

# CORNELL INVASIVE AQUATIC SPECIES PROGRAM

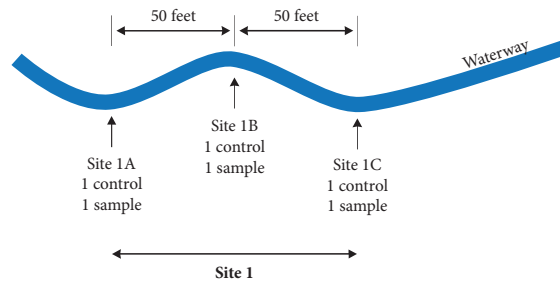
## eDNA Collection Protocol

You will be helping Cornell scientists to look for invasive fish species in waters near where you live. You can find out more about the project and the kinds of fish you will be looking for on the FishTracker website at [fishtracker.vet.cornell.edu](http://fishtracker.vet.cornell.edu)

Collecting samples to test for invading fish is easy, but it is important that you follow the directions below carefully. Collecting will go much more smoothly if you read about how you will collect the samples BEFORE you start. Please make sure you are familiar with all of the steps before you begin. By following the collection instructions carefully, you can insure that the water samples you collect are an accurate representation of what is in the water you are testing.

### Planning your collection

Before you go out to collect samples, the first thing to consider is where you will collect. The edges of streams, lakes, rivers, or ponds are all good places to collect water samples. If possible, plan to work at a site where you will be able to collect water at three side-by-side locations, each about 25-50 feet apart. Here's a map that shows an example of collecting at 3 adjacent locations at one site. If the terrain makes collecting from 3 locations difficult, that's okay, do as many as you can.



### What is in the collection kit?

Once you know where you will collect, check out what is in your kit.

Everything you will need to collect DNA evidence of the presence of invasive fish is provided in the kit, except for a few items like water for the control and a GPS unit to identify where you collect your samples.

The items in the kit are listed below. The list will help you to check that you have all of the materials you need. Find all of the items listed in your kit. Your teacher will go over the directions for using the kit with you. You can practice using the pump and setting up the funnel (without any water) to make sure you know how to assemble and use it before going out to collect your samples.

Keep the kits at room temperature.

Complete eDNA Sampling contents for one site:

1. 3 Polypropylene suction flasks
2. 3 Büchner funnel (2 pieces)
3. \*\*3 Rubber stopper adapter for funnel (replaces black gasket shown in the picture)
4. 6 Glass fiber filter discs
5. 3 Hand held vacuum pump (you may get a different type of pump)



Figure 1. The materials.

6. 3 Ziploc bags for water collection
7. Round toothpicks
8. 2 Forceps (1 for controls, 1 for samples)
9. 6 Collection tubes with buffer for storing filters after use
10. 3 Gloves for water collection (not shown)
11. 1 Black bag for storing used equipment in the field. (Not shown)
12. 1 Small trash bag for disposable waste (not shown)
13. A second black plastic bag for return shipment of washed used equipment (not shown)
14. 2 Red ties for closing the black bags (not shown)

Needed but not provided -

15. Water for control samples - **Not provided** For each control, you will need ~ 300 ml of water. You can use tap or bottled water that is clean and free of fish DNA.
16. GPS unit - **Not provided** (Many cell phone apps will provide this information)
17. Clipboard and waterproof pen/pencil - **Not provided**

Each kit provides materials for sampling one site. Kits will be shipped to you in box all the materials needed to process 3 control sample and 3 field samples (6 samples total) from 3 locations at one site. If you request materials for more than 1 site, the materials for each site will be sent in separate white plastic bags in one box. Materials for each site collection should be kept separate to prevent fish DNA from one site from contaminating water from another site.

### Overview of the collection process

One kit gives you enough material to sample one site. Sampling one site involves collecting and filtering water at up to three side-by-side locations (A, B, and C), each about 25-50 feet

apart. Your white bag contains 6 green-capped tubes and 6 filters for processing your 3 controls and your 3 samples from that site. You will need to use all of them.

Let's go through the general procedure for a single site (Site 1).

1. First, at each location within a site you will filter the control water you bring with you. You need 1 control filter per location to insure that the equipment is not contaminated. The control filters go into the green-capped tubes labeled Site 1 Location A Control, Site 1 Location B Control, and Site 1 Location C Control.

2. After the control is done, at each location (A, B, and C) you will filter 1 sample of about 300 ml of collected water (from your lake, river, stream, or pond) using the same flask and pump you used for the control at that location. For example, you will filter about 300 mL of water collected from Location A using the same flask, funnel, and pump that you used to filter the control water for Location A. The sample filters go into the green capped tubes labeled Site 1, Location A, Sample 1.

3. The same process will be repeated for Location B and Location C, using a separate flask, funnel, and pump for each location. The filters will go into the matching green capped tubes labeled Site 1, Location B, Sample 1 and Site 1, Location C, Sample 1.

That gives you a total of 6 tubes per site, 3 Controls and 3 sample filters, one from each of the three locations within the site. The controls are very important. They are used to make sure you aren't bringing fish DNA in with you as a contaminant, and that the equipment is not contaminated. The control should give a negative (no invasive fish DNA) result. The three samples from multiple locations will be used for rechecking positive results and archiving samples.

If you plan to sample more than one site, you will need one white bag for each site. So, for example, if you plan to sample at one site on a river and then at another site a mile or more away, you will need two white bags (one for each site). You will collect samples at 3 locations at each site, using 6 filters and 6 green-capped tubes for each site (i.e. a total of 12 filters and tubes for two sites).

## Collecting and processing your water samples

### Before you go into the field

1. Organize your materials and select all of your locations before you begin.
2. You can practice assembling and disassembling the filtering apparatus in the classroom before going into the field. Just be sure to keep all of the kit materials together in their white bag.

**NOTE** - The DNA test used to identify invader fish is very specific and will not give a signal in response to any other DNA present in the environment (like human or bacterial DNA). This means that the contents of the kit do not have to be sterile. However, you should still keep things clean and avoid touching the inner surfaces of your equipment as much as possible. Enzymes that break down DNA are found on human skin and could be transferred to the plastic surfaces, where they might break down the DNA you collect.

3. Review where you will collect the 3 water samples and decide who will be responsible for what part of the activity. Someone (with good handwriting!) should be designated to record needed data and to fill in the collection form, including GPS coordinates and a description of the site. Someone needs to collect the water sample, others need to do the actual filtering, which often involves more than 1 person. Someone must be responsible for picking up materials so nothing is left at your site.

4. Review Safety Precautions

### **Safety Precautions**

NEVER collect water samples alone.

Samples can be collected from shore or from a deck or pier over shallow water. Never push or shove near the water, even if it is not deep.

Keep an eye out for potential hazards. Things like slippery ground or docks, submerged or floating electrical cables, equipment, docking boats, and other people are common near the water's edge.

Wear sturdy footwear. Surfaces may be slippery, and there may be obstacles or sharp objects in the collection area.

Check weather forecasts before traveling to the monitoring site and keep in mind that weather conditions can change quickly. Avoid collecting in bad weather and never go out if there is a chance of lightning.

Dress correctly for predicted weather conditions.

**Always follow your teacher's instructions.**

5. Don't forget your control water and GPS unit.

### **In the field**

#### **Before you collect**

1. Review your team organization. Know who is responsible for doing what.

2. Fill in the information requested on the collection form (COLLECTION FORM - CORNELL INVASIVE AQUATIC SPECIES PROGRAM). This what we will use to identify the DNA samples you collect. Make sure that the collection tubes from a site match the information on the collection form - otherwise we will not have any way to identify where the samples come from.

3. Record the GPS readings for your site. Your teacher may do this using a cell phone.

4. Open your kit at a spot well away from the water. The contents for each site are in a separate white bag. **If you are sampling more than one site, the other kits should remain in your vehicle until you are actually at the site where they will be used.**

Organize the kit materials so that everything is easily accessible. Set aside the **black plastic bag and the small trash bag** found inside your kit. The black plastic bag can be laid out to provide a clean surface for the kit materials if needed.

5. Remove the collection flasks, Buchner funnels, filters, forceps, and vacuum pumps. Make sure the materials for each location are together.

**NOTE:** To prevent cross contamination between sites, when you are finished with your collection at one site, all used equipment should be placed directly into the black bag and left in the bag until you return to your classroom. Never reuse equipment once it has been placed into the black bag.

The small trash bag in the kit is for disposal of non-reusable materials like gloves, toothpicks, used water collection bags, and the white bag your kit originally came in. When you are done with your collection, place all disposables in the bag and dispose of properly when you return to school. Please do not leave anything at the collection site.

A glove is provided for each location at a site in case the water is particularly dirty.

### Putting together the filter setup.

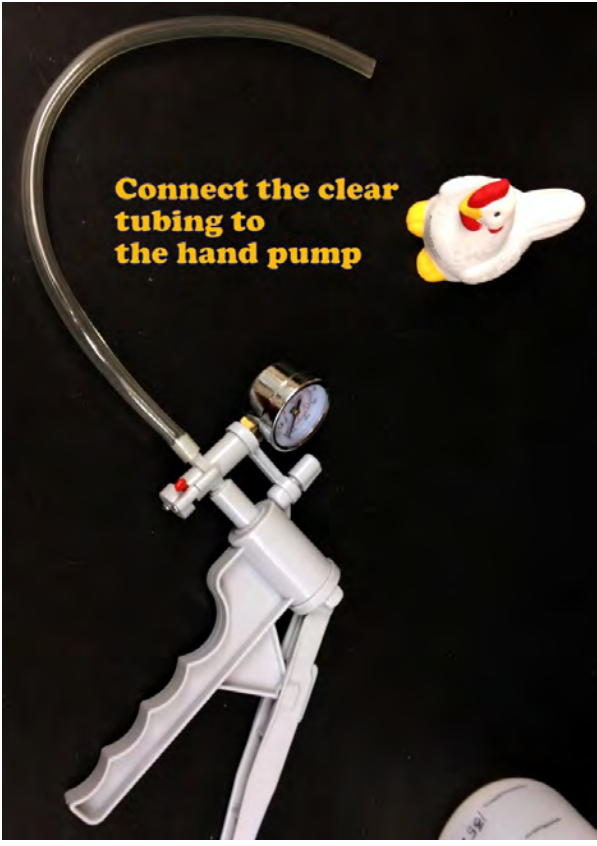
**\*\*RUBBER GASKET NOW REPLACED BY BLACK RUBBER STOPPER**

The pictures below show the parts of the filter setup and how they go together. Our lab chicken will guide you through the process. Try not to touch the inside of the funnel and **handle the filter itself only using the yellow forceps.**

1.



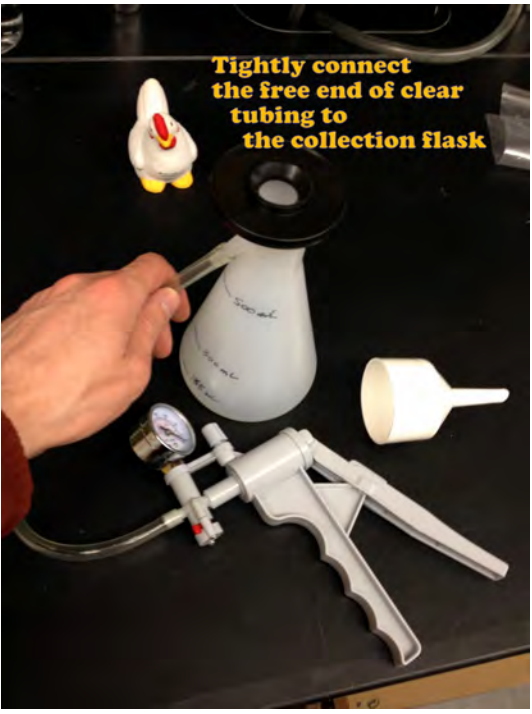
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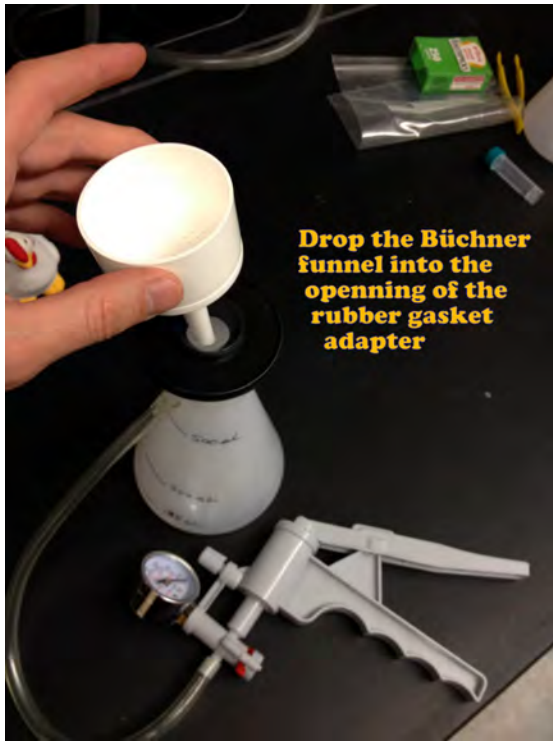
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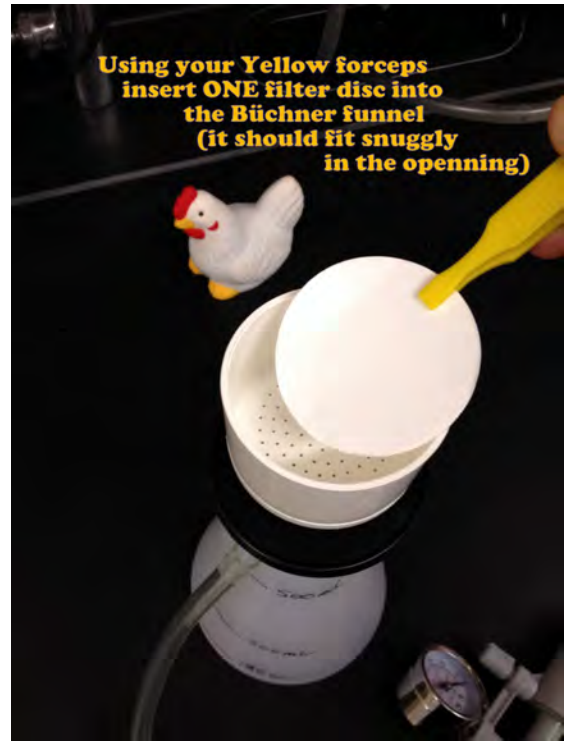
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5.



6.



7.



## Water collection and filtration

Note: This section describes on site processing. It is also possible to collect water, place it on ice and process it back in your classroom within 24 hours. If your sample will be processed back in your classroom, it is very important to keep it **ON ICE OR IN THE REFRIGERATOR** until processing and to process within 24 hours (same day processing is preferred if possible). Please see "Timing of water collection and filtration" on our website for details.

1. Once the apparatus is set up and everything is organized, filter the control water sample you brought with you. Using the forceps, place a single filter into the funnel. Carefully dribble a small amount of water onto the filter, enough to wet the filter and make it stick in place in the funnel, but not enough to make the filter lift up and float. It is easiest if several students work together, one pumping the vacuum, one supporting the flask on a solid surface and putting pressure on the funnel to insure a good vacuum, and one adding water to the funnel.
2. Make sure the vacuum is working before adding more water. Press down on the funnel and stopper as you pump to insure a good vacuum. You can use two hands if it is easier. Add a little more water and start pumping the vacuum pump. Pump smoothly and steadily. It is not necessary to go fast. Make sure the handle goes all the way in and all the way out each time you squeeze. Add water slowly. Don't let it get more than a centimeter or two deep in the funnel. To keep the filter from floating up as the water is added, one student should press gently down on the filter with open forceps while the water is added. If the water is poured too quickly onto the filter, the filter may float loose and allow the water to go through the funnel without passing through the filter, and no cells or DNA will be collected.





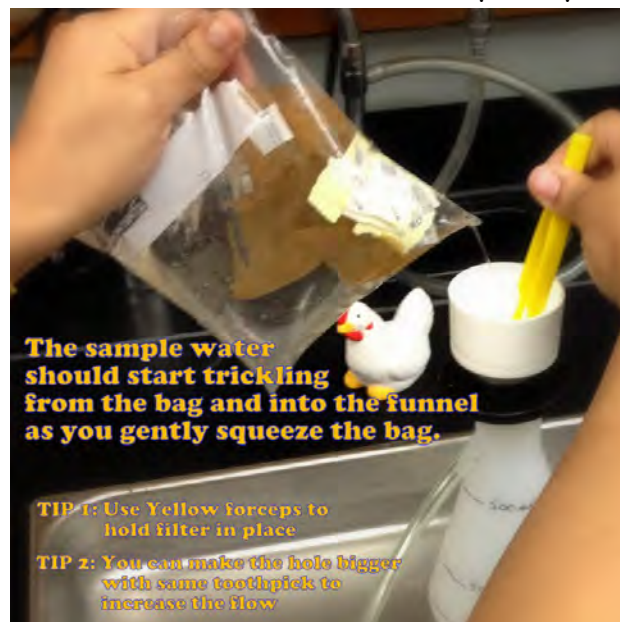
3. To remove the filter, carefully lift one edge with the forceps, and fold the filter in half. Using the forceps, fold the filter a second time (using 2 forceps will make it easier). Do not crumple the filter or bend it any more than necessary to fold it.
4. Place the folded filter in the control vial for your site and screw it firmly shut. The sealed vials should be placed in the small clean Ziploc bag provided. Make sure the code on your collection sheet for this site matches the code on your vial!!



5. Now you are ready to collect your water sample. Collect ~300 ml of water using one of the Ziploc bags provided in the kit. Try to avoid splashing water on you, your shoes, or your clothing.
  - \* Try to get water from throughout the water column (not just from the very top of the water), but try not to stir up dirt or other material from the bottom as you collect. Too much material in the water may clog the filter.
  - \* Try to take your water sample while standing on the water's edge, and avoid actually going into the water if possible.
6. Place a fresh filter in the bottom of the funnel, as you did when you filtered the control.
7. Seal your Ziploc collection bag securely.



8. When everything is ready, turn the bag upside down and punch a small hole in the corner with a toothpick. Turn the bag over again and slowly add the water in a gentle stream directly to the middle of the filter, until it is completely wet it.



9. Start pumping the vacuum pump as before. Pump smoothly and steadily. **It is very important NOT to get water into the pump or pump tubing.** Keep the flask upright while you are pumping. The plastic tubing on the bottom of the funnel will help to direct the water past the opening of the sidearm leading to the pump and help to keep the pump dry.
10. Once the filter is wet and firmly stuck to the funnel surface, carefully and steadily add the remaining water as you pump. If the water flow from the collection bag is very slow, you can make the hole a little bit bigger with your toothpick. Continue pumping the vacuum

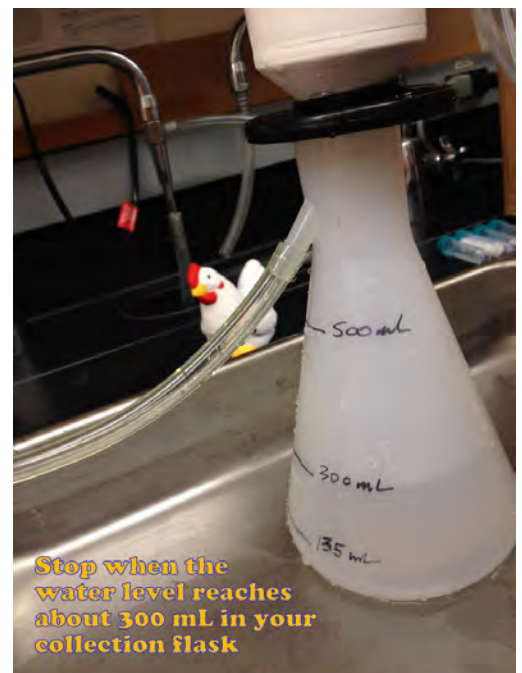
and add the water slowly enough to insure that the filter is not dislodged. Only cells in water that passes directly through the filter will be kept, and only the DNA in the cells captured by the filter will be available for analysis. If the filter comes loose and floats off the funnel surface, let the water drain out of the funnel, reposition the filter with the forceps and start again. Your bag should contain more water than you need, but if not just do a second collection with a new bag.



11. After about 300 ml of water have been filtered into the funnel, stop adding water. The picture at the right shows about how full the flask will be when it has 300 ml in it.

12. Remove the filter as before and transfer it to the green-capped tube labeled for this location, for example Site 1, Location A, Sample 1.

13. If possible, repeat this procedure for 2 more locations within your site (Site 1 Location B, Sample 1, and Site 1 Location C, Sample 1). When you are done you should have 6 tubes for each site, 3 controls and 3 samples.



14. After you have finished filtering the final sample for your site, gently remove the funnel and the tubing attached to the vacuum pump from the filter flask (leave the tubing attached to the vacuum pump). Dispose of any water in the filtration flask, as well as any unused water sample. Carefully place all used equipment in the black plastic bag and seal it with a red tie. **Never reuse equipment once it has been placed into the black bag.** Place all disposable materials (gloves, toothpicks, used water

collection bags, and the white bag your kit originally came in) in the small trash bag provided and dispose of properly when you return to school. **Please do not leave anything at the collection site.**

15. The bag containing the sample tubes and filters should be securely zipped closed and returned to Cornell in the original shipping box along with other reusable materials. **Do not place the vials in the black bag with the used equipment.**

### **After collection**

1. Back in your classroom, green-capped vials containing filters should be stored in the refrigerator until they are shipped back to Cornell.
2. Put the disposable items (Ziploc collection bags, gloves, toothpicks, white plastic bag) in the trash.
3. Place the funnel parts, rubber stopper, filter flask, and forceps in warm soapy water. Do not use any kind of scrub sponges. Rinse well with clean water four times and let air-dry. **(Do not wash the pump!!)**. Place the clean equipment and the vacuum pump and tubing in the clean black bag provided.
4. The black bags containing the used equipment, the small zip lock bags containing the filter vials, and any unused kits in white plastic bags should be returned to Cornell in the original shipping box. Please pack carefully to avoid any damage to the equipment or vials. Upon return, the equipment will be further decontaminated to remove any traces of eDNA and re-used by other students.

### **Results**

1. The results of the testing on your samples will be sent back to you so you can discuss your results in class.
2. Your findings will be added to our state map, along with a tag giving credit to your school for your help in acting as citizen scientists for this project. You can check out the map at [fishtracker.vet.cornell.edu/edna-testing-results/](http://fishtracker.vet.cornell.edu/edna-testing-results/).