# Open Water Wetland Environmental DNA (eDNA) Field Collection Protocols

*Version 2022-03-29*


## Acknowledgements

These protocols were adapted from written sampling methods and in-person training provided by InnoTech Alberta Inc. The filtration method and filter housing were developed by InnoTech Alberta. InnoTech Alberta personnel that contributed to the content include Brian Eaton, Jim Davies and Susan Koziel. The present document was developed by Amanda Schmidt and Jenet Dooley with review help from Stephanie Ball (ABMI).

## Disclaimer

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**Suggested Citation**: Alberta Biodiversity Monitoring Institute, 2022, Open Water Wetland Environmental DNA (eDNA) Field Collection Protocols, 2022-03-29. Alberta Biodiversity Monitoring Institute, Alberta, Canada. Report available at: abmi.ca [Date Cited].



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# Background

ABMI strives to continuously improve our monitoring program by exploring new technologies that are cost and time effective and produce scientifically sound data. In the spring of 2020, ABMI initiated a pilot project to explore the effectiveness of environmental DNA (eDNA) technology for monitoring amphibians and aquatic macroinvertebrates in wetland habitats. The objective of the project was to compare the detection ability of eDNA to the current monitoring methods employed by ABMI. These protocols detail the methods used to collect eDNA through water samples for this project. The methods were originally developed by Brian Eaton's team at InnoTech Alberta and were shared with ABMI through a partnership on this project. It is anticipated that these protocols will be improved and updated as ABMI continues to explore this relatively new technology.

Environmental DNA (eDNA) is the process of obtaining shed genetic data (e.g., hair, skin, mucus, etc.) of species by sampling the surrounding environment (Thomsen & Willerslev, 2015). A variety of habitat elements can be sampled such as, but not limited to, water (Schmelzle & Kinziger, 2016; Stat et al., 2017), flower petals (Thomsen & Sigsgaard, 2019), and topsoil (Buée et al., 2009; O'Brien, Parrent, Jackson, Moncalvo, & Vilgalys, 2005). Depending on the collection methods, eDNA can be used to answer questions about species occurrence (Bohmann et al., 2014; Schmelzle & Kinziger, 2016; Thomsen & Willerslev, 2015) and population structure of species and the community (Bohmann et al., 2018).

Though technically not eDNA, tissue samples (e.g., plant matter, feces) can be collected from an assortment of individuals occupying the local environment and pooled together (pooled DNA) to answer these same questions. Due to the nature of sampling eDNA (requires less exhaustive surveys), the difficulties in identifying certain species, and our requirement for reliable trend data, eDNA may serve to complement or modernize some of our current sampling protocols. eDNA is a new and promising method with the potential to increase the efficiency of monitoring a wide array of taxa.

eDNA typically occurs at low concentrations, and is heterogeneously distributed across both space (differential habitat use) and time (e.g., seasonal habitat occupation) throughout a waterbody (Takahara et al., 2012). The composite sampling layout (Figure 2) and method is an adaptation developed in collaboration with InnoTech Alberta for the 2020 eDNA pilot project. Its aim is to more thoroughly sample heterogeneous habitats in wetlands, increasing the chance for comprehensive eDNA capture.

## *Sample Contamination*

Any approach to collecting eDNA runs the risk of sample contamination; to minimize the chance for contamination, water samples must be collected using strict field protocols. All

eDNA protocols should be completed prior to other wetland protocols (i.e., water chemistry, invertebrate sample, etc.) to avoid contamination by technicians and field gear (Goldberg et al., 2016). There are two types of contamination to worry about: (1) cross-contamination (material from location A gets into samples from location B) and, (2) sediment contamination. Both types of contamination could lead to false positives in an eDNA assay. Bleach, gloves, and multiple layers of packaging are meant to prevent cross-contamination. Control samples are used to indicate if cross-contamination has occurred during the sampling process; they are a critical part of the sampling protocol.

The sampling protocols utilize an extendable pole to prevent sediment contamination. Sediment can contain DNA for very long periods of time. If the filters are contaminated with sediment, detections might represent historic (not current) occurrences of biota at the site. Alternatively, sediment can impede the extraction of eDNA from water samples, so this kind of contamination can cause challenges with analytical steps.

# Sample Collection

### *Site Layout*

Each wetland to be sampled is considered a site. The layout of sampling locations at a site is based on the Autonomous Recording Unit (ARU) location at the wetland. For regular sampling, 3 samples will be collected and there is only 1 sampling location for each of them. Sample locations are spaced so that the collection location for sample 2 falls directly in front of the ARU, if at all possible. Sample 1 is then spaced 50 m counterclockwise from sample 2, and sample 3 is spaced 50 m clockwise (Figure 1).

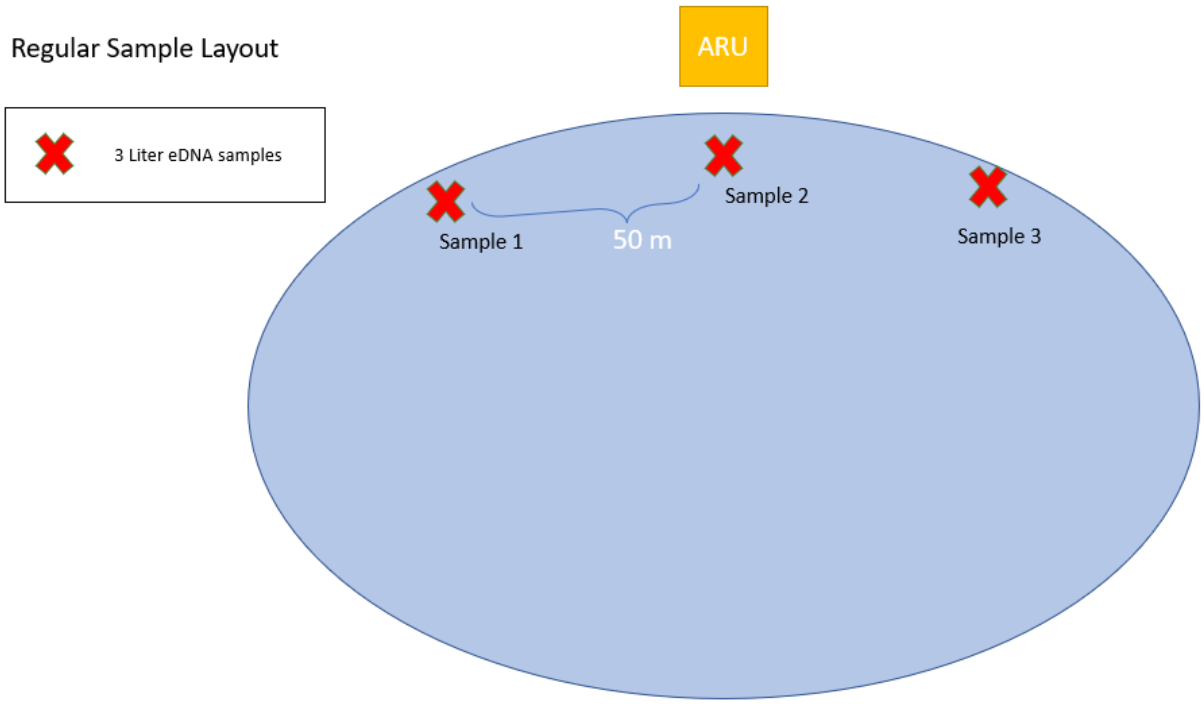


*Figure 1. Regular sample layout showing samples 50 m apart ordered in a clockwise direction with sample 2 centered in front of the ARU*

For sites that include composite sampling, 3 composite samples will also be collected. For each, there will be 3 sampling locations spaced 25 m from all other sampling locations. If space constrained, the samples can be spaced at least 20 m from other sampling locations. They are distributed around regular sampling locations as indicated in Figure 2. Composite samples are named A, B and C, and their subsamples follow as A1, A2 and A3 etc. and are ordered sequentially in the clockwise direction. The location of Composite sample A should be placed somewhere on the opposite shore of the wetland from the ARU (Figure 2), 25 m away from other samples.

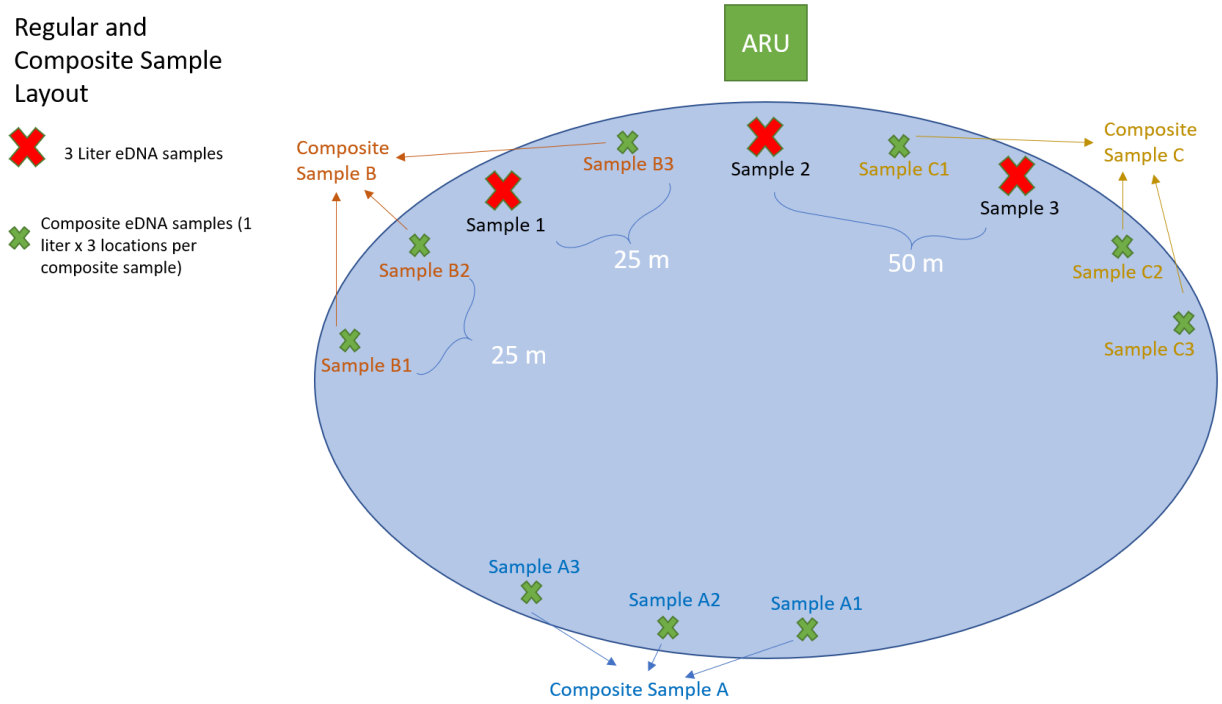


*Figure 2. Regular and composite sampling at the same site. Composite samples are distributed around regular samples as shown, and are ordered sequentially in the clockwise direction. Composite subsamples are at least 20 m from all other sampling locations.*

## Equipment

- GPS unit
- Field-deployable peristaltic pump attached to metal spike
- Cordless drill and extra charged batteries
- Extendable painter's pole with a three-pronged flask clamp attached to end
- Large bucket with 1 L and 3 L markings on the inside
- Spray bottle with 10% bleach solution
- Quat Plus in spray bottles
- Sharpie pens
- Powder-free nitrile gloves (a pair for each person for each sampling location and control sample, + spares)
- 4 L jug of distilled water for each control sample
- Filtration assemblies* with Luer locks and 1 m tubing in large Ziplock bags - control samples
- Filtration assemblies* with Luer locks and 3.5 m tubing in large Ziplock bags - samples
- Extra filtration assemblies - just the Luer lock and filtration assembly
- Desiccant beads in Ziplock bags (~70 g in each bag)

- Hydrolab
- Digital camera
- A garbage bag and spare zip ties

### *Cleaning Equipment Between Sites*

To minimize the transfer of pathogens and organisms between sites, and the chance of cross-contamination, it is important to clean all equipment that contacts the water or wetland soils before going to a new site. The cleaning method used has two steps: 1) all sampling equipment will be sprayed with a 10% bleach solution, and 2) spray bottles of Quat Plus are used for cleaning equipment between sites.

### *Safety Considerations*

Make sure you are following appropriate safety protocols for working around water, particularly in a case where you are working on or around floating mats. Collecting eDNA samples is best completed by two people. The peristaltic pump has moving pinch points for fingers and should be used with care, especially when inserting the filtration tubing.

### *First Site Visit*

On the first visit to a site, all sampling locations must be recorded so that they can be relocated for sampling on subsequent visits. Use a GPS with the given ARU coordinates to navigate to the ARU. Mark a GPS point for reference at the shoreline directly in front of the ARU. Consider the wind direction and start your sampling at the most downwind location so that water carrying any sediment, potentially kicked up while sampling, is not blown towards your next samples. Walk on the shore (not through the water) to the shore adjacent to each sample by using your reference GPS point to navigate the appropriate distance. As you wade into the water (if necessary) to collect your sample, make sure you are extending the sampling pole beyond any area of disturbance created while wading. Use the GPS to double check that you are at least the minimum distance away from other samples (see site layout section). Collect your sample - see the Sampling Procedure section for detailed instructions.

### *Subsequent Site Visits*

The primary difference between subsequent site visits and the first site visit, is that you will use the GPS points collected at the first site visit to navigate to each sampling location on subsequent visits. The rest of the protocol is identical. Stay on the shore as much as possible to get to the GPS location and - only when necessary - wade very carefully into the water, being careful to cause as little disturbance as possible when approaching your sampling point. If you can get within 3 meters of the previous sampling location, a new GPS point is

not necessary. If the water is now too deep, or shallow and you have no choice but to adjust the location of the sampling point, get as close as possible to the old point while maintaining the ability to obtain a good sample. Take a new GPS location after your sampling is complete at that location. Collect the samples as detailed in the Sampling Procedure section. Photos and Water Physiochemistry readings are also collected as described in the Sampling Procedure Section.

# Sampling Procedure

## *Control Sampling Tasks*

A typical sampling day includes both control and environmental samples, and these samples must be taken in the correct order and using the appropriate procedures. Control samples are taken 1) at the beginning of the day, before any site samples are collected, and 2) back at the truck after sampling at each site to test for contamination between sites.

1. Use a 4 L jug of bottled water and a single control filtration assembly. Set up the pump and assembly following Site Sampling Tasks 3-10 and 14-15. You can skip the parts about the extendable pole.
2. While wearing gloves, unscrew the lid of the bottled water and put the upstream end of the tubing inside the jug, and remove the tinfoil from the end of the filtration assembly.
3. Run the pump, until the jug is empty, as described in Sampling Procedure Steps 16-20. If the assembly appears to clog up, check to see if the upstream end of the tubing has sucked itself up tight against the inside of the jug.
4. If one site has been sampled and you are moving to the next, organize all of the sample and the control assemblies for a given sampling location before you drive to the next site. Make sure all bags containing sampling assemblies are appropriately labelled and that you have recorded all the information required about the site.

## *Site Sampling Tasks*

1. Collect all the gear you will need from the truck and transport it to the first sampling location.
2. Once at your sampling location, find a place to secure the peristaltic pump that is at the edge of the water as close to your sampling location as possible. Ensure that you aren't standing on a floating mat.
3. Push the spike attached to the peristaltic pump down into solid ground such that it will not tip over. Figure 3 shows the set-up of the filtration assembly and field-deployable peristaltic pump.

*Figure 3. Set-up of filtration assembly and field-deployable peristaltic pump attached to metal spike for taking a sample. Photo Credit: Jim Davies (InnoTech Alberta)*

4. Place the bucket near the pump to collect filtered water.
5. Carefully remove the tubing clamp (Object 7 in Figure 4 below) and plastic cover from the pump by loosening the nuts denoted by Object 6 in the schematic below. Be careful not to lose the nuts that hold the cover on the pump. Unscrew the nuts with one hand while the other hand forms a cup under the nut to catch any nuts that drop. Store the nuts, pump cover, and tubing clamp in one location every time you install or remove tubing from the pump.

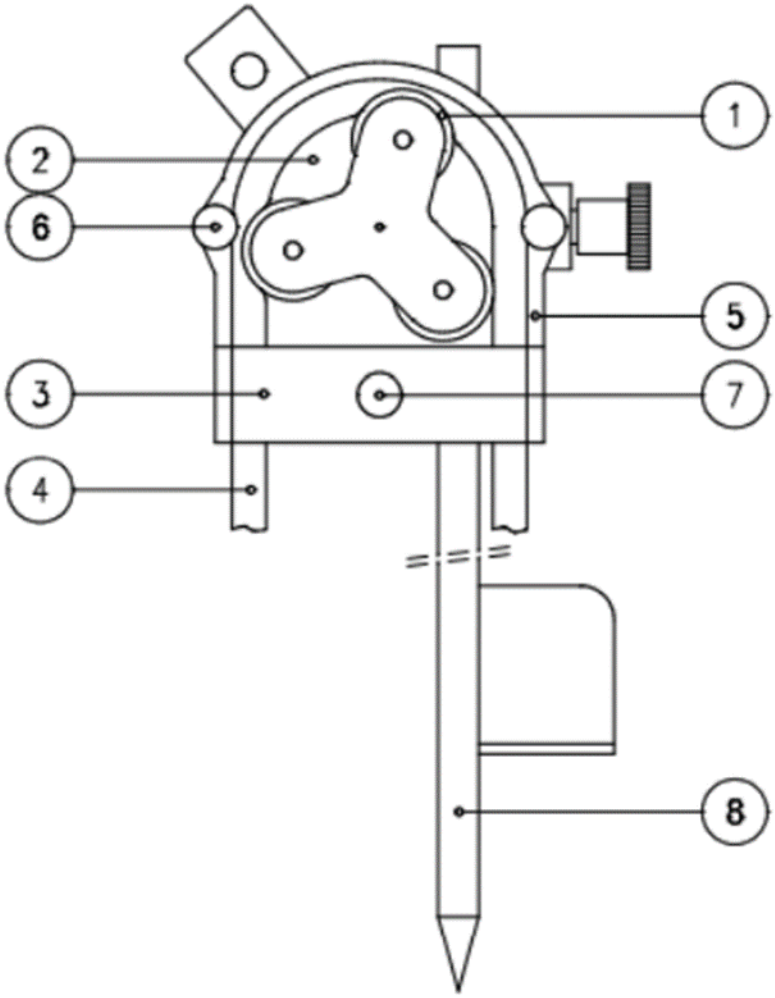


*Figure 4. Schematic of the filtration assembly and field-deployable peristaltic pump attached to metal spike. Figure modified from the operational manual for the Eijkelkamp hand operated peristaltic pump (Item M-1223E)*

6. Attach the drill to the driveshaft of the pump and make sure the drill is set to its slower speed (setting 1).
7. Remove the filtration assembly from its Ziploc bag. You can loop the "downstream" end of the tubing (i.e., the end with the filter housing) around the handle of the bucket so that it hangs into the bucket but doesn't fall in. Make sure the filter is not hanging so low that it comes in contact with the water that accumulates in the bucket during the filtration process. Store the Ziploc bag in a clean location so that you can use it again later.

8. Install the tubing in the peristaltic pump. There is no right or wrong direction. Be careful of pinch points for your fingers.
9. Re-attach the tubing clamp (Object 7 in Figure 4 above). This clamp prevents the tubing from "walking" when the pump turns. Check the inside of the tubing clamp to see which direction is up. Tighten it so that it is snug against the tubing but not so much that it squishes the tubing.
10. Re-attach the cover to the pump. The cover ensures that the tubing will not slip out from between the rotors (Object 1, Figure 4) and the pump housing (Object 5, Figure 4).
11. Gently clamp the "upstream" end of the tubing in the forks at the end of the extendable pole. The jaws should be just tight enough to hold the tubing in place without pinching it shut. Check to make sure the screws that hold the forks onto the pole are tight.
12. Stand on shore and have one person extend the pole so that the tubing reaches out into the water. Aim for the tube to collect water from just below the surface of the water. It is important to keep the tubing out of the benthic region of the wetland for the duration of sampling. Don't stand in the water if you can help it.
13. The person not holding the pole (Person 2) will run the drill. They should put on their gloves now.
14. Person 2: Remove the tinfoil from the filter holder that is hanging in the bucket.
15. Person 2: Make sure the drill is running in the correct direction (which can change depending on the "direction" of the tubing in the pump). Run the drill until you get 3 Liters of water in the bucket (there's a mark on the bucket at the 3L level). Note: Depending on how much suspended material there is in the water, the filter might plug up before you get to 3L. If that happens, detach the first filter holder at the Luer lock, place it in the Ziploc bag, attach a "spare" filter housing, and then continue pumping.
16. Once the bucket contains 3L of water, lift the end of the pole out of the water and continue to run the pump until all the water in the tubing and the filter housing has been replaced by air. This will minimize the amount of water that has to be absorbed by the desiccant.
17. Remove the tubing from the pump (steps 8-11 in reverse order).
18. Coil up the filtration assembly and put it back in its original Ziploc bag. If you used more than one filter housing for a sampling replicate (i.e., you used one or more spares) then make sure all filter housings for that sampling replicate are found in the same Ziploc bag.
19. Label the Ziploc bag with the site name, date, sampling time, and sampling location.
20. Dump out the water from the bucket onto the ground behind you.
21. Spray down the bucket and end of the extendable pole that is placed in the water with bleach
22. Put all your gloves and any other trash into the garbage bag.

23. Complete the post sampling tasks detailed below.

## Post Sampling Tasks

1. After you finish collecting eDNA samples at each location, mark a GPS point. Label the points with the Site ID followed by a dash, then "EDNA" followed by another dash, then the sample location ID. For regular samples this is just the sample number. For composite samples this will consist of a letter and number. The naming convention is as follows:

   [Site ID]-["EDNA"]-[Sampling Location]

   For example, at site OGW-ABMI-1113-71-5, the GPS label for a regular sample location could be OGW-ABMI-1113-71-5-EDNA-1, and for a composite sample location could be OGW-ABMI-1113-71-5-EDNA-A1.
2. Take a photo of the area sampled. Try to frame the photograph so that approximately three quarters of the photo is of the water and 1 quarter isof the horizon. This needs to be done at every sample location, so composite samples will have three associated photos, while regular samples will have one. Label each photo as follows:

   [Site ID]_[Month]_["eDNA"]_[Sampling Location].

   For example, at site OGW-ABMI-1113-71-5, the photo label for a regular sample location could be OGW-ABMI-1113-71-5_July_eDNA_1, and for a composite sampling location could be OGW-ABMI-1113-71-5_July_eDNA_A1.
3. Finally, at each location, take water physiochemistry readings. Allow the Hydrolab probe time to equilibrate, and then record the temperature, conductivity, dissolved oxygen, pH and salinity of the water at that location, just below the surface of the water, where the collected sample would have come from.

## Wrapping up at a Site

1. Repeat Site Sampling steps 2-23 and Post Sampling Steps for all sampling locations at a given sampling site.
2. Once you have collected all your samples, screw the tubing clamp and the front plate back onto the pump to avoid losing pump parts between sites.
3. Remove the drill from the pump, pull the spike/pump from the ground, and walk back to the truck with all your gear and trash.
4. Follow steps 1-4 under Control Sampling Tasks above.
5. Spray down the bucket and end of the extendable pole that is placed in the water with bleach or Quat plus.

## Sites with Composite Sampling

Composite sampling will follow the procedure detailed above with slight alterations. Composite sampling locations are distributed over space (See Figure 2). For each composite

sample, you pump 1 L volumes of water through the filtration assembly at 3 sampling locations spread out along the edge of the wetland. In other words, you would still pump 3 L of water through a filtration assembly in total, but would do so over 3 contiguous sampling locations. You would do this 3 times for a given site . At sites with both regular and composite sampling, you collect 3 "regular" samples and 3 "distributed/composite" samples with 3 sampling locations each. At these sites there are 12 sampling locations total. Each sampling location incorporated into the distributed/composite sample should be separated by 25 m or at least 20 m if space constrained.

### *Sample Preservation*

At the end of the day, preserve your samples. The lab, a trailer, or a motel are preferred places to preserve the samples but it can be done on the back of the truck as long as there isn't too much wind or rain. Preservation works by means of desiccation; truly dry samples will last for months so long as they aren't exposed to extreme environmental conditions. In order to minimize the potential for contamination, we dry the membranes without ever completely opening the filter housing.

1. Wash your hands.
2. Sort all the samples into their sites and arrange them by sampling location (I.e., for Site #1 you might have 3 replicates (from sampling location 1, 2, and 3) and two controls).
3. For each replicate or control, use a small Ziplock bag with desiccant beads. The bags should be labelled exactly the same way as the replicates and controls are labelled. There is one exception: if you used a spare filter housing because the first one clogged, then you would need two desiccant bags for one replicate, and you would need to label each desiccant bag such that the lab understands to look for more than one desiccant bag for that sampling location.
4. Pick one filtration assembly or control and its associated desiccant bag.
5. Put on gloves.
6. Open the big Ziplock bag and pull out the filter housing.
7. Use a 17 mm wrench to unscrew both hose barbs and put them back in the big bag.
8. Put the housing inside the desiccant bag and seal the Ziploc.
9. Once inside the bag, attempt to partially unscrew the two halves of the filter housing. Do not completely separate the two halves, just loosen them.
10. Gently tap the housing (while still inside the desiccant bag) with the wrench or on a hard surface to get the internal support platforms to unstick themselves from the shell of the housing.
11. Shake the filter housing around inside the bag so that many desiccant beads find their way inside the housing through the holes on either end, where the hose barbs used to be attached (See Figure 5 below).

*Figure 5. Ziploc bag with desiccant beads inside of the filter housing. Photo Credit: Jim Davies (InnoTech Alberta).*

12. Some of the beads will turn green/blue – that's ok so long as about half of the beads inside the housing are still orange.
13. Throw away the gloves you were using.
14. Repeat step 4-14 until all the filtration assemblies and controls have been processed
15. Store the now processed filter housings separately from the tubing and hose barbs.

## *Back at the lab*

1. Recharge the batteries for the cordless drills.
2. If a battery was dropped in the water, put it in a sealed Ziplock bag full of desiccant beads overnight. Check it the next day to see if it will take a charge and run the drill.
3. Scan your data sheets and send the scan to yourself and anyone else who might be appropriate.
4. Photocopy the data sheets so that you have each data sheet in triplicate; leave one in the field book/clipboard, put one copy with the samples before you submit them, and put one copy in your office.
5. Download the GPS locations to your computer and copy them to a backup location and/or email them to yourself and anyone else who might be appropriate.

6. Make sure the batteries in the GPS carry enough charge for the next sampling trip and charge them if needed.
7. Prepare your next batch of filtration assemblies, control assemblies, and other materials for the next sampling trip including repairing/replacing anything that was broken or lost.