Environmental DNA monitoring for Chinook Salmon, Coho, Rainbow trout and Cutthroat trout on Vashon Island

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Background:

Environmental DNA (eDNA), the DNA that organisms release into the environment, has become an important tool in species monitoring, as it is highly sensitive in comparison to traditional detection methods (Bohman et al. 2014; Goldberg et al. 2013). eDNA methods have been widely applied to monitor occurrence of a species in aquatic systems (Goldberg et al. 2016), mainly focused on detecting fish or amphibian species in freshwater systems (DeJean et al. 2012; Thomsen et al. 2012; Pilliod et al. 2013). Environmental DNA has also been useful in aiding traditional monitoring methods (Baldigo et al. 2017).

In 2001, Washington Trout sampled 73 streams on Vashon Island and found the presence of trout in 16 and coho in 2 streams by electrofishing. Vashon Nature Center has been running a fall Salmonwatching program to count, identify and fin clip adult spawning salmon returning to island creeks since 2015. We have also conducted surveys for juvenile salmon in spring since 2017. These programs have identified coho in 4 creeks, chum in 3 creeks, and cutthroat trout in all creeks surveyed (10). Historically, steelhead and chinook have been recorded using some island streams (but none have been detected in Vashon Nature Center programs except for one possible chinook juvenile in Shinglemill in 2017 (some expert disagreement on ID). eDNA techniques could improve on VNC's initial observational data by employing a non-invasive way of surveying for fish presence in our creeks.

Objectives

We will extract, qPCR and analyze six filters. Samples will be tested for presence/absence of Coho Salmon, Cutthroat Trout, Steelhead and Chinook Salmon.

eDNA Field Collection Methods:

A total of 6 filters were collected at 2 sites on Vashon Island. One sample site was located just downstream of Cedarhurst culvert and the other sample site was located at the old bridge site upstream of Cedarhurst crossing called the Upper bridge site. Each sampling was conducted on a 150 m transect and samples were taken at 0, 50, 150 m. GPS coordinates of the starting points of the sample transects: Cedarhurst – 47.479078, -122.482106 and Upper Bridge-47.473659, -122.480697.

Water sampling was conducted with a peristaltic pump or hand pump. Before sampling began at a site, all equipment (pump, flask, drill, water bottles, etc.) was wiped down with a germicidal bleach wipe (Kemp & Smith 2005; Champlot et al. 2010), to prevent contamination. At each site, we filtered up to one liter of stream water through a 0.45 µM pore size filter, in triplicate, at 0, 50 and 150 meters upstream. Filters were preserved in 15 ml of color indicator silica gel, at room temperature.

eDNA Laboratory Methods:

Experimental Design:

In eDNA analysis of aquatic organisms, water is typically collected and filtered in the field. DNA was extracted from filters, and amplified using species-specific primers to determine if the species of interest is present or absent. We used Quantitative Polymerase Chain Reaction (qPCR) to detect minute levels of DNA, using species-specific primers, and a fluorescently labeled reporter molecule (probe), which yields increased fluorescence with an increasing amount of product DNA (Figure 1). A sample was determined "positive" or "negative," based on whether or not the sample crossed a threshold referred to as the C_t, "Cycling Threshold."

Samples with higher concentration of DNA typically cross the threshold in fewer cycles (~cycle 20-30) than samples with lower concentration (~ cycle 31-40) (Figure 1).

Laboratory Methods:

All laboratory work was performed in AirClean 600 Work Stations (ISC Bioexpress, Utah, USA), which are equipped with HEPA air filters and UV lights. All work surfaces were decontaminated with 50% bleach, and exposed to UV light for at least one hour before work began. Each filter replicate at a site was extracted for DNA. DNA extraction was performed on half of the filter sample, using the Qiagen DNeasy Blood & Tissue and Qiashredder kits (Qiagen, Inc.), as per Pilliod et al. (2013). The other half of the filter was stored for potential future use.

We tested samples for the presence Coho Salmon, Cutthroat Trout, Steelhead and Chinook Salmon using mitochondrial COI species specific qPCR primers (Ostberg unpublished). Post extraction, each filter sample was processed in triplicate. We included an internal positive control (IPC) to detect potential PCR inhibition from organic compounds in the water. qPCR products were obtained by amplifying DNA in 15µl reaction volumes, containing 5 µl of TaqMan gene expression master mix, 0.5 µl primer and probe mix, 2.28 µl of molecular grade water, 1 µl EXO-IPC (Internal Positive Control) Master Mix, 0.22 µl EXO-IPC DNA, and 3 µl of template DNA. Cycling conditions consisted of 2 minutes at 50ºC, then 95 ºC for 10 minutes, followed by 45 cycles of 95 ºC for 15 seconds, and 60 ºC for 1 minute.

For quantification of the salmonid and trout qPCR products, synthetic Gblock gene fragment (Integrated DNA Technologies) of the COI amplicon were synthesized. To assess the amplification success of each qPCR, we developed a standard curve from 1:10 serial dilutions of these synthetic fragments 10^7 to 10^0. The Limit of Quantification (LOQ, the lowest concentration at which at least 90% of the replicates amplified), and the Limit of Detection (LOD, the lowest concentration that was 10-fold below the LOQ) were determined for each assay by running the standard curve dilution with 24 replicates.

Filter samples were considered positive for eDNA detection if >2 qPCRs out of 3 resulted in a positive amplification (e.g. C_t of 45 or below), as per Turner et al. (2014). If qPCR samples were positive for only one, the samples were re-amplified in triplicate. We used the IPC to determine if inhibitors were affecting the PCR. The mean cycle number (Ct) of the IPC for each sample (over three replicates) was compared with the mean Ct of the no-template control. If the mean Ct of the IPC of the sample was more than three cycles later, the sample was considered to be inhibited.

Results/Discussion:

The LOQ of the Coho Salmon, Steelhead and Chinook Salmon assays were 5.51, 302.5, and 14,893 copies/µl, indicating the Chinook Salmon assay was not sensitive enough to detect very low copy numbers of eDNA. We will attempt to fine-tune the assays for a higher sensitivity. Additionally, the Cutthroat standards did not amplify, however this did not affect the efficacy of the samples. The internal positive control (IPC) amplified in every sample and none of the sample IPC C_t values were > 3 cycles later than the no-template control, indicating that inhibition was not present.

A total of 6 filters from 2sites were processed. None of the associated laboratory negative controls amplified, indicating no detectable contamination from the field and laboratory. Coho and Cutthroat trout were detected in every filter at both sampling sites (Table 1). All samples had at least one more positive qPCR replicate, and were determined to be “Detected.” None of the samples amplified for Chinook salmon or Rainbow trout. These results are concordant with visual observations mentioned in the background information.

Literature Cited:

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Table 1. Environmental DNA detection results, per each site. If a site had less than 2 qPCR reactions amplify (out of 3 total), the sample was re-amplified.

Sample	Chinook Salmon Detected?	# Positive qPCRs	Coho Detected?	# Positive qPCRs	Rainbow trout Detected?	# Positive qPCRs	Cutthroat trout Detected?	# Positive qPCRs
C1	Not Detected	0/3	Detected	3/3	Not Detected	0/3	Detected	3/3
C2	Not Detected	0/3	Detected	3/3	Not Detected	0/3	Detected	3/3
C3	Not Detected	0/3	Detected	3/3	Not Detected	0/3	Detected	3/3
UB1	Not Detected	0/3	Detected	3/3	Not Detected	0/3	Detected	3/3
UB2	Not Detected	0/3	Detected	3/3	Not Detected	0/3	Detected	3/3
UB3	Not Detected	0/3	Detected	3/3	Not Detected	0/3	Detected	3/3
Blank Control	Not Detected	0/3	Detected	3/3	Not Detected	0/3	Detected	3/3

Figure 1. Diagram of qPCR real-time output. A sample replicate is deemed Detection, if the sample (blue line) crosses the threshold (dashed line), before the termination of thermal cycling. The point at which the sample crosses the threshold is referred to as C_t.