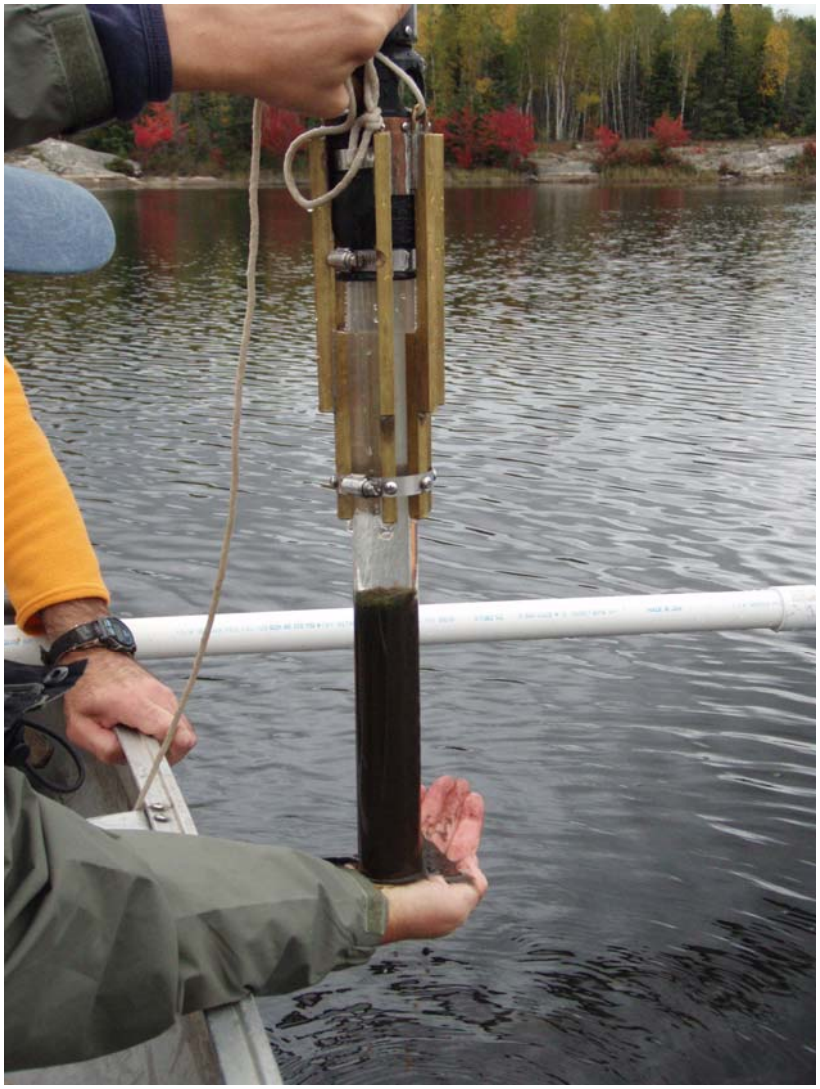
National Park Service
U.S. Department of the Interior

Natural Resources Program Center

Diatom Monitoring Protocol
Great Lakes Inventory and Monitoring Network

Natural Resource Report NPS/GLKN/NRR—2008/068



ON THE COVER
Surface sediment sample collected with a Wiegner corer at Voyageurs National Park. Photograph by: Ray Wise.

Diatom Monitoring Protocol

Version 1.0

National Park Service
Great Lakes Inventory and Monitoring Network

Natural Resource Report NPS/GLKN/NRR—2008/068

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Standard Operating Procedures

SOP #1: Pre-season Preparation
SOP #2: Training and Safety
SOP #3: Using a GPS
SOP #4: Field Methods
SOP #5: Cleaning Sediment Samples
SOP #6: Preparation of Diatom Slides
SOP #7: Analysis of Slides
SOP #8: Archiving Sediment and Diatom Slides
SOP #9: Data Analysis
SOP #10: Reporting
SOP #11: Post-season Procedures
SOP #12: Quality Assurance and Quality Control
SOP #13: Procedure for Revising the Protocol

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 protocol narrative will promptly notify the project manager and/or the Great Lakes Network (GLKN) 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 footer of the document file. For complete instructions, please refer to Revising the Protocol, SOP #13.

Revision History Log:

Previous Version #	Revision Date	Author (with title and affiliation)	Location in Document and Concise Description of Revision	Reason for Change	New Version #
Add rows as needed for each change or set of changes tied to an updated version number					

Acknowledgements

The diatom monitoring project will be closely tied to the water quality monitoring protocols for inland lakes and large rivers. See Elias et al. (2008) and Magdalene et al. (2007), respectively, for additional details on these projects.

1.0 Background and Objectives

This protocol is designed to use diatoms as bioindicators to monitor lakes and large rivers within the National Park Service Great Lakes Inventory and Monitoring Network (Network or GLKN) park units. Network parks encompass a variety of aquatic habitats and biota, including 129 named lakes, totaling nearly 41,000 ha (101,000 acres) (Lafrancois and Glase 2005). Monitoring of diatoms will occur in the lakes selected for GLKN's monitoring of water quality in inland lakes (Elias et al. 2008) and at select sites that are part of monitoring in large rivers (Magdalene et al. 2007). The lakes selected for monitoring span gradients of surface area, depth, visitor use, water chemistry, and spatial distribution within each park. The few river sites selected for diatom monitoring will be lake-like, where sediment deposition occurs. Aquatic resources at each of the parks are described in Elias et al. (2008), Magdalene et al. (2007), and Route and Elias (2005). Throughout the remainder of this protocol we will refer only to the inland lakes protocol to simplify the description, although diatom monitoring also will occur at several sites that are part of the large rivers protocol.

Because diatom monitoring will be conducted in close collaboration between GLKN staff and contracted cooperators, this protocol is intended to address the essential aspects required of the project partners.

1.1 Rationale for Selecting this Resource to Monitor

A variety of stressors such as climate change, environmental contaminants, exotic species, and land and resource uses including shoreline and urban development, recreation, water level management, logging, and agriculture in and near GLKN park units influence the parks' resources. Park managers seek information to make considered decisions in a future certain to bring change.

Bioindicators are critical to long-term monitoring of water quality because (1) they provide an assessment of the actual ecological impact of environmental stressors, (2) it is not possible to measure directly all of the water quality parameters needed to detect unexpected environmental change, and (3) they serve as integrators of changes in water quality that may be missed during routine water chemistry sampling. Physical and chemical measures of water quality provide only a partial picture of ecological impact, and even the full suite of proposed water quality indicators (Elias et al. 2008; Water Quality Monitoring Protocol for Inland Lakes) is likely to miss stressors that are not currently identified or anticipated.

Diatoms (Class Bacillariophyceae) are among the most powerful groups of biological indicators of environmental conditions in aquatic systems (lakes, rivers, wetlands, estuaries; Stoermer and Smol 1999). Diatoms make excellent bioindicators because they are generally abundant in aquatic systems and respond rapidly to changes in their environment. They form a cell wall made of silica, which preserves well in the sediments, and different species can be identified by unique patterns in this silica cell wall. Diatoms sensitively register environmental changes at the base of the food chain and are thus critical to all 'bottom-up' ecological processes. As primary producers, their relationship to environmental stressors is more direct and traceable than those at higher trophic levels *(*e.g., invertebrates, fish, amphibians). The recent discovery that increases in chloride concentration from road-salt application have had a pronounced effect on diatom

assemblages in the Minneapolis/St. Paul metropolitan area as well as more rural parts of Minnesota (Ramstack et al. 2004) is a case in point. Today chloride concentrations in many of these lakes are 1-2 orders of magnitude greater than those in pre-European times. What is unexpected about this finding is not the increase in chloride, although there was no way of assessing the magnitude of the increase without diatoms, but that a conservative anion had produced such a large impact on algal communities and that this change had gone unnoticed.

Analysis of diatoms in surficial lake sediments is a powerful tool to detect recent environmental change, or to place modern conditions in a regional or historical context (Cumming et al. 1992; Ramstack et al. 2003, 2004; Smol and Douglas 2007). We will integrate diatom monitoring in GLKN park units with paleolimnological efforts on a subset of the lakes. Paleolimnology offers unique opportunities for aquatic and terrestrial biomonitoring programs, primarily by providing historical data with which to contextualize modern monitoring in the absence of long-term monitoring data. Paleolimnology is often the only available or practical means for reconstructing ecosystem conditions, because historical water quality records are rarely available and if they are, records do not extend beyond the influence of European settlement. Many, if not most, modern lakes are significantly altered from their natural state; thus the use of reference lakes or computer models to simulate reference conditions may produce questionable results.

By coordinating the diatom monitoring and paleolimnology efforts, it will be possible to place the current lake conditions in a historical perspective. The results will provide a management foundation by determining the natural variability or reference condition of national park lakes and by reconstructing a detailed history of lake response to ecological changes that have occurred in and around the lakes during the last 150 years. Lake-sediment records integrate across both spatial and temporal scales; therefore, the results of the paleolimnological analyses can be used in conjunction with the biomonitoring efforts to quantify modern environmental conditions relative to historical conditions, to detect early ecological change and recent trends, and to evaluate success of management actions.

At the time of this writing, the Network and the St. Croix Watershed Research Station (SCWRS) are collaborating on a paleolimnological project that includes several GLKN park units. As part of this project, a select subset of GLKN lakes will be cored for diatom and geochemical analysis to determine the timing and magnitude of historical environmental change. Results of this project will be available to help interpret and place into context data gathered through diatom monitoring.

1.2 Measurable Objectives

The analysis of diatoms in surficial lake sediments will address the following questions:

- What is the current ecosystem status of a particular lake in relation to other regional lakes?
- Are similar ecological trends occurring across regional lakes? (e.g., related to climate change or atmospheric deposition)
- What is the ecosystem status of this lake in relation to historical environmental change noted in regional sediment cores?
- Has this lake changed since the last time it was sampled? If so, what are the magnitude and direction of that change?

To answer these questions the following steps will be taken:

1. Analyze diatom communities in surficial lake sediments to complement the routine physical and chemical water quality sampling and enhance the potential for detecting long-term changes in water quality.

Rationale for using diatoms as bioindicators include:

- The sensitivity and tolerance of diatoms to environmental variables – including nutrients, organic pollutants, pesticides, heavy metals, salinity (and major ion chemistry), pH, alkalinity, light, temperature, substrate, and depth – are known to vary among species (Battarbee et al. 2001). These species-specific responses can be used to infer environmental conditions and to provide an early-warning of the cumulative impacts of environmental change at the base of aquatic foodwebs. Early detection of change may facilitate park management decisions such that irreversible changes may be avoided.
- Diatoms occur in highly diverse assemblages, and species are relatively easily distinguished. The taxonomic diversity of diatom communities provides a high level of redundancy (confirmation by responses from multiple species) for robust tracking of environmental conditions.
- The combined cost of diatom sampling and analysis is relatively low when compared to other biological indicators (Stevenson and Pan 1999), and diatom samples are easily archived and preserved semi-indefinitely, allowing samples to be re-examined at future dates, if desirable.
- In lake environments, the deep-water sediments provide a highly integrated sample of diatom community structure for the lake as a whole. This means that only a single sample of surface sediment is needed to characterize the entire lake (Anderson 1990), as compared to the numerous samples required to overcome spatial heterogeneity for other bioindicators (e.g., benthic invertebrates).
- Diatoms have been used extensively to assess environmental conditions in freshwaters. Literally dozens of papers are published each year in peer-reviewed journals describing analytical and numerical (statistical) methods, taxonomic refinements, environmental requirements, paleo-environmental constructions, and applications in water-quality issues and policy development (Stoermer and Smol 1999). Diatoms are currently being used in both state (e.g., Minnesota, Montana) and nation-wide (Environmental Monitoring and Assessmeny Program [EMAP], National Water-Quality Assessment [NAQWA]) assessments of water quality.

2. *Analyze contemporary changes in the community composition of diatoms within the context of the range of natural variability, as determined by diatoms present in cores dating back to the time of European settlement.*

The question of natural variability is particularly important to environmental monitoring, which in the final analysis aims to distinguish significant human perturbations from background conditions. Ecological systems are often naturally variable (seasonally and inter-annually), and decades of careful water monitoring may be needed to detect significant trends. Even where long-term datasets are available, it is often difficult to know whether the observed trends are of concern or within the range of natural variation. Comparing the current community composition

of diatoms to the species compositions dating back 150-200 years will allow us to determine whether the current conditions are outside of the range of natural variability of GLKN park lakes.

3. *Compare diatom species assemblages in surficial lake sediments with recent water chemistry measurements to continue to improve our understanding of diatom response to physical/chemical variables.*

Diatom communities are readily compared using multivariate techniques such as ordination because the assemblages are taxonomically diverse. Differences among samples (in space or time) are summarized by dissimilarity metrics such as chord distance or by arraying them along one or a few major axes in multivariate space (using principal components analysis [PCA], correspondence analysis [CA], or similar techniques). If independent water quality measurements have been taken (e.g., pH, total phosphorus, Secchi depth), the ordination can be constrained so that the major axes of diatom variability are aligned with the most explanatory environmental gradients (canonical correspondence analysis); the strength of these relationships can then be summarized statistically as an objective measure of explained variance (Battarbee et al. 2001).

2.0 Sampling Design

Because analysis of diatoms in surficial sediments will be used to augment and integrate water quality monitoring of inland lakes and large rivers, the sampling design is based on these projects (Elias et al. 2008; Magdalene et al. 2007, respectively) and known sedimentation rates. Approximately 30-35 lakes and 5-8 river sites will comprise the routine diatom monitoring sites.

2.1 Number and Location of Sampling Sites

The deep-water sediments of lakes provide a highly integrated sample of diatom community structure from the lake as a whole. Therefore, only a single sample of surface sediment is needed to characterize an entire lake (Anderson 1990). The diatom surface sediment sample at each lake will be collected at the deepest part of the lake, where routine water quality monitoring is conducted (Elias et al. 2008).

2.2 Frequency and Timing of Sampling

Sediment samples for diatom analysis will be collected from lakes selected for water quality monitoring approximately once every five years, depending primarily on sedimentation rate. Although lakes will be sampled annually as part of the water quality monitoring protocol, diatom analysis in surficial sediments will be rotated across parks on an approximate 5-year cycle in an attempt to spread the costs evenly. Additional lakes will be sampled for diatoms and routine water chemistry as funding permits (see Elias et al. 2008 for details).

Surface sediment samples will be collected during the final water quality monitoring event of the season, usually in September or October. Only one sediment sampling event at the end of the field season is necessary because that sample will represent the diatoms that have settled out of the water column over the previous approximately 3-5 years. At each site the top 0-1 cm and 1-2 cm of sediment will be collected, and kept as two separate samples. In most cases only the 0-1 cm sample will be analyzed; however, in lakes that are found to have a high sedimentation rate (this will be determined from the analysis of long cores), the 0-1 and 1-2 cm samples will be combined for analysis. This combined sample will then represent the previous 3-5 years.

2.3 Level of Change that Can Be Detected Given the Sampling Design

Sediment cores collected and analyzed for diatoms as part of the GLKN monitoring program will provide a historical framework for interpreting variability in the samples. Comparing the current community composition of diatoms to the species compositions dating back 150-200 years will demonstrate if the current conditions are outside of the range of natural variability of GLKN park lakes.

Change will be evaluated in two ways; first, the amount of floristic change in the diatom community composition will be measured using a chord distance dissimilarity measure. Second, the change in diatom-inferred water quality estimates will be evaluated using the root mean square error of prediction.

The chord distance dissimilarity measure will be used to quantify the floristic change in diatom community composition between subsequent sampling events. Meaningful change can be assessed using percentile cutoffs (Bennion et al. 2004), or by using the chord distance between

samples from sediment cores as a framework (refer to SOP #9 [Data Analysis] for more information on chord distance).

Methods for evaluating the certainty of inferred water quality estimates are well established. Generally error bars are reported as model error estimates, the jack-knifed or bootstrapped root mean square error of prediction ($RMSEP_{boot/jack}$) of the calibration model (Fritz et al. 1999; Ramstack et al. 2003; Köster et al. 2004) (refer to SOP #9 [Data Analysis] for more information on the diatom calibration model being used for this project). Variability or change in inferred water quality parameters greater than the $RMSEP_{boot/jack}$ represents a measure of significant change.

3.0 Field Methods

This section summarizes the information presented in greater detail in the standard operating procedures (SOPs) #1 (Pre-season Preparation), #4 (Field Methods and Surface Sediment Collection), #7 (Analysis of Diatoms on Microscope Slides), and #9 (Data Analysis). Refer to these SOPs for more information.

3.1 Field Season Preparations and Equipment Setup

Prior to the field season, many preparations must be completed to ensure sampling can be undertaken according to schedule. The field season for collecting surface sediment samples for diatom analysis in GLKN park units is generally late summer/early fall. Because the surface sediment samples will be collected during the final water quality sampling event of the year it will be necessary to coordinate with personnel conducting water quality monitoring of inland lakes.

All details for the season need to be planned well in advance. Field preparations should begin in January to allow enough time for ordering new supplies and equipment and making repairs if necessary. Field equipment should be checked to ensure proper functioning; supplies should be inventoried and orders placed. Refer to SOP #1 (Pre-season Preparation) for a complete list of field season preparations.

For sampling stations located within park boundaries, a Research and Collecting Permit must be obtained before any work can be done. Refer to SOP #1 for details on renewing or obtaining new permits. Permanent diatom slides are considered U.S. government property, and as such, must be curated properly. Consult with the appropriate park representative for advice on compliance with government curation mandates.

Agreements for diatom analysis with cooperators should be reviewed and modified, if necessary. Both parties (NPS and cooperating organization) should agree on the number of samples to be collected in the year, annual timelines, and other expectations.

3.2 Sample Collection

A line-operated gravity corer will be used to collect the upper 0-1 cm and 1-2 cm of sediment for an integrated assessment of lake conditions during the previous approximately 3-5 years (see SOP #4 for details). Collection sites will be precisely located by GPS to allow re-sampling in the same location in subsequent years; sampling sites will target central depositional basins in each lake. Sediment samples can be collected by water quality monitoring personnel during the final round of water quality sampling of the season.

Surface sediment samples will be processed in the field according to SOP #4 and kept refrigerated until they are sent to the analytical laboratory for further processing and analysis. Upon receipt by the analytical laboratory, samples will be homogenized and subsampled for diatom analyses.

3.3 Post-Collection Processing of Samples

Diatom microscope slides will be prepared using standard oxidation and mounting techniques (see SOPs #5 and #6). A total of 400 diatom valves will be counted in each sample using a light microscope fitted with full immersion optics capable of 875-1250X magnification and numerical aperture (N.A.) >1.30. Analyses will follow the enumeration criteria used in the Minnesota calibration set (Ramstack et al. 2003; Edlund and Kingston 2004; Edlund 2005).

Approximately 10% of the samples will be recounted by another analyst. Refer to SOP #12 for details on QA/QC.

Diatoms will be identified using floras and monographs by Hustedt (1927-1966, 1930); Patrick and Reimer (1966, 1975); Collins and Kalinsky (1977); Camburn et al. (1978, 1984-1986); Krammer and Lange-Bertalot (1986, 1988, 1991a, b); Cumming et al. (1995); Reavie and Smol (1998); Camburn and Charles (2000); and Fallu et al. (2000) as well as all other pertinent literature. Diatom taxonomy should be harmonized with the Minnesota (MN) diatom calibration set (Ramstack et al. 2003; Edlund and Kingston 2004; Edlund 2005) because analysis of the GLKN samples will be overlaid on the Minnesota set.

3.4 End-of-Season Procedures

Before storing the gravity corer, be sure it is clean and dry and that all removable parts have been returned to the storage case. Inventory all field and laboratory supplies and replace as necessary as soon as possible. Refer to SOP #11 (Post-season Procedures) for a complete list of end-of-season procedures.

4.0 Data Handling, Analysis, and Reporting

4.1 Metadata Procedures

Metadata allows potential data users to evaluate the quality and usefulness of the data based on an understanding of the complete process under which it was collected and maintained. All of the protocol documentation, including standard operating procedures (SOPs), is part of a dataset's metadata for this reason. A reference to the appropriate version of these documents is part of the metadata for any particular element of a dataset. All data must have associated values for the dates and times they were collected.

Additional metadata associated with diatom monitoring includes geospatial data (location) and biological (species identification). For metadata associated with geospatial data, Executive Order 12906 will be abided by, which mandates that every federal agency document all new geospatial data it collects or produces using the Federal Geographic Data Committee (FGDC) Content Standard for Digital Geospatial Metadata (CSDGM; www.fgdc.gov/metadata/constan.html).

Although not currently required, GLKN will make every effort to complete Biological Data Profiles (www.fgdc.gov/standards/status/sub5_2.html) for diatom datasets and add associated metadata to the National Biological Information Infrastructure Clearinghouse (NBII; www.nbii.gov/datainfo/metadata).

For more details on the Great Lakes Network's overall strategy for metadata generation, management, and distribution see Chapter 8, Data Documentation, of GLKN's Data Management Plan (Hart and Gafvert 2005) and the appendices of that document.

4.2 Overview of Database Design

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

To meet the data flow requirements of NPS-WRD, the Network's version of NPSTORET will be able to export and import water quality data from the master version of STORET maintained by WRD. In addition, GLKN uses the Vital Signs Internet Mapping Service (VSIMS) for data distribution. This service allows users to explore and query monitoring data using spatial and non-spatial parameters. Network versions of NPSTORET are used to update a master version of STORET maintained by NPS WRD. The WRD master copy of STORET data is the data source that is used by the VSIMS to serve water quality data collected by GLKN and other I&M networks.

The Great Lakes Network will maintain one master copy of NPSTORET for each park at the Ashland office on a central server. This is the only copy of NPSTORET that can be used to

export data to other locations (WRD, GLKN's SQL Server). Additional copies of NPSTORET can be used by GLKN personnel stationed at parks, but they can only be used as a conduit for data entry and the importation of data to GLKN's master version of NPSTORET. For analysis, the data from the master copy of NPSTORET, that has passed all QA/QC procedures, must be used.

4.3 Data Entry, Verification, and Editing

Each diatom species will be described by an 8-character species code; all of the codes used in the 145 lake MN calibration set are listed in Appendix 1 of SOP #7 (Analysis of Slides). These codes follow the format used by the Great Lakes Environmental Indicators (GLEI) project (E. Reavie, Natural Resources Research Institute, University of Minnesota, Duluth). The GLEI list will be used as a starting point; however, many taxa and codes will need to be added as the project progresses. A complete and up-to-date list of codes for this project will be maintained by GLKN and the cooperators.

After each sediment sample has been processed and diatoms have been identified, the following information will be recorded:

- The full name, including authority(ies) of each diatom taxon identified
- The appropriate 8-character code for each taxon
- The raw number of valves of each taxon (refer to SOP #7 for details on counting)
- The percentage abundance (relative frequency) of each taxon (number of times a taxon is observed, divided by the total number of diatoms observed)

The cooperators, as subject experts, will verify the data prior to providing them to GLKN. Network personnel will upload the diatom data to NPSTORET and conduct quality assurance/quality control checks prior to transfer to WRD. These procedures protect the integrity of the data and allow the history of each data record to be traced.

4.4 Routine Data Summaries and Statistical Analyses

Multivariate statistical analysis of the diatom data will occur annually, following diatom enumeration. Comparisons of diatom species assemblages with water chemistry measurements will be used to derive an understanding of diatom response to physical/chemical variables. During the 2004-2009 diatom project (Task Agreement #J2105040028), surface sediment samples will be collected from approximately 50-60 lakes within the Great Lakes Network parks. The diatom assemblages from these samples will be appended to the MN diatom calibration set (Ramstack et al. 2003; Edlund and Kingston 2004; Edlund 2005). Subsequent surface sediment samples that are analyzed for diatoms will be plotted passively on this calibration set to look for changes in the lakes. The diatom taxonomy must be harmonized with the MN calibration set prior to statistical analyses.

Changes in the community composition of diatoms will be analyzed in relation to other lakes as well as within the context of the range of natural variability, as determined by diatoms present in sediment cores dating back before the time of European settlement. Refer to SOP #9 Data Analysis, for a detailed discussion of the multivariate statistical methods that will be used in this project.

4.5 Reporting Schedule

One of the Network's goals is to ensure that the results and knowledge acquired through the monitoring programs are shared with all appropriate parties, especially the parks and their natural resource managers. Park managers will be provided in a timely manner with clear, meaningful products. Because the monitoring data will be of interest to a broader scientific community, reports will be provided to the states in which the national park units reside, the NPS I&M Program, and when appropriate, articles will be submitted to peer-reviewed journals for publication. Findings will also be presented orally and in poster format at regional and national meetings. In particular, the Western Great Lakes Research Conference, which is sponsored in part by GLKN, will be a valuable venue for presenting the results of this program.

Annual reports will be prepared by the diatom cooperators in collaboration with the Network's aquatic ecologist. As parks are monitored repeatedly, the reports will become progressively more in-depth and will include syntheses and analyses of data over time. The reports will place the observed results in both a regional and historical context by relating them to other published literature, the significance of the results will be discussed in terms of environmental change, and management recommendations will be provided based on the findings. See SOP #10, Reporting, for more details.

4.6 Recommended Report Format

Reports should follow the format of a typical peer-reviewed journal article. The following outline is a good example of the type of report to be produced.

TITLE PAGE
    (Title, Author(s), Participating Institutions, For Whom Prepared, and Date)
TABLE OF CONTENTS PAGE
EXECUTIVE SUMMARY PAGE (abstract)
1.0 INTRODUCTION
    1.1 Background
    1.2 Justification for Study
    1.3 Objectives
2.0 METHODS
    2.1 Study area(s)
    2.2 Field methods(s)
    2.3 Analytical method(s)
3.0 RESULTS
4.0 DISCUSSION
5.0 MANAGEMENT IMPLICATIONS
6.0 ACKNOWLEDGEMENTS
7.0 LITERATURE CITED (if any)
8.0 TABLES
9.0 FIGURES
1.0 APPENDICES (if any)

Reports should include tabular and graphic displays of data. Tables are appropriate for displaying simple data summaries, but can also be used to show results of more comprehensive analyses. Graphical display of data is especially useful for depicting trends across years (e.g., Figures 1 and 2) or the correlations among multiple variables or lakes (e.g., Figure 3). See SOP # 9, Data Analysis, for more details on presentation of data.

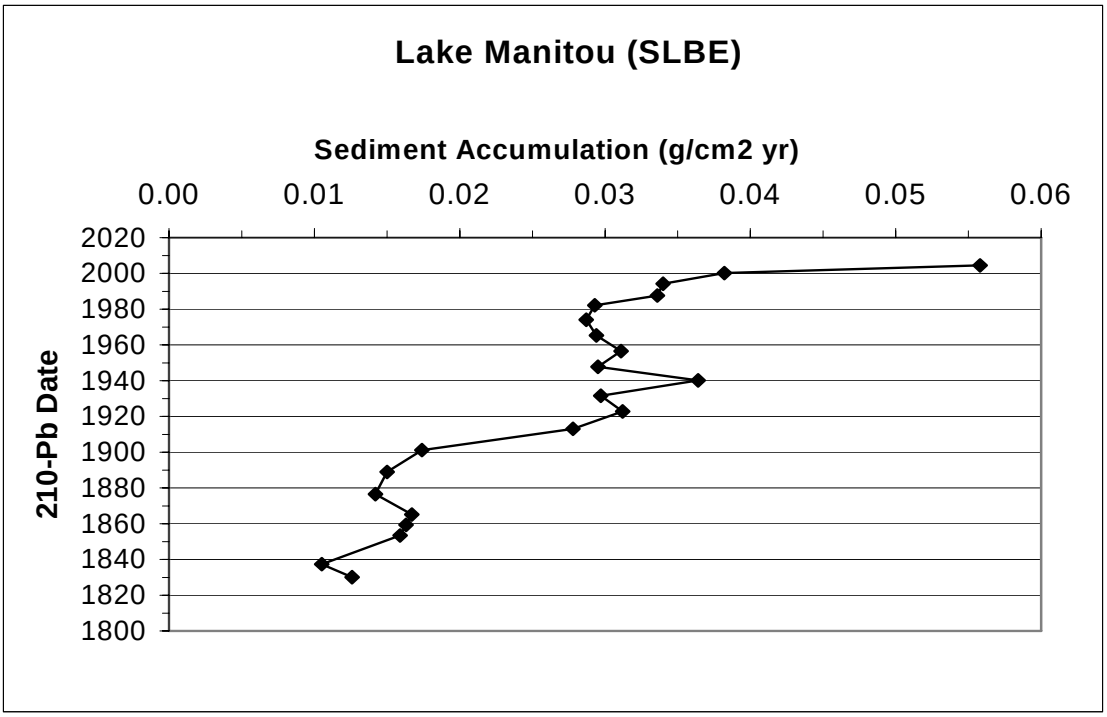

Figure 1. Sediment accumulation rate over time at Lake Manitou, Sleeping Bear Dunes National Lakeshore.

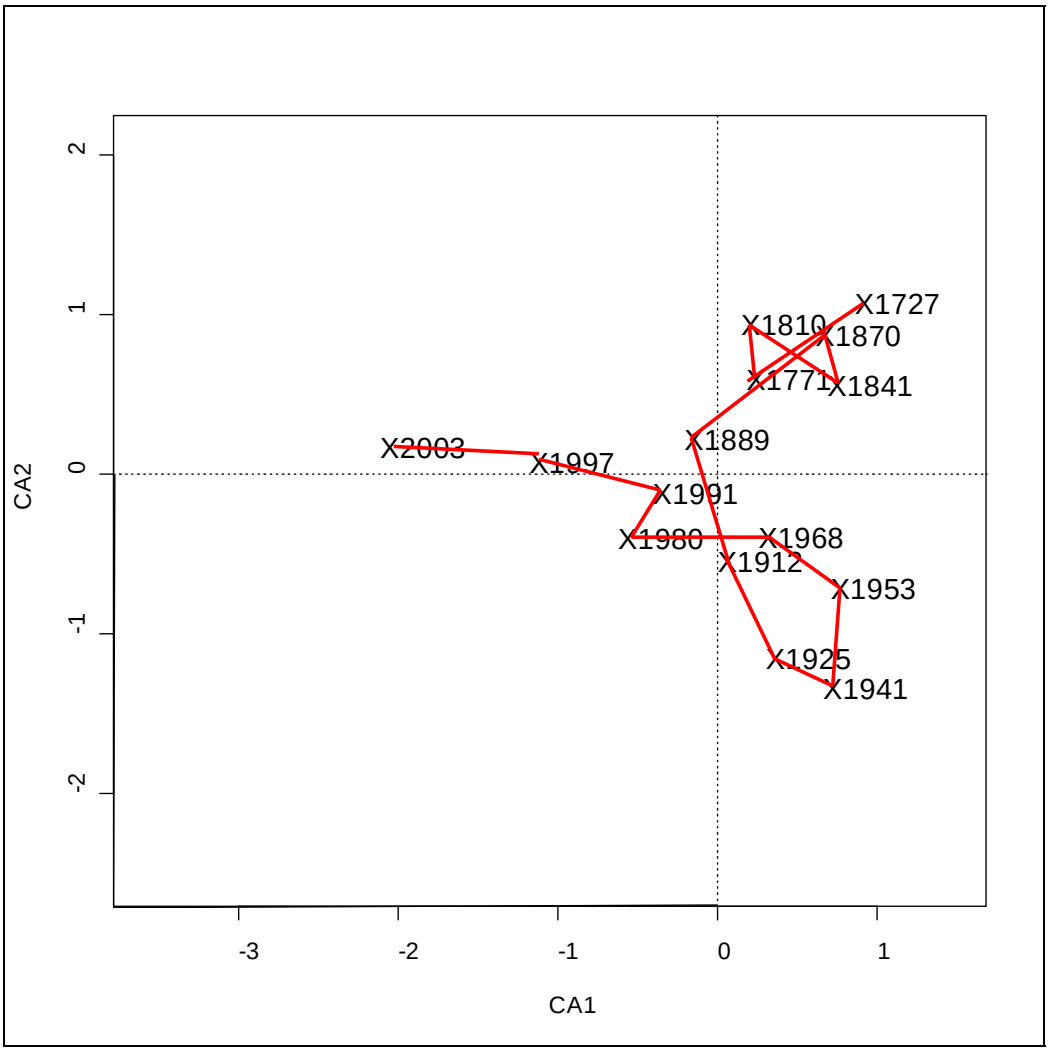

Figure 2. Correspondence analysis of downcore samples from Shell Lake (Sleeping Bear Dunes National Lakeshore) based on diatom species assemblages, from sediments dating from 1727 to 2003.

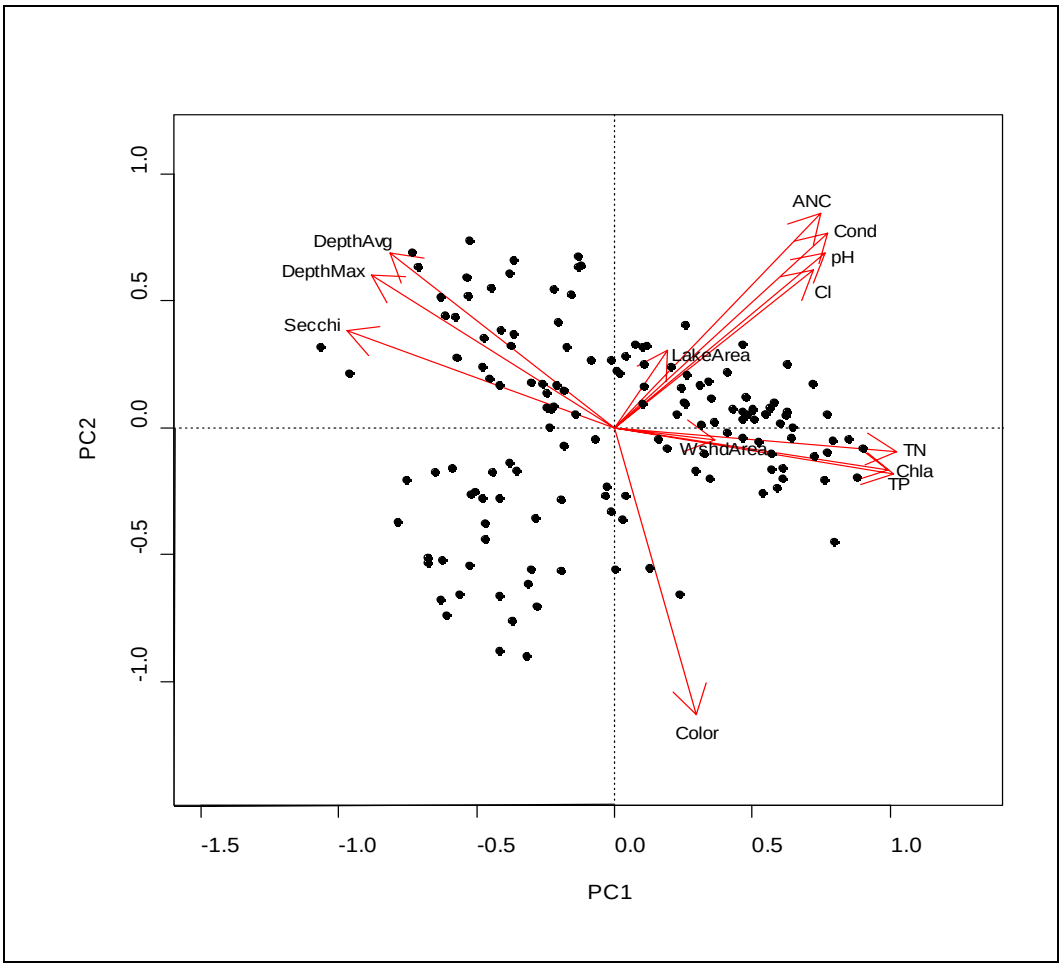

Figure 3. Principal components analysis of environmental data from 145 Minnesota lakes.

4.7 Data Archival Procedures

Data archiving serves two primary functions: it provides a source to retrieve a copy of any dataset when the primary dataset is lost or destroyed, and it provides a data record that is an essential part of the QA/QC process. The cooperators will house the original copies of the diatom count data and will provide copies to the Network.

Both freeze-dried sediment samples and diatom slides will be archived (refer to SOP #8 [Archiving] for details). Researchers will coordinate with each park unit to decide where the collections for each park will be housed. Representatives from each park will also work with researchers to ensure that the archived materials are entered into the appropriate NPS database.

5.0 Personnel Requirements and Training

The requirements of GLKN personnel and cooperators differ; both will be addressed in the following section.

5.1 Roles, Responsibilities, and Qualifications

Field personnel for the GLKN water quality monitoring project will collect the surficial sediment samples. Qualifications of GLKN field staff are detailed in the protocol for monitoring water quality of inland lakes (Elias et al. 2008).

The project manager (GLKN aquatic ecologist) will oversee all aspects of the NPS involvement with the diatom monitoring project. Specific responsibilities of the project manager will be to:

- Hire and train field personnel
- Coordinate field logistics with park staff
- Administer the contract with cooperators for diatom analysis
- Collaborate closely with cooperators on data summaries, reports, and publications
- Ensure collaborators receive water quality monitoring data in a timely manner
- Work with Network data manager to ensure data are entered into NPSTORET, verified, and validated
- Ensure parks and other interested parties are informed of project results

The GLKN will contract with an organization or institution experienced with all aspects of diatom identification, enumeration, and analyses of species assemblages. The cooperators should have the following specific qualifications:

- Experience in multivariate data analysis and paleoecology
- Experience using lake sediment records to explain long-term environmental change, including the effects of human activities on water quality, atmospheric chemistry, and biogeochemical processes
- Extensive experience in diatom taxonomy
- Facilities that include:
    - Research-grade light microscopes with bright field and DIC optics, 875-1200X magnification, numerical aperture (N.A.) >1.3, and image capture
    - Computing facilities with software for multivariate statistical analysis
    - Analytical laboratory
    - Extensive literature on diatom taxonomy
    - Diatom reference collection or stated intent to use specific diatom herbarium (or herbaria) for checking collected specimens against voucher specimens

Specific requirements of the cooperators may vary with the contract language, but in general the cooperators will be responsible for:

- Hiring and training of laboratory and analytical personnel
- Processing sediment samples
- Enumerating and analyzing diatom communities
- Collaborating with GLKN on data summaries, reports, and publications
- Providing raw data to GLKN in a timely manner

- Collaborating with GLKN to ensure parks and other interested parties are informed of results

6.0 Operational Requirements

6.1 Annual Workload and Field Schedule

Surficial sediment samples will be collected annually by GLKN field crews, on a rotational basis across parks such that sampling is repeated on a given lake approximately every 5 years. Approximately 10 sites will be sampled each year. Because the sampling will be conducted during the final round of water quality sampling for the season, little additional field time or travel by GLKN staff is required.

The GLKN project manager will provide cooperators with raw and summarized water quality data in a timely manner and will collaborate on annual reports. The project manager will ensure parks and other interested parties are informed of progress and results. The cooperators will process the sediment samples collected by NPS personnel each year, identify the diatoms, and analyze the species assemblages relative to the GLKN-MN calibration set. Changes in species since the last sampling will be related to changes in water quality. Annual reports will be prepared in collaboration with the Network aquatic ecologist.

6.2 Facility and Equipment Needs

Equipment required by GLKN for collection and storage of sediment samples consists of the following:

- Gravity corers (Wiegner corer), two or more to ensure availability at all parks
- Sample containers
- Container labels
- Pipettes
- Refrigerator space for storing samples

The collaborators will need all laboratory supplies and equipment required for processing of sediment samples, preparation of microscope slides, identification and enumeration of diatoms, and computing facilities with software for multivariate statistical analysis.

6.3 Startup Costs and Budget Considerations

The costs of collecting surficial sediment samples for diatom analysis are minimal if combined with the routine water quality monitoring. Because the Network will make use of water quality monitoring personnel, no additional staff costs are anticipated. Similarly, no additional travel costs are anticipated. The equipment required for collecting the samples is minimal, consisting of a gravity corer, specimen cups, pipettes, and labels. The Network currently has two gravity corers, which cost approximately $1200 each. We expect these corers to remain functional for many years, with annual costs consisting of consumables amounting to approximately $200.

The majority of the costs will be associated with a contract with the cooperators for processing samples, microscopy, analysis, report-writing, supplies, and travel. Because the Network will request bids from interested contractors, the exact cost is not known. However, we expect the cost of the contract to be approximately $15-20,000 annually.

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Standard Operating Procedures

SOP #1: Pre-season Preparation
SOP #2: Training and Safety
SOP #3: Using a GPS
SOP #4: Field Methods
SOP #5: Cleaning Sediment Samples
SOP #6: Preparation of Diatom Slides
SOP #7: Analysis of Slides
SOP #8: Archiving Sediment and Diatom Slides
SOP #9: Data Analysis
SOP #10: Reporting
SOP #11: Post-season Procedures
SOP #12: Quality Assurance and Quality Control
SOP #13: Procedure for Revising the Protocol

Standard Operating Procedure #1: Pre-Season Preparation

Version 1.0

In **Diatom Monitoring Protocol**

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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 will promptly notify the project manager and/or the Great Lakes Network (GLKN) 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 footer of the document file. For complete instructions, please refer to Revising the Protocol, SOP #13.

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Acknowledgements

This standard operating procedure is based on SOP #1, Pre-season Preparation, by Axler et al. (2008), from the Water Quality Monitoring Protocol for Inland Lakes (Elias et al. 2008).

1.0 Introduction

Prior to the field season, many preparations must be completed to ensure sampling can be undertaken according to schedule. The field season for collecting surface sediment samples for diatoms from the Great Lakes Network parks is generally late summer/early fall. Coordination with personnel from the inland lakes water quality monitoring program will be necessary because surface sediment samples will be collected during the final inland lakes monitoring of the year. All details for the season should be planned well in advance. Field preparations should begin in January to allow enough time to order new supplies and equipment, as needed. Table 1 provides a checklist and general guidance for activities conducted prior to sampling. Many of these activities are discussed in more detail in other SOPs.

Table 1. Checklist of activities to be conducted prior to collecting surface sediments for diatom analysis.

	Pre-Sampling Activities Checklist.	Date:
	Prepare calendar of planned field trips	Includes sampling dates, locations, and personnel. It will be necessary to coordinate with personnel from the inland lakes water quality monitoring program.
	Review sampling methods to determine if revision is needed	Check web reference to see if method has been updated from version currently used
	Review checklists of equipment and supplies required; prepare list of items to be ordered	Check each SOP for detailed equipment lists; check expiration dates of chemicals
	Order supplies	Includes specimen cups, centrifuge tubes, chemicals for sediment sample cleaning, microscope slides and coverslips, slide mounting medium
	Clean and test equipment; charge or replace batteries as needed	Includes surface sediment corer, camera, GPS unit, depth finder, cell phone or radio
	Obtain permission for site access, if necessary	
	Confirm research and collection permits for in-park sites	
	Check field vehicle and boat for safety equipment and supplies	Includes flares, flashlight, extra specimen cups, etc.
	Update field folder	Include maps, site information, field forms, sampling procedures
	Prepare headers on field data forms, and specimen cup labels	Header information should be cross-checked with metadata to permit entry into NPSTORET
	Review sample collection, processing, and documentation information	Includes methods, sample collection and processing procedures, and quality control samples
	Make travel reservations and arrangements as needed	
	Provide supervisor with field trip and call-in (check-in) schedule	

1.1 Read the Entire Protocol

Periodically read through the entire protocol, including all standard operating procedures (SOPs). Be sure to understand the purpose for which the various types of data will be collected and review the SOPs for the types of measurements and samples needed. Be alert for portions of the protocol or SOPs that may be in need of revision, and bring them to the attention of the appropriate supervisor.

1.2 Prepare Calendar of Planned Field Sampling

Well in advance of the field season, prepare a calendar of sampling dates for the entire season. Coordinate with personnel from the inland lakes water quality monitoring program because the surface sediment sampling for the diatom monitoring will be done during the last round of water quality sampling of the year. Allow for the possibility of unsuitable weather; sampling may have to be postponed. Include the location of sampling, dates, personnel, and any additional relevant notes (Table 2).

Table 2. Example of calendar of planned field sampling.

Location	Sampling Dates	Personnel	Notes
Indiana Dunes NL	September 15-20	Elias, Wise	
Voyageurs NP	October 1-5	Nauertz, Elias	

1.3 Review Checklists of Equipment and Supplies

Checklists help ensure that equipment and supplies will be ordered on time, data collection activities will be completed appropriately, and data quality objectives will be met. Review the detailed equipment lists that are included with each SOP (Table 3). Note expiration dates on all chemicals. Prepare a list of equipment and supplies that must be ordered and present it to the project manager.

Table 3. Checklists of equipment and supplies for diatom monitoring.

Checklist	Location
Safety equipment	SOP #2
Field supplies and equipment	SOP #4
Laboratory equipment and supplies	SOPs #5 and 6

1.4 Confirm/Apply/Renew Research and Collecting Permits

For sampling stations located within park boundaries, a Research and Collecting Permit must be obtained from each park separately before field work can be done. Submit an application or

renewal application online to: http://science.nature.nps.gov/research/ac/apps/appInstructions. Each park has a research coordinator who will issue a permit from that park. For samples that will be archived, curatorial paperwork may be required by the parks. Work with the appropriate park representative to ensure all necessary archival forms are completed.

1.5 Update Field Folder

Field folders should contain reference information relevant to each sampling station, including maps of site locations and sampling collection and processing procedures. The field folder should be taken along on each sampling trip. Each year, prior to the sampling season, the field folder for each site should be reviewed and the following information updated as needed:

- *Location of sample-collection sites.* Review field notes for any indication that the location for sample collection may need revision. Update protocol if necessary.

- *Name of landowner, tenant, or other responsible party.* If the site is located on private land, ownership may change. Verify.

- *Current copy of research and collection permits (if site is located within NPS boundaries).* Check dates on permits. Renew/apply as described above.

- *Site access instructions (for example, call owner or site operator before arrival at site, obtain key to unlock security gate).* Confirm contact person, procedure, and phone numbers.

- *Photographs to document site conditions.* Take new digital photographs annually.

- *Maps to site (state and local).* Review map for accuracy; update if necessary.

- *Safety information (SOP #2).* Verify/update "Medical Information Form for Field Personnel" and "Emergency Contact Form."

- *Field forms, including data collection field forms and specimen cup labels.* Prepare as much of the field forms as possible in advance (see SOP #4, Appendix A for a field data sheet). For each station, complete the header information, including the park code, lake name, date, etc. Place enough field forms in field folder to last the entire field season.

- *Ensure that copies of the current field procedures are included in the field folder.* Copies of procedures should be on waterproof paper. Use tabs to identify each procedure for ease of access while conducting field activities.

1.6 Clean and Test Equipment

Clean and test all sampling equipment, including surface corer, depth finder, camera, and GPS unit. Start each new field season with fresh batteries and replace spares from the field tool kit.

1.7 Vehicle, Boat, and Safety Gear

Check maintenance schedule of field vehicle and arrange maintenance, if needed. Check boats and vehicle for safety equipment such as flares, spare tires, triangles, cones, and first aid kit. Prepare a list of supplies needed and present list to supervisor. If using a trailer, ensure that tail-lights are in working order. Check that the field tool-kit is complete and replace tools, as needed.

1.8 Training and Safety

Keep current with training and the laboratory requirements associated with your data collection activities. New technicians will need basic skills training, including hands-on training and pilot-testing of equipment.

1.9 Field Reconnaissance

Field reconnaissance trips will not be necessary because water quality sampling will have occurred at least twice at each site before the surface sediment sample is collected. Be sure to check with personnel from the inland lakes water quality monitoring program to get information on boat access, road or trail closings, and any site access peculiarities.

1.10 Travel Arrangements

Make travel arrangements. Because hotel and campground reservations may be difficult-to-impossible to obtain at certain times of the year, it is important to review the sampling schedule and plan ahead. Submit park housing requests well in advance of the sampling season.

1.11 Communicate with Supervisor

Ensure the project manager is informed of supply needs, problems with equipment, changes in sampling schedule, changes in sampling site conditions, and other needs that may have an impact on the program budget, data collection, schedule, or sampling design.

1.12 Literature Cited

Axler, R., E. Ruzycki, and J. E. Elias. 2008. Standard operating procedure #1: Pre-season preparation. *In* Elias, J.E., R. Axler, and E. Ruzycki. 2008. Water quality monitoring protocol for inland lakes, Version 1.0. National Park Service, Great Lakes Network, Ashland, Wisconsin. NPS/MWR/GLKN/NRTR—2008/109. National Park Service, Fort Collins, Colorado.

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Standard Operating Procedure #2: Training and Safety

Version 1.0

In **Diatom Monitoring Protocol**

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Acknowledgements

This SOP is based on SOP #2, Training and Safety, by Elias 2008, from the Water Quality Monitoring Protocol for Inland Lakes (Elias et al. 2008).

The safety related portions of this standard operating procedure were adapted from the Greater Yellowstone Network's SOP on safety (O'Ney 2005), with heavy reliance on the U.S. Geological Survey's manual for collecting water quality data (Lane and Fay 1997).

2.0 Introduction

Prior to collecting surface sediment samples in the field, the field crew members must be trained in the techniques and procedures that will be used throughout the season. Familiarity with the protocol and standard operating procedures (SOPs), as well as with boats, equipment, and basic safety standards, are critical to the success of the diatom monitoring program. The crew must be trained in specific safety procedures to ensure their safety and that of others.

The project manager will conduct or arrange for all necessary training prior to the field season.

2.1 Pre-Season Classroom Training

It is desirable to begin training well before the field season begins to allow adequate time for thorough understanding of field and laboratory procedures.

2.1.1 Understanding the Protocol and Standard Operating Procedures

Reading and understanding the entire protocol and all SOPs are crucial prior to initiating field work. The project manager will allow adequate time for all field crew members to complete this step to ensure success of the project. Field and laboratory related SOPs will also be covered as part of the hands-on training, described below.

2.1.2 First Aid and Cardio-Pulmonary Resuscitation (CPR)

Training in the basic medic/first aid and Heartsaver AED, which includes CPR and use of an automated external defribrillator (AED), is required for all crew members and will be paid for by the Network. Acceptable training should be through the American Red Cross or American Heart Association. Certification is valid for two years. Training and certification should be acquired prior to the field season.

2.2 Hands-On Training

A variety of hands-on training and practice prior to the first sampling period will help ensure high quality sample collection. Familiarity with the use and maintenance of equipment, procedures for collecting and processing sediment samples, techniques for cleaning field and laboratory equipment, and safe use of watercraft are essential to the success of the diatom monitoring project. Field crew leaders are required to complete all of the following training; other field crew members should also complete the training, if possible, although it is not required.

2.2.1 Use of the Wiegner Corer

The Wiegner corer is used for the collection of a surface sediment sample. Refer to SOP #4 for instructions on use and maintenance of the corer.

Crew members will have the opportunity to practice the skills learned prior to the actual sampling until they are comfortable with the use of the corer.

2.2.2 Use of a Global Positioning System (GPS)

Location information must be gathered via GPS for each site during each visit. Training in the use of a GPS will include navigation to a known location, acquiring location information, storing data, and downloading data. Details on the use of a GPS can be found in SOP #3.

2.2.3 Field Data Sheets

In addition to collecting a surface sediment sample, a field data sheet must be completed. Prior to the field season, the field crew will receive training in how to properly complete the field data sheet.

2.2.4 Boat Training

Prior to operating a NPS boat or canoe, training and certification are required. Since the diatom sampling will occur in conjunction with the last inland lakes water quality sampling of the year, the crew leader for the inland lakes project must receive the training and obtain certification; it is highly desirable for the remaining crew members to do so as well.

2.3 Safety Procedures

Safety of field personnel should always be the first concern in conducting a sampling program and in the selection of sampling sites. Numerous safety issues and concerns are associated with implementing a program that includes fieldwork and sampling. Field personnel routinely come into direct and indirect contact with waterborne pathogens, chemicals, and potentially hazardous plants and animals. Fieldwork requires an awareness of potential hazards and knowledge of basic safety procedures. Advanced planning can reduce or eliminate many safety hazards.

2.3.1 USGS Field Manual

This SOP is meant to be used in conjunction with Chapter A9 of the USGS National Field Manual (Lane and Fay 1997), which contains more complete information about potential hazards that personnel may encounter during fieldwork in aquatic environments. The procedures in Chapter A9, when implemented properly, will help ensure the safety and health of field crew members. A copy of this manual is provided to the field crew and may be downloaded from http://water.usgs.gov/owq/FieldManual/Chap9/content.html and taken to the field as a reference. Topics addressed in the USGS document include:

- General references for federal policies and Department of Interior (DOI) safety guidelines
- Safety policies you are required to know and follow under the Occupational Safety and Health Act (OSHA), Environmental Protection Agency (EPA), and Department of Transportation (DOT)
- Understanding and implementing a job hazard analysis (JHA)
- Requirements related to the use of personal protective equipment (PPE) on the job
- Safety training and certification requirements
- Safety issues associated with transportation and operation of vehicles (road vehicles and trailers, watercraft, aircraft, etc.) used to reach sampling sites
- Surface water activities (e.g., wading, working from bridges, boats and cableways, etc.)
- Working around machinery, pumps, and other equipment

- Proper use, handling, transport, storage, and disposal of chemicals
- Handling of contaminated water and limiting exposure to yourself and others
- Environmental conditions caused by extremes in temperature; sun exposure; threats posed by storms, floods, fire, snow, ice, and various animals and plants

In addition to consulting the USGS manual, the field crew should contact individual park's safety officers or resource managers for information on park radio safety procedures and local problems and issues, such as dangerous or nuisance animals (e.g., black bears at VOYA, red fox at ISRO), insect- and tick-borne diseases (e.g., Lyme disease, encephalitis, West Nile disease), and other issues specific to each park.

2.3.2 Basic Safety Preparation

Basic preparations should become routine before every sampling activity. At a minimum, complete a trip plan for each field trip, and leave it at the designated location in the office. The trip plan should include the following information:

- Field trip participants, including guests and observers, with emergency contact information
- Departure and expected return time(s) and date(s)
- Hotel and campground contact information (for overnight trips)
- Basic itinerary, including where and when sampling will occur
- Phone numbers for cellular phones or radio frequencies

Fieldwork should be done in pairs. Always carry a park radio, or if reception is known to be available, a cellular telephone. Carry basic safety equipment, including a first aid kit, flashlight, boots, rain gear, antibacterial soap or hand cleaner, matches or lighter, etc. Be aware of changing weather conditions and the potential for storms. Be aware of potential hazards at a monitoring site. Carry general safety information in each vehicle or boat, including:

- Basic first aid protocols
- Emergency phone numbers
- Locations of emergency facilities (hospitals, police and fire departments, U.S. Coast Guard)
- Maps of the park, surrounding area, and nearest city

2.3.3 Medical Forms and Safety Equipment Checklists

The following pages contain medical forms and equipment checklists for field personnel (adapted from Lane and Fay 1997). Prior to the field season, complete as much of the medical information as possible. Confirm all contact information annually. Medical information sheets should be completed for each individual venturing into the field.

Checklists are helpful for ensuring that personnel have the appropriate safety equipment available during field trips. Field crew members should consider their specific needs and should customize the checklists as necessary. The field crew and project manager will discuss the checklists and determine which items are necessary.

Emergency Contact Form for: (name)_____

Emergency Contacts

#1 Name: _____ Relationship: _____

Phone: (home) (work) _____

#2 Name: _____ Relationship: _____

Phone: (home) (work) _____

Great Lakes Network Contacts

Network Office _715-682-0631 x25___
Apostle Islands _____
Grand Portage NM _____
Indiana Dunes NL_____
Isle Royale NP _____
Mississippi NRRA _____
Pictured Rocks NL _____
St. Croix NSR _____
Sleeping Bear Dunes NL _____
Voyageurs NP _____

Local Emergency Contacts (or call 911)

Hospital Phone: _____

Address: _____

Other medical facility (24-hour care) Phone: _____

Address: _____

Police: _____

Fire: _____

Utility: _____

Health Information Centers

Centers for Disease Control: _____

Information Hotline: _____

Other: _____

Medical Information Form (retain in office)

Employee name: _____ Home phone: _____

Treatment preference: medical _____ other (specify) _____

Doctor: _____ Phone: _____

Other emergency contact: _____ Phone: _____

Allergies and other medical conditions	Medications being taken	Medications to avoid

Relevant medical history:

Special instructions:

General Safety Equipment Checklist

√	**Basic Safety Equipment Checklist**
	Waders, hip boots, rubber knee boots
	Personal flotation device (PFD)
	First aid kit
	Fire extinguisher
	Flashlight and spare batteries
	Park radio and cellular phone
	Rain gear
	Hat, sunscreen, and sunglasses
	Drinking water or sports drinks
	Tool box with basic tools
	Antibacterial soap or hand cleaner
	List of emergency phone numbers and office contacts

Personal Protective Equipment Checklists

Personal Protective Equipment (PPE) must be selected based on the hazards likely to be encountered. The Great Lakes Network is required to supply appropriate PPE, and field personnel are required to use it.

√	**Chemical and disease protection**
	Aprons
	Eye/Face splash guards
	Gloves (vinyl and/or latex or nitrile)
	Protective suits
	Respirators (certification required for use)

√	**Weather and UV protection**
	Boots
	Fluids (*e.g.*, water, sports drinks)
	Hat with a brim
	Insect repellent
	Rain gear
	Sunglasses
	Sunscreen
	Temperature-modifying clothing
	Work gloves

√	**Flotation and reflective protection**
	Orange flotation vests and jackets
	Safety harness

√	**Protection for working around boat motors**
	Hearing protection

Checklists for Vehicles

√	**Communications and Instructions**
	Field folder (including maps, emergency phone numbers for medical facilities, office contacts, family contacts)
	Cellular phone/communication equipment (check that the service is operational for the area to be traveled)

√	**First aid and protective equipment**
	Complete change of clothes (stored in dry area)
	Fire extinguisher (safely secured)
	First aid kit and manual (check for missing or old, expired items and replace if necessary)
	Orange reflective vest

√	**Miscellaneous equipment**
	Bungie cords (to secure loose articles)
	Flagging
	Flares
	Flashlight (including fresh batteries)
	Flexible hose (to vent exhaust away from vehicle)
	Safety cones
	Tool kit
	U.S. Geological Survey TWRI Book 9 Chapter A9

Watercraft Checklists

√	**Instructions and navigation**
	Field folder, with sampling plans
	Charts and maps
	Compass
	Depth finder
	Dead-man's switch
	Navigation lights

√	**Distress and external communication**
	Radio (VHF, AM, FM, and WEATHER)
	Special lighting/flagging (if boat activities might pose a hazard to the public, such as tag line measurements)
	Visual distress signals (Coast Guard approved)
	Whistles or horns
	Type IV throwable rescue device
	Personal flotation devices for each passenger (Coast Guard approved)
	Anchor and lines (spare)
	Bucket for use as a bailer
	Paddle (extra paddle for each canoe or rowboat)
	First aid kit (Coast Guard approved)
	Flashlights and batteries
	Fire extinguishers
	Spare parts (anchor, fuel, propeller, extra lines, cotter pin, starter cord)
	Tool and repair kits
	Extra clothes (hat, foul-weather gear)
	Food and water
	Sunscreen
	Conversion factors and abbreviations

2.4 Literature Cited

Elias, J. E. 2008. Standard operating procedure #2: Training and safety. *In* Elias, J. E., R. Axler, and E. Ruzycki. 2008. Water quality monitoring protocol for inland lakes, Version 1.0. National Park Service, Great Lakes Network, Ashland, Wisconsin. Natural Resources Technical Report NPS/MWR/GLKN/NRTR—2008/109. National Park Service, Fort Collins, Colorado.

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Standard Operating Procedure #3: Using a GPS

Version 1.0

In **Diatom Monitoring Protocol**

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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 will promptly notify the project manager and/or the Great Lakes Network (GLKN) 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 footer of the document file. For complete instructions, please refer to Revising the Protocol, SOP #13.

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Acknowledgements

This standard operating procedure is adapted from SOP #3, Using the Global Positioning System, by VanderMeulen, D., and M. Hart (2008), in the Water Quality Monitoring Protocol for Inland Lakes (Elias et al. 2008).

3.0 Introduction

This Standard Operating Procedure (SOP) provides guidance on some of the more common operations associated with global positioning systems (GPS) units. Although most GPS units are capable of multiple functions, only those operations relevant to the diatom monitoring protocol are discussed.

GPS units currently (2007) used by water quality monitoring staff are the Trimble GeoXT and Garmin 76S. The Trimble GeoXT is an example of a mapping-grade GPS unit, while the Garmin 76S is an example of a recreational-grade unit. Mapping-grade units record data with location accuracy ranging from sub-meter to less than five meters. Recreational-grade units generally are not as accurate, with spatial accuracy less than 15 meters. Mapping-grade units have greater ability to capture spatially referenced metadata (i.e., attributes) than recreational-grade GPS. Recreational-grade GPS units are sufficient for diatom monitoring; therefore a discussion of mapping-grade units is not included in this protocol (see Vandermeulen and Hart (2007) for a detailed discussion of mapping-grade units).

Due to the rapid development of commercial software and hardware capabilities, it is likely that other GPS units or software will be utilized in the future. Therefore, this SOP is meant to act as a working document that is updated periodically as new technology becomes available. Although nomenclature may differ depending on what hard-software is utilized, this document should provide sufficient guidance on the general process of data collection using GPS tools until the SOP is revised. It is strongly recommended that water quality monitoring staff obtain unit-specific GPS training prior to deploying in the field. The training should include hands-on use, and should be designed to test all appropriate functions and operations prior to going out into the field.

Additionally, this SOP is not intended to be exhaustive or simply a regurgitation of operating manuals, but a document that might be carried into the field or periodically reviewed by field technicians and project leaders. The objective of this document is to summarize GPS use guidelines applicable to the diatom monitoring efforts for lakes in GLKN parks.

3.1 Role of GPS in Collecting Samples for Diatom Analysis

GPS units are primarily used in the following ways to support diatom monitoring on lakes:

1) For diatom monitoring on lakes, navigating to and sampling at the position location of previously determined sampling sites is accomplished through a combination of:
    a. Using GPS units to navigate to previously established GPS point locations
    b. Using hardcopy maps and written notes with site descriptions (where available)
    c. Field experience of NPS staff conducting the monitoring
2) Accurate station location descriptions must be recorded and carefully followed by sampling personnel on subsequent field visits. Therefore, once at the sampling location, GPS equipment is used to obtain the sampling coordinates, which allows the user to spatially reference diatom monitoring data to specific geo-referenced locations.

Note that the diatom monitoring efforts will be coordinated with the final field sampling of the season for the inland lakes water quality monitoring program. Therefore, the field crew from the inland lakes monitoring project will have already visited the sites and will be able to assist with navigating to the correct sampling location.

3.2 Using Recreational-Grade GPS Units

Recreational-grade GPS units can be used to acquire location information (generally points) when spatial accuracy is not paramount to the project. Recreational GPS units do not have data dictionaries for storing attribute information with the point location. However, using a recreational-grade unit to capture a waypoint at each sampling site is a reliable means to verify the correct sampling site has been reached, even if a GPS location is not needed.

Personnel that employ recreational-grade GPS units should become familiar with GLKN GPS collection procedures and relevant manufacturer's user guides and operating manuals before GPS operation. For example, prior to using a Garmin 76S (recreational-grade) GPS unit, the following documents should be reviewed:

- GPSMAP 76S Quick Start Guide
- GPSMAP 76S Owner's Manual and Reference Guide
- Garmin MapSource™ User's Manual and Reference Guide

The appropriate user guide and operating manual should be taken into the field during sampling in case the field crew needs to refer to them.

Planning

The sediment sample for diatom analysis will be collected at the same location and at the same time as the final water sampling of the season for the inland lakes monitoring program. Enter the site location coordinates onto a GPS unit prior to departure to the field and use the GPS to navigate to the site. Some recreational grade GPS units have the ability to store and display topographic maps that can aid in navigation. Printed topographic maps of the waypoint locations can also be used to maximize field time and efficiently navigate between waypoints.

Data Collection

Location data are captured by recreational-grade GPS units as *waypoints*. When taking a waypoint, enter the site ID or site designation in the text field provided. Collect reference points at regular intervals as good practice. These reference point positions should be taken at known locations (e.g., trailheads, parking lots, stream confluences) which can later be used in GIS to check the accuracy of waypoint data. Write the site location coordinates on the field data sheet as a backup, even if the data will be downloaded electronically.

3.3 QA/QC

Long-term monitoring is only valuable if users have confidence in the data. Efforts to detect trends and patterns in ecosystem processes require high-quality, well-documented data that minimize error and bias. Data of inconsistent or poor quality can result in loss of sensitivity and lead to incorrect interpretations and conclusions.

NPS Director's Order #11B: Ensuring Quality of Information Disseminated by the National Park Service (www.nps.gov/policy/DOrders/11B-final.htm) specifies that information produced by the NPS must be of the highest quality and based on reliable data sources that are accurate, timely, and representative of the most current information available. Therefore, GLKN will establish and document procedures for quality assurance (QA) and quality control (QC) to identify and reduce the frequency and significance of errors at all stages in the data life cycle. Under these procedures, the progression from raw data to verified data to validated data implies increasing confidence in the quality of those data. Quality assurance and quality control procedures will document internal and external review processes and include guidance for addressing problems with data quality.

Examples of general QA/QC practices include:

- Standardized field data collection forms
- Proper calibration and maintenance of equipment
- Training of field crew and data technicians

Examples of QA/QC practices pertaining to the use of a GPS unit include:

- Ensure that GPS-related software is periodically updated as it becomes available and has been tested.
- Record location data on field data forms as well as with the GPS unit.
- For each monitoring station, compare location positions for different sampling events, including the position recorded during establishment of the monitoring station. This will allow for an assessment of position accuracy over time.
- Ensure that the appropriate coordinate system is used when collecting and exporting data.

3.4 Literature Cited

VanderMeulen, D., and M. Hart. 2008. Standard operating procedure #3, Using the Global Positioning System. *In* Elias, J. E., R. Axler, and E. Ruzycki. 2008. Water quality monitoring protocol for inland lakes, Version 1.0. National Park Service, Great Lakes Network, Ashland, Wisconsin. Natural Resources Technical Report NPS/MWR/GLKN/NRTR—2008/109. National Park Service, Fort Collins, Colorado.

Elias, J. E., R. Axler, and E. Ruzycki. 2008. Water quality monitoring protocol for inland lakes, Version 1.0. National Park Service, Great Lakes Network, Ashland, Wisconsin. Natural Resources Technical Report NPS/MWR/GLKN/NRTR—2008/109. National Park Service, Fort Collins, Colorado.

Standard Operating Procedure #4:
Field Methods

Version 1.0

In **Diatom Monitoring Protocol**

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Acknowledgements

Portions of this standard operating procedure were based on or modified from SOP #6, Procedures for Field Water Column Measurements and Water Sampling, by Axler et al. (2008), from the Water Quality Monitoring Protocol for Inland Lakes (Elias et al. 2008).

4.0 Introduction

Experience with and knowledge of the sampling equipment and the collection and storage of sediment samples for subsequent laboratory analysis is critical for collecting data of high quality. It is highly recommended that new personnel receive training on the operation of the Wiegner corer from an experienced technician. To ensure that consistent, high quality samples are collected, always:

- Maintain a permanent log book for each field instrument for recording problems and repairs. Review the log book before leaving for the field.
- Test each piece of equipment before leaving for the field.
- Follow quality assurance/quality control procedures. Such protocols are mandatory, and include practicing good field procedures and implementing quality-control checks.

4.1 Where and When to Sample

4.1.1 Where to Sample

Sampling locations for the diatom monitoring program will follow the sites selected for the GLKN water quality monitoring program (Elias et al. 2008; the deepest part of the lake is being used as the primary sampling site). Therefore, the GPS location of the water quality monitoring sample site for each lake, as well as the depth at each site, must be obtained before going into the field.

When on the lake, use the GPS in conjunction with the handheld depth finder to navigate to the appropriate location in the lake basin (see SOP #3 Using a GPS).

4.1.2 When to Sample

Sampling for the diatom monitoring program will be conducted in conjunction with the GLKN water quality monitoring program. Collect sediment samples during the final water quality monitoring event of the season, which will usually be in September or October.

4.2 Equipment and Supplies

Refer to the checklist of supplies and equipment needed for field sampling (Table 1) conscientiously prior to each sampling trip. Keep on hand all necessary forms, logbooks, and field data sheets. Copy field data sheets onto waterproof paper. See Appendix A for an example of a blank field data sheet.

4.3 Field Procedures

Use the lake bathymetry map (if available) to orient yourself in the lake basin and use the GPS unit, in conjunction with the handheld depth finder, to navigate to the sampling location (see SOP #3, Using a GPS). Include a note on the water quality monitoring field data sheet when a sediment sample was collected in addition to completing a diatom monitoring field data sheet.

Table 1. Field supplies and equipment checklist required for diatom monitoring.

	Field notebook, pencils, and pen (indelible ink)
	Sharpie® or other permanent markers
	Field data sheets (with header information completed) on waterproof paper
	Specimen cups (with appropriate labels) for collection of surface sediment
	Spatulas, pipettes, Kimwipes®, zip lock bag for garbage
	Tool kit for repairing boat motor and sampling equipment
	Weather radio or barometer
	Extra batteries for all equipment (GPS, etc.)
	Rain gear
	Personal flotation device(s)
	Field trip itinerary
	Cellular phone and/or park radio
	Wiegner corer and adequate length of line
	Anchor and line
	Cooler with ice or cold packs
	Digital camera with extra flash cards, fully charged battery, and extra batteries
	Handheld depth finder
	Map(s); lake bathymetry
	Global positioning system (GPS) and user manual
	GPS coordinates of sampling location (from water quality monitoring program)

4.3.1 Operating the Wiegner Corer and Collecting a Surface Sample

The Wiegner corer falls into the broad category of gravity corers. It is lowered in the set or open position into the lake sediments until penetration stops. The line to the boat is allowed to go slack, and upon retrieval, tension on the line is maintained to create a seal on the core tube. The corer recovers approximately the top 20-35 cm of sediment. The recovered core is extruded out of the top of the core barrel using the included extruder, tools, and sampling tray (optional).

1. Take corer out of case. Locate nut driver, spatula for sampling, plastic sectioning tray (optional), and sectioning setup (the white PVC "X" on stainless steel rod). Unwrap enough line from spool to reach the lake bottom.
2. Before using the corer, loosen the clamps and remove the black rubber protective piece from the top of the corer (this is only to protect the corer during storage, and must be removed before the corer is used).
3. Additional brass plates can be added (or removed) from the corer to increase (or decrease) the weight. Increased weight is desirable when coring firm substrates.
4. Prepare the corer by opening the black stopper and propping it open behind the black rubber band. Place the two arms of the small brass clip in the grooves of the PVC sleeve. Then pull the rope taut to maintain tension on the line and clip. The corer is now in the set position (Figures 1a and b).
5. Lower the corer into the lake and continue lowering until it reaches AND penetrates the sediments. Use a controlled, moderate rate of descent to hit the sediment at about 0.5 m sec^{-1}. Do not allow the corer to free fall.

6. Once the corer has penetrated the sediments and stopped, let the line go slack. The brass clip will slide out of the grooves on the white PVC sleeve (Figure 1c), which will allow the black stopper to close. While maintaining continuous and steady tension on the line, bring the corer back to the surface. As the brass plates break the surface, reach into water and cap the bottom of the corer with the palm of your hand. Keep the corer and core vertical!
7. Bring the core and corer into the boat. Set up the sectioning mechanism as illustrated (Figure 1d), noting that the stopper is flush with the top of the gray PVC and that the small brass pin is inserted into the 3rd or 4th hole from the top of gray PVC. Slide bottom of corer across your hand and onto the black stopper. Push the corer down so the black stopper slides up into the corer until the core barrel hits the brass pin (Figure 1d). If you are using the corer alone, you will want to reach over the side of the boat with the sectioning mechanism and slide the stopper directly into the core barrel as the corer breaks the surface (skipping using your hand).
8. Take the nutdriver and loosen the hose clamp that runs around the black rubber cylinder near the top of the corer. This will allow the black rubber cylinder and the brass plates to be separated from the core tube.
9. Now you will slide the top of the sediments to the top of the core tube for sectioning. Inside one of the arms of the white PVC "X" on the sectioning mechanism is a stainless steel bar. As this stainless bar is depressed, the black stopper will slide up into the core tube and the sectioning mechanism will slide down the stainless rod. Do this slowly. Water will run out of the core tube top and eventually the top of the core will appear at the top of the core tube. Slowly align the top of the mud with the top of the core tube. If using a sectioning tray, place it over the top of the core tube.
10. Reach down and move the brass pin down one or two centimeters (depending on how much mud you want to sample). Then slide the core tube down till it hits the brass pin again. One or two centimeters of sediment will come out of the top of the core tube onto the sectioning tray. Or, if you are not using a sectioning tray, move the core flush with the top of the tube and use a pipette to remove the top 1 cm (or a pipette cut in half such that the bulb portion can be used to scoop out the top 1 cm). If this approach is used, the tube must be pre-marked at 1 cm. Then move the core flush again with the top to collect the next cm. Scrape (or scoop) the sediment into your pre-labeled sampling cup, cap, and place sample in cooler. If additional samples are desired, you can continue to section the entire core.
11. Rinse core tube and sectioning equipment when finished. Reassemble corer using nutdriver and prepare for next core.

Troubleshooting

- Make sure nylon lines threaded through the top of the corer move freely and are not wrapped around anything.
- The stopper on the sectioning mechanism can be tightened or loosened using the wingnut to get a good fit and snug travel up and down the core tube. Test the tightness prior to collecting the core.

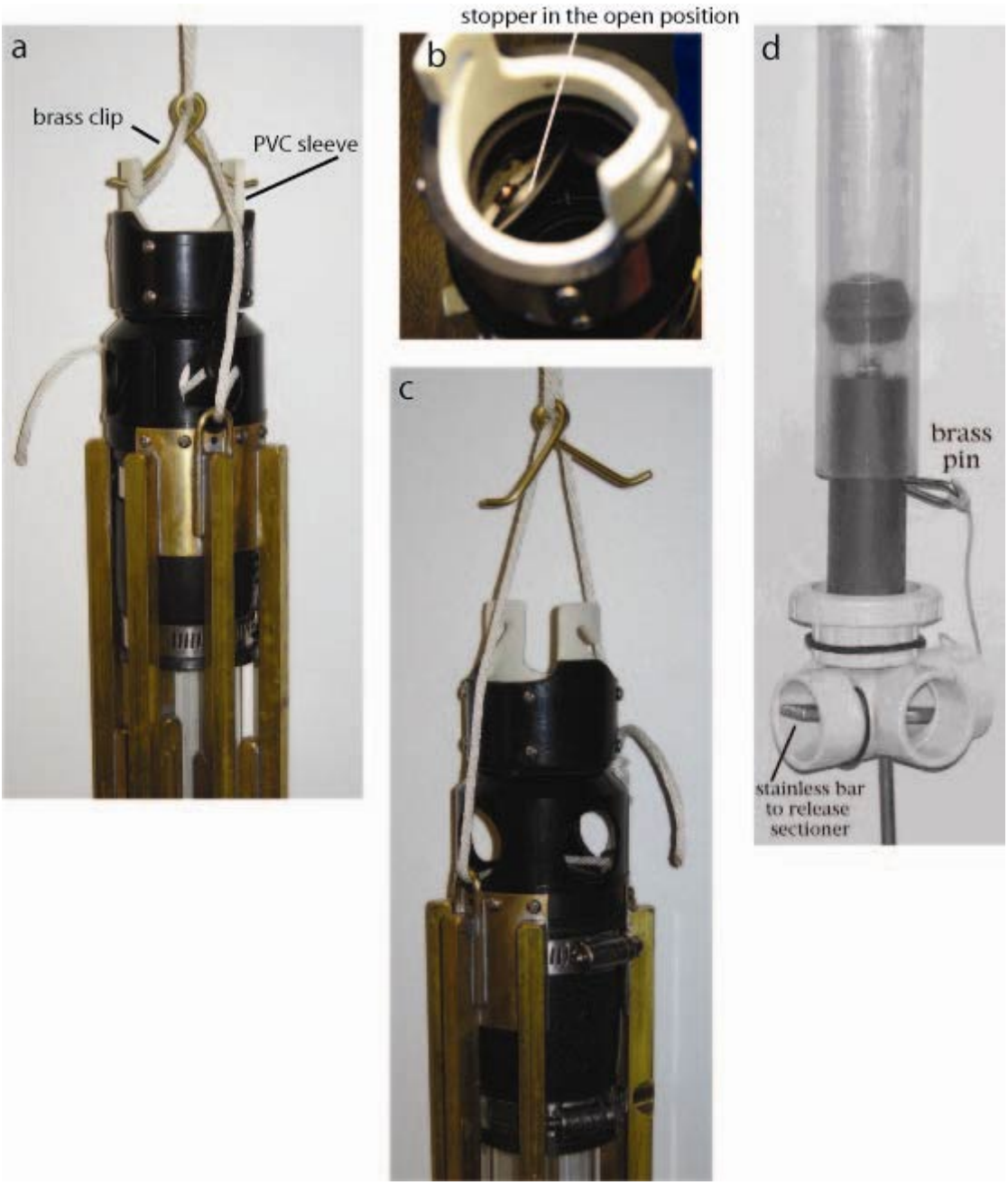

Figure 1. The Wiegner surface corer. a) The corer in the set position, ready to be deployed. b) When deploying the corer, the black stopper must be in the open position, resting against the black rubber band. c) The corer being retrieved from the sediments (brass clip has been released from the white PVC sleeve, and the line has been pulled taut, allowing the black stopper to close. d) The sectioner with the brass pin in place.

4.4 Sample Handling

Place the sediment sample in a specimen cup that is pre-labeled with the date, lake name, GLKN park unit, and the core interval collected (e.g., 0-1 cm). Use a Sharpie marker to label the lid of the specimen cup with the lake name and core interval. On the field data sheet, record the GPS coordinates of the sampling location, the water depth at the sampling location, and the core intervals collected.

Samples should be placed in a cooler while in the field and then transferred to 4°C laboratory storage. Samples should be processed within two weeks of collection.

Upon return to the office, make a photocopy of the data sheet and send the copy to the contractor with the samples. Keep the original data sheet on file at the GLKN office with other field datasheets for the same sites.

4.5 Decontamination of Equipment Between Lakes

As we implement the diatom monitoring protocol we must take precautions against transferring aquatic invasive species from one lake to another. Because the diatom sample will be collected during the final round of water quality monitoring, decontamination of diatom sampling equipment should be coordinated with that of water quality monitoring equipment. The procedures for decontamination vary by species and are likely to change over time. Consult Elias (2008) for specific details regarding decontamination requirements and procedures.

Decontamination to prevent the spread of exotic organisms will also prevent against cross-contamination of the equipment by diatom species from another site.

4.6 Quality Assurance and Quality Control

Quality assurance protocols are means to ensure data collected are as representative of the natural environment as possible. Quality assurance procedures are required in all data collection efforts as part of this monitoring protocol. Many of the key elements of quality assurance have been included in the SOPs where appropriate, as well as detailed in SOP #12. A summary of important QA/QC procedures follows.

- Field staff must be trained in the use of the Wiegner corer. They will be given ample opportunity to practice collecting cores prior to actually collecting a sample.
- Test the Wiegner corer prior to using it to collect a sample. Ensure it is functioning properly.
- All manually recorded field measurement data will be collected on field forms. Hard and electronic copies will be made as soon as possible after sampling and kept at a separate location as backup.
- Complete records will be maintained for each sampling station and all supporting metadata will be recorded appropriately.
- Clean all equipment thoroughly between sampling sites to ensure no cross-contamination.

4.7 Literature Cited

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Elias, J. E., R. Axler, and E. Ruzycki. 2008. Water quality monitoring protocol narrative for inland lakes, Version 1.0. National Park Service, Great Lakes Network, Ashland, Wisconsin. Natural Resources Technical Report NPS/MWR/GLKN/NRTR—2008/109. National Park Service, Fort Collins, Colorado.

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Appendix A. Field data sheet for diatom monitoring.

NPS GLKN DIATOM MONITORING	
Date:	
Park Unit:	
Lake Name:	
Collector:	
GPS coordinates of sampling location	
Water depth at sampling location (m)	
Core interval(s) collected (e.g. 0-1 cm)	
Notes	

Standard Operating Procedure #5: Cleaning Sediment Samples

Version 1.0

In **Diatom Monitoring Protocol**

Prepared by
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August 2008

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Acknowledgements

We consulted Acker et al. (2002) while compiling information for this standard operating procedure.

5.0 Introduction

To accurately identify diatoms at the species level, all cellular organic matter, as well as other organic and carbonate material in the sample, must be removed. Removing the organic matter will allow the details in the structure of the siliceous cell wall to be visible with light microscopy. This protocol describes a method for removing organic material and carbonates from a sediment sample.

5.1 Equipment and Supplies

Refer to the checklists below and ensure adequate equipment and supplies are on hand for cleaning the sediment samples in a safe manner.

√	**Checklist of equipment needed for cleaning sediment samples**
	50 ml NUNC polypropylene fliptop conical centrifuge tubes (NUNC Catalog #362697; available through Fisher Scientific, www.fishersci.com/)
	10% (by volume) reagent grade hydrochloric acid (HCl)
	30% (by volume) reagent grade hydrogen peroxide (H_2O_2)
	De-ionized (DI) water
	Spatula
	Permanent marker to label centrifuge tubes
	Water bath set at 85°C
	Centrifuge capable of holding 50 ml NUNC centrifuge tubes and spinning at 3500 rpm
	Positive-draw fume hood

√	**Checklist of personal protective equipment (PPE) needed for cleaning sediment samples**
	Acid-impervious gloves
	Safety glasses and face shield
	Laboratory coat or apron, acid resistant

5.2 Safety Precautions

Personnel are required to wear the PPE listed in Section 5.1 of this SOP while performing this procedure.

Hydrochloric acid (HCl) is used to oxidize carbonates in the sample; HCl is a corrosive acid and should be used under a fume hood while wearing safety glasses, gloves, and a lab coat. HCl is a corrosive acid; store in a cool, dry, well-ventilated area away from incompatible substances, and keep away from heat, sparks, and flame (refer to the Material Safety Data Sheets (MSDS) in your laboratory for complete storage and handling instructions).

Hydrogen peroxide (H_2O_2) is used to digest organic materials; it is a corrosive material and should be used under a fume hood while wearing a face shield, safety glasses, gloves, and a lab coat. H_2O_2 is corrosive and a strong oxidizer; store in a cool, dry, well-ventilated area away from incompatible substances, and keep away from heat, sparks, and flame (refer to the MSDS in your laboratory for complete storage and handling instructions).

Consult the MSDS for additional information on any of the above chemicals. Be familiar with the location of the MSDS in your laboratory before proceeding with this analysis.

5.3 Methods

1. Sediment samples should be stored in a dark, 4°C, relatively air-tight environment until processed. Process samples within two weeks of collection.
2. Mix each sediment sample well with a spatula to ensure an accurate representation throughout the whole sample.
3. Place approximately 0.5 to 1.0 ml of homogenized sediment in a labeled, 50 mL polypropylene centrifuge tube with a snap cap (tubes should be labeled with the date, GLKN park unit, core interval, and your initials). The samples will not be analyzed quantitatively for diatom concentration, so it is not necessary to record the volume of sample used.
4. To remove carbonates, add 10% hydrochloric acid (HCl) by pipette (one drop at a time) until no further reaction occurs when more acid is added. Samples containing significant amounts of carbonate will bubble and sputter when HCl is added; therefore proceed cautiously with this step and add HCl slowly so that the reaction does not become too violent.
5. Add 10 mL 30% peroxide (H_2O_2), and place in an 85°C water bath (with the centrifuge tube flip-top open) for 3 hours. Monitor the samples closely for the first 20 minutes to make sure the reaction is not too violent. If the samples come close to boiling over, remove them from the water bath and let the reaction proceed at room temperature under a positive-draw fume hood (the samples can be put back in the water bath after the reaction has slowed down, but watch them closely to make sure they do not boil over). Dense samples with lots of silts may need periodic homogenization with a glass stirring rod to ensure that buried organic material gets adequate exposure to peroxide. Samples are fully digested when the solution is clear and the reaction has stopped; if samples are not fully digested after 3 hours they can be left in the water bath for additional time, or left at room temperature under a positive-draw fume hood until the digestion is complete.
6. When cool, fill each centrifuge tube to even levels (to approximately the 50 mL mark on the tube) with de-ionized (DI) water.
7. Centrifuge each sample for 6 minutes at 3500 rpm. Repeat 5-6 times for each sample, decanting supernatant each time and re-filling with DI water. When decanting, pour off as much water as possible without losing any of the sediment.
8. After the last centrifuge run, decant but do not re-fill the centrifuge tubes.
9. The samples can now be used to prepare diatom slides (see SOP # 6).

5.4 Quality Assurance/Quality Control

Diatom frustules are microscopic, and there is a possibility that samples can be contaminated. Laboratory areas where raw or processed samples are handled should be kept as clean as possible and lab bench tops should be kept free of debris.

5.5 Literature Cited

Acker, F., B. Russell, and E. Hagan. 2002. Protocol P-13-42, Diatom cleaning by nitric acid digestion with a microwave apparatus. *In* Protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program. Charles, D. F., C. Knowles, and R. S. Davis (editors). Report No. 02-06, The Academy of Natural Sciences, Patrick Center for Environmental Research – Phycology Section, Philadelphia, Pennsylvania.

Standard Operating Procedure #6: Preparation of Diatom Slides

Version 1.0

In **Diatom Monitoring Protocol**

Prepared by
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August 2008

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Acknowledgements

We consulted Acker et al. (2002) while compiling information for this standard operating procedure.

6.0 Introduction

This protocol outlines the steps to produce high-quality diatom microscope slides from sediment samples cleaned according to SOP #5. Identification of diatom valves under light microscopy requires mounting cleaned material between a glass coverslip and a microscope slide, using a medium that has a refractive index near that of glass. Zrax™ is a commercially available toluene-based mounting media with an appropriate refractive index (R.I. ~1.7). Slides prepared according to this protocol will last for many decades.

6.1 Equipment and Supplies

Refer to the checklists below and ensure adequate equipment and supplies are on hand for preparing diatom slides in a safe manner.

√	**Checklist of equipment needed for preparing diatom slides**
	10% (by volume) reagent grade hydrochloric acid (HCl)
	Disposable plastic pipettes (approx. 3 ml)
	Kimwipe® tissues
	De-ionized (DI) water
	Glass coverslips (22 mm x 22 mm) – No. 1 thickness
	Aluminum drying plate (squares are etched onto the surface, each square is etched with an identifying number)
	Hotplate with temperature control
	Positive-draw fume hood
	Glass microscope slides (1 x 3 inches; 2.5 x 7.5 cm)
	Permanent marker
	Toluene-based mounting medium (information on Zrax™ can be found at http://www.sas.upenn.edu/~dailey/zrax.pdf; Philadelphia, PA USA)
	Forceps
	Rounded toothpicks, disposable wooden stirring sticks, or pencil with eraser
	Single-edged razor blade
	Paper labels for slides

√	**Checklist of personal protective equipment (PPE) needed for preparing diatom slides**
	Acid-impervious gloves
	Safety glasses
	Laboratory coat or apron, acid resistant

6.2 Safety Precautions

Personnel are required to wear the PPE listed in Section 6.1 of this SOP while performing this procedure.

Hydrochloric acid (HCl) is a corrosive material and should be used under a fume hood while wearing safety glasses, gloves, and a lab coat.

The toluene-based mounting medium should be considered hazardous. Toluene is an organic solvent which volatilizes readily when heated. Therefore, only heat the mounting medium under a positive-draw fume hood and wear safety glasses and protective hand wear when working with the mounting medium (until the slides have cooled).

Hotplate temperatures required for this procedure are high enough to cause severe burns; use extreme care when manipulating slides on the hotplate.

Consult the Material Safety Data Sheets (MSDS) for additional information on any of the above chemicals. Be familiar with the location of MSDS in your laboratory before proceeding with this analysis.

6.3 Methods

Note that all slides will be prepared in duplicate, that is, two slides will be prepared from each cleaned sediment sample. The two slides should be designated with the letters ‘a’ and ‘b’. The remaining label information will be the same for the two slides (see section 6.3.2 part 9, below).

6.3.1 Preparing Coverslips

Coverslips will be prepared from sediment samples that have been cleaned according to SOP #5. Note that once the coverslip preparations begin, one will not be able to move the aluminum drying plate. Before beginning the following process, find a place in the laboratory where the aluminum drying plate can be left overnight (somewhere that will be undisturbed and free of vibrations and drafts).

1. Use a Kimwipe® to wipe each coverslip as you remove it from the box. Place each coverslip on a marked space of the aluminum drying plate. Be sure the aluminum drying plate is clean and dry to avoid cross-contamination. NOTE: All slides will be made in duplicate, so prepare two coverslips for each sample.
2. Under the fume hood, add one small drop of 10% HCl to each centrifuge tube to create a better distribution of diatoms.
3. Add de-ionized (DI) water to each centrifuge tube until the liquid is a slightly translucent grey color. This means that more water will be added for samples with a high diatom abundance and less water for samples that have a lower density.
4. If the solution is still overly saturated with diatoms (either the solution is still fairly dark grey or one has previously tried to make a slide from a similar sample that was too dense to count), put a large drop of distilled water on the coverslip before transferring the diatom suspension.
5. Agitate the sample to a uniform dispersion and use a plastic pipette to add the diatom solution to each coverslip. Fill each slip corner-to-corner and to the maximum surface

tension of the coverslip. If the coverslip overflows, discard it and repeat the procedure with a freshly cleaned coverslip. Use a new pipette for each sample.

6. Once the aluminum drying plate is loaded with coverslip preparations, allow coverslips to dry undisturbed overnight at room temperature. The drying plate should be on a surface where vibrations from surrounding equipment are minimal and free of drafts so that the distribution of diatoms on the coverslip is even. It is highly recommended to draw a map of the sample locations on the drying plate to ensure that samples do not get mixed up.

7. Once coverslips have dried, a diatom density check can be performed on unmounted coverslips. Coverslips can be placed, diatom side up, on a slide and observed at 400X under a microscope. If diatoms are too dense the coverslip can be discarded and remade (when remaking, dilute the sample either by adding more DI water to the centrifuge tube, or adding more DI water to the coverslip before transferring the diatom suspension). If the diatoms are not dense enough, more of the suspension can be added to the same coverslip and dried again. A final density check will be made after the coverslips are mounted, but performing this preliminary check before making permanent slides will save time and materials.

6.3.2 Mounting Coverslips on Slides

1. After the coverslips have dried, place the aluminum drying plate full of coverslips on a hotplate to drive off hydroscopic water (for 1 hour at 225^{o}F). The hotplate should be under a fume hood in preparation for the following steps.
2. Label slides and clean with a Kimwipe® (slides can be labeled with a Sharpie marker at this stage, a paper label will be added at the end of the process).
3. Turn on the fan of the fume hood. Once coverslips have been dried, add a small drop of mounting medium to a slide by placing the slide over the top of the bottle and inverting, or by using a disposable pipette (the volume of mounting medium on the slide should be equivalent to approximately 2 to 4 drops of water). Place the drop of mounting medium just off-center of the slide to leave room for a label on the other side of the slide. Remove the appropriate coverslip from the aluminum plate with forceps, being careful to handle the coverslip only at the extreme corners. Invert the coverslip and place it gently on the portion of the slide that is covered with mounting medium.
4. Place the slide (keep the coverslip-side up) on the hotplate (which is still set at 225^{o}F within the fume hood, with the fan turned on). Bubbles will soon result from the evaporation of the toluene; keep the slide on the hotplate until the bubbles significantly diminish.
5. Remove the slide from the hotplate; using a rounded toothpick, wooden stirring stick, or eraser end of a pencil, gently position the coverslip and press down on it to form a thin layer of mounting medium beneath the entire coverslip. Press down firmly, but not so hard as to damage the coverslip. The mounting medium will harden quickly; if it is necessary to reposition the coverslip after the medium hardens, the slide will have to be put back on the hotplate for a few seconds to soften the mounting medium.

6. If there are no bubbles under the coverslip and the mounting medium sufficiently covers the area of the coverslip (i.e., no edges of the coverslip are free of mounting medium), then set the slide aside to cool. If you are unable to remove all of the bubbles, put the slide back on the hotplate for a few seconds and repeat step 5. If there is not enough mounting medium on the slide, put the slide back on the hotplate and add a small amount of mounting medium to the edge of the coverslip (it will be pulled underneath as it heats) with a disposable pipette and repeat steps 4 through 6.
7. Once the slide has cooled, scrape the excess mounting medium from the edges of the coverslip with a razor blade. Always start in the center of the slide and work outward to avoid popping the coverslip loose from the slide; be sure that the blade is aimed away from your fingers.
8. Look at the completed slides on the microscope under medium to low magnification (100x to 450x) to confirm that there is an even distribution of diatoms and that most the diatom frustules do not overlap, are not too dense, or too dilute. Remake any slides that have problems with the diatom dispersion or density that would interfere with the quality and accuracy of the analysis.
9. Label all completed slides with a printed paper label that contains the name of the state, the county, the name of the park, the name of the lake, the GPS coordinates of the sample location, the core interval (e.g., 0-1 cm), the collector's name, and the date (designate the duplicates of each sample as 'a' and 'b'). Use a dating convention in which the day and month are readily distinguishable (e.g., 21 Sept 2008 or 21 IX 2008) to avoid confusion between European and North American abbreviation styles. A glass etching pen can also be used to label slides with the lake name and interval, in case the paper labels ever fall off.

6.4 Quality Assurance/Quality Control

Diatom frustules are microscopic, and there is a possibility that samples can be contaminated. Laboratory areas where raw or processed samples are handled should be kept as clean as possible, and lab bench tops should be kept free of debris. Disposable pipettes should be used when possible.

All slides will be prepared in duplicate: set 'a' will be counted, set 'b' will be archived and available for loan to other laboratories.

6.5 Literature Cited

Acker, F., B. Russell, and E. Morales. 2002. Protocol P-13-49, Preparation of diatom slides using Naphrax™ mounting medium. *In* Protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program. Charles, D.F., C. Knowles, and R.S. Davis (editors). Report No. 02-06, The Academy of Natural Sciences, Patrick Center for Environmental Research – Phycology Section, Philadelphia, Pennsylvania.

Standard Operating Procedure #7: Analysis of Slides

Version 1.0

In **Diatom Monitoring Protocol**

Prepared by
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August 2008

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We consulted Clason et al. (2002) while compiling information for this standard operating procedure.

7.0 Introduction

The purpose of this SOP is to describe the procedure for analyzing diatoms on microscope slides.

7.1 Preview Slides

Permanent slides should be previewed before identifying and enumerating diatoms to ensure that an even distribution of diatoms exists and that the abundance of diatoms is neither too dense nor too sparse for counting. Overlapping of frustules should be minimal. The preferred density of diatoms will vary among analysts, however the valves should not be so dense that the details of individual valves are obscured, and should not be so sparse that it is not possible to identify 400 valves. If the density or distribution of diatoms on the slide is not appropriate the slide must be remade according to SOP #6.

7.2 Counting and Taxonomy

A total of 400 diatom valves will be counted along random transects from the 'a' slide using a light microscope fitted with full immersion optics capable of magnification within the range of 875-1250X and numerical aperture (N.A.) >1.30. Analyses will follow the enumeration criteria used in the Minnesota (MN) calibration set (Ramstack et al. 2003; Edlund and Kingston 2004; Edlund 2005), that is, diatoms will be counted when over 50% of the valve is present or visible within the field of view or when a distinct valve fragment is present (e.g., central area of *Amphora libyca* or head pole of *Asterionella formosa*). If a sample with poor diatom preservation is encountered, a maximum of 10 transects will be counted. Unknown taxa will be give a unique name and 8-character code, and photos will be taken.

Diatoms will be identified to the lowest taxonomic unit using floras and monographs by Hustedt (1927-1966, 1930); Patrick and Reimer (1966, 1975); Collins and Kalinsky (1977); Camburn et al. (1978, 1984-1986); Krammer and Lange-Bertalot (1986, 1988, 1991a, b); Cumming et al. (1995); Reavie and Smol (1998); Camburn and Charles (2000); and Fallu et al. (2000); as well as all other pertinent literature. As slides are being counted, taxonomy should be harmonized with the MN diatom calibration set (Ramstack et al. 2003; Edlund and Kingston 2004; Edlund 2005), because these NPS samples will ultimately be combined with the MN set.

Any new technicians introduced into the program will need to be trained by an experienced taxonomist. To ensure internal taxonomic consistency, photographic databases will be constructed for all predominant diatom taxa; this collection of photographs will be maintained at the St. Croix Watershed Research Station (SCWRS). (Note: should another contracting organization be conducting the work, replace all reference to SCWRS with the appropriate contractor's name.)

Each year, 10% of the samples collected will be recounted by another analyst in the program. The primary analyst will etch a line in the slide to mark the transect counted, and will etch circles at the start and end points of the area counted along the transect. The QC analyst will then use

the etchings to count the exact same portion of the slide. Refer to SOP #12 (Quality Assurance and Quality Control) for details on assessing percent difference between the two counts.

7.3 Entering Data

Each taxon will be described by an 8-character code; all of the codes used in the 145 lake MN calibration set are listed in Appendix A. These codes follow the format used by the Great Lakes Environmental Indicators (GLEI) project (E. Reavie, Natural Resources Research Institute, University of Minnesota, Duluth); the GLEI list will be used as a starting point, however many taxa and codes will need to be added as the project progresses. A complete and up-to-date list of codes for this project will be maintained in a database at the SCWRS.

After a sample has been analyzed, the following information should be entered into an Excel spreadsheet:

- The park unit, lake name, sediment interval, transect counted, date, and the analyst
- The full taxon name
- The appropriate 8-character code for that taxon
- The raw count for each taxon (number of valves of a taxon counted in a given sample)
- The percentage abundance (relative frequency) of each taxon (number of valves of each taxon divided by the total number of valves counted in that sample)
- Any relevant notes

All of the Excel datasheets for this project will be housed at the St. Croix Watershed Research Station. Researchers will communicate with the individual parks to make these data available to them

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Appendix A. Diatom codes used in the Minnesota calibration set.

Code	Genus	Species	Variety	Author
ACEGRANA	Achnanthes	grana		Hohn & Hellerman
ACECONSP	Achnanthes	conspicua		A. Mayer
ACECATEN	Achnanthes	catenata		Bily & Marvan
ACHMINUT	Achnanthidium	minutissimum		(Kütz.) Czarn.
AMPSUBCO	Amphora	subcostulata		Stoermer & J.J.Yang
AMPINARI	Amphora	inariensis		Krammer
AMPLIBYC	Amphora	libyca		Ehrenb.
AMPPEDIC	Amphora	pediculus		(Kütz.) Grunow
AMPVENET	Amphora	veneta		Kütz.
AMPOVALI	Amphora	ovalis		(Kütz.) Kütz.
ANOSPHCO	Anomoeoneis	sphaerophora	fo. costata	(Kütz.) A.M.Schmid
ASTFORMO	Asterionella	formosa		Hass.
AULSUBAR	Aulacoseira	subarctica		(O.Müll.) E.Y.Haw.
AULPFAFF	Aulacoseira	pfaffiana		(Reinsch) Krammer
AULLIRBI	Aulacoseira	lirata	biseriata	(Grunow) E.Y.Haw.
AULITATE	Aulacoseira	italica	tenuissima	(Grunow) Simonsen
AULITALI	Aulacoseira	italica		(Ehrenb.) Simonsen
AULGRANU	Aulacoseira	granulata		(Ehrenb.) Simonsen
AULDISTA	Aulacoseira	distans		(Ehrenb.) Simonsen
AULCCREN	Aulacoseira	crenulata		(Ehrenb.) Thwaites
AULAMBIG	Aulacoseira	ambigua		(Grunow) Simonsen
BELBEROL	Belonastrum	berolinensis		(Lemmermann) Round & Maidana
BRABREBI	Brachysira	brebissonii		R.Ross in B.Hartley
BRAVITRE	Brachysira	vitrea		(Grunow) R.Ross in B.Hartley
COCPLACE	Cocconeis	placentula		Ehrenb.
COCPEDIC	Cocconeis	pediculus		Ehrenb.
COCPLAEU	Cocconeis	placentula	euglypta	(Ehrenb.) Grunow
COCPLALI	Cocconeis	placentula	lineata	(Ehrenb.) VanHeurck
COCNEODI	Cocconeis	neodiminuta		Krammer in Krammer & Lange-Bert.

Code	Genus	Species	Variety	Author
COCNEOTH	Cocconeis	neothumensis		Krammer
CRAHALOP	Craticula	halophila		(Grunow) D.G. Mann in Round, Crawford & Mann
CRACUSPI	Craticula	cuspidata		(Kütz.) D.G.Mann in Round, Crawford & Man
CSPINVIS	Cyclostephanos	invisitatus		(Hohn et Hellermann) Theriot, Stoermer et Håk.
CSPTHOLI	Cyclostephanos	tholiformis		Stoermer, Håk. & Theroit
CSPDUBIU	Cyclostephanos	dubius		(Fricke) Round in Theriot, Håkansson, Kociolek, Round & Stoermer
CYCCOMRA	Cyclotella	comta	radiosa	Grunow
CYCCOMEN	Cyclotella	comensis		Grunow
CYCBODLE	Cyclotella	bodanica	lemanica	(O.Müll. ex Schroter) Bachmann
CYCSTELL	Cyclotella	stelligera		(Cleve & Grunow) VanHeurck
CYCROSSI	Cyclotella	rossii		Håk.
CYCPSEUD	Cyclotella	pseudostelligera		Hust.
CYCOCELL	Cyclotella	ocellata		Pant.
CYCMENEG	Cyclotella	meneghiniana		Kütz.
CYCMICHI	Cyclotella	michiganiana		Skvortsov
CYCDISTI	Cyclotella	distinguenda		Hust.
CYCKRAMM	Cyclotella	krammeri		Håk.
CYCGORDO	Cyclotella	gordonensis		Kling & Håk.
CYTELLIP	Cymatopleura	elliptica		(Bréb. & Godey) W.Sm.
CYMAFFIN	Cymbella	affinis		Kütz.
CYMCISTU	Cymbella	cistula		(Ehrenb. in Hemprich & Ehrenb.) Kirchn. in Cohn
DIATENEL	Diatoma	tenue	elongatum	Lyngb.
ENCSILES	Encyonema	silesiacum		(Bleisch in Rabenh.) D.G.Mann
ENCTRIAL	Encyonema	triangulum		(Ehrenb.) Kütz.
ENPMICRO	Encyonopsis	microcephala		(Grunow) Krammer
EPIARGUS	Epithemia	argus		(Ehrenb.) Kütz.
EPIADNAT	Epithemia	adnata		(Kütz.) Bréb.
EUNBILUN	Eunotia	bilunaris		(Ehrenberg) Mills
EUNZASUM	Eunotia	zasuminensis		(Cab.) Körner
FRACAPUC	Fragilaria	capucina		Desm.
FRACROTO	Fragilaria	crotonensis		Kitton

Code	Genus	Species	Variety	Author
FRACONV2	Fragilaria	construens	var. 2	provisional name
FRACAPVR	Fragilaria	capucina	rumpens	(Kütz.) Lange-Bertalot
FRACAPGR	Fragilaria	capucina	gracilis	(Oestrup) Hust.
FRAVAUCH	Fragilaria	vaucheriae		(Kütz.) J.B.Petersen
FRAEXIGU	Fragilaria	exigua		Grunow in Cl. & Möll.
FRANANAN	Fragilaria	nanana		Lange-Bert.
FRATENER	Fragilaria	tenera		(W. Smith) Lange-Bert.
FRACAPME	Fragilaria	capucina	mesolepta	Rabenh.
GOMPUMIL	Gomphonema	pumilum		(Grunow) Reich. & Lange-Bert.
GOMPARVU	Gomphonema	parvulum		(Kütz.) Kütz.
GOMTRUNC	Gomphonema	truncatum		Ehrenb.
GOMINSIG	Gomphonema	insigne		Gregory
GOMINTRI	Gomphonema	intricatum		Kütz.
GOMGRACI	Gomphonema	gracile		Ehrenb. emend. VanHeurck
GOMDICHO	Gomphonema	dichotomum		Kütz.
GOMANGUS	Gomphonema	angustatum		(Kütz.) Rabenh.
HANAMPHI	Hantzschia	amphioxys		(Ehrenb.) Grunow
HIPCAPIT	Hippodonta	capitata		(Grunow) Lange-Bert., Metzelin & Witkowski
HIPHUNGA	Hippodonta	hungarica		(Grunow) Lange-Bert., Metzeltin & Witkowski
KARCLEVE	Karayevia	clevei		(Grunow in Cleve & Grunow) Round & Bukht.
MARMARTY	Martyana	martyii		(Héribaud) F.E.Round in Round, Crawford & Mann
NAVVITIO	Navicula	vitiosa		Schimanski
NAVTRIVI	Navicula	trivialis		Lange-Bert.
NAVSUBMA	Navicula	subminuscula		Manguin
NAVSEMIN	Navicula	seminulum		Hust.
NAVREINH	Navicula	reinhardtii		Grunow
NAVRADIO	Navicula	radiosa		Kütz.
NAVPHYLL	Navicula	phyllepta		Kütz.
NAVMINUS	Navicula	minuscula		Grunow
NAVMINIM	Navicula	minima		Grunow in Van Heurck
NAVLUNDI	Navicula	lundii		Reichardt

Code	Genus	Species	Variety	Author
NAVLIBON	Navicula	libonensis		Schoeman 1970
NAVLEPTO	Navicula	leptostriata		Jorgensen
NAVPSEUV	Navicula	pseudoventralis		Hust.
NAVCRYPT	Navicula	cryptotenella		Lange-Bert.
NAVCRYCP	Navicula	cryptocephala		Kütz.
NAVCINCT	Navicula	cincta		(Ehrenb.) Ralfs
NAVCAPRA	Navicula	capitatoradiata		Germain
NAVACCOM	Navicula	accomoda		Hustedt
NAVDENSL	Navicula	densilineolata		(Lange-Bert.) Lange-Bert.
NITINCOG	Nitzschia	incognita		Krasske
NITPERMI	Nitzschia	perminuta		(Grunow) M.Perag.
NITSUBLI	Nitzschia	sublinearis		Hust.
NITSUBAC	Nitzschia	subacicularis		Hust.
NITPUSIL	Nitzschia	pusilla		Grunow
NITPALEC	Nitzschia	paleacea		Grunow
NITPALEA	Nitzschia	palea		(Kütz.) W.Sm.
NITLINVS	Nitzschia	linearis	subtilis	(Grunow) Hustedt
NITLIEBE	Nitzschia	liebetruthii		Rabenh.
NITACICO	Nitzschia	acicularioides		Hust.
NITFONTI	Nitzschia	fonticola		Grunow
NITDENTI	Nitzschia	denticula		Grunow
NITARCHI	Nitzschia	archibaldii		Lange-Bert.
NITAMPHI	Nitzschia	amphibia		Grunow
NITRADIC	Nitzschia	radicula		Hust.
NITAGNIT	Nitzschia	agnita		Hust.
NITACICU	Nitzschia	acicularis		(Kütz.) W.Sm.
NITGRACI	Nitzschia	gracilis		Hantzsch
PINVIRID	Pinnularia	viridis		(Nitzsch) Ehrenb.
PINBICPU	Pinnularia	biceps	pusilla	Camburn & Charles
PINBRAUN	Pinnularia	brauniana		(Grunow) Cleve
PLAROSTR	Planothidium	rostratum		(Østrup) Round et L.Bukhtiyarova

Code	Genus	Species	Variety	Author
PLAFREQU	Planothidium	frequentissimum		(Lange-Bertalot) Round et L.Bukhtiyarova
PLALANCE	Planothidium	lanceolatum		(Bréb.) Round & Bukht.
PSASUBAT	Psammothidium	subatomoides		(Hust.) L.Bukhtiyarova et Round
PRABREVT	Pseudostaurosira	brevistriata	"triangular"	provisional name
PRABREVI	Pseudostaurosira	brevistriata		(Grunow in Van Heurck) D.M.Williams et Round
PRABREBC	Pseudostaurosira	brevistriata	capitata	(Héribaud) N.A.Andresen & Kreis
PRABREIN	Pseudostaurosira	brevistriata	inflata	(Pant.) M.B.Edlund
PRAMICRO	Pseudostaurosira	microstriata		(Marciniak) provisional placement in genus
RHOCURVA	Rhoicosphenia	curvata		(Kütz.) Grunow
RHPGIBBA	Rhopalodia	gibba		(Ehrenb.) O.Müll.
ROSLINEA	Rossithidium	linearis		(W.Sm.) Round & Bukht.
ROSLINCU	Rossithidium	linearis	fo. curta	provisional name
SELPUPUL	Sellaphora	pupula		(Kütz.) Mereschk.
SELLAEVI	Sellaphora	laevissima		(Kütz.) D.G.Mann
SELVITAB	Sellaphora	vitabunda		(Hust.) D.G.Mann
STAANCEP	Stauroneis	anceps		Ehrenb.
STAANCGR	Stauroneis	anceps	fo. gracilis	Rabenh.
SRACONVE	Staurosira	construens	venter	(Ehrenb.) Hamilton
SRACONBI	Staurosira	construens	binodis	(Ehrenberg) P.B. Hamilton in Hamilton, Poulin, Charles & Angell
SRACONPU	Staurosira	construens	pumila	(Grunow in Van Heurck) Kingston
SRAELLIP	Staurosira	elliptica		(Schum.) D. M. Williams & Round
SRACONST	Staurosira	construens		(Ehrenb.) D.M.Williams et Round
SLLPINVA	Staurosirella	pinnata	acuminata	A. Mayer
SLLANSAT	Staurosirella	ansata		(M.H.Hohn & Hellerm.) Kingston
SLLPINNA	Staurosirella	pinnata		(Ehrenb.) D.M.Williams & Round
SLLPINLA	Staurosirella	pinnata	lancettula	(Schumann) E.Y.Haw. et M.G.Kelly
SUSMINUS	Stephanodiscus	minutulus		(Kütz.) Cleve & J.D.Möll.
SUSALPIN	Stephanodiscus	alpinus		Hust.
SUSHANTZ	Stephanodiscus	hantzschii		Grunow
SUSHANTE	Stephanodiscus	hantzschii	fo. tenuis	(Hust.) Håk. & Stoermer
SUSMEDIU	Stephanodiscus	medius		Håk.

Code	Genus	Species	Variety	Author
SUSNIAGA	Stephanodiscus	niagarae		Ehrenb.
SUSPARVU	Stephanodiscus	parvus		Stoermer & Håk.
SUSVESTI	Stephanodiscus	vestibulus		Håk., Stoermer & Theriot
SUSOREGO	Stephanodiscus	oregonicus		(Ehrenb.) Håkansson
SUROVALI	Surirella	ovalis		Bréb.
SYNDELIC	Synedra	delicatissima		W.Sm.
SYNACUAN	Synedra	delicatissima	angustissima	Grunow
SYNRADIA	Synedra	radians		Kütz.
SYNRUMFA	Synedra	rumpens	familiaris	(Kütz.) Hust.
SYNULNA	Synedra	ulna		(Nitzsch) Ehrenb.
SYNACUS	Synedra	acus		Kütz.
SYLPARAS	Synedrella	parasitica		(W. Smith) Round & Maidana
TABFLOC4	Tabellaria	flocculosa	IV	Koppen
TABFLOC3	Tabellaria	flocculosa	III	Koppen
TABQUADR	Tabellaria	quadriseptata		Knuds.
TRYHUNGA	Tryblionella	hungarica		(Grunow) D.G.Mann
TRYANGUS	Tryblionella	angustata		W.Sm.

Appendix B. Diatom count sheet.

NPS I&M Diatom Monitoring Program

Park Unit

Lake

Interval

Transect Counted

Date

Analyst

Taxon Code	Taxon Name	# of Valves	Relative Abundance	Notes

Standard Operating Procedure #8: Archiving Sediment and Diatom Slides

Version 1.0

In **Diatom Monitoring Protocol**

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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 will promptly notify the project manager and/or the Great Lakes Network (GLKN) 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 footer of the document file. For complete instructions, please refer to Revising the Protocol SOP, #13.

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

8.0 Introduction

The purpose of this SOP is to outline the procedure for preserving surface sediment samples and archiving sediment samples and diatom slides.

8.1 Freeze-Drying Sediment Samples

After diatom slides have been made, the remaining sediment should be freeze-dried and placed into a vial labeled with: the project name, the lake name, the GLKN park unit, the GPS coordinates of the sample location, the sediment interval (e.g., 0-1 cm), the collector's name, and the date.

Note that if for any reason microscope slides need to be remade at some time in the future, freeze dried sediment can be cleaned following the same procedure that was used for wet sediment (SOP #5). When cleaning freeze dried sediment, approximately 100-200 mg of dry sediment should be used.

8.2 Archiving Freeze-Dried Sediment and Diatom Slides

Both freeze-dried sediment samples and diatom slides will be archived. Freeze-dried sediment samples will be stored in vials and labeled as described above (Section 8.1); diatom slides will be labeled as described in SOP #6, Section 6.3.2. Researchers will coordinate with each park unit to decide where the collections for each park will be housed. Representatives from each park will also work with researchers to ensure that the archived materials are entered into the appropriate NPS database.

All freeze-dried sediment samples from a given year will be stored together, and all slides from a given year will be stored together. Vials containing freeze-dried samples will be stored in boxes, with the tops and sides of the boxes clearly labeled for easy retrieval from storage. Slides will be stored in labeled microscope slide boxes and in the dark. Both freeze-dried samples and slides will be stored at room temperature. Archived slides and samples will be checked periodically to be sure that they have not been damaged while in storage.

Standard Operating Procedure #9:
Data Analysis

Version 1.0

In **Diatom Monitoring Protocol**

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Revision History Log

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

Acknowledgements

In writing this SOP we relied heavily on the lecture notes and course materials from Steve Juggins' Analysis of Environmental Data course that he taught at Iowa Lakeside Laboratory in July 2006. Many thanks to Steve for all he has taught us about multivariate data analysis and all of the questions he has answered for us over the years. Gavin Simpson was extremely helpful in providing information on chord distance. Thanks to Avery Cook Shinneman for helpful discussions and reviews of this SOP.

9.0 Introduction

Diatoms are being analyzed in surficial lake sediments to address the following questions:

- What is the current ecosystem status of a particular lake in relation to other regional lakes?
- Are similar ecological trends occurring among regional lakes?
- What is the ecosystem status of this lake in relation to historical environmental change noted in regional sediment cores?
- Has this lake changed since the last time it was sampled? If so, what is the magnitude and direction of that change?

This SOP describes how the diatom species data will be analyzed to answer the above questions. Multivariate statistical analyses will be used to compare diatom species assemblages with water chemistry measurements to derive an understanding of diatom response to physical/chemical variables. In addition, changes in the community composition of diatoms will be analyzed in relation to other lakes as well as within the context of the range of natural variability, as determined by diatoms present in cores dating back to the time of European settlement.

Approximately 50-60 surface sediment samples will have been collected from Great Lakes Network (GLKN) parks between 2005 and 2009 as part of a project to analyze diatoms to understand historical and current water quality conditions (contract # J21050428). The diatom assemblages from these samples will be appended to a diatom calibration set that exists for Minnesota lakes (henceforth referred to as "the MN calibration set"; Ramstack et al. 2003; Edlund and Kingston 2004; Edlund 2005). After this amendment to the calibration set, analyses of diatom samples will no longer be appended to the calibration set, but will be plotted passively on this set to look for changes in the lakes. It is important to note that before any statistical analyses are done, the diatom taxonomy should be harmonized with the MN calibration set. Table 1 outlines the sequence of steps that will be used in the diatom data analysis; each of these steps is described in detail in this SOP.

The majority of examples used throughout this SOP refer to a diatom calibration set with data from 145 MN lakes. This full calibration set is used to illustrate the various statistical analyses described in this SOP; however, once data from the 50-60 GLKN lakes are available, it may prove more effective to append these to a smaller subset of the MN calibration set. For example, it might make sense to remove shallow lakes from the calibration set, or lakes from certain ecoregions (e.g., prairie/agricultural). This decision will have to be made after merging the datasets and determining if the full suite of MN lakes, or some subset of the calibration set is most useful for reconstructing variables of interest in the Great Lakes Network lakes.

9.0.1 Recommended Reading

Jongman et al. (1995), Leps and Šmilauer (2003), McCune and Grace (2002), and Shaw (2003) provide detailed discussions of the multivariate statistical methods covered in this SOP.

Table 1. Sequence of steps used in diatom data analysis.

Analyses to be conducted when the NPS calibration set is complete • Passively plot new species data on the calibration set • Use the calibration set to predict environmental variables from diatom species assemblage

Analyses to be conducted for any lakes with downcore diatom data
• Exploratory detrended correspondence analysis (DCA) of combined downcore and surface diatom species data
  • If gradients are short in DCA, use principal components analysis (PCA) of species data
  • If gradients are long in DCA, use correspondence analysis (CA) or DCA of species data

9.0.2 Software Suggestions

Currently, several statistical packages are available for these analyses; the most common are CANOCO (ter Braak and Šmilauer 1998), R (R Development Core Team 2006), and PC-ORD (McCune and Mefford 1999). CANOCO and PC-ORD offer a Microsoft Windows-type menu-driven approach, while R requires the user to write small programs (referred to as scripts) in the S computing language. Although R is not as immediately user-friendly as the other alternatives, some advantages are that the software is available as a free download, it is supported by comprehensive help windows and a large community of users, it is easy to replicate analyses, and it produces high quality graphics that the user can customize (e.g., all of the graphs in this SOP were produced in R). Download of the free text editor, Tinn-R (Faria et al. 2007), is highly recommended when using R; Tinn-R is designed to interface with R and allows users to save all of the scripts they have written.

9.1 The GLKN Diatom Calibration Set

9.1.1 Species Data

Diatom species are listed by their 8-character taxon codes; (see SOP #7 Analysis of Slides, Appendix 1, for a list of all codes used in the 145 lake Minnesota calibration set). These codes follow the format used by the Great Lakes Environmental Indicators (GLEI) project (E. Reavie, Natural Resources Research Institute, University of MN, Duluth); the GLEI list will be used as a starting point, however many taxa and codes will need to be added as the project progresses. A complete and up-to-date list of codes for this project will be maintained in a database at the St. Croix Watershed Research Station (SCWRS). (Note: if a different contractor is conducting the work, replace each reference to SCWRS with that of the contractor's name.)

Taxa that occurred in at least two samples with a maximum abundance greater than or equal to 1% of the diatom sum for that sample are included in the analysis for construction of the calibration set. In addition, if a species occurred in only one sample, but at more than 5% of that lake's diatom assemblage, it is included. Note that these criteria only apply to building the calibration set. For analyses after the calibration set is complete, data for all species will be used.

When performing any multivariate analyses (building the calibration set, or analyses afterward) diatom species data will be entered as the percent abundance of that species relative to the total diatom counts in the sample (not the raw counts).

9.1.2 Environmental Data

The top 1 cm of surface sediment that is used for diatom analysis represents the diatoms that have settled out of the water column over the previous 3-5 years. Therefore, it is appropriate to compare the diatom counts to the average water chemistry conditions for the ice-free season during the year that the sediment sample is collected. When the calibration set is built, water quality data from each of the season's samplings will have been averaged, and those averages will have been used for comparison with the diatom counts.

For the statistical analyses, it is important that the environmental data are normally distributed and have similar scaling. Therefore, many of the environmental parameters in the calibration set are transformed (e.g., log10, square root) to achieve a normal distribution of data.

9.2 Multivariate Analyses

The following section is an overview of commonly used multivariate analyses, using the MN diatom calibration set as an example. The purpose of this section is to describe the analyses that were used in constructing the MN diatom calibration set, and is not intended to be an exhaustive review of all available multivariate analyses. For example, nonmetric multidimensional scaling (NMS) is an indirect (unconstrained) ordination method that could be used to examine species data in place of correspondence analysis, which is discussed below (see McCune and Grace (2002) for a detailed discussion of NMS). However, the main focus of this SOP is on the diatom calibration set; the calibration set approach requires a direct (constrained) method of ordination such as canonical correspondence analysis (CCA). In addition, as the project progresses, additional statistical methods may be necessary to explain trends and make management decisions (e.g., Sgro et al. 2007).

9.2.1 Principal Components Analysis

Principal components analysis (PCA) is an indirect ordination method. One of the uses of PCA is to show variation in the lakes in terms of environmental variables alone, not being constrained by a secondary dataset (see Table 2 for a classification of ordination methods). A linear method of indirect ordination is used in this case because environmental data generally exhibit linear relationships with each other and the underlying gradients, as opposed to diatom species data which often have a unimodal response to an underlying gradient (with an optimum and tolerance range for each species along that gradient). Figure 1 is an example PCA of MN lakes based on environmental variables. Axis 1 represents the maximum variation in the dataset; this is a theoretical variable, not necessarily something that was measured. Axis 2 represents the second most explanatory gradient in the dataset. The vectors represent the environmental variables that were included in the analysis, with the value of the variable increasing in the direction of the arrow, and decreasing in the opposite direction (the origin represents the average value for each variable). The absolute length of the vector is not meaningful, but the relative length of the vector in relation to the other vectors is important (the longer the vector the greater the increase). The vectors that are most correlated with Axis 1 are those that best explain the maximum

variation in the dataset, and the vectors most closely correlated with Axis 2 best explain the secondary gradient in the dataset.

9.2.2 Correspondence Analysis and Detrended Correspondence Analysis

The species data can also be examined with an indirect model; this will show the patterns in the lakes based on species assemblage alone. First, the analyst must determine, using gradient lengths, if it is appropriate to use a linear or unimodal model with a given set of species data. If the gradients are short (generally less than 2 standard deviation units is used as a cutoff; however, both methods are effective for standard deviations in the range of 1.5-3), then many of the species responses to the underlying gradients will be linear and it is appropriate to use a PCA (if this is the case, it would be appropriate to use the same approach that was used with the environmental data in Section 9.2.1). If the gradients are long (greater than 2 standard deviation units), then the gradients are generally long enough to have captured most of the tolerance range of a given species and many or most of the species responses to the underlying gradients will be unimodal. In this case, it is appropriate to use correspondence analysis (CA) or detrended correspondence analysis (DCA).

To determine the gradient lengths for a given model, DCA is used as a means to compute axis lengths in standard deviation units. If the gradients are long then CA is used to examine the variation in the lakes based on the species assemblages. The sites, species, or both can be plotted. Occasionally after running a CA, the data have a distinct arch when plotted. The arch is present when the first ecological gradient is much longer than the second gradient; an arch artifact occurs because CA constructs the second gradient as a non-linear function of the first. If this is the case, DCA can be used because it removes this arch effect by detrending and rescaling the data. Since the arch results in shrinking at the ends of the ordination, removing the arch is desirable for accurate interpretation of distances among points.

In the MN dataset, a DCA shows that the gradients in the species data are long (greater than 2 standard deviation units), so a unimodal model is appropriate. There is a strong arch in the data when a CA is run; this is evident in both the plot of sites (Figure 2a) and the plot of species (Figure 2b). Figure 3 demonstrates that running a DCA removes this arch effect in the data.

9.2.3 Canonical Correspondence Analysis

Biological datasets often contain a large amount of variation, some of which can be attributed to ‘noise,’ or to other gradients that were not measured. In order to examine the patterns in the dataset that can be attributed to the variables that were measured, a constrained ordination is used. In the MN dataset, the gradients in the species data are long (see Section 9.2.2 for how this is determined), so it is appropriate to use canonical correspondence analysis (CCA), which is a unimodal direct ordination method (if the gradients were short, then a linear model such as redundancy analysis [RDA] would be appropriate [Table 2]). It is important to note that CCA generally removes the arch that is sometimes present in a CA, because now the axes are being constrained by the measured environmental variables. However, if the data have a long primary gradient it is possible that there will still be an arch in the CCA; if this is the case, detrended CCA (DCCA), which is available in CANOCO, can be used to remove the arch.

Often, many of the environmental variables measured are highly correlated. Therefore, the next step is to determine the best subset of environmental variables that independently explain the variation in the species data. Some statistical packages (for example, CANOCO) will perform this forward selection of variables automatically, or this can be done manually by performing a series of partial CCAs to determine the best subset of variables to use. The result of this forward selection step is a subset of variables that independently explain almost as much variation as the full set of variables. Refer to Ramstack et al. (2003), Edlund and Kingston (2004) and Reavie et al. (2006) for examples of how calibration sets are developed using forward selection.

Figure 4 is a CCA biplot of the 145 lakes MN calibration set, with all of the environmental variables included. In this plot, the sites (lakes) are displayed, but as with CA, the species could be displayed. As in PCA, the variable increases in the direction of the vector, and decreases in the opposite direction. Again, the absolute length of the vector is not important, but its relative length and position compared to the other vectors is meaningful; the variables with the longer vectors are more important in determining that gradient, and variables for which the vectors are situated close together are more closely related to one another.

Figure 5 shows a CCA biplot of the 145 lakes MN calibration set with only the forward selected variables included in the analysis, and gives an example of different ways that results can be displayed. For example, in this plot the environmental vectors have been plotted as a separate biplot to make things easier to read. Also, the ecogregions have been plotted as centroids, both in a separate biplot and as large symbols in the main biplot. Notice that in Figure 5 some of the highly correlated variables have been removed. For example, total phosphorus (TP), total nitrogen (TN), and chlorophyll-*a* (Chla) were highly correlated (Figure 4) and forward selection determined that Chla was the best of the three variables to independently explain a significant amount of variation.

Table 2. Summary and classification of ordination methods (modified from Juggins 2006).

Role of explanatory variables in analysis	Response Model: Linear	Response Model: Unimodal
Indirect (variation *is not* constrained by a secondary dataset)	Principal Components Analysis (PCA)	Correspondence Analysis (CA) & Detrended Correspondence Analysis (DCA)
Direct (variation *is* constrained by a secondary dataset)	Redundancy Analysis (RDA)	Canonical Correspondence Analysis (CCA) & Detrended Canonical Correspondence Analysis (DCCA)

9.3 Analysis After the Calibration Set is Complete

Diatom samples collected during routine monitoring can be passively plotted on the existing calibration set, and the calibration set can be used to determine the environmental conditions in the lake based on the diatom species assemblage. Before beginning these analyses, diatom taxonomy should be harmonized with the MN/GLKN calibration set. Diatom species should be coded with the same 8-character taxon codes used in the calibration set (see Section 9.1.1). When performing the following analyses, the diatom species data should be entered as the percent of that species occurrence relative to the total diatom count for that sample (not the raw counts).

9.3.1 Passively Plotting on the Calibration Set

After the calibration set has been established, subsequent diatom data from surface sediment samples will be passively plotted onto the calibration set to see which lakes they are most similar to, and to see where they plot along the environmental axes. As lakes are revisited, analyses will show whether a given lake's diatom assemblage has changed since the lake was last sampled, and if it has changed, with which environmental axis (or axes) the change is correlated.

For example, Figure 6 is a CCA biplot of a subset of the MN calibration set containing 89 lakes. A modern surface sample from a Twin Cities metro area lake and a downcore sample from that same lake are passively plotted on this calibration set. The plot shows that over time the lake's diatom assemblage has changed (if it had remained stable, both samples would have plotted on top of each other), and this change follows the trajectory of the pH and chloride (Cl) axes. From this plot one can infer that over time this lake likely had an increase in pH and Cl levels. This same method can be applied to GLKN lakes.

9.3.2 Dissimilarity Measure

To determine the amount of floristic change in the diatom community between subsequent sampling events, the chord distance dissimilarity measure will be used. The chord distance provides a quantitative measure of the amount of change in the diatom species assemblage between two samples. So, beginning with the second round of sampling, the chord distance can be used to quantify the change in the diatom community for a given lake since the previous sampling event. The chord distance ranges from 0 (samples are exactly the same) to 1.41 (samples are completely different). Meaningful change can be assessed using percentile cutoffs according to the approach used in Bennion et al. (2004). In addition, lakes which have been cored can be used to determine how large of a chord distance is meaningful; changes in downcore chord distance can put current measurements into context (see Section 9.4).

9.3.3 Environmental Reconstructions

After establishing the calibration set, the environmental variables that were selected in the forward selection step can be predicted for a new species assemblage. This is generally referred to as 'reconstructing' environmental conditions from fossil diatoms in a sediment core, but this same technique can be applied to new surface sediment samples after the calibration set has been established. If, for example, TP was one of the variables found to be significant after forward selection with the new calibration set (or if independent, constrained testing of TP demonstrated an important relationship to the diatom gradient), then a TP value can be determined from a

subsequent diatom surface sediment sample in one of the GLKN lakes. The statistical program C2 (Juggins 2003) is widely used for this step.

9.4 Analyses for Lakes with Downcore Diatom Data

As part of the 2004 - 2009 GLKN diatom project (Task Agreement #J2105040028), a select subset of GLKN lakes was cored for diatom and geochemical analysis to determine the timing and magnitude of historical environmental change. For the lakes where these data are available, an indirect ordination of the diatom species assemblage can be plotted and any subsequent diatom surface sediment samples can be added to this plot to see how the species assemblage is changing (see Section 9.2.2 for instructions on how to decide between a linear or a unimodal model for indirect analysis). Figure 7 is an example of a CA from Shell Lake (Sleeping Bear Dunes National Lakeshore) based on diatom species assemblage. This shows that the diatom community began to change in the late 1800s/early 1900s, and has also been changing along a new trajectory in the past decade or so. When this lake is re-sampled for diatoms in the future, the new sample can be added to this plot and it can be determined if the diatom species assemblage in this lake is continuing to change along the trajectory established in the last decade, if the community is remaining stable, or if it is returning to what it looked like at some point in the past, etc. This can provide an early warning sign of change in the GLKN lakes based on biological communities. To determine which environmental variables are causing the change, the core samples can be passively plotted on the calibration set (follow the method used in Section 9.3.1 and Figure 6).

To determine the amount of floristic change throughout the core, the chord distance can be calculated between each core section. The chord distances throughout the core can be used to put modern distances into context. For example, the chord distance between two samples dating before European settlement could be used as a measure of low floristic change, which could then be compared to the chord distance between two modern samples.

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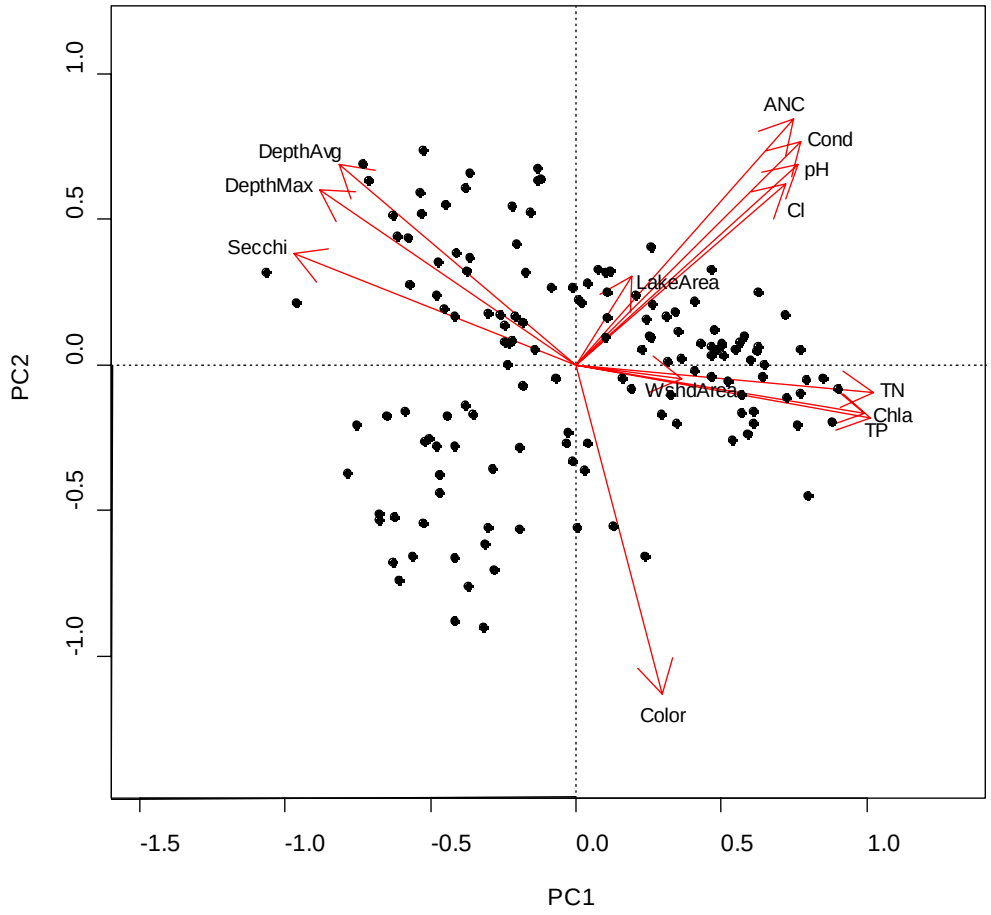
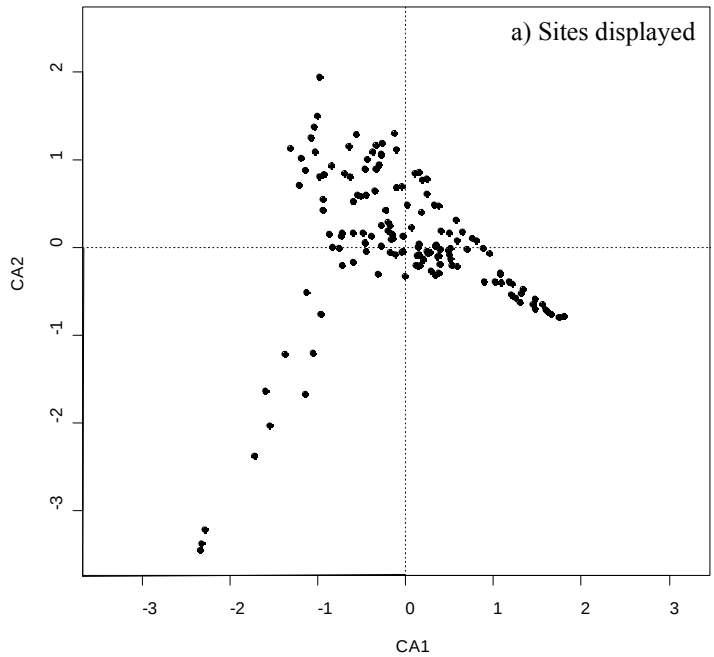


Figure 1. PCA of environmental data (ecoregion excluded) from the 145-lake MN calibration set; the dots represent sites (lakes).



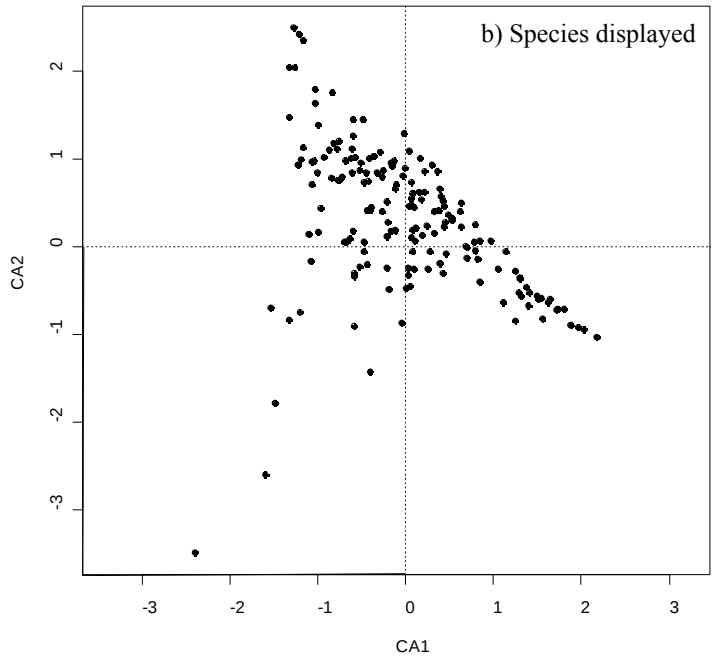
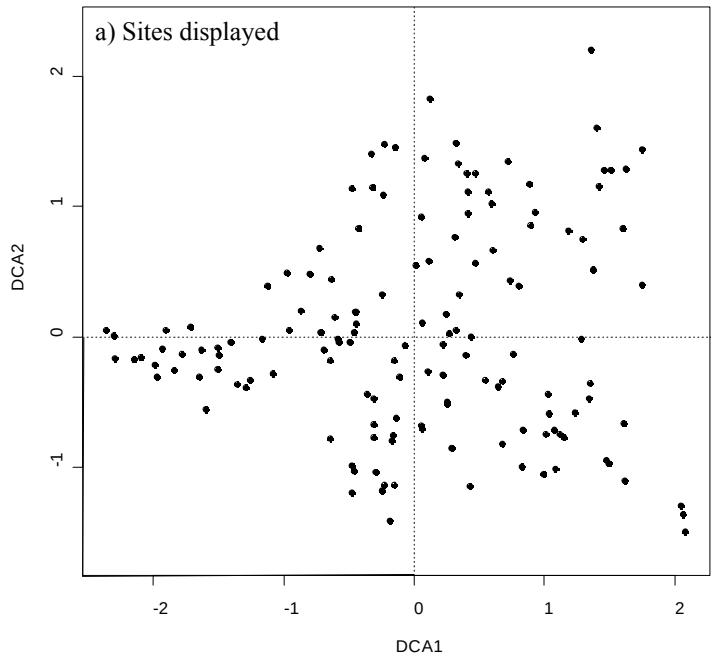


Figure 2. CA biplots of the 145-lake MN calibration set with a) sites displayed and b) species displayed. Note that there is an arch in the data.



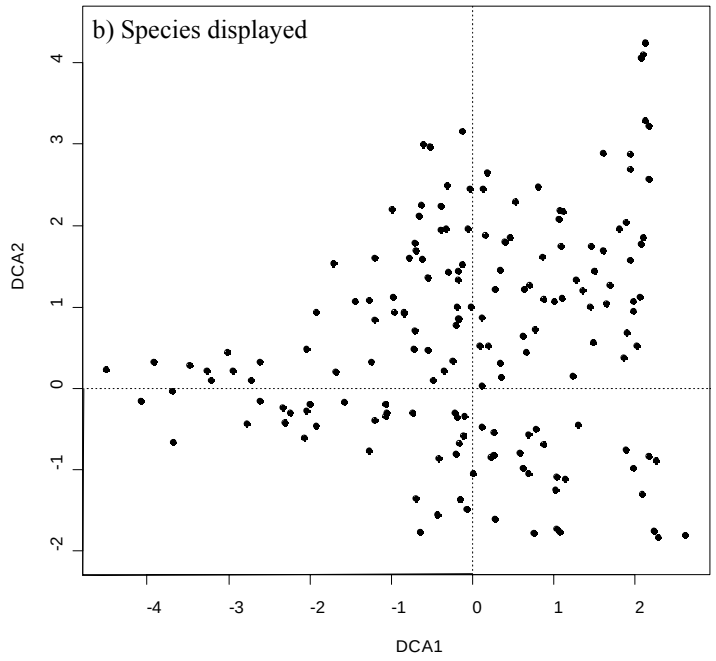


Figure 3. DCA biplots of the 145-lake MN calibration set with a) sites displayed and b) species displayed. Note that the DCA removes the arch effect that was present in the data in the CA (Fig. 2).

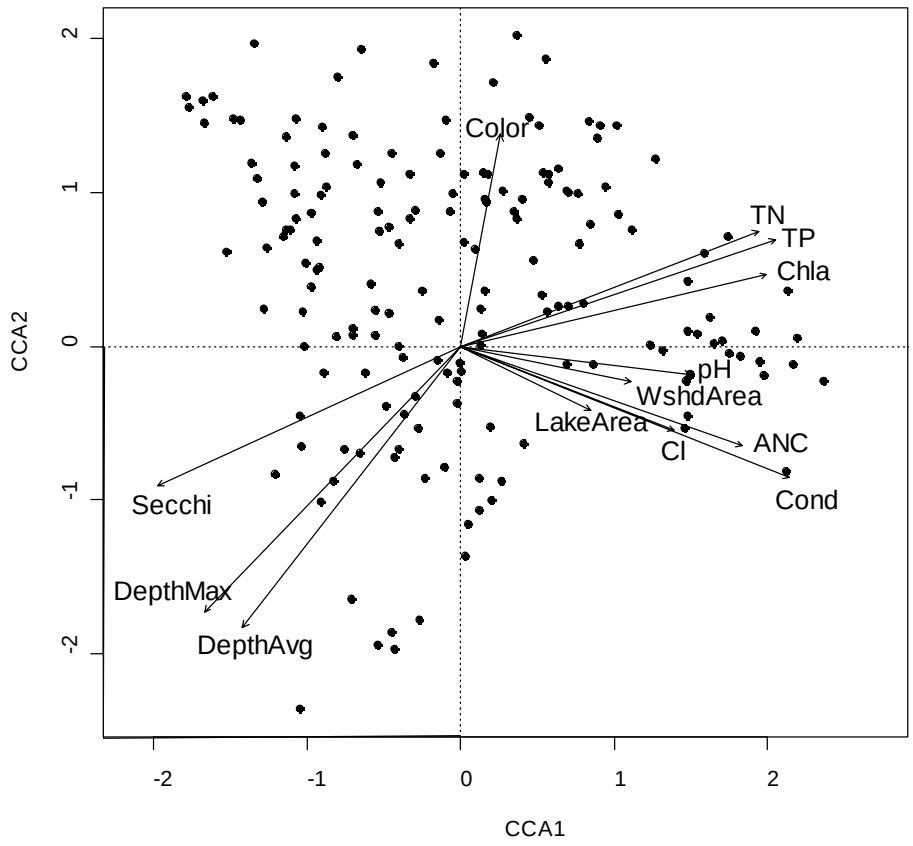


Figure 4. CCA of the 145-lake MN calibration set, with all of the environmental variables included (sites plotted).

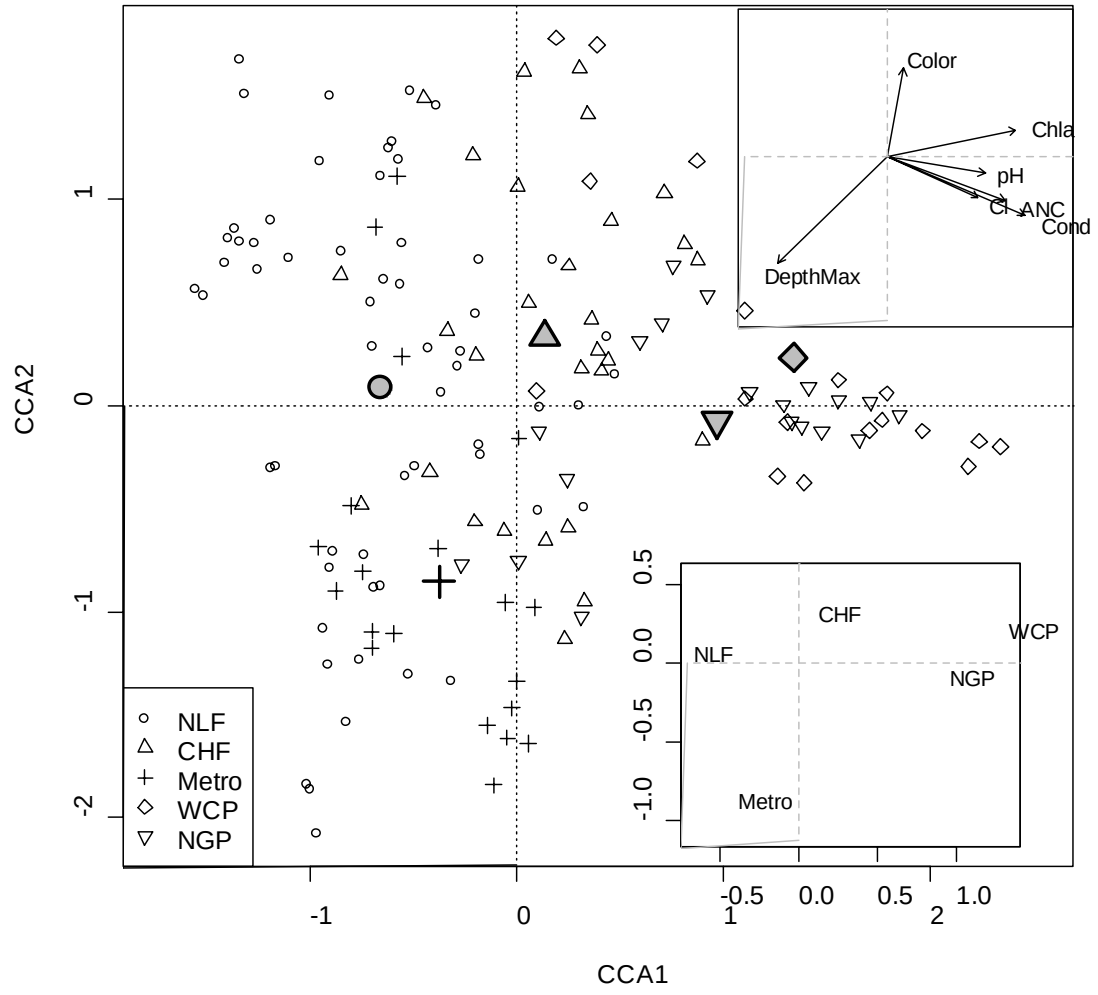


Figure 5. CCA of the 145-lake MN calibration set with the forward selected variables. The environmental vectors have been plotted as a separate biplot to make the plot easier to read. The ecoregions have been plotted as centroids, both in a separate biplot and as large symbols in the main plot. Ecoregion codes: NLF=Northern Lakes and Forests, CHF=Central Hardwood Forests, Metro=Twin Cities Metropolitan Area, WCP=Western Corn Belt Plains, NGP=Northern Glaciated Plains.

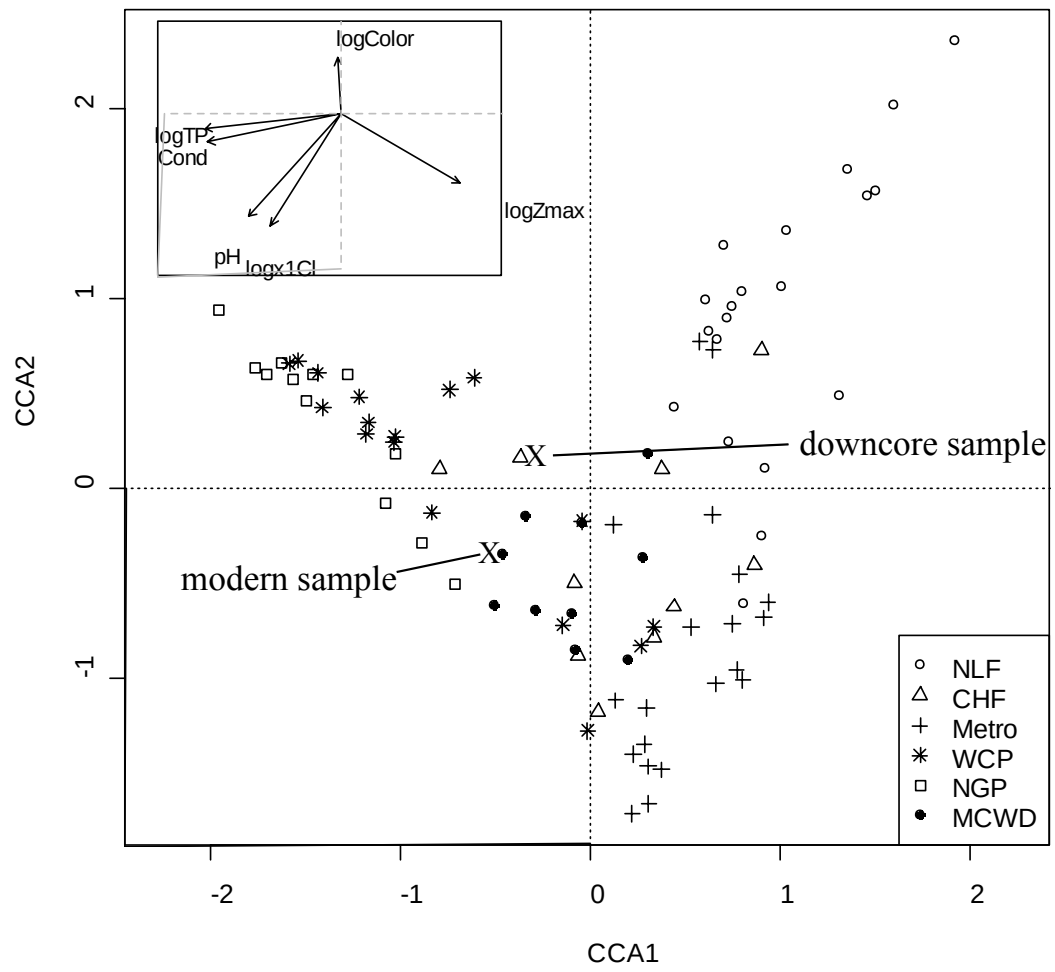


Figure 6. Subset of the MN calibration set (89 lakes), showing a modern and downcore assemblage from a Twin Cities metro lake passively plotted. This plot demonstrates that over time this lake has likely experienced an increase in pH and Cl, and a slight increase in TP and conductivity. Ecoregion codes: NLF=Northern Lakes and Forests, CHF=Central Hardwood Forests, Metro=Twin Cities Metropolitan Area, WCP=Western Corn Belt Plains, NGP=Northern Glaciated Plains, MCWD=Minnehaha Creek Watershed District.

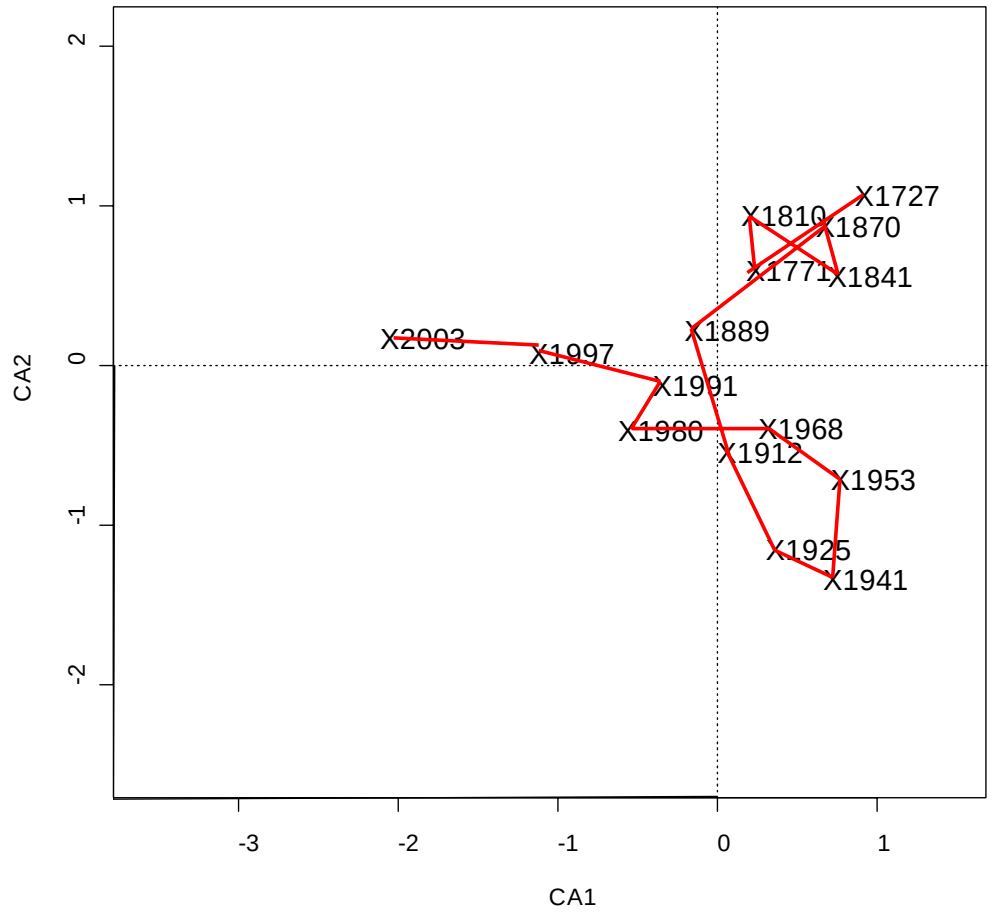


Figure 7. CA of downcore samples from Shell Lake (Sleeping Bear Dunes National Lakeshore) based on species assemblages from sections dating from 1727 to 2003.

Standard Operating Procedure #10: Reporting

Version 1.0

In **Diatom Monitoring Protocol**

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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 will promptly notify the project manager and/or the Great Lakes Network (GLKN) 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 footer of the document file. For complete instructions, please refer to Revising the Protocol, SOP #13.

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

Acknowledgements

This standard operating procedure is based on SOP #10, Reporting, by Sanders and Elias (2008), from the Water quality monitoring protocol for inland lakes (Elias et al. 2008).

10.0 Introduction

A primary goal of the NPS Inventory and Monitoring (I&M) Program is to ensure that the results and knowledge gleaned from monitoring are shared with all appropriate parties, especially the parks and their natural resource managers. Because the Great Lake Network's (GLKN) main focus is to assist parks with monitoring needs, we will strive to provide park managers with clear, meaningful products to convey our findings.

While GLKN primarily addresses concerns of the parks, its monitoring program has the potential to serve a much broader community. For example, monitoring projects can provide a starting point for external scientific research (especially to establish cause-effect relationships) and can provide insights for adaptive management on other public lands. The GLKN is also accountable to multiple organizations within the federal government, including the NPS I&M Program and the U.S. Congress. To ensure accountability and to meet the requests of all parties, we will provide the types of reports and communications detailed below.

10.1 Annual Report

The contractors will write annual reports in collaboration with GLKN's aquatic ecologist. The reports will be written using a typical scientific format and will contain:

- a brief introduction that describes why we are using diatoms for monitoring
- an outline of the sampling strategy, including the number and location of sites sampled
- multivariate statistical summaries of the diatom and water quality data, including tables and figures to enhance visual presentation, as well as a text explanation of the findings
- any other relevant or significant findings
- a discussion section in which important results are interpreted
- literature cited

The analyses and synthesis reports will become progressively more in-depth as lakes in the National Park units are monitored repeatedly. The reports will place the observed results in both a regional and historical context by relating them to other published literature. The significance of the results will be discussed in terms of environmental change, and management recommendations will be provided based on the findings.

The target audience of these reports will be the parks (primarily the natural resource managers), GLKN staff, and both regional and Servicewide I&M. Outside of the NPS, the target audience includes the four state departments of natural resources (Indiana, Michigan, Minnesota, Wisconsin), the St. Croix River Interagency Basin Team, the Twin Cities Metropolitan Council, and the broader scientific community.

10.2 Scientific Journal Articles

Diatom monitoring results are expected to be highly defensible and meet the standards of the peer-review process. The publication of diatom monitoring results in scientific journals will

allow GLKN to reach the scientific community in a way that internal NPS reports cannot. Further, peer-reviewed publications can promote collaborative investigation by members of the scientific community, either independently or in cooperation with GLKN. Ultimately, this process should foster a greater understanding of ecosystem components and processes.

For these reasons, the Great Lakes I&M Network will strive to publish analysis and synthesis reports in peer-reviewed scientific journals. The preparation of manuscripts will be encouraged, and reviewers of analysis and syntheses reports will be asked to recommend whether publication is warranted and suggest appropriate journals.

10.3 Other Communications

While reports are a definitive method of documenting the progress of each program, other means of communication can further disseminate information to a broader audience. To this end, the following additional types of communications will be provided:

Briefings to park biologists
The NPS project manager will present the findings from the water quality monitoring program to the biologists from the parks in which monitoring was conducted the previous year. These presentations, which will likely occur at the annual technical committee meeting in March, will provide a concise synopsis of monitoring results as well as management considerations.

Conference presentations
When possible, the researchers and the NPS project manager will present monitoring results at regional and national scientific conferences. Such presentations will allow GLKN to reach the broader scientific community, as well as land managers and conservation practitioners. Potential conferences include those sponsored by the Ecological Society of America, Society for Conservation Biology, The Wildlife Society, the Natural Areas Association, the NPS Water Professionals Meeting, and the George Wright Society. At a more local scale, the Western Great Lakes Research Conference, which is sponsored in part by GLKN, is a valuable venue for information exchange.

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Standard Operating Procedure #11: Post-Season Procedures

Version 1.0

In **Diatom Monitoring Protocol**

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11.0 Introduction

The proper maintenance and storage of field and laboratory equipment will prolong the life of the gear as well as simplify start-up procedures for the next field sampling season.

11.1 End of Season Procedures

11.1.1 Field Equipment

Clean and dry the surface corer and return removable parts to the storage case before storage. Inventory all supplies (such as specimen cups) and replace supplies as needed as soon as possible.

11.1.2 Laboratory Equipment

Inventory all supplies (microscope slides, coverslips, chemicals, etc.) and replace, if necessary, as soon as possible.

11.2 Data Management

Complete and accurate record keeping of field-derived data is extremely important. At the end of each field season, all field notes should be either scanned or photocopied. Field technicians, crew leaders, and project leaders share responsibility for collecting, verifying, and documenting data according to the guidelines in this monitoring protocol and all applicable standard operating procedures. Refer to the GLKN Data Management Plan for overall guidance (Hart and Gafvert 2005).

11.3 Quality Assurance/Quality Control

Proper care of all field and lab instrumentation and sampling gear is a fundamental part of any QA/QC program.

11.4 Literature Cited

Axler, R., E. Ruzycki, and J. E. Elias. 2008. Standard operating procedure #11, Post-season procedures. *In* Elias, J. E., R. Axler, and E. Ruzycki. 2008. Water quality monitoring protocol for inland lakes, Version 1.0. National Park Service, Great Lakes Network, Ashland, Wisconsin. Natural Resources Technical Report NPS/MWR/GLKN/NRTR—2008/109. National Park Service, Fort Collins, Colorado.

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Standard Operating Procedure #12: Quality Assurance and Quality Control

Version 1.0

In **Diatom Monitoring Protocol**

Prepared by
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Revision History Log

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12.0 Introduction

Long-term monitoring is only valuable if users have confidence in the data. Efforts to detect trends and patterns in ecosystem processes require high-quality, well-documented data that minimize error and bias. Data of inconsistent or poor quality can result in loss of sensitivity and lead to incorrect interpretations and conclusions.

NPS Director's Order #11B: Ensuring Quality of Information Disseminated by the National Park Service (www.nps.gov/policy/DOrders/11B-final.htm) specifies that information produced by the NPS must be of the highest quality and based on reliable data sources that are accurate, timely, and representative of the most current information available. Therefore, GLKN will establish and document procedures for quality assurance (QA) and quality control (QC) to identify and reduce the frequency and significance of errors at all stages in the data life cycle.

Under these procedures, the progression from collection of samples in the field to verified data to validated data implies increasing confidence in the quality of those data. Quality assurance and quality control procedures will document review processes and include guidance for addressing problems with data quality.

Examples of general QA/QC practices include:

- Standardized field data collection forms
- Proper maintenance of equipment
- Training of field crew and laboratory technicians
- Duplicate sampling

12.1 QA/QC Specific to Other SOPs

12.1.1 Field Methods

The Wiegner corer, used to collect surface sediment samples, is not a complicated instrument, though collection of a good sediment sample depends on its proper use. Ensure field personnel are adequately trained in the proper use of the corer and have ample opportunity to practice taking samples prior to taking an actual sample. To ensure cross-contamination of samples does not occur, thoroughly clean corer and all parts between sampling sites.

Collect the sample during the final round of water quality sampling at the proper location. Examine the core in the field to ensure it is a good sample (e.g., depositional sediment rather than sand, and an undisturbed sediment-water interface). Use new disposable pipettes for dispensing each sample into sample cups to avoid cross-contamination. Record the sampling location on the field form and ensure all other information is recorded completely and accurately. Send a copy of the field form to the contractor and keep the original on file in the GLKN office. Store the sample in the dark on ice.

Only one field sample will be collected. In lake environments, the deep-water sediments provide a highly integrated sample of diatom community structure for the lake as a whole. Sedimentary processes collect and mix diatoms from all habitats within the lake (plankton, periphyton, epilithic, epipelic) and deposit them in a uniformly mixed assemblage across the deeper regions

of the lake. This means that only a single sample of surface sediment is needed to characterize the entire lake (Anderson 1990) – as compared to the numerous samples required to overcome spatial heterogeneity for other bioindicators (e.g., benthic invertebrates). The uniformity of sedimentary diatom samples has been repeatedly verified from multiple cores and transects of surface samples taken from individual lakes (e.g., Anderson 1990). Although there is a tendency for benthic species to be better represented in near-littoral areas, this does not present a problem for surface sediments if roughly the same locations are resampled (which is easily accomplished with GPS). Resampling will be done within meters of a station's fixed location to minimize variability across the depositional basin. At this sampling resolution, difference in cores and surface samples will be minimal (Charles et al. 1991, Petterson et al. 1993).

12.1.2 Cleaning Samples and Preparing Slides

Process the samples within two weeks of collection to ensure samples are representative of the diatom assemblage at the time of sampling. Avoid cross-contamination of samples during every step of handling by maintaining clean work surfaces and using new disposable pipettes for each sample.

All slides will be prepared in duplicate: set 'a' will be counted, set 'b' will be archived and available for loan to other laboratories. To ensure duplicates are as similar to each other as possible, mix each sample thoroughly prior to preparing the slides. Follow procedures detailed in SOP #8 for the archival of duplicate slides.

12.1.3 Counting Slides

Any new technicians introduced into the program will need to be trained by an experienced diatom taxonomist. To ensure internal taxonomic consistency, photographic databases will be constructed for all predominant diatom taxa; this collection of photographs will be maintained at the St. Croix Watershed Research Station (SCWRS).

Each year, 10% of the samples collected will be recounted by another analyst in the program. The primary analyst will etch a line in the slide to mark the transect counted, and will etch circles at the start and end points of the area counted along the transect. The QC analyst will then use the etchings to count the exact same portion of the slide. The percent difference between the two counts will be calculated as follows:

$$\%\text{Difference} = (1 - \Sigma \min(a,b)) * 100$$

Where a and b are the relative proportions recorded for a given taxon by the primary taxonomist (a) and the QC taxonomist (b).

The two counts should have a percent difference of less than or equal to 30% (this is the criteria being used by the EPA National Lakes Assessment program; K. Manoylov, pers. comm.). If the percent difference exceeds 30%, the two taxonomists will discuss the discrepancies between the samples. If significant differences are found to exist in how the two analysts have been identifying diatoms, samples may have to be recounted by the primary taxonomist.

Duplicate counts will be used only for taxonomic control; only the original counts will be used in data analysis.

12.1.4 Archiving

Archived slides and samples will be checked periodically to be sure that they have not been damaged while in storage.

12.2 Literature Cited

Anderson, N. J. 1990. Variability of diatom concentrations and accumulation rates in sediments of a small lake basin. Limnology and Oceanography **35**:497-508.

Charles, D. F., S. S. Dixit, J. P. Smol and B. F. Cumming. 1991. Variability in diatom and chrysophyte assemblages and inferred pH: paleolimnological studies of Big Moose Lake, N.Y. Journal of Paleolimnology **5**:267-284.

Petterson, G., I. Renberg, P. Geladi, A. Lindberg, and F. Lindgren. 1993. Spatial uniformity of sediment accumulation in varved lake sediments in northern Sweden. Journal of Paleolimnology **9**:195-208.

Standard Operating Procedure #13: Procedure for Revising the Protocol

Version 1.0

In **Diatom Monitoring Protocol**

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

Acknowledgements

This standard operating procedure is based on SOP #12, Procedure for revising the protocol, by Sanders and Elias (2008), from the Water quality monitoring protocol for inland lakes (Elias et al. 2008).

13.0 Introduction

Long term monitoring programs like that of the National Park Service must be able to accommodate change. Refined field methods, advances in analytical techniques, and feedback from field crews and project managers can all contribute to the improvement of the monitoring protocols. The purpose of the current SOP is to define a systematic and routine process for incorporating these changes into the protocol.

13.1 Steps for Revising the Protocol

Participants in this diatom monitoring project who see a need for change should:

1. Attempt to incorporate the changes by first modifying only the SOP(s), without making changes to the protocol narrative. However, if it is clear that changes are needed in the narrative, it should be revised as well.

2. Make all revisions using the Track Changes feature of Microsoft Word. For minor changes, at least one other person must review the revision. If the change is more extensive, a discussion by Network staff is warranted before acceptance of the revision. For major changes, review from outside of the Network should be sought. Examples of major changes include modifications of the sampling design, significantly altered field methods, and revised analysis techniques.

3. Record the changes in the revision history log of the SOP and/or in the narrative, as appropriate. Include the date of revision, full name(s) and affiliation(s) of author(s), description of and reasons for the changes, and section of SOP or narrative where changes were made.

4. Rename the version of the SOP and/or narrative. For minor changes, only revise the version number after the decimal point (e.g., change V. 1.1 to V. 1.2). For major changes, revise the number before the decimal point (e.g., V. 2.3 to 3.0). Also change the version number of the SOP or protocol in the header or footer, as appropriate.

5. Notify the data manager of the change(s) so that the metadata of the project database will be updated.

6. Distribute the revised version to all appropriate parties, including the members of the field crew and appropriate GLKN staff. The revised version must also be posted on the Network's website.

7. Maintain a library of previous versions. Such historical information may be crucial for understanding, interpreting, and analyzing data.

13.2 Literature Cited

Elias, J. E., R. Axler, and E. Ruzycki. 2008. Water quality monitoring protocol narrative for inland lakes, Version 1.0. National Park Service, Great Lakes Network, Ashland, Wisconsin. Natural Resources Technical Report NPS/MWR/GLKN/NRTR—2008/109. National Park Service, Fort Collins, Colorado.

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