Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

UofS-ETL-EDNA-11

Field Collection of Freshwater Macroinvertebrates

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APPROVAL PAGE

Revisions to an existing SOP, addition of a 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:	John P. Giesy	Date: 04/30/2018
Reviewed By: (QA Coordinator)	_____	Date: _____

DEFINITIONS AND ACRONYMS

ETL	Environmental Toxicology Laboratory (University of Saskatchewan)
DQO	Data Quality Objective
DHSE	Department of Health Safety and Environment (University of Saskatchewan)
QA	Quality Assurance
QC	Quality Control
QAPP	Quality Assurance Project Plan
SOP	Standard Operating Procedure
GWF	Global Water Futures
FSP	Field Sampling Plan
SAP	Sampling and Analysis Plan
CABIN	Canadian Aquatic Biomonitoring Network

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1.0 PURPOSE

The primary purpose of this protocol is to describe the method that will be used to collect freshwater benthic invertebrates in order to construct genetic database and biomonitoring macroinvertebrates using DNA metabarcoding. The measurement of biological effect may detect impacts on the aquatic ecosystem that cannot be measured with traditional physical-chemical monitoring such as changes in water quantity, presence of invasive species, and habitat degradation. Aquatic biomonitoring can indicate preceding river conditions for weeks or months prior to collection. Benthic macroinvertebrates are used in aquatic biomonitoring since they are common inhabitants of lakes and streams and are important in moving energy through food webs. Samples will be collected at locations and during sampling periods as defined in the Work Plan or Field Sampling Plan (FSP).

2.0 SCOPE AND APPLICATION

This section describes the species applicability, temporal applicability, and spatial applicability of the methodology described in this protocol. This protocol was designed to construct genetic database and DNA metabarcoding of macroinvertebrates. Benthic macroinvertebrates are operationally defined as those aquatic invertebrates that inhabit the sediments of a body of water and which are retained by a sieve with a 200 - 500 µm mesh. This SOP includes three sample collection methods: kick net sampling for wadeable streams, sweep net sampling for wetlands (area of 0.2-1.0 m depth) and grab sampling for sediment-associated macroinvertebrates for rivers and lakes. The grab sampler retrieves the top sixteen centimeters which contains the largest, most active benthic macroinvertebrate population susceptible to predation by higher trophic level organisms.

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.

4.0 PERMITTING AND NOTIFICATION

Contact the appropriate Department of Natural Resources and Fish and Wildlife Service offices to fulfill any permitting requirements before commencing fieldwork. A memorandum indicating sampling dates and locations must be sent to the appropriate offices prior to sampling.

5.0 EQUIPMENT, MATERIALS, AND REAGENTS (USE CHECKLIST ATTACHED)

- Global Positioning Unit (GPS)
- Calibrated probes to measure DO, temperature, pH, conductivity
- Turbidity meter
- Camera
- site health and safety plan (HASP)
- this SOP
- any necessary permits
- detailed map of the sampling area
- D-frame Kicknet (mesh size: 400-µm)
- Sampling net with 400-µm mesh size
- waterproof hiking boots and/or rain boots
- appropriate field clothing
- duct tape
- boat and electric outboard motor
- Sediment Grab sampler
- mallet
- Stopwatch
- Squeeze Bottle
- Ladle
- One pair chest waders
- Hats with insect netting
- Two waterproof field
- notebooks and two clipboards with waterproof paper
- 10 grams of certified clean sand (field blanks) each site
- 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 15-mL tube rack
- Disposable 1250-ul plastic pipettes

- One 1000-uL adjustable pipette
- 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)
- Spoon/tweezers
- Two Bucket: a light weight and light colored bucket that is 30-40 cm in height, 20-30 cm diameter, circular, with a handle and has a spout to pour off the sample.
- Two Sieve (mesh size: 200-µm)
- Two Sieve (mesh size: 400-µm)
- Two White tray
- Sample jars
- Tightly sealed container for sample jars & Formalin
- labels
- Plypropylene bottles

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 appropriate safety training. The objective of this section is to ensure that all of the necessary preparatory work is undertaken 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 list 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. Finally, sample labels will be printed in advance of the fieldwork.

6.2 Sampling Objectives

The primary objective of the field-sampling portion of a project is to collect eDNA from benthic invertebrates 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.

Note: We recommend that benthic macroinvertebrates sampling is conducted in late summer or fall.

6.3 Sampling Locations

Samples should be collected from various locations within a study area as defined in the work plan. The sampling method employed is based on the systematic probability sampling strategy, with site selections made using a stratified random method. Once field samples are collected, each location will be plotted and numbered on the most recent maps of the area.

For wadeable stream, select the kick area and path in the sampling location before entering the stream. Inform field team members to keep the selected area from distribution.

For wetland, select the area of 0.2-1.0 m depth with emergent and submergent aquatic plants which is most representative of the wetland and has safe wading conditions. Define the sweep area path before entering the wetland. Inform field team members so that this area is not disturbed.

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 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 Collecting sample using a kick net in wadeable stream

NOTE: Adapted from CABIN field protocol

A. At the downstream end of the kick area, place the kick net downstream of the sampler, flat side of the triangle resting on the substrate of the stream.
B. Walk backward in an upstream zigzag direction, dragging the net along the bottom of the stream while walking.
C. Kick the substrate to disturb it to a depth of ~5-10 cm if possible. For large cobble, turn over and rub your foot over the surface to dislodge macroinvertebrates clinging to the interstitial spaces. Brush the surface of large boulders with your hand or foot.
D. The net should always be held close to the area that is being disturbed to ensure that most of the disturbed substrate and organisms are swept into the net by the current.
E. Continuously zigzag over the stream bottom from bank to bank in an upstream direction for a period of 3 minutes.
F. If the sampler needs to stop to get around an obstruction, take a rest, or remove large cobbles from net, the timer pauses the stopwatch while the sampler lifts the mouth of the net from the water. The stopwatch is then restart when the sampler is ready to continue sampling by placing the net back in the stream.
G. The timers spots the sampler and alerts them of any upcoming obstructions while the sampler is traveling backwards and can't always see where they are going.
H. Transfer sample into sample jars 6.4.6.

6.4.4 Collection benthic macroinvertebrates using a sweep net in wetland

NOTE: Adapted from CABIN wetland sampling protocol

A. To begin, dip the net into the water just above the substrate and start the timer.
B. Walk forwards in a zigzag pattern, moving the net up and down in the water column from just above the substrate to just below the surface of the water. A tapping motion on the substrate with the bottom of the net may be helpful to stir up benthic macroinvertebrates which would otherwise be missed.
C. Move carefully to avoid entraining too much sediment in the net.
D. Keep motion steady and consistently in one direction to ensure that most of the disturbed substrate and organisms are swept into the net.

E. Continue to zigzag through the emergent and submergent aquatic plants for 2 minutes.
F. If the sampler needs to stop to take a rest or adjust his/her footing, the timer pauses the stopwatch while the sampler lifts the mouth of the net from the water. Restart the stopwatch when the sampler is ready to continue sampling and submerges the mouth of the net.
G. Transfer sample into sample jars 6.4.6.

6.4.5 Collection of lake or river invertebrates: grabbing sediment method

Techniques for acquiring macroinvertebrates vary based on the habitat available to biota. For example, if woody debris is present at a sampling location, it should be inspected while using large forceps to remove macroinvertebrates. Sediment associated benthic macroinvertebrates can be collected concurrent with sediment sampling using a grab sampler. In this case, five to ten samples of the top 16 cm will be collected.

1) The sampler should be "set" according to the manufacturer's instructions and lowered through the water column. Dredges should never be allowed to free fall into the substrate. The sampler should be carefully lowered the last few feet to minimize dispersal of fine material due to a sampler induced shock wave.
2) The sampler should be slowly raised through the water column and placed in an appropriate container.
3) If additional weights do not help in the collection of a sample, then the sampling equipment and techniques should be reevaluated for the type of sediment encountered.
4) Transfer sediments to a sieve bucket to remove sediment and debris following Section 6.4.7.

NOTE: Some disadvantages to the use of surface sediment samplers (dredges) include: possible shock wave and loss of very fine grained surface deposits; potential for water column contamination and nearby downcurrent sediment redeposition; larger materials such as twigs and stones prevents jaw closure.

6.4.6 Transferring sample from the net to sample jars

NOTE: Adapted from CABIN wetland sampling protocol

After kick net sampling or sweeping sampling, transfer sample from the net in to sample jars.

A. Splash the side of the net in the river to transfer all material to the collection cup at the end of the net (ensure that the mouth of the net is out of the water).

B. Remove the collection cup attached to the end of the net and empty the contents directly into a wide-mouth plastic sample jar, pail or sieve. Always work over a pail or tray in case of an accidental spill.
C. Wash any material remaining in the cup/net into the sample jar/pail/sieve using a squeeze bottle and forceps to remove any clinging animals.
D. Carefully rinse and discard any stones and large green leaves that have freshly fallen into river and are not invertebrate habitat.
E. Transfer sample from pail/sieve (if using) to sample jar. Check pail/sieve to ensure that no organisms remain.
F. Leave room in the sample jars for Ethanol. Use extra jars if needed.
G. Label the inside, outside and top of jar. The inside label should be written on waterproof paper marked by soft pencil. The outside of the jar should be in waterproof pen. All labels should have the following information: site code, date, and sample jar number.
H. If the amount of sand and gravel in your net is extensive and will likely require the use of many sample jars, elutration using a bucket swirling method may be applied to reduce this material (see bucket swirling section below).
NOTE: Seams and folds should be checked carefully for hidden organisms. The sample collected may require more than one jar, in which case it is critical to number and label the jars accordingly.

6.4.7 Bucket Swirling and Sieving to remove excess debris (only used in special situations)

NOTE: Adapted from CABIN wetland sampling protocol

Bucket swirling or elutriation, is a common method used by to remove large amounts of inorganic material (sand/gravel) from a sample.

A. Place the entire sample in the bucket. Add stream water to a depth of less than half the depth of the container.
B. Wash and scrape large stones and pebbles over the bucket to remove the macroinvertebrates then discard.
C. Swirl the water in the bucket to achieve a vortex. The organisms and fine particulate matter are lighter than the substrate and will float up.
D. Pour off the water that contains the organisms and fine particulate matter into a sieve while it is still moving, before it resettles.
E. Repeat step A-D until there is no organic matter rinsing out of the sand/gravel. Gently swirling the sand/gravel by hand may also help to suspend organic matter and organisms into the water column.

F. The remaining inorganic material should be examined for organisms, particularly caddisflies in heavy stone cases. Use forceps to pick out any remaining organisms and put them in the sample jar. If organism are still present in the elutriation, continue the bucket swirling process until all organisms have been added to the sample jar.
G. A general rule of thumb is to continue bucket swirling until the water runs clear.
H. Transfer the material in the sieve to the sample jar(s). Label and preserve appropriately.
I. Finally, it is recommended that if this is a field protocol utilized for a study, a subset (1 in 10 samples) of the gravel be retained in a separate jar and preserved and labeled appropriately for QA/QC purposes.

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.

8.0 SAMPLE PRESERVATION

Wear protective gloves and goggles.

1) For DNA barcoding or metabarcoding, frozen below -20 ^{o}C is recommended for animal DNA preservation.
2) For barcode database construction, use non-denatured ethanol to preserve invertebrate samples for both morphological identification and DNA barcoding. NOTE: DO NOT shake the jar as large gravel and rocks in the sample will damage the organisms.
3) For conventional identification, add Formalin into jar at a 1:3 ratio (1 part Formalin to three parts sample). Cap jar, gently swirl the sample to distribute the Formalin. NOTE: DO NOT shake the jar as large gravel and rocks in the sample will damage the organisms.

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.

10.0 REFERENCES

Canadian Aquatic Biomonitoring Network, Environment Canada. Field Manual: Wadeable streams. 2012, Cat. No.: En84-87/2012E-PDF.

Canadian Aquatic Biomonitoring Network, Environment Canada. CABIN WETLAND MACROINVERTEBRATE PROTOCOL, 2017, CW66-571/2018E-PDF.

Environmental Protection Agency, State of Ohio, USA. Sediment Sampling Guide and Methodologies, 2nd edition, 2001.

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 COLLECTION FOR DNA METABARCODING OF MACROINVERTEBRATES

Statue Number Item

- ☐ _____ Global Positioning Unit (GPS)
- ☐ _____ Calibrated probes to measure DO, temperature, pH, conductivity
- ☐ _____ Turbidity meter
- ☐ _____ Camera
- ☐ _____ site health and safety plan (HASP)
- ☐ _____ this SOP
- ☐ _____ any necessary permits
- ☐ _____ detailed map of the sampling area
- ☐ _____ D-frame Kicknet (mesh size: 400-µm)
- ☐ _____ Sampling net with 400-µm mesh size
- ☐ _____ waterproof hiking boots and/or rain boots
- ☐ _____ appropriate field clothing
- ☐ _____ duct tape
- ☐ _____ boat and electric outboard motor
- ☐ _____ Sediment Grab sampler
- ☐ _____ mallet
- ☐ _____ Stopwatch
- ☐ _____ Squeeze Bottle
- ☐ _____ Ladle
- ☐ _____ One pair chest waders
- ☐ _____ Hats with insect netting
- ☐ _____ Two waterproof field
- ☐ _____ notebooks and two clipboards with waterproof paper
- ☐ _____ 10 grams of certified clean sand (field blanks)
- ☐ _____ 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
- ☐ _____ Disposable 1250 ul plastic pipettes
- ☐ _____ 1000 uL adjustable pipette
- ☐ _____ 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)
- ☐ _____ Spoon/tweezers
- ☐ _____ Two Bucket: a light weight and light colored bucket that is 30-40 cm in height, 20-30 cm diameter, circular, with a handle and has a spout to pour off the sample.
- ☐ _____ Two Sieve (mesh size: 200-µm)
- ☐ _____ Two Sieve (mesh size: 400-µm)
- ☐ _____ Two White tray
- ☐ _____ Sample jars
- ☐ _____ Tightly sealed container for sample jars & Formalin
- ☐ _____ labels
- ☐ _____ Plypropylene bottles