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Schoodic Institute/Acadia Learning Program
National Park Service (NPS) – Air Resources Division (ARD)*

SAMPLING GUIDE FOR THE COLLECTION OF DRAGONFLY LARVAE, WATER, AND SEDIMENT SAMPLES FROM NATIONAL PARKS FOR MERCURY ANALYSIS

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Revised April 2015

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A student searches for dragonfly larvae samples at Rocky Mountain National Park (CO) (NPS photo).

Scope and Application

Mercury (Hg) is a globally distributed contaminant that can harm human and wildlife health. In its toxic methylated form, mercury bioaccumulates (builds up) in aquatic and terrestrial foodwebs. Effects can include reproductive and neurological impairment. Due in part to emissions from coal-burning power plants, even remote national park environments receive mercury deposition from the atmosphere. (See <http://www.nature.nps.gov/air/AQBasics/mercury.cfm> for background on mercury in national parks.)

Mercury threatens natural resources the National Park Service is charged with protecting. This citizen science project encourages students and visitors in national parks to collect dragonfly larvae for mercury analyses. Dragonfly larvae (Odonata: anisoptera) could serve as indicators of ecosystem health by characterizing the risk and potential toxic effects of mercury. These aquatic macroinvertebrates are long-lived (up to 5 years as larvae) before emerging as adult dragonflies, widespread across the U.S., predatory (i.e., prone to higher concentrations of mercury), important prey for fish species, and they reflect the mercury sensitivity of a specific watershed. Moreover, they are relatively easy to collect!

The study connects people to parks, advances the NPS educational mission, fosters biodiversity discovery opportunities, and provides baseline data to better understand the spatial distribution of mercury contamination in national parks. This project (http://www.nature.nps.gov/air/Studies/air_toxics/dragonfly/index.cfm) expands the geographic scope of research previously conducted by scientists and citizen scientists, and provides data that can be compared across parks. Early data are being used to develop hypotheses regarding whether mercury varies with odonate larval body size or by family, or whether a site's landscape setting drives variability in mercury in odonate larvae. Educational content and lesson plans for use by teachers and NPS staff are also available, (<http://participatoryscience.org/>) ultimately helping to raise awareness about mercury impacts. This study enlightens a new generation of citizen scientists about the connection of all living things and the influence humans have upon natural systems, and how environmentally-responsible decisions can protect our parks and the planet.

The citizen science effort to collect dragonfly larvae from national parks for mercury analysis started in 2011 with four NPS units in the Northeast and Great Smoky Mountains, expanded in 2012 to include a total of approximately 14 parks, and in 2013 to include 22 parks across all seven NPS regions: Northeast, Southeast, National Capitol, Midwest, Intermountain, Pacific West, and Alaska. In 2014, 35 parks from around the nation sampled approximately 3,500 dragonflies from 122 sites, involving over 800 citizen scientists! In 2015, we are continuing the effort by sampling some new parks and supporting the resampling of some parks that have already participated. Citizen scientists involved include students ranging from elementary school to college-aged, park visitors, and bio-blitz participants. In addition to collecting dragonfly larvae, a subset of parks also collect a water sample for mercury-relevant water chemistry (i.e., dissolved organic carbon (DOC), pH, sulfate), and a sediment sample for mercury analysis. These samples will allow the project scientists to identify how mercury concentrations in dragonflies relate to concentrations in other parts of their environment. If your park has been selected for collection of water and sediment samples you will be contacted by either the USGS or University of Maine scientists and provided additional sampling materials.

The optimal study design is 3 sites per park that represent a gradient in mercury-relevant chemistry (as defined above); or a gradient in landscape conditions, such as a high and low elevation, amount of wetlands adjacent to or upstream from the site, or forested versus urban landcover. Consider scoping the sampling locations in advance of collecting samples. Does the proposed sampling location have a healthy population of dragonflies? (A sample size of 20 dragonfly larvae per site is preferred.) Will nearby riparian flora and fauna get trampled? Is the site spacious enough for a group?

A park research permit will be needed to conduct this project. In addition, in some cases, a state permit may be needed to collect dragonfly larvae. Example text for the permit application can be found in Appendix A of this sampling protocol. The project's fact sheet and other materials will provide further assistance. (See NPS Dragonfly Larvae web page as above and additional documents on Sharepoint

http://share.nps.gov/NRSS/ard/Dragonfly_Mercury_Project/Forms/AllItems.aspx.)

Field work carries some inherent risks, and the safety of study participants is a primary concern. The varied and spectacular resources of our national parks, and the engagement of citizen scientists who are potentially less skilled in field work and the associated hazards, present a challenge to safely conduct this project. Further, travel in remote areas may be required to conduct science that has meaningful inference to the parks' diverse landscapes, increasing risks. Be attentive of the possible risks and refer to a detailed Safety SOP or to a Job Hazard Analysis (JHA) that is developed specific to surface water sampling, backcountry work, traveling in high elevations, poison ivy, tickborne disease, etc. Riparian edges can be very slippery, as are stream and lake bottoms. High water levels and turbulent flows may cause an individual to lose balance in the water. Collectively define, communicate, and enforce your park's safety philosophy, standards, and guidelines; take the Operational Leadership training, and other relevant safety trainings; allow sufficient time for oversight and interaction with your group; and follow Departmental guidelines on the use of personal protective equipment, such as PFDs. Nothing in this program's mission, project goals, or day-to-day objectives is so important as to compromise the health and well-being of those participating in this project.

OVERVIEW

This protocol has several sections. Each sampling procedure is fairly straightforward, after you've gotten the hang of it. You should read each section's specific instructions on the following pages. The general structure is as follows:

- 1. Prepare by gathering field gear.** Some gear is provided in the sampling kit, but you'll need to round up a few items. Check what's needed several weeks before sampling (in case you need to order something).
- 2. Collect samples*:**
 -  **First, if you were provided water sampling materials, collect a water sample for mercury and other chemistry.** This sample is the most sensitive to contamination (and stirring up the site by wading in). Often it is useful to collect water samples from all sites within a park in a single separate trip prior to dragonfly sampling in order to avoid contamination and to facilitate immediate shipment. It is important to note that water samples **must** to be shipped to the coordinating lab (UMaine in the east, USGS in the west) within **24hrs** of collection. See water sampling protocol for more details. You need the white, powder-free gloves and the double-bagged 2-liter PET® bottle. **DON'T** open their bags before going out in the field!
 -  **Second, if you were provided sediment sampling materials, collect a surface sediment sample.** Walking around in the water will stir up the sediment we are trying to sample. So, do this before everyone gets in the water, or in a location that is undisturbed. Depending on your site characteristics you will either take 3 mini-cores using short pieces of tubing or collect 3 small scoops of sediment from around each site.
 -  **Third, collect dragonfly larvae!** Many hands sampling makes this go quicker. Larvae are collected using nets and temporarily stored in a small tote or dishpan with lake water until being sorted. Once at least 10 larvae (but ideally 20+) have been collected, samples are sorted, and up to 20 individuals are selected for mercury analysis. These are measured, identified to family, then individually bagged to prevent contamination
 -  **Fourth, observations about the site.** Many are qualitative/visual, but they are still very useful data. You know your site best and you can help the project scientists understand your watershed.
- 3. Ship your samples.** Water samples must **NOT** be frozen. They are stored in a **cool dark place** (a refrigerator is ideal), and **MUST** be shipped to the coordinating lab (UMaine in the east, USGS in the west), via FedEx overnight (we cover this cost), **within 24 hours of collection.** Because of the short holding time for the water sample it is important to communicate when you expect to collect and ship water samples with the coordinating lab well ahead of time (UMaine in the east, USGS in the west).

You'll ship water samples overnight in a cooler with ice packs, (or wet ice well-sealed in bags to prevent leaking), and you will have a second cooler for dragonflies and sediment that will be shipped with dry ice. Dragonflies and sediment tubes should go in a freezer after the sampling trip is over. They can stay in your freezer for several weeks, if you have multiple sites to sample over extended time periods.

* When possible, it is ideal to collect all of the water samples (and possibly sediment as well) on a separate trip from the dragonfly sampling trip, but during a similar time period (within 2 weeks). Especially when time is limited or dragonflies will be collected over several days/weeks, it's efficient to get all the water samples collected and shipped all at once—especially given the need to ship within 24 hours of collecting water. Get in touch if you need help with these logistics.

1. Prepare

The following sampling supplies are needed to collect dragonfly larvae samples, sediment, and a water sample from each site. All supplies will be provided in the sampling kit, with the exception of those materials in **bold>. Participating parks must provide those items separately.**

Materials

Water sampling:

- PET® bottle for mercury in water sample
- Powder free Vinyl gloves (white color, pre-bagged to keep ultra clean) – 2 pair

Sediment sampling:

- Three (3) mini-corer tubes (pre-bagged to keep clean), with two end caps per tube.
- Large bag in which to place sample tubes for storage, shipping
- Powder free Nitrile gloves (blue or purple color)
- Scoops for alternate protocol

Dragonfly sampling:

- Nets* – D Nets or dip nets**
- Clean, white dishpan, bucket, or ice cube trays**
- Clean, new plastic spoons
- Brand new zipper-seal bags – 40 small size + 5 large size
- Powder free Nitrile gloves (blue or purple color)
- A plastic ruler with mm scale – clear is best
- Tags for outer bag, pre-printed with ID codes

General field supplies:

- Sharpie marker and pencil/pen
- Field sheets
- Two coolers containing **wet ice**
- Trash bag**
- Optional – hand lens, GPS, camera, macroinvertebrate field guide, waders**
- Citizen science groups must provide their own PFDs (personal flotation devices)**

Shipping:

- Wet ice for water sample**
- Dry ice for dragonflies, sediment**
- Dry ice label and pre-paid shipping labels for return shipping



Note: sediment tube cap color may vary.



*More information on nets in Appendix B.

2. Collect samples

- Collect (1) water (if collecting), (2) sediment (if collecting), (3) dragonfly larvae.
- When possible, **it is ideal to collect all of the water samples (and possibly sediment as well) on a separate trip from the dragonfly sampling trip.** Especially when time is limited or dragonflies will be collected over several days/weeks, it's efficient to get all the water samples taken and shipped all at once, **since they need to be shipped within 24 hours of sampling.** This also allows you to maximize time with the group in the water, discovering the aquatic biota. Ideally, this separate trip would happen in the same general timeframe as the dragonfly sampling, within ~2 weeks of the dragonfly sampling effort.
- Repeat the sampling for 3 water bodies per park when available.
- We highly encourage you to take photos of the sampling sites and of the collection efforts. Images of engaged NPS employees (in uniform) with citizen scientists are especially preferred.



USGS employee Colleen Emery demonstrates water sampling procedure, 2014.



A. Water Sample

- *For environmental mercury sampling in the field, US EPA generally recommends: 1) wearing pre-cleaned and clean-bagged wind suits (a long-sleeved pants and jacket suit constructed of nylon or other synthetic fiber); 2) double bagging all sample containers and apparatus; 3) using the ‘clean hands-dirty hands’ technique, where only the ‘clean hands’ technician contacts the sample container and inner bag; 4) wearing non-talc, vinyl gloves (shoulder-length for the ‘clean hands’ person); and 5) approaching the sample site from down-current and downwind (EPA Method 1669, 1996). **For this research, a performance-based field method (e.g., Keep It Clean! technique) has been used to streamline the recommended sampling procedure** (Louch 2003, Johnson, 2002). Field technicians wear non-talc gloves, use the ‘clean-hands, dirty-hands’ technique, and double bag all bottles to transport them to and from the clean room, but wind suits and shoulder---length gloves are not worn, nor is a downwind approach always taken due to site location. The semi-clean methodology has produced results that were not significantly different than those obtained using the stricter methodology in replicate samples (Louch 2003, Johnson, 2002).*
- *The sampling procedure below is based on full guidelines published by the US EPA in Method 1669, and Method 1631. A more reader-friendly overview of these methods was published in 2003 by Louch and is available by contacting the National Council for Air and Stream Improvement, Inc. (www.ncasi.org).*

Procedures – Collecting Water Sample

1. **This sample is the most sensitive to contamination** and needs to be done before anyone goes in the water and before any possible stirring up of the site. Therefore, collect the water sample **FIRST, BEFORE** any other field work at the site (including sampling any invertebrates and sediment). Again, you might decide to do this on a separate date (within ~2 weeks of dragonfly sampling), without the large group.
2. For lakes, paddle (or reach, or wade without stirring up sediment) a few feet from shore into at least 1 meter of weed-free water. For streams, reach into a deeper part of the stream, preferably a pool below a riffle.
3. If you mistakenly wade right in before collecting this sample, move a bit upstream or wade far enough out to avoid the disturbed area – or, return another day for water samples!
4. **Two people are needed to sample.**
5. **Note observations and complete the field sheet. Record the code from the water bottle on the field datasheet** as well as any field conditions (weather, temperature, and preceding climatological conditions such as recent rainfall, snow cover if appropriate). Take coordinates (or later get them from Google Earth).



Keep it Clean! Water Sampling Protocol

It's easy to contaminate samples with mercury because it's all around us: in our hair, in soil, leaves, or even in the talcum powder from the wrong type of sample gloves. So we need to keep everything ultra-clean; **particularly when sampling water**. It is imperative to follow the specified sampling guidelines.

- Work in pairs and use the “**Clean Hands–Dirty Hands**” method:
 1. Designate one person as “Clean Hands” and the other person as “Dirty Hands.” Dirty Hands’ job is to deal with everything so that Clean Hands only touches the inner bag and the sample bottle.
 2. First, Dirty Hands opens the glove bag and lets Clean Hands pull out a pair of gloves and put them on. Gloves should arrive with the wrist side facing up, so that you can pull them from the bag with only minimally touching the wrist part (not the palm or fingers that will then contact the sample container). Once gloves are on, Clean Hands is careful not to touch the outside of the gloves or anything else – **pretend you’ve just done a surgical scrub!**
 3. Dirty Hands puts on the other pair of gloves. Dirty Hands opens the outer bag and **DOES NOT TOUCH ANYTHING INSIDE THE OUTER BAG.**
 4. Clean Hands reaches in and unzips the inner bag, and pulls out the sample bottle.
 5. Clean Hands takes the bottle to the sample site, rinses (fills then empties bottle) three times with the bottle and lid completely under water, then fills the bottle completely and caps it. Clean Hands returns the bottle to the inner bag, seals the inner bag, and pushes it inside the outer bag.
 6. Seal the outer bag. Typically, Dirty Hands does this but since sampling is complete, Clean Hands could do the final sealing as well.
 7. A barcode will already be on the bottle, and this code needs to be recorded on the field sheet so we can match the sample to the code in the lab.
 8. Put sample – in the double bags – on ice in cooler to store while in the field; upon return to laboratory or office, store in a refrigerator until shipping.
 9. Dispose of gloves – a fresh set must be used for each sample.
 10. Fill out the field sheet. Notes might include any irregularities or uncertainty about the sample or the procedure. We all make mistakes and the important thing that will help us interpret the data is to write down anything that might have gone less---than--- smoothly.

- See a **video demonstration** of these water sampling procedures for analysis of total mercury: <http://www.youtube.com/watch?v=BIHJFO4pfpI>



B. Sediment sample

What is sediment?

Sediment, in our project, can be thought of as the mud, sand, dead plants, rocks, or other materials that settle to the bottom of a lake or wetland, or slow-moving section of a stream or river.

Scientists are interested in sediment because it can act like a tape recorder. If you dig down in the sediment, you are essentially going back in time.

Scientists call things like sediment cores “proxy” measurements, because they tell us about the environment in times past, when we weren’t there to observe. You might be more familiar with the idea of coring a glacier or deep ice to figure out something about the Earth in past centuries or millennia; here’s a resource on ice cores:

http://climatechange.umaine.edu/icecores/IceCore/Ice_Core_101.html.

We are interested in the most recent sediment for this project, because we think it could affect mercury in the dragonfly larvae’s environment. Logically, you can reason that the top layer of sediment is generally the newest – because sediment deposits (builds up) in layers through time. So we don’t need to take a deep core of the sediment at each site for this project.

Here are some good resources that describe sediment in general:

http://education.nationalgeographic.com/education/encyclopedia/sediment/?ar_a=1

<http://water.usgs.gov/edu/sediment.html>

<http://www.nature.nps.gov/geology/usgsnps/rxmin/sediment.html>

Procedures - Collecting Sediment Sample

Getting ready to sample:

1. Before the group gets in the water, but **AFTER** carefully taking water samples, look around at your site. See if you can tell, by looking, what the material at the bottom of the lake, wetland, or river/stream is like. Is it mucky? Rocky? Are there plants everywhere? If you had to choose one type of sediment that best represents your site, what would it be? Make a note on your field sheet about the sediment types you see, and the relative coverage of each type. For example: is about 60% of the area you’re sampling is sandy, and the other 40% looks like it’s muddy with some rooted plants? Be careful not to stir up all the sediment as you look around.
If it’s hard to see underwater and if you have one available, you can put on a set of swim goggles to see underwater, or make a cheap and easy version by wrapping clear plastic wrap around the end of an empty cardboard toilet paper or paper towel roll with an elastic band.
2. Plan for sampling: based on the coverage you saw, select a spot where you will sample sediment that’s most representative of your site. If your site is like the example above, you might take 2 subsamples from the sandy area and the third from the muddy area.
3. **Each kit includes 2 types of sediment sampling equipment.** If possible you should use the 3 mini-corers provided; however, these may not work well if your site’s sediment is loose or gravelly. If the mini-corers do not work, samples can be taken using the scoop provided for the alternative method.



Taking the sample – mini-core method:

4. Put on purple nitrile gloves. It's helpful to have a second person nearby with gloves on who can help seal the sample tube and hold open the larger bag into which you'll put the full, capped sample tubes. Collecting sediments using the mini-corers takes some practice so you might test the corer in an area of your site where you will not contaminate the entire site if your initial attempts are not successful. If your core doesn't come out well (for example, little sediment is collected or it all slides out as soon as you lift up) empty the tube and try again, moving just enough to avoid any areas you disturbed with your previous attempt.
5. Each mini-corer is composed of a sampling tube, a plastic skirt surrounding the tube, and two end caps (see photo, below). Each mini-corer comes pre-bagged with the skirt and one cap on the tube. The tube is encircled by a line 2cm from one end of the tube (the one without the end cap on) and the skirt should be positioned near this line. The skirt helps you feel the sediment boundary, but if it is getting in the way (if you are trying to fit the corer between roots or rocks for example) you can remove it. There is also a small hole drilled in the side of the tube slightly above the 2cm line.
6. To collect a sample, leave the top (the end opposite the 2cm line) capped and, making sure not to cover the small side hole, embed the tube in the sediment to just above the 2cm line.
7. Cover the side hole tightly with your gloved finger to ensure a vacuum in the tube (this is similar to when you have a straw in a glass of soda, and you cover the top opening of the straw to keep the soda in the straw as you pull the straw out of the glass) and gently lift the tube enough to slip the other cap on the bottom of the tube.
8. Once the bottom cap is secured, slowly loosen the **top** cap taking care not to disturb the sediment. Removing the top cap will allow excess water to drain out the side hole. We want to keep the sediment core intact, and we want the water that's right on top of the sediment (this is an important part of the sample, where there's a lot of suspended material that could be mercury-rich), **so keep the tube upright – you are not trying to drain out all of the water, just the top centimeter or two.** Gently put the top cap back on (some additional water will come out of the side hole).
9. Carefully apply a couple of wraps of the provided Teflon tape to seal the side hole then cover these wraps by tightly stretching a piece of Parafilm (the stretchy, waxy material – peel it away from the paper backing) around the tube to ensure the sample is sealed.
10. Put the mini-core in a large bag. Label the bag with your park, site name and the date, using the Sharpie. Make notes about the sediment on the field sheet.
11. Repeat this in the same area. We want three mini-core tubes from your site. Just reach over a little and start again with a new tube/endcaps. No need to change gloves in between.
12. Sediment samples are frozen like the dragonfly samples, and shipped on dry ice. They can be kept in the freezer until all your sites are done – no need to rush back to ship immediately after sampling (unlike the water samples).

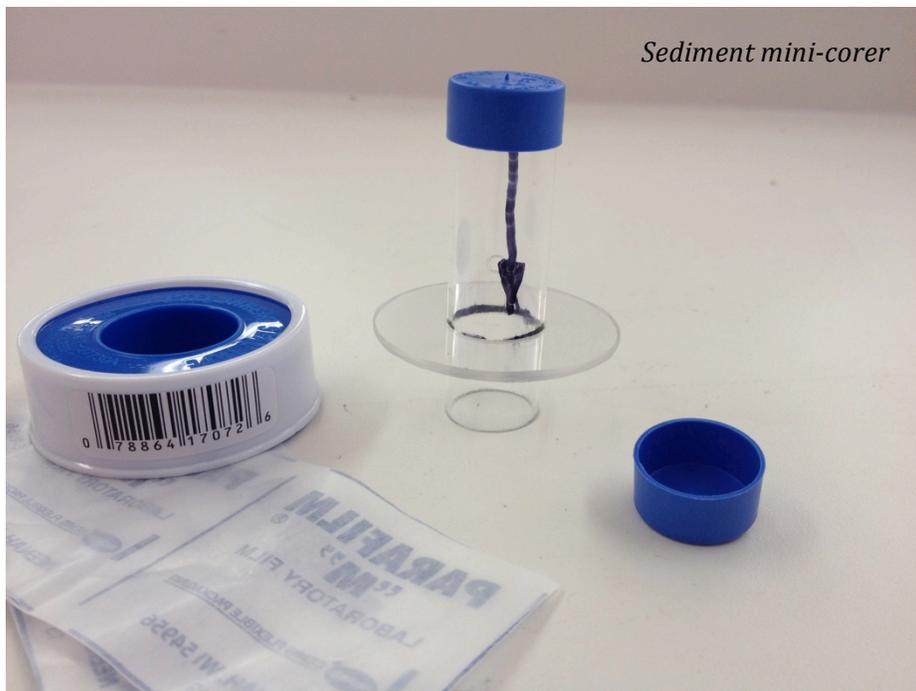


Taking the sample – alternative method:

13. If you can't keep sediment in the tube (which could happen if it's too sandy or loose), use the small plastic scoop provided in the alternative sediment kit. Scoop the top 2 cm of sediment as best you can and deposit the scoopful of sediment into a small zipper bag. Repeat this in three areas of the site and put all zipper bags of scooped sediment from a site in one large bag, and don't forget to label it.

What to do if...

- *Your water body has nothing but rocks.* Lift up a few cobbles/rock gently and see if there's mud or sand underneath, and sample that. Make a note/take a picture of how rocky it is. If you are unable to find an area with sediment to sample make a note on the datasheet that sediment samples were not taken
- *Your water body has lots of plants.* This happens a lot. We don't want a mess of plant roots in the sample, so you might have to try a few times to get a good mini-core. Try to get samples in between the roots, and look at what you've got before you decide to put it in the sampling container. Are there lots of roots/big plant pieces? Reject that mini-core and try again.
- *You make a muddy mess of the site before sampling.* See if you can wade over a bit and try another spot. Make a note if the disturbance seems to affect your samples. Try reaching as far as you can away from your feet, to a spot that's less affected.





C. Dragonfly Larvae Sample

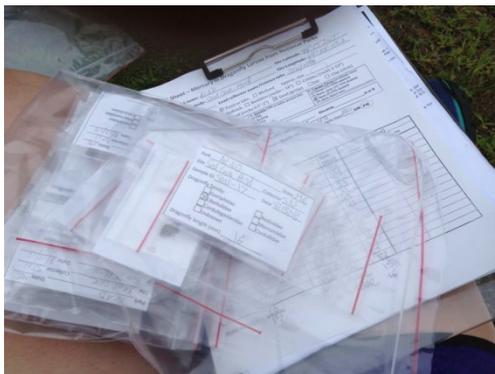
1. **Look around.** Locate likely habitats for dragonflies: vegetated bank margins, snags and logs, aquatic vegetation and decaying organic matter, silt/sand/gravel substrate.
2. **Note observations and finish completing the field sheet.** Take a site photo!
3. **Rinse the nets and dishpans** with water from the site you are sampling.
4. **Partly fill dishpan** or bucket with water from the site. Ice cube trays are nice sorting containers too – they can be filled with stream/pond water as well.
5. **Jab** your net into the likely habitats. Start downstream first, and work upstream if in flowing water. Jab a few times then sweep the net up to the surface. Empty the net into the dishpan or bucket – either by tipping it into the bucket or plucking (with a Nitrile-gloved hand or a plastic spoon) larvae directly from the net. Spend a few minutes jabbing and emptying the net. If you don't find much after a few minutes, move to another area within the same waterbody.
 - See a video demonstration of collecting dragonfly larvae: <http://youtu.be/psTu10uwdOg>
6. **Disinfect** waders, nets, and any other gear that has been in the water before moving to your next site. **Consult your permit** since some parks have different protocols for disinfection. Most but not all) will specify a 5% or 10% bleach solution, which is a mixture of household bleach and water.



Left: A student searches for dragonfly larvae in a collection net at Marsh---Billings---Rockefeller National Historic Park (VT).



Right: A citizen scientist measures a dragonfly larva sample for the study at Acadia National Park (ME)



Left: Completed, double-bagged dragonfly samples with labels prepared for storage in freezer or on ice, and shipment.



Right: Dishpan 'holding tank' at the lakeside with larvae that have been collected, prior to selection of specimens to be bagged and submitted.



Keep it Clean! Dragonfly Larvae Sampling Protocol

It's easy to contaminate samples with mercury because it's all around us: in our hair, in soil, leaves, etc. So we need to keep everything ultra---clean.

- Do not touch the samples (individual dragonfly larvae) with anything **except** a clean plastic spoon or powder---free nitrile glove (purple gloves).
- **Never touch the inside** of the inner bag – the first bag you put the sample into.
- Think of each individual sample as a fresh start – everything needs to be **clean between samples** and be treated just like the first sample you took.
- Work in pairs and use the “**Clean Hands–Dirty Hands**” method:
 1. One person is in charge of the sample bags (“Dirty Hands”) and the other will pick up and place the samples in the bags (“Clean Hands”).
 2. Clean Hands puts on a glove (one gloved hand should suffice to pick and bag larvae). Once the glove is on, Clean Hands touches nothing but the sample and any pre---cleaned supplies. Unlike with water sampling, Dirty Hands does not need to wear gloves.
 3. **Choose, measure, & bag up to 20 individuals per waterbody/site.**
 4. Using a nitrile-gloved hand, or plastic spoon, Clean Hands places one dragonfly into a sample bag while Dirty Hands holds the bag. Nothing should touch the inside of the bag except the dragonfly larva. Clean Hands seals the bag.
 5. Dirty Hands uses the plastic ruler, held up to the individual in the bag, and **measures the body length** in millimeters (mm) – from the front of head to end of tail spine. Most larvae will range between about 10–40 mm, as a reference.
 6. Dirty Hands **fills out a tag** for the bagged individual and slips it and the inner bag into the outer bag. Your samples have an ID code pre-printed on the labels. Use these codes to record the lengths on the field sheet and to refer to a particular sample in your notes.
 7. Dirty Hands **seals the outer bag.**
 8. **Be ‘clean’ with your spoon and/or gloves.** If they have been tossed on the ground or contaminated – say, by brushing back your hair – start with a fresh one. Swish your spoon or gloved hands in pond water (in the dishpan) between samples to ensure there’s no carryover from one sample to the next.
 9. Go to the next individual and repeat. Place all of the small double bags into one or more of the larger zipper seal bags for storage and to keep them safe and together. *(See Section 4)*
 10. Gently return extra site water and invertebrates to the sample site.
 11. Pack out all trash, disinfect gear.



3. Identify

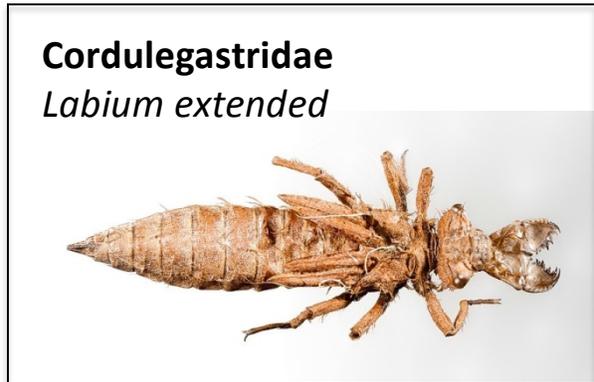
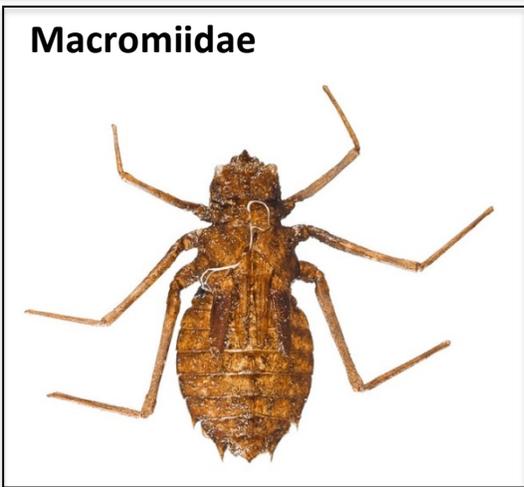
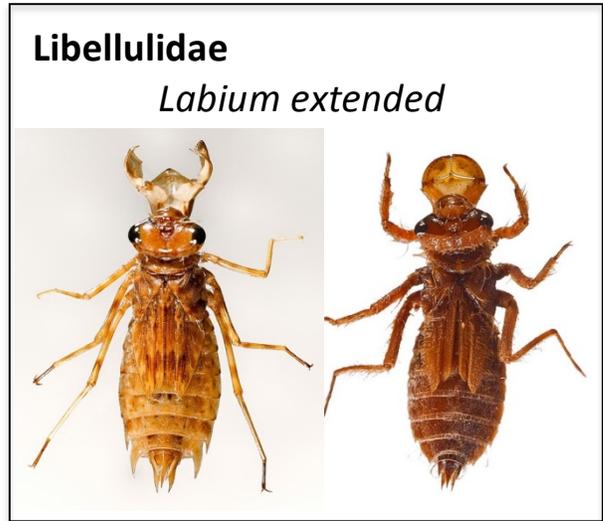
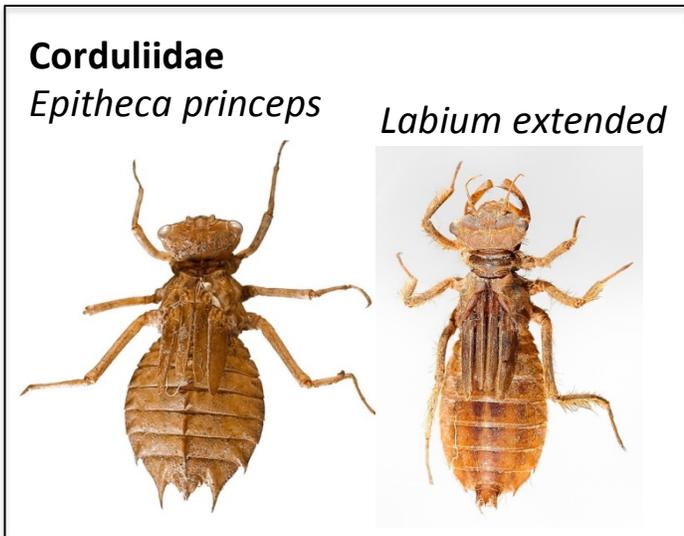
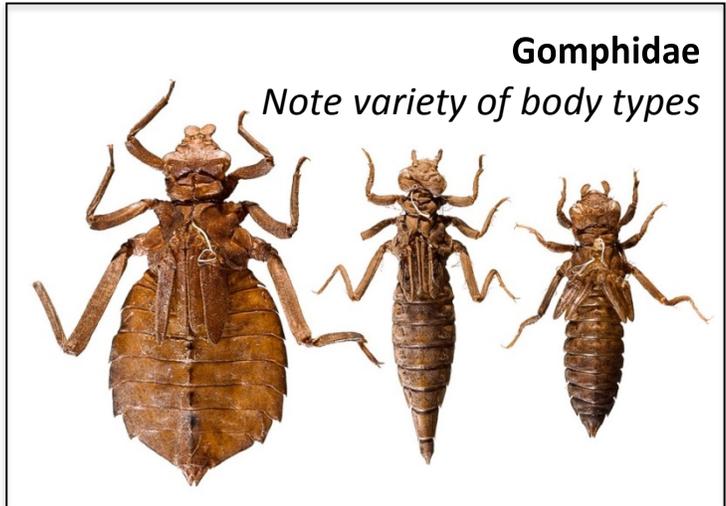
Please identify dragonfly larvae to family if you are comfortable doing so. There are only 6 major families in most places (see following pages). Most identifications can be accomplished in the field with a hand lens and guide book, or you can bag samples (see Section IV) and bring them to a clean indoor workspace to look more closely. The online key available at <http://parkcitizenscience.org/dragonfly/> will assist efforts. The online video “Identifying Maine Dragonfly Larvae to Family” (<http://vimeo.com/76713446>) will also assist with identification because similar larvae families are found outside of Maine.

Identifying to family requires careful inspection. The ID will be checked once the laboratory receives samples, and some samples will be further identified to species through genetic barcoding or a taxonomist, but identifying samples when they are fresh is helpful in the event of damage during shipping.

- A. *It is helpful to distinguish between the two Suborder levels: **Dragonflies (Anisoptera) vs. Damselflies (Zygoptera)**. Although both are predators, the Anisoptera bodies are more robust and may therefore consume and store more mercury. Anisoptera also tend to have longer aquatic lifespans (up to 5 years) than Zygoptera (usually 1 year). The online key <http://parkcitizenscience.org/dragonfly/> begins with the basic split of Anisoptera versus Zygoptera.*
- B. *It takes specialized training to classify dragonfly larvae to the species level; we will do this for some individuals in the lab and provide the identification information to you along with the mercury and other data. This information is especially useful for biodiversity discovery activities!*
- C. *Related websites for additional information on classification and identification of dragonflies:*
 - www.odesforbeginners.com/larvae/larval_id.aspx (for beginners)
 - www.umd.umich.edu/eic/aquatic_insecta/odonata/odonata_key.htm (for beginners)
 - insects.umz.lsa.umich.edu/MICHODO/test/Suborders.htm (real key in traditional sense)
 - www.odeneews.org/NElarvaeGenusKey.pdf (genus level)
 - www.odeneews.org/NEAnisopteraSpeciesKey.pdf (species level - detailed, excellent resource)
 - www.dragonflies.org/catalog.htm (adult dragonflies)
- D. *A nice, readable web site with information about dragonfly life stages and some behavior in each stage: <http://citizenscientistsleague.com/2011/12/15/dragonfly-life-cycle-and-metamorphosis/>*

4. Choose, Measure, & Bag

- Look at the samples you’ve collected. If you have identified to family already, please choose at least 3 individuals in each of the families you found. If you only found one individual in a certain family, please don’t submit that one. Ideally, at least 10 and up to 20 individual larvae will be selected per site. We would like to analyze samples from across the range of sizes present at a site, however, larvae larger than 15 mm are easiest to measure mercury in—please choose medium to larger samples over very small ones when possible.
- Given the risk of contamination, it is very important to follow the **Keep it Clean!** Protocol (above) while choosing, measuring, & bagging dragonfly larvae samples, and through the whole sampling outing.
- In sum, once samples have been collected, identified, and selected, each dragonfly larvae is double-bagged; placed first in an inner zipper bag, then with label placed in an outer bag, and stored on wet ice.



Photos of exuviae: A. Anderson, Kingfisher Photography, Old Town, ME



GENERAL ANATOMY OF NYMPH :

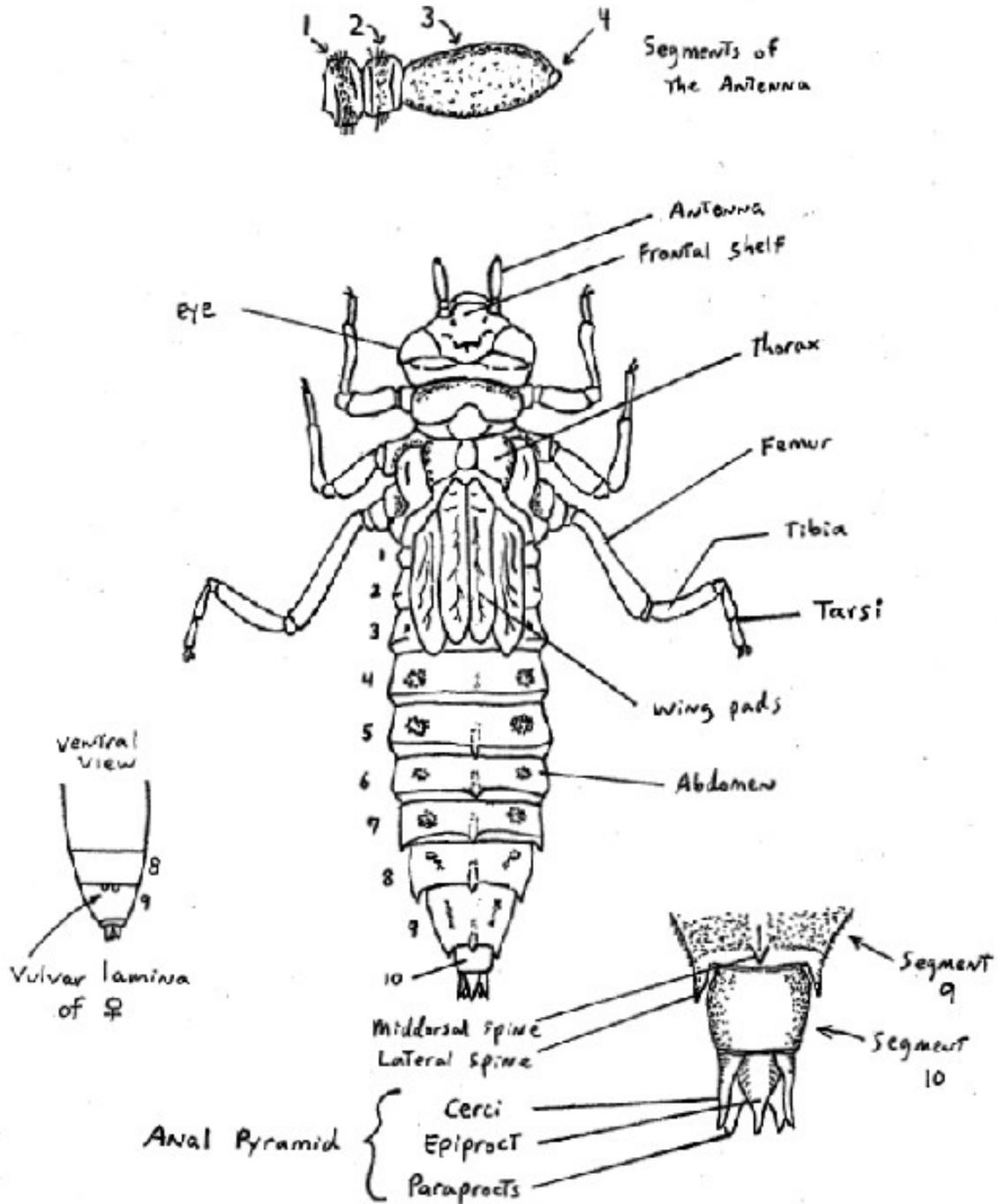


Diagram: Ken Soltesz

Note: The illustration shows a typical Gomphid, with paddle-shaped antennae that have fewer segments; most larvae have long, filamentous antennae with more segments.

5. Store & Send

- In the field, put water, sediment, and dragonfly sample bags into a cooler with ice packs or **wet ice**, being careful not to squish dragonfly larvae.
- Upon return to the laboratory or office, **store dragonfly and sediment samples in a freezer** until shipping. **DO NOT FREEZE THE WATER SAMPLES.** Instead store water samples in a **refrigerator** until shipping.
- **Do not open the inner dragonfly bags!** If you want to check your identification, do so quickly, before freezing, and through the inner bag. Do not allow dragonfly samples to thaw and refreeze; it is important that they remain frozen once placed in the freezer.
- **Water samples MUST be shipped immediately**, with ice packs or very well contained wet ice (it's helpful to double bag the wet ice in a zipper-seal bags to prevent contamination of the bottle with melt water and leaking from the cooler), overnight via FedEx.
- Dragonfly and sediment samples can be stored until all sites are sampled, then **must be shipped in a cooler on DRY ICE.** Include a copy of your field sheet in the shipment, and keep a copy for yourself. Alternatively, the datasheets can be scanned and emailed to the coordinating lab with the originals included in the shipment. Contact us if you have trouble finding dry ice, or have questions about it.
- Note: When shipping samples on dry ice, you must **check the dry ice box on the FedEx® label.** Additionally, the cooler must have the **Dry Ice label** (included in sampling kit) affixed with waterproof tape.
- **Contact your coordinator when a shipment is ready** to verify that someone is available to accept the shipment. Shipment of coolers should be sent FedEx overnight (with pre-paid return label included in kit).

Eastern Parks:

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Orono, ME 04469-5710

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Western Parks:

James Willacker

USGS Forest and Rangeland Ecosystem Science Center

3200 SW Jefferson Way

Corvallis, OR 97331

(jwillacker@usgs.gov); 541-750-0957 (office) or 541-243-3606 (cell)

Not sure if you're east or west? Just email both of us and we'll let you know!

6. Use the data!

We aim to make preliminary park-specific data available ~1 year after the samples have been collected. Work with your citizen scientists to describe the findings at your park. Submit entries to the local science fair. Feel free to contact us if you want to discuss your park's data and how to incorporate it into park programs. UMaine and USGS will interpret the results for the national parks each project year, through webinars or other outlets.

Appendix A. Nets

Participating parks must provide their own sampling nets. There are many options for nets, ranging from expensive D-nets to modified inexpensive baitwell nets. See a few options below. None is necessarily better than the other, so choose according to substrate (if known), resource availability, and group size.

1. The D---Net: LaMotte D---Net, \$70, item #138658 at www.benmeadows.com



#1

2. For smaller mountain streams – or to facilitate group management – aquarium-sized nets may be more useful than a large D-Net. These nets can be purchased for as low as \$4 (see examples at www.carolina.com). A helpful technique may be to allow each citizen scientist or small group one net. Each group or individual then searches a designated section of the stream/shore, bringing potential positive samples back to a central location (i.e., bucket) where the larger group can collectively observe, identify, and select.



#2

3. A good, inexpensive net (specific to dragonfly larvae and not good for quantitative biomonitoring) can be made by modifying a baitwell net – stretch the net across like a pool skimmer and attach with plastic zip ties or small nylon strings. This works well because it won't plug up with sediment (drains quickly) and retains large invertebrates like dragonfly larvae. Cost is \$20-30 and they are available online (http://www.forestry-suppliers.com/product_pages/View_Catalog_Page.asp?mi=5061) or at local fishing shops. These nets are lightweight as well.



#3

Appendix B. How clean is “clean”?

Mercury is all around us: in our hair, in soil, leaves, etc. We need to keep everything (ultra-)clean so to not contaminate our water, sediment, and dragonfly larvae samples. Read on for recommendations on getting gear ready....

How we are using terms in this project:

- **Clean:** Materials and supplies that are prepared in such a way that they would not have contamination that could affect our analyses and interpretation of mercury.
- **Ultra-clean:** We only use this specific term once or twice here. For water samples, which typically have between 10,000---100,000 times less mercury than dragonfly larvae or sediment. The materials you receive for water sampling are specially prepared to be clean enough to allow detection of mercury at these “ultra---trace” concentrations.
- **New:** We use this term when referring to items like plastic spoons and dishpans. By “new”, we mean that the materials are either brand new from the store, or you know they have only been used to hold natural water and invertebrates, similar to what is done in this study. For instance, dishpans might have been used before in your field sampling for macroinvertebrates, which is fine. What we mean by “new” is that this is a designated field supply, not an old dishpan or spoon that was used with food, or materials that you really don’t know about.

Level of cleaning needed for each field item:

Many supplies are “**disposable**”: plastic spoons, gloves, and extra bags. They come to you pre-cleaned and ready to take into the field to use just as they are. These should be marked as “used” when you are done at one site and heading to the next. Each site has its own sampling kit and you should start fresh at each new site. A good practice is to draw an “X” on the trash bag with any supplies that have been used at a site, to keep them from being confused with fresh, un-used supplies.

Before you go in the field:

- For new supplies (e.g., if you bought a dishpan), rinse it out three times with hot tap water before taking it out in the field. Then rinse it in site water as with items you are re-using (see above bullet).
- You can use new garbage bags to transport large items into the field (to minimize road/trail dirt), and to store equipment between sampling days. Just be sure everything is dry first to avoid mildew.

Before your first site or between sites (even with new items): If you are heading from Site A to Site B, you have some field gear that will contact the samples that needs to be **re-used**: nets, and dishpans. What do you do to ensure you are not introducing Site A contamination at Site B? And for new items, how do you ensure that there’s limited contamination from the store or from transit?

- First, you will probably have disinfected your nets, boots/waders, and dishpans after you finished work at Site A. Many parks require disinfection to reduce the spread of amphibian disease or other aquatic-borne pests from site to site. Each park has its own policy and requirements. Often (but not always) this means soaking or spraying nets, boots/waders, and dishpans with a bleach-water solution. Check your permit for procedures.
- Then, you will probably toss your wet, disinfected gear in the back of a vehicle and maybe drive down dusty roads or hike through the woods to get to Site B.
- When you get to Site B (or your first site, for new gear), first figure out where you will do your water and sediment sampling. Locate an area of the pond away from that target sampling location, or downstream of it in the case of a river/stream, and rinse your waders so you can do the water and sediment sampling. Then take those samples.
- After water and sediment sampling, have the rest of the crew rinse their boots, waders, and nets in pond/stream water away from the probable sampling site or downstream. Dunk gear in the water three times to rinse. Then proceed with sampling.

Within a site: If you are at Site A, and you are bagging all of your dragonfly larvae from Site A, you can rinse your plastic spoon in lake/streamwater between samples. The same is true for your Nitrile (purple) gloves. But don’t use those Site A spoons or gloves at Site B – they are contaminated with Site A materials.

Appendix C. Research Permit Application – example RPRS text

Project title: Linking freshwater mercury concentrations in parks to risk factors and bio-sentinels: a national-scale research and citizen science partnership.

Purpose: Mercury, in its toxic methylated form, is a potent neurotoxin that is delivered to ecosystems via deposition from a global atmospheric pool, and ultimately bioaccumulates in aquatic and terrestrial foodwebs. In the northeastern U.S., research sites in ‘pristine’ areas have fish and other biota that exceed thresholds considered safe for human consumption or wildlife protection. All New England states, and many other states, have statewide fish consumption advisories with respect to mercury because, in large part, of the difficulty in predicting which systems are likely to be most affected. This study will be part of ongoing citizen science research that is evaluating the utility of dragonfly larvae (Odonata: Anisoptera) as indicators of mercury status. Our early data are being used to develop hypotheses regarding whether mercury varies with odonate larval body size or by family; or whether a site’s landscape setting drives variability in mercury in odonate larvae. To date, research has been primarily carried out by citizen scientists in national parks in the pilot program; this permit request is in support of an effort to expand the work beyond the pilot parks to identify broader-scale spatial patterns and better understand the utility of this bio-sentinel.

Summary of proposed field methods and activities: Aquatic macroinvertebrates are typically collected using standard D-nets with 500 micron mesh or dip nets with larger mesh (if dragonfly larvae only are targeted), and/or by inspection of cobbles, submerged or emergent vegetation, and woody material. Individual dragonfly larvae are picked (with gloves or pre-cleaned forceps or spoons) from nets and double bagged in zipper seal bags. Individuals are frozen until shipment and analysis. Body length of individuals and identification to taxonomic family may be done at the field site or after received in the lab, by prior arrangement. At the field site, individuals not selected for analysis are immediately returned to the waterbody of origin. Surface water samples (2 L per site) are also collected and analyzed in the lab and surface sediment samples (upper 2 cm of sediment near shore; 3 mini-cores (<2cm in diameter) collected from each water body).

Repository Type: Will be destroyed through analysis or discarded after analysis

Objects Collected:

- Odonata: anisoptera (dragonfly) nymphs; 60 per park per sampling outing. (20 per site, 2–3 sites).
➤➤ *NOTE: most parks will sample once per year. A few parks will sample repeatedly to help determine if there is seasonal variability.*
- Surface water sample will be collected (2 L total).
- Sediment sample

Location Information:

- List sites: *(Specific to each park; to be determined.)*
- Access: *(Specific to each park; access by foot on roads or trails.)*

Where will data, maps, photos, etc. (not specimens) reside upon completion of this project?

Data are stored in original laboratory files, on the project PIs computer (and associated backups), and will be uploaded to IRMA after final quality assurance and publication of results. Data collected by students/teachers from sites in the pilot studies are already available on IRMA.