Using environmental DNA to compare aquatic invertebrate prey community among creeks of Bellingham, WA

**Abstract**

Environmental DNA (eDNA) has become an increasingly popular sampling tool to monitor target taxa and community composition, though its applications in population dynamics research are only beginning to be explored. The following project will examine whether eDNA is an appropriate sampling tool to detect predator-prey dynamics between coho salmon and macroinvertebrate prey taxa. Barriers to fish passage in Padden Creek, Washington State provide a unique opportunity to monitor seasonal variation of the same aquatic insect community in the presence and absence of migratory juvenile salmon. This project will provide valuable insight on the seasonal fluctuations of taxa essential to freshwater ecosystems and habitat connectivity, and the viability of eDNA methods in studies of population dynamics.

Introduction

Sampling techniques involving environmental DNA (eDNA) were initially popularized by their ability to detect cryptic (Jerde et al. 2011), invasive (Ficetola et al. 2008) or endangered species (Olson et al. 2012) that traditional survey methods struggle to identify. In recent years, eDNA’s applications have grown to include the identification of temporal trends in the abundance of target taxa (Duda et al. 2021) or community composition (Bista et al. 2017), rather than presence/absence alone. Among other factors, differences in laboratory protocols (Kelly et al. 2019), spatial scale (Shelton et al. 2019), and the target taxon’s DNA shedding rates (Levi et al. 2019) introduce variability among eDNA samples, limiting eDNA’s ability to measure absolute biomass and abundance. Nonetheless, eDNA remains robust to directional changes, endorsing its value in tracking expected and unexpected fluctuations of a target population: Suchan et al. (2018) and Chang et al. (2018) used DNA from pollen samples to map the routes of migratory insects; Burgoa Cardás et al. (2020) developed eDNA protocols meant to track temporal variation of threatened populations of European eel (*Anguilla anguilla*); and Maruyama et al. (2018) used eDNA from water samples to document the reproductive migration of cyprinid *Opsariichthys uncirostris uncirostris.*

The following project will use eDNA to analyze the population dynamics of coho salmon (*Oncorhynchus kisutch*) and the insect species they prey upon. Coho salmon are an indispensable component of the Pacific Northwest’s cultural and ecological landscape. They are an integral part of Indigenous fishing sovereignty, as members of the Coast Salish tribes have managed salmon for thousands of years (Caldwell et al. 2012). Coho salmon also establish connectivity between marine, freshwater, and terrestrial ecosystems, supporting complex food webs (Schindler et al. 2003). Juvenile salmon feed primarily on invertebrate prey at the water’s surface (Buehrens et al., 2014), whose composition can be used as an indicator of stream health (Masese and Muchiri 2009). Thus, monitoring approaches that target both coho and their prey have high value in the management of freshwater ecosystems. The following project will ask: can a single eDNA sampling framework accomplish this goal?

To improve the health of salmon populations in the Pacific Northwest, the 9th U.S. Circuit Court of Appeals (and later, the U.S. Supreme Court) confirmed that Washington state was financially responsible for removing hundreds of culverts that would otherwise block migratory fishes, including coho salmon (Pease and White 2019). To document community and fish passage changes associated with a recent culvert removal project in Padden Creek, WA, researchers in the Kelly Lab of the University of Washington have collected water samples containing eDNA from Padden Creek and additional creeks (restored or unrestored) in the surrounding watershed.

Sampling conducted above and below a barrier to fish passage in Padden Creek presents a unique opportunity to capture aquatic insect abundance in the presence and absence of coho salmon. Juvenile coho salmon migrate downstream during late spring (April—June) while consuming aquatic and terrestrial vertebrates; spawning adults do not eat during their run upstream (Logerwell et al. 2003). This project will use eDNA collected from water samples upstream of the culvert at Padden Creek to assess how the abundances of common aquatic insect prey types of coho salmon are affected by different densities of juvenile salmon, and subsequent predation, throughout the year. Seasonal stochasticity in prey abundance, in the absence of coho salmon, will be documented downstream of the culvert within the same time frame.

Hypotheses

*Alternative*: Abundance indices derived from eDNA samples will reveal a predator-prey relationship between coho salmon and select insect prey species, where the abundance of one group is inversely proportional the other. Fluctuations in the abundance of insects exposed to coho salmon are unexplained by stochasticity in insect abundance where salmon are absent.

*Null*: Abundance indices derived from eDNA samples do not show an inversely proportion relationship between coho salmon and select insect species that departs from fluctuations in insect abundance where salmon are absent.

Methods

Sample Collection, Annotation & Target Species

Beginning in March 2020, water samples were collected monthly from Padden, where each sample corresponds to a stream reach (upstream, downstream), stream site, and date. To assign taxonomic designations for the DNA in a given sample, extracted DNA was amplified with PCR using COI Leray and MiFish genetic markers (targeted at eukaryotes and fishes, respectively). After the resulting amplicons were sequenced, a reference database was used to assign each sequence to the highest taxonomic resolution possible. For each unique sample, the final product is a list of species and associated number of amplicons, which were analyzed in the context of that sample’s “read depth” (total number of amplicons). ­Target species of my project include coho salmon and ten aquatic insect prey types in the Padden Creek watershed, including: *Ezza just sent me the asv and annotation files, so I still need to comb through those to decide on taxa.*

Data analysis

For all analysis, I will use read proportions—calculated as the number of reads per target species within a sample divided by the total number of reads within that sample—rather than raw reads to standardize samples with differing read depths. I will calculate this index for target species using data from Padden Creek water samples from March 2020 through December 2020. Because adult Coho salmon return in fall and winter to spawn without preying upon invertebrates, I will ignore Coho salmon detections past July 2020. Coho salmon and select aquatic invertebrate species will be tracked separately in the upstream and downstream portions of Padden Creek, with the downstream area serving to mirror conditions for select insect species in the absence of Coho salmon (environmental stochasticity, other sources of predation, etc.). The use of data from the downstream reach as a control relies on the assumption that fish predator assemblages (excluding coho salmon) are similar upstream and downstream.

For each prey type, I will calculate a correlation coefficient using eDNA proportion values for coho salmon and that prey type in the upstream reach across the time series to test for a negative relationship. To test whether this relationship is significantly different from baseline fluctuations of that given prey type, I will use a generalized linear mixed model to explore trends in prey abundance (for each species, using eDNA proportions as a proxy) between the upstream and downstream reaches, and compare prey trajectories from both reaches. Month will be discretized into a factor to indicate the period where juvenile coho salmon are expected upstream (March–June) or not (July–December) (hereafter indicated as “phase”).

I will test three models to explain variation in read proportion for a given prey type *p,* drawn from a beta distribution: (1) a null model testing for a step change of *p* between phases across reaches; (2) a model testing for step changes of *p* between phases that are unique to each reach; and (3) a model testing for different reach-specific trends before and after the phase change. Additionally, I will include a random effect for month to address temporal autocorrelation.

1. *p* ~ Phase
2. *p* ~ Reach + Phase\*Reach
3. *p* ~ Reach + Phase\*Month\*Reach

I will compare data support for models based on AICc to account for small sample size (Akaike 1973; Brewer et al. 2016). Support for the null model will indicate that upstream trends in my index of insect abundance—profiling insect species exposed to juvenile salmon—do not differ significantly from natural fluctuations of insect abundance downstream. Support for my most complex model will indicate that trends of insect abundance (as measured by my index) differ before and after the juvenile salmon out-migration, and those associated with the upstream reach differ from the downstream reach, my ecological baseline. Data manipulation will be carried out using R statistical software (packages *glmmTMB* and *DHARMa* used for model analyses).

Products

1. Project proposal with short presentation on study aims and methodology.
2. Publication quality manuscript.
3. PowerPoint slideshow and oral presentation of completed project.

Timeline

* October – December 2021: Perform initial background literature review
* December 2021: Submit final proposal, proposal presentation; submit FISH 494 contract
* November – April 2022: Data analysis (periodic check-ins with Dr. Kelly)
* April 2022: Complete rough draft and submit to advisor/SAFS faculty for revision
* May 2022: Prepare final paper, poster, and presentation; evaluation meeting
* June 2015: Present findings at Capstone Symposium and submit final paper

Signatures

We have read and discussed the above proposal and we believe this is an achievable project for the student named.

Student: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_



Faculty Sponsor: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_

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