National Park Service
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Natural Resource Stewardship and Science

Protocol Implementation Plan for Dragonfly Larvae Sampling and Mercury Analysis

A contribution of the Dragonfly Mercury Project

Natural Resource Report NPS/NRSS/ARD/NRR—2022/2386

ON THIS PAGE
Citizen scientists look for dragonfly larvae at Glacier National Park (MT).
NPS Photo

ON THE COVER
Public participants in the Dragonfly Mercury Project investigate the content of nets at Saguaro National Park (AZ); image of dragonfly larva in inset photo.
NPS Photo

Protocol Implementation Plan for Dragonfly Larvae Sampling and Mercury Analysis

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Natural Resource Report NPS/NRSS/ARD/NRR—2022/XXXX

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Abstract

This protocol outlines a framework for monitoring mercury in dragonfly larvae and how data are collected, managed, analyzed, and reported for the Dragonfly Mercury Project (DMP). The DMP is a dynamic national-scale study that engages citizen scientists in the collection of dragonfly larvae for mercury analysis. The DMP aims to link mercury deposition with potential risks to aquatic resources and assess changes in mercury concentrations over time to inform resource management decisions and mitigation tools. The community and citizen science approach engages the public in critical conservation science while reducing monitoring costs by more than half compared to sampling by professional organizations, yet still results in high-quality data to support resource management and environmental protection. The DMP is jointly managed by the National Park Service and U.S. Geological Survey with additional leadership from Appalachian Mountain Club and Dartmouth College. This protocol is adapted from a broader protocol for monitoring mercury in aquatic organisms, developed by the National Park Service Great Lakes Inventory & Monitoring Network.

Acknowledgements

Support for the Dragonfly Mercury Project is provided by the National Park Service (NPS), the U.S. Geological Survey (USGS-NPS Water Quality Partnership, Environmental Health Contaminant Biology and Toxics Substances Hydrology Programs, and Ecosystems Mission Area), and the National Park Foundation. Earlier project support was provided by the University of Maine and Dartmouth Superfund Research Program (Celia Chen). We thank Colleen Emery and staff at the U.S. Geological Survey Contaminant Ecology Research Lab for field, lab, and data management assistance. David Krabbenhoft, USGS Upper Midwest Water Science Center, continues to provide knowledgeable insight. We also value the early contributions of Roger Haro (University of Wisconsin-La Crosse), and support offered by Megan Hess and Amanda Klemmer (University of Maine). Schoodic Institute at Acadia National Park (Hannah Webber) provided educational materials that served as the basis for the experiential opportunities afforded to national parks and citizenry across the country. Further, we are particularly indebted to the numerous dedicated National Park Service staff across the country for their engagement in this effort, and for the thousands of community and citizen scientists that volunteered their time and energy to collect dragonfly larvae for this project. We thank Kat Ko of the National Park Service for coordinating park engagement and assisting with report formatting, Kirsten Gallo (formerly) of the National Park Service Inventory & Monitoring Division for an internal review, and the following external peer reviewers: Krista Bartz, Southwest Alaska I&M Network, and Andrea Blakesley, Denali National Park and Preserve. This protocol was adapted from similar contaminant protocols developed by the NPS Great Lakes Inventory & Monitoring Network. We wish to acknowledge the work of the authors as well as their willingness to share that work.

List of Abbreviations and Acronyms

AMC: Appalachian Mountain Club

ANCOVA: analysis of covariance

ANOVA: analysis of variance

ARD: Air Resources Division

CERL: Contaminant Ecology Research Laboratory

CSDGM: Content Standard for Digital Geospatial Metadata

DF: dragonfly

DMP: Dragonfly Mercury Project

dw: dry weight

FGDC: Federal Geographic Data Committee

FRESC: Forest and Rangeland Ecosystem Science Center

GLKN: Great Lakes Inventory and Monitoring Network

Hg: mercury

I&M: Inventory & Monitoring

IAA: interagency agreement

IAR: Investigator's Annual Report

IMD: Inventory & Monitoring Division

MeHg: methylmercury

NPS: National Park Service

NRSS: Natural Resource Stewardship and Science Directorate

NWIS: National Water Information System

PFD: personal flotation device

PI: principal investigator

PIP: protocol implementation plan

QA/QC: quality assurance/quality control

RPD: Relative Percent Difference

SOP: standard operating procedure

TEL: Trace Element Lab

THg: total mercury

UMaine: University of Maine

USEPA: U.S. Environmental Protection Agency

USGS: U.S. Geological Survey

ww: wet weight

Background and Objectives

Introduction

The Dragonfly Mercury Project (DMP or project, herein) is a dynamic national-scale study that engages community and citizen scientists in monitoring mercury risk to aquatic ecosystems using dragonfly larvae as biosentinels. Mercury (Hg) is a globally distributed element that, when converted to methylmercury, biomagnifies through food webs and is highly toxic to fish and wildlife. For 10 years, the project has linked scientists with resource managers, interpreters, and volunteers to sample more than 500 water bodies in more than 120 U.S. National Park Service (NPS) units spanning 47 states (Figure 1). More than 5,000 public participants contributed to data collection to date, revealing some areas of high or severe Hg risk, highlighting the need for continued monitoring at both established and new locations. Findings show that dragonfly larvae provide an effective model for understanding the distribution of mercury across the nation and determining the important factors of mercury bioaccumulation that can be used to estimate potential ecosystem impairment (Eagles-Smith et al. 2020, 2016; Flanagan Pritz et al. 2020, Flanagan Pritz and Nelson 2017, Nelson et al. 2015, VanderMeulen et al. 2018).

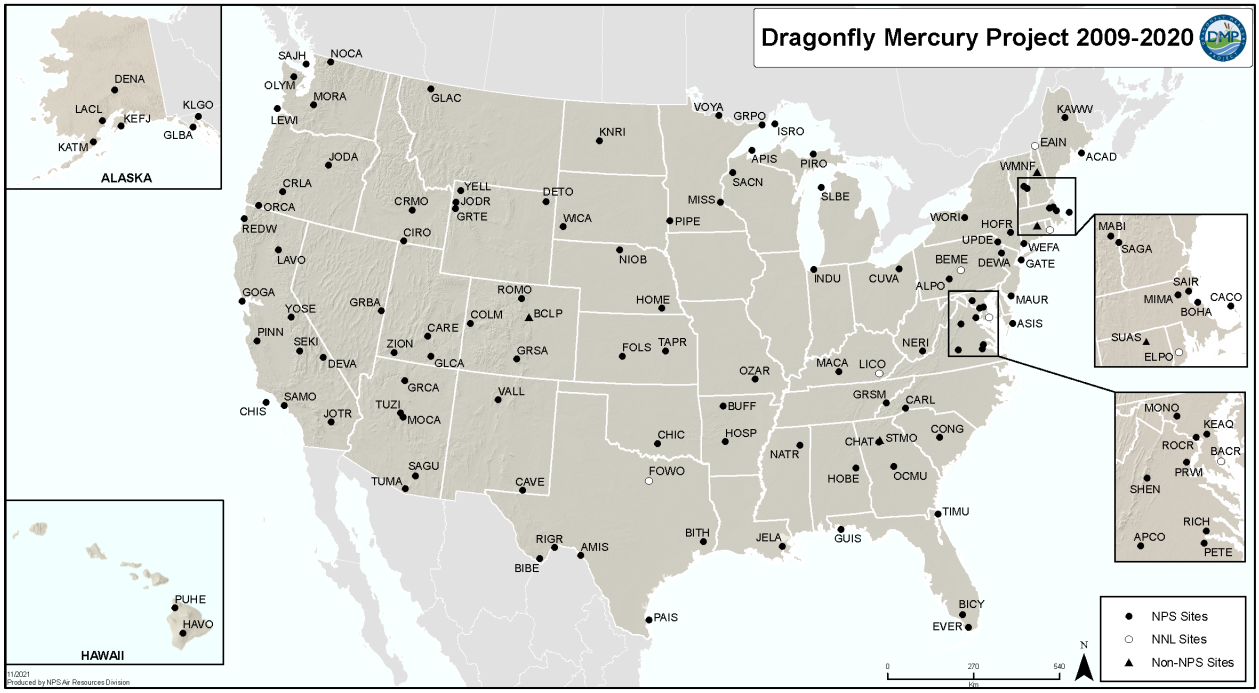

Figure 1. Dragonfly Mercury Project sampling locations across more than 120 U.S. national parks and other protected places, 2009-2020.

The DMP is unlike any other NPS-wide study in that it combines easy to achieve field work with sophisticated laboratory and computational analyses to shed light on the risk of Hg contamination across varied ecosystems of our national parks (Figure 2). In addition to advancing our scientific understanding of Hg risk, informing policy and management decisions, and enhancing protected land surveillance, the project has the potential to create next-generation natural resource stewards.

Participation enables the NPS to foster teachable moments on the management of air, water, and biological resources and connects people to parks and other lands using parks as outdoor classrooms and living laboratories. Community and citizen scientists gain new perspectives and practice civic skills while project scientists gain additional data and insights on Hg in the food web.

As a national-scale monitoring, research, education, and public engagement initiative, the DMP aligns with several central NPS tenets, including:

- The protection of park resources and values, the core mission of the NPS.
- The four pillars of the NPS Natural Resource Stewardship and Science Directorate (NRSS), i.e., holding the line, managing amid continuous change, leveraging for conservation at scale, and enhancing stewardship and science access and engagement (NPS 2016).
- Connecting people to parks and advancing the NPS educational mission, values inherent in the Call to Action issued in advance of the Centennial celebration of the National Park Service in 2016 (NPS 2011).

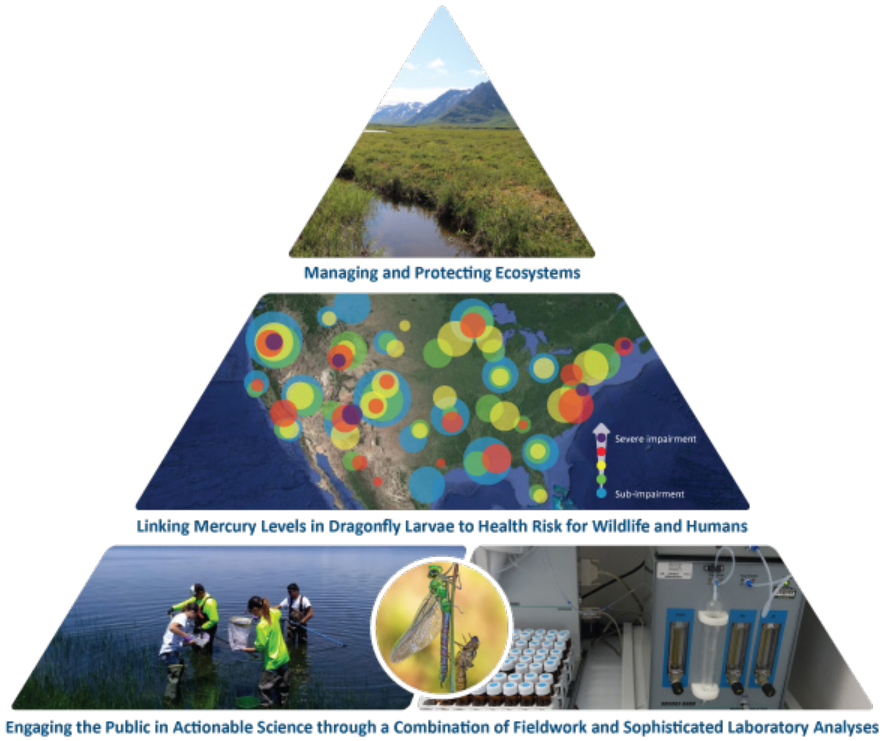

Figure 2. The Dragonfly Mercury Project is rooted in landscape-scale surveillance efforts, within a community and citizen science framework, that has been centered on U.S. national parks to date. Through the collection of dragonfly larvae, the public connects with professional scientists, who analyze mercury in larval samples at the laboratory (bottom level). Dragonfly mercury concentrations, correlated positively with mercury concentrations in a variety of freshwater taxa, provide a proxy for the risk of mercury to human and wildlife health (middle level). This information then serves as the underpinning for actionable science to guide management techniques and mercury risk mitigation options (top level).

Utility of Dragonfly Larvae as Mercury Biosentinels

Dragonfly larvae, the immature form of the familiar flying adult insects, are effective Hg biosentinels (organisms used to gauge pollutant concentrations and inform estimates of risk to human and wildlife health). As predatory invertebrates, dragonfly larvae link Hg concentrations at the base of food webs to those of fish and other predatory taxa (Eagles-Smith et al. 2020). They encompass a geographically widespread distribution across six continents and occupy varied habitat types (Corbet 1999). They are also easy and cost-effective to collect and when analyzed provide high-quality data that reflect Hg exposure in fish and other wildlife.

Early study designs of the DMP sought to determine if dragonfly larvae were appropriate to serve as Hg biosentinels (Nelson et al. 2015). Through that work we found that dragonfly larvae Hg concentrations are well-correlated with those in fish and other aquatic-dependent wildlife (Figure 3), linking dragonflies to both environmental Hg concentrations and potential human exposure pathways (Eagles-Smith et al. 2020). As a result of their utility, dragonfly larvae have been adopted by the NPS Great Lakes Inventory & Monitoring Network (GLKN; VanderMeulen et al. 2018) as the primary means to monitor Hg in nine national parks in the upper Midwest, USA.

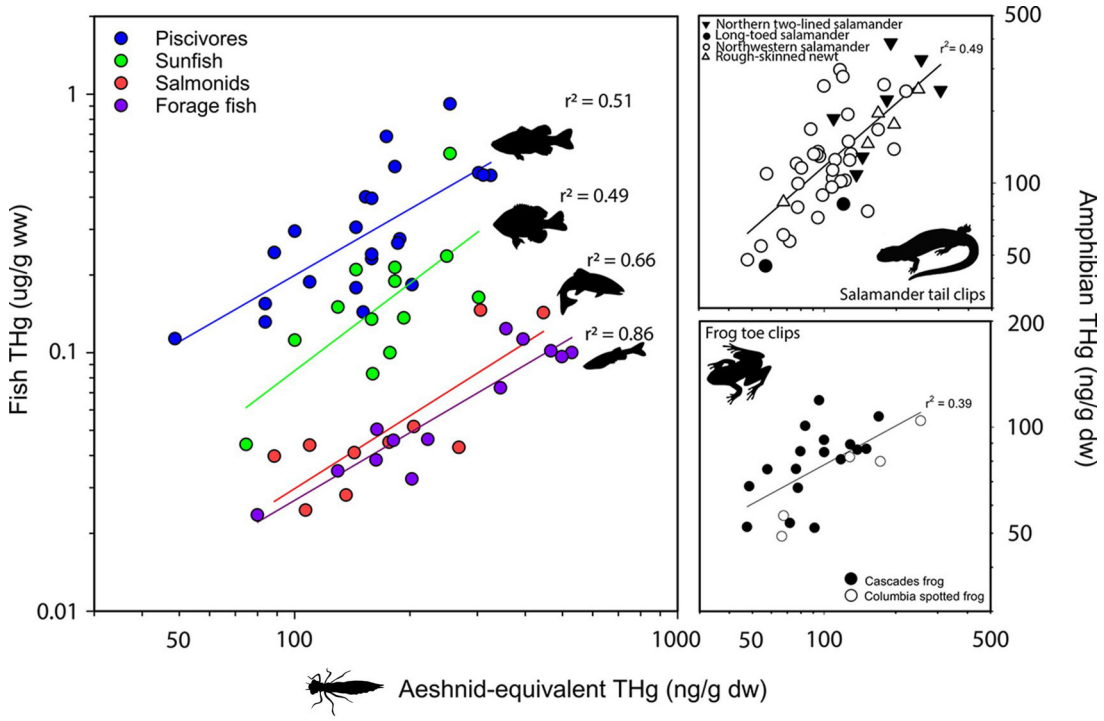

Figure 3. Relationship between Aeshnid-equivalent dragonfly larvae total mercury (THg) concentrations (ng/g dry weight (dw)) and THg concentrations (µg/g wet weight (ww)) in four fish guilds (left panel), salamander tail clips (ng/g dw; top right panel), and frog toe clips (ng/g dw); bottom right panel. Normalized to a consistent family, the Aeshnid-equivalent THg concentration allows for more-representative comparisons across sites and time periods without confounding effects of taxonomy. Each data point represents the paired geometric mean THg concentrations for sites where both dragonfly larvae and either fish or amphibians were sampled. Fish THg concentrations are reported in wet weight to facilitate comparisons to common health benchmarks, whereas dragonfly larvae and amphibian

concentrations are reported on a dry weight basis to reduce variance associated with external moisture. (Eagles-Smith et al. 2020)

State of the Science

The DMP is the most comprehensive assessment of Hg contamination and environmental risk in the nation's protected lands (Eagles-Smith et al. 2020). This foundational work:

- Established a surveillance network for Hg in protected lands across the United States, by development of a robust and effective biomonitoring tool – charismatic mini-fauna (dragonflies).
- Engaged youth and citizenry in meaningful research and experiential learning opportunities that provide community learning and important scientific contributions at an exceptional cost savings to the public in comparison to more common approaches.
- Quantified taxonomic relationships in Hg concentrations among dragonfly families – facilitating development of a consistent reporting unit across the landscape, even when different taxa are sampled.
- Developed an index that informs wildlife and human health risk to Hg based on dragonfly concentrations (Figure 4).
- Determined that risk from Hg differed among habitats, with rivers and streams being higher than lakes and ponds, and that substantially higher Hg was associated with habitats surrounded by wetlands.
- Found evidence that local ecosystem factors confound the direct linkage between atmospheric Hg deposition and patterns of Hg risk to fish and wildlife.
- Found 10 percent of sites were below known impairment benchmarks (low risk); whereas 12 percent of sites posed high or severe risk of impairment of fish, wildlife, and humans (Figure 5).
- Contributes to assessing Hg trends over time and therefore the effectiveness of the Minamata Convention on Mercury, a global treaty to reduce Hg exposure to fish, wildlife, and humans (UNEP 2021).

Implemented under a citizen science framework, the DMP's multi-tiered approach benefits diverse partners and stakeholders—e.g., federal and state agencies, local municipalities, resource managers, and subsistence and recreational consumers of freshwater fish—with different interests and capacities. Educators have benefitted from the "authentic science" approach and applicability of the project to multiple cross-cutting themes (e.g., data literacy, scientific habits of mind, research methods) and disciplinary (e.g., chemistry, biodiversity, environmental science) curricula (Zoellick et al. 2012). Ultimately data from the study help resource managers make informed decisions to safeguard human and wildlife health.

The project is rooted in a community and citizen science framework and utilizes an integrated design, or a "network of networks", where individual groups of resource managers, scientists, and public participants are interconnected (Figure 6). Data integrity is accomplished through iteratively-vetted,

standardized sampling procedures coupled with a centralized laboratory that employs robust quality assurance measures and high-quality data documentation. The community and citizen science approach engages the public in critical conservation science while reducing monitoring costs by more than half compared to sampling by professional organizations, yet still results in high-quality data to support resource management and environmental protection.

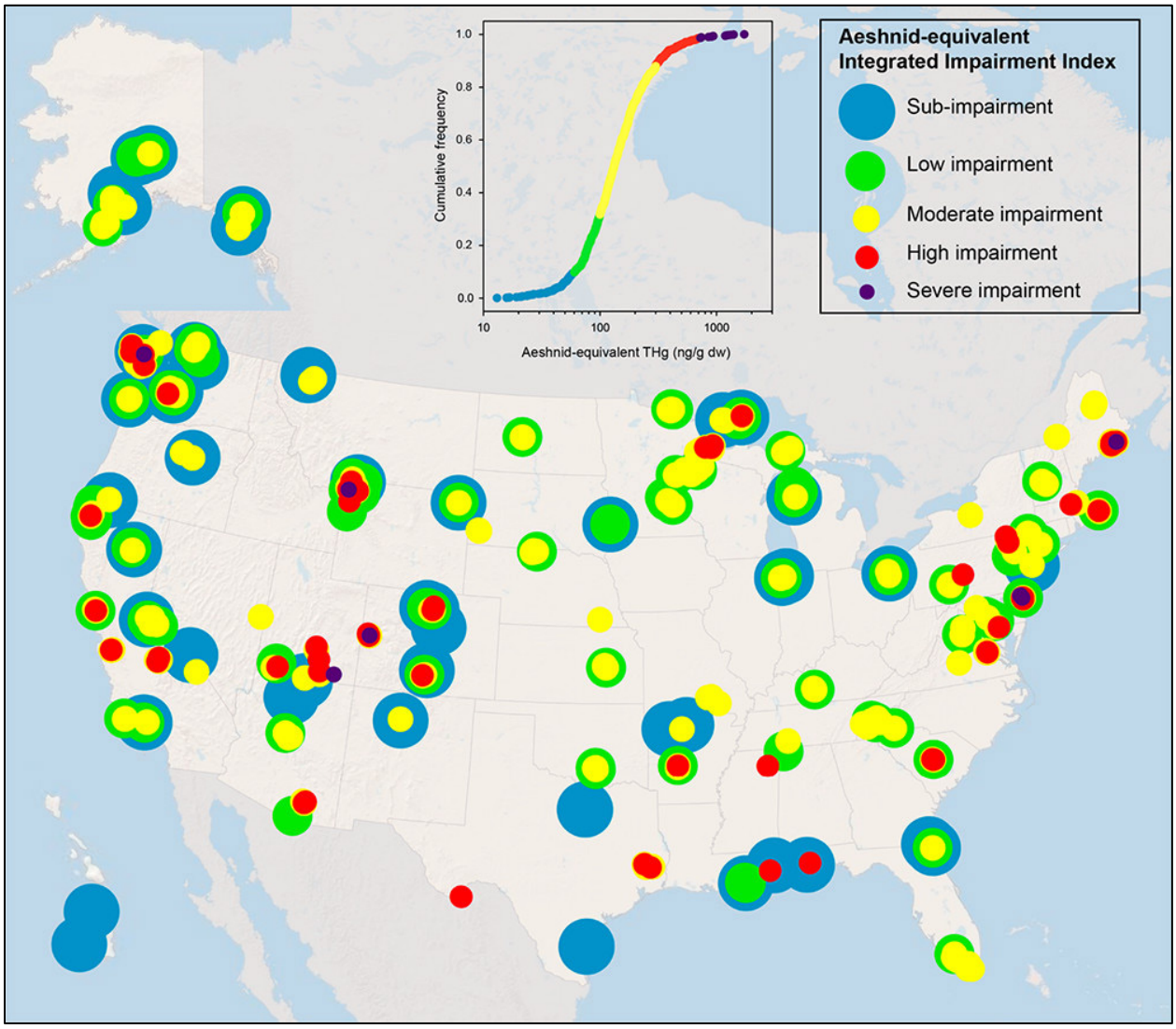

Figure 4. Integrated impairment indices for all 457 sites sampled between 2009 and 2018. Integrated impairment indices are derived from Aeshnid-equivalent geometric mean total mercury (THg) concentrations (ng/g dry weight (dw)) for each site-year and their corresponding association with mercury exposure in fish and other wildlife. The inset for the cumulative frequency distribution illustrates the proportion of sites and years that fall into each of the five categories. (Eagles-Smith et al. 2020)

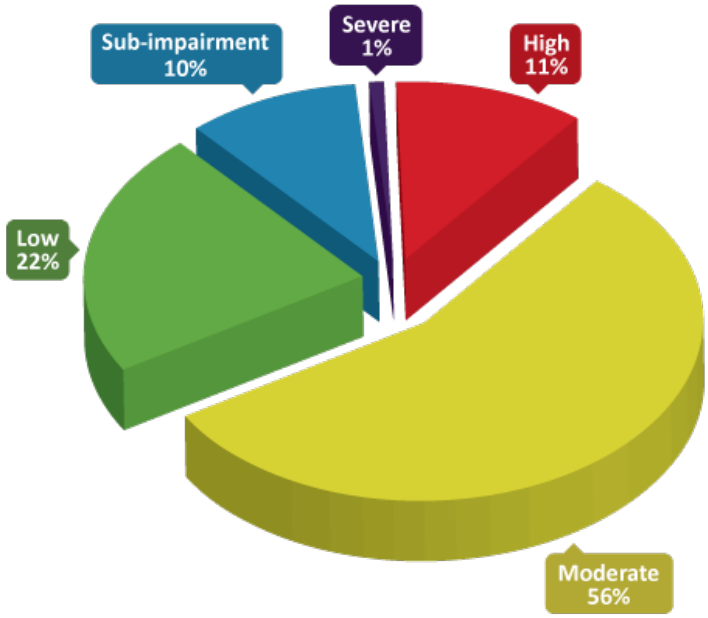

Figure 5. Percentage of all 457 sites sampled between 2009 and 2018 with dragonfly mercury concentrations that correspond with each Integrated Impairment Index risk category.

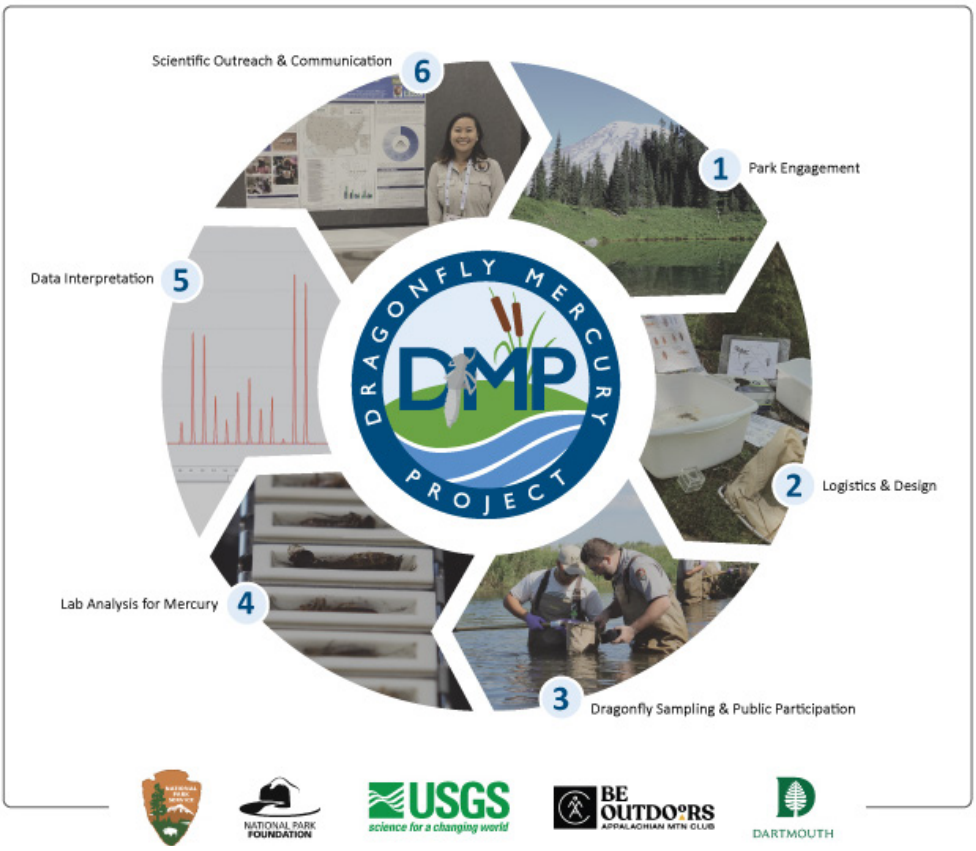

Figure 6. Process and information flow pathways of the Dragonfly Mercury Project. Each step communicates directly with all the others to produce robust data products for science applications, resource management, and community education.

This structure is scalable and adaptable to local circumstances. The train-the-trainer model allows local resource managers and educators to oversee sampling and provisioning of training materials. These staff liaisons work collaboratively with project scientists to select water bodies relevant to local issues, provide guidance on sampling and sample handling protocols, and as appropriate, invest additional time supplementing citizen science field sampling with educational programs.

Public Participation

The DMP began in 2009 as an inquiry-based education program for high school students in Maine. Teachers partnered with project researchers from the University of Maine and Schoodic Institute to develop the field protocols, collect pilot data, expand the project into two national parks in Vermont and New Hampshire, and importantly, develop educational materials that were designed to scaffold student understanding about laboratory and field research, data literacy (Webber et al. 2014), numeracy, scientific habits of mind, and the process of science. This work resulted in a new framework for participatory science and participatory education that defined how citizen science can operate effectively in formal science education (Zoellick et al. 2012).

In 2013, the DMP shifted from education research with Schoodic Institute to scientific investigation designed to understand more about Hg at a national scale, using national parks as field sites. Concomitantly, the project's citizen science engagement shifted from a co-created project to a collaborative project (as core park participants refined protocols and engagement), to its current mode as a primarily contributory citizen science program. (Modes of engagement defined in Shirk et al. [2012] and Watkins et al. [2018]). Nonetheless, the DMP is a model of highly-engaged citizen science and in annual feedback sessions, park leads report deep engagement by students and citizens, and teachable moments that transcend traditional in-classroom activities.

Goals and Objectives

This protocol implementation plan (PIP) provides a framework and description of the field sampling, laboratory processing and analysis, and data evaluation behind the DMP. The protocol is comprised of a narrative (this document), supporting standard operating procedures (SOPs), and USGS Contaminant Ecology Research Laboratory (CERL) data quality and data management documents, or Quality Assurance Project Plan (QAPP; Eagles-Smith et al. 2018). The protocol narrative gives the history and justification for doing the work, outlines the design used to accomplish the stated objectives, and summarizes sampling methods, data management, and analysis methods, but does not provide all methodological details. The QAPP (Eagles-Smith et al. 2018) provides details on the quality assurance/quality control considerations as they relate to both fieldwork and laboratory procedures and performance requirements. Together, these documents provide a framework for obtaining data that will offer park managers and planners with information on the spatial patterns, temporal trends, and potential ecotoxicological significance of mercury contamination of dragonfly larvae. The sampling and analysis of dragonfly larvae, as outlined in these documents, will address the following specific objectives:

Objective 1: Quantify the extent of potential Hg impairment to parks. Identify parks and surface waters where Hg concentrations could pose a risk to fish, fish-eating wildlife, and humans.

Objective 2: Assess spatial patterns in Hg contamination of dragonfly larvae.

Objective 3: Evaluate temporal trends in Hg contamination of dragonfly larvae and explore linkages to other Hg monitoring efforts (e.g., Hg trends in atmospheric deposition, surface waters, other biota, etc.).

Sources and Toxicity of Mercury

Mercury is a globally distributed contaminant released via anthropogenic and natural sources (Obrist et al. 2018). Atmospheric transport delivers Hg to watersheds and aquatic ecosystems. Mercury is responsible for 80% of all fish consumption advisories in the U.S., with advisories in all 50 states. Even the most remote and minimally impacted ecosystems, such as many NPS lands, are threatened by Hg because it is highly toxic and biomagnifies through food webs, affecting animal and human health at environmentally relevant concentrations (Eagles-Smith et al. 2014). Consumption advisories issued for Hg can reduce visitor experience, impact recreational fishing, and threaten Tribal communities dependent on subsistence fishing.

Although there are natural sources of Hg in the environment, fossil fuel combustion currently represents about 50 percent of Hg emissions related to human activities and has resulted in at least a 3-fold increase in atmospheric Hg concentration over the past 150 years. Once released to the atmosphere, Hg can travel long distances before being deposited on the Earth's surface. Deposited Hg that enters waterways can be converted to a more toxic and bioavailable form, methylmercury (MeHg), by certain bacteria when biogeochemical conditions in the environment facilitate their activity (Eagles-Smith et al. 2018). This capacity for Hg to distribute widely, coupled with its propensity to be methylated, makes it a serious concern for protected areas such as national parks, even those that are remote and seemingly without local sources of Hg pollution. As a result, Hg contamination is widespread throughout national parks in the United States, affecting fish and wildlife, as well as posing potential risks for humans consuming fish (Eagles-Smith et al. 2014).

The severity of health risks posed by Hg has prompted efforts to reduce environmental Hg releases and human exposure. However, Hg cycling is complex (Figure 7), and Hg bioaccumulation is often decoupled from environmental Hg contamination (Eagles-Smith et al. 2016), meaning that source reductions alone may not consistently reduce Hg risk. This stems from Hg interactions with ecological processes in the environment such that inorganic Hg (the form that transports globally) requires microbial conversion to MeHg to become bioavailable and toxic. This occurs in specific environmental conditions that support the microbes. Thus, where environmental conditions favor MeHg production (e.g., relatively high dissolved organic carbon (DOC), low pH, higher sulfate, anaerobic water, wetting and drying of sediment), animals can have elevated MeHg accumulation even if there is relatively little inorganic Hg contamination. The opposite can also be true where Hg loading is high. The distribution of Hg in aquatic food webs is widespread, but its disconnect from inorganic Hg contamination complicates predicting the wildlife and human health exposure risks of Hg directly from atmospheric or watershed loading estimates alone. Because of this disconnect between environmental loadings and Hg risk, biosentinel-based monitoring and exposure risk modeling are more effective at predicting Hg risk than monitoring of Hg sources or environmental concentrations.

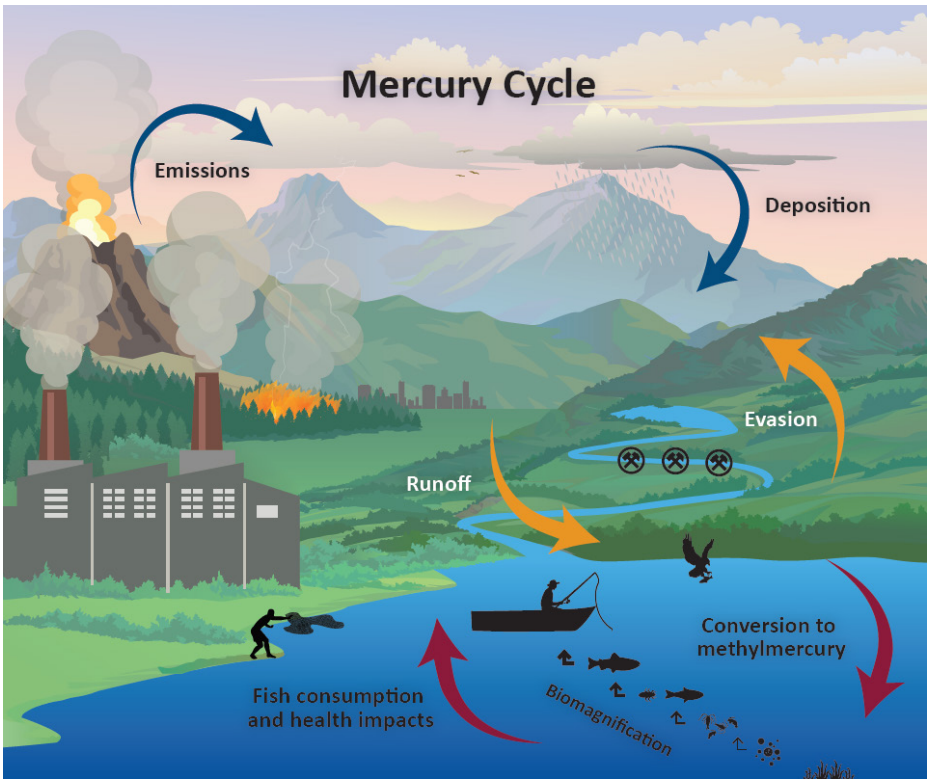

Figure 7. A simplified mercury cycle showing how mercury enters and cycles through ecosystems, bioaccumulates in fish and wildlife, and biomagnifies up the trophic levels of a food web. Evasion occurs when gaseous mercury in water surfaces is re-emitted to the atmosphere. Mining symbols along the river corridor imply a potential source of mercury from historic or current mining practices.

Monitoring and Assessment Questions

The following questions emanate from the monitoring and assessment objectives listed above, and from questions frequently raised in DMP Science Team and Steering Committee meetings:

1. What is the extent of the Hg impairment to parks? Which park units and surface waters have concentrations of Hg in dragonfly larvae high enough to pose potential risks to humans and upper trophic-level wildlife?
2. What are the spatial patterns in Hg contamination in dragonfly larvae within parks?
3. What is the general direction and magnitude of change in the concentration of Hg in dragonfly larvae in parks?

This strategy underscores an ongoing and expanded data collection effort that offers partnering opportunities with other entities to address the Hg risks to protected lands. These opportunities may include targeted assessments of areas where risk estimates are elevated. Likewise, data results can inform potential mitigation strategies and improve communications to park visitors and partners about the threats posed by Hg.

Monitoring and Assessment Strategy

This protocol provides a framework for monitoring and assessing spatial patterns and temporal trends in dragonfly Hg concentrations and ecological risk from Hg. With this protocol we are building upon a compilation of Hg data in larval dragonflies that began in 2009 and is ongoing (Eagles-Smith et al. 2020, Nelson et al. 2015).

This protocol contains three components: (1) risk determination and modeling, (2) spatial analysis, and (3) temporal/trend analysis, each of which inform and are informed by process-based research (Figure 8). The spatial and temporal analyses, which address Objectives 2-3, will assess spatial patterns in Hg in larval dragonflies to identify parks and specific surface waters where exposure may pose a health risk to fish, wildlife, and humans. The temporal/trend analysis will examine temporal patterns in Hg among parks and individual water bodies to identify potential responses in dragonfly Hg to local, regional, and global mitigation efforts. The risk determination, which addresses Objective 1, will evaluate the factors associated with variation in dragonfly larvae Hg concentrations. These factors include Hg sources and deposition, water chemistry and biogeochemical parameters, as well as landscape structure and land cover.

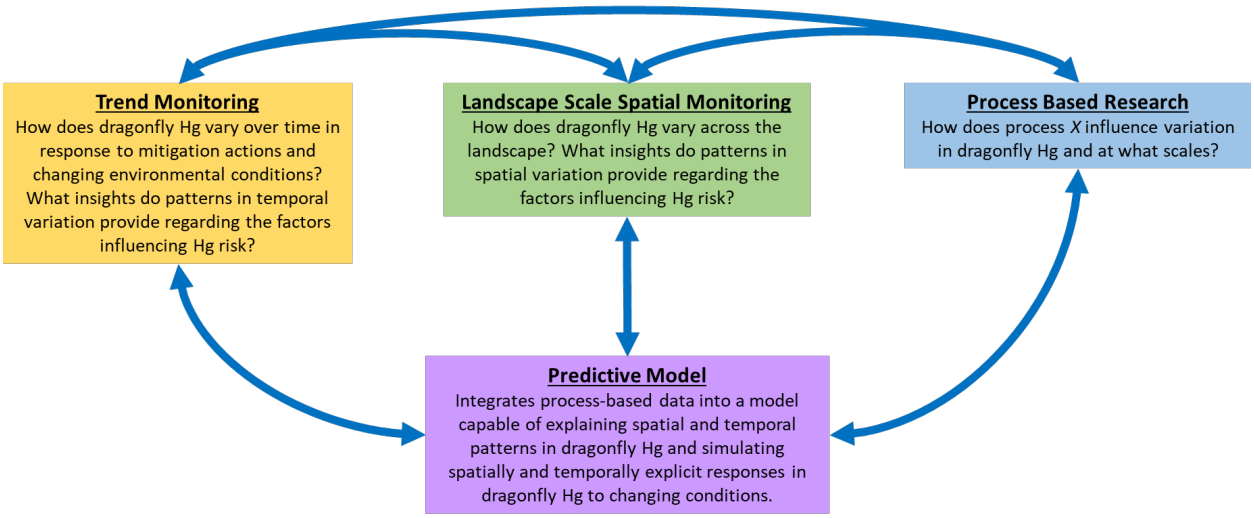

Figure 8. Conceptual model depicting the interactions between monitoring, process-based research, and model development and the ways each component informs the others within the framework of the Dragonfly Mercury Project, feeding into predictions of dragonfly mercury (Hg) and potential risk.

Sampling Schedule

Because of the national scope and citizen science framework of this project, sampling of dragonfly larvae is flexible and variable among regions across the U.S. However, most sampling has historically occurred between May and September, with few parks sampling outside of those months (Eagles-Smith et al. 2020).

Number and Location of Sampling Sites

The selection of aquatic sites to include in this mercury monitoring protocol must be based on the protocol objectives without jeopardizing the safety of field personnel and community volunteers. Monitoring all surface water resources is not logistically or financially feasible. Answering questions about surface water resources within a park or across the NPS requires either a complete census of aquatic sites or a random selection of aquatic sites, which allows inference to the population of aquatic sites as a whole. A complete census of aquatic sites is not feasible, and a solely random selection of aquatic sites is not desirable because many are inaccessible, complicating sample integrity and community science activities associated with this work. The design takes these limitations into account by using a hybrid design of fixed sites distributed across regions for trend monitoring, along with new and rotating sites that are selected based on funding availability and representative aquatic habitat types. This approach will support development, validation, calibration, and maintenance of a predictive model for estimating dragonfly Hg concentrations based on land cover, water chemistry, and other environmental parameters (Figure 9).

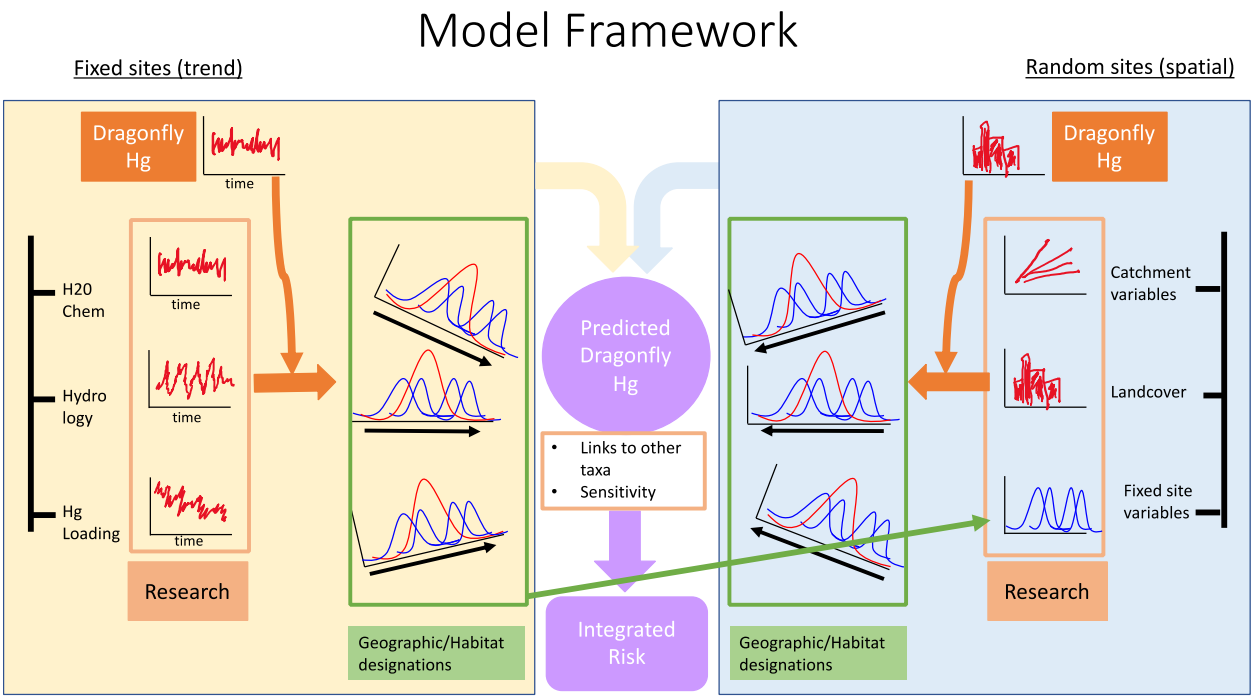

Figure 9. Predictive dragonfly Hg model framework, illustrating how the modeling framework is dynamically integrated with existing and future monitoring and research activities.

The proposed modeling framework is built around integrating existing long-term DMP monitoring (fixed sites) with short-term expanded data collection from randomly-distributed sites within each regional construct. Doing so will ensure that the data feeding the model capture as much variation in both Hg exposure and predictive variables as possible.

Considering budget and logistical constraints, we will target sampling at three fixed sites from each Inventory and Monitoring (I&M) network annually. In cases where multiple parks prefer hosting

fixed sites, we may expand beyond three per network. To the extent possible, each site within a network will represent a different aquatic habitat type (e.g., lake, pond, stream, river, wetland, etc.). Site selection will be based on past data availability, site accessibility, and overlap with other water quality monitoring activities. In addition to the fixed monitoring locations, an additional three or more sites per I&M network will be sampled annually and selected through a stratified random design that accounts for accessibility and habitat type. The goal for these sites is to capture the full extent of the distribution in the biogeochemical, landscape, and hydrologic parameters that control Hg cycling. This will provide information on the extent of Hg risk across parks and networks, as well as build a robust dataset to facilitate risk model development and maintenance. It will also enable managers to respond quickly with increased monitoring and/or health advisories if areas of particularly elevated Hg are discovered. From one year to the next, the selection of the variable sites will largely be at the discretion of park natural resource managers (in collaboration with DMP staff), who will take into account site selection factors previously described and other management considerations such as level of recreational fishing, desire to monitor Hg in fishless surface waters, etc. Efforts will be made to maximize spatial overlap between dragonfly Hg monitoring and other aquatic monitoring by NPS staff to reduce logistical obstacles to sampling and increase overlap where other water quality and habitat assessments are conducted.

Sample Size and Level of Change That Can Be Detected

Analysis of 15 larval dragonflies from a given water body and year will conservatively allow for an 80% probability of detecting a 20% change in mean Hg concentration over 10 years with a Type I error of 0.05 (VanderMeulen et al. 2018). This is based on power analyses by Nelson et al. (2015) and a robust assessment of dragonfly Hg data across more than 120 NPS units (Eagles-Smith et al. 2020). Additionally, Wiener et al. (2009) proposed that analyses of 15 to 25 biosentinel organisms per species of prey fish or large larval dragonflies from a given water body annually would provide a sufficient sample size for a defensible statistical evaluation of either spatial or temporal patterns in Hg concentration. Last, Haro et al. (2013) found that a sample size of ten individually analyzed Gomphid dragonfly larvae from each water body would be sufficient to detect a 20% difference in mean total Hg (THg) concentrations with a Type 1 error of 0.05 across 17 out of the 26 water bodies included in the power analysis using the same dataset.

Sampling Design, Monitoring Schedule, and Objectives

Sampling will consist of a combination of fixed and variable sites across all participating I&M networks. Considering budget and logistical constraints, we will sample three fixed sites from each I&M network annually. With participation by all 32 I&M networks we will sample at least 96 fixed sites. Efforts will be made to maximize spatial overlap between dragonfly Hg monitoring and other aquatic monitoring by NPS staff to reduce logistical obstacles to sampling and leverage other water quality and habitat assessments.

Guidelines for Assessing Ecological and Health Risks of Mercury

This monitoring effort is designed to document spatial and temporal patterns in concentrations of Hg in selected biosentinel organisms, providing an indicator of Hg contamination of aquatic food webs—the primary pathway for exposure of fish and wildlife. This protocol will not directly examine

effects of MeHg exposure on wildlife or humans in the parks. However, we will compare the concentrations of Hg in biosentinel organisms to estimated benchmark values in published field and laboratory studies (below) in which causal relations have been documented. Unlike Hg in sport fish, Hg in larval dragonflies has not yet been related to human or wildlife health. However, Haro et al. (2013) found that THg and MeHg in larval dragonflies could be used to predict THg in both prey and predatory fish (R^2=0.70, p=0.0001; R^2=0.63, p=0.002; using total and MeHg in *Gomphus* spp. from 13 lakes, respectively). Additionally, Eagles-Smith et al. (2020) found that dragonfly larvae total Hg concentrations were correlated with total Hg concentrations in four fish guilds and multiple salamander and frog species. Using these relationships, an Integrated Impairment Risk categorization was developed that considered the number and severity of health benchmarks exceeded when dragonfly larvae had various THg concentrations (Table 1, Figure 10). As such, sampling according to this protocol will allow assessment of the potential adverse ecological effects of Hg using dietary threshold or benchmark concentrations, above which MeHg causes ecologically significant adverse effects in piscivorous fish and wildlife. Consumption of prey fish by piscivorous fish and wildlife is the dominant pathway for MeHg uptake and exposure (e.g., Depew et al. 2012a, 2012b, 2013).

Table 1. Aeshnid-equivalent THg concentrations categorized into Integrated Impairment Indices.

Concentration range ng Hg/g tissue dry weight	**Benchmark Category**
<60 ng/g	Sub-impairment
60-100 ng/g	Low hazard impairment index
100-300 ng/g	Moderate hazard impairment index
300-700 ng/g	High hazard impairment index
>700 ng/g	Severe hazard impairment index

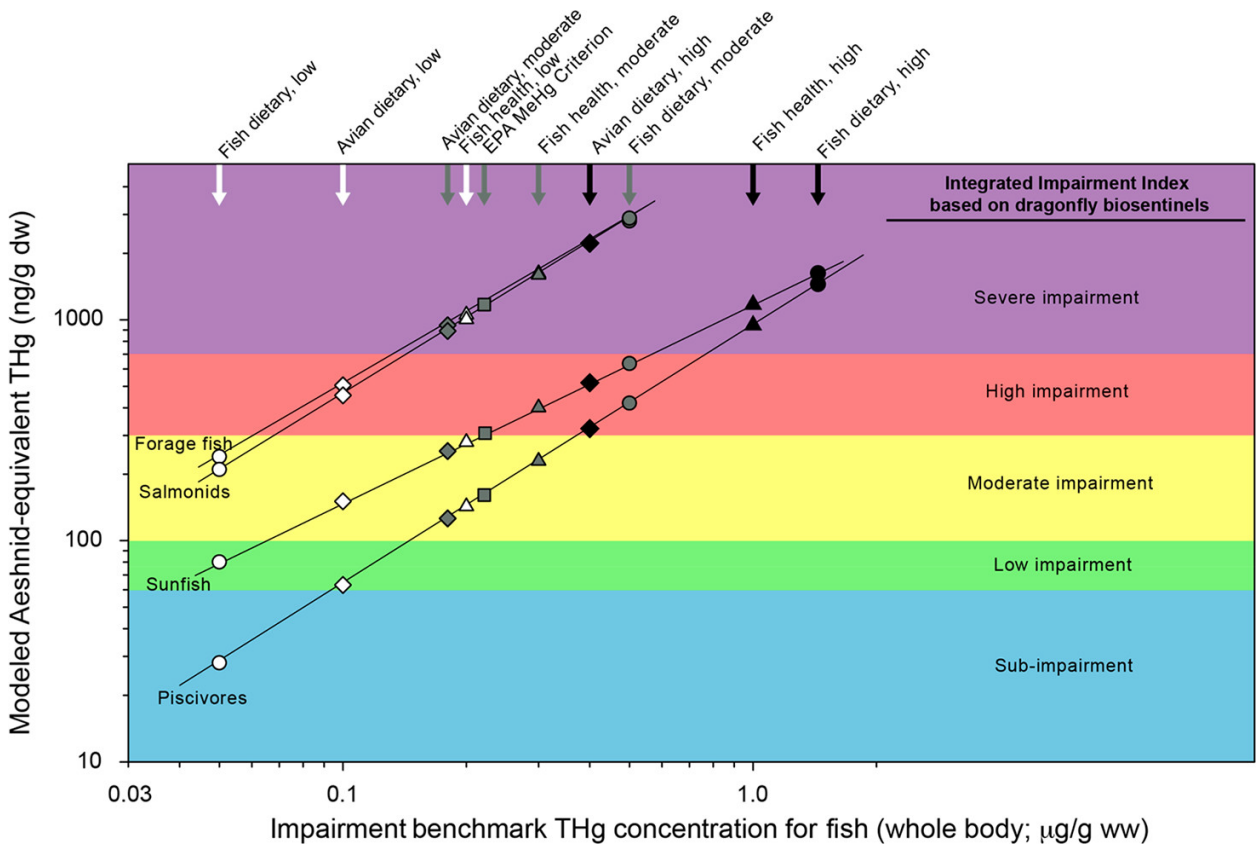

Figure 10. Integrated impairment indices for potential ecosystem risk to mercury, in Aeshnid-equivalent units (ng/g dry weight total mercury (THg)). Aeshnid-equivalent concentrations normalize across taxonomic families using relationships established in Eagles-Smith et al. (2020). Integrated impairment index categories (specified by different colored boxes) represent concentrations corresponding to exceedances of a range of individual published toxicity benchmarks across several fish guilds (piscivores, sunfish, salmonids, and forage fish). Within each of these guilds, the potential impairment was assessed based on (1) fish diet (i.e., risk to other fish as prey), (2) fish tissue (labeled as fish health; i.e., risk to fish themselves based on their tissue concentrations), and (3) avian diet (i.e., risk to fish-eating birds as prey), with three possible levels of severity (low, moderate, and high as white, grey, and black arrows, respectively) for each of these benchmark types. Potential human risk is incorporated through the U.S. Environmental Protection Agency (USEPA) methylmercury criterion for the protection of human health, which was converted to a whole-body equivalent. The integrated impairment indices are defined by the number and severity of benchmarks exceeded as well as the number of guilds exceeding them. The symbols and lines represent the modeled Aeshnid-equivalent THg concentrations associated with the THg concentrations of each impairment benchmark for each fish guild. Circles represent fish dietary benchmarks, triangles represent fish health benchmarks, diamonds represent avian dietary benchmarks, and squares represent USEPA MeHg criterion. White, gray, and black symbols reflect low, moderate, and high-severity benchmarks, respectively. (Eagles-Smith et al. 2020).

What and How: Overview of Dragonfly Larvae Sampling and Analytical Methods

The citizen science framework around which the DMP was built integrates a collaborative team of scientists, resource managers, and engaged citizens and community partners (go.nps.gov/dragonflymercury). Each year, the science team works with resource managers to identify parks and sites that represent a broad distribution of habitats and ecoregions. The team then engages natural resource staff at each park unit and community partners for training in appropriate sampling and data collection protocols. Interested citizen groups are organized to conduct the sampling with professional oversight.

Pre-Season Preparations

DMP field personnel and NPS staff must prepare in advance of the field season to ensure sampling can be undertaken in parks according to schedule. The field season for collecting larval dragonflies is generally from May through October, though the sampling season for desert parks often runs earlier (March through May) and the sampling season for tropical parks can be later (November through January).

Field preparations should begin four months in advance of the field season to allow enough time for coordinating logistics and ordering supplies and equipment, if necessary. Preparations for field work include communication between the DMP Team and participating NPS units to arrange logistics and solidify site selection, acquisition of needed scientific collector's permits, procurement of supplies, recruitment of personnel for field crews, and the coordination of sampling schedules and logistics among participating personnel (volunteers and/or academics, NPS staff). Pre-season preparations for sampling at the parks, including checklists of gear, logistical arrangements, travel preparations, and equipment lists, are described in detail in SOP #1, Pre-Season Preparation. Further, SOP #1 describes the tasks undertaken by USGS and NPS Project Managers and Assistant Project Managers pertaining to the pre-season coordination and management of the DMP. Duties include participating in quarterly core team and science team calls, recruiting new parks, coordinating sampling logistics, and assembling and shipping sampling supplies and kits. Preparations concerning emergency contacts and safety procedures during sampling are described in SOP #2, Training and Safety.

Collection and Handling of Samples in the Field

Larval dragonflies will be collected from open-water benthic substrates (i.e., sand, silt, gravel, and cobble) and from moderately vegetated littoral or wetland habitats using long-handled, coarse-mesh nets. Once captured, all dragonflies will be placed in containers with site water and separated from detritus and debris. Sampling groups will then utilize identification guides (SOP #3, Fieldwork – Dragonfly Larvae) and field keys (SOP #3) to determine taxonomic family. Once identified, the total length of each larva is recorded to the nearest mm using a plastic ruler. Dragonfly larvae are placed in zip-seal bags and kept cool on wet or dry ice while in the field and during transport to the field laboratory. There, samples are stored frozen and ultimately shipped to the U.S. Geological Survey Contaminant Ecology Research Laboratory (CERL) in Corvallis, OR. Detailed methods for

collection and handling of larval dragonflies using clean procedures to minimize contamination during sampling trips are provided in SOP #3.

Sampling should target larger larvae (>15 mm) and be constrained to either a single family or a mixture of families as long as at least three individuals of each family are collected. Larval dragonflies will typically be collected from May through September, in some instances simultaneous with other aquatic-based monitoring (e.g., water quality monitoring). If necessary or as ecoregional climatic conditions allow, collections can occur earlier in the spring or into the fall.

Site observations are noted on field data collection sheets and must include accurate geospatial coordinates. iNaturalist can also be used to submit photos and observations of organisms (SOP #3).

Preparation and Analysis of Samples in an Analytical Laboratory

All larval dragonfly samples will be shipped as described in SOP #4, Shipping, to the USGS Contaminant Ecology Research Laboratory (CERL; Corvallis, OR) for Hg determination. All taxonomic identifications and length measurements will be verified, validated, and if needed, corrected by professional laboratory staff. In the laboratory, each dragonfly larva will be identified to family, measured, weighed, and dried in preparation for Hg analysis.

Total Hg concentrations will be determined following USEPA method 7473 (U.S. Environmental Protection Agency 2000). Additionally, a subset of samples will be analyzed for MeHg concentrations following USEPA method 1630 (U.S. Environmental Protection Agency 2001).

Quality Assurance and Quality Control for Mercury Determinations

The DMP utilizes in depth QA/QC protocols as described in SOP #5, Data Entry and Management, SOP #6, Data Analysis, and the Quality Assurance Project Plan (Eagles-Smith et al. 2018).

Data Management, Analysis, and Reporting

Overview of Database Design

The USGS CERL developed and maintains a database specifically for the DMP that is readily compatible with several other federal databases, including the USEPA STORET database and USGS National Water Information System (NWIS).

The master copy of the CERL database is hosted on a USGS server in Corvallis, OR, and serves as the only primary data source for exporting data to other locations. Additionally, the final authoritative host location of data will be publicly available on ScienceBase (www.sciencebase.gov).

Metadata Procedures

Metadata allow potential data users to evaluate the quality and usefulness of the data based on an understanding of the complete process under which they were collected and maintained. All metadata will be hosted with the public data release on ScienceBase.

For metadata associated with geospatial data, we will abide by Executive Order 12906, which mandates that every federal agency document all new geospatial data it collects or produces using the Federal Geographic Data Committee (FGDC) Content Standard for Digital Geospatial Metadata (CSDGM; https://www.fgdc.gov/metadata/csdgm-standard). All GIS data layers will be documented with applicable FGDC metadata standards.

Data Entry, Verification, and Editing

Instructions for the data entry procedures for this protocol are given in SOP #5, Data Entry and Management. As described above, three general classes of data are collected. The first is field observations and measurements that are recorded on data sheets in the field. These field sheets will be entered into a digital file. An example of a field sheet is shown in SOP #3. The second class of data is the laboratory processing in preparation for chemical analysis. The third class of data is the chemical analysis of dragonfly larvae for THg (and MeHg) concentrations.

Data verification starts with the QA/QC steps that are outlined in the SOPs associated with this protocol. Data that do not pass QA/QC requirements are flagged and prevented from entry into the CERL database until corrected. Data that are outside the expected range for a parameter based on previous records for that parameter will be flagged for further review.

Quality assurance/quality control checks are performed prior to entry into the CERL database. The DMP's data records are regarded as being in provisional status until they undergo USGS Fundamental Science Practices approval and are released to ScienceBase. At that time, the dataset is officially considered certified and can only be modified by the USGS Project Chief and republished with full documentation of modifications to protect the integrity of the data and allow the history of each data record to be traced.

Data Archival Procedures

Data archiving serves two primary functions: it provides a source to retrieve a copy of any dataset when the primary dataset is lost or destroyed, and it provides a data record that is an essential part of

the QA/QC process. The unedited files are the original data for digital data. The archive of the printed data forms for this protocol is described in SOP #5, Data Entry and Management.

USGS CERL will create duplicate files of all digital data at the earliest opportunity. At least two complete copies of any dataset are required, including digital replicas (scanned versions) of hard copy data sheets. Digital field data that are entered directly into a field computer will be backed up to a second medium at the earliest possibility. The data files on field computers must not be erased until the integrity of these data files is verified on the duplicate storage medium.

The USGS CERL's master database is maintained on a central server in Corvallis, OR, that is backed up daily and backed up off-site weekly.

Quality Assurance and Quality Control for Data Management

Quality assurance and quality control procedures are crucial during every step of data entry and data management. Details of such QA/QC regarding data management are provided in SOP #5, Data Entry and Management, and are summarized in Table 2.

Table 2. Summary of QA/QC procedures pertaining to data management.

Procedure	Description
Instrument calibration logs	Each instrument must have a calibration log.
Field and lab forms	Field and lab forms are the only written record of field measurements, so copies are placed in project binders and originals (hardcopy and electronic) must be kept on file indefinitely.
Estimating precision	The precision measurement is calculated using the Relative Percent Difference (RPD) between duplicate sample results per analyte. Precision estimates should be performed by the analytical laboratory as part of their routine QA/QC procedures.
Data entry	Approximately 10% of electronically transferred data should be spot-checked on a random basis for errors. If errors are found, another 10% are spot-checked. 100% of manually entered data will be checked by a second person.
Data archiving	Program sampling data and associated records are archived in boxes and stored at the USGS CERL Corvallis office.
Data validation	Data validation is the process that determines whether data collection quality control objectives were met.
Data validation reports	Data validation reports provide a narrative that discusses any deviations from QA/QC procedures and the impacts of those deviations.

Table 2 (continued). Summary of QA/QC procedures pertaining to data management.

Procedure	Description
Data verification	Data verification demonstrates that a dataset will qualify as credible data.
Data certification	Data certification demonstrates that 1) data are complete for the period of record; 2) they have undergone and passed the quality assurance checks; and 3) they are appropriately documented and in a condition for archiving, posting, and distribution as appropriate.
Data verification reports	Data verification reports document the results of the data verification procedure.
Data qualification codes	Data must be fully qualified before uploading to the USGS CERL database.

Routine Data Summaries

After initial QA/QC procedures have been completed, the analytical results should be summarized annually for each park unit sampled and for the DMP in its entirety, and scanned for outliers. Descriptive statistics for Hg concentration should include geometric mean, median, maximum and minimum values, and measures of variability (e.g., coefficient of variation, standard error, or variance). Periodically (e.g., about every five years) data should be subjected to parametric and nonparametric statistical analyses, as appropriate, to test hypotheses about relationships between Hg concentrations and spatial, temporal, environmental, and human factors (e.g., Gilbert 1987).

Data summaries should also examine the proportion of measurements that exceed defined concentration benchmarks of concern. Geographic variation in concentrations of contaminants should be displayed cartographically to facilitate comparison among sampling sites and to highlight locations where concentrations in biosentinel organisms exceed levels of concern.

Data Analyses

Mercury concentrations in dragonfly larvae will be compared to benchmarks used to assess risks of MeHg to fish, wildlife, and humans described in Figure 10, and in SOP #6, Data Analysis.

Similar to other studies of Hg in larval dragonflies (e.g., Nelson et al. 2015) where data are left skewed, log-transformation will likely be necessary in order to meet the assumption of normally distributed data in parametric statistical methods. Within a given population, concentrations of some contaminants, such as Hg, typically increase with increasing body size or age. Consequently, information on taxa, length, weight, or age should be incorporated as a covariate into statistical analyses when examining spatial and temporal patterns in Hg concentration in larval dragonflies and fish (Tremblay et al. 1998). Simple correlation analysis such as Pearson's will be used to determine significant bivariate correlations in the dataset (e.g., total Hg concentration and body length for larval dragonflies).

We will use one-way analysis of variance (ANOVA) to test the null hypothesis of equality of mean concentrations of Hg among study sites (spatial analysis) or among years (trend analysis) within a study site. When appropriate, we will 1) include historical data collected through similar methodology as described in this protocol, and 2) utilize analysis of covariance (ANCOVA) for combined analysis across sizes/ages/family/species. For significant ANOVA, multiple comparisons among means will be made with Tukey's hsd test, which has an experiment wise error rate of 0.05. A Type I error (α) of 0.05 will be used to judge the significance of statistical tests, which meets and exceeds *a priori* design objectives.

Linear mixed effects analysis can also be used to assess differences among larval dragonfly guilds, where guilds are grouped by family (burrowers—Gomphidae and Cordulegastridae; sprawlers—Corduliidae, Libellulidae, and Macromiidae; claspers—Aeshnidae) and controlling for sites as nested within parks. Body length can also be included in mixed-effects or other multivariate models. Water body (site) will be included as a random effect in statistical models when analyzing broad spatiotemporal trends.

If ancillary data such as water chemistry for study sites are sufficient, we will use information-theoretic modeling (Akaike Information Criteria) to construct linear models with predicted variables pertaining to Hg in biosentinel organisms and predictor variables pertaining to ecosystem factors and other variables that can influence the production and abundance of MeHg (Anderson et al. 2001). In accordance with the information-theoretic approach, we will apply judgment based on the state of scientific understanding of factors and processes controlling the abundance of Hg in selecting predictor variables (e.g., Wiener et al. 2006). Additional information on data analysis is in SOP #6.

Reporting Schedule and Formats

One of the DMP's main goals is to ensure that the results and knowledge acquired through the investigation of Hg in larval dragonflies are shared with all appropriate parties, especially the parks and their natural resource managers. We will work in concert with the NPS Inventory & Monitoring Division and strive to provide park managers with clear, meaningful products in a timely manner to convey our findings. As a collaborator, each participating NPS I&M network may publish their own reports and fact sheets based on the dragonfly Hg results from their network parks. Because the DMP monitoring data will be of interest to a broader community, we will also provide our reports to natural resource, wildlife, and public health officials at the state government level, the NPS I&M Division (IMD), upper-level management in the NPS Washington Office, citizen science groups, and when appropriate, submit them to peer-reviewed journals for publication. We will also present our findings orally and in poster format at regional, national, and international meetings when logistically feasible.

As mentioned above, routine data summaries will be conducted annually for sites and parks that are sampled within that year. The summaries will be compiled from data that have been uploaded to ScienceBase.

More comprehensive analysis and synthesis reports will be produced approximately every five years, with the primary audience being the parks, regional and Servicewide I&M staff, and the broader

scientific community. Drafts of these reports will follow USGS Fundamental Science Practices review prior to release.

Personnel Requirements and Training

Roles and Responsibilities

The DMP is managed by the NPS NRSS Air Resources Division (ARD; Lakewood, CO) and USGS Forest & Rangeland Ecosystem Science Center (FRESC; Corvallis, OR), with collaborative engagement from the Appalachian Mountain Club (AMC) and Dartmouth College who serve with NPS and USGS co-PIs as the DMP Core Science Team. Project staff are situated both at NPS and USGS: at NPS, one project manager (ARD ecologist, GS-12) and one assistant project manager (ARD biologist, GS-5); and at USGS, one project manager (Supervisory Research Ecologist, GS-15) two assistant project managers (USGS ecologists, GS-12 and GS-9/11), and a data manager (USGS ecologist, GS-9). The NPS and USGS project managers serve as co-Principal Investigators (PIs) of the DMP and provide scientific oversight, data interpretation, project communication, and project partnerships. The co-PIs are permanent full-time employees and manage the project as part of their collateral duties. The assistant project managers are term full-time employees. The NPS assistant project manager has many overlapping responsibilities throughout the year, but primarily focuses on coordinating field sampling with participating parks, facilitating fieldwork, and at select units, public engagement aspects of project participation. The USGS assistant project managers primarily focus on sampling logistics, coordination, data management, data analysis, and reporting, with some overlapping duties on other contaminant ecology monitoring initiatives during the field season and throughout the year. The USGS assistant project manager(s) also supervise(s) at least one seasonal or term field crew member or lab tech at the GS-4 or GS-5 level, and, along with other crew members or lab technicians, assist(s) with data collection and analysis. USGS field crews will occasionally conduct monitoring activities for this protocol during the field season, at times concurrent with other contaminant monitoring activities.

As a collaborative partner, the NPS IMD will define a I&M liaison between the DMP and the 32 networks, who would represent I&M networks on the DMP as a subject matter expert and serve as a DMP partner.

The project relies on NPS staff and citizen scientists as the experts in their own ecosystems, drawing from their local knowledge, rather than funding professional scientists to travel to and sample each site. The DMP Core Science Team at the NPS and USGS enlists a liaison within each national park or in some instances, partner within the community. The DMP liaison then coordinates, trains, and leads citizen scientists in actively collecting samples of dragonfly larvae from waters within the parks and sends them to the USGS laboratory for mercury analysis. Field personnel at the NPS units who conduct the sampling and collect field data consist of 1) park and/or I&M staff who serve as the DMP liaisons; and at most NPS units, 2) public participants who serve as a field crew of community volunteers and citizen scientists. The scope and broad geographic coverage of this sampling is only possible because of park and public participation.

This structure is scalable and adaptable to local circumstances. The train-the-trainer model allows local resource managers and educators to serve as the DMP liaison and oversee sampling and provisioning of training materials, and to ensure that samples are collected by the field crew

following strict protocols to avoid contamination. These staff liaisons work collaboratively with project scientists to select water bodies relevant to local issues and, as appropriate, invest additional time supplementing citizen science field sampling with educational programs. Technical staff, including the DMP Project Managers and Assistant Project Managers at USGS and NPS, are available remotely to help select sampling sites, locate dragonfly larvae, and to answer any questions the project leaders may have, even while in the field.

Project Managers

The DMP is jointly managed by PIs at the NPS and USGS. The role of both project managers is to provide scientific oversight in coordination with the Core Science Team and serve as liaisons among other related contaminant monitoring projects conducted by other agencies and entities (federal and non-federal), partners (researchers, state monitoring programs, etc.), NPS staff (program, park, network), USGS staff, and public participants or community-driven scientists.

National Park Service

Specific responsibilities of the NPS project manager include the following:

- Engage interest among old and new participants in the DMP and coordinate field sampling and logistics in NPS units with park staff.
- Collaborate with USGS in selecting appropriate park site locations for monitoring and sample collection.
- Coordinate with resource management and/or interpretive staff at the parks to ensure parks are informed of monitoring activities.
- Ensure collection of dragonfly larvae and shipment to the USGS lab using lab provided materials.
- Review and comment on any draft products (reports, maps, etc.), and provide input from initiation to completion of any project.
- Facilitate peer review, if necessary, of products developed by the DMP.
- Coordinate the dissemination of results to parks and the public.
- Develop communication materials, including fact sheets, web articles, and videos.
- Develop a training program for field personnel.
- Foster the development of educational materials.
- Issue quarterly email updates.
- Lead logistics for quarterly DMP core team meetings, the annual DMP in-person core team meeting, and DMP steering committee meetings.
- Consult with USGS on scientific decisions and study design.
- Provide financial support to the USGS for work performed as outlined as tasks/responsibilities.

U.S. Geological Survey

Specific responsibilities of the USGS project manager include the following:

- Provide scientific oversight, project tracking, data analysis, data management, data interpretation, data visualization tools, and reporting.
- Collaborate with NPS to develop field collection protocols for environmental samples.
- Answer questions from parks, regions, and networks regarding sample collection.
- Coordinate field schedules, availability of supplies, and shipment with field personnel.
- Coordinate laboratory analysis of samples.
- Perform data analysis and develop quality assured databases of research results.
- Supervise or perform data entry, verification, and validation.
- Manage data and serve as the main point of contact concerning data content.
- Develop, document, and oversee the implementation of standard procedures for field data collection and data handling.
- Share resulting data with parks, other partners, and collaborators.
- Complete exit interviews with participating parks regarding successes and failures, and track citizen science involvement and educational engagement.
- Collaborate with analytical laboratories for analysis of environmental samples that address new research questions, and ensure lab results meet program needs (e.g., QA/QC procedures, meaningful minimum detection limits, adequate reproducibility of replicate samples).
- Develop QA/QC measures for the project and supervise staff training.
- Identify sensitive information that requires special consideration prior to distribution.
- Participate on the DMP steering committee and in the DMP core team meetings.
- Collaborate on outreach products including a data visualization/Data Web Map tool, StoryMaps, and DMP communication plan.
- Foster the development of educational materials.
- Expand the utility of biosentinel monitoring by developing impairment thresholds for dragonfly larvae exposed to MeHg.
- Summarize and analyze data and prepare reports.
- Report results in the form of 508-compliant written reports for the NPS (e.g., Open-File Report, Natural Resource Report Series, and peer-reviewed journal articles), webinars for the parks, and fact sheets for park management/citizen scientists (i.e., Annual Data Summary).

Assistant Project Managers

Assistant project managers from NPS and USGS are largely responsible for implementing DMP monitoring protocols. Specific responsibilities include:

- Organize sampling materials and equipment.

- Answer questions from field personnel regarding sample collection.
- Assist with coordination of field schedules and supplies.
- Coordinate field schedules, availability of supplies, and shipment with field personnel.
- Assist parks with site selection.
- Coordinate shipment, receipt, and proper storage of samples.
- Provide access to training materials for field personnel.
- Coordinate logistics with park staff.
- Assist with engagement of public participants.
- Help ensure all aspects of QA/QC are met.
- Coordinate data entry, verification, and validation.
- Train field personnel using established protocols and answer questions regarding methods or techniques.
- Prepare and process samples for laboratory analysis.
- Coordinate laboratory analyses, data validation, and data archiving.
- Conduct post-sampling debrief with park participants.
- Assist with data analysis, data interpretation, and report writing.
- Participate in and travel to DMP core team meetings.
- Present papers utilizing the DMP data.

Field Personnel

The role of field personnel is to conduct field work related to the monitoring project. Field personnel include park and/or I&M staff who serve as DMP liaison(s), public participants, and in some cases, DMP staff. The DMP assistant project managers are responsible for contacting the parks prior to each sampling event to ensure logistical requirements will be met. Park staff must coordinate with DMP assistant project managers and public participants. Responsibilities for DMP field personnel include the following:

- Complete all training for field sampling, sample handling, and boat operation, if required by park.
- Complete all phases of field season preparation, including the acquisition of any necessary sampling permits.
- Engage public participants and oversee sample collection efforts.
- Collect data and samples according to developed protocols.
- Pack and ship samples to analytical laboratory.
- Maintain accurate field and office notes.
- Ensure that all QA/QC procedures are implemented.

- Communicate progress and accomplishments with the assistant project manager(s) before, during, and after sampling at each park unit and report any deviations from sampling protocols.
- Maintain documentation of important details of each field data collection period, including explanations of all deviations from standard procedures and confirmation of geographic locations.
- Maintain hard copies of data forms and send original data forms to archive on a regular basis.
- Submit any documentation that may be required by collection permits, e.g., Investigator's Annual Reports
- Facilitate communication with the DMP, park staff, and the public.

Data Manager

The data management aspect of the monitoring effort is the shared responsibility of the NPS data collectors first, then the USGS manager(s), and finally the USGS project manager and data manager. Typically, NPS field personnel are responsible for data collection. The USGS data manager is responsible for data verification and validation, data archiving, data security, dissemination, and database design. In collaboration with the NPS and USGS project managers and NPS assistant project manager, the USGS manager(s) also develop data entry forms and other database features (as part of quality assurance) and automate report generation.

The data manager is responsible for the following capacities:

- Complete project documentation (i.e., metadata) in appropriate databases.
- Ensure data verification and validation measures for quality assurance.
- Ensure staff are trained in the use of database software and quality assurance procedures.
- Coordinate changes to the field data forms and the user interface for the project database.
- Manage the archival process to ensure regular archival of project documentation, original field data, databases, reports and summaries, and other products from the project.
- Define how project data will be transformed from raw data into meaningful information and create data summary procedures to automate and standardize this process.

Scientific Partners

While the DMP is jointly managed by the NPS and USGS, it receives additional leadership from AMC and Dartmouth College. Both institutions serve as scientific partners. Taken together, the Core Science Team manages the technical, logistical, and outreach aspects of DMP operations, including goals, objectives, existing data and findings, and priority science questions, ranging from big-picture spatial and temporal analyses to more narrow process or foundational questions including information needs and design considerations. DMP scientific partners may also assess or analyze citizen science data or outcomes and report on these in presentations, scholarly publications, or outreach materials.

Further, the NPS IMD, as a DMP partner, serves not only as a project collaborator, but also a user of the data. Therefore, the IMD liaison contributes both to the DMP science team and the DMP Steering Committee.

Steering Committee

The multi-agency DMP Steering Committee represents diverse resource management and regulatory agencies and currently includes staff from the U.S. Fish and Wildlife Service, US EPA, USGS, NPS, AMC, and Dartmouth College. The Committee outlines strategic actions to maximize the benefit of the DMP to resource management, the protection of public health, and the engagement of the public in conservation. Members provide strategic vision, review products and outcomes, and advise the project as it grows.

Personnel Qualifications

The NPS field personnel serving as DMP liaisons should be familiar with the local biology and have experience collecting physical or biological data. Ideally, field personnel should also have a bachelor's or advanced (graduate) degree in biology, chemistry, or a related physical or biological science. If the DMP liaison lacks this experience or background, he or she should be in close consultation with an experienced natural resource professional, ideally one who is deeply familiar with the local environment. The DMP liaison should also have prior leadership experience and good decision-making skills, and be trained in Operational Leadership (SOP #2, Training and Safety). If specialized equipment (e.g., boats, horses, off highway vehicles, etc.) is necessary to access the sampling area, the DMP liaison is responsible for ensuring all participants have proper training and experience to safely use the equipment. While the public participants may not have backgrounds in biology, chemistry, or other related physical or biological science, prior field experience with sampling of fish, benthic invertebrates, zooplankton, water, and/or sediment may be helpful. Those public participants with no prior field experience should be encouraged to engage, as this experience provides a rare opportunity for the layperson to participate in a scientific study in a meaningful way.

Analytical laboratory personnel responsible for analyzing samples for Hg at USGS must be trained on and experienced with the specific methods and equipment necessary to conduct the analyses. If other contract or partner analytical Hg laboratories are employed, outside of USGS, the qualifications and experience of laboratory personnel and QA/QC and performance requirements will be assessed by the USGS Project Manager prior to contracting with an analytical laboratory. Persons overseeing those who are analyzing samples for Hg and involved with data interpretation should have substantial experience in investigations of Hg contamination of aquatic resources, preferably including assessment of the effects of MeHg exposure on aquatic biota. These individuals should possess experience in the collection, handling, and analysis of biological and environmental samples for Hg, and have access to a Hg laboratory with proven analytical reliability. These qualifications should be reflected by accurate measurements of Hg in environmental samples and publication of scientific papers in refereed journals.

Training Procedures

Before participation in data collection, NPS field personnel and DMP liaisons must become familiar with the SOPs and any equipment to be used in the field. Training procedures for new field personnel will include the following:

- Review of this protocol and associated SOPs, specifically #1-4 (Pre-Season Preparation, Training and Safety, Fieldwork – Dragonfly Larvae, Shipping) and #7 (Post-Season Procedures).
- Review of safety procedures and emergency contacts.
- Familiarity with field data forms and sample labels.
- Familiarity with methods for measurements and sample collection.
- Familiarity with methods for handling, preserving, and shipping samples.
- Familiarity with procedures for operation and maintenance of equipment (e.g., boats).
- Park-specific training, provided on-site by park staff (e.g., use of radios).

USGS lab personnel must be familiar with SOP #5, Data Entry and Management, and SOP #6, Data Analysis.

Public participants in the sampling will receive on-site instructions in the methods of collecting and handling biological samples and in the completion of field data forms. Field crew members – including community volunteers and citizen scientists – who collect larval dragonflies must be trained in the use of "clean hands-dirty hands" techniques, which involve the proper use of plastic gloves, zip-seal bags, and sampling gear. Briefly, the sampler coming into direct contact with the samples *must wear clean gloves at all times*, and not come into contact with any surfaces other than the sample. This "clean hands" person also handles the containers into which samples are placed. A "dirty hands" sampling partner handles the equipment and the exterior bag or container that encloses the clean interior bag (SOP #3, Fieldwork – Dragonflies).

Operational Requirements

Annual Workload and Schedule

The annual workload and schedule for monitoring Hg in dragonfly larvae can be viewed both within and outside the context of other planned aquatic monitoring activities. We prepared the estimated workload and schedule for monitoring assuming, for at least some parks/sites, that fieldwork will be concurrent with other monitoring. Workloads are likely to change from year to year depending on availability of staff and availability of funds to allow for monitoring of additional sites or media (e.g., water, sediment, air). Larval dragonflies will be sampled annually across all participating DMP park units. Monitoring will take place in a variety of aquatic habitats. In general, for this monitoring protocol, site accessibility and sampling logistics range from relatively easy to difficult. The time it takes to conduct fieldwork is also weather dependent. Sampling can be delayed, and field crews can be stranded for days due to weather conditions beyond our control. We estimate sampling for larval dragonflies to take from less than one day to as much as seven or more days per site, including travel time. Initial estimates of time required to sample at each park (explained in more detail below, under staff salaries) assume minimal weather-related delays.

Facility and Transportation Needs

At each park, the field crew will need a facility with a sink and countertop space where they can clean equipment and process and prepare samples for shipment, and a freezer for storing samples prior to shipment. Preferably, this will be a field laboratory.

We anticipate that most NPS field personnel and DMP liaisons will be park-based, but some travel by regional or network aquatic ecologists, or DMP project staff, to train or lead the field crews (e.g., community volunteers, citizen scientists) will be necessary. Field crews may travel from local schools, distant universities, or regional conservation groups. Sites will be accessed by on-road vehicles and/or walking trails; however, some sites may require off-trail travel or access via alternative means including horse, helicopter, airplane, ferry, motorboat, or unmotorized boat.

Budget Considerations

This section considers estimated costs associated with equipment and supplies, salary, and analytical laboratory expenses as they relate to monitoring Hg in dragonfly larvae annually in 32 I&M networks. While these dollars are not yet defined at the IMD scale, the following outlines the baseline funding need for the minimum design of three fixed and three random/variable sampling locations per network. Parks, networks, or regions may support additional sites as needed, at a reduced cost that is supported by efficiencies of the baseline support. Importantly, although all cost estimates provided below are accurate at the time of this document's publication (2022), they may differ from current or local costs.

Equipment and Supplies

Limited equipment and supplies are needed for monitoring Hg in larval dragonflies, and sampling gear is already owned by some parks/networks. Other parks/networks may need to purchase sampling nets, waders, coolers, sorting pans, and/or PFDs, and future costs will be associated primarily with replacing equipment as it wears out or becomes obsolete. Consumable supplies (dry

ice, nitrile gloves, Ziplock bags, barcodes, etc.) require purchase and preparation each year, and shipping costs for samples and sampling supplies are also an annual expense. Based on past annual expenses from ongoing DMP efforts, we estimate that annual expenses for equipment, supplies, and shipping for this protocol will range from $5,000 to $7,000 at a national scale.

Staff Salaries

Most staff salaries for this protocol are associated with coordination, laboratory analysis, data management, and product delivery. We anticipate that field sampling can be leveraged from existing NPS efforts through either I&M or park staff. Annual salaries for the components listed above, assuming baseline design of six sites (three fixed, three random/variable) per network are estimated to fall between $130,000 and $160,000. These estimates – according to a 2-week pay period – include the following:

- 15-20 pay periods for assistant project managers
- 7-9 pay periods for data analyst and statistician
- 5-7 pay periods for data manager
- 7-9 pay periods for lab technician
- 5-7 pay periods for park liaison and field coordinator.

Salary for NPS and USGS project managers are provided in-kind from their respective agencies. The salary estimates include staff time for project management; training; pre-season preparation; processing samples; packing, shipping, and receiving samples and supplies; data entry; analysis; reporting; and other various tasks associated with the monitoring effort.

Vehicles and Travel

Overall travel costs are difficult to predict and vary widely among parks and I&M networks. In many cases existing travel efforts can be leveraged. However, to account for the limited situations where that is not the case, we anticipate additional travel expenses for routine sampling to be approximately $5,000 annually across parks. In addition, we anticipate roughly $4,000 in travel costs annually for project leads to travel to parks on a rotational basis to assist with and oversee monitoring activities.

Analytical Laboratory Costs

The USGS CERL laboratory provides cost-leveraged advanced analytical services to the DMP that include:

- Checking of field identifications to family for each individual sample.
- Checking measurements (body length, stage of instar development, etc.) of each individual sample.
- Wet weight and dry weight determinations for each individual sample.
- THg analysis for each individual sample; MeHg analysis for a subset of individuals.
- Electronic data delivery in an agreed-upon format that includes results of relevant laboratory QA/QC (i.e., a QA/QC report).

The combined cost of these services is $800 per site, which is equivalent to $153,600 annually (32 I&M networks × 6 sites/network × $800/site).

This cost is considerably lower than services provided by University of Maine or the Wisconsin State Laboratory of Hygiene, which ranged between $1,000 and $1,325 per site, and is considerably lower than the current DMP rate per site ($1,800). These cost savings are made possible by both leveraged, in-kind USGS laboratory support as well as efficiencies associated with a national-scale program.

Total Estimated Annual Cost

Annual monitoring costs, given the costs outlined above, are expected to range between $288,000 and $310,000 (in 2021 dollars).

Procedures for Revising and Archiving Previous Versions of the Protocol

As our program to monitor mercury in larval dragonflies matures, revisions to the protocol narrative and SOPs are likely. Documenting changes and archiving copies of previous versions of these documents are essential for maintaining consistency in the collection of data and for appropriate interpretation of the data summaries and analyses. The NPSTORET database contains a field for each monitoring component that identifies which version of the protocol was in place when the data were collected. The rationale for dividing a sampling protocol into a protocol narrative and supporting SOPs is based on the following:

- The protocol narrative is a general overview of the protocol that gives the history and justification for doing the work and an overview of the sampling methods but does not provide all methodological details. The protocol narrative will only be revised if major changes are made to the protocol.
- The SOPs are specific step-by-step instructions for performing a given task. They are expected to be revised more frequently than the protocol narrative.
- Usually, when a SOP is revised, it is not necessary to revise the protocol narrative to reflect the specific changes made to the SOP.

All versions of the protocol narrative and SOPs will be archived.

The steps for changing the protocol (narrative, SOPs) are outlined in SOP #9, Procedure for Revising the Protocol. The SOPs contain a Revision History Log that must be updated each time that SOP is revised, to explain why the change was made and to assign a new version number. The new version of protocol narrative or SOP should then be archived in the appropriate folder of the USGS-DMP database structure.

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Appendix A. Standard Operating Procedures

Table A1. Standard Operating Procedures (SOPs) referenced.

Standard Operating Protocol	Citation	Adopted from Existing Protocol, or New	Deviations from Source Protocols
Standard Operating Procedure #1, Pre-Season Preparation	Flanagan Pritz, C.M., C.A. Eagles-Smith, D.D. VanderMeulen, and K. Ko. 2022.	New	–
Standard Operating Procedure #2, Training and Safety	VanderMeulen, D.D., C.M. Flanagan Pritz, and C.A. Eagles-Smith. 2022.	New	–
Standard Operating Procedure #3, Fieldwork – Dragonfly Larvae	Nelson, S. J., C.A. Eagles-Smith, J. Willacker, C.M. Flanagan Pritz, D.D. VanderMeulen, and C. Emery. 2022.	New	–
Standard Operating Procedure #4, Shipping	Willacker, J., C. Emery, C.A. Eagles-Smith, and K. Ko. 2022	New	–
Standard Operating Procedure #5, Data Entry and Management	Eagles-Smith, C.A., J. Willacker, C. Emery, D.D. VanderMeulen, and C.M. Flanagan Pritz. 2022.	New	–
Standard Operating Procedure #6, Data Analysis	Eagles-Smith, C.A., J. Willacker, D.D. VanderMeulen, S.J. Nelson, A. Klemmer, and C.M. Flanagan Pritz. 2022.	New	–
Standard Operating Procedure #7, Post-Season Procedures	Flanagan Pritz, C.M., C.A. Eagles-Smith, K. Ko, D.D. VanderMeulen, and C. Emery. 2022.	New	–
Standard Operating Procedure #8, Reporting	Flanagan Pritz, C.M., C.A. Eagles-Smith, D.D. VanderMeulen, and K. Ko. 2022.	New	–
Standard Operating Procedure #9, Procedure for Revising the Protocol	Sanders, S., D.D. VanderMeulen, C.M. Flanagan Pritz, and C.A. Eagles-Smith. 2022.	Adopted	–

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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