Appendix G.12

Environmental DNA – Certificate of Analysis,

Bureau Veritas Laboratories

Attention: **Melanie MacDonald**
McCallum Environmental Ltd
2 Bluewater Road, Suite 115
Halifax, NS
Canada B4B 1G7

Client Project #: 17-191
Site Location: FMS
C.O.C. #: 20200529
Quote #: N/A
PO#: N/A

Report Date: 2020/06/05
Report #: ME20200605
Version: 1

ENVIRONMENTAL DNA - CERTIFICATE OF ANALYSIS

BV JOB #: E20200529
Received: 2020/05/29, 9:05 AM

Sample Type: Cellulose Nitrate (CN) filter, preserved in silica
Samples Received: 22

Analyses (eDNA Isolation - Species)	Test Requested	Test Performed	Date eDNA Extracted	Date Analyzed IntegritE-DNA™	Date Analyzed Target Species	Laboratory Method	Analytical Method (qPCR Primer/Probe set)
eDNA Isolation and IntegritE-DNA™	22	22	2020/06/01 2020/06/02	2020/06/02 2020/06/03 2020/06/04	N/A	GUE SOP-00056	ePlant5
General Fish assay (eFish)	22	21	N/A	N/A	2020/06/03 2020/06/04	GUE SOP-00056	eFish1

Remarks:

Bureau Veritas Laboratories (Animal DNA Department, DNA Services) is accredited to ISO17025:2017 for eDNA testing.

All work recorded herein has been done in accordance with procedures and practices ordinarily exercised by industry professionals using accepted testing methodologies, quality assurance and quality control procedures (except where otherwise agreed by the client and Bureau Veritas Laboratories in writing). All data has met quality control and method performance criteria unless otherwise noted.

Bureau Veritas Laboratories' liability is limited to the actual cost of the requested analyses, unless otherwise agreed in writing. There is no other warranty expressed or implied. Bureau Veritas Laboratories has been retained to provide analysis of samples provided by the Client using the testing methodology referenced in this report. Interpretation and use of test results are the sole responsibility of the Client and are not within the scope of services provided by Bureau Veritas Laboratories unless otherwise agreed in writing. Bureau Veritas Laboratories is not responsible for the accuracy or any data impacts that result from the information provided by the customer or their agent.

Results relate to supplied samples tested. This Certificate should not be reproduced except in full, without the written approval of the laboratory.

eDNA tests are used to confirm presence of eDNA in samples for the targeted species / species groups.

Collected eDNA samples will contain eDNA at various stages of degradation, being subject to environmental forces that breakdown DNA, including microbial activity, ultraviolet radiation, heat, hydrolysis, and enzymatic activity. eDNA is first evaluated for eDNA quality and presence of qPCR assay inhibitors using the IntegritE-DNA™ assay before testing for target species or genera to confirm that the eDNA is of sufficient quality for testing and to identify and address qPCR inhibition (if present) to avoid false negatives.

SAMPLE RETENTION: Samples and DNA extracts generated from the samples will be retained by Bureau Veritas Laboratories for a period of 90 days after which time they will be discarded unless prearrangement has been made by client with Bureau Veritas Laboratories for longer storage.

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ENVIRONMENTAL DNA - CERTIFICATE OF ANALYSIS

BV JOB #: E20200529
Received: 2020/05/29, 9:05 AM

Methodology for Sample Analysis

Samples received to the laboratory are entered into the Laboratory Information Management System (LIMS) upon receipt. Samples were inspected and assessed for amount of silica beads, silica bead saturation level, coin envelope condition and number of coin envelopes in each bag. Samples were frozen at -20°C until processing in the laboratory. Sample analysis is completed within 10 or 15 business days (as indicated by the client on the COC) following receipt of samples by the testing laboratory.

eDNA isolation is completed using the DNeasy Blood & Tissue Kit™ (QIAGEN). A negative control is included as a blank filter sample with each batch of eDNA isolation to monitor for potential laboratory contamination during the eDNA isolation process.

Following eDNA isolation from the filter, the IntegritE-DNA™ assay[1] is used to avoid the potential of a false negative (Type II error) during target species or genera testing. The IntegritE-DNA™ assay evaluates the integrity of eDNA for suitability for qPCR and for presence of qPCR inhibitors which may reduce the effectiveness of the qPCR assay for target species or genera. This assay evaluates the quality of eDNA to assess whether it is amplifiable using a qPCR assay that targets the chloroplast genome derived from plants/algae that are ubiquitously found in fresh water systems. Four technical replicates per eDNA sample, four technical replicates of negative control (Ultrapure water), and two technical replicates of positive control are used for the IntegritE-DNA™ assay. The cut-off Ct (qPCR cycle threshold) value for the IntegritE-DNA™ assay is 30. If the IntegritE-DNA™ assay produces a positive detection frequency of Ó2 of the 4 technical replicates, this indicates that the eDNA for the target taxa is likely to be of sufficient quality to be detected (if present) with the target assay. If the IntegritE-DNA™ assay produces a positive detection frequency < 2 of the 4 technical replicates (eDNA is degraded or qPCR inhibitors are present), then sample cleanup is completed using the OneStep PCR Inhibitor Removal Kit™ (ZYMO Research) to remove potential qPCR assay inhibitors from the isolated eDNA. Subsequent to inhibitor removal, the IntegritE-DNA™ assay is repeated to re-assess whether the eDNA is of sufficient quality for qPCR. If a sample fails at the IntegritE-DNA™ assay for the second time the client will be informed that the quality of the sample is insufficient for the qPCR assay. eDNA indicator (IntegritE-DNA™) in the sample suggests that degradation has taken place and therefore the target species assay may be ineffective. Once a sample passes the IntegritE-DNA™ assay, then the target species or genera assay is performed. Eight technical replicates per eDNA sample, eight technical replicates of the negative control (Ultrapure water), and two technical replicates of positive control (total DNA or synthetic DNA) are used for the target species or genera assay to assess the detection or non-detection of DNA of the target species or genera. The cut-off Ct value for target species assay is 50.

[1] Hobbs J, Round JM, Allison MJ, Helbing CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849.

BECKY HENDERSON
Senior Customer Service Representative, Bureau Veritas Laboratories, DNA Services
Email: Becky.Henderson@bvlabs.com
Phone #: (519) 836 2400 Ext. 7067714

Please direct all questions regarding this Certificate of Analysis to your Customer Service Representative above.
==========
For Service Group specific validation please refer to the Validation Signature Page.

Total Cover Pages: 2

BV JOB #: E20200529
Report Date: 2020/06/05
Report #: ME20200605

Client Name: McCallum Environmental Ltd
Client Project #: 17-191
Site Location: FMS
Sampler Initials: MMD

RESULTS - General Fish assay (eFish)

Client Sample ID	BV Case ID	Sampling Date	Preservation Type	COC Number	IntegritE-DNA™ Positive detection (Ct≤30)[1]	QC Batch	Cleanup required	IntegritE-DNA™ Positive detection (Ct≤30) after cleanup	QC Batch	Analytical Method (qPCR Primer/Probe set)	Target Species eDNA Positive detection (Ct≤50)[2]	QC Batch
1-A	ME20200020	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1[5]	8/8	200603Q1
1-B	ME20200021	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	8/8	200603Q1
1-C	ME20200022	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	8/8	200603Q1
2-A	ME20200023	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	0/8	200603Q1
2-B	ME20200024	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	1/8	200603Q1
2-C	ME20200025	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	1/8	200603Q1
3-A	ME20200026	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	1/8	200603Q1
3-B	ME20200027	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	1/8	200603Q1
3-C	ME20200028	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	0/8	200603Q1
4-A	ME20200029	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	0/8	200603Q1
4-B	ME20200030	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	1/8	200603Q3
4-C	ME20200031	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	0/8	200604Q2
5-A	ME20200032	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	0/8	200604Q2
5-B	ME20200033	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	0/8	200604Q2
5-C	ME20200034	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	2/8	200604Q2
6-A	ME20200035	2020/05/25	Silica	20200529	0/4[3]	200603Q2	Yes[3]	4/4	200604Q1	eFish1	8/8	200604Q2
6-B	ME20200036	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	8/8	200604Q2
6-C	ME20200037	2020/05/25	Silica	20200529	0/4[3]	200603Q2	Yes[3]	4/4	200604Q1	eFish1	8/8	200604Q2
7-A	ME20200038	2020/05/25	Silica	20200529	0/4[3]	200603Q2	Yes[3]	4/4	200604Q1	eFish1	0/8	200604Q2
7-B	ME20200039	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	0/8	200604Q2
7-C	ME20200040	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	1/8	200604Q2
Field Blank	ME20200041	2020/05/25	Silica	20200529	0/4[3]	200603Q2	Yes[3]	0/4[4]	200604Q1	eFish1	N/A	N/A

[1] **IntegritE-DNA™ Assay:** Four technical replicates were assayed for each eDNA sample. The cut-off Ct value for IntegritE-DNA™ assay was 30. Results are reported as the number of positive detections (n) out of a total of 4 technical replicates, n/4.

[2] **Target Species Assay:** Eight technical replicates were assayed per eDNA sample. The cut-off Ct value for target species assay was 50. Results are reported as the number of positive detections (n) out of a total of 8 technical replicates, n/8.

[3] The IntegritE-DNA™ assay failed and cleanup is required.

[4] Quality of the samples are insufficient for the qPCR assay.

[5] eFISH1: qPCR primer/probe assay to assess the presence of Fish species eDNA (confirmed to detect several fish including 19 species; Sockeye Salmon (*Oncorhynchus nerka*), Pink Salmon (*Oncorhynchus gorbuscha*), Chum Salmon (*Oncorhynchus keta*), Arctic Grayling (*Thymallus arcticus*), Cutthroat Trout (*Oncorhynchus clarkii*), Rainbow Trout (*Oncorhynchus mykiss*), Chinook Salmon (*Oncorhynchus tshawytscha*), Coho Salmon (*Oncorhynchus kisutch*), Atlantic Salmon (*Salmo salar*), Dolly Varden (*Salvelinus malma*), Round Whitefish (*Prosopium cylindraceum*), Slimy Sculpin (*Cottus cognatus*), American Eel (*Anguilla rostrata*), Northern Pike (*Esox lucius*), Smallmouth Bass (*Micropterus dolomieu*), Largemouth Bass (*Micropterus salmoides*), Bull Trout (*Salvelinus confluentus*), Eulachon *(Thaleichthys pacificus*)). This assay is designed to be non-specific. It may detect eDNA from other fish species in addition or instead of the specific species listed here, which the assay has been validated for.

GENERAL COMMENTS

The IntegritE-DNA results for Blank sample (BV case ID, ME20200041) were negative before and after clean-up as expected.

Results relate only to the items tested.

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BV JOB #: E20200529
Report Date: 2020/06/05
Report #: ME20200605

Client Name: McCallum Environmental Ltd
Client Project #: 17-191
Site Location: FMS
Sampler Initials: MMD

QUALITY ASSURANCE REPORT

			eDNA Isolation Negative Control[1]		qPCR Positive Controls[2]		qPCR Negative Controls[3]	
QC Batch	Parameter	Date	Detection at:	Pass/Fail	Detection at:	Pass/Fail	Detection at:	Pass/Fail
200602Q1	IntegritE-DNA	2020/06/02	0 of 4 technical replicates	Pass	2 of 2 technical replicates	Pass	0 of 4 technical replicates	Pass
200603Q2	IntegritE-DNA	2020/06/03	0 of 4 technical replicates	Pass	2 of 2 technical replicates	Pass	0 of 4 technical replicates	Pass
200604Q1	IntegritE-DNA	2020/06/04	eDNA Isolation Negative Control is assessed using IntegritE-DNA only once for each extraction batch.	N/A	2 of 2 technical replicates	Pass	0 of 4 technical replicates	Pass
200603Q1	eFish1	2020/06/03		N/A	2 of 2 technical replicates	Pass	0 of 8 technical replicates	Pass
200603Q3	eFish1	2020/06/03		N/A	2 of 2 technical replicates	Pass	0 of 8 technical replicates	Pass
200604Q2	eFish1	2020/06/04		N/A	2 of 2 technical replicates	Pass	0 of 8 technical replicates	Pass

[1]**eDNA Isolation Negative Control:** Blank filters were included for each batch of eDNA extraction to monitor for laboratory contamination during eDNA isolation. eDNA Isolation Negative Control is assessed using IntegritE-DNA™ only. QC results show no eDNA was isolated from the negative control, therefore there was no indication of sample contamination during handling. Acceptance criteria: 0 of 4 technical replicates

[2]**qPCR Positive Controls:** Two technical replicates of isolated eDNA from freshwater sample were used as positive controls for IntegritE-DNA™. Two technical replicates of total DNA or synthetic DNA from the target species were used as positive controls for eDNA assays. Results show that 100% of the technical replicates amplified the positive control eDNA as expected, therefore an observation of negative result in eDNA samples is not related to the qPCR performance. Acceptance criteria: 2 of 2 technical replicates

[3]**qPCR Negative Controls (Ultrapure water):** Four technical replicates for IntegritE-DNA™ and eight technical replicates for target species or genera were used to monitor for laboratory contamination. Results show that 0% of the technical replicates in the negative controls had amplified eDNA, indicating no contamination was detected. Acceptance criteria: 0 of 4 technical replicates for IntegritE-DNA™, and 0 of 8 technical replicates for other assays.

LABORATORY RESULTS VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).

Reporter: ALI MIRABZADEH, M.Sc.
Senior Analyst, Bureau Veritas Laboratories, DNA Services

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Reviewer: HEATHER ALLEN, M.Sc.
Supervisor, Bureau Veritas Laboratories, DNA Services

==========

BV JOB #: E20200529
Report Date: 2020/06/05
Report #: ME20200605

Client Name: McCallum Environmental Ltd
Client Project #: 17-191
Site Location: FMS
Sampler Initials: MMD

Fish Species Assay Validation Information

eDNA assay Validation

All eDNA assays are validated through a rigorous multistep evaluation protocol that includes tests of DNA target specificity and amplification sensitivity. All eDNA tests available at Bureau Veritas Laboratories have been validated for performance using interlaboratory verification.

General eDNA Assay Information

Target Species	Various Fish Species	eDNA qPCR Primer/Probe set	eFish1
Species Abbreviation	Fish	eDNA qPCR Format	TaqMan

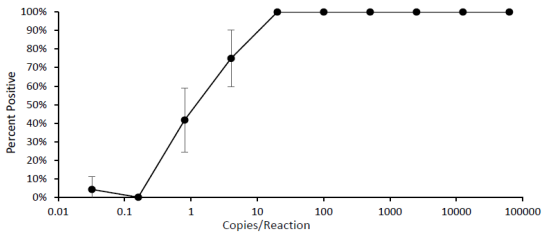
eDNA Assay Specificity Tests

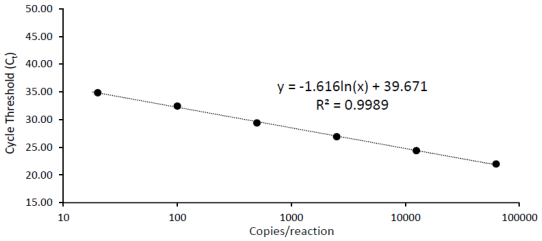
A. qPCR Activity: Multi-species analysis of eDNA assay efficiency. This assay is designed to be non-specific. It may detect eDNA from other fish species in addition or instead of the specific species listed here, which the assay has been validated for. Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA. Technical replicates: n=25

Species:	ANRO	COCO	ESLU	HOSA	LICA	MIDO	MISA	ONCL	ONGO	ONKE	ONKI
Detection:	Yes	Yes	Yes	No	No	Yes*	Yes	Yes	Yes	Yes	Yes
Species:	ONMY	ONNE	ONTS	PRCY	SACO	SAMA	SASA	THAR	THPA	NTC	
Detection:	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes*	No	

* This tool weakly detects Smallmouth Bass and Eulachon DNA

eDNA Assay Sensitivity Test using gBlocks™ synthetic DNA





>100 copies/reaction were tested with n=8 technical replicates.
Ć100 copies/reaction were tested with n=24 technical replicates.
The eFish1 gBlocks sequence is based on Chinook Salmon (Oncorhynchus tschawytscha)

The relationship between Cycle Threshold and Copy Number does not necessarily remain linear when fewer than 100% of technical replicates are positive.

eDNA Assay Sensitivity Test using tissue-derived gDNA

COCO

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	96%	7%
1	96%	7%
0.2	32%	16%
0.04	8%	10%
0.008	0%	0%
0	0%	0%

ONCL

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	20%	14%
0.2	0%	0%
0.04	0%	0%
0.008	0%	0%
0	0%	0%

ONGO

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	96%	7%
1	100%	0%
0.2	100%	0%
0.04	92%	10%
0.008	20%	14%
0	0%	0%

ONKE

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	100%	0%
0.2	40%	17%
0.04	16%	13%
0.008	0%	0%
0	0%	0%

ONKI

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	40%	17%
0.2	0%	0%
0.04	0%	0%
0.008	0%	0%
0	0%	0%

ONMY

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	88%	11%
0.2	36%	17%
0.04	16%	13%
0.008	0%	0%
0	0%	0%

ONNE

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	96%	7%
1	4%	7%
0.2	0%	0%
0.04	0%	0%
0.008	0%	0%
0	0%	0%

ONTS

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	64%	17%
0.2	20%	14%
0.04	0%	0%
0.008	4%	7%
0	0%	0%

PRCY

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	4%	7%
0.2	0%	0%
0.04	0%	0%
0.008	0%	0%
0	0%	0%

SASA

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	96%	7%
1	28%	16%
0.2	12%	11%
0.04	0%	0%
0.008	0%	0%
0	0%	0%

THAR

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	100%	0%
0.2	68%	16%
0.04	20%	14%
0.008	8%	10%
0	0%	0%

Abbreviations

American Eel (*Anguilla rostrata*)	ANRO	Pink Salmon (*Oncorhynchus gorbuscha*)	ONGO	Dolly Varden (*Salvelinus malma*)	SAMA
Slimy Sculpin (*Cottus cognatus*)	COCO	Chum Salmon (*Oncorhynchus keta*)	ONKE	Atlantic Salmon (*Salmo Salar*)	SASA
Northern Pike (*Esox lucius*)	ESLU	Coho Salmon (*Oncorhynchus kisutch*)	ONKI	Arctic Grayling (*Thymallus arcticus*)	THAR
Human (*Homo sapiens*)	HOSA	Round Whitefish (*Prosopium cylindraceum*)	PRCY	Eulachon (*Thaleichthys pacificus*)	THPA
Bullfrog (*Lithobates (Rana) catesbeiana*)	LICA	Rainbow Trout (*Oncorhynchus mykiss*)	ONMY	qPCR no template control	NTC
Smallmouth Bass (*Micropterus dolomieu*)	MIDO	Sockeye Salmon (*Oncorhynchus nerka*)	ONNE	quantitative real-time polymerase chain reaction	qPCR
Largemouth Bass (*Micropterus salmoides*)	MISA	Chinook Salmon(*Oncorhynchus tshawytscha*)	ONTS	environmental DNA	eDNA
Cutthroat Trout (*Oncorhynchus clarkii*)	ONCL	Bull Trout (*Salvelinus confluentus*)	SACO		

References

1. Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, Ascaphus montanus, in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 00: 1 12. https://doi.org/10.1002/edn3.82
2. Hobbs, J, Round, JM, Allison, MJ, Helbing, CC (2019) Expansion of the known distribution of the coastal tailed frog, Ascaphus truei, in British Columbia, Canada, using robust eDNA detection methods. PLOS ON 14(3): e0213849.
3. Klymus, KE, Merkes, CM, Allison, MJ, Goldberg, CS, Helbing, CC, Hunter, ME, Jackson, CA, Lance, RF, Mangan, AM, Monroe, EM, Piaggio, AJ, Stokdyk, JP, Wilson, CC, Richter, CA (2019) Reporting the limits of detection and quantification for
4. Veldhoen N, Hobbs J, Ikonomou G, Hii M, Lesperance M, Helbing, CC (2016) Implementation of novel design features for qPCR-based eDNA assessment. PLOS ONE 11(11): e0164907. https://doi.org/10.1371/journal.pone.0164907

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Report Date: 2020/06/05
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Client Name: McCallum Environmental Ltd
Client Project #: 17-191
Site Location: FMS
Sampler Initials: MMD

Bureau Veritas Laboratories
GUE FCD-00441/7
CHAIN OF CUSTODY RECORD

From Canada, send to:
Bureau Veritas Laboratories, DNA Services
335 Laird Rd #2
Guelph, ON N1G 4P7
eDNA@bvlabs.com

From USA, send to:
Bureau Veritas Laboratories
240 Portage Rd
Po Box 670, PMB 19
Lewiston NY 149092-1604

ENVIRONMENTAL DNA (eDNA) CHAIN OF CUSTODY RECORD

Page 1 of 2

«««An incomplete or incorrect form may lead to delays in testing»»»

COC # 20200529

1 Invoice Information (Required)		2 Report Information (if differs from invoice)		3 Project Information (where applicable)		4 Turnaround Time (TAT) (Required)
Company Name:	McCallum Environmental Ltd	Company Name:	McCallum Environmental Ltd.	Quotation #:		Regular TAT (Most analyses)
Contact Name:	Olena Kharytonova	Contact Name:	Melanie MacDonald	P.O. #:		* 10 business days (Sample # ≤ 50) * 15 business days (Sample # > 50) From date received
Address:	2 Bluewater Road, Suite 115 Halifax, NS, B4B 1G7	Address:	Same	Project #:	17-191	PLEASE REQUEST RUSH FROM CUSTOMER SERVICE
				Site Location:	FMS	
				Sampled By:	MMD	Rush TAT (Surcharges will be applied)
				Note:1		* 5 business days (Sample # ≤ 50) * 10 business days (Sample # > 50) From date received
Phone:	782-233-1717 Fax:	Phone:	(902) 817-2444 Fax:			
Email:	olena@mccallumenvironmental.com	Email:	melanie@mccallumenvironmental.com			

eDNA ANALYSIS

5 IMPORTANT INFORMATION

- Water samples should be kept cool and filtered as soon as possible (within 24 hours of collection).
- Cellulose Nitrate (CN) filter is recommended to use for eDNA test because of higher eDNA recovery.
- Preserve filter in self-indicating silica beads (2-4 mm diameter) or molecular grade ethanol (95 to 100%) immediately following sample filtration.

6 CLIENT SPECIAL INSTRUCTIONS

1

Number	7 Sample identification	8 Date Sampled (YYYY/MM/DD)	9 Date Filtered and Preserved (YYYY/MM/DD)	10 Filter Material	11 Filter Size (Diameter)	12 Filter Pore Size (µm)	13 Preservation Method (Ethanol / Silica)	14 Assays Requested[1]	15 Comments
1	1A	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
2	1B	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
3	1C	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
4	2A	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
5	2B	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
6	2C	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
7	3A	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
8	3B	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
9	3C	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
10	4A	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
11	4B	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
12	4C	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
13	5A	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
14	5B	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
15	5C	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	

filter & DNA data will be emailed to Anna when

16 RELINQUISHED BY: (Signature/Print)	17 DATE: (YYYY/MM/DD)	18 TIME: (HH:MM)	RECEIVED BY: (Signature/Print)	DATE: (YYYY/MM/DD)	TIME: (HH:MM)	CASE RANGE
Melanie MacDonald	2020-05-28		Nancy Horton	20201057129	9:05 am	For Lab Use Only

[1] **Available Assays at Bureau Veritas Laboratories: AMMV**[2] (Western tiger salamander), **ANBO** (Western toad), **ASMO** (Rocky mountain tailed frog), **eFish**[3] (General fish assay), **LICA** (North American bullfrog), **ONCL** (Cutthroat trout), **ONKI** (Coho salmon), **ONMY** (Rainbow trout - Steelhead trout), **ONNE** (Sockeye Salmon), **ONTS** (Chinook salmon), **RAAU** (Northern red-legged frog), **RAPR** (Oregon spotted frog), **SOBE** (Pacific water shrew), **THAR** (Arctic grayling), **ASTR** (Pacific (Coastal) tailed frog), **MISA** (Largemouth Bass) and **ESLU** (Northern Pike)

[2] **AMMV** assay also detects *Ambystoma tigrinum* (AMTI) Tiger Salamander.

[3] **eFish** assay can detect DNA from 12 fish species (Sockeye salmon, Pink salmon, Chum salmon, Arctic grayling, Cutthroat trout, Rainbow trout, Chinook salmon, Coho salmon, Atlantic Salmon, Dolly Varden, Round Whitefish and Slimy Sculpin). This assay is designed to be non-specific. It may detect eDNA from other fish species in addition or instead of the specific species listed here, which the assay has been validated for.

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www.bvlabs.com

BV JOB #: E20200529
Report Date: 2020/06/05
Report #: ME20200605

Client Name: McCallum Environmental Ltd
Client Project #: 17-191
Site Location: FMS
Sampler Initials: MMD

Bureau Veritas Laboratories
GUE FCD-00441/7
CHAIN OF CUSTODY RECORD

From Canada, send to:
Bureau Veritas Laboratories, DNA Services
335 Laird Rd #2
Guelph, ON N1G 4P7
eDNA@bvlabs.com

From USA, send to:
Bureau Veritas Laboratories
240 Portage Rd
Po Box 670, PMB 19
Lewiston NY 149092-1604

ENVIRONMENTAL DNA (eDNA) CHAIN OF CUSTODY RECORD

«««An incomplete or incorrect form may lead to delays in testing»»»

COC # 20200529

1 Invoice Information (Required)		2 Report Information (if differs from invoice)		3 Project Information (where applicable)		4 Turnaround Time (TAT) (Required)
Company Name:	McCallum Environmental Ltd	Company Name:	McCallum Environmental Ltd.	Quotation #:		Regular TAT (Most analyses)
Contact Name:	Olena Kharytonova	Contact Name:	Melanie MacDonald	P.O. #:		* 10 business days (Sample # ≤ 50) * 15 business days (Sample # > 50) From date received
Address:	2 Bluewater Road, Suite 115 Halifax, NS, B4B 1G7	Address:	Same	Project #:	17-191	PLEASE REQUEST RUSH FROM CUSTOMER SERVICE
				Site Location:	FMS	
				Sampled By:	MMD	Rush TAT (Surcharges will be applied)
Phone:	782-233-1717 Fax:	Phone:	(902) 817-2444 Fax:	Note:1		* 5 business days (Sample # ≤ 50) * 10 business days (Sample # > 50) From date received
Email:	olena@mccallumenvironmental.com	Email:	melanie@mccallumenvironmental.com			

eDNA ANALYSIS

5 IMPORTANT INFORMATION	6 CLIENT SPECIAL INSTRUCTIONS
• Water samples should be kept cool and filtered as soon as possible (within 24 hours of collection). • Cellulose Nitrate (CN) filter is recommended to use for eDNA test because of higher eDNA recovery. • Preserve filter in self-indicating silica beads (2-4 mm diameter) or molecular grade ethanol (95 to 100%) immediately following sample filtration.	1

Number	7 Sample identification	8 Date Sampled (YYYY/MM/DD)	9 Date Filtered and Preserved (YYYY/MM/DD)	10 Filter Material	11 Filter Size (Diameter)	12 Filter Pore Size (µm)	13 Preservation Method (Ethanol / Silica)	14 Assays Requested[1]	15 Comments
1	6A	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
2	6B	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
3	6C	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
4	7A	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
5	7B	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
6	7C	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
7	Blank	2020-05-25	2020-05-25	CN	as provided by BV	45	Silica	eFish	
8									
9									
10									
11									
12									
13									
14									
15									

16 RELINQUISHED BY: (Signature/Print)	17 DATE: (YYYY/MM/DD)	18 TIME: (HH:MM)	RECEIVED BY: (Signature/Print)	DATE: (YYYY/MM/DD)	TIME: (HH:MM)	CASE RANGE
Melanie MacDonald	2020-05-28			For Lab Use Only		
			Manuel Hamond	2020/05/29	9:05 am	

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