

Environmental DNA Protocols

January, 2017



Field Collection Procedure for Aquatic Environmental DNA sample collection and analysis

Procedure prepared by:

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MATERIALS

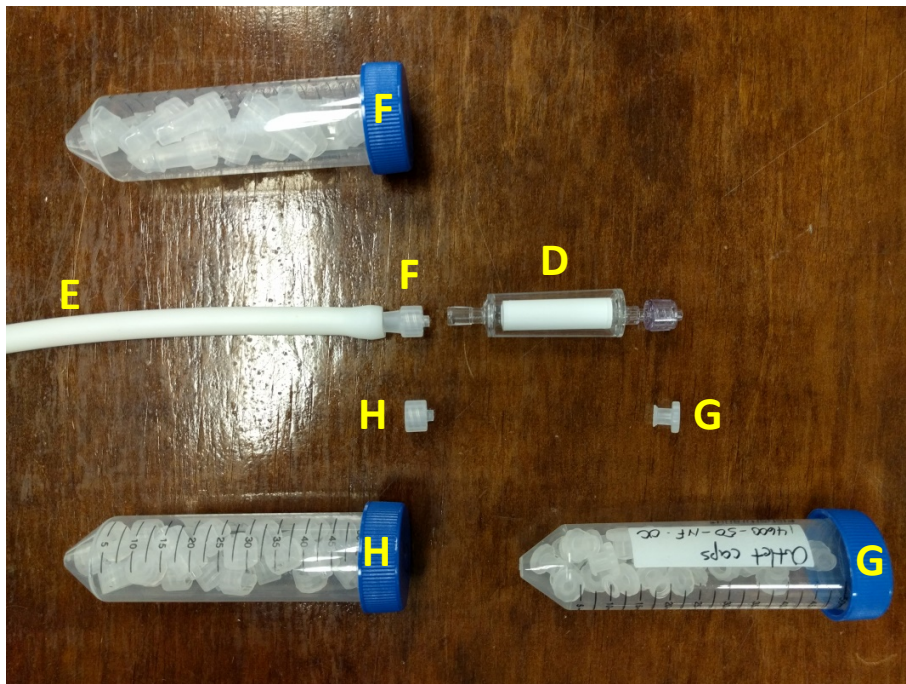
Material List

- 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
- 5) USEPA approved Millipore Sterivex™ 0.45 µm sterile filter units (EPA# 90260-ITA-001)
- 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 and chestpack.
- 15) GPS with extra batteries
- 16) Cooler with blue ice pack
- 17) Ice chest with blue ice packs
- 18) Garbage bags



Pump Apparatus

- A) Cordless drill (brushless)
- B) Pump 1/2" driver bit
- C) Peristaltic Pump mounted to 2x6



Filter Apparatus

- D) Sterivex™ filter (0.45um)
- E) Silicon tubing
- F) Tube adaptor
- G) Inlet caps
- H) 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 field negative controls

General protocol for sterilizing instruments and equipment for analyzing genetic samples

Materials needed:

- 20% household bleach solution (mixed with tap water)
 - DI water in wash bottle
 - 70% ETOH in wash bottle
 - 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 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) Remove items from bleach and rinse with DI water. Make sure to remove all bleach.
 - 4) Rinse all items with 70-100% ETOH.
 - 5) Thoroughly dry items with a fresh Kimwipe, paper towel or air dry on clean paper towels.

SAMPLE COLLECTION

Protocol reference:

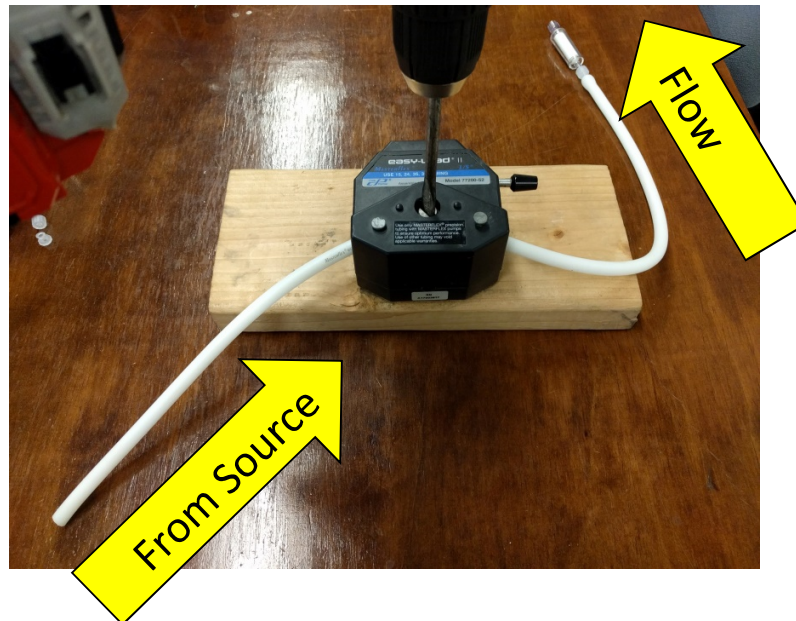
Bergman, P.S., G. Schumer, S. Blankenship, and E. Campbell. 2016. Detection of Adult Green Sturgeon Using Environmental DNA Analysis. PLOS ONE 11(4): e0153500.

Water Collection and Filtration

- 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 useful for holding tubing under water and away from user.



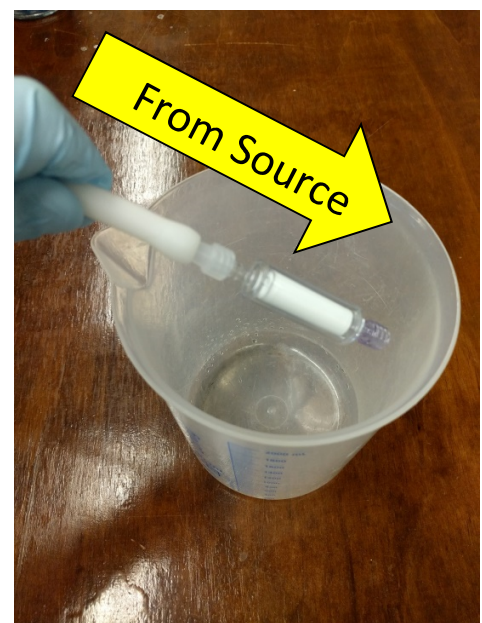
- 3) Sterile Masterflex spooled peroxide-cured silicon tubing, L/S 15 with an internal diameter of 4.8 mm is used in association with a portable Masterflex1 L/S Easy-Load II Model#77200-52 peristaltic pump powered by a cordless hand drill. The tubing is clamped within the pump, so the pump never comes into contact with the water. Peristaltic action draws water from the source.



- 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 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 process will work. Note: 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 assembly. Note: it is very important to remove water from inside filter. If pumping is ineffective the filter can be manually shaken to remove water.



Genidaqs aquatic eDNA sampling procedure

- 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, 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.



- 10) Tubing and gloves are immediately disposed of after each use into a sealed trash bag.
- 11) All filters are kept on in a cooler for the duration of the sampling day, after which they are transferred to a -20 °C laboratory freezer. The filters are stored within individually sealed secondary containers until DNA extraction.

Genidaqs aquatic eDNA sampling procedure

Pro tip: Filters can be wiped with bleach to sterilize outside of 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.

- 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.

Sampling Variation

Genidaqs does not recommend grab sampling, but at any given time this may be necessary due to access issues. In place of grab samples, we have found use of a sump pump beneficial for sampling large water features, as it increases both the water sampling space survey for eDNA and the distance from source to pump apparatus. In the image shown (right) a submersible pump located 100 ft. away delivered water to orange bucket. Water filtration occurred from within the bucket, with both the filtered volume and pumped volume recorded.



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.