United States
Department of
Agriculture

Forest Service

Rocky Mountain Research
Station

General Technical Report
RMRS-GTR-278WWW

April 2012

Forest Service National Protocols for Sampling Air Pollution-Sensitive Waters

Prepared for the USDA Forest Service's
Air Resource Management Program

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Acknowledgments

These protocols were prepared by E&S Environmental Chemistry, Inc., under contract to the U.S. Forest Service, Air Resources Management Program, under the direction of A. Mebane. Protocol development benefited greatly from extensive review comments received on earlier drafts from Forest Service staff. In particular, helpful guidance and suggestions were provided by A. Mebane, C. Huber, J. Gurrieri, B. Gauthier, S. Grant, B. Roper, M. Hudy, C. Carlson, and W. Shaw. The final document was prepared by J. Charles. Field testing of the draft sampling protocols was kindly provided by C. Huber, T. Porwoll, and T. Wickman.

Cover Photo

Todd McDonnell, E&S Environmental Chemistry, Inc., Corvallis, OR.

Citation

Sullivan, T.J., ed. 2012. **USDA Forest Service national protocols for sampling air pollution-sensitive waters**. Gen. Tech. Rep. RMRS-GTR-278WWW. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 334 p.

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Table of Contents

Section 1. Water Chemistry Field Sampling Protocols 1

1.1 BACKGROUND 1

1.1.1 Resources Sensitive to Atmospheric Deposition 2

1.1.2 Study Purpose and Objectives 7

1.2 STUDY DESIGN 11

1.2.1 Lake or Stream Characterization 12

1.2.2 Synoptic Survey 14

1.2.3 Long-Term Monitoring 14

1.2.4 Characterization of Stream Chemistry During Hydrologic Events 15

1.2.5 Other Uses of Resulting Data 15

1.3 WHERE, WHAT, AND WHEN TO SAMPLE 16

1.3.1 Where to Sample 18

1.3.2 What to Measure 24

1.3.3 When to Sample 31

1.4 FIELD METHODS 34

1.4.1 Pre-Trip Preparations 35

1.4.2 Sample Collection 40

1.4.3 On-Site Measurements 48

1.4.4 Post Collection Sample Processing, Documentation, and Cleanup 54

1.4.5 Sample Packaging and Transportation 54

1.4.6 Sequence of Field Activities 57

1.4.7 Safety in Field Activities 57

Section 2. Laboratory Protocols 59

2.1 INTRODUCTION 59

2.2 LABORATORY PREPARATION PRIOR TO SAMPLE ANALYSIS 59

2.2.1 Bottle Cleaning 59

2.2.2 Sample Processing, Preservation, and Storage 63

2.3 CHEMICAL ANALYSIS 67

Section 3. Quality Assurance/Quality Control Protocols 71

3.1 INTRODUCTION 71

3.2 ATTRIBUTES OF DATA QUALITY 72

3.2.1 Method Detection and Reporting Limits 72

3.2.2 Precision and Accuracy 76

3.2.3 Completeness 77

3.2.4 Comparability 78

3.2.5 Representativeness 78

3.2.6 Recommended Laboratory Data Quality Objectives 78

3.3 QA/QC SAMPLE TYPES 79

3.3.1 Filter Blanks, Analytical Blanks, and Field Blanks 80

3.3.2 Replicate Environmental Samples 81

3.3.3 Spiked Project Samples 82

3.3.4 External Quality Assurance Samples 82

3.4 FIELD QUALITY ASSURANCE 84
3.4.1 Sample Containers 84
3.4.2 Field Measurements 84
3.5 REPORTING QUALITY ASSURANCE DATA 85
3.6 LABORATORY AUDITS AND CERTIFICATION 87
3.7 DATA ENTRY 88
3.8 SUMMARY 88
Section 4. Data Analysis Protocols 91
4.1 BACKGROUND AND OBJECTIVES 91
4.2 EVALUATION OF DATA QUALITY 93
4.2.1 Charge Balance 93
4.2.2 Calculated Versus Measured Conductivity 97
4.2.3 Calculated Versus Measured ANC 98
4.2.4 Other Validation Procedures 100
4.2.5 Final Data Quality Determination 101
4.3 APPLY PROCEDURES TO PREPARE RAW DATA FOR GRAPHICAL AND STATISTICAL ANALYSIS 102
4.3.1 Censored Data 102
4.3.2 Outliers and Missing Values 102
4.3.3 Multiple Observations 105
4.3.4 Treatment of Zeros and Negative Values 106
4.3.5 Treatment of Seasonality 106
4.4 CONDUCT EXPLORATORY ANALYSES 107
4.4.1 Analysis of Water Quality Status 107
4.4.2 Graphing and Quality Analysis 108
4.4.3 Recommended Data Analyses 109
4.5 CONDUCT STATISTICAL ANALYSES 128
4.5.1 Statistical Tests for Difference 128
4.5.2 Trend Detection 130
4.5.3 Statistical Power 133
4.6 REPORT DATA IN STANDARDIZED FORMATS 134
4.7 SUMMARY AND CONCLUSIONS 134
Section 5. Field Sampling Protocols for Aquatic Biota 135
5.1 BACKGROUND 135
5.1.1 Lake Zooplankton 135
5.1.2 Stream Macroinvertebrates 136
5.2 STUDY DESIGN 137
5.3 SITE SELECTION 138
5.3.1 Laying Out the Support Reach for Stream Macroinvertebrates 138
5.3.2 Lake Selection for Zooplankton Sampling 141
5.4 PRE-TRIP PREPARATION 143
5.4.1 Equipment and Supplies 143
5.4.2 Equipment Cleaning Protocols 144

5.5 COLLECTION PROCEDURES 145
5.5.1 Stream Benthic Macroinvertebrates 145
5.5.2 Lake Zooplankton 149
5.6 SAMPLE PROCESSING, PRESERVATION, AND HANDLING 151
5.6.1 Stream Benthic Macroinvertebrates 151
5.6.2 Lake Zooplankton 153
5.7 DOCUMENTATION AND TRACKING 154
5.8 LABORATORY ANALYSIS 154
5.9 QUALITY ASSURANCE 155
5.9.1 Sample Replication 155
5.9.2 Taxonomy 155
5.10 INTERPRETATION 156
5.10.1 Biological Metrics 156
5.10.2 Multimetric Indices and Predictive Modeling 157
5.10.3 Taxonomic Resolution 157
5.10.4 Index of Biotic Integrity 158
5.10.5 Biotic Effects Analysis 161
Section 6. Transition Plan 167
6.1 BACKGROUND 167
6.2 TRANSITION STEPS 167
6.3 ANTICIPATED PROTOCOL CHANGES 170
6.4 DECISION OF WHETHER OR NOT TO CHANGE PROTOCOLS 171
Section 7. Training Plan 173
7.1 BACKGROUND 173
7.2 PLANNING THE TRAINING SESSIONS 174
7.3 CLASSROOM TRAINING 176
7.3.1 Handouts 177
7.3.2 Questions 177
7.3.3 Prepare for Field Training 177
7.4 FIELD TRAINING 177
Section 8. References 179

Appendices..... 189

APPENDIX A. ACRONYMS AND ABBREVIATIONS

APPENDIX B. GLOSSARY

APPENDIX C. SAMPLING PROCEDURE FOR SURFACE WATER CHEMISTRY

APPENDIX D. STANDARD OPERATING PROCEDURES FOR FIELD SAMPLING ACTIVITIES

APPENDIX E. DATA ENTRY FORMS AND INSTRUCTIONS FOR FIELD SAMPLING ACTIVITIES

APPENDIX F. LABELING INSTRUCTIONS FOR FIELD SAMPLE CONTAINERS

APPENDIX G. EXAMPLE STANDARD OPERATING PROCEDURES FOR LAB ANALYSIS PROTOCOLS

APPENDIX H. TRAINING CHECKLISTS

APPENDIX I. JOB HAZARD ANALYSIS (JHA)

List of Tables

Table 1-1. Recommended sensitive receptors and indicators for air quality related values affected by atmospheric deposition of air pollutants.....3

Table 1-2. General guidelines for evaluating the likelihood of aluminum toxicity in fresh water.....6

Table 1-3. Common management issues for FS ARM program staff, with associated field study approaches.....8

Table 1-4. Elements to be considered in formulating sampling questions.....10

Table 1-5. Examples of questions that could be used to guide inventory, characterization, and monitoring study design.....11

Table 1-6. General guidance regarding water quality study design.....12

Table 1-7. Parameters to consider for possible inclusion in surface water acidification studies.....27

Table 1-8. Parameters to consider for possible inclusion in studies of atmospheric nutrient nitrogen enrichment of fresh waters.....28

Table 1-9. Suggested timing of surface water samples for evaluation of sensitivity to and effects from atmospheric deposition.....31

Table 1-10. Key issues to consider when initiating and implementing an inventory of lake or stream water chemistry to assess the effects of atmospheric deposition.....36

Table 1-11. Key issues to consider when initiating and implementing a long-term monitoring effort to document and quantify trends in lake or stream chemistry over time in response to inputs of atmospheric deposition.....36

Table 1-12. Key issues to consider when conducting modeling using the MAGIC model (Cosby et al. 1985) to estimate critical load or to calculate changes in lake/stream chemistry in response to future emissions controls.....36

Table 1-13. Ancillary measurements that may help in interpretation of lake or stream water chemistry data.....53

Table 2-1. Summary of a typical bottle rinsing protocol for low-nutrient, low-ionic strength samples...61

Table 2-2. Example laboratory aliquot schedule for a particular project.....64

Table 2-3. Recommended laboratory holding times.....65

Table 2-4. Example low-volume sample schedule.....67

Table 3-1. Recommended DQOs for detection limits, precision, accuracy, and completeness.....76

Table 3-2. Recommended laboratory quality control samples.....80

Table 3-3. Field quality control samples.....84

Table 4-1. Some example approaches for FS ARM program data analysis, tied to the purpose and general approach of the field study.....92

Table 4-2. Factors for converting mg/L units or pH units to μeq/L units.....94

Table 4-3. Data validation quality control procedures.....96

Table 4-4. Critical Q-values for Dixon's outlier Q-test, at the 0.95 confidence level.....105

Table 4-5. Example variables with which to subset water quality data, according to measured water chemistry for analysis. 110

Table 4-6. Example variables with which to subset water quality data for analysis according to features other than measured water chemistry. 110

Table 4-7. Values of *t*. 131

Table 5-1. Example approaches for FS ARM program biological sampling, tied to the purpose of the field study. 137

Table 5-2. Equipment and supplies for benthic macroinvertebrates. 143

Table 5-3. Equipment and supplies for collecting zooplankton samples. 144

Table 5-4. List of metrics in each category used in the EPA National Wadeable Stream Assessment. 160

List of Figures

Figure 3-1. Schematic illustration of precision and accuracy. 77

Figure 3-2. Results from analysis of low-concentration QC samples for nitrate analysis. 86

Figure 3-3. Low-concentration QC samples for sulfate analysis. 87

Figure 4-1. Cation sum versus anion sum. 95

Figure 4-2. Calculated versus measured conductivity. 98

Figure 4-3. Calculated carbonate alkalinity versus laboratory titrated ANC with no obvious outliers. 99

Figure 4-4. Patterns in DOC concentration in streams within a particular region. 101

Figure 4-5. Minimum stream ANC sampled at each site during each year versus median spring ANC for all samples collected at that site during that spring season. 107

Figure 4-6. Box plots comparing hypothetical lake ANC values measured in samples collected during the spring versus the fall season. 108

Figure 4-7. Changes in the concentration of major water chemistry constituents during a hypothetical hydrological episode in one stream. 112

Figure 4-8. Time series of major ions and discharge in Treasure Lake in the Sierra Nevada during snowmelt in 1993. 113

Figure 4-9. Daily discharge (A) and nitrate concentration (B) in Icy Brook and Andrews Creek within the Loch Vale watershed, Rocky Mountain National Park, in April-September 1992. 114

Figure 4-10. Ratio of NO_3^-:(SO_4^{2-} + NO_3^-) concentration versus ANC in stream water samples collected during hydrological episodes in four streams included in the Adirondack region of EPA's Episodic Response Program. 115

Figure 4-11. Map of summer NO_3^- concentrations in drainage lakes sampled by the Adirondack Lakes Survey Corporation in the Adirondack region of New York. 116

Figure 4-12. Example space-for-time substitution analysis for four variables across a gradient of expected response. 117

Figure 4-13. Results of a classification system devised to indicate expected low-ANC streams (in this example, based on geology and elevation) compared with actual low-ANC streams, which are represented as dots on the map. 118

Figure 4-14. A histogram of the frequency of occurrence within a region (or forest) of surface water SO_4^{2-} concentration can reveal the typical distribution of the range of values that may be attributable to broad patterns of regional atmospheric inputs versus the more sporadic occurrence of high values that more likely derive from geological sources of S. 119

Figure 4-15. Critical load simulated by the MAGIC model to protect streams in Shenandoah National Park against acidification to ANC below 0 (top panel) and 20 μeq/L (bottom panel) by the year 2040 is plotted as a function of 1990 ANC. 120

Figure 4-16. Example trends analyses of ANC in lake and stream waters, based on data from EPA's Long Term Monitoring program. 122

Figure 4-17. Plot of ANC versus ion ratio of SO_4^{2-} concentration divided by the sum of base cation concentrations (SBC) for low ANC (≤ 200 μeq/L) surface waters in (a) Adirondacks, (b) Catskill Mountains, and (c) Cascade Mountains. 124

Figure 4-18. Relationship between lake size and lake ANC in the Adirondack Mountains..... 125

Figure 4-19. Nitrogen outputs in soil water or stream water versus N deposition inputs throughout Europe. 126

Figure 4-20. Observed relationship between NO_{3-} leaching loss in runoff and mean air temperature at an experimental watershed site at Gårdsjön, Sweden. 127

Figure 4-21. Example patterns of NO_{3-} concentration in surface water at four sites at various stages of watershed N-saturation. 128

Figure 4-22. Schematic representation of data normality. 129

Figure 4-23. Plot, with regression line, of SO_{42-} concentration in Deep Run, Shenandoah National Park, over the period of monitoring record. 130

Figure 5-1. Equipment, supplies, and sequence for collecting benthic macroinvertebrate samples..... 141

Figure 5-2. Modified D-frame kick net. 146

Figure 5-3. Wisconsin net and collection bucket diagram. Some microzooplankton nets have a reducing collar attached. 150

Figure 5-4. Location of the nine ecoregions used in Wadeable Streams Assessment IBI development. 159

Figure 5-5. Schematic depicting mayfly richness over time in response to changes in stream chemistry. 162

Figure 5-6. Average number of families of aquatic insects for each of 14 streams in Shenandoah National Park versus the mean (left) or minimum (right) ANC of each stream. 164

Figure 5-7. Zooplankton taxonomic richness versus ANC for a combined Adirondack dataset, based on 111 lake visits to 97 lakes in the EMAP, ELS, and STAR zooplankton surveys. 166

SECTION 1.
WATER CHEMISTRY
FIELD SAMPLING PROTOCOLS

T.J. Sullivan, J.R. Webb, A.T. Herlihy, and G.B. Lawrence

1.1 BACKGROUND

The first step in designing a surface water sampling program is identifying one or more problems or questions that require information on water quality. Common water quality problems include nutrient enrichment (from a variety of causes), effects of atmospheric deposition (acidification, eutrophication, toxicity), and effects of major disturbances such as fire or pest infestations. Once the problems or questions have been clearly defined, a sampling program can be designed that addresses where to sample, what to measure, and when and how to conduct the sampling. The selection of measurements should be tailored to specific study objectives and to the study design, which guides the specifics of field, laboratory, and data analysis protocols.

A variety of air pollutants have the potential to stress aquatic ecosystems through contributions from the atmosphere to the Earth's surface. The main focus of this protocol is on atmospheric pollutants that contribute to surface water acidification and eutrophication (nutrient enrichment). Both atmospheric sulfur (S) and nitrogen (N) have the potential to cause acidification. Atmospheric N can also cause eutrophication of aquatic ecosystems in which the N supply is limiting for algal or plant growth. Sampling for atmospherically deposited toxic materials is also addressed, but with lesser coverage.

Acidification

When fossil fuels are burned, as in the combustion of coal in power plants, sulfur and nitrogen are emitted into the atmosphere. The sulfur and nitrogen can contribute to the acidification of surface waters and soils in the ecosystem.

Large areas of the Northeast, Appalachian Mountains, northern Florida, Upper Midwest, and mountainous portions of the Western United States have many lakes and streams with low acid neutralizing capacity and are therefore potentially sensitive to acidification from atmospheric deposition of sulfur and/or nitrogen.

There are already many acidified surface waters in the Eastern United States, while in the Western United States many surface waters are susceptible to acidification, but levels of acidic deposition are relatively low and acidic surface waters are rare. Many of the areas having acid-sensitive surface waters, especially in the Northeastern United States and Appalachian Mountains also contain extensive areas with acid-sensitive soils.

Many fresh waters in the United States are thought to be phosphorus (P)-limited (U.S. EPA 2008). In such waters, the addition of P would be expected to increase plant and/or algal growth, whereas the addition of N would not. Nevertheless, there are also fresh waters considered to be N-limited or N and P co-limited.

The U.S. Forest Service (FS) collects and analyzes data to identify ecosystems and resources sensitive to air pollution and to determine if these resources are being impacted. Such studies are often conducted at the individual forest level. This protocol provides FS staff with a consistent framework regarding decisions of where, when, and how to conduct water sampling for the purpose of evaluating and monitoring air pollution effects on aquatic ecosystems. It is based on protocols developed by the U.S. Environmental Protection Agency, U.S. Geological Survey, and Forest Service, including documents prepared by Herlihy (1997), Turk (2001), Webb et al. (2004), Eilers (2007), and Sullivan and Herlihy (2007). This framework allows the user to build a site-specific project plan based on the relevant management questions. The primary focus of this section is on monitoring effects from atmospheric pollutants that contribute to surface water acidification and eutrophication (nutrient enrichment). References that provide more details on these measurements are also included. Additional sections address protocols for quality assurance/quality control (QA/QC) for water chemistry sampling, laboratory analyses, data analysis, and protocols for sampling and analyzing aquatic biota.

Nutrient Enrichment

In N-limited and co-limited aquatic systems, atmospheric N deposition can influence algal growth, trophic state, and the distribution and abundance of diatoms and other aquatic species. Lakes and streams that are wholly or partly N-limited are most likely to occur in remote regions with naturally oligotrophic surface waters that have not received high levels of atmospheric N deposition in the past. Within the United States, such lakes and streams are most common in the mountainous West.

1.1.1 RESOURCES SENSITIVE TO ATMOSPHERIC DEPOSITION

AIR QUALITY-RELATED VALUES

There are many approaches that can be used by the FS to assess the: 1) current condition of surface waters; 2) sensitivity of aquatic natural resources to potential degradation from atmospheric deposition of S, N, or toxic materials; and 3) extent to which sensitive aquatic natural resources have been harmed in the past or might be harmed in the future under scenarios of future air pollution and atmospheric deposition. A number of approaches for monitoring aquatic resources are highlighted in this protocol; those expected to be most useful to the FS Air Resource Management (ARM) program are listed in Table 1-1. This protocol recommends that such approaches be routinely considered in making evaluations regarding atmospheric deposition sensitivity and/or effects. Site-specific studies can be further customized to fit particular regional or local ecosystem conditions and stressors.

Table 1-1. Recommended sensitive receptors and indicators for air quality related values affected by atmospheric deposition of air pollutants. (Source: modified from Sullivan and Herlihy 2007.)

AQRV	Sensitive Receptor	Indicator/Metric	Potential Criteria*
Flora	red spruce (East)	growth decline	change in diameter change in extent of damage
	sugar maple (East)	growth decline	change in diameter change in extent of damage
	lichens	community composition	loss of sensitive taxa
Soil	soil chemistry	base saturation exchangeable Ca^{2+} exchangeable $Ca^{2+} + Mg^{2+}$ C:N molar ratio	BS < 10% % change over time % change over time C:N < 0.2
	soil solution chemistry	Ca:Al molar ratio $[Ca^{2+} + Mg^{2+} + K^{+}]$:Al molar ratio NO_3^- concentration	Ca:Al < 1.0 BC:Al < 1.0 NO_3^- > 20 µeq/L during growing season
Water	water chemistry	ANC (acid neutralizing capacity) NO_3^- concentration SO_4^{2-} concentration	ANC < 50 µeq/L NO_3 > 10 µeq/L change over time
	water productivity	chlorophyll *a* clarity (lakes)	change over time change over time
	fish	salmonid species presence fish species richness fish condition factor fish Hg concentration fish pesticides(s) concentration	loss over time change over time change over time Hg > 0.3 ppm above threshold values
	zooplankton (lakes)	total zooplankton richness crustacean taxonomic richness rotifer taxonomic richness	change over time change over time change over time
	benthic macroinvertebrates (streams)	mayfly taxonomic richness Index of Biotic Integrity	loss of sensitive taxa deviation from reference
	diatoms	community composition	historical change from paleolimnological reconstruction

* Metrics can be represented in multiple ways, often as change over time detected in a monitoring program or as exceedence above or below a threshold value. Typically, multiple threshold values are possible. For example, surface water target ANC thresholds are commonly set at 0, 20, or 50 µeq/L to achieve different levels of protection.

Atmospheric deposition can contribute to toxicity responses in aquatic systems in several ways. Water acidification entails chemical changes including reduced pH (increased hydrogen ion $[H^+]$ activity), decreased acid neutralizing capacity (ANC), increased inorganic monomeric aluminum (Al_i) concentration, and changed (increased or decreased depending on the extent of acidification) concentrations of calcium (Ca^{2+}) and other base cations (BC). Hydrogen ion and Al_i can be toxic to many aquatic species at sufficiently high concentrations. Other atmospheric pollutants of concern include mercury (Hg) and some pesticides. Atmospheric deposition is an important

component of Hg cycling and biogeochemistry. Mercury in its methylated form is known to bioaccumulate in aquatic organisms, reaching potentially high concentrations in larger piscivorous fish and species that consume them.

Air quality related values (AQRVs) are resource elements that may be damaged by air pollution or atmospheric deposition. There are many possible sensitive receptors for each AQRV. Sensitive receptors monitored for the AQRV water often include its chemistry, which influence its suitability to support various aquatic species and life forms. Acid neutralizing capacity is one indicator of change for the sensitive receptor water chemistry. There are also sensitive biological receptors, which reflect the suitability of the lake or stream water for supporting aquatic organisms that might be sensitive to acidification or eutrophication. These could include specific species of fish, zooplankton, macroinvertebrates, or diatoms. A sensitive receptor can be evaluated by measuring indicators of injury or ecosystem change.

A limited list of key variables does not exist with which to measure ecosystem condition, or ecosystem response to stressors, such as those associated with atmospheric deposition (e.g., acidification, eutrophication, toxicity). Ecosystems are highly complex and cannot be represented by a handful of variables. Nevertheless, some variables are considered reflective of the general level of ecosystem harm associated with atmospheric deposition. This protocol proposes a set of consistent AQRVs and associated sensitive receptors for aquatic ecosystems that could be used by the FS nationwide for evaluation of resource sensitivity to, and effects from, atmospheric deposition (Table 1-1; Sullivan and Herlihy 2007). Individual forests may wish to augment these listed items to emphasize other AQRV receptors that are especially important to a particular forest or region, or for which that forest has specialized expertise. Nevertheless, the recommended sensitive receptors summarized in Table 1-1 are broadly applicable and reflect a range of aquatic effects of atmospheric deposition. Identification of these receptors and indicators helps to determine the protocols that will be needed by the FS ARM program for nationwide inventory and monitoring.

Detailed protocols should be an important part of any resource characterization and/or monitoring program intended to evaluate atmospheric deposition impacts on AQRVs. Standard protocols help to ensure that measured differences among locations or changes over time at one location actually occur and are not a reflection of different methods, sampling personnel, or timing of sample collection. Protocols are necessary to ensure that the data collected are appropriate to the question(s) asked and are of sufficient quality to allow development of meaningful answers. It must, however, be recognized that there will not be a single appropriate protocol in every situation that will efficiently characterize an important attribute nationwide. In limited circumstances, some attributes and site characteristics are sufficiently variable from region-to-region so that supplemental or amended protocols may be justified. Nevertheless, adoption of national-scale procedures for data gathering, analysis, and required core data elements will allow data to be compared across forest- and regional-level boundaries and will provide information that is needed for national assessments and decision-making. Aquatic effects inventory and monitoring for atmospheric deposition effects on FS lands have historically focused on both lakes (mainly in the western United States and streams (mainly in the eastern United States). To the extent practical, this protocol describes attributes and methods that are applicable to both lakes and streams, and that can be applied throughout most or all regions of the United States. This

protocol addresses the sensitive surface water receptors, and associated field, laboratory, and data analysis approaches that are most useful for meeting FS air program objectives.

SENSITIVE CHEMICAL INDICATORS OF WATER QUALITY

Sensitivity to acidification and its effects are commonly evaluated using several chemical criteria, especially ANC, pH, and Al_i. Sensitivity of surface waters to chronic and episodic acidification depends on watershed soil characteristics, mineralogy, and hydrologic flow paths within the watershed (Chen et al. 1984, Cosby et al. 1985), as well as on the current and historic atmospheric deposition loadings of acids and bases. Surface water ANC provides an initial baseline point from which to quantitatively assess the status of stream or lake chemistry. Biological effects of acidification have been associated with a variety of ANC benchmarks, the most common of which are ANC's equal to 0, 20, and 50 microequivalents per liter (μeq/L). Waters with ANC $\leq$ 0 μeq/L have no capacity to neutralize acid inputs; they are acidic by definition. Lakes and streams with ANC chronically less than 0 μeq/L are often fishless or contain few species of fish. Brook trout, which are relatively acid-tolerant, have been shown to be sensitive to episodic acidification[1] when chronic stream ANC is less than about 20 μeq/L. A general benchmark for sensitivity of other types of aquatic biota is often established at ANC equal to 50 μeq/L (Driscoll et al. 2001). Some species may be affected at higher ANC values, even at levels of 100 μeq/L or above. Generally, surface waters with ANC $\leq$ 50 μeq/L are considered prone to episodic acidification (DeWalle et al. 1987, Eshleman 1988), especially where seasonal snowpack accumulations are substantial. Such low ANC waters may also be susceptible to future chronic acidification at current or increased rates of acidic deposition.

Common reference values for pH are 5.0, 5.5, and 6.0. Such values are often used to evaluate the possible extent of adverse effects on fish and other aquatic organisms. Threshold pH levels for adverse biological effects have been summarized for a variety of aquatic organisms (Haines and Baker 1986, Baker et al. 1990). The effects of low pH are specific to the organism and region under consideration and depend upon the concentrations of other chemical constituents in the water, notably Al_i and Ca^{2+}. Lakes or streams having pH less than about 5.0 generally also have ANC less than 0 μeq/L and often do not support fish. Depending on the region, waters having pH greater than about 6.5 and ANC greater than about 50 to 100 μeq/L support large, but variable, numbers of species. Populations of salmonid fish are generally not found at pH levels less than 5.0, and smallmouth bass (*Micropterus dolomieu*) populations are usually not found at pH values less than 5.2 to 5.5 (Haines and Baker 1986). A number of synoptic surveys indicate loss of species diversity and absence of many other fish species in the pH range of 5.0 to 5.5 (Haines and Baker 1986). Levels of pH less than 6.0 to 6.5 have been associated with adverse effects on populations of dace, minnows, and shiners (family Cyprinidae), and bioassays suggest that, given sufficient Al_i concentrations, pH less than 6.5 can lead to increased egg and larval mortality in blueback herring (*Alosa aestivalis*) and striped bass (*Morone saxatilis*; Klauda et al. 1987, Hall 1987).

Aluminum (Al) toxicity to aquatic organisms is caused primarily by inorganic forms of Al rather than organically complexed Al (Al_o); Driscoll et al. 1980, Baker and Schofield 1982, Havas

[1] Episodic acidification refers to the temporary (usually hours to days) decrease in lake, or especially stream, ANC that occurs in response to hydrologic events such as rainfall or snowmelt.

1985). There is limited evidence of biological effects at Al_i less than 50 µg/L (~ 2µM). Free Al concentrations (Al_3^+; roughly equivalent to Al_i concentrations at pH values substantially below 5.0) between 50 and 200 µg/L have been shown to reduce the growth and survival of various species of fish (Muniz and Leivestad 1980, Baker and Schofield 1982). Concentrations of Al_i greater than 200 µg/L are generally considered to have toxic effects to the majority of freshwater fish species (Table 1-2).

Table 1-2. General guidelines[1] for evaluating the likelihood of aluminum toxicity in fresh water.

Concentration of Inorganic Monomeric Al	Expected Response of Aquatic Biota
< 50 µg/L	Biological effects not common in most forms of aquatic biota.
50 to 200 µg/L	Reduced growth and survival of various species of fish, including brook trout, and likely other aquatic life forms.
> 200 µg/L	Adverse effects likely for most freshwater fish species.

[1] Variability is high with species, life stage, and various aspects of water chemistry, including Ca^{2+} concentration, dissolved organic carbon (DOC), and total fluorine (F) concentration.

Sensitivity of surface waters to eutrophication and the nutrient status of lakes and streams are typically evaluated on the basis of concentrations of N and P. These nutrients can be assessed as total N and/or total P, or as one or more of the various forms that commonly occur in surface waters, such as nitrate (NO_3^-) and soluble reactive phosphorus (SRP). The EPA has provided guidance to States for setting nutrient criteria for total N and P concentrations in U.S. lakes (U.S. EPA 2000a), and streams and rivers (U.S. EPA 2000b). Different nutrient criteria are being developed for each of 14 different nutrient ecoregions throughout the country. Nutrient ecoregions are based on aggregations of level III Omernik ecoregions. Draft nutrient guidelines are available on the Web at <*http://www.epa.gov/waterscience/criteria/nutrient/ecoregions/sumtable.pdf*>.

In some areas, the concentrations of potentially toxic substances in surface waters may be of concern. This issue is likely to be of greatest interest to FS staff in areas downwind of substantial emissions sources of pesticides, or where atmospheric deposition of Hg (or other trace metals) is known to be elevated. Monitoring of pesticides in surface waters may be advisable on FS lands directly downwind of intensive agricultural development.

Studies of Hg concentrations in fish tissue may be warranted in areas that are downwind of known Hg emissions sources, especially where such areas co-occur with probable geologic sources of Hg. Regional Hg deposition attributable to long range atmospheric transport is also of concern. In general, this protocol does not recommend that the FS ARM program include routine monitoring of surface waters for Hg concentrations in water. Nevertheless, Hg is of interest to the ARM program because it is atmospherically deposited and it bioaccumulates in aquatic environments reaching potentially high concentrations in large piscivorous fish. Mercury can pose a health risk to humans or wildlife (e.g., bald eagle, osprey, loon, and river otter) that consume large quantities of mercury-contaminated fish. This protocol recommends that a more effective way to evaluate Hg contamination in surface waters is to analyze or monitor concentrations of Hg in fish tissue, rather than in water. Of particular concern are the larger, older, piscivorous fish, such as bass, pike, and some species of trout.

POTENTIAL CONFOUNDING FACTORS

In developing and implementing a national field sampling program, it is important to consider numerous potentially confounding factors. Some of the important considerations that can complicate aquatic inventory and monitoring assessments include:

- Low signal-to-noise ratio[2], especially for dilute aquatic ecosystems;
- Variation in watershed properties such as slope, aspect, underlying bedrock composition, extent and mineralogy of glacial till, depth and composition of soils, distribution of vegetative cover, role of ground water, and presence and hydrologic connectedness of wetlands;
- Interacting stressors especially climate, introduced species, and legacy effects of fire or past land use and exposure to pollutants;
- Constraints of sampling in designated wilderness areas where land-use rules prohibit access via mechanized equipment and installation of fixed equipment;
- Constraints regarding laboratory analytical holding times;
- National and regional applicability;
- Cost and training constraints; and
- Quality control issues and the need for peer review.

1.1.2 STUDY PURPOSE AND OBJECTIVES

Before selection of study sites and parameters to measure, it is important to determine the purpose of the sampling program. For example, the main purpose could encompass any of these:

- Evaluate nutrient limitation;
- Document temporal variability (i.e., diurnal, episodic, seasonal, annual, inter annual) in water chemistry;
- Evaluate spatial extent of acid-base status;
- Parameterize interpretive and predictive models[3];
- Determine sensitivity of resources to potential damage; or
- Estimate the magnitude of impact on water quality.

Variation in purpose dictates variation in general approach (Table 1-3), which in turn influences the selection of appropriate protocols.

[2] Natural and sampling variability are high relative to the magnitude of change that has occurred in response to atmospheric deposition.

[3] To apply a process-based effects model to a particular site various input data are needed depending on the selected model. Such data might include characterization of soils, hydrology, vegetation, and/or historical documentation of land use or atmospheric deposition.

Table 1-3. Common management issues for FS ARM program staff, with associated field study approaches.

Purpose	General Approach
Determine whether one lake or stream or a group of lakes or streams is N-limited for algal growth.	Sample water and determine nutrient and chlorophyll *a* concentrations on multiple occasions (at least monthly during the snow-free season) during multiple years. Consider also nutrient (N, P) addition experiments in the laboratory and/or field enclosures.
Quantify episodic excursions from base-flow conditions in surface water chemistry (i.e., ANC, pH, Al_i, NO_3^- concentrations) during hydrologic events.	Sample water and measure full ion chemistry during rainstorms, snowmelt, and/or rain-on-snow events, with hourly to weekly periodicity.
Determine the distribution of lake or stream water chemistry (i.e., ANC, pH, NO_3^- concentration) across a particular forest or wilderness.	Conduct a statistically based or systematic synoptic survey of lake or stream chemistry.
Quantify long-term changes in lake or stream ANC (or other variables) over time in a particular lake or stream.	Sample at least annually (preferably monthly or seasonally during the open water season) over a period of at least 8 years. Consider restricting sampling times to common hydroperiods or other approaches to standardize timing of sample collection among years. Length of time required to continue monitoring to document statistically significant changes will depend on temporal variability in water chemistry and extent of long-term changes that occur. In general, at least 8 years of data will likely be required.
Determine to what extent air pollution is currently affecting the water resources in a particular forest or wilderness.	Multiple approaches can contribute to this evaluation: 1) Characterize index chemistry for multiple lakes and/or streams expected to be highly sensitive. 2) Conduct a synoptic survey (preferably using a stratified random selection process) of waters in the forest/wilderness. 3) Use a dynamic process-based watershed model to hindcast past changes in acid-base chemistry. 4) Collect and analyze diatom remains in a sediment core from the deepest part of one or more of the presumed most acid-sensitive lakes. 5) Use a steady state or dynamic process-based-watershed model to quantify the critical load of S and/or N deposition.
Evaluate whether the current condition of acid or nutrient sensitive waters warrants mitigation.	Multiple approaches can contribute to this evaluation: 1) Characterize index chemistry for multiple lakes and/or streams expected to be highly sensitive. 2) Conduct synoptic survey (preferably using a stratified random selection process) of waters in the forest/wilderness. 3) Use a dynamic process-based watershed model to hindcast past changes in acid-base chemistry. 4) Collect and analyze diatom remains in sediment cores from the deepest part of one or more of the presumed most acid-sensitive lakes. 5) Use a steady state or dynamic process-based-watershed model to quantify the critical load of S and/or N deposition. 6) Use a dynamic process-based-model to evaluate likely future responses to reduced levels of acidic deposition.

The management needs that the field study is intended to address will help determine the type of field study that is most appropriate. The management needs will lead into a series of questions, which in turn will guide the sampling effort:

- What kinds of sampling are required to support the management needs?
- What are the protocols to meet those sampling requirements?
- What are the standard operating procedures (SOPs) to implement those protocols?

In designing the field study, there are 10 basic questions to consider. Each of these questions should be addressed to avoid the risk that the sampling program will fail to yield the data required to meet the program's needs. This protocol provides guidance regarding how-to answer these questions (modified from <*http://www.epa.gov/owow/monitoring*>):

1. Why is the sampling taking place?
2. Who will use the resulting data, and how will that influence the level of quality assurance that will be required?
3. How will the data be used, and how will the intended use influence data requirements?
4. What parameters or conditions will be measured?
5. How good does the data need to be in terms of accuracy, representativeness, completeness, and intrasite and intersite comparability?
6. What methods should be used?
7. Where are the sampling sites?
8. When will the sampling occur?
9. How will the data be managed?
10. How will the program ensure that the data are credible?

The most important aspect of any inventory and monitoring plan is specification of the objectives and questions to be answered using the resulting data. Once the objectives and questions are conceived and refined and some preliminary data are collected with which to evaluate data variability issues, it is possible to specify a plan that will have a high probability of success. The greatest challenge in developing a monitoring or synoptic survey plan is asking the most appropriate questions. It is important to decide what you want to know and what uncertainty you are willing to accept in your answers. Many field sampling programs are compromised from the beginning because they were not specific about what questions the program was intended to answer. Specificity regarding the questions can lead to specificity regarding the sampling design and result in the collection of data suitable for providing the desired answers.

Because it is not possible to sample at all locations at all times for all parameters, it is important to consider in advance how to make the best choices regarding expenditure of limited funds for field sampling. The most important aspect of sampling design is setting specific objectives, and linking the objectives to specific questions. The questions should consider elements of subject, location, time, trend, and degree (Table 1-4).

Table 1-4. Elements to be considered in formulating sampling questions.

Element	Example
Subject	Stream NO_3^- concentration.
Location	Spring Creek, 50 m below its confluence with Sparks Creek.
Time	During spring snowmelt.
Trend	Is stream NO_3^- concentration increasing from year-to-year during the spring high flow period?
Degree	Is it changing by a statistically significant amount, or a biologically meaningful amount?
Population of Interest	First through third order (at 1:100,000 scale) streams in the Blue Ridge ecoregion in Virginia.

A well-conceived plan for water quality sampling should be:

- Relevant to the beneficial uses of the waters;
- Specific with respect to sampling locations, depths, parameters, schedule, and methods;
- Consistent with approved methods;
- Specific with respect to recommendations for quality assurance, data analysis, and reporting; and
- Designed to maintain continuity to the extent possible with the existing sampling efforts, especially if trend analyses will be conducted using the data.

Within the context of characterization and monitoring studies to measure or document air pollution effects on surface waters, there is a multitude of questions that could be an appropriate focus for field studies. A partial list is given in Table 1-5. Selection of the most relevant questions depends to a substantial degree on location. Key questions can be influenced by the extent of historical acidic and nutrient deposition, inherent sensitivity of the resources present, hydrologic characteristics, types of aquatic resources of greatest interest (e.g., drainage lakes, seepage lakes, low-order streams, moderate-order streams), topography, as well as many other factors.

Monitoring of lake and stream water quality is performed to provide resource managers with information on possible water quality problems that may require intervention to determine the susceptibility of lakes and streams to potential stressors and to document changes (improvement or deterioration) in key parameters of interest or in known problem areas. For example, FS managers should know surface water sensitivity to acidification when they review emissions permit applications. Information from a well-designed and properly-executed monitoring plan may also allow evaluation of the effectiveness of emissions controls or other best management practices and the potential need for other actions that might be warranted.

Table 1-5. Examples of questions that could be used to guide inventory, characterization, and monitoring study design.

Subject	Question
Inventory	What is the distribution of lake water ANC (or alternatively pH, inorganic monomeric Al, Ca, NO_3^-, or SO_4^{2-}) across high-elevation lakes in a particular Wilderness area?
	What is the annual average (or index) water chemistry of the most acid-sensitive streams in a particular National Forest (expressed as 5th percentile of sensitivity of the population of streams, or the five most sensitive streams known to exist in the forest)?
	What are the concentrations of stream water NO_3^- (or ANC, pH, Al_i) during snowmelt at selected long-term monitoring locations in a particular National Forest, and how do they compare with summer or fall index NO_3^- concentration in these streams?
Characterization	What is the extent of episodic chemical change (decrease in ANC, pH; increase in inorganic monomeric Al, NO_3^-) during the peak of snowmelt at selected long-term monitoring stream sites?
	What landscape characteristics (i.e., lithology, soil type, elevation, ecoregion, stream order, etc.) are associated with the occurrence of streams having spring-base flow ANC below 50 µeq/L within the National Forests of NC, TN, and SC?
Monitoring	What is the long-term trend in lake water NO_3^- (or other variable) concentration for FS long-term monitoring sites in the Rocky Mountains over the period of monitoring since 1990, as measured during the summer index period, and what are the characteristics of the sites that show the largest positive trends?
	Given the observed temporal variability in spring-base flow ANC in a particular stream, how long would monitoring need to be conducted to document a statistically significant increase in stream ANC if the average actual increase in ANC was 1 µeq/L/yr?
	Do long-term trends in spring-base flow stream water Ca^{2+} concentrations in second- and third-order streams in XYZ Wilderness area since 1990 suggest the potential for Ca-deficiency in the soils of higher elevation forests in this wilderness?

1.2 STUDY DESIGN

Water quality studies for evaluating aquatic effects of atmospheric deposition are most commonly designed as lake or stream characterization studies, synoptic surveys of the chronic chemistry of lakes or streams in a particular forest or region, characterization of episodic variations in chemistry in response to rain storms and/or snowmelt, or long-term monitoring studies to document and quantify changes in chemistry over time. Each type of design is described below. In selecting an appropriate design, determine in advance precisely what you would like to know. Subsequently, you can determine the type of study design that will be most useful (Table 1-6).

One of the most important, and most frequently overlooked, aspects of study design is that it should incorporate the data requirements of the statistical procedures that will be used to analyze the data. Consulting with a statistician can aid in the development of a study design. In addition, you should determine what you intend to do with the data before beginning study design.

Study design should also coordinate with existing efforts by other FS programs, as well as other governmental agencies, where possible. In some cases, another FS program, the U.S. Environmental Protection Agency, the U.S. Geological Survey, or a State agency may have ongoing or planned sampling programs that overlap with the FS Air Program projects. This

coordination effort may be as simple as collecting some additional data that might be shared, or pursuing joint funding of a desired sampling effort.

Table 1-6. General guidance regarding water quality study design.

If what you want to know is:	You should consider the following kind(s) of study design:
Number of lakes, length of streams, or percent of the regional population of lakes or stream length that is above or below a particular criteria value (i.e., ANC ≤ 50 µeq/L).	Some form of stratified random sampling that will allow extrapolation of results from individual sites to the larger area.
Status of the acid-base chemistry of the most (or some of the most) sensitive lakes or streams in an area.	Non-statistical survey of selected lakes and/or streams in portions of the study area and landscape positions expected to contain the most sensitive aquatic resources.
General assessment of lake or stream chemistry in an area, with identification of some of the more sensitive water bodies in the area.	Statistical or non-statistical screening of a relatively large number of water bodies across the expected gradient of sensitivity, measuring specific conductance and/or pH in the field for making a rough assessment of condition, and collecting samples for full laboratory analyses for a subset of those samples.
Estimate of seasonal or episodic variability in the chemistry of an acid-sensitive lake or stream.	Frequent interval sampling during the period of interest. Sampling can range from hourly to monthly during the season or period of interest and can include multiple years to capture the range of variation.
Analysis of long-term changes in water chemistry over time.	Periodic sampling (usually monthly to annually) over a period of usually at least 8 years focused on an index period or standardized by hydroperiod. More robust studies (with greater statistical power to detect trends) will entail more frequent sampling (weekly to seasonally) and/or will extend for longer than 8 years.
Assessment of temporal variability in water chemistry of a particular lake or stream.	Frequent interval (hourly to seasonally) sampling that captures major changes in hydrology during the season(s) of interest. Should include multiple years.
Determination of whether and to what extent water resources in a particular forest or wilderness have been adversely affected by atmospheric deposition to date.	Multiple designs will be needed using a weight-of-evidence approach. They might include: • Synoptic survey (statistical survey preferred) • Characterization of multiple representative sensitive lakes and/or streams • Long-term monitoring • Assessment of seasonal and episodic variability • Hindcast chemistry using dynamic process based model(s).
Determination of the prognosis for future recovery of damaged aquatic resources or quantification of the atmospheric deposition levels that will be protective of sensitive resources.	Model scenario and critical loads analysis.

1.2.1 LAKE OR STREAM CHARACTERIZATION

There are multiple approaches to characterize the chemistry of a lake or stream. Some studies are based on only one or a few samples. Most commonly, these are collected as index samples. Decisions should be made concerning the frequency and timing of sampling. Springtime base-flow samples are often regarded as a good representation of annual average flow-weighted stream water quality in the southeastern United States when only single samples can be collected. Summer or fall index chemistry (commonly avoiding large rainstorm events) is often regarded as

a good representation of annual average lake-water chemistry. Lake sampling after fall overturn can yield results for fully mixed conditions, but may require measuring the lake temperature profile to verify that turnover has occurred. Selection of an index period has implications for the temporal stability of the water quality and for the degree of impact that might be revealed by that water quality. Water quality is more likely to be stable (and thus comparable among water bodies if a survey is conducted of multiple lakes or streams) during summer and fall. However, in many regions that contain acid-sensitive waters, the lowest pH and ANC and the highest Al_i concentrations are more likely to occur during spring.

Sampling to Support Modeling

If modeling is anticipated using the collected data, consider which water chemistry variables will be needed to calibrate the model. For example, application of the MAGIC model (Cosby et al. 1985), sometimes used in acidic deposition assessments, requires analyses of nine stream water constituents:

- pH;
- Three acid anions: sulfate, nitrate, and chloride;
- Four base cations: calcium, magnesium, potassium, and sodium; and
- Ammonium.

It is desirable, but not necessary, to also provide Gran titrated ANC. If modeling is to be done to estimate future pH, then it will also be necessary to measure dissolved organic carbon, and if pH is below 5.5, to determine total monomeric aluminum and organic monomeric aluminum. If pH is below 5, measure total fluoride, which will be needed to calculate aluminum speciation. Thus, the additional analyses needed to support MAGIC modeling of future pH (in addition to those listed above as required) include:

- Gran ANC;
- Dissolved inorganic carbon;
- Total monomeric Al; and
- Organic (non-labile) monomeric Al.

These additional parameters are not needed to support MAGIC modeling of future ANC. Other models may require different or additional parameters.

Better representation of annual conditions in both streams and lakes can be obtained with seasonal or other periodic sampling, as opposed to collection of only one sample to represent a given year. Selecting a sampling period when flows are low and least variable may provide data that are generally comparable among a group of waters or in a given water body between years, but may not represent the conditions of interest. For example, surveys to assess the effects of acidic deposition on surface water chemistry can substantially underestimate impacts if low-flow periods are used for sampling (Lawrence et al. 2008b). Some studies endeavor to collect one or more samples during high-flow periods (heavy rain or snowmelt) to augment index chemistry sampling. High-flow periods tend to cause:

- Relatively high NO_3^-, Al_i, and P concentrations;
- Relatively low pH and ANC; and
- Variable concentrations of SO_4^{2-} and base cations (depending on local characteristics).

The most stressful conditions (to aquatic biota) generally occur during high-flow periods. The range of difference between high-flow and low-flow chemistry varies by region, with drought cycles, and by individual water body. In general, this protocol recommends that characterization of lakes and streams be represented by one or more index samples for a given year, plus at least two additional samples during snowmelt or rainstorms to partially characterize variability. It is preferable to collect data for at least 2 years and to document inter-annual variability associated with wet/dry cycles.

1.2.2 SYNOPTIC SURVEY

Synoptic surveys of lake or stream chemistry within a forest or within a designated region are usually conducted at times expected to exhibit fairly stable water chemistry. For acidic deposition monitoring, this is usually spring base flow for streams in the Southeastern United States, and the summer or fall index period[4] for lakes or streams in regions that typically develop substantial snow pack. For backcountry sampling within the FS ARM program, typically one sample with replicate(s) is collected for each lake or stream that was selected for sampling. Synoptic surveys are ideally, but not always, statistically based, which allows for extrapolation of sample results from individual water bodies to the regional population of interest. At the time of sample collection, standardize for consistent weather and runoff conditions as much as possible at each site. Periods of high temporal variability, such as heavy rain and periods of rapid snowmelt, or periods with heavy smoke from wildfires, are typically avoided during a synoptic survey. However, if assessment of acidic deposition effects is the goal, avoiding high-flow conditions can result in a substantial underestimation of the magnitude of impact. The extent of this underestimate can be quantified by conducting additional seasonal and episodic sampling for at least a subset of the sampling sites.

1.2.3 LONG-TERM MONITORING

Long-term monitoring of stream or lake chemistry usually involves collection of water samples at regular intervals from weekly to quarterly or even annually, with the primary purpose to detect trends that reflect an environmental change over time. How quickly a trend can be detected depends on the strength of the trend (the rate of change) and the amount of intra annual and inter annual variability in the water chemistry. It is generally possible to detect a change of slight magnitude under conditions of low variability and with a longer period of record. The likelihood of detecting a significant trend in the concentration of a given water chemistry variable will be determined in large part by the length of the monitoring period. In the event of a small to moderate change in chemistry, it may take 10 to 20 years or more of monitoring data to document a significant change.

An effective monitoring plan stems from a series of questions and constraints that sequentially focus the plan into specific elements that are well-defined and unambiguous. Because information is gained during implementation of a monitoring plan, it is often desirable to revisit plan elements to continuously refine and update the monitoring activities. In addition, external factors such as

[4] Index period is a relatively narrow period of time for synoptic sampling (often a 2-month window during either spring, summer, or fall) intended to represent the lake or stream chemistry for that year. Typically, rain or snowmelt conditions are avoided when collecting index samples.

changes in monitoring technology, analytical methods, and regulations will often impinge on the design and execution of the monitoring. For these reasons, routine (e.g., annual) reviews of the results and methods should be incorporated into the monitoring plan. However, if trend detection is one component of the plan, care should be exercised before making changes to the program that might compromise the integrity of the data and the ability to use earlier data to infer statistically significant changes in water quality.

1.2.4 Characterization of Stream Chemistry During Hydrologic Events

Hydrologic events, high flows caused by rainstorms and/or rapid snowmelt, are episodic and can last from a few hours to a few weeks. Although these events occur over a relatively small fraction of the year, they often represent the majority of total annual flow (Likens et al. 1977) and constituent flux. Events can happen throughout the year in most regions of the United States but are most common during seasons of high precipitation and during spring snowmelt in regions where snow accumulates and are generally least common during summer when high evapotranspiration reduces soil moisture.

Stream-water chemistry during hydrological events is important to characterize because high flows often lead to extreme chemical conditions. Effects on stream biota from episodic variations in stream chemistry can be as severe as chronic effects associated with base-flow chemistry. For example, episodic acidification can result in the elimination of an annual age class of fish (McComick and Leino 1999) when the event occurs during sensitive life stages. Episodic acidification can also affect other forms of aquatic life, such as diatom communities, which have been found to be less diverse in an episodically acidified stream than in a nearby chronically acidified stream (Passy et al. 2006).

Manual water sampling is generally not effective at characterizing chemical variability over the course of a hydrologic event because the timing and shape of the hydrograph is difficult to predict and may occur at inconvenient times for the sampler. Automated water sampling triggered by changes in water level, often referred to as stages, provides an effective solution to this problem. Automatic samplers can collect water at predetermined time intervals or at intervals based on the rate of change in water level. Samples collected during events can then be evaluated using the flow measurements recorded at the times of sample collection to optimize the selection of samples for chemical analysis. This approach offers the opportunity to greatly reduce analytical costs with minimal loss of information. However, the use of automatic sampling equipment is restricted in wilderness.

1.2.5 Other Uses of Resulting Data

Surface-water-quality data can also be used to support process-based modeling studies using a watershed model such as MAGIC or PnET-BGC. Such models can be used to hindcast pre industrial chemistry to determine whether and to what extent a lake or stream has acidified since the Industrial Revolution. A second general approach is to conduct scenario modeling to estimate future changes in water chemistry in response to one or more scenarios of emissions control and deposition. A third modeling approach is simulation of the critical and/or target loads of

atmospheric deposition to protect or restore acid-sensitive or nutrient-sensitive aquatic resources (see side box on Critical and Target Loads). All of these modeling approaches require compilation of model-input data. These can include, depending on the selected model, data on soil and water chemistry, hydrology, vegetative characteristics, and estimates of historic and current deposition.

1.3 WHERE, WHAT, AND WHEN TO SAMPLE

Data that provide information on the quality of surface water can be used to evaluate the following kinds of issues:

- Short-term episodic changes (scale ~hours to days) in water quality;
- Long-term chronic changes (scale ~years to decades) in water quality;
- Types of water quality changes;
- Likely cause(s) of water quality changes;
- Longitudinal variation of water quality along streams or depth variation within lakes;
- Status and extent of chemical and biological condition across populations of lakes and streams; and
- Biological effects of water quality changes.

The ability to assess these issues will be limited largely by the extent and intensity of the sampling effort. Three of the most critical aspects of water-quality-sampling design (cf., Green 1979) include:

- Stating the questions to be addressed concisely and precisely;
- Conducting a preliminary pilot sampling; and
- Replicating sampling in time and in space.

The questions to be addressed will come from the project objectives, which should be agreed upon in advance of field sampling.

Sampling can be conducted at different intensity levels depending on study design, questions to be addressed, and the intended use of the resulting data. The level of intensity will influence decisions about how, where, what, and when to sample and will affect associated quality assurance, laboratory analysis, data analysis objectives, and standard operating procedures. For example, if the objective is to gain a general understanding of the distribution of potentially acid-sensitive streams within a particular wilderness, then a low level of sampling intensity may be acceptable. This may, for example, entail only a summer survey of specific conductance, pH, and/or ANC, with no additional measurements. If, however, the objective is to more fully characterize the acid-base chemistry of one or more streams or lakes or to quantify long-term trends in water chemistry, then a higher level of intensity will be required. Sampling that is intended to support regulatory decision-making or that will likely be used in permitting or litigation demands the highest level of intensity. There is not a one-size-fits-all approach to establishing water sampling protocols.

Critical and Target Loads

Modeling of critical and/or target loads requires that a number of decisions be made before initiating modeling. These decisions determine what resources are to be protected, at what level, and over what time period. Sensitive resources to be protected by a given critical or target load can include fish or other types of aquatic biota, or various terrestrial resources such as vegetative or soil condition.

To protect these resources, one or more chemical indicators are typically chosen. Often, ANC is used as the indicator for protecting aquatic resources from acidification. One or more critical ANC levels must be selected (e.g., ANC 20 or 50 µeq/L), typically in association with known or suspected dose/response relationships for various sensitive species. Different critical ANC levels are expected to protect different species of aquatic life.

Finally, one selects a steady-state approach or specifies the time period over which the sensitive resources are to be protected or over which the damaged resources are expected to recover. Steady-state critical loads are determined irrespective of time. Dynamic critical loads, or target loads, may be determined for various endpoint years, for example, 2050 and/or 2100.

Each decision that must be made to simulate critical or target loads has an influence on the resulting model-simulated values. A target load can be selected that is higher than the modeled critical load if the objective is to make some limited progress towards reaching the critical load. Conversely, a target load can be selected that is lower than the critical load to ensure that the sensitive ecosystem is fully protected given modeling uncertainty or to attain the targeted threshold chemistry more quickly for resources that have already been damaged.

The ability to determine the existence of a statistically significant trend in water quality over time is influenced by the: 1) magnitude of change that occurs in the parameter and water body of interest; 2) temporal variability that occurs in that water quality parameter and water body; and 3) the number and temporal distribution of samples collected. To design a monitoring plan to detect the existence of a statistically significant change in lake or streamwater chemistry parameters, the level of change that one wishes to be able to detect, in conjunction with the known or expected temporal variability of the parameter in that lake or stream must be considered. Before initiating a monitoring effort that is intended to evaluate change over time (trends detection), it is helpful to: 1) consult with the person who will be responsible for the eventual statistical analysis of the resulting monitoring data or someone knowledgeable in statistics and 2) conduct a pilot study to determine the temporal variability that occurs in the parameter(s) of interest in that water body or, at a minimum, in a water body thought to be similar in its chemistry and temporal variability.

The overall quality assurance objectives for a water-quality-sampling project are 1) to implement quality control procedures and requirements for field sampling and laboratory analysis that will provide data of sufficient quality to achieve the program objectives and 2) to follow procedures that will provide data of known quality in terms of precision, accuracy, completeness, representativeness, and comparability. Quality assurance and quality control (QA/QC) issues are covered in detail in the QA/QC section (Section 3) of this report. It is important to note that certain aspects of the QA/QC protocols that are adopted for a particular study will affect choices made in designing the sampling program. In particular, it is important to determine, in advance of initiating field work, what the data quality objectives (DQOs)will be with respect to the selected

targets for analytical detection limits, precision, accuracy, and completeness. In addition, plans need to be made concerning how many and which of the field samples to be collected will be replicated in the field. Some sampling programs replicate all samples in the field.

1.3.1 WHERE TO SAMPLE

SELECTION OF SAMPLING LOCATIONS

Selection of sites for water quality sampling should be based on systematic and documented criteria. One of the most important criteria is having a well-defined population of interest. The criteria should be chosen with consideration of watershed factors. These can include representation of bedrock, soil type, geographic distribution of surface waters, presumed sensitivity to stressor(s) of interest, elevation, watershed area, lake area or stream order, site accessibility, and avoidance of watersheds with other elements of human disturbance that might influence surface water composition. Approximate sampling site locations can be identified on a preliminary basis from examination of available mapped data before initial sampling trips, with specific site selection and further documentation developed in association with sampling.

Features of Landscape Associated with Acid-Sensitivity of Surface Waters

It is not possible to define features of the landscape that will be most closely associated with surface water acid sensitivity nationwide. Such relationships are highly variable across the landscape and vary from region-to-region, and often from forest-to-forest. Nevertheless, for a given region or forest it is often possible to identify certain landscape features, such as lithology, elevation, watershed area, and watershed slope, which correlate with acid sensitivity or effects. Sometimes vegetation type (e.g., coniferous forest, alpine, and subalpine vegetation), soil type, or one or more regional soils variables (e.g., pH, depth, or percent clay fraction) may be helpful. Ecoregion designations incorporate many of these variables and can also be useful. In general, the most acid sensitive lakes and streams are expected to occur under the following conditions:

- Bedrock that is not basaltic and does not contain appreciable amounts of carbonate;
- Relatively high elevation;
- Steep terrain;
- Small watershed;
- Thin soils;
- Low soil clay content and low soil pH; and/or
- Flashy hydrology.

In the Southeastern United States, acid-sensitive streams are often associated with siliciclastic bedrock lithology (cf., Sullivan et al. 2007). In glaciated regions, the presence of varying amounts of glacial till can obfuscate relationships between lithology and surface water chemistry. Acid-sensitive lakes and streams often, but not always, occur at relatively high elevation, on steep slopes, and in relatively small watersheds. Knowledge of such relationships, especially if regionally specific, can aid in selection of surface waters for inclusion in inventory and monitoring programs.

Random Versus Non-Random Site Selection

One of the most important considerations in site selection is the determination of whether or not the sampling sites will be selected using a randomized sampling design. Streams or lakes should be randomly selected for sampling if the goal is to characterize populations of surface waters for a defined area too big or impractical to census. Doing so enables the statistics obtained for the sampled waters to be applied to the full population of waters in the designated sample frame within the area.

For a statistically based survey of surface waters some form of stratified random sampling is generally used as this approach allows the sample population to be stratified such that the streams or lakes of greatest interest can be included in amounts that are disproportionate to their frequency of occurrence. Such a stratified random sampling process preserves the ability to make population-level extrapolations while maximizing the collection of data for the sites of greatest interest. A carefully targeted and stratified random sampling does not necessarily have to entail a large and expensive sampling program.

Random surveys of aquatic resources conducted by EPA have often been large efforts that sampled hundreds to more than a thousand water bodies. These have included the Wadeable Stream Survey, National Lake Assessment, National Surface Water Survey, and various surveys conducted as part of the Environmental Monitoring and Assessment Program (EMAP). Nevertheless, smaller surveys could also be conducted using a random sampling structure, thereby allowing extrapolation to a population of waters of particular interest.

If all streams or lakes are included for potential sampling, accessibility may complicate a totally randomized sampling design. Remote sites may require extended periods of time to reach, which lengthens the period over which the survey is conducted and may introduce complications regarding sample-holding times and costs. This can also be problematic because environmental sampling conditions (e.g., stream flow) may vary during the survey if some of the sites take several days to access. Data

Nutrient Status

A rough evaluation of the nutrient status of a lake or stream can be made on the basis of the molar ratio of total N to total P in solution. This determination was formerly based on the Redfield N:P ratio equal to 16. More recent compilations of experimental data (cf., Elser et al. 2009) suggest a cutoff near 44 for N versus P limited fresh water lakes.

If the ratio is above about 44, the water body is presumed to be P-limited, and further addition of N would not be expected to have a large effect on primary productivity. If the ratio is below about 44, the water body is presumed to be N-limited and therefore may be sensitive to nutrient enrichment effects from N addition.

Such a rough evaluation should be based on multiple samples (at least 10) collected across multiple seasons (ideally spring through fall), as the nutrient status can change with season and/or with short term changes in flow or other conditions. Because this is an area of active research, such interpretation should be considered uncertain and subject to change.

A more complete evaluation of nutrient status should be based on laboratory, and perhaps also *in situ* nutrient addition experiments that add N and P, individually and combined, to laboratory flasks or *in situ* enclosures, and then measure nutrient concentrations and chlorophyll *a* or some other measure of primary productivity.

collected from surface waters sampled during low-flow conditions are generally not comparable to those collected during high-flow conditions.

Within a randomized sampling design, candidate sampling sites can be stratified according to accessibility. This can help ensure that a limited number of remote sites will be included. However, use of this approach can result in lower precision in quantifying conditions of remote sites.

Selection of streams or lakes for sampling may be non-random if information is needed on specific waters or watersheds, rather than on a population of waters or watersheds. Non-random sampling may be acceptable for certain studies. Nevertheless, one should carefully consider that the gains realized in ease of sampling and/or availability of data collected previously in a non-random study must be weighed against the loss of the ability to extrapolate conclusions directly from the sampling sites to the population of interest.

For non-random sampling, especially if the lake or stream is intended to be part of a long-term monitoring effort, it may be desirable to select water bodies that exhibit particular characteristics. For example, it makes little sense to spend years monitoring a body of water that is not acid-sensitive if the objective is to evaluate acidification response. Thus, one might purposely select one or more highly sensitive sites for monitoring or for detailed study. Similarly, it would be illogical to focus a study of atmospheric nutrient N enrichment on a surface water body that is P-limited.

For certain studies, it is logical to select sites that are highly sensitive to the stressor in question. Nevertheless, it can be difficult to interpret the results of such studies without first determining where the studied sites fall within the distribution of site sensitivities across the forest or across the region. Such distributions of regional site characteristics can sometimes be provided by various statistically-based large synoptic sampling programs such as EPA's Wadeable Stream Survey, National Stream Survey, National Lake Survey, or EMAP.

Statistically-based survey data can be used to aid in selection of sites for long-term monitoring. Long-term monitoring sites may be chosen at random from among randomly selected survey sites so that the resulting monitoring data will be representative of the entire population of interest. This approach was taken in EPA's Temporally Integrated Monitoring of Ecosystems (TIME) project (Kahl et al. 2004).

Connection of survey or monitoring sites to the broader regional population of lakes or streams is always highly desired. This connection allows extrapolation, whether statistically or semi-quantitatively, of results to more bodies of water than just the one(s) sampled. Ideally, study sites should be statistically selected. If this is not possible, it may be possible to express the results for a given study site relative to the broader population by quantifying its chemistry relative to the population of lake or stream chemistry determined in one of the larger regional or national surveys, such as those conducted by EPA. Alternatively, the feasibility of conducting a synoptic survey targeted to the specific forest or region should be considered. Such a survey could range, depending on resource availability, from sampling a few variables to a study of full water chemistry. A screening survey to identify candidate sites for further study could be focused mainly on such parameters as specific conductance and field pH, with full laboratory chemistry conducted on only a subset of the sites.

Candidates for Inclusion

Before conducting either a random or non-random survey of lakes or streams, determine what kinds (classes) of lakes or streams should be included as candidates for sampling. Pre-selection of classes of water bodies to include may change the population frame in a statistically based sampling or change the waters that are candidates for selection in a non-random design. Candidate lakes might be restricted by hydrologic type (to drainage lakes or seepage lakes, for example), lake size, topographic position, ease of access, or depth. Candidate streams might be restricted to certain stream orders[5], or otherwise constrained according to watershed area, elevation, presence/absence of fish, or presence or absence of watershed disturbance. For non-random surveys, intended to identify and characterize the most acid-sensitive surface waters in a particular region, forest, or wilderness, this protocol recommends focusing on the following types of lakes and streams:

- Perched, seepage lakes;
- Small (less than about 50 to 100 ha) drainage lakes occupying relatively high landscape position and having average depth greater than about 1 m[6]; and
- Low-order streams (first through third order).

In some cases, a systematic approach to pre-selection of sampling sites may reduce the number of candidate sites to such a degree that all or most of the high-interest candidate sites can be sampled.

Selection of Specific Sampling Sites

The sampling site in a lake is generally selected on the basis of logistical considerations. The preferred site is the deepest area of the lake, but this requires use of a boat, raft, or float tube. If it is not possible to sample at the deepest area of the lake, then use the lake outlet or, as last choice, a shoreline location near the outlet.

A number of factors should be considered when selecting the specific sampling point. Any sampling point along a stream will be affected by features of the upstream watershed. The following is a list of landscape features that can affect water quality:

- Impoundment structure;
- Wetland;
- Tributary-stream junction;
- Dramatic change in slope;

[5] Stream order refers to a system of classifying streams based on their branching pattern. The smallest headwater streams are first order. When two first-order streams come together they form a second-order stream. As more first-order streams flow into the second-order stream, its order is not affected; it is still second order. When two second-order streams combine, it becomes third order. The process continues to progressively higher orders. The scale of the mapped data used to designate stream order has influence on the classification. Most acid-sensitive streams tend to be relatively low order (often first through third order at 1:100,000 scale or first through fourth order at higher resolution).

[6] Lakes that are less than about 1 m deep grade into wetlands. Some studies of lakes only include those deeper than 1 m.

- Abrupt change in vegetation, soil type, bedrock type;
- Groundwater discharge (spring);
- Upslope disturbance (fire, mining, camping areas or trails, logging, heavy grazing, windthrow); and
- Upslope human activity (agriculture, residential development, road building).

Specific information on the watershed is necessary for determining if the stream is appropriate for sampling a particular sensitive receptor, and, if so, where the sampling point should be located on the stream reach. The upstream drainage should also be assessed to determine if disturbances, such as fire, mining, or logging, have occurred or if the stream has been influenced by erosion from the stream bank or from adjacent roads or land disturbance. In addition, the presence of other human activity in the watershed, such as agriculture or residential development, may affect downstream water quality.

Proximity to trails or roads can be considered in selecting a sample location. Roads and trails provide accessibility and a bridge can be used for sampling larger streams and taking flow measurements. If there is an existing stream gage in the area of interest, co-locating the stream water sampling site with the gage will provide flow data that would be valuable for interpreting the chemistry data.

As described above, the general sampling location can, and should, be specified in advance of sending sampling personnel into the field. However, the precise sampling location can be selected by the field personnel when a site is first sampled within a non-random survey or monitoring program. Random stream sampling requires that the crews sample at the specified random sampling point; if that is not possible, then the site is classified as Not Sampled and the portion of the population that it represents is categorized as Not Assessed.

Any subsequent sampling of a given site should rely on global positioning system (GPS) coordinates, site photographs, detailed maps, and written description of the site location to return to precisely the same location each time that site is sampled. Where allowed, if a site is intended to be sampled repeatedly, the placement and documentation of uniquely numbered metal tags at the base of a tree or on a rock adjacent to the sampling site can provide confirmation of site location.

The field crew should follow these guidelines in selecting new non-random sites for repeated long-term sampling:

- The best point to sample a lake is over the deepest area of the lake. This requires use of a boat, raft, or float tube. If it is not possible to collect such an open-water sample, the next best option is to sample the largest flowing outlet from the lake; the outlet should be sampled, using stream sampling procedures, as close to the lake as is practical. The third, and least desirable option, is to sample the lake from the shoreline. Shoreline sampling should be conducted, if possible, from a large rock or by wading a short distance from the shore. Care must be taken to avoid disturbing the sediment in any way that could affect the quality of the sampled water. Proximity to logs and aquatic vegetation should be avoided. If possible, use wind currents to advantage by collecting the sample from an area that receives wind-driven surface water movement from the larger lake.

- The best point to sample a stream is where the water is flowing fast or falling, where there are no eddies, and where the depth is at least 15 cm (6 in). Ideally, the sampling point is one that can be reached during most flow conditions while kneeling on the stream bank or on stable rocks downstream from the sampling point. Where possible, sites should be selected that allow the sampler to avoid standing or stepping in the water to reach the sampling point and to avoid any disturbance of the streambed upstream from the sampling location. Ideally, sites should be selected that allow the sampler to reach upstream to collect the sample; well upstream of his or her immediate location and well upstream of any location that has been disturbed.
- Stream-sample sites should be identifiable by reference to semi-permanent landmarks such as confluence points of major tributaries, well-marked boundary lines, and stream crossings by permanent roads or well-marked trails if they occur in proximity to the selected site.
- Stream sample sites should be selected to avoid direct runoff from roads and trails, as well as unmixed flow from tributaries, unless the goals of the sampling include those conditions. This will be achieved for most small streams by selecting sampling sites at least 50 m above road or trail crossings or 50 m above or below inflowing tributaries.

ESTABLISHING AND LOCATING SAMPLING SITES

For sites that are or will be subject to periodic or routine monitoring, a site information folder or report should be established for each site, and provided to the field crew in advance of each sample collection visit. The site information folder should contain:

- Driving and site access directions;
- Maps, including U.S. Geological Survey (USGS) 1:24,000 quadrangle maps and site maps;
- Estimated travel time from the base location to the sampling site;
- Overnight lodging and/or camping information;
- Local contact personnel, if applicable;
- Data collection forms;
- Permission letters for access, if needed;
- Site coordinates and elevation;
- Site-tag numbers for long-term monitoring sites that are marked with a tree tag and locations (not allowed in wilderness);
- Site photographs; and
- Other relevant information.

Maps provided in the site information folder may also include forest recreation maps to help navigate to the area. Maps generated using geographic information systems (GIS) could also be included to show where the project manager has selected potential sites to sample, spatial patterns in the distribution of vegetation types or other landscape properties (e.g., soil or bedrock distribution), or locations where sites were sampled in a previous study.

Lake or Stream Sampling Record data sheets will serve for documenting site information, sample locations, and field measurements. These forms should be printed on waterproof paper. Copies of

these data sheets are provided in Appendix E and are also located on the FS website at <*http://www.fs.fed.us/air*>.

In addition to the material described above for inclusion in site documentation folders, site documentation materials can also include:

- Uniquely numbered aluminum tags (where allowed) for sites sampled repeatedly (i.e., monitoring sites) or for replacement of missing tags at previously established sites;
- Nails and a small hammer for tag placement;
- Flagging tape;
- A camera with a date/time stamp for site photographs;
- A GPS unit for determining geographic coordinates in decimal degrees; and
- Waterproof pens for completing forms in the field.

Depending on the objectives of the field data collection, field crews may be collecting water samples as part of a synoptic survey, or they may be repeating sampling at the same locations in a monitoring effort to examine changes in water chemistry over time. The extent of documentation required by the field crew will depend on whether the site is new or previously established.

1.3.2 WHAT TO MEASURE

This report focuses on protocols for water-quality sampling to quantify the effects of atmospheric deposition on aquatic ecosystems. The atmospheric deposition pollutants addressed in this protocol are S, N, and, to a lesser extent, toxics. Toxics are those air pollutants that are known or suspected to cause cancer or other serious health effects, such as reproductive and birth defects. Straightforward guidance regarding sampling constituents associated with characterization or monitoring of the acidification and nutrient enrichment effects of S and N deposition is possible. However, the constituents to monitor for studies of the effects of toxics are more variable depending on the objectives of the particular study and are less subject to generalization.

The primary water-quality variables to be sampled can include physical, chemical, and/or biological attributes. Choice of variables depends on the potential environmental risks, logistical issues associated with sampling, and costs.

Water-quality survey or monitoring on National Forest System lands to evaluate sensitivity to or responses to atmospheric deposition can involve a number of parameters. The choice of parameters should clearly relate to the water-quality concerns and should be measurable in a routine sampling program. The challenge is to select parameters that are most important with respect to revealing key features of ecological integrity and that can be determined in a relatively straight-forward and cost-effective fashion. For some studies, samples are needed at sufficient frequency and temporal resolution to allow appropriate characterization or statistical trend detection.

The choice of what to measure depends on the type of study: acidification, eutrophication, bioaccumulation, and/or toxicity. Parameters to include in each of these kinds of studies are discussed below.

ACIDIFICATION STUDIES

Atmospheric inputs of both S and N can cause acidification of soil, soil water, and fresh drainage water (lakes, streams). In most regions of the United States that have experienced acidification impacts from air pollution, the impacts have been primarily due to S deposition. There are also some regions, especially in the Western U.S., where resources are more threatened by N than by S inputs. This is partially due to the very low levels of S deposition received in many Western locations. There are also regions (portions of the Northeast, West Virginia, high elevations in North Carolina and Tennessee) where both atmospheric S and N contribute substantially to the observed acidification in some lakes and streams. National forests occupy significant portions of these regions.

Acidification from S and N deposition can have several important chemical and biological effects. In particular, changes in the acid-base status of surface and soil water can cause short-term or long-term toxicity to aquatic or terrestrial biota.

Watershed processes control the extent of ANC contribution from soils to waters as drainage water moves through terrestrial systems. These processes regulate the extent to which drainage waters will be acidified in response to acidic deposition. Of particular importance is the concentration of acid anions in solution, including sulfate (SO_4^{2-}), nitrate (NO_3^-), and organic acid anions. Naturally-occurring organic acid anions, produced in upper horizons of acid sensitive soils, usually are removed from solution as drainage water percolates into the deeper mineral soil horizons. In some regions, organic acids can dominate the acid-base chemistry of a lake or stream (as indicated by color and dissolved organic carbon concentration) due to the occurrence of hydrologically connected wetlands. Organic acids from wetlands, although they acidify a lake, also serve as buffers against further pH depression from acidic deposition. Acidic atmospheric deposition causes natural soil acidification, anion mobility, and cation leaching processes to occur at greater depths in the soil profile, allowing water that is rich in SO_4^{2-} or NO_3^- to flow from mineral soil horizons into drainage waters. If these anions are charge-balanced by H^+ and/or Al_i cations, the water will have low pH and could be toxic to aquatic biota. If they are charge-balanced by base cations, the pH of the water will be higher but the base cation reserves of the soil can become depleted over time.

Nitrate (and also ammonium [NH_4^+] that can be converted to NO_3^- within the watershed) has the potential to contribute to acidification of surface waters. Additionally, N is also a limiting nutrient for plant and microbial growth in most terrestrial and some aquatic ecosystems. Atmospheric N deposition has the potential to contribute to increased productivity, eutrophication, and N-saturation in some surface waters. This occurs in estuaries and near-coastal marine waters and in fresh waters in remote locations where historic atmospheric N deposition has been low. Many of these remote fresh waters are on National Forests.

High concentrations of lake or stream water NO_3^-, which may indicate ecosystem N saturation, have been found at a variety of locations throughout the United States. Locations include the San Bernardino and San Gabriel Mountains within the Los Angeles Air Basin (Fenn et al. 1996), the Front Range of Colorado (Baron et al. 1994, Williams et al. 1996), the Allegheny Mountains of West Virginia (Gilliam et al. 1996), the Catskill Mountains of New York (Murdoch and Stoddard 1992, Stoddard 1994), and the Great Smoky Mountains of Tennessee (Cook et al. 1994).

Sulfur deposition moves through watershed soils and into surface waters in anionic form as SO_4^{2-}. Sulfate is the most important anion contributed by acidic deposition in most parts of the United States. In some regions, notably the glaciated Northeast, Upper Midwest, and West, much of the deposited S moves readily through soils into streams and lakes. Although SO_4^{2-} has been classified as a mobile anion (Seip 1980), it is less mobile in some areas, most notably the unglaciated southeastern United States. Sulfate mobility is an important factor governing the extent to which S deposition contributes to soil and water acidification, base cation depletion, and Al mobilization, each of which can harm sensitive ecosystems.

Aluminum occurs naturally in soils and has a pH-dependent solubility in water. Solubility increases dramatically at pH values below about 5.5. One of the most important effects of acidic deposition on watersheds is increased mobilization of Al from soils to surface waters (Cronan and Schofield 1979). Aluminum concentrations in acidified drainage waters are often an order of magnitude higher than in more neutral waters. Effects of Al mobilization to surface and soil waters include toxicity to aquatic biota (Schofield and Trojnar 1980, Muniz and Leivestad 1980, Baker and Schofield 1982, Driscoll et al. 1980), toxicity to terrestrial vegetation (Ulrich et al. 1980), alterations in nutrient cycling (Dickson 1978, Eriksson 1981), and pH buffering effects (Driscoll and Bisogni 1984). Inorganic monomeric aluminum concentrations often increase with decreasing pH, and reach potentially toxic concentrations (> ~2 μM) in surface drainage waters having pH less than about 5.5.

SELECTION OF ANALYTES FOR ACIDIFICATION STUDIES

There can be substantial leeway in terms of selection of parameters to measure in a field study of surface water acidification. Analytical costs must be weighed against the value contributed by each constituent that can be analyzed in the laboratory. In general, this protocol recommends that the parameters in Table 1-7 be considered the core for inclusion in the suite of analytes to be measured in any study of surface water acid-base chemistry. When budgets allow, add DOC to all of the parameters listed in Table 1-8 of high importance. Although DOC should be measured in all acid-base chemistry studies, color could be substituted as an inexpensive alternative. If pH is below 5.5, this protocol recommends also analyzing for total monomeric aluminum (Al_m) and non-labile (organic) monomeric aluminum (Al_o). The concentration of the potentially toxic Al_i is then obtained by subtracting Al_o from Al_m. Measurement of dissolved inorganic carbon (DIC) is optional; this measurement can be used in estimating bicarbonate (HCO_3^-) concentration, which is important as part of the charge balance. Some studies might choose to analyze silicon (Si) or total fluorine (F), but these are often not needed for a standard acid-base chemistry assessment. The concentration of Si can be useful in evaluating the extent of groundwater influence on surface water chemistry and in discriminating between perched and flow-through seepage lakes. It can also provide useful information when

Seepage Lakes

Seepage lakes are lakes that do not have either inlet or outlet streams. There are two general types: "perched" and "flow-through."

Perched seepage lakes are raised above the surrounding terrain, often by build-up of organic deposits; they are often precipitation-dominated in their hydrology.

Flow-through seepage lakes receive considerable groundwater inputs and generally have higher ANC and Si concentrations than do perched seepage lakes.

interpreting diatom data, as Si or can be limiting to diatom growth. Measurement of total dissolved fluorine (F) is needed to calculate the speciation of Al_i into various components, such as $Al(OH)_2^+$, $Al(OH)^{2+}$, AlF_2^+, $Al(F)^{2+}$, Al^{3+}, etc. This can be important because the Al-fluoride species are thought to be less toxic to aquatic biota than the Al-hydroxide species and free aluminum (Al^{3+}).

Table 1-7. Parameters to consider for possible inclusion in surface water acidification studies.

Parameter	Preferred Unit	Importance	Rationale
ANC	µeq/L	High	ANC is the master acid-base chemistry variable in aquatic systems.
pH	--	High	Biota respond strongly to pH.
SO_4^{2-}	µeq/L	High	Usually the major acid anion from atmospheric deposition.
NO_3^-	µeq/L	High	Sometimes an important acid anion.
Ca^{2+}	µeq/L	High	Usually the major base cation.
Mg^{2+}	µeq/L	Moderate	Usually an important base cation.
K^+	µeq/L	Moderate	Base cation, usually in low concentrations.
Na^+	µeq/L	Moderate	Indicator of road salt contamination, geological sources, or sea salt inputs.
Cl^-	µeq/L	Moderate	Indicator of road salt contamination, geological sources, or sea salt inputs.
NH_4^+	µeq/L	Moderate	Potential indicator of agricultural influence or anaerobic conditions.
Specific Cond.	µS/cm	Moderate	Useful in QA evaluation of internal data consistency; potential general screening variable to identify low ionic strength waters.
DOC	µM	Variable	Indicator of organic acidity.
Al_m	µM	Variable	Used with Al_o to estimate potentially toxic Al_i.
Al_o	µM	Variable	Used with Al_m to estimate potentially toxic Al_i.
Si	µM	Variable	Potential indicator of lake hydrologic type and groundwater inputs; may explain some patterns in diatom presence and abundance.
DIC	µM	Low	Used to estimate HCO_3^- concentration.
Total dissolved F	µM	Low	Used for Al_i speciation.

Important aspects of the water chemistry QA/QC evaluation include determining the charge balance and comparing measured and calculated conductivity, the sum of anions and cations, and comparing titrated and calculated ANC.

Charge balance calculations can also be used to determine the charge density (organic anion concentration per mole of DOC) of DOC in surface waters. To permit these QA/QC checks to be conducted, all parameters listed in Table 1-8 are required except Si. Thus, the full list of parameters should be analyzed if funding permits. It is possible to perform these evaluations without a measurement of total dissolved F if one is willing to make certain assumptions about the Al_i speciation.

Table 1-8. Parameters to consider for possible inclusion in studies of atmospheric nutrient nitrogen enrichment of fresh waters.

Parameter	Preferred Unit	Importance	Rationale
Total N	µM	High	Reflects all forms of N in the system.
NO_3^-	µM	High	Biologically available form of N.
NH_4^+	µM	High	Biologically available form of N.
Dissolved organic N (DON)	µM	Moderate	Potentially available form of N.
Total P	µM	High	Reflects all forms of P in the system.
Soluble reactive P (SRP)	µM	High	Biologically available P.
Chlorophyll *a*	µg/L	High	Reflects primary productivity.
Fe	µM	Variable	May bind with P, influencing its bioavailability and transport.
Total Al	µM	Variable	May bind with P, influencing its bioavailability and transport.
Ca^{2+}	µM	Variable	May bind with P, influencing its bioavailability and transport.
Si	µM	Variable	May be limiting to diatoms under some conditions.
Dissolved oxygen (DO)	mg/L	Variable	May decrease to biologically stressful levels under extreme conditions of nutrient inputs (under most conditions of atmospheric nutrient deposition, decreased DO is not an important issue).
Total suspended solids (TSS)	mg/L	Variable	May be an erosional source of P to streams.
Turbidity	standard units	Variable	May be used to estimate TSS.
Secchi depth	m	Variable	Can reflect algal abundance in lakes.

SELECTION OF ANALYTES FOR EUTROPHICATION STUDIES

Eutrophication, or nutrient enrichment, is a potential consequence of N deposition to aquatic ecosystems. Many freshwater ecosystems are P-limited and would not be expected to increase primary productivity in response to increased atmospheric inputs of N. However, there are also many examples of fresh waters that appear to be N-limited or N and P co-limited (e.g., Baron 2006, Elser et al. 2009). In such aquatic systems, atmospheric inputs of N would be expected to increase productivity and/or alter biological communities such as phytoplankton.

Atmospheric deposition of N is expected to increase in the future in remote areas that are situated down-wind from centers of agricultural and/or human population growth. Surface waters in such areas may be N-limited. As a consequence, N additions can contribute to nutrient enrichment (eutrophication), including changes in algal species distribution and abundance. In particular, high-elevation areas in the Sierra Nevada and Rocky Mountains, and perhaps portions of the Cascade Mountains, are susceptible to such increases in nutrient N deposition (Fenn et al. 2003, Sickman et al. 2003b). In some areas, atmospheric N deposition has been linked with eutrophication of high-elevation lakes (cf., Sickman et al. 2003a, Melack et al. 1989).

Estuaries and other coastal ecosystems are also susceptible to nutrient enrichment, especially from N. Estuarine and marine waters tend to be N-limited. Land clearing, agricultural land uses, sewage treatment discharge, and atmospheric deposition can all result in high loadings of N to coastal zones. Excessive N inputs can contribute to a range of impacts, including enhanced algal

blooms, decreased distribution of seagrasses, and decreased dissolved oxygen (DO) concentration (Valiela et al. 1992, Nixon 1995, Borum 1996, Bricker et al. 1999, Kopp and Neckles 2004). Because of human population growth and the great popularity of coastal areas, there is substantial potential for increased N loading to coastal ecosystems. Atmospheric deposition of N contributes to that load, but is generally not the major source of estuarine N. Air Quality Related Values for protection of estuarine ecological conditions are beyond the scope of this protocol and are not addressed.

In a study of potential eutrophication of lake or stream water, a variety of measurements may be useful (Table 1-8). In general, measures of N, P, and chlorophyll *a* are of greatest importance. This protocol recommends, at a minimum, that water samples be analyzed for total N, NO_3^-, NH_4^+, total P, soluble reactive phosphorus (SRP), and chlorophyll *a*. Dissolved organic N (DON) may also be of interest. Measurement of SRP is intended to reflect the forms of P in surface waters that are most readily available to aquatic biota. Nevertheless, P forms are interchangeable within the water column and stream/lake sediment. Therefore, measured total P, which includes both soluble and particulate forms, is also of interest in evaluating potential nutrient limitation and growth responses. NH_4^+ and NO_3^- are primarily biologically available forms of N. Nevertheless, DON may be converted to NH_4^+ and NO_3^- or used directly by some producers and thus, measured total N is also of interest.

Additional physicochemical parameters that can be useful in evaluation of nutrient status include iron (Fe) (and perhaps other metals), Si (lakes only), DO, total suspended solids (TSS), turbidity, calcium (Ca^{2+}), total Al, and Secchi depth (a physical, rather than a chemical, measurement). Iron, Ca^{2+}, and Al can bind to P and influence its cycling between sediment and water and also its bioavailability. Silica can be limiting or co-limiting, along with P and N, to diatom productivity. High productivity in response to nutrient enrichment can lead to reduction in DO. This effect is generally associated with rather extreme eutrophication, well above the levels that might be expected to occur in response to atmospheric deposition inputs to fresh waters on National Forest System lands. Total suspended solids concentration is useful because eroded sediments, especially the smaller clay-sized particles, can be relatively enriched with adsorbed P, depending on local geology and land use. Thus, eroded sediments contribute to the total P in surface waters, especially in streams during high-flow periods.

It is therefore helpful to evaluate human activities throughout the entire watershed, especially land-use actions that contribute substantial erosion to surface waters. Turbidity can sometimes be used, along with the appropriate training set that includes simultaneous measures of TSS and turbidity, to estimate TSS in stream water. It is possible to rely upon routine measurement of turbidity and infrequent measurement of TSS (a more expensive laboratory analysis), along with an empirical relationship between the two to estimate TSS. Depending on the stream, this approach can yield relationships that are more or less robust. Secchi depth provides an indication of relative algal density in lake water, which is one biological response variable.

BIOACCUMULATION AND TOXICITY STUDIES

Atmospheric deposition can contribute to toxicity responses in several ways. The atmospheric pollutants of concern, in addition to H^+ and Al_i associated with acidification toxicity, are primarily Hg, pesticides, and trace metals.

Mercury

Atmospheric deposition is an important component of Hg cycling and biogeochemistry. Mercury is naturally occurring and is found throughout the environment. Mercury is also present in fossil fuels, is released into the atmosphere during combustion, and is subsequently deposited to the Earth's surface. Mercury enters lakes and rivers from atmospheric deposition emitted by air pollution sources and also from nonpoint sources via erosion and runoff.

Mercury is known to bioaccumulate in aquatic organisms, reaching potentially high concentrations in larger, piscivorous fish and the species that consume them such as loons and river otters. Mercury causes physiological and central nervous system effects on piscivorous birds, piscivorous mammals, and humans who consume large quantities of fish or seafood that have accumulated high levels of Hg. Fish consumption advisories for various lakes and rivers have been issued in most States throughout the United States.

Monitoring studies to evaluate the extent to which Hg deposition affects aquatic ecosystems could focus on the concentrations of total and/or methyl Hg in water, or in tissue of invertebrates, fish, or piscivorous birds and mammals. Alternatively, methylation rates within different environmental compartments might be quantified.

This protocol does not recommend that the FS ARM program initiate widespread efforts to measure and/or monitor Hg concentrations in surface waters. Rather, focused studies are recommended in areas where atmospheric deposition of Hg is known or suspected to be high. Such studies should begin by investigating Hg concentrations in muscle tissue of large piscivorous fish as these data would likely be more useful than measurement of Hg concentrations in water for preliminary judgments regarding Hg cycling and toxicity issues. Furthermore, measurement of ambient Hg concentrations in water is technically difficult and requires advanced training of field crews.

Pesticides and Other Toxics

Pesticides and other toxics can also be deposited from the atmosphere and some of these can bioaccumulate in predator species. The degree of bioaccumulation is generally a function of the age of the organism and its position in the food web. In general, older individuals at the top of the food web have bioaccumulated more toxic materials than have younger individuals nearer to the bottom of the food web.

Pesticides applied to agricultural crops can become volatized or suspended in the atmosphere with dust particles, and eventually be transported by wind to remote areas. Organophosphates have been detected in precipitation at elevations up to 1,920 m in Sequoia National Park (Zabik and Seiber 1993) and measured in plant foliage across a range of elevations (Aston and Seiber 1997). Although the effects of atmospheric deposition of pesticides at remote locations are poorly known, there is particular concern that fungicide deposition could harm sensitive lichen species (McCune et al. 2006).

A variety of other toxic chemicals can be atmospherically deposited, some of which have the potential to bioaccumulate. These include trace metals, polychlorinated biphenyls (PCBs), and some fire retardant chemicals.

1.3.3 WHEN TO SAMPLE

There is no one-size-fits-all answer to the question of when to collect samples of surface water for evaluating potential effects of atmospheric deposition. The answer depends on the type of study and its specific objectives. A suggested breakdown of sample timing is given in Table 1-9. It is fairly standard procedure to target sampling to a particular season under base flow conditions. Lakes are often sampled during summer or fall. Streams are often sampled during spring base flow for acidic deposition research and summer for nutrient work. Streams in the southeastern United States, where snowmelt is not a major hydrologic factor, are often sampled during spring. Because of the possibility for either episodic or incremental degradation of water quality, it may be important to implement a program for monitoring both short- and long-term changes.

Table 1-9. Suggested timing of surface water samples for evaluation of sensitivity to and effects from atmospheric deposition.

Type of Study	Suggested Timing of Sample Collection
Lake or stream characterization	Index period – at least one sample each year for at least 3 years, *and* High-flow period (snowmelt and/or rainstorm) - At least two samples, if possible.
Synoptic survey	Index period – At least one sample at each site within a relatively narrow time period; avoid high-flow conditions.
Characterization of episodic chemistry during hydrologic events	High-flow period – At least three samples during each of at least three hydrologic events, including at least one large storm (1-year storm or larger) and (if applicable) one substantial snowmelt event.
Long-term monitoring	Acceptable Approach Index period – At least one sample per year within a relatively narrow time period or hydroperiod; avoid high-flow conditions. Preferred Approach Index period – At least one sample per season during the open-water seasons, within relatively narrow time periods or hydroperiods.
Modeling with MAGIC or PnET-BGC model	Acceptable Approach Index period – At least one sample. Preferred Approach Index period – At least one sample during each of the open-water seasons during at least 3 years.

For some studies, it may be desirable to avoid sampling during abnormally low or high runoff conditions. Other studies may be focused on extreme flow conditions. USGS discharge data (for example, North Carolina data are found at: <http://nc.water.usgs.gov/info/h2o.html>) can be examined before going into the field to evaluate current stream flow from stream gages in the general area of the sample site. This precaution is more important when sampling stream chemistry (as opposed to lake chemistry) and when water characterization will be based on a single sample rather than multiple samples collected at different times. It is also important to consider the potential influence of climatological wet/drought cycles on the chemistry of surface waters.

LAKE OR STREAM CHARACTERIZATION

The water chemistry of lakes or streams is often characterized on the basis of a single sample, collected during the index season (spring or summer for streams; summer or fall for lakes). However, it is preferable to base surface-water characterization, assessment, or modeling on multiple samples collected over several years either throughout the annual cycle or during the index season.

SYNOPTIC SURVEY

The time at which the water sample is collected during a synoptic survey can influence the resulting chemistry and the ways in which the data can be used. Stream-water chemistry, and to a lesser extent lake-water chemistry, can vary diurnally (Burns 1998), seasonally (Lawrence et al. 2004), and annually (Murdoch and Shanley 2006). Stream-water chemistry can also vary hourly in response to changes in flow (Lawrence 2002). If the objective is to conduct a synoptic survey to compare measurements among different streams or lakes within the same region (a spatial assessment), all sites should be sampled in as short a time period as possible. Even this approach might not ensure the same sampling conditions if a localized storm was affecting only part of the study region. Nevertheless, approaches can be adopted to minimize the effects of temporal variability when conducting synoptic sampling.

The major causes of temporal variability are generally flow and weather. Diel changes in pH and metal concentrations due to patterns of photosynthesis and respiration can also be important. Climate is particularly important in regions where snow accumulation occurs in winter, and water chemistry is affected by snowmelt (Campbell et al. 1995, Lawrence et al. 2004). Seasonal effects can be addressed by restricting the sampling to a period that falls within a single season. However, the choice of season can also affect the frequency and magnitude of flow variations. For example, in some regions, summer is the season that typically has the lowest flows and the fewest rain- or snowmelt-driven hydrological events. Scheduling a synoptic survey during a period of stable flows in such regions is therefore more easily accomplished during the summer as compared with other seasons.

The choice of sampling season may necessitate collection of samples when flow variations are relatively frequent and substantial. Extreme chemical conditions are often associated with extreme flows. This is the case with some acidic deposition effects, which tend to be most severe in some regions during the high, fluctuating flows of spring snowmelt or the large rainstorms associated with hurricanes or other major storm systems. Although these are the conditions most difficult to characterize, they are often highly relevant for assessing biological impacts.

Two approaches can be used in synoptic studies to address flow variations within a season, but each requires stream-flow gages within the sampling region to monitor flow conditions during the sampling period. In the first, sampling of each stream is repeated multiple times during the season under a variety of flow conditions. If the mean and distribution of flows at the collection times are not statistically different among the streams, the mean or median chemical concentration at each site can be used as the representative value for comparing streams. This approach was used successfully in the first large-scale stream survey to assess acidic deposition effects in the United States (Colquhoun et al. 1984).

The second approach involves collection of samples at all sites during a period of time that has limited variation in flow. This approach requires close monitoring of weather conditions coupled with the ability to initiate or interrupt the sampling on short notice. This approach was successfully used in two snowmelt surveys by Lawrence et al. (2008b) in the Adirondack region of New York. Collection of all stream samples was done over 3 days when flows were elevated but stable (higher than 90% of the year in one survey and higher than 84% of the year in a second survey). This approach can be challenging to implement with stream sampling sites that are difficult to access or far apart.

Neither of these approaches is likely to collect samples during the most extreme conditions. However, the stream gage(s) flows can be used to determine how the flow conditions during the sampling window relate to conditions for the overall year and previous years. If information is available to show that the chemical measurement of interest is statistically related to flow, then it is possible to estimate the measurement for more extreme flow conditions based on this relationship and available flow data. For streams where flow measurements are not available, chemical concentrations for flows higher than those at the time of sampling can be approximated from an index stream where flow and chemical concentrations are monitored on a regular basis throughout the year. Examples of this approach are given in Eshleman (1988) and Lawrence et al. (2008b).

CHARACTERIZATION OF EPISODIC CHEMISTRY DURING HYDROLOGIC EVENTS

Characterization of episodic chemistry (generally of streams, occasionally also of lakes) during hydrologic events is challenging under the best of circumstances. Given the additional complications of access difficulties for remote sites, episodic sampling in backcountry settings is seldom attempted. Nevertheless, such sampling can yield important information to aid in interpretation of surface water acid-base and nutrient chemistry.

Because of the transient and unpredictable nature of hydrologic events, precise timing of sample-collection occasions is generally not possible. In particular, it cannot be assumed that such samples necessarily capture the most extreme chemical conditions. For that reason, multiple high-flow samples should be collected, if possible, and they should be distributed across multiple years.

LONG-TERM MONITORING

If a long-term monitoring program for lakes is intended to represent a particular time of year, inter-annual variation in lake hydroperiod (periodicity in lake conditions that reflect the changes of the seasons, including water and air temperature, snowmelt, and vegetative development) can introduce substantial variability. This can be especially problematic for high-elevation lakes. The chemistry of such lakes can change, sometimes abruptly in response to spring snowmelt or fall overturn. Such changes are largely governed by the depth of the snowpack, patterns of rainfall, and temperature. Thus, a program that entails, for example, sampling the first week of July each year, while reducing some aspects of inter-annual variability, may still yield considerable year-to-year variability as a consequence of inter-annual differences in snowmelt hydrology. One potential solution is to target sampling to a specific degree day, which is calculated based on maximum and minimum daily temperature (cf. <*http://pnwpest.org/wy/index.html*>). Standardization of sampling timing on the basis of degree day can partially adjust for inter-annual

differences in snowmelt and the transition to summer weather. It should therefore eliminate some, but not all, of the variability associated with standardization based on the calendar for certain types of lakes. Such an approach should not, however, ignore the influence of flow conditions.

One can quantify changes over time in the concentration-discharge relationship. Using this approach, one compares differences between the concentration-discharge relationship determined during one period of time (several months to several years) and the same relationship determined during a later period of time of similar length. If, for example, the concentration of stream ANC is higher at a given flow condition this year than it was several years ago, the change may suggest that ANC may be increasing at times represented by that flow regime, independent of any changes in flow.

Analysis of trends is often done on an annual basis using one of several approaches that incorporate seasonal effects (Helsel and Hirsch 1992; Lawrence et al. 2004). These approaches are most effective if multiple samples are collected for each season. Weekly sampling provides a sufficient number of samples to account for within-season variability and is likely to enable a trend to be detected sooner than data collected at longer intervals. Annual or quarterly sampling is less expensive than weekly sampling, but does not account for within-season variability and can substantially increase the length of time needed to detect a trend (Murdoch and Shanley 2006). However, annual or quarterly sampling may free-up resources to monitor more sites to get a better picture of regional patterns. Thus, the intended eventual use of the data is important for making sampling decisions, as is the length of time one is willing to wait before being able to document with statistical certainty that a change has taken place.

Because long-term monitoring of surface-water chemistry is usually based on sampling at a constant frequency, most samples are typically not collected during high-flow periods. However, long-term trends in stream chemistry may first become apparent during high flows. An approach for separate trend analysis of high, medium, and low flows has recently been developed (Murdoch and Shanley 2006). This method uses annual or grouped years of data to develop concentration-discharge relations that enable concentrations to be predicted for various flow conditions throughout the year. An annual value can then be derived for upper, medium, or low flow ranges so that long-term trends can be determined for each specific flow range. This type of approach requires that: 1) flow is monitored for the stream site of interest; 2) the solute of interest is statistically related to flow; and 3) sufficient data are available to develop the concentration-discharge relations.

1.4 FIELD METHODS

The methods outlined here are appropriate for analysis of low ionic-strength stream and lake waters associated with forested and alpine watersheds in lands that are sensitive to acidification, toxicity, and/or nutrient enrichment impacts from atmospheric deposition. Because stream and lake waters in these sensitive areas can be extremely dilute and easily contaminated, care must be taken in all phases of sample collection and analysis to ensure that data will be of sufficient quality to support the intended assessment purposes. Each of the important aspects of field sampling is discussed here, with an explanation of the reasons why certain steps should be taken

or avoided in the sampling program. The intent is to provide a general understanding of sampling issues. The specific, step-by-step instructions to the field personnel should be adapted by the project manager from the SOPs included in the appendices for the relevant protocol. The protocol provides core attributes and standard sampling design and procedures across the country, which should then be used by local FS personnel to develop a site-specific project plan that includes the SOP with the specific steps to be followed by field staff. The project plan and SOPs may vary from study to study, but the overall protocol remains constant. General SOPs are provided in Appendix D, which should be modified as needed.

1.4.1 PRE-TRIP PREPARATIONS

A field data sheet should be prepared in advance for each sampling site. The lake and stream sampling record data forms are provided in Appendix E. The appropriate data forms should be completed to the extent possible before the sampling trip. For previously established sites, the available site information, site tag number, and description of the tag tree (where applicable) should be filled out on each form.

Pre-trip preparation should include an evaluation of sample-holding-time issues. Determine in advance what parameters will be analyzed in the laboratory, and then check the laboratory protocol to determine the holding-time requirements of these measurements. The requirements may influence sample-collection scheduling and/or in-field sample aliquot preservation decisions. Coordinate with the laboratory regarding the sample delivery timing. In general, sampling should not be done late in the week or in advance of a holiday unless arrangements have been made with the laboratory to receive samples on weekends or holidays.

The most important issues to consider before going to the field can vary depending on the type of study. A checklist of important issues to consider in advance of initiating and implementing an inventory or monitoring field effort is provided in Table 1-10. Additional issues to consider before initiating a long-term monitoring effort are listed in Table 1-11. Table 1-12 provides a list of issues to consider when conducting a study that will involve critical load or emissions scenario modeling.

Table 1-10. Key issues to consider when initiating and implementing an inventory of lake or stream water chemistry to assess the effects of atmospheric deposition.

Issue	Question
Site-Selection	Is the lake/stream representative of other lakes/streams in the wider regional or National Forest System land population? How sensitive to the stressor(s) of interest is it expected to be? Is the sampling site and its upstream drainage basin reasonably free of disturbances other than atmospheric deposition (i.e., acid mine drainage; geological S; fertilizer application; heavy livestock influence; riparian, in-channel, or shoreline disturbance)? Is the sample site representative of the body of water being sampled? In other words, is the lake sampling site in the deepest part of the lake or in the outlet stream? Is the stream sampling site in the thalweg well below the nearest up-stream confluence?
Implementation	Have arrangements been made with the laboratory? Are any required permits or property access permissions obtained? Have issues associated with laboratory holding times and length of time needed for -site access and delivery of samples to the laboratory been addressed? Have clean, appropriately sized bottles and (if required) syringes been obtained? Have decisions been made about field QA/QC activities including use of sample replication and field blanks? Have all required sampling equipment and supplies been assembled and checked? Have all safety procedures been reviewed? Is the timing of sample collection standardized and appropriate to the research questions? For example, is sampling focused on a summer or fall index period for lakes? Is sampling linked to seasonal climatic shifts? How many samples are collected from each site each year, and how are they distributed in time?

Table 1-11. Key issues to consider when initiating and implementing a long-term monitoring effort to document and quantify trends in lake or stream chemistry over time in response to inputs of atmospheric deposition. Issues include all of those listed in Table 1-10, plus the following.

Issue	Question
Expertise	Has a statistician or someone knowledgeable about statistics been consulted in planning the monitoring effort?
Temporal variability	Has temporal variability in the lake or stream been characterized before including that body of water in the monitoring program? This might include, for example, collection of weekly or seasonal samples within the index period during 1 or 2 years. Has an analysis been conducted to determine, given the amount of temporal variability documented in this water body, how large a change over what period of time would allow unambiguous, statistically significant demonstration of change over time in key water chemistry parameter(s)?

Table 1-12. Key issues to consider when conducting modeling using the MAGIC model (Cosby et al. 1985) to estimate critical load or to calculate changes in lake/stream chemistry in response to future emissions controls. Issues include all those listed in Table 1-10, plus the following.

Issue	Question
Soils	Are soils data available for the subject watershed? Although soils protocols are beyond the scope of this document, MAGIC requires soil chemistry data from the upper mineral B soil horizon (often the top 20 cm of the B horizon). Two to three soil pits per watershed are generally recommended. Soil parameters needed for MAGIC include pH; cation exchange capacity (CEC); exchangeable Ca, Mg, K, Na, and Al; exchangeable acidity; bulk density; loss on ignition; and an estimate of soil depth.
Discharge	Is annual discharge available from a stream gage at or near the location of sample collection? If not, can discharge or runoff be estimated from regional data?
Deposition	Are estimates available for total (wet plus dry plus occult) deposition of S, oxidized N, and reduced N?

Table 1–12. Continued.

Issue	Question
Models	If models other than MAGIC are to be used, have the required inputs been determined and are appropriate input data available? If critical and/or target loads are to be modeled, have decisions been made regarding the resource(s) to be protected, chemical indicators of biological effects, critical levels of those chemical indicators, and time period at which protection is to be evaluated?

PERMITS AND ACCESS

Sampling in designated wilderness areas may require obtaining special permission or permits. Typically, if the monitoring plan involves installation of equipment or motorized access, special authorization will have to be obtained. It may not be possible to obtain permission to install equipment, such as a stream gage, in the most highly protected areas. Early coordination with forest managers and staff regarding all monitoring programs is recommended.

Sampling on lands outside National Forest boundaries or obtaining access across other lands may require additional coordination and planning. Make sure you plan well ahead to obtain any permits or permissions necessary to conduct the project before finalizing your plans. Consider selecting back-up sample locations to be used only if access is not available to the intended sampling site(s). Such an approach may be useful if, for example, road or trail access is blocked by late snowpack, road washout, avalanche, land slide, or other impediment to access of remote sites.

LABORATORY AND SAMPLE BOTTLE ARRANGEMENTS

Appropriate agreements should be made or contracts established with a qualified water chemistry laboratory well in advance of field sampling. If waters are expected to be dilute, the laboratory must be able to implement low-ionic strength methods to achieve the necessary data quality objectives. The laboratory, or another entity, should prepare and provide sample bottles, insulated shipping containers, and refrigerant. Historically, the FS Air Resource Management Laboratory (ARML) in Fort Collins, Colorado, has performed many of the analyses on water chemistry samples collected on National Forest System lands to evaluate sensitivity to and effects from atmospheric deposition.

This protocol recommends that plastic ware and plastic aliquot bottles should be high density polyethylene (HDPE), low density polyethylene (LDPE), or polypropylene. The sample bottle must be made of a material that is nonreactive with the chemical constituents to be measured. Polyethylene and polypropylene are commonly assumed to be essentially inert with respect to most dissolved substances. Harder plastics, such as polycarbonate, tend to be less reactive but will crack more easily than softer plastics. Glass is generally preferred for measurement of low concentrations of dissolved carbon. Teflon®, the most inert plastic but also the most expensive, can be used for measurements of trace concentrations of highly reactive substances.

New bottles should be soaked in deionized water (DIW) before use. Samples can also be collected into previously used bottles that have been rinsed with a dilute wash acid (e.g., hydrogen chloride [HCl] 2%) and soaked in DIW for at least 24 hr. The laboratory should follow a procedure to check acid-washed bottles to ensure that all traces of the acid are undetectable in a

chemical analysis. The ARML does not acid wash bottles. Instead, bottles are washed and subsequently checked with DIW blanks to ensure that cleaning is adequate.

Generally, the laboratory is responsible for providing contamination-free bottles for the sampling. Sample bottle preparation should involve a triple-rinsing of each bottle with DIW. The bottles should then be stored overnight or longer filled with DIW, followed by another rinse with DIW. Ideally, each bottle should then be filled with DIW (which can be poured out after 24 hours or at the time of sample collection). Treating the sample bottles in this manner will help ensure a contamination-free sample. Laboratory conductivity analyses of blank samples typically employ a standard acceptance criterion of less than about 1.2 to 2 µS/cm.

Note that, if water samples are to be collected for analysis of Hg concentrations, sample bottles will need to be Teflon or glass (with Teflon-lined caps), and special bottle cleaning procedures will need to be followed, including prolonged heating in an acid solution. Check with the analytical laboratory for specific requirements.

Sample Bottles and Labels

The size of sample collection bottles can vary depending on the parameters to be analyzed, but should usually be 500 ml or 1000 ml (large enough to allow reanalysis, if necessary), wide-mouth HDPE or LDPE bottles. For some studies, it may be possible to use smaller bottles; some studies collect 2 L of water, but a full bottle weighs over 4 pounds (2 kg).

Pre-printed labels with prompts for all required information associated with the sample should be affixed to each sample bottle as part of the bottle preparation (see Appendix F). At a minimum, this information should include:

1. collection date and time;
2. lake or stream name and site location (inlet/outlet/deep);
3. name of the person who collected the sample (first initial and complete last name);
4. Sample ID/Barcode; and
5. whether the sample is a regular, replicate or blank sample.

The field crew must take precautions to ensure that no bottle mouth is contaminated with leaking refrigerant, tap water, dirt, handling contact or other foreign substance. Package each processed bottle individually in a zipper-lock type plastic bag. Refrigerant should be in double zipper-lock bags. This precaution is especially important if the laboratory analysis indicates that sample contamination has been an issue in the past.

The following steps need to be completed before going to the field:

- Obtain the necessary sample bottles and (if required) syringes for each site to be sampled. Depending on the intended laboratory analyses and sample replication requirements, this can range from one to several bottles and syringes per site. Preprocessed bottles, often filled with DIW, with a preprinted or blank label tape affixed, should be provided by the analytical laboratory. It is a good idea to carry a few extra bottles beyond those needed for the intended sampling.
- Field studies often, but not always, include some sample replication (often 5 to 10% of samples) for quality assurance purposes. Replication is generally desired to assess the

repeatability of the sampling procedure, sample holding and treatment, and laboratory analysis. The amount of replication will be dependent on the sampling design and the QA/QC program. Some studies, including some ARM programs, replicate all samples, but may only pay for analysis of a portion of these, keeping the others as "insurance" against contamination, leakage, or shipping problems. This should be done in areas where access to the site is difficult and travel to the site is the biggest expense involved in the sample collection and analysis.

- Obtain ice blocks and/or frozen refrigerant. Ice blocks generally work better for shipping samples to the lab unless a large number of refreezable packs are used. If using refrigerant, be sure that it has been in the freezer at least two days before the day of sampling. If using block ice, it must be placed in two securely sealed plastic bags to prevent leakage. The outer bag should be clearly marked "ice". If using refrigerant, place each refrigerant container in two securely sealed zipper-type plastic bags to prevent sample bottle contamination in the event of leakage. Place the refrigerant containers into insulated container(s) that will be used for sample holding and transport. Provide enough refrigerant to keep the samples cold until delivery to the lab or until placement in a refrigerator if samples are to be stored at a staging area before shipping.
- Transport the sample bottles and syringes, including the replicate bottles and process blank bottles (if applicable), in the cooler that will be used to store the samples. Bottles can be transferred to a small cooler, suitable for carrying in a backpack, before departing from the trailhead to access a site.

Note that, if sample filtering is to be performed in the field (not recommended in this protocol), then field blanks should be transported to the field, filtered in the field, and returned to the laboratory for analysis due to the increased potential of contamination with field filtering.

ACQUISITION OF EQUIPMENT, SUPPLIES, AND DATA FORMS

Each person or sample-collection team should typically be provided the following equipment and materials:

- Site information folders (including maps and stream water field data forms);
- Site documentation materials;
- Sampling protocol (this document);
- SOPs document(s) for sampling and sample handling;
- Sampling bottles (with label affixed, in a zipper-lock plastic bag) and syringes, if applicable (in a light-weight plastic box with snap-on lid, large enough to hold multiple syringes with plunger pulled three-fourths of the way out);
- Plastic gloves, stored in a secure plastic bag;
- Insulated containers, refrigerant, and backpacks;
- Thermometer appropriate for use in air or water;
- Wrist watch;
- Survey-grade global positioning system and compass;

- 50- or 100-meter tape (and, if available, laser rangefinder [optional]) to measure distances;
- Waterproof labels and markers;
- Notebooks and number 2 pencils or write-in-rain-type pens;
- Digital camera and extra memory cards and battery;
- Heavy-duty aluminum tags, aluminum nails, and a hammer if the sites are being established for long-term monitoring of water chemistry and this type of marking is permitted;
- Backpack with waterproof cover (if the site is not accessible by vehicle);
- Van Dorn sampler if sampling a lake;
- Cable and instrumentation for lake "at-depth" measurements;
- Raft or float tube for in-lake sampling;
- First aid kit; and
- Locally appropriate safety equipment.

Depending on the study, other materials may also be required.

Sufficient time should be allocated in advance of field work for assembling and checking all equipment needed for the sampling program and to make sure that field personnel are thoroughly familiar with all field equipment. Arrangements will need to be made in advance for a vehicle that is suitable for carrying capacity needs (people and equipment) and anticipated road conditions.

Supplies must be assembled for sample collection and transport. These include sample bottles (usually provided by the laboratory), sample syringes (if applicable), refrigerant, coolers or other sample containers for transport of samples from the field to the vehicle, coolers for transport of samples from the vehicle to field staging areas, and packaging materials (including refrigerant) for shipping samples to the laboratory.

PLAN FOR STAFFING

Field sampling staffing needs should be determined well in advance of sampling activities, allowing an adequate time buffer for possible extension of the sampling effort in the event of inclement weather or unforeseen circumstances. Field efforts frequently require more time than is initially estimated. In addition, it can be advantageous to identify at least one back-up field person in the event of sickness or injury.

Field staff should be current on first aid training and local emergency procedures before heading to the field. Lead time of several months may be required for staff to obtain proper first aid training.

1.4.2 SAMPLE COLLECTION

Sample bottles should be labeled using label tape and indelible ink (Appendix F). Information on the bottle label should also be recorded on a chain-of-custody record (Appendix E), along with information about the desired analyses and the identity (ID) of the sample collector. A field logbook should be kept in which station identification codes, dates and times of sampling, and all

field data are recorded. Notes on any unusual conditions at the sample sites or any circumstances that may have caused deviation from normal procedures should be recorded on the Lake or Stream Sampling Record Form and described in the field data logbook.

Each sample should be labeled uniquely with site identifier (site name and ID number), date, and unique barcode or ID in the field. An additional lab number is typically added at a later time to each aliquot in the laboratory. The sampler should fill out the bottle label at the time of sample collection. The Chain of Custody Record Form should include the sample name, date, time of day, analyses requested, comments, and appropriate signatures. Collection time should include whether the time recorded was in Daylight Savings Time or Standard Time. (Most electronic data recording, including stream stage, is recorded in Standard Time, year round. Therefore, this information is needed to relate the water sample to the electronic data.) A chain-of-custody form is shown in Appendix E.

This protocol recommends that aliquots of samples be collected in syringes or glass bottles with septum caps along with the standard bottles and that the syringe or septum-capped samples be used for analysis of pH and DIC. This precaution is considered more important for streams than it is for epilimnetic samples from lakes. If samples for these analyses are collected into bottles (with or without septum caps) in the field, it is especially important that no head space be left in the bottle (i.e., that the bottle is filled completely to the top) and that laboratory procedures limit the opportunity for CO_2 degassing in the laboratory before and during analysis of these parameters. Filled syringes should be transported from the field to the lab in plastic containers that minimize disturbance of the seals of the syringes and protects them from breakage.

Dissolved inorganic carbon (DIC) concentrations and pH are the measurements that are typically analyzed without contact with the atmosphere. Water sample aliquots for DIC and pH from streams should be collected in the field in sealed syringes or glass bottles with septum caps for some analytes to minimize contact with the atmosphere in the event that the dissolved carbon dioxide partial pressure is considerably higher than that of the atmosphere; in these cases, the concentration of some analytes can change if the water sample equilibrates with atmospheric carbon dioxide partial pressure before analysis. This is a common occurrence in surface waters. Collection into a bottle having a septum cap is done by immersing and capping the bottle underwater. Throughout the water chemistry sampling process, it is vital to take precautions to avoid contaminating the sample. Many surface waters in regions of the United States considered sensitive to effects of atmospheric deposition have low ionic strength (i.e., low levels of chemical constituents). Samples from such waters can be contaminated quite easily by perspiration from hands, sneezing, smoking, suntan lotion, insect repellent, fumes from gasoline engines, or chemicals used during sample collection.

For quality assurance, sample collection should be routinely replicated so that the variability introduced by the collection process can be quantified. Although duplicate collection of samples from a subset of the sampling sites is sometimes done, the collection of three replicate samples from a subset of pre-specified sites is an alternative approach for characterizing variability. The entire collection process should be repeated for the duplicate pairs or triplicates so that either two or three sample bottles representing the same sample location and taken at approximately the same sample time are returned to the laboratory. The frequency of replicate sampling is dependent on the overall structure and requirements of the quality assurance program. Some

studies replicate all samples in the field but analyze only a subset of the replicates in the laboratory. This protocol recommends collection and laboratory analysis of duplicate pairs for 5% to 10% of the field samples in a given study.

Water temperature should be measured at the location of sample collection. Place the thermometer in the water at the sampling point and wait for the reading to stabilize. If this is not practical, temperature can be measured in a sampling bottle designated for this purpose and labeled as such. In the latter case, cool the bottle to ambient stream temperature before filling it with stream or lake water for temperature measurement. An example localized SOP illustrating surface water chemistry sampling procedures for lake and streams can be found in Appendix C.

COLLECTION OF STREAM WATER

The stream water sample should be representative of stream water at the location of interest with respect to the measurement(s) of interest. Collection of water at a single point will provide a representative sample of the channel cross-section if the stream is uniformly mixed. The mixing of stream water increases with increasing flow velocity and roughness of the channel bottom. Streams are generally well mixed with regard to dissolved substances if flow is turbulent and there are no close upstream tributaries or nearby point sources of contamination. To verify that the stream is uniformly mixed, sampling can be done for measurement of specific conductance (for example, using a meter in the field) and perhaps other parameters at several points along the cross-section and at different depths. If the measurements do not vary beyond the expected analytical variability, sampling at a single point can be done thereafter. If the required sampling location is not well mixed along the cross-section, depth-integrated samples will have to be collected at multiple points along the cross-section or the sampling site might be moved to a different location. In general, stream sampling within the FS ARM program will involve sampling relatively fast-flowing small streams free of point source impacts. The water in such streams should generally be well-mixed. This protocol therefore recommends sampling at a single point in the main area of flow across the stream cross-section unless local conditions suggest the likelihood of incomplete mixing of water in the stream.

A stream sampling training video has been produced by the Forest Service. Field personnel should review this video as part of their field training program. It is available on the Web via <*http://www.fs.fed.us/air*>.

Manual Sampling

At many sites, the sample bottle may also serve as the collection device by simply dipping the sample bottle into the stream by hand. This avoids the need for a collecting device, thereby reducing equipment needs and the chance for sample contamination. At stream sites that are hazardous to access because of steep banks and/or high flows, a sampling pole (a long pole with a bottle attachment point on the end) or a weighted bottle holder can be used so that the collection bottle can be extended out to the stream or lowered into the stream from above.

With the weighted bottle approach, an open sample bottle is placed within the weighted holder and lowered into the water with a handline. Discrete volume samplers (such as a Van Dorn sampler) can also be used to collect the water sample, but are usually not necessary in relatively

small streams with well-mixed flows where the objective is measurement of dissolved constituents in stream water.

There are many acceptable methods of collecting water samples. Some, such as flow integrated stream samples, are complex and beyond the scope of this protocol; studies requiring such sampling should consult appropriate references (e.g., Wilde et al. 1999). Methods likely to be used to collect samples for most stream studies of atmospheric deposition effects are described below. The information in this section is taken largely from the recommendations of Turk (2001).

Grab/Hand Samples

Once the sampling site has been selected, bottles should be assembled and necessary information added to the labels to unambiguously identify the samples. If the bottles contain DIW, this should be discarded away from the shore so it does not disturb the sampling site.

Avoiding disturbance that can affect the water being sampled is especially important. For grab samples, the most likely disturbances are stirring up sediment or incorporating surface debris into the sample; each can significantly change analytical results.

Personnel falling into the stream is not only a major disturbance of sediment but can also pose a safety risk; thus, selection of a stable place to wade or a shore location from which to reach the sample location is critical. Suitable sampling sites are often slippery due to water, ice, algae, mud, or an unstable substrate, such as loose boulders or poorly stabilized logs. If the sampler tries to use both hands for handling bottles while leaning over the water, sudden loss of balance can occur.

The sample should not be collected where the sampler has waded or fallen. If the sampler is holding the bottles in hand, powder free gloves can minimize contamination from sweat, etc. Laboratory gloves generally cover hands to the wrist, but longer gauntlet-style gloves cover to the elbow and should be used if the sample is collected by hand at a depth greater than a few inches. In addition to salts in sweat, common contaminants include sunscreen and insect repellent. All of these potential contaminants can be minimized by thoroughly rinsing hands and arms before collection and also rinsing the gloves at a site far enough away (and downgradient) such that the sampling site itself is not contaminated by the rinse water.

For cleaning before sample collection, bottles should be individually uncapped underwater, partially filled with stream water, capped and shaken, and the rinse water discarded away from the location where the samples are to be collected (e.g., onshore or downstream). Rinse water should be poured over the cap as the water is being discarded. Three rinses for each bottle are required unless protocols specify otherwise. The bottles are then filled completely and capped underwater. The sampling depth should be consistent for all samples and documented. In general, for streams that are less than about 2 m deep, where possible, sample at a depth of about 0.3 m or mid-way between the water surface and the water/sediment interface, whichever is closest to the surface. It often is impractical and/or unsafe to collect samples deeper than about 0.3 m without a sampling device of some kind.

Shallow Samples

If the water at the site is very shallow, which may be the case for many small streams and the outflow of some lakes, it may not be possible to sample very far below the surface. Very shallow streams and seeps may require creative approaches to collecting samples without disturbing sediment. It may be necessary to create a small dam that allows water to drop into the bottle. The bottle cap, pipettes, syringes, or even plastic basters used for cooking can be cleaned and used to transfer samples from the stream to the bottle in extreme cases. One option, using a syringe (a new syringe at each sampling site), is as follows:

- Rinse the syringe three times with stream water, downstream of the sample site, as usual.
- Use the syringe to put stream water in the sample bottle and rinse the sample bottle three times.
- Finally, use the syringe to fill the bottle to the brim with stream water at the sample site. Cap the bottle and proceed as normal.

Pole Samples

An alternative to collecting grab samples by hand is the use of a bottle attached to a pole made of non-contaminating material such as smooth fiberglass or painted aluminum. This approach is safer than leaning over the water surface or wading and often allows the sample to be collected farther from shore and at greater depth than can be done by hand. This approach is not suitable for streams with significant velocity because of excessive drag from the assembly.

The collection bottle can be larger than the sample bottle and can therefore contain sufficient water to rinse and fill it. Alternatively, the sample bottle itself can be directly attached to the pole. Because of buoyancy and leverage, it may be impractical to use a bottle larger than about 500 ml capacity and a pole longer than about 3 to 4 m. The bottle can be attached to the end of the pole with stainless steel hose clamps or laboratory three-finger style bottle clamps. To minimize the possibility of contamination with surface debris or floating slush and to allow collection at a specific depth, the bottle can be plugged with a non-contaminating silicone stopper attached to a line that the sampler pulls when the bottle is at the proper depth. The depth can be estimated or can be measured with a simple float and line attached to the pole near the bottle. Care must be taken to avoid introducing soil or other debris that may have accumulated on the pole into the sample; the pole should be rinsed in an area away from the sampling site before use.

Deep Samples

If the stream is deeper than about 2 m, it is recommended that samples be taken about 0.5 m below the surface if logistics allow or that a shallower sampling site be selected a short distance further upstream or downstream. Sample collection at a depth of 0.5 m can sometimes be achieved using a pole sampler (described above) or a Van Dorn sampler (described in the section on the collection of lakewater samples). The choice will depend on site location and sample collection logistics. Use of a Van Dorn sampler requires low to moderate stream velocity and a stable position from which to collect the sample, such as a bridge, raft, or float tube.

The water sample should be collected from a point where flow velocity is high relative to other points along the cross-section at the sampling location and water depth is sufficient to submerse a

collection device without disturbing bottom sediments. Side pools with low velocity or eddies should not be used for sampling. Powderless latex, polyethylene, or nitrile gloves should be worn while handling sampling equipment and collecting the sample to reduce the chance of contamination. Care must be taken to avoid touching potential contaminating surfaces while wearing the gloves. For personal safety reasons, field personnel should be alert to the possibility that some individuals are allergic to contact with these glove materials.

A stream water sample can be collected from a deep stream at a specific point along the channel cross-section by 1) lowering a weighted collection bottle with a handline, 2) collecting the sample with a discrete-volume water sampler, or 3) drawing the water sample with a suction pump through a tube that is lowered into the water. The sample bottle should be rinsed with stream water three times by partially filling the bottle, capping and shaking it, and dumping the rise water away from the sample site. If wading is required and if it is both practical and safe at the sampling location, the sampler must stand downstream of the point of collection and avoid collecting particulates resuspended by wading or bumping the streambed with the collector. Sample collection with a tube and peristaltic suction pump can be useful when large sample volumes are needed, but the tube and pump must be well rinsed before actual collection of the sample.

Auto Sampling

If high-flow events need to be sampled in a non-wilderness setting, in most cases, auto-samplers should be used. However, if auto sampling is done during the winter at a location that experiences below-freezing temperatures, the auto-sampler should be kept in a heated shelter to prevent collected samples from freezing. In addition, the sampling tube should be buried between the auto-sampler house and the stream to prevent formation of ice plugs in the tube.

Auto-sampling can be done with one of several types of commercially available auto-samplers. All operate similarly and consist of a controller, peristaltic pump, sample tubing that extends into the stream, and space-efficient custom-shaped sample bottles. The water sample is drawn through a weighted suction head attached to the end of the tubing. The auto-sampler can be set to collect at specific time intervals. This can reduce the frequency of site visits. However, to collect samples timed to the hydrograph, the auto-sampler must be controlled by a programmable datalogger that monitors water level changes.

Water level is most often measured by a pressure transducer installed in a deep portion of the stream so that it will remain below-water during low flows. A large number of pressure transducers are commercially available and vary in design and price. The water level measured by the pressure transducer is typically recorded by the datalogger at 15-minute intervals. The datalogger transfers this information to a data storage module for retrieval during site visits. The datalogger can be programmed to trigger the auto-sampler based on the rate and direction of change of the water level so that samples can be collected on ascending and descending limbs of the hydrograph as well as at the peak. Programming the datalogger for this type of sampling will require some knowledge of flow variability in the stream being monitored. A weatherproof box is required to protect the datalogger, storage module, and battery needed to run the datalogger and auto-sampler. Auto samplers are usually kept in a shelter to limit the risk of vandalism.

For automatic collection of samples, the auto-sampler should be placed on the bank where there is no risk of its being washed into the stream during high flows. This also allows the sample tube to drain freely after sample collection. Auto sampling requires that the sampling tube extend into the stream and that the suction head at the end of the tube is anchored in a deep, well-mixed portion of the channel where it will not be easily dislodged by high flows. The suction head should be positioned so that it will not draw in sediment from the bottom. The entire section of the sampling tube under water also needs to be well anchored. The pressure transducer and its line to the datalogger should be similarly anchored on the stream bottom.

Flow-activated auto-samplers should be visited promptly after hydrological events to retrieve the samples and reset the auto-sampler. Disposable, powderless latex or nitrile gloves should be worn while handling sampling equipment to reduce the chance of contamination. Care must be taken to avoid touching potential contaminating surfaces while wearing the gloves.

Upon arrival at the site, bottles in the auto-sampler that contain samples should be capped and labeled with the date, site, and their position number in the auto-sampler. These bottles can then be removed and replaced with clean, empty bottles with caps removed. The auto-sampler is then prepared for sampling by resetting the counter and sampler spout to sample position number one. Data can then be downloaded from the datalogger. These data provide the date, time, and water level associated with each collected sample. This information can then be used to select samples for chemical analysis.

COLLECTION OF LAKE WATER

Lake water sampling should typically be done by boat, raft, or float tube over the deepest part of the lake. Such a sample is intended to represent average lake chemistry. If boat-based sampling is not possible, an alternative protocol is to sample the outlet stream, if one is present, close to the lake. Outlet stream chemistry should closely approximate average lake chemistry unless there are major perturbations in the vicinity of the outlet stream. Note that, even though this sample is actually collected from the outlet stream, it is intended to represent the chemistry of the lake. Thus, the sample should be labeled and documented as a lake sample rather than a stream sample. In general, this protocol recommends that lake samples should not be collected from the shore line for the purpose of characterizing overall lake chemistry as the chemistry of this water may be different from the lake average, due in part to differences in temperature and biological productivity. Nevertheless, if there is no outlet present or if the outlet is not flowing at the time of sample collection, an alternative, less desirable approach is to collect the sample from the shoreline. For this approach, a shoreline sample collection location should be selected that satisfies as many of the following criteria as possible:

- As close to the outlet as possible;
- Near the lowest point of land around the perimeter of the lake;
- From a bedrock outcropping or otherwise rocky area; and
- From the deepest accessible point.

Water must be deep enough so that surface scum and sediments are not collected into the bottle, preferably in a wind-exposed area so that the water is relatively well-mixed. Avoid sampling in locations having emergent vegetation and/or downed logs or other woody debris.

Lake samples collected from the deepest lake location are normally collected using a Van Dorn sampler at a pre-specified depth. The Van Dorn sampler and any associated tubing should be rinsed three times with lake water, emptied, and then lowered to the specified sampling depth for sample collection. The sampler should be held at the sampling depth for approximately 1 minute to allow equilibration with the water at that depth. A weighted messenger is used to trigger closure of the sampler doors for retrieval of the sample. Water from the Van Dorn is then used to rinse the sample bottle and cap (and syringe[s] if applicable) three times before filling the bottle.

Sample depth for lakes should be standardized to the extent possible across all lakes included within a particular program. For lakes deeper than 2 m, a sample depth of 1.0 or 1.5 m is commonly specified. For lakes less than 2 m deep and for lakeshore sampling (where required), the single-depth sample should be collected at 0.5 m depth. An alternate approach (not detailed in this protocol) is to collect a depth-integrated sample using a 2 m-long tube. The EPA's National Lake Assessment project collected depth-integrated samples of the euphotic zone, estimated as two times the Secchi depth, to a maximum of 2 m.

Normally, a surface sample (i.e., 1.5 m depth) from the deepest part of the lake, with or without replicate(s), is used to characterize lake chemistry at the time of sampling. For some studies, additional samples may be required. These might include samples of particular portions of the lake, littoral zone samples, or samples at different depths. Even if samples are not collected at different depths, lake sampling should ideally be accompanied by measurements of the temperature profile in order to determine if the lake is stratified and to characterize the location and depth of the thermocline, the epilimnion, and the hypolimnion. See the SOP for depth measurements in Appendix D. If samples are collected at different depths, the water temperature should be measured at each sampled depth in conjunction with measuring the temperature profile. Water samples cannot be determined to be within a specific littoral zone unless a depth profile is taken. Surface samples without temperature profile should be labeled as water surface samples rather than epilimnetic samples to avoid possible confusion.

Point samples are those collected at a specific depth. Van Dorn cylinders and Kemmerer bottles are the most common point samplers for lakes. The Van Dorn has some advantage in allowing better circulation of water through the sample container. In general, use of a Van Dorn sampler is recommended for lake sampling. In either case, the sampler is difficult to keep clean unless it is kept in a plastic bag between sites. The sampler should be soaked in the lake before use. At the sample site, the sampler should be raised and lowered several times just below the surface to further rinse the container. It is then lowered to the desired depth, held to stabilize it, and triggered, usually with a weight that slides down the line holding the sampler. When sampling the hypolimnion, care should be taken not to touch the bottom as this will disturb sediment that could contaminate the sample.

Because of water drag on long lengths of rope and on the sampler itself when submerged, both Van Dorn and Kemmerer samplers are prone to sampling at shallower depths than indicated by the length of the rope. If the boat is drifting due to current or wind, deep samples may be in error for reported depth. This error can be avoided by anchoring the sampler or tying the rope to a fixed buoy. These samplers also tend to plane while being lowered; allowing the rope to straighten before triggering the bottle can help minimize this error.

Pumps and tubing are sometimes used to collect point samples from lakes and streams or to integrate samples from lakes. The use of tubing offers the ability to collect an integrated sample of the water column in lakes by lowering the tubing at a constant rate of travel while collecting samples downward to near the bottom and then back up to the surface. In general, however, lake water sampling for inventory and monitoring of the effects of atmospheric deposition does not require collection of depth-integrated samples with a tube.

The primary concern in using these devices is keeping the tubing clean. It is impossible to thoroughly clean the inside of a tube in order to eliminate bacterial growth. Sampling tubes can generally be kept relatively clean by storing them filled with DIW and in a black plastic bag (to reduce light and inhibit bacterial growth) and avoiding disturbance and uptake of sediment while sampling near the bottom. The tubing also can be easily blocked with slush during freezing conditions.

At the time of this writing, a lake sampling video is being prepared for use in field-staff training. All field personnel who will be involved in lake sampling should view this video, which can be found on the FS ARM program website: <*http://www.fs.fed.us/air*>.

1.4.3 On-Site Measurements

Evaluation of Site Characteristics

The field notebook and all field forms should be filled in while personnel are at the sampling location so the sample can be accurately linked to field data and observations. Field notebooks are very helpful at sites used for long-term monitoring to provide easy access to location information and maps, historical information on site characteristics, and field data collected during previous years.

Observations and impressions made by the field teams at the sampling location and elsewhere on the target stream or lake are extremely useful for ecological value assessment, evaluation of general water body condition, and data verification and validation. Thus, it is important that observations made by the field team about lake, stream, or watershed characteristics and conditions be recorded while the field personnel are in the field. Field data forms and field notebooks are provided for this purpose. The forms are designed as a guide for recording pertinent field observations. Field data entry forms are never considered to be comprehensive; any additional observations made by the field crew that might eventually be useful in making a site condition assessment should be recorded in the "Comments" section or the field notebook. Team members complete the form at the end of the sampling, taking into account all observations made while on site.

Stream Stage and Discharge and Lake Level

Stream Stage and Discharge

The most valuable non-chemical measurement for interpreting stream chemistry data is often stream flow (also referred to as discharge), which is measured as volume of water per unit time. Variations in stream flow reflect precipitation and the different pathways water takes to reach the stream channel.

During low flow conditions, water discharging into the stream channel has usually had opportunity to pass well below the surface soil into deeper soils, till, and/or bedrock. Such deeper flow paths provide greater contact between water and the soils and geologic materials in the watershed. As a consequence, base flow commonly receives larger quantities of weathering products that can buffer acidity, raise pH, and increase concentrations of base cations in solution.

During high flow periods, some water enters the stream channel through shallow flow paths that more clearly reflect the chemistry of upper soil horizons. Shallow flow paths tend to result in lower concentrations of base cations in drainage water, less acid neutralization (sometimes reflected in increased water acidity), and higher concentrations of DOC than deeper flow paths.

The United States Geological Survey (USGS) is the recognized leader in development and implementation of flow measurements. USGS protocols and recommended equipment for measuring flow are detailed in Rantz (1982). The following provides a synopsis of this material. In addition, USGS has produced a training video for measuring discharge. It is available on the Web at <*http://wwwrcamnl.wr.usgs.gov/sws/SWTraining/WRIR004036/Index.html*>. Field personnel should review this video as part of their field training program.

To measure stream flow, some type of channel control is necessary. This control may be constructed as a temporary feature, such as a weir or dam, or a natural control, such as a bedrock outcrop or channel-width restriction. An effective control provides a predictable relationship between water level (stage) and flow that does not change over time. A pressure-transducer installed in the deepest part of the stream channel, just upstream of the channel control, can be used to record the water level (commonly at 15 minute intervals). A line must be secured in the stream to transmit the response of the pressure transducer to a datalogger. The pressure transducer measures changes in stage; these stage measurements then must be converted to estimates of flow through the channel control. Alternatively, stage can be measured using a measuring rod or yardstick held vertically in place at a specific location.

To establish the relationship between stage and flow (referred to as a rating curve), simultaneous stream stage and flow measurements are needed over as wide a range of stream flows as possible. To conduct the stream flow measurements, a cross-section is chosen in the general vicinity of the location where the water level measurements are taken. The ideal cross-section chosen for measurement should provide a regular cross-sectional channel shape that provides laminar flow throughout the channel. The more closely these conditions are met, the more accurate will be the resulting estimates of discharge.

Stream flow measurement determined with a single stream velocity measurement is not sufficient for obtaining an accurate representation of discharge. The cross-section is divided into intervals such that at least one pair of depth and stream velocity measurements can be made in each interval. The number and width of the intervals are dependent on the shape of the cross-section.

An alternative, common method of estimating stream velocity relies on measuring the velocity of a neutrally buoyant object, such as a small orange, traveling downstream. This approach can provide grossly inaccurate flow estimates as the object can follow preferentially rapid flow paths or, conversely, be temporarily impeded by stones or wood in the channel. This protocol does not recommend use of this method for estimating stream discharge.

The stream velocity can vary considerably through the cross-section by course and depth, requiring a number of velocity measurements to obtain an accurate flow measurement. A large variety of stream velocity meters with varying precision and accuracy are available commercially. The velocity of each interval is multiplied by the cross-sectional area of that interval and the products of all intervals are summed to provide the estimate of stream flow.

The salt dilution gaging method also provides an approach for estimating stream flow at locations where reliable measurements with flow meters are not possible. Such conditions occur in streams that are highly turbulent or have irregular channels,, in small streams, and during low flow under conditions when a large part of the flow travels through gravel and rocks in the stream bed. For such conditions, salt dilution gaging provides a more reliable method for discharge measurement. Salt dilution gaging involves the addition of a known quantity of salt upstream of the gaging site, either by a single addition or by continuous injection. Discharge is computed based on the concentration or dilution of the salt, determined by conductivity measurements, as it passes the gaging site.

Making flow measurements during periods of high flow may not be safe or it may not be possible to wade into the stream under such conditions. If there is any doubt about the safety of wading under the existing flow conditions, field staff should not enter the stream.

If information on flow is needed to aid in the interpretation of stream chemistry measurements but neither installation of a stream gage nor collection of flow measurements are feasible, water level can be manually recorded from a staff gage at the time that the water sample is collected. This will provide data on stage, though not on discharge. For some research or monitoring project objectives, relative differences in stream stage may be sufficient in place of the more quantitative discharge data.

The staff gage should be located just upstream of an effective control. The staff gage is usually comprised of a pressure-treated post or metal fence post anchored in the stream with a large ruler attached. The elevation of the ruler should be surveyed in reference to an object near the bank that would be considered immovable. This enables future verification that the staff gage has not moved. If the chemical concentrations that are being measured are statistically related to flow, they are also related to stage, although the relationship can differ. Changes in chemical concentrations of flow-dependent constituents can be estimated from stream stage measurements in a manner similar to that done with flow measurements.

Another approach to estimating discharge is to characterize regional flow conditions based on nearby gages on similar watersheds. Using this approach, it is possible to obtain a general idea of the likely flow conditions at the sampling site at the time of sample collection without the need for site-specific measurements. It is important to note that, although discharge can be a very useful parameter in evaluating the effects of atmospheric deposition, it is not absolutely essential. One should not choose to avoid sampling a site for water chemistry simply because it is impractical or impossible to collect parallel data on discharge.

Lake Level

For interpreting lake chemistry data, the lake level can be especially useful. Of particular importance is the likelihood that lake chemistry will vary with precipitation cycles. During drought periods, a higher proportion of inflowing water may follow relatively deep flowpaths, allowing for greater acid neutralization and base cation mobilization. During wet periods, drainage water may preferentially follow shallow flowpaths, allowing less contact with soils and geologic materials and therefore limited acid neutralization. Seepage lakes may receive proportionately greater inflow of groundwater (which, depending on geological and soil conditions, may be rich in base cations) during drought periods. It is also possible that some seepage lakes might lose their connection with the groundwater during drought, causing the opposite effect.

The extent of such influences on hydrology and consequent acid neutralization are expected to be region- and watershed-specific. Measurement of lake level at the time of sampling can provide critical data to help identify such effects. The simplest way to collect such data is to install a fixed staff gage in the lake and record the lake level at each time of sampling. In wilderness settings or other locations where installation of a staff gage is not allowed or is impractical, relative lake level can be documented by measuring the vertical height of the lake surface below a fixed landmark: for example, a large shoreline rock or tree root.

ANCILLARY DATA

Ancillary data is chemical and physical information that is not critical, but may be useful for analysis. This includes both chemical and physical data.

Chemical Data

Physico-chemical data can be collected on site with field equipment for measurements such as pH, specific conductance, DO, and turbidity. This information can be useful for reconnaissance work and sample site selection or for other investigations in which real-time information is needed to direct field activities. In general, however, this protocol recommends that assessments or monitoring of acid-base chemistry or nutrient status should be made using chemical analyses conducted in the laboratory rather than the field. Commercially available field equipment can produce data of quality similar to that of laboratory equipment for some variables; however, reproducing the clean, controlled environment of a laboratory in the field is difficult. Therefore, if real-time data are not required in order to satisfy the objectives of a particular study or other requirements[7], conducting the chemical analysis in the laboratory is recommended to ensure high data quality. If chemical analysis will be required in the field, pre-mobilization and field calibration checks should be conducted. Other types of chemical measurements can also be made in the field with commercially available analysis kits, but the data obtained using these methods typically only provide a rough approximation that may not be sufficiently accurate or precise for inventory or monitoring.

[7] For example, *in situ* measurement of pH is sometimes required for data used by state 303(d) water quality assessment programs to determine waters to be classified as water quality limited according to the Clean Water Act.

Meters with probes that continuously monitor pH and specific conductance are also available. These probes effectively characterize temporal variability but lack the precision and accuracy of laboratory measurements. The need for temporal resolution should be weighed against data quality objectives, logistics, and costs to determine if *in situ* monitoring is advantageous.

Physical Data

Interpretation of water chemistry data can be significantly aided by ancillary physical measurements, such as air and water temperature, weather conditions, recent precipitation, and snow water equivalence (see the list of Natural Resource Manager Air application [NRM Air] minimum database requirements in Section 1.4.5, Sample Documentation, below). Additional data might also be collected at the sampling site, depending on the study.

For example, it can be useful to develop lake thermal profiles to evaluate the extent of lake stratification. For stratified lakes, it can be useful to collect water samples from the hypolimnion. Such data can be used in evaluating sulfur reduction in the lake sediment, hypolimnetic DO depletion, or the dynamics of phosphorus retention and release in lake sediments. Unless detailed analyses of sulfur or phosphorus cycling are to be conducted, however, hypolimnetic samples are generally not needed.

Studies of temporal trends in surface water quality or characterization of water quality conditions within a specific lake or stream or across a forest can be designed to assess a variety of parameters and changes in those parameters over time. For this to be successful, information is often needed on watershed aspects that can impact or aid in interpretation of water quality. These can include, for example, the variables listed in Table 1-13.

The ecological significance of aquatic ecosystem degradation and loss due to physical habitat alterations can exceed the effects of atmospheric deposition or human activities on water chemistry. Therefore, physical habitat surveys of lake shore areas, littoral zones, stream channels, and riparian zones can be useful in conducting overall habitat condition assessments and in interpreting water chemistry data. Habitat information is helpful in estimating what lake or stream biological assemblages "should" be in the absence of many types of anthropogenic impacts. The physical evaluation can provide a reproducible, quantified estimate of habitat condition, serving as a benchmark against which to compare future habitat changes from anthropogenic activities or extreme events. Furthermore, habitat information can aid in the diagnosis of probable causes of ecological impairment in lakes or streams.

In addition to information collected in the field by the shoreline, stream channel, or littoral zone surveys, the physical habitat description of each lake or stream can include many map-derived or measured variables, such as lake surface area, shoreline length, stream width-to-depth ratio, and habitat integrity or complexity. Furthermore, ancillary information, including watershed topography and land use, supplements the physical habitat information. The shoreline, channel, and littoral surveys concentrate on information best derived "on the ground." As such, these survey results provide part of the linkage between large watershed-scale influences and those forces that directly affect aquatic organisms day-to-day. Together with water chemistry, the habitat measurements and observations describe the variety of physical and chemical conditions that are necessary to support biological diversity and foster long-term ecosystem stability.

Table 1-13. Ancillary measurements that may help in interpretation of lake or stream water chemistry data.

Location	Measurements
Streams	Discharge and/or stage
	Water temperature
	Air temperature
	Snowpack depth and snow water equivalence
	Precipitation
	Watershed morphometry
	Fish stocking and management
	Watershed disturbance[1]
Lakes	Secchi disk transparency
	Thermal profile
	Chlorophyll *a*
	Level (if lake is to be sampled multiple times)
	Hypolimnetic water samples
	Littoral zone water samples
	Dissolved oxygen
	Presence of inflowing or outflowing streams
	Watershed morphometry
	Fish stocking and management
	Watershed disturbance

[1] Watershed disturbance can be evaluated by field reconnaissance, examination of aerial photos, regional land cover data sets, etc.

Habitat surveys should not be considered a necessary component of inventory and monitoring. They require an additional commitment of time and resources. Nevertheless, the data collected in such surveys can be very helpful in the subsequent interpretation of effects, especially if the documentation or quantification of effects relies on collection of biological (i.e., phytoplankton, zooplankton, benthic invertebrates, and fish) as well as chemical variables.

The shoreline and littoral habitat surveys conducted by EPA in the EMAP program employed a randomized, systematic design with 10 equally spaced observation stations located around the shore of each sample lake. Teams went to the field with pre-marked lake outlines showing the station locations. The observations at each station included quantitative and semi-quantitative observations of vegetation structure, anthropogenic disturbances, and bank substrate. In-lake littoral measurements and onshore observations dealt with littoral water depth, bottom substrate, near-shore fish cover, and aquatic macrophyte cover. With quantifiable confidence, investigators condensed these observations into descriptions applicable to the whole lakeshore and littoral zone. For example, team observations led to quantitative descriptions, such as the mean canopy or aquatic macrophyte cover along the lakeshore, the extent of shoreline disturbed by various human activities, and the dominant littoral substrate in the lake. There are similar physical habitat evaluation procedures for streams developed for EPA's national surveys, such as the Wadeable Stream Survey.

1.4.4 Post Collection Sample Processing, Documentation, and Cleanup

In general, this protocol does not recommend filtering lake or stream samples in the field except where immediate filtering is required for a particular measurement. To avoid the possibility of sample contamination, it is generally preferable to perform this step within the controlled conditions of a laboratory. Similarly, measurement of pH in the field is not recommended for most studies. This measurement is best performed in the laboratory under controlled conditions.

For most chemical constituents of interest for atmospheric deposition studies, sample preservation in the field is not necessary. Types of sample preservation may include addition of chemicals or filtering to remove particulates. Preservation procedures are generally done for a specific measurement and usually render the sample unusable for other measurements. Therefore, if preservation in the field is needed for a specific measurement, an aliquot will need to be removed from the sample before preservation. The volume of the aliquot will be dependent on the analytical requirements of the measurement.

Most samples contain dissolved and/or particulate organic matter and associated microbes that can change sample chemistry through decomposition and assimilation. All samples should therefore be placed out of the sunlight and in a cooler with ice as soon as possible for transport back to the laboratory, where they can be refrigerated. This procedure is usually sufficient to slow biological processes enough to prevent measureable changes in chemical concentrations.

As a rule of thumb, samples should be returned from the field as quickly as possible to enable processing in the laboratory, and filtration (if needed) should be performed in the laboratory. An exception applies to the collection and analysis of samples for measurement of chlorophyll *a:* those samples are most commonly filtered in the field and the filter (not the filtrate water) is transported in a zipper lock bag on ice in the dark to the laboratory for analysis. It is essential to record in the Notes section of the sampling record forms the volume of water that was filtered for chlorophyll *a* measurement.

1.4.5 Sample Packaging and Transportation

Filled sample bottles should be placed in zipper lock bags before transport from the field site. Syringe stopcocks should be taped shut to keep from leaking, and syringes should be secured from damage (e.g., placed in a paper towel roll) and packed in a plastic box with snap-on lid for transport and shipping. Each syringe box needs to be long enough to hold syringes that are ⅔ to ¾ filled with sample water and wide enough to hold multiple syringes. The bagged sample bottles and boxed syringes should be packed with double-bagged ice or frozen refrigerant for transport (Figure 1–1).

Figure 1–1. Sample syringe packaging for transportation to the lab. Top left: stopcock taped; top right: syringes and packing tubes; bottom: packing tubes packed in a shipping box.

Insulated containers, with double-bagged chemical refrigerant ("blue-ice") or (preferably) with double-bagged ice blocks, are needed for transport of collected samples between the field and other staging locations and, eventually, the laboratory. Ice works better for shipping unless large numbers of chemical refrigerant packs are used. Small insulated containers that will fit into backpacks can be used to carry and protect the samples in the field. Coolers can be used for assembly and transport of samples in vehicles.

Chemical refrigerant containers should be packaged in two zipper-lock plastic bags to minimize the possibility of sample contamination through refrigerant leakage. Field crews will need to make sure that the chemical refrigerant was placed in a freezer at least two days before sampling to completely freeze the refrigerant.

SAMPLE DOCUMENTATION

Sample documentation should be completed in the field. Documentation includes completing and affixing all sample labels, completing all field and chain-of-custody forms, and recording field observations and site condition information. A list of minimum database requirements for the FS's NRM Air database are:

- Forest name;
- Wilderness name (if applicable);
- Official USGS lake/stream name;
- Site name;
- Site ID;
- Field team leader and contact information;
- Latitude and longitude (decimal degrees);

- Datum used (use NAD83 if possible);
- Monitoring project name;
- Date of visit;
- Time samples were collected;
- SampleID;
- Barcode;
- QA sample type (regular, duplicate, blank, split, etc.);
- Sample measurement location (e.g., inlet, outlet, deep, shore (lake), bank (stream)); and
- Sample method (e.g., grab, pole).

There may be other requirements according to the individual study. Note that this protocol does not *require* collection of temperature or stream flow data, although it does recognize that such data can be very useful in interpreting the results of chemical analyses.

Documentation should also include a review of sampling procedures used, labeling, and photographic/written documentation. Key issues include:

1. Were the samples collected at the designated depth and free of influence from any sediment that could be disturbed during sample collection?
2. Were all sample bottle and sample syringe labels fully completed with all required information?
3. Was the sampling fully documented, including site photographs (if appropriate), completed field and chain-of-custody forms, and field notes?
4. Were any conditions or circumstances noted that could potentially compromise or influence the chemistry of the sample?

POST-SAMPLING EQUIPMENT CLEAN-UP

Clean-up is important to minimize the possibility of transporting pathogens, noxious species, or invasive species from one sampling location to another. Risks vary from region to region and location to location. Field personnel should consult with regional and local FS offices for specific problem identification and appropriate precautions and cleaning protocols. A variety of forest pathogens and aquatic and terrestrial invasive species may be of concern, depending on location. Personnel and their boots, vehicles, boats, and equipment can serve as transport vehicles for problematic species; thorough cleaning of equipment, boots, etc. should be done before leaving the site. Wherever a risk exists, field personnel should take additional site-specific appropriate risk-management precautions.

1.4.6 SEQUENCE OF FIELD ACTIVITIES

The recommended sequence of field activities to be conducted by field personnel at the sampling site is as follows:

1. Select/verify sampling site location.
2. Take photographs.
3. Fill out and affix the label for each sampling bottle to be filled at the site.
4. Evaluate and document site conditions.
5. Determine how many and what kinds of samples will be collected (e.g., QA replicates, single versus integrated samples, or special aliquots to be collected into glass bottle or syringe).
6. Collect water sample(s).
7. If required, preserve or filter (if necessary) or transfer selected sample aliquots to glass bottles or syringes (as appropriate).
8. Place collected samples in cold, dark storage container.
9. Collect any needed ancillary data.
10. Determine (if appropriate) stream discharge or stage.
11. Complete all site documentation and chain-of-custody forms.
12. Record all field observations in a field notebook.
13. Clean equipment, clothing, and boots to prevent spreading invasive species to another site.

1.4.7 SAFETY IN FIELD ACTIVITIES

KEY SAFETY CONSIDERATIONS

For safety reasons, an emergency contact individual who is not part of the field crew should always know where the field crew is going each day and by what route. This person should be contacted by the field crew immediately upon return from the field each day.

For sampling remote locations, safety equipment should include, but should not necessarily be limited to, the following:

- Two-way radios and/or cellular telephones (if cell phone access is available in the study area);
- extra batteries for GPS units and radios;
- Rain gear;
- Emergency shelter blankets;
- An adequate supply of drinking water or an appropriate water filtration system;
- Sunscreen;
- First aid kit; and
- Locally required safety equipment.

All field personnel should have current first aid and CPR certificates. Field personnel should never enter a deep or fast-flowing stream without wearing a personal floatation device (PFD). A PFD should also be worn when working close to a stream during high flow conditions.

When sampling a lake at the mid-lake location it is advantageous for one person to remain on shore to provide logistical support, to record data, and serve as a safety precaution. If necessary, a two-way radio can be used to facilitate communication between mid-lake and shore personnel. Sampling from a boat typically requires two people in the boat, one maintaining the position at the sampling site and the other collecting the samples and measurements. The sampler can monitor present position by keeping two appropriate on-shore landmarks in line. The on-shore person should continuously monitor the float tube location in a similar way.

JOB HAZARD ANALYSIS

Field personnel should review the Job Hazard Analysis (JHA) before going into the field and construct a field itinerary before field work. The itinerary should include:

- Departure date and time;
- Expected return date and time; and
- Expected route of travel (roads, trailheads, trails, and destinations).

An example JHA is provided in Appendix I. A location-specific JHA should be created and periodically updated by the project.

SECTION 2.
LABORATORY PROTOCOLS

G.B. Lawrence, J.R. Webb, T.J. Sullivan, A.T. Herlihy

2.1 INTRODUCTION

Surface water samples collected in watersheds on forest lands with minimal recent influence from development or agriculture tend to have low concentrations of nutrients. Because these types of watersheds are often located in upland areas with rocky, infertile soils, their surface waters may have low ionic strength, which means most other dissolved constituents are also low in concentration. Chemical analysis of dissolved materials at low concentrations sometimes requires modification of the standard methods used for sample preparation and analysis. Therefore, the laboratory selected for analyzing low-nutrient, low-ionic strength surface water samples should not only be experienced with these types of samples, but should 1) analyze them on a routine basis, and 2) provide data with reporting limits sufficiently low for project needs.

To produce high-quality data, a laboratory should have effective procedures in place for each of the following elements:

- Bottle cleaning;
- Sample processing; and
- Chemical analysis.

Each of these elements is described in the sections that follow. A fourth element, QA/QC, is covered in the QA/QC section of this document (Section 3). In addition, the laboratory should follow effective procedures for documentation of method implementation and method changes. This report provides information on all major aspects of successfully operating a laboratory for low-nutrient, low-ionic strength water analysis.

2.2 LABORATORY PREPARATION PRIOR TO SAMPLE ANALYSIS

2.2.1 Bottle Cleaning

All bottles used for sample collection and partitioning the sample into aliquots for transport to the laboratory must be clean and free of any contamination. Generally, it is the responsibility of the laboratory to provide clean bottles to the field crew in advance of field sampling. Low-nutrient,

low-ionic strength samples can be easily contaminated by improperly washed bottles because their low concentrations can be measurably altered by trace amounts of contaminants. Therefore, rigorous cleaning procedures must be used, which are often specific to the intended use of the bottle. Laboratories experienced with low-nutrient, low-ionic strength surface water samples have adopted various methods for cleaning laboratory plasticware and glassware. Washing with dilute acid (usually 1 or 2%) is generally preferred to solubilize potential contaminants that can then be removed with multiple DIW rinses. Acid washing should be done in a dedicated room with a negative pressure ventilation system.

Rinse water should meet the specifications of Type III water (American Society for Testing and Materials [ASTM] 1984). A typical water deionization system that produces Type III water includes, in sequence, a carbon removal tank, 1 μm filtration, and two mixed-bed cation-anion removal tanks. To ensure consistent water quality, specific conductance (or resistance) of the water should be monitored between the two primary treatment modules, in this case the mixed-bed tanks. If the specific conductance exceeds the preset limit of 1.0 μS/cm, an indicator light (which is normally on) is deactivated. The system is checked daily, and if the indicator light is off, the first tank is removed and replaced by the second tank. A new tank is then placed in the second position. By monitoring the specific conductance of the two tanks, the initial tank can serve as a first level treatment and the second tank as a polisher. By switching the polisher to the first position when the first level tank no longer meets the standard, you can extend the lifespan of the tanks and, most importantly, prevent substandard water from being used for sampling. The functionality of the tanks degrades gradually, so the two-stage approach is needed to maintain a consistent level of water quality. The filter is replaced with every tank change, and the carbon tank is replaced in response to the volume of water being treated and the organic carbon concentration of the influent. Once every six months is a typical replacement frequency[8].

To ensure that the wash acid does not itself become a contaminant, repeated rinsing is followed by leaching the bottle with DIW. This is done by filling the rinsed bottle with DIW and storing it for 24 hours or longer (Table 2-1). This step is necessary because acid and other contaminants (such as those from a previous sample) can migrate into the plastic matrix of the bottle wall. Over time, the contaminants can slowly leach out and affect the sample concentration. The level of contamination caused by this process is usually low but can be sufficient to cause measureable increases in low-nutrient, low-ionic strength samples. Simple rinsing does not necessarily eliminate this type of contamination.

Finally, each bottle is filled with DIW before shipping the bottles to the project location. Measurement of specific conductance of DIW stored in sample bottles, with an acceptance criterion of < 1.2 μS/cm[9], provides quality assurance for this procedure. Specific conductance testing can be done on selected sample bottles that are treated as sample blanks.

Aliquot bottles that have had acid added for sample preservation are more likely to have contamination from bottle leaching than those that are in contact with wash acid for 30 minutes or

[8] Note that appropriate methods for tank replacement are to some degree equipment-specific. The procedure described here is one possible approach to producing thoroughly rinsed bottles. Other procedures may also be acceptable.

[9] We recommend using an acceptance criterion of ≤ 1.2 μS/cm, but we recognize that some laboratories use a different criterion: as high as about 2.0 μS/cm.

less. Therefore, preserved sample aliquot bottles require longer periods of DIW leaching. Plastic bottles should not be used if the caps have liners because the liners can become a source of contamination. Glass bottles, such as those needed for DOC aliquots, usually have caps with removable plastic liners. The plastic liners must be removed from the caps for washing and be soaked in DIW for the same length of time that the bottle is soaked before being replaced in the cap.

Because the required cleaning procedure depends on the specific use of the bottle, a set of cleaning procedures needs to be developed and documented by the laboratory. Appropriate procedures to clean the various types of collection and aliquot bottles are listed below and summarized in Table 2-1. Although different laboratories may use different or modified procedures, it is critical to document the specific procedures used and to provide assurance that sample bottle contamination has been avoided.

Additional precautions may be needed when washing with acid solutions such as hydrochloric acid (HCl). Local regulations may require separate disposal of acid (or basic) waste products (depending on the concentration), rather than pouring them down the sink. The possible need for special waste disposal procedures may affect the laboratory budget and may influence decisions regarding bottle washing procedures.

Table 2-1. Summary of a typical bottle rinsing protocol for low-nutrient, low-ionic strength samples.

Bottle	Acid-Wash?	Rinses	DI Soak Period
60 mL plastic (cations, acidified)	Yes	4	1 wk, 2X rinse, 24 hr soak
30 mL plastic (anions)	No	6	24 hr
250 mL plastic (pH, ANC,…)	Yes	4	24 hr
30 mL plastic (total Al, acidified)	Yes	4	1 wk, 2X rinse, 24 hr soak
30 mL plastic (NH4 or DON)	No	6	24 hr
40 mL glass (DOC)	Yes	4	24 hr
DOC caps and liners	Yes	6	24 hr
1 L plastic (field, manual collection)	Yes	4	24 hr
500 mL plastic (field, autosample)	Yes	4	24 hr
1L plastic (field, autosample)	Yes	4	24 hr

CLEANING BOTTLES USED FOR SAMPLE COLLECTION IN THE FIELD

1. Remove sediment and other particles from the bottle with tap water, using a soft plastic brush[10] if necessary, then rinse once with deionized (DI) water.
2. Fill with 2% HCl[11] and let stand for 15 to 30 minutes in a separate bottle-washing room, away from analytical instrumentation.
3. Pour out the HCl and rinse thoroughly four times with DIW.
4. Fill with DIW and store for at least 24 hours.
5. To prepare for transport to the field, empty and rinse once with DIW, then fill with DIW for transport to the field.
6. The bottle should subsequently be rinsed three times in the field with the sample that is being collected before filling the bottle.

CLEANING BOTTLES USED FOR GENERAL LABORATORY USE, UNACIDIFIED ALIQUOTS

1. Empty any remaining sample.
2. Fill with 2% HCl and let stand for 15 to 30 minutes.
3. Pour out the HCl and rinse thoroughly four times with DIW. (HCl can be reused for multiple washings but should be replaced and properly disposed of when it becomes discolored.)
4. Fill with DIW and store for at least one week.
5. To prepare for use, empty and then rinse twice with DIW.

CLEANING BOTTLES USED FOR GENERAL LABORATORY USE, ACIDIFIED ALIQUOTS

1. Pour out any remaining sample.
2. Rinse four times with DIW.
3. Fill with 2% HCl and let stand for 15 to 30 minutes.
4. Pour out the HCl and rinse thoroughly four times with DIW.
5. Fill with DIW and store for at least 24 hours.
6. To prepare for use, empty and rinse once with DIW.

Some labs avoid the acid wash step for aliquots to be analyzed for Cl^-, NH_4^+, and/or DON, and instead rely on DIW leaching to remove any contaminants from the bottles. Depending on the intended use of sample aliquot bottles, the recommended number of rinses can vary. These differences are summarized in Table 2-1. As indicated above, specific conductance should be

[10] Note that use of a stiff bottle brush can scour the inside of the bottle, allowing contaminants to more easily adsorb to the bottle wall.

[11] Note the need to use HCl in a separate bottle washing room, away from analytical instrumentation. HCl can become volatilized and thereby contaminate nearby samples and, eventually, damage equipment.

measured for DIW stored in sample bottles as a quality assurance measure. Such analyses should be conducted, at a minimum, on a subset of bottles before field use and on sample blanks during laboratory analysis.

2.2.2 Sample Processing, Preservation, and Storage

When samples arrive at the laboratory, they need to be accompanied by proper documentation using a chain-of-custody form. This form provides field information that includes project identification: when, where, how, and by whom the sample was collected; and information on the chain-of-custody that was followed. This form will need to have been checked by the field sampler against the information written on the sample bottle label to ensure that the information matches. A format for recording sample information in the field has been developed (Appendix E). The transfer of custody from field personnel to lab personnel must be documented by dated signatures on this form. A copy of the signed form should be kept by both project and laboratory personnel. This procedure is needed to ensure that samples were collected and transferred to the laboratory. Samples can get misplaced before arriving at the laboratory or within the laboratory before processing, particularly if there was a sample labeling error.

Prior to the start of sample processing in the laboratory, a unique code or sample serial number (SSN) is typically assigned by the laboratory and added to the laboratory data sheet. A single person (plus a trained backup) is generally assigned the responsibility to ensure that an SSN is not accidently used for more than one sample. If the SSN is assigned in the field, it is important that it is unique. The SSN will be used by the laboratory to track the sample through the steps of sample processing, chemical analysis, and data management. The information on the chain-of-custody form and laboratory data sheet will be entered into an electronic database through the use of a Laboratory Information Management System (LIMS). A variety of LIMS software is available commercially, though some laboratories develop their own database system.

Each sample will typically be analyzed for a variety of constituents that are chosen to meet stated project goals and require specific processing procedures. Different results may be obtained from the same analysis if samples are prepared for analysis using different procedures. For example, results may differ if samples are filtered with filters of different pore sizes or of different materials. Sample processing can involve both preparation for analysis and preservation of the sample, and therefore, varies among analyses. The details of processing and analyses must be established to ensure that both sample processing and analyses done by the laboratory meet project needs.

Processing is generally accomplished by dividing the sample into several aliquots, each with its own filtration/no filtration, preservation, storage, and process and handling time requirements. For example, analysis for concentrations of base cations (calcium, magnesium, sodium, and potassium) may require filtration through a 0.45-µm polycarbonate filter, whereas analysis of DOC may require filtration through a glass fiber filter in order to avoid possible organic contamination from the polycarbonate filter. Preservation of samples for analysis of base cation and other metals that could form precipitates at non-acidic pH values usually involves the addition of nitric acid. However, addition of chemicals to samples should be avoided unless necessary to reduce the potential for sample contamination or alteration.

All samples that require filtering to remove particulate matter are normally filtered as soon as possible after arrival at the laboratory. Prompt filtration after sample collection is normally done for the purpose of removing bacteria, which can alter sample chemistry through their metabolic processes. Samples should be chilled as soon as possible after collection and kept refrigerated up to the time of processing and preservation in order to retard microbial activity. Some aliquots will continue to be refrigerated until analysis (Table 2-2). Freezing is not necessary for most analytes but is recommended for DON and NH_4^+.

The containers used to store aliquots before analysis also vary by analyte. Aliquots for base cation analysis are generally stored in polyethylene or polypropylene bottles, which are economical and considered sufficiently inert with respect to base cations, whereas aliquots for DOC analysis are usually stored in glass bottles to avoid organic contamination from plastic. An example of a typical sample processing schedule for low-nutrient, low-ionic strength samples is shown in Table 2-2. It is important to establish this type of schedule with laboratory personnel to ensure that all samples from a particular project will receive timely processing. In this example, reminders are included to tape bottle caps and not fill bottles completely for aliquots that are preserved by freezing.

Table 2-2. Example laboratory aliquot schedule for a particular project.

Aliquot[1]	Container	Filter	Treatment	Storage
A	250 mL polyethelene	None	None	Refrigerator (4°C)
B	30 mL polyethelene	0.45 µm polycarb.	None	Refrigerator (4°C)
C	40 mL glass[2]	Glass fiber filter (GFF)	None	Refrigerator (4°C)
D	60 mL polyethelene	0.45 µm polycarb.	0.3 µL HNO_3	Room temp.
E	30 mL polyethelene (taped)	GFF - fill 2/3 full	None	Freezer (label DON)
F	30 mL polyethelene (taped)	None - fill 2/3 full	None	Freezer (label NH_4)[3]

[1] A - pH, ANC, specific conductance, Al_m, organic monomeric Al
B - Sulfate, nitrate, chloride
C - Dissolved organic carbon
D - Calcium, magnesium, sodium, potassium, silicon
E - DON
F – NH_4^+

[2] Use of glass for storing samples in the laboratory before DOC analysis is preferred but not essential.

[3] Ammonium concentrations (although typically very low in natural waters) are very unstable. The sample should be analyzed immediately upon arrival at the laboratory or frozen until time of analysis.

Recommended laboratory holding times are given in Table 2-3. Those laboratory holding times should be considered guidelines. Measurement of a sample analyte past the holding time is not justification for excluding that concentration value from the database. Nevertheless, sample measurements taken beyond the specified holding time should be flagged as such in the database. In general, laboratories should strive to complete analyses within the holding time windows, or as soon as possible thereafter.

Table 2-3. Recommended laboratory holding times. (Source: USGS Troy Laboratory.)

Constituent	Holding Time
pH	2 weeks
conductivity	2 weeks
ANC	2 weeks
ammonium	3 months[1]
dissolved nitrogen	3 months[1]
Al_m	2 weeks
organic monomeric aluminum	2 weeks
calcium	6 months
magnesium	6 months
silicon	6 months
sodium	6 months
potassium	6 months
chloride	1 month
nitrate	1 month
sulfate	1 month
DOC	2 weeks
turbidity	2 days

[1] Samples for NH_4^+ and dissolved N are preserved by freezing and analyzed in batches.

The temperatures of refrigerators and freezers that are used for sample storage must be electronically monitored around the clock to ensure that malfunctions do not result in temperature increases that compromise the samples. Various types of temperature monitors are commercially available to notify laboratory personnel via cell phone or computer that the temperature of a refrigeration unit has exceeded a preset threshold. With this notification, samples can then be quickly transferred to another unit until repairs can be completed.

Each laboratory should have detailed documentation of the steps used in sample processing. An example list of sample processing steps follows:

1. Obtain chain-of-custody forms that have assigned SSNs and aliquot labels that correspond to the processing selected for the project. Initiate laboratory data sheet.
2. Retrieve clean aliquot containers and place appropriate numbered dots and/or label the tape on them. Put on gloves, empty the containers, and rinse them with DIW, if applicable.
3. Retrieve the field samples to be filtered from the refrigerator. If there is not sufficient sample volume to prepare all of the required aliquots, be sure to follow the procedure for low-volume samples.

4. Shake the field sample bottle. Rinse the aliquot bottles with a small volume of the sample. Fill aliquot containers with raw sample for the aliquots that do not require filtering. Fill in the letter code that corresponds to the aliquots on the laboratory data sheet to document the processing method.
5. Retrieve the filtering apparatus from the DIW soak and rinse it well with DIW. Set-up the filtering apparatus on the vacuum manifold.
6. Place an appropriate filter (handling the edge of the filter only) on the filter apparatus with tweezers that have been rinsed with DIW. For 0.4 and 0.1 micron filters, place the shiny side up when appropriate (filters are sometimes packaged shiny side down). Filter 10 mL of DIW through the apparatus and into a waste container, then filter 10 mL of the sample through the apparatus and into a waste container. Discard the filtrate.
7. Place proper aliquot container under filtering apparatus. Filter 5-10 mL of sample into container, rinse, and then discard the filtrate.
8. Filter appropriate amount of sample into container. If another aliquot of the same sample requires the same filter, repeat starting at step 7. Fill in the letter code that corresponds to the aliquots on the laboratory data sheet. Discard the used filter and rinse the filtering apparatus with DIW.
9. Repeat steps 6 through 8 for each sample aliquot. If the filter clogs, replace with a new filter following step 6, then go to step 8.
10. After samples have been processed, rinse the filtering chambers with DIW and place them in DIW soak buckets. Replace the DIW in the buckets weekly.
11. Store the remaining sample volume for possible re-analysis, at least until QA/QC analyses have been completed. Once it has been determined that the analysis meets data quality objectives (DQOs), discard remaining sample in field sample bottles and bring the bottles to the bottle washing room.
12. Aliquots that require acidification should be acidified in the hood using the appropriate acid dispenser.
13. Aliquots should be stored in the appropriate places as described in the specific project sample processing schedule.
14. Date and processor's initials must be recorded on the laboratory data sheet. Completed chain-of-custody forms and laboratory data sheets should be filed in a safe location. Information on these forms will need to be entered into the LIMS.

In some situations, it is possible that the sample bottle was not completely filled in the field. If there is insufficient sample volume for all aliquots, analysis of the sample volume available must be prioritized based on the objectives of the project. If it is anticipated that some low-volume samples will be collected, a low-volume schedule should be prepared and made available to laboratory personnel. An example of a low-volume schedule is provided in Table 2-4.

Table 2-4. Example low-volume sample schedule.

Priority	Aliquot Type[1]
1	B - 15 mL
2	D - 15 mL
3	A - 50 mL
4	C - 15 mL
5	H - 15 mL
6	G - 20 mL

[1] See Table 2-2 for the description of aliquot types. Fill aliquot bottles from the available sample volume to the appropriate aliquot volume in the listed order of priority.

2.3 CHEMICAL ANALYSIS

The constituents that need to be measured in a water sample will be determined by the specific objectives of the project. Each method of chemical analysis (method) will provide a constituent concentration with a certain level of accuracy and precision over a finite concentration range that is specific to that method. Low-nutrient, low-ionic strength waters generally require methods that are effective at the lowest concentration ranges. A variety of methods are usually available to determine the concentration of a given constituent, even at low concentration ranges. The method selected must 1) be appropriate for the expected concentration range, 2) provide the data with the accuracy and precision necessary to successfully achieve the data quality objectives specified in the Quality Assurance Plan, and 3) not exceed logistical limitations with regard to sample collection, sample preparation, or laboratory capabilities. For example, a method for determining ammonium concentrations might provide data over the necessary concentration range with a sufficiently high level of accuracy and precision but may require that the analysis be done within 12 hours of collection. Such a short holding time might not be feasible for samples collected from remote sites or might be beyond the processing capabilities of the laboratory.

Currently, the ARML performs most of the chemical analysis for the stream and lake water samples collected in the FS ARM program (see <*http://www.fs.fed.us/air*>). The following is a summary listing of instrumentation and techniques employed for sample analysis. Equivalent instrumentation and techniques can be substituted. Results should be reported by the laboratory using raw instrument units (which must be clearly specified) and converted to the recommended units (µeq/L for most analytes) at a later time. These raw instrument units will most commonly be µS/cm for conductivity, µeq/L for ANC, µg/L for Al, standard pH units, and mg/L for other analytes.

pH (hydrogen ion)

- Method: SM 4500-H+ B/EPA 150.1
- Instrumentation: Metrohm/Brinkmann Titrator
- Technique summary: Standard pH electrode
- Reporting units: standard pH units

Acid Neutralizing Capacity

- Method: USGS NFM 6.6.4.C
- Instrumentation: Metrohm/Brinkmann Titrator
- Technique summary: Gran analysis technique (Gran 1952)
- Reporting units: µeq/L

Conductivity

- Method: SM 2510 B/EPA 120.1
- Instrumentation: Metrohm/Brinkmann Titrator
- Technique summary: Electrometric (APHA 1998)
- Reporting units: µS/cm

Sulfate, Chloride, Nitrate

- Method: SM 4110 B/EPA 300.0
- Instrumentation: Metrohm_MetrosepA
- Technique summary: Ion Chromatograph (IC) with separator column for anions (APHA 1998)
- Reporting units: mg/L (must specify whether units are reported as S versus as SO_4^{2-} and as N versus NO_3^-)

Calcium, Magnesium, Potassium, Sodium

- Method: ASTM D6919 - 03
- Instrumentation: Metrohm IC_Cation_M/D
- Technique summary: Ion Chromatograph (IC) with separator column for monovalent/divalent cations
- Reporting units: mg/L

Fluoride

- Method: SM 4110 B/EPA 300.0
- Instrumentation: Metrohm_MetrosepA
- Technique summary: Ion Chromatograph (IC) with separator column for anions (APHA 1998)
- Reporting units: mg/L

Neither monomeric Al nor DOC is currently analyzed at the ARML. Appropriate techniques for those analyses include the following:

Aluminum, Total Monomeric and Nonlabile Monomeric

- Method: McAvoy et al. (1992)
- Instrumentation: Lachat Flow Injection Analyzer
- Technique summary: Colorimetric detection with open-system samples by pyrocatechol violet technique. Fractionation with ion-exchange resin (Driscoll 1984, McAvoy et al. 1992).

Labile (inorganic) monomeric Al is determined by subtracting the nonlabile monomeric Al concentration from the total monomeric Al concentration.

- Reporting units: µg/L

Dissolved Organic Carbon

- Method: U.S. EPA (1987)
- Instrumentation: Dohrmann Carbon Analyzer
- Technique summary: Persulfate/UV oxidation with infrared detection (U.S. EPA 1987).
- Reporting units: mg/L

The above methods will be suitable for water quality studies in a number of National Forests. Additional methods for sample processing and analysis for low-nutrient, low-ionic strength waters have been described by U.S. EPA (1987), Morrison (1991), Paulsen (1997), and Eilers (2007).

Other methods can be adopted depending on the type of water that is studied and the program objectives. For example, it may be important to obtain water quality data that contribute to state water quality management programs in order to ensure that National Forest waters are included in regional water quality assessments and remediation efforts. In many cases, this will require laboratory adherence to methods specified by the U.S. EPA for use in state implementation of Clean Water Act programs. These methods, however, may apply to standards or criteria associated with water quality issues and sample volumes that differ from particular National Forest concerns and water quality monitoring objectives.

Selection of a method that is capable of meeting the required data accuracy and precision specified in the DQOs does not ensure that this level of data quality will be achieved. Rigorous QA/QC procedures must be followed as part of the method implementation. In this usage, quality control refers to procedures that identify results during chemical analysis that do not meet DQOs, thereby triggering immediate corrective action that usually involves reanalysis of that sample. Data quality objectives are generally based on the precision and accuracy levels required by the project and the laboratory and the analytical limits of the methods used. A key component of QC is the introduction of artificial samples of known concentration, which are associated with a specific set of project samples. Quality control procedures are generally focused on instrument performance.

Additional procedures related to QA, which are also evaluated by DQOs, are used to document laboratory performance through the introduction of artificial and natural samples that are not associated with a specific set of project samples but reflect the accuracy and precision of sample preparation and analysis, including instrument performance. Protocols for QA and QC are described in the QA/QC section of this report.

Each method used to determine a chemical concentration involves a complex set of procedures, reagents, and instrumentation. Any variation in these factors can potentially change the result, yielding a different concentration value. Therefore, each method requires that the analyst adhere to a strict SOP each time that the method is implemented. All details of the method must be documented in the SOP, which must be available to any potential user of the data for review.

Each SOP must be dated, signed, and approved. Any change to an SOP must also be dated, signed, and approved.

We have not attempted to recommend specific SOPs for implementation of analysis methods here. Prescription of specific detailed SOPs is neither desirable nor practical for long-term monitoring programs. SOPs are specific to individual laboratories and instrumentation. Analytical methods, and thus SOPs, will inevitably change as instrumentation and technology improve. Moreover, as indicated above, the specific analyses, methods, and details of SOPs should be determined by program objectives and DQOs. Rather than recommending specific SOPs for the FS ARM program, we recommend that each National Forest monitoring program adopt and adhere to SOPs that assure and document attainment of appropriate DQOs through all phases of data acquisition, including sample collection, handling, analysis, and reporting. Selection of DQOs and development of quality assurance plans is discussed in the QA/QC section of this report.

Example SOPs for laboratory analysis of low-nutrient, low-ionic strength surface waters are provided in Appendix G. Alternative SOPs may be used so long as QA/QC objectives are satisfied.

SECTION 3. QUALITY ASSURANCE/QUALITY CONTROL PROTOCOLS

A.T. Herlihy, T.J. Sullivan, G.B. Lawrence, and J.R. Webb

3.1 INTRODUCTION

The constituents to be measured in a water sample will be determined by the specific objectives of the project. Each method of chemical analysis (method) will provide a constituent concentration with a certain level of accuracy and precision over a finite concentration range that is specific to that method. Low-nutrient, low-ionic strength waters, such as those commonly included in acidic deposition or nutrient enrichment studies, generally require methods that are effective at the lowest concentration ranges. A variety of methods are usually available to determine the concentration of a given constituent, even at low concentration ranges. The method selected must 1) be appropriate for the expected concentration range in the water bodies of interest, 2) provide data with the accuracy and precision to successfully achieve project objectives, and 3) not exceed logistical limitations with regard to sample collection, sample preparation, or laboratory capabilities. For example, a method for determining ammonium concentration might provide data over the necessary concentration range with a sufficiently high level of accuracy and precision but may require that the analysis be done within 12 hours of collection. Such a short holding time might not be feasible for samples collected at remote sites or might be beyond the capabilities of the laboratory.

Often, a variety of laboratory methods and instruments are capable of providing data of suitable quality for a particular study. Nevertheless, selection of a method that is capable of meeting the required data accuracy and precision for a given project does not ensure that this level of data quality will be achieved. Rigorous QA/QC procedures must be followed as part of the method implementation. Application of QA/QC procedures will provide the basis for determining the quality of the resulting data. In the absence of appropriate QA/QC procedures, it is impossible to judge whether the data are of adequate quality to meet the needs of the project.

In this usage, QC refers to procedures that identify data during the chemical analysis that do not meet DQOs, thereby triggering immediate corrective action that usually involves reanalysis of that sample. Data quality objectives are generally based on the precision and accuracy levels required by the project and the laboratory and the analytical limits of the method used. A key component of QC is the analysis of synthetic samples of known concentration, which are

analyzed along with a specific set of project samples. Quality control procedures are primarily focused on instrument performance.

Additional procedures, referred to as QA, are used to document laboratory performance through the introduction of artificial and natural samples that are not associated with a specific set of project samples, but reflect the accuracy and precision of sample preparation and analysis, including instrument performance. Thus, QA and QC procedures are important parts of any field and laboratory sampling program.

There are three primary components to QA for the project laboratory:

- Routine evaluation of laboratory analytical performance relative to DQOs.
- Strict adherence to project SOPs including sample bottle preparation, sample collection, sample processing, and analysis methods.
- Submission of measurement data QA results along with reported analytical data.

3.2 ATTRIBUTES OF DATA QUALITY

The goal of any field monitoring project is to produce sound analyses and high quality data. Establishment of DQOs and development of a QA plan are important to ensure that data meet the established objectives for precision, accuracy, representativeness, completeness, and comparability. Each type of QA/QC sample or process is generally associated with a DQO. The value of the DQO for each analyte is set by project objectives and is usually method-specific. If the range of acceptable values measured for a sample of known concentration and defined by a DQO is exceeded, the method is considered to be out of control limits and remedial action must be taken in the laboratory. The various attributes of data quality and how they are evaluated are described below.

3.2.1 METHOD DETECTION AND REPORTING LIMITS

METHOD DETECTION LIMIT

For chemical measurements, requirements for the method detection limit (MDL) must be established. The term “detection limit” has been used in various ways when referring to the lower limit of a method concentration range. This lower limit can be a function of instrument capability, chemical reactions that are part of the method, or both. The most basic definition of a detection limit is the threshold below which measured values are not considered statistically different from a blank value (Helsel 2005). Blank values are measurements of samples of DIW (water containing no other ions). Thus, measured concentrations below the detection limit are not statistically different from zero.

The repeated measurement of a sample with a known concentration that is at or near the detection limit will exhibit considerable variability, which is assumed to be normally distributed. Therefore, in this range of measurement, separating a true concentration value from a value resulting from analytical noise is problematic. This is of particular consequence for research or monitoring objectives that involve the detection of trace contaminants, such as Hg or organic contaminants and low levels of nutrients or other analytes in dilute waters.

The MDL is defined as the lowest level of analyte that can be distinguished from zero. The first step in determining the detection limit is to make a best estimate of the value of the MDL. A set of standards is then defined with sequentially decreasing concentrations that extend above and below the estimate of the MDL. Each concentration should be analyzed seven times to provide a mean and standard deviation for each concentration value (Helsel 2005).

The true MDL will occur at a concentration that is not statistically different from the next lowest concentration. This is determined by running t-tests between the paired concentrations, starting with the two highest concentrations. A t-test is run between the second highest concentration and the third highest concentration, working downward until a pair of concentrations that are not statistically different are reached. For example, it may be found that the fourth and fifth lowest concentrations were not statistically different (the method could not detect the difference between these two concentrations); the third lowest concentration would then be the true MDL. Determination of the method detection limit is demonstrated in the following example.

1. Assume that the best estimate of the MDL for a particular method equals 0.01 µg/L.
2. Make up a set of solutions with the following concentrations that bracket the estimated MDL, numbered from highest concentration to lowest concentration:
    1. 0.06 µg/L
    2. 0.04 µg/L
    3. 0.02 µg/L
    4. 0.01 µg/L
    5. 0.005 µg/L
    6. 0.003 µg/L
3. Analyze each solution seven times. Calculate a mean and standard deviation from the seven measured values obtained for each solution. The mean values might look like the following:
    1. 0.055 µg/L
    2. 0.039 µg/L
    3. 0.022 µg/L
    4. 0.011 µg/L
    5. 0.014 µg/L
    6. 0.013 µg/L
4. Run t-tests sequentially between each pair of concentrations (1 versus 2, 2 versus 3, 3 versus 4, etc.).
5. Suppose that results for solutions 1 versus 2, 2 versus 3, and 3 versus 4 are statistically different, but results for solutions 4 versus 5 are not statistically different: this means that the concentration of solution 3 (0.02 µg/L) is your MDL.

A variety of less rigorous methods for determining detection limits are also used. One of the most common methods determines the MDL by multiplying the standard deviation of repeated measurements of the estimated MDL by a factor of three. The accuracy of the detection limit determined in this manner will depend on the accuracy of the estimated MDL, which is unknown. Therefore, we recommend the stepwise determination described above.

The MDL can vary from run to run and over time in response to such issues as a change in analyst, new instrumentation, or the aging of instrumentation. Therefore, the initial analysis to determine the MDL should be repeated three times over several weeks and at least annually thereafter for constituents with concentrations in water samples that commonly occur near or below the MDL. Laboratories that focus on the measurement of trace contaminants may determine MDLs more frequently, but otherwise, some unmeasured variation in the MDL won't negatively affect data quality. If a new analyst is appointed or equipment is replaced, a new MDL value should be determined regardless of the length of time since the last MDL was determined.

The MDL is not to be confused with the upper limit of the concentration range of a particular method. At concentrations above the method range, the relationship between measurements of standards and the known concentrations of these standards can change, thereby requiring a different standard curve.

Measurements that fall below the MDL are considered to be non-detects and are often set to zero in the database (Helsel 2005). Establishing an MDL addresses the problem of distinguishing between false positives and real values at concentrations near the MDL.

REPORTING LIMIT

The reliability of measurements that are above but near the detection limit is lower than the reliability of measurements at higher concentrations. The concentration above which measurement variability becomes acceptably low defines the threshold referred to as the "reporting limit." Like MDLs, reporting limits are low concentrations, but they are always higher than the MDL, at least by a small amount. At measured concentrations above the reporting limit, the method is considered reliable and therefore subject to DQOs established for precision and accuracy.

Measurements that fall in the narrow range below the reporting limit but above the MDL may not consistently meet the DQOs for reproducibility or accuracy and should be flagged in the database. We recommend retaining these values in the database because they indicate low, non-zero concentrations and therefore provide information that could be useful. The flag should caution the user that the precision and accuracy of these low measured values is uncertain and likely to be higher than measurements that fall above the reporting limit.

Reporting limits are determined for each chemical analysis by establishing the precision and accuracy of measurements in the lower portion of the method concentration range. To determine the reporting limit for a particular analysis, the steps outlined below should be followed:

1. Select a relative DQO (± %) for both precision and accuracy. A value of 10% is commonly used for most analytes.
2. Make an estimate of the concentration that defines the reporting limit. The values for reporting limits listed in Table 3-1 can be used to provide these estimates. From Table 3-1, for example, the estimated reporting limit for nitrate analysis is 0.1 mg/L.
3. Create three solutions of known concentrations that are higher than the estimated reporting limit, one solution with a concentration equal to the estimated reporting limit, and three solutions of known concentrations that are lower than the estimated reporting limit but higher than the MDL (listed as 0.03 mg/L for nitrate in Table 3-1). For example, the nitrate concentrations in the test solutions might be as follows:
    1. 0.6 mg/L
    2. 0.4 mg/L
    3. 0.2 mg/L
    4. 0.1 mg/L
    5. 0.08 mg/L
    6. 0.06 mg/L
    7. 0.04 mg/L
4. Analyze each solution five times and calculate the mean and standard deviation of the five values at each concentration level.
5. Using the mean values, calculate the accuracy (expressed as % error) and precision (expressed as the coefficient of variation or CV) following the procedures given below in the next section. Resulting data may look something like this:
    1. 0.6 mg/L; % error = 5.4 precision = 6.8
    2. 0.4 mg/L; % error = 6.2 precision = 4.9
    3. 0.2 mg/L; % error = 7.5 precision = 6.3
    4. 0.1 mg/L; % error = 7.2 precision = 7.5
    5. 0.08 mg/L; % error = 8.1 precision = 9.5
    6. 0.06 mg/L; % error = 21.8 precision = 25.0
    7. 0.04 mg/L; % error = 45.6 precision = 39.3
6. Determine the test concentration above which the measurements of error and precision are both less than or equal to 10%. For this example, the concentration is 0.06 mg/L. The next-highest test concentration in the series is then designated as the reporting limit. Based on these data and a DQO of 10% for both accuracy and precision, the reporting limit would be 0.08 mg/L.

Table 3-1. Recommended DQOs for detection limits, precision, accuracy, and completeness[1].

Variable or Measurement	Method Detection Limit	DQOs for Precision and Accuracy		Reporting Limit	Completeness
		Relative DQOs (± %)	Absolute DQOs (± concentration value)		
Oxygen, dissolved	NA	NA	0.5 mg/L	NA	95%
Temperature	NA	NA	1 oC	NA	95%
pH, closed system and equilibrated	NA	NA	0.15 pH units	none	95%
Acid Neutralizing Capacity	NA	15%	6 µeq/L	none	95%
Carbon, dissolved inorganic, closed system	0.10 mg/L	10%	0.1 mg/L	0.5 mg/L	95%
Carbon, dissolved organic	0.1 mg/L	10%	0.1 mg/L	0.5 mg/L	95%
Conductance	NA	10%	1 µS/cm	none	95%
Aluminum, total dissolved, total monomeric, and organic monomeric	10 µg/L	10%	0.02 mg/L	27 µg/L	95%
Major Cations:					95%
Calcium	0.02 mg/L	10%	0.02 mg/L	0.08 mg/L	
Magnesium	0.01 mg/L	10%	0.02 mg/L	0.02 mg/L	
Sodium	0.02 mg/L	10%	0.02 mg/L	0.03 mg/L	
Potassium	0.04 mg/L	10%	0.04 mg/L	0.05 mg/L	
Ammonium	0.02 mg/L	10%	0.02 mg/L	0.04 mg/L	95%
Major Anions:					95%
Chloride	0.03 mg/L	10%	0.03 mg/L	0.1 mg/L	
Nitrate	0.03 mg/L	10%	0.03 mg/L	0.1 mg/L	
Sulfate	0.05 mg/L	10%	0.05 mg/L	0.2 mg/L	
Silica	0.05 mg/L	10%	0.05 mg/L	0.4 mg/L	95%
Phosphorus, total	1 µg/L	10%	0.002 mg/L	4 µg/L	95%
Nitrogen, total	0.07 mg/L	10%	0.03 mg/L	0.15 mg/L	95%
True Color	NA	10%	5 PCU	none	95%
Turbidity	NA	10%	2 NTU	none	95%
Total Suspended Solids	0.1 mg	10%	1 mg/L	0.4 mg/L	95%

[1] NA = not applicable. DQOs for precision and accuracy are expressed two ways: in relative terms (± % of measured value) and in absolute terms (± actual measured concentration). The DQO is considered to be met if *either* of these criteria is satisfied.

3.2.2 Precision and Accuracy

Precision and accuracy are estimates of random and systematic error in a measurement process. Together they provide an estimate of the total error or uncertainty associated with an individual measurement. Precision is measured by repeated analysis of a single sample. The variation of these measurements indicates the level of method precision. Accuracy is an indication of how closely the measurements match the true concentration of the sample. An illustration of the distinction between precision and accuracy is shown in Figure 3-1.

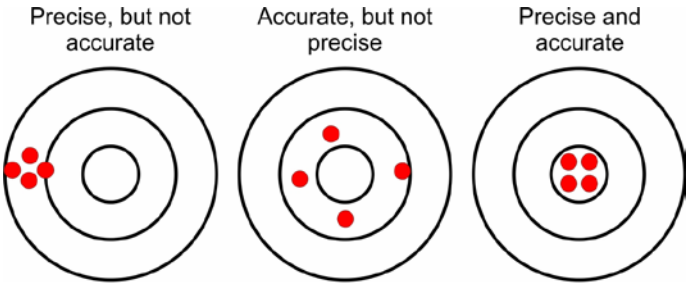

Figure 3-1. Schematic illustration of precision and accuracy.

Accuracy can be determined from measurements of solutions of known composition or from the analysis of samples that have been fortified by the addition of a known quantity of analyte. Accuracy is quantified by relating the measured value of a QC sample to the known value of that QC sample. It is usually expressed as a percent error. For QC samples, the DQO objective is defined as the value of the percent error. If the measured concentration is greater than the known value plus the DQO or lower than the known value minus the DQO, the method is considered to be out of the control limits. The percent error is calculated as shown below:

$$\% \text{ error} = \frac{\text{known concentration - measured concentration}}{\text{known concentration}} \times 100 \qquad (1)$$

Accuracy can also be quantified through analysis of interlaboratory reference samples. The USGS Standard Reference Program provides a most-probable value that can be used to calculate a percent error in the same manner that known values for QC samples are used. The same approach can be used with the Environment Canada's National Water Research Institute Program (NWRI), which provides a most probable value, D as follows:

$$D = [(AV - MCN)/MCV] \times 100 \qquad (2)$$

where AV = analyzed value, and
MCV = mean concentration value (most-probable value for source material)

Method precision is evaluated by analyzing multiple, often duplicate or triplicate, project samples. Ideally, each time a sample is reanalyzed, the same concentration value should be reproduced. Precision is typically quantified by the CV. The DQO is defined as the CV above which the method is out of control. The CV is calculated as:

$$CV = \frac{s}{\overline{X}}(100) \qquad (3)$$

Where s is the standard deviation, and $\overline{X}$ is the arithmetic mean of replicate samples.

It should be noted that relative precision (e.g., CV) is not independent of concentration. For low concentrations, criteria for both bias and precision are typically expressed in terms of absolute rather than relative error.

3.2.3 COMPLETENESS

Completeness requirements are established and evaluated as the percent of valid data obtained versus the amount of data expected. Thus, completeness quantifies the extent to which data are missing. Completeness objectives are usually designated as over 95% for each variable.

3.2.4 Comparability

Comparability is defined as the confidence with which one data set can be compared to another. Comparability is enhanced by the use of standardized field and laboratory sampling procedures. Comparability of data is also facilitated by implementation of standardized QA and QC techniques. For all measurements, reporting units and formats are specified in advance and recorded on field forms and laboratory databases in these units and formats. Comparability is also addressed by providing QA data on detection, precision, and accuracy and by conducting methods comparison studies when necessary and participating in interlaboratory performance evaluation studies, such as those conducted by the USGS and NWRI. In order to provide estimates of trends in any analyte or indicator, data collected each year must be comparable to data collected in all prior and subsequent years. Comparability can be quantified through comparison of precision and accuracy estimates obtained from QA samples.

3.2.5 Representativeness

Representativeness is the degree to which the data accurately and precisely represent the environmental attribute of interest. Although representativeness is not a laboratory QA/QC issue, it is affected by problems in all other attributes of QA. A representative sample requires that the sample site be reflective of the study population of interest and that the sample itself is representative of the system of interest (for example, that the water sample collected in the field reflects the condition in the subject lake or stream). Representativeness is ensured by following all field and laboratory sampling procedures and holding time requirements to ensure that analytical results are representative of the conditions at the time of sampling. Use of QA and QC samples similar to the type of environmental samples being analyzed provides estimates of precision and bias that are applicable to the collected data.

3.2.6 Recommended Laboratory Data Quality Objectives

Each laboratory must also have its own set of DQOs that pertain to the quality of the analytical data produced by the laboratory. Projects also have data quality requirements that are based on the objectives and resources of the project. Therefore, the laboratory DQOs must be evaluated to ensure that the laboratory is capable of delivering the accuracy and precision that the project requires. In general, we recommend DQOs for detection, accuracy, and precision as specified in Table 3-1. These DQO values are used by the USGS New York Water Science Center Water and Soil Analysis Laboratory, in Troy, NY, which specializes in the analysis of low-ionic strength waters for air pollution effects research projects.

These recommended guidelines for precision and accuracy DQOs given in Table 3-1 may not be appropriate for all projects. Forest Service staff might determine, for a specific analyte and project, that one or more recommended guideline(s) can be relaxed, especially if the laboratory is unable to achieve the recommended level of data quality and if the project does not require such high levels of precision and accuracy. Conversely, FS staff might determine that a particular project requires higher standards of precision and accuracy. In general, the values presented in Table 3-1 should satisfy the needs of most anticipated FS ARM program water quality sampling projects.

As represented in Table 3-1, we recommend application of DQOs for precision and accuracy that are calculated two ways: based on relative percent variation and based on absolute variation. A given DQO can be considered to be met if either of these two conditions is satisfied. In general, conformance with the DQO for accuracy and precision will be determined by evaluation of relative variation. However, at low concentration values, the relative DQOs can be difficult or impossible to achieve. For example, if the ANC of a particular stream is 10 μeq/L, the relative DQO for precision and accuracy of the ANC measurement is 15% (Table 3-1), or 1.5 μeq/L. There is no laboratory that can achieve that level of accuracy and precision in measuring ANC. For a sample having such low ANC, however, the absolute DQO (6 μeq/L, Table 3-1) is considered to be achievable. As long as the absolute DQO criterion is satisfied, the DQO for precision and accuracy is considered to be met.

For most analytes, our recommended relative DQO for precision and accuracy is 10%. Nevertheless, most laboratories should be able to do better than that. A good target DQO in most cases is ±5%; this is the level of precision and accuracy that the laboratories and projects should strive for.

3.3 QA/QC SAMPLE TYPES

The following sections describe the various types of samples and DQOs that are typically used for QC and QA in laboratories that specialize in analysis of low-nutrient, low-ionic strength waters. There is no definitive rule regarding how many QA/QC samples should be included in a given project. This will be determined, in part, by the intended use of the data and the available budget. In general, we recommend that at least 30% of the samples analyzed in the laboratory for a given project be QA or QC samples, distributed among the types of samples discussed in the sections that follow.

Quality-control samples are used to measure the accuracy of an instrument's calibration and to detect variations in instrument response within an analytical run. Types of laboratory QC samples are summarized in Table 3-2. These samples are made up in the laboratory using Type I DIW and purchased chemicals. Source material for all QC samples is either obtained from a manufacturer other than the producer of the source material used to make calibration standards, or is obtained from a lot other than the source material used to make calibration standards.

Quality control-high and QC-low samples are analyzed within a given laboratory run for most constituents. Exceptions are ANC, pH, and specific conductance. Either the QC-high sample or QC-low sample is analyzed within an ANC, pH, and specific conductance run, depending upon the expected concentration range of the environmental samples. This reduces the chance of carryover from a low pH (or low ANC or specific conductance) QC sample to a high pH project sample through the transfer of the electrode between samples.

We recommend that QC samples be analyzed immediately after instrument calibration, once after every 10 project samples, and at the end of each run. QC samples that do not meet DQOs for accuracy are rerun. If the value is then acceptable, the run is continued. If the rerun QC sample value is unacceptable, the project sample data preceding it are considered to be out-of-control: the data are rejected, and the instrument is recalibrated. Only accepted QC-sample and project sample

data are entered into the database. The analytical results of QC samples should be recorded to indicate the frequency of out-of-control data that are not rerun and biases and trends of control data.

Table 3-2. Recommended laboratory quality control samples.

QC Sample Type (Analyte), and Description	Frequency	Acceptance Criteria	Corrective Action
Laboratory Blank: (all analyses except pH and total suspended solids [TSS]) Reagent Blank: (DOC, Al [total, monomeric, and organic monomeric], ANC, NH_4^+, SiO_2)	Once per batch before sample analysis	Control limits < ±MDL or < 1 µM, whichever is least restrictive	Prepare and analyze new blank. Determine and correct problem (e.g., reagent contamination, instrument calibration, or contamination introduced during filtration) before proceeding with any sample analyses. Reestablish statistical control by analyzing three blank samples.
Filtration Blank: (all dissolved analytes, excluding syringe samples) ASTM Type II reagent water processed through filtration unit	Prepare once per week and. archive	Measured concentrations < MDL	Measure archived samples if review of other laboratory blank information suggests source of contamination is sample processing.
Detection Limit Quality Control Check Sample (QCCS): (all analyses except true color, turbidity, and TSS), prepared so concentration is approximately four to six times the required MDL	Once per batch	Control limits < ±MDL	Confirm achieved MDL by repeated analysis of appropriate standard solution. Evaluate affected samples for possible re-analysis.
Calibration quality control check sample (CQCCS)[1]	Before and after sample analyses	Control limits < precision objective: Mean value < bias objective	Repeat CQCCS analysis. Recalibrate and analyze CQCCS. Reanalyze all routine samples (including performance evaluation and field replicate samples) analyzed since the last acceptable CQCCS measurement.
Internal Reference Sample: (suggested when available for a particular analyte)	One analysis in a minimum of five separate batches	Control limits < precision objective. Mean value < bias objective	Analyze standard in next batch to confirm suspected imprecision or bias. Evaluate calibration and CQCCS solutions and standards for contamination and preparation error. Correct before any further analyses of routine samples are conducted. Re-establish control by three successive reference standard measurements that are acceptable. Qualify all sample batches analyzed since the last acceptable reference standard measurement for possible reanalysis.
Laboratory Replicate Sample: (all analyses) For closed headspace syringe samples, a replicate sample represents a second injection of sample from the sealed syringe.	One per batch	Control limits < precision objective	If results are below MDL: Prepare and analyze split from different sample (volume permitting). Review precision of CQCCS measurements for batch. Check preparation of split sample. Qualify all samples in batch for possible reanalysis.
Matrix spike samples: (only prepared when samples with potential for matrix interferences are encountered)	One per batch	Control limits for recovery cannot exceed 100±20%	Select two additional samples and prepare fortified subsamples. Reanalyze all suspected samples in batch by the method of standard additions. Prepare three subsamples (unfortified, fortified with solution approximately equal to the endogenous concentration, and fortified with solution approximately twice the endogenous concentration).

[1] For turbidity, a CQCCS is prepared at one level for routine analyses (U.S. EPA 1987). Additional CQCCSs are prepared as needed for samples having estimated turbidities greater than 20 nephelometric tubidity units (NTU). For total suspended solids determinations, CQCCS is a standard weight having mass representative of samples.

3.3.1 Filter Blanks, Analytical Blanks, and Field Blanks

Blanks are aliquots of DIW that are processed and analyzed in the same manner as project samples. Filter blanks are analyzed only for constituents that require filtration. Filter-blank analysis indicates whether detectable contamination has occurred during any step in sample handling that occurs in the laboratory, including bottle-washing procedures, filtration, sample

preservation, and chemical analysis. Analytical blanks are aliquots of Type I DIW (ASTM 1984) that are processed and analyzed as project samples, except that the filtration step is omitted. Contamination of analytical blanks may be attributed to any step in sample-handling other than filtration, including the quality of DIW. The use of an analytical blank together with a filter blank therefore enables contamination from filtration to be isolated from contamination during DIW preparation or other phases of sample preparation and analysis. The use of both a filter blank and an analytical blank is recommended because the filtration process poses the greatest single source of potential sample contamination. A filter blank and an analytical blank should be included as a QC pair in the sample stream at a frequency of at least 1 per 50 project samples.

Some programs require a QA sample referred to as a field blank. The field blank is prepared by bringing DIW into the field and transporting it back to the analytical laboratory, with or without transferring it to a sample bottle. From that point forward, the DIW in the sample bottle is treated as any other sample collected in the field. It is not clear what information this procedure provides because the action involved does not replicate any aspect of the actual field sampling. We therefore do not recommend the collection of field blanks unless some specific project objective requires field filtration of water samples, which could introduce the potential for sample contamination.

3.3.2 Replicate Environmental Samples

An environmental replicate set generally consists of either two duplicate samples or three triplicate samples. The replicated samples are collected at the same field site, following the same collection procedure and as close as possible to the same time, as the original sample. The purpose of replicate samples is to document sampling and analytical precision using samples that reflect the chemistry of actual project samples. The results of analysis of sample replicates provide useful information regarding the overall ability of the field and laboratory program to quantify the constituents of interest. Differences in measured values between or among replicates reflects fine-scale temporal and spatial variability in water quality at the sample site location plus any variability or error introduced in the sample collection, sample processing, and/or laboratory analysis procedures. Ideally, replicates are collected and analyzed as part of the sampling protocols of every project. For some programs, replicates are collected (as back-up) from every site, but only a subset of those replicates are analyzed. Environmental samples provide a better test of precision than artificial samples because they include natural constituents that could alter the reproducibility of a given laboratory method. Precision can also be affected by bottle washing, sample-collection, sample-processing procedures, and analysis.

In long-term monitoring studies, project sites should be selected for replicate collection on a rotating basis to evaluate precision within the full variability of project samples being analyzed. For the analysis, the laboratory should alternate between analyzing a replicate set consecutively (within the same analytical run) and separating the replicate samples in analytical runs that occur on different days or at the beginning and end of the analytical run. One set of replicate project samples should be included in the sample stream at a frequency of at least 1 per 50 project samples. More commonly, this frequency should be 1 per 20 project samples if the budget allows.

3.3.3 Spiked Project Samples

Surface water samples tend to contain a wide variety of chemical constituents with concentrations that can be highly variable. As a result, there is the potential for one constituent to interfere with the analysis of another constituent. For example, a sample with a high concentration of DOC (which imparts a brown color to the water) would interfere with some analyses that rely on colorimetric measurement to determine concentrations. Well-documented methods specify which constituents may interfere with a given analysis and at what concentration range. However, these specifications should be verified for the samples within a specific project and its testing laboratory to ensure the accuracy of the measurements. If sample concentrations are being measured in a range that could cause interference with the measurement of another constituent, the sample should be run twice: once untreated and once after being spiked with a known amount of the constituent of concern. The measured value of the samples, including the spike, should fall within the range of the method. If the concentration of the spiked sample equals the concentration of the unspiked sample plus the added amount, then recovery is complete and it can be assumed that there is no interference. To express this relationship in terms of percent recovery, the equation below can be used:

$$\%\ recovery = \frac{concentration_{spikedsample} - concentration_{unspikedsample}}{concentration_{added}} \times 100 \qquad (4)$$

The full range of an interfering constituent may not be known in the early stages of project sampling. Therefore, including spiked project samples in the sample stream is recommended until it is determined that interference is not a concern. Also, when a laboratory is starting to use a new method, the inclusion of spiked project samples is recommended to verify method specifications.

Forest Service staff should ask the laboratory to identify requested analyses that might be subject to interference with the types of samples that will be analyzed, intended methods, and analytes. It may not be possible, however, for laboratory staff to make that determination until after a given water body has been sampled and analyzed. If interferences are probable, then FS staff should request analyses of spiked samples to determine the extent of interference.

3.3.4 External Quality Assurance Samples

A variety of programs exist that provide reference samples for laboratory analysis. These programs develop bulk samples that can be subsampled and sent to participating laboratories on a set frequency. Usually these samples comprise some type of environmental sample that is chemically similar to the samples that a laboratory typically analyzes. Results from all the participating laboratories are compiled, statistically summarized, and provided to the laboratories (usually without specific identification). Participation in a reference sample program provides the opportunity for a laboratory to compare their performance with other laboratories. Program participation should be included in the QA/QC program of any laboratory that analyzes environmental samples.

In particular, laboratories that analyze low-nutrient, low-ionic strength water samples should participate in reference programs that provide these types of samples. The USGS Standard Reference Sample (SRS) Project conducts a national interlaboratory analytical evaluation

program semi-annually. The program includes three types of samples: low-ionic strength, nutrient, and trace constituents. Typically, the reference samples consist of snow, rain, surface water, or DIW that is collected, filtered, and possibly spiked with reagent-grade chemicals to meet the goals of the program. Reference samples for low-ionic strength constituents are analyzed for calcium, chloride, magnesium, pH, potassium, sodium, specific conductance, and sulfate. Reference samples for nutrient constituents are analyzed for ammonium and nitrate. Reference samples for trace constituents are analyzed for aluminum, calcium, magnesium, potassium, silicon, and sodium. Laboratory personnel are aware of the presence of the SRS sample at the time of analysis but do not know the constituent concentrations until results are posted on the SRS Project website after the conclusion of each study. The most probable value (MPV) for each constituent is equal to the median value calculated from the results submitted by participating laboratories. Laboratory results are compared with the MPV for each constituent, and a percent difference is calculated and reported.

A second standard reference program is operated by Environment Canada's NWRI. This program sends a set of 10 samples to a group of participating laboratories twice a year. The samples are obtained from predominantly low-ionic strength waters representing several sources, such as precipitation, snow, lake water, and stream water throughout North America. The concentrations of the constituents in the NWRI samples are similar to those of the environmental samples analyzed by laboratories that specialize in low-nutrient, low-ionic strength samples. Laboratory results are compared with a median concentration value (MCV) calculated from results from all participants in the NWRI program. (The USGS MPV and NWRI MCV are the same statistic, although named differently.) Laboratory personnel are aware of the presence of NWRI samples at the time of analysis but do not know the MCV of the constituents until Environment Canada publishes a report at the conclusion of each study.

A drawback to standard reference sample programs is that the analyst knows that this is a "high priority" sample and therefore may give extra attention to its analysis. Therefore the results might not fully reflect those obtained in the analysis of routine project samples. This type of analyst bias can be avoided with blind reference samples.

Blind reference samples are processed and analyzed as environmental samples and therefore appear to the analyst to be project samples. Ideally, these samples would originate from an interlaboratory reference program so that known concentration values would have been or would be established for the sample. Implementation of a blind reference sample program requires the participation of one person who works in the laboratory. The reference samples must be coded and prepared by this person so that they cannot be distinguished from routine samples by the analyst. This person is also responsible for retrieving the analysis data from the laboratory database and recoding it as QA data. One blind reference sample per 50 project samples is recommended.

When evaluating candidate laboratories, their participation and performance in an interlaboratory reference program is a very useful decision criterion. We recommend that, where practical, the FS avoid using laboratories that do not participate in such a program. FS staff should request and review performance results before making arrangements to use a particular laboratory.

3.4 FIELD QUALITY ASSURANCE

3.4.1 Sample Containers

The required sample containers and cleaning procedures are described in detail in the laboratory protocols. At least 2% of the cleaned containers (randomly selected) must be given a specific conductance check, which entails measuring the conductance of DIW in the sample container after the 48-hour soak period. Conductance should be lower than 1.2 μS/cm. If the conductance is greater than 1.2 μS/cm, re-rinse all the containers cleaned since the last acceptable check. If contamination is found, then 25% of the sample containers in subsequent batches should be monitored until all monitored containers in a batch pass the conductance test.

3.4.2 Field Measurements

Measurements of dissolved oxygen, temperature, conductance, and pH are often made in the field. If these measurements are made in the field, they require field QA procedures and the use of both performance evaluation (PE) and QC samples as described in Table 3-3. These samples confirm that the measuring devices (often field meters) are functioning properly and are within control limits over the entire length of the study.

Table 3-3. Field quality control samples. (Source: Paulsen 1997.)

Measurement	QC Sample Type	Description	Frequency	Acceptance Criteria	Corrective Action
Dissolved Oxygen	PE Sample[1]	Concurrent determination of sample by Winkler titration	Once per meter per field season	Measured O_2 within ±1 mg/L of O_2 estimated by Winkler titration	Replace meter and/or probe
	QC Check Sample	Water-saturated air	Daily (at base station)	Instrument can be calibrated to theoretical value	Replace meter and/or probe
Temperature	PE Sample	Concurrent measurement of 0 °C and 25 °C solutions with NIST-traceable thermometer	Once per meter	Within ±1 °C of thermometer reading	Replace probe and/or meter
	QC Check Sample	Concurrent measurement of sample with field thermometer	Weekly	Within ±1 °C of thermometer reading	Replace probe and/or meter
Conductance	QC Check Sample	Solution of known conductance	Weekly	Within 10 μS/cm of theoretical value	Re-calibrate meter using NIST-traceable standards; replace probe and/or meter
pH	QC Check Sample	Solution of known pH	Daily	Within 0.1 pH unit of theoretical value	Re-calibrate meter and probe or replace probe

[1] PE is performance evaluation sample.

Peck and Metcalf (1991) developed a stable and well-quantified (both theoretically and analytically) QC check sample for conductance, pH, and ANC measurements in dilute surface waters. It is a 1:200 dilution of the National Institute of Standards and Technology (NIST) 0.025 mol/kg KH_2PO_4 and 0.025 mol/kg Na_2HPO_4 standard pH stock solution. It has a pH of 6.89, a conductance of 37.6 μS/cm, and an ANC of 125 μeq/L. This solution is recommended as a QC

check for studies doing field pH and/or conductance measurements in relatively well-buffered waters.

Unpublished data from EPA's National Surface Water Survey showed that pH can be measured more precisely in the laboratory using water samples that have been collected in sealed 60 mL syringes with no headspace than in the field using portable pH meters. In general, measurements made under controlled laboratory conditions are more precise and accurate than those made in the field where contamination, weather, and fatigue can induce variability. Thus, we recommend that pH and conductance measurements be made in the laboratory.

Analytes that are sensitive to changes in CO_2 concentrations (e.g. pH, DIC) should ideally be measured in samples collected in the field into glass bottles having septum caps or into syringes with no air bubbles and analyzed within 72 hours of collection if the sample CO_2 concentration is likely to be supersaturated with respect to the atmospheric CO_2 concentration. Stream samples affected by discharging ground water (springs) and lake samples from the hypolimnion of stratified lakes are especially likely to be supersaturated with CO_2. Typically, you need one syringe for pH and DIC. Collecting an extra syringe is recommended in case additional sample volume is needed in the laboratory.

Temperature measurements must be made in the field. Dissolved oxygen measurements are also made in the field using a meter.

3.5 REPORTING QUALITY ASSURANCE DATA

Before selecting a laboratory, their QA results should be evaluated to ensure that the data quality delivered by that laboratory will be suitable for the planned project. The most common and perhaps most effective method of reporting QA data is through the use of control charts, which plot QC or QA data through time. The control charts 1) indicate whether the laboratory DQOs are met for individual QC samples, 2) reveal long-term biases or trends within and outside the control limits, and 3) provide comparisons with results from other laboratories. Each constituent has prescribed control limits that are set by the laboratory (Table 3-1). Ideally, when no bias is present, half the data points in a control chart would be above and half below the target value line. Although QC samples are used to evaluate data quality and identify samples that need to be rerun during the analysis, when plotted on control charts, QC samples also provide useful data to evaluate method performance over time, thereby also providing QA information.

Results from the analysis of QC samples are plotted on control charts in which the central line is equal to the target value (known concentration) of the control sample (Figure 3-2). Both a high and a low concentration QC sample, relative to the expected concentration distribution of the project samples, should be analyzed. If the QC sample is a blank, the target value is set to zero. A constituent analysis is considered biased if 70% or more of the data points on a chart are either above or below the target value line. The upper and lower control-limit lines on each chart represent the range of satisfactory data based on the DQOs. The QC-high and QC-low samples are plotted on separate graphs by constituent and date of analysis, and the control charts are evaluated for trends and/or bias and precision.

Figure 3-2 provides 3 years of data for a QC sample used for low concentration measurements of nitrate. Virtually all of the data fall within the control limits without any indication of trends or bias.

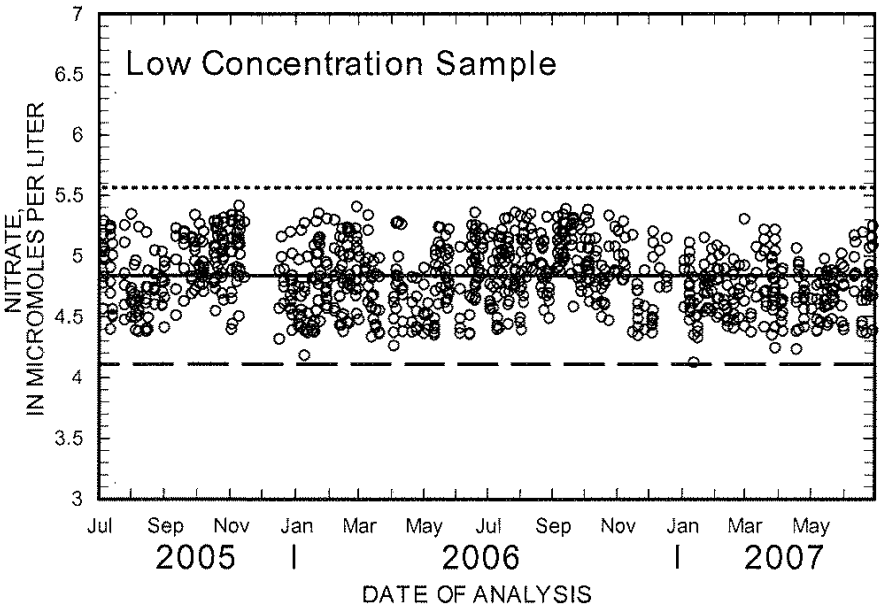

Figure 3-2. Results from analysis of low-concentration QC samples for nitrate analysis. The target value of the control sample is represented by the central line; the upper and lower dotted and dashed lines represent the range of satisfactory data based on the DQO.

Results of the QC sample analysis shown in Figure 3-3 also indicate a reliable method; only one value fell outside the control range. However, within the control range, an upward trend occurred in 2006, followed by a downward trend in 2007. If either of these trends had continued, the data would have drifted out of control limits. To ensure early detection of trends in QC, control charts should be updated daily to monthly depending on the sample load.

For the analysis of filter blanks and analytical blanks, the control range is defined by zero and the DQO threshold. For replicate sample concentrations, the CV of the two or three replicate samples is plotted and the control limits are determined by ± the DQO for accuracy and precision. Control charts can be used to show results from interlaboratory comparisons by plotting the percent difference from the most probable value. Control limits are defined by the acceptable percent difference from the most probable value, which might be designated to be 10%, for example, unless the concentration of the test solution is low, in which case a higher value should be selected.

Documentation of QA data should be readily available to projects that use or contemplate using a specific laboratory. Forest Service staff should request, from the laboratory, in advance of starting a project, QA results that would be applicable to the planned project. These results should include, at a minimum, QC results plotted on control charts and comparisons with results from other laboratories.

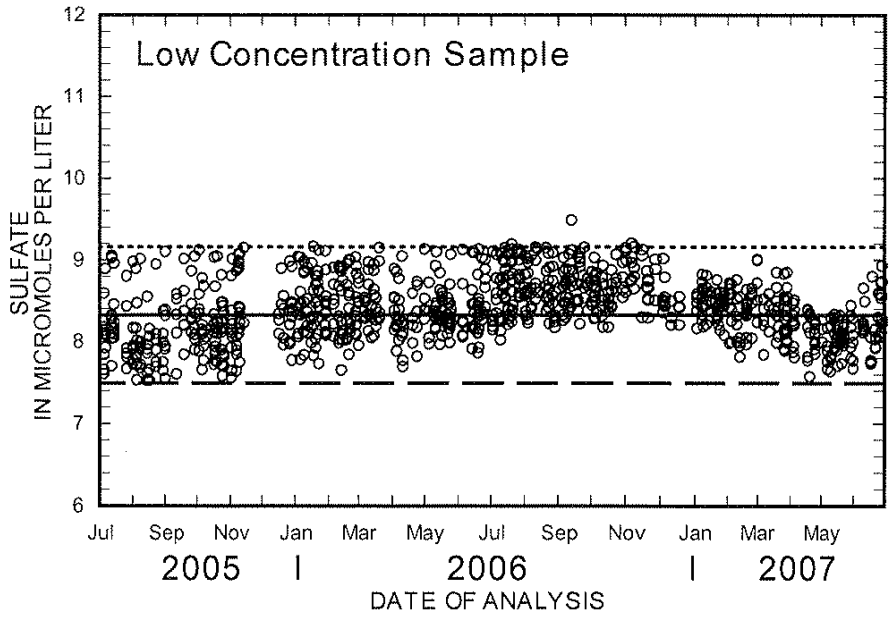

Figure 3-3. Low-concentration QC samples for sulfate analysis. The target value of the control sample is represented by the central line; the upper and lower dotted and dashed lines represent the range of satisfactory data based on the DQO.

3.6 LABORATORY AUDITS AND CERTIFICATION

All laboratories conducting chemical analyses should be periodically audited by a qualified external body on a set frequency that does not exceed 3 years. These audits should be comprehensive in covering every aspect of laboratory activities. Documentation of audit results, recommendations, and actions taken by the laboratory should be maintained and available to projects that use the laboratory. Ideally, audits are conducted as a component of a certification program. In addition, round-robin programs, in which multiple labs analyze the same set of performance evaluation samples and compare results, are an excellent way to evaluate the performance of an individual laboratory and to ensure that it provides high-quality data. If a laboratory is not able to document that it provides high-quality data, then the FS may wish to find an alternate laboratory for analyzing samples considered to be important from a regulatory or decision-making perspective. Any laboratory chosen for important project work should be able to provide documentation of audits within the last 2 years of their laboratory procedures.

There is currently one national accreditation program for laboratories that analyze environmental samples. This program is administered through the National Environmental Laboratory Accreditation Conference (NELAC), formed on the recommendation of the EPA. A cooperative association of state and federal agencies, NELAC was formed to establish and promote mutually acceptable performance standards for the operation of environmental laboratories. The standards cover both analytical testing of environmental samples and the laboratory accreditation process. To accomplish these goals, NELAC developed the National Environmental Laboratory Accreditation Program (NELAP). This program recognizes state programs as accrediting authorities that administer the program. For example, a laboratory headquartered in New York State would apply to New York State's Environmental Laboratory Accreditation Program

(ELAP). As each laboratory becomes accredited under a NELAP-recognized accrediting authority, the laboratory and its accredited scope of testing will be entered into a national database. One of the fundamental principles of NELAC is that of reciprocity among NELAP accrediting authorities. For example, once a laboratory is accredited in one state for testing under a specific EPA program, it can be accredited in another state for that EPA program without having to meet additional accreditation requirements. We recommend that the FS use accredited laboratories or, at a minimum, laboratories that can demonstrate their ability to produce high-quality data, as described above.

3.7 DATA ENTRY

Field crews will be required to enter the field data directly into a Web-based form for inclusion of the data into NRM Air. Information such as SampleID, Location, Date/Time, and other field data will be entered into the form. When laboratory analyses are complete, lab results will be merged with the field data and brought into the database. National Resource Manager Air will support two import formats for lab results: 1) an Access database in a STORET-compatible format and 2) an Excel template that can be output by an NRM Air tool. Data that pass the QC checks are electronically transferred into the laboratory database, where they can be reviewed for errors that could result from mislabeling, data entry mistakes, misidentification of samples, contamination, or a number of other potential sources of error. Once the data have been verified, they should be placed in a location accessible to project personnel for downloading. All types of data storage should be backed up on a daily basis.

3.8 SUMMARY

As discussed in the preceding sections, there are many types of QA/QC data and a variety of ways to evaluate the reliability of the data collected for a particular project. In the absence of such QA/QC analyses, it is impossible to determine whether or not the collected data can meet project objectives. Key elements of the QA/QC program that should be used in evaluating a laboratory for possible use in analyzing samples from dilute lakes or streams are summarized as follows:

1. Develop project DQOs. These are project specific goals for data quality for each measured variable. Table 3-1 is a good starting point but will not be optimal for all projects. Add or subtract any variables that are not pertinent to the project at hand. Revise criteria based on specific project needs.

    1a. Detection Limits. The detection limit is the threshold below which measured values are not considered different from zero concentration. Detection limits must be evaluated in relation to levels of ecological concern. If the detection limit is near or above the level of concern, then the usefulness of the data might be limited.

    1b. Precision and Accuracy. Precision is measured by repeated analysis of the same sample to determine the variability in the analytical data for each variable. Accuracy is measured by blind analysis of samples with known concentration. The deviation of analytical measurements from the true known concentration is called “bias.” The need

for precision and accuracy for a project is dependent on the magnitude of the effect being studied: if you are trying to quantify small differences among groups or small changes over time, then you will need higher precision and accuracy of measurement.

1c. Comparability. Data need to be comparable to what is being measured at other locations and times. To help ensure comparability, the analytical laboratory should be certified and/or participate in sample "round-robin" programs in which the same samples are analyzed by multiple laboratories and results compared.

1d. Completeness. Ideally, all sites intended for sampling and all measurements intended at each site will be made, constituting 100% completeness. In reality, completeness is typically somewhat less than 100%. Minimum completeness goals should be set depending on how much of a problem missing data will be in the final analysis of the data.

2. Evaluate laboratory QA data to see how well they meet your DQOs.

2a. Evaluate analytical results for blank samples.

2b. Determine results for sample replicates.

2c. Quantify expected versus observed results for spiked samples.

2d. Examine laboratory performance for external QA audit samples.

3. Evaluate field sampling QA procedures and data.

3a. Sample bottles may be provided already cleaned by the laboratory or they may need to be cleaned and tested as described in the Field Protocol.

3b. We recommend that water sample filtration and pH measurements be made in the laboratory, where precision is higher and risk of contamination lower, rather than in the field. If field filtration is done, field blank samples can indicate potential sample contamination. Water for pH measurements in the lab should preferably be collected in 60 mL syringes, avoiding the introduction of any air bubbles, and then sealed in the syringes with a stopcock.

3c. Evaluate results of field QC check sample analyses for any field measurements as described in Table 3-3.

SECTION 4.
DATA ANALYSIS PROTOCOLS

T.J. Sullivan, A.T. Herlihy, G.B. Lawrence, and J.R. Webb

4.1 BACKGROUND AND OBJECTIVES

A data analysis protocol (DAP) provides the basis for translating water quality data generated in the analytical laboratory into meaningful guidance for data analysis and interpretation. It connects the raw data to the information goals for which the data were collected. There are a number of information goals that are relevant to water sampling efforts within the FS ARM program, as described in Section 1 of this protocol. These goals stem from specific questions that are formulated to inform management decision making.

The purpose of this DAP is to describe graphical, statistical, and other approaches to be used in validating, presenting, analyzing, and understanding water quality data. The DAP can support analytical efforts by FS personnel who do not have advanced training in chemistry and statistics. However, we recommend that, whenever possible, data analysis should be conducted by staff members who have a good grounding in water chemistry and a basic understanding of statistics. In particular, staff involved in trends analysis should have had formal training in statistics and/or consult with a trained statistician while conducting the trends analysis.

The DAP is divided into sections, as follows:

1. Develop a statement of the objectives of the data analysis.
2. Evaluate and assure the quality of the dataset.
3. Prepare raw data for graphical and statistical analysis.
4. Conduct exploratory analyses.
5. Conduct, if needed, formal statistical analyses.
6. Report data in standardized formats.

As described in the Surface Water Sampling Protocol, collection of surface water chemistry data should always have a purpose. Specific questions need to be formulated, and the nature of these questions will guide the design of the study, including what, where, when, and how to sample. To some degree, these questions will also help guide data analysis.

Some example approaches for data analysis that we recommend for FS staff consideration are outlined in Table 4-1. Each example data analysis approach given in the table is tied to a specific purpose.

Table 4-1. Some example approaches for FS ARM program data analysis, tied to the purpose and general approach of the field study.

Purpose	General Approach	Example Data Analysis[1]
1. Determine whether lake or stream is N-limited	A. Measure N, P, and chlorophyll *a* during snow-free season	a. Plot molar N:P ratio over time relative to published ratios that have been shown to be associated with N-limitation. Compare to changes in chlorophyll *a* concentrations.
2. Quantify episodic excursions from base flow chemistry	A. Measure water chemistry during rainstorms and/or snowmelt	a. Plot changes during episodes in discharge and selected water chemistry parameters to reveal episodic changes in ANC, pH, and Al_i, and to illustrate changes in potential drivers of those parameters. b. Plot changes over time during spring and summer in discharge and selected water chemistry parameters. c. Plot changes over time (including episodes) in the ratio of NO_3^- concentration to $[NO_3^- + SO_4^{2-}]$ concentration versus ANC to illustrate the relative importance of NO_3^- as a mineral acid anion in driving episodic ANC changes.
3. Determine spatial patterns in water chemistry across a forest or wilderness	A. Conduct regional survey of water chemistry	a. Map various chemical concentrations across the landscape to reveal spatial patterns. Correlate those to landscape features (geology, elevation, etc.). b. Examine patterns in the data across spatial gradients. c. Construct histograms to identify regional (or forest-level) outliers in concentrations of key variables.
4. Quantify long-term changes over time in water chemistry	A. Sample over an extended period of time (at least 8 to 10 years), preferably monthly or seasonally during open-water seasons	a. Standardize sampling to account for episodic changes or otherwise address variability. b. Conduct trends analyses. c. Compare trends in biologically relevant variables (ANC, pH, Al_i) with trends in potential drivers and buffers (SO_4^{2-}, NO_3^-, SBC, DOC).
5. Determine extent to which air pollution is affecting water resources	A. Characterize index chemistry for multiple lakes or streams expected to be sensitive	a. Plot central tendency and variability (i.e., box and whisker plots) in key variables across sites. b. Compare with common benchmark thresholds for ANC (0, 20, 50, 100 µeq/L), pH (5.0, 5.5, 6.0), and Al_i (2, 7 µM) and calculate the percent of sites that exceed thresholds.
	B. Conduct survey of waters in study area	a. Plot central tendency and variability in key parameters. b. Compare to common benchmark thresholds. Map chemical concentrations.
	C. Use dynamic model to hindcast past changes in water chemistry since about 1900 or earlier	a. Map simulated changes in chemical concentrations. b. Compare simulated changes with changes derived by other means (i.e., long-term monitoring, paleolimnology, space-for-time substitution). c. Plot simulated changes in ANC versus aspects of current chemistry (i.e., ANC, ANC/SO_4^{2-}). d. Estimate proportional changes in ANC versus SO_4^{2-}, SBC versus SO_4^{2-}, ANC versus $(SO_4^{2-} + NO_3^-)$, etc. to reveal interactions among variables.
	D. Collect and analyze diatom remains in sediment core(s)	a. Analyze as described above for dynamic model hindcasts a through c.
	E. Simulate critical load of S and/or N deposition	a. Map critical loads and exceedances (amount by which ambient deposition exceeds critical load). b. Plot critical load as function of water chemistry (i.e., ANC, ANC/SO_4^{2-}, etc.). c. Develop procedures with which to extrapolate critical load on basis of water chemistry and/or landscape characteristics.
6. Evaluate possible need for mitigation	A. Follow approaches similar to 5	a. Follow approaches similar to 5.

[1] The following abbreviations are used here: nitrogen (N); phosphorus (P), acid neutralizing capacity (ANC), inorganic monomeric aluminum (Al_i), nitrate (NO_3^-), sulfate (SO_4^{2-}), sum of base cations (SBC), dissolved organic carbon (DOC).

4.2 EVALUATION OF DATA QUALITY

The first step in analyzing any raw dataset provided by an analytical laboratory should always be to conduct an evaluation of the quality of the data. This should include reviewing the QA/QC data provided by the laboratory, determining if DQOs have been met by the laboratory, and conducting or reviewing the results of data validation.

There are a variety of protocols that can be used to evaluate and confirm overall analytical data quality. These include comparing measured with calculated variables when both are intended to represent the same parameter. If measured and calculated values are similar (within an expected range of error), then there is an increased likelihood that the data used in the comparison are of high quality. If measured and calculated values of the same parameter differ by more than the expected variability, then it can be inferred that one or more of the values in the calculations represented in that comparison may be in error. This approach can be helpful in flagging certain samples or measurements for re-examination to determine if there were recording errors or some other kind of error that might be identified and corrected.

Data validation protocols also include constructing plots of variables that might be expected to correlate with each other. Any sample that deviates substantially from the expected relationship might be further examined for potential error or flagged in the dataset.

Sample contamination, analytical error, and/or reporting error can lead to incorrect data values that are not representative of conditions in the field. Many such errors can be identified and, in some cases, corrected through data validation. Data validation is the process of checking for internal consistency among data values using the ionic relationships among the analytes in the dataset. If data validation is done in a timely manner, problematic values can sometimes be reanalyzed. If nothing else, values that fail validation checks can be flagged in the dataset and considered potentially suspect in various data analyses. Sample analysis results from the laboratory are considered preliminary until the internal consistency checks described below are performed.

4.2.1 CHARGE BALANCE

The sum of positively charged ions (cations) in water must equal the sum of those with negative charge (anions). Major discrepancies between the sum of measured anions and cations thus reflect analytical errors, failure to measure all ions with significant concentrations, or a combination of both. Although charge balance calculation and comparison alone cannot necessarily identify the cause of a charge imbalance, they can serve as a QC check on the completeness and accuracy of the ion chemistry data. A high-quality dataset will show reasonable agreement between the calculated cation and anion sums after accounting for the failure to measure all ions in solution.

To assess the quality of the data for the ionic species in water, ion charge balances involving a comparison of the sum of cations to the sum of anions should be calculated for all water samples. If the data are provided in mass units (e.g., mg/L), then they must be first converted into equivalence units (i.e., microequivalents per liter; µeq/L) by multiplying the concentration in mg/L by the appropriate factor in Table 4-2. Anion and cation sums in units of µeq/L are then approximated as defined in Equations 1 and 2 below:

$$Sum\ of\ cations = Ca^{2+} + Mg^{2+} + Na^{+} + K^{+} + NH_4^{+} + H^{+} \quad (1)$$

$$Sum\ of\ anions = SO_4^{2-} + NO_3^{-} + Cl^{-} + F^{-} + (ANC + H^{+}) \quad (2)$$

Table 4-2. Factors for converting mg/L units or pH units to µeq/L units.

Analyte	To Convert from mg/L to µeq/L, multiply by:
Ca^{2+}	49.90
Mg^{2+}	82.29
Na^{+}	43.50
K^{+}	25.58
NH_4^{+} (mg NH_4/L)	55.44
NH_4^{+} (mg N/L)	71.39
SO_4^{2-}	20.82
SO_4^{2-} (mg S/L)	62.38
ANC (mg $CaCO_3$/L)	19.98
Cl^{-}	28.21
F^{-}	52.63
NO_3^{-} (mg NO_3/L)	16.13
NO_3^{-} (mg N/L)	71.39
Al^{n+} (inorganic monomeric)	74.13[1] (assumes a +2 charge)
DOC[2]	5 to 10 (rough approximation)
To convert pH to H^{+} in units of µeq/L: H^{+} (µeq/L) = 10^{-pH} x 1,000,000	

[1] The factor given for conversion of Al concentration from mass units to equivalence units assumes an average charge of +2 on the inorganic Al species present in the water. If the water sample has very low pH (less than about 4.8), then use a factor of 111.19 instead of 74.13 (assumes average charge of +3).

[2] To convert DOC in mg/L to DOC concentration in µmol/L, multiply by 83.33. To estimate the equivalent concentration of organic acid anions from the DOC, multiply DOC in mg/L by a value of 5 to 10 to generate a very rough estimate of organic acid anion concentration in µeq/L.

The (ANC + H^{+}) term in the anion sum is determined by laboratory measurements of ANC and pH. The hydrogen ion concentration (H^{+}) is calculated from pH as described in Table 4-1. To make this conversion on a calculator: take the pH value; change the sign to negative; hit the inverse $\log_{10}$ key; and multiply by 10^6.

A charge balance plot should be made by plotting the sum of anions versus the sum of cations (Figure 4-1). Some deviation from the one-to-one line (i.e., y = x) is expected because of analytical errors associated with the measurement of the individual anions and cations. Although random analytical errors would tend to cancel out in calculating the sum of anions or cations, the analytical accuracy and precision and their relative contribution to the ion sum differ for each of the ions measured. Some charge imbalance may occur because of differences in analytical precision and accuracy. Thus, the calculated charge balance is an imprecise measure of data quality. It is useful as a tool for determining rather large deviations from the expected relative concentrations of anions and cations.

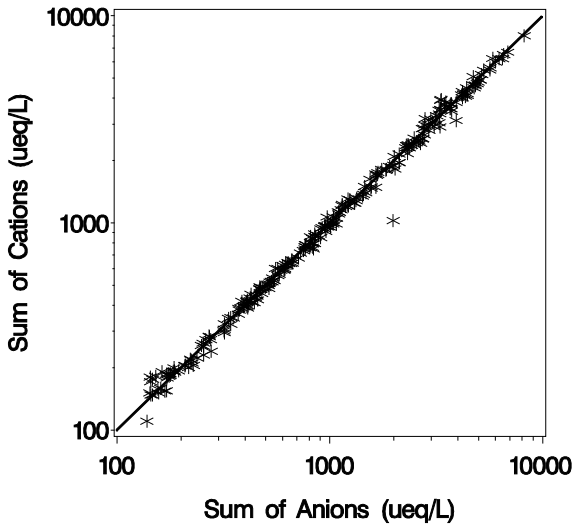

Figure 4-1. Cation sum versus anion sum. In this figure, two potential outliers warrant further investigation to determine if an error was made in analyzing or reporting the concentration of one or more ions.

Percent ion balance differences (% IBD; [cation sum - anion sum]/[cation sum + anion sum] x 100) should be calculated for all samples. As a general guideline, we recommend the following criteria for % IBD (Table 4-3):

If the sum of anions + cations ≤ 100 μeq/L, % IBD should be ≤ 25%.

If the sum of anions + cations > 100 μeq/L, % IBD should be ≤ 10%.

For any samples that do not satisfy these criteria, the analytical data should be reviewed to determine if the cause of the imbalance is due to data entry error, analysis error, or some other identifiable error. If laboratory analysis error is discovered, the sample should be reanalyzed for those analytes that do not exceed laboratory holding times. If no error can be determined through data review and/or reanalysis, the results can be finalized without change, assuming that the imbalance is due to unmeasured ions.

Although the calculated charge balances do not include all ions that could potentially contribute to the sum of the cations and anions, those that are included contribute most to the overall anion and cation sum in most dilute freshwater environments. Inorganic ions not included, such as P and trace metals, are generally present in relatively low concentrations in most low-ionic strength waters and are not significant contributors to the total ion balance. Silica is not included in the charge balance because in most natural waters it exists predominantly in an uncharged form and does not contribute to either the anion or cation charge balance.

Table 4-3. Data validation quality control procedures. (Source: Paulsen 1997.)

Activity or Procedure	Requirements and Corrective Action
Range checks, summary statistics, frequency distributions, and/or other exploratory data analysis (e.g., box and whisker plots).	Identify suspect values. Review field notes and laboratory data for possible problems or errors. Correct reporting errors or qualify as suspect or potentially invalid.
Ion balance. Calculate percent ion balance difference (% IBD) using data from cations, anions, pH, and ANC.	If total ionic strength[1] ≤ 100 µeq/L, % IBD should be ≤ 25%. If total ionic strength > 100 µeq/L, % IBD should be ≤ 10%. Determine, if possible, which analytes are the largest contributors to the ion imbalance. Review suspect analytes for possible analytical error and reanalyze any samples for which analytical error appears likely. If analytical error is not indicated, qualify sample to attribute imbalance to unmeasured ions. Reanalysis is not required.
Conductivity check. Compare measured conductivity of each sample to a calculated conductivity based on the equivalent conductances of all major ions in solution.	If measured conductivity ≤ 25 µS/cm, ([measured - calculated] ÷ measured) should be ≤ ±25%. If measured conductivity > 25 µS/cm, ([measured - calculated] ÷ measured) should be ≤ ±15%. Determine, if possible, which analytes are the largest contributors to the difference between calculated and measured conductivity. Review suspect analytes for analytical error and reanalyze any samples for which analytical error appears likely. If analytical error is not indicated, qualify sample to attribute conductivity difference to unmeasured ions. Reanalysis is not required.
Aluminum check. Compare results for organic monomeric aluminum and total monomeric aluminum.	[Organic monomeric] should be < [total monomeric]. Review suspect measurement(s) to confirm if analytical error is responsible for inconsistency.
ANC check: 1) Calculate carbonate alkalinity based on pH and dissolved inorganic carbon (DIC). Compare to measured ANC. 2) Calculate charge balance ANC and compare with laboratory measured (titrated) ANC.	Review suspect measurements for samples with titrated ANC < carbonate alkalinity or those with differences > 15% or >15 µeq/L for samples with ANC < 150 µeq/L. Determine if data entry error, analytical error, or non-carbonate alkalinity is likely to be responsible for lack of agreement. Review samples having ([measured – calculated] ÷ measured) > ± 15% (for low DOC waters) to 20% (high DOC waters). Determine if observed discrepancy can be attributed to organic anions. Strong organic acid anions are expected to decrease titrated ANC, compared with calculated charge balance ANC, by an amount equal to approximately (as a crude approximation) five times the DOC concentration in mg/L for acidic waters (ANC < 0 µeq/L) to 10 times the DOC concentration in mg/L. Determine, if possible, if data entry error or analytical error is likely to be responsible for the observed inconsistency.

[1] Total ionic strength is calculated as the sum of cations (Equation 1) added to the sum of anions (Equation 2)

Two types of water bodies, however, often have charge imbalance due to ions that are not included in Equations 1 and 2. In acidic waters (pH less than about 5.5), aluminum (Al), which becomes more soluble with decreasing pH, may be a major contributor to the cation sum. Also, in waters with relatively large amounts of dissolved organic carbon (DOC higher than about 3 mg/L [250 µmol/L] to 5 mg/L [417 µmol/L]), organic anions can be a major contributor to the anion sum.

In acidic waters, failure to include Al in the charge balance may cause a cation deficit (anion sum higher than cation sum). At pH greater than 5.5, Al concentrations are typically so low as to be unimportant in the overall ion balance. At lower pH, however, Al should be incorporated into the cation sum for charge balance checks. Typically, there are several different forms (species) of inorganic Al, and they can have different charges. The concentration of Al in μeq/L can be approximated by converting measured values of Al_i in mg/L to equivalence units using the conversion factor in Table 4-2, which assumes a +2 average charge for Al. For highly acidic waters (pH less than about 4.8), an average charge of +3 should be assumed for Al (as given in the footnote to Table 4-2). Alternatively, if Al is not measured, a cation deficit (anion sum higher than cation sum) in acidic waters (pH less than about 5.2) should not necessarily be interpreted as a QA problem as it can be assumed that some or all of the cation deficit results from unmeasured Al.

Naturally occurring organic anions (derived from organic acids) contribute to the overall anion sum. Because there is no direct measure of organic anions, they are typically not included in the anion sum as represented in Equation 2. Where they are present in significant concentrations, the charge balance will show an anion deficit (cation sum higher than anion sum). Dissolved organic carbon concentration may be used as a surrogate variable for organic anions to check whether any observed anion deficit could be related to organic acids. In general, when DOC is less than about 3 mg/L (250 μmol/L) to 5 mg/L (417 μmol/L) organic anion contributions to the ion balance are relatively minor and can be ignored. When DOC is greater than about 5 mg/L, there should be an appreciable anion deficit, calculated with the following equation:

$$\text{Anion deficit} = (\text{cation sum}) - (\text{anion sum}) \quad (3)$$

The anion deficit should be roughly proportional to the DOC, with higher anion deficit in samples having higher DOC, and to some extent also higher pH. In general, the slope of the plot of anion deficit (in μeq/L; y-axis) versus DOC (in mg/L; x-axis) should be about 5 to 10 μeq of anion deficit per mg of DOC.

4.2.2 Calculated Versus Measured Conductivity

The presence of ions in water increases the electrical conductivity (also called specific conductance) of that solution. Conductivity, therefore, provides an indication of total ion concentration. Further, since the relationship between ion concentration and conductivity is known for most ionic species, the measured conductivity of a water sample can be used as an internal check on both the accuracy and the completeness of the measurements of ionic species by comparing the measured and expected conductivity. The expected conductivity is calculated as the sum of the product of the ionic concentration times the equivalent conductances of each of the measured ions in water. For relatively dilute waters (conductivity below 200 μS/cm), Equation 4 is used. For higher conductivity waters, a more complex equation is used that adjusts for high concentration effects. All waters that are sensitive to acidification from acidic deposition, and most waters that are sensitive to nutrient enrichment effects from atmospheric N deposition will have conductivity less than 200 μS/cm. Thus, we recommend use of Equation 4 and do not present the more complex equation. All of the concentrations in the equation need to be expressed in units of μeq/L. Conversion factors to convert from mass units to equivalence units are given in Table 4-2. For samples having conductivity lower than 200 μS/cm:

Calculated conductivity = ((Ca^{2+} *x 59.47) + (*Mg^{2+} *x 53.0) + (*K^{+} *x 73.48) +*
(Na^{+} *x 50.08) + (*NH_4^{+} *x 73.5) + (*H^{+} *x 349.65) + (*SO_4^{2-} *x 80.0) +*
((ANC+H^{+}*-* OH^{-}*) x 44.5) + (*Cl^{-} *x 76.31) + (*NO_3^{-} *x 71.42) +*
(OH^{-} *x 198)) / 1000* *(4)*

Calculated conductivity should be plotted against measured conductivity as a first step to look for gross outliers (data values that fall well outside the normal range; Figure 4-2). As a more quantitative QA check, the % conductivity difference should be calculated as:

% conductivity difference = (calculated-measured)/measured x 100 *(5)*

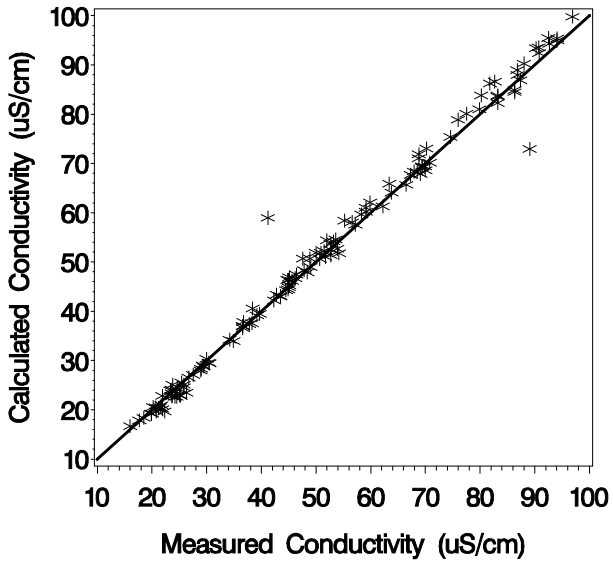

Figure 4-2. Calculated versus measured conductivity. Two obvious outliers warrant further investigation to determine if an error was made in analyzing or reporting the concentration of one or more ions.

As a general guideline, we recommend careful review of samples for which the % conductivity difference exceeds 25% for samples in which measured conductivity is less than 25 µS/cm. We further recommend careful review of samples for potential data entry or analysis error if the % conductivity difference exceeds 15% for samples in which measured conductivity is more than 25 µS/cm (Table 4-3).

4.2.3 Calculated Versus Measured ANC

Carbonate Alkalinity Versus Titrated ANC

There are two methods for evaluating the internal consistency of the dataset on the basis of observed differences between laboratory measurements (titrations) of ANC and calculated ANC or carbonate alkalinity using various ion measurements. The first involves comparisons between calculated carbonate alkalinity and laboratory measures of ANC made by acid titration. In almost all surface waters, the vast majority of ANC is made up of carbonate alkalinity (Figure 4-3). Carbonate alkalinity ([Alk_c]) is calculated directly from laboratory measurements of pH and DIC concentrations (Hillman et al. 1987) and is a measure of just the carbonate ions (HCO_3^- and CO_3^{2}) in the sample that would react with acid during an ANC titration. Alk_c in µeq/L is calculated from the equation:

$$Alk_c=((DIC/12011) \times ((H_{molar} \times K_1 + 2 \times K_1 \times K_2)/(H_{molar} \times H_{molar} + H_{molar} \times K_1 + K_1 \times K_2))) (KW/H_{molar}) - H_{molar}) \times 10^6 \quad (6)$$

where DIC is in mg/L, $H_{molar} = 10^{-pH}$, $K_1=4.4463x10^{-7}$, $K_2=4.6881x10^{-11}$, and $KW=1.01x10^{-14}$.

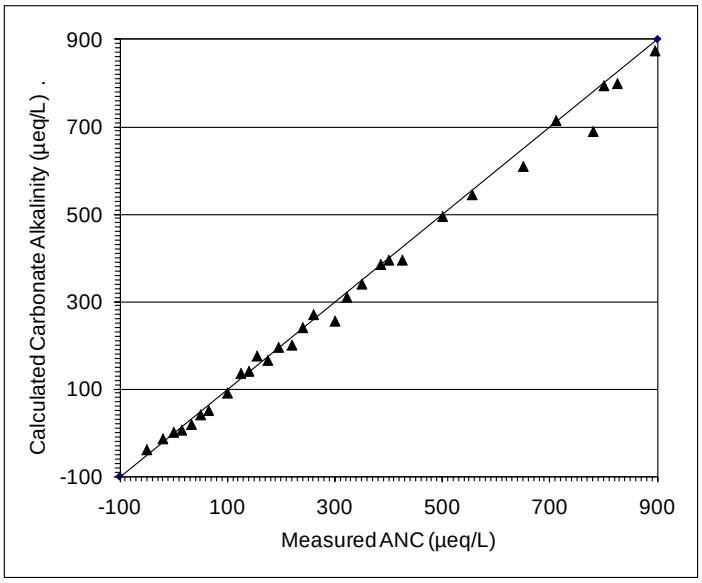

Figure 4-3. Calculated carbonate alkalinity versus laboratory titrated ANC with no obvious outliers. In general, calculated carbonate alkalinity is slightly lower than measured ANC (as it should be).

ANC is a measure of all ions that react with acid during the acid titration. It includes all the carbonate ions that are represented in Alk_c. Thus, ANC must be greater than or equal to Alk_c. If calculated Alk_c is higher than the titrated ANC, the discrepancy must be due to analytical error(s) in the measurement of ANC, pH, or DIC; the presence of noncarbonate ions that react with acid during the titration; or a combination of both. Comparisons of the Alk_c and titrated ANC can serve as a QC check on the measured pH, DIC, and ANC. In nonacidic (ANC greater than 0 µeq/L) waters with low DOC, samples that have ANC less than Alk_c or those with (ANC-Alk_c) differences greater than 15% (greater than 15 µeq/L for samples with ANC less than 150 µeq/L) should be carefully reviewed for potential QA problems (Table 4-3). Acidic waters (ANC less than 0 µeq/L) and higher DOC waters (above about 3 mg/L [250 µmol/L] to 5 mg/L [417 µmol/L]) often have other ions (Al, weak organic acid anions) that react with acid during the titration. Therefore, [ANC-Alk_c] differences do not necessarily indicate a QA problem, but they do suggest that the data should be reviewed for potential errors.

CALCULATED VERSUS TITRATED ANC

The second QA check of ANC values compares laboratory titrated ANC with a charge balance definition of ANC, calculated as:

$$ANC_G = Ca^{2+} + Mg^{2+} + K^+ + Na^+ + NH^{4+} + Al^{2+} - SO_4^{2-} - NO_3^- - Cl^- \quad (7)$$

where ANC_G is laboratory Gran titrated ANC, and all parameters are expressed in units of µeq/L.

For Al species, assume an average charge of +2 (thus, Al concentration in μeq/L equals Al concentrations in μmol/L times 2) for waters having pH above about 4.8. For waters having lower pH, assume an average charge on the Al species of +3 (thus, Al concentration in μeq/L equals Al concentration in μmol/L times three). For low DOC (less than about 3 mg/L [250 μmol/L] to 5 mg/L [417 μmol/L]) waters, laboratory titrated and calculated charge balance ANC should be approximately equal, plus or minus an allowance for analytical errors. In general, the errors on the individual ions should cancel each other out and the two estimates of ANC should be within about 15% of each other (or within about 15 μeq/L for relatively low ANC [less than 50 μeq/L] waters). If they differ by more than this amount, it suggests errors in one or more of the measurements that go into the calculations and the comparison.

For higher DOC waters (greater than about 3 mg/L [250 μmol/L] to 5 mg/L [417 μmol/L]), laboratory titrated ANC should be lower than calculated charge balance ANC by an amount equal to the concentration of strong organic acid anions in solution. That concentration of strong organic acid anions can be roughly approximated by multiplying the DOC concentration, expressed in mg/L, by the estimated organic acid charge density (average charge per mg of DOC). Thus, for high DOC waters, DOC-adjusted titrated ANC is calculated as:

$$\text{DOC-adjusted } ANC_G = Ca^{2+} + Mg^{2+} + Na^{+} + K^{+} + NH^{4+} - SO_4^{2-} - NO_3^{-} - Cl^{-} - A^{-} \quad (8)$$

where A^- is the estimated strong organic acid anion concentration (defined as those with acid dissociation constants giving them an equilibrium pH less than about 4), which is very roughly approximated by:

$$A^{-}\ (\mu eq/mg) \approx DOC\ (mg/L) \times 4\ \mu eq/mg \quad (9)$$

If the DOC-adjusted laboratory titrated ANC differs from calculated charge balance ANC by more than about 20% (or 20 μeq/L for relatively low [≤ 50 μeq/L] ANC samples) in high DOC waters, that suggests the possibility of data entry error or analytical error in one or more of the parameters that enter into the calculations. In that case, laboratory and data entry records should be reviewed for possible errors.

Note that these methods for estimation of the equivalent concentrations of Al and strong organic acid anions are crude approximations for the purpose of evaluating the internal consistency of the dataset and for identifying possible incorrect values for further examination. For high-DOC waters in particular, lack of agreement between calculated and titrated ANC does not necessarily mean that there are errors in the dataset. More rigorous approaches are available for calculating the equivalent concentrations of Al and organic anions, but these are not needed for the purpose of dataset validation.

4.2.4 Other Validation Procedures

The dataset should be examined in other ways to look for outliers (data values that fall well outside the normal range for that water body over multiple samplings or for multiple water bodies within a forest or region). The range of values in the dataset and/or a histogram plot (Figure 4-4) should be used to look for outliers in all variables. Outliers may also be identified by plotting each variable by sample date to look for isolated gross variations over time. Analysis and sample collection records should then be reviewed to determine if the cause of any outlier is likely due to

data entry error, analytical error in the laboratory, or sampling error in the field. If errors are discovered, samples can be reanalyzed or rejected. If no error can be determined, the results should be assumed to be correct and accepted without change. Outliers should not be rejected unless there is a strictly objective basis for rejection. If there is a clearly identified error, the result should be rejected and, if possible, corrected; if there is an unexplained anomaly, the data should be retained.

Another useful procedure is to plot variables in the dataset against each other for variables that are known to be highly correlated. Examples of strongly correlated variables can include Ca^{2+}–Mg^{2+}, Ca^{2+}–ANC, Na^{+}–Cl^{-}, DOC–color, and N–P. Data points that fall outside the cloud of data points defining the general relationship warrant closer examination.

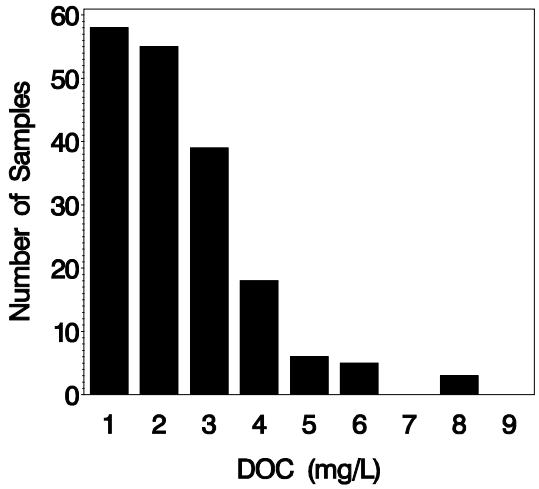

Figure 4-4. Patterns in DOC concentration in streams within a particular region. The data are not normally distributed; rather, they are skewed towards lower concentrations (≤ 3 mg/L).

4.2.5 Final Data Quality Determination

Each of the dataset internal consistency checks outlined above provides an opportunity to identify potential problems in the data related to sampling error, data entry error, and/or laboratory error. Some of the problems identified through these analyses might be corrected by reanalysis or simply by replacing a value that was entered incorrectly into the dataset. In other cases, the cause of the anomaly will be unknown and will represent an error of some sort or the presence of one or more unmeasured analytes. Such unexplained deviations from expected patterns should generally not be altered or deleted from the dataset. As described in the preceding sections, the final validated dataset should show:

- Good charge balance agreement;
- Good agreement between measured and calculated conductivity;
- Good agreement between laboratory titrated and defined ANC based on various ion measurements;
- Reasonable (readily understandable) distribution of parameter values as reflected in frequency distributions across space and/or across time; and

- Clear patterns between paired variables that are known to be strongly correlated with each other.

Note that there is generally no clear-cut definition of what constitutes "good" or "reasonable" agreement, although targets for percent and absolute variation are presented where applicable. The purpose of these analyses is not to discard measurements that are not completely understandable but rather to identify the samples and/or measurements that appear to have a higher likelihood of some kind of error. In the best of cases, the error is identified and corrected. In other cases, the error remains unknown or there may not be an error at all but rather an aspect of the water chemistry that is not fully understood.

Unless these internal consistency checks are conducted and unless the dataset is found to be generally internally consistent, it is not possible to determine whether analysis of these data will yield meaningful and representative results. To the extent that the water chemistry data make sense, greater confidence can be placed in conclusions drawn from analysis of those data.

4.3 APPLY PROCEDURES TO PREPARE RAW DATA FOR GRAPHICAL AND STATISTICAL ANALYSIS

4.3.1 Censored Data

Data that have reduced certainty are often censored for reporting or analysis purposes or both. Examples can include measured values below the MDL or measured values below the reporting limit (e.g., if nitrate concentration is reported as being less than 1 µeq/L). Censored data can cause problems in statistical calculations if there is no real number that can be used in the calculations. Other problems can arise in deciding what to do with censored data when reporting limits are approximately the same as analyte concentrations of ecological concern. If detection and reporting limits are well below any real level of concern, there is generally no substantial problem with interpretation or treatment of censored data. However, when detection or reporting limits are approximately at or below the same level as the level of concern, then interpretation based on censored data may be problematic regardless of how censored data are handled.

We recommend that laboratory measurements below the MDL be reported by the laboratory as zeros. These measurements are not statistically different from zero. We recommend that measured values above the MDL but below the reporting limit be flagged as having potentially higher uncertainty than do values above the reporting limit. Nevertheless, these values are entered as measured into the database and used as measured in data analyses, with no adjustment. We do not recommend reporting any data values in the dataset as being less than a particular value. Rather we recommend reporting actual values, including zeros for samples measured as below the MDL and including measured values that are less than the reporting limit but above the MDL.

4.3.2 Outliers and Missing Values

Outliers can be difficult to identify and interpret. Their importance is dependent on the type and objective of the analysis being conducted. There are a number of statistical outlier tests that one could apply but we caution against removing any outlier unless there is a good argument for removal based on a clearly identified analytical error or data management issue (e.g.,

typographical error). It is often useful to run the statistical analysis with and without any suspected outliers to see if the results are substantially different. If not, then the point is moot and it doesn't matter. If the outlier causes a big change in the results, then it will require some careful reexamination of the data before deciding what to do. In general, any conclusion that relies on the presence of one or a few extreme values should not be considered a robust conclusion. If it is determined that an important conclusion does depend on the inclusion of one or a few data outliers, we recommend not drawing that conclusion, but rather going back into the field to collect additional data.

In general, because they are not routinely removed from the dataset, outliers can be subjectively identified visually, without a formal statistical test. However, if a measured value appears to be an outlier, the analyst may wish to eliminate this deviant value and not include it in various calculations, data analyses, or data presentations. This generally should not be done unless it can be objectively determined that the questionable value is indeed likely to be erroneous. We recommend Dixon's Q-test as a relatively simple test to determine outlier status. The test is conducted as follows:

1. Arrange the values of the observations in ascending order.
2. Calculate the experimental Q-statistic (Q_{exp}) as the ratio of the difference between the suspect value and the value of its nearest neighbor (in the ascending series of values that comprise the dataset) divided by the range of values in the dataset. For example, to test whether the lowest value (x_1) is an outlier, calculate Q_{exp} as:

$$Q_{exp} = \frac{X_2 - X_1}{X_N - X_1} \qquad (10)$$

   where x_1 is the lowest value in the series
   x_2 is the second lowest value in the series
   x_N is the highest value in the series

3. Similarly, to test whether the highest value is an outlier:

$$Q_{exp} = \frac{X_N - X_{N-1}}{X_N - X_1} \qquad (11)$$

   where x_{N-1} is the second highest value in the series and the other terms are as defined above.

4. Compare the calculated Q_{exp} to a critical Q-value (Q_{crit}) that is taken from a table (see, for example, Table 4-4, Rorabacher 1991). You must first choose your confidence level (CL). We recommend a 95% CL. For example, as shown in the table, at the 95% CL and a total number of measured values equal to 9, the Q_{crit} is 0.493.
5. If $Q_{exp} > Q_{crit}$, the questionable value can be designated as an outlier.

Missing values are a fact of life in most statistical analyses of environmental data. They are more problematic in parametric tests such as regression or analysis of variance than in non-parametric tests. Parametric tests are used for estimating parameter values and testing hypotheses concerning them when the form of the underlying data distribution is known (typically, the data are normally

distributed). For tests of data for which we do not know the underlying data distribution, including those that are not normally distributed, nonparametric tests must be used. These tests compare the distributions, rather than the parameters.

Missing values can be synthesized from other data, but we would not recommend this approach as a general procedure without careful consideration. The EPA's National Surface Water Surveys synthesized a small number of missing values using regional regression models in order to make complete regional population estimates. The general approach is to substitute for the missing value a synthetic value developed from the remainder of the dataset or published relationships. The synthetic value can be calculated as the median of the existing measured values for that parameter or using a regression relationship based on one or more other variables. For example, if a measured value of Na^+ is missing, one can estimate the missing Na^+ concentration from the measured Cl^- concentration using a regression approach based on Na^+ and Cl^- measurements in the dataset. Thus, the regression equation, developed from the existing data, with which to estimate the Na^+ concentration from the measured Cl^- concentration should be used to estimate any missing values of Na^+ concentration. Similarly, Al_i concentration can be estimated from pH or H^+ concentration using a linear regression approach.

The median of existing measured values can also provide a reasonable substitute for a missing value. However, one should be careful to avoid using the median of data points known to exhibit a wide range of values, especially when there is an opportunity to reduce that variability. For example, if a Ca^{2+} concentration measurement is missing from a dataset containing first- through fifth-order streams and where the Ca^{2+} concentration varies strongly with stream order, it would be better to take the median of all streams in the dataset that are of the same order as the stream having the missing value rather than the median of all streams of all stream orders.

It can be considered acceptable to create synthetic substitutes for a small number of missing values, but these should generally not constitute more than 5% of the data for any variable. In general, we recommend not creating synthetic substitutes for missing values unless these missing values prevent the use of a particular analysis needed for a project objective. For example, a principal components analysis cannot be performed on lake ion chemistry using samples that have one or more missing variable values. Thus, any sample that has even one missing value cannot enter into the analysis unless the void is first filled with a synthetic value. Furthermore, some missing values may not be particularly important to interpretation of the data (for example, a missing NH_4^+ concentration in a lake that is expected to be very low). It is advisable to avoid, if possible, deleting that entire sample from the analysis simply because the NH_4^+ measurement is missing. If synthetic values are to be constructed, we recommend using the most robust empirical approach that can be developed from that particular dataset.

Table 4-4. Critical Q-values for Dixon's outlier Q-test, at the 0.95 confidence level. (Source: Rorabacher 1991.)

Number of Measurements	Q_{crit} (CL: 95%)	Number of Measurements	Q_{crit} (CL: 95%)
3	0.970	17	0.365
4	0.829	18	0.356
5	0.710	19	0.349
6	0.625	20	0.342
7	0.568	21	0.337
8	0.526	22	0.331
9	0.493	23	0.326
10	0.466	24	0.321
11	0.444	25	0.317
12	0.426	26	0.312
13	0.410	27	0.308
14	0.396	28	0.305
15	0.384	29	0.301
16	0.374	30	0.298

4.3.3 Multiple Observations

We do not recommend averaging the results of replicate (duplicate or triplicate) samples in the dataset. Rather, the first sample collected at a given site and sample occasion is considered to be the normal sample. It is used in statistical and other data analyses to represent the chemistry of that lake or stream on that sampling occasion. Any second or third sample (replicate) collected on that sampling occasion is used only for QA purposes to assist in quantifying the cumulative variability and error associated with the collection and analysis of the water in that lake or stream. The replicate sample result is not used in routine data analyses.

If multiple samples are collected within a given rainstorm, season, or year, results of analyses of those samples are maintained as separate values in the dataset. Depending on the objectives of a particular study or analysis, they might be averaged in the process of analyzing the data. For example, if the objective is to compare spring base-flow chemistry across streams in a particular forest, one may choose to average all samples collected during the spring season (avoiding rainstorm and snowmelt periods) over a finite period of time (perhaps 5 years). Such an approach is appropriate if, for example, some streams in the forest were sampled only once and others multiple times within that 5-year period. If there is reason to believe that stream chemistry changed appreciably during that 5-year period, then it may not be advisable to average the data across multiple years. Instead, one may choose to use the spring base-flow sample collected at the time closest to April of a particular year, for example. One should be particularly careful about averaging multiyear data if part of the sampling window occurred during a wet year(s) and part during a dry year(s).

If most or all sample sites were sampled during multiple years, an analysis of the spatial distribution of water chemistry across a forest will often be conducted using 3- or 5-year averages of chemistry to represent each site. Such averages should not combine samples collected during different seasons unless it is clear that seasonality is not an important issue.

4.3.4 Treatment of Zeros and Negative Values

For studies of dilute surface waters potentially impacted by air pollutants, the only major variable expected to have negative values is ANC. Some, but not all, lake or stream datasets will have some negative ANC values. Because negative ANC values are real measurements, they must be left as negative numbers. However, some transformations (e.g., log transforms) required for some statistical analyses may only be applicable to non-negative and non-zero numbers. If that becomes an issue for an analysis planned for a particular dataset, add a constant whole number just larger than the largest negative number in the data (i.e., add 50 μeq/L if the lowest ANC is -49) to all ANC measurements so that there are no longer any negative numbers in the analysis. This should be done only for that particular analysis. Designate the new variable as [ANC + 50 μeq/L]. This manipulation of the data must be taken into consideration in interpreting the results of the analysis. For zero values, we recommend adding one to all values of that variable when almost all the data are greater than one, and changing the name of the revised variables to be used in the analysis to, for example (sodium + 1 μeq/L). This works well for $\log_{10}$ transforms as when x=0, $\log_{10}(x+1)=0$. When many of the data values are less than one, add a constant number that is smaller than almost all the data values to each zero value in the dataset. For example, zero values for NH_4^+ concentration, which may be fairly common in many surface water datasets, may be adjusted by adding a constant of 0.001 μeq/L before transformation.

4.3.5 Treatment of Seasonality

Seasonal variation in water chemistry data can affect data analysis and interpretation in two fundamental ways. First, chemical parameters that affect the suitability of the water to support various species and biological communities tend to vary with season. This is the case in many waters with respect to pH, ANC, Al_i, DOC, NO_3^-, SO_4^{2-}, and base cation concentrations. Thus, the chemical conditions that are most stressful to biota may occur to a greater or lesser degree depending on season. These seasonal differences are most pronounced in regions that experience substantial seasonal changes in rainfall or temperature. Interpretation of chemical parameter values above or below known biological stress thresholds will be highly influenced by when the samples were collected.

Second, seasonality in the data can affect certain statistical tests (such as trends analysis, for example). A dataset having substantial seasonality may require use of different statistical tests as compared with a dataset lacking seasonality. This is further discussed in Section 4.5, Conduct Statistical Analysis, of this DAP.

This DAP does not recommend the need for any particular adjustment of seasonal data. Some sampling studies may choose to reduce the effects of seasonality on the data by careful timing of field activities. Other studies may strive to quantify the seasonality that occurs. It can also be very useful to quantify the relationship between annual average or base-flow chemistry and observed extreme values that are influenced by season and/or episodic processes. For example, Sullivan et

al. (2003) illustrated the relationship between median spring season ANC and the minimum ANC reported in the data record for streams in Shenandoah National Park (Figure 4-5). Such an analysis could also be conducted to compare spring median or spring minimum ANC to summer or fall index ANC. These kinds of relationships can be useful in evaluating the likelihood of experiencing extreme values that exceed various response thresholds for expected biological effects.

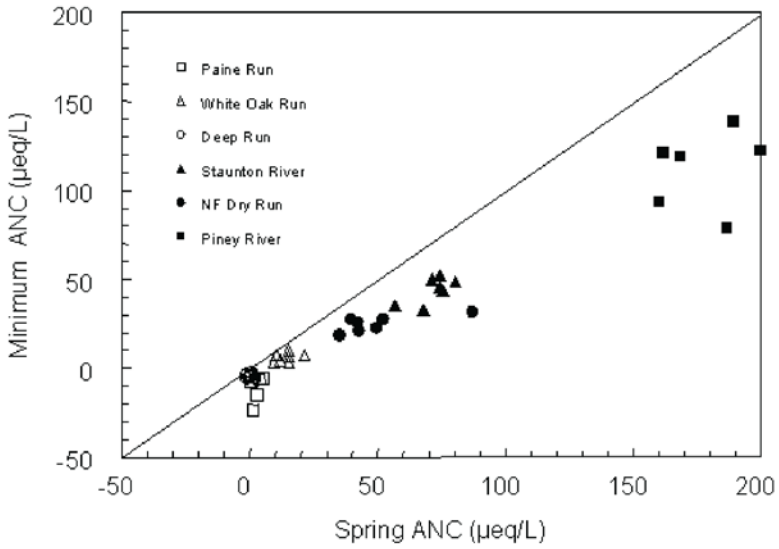

Figure 4-5. Minimum stream ANC sampled at each site during each year versus median spring ANC for all samples collected at that site during that spring season. Data are provided for all intensively studied streams within Shenandoah National Park during the period 1993-1999. A 1:1 line is provided for reference. The vertical distance from each sample point upwards to the 1:1 line indicates the ANC difference between the median spring value and the lowest sample value for each site and year.

4.4 CONDUCT EXPLORATORY ANALYSES

4.4.1 Analysis of Water Quality Status

Various graphical and statistical methods are available for describing ambient water quality and assessing differences in water quality across a forest or region. Current status of water quality should be compared among sites, with previously obtained data for individual sites, with criteria values or standards used in water quality assessments, and with values that represent ecological thresholds.

There is no standard procedure for the statistical analysis of water quality data for the purpose of evaluating sensitivity to, or effects from, atmospheric deposition. Rather, there exists a range of options for depicting results and/or analyzing differences over space or time. Selection of methods will depend on a host of issues, including project objectives; the quantity and quality of the data; number of sampling locations; length of the period of record; extent to which samples were collected across years, seasons, and hydrological episodes; and specifics of the resulting dataset. Important dataset issues include the presence and abundance of extreme outliers, censored data, and negative values; normal versus non-normal distribution of the data; seasonality

and episodicity of the data; and extent to which data values are missing and/or are lower than reporting limits. It is generally advisable to consult with a statistician or a staff member who is knowledgeable in statistics before conducting trends analyses and other complex statistical analyses. Nevertheless, there are some commonly used and accepted data analysis approaches and statistical tests that are often applicable to the types of data analyses needed by the FS ARM program. These are described in the sections that follow.

4.4.2 Graphing and Quality Analysis

Graphics used to visualize water quality data include scatter plots of values for single or multiple sites by date. Water quality should also be examined relative to continuous variables, such as elevation, watershed area, or discharge. The range and distribution of data for different periods of time or for different lakes or streams can be depicted with histograms (Figure 4-4) or box-and-whisker plots (Figure 4-6). The box plot graphically represents the central tendency and variability in a dataset. The range indicated by the box (top to bottom) represents the middle half of the data and is bisected by a line that represents the median value of the data. Because the bottom of the box represents the lower-quartile (25th percentile) of the data and the top of the box represents the upper-quartile (75th percentile) of the data, the vertical length of the box represents the "interquartile range" (IQR) of the data. The end of each whisker represents the last value from the dataset that is no more than 1.5 times the IQR. The outliers (values beyond 1.5 times the IQR) are all shown on the plot. Data points marked with a star are greater than 1.5 times but less than three times the IQR and are considered possible outliers; those that are marked with an open circle are greater than three times the IQR and are considered probable outliers.

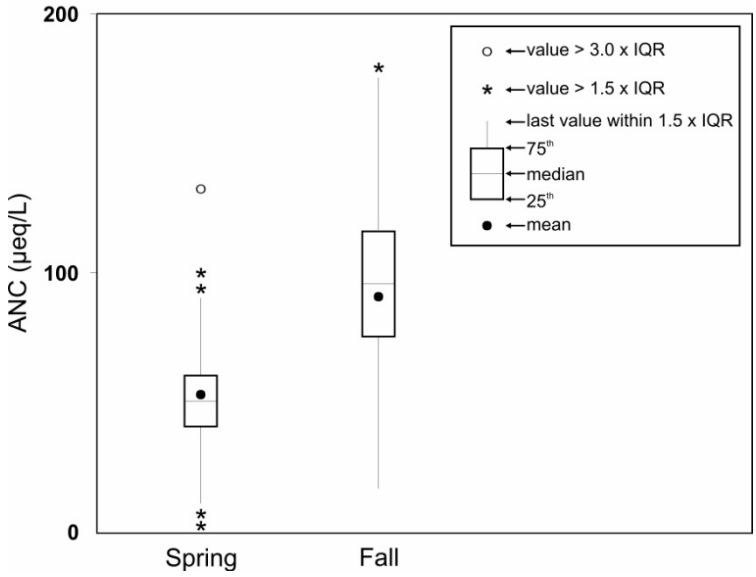

Figure 4-6. Box plots comparing hypothetical lake ANC values measured in samples collected during the spring versus the fall season.

Graphics should be used to examine temporal variation in data for individual sites, including patterns associated with season or discharge, as well as gradual or more sudden changes in values. Spatial variation among multiple sites can also be represented graphically, including variation related to differences in watershed properties, land use, or exposure to pollutants. Even

when a more quantitative statistical analysis of water quality data is desired, qualitative visual data examination is recommended as a preliminary step.

The steps that one should take in analyzing the dataset will depend to a large degree on the specifics of the dataset itself and the purpose of the analysis. Common management issues for FS ARM program staff that involve analysis of surface water field sampling data are outlined in Table 4-1. That table identifies six major approaches (each tied to a purpose):

1. Determine whether one lake or stream, or a group of lakes or streams, is N-limited for algal growth.
2. Quantify episodic excursions from base flow conditions in surface water chemistry during hydrologic events.
3. Determine the distribution of lake or stream water chemistry across a particular forest or wilderness.
4. Quantify long-term changes in lake or stream ANC (or other variable) over time in a particular lake or stream.
5. Determine to what extent air pollution is currently affecting the water resources in a particular forest or wilderness.
6. Evaluate whether the current condition of acid or nutrient sensitive waters warrants mitigation.

The analyses that could or should be conducted will depend in part on which approach is required to answer particular management questions.

Every dataset will offer its own challenges and, if sufficiently examined, reveal its own, often unique, patterns. Regional differences are important. Furthermore, water quality data analysis is exploratory in nature. To properly analyze a water quality dataset, the analyst must experiment with different approaches and eventually find some that work both with that dataset and those specific analysis objectives.

Despite these difficulties and the site-specificity of water quality data analysis, it is possible to offer recommendations and examples of steps to be considered by FS ARM program staff. A successful analysis method will develop through trial and error. The example analyses illustrated in this section of the DAP show some of the approaches that we have found to be useful, and FS staff may find some of these examples to be successful in some cases. Nevertheless, an analyst should always explore multiple options to determine what works best for a particular dataset. If the data are of high quality, it is likely that they will tell a story. Some creativity may be required to reveal that story.

4.4.3 Recommended Data Analyses

We recommend various types of data analysis in this section. These recommendations are specific to the purpose of the data analyses outlined in Section 1 of this document. Forest Service staff may find alternative approaches to be as or more successful than those provided here. There is no one clear choice of how to approach exploratory data analysis.

SUBSET THE DATA

Exploratory data analyses should be conducted using all of the available data. In addition, however, it is often very helpful to also create various subsets of the data and analyze them individually. This is because inherent variability can obscure the patterns that might exist in the particular subset of the data that represents the more sensitive or affected bodies of water, time of year, hydrological conditions, geological settings, etc. Therefore, FS staff should explore various ways to subset their data before conducting exploratory analyses in order to determine whether some patterns are only evident or are strongest for one or more subsets when compared with the dataset as a whole.

Data can be subset for exploratory analysis using water chemical criteria (Table 4-5). In addition to using chemical criteria, data can be subset using features of the landscape, hydrology, or morphology (Table 4-6). This is an opportunity for the analyst to be creative. Try different approaches, and see what works. The objective is to improve your understanding of the data and the story that they have to tell.

Table 4-5. Example variables with which to subset water quality data, according to measured water chemistry for analysis.

Variable	Possible Cutoff Values for Designating Lake or Stream Classes
ANC	0, 20, 50, 100 µeq/L
NO_3^-	5, 10, 15 µeq/L
DOC	200, 400, 500 µM
pH	5.0, 5.5, 6.0, 6.5
Al_i	2, 7 µM
Ca + Mg[1]	Highly region/forest specific
SO_4^{2-}	Highly region/forest specific

[1]Can be analyzed individually or in combination; in some cases (where they are quantitatively important), Na and/or K might also be included.

Table 4-6. Example variables with which to subset water quality data for analysis according to features other than measured water chemistry.

Variable	Possible Lake or Stream Classes
Geologic class	For example: siliciclastic, granitic, argillaceous, etc.
Elevation	Can use above or below a specific cutoff, or as discrete elevational bands
Lake type	Drainage, seepage, type of seepage lake (perched or flow-through)
Stream Strahler order	Can combine into classes (i.e., 1st plus 2nd, 3rd plus 4th, etc.) or analyze as individual orders

DETERMINE WHETHER ONE LAKE OR STREAM, OR A GROUP OF LAKES OR STREAMS, IS N-LIMITED FOR ALGAL GROWTH

Productivity of surface water can be limited by a multitude of factors. For example, small streams are commonly limited by available sunlight; if the stream is highly shaded by riparian vegetation, then primary productivity may be low even if nutrient concentrations are high enough to support

algal growth. Streams can also be limited by substrate type. For example, if suitable substrate is not available for attachment, then algal productivity may be low relative to nutrient availability. Streams and lakes can also be limited by presence of a nutrient, most commonly P and/or N; if N is limiting, then atmospheric contributions of N can enhance productivity, contribute to eutrophication, and perhaps alter species composition and abundance.

The relative importance of N and P as potentially limiting or co-limiting nutrients can be evaluated by conducting a rough screening analysis based on the molar ratio of N:P concentrations in surface water. This ratio ideally should include all forms of N and P, both particulate and dissolved, both organic and inorganic. Thus, total N is the sum of the concentrations of NO_3^--N, NH_4^+-N, and organic N. Units are in µmol/L for both elements. Phosphorus is measured as total P. If measurements of total N are not available, one can use an estimate of total inorganic N (TIN), calculated as the sum of the molar concentrations of NO_3^- and NH_4^+. Note that the concentrations of NO_3^--N in µmol/L are the same as the concentrations in µeq/L; no conversion is needed. Total N in µmol/L is equal to the concentration of total N in mg/L times 71.38. Total P in µmol/L is equal to the concentration in mg/L times 32.29.

Based on available experimental data, a large majority of lakes that have total N:total P below about 44 have been found to indicate N-limited phytoplankton growth (Elser et al. 2009). This is an area of active research, and interpretations may be subject to change in the near future. Lakes and streams that are nutrient (N and/or P)-limited, rather than limited by light or some other factor, may change in their limitation status over time, perhaps with season. Therefore, temporal patterns in the N:P molar ratio could be examined over time. In addition, we recommend evaluation of spatial patterns in N:P to determine, for example, if water bodies in a forest tend to be N-limited primarily at certain elevations, on certain geological types, or in certain vegetation communities. Thus, N:P ratios could be mapped relative to landscape condition to reveal such patterns, if they occur.

Determination that a water body or a group of water bodies exhibits potential N-limitation based on the N:P ratio is not sufficient evidence to indicate that the system is, in fact, N-limited. Furthermore, such an analysis does not necessarily identify any variability that might occur in that nutrient status throughout the growing season. These recommended N:P ratio analyses are screening-level analyses that may suggest the possibility of N-limitation.

The next step in the assessment process is to conduct laboratory studies to determine N versus P limitation. These could involve collection of multiple liters of lake water, which is then shipped on ice to the laboratory. The water is dispersed into flasks (typically, at least three flasks per treatment). Treatments may involve multiple light levels and varying (low and high) nutrient additions of N only, P only, N+P, and control (no nutrient addition). Incubation, with continuous or periodic mixing, is conducted under approximate ambient lake temperature conditions. Algal growth can be tracked daily by measuring the chlorophyll *a* concentration in an aliquot of water from each flask.

Based on the results of the laboratory incubation studies, and the degree of rigor required for the project in the determination of nutrient limitation, it may be desirable to progress to *in situ* incubation studies. Such experiments should involve *in situ* incubation of water over a period of time during the growing season in multiple containers suspended in the lake or stream. The

containers should include a control (no nutrient addition), multiple N addition containers (at least two levels: high and low), multiple P addition containers, and multiple N + P addition containers. For example, one may double and triple the ambient nutrient concentrations in the two containers for each type of nutrient input. Changes in the concentration of chlorophyll *a* over time in the treatment containers, relative to the control, indicate productivity responses to nutrient addition. Such experiments can verify whether or not, and when, a lake or stream (or a group of lakes or streams) may be susceptible to eutrophication effects associated with atmospheric N deposition.

QUANTIFY EPISODIC EXCURSIONS FROM BASE FLOW CONDITIONS IN SURFACE WATER CHEMISTRY DURING HYDROLOGIC EVENTS

Changes in the concentrations during episodes of major ions, pH, and ANC should be evaluated for a given lake or stream by plotting individual measured values during multiple storms. An example for one lake or stream during one storm or snowmelt episode is shown in Figure 4-7.

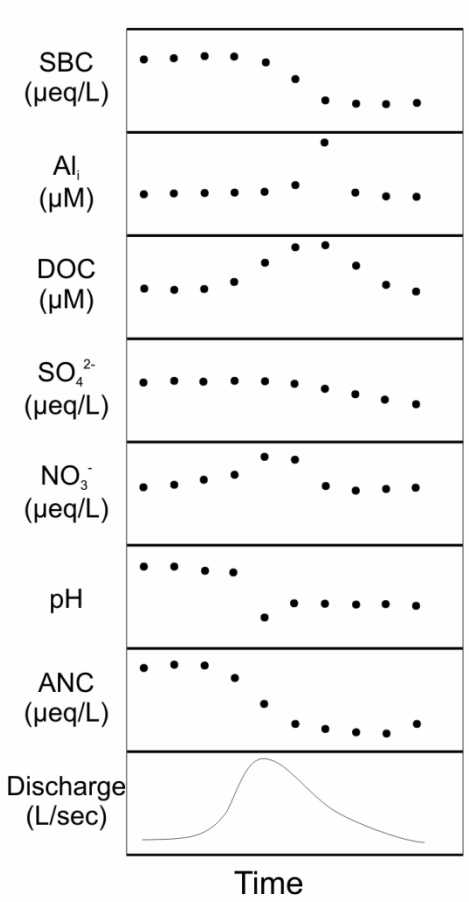

Figure 4-7. Changes in the concentration of major water chemistry constituents during a hypothetical hydrological episode in one stream. Data for each variable of interest should be plotted along the same time axis and compared at the same scale relative to the pattern of discharge.

The extent to which ANC and pH decrease and the extent to which Al_i increases in response to hydrological episodes provides an indication of the chemical extremes aquatic biota are exposed to during hydrological episodes. Patterns of episodic responses of the sum of base cations (SBC),

DOC, SO_4^{2-}, and NO_3^- concentration can reveal important information regarding the causes of episodic excursions of ANC, pH, and Al_i. Both ANC and pH can decrease and Al_i can increase, in response to base cation dilution (decreased SBC), NO_3^- leaching, SO_4^{2-} leaching, and DOC mobilization. The relative importance of these potential drivers varies by watershed, region, season, and hydrologic event. Examination of the kinds of plots shown in Figure 4-7 can reveal these patterns in the various potential drivers at one site during one event. It may be necessary to sample and analyze multiple sites and multiple events.

Similarly, temporal patterns of changing water chemistry in a given lake or stream should be examined across the annual or seasonal cycle. In regions having marked snowpack development, such an analysis should include the entire snowmelt period, as is shown in Figure 4-8.

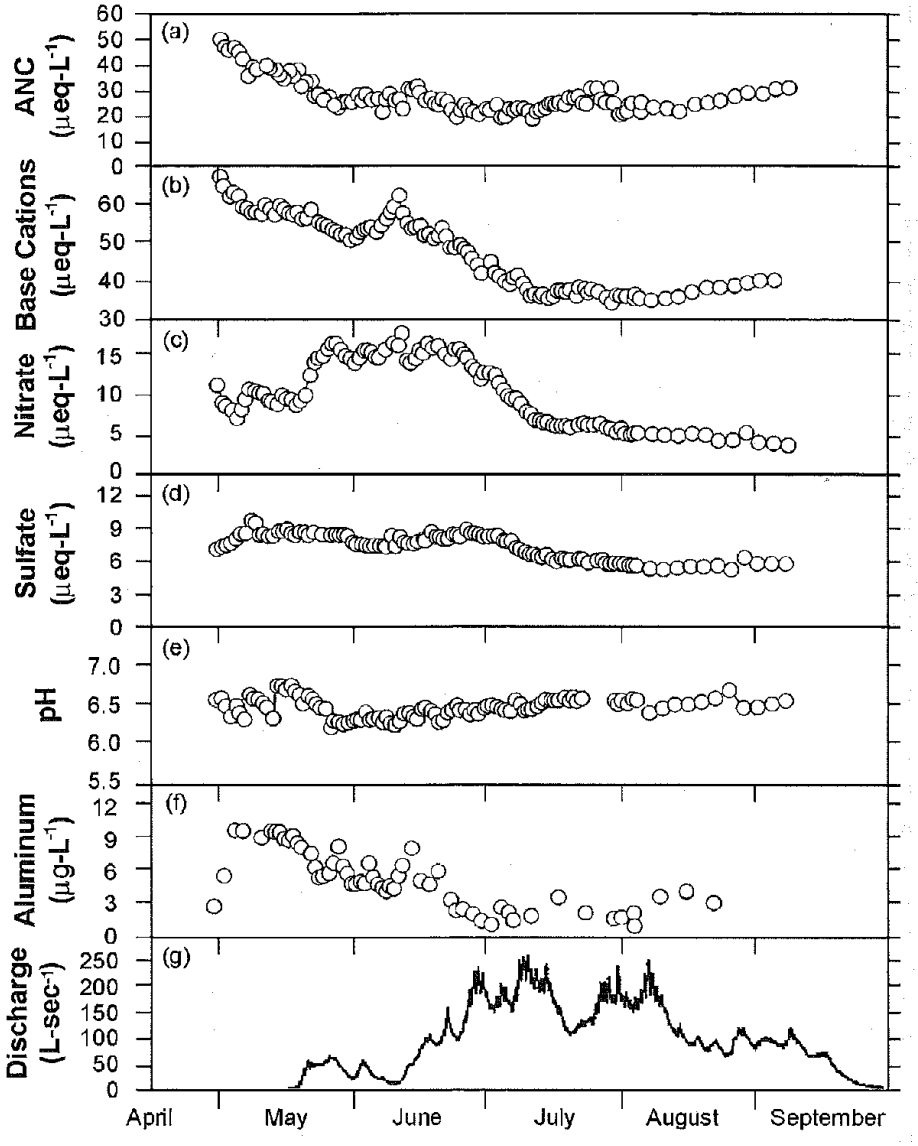

Figure 4-8. Time series of major ions and discharge in Treasure Lake in the Sierra Nevada during snowmelt in 1993. (Source: Stoddard 1995.) Seasonal and episodic changes in surface water chemistry can be examined using these simple time series plots.

Intensive time series data, where available, provide finer resolution of episodic changes in chemistry. For example, Figure 4-9 shows dramatic changes in NO_3^- concentration in two alpine streams in Rocky Mountain National Park in response to seasonal and snowmelt patterns. In this example, peak stream water NO_3^- concentrations occurred relatively early in the snowmelt process in May and June. Subsequently, there was an extended period of declining NO_3^- concentrations as snowmelt proceeded throughout the summer, followed by an increase during the fall.

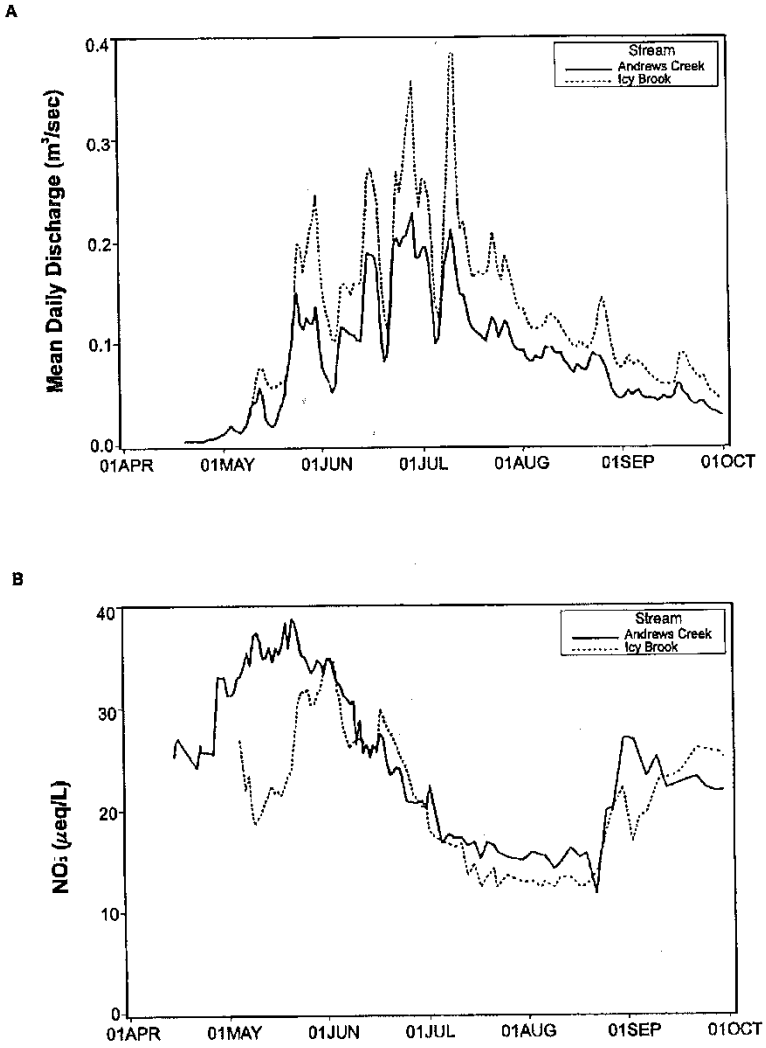

Figure 4-9. Daily discharge (A) and nitrate concentration (B) in Icy Brook and Andrews Creek within the Loch Vale watershed, Rocky Mountain National Park, in April-September 1992. (Source: Campbell et al. 1995.). This graphic shows an approach for displaying data from repeated sampling of two streams for the purpose of documenting changes in NO_3^- concentration as snowmelt proceeds within a given year.

An analysis of surface water NO_3^- concentration as a fraction of the total combustion-related mineral acid anion (SO_4^{2-} plus NO_3^-) concentration can reveal the relative roles of NO_3^- and SO_4^{2-} in influencing surface water chemistry. In many surface waters, under certain hydrological conditions, SO_4^{2-} dominates the total mineral acid anion concentration. When this occurs, NO_3^-

has relatively little influence on the ANC or pH of the water. There can, however, be times when NO_3^- is also important to the acid-base status of the water. For example, data collected from four Adirondack Mountain streams during hydrological episodes (Figure 4-10) illustrated that, for the study streams, NO_3^- generally provides less than half of the contribution of mineral acid anions from the atmosphere (SO_4^{2-} provides the majority), but the relative importance of NO_3^-, compared to SO_4^{2-}, increases at lower ANC values (which occur during high flow periods).

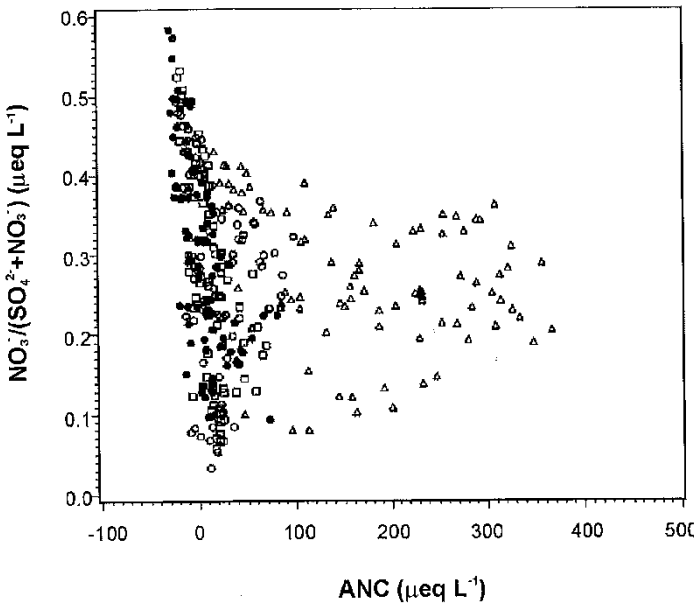

Figure 4-10. Ratio of NO_3^-:(SO_4^{2-} + NO_3^-) concentration versus ANC in stream water samples collected during hydrological episodes in four streams included in the Adirondack region of EPA's Episodic Response Program. The different symbols on the graph represent different streams. (Source: Sullivan et al. 1997.)

DETERMINE THE DISTRIBUTION OF LAKE OR STREAM WATER CHEMISTRY ACROSS A PARTICULAR FOREST OR WILDERNESS

Patterns in water chemistry should be mapped to illustrate spatial patterns in the data. Figure 4-11 shows one way to do that (in this case, for lake water NO_3^- concentration in Adirondack lakes). Each bar represents one lake; the base of the bar reflects the lake location, and the height of each bar is proportional to the NO_3^- concentration. In this example, concentrations of NO_3^- are highest in the southwestern portion of the Adirondack Park and in the central High Peaks region, the general locations where N deposition and precipitation amounts are highest. Thus, spatial patterns in surface water chemistry should be compared with various factors that are known or suspected to be associated with water chemistry. These might include geology, soils types, elevation, atmospheric deposition, precipitation amounts, vegetation types, etc.

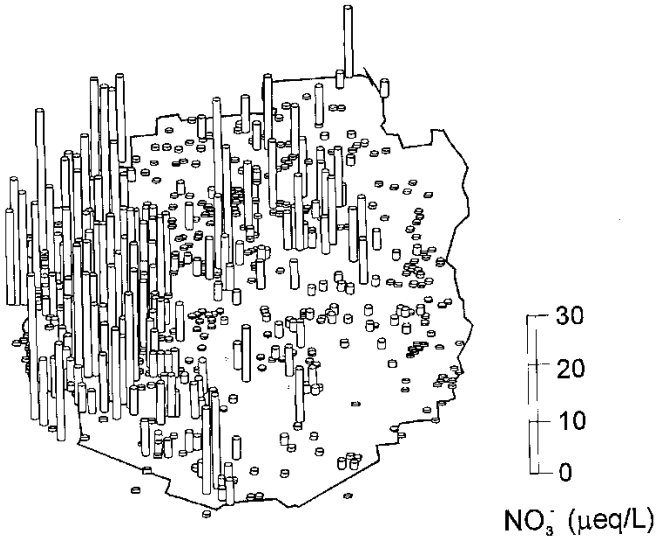

Figure 4-11. Map of summer NO_3^- concentrations in drainage lakes sampled by the Adirondack Lakes Survey Corporation in the Adirondack region of New York. (Source: Sullivan et al. 1997.) Maps such as this can reveal spatial patterns in the concentration of any surface water variable across a study area.

Spatial patterns can also be analyzed across a gradient of deposition or across a gradient of expected resource sensitivity using space-for-time substitution analysis. In this approach, it is assumed that the lakes or streams across the study area were initially relatively homogeneous in their chemistry, and furthermore, that differences observed across space at the present time correspond with changes that occurred in the past. Figure 4-12 shows an example space-for-time substitution analysis across a gradient in atmospheric deposition across the Upper Midwest. Such an analysis could be conducted across a gradient in elevation, deposition, slope steepness, etc. rather than, or in addition to, across a gradient in deposition. In this case, the data show decreases in $[HCO_3^- - H^+]$, DOC, and [Ca + Mg] over a gradient of increasing acidic deposition. Similarly, the ratio of SO_4^{2-} to SBC concentrations increases with increasing acidic deposition.

Furthermore, the slopes of the data on these graphs provide estimates of the quantitative importance of the various changes. Such quantitative estimates can be combined with model estimates of changes at selected locations in a weight-of evidence assessment.

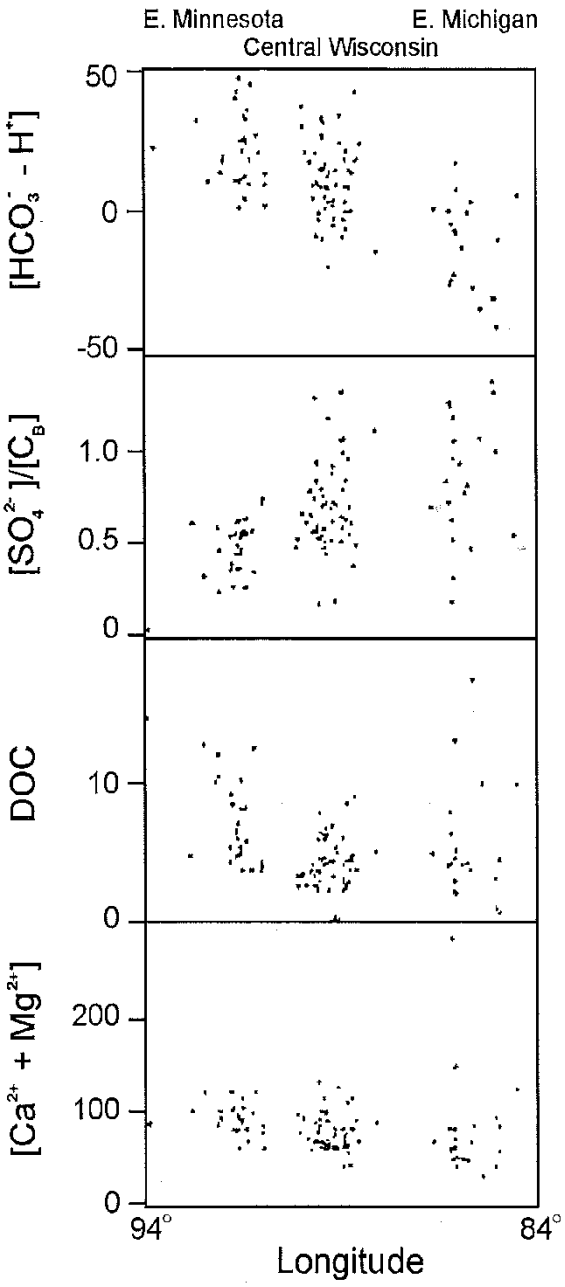

Figure 4-12. Example space-for-time substitution analysis for four variables across a gradient of expected response. This example is based on subsetting the available data, across a gradient of acidic deposition in the Midwest, to include only seepage lakes having low Cl^- concentration (to remove road salt influence) and those having ANC below 50 µeq/L. Note that the variable $[HCO_3^- - H^+]$ is one representation of ANC. (Source: Sullivan 1990.)

Portions of a region or a forest within which most of the acid-sensitive waters are expected to occur can sometimes be identified and mapped. In an example from the Southern Appalachian Mountains Initiative study, the area delimited by an acidification sensitivity classification scheme is shown in Figure 4-13. The darkly shaded area includes the siliceous geologic sensitivity class surrounded by a 750 m buffer. In addition, all areas lower than 400 m elevation have been deleted

and areas higher than 1,000 m elevation have been added. The area thus circumscribed includes 95% of the known acidic streams and 88% of the known streams having ANC ≤ 20 µeq/L (of more than 900 streams surveyed) within the region. Furthermore, all known streams having ANC ≤ 20 µeq/L are in close proximity to the final mapped area. FS staff can use an approach such as this to circumscribe portions of a forest thought to contain most of the sensitive or impacted water bodies.

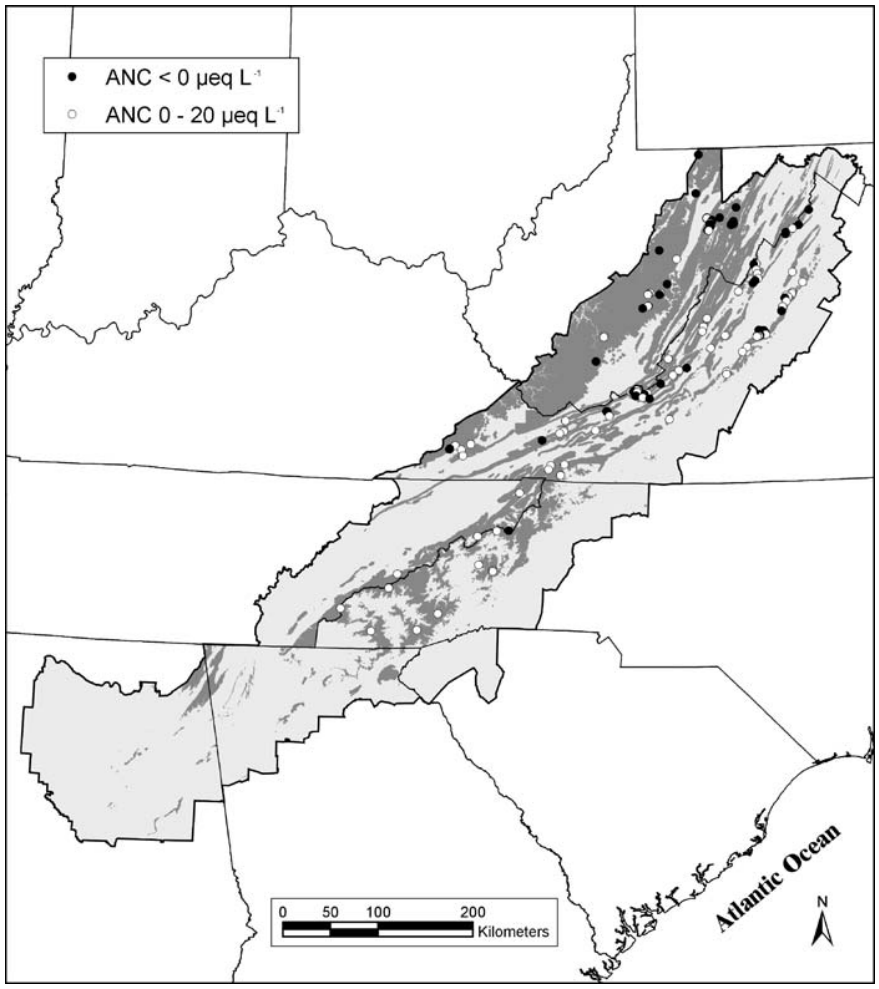

Figure 4-13. Results of a classification system devised to indicate expected low-ANC streams (in this example, based on geology and elevation) compared with actual low-ANC streams, which are represented as dots on the map. (Source: Sullivan et al. 2007.)

The distribution of data values across a given study region can reveal important information about the source of a constituent. For example, it can be helpful to plot the frequency distribution of surface water SO_4^{2-} concentrations within a relatively small study area. Atmospheric S contributions to watersheds are expected to yield a reasonably well-defined bell-shaped or half bell-shaped curve in surface water SO_4^{2-} concentrations. Differences from one study watershed to another in such features as elevation, aspect, vegetation type, and topography contribute to variability, but the overall patterns should be relatively homogeneous if the study area is relatively homogeneous. The observed outlier lakes or streams having much higher concentrations of SO_4^{2-} than the population of lakes or streams at large can be presumed to

receive contributions of geological S unless there is a good reason why atmospheric deposition should be markedly higher at those outlier locations. For example, histograms showing the frequency of occurrence of SO_4^{2-} concentration in lakes in four regions of EPA's Western Lakes Survey show clear outlier values in the Sierra Nevada, NW Wyoming, and the Colorado Rocky Mountains (Figure 4-14). These lakes likely receive geological S from their watersheds. Lakes or streams that contain appreciable geological S are not good candidates for monitoring or study to quantify effects from atmospheric sources of S.

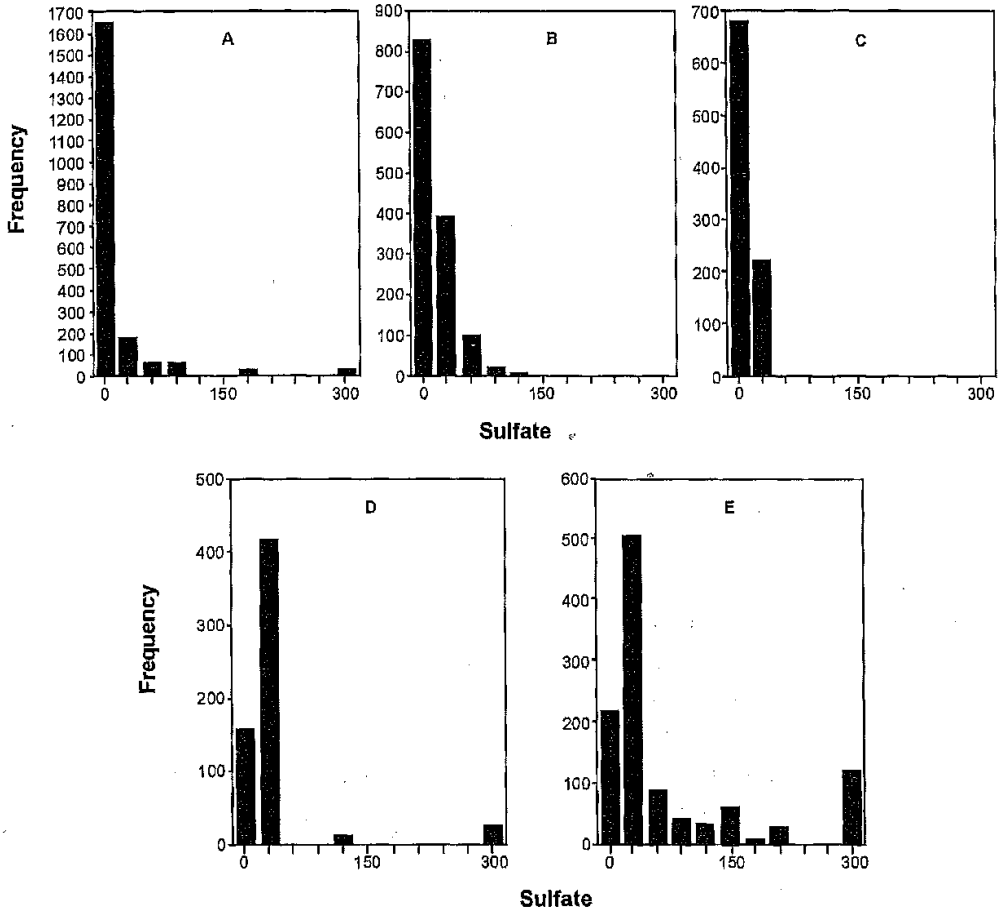

Figure 4-14. A histogram of the frequency of occurrence within a region (or forest) of surface water SO_4^{2-} concentration can reveal the typical distribution of the range of values that may be attributable to broad patterns of regional atmospheric inputs versus the more sporadic occurrence of high values that more likely derive from geological sources of S. In this example, the concentrations of SO_4^{2-} (µeq/L) in lakes in (A) Sierra Nevada, (B) Cascade Mountains, (C) Idaho Batholith, (D) NW Wyoming, and (E) Colorado Rocky Mountains are examined (Western Lake Survey). If a given study area receives relatively homogenous levels of atmospheric S deposition, then it can be assumed that the observed outlier high concentrations in lake water represent largely non-atmospheric input. (Source: Sullivan 2000.)

Spatial patterns in water chemistry and/or landscape characteristics can also be used to aid in extrapolating results from a relatively few intensively studied sites to the larger region. In many cases, model simulations of future chemistry or critical deposition load may be available for only a small subset of the lakes or streams in a given forest. Such results can sometimes be extrapolated to the wider population of waters using relationships with water chemistry (such as, for example, ANC in the example shown in Figure 4-15) and/or landscape features that correlate with sensitivity.

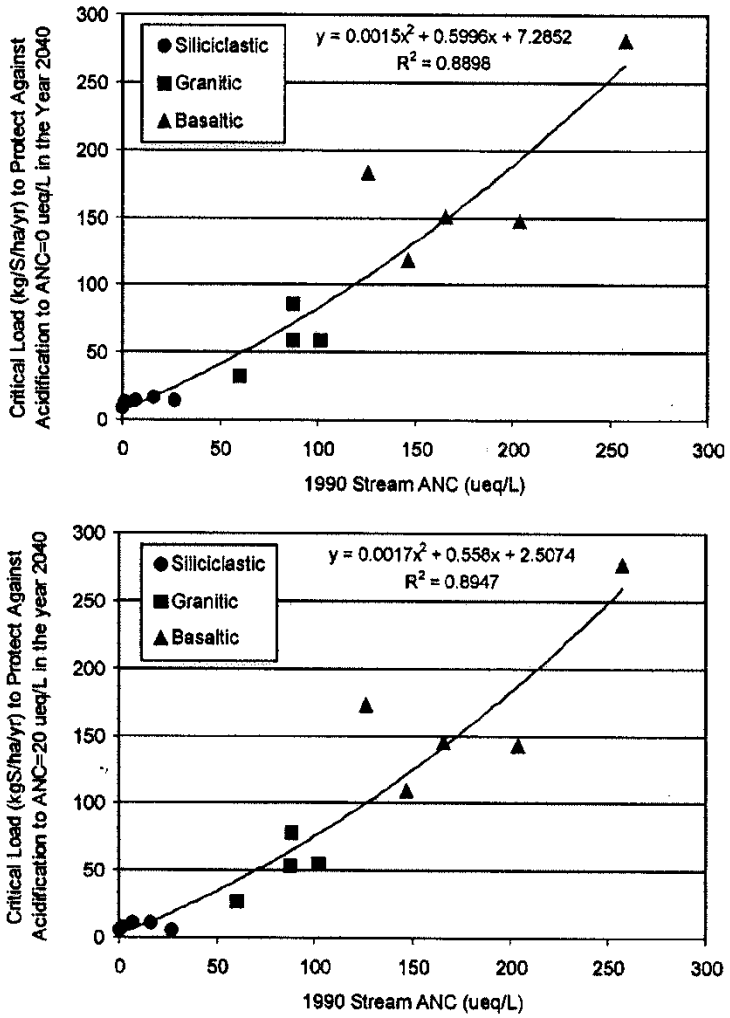

Figure 4-15. Critical load simulated by the MAGIC model to protect streams in Shenandoah National Park against acidification to ANC below 0 (top panel) and 20 µeq/L (bottom panel) by the year 2040 is plotted as a function of 1990 ANC. Stream sites are coded to show differences in geology. (Source: Sullivan et al. 2008.) This approach yields a predictive equation for estimating the model projected value (in this example of critical load) for a specific stream based on the measured value of ANC in that stream.

QUANTIFY LONG-TERM CHANGES IN LAKE OR STREAM CHEMISTRY OVER TIME

Detection of trends in water quality over time can be complicated by analytical error and measurement uncertainty that contribute to "scatter" in time series data. In addition, inter- and intra-annual variability contribute additional scatter. In particular, seasonal and episodic variability (which are largely driven by climate and hydrology) often contribute to short-term changes in water chemistry by acidification (Figure 4-16). Therefore, many years (we recommend at least eight) of monitoring data may be needed to reveal a probable trend. In general, it is helpful to collect data multiple times each year to measure the seasonal variability that exists and to discern the trend that exists within the noise of that variability. In other cases, it can be helpful to standardize time series data to minimize the influence of hydrological differences in constituent concentrations; the purpose in this is to minimize or eliminate the variability associated with seasonality in the data. This can be accomplished in multiple ways, including:

- Focusing on summer or fall index chemistry, with collection of samples under conditions having minimal influence of snowmelt or rainstorm events;
- Representing the available data as discharge-weighted average values if discharge values are available for the site in question or can be estimated or indexed from a nearby site that is gaged; or
- Focusing only on the minimum (i.e., ANC) or maximum (i.e., Al_i) concentration measured during each year; in this case, the plot would be of the lowest of multiple measured ANC values for each year over a period of 8 or more years.

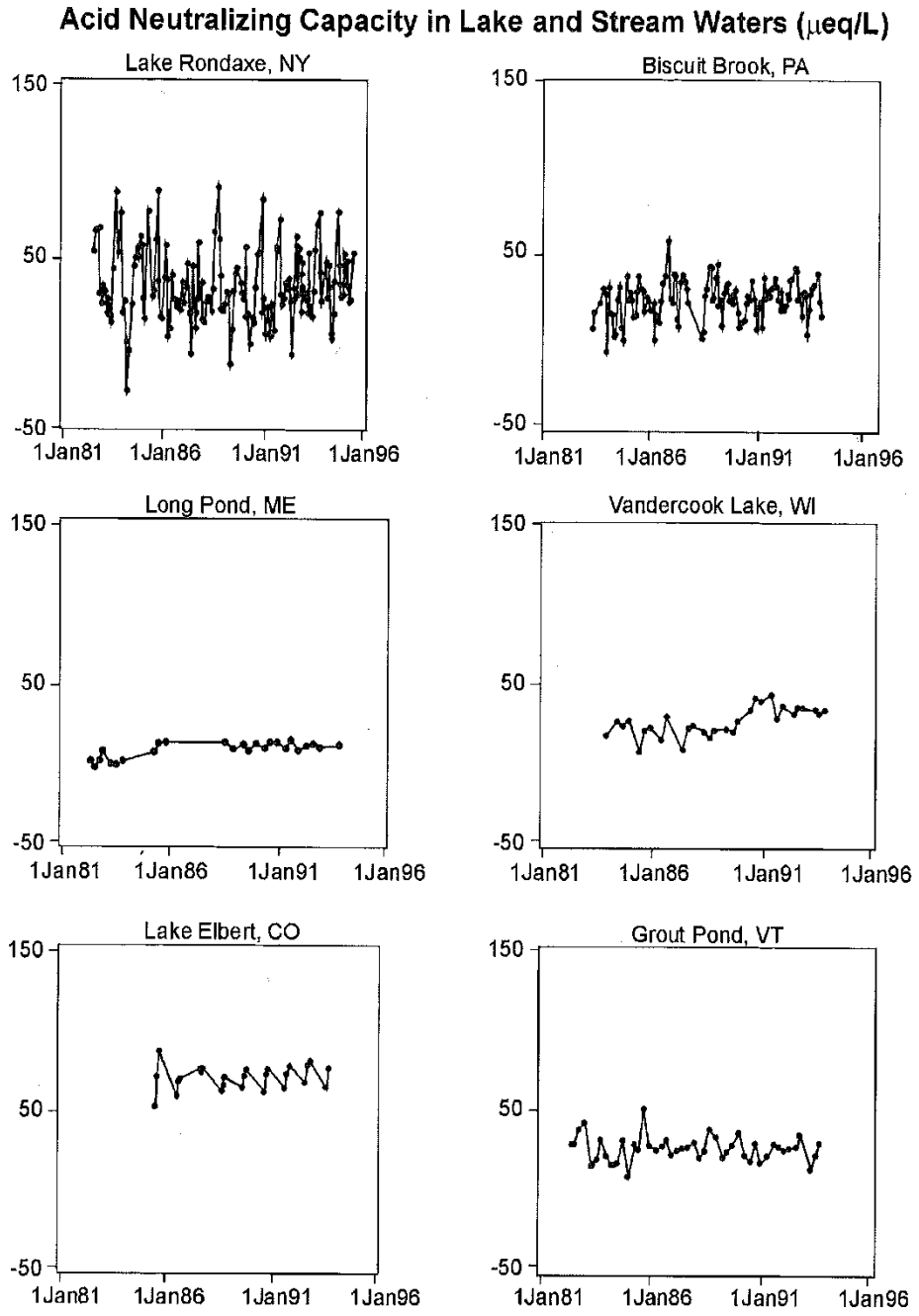

Figure 4-16. Example trends analyses of ANC in lake and stream waters, based on data from EPA's Long Term Monitoring program. (Source: Sullivan 2000.) These examples illustrate that inter annual and intra annual variability can sometimes be larger than the change over time in the variable of interest.

DETERMINE TO WHAT EXTENT AIR POLLUTION IS CURRENTLY AFFECTING THE WATER RESOURCES IN A FOREST OR WILDERNESS

There are many ways to analyze data to shed light on this question. In general, no single approach should be considered definitive. When multiple approaches converge to provide similar conclusions, there is greater confidence in the validity of that conclusion. Some of the figures presented previously (e.g., Figures 4-7 through 4-12) can provide useful information. Another approach entails plotting the relationships between the ratio of SO_4^{2-} (or $SO_4^{2-} + NO_3^-$) to the SBC concentrations versus ANC (Figure 4-17). A clear pattern across sites of decreasing ANC as the ratio of mineral acid anion (presumed to have been derived from acidic deposition) to base cation increases (reflective of ecosystem acid buffering) suggests that ANC has decreased in response to acidic deposition. The concentration of DOC can alter the relationship by decreasing ANC below what would otherwise be expected at a given SO_4^{2-}/SBC ratio. This is clearly evident in the top panel (Adirondack mountain lakes). Streams in the Catskill Mountains (middle panel) tend to have uniformly low DOC, and so subsetting on DOC concentration is not necessary. Because NO_3^- concentration is relatively high at some Catskill stream sites, the ratio includes NO_3^- in the middle panel and is presented for that region as $[SO_4^{2-} + NO_3^-]$:SBC. In the Cascade mountain lakes (bottom panel) there is little evidence of a regional acidification signal.

Assessments of acidic deposition effects and recovery generally rely on ANC and pH as the primary chemical indicators. However, both of these measurements can be influenced by naturally produced organic acidity associated with DOC, which can be abundant in streams and lakes draining wetlands and coniferous forests. In waters with significant concentrations of DOC, acidity from acidic deposition can be distinguished from natural organic acidity using the base-cation surplus (BCS). The BCS is an index that is based on the mobilization of toxic inorganic aluminum within the soil. In the absence of acidic deposition or geological S, inorganic Al remains in the soil in a non-harmful form. However, acidic deposition dissolves soil Al in a form that moves from soils into surface waters and harms both terrestrial (Minocha et al. 1997, Long et al. 2009) and aquatic life (Baldigo et al. 2007 and 2009 and Lawrence et al. 2008a). A BCS value less than 0 μeq/L in surface water generally indicates that the soil has become sufficiently acidified by acidic deposition to enable toxic forms of aluminum to be mobilized (Lawrence et al. 2007, Lawrence et al. 2008b). A negative BCS value could also occur from acid mine drainage or where drainage waters pass through geologic deposits rich in sulfide-bearing minerals. A BCS value between 0 and 50 μeq/L indicates a watershed with low calcium availability, which is at risk of future acidification from continued acidic deposition and can limit the productivity of aquatic (Jeziorski et al. 2009) and terrestrial (Long et al. 2009) ecosystems.

The BCS can be calculated using variables typically measured in low-ionic strength waters at risk from acidic deposition:

$$BCS = (Ca^{2+} + Mg^{2+} + Na^{+} + K^{+}) - (SO_4^{2-} + NO_3^{-} + Cl^{-} + RCOO^{-}_s) \quad (12)$$

$$RCOO^{-}_s = 0.071(DOC) - 2.1 \quad (13)$$

where all concentrations used in Equation 12 are in μeq/L and the concentration of DOC in Equation 13 is expressed in μmol/L.

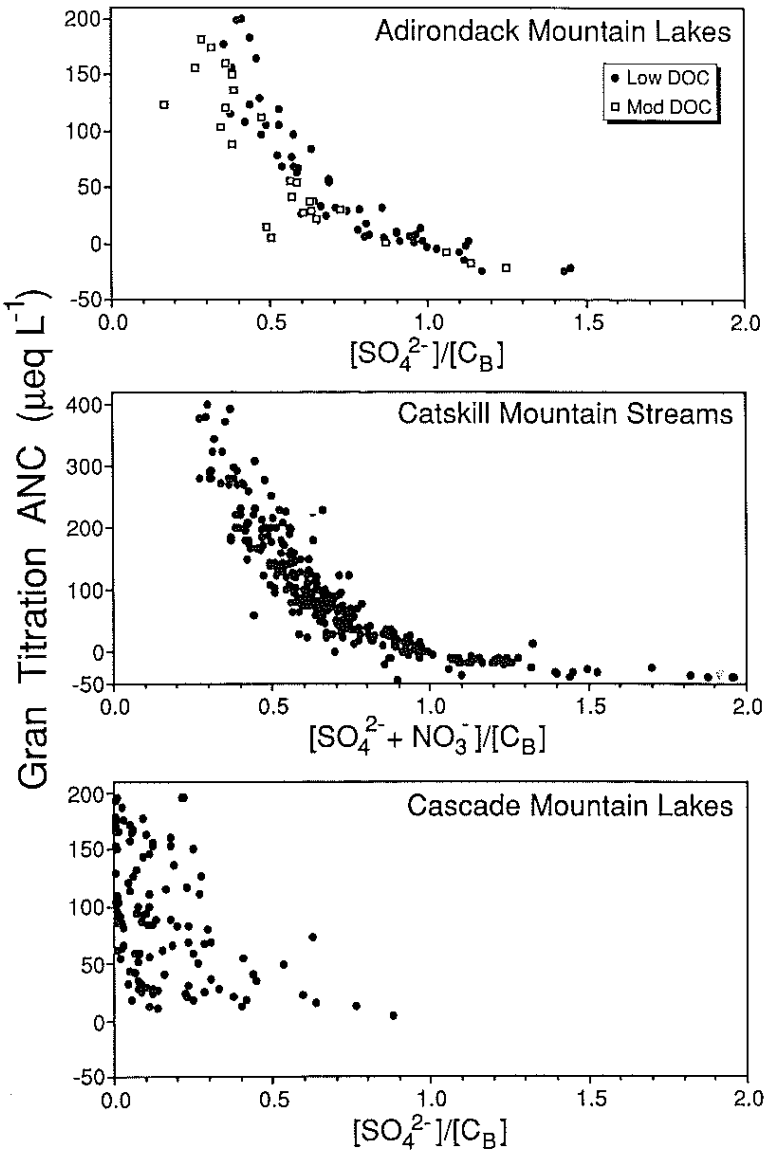

Figure 4-17. Plot of ANC versus ion ratio of SO_4^{2-} concentration divided by the sum of base cation concentrations (SBC) for low ANC (≤ 200 µeq/L) surface waters in (a) Adirondacks, (b) Catskill Mountains, and (c) Cascade Mountains. The base cation sum includes Ca^{2+} + Mg^{2+} + Na^{+} + K^{+}. This plot reveals that surface water ANC is strongly related to the ratio SO_4^{2-}/SBC in the Adirondack region, and the ratio (SO_4^{2-} + NO_3^{-})/SBC in the Catskill region, but weakly related to the ratio SO_4^{2-}/SBC in the Cascade Mountains region. Such an analysis sheds light on factors controlling surface water ANC. (Source: Sullivan 1990.)

Perhaps the most straight-forward way to determine whether or not and to what extent a given lake or stream has acidified is to construct a model hindcast of past water chemistry. The two models most commonly used for such purpose are MAGIC (Cosby et al. 1985) and PnET-BGC (Gbondo-Tugbawa et al. 2001). Each of these models has been widely used across the United States to model watershed acid-base chemistry, including hindcasts of past chemistry, forecasts of

future chemistry under differing future deposition rates, and to estimate critical loads of deposition to provide resource protection or to allow damaged resources to recover.

In addition, paleolimnological reconstructions of past lakewater chemistry can be constructed from the fossil remains of algal diatoms or chrysophytes (cf. Charles et al. 1990a and 1990b) in dated lake sediment cores. If both process model hindcast simulations and paleolimnological reconstructions suggest past acidification (especially if the estimates of change are quantitatively similar), then there is increased confidence in the validity of that conclusion.

It can be helpful to discern what types of lakes and/or streams within a forest have the lowest ANC, highest NO_3^- concentration, highest Al_i concentration, etc. Such kinds of waters become potentially important sites for further study or enhanced protection. For example, it may be that the lakes or streams in a forest having particularly low ANC may be generally, or entirely, small. In the Adirondack Mountain region, lake ANC is related to lake area (Figure 4-18). In this example, small lakes are more likely to be both low and high in ANC; intermediate-sized lakes tend to have more intermediate chemistry. Few lakes larger than 20 ha are acidic in this region. Similarly, it can be helpful to examine relationships between lake or stream chemistry and other morphometric features of the landscape, such as watershed area, stream order, lake depth, watershed slope, etc.

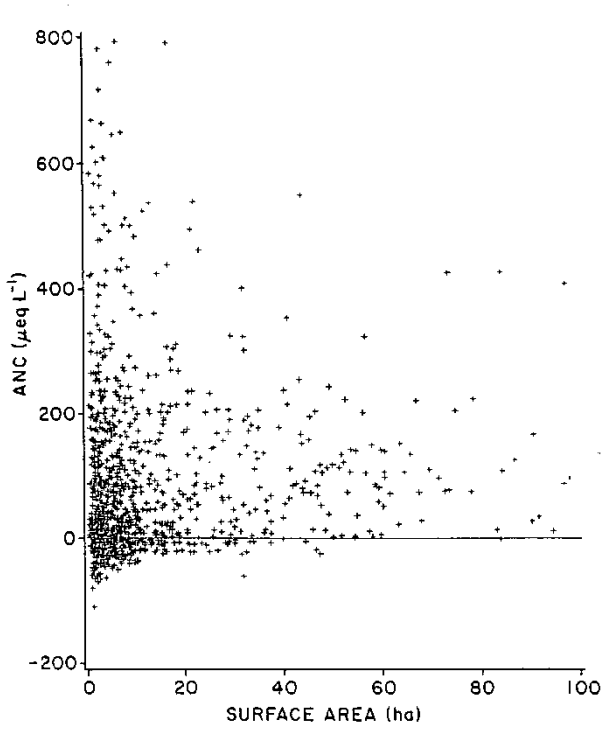

Figure 4-18. Relationship between lake size and lake ANC in the Adirondack Mountains. (Source: Sullivan et al. 1990.)

Across a given region, the leaching loss of NO_3^- in drainage water, which can be expressed as NO_3^- (sometimes including also NH_4^+) outputs in units of mass of N per unit watershed area per year, is related to N inputs in deposition (expressed in the same units). For example, this was shown for research sites across northern Europe (Figure 4-19). In this example, N output was

very low at N deposition levels less than about 9 kg N/ha/yr. Here, N leaching became pronounced for some, but not all, sites at N deposition greater than 9 kg/ha/yr. In addition, N leaching became consistently high at N deposition greater than 25 kg/ha/yr. Within a given forest or FS region, it might be possible to use this analysis approach to identify at what level of N deposition leaching of N to stream or lake water becomes pronounced.

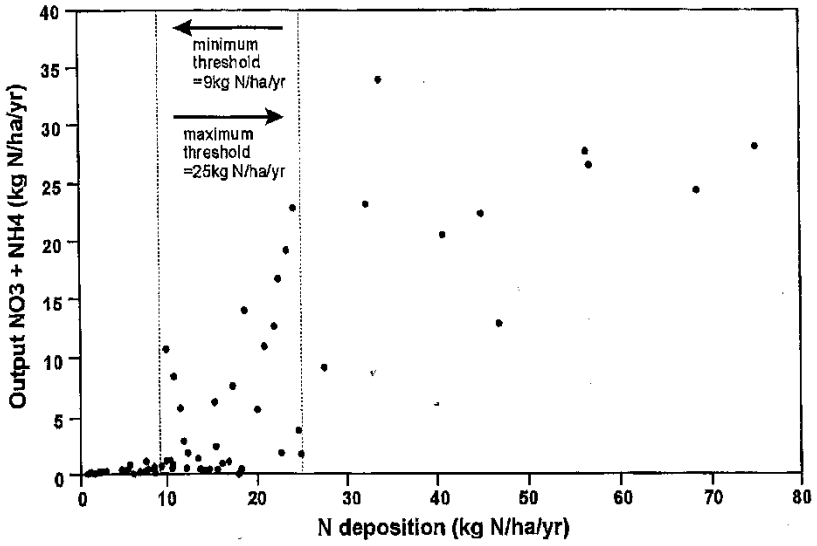

Figure 4-19. Nitrogen outputs in soil water or stream water versus N deposition inputs throughout Europe. (Source: Dise and Wright 1995.)

Nitrogen leaching is not always governed entirely, or even mainly, by N deposition. Other factors, especially climatic factors, can also be important. For example, Moldan and Wright (1998) showed a strong relationship between N leaching and air temperature at a research site in Sweden (Figure 4-20). This analysis suggests that N dynamics at this research site might be strongly controlled by climatic condition, in this case air temperature. Precipitation and/or snowpack condition could similarly be important.

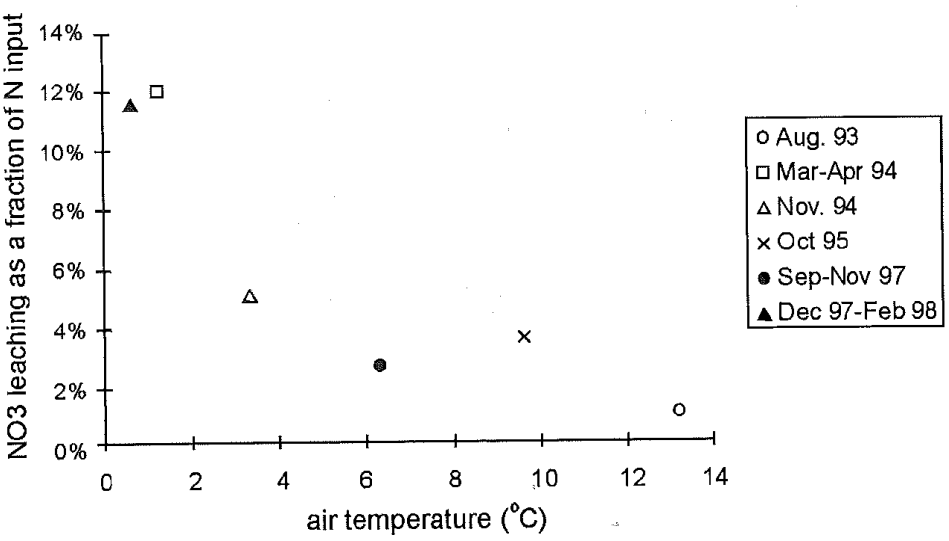

Figure 4-20. Observed relationship between NO_3^- leaching loss in runoff and mean air temperature at an experimental watershed site at Gårdsjön, Sweden. Each point represents an average of data collected over a period of 14 to 90 days. (Source: Moldan and Wright 1998.)

Nitrogen saturation of aquatic ecosystems has been described in stages, from Stage 0, which reflects relatively pristine, unimpacted conditions, to Stage 3, which reflects advanced N saturation (Stoddard 1994). Seasonal surface water nitrate concentration peaks at Stage 0 are generally rather low (less than about 25 μeq/L) and of relatively short duration (Figure 4-21). At Stage 1, the peaks in surface water NO_3^- concentration are higher and the period of elevated NO_3^- concentration is more extensive. Under Stage 2 N saturation, NO_3^- concentrations remain elevated throughout the annual cycle but some seasonality is still evident. At Stage 3, the N output may actually be greater than the N input (when expressed as mass per unit area per year). The temporal pattern of NO_3^- concentration in a given lake or stream can indicate the probable stage of N saturation of the watershed. For a given stream or lake, this type of analysis can illustrate the stage of N saturation of that water body as well as its drainage area.

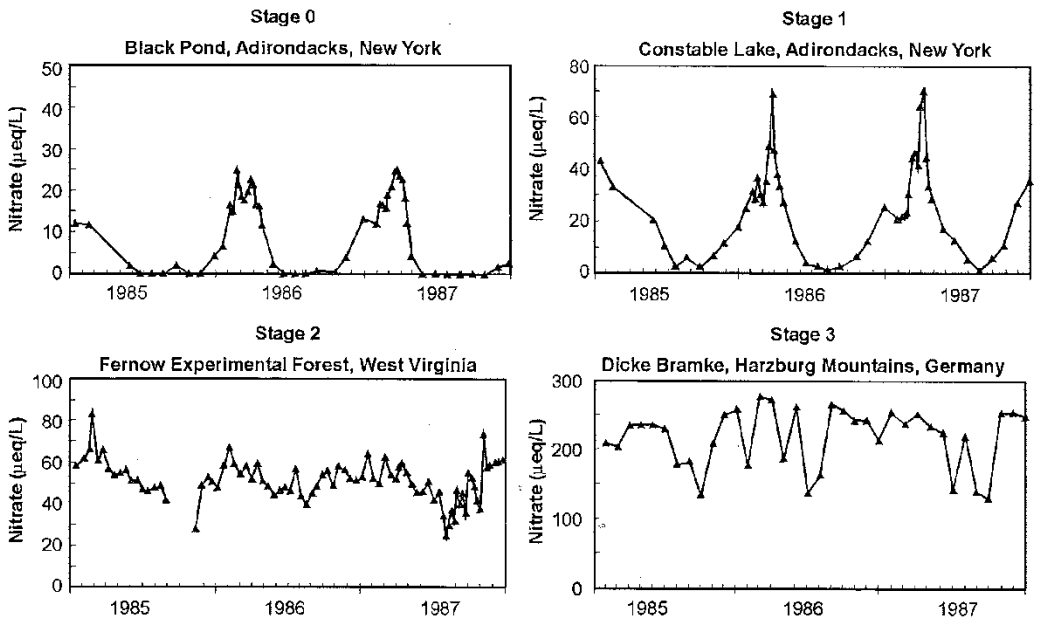

Figure 4-21. Example patterns of NO_3^- concentration in surface water at four sites at various stages of watershed N-saturation. (Source: Stoddard 1994.)

EVALUATE WHETHER THE CURRENT CONDITION OF ACID- OR NUTRIENT-SENSITIVE WATERS WARRANTS MITIGATION

There is no standard analysis approach that will answer this question. This is a management judgment that should be based on a variety of analyses, as outlined above. Potential mitigation strategies can include imposing tighter controls on atmospheric emissions of S and/or N and adding base cations to waters or watersheds by liming. Virtually all of the approaches suggested above can contribute to such decision-making.

4.5 CONDUCT STATISTICAL ANALYSES

4.5.1 Statistical Tests for Difference

It should not be automatically assumed that formal statistical tests are needed in analyzing a dataset. Much can be gained by conducting routine exploratory data analyses, such as those outlined in the previous section, without adding the complexity of formal statistical tests. In many cases, the assistance of a statistician or other person who is quite knowledgeable about statistics will be needed for conducting such tests.

Statistical tests are often used to determine the existence of significant differences between groups of sites or samples. The most common tests are parametric, and include t-tests and analysis of variance (ANOVA) that use means and variances to determine significant differences among group means. These parametric tests, however, make assumptions about data normality and independence that need to be examined before using them. Data are normally distributed if the various concentrations measured at different times for the same site or at different sites are bell-shaped (Figure 4-22). If the data are not normally distributed, they must be transformed

before parametric analysis or they must be analyzed using a non-parametric test. For water chemistry, variables are often log-transformed to achieve a normal distribution. There are a number of statistics that can test for normality (e.g., the Kolmogorov-Smirnov test), but we recommend plotting histograms or some other type of frequency plot and visually inspecting the graph to see if the distribution deviates grossly from a bell-shaped normal distribution.

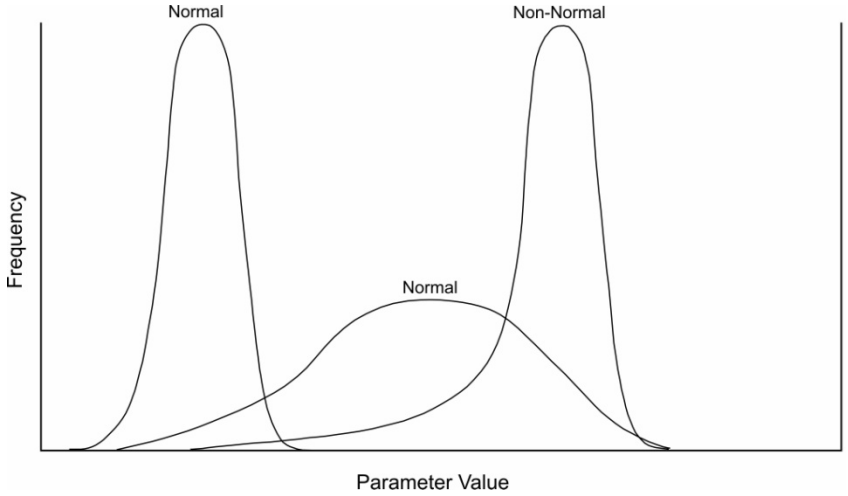

Figure 4-22. Schematic representation of data normality.

Non-parametric or distribution-free statistics are used to test for group differences in skewed (not-normally-distributed) data or when the analyst is not comfortable with assumptions about normality. Water chemistry data are commonly not normally distributed. The non-parametric tests are based on sample ranks (rank order number from low to high) and not actual data values. Because the test is based on rank, no assumption is made about the underlying data distribution. The best known non-parametric test for group difference is the Mann-Whitney U test, or as it is also known, the Wilcoxon rank-sum test.

Non-parametric tests, including the rank-sum test, tell you nothing about the magnitude of the difference between groups: just whether the group differences are significant. Analysts should be cautioned, however, that with large enough sample sizes, groups can be statistically different but such differences may have little ecological significance. The magnitude of any differences needs to be examined; running a statistical test to determine if differences are statistically significant is not sufficient.

There are multiple forms of the rank-sum test, and which form to use is a complex choice (Helsel and Hirsch 1992). We recommend that this test not be applied by persons lacking formal training in statistics. The rank-sum test determines whether one group of measurements tends to produce higher values than another group of measurements. In other words, the test determines if both groups of data are from the same population. The groups might represent different lake types, different periods of time, different seasons, etc.

For comparing more than two independent groups of data points, the Kruskal-Wallis test is often used. It can be computed by an exact method used for small sample sizes (typically five or fewer samples per group), a large-sample (chi-square) approximation, or by ranking the data and performing a parametric test on the resulting ranks (Helsel and Hirsch 1992). The latter two

methods only produce valid *p*-values when sample sizes are large. The null hypothesis for all variations of this test specifies that all of the groups have identical data distributions (or have the same median value); the alternate hypothesis specifies that at least one group differs from the others with respect to its data distribution or its median value.

As in a rank-sum test, all observations are combined and ranked from lowest (1) to highest (N). The average group rank ($\overline{R}_j$) is compared to the overall average rank to calculate the test statistic.

And like the-rank sum test, we recommend that this test should be conducted by someone who has had formal training in statistics.

4.5.2 Trend Detection

Trends in water chemistry over time can be evaluated using simple linear regression (SLR) or using a more sophisticated statistical approach such as the non-parametric seasonal Kendall tau test (SKT; Hirsch and Slack 1984) for determining monotonic trends in seasonally varying water quality. The SLR approach is simpler to apply and will sometimes yield nearly identical estimates of slope as the SKT (Sullivan et al. 2003). An example SLR analysis for SO_4^{2-} concentration in Deep Run, a small acid-sensitive stream in Shenandoah National Park, is shown in Figure 4-23. The slope of the regression (-0.57 μeq/yr) indicates the rate of change (in this case a decrease) in the variable (SO_4^{2-}) over time. Whether or not the relationship between SO_4^{2-} concentration and time is statistically significant can be determined using the *p*-statistic. We recommend a *p*-value of less than or equal to 0.05 as the benchmark for determining statistical significance. The r^2-statistic can be used to determine the percent of the variation in SO_4^{2-} concentration that is explained by the variable time.

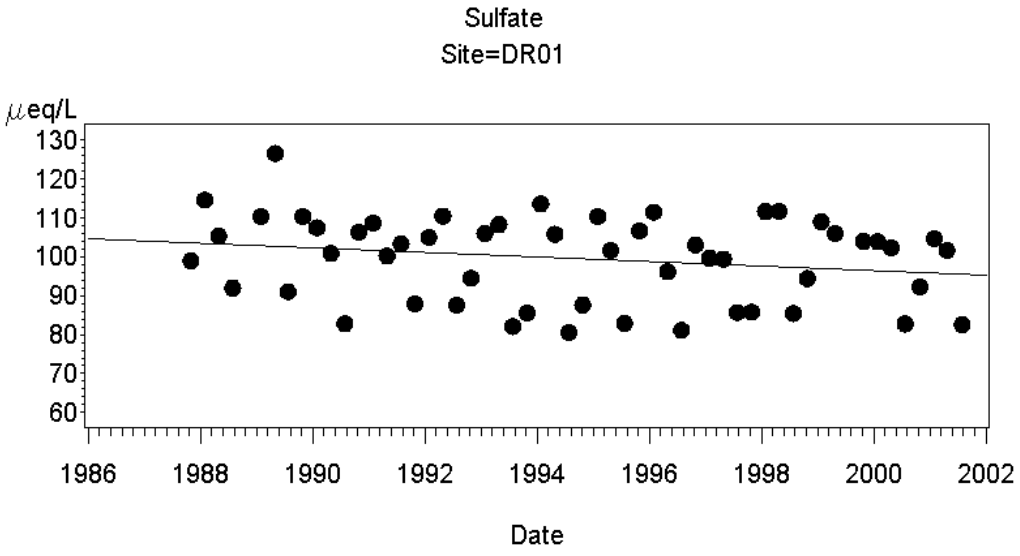

Figure 4-23. Plot, with regression line, of SO_4^{2-} concentration in Deep Run, Shenandoah National Park, over the period of monitoring record. (Source: Sullivan et al. 2003.)

If the regression relationship is statistically significant at $p \leq 0.05$, the next step is to conduct a test to determine whether the slope of that relationship is significantly different from zero. If it is determined that the slope is statistically either greater or less than zero, then it can be concluded that the parameter in question is truly increasing or decreasing over time.

The test statistic for determine if the slope is different from zero is expressed as:

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}} \qquad (14)$$

where r is the correlation coefficient of the regression and n is the number of data points.

The null hypothesis that the slope of the regression equals zero is rejected if the absolute value of t is higher than t_{crit}, where t_{crit} is the point on the Student's t-distribution with (n-2) degrees of freedom and with a probability of exceedance of α/2. The Student's t-distribution for that confidence level is given in Table 4-7.

Table 4-7. Values of *t.* (Source: Steel and Torrie 1980.)

df	Probability (α=0.05) of a numerically larger value of *t*	df	Probability (α=0.05) of a numerically larger value of *t*
1	12.706	18	2.101
2	4.303	19	2.093
3	3.182	20	2.086
4	2776	21	2.080
5	2.571	22	2.074
6	2.447	23	20.69
7	2.365	24	2.064
8	2.306	25	2.060
9	2.262	26	2.056
10	2.228	27	2.052
11	2.201	28	2.048
12	2.179	29	2.045
13	2.160	30	2.042
14	2.145	40	2.021
15	2.131	60	2.000
16	2.120	120	1.980
17	2.110	∞	1.960
Probability (α/2=0.025) of a larger positive value of t			

In general, we recommend that FS staff conduct SLR analyses as the routine approach to determine changes in water chemistry over time. If it is deemed necessary to obtain a more rigorous trends estimate, then a more complex statistical analysis can be conducted.

In some cases, there may be a visually obvious change in the slope of the data points in the midst of the time series. For example, some water bodies in the United States experienced acidification

during the 1980s and 1990s, but recovery (decreasing ANC) is evident after about the late 1990s. In such a situation, it can be helpful to visually split the data into two time periods (acidification and recovery) and perform a SLR separately on each time period to determine if there has been a change over time.

Simple linear regression analysis is a good first approach for analyzing temporal data and can be performed using common spreadsheet and statistical software. However, regression analysis is sensitive to data normality and other assumptions and is often not the most robust method to quantify the statistical significance of temporal trends. Loftis et al. (1989) evaluated a number of different trend detection methods under a number of different conditions and found that there is no one method that outperforms the others under all conditions. However, they found that the most powerful methods under most conditions were non-parametric tests that looked at the correlation between rank order and time. For annual data, the Kendall-tau (also called Mann-Kendall test for trend) test was generally the most powerful. For seasonal data, the SKT or seasonal analysis of covariance (ANCOVA) on ranks were the most powerful tests. These non-parametric tests performed about as well as parametric tests with normal data and outperformed them when the data were non-normal. We recommend their use for testing the statistical significance of temporal trends when a more rigorous statistical application than SLR is desired. Note that these tests are the most powerful for testing whether or not water quality is changing over time; they do not, however, quantify the magnitude of change.

Trend detection in surface waters can be conducted using complex statistical tests that attempt to adjust for natural variation related to seasonality and variations in discharge. Different methods have been developed for assessing trends or the effect of time, with proper method selection dependent on assumptions related to the distribution and independence of the data and on whether change occurs monotonically or as a step change. It is also possible to construct complicated models that incorporate flow, temperature, and/or other environmental factors in addition to the time variable to quantify trends. These regression-based approaches, however, are sensitive to issues of data normality and independence. In addition, different methods are available for assessing trends for individual streams or lakes, as well as for assessing regional trends associated with classes or populations of streams and lakes. These methods are described by Helsel and Hirsch (1992), Stoddard et al. (2003), and Irwin (2008). In general, we do not recommend application of such tests for routine water chemistry assessment in the FS ARM program.

If a more complicated test is to be conducted, trends in time-series data collected at quarterly, monthly, or weekly intervals for individual surface waters are most commonly assessed using the SKT developed and described by Helsel and Hirsch (1992). The non-parametric SKT is based on the correlation between the ranks of the dependent variable (concentration) and an evenly spaced time interval. The SKT is popular because of its relative simplicity compared to other approaches and minimal data assumptions. It is appropriate for data showing seasonal cycles, and it is robust with respect to issues of normality, missing or censored data, and serial correlation. It can be applied on unadjusted chemical concentration data or on residuals from ordinary least-squares regression of concentration on estimated discharge, thus accounting for the effects of changes in discharge. An alternative approach is to remove the seasonality from the dataset (for example by subsetting to only include samples collected during summer base-flow) and then analyzing the subsetted data as an annual, rather than seasonal, dataset. The SKT provides the significance and

direction of any trend. A different test based on the median of the set of slopes calculated for all possible pairs of points in the time series is commonly applied to calculate the slope or rate of change associated with the overall trend (Sen 1968).

Regional trends associated with classes or populations of surface waters can be assessed using a median trend test (SAS 1988, Altman et al. 2000, Stoddard et al. 2003). This test, a meta-analysis, is based on the median of slopes obtained for linear regression of concentrations with time for the individual surface waters in the class or population of concern. Regional trend significance is tested by estimating confidence intervals around the median values, with median slopes significantly different from zero taken to indicate regional increasing trends (positive median slope) or regional decreasing trends (negative median slope). This test allows determination of trends for a resource management or geographic unit as a whole. It can be applied to include multiple predictor variables in the regression models, thus accounting for other factors, such as discharge, in addition to time.

4.5.3 Statistical Power

For purposes of inferring differences or change, data analysts and resource managers will have to make decisions concerning acceptable error levels, and such decisions should be based on the allocation of risk, given the relative importance of ensuring resource protection compared to the cost of potentially unnecessary responses. These decisions are typically made in terms of statistical power and significance. Statistical power refers to the avoidance of false negatives, or wrongly concluding that a change or impact has not occurred when, in fact, a change or impact has occurred (type II error). Statistical significance refers to the avoidance of false positives, or wrongly concluding that a change or impact has occurred when, in fact, no change or impact has occurred (type I error).

Statistical power and significance levels are stated as percentages. A statistical power level of 90%, for example, would mean that, 90% of the time, an effect of a specified size (whatever it is) will be correctly identified. A statistical significance level of 5%, for example, would mean that only 5% of the time would an identified effect be incorrectly identified (or that there is a 95% probability that the identified effect is, in fact, correctly identified). Ideally, statistical power and significance objectives are established as part of the initial study or monitoring program design and not during data analysis. They will be stated in terms of the project's ability to detect a specific effect or a specified trend (a magnitude of change within a specified time period).

The ability of a monitoring program to detect temporal trends is a function of a number of different factors. For detecting a trend at a single site, for a specified type I and type II error rate (for example, a 95% confidence level), trend detection is determined by the magnitude of the actual trend you wish to detect, how long you have to detect the trend, and the variability of the water quality parameter being assessed. For water chemistry assessment, trend magnitude is usually expressed as a percent change in the variable of interest per year. Evaluation of the magnitude of the trend one wishes to be able to document is usually based on program objectives related to ecologically significant changes in water quality values within a time period of policy relevance. There is no standard procedure for this, but in very general terms, one typically wishes to be able to document, for acid-base chemistry monitoring, a change in ANC of at least 1-2 μeq/L/yr over a period of about 10 years.

The ability to detect a trend is also related to how long a monitoring program is continued. Small trends that are not detectable with 5 years of data can be obvious in 100 years. It requires a very large trend magnitude for a trend to be detectable in a short amount of time. Trend detection is dependent on water-quality parameter variability both in terms of analytical precision and natural (e.g., climatic) temporal variability. For a given trend magnitude of interest, it will take longer to detect a trend in a noisy, highly varying indicator than in a more precise and temporally stable indicator. Similarly, to detect a trend over a fixed amount of time, smaller trend magnitudes can be detected in stable indicators more readily than in noisy indicators.

For detecting regional trends (average trend across a number of sites), the number of regional sample sites is also an important factor. Regional trend detection ability increases with the number of sample sites. Thus, you can enhance your ability to document a trend by: 1) monitoring over a longer time period, 2) reducing short-term variability due to seasonality, episodes, or data quality, and/or 3) monitoring more sites. Larger trends will be easier to document than smaller trends.

4.6 REPORT DATA IN STANDARDIZED FORMATS

Data is managed in NRM Air. Specific requirements for incorporation into NRM Air are detailed in the NRM Air User Guide available at <http://fsweb.nris.fs.fed.us/products/air/documentation.shtml>.

4.7 SUMMARY AND CONCLUSIONS

It is not possible to specify a routine set of data analyses that should be conducted for every water chemistry data set assembled by the FS ARM program. Decisions regarding analysis of the data will be influenced by the distribution of the collected data and the objectives of the particular investigation. Nevertheless, we do suggest many of the kinds of analyses that should be considered for the datasets typically collected within the FS ARM program.

The first step in analyzing a water chemistry dataset is to evaluate the overall quality of the data. In the process of evaluating data quality, it is often possible to identify data that are incorrect and sometimes to reanalyze or otherwise correct the identified errors. Next is a series of steps to prepare the raw data for graphical and statistical analysis. This involves applying procedures to deal with such potentially confounding issues as censored data, outliers, missing values, multiple observations, and treatment of zeros and negative values. Recommendations are provided.

A range of kinds of exploratory data analyses are illustrated with examples from the published literature. These include suggestions regarding how to subset the data in order to increase data analysis efficiency. Specific analyses suited to various study objectives are provided as examples.

Finally, the role of formal statistical analysis is considered. Frequently, such analyses will require the assistance of someone with formal training in statistics. In many cases, however, formal statistical analyses are not required. Much can be gained via routine exploratory analyses and application of simple graphical and analytical procedures.

SECTION 5.
FIELD SAMPLING PROTOCOLS FOR AQUATIC BIOTA

A.T. Herlihy and T.J. Sullivan

5.1 BACKGROUND

Aquatic invertebrates can be good indicators of water quality and can provide documentation for ecological effects of changing water quality. Bottom dwelling (benthic) invertebrates have been used extensively to assess biological conditions in streams. Benthic macroinvertebrates can also be used in assessing lake biology, but their use for this purpose has not been common in the United States. More commonly in this country, biological conditions in the epilimnion of thermally stratified lakes are evaluated using zooplankton. Both stream macroinvertebrate and lake zooplankton data can provide useful information to reveal some of the ecological effects that result from atmospheric deposition, and subsequent alterations of surface water chemistry.

5.1.1 Lake Zooplankton

Zooplankton are important components of the biological community of lakes. There may be as many as 200 species or more that occur within lakes in a given region. They constitute key portions of the aquatic food web, and play a major role in transferring energy from the primary producers (mainly phytoplankton) to predatory invertebrates and to fish and other vertebrates.

Individual zooplankton species and the zooplankton community as a whole respond to a number of environmental stressors. These include acidification, nutrient enrichment, sedimentation, fish stocking, and habitat manipulation. Effects of these environmental stressors can sometimes be revealed by evaluating changes in the presence/absence of known regional indicator species, overall species composition, biomass, body size distribution, and/or the structure of the food web.

Lake zooplankton include crustaceans, rotifers, pelagic insect larvae, and aquatic mites. Many species, especially crustaceans and rotifers, are known to be sensitive to changes in chemistry (cf. Gerritsen et al. 1998, Melack et al. 1989, Sullivan et al. 2006). Nevertheless, the species composition and trophic structure of zooplankton communities are controlled by multiple factors, of which aquatic acid-base chemistry is only one. Populations of zooplankton can be strongly influenced by changes at both lower and higher trophic levels because zooplankton are sensitive to changes in the distribution and abundance of both algae and predators. Predation occurs by planktonic predators and by fish. Thus, the presence or absence of plankton-feeding fish can have

a large influence on the presence, abundance, and body size of various species of zooplankton. It can therefore be difficult to infer the cause(s) of observed changes in the zooplankton community over time unless data are also available regarding the status of the fish populations in the lake(s) under study. Therefore, if fish population data are available for lakes within a given forest or wilderness, it can be helpful to select lakes for zooplankton sampling that also have data on fish. In addition, both intra- and inter-annual variability in zooplankton species distributions can be high. In general, the greatest development of zooplankton occurs from June to mid-October, and mid-summer is considered to be a relatively stable period for zooplankton monitoring.

5.1.2 Stream Macroinvertebrates

Benthic macroinvertebrates inhabit the bottom substrates of streams and provide a good indication of overall biological condition (Kerans and Karr 1994, Barbour et al. 1999, Reynoldson et al. 2001, Klemm et al. 2002 and 2003, Clarke et al. 2003, Bailey et al. 2004, Griffith et al. 2005). Monitoring these assemblages is useful in assessing the status of the water body and investigating the possibility of trends over time in ecological condition. Benthic macroinvertebrate species respond to a variety of stressors in different ways, and it is often possible to determine the type of stress that has affected the macroinvertebrate assemblage (e.g., Klemm et al. 1990).

Because many stream macroinvertebrates have life cycles of a year or more and are relatively immobile, macroinvertebrate assemblage structure is a function of present and past conditions and provides an integration of the variability that typically occurs in stream condition with season and with changing hydrology (Barbour et al. 1999, Peck et al. 2006). For general bioassessment purposes, stream macroinvertebrates are typically sampled in summer. However, for assessing acidic deposition impacts, we generally recommend sampling during spring base-flow as that is the season of maximum impact (lowest ANC/pH). At high elevation in regions that experience substantial snowpack development, spring sampling may not be feasible; at such locations, summer sampling is recommended.

The insect order Ephemeroptera (mayflies) is an excellent indicator taxa for acidification effects. However, it comprises just one order of benthic invertebrates, and there are many other taxonomic groups that make up the benthic stream community that should be considered for a full biological assessment. These other taxa also contribute a great deal of information about stream condition. In addition to acidification impacts, macroinvertebrates are excellent indicators of substrate alteration (e.g., sedimentation), nutrient enrichment, metal pollution, and habitat alteration. In the EPA National Wadeable Stream Assessment, the greatest risk of poor stream macroinvertebrate condition was found in streams with excess sediment (enhanced erosion) and/or with nutrient enrichment (Van Sickle and Paulsen 2008).

Some studies have found that acidified streams host fewer invertebrate taxa than streams with higher acid neutralizing capacity (ANC) and pH (e.g., Feldman and Connor 1992, Kaufmann et al. 1999, Sullivan et al. 2003). This is especially true for mayflies (order Ephemeroptera) and, to a lesser extent, caddisflies (order Tricoptera) and stoneflies (order Plecoptera). Aquatic insect status is sometimes evaluated on the basis of these three orders using what is known as the EPT (Ephemeroptera-Plecoptera-Tricoptera) Index (EPT taxa richness).

5.2 STUDY DESIGN

Aquatic invertebrates can be collected and analyzed as part of lake or stream characterization studies, synoptic surveys, long-term monitoring, or used to augment model projections of future chemical conditions. As is described in detail in the Water Chemistry Field Sampling Protocols (Section 1), the design of an aquatic biota study should be a function of the study purpose and questions being asked. Some example approaches for biological characterization or monitoring are outlined in Table 5-1. Each example approach is tied to a specific purpose. The reader is also referred to the discussion with examples, provided in Section 1.1.2, Study Purpose and Objectives.

Table 5-1. Example approaches for FS ARM program biological sampling, tied to the purpose of the field study.

Purpose	Approach
1. Determine spatial patterns in biological assemblages relative to chemical and/or deposition gradients.	a. Conduct survey of lake zooplankton or stream benthic macroinvertebrate communities in waters that exhibit varying water chemistry and/or that receive varying atmospheric deposition levels.
2. Quantify long-term changes over time in biology.	a. Conduct trends analysis in species richness. b. Compare trends in biota with trends in water chemistry.
3. Determine extent to which air pollution is affecting water resources.	a. Characterize biology of multiple lakes or streams expected to be sensitive. b. Plot changes in species richness versus changes in ANC or NO_3^- concentration.

Evaluation of the status of the aquatic invertebrate biota can be used, along with assessment of chemical status and/or change, to estimate the impacts of nutrient enrichment, acidification, and various kinds of habitat disturbances. In general, changes in aquatic chemistry are more easily documented than are changes in aquatic biology. Nevertheless, the concerns on the part of land managers and the public regarding changes in chemistry are fundamentally rooted in widespread concerns about protecting resources against biological damage. The ultimate purpose of studying or monitoring aquatic chemistry on federal lands is often mainly to aid in the protection of biological resources. Also, biota reflect conditions over longer time periods than the single point in time represented by a water sample. Therefore, there is additional power in the inclusion of a biological component in the investigation or monitoring of chemical conditions.

For evaluation of biological responses to acidification, study designs most commonly include 1) documentation of relationships between water chemistry (usually ANC; also pH, Al_i, or NO_3^- concentration) and macroinvertebrate taxonomic composition across sites (lakes or stream reaches) within a reasonably small area (i.e., National Forest or wilderness), or 2) evaluation of changes over time in water chemistry and macroinvertebrate taxonomic composition. Such studies often focus on stream aquatic insects in the orders Ephemeroptera (mayflies), Tricoptera (caddisflies), and Plecoptera (stoneflies). For a more rapid and less expensive stream assessment, the analysis can be restricted to only the order Ephemeroptera, which is the order most susceptible to acidity. However there may be no mayfly species present if the ANC in a given study stream is especially low (near or below zero). For lakes, acidification studies typically focus on zooplankton, mainly crustaceans and rotifers. We recommend, if available funding allows, that

such studies be conducted on a suite of acid-sensitive lakes or streams within a given National Forest or region, if lake or stream acidification is believed to be an important issue. If, after a prolonged period of monitoring, a trend is indeed documented, a decision will have to be made regarding whether or not to continue monitoring. In general, we recommend continued monitoring. Data collected will help to identify long-term and future interactions between climate change and air pollution effects.

For a more complete assessment of biological condition, we recommend use of an Index of Biotic Integrity (IBI). Such an index provides a more complete assessment of biological condition than the rapid single order or EPT assessments discussed above. An IBI can provide a more rigorous assessment of biological conditions and response to multiple stressors where in-depth study is warranted as it requires compiling available information about the feeding groups, pollution tolerance, and habits of the taxa in the study waters. It is somewhat more expensive, however, and requires more specialized taxonomic and autoecological (individual species ecology) expertise. For this reason, we do not recommend implementation of an IBI as a routine procedure on FS lands.

5.3 SITE SELECTION

In general, criteria for site selection for the purpose of conducting a biological assessment are the same as criteria for site selection for the purpose of investigating aquatic chemistry: random versus non-random site selection, and the establishment and documentation of the stream reach(es) and/or lake(s) to sample. See the discussion in Section 1.3 in the Water Chemistry Field Sampling Protocols for a more complete discussion of these issues.

By necessity in many cases, the sites included in a biological study will be only a subset of the sites included in the chemical investigation. It is important to choose this subset wisely. In general, one may wish to avoid sites having substantial disturbance other than atmospheric deposition that may influence the acid-base chemistry or nutrient status of drainage waters (i.e., geological sulfur, forest fire, insect infestation, tree disease, large windthrow, or other substantial disturbance). In general, one should include biological characterization for monitoring sites that are expected to be sensitive to the stresses of interest. For acidification studies, these are usually the lakes or streams having ANC less than about 50 to 100 μeq/L. For nutrient enrichment studies, these are usually N-limited water bodies. Short of direct experimentation, it is difficult to predict which water bodies might be N-limited. The Redfield ratio, based on the molar N:P ratio in phytoplankton, suggested that water bodies might be N-limited if the molar N:P ratio was less than 16. Subsequently, experimental studies suggested higher cutoffs. Recent research suggests that N-limited lakes generally include lakes having the molar ratio of N:P less than about 44 (Guildford and Hecky 2000, Schindler et al. 2008, Elser et al. 2009).

5.3.1 LAYING OUT THE SUPPORT REACH FOR STREAM MACROINVERTEBRATE SAMPLING

Unlike chemistry, which can be measured at one point, characterizing stream biota requires sampling a length of a stream to capture the range of available habitat. There are a large number of field protocols for sampling stream macroinvertebrates for bioassessment, and they all specify

collecting a number of different net samples (kick, Hess, or Surber) from different places along the stream sample reach and compositing them into either a single composite sample or habitat type (e.g., riffle) composite sample. The procedures that we recommend (summarized here) are based on the procedures developed by the EPA for the Environmental Monitoring and Assessment Program Surface Water (EMAP-SW) sampling program (Peck et al. 2006). These protocols have been used in studies of streams across the entire U.S., and they work in a wide variety of stream types. They were also designed to be implemented by different types of field crews and require a minimum of field decision-making.

At each selected stream sampling location (called the X-site) the support reach (the length of stream to be sampled at the sampling location) must be laid out. The support reach must be sufficiently long to represent the biological community being sampled. Based on several studies (Robison 1998, Li et al. 2001, Reynolds et al. 2003), a support reach with a length of 40 times the average wetted channel width measured near the X-site is sufficient for almost all sampling. The support reach is established about the X-site using the procedures described below. Field staff should reconnoiter the support reach to make sure it is clear of obstacles that would prohibit sampling and data collection activities. Record the channel width used to determine the support reach length and identify the support reach length upstream and downstream of the sample-site. Figure 5-1 illustrates the principal features of a hypothetical support reach, including the location of 11 cross-section transects from which the samples will be collected.

To lay out the support reach (from Peck et al. 2006):

1. Use a surveyor's rod or tape measure to determine the wetted width of the channel at three to five places considered to be of *typical wetted width* within approximately five channel widths upstream and downstream from the X-site. Average the readings together and round to the nearest 0.5 m. If the average width is less than 3.5 m, use 150 m as a minimum support reach length. Record this width on the Stream Verification Form.

   For channels with *interrupted flow*, estimate the width based on the unvegetated width of the channel (again, with a 150 m minimum).

2. Check the condition of the stream upstream and downstream of the X-site by having one team member go upstream and one downstream. Each person goes until he/she has visited the candidate sample reach to a distance of 20 times the average channel width in each direction (equal to one-half the support reach length, but a minimum of 75 m) determined in step 1 from the X-site.

   For example, if the support reach length is determined to be 150 m, each person would go 75 m from the X-site to lay out the reach boundaries.

3. Determine if the support reach needs to be adjusted about the X-site due to confluences with higher order streams (downstream), lower order streams (upstream), impoundments (lakes, reservoirs, ponds), physical barriers (e.g., falls, cliffs), or because of access restrictions to a portion of the initially-determined support reach.

   If such a confluence, barrier, or access restriction is present, note the distance and flag the confluence, barrier, or the limit of access as the endpoint of the reach. Move the other endpoint of the support reach an equivalent distance away from the X-site. *The X-site must*

*still be **within the support reach** after adjustment*. The total support reach length does not change, but the support reach is no longer centered on the X-site.

Note: If the sampling sites are statistically (randomly) selected, **do not** slide the support reach to avoid man-made obstacles such as bridges, culverts, rip-rap, or channelization, or in streams with interrupted flow to obtain more inundated areas to sample. If the sites are not statistically selected, it is recommended to avoid sites that are influenced by substantial human-caused channel disturbance.

4. Starting back at the X-site (or the new midpoint of the reach if it had to be adjusted as described in step 3), measure a distance of 20 channel widths downstream on one side of the stream using a tape measure. Be careful not to "cut corners." Enter the channel to make measurements only when necessary to avoid disturbing the stream channel before sampling activities. This endpoint is the downstream end of the support reach, and is flagged as the location of transect A.

5. Using the tape measure, measure 1/10 (4 channel widths in big streams or 15 m in small streams) of the required stream length upstream from the start point (transect A). Flag this spot as the next cross-section or transect (transect B). For transect A, roll one die to determine if it is a left (L), center (C), or right (R) sampling point (facing downstream) for collecting benthic macroinvertebrate samples. A die roll of 1 or 2 indicates L, 3 or 4 indicates C, and 5 or 6 indicates R—or use a digital wristwatch and glance at the last digit to determine the sampling point (1-3=L, 4-6=C, 7-9=R). Mark L, C, or R on the transect flagging.

6. Proceed upstream with the tape measure and flag the positions of nine additional transects (sequentially labeled "C" through "K") as you move upstream at intervals equal to 1/10 of the reach length. Assign sampling spots to each transect in order as L, C, or R from the first random selection.

   For example, if the sampling spot assigned to transect A was *C*, transect B is assigned *R*, transect C is *L*, transect D is *C*, etc.

There are some conditions that may require adjusting the end-points of the support reach about the X-site (i.e., the support reach will be shifted either upstream or downstream so that the X-site will no longer be located at the midpoint of the support reach) to avoid features we do not wish to (or physically cannot) sample across. The full length of the support reach should be of the same stream order as the X-site. Do not extend the support reach upstream if the stream order decreases or downstream if the stream order increases. If you encounter an impoundment such as a lake, reservoir, or pond or an impassible barrier (e.g., a waterfall or a cliff) while laying out the support reach, adjust the reach such that the barrier is at one end. Adjusting or sliding the support reach involves noting the distance of the confluence, barrier, or other restriction from the X-site, flagging the confluence, impoundment, or barrier as the endpoint of the reach, and adding the distance to the other end of the reach such that the total support reach length remains the same though it is no longer centered about the X-site. In cases where you are denied access permission to a portion of the support reach, you can adjust the reach to make it entirely accessible; use the point of access restriction as the endpoint of the reach.

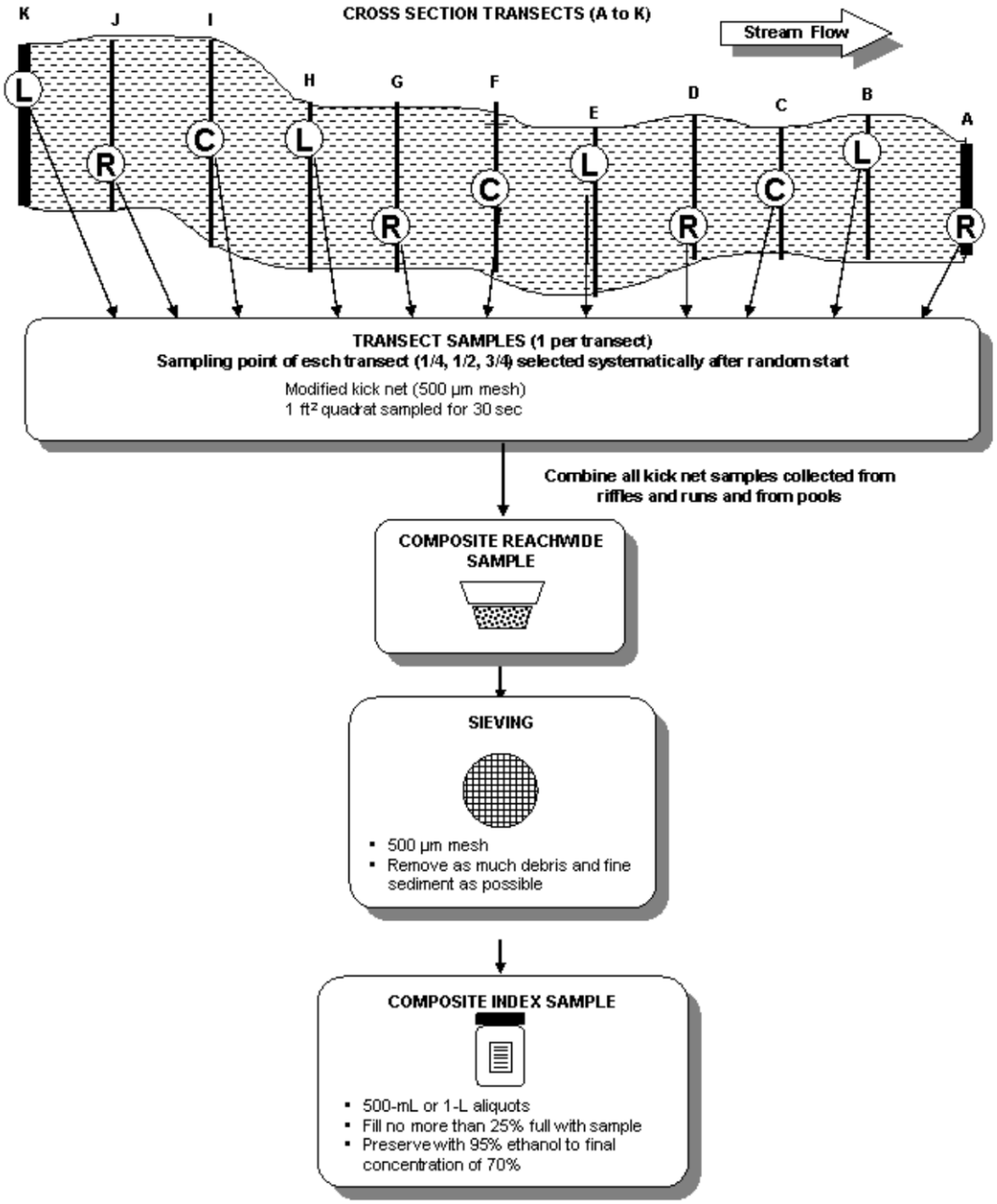

Figure 5-1. Equipment, supplies, and sequence for collecting benthic macroinvertebrate samples. (Source: Peck et al. 2006.)

5.3.2 Lake Selection for Zooplankton Sampling

Acidification effects on individual species of zooplankton and on the zooplankton community in general may occur across a rather wide spectrum of lake pH, ANC, and Al_i concentrations. Effects are usually observable at ANC values below about 50 to 100 µeq/L and pH below about 6.0 to 6.5. Such ANC and pH cutoff values generally correspond with Al_i concentrations near 2

μM. Lakes having pH below 6.0 and/or ANC below about 50 to 100 μeq/L have an increased likelihood of having Al_i above this general response threshold. Nevertheless, it is possible that effects on zooplankton also occur at somewhat higher pH and ANC (and lower Al_i) values.

A complicating factor relates to the influence of lake size and watershed area on lake biology. In general, smaller lakes in smaller watersheds are more likely to be lower in pH and ANC and to have less diverse zooplankton communities than larger lakes in larger watersheds. To some degree (often to a large degree) this relationship is controlled by the effects of lake chemistry on biota; but additionally, smaller lakes in smaller watersheds might be expected to have less diverse biotic assemblages than larger lakes in larger watersheds as a consequence of their physical simplicity and reduced number of available niches. Thus, the often-observed patterns of changing zooplankton species composition and taxonomic richness among lakes are likely only partly due to water acid-base chemistry. This makes it difficult to tease out the effects of changing water chemistry when evaluating changes over time in the biological community. For these and other reasons, it is important to give careful consideration to site selection for zooplankton monitoring or characterization. It can be helpful to include multiple lakes in the study, selected to cover a range of acid-base chemistry and perhaps within rather narrow windows regarding lake and watershed areas. In addition, it can be helpful to study the zooplankton communities of lakes that are also being studied with regard to their fish and algal communities; this may allow an improved opportunity to sort out what may be a multitude of factors that simultaneously influence the lake zooplankton community.

Biological effects are more likely to be observable in lakes that have relatively low ANC (< 50 μeq/L) and pH (< 6.0). Nevertheless, having lakes in the study with somewhat higher ANC and pH is also important, especially for evaluating effects on taxonomic richness or the presence/absence of particular indicator species. This will help to make sure that a sufficient range of response occurs in the dataset so as to increase the likelihood of being able to document what may be a very “noisy” relationship. Further discussion of the interpretation of zooplankton data is provided in Section 5.10, Interpretation. It is likely that study of the zooplankton community will be less helpful for evaluation of effects related to nutrient N enrichment: such effects on zooplankton communities have not been as well documented as the effects from acidification.

Zooplankton samples should generally be collected at the lake index location for water chemistry sampling. This should be the deepest part of the lake. The index location is described in Section 1.3.1, Where to Sample. It is important to collect the zooplankton tows in the deepest part of the lake because, especially in mid- to late-summer, the size of the cold-water hypolimnion can be reduced substantially. Missing the deep spot can cause exclusion of individuals occupying the cold water stratum, thereby confounding interpretation of the true zooplankton assemblage in the lake.

Zooplankton tows can be compromised by high algal production and/or high DOC because of algal or organic particle fouling of the net. This problem can be partially ameliorated by using a reducing collar. Alternatively, if fouling is a major problem, zooplankton can be collected in integrated water column samples using a hose and pump system.

5.4 PRE-TRIP PREPARATION

5.4.1 EQUIPMENT AND SUPPLIES

Table 5-2 shows the checklist of equipment and supplies required to complete the collection of benthic macroinvertebrates from streams. Use this checklist to ensure that equipment and supplies are organized and available at the stream site in order to conduct the activities efficiently. Similarly, Table 5-3 provides the checklist for zooplankton sampling. These checklists can also be found in Appendix H.

Table 5-2. Equipment and supplies for benthic macroinvertebrates. (Source: Peck et al. 2006.)

Quantity	Item	✓
1	Modified kick net (D-frame with 500 µm mesh) and 4-ft handle	
	Spare net(s) and/or spare bucket assembly for end of net	
1	Watch with timer or a stopwatch	
2	Buckets, plastic, 8- to 10-qt capacity (collapsible for back country)	
1	Sieve with 500 µm mesh openings or sieve-bottomed bucket, 500 µm mesh openings	
2 pr.	Watchmakers' forceps (straight and curved)	
1	Wash bottle, 1-L capacity, labeled *STREAM WATER*	
1	Small spatula, spoon, or scoop to transfer sample	
1	Funnel with large bore spout	
4 to 6 Each	Sample jars, HDPE plastic, with leakproof screw caps, 500-ml and/or 1-L capacity, suitable for use with ethanol	
2 gal	95% ethanol in a proper container (smaller amounts can be carried in for back country work or ethanol can be added to sample containers at the vehicle after returning from the field)	
2 pr.	Rubber gloves suitable for use with ethanol	
1	Cooler (with suitable absorbent material) for transporting ethanol and samples in the vehicle	
2	Preprinted benthic sample labels with sample ID numbers	
4	Preprinted benthic sample labels without sample ID numbers	
6	Blank labels on waterproof paper for placing inside of jars	
1	Sample Collection Form for the site(s)	
	Soft (#2) lead pencils	
	Fine-tip indelible markers	
1 pkg.	Clear tape strips	
4 rolls	Plastic electrical tape	
1	Knife, pocket, with at least two blades	
1	Scissors	
1	Pocket-sized field notebook (optional)	
1 pkg.	Kim wipes in small resealable plastic bag	
1 copy	Field operations and methods manual	
1 set	Laminated sheets of procedure tables and/or quick reference guides for collecting benthic macroinvertebrates	

Table 5-3. Equipment and supplies for collecting zooplankton samples. (Source: U.S. EPA 2007.)

Quatity[1]	Item	✓
2	Wisconsin fine mesh (80 µm[2]) net with attached collection bucket	
2	Wisconsin coarse mesh (243 µm[2]) net with attached collection bucket	
2	Sample line, marked at 0.5 m increments	
2	Secchi disk with cable	
2/site+	125 ml wide-mouth polyethelene sample jars (two per site, plus additional jars for replicates and other back-up sampling)	
1	Squirt bottle with DIW	
	95% ethanol	
2/site+	CO_2 tablets	
1/site+	500 ml wide-mouth container	
2	Two lids converted to form strainers (one with 80 µm, one with 243 µm mesh), made by drilling two holes in each lid and gluing a piece of the netting to the inside of the lid using silicone glue	
2/site+	Zipper-lock plastic bag	
	Clear tape for covering labels	
	Electrical tape	
1/site+	Zooplankton Sample Data Form	
	Pencils and permanent markers	
	Mild (10%) bleach solution for cleaning net and strainer lids between lakes and backwashing net (with a garden hose) after use	

[1] It is advisable to include some extras beyond what is needed for the number of sites to be sampled.
[2] These two mesh sizes (80 and 243 µm) are general guidelines. Other sizes could be used.

5.4.2 Equipment Cleaning Protocols

Field survey personnel and/or their equipment can serve to transport pathogens and invasive species among water bodies. Field personnel should take appropriate precautions to minimize or eliminate this risk. General equipment cleaning guidelines are provided below. In addition, field staff should consult with local experts to determine if local conditions require any additional specific precautions. Between sample sites and at the duty station subsequent to field sampling, all gear that was exposed to stream or lake water should be thoroughly cleaned. Clothing, skin, and fingernails should also be cleaned. Gear should be disinfected using a 10% bleach solution or a solution of an alternative disinfectant and then thoroughly rinsed. Use of a high-pressure hose can be helpful. Gear should then be completely dried or allowed to dry before re-use at another lake or stream.

It is important to follow appropriate safety precautions when working with disinfectant products, especially as concentrated solutions. Such precautions include appropriate ventilation, use of impervious gloves and splash goggles, and access to eye wash stations.

5.5 COLLECTION PROCEDURES

5.5.1 Stream Benthic Macroinvertebrates

The procedures recommended in this protocol for the collection and preservation of stream macroinvertebrates are based largely on the protocol designed by EPA for the EMAP-SW surface water sampling efforts. EMAP-SW protocols for invertebrate sampling are described in detail by Peck et al. (2006).

The EMAP-SW benthic macroinvertebrate protocol was designed to evaluate the biological condition of wadeable streams in the United States for the purpose of detecting stresses on assemblage structure and assessing the relative severity of these stresses (Peck et al. 2006). It is based on the Level III procedure for benthic macroinvertebrates of the EPA Rapid Bioassessment Protocol (Plafkin et al. 1989, Barbour et al. 1999), which has been adopted for use by many states.

Benthic macroinvertebrates are collected at each of 11 equidistant transects spaced throughout the support reach to ensure distribution of individuals among available major habitat types, eliminate individual sampler bias, and provide a comparable and consistent sample from every reach. All 11 transect samples are combined into a single composite sample to characterize the support reach and reduce the cost and effort in processing and analysis (Patil et al. 1994, Barbour et al. 1999, Roth et al. 2002). The number of individual field collections is expected to provide a composite sample having a sufficient number of individuals to characterize the taxonomic composition and relative abundance of the stream assemblage (e.g., Larsen and Herlihy 1998).

Samples are collected from each support reach with a D-frame kick net that can generally be used in the stream by one person (Figure 5-2). Typically, a field crew of two people collects kick net samples for benthic macroinvertebrates. One person typically collects the samples while the second person times the collection of samples and records information on the field data form. However, in swift waters, two people may be needed to collect the samples.

Each kick net sample is collected at each of the 11 cross-section transects (transects *A* through *K*) at an assigned sampling point (*Left*, *Center*, or *Right*) as illustrated in Figure 5-1. Assign the *Left*, *Center*, or *Right* sampling point at transect *A* at random. Once the first sampling point is determined, assign points at successive transects in order (*Left*, *Center*, *Right*). At transects assigned a *Center* sampling point where the stream width is between one and two net widths wide, pick either the *Left* or *Right* sampling point instead. If the stream is only one net width wide at a transect, place the net across the entire stream width and consider the sampling point to be *Center*. If a sampling point is located in water that is too deep or otherwise unsafe to wade, select an alternate sampling point nearby. Never sample at an unsafe location.

Collect a kick net sample at each transect as described below, beginning at the transect that is furthest downstream. ***Never collect benthic macroinvertebrates from a streambed location which you have recently disturbed (e.g., walked in).*** If a replicate composite sample is to be collected, do so at each transect within that support reach before moving upstream to the next transect.

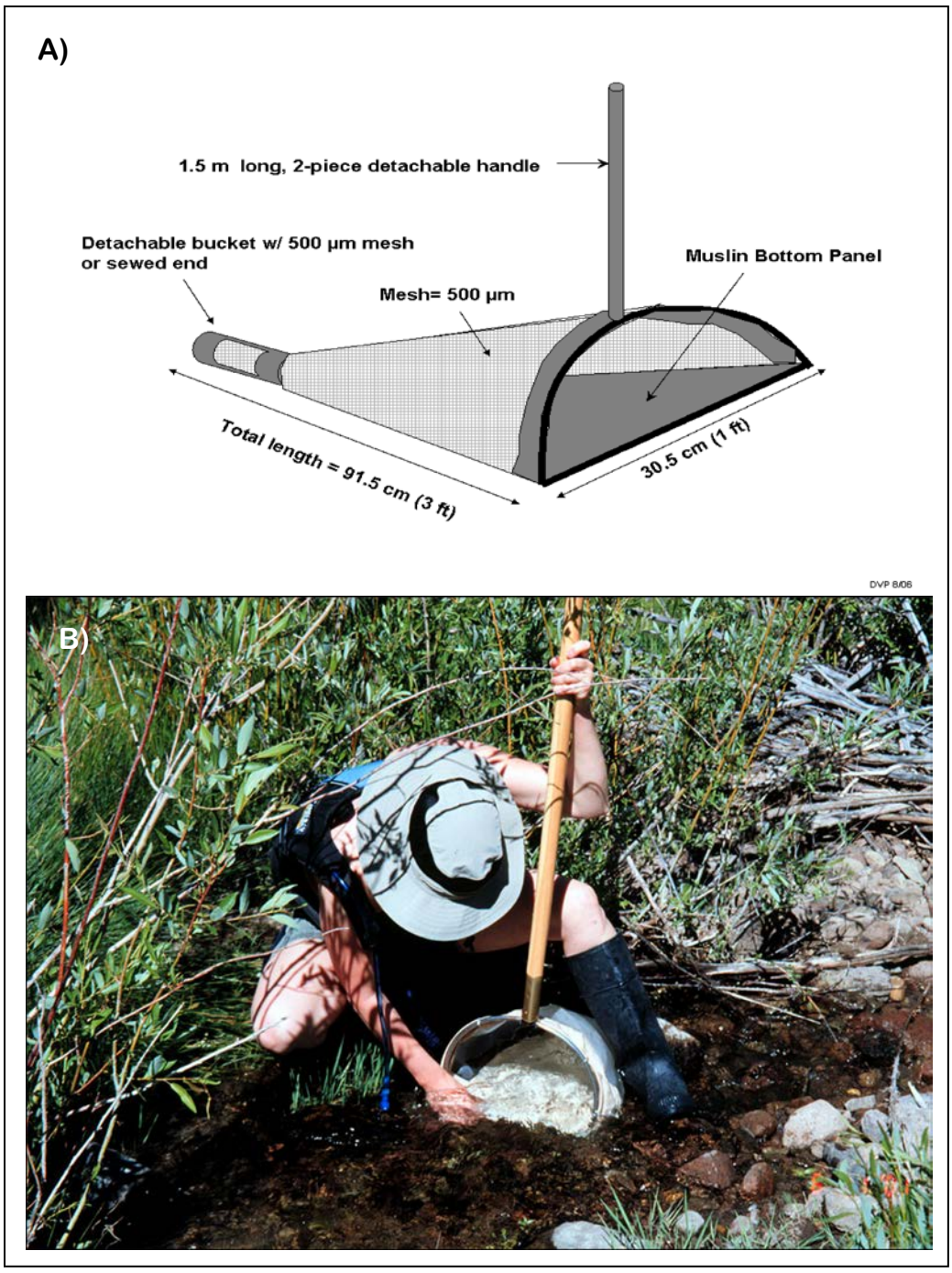

Figure 5-2. Modified D-frame kick net. A): schematic drawing (not drawn to scale). (Source: Peck et al. 2006.) B): photograph showing EMAP crew member sampling a macroinvertebrate transect with modified D-frame net in Utah. (Photo: A. Herlihy.)

At each sampling point, determine if the flowing water or the slack water procedure is to be used based on whether or not there is enough current to extend the net. For each kick net sample, record the dominant substrate type: fine/sand, gravel, coarse substrate (coarse gravel or larger), or other (e.g., bedrock, hardpan, wood, aquatic vegetation, etc.), and the habitat type (pool, glide, riffle, or rapid) on the Sample Collection Form. Note that these substrate types and habitats are

defined in the table. Collect only from the upper 4 to 5 cm (1.5 to 2 in) of the substrate. As you go upstream from transect to transect, combine all the kick net samples into a single container, whether they were collected using the flowing water or slack water procedure.

To collect kick net samples for the reach-wide composite sample (taken from Peck et al. 2006):

1. At each cross-section transect, beginning at the downstream end of the reach with transect *A* (see Figure 5-1), locate the assigned sampling point (Left, Center, or Right as you face downstream) as 25%, 50%, and 75% of the wetted width, respectively. If you cannot collect a sample at the designated point because of deep water or unsafe conditions, relocate the point nearby on the same transect.
2. Attach the handle to the kick net. Make sure that the handle is on tight or the net may twist in a strong current, causing the loss of part of the sample.
3. Determine if there is sufficient current in the area at the sampling point to extend the net fully. If so, use the *flowing water* procedure (go to step 4). If not, use the *slack water* procedure (go to step 10).

   For vegetation-choked sampling points where neither procedure can be used, sweep the net through the vegetation within a 0.09 m^2 (1 ft^2) quadrat for 30 seconds. Place the contents of this hand-swept sample into the sampling container. Go to step 14.

Flowing Water Procedure:

4. With the net opening facing upstream, position the net quickly and securely on the stream bottom to eliminate gaps under the frame. Avoid large rocks that prevent the sampler from seating the net properly on the stream bottom.

   Note: If there is too little water to collect the sample with the kick net, randomly pick up 10 rocks from the riffle and pick and wash the organisms off them into a bucket which is half-full of water.

5. Holding the net in position on the substrate, visually define a rectangular quadrat that is one net width wide and one net width long upstream of the net opening. The area within this quadrat is ~0.09 m^2 (1 ft^2). Alternatively, place a wire frame of the correct dimensions in front of the net to help delineate the quadrat to be sampled.

6. Hold the net in place with your knees. Check the quadrat for heavy organisms, such as mussels and snails. Threatened, endangered, or sensitive (TES) species must be left in place and not removed but they need to be recorded on the field form. Remove any heavy non-TES species from the substrate by hand and place them into the net. Pick up any loose rocks or other larger substrate particles in the quadrat. Use your hands or a small scrub brush to dislodge organisms so that they are washed into the net. Scrub all rocks that are golf ball-sized or larger and which are situated over halfway into the quadrat. Large rocks that are less than halfway into the sampling area are pushed aside. After scrubbing, place the substrate particles outside of the quadrat.

7. Keep holding the sampler securely in position. Start at the upstream end of the quadrat, use your foot and toes to **vigorously** kick the **upper 4 to 5 cm (1.5 to 2 in**) of the remaining

finer substrate within the quadrat for 30 seconds (use a stopwatch). Avoid going too deep into the substrate with your kicking.

Note: For samples located within dense beds of long, filamentous aquatic vegetation (e.g., algae or moss), kicking within the quadrat may not be sufficient to dislodge organisms in the vegetation. Usually, these types of vegetation lie flat against the substrate due to current. Use a knife or scissors to remove *only the vegetation that lies **within** the quadrat* (i.e., not entire strands that are rooted within the quadrat but extend beyond it) and place it into the net.

8. Pull the net up out of the water. Immerse the net in the stream several times to remove fine sediments and to concentrate organisms at the end of the net. Avoid having any water or material enter the mouth of the net during this operation.
9. Go to step 14.

Slack Water Procedure:

10. Visually define a rectangular quadrat that is one net width wide and one net width long at the sampling point. The area within this quadrat is ~0.09 m^2 (1 ft^2). Alternatively, lay a wire frame of the correct dimensions in front of the net at the sampling point to help delineate the quadrat.

    Note: If there is not enough water present to use the net, spend 30 seconds collecting and examining pieces of substrate from about 0.09 m^2 (1 ft^2) of substrate at the sampling point.

11. Inspect the stream bottom within the quadrat for any heavy organisms, such as mussels and snails. Remove these organisms by hand and place them into the net or into a bucket. Pick up any loose rocks or other larger substrate particles within the quadrat and hold them in front of the net. Use your hands (or a scrub brush) to rub any clinging organisms off of rocks or other pieces of larger substrate (especially those covered with algae or other debris) into the net. After scrubbing, place the larger substrate particles outside of the quadrat.
12. Use your foot and toes to **vigorously** kick the **upper 4 to 5 cm (1.5 to 2 in)** of the remaining finer substrate within the quadrat while dragging the net repeatedly through the disturbed area just above the bottom. Continuously move the net forward so that the organisms trapped in the net do not escape. Continue kicking the substrate and moving the net for 30 seconds.

    Note: If there is too little water to use the kick net, **vigorously** stir up the substrate with your gloved hands and use a sieve with 500 μm mesh size to collect the organisms from the water in the same way the net is used in larger pools.

13. After 30 seconds, remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net.

All samples:

14. Invert the net and transfer the contents into a bucket or wide-mouthed container with a lid labeled *REACHWIDE*. Inspect the net for any residual organisms clinging to the net and

deposit them into the *REACHWIDE* container. Use a squirt bottle with stream water and watchmakers' forceps if necessary to remove organisms from the net. Carefully inspect any large objects (such as rocks, sticks, and leaves) in the bucket and wash any organisms found off of the object and into the bucket before discarding the object. Remove as much detritus as possible **without losing any organisms**. Replace the lid on the bucket or container.

15. Determine the **predominant** substrate size/type you observed within the sampling quadrat. Place an *X* in the appropriate substrate type box for the transect on the Benthic Macroinvertebrate Sample Collection Form.

    Note: If there are co-dominant substrate type(s), you may check more than one box; note the co-dominants in the comments section of the form.

    Fine/sand: not gritty (silt/clay/muck < 0.06 mm diam.) to gritty, up to ladybug sized (2 mm diam.).

    Gravel: fine to coarse gravel (ladybug to tennis ball sized; 2 mm to 64 mm diam.).

    Coarse: cobble to boulder (tennis ball to car sized; 64 mm to 4000 mm).

    Other: bedrock (larger than car sized; > 4000 mm), hardpan (firm, consolidated fine substrate), wood of any size, aquatic vegetation, etc. Note type of "other" substrate in comments on field form.

16. Identify the habitat type where the sampling quadrat was located. Place an "X" in the appropriate channel habitat type box for the transect on the Sample Collection Form.

    Pool: still water; low velocity; with smooth, glassy surface; usually deep compared to other parts of the channel.

    GLide: water moving slowly, with smooth, unbroken surface; low turbulence.

    RIffle: water moving with small ripples, waves, and eddies; waves not breaking, and surface tension is not broken; "babbling" or "gurgling" sound.

    RApid: water movement is rapid and turbulent; surface with intermittent "white water" with breaking waves; continuous rushing sound.

17. Proceed upstream to the next transect (including all transects in sequence through transect *K*, the upstream end of the support reach) and repeat steps 1 through 16. Combine all kick net samples within the sample reach into the *REACHWIDE* container.

18. Thoroughly rinse the net with stream water before proceeding to the next sampling location. It is also extremely important that all equipment, including waders, be cleaned between sites to avoid transmission of non-native invasive species.

If the kick net cannot be used to collect a sample at a flowing water sampling point, select the number of rocks necessary to cover approximately 0.09 m^2 (1 ft^2) of the streambed from the area near the sampling point within the area of flowing water. Inspect and remove any organisms found on each rock and place them into the sampling container. If the kick net cannot be used at a slack water habitat due to insufficient depth of water, spend about 30 seconds picking up pieces of substrate from a 0.09 m^2 (1 ft^2) area at the sampling point. Inspect and remove any organisms found on each piece of substrate and place them into the sampling container. At vegetation-

choked sampling points where neither procedure can be used, sweep the net through the vegetation for 30 seconds and place the contents into the sampling container.

5.5.2 Lake Zooplankton

The procedures recommended in this protocol for collection and preservation of lake zooplankton are based largely on the protocols designed by EPA for the National Lakes Survey conducted in 2007 (U.S. EPA 2007) using a "Wisconsin" plankton net. General and detailed procedures are as follows.

Two vertical plankton tow samples are collected at the lake sampling index site location. One sample is typically collected using a fine mesh (typically ~ 50 to 80 μm) and one sample is collected using a coarse mesh (typically ~ 200 to 250 μm) plankton net (Figure 5-3). We recommend a 80 μm Wisconsin net for the fine mesh and a 243 μm Wisconsin net for the coarse mesh. Each net is attached to a collection bucket. Some of the larger species of zooplankton can swim fast enough to avoid being caught in the net. The coarse mesh net optimizes capture of the faster-swimming macrozooplankton because the pressure wave above the net is minimized. The fine mesh net optimizes capture of the microzooplankton. The two samples are collected and analyzed separately.

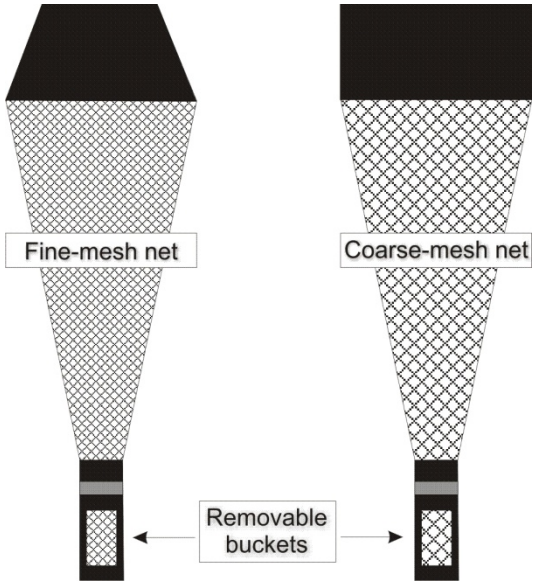

Figure 5-3. Wisconsin net and collection bucket diagram. Some microzooplankton nets have a reducing collar attached. (Source: U.S. EPA 2007.)

Each tow is collected by pulling the sampling apparatus from a depth of about 1 m above the lake bottom to the lake surface. It is important to avoid touching lake sediments with the sampling apparatus, as that can clog the net pores and compromise the integrity of the sample. The net should be raised steadily, but rather slowly (~ 1 ft/sec), to reduce the pressure wave that can build up at the top of the net during retrieval. Some species can detect this wave and swim out of the path of the net. Use of a wide net aperture (30 to 50 cm) can be helpful to avoid missing fast-swimming taxa such as Chaoborus, Leptodora, and Mysis.

If the lake depth at the sampling site is less than 2.0 m and the Secchi disk is visible at the bottom, a second vertical tow is made with each net (fine and coarse mesh) and the original (first) and the second samples are combined. Note that the samples collected using the fine and coarse mesh nets are not combined. If the net or attached collection bucket touches the lake sediment, field personnel should retrieve and rinse the apparatus and repeat the process. Description of the procedures to be followed for sample collection is provided above. If other mesh sizes are used instead of those that we recommend, it is important to standardize these mesh sizes such that there is consistency among sites and over time in the sampling program.

To collect zooplankton sample (taken from U.S. EPA 2007):

1. Fill out sample label.
2. Measure lake depth at sample location.
3. Clean and thoroughly rinse the inside surfaces of the nets and collection buckets with DIW.
4. Inspect nets and buckets for possible holes or tears.
5. Attach collection bucket to small end of each net.
6. Attach marked (every 0.5 m) lines to large end of coarse net.
7. Lower coarse net in constant upright position over side of boat until the mouth of the net is about 1.0 m above the lake bottom. If the lake depth is less than 2 m and the Secchi disk can be seen at the bottom, collect a second tow with the coarse net and combine the replicated samples (make note of this on data form).
8. Retrieve net to surface at constant rate (about 1 ft per second) without stopping.
9. At the surface, slowly move the net up and down in the water column, without submersing the net mouth, in order to flush zooplankton from the net into the collection bucket.
10. Further rinse contents from net into collection bucket by spraying net from outside to inside with squirt bottle containing DIW.
11. Holding collection bucket in vertical position, detach it from net.
12. Swirl the bucket without spilling the contents in order to filter excess water out of the bucket through the screened sides.
13. Repeat steps 6 through 12 with the fine mesh net on the opposite side of the boat.

5.6 SAMPLE PROCESSING, PRESERVATION, AND HANDLING

5.6.1 STREAM BENTHIC MACROINVERTEBRATES

After collecting kick net samples for the reachwide samples, prepare a composite index sample from the contents of the container as described below. You will need to record tracking information for each composite sample on the Sample Collection Form. Check to be sure that the completed label on each jar is covered with clear tape and that a waterproof label is placed in each jar and filled in properly. Confirm that the inside and outside labels describe the same sample. Replace the lid on each jar and seal with plastic electrical tape. It is helpful to mark the lid of each jar with the site number; use a permanent marker or write on a piece of light-colored

tape (or a small blank address label) and attach it to the lid. Place the sample jars in a cooler or other secure container for transporting and/or shipping it to the laboratory. The container and absorbent material placed between the jars both should be suitable for transporting ethanol. Check to see that all equipment is returned to the vehicle. Samples do not need to be kept on ice or cooled after they are preserved with ethanol.

Procedure for preparing composite samples for benthic macroinvertebrates (taken from Peck et al. 2006) are:

1. Pour off the water from the reachwide bucket through a sieve (or sieve bucket) with 500 µm mesh size. Remove any large objects such as sticks, rocks, or large plant material from the bucket or container. Inspect these objects carefully and dislodge any clinging organisms back into the sample bucket or container before discarding.
2. Estimate the total volume of the sample in the sieve and determine the size (500-ml or 1-L) and number of jars that will be needed for the sample. Avoid using more than one jar for each of the composite samples if possible, but don't fill the jar more than ¼ full with each composite sample.
3. Fill in a sample label with the stream ID, date of collection, and other required information. Attach the completed label to the jar and cover it with a strip of clear tape. Record the sample ID number for the composite sample on the Sample Collection Form. For each composite sample, make sure the number on the form matches the number on the label.
4. Wash the contents of the bucket or container to one side. Transfer the sample from the bucket or container into a jar, using a large-bore funnel if necessary. Use as little water from the wash bottle as possible to help transfer material. If the jar becomes too full of liquid, carefully pour off the water through the sieve. Continue to transfer sample material to the jar until it is not more than ¼ full of solid material. Use additional jars for the remaining sample. Carefully examine the bucket or container for any remaining organisms and use watchmakers' forceps to place them into the sample jar.

   If a second jar is needed, fill in a sample label that does not have a pre-printed ID number on it. Record the ID number from the pre-printed label prepared in step 4 in the SAMPLE ID field of the label. Attach the label to the second jar and cover it with a strip of clear tape. Record the number of jars required for the sample on the Sample Collection Form. Make sure the number you record matches the actual number of jars used. If possible, write Jar N of X (N being the sequential jar number and X being the total number of jars for the sample) on each sample label using a waterproof marker.

5. Place a waterproof label with the following information inside each jar:
   - Stream ID number
   - Name of stream
   - Date of collection
   - Collector's initials

6. Remove as much water as you can from each sample jar without removing any sample material by pouring it through the sieve. If possible, completely fill each jar with 95% ethanol (no headspace) so that the final concentration of ethanol is between 75 and 90%. It is very important that sufficient ethanol be used or the organisms will not be properly preserved. Do not freeze samples to preserve them.

   Note: For backcountry work, prepared composite samples should be transported back to the vehicle before adding ethanol. In that case, fill each jar with stream water and a minimal amount of ethanol to cushion the sample from the grinding action of non-biological material in the sample during transport. Replace the water with ethanol at the vehicle as soon as possible.

7. Replace the lid on each jar. Slowly tip the jar to a horizontal position, then gently rotate the jar to mix the preservative. Do not invert or shake the jar. After mixing, seal each jar with plastic tape.

8. Store the labeled sample jars in a container with absorbent material that is suitable for use with 95% ethanol until transport or shipment to the laboratory.

5.6.2 Lake Zooplankton

After rinsing the outside of the plankton net using the squirt bottle with DIW, transfer the sample to one (or more, if needed) sample jars. The collected zooplankton are doped by adding CO_2 tablets or alka-seltzer to stop their movement and then preserved. Detailed procedures for sample preservation are described below. Zooplankton samples, once preserved, do not need to be stored on ice and can be shipped via ground transport to the laboratory. Field personnel should include one copy of the data form along with the samples (each in its own plastic bag) when they are shipped to the laboratory. Take one copy of the data form back to the office in the lake-data folder. The development and use of a folder for each study lake is described in Section 1.3.1 of the Water Chemistry Field Sampling Protocols, Establishing and Locating Sampling Sites. The sample jars should be surrounded with packing material before shipping.

Detailed procedures for processing zooplankton samples (taken from U.S. EPA 2007) are:

1. Place bucket that had been attached to the coarse net into a 500 ml container filled three-fourths full with lake water, to which a CO_2 tablet has been added (alternatively, Alka-Seltzer or club soda can be used). Wait until zooplankton have been narcotized and stop moving (about 1 minute).

2. Transfer contents of bucket into a 125 ml polyethylene jar using DIW from the squirt bottle. Continue to rinse the bucket until the majority of the collected zooplankton are transferred to the jar.

3. Drain much of the excess water out of the jar by attaching a lid modified to create a strainer that has been prepared in advance by cutting two holes in the lid and gluing small pieces of the appropriate (large or small) mesh material to the inside of the lid to cover the holes. Carefully decant the excess water out of the jar while retaining the zooplankton inside the jar.

4. Fill the jar a little more than half full with 95% ethanol[12]. If the volume of zooplankton collected fills the jar more than half full, use a second (and third, if necessary) jar to preserve the additional sample volume. Record the number of jars used on the Zooplankton Sample Data Form. Label each jar identically and then add to the labels, as appropriate, "1 of *x*", "2 of *x*", etc., with *x* being the total number of jars used for the sample.
5. Record the length of tows collected on the Zooplankton Sample Data Form. Verify that all required information is provided on sample labels and data form. Cover each label with clear tape.
6. Seal jar lids by wrapping electrical tape around the juncture of the lid in a clockwise direction so the lid is pulled tight as tape is stretched around it.
7. Place each jar in a zippered plastic bag.
8. Repeat steps 1 through 7 for the second (fine mesh) sample collected.
9. Thoroughly clean and rinse all equipment and the strainer lids before transporting them to another lake.

5.7 DOCUMENTATION AND TRACKING

Data collection forms for stream macroinvertebrate sampling and lake zooplankton sampling can be found on the Utah State University BugLab website (<*http://www.usu.edu/buglab/*>). Example labels for stream benthic macroinvertebrate samples and lake zooplankton samples are shown in Appendix F. Data collection forms and labels should be carefully filled out and double-checked for completeness and accuracy before leaving the field site.

5.8 LABORATORY ANALYSIS

Biological samples will need to be sent to a contract or agency laboratory, where experts will enumerate and identify the individual organisms in the composite sample. Taxonomic richness results are very sensitive to both counting effort (e.g., how many individuals are counted) and taxonomic resolution. Thus, it is imperative that a consistent laboratory counting protocol be used when multiple labs are involved or samples are analyzed over a period of time for trends determination. For the stream benthic IBI assessment, we recommend a 500 fixed organism count protocol using a gridded sorting tray (typically, a 5x6 grid of 30 cells). Individual grid cells from the tray are selected at random and completely processed until more than 500 organisms are enumerated. The percent of the sample processed is calculated as number of grids processed/total number of grids and this number used to infer the total number and density of individuals in the composite sample. For counts of the EPT taxa only, grid cells should be processed either until 100 individuals of the EPT taxa have been enumerated or until 500 total organisms have been enumerated.

[12] For backcountry sampling, add only a small amount of ethanol in the field and then fill jars to near the top with water. Upon returning to the vehicle, discard the water and replace with ethanol.

Benthic organisms should be identified, if possible, to the genus level except for the following non-insect taxa: oligochaetes, polychaetes, and arachnids to family; nematodes and platyhelminthes to phylum. In most cases, identification to family should be considered the minimal requirement. For a basic EPT taxa richness assessment, the EPT orders should be identified to genus. For lake zooplankton, individuals should be identified to the species level, where possible. Each net sample is counted independently and at least half the sample volume examined. Subsamples are examined and counted until no new species are found or until a total of 300 to 500 individuals have been counted. Either approach is acceptable; the choice should be based on the standard protocol of the laboratory.

5.9 QUALITY ASSURANCE

5.9.1 Sample Replication

It is always advisable to replicate a portion of the samples, regardless of whether they are chemical or biological. This offers an opportunity to evaluate variability that may be introduced in the course of sampling, preserving, and analyzing the samples. Although we do not recommend that replicate zooplankton or benthic macroinvertebrate sampling should necessarily be required, we do think it is a good idea. Our recommendation is that about 5% to 10% of the sampled lakes or streams be replicated. Sample information provided on the data form for the replicate in this case will be identical to that of the original sample except for the sample ID and the time of sampling, which will differ slightly between the first and second samples at a given site.

The replicate zooplankton sample should be collected at the same general location as the primary (first) sample, on the opposite side of the boat. If a stream site is to be replicated, the additional (replicate) sample is collected at each transect location to yield a pooled composite replicate, comparable to the composite normal sample. The replicated stream benthic macroinvertebrate sample at each transect location along the sample stream reach should be collected at a different stream location from the normal sample. For the replicate, move the transect sample location from L to C; from C to R; and from R to L. Check the box on the data form indicating whether or not the sampling was replicated at this site. If the stream is not wide enough to accommodate collection of a second (replicate) sample, slide the replicate site upstream about 10 m and collect the replicate sample there. With replicate sample, extra caution must be taken to not disturb any of the actual sampling sites by walking in them before sampling.

5.9.2 Taxonomy

A major potential pitfall in any aquatic invertebrate study is the inherent variability and uncertainty in taxonomy among aquatic entomologists and among laboratories. This can be especially problematic in a long-term monitoring study if different laboratories or laboratory staff are involved in the identification of collected organisms over the course of the study. We recommend choosing a highly experienced taxonomic laboratory and trying to maintain consistency throughout the project. When multiple labs are involved, interlaboratory QA is essential. In EPA's National Stream Survey, which used eight different laboratories, 10% of the samples were randomly selected for QC re-identification and sent to an independent taxonomist

in a separate laboratory for comparison (Stribling et al. 2008). The results of the sample-based comparisons were summarized as percent taxonomic disagreement (PTD) and percent difference in enumeration (PDE). Percent difference in enumeration differences among labs were minor (<3%) but PTD were on the order of 20%. Having lab taxonomists intensively interact, resolve differences, and update the data after the first round of identifications was important, and improved PTD substantially in the EPA survey. We also recommend that at least 5% to 10% of samples be sent to an alternate laboratory or alternate entomologist to evaluate any differences that might arise in taxonomic identification. Such differences should be resolved, if possible, before finalizing the dataset.

5.10 INTERPRETATION

Analysis of lake or stream water quality data can provide critical information regarding the status, or change over time, in biologically relevant water chemistry. Thus, it may be known or suspected based on measured water chemistry that in-lake or in-stream biota respond to a given concentration (or change in concentration) of ANC, pH, Al_i, etc. Nevertheless, there is always some degree of uncertainty regarding the biological effects that actually occur under a given suite of water chemistry. Federal land managers can draw stronger inferences about biological effects if the biological resource itself is characterized or monitored. This can be important in setting target deposition loads, pursuing litigation, and evaluating damage or recovery scenarios. Substantial value can be gained by sampling biota in addition to water chemistry.

5.10.1 Biological Metrics

Biological assemblage data are typically analyzed by calculating metrics from the list of the species, genera, or families identified and their abundances. For example, the number of different mayfly genera in the sample can be tallied and this number becomes the mayfly genus richness metric. Richness metrics can be calculated for any defined taxonomic group (e.g., mayflies, rotifers, or insects) as well as total sample richness. Similarly, richness can be calculated for any other autecological attributes such as functional feeding groups (shredder richness), habitat preference (swimmer richness), or tolerance to various pollutants. In addition, the same type of metrics can be calculated based on percent of individuals in the sample (e.g., percent mayfly individuals or percent shredder individuals). There are also metrics to reflect overall sample diversity that are based on equations that aim to mathematically express diversity as a combination of overall sample richness (number of different taxa) and evenness (equal number of individuals across taxa).

A simple assessment can be made based on a single metric that is responsive to specific pollutants of interest. For example, mayflies are very sensitive to pH, and mayfly taxonomic richness is therefore a good metric to use for acidic deposition studies. Total sample richness or diversity may also be used as a single overall measure of biological condition. The most robust measures of biological condition, however, require modeling or combining multiple metrics into one overall multi-metric index. Application of a multi-metric index or model requires some expertise in the biotic assemblage being assessed. In particular, gathering the necessary autecological

information can be time-consuming and require detailed knowledge of the different species that occur within the study region.

5.10.2 Multimetric Indices and Predictive Modeling

There are two major assessment approaches for quantifying whole community biological condition: multimetric indices (e.g., IBI) or predictive modeling (e.g., the observed/expected or "O/E" approach). A multi-metric index, such as the IBI, is developed by selecting the best 5 to 15 metrics that quantify condition over a suite of different aspects of biotic integrity and then summing individual metric scores into a single index of condition. Metric selection and interpretation of metric values at the sampling site are usually based on values observed at least-disturbed reference sites in similar settings.

The predictive-modeling approach uses reference sites to assemble lists of taxa that appear to be indicative of a least-disturbed reference condition (the expected or "E" list). Taxa lists from a specific study site comprise the observed, or "O," list. The proportion of the expected taxa found in the observed list (O/E ratio) is a measure of the proportion of the taxa expected to be at an undisturbed site that are actually present at the study site. An O/E ratio of one indicates a high-quality site (all expected taxa present). An O/E ratio of <0.5 means that less than half the expected reference taxa are present at the site. In practice, the E list is developed for each study site by statistical modeling (cluster analysis and discriminate function analysis) of reference site data to take into account natural differences in expected taxa distributions.

This modeling approach was pioneered in Great Britain (Moss et al. 1987) and has been applied in many different locations throughout the world. These predictive models require statistical expertise to develop in new regions and a large number of reference sites as the basis for the modeling. Study-site and reference-site data must be collected with comparable field protocols, lab protocols, and taxonomic resolution. Due to its complexity and data requirements, we do not recommend the O/E approach as a routine tool for biological assessment on FS lands potentially influenced by atmospheric S and N deposition. Nevertheless, this can be a powerful tool for stream biological assessment if one is willing to develop the modeling approach and reference-site database for a given region or study area.

5.10.3 Taxonomic Resolution

In conducting any biological assessment, the level of taxonomic resolution (e.g., order, family, genus, or species) is an important consideration. In general, identifications to lower taxonomic levels cost more but provide more information. For stream macroinvertebrates, identification is usually taken to either the family or genus level. Some organisms can be identified to species, but, in a given sample, most of the organisms can only be identified to genus due to the lack of sample keys for many taxa and the small size of early life history stages of many of the individuals. With stream macroinvertebrates, the major laboratory effort involves picking the organisms out of the sample matrix rather than identifying them. Therefore, the laboratory cost difference between family- and genus-level analyses may not be substantial. In terms of information content, Waite et al. (2004) found that family- and genus-level stream macroinvertebrate data were similar in their ability to distinguish among the coarse impacts (e.g., most severe versus least severe impact classes).

Genus data, however, often distinguished the subtler differences in mid-Atlantic streams (e.g., mixed/moderate impacts versus high or low impacts) better than family level data. In their analysis, acidic deposition impacts were considered a moderate impact and not a severe impact. Ordination analysis showed that both family and genus levels of analysis responded to similar suites of environmental variables.

We suggest that identification to the family level can be sufficient for many bioassessment purposes. However, identifications to genus do provide more information, especially in genera-rich families like Chironomidae. Genus or finer levels of identification are important for investigating natural history, stream ecology, biodiversity, and indicator species. Decisions about the taxonomic level of identification need to be study specific and depend on available resources (cost) and study objectives.

5.10.4 Index of Biotic Integrity

The IBI is a multi-metric index that has been used extensively in streams to characterize fish, macroinvertebrate, and periphyton condition. Note that we do not recommend application of an IBI as part of the routine process of evaluating biological response to atmospheric deposition stressors on FS lands.

In general, individual analyses for one or more of the EPT orders or application of an EPT Index is often sufficient. If, however, there is a need to more fully characterize biological conditions in a particular stream reach and if appropriate invertebrate taxonomic and autecological expertise is available to the project team, then a stream benthos IBI is an appropriate way to proceed. Once the samples have been collected, there is not usually a dramatic difference in cost to enumerate all taxa (for implementation of an IBI) as opposed to just the insect orders included in the EPT. Nevertheless, application and interpretation of the IBI does require that more specialized taxonomic and autoecological expertise be available to the project.

There are a number of different approaches to calculating IBIs, but they all follow a similar process. First, the metrics that best reflect condition are selected from the set of candidate metrics. IBIs typically are comprised of 5 to 15 different metrics. Metric values are then scored to a consistent scale (e.g., 0-10 points) and summed to calculate the one overall IBI value.

A wide variety of IBIs have been developed for different stream types and regions around the world. For assessing stream benthos, we recommend as a starting point the macroinvertebrate IBI developed by EPA for the National Wadeable Streams Assessment, in part because it was designed to be applied nationwide (Stoddard et al. 2008). The Wadeable Streams Assessment IBI is formulated differently for each of nine different ecoregions in the United States (Figure 5-4).

Candidate metrics were divided into six different categories and the best performing metric in each category was selected for inclusion in the regional IBI. The six metrics in each regional IBI are listed in Table 5-4. The six metrics values were then each scored on a 0-10 scale and summed into a final IBI score (see Stoddard et al. 2008 for calculation details). There has been much less work done on developing IBIs for lake systems. We do not recommend calculating an IBI for lake zooplankton at this time as we are not aware of any existing IBIs that are ready for use.

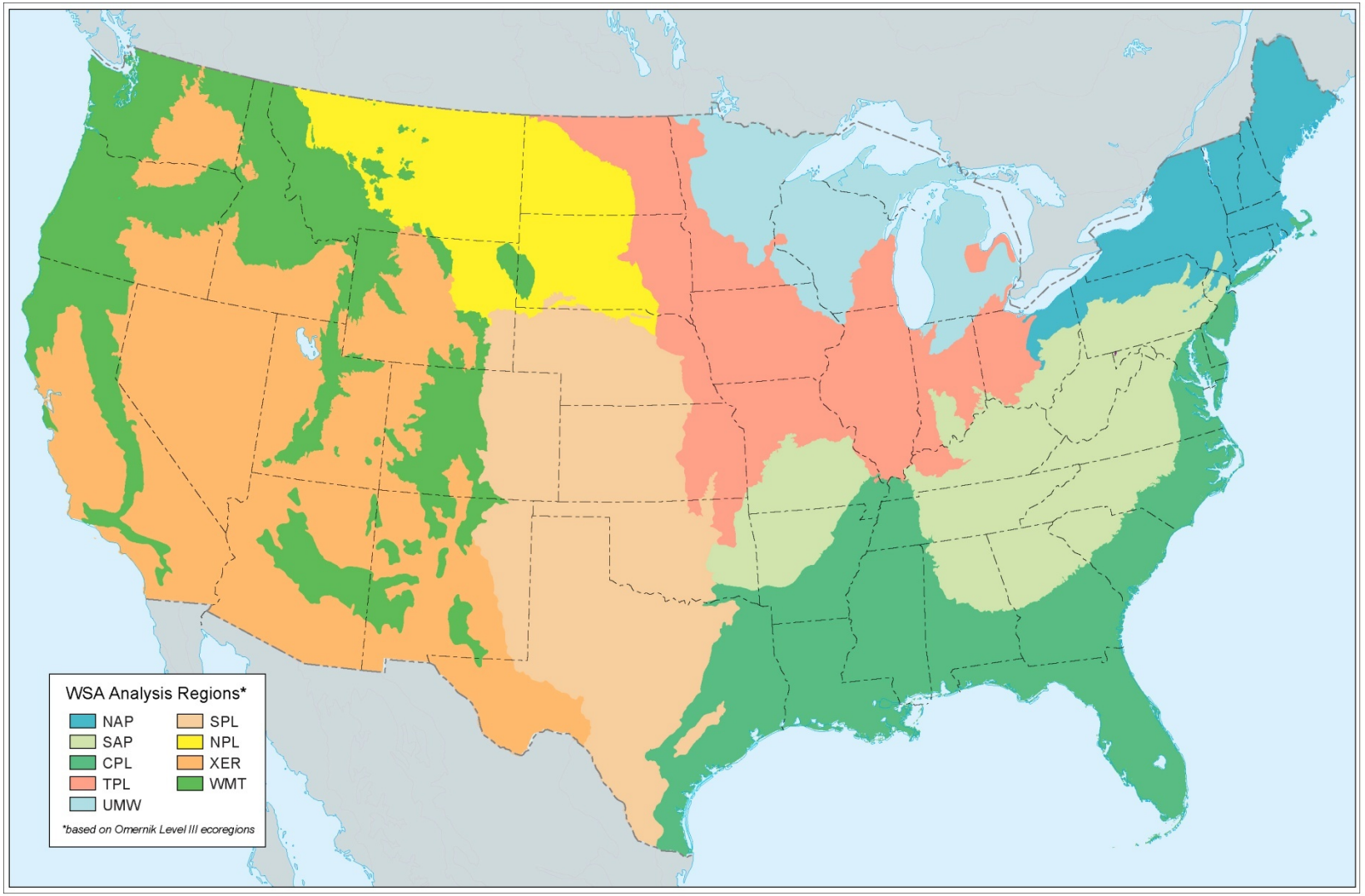

Figure 5-4. Location of the nine ecoregions used in Wadeable Streams Assessment IBI development (see Table 5-4 for ecoregion abbreviations). (Source: USEPA 2006.)

Table 5-4. List of metrics in each category used in the EPA National Wadeable Stream Assessment. (Source: Stoddard et al. 2008). Metrics were selected and scored separately for each of nine aggregate ecological regions: NAP (Northern Appalachians); SAP (Southern Appalachians); CPL (Coastal Plain); UMW(Upper Midwest); TPL (Temperate Plains); NPL (Northern Plains); SPL (Southern Plains); WMT (Western Mountains); and XER (Xeric).

Metric Category	Individual Metrics	Aggregate Ecological Regions								
		NAP	SAP	CPL	UMW	TPL	NPL	SPL	WMT	XER
Composition	% EPT Taxa	X					X		X	
	% EPT Individuals					X		X		
	% Non-Insect Taxa									X
	% Non-Insect Individuals			X						
	% Ephemeroptera Taxa		X							
	% Chironomid Taxa				X					
Diversity	Shannon Diversity		X	X	X	X		X		
	% Individuals in top 5 taxa	X							X	X
	% Individuals in top 3 taxa						X			
Feeding	Scraper Richness	X	X			X	X	X	X	X
	Shredder Richness			X	X					
Habit[b]	% Burrower Taxa		X		X		X	X		
	% Clinger Taxa	X		X					X	X
	Clinger Taxa Richness					X				
Richness	EPT Taxa Richness	X	X	X	X			X	X	X
	Ephemeroptera Taxa Richness					X				
	Total Taxa Richness						X			
Tolerance	Intolerant Richness						X	X		
	% Tolerant Individuals		X	X					X	X
	% PTV[a] 0-5.9 Taxa	X								
	% PTV 8-10 Taxa				X	X				

[a] PTV = Pollution Tolerance Value

[b] Habit reflects the life strategy of the various taxa with respect to maintaining position in the stream (i.e., burrowing, clinging)

5.10.5 Biotic Effects Analysis

If an atmospheric deposition effects study design calls for biological characterization—for example, in conjunction with a chemical characterization or monitoring effort—we recommend analysis of taxonomic richness of stream benthic macroinvertebrates and/or lake zooplankton for a group of streams or lakes across a gradient of acid-base chemistry. Such an analysis should be based on at least 10 water bodies and preferably more. The preferred chemical metric is usually ANC; the analysis should also be conducted for pH, Al_i, and/or NO_3^- concentration. The preferred biological metric is species richness or genus richness; in some cases family richness is the best that can be done because of taxonomic uncertainties. The taxonomic groups to be considered can include crustaceans and/or rotifers for lakes; mayflies, caddisflies, and/or stoneflies for streams; and/or some combination of the above.

The basic data analysis for studying the effects of stressors on biological condition involves plotting biological metric scores or IBI scores versus water chemistry, as shown schematically in Figure 5-5A for mayfly genera richness. The strength of the relationship can be evaluated using the r^2 statistic. This analysis provides useful information on the extent to which invertebrate biological assemblages are associated with water acid-base chemistry. Trends analysis cannot be used to interpret biological change in response to improved or declining acid-base chemistry unless this basic analysis is performed and yields a meaningful relationship.

For water bodies that are included in long-term chemical monitoring, one should also consider subjecting at least a subset of those water bodies to biological monitoring. The biological monitoring candidates should preferably be relatively low in ANC and pH, exhibit chemistry that is not excessively variable within and among years, and exhibit reasonably rich biological assemblages. Selection of two to four waters, spread across the ANC gradient (to the extent that such a gradient occurs) between about -50 μeq/L and 50 or 100 μeq/L, would be appropriate. Resulting monitoring data should be analyzed as shown schematically in Figure 5-5B (or some variation thereof). This analysis allows determination of the extent to which chemistry and biotic richness are deteriorating or improving over time, and the degree to which those trends are linked.

Variability in any of the figures used to examine relationships between stream or lake chemistry and biological community metrics can be caused by changes in environmental conditions, especially hydrological conditions. For that reason, it is always advisable to examine the influence of weather/hydrology on the observed relationships. This can easily be done by coding the points on any of these figures according to hydrological conditions (in discrete classes). This can be based on inlet or outlet stream discharge (i.e., cumulative seasonal or annual stream flow), seasonal or annual precipitation, date of snowmelt, or other variable constructed to represent the differences between wet years or seasons and dry years or seasons. This allows the analyst to determine to a first approximation the extent to which the observed relationships between chemistry and biology are influenced by hydrological differences.

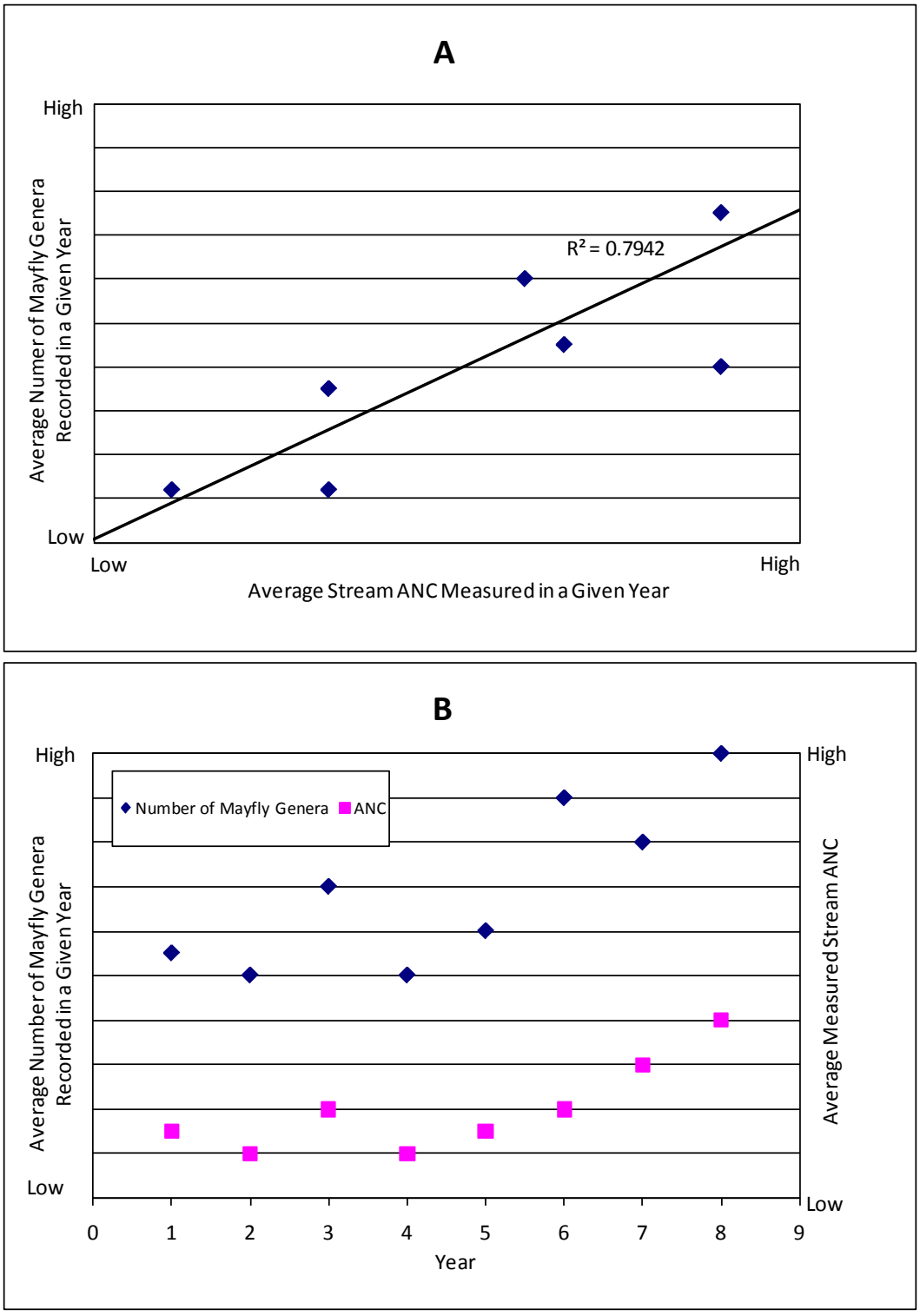

Figure 5-5. Schematic depicting mayfly richness over time in response to changes in stream chemistry. Plot A shows richness plotted against chemistry; Plot B shows both richness and chemistry plotted against time.

STREAMS

For documenting biological effects in streams in response to changes in atmospheric deposition, we recommend analyzing the quantitative relationships between invertebrate community metrics and stream ANC in multiple streams selected across an ANC gradient within a given forest or wilderness. The same analyses could also be done using the variables pH, NO_3^-, and Al_i. For an initial analysis, we further suggest that, for studies of response to acidic deposition, the analysis can be limited to insects (class Insecta of the phylum Arthropoda) of the orders Ephemeroptera, Plecoptera and Trichoptera (EPT, or mayflies, stoneflies, and caddisflies) because of their general importance to stream ecology and their demonstrated responsiveness to changes in acid-base chemistry.

We recommend examination of the number of genera (or if that is not possible, families) present within each of these three orders, both individually and combined, in relation to differences among streams in stream chemistry. Figure 5-6 shows an example of this analysis for mayflies in streams in Shenandoah National Park (Sullivan et al. 2003). The analysis was based on both the minimum ANC and the average ANC of multiple measurements in a given stream. The same type of analysis can be conducted for a single chemistry measurement from each stream, if that is what is available. The analysis shown in Figure 5-5 should be conducted for all three of the principal insect orders, plus for the EPT Index.

The EPT Index is calculated either as the total number of genera or the total number of families present in a given stream from the orders Ephemeroptera, Plecoptera, and Tricoptera. It represents the number of genera or families among all three orders enumerated in a single sample or in the average of multiple samples. In general, we recommend basing an EPT Index on the number of genera present. If that is not possible, the calculation can be based on the number of families present. The data shown in Figure 5-6 illustrate, as is often the case, that relationships between mayfly richness and stream chemistry are typically stronger than relationships for caddisflies. Stoneflies alone are often not very sensitive to changes in ANC and pH.

For trends analysis of change in benthic insect diversity over time, we recommend plotting the number of genera or families (within each of the three orders individually, and combined as an EPT Index) recorded for one or multiple (averaged) samplings from a given stream each year over a period of at least 8 years. This will provide an assessment of possible changes in benthic insect richness over time that can then be related to possible changes in stream ANC or some other variable. For example, the average number of genera or families of mayfly recorded during various samplings in a given year (y-axis) should be plotted against the average ANC (x-axis) determined for those same sampling occasions over the period of study. In addition, both the average number of genera or families of mayfly and the average stream ANC should be plotted over time (across the years of record) using the same time scale (cf. Figure 5-5). Such analyses allow evaluation of the extent to which changes in biota are associated with change in chemistry and the degree to which either or both are changing over time.

If the result of application of an EPT index is not clear, that result may be attributable to a lack of invertebrate response or it may be that the index is not sufficiently sensitive to illustrate the biological response that has occurred. In such a situation, FS staff should consider the possibility of applying an IBI, which may be a more powerful approach.

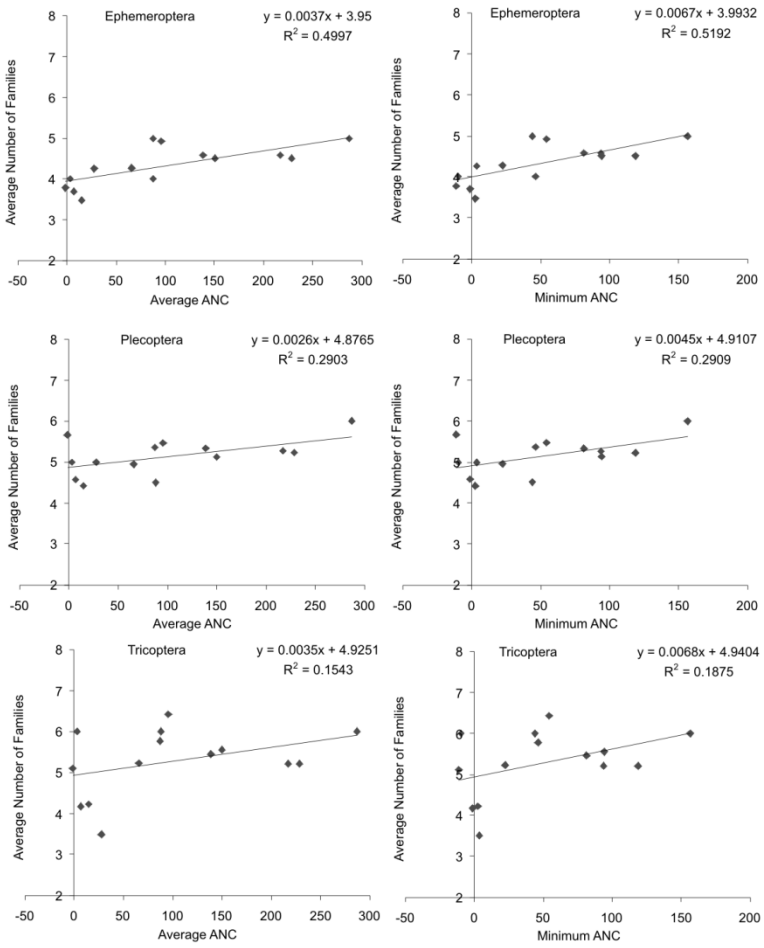

Figure 5-6. Average number of families of aquatic insects for each of 14 streams in Shenandoah National Park versus the mean (left) or minimum (right) ANC of each stream. The stream ANC values are based on quarterly samples from 1988 to 2001. The invertebrate samples are contemporaneous. Results are presented for the orders Ephemeroptera (top), Plecoptera (center), and Tricoptera (bottom). The regression relationship and correlation are given on each diagram. (Source: Sullivan et al. 2003.)

LAKES

We recommend, for lake characterization studies focused on acidification, analyzing zooplankton data for more than 1 and preferably 10 or more lakes across an ANC gradient to determine whether any relationships exist between zooplankton richness and lake ANC. Parallel analyses can also be conducted for pH and Al_i in addition to ANC. These analyses should be conducted for all zooplankton groups combined (total zooplankton) and for discrete groups of zooplankton. The discrete groups should include crustaceans and rotifers at a minimum and could also include large cladocerans. An example for Adirondack lakes in New York, showing the number of zooplankton species versus ANC at the time of zooplankton survey, is shown in Figure 5-7.

It is generally expected that variation (or scatter) in the relationships between lake chemistry and taxonomic richness may increase as the size of the study area increases. An analysis such as that shown in Figure 5-7 for a large region may yield so much variability that patterns are not clear. It

may be necessary to restrict analyses such as this to a specific wilderness or to a designated subset of a region or forest, such as a certain geological type, ecoregion, or elevational band. It is advisable to examine differences in the relationships between zooplankton and lake chemistry under varying schemes for subsetting the data into groupings of lakes that are generally more similar to each other than the group of all lakes across a given region.

If there are clear relationships between zooplankton species richness and lake chemistry across a wilderness, forest, or designated subset of lakes within a wilderness, forest, or region, then it can be useful to develop a time-series database for one or more presumed acid-sensitive lakes (having ANC between about -20 and +50 μeq/L). Such a database would entail contemporaneous zooplankton species richness and lake ANC measurements over a period of time of at least 8 years. Plots can then be constructed to determine, for a given lake, the relationship between ANC and zooplankton richness and changes in both of these variables over time using plots such as those depicted in Figure 5-5. We do not recommend this as a standard component of long-term chemical monitoring efforts. However, if it is important to document changes in biological effects in response to anticipated changes in lake chemistry, then a time series of zooplankton richness (for crustaceans, rotifers, total zooplankton, or other taxonomic grouping) may be the most straight-forward and cost-effective strategy.

Biotic assemblages in lakes vary at both temporal and spatial scales influenced by such factors as climate, vegetative cover, and disturbance. Therefore, environmental indicators exhibit variability that has a great influence on our ability to estimate biological status or trends over time. Stemberger et al. (2001) attempted to quantify the various contributions to the variance in zooplankton status as part of EPA's EMAP sampling program in the northeastern United States. Variance in zooplankton indicators was attributed primarily to four components of variance:

1. Lake variance: the lake-to-lake variability in zooplankton indicators in the study population. This depends largely on such factors as lake size, depth, fish presence/absence, pH, thermal characteristics, and productivity (Dodson et al. 2000).
2. Year variance: coherent variation from year-to-year across all lakes, due, for example, to an unusually warm or wet weather pattern.
3. Lake-by-year interaction variance: independent year-to-year variation at each lake due to site-specific forcing factors, such as variation in nutrient inflows or mixing regime.
4. Index variance: local spatial and temporal variance due, for example, to within-index period temporal changes, measurement error, or differences among crews or laboratories in application of the protocols (Stemberger et al. 2001).

In general, Stemberger et al. (2001) found lake variance to be the largest component of variance for zooplankton in the northeastern United States, followed by index variance. Efforts to reduce the magnitude of the factors that contribute to zooplankton variance can maximize one's ability to detect differences or trends in the data.

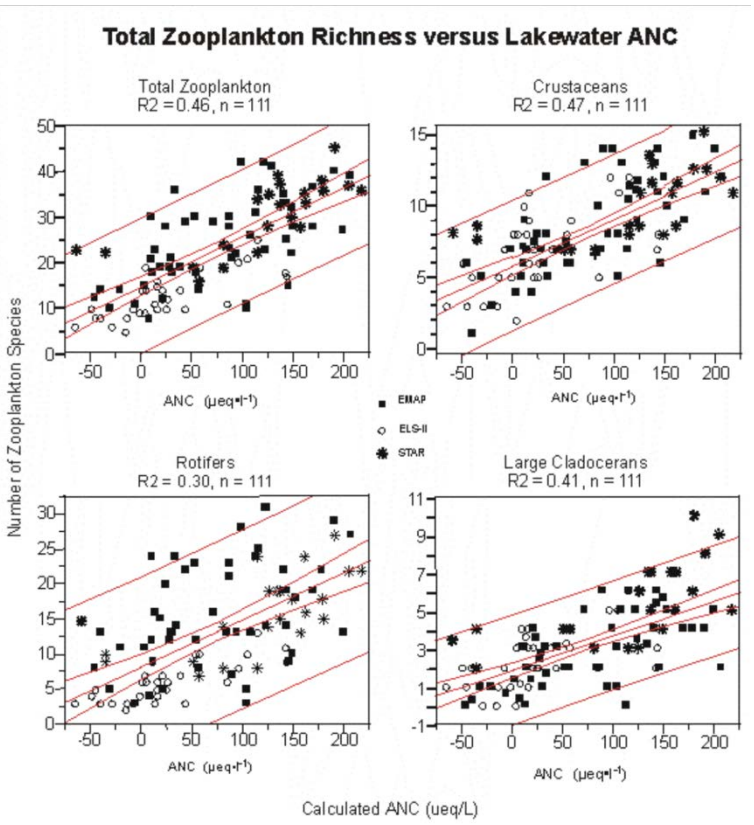

Figure 5-7. Zooplankton taxonomic richness versus ANC for a combined Adirondack dataset, based on 111 lake visits to 97 lakes in the EMAP, ELS, and STAR zooplankton surveys. (Source: Sullivan et al. 2006.)

SECTION 6. TRANSITION PLAN

T.J. Sullivan and G.B. Lawrence

6.1 BACKGROUND

Under contract to the Forest Service, E&S Environmental Chemistry, Inc., and its research partners at Oregon State University, U.S. Geological Survey, and the University of Virginia have prepared new national FS protocols for the sampling, analysis, and quality assurance of the chemistry of lakes and streams on federal lands that are potentially sensitive to adverse impacts from atmospheric deposition of air pollutants. The protocols are also accompanied by a Training Plan to train field sampling staff in the field components of the new protocols (Section 7). The intention is to shift, where practical, FS survey and monitoring program efforts to the new protocols. It is hoped that this standardization of sampling and analysis protocols will improve comparability of the resulting data within and among regions and will improve the overall quality of the water chemistry data collected and analyzed for lakes and streams on National Forest System lands.

It must be recognized, however, that there are risks associated with changing the protocols of an ongoing sampling program. In some cases, there may be a substantial period of record established for a particular lake or stream that is based on pre-existing protocols, and multiple waters within a particular wilderness may already have been surveyed and characterized with a particular set of protocols which may differ in important ways from the newly developed protocols. A change in approach may introduce bias into future efforts to examine patterns in water chemistry across time or across space. Therefore, changes must be carefully considered, and the likely results of those changes (if any) must be evaluated before making a wholesale change in sampling and/or analysis methods. This Transition Plan provides a framework for considering such protocol changes and their likely effects on the resulting data before full implementation of the new protocols.

6.2 TRANSITION STEPS

This Transition Plan is divided into sequential steps to be followed in order to ascertain the likelihood that protocol changes might introduce bias into ongoing monitoring or characterization efforts. In some cases, the preferred approach might be to continue to monitor surface waters using existing protocols or to augment these protocols with additional elements from the new

protocols while retaining the basics of the existing protocols. In other cases, it might be best to transition to the new protocols after first evaluating the ramifications of methods changes.

1. **Read and become familiar with the new national protocols.** Each FS field staff should read the field sampling protocol. Each FS staff person involved with the analysis, quality assurance, and/or interpretation of lake and stream water data should read all relevant sections from the National Protocol (field sampling, laboratory, QA/QC, data analysis, and biology). Each staff person should become thoroughly familiar with the portions of the new protocols that are relevant to his or her work duties and review existing SOPs in local FS survey and monitoring programs to determine all significant ways in which the new procedures differ from current practices.

2. **Attend a field sampling protocol training session.** Anyone who is involved with the collection of water samples in the field should attend a training session to receive hands-on classroom and field training in the new field sampling protocols.

3. **Determine if any existing lake or stream monitoring sites will be dropped from the sampling program or if any new sites will be added.** The new protocol emphasizes the need for matching sampling sites (and sampling schedules) with research questions and needs. Project managers should review existing monitoring programs to determine whether and to what extent the sites being sampled provide information required to achieve program goals. In some cases, a lake or stream may be included in a long-term monitoring effort, but available data might indicate that the water body is not very sensitive to the stressor(s) of concern, receives substantial inputs of geological S (which confound evaluation of effects of atmospheric S deposition), or is impacted by some form of disturbance to such an extent that it is not possible to ascertain the influence of air pollutants. Thus, based on considerations and priorities outlined in the National Protocols, the decision could be made to drop one or more sites from the monitoring program and/or to add others that might better meet program needs. However, before dropping a site from a long-term monitoring effort, one must carefully weigh the value of data that have been collected from that site to date versus the benefit of replacing that site with a new site that may have little or no data associated with it but will provide more useful information for the program in the future.

4. **Determine if sample collection protocols need to be changed.** The new protocols could involve changes in any or all of the elements in where, what, when, and how to collect water samples. A change in collection location (Where) could affect the data even if this location is still considered the same site. Furthermore, a sample collected from a lake outlet could provide different data than a sample from the upper water column at the deepest portion of that same lake. If new field measurements or types of sample collection are added (What), the new procedures should be evaluated to ensure that they will not interfere with the existing field measurement and sample collection procedures. For example, if the existing sample collection location in a stream is downstream of the cross-section for new flow velocity measurements, the water sample should be collected before the velocity measurements are taken to ensure that the water sample is not contaminated with suspended solids caused by stream wading. Changing the time of year or frequency (When) that the sample is collected could change the data record. Switching from spring sampling to summer sampling could bias the data towards lower flow conditions that might prevail

during summer and that are typically less acidic than higher spring flow conditions. Switching from weekly sampling to monthly sampling would lower the sensitivity for detecting long-term trends. The procedures used to collect the samples (How) could also change the data. Water sampling devices that integrate flow or depth can produce different results than dipping a bottle. Collection and transport via syringe may yield different values for some parameters (i.e., pH and DIC) than collection and transport via bottle.

Influences such as those described above should be evaluated in conjunction with changing methods in the middle of a monitoring program to determine if a sampling bias will be introduced. This should be done by performing the collection procedures with both the existing and new procedures for a length of time to account for the full variation in sampling conditions. In many cases, this could require a year or more of duplicating procedures. Results obtained using the original protocol should be compared to results obtained using the new protocol using a scatterplot with a 1:1 line added. If one approach yields results that are consistently either higher or lower than the other approach, the data points will plot consistently either above or below the 1:1 line. If there is no bias introduced by the change in protocol, the data points will be approximately evenly distributed above and below the 1:1 line. If it is determined that there is a bias introduced by the method change, then a decision will need to be made regarding whether to:

a. Stick with the original protocol;
b. Shift to the new protocol and ignore the difference if it is judged that the difference is too small to be of consequence to the intended use of the data; or.
c. Develop a regression approach to "correct" the data points obtained using the original method to more closely approximate the results obtained with the new (and presumably improved) method.

In general, we recommend the first option unless there is a compelling reason to change. This is a judgment call, however, and any of these options can be reasonable depending upon circumstances.

Changes in the manner in which the sample is collected that improve precision without adding bias, such as additional steps to prevent possible sample contamination during sampling, should not influence the decision as to whether or when to shift to the new protocols. An example of this type of change is in instituting the use of latex gloves during the sample collection. Any sampling procedure change that is expected to reduce the likelihood of sample contamination should be viewed as a positive step that should be implemented as soon as is practical.

5. **Evaluate the need for change in chemical measurements done in the laboratory or a change in the data quality objectives for the methods being used.** If a new measurement is needed, the method should be fully evaluated to ensure that the desired results will be obtained. Also, if it is determined that a laboratory is unable to meet the data quality objectives needed for a FS characterization or monitoring program, an alternative laboratory will need to be found. If the laboratory needs to upgrade or replace existing instrumentation or to modify existing SOPs, they must provide duplicate results using

samples that are representative of the relevant study sites for a minimum of 100 samples to document that the changes have not introduced a bias. Because of potential methods interferences due to the mix of chemical constituents in a water sample, a laboratory methods change might alter results for one type of surface water but not another.

6. **Conduct side-by-side sampling and/or analysis to compare results obtained using initial protocols with results obtained using new protocols.** Such side-by-side comparisons should be conducted when potentially significant changes are made in either field or laboratory protocols that could affect long-term continuous records. In this situation, the data measured with previous protocols will need to be married with the new data without bias, which could be misinterpreted as a real change over time. The timeframe over which the side-by-side comparisons should be conducted should include the full range of variability in the parameters of interest. For marrying long-term records for trends analysis, the minimum recommended length of time is 1 year. However, the length of time required for duplicate analyses is affected by the sampling frequency. Running duplicate sampling and analysis for 1 year would be adequate for a weekly sampling program but certainly not for a seasonal sampling program that collects only four times per year. Our overall recommendation is a minimum of 1 year and a minimum of 100 samples distributed across the various sampling sites. The side-by-side comparisons should be evaluated after sufficient data have been collected. The original protocol should not be dropped until the analysis of the side-by-side comparison is completed and the results indicate that the datasets based on the new and the old protocols can be married without creating artifacts in the record.

7. **Provide proper documentation to eliminate ambiguity in protocol applications.** Clearly label samples collected with the new protocols and samples collected with the old protocols in such a way that the differences are documented and unambiguous. For example, if the decision is made to replace bottle sampling with syringe sampling for the measurement of water pH, procedures must be in place to document this change in the database; in this instance, the documentation for that sample in the collection method field of the database would indicate “lab pH - bottle” or “lab pH - syringe.” Details of each sampling method should also be documented in the field sampling protocols.

6.3 ANTICIPATED PROTOCOL CHANGES

We anticipate that a number of protocol changes will occur in the sampling that is conducted within the various FS regions as a result of adoption of the new National Protocols by the FS ARM program. The anticipated changes expected to be the most significant in terms of potentially introducing bias into the data and/or altering conclusions drawn from the data include the following:

FIELD SAMPLING

- Sample at index site near deepest portion of lake.
- Sample for pH and DIC using syringe or glass bottle with septum cap.
- Include discharge measurement.

LABORATORY

- Shift from field pH to lab pH measurements.
- Perform filtration in the laboratory.
- Change laboratory instrument(s) or analysis procedure(s).
- Provide documentation of detection and reporting limits.
- Provide full suite of QA and QC.

BIOLOGICAL MEASUREMENTS

- Change net mesh size.
- Change lab counting protocol.
- Change taxonomic resolution.

6.4 DECISION OF WHETHER OR NOT TO CHANGE PROTOCOLS

FS staff should be very careful about methods changes that potentially could influence chemical results while in the midst of an inventory or monitoring program. Especially if trends analyses are planned for the resulting data, it is always important to "compare apples with apples." In many cases, existing protocols, while not necessarily the preferred way of doing things, might best be left in place throughout the duration of a multi-year survey or of a long-term monitoring effort.

Staff must recognize that a methods change with unquantified impacts on sampling results will compromise their ability to make comparisons across space and across time. Especially when a long period of monitoring record already exists, potential methods changes must be very carefully considered and thoroughly documented with side-by-side sampling and analysis in order to preserve the integrity of future data comparisons. If it is determined that a change in protocols will require an adjustment of data values obtained using the original protocol, then FS staff should consult with a statistician or scientist well-versed in this kind of data adjustment.

SECTION 7.
TRAINING PLAN

T.J. Sullivan

7.1 BACKGROUND

Training people to collect water samples properly and to record all needed ancillary data is one of the most important aspects of a water survey, water quality characterization, or monitoring effort. This Training Plan provides guidance on how to conduct an effective training session for water sampling. It must be recognized that considerable personnel and financial resources go into field sample collection, laboratory analysis, QA/QC, and data analysis using the resulting data. If field staff are not attentive to detail or have not been properly trained, all this effort and expense can be wasted. All activities down the chain are dependent on collection of a good representative sample.

This Training Plan is based on National Water Chemistry Field Sampling Protocols for Air Pollution Sensitive Waters, developed for the FS ARM program by E&S Environmental Chemistry, Inc., and collaborating scientists from Oregon State University, the U.S. Geological Survey, and the University of Virginia. Key elements of the Protocols are summarized in this Training Plan. The Trainer should understand all elements of the Protocols before planning or conducting a training session. The materials in the Protocols are detailed and complex. The Trainer should, therefore, plan to devote considerable time to becoming thoroughly familiar with the materials in the Protocols before conducting a training session.

This Training Plan consists of an outline of the training materials and approach (this document), to be used in combination with a series of PowerPoint slides, handout materials, and three training videos. The slides and training videos serve as supporting materials. The slides will assist the trainer in covering all major issues in the training session. They will help to organize the presentation and facilitate discussion. The training videos are a more visual component and intended to augment the materials covered in the slides.

The protocols address both lake and stream sampling. Consequently, this Training Plan also addresses both types of surface water. A trainer can choose to cover either one or both in the training session. Recognize that, if it is not possible to access the deep water index site at a given lake, lakes are sometimes sampled at their outlet stream location; outlet stream sampling follows stream (not lake) sampling protocols. Therefore, it may be desirable to train all sampling personnel in the stream sampling protocols. National forests in some regions of the country contain few or no lakes; in such regions, it may not be necessary to conduct any training with respect to lake sampling protocols.

Seasonal or temporary field staff and volunteers should attend the full training session before beginning field work. It is recommended that all permanent or long-term field sampling personnel attend at least two full training sessions. Field personnel who sample year-after-year should attend the full session before each of the first two years of sampling. After that, attending annual training sessions could be optional for experienced samplers but they should annually review the PowerPoint slides, the stream sampling video, appropriate SOPs, and the lake sampling and discharge measurement videos (if applicable) at the beginning of each sampling field season.

The training session outlined here consists of two parts: classroom training and field training. The classroom training support materials include a set of PowerPoint slides, series of handouts, a stream sampling video, a lake sampling video, and a discharge measurement video. Classroom training should take about 4 hours, allowing limited time for discussion. The basic field training can also be conducted in about 4 hours if the lake sampling component and the stream discharge aspects are omitted. Thus, it should be possible to complete the full training for stream sampling (omitting discharge measurement), including both classroom and field components, in 1 day. If the training includes field training in lake sampling and/or discharge measurement, a second day may be needed.

7.2 PLANNING THE TRAINING SESSIONS

There are many things to consider before conducting a field training session for water sampling. These include:

- How close to the actual sampling period do you conduct the training?
- Where will the training session be held? (Field training will require a representative sample site for demonstration purposes and practice). Select a training site that is as similar as possible to actual study sites.
- What is an effective trainer/trainee ratio?
- Are the trainees new to water sampling or are they experienced? In other words: is this initial or refresher training?
- All sample teams should have equipment for the training. Will samplers bring their own equipment or will it be distributed at the session?
- How will sample bottles, syringes, and coolers be distributed?
- Is all of the necessary equipment available at the training site?
- Which portions of the training will be included?
- Will the training session last one day or two? If two days of training are anticipated, what arrangements are needed for overnight accommodations?
- How many vehicles will be needed to transport personnel and gear to the field training locations?

The following major steps should be taken to prepare for the training session:

1. Set the date and inform the trainees.
2. Make arrangements for overnight accommodations, if needed.
3. Order bottles and syringes from the lab.
4. Make arrangements for obtaining classroom materials:
    - Stream sampling video;
    - Lake sampling video (optional);
    - Discharge measurement video (optional);
    - PowerPoint presentation;
    - Projection equipment or viewing monitors for videos and PowerPoint;
    - Sample binders with forms and form instructions (one per trainee plus extras);
    - Backpack with sampling equipment, bottles, syringes, zipper lock bags, snap-on lid plastic containers for transporting syringes; and
    - Sample labels.
5. Cull the PowerPoint slides and handouts (if desired) to retain subsets of slides and handouts that are appropriate to your region, sampling needs, available training time, and expectations. Some important issues to consider in culling the slides and handouts include the following:
    - Will you cover stream sampling, lake sampling, or both? Some regions and districts have few or no lakes, and, therefore, lake protocols are probably not relevant. If lakes are to be sampled, the preferred approach is to sample in deep water from a boat or float tube. However, an acceptable backup approach (if deep water sampling is not possible) is to sample from the outlet stream using stream sampling protocols. Therefore, all staff should be trained in stream sampling protocols; not all staff necessarily require training in lake sampling protocols. If you will conduct field training in both stream and lake methods, you will probably need more than a half-day for field training.
    - Will you conduct field training in discharge measurement? If so, you will need to spend several additional hours to do so.
6. Make arrangements for field session material and logistics:
    - Vehicles;
    - Bottles and syringes; bags and plastic boxes for transport;
    - Maps to locate training demonstration site(s);
    - Sample labels;
    - Forms and form instructions;
    - Transport and access to training demonstration site(s);

- Coolers;
- Miscellaneous equipment and supplies; and
- Ice.

7.3 CLASSROOM TRAINING

The classroom training addresses major considerations in why, where, what, when, and how to collect water samples. There are many issues to consider and multiple options for how to proceed. In general, sampling decisions reflect the purpose of the data collection effort. The intended use of the data has a major influence on how one should proceed in designing and carrying out a sampling program. This should be emphasized to trainees. There is a series of optional PowerPoint slides that covers various aspects of study design. You may choose to include all, some, or none of these slides.

The first step in the classroom training is to emphasize to the trainees why it is important that they understand the protocols and why they need to take great care in their sampling and documentation efforts. The following questions should be addressed:

- Why do you have to be careful about site selection?
- Why do you need to be careful about when to sample?
- Why do you have to be careful about how you collect your sample?
- Why is attention to detail important?

After briefly reviewing the issues outlined above, the classroom training should focus on the PowerPoint presentation and/or training videos that can be found on the FS ARM program website (<*http://www.fs.fed.us/air*>). The major additional items covered in the PowerPoint presentation and videos include:

- Pre-trip activities;
- Site documentation;
- Collection of water samples;
- Post sampling actions;
- Logistics of stream sampling and documentation;
- Stream discharge; and
- Logistics of lake sampling and documentation.

All trainees should watch the stream sampling, lake sampling, and/or discharge measurement videos.

7.3.1 Handouts

Handouts are an important part of training. The recommended handout materials for training are the forms and instructions (Appendix E), sample container labeling instructions (Appendix F), and the equipment checklists (Appendix H) at the end of this report.

Go over every line of the checklists, label, and forms with the trainees to ensure that they fully understand what to do before sampling, what to take with them during sampling, and what is required in the way of documentation. Trainees should be given a sample label and each of the water chemistry forms (with detailed instructions) for lake and/or stream sampling, as appropriate. The trainer should carefully go through the label and each of the forms to make sure that trainees fully understand what is required for completing each.

7.3.2 Questions

Throughout the session and at the end of the classroom training session, ask questions of your trainees. Re-emphasize the importance of being careful to follow protocols and collect a good representative water sample.

7.3.3 Prepare for Field Training

Finally, pack up the equipment for the field training session, check to make sure that you have all of the needed equipment, supplies, and forms, and pre-fill out paperwork, as needed, for field sampling. Be sure that each of the following issues is highlighted in both the classroom and field training:

- Importance of attention to details;
- Selection of sampling location;
- How to avoid contaminating your sample;
- How to fill out the forms and labels; and
- Importance of avoiding transmission of invasive species between sites.

7.4 FIELD TRAINING

Field training offers an opportunity to put into practice the things that you learned in the classroom training. The field training program should involve having each trainee conduct all activities that would normally be conducted during an actual sampling event. The field training should include each of the following major elements:

- Pack up gear that will be needed;
- Locate site;
- Collect stream sample;
- Collect lake sample (optional);
- Measure discharge (optional);

- Clean up site, equipment, clothing, and boots;
- Labeling and documentation;
- Sample transport to vehicle; and
- Sample transport to lab.

Note that it may not be necessary to train field personnel in lake sampling protocols in some regions. Training in discharge measurement should also be considered optional. The field training session (without either lake sampling or discharge measurement) should take about four hours, assuming a very short hike to access the site. If you choose to include lake sampling and/or discharge measurement protocols, it might take a full day or two half-days for the field component of the training.

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Appendices

APPENDIX A. ACRONYMS AND ABBREVIATIONS 191

APPENDIX B. GLOSSARY 195

APPENDIX C. SAMPLING PROCEDURE FOR SURFACE WATER CHEMISTRY 203

APPENDIX D. STANDARD OPERATING PROCEDURES FOR FIELD SAMPLING ACTIVITIES 211

APPENDIX E. DATA ENTRY FORMS AND INSTRUCTIONS FOR FIELD SAMPLING ACTIVITIES 247

APPENDIX F. LABELING INSTRUCTIONS FOR FIELD SAMPLE CONTAINERS 271

APPENDIX G. EXAMPLE STANDARD OPERATING PROCEDURES FOR LAB ANALYSIS PROTOCOLS 275

APPENDIX H. TRAINING CHECKLISTS 313

APPENDIX I. JOB HAZARD ANALYSIS (JHA) 319

APPENDIX A. ACRONYMS AND ABBREVIATIONS

Al	Aluminum
Al^{3+}	Free aluminum (uncomplexed, trivalent)
Al_i	Inorganic monomeric aluminum
Al_m	Total monomeric aluminum
Al_o	Non-labile monomeric (presumed organically complexed) aluminum
$Al(F)_2^+$	An aluminum fluoride molecule
AlF^{2+}	An aluminum fluoride molecule
$Al(OH)^{2+}$	An aluminum hydroxide molecule
$Al(OH)_2^+$	An aluminum hydroxide molecule
ANC	Acid neutralizing capacity
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AQRV	Air quality related values
ARML	Air Resource Management Laboratory
BCS	Base cation surplus
BMP	Best management practices
BS	Base saturation of the soil
Ca^{2+}	Calcium
CEC	Cation exchange capacity
Cl^-	Chloride
CL	Confidence level
CO_2	Carbon dioxide
CPR	Cardio-pulmonary resuscitation
CQCCS	Calibration quality control check sample

CV	Coefficient of variation
DAP	Data analysis protocols
DIC	Dissolved inorganic carbon
DIW	Deionized water
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
DQO	Data quality objective
ELAP	Environmental Laboratory Accreditation Program
EMAP-SW	Environmental Monitoring and Assessment Program-Surface Waters
EPA	U.S. Environmental Protection Agency
EPT	Ephemeroptera-Plecoptera-Tricoptera Index
F	Fluorine
Fe	Iron
FS	U.S. Forest Service
GIS	Geographical information system
GPS	Global positioning system
H^+	Hydrogen ion
HCl	Hydrochloric acid
HCO_3^-	Bicarbonate
HDPE	High density polyethylene
Hg	Mercury
IBD	Ion balance difference
IBI	Index of Biotic Integrity
IQR	Interquartile range
JHA	Job Hazard Analysis
K^+	Potassium
LDPE	Low density polyethylene
LIMS	Laboratory Information Management System
m	Meter

MAGIC	Model of Acidification of Groundwater in Catchments – a watershed ion balance model
MCV	Median concentration value
MDL	Method detection limit
Mg^{2+}	Magnesium
ml	Milliliters
MPV	Most probable value
N	Nitrogen
Na^{+}	Sodium
NELAC	National Environmental Laboratory Accreditation Conference
NELAP	National Environmental Laboratory Accreditation Program
NH_4^{+}	Ammonium
NIST	National Institute of Standards and Technology
NO_3^{-}	Nitrate
NRM	National resource manager
NTU	Nephelometric turbidity units
NWRI	Environment Canada's National Water Research Institute
P	Phosphorus
PDE	Percent difference in enumeration
PE	Performance evaluation
PFD	Personal floatation device
PnET-BGC	Photosynthesis and Evapotranspiration – Biogeochemistry Model; A model of water, carbon, and nitrogen balance, coupled with a biogeochemistry model
ppm	Parts per million
PTD	Percent taxonomic disagreement
QA/QC	Quality assurance/quality control
QCCS	Quality control check sample
S	Sulfur
SBC	Sum of base cations
Si	Silicon
SKT	Kendal tau test
SLR	Simple linear regression

SO_4^{2-}	Sulfate
SOP	Standard operating procedures
SRP	Soluble reactive phosphorus
SRS	Standard reference sample
SSN	Sample serial number
TIME	Temporally Integrated Monitoring of Ecosystems program
TOC	Total organic carbon
TSS	Total suspended solids
USFS	U.S. Forest Service
USGS	U.S. Geological Survey
WTRS	Surface water sample depth zone: lake/stream water surface and water subsurface
µeq/L	Microequivalents per liter
µg/L	Micrograms per liter
µM	Micromoles per liter
µS/cm	Microsiemens per cm

APPENDIX B. GLOSSARY

Acid anion	Negatively charged ion that does not react with hydrogen ion in the pH range of most natural waters.
Acid neutralizing capacity (ANC)	The equivalent capacity of a solution to neutralize strong acids. The components of ANC include weak bases (carbonate species, dissociated organic acids, alumino-hydroxides, borates, and silicates) and strong bases (primarily OH^-). ANC can be measured in the laboratory by the Gran titration procedure or defined as the difference in the equivalent concentrations of the base cations and the mineral acid anions. It is a key indicator of the ability of water to neutralize the acid or acidifying inputs it receives. This ability depends largely on associated biogeophysical characteristics.
Acid-base chemistry	The reaction of acids (proton donors) with bases (proton acceptors). In the context of this report, this refers to the reactions of natural and anthropogenic acids and bases, the result of which is described in terms of pH and acid neutralizing capacity of the system.
Acidic deposition	Transfer of acids and acidifying compounds from the atmosphere to terrestrial and aquatic environments via rain, snow, sleet, hail, cloud droplets, particles, and gas exchange.
Acidic lake or stream	A lake or stream in which the acid neutralizing capacity is less than or equal to 0.
Acidification	The decrease of acid neutralizing capacity in water or base saturation in soil caused by natural or anthropogenic processes.
Acidified	Pertaining to a natural water that has experienced a decrease in acid neutralizing capacity or a soil that has experienced a reduction in base saturation.

Algae	Photosynthetic, often microscopic and planktonic, organisms occurring in marine and freshwater ecosystems.
Algal bloom	A reproductive explosion of algae in a lake, river, or ocean.
Alpine	The biogeographic zone made up of slopes above the tree line and characterized by the presence of rosette-forming herbaceous plants and low, shrubby, slow-growing woody plants.
Analyte	A chemical species that is measured in a water sample.
Anion	A negatively charged ion.
Anthropogenic	Of, relating to, derived from, or caused by humans or related to human activities or actions.
Atmosphere	The gaseous envelope surrounding the Earth. The dry atmosphere consists almost entirely of nitrogen and oxygen, together with trace gases, including carbon dioxide and ozone.
Autoecology	Study of the ecology of individual species, as opposed to the entire community of species.
Base cation	An alkali or alkaline earth metal cation (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}).
Base saturation	The proportion of total soil cation exchange capacity that is occupied by exchangeable base cations; i.e., by Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}.
Benthic macroinvertebrates	Animals without backbones that inhabit the bottom substrates of streams
Bioaccumulation	The phenomenon wherein toxic elements are progressively amassed in greater qualities as individuals farther up the food chain ingest matter containing those elements.
Biological effects	Changes in biological (organismal, populational, and community-level) structure and/or function in response to some causal agent; also referred to as biological response.

Calibration	Process of checking, adjusting, or standardizing operating characteristics of instruments or coefficients in a mathematical model with empirical data of known quality. The process of evaluating the scale readings of an instrument with a known standard in terms of the physical quantity to be measured.
Catchment	An area that collects and drains rainwater (also called "watershed").
Cation	A positively charged ion.
Cation exchange capacity	The sum total of exchangeable cations that a soil can adsorb.
Chronic acidification	The decrease of acid neutralizing capacity in a lake or stream over a period of decades or longer, generally in response to gradual leaching of ionic constituents.
Circumneutral	Close to neutrality with respect to pH (neutral pH = 7); in natural waters, pH 6-8.
Climate	Climate in a narrow sense is usually defined as the 'average weather' or more rigorously as the statistical description in terms of the mean and variability of relevant quantities over a period of time ranging from months to thousands or millions of years. These quantities are most often surface variables such as temperature, precipitation, and wind. Climate in a wider sense is the state, including a statistical description, of the climate system. The classical period of time is 30 years, as defined by the World Meteorological Organization.
Coarse stream substrate	Cobble to boulder (tennis ball to car sized; 64 mm to 4000 mm) size substrate.
Critical load	A quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge.
Decomposition	The microbially mediated reaction that converts solid or dissolved organic matter into its constituents (also called decay or mineralization).
Dissolved inorganic carbon	The sum of dissolved (measured after filtration) carbonic acid, bicarbonate, and carbonate in a water sample.

Dissolved organic carbon	Organic (derived from the breakdown of plant or animal material) carbon that is dissolved or unfilterable (0.45-µm pore size) in a water sample.
Drainage lake	A lake that has a permanent surface water inlet and outlet.
Ecosystem	The interactive system formed from all living organisms and their abiotic (physical and chemical) environment within a given area. Ecosystems cover a hierarchy of spatial scales and can comprise the entire globe, biomes at the continental scale, or small, well-circumscribed systems, such as a small pond.
Epilimnion	The layer of water in a thermally stratified lake that lies above the thermocline, is circulating, and remains perpetually warm.
Episodic acidification	The short-term decrease of acid neutralizing capacity from a lake or stream. This process has a time scale of hours to weeks and is usually associated with hydrological events.
EPT Index	Index of taxonomic richness of three insect orders (Ephemeroptera, Plecoptera, and Tricoptera)
Eutrophication	The process whereby a body of water becomes over-enriched in nutrients, resulting in increased productivity (of algae or aquatic plants) and sometimes also decreased dissolved oxygen levels.
Evapotranspiration	The process by which water is returned to the air through direct evaporation or transpiration by vegetation.
Fine/sand stream substrate	Stream substrate not gritty (silt/clay/muck < 0.06 mm diameter) to gritty (up to ladybug sized; 2 mm diameter) substrate.
Glide	Water moving slowly along stream channel, with smooth, unbroken surface; low turbulence.
Gran analysis	A mathematical procedure used to determine the equivalence points of a titration curve for acid neutralizing capacity.
Gravel stream substrate	Fine to coarse gravel (ladybug to tennis ball sized; 2 mm to 64 mm diameter) size substrate.
Ground water	Water in a saturated zone within soil or rock.

Hindcast	To estimate the probability of some past event or condition as a result of rational study and analysis of available data.
Hydrologic(al) event	Pertaining to increased water flow or discharge resulting from rainfall or snowmelt.
Hydrologic flow paths	Surface and subsurface routes by which water travels from where it is deposited by precipitation to where it drains from a watershed.
Hydrology	The science that studies the waters of the earth—their occurrence, circulation, and distribution; their chemical and physical properties; and their reaction with their environment, including their relationship to living things.
Hypolimnion	The layer of water in a thermally stratified lake that lies below the thermocline, is noncirculating, and remains perpetually cold.
Index of Biotic Integrity (IBI)	Provides assessment of biological condition based on a combination of metrics.
Invasive species	A species aggressively expanding its range and population density into a region in which it is not native, often through outcompeting or otherwise dominating native species.
Labile monomeric aluminum	Operationally defined as aluminum that does not pass through a cation exchange column; assumed to represent inorganic monomeric aluminum (Al_i).
Leaching	The removal of soil elements or applied chemicals by water movement through the soil.
Macrophyte	Rooted aquatic plant.
MAGIC	Model of Acidification of Groundwater in Catchments – a watershed ion balance model.
Mitigation	Generally described as amelioration of adverse impacts caused by a stressor such as acidic deposition at the source (e.g., emissions reductions) or the receptor (e.g., lake liming).
Model	An abstraction or representation of a system, generally on a smaller scale.

Monomeric aluminum	Aluminum that occurs as a free ion (Al^{3+}), simple inorganic complexes (e.g., $Al(OH)_n^{3-n}$, AlF_n^{3-n}), or simple organic complexes, but not in polymeric forms; operationally defined as extractable aluminum measured by the pyrocatechol violet method or the methyl-isobutyl ketone method (also referred to as the "oxine" method) is assumed to represent total monomeric aluminum. Monomeric aluminum can be divided into labile and non-labile components using a cation exchange column.
Non-labile monomeric aluminum	Operationally defined as aluminum that passes through a cation exchange column and is then measured by one of the two extraction procedures used to measure monomeric aluminum; assumed to represent organic monomeric aluminum (Al_o).
Occult deposition	The removal of gases and particles from the atmosphere to surfaces by fog or mist.
Organic acids	Heterogeneous group of acids generally possessing a carboxyl (-COOH) group or phenolic (C-OH) group.
Parameter	(1) a characteristic factor that remains at a constant value during the analysis, or (2) a quantity that describes a statistical population attribute.
pH	The negative logarithm of the hydrogen ion activity. The pH scale is generally presented from 1 (most acidic) to 14 (most alkaline); a difference of one pH unit indicates a tenfold change in hydrogen ion activity.
Phytoplankton	The plant-like forms of plankton. These single-celled organisms are the principal agents of photosynthetic carbon fixation in some fresh waters.
Plankton	Small (often microscopic) plant-like or animal species that spend part or all of their lives in open water.
PnET-BGC	Photosynthesis and Evapotranspiration Biogeochemistry Model: a model of water, carbon, and nitrogen balance, coupled with a biogeochemistry model.
Pool	In ecological systems, the supply of an element or compound, such as exchangeable or weatherable cations or adsorbed sulfate, in a defined component of the ecosystem.

Population	For the purpose of this report, 1) the total number of lakes or streams within a given geographical region or the total number of lakes or streams with a given set of defined chemical, physical, or biological characteristics; or 2) an assemblage of organisms of the same species inhabiting a given ecosystem.
Precision	A measure of the capacity of a method to provide reproducible measurements of a particular analyte (often represented by variance).
Primary Productivity	All forms of production accomplished by plants.
Quality assurance	A system of activities for which the purpose is to provide assurance that a product (e.g., data base) meets a defined standard of quality with a stated level of confidence.
Quality control	Steps taken during sample collection and analysis to ensure that data quality meets the minimum standards established in a quality assurance plan.
Rapid	Water movement along stream channel is rapid and turbulent; surface with intermittent "white water" with breaking waves; characterized by a continuous rushing sound.
Reachwide sample	All kick net samples collected at the 11 transects combined into a single composite sample.
Riffle	Water moving along stream channel, with small ripples, waves, and eddies; waves not breaking, and surface tension is not broken; characterized by a "babbling" or "gurgling" sound.
Scenario	One possible deposition sequence following implementation of a control or mitigation strategy and the subsequent effects associated with this deposition sequence.
Sensitivity	For this report, the degree to which a system is affected, either adversely or beneficially, by an effect of NO_x and/or SO_x pollution (e.g. acidification, N-nutrient enrichment, etc.). The effect may be direct (e.g., a change in growth in response to a change in the mean, range, or variability of N deposition) or indirect (e.g., changes in growth due to the direct effect of N consequently altering competitive dynamics between species and decreased biodiversity).

Species richness	The number of species occurring in a given ecosystem, generally estimated by the number of species caught and identified using a standard sampling regime.
Specific conductance	The conductivity between two plates with an area of 1 cm^2 across a distance of 1 cm at 25^o C. Provides an index of the ionic strength of a water sample.
Steady state	The condition that occurs when the sources and sinks of a property (e.g., mass, volume, concentration) of a system are in balance (e.g., inputs equal outputs; production equals consumption).
Stream flow	Water flow within a river channel, for example, expressed in m^3/s or cfs (cubic feet per second). A synonym for river discharge.
Subpopulation	Any defined subset of the target population.
Support reach	The length of stream to be sampled at a sampling location.
Total monomeric aluminum	Operationally defined as the simple unpolymerized form of aluminum present in inorganic or organic complexes.
Turnover	The interval of time in which the density stratification of a lake is disrupted by seasonal temperature variation, resulting in entire water mass becoming mixed.
Variable	A quantity that may assume a numeric value during analysis.
Watershed	The geographic area from which surface water drains into a particular lake or point along a stream.
X-site	Stream sampling location.
Zooplankton	The animal forms of plankton. Zooplankton include crustaceans, rotifers, pelagic (open water) insect larvae, and aquatic mites.

APPENDIX C. SAMPLING PROCEDURE FOR SURFACE WATER CHEMISTRY

Robert Musselman
US Forest Service, Rocky Mountain Research Station

This sampling procedure provides guidance in container handling for stream sampling projects conducted by the Rocky Mountain Research Station. It is intended for training purposes and may be augmented as needed with requirements from formal Standard Operating Procedures (SOPs).

Locate Sample Collection Site

Streams are always sampled upstream from any manmade structure such as a bridge, culvert, or flume. Lakes are sampled at their outlet unless the chemistry of the lake profile is needed. Approach the sampling location from downstream if possible. Choose a sampling spot that is fast-moving, at least 15–20 cm (6–8 inches) deep if possible, and where you can reach it from a solid place on the stream bank (a rocky, not soft or spongy, spot) or from a large rock.

Collect from the same sampling site each time if collections are repeated over time. Check the previous collection field notes for exact sampling location. A GPS identification of the location longitude/latitude/elevation is helpful.

Take Water Temperature

Place a thermometer in the water near the sampling point—preferably downstream. Avoid disturbing the bottom at the sample site. Allow the thermometer to equilibrate (reach stream temperature and stop changing readings) and record the temperature. It may take a couple of minutes for the thermometer to equilibrate. Digital thermometers equilibrate faster and it is easier to determine when they have reached water temperature.

Label Bottle Correctly

Sample bottles are typically brown, 250 ml (about 1 cup volume), high-density polyethylene (HDPE) or low-density polyethylene (LDPE) plastic, unless otherwise provided. Before immersion of the sample bottle, use a black permanent marker to write on the dry bottle the sample location (geographic area name and stream or lake name—e.g., Indian Peaks Wilderness,

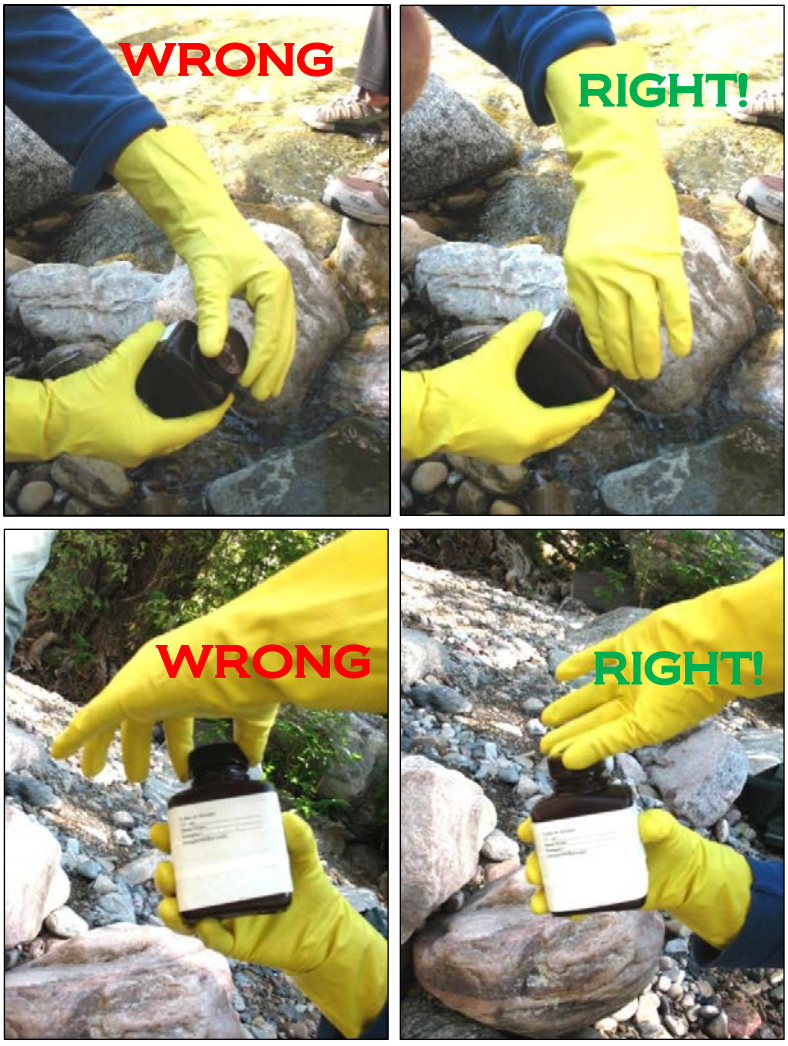
Blue Lake), date and year, time of day (indicate daylight or standard time as MDT or MST for Mountain Daylight Savings Time or Mountain Standard Time), water temperature (add ^{o}F or ^{o}C), and sampler's first and last names.

Put on Gloves and Rinse Bottle and Gloves

After the bottle is labeled, put on the gloves (powder-free latex or nitrile) and rinse the capped bottle and the gloves downstream to remove any possible contaminants. Be careful not to touch your face, the ground, clothing, or anything except the bottle and cap after the gloves and bottle are rinsed. Fingers contain contaminants such as nitrates, and insect repellents, sunscreen, and cigarette smoke are particularly troublesome as contaminants. Avoid breathing directly in/on the bottle or cap during sample collection.

Prepare Bottle for Sampling

First, rinse the inside of the bottle, the inside of the cap, and then rim of the bottle with the deionized water (DIW) that comes in the bottle. To rinse, remove the cap with index finger and thumb, palm facing up. If the cap is not held correctly when removed, there is an increased possibility of pouring water over your hand and into the cap or bottle when rinsing.



To remove the cap for rinsing: DON'T have your palm facing the bottle. DO have your palm facing AWAY from the bottle.

Once the cap is removed, turn your palm down to face the cap up, fill the inside of the cap with DIW, and empty it over the rim and threads of the bottle, pointing the mouth of the bottle downward and being careful not to pour water over your finger and/or thumb or back into bottle.

To hold cap for rinsing: DON'T have your palm facing up; DO have your palm facing DOWN.

To collecting rinse water in cap, keep fingers and palm BELOW and OUT OF THE WAY.

Rinse the rim of the bottle with water from the cap, with fingers and palm OUT OF THE WAY and the bottle opening DOWN.

Rinse the cap and bottle rim about six times, then completely empty the bottle of any remaining DIW. Pour discarded DIW downstream or off-stream on land away from the sampling site. After the final rinse, place cap back on and tighten it ¼ turn, hold the cap on the bottle with index finger of the hand holding the bottle. Now the sample rinses can be collected.

RINSE THE BOTTLE WITH SAMPLE WATER

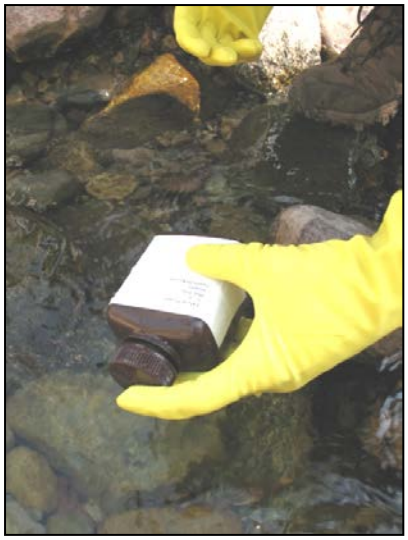
After DIW rinsing, the bottle is rinsed again with water from the sample location to remove any DIW left in the bottle. This ensures that the bottle will eventually contain only sample water for analysis. Collect water for the rinses in the same way as collecting the sample afterward to ensure good habits in practice.

When collecting water on the left side of stream (facing upstream), hold the bottle with your right hand, cap pointing upstream. Remove cap with the index finger and thumb of your left hand, with your palm placed under the bottle (palm and other fingers should not be upstream of the bottle).

When collecting water on the right side of stream (facing upstream), hold the bottle with your left hand, cap pointing upstream. Remove the cap with the index finger and thumb of your right hand, with your palm placed under the bottle (palm and other fingers should not be upstream).

Whether you are on the right or left side of the stream (facing upstream) and whether you are holding the bottle in your right hand or left hand, always be careful that the hand holding the bottle and your palm and fingers of the other hand tightening and loosening the cap are not upstream of the bottle opening.

Move to your sampling point and reach as far into stream as possible. Hold the cap on the bottle with your index finger.



To handle the sample bottle in preparation for sampling, hold the cap on with your index finger.

Immerse the bottle completely about 10 cm (4 inches) deep or half-way to the bottom if the stream is shallow (be sure to write the depth the sample was collected on the field notes after the

sample is collected and secured). If the stream is too shallow to immerse the bottle fully, immerse it as far as possible and collect as much sample water as possible, being very careful not to touch the bottom where sediments can be disturbed and making sure that no surface film flows into the bottle. A syringe (appropriately rinsed at least three times with DIW) may be used to fill the bottle if necessary, but it is best to collect the sample directly into the bottle. If a syringe is used, be sure to document this in the field notes.

Place the bottle flat on its side underwater, pointing the mouth of the immersed bottle upstream. Place the thumb and forefinger of your free hand on the cap before removing the index finger holding the cap on the bottle. Remove the cap with your thumb and index finger, with the palm of that hand placed under or over the bottle to make sure the palm is not upstream of the bottle.

To remove cap under water: DON'T have your hand upstream of the bottle;
DO keep your palm UNDER or BESIDE the bottle.

Be very careful that any water entering bottle does not touch your gloves on its way toward the bottle by making sure your hands and fingers are not held upstream of the bottle when the cap is removed. Move the cap enough to let water enter the bottle. Fill the bottle about half full. It is not necessary to fill the bottle completely for rinses. Place the cap back on loosely (¼-turn) underwater, holding cap on with index finger of the hand holding the bottle. Always remove and replace the cap underwater.

Remove the immersed and capped bottle from the stream and shake it. It is okay if some water leaks out rinsing the rim during shaking. The rinse procedure is then the same as described for DIW.

Rinsing must be conducted downstream of the sample site. Rinse the cap and rim three times, being careful that the rinse water does not touch your gloves. The bottle opening should be pointed downward during rinses to avoid rinse water flowing back into the bottle. This is easier if the bottle is only half-filled (or half-dumped) for rim/cap rinses. After the three separate cap/rim rinses, pour out any remaining rinse-water downstream of the sample point. Do not touch the bottle mouth or the inside of the cap.

COLLECT THE SAMPLE

After three stream-water rinses, collect the final sample on the fourth immersion. Use the same procedure as before, but fill the bottle completely.

Tip the bottle mouth up but still underwater to remove all air bubbles from the bottle before capping. If necessary because the stream is shallow, squeeze the bottle slightly as the cap is tightened so that no air remains in bottle. Be sure that the cap is on tight, first underwater and again when the bottle is taken out of the stream.

Be especially careful not to contaminate the sample with surface film or bottom sediment. If this happens, discard the sample and start the procedure over with three more stream-water rinses.

If the stream is too shallow to immerse bottle fully when tipped up for filling, collect as much as possible, being very careful not to touch the surface or bottom. Note the sampling depth on field notes.

SEAL AND STORE BOTTLE FOR TRANSPORTATION

Once the sample is collected, seal the sample bottle immediately in a zipper-lock bag, place the bag in a cooler, and keep the bag cold with frozen ice-packs or snow (if using snow, place the snow in two nested zipper-lock bags). Do not place snow or ice in the same bag as the sample. Do not expose sample bottles to the sun.

COMPLETE MISCELLANEOUS POST-SAMPLING TASKS

After all samples are secured in the cooler, remove the gloves, rinse them in the stream, and place them in a zipper-lock bag. Before leaving the field site, make sure the field data sheet is completed. Note any noteable weather conditions such as wind or rain. Measure the air temperature (the thermometer must be shaded) and record it. When you return from the field, remove the gloves from their bag, rinse the gloves (with DIW if available), and dry them out for reuse.

SHIPPING INSTRUCTIONS

Samples are filtered in the lab, so no further processing is typically necessary in the field. Hand-delivery to the lab is preferred, either by the sampling team or through lab pick-up arrangements.

Keep samples cool during shipment. Ship them overnight to the pre-assigned lab in a hard-sided cooler with frozen icepacks via FedEx or UPS. Labs are not typically open on weekends or holidays, so do not ship the samples unless they can arrive at the lab on a workday, Monday through Friday. If you need to store the samples before shipping them, keep them refrigerated at 39-40^{o}F (4^{o} C) but do not allow them to freeze. Ship them using an afternoon pickup for next-day arrival if possible to shorten shipping time.

COLLECT DUPLICATE AND BLANK SAMPLES

Collect a duplicate sample if so instructed. Generally, every 10th to 15th sample collected is replicated (duplicated). Sample sites chosen for duplicate sampling are selected at random among streams or lakes sampled. If a duplicate is required for your site, repeat the same procedures as with the normal stream samples. The duplicate is the second of the two samples collected. Write "DUPL" on the sample bottle before the sample is collected. Duplicates document the repeatability of individual sample collections and reproducibility of laboratory results.

Take a field blank sample bottle to the field if so instructed. The field blank (FB) remains unopened. Field blanks are included in the analysis to quantify chemicals from non-sample sources such as water bottles, DIW, filter paper, handling procedures, etc. Write the name of the sample study area, stream or lake name, date and year, sampler's name, and "FB" on the bottle. The FB bottle is never opened in the field; however, the extra FB bottle can be used in place of a sample bottle in an emergency: for example, if a sample bottle or its cap is lost or contaminated. The FB is not as vital to the analysis as the samples.

ACKNOWLEDGEMENTS

Thanks to Andrea Holland-Sears for the photos taken during a training session August, 2011, to Dave Richie for providing the demonstration for those photos, and to Cass Cairns for the rim-rinsing photo.

INFORMATION SOURCES FOR WILDERNESS LAKE SAMPLING

Where to find blank field sampling forms:

Go to: <*http://www.fs.fed.us/air/*> and click on: Forms.

Where to find field sampling protocols:

Go to: <*http://www.fs.fed.us/air/*> and click on: Sampling Protocols.

Where to obtain water bottles and where to ship samples:

Louise O'Deen
Lab Manager
USFS/USGS Water Chemistry Laboratory
2150 Centre Avenue, Building C
Fort Collins, CO 80526-8118

Shipping notes:

Ship your samples by overnight UPS or FedEx Monday through Thursday for workday arrival. Samples should be shipped in a cooler with icepacks. Do not ship on Friday. The lab is not open weekends. Keep samples refrigerated and ship Monday.

For more information, contact:

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APPENDIX D.
STANDARD OPERATING PROCEDURES FOR FIELD SAMPLING ACTIVITIES

D-1 STANDARD OPERATING PROCEDURE FOR STREAM SAMPLING

This Standard Operating Procedure (SOP) provides guidelines for stream sampling within the FS ARM program. It is intended as a Generic SOP, suitable for adoption as a stand-alone procedure, or for modification to fit local program needs. It is divided into individual sections that cover pre-trip activities, sampling site documentation, stream sampling and sample handling, measurement of stream discharge, post-trip activities, and needed equipment and supplies.

D-1.1 Pre-Trip Activities

Field teams conduct a number of activities in their office or at a base site. These include tasks that must be completed both before departure to the sampling site and after return from the site. This section describes pre-trip procedures for office and base site activities that should be carried out in support of stream sampling.

Pre-departure activities include development of sampling itineraries, instrument calibration if appropriate, equipment checks and repair, supply inventories, and sample container preparation. Procedures for these activities are described in the following sections. An example checklist for materials and supplies is given in Table D-1. Use this checklist to ensure that equipment and supplies are organized and available at the stream site in order to conduct the activities efficiently. Remember to take any safety equipment required by your unit (e.g., hard hats, radios, or cell phones).

Table D-1. Checklist of materials and supplies for stream sampling site visits.

Standard Items:	✓
Collection permits and entry permits, if required	
Site documentation forms (for new sites)	
Clipboard	
Site documentation reports (compiled as folders for existing sites)	
Stream sampling record forms	
Insulated container with ice or frozen refrigerant (packed in sealed plastic bags or other containers)	
Small insulated container (with ice) for hike-in sites	
Watch for recording time	
Digital field camera with free memory and extra charged battery	
GPS unit with extra batteries	
Compass	
Field thermometer (with string attached)	
Pre-processed sample bottle(s) with completed sample label attached. Include a second bottle if sampling at that site is to be replicated. Put each bottle in a clean plastic zipper-lock bag.	
Plastic gloves in sealed plastic bag	
60 mL plastic syringes (with Luer type tip) with completed sample labels attached. Plastic container with snap-on lid to hold filled syringes	
Syringe valves (Mininert® with Luer type adapter, or equivalent, available from a chromatography supply company)	
Water Chemistry labels (if not already filled out and attached to sample containers at base site)	
Soft-lead pencils and write in rain type pens for filling out field data forms and notebook entries	
Fine-tipped indelible markers for filling out labels	
Roll or box of tape strips	
Field operations and methods documents	
First aid kit	
Backpack	
Extra zipper-lock bags	
Optional Items (may be required for specific studies):	
60 mL glass bottles with septum caps and with completed sample labels attached	
Calibrated multiparameter sonde, data logger and cable, with extra batteries	
Calibration standards, quality control check samples, DIW, rinse bottles, waste tray and container, calibration cup, and sensor guard for sonde (multiple sensors combined in a unit that is lowered into the water)	
Sonde calibration and post-calibration record forms	
Measurement tape	
Waders or high-top water proof boots for wading	
Clear packaging tape to cover labels	
Dissolved oxygen (DO)/temperature meter with probe	
DO repair kit containing additional membranes and probe filling solution	
Conductivity meter with probe	

PREPARATION OF SAMPLE CONTAINERS

Before leaving the base location, package the sample containers, typically two sample bottles and two 60 mL syringes for each site to be sampled, plus backup bottles and syringes in the event that one is lost or contaminated. Make sure to have plastic containers for transport of filled syringes from the field to the laboratory. Fill out a set of water chemistry sample labels and attach a completed label to each sample bottle and/or syringe. Make sure the syringe labels do not cover the volume gradations on the syringe. Place each sample bottle in a separate zipper lock bag. Finally, make sure that ice and or refrigerant for shipment to the lab is frozen or freezing so that it will be ready when you return from the field to the base location with the samples.

DAILY ITINERARIES

Field sampling efforts should include a Project Leader who guides activities in the field, and a Project Coordinator who remains in the office during the sampling effort. The Project Leader reviews each site folder to ensure that it contains the appropriate maps, contact information, copies of access permission letters (if needed), and access instructions. Additional activities can include confirming the best access routes, calling landowners or local contacts (if applicable), confirming lodging or camping plans and locations (with directions), and coordinating rendezvous locations with individuals who must meet with field teams before accessing a site. This information is used to develop an itinerary.

The Project Leader should provide the Project Coordinator with a schedule for each day of sampling. Schedules include departure time, estimated duration of sampling activities, routes of travel, and estimated time of arrival at the sampling site(s) and return to the base site. Changes that might be made to the itinerary should be relayed by the Project Leader to the Project Coordinator as soon as possible. Miscommunications can result in the initiation of expensive search and rescue procedures and disruption of carefully planned schedules.

INSTRUMENT CHECKS AND CALIBRATION

If appropriate, each field team should test and/or calibrate field instruments before departure for the sampling site. Such testing may be appropriate for dissolved oxygen (DO) meters, global positioning system (GPS) units, and perhaps other instrumentation. Batteries should be checked before departure for field sites. Extra batteries should be carried.

Field personnel should check the inventory of supplies and equipment before departure using project-specific site-visit checklists. Meters, probes, and sampling gear should be packed for transport to the field in such a way as to minimize physical shock and vibration during transport. Rafts or float tubes should be packed for transport so as to minimize the potential for puncture by any sharp object.

D-1.2 SITE DOCUMENTATION

BACKGROUND

This section describes SOPs for establishing and documenting sampling sites on small well-mixed streams or lake outlets. This procedure applies to new sites for which approximate locations have been designated based on program objectives and sampling design. It also applies to previously established sites for which current or updated site documentation is needed.

OBJECTIVE

The objective of this procedure is to establish and document new sampling sites and to update documentation for established sites, providing:

- Site descriptions and notes;
- Travel and access descriptions and notes;
- Site coordinates obtained in the field using a GPS unit;
- Site and access-related photos; and
- Placement or confirmation of numbered site tags (where applicable).

For established sites, existing site documentation will be evaluated for clarity and improved as needed based on conditions observed in the field.

MATERIAL NEEDED FOR USE IN FIELD FOR SITE DOCUMENTATION

1. Available site documentation records for previously established sites:
    a. Site location maps, topographic maps, and road maps;
    b. Site descriptions and access notes;
    c. Site tag numbers and tag tree descriptions (where applicable);
    d. Site coordinates; and
    e. Site photos.
2. Preliminary site documentation for new sites:
    a. Site location maps, topographic maps, and road maps indicating approximate site locations; and
    b. General site descriptions and access notes.
3. General material for site documentation:
    a. Regional-scale topographic and road maps;
    b. Stream water sampling site documentation forms on waterproof paper;
    c. Clipboard or field notebook and pens for use with waterproof paper;
    d. GPS unit with replacement batteries;
    e. Digital camera with charged battery and charged replacement battery;
    f. Site tags, aluminum nails, and hammer (if applicable);
    g. Measuring tape;
    h. Blaze orange material for flagging tag trees in photos (if applicable);
    i. Gate keys (if needed); and
    j. Cell phone with numbers of project staff and management agency offices.

SEQUENCE OF INITIAL SITE DOCUMENTATION ACTIVITIES

1. Initiate the Stream Sampling Site Documentation and Sample Record Forms.
    a. Complete the header information on each form (1-5). See Stream Form instructions in Appendix E for details on completing the forms correctly.
2. Select or locate using GPS the specific sampling site (applies to new sites).
    a. The approximate or preliminary location of new site locations will be indicated on topographic maps. The sample collection team must still determine the exact point on the stream to be sampled.
    b. Avoid establishing sites where streams may not be well mixed, such as locations in close proximity to inflowing tributaries or braided channels. Also avoid locations that may be influenced by runoff from disturbed areas, roads, trails, drainage ditches, or other sources of inflow. Select sites that are upstream rather than downstream of potentially altered inflow. As a general rule, select sites that are at least 25 m above or below confluence points or inflow.
    c. The best point to sample will be where the water is flowing fast or falling, where there are no eddies, and where the depth is at least 8 inches (20 cm). Ideally the sampling point is one that can be reached while kneeling on the stream bank or on stable rocks downstream from the sampling point. If possible, avoid standing in the water to reach the sample point.
3. Obtain coordinates at the site using the GPS unit. These coordinates will be part of the site identification information entered into the national database (NRM Air) and must be documented exactly the same on future visits to the site.
    a. The unit position format should be set to Decimal Degrees (hddd.ddddd). The Datum should be set to NAD83. Distance and elevation should be set to meters. Wide Area Augmentation System (WAAS) should be enabled.
    b. When “Mark Waypoint” is selected, the default GPS site ID (a number) should be changed to the actual Site ID.
    c. Before saving the coordinates, note the estimated accuracy of the measurement on the Site Documentation Form 1.
    d. Save the coordinates in the GPS unit’s memory and record both the coordinates and the elevation on the Sampling Site Documentation Form 1. Do not rely solely on the GPS to store the coordinates.
    e. Confirm that the waypoint has been saved in the GPS unit.
4. Enter the approximate stream depth and width on the Sample Record Form 3.
    a. Enter the approximate average values for stream depth and width observed in the sampling site area (about 5 m upstream and downstream of the sampling site) on the sample site documentation date.
5. Enter site description information on the Site Documentation Form 2.
    a. For existing sites, enter information to improve and update existing site description information.

b. Generally describe the site, referring to proximity to landmarks (trails, bridges, tributaries, trees, landscape features, or other relatively permanent features). Add any additional information, including detailed stream bank and stream structure descriptions, that will help future sample collectors indentify the site. Also, add any information here that might be relevant to water and stream quality, such as cleared land, roads, construction, logging, development, or any earth disturbance, etc. observed above the site in the watershed or in the stream.

c. As a general convention, the right bank and left bank of a stream are determined based on looking downstream. When documenting field observations on the forms, indicate whether the observation was made looking downstream or upstream.

6. Enter travel and access directions on the Site Documentation Form 1.

   a. For existing sites, improve and update existing travel and access information.

   b. Travel and access notes should be sufficient to guide future sample collectors to the site without reliance on GPS units. Not all future sample collectors will necessarily have GPS units.

   c. Access notes should refer to trails, roads, and permanent landmarks, providing distances and, where helpful, compass-based directions. Backtrack if necessary to determine distances. Linear distances and directions from the established site waypoint can be determined using the GPS unit.

   d. In cases where a parking location is not immediately adjacent to the sampling site, use the GPS unit to obtain the coordinates for the parking location and record these in the travel and access information area of the Site Documentation Form 1.

   e. For complicated or long walk-ins, use the GPS unit to record and save a track. But again, do not rely on future sample collectors having access to a GPS unit.

   f. Sketch the route on the back of the Site Documentation Form for scanning and saving as a jpg image if that would be helpful to future sample collectors.

7. Obtain site and access photos.

   a. For site documentation, if the camera allows, set the camera's picture size at 3 megapixels. This will create picture files of about 550-650 kilobytes (Kb). Larger, higher resolution files are not needed for site documentation work. Switch to higher resolution if you are taking pictures for other purposes.

   b. Photos should be obtained providing views looking downstream and upstream of the sampling site, views of the tag tree (if applicable, with blaze orange material attached), and views of other distinguishing features in relation to the site (e.g., trails, roads, notable rocks, trees, landforms, bridges, and signs). Photos should also be obtained to show important aspects of site access (e.g., parking area and forks in the trail).

   c. All photos should be listed on Site Documentation Form 2, including the filename, date, and description. Enter this information at the time that the pictures are taken. Do not rely on memory for later entry of photo descriptions. The entered description should serve as the photo caption for site documentation reporting.

D-1.3 Stream Sampling

Water chemistry data are used to characterize acid-base status, trophic condition, and to classify streams based on their water chemistry. Samples for analysis of most parameters are collected into plastic bottles. Syringe samples or samples collected into glass bottles with septum caps are preferred for collection of sample aliquots for laboratory analysis of pH and DIC where practical. Syringes and septum caps are used to protect samples from exposure to the atmosphere because the measured values for these parameters can change if the stream water sample equilibrates with atmospheric CO_2 subsequent to collection.

Stream samples are obtained at a single sampling location below the water surface in the portion of the stream cross section that appears visually to represent the greatest amount of flow or, alternatively, at mid-channel in an area of flowing water. Spatial variability across the channel of a single stream is expected to be minimal in relatively small wadeable streams as compared to the variability expected among sites, so a composite water chemistry sample is not required.

At each stream, optional on-site water data and streamside measurements are made using field meters and recorded on Sample Record Form 4. Stream water is collected in one or more bottles and two 60 mL syringes or glass bottles with septum caps that are stored on ice in darkness and shipped or driven to the analytical laboratory as quickly as possible after collection. Overnight express mail to the laboratory is required for these samples because the syringe or glass bottle samples need to be analyzed, and some or all of the bottled sample needs to be stabilized (by filtration and/or acidification) within a short period of time (typically 72 hours) after collection. Check with the analytical laboratory in advance of sampling regarding applicable holding times for the parameters to be measured.

These SOPs describe the process for routine sampling and data collection at water quality monitoring sites on streams. Water samples are collected for lab analysis with optional on-site measurements of selected water quality parameters (i.e., water temperature, specific conductance, pH, DO, and turbidity) using a multi-parameter instrument (sonde).

This section describes procedures to be followed for data collection at established water quality monitoring sites. The sites may be part of a synoptic sampling or fixed long-term sample site program for which water quality data and water samples are collected on a scheduled periodic basis.

DOCUMENTATION OF DATA AND SAMPLE COLLECTION

The Stream Sampling Record Forms 3, 4, and 5 are used to document sample collection and record all field data. These forms are used to record the following information:

- The organization, site ID, and site name;
- The date and arrival time for the site visit and specific times of measurements obtained;
- The name, contact information, and affiliation of the individual who is the Collector of Record and responsible for protocol adherence during the site visit;
- Suggested revisions or amendments to site documentation and travel directions;
- A listing of site-related photographs taken, including file name, date, and descriptions;
- Qualitative descriptions of weather, stream discharge level and appearance, and other factors that might influence water quality during the site visit;
- Air temperature;
- Results for all water quality data collected on-site, including:
    a. Numerical results, units, and measurement time; and
    b. Instruments used.

- Identification of calibration and post-calibration sensor check records,
- Results for all discharge data collected, including:
    a. Location of measurement site relative to the sampling and data collection site;
    b. Numerical results, units, and specific time of measurement;
    c. Methods identification; and
    d. Identification of discharge record files.
- A listing of all samples collected, including:
    a. Collection time;
    b. Types of samples collected and number of replicates; and
    c. Method of delivery to analytical lab.

If desired, on-site measurement of one or more parameters can be made using a multi-parameter water quality sonde (a hand held instrument with a probe containing multiple sensors for measuring various physical parameters) that is lowered into the water. Such measurements might include temperature, pH, DO, specific conductance, and/or turbidity. The procedures for such *in situ* data collection will vary with the specific field instrument, but in general require the following steps:

- Initiate water quality sonde field calibration and calibration checks.
- Record results on a water quality instrument calibration and post-calibration record form.
- Confirm that sensor check criteria are met. If criteria are not met, recalibrate, perform sensor maintenance, or replace sonde or sensors as needed to meet the criteria.

Deploy the water quality sonde for the period required to obtain stabilization. Enter the results and time of measurement in the On Site Water Quality Data section of the Stream Sampling Record Form 4.

SEQUENCE OF ACTIVITIES FOR DATA COLLECTION

Collectors are advised to avoid entering or disturbing the stream or stream bank at, or upstream of, the collection site before sample collection and completion of water quality data documentation. The typical sequence of activities on arrival at the sampling site is as follows:

1. Confirm the site location based on information in the Site Documentation Forms, including coordinates, photos, and access notes.
2. Initiate completion of the Sample Record Forms.
3. Complete Site Information and General Observations sections of the Stream Sampling Record Form 3.
4. Enter information needed to improve or correct the site description and travel directions provided on the Sample Record Form 3.
5. Obtain any photographs needed to improve site documentation and enter file names, dates, and descriptions.
6. Note any factors (other than weather and discharge conditions) that might affect water quality (e.g., bank or upstream disturbance or debris in the water).

7. Collect water samples and complete the Water Sample and Replicates section of the Sample Record Form 3. Enter any on-site data into that section of the Sample Record Form 4.
8. Complete the Chain of Custody Form (see Appendix E for the form and instructions).
9. Check to make sure that all of the information recorded on the sample label(s), chain of custody form, and stream sampling record forms match.
10. Obtain discharge measurements or stage height data, if required. Indicate method, time of measurement, result, name of the record file, and location of measurement relative to the data and sample collection site in the Stage and Discharge Data section of the Sample Record Form 5. Note that discharge gaging may be conducted at the same time as other site visit activities if the discharge measurement site is downstream of the water quality and data collection site. Also note that measurements of discharge or stage height are considered optional.

SAMPLE COLLECTION

In the field, make sure that all labels are filled out correctly (see Appendix E for detailed instructions) and that the labels on the bottles (and syringes, if used) are securely attached. Carefully avoid disturbance of water upstream of the sampling point before sample collection. This means: do not walk in the upstream water or on upstream rocks of the sample site.

Collect a water chemistry sample from the middle of the stream channel at the sampling site, unless no water is present at that location. Throughout the collection process, it is important to take precautions to avoid contaminating the sample. Wear gloves provided in sample bag. Rinse all sample containers three times with stream water before filling them with the sample. Many streams have a very low ionic strength and can be contaminated quite easily by perspiration from hands, sneezing, smoking, insect repellent, sunscreen, or chemicals used when collecting other types of samples (e.g., formalin or ethanol). Make sure that none of the water sample contacts your hands before going into the sample bottle or syringe. The chemical analyses conducted using the syringe samples can be affected by equilibration with atmospheric carbon dioxide; thus it is essential that no outside air contact the syringe samples during or after collection.

Document the information from the sample bottle/syringe label on Stream Sample Record Form 3. Note any problems related to possible contamination in the form comments section.

General stream sample collection procedures for water chemistry are as follows. See additional detail under Section 1.4.2.

Collection into Bottle

1. Select sample location in a flowing portion of the channel near the middle of the stream.
2. Put on gloves provided in the sample bag.
3. Always keep the empty sample bottles capped when it enters and leaves the water.
4. Rinse sample bottle and lid three times with stream water, dumping rinse water on the bank or downstream of sampling location.
5. Fill the sample bottle(s) completely, holding the bottle in a tilted position approximately at the midpoint between the water surface and the streambed, being careful not to disturb any sediment before or while collecting the sample.

6. Cap the bottle underwater. Bottles should be filled so that no air bubbles remain in the bottle and capped very tightly while submerged. This may require tipping the bottle up and squeezing the sides of the bottle slightly underwater to help force out any bubbles. This procedure is of extreme importance if a septum cap is to be used.
7. Put the sample bottle into a clean plastic zipper-lock bag.
8. Place the sample bottle(s) in a cooler (on ice or in stream water) and shut the lid. This may be a soft cooler for packing out of the field to the vehicle or a hard cooler in the vehicle. If a cooler is not available, place the bottle(s) in an opaque garbage bag and immerse it in the stream.

Collection into Syringe

1. Rinse the syringe three times with water from the sampling location.
2. Slowly fill the syringe with sample, avoiding generation of air bubbles, until it is two-thirds to three-fourths full. This will help to ensure that the plunger remains inserted far enough into the filled syringe so that it will not be likely become dislodged during transport.
3. Expel any air by tilting the syringe upward and depressing the plunger to force the air out.
4. Repeat procedure using a second syringe.
5. Place the filled syringes into plastic container for transport.

Collection from a Very Shallow Stream

If the stream is too shallow to collect a sample using standard procedures, the following approach can be used using a new clean syringe at each site:

1. Rinse the syringe three times with stream water, downstream of the sample site, as usual.
2. Use the syringe to put stream water in the sample bottle and rinse the sample bottle three times.
3. Finally, use the syringe to fill the bottle to the brim with stream water at the sample site. Cap the bottle and proceed as usual.

SAMPLE COLLECTION PROCEDURE

The sample should be collected on a step-by-step basis as follows:

1. Remove the gloves from the plastic bag and put them on.
2. Remove the sample bottle from the plastic bag. Do not put the bag on the ground.
3. Check to ensure that the correct labels are affixed to each sample bottle and syringe.
4. Rinse the sample bottle in the stream at a location at least 2-3 feet downstream of the sample collection point. Always keep the empty sample bottle capped when entering and leaving the water. The bottle and cap should be rinsed three times. For each rinse, fill the bottle and then pour the rinse water over the inside of the cap, held bottom-side up in the other hand. Pour the rinse water downstream of the rinsing and sampling points and avoid stirring-up the streambed debris during the process.
5. After the rinsing is completed, move upstream to the sampling point and collect the sample by submersing the tilted bottle or syringe to a depth midway between the sediment and the water surface. If collecting in a bottle, remove the cap and fill up the bottle as completely as possible. Cap the bottle while underwater making sure no air bubbles remain. This can be done by tilting the bottle up and squeezing the sides slightly before putting on the cap. This procedure is of extreme importance if a septum cap is to be used. While collecting the sample, avoid stirring-

up streambed debris that might be collected with the sample. Try to avoid generating large bubbles in the bottle while it is being filled. Make sure the sample does not flow over sampler's gloves or the outside of the bottle during collection. This can often be best achieved by sampling rapidly flowing or falling water. If debris may have entered the sample bottle, discard the contents downstream, re-rinse the bottle (or use a clean back-up bottle), and collect a new sample.

6. Immediately after collecting the sample, return the bottle to its plastic bag. Seal the bag.
7. If a sample is to be collected into a syringe, submerge a 60-mL syringe halfway into the stream and withdraw a 15-20 mL aliquot. Pull the plunger to its maximum extension and shake the syringe so the water contacts all surfaces. Point the syringe downstream and discard the water by depressing the plunger. Repeat this rinsing procedure two more times.
8. Submerge the syringe into the stream again and slowly fill the syringe with a fresh sample. Try not to get any air bubbles in the syringe.
9. Invert the syringe (tip pointing up), and cap it with a syringe valve or stopcock. Tap the side of the syringe lightly to detach any trapped air bubbles. With the valve open, expel the air bubbles and a small volume of water, leaving the syringe between two-thirds and three-fourths full. Note that the syringe is transported only partially full to avoid dislodging the plunger during transport. Close the syringe valve. If any air bubbles were drawn into the syringe during this process, discard the sample and fill the syringe again (step 8).
10. Repeat steps 7 through 9 with a second (back-up) syringe. Place the syringes together in a separate plastic bag and place in a plastic container, which is then placed into the cooler (or stream water if that method of cooling is used while still in the field).
11. Complete Stream Sample Record Form 3 while at the sample site.
12. Inspect all equipment, and clean off any plant and animal material before moving to the next sample location. This effort ensures that introductions of nuisance species do not occur between streams. Inspect, clean, and handpick plant and animal remains from any footwear or equipment that may have contacted stream water.

SAMPLE HANDLING

1. Place the bagged sample on double-bagged ice or refrigerant immediately after collection. Note: do not put ice in the plastic bag that contains the sample bottle or in the plastic container that contains the syringes. Ice or refrigerant should be double bagged in plastic bags to avoid possible leakage and contamination of the samples. Samples can be held in a soft-sided cooler until returned to the vehicle.
2. The large sample cooler can be left in the collection team's vehicle. The sample can be transferred to the larger cooler upon return to the vehicle.
3. For sites that are not close to road access, the collection team should make arrangements to keep the sample on ice after collection and during the return hike. One approach would be to use a small soft-pack cooler or other container that will fit in a backpack. Ice, snow, or refrigerant could be placed in a plastic bag in the cooler or container (double bag to avoid leakage and contamination of samples). Samples are transferred to the larger cooler at the vehicle.
4. The samples should be kept in the dark and on ice until delivery to the lab. The ice may need to be replenished during sample transit. Do not place the sample bottle in a refrigerator or cooler with food or in any container that is not clean. Ship the samples as soon as possible, preferably

within 24 hours of sampling. Ship early in the week to ensure the lab receives the samples during the work-week.

5. Note that we do not recommend filtration in the field. If, however, a program filters the samples in the field for chlorophyll *a* measurement, it is important to record on the sampling record form the volume of water filtered. Record this information in the Notes section of the form. The filter is then sent to the analytical laboratory for determination of chlorophyll *a* content.

POST SAMPLING ACTIONS

1. Completely fill out all stream sampling forms. Refer to Appendix E for instructions on filling out the forms.
2. Complete the Chain of Custody Form.
3. Check to make sure that all of the information on the sample label(s), Chain of Custody Form, and all stream sampling forms is consistent.
4. Transport the samples back to the vehicle in a soft cooler on ice or snow.
5. After carrying the samples to the vehicles, place the bottle(s) and syringes in a cooler and surround them with 1 gallon re-sealable plastic bags filled with ice. Double bag the ice to avoid getting cooling water into sample bags.

FIELD MEASUREMENTS

Determine stream temperature with a field thermometer (one that does not use mercury). Determine specific conductance and dissolved oxygen concentration using field meters (optional). Follow instructions provided below. Record the measured values on the Stream Sample Record Form.

Measuring for Specific Conductance

1. Check the batteries and electronic functions (e.g., zero and red line) of the conductivity meter as instructed by the operating manual.
2. If you haven't tested the meter at a base location recently, insert the probe into the RINSE container of the quality control check sample (QCCS) and swirl for three to five seconds. Remove the probe, shake it off gently, transfer it to the TEST container of QCCS, and let it stabilize for 20 seconds. If the measured conductivity is not within 10% of the theoretical value, repeat the measurement process. If the value is still unacceptable, do not use the meter until it can be inspected, problem(s) diagnosed, and repaired.
3. Submerge the probe in an area of flowing water near the middle of the channel at the same location where the water chemistry sample was collected. Record the measured conductivity and any pertinent comments about the measurement on the Field Measurement form.

Measuring for Dissolved Oxygen and Temperature

1. Inspect the probe for outward signs of fouling and for an intact membrane. Do not touch the electrodes inside the probe with any object. Always keep the probe moist by keeping it inside its calibration chamber.
2. Check the batteries and electronic functions of the meter as described in the operating manual.
3. Calibrate the oxygen probe in water-saturated air as described in the operating manual. Allow at least 15 minutes for the probe to equilibrate before attempting to calibrate. Try to perform

the calibration as close to stream temperature (not air temperature) as possible by using stream water to fill the calibration chamber before equilibration.

4. After the calibration, submerge the probe in midstream at mid-depth at the same location where the water chemistry sample was collected. Face the membrane of the probe upstream and allow the probe to equilibrate. Record the measured DO and stream temperature on the Field Measurement form. Record the time that the DO and temperature measurements were made in 24 hour units (e.g., 14:23) on the Sample Record Form 4. If the DO meter is not functioning, measure the stream temperature with a field thermometer and record the reading on the Sample Record Form 4 along with any pertinent comments.

NOTE: Older model dissolved oxygen probes require a **continuous** movement of water (0.3 to 0.5 m/s) across the probe to provide accurate measurements. If the velocity of the stream is appreciably less than that, agitate the probe in the water as you are taking the measurement.

Measuring for Temperature Only (if no field meters are being used)

1. Place a field thermometer ($\pm 1^{o}$ C accuracy) beneath the surface of the stream at the approximate depth of sample collection in an area of flowing water at or near where the water chemistry samples were collected.
2. Record the stream temperature (estimated to the nearest 0.1^{o} C) on the Field Measurement form. Record the time the temperature measurement was made in 24 hour units (e.g., 14:23) on the field form, along with any pertinent comments (e.g., measurement taken in sun or shade).

Steps below describe the equipment cleaning procedures to be followed after measurements are taken. Inspect all equipment and clean off any plant and animal material. This effort ensures that introductions of nuisance species do not occur between streams.

Cleaning Equipment After Sampling

1. Clean any equipment that may have contacted surface water for biological contaminants. If you are moving between sites on the same day, do this before moving to the next site.
2. Clean and dry other equipment before storage. Rinse coolers with water to clean off any dirt or debris on the outside and inside.
3. Inventory equipment and supply needs and relay orders to the Project Coordinator.
4. Remove dissolved oxygen meters, other instrumentation, and GPS units from carrying cases and set up for pre-departure checks and calibration. Examine oxygen membranes of DO meters for cracks, wrinkles, or bubbles. Replace if necessary.
5. Recharge batteries overnight if possible. Replace other batteries as necessary.
6. Recheck field forms from the day's sampling activities. Make corrections and completions where possible and initial each form after review.

POST-TRIP ACTIVITIES

Upon return to a lodging or office location after sampling, the team should review all labels and completed data forms for accuracy, completeness, and legibility. A final inspection should be made of all samples. If information is missing from the forms or labels, the Project Leader should attempt, if possible, to fill in the information accurately. The Project Leader should initial all data forms after review. If samples are missing or not properly labeled, it may be necessary to reschedule the site for complete sampling. Other post-sampling activities include inspection and cleaning of sampling equipment, inventory and sample preparation, sample shipment, and communications.

EQUIPMENT CLEANUP AND CHECK

Inspect, clean, and handpick plant and animal remains from any vehicle, footwear, or equipment that may have contacted stream water.

SHIPMENT OF SAMPLES AND FORMS

Upon completion of data and sample collection, the samples and forms should be shipped or transported to the analytical laboratory in as short a time as is reasonably possible. Call or email the lab to alert them that samples are in transit and tell them what date to expect delivery. Samples should be maintained in insulated containers with refrigerant after collection and during transport. The Chain of Custody Record Form should be maintained and kept with the samples until the samples are logged-in at the analytical laboratory. If samples are to be shipped to the laboratory, an overnight shipping service should be used, and shipping should be avoided when samples would be delayed by transit over a weekend or holiday period.

The field team should ship samples to the lab as soon as possible after collection. Water samples should be shipped in coolers packed with ice. Line each shipping cooler with a large plastic bag. Inside, package the ice separately within numerous (as many as feasible) zipper-lock plastic bags and ensure that the ice is fresh before shipment. Use block ice when available or “blue ice” packaged refrigerant. Block ice should be sealed in two large plastic bags. White or clear bags will allow for labeling with a dark indelible marker; label all bags of ice as “ICE” with an indelible marker to prevent misidentification by couriers of any leakage of water as a possible hazardous material spill.

To prepare the sample bottles and syringes for shipping, line the shipping cooler with a 30-gal plastic bag. Place another plastic bag in the cooler, and place the samples in the second bag. Place filled syringes and/or glass bottles in sturdy containers to prevent damage during transport. Ensure that all label entries are complete and close the bag of samples. Place bags of ice around it. Then close the cooler liner (outer garbage bag). Ship water samples on the day of collection whenever possible. If that is not possible, they should be shipped the next day.

PROCESSING SITE DOCUMENTATION DATA AND INFORMATION

A file system and database with reliable backup should be established for storage of site records and files, map images, and photos. Processing site documentation data and information include the following steps:

1. Retrieve site coordinates (and any tracks) from the GPS unit using the GPS software. Delete any extra coordinate sets (waypoints) and save the file as a *.gdb file.
2. Retrieve photos from the camera.
3. Enter or revise the site record in the site documentation database.
    a. Enter site coordinates obtained in the field.
    b. Enter or revise the site description, travel and access directions.
    c. Enter or revise the tag and tag tree information as needed. If no changes were made, note that the tag placement was confirmed on the particular date. Note that tree tags may not be applied in a wilderness setting.
    d. Add new photos as JPG images with captions to the site record.
4. Create site maps providing both detailed and broader information for access and orientation. Annotate maps and pictures with text and arrows when it would be helpful. Note that the

accuracy of maps may vary and the coordinate-based point on the maps, as well as other information, may be misleading. Add clarifying notes. Save these maps as JPG images in the site record. Add captions as appropriate. Enter site and visit data into NRM Air and attach the JPG images to the data records.

D-1.4 STREAM DISCHARGE

Stream discharge is equal to the product of the mean current velocity and vertical cross sectional area of flowing water. It reflects the volume of water per unit time that passes a particular location (a line drawn at a right angle to the stream channel) on the stream. Discharge measurements can be helpful for assessing trends in stream water chemistry that are sensitive to stream flow differences. Stream discharge information is also useful in interpreting the representativeness of water chemistry data and some physical habitat information.

The location selected for measuring stream discharge should be as close as possible to the location where chemical samples are collected. Variability in stream discharge within the reach of interest is expected to be small compared to variability in stream discharge among streams, so multiple determinations at a site are not required.

No single method for measuring discharge is applicable to all types of stream channels. The preferred procedure for obtaining discharge data for small streams is based on "velocity-area" methods (e.g., Rantz 1982, Linsley et al. 1982). For streams that are too small or too shallow to use the equipment required for the velocity-area procedure, an alternative method, the timed filling procedure, is presented. It is based on timing the filling of a bucket of known volume with water.

VELOCITY-AREA PROCEDURE

Because velocity and depth typically vary greatly across a stream, accuracy in field measurements is achieved by measuring the mean velocity and flow cross-sectional area of many increments across a channel (Figure D-1). Each increment gives a subtotal of the stream discharge, and the whole is calculated as the sum of these parts. Discharge measurements are made at only one carefully chosen channel cross section within the sample reach. It is important to choose a channel cross section that is as much like a canal as possible. A glide area with a U-shaped channel cross section that is free of obstructions provides the best conditions for measuring discharge by the velocity-area method. You may remove rocks and other obstructions to improve the cross-section before any measurements are made. However, because removing obstacles from one part of a cross-section affects adjacent water velocities, you must not change the cross-section once you commence collecting the set of velocity and depth measurements.

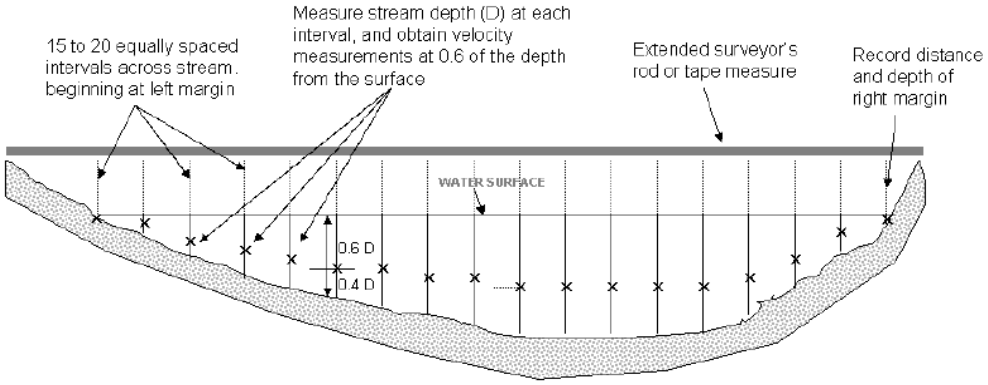

Figure D-1. Layout of a channel cross-section for obtaining discharge data by the velocity-area procedure.

The procedure for obtaining depth and velocity measurements is outlined below (Rantz 1982). Record the data from each measurement in the Stream Discharge section of the Stream Sampling Record Form, giving the distance from the left bank (facing downstream), water depth, measured velocity, and any required flags or notes for each measurement increment.

1. Locate a cross-section of the stream channel for discharge determination that has most of the following qualities:
   - Segment of stream above and below cross-section is straight.
   - Depths mostly greater than 15 cm and velocities mostly greater than 0.15 m/s. Do not measure discharge in a pool.
   - "U" shaped, with a uniform streambed free of large boulders, woody debris or brush, and dense aquatic vegetation.
   - Flow is relatively uniform, with no eddies, backwaters, or excessive turbulence.
2. Lay the surveyor's rod (or stretch a meter tape) across the stream perpendicular to its flow with the "zero" end of the rod or tape on the left bank, as viewed when looking downstream. Leave the tape tightly suspended across the stream, at the bankfull mark or higher. Adjust the tape with the aid of a small bubble level suspended from the rod or tape so that it is, and remains throughout the period of measurement, level.

   Physical indicators of the bankfull stage include: 1) top of highest depositional features, 2) break in the slope of the bank or a change in particle size, 3) staining of rocks, and 4) exposed root hairs below an intact soil.
3. Attach the velocity meter probe to the calibrated wading rod. Check to ensure the meter is functioning properly and the correct calibration value is displayed. Calibrate (or check the calibration) the velocity meter and probe as directed in the meter's operating manual. Place an X in the VELOCITY AREA box on the Stream Discharge Form.
4. Divide the total wetted stream width into 15 to 20 equal-sized intervals. To determine interval width, divide the width by 20 and round up to a convenient number. Intervals should not be less than 10 cm wide, even if this results in less than 15 intervals. The first interval is located at the left margin of the stream (left side, looking downstream) and the last interval is located at the right margin of the stream (right side, looking downstream).
5. Stand downstream of the rod or tape and to the side of the first interval point (closest to the left bank, looking downstream).
6. Place the wading rod in the stream at the interval point and adjust the probe or propeller so that it is at the water surface. Place an X in the appropriate DISTANCE UNITS and DEPTH UNITS boxes on the Stream Discharge Form. Record the distance from the left bank and the depth indicated on the wading rod on the Stream Discharge Form.

   Note that, for the first interval, distance equals 0 cm, and in many cases depth may also equal 0 cm. For the last interval, the distance will equal the wetted width (in cm) and depth may again equal 0 cm.
7. Stand downstream of the probe or propeller to avoid disrupting the stream flow. Adjust the position of the probe on the wading rod so that it is at 0.6 of the measured stream depth below the surface of the water. Face the probe upstream at a right angle to the cross-section, even if local flow eddies hit at oblique angles to the cross-section.

8. Wait 20 seconds to allow the meter to equilibrate, then measure the velocity. Place an "X" in the appropriate VELOCITY UNITS box on the Stream Discharge Form. Record the value on the Stream Discharge Form. Note for the first interval, velocity may equal 0 because depth will equal 0. Note that negative velocity readings are possible; when recording negative values, assign a flag to denote they are indeed negative values.
   - For the electromagnetic current meter (e.g., Marsh-McBirney), use the lowest time constant scale setting on the meter that provides stable readings.
   - For the impeller-type meter (e.g., Swoffer 2100), set the control knob at the mid-position of DISPLAY AVERAGING. Press RESET then START and proceed with the measurements.
9. Move to the next interval point and repeat steps 6 through 8. Continue until depth and velocity measurements have been recorded for all intervals. Note for the last interval (at the right margin), depth and velocity values may equal 0.
10. At the last interval (the right margin), record a Z in the FLAG field on the field form to denote the last interval sampled.

TIMED FILLING PROCEDURE

In channels too small for the velocity-area method, discharge can sometimes be determined directly by measuring the time it takes to fill a container of known volume. "Small" is defined as a channel so shallow that the current velocity probe cannot be placed in the water or where the channel is broken up and irregular due to rocks and debris and a suitable cross-section for using the velocity area procedure is not available. The timed filling method can be extremely precise and accurate but requires a natural or constructed spillway of free-falling water. Because obtaining data by this procedure can result in channel disturbance and can stir up a lot of sediment, wait until after all biological and chemical measurements and sampling activities have been completed before proceeding.

Choose a cross-section of the stream that contains one or more natural spillways or plunges that collectively include the entire stream flow. A temporary spillway can be constructed using a portable V-notch weir, plastic sheeting, or other materials (i.e., rocks or wood) that are available onsite. Choose a location within the sampling reach that is narrow and easy to block when using a portable weir. Position the weir or constructed spillway in the channel so that the entire flow of the stream is completely rerouted through its notch (Figure D-2). Impound the flow with the weir, making sure that water is not flowing beneath or around the side of the weir. Use mud or stones and plastic sheeting to get a good waterproof seal. The notch must be high enough to create a small spillway as water flows over its sharp crest.

The timed filling procedure is presented in below. Make sure that the entire flow of the spillway is going into the bucket. Record the time it takes to fill a measured volume on the Stream Discharge section of the Stream Sampling Record Form. Repeat the procedure five times. Discharge will be calculated as an average of these five measurements. If the cross-section contains multiple spillways, you will need to do separate determinations for each spillway. Clearly indicate which time and volume data replicates should be averaged together for each spillway; use an additional Stream Sampling Record Form if necessary. On the additional form, record a flag value (e.g., F1) on all lines in the Timed Filling section, and explain that the flag means an additional spillway was measured in the comment section.

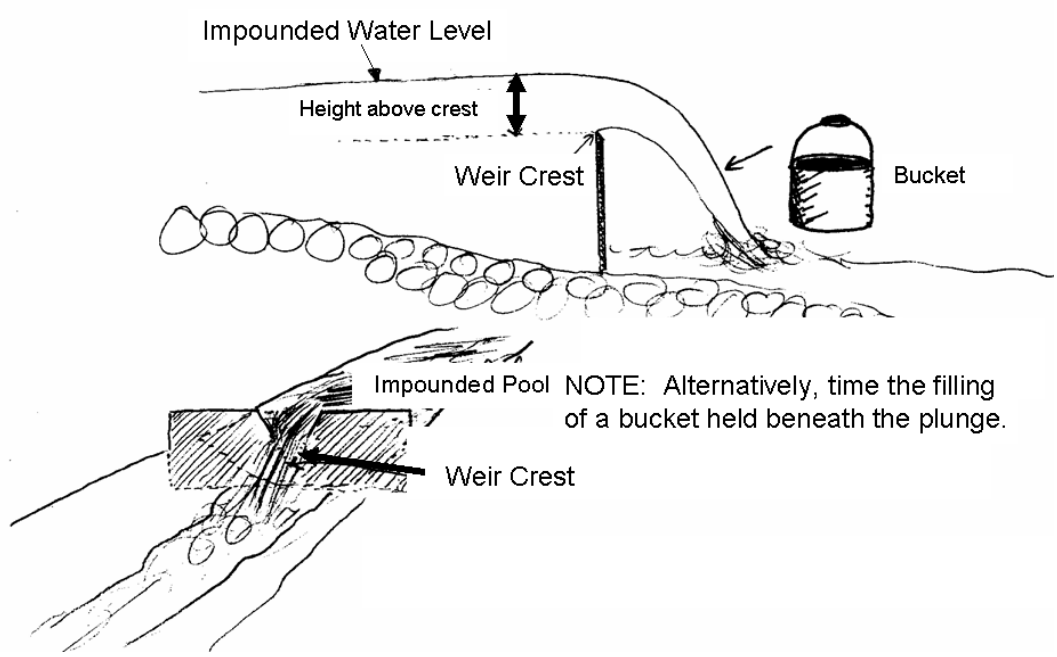

Figure D-2. Use of a portable weir in conjunction with a calibrated bucket to obtain an estimate of stream discharge.

To determine stream discharge through the time-filling procedure:

1. Choose a cross section that contains one or more natural spillways or plunges, construct a temporary spillway using on-site materials, and/or install a portable weir using a plastic sheet and on-site materials.
2. Place an X in the TIMED FILLING box in the stream discharge section of the Stream Discharge form.
3. Position a calibrated bucket or other container of known volume beneath the spillway to capture the entire flow. Use a stopwatch to determine the time required to collect a known volume of water. Record the volume collected (in liters) and the time required (in seconds) on the Stream Discharge form.
4. Repeat step 3 a total of five times for each spillway that occurs in the cross section. If there is more than one spillway in a cross-section, you must use the timed-filling approach on all of them. Additional spillways may require additional data forms.

Note: If measuring discharge by this procedure will result in significant channel disturbance or will stir up sediment, do not determine discharge until all biological and chemical measurement and sampling activities have been completed.

EQUIPMENT AND SUPPLIES

Table D-2 shows the list of equipment and supplies necessary to measure stream discharge. Use this checklist to ensure that equipment and supplies are organized and available at the stream site in order to conduct the activities efficiently.

Table D-2. Equipment and supply checklist for measuring stream discharge.

Quantity	Item	✓
1	Surveyor's telescoping leveling rod (7 m long, metric scale, round cross-section)	
1	50-m fiberglass measuring tape and reel	
1	Small bubble level to make sure that the tape is level	
1	Current velocity meter, probe, and operating manual	
1-2	Extra batteries for velocity meter	
1	Top-set wading rod (metric or English scale) for use with current velocity meter	
1	Portable weir with 60° "V" notch (optional)	
1	Plastic sheeting to use with weir (optional)	
1	Plastic bucket (or similar container) with volume graduations or known total volume	
1	Stopwatch	
1	Clipboard	
	Soft (#2) pencils	
	Stream Discharge forms (one per stream plus extras if needed for timed filling procedure or additional velocity-area intervals)	
1 copy	Field operations and methods documents	
1 set	Laminated sheets of procedure tables and/or quick reference guides for stream discharge	

D-1.5 ACKNOWLEDGMENTS

This SOP is based partly on material developed in the following publications:

Herlihy, A.T. 2006. Section 5. Water chemistry. In: Peck, D.V., A.T. Herlihy, B.H. Hill, R.M. Hughes, P.R. Kaufmann, D.J. Klemm, J.M. Lazorchak, F.H. McCormick, S.A. Peterson, P.L. Ringold, T. Magee, and M. Cappaert. Environmental Monitoring and Assessment Program-Surface Waters Western Pilot Study: Field Operations Manual for Wadeable Streams. EPA/620/R-06/003. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC. pp. 85–98.

Kaufmann, P.R. 2006. Section 6. Stream Discharge. In: Peck, D.V., A.T. Herlihy, B.H. Hill, R.M. Hughes, P.R. Kaufmann, D.J. Klemm, J.M. Lazorchak, F.H. McCormick, S.A. Peterson, P.L. Ringold, T. Magee, and M. Cappaert. Environmental Monitoring and Assessment Program-Surface Waters Western Pilot Study: Field Operations Manual for Wadeable Streams. EPA/620/R-06/003. U.S. Environmental Protection Agency, Office of Research and Development, Washington, D.C. pp. 99–110.

Merritt, G.D.,V.C. Rogers, and D.V. Peck. 1997. Section 3. Base site activities. In: Baker, J.R., D.V. Peck, and D.W. Sutton (eds.). Environmental Monitoring and Assessment Program Surface Waters: Field Operations Manual for Lakes. Report No. EPA/620/R-97/001. U.S. Environmental Protection Agency, Washington DC.

D-2 STANDARD OPERATING PROCEDURE FOR LAKE SAMPLING

This SOP provides guidelines for lake sampling within the FS ARM program. It is intended as a general SOP, suitable for adoption as a stand-alone procedure, or for modification to fit local program needs. It is divided into individual sections that cover pre-trip activities, sampling site documentation, index site location, lake sampling, lake assessment, post-trip activities, and needed equipment and supplies.

D-2.1 PRE-TRIP ACTIVITIES

Field teams conduct a number of activities before departing for the field sampling. These include tasks that must be completed both before departure to the sampling site. This section describes procedures for office and base site pre-trip activities that should be carried out in support of lake sampling.

Pre-departure activities include development of sampling itineraries, instrument calibration, equipment checks and repair, supply inventories, and sample container preparation. Procedures for these activities are described in the following sections.

PREPARATION OF SAMPLE CONTAINERS

Generally, the analytical laboratory supplies properly washed bottles and syringes used in the sampling program. In order to do this, the laboratory needs to know in advance what analytes will be measured in the samples to be collected. Ensure that the proper number, type, and size sampling containers are provided. It is wise to carry a few back-ups.

Before leaving the base location, package the sample containers (typically two sample bottles and two 60 mL syringes or glass bottles with septum caps) for each site to be sampled (plus back-up). Fill out a set of water chemistry sample labels. Attach a completed label to each sample bottle and syringe. Make sure the syringe labels do not cover the volume gradations on the syringe.

DAILY ITINERARIES

Field sampling efforts should include a Project Leader who guides activities in the field and a Project Coordinator who remains in the office during the sampling effort. The Project Leader reviews each site folder to ensure that it contains the appropriate maps, contact information, copies of access permission letters (if needed), and access instructions. Additional activities can include confirming the best access routes, calling landowners or local contacts (if applicable), confirming lodging or camping plans and locations (with directions), and coordinating rendezvous locations with individuals who must meet with field teams before accessing a site. This information is used to develop an itinerary. The Project Leader should provide the Project Coordinator with a schedule for each day of sampling. Schedules include departure time, estimated duration of sampling activities, routes of travel, and estimated time of arrival at the sampling site(s) and return to the base site. Changes that might be made to the itinerary should be relayed by the Project Leader to the Project Coordinator as soon as possible. Miscommunications can result in the initiation of expensive search and rescue procedures and disruption of carefully planned schedules.

INSTRUMENT CHECKS AND CALIBRATION

Each field team should test and/or calibrate field instruments such as, thermometers, DO meters, and GPS units, before leaving for the sampling site. Batteries should be checked before departure for field sites. Extra batteries should be carried.

Field personnel should check the inventory of supplies and equipment before departure using site-visit checklists. Meters, probes, and sampling gear should be packed for transport to the field in such a way as to minimize physical shock and vibration during transport. Rafts or float tubes should be packed for transport so as to minimize the potential for puncture by any sharp object.

SUPPLY INVENTORIES

Develop a checklist of equipment and supplies that will be needed to conduct lake sampling. Check off each item as it is packed and loaded for transport to the field.

A preliminary list of equipment and supplies required to collect lake samples and associated field data is presented in Table D-3. Use and revise this checklist to ensure that equipment and supplies are organized and available at the lake sampling site in order to conduct the activities efficiently.

Table D-3. Checklist of equipment and supplies for sampling water chemistry and Secchi depth.

Quantity	Item	✓
Standard Items:		
1	Field thermometer	
1-2	Sample bottle(s) with completed sample label attached (in clean plastic bag). Include a second bottle if sampling at that site is to be replicated	
2-4	60 mL plastic syringes (with Luer type tip) or glass bottles with septum caps with completed sample labels attached	
1	Plastic container with snap-on lid to hold filled syringes	
2-4	Syringe valves (Mininert® with Luer type adapter, or equivalent, available from a chromatography supply company)	
1	Cooler with 4 to 6 plastic bags (1-gal) of ice or a medium or large opaque garbage bag to store the water sample at shoreline	
1	Lake Sampling Record Form	
1 set	Water Chemistry labels (if not filled out and attached at base site)	
2-4	Soft-lead pencils and write-in-rain-pens for filling out field data forms and notebook entries	
2-4	Fine-tipped indelible waterproof markers for filling out labels	
1 copy	Field operations and methods documents	
2-4	Plastic gloves stored in a secure plastic bag	
1	Survey grade GPS unit and compass	
1	Digital camera with extra memory cards and batteries	
1	Backpack with waterproof cover (if site is not accessible by vehicle)	
1	Van Dorn sampler with messenger and cable	
1	Raft or float tube with pump for inflating, oars, paddles, or flippers	
1-2	Personal flotation devices for each person in the boat or float tube	
1	First aid kit	
1	Locally determined safety equipment	
1	Secchi disk and line (with depth increments)	
1	Tape measure	
Optional Items:		
roll/box	Clear packaging tape to cover labels (tape strips)	
1	Dissolved oxygen (DO)/temperature meter with probe	
1	DO repair kit containing additional membranes and probe filling solution	
1	Conductivity meter with probe	
1	250-mL or 500-mL plastic bottle of conductivity QCCS labeled RINSE (in plastic bag)	
1	250 mL or 500-mL plastic bottle of conductivity QCCS labeled TEST (in plastic bag)	

D-2.2 SITE DOCUMENTATION

BACKGROUND

This section describes SOPs for establishing and documenting sampling sites on small to medium-size lakes, primarily those situated in relatively remote backcountry locations. This procedure applies to new sites for which approximate locations have been designated based on program objectives and sampling design. It also applies to previously established sites for which current or updated site documentation is needed.

Sampling the correct lake is critical to most lake study sampling designs. It is also important to identify, to the extent possible, the index site (deepest point) on a lake. On arriving at a target lake, the GPS unit is a valuable tool for identifying and verifying the location. Nevertheless, site verification must be supported by all available information (e.g., maps, photos, signs, GPS, and expected lake size and shape). Do not sample the lake if there is reason to believe it is the wrong one. Contact the Project Coordinator to resolve discrepancies.

OBJECTIVE

The objective of this procedure is to establish and document new lake sampling sites and to update documentation for established sites, providing:

- Site descriptions and notes;
- Travel and access descriptions and notes;
- Site coordinates obtained in the field using a GPS unit; and
- Site and access-related photos.

For established sites, existing site documentation will be evaluated for clarity and improved as needed based on conditions observed in the field.

MATERIAL NEEDED FOR USE IN THE FIELD FOR SITE DOCUMENTATION

1. Available site documentation records for previously established sites:
    a. Site location maps, topographic maps, and road maps;
    b. Site descriptions and access notes;
    c. Site coordinates; and
    d. Site photos.
2. Preliminary site documentation for new sites:
    a. Site location maps, topographic maps, and national forest maps, indicating approximate site locations; and
    b. General site descriptions and access notes.
3. General material for site documentation:
    a. Regional-scale topographic and road maps;
    b. Lake sampling site documentation forms on waterproof paper;
    c. Clipboard or field notebook and pens for use with waterproof paper;
    d. GPS unit and replacement batteries;
    e. Digital camera with charged battery and charged replacement battery;
    f. Gate keys (if needed); and
    g. Cell phone with numbers of project staff and management agency offices.

SEQUENCE OF SITE DOCUMENTATION ACTIVITIES

1. Complete the header information on each form (1-4) before entering the field. See Lake Form instructions in Appendix E for details on completing the forms correctly.
2. Select or locate the specific sampling site using the GPS unit (applies to new sites).
    a. The approximate or preliminary location of new site locations will be indicated on topographic maps. The sample collection team must still determine the exact point on the lake to be sampled.
    b. The preferred sampling site location is over the deepest area of the lake, which is often, but not always, near mid-lake. This is designated as the "index" site. If it is not feasible to access the index site, or for conducting some types of screening studies, it is acceptable to sample from the principal outlet stream (designated "outlet" sample) rather than at the index site. If there is no available outlet stream or if the outlet stream is not flowing at a sufficient rate to collect a representative sample, then it can be acceptable to collect a sample by reaching into the lake from an appropriate location along the lake shore (designated "shoreline" sample).
3. Obtain coordinates at the site using the GPS unit. These coordinates will be part of the site identification information entered into the national database (NRM Air).
    a. The unit position format should be set to Decimal Degrees (hddd.ddddd). The Datum should be set to NAD83. Resolution should be expressed in meters. Wide Area Augmentation System (WAAS) should be enabled.
    b. When "Mark Waypoint" is selected, the default GPS site ID (a number) should be changed to the actual Site ID.
    c. Before saving the coordinates note the estimated accuracy of the measurement and enter on the Site Documentation Form 1.
    d. Save the coordinates in the GPS unit memory and record the coordinates in decimal degrees, the datum, and the elevation (preferably in meters) on the Sampling Site Documentation Form 1. Use NAD83 to conform to Forest Service standards. Do not rely solely on the GPS unit to store the coordinates.
    e. Confirm that the waypoint has been saved in the GPS unit.
    f. It can be very helpful to establish and document benchmarks on shoreline rocks.
4. Enter (if applicable) the approximate lake water level on the Sampling Site Documentation Form 2.
5. Enter site description information on the Site Documentation Form 2.
    a. For existing sites, enter information to improve and update existing site description information.
    b. Generally describe the site, referring to the proximity of landmarks (trails, bridges, tributaries, trees, shoreline features, landscape features, or other relatively permanent features). Add any additional information that will help future sample collectors indentify the site. Also, add any information here that might be relevant to water quality, such as cleared land, mining (ongoing or historical), roads, construction, logging, heavy grazing,

development, or any earth disturbance observed on or near the shoreline, above the lake in the watershed, or along the inlet stream(s).

6. Enter travel and access directions on the Sampling Site Documentation Form 1.
    a. For existing sites, improve and update existing travel and access information.
    b. Travel and access notes should be sufficient to guide future sample collectors to the site without reliance on GPS units. Not all future sample collectors will necessarily have GPS units.
    c. Access notes should refer to trails, roads, and permanent landmarks, providing distances and, where helpful, compass-based directions. Backtrack if necessary to determine distances. Linear distances and directions from the established site waypoint can be determined using the GPS unit.
    d. In cases where a parking location is not immediately adjacent the sampling site, use the GPS unit to obtain the coordinates for the parking location and record them in the travel and access information entry area of the Sampling Site Documentation Form.
    e. For complicated or long walk-ins, use the GPS unit to record and save a track. But, again, do not rely on future sample collectors' access to a GPS unit.
    f. Sketch the route on the back of the Sampling Site Documentation Form for scanning and saving as a JPG image if that would be helpful.
7. Make a sketch of the lake on Sampling Site Documentation Form 2. Mark on the sketch the launch and sampling site locations.
8. Obtain site and access photos
    a. Photos should be obtained providing views of the sampling site and the shoreline and views of other distinguishing features in relation to the site (bridges, roads, notable rocks, trees, landforms, signage, etc.) Photos should also be obtained to show important aspects of site access (parking area, forks in the trail, etc.).
    b. All photos should be listed on Form 2, including their filenames, date, and descriptions. Enter this information at the time that the pictures are taken. Do not rely on memory for later entry of photo descriptions. The entered description should serve as the photo caption for site documentation reporting.

LAKE VERIFICATION AT THE LAUNCH SITE

Record directions to the sampling site and a description of the launch location for lake sampling on the Lake Sampling Site Documentation Form in the site information folder. This information will be important in the future if the site is revisited by another sampling team. Provide information about signs, road numbers, gates, landmarks, and any additional information you feel will be useful to another sampling team in locating this site. It is also helpful to describe the road distance traveled (in miles) between turns and hiking distance and/or time traveled to reach the sampling or launch site. Additional details can also be helpful. What landmarks are in the vicinity of the site? Is the trailhead well marked?

If a GPS fix is obtained, record the location in decimal degrees and the type of satellite fix (2D or 3D) for the site. Compare the site information folder map coordinates recorded for the site with the GPS coordinates displayed at the site. Check to see if the two sets of coordinates are within a distance that

is approximately equal to the precision of the GPS receiver without differential correction of the position fix. If a GPS fix is not available, do not record fix information but try to obtain the information at a later time during the visit. A fix may be taken at any time during a site visit and recorded on the form. If this is the first visit to this lake, mark the location of the launch site with an "L" on the lake outline that is provided on the Lake Sampling Site Documentation Form. In addition to the GPS unit, use as many of the following methods as possible to verify the location and identity of the site:

1. Obtain confirmation from a local person familiar with the area.
2. Identify confirming trails, roads, and signs.
3. Compare lake shape to that shown on the topographic map included in the site information folder.
4. Determine lake position relative to identifiable topographic features shown on the map.
5. Compare visual evaluation of lake area with available mapped information.

If this is not the first visit to this lake and if the lake shape on the map sketch that appears on the Lake Sampling Site Documentation Form and on the USGS map do not correspond with each other or with the actual lake shape as seen in the field, check "Not Verified" and provide comments on the form. The lake should not be sampled if there are clear, major differences in lake shape or lake area.

INDEX SITE LOCATION

Locate the sampling site in what is approximately the deepest portion of the lake. There are different ways to do this, as follows:

1. If the deepest location had been determined and documented on a previous trip to this lake, based on that documentation and use of GPS unit and/or mapped lake features, navigate to the sampling location.
2. If the sampling location has not previously been documented, locate the deepest part of the lake based on visual examination of the lake shape and surrounding topography, coupled with reconnaissance on foot and/or by boat for up to about one-half hour. Use visual cues and/or soundings with a weighted line to locate what appears to be the deepest part of the lake.

Once the sampling location has been selected at what appears to be the deepest part of the lake, determine the GPS coordinates and record them on the Lake Sampling Site Documentation Form. Mark the sample site with an "x" in the lake drawing. A checklist for lake verification is given in Table D-4.

Table D-4. Lake verification checklist.

Item	✓
Site information folder for lake to be sampled	
Clipboard	
Lake Sampling Site Documentation Form	
Field notebook	
GPS unit with manual, extra battery pack	
50 m line to attach to rock anchor	

D-2.3 Lake Sampling

These procedures cover collection of lake water samples and measurement of Secchi depth (transparency). The lake sampling procedures assume collection of the primary sample from the deepest part of the lake. Measurement of Secchi depth and collection of the deep water index sample will require use of a boat or float tube. If it is not possible to sample the lake by boat or float tube, the next best option is to sample at the principal outlet stream. If a lake outlet sample is to be collected instead of a sample in deep water, follow the procedures outlined in the stream sampling SOP and sample the outlet stream as close to the lake as is practical.

If neither a deep water sample nor an outlet sample can be collected, the third option is to sample from the shoreline, satisfying as many of the following criteria as possible:

- As close to the outlet as possible;
- From a bedrock outcropping or otherwise rocky area; and
- From the deepest accessible point.

Water must be deep enough so that surface scum and sediments are not collected into the bottle. Take samples from a wind-exposed area so that the water is relatively well-mixed. Avoid sampling in locations having emergent vegetation and/or downed logs or other woody debris.

DOCUMENTATION OF DATA AND SAMPLE COLLECTION

The Lake Sample Record Forms 3 and 4 are used to document sample collection and field data. See Appendix E for complete instructions for filling out the forms correctly. These forms are used to record the following information:

- The site ID and site name;
- The date and arrival time for the site visit and specific times of measurements obtained;
- The name, contact information, and affiliation of the individual who is the Collector of Record and responsible for protocol adherence during the site visit;
- Suggested revisions or amendments to site documentation and travel directions;
- A listing of site-related photographs taken, including file name, date, and descriptions;
- Qualitative descriptions of weather, lake level and appearance, and other factors that might influence water quality during the site visit;
- Air temperature;
- Results for all water quality data collected on-site, including:
    a. Numerical results, units, and measurement time; and
    b. Instruments used and methods identification;
- Identification of calibration and post-calibration sensor check records;
- A listing of all samples collected, including:
    a. Collection time; and
    b. Types of samples collected and number of replicates.

SEQUENCE OF ACTIVITIES FOR DATA COLLECTION

The typical sequence of activities on arrival at the lake sampling site is as follows:

1. Confirm the site location based on information in the Lake Sampling Site Documentation Form, including coordinates, photos, and access notes.
2. Initiate completion of the Lake Sample Record Forms (see Appendix E for complete form instructions).
3. Complete Site Information and General Observations sections of the Lake Sample Record Forms.
4. Enter information needed to improve or correct the site description and travel directions provided on the Site Documentation Form 3.
5. Obtain any photographs needed to improve site documentation and enter file names, dates, and descriptions.
6. Note any factors (other than weather and lake level) that might affect water quality (e.g., shoreline or watershed disturbance or debris in water).
7. Collect water samples and complete the Water Sample section of the Sample Record Form 3. Enter any on site water data (optional) into Sample Record Form 4.
8. Complete the Chain of Custody Form.
9. Check to make sure that all of the information recorded on the sample label(s), Chain of Custody Form, and the lake sampling forms is consistent.
10. Obtain lake level measurements, if required.

If desired, *in situ* measurement of one or more parameters can be made using a multiparameter water quality sonde. Such measurements might include temperature, pH, DO, specific conductance, and/or turbidity. The procedures for such *in situ* data collection will vary with the specific field instrument, but in general require the following steps:

- Initiate water quality sonde field calibration and calibration checks.
- Record results on a water quality instrument calibration and post-calibration record form.
- Confirm that sensor check criteria are met. If criteria are not met, recalibrate, perform sensor maintenance, or replace sonde or sensors as needed to meet the criteria.

Deploy the water quality sonde for the period required to obtain stabilization. Enter the results and time of measurement in the *in situ* Water Quality Data section of the Lake Sampling Record Form.

SAMPLE COLLECTION

Deep Water Index Sample

Collect a water sample at the index site using a Van Dorn water sampler from 1.5 m depth (0.5 m if lake depth is less than 2.0 m), using the procedure described below. From the Van Dorn sampler, fill the required number of syringes and/or glass bottles, and one or two 500 ml or 1000 ml sample bottles. Procedures for collecting these samples are presented below. Prior to filling syringes and sample bottles, check the labels on these containers to ensure that all written information is legible and that each container has the same (and correct) site identification number. It can also be a good idea to place clear packing tape over the label and identification code, covering the label completely to seal it against water damage. Record the identification code assigned to the sample set (the syringes and bottles collected from the same site are considered one sample) and have the same code on the Lake Sampling Record Form. Also record the depth from which the sample was collected (usually 1.5 m or 0.5 m) on the form. Enter a flag code and provide comments on the Sample

Collection Form if there are any problems in collecting the sample or if conditions occur that may affect sample integrity. Store samples in the appropriate containers in the dark, and verify that they are carefully packed with plenty of ice bags and properly positioned, sealed, and labeled in the sample coolers. Recheck all forms and labels for completeness.

To use the Van Dorn sampler:

1. Open the Van Dorn sampler by pulling the elastic bands and cups back and securing the latches. Make sure that the mechanism is cocked so that it will be tripped by the messenger weight. Make sure that all valves are closed. Inspect the line for fraying, especially where it connects to the Van Dorn sampler. Do not place hands inside or on the lip of the container; this could contaminate samples. To reduce chances of contamination, wear powder-free latex laboratory gloves.
2. Attach the free end of the messenger line to the boat. This is important to prevent accidental loss of the equipment overboard. Rinse the open sampler by immersing it in the water column three times.
3. Lower the sampler to 1.5 m below the surface (0.5 m in lakes < 2 m deep).
4. Trip the sampler by releasing the messenger weight so that it slides down the line.
5. Raise the full sampler out of the lake. Set it on a clean, flat surface in an upright position. To avoid contamination, do not set the sampler in the bottom of the boat. Applying some body weight to the top of the Van Dorn sampler often will seal minor air leaks and preserve the sample integrity. If air enters the Van Dorn sampler, discard the sample and obtain another (repeat steps 1 to 5).

Use the following procedure for syringe and sample bottle collection. In doing so, wear powder-free surgical gloves while collecting syringe and bottle samples. Syringes may be chilled before use to reduce the occurrence of air bubbles in the sample.

Fill one bottle for each routine lake water sample. Fill a second bottle, with its own unique Sample ID/Barcode, if this site is to be replicated for QA/QC purposes.

1. Make sure that the sample bottle(s) and 60 ml syringes have the same site identification code number (which identifies a single lake) and that the labels are completely covered with clear tape. Record the identification code number on the Sample Collection Form.
2. Unscrew the valve at the top of the Van Dorn sampler. Fit a pre-labeled syringe to the fitting.
3. Slowly draw a 20 mL aliquot into the 60 pre-labeled syringe. Pull the plunger back so that the water contacts all inner surfaces of the syringe. Expel the water from the syringe. Repeat this rinse procedure twice more (there are three rinses for each syringe sample).
4. Reattach the syringe to the Van Dorn sampler and slowly draw 60-mL of water into the syringe. If air enters the Van Dorn sampler during this process, dispose of the sample and obtain another Van Dorn sample.
5. Place the syringe valve on the syringe tip. Press the green button toward the syringe.
6. Hold the syringe with the tip and valve pointed skyward. Tap the syringe to gather air bubbles to the top. Expel all air from the syringe by depressing the plunger and then close the one-way stopcock to seal the syringe with 40 to 50 mL of sample water remaining. Place a piece of tape around the stopcock so that it cannot freely open and package securely to prevent damage during transport (e.g. inside a paper towel cardboard roll). Secure in a container with packing material and ice.

7. Repeat steps 2 to 5 for one to three additional syringes. There should be a total of two syringes for each routine water sample if the protocol specifies that water for both DIC and pH be collected in syringes (four syringes if sample is being replicated).
8. Place the syringes in the solid plastic container and place in the cooler. Use ice contained in sealed 1-gal plastic bags to maintain the sample below 4 µC.
9. Unscrew the top valve of the Van Dorn sampler. Unscrew the lid of the pre-labeled sample bottle.
10. Open the bottom valve of the Van Dorn sampler and partially fill the sample bottle with water (approximately 50 mL).
11. Screw the lid on the bottle. Shake the bottle so that the water inside contacts all sides. Discard the water. Repeat this rinse procedure twice more. Collection of the water sample in the bottle should be preceded by three rinses.
12. Open the Van Dorn valve and completely fill the bottle.
13. Compress the plastic bottle to remove any residual head space. Seal the cap tightly. Holding the glass bottle (if applicable) level; fill it completely to the top. Seal the cap tightly.
14. Place bottle in a cooler with sealed 1-gal plastic bags of ice. Note the depth from which the sample was collected on the Sample Collection Form.

Shoreline Sample

Only collect a shoreline sample if the study objective is to perform a rough screening to identify probable lake chemical conditions or if it is not feasible to collect either a deep water or outlet sample from the subject lake. Collect the shoreline sample as follows. In the field, make sure that the labels all have the sample ID number (barcode), and that the labels on the bottles and syringes are securely attached. Carefully avoid disturbance of water or sediment in the vicinity of the sampling point before sample collection. This means not walking in the water or on loose rocks. If you must walk out to obtain a clean sample, wait for the sediment to settle before collecting the sample.

Collect a water chemistry sample, as described below, in the deepest water possible. Throughout the collection process, it is important to take precautions to avoid contaminating the sample. Rinse all sample containers three times with lake water before filling them with the sample. Many lakes have a very low ionic strength and can be contaminated quite easily by perspiration from hands, sneezing, smoking, insect repellent, sunscreen, or chemicals used when collecting other types of samples (e.g., formalin or ethanol). Make sure that none of the water sample contacts your hands before going into the sample bottle or syringe. The chemical analyses conducted using the syringe or septum bottle samples can be affected by equilibration with atmospheric carbon dioxide; thus, it is essential that no outside air contact the syringe samples during or after collection.

Shoreline Collection into Bottle

1. Rinse the sample bottle and lid three times with water. Discard the rinse on the shore away from the sample location.
2. Check to ensure that the correct labels are affixed to each sample bottle and syringe. Fill the sample bottle(s), holding the bottle in a tilted position approximately at the midpoint between the water surface and the lakebed, being careful not to disturb any sediment before or while collecting the sample. Try to avoid generating large bubbles in the bottle while it is being filled. If a septum cap is being used, place the cap on the bottle under the surface of the water to avoid any contact of the sample with the air.

3. Place the sample bottle(s) in a cooler (on ice or in a bag in the lake) and shut the lid. If a cooler is not available, place the bottle(s) in an opaque garbage bag and immerse it in the stream.

Shoreline Collection into Syringe

1. Submerge a 60-mL syringe halfway into the lake and withdraw a 15-20 mL aliquot. Pull the plunger to its maximum extension and shake the syringe so the water contacts all surfaces. Point the syringe away from the lake and discard the water by depressing the plunger. Repeat this rinsing procedure two more times.
2. Submerge the syringe into the lake again and slowly fill the syringe with a fresh sample. Try not to get any air bubbles in the syringe. If more than one to two tiny bubbles are present, discard the sample and draw another one.
3. Invert the syringe (tip pointing up), and cap it with a syringe valve. Tap the syringe lightly to detach any trapped air bubbles. With the valve open, depress the plunger to expel the air bubbles and a small volume of water, leaving between 40 and 50 mL of sample in the syringe. Close the syringe valve. If any air bubbles are drawn into the syringe during this process, discard the sample and fill the syringe again.
4. Repeat steps 1 through 3 with a second syringe. Fill a total of four syringes if the lake sample is to be replicated. Place the syringes together in the cooler or temporarily (until time to depart from the lake) in the opaque plastic bag immersed in lake water with the sample bottle(s).

Post Sampling Actions

1. Completely fill out all lake sampling forms. Refer to Appendix E for instructions on filling out the forms.
2. Complete the Chain of Custody Form.
3. Check to make sure that all of the information recorded on the sample label(s), Chain of Custody Form, and Lake Sampling Record Form match.
4. Place each filled bottle into a zipper-lock bag; place the filled syringes into a plastic box with snap-on lid. After carrying the samples to the vehicles, place the (bagged) bottle(s) and (boxed) syringes in a cooler and surround them with 1-gallon re-sealable plastic bags filled with ice. Double bag the ice to avoid getting cooling water (melted ice) into the sample bags.

The sample should be collected on a step-by-step basis as follows:

1. Remove the gloves from the plastic bag and put them on.
2. Remove the sample bottle from the plastic bag. Do not set the bag on the ground or any dirty surface.
3. Rinse the sample bottle in the lake at a location at least 10 feet away from the sample collection point. The bottle and cap should be rinsed three times. For each rinse, fill up the bottle and then pour the rinse water over the inside of the cap, held bottom-side up in the other hand. Pour the rinse water away from the lake sampling point and avoid stirring-up lakebed debris during the process.
4. After the rinsing is completed, move to the sampling point and collect the sample by submersing the tilted bottle or syringe to a depth midway between the sediment and the water surface. Fill the bottle completely. While collecting the sample, avoid stirring-up lakebed debris that might be collected with the sample. If debris may have entered the sample bottle, discard the contents (at a different location), re-rinse the bottle (or use a clean back-up bottle), and collect a new sample.

5. Immediately after collecting the sample, place the lid on the bottle (tightly) and return the bottle to its plastic bag. Seal the bag.
6. Complete the Water Sample Collection section of the Lake Sampling Record Form while at the sample site.

Record the information from the sample label on the Lake Sampling Record Form. Note any problems related to possible contamination in the comments section of the form.

1. Place the sample on ice or refrigerant immediately after collection. Note: do not put ice in the plastic bag that contains the sample bottle.
2. For sites that are close to road access, the large sample cooler can be left in the collection team's vehicle. The samples can be placed in the cooler upon return to the vehicle.
3. For sites that are not close to road access, the collection team should make arrangements to keep the samples on ice after collection and during the return hike. One approach would be to use a small soft-pack cooler or other container that will fit in a backpack. Ice, snow, or refrigerant should be placed in a small plastic bag in the cooler or container (double bagged to avoid leakage and contamination of samples).
4. The samples should be kept in the dark and on ice until delivery to the lab. The ice may need to be replenished during sample transit. Avoid letting the sample bottle float in melted ice water. Do not place the sample bottle in a refrigerator or cooler with food or in any container that is not clean. Ship the samples to the laboratory as soon as possible, preferably within 24 hours of sampling.

FIELD MEASUREMENTS

Anchor the boat if possible. After achieving a stable position and determining the site depth, measure Secchi disk transparency using the procedures below. Record the depth of disk disappearance and the depth of reappearance on the Lake Sampling Record Form. If the Secchi disk is visible at the bottom of the lake, check the "clear to bottom" space on the form. Comment on the form if there are any conditions that may affect this measurement (e.g., surface scum, suspended sediments, or weather conditions).

Other field measurements might be made depending on the study. These could include measurements at the sample site and/or measurements at other locations or depths. They might include dissolved oxygen, specific conductance, and/or other parameters.

To measure for transparency using the disk:

1. Remove sunglasses unless they are prescription lenses.
2. Clip the calibrated chain (should already be in 0.5-m increments) to the Secchi disk. Make sure the chain is attached so that depth is determined from the upper surface of the disk.
3. Lower the Secchi disk over the shaded side of the boat until it disappears. If the disk is visible to the lake bottom, check the appropriate space on the form.
4. Read the depth indicated on the chain. If the disappearance depth is <1.0 m, determine the depth to the nearest 0.01 m by marking the chain at the nearest depth marker and measuring the remaining length with a tape measure. Otherwise, estimate the disappearance depth to the nearest 0.1 m. Record the disappearance depth on the Lake Sampling Record Form.
5. Slowly raise the disk until it reappears and record the reappearance depth on the form.
6. Note any conditions that might affect the accuracy of the measurement in the comments field.

D-2.4 General Lake Assessment

Standard operating procedures are summarized here for the site assessment conducted at lake sampling locations. The purpose of this assessment is to record site characteristics that may aid in the interpretation of the chemical and/or biological data collected from the lake.

Team members should complete the Lake Assessment portions of the Site Documentation Forms at the end of lake sampling, recording all observations from the lake that were noted during the course of the visit. This Lake Assessment is designed as a template for recording pertinent field observations. It is not intended to be comprehensive, and any additional observations should be recorded in the comments section. The Assessment consists of three major sections: General Lake Hydrologic Information, Shoreline Characteristics, and Qualitative Macrophyte Survey. Each is described below.

GENERAL LAKE HYDROLOGIC INFORMATION

Observations regarding the general characteristics of the lake are described in Table D-5. The hydrologic lake type is an important variable for defining subpopulations for acidic deposition effects.

Table D-5. General lake information noted during lake assessment. (Source: Herlihy 1997.)

Item	Description
Hydrologic Lake Type	Note if there are any stream outlets from the lake, even if they are not flowing. If no lake outlets were observed, record the lake as a seepage lake. If the lake was created by a man-made dam (not that a dam is present just to raise the water level), record the lake as a reservoir. Otherwise record the lake as a drainage lake.
Outlet Dams	Note the presence of any dams (or other flow control structures) on the lake outlet(s). Differentiate between artificial (manmade) structures and natural structures (beaver dams). Describe in detail the observed flow control structure, providing measurements if possible. Note the material from which the structure is made.
Lake Level	If a lake level reference point is established, examine the lake shoreline for evidence of lake level changes (e.g., “bathtub ring”). If there are none, check "zero"; otherwise try to estimate the extent of vertical changes in lake level from the present conditions based on other shoreline signs.

SHORELINE CHARACTERISTICS

Shoreline characteristics of interest during the lake assessment are described in Table D-6. To estimate the extent of major vegetation types, limit the assessment to the immediate lake shoreline area (i.e., within 20 m of the water). Also estimate the percentage of the immediate shoreline that has been developed or modified by humans.

Table D-6. Shoreline characteristics observed during lake assessment. (Source: Herlihy 1997.)

Characteristics:	Description
Forest/Shrub	Deciduous, coniferous, or mixed forest, including shrub and sapling vegetation
Agriculture	Cropland, orchard, feedlot, pastureland, or other horticultural activity
Open Grass	Meadows, lawns, or other open vegetation
Wetland	Forested and nonforested wetlands (submerged terrestrial vegetation)
Barren	Nonvegetated areas such as beaches, sandy areas, paved areas, and exposed rock
Developed	Immediate shoreline area developed by human activity; this includes lawns, houses, stores, malls, marinas, golf courses, or any other human-built land use
Shoreline Modifications	Actual shoreline that has been modified by the installation of riprap, revetments, piers, or other human modifications

QUALITATIVE MACROPHYTE SURVEY

Macrophytes (aquatic plants large enough to be seen without magnification) can be important indicators of lake trophic status. The most important macrophyte indicator for assessment purposes is often the percentage of the lake area covered with macrophytes. For both "emergent/floating" and "submergent" coverage, choose one of the four percentage groupings (0 to 25 percent, 25 to 50 percent, 50 to 75 percent, or 75 to 100 percent) that best describes the lake. In some cases, it will be fairly easy to estimate the percentage from observations made during sampling. In other cases, it will be an educated guess, especially if the water is turbid. After recording the areal percentage of macrophyte coverage, record the density of the plants in the observed macrophyte beds as dense, moderate, or sparse. Finally, provide any qualitative description (genera present, if known; dominant type—floating, emergent, or submergent) of the macrophyte beds that would be useful for interpreting the trophic status of the lake. All activities described in this subsection are recorded on the Lake Assessment portion of the Lake Sampling Site Documentation Form 1.

D-2.5 POST-TRIP ACTIVITIES

DATA FORMS AND SAMPLE INSPECTION

After all lake sampling and chain of custody forms are completed, one team member must review the data forms and sample labels for accuracy, completeness, and legibility. Confirm that the SiteID is correct on the forms, as well as the date of the visit. Verify that all information has been recorded accurately, the recorded information is legible, and any flags are explained in the comments section. Ensure that written comments are legible and use no "shorthand" or abbreviations. After reviewing the lake forms, the reviewer should initial the lower right corner of each page of the form. Ensure that all samples are labeled, all labels are completely filled in, and each label is covered with clear plastic tape.

LAUNCH SITE CLEANUP

If a boat or inflatable raft or float tube was used for lake sampling, inspect it for evidence of weeds and other macrophytes. Clean the boat or raft as completely as possible before leaving the launch site to minimize the possibility of transporting aquatic plant fragments or aquatic animals to other lakes where these species may not already occur. Clean up all waste material at the launch site and dispose of or transport it out of the site.

PROCESSING SITE DOCUMENTATION DATA AND INFORMATION

A file system and database with reliable backup should be established for storage of site records and files, map images, and photos. Processing site documentation data and information include the following steps:

1. Retrieve site coordinates (and any tracks) from the GPS unit using the GPS software. Delete any extra coordinate sets (waypoints) and save the file.
2. Retrieve photos from the camera.
3. Enter or revise the site record in the database.
    a. Enter site coordinates obtained in the field.
    b. Enter or revise the site description, travel and access directions.
    c. Add new photos as JPG images with captions to the site record.
4. Create site maps providing both detailed and broader information for access and orientation. Annotate maps and pictures with text and arrows when it would be helpful. Note that the

accuracy of maps varies and the coordinate-based points on the maps, as well as other information, may be misleading. Add clarifying notes. Save these maps as JPG images in the site record. Add captions as appropriate.

5. Enter site and visit data into NRM Air. Images and site maps can be attached to the site or visit database records.

ACKNOWLEDGEMENTS

This SOP is based partly on material developed in the following publications:

Baker, J.R., and D.V. Peck. 1997. Section 4. Lake verification and index site location. In: Baker, J.R., D.V. Peck, and D.W. Sutton (eds.). Environmental Monitoring and Assessment Program Surface Waters: Field Operations Manual for Lakes. Report No. EPA/620/R-97/001. U.S. Environmental Protection Agency, Washington DC. 69p.

Baker, J.R., D.V. Peck, and D.W. Sutton, eds. 1997. Environmental Monitoring and Assessment Program Surface Waters: Field Operations Manual for Lakes. Report No. EPA/620/R-97/001. U.S. Environmental Protection Agency, Washington DC. 69p.

Herlihy, A.T. 1997. Section 9. Final lake activities. In: Baker, J.R., D.V. Peck, and D.W. Sutton (eds.). Environmental Monitoring and Assessment Program Surface Waters: Field Operations Manual for Lakes. Report No. EPA/620/R-97/001. U.S. Environmental Protection Agency, Washington DC. 204p.

Linsley, R.K., M.A. Kohler, and J.L.H. Paulhus. 1982. Hydrology for Engineers. McGraw-Hill Book Co. New York. 508p.

Merritt, G.D.,V.C. Rogers, and D.V. Peck. 1997. Section 3. Base site activities. In: Baker, J.R., D.V. Peck, and D.W. Sutton (eds.). Environmental Monitoring and Assessment Program Surface Waters: Field Operations Manual for Lakes. Report No. EPA/620/R-97/001. U.S. Environmental Protection Agency, Washington DC.

Rantz, S.E. 1982. Measurement and Computation of Streamflow: Volume 1. Measurement of Stage and Discharge. U.S. Geological Survey Water-Supply Paper 2175. U.S. Government Printing Office, Washington, DC. Available at <*http://pubs.usgs.gov/wsp/wsp2175/pdf/WSP2175_vol1a.pdf*>.

APPENDIX E.
DATA ENTRY FORMS AND INSTRUCTIONS FOR FIELD SAMPLING ACTIVITIES

E-1 STREAM SAMPLING FORMS AND INSTRUCTIONS

There are two types of records used for collecting stream water samples: one documents sample site characteristics and the other documents that actual water samples. Site characteristics are documented on two forms called Stream Sampling Site Documentation Forms 1 and 2. These forms are used to document new sampling sites and to clarify and update existing site information.

The Stream Sample Records Forms 3, 4, and 5 are used to record each sample site visit. These forms are to be completed each time you visit a sample site and collect data.

Site information is required at the top of each form, but varies slightly.

FORM HEADER INFORMATION

Form headers provide basic site information. Each form header must be completely filled out in order for the water chemistry and field data to be associated to the correct site in a project and the NRM Air database. The minimum information needed is **underlined in bold** below. This information also includes the GPS information (latitude/longitude) on Form 1, under Site Verification.

Headers for Forms 1 and 3

1. **Monitoring Project Name.** Document the name of the project the monitoring site is assigned to (e.g., R8 Wilderness Stream Water Chemistry Survey or R9 Dry River Watershed Monitoring 2002). To ensure all the lab data is associated with the correct project and site it is very important to document the project name as it is displayed in NRM Air. If this is a new project document on the forms exactly what will be entered into NRM Air as the project name.
2. **Forest Name.** Document the National Forest where the sample will be collected.
3. **Wilderness Name (if applicable).** If the sample site is in a wilderness area, document the wilderness name.
4. **Stream Name (USGS).** Document the official USGS stream name where the sample site is located. In some cases, the stream will not have a name; in this case, enter “Unnamed Stream” or “Unnamed Tributary to [name of stream]”.
5. **Stream Name (Local)**. Document the local stream name. In most cases this will be the same as the USGS stream name. In some cases, an unnamed USGS stream may have a local name associated with it.

Site Name. Document the specific site name as there may be more than one site on a single stream there is a need for a unique sample site name. This unique site name should be developed at the project level. For example, you have a stream named "Mill Creek" with three different sample site locations, you may call each site "Mill Creek SS1," "Mill Creek SS2," and "Mill Creek SS3."

6. **Site ID.** Each sample site should have a unique Site ID, developed at the project level. The unique ID can be a mix of letters and numbers in any logical sequence (be sure to document the naming convention). The Site ID can contain characters for the project's national forest and region. Using the example above, for the site name "Mill Creek SS1" on the Smokey Forest in Region 10, you may abbreviate the names and numbers in the ID: for example, in the Site ID "R10SFMILLCKSS1", R10 = Region 10, SF = Smokey Forest, MILLCK = Mill Creek, SS1 = Sample Site 1.
7. **Date of Visit.** Enter the date that you visited the sample site. Check the space according to whether this is the first, "Initial" visit to this site to establish it as part of a survey or monitoring effort or if this site has been visited and documented previously and therefore this is a "Subsequent" visit.
8. **Field Team Leader Information.** Enter the name, affiliation, phone number, and email address of the field person responsible for the sampling.
9. **Access Information.** Check all spaces that apply to the type of access to this site. Document any travel directions (attach a map if available), estimated time of travel, and any additional information that may be helpful in getting to this site.

Headers for Forms 2, 4, and 5

Note that each of these Forms has space near the top to enter information that identifies the stream, sample site, and the date of sampling. This is an abbreviated version of the Basic Site Information described above. It is important to fully complete this "header" information on each form to ensure that identifiers are consistent and related data are kept together.

The instructions for completing the following four attributes are described under Basic Site Information, above.

- Stream Name (Local): see item 4 above.
- Date of Visit: see item 7 above.
- Site Name: see item 5 above.
- Site ID: see item 6 above.

STREAM SAMPLING – SITE DOCUMENTATION FORM 1

Site Verification, GPS Information, and Tagging

Most of this data will be collected and recorded at the sample site.

1. **Stream Verified.** Does the available data match conditions observed on the ground sufficiently to verify that the intended sampling site has been located? Check Yes or No. Check all methods used to verify that you have located the correct site.
2. **GPS Information.** After establishing the sample site location, record the coordinates from the GPS unit: Latitude and Longitude in decimal degrees (if possible to six decimal places); include the Datum of the collected coordinates (e.g., NAD83). Record the Elevation in feet or meters, and, if the GPS unit has the capability, record its given accuracy in feet or meters.

These coordinates will be the ones used in site identification and will be documented on the forms and in the NRM Air database. Each time the site is visited, use the same coordinates.

3. **Site Tag.** Record whether a metal tree tag has been affixed to a prominent tree in proximity to the sampling site. (Many wilderness areas do NOT allow the use of metal tags.) If a tag has been used, indicate the tree species to which the tag is attached. If the species is not known, record "unknown." Record whether the tag was affixed to the tree on a previous trip (an existing tag) or newly placed on this trip (a new tag). Describe the tree and its location relative to the sampling site. Include, in the description, the height above the ground and compass bearing from the tag to the sampling site.

Site Assessment

The site assessment data will be collected from field observations (within 20 meters of the streambank) and recorded at the sample site.

1. **Streambank Character.** Estimate the percentages of vegetation types along the streambank (i.e., forest/shrub, herbaceous, wetland, or barren). Also look at the observed land use in the same area and identify shoreline modifications (e.g., a dock or riprap), development, and/or agricultural use. Use the following classes for these estimates (record the percent range on the form):
    a. Rare (< 5%)
    b. Sparse (5-25%)
    c. Moderate (25-75%)
    d. Extensive (> 75%)
2. **The vegetation Dominant Age Class** (forested areas only). If the site is located within a forested area, estimate the dominant age class of the trees. Check the appropriate age class space (i.e., 0-10 years, 10-25 years, 25-50 years, or > 50 years).
3. **Dominant plant species.** Observe the dominant plant species within the area of the sample site and, if known, record them on Form 1.
4. **Beaver activity.** Document observed beaver activity; check the appropriate spaces on the form.

Watershed Assessment

Most of this information can be obtained in the office through geographic information system (GIS)-based analysis before visiting the sample site. It can be helpful to have this information completed before the first sampling visit. This information will help to locate the targeted sample location. The form is useful for verifying in the field that the office GIS-based analysis appears to be a valid identification of watershed characteristics.

1. **Vegetation cover.** Using ortho-corrected or georeferenced aerial imagery in a GIS, estimate the different vegetation types, including exposed rock and tallus. Document this as a percentage of the watershed area above the sample site. Verify in the field whether this appears to be a valid identification.
2. **Lithology.** If GIS information on bedrock lithology is available, indicate the primary lithology (e.g., granitic, volcanic, or metamorphic). If there is more than one lithology, make note of additional significant lithology.
3. **Watershed area**. Using GIS, digitize the watershed area above the sample site (the watershed size will vary based on the sample site location). Calculate the watershed area in acres or

hectares. It will be helpful to determine the watershed area and boundary before determining other watershed assessment attributes.

4. **Primary watershed aspect.** The primary aspect of the watershed is determined by estimating the direction the watershed is facing based on the direction of stream flow. Indicate the aspect in degrees, with North as 0˚, East as 90˚, South as 180˚, and West as 270˚.
5. **Average watershed slope.** A simple way of calculating average slope percentage within the watershed boundary is to use digital elevation model (DEM) GIS files and Zonal Stats in GIS Spatial Analyst.
6. **Stream order.** Identify the stream order at the sampling site location using the Forest Service's National Hydrography Dataset (NHD).

STREAM SAMPLING – SITE DOCUMENTATION FORM 2

Photo Log

Take two photos at the sampling site, looking both upstream and downstream. On the initial visit to the site, it can be helpful to take additional photos to visually record stream and site characteristics (e.g., land use and vegetation). Record the photo ID or file name, date photographed, and description of the photo. It is recommended that these photos be attached to the site documentation forms in the project files in hardcopy once they are printed.

Description and Sketch of Site

Record a description and make a sketch of the site. This information can help in locating and characterizing the site, and may include access and tree tag information, land marks, and land use indicators.

Additional Notes

Add any additional information that may help to identify, locate, or describe this site.

STREAM SAMPLING – SAMPLE RECORD FORM 3

When collecting water chemistry data, care must be taken to protect samples from contamination. It is recommended that water samples be taken before the general observations (e.g., stream width and depth) are measured; this will ensure stream bottom sediments will not be stirred up before sampling, potentially contaminating the water samples.

Basic Site Information—Suggested Revisions

Examine information recorded on the Stream Sampling Site Documentation Forms for agreement with site characteristics. Indicate any suggested revisions or updates to the site documentation form. Place a check mark next to all types of information requiring revision and explain the suggested revision in the space provided.

Water Samples and Replicates

1. **Time Sampled**. Indicate time of sample collection using a 24-hour clock (e.g., 4 p.m. is "1600"). Note that the time recorded on the bottle(s) and syringe(s) for the replicates should differ from the time recorded for the normal (regular) sample. This is important! The recommended protocol is to separate the sampling times for normal and replicate samples by one minute.

2. **Sample ID.** Enter the unique identification code assigned to the sample (developed at the project level). These Sample ID codes are designed to be familiar to the project personnel and can represent the specific site, sample, type, and date, etc. For example, the Site ID combined with the sample type and/or date (e.g., SITEIDREG for a regular sample or SITEIDREP for a replicate sample). Note: Barcodes provided by ARML are a unique identifier and have a specific location for placement on the form (see item 6 below).

   The sample ID represents a sample of water (bottle(s) and/or syringes) intended to represent conditions at a particular location, on a particular day, at a particular time. Note that multiple containers (bottle and/or syringes) obtained within one time window represent the SAME sample and receive the SAME ID code (and barcode). Replicated samples will receive different ID codes (and barcodes).

3. **Sample Type.** Record the type of sample collected (i.e., regular, replicate, or field blank).
4. **Bottle Type.** Record the type of bottle (or syringe) used for sample collection (i.e., plastic, glass, or syringe).
5. **Number of Bottles.** Record the number of samples (aliquots) collected for the normal (regular) sample and any replicates that may have been collected.
6. **Bar Code.** The barcode (a unique number/letter ID) will be prepared and provided by the ARML. Each year the lab provides new barcodes specific for that year. The barcode sheets will have multiple stick-on copies of the same barcode. These can be organized before field sampling and subsequently be affixed to the Stream Sampling Record Form, the Chain of Custody Form, and to each container (bottle or syringe) for the sample.
7. **Collection Location.** If the sample was not collected at the intended (targeted) location, explain the reason for changing the sample location.

General Observations

1. **Air Temperature.** Record the air and water temperature to the nearest degree and document the time (24hr) when it was measured. Record whether it is expressed in °C or °F.
2. **Weather Condition** (current and previous). Check the spaces that best describe the collection-day weather up to the time of sampling and the average weather over the previous three days (if known).
3. **Stream Depth.** Record the stream depth measured at sample site in mid-channel; check the space for the appropriate measurement units.
4. **Stream Width.** Record the stream width measured at the sample site; check the space for the appropriate measurement units.
5. **Observed Discharge Level.** Indicate the observed level of discharge in the stream at the time of sampling. Check the appropriate space (i.e., no flow, low flow, normal flow, or flood).
6. **Delivery Method.** Record the delivery method type.

STREAM SAMPLING – SAMPLE RECORD FORM 4

On-Site Water Data (optional)

If on-site water data were collected, record the measured values—the time measurements were taken, air temperature, and water temperature (at sample site). Check the space for the appropriate measurement units. Note: For field instrument data, express DO in units of mg/L and, if possible, percent DO. Correct specific conductance to 25^{o} C. Record the measurements, instruments, and methods used for on-site water data collected.

Photo Log

Take two photos of the sampling site looking both upstream and downstream. It can be helpful to take additional photos to visually record stream and site characteristics (e.g., land use and vegetation). Record the photo ID or file name, date photographed, and description of the photo. It is recommended that these photos be attached to the site documentation forms in the project files in hardcopy once they are printed.

Additional Notes

Add any additional information that may help to identify, locate, or describe this sample site.

STREAM SAMPLING – SAMPLE RECORD FORM 5

This form is for collecting stream water stage and discharge data.

General Information

Record the time this data was obtained and indicate what methods were used to collect an estimate of stream stage or discharge (check appropriate spaces). Complete the appropriate stage or discharge section on the form (Stage Measurement Only, Velocity-Area Procedure, or Timed Filling Procedure).

Stage Measurement Only

If stage measurements (estimates) were made in the field, record the measured value and indicate the unit of measure. Describe the location of measurement. Indicate if a rating curve has been developed with which to estimate discharge from stage measurements at this location, and indicate what the stage is referenced to (i.e., fixed staff gage or permanent landscape feature).

Discharge Measurement by Velocity-Area Procedure

If the velocity-area procedure was used to measure discharge, check “Yes” and indicate the units of measurement for water depth and velocity. Record the approximate width of the stream at the sampling location. Record the water depth and velocity in each of up to 20 evenly spaced intervals of the stream cross section.

Discharge Measurement by Timed Filling Procedure

If the timed filling method was used to measure discharge, check “Yes” and indicate the units of measurement for time and water volume. Record the time and volume measurements for five separate trials at each of up to three spillway locations.

USDA Forest Service
National Protocols for Air Pollution Sensitive Waters

STREAM SAMPLING - SITE DOCUMENTATION
FORM 1

BASIC SITE INFORMATION

Monitoring Project Name:

Forest Name: **Wilderness Name** (if applicable):

Stream Name (USGS): **Stream Name** (Local):

Site Name: **Site ID:**

Date of Visit: **Visit:** ____ Initial ____ Subsequent

Field Team Leader:

Affiliation:

Phone: **Email:**

Access: ____ Vehicle ____ Short Hike (< 1 hr) ____ Long Hike (> 1 hr) ____ Overnight Hike

Travel Directions to Stream Sampling Site and Access Information:

SITE VERIFICATION, GPS INFORMATION AND TAGGING

Stream Verified: ____ Yes ____ No

Site has been verified by (check all that apply): ____ GPS ____ Local Contact ____ Signs ____ Vegetation ____ Roads ____ Topo Map ____ Photos ____ Other

GPS Information **Latitude** (DD) ___ ___ . ___ ___ ___ ___ ___ ___ **GPS Accuracy:** ____ ft. ____ m

Datum: **Longitude** (DD) -___ ___ ___ . ___ ___ ___ ___ ___ ___ **Elevation:** ____ ft. ____ m

Site Tag has been Affixed? ____ Yes ____ No ____ New Tag ____ Existing Tag **Tag Tree Species:**

Describe tag tree location relative to stream sampling site:

SITE ASSESSMENT (Observations within 20 m of streambank)

Streambank Character (use % class below)

_____% Forest/Shrub
_____% Open Herbaceous
_____% Wetland
_____% Barren (beach/rock)
_____% Agriculture
_____% Developed
_____% Shoreline Mod. (e.g., riprap)

Rare (< 5%) Moderate (25-75%)
Sparse (5-25%) Extensive (> 75%)

Dominant Age Class (forested areas only)

____ 0 - 10 years
____ 10 - 25 years
____ 25 - 50 years
____ > 50 years

What are the dominant plant species (if known)?

Is there beaver activity near the sample site?

Signs of Beaver: ____ None ____ Rare ____ Common

Beaver Flow Modifications: ____ None ____ Minor ____ Major

WATERSHED ASSESSMENT

What percent of the watershed above the site is covered by each of these?

_____% Hardwoods
_____% Conifers
_____% Mixed Forest
_____% Exposed Rock
_____% Herbaceous/Shrubs
_____% Tallus
_____% *Total*

Primary lithology type:

Note additional significant lithology types:

What is the watershed area above the sample site? _____ ____ ha ____ ac

What is the watershed aspect (degrees)? _____°

What is the average slope of the watershed? _____%

What is the stream order of the sample site (use NHD dataset)? _____

Version 03/2012

USDA Forest Service
National Protocols for Air Pollution Sensitive Waters

STREAM SAMPLING – SITE DOCUMENTATION
FORM 2

Stream Name (Local):	**Date of Visit:**
Site Name:	**Site ID:**

PHOTO LOG

Photo ID/File Name	Date	Description of Photo

DESCRIPTION AND SKETCH OF SITE

ADDITIONAL NOTES

Version 03/2012

USDA Forest Service
National Protocols for Air Pollution Sensitive Waters

STREAM SAMPLING - SAMPLE RECORD
FORM 3

BASIC SITE INFORMATION

Monitoring Project Name:

Forest Name: **Wilderness Name** (if applicable):

Stream Name (USGS): **Stream Name** (Local):

Site Name: **Site ID:**

Date of Visit: **Arrival Time** (24hr): ____ Standard ____ Daylight Saving

Field Team Leader:

Affiliation:

Phone: **Email:**

Are there any suggested revisions to the Site Documentation Forms 1 and 2 (select the area of revision below)?

____ GPS Information ____ Stream Description ____ Site Description ____ Access/Travel Information

Describe suggested revision:

WATER SAMPLES AND REPLICATES

Time Sampled	*Sample ID*	*Sample Type (reg, rep ,blank)*	*Bottle Type (plastic, glass, syringe)*	*# of Bottles or Syringes*	*Bar Code*

Collection Location (explain any deviation from targeted sampling location):

GENERAL OBSERVATIONS

Time Obtained (24 hr): **Air Temperature:** ____ ^{O}C ____ ^{O}F

Water Temperature (at sample location): ____ ^{O}C ____ ^{O}F **Instrument Used:**

What is the weather condition on the day of sampling? ____ Clear ____ Partly Cloudy ____ Overcast ____ Hail ____ Light Rain ____ Occasional Rain ____ Persistent Rain ____ Snow or Sleet

What was the weather for the 3 days prior to day of sampling? ____ Generally Dry ____ Occasional Rain/Snow ____ Generally Wet ____ Very Wet

Average stream depth at sample location: ____ ft ____ in ____ m ____ cm

Average stream width at sample location: ____ ft ____ in ____ m ____ cm

Discharge Level: ____ No Flow ____ Low Flow ____ Normal Flow ____ Flood

Delivery method to laboratory: ____ Vehicle ____ Overnight Shipping ____ Other (explain):

Version 03/2012

USDA Forest Service
National Protocols for Air Pollution Sensitive Waters

STREAM SAMPLING – SAMPLE RECORD
FORM 4

Stream Name (Local):	**Date of Visit:**
Site Name:	**Site ID:**

ON SITE WATER DATA (OPTIONAL)

Time Obtained (24 hr)

Parameter	Value	Equipment (make/model)	Method/Reference (EPA/SM/USGS)
Conductivity	uS/cm Corrected to 25^0 C? ____ Yes ____ No		
pH			
Turbidity	_____NTU		
Dissolved Oxygen	mg/l %DO		
Water Temperature	____ ^oC ____ ^oF		
Other			

PHOTO LOG

Photo ID/File Name	Date	Description of Photo

ADDITIONAL NOTES

Version03/2012

USDA Forest Service
National Protocols for Air Pollution Sensitive Waters

STREAM SAMPLING - SAMPLE RECORD
FORM 5

Stream Name (Local): **Date of Visit:**

Site Name: **Site ID:**

STAGE AND DISCHARGE DATA

General Information

Time Obtained (24 hr): ____ **Not obtained in field.**

Method of determining stage and/or discharge (check all that apply below) ____ **None**

____ Cross section of depth measurements.

____ Velocity-area procedure (number of sets of measurements taken_____).

____ Salt dilution method.

____ Relative stage comparison with nearby fixed gage.

____ Stage measurement with pressure transducer.

____ Stage measurement with staff gage.

____ Timed filling procedure (number of spillways measured_____).

Stage Measurement Only

Stage relative to gage: _____ ____ ft ____ in ____ m ____ cm

Location of fixed gage measurement relative to stream sampling site

Stage measurement with staff gage _____ ____ ft ____ in ____ m ____ cm

Location of fixed gage measurement relative to stream sampling site

Existing ratings curve? ____ Yes ____ No **If yes, referenced to what?**

Mid-channel stream depth at sample location: ____ ft ____ in ____ m ____ cm

Discharge Measurement by Velocity-Area Procedure

Velocity-area procedure? ____ Yes ____ No **Depth:** ____ ft ____ in ____ m ____ cm **Velocity:** ____ m/s ____ ft/s

Stream width at measurement location: ____ ft ____ in ____ m ____ cm

Interval	1	2	3	4	5	6	7	8	9	10
Depth										
Velocity										
Interval	11	12	13	14	15	16	17	18	19	20
Depth										
Velocity										

Discharge Measurement by Timed Filling Procedure

Timed filling procedure? ____ Yes ____ No **Time:** ____ min ____ sec **Volume:** ____ L ____ gal

Spillway Number	Time to Fill Measure Volume									
	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5	
	Time	Volume	Time	Volume	Time	Volume	Time	Volume	Time	Volume
1										
2										
3										

ADDITIONAL NOTES

Version 03/2012

E-2 LAKE SAMPLING FORMS AND INSTRUCTIONS

There are two types of records used for collecting lake water samples: one documents lake characteristics and the other documents the actual water samples. Site characteristics are documented on two forms called Lake Sampling Site Documentation Forms 1 and 2. These forms are used to document new sampling sites and to clarify and update existing site information.

The Lake Sampling Record Forms 3 and 4 are used to record each sample site visit. These forms are to be completed each time you visit a sample site and collect data.

Site Information is needed at the top of each form, but varies slightly.

FORM HEADER INFORMATION

Form headers provide basic site information. Each form header needs to be completely filled out in order for the water chemistry and field data to be associated to the correct site in a project and the NRM Air database. The minimum information required to import this data into the NRM Air database is **underlined in bold** below. This also includes the GPS information (latitude/longitude) on Form 1 under Site Verification.

Headers for Forms 1 and 3

1. **Monitoring Project Name.** Document the name of the project the monitoring site is assigned to (e.g., R2 Long-Term Lake Monitoring or R2_R4 Wind River Mountains Bulk Deposition Monitoring). To ensure all the lab data is associated with the correct project and site, it is very important to document the project name as it is displayed in NRM Air. If this is a new project document on the forms exactly what will be entered into NRM Air as the project name.
2. **Forest Name.** Document the National Forest where the sample will be collected.
3. **Wilderness Name (if applicable).** If the sample site is in a wilderness area, document the wilderness name.
4. **Lake Name (USGS).** Document the lake name where the sample site is located. In some cases the lake will not have a name; in this case, document “Unnamed Lake” or “Unnamed Tributary to [name of lake]”.
5. **Lake Name (Local).** Document the local lake name. In most cases this will be the same as the USGS lake name. In some cases, an unnamed USGS lake may have a local name associated with it.
6. **Site Name.** Document the specific site name as there may be more than one site on a single lake there is a need for a unique sample site name. This unique site name should be developed at the project level. For example, you have a lake named “Deep Lake” with two different sample site locations (e.g., in the outlet and in the middle of the lake), you may call one site “Deep Lake Outlet” and the other site “Deep Lake Mid.”
7. **Site ID.** Each sample site should have a unique identifier (ID) and Site ID, developed at the project level. The unique ID can be a mix of letters and numbers in any logical sequence (be sure to document the naming convention). The Site ID can contain characters for the project’s national forest and region. Using the example above, for the site name “Mill Creek SS1” on the Smokey Forest in Region 10, you may abbreviate the names and numbers in the ID: for example, in the Site ID “R10SFMILLCKSS1”, R10 = Region 10, SF = Smokey Forest, MILLCK = Mill Creek, SS1 = Sample Site 1.

8. **Date of Visit.** Enter the date you visit the sample site. Check the space according to whether this is the first "Initial" visit to this site to establish it as part of a survey or monitoring effort or if this site has been visited and documented previously and, therefore, this is a "Subsequent" visit.
9. **Field Team Leader Information.** Enter the name, affiliation, phone number, and email address of the field person responsible for the sampling.
10. **Access Information.** Check all spaces that apply to the access of this site. Document any travel directions (attach a map if available), estimated time of travel, and any additional information that may be helpful in getting to this site.

Headers for Forms 2 and 4

Note that each of these forms has space near the top to enter information that identifies the lake, sample site, and the date of sampling. This is an abbreviated version of the Basic Site Information described above. It is important to fully complete this "header" information on each form to ensure that identifiers are consistent and related data are kept together.

The instructions for completing the following four attributes are described under Basic Site Information above.

- Lake Name (Local): see item 4 above.
- Date of Visit: see item 7 above.
- Site Name: see item 5 above.
- Site ID: see item 6 above.

LAKE SAMPLING – SITE DOCUMENTATION FORM 1

Site Verification, GPS Information, and Tagging

1. **Lake Verified.** Does the available data match conditions observed on the ground sufficiently to verify that the intended sampling site has been located? Check Yes or No. Check all methods used to verify that you have located the correct site.
2. **GPS Information.** After establishing the sample site location record the coordinates from the GPS unit—Latitude and Longitude in decimal degrees (if possible to six decimal places)—include the Datum of the collected coordinates (e.g., NAD83). Record the Elevation in feet or meters, and if the GPS unit has the capability, record its accuracy in feet or meters. These coordinates will be the one used for site identification and will be documented on the forms and in the NRM Air database. Each time the site is visited, use the same coordinates.

Lake Assessment

The lake assessment data will be collected from field observations and recorded at the lake.

1. **Shoreline Character.** Estimate the percentages of vegetation types within 20 meters of the shoreline (i.e., forest/shrub, herbaceous, wetland, or barren). Also, look at the observed land use in the same area, identify shoreline modifications (e.g., dock or riprap), development, and/or agricultural use. Use the following classes for these estimates (record the percent range on the form):
    a. Rare (< 5%)
    b. Sparse (5-25%)
    c. Moderate (25-75%)
    d. Extensive (> 75%)

2. **Hydrologic Lake Type.** Record the hydrologic type of the lake from the following:
    a. Reservoir (artificial, human-made dam).
    b. Drainage (outlet stream present; may or may not be flowing at time of visit).
    c. Seepage (no outlet stream present, regardless of whether or not it is flowing at the time of visit). If a dam is present, select the appropriate type (e.g., artificial, augmented, or natural). Record the number of inlets to and outlets from the lake (if present).

3. **Macrophyte Observation.** From a quick visual survey of the lake, estimate the percent of lake area covered by "emergent/floating" and "submergent" macrophytes (rooted aquatic plants large enough to be seen without magnification). Select the appropriate percent class (i.e., 0-25%, 25-50%, 50-75%, or >75%). Record the average density of the macrophyte community (i.e., absent, sparse, moderate, or dense). Identify (if known) the one to three most prevalent macrophyte species.

4. **Lake Trophic State.** Eutrophication is the process whereby a body of water becomes over-enriched in nutrients, resulting in increased productivity of algae or aquatic plants (biomass) and sometimes also decreased dissolved oxygen levels. Based on the amount of biomass in the lake, record your estimation of the lake's trophic state from the following classes:
    a. Oligotrophic (low biomass production). Oligotrophic lakes are most common in cold regions underlain by resistant igneous rocks (especially granitic bedrock).
    b. Mesotrophic (moderate or intermediate level of biomass productivity). These lakes are commonly clear water lakes and ponds with beds of submerged aquatic plants and medium levels of nutrients.
    c. Eutrophic (high biomass productivity due to excessive nutrients, subject to algal blooms). Eutrophic waters commonly lack fish species like trout, which require cold, well-oxygenated waters.
    d. Hypereutrophic (very high biomass productivity —nutrient-rich). Lakes are characterized by frequent and severe nuisance algal blooms and low transparency.

Watershed Assessment

Most of this information can be obtained in the office through geographical information system (GIS)-based analysis of aerial photography before visiting the sample site. It can be helpful to have this information completed before the first sampling visit. This information will help to locate the targeted sample location. The form is useful for verifying in the field that the office GIS-based analysis appears to be a valid identification of watershed characteristics.

1. **Vegetation cover.** Using ortho-corrected or georeferenced aerial imagery in a GIS, estimate the different vegetation types including exposed rock and tallus. Document this as a percentage of the watershed area above the sample site. Verify in the field whether this appears to be a valid identification.

2. **Lithology.** If GIS information on bedrock lithology is available, indicate the primary lithology (e.g., granitic, volcanic, or metamorphic). If there is more than one lithology, make note of additional significant lithology.

3. **Watershed area.** Using GIS, digitize the watershed area above the sample site (the watershed size will vary based on the sample site location). Calculate the watershed area in acres or hectares. It will be helpful to determine the watershed area and boundary before determining other watershed assessment attributes.

4. **Watershed aspect.** The primary aspect of the watershed is determined by estimating the direction the watershed is facing based on the direction of stream flow. Indicate the aspect in degrees with North as 0˚, East as 90˚, South as 180˚, and West as 270˚.
5. **Average watershed slope.** A simple way of calculating average slope percent within the watershed boundary is to use digital elevation model (DEM) GIS files and Zonal Stats in GIS Spatial Analyst.
6. **Stream order.** Identify the stream order at the sampling site location using the Forest Service's National Hydrography Dataset (NHD).

LAKE SAMPLING – SITE DOCUMENTATION FORM 2

Establish Lake Level Reference Point

On the initial visit (or subsequent visit) to the lake, establish a reference point for measuring lake level.

If it is allowed, permanently monument the lake level location by marking a shoreline rock (e.g., inserting a bolt). Check the appropriate space (Yes or No), indicating whether there is an established permanent marker. If a permanent monument is not allowed, identify a landmark (e.g., "bathtub ring," rock formation, or outcrop) from which to measure lake level. Record in the space provided the type of monument or landmark used to mark the reference point. Describe the lake level reference point location, with measurements and photos, on Form 2 under Description and Sketch of Lake and Sample Location and under Photo Log.

Measure the lake level at the time that the reference point is established; record the distance from lake level to the reference point—include measurement units (feet, inches, meters, or centimeters).

Description and Sketch of Lake and Sample Location

Record a description and sketch of the lake. This information can help in locating and characterizing the lake, and may include lake access, land marks, and land use indicators. Make sure to include sampling locations (e.g., mid-lake, shoreline, or outlet), lake level reference point location, and photo points.

Photo Log

Take photos of the lake sampling site to help identify the precise location of the sampling location. On the initial visit to the site, it can be helpful to take additional photos to visually record lake and site characteristics (e.g., land use and vegetation). Record the photo ID or file name, date photographed, and description of the photo. It is recommended that these photos be attached to the site documentation forms in the project files in hardcopy once they are printed.

Additional Notes

Add any additional information that may help to identify, locate, or describe this site.

LAKE SAMPLING – SAMPLE RECORD FORM 3

Basic Site Information—Suggested Revisions

Examine information recorded on the Lake Sampling Site Documentation Forms for agreement with site characteristics. Indicate any suggested revisions or updates to the site documentation form. Place a check mark next to all types of information requiring revision; explain the suggested revision in the space provided.

Water Samples and Replicates

1. **Time Sampled.** Indicate time of sample collection, using a 24-hour clock (e.g., 4:00 PM is "1600"). Note that the time recorded on the bottle(s) and syringe(s) for the replicates should differ from the time recorded for the normal (regular) sample. This is important! The recommended protocol is to separate the sampling times for normal and replicate samples by one minute.
2. **Sample ID.** Enter the unique identification code assigned to the sample (developed at the project level). These Sample ID codes are designed to be familiar to the project personnel and can represent the specific site, sample, type, and date, etc. For example, the Site ID combined with the sample type and/or date (e.g., SITEIDREG for a regular sample or SITEIDDUP for a duplicate sample). Note: Barcodes provided by the ARML are a unique identifier and have a specific location for placement on the form (see item 6 below).

   The sample ID represents a sample of water (bottle(s) and/or syringes) intended to represent conditions at a particular location, on a particular day, at a particular time. Note that multiple containers (bottle and/or syringes) obtained within one time window represent the SAME sample and receive the SAME ID code (and barcode). Replicated samples will receive different ID codes (and barcodes).
3. **Sample Depth.** Record the depth at which the sample was collected. Indicate the units of measure (ft or m).
4. **Sample Depth Zone.** Record the limnetic zone (i.e., epilimnion or hypolimnion) in which the sample was collected. If the limnetic zone is not known record either "surface" or "deep".
5. **Sample Type**. Record the type of sample collected (i.e., regular, replicate, or field blank).
6. **Bottle Type.** Record the type of bottle (or syringe) used for sample collection (i.e., plastic, glass, or syringe).
7. **Number of Bottles.** Record the number of sample aliquots (bottles or syringes) collected for the normal (regular) sample and any replicates that may have been collected.
8. **Bar Code.** The barcode (a unique number/letter ID) will be prepared and provided by ARML. Each year the lab provides new barcodes specific for that year. The barcode sheets will have multiple stick-on copies of the same barcode. These can be organized before field sampling and subsequently be affixed to the Lake Sampling Record Form, the Chain of Custody Form, and to each container (bottle or syringe) for the sample.
9. **Collection Location.** If the sample was not collected at the intended (targeted) location, explain the reason for changing the sample location.

General Observations

1. Record the **Time Obtained** (24hr) when the general observations were made.
2. **Air Temperature** and **Water Temperature.** Record the air and water temperature collected to the nearest degree; check the units in °C or °F. Document the instrument used for measurement (e.g., certified thermometer or Clinefinder).
3. **Weather conditions** (current and previous). Check the spaces that best describe the collection day weather up to the time of sampling, and the average weather over the previous three days (if known).
4. **Lake Level Change.** Measure the distance from lake level to the reference point (see initial or previous years established reference point for location) and record the measurement plus or minus (+ / -) the reference point—include measurement units (i.e., ft, in, m, or cm). If there is no change from the reference point, mark the "zero change" space on the form.
5. **Observed Discharge Level.** Indicate the observed level of discharge from the lake at the time of sampling. Check the appropriate space (i.e., no flow, low flow, normal flow, high flow, or flood).

LAKE SAMPLING – SAMPLE RECORD FORM 4

Transparency Data

Record two depth measurements using the Secchi disk, 1) when the disk disappears and 2) when the disk reappears; select the appropriate unit of measurement. If the Secchi disk was visible to the bottom of the lake, check the space for "Yes" beside Clear to Bottom?

Depth/Temperature Profile

1. **Method/Instrument.** Document the EPA/SM/USGS method used for the measurements, as well as the make and model of the equipment used for the profile.
2. **Index Location and Depth.** Indicate whether the depth profile was measured at the index site or not. If the profile was collected in a different location, explain the reason. Record the total depth at the location of the profile and select the appropriate units.
3. **Measurements.** Collect the depth and measurements for that depth; record the depth and measurement values in the profile table for each parameter. Check the appropriate space indicating units of measure (temperature and DO). Indicate whether conductivity measurements are corrected to 25^{o} C.

On-Site Water Data (optional)

If on-site water data were collected, record the measured values: the time measurements were taken, air temperature and water temperature (at sample site). Check the space for the appropriate measurement units. Note: Express DO in units of mg/L and, if possible, percent DO. Correct specific conductance to 25^{o} C. Record the measurements, instruments, and methods used for on-site water data collected.

Photo Log

Take photos of the lake sampling site that will help identify its precise location. It may be helpful to take additional photos to visually record lake and site characteristics (e.g., land use and vegetation). Record the photo ID or file name, date photographed, and description of the photo. It is recommended that these photos be attached to the site documentation forms in the project files in hardcopy once they are printed.

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National Protocols for Air Pollution Sensitive Waters

LAKE SAMPLING - SITE DOCUMENTATION
FORM 1

BASIC SITE INFORMATION

Monitoring Project Name:

Forest Name: | **Wilderness Name** (if applicable):

Lake Name (USGS): | **Lake Name** (Local):

Site Name: | **Site ID:**

Date of Visit: **Visit:** ____ Initial ____ Subsequent

Field Team Leader:

Affiliation:

Phone: **Email:**

Access: ____ Vehicle ____ Short Hike (< 1 hr) ____ Long Hike (> 1 hr) ____ Overnight Hike

Travel Directions to Lake Sampling Site and Access Information:

Sample Site Location: ____ Lake Inlet ____ Lake Outlet ____ Lake Shoreline ____ Lake Index Site (mid-lake)

Describe location of sample collection site:

SITE VERIFICATION, GPS INFORMATION

Lake Verified: ____ Yes ____ No

Site has been verified by (check all that apply): ____ GPS ____ Local Contact ____ Signs ____ Vegetation ____ Roads ____ Topo Map ____ Photos ____ Other

GPS Information **Latitude** (DD) __ __ . __ __ __ __ __ __ **GPS Accuracy:** ____ ft. ____ m

Datum: **Longitude** (DD) -__ __ __ . __ __ __ __ __ __ **Elevation:** ____ ft. ____ m

LAKE ASSESSMENT (Observations within 20 m of shoreline)

Shoreline Character (use % class below)

_____% Forest/Shrub
_____% Open Herbaceous
_____% Wetland
_____% Barren (beach/rock)
_____% Agriculture
_____% Developed
_____% Shoreline Mod. (e.g., riprap)

Rare (< 5%) Moderate (25-75%)
Sparse (5-25%) Extensive (> 75%)

Macrophyte Observation (aquatic plants large enough to be seen without magnification)

Emergent/Floating Coverage (% lake area): ____ 0-25 ____ 25-50 ____ 50-75 ____ >75

Submergent Coverage (% lake area): ____ 0-25 ____ 25-50 ____ 50-75 ____ >75

Density: ____ Absent ____ Sparse ____ Moderate ____ Dense

Macrophyte Species (if known)

Lake Trophic State (estimate amount of biomass in lake)

____ Oligotrophic (little or none) ____ Mesotrophic (intermediate)
____ Eutrophic (large amount) ____ Hyperutrophic (extreme)

Hydrologic Lake Type

____ Reservoir ____ Drainage (outlets present) ____ Seepage (no outlets observed)

Inlets:_____ # Outlets_____ Outlet Dams: ____ None ____ Artifical ____ Augmented ____ Natural

LAKE WATERSHED ASSESSMENT

What percent of the watershed area is covered by each of the following?

_____% Hardwoods
_____% Conifers
_____% Mixed Forest
_____% Exposed Rock
_____% Herbaceous/Shrubs
_____% Tallus
_____% *Total*

Primary lithology type:

Note additional significant lithology types:

What is the watershed area contributing to the lake? _____ ____ ha ____ ac

What is the watershed aspect (degrees)**?** _____°

What is the average slope of the watershed? _____%

What is the stream order of the sample site (use NHD dataset)? _____

Version 03/2012

USDA Forest Service
National Protocols for Air Pollution Sensitive Waters

LAKE SAMPLING – SITE DOCUMENTATION
FORM 2

Lake Name (Local):	**Date of Visit:**
Site Name:	**Site ID:**
Latitude (DD) ___ ___ . ___ ___ ___ ___ ___ ___	**Longitude (DD)** -___ ___ ___ . ___ ___ ___ ___ ___ ___

ESTABLISH LAKE LEVEL REFERENCE POINT

Is reference point permanently monumented? ___ Yes ___ No	If there is not an established "Reference Point": establish a monument either by affixing a permanent marker (if allowed) or identifying a landmark (e.g., bathtub ring, rock formation/outcrop). Describe and document the location with measurements and photos, and include in the lake sketch.
What type of monument/landmark is used to mark the reference point?	
	Lk. Level at time of establishment (dist. from ref point):_____ Units:_____

DESCRIPTION AND SKETCH OF LAKE AND SAMPLE LOCATION

PHOTO LOG

Photo ID/File Name	Date	Description of Photo

ADDITIONAL NOTES

Version 03/2012

USDA Forest Service
National Protocols for Air Pollution Sensitive Waters

LAKE SAMPLING - SAMPLE RECORD
FORM 3

BASIC SITE INFORMATION

Monitoring Project Name:

Forest Name: **Wilderness Name** (if applicable):

Lake Name (USGS): **Lake Name** (Local):

Site Name: **Site ID:**

Latitude (DD) ___ ___ . ___ ___ ___ ___ ___ ___ **Longitude** (DD) -___ ___ ___ . ___ ___ ___ ___ ___ ___

Date of Visit: **Arrival Time** (24hr): ____ Standard ____ Daylight Saving

Field Team Leader:

Affiliation:

Phone: **Email:**

Are there any suggested revisions to the Site Documentation Forms 1 and 2 (select the area of revision below)?

____ GPS Information ____ Stream Description ____ Site Description ____ Access/Travel Information

Describe suggested revision:

WATER SAMPLES AND REPLICATES

Time Sampled	*Sample ID*	*Sample Depth (ft, m)*	*Sample Depth Zone (e.g., epi, hyp, surface)*	*Sample Type (reg, rep ,blank)*	*Bottle Type (plastic, glass, syringe)*	*# of Bottles or Syringes*	*Bar Code*

Collection Location (explain any deviation from targeted sampling location):

GENERAL OBSERVATIONS

Time Obtained (24 hr) **Air Temperature:** ____ ^{O}C ____ ^{O}F

Water Temperature (at sample location) ____ ^{O}C ____ ^{O}F **Instrument Used:**

What is the weather condition on the day of sampling? ____ Clear ____ Partly Cloudy ____ Overcast ____ Hail ____ Light Rain ____ Occasional Rain ____ Persistent Rain ____ Snow or Sleet

What was the weather for the 3 days prior to day of sampling? ____ Generally Dry ____ Occasional Rain/Snow ____ Generally Wet ____ Very Wet

Lake Level Change (dist. from ref point): ____ Zero Change ____ Change is +/-_____ Units:_____

Observed Discharge Level (lake flow at outlet): ____ No Flow ____ Low Flow ____ Normal Flow ____ High Flow ____ Flood

ADDITIONAL NOTES

Version 03/2012

USDA Forest Service
National Protocols for Air Pollution Sensitive Waters

LAKE SAMPLING – SAMPLE RECORD
FORM 4

Lake Name (Local):	**Date of Visit:**
Site Name:	**Site ID:**
Latitude (DD) ___ ___ . ___ ___ ___ ___ ___ ___	**Longitude** (DD) - ___ ___ ___ . ___ ___ ___ ___ ___ ___

TRANSPARENCY DATA

Secchi Disk Depth Measurements

Depth Disappeared_____ ____ ft. ____ m Depth Reappeared_____ ____ ft. ____ m

Clear to Bottom? ____ Yes ____ No Notes:

DEPTH/TEMPERATURE PROFILE

Method:_____ **Instrument Make/Model:**_____

Index Location´ ____ Yes ____ No **If No explain:** **Total Profile Depth**_____ ____ ft ____ m

	Depth	Temp	DO	pH	Cond	Turb		Depth	Temp	DO	pH	Cond	Turb
1							11						
2							12						
3							13						
4							14						
5							15						
6							16						
7							17						
8							18						
9							19						
10							20						

Temp at 60% of Total Depth Profile: _____ **Temp Units:** ____ ^{0}C ____ ^{0}F

Mixed: ____ **Stratified:** ____ Weak ____ Strong

DO units: ____ % ____ mg/L **Conductivity corrected to 25˚C:** ____ Yes ____ No **Turb = NTU**

ON SITE WATER DATA (OPTIONAL)

Time Obtained (24 hr)

Parameter	Value	Equipment (make/model)	Method/Reference (EPS/SM/USGS)
Conductivity	uS/cm Corrected to 25^{0} C? ____ Yes ____ No		
pH			
Turbidity	_____NTU		
Dissolved Oxygen	mg/l %DO		
Water Temperature	____ ^{0}C ____ ^{0}F		
Other			

PHOTO LOG

Photo ID/File Name	Date	Description of Photo

Version 03/2012

E-3 CHAIN OF CUSTODY FORM AND INSTRUCTIONS

1. **Page ___ of ___**. Page number(s) of total number of chain of custody forms sent.
2. **Forest/Wilderness/Park/Other (Circle one)**. Circle one of these options and write the name of the national forest, wilderness area, national park, or other area (e.g., specific unit within the national forest) in the space provided. Provide the name and affiliation of the project contact individual.
3. **Address and Phone Number**. Provide address and phone number of the office of the national forest, wilderness, national park, or other area. In the address, please include the city, state, and zip code.
4. **Shipped to (Lab Name and Address)**. Name, address, and email of the laboratory to which the water samples and original Chain of Custody Form will be sent.
5. **Lab Phone #**. Phone number of the laboratory to which the water samples and original Chain of Custody Form will be sent.
6. **Lab Contact and Email**. Contact person and email address in the analytical laboratory.
7. **Shipped by: UPS/Fed Ex/USPS/Other**. Identify the carrier that you used. (Remember to consider the arrival date of the shipped samples because, on weekends and government holidays, there may not be anyone to receive samples at the laboratory). In general, you should try to ship samples on Monday, Tuesday, or Wednesday in order to arrive before the weekend.
8. **Shipping #**. Tracking number assigned to the shipment by the carrier.
9. **Sampled Date**. Date sample was taken (mm/dd/yyyy).
10. **Sample Time**. Time sample was taken (24-hr: ####).
11. **Site/Sample ID**. The unique identification number assigned to the sample in the field based on the specific protocol. Refer to the lake or stream form instructions for more detail on developing a site ID before field collection (see header instructions, item 6).
12. **Site/Sample Location**. Document the lake or stream sample site name. Refer to the lake or stream form instructions for more detail on developing a site name before field collection (see header instructions item five). Provide the latitude and longitude in decimal degrees.
13. **Sample Type**. Document the type of sample collected, such as normal (regular) water sample, field blank, replicate (duplicate), or split.
    - Replicate samples are collected at the same location as the normal water sample but at slightly different times (typically, one minute apart). Replicates are usually collected for QA purposes or as backup samples should the normal sample be lost or damaged.
    - A field blank is a prepared sample of DIW that is carried into the field and then shipped to the laboratory with the samples.
    - A field split is the second sample bottle when a normal sample has been split in the field into two bottles. The first bottle is labeled as the normal sample; the second is labeled as the field split (S).
14. **Filtered (Y/N). Where?** Was this sample filtered in either the field or field laboratory? If so, where?
15. **Preserved (Y/N/Type)**. Was this sample preserved in the field and, if so, with what kind of preservative (e.g., H_2SO_4)?

16. **Analyses Requested**. Document instructions for the laboratory, requesting the type of analyses to be performed (e.g., ANC, pH, conductivity, major cations, anions, etc.). You may write "same as usual" or leave the space blank if you have an agreement with the laboratory concerning routine analyses.
17. **Bar Code**. The barcode is a unique identifier provided by ARML for tracking purposes. The multiple (4) stick-on copies of the barcode ID labels are prepared to be affixed in the field to multiple forms and sample containers.
18. **Comments**. Any extra remarks or instructions are placed in this space.
19. **Received/Relinquished by:**
    - **Print Name**. Printed name of sampler relinquishing the samples to another person for shipment to the laboratory or directly to the laboratory.
    - **Signature**. Sampler's signature relinquishing the samples to another person for shipment to the laboratory or directly to the laboratory.
    - **Date & Time Relinquished**. Date and time relinquished by the sampler or by person shipping samples to the laboratory.
    - **Date & Time Received**. Date and time samples were received from the sampler.
20. **Received at Laboratory by:**
    - **Print Name**. Printed name of laboratory personnel receiving the samples.
    - **Signature**. Signature of the laboratory personnel receiving the samples.
    - **Date**. Date the samples were received by the laboratory.
    - **Time**. Time the samples were received by the laboratory.

It is extremely important to send this form and accompanying lake or stream sampling record forms to the laboratory with the samples so that proper connections can be made between field and laboratory information and so that relevant data may be entered into the ARM program database.

USDA Forest Service

National Protocols for Air Pollution Sensitive Waters

CHAIN OF CUSTODY

(Also fill out detailed site and sample information on either the Lake or Stream forms)								Page_____ of _____
Forest/Wilderness/Park/Other (Circle one) Name:					Ship to (Lab name and address):			
Contact Individual and Affiliation:								
Address:					Lab contact:			Shipped by: UPS/FedEx/USPS/Other:
					Lab Phone Number:			Shipping #:
Phone Number:					Lab email:			
Sample Date	***Sample Time*** *(24 hour)*	***Site/Sample ID***	***Site/Sample Location; Lake/Stream Name or Latitude/Longitude***	***Sample Type*** *(Normal, Replicate, Blank, Split)*	***Filtered*** *(Y/N)* ***Where?*** *(Field or Field Lab)*	***Preserved*** *(Y/N/Type)*	***Analyses Requested***	***Bar Code***

Comments:

Received/Relinquished by:			
Print Name	***Signature***	***Date & Time Received***	***Date & Time Relinquished***

Received at Laboratory by:			
Print Name	***Signature***	***Date***	***Time***

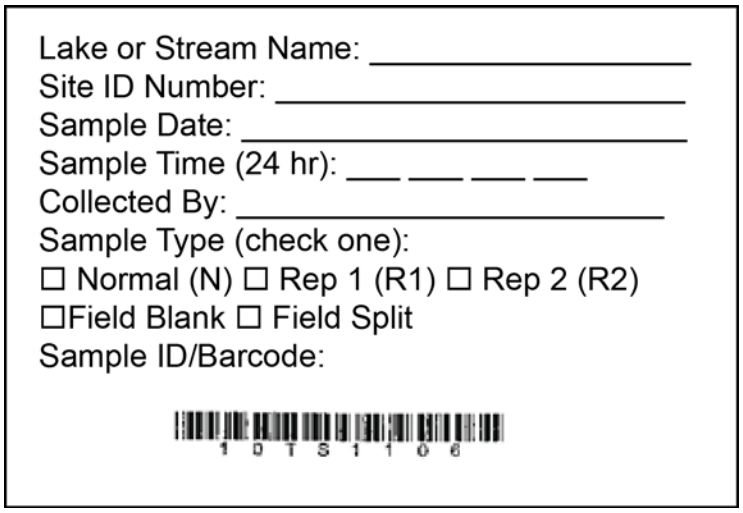
Sender: Please send original of this form and accompanying samples to the contract lab. Keep a copy in local files.

Version 03/2012

APPENDIX F.
LABELING INSTRUCTIONS FOR FIELD SAMPLE CONTAINERS

F-1 WATER SAMPLE

Below is an example of what should be on the label for the water samples. These can be premade labels or filled in on a blank label at time of sample collection. Apply a separate label to each sample container (e.g., bottle or syringe). Placing one or more strips of packing tape over the label once it is on the container can protect the label from damage and subsequent misreading.



Lake or Stream Name. Enter the name of the lake or stream sampled. Provide both the USGS name (from a topographical map) and the local name by which the lake or stream is known.

Site ID Number. Each sample site (location) will be assigned a unique Name and ID number. These are generated locally for the project. The identification number will appear on all sample bottles used for sampling this lake or stream at this location. It is especially important to have a unique Site ID when more than one sample site is located on a given lake or stream.

Sample Date. Enter the date when visiting the sampling site.

Sample Time (24-hr). Indicate time of arrival at sampling location. Use 24-hour (military time) format. Indicate whether it is recorded in standard local time or daylight savings local time.

Collected By. Enter the name and affiliation of the field person responsible for the sampling.

Sample Type. Check the space indicating whether this is a normal sample, a replicate sample (Replicate 1 or Replicate 2), or a field blank or field split sample.

Barcode. Affix the same numbered bar code represented on the form from the sheets supplied by the lab to the associated sample container. It is very important to make sure each sample has a unique bar code. Do not use the same bar code on different samples (e.g., normal and replicate).

F-2 ZOOPLANKTON SAMPLE

Below is an example of what should be on the label for the zooplankton sample containers. These can be premade labels or written on a blank label at time of sample collection. In addition to the label affixed to the outside of the sample bottle, a label must be placed inside the bottle of preserved sample. This internal label, on waterproof paper, must be filled out with a pencil.

Lake Name: _____
Site ID Number: _____
Sample Date: _____
Sample Time (24 hr): ___ ___ ___ ___
Collected By: _____
Sample Depth ☐ m ☐ ft: _____
Net Mesh Size: ☐ 80 µm ☐ 243 µm ☐ Other
Other (specify)_____

Lake Name. Enter the name of the lake or stream sampled. Provide both the USGS name (from a topographical map) and the local name by which the lake or stream is known.

Site ID Number. Each sample site (location) will have a unique Name and ID number assigned to it. These are generated locally for the project. The identification number will appear on all sample bottles used for sampling this lake or stream at this location. It is especially important to have a unique Site ID when more than one sample site is located on a given lake or stream.

Sample Date. Enter the date when visiting the sampling site.

Sample Time (24-hr). Indicate time of arrival at sampling location. Use 24-hour (military time) format. Indicate whether it is recorded in standard local time or daylight savings local time.

Collected By. Enter the name and affiliation of the responsible field person.

Sample Depth. Record the water depth at which the sample was collected. Check whether the depth is being recorded in meters or feet.

Net Mesh Size. Check whether the net used to collect the sample was of mesh size 80 µM, 243 µM, or some other size. If the mesh was of a size other than 80 µM or 243 µM, specify the mesh size used.

F-3 STREAM BENTHIC MACROINVERTEBRATE SAMPLE

Below is an example of what should be on the label for the macroinvertebrate sample containers. These can be premade labels or written on a blank label at time of sample collection. In addition to the label affixed to the outside of the sample bottle, a label must be placed inside the bottle of preserved sample. This internal label, on waterproof paper, must be filled out in pencil.

Stream Name: _____
Site ID Number: _____
Sample Date: _____
Sample Time (24 hr): ____ ____ ____ ____
Collected By: _____
Number of Kick Net Samples Collected: _____

Stream Name. Enter the name of the lake or stream sampled. Provide both the USGS name (from a topographical map) and the local name by which the lake or stream is known.

Site ID Number. Each sample site (location) will have a unique Name and ID number assigned to it. These are generated locally for the project. The identification number will appear on all sample bottles used for sampling this lake or stream at this location. It is especially important to have a unique Site ID when more than one sample site is located on a given lake or stream.

Sample Date. Enter the date when visiting the sampling site.

Sample Time (24-hr). Indicate time of arrival at sampling location. Use 24-hour (military time) format. Indicate whether it is recorded in standard local time or daylight savings local time.

Collected By. Enter the name and affiliation of the field person responsible for the sampling.

Number of Kick Net Samples Collected. Record the number of discrete kick net samples that were collected and pooled to form this one sample being submitted to the laboratory.

APPENDIX G. EXAMPLE STANDARD OPERATING PROCEDURES FOR LAB ANALYSIS PROTOCOLS

G-1 USGS STANDARD OPERATING PROCEDURE FOR LABORATORY ANALYSIS OF ONE ANALYTE (AMMONIUM) IN DILUTE FRESH WATER – FLOW INJECTION ANALYSIS

1. Scope and Application

Analytes: Ammonium

Reporting Limit: 2.0 µmoles/L

Applicable Matrices: This method is used to determine the concentration of ammonium in precipitation, dilute surface waters, and soil waters.

Dynamic Range: The analytical range of the determination of ammonium is from 0.5 µmol NH_4^+/L (as N) to 35.7 µmol NH_4^+/L (as N). Sample concentrations that exceed this range must be diluted and reanalyzed.

2. Summary of Procedure

The ammonium analysis is an automated colorimetric reaction. Samples are systematically introduced into the flow-injection analyzer (FIA) reaction manifold. The sample reacts with phenol and hypochlorite to form an indophenol blue complex. The color intensity of the blue complex is enhanced by mixing with nitroferricyanide. The mixture is heated to 60°C to ensure optimal color development. Prior to entrance into the flow cell, the sample flows through a debubbler to remove any gas bubbles that develop. Absorbance of the color complex is measured at a wavelength of 630 nm.

3. Safety Issues

Chemical Hazards:

1. All strong acids and bases should be mixed in a fume hood.
2. Gloves, safety glasses, and lab coats should be worn when preparing and performing this analysis.

3. For proper handling techniques for specific chemicals, consult the appropriate Material Safety Data Sheets (MSDS).

4. Sample Preservation, Containers, Processing and Analysis Times

Sample Preservation: Samples are frozen after processing. On the day of analysis, samples are thawed in warm water or at room temperature.

Containers: Samples are stored in 30-mL polyethylene bottles. The bottles have been rinsed with DIW.

Processing and Analysis Times:

Sample processing: one week

Lab analysis: three months

LIMS entry: one week

5. Reagents and Standards

General Information: All reagents are commercially purchased and should be stored in the original container. Date the reagent bottles when received and when opened. Note expiration date, if any. No verification of the reagents is necessary.

Reagents:

1. Degassed Deionized (DI) Water
    a. Milli-Q water is degassed by bubbling with commercial-grade helium for about 2 minutes.
    b. Degassed Milli-Q water is used for carrier water and for preparation of all reagents.
2. Sodium Phenolate
    a. Liquid phenol (C_5H_5OH) should be stored in a designated flammable-storage cabinet.
    b. In a 1,000-mL volumetric flask containing about 500 mL Milli-Q water, add 88-mL liquefied phenol.
    c. Add 32 g sodium hydroxide (NaOH); swirl to dissolve and allow to cool.
    d. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
    e. Store in a red glass bottle at 4°C; label and date.
    f. Prepare every other day.
3. Sodium Hypochlorite
    a. Use household bleach containing at least 5.25 percent sodium hypochlorite (NaOCl).
    b. Fill a specially designated 1-L polyethylene bottle to the 500 mL mark with Milli-Q water and then add bleach to the 1-L mark.
    c. Shake thoroughly.
    d. Store in a polyethylene bottle at 4°C; label and date.
    e. Prepare every other day.
4. Sodium Nitroferricyanide
    a. In a 1,000-mL flask containing about 500 mL Milli-Q water, add 3.50 g sodium nitroferricyanide ($Na_2Fe(CN)_5NO•2H_2O$).

b. Swirl to dissolve the solid.
c. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
d. Store in a polyethylene bottle at 4°C; label and date.
e. The solution is stable for 3 months.

5. Cleaning Solution
   a. In a 1,000-mL volumetric flask containing about 700 mL Milli-Q water, carefully add 82.5 mL concentrated hydrochloric acid (HCl).
   b. Allow to cool.
   c. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
   d. Store in a polyethylene bottle; label and date.
   e. Prepare as needed.

Standards:

1. Ammonium Standard Stock Solution, 1,000 mg NH_4^+/L (as N)
   a. Purchased commercially.
2. Ammonium Standard Substock Solution, 5 mg NH_4^+/L (as N)
   a. Pipet 0.5 mL of the ammonium standard stock solution into a designated 100-mL volumetric flask containing about 50 mL of Milli-Q water.
   b. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
   c. Store in the volumetric flask at 4°C; label and date.
   d. Prepare every other day.
   e. To avoid contamination, aliquots of substock solution must not be withdrawn directly from the bottle.
3. Ammonium Working Standards
   a. Pipet desired amount of standard substock into a designated 100-mL volumetric flask.
   b. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
   c. Store in the volumetric flask at 4°C; label and date.
   d. Prepare every other day.

Working Standard	Ammonium Concentration	Standard Substock Added (mL)	Final Volume (mL)
A	35.70 μmol/L (0.500 mg/L)	10.0	100
B	26.77 μmol/L (0.375 mg/L)	7.5	100
C	17.85 μmol/L (0.250 mg/L)	5.0	100
D	10.71 μmol/L (0.150 mg/L)	3.0	100
E	7.14 μmol/L (0.100 mg/L)	2.0	100
F	3.57 μmol/L (0.050 mg/L)	1.0	100
G	1.78 μmol/L (0.025 mg/L)	0.5	100

4. Ammonium Quality-Control (QC) Stock Solution, 1,000 mg NH_4^+/L (as N)
    a. Purchased commercially, this stock must be from a manufacturer or lot different from the standard stock.
5. Ammonium Quality-Control (QC) Substock Solution, 5 mg NH_4^+/L (as N)
    a. Pipet 0.5 mL of the ammonium QC stock into a designated 100-mL volumetric flask containing about 50 mL of Milli-Q water.
    b. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
    c. Store in the volumetric flask at 4°C; label and date.
    d. Prepare every other day.
    e. To avoid contamination, aliquots of substock solution must not be withdrawn directly from the bottle.
6. Ammonium QC Samples
    a. Pipet desired amount of QC substock into a designated 250-mL volumetric flask.
    b. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
    c. Store in the volumetric flask at 4°C; label and date.
    d. Prepare every other day.

QC Sample	Ammonium Concentration	QC Substock Added (mL)	Final Volume (mL)
High	17.85 □mol/L (0.250 mg	12.5	250
Low	7.14 □mol/L (0.100 mg/	5.0	250

6. QC Procedure

1. The standard curve is a linear plot of standard concentration versus peak area. The best-fit line is drawn and the curve is accepted if the correlation coefficient is 0.998 or greater.
2. Quality-control samples are analyzed at the start of a run, after every 10 samples during the run, and at the end of the run.
3. A quality-control sample is acceptable if the analyzed value is within 10 percent of the QC-high range known value and within 15 percent of the QC-low range known value.

4. If one of the QC samples fails the acceptance criteria, the run is stopped and the QC sample is re-run. If the QC sample fails again the run is stopped and the instrument is re-calibrated. Samples associated with the failed QC sample are re-analyzed.

7. Chemical Analysis Procedure:

Instrumentation:

Lachat QuickChem 8000 flow-injection analyzer
Omnion software v 3.0

Timing:

Method Cycle Period - 90 seconds
Auto-sampler Timing Sample Period - 41 seconds
Minimum Probe in Wash Period - 41 seconds

Settings	Ammonium Channel (seconds)
Time to Valve	26
Load Period	36
Inject Period	53
Expected Inject to Peak Start	24
Expected Peak Base Width	86

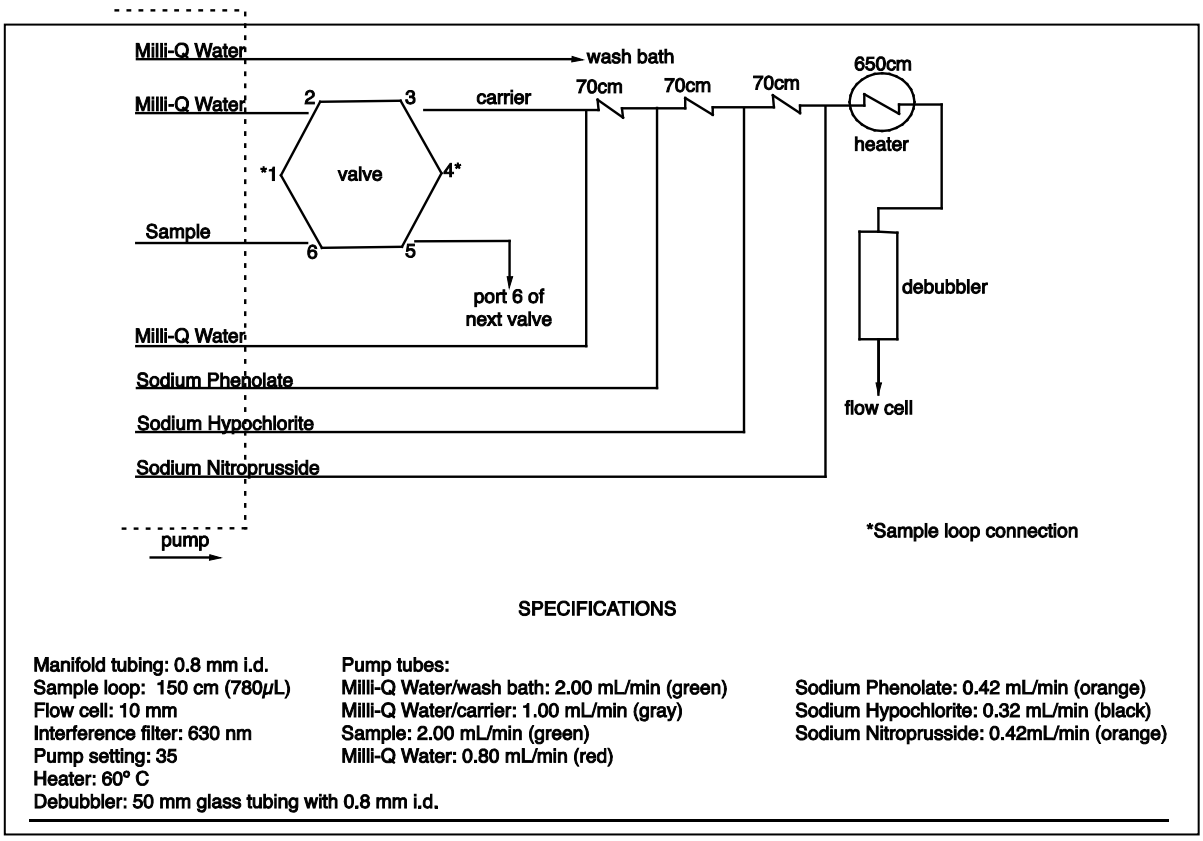


Figure G-1. Flow diagram for ammonium manifold.

Start-Up:

1. Turn the surge protector power on.
2. On the computer desktop, double click the Omnion 3.0 icon.
3. Check the waste container daily and empty if nearing capacity.
4. Fill DIW reagent bottle with degassed Milli-Q water.
5. Place pump tubes in appropriate reagent containers- see diagram. Cover reagent bottle openings with parafilm.
6. Push down cartridges on pump until they click into place. Pull tighteners on pump cartridges all the way to right and then forward one click.
7. Pump speed setting should be 35. Turn pump on.
8. Allow reagents and Milli-Q water to pass through system for at least 10 minutes.
9. Check digital readout on heater- it should read 60°C.
10. Observe module connections for any leaks or clogs.
11. Empty debubbler by turning upside down, then right side up.

Calibration:

1. Click OPEN. Double click the CALIB+QC+NH4.omn file.
2. At the top of the screen the heading should read Omnion – Run 1 (CALIB+QC+NH4.omn).
3. Fill cups on calibration rack with standards and QC samples.
4. Click green START arrow.
5. If the calibration is within acceptance criteria, the instrument will analyze the QC samples. If the calibration fails, a message will pop up, with options to proceed. In most cases choose Recalibrate.
6. If the calibration and QC samples pass, set up a sample tray.

Analysis:

1. Take ammonium samples out of the freezer and thaw in warm water.
2. Enter the sample serial numbers (SSNs) into run worksheet. Use the Auto Sample ID feature if the SSNs are consecutive.
3. From menu bar select RUN. Click EXPORT WORKSHEET DATA. Note date on run worksheet printout.
4. Delete calibration samples from the run worksheet.
5. Fill the auto sampler tubes with the appropriate samples.
6. Empty debubbler.
7. Click green START arrow.
8. Fill QC containers and carrier bottle as needed.
9. QC samples will be run automatically every 10 samples. If all QC samples pass, the run will proceed until the tray is finished.

10. If a QC sample fails, a message will pop up and analysis will cease. Click the Stop Now option. Remove SSNs that have been analyzed from the run worksheet.
11. Click green Start arrow.
12. If QC samples pass, the run will continue. If QC samples fail, remake QC samples from substock and/or recalibrate.
13. Review the sample peaks and analysis data as the run progresses. Note any air bubbles, bad peaks, and/or samples requiring dilutions or re-runs.

Shut Down:

1. Change the pump setting to override standby.
2. Remove pump tubes from reagent bottles and rinse lines and weights with Milli-Q water. Place lines in a beaker of Milli-Q water.
3. After several minutes, place pump tube reagent lines in the cleaning solution. Allow to pump for about 2 minutes.
4. Rinse and place pump tube reagent lines in Milli-Q water again. Allow to pump for several more minutes.
5. Remove lines from beaker and allow to pump air until no more water is moving through manifold.
6. Release tension of cartridges by pushing holders on side of pump and push tension regulators on top of the cartridges all the way to the left.
7. Exit Omnion.
8. Turn the surge protector off.

Maintenance:

1. All pump tubes should be replaced as they become worn or stretched; the frequency depends upon the number of samples analyzed. The sample and wash bath tubes should be changed as they become discolored or clogged. Note dates in instrument notebook.
2. Interference filters should be cleaned with lens paper twice a year or whenever they become dusty or soiled.
3. Manifold tubing should be replaced as it becomes discolored or clogged; note in instrument notebook.
4. Sample tubes are rinsed, soaked in DIW overnight, and oven dried at 60°C between uses.
5. Buildup and clogging in the waste lines may require periodic replacement of the lines; note in instrument notebook.

Data Processing and LIMS Entry:

1. In Microsoft Excel, open all files for the run date. They are stored in C:\Program Files\Lachat\Omnion\Data. All files are in a comma delimited text format.
2. Edit files as needed and copy/paste them all into the same file.
3. Save as a comma delimited file (.csv file extension) named by date.
4. Print a copy and write the filename on the copy and close file.

5. Double click Watershed LIMS icon.
6. Click Import Data.
7. Under the Import drop down, choose Ammonium.
8. Choose and open the desired file.
9. Choose Client, Units, type in Test Date, and choose Analyst.
10. Exclude and/or edit any data necessary.
11. Click Client ID to Sample No.
12. Click Set Data.
13. Investigate problems for data that did not transfer or are duplicated.

8. Archiving

Data: Data files are backed-up daily by an automatic back-up program. Hard copies of the runs are filed and kept indefinitely. The laboratory LIMS system is backed up daily by automatic back-up program.

Samples: Samples are stored at room temperature until data can be verified. Sample bottles are cleaned and reused for new samples.

G-2 UNIVERSITY OF VIRGINIA STANDARD OPERATING PROCEDURE FOR LABORATORY ANALYSIS: SHENANDOAH WATERSHED STUDY/VIRGINIA TROUT STREAM SENSITIVITY STUDY

R.F. Webb, R., F.A. Deviney, and S.W. Maben (unpublished manuscript)

G-2.1 Metrohm Titrando 809 Titration System: pH and ANC

This document describes the current laboratory procedure for measuring pH and ANC using the Metrohm Titrando 809 Titration System. Analytical methods are based on the methods published in U.S. Environmental Protection Agency (1987).

Summary: pH is measured first while the sample is not stirred. The measurement of ANC is a multi-step process. The stirrer comes on, and the sample is titrated with 0.01 N HCl to endpoints of pH 4.5 and 4.2, and the pH and volume of acid are recorded for each endpoint. Then eight equal-volume aliquots of acid are added, and the pH recorded after each addition. Finally the sample is titrated to a pH 3.5, and the pH and volume of acid added is recorded. These 11 pH-volume data pairs are used to calculate the Gran1 ANC.

Section I: Standards

Use purchased NIST-traceable pH 4 and 7 buffer solutions to calibrate the electrode. Use purchased NIST-traceable 0.01 N HCl volumetric solution for the titrant and to prepare the pH 4.60 QCS (Quality Control Sample).

Preparation of pH 4.60 QCS (Quality Control Sample):

All glassware should be Class A volumetric laboratory glassware which has been initially cleaned by acid washing and afterwards cleaned by multiple rinsings with DI (deionized) water.

- Using a volumetric pipette, add 5 mL of 0.01 N HCl to a 2000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.
- Store in volumetric flask at room temperature.

Section II: Operation

Analyzing Samples:

1. Log in to Titrator computer
2. Turn on 730 Sample Changer with on/off switch near the bottom on the side
3. After Sample Changer has initialized, open Tiamo software
4. Click "OK" to "Prepare dosing device" reminder
5. Enter your name as "User" and the date as "Remark"
6. Check level of titrant (0.01 N HCl). Fill reservoir if needed
7. Fill beaker in rack position 13 half-full with fresh DIW
8. Open Manual Control under Tools menu or by clicking on icon (looks like a pointing hand)
9. Move sample changer to rack position 13:
    a. Choose Tower 1
    b. Select Move tab
    c. Enter Rack position: 13, then click Start
    d. Enter Lift position: 190 mm, then click Start
10. Prepare Dosing device:
    a. Choose Dosing device 2 (800)
    b. Select Prepare tab
    c. Click Start (Answer yes to Splash warning). This will take a few minutes.
11. Click "Sample table → Import data" to import a spreadsheet which has been generated by the SWAS/VTSSS LIMS (Laboratory Information Management System) to create the Series list or follow instructions to create Series list manually (see section at end entitled "To create Series List manually")
12. Flush tubing:
    a. Select Add fixed volume tab
    b. Enter Volume: 6 mL
    c. Enter Dosing rate: maximum
    d. Enter Filling rate: 5 mL/min
    e. Click Start (While the 6 mL is being delivered, make sure any bubbles in the tubing are being flushed out.) This will take a few minutes.
13. Fill the following Sample Changer positions: (Rinse each beaker 10 times with DIW, then with a little of the solution.)

a. Rack position 1 = 100 mL pH 7 buffer (approximate volume)
b. Rack position 2 = 100 mL pH 4 buffer (approximate volume)
c. Rack position 14 = Diluted pH 7 buffer for electrode storage (half full)

14. Raise tower head to install electrode:
    a. Choose Tower 1
    b. Select Move tab
    c. Enter Lift position: 0 mm, then click Start
15. Prepare electrode:
    a. Carefully remove electrode from storage sleeve
    b. Open fill hole
    c. Replace filling solution in electrode (3M KCl) weekly or more often as necessary by using a transfer pipette to remove old filling solution and then filling electrode with new filling solution to just below the fill hole
    d. Shake downward gently to remove air bubbles if filling solution was changed
    e. Rinse bottom half of electrode with DIW
    f. Connect electrode and place in tower head
16. Place electrode in diluted pH 7 buffer:
    a. Choose Tower 1
    b. Select Move tab
    c. Enter Rack position: 14, then click Start
    d. Enter Lift position: 190 mm, then click Start
17. Fill Sample Changer positions with samples according to Series list. (You can fill a few positions ahead and then start the Series. Continue to fill the rest once the Series has started.)
    a. Rinse beaker 10 times with DIW
    b. Rinse 100 mL measuring volumetric flask 3 times with DIW
    c. Rinse 100 mL measuring volumetric flask with a little sample
    d. Fill 100 mL volumetric flask with sample as full as possible (will slightly rise over top)
    e. Dry excess liquid sample from sides of flask so that it won't drip into beaker
    f. Carefully transfer all of sample into beaker
18. Put fresh DIW in beakers in rack positions 12 & 13
19. Rinse electrode:
    a. Choose Tower 1
    b. Select Move tab
    c. Enter Lift position: 0 mm, click Start
    d. Enter Rack position: 12, click Start
    e. Enter Lift position: 190 mm, click Start
    f. Choose Stirrer 1 (Tower)
    g. Click Start (let stir for ~ 10 seconds)
    h. Click Stop

20. Close Manual Control
21. Click Start to begin Determination Series
22. Fill the rest of the Sample Changer positions according to the Series list.

Notes:

a. After the calibration is completed, Rack position 12 can be used for samples.
b. Rack position 13 is always for the rinse water throughout the entire run. Replace with fresh DIW occasionally throughout the run.
c. Rack position 14 is always for diluted pH 7 buffer for electrode storage at the end of the run.

Shutdown Procedure:

1. Open Manual Control under Tools menu or by clicking on icon
2. Remove electrode:
    a. Choose Tower 1
    b. Select Move tab
    c. Enter Lift position: 0 mm, click Start
    d. Remove electrode from tower head and disconnect
    e. Rinse bottom half of electrode with DIW
    f. Close fill hole
    g. Carefully place electrode in storage sleeve filled with electrode storage solution. Refill sleeve only with electrode storage solution (not 3M KCl electrode filling solution).
3. Rinse stirrer and tip:
    a. Fill beaker in rack position 12 with fresh DIW
    b. Choose Tower 1
    c. Select Move tab
    d. Enter Rack position: 12, click Start
    e. Enter Lift position: 190 mm, click Start
    f. Choose Stirrer 1 (Tower)
    g. Click Start (let stir for ~ 10 seconds)
    h. Click Stop
4. Move stirrer and tip to storage:
    a. Fill beaker in rack position 13 with fresh DIW
    b. Choose Tower 1
    c. Select Move tab
    d. Enter Lift position: 0 mm, click Start
    e. Enter Rack position: 13, click Start
    f. Enter Lift position: 190 mm, click Start
5. Close Manual Control
6. Exit Tiamo

7. Turn off Sample Changer
8. Log off of computer
9. Rinse all beakers 10 times with DIW and put on rack to dry

Section III: Quality Control

The pH 4.60 QCS is analyzed at the beginning and end of each Series and throughout the Series (approximately every 8 samples).

The Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP gives the following precision objective for pH values less than the transition value of pH 5.75: + 0.075 pH units.

Therefore the pH value of the QCS should be 4.525 – 4.675. If it is not, stop the run to determine the problem. If the QCS fails, all samples run since the last passing QCS must be re-analyzed

To check the measured value of the pH 4.60 QCS during the run:

1. Click on the Database icon on the left side of the Tiamo screen
2. Click on the page arrows to display the results of the current day's run. (Determinations are listed chronologically. It will be at the end.)
3. The pH value is in the RS01 column
4. Note: Click on the Workplace icon on the left side of the Tiamo screen to return to the Series list.

To create Series List manually:

1. Double-click in a box on the first available line of Series (has a * on the method box) to bring up editing window
2. After entering the information, click on "Apply", then use the arrows at the bottom to move to the next line
3. Set up Series as follows:
    a. Begin Series by initiating the calibration with pH 4 & 7 buffers (first line):
        i. Method: pH Cal (Select from dropdown list)
        ii. Sample position: 1
        iii. Station ID: Calibration
        iv. Sample size: 100
        v. Sample size unit: mL
    b. First sample of Series is the pH 4.60 QCS sample:
        i. Method: pH and ANC 2 (Select from dropdown list)
        ii. Sample position: 3
        iii. Station ID: PH 4.60 QCS
        iv. Activity ID: QC
        v. Sample size: 100
        vi. Sample size unit: mL

c. Second sample of Series is a QC sample (DIW):
   i. Method: pH and ANC 2 (Select from dropdown list)
   ii. Sample position: 4
   iii. Station ID: DIW
   iv. Activity ID: QC
   v. Sample size: 100
   vi. Sample size unit: mL

d. Begin analyzing samples with the following format:
   i. Method: pH and ANC 2 (Select from dropdown list)
   ii. Sample position: Enter Sample Changer position for the sample
   iii. Station ID: 4-character Station ID for the sample
   iv. Date: Date sample was collected
   v. Time: Time sample was collected
   vi. Activity ID: Only use this field if the sample has a replicate. Use 1 and 2 to indicate which replicate it is.
   vii. Organization: Organization sample is assigned to (Usually either SHEN for samples collected in Shenandoah National Park or UVAVTSSS for samples collected for the VTSSS project. Leave blank if unsure.)

e. Do not use Sample Changer positions 13 and 14 for samples
f. Include a pH 4.60 QCS sample approximately every 8 samples
g. Always end Series with a pH 4.60 QCS sample

Method	Sample position	Station ID	Date	Time	Activity ID	Organization	ANC ID	pH ID	Sample size	Sample size unit
pH Cal	1	Calibration							100	mL
pH and ANC 2	3	PH 4.60 QCS			QC				100	mL
pH and ANC 2	4	DIW			QC				100	mL
pH and ANC 2	5	PINE	3/23/2009	15:00		SHEN			100	mL
pH and ANC 2	6	NFDR	3/23/2009	16:20		SHEN			100	mL
pH and ANC 2	7	STAN	3/24/2009	9:15	1	SHEN			100	mL
pH and ANC 2	8	STAN	3/24/2009	9:15	2	SHEN			100	mL
pH and ANC 2	9	VT36	4/6/2009	11:15		UVAVTSSS			100	mL
pH and ANC 2	10	VT51	4/6/2009	12:10		UVAVTSSS			100	mL
pH and ANC 2	11	VT 62	4/7/2009	13:40		UVAVTSSS			100	mL
pH and ANC 2	12	PH 4.60 QCS			QC				100	mL

G-2.2 VWR Digital Conductivity Meter

This document describes the current laboratory procedure for measuring specific conductance using the VWR Digital Conductivity Meter. The analytical method is based on the method published in APHA (2005). The meter automatically corrects the reading to 25 °C.

Section I: Standards

Use purchased NIST-traceable conductivity standards to calibrate the conductivity meter following manufacturer's instructions. Choose standards close to the expected range of samples to be analyzed.

Section II: Operation

Analyzing Samples:

1. Turn on conductivity meter to micromho setting
2. Rinse conductivity probe thoroughly with DIW
3. Rinse conical sample beaker 10 times with DIW
4. Fill conical sample beaker with sample
5. Place conductivity probe in sample, gently swirl and then remove probe
6. Discard sample, and then fill conical beaker with fresh sample
7. Place conductivity probe in sample, gently swirl and make sure probe is not touching the bottom or sides of beaker
8. Wait for reading to stabilize and then record reading

NOTE: Always measure and record conductivity of DIW at beginning of each session.

Shutdown Procedure:

1. Turn off conductivity meter
2. Rinse conductivity probe thoroughly with DIW
3. Rinse conical sample beaker 10 times with DIW
4. Place conductivity probe in sleeve with VWR Redi-Stor™ Conductivity Probe Storage Solution

Section III: Quality Control

Calibrate conductivity meter every 6 months with NIST-traceable conductivity standards.

Measure and record the conductivity of DIW as a blank before beginning sample analysis.

G-2.3 Dionex ICS-3000 Ion Chromatography System

This document describes the current laboratory procedure for measuring anions and cations using the Dionex ICS-3000 dual system. Current analytes measured by ion chromatography include:

- Anions – chloride, nitrate, sulfate (Phosphate is included for MIDN project samples.)
- Cations – ammonium, calcium, magnesium, potassium, sodium

Analytical methods for anions are based on EPA Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography (U.S. EPA 1997). Analytical methods for cations are based on ASTM Method D 6919 -03: Determination of Dissolved Alkali and Alkaline

Earth Cations and Ammonium in Water and Wastewater by Ion Chromatography (ASTM International 2003).

Section I: Standards

All glassware should be Class A volumetric laboratory glassware which has been initially cleaned by acid washing and afterwards cleaned by multiple rinsings with DI (deionized) water.

Preparation of Anion Calibration Standards:

Anion Calibration Standards Working Stock:

Prepare as follows from prepared or purchased 1000 mg/L stock solutions.

(Throughout this procedure, use only NIST-traceable stock solutions when using purchased stock solutions.)

Using a volumetric pipette, add the following to a 500 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.

10 mL of 1000 mg/L chloride stock

15 mL of 1000 mg/L nitrate stock

40 mL of 1000 mg/L sulfate stock

25 mL of 100 mg/L phosphate stock - prepared by diluting 1000 mg/L phosphate stock by a factor of 10

The final concentration of the Anion Calibration Standards Working Stock is:

20 mg/L chloride + 30 mg/L nitrate + 80 mg/L sulfate + 5 mg/L phosphate

Anion Calibration Standards:

Dilute the indicated volume of Anion Calibration Standards Working Stock to 500 mL in a volumetric flask and mix well to yield standards of the following concentrations in mg/L:

Standard	Volume of Stock	Cl^-	NO_3^-	SO_4^{2-}	PO_4^{3-}
1	50 mL	2.0	3.0	8.0	0.5
2	40 mL	1.6	2.4	6.4	0.4
3	30 mL	1.2	1.8	4.8	0.3
4	20 mL	0.8	1.2	3.2	0.2
5	10 mL	0.4	0.6	1.6	0.1
6	5 mL	0.2	0.3	0.8	0.05
7	2.5 mL	0.1	0.15	0.4	0.025
8	0 mL	0.0	0.0	0.0	0.0

Preserve all working stocks and standards with chloroform and store refrigerated in prepared polyethylene bottles.

Preparation of Anion Quality Control Standards:

The Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP gives the following Method Detection Limit Objectives:

Chloride:	0.03 mg/L
Nitrate:	0.03 mg/L
Sulfate:	0.05 mg/L

Anion Quality Control Standards Working Stock:

Prepare as follows from purchased or prepared 1000 mg/L stock solutions.

Using a volumetric pipette, add the following to a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.

15 mL of 1000 mg/L chloride stock

15 mL of 1000 mg/L nitrate stock

25 mL of 1000 mg/L sulfate stock

20 mL of 100 mg/L phosphate stock - prepared by diluting 1000 mg/L phosphate stock by a factor of 10

The final concentration of the Anion Quality Control Standard Working Stock is:

15 mg/L chloride + 15 mg/L nitrate + 25 mg/L sulfate + 2.0 mg/L phosphate

Anion MDL (method detection limit) Standard Solution:

Pipette 5 mL of the Anion Quality Control Standards Working Stock into a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well to provide a solution of the following concentrations:

0.075 mg/L chloride

0.075 mg/L nitrate

0.125 mg/L sulfate

0.010 mg/L phosphate

Anion Detection Limit Quality Control Check Sample:

Pipette 5 mL of the Anion Quality Control Standards Working Stock into a 500 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well to provide a solution of the following concentrations:

0.15 mg/L chloride

0.15 mg/L nitrate

0.25 mg/L sulfate

0.02 mg/L phosphate

Anion Calibration Quality Control Check Sample:

Pipette 30 mL of the Anion Quality Control Standards Working Stock into a 500 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well to provide a solution of the following concentrations:

0.90 mg/L chloride

0.90 mg/L nitrate

1.50 mg/L sulfate

0.12 mg/L phosphate

Preparation of Cation Calibration Standards:

Cation Calibration Standards Working Stock:

Prepare as follows from purchased or prepared 1000 mg/L stock solutions.

Using a volumetric pipette, add the following to a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.

5 mL of 1000 mg/L ammonium stock

50 mL of 1000 mg/L calcium stock

25 mL of 1000 mg/L magnesium stock

25 mL of 1000 mg/L potassium stock

30 mL of 1000 mg/L sodium stock

The final concentration of the Cation Working Stock is:

5 mg/L ammonium + 50 mg/L calcium + 25 mg/L magnesium + 25 mg/L potassium + 30 mg/L sodium

Cation Calibration Standards:

Dilute the indicated volume of Cation Calibration Standards Working Stock to 500 mL in a volumetric flask and mix well to yield standards of the following final concentrations in mg/L:

Standard	Volume of Stock	NH_4^+	Ca^{2+}	Mg^{2+}	K^+	Na^+
1	50 mL	0.5	5.0	2.5	2.5	3.0
2	40 mL	0.4	4.0	2.0	2.0	2.4
3	30 mL	0.3	3.0	1.5	1.5	1.8
4	20 mL	0.2	2.0	1.0	1.0	1.2
5	10 mL	0.1	1.0	0.5	0.5	0.6
6	5 mL	0.05	0.5	0.25	0.25	0.3
7	2.5 mL	0.025	0.25	0.125	0.125	0.15
8	0 mL	0.0	0.0	0.0	0.0	0.0

Preserve all working stocks and standards with chloroform and store refrigerated in prepared polyethylene bottles.

Preparation of Cation Quality Control Standards:

The Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP gives the following Method Detection Limit Objectives:

Ammonium:	0.02 mg/L
Calcium:	0.02 mg/L
Magnesium:	0.01 mg/L
Potassium:	0.04 mg/L
Sodium:	0.02 mg/L

Cation Detection Limit Quality Control Standards Working Stock:

Prepare as follows from purchased or prepared 1000 mg/L stock solutions.

Using a volumetric pipette, add the following to a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.

10 mL of 1000 mg/L ammonium stock

10 mL of 1000 mg/L calcium stock

5 mL of 1000 mg/L magnesium stock

20 mL of 1000 mg/L potassium stock

10 mL of 1000 mg/L sodium stock

The final concentration of the Cation Quality Control Standards Working Stock is:

10 mg/L ammonium + 10 mg/L calcium + 5 mg/L magnesium + 20 mg/L potassium + 10 mg/L sodium

Cation MDL (method detection limit) Standard Solution:

Pipette 5 mL of the Cation Quality Control Standards Working Stock into a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well to provide a solution of the following concentrations:

0.05 mg/L ammonium

0.05 mg/L calcium

0.025 mg/L magnesium

0.10 mg/L potassium

0.05 mg/L sodium

Cation Detection Limit Quality Control Check Sample:

Pipette 5 mL of the Cation Quality Control Standards Working Stock into a 500 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well to provide a solution of the following concentrations:

0.1 mg/L ammonium

0.1 mg/L calcium

0.05 mg/L magnesium

0.2 mg/L potassium

0.1 mg/L sodium

Cation Calibration Quality Control Check Sample Working Stock:

Prepare as follows from purchased or prepared 1000 mg/L stock solutions.

Using a volumetric pipette, add the following to a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.

10 mL of 1000 mg/L ammonium stock

20 mL of 1000 mg/L calcium stock

10 mL of 1000 mg/L magnesium stock

40 mL of 1000 mg/L potassium stock

20 mL of 1000 mg/L sodium stock

The final concentration of the Cation Quality Control Standard Working Stock is:

10 mg/L ammonium + 20 mg/L calcium + 10 mg/L magnesium + 40 mg/L potassium + 20 mg/L sodium

Cation Calibration Quality Control Check Sample:

Pipette 30 mL of the Cation Quality Control Check Sample Working Stock into a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well to provide a solution of the following concentrations:

0.3 mg/L ammonium

0.6 mg/L calcium

0.3 mg/L magnesium

1.2 mg/L potassium

0.6 mg/L sodium

Section II: Operation

Start-up Procedure:

1. Rinse and fill eluent reservoirs, autosampler reservoir and external water pump reservoir with fresh DIW. (If system is already on, just fill all reservoirs with DIW.)
2. Log in to IC computer
3. Open Chromeleon software:
    a. Toshiba-User_1 is System 1: Anions
    b. Toshiba-User_2 is System 2: Cations
4. If the Tabset panel “Anions and Cations Panels” does not open, choose it under the Window menu
5. Turn on external water pump by choosing the Detector Compartment tab for the Anion system and changing the DC_ACRelay_1 to closed

6. If both the anion and cation systems are being used, perform steps 7 - 12 for both systems
7. Switch on the pump motor using the Panel (Home tab)
8. Loosen the purge valve screw on the pump one-half turn
9. Switch the pump to Prime using the Panel. The pump will rapidly deliver DIW from the reservoir out the waste line. Watch for bubbles coming through the tubing at the pump and once all bubbles are gone, switch Prime off and tighten the purge valve.
10. Check that the pressure builds and then levels off around 2000 psi
11. Switch on all the other modules on the Panel:
    a. Eluent generator
    b. Trap column
    c. Suppressor
    d. Cell heater
    e. Column heater
12. Sometimes the suppressor will not turn on using the Panel. If this happens:
    a. Select the Detector Compartment tab
    b. Click on Calculate Current
    c. Set the cell temperature to 30.0 °C and click OK
    d. Click on Calculate Current again
    e. Select the suppressor type (either ASRS-4mm for anions or CSRS-4mm for cations) and enter the desired eluent concentration
    f. If this doesn't work either, run a DIW sample using a Sequence, and it will set everything correctly
13. Prime the autosampler syringe using the Prime button under the Autosampler tab. Then flush the syringe using the Flush button.
14. The system will now have to run for several hours until it is stable. For anions, the background conductivity should be about 1.0 uS. For cations, the background conductivity should be about 0.1 uS. To check the background conductivity, select the Conductivity Detector tab on the Panel and see what the Total Signal reading is. After the background conductivity reaches these values and is no longer rising or falling, the system is ready to use for analysis.

Analyzing Samples:

The following steps have to be performed for both Anions and Cations separately.

1. Open the Browser and create a new Sequence by opening a previous Sequence that used the appropriate program and method and saving it under a new name. (Save it under a new name; otherwise all the data from the previous sequence will be lost.)
2. Copy the names and primary keys for the samples and QC samples from the spreadsheet file generated by the SWAS/VTSSS LIMS (Laboratory Information Management System) and save the Sequence. Alternatively, enter the sample names manually.
3. All autosampler vials should be rinsed several times with DIW and preferably filled with DIW and stored overnight before use
4. For each sample:

   a. Pre-rinse autosampler vial with sample by pouring 2-3 mLs of sample into autosampler vial, cap it, shake and then discard sample. Repeat 2-3 times if sample volume permits.
   b. Fill one-half to two-thirds full with sample and cap vial
   c. Load the sample vials into the rack according to the Sequence
5. Start the Sequence with the following steps:
   a. Under the Batch menu, choose Start
   b. Add the Sequence to be started
   c. Click on Ready Check, and if there are any other warnings other than the amount of disk space and eluent needed, these will have to be fixed before the Sequence can be started
   d. Click Start
6. Be sure there is enough DIW in all reservoirs before leaving unattended for long periods of time.

Shutdown Procedure:

1. Turn external water pump off by choosing the Detector Compartment tab for the Anion system and changing the DC_ACRelay_1 to open. Wait 3-5 minutes for the bubbles to clear out of the suppressors before proceeding with step 2.
2. Switch off all the other modules on the Panel for all systems being used:
   a. Eluent generator
   b. Trap column
   c. Suppressor
   d. Cell heater
   e. Column heater

Section III: Quality Control

Sequence Set-up:

Described on pg. 70 in Figure 6-2 of Paulsen, 1997.

1. Calibration standards
2. Laboratory blank (DIW)
3. Detection limit quality control check sample
4. Calibration quality control check sample
5. Samples (insert quality control check samples at regular intervals)
6. Calibration quality control check sample
7. Calibration standards

Note: Analyze the MDL Standard Solution at least once during each sequence

Performance Criteria:

Laboratory blank: Value obtained should be less than the specified MDL objective given in the Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP

Detection limit quality control sample: Value obtained should be within specified limits: True value + MDL objective given in the Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP

Calibration quality control check sample: Value obtained should be within specified limits: True value + precision objective given in the Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP

G-2.4 Technicon AutoAnalyzer II: Silica – SiO_2

This document describes the current laboratory procedure for measuring silica using the Technicon AutoAnalyzer II. Analytical methods are based on the methods published in American Public Health Association (2005).

Summary: Silica in the sample reacts with molybdate ion in acidic solution to form a colored complex. The absorbance of the resulting solution is measured using a spectrophotometer.

Section I: Standards

All glassware should be Class A volumetric laboratory glassware which has been initially cleaned by acid washing and afterwards cleaned by multiple rinsings with DI (deionized) water.

Preparation of Silica Calibration Standards:

Silica Calibration Standards Working Stock: 214 mg/L SiO_2

Prepare as follows from prepared or purchased 1000 mg/L stock solutions.

(Throughout this procedure, use only NIST-traceable stock solutions when using purchased stock solutions.)

Using a volumetric pipette, add 50 mL of 1000 mg/L Si stock solution to a 500 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.

Silica Calibration Standards:

Dilute the indicated volume of Silica Calibration Standards Working Stock to 1000 mL in a volumetric flask and mix well to yield standards of the following concentrations in mg/L:

Std. #	Volume of stock	Concentration (mg/L SiO_2)
1	50 mL	10.7
2	40 mL	8.56
3	30 mL	6.42
4	20 mL	4.28
5	10 mL	2.14
6	5 mL	1.07
7	2.5 mL	0.535
8	0 mL	0.00

Preparation of Silica Quality Control Standards:

The Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP gives the following Method Detection Limit Objective: 0.05 mg/L SiO_2

Silica Quality Control Standards Working Stock: 12.84 mg/L SiO_2

Prepare as follows from prepared or purchased 1000 mg/L stock solutions.

Two steps:

1. Using a volumetric pipette, add 25 mL of 1000 mg/L Si stock solution to a 250 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well. The final concentration of this solution is 214 mg/L SiO_2.
2. Using a volumetric pipette, add 30 mL of the 214 mg/L SiO_2 solution to a 500 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well. The final concentration of this solution is 12.84 mg/L SiO_2.

Silica MDL (method detection limit) Standard Solution:

0.1284 mg/L SiO_2:

Pipette 10 mL of the 12.84 mg/L Silica Quality Control Standards Working Stock into a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.

Silica Detection Limit Quality Control Check Sample:

0.2568 mg/L SiO_2:

Pipette 10 mL of the 12.84 mg/L Silica Quality Control Standards Working Stock into a 500 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.

Silica Calibration Quality Control Check Sample:

2.568 mg/L SiO_2:

Pipette 100 mL of the 12.84 mg/L Silica Quality Control Standards Working Stock into a 500 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.

Section II: Operation

Reagents:

- Ammonium Molybdate reagent: Stable for 2 months refrigerated.
    1. Add 40 mL of 2.5 N sulfuric acid (H_2SO_4) to a cleaned 1000 mL volumetric flask partially filled with DIW and mix
    2. Add 10.0 g ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) and mix until dissolved
    3. Fill to the line with DIW and mix well
    4. Filter or remake if cloudy
    5. Add 1-2 mL of SLS solution* as a wetting agent
- Oxalic Acid reagent: Prepare daily.
    1. Add 17.5 g oxalic acid dihydrate ($H_2C_2O_4.2H_2O$) to a cleaned 250 mL volumetric flask partially filled with DIW and mix until dissolved

2. Fill to the line with DIW and mix well

- Ascorbic Acid reagent: Prepare daily.
    1. Add 12.5 mL acetone (CH_3COCH_3) to a cleaned 250 mL volumetric flask partially filled with DIW and mix
    2. Add 4.4 g ascorbic acid ($C_6H_8O_6$) and mix until dissolved
    3. Fill to the line with DIW and mix well
    4. Add 1-2 mL of SLS solution* as a wetting agent

* To prepare SLS solution: Add 6-8 g of sodium lauryl sulfate to 100 mL deionzed water, mix and then add 5 drops concentrated sulfuric acid.

Colorimeter configuration for silica:

Flow cell: 15 mm

Mode: I

Wavelength filter: 660 nm

Sampling rate: 30/hour

Sample:Rinse ratio: 2:1

Start-up Procedure:

1. Rinse and fill reservoirs for autosampler rinse tubing and reagent tubing with fresh DIW
2. Flush autosampler rinse reservoir with DIW using a squirt bottle
3. Mount auto-analyzer flow-rated pump tubing on pump, place platen on pump and turn motor on
4. Allow DIW to pump to flush system for at least 10 minutes
5. Place labeled reagent lines into appropriate reagent container. (All reagents should be room temperature.)
6. From this point, collect waste in hazardous waste container
7. Turn on strip chart recorder and set to 100 mV
8. Turn on colorimeter
9. Turn the knob on the colorimeter to ZERO and adjust the pen on the strip chart recorder to read zero units using the zero knob on the strip chart recorder
10. Turn the knob on the colorimeter to FULL SCALE and adjust the pen on the strip chart recorder to read 100 units using the var knob on the strip chart recorder
11. Turn the knob on the colorimeter to NORMAL
12. Turn the baseline adjusting knob on the colorimeter so that it is mid-range by turning knob as far as it will go one way and then turn back 5 full turns
13. After reagents have pumped through the colorimeter for about 10 minutes:
    a. Turn the STD CAL knob on the colorimeter to the previous run's setting, if known

   b. Turn the sample light source silver knob so that it is fully open (completely clockwise)
   c. Adjust the reference light source silver knob until the pen on the strip chart recorder is at about 5 units

14. Install pen on strip chart recorder and start paper at 10 mm/min
15. Place a tube of highest concentration standard in the auto sampler and run several times (5-10):
   a. When first peak comes out, adjust STD CAL knob on colorimeter so that the top of the peak is at about 95 units
   b. After adjustment, check for reproducibility. Successive peaks should be within 2 units

Analyzing Samples:

1. Create a Sample list using the spreadsheet file generated by the SWAS/VTSSS LIMS (Laboratory Information Management System). Alternatively record the sample names manually.
2. For each sample:
   a. Rinse autosampler tube with DIW
   b. Pre-rinse each autosampler tube with sample by filling half-full and discarding. Repeat if sample volume permits.
   c. Fill one-half to two-thirds with sample
   d. Load the sample vials into the rack according to the Sample list
3. Throughout the run, check reservoir for autosampler rinse tubing and refill with fresh DIW as needed

Shutdown Procedure:

1. After the autosampler has sampled the last tube, turn off autosampler
2. After the last peak has been recorded on the stripchart recorder, turn off stripchart recorder, and remove and cap pen
3. Turn off colorimeter
4. Remove reagent lines from reagent containers and place in reservoir for reagent tubing filled with fresh DIW
5. Pump DIW through all tubing lines for about 15 minutes until reagents are out of lines
6. Turn pump motor off, remove platen from pump, and release flow-rated pump tubing
7. Cap hazardous waste container and dispose of properly

Section III: Quality Control

Sequence Set-up:

Described on pg. 70 in Figure 6-2 of Paulsen, 1997

1. Calibration standards
2. Laboratory blank (DIW)

3. Detection limit quality control check sample
4. Calibration quality control check sample
5. Samples (insert quality control check samples at regular intervals)
6. Calibration quality control check sample
7. Calibration standards

Note: Analyze the MDL Standard Solution at least once during each sequence

Performance Criteria:

Laboratory blank: Value obtained should be less than the specified MDL objective given in the Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP

Detection limit quality control sample: Value obtained should be within specified limits: True value + MDL objective given in the Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP

Calibration quality control check sample: Value obtained should be within specified limits: True value + precision objective given in the Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP

G-2.5 Technicon AutoAnalyzer II: Total and Organic Monomeric Aluminum

This document describes the current laboratory procedure for measuring total and organic monomeric aluminum using the Technicon AutoAnalyzer II. Analytical methods are based on the methods published in McAvoy et al. (1992).

Summary: Monomeric aluminum in the sample reacts with pyrocatechol violet to form a colored complex. The absorbance of the resulting solution is measured using a spectrophotometer.

Section I: Standards

All glassware should be Class A volumetric laboratory glassware which has been initially cleaned by acid washing and afterwards cleaned by multiple rinsings with DI (deionized) water.

Preparation of Aluminum Calibration Standards:

Aluminum Calibration Standards Working Stock: 0.50 mg/L Al

Prepare as follows from prepared or purchased 1000 mg/L stock solutions.

(Throughout this procedure, use only NIST-traceable stock solutions when using purchased stock solutions.)

Two steps:

1. Using a volumetric pipette, add 25 mL of 1000 mg/L Al stock solution to a 500 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well. The final concentration of this solution is 50.0 mg/L Al.
2. Using a volumetric pipette, add 10 mL of the 50.0 mg/L Al solution to a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.

Aluminum Calibration Standards:

Dilute the indicated volume of Aluminum Calibration Standards Working Stock to 250 mL in a volumetric flask and mix well to yield standards of the following concentrations in ug/L:

Std. #	Volume of stock	Concentration (mg/L Al)
1	50 mL	100
2	40 mL	80
3	30 mL	60
4	20 mL	40
5	10 mL	20
6	5 mL	10
7	2.5 mL	5
8	0 mL	0

Preparation of Aluminum Quality Control Standards:

The Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP gives the following Method Detection Limit Objective: 10 ug/L Al

Aluminum Quality Control Standards Working Stock: 1.0 mg/L Al

Prepare as follows from purchased or prepared 1000 mg/L stock solutions.

Two steps:

1. Using a volumetric pipette, add 25 mL of 1000 mg/L Al stock solution to a 500 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well. The final concentration of this solution is 50.0 mg/L.
2. Using a volumetric pipette, add 20 mL of the 50.0 mg/L Al solution to a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well. The final concentration of this solution is 1.0 mg/L Al.

Aluminum MDL (method detection limit) Standard Solution:

20 ug/L Al:

Pipette 20 mL of the 1.0 mg/L Aluminum Quality Control Standards +Working Stock into a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.

Aluminum Detection Limit Quality Control Check Sample:

40 ug/L Al:

Pipette 20 mL of the 1.0 mg/L Aluminum Quality Control Standards Working Stock into a 500 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.

Aluminum Calibration Quality Control Check Sample:

50 ug/L Al:

Pipette 25 mL of the 1.0 mg/L Aluminum Quality Control Standards Working Stock into a 500 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix well.

Section II: Operation

Reagents:

- Pyrocatechol Violet (PCV) Reagent – Dilute PCV stock 1:10 with DIW:
    1. With a graduated cylinder, add 100 mL of stock to a pre-rinsed 1000 mL volumetric flask
    2. Dilute to the line with DIW and mix well

    PCV stock: Add 1.875 g of pyrocatechol violet ($C_{19}H_{14}O_7S$) to a 1000 mL volumetric flask partially filled with DIW and mix. Dilute to the line with DIW and mix well. Store refrigerated in an amber polyethylene bottle.

- Hydroxylamine hydrochloride-Phenanthroline Reagent – Dilute Hydroxylamine hydrochloride-Phenanthroline stock 1:10 with DIW:
    1. With a graduated cylinder, add 100 mL of stock to a pre-rinsed 1000 mL volumetric flask
    2. Dilute to the line with DIW and mix well

- Hydroxylamine hydrochloride-Phenanthroline stock: Add 300 g of hydroxylamine hydrochloride (NH_2OH . HCl) to a 1000 mL volumetric flask partially filled with DIW and mix. Add 3 g of 1,10-phenanthroline ($C_{12}H_8N_2$) and mix. Dilute to the line with DIW and mix well. Store refrigerated in an amber polyethylene bottle.
- Hexamethylene Tetramine Buffer Reagent:
    1. Add 200 g of hexamethylene tetramine ($(CH_2)6N_4$) to a 1000 mL volumetric flask partially filled with DIW and mix
    2. Dilute to the line with DIW and mix well
    3. Add 1-2 mLs of Triton-X as a surfactant and mix well

<u>Colorimeter configuration for Aluminum:</u>

Flow cell: 50 mm
Mode: I
Wavelength filter: 590 nm
Sampling rate: 10/hour
Sample:Rinse Ratio: 1:3

Start-up Procedure:

1. Rinse and fill reservoirs for autosampler rinse tubing and reagent tubing with fresh DIW
2. Flush autosampler rinse reservoir with DIW using a squirt bottle
3. Mount auto-analyzer flow-rated pump tubing on pump, place platen on pump and turn motor on
4. Allow DIW to pump to flush system for at least 10 minutes

5. Place labeled reagent lines into appropriate reagent container. (All reagents should be room temperature.)
6. From this point, collect waste in hazardous waste container.
7. Turn on strip chart recorder and set to 100 mV
8. Turn on colorimeter
9. Turn the knob on the colorimeter to ZERO and adjust the pen on the strip chart recorder to read zero units using the zero knob on the strip chart recorder
10. Turn the knob on the colorimeter to FULL SCALE and adjust the pen on the strip chart recorder to read 100 units using the var knob on the strip chart recorder
11. Turn the knob on the colorimeter to NORMAL
12. Turn the baseline adjusting knob on the colorimeter so that it is mid-range by turning knob as far as it will go one way and then turn back 5 full turns
13. After reagents have pumped through the colorimeter for about 10 minutes:
    a. Turn the STD CAL knob on the colorimeter to the previous run's setting, if known
    b. Turn the sample light source silver knob so that it is fully open (completely clockwise)
    c. Adjust the reference light source silver knob until the pen on the strip chart recorder is at about 5 units
14. Install pen on strip chart recorder and start paper at 10 mm/min
15. Place a tube of highest concentration standard in the auto sampler and run several times (5-10):
    a. When first peak comes out, adjust STD CAL knob on colorimeter so that the top of the peak is at about 95 units
    b. After adjustment, check for reproducibility. Successive peaks should be within 2 units.

Analyzing Samples:

1. Create a Sample list using the spreadsheet file generated by the SWAS/VTSSS LIMS. Alternatively record the sample names manually.
2. For each sample:
    a. Rinse autosampler tube with DIW
    b. Pre-rinse each autosampler tube with sample by filling half-full and discarding. Repeat if sample volume permits.
    c. Fill one-half to two-thirds with sample
    d. Load the sample vials into the rack according to the Sample list
3. Throughout the run, check reservoir for auto-sampler rinse tubing and refill with fresh DIW as needed

Fractionation Procedure:

To measure the organic fraction of the total monomeric aluminum, the inorganic monomeric aluminum must be removed by passing the sample through a cation exchange column. The resulting fraction is analyzed to quantify the organic monomeric aluminum. The inorganic monomeric aluminum fraction may then be calculated by taking the difference between the total monomeric aluminum concentration and the organic monomeric aluminum fraction.

1. Prepare cation exchange resin:
    a. Tare a large (1-2 liter) plastic beaker on a lab balance
    b. Add the desired amount of Amberlite IR-120+ sodium form ion exchange resin and record the mass
    c. Calculate 1% of the mass of the sodium form. Add this amount of Amberlite IR-120+ hydrogen form ion exchange resin.
    d. Add DIW and mix with a stirring rod
    e. The composition of the cation exchange resin should be such that the pH of the sample does not change significantly after passing through the column. Several different compositions of resin may be necessary if the pH range of the samples is large (pH 4-7). The lower the sample pH value, the lower the pH of the resin should be. Test the resin by fractioning samples of various pH values and observing the pH change of the sample. Adjust the composition of the resin by adding more hydrogen form (to lower the pH) or sodium form (to raise the pH) of Amberlite IR-120+. In this way, prepare various resins appropriate for the samples.
2. Fractionate samples:
    a. Rinse 250 mL separatory funnel, stopper with tube, flow regulator and various pieces of connecting tubing with DIW at least 3 times
    b. Pre-rinse funnel with a few milliliters of sample and discard
    c. Close funnel stopcock and fill funnel with 50 – 75 mL of sample (or less if sample volume is low)
    d. Insert stopper with tube into top of funnel
    e. Prepare cation exchange column:
        i. Replace poly-fiber in end of column tubing daily
        ii. Insert connector into end of column tubing with the poly-fiber in it
        iii. Place the other end of the column tubing into a polyethylene beaker filled with cation exchange resin (Add DIW as needed to make the resin the proper consistency.)
        iv. Using a pipette bulb, pull resin into the column tubing
        v. Remove pipette bulb, and hold thumb over end of tubing
        vi. Remove other end of tubing from resin and quickly hold both ends of tubing up so that resin does not flow out

vii. Check to see that the column is full of well-packed resin with no air pockets. If not, remake column.

viii. Use a DIW squirt bottle to bring level of resin to end of connector to create a 4 cm column

ix. Do not allow water to flow out of column at any time to keep resin well packed. If this happens, make a new column.

f. Close the pinch clamp at the end of the tubing connected to the flow regulator

g. Keeping the column in a U-shape so that resin or water does not flow out, connect the end of the column with the poly-fiber in it to the flow regulator

h. Connect the other end of the column to the tubing at the bottom of the funnel

i. Confirm that the column is wetted, well-packed and without air pockets

j. Open funnel stopcock

k. Squeeze and tap tubing at end of funnel to remove air and ensure free flow

l. Open pinch clamp and allow at least 20 mL of sample to come through to flush column and tubing

m. Check to see that bubbles are coming out of the funnel stopper tube at a consistent rate. If not, be sure stopper is tight at top of funnel.

n. The flow rate out of the column should be 4 mL +/- 0.25 mL per 15 seconds. Check with graduated cylinder and stopwatch. If it is too slow or too fast, adjust rate by opening or closing the flow regulator as needed.

o. Collect sample in polyethylene beaker. Analyze as soon as possible. Store by refrigeration only if absolutely necessary.

Shutdown Procedure:

1. After the autosampler has sampled the last tube, turn off autosampler
2. After the last peak has been recorded on the stripchart recorder, turn off stripchart recorder, and remove and cap pen
3. Turn off colorimeter
4. Remove reagent lines from reagent containers and place in reservoir for reagent tubing filled with fresh DIW
5. Pump DIW through all tubing lines for about 15 minutes until reagents are out of lines
6. Turn pump motor off, remove platen from pump, and release flow-rated pump tubing
7. Cap hazardous waste container and dispose of properly

Section III: Quality Control

Sequence Set-up:

Described on pg. 70 in Figure 6-2 of Paulsen, 1997

1. Calibration standards
2. Laboratory blank (DIW)
3. Detection limit quality control check sample
4. Calibration quality control check sample
5. Samples (insert quality control check samples at regular intervals)
6. Calibration quality control check sample
7. Calibration standards

Note: Analyze the MDL Standard Solution at least once during each sequence

Performance Criteria:

Laboratory blank: Value obtained should be less than the specified MDL objective given in the Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP

Detection limit quality control sample: Value obtained should be within specified limits: True value + MDL objective given in the Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP

Calibration quality control check sample: Value obtained should be within specified limits: True value + precision objective given in the Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP

G-2.6 Teledyne-Tekmar Phoenix 8000 TOC Analyzer

This document describes the current laboratory procedure for measuring DOC using the Teledyne-Tekmar Phoenix 8000 TOC (total organic carbon) Analyzer. Analytical methods are based on the methods published in EPA Method 415.3.

Summary: Inorganic carbon is removed by acidification and sparging with nitrogen gas. Organic carbon in the sample is oxidized to carbon dioxide by simultaneous exposure to persulfate ions and UV (ultraviolet) radiation. The carbon dioxide produced is then measured by an NDIR (nondispersive infrared) detector.

Section I: Standards

All glassware for preparing DOC standards should be Class A volumetric laboratory glassware which has been cleaned as follows:

1. Wash with laboratory detergent (Liquinox) in hot tap water
2. Rinse with hot tap water
3. Fill with (or soak in) 2N HCl overnight
4. Rinse 10 times with DIW
5. Use the glassware immediately (If this is not possible, store the glassware filled with DIW and preferably use within 24 hours.)

Preparation of DOC Calibration Standards:

DOC Calibration Standards Working Stock: 50 mg/L DOC

(Throughout this procedure, use only NIST-traceable stock solutions when using purchased stock solutions.)

Use a purchased 50 mg/L TOC/DOC standard or prepare as follows from a purchased 1000 mg/L TOC/DOC standard:

Using a volumetric pipette, add 25 mL of 1000 mg/L TOC/DOC standard to a 500 mL volumetric flask partially filled with DIW. Acidify with 2.5 mL H_3PO_4 to bring to pH < 2. Dilute to the line with DIW.

DOC Calibration Standards:

All standards must be made at the same time with the same source of DIW.

Prepare standards using volumetric glassware according to the following table:

Volume of stock	Concentration (mg/L)	Total Volume	Vol. H_3PO_4 added
50 mL	10.0	250 mL	1.25 mL
50 mL	5.0	500 mL	2.5 mL
25 mL	2.5	500 mL	2.5 mL
10 mL	1.0	500 mL	2.5 mL
5 mL	0.5	500 mL	2.5 mL
5 mL	0.25	1000 mL	5 mL
0 mL	0.0	500 mL	2.5 mL

Add H_3PO_4 to standards after bringing to final volume.

Store at room temperature in volumetric flasks away from light. May be stored for 30 days.

Preparation of DOC Quality Control Standards:

The Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP gives the following Method Detection Limit Objective: 0.1 mg/L DOC

DOC Quality Control Standards Working Stock: 100 mg/L DOC

Use a purchased 100 mg/L TOC/DOC standard or prepare as follows from a purchased 1000 mg/L TOC/DOC standard:

Using a volumetric pipette, add 50 mL of 1000 mg/L TOC/DOC standard to a 500 mL volumetric flask partially filled with DIW. Acidify with 2.5 mL H_3PO_4. Dilute to the line with DIW.

DOC MDL (method detection limit) Standard Solution:

0.25 mg/L DOC:

Pipette 5 mL of the 100 mg/L DOC Quality Control Standards Working Stock into a 2000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix. Add 10 mL H_3PO_4 and mix well.

DOC Detection Limit Quality Control Check Sample:

0.50 mg/L DOC:

Pipette 5 mL of the 100 mg/L DOC Quality Control Standards Working Stock into a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix. Add 5 mL H_3PO_4 and mix well.

DOC Calibration Quality Control Low Check Sample:

1.5 mg/L DOC:

Pipette 15 mL of the 100 mg/L DOC Quality Control Standards Working Stock into a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix. Add 5 mL H_3PO_4 and mix well.

DOC Calibration Quality Control High Check Sample:

3.0 mg/L DOC:

Pipette 30 mL of the 100 mg/L DOC Quality Control Standards Working Stock into a 1000 mL volumetric flask partially filled with DIW. Dilute to the line with DIW and mix. Add 5 mL H_3PO_4 and mix well.

Section II: Operation

Reagents:

- 21% Phosphoric Acid Reagent: Stable for one month
    1. Rinse a clean (does not have to be acid-washed) laboratory flask multiple times with DI (deionized) water
    2. Add 188 mL of DIW using a graduated cylinder which has been rinsed well
    3. Carefully add 37 mL of 85% phosphoric acid (H_3PO_4) and mix well
- 10% Persulfate/5% Phosphoric Acid Reagent: Stable for one week
    1. Rinse a clean (does not have to be acid-washed) laboratory flask multiple times with DIW
    2. Add 25 g of 98+% sodium persulfate ($Na_2S_2O_8$)
    3. Add 213 mL DIW and mix well
    4. Carefully add 9 mL of 85% phosphoric acid (H_3PO_4) and mix well
    5. Wait 12 hours before using

Note: When analyzing more than 50 samples, double the amount of this reagent.

Start-up Procedure:

Note: If power to Phoenix has been turned off, allow 2 hours for NDIR detector to warm up before analysis.

1. Carefully remove the gas/liquid separator from the instrument and rinse well with DIW. Add a few drops of 21% phosphoric acid reagent, fill to level of sidearm with DIW and then replace. (Perform at least weekly.)
2. Unplug the UV lamp, disconnect lines and carefully remove it from the instrument. Rinse well with DIW and then replace. (Perform at least weekly.)

3. Carefully remove the IC sparger from the instrument. Rinse well with DIW and then replace. (Perform at least weekly.)
4. Remove plug from bottom of mist trap to drain excess water
5. Check the copper in the halogen scrubber. If it has become totally discolored, replace copper and tin in the halogen scrubber
6. Check screw at bottom of syringe and hand-tighten
7. Place appropriate reagent lines in Phosphoric Acid and Persulfate reagents
8. Rinse DIW reservoir a few times and fill with DIW
9. Place waste line into waste container
10. Log in to DOC computer
11. Open TOC Talk for Phoenix 8000 (version 3.6.385.2)
12. Type in User name
13. Type in Password
14. Open valve on nitrogen tank. (Make sure that the tank level is at 500 + psi.)
15. Choose System → Instrument and click to change system status from Standby to Ready
16. On the Instrument Setup/Status screen, check that the gas flow rates (From Detector and To UV Reactor) are 200 cc/min +/- 10% and within 15 psi of each other. Click OK to close window. (It is seldom necessary to adjust the nitrogen tank regulator, but never turn the tank input pressure above 35 psi. Low flow rates usually indicate a leak in the system.)
17. Exit the Instrument Setup/Status screen by choosing OK

Calibrating Instrument:

1. Choose Run and select Sample Setup
2. Choose File → Open
    a. Select appropriate calibration curve file or create a new one as described below
    b. Change Status field to Ready for the first line and then for all samples by choosing Edit → Reset Status for all rows
    c. An example of a file for calibration going up to 10.0 mg/L is:

Pos	Sample ID	Sample Type	Method ID	Reps	Status
1001	Prime	Sample	Prime System	1	Ready
1001	Clean	Sample	Cleaning Procedure	3	Ready
1001	Clean	Sample	Cleaning Procedure	5	Ready
1	0.0 mg/L	TOC Standard	Drinking H2O TOC 0.1-20ppm	3	Ready
2	0.25 mg/L	TOC Standard	Drinking H2O TOC 0.1-20ppm	3	Ready
3	0.5 mg/L	TOC Standard	Drinking H2O TOC 0.1-20ppm	3	Ready
4	1.0 mg/L	TOC Standard	Drinking H2O TOC 0.1-20ppm	3	Ready
5	2.5 mg/L	TOC Standard	Drinking H2O TOC 0.1-20ppm	3	Ready

Pos	Sample ID	Sample Type	Method ID	Reps	Status
6	5.0 mg/L	TOC Standard	Drinking H2O TOC 0.1-20ppm	3	Ready
7	10.0 mg/L	TOC Standard	Drinking H2O TOC 0.1-20ppm	3	Ready

3. Alternatively, to create a new file to use to calibrate: Choose File → New
    a. Make sure that the Rack Style is 40 mL vial
    b. Enter an 8-character Rack ID
    c. Use the down arrow to add more lines
    d. When you select TOC Standard, a pop-up menu will appear to select the appropriate range (usually 0.1 – 20 ppm)
    e. Select the appropriate standard from the list and choose Exit
4. Load the appropriate standards in the autosampler according to the Autosampler list
5. Choose Save/Use
6. Choose System → Instrument and de-select the Auto Shutdown box if necessary
7. Choose Start (note that it will take several hours for the calibration to be completed)

Confirming Calibration:

1. After the standards are finished running, the Calibration Curve screen automatically appears. (To open it manually, select Results ☐ Calibration.)
2. Select the calibration curve file created in step 2 above from the list, if it is not already displayed
3. In the Use column, select the standards that have the current date and de-select all others
4. Choose Recalc to display the new calibration curve
5. Choose Save as New Version
6. Choose Exit

Analyzing Samples:

1. Choose Run and select Sample Setup
2. Choose File → New
3. Build the Autosampler list:
    a. Make sure that the Rack Style is 40 mL vial
    b. Use the arrow key to add more lines
    c. First 2 samples are:

Pos	Sample ID	Sample Type	Method ID	Reps	Status
1001	Clean	Sample	Cleaning Procedure	6	Ready
1001	System Blank	Blank TC Range 2	Blank TC Range 2 Drinking H_2O	5	Ready

    d. Throughout the run, include DOC Quality Control Standards, which must be run as sample type “Calibration Verification”
    e. Enter sample data with the following format:
        Pos = Sample position in autosampler rack

Sample ID = Sample identification (usually site, date and time)

Sample Type = Sample

Method ID = Drinking H2O TOC 0.1 -20ppm (or range being used)

Reps = 2 (may run 3 from a 40 mL vial if desired)

Status = Ready

f. The last sample should be a DOC quality control check sample run as sample type "Calibration Verification"

g. Always finish the run with a cleaning procedure:

Pos	Sample ID	Sample Type	Method ID	Reps	Status
1001	Clean	Sample	Cleaning Procedure	6	Ready

4. To begin the run immediately, choose Save/Use and enter a Rack ID, usually in the following format: YYYYMMDD (YYYY for 4-digit year, MM for 2-digit month and DD for 2-digit day)
   a. If the analyzer is already running, use "Save as" to save the file to use later and then choose Exit
   b. When ready to use the saved file, choose File → Open and select saved file
5. Choose System → Instrument and select the Auto Shutdown box
6. If the Calibration has been confirmed, choose Start to begin analysis

Shutdown Procedure:

1. After the run is complete, Exit the Sample Analysis screen
2. Choose System → Instrument and click to change system status from Ready to Standby and then choose OK
3. Choose System → Exit
4. If the analysis was completed less than 30 minutes ago, if asked, answer "Yes" to the question: "Would you like to leave the gas to the Perm Dryer ON?"
5. Turn off N_2 tank. Wait 30 minutes if the analysis was completed less than 30 minutes for the gas to dry the Perm Dryer.
6. Cap waste container and dispose of waste properly

Long versus Short-time Shutdown:

Short-time Shutdown: Leave the Phoenix 8000 in Standby mode when not in use. Standby mode turns off all components except the detector.

Long-time Shutdown: If the Phoenix 8000 will not be run for over a month, the auto-sampler and analyzer should be turned off with the power switches in the back. When turning the analyzer back on, allow 2 hours for the NDIR detector to stabilize before beginning analyses.

Section III: Quality Control

Sequence Set-up:

Described on pg. 70 in Figure 6-2 of Paulsen, 1997

1. Calibration standards
2. Laboratory blank (DIW)
3. Detection limit quality control check sample
4. Calibration quality control check sample
5. Samples (insert quality control check samples at regular intervals)
6. Calibration quality control check sample

Note: Analyze the MDL Standard Solution at least once during each sequence

Performance Criteria:

Laboratory blank: Value obtained should be less than the specified MDL objective given in the Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP

Detection limit quality control sample: Value obtained should be within specified limits: True value + MDL objective given in the Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP

Calibration quality control check sample: Value obtained should be within specified limits: True value + precision objective given in the Measurement Data Quality Objectives Table in the Project Laboratory Quality Assurance Project Plan of this SOP

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U.S. Geological Survey [USGS]. Standard Operating Procedure for Laboratory Analysis of One Analyte (Ammonium) in Dilute Fresh Water – Flow Injection Analysis. Unpublished manuscript.

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APPENDIX H.
TRAINING CHECKLISTS

Table H-1. Checklist of materials and supplies for stream sampling site visits.

Items:	✓
Standard Items	
Collection permits and entry permits, if required.	
Site Documentation Forms (for new sites)	
Clipboard	
Site Documentation Reports (compiled in folders for existing sites)	
Stream sampling record forms	
Insulated container with ice or frozen refrigerant (packed in sealed plastic bags or other containers)	
Small insulated container (with ice) for hike-in sites	
Watch for recording time	
Digital field camera with free memory and extra charged battery	
GPS unit with extra batteries	
Compass	
Field thermometer (with string attached)	
Pre-processed sample bottle(s) with sample label attached. Include a second bottle if sampling at that site is to be replicated. Put each bottle in a clean plastic zipper-lock bag.	
Plastic gloves in sealed plastic bag	
60 mL plastic syringes (with Luer type tip) with completed sample labels attached. Plastic container with snap-on lid to hold filled syringes	
Syringe valves (Mininert® with Luer type adapter, or equivalent, available from a chromatography supply company)	
Water Chemistry labels (if not already filled out and attached to sample containers at base site)	
Soft-lead pencils and write-in-rain-type pens for filling out field data forms and notebook entries	
Fine-tipped indelible markers for filling out labels	
Roll or box of tape strips	
Field operations and methods documents	
First aid kit	
Backpack	
Extra zipper-lock bags	

Items:	✓
Optional Items (may be required for specific studies):	
60 mL glass bottles with septum caps and with completed sample labels attached	
Calibrated multiparameter sonde, data logger, and cable, with extra batteries	
Calibration standards, quality control check samples, DIW, rinse bottles, waste tray and container, calibration cup, and sensor guard for sonde (multiple sensors combined in a unit that is lowered into the water)	
Sonde calibration and post-calibration record forms	
Measuring tape	
Waders or high-top waterproof boots for wading	
Clear packaging tape to cover labels	
Dissolved oxygen/temperature meter with probe	
DO repair kit containing additional membranes and probe filling solution	
Conductivity meter with probe	

Table H-2. Checklist of material needed for use in field for site documentation.

Items	✓
A. Available site documentation records for previously established sites:	
site location maps, topographic maps, and road maps	
site descriptions and access notes	
site tag numbers and tag tree descriptions (where applicable)	
site coordinates	
site photos	
B. Preliminary site documentation for new sites:	
site location maps, topographic maps, and road maps, indicating approximate site locations	
general site descriptions and access notes	
C. General material for site documentation	
regional-scale topographic and road maps	
Stream or Lake Sampling Site Documentation Forms on waterproof paper	
clipboard or field notebook and pens for use with waterproof paper	
GPS unit with replacement batteries	
digital camera with charged battery and charged replacement battery	
site tags, aluminum nails, and hammer (if applicable)	
measuring tape	
blaze orange material for flagging tag trees in photos (if applicable)	
gate keys (if needed)	
cell phone with numbers of project staff and management agency offices	

Table H-3. Equipment and supply checklist for measuring stream discharge.

Quantity	Item	✓
1	Surveyor's telescoping leveling rod (7 m long, metric scale, round cross-section)	
1	50-m fiberglass measuring tape and reel	
1	Small bubble level to make sure the tape is level	
1	Current velocity meter, probe, and operating manual	
1-2	Extra batteries for velocity meter	
1	Top-set wading rod (metric or English scale) for use with current velocity meter	
1	Portable weir with 60° "V" notch (optional)	
1	Plastic sheeting to use with weir (optional)	
1	Plastic bucket (or similar container) with volume graduations	
1	Stopwatch	
1	Covered clipboard	
	Soft (#2) pencils	
	Stream Discharge forms (one per stream, plus extras if needed for timed filling procedure or additional velocity-area intervals)	
1 copy	Field operations and methods documents	
1 set	Laminated sheets of procedure tables and/or quick reference guides for stream discharge	

Table H-4. Lake verification checklist.

Item	✓
Site information folder for lake to be sampled	
Clipboard	
Lake Sampling Site Documentation Form	
Field notebook	
Sampling permit (if needed)	
GPS unit with manual, extra battery pack	
50-m line to attach to rock anchor	

Table H-5. Checklist of equipment and supplies for benthic macroinvertebrates. (Source: Peck et al. 2006.)

Quantity	Item	✓
1	Modified kick net (D-frame with 500 µm mesh) and 4-ft handle	
	Spare net(s) and/or spare bucket assembly for end of net	
1	Watch with timer or a stopwatch	
2	Buckets, plastic, 8- to 10-qt capacity (collapsible for back country)	
1	Sieve with 500 µm mesh openings or sieve-bottomed bucket, 500 µm mesh openings	
2 pr.	Watchmakers' forceps (straight and curved)	
1	Wash bottle, 1-L capacity, labeled *STREAM WATER*	
1	Small spatula, spoon, or scoop to transfer sample	
1	Funnel, with large bore spout	
4 to 6 Each	Sample jars, HDPE plastic with leakproof screw caps, 500-ml and/or 1-L capacity, suitable for use with ethanol	
2 gal	95% ethanol, in a suitable container (smaller amounts can be carried in for back country work or ethanol can be added at the vehicle after returning from the field)	
2 pr.	Rubber gloves suitable for use with ethanol	
1	Cooler (with suitable absorbent material) for transporting ethanol and samples in vehicle	
2	Preprinted benthic sample labels with sample ID numbers	
4	Preprinted benthic sample labels without sample ID numbers	
6	Blank labels on waterproof paper for placing inside of jars	
1	Sample Collection Form for site	
	Soft (#2) lead pencils	
	Fine-tip indelible markers	
1 pkg.	Clear tape strips	
4 rolls	Plastic electrical tape	
1	Knife, pocket, with at least two blades	
1	Scissors	
1	Pocket-sized field notebook (optional)	
1 pkg.	Kim-wipes in small re-sealable plastic bag	
1 copy	Field operations and methods manual	
1 set	Laminated sheets of procedure tables and/or quick reference guides for benthic macroinvertebrates	

Table H-6. Equipment and supplies for collecting zooplankton samples. (Source: U.S. EPA 2007.)

Quantity[1]	Item	✓
2	Wisconsin fine mesh (80 µm[2]) net with attached collection bucket	
2	Wisconsin coarse mesh (243 µm[2]) net with attached collection bucket	
2	Sample line, marked at 0.5 m increments	
2	Secchi disk with cable	
2/site+	125 ml wide-mouth polyethelene sample jars (two per site, plus additional for replicates and other back-up sampling)	
1	Squirt bottle with DIW	
	95% ethanol	
2/site+	CO_2 tablets	
1/site+	500 ml wide-mouth container	
2	Two lids converted to form strainers (one with 80 µm, one with 243 µm mesh), made by drilling two holes in each lid and gluing a piece of the netting to the inside of the lid using silicone glue	
2/site+	Zipper lock-type plastic bag	
	Clear tape for covering labels	
	Electrical tape	
1/site+	Zooplankton Sample Data Form	
	Pencils and permanent markers	
	Mild (10%) bleach solution for cleaning net and strainer lids between lakes; backwash net with a garden hose after use	

[1] *It is advisable to include some extras, beyond what is needed for the number of sites to be sampled.*
[2] *These two mesh sizes (80 and 243 µm) are general guidelines. Other sizes could be used.*

Table H-7. Checklist of equipment and supplies for sampling lake water chemistry and Secchi depth.

Quantity	Item	✓
Standard Items:		
1	Field thermometer	
1-2	Sample bottle(s) with completed sample label attached (in clean plastic bag). Include a second bottle if sampling at that site is to be replicated	
2-4	60 mL plastic syringes (with Luer type tip) or glass bottles with septum caps with completed sample labels attached	
1	Plastic container with snap-on lid to hold filled syringes	
2-4	Syringe valves (Mininert® with Luer type adapter, or equivalent, available from a chromatography supply company)	
1	Cooler with 4 to 6 plastic bags (1-gal) of ice or a medium or large opaque garbage bag to store the water sample at shoreline	
1	Lake Sampling Record Form	
1 set	Water Chemistry labels (if not already filled out and attached at base site)	
2-4	Soft-lead pencils and write-in-rain pens for filling out field forms and notebook entries	
2-4	Fine-tipped indelible waterproof markers for filling out labels	
1 copy	Field operations and methods documents	
2-4	Plastic gloves stored in a secure plastic bag	
1	Survey grade global positioning system and compass	
1	Digital camera with extra memory cards and batteries	
1	Backpack with waterproof cover (if site is not accessible by vehicle)	
1	Van Dorn sampler with messenger and cable	
1	Raft or float tube with pump for inflating	
1	First aid kit	
1	Locally determined safety equipment	
1	Secchi disk and line (with depth increments)	
1	Measuring tape	
Optional Items:		
roll/box	Clear packaging tape to cover labels (tape strips)	
1	Dissolved oxygen/temperature meter with probe	
1	DO repair kit containing additional membranes and probe filling solution	
1	Conductivity meter with probe	
1	250-mL or 500-mL plastic bottle of conductivity QCCS labeled RINSE (in plastic bag)	
1	250 mL or 500-mL plastic bottle of conductivity QCCS labeled TEST (in plastic bag)	

APPENDIX I.
JOB HAZARD ANALYSIS

The following pages show an example Job Hazard Analysis (JHA). This JHA may be used but should be revised to include any safety concerns specific to your area.

U.S. Department of Agriculture	1. WORK PROJECT/ACTIVITY	2. LOCATION	3. UNIT
Forest Service			
JOB HAZARD ANALYSIS (JHA)	4. NAME OF ANALYST	5. JOB TITLE	6. DATE PREPARED
References-FSH 6709.11 and -12 (Instructions Following Form)			

7. Tasks/Procedures	8. HAZARDS	9. ABATEMENT ACTIONS Engineering Controls * Substitution * Administrative Controls * PPE
Back Country Travel	Overdue	Plan ahead. Leave an itinerary of planned trip and follow carefully. Establish regular check in times. Never travel or work alone in remote areas. Each person should carry: radio with extra batteries or well charged batteries (know proper channels and tones to use), first-aid kit, compass and map, waterproof matches, fire starter, pocket knife, survival kit, flashlight, extra food, extra clothing, rain gear, signal mirror, extra water.
	Disorientation	Find sheltered spot and prepare camp. If unable to orient yourself use signal mirror, flares or fire to attract attention.
	Environmental Hazards	Watch weather conditions. Choose safe travel routes, avoid snag or rock slide areas. Maintain secure footing and working positions. Be on guard against injury from falling trees, snags, limbs, rolling logs, or rocks. Maintain safe distance between people (10 feet). Be sure others know where you are. Wear eye protection.
	Contaminated Water/Giardia	Carry extra water; filter water; use water tablets or boil water before drinking to avoid contamination by giardia.
Horse Travel	Riding horses is dangerous. Death or serious injury may occur. Horses can spook, trip, and fall. Some hazards to be aware of while riding: Rough terrain Rolling debris on trails, dust, brush and limbs Fast-moving water crossings Snow banks Back packers Foot getting stuck in stirrup while getting off Falling from horse	Personal protective equipment (PPE) will be riding boots, long sleeve shirt and pants. Riding helmets recommended for inexperienced riders. Carry a radio at all times and check in and out with the local forest. Be alert, talk to the animals while working with them to make them aware of your presence. Watch for falling debris and wildlife that may spook your animals. When in rough terrain, keep only the balls of your feet in the stirrups, or get off and walk. Check water crossing before entering water. Take extra time on switchbacks to give the pack animals time to round the corners. Snow banks contain air pockets underneath; consider firmness of snow and possible entrapment of stock in snow while crossing. If it is warm, do not cross over deep snow. Talk to backpackers and ask them to step to the side of the trail and stand quietly while you pass.
Vehicle Travel/Driving	Vehicle accidents and associated injury	Always wear safety belts and make sure everyone is buckled up! Drive carefully on heavily travelled roadways. Driving defensively means anticipating the other drivers actions before they happen. Back your vehicle in when parking and use a ground guide when available. Drive carefully in snow and mud, chain up BEFORE you get stuck. Don't attempt accessing remote areas in poor conditions. Roads are narrow, drive defensively, giving yourself enough time/space to react to other drivers. Maintain stopping distance of half the distance you can see. Drive with headlights on. Stop and take a break if you feel sleepy while driving, or let someone else drive (work–rest-ratio: Drive no more than 10 hours in one day and take a 10 minute break every two hours). If possible, remove hazards from roadbed rather than try to drive over or around them.

In the Field	Slips and falls/Balance/Crossing Snow	Use traction devices on shoes and waders. Move slowly, take your time. Use a walking staff to provide a three point support. Snow banks contain air pockets underneath; consider firmness of snow before crossing or avoid crossing snow patches when possible.
	Crossing streams with high flow velocity, drowning	Evaluate a stream before entering. Follow the "rule of 10" 1) if stream is 1 foot deep and flowing @10 ft/sec, it is too hazardous to wade 2) if stream is 2 feet deep and flowing at 5 ft/sec, it is too hazardous to wade. If you do enter a stream and discover it is too dangerous to wade, back out using your wading pole for balance. Secure packs with quick release straps and be ready to discard if an emergency arises. Be prepared to administer CPR.
	Mid-lake water sampling : Float tube	Wear a life vest. Return to shore if you become extremely tired. Have other crew members on shore watch for hazards in the water.
	Mid-lake water sampling: Van dorn samplers	Be cautious when operating sampler, it can trap fingers.
	Exposure Cold weather conditions Hot weather conditions Sunburn Severe weather	Hypothermia: Work in teams of two. Have warming devices available. Wear proper equipment that is in good condition. Be aware of signs of hypothermia, its prevention, detection, and its treatment. Stay in tune to current weather and extended forecasts. Heat exhaustion: maintain adequate water intake by drinking water periodically throughout the day to avoid dehydration. Wear loose-fitting light colored clothing to prevent overheating. Sunburn: Wear protective clothing, hat, sunglasses, and sunscreen (apply frequently). Severe weather: During lightning storms or when a storm is approaching, avoid high points, move to low ground, and stay low, not under a tree.
	Hazard trees/Blow-down/heavy debris	Be aware of your surroundings, including hazard trees with hanging or leaning debris that may be dislodged and fall, especially during high winds. Do not rest or camp under hazard trees or hanging/leaning debris in trees.
	Scrapes and punctures	Wear proper clothing, long sleeved shirts and pants. Use first aid as necessary.
	Eye injuries	Travel with care through heavy brush. Use eye protection in brushy areas.
	Insect bites/stings	Avoid wearing heavy fragrances. Carry first-aid and sting relief kits. Make sure all crew members are informed about others who are allergic and what to do if they need assistance. Carry necessary emergency medication.
	Animal encounters	Bears: store food away from sleeping area. If you encounter one make lots of noise and back away. Rodents: (haunta virus) avoid feces and dust. Store food tightly and dispose if contaminated. Rattle snakes: watch your step on the trail and step over logs/rocks and other obstacles.

10. LINE OFFICER SIGNATURE	11. TITLE	12. DATE

(over)

JHA Instructions (References-FSH 6709.11 and .12)

The JHA shall identify the location of the work project or activity, the name of employee(s) writing the JHA, the date(s) of development,and the name of the appropriate line officer approving it. The supervisor acknowledges that employees have read and understand the contents, have received the required training, and are qualified to perform the work project or activity.

Blocks 1, 2, 3, 4, 5, and 6: Self-explanatory.

Block 7: Identify all tasks and procedures associated with the work project or activity that have potential to cause injury or illness to personnel and damage to property or material. Include emergency evacuation procedures (EEP).

Block 8: Identify all known or suspect hazards associated with each respective task/procedure listed in block 7. For example:

a. Research past accidents/incidents

b. Research the Health and Safety Code, FSH 6709.11 or other appropriate literature.

c. Discuss the work project/activity with participants

d. Observe the work project/activity

e. A combination of the above

Block 9: Identify appropriate actions to reduce or eliminate the hazards identified in block 8. Abatement measures listed below are in the order of the preferred abatement method:

a. Engineering Controls (the most desireable method of abatement). For example, ergonomically designed tools, equipment, and furniture.

b. Substitution. For example, switching to high flash point, non-toxic solvents.

Work Leader
c. Administrative Controls. For example, limiting exposure by reducting the work schedule; establishing appropriate procedures and practices.

d. PPE (least desirable method of abatement). For example, using hearing protection when working with or close to portable machines (chain saws, rock drills portable water pumps).

e. A combination of the above.

Block 10: The JHA must be reviewed and approved by a line officer. Attach a copy of the JHA as justification for purchase orders when procuring PPE.

Blocks 11 and 12: Self-explanatory.

Emergency Evacuation Instructions (Reference FSH 6709.11)

Work supervisors and crew members are responsible for developing and discussing field emergency evacuation procedures (EEP) and alternatives in the event a person(s) becomes seriously ill or injured at the worksite.

Be prepared to provide the following information:

a. Nature of the accident or injury (avoid using victim's name).
b. Type of assistance needed, if any (ground, air, or water evacuation).
c. Location of accident or injury, best access route into the worksite (road name/number), identifiable ground/air landmarks.
d. Radio frequency(s).
e. Contact person.
f. Local hazards to ground vehicles or aviation.
g. Weather conditions (wind speed & direction, visibility, temp).
h. Topography.
i. Number of person(s) to be transported.
j. Estimated weight of passengers for air/water evacuation.

The items listed above serve only as guidelines for the development of emergency evacuation procedures.

JHA and Emergency Evacuation Procedures Acknowledgement

We, the undersigned work leader and crew members, acknowledge participation in the development of this JHA (as applicable) and accompanying emergency evacuation procedures. We have thoroughly discussed and understand the provisions of each of these documents:

SIGNATURE and DATE	SIGNATURE and DATE

Editing, design, and layout by Forest Service Publishing Arts, a division of the Washington Office National Forest System Business Administration Support Services staff.