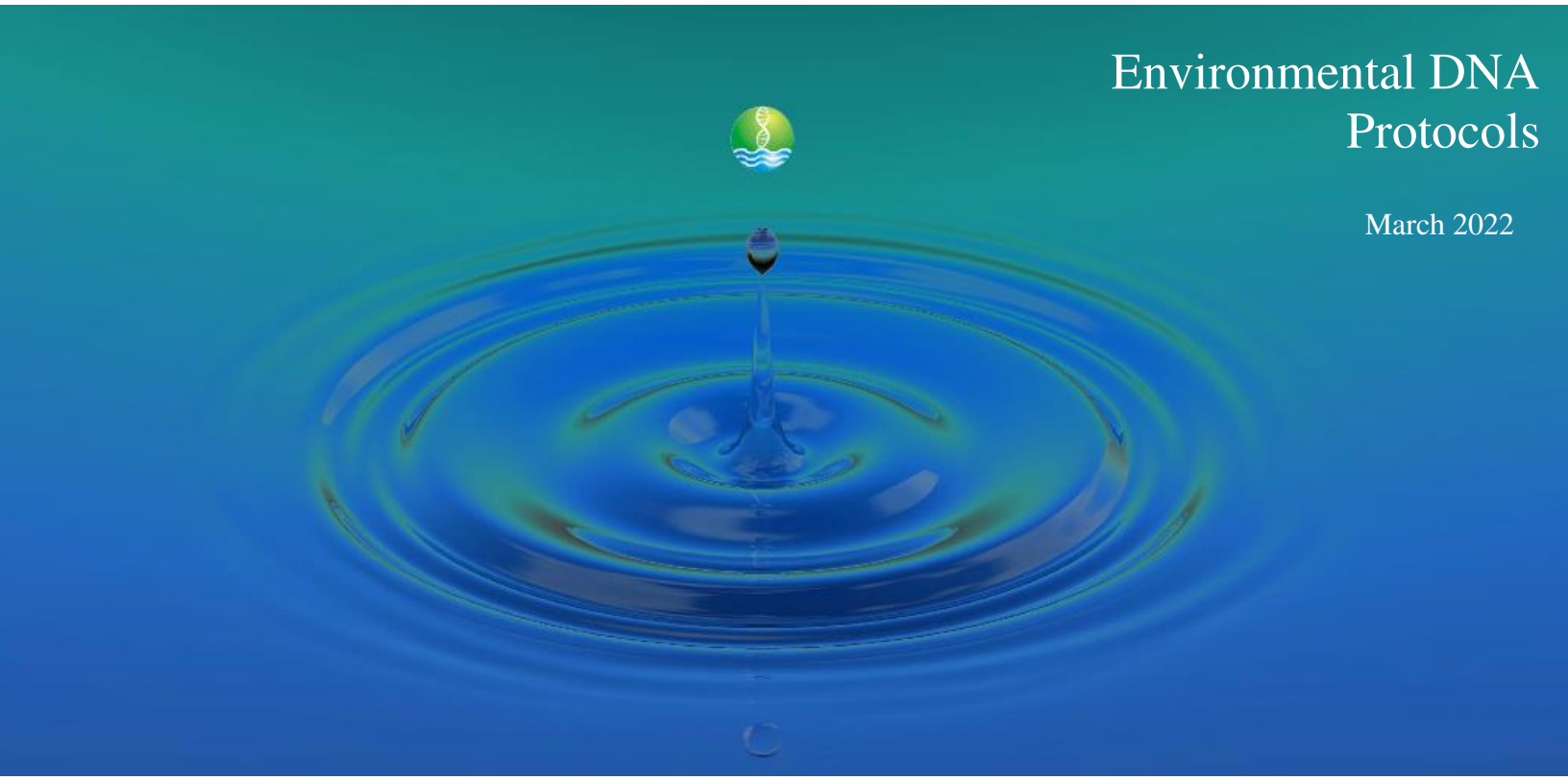

Environmental DNA Protocols

March 2022

Field Collection Procedure for Aquatic Environmental DNA sample collection and analysis

Procedure prepared by:

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MATERIALS

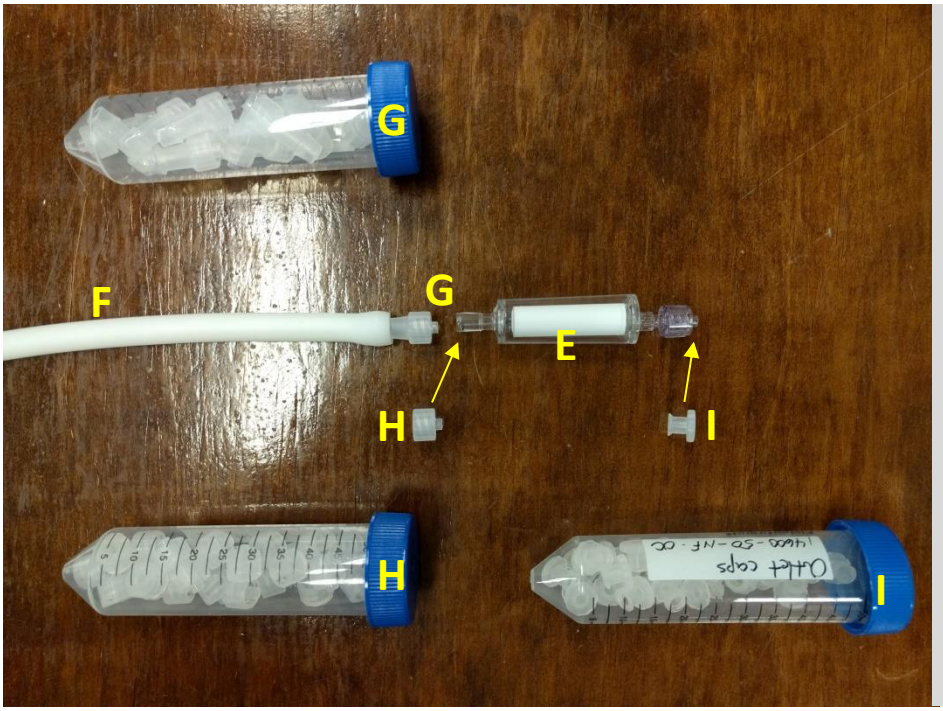
Material List

1) Cordless drill (brushless)
2) Pump driver bit (1/2 inch spade)
3) Backup drill battery and battery charger
4) Cole Parmer Peristaltic Pump MasterFlex Easy Loader II Model#77200-52
    a. Pumps can be chained together when samples collected in unison
5) USEPA approved Millipore Sterivex™ 0.45 µm sterile filter units (EPA# 90260-ITA-001)
    a. Clean scissors if cutting tubing in the field.
6) Masterflex spooled peroxide-cured silicon tubing, L/S 15
7) Tube adaptor (Cole Parmer 30800-22)
8) Inlet caps (Qiagen Mat. No. 1104193)
9) Outlet caps (Qiagen Mat. No. 1104194)
10) Graduated beaker
11) Nitrile gloves (non-powdered)
12) Ziploc bags
13) Permanent markers
14) Backpack or toolbox
15) GPS with extra batteries*
16) Cooler with blue ice pack
17) Ice chest with blue ice packs if many filters are collected in one day*
18) Garbage bags

*Optional, project dependent.

Pump Apparatus

A) Cordless drill (brushless)
B) Pump ½" driver bit
C) Peristaltic Pump mounted to 2x6
D) Pumps in series



Filter Apparatus

E) Sterivex™ filter (0.45um)
F) Silicon tubing
G) Tube adaptor
H) Inlet caps
I) Outlet caps

CONTAMINATION PREVENTION

Guidelines

1) The Genidaqs protocol is crafted to reduce the possibility of contamination, primarily through the use of a filter with filtration material shielded within an external housing. Therefore, the filtration material is never handled by the user. Irrespective of filter, following the procedure ensures the best chances of success.
2) Prior to project initiation, Genidaqs recommends that field personnel be trained on the collection protocols, which in part consists of field personnel verifying that they can reliably generate both negative and positive field collections.
3) The Genidaqs protocol uses pre-packaged sterile material. Material should be transported prior to field use in "clean" containers.
4) Ideally there should be a "clean" person handling the filter after water collection that bags and tags. If a single user is in the field, change sterile gloves at each site.
5) Consumables are considered single use. Following use, filter packaging, silicon tubing, gloves, etc. are immediately placed in "dirty" storage.
6) When sampling a flowing water system, conduct collections moving upstream.
7) Take one negative field control per sampling crew at the start of each sampling day. The negative field control consists of pumping clean water through a fresh filter. A clean, sealed bottled water works well as a field control.
8) Place filters on ice between sites. Store in a freezer when done with field work and until delivering samples to the lab. Transport samples on ice in cooler.

General protocol for rinsing and re-using tubing at multiple sites

1) Upon arriving at the new sampling site, pump water through the tubing for approximately 2 minutes prior to attaching filters to flush out any eDNA from the previous site.
2) When flush is complete, attach filters and begin sampling.
3) If sampling in flowing water (river, stream, tidal, etc.), flushed water can be placed on to shore, into a secondary container or into the water. Flowing water will move any contaminants outside of the sampling area.
4) If sampling in stagnant water (vernal pool, pond, lake, etc.), flushed water should be directed on to shore or into a secondary container to not contaminate the new sampling site. In the rare chance that this is not possible, complete flush close to but not directly at the new sampling location or use new tubing at each site.

General protocol for sterilizing instruments and equipment for analyzing genetic samples

Materials needed:

- 20% household bleach solution (mixed with tap water) in spray bottle or beaker
- DI or tap water
- Kimwipes or paper towels

1) Rinse all tissue and body fluids off of instruments, vials, petri dishes, and any other item being exposed to sample tissue using tap water.
2) Submerge any submergible items in 20% bleach solution for at least 1 minute. Be sure to completely expose all surfaces to bleach, including opening and closing scissors while submerged.
3) Use spray bottle to coat surfaces of larger items (pumps, coolers, etc) that cannot be submerged into bleach solution. Spray paper towel with bleach solution and gently wipe surface of non-submersible items such as pens, drills and batteries.
4) Remove items from bleach and rinse with DI water or tap water. Spray clean water on to paper towel and wipe bleach off of non-submersible items. Make sure to remove all bleach. Thoroughly dry items with a fresh Kimwipe, paper towel or air dry on clean paper towels.

SAMPLE COLLECTION

Protocol reference:

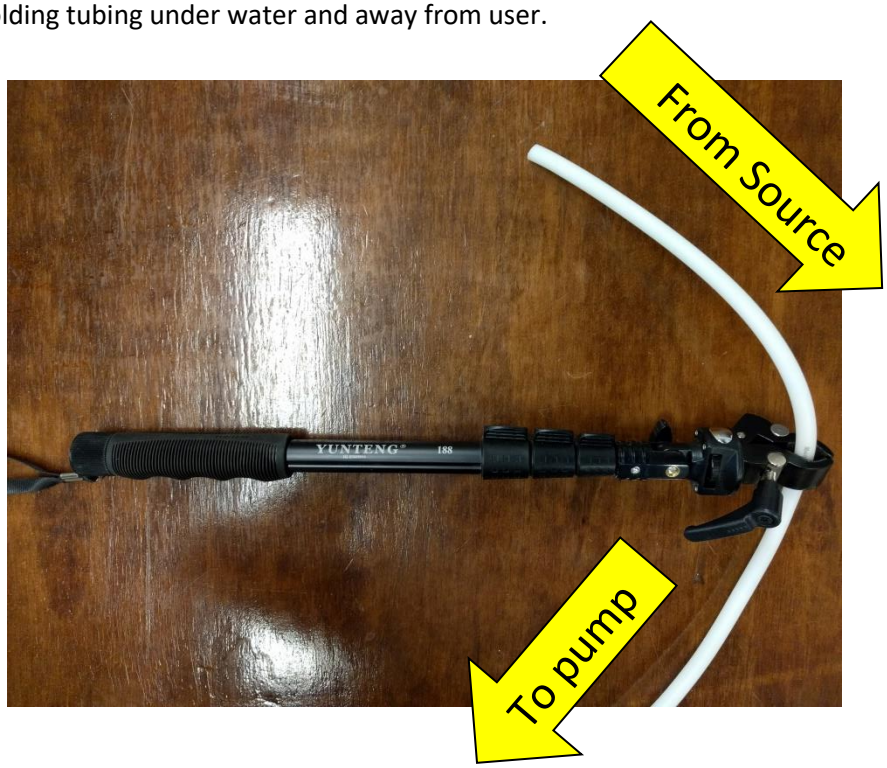
Bergman, P.S., G. Schumer, S. Blankenship, and E. Campbell. 2016. Detection of Adult Green Sturgeon Using Environmental DNA Analysis. PLOS ONE 11(4): e0153500.

Water Collection and Filtration – Point Sampling

Reminders:

- Collect 1 field control at the start of the sampling day per field crew.
- Put on clean gloves at each sampling site. If in doubt of glove cleanliness, switch gloves.

1) Water samples may be collected either from the bank margin, or, where this is infeasible due to dense vegetation or steep topography, by vessel at the channel center.
2) For each sampling event, water is filtered directly from the water body at an approximate depth of 2-6 inches below the surface. We have found a fishing pole or telescoping pole (hiking pole) useful for holding tubing under water and away from user.



3) Sterile Masterflex spooled peroxide-cured silicon tubing, L/S 15 with an internal diameter of 4.8 mm is used in association with a portable Masterflex1 L/S Easy-Load II Model#77200-52 peristaltic pump powered by a cordless hand drill. The tubing is clamped within the pump, so

the pump never comes into contact with the water. Peristaltic action draws water from the source.

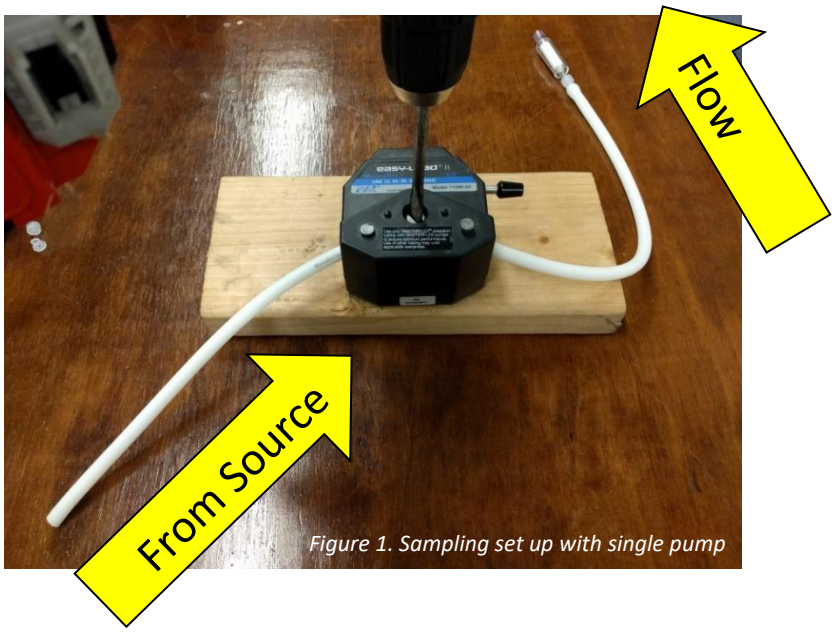


Figure 1. Sampling set up with single pump

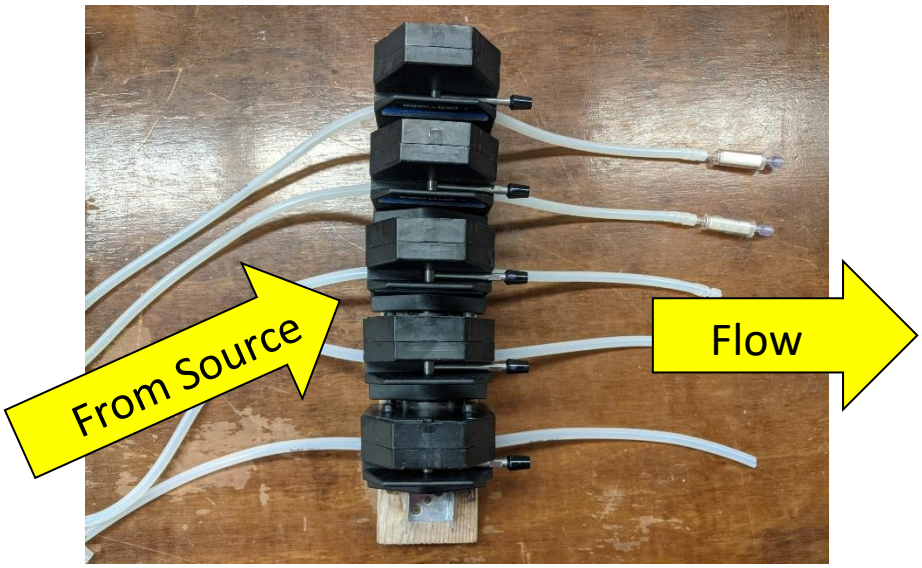
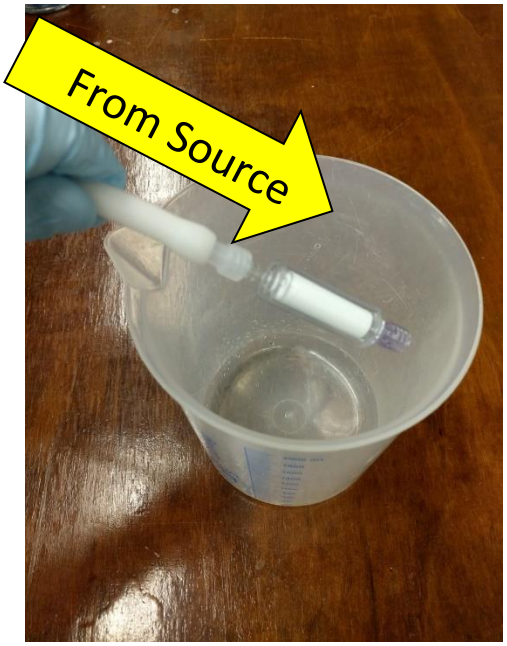


Figure 2. Sampling set up with multiple pumps

4) Water samples are filtered through a Millipore Sterivex™-GP 0.45 µm sterile filter unit. Partially tear open filter packaging to expose filter and connect to tube adapter. The filter only connects to the tube adapter in one way, ensuring proper connection. Note that water is expelled out of the filter and is not sucked into the filter, which is a reversed orientation to that of the commonly used grab samples using disc filter apparatus.



Pro tip: If the tube adapter isn't used (or forgotten) the tube itself can be press fit over the filter inlet and the process will work. However, note that if filter is installed backward, water will simply flow through the filter assembly and not come into contact with the filter material (i.e. no organic material will be captured).

5) All water filtration occurs directly at each site. No water is transported or stored during sampling nor is any water transported between sampling sites.

6) Sample filtrate is captured and measured in a graduated beaker to verify the volume filtered for each sample. Filtration ceases when either the target volume is reached or the filter clogs. Reverse pump to drain remaining water from inside the filter assemble. Note: it is very important to remove water from inside filter. If water is left inside the filter housing, the plastic may break when filters are frozen. If pumping is ineffective the filter can be manually shaken to remove water.

7) In water with high levels of particulates, sediment may accumulate inside filter assembly. This is ok as long as residual water is removed from filter assembly.

8) Filtrate is poured out after completion of sampling at each site.

9) After filtration, the inlet and outlet of filters are capped at each end, the filter is labelled with location ID and volume filtered, placed into a sterile secondary container (Ziploc), sealed, and immediately placed in a cooler. Filter is considered self-contained at this point. Multiple filters from the same site can be placed in the same secondary container (bag). Filters from different site should be stored in separate bags.

10) Gloves are immediately disposed of after each use into a sealed trash bag. Tubing should be disposed of after each site unless the tubing rinsing protocol is being followed.

11) All filters are kept in a cooler for the duration of the sampling day, after which they are transferred to a -20 °C freezer. Filters must be transported or mailed to the Genidaqs laboratory on ice. If mailed, filters must be shipped overnight, and prior notice must be given to Genidaqs staff to ensure timely delivery. The filters are stored within individually sealed secondary containers until DNA extraction.

Pro tip: Filters can be wiped with bleach to sterilize outside or each filter prior to storage and placed into a new secondary container labelled appropriately for project. This bleach step breaks the connection between field and lab environments. In the laboratory, all filters are wiped with DNA neutralizing solution prior to DNA extraction.

12) To ensure that field equipment is free of contamination, DNA field controls are taken for each sampling day. Each field control consists of Sterivex™ filtered water processed in the same fashion as the field samples. The field controls are processed for the presence of target species DNA in parallel with all samples. One field control is collected per sampling team at the start of each sampling day.

Water Collection and Filtration – Transect Sampling

Transect sampling involves using a vessel to move across a landscape while simultaneously collecting an eDNA sample. This sampling technique is preferred for sampling across larger areas or when there is a need to move across stagnant water to capture eDNA particles that may not be encountered when using a stationary point sample. Genidaqs conducts two types of transect sampling styles: (1) moving point sample, and (2) aggregate subsampling with sump.

Moving points sample:

Additional sampling gear:

- Mobile vessel (boat, kayak, raft, etc)
- GPS unit/map with desired transects

1) Plan transects out prior to going into the field. Use GPS with map to orient yourself and find location of transects when in the field.
2) Sample transects from downstream to upstream if there is flow.
3) Using a mobile vessel, follow sampling instructions as written for the point sampling methodology while moving across the pre-planned transect. Typically, one or more staff collect the sample while one or more staff move the vessel.
4) Try to filter water throughout the entirety of the transect. This will involve slowing down the pumping rate (drilling speed) to moderate filtration rate to match transect speed. Ideally, filtration will be complete (filter will clog) close to the end of the transect. Drilling speed will vary depending on turbidity and transect length.
5) Filter replicates can be collected in unison with one transect pass using multiple pumps chained together (Figure 2).

Aggregate subsampling with sump:

Additional sampling gear:

- Boat with power accessory plug
- Inverter (Bestek 300W Power Inverter, model #MRI3011BU)
- GPS unit or map with desired transects
- Submersible pump (sump) (Green Expert 1/6HP sump model# 203617) with hose attachment
- Hose
- Hose flow splitter (Optional. Splitter can be used to slow flow during long transects)
- Anchor (Optional. May be needed to keep pump submerged)
- Large secondary container

1) Plan transects out prior to going into the field. Use GPS to orient yourself and find location of transects when in the field.
2) If flow is present, start at the downstream end of the transect. Collect water into a large secondary container on board the boat for the length of the transect using the sump (Figure 3A).

It may be necessary to calculate the required container volume based on pump flow rate, transect length, and boat speed prior to conducting field work.

3) Once transect is complete, follow the sampling methodologies described in the Point Sampling section to collect eDNA samples from the secondary container (Figure 3B).

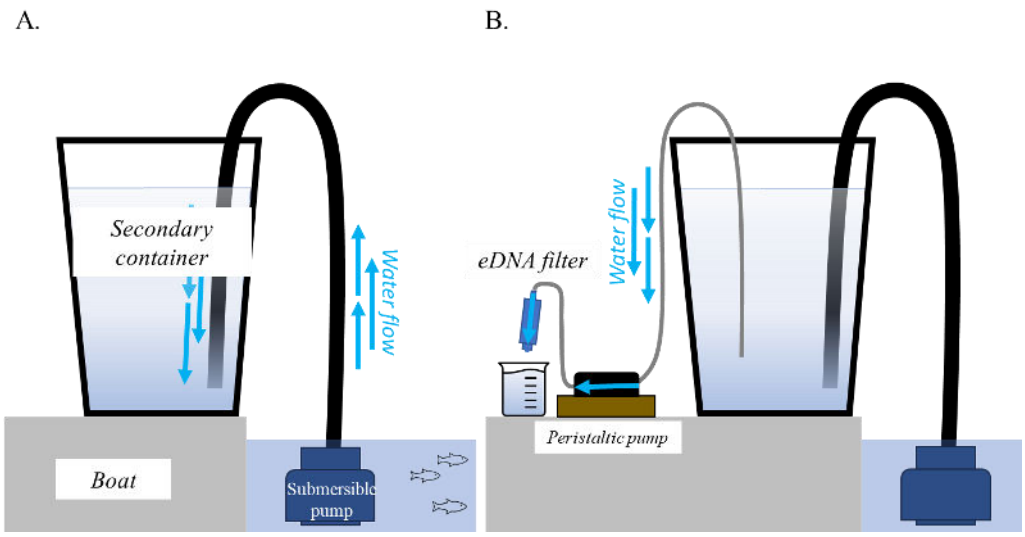
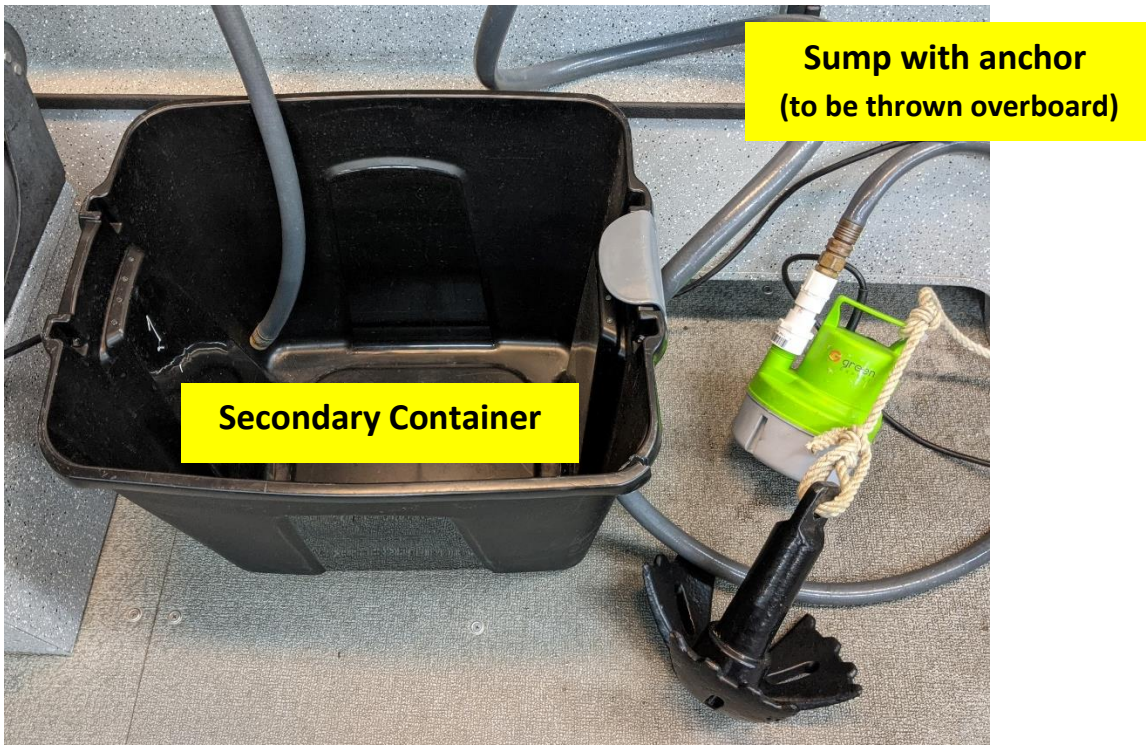


Figure 3. (A) Sump subsampling into secondary container. (B) Sample filtration from secondary container.



4) If multiple transects are to be completed in one day, sump and secondary container can be rinsed in between sites using a similar methodology to the tube rinsing protocol: sump is run for

2 or more minutes after which the sump hose is used to rinse the walls of the secondary container downstream and prior to the start of the next transect to remove eDNA from the previous transect. Separate clean secondary containers can be used for each transect if desired.

FILTER PROCESSING

We will not detail here processing of the sterivex filters and the DNA extraction. DNA from all samples and controls are extracted using PowerWater Sterivex™ DNA Isolation Kit (Mo Bio Laboratories, Inc.) following the manufacturer's recommended guidelines. Please refer to Bergman et al. (2016) for more details.

OTHER RESOURCES

Other procedures available from Genidaqs:

1) Field Collection Procedure for Environmental DNA from Soil.
2) Procedure for field preservation of gut contents for genetic analysis.
3) Tissue sampling and preservation procedures for genetic analysis.
4) Procedure for collection of DNA samples using external buccal swabs.