



*U.S. Geological Survey (USGS) Forest and Rangeland Ecosystem Science Center
Appalachian Mountain Club (AMC) – Research Department
National Park Service (NPS) – Air Resources Division (ARD)*



DRAGONFLY MERCURY PROJECT

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

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**** REMINDERS FOR 2025 FIELD SEASON ****

This page is intended for those who are familiar with protocols from previous sampling efforts to easily navigate to updated material. However, it is suggested for both new participants and returners to read through this sampling guide before each year's sampling effort.

- **Changes to kit shipment procedures:** Dragonfly sampling kits will no longer arrive in little red coolers. Kits will be shipped in a DMP-labeled, red cardboard box.
- **Return sample shipment procedures:** We will continue to use a modified dragonfly sample return shipment procedure to afford more flexibility and better ensure sample integrity. Dragonfly samples are no longer shipped to the lab in little red coolers. Once all dragonfly sampling is complete, contact dmp@nps.gov with a planned sample shipment date. USGS will send a specialized shipper, pre-paid shipping label and ice packs (if not using dry ice) within 1-2 weeks of the anticipated sample shipment date. Packing & shipping details will be provided in post-sampling email follow-ups, on a laminated card in the shipper, and in the sampling guide.
- **Water sampling kits:** Water sampling kits will continue to automatically ship for each planned dragonfly sampling site. Water samples should be shipped to the lab on a Mon or Tues, ideally within 1-week of collection. Replacement Styrofoam coolers for shipping any remaining water samples can be requested by contacting dmp@nps.gov. See water sampling details now included in this sampling guide, starting on page 14.
- **Minimum larvae size:** Small larvae (<10 mm) complicate lab processing. It can also be difficult to generate quality data from them. Therefore, we are requesting that all larvae collected are at least 10 mm in total length.
- **Family identification:** It is relatively difficult to separate Corduliidae and Libellulidae with 100% certainty. The identification information provided will separate most larvae, but it is okay if you are not sure! All identifications are checked in the lab and in most analyses, these two families will be combined.
- **A word on safety:** Please refer to the section on Safety for guidance towards a draft park Job Hazard Analysis (JHA) to consider while creating your own. Nothing in this program's mission, project goals, or day-to-day objectives is so important as to compromise the safety, health, and well-being of those participating. See newly added Appendix F for Personal Floatation Device (PFD) recommendations.

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Park scientists search for dragonfly larvae at Redwood National Park, CA, 2020. NPS Photo.

Scope and Application

Background

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 <https://www.nps.gov/subjects/air/nature-toxics.htm> 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) 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 or more 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 Dragonfly Mercury Project (DMP) 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. The DMP expands the geographic scope of research previously conducted by scientists and citizen scientists and provides data that can be compared across parks. Data from this project are being used to develop hypotheses regarding the characteristics, environments, and processes that influence mercury concentrations in dragonfly larvae. The DMP 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 Great Smoky Mountains NP and four other NPS units in the Northeast. Since then, it has expanded to over 100 NPS units and National Natural Landmarks across all NPS regions. Over 5,000 citizen scientists have participated in the DMP since then! Those involved include students ranging from elementary school- to college-aged, park visitors, and BioBlitz participants.

NPS Project Webpage:

[go.nps.gov/dragonflymercury](https://www.nps.gov/dragonflymercury)

USGS Project Webpage:

<https://www.usgs.gov/centers/forest-and-rangeland-ecosystem-science-center/science/dragonfly-mercury-project>

Data Visualization Dashboard:

[go.nps.gov/dmpmap](https://www.nps.gov/dmpmap)

Study Design

The optimal study design is 3 sites per park that represent a gradient in mercury-relevant chemistry; 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 land cover. Consider scoping the sampling locations in advance of collecting samples and scope a backup site if you have time. 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?

Research Permit

A National Park Service research permit will most likely be needed to conduct this project. In some cases, additional state, Federal or private lands permits may also be needed to collect dragonfly larvae. If collections will occur on, or require access through, lands not directly under the park's jurisdiction, the park should receive written permission from the landowner. Participating parks are responsible for all permits and permissions. Contact the park's natural resource coordinator for more details on permitting. Example text for the permit application can be found in Appendix C of this sampling protocol.

Safety

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. High water levels and turbulent flows may cause an individual to lose balance in the water. Washes may flash flood at a moment's notice. Lakes may be significantly colder than air temperatures, even in summer. Be attentive of the possible risks and refer to a detailed Job Hazard Analysis (JHA) that is developed specific to surface water sampling, backcountry work, traveling in high elevations, poison ivy, tickborne disease, etc. An NPS-approved JHA is needed before heading out into the field. Example JHAs are available in the Safety Resources section on the DMP SharePoint site: <https://doimspp.sharepoint.com/sites/ext-usgs-dragonflymercury>. Define, communicate, and enforce your park's safety philosophy, standards, and guidelines; follow the tenets of Operational Leadership (e.g., brief your team prior to sampling on mission elements: Communication, Contingency Resources, Environment, Planning, Task Complexity, Supervision, Team Fitness, and Team Selection) and other relevant safety trainings such as swift water rescue; allow sufficient time for oversight and interaction with your group; and follow Departmental guidelines on the use of personal protective equipment (PPE), such as personal flotation devices (PFDs). All citizen scientists should be wearing approved PFDs when sampling in all water bodies, regardless of depth or flow. USGS scientists are required to wear PFDs in water more than ankle-deep. Options for PFDs approved by the Department of Interior can be found in Appendix F. Please use caution when sampling. **Nothing in this program's mission, project goals, or day-to-day objectives is so important as to compromise the safety, health, and well-being of those participating.**



Dragonfly Sampling Overview

This protocol has several sections. Each sampling procedure is straightforward, but it may take 30 minutes to get through upon the first time implementing. 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!**
 -  **First, collect dragonfly larvae samples. 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 larvae have been collected, samples are sorted, and 20 individuals (or as many as are able to be collected) at least 10 mm long are selected for mercury analysis. These are measured, identified to family, then individually bagged to prevent contamination.
 -  **Second, 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. Most importantly, **accurate coordinates must be provided** (latitude and longitude) for each site, as we can only accept samples that are properly geo-referenced. We also encourage observations through the iNaturalist Project "Dragonfly Mercury Project" <https://www.inaturalist.org/projects/dragonfly-mercury-project-nps>.
3. **Ship your samples.** You'll ship dragonfly samples overnight in a shipper with either dry ice or provided ice packs. 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.



1. Prepare

The following sampling supplies are needed to collect dragonfly larvae samples from each site. All supplies will be provided in the sampling kit, except for those materials in **bold**. Participating parks must provide those items separately.

Materials

Dragonfly sampling:

- ☐ **Nets*** – D Nets or dip nets (often called “baitwell nets”)
- ☐ **Clean, white dishpan, bucket, or ice cube trays**
- ☐ Clean, new plastic spoons
- ☐ Brand new zipper-seal bags – 20 small + 20 large size
- ☐ Powder free Nitrile gloves
- ☐ 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
- ☐ Dragonfly identification cards
- ☐ **Cooler for samples**
- ☐ **GPS-enabled phone or standalone GPS unit**
- ☐ **Copy of research permit where required**
- ☐ **Trash bag**
- ☐ **Optional – hand lens, camera, photo release forms, macroinvertebrate fieldguide, waders**
- ☐ **Citizen science groups must provide their own PFDs (personal flotation devices) for safety**



Shipping (supplies provided after sampling is complete):

- ☐ **Dry ice** or ice packs for dragonflies
- ☐ Newspaper or other filler paper
- ☐ Shipper
- ☐ Pre-paid return shipping label(s)
- ☐ Dry ice label (if necessary)



Resources

- See the NPS project webpage (go.nps.gov/dragonflymercury) and the USGS project webpage (<https://www.usgs.gov/centers/forest-and-rangeland-ecosystem-science-center/science/dragonfly-mercury-project>) for access to information about mercury and dragonflies, mercury in dragonfly data and reports and an educational toolkit!
- Watch “how-to” videos of sampling and shipping methods on the project SharePoint site: <https://doimsp.sps.sharepoint.com/sites/ext-usgs-dragonflymercury/>
- Follow “Six-Legged Scouts in the National Parks” on Facebook (<https://www.facebook.com/SixLeggedScoutsInTheNationalParks>) and on Instagram (#dragonflymercuryproject)

*More information on nets in Appendix A.

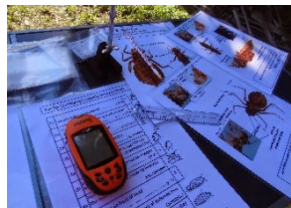


2. Collect samples

- Collect dragonfly larvae.
- Repeat the sampling at each site being sampled.
- 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, wearing the appropriate PPE, are especially preferred.
- Reference Appendix D on “Sacrificing Dragonfly Larvae for Science” in order to address potential ethical concerns raised by your team of citizen scientists.

Procedure

1. **Look around.** Locate likely habitats for dragonflies: vegetated bank margins, snags and logs, aquatic vegetation and decaying organic matter, silt/sand/gravel substrate. (See Appendix E on how to find dragonfly larvae) and take a site picture.
2. **Record the site name and coordinates. IMPORTANT:** USGS requires all samples to be tagged to a specific geographic location. Site coordinates **MUST** be provided, or samples will not be processed.
3. **Note observations and fill-in the field sheet.** In addition to filling out the datasheet, feel free to make notes about the site conditions.
4. **Rinse the nets and dishpans** with water from the site you are sampling.
5. **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.
6. **Jab** your net into the likely habitats. Start downstream first, then 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.
7. **Record information about the number of participants of each type on the datasheet.** Any personnel being paid for their time should be recorded as *Park Staff*, while those not being paid for their time should be recorded as *local/park volunteers*. Record the total time spent at the site.
8. **Disinfect** waders, nets, and all 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.





Procedures – Collecting Dragonfly Larvae Samples

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 pickup 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. 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. Where possible, only larvae > 10 mm in length should be collected for mercury analysis.
 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. Choose, Measure, & Bag)
 10. Gently return extra site water and invertebrates to the sample site.
 11. Be sure to record the site coordinates and fill out your field sheet completely.
 12. 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 guidebook, or you can bag samples (Section 4) and bring them to a clean indoor workspace to look more closely.

Tools to help identify dragonfly larvae by family:

- Dragonfly family identification key: <https://irma.nps.gov/DataStore/DownloadFile/646996>
- Video “Identifying Maine Dragonfly Larvae to Family” <http://vimeo.com/76713446>. Similar families are found outside of Maine.
- Macroinvertebrate website: <https://www.macroinvertebrates.org/taxa-characters/odonata-larva>

Identifying to family requires careful inspection. The ID will be checked once the laboratory receives samples but identifying samples when they are fresh is helpful in the event of damage during shipping.

Consider these tips when identifying:

- It is relatively difficult to separate Corduliidae and Libellulidae with 100% certainty. The identification information provided will separate most larvae, but it is okay if you are not sure! All identifications are checked in the lab and in most analyses, these two families will be combined.
- 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. The key distinguishing characteristic between the two suborders is that Zygoptera have 3 caudal gills extending from the tip of the abdomen. Anisoptera do not. Anisoptera tend to have longer aquatic lifespans (up to 5 years) than Zygoptera (usually 1 year).
- See the “Tips for Improving upon Dragonfly Identification Accuracy Rates” on the “Successes of Citizen Science– Dragonfly Mercury Project” webpage available at: <https://www.nps.gov/articles/citizen-science-success-dmp.htm>

4. Choose, Measure, & Bag

- Look at the samples you’ve collected. If you have identified to family already, ideally choose at least three individuals in each of the families you found. If you found fewer than three individuals in a certain family, exclude the family unless you have fewer than 15 individuals among the other families. Ideally, at least 10 and up to 20 individual larvae will be selected per site. **Larvae larger than 15 mm are easiest to measure mercury in and larvae less than 10 mm may not be able to be analyzed at all.**
- 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. If you make a mistake or think a sample may have been contaminated (someone touched it with bare hands, or some soil fell in the bag), don’t worry! Just make a note on the field sheet about what happened.
- 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.
- If you are taking a photo of the specimen, consider using an iPhone/Android macro lens, if available, and doing so just before the larva is bagged to obtain the clearest picture possible!



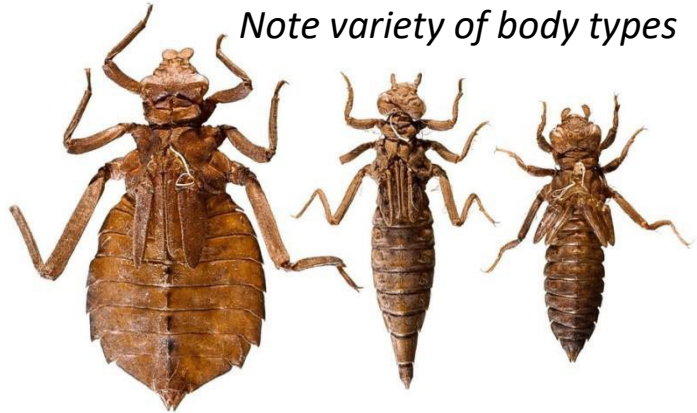


Aeshnidae



Gomphidae

Note variety of body types



Corduliidae

Epitheca princeps

Labium extended



Libellulidae

Labium extended



Macromiidae



Cordulegastridae

Labium extended



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



GENERAL ANATOMY OF NYMPH :

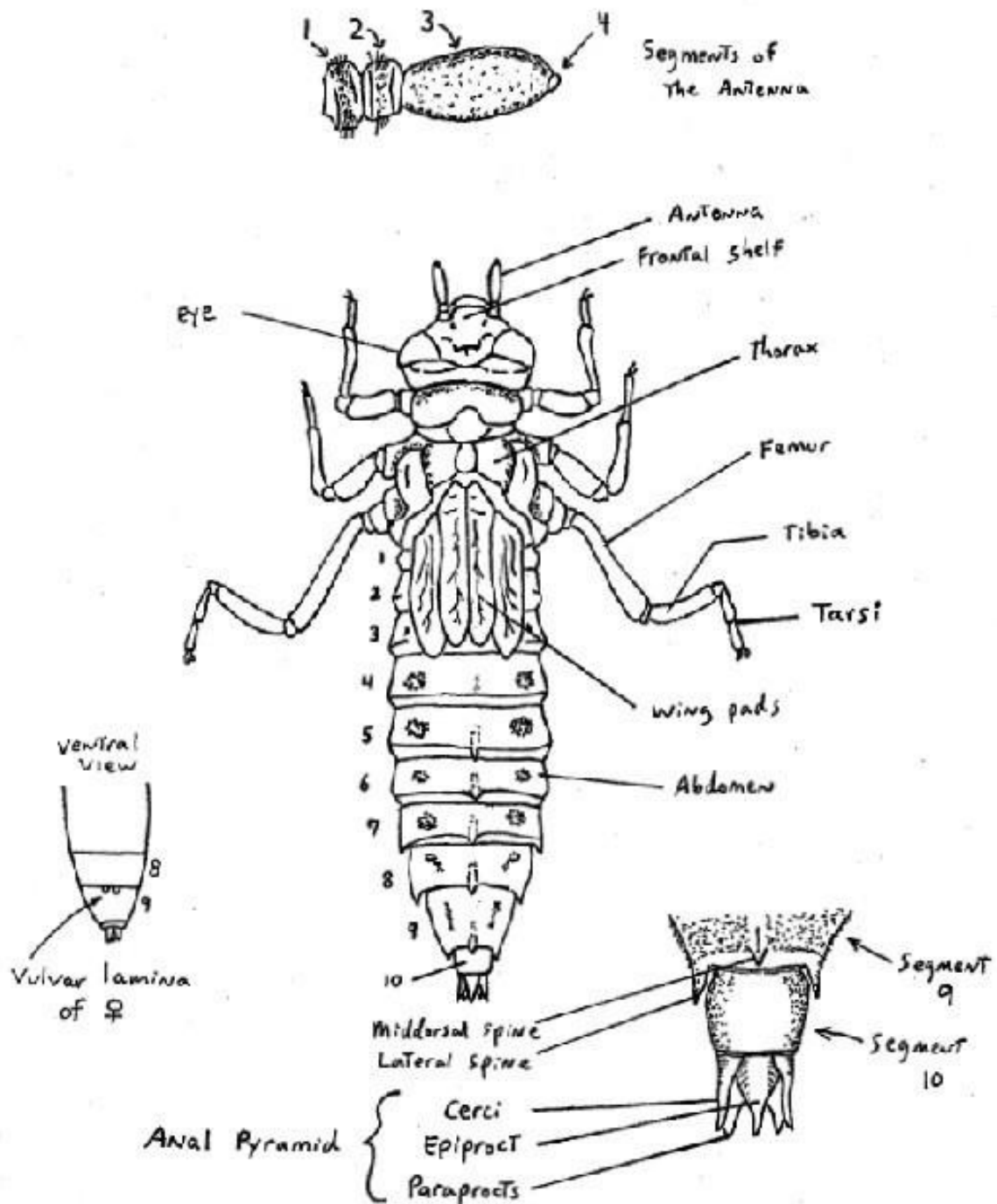


Diagram: Ken Soltesz

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. Storing Samples

- In the field, put 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 larvae in a freezer** 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.
- Dragonfly samples from each site can be stored in a freezer until all sites have been sampled at the park and then shipped all at once.

6. Shipping Samples

All sample shipments must be arranged with USGS prior to being shipped. Please contact the DMP team via the project email (dmp@nps.gov) to arrange a shipping date and request a shipper and pre-paid label for sample shipment to the lab.

- Any sample shipments should be scheduled for **Monday or Tuesday** to ensure samples are received prior to the end of the work week.
- We provide a pre-paid, addressed shipping label for FedEx®. Please fill in your name and address in the *Sender Information* section of the label and **confirm that *PRIORITY overnight service is selected***.
- Samples **must be shipped frozen**. We prefer that samples be shipped on dry ice using the provided labels and shipper; however, if you are unable to locate dry ice locally, please request ice packs to be sent with the shipper.
- **To pack a cooler for shipping with dry ice**, place your solidly frozen samples (with as much air removed from the bags as possible) in the provided padded envelope, on the **bottom of the shipper**, cover the samples with dry ice (we recommend approximately **5-7 lbs of dry ice** for the coolers we provide), and then fill any remaining airspace with crumpled paper. Place completed field sheets on top, then place the lid on the shipper. Seal the outer cardboard box with waterproof tape (do not use duct tape). When shipping samples on dry ice, you must **check the dry ice box on the FedEx® label**. Additionally, the outer cardboard box must have the **Dry Ice label** (included in shipper) affixed with waterproof tape.
- **To pack a cooler for shipping with ice packs**, place one ice pack vertically along each wall of the inner shipper, forming a cube for the samples to be placed in the middle. Place your solidly frozen samples into the middle of the ice pack cube. Place the remaining ice pack on top, like a lid, covering the samples. Place completed field sheets on top, then place the lid on the shipper. Seal the outer cardboard box with waterproof tape (do not use duct tape).
- Ensure data are complete on all field datasheets and that all samples are accounted for in the shipment. Sign and date in the appropriate spot at the bottom of each datasheet. 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 project email (dmp@nps.gov) with the originals included in the shipment.
- Not all FedEx locations will accept a shipment containing dry ice (which is classified as a dangerous good). The [FedEx Location Locator](#) can be used to **find FedEx locations that will accept dry ice** by selecting *Dangerous goods shipping* under the *More options* menu.
- For any additional questions or concerns please contact the DMP team (dmp@nps.gov)

Shipping Address

USGS Forest and Rangeland Ecosystem Science Center
Attn: James Willacker
3200 SW Jefferson Way
Corvallis, OR 97331



7. Use the data!

We aim to make preliminary park-specific data available ~1 year after the samples have been shipped to USGS. 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. Project leads will interpret the results for the national parks each project year, through webinars or other outlets.

The data can be accessed from the following platforms:

- Dragonfly Mercury Project Data Dashboard:
go.nps.gov/dmpmap
- Total Mercury Concentrations in Dragonfly Larvae from U.S. National Parks (Eagles-Smith et al. 2020): <https://doi.org/10.5066/P9TK6NPT>
- Other DMP results and reports can be accessed at:
<https://irma.nps.gov/Datastore/Collection/Profile/4082>



Water Sampling Overview

Paired water sampling is often (but not always) incorporated into the collection process to help better understand how water chemistry influences mercury in dragonfly larvae. If water sampling is going to take place, below are the guidelines for this task.

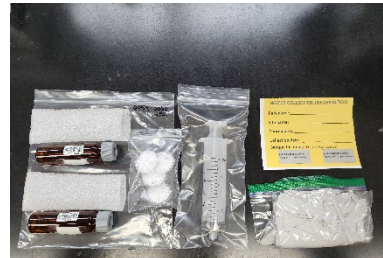
1. Prepare

All needed water sampling supplies will be provided in the water sampling kit, which will arrive in a separate Styrofoam cooler alongside the dragonfly sampling kits. Supplies will cover collection of two sample vials of filtered site water from each site. One for dissolved organic carbon (DOC) and one for cation/anion analysis.

Materials

Water sampling:

- ☐ Collection information card
- ☐ 60ml (or larger) Luer-lok syringe
- ☐ Disposable filters (extra included)
- ☐ Amber glass vials in padded sleeves
- ☐ Sample barcode ID labels + duplicate
- ☐ Nitrile gloves
- ☐ Cooler & ice pack or wet ice
- ☐ Pre-paid return shipment label



General field supplies:

- ☐ Cooler & ice pack or wet ice for samples

Shipping:

- ☐ Single ice pack
- ☐ Styrofoam cooler nested in cardboard box
- ☐ Padded sleeves & filler paper/bubble wrap
- ☐ Pre-paid return shipment label



Resources:

- See the project SharePoint site for a step-by-step video guide to water sampling (<https://doimspg.sharepoint.com/sites/ext-usgs-dragonflymercury/>)

When and Where to Collect Water

- Generally, it is recommended that water collection occur after dragonfly larvae have been collected from a site. This is to prevent water collection at a site where no larvae were harvested. If water is added to the sample vial and dragonfly larvae do not end-up getting collected from the site, the vial cannot be reused.
- Collect water upstream or away from the larvae sampling site to avoid collecting a “dirty” sample; sediment may get stirred up when looking for larvae. If there is no clear “upstream” at the site or dragonfly sampling will muddy the entire accessible area, a “tentative” sample should be taken, following steps 1-2 below, but do not proceed further until after the larvae have been collected. If larvae are not found and collected at the site, the water can be expelled from the syringe and the filter disposed (if a filter has been added to the syringe already). At the next site, you’ll repeat Step 1 “Triple rinse the syringe” to re-prime the syringe with water from the new site.



2. Collect samples

Procedure

1. Triple rinse the syringe
 - a. Rinse the syringe with site water by drawing ~10ml site water into the syringe. Shake and expel water. Repeat 3 times.
2. Draw water for sampling
 - a. Draw water into the syringe until full.
 - b. Lay the syringe on its side in a safe place where it will not be disturbed, and the tip will not touch anything.

-- PAUSE HERE IF DRAGONFLY LARVAE HAVE NOT BEEN SUCCESSFULLY COLLECTED FROM THE SITE ---

3. Record unit name, site name, site coordinates, collection date
4. Remove the duplicate barcode taped to exterior of vial and place it onto the collection information card in the sample incoming id section. If the dragonfly field datasheet is on-hand, record the incoming id found on the barcode label (e.g. 24WATR0000XX1) in the field datasheet site notes section.
5. Triple rinse the vial
 - a. Wearing gloves, attach the filter to the syringe. Make sure water is drawn up into syringe before attaching filter.
 - b. Expel ~5ml of filtered water into pre-labeled amber vial, cap, shake to rinse, then dump water from the vial. Repeat 3 times.
6. Collect water sample
 - a. Fill the amber glass vial to the bottom of the threads with remaining filtered water.
 - i. If water becomes too difficult to push through the filter, discard clogged filter and attach a new one
 - ii. Syringe and filter (if not clogged) can be re-used for additional water samples at the same site
 - iii. Remove filter (being careful not to touch either the connection or nozzle of the filter), refill the syringe with site water, and repeat the sample collection process starting at step 4 to fill another water vial at the same site
 - iv. Save syringe for reuse at additional sites.
 - v. Filters are one-time use per site and can be discarded/packed out with trash.
 - b. Keep the samples cool until they can be refrigerated by placing the vials in a cooler with wet ice (cubed from your freezer is fine) or ice packs.*
 - c. Place the tightly-capped samples in a refrigerator, standing upright, in a secure container that clearly labels them as research samples. If possible, it is best to store them in a separate refrigerator from food or drinks.



3. Store and Send Samples

- Store water samples refrigerated, but not frozen.
- Water samples should ideally be shipped within 1-week of collection, using the provided Styrofoam cooler, ice pack and outer cardboard box. They should remain cool but not frozen.
- All sample shipments must be arranged with USGS prior to being shipped. Please contact the DMP team via the project email (dmp@nps.gov) to arrange a shipping date.
- Any sample shipments should be scheduled for Monday or Tuesday to ensure samples are received prior to the end of the work week.
- We provide a pre-paid, addressed shipping label for FedEx®. Please fill in your name and address in the Sender Information section of the label and confirm that PRIORITY overnight service is selected.
- Ensure each water sample on the field datasheet has been accounted for, the field datasheet has been filled out completely and duplicate barcode labels originally taped on each water vial are placed on the matching field datasheet for that site.
- To pack water, place all water sample vials in foam padded sleeves, then seal in plastic bag. Place bagged sample vials on bottom of cooler. Place provided ice pack on top and fill any remaining space with packing material. Add water field sheets on top, then place lid on top. Seal the outer cardboard box with waterproof tape (do not use duct tape).
- For any additional questions or concerns please contact the DMP team (dmp@nps.gov)

Shipping Address

USGS Forest and Rangeland Ecosystem Science Center
Attn: James Willacker
3200 SW Jefferson Way
Corvallis, OR 97331

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. Dip/Baitwell nets with 3/16 mesh at a 6" net depth are ideal for scooping dragonfly larvae. They are light-weight and can be found with a variety of handle lengths. Longer handles do make the sampling a little more comfortable and help with sweeping vegetation efficiently, however the extra reach provided from a longer handle isn't often necessary for these sampling efforts. Cost is ~\$15 and online purchase options include:

- Item #BN11: <https://www.memphisnet.net>
- Item #77278: <https://www.forestry-suppliers.com>
- SKU: AG-1, replacement net SKU: R-AG-1: <https://www.cumingsnets.com>

2. D-nets are another type of dip net that work well and may already be on-hand from other macroinvertebrate collection efforts.

- ~\$100, item #53788: www.forestry-suppliers.com



Appendix B. How clean is “clean”?

Mercury is all around us: in our hair, in soil, leaves, etc. We need to keep everything clean so to not contaminate our samples. Read on for recommendations on getting gear ready....

How we are using terms in this project:

- **Clean:** Materials and supplies prepared in such a way that they would not have contamination that could affect our analyses and interpretation of mercury.
- **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 “New”).
- 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), 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.
- 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/stream water 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: The Dragonfly Mercury Project – engaging citizen scientists in monitoring mercury contamination in National Parks

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. A critical element of this research is the engagement of citizen scientists to collect larvae and facilitate sampling over a spatial scale that would otherwise be logistically and fiscally impossible.

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.

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

Objects Collected:

- Odonata: Anisoptera (dragonfly) nymphs; 15-20 per site, include number of sites proposed for your park.

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 in an update to the to ScienceBase annually, after final quality assurance and publication of results.

Appendix D. Sacrificing Dragonfly Larvae for Science: What is the Impact?

Dragonflies are typically found in very high densities, and our project samples a relatively tiny number per waterbody.

Evidence:

- Range is 20-200 individuals/m² depending on time of year and species^{1,2}

Dragonflies are prolific reproducers (r strategists): they produce a lot of offspring because it is expected that most won't survive to adulthood.

Evidence:

- Rearing studies in Maine: a single female lays 400-1600 eggs and can do this more than once per season.
- Other studies show that females can lay from 800-2600 eggs over a 9 day period.³
- In nature, >95% of larvae don't survive to adulthood.^{4,5}

Other threats such as vehicle traffic can pose a significant threat to dragonflies:

- To a dragonfly, roads are warm, sunny clearings with ample flying insect prey; thus, dragonflies often use roads as resting and foraging sites.
- In one study at Acadia National Park, researchers found that vehicle collisions with flying adult dragonflies were common near roadways: at least 3.2 Odonata per kilometer of road were struck each day during the breeding season, along high-traffic roadways near freshwaters.⁶ Further, these mortality rates were likely underestimates because researchers were unable to count insects that remained stuck to vehicles, and weather was unusually rainy during the study.

The impact of this project on dragonflies is expected to be minimal.

- We sample the smallest number of individuals that are statistically meaningful.
- Power analyses are used to optimize the number of samples.
- Other factors are responsible for even higher rates of mortality (cannibalism, predation, automobile collisions (see above))
- Dragonflies are ubiquitous and found in so many habitats around your region – even urban sites, irrigation ditches, cattle ponds, cold regions, and nutrient-enriched sites.

¹ Benke, A. C., & Benke, S. S. (1975). Comparative dynamics and life histories of coexisting dragonfly populations. *Ecology*, 56(2), 302-317.

² Benke, A. C. (1976). Dragonfly production and prey turnover. *Ecology*, 57(5), 915-927.

³ Hopper, K. R., et al. Crowley, P. H., & Kielman, D. (1996). Density dependence, hatching synchrony, and within-cohort cannibalism in young dragonfly larvae. *Ecology*, 77(1), 191-200.

⁴ Wissinger, S. A. (1988). Life history and size structure of larval dragonfly populations. *J. N. Am. Benth. Soc.*, 7(1), 13-28.

⁵ McPeck, M. (2012). Dartmouth College, pers. comm.

⁶ Remsburg, A., & Blaker, P. (2009). Odonata communities of Acadia National Park and Mt Desert Island from exuvia and roadside surveys in 2009. Unpubl. project report, Unity College, Unity ME.

Appendix E. Dragonfly Ecology 101: How do I find them?

Dragonflies inhabit a wide range of microhabitats so it may not be completely intuitive where to start sampling. If you are having trouble finding dragonflies one of two factors may play a part:

1. Habitat selection
2. Timing of sampling

Finding buggy habitats:

Dragonfly guilds are separated by their behavior, morphology and microhabitat in which they reside¹

□ **Claspers:** Cling to structure in the water column

- Family*: Aeshnidae
 - Habitat: Aquatic vegetation or snags
 - Collection: Claspers typically climb up onto vegetation, so sweeping macrophytes or grass along the banks is effective for collection of this family
- * Nymphs in the genera *Epithea* (Corduliidae) and *Leucorrhinia* (Libellulidae) are also claspers



□ **Burrowers:** morphology to burrow and resides under sandy substrates

- Family: Cordulegastridae – shallow burrower
 - Habitat: lotic systems within depositional zones containing fine sediments
- Family: Gomphidae – deep burrower
 - Habitat: lentic or lotic systems containing fine sandy sediment
- Collection: Skimming the top inch or so of sandy substrates is effective for collection of this family.
 - You can also look for lines in the sand. Both of these families will keep their posterior end out of sediments to be able to respire, this leads to a line that is drawn out in the sand as they move within substrates. Sweeping your net along these lines can yield specimens.



□ **Sprawlers:** longer legs to stabilize on top littoral sediments or vegetation

- Families: Libellulidae, Corduliidae, Macromiidae
 - Habitat: Lentic-littoral on top of sediments or aquatic vegetation
- Collection: variable



□ **Hiders:** conceal their bodies under leaf litter and detritus

- Species*: Hagenius brevistylus (Gomphidae) - Abdomen strongly flattened dorsoventrally
- Collection: Sweep your net into depositional zones containing high detritus

*A few Corduliid nymphs are hiders



This general guidance is simplified, there are exceptions for each and it's not restricted to these families.

Timing of sampling:

- Depending on your climate, you may be between populations that are emerging and those that are laying new eggs – making the individuals harder to find or even non-existent in that microhabitat
- If you are looking for 20 minutes and find nothing, try another spot along the shore to explore these different microhabitats

¹Corbet, P.S. (1999). Dragonflies: Behavior and Ecology of Odonata. p. 149-155.

Appendix F. PFDs

Participating parks must provide their own PFDs. Different organizations have their own requirements for PFDs. The options below are examples of those approved for use by the Department of Interior (e.g., USGS and NPS) and may not meet the requirements of other entities. Be sure to verify with your own safety programs for approval and clearance for the type of PFD.



Classic Industrial Flotation Vest with SOLAS Reflective Tape – Mustang Survival PRO

<https://pro.mustangsurvival.com/collections/foam-pfds/products/classic-industrial-flotation-vest-with-solas-reflective-tape>



Foam PFDs & Hybrids | Trusted Since 1967 | Mustang Survival – Mustang Survival PRO

<https://pro.mustangsurvival.com/collections/foam-pfds>