A SURVEY OF LAKE CRESCENT FOR ENDEMIC SALMONID SPAWNING SITES USING eDNA

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INTRODUCTION

Olympic National Park, located on the Olympic Peninsula of Washington, encompasses an area of 3750km^2. It includes twelve watersheds, >6000km of streams, 646 high mountain lakes and two large lakes - Lake Ozette and Lake Crescent [1]. Lake Crescent is a glacially carved lake isolated ≈7,000 years ago by a massive landslide which separated it from nearby Lake Sutherland.t

Home to 31 freshwater and anadromous fish species, the park supports 70 unique populations of Pacific salmonids. Salmonids in Lake Crescent became landlocked by the Lyre River Falls, resulting in genetic isolation and the development of two uniquely adapted endemic salmonid populations, the Beardslee trout and the Crescenti cutthroat trout. The Beardslee trout is currently known to spawn in only one location; a 122m stretch above the Lyre River bridge at the outlet of Lake Crescent, from January to May. Numbers of spawning fish typically vary from 30 to 100; however, some years have seen no spawning fish [2][3]. Cutthroats spawn from the Lyre River bridge west to Boundary Creek, September through March. The Barnes Creek cutthroats are similar in size to the Lyre River cutthroats and spawn in Barnes Creek from January to June.

Environmental DNA or 'eDNA' is a complex mixture of genomic DNA from many different organisms found in environmental samples (soil, sediments, water). To detect the presence or absence of a single species from an eDNA sample, species-specific DNA amplification is carried out through quantitative PCR. The goals of this project were to identify salmonid spawning sites using eDNA and to determine the efficacy of this technique as a monitoring tool for endemic salmonids in Lake Crescent.

RESEARCH QUESTION & HYPOTHESIS

The research question for this project was twofold:

1. Can eDNA determine the presence of endemic salmonids, Beardslee trout and Crescenti cutthroat trout, at known spawning sites in Lake Crescent?

2. Is eDNA an effective tool for monitoring Lake Crescent to determine if potential spawning sites exist which have not yet been documented?

I hypothesized that use of eDNA as a tool for identification of spawning sites of Beardslee trout and/or Crescenti cutthroat trout populations in Lake Crescent will show both the locations of previously known sites and possibly a new range of sites around the Lyre River area and southern shoreline of the lake. In addition, eDNA may be an efficient tool for monitoring spawning populations annually as the method allows the presence of a species to be confirmed through a water sample rather than extensive field work.

PROCEDURES

A. eDNA Sampling Protocol

Water Collection Sites

• Collection sites were determined based on known sites and possible sites base on water flow, a schedule was prepared for water sampling based upon salmonid spawning times [1]:

eDNA Sampling

• Water samples were filtered using a 0. 2µm pore size filter and then stored in 2mL cryovial, filled with 100% ethanol and stored at room temperature.

B. Salmonid eDNA Extraction

Salmonid DNA extraction was accomplished through a modified protocol using cell homogenizers (QIAshredders) and a commercial DNA extraction kit [4].

C. Salmonid qPCR Protocol

qPCR is real-time recording of accumulating DNA sequences during the amplification process. This is achieved through continuous measurement of a signal emitted during incorporation of a fluorescent probe in the amplified DNA. This process utilized the TaqMan probe system and protocol from a commercial Qiagen kit[9]. Primer and probe sequences (5'-3') for salmonid assays from mitochondrial gene CO1.

RESULTS

Lyre River Crescenti Cutthroat Trout eDNA

Well	Position	Sample	CT	CT Mean
4	A4	Lyre River 1 [01/05]	37.984	38.653
5	A5	Lyre River 1 [01/05]	Undetermined	38.653
6	A6	Lyre River 1 [01/05]	39.323	38.653
16	B4	Lyre River 2 [01/05]	37.928	38.277
17	B5	Lyre River 2 [01/05]	Undetermined	38.277
18	B6	Lyre River 2 [01/05]	38.626	38.277
40	D4	Unnamed #2 [01/05]	Undetermined	38.666
41	D5	Unnamed #2 [01/05]	38.614	38.666
42	D6	Unnamed #2 [01/05]	38.718	38.666
7	A7	Log Cabin Cr [02/16]	38.164	38.005
8	A8	Log Cabin Cr [02/16]	37.845	38.005
9	A9	Log Cabin Cr [02/16]	Undetermined	38.005
55	C7	Log Cabin Cr [04/14]	34.617	34.374
57	C9	Log Cabin Cr [04/14]	34.383	34.374
59	C11	Log Cabin Cr [04/14]	34.021	34.374
76	G4	Lyre R Bridge 1 [02/16]	40.173	39.562
77	G5	Lyre R Bridge 1 [02/16]	38.908	39.562
78	G6	Lyre R Bridge 1 [02/16]	39.606	39.562
88	H4	Lyre R Bridge 2 [02/16]	36.266	37.046
89	H5	Lyre R Bridge 2 [02/16]	36.441	37.046
90	H6	Lyre R Bridge 2 [02/16]	38.431	37.046
7	A7	Lyre R Bridge [04/14]	38.789	36.416
9	A9	Lyre R Bridge [04/14]	35.867	36.416
11	A11	Lyre R Bridge [04/14]	36.593	36.416

Barnes Creek Crescenti Cutthroat Trout eDNA

Well	Position	Sample	CT	CT Mean
19	B7	Barnes Creek [02/16]	Undetermined	37.571
20	B8	Barnes Creek [02/16]	37.629	37.571
21	B9	Barnes Creek [02/16]	37.512	37.571
151	G7	LaPoel Cr [04/14]	40.121	39.126
153	G9	LaPoel Cr [04/14]	Undetermined	39.126
155	G11	LaPoel Cr [04/14]	38.130	39.126
79	G7	Fairholme [02/16]	36.662	37.527
80	G8	Fairholme [02/16]	36.985	37.527
81	G9	Fairholme [02/16]	38.933	37.527

199	I7	Fairholme [04/14]	35.071	34.660
201	I9	Fairholme [04/14]	34.587	34.660
203	I11	Fairholme [04/14]	34.322	34.660

Beardslee Trout eDNA

Well	Position	Sample	CT	CT Mean
31	B7	Lyre R Bridge [04/14]	35.611	35.916
33	B9	Lyre R Bridge [04/14]	37.116	35.916
35	B11	Lyre R Bridge [04/14]	35.019	35.916
247	K7	Log Cabin Cr [01/05]	37.723	37.443
249	K9	Log Cabin Cr [01/05]	Undetermined	37.443
251	K11	Log Cabin Cr [01/05]	37.164	37.443
13	A13	Log Cabin Cr [02/16]	43.291	42.711
15	A15	Log Cabin Cr [02/16]	42.131	42.711
17	A17	Log Cabin Cr [02/16]	Undetermined	42.711
79	D7	Log Cabin Cr [04/14]	36.967	38.582
81	D9	Log Cabin Cr [04/14]	40.197	38.582
83	D11	Log Cabin Cr [04/14]	Undetermined	38.582

CONCLUSIONS

The purpose of this study was to not only identify salmonid spawning sites in Lake Crescent, Olympic National Park, but to also determine the efficacy of eDNA as a tool for future monitoring of the endemic salmonid species of Lake Crescent, the Beardslee trout and Crescenti Cutthroat trout.

The Lyre River population of Crescenti Cutthroat trout was found to spawn in the headwaters of the Lyre River, in one creek feeding into the Lyre from the south, and in one creek 1 km east of the Lyre River Bridge.

The Barnes Creek population of Crescenti Cutthroat trout was found to spawn at Barnes Creek, at Fairholme (the most southwestern point of the lake and a previously undocumented location) and at LaPoel, where only offshore spawning was thought to occur.

Beardslee Trout were found to spawn in a creek 1km east of the Lyre River, a previously unknown location.

eDNA sampling has not been conducted at Lake Crescent to date. Surveys of spawning sites are currently conducted weekly from January-May by counting redds in stream and riverbeds. Although this method allows researchers to develop trends and abundance of spawning fish, it is lengthy and time consuming. eDNA provides the results needed to pinpoint the presence of species in a creek, river, or area of the lake without extensive on-site field work. eDNA is eminently suitable for monitoring salmonid spawning sites in Lake Crescent. Identification of species is simplified by the presence of only one rainbow and one cutthroat species within the lake, avoiding the need for more extensive preparation of additional assays in order to differentiate the fish. In conclusion, eDNA is effective in determining the presence of endemic salmonids in Lake Crescent while being a valuable tool for future data collection to monitor salmonid spawning sites in support of research and conservation efforts.

REFERENCES

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