

Standard Operating Procedure 5: Data Analysis

Version 1.0

In Protocol for Monitoring Mercury in Resident Lake Fish

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Revision History Log

The following table lists all edits and amendments to this document since the original publication date. Information entered in the log must be complete and concise. Users of this Standard Operating Procedure (SOP) will promptly notify the Southwest Alaska Network (SWAN) project manager and/or data manager about recommended and required changes. The project manager 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 8 (Protocol Revision).

Revision History Log:

Previous Version #	Revision Date	Revision Author	Revision Description and Location in SOP	Justification for Revision	New Version #
—	—	Bartz	Initial creation	—	1.0

Add rows as needed for each change or set of changes tied to an updated version number

Acknowledgements

This SOP was adapted from a similar SOP in the Great Lakes Network (GLKN) protocol for monitoring mercury in dragonflies and fish (VanderMeulen et al. 2018). Many of the principles and concepts discussed in the GLKN SOP were based on SOP 9 from the Network's protocol for monitoring water quality of inland lakes (Elias et al. 2015). Additional data analysis approaches were drawn from Nelson et al. 2015 and from Wiener et al. 2009.

Introduction

Data analysis is the process by which measurements are interpreted meaningfully. It begins after data have been collected and entered into an electronic file or database, and have undergone various checks to ensure that data entry is free of errors and statistical outliers are genuine. Data analysis may include exploration, summarization, and transformation steps, and it concludes with analyses that lead to useful interpretations of the data.

Well-conceived and well-developed monitoring strategies have clear connections between questions of interest, appropriate sampling designs, and resulting analytical approaches (Noon 2003). Accordingly, the utility and robustness of the Southwest Alaska Network's (SWAN) analyses for monitoring mercury (Hg) in resident lake fish are based on ecologically meaningful questions and relationships, which dictate the sampling design that, in turn, prescribes an analytical approach. Increasingly, biometricians recommend that ecologists elucidate and quantify ecologically important phenomena, rather than exclusively pursue statistical significance (Yoccoz 1991; Johnson 1999; Anderson et al. 2001).

Monitoring Overview

This Standard Operating Procedure (SOP) outlines methods for assessing the status and trend of Hg in resident lake fish, in order to address the following three objectives:

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.

As described in the protocol narrative, fish will be sampled once every five years from "Tier 1" lakes in two SWAN parks. The selection of Tier 1 lakes incorporated substantial input from park staff and invited experts in 2004, with the intent of creating a targeted sampling design for water quality monitoring. For the purposes of this protocol, the focus on Tier 1 lakes (1) reduces logistical obstacles to sampling, (2) maximizes spatial overlap between Hg monitoring and water quality monitoring by SWAN, and (3) utilizes study sites with available historical Hg data and pertinent ancillary information on the biological, physical, and chemical characteristics of aquatic resources.

Total mercury (THg) data will be provided from the analytical laboratory in units of $\mu\text{g/g}$ for each individual fish. Subsequent analyses will be based on the assumption that THg provides a valid estimate of methylmercury (MeHg), because nearly all ($\geq 95\%$) of the Hg in the axial muscle tissue (i.e., skinless fillets) of predatory fish is MeHg (Grieb et al. 1990; Bloom 1992). Therefore, analyses of adult lake trout, a top predator species, will use THg as the response variable, under the assumption that THg and MeHg are essentially equivalent. The relationship between THg and MeHg will be analyzed occasionally to verify this assumption.

Quality Assurance/Quality Control Checks

Quality assurance/quality control (QA/QC) procedures are outlined here and in the Data Quality Standards (DQSs) for this protocol (Bartz et al. 2018). All Hg data will undergo several QA/QC procedures (e.g., data-entry filters, removal of logically inconsistent entries) to ensure that they accurately represent the natural environment at the time of sampling. In addition to these procedures, several analytical and data sorting techniques (e.g., scatter plots, box-and-whisker plots, stem-and-leaf plots, sorting values of a given indicator in ascending or descending order, “COUNTIF” statements in Excel) are available to identify potentially erroneous values, whereas tests such as the generalized extreme Studentized deviate test can be used to assess for statistical outliers in log-transformed data (Rosner 1983). During this process, data points that do not meet limitations for precision or bias may be flagged or eliminated from the database. Some statistical measures, such as population mean, are sensitive to extreme atypical values, or outliers. Therefore, to reveal the central tendency of the population, the project manager may elect to remove outliers from the data pool. Such removals should be performed with great caution, and only when it is clear that the outlier truly does not reflect system properties (e.g., the outlier resulted from instrument error, transcription error, or sample contamination).

Data Summarization

Brief characterizations of the data from each lake, each park, and the SWAN as a whole will be performed during sampling years (i.e., once every five years), after all QA/QC procedures have been completed. Summary statistics including geometric mean, median, minimum and maximum values, as well as skew, kurtosis, and measures of variability (e.g., coefficient of variation, standard error, variance) when appropriate, will be used to characterize Hg levels in fish, by lake and park, for single years and multiple years.

Analysis of Spatial Differences and Temporal Trends

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. Similar to studies of Hg in larval dragonflies where data are left-skewed (e.g., Nelson et al. 2015), log-transformation may be necessary 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 tests). A Type I error (α) of 0.05 will be used to judge the significance of all tests. For significant ANOVAs, multiple comparisons among means will be made with Tukey’s HSD test, which has an experiment-wise error rate.

Within a given population of fish, concentrations of some contaminants (such as Hg) typically increase with increasing age or size. Consequently, information on age, length, or weight 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). See Eagles-Smith et al. 2014 for a detailed description of methods.

Typically, temporal trends are analyzed within a site by relating log-transformed contaminant data to year using linear regression. To examine temporal trends in Hg concentrations among several sites, mixed effects models with a linear time trend (fixed effect) and random effects for sites may be used. The random effects allow contaminant concentrations at each site to deviate consistently from the population mean but with a common trend over time for all sites. Mercury concentration data may also be evaluated through nonparametric trend tests (e.g., Mann-Kendall or Seasonal Kendall) and through comparisons of period means. For the latter-most approach, one is often interested in comparing values before and after an important event (e.g., anthropogenic disturbance, natural catastrophe, drought), and considers years within each of the two periods as replicates. We may also examine trends for breakpoints, or changes in slope, which may indicate the timing of an important event, and hence a potential cause.

If ancillary data for study sites 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 ecosystem factors and other variables that can influence the production and abundance of MeHg (Burnham and Anderson 2002). Simple correlation analysis such as Pearson's will be used to determine significant univariate correlations in the data set (e.g., total Hg concentration vs. body length). Body length can also be included in mixed-effects of other multi-variate models. Waterbody (lake) will be included as a random effect in statistical models when analyzing broad spatial-temporal trends. In accordance with the information-theoretic approach, we will apply judgment based on the state of scientific understanding of factors and processes controlling the abundance of Hg in selecting predictor variables (e.g., Wiener et al. 2006).

Assessment of Human Health Risks

To assess the potential for harmful MeHg exposure in humans and top predator piscivores consuming fish from Tier 1 lakes (Objective 3), we will compare the Hg concentrations in lake trout from these lakes to relevant toxicological benchmarks, and then compute the proportion of individuals exceeding each benchmark at each lake, as in Eagles-Smith et al. 2013 and 2014. Specifically, we will compare THg levels in the axial muscle of fish sampled in SWAN lakes to the U.S.

Environmental Protection Agency (EPA) tissue residue criterion for MeHg (0.300 µg/g wet weight (ww)), which was established to protect the health of humans who eat noncommercial fish, such as wild-caught sport fishes (Borum et al. 2001). We will also consider the fish consumption guidelines recommended by the Alaska Department of Health and Social Services (ADHSS) and the Great Lakes Fish Advisory Workgroup (GLFAW). Both organizations' guidelines offer benchmarks for unrestricted consumption by children, nursing mothers, or women who are (or can become) pregnant. Specifically, the ADHSS threshold for Alaska-caught fish is 0.20 µg/g ww (Hamade 2014), and the

GLFAW threshold for Great Lakes sport fish is 0.050 µg/g ww (Table 1; GLFAW 2007). Finally, we will compare THg levels in the axial muscle of fish sampled in SWAN lakes to a benchmark associated with altered biochemical processes, damage to cells and tissues, and impaired reproduction in sport fish (0.500 µg/g ww; Sandheinrich and Wiener 2011). Underlying these comparisons – i.e., of fish tissue concentrations (THg) with toxicological benchmarks (MeHg) – is the assumption that virtually all of the Hg in the fillets of fish is MeHg, so measurement of THg in the axial muscle of fish provides a valid, conservative estimate of MeHg concentration in the edible fillet (Wiener et al. 2007).

Table 1. Guidelines published by the Great Lakes Fish Advisory Workgroup (GLFAW) for human consumption of sport fish containing Hg in their fillets.

Concentration range in fish (µg/g wet weight)	Consumption Guidelines (fish-meal frequency) ¹
≤ 0.050	Unrestricted consumption
0.051 – 0.110	2 meals per week
0.111 – 0.220	1 meal per week
0.221 – 0.950	1 meal per month
> 0.950	Do not eat

¹ Guidelines for the MeHg-sensitive group including pregnant women, women who may become pregnant, and children under 15 years of age, recommended in the protocol for Hg-based fish consumption advice (GLFAW 2007).

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