Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

UofS-ETL-EDNA-02

Water Sample Collection for eDNA: Ethanol Preserved Method

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APPROVAL PAGE

Revisions to an existing SOP, addition of an SOP change form, or preparation of a new SOP must be reviewed, approved, and signed by the following:

Authored By: Yuwei Xie, Abigail DeBofsky, Markus Hecker and John P. Giesy Date: 04/04/2018

Supervisor Review By: John P. Giesy Date: 04/30/2018

Reviewed By: _____ Date: _____
(QA Coordinator)

DEFINITIONS AND ACRONYMS

eDNA Environmental DNA

ETL Environmental Toxicology Laboratory (University of Saskatchewan)

DHSE Department of Health, Safety and Environment (University of Saskatchewan)

PPE Personal Protective Equipment

QA Quality Assurance

QC Quality Control

QAPP Quality Assurance Project Plan

SOP Standard Operating Procedure

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1.0 PURPOSE

This protocol describes procedures for collecting water for environmental DNA (eDNA) based aquatic taxa identification and community composition characterization. This SOP is one optional sampling approach for monitoring local, invasive or endangered animals. eDNA is genetic material left by organisms in their surrounding environment, and which can be obtained by collecting environmental matrices such as soil, sediment, water, etc. without the source organisms having to be visually present. eDNA may originate from any tissue or biological material left behind by an organism such as cells, tissues, urine, hair, skin, mucus and dead individuals leaking genetic material. Samples collected can be subjected to two types of eDNA analyses: 1) Targeted DNA analysis for individual species of interest (e.g., endangered or invasive species); this analysis is based on specific genetic primers/probes that enable detection of the presence of species of interest using quantitative real-time PCR (qPCR); and 2) Non-target biodiversity analysis for monitoring biodiversity; this approach makes use of next-generation sequencing based metabarcoding technology (sequence-by-synthesis).

2.0 SCOPE AND APPLICATION

This SOP applies to the ETL for water samples supplied for the GWF project: "Next generation solutions to ensure healthy water resources for future generations."

3.0 SAFETY CONSIDERATIONS

Safety training and medical monitoring requirements are also consistent among all protocols for field studies and are described in the Health and Safety Plan for the GWF field studies. Personnel should review before collecting samples. The trip leader should hold a tailgate safety briefing before collecting samples. Identify risks and potential mitigative procedures to minimize those risks. For instance, if collecting while wearing waders always wear a belt on the outside of the waders to minimize the potential for the waders to fill with water.

3.1 PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment (PPE), consisting of nitrile gloves will be worn at all times when handling samples.

5.0 EQUIPMENT, MATERIALS AND REAGENTS (USE CHECKLIST ATTACHED)

- Global Positioning Unit (GPS)
- Calibrated probes to measure DO, temperature, pH, conductivity
- Turbidity meter
- Camera
- Bucket
- Ladle
- 12V DC Portable Freezer with 110V plug
- Ice bags (-80 C chilled in Styrofoam box)
- Two Large coolers
- One 1200 watt inverter
- 20-foot extension cord
- DNA free Sandwich bags
- Notebook
- Pencil
- Three Permanent markers (one thick and two thin)
- Chain of custody forms
- One Field Record Sheet per site
- Field collection sheets printed on Rite in the Rain® paper
- Bleach water and rag for wiping down equipment
- Paper towels
- Kim wipes
- Nitrile gloves
- Absolute ethanol
- Non-denatured absolute ethanol (molecular biology level, 200 proof)
- 10% bleach water (bleach, RO water)
- 70% ethanol (absolute ethanol, RO water)
- Sterile DO water
- Spray bottle with Invitrogen™ RNase Away™ Decontamination Reagent
- Spray bottle with 70% ethanol
- Spray bottle with 10% bleach
- Three 250-mL Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure per site
- Four 1-L Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure per site

6.0 METHOD, PROCEDURES, AND REQUIREMENTS

The following field procedures describe methods for collections of samples in a stepwise fashion and explain the reasoning behind the sampling design and techniques. Detailed below are the preparatory procedures, sampling design, sampling techniques, and procedures for sample documentation, preservation, and shipping.

6.1 Mobilization and Training

This section describes requirements for mobilization for the necessary fieldwork, as well as the necessary safety training. The objective of this section is to ensure that all of the necessary preparatory work has been conducted to enable the successful completion of the overall project.

Mobilization for the necessary fieldwork entails procuring and packing equipment and training of field personnel in accordance with the site Health and Safety Plan (HASP).

The project manager will assemble and pack all equipment specified in the Section above. If any items need to be purchased, they will be ordered well in advance to ensure that the schedule is not impacted by equipment needs. Pre-cleaned sample containers will be purchased to minimize the potential for contamination.

6.2 Sampling Objectives

The primary objective of the field-sampling portion of a project is to collect eDNA from water samples to ultimately be used in monitoring programs. The sample should contain eDNA from prokaryotic and eukaryotic species; therefore, prevention of contamination of samples with exogenous DNA is paramount. Detection of false positives due to contamination of samples will limit conclusions that might be drawn from the sample.

6.3 Sampling Locations

Samples should be collected from various locations within a study area as defined in the work plan. Once field samples are collected, each location will be plotted and numbered on the most recent maps of the area.

6.4 Sampling Methodology

This section details the overall sampling methodology, equipment, and techniques to be employed in this sampling effort.

6.4.1 Record geographic information

Utilize a GPS unit to determine precise site locations and document the coordinates.

6.4.2 Measure and record environmental variables

Measure and record environmental variables of interest (DO, temperature, pH, conductivity, turbidity) and observations. Be sure to record the serial number of the probes.

All instruments used for measurements in the field need to be calibrated and the date of calibration logged in the log book for that piece of equipment (See other SOPs for measurements and calibration of instruments).

6.4.3 Collection of water for eDNA

6.4.3.1 Prepare sampling kit

Chill the ice bags in -80 C freezer and store them in Styrofoam boxes.
Clean, sterilize and dry the Nalgene PP bottles for field sampling.
The eDNA water Sampling Kit is designed for field sampling of eDNA from waters in remote sites. Preparing 1 L tubes containing the DNA preservative buffer in the hood in the Biochemistry & Molecular Biology Room (Room 261 in ETL) before field sampling. Using the Cryogenic permanent marker which is alcohol resistant and water resistant, write sample information (station number, replicate number, and date) on caps and tubes and a Ziplock bag for storage.
Note: After water samples are collected, they need to be immediately preserved using the DNA preservation buffer (670 mL of absolute ethanol and 30 ml of sodium acetate (3M) per sampling bottle; provided with the kit). One blank water sample is also included in the eDNA Sampling Kit as field blanks for each site.
Table 1. The content of the eDNA Sampling Kit for each sampling site

	Content	Number
Field Sample	Water sampling kit (1-L small mouth Nalgene Sterile bottle containing DNA preservation buffer; sterile 250 mL sampling bottle for filling 1-L Nalgene bottle, maximum volume: ~ 300 mL)	3
Blank sample	Blank water sampling bottle (field blank) (1-L small mouth Nalgene Sterile bottle with the DNA preservation buffer and 300 mL diH_2O)	1

6.4.3.2 Collecting water

1) Water should be sampled before collecting synoptic samples of sediment.

2) In lotic systems, samples should be collected consecutively in an upstream direction where possible, to avoid re-sampling of water. However, depending on logistics this may not be possible in all cases. According to documentation must be made in the field notebook.
3) To avoid possible contamination from the surveyor boots, or by stirring up sediment, samples should be collected with the surveyor not entering the water body where possible (from a boat, through the ice, standing on the bank/shore) or from a bridge.
4) Sampling process:
   A. Don a pair of gloves (included with your kit). **Note:** Wear a new pair of clean gloves for each sampling site!
   B. Open the field blank water sampling bottle during water sampling and place next to sampling site (on bank/shore or next to whole during sampling through ice).
   C. Use the second smaller sterile 250 mL sampling bottle provided within each kit that does not contain any liquid for scooping up water to fill the water sampling bottle to the 1 L marker highlighted on the Nalgene bottle. Proceed in the same way with replicate sample 2 for each site.
   D. Cap and label the bottle properly (include site name, replicate #, date, initials of person sampling, matrix samples [water]). Mix thoroughly by inverting the bottle ten times. The water sample can be stored and shipped at ambient temperature.
   E. Cap the field blank water sampling bottle after the three water samples were taken. Labe the field blank bottle with the site name, date and initials).
5) Store and ship samples on ice (recommended) or at ambient temperature (within one week).

7.0 RECORDS, DOCUMENTATION, AND QC REQUIREMENTS

Field personnel will document all sampling activities in accordance with the work plan. Before exiting each site, the following information will be recorded on the field record sheet (Appendix A): (a) site name; (b) date and time of collection; (c) the analyses requested (i.e. water quality parameters); (d) the method of preservation; and (e) initials of personnel who conducted sampling. If any additional biological samples such as fish or invertebrates are taken from that site, those should be noted as well.

8.0 SAMPLE PRESERVATION

All samples should be temporally stored and shipped on dry ice to ETL, and stored at -20 ^{o}C or -80 ^{o}C until sample preparing. Note: Analyses should be undertaken within one

month of sample collection. A chain-of-custody form (UofS-ETL-SOP-EDNA-07: Standard Operating Procedure for Maintenance of Sample Chain-of-Custody) will be completed for each sample and be archived. A copy of the form will accompany all samples when transported, relinquished or split. Sample management will also follow a scripted procedure (ETL-SOP-EDNA-09: Management of eDNA sample: Receiving, Preservation, Storage, Documentation, Decontamination, and Disposal) to ensure samples will not be lost or destroyed.

9.0 RESPONSIBILITIES

Project Director — Will oversee and approve all project activities.

Project Manager — Will oversee and approve all project activities; review QA reports; approve final project QA needs; authorize necessary actions and adjustments to accomplish program QA objectives; and act as a liaison between agencies, field staff.

Quality Assurance (QA) Manager — Will oversee all QA activities to ensure compliance with contract specifications; initiate audits on work completed by project personnel and subcontractors, including analytical laboratories and independent data validation contractors; review program QA activities, quality problems, and quality-related requests. In response to the field and analytical findings, this person will approve the corrective actions. This person will report quality non-conformances to the Project Manager and review all pertinent portions of the deliverables before they are transmitted to ensure conformance with QA/QC procedures and quality work product.

Data Manager — Will oversee data management for this project. This person is responsible for the structure, organization, format, implementation, and operation of the project database.

Field Team Leader — Will oversee field activities and supervise the field crews. This person will ensure that proper sample collection, preservation, storage, transport, and COC QC procedures are followed. This person will inform the Project QA Manager when field problems occur, and will communicate and document corrective actions taken. The Field Team Leader will discuss field activities with the Project Manager.

Laboratory Project Manager — The laboratory project manager for this project is the person responsible for assuring that the analysis of all samples is performed in accordance with the QAPP and the laboratory's quality assurance manual. In addition, the Laboratory Project Manager performs the final laboratory review of project data packages for completeness and compliance with project requirements.

10.0 REFERRENCES

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology letters*, *4*(4), 423-425.

THOMSEN, P., Kielgast, J. O. S., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., ... & Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular ecology*, *21*(11), 2565-2573.

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R. A., ... & Williams, P. (2015). Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (Triturus cristatus). *Biological Conservation*, *183*, 19-28.

APPENDIX A: FIELD RECORD SHEET

Field Record Sheet

Book No: ETL-eDNA-FRS Page No.: _____

Project:		
Sample Identification:		
Date:		Time:
Location:	Latitude:	Longitude:
Description of Sample Location/ Observations:		
Conditions:		
Principal Scientist:		
Crew Members:		
Water Depth (m):		Water Temp (°C):
Specific Conductivity (µmoh):		Air Temp (°C):
Dissolved Oxygen(mg/L):		pH:
Container Preparation/Storage:		
Samples Collected and Preserved:		
Sample Distribution:		

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APPENDIX B: CHECKLIST FOR WATER SAMPLE COLLECTION FOR EDNA (ETHANOL PRESERVED METHOD)

Statue Number Item

- ☐ _____ Global Positioning Unit (GPS)
- ☐ _____ Calibrated probes to measure DO, temperature, pH, conductivity
- ☐ _____ Turbidity meter
- ☐ _____ Camera
- ☐ _____ Bucket
- ☐ _____ Ladle
- ☐ _____ 12V DC Portable Freezer with 110V plug
- ☐ _____ Ice bags (-80 C chilled in Styrofoam box)
- ☐ _____ 2 Large coolers
- ☐ _____ 1 1200 watt inverter
- ☐ _____ 20 foot extension cord
- ☐ _____ DNA free Sandwich bags
- ☐ _____ Notebook
- ☐ _____ Pencil
- ☐ _____ 3 Permanent markers (1 thick, 2 thin)
- ☐ _____ Chain of custody forms
- ☐ _____ 1 Field Record Sheet per site
- ☐ _____ Field collection sheets printed on Rite in the Rain® paper
- ☐ _____ Bleach water and rag for wiping down equipment
- ☐ _____ Paper towels
- ☐ _____ Kim wipes
- ☐ _____ Nitrile gloves
- ☐ _____ Absolute ethanol
- ☐ _____ Non-denatured absolute ethanol (molecular biology level, 200 proof)
- ☐ _____ 10% bleach water (bleach, RO water)
- ☐ _____ 70% ethanol (absolute ethanol, RO water)
- ☐ _____ Sterile DO water
- ☐ _____ Spray bottle with Invitrogen™ RNase Away™ Decontamination Reagent
- ☐ _____ Spray bottle with 70% ethanol
- ☐ _____ Spray bottle with 10% bleach
- ☐ _____ Three 250-mL Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure
- ☐ _____ Four 1-L Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure (One for blank water sample)