National Park Service
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Natural Resource Stewardship and Science

Protocol for Monitoring Mercury in Dragonfly Larvae and Fish (Version 1.0)

Great Lakes Inventory and Monitoring Network

Natural Resource Report NPS/GLKN/NRR—2018/1726



ON THE COVER
Larval dragonflies captured from a lagoon in the Apostle Islands National Lakeshore (Wisconsin).
NPS photo/T. Gostomski

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Contents

Page

Figures vii

Tables ix

Appendices xi

Abstract xiii

Acknowledgments xv

List of Abbreviations and Acronyms xvii

Park Units xvii

Others xvii

1. Background and Objectives 1

1.1 Introduction 1

1.2 Goals and Objectives 4

1.3 Sources and Toxicity of Mercury 5

1.4 Biosentinel Organisms for Mercury 6

1.4.1 Larval dragonflies 7

1.4.2 Prey Fish 11

1.4.3 Predatory Fish 14

1.5 Monitoring and Assessment Questions 15

2. Monitoring and Assessment Strategy 19

2.1 Sampling Schedule 19

2.2 Number and Location of Sampling Sites 20

2.3 Sample Size and Level of Change That Can Be Detected 22

2.4 Guidelines for Assessing Ecological and Health Risks of Mercury 25

2.4.1 Benchmarks for Assessing Mercury Risk to Fish 25

2.4.2 Benchmarks for Assessing Mercury Risk to Piscivorous Wildlife 28

2.4.3 Benchmarks for Assessing Mercury Risk to Fish-Eating Humans 29

3. Overview of Sampling and Analytical Methods 31

Contents (continued)

Page

3.1 Pre-Season Preparations 31

3.2 Collection and Handling of Samples in the Field 31

3.2.1 Larval dragonflies 31

3.2.2 Prey fish 31

3.2.3 Predatory fish 32

3.3 Preparation and Analysis of Samples in an Analytical Laboratory 32

3.3.1 Larval dragonflies 32

3.3.2 Prey fish 33

3.3.3 Predatory fish 33

3.3.4 Quality assurance and quality control for mercury determinations 33

4. Data Handling, Analysis, and Reporting 35

4.1 Overview of Database Design 35

4.2 Metadata Procedures 35

4.3 Data Entry, Verification, and Editing 36

4.4 Data Archival Procedures 36

4.5 Quality Assurance and Quality Control for Data Management 37

4.6 Routine Data Summaries 37

4.7 Data Analyses 38

4.8 Reporting Schedule and Formats 39

5. Personnel Requirements and Training 41

5.1 Roles and Responsibilities 41

5.1.1 Project Manager 41

5.1.2 Assistant Project Managers 42

5.1.3 Field Personnel (Field Crew Member/Leader) 42

5.1.4 Data Manager 43

5.2 Personnel Qualifications 43

Contents (continued)

Page

5.3 Training Procedures....44

6. Operational Requirements....45

6.1 Annual Work Load and Schedule....45

6.2 Facility and Transportation Needs....45

6.3 Budget Considerations....45

6.3.1 Equipment and Supplies....46

6.3.2 Staff Salaries....46

6.3.3 Vehicles and Travel....47

6.3.4 Analytical Laboratory Costs....47

6.3.5 Total Estimated Annual Costs....48

6.4 Procedures for Revising and Archiving Previous Versions of the Protocol....48

Literature Cited....51

Figures

Page

Figure 1. National parks in the Great Lakes Inventory and Monitoring Network. 1

Figure 2. Proportional species richness of dragonfly families found in the nine park units of the National Park Service Great Lakes Monitoring and Inventory Network. 7

Figure 3. Relation between mean concentrations of total mercury in whole, 1-year-old yellow perch and methylmercury in whole larvae of the dusky clubtail dragonfly (*Gomphus spicatus*) from interior lakes in Voyageurs National Park sampled in 2002 and 2003..... 9

Figure 4. Linear regression between mean concentration of total mercury (THg) in small, whole yellow perch (<76 mm) and mean concentrations of (a) THg and (b) MeHg in coexisting larval *Gomphus* across 15 lakes in six national parks in the western Great Lakes region collected during 2008–2010 10

Figure 5. Relation between the estimated concentrations of total mercury in axial muscle (skinless fillets) of 55-cm northern pike and mean concentrations in whole age-1 yellow perch from interior lakes in Voyageurs National Park, with concentrations expressed on a dry weight basis 13

Figure 6. Percent of game fishes with concentrations of THg in skinless fillets equaling or exceeding 300 ng g-1 wet weight (the U.S. Environmental Protection Agency fish tissue criterion for MeHg), in relation to the mean concentration of MeHg in coexisting larval Gomphus in 13 lakes within the National Park Service Great Lakes Inventory and Monitoring Network 30

Figure B1. Graphical representation of the power that each park in the GLKN has per number of samples to detect a 20% change over 10 years with a Type I error of 0.05 using a regression analysis for dragonfly larvae. 80

Tables

Page

Table 1. Examples of studies that explore spatial and temporal variations in total mercury (THg) in larval dragonflies, prey fish, and/or predatory fish in Great Lakes Network parks. 16

Table 2. Fixed monitoring sites for mercury in larval dragonflies and fish across Great Lakes Network sites. R.P. = regional park. 21

Table 3. Number of annual samples per water body by park necessary to detect a 20% change in total mercury over 10 years with Type I error of 0.05 and 80% power for three types of biosentinel organisms 23

Table 4. Number of larval dragonflies to be collected annually depending on number of monitoring years and percent change detectable in total mercury with power to detect the change at 80% and a Type 1 error of 0.05 for Angleworm Lake at Isle Royale National Park (data collected from 2008–2012 in six GLKN parks; power analysis details in Appendix B). 24

Table 5. Concentrations of mercury in fish used as benchmarks to assess risks of methylmercury (MeHg) to fish, piscivorous wildlife, and humans. 27

Table 6. Guidelines for human consumption of sport fish containing mercury in their fillets. 29

Table 7. Summary of QA/QC procedures pertaining to data management. 37

Table 8. Total estimated annual costs for monitoring mercury in dragonfly larvae and fish at GLKN parks. 48

Table A1. Dragonfly species recorded for the nine National Park Service units in the Great Lakes Inventory and Monitoring Network[1] from 2008 to 2014, and species likely present based on county (USA) and provincial (Canada) records[2]. 68

Table B1. The number of samples needed per park to determine a 20% change through regression analysis over 10 years with a Type I error of 0.05 and 80% power 81

Table B2. Sample numbers produced from the three different analyses: 1. regression (time) is the number of samples needed each year to detect a 20% change over 10 years with a Type I error of 0.05; 2. ANOVA (time) is the number of samples needed each year to detect a difference among years with a Type I error of 0.05; and 3. ANOVA (per unit) is the number of samples per unit (park or water body) to detect a difference among units (park or water body) with a Type I error of 0.05 82

Appendices

Page

Appendix A. Dragonflies of the Great Lakes Network Parks. 67

Appendix B. Power Analysis of Dragonfly Larvae and Fish Data. 77

Appendix C. Examples of Laboratory Methodologies to Analyze Larval Dragonflies and Fish for Total and Methylmercury. 85

Abstract

This protocol describes a framework for quantifying concentrations of mercury in larval dragonflies and fish in nine national parks within the Great Lakes Inventory and Monitoring Network (GLKN). Mercury is a metal that when methylated biomagnifies to potentially toxic concentrations in upper trophic levels. Atmospheric transport and deposition are the primary pathways for entry of mercury into most watersheds and surface waters in GLKN parks. Mercury levels in biosentinel organisms vary greatly among aquatic ecosystems, including those in close proximity, due to a number of factors that affect mercury methylation and delivery to the base of the aquatic food web and that affect biomagnification. Both larval dragonflies and fish are high on the aquatic food web, bioaccumulate mercury, and as biosentinel organisms and act as integrators of mercury contamination. Mercury concentrations in dragonfly larvae are strongly correlated with mercury concentrations in fish and therefore can be used to assess mercury contamination in the aquatic environment. Through this protocol we will monitor mercury in dragonfly larvae annually at all nine GLKN parks, with fish sampled every five years at each park, on a rotation of two GLKN parks per year, except for the fifth year when only one park will be sampled. Sampling predatory fish will be a higher priority than prey fish, as predatory fish typically have higher levels of mercury than prey fish, are more likely to be caught and eaten by people and from the perspective of protection of human health are of higher interest for monitoring. Monitoring will take place at four sites per park across a variety of waters including inland lakes, rivers, and streams. At each site we will collect 20 dragonfly larvae, and when fish are sampled either 20 prey or 15 predatory fish. Data will be comprised of field observations and measurements that are recorded on data sheets in the field, and the results of testing performed by contract analytical laboratories. The data will be analyzed to determine 1) what parks and surface waters within the GLKN have concentrations of mercury in dragonfly larvae and fish high enough to pose a risk to humans and wildlife in upper trophic levels, 2) what are the spatial patterns in mercury contamination in dragonfly larvae and fish within parks of the GLKN, and 3) what is the general direction and magnitude of change in the concentration of mercury in dragonfly larvae and fish in parks of the GLKN.

Acknowledgments

Major portions of this protocol and its associated standard operating procedures are derived from an earlier draft version that focused on monitoring a wider suite of bioaccumulative contaminants in aquatic food webs in six national parks within the GLKN (Weiner et al. 2009). Sections of this protocol that focus on sources and toxicity of mercury, and guidelines for assessing ecological and health risks of mercury are drawn nearly verbatim from Wiener et al. (2016). M. Brigham and M. Bozak provided constructive comments on earlier drafts, while K. Bartz, A. Blakesley, C. Flanagan-Pritz, and C. Sergeant reviewed and greatly improved the current version of this protocol. Note that all product and company names are trademarks™ or registered® trademarks of their respective holders. Use of them in this protocol narrative and associated standard operating procedures does not imply any affiliation with or endorsement by the U.S. National Park Service.

List of Abbreviations and Acronyms

Park Units

APIS:	Apostle Islands National Lakeshore
GRPO:	Grand Portage National Monument
INDU:	Indiana Dunes National Lakeshore
ISRO:	Isle Royale National Park
MISS:	Mississippi National River and Recreation Area
PIRO:	Pictured Rocks National Lakeshore
SLBE:	Sleeping Bear Dunes National Lakeshore
SACN:	St. Croix National Scenic Riverway
VOYA:	Voyageurs National Park

Others

DDD:	dichloro-diphenyl-dichloroethane
DDE:	dichloro-diphenyl-dichloroethylene
DDT:	dichloro-diphenyl-trichloroethane
DOC:	dissolved organic carbon
dw:	dry weight
GLKN:	Great Lakes Inventory and Monitoring Network
Hg:	mercury
THg:	total mercury
IAR:	investigators annual report
MeHg:	methylmercury
NPS:	National Park Service
NRDS:	Natural Resources Data Summary (report)
Pb:	lead
PCB:	polychlorinated biphenyl
PBDE:	polybrominated diphenyl ethers
PDF:	Portable Document Format
PFC:	perfluorochemical
PFOS:	perfluorooctanesulfonates
PFOA:	perfluorooctanoic acid
QA/QC:	quality assurance quality control
SOP:	standard operating procedure
USEPA:	United States Environmental Protection Agency
UW-L:	University of Wisconsin-La Crosse
UM:	University of Maine
USGS:	United States Geological Survey
ww:	wet weight
YOY:	young-of-the-year

1. Background and Objectives

1.1 Introduction

The National Park Service (NPS) instituted a program to inventory and monitor natural resources at approximately 270 NPS units (parks) across the U.S. (Fancy et al. 2009). The program is being implemented by 32 "networks" of parks that share common management concerns and geography. The Great Lakes Network (GLKN) is comprised of nine parks in four states surrounding the western Great Lakes (Figure 1). These parks include the Apostle Islands National Lakeshore (APIS), Grand Portage National Monument (GRPO), Indiana Dunes National Lakeshore (INDU), Isle Royale National Park (ISRO), Mississippi National River and Recreation Area (MISS), Pictured Rocks National Lakeshore (PIRO), Sleeping Bear Dunes National Lakeshore (SLBE), St. Croix National Scenic Riverway (SACN), and Voyageurs National Park (VOYA). These parks contain abundant and diverse aquatic resources, including intermittent streams, vernal pools, about 1300 km of perennial streams, large river systems, more than 130 inland lakes, more than 300 square kilometers of wetlands, and near-shore areas in Lake Michigan and Lake Superior (Lafrancois and Glase 2005). More than half of the area encompassed by ISRO and VOYA is aquatic habitat.



Figure 1. National parks in the Great Lakes Inventory and Monitoring Network.

The purpose of the NPS national Inventory and Monitoring program is to identify and monitor ecological indicators, referred to as "Vital Signs" of park ecosystem health. Vital Signs are a select group of attributes that are particularly rich in information needed for understanding and managing NPS areas. The GLKN has developed a guiding document that provides the goals, ecological context, selected Vital Signs, including bioaccumulative contaminants, and an implementation schedule for the program (Route and Elias 2007).

Given the hundreds of contaminants present within the water bodies of network parks, and the cost of monitoring all of them, Route et al. (2009) used the following four criteria to identify and select specific pollutants for inclusion in trophic bioaccumulation protocols for the network:

- Contaminants that persist in the environment and bioaccumulate in fish and wildlife,
- Contaminants that have caused a park water body to be listed as polluted under section 303(d) of the federal Water Quality Act (e.g., contaminants that have prompted a fish consumption advisory),
- Contaminants listed as a Level I Substance in Appendix I of the Great Lakes Binational Toxics Strategy (for those parks on the Great Lakes), and
- Contaminants identified by state and federal authorities as new and emerging chemicals of concern.

Based on these criteria, the following contaminants were selected for initial analysis in contaminant monitoring protocols utilizing nestling bald eagles (Route et al. 2009) and other aquatic organisms (fish, larval dragonflies, etc.; Wiener et al. 2009) that were implemented in 2006 and 2008, respectively:

- DDT (dichloro-diphenyl-trichloroethane) and metabolites:
    - DDE (dichloro-diphenyl-dichloroethylene)
    - DDD (dichloro-diphenyl-dichloroethane)
- PCBs (polychlorinated biphenyls; total concentration and 75 congeners)
- PFCs (perfluorochemicals; total concentration and 16 telomers)
- PBDEs (polybrominated diphenyl ethers; total and 9 congeners)
- Lead (Pb)
- Mercury (Hg), including total Hg (THg) and methylmercury (MeHg)

In brief, DDT is an organochlorine compound used as an insecticide. It and its metabolites or breakdown products, DDE and DDD, are persistent in the environment, bioaccumulate in exposed organisms, and biomagnify to high concentrations in organisms in upper trophic levels of food webs (Kidd et al. 2001, Blus 2003, Henny et al. 2009). Polychlorinated biphenyls (PCBs) are a group of synthetic organic chemicals consisting of 209 potential compounds or congeners that are transported in aquatic systems and in the atmosphere, resist degradation in the environment, bioaccumulate in exposed organisms, and biomagnify in aquatic food webs (Rice et al. 2003). Perfluorochemicals (PFCs) are a family of stable, fluorinated organic compounds that are persistent in the environment

and bioaccumulate in exposed organisms (Condor et al. 2008, Houde et al. 2006), including fish (Martin et al. 2003, Holzer et al. 2011). Polybrominated diphenyl ethers (PBDEs) are a class of halogenated flame retardants used in plastics, polyurethane foams, and textiles that are released to the environment during product use and disposal (Vonderheide et al. 2008). They are lipophilic, and some congeners readily biomagnify (Law et al. 2006, Wu et al. 2009). Lead is a metal that in its elemental form can be toxic through chronic or acute exposure. Mercury is also a metal that when methylated biomagnifies to potentially toxic concentrations in upper trophic levels, and is discussed in detail below. A synthesis of aquatics-based contaminants research in network parks prior to ca. 2005 by Lafrancois and Glase (2005) outlines past studies of these and other contaminants.

In 2006, the protocol to monitor these contaminants in bald eagle nestlings was implemented at APIS, MISS, and SACN, with annual sampling occurring through 2011, and again in 2014 and 2015. We found that mercury, DDE, and PCBs declined significantly from 2006 to 2008 through analysis of data collected from those parks, as well as previously published data for bald eagle nestlings along the southern shore of Lake Superior (Dykstra et al. 2010). Most of the 16 PFC analytes monitored, including perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), and total PBDEs (the sum of all nine congeners) also declined through 2011 (Route et al. 2014a, and 2014b), and lead declined from 2006 through 2015 (Bruggeman et al. 2018). The decline in mercury levels in nestling bald eagles appears to have slowed, even though overall mercury concentrations in the environment have declined (Bill Route, unpublished data 2009–2015). Yet, in some areas such as the upper St. Croix River in northern Wisconsin, concentrations are elevated (>7.5 µg/g dry weight [dw] in breast feathers), and may continue to pose some reason for concern.

Through the companion protocol to monitor bioaccumulative contaminants in aquatic food webs, adult predatory fish samples were collected from 2008 to 2012 at GRPO, INDU, ISRO, PIRO, SLBE, and VOYA and analyzed for the six contaminant groups. Prey fish and larval dragonflies were also collected in these parks and analyzed solely for mercury. Key findings from that work include 1) concentrations of legacy (DDT and metabolites, PCBs, and lead) and emerging contaminants (PFCs and PBDEs) in fish were either non-detectable or very low, with only one site at INDU that may be of concern, 2) mercury concentrations in axial muscle tissue (skinless fillets) of adult fish from several water bodies substantially exceeded U.S. Environmental Protection Agency (USEPA) tissue residue criterion (0.3 ppm ww) for methylmercury, 3) fish in some water bodies have mercury concentrations high enough to potentially impair fish health, 4) whole prey fish from some water bodies had concentrations of mercury considered harmful to fish-eating wildlife, 5) because their mercury levels strongly correlate with mercury in fish tissue, larval dragonflies are useful biosentinels of methylmercury in aquatic food webs across the western Great Lakes region (Haro et al. 2013, Wiener et al. 2016).

Separately, in 2011 the NPS Air Resources Division partnered with the University of Maine and 4 national parks across the U.S. and began a citizen-science based pilot project to collect and analyze larval dragonflies for mercury. This work was expanded to 11 parks in 2012. One of the objectives of the project was to evaluate relationships among potential factors that influence mercury accumulation in larval dragonflies and mercury in water across the broad spatial scale of parks in all NPS regions

to determine their utility as biosentinels of exposure (Nelson et al. 2015). Since 2012, 93 parks, including all nine of the GLKN parks, have participated in this project (C. Flanagan-Pritz pers. comm.). Through related work, in 2015 and 2016, fish were also collected from a number of parks in the eastern U.S., including INDU, ISRO, MISS, SACN, and VOYA, and are being analyzed for mercury (Collin Eagles-Smith, USGS, unpublished data).

This protocol, which draws heavily on the protocol for monitoring of contaminants in aquatic food webs by Wiener et al. (2009), focuses on monitoring mercury in dragonfly larvae annually, and fish periodically, across all nine GLKN parks. The contaminants in bald eagle nestlings protocol (Route et al. 2009) is also being revised to monitor contaminants in nestlings on a less frequent basis, though the network will continue to support efforts to monitor bald eagle productivity through aerial nest surveys. Rationale for these changes includes:

- Concentrations of five of the organic contaminant groups (all pesticide or industrial compounds) are declining (eagles) or very low or non-detectable (fish), and except for a few instances are not at levels that exceed toxicological thresholds.
- Laboratory costs for monitoring the organic contaminants, especially contaminants of emerging concern, are high.
- Mercury remains the most pervasive and toxic contaminant in the upper Midwest.
- Mercury levels in eagles and fish at some network parks are of concern.
- Larval dragonflies have been shown to be suitable biosentinels of mercury in aquatic food webs.
- Dragonflies are ubiquitous across network parks and compared to other media (bald eagle nestling feathers, fish tissue, lake sediment, etc.) are relatively simple to collect in the larval stage and represent a cost-effective alternative.

1.2 Goals and Objectives

The protocol is comprised of a narrative (this document), supporting standard operating procedures (SOPs), and a quality assurance plan (QAP; VanderMeulen et al. 2018). The protocol narrative gives the history and justification for doing the work, including the current state of the science, and an overview of sampling methods, data management, and analysis, but does not provide all methodological details. With a few exceptions (e.g., data analysis), the SOPs are specific step-by-step instructions for performing a given task. The QAP describes in detail 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 provide park managers and planners with information on the spatial patterns, temporal trends, and potential ecotoxicological significance of mercury contamination of dragonfly larvae and fish for nine national park units in the GLKN. The sampling and analysis of dragonfly larvae and fish, as outlined in this protocol, will address the following specific objectives:

> *Objective 1*: Identify parks and surface waters within the GLKN where concentrations of mercury may pose a risk to fish, fish-eating wildlife, and humans.

Objective 2: Assess spatial patterns in mercury contamination of dragonfly larvae and fish.

Objective 3: Evaluate temporal trends in mercury contamination of dragonfly larvae and fish.

1.3 Sources and Toxicity of Mercury

Atmospheric transport and deposition are the primary pathways for entry of mercury into most watersheds and surface waters in the western Great Lakes region (Wiener et al. 2006, Evers et al. 2011b, Drevnick et al. 2012, Lepak et al. 2015). Analyses of sediment cores from inland lakes have shown that most (approximately 70%) of this atmospheric mercury is from anthropogenic sources (Swain et al. 1992, Wiener et al. 2006, Drevnick et al. 2012). In addition, a growing body of evidence indicates that atmospheric deposition is the primary source of mercury accumulating as methylmercury in lacustrine food webs and fish in areas like the Great Lakes region that lack a significant geological mercury source (Wiener et al. 2006, Orihel et al. 2007, Munthe et al. 2007, Harris et al. 2007, Evers et al. 2011b, Lepak et al. 2015).

Spatial patterns in wet deposition of mercury across the Great Lakes region during 2002–2008 and in litterfall (an indicator of dry deposition) during 2007–2009 were influenced by regional emissions from anthropogenic sources (Risch et al. 2012a, 2012b). Mean annual inputs of mercury in wet and litterfall deposition were greatest in areas with the greatest emissions from anthropogenic sources (Indiana, Ohio, Illinois, eastern and northwestern Pennsylvania, southern Michigan, and southeastern Wisconsin) and least in areas with few anthropogenic sources (northern Wisconsin, Minnesota, northern Michigan, and Ontario). In 2005, coal-fired power plants accounted for an estimated 57% of total anthropogenic emissions of mercury to the atmosphere in the Great Lakes region (Evers et al. 2011b).

The microbial production of methylmercury strongly affects its concentrations in water and aquatic biota, including fish (Bodaly et al. 1993, Paterson et al. 1998). Landscape factors, including wetland density (Hurley et al. 1995, Chasar et al. 2009, Nagorski et al. 2014) and coniferous forest cover (Drenner et al. 2013, Eagles-Smith et al. 2013), are also associated with methylmercury concentrations in water and fish. Thus, both water bodies and landscapes can differ greatly in their sensitivity to mercury loadings from the atmosphere. Mercury-sensitive ecosystems are those where inorganic mercury is more efficiently converted to methylmercury, and seemingly small inputs of inorganic mercury can cause significant methylmercury bioaccumulation in fish and wildlife (Wiener et al. 2003).

Mercury is a highly toxic metal that has no known essential biological function. Toxicological concerns about mercury pollution of aquatic systems focus appropriately on methylmercury, which can biomagnify to high, sometimes harmful, concentrations in organisms in upper trophic levels (Wiener et al. 2003, Scheuhammer et al. 2007, Sandheinrich and Wiener 2011). Although most of the mercury in atmospheric deposition exists as inorganic forms, nearly all of the mercury accumulated by fish and other top predators is methylmercury (Grieb et al. 1990, Bloom 1992, Hammerschmidt et al. 1999, Van Walleghem et al. 2007), an organic compound produced by anaerobic bacteria that are present in wetlands, sediment, and anoxic bottom waters (Benoit et al. 2003, Colombo et al. 2013, Gilmour et al. 2013, Parks et al. 2013). In fish, methylmercury readily crosses internal and external

biological membranes (Pickhardt et al. 2006), is eliminated very slowly relative to its rate of uptake (Trudel and Rasmussen 1997, Van Walleghem et al. 2007, 2013), and accumulates to concentrations that vastly exceed those in surface water (Wiener et al. 2003). Concentrations of methylmercury in piscivorous fish commonly exceed those in the water in which they reside by a factor of 106 to 107 or more (Wiener et al. 2003).

Aquatic food webs are the principal pathways for exposure of humans and wildlife to methylmercury (Mergler et al. 2007, Scheuhammer et al. 2007, 2012, McKelvey and Oken 2012, Driscoll et al. 2013). Methylmercury is highly neurotoxic, adversely affecting both the adult and developing brain (Clarkson and Magos 2006, McKelvey and Oken 2012). In birds and mammals, methylmercury from reproducing females readily passes to the developing egg or embryo, life stages that are more sensitive than the adult to methylmercury exposure (Wiener et al. 2003, Heinz et al. 2009b). Methylmercury is an endocrine disrupter and impairs reproduction partly by disruption of the hypothalamic-pituitary-gonadal axis (Colborn et al. 1993, Tan et al. 2009). Recent studies have also shown that exposure of fish to environmentally realistic concentrations of methylmercury can adversely affect gene expression, metabolism, reproduction, and other processes (Crump and Trudeau 2009, Sandheinrich and Wiener 2011, Scheuhammer et al. 2012).

Concentrations of mercury in predatory fish from many water bodies in the Great Lakes region exceed not only state and provincial guidelines for fish consumption (Evers et al. 2011a, 2011b), but also the USEPA Fish Tissue Residue Criterion for Methylmercury (established to protect human health; Borum et al. 2001). Most states in the Great Lakes region have issued statewide guidelines advising that women of childbearing age and children limit their consumption of fish, and many states have issued advisories for specific water bodies (USEPA 2011). In the neighboring Canadian province of Ontario, 88% of the fish consumption advisories issued for sport fish in inland waters are due to mercury (Ontario Ministry of Environment 2013). Thus, much of the Great Lakes region can be considered a mercury-sensitive landscape in which atmospheric deposition of mercury has led to high concentrations of methylmercury in predatory fish (Wiener et al. 2003, Evers et al. 2011b). Mercury threshold values above which may be detrimental to wildlife and human health are discussed in detail in Section 2.4.

1.4 Biosentinel Organisms for Mercury

The monitoring and assessment approach outlined in this protocol emphasizes the analysis of biosentinel organisms to identify spatial and temporal patterns in the contamination of aquatic food webs. Biosentinel organisms are those that consistently "integrate" uptake of substances that may be dangerous, and therefore serve as indicators about the environmental condition or health of their ecosystems. This mercury monitoring protocol focuses on three groups of biosentinel organisms—larval dragonflies, small prey fish (often termed "forage fish"), and predatory fish (also referred to as "sport fish" in this protocol) —that are widely distributed in aquatic habitats in parks within the GLKN. These biosentinels are considered relevant, useful, and sufficiently diagnostic to detect spatiotemporal variations in the concentration of mercury, based on published guidelines pertaining to aquatic biological indicators of methylmercury contamination (Wiener et al. 2007).

1.4.1 Larval dragonflies

Dragonflies (Odonata: Anisoptera) are a well-known and conspicuous group of insects. Adults are relatively long-lived at up to one year, and display great agility in flight. Larval dragonflies live from one to four years and are present in a wide variety of freshwater ecosystems.

County records from the North American Odonate Database, which is maintained by the Dragonfly Society of the Americas (Abbott 2007), document 115 species in counties where the nine GLKN park units are located, or adjacent Canadian provinces (Appendix A). State records show an additional two species in these counties, bringing the total to at least 117 dragonfly species likely present in GLKN parks. The 89 larval dragonfly species confirmed from parks (Wiener et al. 2016 and the NPSpecies database) are shown in Appendix A and represent 76% of species likely present in the parks.

Libellulidae and Gomphidae are the most species-rich families of dragonflies in network parks (Figure 2). Based on state and county records, at least 16 species are ubiquitous across all nine park units. Currently, 60% of the confirmed species are known from only one or two of the nine park units, but this is likely limited by prior sampling effort. Based on North American Odonate Database records, GLKN parks appear to span both a north-south (e.g. VOYA-GRPO-ISRO versus SACN-MISS-INDU) and east-west (e.g. VOYA-GRPO-ISRO versus INDU-SLBE) biogeographic region for odonates.

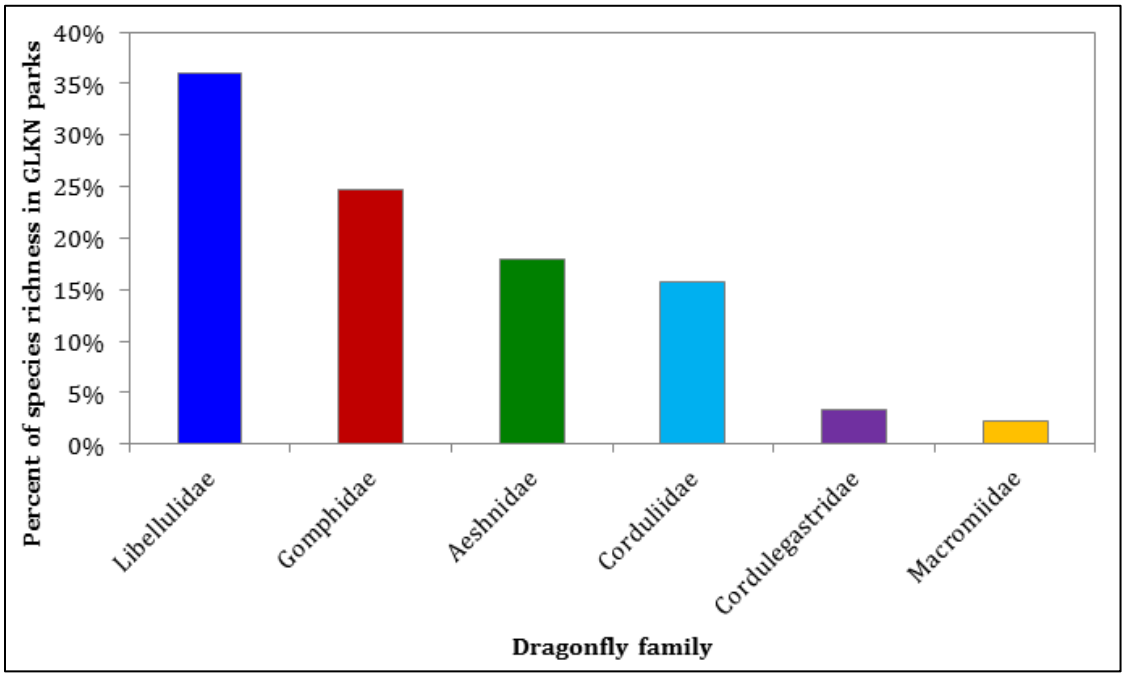


Figure 2. Proportional species richness of dragonfly families found in the nine park units of the National Park Service Great Lakes Monitoring and Inventory Network.

Several dragonflies in the Great Lakes region have special conservation status. One such species, the Hine's Emerald (*Somatochlora hineana*), is federally listed as endangered throughout its range

(Illinois, Indiana, Ohio, and Wisconsin) (USFWS 2017). The Hine's Emerald is known to occur in only one of the nine park units (INDU) and is the only federally listed odonate in the Midwest region. Seventeen additional species are state-listed as "special concern" or "threatened" by Wisconsin, Minnesota, and/or Michigan: *Aeshna sitchensis*, *A. subarctica*, *Boyeria grafiana*, *Rhionaeschna mutata*, *Gomphus lineatifrons*, *G. quadricolor*, *Ophiogomphus anomalus*, *O. howei*, *O. susbehcha*, *Stylurus amnicola*, *S. notatus*, *S. plagiatus*, *Somatochlora brevicincta*, *S. forcipata*, *S. incurvata*, *Williamsonia fletcheri*, and *W. lintneri*.

The ecology of larval dragonflies is well documented at the genus level (Corbet 1999, Tennessen 2007), yet there is a need for species-level information on life history and habitat requirements. All larval dragonflies are obligate, generalist predators. However, the type of prey encountered and their diet is a function of habitat preference and mode of habit (i.e., burrowing, climbing, or sprawling). For example, species in the families Gomphidae and Cordulegastridae are primarily burrowers that feed on benthic macroinvertebrates. Species in the family Aeshnidae are climbers that cling to vertical portions of aquatic vegetation and feed on invertebrates, including zooplankton, that inhabit the water column. These differences are probably important in defining pathways for dietary methylmercury uptake (Tremblay et al. 1996) and need to be taken into account when examining differences in mercury concentrations in larval dragonflies among sites and across GLKN parks (Nelson et al. 2015).

The structure of the dragonfly assemblage in a particular body of water is greatly affected by hydroperiod and by the presence or absence of fish. Wellborn et al. (1996) showed how hydroperiod regulates fish distribution among lentic ecosystems, which can constrain dragonfly species composition in terms of life history and behavior (i.e., activity pattern). For example, ponds inhabited by fish tend to be dominated by dragonfly species that grow rapidly as larvae, possess small terminal body size as adults, and forage as adults via sit-and-wait strategies (Tennessen 2007). Perennial waters without fish possess large-bodied, long-lived dragonfly larvae that are more prone to be active hunters.

For the most part larval dragonflies have typically only been sampled for mercury as part of larger food-web studies, in fish bioaccumulation studies, or in general surveys of macroinvertebrate body burdens (e.g., Goutner and Furness 1997, Hall et al. 1998, Mason et al. 2000, Wiener and Shields 2000, Gorski et al. 2003, Haines et al 2003, Allen et al. 2005, Chasar et al. 2009, Ward et al. 2010, and Jones et al. 2013). Ongoing research (Eagles-Smith et al. 2016) has determined their utility as national-scale biosentinels with investigations of taxonomic differences, landscape factors, and habitat features. Several characteristics and factors contribute to their usefulness as biosentinels, including the following:

1) All species are obligate predators and as such, bioaccumulate methylmercury
2) They persist and reproduce in ecosystems across a range of mercury contamination
3) Larvae are largely restricted to the aquatic systems in which they were hatched
4) Individuals of most species are large enough to provide adequate biomass for whole body analysis of methylmercury and total mercury

5) Many taxa are ubiquitous across ecosystems at the regional level
6) Most species in the western Great Lakes region are long-lived (i.e., semi- or mero voltine)
7) Larvae can be readily obtained with simple, inexpensive, and portable sampling gear
8) Larvae are robust enough for laboratory and field handling, and most mature larvae can be taxonomically identified to species level
9) There is a strong positive correlation between mercury in larval dragonflies and mercury in prey and predatory fish in GLKN lakes

Prior research in interior lakes in Voyageurs National Park (VOYA) showed that the mean methylmercury concentration in the larval gomphid dragonfly *Gomphus spicatus* (common name, dusky clubtail) was correlated with the concentration in both coexisting prey and predatory fish (Knights et al. 2005). In the summers of 2002 and 2003, larval *G. spicatus* were collected in 11 VOYA lakes sampled by the University of Wisconsin-La Crosse and the U.S. Geological Survey. Methylmercury concentrations in this species were strongly correlated with concentrations of total mercury in whole, 1-year-old yellow perch (Figure 3). *Gomphus spicatus* currently occurs in five of the nine park units in the GLKN (Appendix 1). Larvae of this species burrow in silt and are found in both lentic, littoral, and lotic depositional habitats (Tennessen 2007). Adults typically emerge in early June and are often found far from water perched under open sunlight, unless actively undergoing oviposition through mid-July (Mead 2003).

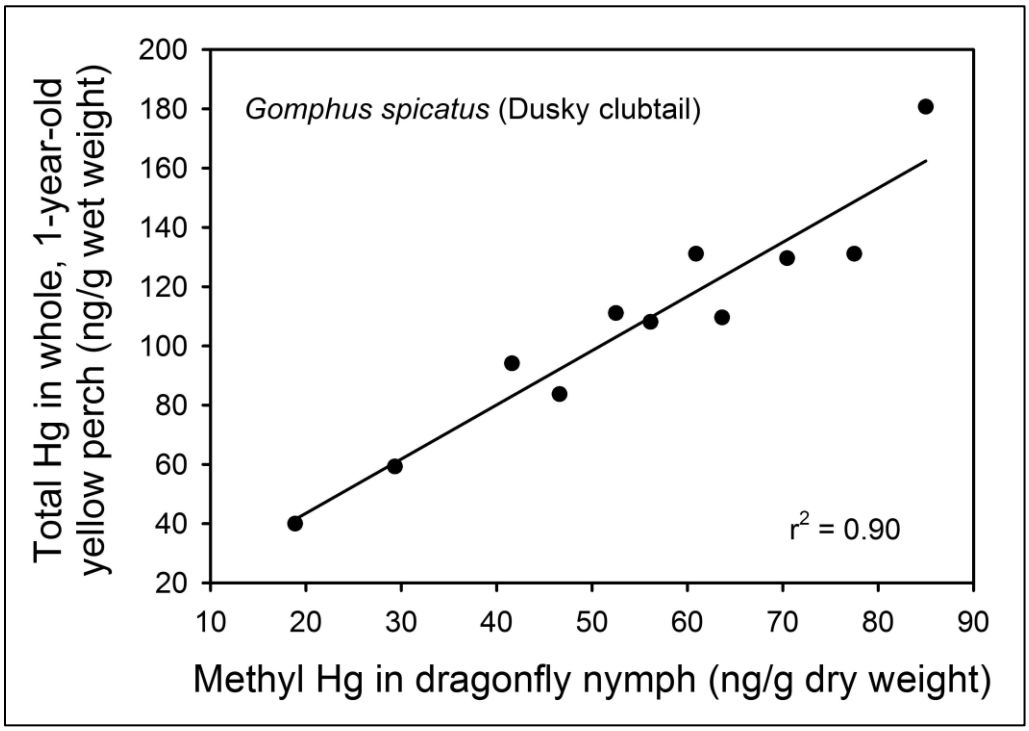


Figure 3. Relation between mean concentrations of total mercury in whole, 1-year-old yellow perch and methylmercury in whole larvae of the dusky clubtail dragonfly (*Gomphus spicatus*) from interior lakes in Voyageurs National Park sampled in 2002 and 2003. Each data point represents mean values for a single lake (from Knights et al. 2005).

Since the earlier study by Knights et al. (2005), additional research in the western Great Lakes region has also shown that larval dragonflies are useful biosentinels of methylmercury in aquatic food webs (Haro et al. 2013, Jeremiason et al. 2016, Wiener et al. 2016). Similar to Knights et al. (2005), methylmercury concentrations in larval *Gomphus* spp., a genus of dragonflies that burrows when in the larval stage, were correlated with concentrations of total and methylmercury in whole, small (<76 mm) yellow perch across 15 lakes in the northwestern Great Lakes region for samples collected during 2008–2010 (Figure 4; Haro et al. 2013). The weaker correlation observed across six parks in Haro et al. (2013) as compared to one park in Knights et al. (2005) could indicate variability in sub-regional factors (e.g., vegetation, topography, geology) that operates when a broader spatial scale is considered or variation due to inclusion of multiple *Gomphus* spp. in the later analysis.

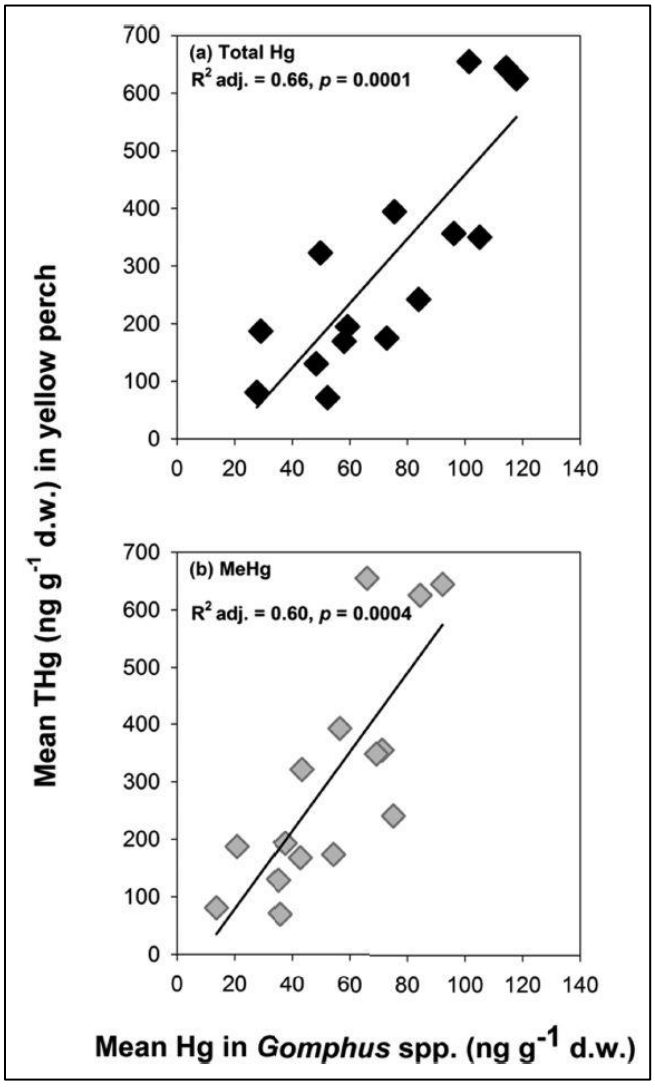


Figure 4. Linear regression between mean concentration of total mercury (THg) in small, whole yellow perch (<76 mm) and mean concentrations of (a) THg and (b) MeHg in coexisting larval *Gomphus* across 15 lakes in six national parks in the western Great Lakes region collected during 2008–2010. Data for all coexisting species of *Gomphus* were combined to estimate the unweighted mean concentrations of THg and MeHg in larvae for each lake. Figure is from Haro et al. (2013).

Importantly, Nelson et al. (2015) found that total mercury is a good proxy for methylmercury in larval dragonflies, where methylmercury was, on average, 88% ± 17% of total mercury in larval dragonflies collected from 23 national parks across the continental U.S. in 2012–2013, with percentages similar to those found in fish tissues. Wiener et al. (2016) also showed that most of the mercury present in larval dragonflies was methylmercury, with percentages ranging from 69% to 93% for samples collected from 2008–2012 at six GLKN parks. These findings indicate that analysis of larval dragonflies for total mercury is appropriate for assessing methylmercury contamination of aquatic food webs, which could represent a significant cost savings compared to that associated with analyzing methylmercury. Ongoing research (Eagles-Smith et al. 2016) is refining interpretation of these biosentinel data to determine which taxonomic differences are important to quantify.

Aquatic invertebrates such as dragonfly larvae are also important food sources and vectors for contaminant exposure to terrestrial food-webs. Recent studies have shown that some insectivorous passerine songbirds that feed in wetland habitats are at risk of methylmercury exposure (Gann et al. 2015, Jackson et al. 2015). Dragonflies emerging as adults from fishless marshes and bogs provide a potentially important pathway for dietary exposure of terrestrial insectivores to methylmercury (Chumchal and Drenner 2015).

1.4.2 Prey Fish

Small prey fish are one of three target biosentinel organisms for this protocol, and 1-year-old yellow perch (*Perca flavescens*) is the preferred target prey fish for parks in the GLKN. Prey fish are generally defined here as small finfish that are consumed whole by predatory fish and aquatic wildlife, such as common loons (*Gavia immer*). In concept, they can be small-bodied adult fish (e.g., minnows, darters), or juveniles of species that grow to larger sizes (e.g., perch, bass, walleye), but the focus in this protocol will be on juvenile yellow perch.

The advantages and rationale for the use of small prey fish as biosentinels in aquatic monitoring and assessment programs for mercury and other bioaccumulative contaminants have been discussed in detail (Yeardley 2000, Lazorchak et al. 2003, Wiener et al. 2007, and Choy et al. 2008). To summarize the rationale: prey fish are widely distributed, common, and important in the transfer of methylmercury to organisms in higher trophic levels, such as piscivorous fish and many fish-eating birds. Moreover, interpreting the ecological consequences of varying methylmercury concentrations in prey fish of uniform age is less susceptible to potentially confounding factors, such as variation in trophic position or size, than are concentrations in long lived, piscivorous or omnivorous fish.

The mean concentration of total mercury in prey fish has been shown to be a useful indicator of methylmercury contamination in food webs supporting sport fishing and wildlife (Ackerman et al. 2015). Thus, the analysis of prey fish provides information relevant to food web contaminant dynamics as well as to the public and the policy community, park managers, and planners. Moreover, due to their abundance, the effects of sampling prey fish on target fish populations would be insignificant in all but the very smallest water bodies.

The analysis of whole prey fish provides ecologically relevant data on whole body concentration and burden. Mercury burden, defined as the total mass of mercury accumulated in a whole fish, is

calculated as the product of body weight and whole-body concentration. In age-1 prey fish, burden is an ecologically relevant measure of bioaccumulation, representing the mass of methylmercury accumulated by a fish during its year of residence in the sampled water body. The burden also represents the mass of mercury that a predator would ingest when eating the prey fish.

Preferred prey fish biosentinels: *Whole, 1-year-old yellow perch.* Analyses of total mercury in yellow perch provide a useful measure of methylmercury concentrations in food webs and of associated risk to piscivorous fish and wildlife in the Great Lakes region (Wiener et al. 2012). Nearly all of the mercury in yellow perch is methylmercury ; on average methylmercury accounts for 99% of the total mercury in yellow perch axial muscle tissue (Grieb et al. 1990, Bloom 1992) and 95% or more of the total mercury in whole yellow perch (Hammerschmidt et al. 1999, Drysdale et al. 2005, Van Walleghem et al. 2007). This species is generally abundant in lentic waters throughout the Great Lakes region (Wiener and Eilers 1987, Kallemeyn 2000, Kallemeyn et al. 2003), and its geographic distribution extends across much of the northcentral, northeastern, and eastern United States, as well as central and eastern Canada (Scott and Crossman 1973, Becker 1983). During their first year, yellow perch have a small gape (jaw opening), which limits their diet largely to small zooplankton and small zoobenthos (Roseman et al. 1996, Lyons et al. 2000). Thus the trophic position of age-1 yellow perch is not expected to vary substantially among aquatic sites.

The yellow perch is a preferred prey of certain piscivores, such as walleye (*Sander vitreus*), northern pike (*Esox lucius*), and common loons, and is therefore an important link in the food web transfer of methylmercury (Colby et al. 1979, Barr 1996, Soupir et al. 2000). Concentrations of total mercury in small yellow perch are strongly and positively correlated with concentrations in co-existing piscivorous fish, including walleye, black bass (*Micropterus* spp.), and northern pike in the GLKN region (Cope et al. 1990, Suns et al. 1987). In Voyageurs National Park, for example, concentrations of mercury in the axial muscle tissue of 55-cm northern pike were strongly correlated with those in co-existing 1-year-old yellow perch (Figure 5). Moreover, statistical analyses have shown strong relations between the total mercury concentration in age-1 yellow perch and ecosystem characteristics (e.g., lake chemistry, wetland influence) or perturbations (e.g., water-level fluctuations, experimental acidification) that are known to influence the production of methylmercury and its abundance in aquatic food webs (Suns and Hitchin 1990, Grieb et al. 1990, Wiener et al. 1990, Simonin et al. 1994, Frost et al. 1999, Sorensen et al. 2005, Wiener et al. 2003, 2006).

Substantial recent data on mercury concentrations in yellow perch (including age-1 and young-of-the-year fish) are available for inland lakes in Isle Royale National Park (Gorski et al. 2003) and Voyageurs National Park (Sorensen et al. 2005, Wiener et al. 2006, and Brigham et al. 2014). Recent data from these and four additional parks in the GLKN are also available on mercury in larger yellow perch and northern pike (Kallemeyn 2000, Gorski et al. 2003, Knights et al. 2005, Drevnick et al. 2007, Wiener et al. 2016), a widespread, largely piscivorous fish that feeds heavily on yellow perch.

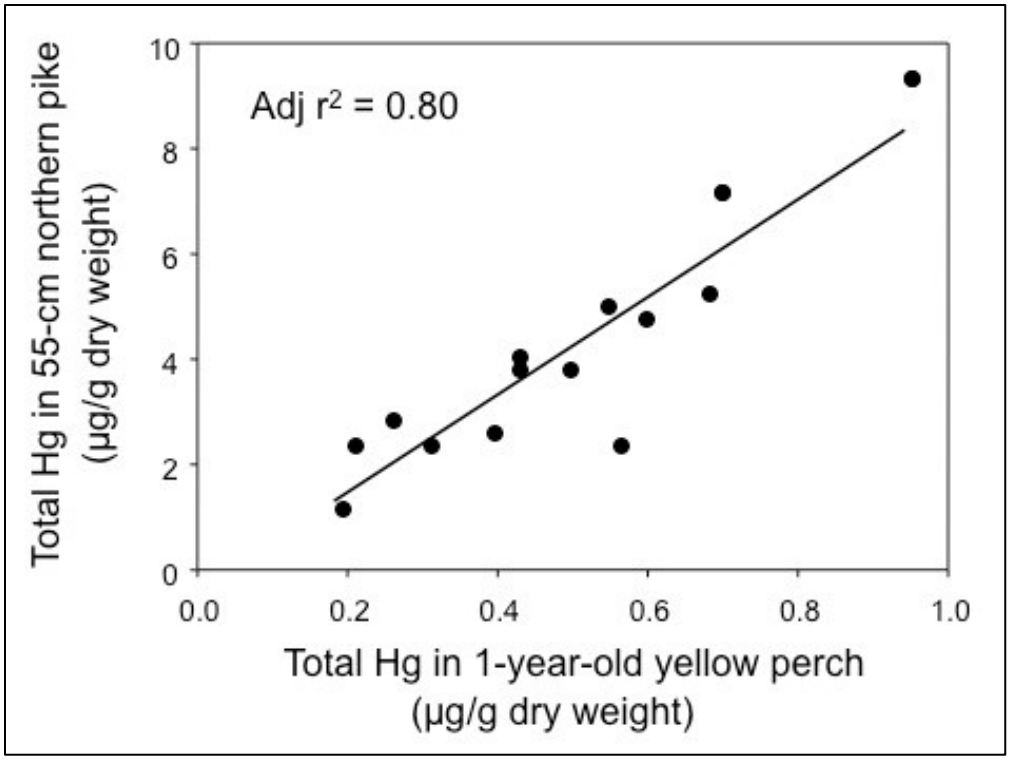


Figure 5. Relation between the estimated concentrations of total mercury in axial muscle (skinless fillets) of 55-cm northern pike and mean concentrations in whole age-1 yellow perch from interior lakes in Voyageurs National Park, with concentrations expressed on a dry weight basis. Each data point represents a single lake sampled during 2001–2003 (from Knights et al. 2005).

One-year-old yellow perch can be sampled in spring with portable active or passive gears fished in littoral habitat without significantly affecting their abundance or year-class strength. Age-1 yellow perch sampled in spring have resided in the sampled water body for about one year. The age of small yellow perch can be accurately determined by examining scales taken near the area of insertion of the left pectoral fin (DeVries and Frie 1996).

Alternative prey-fish species: Because some of the water bodies to be sampled do not contain yellow perch, alternate small-bodied prey fish are required for mercury analysis. Moreover, attempts to obtain small yellow perch from particular water bodies in which they reside are not always successful, due to their low abundance. In such cases, other prey fishes will be sampled and retained for analysis, using the same age and gape criteria applied to age-1 yellow perch. Other prey fish species that are generally widespread and often abundant in waters of the Great Lakes region include members of the following families: Centrarchidae (sunfishes), Cyprinidae (minnows, shiners, and daces), Umbridae (mudminnows), Cottidae (sculpins), and Percidae (perch and darters). Target alternative prey fishes for this project will include the following species:

Green Sunfish:	*Lepomis cyanellus*
Bluegill:	*Lepomis macrochirus*
Pumpkinseed:	*Lepomis gibbosus*

Rock Bass:	*Ambloplites rupestris*
Blacknose Dace:	*Rhinichthys atratulus*
Longnose Dace:	*Rhinichthys cataractae*
Northern Redbelly Dace:	*Phoxinus eos*
Creek Chub:	*Semotilus atromaculatus*
Fathead Minnow:	*Pimephales promelas*
Central Mudminnow:	*Umbra limi*
Mottled Sculpin:	*Cottus bairdii*
Slimy Sculpin:	*Cottus cognatus*
Johnny Darter:	*Etheostoma nigrum*
Iowa Darter:	*Etheostoma exile*
Brook Stickleback:	*Culaea inconstans*

1.4.3 Predatory Fish

For this protocol predatory fish are defined as those in upper trophic levels of aquatic food webs, particularly predatory (sport) fishes that are largely piscivorous as adults and that are commonly sought by anglers. The analyses of axial muscle (skinless fillets) from adult predatory fishes provide information on concentrations of mercury in fish tissue that are commonly eaten by humans. Accordingly, analyses of total mercury in edible fillets of predatory fish provide information useful for human health-risk assessment.

Preferred predatory fish biosentinel: *Adult northern pike*. Northern pike are widely distributed and often abundant in inland lakes, rivers, and streams of the Great Lakes region (Wiener and Eilers 1987, Kallemeyn 2000, Kallemeyn et al. 2003). Moreover, the geographic distribution of northern pike in the northern hemisphere is circumpolar, extending across Russia, Scandinavia, Norway, Europe, and the British Isles providing a wide range of opportunities for comparative analyses (Scott and Crossman 1973). Northern pike, which are largely piscivorous as adults, are a popular sport fish in inland waters of the Great Lakes region that are often caught by anglers and eaten, including those caught at GLKN parks.

The analyses of total mercury in edible fillets of northern pike will not only provide information for assessing the status of fish in park waters, but will also be useful to state agencies for risk assessment and possible issuance of fish-consumption advice for the species and sampled surface waters. Because the concentrations of total mercury in axial muscle are strongly correlated with those in blood (Schmitt and Brumbaugh 2007), the concentrations of total mercury in the axial muscle of predatory fish provide a toxicologically relevant indicator of methylmercury exposure.

Northern pike can be readily sampled by angling, electrofishing, and gill netting. If attempts to obtain adult northern pike from a given water body are unsuccessful, or if northern pike are not present in a sampled water body, we will attempt to obtain another species of predatory or other adult fish from

that water body for analysis of mercury. Other fishes that could be sampled and analyzed in place of northern pike include the following species, ranked in order of diet similarity:

Grass Pickerel:	*Esox americanus*
Walleye:	*Sander vitreus*
Smallmouth Bass:	*Micropterus dolomieu*
Largemouth Bass:	*Micropterus salmoide*s
Yellow Perch (large):	*Perca flavescens*
Black Crappie:	*Pomoxis nigromaculatus*
Bluegill:	*Lepomis macrochirus*
Rock Bass:	*Ambloplites rupestris*
Black Bullhead:	*Ameiurus melas*
Rainbow Trout:	*Oncorhynchus mykiss*
Gizzard Shad:	*Dorosoma cepedianum*
Common Carp:	*Cyprinus carpio*
Creek Chub:	*Semotilus atromaculatus*

1.5 Monitoring and Assessment Questions

The following questions emanate from the monitoring and assessment objectives listed in Section 1.2 (Goals and Objectives) and from questions frequently raised in our communications with managers of federal lands and natural resources:

1) Which park units and surface waters within the GLKN have concentrations of mercury in dragonfly larvae and fish high enough to pose a risk to humans and wildlife in upper trophic levels?
2) What are the spatial patterns in mercury contamination in dragonfly larvae and fish within parks of the GLKN?
3) What is the general direction and magnitude of change in the concentration of mercury in dragonfly larvae and fish in parks of the GLKN?

The first two questions are closely related to the identification of *biological mercury hotspots*, which Driscoll et al. (2007) defined as locations on the landscape that, compared with the surrounding landscape, are characterized by elevated concentrations of methylmercury in biota that exceed criteria for protection of human health or wildlife, as determined by a statistically adequate sample size. The analysis of spatial and temporal patterns in methylmercury concentrations in biosentinel organisms is an important first step in the identification of watershed, aquatic, and human factors that control or influence the abundance of methylmercury and the associated exposure of biota within the park units.

Examples of studies where spatial or temporal patterns in mercury concentrations in dragonfly larvae, and prey and/or predatory fish were examined in one or more GLKN parks are shown in

Table 1. Additional examples are provided in Lafrancois and Glase's (2005) compilation of aquatic studies in GLKN parks.

Table 1. Examples of studies that explore spatial and temporal variations in total mercury (THg) in larval dragonflies, prey fish, and/or predatory fish in Great Lakes Network parks.

Source of variation examined	Biosentinel organism	Data set	Reference
Spatial (lake and watershed factors)	1-year-old yellow perch	17 interior lakes, VOYA (sampled 2001–2003)	Wiener et al. 2006
Spatial (regional atmospheric deposition, lake and watershed factors)	Late-instar larval dragonflies	23 water bodies, six national parks (sampled 2008–2012)	Wiener et al. 2016[1]
Spatial (regional deposition, lake and watershed factors)	Whole prey fish	23 water bodies, six national parks (sampled 2008–2012)	Wiener et al. 2016[1]
Spatial (regional deposition, lake and watershed factors)	Axial muscle tissue predatory fish	23 water bodies, six national parks (sampled 2008–2012)	Wiener et al. 2016[1]
Spatial (regional atmospheric deposition, lake and watershed factors)	Larval dragonflies	23 national parks (sampled 2013)	Nelson et al. 2015
Temporal (water-level fluctuation)	1-year-old yellow perch	Sand Point Lake, VOYA (sampled 1991–2003)	Sorensen et al. 2005
Temporal (regional atmospheric deposition, lake and watershed factors)	1-year-old yellow perch	4 interior lakes, VOYA (sampled 2000–2012)	Brigham et al. 2014
Temporal (water-level fluctuation)	1-year-old yellow perch	6 large lakes, VOYA (sampled 2001–2010)	Larson et al. 2014

[1]These three examples are from a single study that focused on multiple biosentinel organisms.

Many site and habitat characteristics influence concentrations of methylmercury in biota, and the interpretation of spatiotemporal patterns in biosentinel data will be enhanced by considering relevant information about the sampled surface waters and their watersheds (for a review, see Wiener et al. 2007). Important atmospheric metrics for monitored parks include deposition of total mercury and sulfate, and annual rainfall. Data on sulfate and total mercury in wet deposition are available from the National Atmospheric Deposition Program and the Mercury Deposition Network for these systems, respectively (NADP 2017). Watershed metrics include total watershed area and land cover, particularly the abundance of hydrologically connected wetlands. Useful metrics for lentic systems include morphometry (area, maximum depth, mean depth, percent littoral area), water level fluctuations, depth profiles of temperature and dissolved oxygen during summer, and hydrologic type (e.g., seepage or drainage lake). Useful physicochemical metrics for water include dissolved organic carbon or color, pH, sulfate, chlorophyll, acid neutralizing capacity, phosphorus, and Secchi disk depth.

Many of these physicochemical and morphometric parameters are being monitored annually in selected water bodies in park units of the GLKN (Elias et al. 2015, Magdalene et al. 2016). To the extent feasible, the sampling sites in this protocol will be co-located with sampling sites currently monitored by the GLKN's water quality monitoring program (see Section 2.2).

2. Monitoring and Assessment Strategy

This protocol provides a framework for monitoring and assessing spatial patterns and temporal trends in all nine GLKN parks. With this protocol we are building upon a compilation of mercury data collected through implementation of an earlier version of this protocol (focused on assessing trophic bioaccumulation for a wider suite of contaminants in fish, larval dragonflies, and other media), a protocol for monitoring the same contaminants suite but in nestling bald eagles (Route et al. 2009), and monitoring specific to mercury in larval dragonflies that began in 2012 and is ongoing (Nelson et al. 2015, Eagles-Smith et al. 2016).

This protocol contains two basic components: (1) spatial analysis and (2) trend analysis. The spatial analysis, which addresses Objectives 1 and 2 (Section 1.2), will assess spatial patterns in mercury in larval dragonflies and fish to identify parks and surface waters within the GLKN where exposure may pose a risk to fish, wildlife, and humans. The trend analysis, which addresses Objective 3 (Section 1.2), will examine temporal patterns in mercury for parks and individual water bodies. A variety of reliable historical data on mercury in water, sediment, soil, seston, zooplankton, larval dragonflies, fish, and bald eagle nestlings are available for most of the GLKN (Table 1; also see Gorski et al. 2003, Lafrancois and Glase 2005, Knights et al. 2005, Drevnick et al. 2007, Route et al. 2011, and Rolfhus et al. 2011, 2015). Inclusion of these historical data with the "new" data obtained through this protocol will extend the trend analysis for mercury for many of the parks by several years.

2.1 Sampling Schedule

Dragonfly larvae will be sampled annually and prey and/or predatory fish every five years at all nine GLKN parks. In order to maximize efficiency, many parks will collect larval dragonflies during previously scheduled GLKN water quality monitoring, typically during June-August. This schedule is consistent with park and citizen science based larval dragonfly monitoring (Nelson et al. 2015, Eagles-Smith et al. 2016) that has taken place annually at GLKN parks since 2012. From 2008 through 2012 larval dragonflies were sampled annually during April through early June, targeting larger larvae (late instars), which were expected to emerge in a few weeks as winged adults (Wiener et al. 2016). That monitoring was conducted by field crews solely focused on monitoring mercury in a number of media (water, sediment, and biota) and not concurrent with other monitoring (e.g., water quality). Regardless of the timing, field collections of larval dragonflies will focus on larger (length >15 mm) larvae, and include at least three individuals from each family collected.

Fish will be sampled every five years at each park, on a rotation of two GLKN parks per year, except for the fifth year when only one park will be sampled. Although this protocol contains rationale and methodology for sampling both prey and predatory fish, sampling predatory fish will be a higher priority than prey fish. Predatory fish typically have higher levels of mercury than prey fish, are more likely to be caught and eaten by people, and from the perspective of protection of human health are of higher interest for monitoring. However, some water bodies like Legion Lake at PIRO only contain prey fish (Weiner et al. 2016), or in some cases continuation of previously established long-

term monitoring of prey fish may be more desirable (e.g., young-of-year yellow perch from Sand Point Lake at VOYA).

When prey fish are targeted they will be collected in the spring a few weeks after ice out, when growth is slow and temporal variation in mercury concentration is small, to obtain data that are temporally and spatially comparable. Spring sampling of prey fish is imperative because the size of young prey fish varies less at that time of the year. Small prey fish typically grow rapidly during the summer in temperate or southern boreal waters, increasing their biomass by 2-to 5-fold during the growing season (April/May-November). Mercury concentrations are usually increasing or stable during this period of growth; thus, the total body burden (mass) of mercury in individual fish increases substantially during summer (Bodaly and Fudge 1999, Gorski et al. 1999). The timing of within-season sampling of predatory fish is less of a concern, although year-to-year consistency is desirable. Sampling in the early spring would be comparable to that by Wiener et al. (2016) in GLKN lakes from 2008–2012.

2.2 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 (Section 1.2), without jeopardizing the safety of field personnel. To reiterate, dragonfly larvae will be sampled annually at all sites, and prey or predatory fish at some or all of the same sites every five years. It is clear from past work that we cannot address the objectives for all surface water resources in a park or across parks in a statistically adequate manner while staying within our budget. Answering questions about surface water resources within a park or across the GLKN 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, as GLKN parks contain over 1000 lakes, 129 of which are named, 790 miles of perennial streams, 146 miles of intermittent streams, and countless wetlands (Lafrancois and Glase 2005). A random selection of aquatic sites is not desirable because many are inaccessible and would require more than a day of off-trail, backcountry travel to reach.

This realization led to the design that will best provide for assessments of individual aquatic sites. Taking into account GLKN staffing (Section 5.1) and budget (Section 6.3) and constraints, we will sample four aquatic sites per park annually. Three sites from each park will be revisited annually, and the fourth site will be rotated from one year to the next. By having a rotating site we will be able to obtain a better understanding of mercury in surface waters throughout the park, and respond quickly with increased monitoring and/or health advisories if mercury “hot spots” are discovered. From one year to the next the actual locations of the rotating sites will largely be at the discretion of park natural resource managers, who will take into account site selection factors previously described, and other management considerations such as level of recreational fishing, desire to monitor mercury in fishless surface waters, etc.

The selection of sites for annual dragonfly larvae and every-five-year fish resampling within each park unit (Table 2) incorporated substantial input from park personnel as well as the following general criteria: (1) to select study sties with available historical mercury data (Table 1) and pertinent ancillary information on the biological, physical, and chemical characteristics of aquatic resources,

(2) to maximize spatial overlap between mercury monitoring and other aquatic monitoring by GLKN, and (3) to reduce logistical obstacles to sampling. Information such as pH, temperature, dissolved oxygen, water clarity (measured with Secchi disk, transparency tube, or levels of total suspended solids), major ions, nitrogen, phosphorus, bathymetry, water level, hydrology, and landscape variables (drainage area, forest type, and wetlands area) for lakes or rivers routinely monitored for water quality by GLKN can be found in Elias et al. (2015) or Magdalene et al. (2016). For sites not routinely monitored for water quality this information is also available from a variety of reports (e.g., Kallemeyn et al. 2003, Wiener et al. 2016).

Table 2. Fixed monitoring sites for mercury in larval dragonflies and fish across Great Lakes Network sites. R.P. = regional park.

Park	Site Name	Latitude	Longitude
APIS	Little Sand Bay Lagoon	46.94794	-90.885679
	Michigan Lagoon	46.87720	-90.512139
	Outer Lagoon	47.00431	-90.460625
GRPO	Grand Portage Creek (lower reach)	47.96500	-89.684253
	Poplar Creek (south branch)	47.98462	-89.759674
	Snow Creek (lower reach)	47.97654	-89.799211
INDU	Great Marsh	41.67910	-86.987694
	Middle Lagoon	41.61526	-87.272751
	Miller Woods West Pond	41.60722	-87.273074
ISRO	Lake Harvey	48.05043	-88.796117
	Lake Richie	48.04194	-88.699115
	Sargent Lake	48.09039	-88.665195
MISS	Mississippi River/N. Mississippi R.P.	45.04266	-93.282040
	Pickerel Lake/Lilydale R.P.	44.91913	-93.121140
	Lake Rebecca/Jaycee R.P.	44.74637	-92.858480
PIRO	Beaver Lake	46.57220	-86.334800
	Grand Sable Lake	46.64920	-86.032600
	Legion Lake	46.52630	-86.361000
SLBE	Bass Lake (Benzie County)	44.73414	-86.064136
	Bass Lake (Leelanau County)	44.92221	-85.883276
	Loon Lake	44.70747	-86.129251

Table 2 (continued). Fixed monitoring sites for mercury in larval dragonflies and fish across Great Lakes Network sites. R.P. = regional park.

Park	Site Name	Latitude	Longitude
SACN	Namekagon River (Phipps Landing)	46.07200	-91.416856
	Namekagon River (Earl Landing)	45.91588	-91.764180
	St. Croix River (Norway Point)	45.92586	-92.63865
VOYA	Brown Lake	48.51635	-92.796405
	Peary Lake	48.52480	-92.771546
	Ryan Lake	48.51882	-92.707356

2.3 Sample Size and Level of Change That Can Be Detected

On average, analysis of 20 larval dragonflies, 20 prey fish, and 35 predatory fish from a given water body and year will allow for an 80% probability of detecting a 20% change in mean mercury concentration over 10 years with a Type I error of 0.05. This is based on 1) our power analysis (Appendix B) using data collected from 2008–2012 by Wiener et al. (2016), 2) a power analysis completed for an earlier version of this monitoring protocol (Weiner et al. 2009), and 3) that by Haro et al. (2013) on a subset of the 2008–2012 data described in Wiener et al. (2016).

We conducted a power analysis using mercury data collected from 2008–2012 in dragonfly larvae (n= ca. 2,600), prey fish (n= ca. 3,200), and predatory fish (n= ca. 1,400) across six GLKN parks (Appendix B). The purpose was to ascertain the appropriate number of dragonfly larvae, prey fish, and predatory fish samples to collect annually under a variety of scenarios (i.e., number of years, percent change, Type I error, and Type II error [power of the test]). Historically, for trend analysis of a given vital sign in the GLKN, the proposed (*a priori*) objective has been a sampling design that will have an 80% probability (i.e., power) of detecting a 20% change in the measured value over 10 years with a Type I error (α) of 0.10 (Route and Elias 2007).

In brief, three different power analyses were conducted in R (R Core Team 2016) for total mercury in dragonfly larvae and fish. The analyses included: 1) a regression power analysis on the linear trend across years (temporal), 2) an analysis of variance (ANOVA) power analysis on the difference among years (temporal), and 3) an ANOVA power analysis on the difference among units (spatial). Each of the above analyses was conducted for all six parks, for each park, and for each water body within each park. Of the three different analyses, the most conservative analysis to detect change through time for each park is the ANOVA power analysis for differences among years, which in the absence of a linear trend would detect differences among years over the course of 10 years.

Through the ANOVA power analysis on the difference among years for each park, and using 80% probability of detecting a 20% change in mean mercury concentration over 10 years with a Type I error of 0.05 (slightly more conservative than that proposed above), for the six park units on average 14 larval dragonflies, 13 prey fish, and 32 predatory fish would need to be collected for each water

body, annually (Table 3). Unlike the power analysis by Haro et al. (2013), we did not restrict our power analysis to a given dragonfly family, or species of prey or predatory fish, except predatory fish at GRPO, where only rainbow trout were collected. Nor did we account for variation length or weight of larval dragonflies or fish. Therefore, except for predatory fish at GRPO, estimates—especially for predatory fish at other parks—are very conservative and in reality are likely to be lower.

Table 3. Number of annual samples per water body by park necessary to detect a 20% change in total mercury over 10 years with Type I error of 0.05 and 80% power for three types of biosentinel organisms. Data used in analysis are from Wiener et al. (2016). Each category of biosentinel organism (larval dragonflies, prey fish, and predatory fish) represents a mix of species, except predatory fish at GRPO, where only rainbow trout were collected. Park acronyms as previously described.

Park	# of Larval Dragonflies	# of Prey Fish	# of Adult Predatory Fish
GRPO	27	20	4
INDU	4	8	8
ISRO	11	14	65
PIRO	12	15	31
SLBE	16	4	57
VOYA	16	14	28
Average	**14**	**13**	**32**

In an earlier version of this monitoring protocol, 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 mercury concentration. Additionally, 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 mercury 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.

There are tradeoffs in sampling to be able to detect a 1%, 5%, 10%, or 20% change with 5, 10, or 20 years of annual sampling. This is illustrated in Table 4, which shows the number of larval dragonfly samples needed annually to detect change for a variety of scenarios and years, based on total mercury in larval dragonflies from Angleworm Lake at Isle Royale National Park (Wiener et al. 2016). For example, with an 80% probability of detecting a 20% change in mean mercury concentration over 10 years with a Type I error (α) of 0.05, we would need to collect and analyze at least 13 individual larval dragonflies from the lake annually, compared to 197 for an 80% probability of detecting only a 5% change.

Table 4. Number of larval dragonflies to be collected annually depending on number of monitoring years and percent change detectable in total mercury with power to detect the change at 80% and a Type 1 error of 0.05 for Angleworm Lake at Isle Royale National Park (data collected from 2008–2012 in six GLKN parks; power analysis details in Appendix B).

# of Years	Percent Change	# of Larval Dragonflies
5	1	13877
	5	556
	10	139
	20	35
10	1	4910
	5	197
	10	50
	20	13
20	1	2405
	5	97
	10	25
	20	7

To reiterate, based on our power analysis (Appendix B) and that by Weiner et al (2009) and Haro et al. (2013), individual analysis of 20 dragonfly larvae, 20 prey fish, and 35 predatory fish from a given water body and year will allow for an 80% probability of detecting a 20% change in mean mercury concentration over 10 years with a Type I error of 0.05.

Because larval dragonflies will be sampled at all nine GLKN parks annually, application of the findings of the power analysis is most appropriate to dragonfly larvae, rather than fish that will be sampled at each park every five years. Through the power analysis we found that on average 20 larval dragonflies should be collected annually from each water body to allow for an 80% probability of detecting a 20% change in mean mercury concentration over 10 years with a Type I error of 0.05. However, these sample sizes may not be adequate for every park or water body (e.g., 27 larval dragonflies at GRPO as shown in Table 3).

The power analysis gives us a sense of, on average, how many prey (n=20) and predatory (n=35) fish would need to be sampled annually per water body using the same power assumptions as larval dragonflies. However, because of budgetary and logistical constraints, fish will only be collected every five year at each park. Moreover, recent fish collection efforts at GLKN parks by park staff or park partners (i.e., the same field crews that will collect fish using this protocol) have shown that while collection of 20 prey fish per water body is logistically feasible, sampling 35 predatory fish per water body is not, and that 15 predatory fish per water body is a more realistic goal (Collin Eagles-Smith, pers. comm.).

To conclude, we will collect **20 larval dragonflies** per water body annually, and every five years collect **20 prey or 15 predatory fish** per water body, with the understanding that for assessment of temporal mercury trends in fish, data collected through this protocol would need to be paired with past fish mercury data from the same water bodies collected and analyzed with comparable methodology.

2.4 Guidelines for Assessing Ecological and Health Risks of Mercury

This monitoring effort is designed to document spatial and temporal patterns in concentrations of mercury in selected biosentinel organisms, providing an indicator of mercury contamination of aquatic food webs—the primary pathway for exposure of fish and wildlife. This protocol will not directly examine effects of methylmercury exposure on wildlife or humans in the parks. However, we will compare the concentrations of mercury in biosentinel organisms to estimated threshold values in published field and laboratory studies (below) in which causal relations have been documented. Unlike mercury in sport fish, mercury in larval dragonflies has not yet been related to human or wildlife health. However, Haro et al. (2013) found that total and methylmercury in larval dragonflies could be used to predict total mercury in both prey (Figure 4) and predatory fish (R^2=0.70, p=0.0001; R^2=0.63, p=0.002; using total and methylmercury in *Gomphus* spp. from 13 lakes, respectively). Ongoing research by Nelson et al. (2015), Eagles-Smith et al. (2016), and that conducted through this protocol will continue to explore the connection between mercury in dragonfly larvae and fish to better inform the potential ecological and health risks of mercury based on concentrations in larval dragonflies.

For methylmercury, we will use two approaches to assess the potential adverse ecological effects of mercury quantified in fish from the park units. The first is the tissue residue-effects approach, which uses the concentration of mercury in whole fish or axial muscle tissue as a metric to assess the effect of methylmercury exposure on the analyzed organism. The tissue residue-effects approach has been reviewed in detail, and is considered valid for risk assessments of methylmercury (Adams et al. 2011, McElroy et al. 2011, and Sappington et al. 2011). The second ecological assessment approach, applied to mercury concentrations measured in whole prey fish, uses dietary threshold or benchmark concentrations above which methylmercury causes ecologically significant adverse effects in piscivorous fish and wildlife, for which consumption of prey fish is the dominant pathway for methylmercury uptake and exposure (e.g., Depew et al. 2012a, 2012b, 2013). To assess the potential for harmful methylmercury exposure in humans who eat fish from park waters, we will compare total mercury levels in axial fillets of adult fish to the U.S. Environmental Protection Agency's tissue residue criterion for methylmercury (300 ng/g wet weight; Borum et al. 2001).

2.4.1 Benchmarks for Assessing Mercury Risk to Fish

The concentration of mercury in the axial muscle of adult fish is strongly correlated with that in blood (Schmitt and Brumbaugh 2007), enhancing the toxicological relevance of the mercury concentration in axial muscle as an indicator of methylmercury exposure in ecological risk assessment.

To assess the potential for adverse effects of methylmercury exposure on adult fish, we will compare concentrations measured in their axial muscle tissue to a published benchmark concentration

associated with sub-lethal and reproductive effects in fishes. For adult fish, we will use a benchmark value of 500 ng/g wet weight (ww) in axial muscle, based on a critical review of mercury concentrations associated with altered biochemical processes, damage to cells and tissues, and impaired reproduction in fish (Sandheinrich and Wiener 2011). This estimated benchmark is in agreement with the findings of Dillon et al. (2010), who used data from laboratory experiments to develop a dose-response curve for adult fish based on biological endpoints, such as reproductive failure, that are directly related to mortality or are biologically equivalent to mortality. Dillon et al. (2010) estimated that a whole-body concentration of 300 ng/g ww (equivalent to a ww concentration of 500 ng/g in axial muscle) would, on average, injure 8% of the fish.

For adult piscivorous fish, we will also compare concentrations measured in coexisting prey fish to a published benchmark concentration of dietary methylmercury that are associated with reproductive effects in fishes. Reproduction of fish is affected at much lower dietary concentrations of methylmercury than other biological endpoints, such as growth, behavior, and mortality (Dillon et al. 2010, Depew et al. 2012a). We will compare concentrations of total mercury in whole prey fish to an estimated dietary threshold (40 ng/g ww in prey fish) associated with reproductive effects of methylmercury on piscivorous fish (Depew et al. 2012a, 2013). Most (generally ≥95%) of the mercury in whole prey fish is present as methylmercury (Hammerschmidt et al. 1999, Drysdale et al. 2005, Van Walleghem et al. 2007, Greenfield and Jahn 2010); therefore, our analyses for total mercury will yield valid estimates of the methylmercury concentration in whole prey fish.

Some of the water bodies sampled by Wiener et al. (2016) did not yield long-lived piscivorous fishes. To assess risks of methylmercury exposure to whole prey fish at these sites, we will use a whole-body concentration of 200 ng/g ww, an estimated tissue threshold associated with sublethal and reproductive effects in fish (Beckvar et al. 2005). Whole-body concentrations below this threshold value are not expected to adversely affect the growth, reproduction, or survival of freshwater fish. Benchmark concentrations used to assess ecological risks of methylmercury exposure to fish are summarized in Table 5.

Table 5. Concentrations of mercury in fish used as benchmarks to assess risks of methylmercury (MeHg) to fish, piscivorous wildlife, and humans.

Risk group or species	Biological indicator used	MeHg exposure indicated	Benchmark concentration (ng/g wet weight)1	Adverse effect	Reference
Prey fish	whole prey fish	bioaccumulation in whole-body	200	health and reproduction (threshold)	Beckvar et al. 2005
Sport fish	axial muscle (adult fish)	bioaccumulation in muscle tissue	500	health and reproduction	Sandheinrich and Wiener 2011, Dillon et al. 2010
	whole prey fish	concentration in diet	40	reproductive effects (threshold)	Depew et al. 2012b
Belted kingfisher	whole prey fish	concentration in diet	30	health and reproduction (threshold)	Lazorchak et al. 2003
American mink	whole prey fish	concentration in diet	70	health and reproduction (threshold)	Lazorchak et al. 2003
Common loon	whole prey fish	concentration in diet	100	behavior (threshold)	Depew et al. 2012a
	whole prey fish	concentration in diet	180	significant reproductive effects	Depew et al. 2012a
	whole prey fish	concentration in diet	400	complete reproductive failure	Depew et al. 2012a
Humans	axial muscle (adult fish)	concentration in diet	300	Health	Borum et al. 2001

[1] Concentrations are in nanograms per gram (ng/g), which are equivalent to parts per billion.

2.4.2 Benchmarks for Assessing Mercury Risk to Piscivorous Wildlife

Several piscivorous birds and mammals occur in GLKN parks. Avian piscivores that nest in the western Great Lakes region include osprey (*Pandion haliaetus*), bald eagle (*Haliaeetus leucocephalus*), common loon, common merganser (*Mergus merganser*), hooded merganser (*Lophodytes cucullatus*), red-breasted merganser (*Mergus serrator*), common tern (*Sterna hirundo*), belted kingfisher (*Ceryle alcyon*), American bittern (*Botaurus lentiginosus*), and great blue heron (*Ardea herodias*). Mammalian piscivores in the area include American mink (*Mustela vison*) and river otter (*Lontra canadensis*).

We will use the belted kingfisher, common loon, and American mink as model piscivores to assess potential ecological risks of methylmercury in whole prey fish to fish-eating wildlife. Each of these species is distributed across much of the United States and Canada, and each forages in aquatic habitats, with small fish typically composing half (American mink), most (belted kingfisher), or nearly all (common loon) of their diet (Salyer and Lagler 1949, Davis 1982, Barr 1996, Merrill et al. 2005, Evers et al. 2005, Basu et al. 2007a, Kelly et al. 2009). The belted kingfisher and American mink are both considered highly sensitive to methylmercury, and each has been used in assessments of ecological risks of methylmercury in aquatic food webs (Lazorchak et al. 2003, Basu et al. 2007b, Scheuhammer et al. 2007, Walters et al. 2010, Eagles-Smith et al. 2013). We will use the values derived by Lazorchak et al. (2003), 30 ng/g ww for the belted kingfisher and 70 ng/g ww for American mink, as dietary benchmarks to screen for potential risks of methylmercury in whole fish on the health and reproduction of these organisms.

In birds, the embryo is the most methylmercury-sensitive life stage (Scheuhammer et al. 2007, 2012), and methylmercury in the diet of reproducing females is transferred rapidly to the developing egg (Heinz et al. 2009a). The in ovo sensitivity of birds to methylmercury can vary greatly among species. In egg-injection experiments by Heinz et al. (2009b), the sensitivity of embryos to methylmercury varied 20-fold among 23 avian species, with the median lethal concentration (LC_{50}) for egg-injected methylmercury ranging from 0.12 to 2.4 µg/g ww. In the Great Lakes region, Rutkiewicz et al. (2011) found that about 14% to 27% of adult bald eagles may be exposed to mercury levels capable of causing changes in brain chemistry.

Toxicological benchmarks for the common loon can be used to gage the potential risks of methylmercury in fish to fish-eating birds that are moderately sensitive to methylmercury. The ecotoxicological effects of methylmercury have been intensively studied in the common loon, which has been widely used as a bioindicator for assessing ecological risks of mercury in aquatic food webs (Depew et al. 2012b, 2013). The LC_{50} for methylmercury in eggs of common loons is 1.78 µg/g ww (Kenow et al. 2011)—a value near the upper end of the range of LC_{50} reported for the 23 avian species studied by Heinz et al. (2009b). Thus, the common loon is less sensitive to methylmercury than many avian species. The estimated dietary threshold for adverse behavioral impacts of methylmercury in adult loons is 100 ng/g ww in prey fish, whereas a mercury concentration of 180 ng/g in prey fish adversely affects reproductive success of adult loons (Depew et al. 2012b, 2013). Benchmarks used to assess ecological risks of methylmercury exposure to piscivorous wildlife are summarized in Table 5.

2.4.3 Benchmarks for Assessing Mercury Risk to Fish-Eating Humans

Consumption of fish is the primary pathway for exposure of humans to methylmercury (Mergler et al. 2007, McKelvey and Oken 2012). To assess the potential for harmful methylmercury exposure in humans consuming fish, we will compare mercury levels in axial muscle of sport fish to the U.S. Environmental Protection Agency tissue residue criterion for methylmercury (300 ng/g ww), which was established to protect the health of humans who eat noncommercial fish, such as wild-caught sport fishes (Borum et al. 2001). Virtually all of the mercury in the fillets of fish is methylmercury (Grieb et al. 1990, Bloom 1992), and therefore, measurement of total mercury in the axial muscle of fish provides a valid estimate of methylmercury concentration in the edible fillet (Wiener et al. 2007). We will also consider the fish consumption guidelines for mercury recommended by the Great Lakes Fish Advisory Workgroup (2007) (Table 6), a group from the eight Great Lake states with substantial expertise on bioaccumulative contaminants in fishery resources of the Great Lakes region as well as the health risks of contaminants to humans who consume fish.

Table 6. Guidelines for human consumption of sport fish containing mercury in their fillets.

Concentration range in fish muscle (ng/g wet weight)	Consumption advice[1] (fish-meal frequency)
≤50	unrestricted consumption
51–110	2 meals per week
111–220	1 meal per week
221–950	1 meal per month
>950	do not eat

[1] Guidelines for the methylmercury-sensitive group including pregnant women, women who may become pregnant, and children under 15 years of age, recommended in the protocol for mercury-based fish consumption advice (Great Lakes Fish Advisory Workgroup 2007).

Extrapolating concentrations of mercury likely to accumulate in humans back down through the aquatic food web is worth considering in setting targets. As shown in Section 1.4, the concentration of total mercury in age-1 yellow perch, our preferred biosentinel prey fish, is a useful predictor of mercury concentrations in the edible filets of predatory game fish, such as northern pike. The linear regression of total mercury ww concentration in axial muscle of 55-cm (ca. 1-kg) northern pike against the mean ww concentration of total mercury in coexisting whole, age-1 yellow perch in 14 interior lakes in Voyageurs National Park yielded the following equation (Knights et al. 2005):

$$Hg_{np} = -37 + 9.02\ Hg_{yp}$$

where Hg_{np} is the concentration of methylmercury in 55 cm northern pike in ng/g (parts per billion) ww, and Hg_{yp} is the mean concentration of total mercury in whole, age-1 yellow perch in ng/g ww. The equation had a coefficient of determination (R^2) of 0.81, a significant positive slope ($p < 0.001$), and an intercept that did not differ from 0 ($p > 0.7$). The slope and intercept had standard errors of 1.27 and 125, respectively. Thus, we anticipate that the U.S. Environmental Protection Agency's

Tissue Residue Criterion of 300 ng/g ww for methylmercury would be exceeded in adult northern pike in water bodies where total mercury in whole age-1 yellow perch exceeded a mean concentration of **37 ng/g ww**. Similarly, Haro et al. (2013) found that the majority (>50 %) of predatory game fish have total mercury tissue concentrations above 300 ng/g ww when *Gomphus* spp. have methylmercury levels that exceed approximately **40 ng/g dw** (Figure 6).

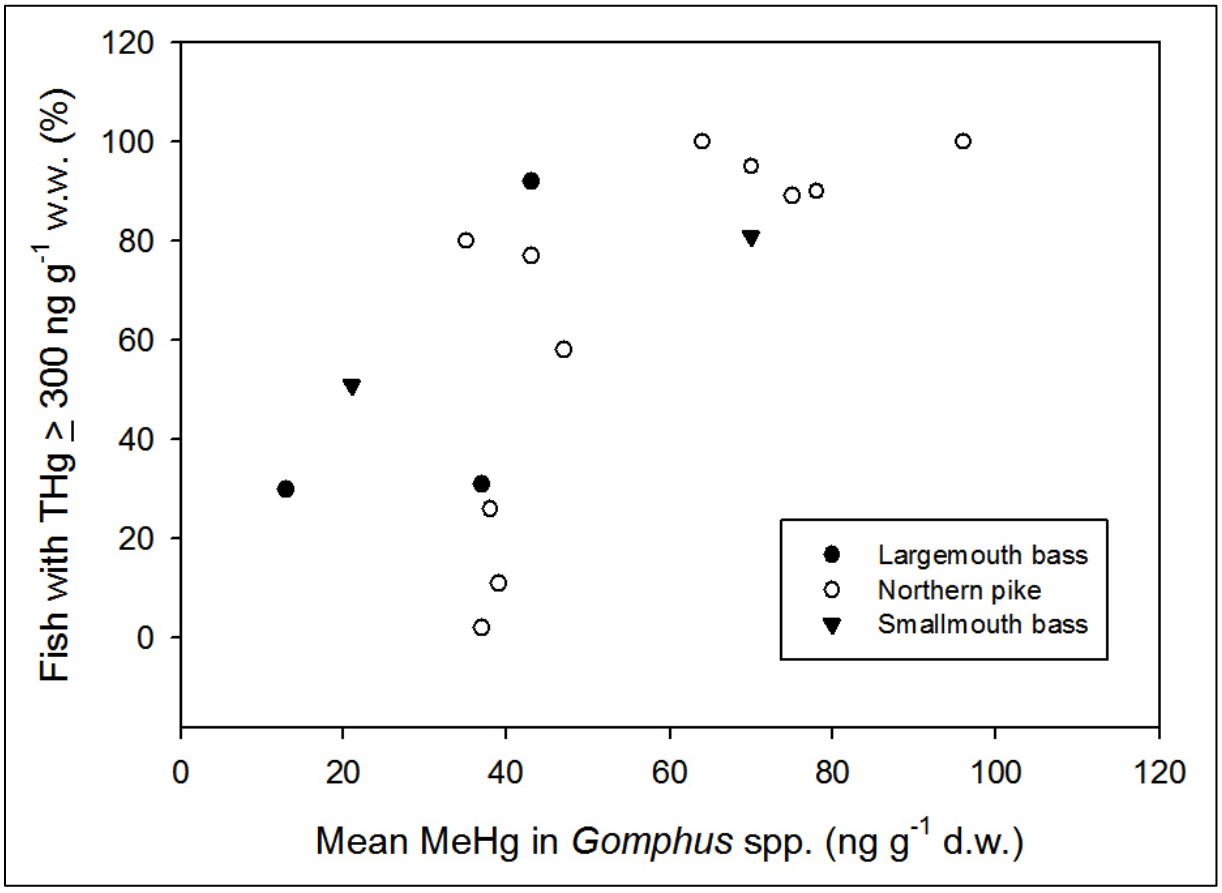


Figure 6. Percent of game fishes with concentrations of THg in skinless fillets equaling or exceeding 300 ng g-1 wet weight (the U.S. Environmental Protection Agency fish tissue criterion for MeHg), in relation to the mean concentration of MeHg in coexisting larval Gomphus in 13 lakes within the National Park Service Great Lakes Inventory and Monitoring Network. Figure is from Haro et al. (2013).

3. Overview of Sampling and Analytical Methods

The methods employed in this protocol will produce data of high analytical reliability that are comparable to information being gathered for other sites in the Great Lakes region and across the U.S. Data will be comprised of field observations and measurements that are recorded on data sheets in the field, and the results of testing performed by contract analytical laboratories. These data will provide a solid foundation for assessing mercury contamination and potential ecological risks to biota that forage in surface waters of GLKN parks. QA/QC considerations as they relate to fieldwork procedures and performance requirements are discussed in detail in the Quality Assurance Plan for Monitoring Mercury in Dragonfly Larvae and Fish (VanderMeulen et al. 2018).

3.1 Pre-Season Preparations

Detailed preparations for sampling at the parks will begin each January and should be completed by mid-April. Preparations for field work include 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 academic, park, and GLKN staff). Pre-season preparations for sampling at the parks, including checklists, logistic arrangements, travel preparations, and equipment lists are described in detail in SOP #1. Preparations concerning emergency contacts and safety procedures during sampling are described in SOP #2.

3.2 Collection and Handling of Samples in the Field

3.2.1 Larval dragonflies

Larval dragonflies will be collected from open-water benthic substrates (i.e., sand, gravel, and cobble) and from moderately vegetated littoral or wetland habitats using D- or dip-nets. Most dragonflies in the western Great Lakes region have relatively long life cycles in which the larval stage can span from one to four years (Hilsenhoff 1995). Sampling will target larger larvae that preferably are longer than 15 mm and where there are at least three individuals for each family represented. Larval dragonflies will typically be collected from June through August, and in many instances simultaneous with other aquatics-based monitoring (e.g., GLKN water quality monitoring). If necessary, collections can occur earlier in the spring or into the fall, as long as minimum length requirements are met. Once collected, dragonfly larvae should be held in zip-seal bags and kept cool on wet ice while in the field and during transport to the field laboratory. There, samples should be frozen while stored, and shipped frozen to an analytical laboratory. Detailed methods for collection and handling of larval dragonflies during sampling trips are provided in SOP #4.

3.2.2 Prey fish

Prey fish will be sampled in spring (a few weeks after ice out) with small-mesh bag seines, back-pack electroshocker, or dip-nets in littoral habitat. Passive gear, such as minnow traps and small-mesh nets, may also be used. The field crew should attempt to obtain age-1 yellow perch or small prey fish of an alternative target species (listed in Section 1.4) if yellow perch are not present or sufficiently abundant in the water body being sampled. In the field, prey fish will be held in sealed, labeled zip-seal freezer bags containing water from the sampled water body. At day's end, fish will be identified, euthanized in the field with an overdose of methane tricaine sulfonate (MS 222), and

placed in individual labeled bags. All bagged fish from a site should then be placed into a larger plastic bag (i.e., a site bag). Multiple fish of a given species from a given aquatic site may be grouped together in a site bag, but fish from multiple species and/or lakes should not be grouped within the same site bag. Fish should be frozen within 12 hours of collection. Samples will be transported in frozen condition to an analytical laboratory and stored at ≤−20ºC until further processing. Detailed methods for sampling, handling, and storage of prey fish in the field are shown in SOP #4.

3.2.3 Predatory fish

Predatory fish will be obtained with hook and line, gill nets, bag seine, or backpack electroshocker and held in surface water or in a portable cooler on ice while in the field. Our target sample size for predatory or other adult fish (northern pike or an alternative species) is 15 individual fish per water body during each year of sampling. Target predatory fish species specific to individual water bodies for many aquatic sites can be found in Wiener et al. (2016). Fish will be euthanized before being placed into a clean, food-grade plastic bag for transport. Detailed methods for collecting, processing, and storage of predatory fish in the field are provided in SOP #4.

3.3 Preparation and Analysis of Samples in an Analytical Laboratory

All larval dragonfly and fish samples will be shipped to an analytical laboratory for mercury analysis. Research laboratories with expertise in larval dragonfly and/or fish taxonomy will re-check identifications made by field crews; commercial laboratories likely will not have the capacity for this. Through a research laboratory GLKN may occasionally require that select dragonfly larvae be identified to species, or that a voucher collection be created and maintained. Additional dragonfly larvae beyond the 20 to be assessed for total mercury from each site will be collected when preservation of voucher specimens is desired. Most larvae present in the Great Lakes region can be identified to species with taxonomic keys by Hilsenhoff (1995) and Needham et al. (2000). Quality control for taxonomic identification of larval dragonflies should follow procedures outlined by Barbour et al. (1999), and questionable specimens should be taxonomically verified by a recognized dragonfly specialist. All individual specimens will be subjected to a variety of measurements (length, ww, dw, etc.) and analyzed for total mercury. Periodically, a subset may be analyzed for methylmercury, to verify the percentage of total mercury that is methylmercury, by region, park, water body, or larval dragonfly guild, family, or species.

It is expected that there will be some subtle methodological differences among analytical laboratories in how mercury is analyzed. Examples of detailed laboratory procedures used by Wiener et al. (2016) to analyze larval dragonflies and fish collected in Great Lakes Network parks from 2008 to 2012 are shown in Appendix C. General descriptions of laboratory procedures follow.

3.3.1 Larval dragonflies

Dragonfly larvae will be thawed on a clean, acid-washed work surface and measured (total length to the nearest millimeter); those with body lengths ≥15 mm should be processed and analyzed individually. Smaller individuals of a given species with body lengths <15 mm can be pooled into composite samples for analysis at the analytical laboratory's discretion, given specific instrument specifications. Individual larvae should be rinsed briefly in deionized water to remove surficial debris then blotted with a lint-free wipe to remove excess surface moisture prior to obtaining a ww weight

(to 0.001 g). Dragonflies can be freeze-dried or dried in a laboratory oven set to 50°C. Once a constant mass is obtained (typically 48 hours) a final dw (to 0.001 g) is recorded. Depending on the size of individual larvae, samples may be analyzed whole or ground using either acid-washed mortar and pestles or glass rods.

3.3.2 Prey fish

In the laboratory, each individual fish should be gradually thawed at room temperature, identified to species, measured (total length to the nearest millimeter), and weighed (to 0.001 g). Samples should then be coarsely chopped to facilitate rapid and complete moisture loss, and dried in a laboratory oven set to 50°C or in a freeze dryer. After drying, double-bagged samples should be stored in a desiccator with sufficient drying agent and vacuum. Each dry sample should be homogenized to a fine powder using an acid mortar and pestle.

3.3.3 Predatory fish

Each fish will be weighed (to 0.001 g), measured (maximum total length), and dissected to remove an approximately 5 g sample of axial muscle tissue. Samples of axial muscle will be stored frozen until drying as described above for whole prey fish. Age of northern pike will be estimated from growth structures on the cleithra, which will be dissected, cleaned of flesh, and examined with a lighted magnifying glass (Casselman 1974). Dried muscle samples will be homogenized to a fine powder using an acid washed mortar and pestle.

Examples of detailed laboratory procedures for taxonomic identification, physical measurements, and preparing samples and analyzing them for total and methylmercury in larval dragonflies and prey and predatory fish are described in detail in Appendix C.

3.3.4 Quality assurance and quality control for mercury determinations

Determinations of total mercury and methylmercury in biological samples will be supported by an array of quality assurance elements to characterize precision and accuracy. These include the use of (1) analytical and procedural blanks, (2) sample replication, (3) certified reference materials, (4) standard addition spike recoveries, (5) periodic checks of standards during analysis, as well as evaluation of calibration linear regression. QA/QC considerations as they relate to laboratory procedures, methods, and performance requirements are discussed in detail in the protocol QAP (VanderMeulen et al. 2018).

4. Data Handling, Analysis, and Reporting

4.1 Overview of Database Design

The NPS Water Resources Division (WRD) has established a policy that all I&M water quality monitoring data will be made compatible with, and be uploaded to, the U.S. Environmental Protection Agency's (EPA) STORET database. The WRD developed a Microsoft Access database tool, NPSTORET, which duplicates most of the EPA data and table structures to facilitate easier movement of GLKN's water quality data into EPA Water Quality Exchange framework. We will use NPSTORET as the primary data entry tool and data transfer mechanism to WRD.

The GLKN will maintain one master copy of NPSTORET at the Ashland office on a central server. This is the only copy of NPSTORET that can be used to export data to other locations (WRD). Additional copies of NPSTORET can be used by GLKN staff or cooperators, but they can only be used as a conduit for data entry and the importation of data to GLKN's master version of NPSTORET. For analysis, the data from the master copy of NPSTORET must be used. The GLKN will continue to improve tools for automating analysis and visualization of the information contained in the NPSTORET dataset.

4.2 Metadata Procedures

Metadata allows potential data users to evaluate the quality and usefulness of the data based on an understanding of the complete process under which it was collected and maintained. In this respect, all of the protocol documentation, including the SOPs and the QAP, is part of a dataset's metadata. A reference to the appropriate version of these documents is part of the metadata for any particular element of a dataset. Although perhaps obvious, all data must have an associated value for the date and time they were collected.

Most of the remaining metadata will be recorded directly in the protocol-specific databases and tables. We will enter or import all required metadata for the NPSTORET database; the data and metadata will ultimately be moved to the EPA STORET database maintained by NPS-WRD staff in Ft. Collins, Colorado.

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 and NPS metadata standards. The network will also generate FGDC-style metadata for non-spatial datasets that meet this standard, absent only the geospatial-specific elements.

For more details on the GLKN's overall strategy for metadata generation, management, and distribution see Chapter 8, Data Documentation, of GLKN's Data Management Plan (Hart and Gafvert 2006) and the appendices of that document.

4.3 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 (Section 3, Overview of Sampling and Analytical Methods), two 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 #4. The second class of data is the result of testing performed by contract analytical laboratories. GLKN will develop formatting routines to be applied to the digital files prior to importation of data into NPSTORET.

Data verification starts with the QA/QC steps that are outlined in the SOPs and QAP associated with this protocol. If data being imported into NPSTORET do not pass a QA/QC test, NPSTORET prompts the user to make corrections and re-import the data. Data that are outside the expected range for a parameter based on previous records for that parameter will be flagged for further review by an expert.

Quality assurance/quality control checks are performed as data are imported into NPSTORET and again when the data are transferred to WRD. The GLKN's data records are regarded as being in provisional status until they are returned to GLKN from WRD, or are accepted by WRD without changes after the final QA/QC steps. Once returned to GLKN by WRD, and after appropriate documentation is completed, the dataset is officially considered certified. Only qualified users who have been trained and given edit permissions are allowed to edit data in NPSTORET. These procedures protect the integrity of the data and allow the history of each data record to be traced.

4.4 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 archival of the printed data forms for this protocol is described in SOP #5.

The GLKN will create duplicate files of all digital data at the earliest opportunity. At least two complete copies of any dataset are required by WRD, 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 GLKN's master version of NPSTORET is maintained on a central server in the Ashland Office that is backed up daily, and backed up off-site weekly. Complete details of the GLKN server archiving procedure are found the Infrastructure chapter of GLKN's Data Management Plan (Hart and Gafvert 2006); the general strategy for data archiving is also described in this plan and its appendices.

4.5 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, the QAP, and are summarized in Table 7.

Table 7. Summary of QA/QC procedures pertaining to data management.

Procedure	Description
Instrument calibration logs	Each instrument must have a calibration log.
Field forms	Field 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 GLKN Ashland office. Boxes are numbered consecutively by year, project, and station number.
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.
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) that 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 NPSTORET

4.6 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 GLKN as a whole and also scanned for outliers. Descriptive statistics for mercury 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 mercury 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 thresholds 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.

4.7 Data Analyses

Mercury concentrations in prey and predatory fish will be compared to benchmarks used to assess risks of methylmercury to fish, wildlife, and humans described in Section 2.4, and in SOP #6, Data Analysis. Building on the work of others (e.g., Haro et al. 2013), we will establish statistically-robust positive relationships between mercury in larval dragonflies and mercury in prey or predatory fish, which will allow us to predict when benchmarks used to assess risks of methylmercury to fish, wildlife, and humans are likely being exceeded.

Similar to other studies of mercury 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 mercury, 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 mercury concentration in larval dragonflies and fish (Tremblay et al. 1998, Wiener et al. 1997). Simple correlation analysis such as Pearson's will be used to determine significant univariate correlations in the dataset (e.g., total mercury 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 mercury among study sites (spatial analysis) or among years (trend analysis) within a study site for equal age/size fish. When appropriate, we will 1) include historic 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. 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 for the GLKN.

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 of other multivariate models. Water body (site) will be included as a random effect in statistical models when analyzing broad spatial-temporal trends.

If ancillary data for study sites are sufficient, we will use information-theoretic modeling (Akaike Information Criteria) to construct linear models with predicted variables pertaining to mercury in biosentinel organisms and predictor variables pertaining to ecosystem factors and other variables that can influence the production and abundance of methylmercury (Anderson et al. 2001, Burnham and Anderson 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 mercury in selecting predictor variables (e.g., Wiener et al. 2006). Additional information on data analysis is in SOP #6.

4.8 Reporting Schedule and Formats

One of the GLKN's main goals is to ensure that the results and knowledge acquired through the mercury in larval dragonflies and fish monitoring program are shared with all appropriate parties, especially the parks and their natural resource managers. We will strive to provide park managers with clear, meaningful products in a timely manner to convey our findings. Because our monitoring data will be of interest to a broader community, we will also provide our reports to the states, the NPS I&M Division, and when appropriate, submit them to peer-reviewed journals for publication. We will also present our findings orally and in poster format at regional meetings when logistically feasible, such as the Western Great Lakes Research Conference, the St. Croix Research Rendezvous, or the Lake of the Woods Research Conference.

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 the EPA's STORET database by NPS-WRD.

The target audience of more comprehensive analysis and synthesis reports will be produced about every five years, with the primary audience being the parks, the GLKN, both regional and Servicewide I&M, and the broader scientific community. Drafts of these reports will be reviewed internally and sent to the parks, and possibly outside sources, for further review. The extent of review will depend on how analytically complicated the methods are and the gravity of inference and recommendations.

Both annual summaries and technical reports that include detailed analyses of trends should adhere to Servicewide I&M reporting guidelines for Natural Resource Data Summary reports (for annual summaries) and Natural Resource Reports (for detailed analysis reports). Refer to the Natural Resource Publications Management website for the most up-to-date guidelines and formats (https://www.nps.gov/im/publication-series.htm). Reports should include tables and figures appropriate for the data and for the intended audience. Additional information can be found in SOP #7, Reporting.

5. Personnel Requirements and Training

5.1 Roles and Responsibilities

The aquatics program at the GLKN is staffed by a project manager (GLKN aquatic ecologist, GS-11) and two assistant project managers (GLKN aquatic ecologists, GS-9s). The project manager and the assistant project managers are permanent full time employees. The assistant project managers primarily focus on leading fieldwork for other aquatics monitoring protocols (diatoms, inland lakes, and large rivers) during the field season, but also have many overlapping responsibilities throughout the year. The assistant project managers will each supervise at least one seasonal crew member at the GS-4 or GS-5 level, and, along with the crew members, may be stationed at one of the parks.

Field crews will conduct monitoring activities for this protocol during the field season, at times concurrent with other aquatic monitoring activities. The GLKN will explore the possibility of sharing seasonal positions with the parks. When a park has an aquatic person on staff, the GLKN will make use of such existing staff expertise on the crew when possible, paying for the time spent on I&M monitoring activities, and will provide the same training to the park person as to the rest of the crew members.

5.1.1 Project Manager

The role of the project manager is to serve as a liaison among other related aquatics monitoring projects conducted by partners (researchers, state monitoring programs, etc.), park staff, other network staff (field personnel, data manager), contracted analytical laboratories, and other GLKN project managers. The individual will coordinate with resource management staff at the parks to ensure parks are informed of monitoring activities. Specific responsibilities of the project manager include the following:

- Coordinate field schedules and availability of supplies with field personnel.
- Develop a training program for field personnel.
- Develop, document, and oversee the implementation of standard procedures for field data collection and data handling.
- Coordinate logistics with park staff.
- Develop QA/QC measures for the project, supervise staff training, and conduct quality assurance checks of field sampling techniques at least once, mid-season, with each field crew.
- Contract with analytical laboratories for analysis of environmental samples, ensure lab results meet program needs (e.g., QA/QC procedures, meaningful minimum detection limits, adequate reproducibility of replicate samples).
- Supervise or perform data entry, verification, and validation.
- Summarize and analyze data, and prepare reports.
- Serve as the main point of contact concerning data content.

The project manager will also work closely with the data manager in the following capacities:

- Complete project documentation (i.e., metadata) in appropriate databases.
- Develop data verification and validation measures for quality assurance.
- Establish and implement a procedure to officially certify datasets.
- 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.
- Identify sensitive information that requires special consideration prior to distribution.
- 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.
- Establish meaningful liaisons with state counterparts to promote sharing of data on a timely basis.

5.1.2 Assistant Project Managers

Assistant project managers are largely responsible for implementing GLKN aquatics monitoring protocols. Specific responsibilities include:

- Assist with coordination of field schedules and supplies.
- Supervise and train field personnel.
- Coordinate logistics with park staff.
- Help ensure all aspects of QA/QC are met.
- Perform data entry, verification, and validation.
- Train other staff in the use of database software.
- Assist with data analysis and report writing.

5.1.3 Field Personnel (Field Crew Member/Leader)

The role of field personnel is to conduct all field work related to the monitoring project. Field personnel will include both a crew leader and a crew member. The crew leader is responsible for contacting the parks prior to each sampling event to ensure logistical requirements will be met. Crew leaders and crew members may be park staff that coordinate with their respective parks and the GLKN project manager. Responsibilities for GLKN or park crew leaders and crew members 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.
- 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.
- Maintain and calibrate equipment according to protocols and manufacturers' directions.
- Communicate progress and accomplishments with the project manager during and after sampling at each park unit, and report any deviations from sampling protocols.
- Download, enter, and verify data into databases as required.
- Maintain documentation of important details of each field data collection period, including explanations of all deviations from standard procedures.
- Maintain hard copies of data forms and send original data forms to archive on a regular basis.
- Facilitate communication among the GLKN, park staff, and the public.

5.1.4 Data Manager

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

5.2 Personnel Qualifications

The field crew leader must have a bachelor's or advanced (graduate) degree in biology, chemistry, or a related physical or biological science. The crew leader should also have prior leadership experience and good decision-making skills, as well as experience in the use of boats, motors, and canoes. Members of field crews should have a background in biology, chemistry, or other related physical or biological science, although an undergraduate degree is not required. Prior field experience with sampling of fish, benthic invertebrates, zooplankton, water, and sediment and with the use of boats, outboard motors, and canoes is highly desirable.

Analytical laboratory personnel responsible for analyzing samples for mercury must be experienced and be trained on the specific methods and equipment necessary to conduct the analyses. In addition to QA/QC and performance requirements, the qualifications and experience of laboratory personnel will be assessed by GLKN staff prior to contracting with an analytical laboratory.

Persons overseeing those who are analyzing samples for mercury and involved with data interpretation should have substantial experience in investigations of mercury contamination of aquatic resources, preferably including assessment of the effects of methylmercury exposure on aquatic biota. These individuals should possess experience in the collection, handling, and analysis of biological and environmental samples for mercury, and access to a mercury laboratory with proven analytical reliability. These qualifications should be reflected by accurate measurements of mercury in environmental samples and publication of scientific papers in refereed journals.

5.3 Training Procedures

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

- Review of this protocol, associated SOPs, and the QAP
- Familiarity with procedures for calibration, operation, and maintenance of equipment
- Review of safety procedures and emergency contacts
- Familiarity with methods for measurements and sample collection
- Familiarity with methods for handling and preserving samples
- Completion of field data forms and sample labels
- Completion of field and calibration logbooks
- Park-specific training, provided on-site by park staff (e.g., use of radios)

Field crews who participate 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. Crew members 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.

6. Operational Requirements

6.1 Annual Work Load and Schedule

The annual workload and schedule for monitoring mercury in dragonfly larvae and fish must be viewed within the context of the other planned aquatic monitoring activities. We prepared the estimated workload and schedule for monitoring assuming that 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, whether or not fish will be sampled, and availability of funds to allow for monitoring of additional sites or media (e.g., water, sediment, seston, or zooplankton).

Larval dragonflies will be sampled annually and prey and/or predatory fish every five years at all nine GLKN parks. Monitoring will take place across a variety of waters including inland lakes, rivers, streams, and potentially wetlands. In general, for this and other GLKN aquatic monitoring protocols, site accessibility and sampling logistics range from difficult for APIS, ISRO, and VOYA, moderate for GRPO, MISS, SACN, and SLBE, and relatively easy for INDU and PIRO. The time it takes to conduct fieldwork is always weather dependent, and this is especially true at parks where travel on Lake Superior or Lake Michigan is required (SLBE, ISRO, and APIS). Sampling can be delayed and field crews can be stranded for days when wind and waves prohibit boat travel. We estimate sampling for larval dragonflies to take from one day at INDU, to as much as seven or more days at ISRO and VOYA, 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. Periodic sampling for fish will always take place independent of larval dragonflies or other monitoring, and is logistically much more challenging and time consuming.

6.2 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, which all park units have except for APIS. The GLKN office is located near APIS, however, and serves as a field laboratory for samples collected there.

We anticipate that most field crews will be park-based but some travel by GLKN's aquatic ecologists to train or lead field crews will be necessary. Isle Royale National Park in Lake Superior will be accessed by the NPS ferry operating out of Houghton, Michigan, or by commercial ferry operating from Grand Portage, Minnesota. North Manitou Island (SLBE) on Lake Michigan will be accessed by commercial ferry operating from Leland, Michigan. Motorboats will be needed to access sampling sites or portage points to sampling sites in APIS, ISRO, and VOYA. Sites at GRPO, INDU, MISS, PIRO, SACN, and SLBE are accessible by road or trail.

6.3 Budget Considerations

This section considers estimated costs associated with equipment and supplies, salary, and analytical laboratory expenses as they relate to monitoring mercury in dragonfly larvae annually at all nine GLKN parks, and in prey or predatory fish at two (or one on the fifth year) GLKN parks. If funds are limiting for any given year then monitoring dragonfly larvae will be a higher priority than fish.

6.3.1 Equipment and Supplies

Most of the equipment and supplies for monitoring mercury in larval dragonflies have already been purchased during the pilot phase of this protocol. Future costs associated with purchase of equipment and supplies related to fieldwork are now primarily associated with replacing equipment as it wears out or becomes obsolete, or when supplies need to be replenished. Some of the parks already had some of the necessary sampling gear and equipment when this protocol was first implemented, and continue to replace gear and equipment as necessary. When possible, we will coordinate with the parks in the use of their equipment. Sampling at APIS and ISRO requires large boats for travel on the Great Lakes. The GLKN owns two boats, with motors, trailers, and other necessary equipment, appropriate for use on Lake Superior at APIS and ISRO. SLBE owns boats appropriate for traveling to North and South Manitou islands and will provide transportation for field crews as necessary. The GLKN and VOYA jointly own a boat, motor, and trailer to be shared by park and GLKN staff at that park. Boats or canoes appropriate for inland lakes are available to the GLKN at all the parks, though GLKN-owned crafts may be required in the future. The GLKN purchases supplies related monitoring under this protocol, and for some parks provides funds to help cover fuel costs and boat operator salaries when parks assist in transporting GLKN staff. Based on past annual expenses for other aquatic monitoring protocols, we estimate that annual expenses for equipment, supplies, and fuel for this protocol will range from $1,500 to $3,000.

6.3.2 Staff Salaries

We anticipate that many if not all of the same GLKN and park staff who oversee and implement other GLKN aquatics protocols will implement this protocol. From 2011 to 2014 annual salary expenses related to implementation of other GLKN aquatic protocols ranged between $190,000 and $215,000, taking into account the following:

- annual salaries for the GLKN project manager and assistant project managers,
- six-to-eight pay periods for a GLKN seasonal crew member duty stationed at ISRO,
- six-to-ten pay periods for a VOYA permanent crew leader shared with the GLKN, and
- various salary expenses for park staff at other parks who conduct aquatic monitoring work for the GLKN.

The project manager's annual salary is divided between the I&M Division and WRD, with the majority of salary typically assigned to WRD. The GLKN data manager's salary is covered entirely by the I&M Division and is not reflected in the annual salary expenses above. The salary estimates include staff time for project management, training, pre-season preparation, sampling, processing samples, packing and shipping samples, data entry, analysis, reporting, and other various tasks associated with the monitoring effort.

Although we do not anticipate this to be the case, if this protocol were to be implemented as a stand-alone effort, and taking into account that unlike our other aquatics protocols where sites are visited multiple times in a season, for this protocol sites are only visited once per season, then a conservative range for annual salary expenses would be from $60,000 to $75,000.

6.3.3 Vehicles and Travel

We expect travel expenses to be approximately $5,000 annually. This estimate includes GSA vehicles and travel (lodging and per diem), and is based on the following assumptions:

1) GSA vehicles will be shared with other monitoring projects or parks, when possible.
2) Park housing will be available at ISRO, VOYA, SLBE, and PIRO.
3) The project manager will travel to GLKN parks on a rotational basis to assist with and oversee monitoring activities.
4) Park staff that serve as crew leaders will work with the project manager and assistant project managers, and will occasionally travel to other parks as needed.

6.3.4 Analytical Laboratory Costs

The GLKN will assess the differences in detection and reporting limits, past performance evaluations, cost estimates, and other criteria, prior to selecting a contract laboratory. The laboratory selected by GLKN must be able to detect and report concentrations appropriately low such that changes in mercury concentrations can be detected early. The laboratory selected must meet the detection limits outlined in the protocol QAP and have a rigorous internal QA/QC plan.

For the purpose of estimating annual analytical expenses for monitoring mercury in larval dragonflies we will consider the following two scenarios:

Scenario 1 – Advanced Laboratory Services:

- Checking of field identifications to family for each individual
- Checking or updating measurements (body length, stage of instar development, etc.)
- Wet weight and dry weight determinations for each individual
- Total mercury analysis on each individual; methylmercury 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)

Scenario 2 – Basic Laboratory Services

- Wet weight and dry weight determinations for each individual
- Total mercury analysis on each individual
- Electronic data delivery in an agreed-upon format that includes results of relevant laboratory QA/QC (i.e., a QA/QC report)

Since 2012 the University of Maine has supplied advanced services shown in Scenario 1 at a rate of $1,000 to $1,350 per site, which would amount to $48,600 annually (9 parks × 4 sites/park × $1,350/site). On a few occasions we have utilized the Wisconsin State Laboratory of Hygiene. They deliver the basic services shown in Scenario 2 at a rate of $1,280 per site, which would amount to $46,080 annually. Both scenarios assume that 20larval dragonflies are collected per site. For planning purposes we will estimate annual analytical laboratory expenses for larval dragonflies to be $50,000.

Unlike dragonfly larvae, the cost for which are estimated per site, cost for analyzing total mercury in prey or predatory fish are per individual, and range from $75–$90 for each individual sample (whole fish for prey fish or axial muscle fillet for predatory fish). For planning purposes we will estimate annual analytical laboratory expenses for fish to be $10,800 (2 parks × 4 sites/park × 15 individuals/site × $90/sample).

6.3.5 Total Estimated Annual Costs

Annual monitoring costs (up to $144,000; Table 8) for mercury in dragonfly larvae and fish are high and are nearly what the GLKN receives from WRD (approx. $150,000). Non-salary annually reoccurring expenses are estimated to be $68,500 to $70,000. Monitoring water quality of inland lakes (Elias et al. 2015) large rivers (Magdalene et al. 2016), diatoms (Ramstack et al. 2008), and wadeable streams (protocol in preparation) are not included in these estimates, putting the total cost of all aquatic monitoring by GLKN well beyond the funding WRD provides. Because of the importance of monitoring mercury in GLKN parks, the GLKN is contributing substantial I&M funds to implement these aquatic monitoring protocols.

Table 8. Total estimated annual costs for monitoring mercury in dragonfly larvae and fish at GLKN parks.

Item	Cost
Annual equipment and supplies	$1,500–$3,000
Salary and benefits	$60,000–$75,000
Travel	$5,000
Laboratory analyses – larval dragonflies	$50,000
Laboratory analyses – fish	$11,000
Total	**$127,500–$144,000**

6.4 Procedures for Revising and Archiving Previous Versions of the Protocol

As our program to monitor mercury in larval dragonflies and fish matures, revisions to the protocol narrative, SOPs, and QAP 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, supporting SOPs, and a QAP 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 or the QAP.
- The QAP describes in detail QA/QC considerations as they relate to both fieldwork and laboratory procedures and performance requirements.
- 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, SOPs, and QAP will be archived.

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

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Appendix A. Dragonflies of the Great Lakes Network Parks.

Dragonfly species recorded for the nine National Park Service units in the Great Lakes Inventory and Monitoring Network from 2008 to 2014, and species likely present based on county (USA) and provincial (Canada) records presented below (Table A1). Unit acronyms: (APIS) Apostle Islands National Lakeshore, (GRPO) Grand Portage National Monument, (INDU) Indiana Dunes National Lakeshore, (ISRO) Isle Royale National Park, (MISS) Mississippi National River and Recreation Area, (PIRO) Pictured Rocks National Lakeshore, (SACN) Saint Croix National Scenic Riverway, (SLBE) Sleeping Bear Dunes National Lakeshore, and (VOYA) Voyageurs National Park. Survey effort varies by number of water bodies and years sampled among parks from 2008 to present. Identification of dragonfly species collected from 2015 to present is pending and includes all samples collected to-date for APIS and MISS.

Table A1. Dragonfly species recorded for the nine National Park Service units in the Great Lakes Inventory and Monitoring Network[1] from 2008 to 2014, and species likely present based on county (USA) and provincial (Canada) records[2].

Family	Species[3]	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
Aeshnidae	*Aeshna canadensis*	X	X[1]	X	X	X	X[1]	X[1]	X[1]	X[1]
	A. clepsydra	–	–[1]	X[1]	X	–	MI	X	X[1]	–[1]
	A. constricta	X	–	X[1]	X	X	X	X[1]	X[1]	X[1]
	A. eremita	X	X	–	X[1]	X	MI[1]	X	–	X[1]
	A. interrupta	X	X[1]	–[1]	X[1]	X	MI[1]	X	X	X[1]
	A. juncea	–	X	–	X[1]	–	–	–	–	X
	A. sitchensis	WI	X	–	X	–	X	–	–	X
	A. subarctica	WI	X	–	X	–	–	X	–	X
	A. tuberculifera	X	X	–	X	X	MI	X	X[1]	X
	A. umbrosa	X	X[1]	X[1]	X[1]	X	X[1]	X[1]	X[1]	X[1]
	A. verticalis	X	–[1]	X	MI	X	X	X	MI[1]	X[1]
	Anax junius	X	X	X[1]	X	X	X[1]	X[1]	X[1]	X[1]
	Basiaeschna janata	X	X	–	X[1]	X	X[1]	X[1]	X[1]	X[1]
	Boyeria grafiana	–	X[1]	–	X	–	–	–[1]	–	X
	B. vinosa	X	X[1]	X	X	X	X[1]	X[1]	X[1]	X[1]

[1] Based on confirmed records from the NPSpecies database and research conducted within the parks (also shaded).

[2] County and provincial records from www.odonatacentral.org, indicated by "X", with records vetted by voucher specimens and reviewed by subject and locality experts. Counties and provinces by park include: APIS (Ashland, Bayfield), GRPO (Cook [MN]; Ontario), INDU (Lake, LaPorte, Porter), ISRO (Keweenaw [MI]; Cook [MN]; Ontario), MISS (Anoka, Dakota, Hennepin, Ramsey, Washington), PIRO (Alger), SACN (Bayfield, Burnett, Douglas, Pierce, Polk, St. Croix, Sawyer, Washburn [WI]; Chisago, Pine, Washington [MN]), SLBE (Benzie, Leelanau), and VOYA (Koochiching, St. Louis [MN]; Ontario). Due to Ontario's size, only records from near the border parks of GRPO, ISRO, and VOYA were included. Additional Wisconsin Department of Natural Resource records are indicated by "WI"; records from 2017 Michigan Odonata Survey maps are indicated by "MI". No additional publically available Odonata databases were found for Minnesota or Indiana.

[3] Taxonomic nomenclature checked against Paulson and Dunkle (2016).

Table A1 (continued). Dragonfly species recorded for the nine National Park Service units in the Great Lakes Inventory and Monitoring Network[1] from 2008 to 2014, and species likely present based on county (USA) and provincial (Canada) records[2].

Family	Species[3]	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
Aeshnidae (continued)	*Epiaeschna heros*	WI	–	X[1]	–	–	–	–	–	–
	Gomphaeschna furcillata	X	–	–	–	–	X	X	X[1]	–
	Nasiaeschna pentacantha	X	X	–	X	–	–	X	MI	X[1]
	Rhionaeschna multicolor	–	–	–	–	X	–	–	–	–
	R. mutate	–	–	X	–	X	–	X	X	–
Gomphidae	*Gomphus spicatus*	X	–	–	X[1]	–	X[1]	X[1]	X[1]	X[1]
	A. furcifer	X	–	X	–	X	–	X	X	–
	A. submedianus	–	–	–	–	X	–	–	–	–
	A. villosipes	–	–	X	–	X	–	–	X	–
	Dromogomphus spinosus	X	X	–	X[1]	X	X[1]	X[1]	X[1]	X[1]
	Gomphus adelphus	X	X	–	X	X	X[1]	X[1]	X[1]	X[1]
	G. exilis	X	X	–	X[1]	–	X[1]	X	X[1]	X[1]
	G. externus	–	–	–	–	X	–	X	–	–
	G. fraternus	X	–	–	–	X	–	X[1]	–	X
	G. graslinellus	–	X	–	X	X	–	X	MI[1]	X[1]

[1] Based on confirmed records from the NPSpecies database and research conducted within the parks (also shaded).

[2] County and provincial records from www.odonatacentral.org, indicated by "X", with records vetted by voucher specimens and reviewed by subject and locality experts. Counties and provinces by park include: APIS (Ashland, Bayfield), GRPO (Cook [MN]; Ontario), INDU (Lake, LaPorte, Porter), ISRO (Keweenaw [MI]; Cook [MN]; Ontario), MISS (Anoka, Dakota, Hennepin, Ramsey, Washington), PIRO (Alger), SACN (Bayfield, Burnett, Douglas, Pierce, Polk, St. Croix, Sawyer, Washburn [WI]; Chisago, Pine, Washington [MN]), SLBE (Benzie, Leelanau), and VOYA (Koochiching, St. Louis [MN]; Ontario). Due to Ontario's size, only records from near the border parks of GRPO, ISRO, and VOYA were included. Additional Wisconsin Department of Natural Resource records are indicated by "WI"; records from 2017 Michigan Odonata Survey maps are indicated by "MI". No additional publically available Odonata databases were found for Minnesota or Indiana.

[3] Taxonomic nomenclature checked against Paulson and Dunkle (2016).

Table A1 (continued). Dragonfly species recorded for the nine National Park Service units in the Great Lakes Inventory and Monitoring Network[1] from 2008 to 2014, and species likely present based on county (USA) and provincial (Canada) records[2].

Family	Species[3]	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
Gomphidae (continued)	*G. lineatifrons*	WI	–	–	–	–	–	X[1]	X[1]	–
	G. lividus	X	X	–	X[1]	–	X	X[1]	X[1]	X[1]
	G. quadricolor	X	X	–	X	X	–	X[1]	X	–
	G. spicatus	X	X	–	X[1]	X	X[1]	X[1]	X[1]	X[1]
	G. vastus	X	–	–	–	X	–	X[1]	X	X
	G. ventricosus	X	–	–	–	X	–	X[1]	–	X
	G. viridifrons	X	–	–	–	X	–	X[1]	–	X
	Hagenius brevistylus	X	X	–	X[1]	X	X[1]	X[1]	X[1]	X[1]
	Ophiogomphus anomalus	WI	X	–	X	–	–	X[1]	–	X
	O. carolus	X	X	–	X	–	X	X	–	X
	O. colubrinus	X	X	–	X	–	X[1]	X[1]	X	X
	O. howei	X	–	–	–	–	–	X[1]	–	–
	O. rupinsulensis	X	X	–	X	X	MI	X[1]	X[1]	X
	O. smithi	–	–	–	–	–	–	X	–	–
	O. susbehcha	–	–	–	–	X	–	X[1]	–	–

[1] Based on confirmed records from the NPSpecies database and research conducted within the parks (also shaded).

[2] County and provincial records from www.odonatacentral.org, indicated by "X", with records vetted by voucher specimens and reviewed by subject and locality experts. Counties and provinces by park include: APIS (Ashland, Bayfield), GRPO (Cook [MN]; Ontario), INDU (Lake, LaPorte, Porter), ISRO (Keweenaw [MI]; Cook [MN]; Ontario), MISS (Anoka, Dakota, Hennepin, Ramsey, Washington), PIRO (Alger), SACN (Bayfield, Burnett, Douglas, Pierce, Polk, St. Croix, Sawyer, Washburn [WI]; Chisago, Pine, Washington [MN]), SLBE (Benzie, Leelanau), and VOYA (Koochiching, St. Louis [MN]; Ontario). Due to Ontario's size, only records from near the border parks of GRPO, ISRO, and VOYA were included. Additional Wisconsin Department of Natural Resource records are indicated by "WI"; records from 2017 Michigan Odonata Survey maps are indicated by "MI". No additional publically available Odonata databases were found for Minnesota or Indiana.

[3] Taxonomic nomenclature checked against Paulson and Dunkle (2016).

Table A1 (continued). Dragonfly species recorded for the nine National Park Service units in the Great Lakes Inventory and Monitoring Network[1] from 2008 to 2014, and species likely present based on county (USA) and provincial (Canada) records[2].

Family	Species[3]	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
Gomphidae (continued)	*Progomphus obscurus*	–	–	–	–	–	–	X[1]	X[1]	–
	Stylogomphus albistylus	X	X	–	X	–	MI	X	–	X
	Stylurus amnicola	X	–	–	–	X	–	X[1]	–	X
	S. notatus	–	–	–	–	X	–	X	X	X
	S. plagiatus	–	–	–	–	X	–	X	–	–
	S. scudderi	X	–	–	MI	–	X	X	X	X
	S. spiniceps	X	–	–	–	–	–	X[1]	–	X
Cordulegastridae	*Cordulegaster bilineata*	–	–	–	–	–	–	–	X	–
	C. diastatops	–	–	–	X	–	X	–	–[1]	–
	C. maculate	X	X[1]	–	X	X	X[1]	X[1]	X	X
	C. obliqua	X	–	–	X	X	X	X[1]	–	X
Macromiidae	*Didymops transversa*	X	X	–	X[1]	X	X[1]	X[1]	X[1]	X[1]
	Macromia illinoiensis	X	X	–	X[1]	X	X[1]	X[1]	X[1]	X[1]
Corduliidae	*Cordulia shurtleffii*	X	X[1]	–	X[1]	X	X[1]	X	X[1]	X[1]
	Dorocordulia libera	X	X	–	X	X	X	X[1]	X[1]	X

[1] Based on confirmed records from the NPSpecies database and research conducted within the parks (also shaded).

[2] County and provincial records from www.odonatacentral.org, indicated by "X", with records vetted by voucher specimens and reviewed by subject and locality experts. Counties and provinces by park include: APIS (Ashland, Bayfield), GRPO (Cook [MN]; Ontario), INDU (Lake, LaPorte, Porter), ISRO (Keweenaw [MI]; Cook [MN]; Ontario), MISS (Anoka, Dakota, Hennepin, Ramsey, Washington), PIRO (Alger), SACN (Bayfield, Burnett, Douglas, Pierce, Polk, St. Croix, Sawyer, Washburn [WI]; Chisago, Pine, Washington [MN]), SLBE (Benzie, Leelanau), and VOYA (Koochiching, St. Louis [MN]; Ontario). Due to Ontario's size, only records from near the border parks of GRPO, ISRO, and VOYA were included. Additional Wisconsin Department of Natural Resource records are indicated by "WI"; records from 2017 Michigan Odonata Survey maps are indicated by "MI". No additional publically available Odonata databases were found for Minnesota or Indiana.

[3] Taxonomic nomenclature checked against Paulson and Dunkle (2016).

Table A1 (continued). Dragonfly species recorded for the nine National Park Service units in the Great Lakes Inventory and Monitoring Network[1] from 2008 to 2014, and species likely present based on county (USA) and provincial (Canada) records[2].

Family	Species[3]	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
Corduliidae (continued)	*Epitheca canis*	X	X[1]	–	X	X	X[1]	X[1]	X[1]	X[1]
	E. cynosura	X	X	X[1]	X	X	X[1]	X[1]	X[1]	X[1]
	E. princeps	X	–	X[1]	–	X	–[1]	X[1]	X[1]	X[1]
	E. spinigera	X	X	–[1]	X[1]	X	X[1]	X[1]	X[1]	X[1]
	Neurocordulia molesta	–	–	–	–	X	–	X[1]	–	–
	N. yamaskanensis	–	–	–	–	X	–	X[1]	MI	X
	Somatochlora brevicincta	–	–	–	–	–	–	–	–	X
	S. cingulata	–	X	–	X	–	MI	–	–	X
	S. elongata	X	X[1]	–	X	–	X	X	X	X
	S. ensigera	–	X	–	X	X	–	WI	–	X
	S. forcipata	X	X	–	X	–	X	X	MI	X
	S. franklini	X	X	–	X	–	X	X	–	X[1]
	S. hineana	–	–	X	–	–	–	–	–	–
	S. incurvata	WI	–	–	X	–	X	WI	–	–

[1] Based on confirmed records from the NPSpecies database and research conducted within the parks (also shaded).

[2] County and provincial records from www.odonatacentral.org, indicated by "X", with records vetted by voucher specimens and reviewed by subject and locality experts. Counties and provinces by park include: APIS (Ashland, Bayfield), GRPO (Cook [MN]; Ontario), INDU (Lake, LaPorte, Porter), ISRO (Keweenaw [MI]; Cook [MN]; Ontario), MISS (Anoka, Dakota, Hennepin, Ramsey, Washington), PIRO (Alger), SACN (Bayfield, Burnett, Douglas, Pierce, Polk, St. Croix, Sawyer, Washburn [WI]; Chisago, Pine, Washington [MN]), SLBE (Benzie, Leelanau), and VOYA (Koochiching, St. Louis [MN]; Ontario). Due to Ontario's size, only records from near the border parks of GRPO, ISRO, and VOYA were included. Additional Wisconsin Department of Natural Resource records are indicated by "WI"; records from 2017 Michigan Odonata Survey maps are indicated by "MI". No additional publically available Odonata databases were found for Minnesota or Indiana.

[3] Taxonomic nomenclature checked against Paulson and Dunkle (2016).

Table A1 (continued). Dragonfly species recorded for the nine National Park Service units in the Great Lakes Inventory and Monitoring Network[1] from 2008 to 2014, and species likely present based on county (USA) and provincial (Canada) records[2].

Family	Species[3]	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
Corduliidae (continued)	*S. kennedyi*	X	–	–	–	X	X	X[1]	–	X
	S. minor	X	X[1]	–	X	–	MI[1]	X	–	X
	S. tenebrosa	–	–[1]	–	–	–	–[1]	–	X	–
	S. walshii	X	X	–	X	X	X	X	–	X
	S. williamsoni	X	X[1]	–	X[1]	X	X[1]	X	X[1]	X[1]
	Williamsonia fletcheri	WI	–	–	–	–	X	X	–	–
	W. lintneri	–	–	–	–	–	MI	–	–	–
Libellulidae	*Celithemis elisa*	X	–	X[1]	X	X	X	X[1]	X[1]	X[1]
	C. eponina	WI	–	X[1]	–	X	–	X	MI	–
	C. fasciata	–	–	X	–	–	–	–	–	–
	Erythemis simplicicollis	X	–	X[1]	–	X	–	X	X	X
	Ladona julia	X	X	X	X[1]	X	X[1]	X[1]	X[1]	X[1]
	Leucorrhinia frigida	X	X[1]	X[1]	X[1]	X	X[1]	X	X[1]	X[1]
	L. glacialis	X	X[1]	–	X	X	X[1]	X	X[1]	X
	L. hudsonica	X	X	–	X	X	X	X	X[1]	X[1]

[1] Based on confirmed records from the NPSpecies database and research conducted within the parks (also shaded).

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Table A1 (continued). Dragonfly species recorded for the nine National Park Service units in the Great Lakes Inventory and Monitoring Network[1] from 2008 to 2014, and species likely present based on county (USA) and provincial (Canada) records[2].

Family	Species[3]	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
Libellulidae (continued)	*L. intacta*	X	–[1]	X[1]	X[1]	X	X[1]	X[1]	X[1]	X[1]
	L. proxima	X	X	–	X	X	X	X	X[1]	X[1]
	Libellula cyanea	–	–	X	–	–	–	–	–	–
	L. incesta	–	–	X[1]	–	X	–	X	X[1]	–
	L. luctuosa	X	–	X[1]	–	X	MI	X[1]	MI[1]	X
	L. pulchella	X	X	X[1]	X	X	X	X	X[1]	X
	L. quadrimaculata	X	X[1]	X[1]	X	X	X[1]	X[1]	X[1]	X[1]
	L. semifasciata	–	–	X[1]	–	–	–	–	–	–
	L. vibrans	–	–	X[1]	–	–	–	X	–[1]	–
	Nannothemis bella	X	–	X	–	–	X	X	X[1]	X
	Pachydiplax longipennis	X	–	X[1]	–	X	–	X	MI[1]	X
	Pantala flavescens	X	X	X[1]	X	X	MI	X	X[1]	X
	P. hymenaea	X	–	X[1]	–	X	–	X	X[1]	X
	Perithemis tenera	X	–	X[1]	–	X	–	X	–	–
	Plathemis lydia	X	X	X[1]	X	X	X[1]	X[1]	X[1]	X

[1] Based on confirmed records from the NPSpecies database and research conducted within the parks (also shaded).

[2] County and provincial records from www.odonatacentral.org, indicated by "X", with records vetted by voucher specimens and reviewed by subject and locality experts. Counties and provinces by park include: APIS (Ashland, Bayfield), GRPO (Cook [MN]; Ontario), INDU (Lake, LaPorte, Porter), ISRO (Keweenaw [MI]; Cook [MN]; Ontario), MISS (Anoka, Dakota, Hennepin, Ramsey, Washington), PIRO (Alger), SACN (Bayfield, Burnett, Douglas, Pierce, Polk, St. Croix, Sawyer, Washburn [WI]; Chisago, Pine, Washington [MN]), SLBE (Benzie, Leelanau), and VOYA (Koochiching, St. Louis [MN]; Ontario). Due to Ontario's size, only records from near the border parks of GRPO, ISRO, and VOYA were included. Additional Wisconsin Department of Natural Resource records are indicated by "WI"; records from 2017 Michigan Odonata Survey maps are indicated by "MI". No additional publically available Odonata databases were found for Minnesota or Indiana.

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Table A1 (continued). Dragonfly species recorded for the nine National Park Service units in the Great Lakes Inventory and Monitoring Network[1] from 2008 to 2014, and species likely present based on county (USA) and provincial (Canada) records[2].

Family	Species[3]	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
Libellulidae (continued)	*Sympetrum ambiguum*	–	–	X[1]	–	–	–	–	MI	–
	S. corruptum	X	X	X[1]	X	X	MI	X	X[1]	X
	S. costiferum	X	X	X[1]	X	X	X	X	X[1]	X
	S. danae	X	X	–	X	X	MI	X	X[1]	X
	S. internum	X	X	–	X	X	MI	X	X[1]	X
	S. madidum	WI	–	–	–	–	–	WI	–	–
	S. obtrusum	X	X	X[1]	X	X	X	X	X[1]	X[1]
	S. rubicundulum	X		X[1]	X	X	X	X	X[1]	–
	S. semicinctum	X	X	X	X	X	MI	X	X	X
	S. vicinum	X	X	X[1]	X	X	X	X	X[1]	X[1]
	Tramea carolina	–	–	X[1]	–	–	–	X	–	–
	T. lacerata	X	X	X[1]	X	X	–	X	X[1]	X
	T. onusta	WI	–	X[1]	–	X	–	X	X	X

[1] Based on confirmed records from the NPSpecies database and research conducted within the parks (also shaded).

[2] County and provincial records from www.odonatacentral.org, indicated by "X", with records vetted by voucher specimens and reviewed by subject and locality experts. Counties and provinces by park include: APIS (Ashland, Bayfield), GRPO (Cook [MN]; Ontario), INDU (Lake, LaPorte, Porter), ISRO (Keweenaw [MI]; Cook [MN]; Ontario), MISS (Anoka, Dakota, Hennepin, Ramsey, Washington), PIRO (Alger), SACN (Bayfield, Burnett, Douglas, Pierce, Polk, St. Croix, Sawyer, Washburn [WI]; Chisago, Pine, Washington [MN]), SLBE (Benzie, Leelanau), and VOYA (Koochiching, St. Louis [MN]; Ontario). Due to Ontario's size, only records from near the border parks of GRPO, ISRO, and VOYA were included. Additional Wisconsin Department of Natural Resource records are indicated by "WI"; records from 2017 Michigan Odonata Survey maps are indicated by "MI". No additional publically available Odonata databases were found for Minnesota or Indiana.

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Appendix B. Power Analysis of Dragonfly Larvae and Fish Data.

Introduction

Dragonfly larvae (n = ca. 2,600) were sampled from 2008 to 2012 in 26 water bodies across six national parks in the Great Lakes Inventory and Monitoring Network (GLKN). Parks sampled include Grand Portage National Monument (GRPO), Indiana Dunes National Lakeshore (INDU), Isle Royale National Park (ISRO), Pictured Rocks National Lakeshore (PIRO), Sleeping Bear Dunes National Lakeshore (SLBE), and Voyageurs National Park (VOYA). Prey (n = ca. 3,200) and predatory fish (n = ca. 1,430) were also sampled from 28 water bodies at the same parks and in the same years. All samples were analyzed for total mercury (THg) and other organic contaminants. A complete description of the sampling effort and results are in Wiener et al. (2016).

The purpose of this project was to conduct a power analysis using the THg data in order to ascertain the appropriate number of larval dragonfly, prey fish, and predatory fish samples to collect annually under a variety of scenarios (i.e., number of years, percent change, Type I error, and Type II error [power of the test]). In contrast with previous power analyses conducted using the same GLKN dataset, this analysis considered all dragonfly families and all fish species combined rather than focusing on a specific family or species. Thus, this power analysis provides a more conservative (i.e., not adjusted for taxonomic differences) estimate of the number of samples needed to detect spatial or temporal differences.

Power Analyses

Fish data were spread across two dataframes containing prey and predatory fish. These two dataframes were combined to form one large 'fish' dataframe with a new variable 'trophic level' that described whether the fish were 'prey' or 'predator'.

All power analyses were completed on non-transformed total mercury (THg) data from both dragonfly larvae and fish. Separate analyses were conducted on total combined fish, as well as prey and predator fish separately.

Three different power analyses were conducted for the dragonfly larvae and fish. The analyses included: 1) a regression power analysis on the linear trend across years (temporal); (2) an analysis of variance (ANOVA) power analysis on the difference among years for the whole GLKN, for each park, and for each water body (temporal); and 3) an ANOVA power analysis on the difference among parks or water bodies (spatial). All power analyses were conducted using the 'pwr' package (Champely 2016) in R (R Core Team 2016) and are further described below.

Regression – linear trend across years

Regression power analyses for temporal trends were conducted using a modification of the 'power.t.test' function with the 'pwr' package (Champely 2016) in R (R Core Team 2016). The new R function, which is a modification of the t-test function in 'pwr', is stored in a separate R code file called 'power_functions.R'.

The first analysis evaluated the number of samples needed to detect a linear trend in the whole GLKN. This analysis used the average of yearly standard deviation of THg for the whole GLKN. There were three scenarios for the number of years over which to detect the trend: 5, 10, and 20 years. There were four scenarios for the percent change to detect over those periods of years: 1%, 5%, 10%, and 20% change. Two scenarios for each set of conditions were run using a Type I error of 0.05 and 0.10. The results from this analysis are included in the dragonfly, fish, predator fish, and prey fish spreadsheets, with dragonflies under the tab 'dfly_samples_reg_GLKN', fish under 'fish_ samples_reg_GLKN', predatory fish under 'pred_ samples_reg_GLKN', and prey fish under 'pred_ samples_reg_GLKN'.

The second analysis evaluated the number of samples needed to detect a linear trend in each park. This analysis used the average of yearly standard deviation of THg for each park. As for the first analysis, the same three scenarios of time and four scenarios of percent change were used, with two scenarios run using a Type I error of 0.05 and 0.10. The results from this analysis are also included in the dragonfly, fish, predator fish, and prey fish spreadsheets under the tabs 'dfly_samples_reg_park' (dragonflies), 'fish_ samples_reg_park' (fish), 'pred_ samples_reg_park' (predator fish), and 'prey_ samples_reg_park' (prey fish).

The third analysis determined the number of samples needed to detect a linear trend in each water body. This analysis used the average of yearly standard deviation of THg for each water body. The same scenarios were used, with two scenarios run using a Type I error of 0.05 and 0.10. The results from this analysis are in the spreadsheets under the tabs 'dfly_samples_reg_water body' (dragonflies), 'fish_ samples_reg_water body' (fish), 'pred_ samples_reg_water body' (predatory fish), and 'prey_ samples_reg_water body' (prey fish).

ANOVA – difference among years

ANOVA power analyses to detect temporal differences were conducted using the 'power.anova.test' function with the 'pwr' package (Champely 2016) in R (R Core Team 2016). The first analysis determined how many samples were needed to tell the difference among years for dragonfly larvae and fish in the whole GLKN. This analysis used the variation within a year and the variation among years for all samples in the GLKN. There were four scenarios for the number of years: discerning difference in THg among 2, 5, 10, and 20 years. The test indicates how many samples would be needed to tell if there is a difference among years, within that time span, but not which year is significantly different. The number of samples to compare two individual years to one another, which would allow for post-hoc tests on which years were significantly different from each other, the sample size for 2 years would be required. Two scenarios were run using Type I errors of 0.05 and 0.10. The results from this analysis are included in the dragonfly, fish, predator fish, and prey fish spreadsheets, with dragonflies under the tab 'dfly_samples_per_GLKN', fish 'fish_ samples_per_GLKN', predatory fish 'pred_ samples_per_GLKN', and prey fish 'prey_ samples_per_GLKN'.

The second analysis determined how many samples were needed to discern difference among years for dragonfly larvae and fish in each park. This analysis used the variation within a year for each park and the variation among years for each park. There were four scenarios for the number of years:

determining difference in THg among 2, 5, 10, and 20 years. Two scenarios were run using a Type I error of 0.05 and 0.10. The results from this analysis are included in the dragonfly, fish, predator fish, and prey fish spreadsheets, with dragonflies under the tab 'dfly_samples_per_park', fish 'fish_ samples_per_park', predatory fish 'pred_ samples_per_park', and prey fish 'prey_ samples_per_park'.

The third analysis determined many samples were needed to tell the difference among years for dragonfly larvae and fish in each water body. This analysis used the variation within a year and the variation among years for each water body. There were four scenarios for the number of years: being able to discern difference in THg among 2, 5, 10, and 20 years. Two scenarios were run using a Type I error of 0.05 and 0.10. The results from this analysis are included in the dragonfly, fish, predator fish, and prey fish spreadsheets, with dragonflies under the tab 'dfly_samples_per_water body', fish 'fish_ samples_per_water body', predatory fish 'pred_ samples_per_water body', and prey fish 'prey_ samples_per_water body'.

ANOVA –difference among parks and water bodies

ANOVA power analyses to detect spatial differences were conducted using the 'power.anova.test' function with the 'pwr' package (Champely 2016) in R (R Core Team 2016). The first analysis evaluated how many samples would be needed to determine a significant difference in THg of fish or dragonfly larvae among parks within the GLKN. This analysis used the variation within parks and the variation among parks to predict the number of samples. Two scenarios were run, using a Type I error of 0.05 and 0.10. The results from this analysis are included in the dragonfly, fish, pred fish, and prey fish spreadsheets with dragonflies under the tab 'dfly_samples_diff_park', fish 'fish_samples_diff_park', predatory fish 'pred_samples_diff_park', and prey fish 'prey_samples_diff_park'.

The second analysis evaluated how many samples would be needed to determine a significant difference in THg of fish or dragonfly larvae among water bodies within a park. This analysis used the variation within the water bodies and the variation among water bodies of a particular park to predict the number of samples. Two scenarios were run for each park, using a Type I error of 0.05 and 0.10. The results from this analysis are included in the dragonfly, fish, predator fish, and prey fish spreadsheets, with dragonflies under the tab 'dfly_samples_diff_wb', fish 'fish_samples_diff_wb', predatory fish 'pred_samples_diff_wb', and prey fish 'prey_samples_diff_wb'.

Further scenarios

For all above analyses, any of the following predictor variables within the power analyses can be modified: number of years, percent change, Type I error, Type II error (power of the test).

Results and Discussion

Some parks within the GLKN have more power to detect change than others, depending on the analysis and type of sample (dragonfly, fish [predatory or prey]) being collected. For example, when looking at dragonfly larvae samples needed per park with a 20% change over 10 years with Type I error of 0.05, GRPO has the greatest power to detect change with the smallest number of samples

(Figure B1). This is due to high among-year difference (standard deviation) combined with low within year difference (standard deviation) of total mercury values in dragonfly larvae in the park (Table 1). The high standard deviation among years would make it easier to detect change from year to year. In contrast, SLBE has the least power to detect change per number of samples collected (Figure 1). This is due to low among-year difference combined with higher within year difference (Table 1), which would make it harder to detect change through time.

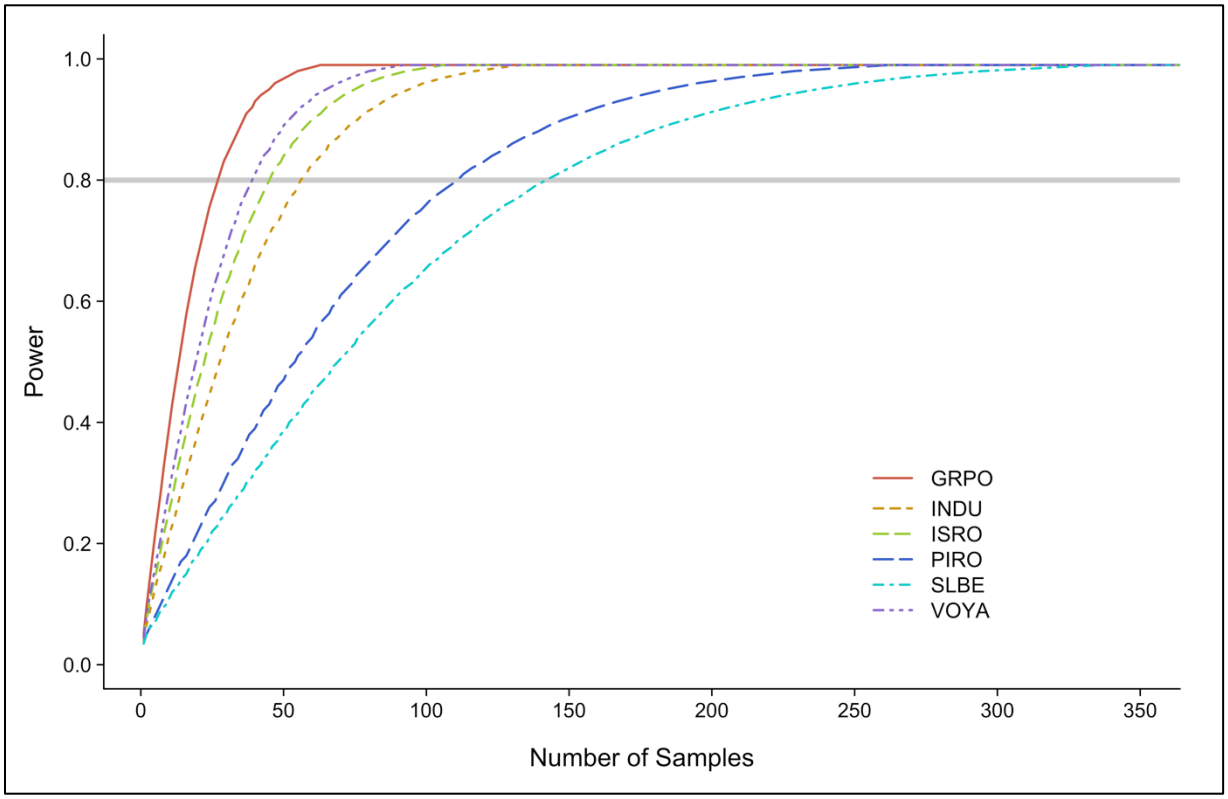


Figure B1. Graphical representation of the power that each park in the GLKN has per number of samples to detect a 20% change over 10 years with a Type I error of 0.05 using a regression analysis for dragonfly larvae.

On average, the number of dragonfly larvae samples needed per water body in each park to detect a 20% change over 10 years with a Type I error of 0.05 and 80% power is 17. Looking at individual parks, depending on the variation within years and among years, certain parks require more dragonfly samples than others (Table B1). The highest number of samples required in a park is 142 at SLBE, whereas the lowest number needed in a park is 27 at GRPO. The average number of fish samples needed per water body in each park is 58 (Table B1). This high number per water body is likely due to the very large standard deviation within and among years for fish samples. When predatory and prey fish are analyzed separately, the standard deviation decreases; however the number of samples needed is still larger than for dragonfly larvae (Table B2).

Table B1. The number of samples needed per park to determine a 20% change through regression analysis over 10 years with a Type I error of 0.05 and 80% power. Results for both dragonfly larvae (dfly) and combined predatory and prey fish (fish) are presented. “Within_stddev” is the standard deviation within a year for all organisms sampled in a park. “Among_stddev” is the standard deviation among years for all organisms sampled within a park. “Mean_per_wb” is the number of samples per park divided by the number of water bodies in that park. Variable names used in R code are given in parentheses.

Park	N of water bodies in park (#_of_wb)	Std. Dev. within year (within_stddev)	Std. Dev. among years (among_stddev)	Number of samples needed – park (num_samples)	Number of samples needed - water body (mean_per_wb)	Organism
GRPO	5	53.97	18.75	27	5	dfly
INDU	3	21.75	14.85	56	19	dfly
ISRO	4	28.20	8.78	45	11	dfly
PIRO	5	80.32	20.18	111	22	dfly
SLBE	4	38.71	12.00	142	36	dfly
VOYA	5	48.48	17.28	39	8	dfly
GRPO	5	184.60	14.63	83	17	fish
INDU	3	204.11	33.82	200	67	fish
ISRO	4	1400.73	301.62	326	81	fish
PIRO	5	1098.95	459.71	227	45	fish
SLBE	4	949.21	538.60	251	63	fish
VOYA	5	2196.54	117.62	387	77	fish

Table B2. Sample numbers produced from the three different analyses: 1. regression (time) is the number of samples needed each year to detect a 20% change over 10 years with a Type I error of 0.05; 2. ANOVA (time) is the number of samples needed each year to detect a difference among years with a Type I error of 0.05; and 3. ANOVA (per unit) is the number of samples per unit (park or water body) to detect a difference among units (park or water body) with a Type I error of 0.05. Number of samples or mean (standard deviation) number of samples are reported for the whole GLKN, per park in the GLKN, and per water body in each park.

	Regression (time)				ANOVA (time)				ANOVA (per unit)			
Network or Park	**Dragonfly**	**Fish**	**Fish (pred)**	**Fish (prey)**	**Dragonfly**	**Fish**	**Fish (pred)**	**Fish (prey)**	**Dragonfly**	**Fish**	**Fish (pred)**	**Fish (prey)**
GLKN	121.00	455	164	126	70	30	71	50	4	14	4	13
GLKN mean per park	70 (46)	246 (105)	108 (25)	101 (60)	18 (9)	170 (238)	123 (21)	68 (103)	40 (57)	23 (8)	7 (4)	7 (6)
GRPO mean per wb	17 (9)	44 (47)	17 (16)	57 (60)	27 (23)	19 (19)	4 (0)	20 (18)	11	4	3	10
INDU mean per wb	38 (14)	116 (72)	78 (0)	42 (14)	4 (3)	13 (5)	8 (0)	8 (2)	149	31	6	17
ISRO mean per wb	19 (7)	165 (24)	67 (36)	79 (0)	11 (8)	13 (15)	65 (100)	14 (0)	4	11	5	4
PIRO mean per wb	46 (32)	140 (78)	61 (14)	52 (27)	12 (6)	16 (20)	31 (40)	15 (19)	6	18	7	5
SLBE mean per wb	131 (156)	188 (44)	63 (7)	47 (0)	16 (15)	7 (5)	57 (98)	4 (0)	58	29	10	5
VOYA mean per wb	33 (19)	275 (151)	54 (19)	14 (3)	16 (13)	189 (98)	28 (34)	14 (17)	12	27	18	3

The most conservative analysis to detect change through time for each park would be the ANOVA power analysis for differences among years (i.e., ANOVA (time) in Table B2), which in the absence of a linear trend would detect differences among years over the course of 10 years. For dragonfly larvae, an average of 18 samples collected per park per year would allow for the detection of a difference among years over 10 years (Table B2). To investigate the difference among years for particular water bodies within each park, the number of dragonfly samples needed ranges from 4 to 27 with an average of 14 samples per water body (Table B2). For fish, because of the high standard deviation, on average 170 samples would be needed per park (Table B2). When looking at the average number of samples needed per water body in each of the parks, most of the parks are within the 15 fish samples per year per water body range, with the exception of VOYA (Table B2).

Last, the number of samples needed to detect a change among either parks within the GLKN or among water bodies in each of the parks is in the ANOVA (spatial) analysis (Table B2). The average number of dragonfly larvae samples needed per park to detect differences among parks is 40 (Table B2). Interestingly, the park that has the highest number of samples needed to detect differences among water bodies within the park is INDU. This is likely because of relatively small differences in THg in dragonfly larvae among the water bodies within the park. For fish, the average number of samples needed per park to discern difference among parks is 23 (Table B2). This is low compared to the other sample estimates for fish. This is likely due to high THg differences in fish among parks; therefore it will be easier to detect a difference among parks in the future.

In conclusion, collecting 20 dragonfly larvae per water body will be sufficient to conduct multiple types of analyses to detect differences through time or among park or water body units for most parks. Importantly, Haro et al. (2013) found that a sample size of 10 individually analyzed gomphid larvae from each water body would be sufficient to detect a 20% difference in mean THg concentrations with a Type 1 error of 0.05 across 17 out of the 26 water bodies in this power analysis. Because gomphids are relatively abundant throughout the Great Lakes region it is reasonable to assume that in general the sample size suggested by Haro et al. (2013) for gomphids will be met by collecting 20 larval dragonflies per water body. However, for fish, sampling numbers may need to be specific for each park, depending on the type of analyses conducted in the future.

Possibilities for further analyses include methylmercury for dragonfly larvae, log-transformed THg, and limiting to specific taxa (e.g., Gomphidae as in Haro et al. 2013).

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Appendix C. Examples of Laboratory Methodologies to Analyze Larval Dragonflies and Fish for Total and Methylmercury.

Procedure

Page

Analyses of larval dragonflies, water, sediment, seston, and zooplankton for total mercury and methylmercury. 86

Analysis of Prey Fish and Predatory Fish for Total Mercury. 96

Technical Operating Procedure:
Analyses of larval dragonflies, water, sediment, seston, and zooplankton for total mercury and methylmercury.

Scope

This technical operating procedure describes the analytical procedures for methylmercury and total mercury analysis of water, sediment, seston, zooplankton, and larval dragonflies.

Principle

Samples are collected and stored frozen according to SOP GLRI-04 (see Wiener 2009), followed by a variety of digestion methods to liberate mercury species from their matrix. Mercury species are then determined using the Brooks-Rand MERX Automated Mercury Analyzer, which has the capability to measure both total (THg) and methylmercury (MeHg) in samples. All sample processing and analyses should be conducted in a room where access is controlled—dust from external sources and clothing must be minimized by wearing clean-room-dedicated shoes and outer garments such as Tyvek clean lab smocks. Sample digestates and distillates must be prepared in this clean environment, preferably in a HEPA filter enclosure. Solid samples must be stored as lyophilized homogenates in double-wrapped Ziploc bags. Water samples must be stored in a refrigerator, acidified to 1% v/v trace metal grade HCl in Teflon bottles that are double-bagged.

Safety Precautions

Strong acids, bases, and oxidizers are routinely utilized in the analytical process. Normal precautions must be taken to prevent exposure to chemical reagents, including use of face-fitting goggles, gloves, and gown.

Reagents

<u>Analysis of Total Mercury</u>

- Hydrochloric acid, HCl, trace metal grade HCl, 12.1 M (Fisher Scientific)
- Mercuric nitrate, $Hg(NO_3)_2$, 1.0 ppm (Brooks Rand # 3600)
- Deionized water (>18 $M\Omega\text{-}cm^{-1}$)
- Argon gas, compressed (Grade 5.0)
- Nitrogen, compressed (Grade 5.0)

Stannous chloride, $SnCl_2$, 20% w/v: Add 100 g $SnCl_2$ to 50 mL trace metal grade concentrated HCl in a 500 mL Teflon bottle. Add 450 mL deionized water. Purge for 1 hour with Hg free N_2 at 100 mL/min. Prepare freshly every 6 months.

Hydroxylamine hydrochloride, $NH_2OH.HCl$, 30% w/v: Dissolve 30 g of $NH_2OH*HCl$ in a Teflon bottle containing 100 mL of deionized water. The solution should be purged with Hg-free N_2 gas at 100 mL/min for 1 hour. Prepare fresh every 6 months.

Bromine monochloride, BrCl: Dissolve 27 g of reagent grade potassium bromide (KBr) in a 2.5 L bottle of trace-metal grade concentrated HCl (12.1 M). Place a Teflon coated stir bar into the bottle and stir for 1 hour. Slowly add 38 g reagent grade potassium bromate ($KBrO_3$) to the bottle while stirring. CAUTION: This needs to be done slowly and in a fume hood because large quantities of

free halogens are produced. As you add the $KBrO_3$ to the solution, the color should change from yellow to red to orange. Cap bottle loosely and allow to mix for an additional hour. The BrCl is analyzed for Hg prior to adding to samples.

Analysis of Methylmercury

- Hydrochloric acid, Trace Metal Grade HCl, 12.1 M (Fisher Scientific)
- Methylmercuric hydroxide, CH_3HgOH, 1.0 ppm (Brooks Rand # 6601)
- Deionized water (>18 $M\Omega\text{-}cm^{-1}$)
- Argon gas, compressed (Grade 5.0)
- Nitrogen, compressed (Grade 5.0)
- Nitric acid, trace metals-grade HNO_3, 4.5 M
- Potassium hydroxide, KOH, 4.5 M
- Copper sulfate, $CuSO_4$, 25% w/v

Acetate buffer (2 M): measure approximately 50 mL deionized water, 47.2 mL glacial acetic acid, and 108.8 g anhydrous sodium acetate into a 500 mL Teflon bottle. Bring up to 400 mL volume and shake until all solids dissolve. For a working solution, fill a 125 mL Teflon bottle with stock buffer and shake well before use.

Sodium tetraethylborate, $NaB(CH_2CH_3)_4$ ("NaTEB"): Pure solid NaTEB is purchased in 1 g sealed glass vials (stored under N_2 gas) and kept in the freezer until use. To dilute NaTEB to a 1% w/v working solution, dissolve 2 g of KOH in 100 mL of reagent water in a 125 mL Teflon vial and chill to sub-freezing temperatures. Check the condition of the solution often. As soon as the KOH solution begins freezing, remove the vial of NaTEB from the freezer and score the neck of the bottle with a glass cutter or the back of a ceramic knife. Wrap the vial in a lab wipe and break the neck of the vial. It is best to work quickly at this point as to keep the pure NaTEB cold and to limit its exposure to oxygen to reduce the risk of combustion. Immediately dump the pure NaTEB into the 2% KOH solution and gently swirl to dissolve. Rinse the glass vial with the solution if any significant amount of NaTEB remains in the vial. When the NaTEB solution is almost entirely melted, homogenize, and pour equally into 20 clean, chilled 5 mL Teflon vials. Cap the vials, store in a sealed bag, and record the date prepared. This solution should be kept frozen and made fresh every 2 weeks. Never use NaTEB solid or solutions that are yellow in color. Following use, NaTEB should be stored in an appropriately labeled and sealed bag in the freezer until the solution can be disposed of properly. To dispose of old or unused portions of the 1% w/v NaTEB solutions, thaw the vials and pour into a beaker under a fume hood. Fill the beaker with an equivalent volume of 6 M HCl (50% concentrated solution), place on a hotplate, boil down to half-volume, and then discard the remaining solution as acid waste. Never dispose of concentrated NaTEB in this fashion, as that it will combust, but rather dilute to a 1% w/v concentration with water and then process as previously described.

Equipment

1. MERX-M,T Instrument (Brooks Rand Co., Seattle WA)
2. MERX Autosampler Vials, Caps, (Brooks Rand # 9124)

3. Distillation Block (Environmental Express # SC151)
4. Teflon Vials, 22 mL, with Teflon cap (Savillex Co. # 200-022-20, 600-033-01); shipped with 10-position plastic rack.
5. Teflon Vials, 30 mL, with Teflon cap (Savillex Co. # 200-030-20, 600-033-01); shipped with 10-position plastic rack.
6. Eppendorf research-grade pipettes (Fisher Scientific)
7. Standard Reference Materials (Mussel tissue: National Institute of Standards and Technology (NIST) "Mussel 2976"; Lobster hepatopancreas: National Research Council Canada (NRCC) "TORT-2"; Estuarine sediment: NRCC "MESS-3")
8. HEPA Filter Hood (Purair # P5-36, ASTS-030, or equivalent)
9. Teflon Spatula (Fisher Scientific # NC9979753)
10. PC Computer

Procedure

Methylmercury Analysis: Zooplankton and Larval Invertebrates

Portions of this method were reproduced or adapted from the U.S. Geological Survey Document, "Analysis of Methylmercury in Biological Samples by Cold Vapor Atomic Fluorescence Detection with the Brooks-Rand "MERX" Automated Methylmercury Analytical System", J. Ogorek and J. Dewild (USGS, Middleton, WI).

1. Solid, lyophilized samples of zooplankton or larval invertebrates are ground with an acid-cleaned mortar and pestle to a homogenized powder, then returned to the bag until analysis.
2. Digestion batches consist of a set of procedural reagent blanks, standard reference materials (SRMs), replicate samples, spiked samples, and unreplicated samples. For example, a typical digestion batch is composed of:

    3 Procedural blanks

    6 SRMs (triplicates of 2 unique SRMs)

    3 replicates of a single sample

    3 spiked replicates to assess matrix interference

    5 Calibration standards (working range 0, 25, 50, 100, 150 pg MeHg)

    25–30 unique samples

    45–50 samples total
3. For biological tissue samples and SRMs, approximately 40–60 mg of dried, homogenized sample is added to an acid-cleaned Teflon 22-mL screw-cap vial using a Teflon spatula.
4. 7.0 mL of 4.5 M HNO_3 is added to each vial by re-pipettor (bottle-top dispenser), and the vial vigorously inverted and shaken.
5. Vials are placed into plastic racks and triple-bagged with 12″×15″ Ziploc bags. The digestion batch is heated in a drying oven for 12 hours at 60ºC.

6. Analytical batches consist of the digested samples plus calibration standards, instrument rinses, and periodic check standards. For example:

    3 Conditioning rinses

    3 Procedural blanks

    5 Calibration standards (working range 0, 25, 50, 100, 150 pg MeHg)

    4 check standards (varied mass)

    6 SRMs (2-triplicate analyses)

    3 replicates of a single sample

    3 spiked replicates to assess matrix interference

    25–30 unique samples

    55–60 samples total

7. MERX vials for MeHg analysis (40 mL) are prepared by adding approximately 35–37 mL of deionized water (>18 MΩ-cm^{-1}), 0.20 mL of acetate buffer, and 0.10 mL of the digested sample.
8. During the MERX vial preparation in step 1.7, a 5.0 mL vial of 1% w/v frozen NaTEB is allowed to partially thaw in the fume hood. While in this two-phase state, 0.050 mL of NaTEB is added to each MERX vial. NaTEB is an unstable reagent and must always remain at or near freezing temperatures to slow degradation. Begin thawing several minutes before use but always make sure that some frozen NaTEB remains in the vial. Promptly cap and return the vial of NaTEB to the freezer after use. NaTEB is toxic and spontaneously combustible in air. Only open vials and dispense NaTEB under a fume hood. Add NaTEB directly to the sample mixture (not to the glass surface inside the vial) to reduce volatilization.
9. Additional deionized water is slowly and carefully added to the vial to create a convex meniscus surface at the top of the vial. The cap/septa enclosure is carefully and tightly closed over the top, caring to minimize spills or drops. The vial should have no air space—the vial must be re-filled if small bubbles are observed through the vial or septa wall. Rigorously invert the closed vial to mix the reagents.
10. MERX racks consist of 24 positions—we typically fill 2–3 racks of 24 during a complete analytical run. Quality assurance samples are interspersed among the samples throughout the batch. The first 16 vials follow a set QA schedule:

    Vials 1–3: Rinses to clean the autosampler needle and tubing (contain water only)

    Vials 4–6: Analytical blanks (contain water, acetate buffer, NaTEB only)

    Vials 7–10: Calibration standards (25, 50, 100, 150 pg MeHg, contain water, acetate buffer, NaTEB, and aqueous MeHg standard)

    Vials 11–13: SRM #1 in triplicate

    Vials 14–16: SRM #2 in triplicate

The balance of the analytical batch includes individual samples, 10% of samples run in triplicate, and another triplicate set spiked with MeHg. Spike levels are typically 2–3 times the expected mass of the sample into which it was spiked. These samples and QA samples are randomly mixed in analysis order to account for drift through the analytical run. Additionally, aqueous check standards are included every 10 samples to assess recovery drift throughout the run.

11. MERX Instrument Operation: instructions for normal operation of the MERX instrument and its Guru4 Software (control program) are supplied with the instrument (Brooks Rand, Co., Seattle WA).
    a. The night before analysis, adjust the sensitivity of the detector so that the baseline offset is approximately 55,000–60,000 units by changing the photomultiplier tube (PMT) detector value using the up/down arrows on the front of the detector. When the PMT value is changed, the offset value will go blank, and the new offset value will temporarily appear in the signal field.
    b. Open the Mercury Guru4 software with the shortcut on the desktop.
    c. Open a new analytical template (".brt" file) for the planned analysis. Template files are designed by the user—these control files are initially supplied by Brooks Rand upon initial setup.
    d. Save the file as "data" type (from the "File" dropdown menu) in the clean lab GLRI data folder. Name the new run file by date and sample description (MMDDYY.brd).
    e. From the "Instrument" dropdown menu, select "Connect", prompting a popup window displaying three communication ports. Select the appropriate ports (CVAFS = COM#, Purge and Trap = COM#, and autosampler = COM#) and click "Accept". The communication status at the top of the screen will turn green indicating connection with each module. COM values depend upon which USB ports on the PC are connected to the MERX components.
    f. Press the autozero button when the signal value is approximately 55,000-60,000 units. Once the offset value stabilizes (2–3 minutes), measure the instrument noise (found in the "File" dropdown menu). Record the new offset, PMT, and noise values in the instrument log notebook.
    g. Fill out the Guru4 sample worksheets, indicating sample type, vial position, # of vials, and sample name.
    h. Check that all modules of the instrument have power and the Ar and N_2 gases supply is turned on.
    i. Check the lamp noise.
    j. Press the start button to initiate the analytical run.
12. During the course of the analytical run, values reported by MERX are entered by permanent marker into a laboratory book. The following data are included: title, date, analyst initials, sample name/ID, run #, MERX trap #, volume of sample analyzed, MeHg peak area, and any

comments relevant to the sample analysis. The MERX unit employs three traps for MeHg analysis, so trap # is 1, 2, or 3.

13. QA Acceptance Criteria and response: Certain QA criteria must be met to deem the analysis "acceptable". We have developed a set of QA criteria to evaluate, as well as corrective actions taken:
    a. SRM Results: SRM replicates should be within the reported 95% confidence interval, as reported by NIST (U.S.) or NRCC (Canada). The SRMs we typically employ for zooplankton and invertebrate analysis are NRCC Lobster hepatopancreas (TORT-2) and NIST Mussel Tissue (MUSS-2976).
    b. Spiked-sample Recoveries: 75%–125%.
    c. Check Standard Recoveries: 75%–125%.
    d. Precision of Triplicates: ≤15% coefficient of variation (%CV=rsd × 100)
    e. Detection Limit: Sample concentrations reported as numeric values must be above the detection limit. This value is calculated as 3× the standard deviation of the analytical blank signal, divided by the slope of the calibration curve. Any samples falling below this value are reported as "< DL". Typical MeHg detection limits for this method are 0.040 ng/L (water) and 0.25 ng/g dry weight (tissues).
    f. Analytical batches are evaluated holistically, meaning that failure of one QA criterion does not necessarily fail the entire run. Failure of an entire run may be the result of the following:
        i. Fewer than ⅔ of replicates, samples, or check standards from two or more QA categories (# 1–5 above) meet their expected criterion.
        ii. A single QA category (# 1–5 above) fails all of its QA expectations.
        iii. Fewer than half of SRM replicates fall within the expected 95% confidence interval.
        iv. Exceptions to these rules may be due to unique sample type or concentration. For example, low-level samples typically exhibit much higher % CV than do normal samples.
14. We do not use the Guru4 MERX interface software to determine sample concentration. Raw data are manually entered into a Microsoft Excel spreadsheet. This spreadsheet calculates sample concentration, spiked-sample recovery (%), precision (%CV), SRM recovery, procedural blank mass (pg), and a graphical representation of the calibration curve (slope, y-intercept).
15. Data management: upon completion of the spreadsheet, data are checked by two personnel to ensure agreement between the Guru4 MERX software output, lab notebook hardcopy, and the Excel spreadsheet. Accepted MERX files and their corresponding Excel spreadsheets are backed up and stored electronically.

16. Used MERX vials may be cleaned for subsequent use by acid washing in hot (65ºC) 6 M HCl for 12 h, followed by four rinses with deionized water. The cap/septa are single-use only, and not re-cleaned.

Total Mercury Analysis: Zooplankton and Larval Invertebrates

Portions of this method were reproduced or adapted from the U.S. Geological Survey document, "Determination of Total Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry", M.L. Olson and J.F. De Wild, SOP001 Revision 4, USGS Middleton, WI.

1. Change the PMT setting on the MERX detector to 10,000-12,000 units during the night prior to analysis.
2. After MeHg analysis has been performed on the digestates of zooplankton and dragonfly larvae, 2.00 mL of BrCl are added to each 22 mL digestion vial. The vials are gently swirled and allowed to sit in the fume hood for 1 minute (this addition is exothermic and produces Br_2 and Cl_2 gas).
3. Cap the digested samples tightly and place back into their plastic racks. Triple bag the vials/rack with 12″×15″ Ziploc bags, and digest in a drying oven at 40ºC for 12 hours.
4. After samples have cooled, vials are checked to see if the distinct reddish-tint of the BrCl remains (it is added in excess to convert all organic forms of Hg to inorganic Hg(II)). If the reddish-tint persists, the samples are ready for analysis. If any of the samples have cleared, add 1.00 mL more BrCl and re-digest for another 12 hours at 40ºC.
5. Just prior to preparing the MERX autosampler vials for THg analysis, add 0.20 mL of 30 % w/v hydroxylamine hydrochloride to each vial. A visual clearing of the reddish-tint should occur, as this reducing agent rids the solution of oxidizing Br and Cl free radicals. This step should be conducted in a fume hood.
6. Change the MERX autosampler needle to the THg needle (from the MeHg needle), and re-connect N_2 and Ar gas lines to the MERX THg purge/trap unit. Connect the THg purge/trap unit output line to the MERX detector inlet port.
7. THg MERX vial preparation: add approximately 25 mL of deionized water to each vial. 1.00 mL of digestate is added by pipette—these additions are also weighed on an analytical balance and recorded in the analysis book.
8. 0.100 mL of 20% w/v SnCl2 is added to each vial, and the septa/cap tightly secured.
9. Racks are set up according to the sample schedule outlined in Step 10 of methylmercury analysis procedure (above).
10. Analyze the sample batch, following Steps11 through 15 in the methylmercury analysis procedure above.
11. The QA acceptance criteria for THg are identical for that of MeHg (Step 13 above).

Methylmercury Analysis: Water, Seston, and Sediment

Portions of this method were reproduced or adapted from "Standard Operating Procedure for the Determination of Methyl Mercury in Water and Suspended Solids by Aqueous Phase Ethylation, Followed by Gas Chromatography Separation with Cold Vapor Atomic Fluorescence Detection", J.F. De Wild and M.L. Olson, U.S. Geological Survey, Middleton, WI (WDML SOP005), and from EPA Method 1630, "Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS" (available as .pdf download at the EPA website).

1. The method entails the aqueous steam distillation of water samples or frozen seston filters in order to remove matrix interferences for the subsequent ethylation step prior to analysis. Samples are distilled from Teflon "From" vials into "Receiver" vials.
2. Sample distillation procedure (text adapted from EPA 1630):
    a. Water: Weigh an approximately 90-mL aliquot from a thoroughly shaken, acidified sample, into a 120-mL Teflon distillation "from" vial. Add 1.0 mL of 25% w/v $CuSO_4$ to each vial.
    b. Seston and Sediment: Weigh approximately 90 mL of water into a "from" vial, and add a single frozen seston filter or 0.25-g–2.0-g lyophilized sediment (how much sediment depends upon its organic matter content, highly organic is lower mass, less organic is higher mass). Add 1.0 mL of 25% w/v $CuSO_4$, 1.0 mL of 50% v/v H_2SO_4, and 0.50 mL of 25% w/v KCl to each vial.
    c. For each sample, prepare a 120 mL distillate "receiver" vial. Add approximately 20 mL reagent water to each "receiver" vial and replace the cap so that the tubing extends into the water layer.
    d. Record the sample ID associated with each "from" and "receiver" vial.
    e. It is important to develop an unambiguous tracking system, such as the use of engraved vial numbers, because the distillation vials themselves cannot be labeled (due to the heat).
    f. Place each prepared "from" vial into one of the holes in the heating block and attach the ⅛″ OD Teflon tubing to the incoming gas supply from the flowmeter manifold. Adjust the N2 gas flow rate through the bubbler to 60 ± 20 mL/min.
    g. As each "from" vial with sample is placed into the heating block, place the corresponding labeled "receiver" vial into the ice bath immediately adjacent to the heating block. Attach the tubing from the receiving vessel to the port of the distillation vessel.
    h. Turn on the temperature controllers to the 35-position heating block to a pre-set block temperature of 120ºC.
    i. Distill the samples until each "from" vial contains only about 20% of its original volume. This time period will be approximately 2.5 h to 5 h depending upon exact temperatures, gas flow rates, and water characteristics.

j. Different samples and locations on the block will distill at somewhat different rates, so after about 2 h, all of the tubes should be monitored frequently to avoid over-distillation. As the individual samples fill to the line, they should be removed from the distillation unit. Over-distillation is the greatest potential risk for poor recoveries by this method. If more than the prescribed amount of sample distills over, the risk of HCl fumes co-distilling increases. Chloride and low pH are interferences with the ethylation procedure.

k. Once all of the "from" vials are distilled into the "receiver" vials, the distillates are stored in a refrigerator for up to 4 days before analysis.

l. The distillation-side (dirty) vials must be scrubbed thoroughly with a test-tube brush and detergent, then rinsed in acetone and reagent water to remove organics prior to acid cleaning.

3. Distillates obtained using this method are analyzed by the MERX MeHg instrument as per Steps 11 through 15 in the methylmercury analysis procedure above. Approximately 35–38 mL of the distillate sample in the "receiver" vial is added to the MERX vial, followed by acetate buffer and NaTEB as before.

Total Mercury Analysis: Water

1. Acidified samples from the field will at this point have had a volume of water removed for MeHg analysis. Record the remaining sample mass (g).
2. Add 2.0 mL BrCl (in a fume hood) to each sample bottle. Triple bag the samples in Ziploc bags and digest all samples at 40ºC for 12 h. If the cooled samples are still of reddish tint, they are fully oxidized and ready for analysis. If the tint has faded back to a clear color, add another 1.0 mL of BrCl and re-digest for another 12 h. Repeat this process until every water sample retains a reddish tint.
3. Just prior to preparing the MERX autosampler vials for THg analysis, add 0.20 mL of 30 % w/v hydroxylamine hydrochloride to each vial. A visual clearing of the reddish-tint should occur, as this reducing agent rids the solution of oxidizing Br and Cl free radicals. This step should be conducted in a fume hood.
4. Change the MERX autosampler needle to the THg needle (from the MeHg needle), and re-connect N_2 and Ar gas lines to the MERX THg purge/trap unit. Connect the THg purge/trap unit output line to the MERX detector inlet port.
5. THg MERX vial preparation: add approximately 25–30 mL of water sample to each vial—these additions are also weighed on an analytical balance and masses recorded in the analysis book.
6. 0.10 mL of 20% w/v SnCl2 is added to each vial, and the septa/cap tightly secured.
7. Racks are set up according to the sample schedule outlined in Step 10 of MeHg analysis procedure above.
8. Analyze the sample batch, following Steps 11 through 15 in MeHg analysis procedure above.
9. The QA acceptance criteria for THg are identical for that of MeHg (Step 13).

Total Mercury Analysis: Seston

1. Place a folded, frozen seston filter sample into an acid-cleaned 40-mL Teflon vial, and add enough water to cover the filter. Measure the mass of water added.
2. Add 2.0 mL BrCl (in a fume hood) to each sample vial. Triple bag the vials and plastic racks in Ziploc bags and digest all samples at 40ºC for 12 h. If the cooled samples are still of reddish tint, they are fully oxidized and ready for analysis. If the tint has faded back to a clear color, add another 1.0 mL of BrCl and re-digest for another 12 h. Repeat this process until every seston filter retains a reddish tint.
3. Just prior to preparing the MERX autosampler vials for THg analysis, add 0.20 mL of 30 % w/v hydroxylamine hydrochloride to each vial. A visual clearing of the reddish-tint should occur, as this reducing agent rids the solution of oxidizing Br and Cl free radicals. This step should be conducted in a fume hood.
4. Change the MERX autosampler needle to the THg needle (from the MeHg needle), and re-connect N_2 and Ar gas lines to the MERX THg purge/trap unit. Connect the THg purge/trap unit output line to the MERX detector inlet port.
5. THg MERX vial preparation: add approximately 25–30 mL of the digested filter vial (not the filter) to each MERX THg vial—these additions are also weighed on an analytical balance and masses recorded in the analysis book.
6. 0.10 mL of 20% w/v $SnCl_2$ is added to each vial, and the septa/cap tightly secured.
7. Racks are set up according to the sample schedule set up Step 10 of the MeHg analysis procedure above.
8. Analyze the sample batch, following Steps 11 through 15 of the MeHg analysis procedure above.
9. The QA acceptance criteria for THg are identical for that of MeHg.

Total Mercury Analysis: Sediment

1. Lyophilized, homogenized sediment is analyzed via GLRI SOP-9 (see Wiener et al. 2009), “Analysis of prey fish and predatory fish for total mercury”. This method does not involve acid digestion; rather, it is a dry thermal combustion technique that involves a catalyst. Typical masses analyzed with the Milestone DMA-80 instrument are 0.25–2.0 grams for sediment samples. All other calibration and analytical procedures are identical to that of fish tissues.
2. The SRM for THg in sediment is “Estuarine Sediment”: NRCC MESS-3.

Technical Operating Procedure:
Analysis of Prey Fish and Predatory Fish for Total Mercury.

Scope
This standard operating procedure describes methods for the analysis of fish tissue for determination of total mercury. This procedure uses U.S. Environmental Protection Agency (EPA) Method 7473 "Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry."

Principle
Precise measuring, recording, handling, and clean-technique procedures are essential for high accuracy and precision.

Safety Precautions
Concentrated hydrochloric acid is used to preserve standard solutions, and labware is acid-washed in 50% concentrated nitric acid. Avoid contact and inhalation of fumes while working with corrosives. While handling acids, wear a lab coat, face shield or safety goggles, vinyl or nitrile gloves, and an acid-resistant apron, and conduct work in a fume hood. Mercury can be toxic if inhaled, ingested, or absorbed through skin; exercise extreme caution while handling concentrated mercury standards. The analyzer utilizes compressed oxygen; keep highly flammable materials away from the oxygen cylinder and line.

Reagents
Acids used in the formulation of standards must be the equivalent of Instra-analyzed® (J.T. Baker) grade or better and designated for use in mercury determination. A commercially prepared 1000-ppm Hg stock solution is used to formulate mercury standards. Reagent-grade water must have a nominal resistance of $\geq$15 MΩ-cm^{-1}. Standard (welding) grade compressed oxygen is used to facilitate combustion of the sample and as a carrier gas.

Equipment
A Milestone DMA-80 direct mercury analyzer is used for mercury determinations. A Mettler Toledo XS-64 balance with an anti-static electrode is used for weighing of samples and standards. Acid-washed, grade-A volumetric flasks are used for the formation of working standards. Acid-washed plastic spoons are used to weigh samples into quartz sample boats. A Fisher Isotemp muffle furnace is used to clean the quartz sample boats by heating to 65ºC. Microsoft Excel and Access software are used for management and storage of generated data.

Procedure

I. Preparation

A. Carefully read both EPA Method 7473 and the manufacturer's operator manual for the DMA-80 analyzer for detailed instructions on method application and instrument operation.

B. Clean the quartz sample boats by rinsing with de-ionized (DI) water and place in a muffle furnace for 1 minute at 65ºC. (Sample boats may be placed in a cool oven with the timer set

for 1 hour, which allows the oven to reach temperature before turning off; remove boats the following day when cooled).

II. Instrument calibration

A. Perform a primary calibration as detailed in EPA Method 7473.

1. Prepare standard solutions from the 1000-ppm mercury stock solution. Typically, 1 ppm and 0.1 ppm working standards are prepared; however, other concentrations may be used. Prepare the working standards gravimetrically in 50-mL volumetric flasks and stabilize in 2% HCl. Refer to page 73 of the DMA operator manual for detailed instructions for preparation of the working standards.
2. Pipette appropriate volumes of the working standards into quartz sample boats and enter the weight into the DMA sample measurement file. Select the "standard" method and analyze each calibration standard in duplicate. Prepare and weigh calibration standards singly and analyze each immediately; loss of mercury can occur, particularly from low concentration standards, if sample boats sit even for a few minutes before analysis. Perform a calibration across the anticipated range of relevant concentrations. The instrument utilizes two cells for best sensitivity, and therefore two separate curves may be required. The range of cell 1 is approximately 0–25 ng; the range of cell 2 is approximately 25–1,000 ng. Refer to EPA Method 7473 and the operator manual for more detail on generation of the primary calibration curve. For blanks, use a clean, empty sample boat. Select the "square" algorithm method to fit a polynomial regression to the calibration curve(s). The curve is considered acceptable if the R^2 value is ≥ 0.999.

B. At the beginning of each analysis day, analyze a minimum of one low and one high concentration standard for the relevant working range. If recovery of these check standards is within ±10 % of the true value, the existing primary calibration curve can be considered valid and used for the subsequent analyses.

III. Sample analysis

A. Weigh 35 to 50 mg of lyophilized, homogenized fish tissue (to ± 0.0001 g) into a tared quartz sample boat. Record the weight in the DMA software data file.

B. Select the relevant calibration file and method ("fish") and proceed with sample analysis.

C. Save data often as the software does not automatically save the results of sample analysis.

IV. Quality assurance and quality control (QA/QC)

A. Maintain sample integrity

1. Protect lyophilized samples from moisture before weighing. Keep lyophilized samples in a desiccator, and return samples to desiccators promptly after weighing.
2. Avoid contamination of samples. Wear new, clean gloves when handling sample boats. Use acid-washed spoons for weighing samples.

B. Blanks and check standards

1. After the analysis of every 10 samples, a method blank should be analyzed. The measured concentration of Hg in the method blank should be less than the limit of quantification or less than 10% of the lowest sample analyzed, or the previous 10 samples must be reanalyzed.
2. After the analyses of 10 samples, a mid-range check standard should be analyzed. The check standard should be ±10% of the true value, or the previous 10 samples must be reanalyzed.

C. Standard reference materials (SRMs)

1. For each batch of samples analyzed, at least two SRMs should be analyzed in triplicate as a measure to validate the calibration curve. Select SRMs with certified concentrations relevant to the anticipated range of sample concentrations. Appropriate SRMs include NIST Mussel 2976 and NRCC DORM-3 and DOLT-4. Acceptance criteria are mean measured concentrations within the certified range.

D. Matrix interference

1. Ten percent of the samples should be analyzed in triplicate. The acceptance criterion is a mean RSD that is ≤10%
2. For each sample analyzed in triplicate, triplicate spiked samples should also be analyzed. The acceptance criterion is a mean percent recovery in the range of 90% to 110%.

V. Data summarization, review, and acceptance

A. Save analysis data and transfer data to an Excel spreadsheet.

B. Summarize QA/QC samples with calculated means, RSDs, and percent recoveries.

C. Provide a QA/QC summary to a project investigator for approval or rejection. QA/QC measures are considered as a group when deciding whether to accept or reject an analysis (i.e. if only one measure has not met acceptance criteria, the batch is not automatically rejected).

D. Provide approved data to database manager for import into project database.

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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