Great Lakes Inventory and Monitoring Network

PROTOCOL FOR MONITORING AND ASSESSING METHYLMERCURY AND BIOACCUMULATIVE ORGANIC CONTAMINANTS IN AQUATIC FOOD WEBS

Prepared by

James Wiener[1], Roger Haro[1], Kristofer Rolfhus[1], Mark Sandheinrich[1], and Bill Route[2]

[1]University of Wisconsin-La Crosse
River Studies Center, 1725 State Street
La Crosse, Wisconsin 54601

[2]National Park Service
Great Lakes Inventory and Monitoring Network
2800 Lake Shore Drive East
Ashland, Wisconsin 54806

for the

PREFACE

This protocol outlines a framework for quantifying concentrations of methylmercury, total mercury, and bioaccumulative organic contaminants in aquatic organisms selected as biosentinels of localized contamination of aquatic food webs in six parks of the Great Lakes Network. This protocol, together with the companion protocol for monitoring bioaccumulative contaminants in nestling bald eagles (Route, Bowerman, & Kozie 2008), provide contaminant monitoring across the nine park units of the Great Lakes Inventory and Monitoring Network.

The protocol focuses on three groups of biosentinel organisms (small prey fish and larval dragonflies for mercury, and predatory fish for organic contaminants) that are widely distributed in aquatic habitats in parks within the Great Lakes Network. The selection of these biosentinel organisms for monitoring and assessment was based on published recommendations, on inferences drawn from existing data for park units in the Great Lakes Network, and on the authors' collective experience with investigations of mercury and bioaccumulative organic contaminants in aquatic biota in Parks and other surface waters within the region. Other biosentinel organisms should be considered for inclusion in the protocol when needed to enhance the application and effectiveness of this protocol for assessing contamination of aquatic food webs in the Great Lakes Network.

The authors recommend that the first 3 years of this inventory and monitoring program, 2008-2010, be devoted to three tasks: (1) the collection and analysis of samples from the six park units; (2) statistical analysis, interpretation, and reporting of the data; and (3) the further evaluation of data variability, sampling design, operational costs, and logistical factors. The results from this initial 3-year effort should be used to refine this strategy, as needed.

ACKNOWLEDGMENTS

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LIST OF ABBREVIATIONS AND ACRONYMS USED IN THIS DOCUMENT

Park Units
APIS	Apostle Islands National Lakeshore
GLKN	Great Lakes Inventory and Monitoring Network
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
DDT	dichloro-diphenyl-trichloroethane
DOC	dissolved organic carbon
Hg	mercury
MeHg	methylmercury
NPS	National Park Service
PCB	polychlorinated biphenyl
PBDE	polybrominated diphenyl ethers
PFOS	perfluorooctanesulfonates
PFOA	perfluorooctanoic acid
SOP	standard operating procedure
USEPA	United States Environmental Protection Agency
YOY	young of the year

REVISION HISTORY LOG

The following table lists all edits and amendments to this document since the original date of publication. Information entered in the log must be complete and concise. Users of this protocol and attached standard operating procedures should promptly notify the project leader or the data manager about recommended and required changes. The project leader must review and incorporate all changes, complete the revision history log, and change the date and version number on the title page and in the header of the document file. For complete instructions, please refer to SOP 1 (Revising the protocol).

Table 1. Original Publication Name, Version and Date: Protocol for Monitoring and Assessing Methylmercury and Bioaccumulative Organic Contaminants in Aquatic Food Webs (Version 1.0, April 18, 2008)				
Date and Number of Previous Version	Date of Revision	Author(s) of Revision (with title and affiliation)	Location in Document and Concise Description of Revision	Reason for Change
Add rows as needed for each change or set of changes tied to an updated version number				

PROTOCOL FOR MONITORING AND ASSESSING METHYLMERCURY AND BIOACCUMULATIVE ORGANIC CONTAMINANTS IN AQUATIC FOOD WEBS

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Prepared by

James Wiener, Roger Haro, Kristofer Rolfhus, Mark Sandheinrich, and Bill Route

EXECUTIVE SUMMARY

This protocol provides a framework for monitoring and assessing spatial patterns and temporal trends in methylmercury and organic contaminants in aquatic food webs in six park units within the National Park Service Great Lakes Inventory and Monitoring Network (GLKN). Methylmercury and certain persistent organic contaminants readily bioaccumulate and can biomagnify to concentrations that can adversely affect the health and reproduction of organisms atop aquatic food webs, which are the key pathways for exposure of humans and wildlife to these compounds. Concentrations of methylmercury and certain bioaccumulative organic contaminants in fish are elevated across parts of the Great Lakes region, and concentrations of methylmercury commonly exceed federal, state, and tribal criteria established for the protection of human health. This protocol, together with a companion protocol for monitoring bioaccumulative contaminants in nestling bald eagles (Route et al. 2008), supports contaminant monitoring in all nine park units of the Network.

The monitoring and assessment approach involves the sampling and analysis of biosentinel organisms to assess spatial and temporal patterns in contamination of aquatic food webs. For methylmercury, we will target 1-year-old prey fish, with yellow perch (*Perca flavescens*) as our biosentinel organism of choice. Other species, including green sunfish (*Lepomis cyanellus*) and larval dragonflies (Insecta: Odonata), will be sampled at sites where yellow perch are unavailable. We will target adult northern pike (*Esox lucius*) as the biosentinel of choice for organic contaminants, which include DDT, PCB, PBDE, PFOS, and PFOA. Sampling will be done at sites where water quality is being monitored annually by GLKN, to facilitate the statistical analysis of contaminant data in relation to physicochemical and landscape metrics of surface waters and watersheds, to strengthen interpretation, and to develop predictive models. Information from the mercury analyses can be used to identify locations on the landscape characterized by concentrations of methylmercury in biota that exceed criteria for protection of human health or wildlife. Moreover, the analysis of spatial and temporal patterns in concentrations of methylmercury and organic contaminants in biosentinel organisms is an important step in identifying landscape, aquatic, and human factors that control the abundance of these contaminants and the associated exposure of biota within park units.

1. BACKGROUND AND OBJECTIVES

1.1 INTRODUCTION

The National Park Service (NPS) has instituted a program to inventory and monitor natural resources at approximately 270 NPS units (parks) across the nation (Fancy 2004). The program is being implemented by forming 32 "Networks" of parks that share common management concerns and geography. The Great Lakes Network (GLKN) includes nine parks in four states surrounding the western Great Lakes. These 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).

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 Great Lakes Network has developed a guiding document that provides the goals, ecological context, the selected Vital Signs, and an implementation schedule for the program (Route and Elias 2007).

One of the Vital Signs identified for parks in the Great Lakes Network is Trophic Bioaccumulation, or the bioaccumulation of contaminants that biomagnify in food webs, are highly toxic, and constitute a threat to organisms in upper trophic levels. Methylmercury and certain persistent organic contaminants readily bioaccumulate in exposed organisms and can biomagnify to concentrations in upper trophic levels that can adversely affect the health and reproduction of organisms atop aquatic food webs (Wiener et al. 2003, Scheuhammer et al. 2007). High concentrations of methylmercury have been widely reported in fish and aquatic wildlife inhabiting inland waters within the Great Lakes region (Grieb et al. 1990, Meyer et al. 1998, Wiener et al. 2006, Rasmussen et al. 2007).

This protocol and the associated standard operating procedures (SOPs) provide the rationale and methods for assessing and monitoring methylmercury and organic contaminants in aquatic food webs of six park units in the Great Lakes Network (GRPO, INDU, ISRO, PIRO, SLBE, and VOYA). This is one of two protocols developed to assess trophic bioaccumulation in the Network, given that Route, Bowerman, and Kozie (2008) have developed and implemented a protocol for monitoring bioaccumulative contaminants in nestling bald eagles at three park units (APIS, MISS, and SACN). Together, the two protocols provide contaminant monitoring across the nine park units of the Great Lakes Network.

1.2 GOALS AND OBJECTIVES

This monitoring and assessment protocol provides a framework for obtaining data that will provide park managers with information on the spatial patterns, temporal trends, and potential ecotoxicological significance of methylmercury and selected bioaccumulative organic contaminants in aquatic ecosystems of park units in the Great Lakes Network. The sampling and analysis of aquatic biosentinel organisms, as outlined in this protocol, will address the following specific objectives.

Objective 1: To assess spatial patterns in contamination of aquatic food webs in parks of the Great Lakes Network.

Objective 2: To identify parks and surface waters within the Great Lakes Network where concentrations of methylmercury or bioaccumulative organic contaminants in aquatic food webs may pose a risk to organisms atop aquatic food webs.

Objective 3: To evaluate temporal trends in contamination of aquatic food webs in parks of the Great Lakes Network.

1.3 RATIONALE FOR MONITORING METHYLMERCURY AND BIOACCUMULATIVE ORGANICS IN AQUATIC FOOD WEBS

Methylmercury is a bioaccumulative toxic compound that can biomagnify to high, sometimes harmful, concentrations in organisms in upper trophic levels (Wiener et al. 2003, Scheuhammer et al. 2007). Atmospheric deposition is the primary source of the mercury accumulating in watersheds and surface waters in the Great Lakes region, and analyses of sediment cores from lakes in this and other regions have conclusively shown that most (~70%) of this atmospherically deposited mercury is derived from anthropogenic sources (Swain et al. 1992, Engstrom and Swain 1997, Lockhart et al. 1998, Lorey and Driscoll 1999, Lamborg et al. 2002, Wiener et al. 2006, Engstrom et al. 2007). In addition, a growing body of evidence indicates that atmospheric deposition is the primary source of mercury accumulating as methylmercury in aquatic food webs and fish in lakes (Fitzgerald et al. 1998, Wiener et al. 2006, Orihel et al. 2007, Munthe et al. 2007, Harris et al. 2007).

Concentrations of methylmercury and some bioaccumulative organic contaminants in predatory fish from many water bodies in the United States and Canada, including the Great Lakes region, exceed state, provincial, tribal, and federal criteria for the protection of human health (USEPA 2001*a*, 2005). In the United States, mercury was responsible for 76% of the 3,221 fish-consumption advisories posted in 2004, when 44 states, 1 territory, and 2 tribes had advisories attributed to mercury and more than 53,300 km^2 of lake area and 1,230,000 km of rivers were under advisory for mercury (USEPA 2005). In Canada, more than 97% (2,572) of all fish-consumption advisories listed in 1997 were attributed to mercury (USEPA 2001*a*). Seven states in the Great Lakes region have issued statewide fish-consumption advisories for mercury in lakes (Illinois, Indiana, Michigan, Minnesota, Ohio, Pennsylvania, and Wisconsin), and five of these (Illinois,

Indiana, Ohio, Pennsylvania, and Wisconsin) have statewide fish-consumption advisories for mercury in rivers (USEPA 2005). 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 (e.g., Wiener et al. 2003). Two of the seven states in the Great Lakes region, Indiana and Minnesota, have issued statewide fish-consumption advisories for organic contaminants—both for PCBs (USEPA 2005)

Toxicological concerns about mercury pollution of aquatic ecosystems focus appropriately on methylmercury (Wiener et al. 2003, Scheuhammer et al. 2007). Although most of the mercury in atmospheric deposition exists as inorganic forms, nearly all of the mercury accumulated by fish and higher trophic levels is methylmercury (Grieb et al. 1990, Bloom 1992, Hammerschmidt et al. 1999). Methylmercury readily crosses external and internal biological membranes (Pickhardt et al. 2006), is eliminated slowly relative to its rate of uptake (Trudel and Rasmussen 1997, Van Walleghem et al. 2007), and accumulates to concentrations in aquatic organisms that vastly exceed those in surface water. In fish, for example, concentrations of methylmercury commonly exceed those in the water in which they reside by a factor of 10^6 to 10^7 or more (Wiener et al. 2003). Direct uptake from water is important for organisms, such as algae, in the lowest trophic levels (Pickhardt et al. 2002, Gorski et al. 2006), whereas aquatic organisms, such as fish, in upper trophic levels obtain methylmercury almost entirely from the diet (Rodgers 1994, Hall et al. 1997, Harris and Bodaly 1998). The concentration of methylmercury increases up the food web from water and lower trophic levels to fish and piscivores, and the greatest increase in concentration occurs in the trophic step between water and algae (Wiener et al. 2003). In contrast to methylmercury, inorganic mercury in natural waters is not readily transferred through successive trophic levels and does not biomagnify in food webs (Watras et al. 1998, Pickhardt et al. 2002).

In a toxicological sense, the primary problem with mercury in aquatic ecosystems results from *biotic exposure to methylmercury* (Wiener et al. 2003). Aquatic food webs are the primary pathway for exposure of humans and wildlife to methylmercury (National Research Council 2000, Wiener et al. 2003, Mergler et al. 2007, Scheuhammer et al. 2007). Processes that affect the mass of methylmercury in aquatic ecosystems or its concentration at the base of the aquatic food web strongly affect its concentration in all trophic levels, including predatory fish and wildlife (Paterson et al. 1998, Benoit et al. 2003, Wiener et al. 2003). The production of methylmercury via the microbial methylation of inorganic Hg(II) by sulfate-reducing bacteria is a key process affecting concentrations of methylmercury in organisms of all trophic levels (Benoit et al. 2003). Anaerobic zones in sediments and wetlands are widely considered the most important sites of microbial methylation, and a water body can receive methylmercury from both internal and external sites (Watras et al. 1994, Hurley et al. 1995, St. Louis et al. 1996, Sellers et al. 2001).

Methylmercury is highly neurotoxic, adversely affecting both the adult and developing brain (Clarkson and Magos 2006). In birds and mammals, methylmercury from reproducing females readily passes to the developing egg or embryo, life stages that are

much more sensitive than the adult to methylmercury exposure (Wolfe et al. 1998, Wiener et al. 2003). In birds, for example, the dietary concentrations of methylmercury that significantly impair reproduction are only one-fifth those that produce overt toxicity in the adult (Scheuhammer 1991). Reproductive impairment has been associated with methylmercury exposure in field studies of several aquatic and marsh birds (Wiener et al. 2003, Heath and Frederick 2005, Schwarzbach et al. 2006, Scheuhammer et al. 2007, Evers et al. 2008, Burgess and Meyer 2008). In laboratory experiments with birds and mammals, methylmercury adversely affects reproductive success, behavior, cellular development, and adult survival, and causes teratogenic effects (Meyer et al. 1998, Wolfe et al. 1998, Wiener et al. 2003, Scheuhammer et al. 2007). Recent experiments and field studies have also shown that exposure of fish to environmentally realistic concentrations of methylmercury can adversely affect foraging efficiency, gene expression, metabolism, reproduction, and health (Fjeld et al. 1998, Latif et al. 2001, Hammerschmidt et al. 2002, Drevnick and Sandheinrich 2003, Moran et al. 2007, Larose et al. 2008). Diminished reproductive success could have adverse population-level consequences for fish and wildlife species exposed to methylmercury.

It would be prohibitively expensive to monitor the hundreds of contaminants that are present within water and park units of the Great Lakes Network. Route et al. (2008) used the following four criteria to identify and select specific pollutants for inclusion in trophic bioaccumulation protocols for the Network.

1) Contaminants that persist in the environment and bioaccumulate in fish and wildlife;
2) 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);
3) 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
4) Contaminants identified by state and federal authorities as a new and emerging chemicals of concern.

Based on these criteria, the following contaminants were selected for initial analysis in this protocol.

MeHg (methylmercury)
Alkyl-lead
DDT (dichlorodiphenyltrichloroethane and derivatives)
Dioxins
PCBs (polychlorinated biphenyl)
PFOS (perfluorooctanesulfonates)
PFOA (perfluorooctanoic acid)
PBDE (polybrominated diphenyl ethers)

We will examine the concentrations of the organic contaminants during pilot work in 2008, and apply data from this initial sampling, as well as information emanating from

new assessments of new and emerging contaminants from other programs, to identify analytes for future sampling.

1.4 BIOSENTINEL ORGANISMS FOR MONITORING AND ASSESSMENT

The monitoring and assessment approach outlined in this protocol recommends the analysis of biosentinel organisms to identify spatial and temporal patterns in the contamination of aquatic food webs. For mercury, the protocol focuses on two groups of biosentinel organisms, small prey fish (often termed "forage fish") and larval dragonflies, which are widely distributed in aquatic habitats in parks within the Great Lakes Network. These biosentinels are considered relevant, useful, and sufficiently diagnostic to detect spatio-temporal variations in the concentration of mercury, based on published guidelines pertaining to aquatic biological indicators of methylmercury contamination (Wiener et al. 2007). For organic contaminants, we will target adult northern pike (*Esox lucius*) as the biosentinel organism of choice.

Biosentinels for Methylmercury

Prey Fish. Prey fish are here defined as small finfish that are consumed whole by predatory fish and aquatic wildlife, such as common loons (*Gavia immer*). One-year-old prey fish are the primary target biosentinel organism for this protocol, and 1-year-old yellow perch (*Perca flavescens*) is the preferred target prey fish for parks in the Great Lakes Network. 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, Choy et al. 2008). To summarize, 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, methylmercury concentrations in prey fish of uniform age are much less susceptible to certain, potential confounding factors, such as variation in trophic position, than are concentrations in long-lived, piscivorous fish. The mean concentration of total mercury in prey fish is a useful indicator of methylmercury contamination in food webs supporting the production of sport fish and wildlife. Thus, the analysis of prey fish provides information relevant to the public and the policy community, as well as park managers. The effects of removal sampling of 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. Prey fish should be analyzed individually, and ancillary measurements include the total length (to nearest

millimeter), fresh weight (to 0.01 g), and age of individual fish. Age can be estimated by examination of scales or other bony structures (DeVries and Frie 1996).

Preferred biosentinel: 1-year-old yellow perch. Analyses of total mercury in whole age-1 yellow perch has provided a useful measure of methylmercury concentrations in food webs of many North American waters. This species is generally present and abundant in lentic waters within the Great Lakes region (Wiener and Eilers 1987, Kallemeyn 2000, Kallemeyn et al. 2003), and its geographic distribution extends across much of the north-central, northeastern, and eastern United States, as well as the central and eastern provinces of 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, and common loons, and is therefore an important link in the food-web transfer of methylmercury (Colby et al. 1979, Barr 1996). 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 (Cope et al. 1990, Suns et al. 1987). In Voyageurs National Park, for example, concentrations of mercury in the axial muscle tissue of northern pike are strongly correlated with those in co-existing 1-year-old yellow perch (Figure 1). Moreover, statistical analyses have shown strong relations between the total-mercury concentration in age-1 or young-of-the-year 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 Royal National Park (Gorski et al. 2003) and Voyageurs National Park (Sorensen et al. 2005, Wiener et al. 2006). Recent data from these two parks 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, Mark B. Sandheinrich, University of Wisconsin-La Crosse, unpublished data), a widespread, largely piscivorous fish that feeds heavily on yellow perch.

One-year-old yellow perch can be readily 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 1 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).
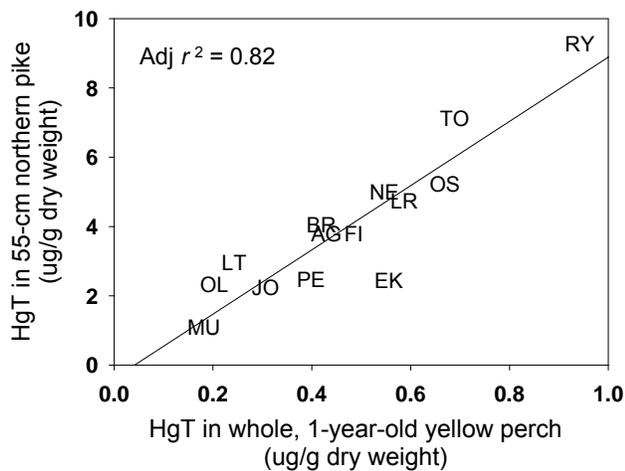
Figure 1. Relation between estimated concentrations of total mercury (HgT) in axial muscle of 55-cm northern pike and whole age-1 yellow perch from interior lakes in Voyageurs National Park. Data points for individual lakes are plotted by two-letter lake identification code (from Knights et al. 2005).

Other (alternative) prey fish. If attempts to obtain small yellow perch from a given water body are unsuccessful, other prey fishes should be sampled and retained for possible analysis. Other prey fish species that are generally widespread and often abundant in waters of the Great Lakes region include members of the centrarchid (sunfishes), cyprinid (minnows, shiners, and daces), and percid (perch and darters) families. Target alternative prey fishes include green sunfish (*Lepomis cyanellus*), pumpkinseed (*Lepomis gibbosus*), bluegill (*Lepomis macrochirus*), blacknose shiner (*Notropis heterolepis*), finescale dace (*Phoxinus neogaeus*), northern redbelly dace (*Phoxinus eos*), Johnny darter (*Etheostoma nigrum*), and Iowa darter (*Etheostoma exile*).

Larval dragonflies. Dragonflies (Ansioptera: Odonata) are a well known and conspicuous group of insects. Adults are relatively long-lived and display great agility in flight. Larval dragonflies are present in a wide variety of freshwater ecosystems. Compilation of county records from the North American Odonate Database, which is maintained by the Dragonfly Society of the Americas (Abbott 2007), indicate that 116 species have been documented in one or more of the counties where the nine park units in the Great Lakes Inventory and Monitoring Network are located (Appendix 1). The Libellulidae and Gomphidae are expected to be the most diverse families of dragonflies (in number of species) in Network parks (Figure 2). Two aeshnid species (*Aeshna canadensis* and *A. umbrosa*) and three libellulid species (*Libellula quadrimaculata*, *Sympetrum costiferum*, and *S. obtrusum*) are ubiquitous across all nine park units. About one-fourth (27%) of the species in the assemblage probably occur in only one or two of the nine park units.

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Several dragonflies in the Great Lakes region have special conservation status. One species, the Hine's Emerald (*Somatochlora hineana*), is federally listed as endangered throughout its range (IL, IN, OH, and WI) (Anonymous 2007). The Hine's Emerald is known to occur in only one (INDU) of the nine park units (Appendix 1). Five other species are currently listed as threatened or endangered by the State of Wisconsin. These include *Ophiogomphus anomalus*, *O. howei*, *O. susbehcha*, *Rhionaeschna mutate*, and *Somatochlora incurvata*. These species have limited ranges, each being restricted to just one or two park units (Appendix 1).
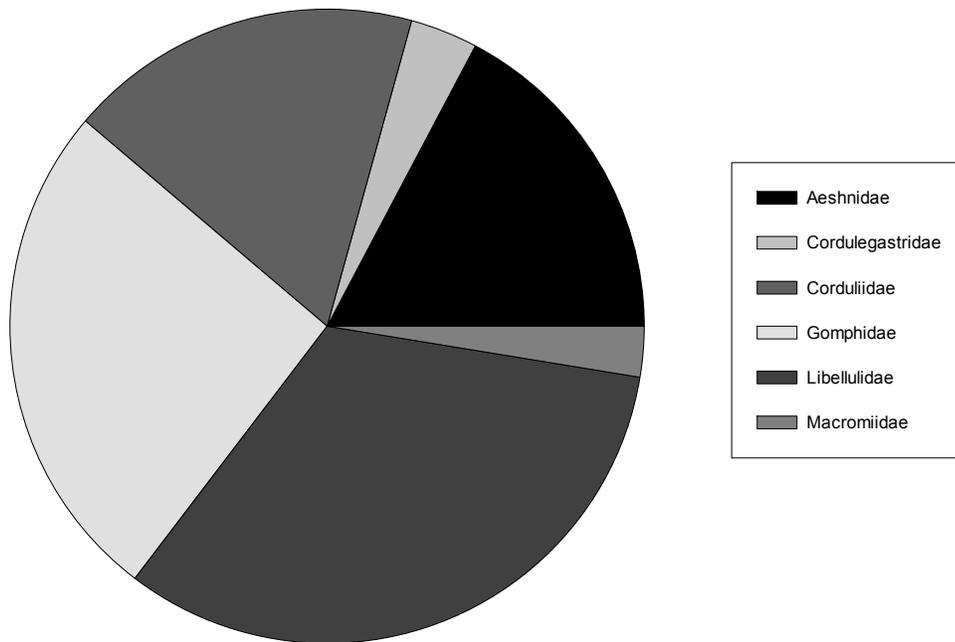
Figure 2. Species richness by Family among dragonflies found in the nine National Park Service units of the Great Lakes Monitoring and Inventory Network.

The ecology of larval dragonflies is well documented at the genus level (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 macroinvertebrates, including zooplankton, that inhabit the water column. These differences are probably important in defining pathways for dietary methylmercury uptake (Tremblay et al. 1996).

The structure of the dragonfly assemblage in a particular body of water is greatly affected by disturbance (hydroperiod) and by the presence or absence of fish. Wellborn et al. (1996) showed how hydroperiod regulates fish distribution among lentic ecosystems,

which, in turn, 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, possess small terminal body size, and forage via sit-and-wait strategies. Permanent aquatic ecosystems without fish possess large-bodied, long-lived dragonfly larvae that are more prone to be active hunters.

Larval dragonflies have been used as sentinel organisms (Johnson et al. 1993) to detect and monitor heavy metal pollution in a number of freshwater ecosystems. 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 low to moderately contaminated ecosystems.
3. Larvae are restricted to the ecosystems in which they were hatched.
4. Individuals of most species are large enough to provide adequate tissue for whole-body analysis of methylmercury.
5. Many species 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 collected with simple, inexpensive sampling gear.
8. Larvae are robust enough for laboratory and field handling, and most mature larvae can be taxonomically identified to species level.

Analysis of dragonfly larvae from interior lakes in Voyageurs National Park indicates that the mean methylmercury concentration in the larval gomphid dragonfly *Arigomphus cornutus* (commonly known as the horned clubtail) is correlated with the concentration in coexisting predatory fish (Knights et al. 2005). In the summer of 2002 and 2003, larval *A. cornutus* were collected in 10 of 13 VOYA lakes sampled by the University of Wisconsin-La Crosse and US Geological Survey. Methylmercury concentrations in this species were significantly correlated with concentrations of total mercury in coexisting northern pike (Figure 3). *Arigomphus cornutus* currently occurs in five of the nine park units in the Great Lakes Network (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 around small to medium size streams with marshy or boggy edges through late July (Mead 2003).
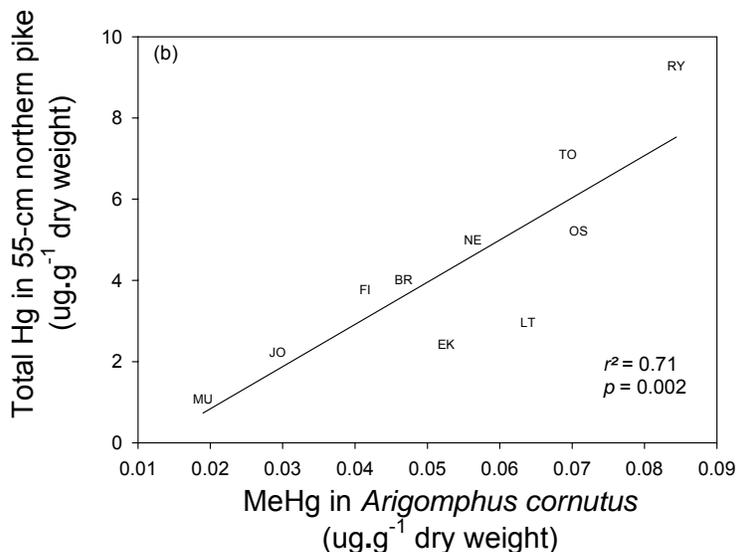
Figure 3. Relation between concentrations of total mercury (HgT) in axial muscle of 55-cm northern pike and methylmercury (MeHg) in whole larvae of the horned clubtail dragonfly (*Arigomphus cornutus*) from interior lakes in Voyageurs National Park. Data points for individual lakes are plotted by two-letter lake identification code (from Knights et al. 2005).

Biosentinels for Organic Contaminants

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 (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 and caught fish are often eaten. The analysis of organic contaminants in edible fillets of northern pike will, therefore, provide information useful to state agencies for risk assessment and possible issuance of fish-consumption advice for the species and sampled surface waters. 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, we will attempt to obtain another species of predatory fish from that water body for analysis of organic contaminants. Other predatory fish species that could be sampled and analyzed in place of northern pike include grass pickerel (*Esox americanus*), walleye (*Sander vitreus*), smallmouth bass (*Micropterus dolomieu*), and largemouth bass (*Micropterus salmoides*).

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1.5 MONITORING AND ASSESSMENT QUESTIONS

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

1. What are the spatial patterns in contamination of aquatic food webs in parks of the Great Lakes Network?

2. Which park units and surface waters within the Great Lakes Network have concentrations of methylmercury or organic contaminants in aquatic food webs high enough to pose a risk to humans and other organisms in upper trophic levels?

3. What is the general direction and magnitude of change in the concentration of methylmercury and organic contaminants in aquatic food webs in parks of the Great Lakes Network?

For mercury, 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.

Two recent studies, both in Voyageurs National Park, illustrate the utility of small yellow perch as bioindicators of methylmercury in aquatic food webs (Table 2). Wiener et al. (2006), who analyzed 1-year-old yellow perch from 17 interior lakes, found that spatial variation in the mean concentration of mercury was strongly related to lacustrine and watershed variables known to affect the microbial production and abundance of methylmercury. These variables included pH, dissolved sulfate, and total organic carbon in lake water (an indicator of wetland influence). Sorensen et al. (2005), who analyzed young-of-the-year (YOY) yellow perch sampled from Sand Point Lake during 1991-2003, showed that temporal variation in mean concentration was strongly correlated with maximum water level ($r = 0.80$, $p < 0.01$) and with change in maximum water level from March to July ($r = 0.86$, $p < 0.01$). Moreover, their data for Secchi disk visibility and color of lake water strongly suggest that inputs of dissolved organic matter (and associated inorganic mercury and methylmercury) increased concomitantly with increasing maximum water level, which is an indicator of inundated land area in the watershed.

Table 2. Temporal and spatial variations in mean concentrations of mercury (Hg) in small yellow perch in Voyageurs National Park.

Source of variation examined	Biosentinel fish	Data set	Mean concentration (ng Hg/g wet wt)		Reference
			median	*range*	
Spatial (lake and watershed factors)	1-year-old perch, sampled in May	17 interior lakes (sampled in 2001-2003)	107	38-180	Wiener et al. 2006
Temporal (water-level fluctuation)	YOY perch, sampled in late Sept or Oct	Sand Point Lake (12 annual samples during 1991-2003)	~90	32-200	Sorensen et al. 2005

Many factors can strongly influence the concentrations of methylmercury in biota, and the interpretation of spatiotemporal patterns in biosentinel data will be enhanced by consideration of relevant information on 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, respectively. 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 Great Lakes Network (Elias et al. 2007). To the extent feasible, the sampling sites in this protocol will be co-located with sampling sites being monitored by the Great Lakes Network's water quality monitoring program.

2. MONITORING AND ASSESSMENT STRATEGY

This protocol provides a framework for monitoring and assessing spatial patterns and temporal trends in methylmercury and organic contaminants in aquatic food webs in six park units within the National Park Service Great Lakes Inventory and Monitoring Network (GRPO, INDU, ISRO, PIRO, SLBE, and VOYA). This is one of two protocols developed to assess trophic bioaccumulation in the Network; Route et al. (2008) have developed and implemented a protocol for monitoring bioaccumulative contaminants in nestling bald eagles at the other three park units (APIS, MISS, and SACN) in the Network. Collectively, the two protocols provide monitoring of bioaccumulative contaminants in all nine park units within the Great Lakes Network.

This protocol contains two study 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 methylmercury and organic contaminants in aquatic food webs to identify parks and surface waters within the Great Lakes Network where exposure may pose a risk to biota. The *trend analysis*, which addresses Objective 3 (section 1.2), will examine temporal patterns in methylmercury and organic contaminants in lacustrine food webs in the six parks. Reliable historical data on methylmercury in fish, including small yellow perch, are available for two of the six parks included in this protocol, ISRO (Gorski et al. 2003, Drevnick et al. 2007) and VOYA (Knights et al. 2005, Sorensen et al. 2005, Wiener et al. 2006). Inclusion of these historical data with the "new" data obtained in 2008-2010 via this protocol will extend the trend analysis for mercury for these two park units by several years preceding the implementation of this protocol. For example, 6 years of historical data on total mercury in age-1 yellow perch are available for Brown, Ryan, and Peary lakes at VOYA, providing a foundation for trend analysis of mercury in interior lakes in the park. These samples of age-1 yellow perch were collected in 2000-2001 and 2003-2006 and analyzed by the University of Wisconsin-La Crosse.

2.1 SAMPLING DESIGN

Aquatic biosentinel organisms will be sampled at all six park units during the first 2 years of this study (2008-2009). We will sample at INDU, PIRO, and SLBE in 2008, 2011, 2014, and 2017 and at GRPO, ISRO, and VOYA in 2009, 2012, 2015, and 2018. With this design, all six parks will be sampled once every 3 years. The first two years of each 3-year sampling rotation will be devoted to the collection and chemical analysis of samples. The third year of each 3-year sampling rotation will be devoted to completion of chemical analysis of samples, to statistical analysis of data, and to interpretation and reporting of results.

2.2 SAMPLING LOCATIONS

We propose to sample from two to (usually) four aquatic sites in each of the six park units (section 2.1) during the first two years of each 3-year sampling rotation. In general, the number of water bodies sampled will depend on the effort required to obtain target samples and on the availability of park unit personnel to assist in the field with transit of field crews and sampling.

Candidate sites for biosentinel sampling in each of the park units are listed in Table 3. The proposed list of sampling sites within each park unit can be modified and refined, as desirable and appropriate, to meet the following general goals: (1) to maximize spatial overlap between contaminant monitoring and water-quality monitoring by GLKN, (2) to incorporate input on site selection from park personnel, (3) to reduce logistical obstacles to sampling, and (4) to select study sites with available historical contaminant data and pertinent ancillary information on the biological, physical, and chemical characteristics of aquatic resources. Available information for lakes sampled by the GLKN water quality program include lake pH, temperature, dissolved oxygen, Secchi disk visibility, major ions, nitrogen, phosphorus, bathymetry, hydrology, and landscape variables (drainage

area, forest type, and wetlands area). To strengthen and enhance the trend analysis of mercury data, sampling of age-1 yellow perch at ISRO and VOYA will focus on lakes with both water quality and recent historical data on mercury in age-1 perch .

Table 3. Proposed schedules, candidate biosentinel organisms, and candidate water bodies to be sampled in the six park units.

Park unit*	Sample years	Candidate organism	Candidate sites	Comments
INDU	2008 2011 2014 2017	*Hg:* 1-year-old green sunfish and dragonfly larvae *Organics:* Grass pickerel	Long Lake Grand Marsh	Target green sunfish & dragonfly larvae from Long Lake, which is sampled by GLKN water quality program. Target dragonfly larvae & grass pickerel (*Esox americanus*, to be sampled in lieu of northern pike) from Grand Marsh.
SLBE	2008 2011 2014 2017	*Hg:* 1-year-old yellow perch and dragonfly larvae *Organics:* northern pike	Bass (Benzie Co., in south-central park) Bass (Leelanau Co., at north end of park) Manitou Lake Round Lake (at extreme southern end of park)	Manitou Lake (N. Manitou Island, access difficult) receives light fishing pressure, whereas Bass (Leelanau Co.), Bass (Benzie Co.) and Round lakes receive moderate to high fishing pressure. All four lakes are sampled by GLKN water quality program. We may also sample northern pike at Tucker Lake, which is contaminated by an old dump site.
PIRO	2008 2011 2014 2017	*Hg:* 1-year-old yellow perch and dragonfly larvae *Organics:* northern pike	Beaver Lake Chapel Lake Grand Sable Lake Miners Lakes	All four lakes are sampled by GLKN water quality program. Many waters at SLBE have high color & DOC, variables associated with high MeHg levels in biota. High Hg concentrations reported in piscivorous fish.
GRPO	2009 2012 2015 2018	*Hg:* Dragonfly larvae and small prey fish if available *Organics:* predatory fish if available	Snow Creek and either Grand Portage Creek or Poplar Creek--streams draining portions of the Grand Portage Highlands	GRPO has limited aquatic and fisheries resources (Lafrancois & Glase 2005), and sampling may need to focus on other biosentinel organisms. Snow Creek drains a wetland, a potential site of Hg methylation.
VOYA	2009 2012 2015 2018	*Hg:* 1-year-old yellow perch and dragonfly larvae *Organics:* northern pike	Brown Lake Ek Lake Peary Lake Ryan Lake	All four lakes are sampled by GLKN water quality program. Recent historical data on Hg in 1-year old yellow perch and northern pike are available from all four lakes.
ISRO	2009 2012 2015 2018	*Hg:* 1-year-old yellow perch and dragonfly larvae *Organics:* northern pike	Angleworm (or Eva) Harvey Richie Sargent	Recent historical fish-Hg data are available for all four lakes. P.E. Drevnick observed symptoms of Hg toxicity in northern pike from Angleworm Lake. Hg concentrations in northern pike from Eva Lake were the highest reported for 25 ISRO lakes by Kallemeyn (2000). Harvey, Richie, and Sargent lakes are being sampled by the GLKN water quality program.

*GRPO = Grand Portage National Monument, INDU = Indiana Dunes National Lakeshore, ISRO = Isle Royale National Park, PIRO = Pictured Rocks National Lakeshore, SLBE = Sleeping Bear Dunes National Lakeshore, VOYA = Voyageurs National Park.

2.3 SAMPLE SIZE

Methylmercury. Individual analyses of 25 biosentinel organisms (prey fish or large larval dragonflies) from a given water body and year provides a sufficient sample size for a defensible statistical evaluation of either spatial or temporal patterns in mercury concentration (Appendix 2). Moreover, in most situations statistical power does not improve substantially if sample size is increased above 25. A detailed evaluation of statistical considerations pertaining to sampling design is presented in Appendix 2 of this protocol.

Organic Contaminants. To reduce the total cost of contractual analysis of samples, the determination of organic contaminants will be done on composite samples of axial muscle tissue of northern pike (or an alternative predatory species; see section 1.4, page 11). The composite sample for each sampled water body will contain equal masses of tissue from 8 individual fish of a single species that fall within a given total-length interval (e.g., 40-50 cm or 45-55 cm for northern pike). A composite sample will be analyzed from each of nine water bodies during each year of sampling. We will create a duplicate composite sample for one of the study sites for quality assurance purposes, yielding a total of 10 composite samples per year and 20 composite samples during each 3-year sampling rotation for analysis of organic contaminants.

2.4 TIME AND FREQUENCY OF SAMPLING

Time of Sampling. The size of young prey fish can vary seasonally because measurable growth occurs throughout much 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. 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). We recommend that prey fish be sampled in spring (2 to 3 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. Sampling of larval dragonflies should also be done in spring (2 to 3 weeks after ice out), before the older, larger larvae transform and emerge as adults (see section 3.2).

Spatial Analysis. Sampling of biosentinel organisms will be done once at each of the seven park units during this study, with the proposed year of sampling in each park unit shown in Table 3.

2.5 Level of Change that can be Detected

For trend analysis of mercury in the Great Lakes Inventory and Monitoring Network, the proposed (*a priori*) objective for sampling design is a sampling program that will have an *80% probability of detecting a 20% change in mean mercury concentration over 10 years with a Type I error (α) of 0.10.* Statistical considerations pertaining to sampling design, based largely on data sets for mercury in *adult* fish, indicate that the GLKN statistical design objectives would be difficult to achieve in a trend-analysis program for methylmercury in adult fishes (Appendix 2). Given conditions of small sampling variability and small analytical variability, the smallest annual change in log mercury concentration that may be detected in adult fish is estimated to be 3.9% (Appendix 2). The use of biosentinel organisms, such as small prey fish of uniform age (1 year), should significantly enhance our ability to statistically detect spatial and temporal patterns in methylmercury concentration. During the experimental acidification of Little Rock Lake (Wisconsin), for example, annual sampling and analysis of 30 age-1 yellow perch allowed statistical detection (α = 0.05) of treatment effects on mercury concentration as low as 12% between the treatment and reference basins (Wiener et al. 1990).

2.6 Guidelines for Determining Management Concerns

This assessment and monitoring effort is designed to document spatial and temporal patterns in mercury concentrations in selected biosentinel organisms, providing an indicator of methylmercury contamination of aquatic food webs--the primary pathway for exposure to methylmercury. The first 3 years of this study were not intended to examine effects of methylmercury exposure on wildlife or humans in the parks. However, we will compare the concentrations of methylmercury in biosentinel organisms to estimated threshold values in published field and laboratory studies in which cause-effect relations have been documented. We will maintain an awareness of new toxicological findings, standards, and criteria for methylmercury that emanate from scientific and governmental institutions in the US and Canada, and apply such information to the interpretation of findings in reports to park managers.

Wildlife Health. There is no single tissue residue criterion for methylmercury established to protect the health of piscivorous fish and wildlife. However, evidence from laboratory and field studies is sufficient to indicate that dietary methylmercury concentrations exceeding 0.3 mg/kg wet weight negatively affect both birds and mammals. Kenow et al. (2007), for example, reported that dietary concentrations of 0.4 mg/kg reduced the immune response of common loon chicks by 58%. In nestling great egrets, dietary methylmercury of 0.5 mg/kg altered behavior (Bouton et al. 1999), food consumption and growth (Spalding et al. 2000*a*), blood and organ biochemistry (Hoffman et al. 2005), and organ histology (Spalding et al. 2000*b*). In nestling snowy egrets, dietary concentrations of 0.39 mg/kg altered protein metabolism (Shaw-Allen et al. 2005). Basu et al. (2007) found that levels of neuroreceptors in the brain of wild mink were negatively correlated with the concentration of methylmercury in the brain. A subsequent dosing study in the laboratory corroborated the conclusions that methylmercury was the cause of the reduction in neuroreceptor density and showed that

dietary methylmercury as low as 0.1 mg/kg significantly reduced the level of neuroreceptors in the brain of mink.

Human Health. The US Environmental Protection Agency's Tissue Residue Criterion for methylmercury, established to protect the health of humans who eat noncommercial fish, is 0.3 mg/kg (parts per million) wet weight (Borum et al. 2001). Nearly all of the mercury present in the skeletal muscle of fish is methylmercury (Bloom 1992), and measurement of total mercury in the axial muscle of fish provides a valid estimate of methylmercury concentration (Wiener et al. 2007).

The concentration of total mercury in age-1 yellow perch, our preferred biosentinel organism, provides a useful predictor of mercury concentrations in the edible filets of predatory game fish, such as northern pike (Figure 1). The linear regression of total mercury concentration (wet weight) in axial muscle of 55-cm northern pike (~1 kg fish) against the mean wet-weight concentration of total mercury in coexisting whole, age-1 yellow perch in 14 interior lakes in Voyageurs National Park yielded the following equation.

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

where Hg_{np} is the concentration of methylmercury in 55-cm northern pike in ng/g (parts per billion) wet weight, and Hg_{yp} is the mean concentration of total mercury in whole, age-1 yellow perch in ng/g wet weight. 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.

The US Environmental Protection Agency's Tissue Residue Criterion for methylmercury equates to 300 ng/g wet weight. Thus, we anticipate that the Criterion would be exceeded in adult piscivorous fish, such as northern pike, in water bodies where total mercury in whole age-1 yellow perch exceeded a mean concentration of 30 ng/g wet weight.

3. OVERVIEW OF SAMPLING AND ANALYTICAL METHODS

The methods employed in this project will produce data of high analytical reliability that are comparable to information being gathered for other sites in the Great Lakes region and the Nation. These data will provide a solid foundation for assessing methylmercury contamination and potential ecological risks to biota that forage in surface waters of park units in the Great Lakes Inventory and Monitoring Network.

3.1 FIELD SEASON PREPARATIONS

Detailed preparations for sampling of biosentinel organisms will begin in early winter (January through February). Field preparations will include acquisition of needed scientific collector's permits, procurement of field supplies, and the coordination of sampling schedules among participating personnel of the University, the Great Lakes Inventory and Monitoring Network, and the park units to be sampled that calendar year (see SOP 10).

3.2 FIELD MEASUREMENTS AND SAMPLING

Prey fish. Prey fish should be sampled in early spring (2 to 3 weeks after ice out) with small-mesh bag seines or with a back-pack electroshocker deployed in littoral habitat. Passive gear, such as minnow traps and small-mesh traps nets, may also be used to obtain fish. 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.

The size range of age-1 yellow perch (or other target species) may be estimated in the field by measuring and recording the total length (distance from the tip of the snout to the tip of the compressed tail) of about 100 to 200 randomly selected fish and plotting the data on a length-frequency histogram. In general, the first distinct bell curve below a total length of 90 mm on the length-frequency distribution will represent age-1 fish. If the size range of age-1 yellow perch is known from prior sampling or can be inferred in the field from the length-frequency distribution, the field crew should randomly select and retain 38 to 40 fish within this size range for processing. If the size range of age-1 fish cannot be inferred from the length-frequency distribution because of a non-distinct length-frequency curve, the crew should retain 50 to 75 small target fish from each site. Fish may be euthanized in the field with an overdose of methane tricaine sulfonate (MS 222) or by other agency-approved means of euthanasia. In the field, prey fish should be held in labeled zip-seal freezer bags containing water from the sampled water body, and placed on ice as soon as feasible. Detailed methods for sampling and processing of prey fish in the field are provided in SOP 2.

Larval dragonflies. If attempts to sample prey fish are unsuccessful, larval dragonflies should be collected with D-frame nets from open benthic substrates (i.e., sand, gravel and cobble) and from moderately vegetated littoral or wetland habitats. Most dragonflies in the western Great Lakes region have relatively long lifecycles, in which the larval stage can span from 1 to 4 years (Hilsenhoff 1996). Sampling should target larger larvae that are approaching emergence as adults, given that the concentration of methylmercury is expected to be greatest in older individuals. Sampling should be done in early spring (2 to 3 weeks after ice out) before older larvae transform and emerge as adults. Detailed methods for sampling and processing of larval dragonflies in the field are provided in SOP 3.

3.3 SAMPLE STORAGE

Prey fish. In the field, prey fish should be held in sealed, labeled zip-seal freezer bags containing water from the sampled water body. At day's end, these samples should be sorted by species (or taxon) and grouped by species (taxon) and water body. Each species-waterbody group should be placed into double, labeled zip-seal freezer bags containing tap water or surface water. These bagged samples should be frozen at ≤20°C within 12 to 24 hours of collection. Field data for each group of samples should be entered onto pre-printed field data sheets on Rite-in-the-Rain® paper. When each sampling trip has been completed, samples should be transported in frozen condition to the University of Wisconsin-La Crosse, River Studies Center, where they will be stored in an ultra-cold (-80°C) freezer in double-sealed zip-seal plastic bags until further analysis. Detailed methods for processing and storage of prey fish are provided in SOP 2.

Larval dragonflies. When feasible, samples should be pre-sorted visually in the field by taxon and size. Dragonfly larvae should be placed in aerated containers filled with water from the sampled water body while in the field and during transport back to the field laboratory. Larvae should be held in aerated water from the sampled water body at room temperature for 12 to 15 hours to allow for the defecation of gut contents. Each larva can then be blotted dry on Whatman® filter paper, placed into a pre-labeled, sterile Whirl-Pak® bag, and frozen. Field data for each sample should be entered onto pre-printed field data sheets on Rite-in-the-Rain® paper. When each sampling trip has been completed, samples should be transported in frozen condition to the University of Wisconsin-La Crosse, River Studies Center, where they will be stored in an ultra-cold (-80°C) freezer in double-sealed zip-seal plastic bags until further analysis. Detailed methods for processing and storage of larval dragonflies are provided in SOP 3.

3.4 PREPARATION AND ANALYSIS OF SAMPLES

Prey fish. In the laboratory, each fish should be gently thawed, identified to species, measured (total length to the nearest millimeter), and weighed (to 0.01 g). From 8 to 20 scales should be taken with a scalpel from the area of insertion of the left pectoral fin and placed into a labeled scale envelope for eventual age estimation. Each fish should be placed into a labeled zip-seal plastic bag, and stored in an ultra-cold freezer until lyophilization. Samples are lyophilized from the frozen state in a VirTis Freeze Drier unit that achieves a constant vacuum of <10 mTorr and condenser temperature of -90°C for about 3-4 days. Selected samples are periodically removed and weighed to assure a constant mass after day 3, and dried further if sample mass has changed more than 5% during the last 24 hours of lyophilization. After lyophilization, double-bagged samples may be stored indefinitely (years to decades) in a glass dessicator with sufficient drying agent and vacuum. Ideally, samples should be stored in the dark. Lyophilized whole fish should be homogenized individually with an acid-cleaned stainless steel blender cup that attaches to a conventional blender. Samples are homogenized until the tissue becomes a fine powder, which is stored in the same zip-seal bag used for the whole fish.

The total mercury content of whole prey fish is determined with methods adapted from U.S. Environmental Protection Agency Method 1631 (USEPA 2001*b*) and from Hammerschmidt et al. (1999). Lyophilized and homogenized samples are weighed into plastic tubes, and then digested in a strong acid solution to free mercury from its organic matrix. Additional chemical digestion with a free radical source is used to aid in organic matrix oxidation. Digested samples are then placed into an autosampler and analyzed by adding a reducing agent that converts inorganic mercury to gas phase Hg^o, which is purged onto gold pre-concentration traps and analyzed by cold-vapor atomic fluorescence spectrophotometry. A Leeman Labs Hydra AF Gold Plus analyzer is used to automate the entire analytical procedure for total mercury. Most of the mercury in whole prey fish is present as methylmercury; therefore, the analyses of prey fish for total mercury yields a valid estimate of methylmercury concentration (Wiener et al. 2007). Detailed methods for analysis of prey fish for total mercury are provided in SOP 4. A randomly selected subsample (5% to 10%) of the prey fish analyzed for total mercury will also be analyzed for methylmercury (SOP 5).

Larval dragonflies. In the laboratory, samples of dragonflies in Whirl-Pak® bags should be thawed for taxonomic identification, and processed soon after thawing to limit sample degradation on a clean, acid-washed work surface. Most late-instar dragonfly larvae found in the Great Lakes Network can be identified to species. Larval dragonflies should be identified to species or genus with taxonomic keys by Needham et al. (2000) and Hilsenhoff (1999). After taxonomic identification, specimens grouped by water body and sampling date should be weighed individually (wet weight) and measured (body length). Wet weight can be determined on a top-loading balance, with in a pre-weighed Teflon Petri dish used as a weighing boat. The identification, length, and fresh weight of each individual should be entered into a standard SQL database (e.g., Microsoft Access) with a unique specimen identification number.

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. Species lists from the voucher collection should be created and maintained, and a representative specimen of each species obtained from a particular sampling location should be preserved with 70% ethanol and stored in labeled 25-ml glass vials.

Large dragonfly larvae, weighing ≥150 mg, should be processed and analyzed individually. Smaller individuals of a given species can be pooled into composite samples to obtain the dry mass of sample (~25 mg) needed for analysis. Individual and composite sample of dragonflies should be placed refrozen before lyophilization. Samples of dragonflies should be lyophilized in the frozen state in a VirTis Freeze Drier unit that achieves a constant vacuum of <10 mTorr and condenser temperature of -90^oC for about 3-4 days. Selected samples should be periodically removed and weighed to ensure a constant mass after day 3, and dried further if sample mass has changed more than 5% during the last 24 hours of lyophilization. After lyophilization, double-bagged samples can be stored for years to decades in a glass dessicator with sufficient drying agent and vacuum. Ideally, samples should be stored in the dark. Each lyophilized

individual and composite sample should be homogenized with an acid-washed mortar and pestle, and the homogenate returned to the original bag until digestion and analysis.

Methylmercury in dragonflies will be determined by proven, published procedures adapted from Liang et al. (1994) and Hammerschmidt and Fitzgerald (2005). Homogenized, lyophilized samples are added to Teflon vials in sub-gram quantities. Methylmercury is extracted from samples and freed from its organic matrix via digestion with dilute nitric acid. Methylmercury is then converted to a volatile form, purged onto a pre-concentration trap for organic volatiles, and desorbed into a carrier gas stream. The various volatile mercury species are then separated by gas chromatography, and the methylmercury is thermally decomposed to elemental mercury (Hg^o), which is can be quantified by cold vapor atomic fluorescence spectrophotometry at picogram levels. Details on the purge-trap method and detection by cold vapor atomic fluorescence spectrophotometry are described in Horvat et al. (1993) and Olson et al. (1997). SOP 5 provides detailed descriptions of procedures for the preparation and analysis of larval dragonflies for methylmercury.

Quality assurance and quality control for mercury determinations. Total and methylmercury determinations in prey fish and dragonflies invertebrates should be supported by a full array of quality-assurance elements characterizing precision and accuracy. These include the use of (1) analytical and procedural blanks, (2) evaluation of calibration linear regression, (3) sample replication, (4) certified reference materials, (5) standard addition spike recoveries, and (6) periodic check standards during analysis. About ten percent of the biosentinel samples should be analyzed in triplicate to assess sample precision, and recoveries from (standard-addition) spiked samples should be done for matrix evaluation. The data acceptance/rejection criteria and the order of quality-control elements for consideration are described in SOP 6.

4. DATA HANDLING, ANALYSIS, AND REPORTING

4.1 METADATA PROCEDURES

Metadata allow potential data users to evaluate the quality and usefulness of the data based on an understanding of the complete process under which data were collected and maintained. Thus, all of the protocol documentation, including standard operating procedures (SOPs), are part of the metadata for this inventory and monitoring effort, and a reference to the appropriate version of these documents is part of the metadata for any particular element of a dataset. All data must, therefore, have an associated value for the date and time on which they were collected.

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 with the Federal Geographic Data Committee (FGDC) Content Standard for Digital Geospatial Metadata (CSDGM; www.fgdc.gov/metadata/contstan.html). 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, absent only the geospatial-specific elements.

More details on the Network's overall strategy for metadata generation, management, and distribution are provided in chapter 7 (Data Documentation) of the GLKN Data Management Plan (Hart and Gafvert 2006).

4.2 OVERVIEW OF DATABASE DESIGN

The Water Resource Division of the National Park Service requires that all Inventory and Monitoring data on water quality be compatible with, and uploaded to, the US Environmental Protection Agency (USEPA) STORET database. The Water Resource Division developed a Microsoft Access database tool, NPSTORET, which duplicates most of the data and table structures in USEPA STORET to facilitate easier movement of the water quality data into USEPA STORET format. Data collected under this protocol are ecologically linked to aquatic health, and we will therefore use NPSTORET as the primary data entry tool for uploading to the USEPA STORET system. Internally, the Network will also migrate the data into a Microsoft SQL Server relational database management system for integration with other Great Lakes Network data and to generate summary reports and drive an Internet Mapping System (IMS) used for exchange and visualization of the monitoring and assessment data.

The Great Lakes Network will maintain one master copy of NPSTORET at the Ashland (WI) office on a central server. This is the only copy of NPSTORET that will be used to export data to other locations. Additional copies of NPSTORET will be used by Network staff or cooperators, but they will only be used as a conduit for data entry and the importation of data to GLKN's master version of NPSTORET. During analysis, the data from the master copy of NPSTORET, or the mirrored tables in GLKN's SQL Server geodatabase must be used.

4.3 DATA ENTRY, VERIFICATION, AND EDITING

Detailed instructions for the data entry procedures for this protocol are given in SOP 9 (Data entry and management). Two general classes of data will be obtained. The first includes field observations and measurements recorded on printed data sheets in the field. These field sheets will be entered into a digital form in NPSTORET that is a visual replica of the printed forms. The second class of data includes the analytical results of determinations of total mercury and methylmercury done at the University of Wisconsin at La Crosse. An import routine will be created in NPSTORET to bring in laboratory results and duplicate the quality assurance and quality control (QA/QC) procedures that would be performed in NPSTORET if these data had been entered through the form interface.

Data verification starts with the QA/QC steps outlined in the accompanying SOPs. If data being entered into NPSTORET do not pass a form-based QA/QC test, NPSTORET will prompt the user to make corrections and re-enter the data. Data that are outside the

expected range for a parameter based on previous records and scientific literature will be flagged for further review.

Quality assurance/quality control checks are performed as data are entered into NPSTORET and again when the data are transferred to the Water Resource Division of the National Park Service. The Network's water quality data records, including those from this protocol, are considered provisional until they are returned to the Network from the Water Resource Division, or are accepted by the Division for upload to USEPA STORET without changes. Only qualified users who have been trained and given editorial 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: (1) to provide a source for retrieval of any dataset when the primary dataset is lost or destroyed, and (2) to provide a data record that is an essential part of the QA/QC process. For digital data (e.g., data maintained electronically in a GPS or laptop computer and transferred electronically), the unedited files are considered the original data.

All field data will be recorded initially on hardcopy data forms to conform to USEPA standards. Hardcopy forms will be reviewed by the project manager within 30 days of collection in the field. Corrections will be made without erasing original data, dated, and initialed. We will make one photocopy and one digital replica (scanned version) of these corrected field data sheets. Following data entry and initial QA/QC in NPSTORET, we will create duplicate files of all digital data.

The Network's master version of NPSTORET and the SQL Server geodatabase will be maintained on a central server in the Ashland (WI) Inventory and Monitoring Office that is backed up daily, and backed up off-site weekly. Complete details of the GLKN Server archiving procedure, as well as the general strategy for data archiving, are found in the Chapter 4 (Data Management Infrastructure) of GLKN's Data Management Plan (Hart and Gafvert 2006).

4.5 QUALITY ASSURANCE AND QUALITY CONTROL FOR DATA MANAGEMENT

Quality assurance and quality control (QA/QC) procedures are crucial during all steps of data entry and management. The QA/QC procedures applied in the management of monitoring data at the Great Lakes Inventory and Monitoring Network are summarized in Table 4.

Table 4. Summary of quality assurance and quality control procedures applied in the management of monitoring data at the Great Lakes Inventory and Monitoring Network.

Procedure	Description
Instrument calibration logs	Each instrument, such as a GPS, must have a permanently bound logbook with results of annual calibrations.
Field forms	Field forms are the only written record of field data. Copies will be placed in site binders and originals kept on file indefinitely.
Estimating precision	Method precision will be quantified as Relative Standard Deviation (RSD, calculated from analyses of triplicate subsamples) or as Relative Percent Difference (RPD, calculated from analyses of duplicate subsamples). Precision will be estimated within 7 days of receipt of laboratory results.
Manual data entry	Data will be manually entered into computer forms that mimic the field form, to reduce transcription error.
Data verification	All (100%) manual entries from hardcopy field forms to NPSTORET will be checked in the first year. Errors will be corrected and documented. The percentage of entries verified after the first year may be reduced, depending on the error rate, but will never be less than 25%.
Data verification reports	A data verification report will be prepared each year to document transcription errors and steps to be taken to reduce such errors.
Data validation	Data validation is the checking of the data against known ranges for outliers. This will be done during verification (above), electronically within NPSTORET, and during upload to USEPA STORET.
Data validation reports	A data validation report will be produced annually. The report will document deviations, if any, from QA/QC procedures and objectives and discuss the impacts of those deviations.
Data qualification codes	Data will be coded when it is fully qualified before they are sent to WRD for upload to USEPA STORET.
Data archiving	Data and associated records will be archived at the Great Lakes Inventory and Monitoring office in Ashland (WI) in boxes numbered consecutively by year, project, and park.

4.6 ROUTINE DATA SUMMARIES

After QA/QC procedures have been completed, the mercury concentrations in biosentinel organisms should be summarized annually for each park sampled and for the Great Lakes Network as a whole. Descriptive statistics for mercury concentration should include mean, median, maximum and minimum values, skewness, kurtosis, and measures of variability (e.g., coefficient of variation, standard error, variance). Data should also be subjected to parametric and nonparametric analyses, as appropriate, to test statistical

hypotheses related to mercury concentrations and their potential relation to 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 mercury concentrations should be displayed cartographically, to facilitate comparison among sampling sites and to highlight locations where concentrations in biosentinels exceed levels of concern.

4.7 METHODS FOR ANALYSES OF SPATIAL AND TREND DATA

Within a given population, concentrations of methylmercury in fish typically increase with increasing body size or age. Consequently, information on the length, weight, or age of the fish analyzed must be incorporated into statistical analyses when examining spatial and temporal patterns in mercury concentration in samples of fish that vary in size and age composition (Tremblay et al. 1998, Wiener et al. 1997). In contrast, mercury concentrations in 1-year-old prey fish do not vary with body size--either within or among water bodies (e.g., Wiener et al. 2006). Thus, concentrations of mercury in a given species of age-1 prey fish can be compared directly among sites or years, without including metrics for body size in the statistical analysis.

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 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 0.10 will be used to judge the significance of statistical tests, in accordance with *a priori* design objectives for the Great Lakes Inventory and Monitoring Network.

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 methylmercury in selecting predictor variables (e.g., Wiener et al. 2006). All statistical analyses will be done on a personal computer with SAS software (SAS Institute Inc., Cary, NC).

4.8 REPORTING SCHEDULE

Data will be summarized annually in a single Annual Summary Report containing results for all Network parks where sampling was done during the year being reported. The primary audience for these reports is park managers. A draft Annual Summary Report will be submitted to the Program Coordinator of the NPS Great Lakes Inventory and Monitoring Network (Ashland, Wisconsin) for internal review by January 31 of the year

after sampling. After review and revision, a final report will be provided to Network parks and partners by April 15.

A comprehensive Analysis and Synthesis Report with results for all Network parks will be prepared after the initial 3 years of sampling. The target audience for the Analysis and Synthesis Report includes the parks within the Network, both regional and Service-wide Inventory and Monitoring Offices, and the broader scientific community. A draft Analysis and Synthesis Report will be submitted to the Program Coordinator of the NPS Great Lakes Inventory and Monitoring Network by February 15 of the year following the third year of sampling. The draft will be reviewed internally and sent to the parks and possibly to external reviewers, at the discretion of the regional Program Coordinator. After review and revision, the final report will be provided on April 15. Publication of monitoring and inventory results in a peer-reviewed scientific journal is encouraged, to enhance the dissemination of information and to obtain additional external evaluation of program methods, data, and interpretations. Results can also be communicated to the scientific and resource-management communities in presentations at technical conferences and workshops.

4.9 REPORT FORMAT

Both the Annual Summary and the Analyses and Synthesis reports will follow the format of a typical article in a peer-reviewed journal. These reports will be included in the ongoing series of Technical Reports produced by the Great Lakes Network and its collaborators. All final reports will be available on the Web in pdf format.

The final reports will contain a brief review of pertinent literature, a description of methods used in the study, a summary of quality-assurance results, statistical analyses and interpretations of data, tabular and graphic displays of summary data, and interpretations of findings. Detailed additional information and lengthy tables will be attached as appendices or data supplements.

5. PERSONNEL REQUIREMENTS AND TRAINING

5.1 ROLES AND RESPONSIBILITIES

Either a faculty member from the University of Wisconsin-La Crosse (UW-L) or the GLKN Program Coordinator will serve as crew leader on sampling trips. Other members of the field crew will include an Associate Researcher and one or two faculty members. The duties of the UW-L Associate Researcher include preparation for the field season, participation in the collection of samples and other field data, preparation and analysis of samples for total mercury and methylmercury, entry of all field and laboratory data, and statistical summarization of data for the Annual Summary Report and the Analysis and Synthesis Reports. All involved UW-L faculty members will participate in the sampling of aquatic biosentinel organisms during the course of the project.

Professor Wiener will serve as the University's primary point of contact with the GLKN Program Coordinator and other program personnel, and will represent the University in NPS meetings or workshops concerning the project or the overall Inventory and Monitoring Program. Professor Wiener will have lead responsibility for preparation and submission of project data, reports, and any related documents to the GLKN Program Coordinator. He will also be responsible for making any needed revisions to this protocol, given concurrence from the GLKN Program Coordinator. Professor Sandheinrich will be responsible for oversight of the total-mercury laboratory, including oversight of the preparation and analyses of biosentinel organisms for total mercury. Professor Rolfhus will be responsible for oversight of the methylmercury laboratory, including oversight of the preparation and analyses of biosentinel organisms for methylmercury. Professor Haro will be responsible for oversight of the taxonomic identification and processing of dragonflies and any other aquatic invertebrates collected during the monitoring and inventory project.

5.2 CREW QUALIFICATIONS

The leader of the field crew 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 and benthic invertebrates and with the use of boats, outboard motors, and canoes, is highly desirable.

Mercury analysts must have at least two years of undergraduate laboratory course experience, and preferably at least six months of research experience with a faculty mentor. Undergraduates must be working towards a major in chemistry, biology, or related physical science, and must have already finished an applicable laboratory course, such as quantitative analysis.

Persons performing or providing oversight of analytical determinations of total mercury and methylmercury and involved with the interpretation of data should have substantial experience in investigations of methylmercury 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 total mercury and methylmercury in environmental samples and publication of scientific papers in high-impact refereed journals. The qualifications of participating UW-L faculty are described further in Appendix 3.

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 and all SOPs;
- 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, sample labels, chain-of-custody forms;
- Data entry into NPSTORET;
- Completion of field and calibration logbooks; and
- Park-specific training, provided on-site by park staff (e.g., boat operation, navigation, radios)

Park staff participating in the sampling of biosentinel organisms will be instructed in the methods of collecting and handling biological samples and in the completion of data forms. Samplers 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.

Analytical training. One month of full-time experience is usually required to achieve proficiency in determinations of total mercury and methylmercury, and three months of experience are generally required to work independently and to troubleshoot the analytical system. The primary characteristics of a successful analyst are patience, organization, attention to detail, and the ability to logically isolate variables during troubleshooting activities. The most efficient analyses are conducted during long continuous periods, typically 10 hours or more. Thus, laboratory analysts must demonstrate an ability to stay focused during long periods of time.

6. OPERATIONAL REQUIREMENTS

6.1 ANNUAL WORK LOAD AND SCHEDULE

Detailed preparations for annual sampling trips will begin in early winter (January through February), as described in section 3.1 (Field Season Preparations) of this protocol. Sampling of biosentinel organisms will be done in May and early June, and is

expected to require about 3 weeks (15-18 days) of field work each year. The annual preparation and analysis of samples will begin in June and is expected to require 6 months of effort in the laboratory. Schedules for reporting of annual and three-year results to the National Park Service are provided in section 4.8 of this report.

6.2 FACILITY AND MAJOR EQUIPMENT NEEDS

This section outlines the combined facilities and equipment needed by the University of Wisconsin-La Crosse (UW-L) and the Great Lakes Inventory and Monitoring Network (GLKN) for accessing study sites and sampling biosentinel organisms at parks in the Network. Sampling can normally be conducted by a two-person field crew, however, at some parks additional logistical support will be required. For example, access to sample areas at ISRO and VOYA will require a boat operator from the Network office or the park. It is anticipated that field crews during sampling of biosentinel organisms will include one or two UW-L personnel and one,NPS employee from either GLKN or the park unit being sampled. Additional quality control visits will be conducted on a periodic basis. All UW-L personnel are stationed in La Crosse, and GLKN personnel are stationed in Ashland (WI).

Travel from La Crosse and Ashland to park units will be done with rented minivan, GSA lease vehicle, or privately owned vehicle. Isle Royale National Park in Lake Superior will be accessed by commercial vessel (ferry) operating from Grand Portage, Minnesota. Motor boats will be needed to access sampling sites or portage points to sampling sites in Isle Royale National Park (ISRO) and Voyageurs National Park (VOYA). When feasible, we will use Park personnel, boats, laboratory space, and lodging facilities during sampling trips.

Network motor boats will be used to access portage points to sampling sites at VOYA and ISRO. A boat operator from either the Network or the park will be needed to assist with access to the sample sites. We will not attempt to sample on the Great Lakes when lake and weather conditions are considered too dangerous.

Access to the inland lakes being sampled at INDU, SLBE, PIRO, and GRPO are accessible by road. Wet labs are available at VOYA, GRPO, ISRO, PIRO, SLBE, INDU, and the Network office in Ashland.

Park lodging is available with advanced notice and planning at VOYA, GRPO, ISRO, PIRO, SLBE, and INDU. This lodging is often on a first come first serve basis and must be coordinated closely with the park. Occasional lodging at hotels may be required.

Biosentinel samples should be frozen within 24 hours after collection. To facilitate this, UW-L will purchase a small, portable freezer, which will be used for sample storage and transport during sampling trips to most parks. Samples obtained at ISRO will be stored in freezers at NPS facilities or held on dry ice in portable coolers. At the end of each sampling trip, all samples will be transported to the University of Wisconsin-La Crosse for storage, preparation, analysis, and archiving.

6.3 START-UP COSTS

We expect the initial investment in training to total about $5,876 (Table 5). Initial start-up costs for equipment and supplies are estimated at $7,583 (itemized in Table 6). One-time purchases exceeding $250.00 include a GPS unit, a freezer for dedicated sample storage, fish-sampling gear, and D-nets for benthic invertebrate sampling. The annual estimated total cost of replenishing expendable supplies is $2,831 (Table 6).

Table 5. Total start-up costs for training to sample, identify, process, and analyze biosentinel organisms from parks of the Great Lakes Network. Analytical training will include sampling, preparation, and digestion of samples as well as demonstrated proficiency in the determination of total mercury and methylmercury.

Item	Time required (hours)	Cost per unit of time (salary & benefits)	Cost for item
(1) Training in sampling techniques and field procedures (e.g., GPS)	16	$21.60	$346
(2) Training in identification and measurement of biosentinel organisms	16	21.60	346
(3) Analytical training in the preparation and analysis of prey fish for total mercury	80	21.60	1,728
(4) Analytical training in the preparation and analysis of dragonfly larvae for methylmercury	160	21.60	3,456
Total cost	**272**		**$5,876**

Table 6. Estimated costs of equipment and supplies for sampling and analysis of biosentinel organisms for mercury and bioaccumulative organic contaminants at six park units in the Great Lakes Inventory and Monitoring Network. An asterisk (*) indicates a one-time startup expense; other costs are expected to recur annually.

Item	Start-up cost for item	Annual replacement cost for item
Portable electric freezer for field trips*	$200	--
Freezer for sample storage at UW-L*	650	--
Frame back pack*	140	--
GPS unit*	350	--
Portable coolers (2)*	60	--
Small-mesh bag seines (3 @ $350 each)*	1,050	--
Sampling gear for northern pike	200	25
D-nets for benthic invertebrate sampling (2)*	264	--
Wildco© wash bucket with 500-µm mesh*	99	--
Clipboard*	26	--
Rite-in-the-Rain paper	26	26
Sharpies & permanent markers	15	15
Zip-Loc bags	25	25
Envelopes for fish scales	25	25
Polyethylene vials for invertebrates	100	25
Stainless steel scalpels and forceps*	50	25
Mercury standards, certified reference materials, & analytical reagents for total Hg	520	520
Mercury standards, certified reference materials, & analytical reagents for methyl Hg	2,708	1,170
Expendable laboratory supplies (total Hg)	775	775
Expendable laboratory supplies (methyl Hg)	300	200
Total	**$7,583**	**$2,831**

6.4 TOTAL ANNUAL BUDGET

The expected total cost of the program to GLKN during the first 3 years (2008-2010) is $178,532 (Table 7). This estimate does not include in-kind support provided by Network parks during sampling of biosentinel organisms in the field or the salaries and benefits for the GLKN Program Coordinator and Data Manager (both in Ashland, WI).

The University will annually contribute an estimated $22,460 of in-kind support (the total estimated cost of salaries and benefits for time donated annually by the four faculty members), which represents about 50 percent of the total direct costs to the University (Table 8). Most of the estimated total direct costs of the project will be expended for salaries, wages, and benefits of University personnel and students involved with the sampling, preparation and analyses of samples, analysis of data, and reporting of project results. We estimate that the UW-L Associate Researcher will devote 9 full months of time annually to the project, which is based on the UW-L River Studies Center's extensive experience with the effort required to prepare for sampling trips and to sample,

process, and analyze small fish and invertebrate organisms for total mercury and methylmercury.

Table 7. Estimated annual budget during Years 1 and 2 for monitoring and inventory of mercury and bioaccumulative organic contaminants at six park units in the Great Lakes Network, excluding costs of the Program Coordinator, Data Manager, participating personnel at the park units, and contractual analysis of organic contaminants in fish. Tabulated costs apply to years when collection, processing, and analyses of samples are being done. During Year 3, which is devoted to data analysis and preparation of reports to GLKN, direct costs are $11,000 for faculty time plus 17.5% indirect costs. Startup costs for training and purchasing of equipment and supplies are itemized in Section 6.3.

Budget category & item	Rate	Annual cost (dollars)	Annual in kind contribution (dollars)
Salaries, wages and benefits (UW-L)			
Associate Researcher	$2,500/mo + 44.5% benefits for 9 mo/yr (0.75 FTE allocated to project annually)	$32,513	0
Faculty (Drs. Rolfhus, Haro, Sandheinrich, and Wiener)	$2,750 per faculty member per year	11,000	$22,460
Student labor (technical support in laboratory)	$10.00/hour + 2.5% benefits for 160 hr/yr	1,640	0
Subtotal		**$45,153**	**$22,460**
Expendable supplies	Annual costs for expendable field and laboratory supplies, as itemized in Table 6	**$2,831**	0
Travel (UW-L)	42 person days @ $96/day (for sampling trips and meetings with NPS)	**4,032**	0
Vehicle rental and gasoline	Annual rental of mini-van for 2 weeks @ $319/wk for transport of personnel and gear from La Crosse to sampling sites plus estimated cost of gasoline for 2000 miles @ $0.20/mile plus annual transport of fish samples for organic analyses to Wisconsin Laboratory of Hygiene via rental car (La Crosse to Madison, WI, and return; $75.00)	**1,113**	0
Commercial ferry to Isle Royale	Transport of 3 field crew members ($57 each way per person) and a canoe ($29 each way) from Grand Portage (MN) to ISRO	**400**	0
Subtotal annual direct costs (UW-L)		**$53,529**	**$22,460** (total in-kind contribution)
Grand total to UW-L	Including indirect costs at 17.5% CESU rate	**$62,897**	

The estimated total costs for the first three years of this project are $178,532, with $154,532 of these funds directed to the University of Wisconsin-La Crosse and $24,000

directed to the Wisconsin Laboratory of Hygiene (Table 8). The University of
Wisconsin-La Crosse is a member the Great Lakes-Northern Forest Cooperative
Ecosystem Studies Unit (CESU), and Professor Wiener serves as the University's
Technical Representative to the CESU. The indirect costs tabulated in Table 8 are based
on a transfer of funds from the National Park Service via a Cooperative Agreement
through the Cooperative Ecosystem Studies Unit at the member rate of 17.5%. The total
cost ($24,000) for contractual analysis of fish samples for organic contaminants at the
Wisconsin Laboratory of Hygiene is based on analysis of 10 samples per year during 2
years at a unit cost of $1,200 per composite sample. Payment to the contract laboratory
will be made directly by GLKN.

Table 8. Estimated total cost for monitoring and inventory of mercury and bioaccumulative
organic contaminants at six park units in the Great Lakes Network, during the first 3 years of the
project (2008-2010).

Item	Cost (dollars)	Totals (dollars)
Start-up costs		
Training	$5,876	
Equipment and supplies	7,583	
Subtotal		$13,459
Annual direct costs		
Year 1	53,529	
Year 2	53,529	
Year 3	11,000	
Subtotal		$118,058
Total direct cost at UW-La Crosse		$131,517
Indirect costs (@17.5% CESU rate)		23,015
Contractual analysis of fish samples (paid by GLKN to WI Lab Hygiene)		
Year 1	12,000	
Year 2	12,000	
Subtotal		24,000
Grand total during first 3 years		$178,532

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Appendix 1.

Appendix 1. Dragonfly species recorded for the nine National Park Service units in the Great Lakes Monitoring and Inventory Network. Species records were derived from county records. Unit acronyms are as follows: (APIS) Apostle Islands National Lakeshore, (GRPO) Grand Portage National Monument; (INDU) Indiana Dunes National Lakeshore, (ISRO) Isle Royale National Park, (MISS) Mississippi National Riverway and Recreation Area, (PIRO) Pictured Rocks National Lakeshore, (SACN) Saint Croix National Scenic Riverway, (SLBE) Sleeping Bear Dunes National Lakeshore, and (YOYA) Voyageurs National Park.

TAXA	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
AESHNIDAE									
Aeshna canadensis	X	X	X	X	X	X	X	X	X
A. clepsydra			X	X			X	X	
A. constricta	X		X		X	X	X	X	
A. eremita	X	X		X	X		X		
A. interrupta interrupta		X			X		X		X
A. interrupta lineata					X				X
A. sitchensis				X		X			X
A. subarctica				X			X		X
A. tuberculifera	X			X	X		X	X	
A. umbrosa	X	X	X	X	X	X	X	X	X
A. verticalis	X				X	X	X		
Anax junius	X	X	X	X	X	X	X	X	
Basiaeschna janata	X	X		X	X	X	X	X	
Boyeria grafiana		X							
B. vinosa	X		X	X		X	X	X	
Epiaeschna heros			X						
Gomphaeschna furcillata						X	X	X	
Nasiaeschna pentacantha							X		
Rhionaeschna mutata								X	
CORDULEGASTRIDAE									
Cordulegaster bilineata								X	
C. diastatops				X		X			
C. maculata	X	X		X		X	X	X	X
C. obliqua	X				X	X	X		
CORDULIIDAE									
Cordulia shurtleffii	X	X		X	X	X	X	X	
Dorocordulia libera	X	X		X	X	X	X	X	X
Epitheca (Tetragoneuria) canis	X	X		X	X	X	X	X	X
Epitheca (Epicordulia) princeps	X				X		X	X	

Appendix 1, continued.

TAXA	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
CORDULIIDAE									
Epitheca. (Tetragoneuria) cynosura	X	X			X	X	X	X	
E. (Tetragoneuria) spinigera	X	X		X	X	X	X	X	
Neurocordulia molesta							X		
N. yamaskanensis					X		X		X
Somatochlora elongate	X	X		X		X	X	X	
S. ensigera		X							
S. forcipata				X		X	X		X
S. franklini				X		X	X		X
S. hineana			X						
S. incurvata				X		X			
S. kennedyi	X				X	X	X		
S. minor	X	X		X			X		X
S. tenebrosa								X	
S. walshii					X	X	X		X
S. williamsoni	X	X		X	X	X	X	X	
Williamsonia fletcheri						X	X		
GOMPHIDAE									
Arigomphus cornutus	X				X	X	X		X
A. furcifer			X		X		X	X	
A. villosipes					X			X	
Dromogomphus spinosus	X	X		X		X	X	X	
Gomphus (Gomphurus) externus					X		X		
G. fraternus	X				X		X		X
G. lineatifrons							X	X	
G. vastus	X				X		X	X	X
G. ventricosus	X				X		X		X
Gomphus (Gomphus) exilis	X	X		X		X	X	X	X
G. graslinellus		X					X		X
G. lividus	X	X				X	X	X	

Appendix 1, continued.

TAXA	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
GOMPHIDAE									
Gomphus (Gomphus) quadricolor	X				X		X	X	
G. spicatus	X	X		X	X	X	X	X	X
G. (Hylogomphus) adelphus	X	X			X	X	X	X	
G. viridifrons	X				X		X		X
Hagenius brevistylus	X	X		X	X	X	X	X	X
Ophiogomphus anomalus		X					X		
O. carolus	X	X				X	X		
O. colubrinus	X	X		X		X	X	X	X
O. howei	X						X		
O. rupinsulensis	X	X		X	X		X	X	X
O. smithi							X		
O. susbehcha					X		X		
Progomphus obscurus							X	X	
Stylogomphus albistylus	X						X		
Stylurus amnicola	X				X		X		X
S. notatus					X		X	X	X
S. scudderi	X					X	X	X	X
S. spiniceps	X						X		X
LIBELLULIDAE									
Celithemis elisa	X		X	X	X		X	X	
C. eponina			X		X		X		
C. fasciata			X						
Erythemis simplicicollis simplicicollis	X		X		X		X	X	
Ladona julia	X	X	X	X	X	X	X	X	
Leucorrhinia frigida	X	X		X	X	X	X	X	
L. glacialis	X	X			X	X	X	X	
L. hudsonica	X	X		X	X	X	X	X	X
L. intacta	X		X	X	X	X	X	X	X
L. patricia							X		
L. proxima	X	X		X	X	X	X	X	X

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Appendix 1, continued.

TAXA	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
LIBELLULIDAE									
Libellula cyanea			X						
L. incesta								X	
L. luctuosa			X		X		X		
L. pulchella	X		X		X	X	X	X	X
L. quadrimaculata	X	X	X	X	X	X	X	X	X
L. semifasciata			X						
L. vibrans			X						
Nannothemis bella	X					X	X	X	
Pachydiplax longipennis	X		X		X		X		
Pantala flavescens	X	X	X		X		X	X	
P. hymenaea	X		X		X		X	X	
Perithemis tenera	X				X		X		
Plathemis lydia	X		X	X	X	X	X	X	
Sympetrum ambiguum			X						
S. corruptum	X	X	X		X		X	X	
S. costiferum	X	X	X	X	X	X	X	X	X
S. danae	X			X	X		X	X	X
S. internum	X	X		X	X		X	X	X
S. obtrusum	X	X	X	X	X	X	X	X	X
S. rubicundulum	X		X	X	X	X	X	X	
S. semicinctum fasciatum					X				
S. vicinum	X	X	X		X	X	X	X	X
Tramea carolina			X				X		
T. lacerata			X		X			X	
T. onusta			X		X		X	X	
MACROMIIDAE									
Didymops transversa	X	X		X	X	X	X	X	
Macromia illinoiensis illinoiensis						X	X	X	

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Appendix 2. Statistical considerations pertaining to sampling design.

Computing Sample Size Required to Detect a Linear Trend

An objective of many contaminant monitoring programs is to detect and describe temporal change in contaminant concentrations in fish or other organisms. The probability of detecting changes or trends of a specified magnitude in contaminant concentration is referred to as *statistical power* (Riget et al. 2000). Statistical power is influenced by the magnitude of the temporal trend in contaminant concentrations, the number of years over which sampling occurs, the number of samples collected each year, within- and between-year sampling and analytical variability, and the significance level of the applied test (e.g., simple linear regression; Bignert et al. 2004). Moreover, the pattern of change also greatly affects the power of a monitoring program to detect changes in contaminant concentration. Nicholson and Fryer (1992) provided an example of six scenarios with the same magnitude, but with different patterns, of change in contaminant concentrations over 10 years (Figure A2.1) and the relative ability (i.e., power) to statistically detect change in contaminant concentration (Table A2.1). For example, they showed that, if there was a 90% probability of detecting a change in contaminant load with immediate uptake (scenario on the upper left corner in Figure A2.1), then there was only a 46% chance of detecting the same magnitude of change if it occurred in a linear fashion (scenario on the upper right corner of Figure A2.1).
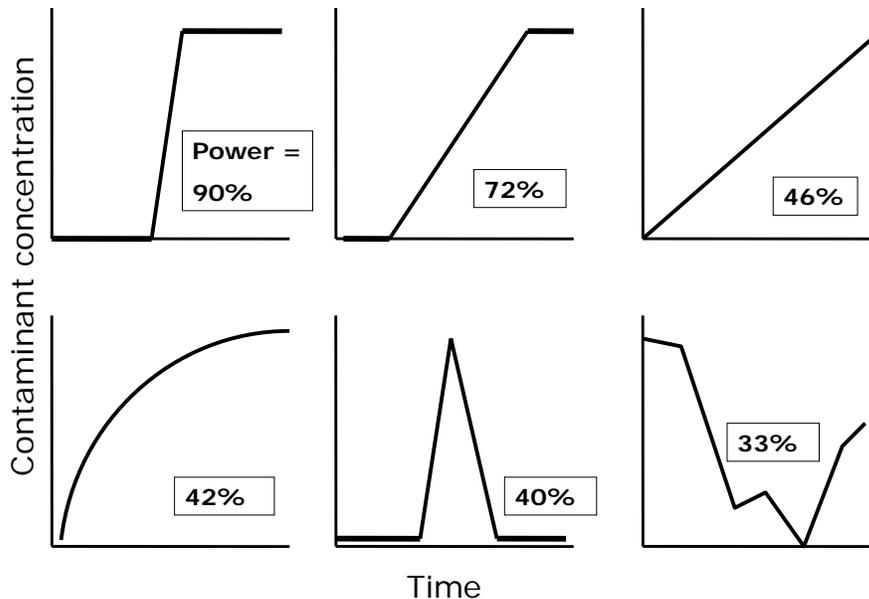
Figure A2.1. Changes in contaminant levels with time for six hypothetical scenarios. The magnitude of change is identical for each scenario (from Nicholson and Fryer 1992).

Table A2.1. Power achieved under various scenarios, as illustrated in Figure A2.1, for a change in contaminant level giving 90% power for scenario 1. The right-hand column gives the factor by which the change in contaminant level would need to increase for the corresponding scenario to have a power of 90% or 0.90 (from Nicholson and Fryer 1992).

Scenario	Power (%)	Required increase
1. Change in load with immediate uptake	90	1.00
2. Change in load with gradual uptake	72	1.21
3. Linear change in load and uptake	46	1.57
4. Exponential change in load and uptake	42	1.63
5. Incident with immediate recovery	40	1.67
6. Randomly fluctuating levels	33	1.83

If the power and significance level of the statistical test is specified, it may be possible to determine the number of samples of fish or other biota that need to be collected annually to detect a temporal trend in contaminant concentrations with a specific magnitude and pattern of change. **The *a priori* proposed objective is to determine the number of samples that need to be collected annually to have an 80% probability of detecting a 20% change in mean contaminant concentration over 10 years with a Type I error (α) of 0.10.** In the absence of pilot data or other information, we will assume that contaminant concentrations in fish or other biota change annually by a constant percentage. The change in contaminant level can, therefore, be described as a log-linear relationship after log transformation of the concentration data, with the slope of the line (*b*) equal to the mean annual change in contaminant concentration. Therefore, a 20% change in concentration over 10 years is equivalent to an average annual change in log-concentration of 1.1% ($b = 0.011$). In contaminant monitoring programs, annual mean log-concentrations in biota are typically normally distributed with homogeneous within-year variance (Nicholson et al. 1995) and, therefore, parametric statistical tests can be applied during analysis of the data. In addition, we will assume that sampling occurs once per year and that sampling occurs annually. The ability (i.e., power) to detect trends in contaminant concentrations is greatly reduced if sampling is done at a frequency less than once a year (Bignert et al. 2004).

Nicholson et al. (1997) presented equations to calculate power for detecting temporal trends in contaminant monitoring programs. Those equations, as well as modifications of those equations, will be used in the calculation of sample size. If we consider changes in contaminant concentrations to be a linear trend with time, then

$$\text{Log } y_t = a + bt \tag{1}$$

where y_t is the expected mean contaminant concentration in year *t*, and
 b is the linear trend (i.e., change) in the mean log concentration per year.

If the mean log-concentrations are normally distributed about the linear trend with constant variance ($Var[y_t]$), then

$(Var[y_t]) = \Psi^2$ (2)

Simple linear regression of contaminant concentrations in biota versus time is used to establish the linear trend and an *F*-test with 1 and T - 2 degrees of freedom (where $T =$ total number of years contaminant levels are measured) is used to test the null hypothesis of $b = 0$.

An estimate of the total variance, Ψ^2, can be obtained from the residual sum of squares of the regression of y_t on t. But to determine sample size, R, we must further subdivide the total variance into its sub-components. If each biosentinel organism is analyzed individually, then for the mean log-concentration, the total variance can be expressed as

$\Psi^2 = \sigma^2_y + \sigma^2_w / R + \tau^2_y + \tau^2_w / R$ (3)

where σ^2_y is the between-year sampling variance (i.e., variability introduced when animals are collected a year apart after accounting for any systematic trend),
σ^2_w is the within-year sampling variance (i.e., variation in animals collected at the same time),
τ^2_y is the analytical variation for the same samples measured a year apart, and
τ^2_w is the analytical variation between replicated determinations made at the same time.

Equation (3) can be re-arranged as follows to solve for R,

$R = (\sigma^2_w + \tau^2_w)/(\Psi^2 - (\sigma^2_y + \tau^2_y))$ (4)

Before proceeding, it is instructive to note from the denominator of equation (4) that between-year sampling and analytical variance $(\sigma^2_y + \tau^2_y)$ must be less than the maximum allowable total variance, Ψ^2, for a pre-defined level of power, alpha, sampling period, and linear trend. If between-year sampling and analytical variance exceeds the total variance, then even an infinite number of samples will be insufficient to detect a trend under the pre-defined conditions.

The four components of variance might be obtained from a pilot study specifically designed to obtain this information or from published sources. Nicholson et al. (1997) estimated τ_w and τ_y from a laboratory inter-calibration study of metals in fish tissue. Estimates of sampling variance, σ_w and σ_y, were made from approximately 90 trend series of mercury in fish and mussel tissue. The estimates of each of the variance components were ranked by type and divided into three equal groups representing low, medium, and high variability. The median of each group was calculated (Table A2.2) and subsequently used in their assessment of how these sources of variance affected power.

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Table A2.2. Median coefficients of variance for low, medium, and high sampling and analytical variability (from Nicholson et al. 1997).

Group	Sampling variability between years (σ_y)	Sampling variability within years (σ_w)	Analytical variability between years (τ_y)	Analytical variability within years (τ_w)
Low	0.082	0.255	0.087	0.039
Medium	0.255	0.306	0.134	0.052
High	0.516	0.443	0.235	0.103

By re-arranging equations used by Nicholson et al. (1997) for power calculation, it is possible to estimate total maximum allowable variance, Ψ^2, for a pre-defined level of power, alpha, sampling period and linear trend.

Power can be calculated from a non-central F-distribution on 1 and T-2 degrees of freedom, with non-centrality parameter

$$\delta \ = \ b^2\frac{(T\text{-}1)\ T\ (T+1)}{12\ \Psi^2} \tag{5}$$

Equation (5) can be re-arranged to solve for Ψ^2,

$$\Psi^2 = b^2\frac{(T\text{-}1)\ T\ (T+1)}{12\ \delta} \tag{6}$$

The non-centrality parameter, δ, can be computed with the SAS (Statistical Analysis System, Inc.) functions FNONCT and FINV.

$$\delta \ = \text{FNONCT}\ (F_{1\text{-}\alpha},\ 1,\ T\text{-}2,\ 1-\text{power}) \tag{7}$$

where α is the significance level of the test and $F_{1\text{-}\alpha}$ is the $100(1\text{-}\alpha)$th percentile from the F distribution with 1 and T - 2 degrees of freedom determined by

$$F_{1\text{-}\alpha} = \text{FINV}(1-\alpha,\ 1,\ T-2,\ 0) \tag{8}$$

Recalling that for our sampling objective we have defined $\alpha = 0.10$, power $= 0.80$, $b = 0.011$, and $T = 10$, and solving for $F_{1\text{-}\alpha}$ and δ, equation (6) then becomes

$$\Psi^2 = (0.011)^2\frac{(10\text{-}1)\ 10\ (10+1)}{12\ (7.434)} \tag{9}$$

$$\Psi^2 = 0.00134$$

From Table A2.2, for an optimal situation with low sampling and analytical variation, $\sigma^2_y + \tau^2_y = 0.0143$. Because this value exceeds the calculated allowable maximum variance,

Ψ^2, for our pre-defined sampling objective, it is unlikely that an annual trend as small as 1.1% could be detected after 10 years of sampling even with an infinite number of samples

Given this, it is informative to re-state the sampling objective and instead determine what minimal trends may be detected with 80% probability and $\alpha = 0.10$ after 10 years with different combinations of R and sampling and analytical variation (Table A2.3). For example, under optimal conditions of low sampling and analytical variability, the smallest annual change in log concentration that may be detected is 3.9% (Table A2.3). As demonstrated by Nicholson et al. (1997), increasing R reduces the number of years required to detect a specified trend or reduces the minimum trend that can be detected within a specified period, but the effect is small when sample sizes exceed 25. Moreover, changes in sample size have greatest effect when sampling variability is low or medium.

Table A2.3. Minimum annual trend (percent change in log concentration) that can be detected in 10 years with annual sample sizes (R) of 5 or 25 organisms, power = 0.80, $\alpha = 0.10$ and different combinations of low, medium, or high sampling variability.

Sampling variability (σ)	Analytical variability (τ)					
	Low		Medium		High	
	R=5	R=25	R=5	R=25	R=5	R=25
Low	5.0	3.9	5.9	5.0	8.3	7.7
Medium	9.1	8.3	9.6	8.8	14.1	10.6
High	16.8	15.9	17.1	16.2	18.1	17.2

Computing Sample Size Required to Detect Differences in Contaminant Concentration in Fish between Two Water Bodies or between Two Times in the Same Water Body

Recent work in the Voyageurs National Park demonstrates that concentrations of methylmercury in fish may vary markedly both spatially and temporally (Sorensen et al. 2005, Wiener et al. 2006). For example, standardized mercury concentrations in fillets of 55-cm northern pike (*Esox lucius*) vary almost 10-fold, and concentrations in whole, age-1 yellow perch vary more than 5-fold among the small interior lakes in the Park (Knights et al. 2005, Wiener et al. 2006). Within a single lake in Voyageurs National Park, mercury concentrations in age-1 yellow perch varied more than four-fold during 6 years of sampling (M.B. Sandheinrich, University of Wisconsin-La Crosse, unpublished data). Consequently, given a target value for power, it is desirable to determine the minimum sample size required to detect differences in mean concentrations of mercury in fish between two lakes or between two years within a given lake.

Numerous statistical texts provide formulae to calculate sample size based on the standard normal (*z*) distribution (e.g., Snedecor and Cochran 1989) or the *t* distribution (e.g., Zar 1999). Gerow (2006) discussed the shortcomings of these formulae, advocated the use of a non-central *t* distribution, and provided an Excel tool (available for free

download at www.statsalive.com) for calculation of sample size requirements for situations commonly encountered by fisheries biologists.

We applied the Excel tool (Gerow 2006) to data on mercury concentrations in age-1 yellow perch sampled from 17 inland lakes of Voyageurs National Park in 2000, 2001, and 2002 (Wiener et al. 2006) to estimate the sample size required to detect a 20% difference in mean mercury concentrations between two samples with a power of 0.80 and alpha = 0.10. The standard deviation of each sample was assumed to be proportional to the mean; a two-sided test was used and samples were assumed to be independent and equal. For the 32 samples collected in Voyageurs National Park, the average coefficient of variation of the mean mercury concentration in yellow perch was 0.16 (median = 0.15) and ranged from 0.08 to 0.31. Based on the average coefficient of variation, a minimum of 11 fish in each sample would need to be collected to detect a 20% difference in mean mercury concentrations. Given the maximum coefficient of variation (0.31), a minimum of 37 fish in each sample would be required.

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