COMPARING ENVIRONMENTAL DNA AND TRADITIONAL MONITORING APPROACHES TO ASSESS THE ABUNDANCE OF OUTMIGRATING COHO SALMON (*ONCORHYNCHUS KISUTCH)* IN CALIFORNIA COASTAL STREAMS.

By

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22 ABSTRACT

23 COMPARING ENVIRONMENTAL DNA AND TRADITIONAL MONITORING
24 APPROACHES TO ASSESS THE ABUNDANCE OF OUTMIGRATING COHO
25 SALMON IN CALIFORNIA COASTAL STREAMS.
26

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28

29 Environmental DNA (eDNA) has the potential to dramatically increase the
30 information available to managers regarding species distribution and abundance.
31 Collection of reliable survey information on fish abundance is essential to monitor
32 population trends and restoration efforts for endangered and threatened species. In
33 Northern California, coho salmon are a federally listed species and a focus of ongoing
34 monitoring programs and restoration projects. I examined the feasibility of using eDNA
35 to supplement, or replace, traditional outmigrating juvenile coho salmon monitoring
36 approaches currently used at two existing coho salmon life-cycle monitoring stations.
37 Over the spring of 2018 and spring of 2019, I collected water samples, water quality, and
38 flow information during the coho salmon smolt migration season at cross-sections of two
39 creeks in Northern Humboldt County, California concurrently with daily downstream
40 migrant trapping. In addition, I compared differences in the amount of eDNA filtered from
41 water samples collected and filtered through multiple filter sizes and material. Extracted
42 DNA was amplified using qPCR and a species-specific assay. Results of model selection
43 using weekly and daily abundance estimates and Flow Corrected eDNA indicate high

44 variability of eDNA concentration both within sites and between sites for each creek. The
45 best-fit models did not include Flow Corrected eDNA; a measure of eDNA concentration
46 adjusted for stream flow. However, when using Flow Corrected eDNA values to generate
47 an additional measure of abundance, Area Under the Curve (AUC), the predictive ability
48 of the models increased significantly on both Prairie and Freshwater Creek. A linear
49 regression resulted in a significant positive relationship that explained 71% of the
50 variation between AUC and the downstream migrant coho salmon estimates on Prairie
51 Creek and 88% of the variation in Freshwater Creek in 2018. Additionally, there was a
52 significant relationship between AUC and the downstream migrant coho salmon estimates
53 in 2019 for only one of the filter sizes tested. These results imply that this approach shows
54 promise for elucidating relationships between eDNA and juvenile coho abundances, but
55 more research is necessary to determine what sampling methods, and analytical
56 approaches, to use in these small lotic systems.
57

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158

159 # INTRODUCTION

160 Coho salmon (*Oncorhynchus kisutch*) populations inhabiting the west coast of
161 North America have been subjected to human-caused stressors through ecosystem changes
162 that have negatively affected salmon abundances over recent centuries. In northern
163 California, coho salmon are extremely valuable both ecologically and culturally and they
164 comprise a fundamental component of redwood forest ecosystems. Therefore, the
165 monitoring, conservation, and restoration of these fish populations is of paramount
166 importance to a properly functioning watershed. Unfortunately, coho salmon populations
167 within the Southern Oregon Northern California Coast (SONCC) Environmentally
168 Significant Unit (ESU) are listed as “threatened” under the Endangered Species Act (ESA)
169 (NMFS, 2014). Listing of coho salmon under the ESA mandates regulatory agencies to
170 develop recovery plans that assess potential barriers to population recovery and
171 recommends comprehensive management actions. Collection of reliable and timely survey
172 information on population abundance and distribution is essential to demonstrating the
173 success or failure of conservation efforts. In this thesis, I evaluate the potential of using
174 environmental DNA (eDNA), an emerging monitoring technique being used in ecological
175 research, to assess the abundance of juvenile coho salmon out-migrating from two
176 Northern California coastal watersheds.

177 There are several challenges in determining the abundance and distribution of fish
178 in lotic systems. Primarily, there is a need to be able to collect accurate scientific data
179 (Thomsen and Willerslev, 2015). To accomplish this, traditional fisheries methods rely on

180 the capture, or observation, of fish with the use of several sampling types including nets,
181 traps, electrofishing, angling, hydroacoustics, and visual observation (Pope and Willis
182 1996, Bonar et al., 2009). However, these methods can provide incomplete and inadequate
183 information for a number of reasons including: 1) issues with low detection probabilities,
184 2) identification of similar species and sampling bias, and 3) the challenges of executing
185 fieldwork in riverine environments (Mackenzie and Royle, 2005; Bonar and Hubert,
186 2011). Often these methods are resource intensive and potentially harmful to fish
187 populations when handling species that are rare or endangered. Equipment, infrastructure,
188 and personnel are all expensive for resource managers. The funding it takes to operate
189 traditional fisheries sampling equipment limits the area scientists are able to study. Due to
190 the relative simplicity and lower costs of eDNA, there is potential to use it as a viable tool
191 to complement or even replace traditional sampling methods and provide managers and
192 conservation organizations with a rapidly deployable survey method to quantify the status
193 of target species.

194 Environmental DNA, commonly referred to as eDNA, is a term used to describe
195 genetic material that organisms are constantly shedding from their bodies into the
196 surrounding environment. This material comes in the form of urine, feces, mucus, and
197 sloughed epidermal cells. These short fragments of genetic sequences are released from
198 the organism and end up in detectable forms in soil, air, ice, plant surfaces, predator
199 stomachs, or in water (Walker et al., 2007, Andersen et al., 2012, Epp et al., 2012, Folloni
200 et al., 2012). The material can then be extracted, purified, and analyzed to reveal
201 information about the recent presence or absence of organisms (Schwartz et al., 2007,

202 Dejean et al., 2012, Thomsen et al., 2012, Bohmann et al., 2014). The use of eDNA
203 analysis has led to a better ability to detect rare, cryptic, and difficult to capture organisms
204 when traditional monitoring approaches have been unsuccessful (Thomsen et al., 2012,
205 Laramie et al., 2015).

206 Monitoring of populations with eDNA could also provide a means for managers,
207 researchers, and conservation organizations to assess the success of habitat restoration
208 projects. These projects involve huge amounts of planning, cost, and time and are a
209 common mandate of recovery plans for listed species. With so many resources involved,
210 monitoring before, during, and after restoration is essential. Often, assessing the success of
211 restoration projects can be undermined by inadequate funding, access issues, and the lack
212 of available sampling infrastructure. Conservation groups and small community resource
213 agencies often lack the equipment, training, and time to effectively monitor restoration
214 projects for fish. Using eDNA, surveyors will be able to survey a larger area of complex
215 river and stream systems with fewer funds than would be necessary with traditional
216 sampling methods. This may allow these groups to better assess the watershed scale
217 effectiveness of restoration activities on aiding the recovery of salmon populations.
218 Providing organizations with an easily adopted sampling technique that requires no
219 infrastructure could increase the amount of information gleaned from restoration projects
220 and their benefits to target fish species.

221 The use of eDNA to assess the distribution and presence or absence of
222 macroscopic organisms is a relatively new application of the technique. In 2008, Ficetola
223 et al., published a paper demonstrating the utility of eDNA analysis in the assessment of

224 distribution of a living species. Using custom designed, species-specific genetic primers,
225 mitochondrial sequences were amplified to detect the presence or absence of a frog (*Rana*
226 *catesbeiana)* in natural and laboratory experiments. The results showed agreement
227 between the presence or absence of the organism using traditional sampling techniques
228 and the amplification, or lack thereof, of DNA within collected water samples. This
229 technique showed a proof-of-concept and laid the foundation for continued work into the
230 practicality of eDNA monitoring. Since then, the procedure has been used to evaluate
231 distributions of target species and biodiversity in a wide variety of aquatic ecosystems.
232 Examples include lakes (Ficetola et al., 2008; Thomsen et al., 2012; Takahara et al.,
233 2013), rivers (Minamoto et al., 2012; Fukumoto et al., 2015; Wilcox et al., 2016), and
234 marine systems (Foote et al., 2012; Thomsen et al., 2012). Presently, analysis of
235 environmental DNA has come to be relied on as a valid survey technique for determining
236 the presence and absence of organisms, and it has been found to be robust in the detection
237 of species even at low densities in lentic and lotic systems (Ficetola et al. 2008; Goldberg
238 et al. 2011). The next goal of many researchers has been to extend this process to estimate
239 the relative abundance of individuals within systems (Lacoursière-Roussel et al., 2016,
240 Doi et al., 2017, Levi et al., 2018, Rice et al., 2018). Within aquatic mesocosms,
241 monitoring of eDNA concentrations has resulted in a general correlation between
242 abundance and eDNA concentrations (Doi et al., 2017, Lacoursière-Roussel et al., 2016).
243 However, use of eDNA concentration to estimate relative abundance of organisms in
244 natural systems has been investigated in only a few cases (Pilliod et al., 2013, Klobucar et
245 al., 2017, Levi et al., 2018, Shelton et al., 2019).

246 Recently, studies have looked to determine if a relative abundance measurement
247 from concentrations of eDNA correlates with traditional measures of fish abundance. For
248 example, Levi et al. (2018), published work utilizing a cross-channel research weir to
249 enumerate migrating salmonids in conjunction with an eDNA monitoring program and
250 investigated correlations between migrating fish abundance and measured concentrations
251 of eDNA. Levi et al.'s study site is heavily managed and produces some of the most
252 reliable salmon census data in Alaska. The results of their study showed a tight tracking of
253 local eDNA signal and salmon that had been enumerated the previous day for both
254 upstream migrating adults and downstream migrating smolts. To better account for
255 changes in flow and its effects on eDNA concentrations, Levi et al. (2018), produced a
256 "Flow-Corrected eDNA" or "eDNA Flow Rate" by multiplying streamflow and eDNA
257 concentration and used this value to correlate with trap catches. The researchers found
258 statistically significant relationships between adult Sockeye Salmon, total Coho Salmon,
259 and Sockeye Salmon smolts and their respective flow-corrected eDNA rates. However,
260 other studies have found poor relationships between local eDNA concentrations and the
261 local abundance of targeted organisms (Rice et al., 2018, Lacoursière-Roussel et al.,
262 2016).
263 Environmental DNA that is released by an organism is immediately subject to
264 degradation through biotic and abiotic mechanisms. Ultraviolet radiation, temperature,
265 time, water chemistry, and biotic interactions are all acting on the free-floating strands of
266 genetic material. Persistence of eDNA has only been minimally investigated (Dejean et
267 al., 2011, Merkes et al., 2014, Piaggio et al., 2014) with all of the focus on how long

268 material is detectable once released from an organism. However, detection probability
269 may also be affected by physical water quality parameters. Fluctuations through time in
270 temperature and dissolved oxygen may alter environmental DNA fate dynamics and its
271 detectability or relationship to abundance (Strickler et al., 2015). One way to measure the
272 detectability of eDNA is to monitor physical water quality parameters concurrently with
273 eDNA samples throughout the sampling season to observe if there is a correlation between
274 detectability and water quality.

275 My goal in this study was to use existing fish monitoring infrastructure in two
276 local Humboldt County watersheds to compare daily and weekly measurements of
277 abundance of out-migrating Coho Salmon with eDNA concentrations taken from water
278 samples at the trap site. I conducted two years of sampling, the first year on two separate
279 creeks using a single filter size and the second year I sampled a single creek with multiple
280 filter sizes. For the second year, I adjusted my sampling protocol to more narrowly focus
281 in on possible sources of error and inconsistency in the first year. Specifically, my
282 objectives for this project were to determine if eDNA concentration were correlated with:
283 1) daily, or weekly, out-migrating smolt abundance, and 2) water-quality parameters?
284
285

286 # MATERIALS AND METHODS

287 ## Sampling Sites

288 Two sampling sites in Northern California, Humboldt County were selected for an
289 eDNA monitoring program. Both sites were chosen based on on-going monitoring of
290 salmon populations within the watersheds via life-cycle monitoring stations (LCMS).
291 These LCMS utilize in-stream infrastructure (e.g. Passive Integrated Transponder (PIT)
292 antennas, weirs, down-stream migrant traps, and spawning ground surveys) to evaluate the
293 population dynamics of salmonids within the watershed. These LCMS provided historical
294 information on run-timing and size of the Coho salmon outmigration season and were
295 used to best determine beginning and end dates for eDNA sampling.

296 The first sampling site was located in Prairie Creek, a sub-basin of Redwood Creek
297 in Humboldt County, California (Figure 1). The Prairie Creek watershed encompasses an
298 area of 103 km^2, provides 38 km of anadromous habitat, and lies almost entirely within the
299 boundaries of Redwood State and National Park. Prairie Creek produces the majority of
300 coho salmon within the Redwood Creek basin (Brown, 1988). Within the riparian zone of
301 the upper-basin, the overstory is composed of near-pristine old-growth coastal redwood
302 (*Sequoia sempervirens*), douglas-fir (*Pseudotsuga menziesii*), and sitka spruce (*Picea*
303 *sitchensis*), while the lower section of the creek has been subject to some human alteration
304 and is composed of mostly second-growth red alder (*Alnus rubra*), douglas fir, and coastal
305 redwood. Understory plants include redwood sorrel (*Oxalis oregano*), western white

306 trillium (*Trillium ovatum*), red huckleberry (*Vaccinium parvifolium*), and wood fern
307 (*Dryopteris spp.*). Prairie Creek supports self-sustaining populations of 4 species of
308 salmonid fishes: chinook salmon (*Oncorhynchus tshawtyscha*), coho salmon
309 (*Oncorhynchus kisutch*), resident and anadromous steelhead trout (*Oncorhynchus mykiss*),
310 and resident and anadromous coastal cutthroat trout (*Oncorhynchus clarki*). Prairie Creek
311 was sampled for eDNA concentrations during only the 2018 season.

312 The second sampling site was located in Freshwater Creek (Figure 3). The
313 Freshwater Creek watershed empties into Humboldt Bay in Humboldt County, CA. The
314 watershed spans an area of 92 km^2 with approximately 14.5 km accessible to anadromous
315 fish in the mainstem (Ricker and Anderson, 2011). The terminal part of the basin (river
316 km 10 and below) is a low-gradient stream with considerable development on the first six
317 river kilometers, consisting of levees, agricultural land, and cattle pasture. The riparian
318 zone of the lower stretches of the creek is characterized by willow (*Salix spp.*), blackberry
319 (*Rubus ursinus*), and red alder. The upper basin contains well-developed forest community
320 structure of red alder, willow, as well as coastal redwood, douglas-fir, and salmonberry
321 (*Rubus spectasbilis*) (Moore and Ricker, 2012). Freshwater Creek supports self-sustaining
322 populations of four species of salmonid fishes: chinook salmon, coho salmon, resident and
323 anadromous steelhead trout, and resident and anadromous coastal cutthroat trout.

324 Fish Sampling

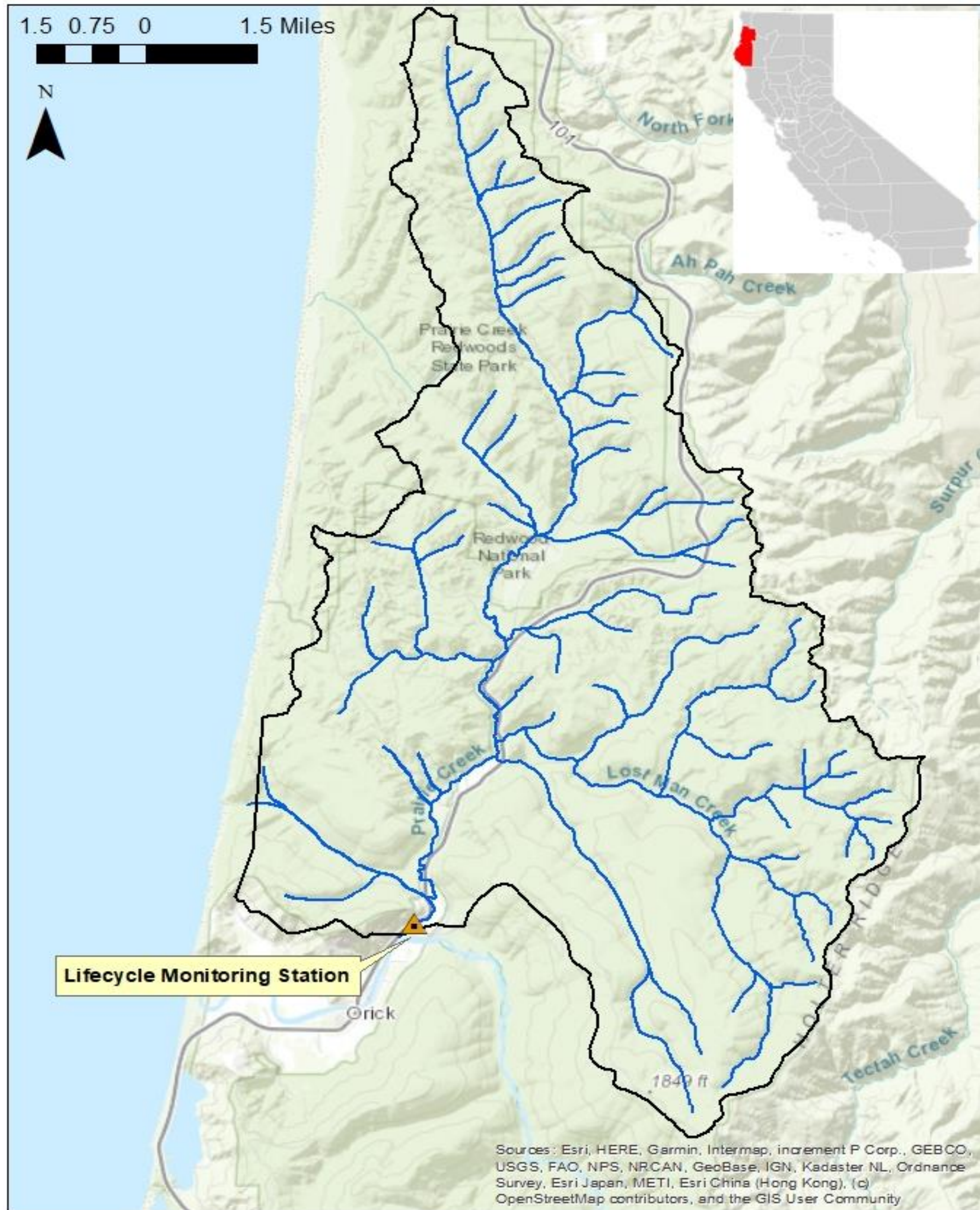
325 Spring smolt trapping of salmonids on Prairie Creek takes place immediately
326 upstream of the confluence of Prairie Creek and Redwood Creek (Figure 1). At Prairie

327 Creek, a Rotary Screw Trap (RST) is operated by the California Cooperative Fish and
328 Wildlife Unit at Humboldt State University (HSU). At Freshwater Creek, a modified weir
329 box-trap design is used to capture downstream migrants along the lower mainstem of the
330 creek (Figures 2 and 3). Trap checks begin during early March as flows permit and
331 continue daily until migration is no longer observed in late spring. All fish in the trap are
332 enumerated and a subset are tagged and transported upstream for release and trap
333 efficiency estimates based on recaptures of marked fish. Length and weight measurements
334 are taken for a subset of coho salmon present in the trap, as well as other salmonids. Fish
335 sampling on Freshwater Creek is similar. Trap checks are carried out seven days a week
336 from early March through June. Coho salmon abundances are recorded and fish are
337 released downstream, or returned upstream for trap efficiency estimates based on
338 recaptures of marked fish. Producing these trap efficiency estimates allow the monitoring
339 programs to estimate weekly abundances of fish moving downstream.

340 These capture data were organized into strata and analyzed using the Darroch
341 (1961) stratified Petersen estimator to produce an estimated abundance. This analysis was
342 implemented using the program DARR (Bjorkstedt, 2005). The strata for these capture
343 data are commonly weeks for fisheries downstream migrant applications to account for
344 variations in capture efficiency, but other temporal scales can also be used. I used the
345 DARR program to generate both weekly and daily abundance estimates of Coho Salmon
346 using mark-recapture data. Daily estimates provide a higher resolution to test the
347 relationship between direct daily eDNA measurements and DARR estimates based on trap
348 catches and may better represent day to day variations. However, the weekly scale allows

349 the mark recapture estimates to be compounded and daily eDNA concentrations to be

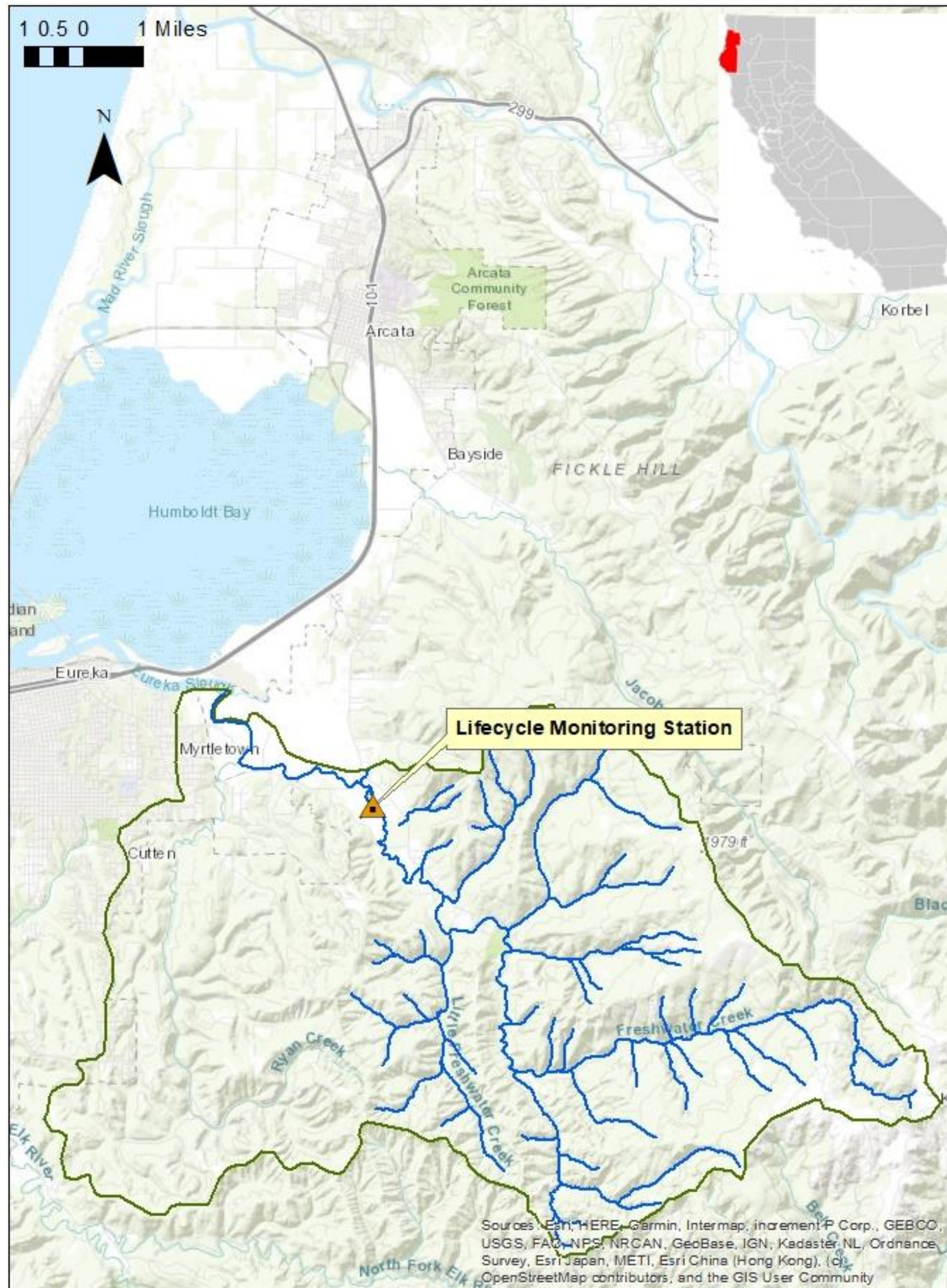
350 averaged out, reducing the variation and error of both measurements.



351

352 Figure 2: Site map of Prairie Creek showing coho salmon life monitoring station and

353 approximate location of eDNA sampling site.



354
355 Figure 3: Site map of Freshwater Creek showing coho salmon lifecycle monitoring station
356 and approximate location of eDNA sampling site.

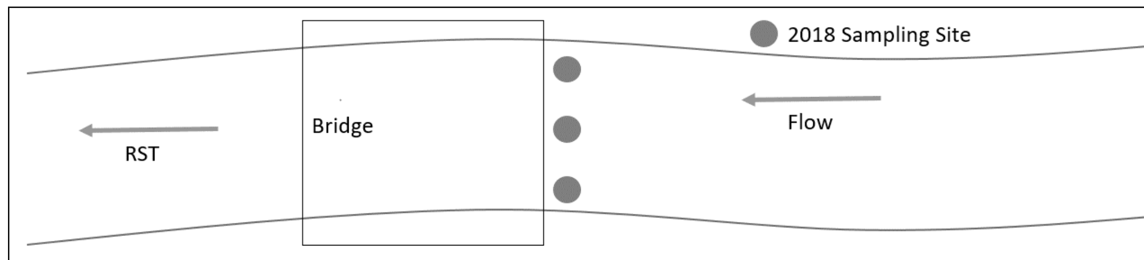
357

358 Figure 4: Modified weir box trap deployed seasonally on the mainstem of Freshwater
359 Creek in Northern Humboldt County, CA.,

360

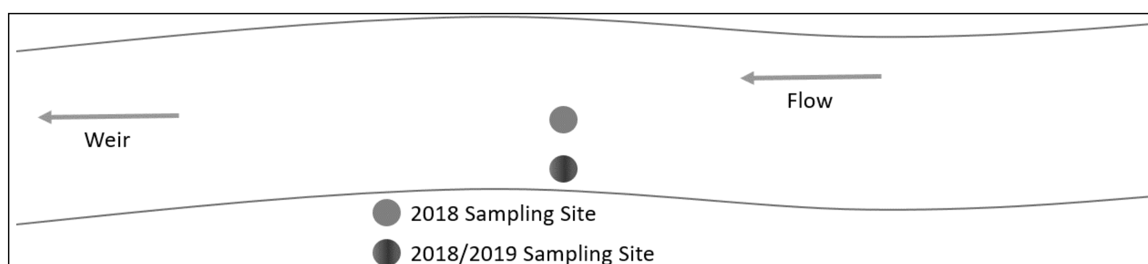
361 eDNA sampling

362 During the 2018 season, I collected water samples every other day for 15 weeks
363 beginning March 1, 2018 to coincide with RST sampling at Prairie Creek and trap
364 sampling on Freshwater Creek. I collected water samples for molecular analysis at
365 approximately the same time for each event (1000-1200); this was immediately after, or
366 during fish processing and measurements at the LCMSs. Water was collected from
367 multiple sample sites in a transect approximately 100 meters above the traps (Figure 4).

368 

369 Figure 5: 2018 Prairie Creek sampling locations. Immediately above Bald Hills Rd

370 Bridge.

371 

372 Figure 6: 2018 and 2019 Freshwater Creek sampling locations.

373 By sampling above the traps, I measured the change in concentrations over time

374 without interference from fish within the trap. At each location, I collected two replicates

375 taken per sampling event. I collected water in labeled 2-L WhirlPak bags and filtered

376 sample volumes of a maximum of 1.5-L. After 1.5-L we found filtering difficult due to

377 clogged filters. Sample bags were wiped with paper towels and 50% bleach solution to

378 eliminate surface contamination. Samples were transported on wet ice to Humboldt State

379 University where filtration was carried out within 2h of sampling to prevent degradation

380 of DNA. Field controls were used with each sampling event. Field controls are water

381 samples from a known eDNA-free environment that were present and processed in the

382 same conditions as all other samples to identify possible contamination issues during

383 sampling.

During the 2019 season, water samples were collected at Freshwater Creek every weekday for 15 weeks beginning March 1, 2019 to coincide with trap sampling. The 2019 sampling design was the same as 2018, with a few exceptions. Preliminary analysis did not indicate there was a difference in sample location, so samples were collected in duplicate from a single stream location immediately above the trap. Water was collected in labeled 2-L WhirlPak bags and 1-L sample volumes were filtered. Difficulties were encountered when filtering large volumes of water and therefore 1-L was used so each sampling event would have the same volume of water filtered.

Filtration

Samples were filtered within lab facilities at Humboldt State University. Lab benches were cleaned with 50% bleach solution before and after each filtration event. During 2018, a Welch brand vacuum pump model WOB-L 2522B-01 was used to filter water samples. Pump trials were run until 1.5-L of water had been filtered through a 3.0µM GE Healthcare Whatman® Polycarbonate Filters and the time recorded. Filters were stored in 2mL tubes at -20°C until extraction.

During 2019, two filter types were used. Results from 2018 suggested that DNA yield may be low utilizing 3.0µM Polycarbonate filters, prompting the development of a hypothesis that using a filter with smaller pore size would increase the DNA yield and improve correlations with trap abundance. Therefore, one water sample was filtered through a 3.0µM GE Healthcare Whatman® Mixed Cellulose Ester (MCE) filter and the

405 other water sample was filtered through a 0.45µM GE Healthcare Whatman® Cellulose
406 Nitrate (CN) filter. Due to susceptibility of the 3.0µM Polycarbonate filters to splitting, I
407 used a one-gallon fluid evacuator hand pump to process water samples through these
408 filters. For the 0.45µM, I used a GE ⅓ horsepower vacuum pump.

409 In both years, filters were manipulated using sterile gloves and forceps. Filters
410 were taken from pump stations, rolled, and immediately stored in 2mL tubes at -20̊C until
411 extraction.

412 eDNA molecular methods

413 The primary steps for eDNA analysis – water filtration, DNA extraction, and
414 qPCR – were all conducted in separate facilities dedicated to each activity to minimize
415 contamination. Extraction facilities were UV-irradiated and wiped down with RNAse
416 AWAY™ spray prior to extraction procedures. DNA extractions were carried out using a
417 QIAGEN DNeasy Blood and Tissue Kit according to the manufacturer's directions except
418 as noted by Schmelzle and Kinziger (2016). Lysis solutions were prepared and incubated
419 in thermomixers for 24 hours at 56 °C before extraction. After incubation, 200µL of lysis
420 product was used in the extraction protocol. An elution volume of 100µL was used in the
421 final step of extraction. Extracted DNA was then stored in 1.5 mL cryo-vials at -20̊C until
422 analysis.

423 Amplification of Coho Salmon eDNA was done using a TaqMan™ Real-Time
424 PCR assay (Thermofisher, Waltham, MA) specifically designed to detect Coho Salmon. A
425 QuantStudio 3™ Real-Time PCR machine (Thermofisher, Waltham, MA) was used to

426 amplify 96-well optical PCR plates containing field samples, negative and positive
427 controls, and serial dilutions. We used a primer and probe species-specific set developed
428 and applied to Coho Salmon in eDNA surveys by Pilliod and Laramie (2016), the
429 sequence is reported in Table 1. This assay targeted a fragment of the cytochrome b gene
430 and exhibited a high degree of specificity for coho salmon and did not exhibit positive hits
431 for closely related salmonid DNA (Figure 6). Reactions were carried out at a total volume
432 of 25µL using 2µL of extracted DNA. Each qPCR was run in triplicate, with negative and
433 positive controls included for all qPCR reactions. A positive control of extracted coho
434 tissue was used on all qPCR plates and a negative control of DNA free water. DNA
435 concentration was calculated using a 5-step 1:10 serial dilution of DNA extract from coho
436 tissue and the use of a NanoDrop™ Spectrophotometer to develop a standard curve
437 relationship between Ct values and DNA concentration (Figure 7).

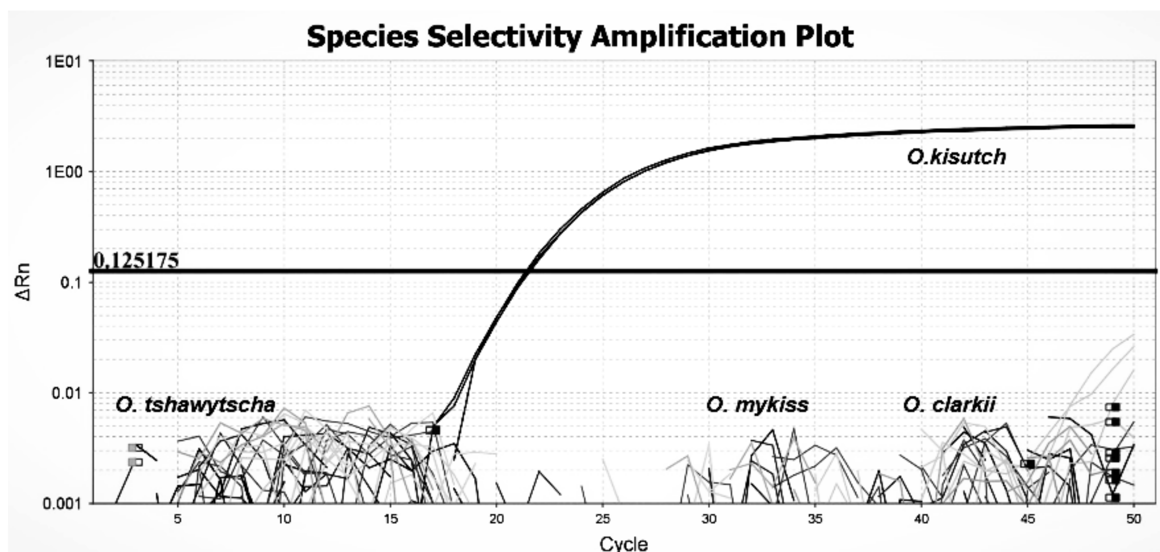
438 ## Environmental Covariates

439 To describe the abiotic factors influencing the concentrations of eDNA in the
440 stream channel over time, physical water quality measurements were taken during each
441 sampling event using a YSI® multiparameter water quality meter. Temperature, dissolved
442 oxygen, and conductivity were recorded both on datasheets and internally inside the unit
443 during each sampling event. Temperature was recorded in degrees Celsius, dissolved
444 oxygen was recorded as mg•L^{-1}, and conductivity was recorded as microsiemens•cm^{-1}.
445 Water quality meter measurements were taken 30cm below the surface of the water, or
446 just off the creek bed when sampling in depths less than 30cm.

447 Discharge measurements of Prairie Creek were recorded at a USGS gaging station
448 that is maintained on the mainstem and downloaded via the USGS web portal
449 (https://waterdata.usgs.gov/nwis/). Data were checked graphically for any potential
450 anomalies, and approximately 1 month of data was lost due to high winter flows and
451 equipment malfunction on Prairie Creek in 2018. Therefore, estimates of eDNA flow-rate
452 were not calculated for the period of missing data. Freshwater Creek has a previously
453 developed rating curve for relation to a USGS station on Little River, a neighboring
454 watershed. These measurements do not capture all possible flow scenarios due to the
455 location of the gaging stations relative to the sampling site; however, I assume that relative
456 flow is sufficient to model fish and eDNA movement.
457
458 Table 1: Coho salmon (O. kisutch) qPCR assay. Table shows region of target
459 amplification (Cytochrome B) and sequences for forward primer (F-primer), reverse
460 primer (R-primer), and probe.

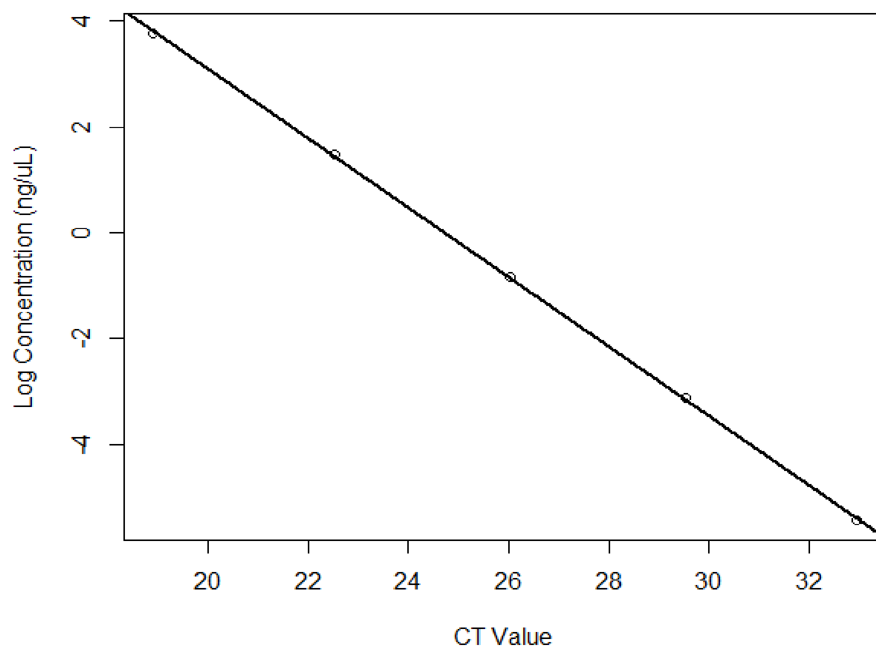
Target Species	**Region**	**Base pairs**	**F-Primer**	**R-Primer**	**Probe**
O. kisutch	Cytochrome B	114	CCT TGG TGG CGG ATA TAC TTA TCT TA	GAA CTAG GAA GAT GGC GAA GTA GAT C	6FAM-TGG AAC ACC CAT TCA T-MGBNFQ

461



462
463 Figure 7: Amplification plot showing qPCR results when testing coho salmon against

464 closely related species.



465
466 Figure 8: Standard curve plot relating qPCR thermo-cycle value (CT value) to

467 concentration of eDNA (ng/uL).

468 Statistical analysis

469 Correlation between eDNA and trap estimates

470 Management and manipulation of data was completed using Google Suite and R
471 Computing Software Version 3.6.1. Relationships between measurements of smolt
472 abundance and eDNA concentrations in the water were assessed using generalized linear
473 models with a negative binomial error distribution using the Stats package (R Core Team,
474 2019). Additionally, I calculated a metric of "eDNA Flow-Rate" following procedures
475 from Levi et al. (2018), whereby the Average-Daily-Flow (CFS) was multiplied by the
476 calculated eDNA concentration ($ng\bullet uL^{-1}$) for that day. This calculation eliminated the
477 volume units and thus was a rate of DNA moving down the watershed ($ng\bullet sec^{-1}$).
478 To test the hypothesis that weekly and daily smolt estimates within a trap could be
479 modeled as a function of Flow Corrected eDNA, I generated candidate models for each
480 creek in each year. The first analysis used methods developed in Levi et al., 2018 and used
481 a negative binomial error structure to account for the count nature of the smolt abundance
482 data while helping to correct for possible overdispersion. Covariates were examined for
483 collinearity and I only included a single covariate from any pair with a correlation greater
484 than 0.7. I built models with two different smolt estimate responses for each creek in each
485 year: 1) daily DARR estimates, and 2) weekly DARR estimate. I did not include any
486 interactions in the global model because I did not have any *a priori* hypotheses for why
487 there should be an interaction between any covariates nor did I find any evidence for an
488 interaction in my preliminary data exploration. All potential combinations of temperature

489 and Flow Corrected eDNA in the global model were fit and the most appropriate model
490 was selected using the model with the lowest AIC (Burnham & Anderson, 2004). Variable
491 values were averaged for the entire week for the weekly model and daily averages were
492 used for daily DARR estimate models. Results are displayed in response plots showing the
493 relationship between DARR estimates and significant predictors.

494 The second analysis took the Flow Corrected eDNA values and the weekly DARR
495 estimates from the first analysis to estimate a total weekly abundance using Area Under
496 the Curve (AUC) (Pochardt et al., 2019). AUC is the area under the curve between the
497 time-series of Flow Corrected eDNA and sample date. The AUC values for each week
498 were calculated using the DescTools package (Signorell, 2019). This function uses
499 trapezoidal interpolation to integrate the area under the curve and estimate any missing x-
500 values. The values of Flow Corrected eDNA were averaged across all sampling locations
501 within each creek in 2018. In 2019, I had only one sampling location, but separate values
502 were calculated for each filter type. Once the AUC values were calculated, these values
503 were regressed against the weekly DARR estimates. The only predictor used in the model
504 was AUC of the Flow Corrected eDNA. To determine whether AUC was a reasonable
505 predictor of the weekly DARR estimates, I compared the AIC of this model to that of the
506 null model.

507

508 Effects of water quality of eDNA flow rate

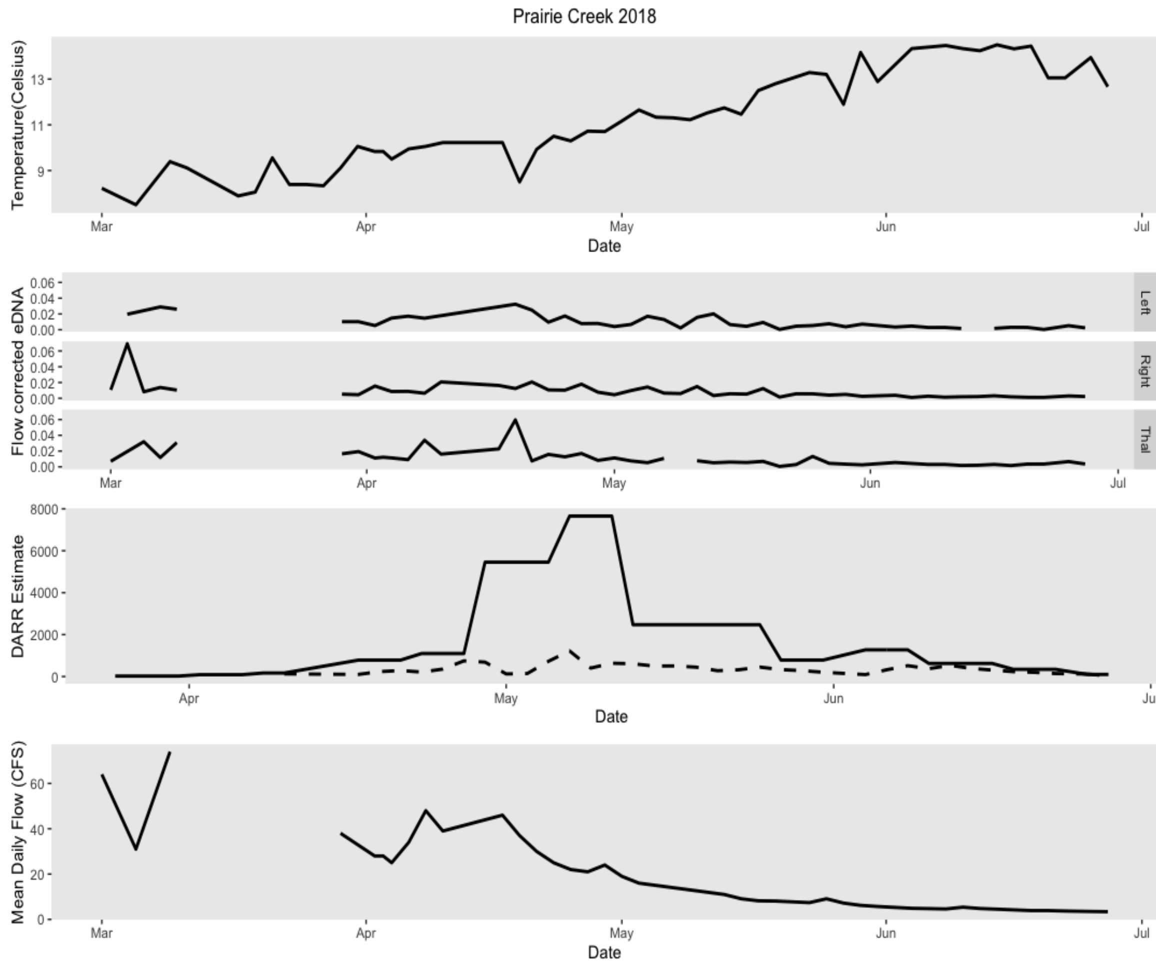
509 Environmental DNA movement downstream is likely affected by water quality.
510 To investigate these factors, data collected with a YSI© multi-parameter water quality

511 meter were included in the models to determine any significant effects of temperature,
512 conductivity, and dissolved oxygen on eDNA concentration. Water quality parameters
513 may increase or decrease eDNA detectability. For example, temperature increases may
514 lead to higher rates of eDNA degradation. However, temperature also increases metabolic
515 activity and the increased rates of eDNA degradation may be attenuated by the increased
516 sloughing of eDNA from organisms. To test the hypothesis that changing environmental
517 covariates over the migration season have effects on the concentration of eDNA, I used
518 water quality data collected across the migration season. Measurements were collected at
519 every sampling location during each sampling event. Values used for analysis were the
520 average measurements of all sampling locations for each sampling event. The variables
521 were then, as in the prior analysis, tested for collinearity. I then built candidate models to
522 determine the relationship between water quality measurements and Flow Corrected
523 eDNA values.

524 # RESULTS

525 During the 2018 outmigration season, Prairie Creek displayed a seasonal increase
526 in temperature (Figure 8). In 2018, a partial loss of flow data on Prairie Creek occurred
527 due to equipment malfunction and therefore concentrations were not calculated from
528 approximately mid-March to early April (Figure 8). The migration of smolts primarily
529 occurred from late May through early June (Figure 8), thus, I do not believe this loss of
530 data greatly affected my ability to see how eDNA concentration were able to predict Coho
531 Salmon abundances.

532 A total of 164 eDNA samples were collected from Prairie Creek from March 1-
533 June 27 2018. Concentrations of environmental DNA ranged from $5.27\text{x}10^{-5}$ ng•uL^{-1} -
534 $4.89\text{x}10^{-3}$ ng•uL^{-1}. Weekly estimates of Coho Salmon ranged from 22-7655 individuals
535 and daily estimates measurements 28-1221. Furthermore, no significant differences were
536 found between sampling sites within the creek (p-value=0.251).



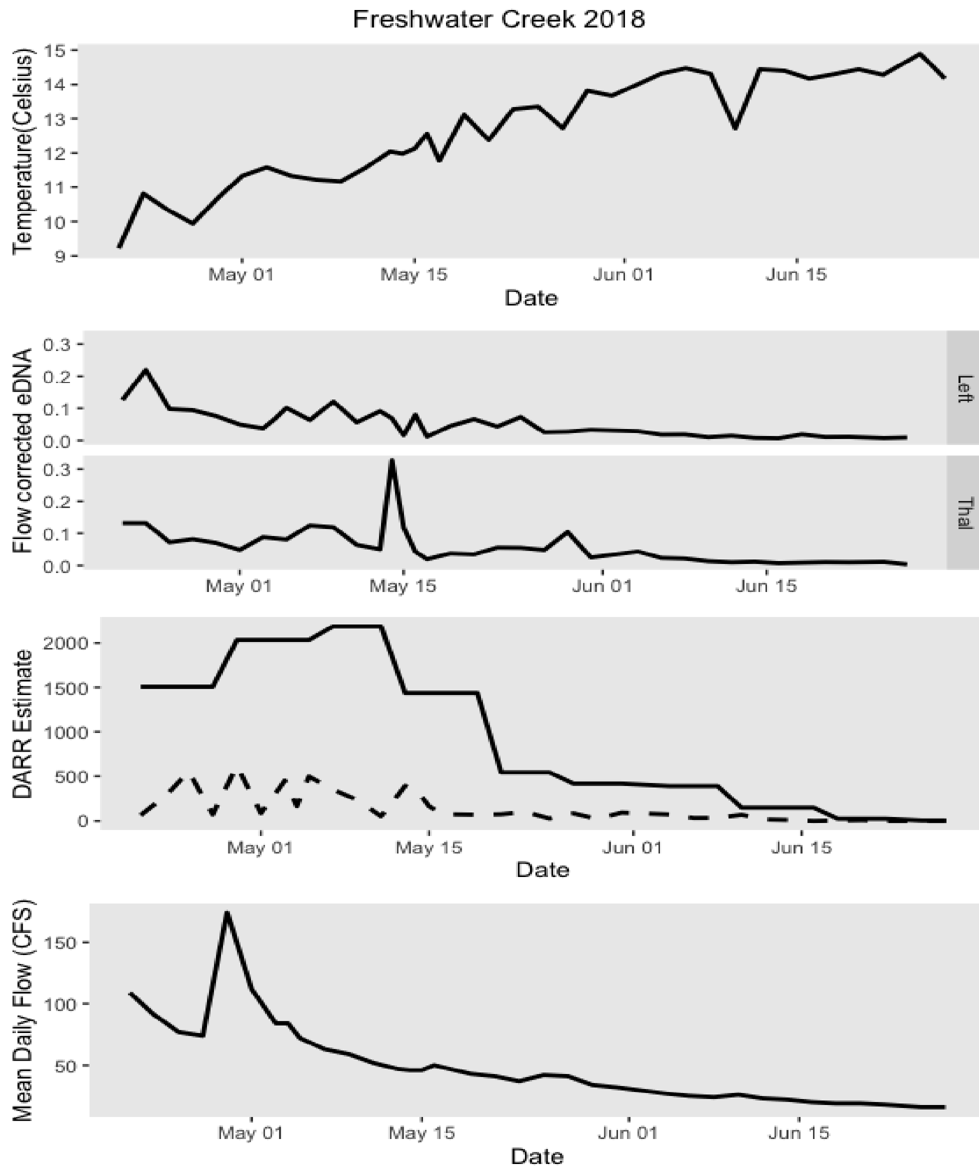
537

538 Figure 9: Top to bottom: Temperature measurements on Prairie Creek in 2018. eDNA

539 Flow Rate from March-June at each sampling location. Darr estimates by week (solid line)

540 and day-pairs (dashed line). Mean daily flow measurements.

541 On Freshwater Creek in 2018, a total of 70 samples were collected from April 21-
542 June 27. Concentrations of eDNA ranged from $2.20x10^{-4}$ ng·uL^{-1} - $7.10x10^{-3}$ ng·uL^{-1}.
543 Weekly estimates of Coho Salmon in the trap ranged from 5-2815 individuals and daily
544 estimates ranged between 0 and 616. I found that patterns from Prairie Creek were similar
545 in Freshwater Creek in 2018, with the greatest numbers of smolts estimated during May,
546 and the single highest abundance of coho salmon occurring in early May (Figure 9).
547 Sampling sites in the creek were not statistically significantly different from each other (p-
548 value=0.296, F=1.107, df=1).
549



550

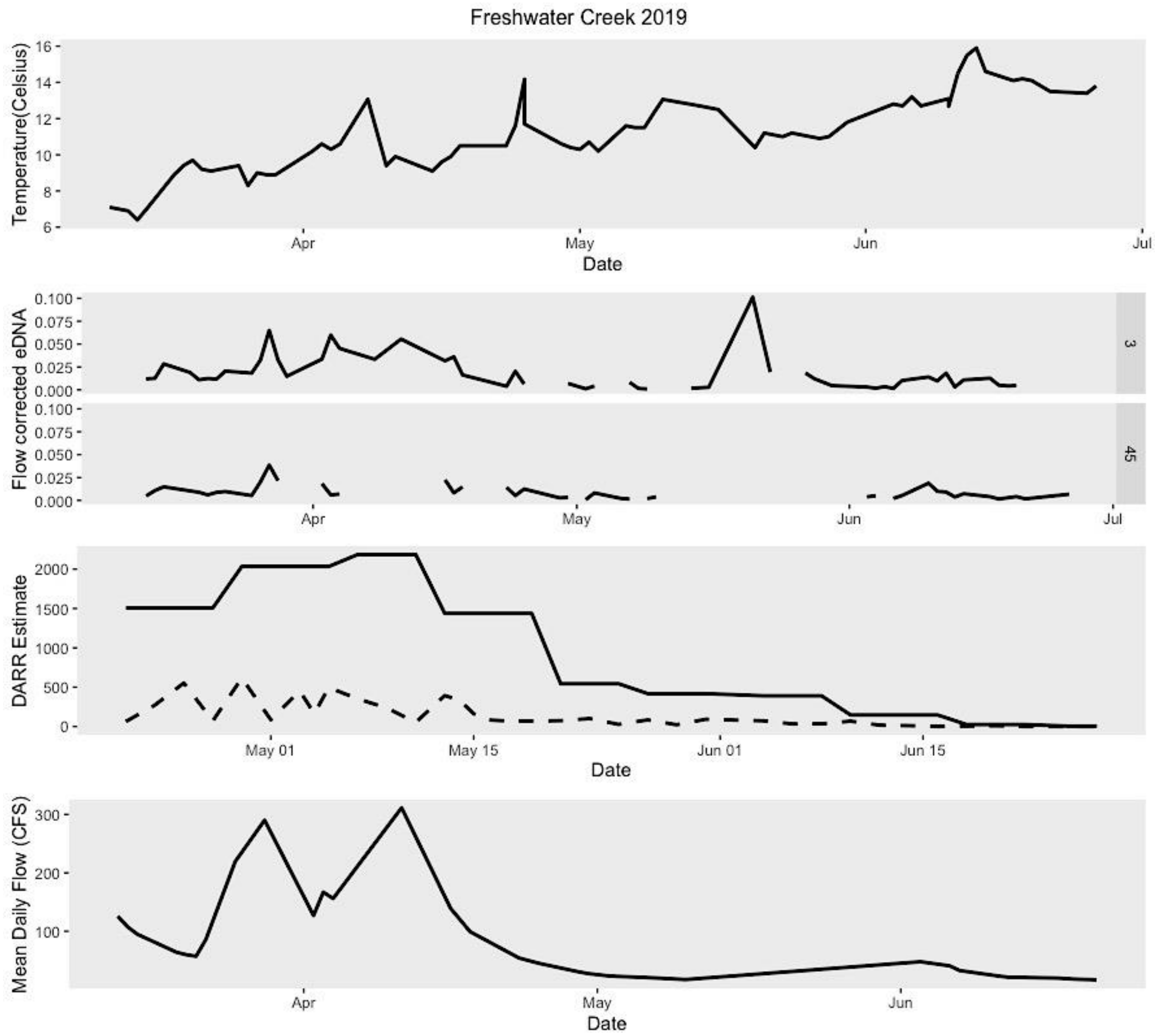
551 Figure 10: Top to bottom: Temperature measurements on Freshwater Creek in 2018.

552 eDNA flow rate values from April-June at each sampling location. Coho salmon estimates

553 by day (dashed line) and week (solid line). Mean daily flow measurements.

554

555 During 2019, a total of 108 samples were collected, filtered, and analyzed from
556 Freshwater Creek on the left bank, beginning March 13 and ending on June 26.
557 Concentrations of eDNA ranged from 2.45×10^{-5} $ng \cdot uL^{-1}$ - 8.65×10^{-4} $ng \cdot uL^{-1}$ across the two
558 different filter types (i.e., 0.45 µM and 3.0 µM). During the 2019 sampling season, the
559 trends of water quality and trap catches in Freshwater Creek were similar to those in 2018;
560 however, a late season storm at the end of May lead to an increase in dissolved oxygen
561 saturation, a decrease in temperature, and an increase in conductivity. Daily estimates of
562 coho salmon ranged from 0-662 individuals and weekly estimates range from 0-2377
563 (Figure 10). Concentrations from samples of different filter pore sizes did not yield a
564 significant difference when assessed using a Student's t-test (F-value=3.177, df=1,
565 p=0.0778) (Figure 11).

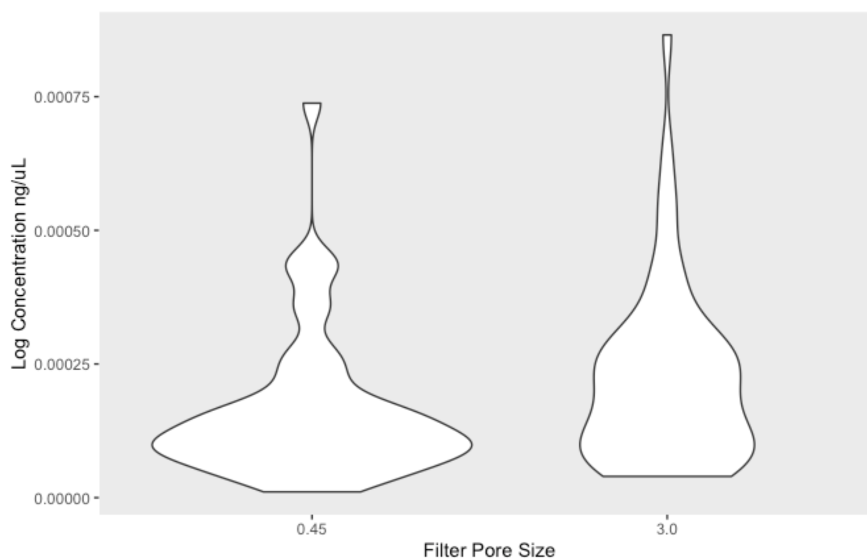


566

567 Figure 11: Top to bottom: Temperature measurements on Freshwater Creek in 2019.

568 eDNA flow rate values from March-June at each sampling location. Coho salmon

569 estimates by day (dashed line) and week (solid line). Mean daily flow measurements.



570
571 Figure 12: Violin plot showing concentrations of eDNA attained during the 2019 season
572 Freshwater Creek between 0.45u and 3.0u filter types.
573

574 Model Performance – eDNA and Weekly and Daily trap estimates

575 The colinearity analysis found that all the water quality parameters (i.e.,
576 temperature, conductivity, and dissolved oxygen) were highly correlated (r=0.75), thus, I
577 selected temperature as my representative water quality parameter. For each creek, in each
578 year, we fit models with all additive combinations of model covariates but without
579 interactions, which left the following global model equations:

580 $$\text{WeeklyDARR} = \beta_0 + \beta_1\text{FlowDNA}_i + \beta_2\text{Temperature}_i + \epsilon$$

581

582 $$\text{DailyDARR} = \beta_0 + \beta_1\text{FlowDNA}_i + \beta_2\text{Temperature}_i + \epsilon$$

583 Where β_x is the model estimate parameter, WeeklyDARR and DailyDARR is the Coho
584 Salmon weekly or daily estimate, FlowDNA is the flow corrected eDNA measurement
585 from the water sample, Temperature is the mean weekly or daily temperature, i is the
586 sampling event, and ϵ is the residual error. Models for each creek in each year were
587 developed using DARR estimates that were generated weekly and daily.

588 With the exception of daily DARR estimates on Freshwater Creek in 2019, all of
589 the models tested selected temperature as a significant predictor variable for determining
590 drivers of coho smolt abundances over the migration season. In all significant cases, Flow
591 Corrected eDNA measurements were consistently shown to have a negative relationship
592 with temperature. For Prairie Creek in 2018, the model selection process resulted in
593 temperature as the only selected predictor variable when using the weekly DARR
594 estimates, and the null model resulting in the best-fit model when using the daily DARR
595 estimates (Table 2 and 3, Figure 12). On Freshwater Creek in 2018, the model selection
596 process resulted in mean daily temperature being selected as the only significant variable,
597 with a negative relationship with weekly and daily abundance estimates (Table 4 and 5,
598 Figure 13). In 2019 on Freshwater Creek, AIC model selection process resulted in Flow
599 Corrected eDNA Rate and temperature being included in the best-fit model when
600 predicting weekly abundance estimates of coho; however, with daily abundance estimates,
601 only Flow Corrected eDNA was selected. The relationships for both daily and weekly
602 DARR estimates were negative (Table 6 and 7, Figures 14 and 15).

603

604 Table 2: Table displaying Daily DARR estimate model parameters and values for Prairie

605 Creek 2018.

Intercept	FlowDNA	Temperature	df	logLik	AICc	Delta	Weight
7.52		-0.43(0.12)	3	-800.375	1607	0	0.669
7.52	-0.088(0.16)	-0.48(0.16)	4	-800.278	1609	1.99	0.247
7.56			2	-804.047	1612.2	5.21	0.049
7.55	0.20(0.12)		3	-803.348	1613	5.95	0.034

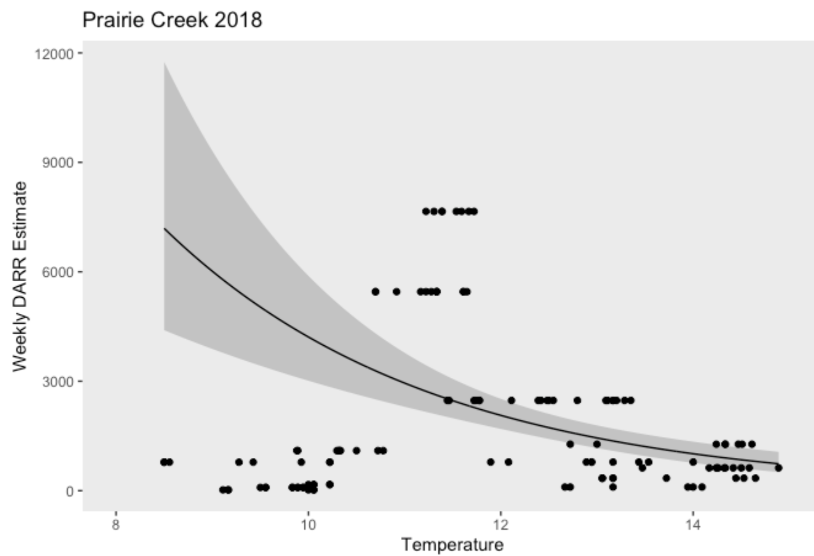
606

607 Table 3: Table displaying Weekly DARR estimate model parameters and values for

608 Prairie Creek 2018.

Intercept	FlowDNA	Temperature	df	logLik	AICc	Delta	Weight
5.92			2	-596.707	1197.6	0	0.379
5.92		-0.104(0.07)	3	-595.791	1197.9	0.31	0.325
5.92	0.075(0.07)		3	-596.345	1199	1.42	0.187
5.91	-0.016(0.10)	-0.11(0.10)	4	-595.781	1200	2.49	0.109

609



610

611 Figure 13: Response plot of Temperature and Weekly DARR estimates on Prairie Creek in

612 2018. Daily DARR estimate model resulted in the null model selected as the best-fit and is

613 therefore not included.

614 Table 4: Table displaying Weekly DARR estimate model parameters and values for

615 Freshwater Creek in 2018.

Intercept	FlowDNA	Temperature	df	logLik	AICc	Delta	Weight
6.57		**-1.03(0.10)**	**3**	**-502.841**	**1012.1**	**0**	**0.686**
6.57	**0.10(0.13)**	**-0.97(0.13)**	**4**	**-502.49**	**1013.6**	**1.56**	**0.314**
6.77	**0.75(0.12)**		**3**	**-520.715**	**1047.8**	**35.75**	**0**
6.91			**2**	**-529.716**	**1063.6**	**51.56**	**0**

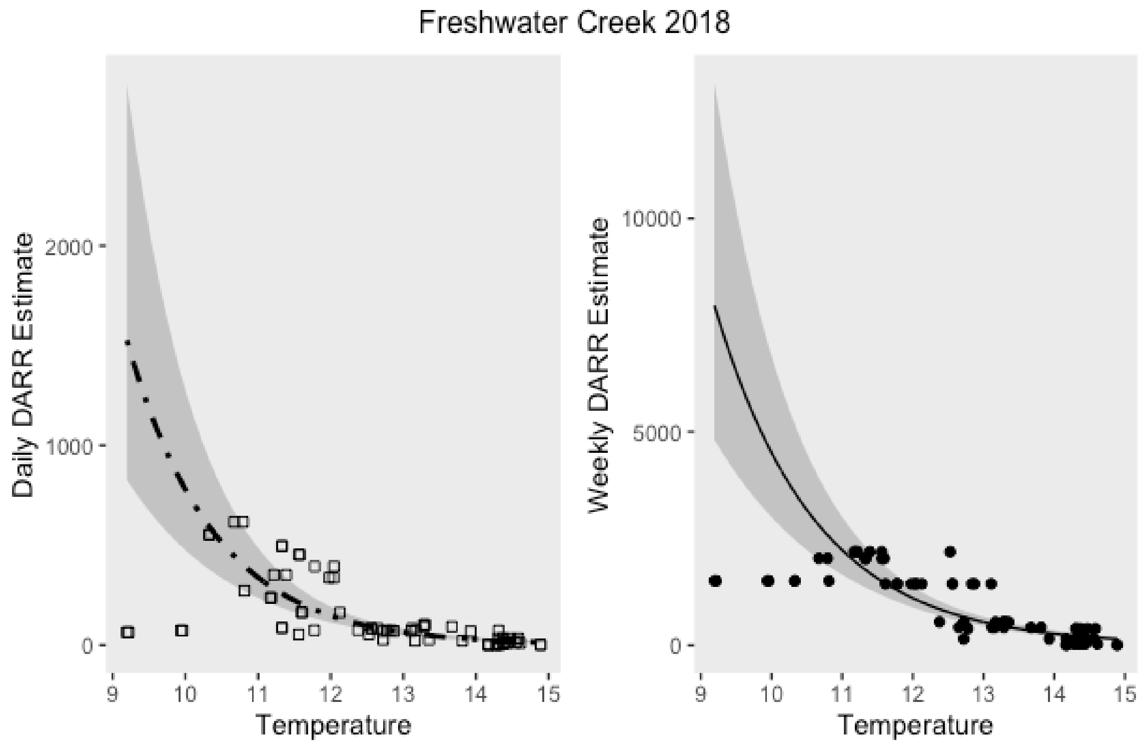
616

617 Table 5: Table displaying Daily DARR estimate model parameters and values for

618 Freshwater Creek in 2018.

Intercept	FlowDNA	Temperature	df	logLik	AICc	Delta	Weight
4.48		**-1.23(0.12)**	**3**	**-367.482**	**741.3**	**0**	**0.562**
4.47	**0.19(0.16)**	**-1.11(0.16)**	**4**	**-366.598**	**741.8**	**0.5**	**0.438**
4.73	**1.02(0.15)**		**3**	**-382.407**	**771.2**	**29.85**	**0**
4.96			**2**	**-392.199**	**788.6**	**47.24**	**0**

619



620

621 Figure 14: Response plot of Flow Corrected eDNA and Daily DARR estimates (left, open

622 boxes, dashed line) and Weekly DARR estimates (right, solid circles, solid line) and

623 Freshwater Creek in 2018.

624 Table 6: Table displaying Weekly DARR esimtate model parameters and values for

625 Freshwater Creek in 2019.

Intercept	FlowDNA	Temperature	df	logLik	AICc	Delta	Weight
6.78	**-0.27(0.13)**	**-0.34(0.13)**	**4**	**-621.604**	**1251.7**	**0**	**0.905**
6.80		**-0.20(0.12)**	**3**	**-625.751**	**1257.8**	**6.08**	**0.043**
6.81	**-0.14(0.12)**		**3**	**-626.144**	**1258.6**	**6.86**	**0.029**
6.82			**2**	**-627.502**	**1259.2**	**7.42**	**0.022**

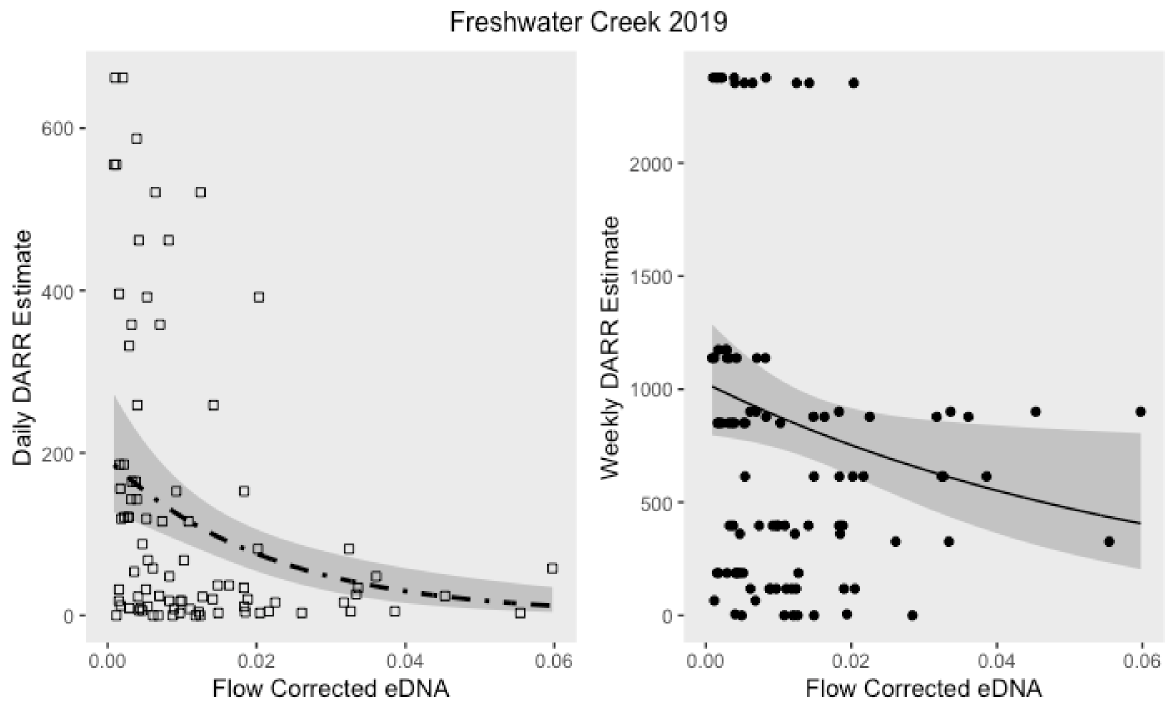
626

627 Table 7: Table displaying Daily DARR estimate model parameters and values for

628 Freshwater Creek in 2019.

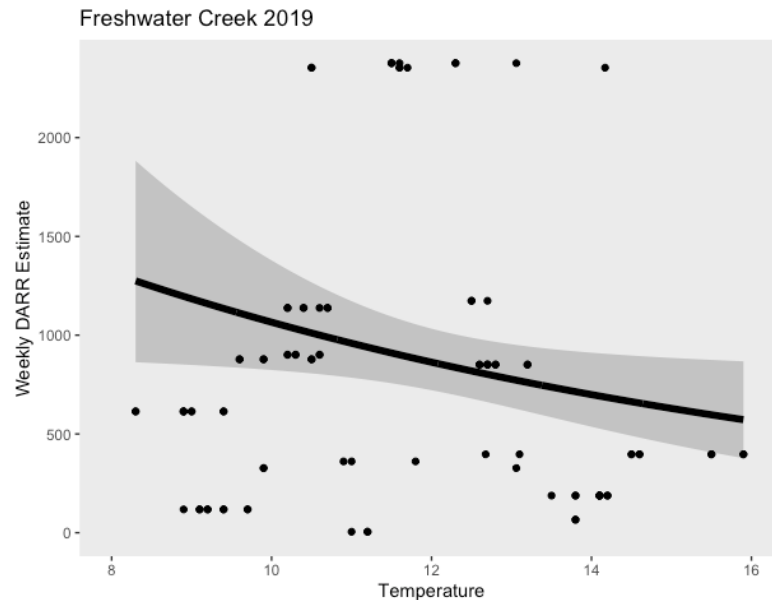
Intercept	FlowDNA	Temperature	df	logLik	AICc	Delta	Weight
4.76	**-0.66(0.15)**		**3**	**-461.245**	**928.8**	**0**	**0.73**
4.76	**-0.67(0.17)**	**-0.08(0.16)**	**4**	**-461.136**	**930.8**	**2**	**0.269**
4.93			**2**	**-469.435**	**943**	**14.22**	**0.001**
4.93		**0.10(0.16)**	**3**	**-469.295**	**944.9**	**16.1**	**0**

629



630

631 Figure 15: Response plot of Flow Corrected eDNA and Daily DARR estimates (left, open
632 boxes, dashed line) and Weekly DARR estimates (right, solid circles, solid line) on
633 Freshwater Creek in 2019.



634

635 Figure 16: Response plot of Temperature and Weekly DARR estimates on Freshwater

636 Creek in 2019.

637

638 Model Performance – Area Under Curve eDNA and Weekly trap estimates

639 Model results indicate a significant predictive relationship between Area

640 Under the Curve of Flow Corrected eDNA (AUC) and Weekly DARR estimates. In 2018

641 on Prairie Creek, the AUC variable was calculated using the average Flow Corrected

642 eDNA concentrations across all sampling sites. The linear regression resulted in a

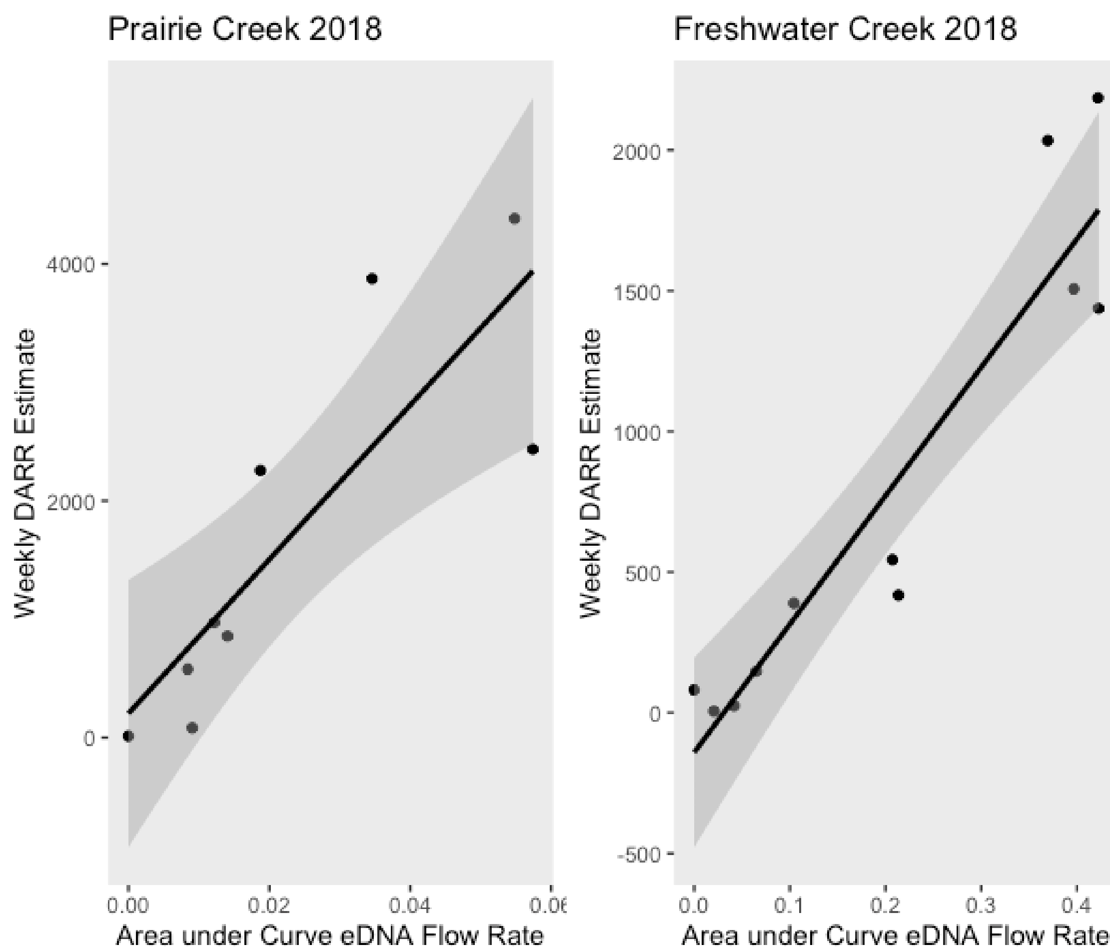
643 significant positive relationship that explained 71% of the variation between AUC and the

644 weekly DARR estimate on Prairie Creek (estimate= 4564, se= 568, p<0.0001) (Figure 16).

645 In 2018 on Freshwater Creek, the AUC calculations again resulted in a significant,

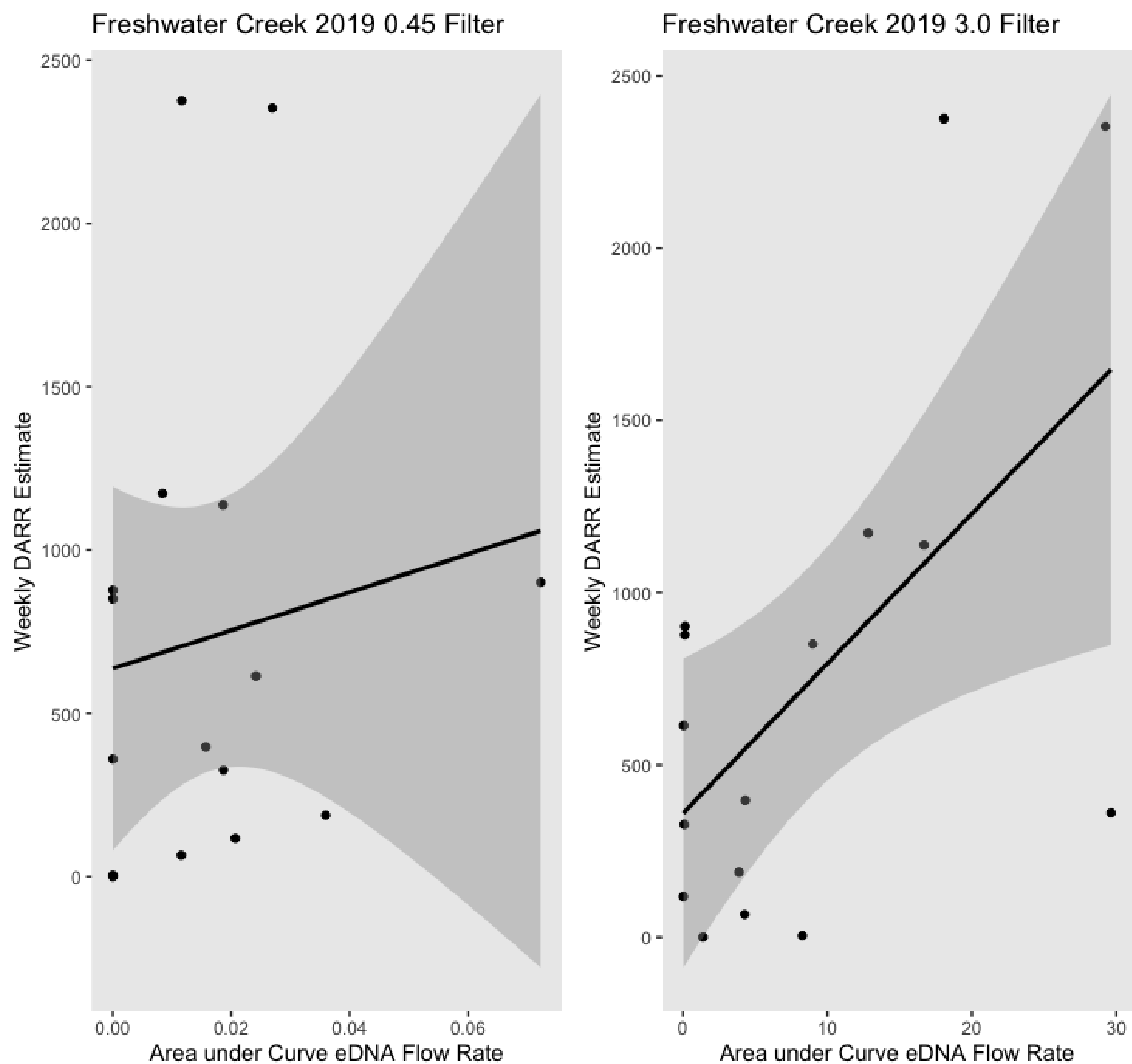
646 positive relationship of AUC and Weekly DARR estimates that explained 88% of the

647 variation in the data (estimate= 65111, se= 15686, $p<0.01$) (Figure 16). On Freshwater
648 Creek in 2019, a separate model was developed for each filter pore size. Freshwater Creek
649 in 2019 generated differences between filter types. The 3.0 micron filter model explained
650 34% of the variaton in the data (estimate= 43, se= 16, $p=0.0173$), while the 0.45 micron
651 filter model was not strong a strong predictor of Weekly DARR estimates of coho salmon
652 smolts (estimate= 5832, se= 10649, $p=0.59$) (Figure 17).



653

Figure 17: Left: Data and linear model of AUC Flow Corrected eDNA values and Weekly DARR estimates on Prairie Creek (r-squared=0.71). Right: Data and model of AUC Flow Corrected eDNA and Weekly DARR estimates on Freshwater Creek in 2018 (r-squared=0.88).



654

655 Figure 18: Left: Data and linear model of AUC Flow Corrected eDNA and Weekly DARR

656 estimates with a 0.45u filter (r-squared=0.02). Right: Data and linear model of AUC Flow

657 Corrected eDNA and Weekly DARR estimates with 0.45u filter (r-squared=0.34)

658 Freshwater 2019.

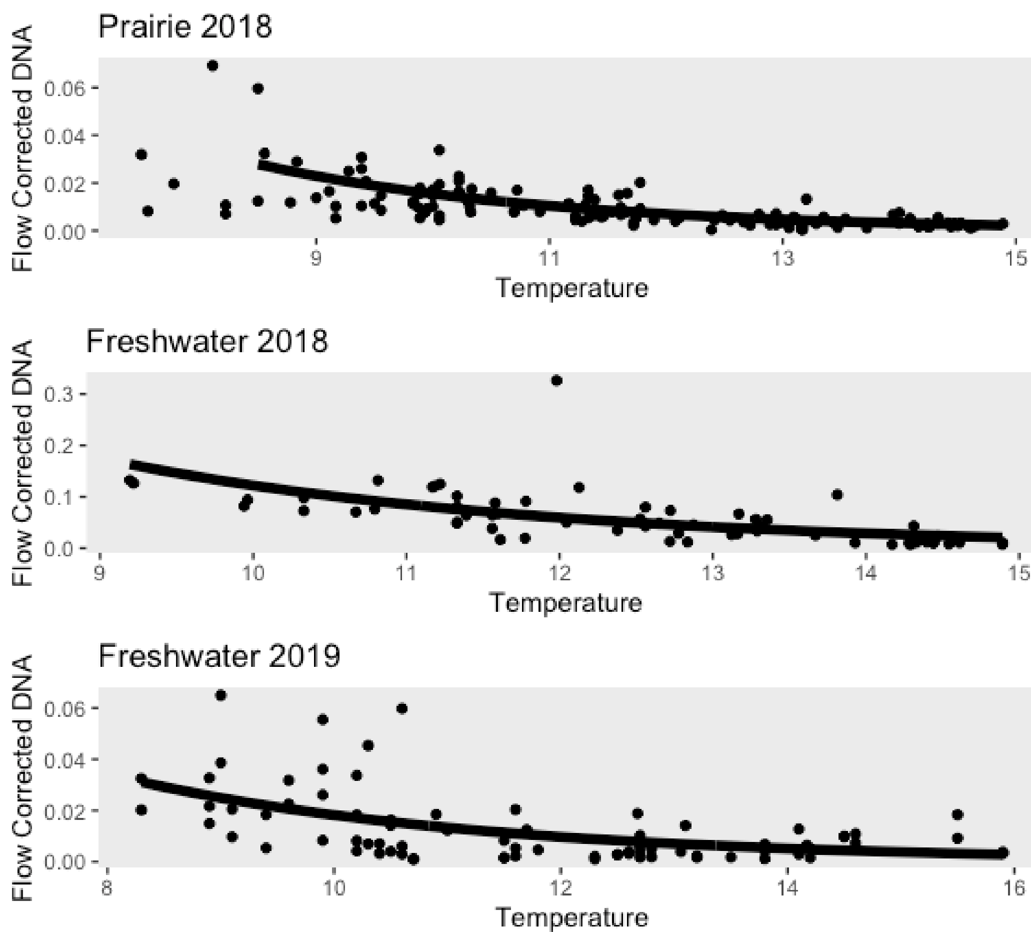
659 Effects of water quality of eDNA flow rate

660 Candidate models included dissolved oxygen content, water temperature, water
661 conductivity, and flow. However, correlation plots revealed high degrees of correlation
662 between environmental variables over the season. Therefore, for the purposes of this
663 analysis, only temperature was reported as the final result. Generalized linear model
664 structure was used from the Stats package in R (R Core Team, 2019). The equation for
665 the model is as follows:

666 $$FlowDNA = \beta_0 + \beta_1 Temperature_i + \epsilon$$

667 Where β_x is the model parameter estimate, Temperature is the YSI measured water
668 temperature, i is the sampling event, and ϵ is the residual error.

669 Temperature was found to have a statistically significant negative relationship with
670 the concentrations of Flow Corrected eDNA for all creeks in both years. This effect was
671 seen on Freshwater Creek in 2018 (estimate=-0.0029, r-squared=0.27, $p<0.001$), Prairie
672 Creek in 2018 (estimate=-0.699, r-squared=0.29, $p<0.001$), and Freshwater Creek in 2019
673 (estimate=-0.0023, r-squared=0.27, p=0.001075).



674
675 Figure 19: Response plot of Flow Corrected eDNA concentrations across increasing
676 temperature on Prairie Creek 2018 (Top), Freshwater Creek 2018 (Middle), and
677 Freshwater Creek 2019 (Bottom).

678 # DISCUSSION

679 The predictive relationship between Flow Corrected eDNA and coho salmon
680 downstream migrant abundance depended on the sampling and analytical methods I used
681 to quantify the abundance of eDNA. I did not observe a strong predictive relationship
682 when using Flow Corrected eDNA values alone to predict weekly or daily DARR
683 estimates of juvenile salmon abundance, as was done by Levi et al. 2018. For these
684 models, the most parsimonious model for both Prairie and Freshwater Creeks nearly
685 always included mean daily temperature, but not Flow Corrected eDNA. The relationship
686 with water temperature was negative, suggesting that the persistence of eDNA declined as
687 water temperature increased. However, after calculating Area Under the Curve values for
688 Flow Corrected eDNA and using a simple linear model to assess the relationship with
689 weekly DARR estimates of abundance, I found significant predictive relationships on
690 Prairie and Freshwater Creek in 2018, and for 3.0 micron filters on Freshwater Creek in
691 2019. These results imply that this approach shows promise for elucidating relationships
692 between eDNA and juvenile salmon abundances, but more research is necessary to
693 determine under what conditions it is appropriate to use these methods in lotic systems.

694 Area Under the Curve calculations are aggregate summaries of the contribution of
695 each weeks Flow Corrected eDNA measurements across the sampling seasons. For this
696 study, using AUC allowed the eDNA variation across sampling dates to be condensed into
697 weekly measurements. These weekly measurements can then be compared to the weekly
698 DARR abundance estimates. This process is in contrast to using the raw calculated

699 measurement of Flow Corrected eDNA for the trapping week. The differences in the
700 detectability and concentrations of eDNA and the relationship with DARR estimates
701 frequently used by resource managers across creeks and years need to be considered
702 further. On Freshwater Creek in 2019, different filter types yielded differences in the
703 strength of the relationship. One noticeable issue with the 0.45 micron filters is the fact
704 that it's much more difficult to filter large volumes of water. This study encountered
705 several issues with filters clogging, potentially reducing the quality of the sample. Other
706 eDNA studies have used a wide range of volumes of water in their research and this study
707 is inline with the notion that water quality must be considered when deciding standard
708 sample volumes. Additionally, the drop in the predictive nature of the relationship in 2019
709 may be due to a change in the sampling methodology. In 2019, the sampling scheme was
710 altered from using duplicate samples across multiple sampling locations to two samples,
711 each filtered through a different filter size, from a single site. This change in methodology
712 was made in response to the initial analysis that showed no statistical difference between
713 flow corrected eDNA concentrations from different sampling locations. However, using a
714 single sample and sampling location for Freshwater Creek in 2019 did not capture the
715 variation in eDNA concentrations within a sampling site and across the width of the creek.
716 This may be the reason for the decrease in predictive ability of the AUC model on
717 Freshwater Creek in 2019. If this project were to be undertaken again, I would ensure that
718 all samples are collected in duplicate and I would take samples from multiple locations
719 across the stream channel. In fact, when using only a single replicate from a single
720 sampling location on Freshwater Creek in 2018, the model r-squared was only 0.43, which

721 was comparable to linear regression for 2019. Similarly, the regressions fit for individual
722 sample sites in Prairie Creek in 2018 each had a lower r-squared value than that for the
723 model fit after data from all sites were combined. Another question that should be tested
724 based on the change in methodology between 2018 and 2019 is whether the filter material
725 (Polycarbonate vs. mixed cellulose) affects the results. The information gained from this
726 study is important to resource managers and this study attempts to provide more insight
727 regarding the dynamics of eDNA in lotic systems. This study adds to the growing
728 knowledge base of information eDNA and its utility in ecological research.

729 Previous environmental DNA studies have had conflicting results regarding the
730 ability to predict species abundances based on eDNA concentrations (Levi et al., 2018,
731 Rice et al., 2018, Doi et al., 2017, Lacoursière-Roussel et al., 2016). Studies that involved
732 the use of laboratory experiments or mesocosms (Doi et al., 2017, Lacoursière-Roussel et
733 al., 2016) were able to establish significant predictive relationships using eDNA
734 concentrations to quantify fish abundance. Mesocosms and laboratory study allow
735 researchers to determine abundance absolutely under standard environmental conditions.
736 Maintaining near-static conditions may allow the detection of eDNA concentration with a
737 high-level of confidence. Additionally, Levi et al. (2018) was able to predict relative run
738 size over the season based on near daily or daily eDNA concentration measurements. In
739 that study, researchers used a weir that was able to capture the entirety of the stream
740 channel, allowing them the ability to enumerate water flow and migrating salmon
741 abundances precisely. This was not the case with our study. Issues involving the ability of
742 the trap to enumerate fish restricted our ability to accurately determine the absolute

743 number of fish moving through the system. Spring rainstorms cause increased volumes of
744 water to move downstream, disrupting the ability of downstream migrant traps to capture
745 fish efficiently and in some cases, causing all trapping to cease until high flows recede.
746 Water flow data for Prairie Creek was available from a United States Geological Survey
747 (USGS) gage operated multiple kilometers upstream of our sampling site. This flow
748 measurement did not include tributaries that entered into mainstem Prairie Creek, above
749 our sampling site but below the gage. For flow measurements at Freshwater Creek, no
750 gage exists within the watershed. Therefore, it was necessary to use a relative flow value
751 adopted from a nearby creek where flow data is taken.

752 If this project were to be undertaken again, it may be advantageous to sample
753 within the trap box, immediately upstream of the trap, and at the sampling location used in
754 this site. This would provide a way to better understand the correlations between eDNA
755 and absolute number of fish in this particular case. Additionally, looking to other research
756 suggests alternative sampling regimes. Other studies that found positive correlations
757 between fish abundance and eDNA concentrations (e.g., Shelton at el. 2019, Levi 2018)
758 tested different water systems with a different species, and a different sampling design.
759 Shelton (2019) did their eDNA and fish sampling within an estuary and at several
760 different locations, providing several sampling sites within the populations range.
761 Sampling multiple sites in a downstream transect may provide information of the
762 movement of eDNA. In Levi et al. (2018), their predictive model worked well for a
763 salmon species with a concise life-history, but did not work as well when attempting to
764 model coho salmon abundance. Coho salmon are a species with multiple life stages in the

765 creek during any given time. In the spring when my experiment was run, smolts were
766 outmigrating, however, individuals born that year were also present in the creek.
767 Additionally, adult carcasses left over from the winter may still have been present in the
768 creek and releasing eDNA. High flow events can redistribute these individuals and change
769 their relation to the sampling site and may contribute to eDNA signal and detectability
770 over the season. Designing future studies that can offer answers to these complicated
771 factors may help to provide more utility in monitoring abundances using eDNA.

772 Using environmental DNA to correlate with fish abundance via a traditional
773 monitoring approach such as downstream migrant trapping presents a suite of challenges
774 that may make observing a predictive relationship difficult. One possible difficulty is
775 gaining reliable estimates of eDNA concentration using qPCR. While qPCR has been
776 found to be highly sensitive to detection of eDNA, there is evidence that this molecular
777 technique is not as reliable as other detection platforms (Nathan et al., 2014). Within
778 sample variability and across sample variability represents error in the molecular
779 technique, but may also be representative of the localization of the eDNA signal. Dejean
780 et al., 2011 found there was rapid deterioration in the detectability of an eDNA signal in
781 lotic systems when moving downstream from the source of genetic material. The
782 persistence of genetic material within the water column is not yet well understood and the
783 influencing factors on the environmental fate of DNA in field settings warrants further
784 investigation. Increased temperatures create a higher metabolic demand in fish and may
785 increase the output of genetic material through the release of mucus and other bodily
786 fluids. However, increased temperature due to UV radiation may be attenuating eDNA

787 concentrations at the same time, leading to lower concentrations of detectable genetic
788 material (Strickler et al., 2015). This falls in line with the results I found. The temperature
789 increase over the migration season may have had an effect on the ability to detect accurate
790 eDNA concentrations. The questions surrounding the dynamics of eDNA once released
791 from organisms into the environment warrant further investigation if this method is to be
792 used in lieu of, or in addition to, traditional monitoring approaches.

793 The traditional monitoring approach of trapping downstream migrants relies on the
794 ability to capture fish, throughout the migration season, under variable weather conditions
795 and flows. The population estimates for this technique are derived from mark recapture
796 programs necessary to estimate a weekly trap efficiency and weekly abundance estimate.
797 The counts taken each day at the trap are a function of the number of fish passing the
798 downstream migrant traps and the capture probability. Thus, because there is error
799 associated with fish capture probability, correlating eDNA concentrations with only a
800 subset of individuals found within the trap each day may not yield relationships. This may
801 be due to the ability of fish to move freely between the eDNA sampling location and the
802 trap. Fish may be moving throughout the study location and releasing eDNA but not being
803 captured in the trap. Other factors, including flow, compound this possibility. Changes in
804 flow throughout the migration season and in relation to sampling events may cause
805 dilution of eDNA concentrations and alter fish movement behavior.

806 The most comparable studies (Levi et al., 2018, Tillotson et al., 2018) both discuss
807 the need for well-characterized study sites when assessing salmon abundances from eDNA
808 concentrations. Our characterization of study sites was limited to pre-existing

809 infrastructure and data collection protocols that were ongoing. Expanding the scope of
810 data collected by monitoring programs interested in adopting eDNA sampling protocols
811 may be necessary to provide sufficient information to correlate with eDNA
812 concentrations.

813 Although infrastructure and data collection may contribute to the differences in the
814 results from this study to previous research, another factor present is the scale of the
815 watershed and scale of fish abundance. The number of fish in a river, the size of the
816 watershed, and therefore the concentration of eDNA, likely contribute to the ability to
817 accurately quantify the amount of eDNA in the water. Studies such as Levi et al. 2018 and
818 Pochardt et al. 2019 both used systems that contain hundreds of thousands or millions of
819 fish in contrast to the systems in this study that had numbers of fish at least an order of
820 magnitude lower. There may be a threshold of abundance that needs to be surpassed
821 before a linear relationship between Flow Corrected eDNA and abundance can be
822 observed. In systems where fish abundances are threatened or endangered, the
823 environmental DNA may be too dilute, or too rare, to show variations great enough for a
824 significant relationship with abundance measurements. In these situations, relating species
825 abundances to eDNA concentrations may require alternative analytical approaches, such
826 as the AUC analysis presented in this thesis. Continuing to conduct studies that look at
827 different size watersheds and different concentrations of fish will help to solidify these
828 questions and help managers know what survey methods and analytical approaches using
829 eDNA monitoring are the most appropriate.

830 The use of environmental DNA in fisheries has been rapidly expanding over the
831 past decades. Each year, new research papers reveal insights into how this molecular
832 technique can provide new and interesting information on the distribution, occupancy, and
833 abundance of species of concern. While environmental DNA can provide information not
834 easily gained with traditional monitoring approaches, it is necessary to understand its
835 limitations in the field. This study used environmental DNA concentrations taken over 2
836 seasons of outmigrating Coho Salmon smolts in conjunction with two downstream
837 migrant traps to attempt to build a relationship relating eDNA concentrations to predict
838 abundance. Results from this study were varied and highlight the need for continued
839 research into the field. The dynamics of eDNA within the water column are not well
840 understood and our evidence, when related to what other researchers have found, reveals
841 the need for high-quality site characterization when attempting to determine abundance
842 via highly transient eDNA concentrations moving downstream.

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