GEN-FISH
Genomic Network for Fish Identification, Stress and Health
University of Windsor and Partners

eDNA Collection Data Sheet Instructions

GEN-FISH, University of Windsor, Windsor, ON

Please complete one eDNA Collection Data Sheet per sample site

ALWAYS TAKE eDNA WATER SAMPLES FIRST BEFORE DEPLOYING OR USING CONVENTIONAL SURVEY EQUIPMENT

*Traditional survey equipment can introduce DNA that can contaminate environmental samples
*Do **NOT** take samples in close proximity to traditional survey equipment

ALWAYS SAMPLE DOWNSTREAM SITES FIRST IF COLLECTING FROM MULTIPLE SITES IN SAME LOTIC SYSTEM

*Collecting samples at upstream locations can introduce contaminating DNA into the environment that can be carried downstream and subsequently collected

CHILL AND/OR FREEZE FILTERS ASAP

*Immediately place filters in chilled storage container for transportation after filtering (e.g. on freezer packs cleaned with bleach), and place in a *clean* freezer (-20C) as soon as logistically possible. If a clean freezer (i.e. a freezer lacking DNA or tissue samples) is unavailable, please place samples in an extra bag layer (i.e. one or two Ziploc bags) to protect them from contamination.

Colour Code:

- **Critical Information –** sample will be compromised without this data
- **Important Information –** important data for modelling/biological inferences
- **Informative, but not crucial –** data that would improve modelling, but not essential

Site Identification and Team Details:

- **Project Name**
- **Site Code:** Use three initials of principal member of field crew, last two numbers of year, and three numbers representing collection's chronological place in that year.
  Example: *FBC-95-001* or *FBC-95-Jun-001*
- **Collector:** Record name of the member of the field crew conducting sampling.
  Example: *F Cross, C Darwin, and E Ricketts* or *NE Mandrak and ichthyology class.*
- **Recorder:** Record name of the member of the field crew completing most or all of field sheet.
- **Other Crew:** Record name of any other crew members
- **Date:** (dd/mm/yy)
- **Arrival Time, Departure Time:** Record the time when arrived at, and left, field site, using the 24-hour system (i.e., "military time").
  Example: *Start Time: 1300h Stop Time: 1500h*
- **Start Time, Stop Time:** Record the time when fieldwork began and ended at the site, using the 24-hour system (i.e., "military time").

Example: Start Time: 1300h Stop Time: 1500h

- **Starting Location:** Record the name and address of the location where your crew begins their work day before travelling to sampling sites (e.g. University of Windsor, 401 Sunset Ave, Windsor, ON)
- **Waterbody Name:** Do not abbreviate principal name of river, creek, wetland, lake, etc. Compass points used as adjectives of the principal name may be abbreviated. The following
- abbreviations for the types of waterbody may be used, but all others must be spelled out: R. = River; Cr. = Creek; Res. = Reservoir.
  Example: *Solomon River* or *N. Fork Solomon R.* or *Wilson Reservoir* or *Cedar Bluff Res.*
- **Latitude, Longitude** (dd.ddddd, -dd.ddddd): take using GPS as close to sampling location as possible.
- **Narrative Locality Description:** Give the distance north or south and then east or west from a sizable town (e.g., county seat) or some other prominent feature nearby to the study site.
  Example: *26 km and 8 km E of Hays on FAS 235 (Saline River Road) E of US Hwy 183.*

eDNA Sampling Method:

- **Gear Type** – OSMOS, manual, other (be specific)
- **When Filtered?** – date (dd/mm/yy); military time (xx:xx)
- **Where Filtered? -** onsite, lab, other (e.g. motel)
- **Time Until Sample Frozen** – Record how long (in hours) between when samples were collected and placed in a freezer for storage.
  *Note: samples should be frozen as soon as logistically possible
- **Preservation Method** – how samples were preserved (RNAlater or silica beads).
  - RNAlater is ***required*** for preservation if study objectives include analysis of RNA from environmental samples (e.g. microbial community transcriptome, eRNA, etc.)
  - Silica beads are a preferred option when *only* analysis of DNA data is required – silica beads produce high-quality eDNA and are a 'chemistry-free' preservation method, reducing the impact of preservation on downstream processing (e.g. extraction, quantification, etc.) and improving standardization. Note, however, that silica bead preservation *will degrade any RNA collected in the sample* – you will not be able to recover eRNA or microbial RNA at a later time-point
    - Note: Dry filter on manifold (i.e. maintain suction after water has been completely filtered) for 30 seconds to minimize the amount of water retained on filter prior to storage with silica beads. Silica beads will become saturated and ineffective if exposed to too much moisture. Note that beads change colour when saturated – if complete saturation of beads occurs, exchange with new, dry beads.
- **Sample No. –** Record how many replicates were created (3 replicates are typically requested)
- **Volume Filtered** (ml) – volume of water filtered
- **Pre-filter** – was water sample pre-filtered?

- **Was a blank created? –** Yes or No. Field blanks are required every 3 sampling sites if sampling from the same water body or every time you move by vehicle to sample a new location.
- **Filtering Time** (sec) – time taken to filter sample
- **Filter Pore Size -** Record the filters being used, as well as material (typically 1.5um GF filters)
- **Other:** Record any other observations you think are important

If Lentic:

Lake Site Dimensions:

- **MAX Site Depth (m)**: depth at exact sample location using meter stick, graduated line, handheld sonar.
- **Distance from Shore Sampled (m)**: distance sample taken from shore.
- **Depth Sampled (m)**: depth at which sample taken (using meter stick, graduated line, etc.

Habitat Classification:

- Circle the general habitat classification/type that best describes habitat from which sample was collected.

Thermocline:

- Circle whether the lake is stratified (yes | no | unknown) and, if yes, whether the sample was collected above the thermocline (yes | no | unknown)

If Lotic:

*Note: If study objectives include estimating eDNA concentrations/quantity, then estimating discharge is **critically** important. The 'Colour Code' for the '**Depth Measurements**' and '**Stream Velocity Measurements**' data therefore changes from (•) to (•)

Stream Site Dimensions:

- **Stream Width (m)**: use laser range finder, tape measure.
- **Habitat Type (Riffle | Run| Pool)**: Circle habitat type describing to sample collection location
- **Distance from Shore Sampled (m)**: distance sample taken from shore.
- **Depth Sampled (m)**: depth at which sample taken using meter stick, graduated line.
- **MAX Site Depth (m)**: depth at exact sample location using meter stick, graduated line, handheld sonar.

Stream Flow:

- **Stream Flow (None | Slow | Medium | Fast)**: estimated if not measured.
- **Flow Meter Used**: e.g. Swoffer, orange, etc. (if measured).

- **Stream Velocity Measurements:** Minimum of one stream velocity measurement in stream thalweg. If possible, take three measurements along horizontal stream transect equidistant from each other and shore line, with middle estimate taken mid-stream

- **Flow Relative to Typical Levels**: are stream levels low, typical, or high *for that season* (circle one) – record only if familiar with historical site conditions

<u>For all Sites (both Lentic and Lotic):</u>

Site Characteristics:
Measure water-quality parameters at exact location and depth of water sample. Repeat measurements at same depth 1 m to the left (or upstream in flowing waters) and 1 m to the right (or downstream). If measurements vary by more than 5%, confirm that equipment is working properly and repeat measurements. Record instrument used.

- **Water Temperature, Air Temperature:** Record the values for each.
- **Conductivity:** Record the value from a conductivity meter.
  Example: *540 µS/cm or µmhos/cm at 18°C or 25°C*
- **pH:** Record the value to the nearest 0.1 standard pH unit.
- **Dissolved Oxygen:** Record the value, if determined.
- **Turbidity:** Record the value from a turbidimeter (values other than NTU should be noted).
- **Other Chemical Measurements:** List other chemical attributes tested, if any, and their values and units.

Biological Components:

- **Aquatic Animals**: record any animals observed (e.g. snails, leeches) and their number.

Weather:

- Record the general conditions during the visit
- **Wind Speed**: Provide only if have instrument, e.g. Kestrel.
- **Wind Direction**: Use ordinal directions to two levels (e.g. NE).

Photographs:
Please take a digital photograph of each sampling site. Record photograph file numbers for each category.

- Site
- Filter after filtering
- Completed field sheets