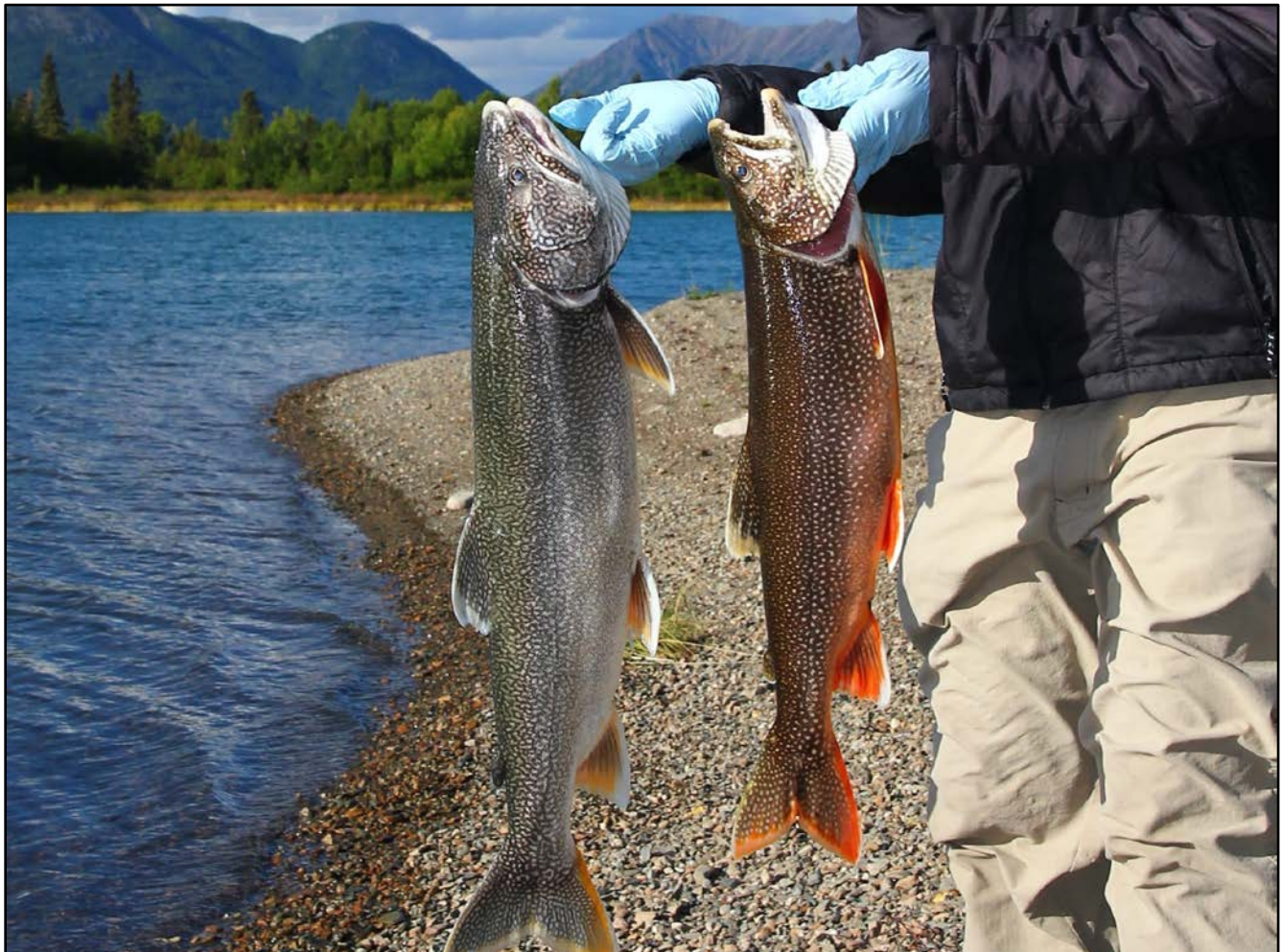




Protocol for Monitoring Mercury in Resident Lake Fish - Version 1.0

Southwest Alaska Inventory and Monitoring Network

Natural Resource Report NPS/SWAN/NRR—2018/1734



ON THE COVER

Lake trout collected for mercury analysis from Lake Clark National Park and Preserve, Alaska.

NPS / STEVE HUFFMAN

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Southwest Alaska Inventory and Monitoring Network

Natural Resource Report NPS/SWAN/NRR—2018/1734

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Executive Summary

Generally, monitoring protocols written for the National Park Service Inventory and Monitoring Program contain a narrative and a supporting set of Standard Operating Procedures (SOPs) and Data Quality Standards (DQs). The narrative describes the context and rationale for monitoring, and provides an overview of how the data will be collected, managed, analyzed, and reported. The SOPs are specific step-by-step instructions for performing a given task. The DQs are an abbreviated quality assurance plan.

This protocol outlines a framework for monitoring mercury (Hg) concentrations in resident lake fish within Southwest Alaska Network (SWAN) parks. It is adapted from a broader protocol for monitoring Hg in aquatic organisms, developed by the Great Lakes Network (GLKN; VanderMeulen et al. 2018). The primary goal of this monitoring is to understand the spatial differences, temporal trends, and health ramifications of Hg contamination in resident lake fish from the SWAN. Three objectives will be addressed by this monitoring:

1. assess spatial differences in Hg contamination of resident lake fish, among waterbodies;
2. evaluate temporal trends in Hg contamination of resident lake fish, within waterbodies; and
3. identify waterbodies where Hg contamination of resident lake fish may pose a risk to humans and top predator piscivores by surpassing relevant toxicological benchmarks.

For each of these objectives, our monitoring will rely on total Hg in fish axial muscle as an indicator of methyl Hg exposure. It will target four high priority lakes which vary in size, depth, wetland cover, glacial influence, and Hg concentration. The focal species will be lake trout, a widespread, long lived, top predator in SWAN lakes. In short, the SWAN will sample 20 lake trout from all 4 lakes, once every 5 years.

Two broad types of data will be generated from this protocol. The first type includes observations and measurements that are recorded on field forms while sampling and processing fish, as well as photographs and coordinates that are taken. The second type includes results of analyses performed by contract laboratories. We will use NPSTORET as the primary data entry tool and data transfer mechanism.

After undergoing initial quality assurance/quality control checks, the data will be summarized for each lake. Summaries will include descriptive statistics and simple graphs related to total Hg concentrations. Upon completion of these summaries, data will be subjected to parametric and/or nonparametric analyses, as appropriate, to answer questions related to the objectives for this protocol. Results will be incorporated into synthesis reports and several other means of communication. We will strive to provide clear, concise, and meaningful products in a timely manner.

Finally, the estimated annual operating cost for implementing this protocol during sampling years is \$26,900 (based on FY2017 dollars). The protocol could be scaled up in the future with additional lakes or analytes, if funding, time, and capacity permit.

Acknowledgments

This protocol was adapted from similar contaminants protocols developed by two other Inventory and Monitoring networks: Great Lakes Network (VanderMeulen et al. 2018) and Southeast Alaska Network (Sergeant et al. 2018). We wish to acknowledge the work of the authors, as well as their willingness to share that work. Additionally, we thank National Park Service staff who helped advance this monitoring during the past decade, especially D. Young, T. Hamon, J. Shearer, and E. Booher. We also thank our collaborators from other agencies, particularly R. Gerlach, H. Teas, and C. Furin (Alaska Department of Environmental Conservation), and D. Krabbenhoft (United States Geological Survey), for their insight and advice on monitoring mercury in fish. Finally, we wish to thank several colleagues who improved this document with their reviews, including A. Miller, J. Lawler, C. Miller, and C. Flanagan-Pritz (National Park Service), and R. Gerlach and C. Furin.

List of Acronyms

ALAG:	Alagnak National Wild River
ANIA:	Aniakchak National Monument and Preserve
As:	Arsenic
Cd:	Cadmium
Cr:	Chromium
Cu:	Copper
DDE:	Dichlorodiphenyldichloroethylene
DDT:	Dichlorodiphenyltrichloroethane
DQs:	Data Quality Standards
dw:	dry weight
EPA:	Environmental Protection Agency
FY:	Fiscal Year
FTE:	Full Time Equivalent
GLKN:	Great Lakes Network
ha:	hectare(s)
Hg:	Mercury
I&M:	Inventory and Monitoring
JHA:	Job Hazard Analysis
KATM	Katmai National Park and Preserve
KEFJ:	Kenai Fjords National Park
km:	kilometer(s)
LACL:	Lake Clark National Park and Preserve
m:	meter(s)
MeHg:	Methylmercury

Ni:	Nickel
NPS:	National Park Service
PAHs:	Polycyclic aromatic hydrocarbons
Pb:	Lead
PCBs:	Polychlorinated biphenyls
QA:	Quality assurance
QC:	Quality control
SEAN:	Southeast Alaska Network
SOCs:	Semi-volatile organic compounds
SOPs:	Standard Operating Procedures
STORET:	STOrage and RETrieval
SWAN:	Southwest Alaska Network
WRD:	Water Resources Division
ww:	wet weight
Zn:	Zinc
µg/g:	micrograms per gram

Background

In 1998, the National Park Service (NPS) initiated a natural resource Inventory and Monitoring (I&M) Program. The purpose of the program was to develop a baseline inventory of significant natural resources in national parks, and to monitor key ecological indicators – known as Vital Signs – over time. Now the program includes more than 270 park units organized into 32 regional networks. The Southwest Alaska Network (SWAN) is one of four networks established within Alaska under the Inventory and Monitoring Program. It consists of five park units: Lake Clark National Park and Preserve (LACL), Katmai National Park and Preserve (KATM), Kenai Fjords National Park (KEFJ), Alagnak National Wild River (ALAG), and Aniakchak National Monument and Preserve (ANIA). Collectively, these park units comprise 3.8 million ha and extend over 650 km of the Alaska and Kenai Peninsulas (Figure 1).

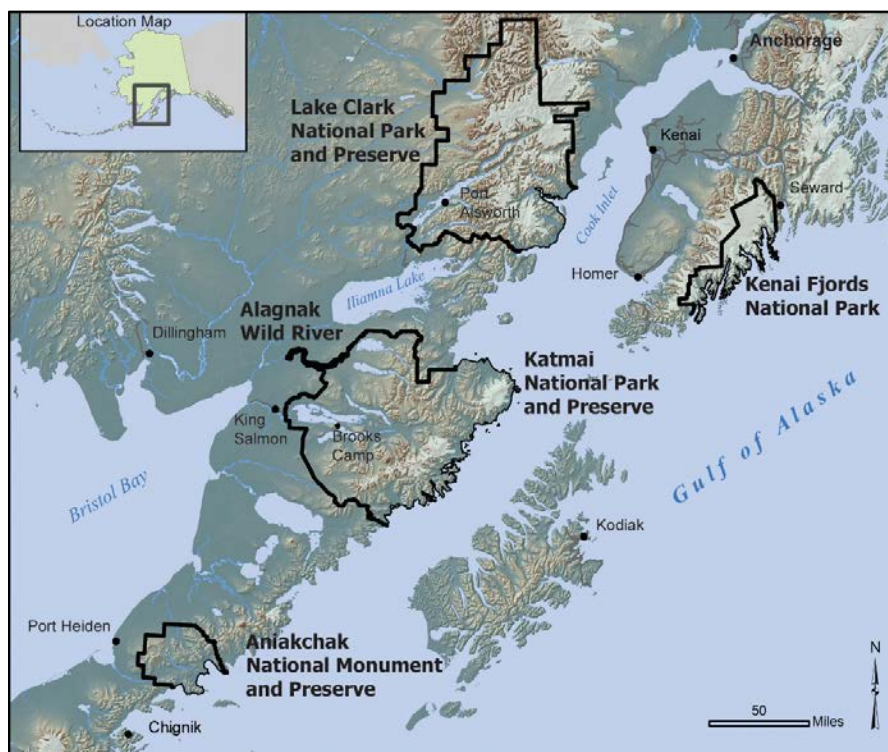


Figure 1. Map of five park units included in the Southwest Alaska Network.

Freshwater resources in the SWAN are abundant, featuring two of the largest lakes in the National Park system: Naknek Lake (58,824 ha) in KATM and Lake Clark (31,117 ha) in LACL, numerous multi-lake systems, and thousands of kilometers of rivers, including five designated “Wild Rivers.” The Naknek Lake and Lake Clark systems are so extensive that their watersheds cover 48.6% and 31.6% of the land area within their respective parks. In establishing these parks, Congress recognized the cultural, ecological, recreational, and economic importance of freshwater resources, with reference to protecting and maintaining rivers and/or lakes in their natural state in the enabling legislation (ANILCA 1980).

During a series of scoping workshops in 2002-2003, experts within and outside the NPS identified resident lake fish as a Vital Sign that warranted long-term monitoring in SWAN park units (Bennett et al. 2006). The rationale for monitoring these non-migratory freshwater fish species was multifaceted. They occupy a range of trophic levels and hence reflect changes in the food web. They are important to subsistence and recreational users. They also provide an indicator of environmental contaminants in aquatic systems. Given this rationale, four measurable objectives for resident lake fish monitoring were developed (Bennett et al. 2006). The first three objectives, which pertained to species composition and abundance, were tabled after field tests of a draft protocol proved intractable. The last objective, related to contaminant levels, was implemented in 2005 without a formal protocol, largely through opportunistic sampling supported by small grants or broader studies. In all, from 2005 through 2016, 9 fish species were sampled from 18 lakes in 2 parks, and then analyzed for organic or inorganic contaminants (Table 1). The results of this sampling establish a baseline for contaminant concentrations in select species and locations. This baseline suggests that concern over some contaminants is warranted, as described below.

Table 1. Past studies of contaminants in resident lake fish sampled from SWAN park units.

Year(s)	Analyte(s)	Park(s)	Lake(s)	Species	N ¹	Reference(s) ²
2005	Hg, metals	LACL	Clark	Arctic grayling, burbot, northern pike	14	A
2005	Hg, metals, SOCs	LACL	Clark	Lake trout	9	A
2009, 2010	Hg	KATM	Naknek, Brooks, Idavain	Dolly Varden lake trout, rainbow trout	15	B
2009, 2010	Hg	LACL	Clark	Lake trout, round whitefish, humpback whitefish, arctic grayling	22	B
2011	SOCs	KATM	Brooks, Idavain	Lake trout, arctic char	20	C, D
2011	SOCs	LACL	Kijik, Kontrashibuna	Lake trout	20	C, D
2011	Hg, metals	KATM	Naknek, Brooks, Idavain, Jojo	Lake trout, arctic char, northern pike	40	E
2011	Hg, metals	LACL	Clark, Kontrashibuna, Pickerel	Lake trout, northern pike, humpback whitefish	65	E
2011, 2012	Hg	LACL	Clark, Kontrashibuna, Telaquana	Lake trout	59	F
2015, 2016	Hg	KATM	Brooks, Grosvenor, Hammersly, Kulik, Nonvianuk, Kukaklek	Lake trout	60	G

¹ N indicates the total number of fish collected. Note that 30 of the 130 fish analyzed in 2015 and 2016 were archived samples collected in 2011 and 2012.

² A = H. Teas and L. Verbrugge, unpublished data; B = H. Teas, unpublished data; C = Simonich et al. 2013; D = Flanagan Pritz et al. 2014; E = Shearer and Young 2012; F = Eagles-Smith et al. 2014; G = Bartz et al., unpublished data.

Table 1 (continued). Past studies of contaminants in resident lake fish sampled from SWAN park units.

Year(s)	Analyte(s)	Park(s)	Lake(s)	Species	N ¹	Reference(s) ²
2015, 2016	Hg	LACL	Kontrashibuna, Kijik Crescent, Lachbuna, Snipe, Turquoise, Telaquana	Lake trout	70	G

¹ N indicates the total number of fish collected. Note that 30 of the 130 fish analyzed in 2015 and 2016 were archived samples collected in 2011 and 2012.

² A = H. Teas and L. Verbrugge, unpublished data; B = H. Teas, unpublished data; C = Simonich et al. 2013; D = Flanagan Pritz et al. 2014; E = Shearer and Young 2012; F = Eagles-Smith et al. 2014; G = Bartz et al., unpublished data.

Semi-Volatile Organic Compounds

Semi-volatile organic compounds (SOCs) are a broad class of carbon-based contaminants that persist in the environment, bioaccumulate in food chains, and ultimately impair health in wildlife and humans (Colburn et al. 1993, Longnecker et al. 1997, Carpenter 2006). SOCs include pesticides (e.g., DDT), industrial pollutants (e.g., PCBs), and combustion by-products (e.g., PAHs). In 2011, the SWAN sampled 40 fish from 4 lakes in KATM and LACL as part of a study of SOCs in fish from remote National Parks in the western United States (Flanagan Pritz et al. 2014; Table 1). A majority of the 44 SOCs analyzed were detected in at least 1 of the 10 fish sampled in each lake. Furthermore, concentrations of three historical-use pesticides (chlordane, dieldrin and p,p'-DDE) exceeded EPA thresholds for fish consumption by subsistence users in one or more lakes.

Mercury

Mercury (Hg) is a toxic metal that has no known essential biological function. Although it occurs naturally in cinnabar ore and is released during volcanic eruptions (AMAP 2013), Hg is also emitted from the combustion of coal for human energy needs (Streets et al. 2011). Elemental Hg exists in three different forms: Hg vapor (Hg^0), mercurous (Hg^{1+}), and mercuric (Hg^{2+}). Hg^0 can be transported atmospherically to areas far from original emission sources. It also can be converted to Hg^{2+} and deposited on the earth's surface via rain or snow. Under anaerobic conditions, typically in the sediments of wetlands, bacteria can convert Hg^{2+} to methylmercury (CH_3Hg^+ ; hereafter, MeHg), which is 5-10 times more toxic than elemental Hg (Wolfe et al. 1998). Because of the tendency of Hg to methylate in aquatic ecosystems, bioaccumulate in aquatic organisms, and biomagnify in aquatic food webs, MeHg tends to exist at greater concentrations in aquatic biota than terrestrial. In fish, for example, MeHg readily crosses biological membranes (Pickhardt et al. 2006), binds various proteins (NRC 2000), excretes slowly relative to its rate of uptake (Trudel and Rasmussen 1997), and accumulates to concentrations vastly exceeding those in surface water (Wiener et al. 2003). Concentrations of MeHg in piscivorous fish commonly exceed those in the water in which they reside by a factor of 10^6 or more (Wiener et al. 2003).

In 2011 and 2012, the SWAN sampled 59 fish from 3 lakes in LACL as part of a study of Hg in fish from 21 National Parks in the western United States (Eagles-Smith et al. 2014; Table 1). Results indicated that Hg concentrations in fish from LACL were among the highest, although considerable variation existed among the three lakes. A subsequent study of 130 fish from 13 lakes in LACL and

KATM confirmed this wide variation among lakes (Table 1) and showed that median Hg concentration in 6 of the 13 lakes exceeded the State of Alaska fish consumption threshold (0.20 µg/g wet weight (ww); Hamade 2014). The four lakes with the highest median Hg concentrations were all in KATM.

Other Metals and Metalloids

Metals and metalloids other than Hg also may be considered contaminants. These include elements such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn). Some of these elements are essential nutrients, required in small concentrations for stabilizing the body's internal environment (e.g., Cu and Zn); others are non-essential metals, which can be toxic even in small concentrations (e.g., As and Pb). In 2011, the SWAN sampled 105 fish from 7 lakes in KATM and LACL as part of a statewide assessment of contaminants in fish species (Shearer and Young 2012; Table 1). Results reflected total concentrations of each element. Concentrations of Pb were below the detection limit for all but three fish. Concentrations of Cd were also undetectable for the majority of fish, whereas concentrations of As and Cu were mostly detectable but low, ranging from 0.04 to 0.17 for As and from 0.18 to 0.99 for Cu (both ranges expressed in µg/g ww for axial muscle samples). The majority of fish total As is organic, which is less toxic than inorganic As.

Given the results of past sampling (described above), the costs of analyzing a suite of SOC's (~\$1000/fish), and the constraints on capacity and funding, future monitoring of contaminants in resident lake fish by the SWAN will focus solely on Hg. This protocol outlines the means by which Hg data will be collected, managed, analyzed, and reported. It is modeled upon a peer-reviewed protocol published by the Great Lakes Network (GLKN) after 10 years in development (VanderMeulen et al. 2018). The GLKN protocol describes a more comprehensive monitoring program, addressing Hg in multiple species at three trophic levels (larval dragonflies, prey fish, and predatory fish). The SWAN will implement a scaled-down version of GLKN's monitoring program by focusing on Hg in long lived, top predator fish.

Conceptual Framework for Monitoring

Mercury contamination in resident lake fish likely involves multiple sources and pathways. The sources may be distant or local, manmade or natural, and the pathways of transmittal may be simple or complex. Understanding the relative importance of these sources and pathways and anticipating potential impacts on human health are ongoing challenges for park managers in the SWAN. This is particularly true since few if any of these sources and pathways can be altered by management actions within park boundaries, leaving parks with few feasible options to limit Hg build-up in fish.

Factors driving fish Hg levels can be grouped into four broad categories: loading, methylation, bioaccumulation, and biomagnification. Loading pertains to the source of the Hg, which can be distant or local. Methylation is determined by local ecological conditions, and bioaccumulation and biomagnification by local fish biology and food web differences, respectively. Based on our understanding of the dynamic interactions between aquatic, terrestrial, and atmospheric ecosystems, we hypothesize that at least seven key factors could be at play in the SWAN (Table 2).

Table 2. Distant and local factors hypothesized to drive fish Hg concentrations in SWAN parklands, and potential measures for Vital Signs monitoring.

Scale	Category	Factor	Specific Example	Potential Measures
Distant	Loading	Atmospheric transport/deposition	Hg-rich emissions from coal-fired power production are transported and deposited to distant habitats	Total Hg in lake water; total Hg in precipitation; <i>sulfate in precipitation</i> ¹
Local	Loading	Underlying geology	Bedrock and surface soils may be enriched in Hg where natural deposits or active volcanoes exist	Total Hg in soils; proximity to active volcanoes
	Loading	Melting glaciers	Glaciers contain latent reservoirs of atmospherically deposited Hg, as well as entrained geologic materials	Total Hg in meltwater; % <i>glacier cover</i> ¹ ; % <i>glacial loss</i> ¹
	Loading	Spawning salmon	Adult salmon import not only marine derived nutrients but also bio-available Hg to lacustrine food webs	Total Hg in salmon; <i>spawner abundance and density</i> ¹
	Methylation	Wetland cover	Wetlands provide favorable conditions for methylation and yield compounds that increase Hg bioavailability	% <i>wetland cover</i> ¹ ; abundance of connected wetlands
	Methylation	Water quality	Water quality conditions are believed to control the bioavailability of inorganic Hg	Sulfate; dissolved organic carbon; <i>pH</i> ¹ ; <i>Secchi depth</i> ¹
	Bioaccumulation	Fish age	Hg absorption into tissues outpaces removal, so Hg levels increase over time in individual fish	Otolith analysis ² ; length and weight of resident lake fish ²
	Biomagnification	Fish trophic position	Top predators tend to have elevated Hg because concentrations increase toward the top of the food chain	Carbon and nitrogen isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) in resident lake fish

¹ Measures addressed through other NPS monitoring efforts (also in italic font).

² Measures included in this protocol (also in bold font).

Most of these factors are included in conceptual models developed for the SWAN Vital Signs Monitoring Plan (Bennett et al. 2006). For example, atmospheric deposition and bioaccumulation are pictured in the model of human activities (Figure 2); volcanic action and glacial melt are depicted in the model of ecosystem interactions (Figure 3); and resident fish and migrating salmon are portrayed in the model of biotic interactions (Figure 4). The fact that no single model covers all factors underscores the complexity of the Hg cycle. Indeed, the potential measures listed in Table 2 draw from many of the Vital Signs identified by Bennett et al. (2006), including air quality, water quality, land cover, glacial extent, volcanic activity, and salmon.

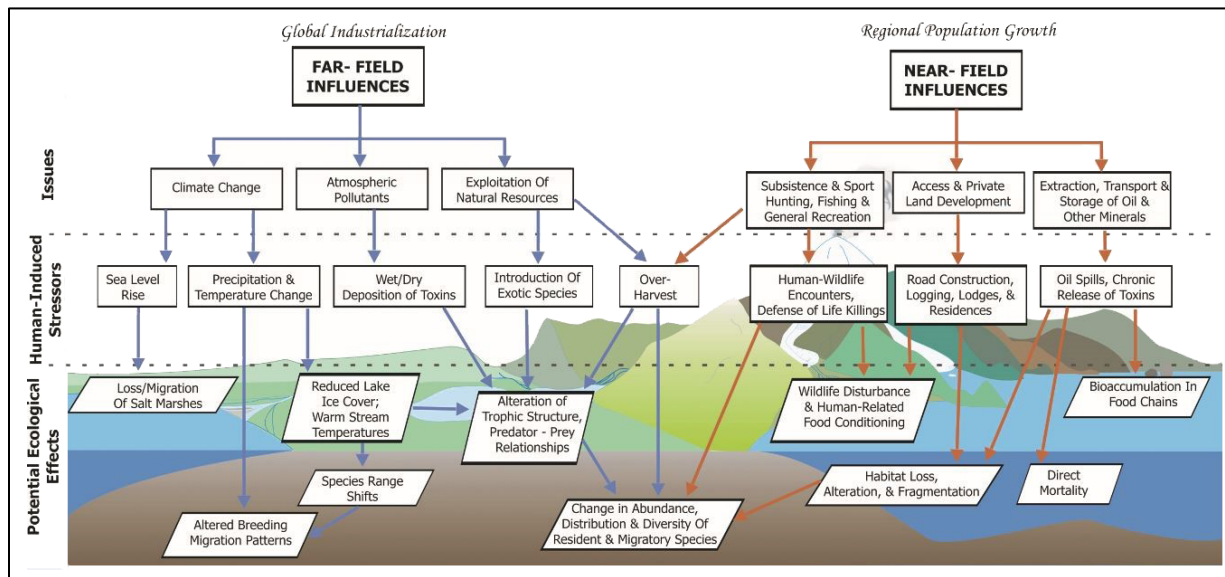


Figure 2. Conceptual model of human activities from the SWAN Vital Signs Monitoring Plan.

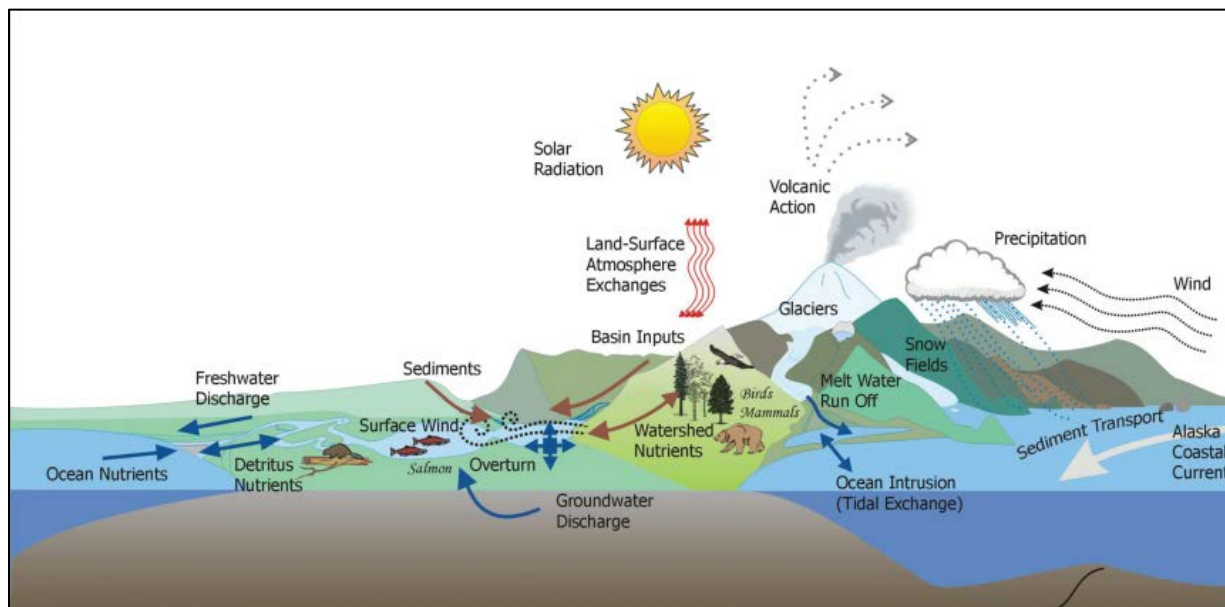


Figure 3. Conceptual model of ecosystem interactions from the SWAN Vital Signs Monitoring Plan.

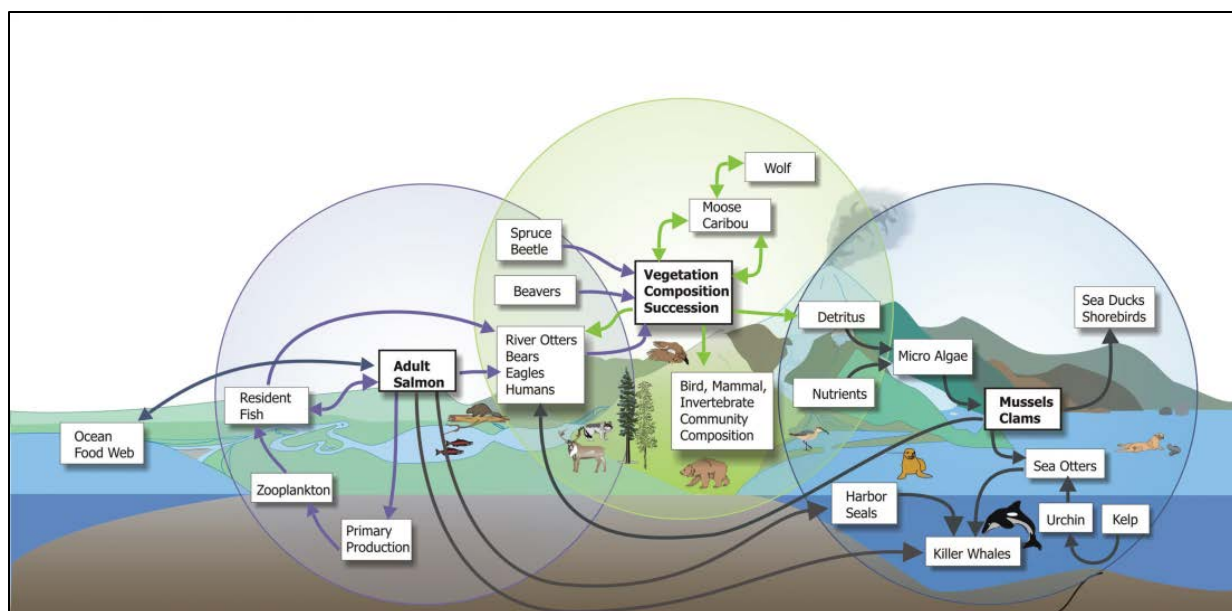


Figure 4. Conceptual model of biotic interactions from the SWAN Vital Signs Monitoring Plan.

Measurable Objectives

The primary goal of this monitoring is to understand the spatial differences, temporal trends, and health ramifications of Hg contamination in resident lake fish from park units in the SWAN. The following specific objectives will be addressed by this monitoring:

1. assess spatial differences in Hg contamination of resident lake fish, among waterbodies;
2. evaluate temporal trends in Hg contamination of resident lake fish, within waterbodies; and
3. identify waterbodies where Hg contamination of resident lake fish may pose a risk to humans and top predator piscivores by surpassing relevant toxicological benchmarks.

These objectives are adopted from the GLKN protocol (VanderMeulen et al. 2018) with minor modifications in wording to specify that our focal taxa are limited to fish, and do not include dragonflies. As described in the GLKN protocol, these objectives are related to a parallel set of questions that will be informed by this monitoring:

1. Do Hg concentrations of resident lake fish differ among select waterbodies within the SWAN?
2. What are the general direction and magnitude of change in Hg concentrations of resident lake fish from individual waterbodies within the SWAN?
3. Which waterbodies within the SWAN have Hg concentrations of resident lake fish that surpass relevant toxicological benchmarks for humans and top predator piscivores?

For both the objectives and the related questions, our monitoring will target total Hg (THg) in fish axial muscle as an indicator of MeHg exposure. Almost all of the Hg present in the muscle of top predator fish is MeHg (Grieb et al. 1990, Bloom 1992, Harris et al. 2007). Therefore, measurement of THg in fish axial muscle is a valid, conservative proxy for MeHg in the edible fillet (Wiener et al. 2007).

Several toxicological benchmarks are available for assessing the risk of Hg exposure to humans who eat fish (Objective 3). For the purposes of this protocol, we will consider three. The least conservative benchmark is the U.S. Environmental Protection Agency (EPA) tissue residue criterion (0.300 µg/g ww; Borum et al. 2001). We will also consider two benchmarks for unlimited consumption by children, nursing mothers, or women who are (or can become) pregnant: the Alaska Department of Health and Social Services threshold for Alaska-caught fish (0.20 µg/g ww; Hamade 2014), and the Great Lakes Fish Advisory Workgroup threshold for Great Lakes sport fish (0.050 µg/g ww; GLFAW 2007).

Various toxicological benchmarks are available for assessing potential adverse effects of Hg exposure to fish and wildlife (Table 3), but the majority are irrelevant to this protocol. This is because most of these benchmarks pertain to piscivores, like the belted kingfisher (*Megasceryle alcyon*) and American mink (*Neovison vison*), that favor smaller forage fish, not adult top predators. Furthermore, the benchmarks apply to whole body composite samples, rather than axial muscle tissue. For these reasons, we will consider only one benchmark (0.500 µg/g ww), which is applicable

to axial muscle tissue in adult sport fish species, based on a critical review of Hg concentrations associated with altered biochemical processes, damage to cells and tissues, and impaired reproduction in fish (Sandheinrich and Wiener 2011). Essentially, this benchmark assesses whether Hg contamination of adult sport fish poses a risk to the sport fish themselves.

Table 3. Concentrations of Hg in fish used as benchmarks in other studies when assessing risks to fish and wildlife.

Risk Group or Species	Biological Indicator Used	Type of Exposure Indicated	Benchmark Concentration (µg/g ww)	Adverse Effect(s)	Reference(s)
Sport fish	Axial muscle (adult fish)	Bioaccumulation in muscle tissue	0.500	Health and reproduction	Sandheinrich and Wiener 2011
Sport fish	Whole prey fish	Concentration in diet	0.040	Reproduction	Depew et al. 2012a
Prey fish	Whole prey fish	Bioaccumulation in whole body	0.200	Health and reproduction	Beckvar et al. 2005
Belted kingfisher	Whole prey fish	Concentration in diet	0.030	Health and reproduction	Lazorchak et al. 2003
American mink	Whole prey fish	Concentration in diet	0.070	Health and reproduction	Lazorchak et al. 2003
Common loon	Whole prey fish	Concentration in diet	0.100	Behavior	Depew et al. 2012b
Common loon	Whole prey fish	Concentration in diet	0.180	Reproduction ¹	Depew et al. 2012b
Common loon	Whole prey fish	Concentration in diet	0.400	Reproduction ²	Depew et al. 2012b

¹ Significant reproductive effects.

² Complete reproductive failure.

Sampling Design

Spatial Sampling Design

A sampling design should determine the appropriate timing, frequency, and spatial scale at which data are collected to meet monitoring objectives in terms of both statistical and ecological relevance. Obtaining accurate and precise natural resource information can be expensive due to operational costs associated with staffing, travel, and equipment, and natural variables such as inclement weather. This is especially true in Alaska as most park lands are located in remote, wilderness settings where float planes, helicopters or boats provide the only feasible means of access (Bennett et al. 2006). Thus, logistical and budget considerations must be taken into account for any sampling design within SWAN park units.

Typically, three broad categories are considered for sampling designs: census, targeted, and probabilistic. Census sampling designs include all units within a population (e.g., all lakes within a park). Targeted sampling designs include a subset of units within the population, based on judgment and/or a variety of selection criteria (e.g., lake size, salmon presence, ease of access). Probabilistic sampling designs ensure that the sample unit represents a random subsample of the population, thus allowing results to be extrapolated beyond the sample unit.

In 2004, park staff and invited experts developed a targeted sampling design for freshwater monitoring in which major lakes within SWAN park units were prioritized into “tiers.” This design was intended to ensure that key watersheds would be monitored annually even if I&M funding was greatly reduced. Prioritization criteria incorporated level of use, management issues, spatial coverage, ease of access, and both physical and ecological attributes. Physical attributes included geologic source inputs (i.e., glacial and non-glacial sources), which are likely to respond differently to climate change. Ecological attributes included the presence/absence of anadromous salmon, which can be used to estimate the contribution of marine-derived nutrients and contaminants in freshwater systems.

Tier 1 (high priority) lakes offer relatively easy access. They therefore receive relatively heavy use and significant management concern. In KATM, Naknek Lake and Lake Brooks were selected as Tier 1 lakes (Figure 5). Tier 1 lakes in LACL included Lake Clark and Kijik Lake (Figure 6). All four Tier 1 lakes support anadromous salmon. Tier 2 (lower priority) lakes are less accessible than their Tier 1 counterparts. Tier 2 lakes are important for expanding spatial coverage of freshwater monitoring beyond Tier 1 lakes — that is, for comparing status and trends observed at Tier 1 sites with other watersheds in the parks.

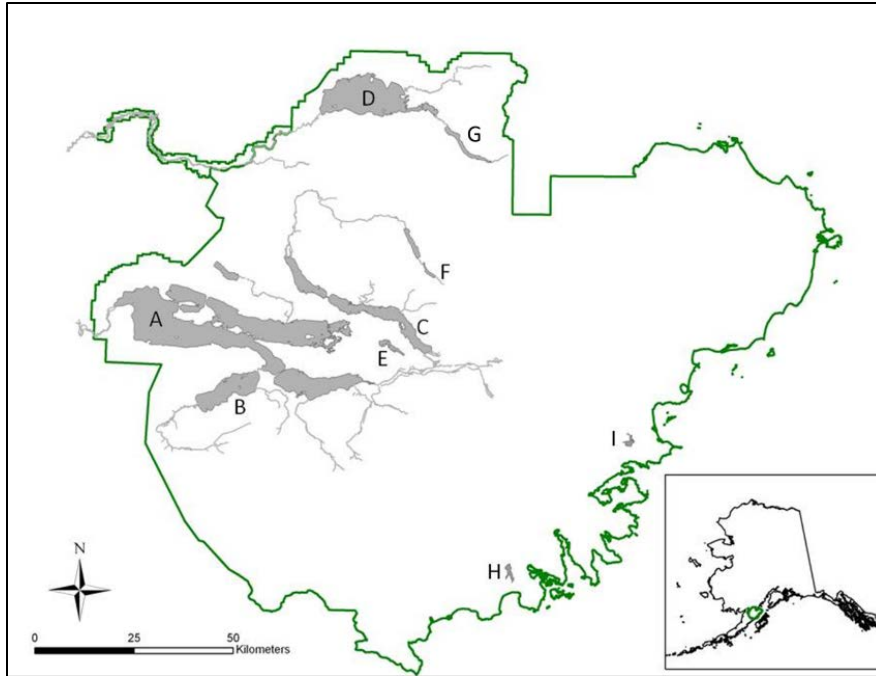


Figure 5. Lakes within KATM prioritized as either Tier 1 or Tier 2. Tier 1 lakes include Naknek Lake (A) and Lake Brooks (B). Tier 2 lakes include Lake Grosvenor (C), Kukaklek Lake (D), Jojo Lake (E), Murray Lake (F), Battle Lake (G), Dakavak Lake (H), and Hallo Glacier Lake (I), among others.

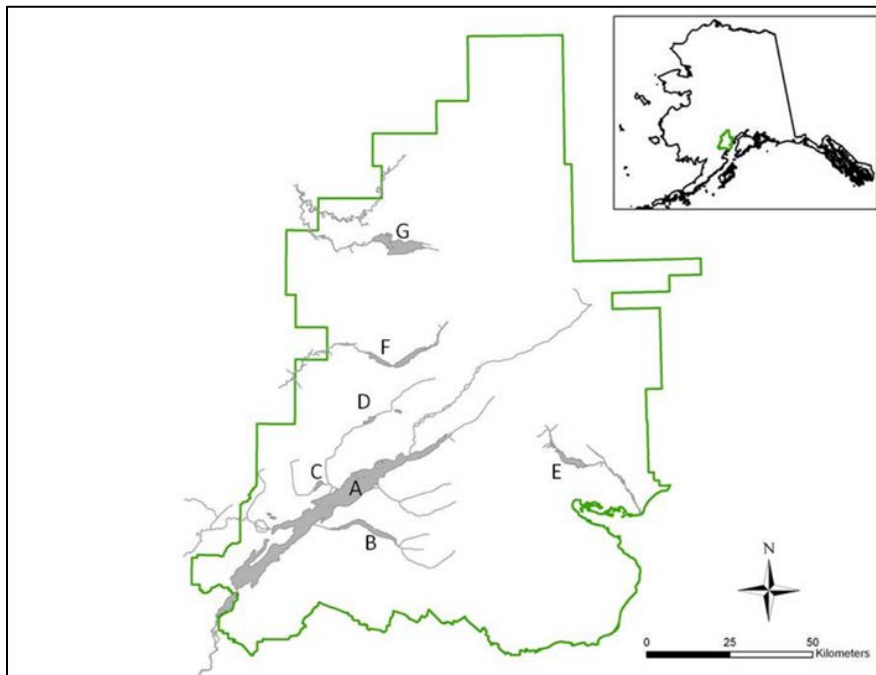


Figure 6. Lakes within LACL prioritized as either Tier 1 or Tier 2. Tier 1 lakes include Lake Clark (A) and Kijik Lake (C). Tier 2 lakes include Kontrashibuna Lake (B), Lachbuna Lake (D), and Crescent Lake (E), Lower and Upper Twin Lakes (F), and Telaquana Lake (G), among others.

Given the logistical challenges and costs associated with accessing SWAN lakes, our monitoring of fish Hg will target the four Tier 1 lakes in KATM and LACL (Table 4) and should provide an index of fish Hg levels in these lakes over time. We realize that this approach will inhibit the ability to make statistical inferences to lakes beyond those being monitored. However, we believe it is a sustainable option, considering current constraints on time, travel, and funding. Furthermore, these lakes represent a broad range in size, depth, wetland cover, glacial influence, and Hg concentration (Table 4, Figure 7), if not geographical location. In the future, if constraints ease, samples may be collected opportunistically at Tier 2 lakes to broaden the geographic scope of monitoring.

Table 4. Four Tier 1 lakes targeted for monitoring Hg in resident lake fish in the SWAN.

Park	Lake	Water Type	Size (ha)	Elevation (m)	Depth (m) ¹	Glacier Cover (%) ²	Wetland Cover (%) ³	Latitude	Longitude
KATM	Naknek	Glacial	58,824	12	175	2.7	14.5	58.65064	-156.11916
KATM	Brooks	Clear	7,557	22	60	0.0	4.3	58.50466	-155.92045
LACL	Clark	Glacial	31,337	80	262	5.9	18.7	60.21523	-154.33128
LACL	Kijik	Clear	453	115	108	0.0	0.9	60.30074	-154.32701

¹ Maximum lake depth estimated from the SWAN's 5-m contour bathymetry data layer (NPS 2006).

² Glacial cover in watersheds estimated from an Alaska glaciers geodatabase (Loso et al. 2014).

³ Wetland cover in watersheds estimated from a polynomial relationship between average watershed slope (NPS 2009) and percentage of wetlands (USFWS 2011).

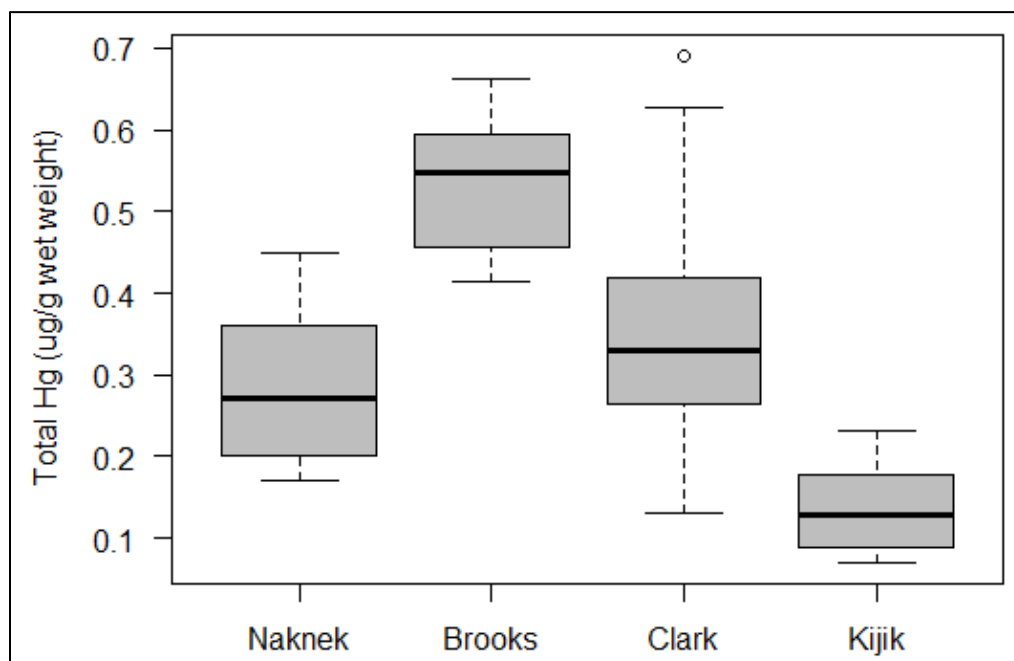


Figure 7. Total Hg concentration in fish sampled from four Tier 1 lakes in KATM and LACL. Fish from Lake Brooks (n = 10), Lake Clark (n = 48), and Kijik Lake (n = 10) are lake trout; whereas fish from Naknek Lake are northern pike (n = 10), because only one lake trout has been analyzed for Hg from this lake (total Hg = 0.15 $\mu\text{g/g ww}$).

Within each Tier 1 lake, sampling locations will be selected on the basis of their logistical feasibility and conformance to the habitat preferences of our focal species (see below). In particular, we will target locations where depth increases rapidly (e.g., lake shelf edges) and/or where salmon smolts are relatively concentrated (e.g., stream mouths). This spatial sampling design assumes that the focal species' population in each Tier 1 lake is panmictic, until proven otherwise. See SOP 3 for details.

Temporal Sampling Design

According to the SWAN Vital Signs Monitoring Plan, the sampling schedule for contaminants in resident lake fish was intended to occur “every 5 years within high priority lakes and every 10–15 years within lower priority lakes in KATM and LACL” (Table 5-2 in Bennett et al. 2006). In keeping with this intent, the SWAN will sample fish from Tier 1 lakes once every 5 years. All lakes will be visited during the same year, beginning in 2018 or 2019, to coincide with off-years for vertical profile sampling of lower priority (Tier 2) lakes (Shearer et al. 2015). Fish will be collected in early summer, after ice out but before the arrival of adult sockeye salmon. This timing should minimize both temporal variability and sampling effort.

Focal Species

Resident lake fish in southwest Alaska consist of a variety of species. Those targeted by subsistence or recreational users include humpback whitefish (*Coregonus pidschian*), round whitefish (*Prosopium cylindraceum*), least cisco (*C. sardinella*), northern pike (*Esox lucius*), arctic grayling (*Thymallus arcticus*), arctic char (*Salvelinus alpinus*), rainbow trout (*Oncorhynchus mykiss*), and lake trout (*S. namaycush*). For the purposes of this protocol, we will focus on sampling lake trout. Lake trout is a widespread, long lived, top predator species in SWAN lakes. It also comprises more than half of the samples in our historical baseline dataset (Table 1), which could enable an extended period of record for the purpose of trend analysis.

Sample Size

We conducted power analyses using Hg data for 78 resident lake fish sampled from 2005 to 2013 in Tier 1 lakes (Appendix A). The purpose was to determine the power to detect a difference in fish Hg levels (a) between lakes at a single time, and (b) within a single lake over time, under a variety of scenarios (i.e., years monitored, lakes included, percent change detected, Type I error (α), and sample size (n)). In brief, three different power analyses were conducted in R (v 3.4.2; R Core Team 2017) using code developed for comparable analyses by the SEAN (Sergeant et al. 2018, Appendix A) and GLKN (VanderMeulen et al. 2018, Appendix B). Analyses addressed the following questions:

1. For sample sizes ranging from 2 to 50 fish, what is the power to detect a difference in fish Hg levels between two sites sampled at a single point in time?
2. For monitoring programs running from 10 to 50 years, what is the power to detect a linear long-term trend in fish Hg levels at a single site?
3. How many fish need to be collected annually to detect a linear long-term trend in fish Hg levels at a single site?

Of these analyses, the first and third addressed the issue of sample size. Results from the first analysis indicate that a sample size of 10 fish generally produced high power (i.e., ≥ 0.80) to detect

between-lake differences in Hg concentrations measured at a single point in time. However, this sample size yielded low power for two scenarios (Naknek Lake vs. Lake Clark and KATM vs. LACL), which had “flatter” curves and thus needed 40+ fish to attain power comparable to that of other scenarios with a sample size of 10. Note that the results for Naknek Lake relied on the use of a proxy species to bolster sample size and, thus, should be interpreted cautiously.

Results from the third analysis indicate that the sample size needed to detect a 20% change in Hg over 10 years with a Type 1 error rate of 0.05 and power of 0.80 was 27 fish at Lake Clark. This sample size decreased to 13 or 6 fish when the time horizon was extended to 20 or 50 years, respectively. Other Tier 1 lakes could not support this analysis because they did not contain multiple years of data. Therefore, these results (like those from the first analysis) should be interpreted cautiously.

Given the varied results, the accompanying caveats, and the current constraints on time and funding, the SWAN will sample 20 fish per Tier 1 lake once every 5 years, with the expectation that differences between certain sites will not be evident at this sample size (first analysis), and a 20% change in Hg will not be detectable until the time horizon reaches at least 20 years (third analysis).

Overview of Field Procedures and Laboratory Methods

The field procedures and laboratory methods employed in this protocol will produce data of high analytical reliability, comparable to those being gathered by other networks and agencies across the country. Data will be comprised of observations and measurements recorded in the field, and analytical results of testing performed by contract laboratories (Table 5). These data will provide a solid foundation for assessing Hg contamination in resident lake fish, and the potential risks to humans who eat those fish. Quality assurance and quality control (QA/QC) considerations are detailed in the Data Quality Standards (DQSs) and Standard Operating Procedures (SOPs) for this protocol.

Table 5. Monitoring objectives, sampling methods, and data collected while monitoring Hg in resident lake fish.

Monitoring Objectives	Sampling Methods	Data Collected - Field	Data Collected - Laboratory	Analysis Tools
1. Assess spatial differences 2. Evaluate temporal trends 3. Identify lakes where contamination exceeds thresholds	Angling or gill netting followed by post-field processing, including measurements and dissections	Date and location caught; fish length (mm), weight (g), gender, species, and stomach contents; bycatch	Total Hg concentration ($\mu\text{g/g}$ dw and ww) and moisture content (%) in muscle tissue; fish age (yrs)	Age analysis of the otoliths; chemical analysis of the tissue samples; statistical analysis of all data, in combination with other covariates

Pre-Season Preparations

Pre-season preparations will begin in January and last through May during years when sampling is scheduled. Preparations include acquisition of research permits, procurement of sampling supplies, recruitment of field personnel, and coordination of sampling schedules and logistics. Steps required for pre-season preparations are outlined in SOP 1. For preparations concerning training and safety, see SOP 2.

Field Procedures

Fish sampling will be conducted from a boat, float plane, or lake shore by teams of two or more people. The target sample size is 20 fish per lake, and the target fish species is lake trout. Ideally, fish will be ≥ 400 mm long with genders divided evenly between males and females. Fish will be caught via angling or gill nets, following state and federal fishing regulations. Once caught, fish will be held in a net pen underwater, or in a portable cooler on ice. Prior to departure, each fish will be euthanized, placed in a labeled plastic bag, and stored under refrigeration until dissection. Detailed methods for the collection and storage of fish are provided in SOP 3.

Post-Field Processing

Fish will be processed within 24 hours of euthanization. Each fish will be photographed, weighed, measured, sexed, and dissected by removing otoliths (fish ear bones) and axial muscle tissue.

Specifically, we will remove three tissue subsamples per fish, with each subsample weighing ~10 g. Triplicate subsamples will be placed in separate bags and stored in a -20°C freezer. Otoliths will be stored in vials at room temperature. Dissections will be performed wearing clean nitrile gloves and using equipment that is washed between dissections with laboratory grade soap. After dissection, carcasses will be disposed of as specified in the collection permit. Additional details regarding fish processing are provided in SOP 3.

Laboratory Methods

The SWAN will rely on qualified contractors or government laboratories that meet monitoring program specifications and nationally accepted standards to perform laboratory analyses. Tissue samples will be shipped on dry ice to an analytical laboratory for Hg analyses. Total Hg analysis will follow EPA Method 7473 (SW-846) and will be reported as µg/g dry weight (dw), in order to control for differences in moisture content among individual fish (Eagles-Smith et al. 2013). However, the moisture content of each sample will be recorded to enable conversion to wet weight, facilitating comparison with toxicological benchmarks and other data sets. Periodically, a subset of tissue samples may be analyzed for MeHg concentration, to verify the percentage of total Hg that is MeHg. Otoliths will be shipped separately to facilities with expertise in age analysis, where they will be set, sectioned, and mounted on microscope slides prior to reading, as described in Campana et al. 2008.

Quality Assurance and Quality Control

Determinations of total Hg and MeHg in fish samples will be supported by an array of quality assurance elements to characterize precision and accuracy. These include the use of (1) analytical and procedural blanks, (2) evaluation of calibration linear regression, (3) sample replication, (4) certified reference materials, (5) standard addition spike recoveries, and (6) periodic checks of standards during analysis. QA/QC considerations as they relate to laboratory procedures and performance requirements are discussed in detail in the DQSs (Bartz et al. 2018).

Data Management, Processing, Analysis, and Reporting

Data Management and Processing

Database Overview

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

The SWAN will maintain one master copy of NPSTORET at the Anchorage office on a central server. This will be the only copy of NPSTORET used to export data to other locations. Additional copies of NPSTORET can be used by SWAN staff or cooperators, but only as a conduit for data entry or import to SWAN's master copy of NPSTORET. For analysis, the data from the master copy of NPSTORET must be used.

Metadata Overview

Metadata allow potential users to evaluate data quality and utility based on an understanding of the complete process under which data were collected and maintained. In this respect, the protocol narrative, DQs, and SOPs, are all considered metadata. Other types of metadata will be entered or imported into the NPSTORET database. For details on the SWAN's strategy for metadata generation, management, and distribution, see Chapter 8 in the network's Data Management Plan (Mortenson 2006), and the appendices of that document.

Data Entry, Verification, and Editing

Two broad types of data are generated during the field- and lab-based work described above. The first type includes observations and measurements that are recorded on field forms while sampling and processing fish, as well as photographs and coordinates that are taken. The second type includes results of analyses performed by contract laboratories. Field forms will need to be converted from hard copies to electronic files. Laboratory results will already be digitized, but may require additional formatting prior to importation into NPSTORET. Additional instructions for data entry procedures are outlined in SOP 4.

Data verification starts with the QA/QC steps that are outlined in the SOPs and DQs associated with this protocol. If data fail a QA/QC test during import into NPSTORET, the database will prompt the user to correct and re-import the data. Data that are outside the expected rate of change for a given parameter (based on previous records) will be flagged for further review. QA/QC checks are also performed when data are transferred to WRD. Data will be regarded as provisional until they are returned by WRD, or are accepted by WRD without changes after the final QA/QC steps. Once returned by WRD, and after appropriate documentation is completed, data will be considered certified.

Only qualified users who have been trained and given edit permissions are allowed to edit data in NPSTORET. These procedures protect the integrity of the data and allow the history of each data record to be traced.

Data Archiving

Data archiving serves two primary functions: it provides a source to retrieve a copy of a dataset when the original is lost or destroyed, and it provides a data record that is an essential part of the QA/QC process. Archiving procedures for both printed and digital data are described in SOP 4.

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

The SWAN's master version of NPSTORET is maintained on a central server in the Alaska Regional Office in Anchorage, Alaska that is backed up daily, and backed up off-site weekly. Complete details of this central server's archiving procedures are found in Chapter 11 of the SWAN's Data Management Plan (Mortenson 2006).

Data Analysis and Reporting

Routine Data Summaries

After initial QA/QC procedures have been completed, the analytical results will be summarized for each lake. Summaries should include descriptive statistics for THg concentration, such as geometric mean, median, minimum, and maximum values, skewness, kurtosis, and measures of variability (e.g., standard error, coefficient of variation, etc.). Summaries should also include simple graphics, such as biplots of fish age vs. THg, and boxplots of THg at different sites.

Data Analyses

Within a given population, concentrations of some contaminants, such as Hg, typically increase with increasing body size or age. Consequently, information on length, weight, or age should be incorporated into statistical analyses – as a means of standardization or as a covariate – when examining differences in fish Hg concentration among lakes (Tremblay et al. 1998).

Upon completion of routine summaries, data should be subjected to parametric and/or nonparametric analyses, as appropriate, to answer questions related to the objectives for this monitoring. Data transformation may be needed in order to meet the assumptions of parametric statistical methods. If assumptions are met, we will use one-way analysis of variance (ANOVA) to test the null hypothesis of equality of mean THg concentrations among lakes within a year (Question 1) or among years within a lake (Question 2) for equal age/size fish. If assumptions of parametric analyses cannot be met, we will use equivalent nonparametric analyses (e.g., Kruskal Wallis). A Type I error (α) of 0.05 will be used to judge the significance of all tests. For Question 3, we will compare the Hg concentrations in lake trout to relevant toxicological benchmarks (described above), and then compute the proportion of individuals exceeding each benchmark at each lake, as in Eagles-Smith et

al. 2013 and 2014. To address risks to human health, we will also use fish consumption advice for the State of Alaska (Table 6 in Hamade 2014) to calculate a safe number of meals per month of lake trout from a given lake, based on the observed range of THg concentrations in that lake.

If ancillary data are sufficient, we will use information-theoretic modeling to construct linear models with predicted variables pertaining to Hg in fish and predictor variables pertaining to drivers of fish Hg (Table 2). In accordance with the information-theoretic approach (Burnham and Anderson 2002), we will apply judgment based on the state of scientific understanding of factors and processes controlling the abundance of mercury in selecting predictor variables (e.g., Wiener et al. 2006). Additional information on data analysis is detailed in SOP 5.

Reporting Schedule and Formats

A key goal of this monitoring is to ensure that the results are shared with all parties, especially park managers and subsistence users. Because the results may be of interest to a broader community, we will also communicate them to state agencies (e.g., Alaska Department of Fish and Game) and to other I&M networks (e.g., SEAN, GLKN). Findings will be presented at local and regional subsistence meetings (Regional Advisory Council) and, when appropriate, submitted to peer-reviewed journals for publication. We will strive to provide clear, concise, and meaningful products in a timely manner.

Routine data summaries and in-depth analyses will be conducted after each sampling cycle, once every five years. Results will be incorporated into synthesis reports in order to document monitoring of fish Hg within the SWAN. These reports will be formatted as scientific journal articles, targeted toward park managers, I&M staff, subsistence councils, state agencies, and the scientific community. Reports will be posted on the SWAN website (<https://www.nps.gov/im/swan/index.htm>). Synthesis reports should adhere to I&M reporting guidelines for Natural Resource Reports. Refer to the Natural Resource Publications Management website for the most up-to-date guidelines and formats (<https://www.nps.gov/im/publication-series.htm>).

While synthesis reports are a definitive method of documenting the progress of a monitoring program, other means of communication can disseminate information to a broader audience. To this end, several additional types of communications will be provided periodically, including resource briefs, oral presentations, online newsletters, and permit reports. Additional guidance on reporting can be found in SOP 6.

Personnel Requirements and Training

Roles and Responsibilities

Freshwater monitoring at the SWAN is currently staffed by a project manager (Aquatic Ecologist, permanent full time equivalent (FTE)), a crew leader (Hydrologic Technician, permanent seasonal) and a crew member (Hydrologic Technician, seasonal). This contrasts with staffing at the GLKN, which includes two assistant project managers (permanent, FTE), in addition to the project manager and field crew. At the SWAN, the roles and responsibilities of these assistant project managers are divided among the project manager and the crew leader. Otherwise the roles and responsibilities outlined by VanderMeulen et al. (2018) in the GLKN Hg protocol are identical to those envisioned for monitoring Hg in resident lake fish at SWAN parks (Table 6).

Table 6. Roles and responsibilities of various SWAN staff members involved in monitoring Hg.

SWAN Staff	Roles and Responsibilities
Project manager (Aquatic Ecologist)	Completes all phases of field season preparation
	Coordinates field schedules, logistics, and purchasing
	Develops a training program for field personnel
	Sets up and facilitates contracts with laboratories for sample analysis
	Develops, documents, and oversees data collection and subsequent data handling
	Develops QA/QC measures and conducts QA checks of field sampling techniques
	Supervises or performs data entry, verification, and validation
	Summarizes and analyzes data, and prepares reports
	Serves as the main point of contact concerning data content
Data manager (alongside the project manager)	Uploads project data and metadata to the appropriate databases
	Develops data verification and validation measures for QA
	Establishes and implements a procedure to certify datasets
	Coordinates changes to the field data forms and the user interface for the database
	Manages the archiving process to ensure regular archival of project products
Crew leader (Hydrologic Technician)	Assists with coordination of field schedules, logistics, and supplies
	Completes pre-season training and helps to train other field personnel
	Collects fish samples and accompanying data according to the protocol
	Communicates progress and deviations from the protocol to the project manager
	Maintains accurate field and office notes
	Performs data entry, verification, validation; helps ensure all QA/QC aspects are met
	Facilitates communication among the SWAN, park staff, and the public
Crew member (Hydrologic Technician)	Completes pre-season training
	Collects fish samples and accompanying data according to the protocol
	Communicates progress and deviations from the protocol to the project manager
	Maintains accurate field and office notes
	Performs data entry, verification, validation; helps ensure all QA/QC aspects are met

Training Procedures

Before participation in data collection, personnel must become familiar with the SOPs and equipment used in the field. Training procedures for new personnel will include the following (in addition to mandatory training related to safety):

- Review of this protocol and the associated SOPs and DQs
- Familiarity with procedures for 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 forms and sample labels
- Park-specific training, provided on-site by park staff (e.g., use of radios)

Field crews who participate in the sampling will receive on-site instructions in the methods of collecting and handling biological samples and in the completion of field forms. Crew members must be trained in the use of “Clean Hands – Dirty Hands” techniques, which involve the proper use of nitrile 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. See SOP 2 (Training and Safety) and SOP 3 (Field Work) for additional details.

Budget

This section lists basic costs associated with personnel, equipment and supplies, logistics and travel, and laboratory analyses, as they relate to monitoring Hg in SWAN resident lake fish. Because field work for this monitoring will be conducted on a five-year sampling cycle, annual costs will vary from year to year. Here, costs are described in FY2017 dollars for years during which sampling occurs (Table 7).

Table 7. Estimated annual operating cost (based on FY2017 dollars) for implementing this protocol at SWAN parks during sampling years. Sampling interval is expected to be once every five years.

Category	Item	Cost (\$)	Notes
Personnel	SWAN Aquatic Ecologist	9,760	GS-11 – 2 pay periods (benefits included)
	SWAN Data Manager	1,980	GS-11 – 0.5 pay period (benefits included)
	SWAN Hydrologic Technician	3,670	GS-07 – 1.5 pay periods (benefits included) ¹
	SWAN Hydrologic Technician	2,010	GS-05 – 1.5 pay periods (benefits included) ¹
	Subtotal	17,420	–
Equipment and Supplies	Equipment	0	Assumes use of park boats is free of charge
	Supplies	640	Fuel costs not included; paid in total separately
	Subtotal	640	–
Logistics and Travel	Logistics	1,140	Assumes 4 flights ²
	Travel	1,160	Assumes park housing is free
	Subtotal	2,300	–
Laboratory Analyses	THg	5,000	4 lakes x 20 fish per lake x \$62.5 per fish ³
	Age	1,400	4 lakes x 20 fish per lake x \$17.5 per fish ³
	Shipping	140	Assumes government-rate FedEx account will continue
	Subtotal	6,540	–
Total		26,900	Estimated total cost per sampling year

¹ The GS-07 is a career seasonal position; the GS-05 is a seasonal position.

² Two flights in a Cessna-185 and two flights in a Cessna-206.

³ Cost per sample represents the midpoint of the range estimated in the text.

Personnel

Salary estimates include staff time for project management, pre-season preparation, training, sampling, processing samples, shipping samples, data entry, analysis, reporting, and various other tasks associated with this monitoring effort. These tasks will be shared by four SWAN employees: the Aquatic Ecologist, Data Manager, and two Hydrologic Technicians. We expect that salary expenses will total approximately \$17,420 annually, during sampling years.

Equipment and Supplies

Equipment needed to conduct this monitoring includes motorized boats (5-10 m in length) capable of travel on large lakes. The SWAN does not own motorized boats of this size, but it has a history of coordinating with parks to use theirs. Supplies include a variety of smaller items, such as fishing licenses and rods, lures, gloves, scales, and bags. Some of these items are already on hand, having been purchased for past projects; others need to be replenished yearly. We estimate that supplies for this protocol will cost from \$410 to \$880 annually (during sampling years), depending on which items need to be replaced.

Logistics and Travel

Logistics needed to support this monitoring include four flights in park planes per sampling year, specifically King Salmon to Port Alsworth and back, and Port Alsworth to Kijik Lake and back. Travel covers 14 days of per diem for a 2-person field crew, as well as a 5-day trip by the Aquatic Ecologist to assist with sampling activities. We expect logistics and travel expenses to be approximately \$2,300 annually, during sampling years.

Laboratory Analyses

Analytes of interest include THg in fish fillets and age from fish otoliths. The SWAN will confer with other networks monitoring Hg in fish (e.g., GLKN and SEAN) before selecting contract laboratories. Selected laboratories must meet the guidelines outlined in the DQSs and have rigorous internal QA/QC plans. Estimated costs of analysis per individual fish range from \$50 to \$75 for THg, and from \$15 to \$20 for otolith age. Funds permitting, stable isotopes of carbon and nitrogen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) could also be analyzed for the purpose of quantifying fish trophic position as a covariate of THg. Stable isotope analyses range from \$15 to \$25 per sample for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, combined. Likewise, concentrations of MeHg could be analyzed using stored replicate tissue samples, if and when funding becomes available, as could concentrations of selenium – an element that counteracts MeHg toxicity (Ralston and Raymond 2010).

Safety

Implementation of this protocol entails multiple complex risks, hence safety of personnel must be the uppermost concern. The greatest risks encountered in sampling SWAN lakes are those associated with boat and plane transport. Other important risks include wildlife encounters and severe weather. Field work requires situational awareness of these risks and knowledge of basic safety procedures. Planning, training, and a safety-oriented attitude can reduce or eliminate many risks.

A job hazard analysis (JHA), prepared by the project manager, will be reviewed with field personnel at the start of the season. The JHA covers safety concerns and mitigation strategies specific for this monitoring protocol. Concerns include:

- Boating on large, cold lakes and streams
- Flying in small park planes
- Using sharp equipment, such as lures and knives
- Working, sometimes overnight, in remote backcountry areas where communication with dispatch can be challenging, encounters with bears can be frequent, and emergency rescue can be slow

Based on the JHA and associated mitigation strategies, it was determined that this protocol can be safely implemented, provided that staff execute it in accordance with SOP 2 (Training and Safety) and the recommended mitigation strategies. Personnel must review the JHA, SOP, and relevant safety plans (Table 8) before conducting field work. These documents will be reviewed annually and updated as necessary to ensure that they adequately mitigate risks to personnel, property, and the public.

Table 8. Relevant documents and locations on the central server in the NPS Alaska Regional Office.

Relevant Document	File Path on the Central Server
JHA – Resident Lake Fish Sampling	T:\SWAN\Program_Management\Field_Operations\Safety\JHA_Job_Hazard_Analyses
SOP 2: Training and Safety	T:\Vital_Signs\Water_Fish\Protocols\Fish\SOPs\SOP2
ARCC Backcountry Itinerary	T:\Vital_Signs\Water_Fish\Administrative\Logistics\ARCC_Forms\Blank_Forms
ARCC Float Plan	T:\Vital_Signs\Water_Fish\Administrative\Logistics\ARCC_Forms\Blank_Forms
ARCC Flight Plan	T:\Vital_Signs\Water_Fish\Administrative\Logistics\ARCC_Forms\Blank_Forms

In addition to the documents that have been developed to safely implement this protocol (Table 8), safety-related checklists have been created, and safety-oriented training has been identified (see SOP 2). This training includes the Motorboat Operator Certification Course (MOCC), Basic Aviation Safety (A-100), Aviation Transportation of Hazardous Materials (A-110), Aircraft Mishap Review

(A-200), Water Ditching and Survival (A-312), Operational Leadership, and Bear Safety and Firearms. To increase overall levels of safety and awareness, obtaining additional levels of training beyond basic certifications will be encouraged.

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Appendix A. Power Analysis

Introduction

Contaminants monitoring from 2005 onward built a baseline of tissue samples of >300 fish, representing 9 species from 20 lakes in 2 parks within the Southwest Alaska Network (SWAN). We used these data to ascertain the power to detect a difference in fish mercury levels (a) between sites at a single time, and (b) at a single site over time. Specifically, we conducted power analyses to address the following questions, under various scenarios:

1. For sample sizes ranging from 2 to 50 fish, what is the power to detect a difference in fish mercury levels between two sites sampled at a single point in time?
2. For monitoring programs running from 10 to 50 years, what is the power to detect a linear long-term trend in fish mercury levels at a single site?
3. How many fish need to be collected annually to detect a linear long-term trend in fish mercury levels at a single site?

Methods

Total mercury (THg) concentrations were measured in fish axial muscle samples and reported in units of microgram per gram wet weight ($\mu\text{g/g ww}$; Table A1). Samples originated from four lakes in two parks: Katmai National Park and Preserve (KATM) and Lake Clark National Park and Preserve (LACL). Each lake is a high priority “Tier 1” site according to the SWAN freshwater monitoring protocol (Shearer et al. 2015). Samples from three of the four lakes were lake trout (*Salvelinus namaycush*). The fourth lake had only one lake trout sample on record, so an alternate top predator species with a similar distribution of THg was used as a substitute: northern pike (*Esox Lucius*; Figure A1).

Table A1. Summary of total mercury (THg) data used to perform power analyses.

Park	Lake	Fish Species	Sample Size	Year(s) Sampled	THg – Mean ($\mu\text{g/g ww}$)	THg – SD ($\mu\text{g/g ww}$)
KATM	Naknek Lake	Northern Pike	10	2011	0.279	0.091
KATM	Lake Brooks	Lake Trout	10	2011	0.531	0.085
KATM	Mixture ¹	Mixture ¹	20	2011	0.405	0.155
LACL	Lake Clark	Lake Trout	48	2005, 2010-2012	0.138	0.057
LACL	Kijik Lake	Lake Trout	10	2013	0.279	0.091
LACL	Mixture ¹	Lake Trout	58	2005, 2010-2013	0.313	0.137

¹ Mixture of two lakes or two species within a given park (KATM or LACL).

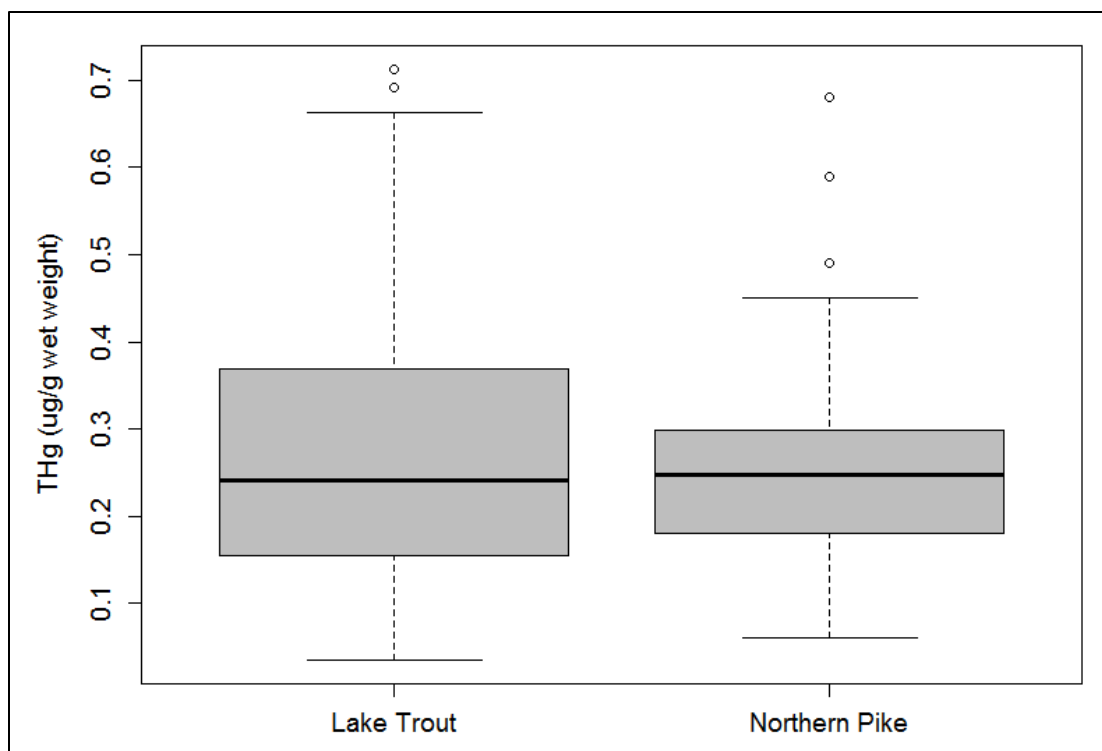


Figure A1. Distribution of total mercury (THg) in samples of two resident lake fish species collected network-wide since 2005.

Power analyses were performed on untransformed THg data, after confirming that the data were normally distributed via Shapiro-Wilk tests. Analyses were conducted at two spatial scales: lakes and parks. All analyses were conducted in R (v 3.4.2; R Core Team 2017) using code developed for similar analyses in the Southeast Alaska Network (SEAN; Sergeant et al. 2018) and Great Lake Network (GLKN; VanderMeulen et al. 2018).

Question 1

To evaluate the power to detect a difference in THg levels between fish from two sites sampled at a single point in time, we implemented the *pwr.t.test* function from the *pwr* package (v. 1.2-1; Champely 2017) in R. Seven scenarios, representing all possible pairs of lakes and parks, were run (Table A2). All scenarios used two-tailed t-tests with a vector of possible sample sizes (ranging from 2 to 50) and a Type I error rate of 0.05 (α). From this and the effect size (d), *pwr.t.test* calculated statistical power (1 minus the Type II error rate). Generally, the larger the effect size, the more power there is to discern a difference at a given sample size.

Table A2. Parameter inputs for the *pwr.t.test* function in the R statistical package *pwr*. Site pairs were either lakes or parks.

Site Pair	Effect Size (d) ¹	Sample Sizes (n)
Naknek vs. Brooks	2.858	2, 5, 10, 15, 20, 30, 40, 50
Naknek vs. Clark	0.667	2, 5, 10, 15, 20, 30, 40, 50
Naknek vs. Kijik	1.866	2, 5, 10, 15, 20, 30, 40, 50
Clark vs. Kijik	2.275	2, 5, 10, 15, 20, 30, 40, 50
Clark vs. Brooks	1.750	2, 5, 10, 15, 20, 30, 40, 50
Kijik vs. Brooks	5.436	2, 5, 10, 15, 20, 30, 40, 50
KATM vs. LACL	2.858	2, 5, 10, 15, 20, 30, 40, 50

$$^1 \text{Where the effect size (d)} = \frac{(\text{Mean}_{\text{Site 1}} - \text{Mean}_{\text{Site 2}})}{\sqrt{\frac{(\text{SD Site 1})^2 + (\text{SD Site 2})^2}{2}}}$$

Question 2

To evaluate the power to detect a linear long-term trend in fish THg levels at a single site, we implemented the *powertrend* function from the *fishmethods* package (v 1.10-4; Nelson 2017) in R. Six scenarios, representing individual lakes or parks, were run (Table A3). Within each scenario, the maximum time over which to detect a trend was set to 10, 20, or 50 years, and the percent change in THg detected over those time periods was set to 5, 10, 15, or 20%. All tests were two-tailed with $\alpha = 0.05$.

Table A3. Parameter inputs for the *powertrend* function in the R statistical package *fishmethods*. Sites were either lakes or parks.

Site	Starting Mean ($\mu\text{g/g ww THg}$) ^{1, 3}	Proportional Standard Error ($\mu\text{g/g ww THg}$) ^{2, 3}
Naknek	0.279	0.103
Brooks	0.531	0.051
Clark	0.138	0.049
Kijik	0.279	0.130
KATM	0.405	0.086
LACL	0.313	0.057

¹ A1 in the *powertrend* function.

² PSE in the *powertrend* function = $[SE(A1)]/A1$.

³ The relationship between A1 and PSE (i.e., *pserel* in the *powertrend* function) was set to the default value of $1/\sqrt{A1}$.

Question 3

To evaluate the sample size needed annually to detect a linear long-term trend in fish mercury levels at a single site, we used a modification of the *pwr.t.test* function from the *pwr* package in R. The modification was developed for the GLKN by S. Hafner (VanderMeulen et al. 2018). It employed the standard deviation (SD) within and among years to run a regression power analysis. Because only

one of our lakes contained multiple years of THg data with which to compute SD among years, our application of this analysis was limited to Lake Clark in LACL. The maximum time over which to detect a trend was set to 10, 20, or 50 years, and the percent change in THg detected over those time periods was set to 5, 10, or 20%. All tests used a Type 1 error rate of 0.05.

Results and Discussion

The power analyses presented below utilize limited data and carry multiple assumptions (e.g., normal distributions, linear trends, comparable species). Hence, results should be interpreted cautiously.

Question 1

For most scenarios, a sample size of 10 fish produced high power (i.e., >0.80) to detect between-site differences in THg concentrations measured at a single point in time (Figure A2). However, this sample size yielded low power for two scenarios (Naknek Lake vs. Lake Clark and KATM vs. LACL), which had “flatter” curves and thus needed 40+ fish to attain power comparable to that of other scenarios with a sample size of 10. This suggests that an n between 10 and 40 fish per site is a reasonable target sample size.

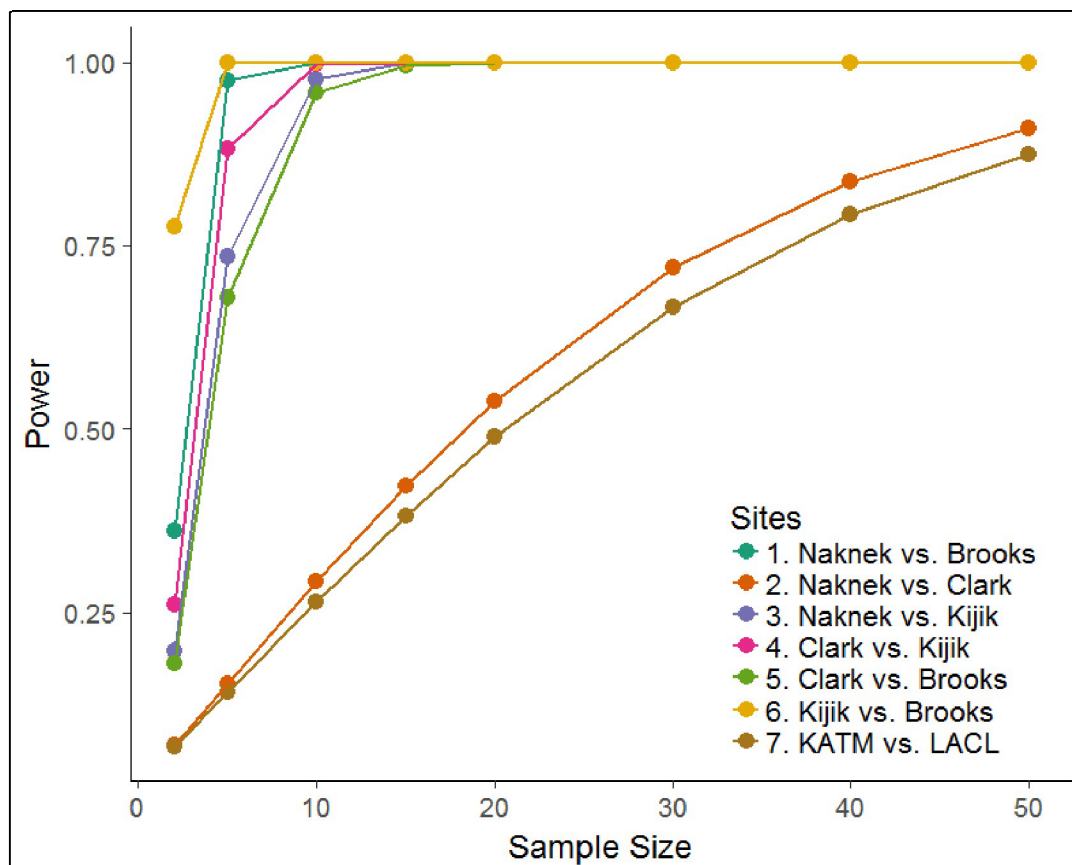


Figure A2. Power to detect a statistical difference in two site means, based on sample sizes ranging from 2 to 50 and $\alpha = 0.05$.

Question 2

The power to detect a linear long-term trend in fish mercury at a single site depended largely on the proportional standard error (PSE) of the site. In general, the lower the PSE the higher the power, at a given percent change (5, 10, 15, or 20%) and maximum time (10, 20, or 50 years). For example, Lake Clark and Kijik Lake exhibited the lowest and highest PSEs in our analysis, respectively (Table A3). Lake Clark had a 93% probability of detecting a 20% change in THg concentration over 10 years while Kijik Lake had a 26% probability (i.e., power = 0.93 and 0.26; Figure A3 and Table A4). To reach GLKN's stated objective (80% probability of detecting a 20% change in THg concentration at $\alpha = 0.05$), half of our sites would require 10 years of monitoring, and the other half would require 50 years.

Question 3

The sample size needed to detect a 20% change over 10 years with a Type 1 error rate of 0.05 and power of 0.80 was 27 fish at Lake Clark (Table A5). This sample size decreased to 13 or 6 fish when the time horizon was extended to 20 or 50 years, respectively. Other lakes could not support this analysis because they did not contain multiple years of data. It did not make intuitive sense to run this analysis at the park scale, given the high variability in mean THg levels among sites.

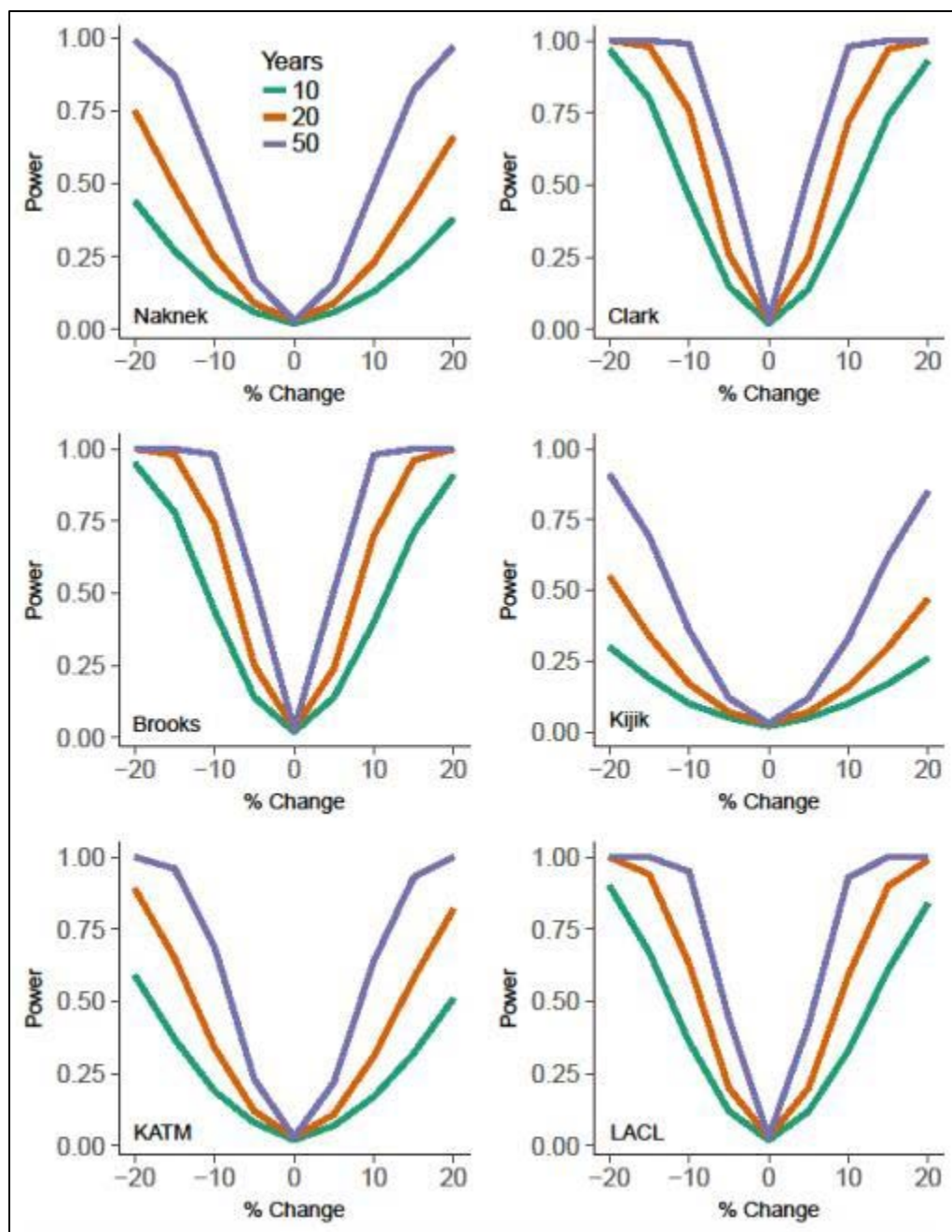


Figure A3. The power to detect a linear long-term trend in total mercury at a single site (i.e., lake or park), given the percent change detected over a period of years.

Table A4. Statistical power to detect a 10% or 20% change in total mercury concentration at a single site after 10, 20, or 50 years of monitoring.

Site	10 Years		20 Years		50 Years	
	10% Change	20% Change	10% Change	20% Change	10% Change	20% Change
Naknek Lake	0.13	0.38	0.23	0.66	0.49	0.97
Lake Brooks	0.40	0.91	0.70	1.00	0.98	1.00
Lake Clark	0.42	0.93	0.72	1.00	0.98	1.00
Kijik Lake	0.10	0.26	0.16	0.47	0.33	0.85
KATM	0.17	0.51	0.31	0.82	0.64	1.00
LACL	0.33	0.84	0.59	0.99	0.93	1.00

Table A5. Sample size needed to detect a 10% or 20% change in total mercury concentration at Lake Clark after 10, 20, or 50 years of monitoring.

Site	10 Years		20 Years		50 Years	
	10% Change	20% Change	10% Change	20% Change	10% Change	20% Change
Naknek Lake	– ¹	– ¹	– ¹	– ¹	– ¹	– ¹
Lake Brooks	– ¹	– ¹	– ¹	– ¹	– ¹	– ¹
Lake Clark	105	27	52	13	21	6
Kijik Lake	– ¹	– ¹	– ¹	– ¹	– ¹	– ¹
KATM	– ¹	– ¹	– ¹	– ¹	– ¹	– ¹
LACL	– ¹	– ¹	– ¹	– ¹	– ¹	– ¹

¹ Not enough data to compute SD among years – a necessary input parameter for this analysis.

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