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

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Scope and Application.

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. The study connects people to parks, advances the educational mission, fosters biodiversity discovery opportunities, and provides baseline data to better understand the spatial distribution of mercury contamination in national parks.

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 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.)

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!

This citizen science 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 interpreters, resource managers, or research learning center staff are also available. Ultimately, this project also helps raise awareness about mercury impacts.

The citizen science effort to collect dragonfly larvae from national parks for mercury analysis expanded in 2012 to include a total of approximately 14 parks across the following NPS regions: Northeast, Southeast, Midwest, Intermountain, Pacific West, and Alaska. Citizen scientists involved include students ranging from elementary-aged to college, park visitors, and BioBlitz participants. In addition to collecting dragonfly larvae, all parks are collecting a water sample for mercury-relevant water chemistry (i.e., dissolved organic carbon (DOC), pH, sulfate) and a water sample for total mercury. Most parks will sample once per year but a few parks will sample twice per year to help determine if there is seasonal variability. Data results will be available in 2013/2014.

The optimal study design is 2–3 sites per park that represent a gradient in mercury-relevant chemistry (as defined above); or a gradient in landscape condition, 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 retain a healthy population of dragonflies? (A sample size of 15 dragonfly larvae per site is preferred.) Will nearby riparian flora and fauna get trampled? Is the site spacious enough for a group? Safety of the participants involved is extremely important. 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. Be attentive of the possible risks.

A park research permit will be needed to conduct this project; the dragonfly and water samples collected for this project will be destroyed through analysis, or discarded after analysis. 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://sharenrss/ard/Dragonfly_Mercury_Project/Forms/AllItems.aspx.)

OVERVIEW

This protocol has several sections. Each sampling procedure is fairly straightforward, after you've gotten the hang of it. Here is the general structure:



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

- 1. Prepare by gathering field gear.** Some is provided in the sampling kit, but you'll need to round up a few items. Check what's needed a couple of weeks before sampling (in case you need to order something).
- 2. Sample:**
 - First, a water sample for mercury.** It is the most sensitive to contamination. You need the white, powder-free gloves and the double-bagged Teflon® bottle. **DON'T** open their bags before going out in the field!
 - Second, a water sample for other chemistry.** No gloves needed, it's the HDPE (whitish) bottle in the kit.
 - Third, dragonfly larvae!** Many hands sampling makes this go quicker. Read the protocol before handling samples – we need to keep them clean (contamination-free).
 - 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. Label, measure, identify your samples (bagging them individually as you go).** We will check this information at the lab as well, but it's most useful if you can do it on site when samples are fresh.
- 4. Ship your samples.** Dragonflies 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. Water samples are stored in a refrigerator, and should be shipped within 24 hours of collection. This might mean one shipment for water samples, and maybe a later shipment for dragonflies. Get in touch if you need help with these logistics.

I. Prepare.

- *The following sampling supplies are needed to collect dragonfly larvae samples and a water sample from each site. All supplies will be provided in the sampling kit, or are already provided within this protocol (as is the case for the field sheet and tags), with the exception of those materials in **bold**. Participating parks must purchase/provide those items separately; funds are provided for dry ice and bottled water in the sampling kit.*

Materials:

Dragonfly sampling:

- Net(s)* - D Net or dip net**
- Bottled spring water** (1 liter) – see Appendix B for the list of regionally-available bottled waters meeting criterion
- Clean squirt bottle** (an empty spring water bottle with squirt top works; make sure it has not been in contact with mouth – it needs to be clean)
- Clean, new plastic spoons
- Clean, new white dishpan or bucket**
- Brand new Ziploc bags – 30 snack size + 5 quart size
- Powder free Nitrile gloves (blue or purple color)
- A plastic ruler with mm scale – clear is best
- Tags for outer bag (print sheet below)

Water sampling:

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

General field supplies:

- Sharpie marker (not ultrafine – regular felt-tip) and pencil/pen
- Field sheet (print sheet below)
- Ice - wet and dry**
- Cooler
- Dry ice label and pre-paid shipping label for return shipping
- Trash bag**
- Optional - hand lens, GPS, camera, macroinvertebrate field guide, waders**

- **Gather supplies.**
- **Print** Field sheet and Tags before going in to the field. Cut tags apart.
- **Label** each HDPE plastic bottle (for the water sample) with site name, date, and your initials before getting the containers wet. Use the Sharpie permanent marker.

*More information on nets on following page.



Selected sampling supplies required for the collection of dragonflies. Some of these materials are provided in the sampling kit (Ziplocs, gloves, spoons, Sharpie, ruler).

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

II. Collect.

- *Collect samples in the order listed: water for mercury, water for mercury-relevant chemistry, dragonfly larvae.*
- *Repeat dragonfly larvae and water sampling for 2–3 waterbodies/sites per park.*
- *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.*



A high school student collecting water for mercury analysis at Great Smoky Mountains National Park, North Carolina, 2012.

A. Water Sample for Mercury Analysis

- *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 below sampling procedure 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 for Mercury Analysis

1. Collect water for total mercury **FIRST, BEFORE** any other field work at the site (including sampling any invertebrates and sampling water for mercury-relevant chemistry). **This sample is the most sensitive to contamination** and needs to be done before anyone goes in the stream/pond and before any possible stirring up of the site.
2. If you mistakenly wade right in before collecting this sample, either move a little upstream or wade/paddle far enough out to avoid the disturbed area – or, return another day for the water samples!
3. **NOTE:** the Teflon bottles for mercury collection are stored with a 0.1% HCl (hydrochloric acid) solution. Though it won't burn technicians or acidify water, it should be poured out on the ground where it will be immediately neutralized by soil, not directly in the stream, for safest disposal.
4. **Two people are needed to sample.**

Keep it Clean!

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 for total mercury**. 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. 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, pulls out the sample bottle, and disposes of the storage solution.
5. Clean Hands takes the bottle to the sample site, rinses 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. Dirty Hands seals the outer bag.
7. The bottle number (usually capital letters and numbers) is written on the outer bag. Record that bottle number on the field sheet for each site. **Nothing must be stuck on or written inside the outer bag.**
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. On the field sheet, record sample date, time, site name, field technician names, and any notes about the site, weather, sample or procedure, or other irregularities should be recorded while in the field.

- See a **video demonstration** of these water sampling procedures for analysis of total mercury: <http://www.youtube.com/watch?v=BIHJFO4pfpl>

B. Water Sample for Mercury-Relevant Chemistry

1. Collect water for basic, mercury-relevant chemistry (below, steps 2–4) **BEFORE** sampling any invertebrates so that there is no sediment stirred up in the water column.
2. **Note observations and complete the field sheet.** Record 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).
3. 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, and **rinse the HDPE plastic bottle provided twice** with lake water. For streams, reach into a deeper part of the stream, preferably a pool below a riffle, and rinse the bottle twice. **Fill the bottle and cap it** to allow it to equilibrate a few minutes with sample water (this is a third rinse, but let the water sit in the bottle a couple of minutes).
4. Finally, **empty the bottle and collect samples** by placing cap loosely on the bottle and quickly inserting it to about ½ meter depth (1.5 feet) (or about halfway to the bottom for shallow streams – the goal is to submerge the entire bottle without stirring up any sediment or plant matter on the bottom). **Fill and cap underwater**, eliminating air in the bottle. Avoid collecting the surface film on the water, and avoid contaminating samples with salt from your skin. **Tighten bottle caps completely.**

C. Dragonfly Larvae Sample

1. **Look around.** Locate likely habitats for macroinvertebrates: vegetated bank margins, snags and logs, aquatic vegetation beds and decaying organic matter, silt/sand/gravel substrate.
2. **Note observations and finish completing the field sheet.** Take a site photo!
3. **Fill** squirt bottle and partly fill dishpan or bucket with water from the stream or pond you are sampling.
4. **Jab** your net into the likely habitats. Jab 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 and squirt the backside with the squirt bottle to clear the net*. Spend about 20 minutes jabbing and emptying the net at most. If you don't find much after 20 minutes, move to another site within the same reach.
 - See a video demonstration of collecting dragonfly larvae: <http://youtu.be/psTu10uwdOg>



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

** Some samplers find that it's easier to skip the step of emptying the net into the bucket or dishpan, and simply pluck or scoop larvae directly from the net into clean sample bags (see Section IV, below). This is fine, but to get a good sense of the types and sizes of larvae at your site, you might want to try the bucket method to give you a better collection of dragonfly larvae from which to choose.*

Procedures – Collecting Dragonfly Larvae Samples

Keep it Clean! 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** the pre-cleaned plastic spoon or powder-free nitrile 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 **cleaned 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 pair of gloves (one-gloved hand may suffice). Once the gloves are on, Clean Hands touches nothing but the sample and any pre-cleaned supplies.
 3. Using a nitrile-gloved hand, or pre-rinsed plastic spoon, Clean Hands places one dragonfly into a sample bag while Dirty Hands holds the bag. Clean Hands seals the bag.
 4. Dirty Hands puts this bag into a clean outer bag.
 5. Dirty hands uses the plastic ruler, held up to the individual in the bags, and **measure the body length** in millimeters (mm) – from the tip of the mentum (front of head) to end of tail spine. Most will range between about 10–40 mm, as a reference.
Optional: look more closely at the wing pads to identify which instar (developmental stage, like a molt) the dragonfly larva is in. See Appendix C.
 6. Dirty hands **fills out a tag** (see below) for the bagged individual and slips it and the inner bag into the outer bag.
 7. **Give your samples a Sample ID:** Your waterbody name and a unique number (for example, if your site is the Colorado River, call your first sample “Colorado River-1”, second sample “Colorado River-2”, etc.). Dirty hands seals the outer bag.
 8. **Clean up:** rinse your spoon with the bottled spring water 3 times. Rinse anything else that contacted the sample with the bottled spring water 3 times, and if you are using gloves to handle the samples, put on a fresh glove for the next sample. Treat each individual like a brand-new item.
 9. Go to the next individual and repeat. **Choose, measure, & bag 15 individuals per waterbody/site.** Place all of the small double bags into one or more of the larger Ziplocs® for storage and to keep them safe and together. *(See Section IV)*
 10. Gently return extra streamwater and invertebrates to the sample site.
 11. Pack out all trash.

III. Identify.

Please identify dragonfly larvae to family if you are comfortable doing so. There are only 6 major families in most places. Identification 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/> would assist efforts. Identifying to family might require careful inspection. The ID will be checked once the UMaine laboratory receives samples, and most samples will be further identified to species by a Maine taxonomist, but identifying samples when they are fresh is helpful in the event of damage during shipping.



The 6 major families of dragonfly larvae, from left to right: Corduliidae (Emerald Family), Macromiidae (Cruiser Family), Cordulegastridae (Spiketail Family), Libellulidae (Skimmer Family), Aeshnidae (Darner Family), Gomphidae (Clubtail Family).

*Gomphids have many body forms (they can also have a long, pointed abdomen); key features are a small head and short legs as compared to most Corduliidae, Macromiidae, and Libellulidae.

- 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 in the lab in Maine and provide the identification information to you along with the mercury and other data.
- C. Related websites for additional background 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.ummz.lsa.umich.edu/MICHODO/test/Subordrs.htm (real key in traditional sense)
 - www.odenews.org/NElarvaeGenusKey.pdf (genus level)
 - www.odenews.org/NEAnisopteraSpeciesKey.pdf (species level - detailed, but an 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/>

IV. Choose, Measure, & Bag.

- *Look at the samples you've collected. If you have identified to family already, **please choose Gomphids first, then Aeshnids; if you are in a pond, you may be more likely to find Corduliidae and Libellulidae – choose these first if they are most abundant.** Within a family, see if you can get several specimens about the same size. 15 individual larvae will be selected per site. Ideally, we would like to analyze samples larger than 15 mm – please choose larger samples over smaller ones if possible.*
- *Given the risk of contamination, it is very important to follow the **Keep it Clean!** Protocol (below) 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 Ziploc® bag, then with label placed in an outer bag, and stored on wet ice.*



Left photo: A high school student measures a dragonfly larvae sample for the study at Great Smoky Mountains National Park, North Carolina.

Right photo: A completed, double-bagged dragonfly sample with labels prepared for storage in freezer or on ice, and shipment.

V. Store & Send.

- In the field, put both the water sample and your dragonfly sample bags **on wet ice** in a cooler.
- Upon immediate return to the laboratory or office, **store dragonfly samples in a freezer** until shipping. **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 bag if at all possible.
- **Samples must be shipped in a cooler, preferably on dry ice.** Include a copy of your field sheet in the shipment, and keep a copy for yourself.
- Note: If you ship the 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. If you are unable to ship with dry ice, please contact Sarah to discuss an alternate shipping option.
- **Contact Dr. Sarah Nelson when shipment is ready** (sarah.j.nelson@maine.edu, 207-581-3454) 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) to:

University of Maine
George J. Mitchell Center
c/o Dr. Sarah J. Nelson
5710 Norman Smith Hall
Orono, ME 04469-5710

Mercury in Dragonfly Larvae from National Parks – Sampling Guide

Tags to go in outer bags. Print 1+ copy per site and cut apart the tags before collecting.

<p>Park _____ State _____</p> <p>Site _____ Collector _____</p> <p>Sample ID _____ Date _____</p> <p>Dragonfly family:</p> <table><tr><td><input type="checkbox"/> Gomphidae</td><td><input type="checkbox"/> Aeshnidae</td></tr><tr><td><input type="checkbox"/> Libellulidae</td><td><input type="checkbox"/> Macromiidae</td></tr><tr><td><input type="checkbox"/> Cordulegasteridae</td><td><input type="checkbox"/> Corduliidae</td></tr><tr><td><input type="checkbox"/> Unknown</td><td></td></tr></table> <p>Dragonfly length (mm) _____</p>	<input type="checkbox"/> Gomphidae	<input type="checkbox"/> Aeshnidae	<input type="checkbox"/> Libellulidae	<input type="checkbox"/> Macromiidae	<input type="checkbox"/> Cordulegasteridae	<input type="checkbox"/> Corduliidae	<input type="checkbox"/> Unknown		<p>Park _____ State _____</p> <p>Site _____ Collector _____</p> <p>Sample ID _____ Date _____</p> <p>Dragonfly family:</p> <table><tr><td><input type="checkbox"/> Gomphidae</td><td><input type="checkbox"/> Aeshnidae</td></tr><tr><td><input type="checkbox"/> Libellulidae</td><td><input type="checkbox"/> Macromiidae</td></tr><tr><td><input type="checkbox"/> Cordulegasteridae</td><td><input type="checkbox"/> Corduliidae</td></tr><tr><td><input type="checkbox"/> Unknown</td><td></td></tr></table> <p>Dragonfly length (mm) _____</p>	<input type="checkbox"/> Gomphidae	<input type="checkbox"/> Aeshnidae	<input type="checkbox"/> Libellulidae	<input type="checkbox"/> Macromiidae	<input type="checkbox"/> Cordulegasteridae	<input type="checkbox"/> Corduliidae	<input type="checkbox"/> Unknown	
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<input type="checkbox"/> Unknown																	

Field Sheet – Mercury in Dragonfly Larvae from National Parks

National Park name: _____ Site Latitude: _____

Site name/Waterbody: _____ Site Longitude: _____

Date: _____ Lead collector name/Contact info: _____

About the site: Stream Pond or lake Wetland Approx. size _____

Stream/pond substrate: Bedrock Boulders (rocks > 10") Cobbles (rocks 2-10")
 Gravel (rocks 0.1"-2") Sand (gritty) Silt Clay (slick)

1. Land Use (500 m radius around site) <input type="checkbox"/> Urban <input type="checkbox"/> Upland conifer <input type="checkbox"/> Cultivated <input type="checkbox"/> Swamp hardwood <input type="checkbox"/> Pasture <input type="checkbox"/> Swamp conifer <input type="checkbox"/> Upland hardwood <input type="checkbox"/> Marsh	2. Terrain (500 m radius around site) <input type="checkbox"/> Flat <input type="checkbox"/> Rolling <input type="checkbox"/> Hilly <input type="checkbox"/> Mountains	3. Canopy Cover (overhead view) <input type="checkbox"/> Dense (75-100% shaded) <input type="checkbox"/> Partly open (25-75% shaded) <input type="checkbox"/> Open (0-25% shaded) (% daily direct sun) _____
--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

4. Physical Characteristics of Site (Make notes here)			
Slope _____	Soil _____	Elevation _____	m or ft
Overstory vegetation/forest _____	Understory vegetation/Forest _____		

Water sample collected for mercury analysis – Bottle ID: _____ Sample time: _____ AM PM

Notes about the water samples or site information:

Dragonfly Samples collected:

#	Sample ID	Family	Size (mm)	(Optional)	
				Instar	Species
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					

Notes about the samples:

Appendix A. Research Permit Application – example RPRS text

Project title: Developing a citizen science program for mercury in National Parks: Can dragonfly larvae both inform science and engage Park audiences?

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 in the Northeast region. 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 (under scientist supervision) in Maine, and in national parks in the pilot program ; this permit request is in support of an effort to expand the work beyond the Northeast and the existing 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 Ziploc® 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 (~500 mL per site) are also collected and analyzed in the lab.

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

Objects Collected:

- Odonata: anisoptera (dragonfly) nymphs; 45 per park per sampling outing. (15 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 (~1 L total).
 - *NOTE: we have funding to take a water sample for Hg and Hg-relevant chemistry (DOC, pH, sulfate) at all parks/sites.*

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 or to a web site that houses citizen science data, www.participatoryscience.org – on a special page that will include Park info that is in development in 2013. Data collected by students/teachers from sites in the Northeast U.S. are already available on this site.

Appendix B. Acceptable Bottled Spring Waters*

Based on water quality reports at <http://www.nestle-watersna.com/QualityReports.htm>

Visit map at: <http://www.myspringwater.com/GetQualityWater/RegionalMap.aspx>

Brand	Specific product	Region served	MRL	MCL	Mercury (mg/L)	Report date
Arrowhead®	Mountain Spring Water	West & Rockies	0.0002	0.002	ND	12/2011
Deer Park®	Natural Spring Water	Southeast	0.0002	0.002	ND	12/2011
Ozarka®	Natural Spring Water	Gulf coast	0.0002	0.002	ND	12/2011
Ice Mountain®	Natural Spring Water	Midwest	0.0002	0.002	ND	12/2011
Zephyrhills®	Natural Spring Water	Florida	0.0002	0.002	ND	12/2011
Poland Spring®	Natural Spring Water	Northeast	0.0002	0.002	ND	12/2011

MCL - Maximum Contaminant Level. The highest level of a substance allowed by law in drinking water (bottled or tap water). The MCLs shown are the federal MCLs set by the U.S. Environmental Protection Agency and the Food and Drug Administration, unless no federal MCL exists;

MRL - Minimum Reporting Limit. Where available, MRLs reflect the Method Detection Limits (MDLs) set by the U.S. Environmental Protection Agency or the Detection Limits for Purposes of Reporting (DLRs) set by the California Department of Health Services. These values are set by the agencies to reflect the lowest concentration of each substance that can be accurately quantified by applicable testing methods, and are also the minimum reporting thresholds applicable to the Consumer Confidence Reports produced by tap water suppliers;

ND - Not detected at or above the MRL

Why are these waters acceptable? Published water quality reports are available for these waters, and compare across the types because they are managed by a single parent company. The maximum contaminant level (MCL) allowed in these waters is 0.002 mg/L, which equals 0.002 ppm (parts per million). We can convert this to parts per billion (ppb) by multiplying by 1000; this gives us a maximum concentration of 2 ppb in the spring waters. We are measuring aquatic invertebrates at mercury concentrations ranging from 10–1000 ppb, dry weight (parts per billion). Dragonfly larvae contain ~50–600 ppb, dry weight and average 250 ppb, dry weight. This means that the concentration in the spring water used to rinse equipment could contribute, at most, between 0.3–4% of the mercury burden found in an individual dragonfly. This assumes that mercury in the water is right at the MCL, which may or may not be the case. Typically, error margins allowed in most laboratory methods are 10% or less for most quality control parameters. Therefore, we are comfortable with using these spring waters because the probable error they introduce is reasonable in comparison to the target organisms' typical mercury concentrations.



Map courtesy of Staples.com

*The suggestions herein do not represent an official endorsement by the U.S. Government.

Mercury in Dragonfly Larvae from National Parks – Sampling Guide – Appendix B.
Acceptable Bottled Spring Waters

Appendix C. Optional – Instar Identification

[In progress]