Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

UofS-ETL-EDNA-06

Water Collection for DNA Metabarcoding of Phytoplankton

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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:

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Supervisor Review By: _____ Date: 04/30/2018

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(QA Coordinator)

DEFINITIONS AND ACRONYMS

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

GWF Global Waters Futures

HASP Health and Safety Plan

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1.0 PURPOSE

This protocol describes the procedures for collecting and filtering water samples for phytoplankton for DNA/RNA metabarcoding of freshwater algal communities in streams and rivers. DNA metabarcoding is a rapid and comparable method of high-throughput, DNA-based identification of multispecies.

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
- 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
- 2 15mL tube rack
- Sharps container
- Box of scalpel blades
- DNA free Sandwich bags
- Burner
- Butane gas for decontamination of stainless steel manifold (Caution: EXPLOSIVE, AWAY FROM FLAMMING!!!)
- Sterile DNase-free and RNase-free 2 mL tubes
- Greiner Bio-One™ Cryo.s™ Freezing Tubes
- 5-mL Axygen™ Screw Cap Transport Tubes (SCT-5ML-S)
- EMD Millipore™ Durapore™ PVDF Membrane Filters, pore size 0.45 µm, diam. 47 mm (Cat. No.: HVLP04700)
- EMD Millipore™ Durapore™ PVDF Membrane Filters, pore size, 0.65µm, diameter, 47mm (Cat. No.: DVPP04700)
- EMD Millipore™ MF-Millipore™ Mixed Cellulose Ester Membranes, pore size, 1.2µm, diameter, 47mm (Cat. No.: RAWP04700)
- 50-mL sterile Graduated Conical-Bottom Tubes
- Disposable 1250-ul plastic pipette tips
- One 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
- Four Sets of sterile forceps per site
- Four 1 L Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure for each site
- Four 1 L Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure for each site

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 water samples to ultimately be used in monitoring programs. The sample should contain eDNA from prokaryotic (bacteria) 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 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 phytoplankton

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 Nalgene PP bottles for field sampling.

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.

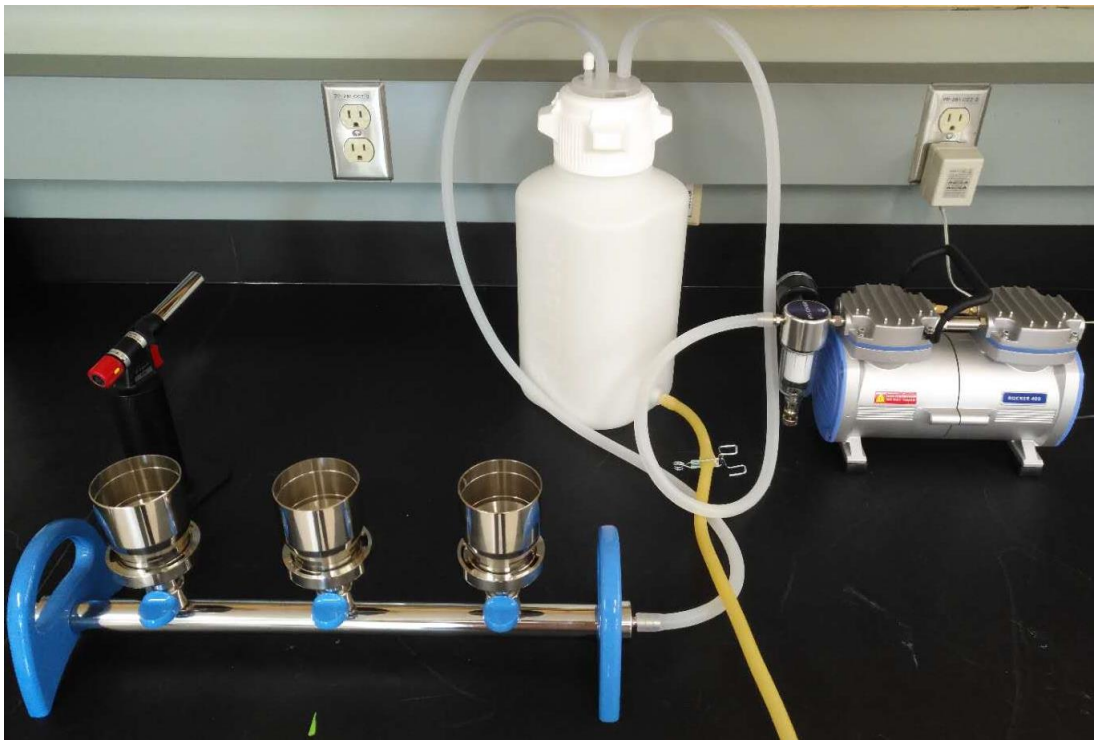
4) Test the MultiVac Vacuum Filtration System, Hand-Operated Vacuum Pumps and portable freezer.

5) Prepare one blank water sampling bottle with 500 mL filtered tape water with 0.2 μm membrane filter 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.

6.4.3.2 Water sampling

1) Water should be sampled prior to sediment sampling.
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 documentation must be made in the field note-book.
3) 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.
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. Submerge Sterile Nalgene™ collection bottle in water to collect desired volume of water. Proceed in the same way with replicate sample 2 for each site.
   D. Cap and label the bottle properly (include site name, replicate # and date).
   E. Cap the field blank water sampling bottle after the three water samples will be taken. Labe the field blank bottle with the site name and date).
   F. To minimize degradation of DNA, filter the water samples as soon as possible. The water sample can be stored on ice in the cooler for short-term storage (within 12 hours) for delivery to sample preparing base or ETL. Note: DO NOT FREEZE.
5) Pump Assembly

Connect pump head, manifold, Sterile Disposable Filter Units, and Waste Bottle with 2m Silicone Tubing. Secure filter funnel to adapter stem, creating airtight seal. Waste Bottle does not need to be sterile (but if it is not decontaminated between sites, discharge water should not be emptied in aquatic environments). Connect pump to 120-V AC power source such as a wall outlet. Pump can also be powered by a 12-V DC battery (such as a vehicle battery) with the use of a power inverter (12-V DC to 120-V AC).



6) Decontaminate funnel, membrane filter holder and forceps before and after each use
   A. Clean funnel, adapter stem and forceps with tap water.
   B. Spray funnel, adapter stem and forceps 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 funnel, adapter stem and forceps again with 70% ethanol.
   E. Decontaminate the surface of funnel, membrane filter holder and forceps with burner for 10 seconds burning. Cool them using sterile RO water.
   F. Spray RNase Away to remove RNase and residual DNA.
7) Filter assembly and water filtration
   A. Assemble proper membrane filter to the funnel on the manifold.

      Note: Phytoplankton of non-targeting eDNA/eRNA waters: 0.45 µm or 0.7 µm membrane filter depending on the algal density. If the turbidity is high, use stack filter method (put 1.2 µm membrane filter on the top of 0.7 µm or 0.45 µm membrane filter); the blank water sample: 0.2 µm membrane filter.

   B. Filter funnel with water sample and check the leak. Turn the vacuum pump on.

      Note: If there is a leak, re-assembly the tubes, funnel and adaptor stem.

C. After the water has been passed through the filter, dry the membrane filter as much as possible using vacuum, then turn the vacuum pump off.

a) For disposable Filter Units, cut the filter out of the apparatus using a new scalpel blade, cutting along the inside of the rubber seal, about 2 mm from the edge.

b) For manifold funnel, remove the funnel.

D. Using two sets of sterile forceps, pick up the filter membrane at opposite edges and roll the filter into a pre-labeled 5 mL tube. Do not tightly roll or fold the filter membrane. To see a video, please visit https://youtu.be/KUT6nKJPj4s.

8) Preserve the membrane filter during delivery or short-time storage.

A. LifeGuard Solution is recommended for preserving the membrane filters for RNA analysis. To reduce the volume of LifeGuard solution, insert one 2 mL freezing tube in the 5 mL tube. Then, add 2 mL LifeGuard solution into the 5 mL tube. Let 5 mL tube sit on ice during delivery and be stored in freezer for long-term analysis. If the cooler is unavailable, the LifeGuard preserved RNA can keep stable for one day under 37 C, 1 week at room temperature, 2 weeks at 2-8 C and one month at -20 C.

B. Frozen is recommended for preserving the membrane filters for DNA analysis without any preservation buffers, if the -20 C or -80 C freezer is available.

C. Non-denatured ethanol can be used for the membrane filters for DNA analysis. Add 5-mL non-denatured ethanol into the 5-mL tube. Let 5-mL tube sit on ice during delivery and be stored in freezer for long-term analysis. If the cooler is unavailable, the ethanol preserved NNA can be delivered under ambient temperature during delivery. Then store the ethanol preserved membrane in freezer for long-term storage. Ensure that the label will not be washed off with the addition of ethanol.

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 taking the sample (i.e. water quality parameters); (d) the method of preservation; and (e) initials of personnel. 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

Filters for DNA metabarcoding will be placed in preservation buffer under -20 ^{o}C or -80 ^{o}C for long-term storage. Samples for RNA metabarcoding should be preceded as soon as possible according to the preservation buffer and storage condition. A chain-of-custody form (UofS-ETL-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 COLLECTING PHYTOPLANKTON FOR METABARCODING

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
- ☐ _____ 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
- ☐ _____ Sharps container
- ☐ _____ Box of scalpel blades
- ☐ _____ DNA free Sandwich bags
- ☐ _____ Burner
- ☐ _____ Butane gas for decontamination of stainless steel manifold (Caution: EXPLOSIVE, AWAY FROM FLAMMING!!!)
- ☐ _____ Parafilm
- ☐ _____ Sterile DNase-free and RNase-free 2 mL tubes
- ☐ _____ Greiner Bio-One™ Cryo.s™ Freezing Tubes
- ☐ _____ 5 mL Axygen™ Screw Cap Transport Tubes (SCT-5ML-S)
- ☐ _____ Whatman® nitrocellulose membrane filters white gridded, pore size 0.45 µm, diam. 47 mm, sterile (Cat. No.: WHA7141104 ALDRICH™)
- ☐ _____ EMD Millipore™ EZ-Pak™ Membranes, pore size, 0.7µm, diameter, 47mm (EMD Millipore™ EZHCWG474)
- ☐ _____ EMD Millipore™ MF-Millipore™ Mixed Cellulose Ester Membranes, pore size, 1.2µm, diameter, 47mm (Cat. No.: RAWP04700, Fishersci)
- ☐ _____ 50 mL sterile Graduated Conical-Bottom Tubes
- ☐ _____ 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
- ☐ _____ Three sets of sterile forceps per site
- ☐ _____ Three 1 L Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure for each site
- ☐ _____ Three 1 L Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure for each site