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Natural Resource Stewardship and Science

Citizen Scientists Study Mercury in Dragonfly Larvae

Dragonfly larvae provide baseline data to evaluate mercury in parks nationwide

Natural Resource Report NPS/NRSS/ARD/NRR—2015/938





ON THIS PAGE
Citizen scientist sampling at ROMO: a dragonfly exuviae (shed exoskeleton) on a blade of grass, with citizen scientist in the background; inset photo shows a citizen scientist inspecting a captured larva.
Photograph courtesy Rocky Mountain National Park

ON THE COVER
Citizen scientists (here, YCC crews) sample Obsidian Creek for dragonfly larvae.
Photograph by: Matt Ohlen

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Dragonfly larvae provide baseline data to evaluate mercury in parks nationwide

Natural Resource Report NPS/NRSS/ARD/NRR—2015/938

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Executive Summary

This citizen scientist study of mercury in dragonfly larvae from national parks project engages students, teachers, and park visitors in the collection of dragonfly larvae for mercury analysis and species identification. It supports the Call to Action (C2A) #7: Next Generation Stewards and #16: Live and Learn (http://www.nature.nps.gov/air/Studies/air_toxics/dragonfly/). In partnership with 22 national parks across the U.S., we are developing the use of dragonfly nymphs as bio-sentinels for mercury (Hg) in aquatic foodwebs. Dragonfly larvae can serve as indicators of ecosystem health by characterizing the risk and transfer of mercury around food webs. High concentrations of Hg accumulate in these aquatic insects because they are predatory and are long-lived underwater. Dragonfly larvae are also widespread across the U.S., allowing comparisons among parks. The larvae are important prey for fish species and can be used as a surrogate for mercury levels in fish. Data on fish mercury are useful for the National Park Service (NPS) to understand park- and site-specific threats to human and wildlife health.

Mercury is a globally distributed, toxic contaminant that threatens resources the NPS is charged with protecting. The main source of human-caused mercury in remote national park environments is atmospheric deposition from coal-burning power plants. This project directly implements the C2A by enlightening 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.

To validate the use of these sentinels, and gain a better understanding of the connection between biotic and abiotic pools of Hg, this project also includes collection of landcover/landscape data, surface water chemistry including Hg and Hg-relevant chemistry (pH, sulfate (SO_4^{2-}), and dissolved organic carbon (DOC)). Over 775 dragonfly larvae samples were collected from 61 sites by approximately 265 citizen scientists and analyzed for mercury by the University of Maine. Results from 2012 and 2013 indicated significant differences in mercury concentrations among sites, even within a park, suggesting that dragonfly larvae can describe fine-scale differences in mercury risk.

Because of the wide geographic scope of the research, the project also provides a nationwide snapshot of Hg in biota and water, primarily in undeveloped watersheds. In data from 23 parks (including 1 state park) sampled in 2013, total Hg (THg) in water (unfiltered) ranged over two orders of magnitude (0.16–28 ng/L; median=2.2 ng/L), and pH ranged from 5.5–9.2 (mean=7.7). In dragonfly larvae, THg concentrations averaged 112±124 (mean ± SD) ppb, dw. Total Hg in dragonfly larvae was weakly positively correlated with both water THg (r=0.31) and DOC (r=0.36). Sites with the greatest dragonfly larvae THg concentrations were located in New England as well as some western sites (e.g., YELL, GOGA) that could be influenced by global Hg sources as well as regional to local influences such as geothermal activity, wildfires, and urbanization. Importantly, although atmospheric sources of Hg are often the primary inputs to watersheds, many of the drivers of methylmercury production operate within a waterbody or particular food web, and complicate prediction of Hg at the landscape scale.

Although eastern parks generally had greater concentrations of Hg than those in the west, local and regional sources may be especially influential at GOGA and YELL. Site-to-site variability within a park was high. For instance, one park (YELL) included a site with the greatest concentrations of THg in dragonflies and fourth-highest concentration of THg in water, but also a site where dragonfly THg fell in the lowest quartile of all the data. Some Hg in YELL could be contributed via geothermal features (as reported by Hall et al. 2006) or wildfires upwind of the park, in addition to atmospheric deposition from distant sources; however, as mentioned above, in-lake or in-stream processes are likely to be the primary drivers of methylation. Multivariate models using three techniques (CART, MARS, stepwise regression) all found DOC, THg in water, and pH to be important variables, with some influence of topography/wetlands, east-west position, and habitat guild. These findings support ongoing focus on DOC and acidity as well as landscape factors in determining relative dragonfly Hg across sites.

Our ongoing research is examining the factors that influence water and dragonfly Hg, as well as sample collection across a broader set of parks (44 in 2014), and evaluation of temporal trends in dragonfly and water Hg within a year. There are no established thresholds for mercury toxicity that would affect dragonfly larvae health or pose risk to their predators. However, related work conducted by USGS will define mercury benchmarks related to dietary risk of predators that might consume dragonfly larvae.

A summary Project Brief describing the study and results for 2013 can be found at the project Web site, http://www.nature.nps.gov/air/Studies/air_toxics/dragonfly/.

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We thank NPS staff (and Stone Mountain State Park staff) and partners and many, many citizen scientists at the 23 participating parks for their contributions of time, effort, and ideas that helped to make this work happen, and continue to improve. Andrea Blakesley, Luke Gommerman, Cynthia Ocel, Shannon Trimboli, and Zoe Unruh served as an advisory committee who evaluated interpretive products that were aimed at improving citizen scientist engagement. The University of Maine's Sawyer Water Research Laboratory provided support and analysis for the project; in particular, we thank Marianne Lagerklint, Clive Devoy, the late MaryLou Dorr, and numerous students who prepared samples. We also thank Celia Chen, Kate Buckman, and the staff at the Dartmouth Trace Element Analysis Core laboratory. The Dartmouth Trace Element Analysis Core is partially supported by NIEHS P42 ES007373, NIEHS P01 ES022832, EPA RD83544201. Marissa Giroux managed shipments of supplies and incoming samples, and provided invaluable assistance coordinating the program during Summer 2013. Acadia Learning teachers Jen Stainton, Erica Ferland, Ed Lindsey, and Emily Sherman were instrumental in developing the project as a potential citizen science program and developed and field-tested many of the protocols. Ed Lindsey and Alex Baron carried out collaborative research to investigate effects of depuration and provided data on survival rates of dragonfly larvae. Aaron Weiskittel and Bill Halteman (UMaine) provided crucial assistance with R code and statistical interpretation. Finally, Bronco Quick not only performed species identification for the project, but also provided guidance and information – and example specimens – that were fundamental to our understanding of dragonfly larvae in context. Two reviewers provided constructive comments that greatly improved this report.

List of Acronyms – Park Abbreviations

ACAD - Acadia National Park
BICY - Big Cypress National Preserve
CACO - Cape Cod National Seashore
CUVA - Cuyahoga Valley National Park
DENA - Denali National Park & Preserve
GOGA - Golden Gate National Recreation Area
GRSM - Great Smoky Mountains National Park
JELA - Jean Lafitte National Historic Park & Preserve
KEAQ - Kenilworth Park & Aquatic Gardens
KNRI - Knife River Indian Villages National Historic Site
MABI - Marsh-Billings-Rockefeller National Historic Park
MACA - Mammoth Cave National Park
MONO - Monocacy National Battlefield
NIOB - Niobrara National Scenic River
NOCA - North Cascades National Park
OZAR - Ozark National Scenic Riverways
PIRO - Pictured Rocks National Lakeshore
ROMO - Rocky Mountain National Park
SACN - Saint Croix National Scenic Riverway
SAGA - Saint-Gaudens National Historic Site
STONE - Stone Mountain State Park, Georgia
VOYA - Voyageurs National Park
YELL - Yellowstone National Park

Introduction

Background and Justification

Mercury (Hg) is a globally distributed, toxic contaminant that threatens resources the National Park Service (NPS) is charged with protecting. Fish and wildlife with Hg concentrations exceeding human and wildlife consumption guidelines have been documented in many national parks across the US (e.g., Brigham et al. 2014, Eagles-Smith et al. 2014, Haro et al. 2013, Bank et al. 2007). Resource managers' efforts to target fish consumption advisories for humans and focus conservation efforts for fish-eating species are hampered by significant variability in Hg concentrations from site to site – often in neighboring lakes, streams, or wetlands. Although recent research has explained patterns and correlates of Hg in lake and stream water (e.g. Driscoll et al. 2007, others; Figure 1), concentrations in water are often de-coupled from those in foodwebs due to complex, in-lake or in-stream Hg transformations. Therefore, invertebrate Hg concentrations are better-integrated indicators of Hg variation among locations and more appropriate proxies for humans and wildlife risk than water concentrations, and are more easily captured and potentially more representative of site-specific Hg than fish (Haro et al. 2013, Sackett et al. 2009, Simonin et al. 2008, Knights et al. 2005).

This project asserts that dragonfly larvae (*Odonata: Anisoptera*) can serve as indicators of ecosystem health by characterizing the risk and transfer of mercury through food webs. Until recently[1], dragonfly larvae have typically only been sampled for mercury as part of larger foodweb studies, in fish bio-accumulation studies, or in general surveys of macroinvertebrate body burdens (e.g., Jones et al. 2013, Ward et al. 2010, Chasar et al. 2009, Allen et al. 2005, Gorski et al. 2003, Haines et al. 2003, Mason et al. 2000, Wiener & Shields 2000, Hall et al. 1998, Goutner and Furness 1997). More recent research has begun to focus on aquatic macroinvertebrates, and specifically dragonfly larvae, as potential

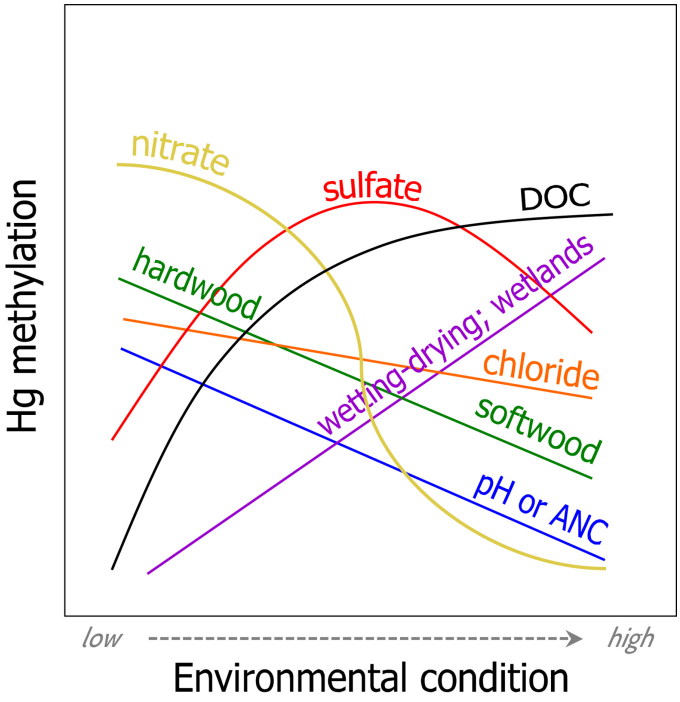


Figure 1. Emerging relationships between Hg methylation and environmental conditions or gradients. Water chemistry and site characteristics measured in this study allow for testing of some of these responses. From: Eagles-Smith et al. 2013.

[1] Exceptions: a very early, extensive study of mercury in dragonfly larvae in Canadian wetlands was conducted in 1993 and published only in the gray literature (Headon & Hall, 1997). Parkman and Meili (1993) also evaluated landscape influences on macroinvertebrate Hg concentrations in Sweden in the early 1990s.

indicators or bio-sentinels for Hg (Haro et al. 2013, Riva-Murray et al. 2011), as vectors for Hg bio-magnification (Buckland-Nicks et al. 2014), or as indicators of heavy metal pollution more generally (Tollett et al. 2009, Nummelin et al. 2007). Current research is quantifying relationships among Hg in dragonfly larvae, water chemistry, and sediment Hg (Flanagan Pritz et al. 2014, Eagles-Smith et al. 2013, this study) and the relationship between dragonfly larvae Hg and fish Hg (Haro et al. 2013), and supports their use as bio-sentinels that describe Hg spatial patterns.

In the U.S., there are seven families of dragonflies: Aeshnidae, Gomphidae, Cordulegasteridae, Corduliidae, Libellulidae, Macromiidae, and Petaluridae. Petaluridae are rare and have not been found in this research, so in effect there are six families of interest here. There is some debate within the entomological community regarding whether Corduliidae and Libellulidae are truly distinct families, or are sub-families of a larger family that some believe should be called "Corduliinae". This project does not support one or the other of these viewpoints, but we use a standard reference text (Needham, Westfall, and May, 2000) and refer to families as described therein. There are hundreds of dragonfly (Anisoptera) species in the U.S., and thousands worldwide. Although habitat guilds or feeding guilds vary by species, in general, the Gomphidae and Cordulegasteridae are burrowers, Aeshnidae (perhaps the most voracious predators) are here considered claspers, and Corduliidae, Libellulidae, and Macromiidae are considered sprawlers in this research. We considered guild as a potential factor that could affect Hg accumulation due to potential differences in diet among guilds, but this aspect of the research is early-stage, and future work will better define guild effects. Similarly, we identified instar (the developmental stage) and size (mass and length) of individual larvae in order to outline how size and/or growth could affect larvae Hg.

Dragonfly larvae are useful sentinels because they are widespread in freshwaters across the US, long-lived, exhibit site fidelity, contain almost all of their Hg as MeHg, and have concentrations of Hg that are meaningful for laboratory and statistical analyses. While fish are perhaps the most commonly sampled taxon because they occur across a wide geography and provide strong linkages to human and wildlife health, dragonfly larvae are relatively easier to collect, and represent vulnerability to mercury in fishless ecosystems like shallow ponds, ephemeral pools, and marshes – some of the most productive and ecologically important aquatic habitats. Data that quantify fish Hg are useful for the NPS to understand park- and site-specific threats to human and wildlife health.

Importantly for this project, dragonfly larvae (and adults) are charismatic biota that garner great interest from park visitors, students, and citizen naturalists. This interaction can spark further discussion regarding Hg sources and effects, fostering public awareness of Hg as an airborne pollutant. Although the largest single source of human-caused mercury in remote environments such as national parks is atmospheric deposition from coal-burning power plants (excluding artisanal gold mining, unlikely to be a source in US National Parks; UNEP 2013), engaging the public in 'invisible', ultra-trace level air contaminants can be challenging. Additionally, the sources of inorganic Hg to ecosystems are likely to be less important (in terms of toxicological risk) at the individual waterbody scale than specific characteristics of the aquatic system that transform Hg to the more toxic methyl form. Further, ultra-clean handling procedures needed for air and water sampling are beyond the scope of most interpretive programming conducted with citizen scientists.

In 2012, 12 national parks participated in a pilot dragonfly Hg study (Flanagan et al. 2012, http://www.nature.nps.gov/air/Studies/air_toxics/dragonfly/). Over 200 dragonfly larvae samples were collected from 25 sites by about 200 citizen scientists and sent to the University of Maine for mercury analysis. Preliminary results indicated significant differences in Hg concentrations among sites, even within a park, suggesting that dragonfly larvae can describe fine-scale differences in Hg bioaccumulation. The pilot effort also demonstrated the feasibility of citizen scientists as field samplers for this Hg study, and our ability to deliver training, materials, and content remotely using videos, webinar, e-mail, and phone contact with interpreters and park staff across the US.

Dragonflies and other macroinvertebrates are often subjects of existing NPS interpretive programming and resource management activities. This project builds on existing park programs and interests to expand a successful 2012–2013 pilot effort to engage citizen scientists in collection of dragonfly larvae for Hg research. The 2013 study analyzed 775 dragonfly larvae samples collected from 60 sites at 22 national parks (and 1 state park), by 265 citizen scientists. This project directly implements Call to Action (C2A) #7: Next Generation Stewards and #16: Live and Learn by enlightening 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 (NPS 2011).

Objectives

This citizen scientist study of mercury in dragonfly larvae project engages students, teachers, and park visitors in the collection of dragonfly larvae for Hg analysis and taxonomic identification. This project continued sampling efforts in the existing network of 12 pilot parks, expanded the study to total 22 national parks (including urban parks) plus one state park, enhanced larval identification protocols for biodiversity discovery activities (C2A #7), and developed complementary interpretive materials for parks to foster teachable moments (C2A #16). Our objectives supported field sampling and discovery activities, scientific research, and outreach, as follows.

Field sampling and discovery objectives:

1. Refine existing citizen science sample collection protocols to allow for better citizen taxonomic identification to family level and instar (larval development stage) (C2A #7).
2. Work with a Maine entomologist to check field identifications, identify a subsample to species, measure larvae, and determine instar (growth stage) upon receipt of samples.
3. Collect water and dragonfly larvae samples in 2–3 water bodies in each park for analysis of total Hg (dragonfly larvae and water), Hg-relevant chemistry (water: dissolved organic carbon, pH, sulfate), and methylmercury (MeHg), the form of Hg that bio-magnifies and is more toxic (subset of dragonfly larvae).

Scientific objective:

4. Evaluate the relationships among potential factors that influence Hg bioaccumulation in dragonfly larvae and Hg in water across the broad spatial scale of parks in all NPS regions to determine their utility as bio-sentinels of exposure.

Outreach and interpretive objectives

5. With Schoodic Institute staff (headquartered at Schoodic Education and Research Center at Acadia National Park) and NPS Interpretive staff, develop complementary outreach materials related to mercury in parks to foster teachable moments (C2A #16).
6. Share the project database, supporting materials, and project outputs with citizen scientists, NPS interpreters, and the public audience via regular science updates (email), webinars, and the project Web site: http://www.nature.nps.gov/air/Studies/air_toxics/dragonfly/index.cfm.

Methods

Citizen scientist engagement

This project uses a "train the trainer" approach, whereby staff or partner(s) at each park (hereafter, "park leader") act as the primary point of contact for the project team. Each park leader is provided a sampling kit that includes supplies used in sampling, field sheets, paperwork for shipping, and the sampling protocol. The park leader reviews this sampling protocol prior to sampling. At a final check-in prior to sampling, the project lead contacts each park leader to answer any questions and ensure that the park leader is ready for sampling.

Typically, each park leader customizes the implementation of the project to best fit the available citizen science group, their park's specific outreach or education mission, and access to the sampling sites. The three most common modes of implementation are: (1) a corps of park volunteers/staff such as the Youth Conservation Corps or Teacher-Ranger-Teachers are the citizen scientists for the project; (2) a school group (middle school through undergraduate) visits the park and this sampling excursion is the sole focus or one focus of a day of experiential learning in the park; (3) adult citizen volunteers who have an interest in aquatic ecology perform sampling, with minimal guidance from the park leader.

Following field sampling and after receipt of dragonfly and water samples, each park leader is asked to provide information regarding the name of the citizen science group that participated, number of participants, estimated citizen science hours and park staff hours contributed to the project, and provide feedback to improve the project in future years.

Feedback informed the development of interpretive materials used by the park leaders to deepen participant understanding of dragonflies, sampling, and the research project. Park leaders attended a project webinar, January 23, 2014. General accumulated feedback was discussed and, from the list of needs identified in feedback, educational materials prioritized. Dragonfly field identification cards, one-page brief field-sampling instructions, and a research flowchart were developed with a core group of park staff—Andrea Blakesley (DENA), Luke Gommermann (BICY), Shannon Trimboli (MACA), Cynthia Ocel (ACAD), and Zoe Unruh (Alice Ferguson Foundation/KEAQ, MONO) refining all products.

Site selection and sample collection

Details of the sampling procedure are found in the Sampling Protocol, available on the project Web site (http://www.nature.nps.gov/air/Studies/air_toxics/dragonfly/). Briefly, in 2013, each park was asked to choose 2-3 sites that were representative of freshwaters in the park, and that varied across some environmental gradient – for example, high to low elevation, high to low concentration of a chemical constituent (e.g., dissolved organic carbon (DOC), acidity), landcover differences (e.g., forest type, presence of wetlands), urban to rural setting, or flat to steep topography. Sites were evaluated to determine presence of dragonfly larvae and sites were chosen in consultation with the project PI when there were additional considerations. Some parks only contained one waterbody (e.g., MABI), so only one site was sampled.

At each site, the park leader and citizen scientists first collected a water sample for THg analysis, using the modified US EPA "Clean Hands-Dirty Hands" technique (EPA Method 1669, 1996), and collecting into a pre-cleaned, double-bagged 250 or 500 mL Teflon® bottle using powder-free vinyl gloves. Then a water sample was collected for non-Hg chemistry analysis (air-equilibrated pH (EqpH), DOC, sulfate (SO_4^{2-}). Citizen scientists then used a variety of nets (often D-nets, baitwell nets, or kicknets) to collect dragonfly larvae at each site. Net type was not important to standardize across sites because the project does not seek to quantify sampling effort, neither in terms of people-time nor area sampled. Dragonfly larvae were identified to family in the field when feasible, measured to the nearest millimeter (mm), and individually bagged in MiniGrip® polyethylene zipper-seal bags. A label for each sample was filled out and included inside a second, outer MiniGrip® bag. Fifteen larvae per site were requested for laboratory analysis, and samples were selected using the following criteria, if more than 15 were gathered: first, individuals should be greater than 10 mm length; second, samples should be representative of the overall family composition and abundance at the site; third, whenever possible, three or more individuals in each family found should be submitted. Finally, citizen scientists and/or the park leader recorded information about each sampling site, including site coordinates, landscape characteristics, and observations about stream, pond, or wetland basic morphometry and habitat.

Sample storage and shipping

Water samples were stored locally in a refrigerator, and dragonfly larvae samples were stored locally in a freezer, until shipment. Samples were shipped overnight on dry ice to the University of Maine for analysis. During 2013, the laboratory analyses to be performed were relatively robust to freezing[2], so both water and dragonfly larvae samples were shipped in the same cooler with dry ice. The 2013 analyses were also relatively robust to extended storage times, but we requested that water samples were shipped the same day as the sampling event. Often this meant the park leader would return to the site(s) sampled for dragonfly larvae earlier in the project to collect a round of water samples, then ship all samples together. Exceptions to the shipping procedure were: (1) DENA samples took two days to arrive in Maine, given the great distance between the two locations; (2) KNRI was remote to any dry ice supplier, so samples were shipped late in the day, packed in large amounts of wet ice, and water samples were pre-frozen to maximize cold temperature in the dragonfly larvae samples.

Sample preparation and laboratory analyses

All water samples were analyzed at the Sawyer Laboratory at the University of Maine, and all dragonfly larvae samples except those from MABI and SAGA were analyzed at the Sawyer Lab. Dragonfly larvae samples from MABI and SAGA were analyzed at Dartmouth College's Trace Element Analysis (TEA) Core Laboratory, because of long-standing collaborative research with

[2] There is limited information regarding the potential effect of freezing on samples to be analyzed for DOC analysis. Fellman et al. (2008) found a low bias in samples frozen prior to DOC analysis if concentrations of DOC were >5.0 mg/L. The effects of freezing could be more important when evaluating spectral properties of dissolved organic matter (Fellman et al. 2008, Griffin et al. 2012). We regard DOC concentrations >5 mg/L as potentially biased low in this dataset, but further confirmation of the effect of freezing on DOC is needed.

Celia Chen, Dartmouth staff, and the Dartmouth TEA Core that included citizen science engagement through teachers at these two parks. The Sawyer Lab has been the analytical laboratory for over 30 years of research and long-term monitoring for projects that include US EPA Long-Term Monitoring (e.g., Strock et al. 2013), the Bear Brook Watershed in Maine (e.g., Norton et al. 2010), and Acadia National Park paired research watersheds (e.g., Nelson et al. 2013, Kahl et al. 2007), as well as many state and federal agency research and monitoring programs.

Water samples

A summary of sample preparation and analytical methods are given in Tables 1 and 2. Details of laboratory preparation and analysis are as follows:

Container preparation. For air-equilibrated pH (EqpH), DOC, and SO_4^{2-}, plasticware and aliquot bottles were high-density polyethylene (HDPE), which were washed with hot deionized (DI) water. After washing, the bottles were rinsed with DI water and set aside to soak filled with DI water for 48 hours. Each batch of bottles was verified by selecting 10% of the batch for a conductivity check. Equipment for sampling was prepared at the Sawyer Lab in a Hg clean room to eliminate contamination by ambient Hg sources (US EPA 2002). Teflon® bottles were rinsed with hot tap water; soaked overnight in 2% Contrad 70; rinsed with hot tap water, then 2–3x with DI water, then 2x with 2% aqua regia; filled with 2% aqua regia to stand overnight; rinsed 2x with DI water, then rinsed 2x and re-filled with aqua regia; and finally rinsed 6x with DI water. Bottles were then filled with DI water and hydrochloric acid (Optima) to 0.1%. Bottles were double-bagged with an ID etched on the bottle and marked on the outside of the bag.

Sample preparation and storage. Samples for pH and water THg were not filtered; aliquots for DOC and SO_4^{2-} were filtered through 0.4 µm Nucleopore filters. Samples were filtered using vacuum filtration with a plastic magnetic filter apparatus and collection flask. Filters and filter funnel were rinsed first with at least 25 ml of sample. These rinses also served to rinse the collection flask. A new filter was used for each sample. DOC and SO_4^{2-} aliquots were stored in DI cleaned 60 ml HDPE bottles. THg (water) samples were stored, preserved, in their original sampling container.

Instrumentation. Closed cell pH was determined on an Orion 8104 Ross Combination pH electrode. Dissolved inorganic carbon (DIC) and DOC were measured with an OI Corporation Analytical Model 1010 Wet Oxidation Total Organic Carbon (TOC) Analyzer. Anions were measured by ion chromatography with a Dionex Model 2010I system. Total Hg in water (unfiltered) was determined using a Tekran 2600 system.

Dragonfly samples – THg and MeHg preparation and analyses

Dragonfly larvae samples were analyzed on an individual basis to ensure maximum statistical power; however, in some cases, individuals were too small to ensure that results would be detectable; in those cases, individuals were paired with another specimen from the same site of the same size and family and analyzed together. Each individual was first patted with a fresh Kimwipe to remove surface moisture, then weighed to determine fresh weight (wet weight). Individuals were then freeze-dried to constant moisture content and final dry weight was recorded. Samples were photographed after drying to assist with future identification and data interpretation. The University of Maine Sawyer Lab determined THg concentrations in dragonfly larvae using a Milestone DMA-80 mercury

analyzer (US EPA method 7473). The Dartmouth Trace Element Analysis (TEA) Core Laboratory analyzed dragonfly larvae from MABI and SAGA using a DMA and EPA method 7473 as well.

A subset (typically, three individuals with large body size) of dragonfly larvae at each of the sites was analyzed for MeHg concentrations to assess MeHg:THg ratios, which indicate Hg trophic transfer potential. All samples for MeHg analysis (except for MABI and SAGA samples) were processed at the University of Maine Sawyer Lab, as above, and were then split in half longitudinally, with one half analyzed for THg at the Sawyer Lab. The remaining halves of individual samples were shipped to the Dartmouth TEA core for Hg speciation analysis using isotope dilution gas chromatography-ICPMS (Taylor et al. 2011). Details of sample preparation, storage, and analytical methods are summarized in Tables 1 and 2.

Table 1. Target holding times, method detection limits, and precision and accuracy objectives for surface water and dragonfly larvae samples.

Analyte	Holding time[1]	Detection limit[2]	Applicable range	Precision objective	Accuracy objective
EqpH	28 days	n/a	≤ 5.74 SU	± 0.075 SU	± 0.025 SU
			> 5.75 SU	± 0.15 SU	± 0.05 SU
DOC	28 days	0.1 mg/L	≤ 2 mg/L	± 0.1 mg/L	± 0.1 mg/L
			≤ 2 mg/L	± 5%	± 5%
SO_4^{2-}	28 days	1.0 μeq/L	≤ 20 μeq/L	± 1.0 μeq/L	± 0.8 μeq/L
			> 20 μeq/L	± 5%	± 4%
THg – Water	28 days[3]; 90 days[4]	0.02 ng/L[5]	n/a	± 10%	± 10%
THg – Tissue					
UMaine	n/a	<1 ng[6]	n/a	± 10%	± 10%
Dartmouth	n/a	<1 ng[6]	n/a	± 20%	± 25%
MeHg – Tissue					
Dartmouth	n/a	<0.05 ng	n/a	± 20%	± 25%

[1] Holding time is the time from sample collection to analysis;

[2] The method detection limit is determined as a one-sided 99 percent confidence interval from repeated measurements of a low-level standard across several calibration curves.

[3] If sample is to be oxidized in the original sample container, as was done in this project, it can be held up to 28 days prior to preservation. If not preserved in the original sample container (not relevant to this project), the sample must be preserved within 48 hours of collection (US EPA Method 1631, rev. E)

[4] Once preserved, samples can be held for 90 days (US EPA Method 1631, rev. E)

[5] For THg in water, the MDL is calculated annually. For this project, it was 0.02 ng/L as of January 25, 2013.

[6] The detection limit using the DMA-80 is not a concentration, but a total mass of Hg detected in the sample.

Table 2. Sample processing, storage, and analytical methods.

Analyte	Preparation	Storage	Description	Method or Reference
EqpH	none	store in original 500 ml HDPE bottle at 4°C for no more than 7 days	Electrode	Hillman et al.[1], EPA 150.1[2]
DOC	filter through 0.4 µm polycarbonate	store at 4°C for no more than 28 days	High temperature combustion; IR Spectrophotometry	EPA 415.1[2]
SO_4^{2-}	filter through 0.4 µm polycarbonate	store frozen (-20°C) for no more than 14 days	High temperature combustion; IR I on chromatography	EPA EPA 300.0[3]
THg – Water	0.5% BrCl added to original sample container (within 28 days)	Store up to 90 days after collection once preserved	Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry	EPA 1631
THg – Tissue				
UMaine	Dried to constant mass	Frozen until dried, then in vials kept in dessicator	Thermal decomposition, amalgamation, and atomic absorption spectrophotometry	EPA 7473
Dartmouth	Dried to constant mass	Frozen until dried, then in vials kept in dessicator	Thermal decomposition, amalgamation, and atomic absorption spectrophotometry	EPA 7473
MeHg – Tissue				
Dartmouth	Dried to constant mass	Frozen until dried, then stored in vials kept in dessicator	Isotope dilution gas chromatography-ICPMS	

Method references:

1. Hillman,D.C., J. Potter, and S. Simon, 1986. Analytical methods for the National Surface Water Survey, Eastern Lake Survey. EPA/600/4-86/009, EPA Las Vegas.

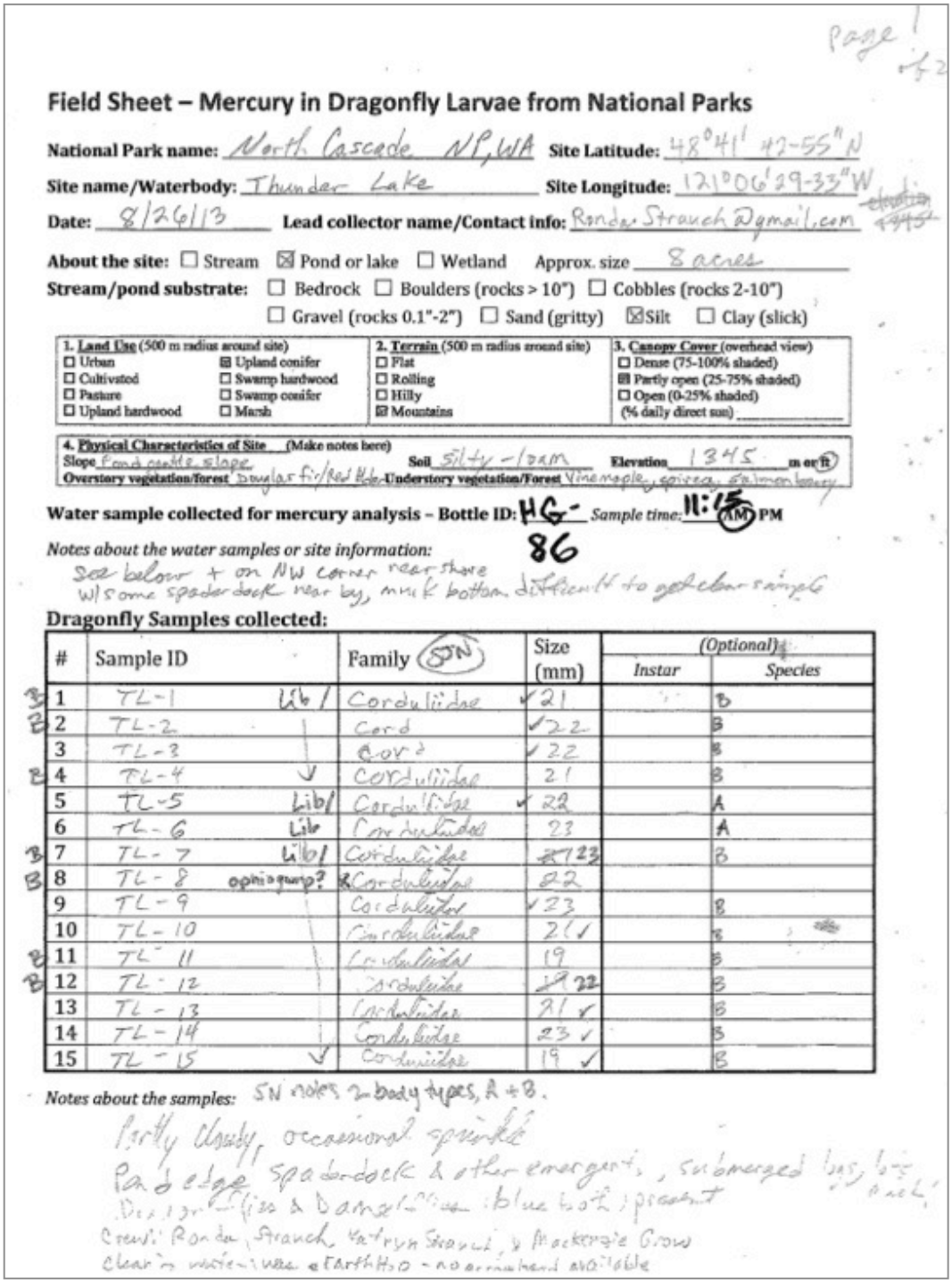
2. Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020, 1979, Revised 1983.

3. Methods for the Determination of Inorganic Substances in Environmental Samples, EPA 600/R-93-100, 1993.

Dragonfly species and instar identification

Upon receipt of samples, UMaine lab staff measured all individual dragonfly larvae, and checked or updated measurements reported by park leaders and citizen scientists. We also identified each individual to the family level, through the inner zipper-seal bag, using a hand lens (loupe) with 10X magnification when needed. These family identifications were also checked against those reported by field crews at the sites, and were edited or updated as needed by annotating the field sheets (see Figure 2 for an example edited sheet). Representative individuals from each site were selected for species identification; these individuals were not analyzed for Hg. When multiple families were present, one individual from each family was selected for species ID; if there appeared to be more than one body type/species within a given family, at least one of each probable species-type was selected for species analysis. Although it is not possible to determine whether all species were selected for analysis, the majority of sites only included two to three families, and only rarely were

more than one species within a family present. When multiple body types within a family were present, the field data sheet was updated to show which individuals from that family were likely to be species A versus species B (Figure 2).



Page 1 of 2

Field Sheet – Mercury in Dragonfly Larvae from National Parks

National Park name: North Cascade NP, WA **Site Latitude:** 48°41' 42-55"N

Site name/Waterbody: Thunder Lake **Site Longitude:** 121°06'29-33"W

Date: 8/26/13 **Lead collector name/Contact info:** Ronda.Strauch@gmail.com

About the site: ☐ Stream ☒ Pond or lake ☐ Wetland Approx. size 8 acres

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)		2. Terrain (500 m radius around site)	3. Canopy Cover (overhead view)
☐ Urban	☒ Upland conifer	☐ Flat	☐ Dense (75-100% shaded)
☐ Cultivated	☐ Swamp hardwood	☐ Rolling	☒ Partly open (25-75% shaded)
☐ Pasture	☐ Swamp conifer	☐ Hilly	☐ Open (0-25% shaded)
☐ Upland hardwood	☐ Marsh	☒ Mountains	(% daily direct sun)

4. Physical Characteristics of Site (Make notes here)
Slope Pond gentle slope Soil silty - loam Elevation 1345 m or ft
Overstory vegetation/forest Douglas fir/red Alder Understory vegetation/Forest Vine maple, spirea, Salmonberry

Water sample collected for mercury analysis – Bottle ID: HG-86 *Sample time:* 11:15 AM PM

Notes about the water samples or site information:
See below + on NW corner near shore
W/some spadderdock near by, muck bottom. difficult to get clear sample

Dragonfly Samples collected:

#	Sample ID		Family (SJN)	Size (mm)	(Optional) Instar	(Optional) Species
B 1	TL-1	Lib	Corduliidae	✓21		B
B 2	TL-2		Cord	✓22		B
3	TL-3		Cor?	✓22		B
B 4	TL-4	↓	Corduliidae	21		B
5	TL-5	Lib	Corduliidae	✓22		A
6	TL-6	Lib	Corduliidae	23		A
B 7	TL-7	Lib	Corduliidae	~~23~~ 23		B
B 8	TL-8	ophiogomp?	Corduliidae	22		
9	TL-9		Corduliidae	✓23		B
10	TL-10		Corduliidae	21 ✓		B
B 11	TL-11		Corduliidae	19		B
B 12	TL-12		Corduliidae	~~22~~ 22		B
13	TL-13		Corduliidae	21 ✓		B
14	TL-14		Corduliidae	23 ✓		B
15	TL-15	↓	Corduliidae	19 ✓		B

Notes about the samples: SN notes 2 body types, A + B.
Partly cloudy, occasional sprinkle
Pond edge spadderdock & other emergents, submerged logs, lots rock
Dragonflies & Damselflies: blue tail present
Crew: Ronda Strauch, Katryn Strauch & Mackenzie Crow
Clear in water-view, Earth H2O - no armband available

Figure 2. Example field sheet, originally completed by the park leader and/or citizen scientists, and annotated with corrections to individual size and family identification by UMaine PI and staff (SN, SJN initials note additions). Under "species", the designations A and B denote different body types/probable species (or here, family) types within one initial family ID.

Individuals selected for identification to species and instar determination were removed from zipper seal bags and placed in plastic scintillation vials with 95% alcohol for preservation for taxonomic identification. They were transferred to Bronco Quick, dragonfly taxonomist who has performed identification for several University of Maine studies and the Maine Department of Environmental Protection's MDDS (Maine Dragonfly and Damselfly Study), as well as led field courses regarding dragonfly ecology and identification. Quick used the key of Needham, Westfall and May (2000) and consulted Needham and Westfall (1955) as well as Soltesz (1996) to identify each individual to species, when possible. Some individuals could not be identified to species because of their small size. Quick also identified individuals with respect to likely instar, determining that three classes (early-, mid-, and late- instar) were most appropriate for the project. Because of high variability in metrics such as wingpad index across species, we reduced uncertainty by grouping instars more broadly.

Data QA/QC and data management

Quality Control (QC)

In general, precision is the agreement of independent data resulting from repeated measurement under specified conditions (Taylor, 1987). Accuracy is the degree of agreement of a measured value with the true or expected value (Taylor, 1987). Variance from accepted values is required to be 5-10% or less, depending on the analyte and the sample range (Table 1). Bias is avoided by using control charts to document performance on standards and standard reference material. Data for this project met the data quality objectives listed in Table 1.

Quality assurance (QA)

Quality assurance looks across the dataset and uses established normal ranges, known relationships (e.g., pH:acid neutralizing capacity), and knowledge of the sampled media and sampling locations to determine the validity of each observation within the dataset. Without full ion chemistry, the suite of potential QA analyses is limited, but QA evaluation was done to the extent possible. The range of measured concentrations in each variable was evaluated, and values that appeared to be extreme were carefully scrutinized, and in some cases, analyses were repeated in the laboratory to confirm results. Field and laboratory notes and photographs of samples were used as evidence to confirm seemingly anomalous results, or to flag such results as unreliable. Follow-up with park leaders often helped to identify site or sample collection factors that could result in anomalous results (for example, shallow depth in a heavily sediment-influenced site could result in a high THg in water concentration, due to included sediment particles in a water sample).

Statistical methods

The goal of statistical analyses in the project were exploratory; specifically to (1) summarize the existing dataset for each park, as a baseline dataset, and (2) explore potential relationships among dragonfly larvae THg, landscape characteristics, and surface water chemistry, as areas for development in future research. For goal (1), analyses were straightforward; summary statistics were calculated using SYSTAT statistical software, via "Analyses > Basic Statistics" and using the "By Groups" option to group observations by park; or using basic functions in the statistical package R (version 3.1.1; R Core Team 2014), in particular, the package `psych` in R. Because chemical

concentrations are often left-skewed, we computed the medians as well as means; medians are a better measure of center for skewed distributions.

For goal (2), data exploration and pattern detection across sites and parks, we developed and compared parametric and non-parametric statistical models using R (version 3.1.1; R Core Team 2014). Only 2013 data were used in these analyses. To meet the assumption of normally distributed data in parametric statistical methods, we log-transformed variables that were left-skewed (e.g., DOC, THg in water, THg in dragonfly larvae). At SACN, ROMO (McGraw), and GRSM (Abrams), site coordinates were not provided, so they were determined using Google Earth. We simplified landscape data reported on the field sheets into fewer classes for the variables terrain, cancov (canopy cover), and landcov (land cover); grouped dragonfly larvae into guilds by family (burrowers (Gomphidae, Cordulegastridae), sprawlers (Corduliidae, Libellulidae, Macromiidae), and claspers (Aeshnidae); and re-coded categorical variables for uniformity prior to model development.

Parametric statistics and models

We used R (R Core Team, 2012) and the package `nlme` (version 3.1-117) to perform a **linear mixed effects (LME) analysis** that evaluated whether there were differences among guilds, when controlling for sites as nested within parks (earlier one-way ANOVA analysis confirmed differences among sites and parks; among sites ($F(62,429) = 19.1$, $p < 0.001$); and among parks ($F(22,466) = 16.6$, $p < 0.001$). As a fixed effect, we entered guild. As random effects, we had intercepts for sites nested within parks. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. **Correlations** (Pearson's) between pairs of variables were assessed using simple correlation analyses (`cor` in R), on log-transformed data as necessary, to determine whether there were any significant univariate correlations in the dataset (for example, between dragonfly THg concentration and dragonfly body mass). Graphics were produced using the package `PerformanceAnalytics`, and `boxplot`.

Stepwise linear regression, a form of multiple linear regression, although somewhat controversial in that models can be overfit, is useful in this exploratory stage of this research as a complement to the nonparametric methods listed below. Data must be normally distributed – thus we used log-transformed variables in this analysis – and variables should not be collinear. Because there were many dragonfly larvae collected at each site, yet only one water sample or landscape characterization per site, the problem of pseudoreplication would occur in this analysis. However, because multiple dragonfly families and sizes existed at each site, we did not create a single mean concentration by site, because it would mean these dragonfly-specific variables would have to be eliminated from the potential model. In stepwise regression, variables can be continuous or categorical. Stepwise linear regression computes multiple regressions iteratively, each step adding or subtracting a predictor until the model seems to be the best fit for the response variable. We used the function `lm` and subsequently `stepAIC` in R to develop and evaluate (using AIC – Akaike's Information Criterion) a stepwise regression model. Only complete cases were used. The model was run in both directions (forward and backward). The package `sjPlot` was used to create the forest plot of model estimates.

Nonparametric statistical analyses.

Multivariate Adaptive Regression Splines (**MARS©**) is a technique that creates regression surfaces and automatically incorporates non-linearities in the data (Friedman 1991). Conceptually, MARS is an extension of regression analysis, but allows for hinge functions (where data are non-linear and the regression 'bends' at a 'knot' – like a piecewise regression but with multiple predictor variables) and models interactions among variables. Therefore, it can create a model that might describe a system where, for example, Variable X does not affect Variable Y unless Variable Z is low. MARS is well suited to this dataset because it does not assume any specific distribution in the data, and it can include both continuous and categorical variables. MARS builds models using both a forward pass (adding terms to the model) and a backward pass (deleting terms, or pruning the model), and in the backward pass, generalized cross validation (GCV) is used to compare and determine parsimony in the subsets of models that are produced. For this project, we used the `earth` package in R, and modeled dragonfly THg (response variable) as a function of the potential explanatory variables listed in Table 3. Notably, `earth` cannot handle any missing values (`NA`) in the dataset, so only complete cases can be used in creating the model and observations with missing values were deleted (creating a new dataset) prior to running `earth`.

Table 3. Variables used in statistical analyses and models. "X" symbols in boldface text were those that were important in the various models and analyses, as discussed in Results, below.

Variable	Description	Structure	LME	CART	MARS	Stepwise
dfthg	Dragonfly larvae THg (response variable)	Numeric	-	-	-	-
guild	Dragonfly habitat guild	Factor w/ 3 levels	**X**	**X**	X	**X**
dw	Dragonfly larvae dry weight	Numeric		X	**X**	X
length	Dragonfly larvae length	Numeric		X	X	X
cancov	Canopy cover class	Factor w/ 3 levels		X	X	**X**
EQPH	Water sample equilibrated pH	Numeric		**X**	**X**	**X**
DOC	Water sample DOC	Numeric		**X**	X	
logdoc	ln-transformed DOC (water)	Numeric		X	**X**	**X**
SO4	Water sample SO_4^{2-}	Numeric		X	X	
logso4	ln-transformed SO_4^{2-} (water)	Numeric		X	X	X
THG	Water sample THg	Numeric		**X**	X	
logthg	ln-transformed THg (water)	Numeric		X	**X**	**X**
park	Park abbreviation	Factor w/ 23 levels	**X**			
site	Site ID	Factor w/ 67 levels	**X**			
lat	Site latitude	Numeric		X	X	**X**
long	Site longitude	Numeric		**X**	**X**	X
elev	Site elevation	Integer		X	**X**	X
landcov	Landcover class	Factor w/ 10 levels		X	X	**X**
terrain	Terrain (topography)	Factor w/ 4 levels		X	**X**	**X**
wtrbdy	Waterbody type	Factor w/ 3 levels		X	X	**X**

Classification and Regression Tree analysis (**CART**) is a recursive partitioning approach to the same type of dataset (e.g., De'ath and Fabricius 2000). Also called a decision tree analysis, CART recursively splits the data set into groups (or 'nodes') based on one variable at each split, and grouping the response variables according to within-group similarity at each node. Each variable used to the split the data is not discarded, but can appear again to split the model in later steps (thus, "recursive"). Because the response variable in this dataset is continuous, our analysis is technically a regression tree (as opposed to classification tree, when the results are used to place data into classes). As with MARS, there are no assumptions regarding the distribution of data, and both continuous and categorical data can be used. We used the R package `rpart` for the CART analysis, and `rpart.plot` for creation of the tree graphic.

Because the goal of this analysis was data exploration, the most valuable analysis lies in comparing the parametric and non-parametric models to determine which variables appear as the strongest predictors across all three analyses (Stepwise, MARS, CART). As appropriate for each technique, cross-validation (to determine the most parsimonious model within one of the analyses), bootstrapping (to determine a confidence interval around the model prediction) and/or sensitivity analyses (to determine which variables have greater or lesser effects on model fit) were performed and reported in the results section for each analysis.

Related research: Old Town High School Collaboration

After participating in an inquiry-learning program (Acadia Learning, see http://participatoryscience.org/) for several years, teacher Ed Lindsey began a two-year program of collaborative research, where high school students conducted independent research with input from PI Nelson. Though funded largely through other means, the projects were designed to better define underlying assumptions of this dragonfly mercury research, The projects addressed the following three questions: 1) at what rate do dragonfly larvae accumulate Hg as they develop, from hatching to emergence? 2) should we depurate (purify) live samples before submitting?; 3) what are survival estimates for dragonfly larvae? Because each project (or several sub-projects within each project focus) was an encapsulated study, methods for each of the inquiries are presented – very briefly – in the Results section and students' final products with details of methodology are included as figures in the text. Hg laboratory analysis methods and handling procedures were identical to those used in the NPS study. Equipment and supplies that were new to this research were soaked in ultrapure DI water for 72 hours and the solution was analyzed for THg on the Tekran 2600, to ensure that blank concentrations were below the detection limit, or sufficiently low to warrant using those materials (e.g., closed-cell foam and plastic screen used to build floating dragonfly cages ('boats') used for rearing studies).

Results

Citizen scientist engagement

During 2013, 15 of the 23 parks reported information about their citizen scientist groups, and hours of citizen and staff engagement in the project. These 15 parks reported 266 citizen scientists, from elementary school students to adult volunteers, and 1,756 volunteer hours. Many parks and partners had press coverage or created their own news articles regarding the sampling events (see links on the project Web site, http://www.nature.nps.gov/air/Studies/air_toxics/dragonfly/).

Park leaders reported that the groups they worked with were excited to participate in 'real science'. Participants wanted to know about dragonflies as study organisms, where their efforts fit into the research process, and if or when they would be able to see the results from their sampling efforts. Park leaders also reported that the biggest obstacles were identifying dragonfly larva and maintaining organization while sampling.

Interpretive materials

On January 23, 2014 park leaders met, via webinar, with project leaders. There were an estimated 40-50 individuals who participated in the webinar, which was designed to (1) give an overview of the project, (2) give a brief science update, and (3) allow interpreters and resource management staff to provide input on the types of materials that would better support them in project implementation. Based on this feedback, interpretive materials that would be useful in future iterations of the project were prioritized for development and inclusion. The materials that were developed as a result of this input (available on the project Web site, http://www.nature.nps.gov/air/Studies/air_toxics/dragonfly/) were:

1. Field identification cards

   Identifying dragonfly larvae is difficult. Park leaders expressed the need for field-ready dragonfly larvae identification cards. The resources for field identification had ranged from park to park, from multi-page scientific dichotomous keys to one-page photocopies. With input from core park leaders we developed 4x6 identification cards, designed to be printed and laminated for field use. These, like the rest of the materials, are available for park leaders as well as citizen scientists. The cards are used in the field, but are also used before the field event for familiarization.

2. One-page condensed field sampling protocols

   The entire field sampling protocol is detailed and covers the entire sampling process. The park leaders asked for a brief, one page field protocol to share with citizen scientists before and during the field sampling. The sampling process could then be concisely referred to in the field. We developed one-page 'tear sheet' protocols for field use.

3. Research flowchart

   Many park leaders reported that citizen scientists often asked, while sampling or after sampling, "What happens to my samples next?" In the January 2014 webinar we found that what was needed was a pictorial guide showing the role the citizen scientists play in the entire research arc. We developed a flowchart titled, "How citizen scientists are helping the dragonfly project".

Other products identified for future development were:

- Identification video-used for familiarizing citizen scientists to the identification of dragonfly larvae to family
- One-page dragonfly larvae key- for identifying dragonfly larvae in the field.
- Basic sampling protocol videos- for use by Park staff or interns, and teachers, to ensure comfort with the protocols.

Mercury data and analyses

Summary: THg in dragonfly larvae

Twenty-two national parks (DENA, OZAR, SAGA, MABI, NOCA, NIOB, ROMO, KEAQ, MONO, GRSM, KNRI, JELA, MACA, VOYA, PIRO, CACO, YELL, ACAD, CUVA, GOGA, SACN, BICY) plus one state park (STONE) collected and submitted samples for analysis (Figure 3). Several other parks planned to sample or attempted sampling, but were unable to contribute samples: APIS, GRPO, GRSA, and BIBE planned to sample, but were unable to complete the sampling due to changes in weather and/or the 2013 government shutdown; MEVE and GRCA scoped field sites but inadequate samples or site conditions precluded sampling that year. In addition to BICY, which has warm weather year-round and sampled late in the season (Nov. 2013), a state park in Georgia (Stone Mountain State Park in GA) sampled at their cost and samples were received in March 2014, adding to geographic coverage in the southeastern region. The 2014 STONE samples are included with the 2013 dataset in statistical analyses.

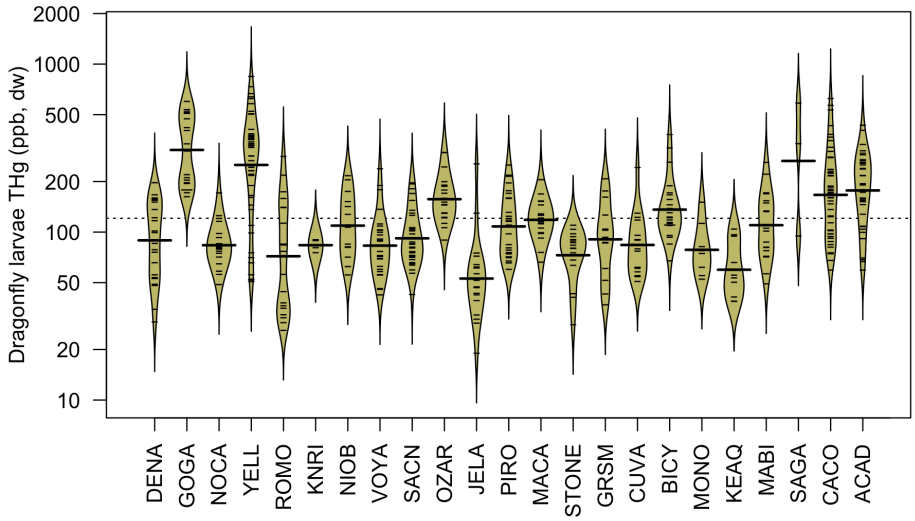


Figure 3. Beanplot showing THg in dragonfly larvae (ppb, dw) for each park in 2013. The graph is ordered from west to east, reading left to right. Each 'bean' is a density trace; thin black lines show the actual observations; thicker black line is the mean for each park. The dotted line shows the overall mean for 2013.

THg concentrations in dragonfly larvae ranged from below the detection limit to 844 ppb, dry weight (Figures 3 and 4; Table 4). The overall median concentration was 111.9±124.3 ppb, dw (Geometric

mean: 120.6 ppb, dw). Only 8 (of 775) samples had values that were below the detection limit. Percent moisture ranged 55-96% (Mean±SD: 81±5.7%).

Table 4. Summary statistics for THg in dragonfly larvae in 22 national parks plus one state park for 2013. "SD" is standard deviation. Samples with values below the detection limit were not included in calculations.

Park	Mercury concentration (ppb, dry weight)					
	Mean	Median	SD	Min	Max	N
ACAD	199	192	91.3	59.4	433	38
BICY	149	122	75.3	67.3	381	23
CACO	201	166	134	59.4	624	48
CUVA	92.3	79.4	48.9	50.8	242	15
DENA	102	99.3	49.8	29.2	197	23
GOGA	343	319	157	162	599	20
GRSM	103	93.1	52.7	36.9	208	13
JELA	64.2	49.4	53.5	19.0	255	18
KEAQ	63.9	54.4	25.3	38.7	104	10
KNRI	83.8	84.8	7.0	75.4	90.1	4
MABI	121	105	56.5	49.2	260	19
MACA	123	118	33.7	66.2	205	18
MONO	83.3	76.1	33.2	52.4	151	8
NIOB	119	109	58.7	52.2	217	14
NOCA	87.4	80.0	29.0	48.7	171	19
OZAR	165	148	58.1	89.6	298	20
PIRO	118	113	52.8	60.0	251	30
ROMO	94.7	76.8	73.3	26.0	282	19
SACN	96.9	83.0	41.4	38.3	196	34
SAGA	339	336	246	94.7	586	3
STONE	77.1	81.9	23.2	28.1	110	16
VOYA	91.1	80.1	44.6	42.3	238	30
YELL	314	322	189	50.7	845	50

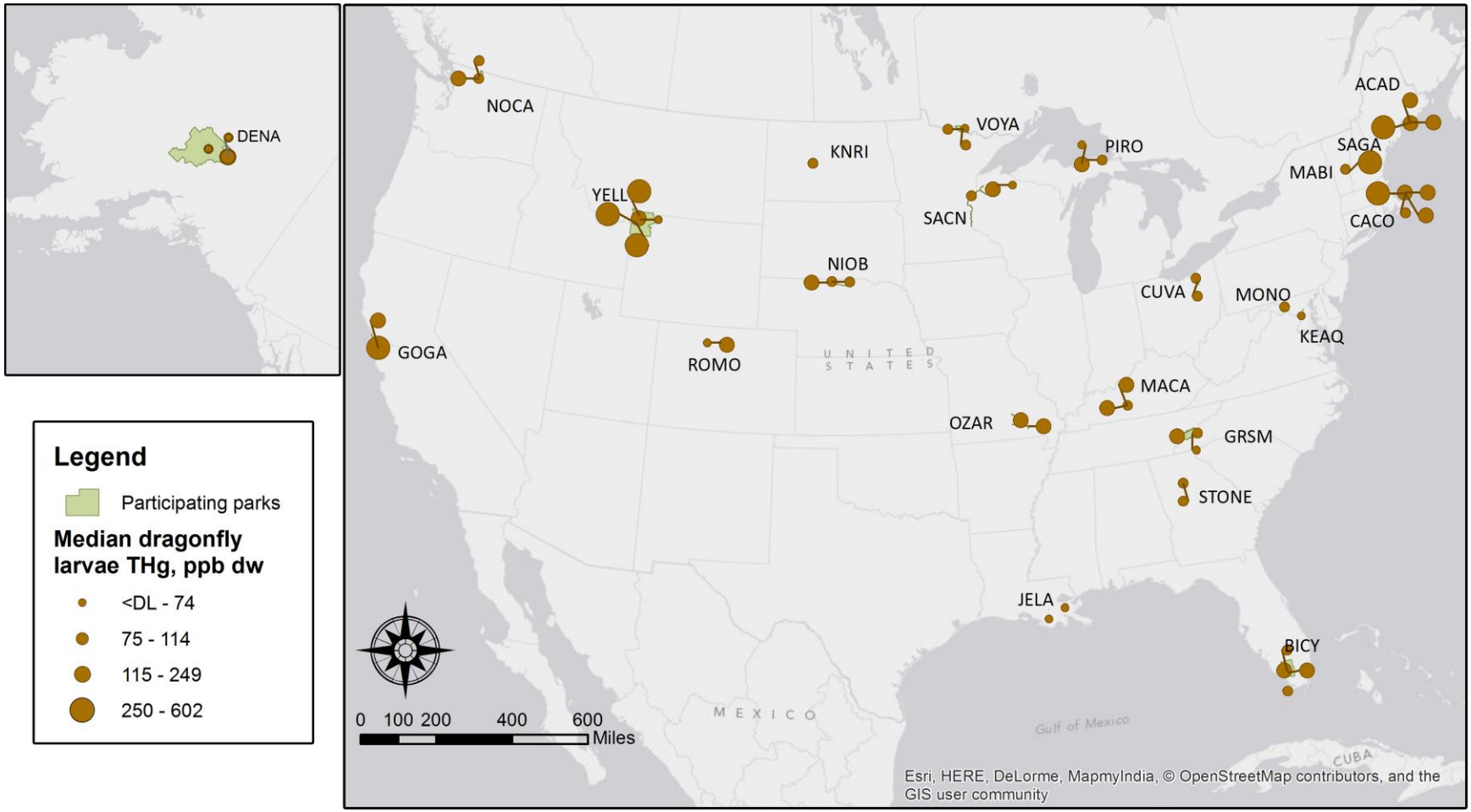


Figure 4. Total mercury (THg) concentrations in dragonfly larvae collected from 22 national parks (plus one state park) during 2013. Most parks had multiple sampling sites, shown on the map as multiple dots. Even within a park and in close proximity, THg concentrations in dragonfly larvae could be highly variable and site-specific. Gray background map: ESRI (World Light Gray Base), Sept. 18, 2014. Sources: ESRI, DeLorme, HERE, MapmyIndia, © OpenStreetMap contributors, and the GIS community.

Water Chemistry

Table 5. Summary statistics for water chemistry in all participating parks, 2013.

Variable	Mean	Median	SD	Min	Max	N
EqpH	7.63	7.93	0.86	5.43	9.25	78
DOC (mg/L)	6.9	4.1	7.2	0.2	31.5	78
SO_4^{2-} (μeq/L)	400	80.5	1637	<DL (<2)	13660	76
THg (ng/L)	3.6	2.2	4.7	<DL (<0.5)	27.9	62

Water THg ranged from below the detection limit (<DL) to 27.9 ng/L. Median THg in water was 2.2 ng/L (Table 5). Mean pH was 7.63; however, western sites (west of approximately -95° longitude, or from the Mississippi River eastward) always had pH > 7.4, and often EqpH was >8 in these western sites. Eastern sites had EqpH ranging from 5.43 - ~8.5. Sulfate had an extremely wide range, up to 13,660 μeq/L at KNRI; however, only one site was sampled, and so further sampling should be conducted to determine the validity of this exceptionally high concentration. Sulfate in DENA, CUVA, and GOGA were also one or two orders of magnitude greater than all other parks, and should be validated with 2014 sampling. GOGA could be influenced by proximity to the ocean; one site is part of an estuary and is most likely marine-influenced. DOC ranged 0.2–31.5 mg/L, a fairly typical range for natural surface waters. The greatest DOC concentrations were in DENA; GOGA, an urban site with an extreme slope surrounding the site; and in VOYA, where boreal wetlands could have influenced DOC concentrations. Taken together, the range in chemical concentrations and combinations of variables should provide a sufficiently variable dataset for testing potential predictors of Hg across the landscape.

MeHg results

In 2013, 159 samples were analyzed for MeHg; results ranged from 1.2 – 575 ppb, dw with mean ± SD concentrations of 138 ± 104 ppb, dw. The median MeHg concentration in dragonfly larvae was 104 ppb, dw. A key objective of measuring MeHg in dragonfly larvae was to confirm or reject the hypothesis that THg could be used to represent the body burden of Hg in dragonfly larvae. Put more simply, could we measure THg in future studies and assume that it represents MeHg available for trophic transfer? If the proportion of Hg within each dragonfly larva that is MeHg is near 1.0 (100%), then THg is a good proxy for MeHg, making use of dragonfly biosentinels more cost-effective. In 154 samples for which both THg and MeHg data were available to compute this proportion, we found that 88 ± 17% of THg was in the form of MeHg (median: 95%). The MeHg:THg proportions reported here for dragonfly

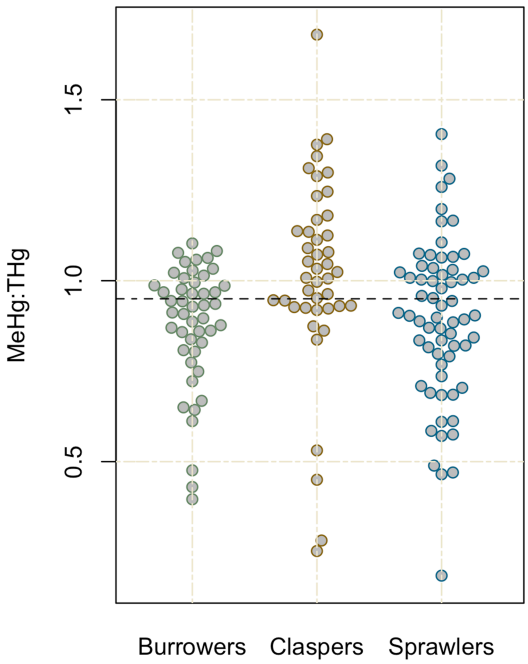


Figure 5. Methylmercury as a proportion of THg in dragonfly larvae samples, grouped by guild. The dashed line indicates the median MeHg: THg (0.95). Values greater than 1 occur due to variability in MeHg and THg results that is expected during laboratory analysis and is magnified by computation of ratios. (Graph: `beeswarm` package for R, v. 0.1.6)

larvae are similar to those reported for fish muscle (71-89% in finescale dace, Bodaly et al. 1999; >95% in muscle across 229 fish samples, Bloom et al. 1992; also see Sandheinrich & Wiener 2011) and other fish tissues (84-97% in yellow perch eggs and carcasses, Hammerschmidt et al. 1999).

Species and instar identification

Of 775 dragonfly samples in the 2013 project database, 285 (37 percent) were identified to genus only, and 169 (22 percent) identified to species. One hundred percent of the dragonfly samples were identified to family. Instar (developmental stage of larvae) was estimated for the 285 individuals, and the project PIs plan to evaluate whether size (body length, mass) can be used as a proxy for instar, as instar can be challenging to determine. Identifications (number of species or genera in each family) were as listed in Table 6. The full species list is in Appendix A.

Table 6. Generic and species richness of dragonfly larvae collected during the 2013 field season across 23 parks. Approximately 22% of the total number individuals collected were identified to species (n=169).

Family	Number of genera	Number of species
Aeshnidae	5	8
Cordulegastridae	1	2
Corduliidae	6	7
Gomphidae	7	10
Libellulidae	8	18
Macromiidae	1	2
TOTALS:	**28**	**47**

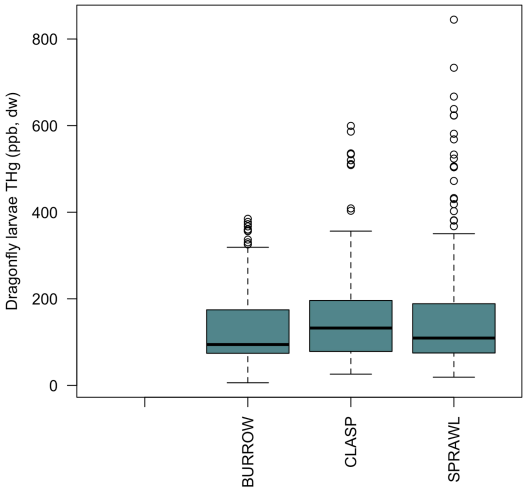


Figure 6. Boxplot showing distribution of dragonfly THg data among three habitat guilds: burrowers, claspers and sprawlers.

Simple associations: LME and Correlation

Controlling for site, which was nested within park, there were significant differences among the three dragonfly larvae guilds (mixed-effects linear model, df=428, n=491, number of groups=61; for claspers, $p<0.001$; for sprawlers, $p=0.02$, as compared to burrowers). Claspers, which include the family Aeshnidae, had the greatest median THg concentration (Figure 6). One potential explanation of this greater Hg concentration could be that Aeshnidae are voracious predators, and might eat prey that are larger or higher order in their local foodweb due to their aggressive feeding strategy. Future research using stable isotopes would elucidate trophic position and thus help to define mechanisms for this possible pattern.

Correlations (Pearson's) between dragonfly larvae THg and each elevation, DOC, THg (aq), SO_4^{2-}, and dw (dragonfly dry weight) were highly significant ($p<0.001$) but weak ($r = 0.18–0.37$) for the following variables, in descending order: elevation (elev), logdoc (log of DOC in water), logthg (log of THg in water), logso4 (log of SO_4^{2-} in water), dw (dragonfly larva dry weight). All correlations were positive except logdoc; although DOC and THg in water were positively (weakly) correlated, the relationship between DOC in water and Hg in dragonfly larvae was not linear, and although there was an increase in dragonfly THg initially, it then declined or became more variable. This suggests that at some sites that had high DOC, there could be

dragonflies with relatively low Hg, and warrants further investigation. The results of simple, univariate correlation analysis make clear that multivariate approaches are necessary for this dataset.

Stepwise Regression

Stepwise linear regression yielded a final model that included the terms guild (habitat guild), EQPH (EqpH), logdoc (log of DOC in water), logthg (log of THg in water), lat (latitude), landcov (landcover class), terrain (terrain class), cancov (canopy cover class), wtrbdy (waterbody type). Diagnostic plots for the stepwise regression suggest reasonably good results in terms of normality of the data and residuals (Figure 7). However, because stepwise regression can overfit models and are not always the tool of choice for these types of analyses, results should be interpreted cautiously. Here we are simply noting the relative importance of the proposed predictor variables, to compare with those resulting from CART and MARS analyses. Variables considered "important" in each model type are noted in Table 3 with boldface 'X' symbols. The forest plot of model estimates (Figure 8) illustrates the relative significance and plots the effects (β_i) and confidence intervals of the model.

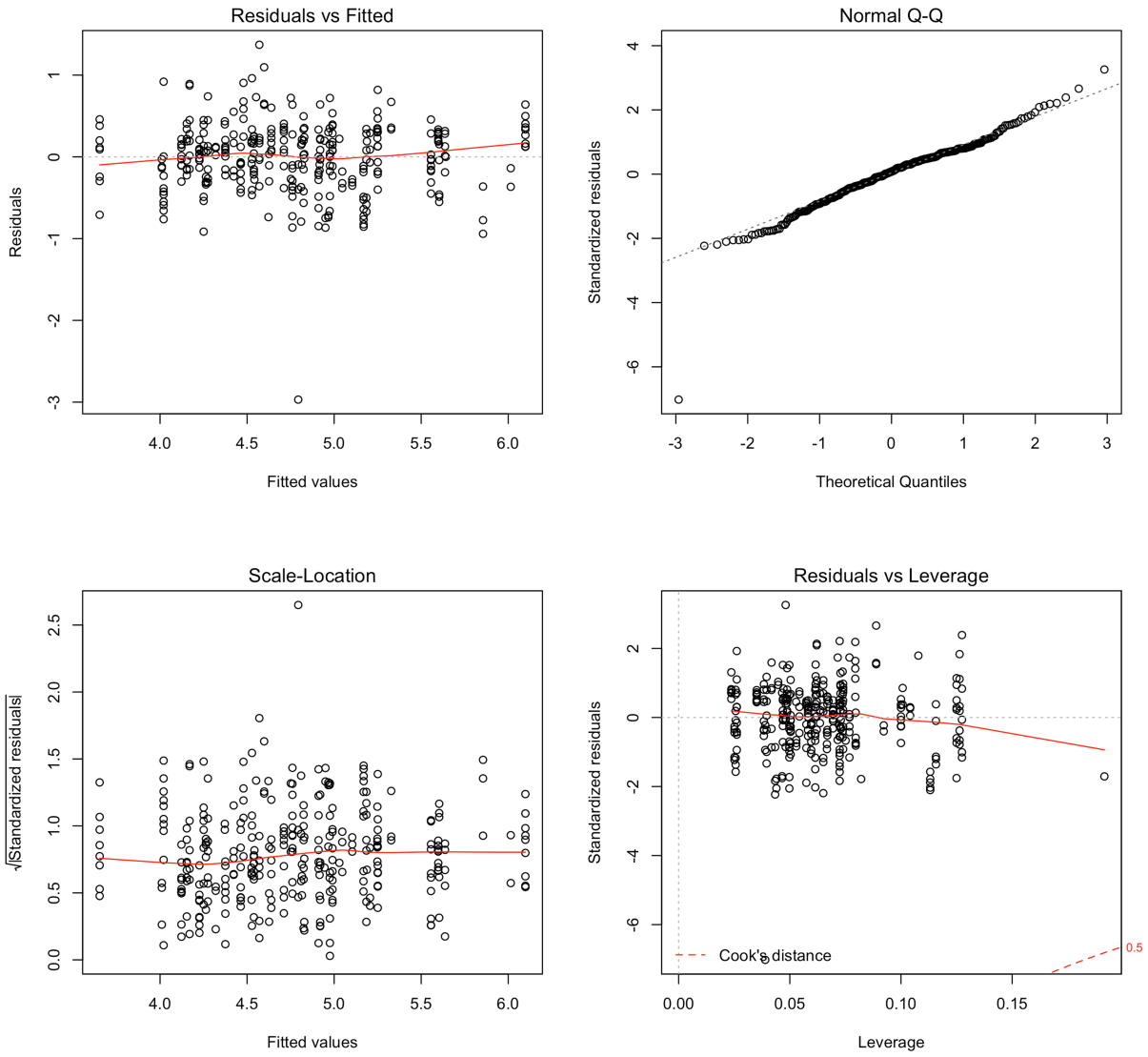


Figure 7. Diagnostic plots for the stepwise linear model described above.

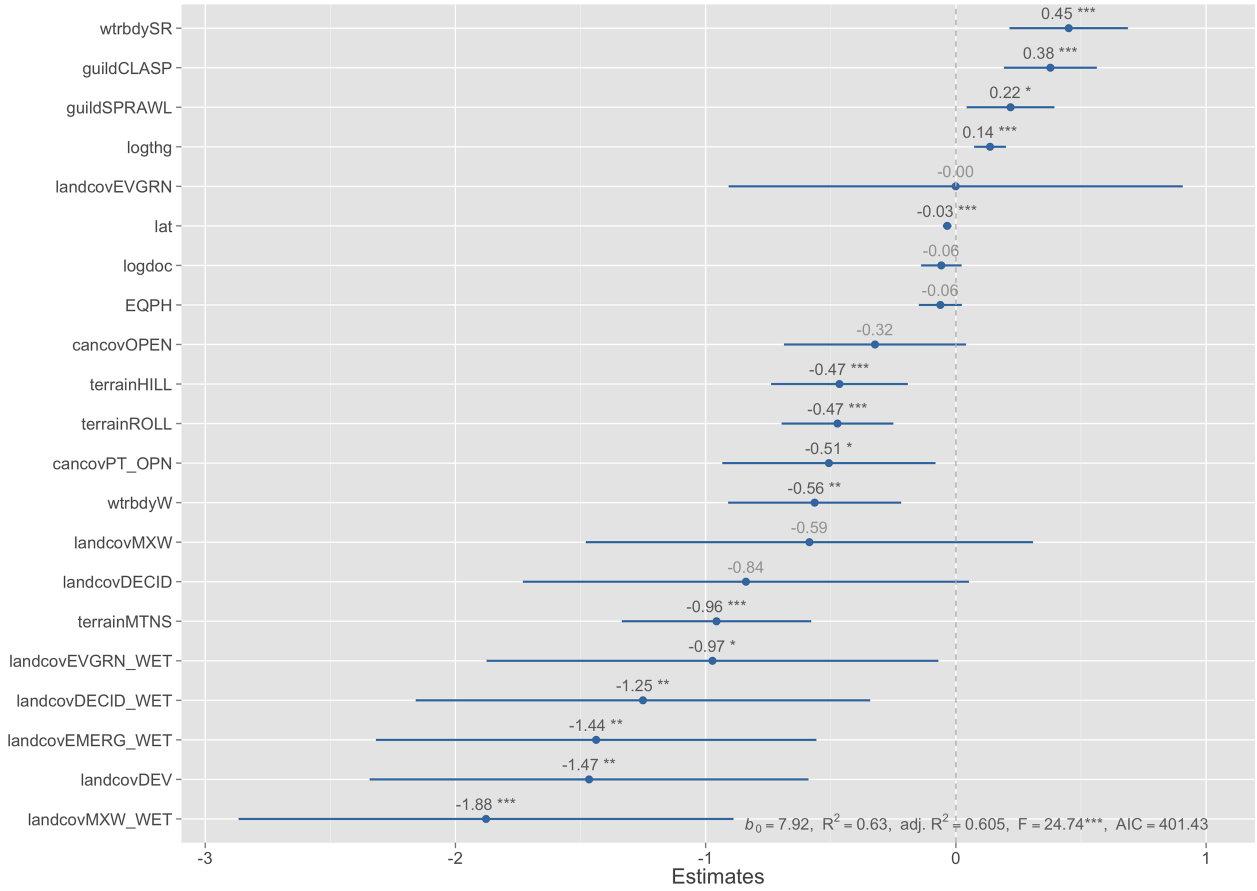


Figure 8. Forest plot displaying effects and confidence intervals for each model term in the stepwise regression.

MARS

MARS analysis was run using `earth`, and the same set of potential predictor variables as were used in stepwise regression. The `earth` package in R includes a function that lists relative importance of variables selected by the model; though instructive, the function is meant to be used simply as a rough estimate of "variable importance" (see Millborrow 2014 for a more complete discussion). In the final MARS model that best represents the 2013 dragonfly larvae THg response data, the following variables were considered to be important, in descending order of importance: logdoc, logthg, long, EQPH, elev, terrainHILL, dw, guildCLASP, landcovMXW_WET, wtrbdyW (wetlands), logso4, lat. Some of these variables are collinear (dry weight and length tended to be strongly correlated, for example; variables like wtrbodyW (waterbody type is wetland) overlaps with landcovMXW_WET (mixed wetland landcover). In future research, these variables should be reduced to avoid overly complicated models. The `earth` package includes built-in cross validation procedures; evaluation of the resulting plot (Figure 9) suggests that fewer terms could have been included to create a more parsimonious model; perhaps seven terms were needed. The seven most "important" variables are noted in Table 3 with bold-face 'X' symbols; others might interpret the results differently and include more or fewer terms.

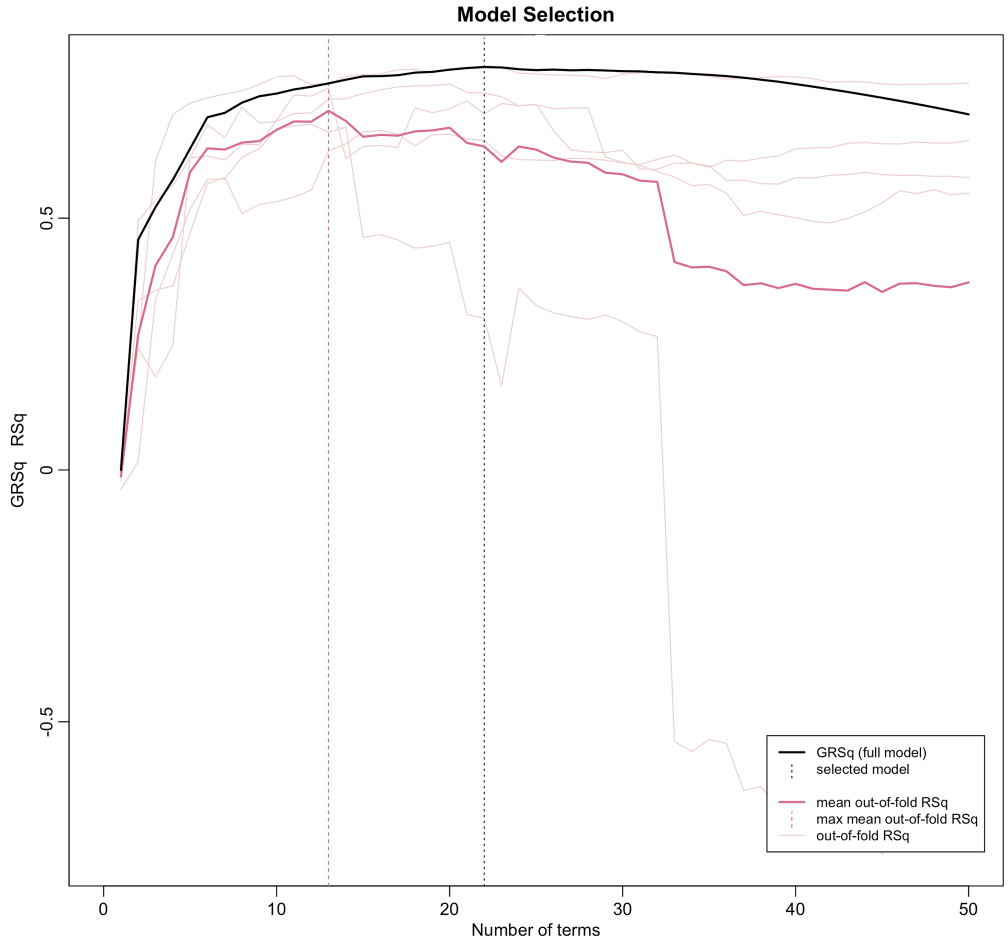


Figure 9. Cross-validation results from MARS analysis that modeled effects of landscape, chemical, and dragonfly variables on dragonfly larvae THg (via earth in R). The dark black line shows model improvement with addition of terms.

CART

For the CART analysis, we included all possible predictor variables, not just the set of variables used in stepwise regression and MARS. CART is more easily limited by stopping rules (here, defaults were used) and the resulting model was reasonably simple, even with the full variable set. The resulting tree (Figure 10) begins with a split on DOC in water, and proceeds to splits based on long (longitude) and THG (THg in water). THG, DOC, and long then re-enter the model at subsequent splits, and final splits on terrain and guild complete the most complicated paths to terminal nodes. One terminal node with dragonfly larvae THg centered around 613 ppb, dw results from a condition where water DOC is low, yet THg in water is quite high. This node represents the samples from YELL, where potential sources of Hg could be geothermal features (as reported by Hall et al. 2006) or wildfires upwind of the park, in addition to atmospheric deposition from distant sources. Relatively high inorganic Hg in waterbodies combined with low DOC concentrations is a unique combination present in YELL. Notably, some sites in YELL had relatively low THg concentrations. Again, important splitting variables are noted in bold text in Table 3.

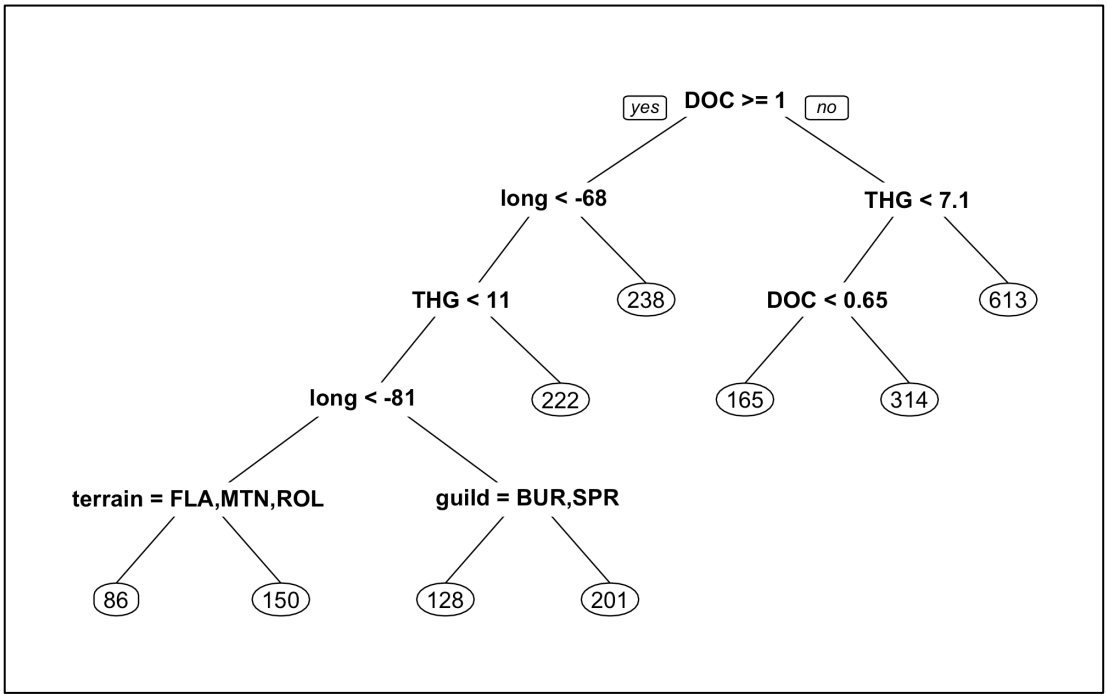


Figure 10. Regression tree with nine terminal nodes describing dragonfly THg concentrations (in ppb, dw) in each path. Note that "yes" responses proceed to the left branch, whereas "no" responses proceed to the right branch at each node.

Related research: Old Town High School Collaboration

Collaborative Research with Old Town High School and the University of Maine carried out in laboratory mesocosms or other in-lab treatments was designed to address three main questions. Each question, and a summary of student findings, follows.

1) *At what rate do dragonfly larvae accumulate Hg as they develop, from hatching to emergence?*



Figure 11. Emergence jars.

Wild-caught nymphs from Baker Brook (in Sunkhaze National Wildlife Refuge, Maine; a site studied for several years by Ed Lindsey and PI Nelson) were placed individually in emergence jars (Figure 11). In each jar, a few small stones and a stick for emergence, collected from the site, were included. A small piece of screen capped the top of the apparatus, precluding emergent dragonflies from escaping. A sample (n~15, hereafter "F0" nymphs) of the largest nymphs were refrigerated alive and stored until the larvae kept in emergence jars had emerged. Wing pads on exuviae of nymphs that had emerged were examined to compare with wing pad size in the F0 (refrigerated) nymphs. The F0 samples were then frozen for storage until THg analysis. As dragonflies emerged, teneral (newly-emerged) adults along with their shed exuviae (exoskeletons) were frozen for THg analysis. Of the three sample types (nymph, exuviae, teneral adults), teneral

adults had the greatest concentration of THg (Figure 12). This is most likely because teneral adults had shed a mass of relatively low-Hg in the exuviae, and retained most of the Hg in tissue. This finding is significant because teneral adults are very vulnerable to predation as they 'dry out' before flight, and could represent a heretofore un-studied vector for Hg trophic transfer. In related work, students raised dragonfly nymphs from eggs in laboratory mesocosms, and examine nymph vs. exuviae concentrations after three (of ~12-14) molts. In early stages, the exuviae did have relatively large concentrations of THg, but this pattern reversed after the 9th molt.

2) should we depurate (purify) live samples before submitting?

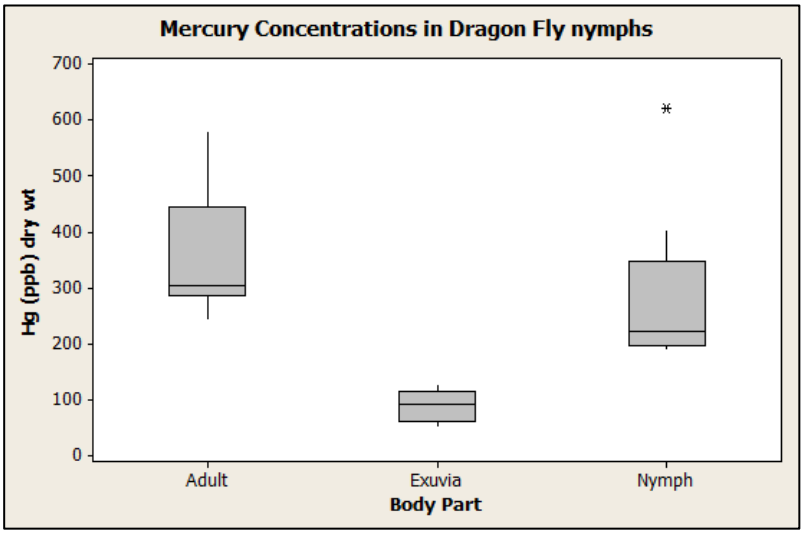


Figure 12. Boxplot with dragonfly nymph, exuviae, and teneral adult THg concentrations from larvae harvested during the emergence study.

Student Alex Baron captured dragonfly larvae and frozen and submitted them to the laboratory in three treatments: immediately frozen, frozen after being unfed and allowed to depurate for 32 hours, and unfed and depurated for one week. This side study quantified, on a small scale for one species of dragonfly, potential bias in submission of non-depurated larvae. In the servicewide research, samples are submitted without allowing for depuration, but samples in Haro et al. (2013) were depurated prior to freezing and analysis. This student also participated as a citizen scientist during summer 2013 at ACAD's dragonfly larvae sampling event. Using one-way ANOVA, we did not find significant differences among the three treatments in this study, suggesting that there is no difference between depurated and non-depurated larvae (Figure 13).

3) What are survival estimates for dragonfly larvae?

The Collaborative Research has also provided an estimate of survival of larvae to adulthood. Earlier estimates, based on damselflies (Odonata:Zygoptera) were that ~95% of larvae were not likely to survive to adulthood (M. McPeek, Dartmouth College, pers. comm.); however, there were no available data for dragonfly larvae. A rearing study in the Collaborative program where larvae were not isolated from each other or from natural sediment that presumably contained other predators resulted in mortality rates of 95% (~80 of 1,600 eggs that hatched survived after ~2 months in a mesocosm). This result, coupled with egg-laying rates of 400-1,600 eggs per individual female observed in the Collaborative Research program, suggests that our taking of 15-20 individuals per waterbody is unlikely to damage dragonfly populations to a greater extent than natural processes. (Lindsey & Baron 2013, unpubl. data).

Summary statistical findings of dragonfly larvae depuration study

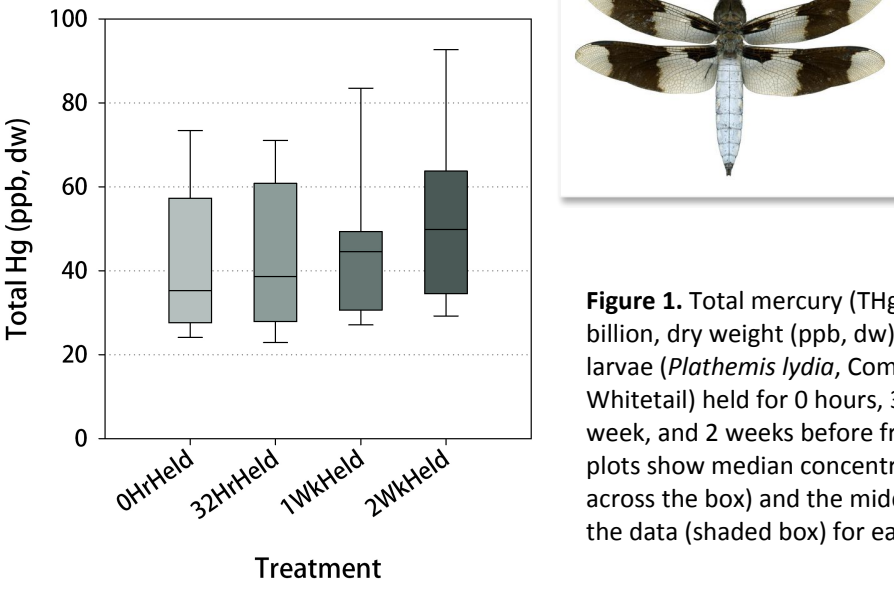
A. Baron[1], E. Lindsey[1], S. Nelson[2]
[1] Old Town High School, Old Town, ME; [2] University of Maine, Orono, ME

January 22, 2014

A one-way ANOVA of total Hg in dragonfly larvae yielded no significant differences among groups with respect to length of time they were held prior to freezing ($F(3, 54)=0.903$, $p=0.45$). (Note: ANOVA was performed on log-transformed data to meet the assumption of normality).

Table 1. Mean, n, and standard deviation for THg in ppb, dry weight for dragonfly larvae collected in Orono, Maine and held live in aerated water for 0, 32, 168, and 336 hours prior to freezing. Descriptive statistics for larval body length, dry weight, wet weight, and percent moisture are also given. Wingpad index was 5 for 10 of the individuals, 6 for 43 individuals, and 7 for one individual.

Treatment	Statistic	THg (ppb, dw)	Length (mm)	Wet wt. (g)	Dry wt. (g)	Percent moisture
0Hr-Held	n	13	13	13	13	13
	Mean	42.6	20.5	0.3503	0.07	80.1
	Std Dev	17.5	1	0.0268	0.0115	2.35
32Hr-Held	n	13	14	13	13	13
	Mean	43.4	21	0.3505	0.0767	78.2
	Std Dev	17.2	1	0.0328	0.0112	1.6
1Wk-Held	n	14	15	14	14	14
	Mean	44.9	20.9	0.354	0.073	79.3
	Std Dev	20.3	0.8	0.0374	0.0092	1.9
2Wk-Held	n	14	15	14	14	14
	Mean	53	21.5	0.3838	0.0727	81.0
	Std Dev	22.2	0.9	0.0365	0.0109	2.5



J. Ware, 2011. *Plathemis lydia* adult. Accessed Jan. 2013, available: Encyclopedia of Life, http://eol.org/pages/131397/overview

Figure 1. Total mercury (THg) in parts per billion, dry weight (ppb, dw) in dragonfly larvae (*Plathemis lydia*, Common Whitetail) held for 0 hours, 32 hours, 1 week, and 2 weeks before freezing. Box plots show median concentrations (line across the box) and the middle 50% of the data (shaded box) for each group.

Figure 13. Student summary of findings from depuration study, conducted as part of Old Town High School (Maine) collaborative research.

Discussion

The analyses of Hg in dragonfly larvae and surface waters in these national parks were conducted to explore potential relationships among landscape factors, aquatic habitat, and dragonfly larval characteristics. Because this project is the first at this national scale to evaluate use of dragonfly larvae as bio-sentinels, our goals were to determine the extent to which dragonfly larvae indicated relative Hg across waterbodies, and indicate variables that should and should not be further investigated for use as potential predictors of Hg risk in future research. This project also evaluated whether dragonfly larvae were appropriate bio-sentinels for Hg by determining the percent of THg that was MeHg in a subset of samples (n=154). MeHg was, on average, 88 ± 17% of THg in these dragonfly larvae samples, similar to percentages found in fish muscle and other tissues. Given that: (1) these dragonfly larvae are analyzed as whole-body samples; (2) samples in this study represent a broad geographic range, array of habitats and feeding strategies, and sizes and species of dragonflies; and, (3) exuviae (exoskeletons) are low in Hg and are likely to contain little Hg as MeHg, we concluded that THg is generally a good proxy for MeHg in dragonfly larvae based on these samples.

What influences THg in dragonfly larvae?

Dragonfly larvae THg concentrations were significantly different among sites and among parks, as well as among three guilds (sprawlers, climbers, burrowers) when sites and parks were taken into account in a linear mixed-effects model. No single variable adequately described the pattern of THg in dragonfly larvae. Three multivariate statistical models (CART, MARS, stepwise regression) were used in exploratory analyses to evaluate potential drivers of THg in dragonfly larvae, based on water chemistry, landscape factors, and larvae characteristics. These models pointed to DOC, THg in water, and pH as important variables across all three model types, with some influence of topography/wetlands, east-west position, and habitat guild. These findings support ongoing focus on DOC and acidity as well as landscape factors in determining relative Hg across sites (e.g., Krabbenhoft et al., 2011). However, unlike studies of fish Hg that found no to limited association between fish Hg and water Hg, this study did find that water THg was important in describing some of the variability in the dragonfly larvae THg data (~31% in stepwise regression; also an important variable in MARS and CART). Haro et al. (2013) also noted an association between dragonfly larvae THg and water Hg. Given the framework provided by this exploratory analysis, future statistical analyses should use hierarchical models and control for the effects of guild as well as sites and parks to develop waterbody-specific estimates of Hg that can be used to more effectively test the influence of water Hg on dragonfly larvae Hg.

Implications for continuing research

How can we make the project more valuable for citizen scientists?

Park staff already involved with the project have enjoyed the project as it allows them to engage park visitors in real science. However, park staff and park visitors often have questions before, during, and after their engagement with the field portion of the project. Questions have included, “How does mercury affect the plants and animals of my park?” or, “What do data from my Park mean?”. Future interpretive products will address these questions. Working with the project scientists we are

developing cohesive take away messages to answer the larger programmatic questions "What can I do?" and "What is mercury doing to Park wildlife?"

Additionally, we are developing a series of "train the trainer" videos that present sampling methods for each component of the project. These videos are geared toward interpreters/staff who are leading the field excursions, but will be accessible to citizen scientists and the public. The videos will serve to empower the citizen scientists to work more as partners with the park leaders. Finally, we are developing a training module for park staff and visitors to interpret their park's data and tell the story of the data.

How will these data help us understand risks to human or wildlife health?

There is not yet a definitive answer to questions about how the dragonfly data relate to human or wildlife health. The data are best regarded as "screening"-type data — if a park or site seems to have a set of data points much greater than the median for the overall dataset, then that park or site should consider additional studies of Hg in other biota, sediment, or water to evaluate whether there is any cause for concern. For many parks, there is only one year of data, so conclusions regarding relative sensitivity of biota to Hg are limited. Ongoing work in these and other parks will build confidence in the data as the research builds multi-year datasets. In one park (MABI) where sampling has occurred every autumn since 2010, there has been very little year-to-year variability; however, we are unsure if that pattern will hold at other sites.

Citizen science is emerging as a potential tool for collection of data not only for scientific research (e.g., National Phenology Network, eBird), but also potentially for use in policy and management decision-making. Agencies and scientists are working toward producing guidelines to ensure best practices and data quality (e.g., Hines et al. 2013). A few studies (and one legal opinion: (Smith 2014)) have begun evaluating the role that citizen science might play in resource management and policymaking (McKinley et al. 2014, Pocock et al. 2013, McKinley et al. 2012, Oscarson et al. 2007), and the reliability of citizen scientist-collected data (e.g., Galloway et al. 2006). Further, some agencies are beginning to set up resources for citizen scientists to directly contribute data and observations (e.g., this project, US EPA's MyEnvironment tool), but more work is needed in this developing field.

How could we make the data more instructive about understanding Hg?

Haro et al. (2013) sampled dragonfly larvae and fish in six Great Lakes parks, finding that dragonfly larvae could be used as surrogates for fish Hg. This research expands the scope of the Haro et al. study nationwide. Sampling in 2014–2015 in 50 parks will link water, sediment, and dragonfly data plus available fish data and help us determine how closely dragonfly mercury concentrations are coupled with these other media. These data provide a baseline that could be used in foodweb studies designed to test hypotheses about biomagnification in a variety of ecosystem types. Specifically, these baseline data, along with those being collected in 2014-2015, provide a snapshot of Hg concentrations in water and biota across the U.S. prior to implementation of MATS (the Mercury Air Toxics Standards), expected to take effect in Spring 2015. The data can also be compared to modeled methylmercury sensitivity maps being developed by Krabbenhoft et al. (2011) for National Parks as well as large basins nationwide.

Future work and data needs

Ongoing research (Flanagan Pritz et al. 2014) is expanding the geographic scope of this research, as well as investigating the relative importance of sediment and the dissolved phase of Hg and MeHg in water samples. In some parks, the ongoing research will result in a data record in excess of five years, but others will only have one or two years' worth of data. Long-term monitoring of ecosystem changes that are affected by non-point source pollution requires many years of data; in evaluating the effectiveness of the Clean Air Act on lake acidification, over a decade of data was required before clear declines were revealed (e.g., Strock et al. 2014), and the extent of biological 'recovery' of these ecosystems is still unclear. Long-term data collection, coupled with the extended geographic reach and enhanced depth of research in multiple media, could enable the project to provide long-term monitoring information as well as basic scientific research regarding mercury pattern and process in the national parks.

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Appendix A: Dragonfly larvae species identifications

The following table lists genus and species identifications for each park, determined for samples submitted during 2013 (and 2014 for STONE). Identifications followed by "?" are uncertain. When species is missing, identification was only possible to the genus level.

Park	Family	Genus	Species
ACAD	Aeshnidae	*Aeshna*	
	Aeshnidae	*Basiaeshna*	*janata*
	Corduliidae	*Dorocordulia*	*lepida*
	Corduliidae	*Tetragoneuria*	*epitheca spp?*
	Gomphidae	*Gomphus*	*borealis*
	Gomphidae	*Gomphus*	*exilis*
	Libellulidae	*Libellula*	*auripennis*
	Libellulidae	*Libellula*	*incesta*
BICY	Aeshnidae	*Nasiaeshna*	*pentacantha*
	Corduliidae	*Epicordulia*	*princeps*
	Corduliidae	*Tetragoneuria*	*cynosura*
	Libellulidae	*Libellula*	*auripennis*
	Libellulidae	*Perithemis*	*tenera*
	Macromiidae	*Macromia*	
CACO	Corduliidae	*Tetragoneuria*	*cynosura*
	Gomphidae	*Gomphus*	*exilis*
	Gomphidae	*Gomphus*	*spicatus*
	Libellulidae	*Leucorrhinia*	*frigida*
	Libellulidae	*Leucorrhinia*	*intacta*
	Libellulidae	*Libellula*	*auripennis*
	Macromiidae	*Macromia*	*illinoiensis*
CUVA	Aeshnidae	*Aeshna*	*vinosa*
	Aeshnidae	*Anax*	
	Aeshnidae	*Boyeria*	*vinosa*
	Gomphidae	*Arigomphus*	*furcifer*
	Libellulidae	*Libellula*	*pulchella*
DENA	Aeshnidae	*Aeshna*	*subarctica or juncea*
	Corduliidae	*Cordulia*	*shurtleffi*
	Libellulidae	*Leucorrhinia*	*glaciacis?*
	Libellulidae	*Pachydiplax*	*longipennis?*
GOGA	Aeshnidae	*Aeshna*	
	Libellulidae	*Sympetrum*	*illotum*
GRSM	Cordulegastridae	*Cordulegaster*	*erronea*
	Cordulegastridae	*Cordulegaster*	*maculata or diastatops*

Park	Family	Genus	Species
	Gomphidae	*Gomphus*	*descriptus?*
	Gomphidae	*Lanthus*	*parvulus or vernalis*
JELA	Libellulidae	*Erythemis*	*simplicicollis*
	Libellulidae	*Pachydiplax*	*longipennis*
KEAQ	Libellulidae	*Libellula*	*incesta*
	Libellulidae	*Pachydiplax*	*longipennis*
KNRI	Gomphidae	*Gomphus*	*stylurus*
MACA	Aeshnidae	*Nasiaeshna*	*pentacantha*
	Corduliidae	*Erithemis*	*simplicicollis*
	Libellulidae	*Leucorrhinia*	*proxima*
	Libellulidae	*Libellula*	
	Libellulidae	*Pachydiplax*	*longipennis*
	Libellulidae	*Plathemis*	*lydia*
MONO	Libellulidae	*Pachydiplax*	*longipennis*
NIOB	Gomphidae	*Gomphus*	
NOCA	Aeshnidae	*Aeshna*	
	Corduliidae	*Cordulia*	*shurtleffi*
	Corduliidae	*Somatochlora*	*franklinii*
	Libellulidae	*Ladona*	*julia*
	Libellulidae	*Leucorrhinia*	*glacialis*
	Libellulidae	*Libellula*	*needhami?*
OZAR	Gomphidae	*Gomphus*	
	Gomphidae	*Hagenius*	*brevistylus*
	Macromiidae	*Macromia*	*illinoiensis*
PIRO	Aeshnidae	*Aeshna*	*eremita*
	Aeshnidae	*Anax*	*junius*
	Aeshnidae	*Basiaeshna*	*janata*
	Aeshnidae	*Basiaeshna*	
	Gomphidae	*Arigomphus*	
	Gomphidae	*Gomphus*	*stylurus*
	Gomphidae	*Ophiogomphus*	
	Macromiidae	*Macromia*	
ROMO	Aeshnidae	*Aeshna*	
	Libellulidae	*Sympetrum*	*madidum*
SACN	Aeshnidae	*Aeshna*	
	Aeshnidae	*Boyeria*	*grafiana*
	Gomphidae	*Dromogomphus*	*spinosus*
	Gomphidae	*Gomphus*	
	Gomphidae	*Hagenius*	*brevistylus*
	Gomphidae	*Ophiogomphus*	

Park	Family	Genus	Species
	Gomphidae	*Stylurus*	*spiniceps*
	Macromiidae	*Macromia*	*illinoiensis*
	Macromiidae	*Macromia*	*taeniolata*
STONE	Corduliidae	*Tetragoneuria*	*spinigera*
	Libellulidae	*Ladona*	*deplanata*
VOYA	Aeshnidae	*Aeshna*	
	Corduliidae	*Cordulia*	*shurtleffi*
	Corduliidae	*Tetragoneuria*	*spinosa*
	Libellulidae	*Leucorrhinia*	*frigida*
	Libellulidae	*Leucorrhinia*	*intacta*
YELL	Aeshnidae	*Aeshna*	
	Corduliidae	*Cordulia*	*shurtleffi*
	Corduliidae	*Somatochlora*	
	Gomphidae	*Ophiogomphus*	
	Libellulidae	*Libellula*	*saturata*

The Department of the Interior protects and manages the nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its special responsibilities to American Indians, Alaska Natives, and affiliated Island Communities.

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