine surveys is to generate estimates of Chinook salmon abundance at the scale of Skagit Bay. We can use our estimates of numbers, biomass, and DNA concentration at each month-site combination to provide spatial averages provide three abundance indices for Skagit Bay from February to June. Comparing information from di erent sources (i.e., beach seine and qPCR surveys) involves comparing measures expressed in di erent units (i.e., number, biomass, and DNA concentration). To facilitate comparison among these measures, we construct a dimensionless index of abundance for each measure. We scaled results from each methodology to its average value in February 2017. Each index, therefore, has an average value of 1 in February 2017. Subsequent months have values that reflect multiples of the February value (e.g., a value of 2 would indicate a doubling). As all three indices are dimensionless, this rescaling allows direct comparison among indices of abundance for qPCR and beach seine samples over time.

3. Results

3.1. Detection of Chinook salmon and Chinook salmon eDNA

We included sampling data from 38 site-month combinations (Table S2.2) representing 76 beach seine sets. Paired with these seine sets, we conducted qPCR analyses on a total of 155 1L water bottles. In addition to water samples, we ran PCRs on 6 replicate negative controls per PCR plate to test for contamination; no negative controls had detectable Chinook salmon DNA. Beach seines captured one or more Chinook salmon at 31 of 38 site-month combinations; Chinook salmon were observed in at least 3 sites in all months and in all five months at 2 sites. In contrast, all 38 site-month combinations had at least one qPCR reaction in which amplification of Chinook salmon DNA was detected. Comparisons of estimated DNA concentration for each bottle at each site-month combination showed no clear evidence that sample order a ected DNA concentration (Supplement S2; Fig. S2.5). A parallel examination of beach seine sets showed limited di erences between Chinook salmon catches between the two sets (Fig. S2.6)

273 Examination of standard curves showed that detection of qPCR amplification occurred at a minimum DNA
274 concentration of 1.7×10^{-6} μg μL^{-1} (Fig. S2.1). Using posterior estimates from the occurrence component
275 of the statistical model, we calculated the limit of detection probabilistically and conclude that our qPCR
276 assay will detect a DNA concentration of 1.4×10^{-6} μg μL^{-1} 5% of the time (Supplement S2).

277 3.2. Comparing eDNA and Seines at multiple spatial scales

278 There is no *a priori* reason why two independent indices of organismal abundance should be identical, given
279 that each is likely to have its own suite of attributes. Nevertheless, agreement between methods does improve
280 confidence in the underlying biological dynamics we are trying to observe, while the di erences allow us to
281 understand the attributes of each sampling method relative to one another. Here, we compare indices of
282 salmon abundance and biomass from beach seines with qPCR at two spatial scales.

283 3.2.1.Site-scale: Sites within Skagit Bay

284 The finest scale possible to compare the methods is at a single site-month. Across all sites and month
285 combinations, qPCR estimates are positively correlated with both abundance and biomass (0.409 [0.202,0.581]
286 and 0.508 [0.319,0.658], respectively; mean Pearson correlation [95% CI]; Fig. 2). This indicates both the
287 number and biomass of fish captured locally is related to the DNA concentration estimated but there is
288 abundant scatter in both relationships (Fig. 2). When individual sites are disaggregated, the relationship
289 between qPCR and abundance is less clear (Fig. S2.3); the relationship between qPCR and biomass is similar
290 (data not shown).

291 3.2.2. Population-Scale: Skagit Bay

292 At the scale of Skagit Bay, we constructed dimensionless indices derived from qPCR and both abundance
293 and biomass derived from seine samples (Fig. 3). Again, these values represent changes in magnitude relative
294 to the February sampling date and can be directly compared among indices. At this larger scale, all indices
295 show a broadly similar temporal pattern with minimum values in February and a peak in March before a
296 decline in April with subsequent increases in May and June. However, qPCR estimates are notably more
297 precise for a given month and have lower variability among months (range of mean estimate: 1 to 9.5) than
298 either numbers (range: 1 to 19.2) or biomass (range: 1 to 216.4) derived from beach seines (Fig. 3; note the
299 value of the seine estimate in June is beyond the y-axis values to maintain readability of other values).

300 At the population-scale, correlations between indices of qPCR and abundance as well as qPCR and biomass

were stronger than at the local site level (Pearson correlations of 0.84[0.42,0.98] and 0.94[0.82,0.99], respectively). Correlations have notably larger uncertainty intervals at the population scale than at the site level because this model includes uncertainty across five sampling months. The relationship between qPCR and the abundance index appears to be largely linear whereas the relationship between qPCR and the biomass index appear to be log-linear (Fig. 3); this may suggest a saturating relationship as DNA concentration increases with increasing Chinook salmon biomass.

3.3. Sources of variation

The qPCR assay shows consistently small variability among PCR standards (mean SD[90%CI] = 0.18[0.15,0.21]; variability around the qPCR standard curve using a dilution series of Chinook salmon DNA; Fig. 4), among PCR reactions from a single water sample (0.29[0.27,0.31]), and among bottle samples take from a single site-month (0.21[0.18,0.24]). These three components can be combined to generate an estimate of total, within-site variability ("PCR+Bottles", mean SD[90%CI] = 0.41[0.39,0.43]; Fig. 4). We found no evidence that within a site the order in which bottles were collected – including before or after beach seines – a ected estimated eDNA concentration (Fig. S2.5).

Total within-site variability is notably smaller than either the average variability in eDNA among months at each site (0.49[0.45,0.54]) or the average variability among sites during each month (0.54[0.49,0.59]). Thus, the variability in DNA concentration due to processes occurring within a site – including variation in DNA concentration at small temporal and spatial scales and variability due to laboratory processing – is lower than larger scale (among site or among month) variation. Therefore, we can confidently identify di erences in eDNA concentrations over space and time.

For beach seine surveys, replicate beach seines provide insight into within-site variation. Constraints on time and labor meant that only two replicate beach seines are taken at each site-month. In contrast with eDNA results, variability between replicate beach seines (15.9[7.2,33.6]; mean SD[90% CI]) is on the same order or slightly larger than among site (11.6[6.5,20.2]) or among month variability (9.8[5,18.2]; Fig. 4). Consequently, the level of variability within sites due to replicate beach seines makes it more challenging to identify site-to-site and month-to-month variation in Chinook abundance than with eDNA methods.

Note that the units for beach seine surveys are individual fish while the units of eDNA surveys are concentrations of DNA so it is not appropriate to directly compare estimates across methods, but patterns of variation at various scales are comparable between methods.

4. Discussion

After hundreds of years of use, we generally understand the sampling attributes of traditional methods such as beach seines. In contrast, we are only beginning to understand the attributes of eDNA sampling. As there are many reasons to expect results from eDNA and net samples to differ, direct comparisons between eDNA and traditional survey methods are not necessarily an effective way to determine the validity of eDNA methods. Our results suggest that eDNA surveys are capable to support the management of this particular threatened species. In a larger sense, our results provide quantitative information about the systematic ways in which eDNA behaves differently than traditional sampling methods, and thereby begin to circumscribe the potential uses of eDNA methods in ecological and management applications.

4.1. Comparing eDNA and Seines

Both eDNA and seine indices track the outmigration of Chinook salmon through the estuarine environment, showing parallel temporal trends of different magnitudes (Fig. 3). Thus, eDNA provides very similar information to traditional surveys in this case, although the quantitative relationship between indices varies by spatial scale. At the population scale – the scale of conservation and management – an eDNA-Biomass correlation of 0.94 is striking; eDNA and seines provide essentially identical information. At the site scale, this correlation is reduced by approximately half. The different degrees of correlation at the two spatial scales suggests that eDNA and seine nets are reflecting distinct information about the site-scale but that either method can be scaled up to yield information about the population-scale.

It is important to note that there are additional attributes contributed by beach seine sampling that eDNA cannot provide. For example, physical capture of the fish can provide information on age, size, evidence of disease, the presence of tags or markers of hatchery origin, genetic samples, diet information, etc. All of these aspects are important measures of the population that require physical specimens in hand; eDNA cannot replace this invaluable information at present. Furthermore, results from beach seine surveys are available in almost real time; eDNA results take, at minimum, two or three days to process. In practice, eDNA samples are frequently processed at one time at the end of the season to take advantage of economies of scale in laboratory processing. Thus eDNA may not be as amenable to applications in which near real-time results are vital.

4.2. Apportioning Variance in eDNA and Seine Surveys

Estimates of eDNA indices were more precise than those derived from beach seines, in that the variance among-replicates (within-site-month) was substantially smaller for eDNA than seines. Indeed eDNA variation among water samples within a site-month is surprisingly low ("Bottles" in Fig. 4), while the contribution of qPCR methodology to observed within-site variation is small but non-trivial. Within-site variability for eDNA is relatively small compared to among-site or among-month variability of the same technique, indicating relatively high power to detect biological changes over space or time. By contrast, within-site variability in seines is on the same order as (or larger than) among-site and among-month variability, with concomitantly lower power to detect changes at the spatial and temporal scales sampled. For the beach seines, this by design in part – the primary metric of interest is the population-scale, not local scale.

To improve the precision of a survey, one must either alter the sampling technique itself or increase replication to decrease the standard error of the population estimate. To the extent that we might improve qPCR sample-processing – for example via more accurate pipetting of dilution-series standards – it is likely possible to improve the precision of qPCR-derived sampling estimates. In contrast, absent dramatic developments in beach seine technology, there is no obvious avenue for improving the fundamental attributes of beach seine catches for salmon.

With regard to increasing replication to improve precision, we could increase eDNA-sampling replication by collecting additional bottles of water, which affects sampling speed and effort only trivially in the field, although it increases lab processing effort to a somewhat greater degree. For beach seines, additional seines would have to be conducted at the cost of substantial time and effort. At present, the highly efficient sampling crews working with the Skagit River System Cooperative can sample up to 10 or 12 sites (20–24 beach seines) on a given day. Simply increasing the number of beach seines by one per site-month to improve overall precision would increase the associated field effort by nearly 50%; this is not a practical option.

4.3. eDNA as an Environmental "Smoother"

Nearshore fish are patchily distributed in space and time. Seine nets capture fish that are simultaneously 1) present in the precise area being strained by the net, 2) within the size-class captured by that net, and 3) behaviorally likely to be captured. Thus, the natural patchiness of fish distributions interacts with beach seine sampling to produce hit-or-miss fish counts. By contrast, the residual genetic information left behind by those same fish is likely to have a less patchy distribution than the fish themselves: shed cells are distributed throughout water surrounding a fish, and they persist in the environment, moving and mixing for some

period of time during which they can be observed. The result is therefore a smoother distribution (in space and time) of eDNA than of the fish themselves; eDNA integrates information from a larger area than the average 0.486 ha sampled by beach seines. Our dataset appears to support this view: within a site-month, eDNA samples had relatively low variance among replicates, while replicate seine samples were more variable. This could be explained by generally homogeneous eDNA concentrations in space and time – as one might expect in a mixed estuary – except that we see di erences in eDNA concentration among sites and months (Fig. 2). Moreover, both seines and eDNA surveys captured the change in Chinook transiting through the habitat over the five sampled months, indicating that the two methods are indeed reflecting a common phenomenon to some extent. We therefore suggest that eDNA is acting as a smoothing function for fish surveys in this context, creating a more continuous sampling distribution than is produced by the seine net. This is consistent with the suggestion of Pont and colleagues (2018), who in the context of a large river found that “eDNA integrated a larger space than the classical sampling” technique.

4.4. Interpreting eDNA Results, and Further Implications

We found di erent apparent scaling relationships between eDNA and fish abundance or fish biomass (as estimated by the seines), respectively: indices of abundance and eDNA appear to be linearly related while biomass and eDNA appear to be log-linearly related. These relationships provide insights about the biological processes that each survey method is actually sampling. For example, a log-linear relationship with Chinook salmon biomass suggests that observed eDNA may saturate with increasing biomass. Such a relationship would suggest that eDNA sampling may be a powerful tool for detecting and discriminating at low population biomass but have reduced discriminatory power when biomasses are large. The value of eDNA for detecting species at low abundances is further supported by our detection of eDNA from Chinook salmon in at least one of the PCR replicates at each site-month, and has been reported in work by other researchers (Wilcox et al., 2016; Boussarie et al., 2018).

Ecological sampling is geared toward quantifying organisms in some way, and it is often unclear how to interpret results with units reflecting DNA concentration (e.g., $\mu g \ \mu L^{-1}$) relative to the more familiar units of abundance and biomass. For traditional survey methods it is possible convert observed catches to abundance given the area sampled and estimates of capture efficiency, although such calculations often depend upon an unknown or assumed constant of proportionality. In our Skagit Bay application, seines average 0.486 ha sampled and the capture efficiency for Chinook salmon derived from mark-recapture experiments is approximately 85% (Beamer et al., 2005) leading to a straightforward calculation for total abundance: expand

the number of Chinook within the sampled site to account for the 15% of Chinook that are missed by beach seines and expand the average density to the total area in Skagit Bay. For eDNA, the sampled volume of a water bottle is 1L, but we currently lack sufficient information about the variability in eDNA concentration (relative to the fish themselves) to develop an analogous measure of capture efficiency. Given the imperfect correlation between beach seine and eDNA (Fig. 2) at the site level, the lower variance within sites for eDNA (Fig. 4), and that eDNA from an organism can be present even when the organism is itself not present at that same instant, we can conclude that eDNA "sites" represent some larger volume of space-time than that sampled by the beach seine (Pont et al., 2018; Knudsen et al., 2019).

An intriguing and productive avenue of future research is that an eDNA survey might be conducted in conjunction with seines (or other traditional methods) with the eDNA being collected more frequently than beach seines. Such an approach could provide physical specimens at appropriate intervals to provide information on age, size and other information while reducing the number of total fish impacted by the nets. This is particularly appealing to decrease potential negative impacts of sampling on populations of conservation concern.

5. Conclusions

ESA-listed species and populations require substantial time and money to monitor and manage. Our results indicate eDNA surveys are a practical complement to traditional sampling, and can outperform aspects of traditional seine sampling: eDNA provides less-variable indices of abundance with less field sampling effort, and at a spatial scale reflecting the whole population, these indices are directly comparable to seine-derived indices. However, eDNA cannot at present replace the many data streams derived from physical sampling. Hence, optimizing eDNA sampling in conjunction with traditional sampling holds the promise of reducing uncertainty of abundance estimates while reducing sampling costs and handling impacts on a threatened species. Such parallel streams of data can then be combined formally to improve population estimates (e.g., Maunder & Punt, 2013; Robinson et al., 2014). We believe that eDNA should begin to be regarded like other survey technologies (e.g., camera traps (Burton et al., 2015) or remote sensing (Pimm et al., 2015)) that have improved ecological surveys immensely in recent years.

We have shown the ways in which eDNA and traditional surveys can differ systematically and yet both provide important information about a target species. These differences likely stem from the different biological processes sampled by the two techniques, and we suggest that eDNA may usefully smooth an otherwise highly patchy organismal distribution over scales of tens to hundreds of meters and of minutes to hours. We

also point out that our information is currently limited to one system and one species. We expect future applications to more fully circumscribe both the potential and limitations of eDNA methods for applied problems.

Finally, we emphasize that the absence of a direct method for translating information from eDNA into units available from traditional methods like beach seines does not hinder the use of eDNA information. We do not yet understand the many mechanics contributing to the spatial and temporal distribution of eDNA in relation to the physical individuals – rates of DNA production, degradation, mixing and transport all remain highly uncertain under field conditions – and are likely to remain that way for most species in most environments. However, there is information present in the observed patterns of eDNA itself that can be useful in understanding both the attributes of eDNA in field settings and for application to applied problems.

Author Contributions

AS, RK, JO, LP, and CG conceived ideas and designed methodology; AS and JO collected field eDNA samples; EB directed beach seine sampling design and staffing; RH led field beach seine sampling; JO and PS performed laboratory analyses; AS, JO, and RK analyzed data; AS and RK led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Acknowledgments

We thank the many individuals who aided us during field collections of water in challenging weather conditions including B. Feist, R. Gallego, E. Iwamoto, K. Richerson, A. Wells, and G. Williams. A. Wells helped with laboratory processing. We thank the sta of the SRSC for their help in understanding beach seine sampling and for coordination with sampling crews. Partial support for this project was provided by NOAA's Advanced Sampling Technology Working Group, Washington State Department of Ecology's Intensively Monitored Watersheds Program, and the Bureau of Indian A airs. M. Ford, J. Samhouri, W. Satterthwaite, and three anonymous reviewers provided helpful comments on earlier versions of the manuscript.

470 **Figure Captions.**

471 **Figure 1.** Map of the study area. **Right Panel** Sampling locations are shown as dots and the town of La
472 Conner, Washington is noted.

473 **Figure 2.** Correlation between environmental DNA and estimated Chinook numbers (left panel) and Chinook
474 biomass (right panel).Each point represents a site-month combination and error bars show 90% CI.

475 **Figure 3.** Indices of abundance for Chinook salmon in Skagit Bay derived from environmental DNA and
476 beach seine surveys. *Top* Indices for qPCR, beach seine numbers, and beach seine biomass for each month.
477 Each index is standardized relative to its value in February (month 2). All three indices have a mean value of 1
478 in February (90% CI shown) and values in subsequent months represent multiples of February abundance. For
479 example, a value of 10 would indicate 10 times the abundance in February. Due to an extremely large value
480 for seine biomass in month 6 the mean and 90% CI are provided on the plot. *Middle* Relationship between
481 the indices for qPCR and beach seine number for Chinook salmon in Skagit bay. Each point corresponds to
482 the index for a single month and 90% CI. *Bottom* Relationship between the indices for qPCR and log[beach
483 seine biomass] for Chinook salmon in Skagit bay. Each point corresponds to the index for a single month and
484 90% CI.

485 **Figure 4.** Partitioning sources of variability for qPCR (*top*) and beach seine surveys (*bottom*) of Chinook
486 salmon. For each category, grey points show estimated value for individual replicates and black points
487 show among-replicate means (± 90% CI). For qPCR we can partition variability among three processes
488 occurring at the sub-site level which correspond to: 1) the variability attributable to the the use of known
489 Chinook salmon DNA concentration to develop a standard curve for qPCR (“PCR standards”“); 2) variability
490 attributable to processing of water samples for qPCR processing (”PCR samples“); 3) small scale spatial and
491 temporal variation in DNA concentration at each site-month combination as observed by replicate water
492 bottles (”Bottles“). For both qPCR and beach seine surveys, we calculate 1) the total variability within
493 each site-month combination (”PCR + Bottles" for qPCR; “Seines” for beach seine samples); 2) the among
494 site variation in estimated abundance for each month (“Months”), and 3) the among month variation in
495 estimated abundance at each site (“Sites”). The dashed vertical line separates processes contribution to
496 variation within a sample or month-site (left of the line) while total within month-site variability and larger
497 scale factors are on the right side of the line. Similar processes for the eDNA and seine methodologies are
498 aligned along the x-axis to ease comparisons between methods.

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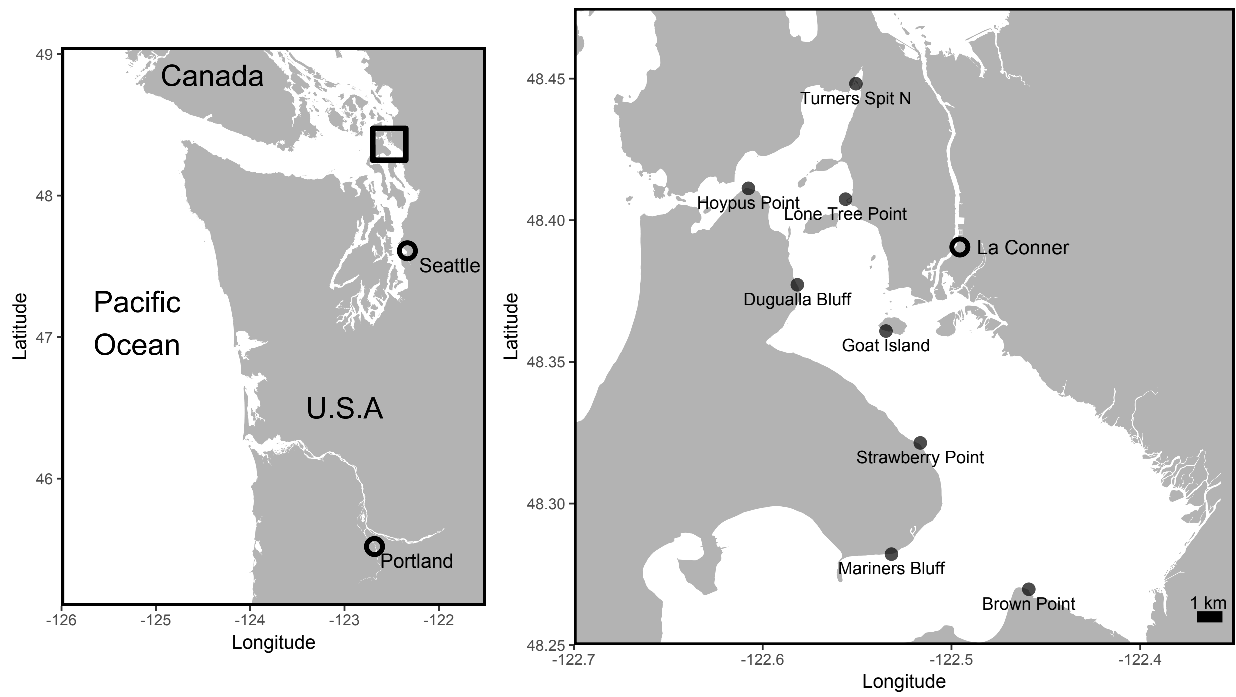

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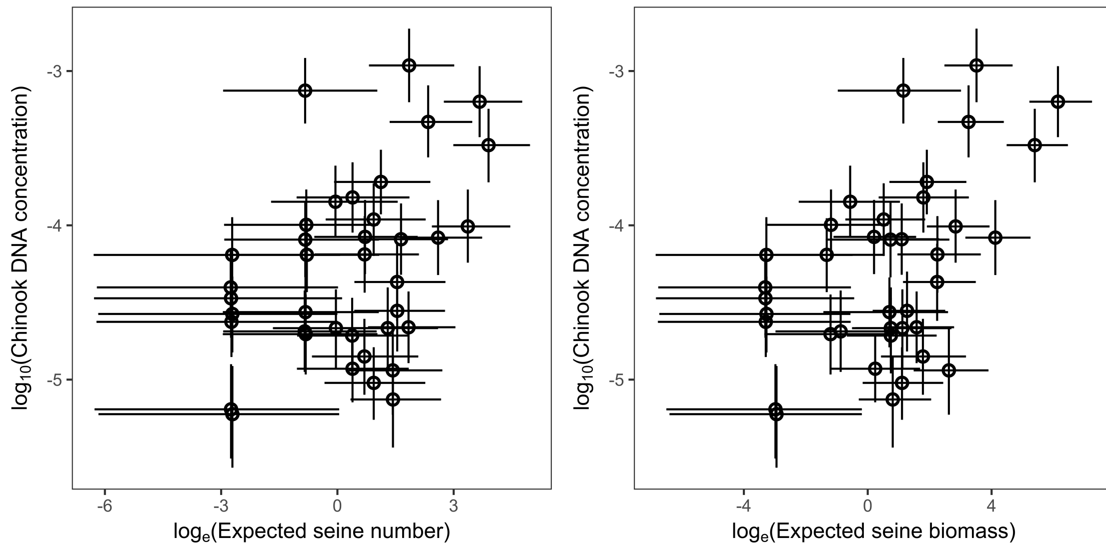

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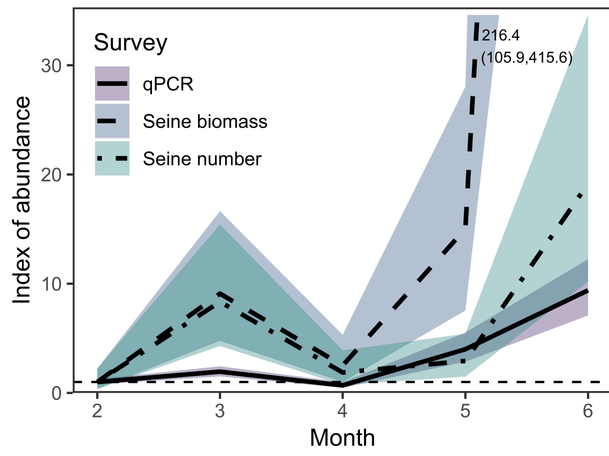

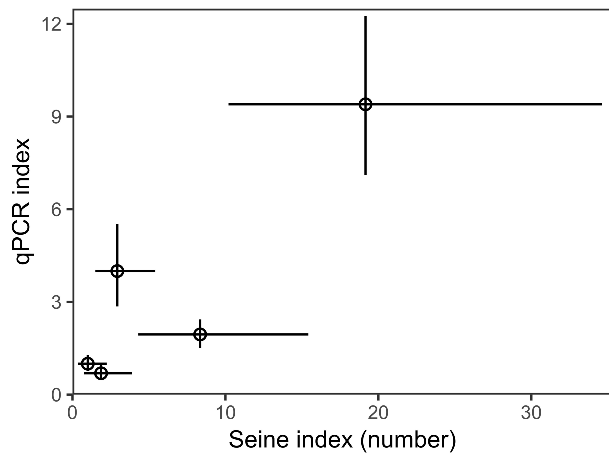

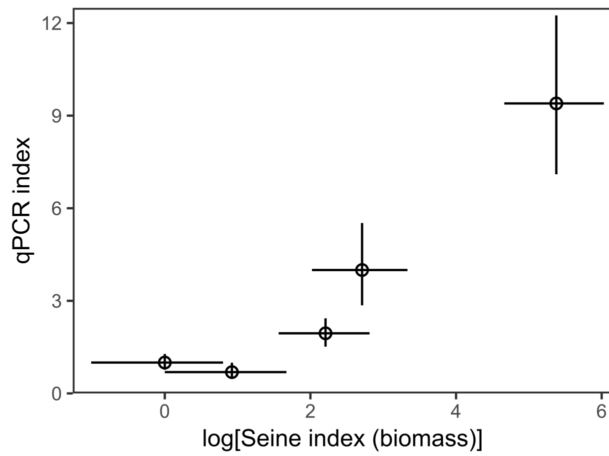

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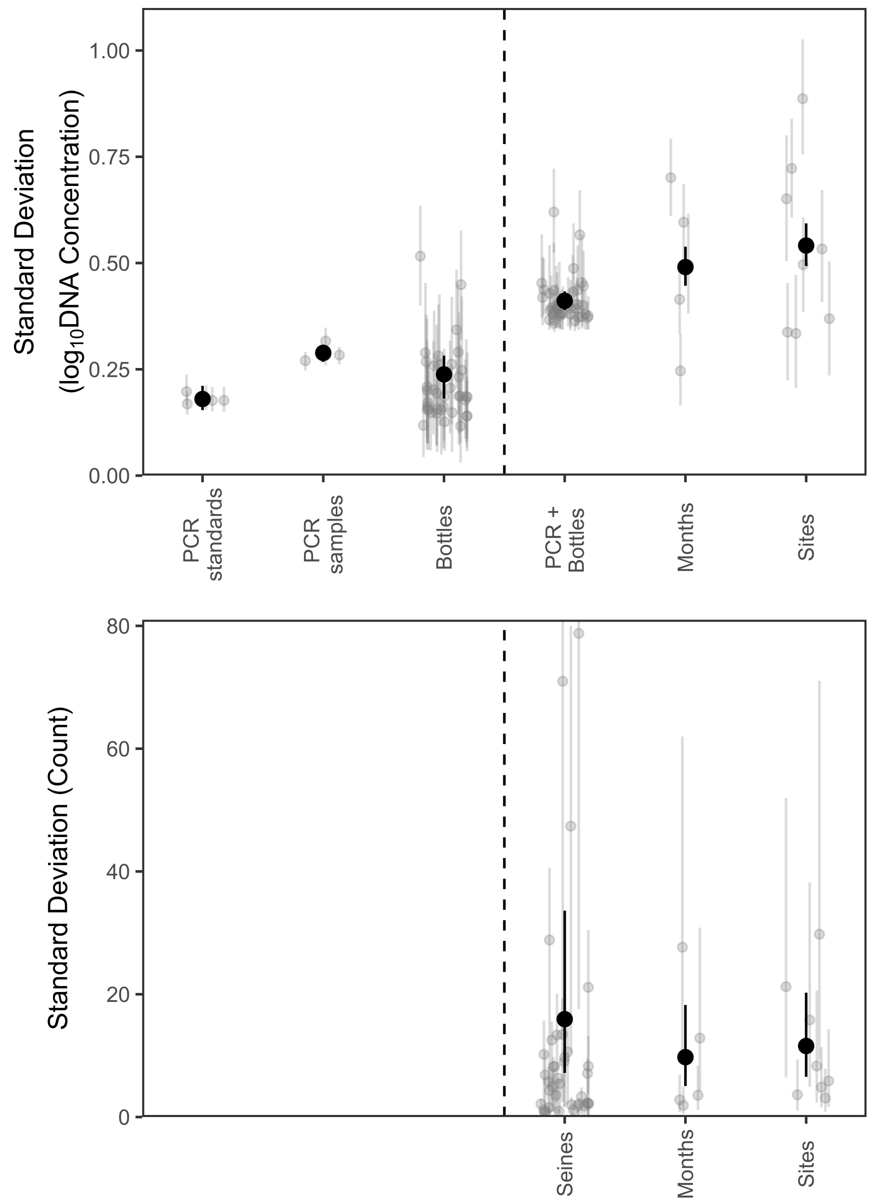

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