

Copper Fox Metals Inc.

Schaft Creek Project: Fisheries Baseline 2008 – Addendum



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SCHAFT CREEK PROJECT: Fisheries Baseline 2008 – Addendum

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Prepared for:



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1. Introduction

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The Schaft Creek Project Fisheries Baseline 2008 was produced in February 2010 and contained the results from genetic analyses of unidentified salmonids captured in Mess Lake. The unidentified salmonids were positively identified as *Oncorhynchus nerka*, or Kokanee salmon, a freshwater form of sockeye salmon. While the results from these analyses were reported in the baseline report, the supporting lab results were not included in the appendices. This addendum presents a review of the methods used to genetically identify the specimens from Mess Lake, as well as the original lab results. The lab results are presented in the attached appendix.

2. Methods

2. Methods

Genetic samples were sent to Dr. Eric Taylor at the University of British Columbia Department of Zoology for identification. DNA was extracted from ethanol-stored fin tissues using standard spin column protocols (Qiagen Ltd). The DNA of each fish was subject to polymerase chain reaction (PCR) amplification and sequencing of the cytochrome oxidase I (CO1) gene of the mitochondrial DNA genome. This gene is the standard used for fishes for molecular identification using "DNA bar-coding". Resultant sequences (about 853 base pairs) were submitted to the Barcode of Life Database (BOLD) (Hubert *et al.* 2008, Ratnasingham and Hebert 2007) to identify the DNA samples to species.

References

References

Hubert, N., R. Hanner, E. Holm, N.E. Madrak, E.B. Taylor, M. Burrige, D. Watkinson, P. Dumont, A. Curry, P. Bentzen, J. Zhang, J. April and L. Bernatchez. 2008. Identifying Canadian Freshwater Fishes through DNA Barcodes. PLoS ONE, 3: e2490.

Ratnasingham, S. and P.D.N. Hebert. 2007. Barcode of Life Data Systems v2.5. Biodiversity Institute of Ontario. Accessed September 2008 at <http://www.boldsystems.org/views/login.php>.

Appendix 1

Genetic Results for Fish Captured in Mess Lake

DNA Species Identification Report

Eric Taylor

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October 2, 2008

Locality: Mess Lake (tributary of Stikine River, in Mt. Edziza Park)

Number of samples: 24

Date: Assayed September, 2008

Methods: DNA was extracted from ethanol-stored fin tissues using standard “spin-column” protocols (Qiagen Ltd). Each fish’s DNA was subject to polymerase chain reaction (PCR) amplification and sequencing of the cytochrome oxidase I (CO1) gene of the mitochondrial DNA genome. This gene is the standard used for fishes for molecular identification using “DNA barcoding”. Resultant sequences (about 853 base pairs) were submitted to the Barcode of Life Database (BOLD, see <http://www.boldsystems.org/views/login.php>) to identify the DNA samples to species.

Reference: Hubert N, Hanner R, Holm E, Mandrak NE, Taylor E, et al. 2008. Identifying Canadian Freshwater Fishes through DNA Barcodes. *PLoS ONE* 3(6): e2490. (plus appendices).

General results (see attached spreadsheet for details).

All fish except five were successfully assayed and all of these fish were positively identified (> 99% sequence similarity) as *Oncorhynchus nerka*. An example of specimen similarity to *O. nerka* can be seen in the attached phylogenetic tree (“final-tree 4-4) based on the sequence submitted (specimen July 4, No. 4)) to the BOLD database.

The five specimens (5-12, 5-14, 5-16, 5-18, 5-22) that could not be assayed (despite several attempts) appeared to have had their tissues stored in clove oil rather than ethanol. Upon opening the tubes containing these tissue samples, there was an unmistakable and strong odour of cloves. The storage medium was also a golden-yellow colour.

Location	Date	Length	Weight	Sample #	Sample #	Comments	Species
Mess Lake	4-Jul-08	72	n/a	1	2	MT101	<i>O. nerka</i>
Mess Lake	4-Jul-08	116	n/a	3	4		<i>O. nerka</i>
Mess Lake	4-Jul-08	103	n/a	5	6		<i>O. nerka</i>
Mess Lake	4-Jul-08	102	n/a	10	11		<i>O. nerka</i>
Mess Lake	4-Jul-08	117	n/a	12	13		<i>O. nerka</i>
Mess Lake	4-Jul-08	105	n/a	14	15		<i>O. nerka</i>
Mess Lake	4-Jul-08	125	n/a	18	17		<i>O. nerka</i>
Mess Lake	4-Jul-08	116	n/a	19	20		<i>O. nerka</i>
Mess Lake	5-Jul-08	105	13	2	3		<i>O. nerka</i>
Mess Lake	5-Jul-08	124	26	5	4		<i>O. nerka</i>
Mess Lake	5-Jul-08	102	16	7	6		<i>O. nerka</i>
Mess Lake	5-Jul-08	125	21	9	8		<i>O. nerka</i>
Mess Lake	5-Jul-08	104	15	11	10		<i>O. nerka</i>
Mess Lake	5-Jul-08	102	13	13	12	stored in Clove oil?	<i>unknown</i>
Mess Lake	5-Jul-08	101	16	15	14	stored in Clove oil?	<i>unknown</i>
Mess Lake	5-Jul-08	108	16	17	16	stored in Clove oil?	<i>unknown</i>
Mess Lake	5-Jul-08	108	15	19	18	stored in Clove oil?	<i>unknown</i>
Mess Lake	5-Jul-08	107	18	23	22	stored in Clove oil?	<i>unknown</i>
Mess Lake	5-Jul-08	114	18	44	43		<i>O. nerka</i>
Mess Lake	6-Jul-08	109	16	1	2		<i>O. nerka</i>
Mess Lake	6-Jul-08	103	16	3	4		<i>O. nerka</i>
Mess Lake	6-Jul-08	95	12	5	6		<i>O. nerka</i>
Mess Lake	6-Jul-08	58	1.6		7		<i>O. nerka</i>
Mess Lake	6-Jul-08	113	16.8	8	9		<i>O. nerka</i>

BOLD TaxonID Tree

Title : COI REFERENCE DATABASE Tree
Date : 26-September-2008
Data Type : Nucleotide
Distance Model : Kimura 2 Parameter
Codon Positions : 1st, 2nd, 3rd
Labels : Country & Province, ProcessID, Family,
Colorization :

Sequence Count : 100
Species count : 8
Genus count : 2
Family count : 1
Unidentified : 1

