Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

UofS-ETL-EDNA-03

Surficial Sediment Collection for eDNA Analysis

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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

eRNA Environmental RNA

GWF Global Waters Futures

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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Section Heading Page

1.0 PURPOSE

This standard operating procedure (SOP) specifies requirements for collection of surficial sediment for identification and quantification of environmental DNA (eDNA) in surficial sediment. Sampling collection for sedimentary environmental RNA (eRNA) is also included in this protocol. 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 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
- 1 set MultiVac 300-MB-T, Vacuum Filtration System, 110V (Rocker 400 Vacuum Pump, Manifold, Burner, Waste Bottle, Filters and 2m Silicone Tubing)
- 2 Nalgen Repairable Hand-Operated PVC Vacuum Pumps with Gauge (backup)
- Bucket
- Ladle
- Sediment corer or grabber
- 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
- Burner
- Butane gas for decontamination of stainless steel manifold (Caution: EXPLOSIVE, AWAY FROM FLAMMING!!!)
- Parafilm
- Sterile 0.1 mm glass beads
- Sterile 0.5 mm glass beads
- 50 mL sterile Graduated Conical-Bottom Tubes
- LevGo smartSpatula™ Disposable Polypropylene Spatula
- Disposable 1250 ul plastic pipet tips
- 1000 uL adjustable pipette
- 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)

- Qiagen™ LifeGuard Soil Preservation Solution®
- 10% bleach water (bleach, RO water)
- 50% 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
- Spray bottle with 50% bleach

6.0 METHOD, PROCEDURES, AND REQUIREMENTS

The following field procedures describe methods for collection 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 surficial sediment to ultimately be used in bio-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.
This sample should be collected at a location that is representative of the water in the pond or lake. This is most often found at a deeper location away from these sources of water (i.e. away from the stream, spring or other source of water feeding into the pond or lake). Two good locations to collect the sample would be from a dock or swimming platform or at the pipe or stream leading out of the pond/lake. More sample size can increase the detective rate of rare animals.

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 the 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 calibrates 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 sediment for eDNA

To prevent contamination, any surficials that will come into contact during the procedure should first be cleaned with 70% ethanol. Prior to sampling, a fresh pair of gloves must be donned and forceps and scalpels must be cleaned in a 1:10 bleach/RO water solution for approximately 1 minute then sprayed with RNase Away. Tools should be dried with Kim wipes and placed in a clean, covered location, such as between two Kim wipes. To prevent contamination, separate clean and dirty tools as much as possible.

6.4.3.1 Prepare sampling kit

1) Chill the ice bags in -80 C freezer and store them in Styrofoam boxes.

2) Clean, sterilize and dry the sediment corer or grabber before and after each use.
   A. Clean corer with tap water.
   B. Spray corer with 50% bleach. Let bleach remain on surfaces for 15 minutes.
   C. After 15 minutes, use a DI water-dampened Kim Paper to remove bleach residue.
   D. Spray corer again with 70% ethanol.
   E. Decontaminate the corer with burner for 10 seconds burning. Cool them using sterile RO water.
   F. Spray RNase Away to remove RNase and residual DNA.
2) Prepare one blank sediment sampling 50 mL tube with 10 mL sterile di-water, 0.2 mL 0.1 mm and 0.2 mL 0.5 mm glass beads for each sampling location in the hood in the Biochemistry & Molecular Biology Room (Room 261 in ETL) before field sampling. The blank sample is treated as sample.
3) Using the Cryogenic permanent marker which is alcohol resistant and water resistant, write sample information (Location number, replicate number, pore size, preserve method, sampler, and date) on tubes, bottles and a Ziplock bag for storage for each sampling site.

6.4.3.2 Collecting sediment

1) Sediment sampling should be after water sampling.
2) 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) to avoid possible contamination from the surveyor boots, or by stirring up sediment.
3) Don a new pair of clean gloves (included with your kit). **Note:** Wear a new pair of clean gloves for each sediment sampling!
4) Sampling process:
   A. Open the field blank sediment sampling tube during sediment sampling and place next to sampling site (on bank/shore or next to hole during sampling through ice).
   B. Use a sediment corer (Please read SOP ETL 4012: Standard Method for Field Collection of Sediments for Chemical Analyses) to collect sample and then use the sterile spatula provided within each kit to collect the top 1-2 cm of the surface sediment (avoid collecting from the edges where the sediment had contact with the 50 mL sampling tube) and transfer approximately 5 mL to a sterile sediment sampling tube provided with the kit. Proceed in the same way with replicate samples 2 and 3 for each site.
   C. Cap and label the sediment sampling tube properly (include site name, replicate #, date, initials of person sampling, matrix samples [sediment]).

Note: For eRNA analysis, the sediment samples should be preserved using LifeGuard Solution, three volumes (15 mL) LifeGuard Solution for one volume sediment (5 mL).

The sediment sample should be frozen at or below -20^{o}C shortly after sampling, and then be stored and shipped at -20^{o}C.

D. Cap the field blank sediment sampling tube after all replicate samples will be taken. Label the field blank sediment sampling tube with the site name, date and initials.

E. The 'corer negative control' consisted of 5 mL of water collected below an internal/external rinsing (please see 5.4.3.1 2) section) of a decontaminated and reassembled corer (corer, liner tube, nosepiece) and added to 10 mL of CTAB in a 50-mL centrifuge tube. The corer negative control is treated as sample.

4. Store and ship samples at or below -20^{o}C.

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 taking the sample. If any additional biological samples such as fish or invertebrates are taken from that site, those should be noted as well. The wet weight of each sample was measured by weighing the sample in its tube and subtracting off the previously measured weight of the tube and CTAB.

8.0 SAMPLE PRESERVATION

Sediment samples are shipped at or below -20^{o}C, and stored at -80 ^{o}C for long-term storage. Samples for eRNA should be preceded as soon as possible according to the preservation buffer and storage condition. 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 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 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.

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 EDNA SAMPLING OF SURFACAL SEDIMENT

Statue Number Item

- ☐ _____ Global Positioning Unit (GPS)
- ☐ _____ Calibrated probes to measure DO, temperature, pH, conductivity
- ☐ _____ Turbidity meter
- ☐ _____ Camera
- ☐ _____ One set MultiVac 300-MB-T, Vacuum Filtration System, 110V (Rocker 400 Vacuum Pump, Manifold, Burner, Waste Bottle, Filters and 2m Silicone Tubing)
- ☐ _____ Two Nalgen Repairable Hand-Operated PVC Vacuum Pumps with Gauge (backup)
- ☐ _____ Bucket
- ☐ _____ Ladle
- ☐ _____ Sediment corer or grabber
- ☐ _____ 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
- ☐ _____ Burner
- ☐ _____ Butane gas for decontamination of stainless steel manifold (Caution: EXPLOSIVE, AWAY FROM FLAMMING!!!)
- ☐ _____ Parafilm
- ☐ _____ Sterile 0.1 mm glass beads
- ☐ _____ Sterile 0.5 mm glass beads
- ☐ _____ 50 mL sterile Graduated Conical-Bottom Tubes with racks
- ☐ _____ LevGo smartSpatula™ Disposable Polypropylene Spatula
- ☐ _____ Disposable 1250 ul plastic pipettes
- ☐ _____ 1000 uL adjustable pipette
- ☐ _____ Notebook
- ☐ _____ Pencil
- ☐ _____ Three Permanent markers (1 thick, 2 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)
- ☐ _____ Qiagen™ LifeGuard Soil Preservation Solution®
- ☐ _____ 10% bleach water (bleach, RO water)
- ☐ _____ 50% 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
- ☐ _____ Spray bottle with 50% bleach