



Integrated aquatic community and water quality monitoring of mountain ponds and lakes in the Klamath Network

Natural Resource Report NPS/KLMN/NRR—2012/484



ON THE COVER

One of the Cluster Ponds at Lassen Volcanic National Park, September 2008
Photograph by: Aaron Maxwell

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January 2012

U.S. Department of the Interior
National Park Service
Natural Resource Stewardship and Science
Fort Collins, Colorado

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Please cite this publication as:

Dinger, E. C., D. A. Sarr, S. R. Mohren, and R. L. Hoffman. 2012. Integrated aquatic community and water quality monitoring of mountain ponds and lakes in the Klamath Network. Natural Resource Report NPS/KLMN/NRR—2012/484. National Park Service, Fort Collins, Colorado.

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Executive Summary

The long-term sampling protocol for mountain lakes and ponds in the Klamath Inventory and Monitoring Network is the result a collaborative effort of park personnel, USGS aquatic ecologist, and the Network staff.

Key steps covered in the narrative include a brief history of “water quality” monitoring and justification for use of key parameters as biomonitoring tools. Invertebrates and plankton can respond to short-term impacts, while fish and amphibians can show long-term signals. The background of sampling done prior to this protocol in both Lassen Volcanic National Park and Crater Lake National Park is also covered; sampling at Lassen Volcanic National Park has been sporadic, and Crater Lake has been studied and monitored extensively, but is outside the scope of this particular protocol. The narrative links the methodology developed here to both national (e.g., United States Environmental Protection Agency and United States Geological Survey) methods and other Inventory and Monitoring Networks methods (e.g., North Coast Cascades Network). In sum, methods have been kept as comparable as possible, even though sampling designs and revisit patterns vary among national and NPS programs.

An always revisit panel design was chosen over more complicated designs based on: (1) Logistics of site establishment, (2) Trend detection, (3) Conceptual simplicity, and (4) ease of data analysis. Thirty sites will be monitored every three years at Lassen Volcanic National Park, and between six and ten sites will be sampled at Crater Lake National Park. Sampling will commence in 2013. Sampling will occur in summer, after snowmelt; starting at Lassen Volcanic National Park and then in Crater Lake National Park. One lagoon in Redwood National and State Parks will be sampled at the conclusion of the season.

Parameters were selected for:

- It directly or indirectly addresses protocol objectives.
- It is mandated by National Park Service Water Resources Division.
- It can be used to develop or derive an index or indices that address protocol objectives.
- It places other parameters in a context to better address protocol objectives.
- It assists in making correlative statements between response variables and stressors.
- It is a cost-effective alternative to other parameters.

Parameters selected were: temperature, pH, conductance, dissolved oxygen, water level, acid neutralizing capacity, anions/cations, dissolved organic carbon, nutrients, oxidation/reduction potential, turbidity, lake substrate, lake area, shoreline length, water clarity, chlorophyll, and aquatic communities (vertebrates, benthic and planktonic invertebrates). The protocol will also make use of a number of derived indices: Observed/Expected ratios of biodiversity, fish condition index, Hilsenhoff biotic index, etc.

Power analyses on multivariate community data, the sort collected in this protocol are problematic, but high power was found in univariate measures based on community data (e.g., species richness). Power analyses were moderate to low for chemical parameters.

Twenty standard operating procedures are provided that detail all aspects of implementing the protocol, from the initial hiring and preparation, to the reporting of data and how to revise the protocol if revisions are needed.

The standard operating procedure includes a detailed Quality Assurance Project Plan (QAPP) that have been written to meet the requirements of the National Park Service, Water Resources Division, the State of California Surface Water Ambient Monitoring Program Quality Assurance Program, and the Klamath Network Data Management Plan. The QAPP addresses the needs of measurement quality objectives, sample contamination, field measurements, sample handling, instrumentation testing and calibration, and audits. The QAPP also include information on the preferred method to document cumulative bias, which arises when personnel, equipment, or contract laboratories are changed. The cumulative bias procedures will allow the crosswalk of data before and after changes are made to maintain the integrity of the data.

Data management of the data collected from this protocol is also detailed within the standard operating procedures, including database use, data entry, metadata guidelines, and archiving the data.

Reporting will consist of two primary report formats: Annual reports, produced every three years, and covering the basic summaries of the physical, chemical, and biological data collected for that year; and Analysis and Synthesis reports that will provide in-depth analyses covering classification and characterization of the lakes, as well as trend analyses.

Sixteen appendixes are provided, covering important topics such as field safety (safety handbook and Job Hazard Analyses), field forms, equipment manuals, identification guides, and tolerance values used in analyzing the data.

Taken together, this protocol sets the standards for water quality monitoring of mountain lakes and ponds for future monitoring in the Klamath Inventory and Monitoring Network.

Revision History Log

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version #

1.0 Background and Objectives

1.1 Rationale for Integrated Monitoring of Ponds and Lakes

The National Park Service (NPS) recognizes that “aquatic resources are some of the most critical and biologically productive resources in the national park system” and that they “are vulnerable to degradation from activities both within and external to parks” (NPS 2000). The ponds and lakes (hereafter, “lakes” is used to refer to both lakes and ponds) present in Crater Lake National Park (CRLA) and Lassen Volcanic National Park (LAVO) are integral components of the Klamath Network landscape. In general, these ecosystems are complex adapting systems with characteristics that are influenced by local as well as regional environmental conditions (Larson et al. 1994, 1999; Allan and Johnson 1997). Due to their aesthetic value, lakes act as factors that influence or even drive local and landscape-level ecosystem modification by attracting and increasing human activity around and near them (Walsh et al. 2003). Therefore, lakes can be useful indicators of impacts or changes due to various types of environmental perturbation across the landscape, including near-field impacts such as visitor use and far-field impacts such as atmospheric deposition of pollutants and nutrients originating from agricultural activities and climate change (Stow et al. 1998, Vinebrooke and Leavitt 2005).

The Klamath Network vital sign selection process resulted in the identification of two aquatic resource vital signs for monitoring: Aquatic Communities and Water Quality (Sarr et al. 2007). Prioritization of these vital signs was driven by potential natural and anthropogenic stressors on water resources (including physical, chemical, and biological characteristics) of freshwater habitats. Identified stressors of aquatic resources included (1) climate change, (2) atmospheric deposition of pollutants and nutrients, (3) introduced and invasive species, (4) recreational visitor use, and (5) land use, including park maintenance activities. Because aquatic communities and water quality are intrinsically related (see [section 1.4](#)), these vital signs have been integrated into a single protocol. In the words of Dr. Robert Wetzel, late Professor of Aquatic Ecology, University of North Carolina, Chapel Hill, “Water quality is biological” (Wetzel 2001).

The use of water quality defined by human needs works well for municipalities concerned about drinking water supplies. However, in this protocol we strive to use “water quality” in terms of the “natural conditions,” and not just human needs. This is aligned with the broad purpose of the National Park Service in maintaining natural conditions “unimpaired for future generations.”

Initial selection of aquatic communities and water quality did not discern between lentic (standing water – e.g., lakes) versus lotic (running water – e.g., streams) habitats. Fundamental differences in the structure of these two general ecosystem types dictated that a basic division be made in how these habitats are sampled. Protocols on sampling lotic habitats are covered in a separate publication (Dinger et al. 2011, in revision).

Lakes are integral ecosystems in the overall landscape of many parks. Not only are lakes attractive park visitor destinations, but they also serve as watering holes for park wildlife. Lake ecosystems have been studied over the history of science, both as isolated “mesocosms” (i.e., stand alone self-contained systems) and as integral parts of the landscape, and have provided a rich context for understanding the linkages between stressors and their effects. Consequently, aquatic habitats are known to respond to physical and biological stressors in predictable ways.

For instance, climate change and altered precipitation cycles can alter the amount of available lake habitat and productivity (Gleick 1998). Deposited atmospheric nutrients and resulting eutrophication can cause increased algal blooms, leading to anoxic conditions, fish die-offs, and other water quality problems (Jassby et al. 1994, Wetzel 2001, Jassby et al. 2003, Sickman et al. 2003). Introduced non-native fish and bullfrogs can radically alter the native food webs, eliminating native, resident species (Adams 1999, Vander Zanden et al. 1999). The strength and predictability of relationships between lake ecosystems and the surrounding environment makes them excellent bellwethers for tracking natural and human-caused environmental changes.

The overall aquatic community of a lake serves as an integrated biomonitoring tool, encompassing short-term and long-term responses to impacts (Rosenberg and Resh 1993). Invertebrates (benthic and planktonic) can rapidly respond to impacts, while fish and amphibians (with longer life cycles) will demonstrate time-integrated responses. Trends in aquatic communities reflect changes in water and habitat quality, so that monitoring aquatic communities and associated habitat characteristics will detect changes in ecological integrity over time. We have chosen to monitor multiple indicators of the lake ecosystem, encompassing key attributes of habitat, chemistry, and biology. The use of multiple indicators for measuring potential ecosystem change will provide us with an integrated and robust system for detecting trends and impacts over time.

Attributes of the lake ecosystems serve as indicators to park landscape impacts as a whole; for example, changes to the hydrologic cycle which will manifest in snowpacks and soil moisture content also will affect the water quality of the lakes. Effects of atmospheric deposition of pollution and nutrients which can infiltrate the terrestrial vegetation, lichen, and soil may be apparent in effects to the trophic status of lakes. In sum, monitoring of lakes serves as a comprehensive approach to address current and future issues for management of park resources.

1.2 Link to National and Regional Strategies

Lake monitoring is being conducted by several other western U.S. National Park Service Inventory and Monitoring networks. These networks include the North Coast and Cascades Network to the north as well as the Sierra Nevada Network to the south. Combined, these Inventory and Monitoring networks will be monitoring mountain lakes from the Canadian border to central California. In developing this protocol, draft protocols from both the Sierra Nevada and North Coast and Cascades Networks have been consulted to ensure that methods and results are as comparable as possible. However, differences in sampling scheme and protocol purpose necessitate differing protocols: the North Coast and Cascades Network has a sampling scheme directed towards a lower number of replicates per park (six) but repeated each year; the Sierra Nevada Network has chosen a sampling scheme emphasizing the measurement of inter-annual trends at two sites and a split panel design to sample a total of 76 lakes every 3 years; the Klamath Network (this protocol) samples a total of 31 lakes over 30 years. The goals of the North Coast and Cascades Network protocol and ours are very similar: to conduct a holistic ecosystem sampling approach that includes water chemistry, fish, aquatic macroinvertebrates, zooplankton, amphibians, and physical habitat. The Sierra Nevada Network is focusing on water chemistry, amphibians, and lake outflow. However, where there is overlap, similar protocols have been utilized throughout. Although an exact overlap is impractical due to differing network logistics and budgets, similar measures are being conducted at the three networks. Trends in the

Sierra Nevada Network and North Coast and Cascades Network will be comparable to observed trends in the Klamath Network, thereby facilitating the development of a regional understanding of condition and trends in high elevation lake and pond ecosystems.

We also have incorporated aspects of several national lake monitoring strategies. The United States Environmental Protection Agency (USEPA) has published three separate manuals on Standard Operating Procedures for lake sampling, all from different programs: (1) Environmental Monitoring and Assessment Program (EMAP) Surface Waters, Field Operations Manual for Lakes (Baker et al. 1997); (2) Lake and Reservoir Bioassessment and Biocriteria (USEPA 1998); and (3) Survey of the Nation's Lakes – Field Operations Manual (USEPA 2007). An additional program with the United States Geological Survey (USGS) was investigated (the National Water Quality Assessment Program [USGS 2002]) and although their methods are directed towards wadeable and non-wadeable streams, commonalities were examined and reviewed for inclusion in our protocols (e.g., Quality Assurance Project Plan aspects and water quality sampling procedures). With state agencies, there is no known program within Oregon for lake monitoring and the California Surface Water Ambient Monitoring Program has identified lakes and reservoirs as a high priority for monitoring but allocations of efforts to date have focused on the bioaccumulation of pollutants in the higher trophic levels (e.g., fish).

Methodology has been utilized from all sources; however, many of the above protocols are designed for a differing sampling frame and may not be entirely applicable. For example, the Survey of the Nation's Lakes recent protocol and report (USEPA 2007, 2009) was based on only sampling lakes greater than 4 hectares in area, whereas our protocol samples both large lakes and the smaller ponds of the Klamath Network.

Further aspects of our protocols have been developed using established techniques in the primary scientific literature. Specifically, we have studied the methodology of principal investigators who have utilized Observed/Expected ratios of biological diversity to develop a quantitative index of lake health (Johnson 2003, Knapp et al. 2005, USEPA 2007). By incorporating these methods, we are engaging in both regional (Knapp et al. 2005), national (USEPA 2007), and international (Johnson 2003) efforts to develop Observed/Expected ratios for lentic communities (see section 2.5.5 for more on derived indices).

1.3 Monitoring History

Lakes in LAVO and CRLA have been inventoried in the past, but overall sampling has not been coordinated or systematic. The exception is long-term monitoring of Crater Lake proper, which has been ongoing since 1983 (Larson et al. 2007). Because the Crater Lake Monitoring Program is base-funded and managed by the park and the lake arguably unique, it is not included in the sampling frame of this protocol, although there is some overlap (e.g., vertical profiles, Chlorophyll *a*, Secchi Disk readings, etc.). Other monitoring of CRLA lentic habitats has been sparse, with a single known survey of the Whitehorse Ponds area that collected limited chemical and biological information (Salinas et al. 1994).

A substantial amount of inventory data has been accumulated for LAVO. The Water Resources Division of the National Park Service has completed the Baseline Water Quality Data Inventory

and Analysis report for LAVO (NPS-WRD 1999). The report details results from a total of 218 water quality stations (including streams, lakes, and hot springs) from the park. However, only 25 lakes were found to have existing data. Only three of 218 stations have long-term data, and only one (a stream) has data going back to pre-1985. Additional surveys of LAVO include the Level 1 Baseline Water Quality Report (Currens et al. 2006), which sampled dissolved oxygen, pH, and conductance for 23 lakes. Biological surveys for fish, amphibians, and invertebrates were done by Parker (2008) and Stead et al. (2005). In addition, there has been considerable interest and past research focused on the unusual fumaroles and hot springs of the park (Ingebritsen and Sorey 1985, Siering et al. 2006). In sum, the existing data and knowledge of LAVO waters are either haphazard or sporadic (e.g., NPS-WRD 1999), specialized habitats (e.g., Ingebritsen and Sorey 1985), or recent inventories (e.g., Stead et al. 2005, Parker 2008).

Existing water quality data will be integrated into Analysis and Synthesis reports, as appropriate, but due to the lack of any prior, systematic, established monitoring program, the prior sporadic water quality inventory work was not a large influence in development of this protocol.

1.4 Integrated Conceptual Model of Aquatic Communities and Water Quality

The Klamath Network presented graphical conceptual models supporting its overall monitoring design in their vital signs monitoring plan (Sarr et al. 2007). These models support the conceptual approach of integrating the water quality characteristics (i.e., physical, chemical, and biological) and aquatic communities into a single protocol (Figure 1), as well as the focus on integrating ecosystem composition, structure, and function (Figure 2). In this protocol, we integrate aspects of structural, compositional, and functional measures of the ecosystem. For example, we will monitor the ecosystem structure of lakes (e.g., shoreline habitat and lake morphometry) and aquatic communities (fish, amphibians, zooplankton, and macroinvertebrates). Combined with multiple water chemistry parameters (e.g., pH, alkalinity, and nutrients), we will be able to address functional aspects of the trophic structure of these ecosystems.

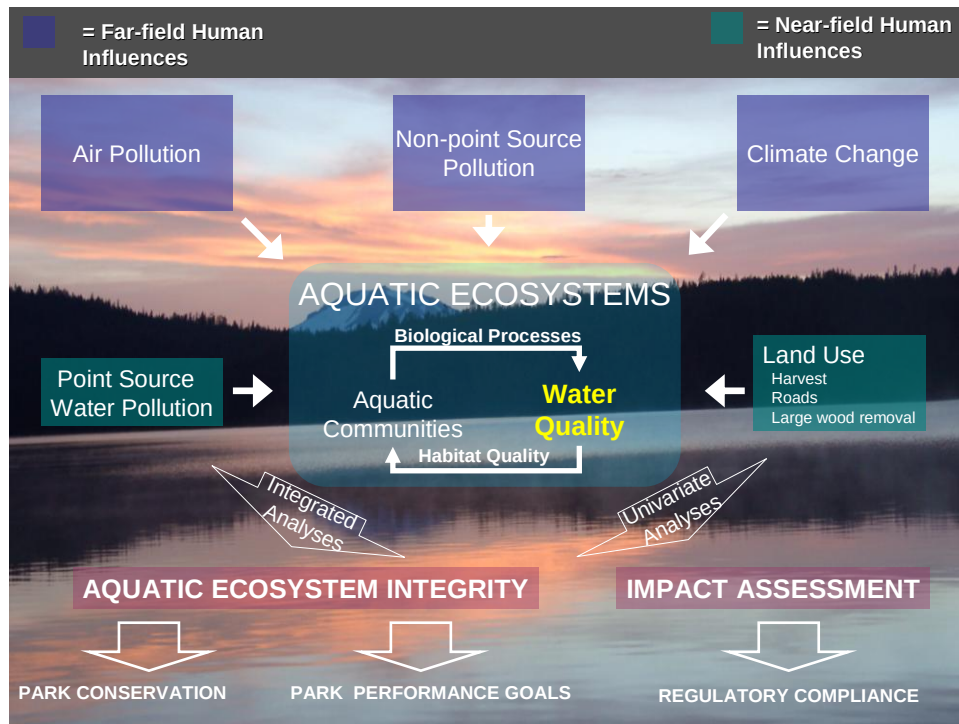


Figure 1. Conceptual ecological model showing the integral relationships between water quality and aquatic communities in aquatic ecosystems.

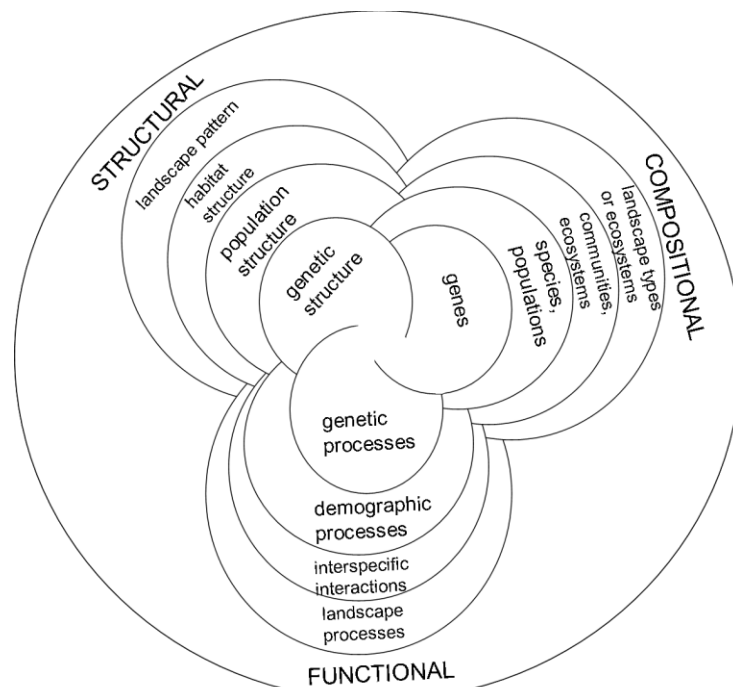


Figure 2. Conceptual model of the multiscale hierarchy of biodiversity indicators that describe composition, structure, and function at each level of organization (from Noss 1990).

By sampling multiple components of our ecosystem models, we utilize a diversified, multispecies approach that is the most comprehensive and robust way to ensure that important trends are detected, as argued by Manley et al. (2004). Monitoring multiple species and attributes together will track changes in ecosystem composition, function, and structure better than single species approaches or schemes focusing on a single aspect (e.g., water chemistry).

1.5 Existing and Potential Ecosystem Stressors

As mentioned above, five basic stressors, identified as part of the vital signs scoping process, that could impact the water quality and aquatic communities of Klamath Network lakes include: (1) climate change; (2) atmospheric deposition of pollutants and nutrients; (3) presence or introduction of non-native and invasive biota; (4) non-recreational land use practices within and external to parks; and (5) visitor recreational activities within parks. These stressors were considered in the selection of parameters to monitor.

1.5.1 Climate Change

Concerns about the potential impacts of climate change are well documented (IPCC 2007). Researchers have documented that various physical, chemical, and biological characteristics of lakes (e.g., water-level, temperature, nutrient concentration, pH, dissolved oxygen, productivity, and composition of the macroinvertebrate community) can be good indicators of impacts due to climate change (McKnight et al. 1996, Arnott et al. 2003, and O'Reilly et al. 2003). Even modest temperature increases in the western United States may cause significant changes to the hydrologic cycle, as manifested in earlier snowmelt, earlier ice-out on lakes, reduced summer base flows (Dettinger et al. 2004), a lower snowpack volume at lower to mid elevations (Knowles and Cayan 2001), and increased flooding due to rain-on-snow events (Heard et al. 2009).

1.5.2 Atmospheric Deposition

Atmospheric contaminants have been recognized as a potential stressor of ecosystems, both aquatic and terrestrial, for several decades (Schindler 1987, Landers et al. 2008). A classic example is acid rain, where SO_x and NO_x precursors from combustion are transported thousands of kilometers from their source and deposited by precipitation, causing sensitive ecosystems to acidify (Likens et al. 1979). Similar concerns with nutrients (e.g., from agricultural fertilizers) and pollutants (e.g., volatile organic chemicals, toxicants, etc.) can also perturb ecosystems by eutrophication processes or toxicity effects (Landers et al. 2008).

1.5.3 Non-native and Introduced Species

Introduced, non-native, and exotic species can cause large changes to native biodiversity and the trophic dynamics of lakes (Vander Zanden et al. 1999, Knapp et al. 2001, Parker et al. 2001, Schindler and Parker 2002, Boersma et al. 2006). In parks of the Klamath Network, the introduction of kokanee (land-locked sockeye salmon [*Onchorhynchus nerka*] and Rainbow trout [*Onchorhynchus mykiss*]) in CRLA, and Brook trout (*Salvelinus fontinalis*) in LAVO are potential ecosystem stressors. Emerging, potential invasives also include both vertebrate (e.g., American bullfrogs [*Rana catesbeiana*]) and invertebrate (e.g., New Zealand mudsnails [*Potamopyrgus antipodarum*]) taxa. Considerable threats also exist from emerging diseases, such as the chytrid fungus (*Batrachochytrium dendrobatidis*) that affects native amphibians and whirling disease (*Myxobolus cerebralis*) that impacts native salmonids.

1.5.4 Non-recreational Land Use Practices within and External to Parks

Land use practices that include potential stressors relevant to Klamath Network parks include: park operations (e.g., construction and road maintenance), past mining operations, dam operations, sewage and wastewater, fire management, timber harvest, geothermal explorations, and trespassing livestock grazing (Hoffman and Sarr 2007). Additional concerns stem from development and the cultivation of illegal crops (especially marijuana) within park boundaries. The potential pathways whereby these stressors manifest themselves in aquatic ecosystems is beyond the scope of this narrative, but increased sediments, pollutants, and hydrologic changes from both direct and indirect impacts are possible.

1.5.5 Visitor Recreational Activities

Potentially damaging recreational uses include camping, packstock use, boating, and fishing. Recreational impacts may include mechanisms from the above stressor categories. For example, camping can cause the input of nutrients from improper disposal of camper waste, or anglers and the use of boats can contribute to the introduction and dispersal of non-native species.

1.6 Vital Signs Objectives

The programmatic goals of the Klamath Network are (from Sarr et al. 2007):

- To determine status and trends in selected indicators of the condition of park ecosystems to allow managers to make better informed decisions
- To provide early warning of abnormal conditions and impairment of selected resources to help develop effective mitigation measures and reduce costs of management
- To provide data to foster better understanding of the dynamic nature and condition of park ecosystems and to provide reference points for comparisons with other altered environments
- To provide data to meet legal and Congressional mandates related to natural resource protection and visitor enjoyment
- To provide means of measuring progress towards performance goals
- To support park interpretation and educational programs

Applications of these programmatic goals, and the specific wadeable streams objectives to meet these goals, were largely determined at scoping meetings with Network ecologists, USGS specialists, and park resource experts. Refinement based on feasibility, logistics, and budgetary realities determined during the pilot project (Appendix A) were also taken into consideration.

1.6.1 Monitoring Objectives

Objective 1: Determine the status and trends of ecological conditions in Klamath Network mountain ponds and lakes. Through careful selection of indicators, we can inform managers to help with decision making, warn of abnormal conditions, and gain understanding of the park ecosystems. Through quality control, data analysis, and multiple reporting formats, we can meet legal requirements, measure performance goal progress, and help education programs. Together, this will meet the Network's programmatic goals.

To meet this goal, several terms must be clearly defined:

- “Ecological condition” – From the EPA Report on the Environment, “ecological condition” is defined as “the state of the physical, chemical and biological characteristics of the environment, and the processes and interactions that connect them (USEPA 2008).”
- “Status” – “defined as some statistic (e.g., a mean, median, proportion, etc.) of a parameter over all monitoring sites within a single or well-bounded window of time. Status will always have some measure of statistical precision (e.g., a confidence interval, standard error, variance)...(Sarr et al. 2007).”
- “Trend” – “defined as a non-cyclic, directional change in a response measure that can be with or without pattern (Urquhart et al. 1998).”

The very definition of ecological condition speaks to the need for integrating indicators from a range of physical, chemical, and biological characteristics.

Objective 2: Assist parks with “impaired quality waters,” also known as “303d” lists as defined by the Clean Water Act. The method of assisting should be in two functions:

- a. Gather information on the pollutants that exceed standards that will assist the park and the state to design specific pollution prevention or remediation programs through Total Maximum Daily Loads.
- b. Determine whether the overall program goal of improved water quality is being achieved after the implementation of effective pollution control actions.

Currently, there are no natural lakes or ponds in the Klamath Network that are 303d listed as impaired quality waters. However, monitoring under this protocol should serve as an early detection system for emerging degrading conditions that could eventually necessitate listing.

Objective 3: Assist parks with monitoring of “Outstanding National Resource Waters” or Tier 3 waters as defined by the Clean Water Act. The method of assisting should be in two functions:

- a. Allow characterization of existing water quality and to identify changes or trends in water quality over time.
- b. Identification of specific existing or emerging water quality problems.

Currently, there are no Outstanding National Resource Waters in any of the parks of the Klamath Network. This specific objective and functions dictated by the Water Resources Division are met by monitoring the lakes and streams of the Network parks.

1.6.2 Measurable Objectives

Measurable objectives to meet the objectives of this protocol (see relevant SOPs for details) include:

- Use probabilistic sampling to establish accessible lake sites within LAVO. Establish sampling sites at every perennial lake outside the Crater Lake caldera in CRLA.
- Measure physical environment parameters at each lake site: substrate composition and percentage, depth, area, shoreline complexity, qualitative water level, etc.
- Collect core water quality parameters in a vertical profile at the deepest portion of each lake: dissolved oxygen, temperature, specific conductivity, turbidity, and pH.
- Measure stream water anions, cations, dissolved organic carbon, and nutrients in the hypolimnion (0.5 meters above the lake bottom) and epilimnion zones (0.5 meters deep) of deeper lakes, and at a single midpoint of shallower lakes (<1.0 meters deep).
- Collect measures of algal biomass measured at Chlorophyll *a* concentrations in the hypolimnion and epilimnion of deep lakes and midpoint of shallow lakes.
- Collect semi-quantitative samples of littoral zone benthic macroinvertebrates.
- Conduct Visual Encounter Surveys for amphibians to develop species lists.
- Survey for fish populations using gill nets deployed to determine presence and catch per unit effort in deeper lakes (>1.0 meters deep)
- Photograph lakes so images can provide visual comparisons over time.
- Develop and maintain a database and associated metadata derived from the sampling procedures.
- In an Annual Report, report status of key parameters for each park surveyed that year.
- Write an in-depth Analysis and Synthesis reports every sampling year that explore relevant topics in depth. Specifically, individual Analysis and Synthesis reports will detail trends in core parameters and species composition and abundances, explore data patterns to relate stressors to observed trends, utilize observed/expected models for species assemblages, and utilize indices of biotic and ecological integrity.

2.0 Sampling Design

The two parks that are the focus of this protocol, LAVO and CRLA, are the only parks in the Network with mountain lakes. LAVO has at least 239 known lakes, whereas CRLA only has 38 potential lakes, with many known to be ephemerally-flooded wetlands (an estimated 83% are not perennial, see below). We will implement an *always revisit* survey of 30 spatially balanced but random sites for LAVO (along with a single judgment site), but will be conducting a complete census of the approximate six to ten perennial lakes in CRLA. A single judgment site at Redwood National and State Parks (REDW) is also done (Table 1).

2.1 Rationale for Selection of Sampling Design

The Klamath Network has chosen an always revisit sampling design to monitor water quality and aquatic communities in LAVO. This design will maximize our statistical power to detect trends and allow us to track both park-based measures (e.g., the average number of zooplankton species) and lake-specific measures (e.g., the number of zooplankton species at Reflection Lake). The strengths of this design are: 1) the conceptual simplicity, 2) ease of data analysis, 3) logistical ease of site establishment, and 4) the aforementioned trend detection. The weaknesses are: 1) the number of unique sites is fixed by the initial sample size, and 2) the possibility that the sample selection does not represent the population of lakes (or that the unsampled portion of the lake population may change whereas the sampled does not [and vice-versa]). These two weaknesses are mitigated by a reasonable sample size ($n = 30$) and using a randomized but spatially balanced site selection procedure.

Alternative sampling designs (e.g., rotating split panels of revisit and non-revisit sites) were considered by the Network, but were deemed to be overly complex; although ideal for status assessment on a year to year basis, statistical methodology for split panel trends is still developing. A secondary design, where all sites are revisited but with different frequencies, was also considered but rejected for similar reasons.

2.1.1 Judgment Sites

Two sites to be sampled in this protocol are “judgment” sites. These are sites that are subjectively selected because either: (1) they have a history of sampling, (2) they are accessible, or (3) the target population is very specialized or unique. Another justification is that certain sites may be facing specific threats and monitoring for these threats is best concentrated at such sites.

Table 1. Sample size for park units to be monitored under this protocol. Note that one sample site in Lassen Volcanic National Park includes a single judgment site.

Park Unit	Sample size
Lassen Volcanic National Park	31
Crater Lake National Park	6-10
Redwood National and State Parks	1
All units	38-42

The continuation of existing monitoring or focused monitoring for special populations or threats is valuable in its own right, but because such sites are usually not probabilistic, they can only be used to make inferences to the specific sites in question. Judgment sites were minimized so that most sampling efforts could be focused on the probabilistic sample. Two sites were selected with input from individual park specialists at protocol scoping meetings:

- Lake Helen Judgment Site (LAVO) - this site was selected as a monitoring priority from park resource personnel because it: (1) is a high use site; (2) receives year-round use; (3) has very little surrounding vegetation and a major park roadway next to it; (4) has high levels of illegal boating activity, increasing risk of invasive species; and (5) has a low buffering capacity against acid deposition. In relation to other lakes of LAVO, this buffering capacity is especially low (average August 2005 alkalinity measurement = 3.6 mg/l, park average = 21.3 mg/l measured as mg/l CaCO₃ (Currens et al. 2006).
- Freshwater Lagoon Judgment Site (REDW) - this site was selected owing to several unique features: (1) it is no longer a proper functioning lagoon, having been cut off from the Pacific Ocean by US Highway 101; (2) it saddles the park boundary, with half on state lands, and half on NPS land; (3) the state portion has been extensively and currently stocked with exotic fish (e.g., largemouth bass, *Micropterus salmoides*; Japanese pond smelt, *Hypomesus olidus*; and brown bullhead, *Ictalurus nebulosus*) as well as being invaded by exotic vegetation (Brazilian waterweed, *Egeria densa*) and exotic invertebrates (apple snail, probably *Pomacea* sp.). It is also a likely place for emerging new aquatic invasive organisms and hence early detection here may warn park staff of new threats to the rest of the park.

2.2 Target Sample Site Population

Mountain lakes in the Klamath Network occur exclusively in LAVO and CRLA. Additional lentic habitats within the Klamath Network include several coastal freshwater lagoons and artificial ponds in REDW and a large reservoir (Whiskeytown Lake) located in Whiskeytown National Recreation Area.

Outside of the judgment sites, the target sample site population for this protocol is all mountain lakes that are:

- Perennial – This selection criterion is applied to remove habitats that are influenced by seasonal desiccation which could mask other stressors of interest. It also ensures that data collection can always occur at the sites, assisting in data completion goals (SOP #16: Quality Assurance Project Plan).
- <25 m maximum depth – This selection criterion removes large lakes that are characterized by different physical and environmental processes. While monitoring these lakes is valuable, they would require separate methodology to adequately assess and they are a numerical minority of sites within the Klamath Network.
- <1000 m from a travelable road or trail – This selection criterion reduces logistical constraints to field crews, such as travel time, to ensure that each site can be sampled in the allotted time frame for achieving sampling objectives.

- In topographies with slope <30 degrees – This selection criterion ensures crew safety and that access to lakes is feasible.

Note that this “population” is different from a statistical population (SOP #19: Data Analysis and Reporting contains details on the statistical population). This target sample site population defines the lakes that comprise the list for choosing sampling sites. Like a statistical population, inferences from the monitoring of these lakes should only be made to lakes that fit these criteria.

2.3 Sample Site Selection

2.3.1 Lassen Volcanic National Park

Sites in LAVO are chosen using a Generalized Random Tessellation Stratified design (GRTS – pronounced “grits”) (Stevens and Olsen 1999, 2004). This design employs a systematic sampling technique to obtain a spatially balanced probabilistic sample. A particularly attractive feature of GRTS is the ability to accommodate unequal probability sampling by allowing the probability of individual sampling units to vary. In the case of the lake and pond selection, sites that do not fit the criteria in section 2.2 are assigned a probability of zero. This procedure also produces a spatially balanced over-sample (i.e., a list of additional sites to sample if sample points need to be replaced or added). Since the GRTS method creates spatially balanced and dispersed sample sites, it minimizes spatial autocorrelation among sites and maximizes the effective sample size for a given number of sample sites, which should help increase statistical power.

We have focused on a spatially balanced design because a simple random sample, although the most conceptually easy and statistically simple design to implement, can produce a cluster distribution of sites. The GRTS output is a list of sites, that when visited in order, produces a spatially balanced selection across the landscape.

The National Hydrography Dataset (NHD), containing geospatial hydrologic data that enumerate all lentic habitats within the park, was used to populate the Geographical Information System (GIS) database for running the GRTS draw with a custom script in the statistical software program *R*. Prior to running the draw, potential site locations not fitting the criteria described in section 2.2 were removed, as were prior selected judgment sites. The *R* script “spsurvey” was used to draw the site list from remaining locations in each park (Kincaid 2006). Table 2 provides a summary of the numbers and proportions of lakes available for inclusion, excluded by the criteria, and total number sampled through the program.

Step by step site selection procedures using GRTS are further outlined in SOP #3: Site Selection, as are the results of the GRTS draw. However, the process of using the computer program *R* is beyond the scope of this protocol. Note, however, that SOP #3: Site Selection should only have to be used at the initiation of the protocol prior to the first field season and will not need to be done prior to every field season. It is provided to give the field crews and incoming Project Leads the proper context for the survey design and rationale. It is also possible that a new GRTS draw may be necessary if the sampling population changes over time (as in Irwin 2008).

Table 2. Lakes (“Sites”) within Lassen Volcanic National Park showing results of criteria filters and total lakes sampled through protocol implementation. NHD = National Hydrography Dataset.

	# of sites	% of total sites
Sites in NHD GIS file	239	100%
Sites excluded by selection criteria	36	15%
Sites available for GRTS draw	203	85%
Sites not sampled in protocol:	208	87%

Example photos of lakes included in the sampling frame are presented in Figure 3. These sites encompass a wide variety of environments, from meadow ponds to high elevation alpine lakes. The figure is presented to give the reader an overview of the lakes/ponds and is not intended to be an exhaustive presentation of sites.

2.3.2 Crater Lake National Park

Initial scoping of available sites in CRLA included examination of the National Wetlands Inventory dataset for this park unit, which detailed 139 sites, including both emergent palustrine (marsh and swamp wetlands) and lacustrine (lake/pond) sites. However, the dataset only identified sites within the rim of Crater Lake caldera as being lacustrine (i.e., are a part of Crater Lake proper). A separate dataset provided by park specialists only identified 38 potential lakes, not including wetlands.

A preliminary site visit to 23 of these potential sites on 28-30 October 2008 revealed that only four of these sites were potentially perennial (see Figure 4 for examples of habitats encountered). Assuming the ratio of 17% (4 of the 23 visited) being perennial, the total number of perennial sites will be six or seven (17% of 38 potential sites). For planning and budgetary purposes, we have assumed that a minimum of six to a maximum of ten sites will be perennial.

Sites determined to be ephemeral in the preliminary site visit will be excluded from the list of sites to be visited. Sampling will start with the four sites known to have water during the 2008 site visits, and then sampling will commence at the rest of the unknown sites (i.e., those not visited during the site visits of October 2008). As these sites are shown to be ephemeral, they will be removed from the sampling list. If future follow-up sites are shown to be ephemeral, they will be removed from the sampling list, even if prior year(s) sampling had occurred.



Figure 3. Lake and pond habitats of Lassen Volcanic National Park. Clockwise, from upper left: Cluster Pond #4 (10654), Cluster Lake #4 (10443), Lake Helen (11076), Unnamed Lake (11068), Cliff Lake (10980), and Cluster Lake #3 (10452). Numbers in parentheses are unique site ID numbers (SOP #1: Preparations, Equipment, and Safety). Photos by Aaron Maxwell.



Figure 4. Examples of Crater Lake National Park lakes, including probable ephemeral sites. All sites are unnamed locations. Only the lower left is likely a permanent water body. Photos by Aaron Maxwell.

2.4 Frequency and Timing of Sampling

2.4.1 Sample Frequency

Sampling of lakes will occur every 3 years as a part of the overall design of integrated aquatic communities and water quality (Table 3). In between these sampling periods, wadeable streams (covered in a separate protocol) will be implemented.

Table 3. Rotational pattern of sampling frequency of integrated water quality and aquatic communities for both lakes and wadeable streams. After 2015, the pattern continues.

Habitat type	Park units	2011	2012	2013	2014	2015
Lakes	Lassen Volcanic National Park			X		
	Crater Lake National Park			X		
	Redwood National and State Parks			X		
Wadeable Streams	Whiskeytown National Recreation Area	X			X	
	Lassen Volcanic National Park	X			X	
	Oregon Caves National Monument	X			X	
Wadeable Streams	Redwood National and State Parks		X			X
	Crater Lake National Park		X			X
	Oregon Caves National Monument		X			X

2.4.2 Sample Timing

Timing of sampling efforts at individual sites across years will be kept as constant as logistically possible. This will help to reduce inter-annual variation that may be due to phenological characteristics of the lakes being sampled. For example, if Lake Helen is sampled on 15 July 2010, we will attempt to repeat the visit between 14-16 July 2013. Sampling Lake Helen later in the field season, such as in late August or early September, will introduce variation from such things as insect emergence, zooplankton cycles, lake turnover, etc. After the first field season, dates for sites visits will be used for planning the next sampling period in perpetuity.

Another aspect of sample timing that can affect results is diurnal shifts in parameters. For instance, primary production may peak during times of higher solar radiation (middle of the day), so that dissolved oxygen correspondingly increases as these times. Measurements of dissolved oxygen taken at mid-day may thus differ than values taken at dawn. Another variable aspect can be the behavioral differences in amphibians from mid-day to dusk activities that can affect detectability during surveys. To reduce variability in these parameters, field crews will perform all sampling during standard daylight hours; generally between 10 a.m. and 3 p.m. Obviously, logistics of site access will dictate actual start times, but crews cannot start sampling pre-dawn. Additionally, time of sampling will be recorded for measured parameters to help in the interpretation of variation relative to time of sampling.

Seasonal sampling will commence in LAVO, with the anticipation that snowmelt will occur at this park before snowmelt occurs in CRLA. Because there is the possibility that the first sampling period may be an unusually early snowmelt, several weeks will be added to the actual start period. The logic for this is that the initiation of the protocol based on an unusual dry year

may result in an inability to access lakes at the scheduled times in years characterized by a heavier snowpack. After completion of sampling sites in LAVO, crews will initiate sampling of sites in CRLA. The Freshwater Lagoon in REDW will be the final site sampled of the year. The rationale for finishing in the Freshwater Lagoon is that the numerous exotic organisms of this habitat would pose a threat to the other parks of the Network, and by finishing there, the risk of transmission of these non-native species will be minimized. Also, the biological activity at this coastal site is unlikely to decrease materially by September, due to its much milder climate. The general schedule of field work is shown in Figure 5.

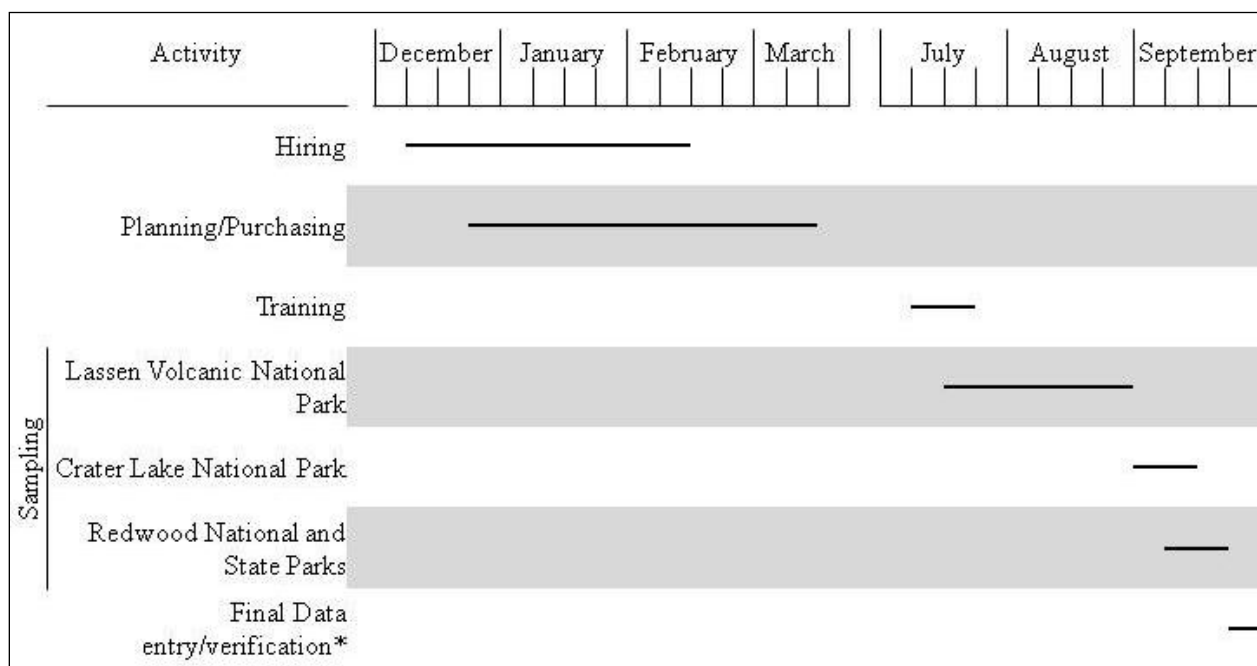


Figure 5. Timeline for protocol hiring, planning, training, and sampling. *Final data entry is for any remaining sites to be entered into the database, and final verification of data by the Project Lead. Actual data entry during the sampling season is done concurrent to sampling.

2.5 Rationale for Selection of Parameters

Each parameter to be measured was chosen for the following reasons:

- It directly or indirectly addresses protocol objectives.
- It is mandated by National Park Service Water Resources Division.
- It can be used to develop or derive an index or indices that address protocol objectives.
- It places other parameters in a context to better address protocol objectives.
- It assists in making correlative statements between response variables and stressors.
- It is a cost-effective alternative to other parameters.

In this context, parameters are defined as features allowing quantitative or semiquantitative measurements (e.g., numerical, ordinal, or categorical data) through field visits or laboratory analyses. Table 4 provides a summary overview of parameters to be monitored; the text below explores the context and rationale for each parameter in more detail.

2.5.1 Core Parameters

The core parameters represent a set of water quality attributes that will be measured as part of all NPS Water Resource Division funded water quality monitoring protocols. As such, these attributes contribute some measure of consistency and comparability of water quality conditions among multiple monitoring programs (Penoyer 2003). With the exception of water level, all core parameters will be measured in a profile at the deepest part of the lake (determined using a hand-held sonar unit). However, the use of the word “core” does not imply that these parameters are more or less important than other parameters.

Water temperature is a critical variable controlling many ecosystem processes, both physical and biological, and it can impact almost all functions within an ecosystem. For instance, temperature controls aquatic biota metabolisms and affects the breakdown of organic matter, nutrient uptake, and primary production (Allan and Castillo 2007). Temperature also affects dissolved oxygen, with denser colder water being able to sustain higher amounts of dissolved oxygen. It also affects physical processes, such as lake stratification, which has important implications for nutrient availability and the “growing zone” of a lake (Cole 1994). Lake water temperature is also a critical parameter for tracking climate change’s manifestation in these important park ecosystems.

Water pH, the measure of hydrogen ion concentration in the water column, is a critical attribute of any water body with many physical and biological effects. Low pH (<7) indicates acidic waters, and high pH (>7) indicates basic water. Most aquatic plant and animal species occur within specific habitat envelopes of pH conditions and changes in pH will likely result in changes in species relative abundance or overall species composition. In addition, the pH of the water will determine the solubility of many heavy metals, which can have negative impacts on invertebrate biodiversity (Wiederholm 1984, Allan and Castillo 2007). Acidification of water also has deleterious effects on zooplankton communities (Locke 1990).

Specific conductance, or simply *conductance*, the ability of a water body to conduct an electric current, is directly correlated with the dissolved ion concentration in a body of water. In essence, the “purer” the water, the lower the concentrations of dissolved salts and thus the lower the conductance. Changes in conductance will detect changes in major ions or nutrients, such as overall potassium, calcium, and other anions and cations.

Dissolved oxygen, a critical element for the aquatic biota of a lake, is closely linked to physical and biological processes. For instance, respiration, photosynthesis, and atmospheric exchange are the principle processes that affect or are affected by dissolved oxygen concentrations. In addition to warm water temperatures, high microbial activity, driven by organic pollution, can drive up the demand for dissolved oxygen resulting in anoxic conditions.

Water level is an additional parameter to be measured dictated by the Water Resources Division. Water level is indicative of annual changes in the water cycle, the amount of winter precipitation an area may have received, and likely to be impacted by climate change. In the Klamath Network, estimates of lake water level will be based on elevation readings of the wetted perimeter, and qualitative estimates by the field crew.

Table 4. Parameters to be measured under this protocol.

Parameter	SOP #	Where ¹	Methodology ¹
Water chemistry - field			
Dissolved oxygen	10	Deepest portion of lake	Multiprobe water quality sonde profile
Oxidation/reduction potential			
pH			
Specific conductivity			
Temperature			
Turbidity			
Acid Neutralizing Capability	9	0.5 m below surface, 0.5 m above lake bottom at deepest portion	Field titration kit
Water chemistry - Lab			
Anions (Cl, SO ₄)	8,9	0.5 m below surface, 0.5 m above lake bottom at deepest portion	Ion chromatography
Cations (Na, Ca, K, Mg)			Spectrophotometry
Dissolved organic carbon			Combustion-Infrared
Total nitrogen			Persulfate Digestion
Total phosphorous			Spectrophotometry, Persulfate, sulfuric acid digestion
Chlorophyll a ²			Fluorometer
Lake Environment			
Percent Substrate type	11	Wetted perimeter	Shoreline walk-around, entry into GPS unit, extraction of data using GIS
Area			
Maximum length			
Maximum width			
Shoreline length			
Relative water level			
Water clarity	8	Deepest portion of lake	Secchi Disk
Maximum depth			Hand-held sonar
Aquatic Community			
Zooplankton	8	Deepest portion of lake	Zooplankton tows
Benthic macroinvertebrates	11	Littoral zone	Semi-quantitative sweep net
Amphibians	11	Littoral zone/water-terrestrial interface	Visual encounter survey
Fish	7	Open pelagic zone	2 hr gill net

¹= See SOPs for more details; "where" indicates actual location *within* site that parameter will be taken. ²= actually "biological," but collected with water chemistry sample.

2.5.2 Water Chemistry Parameters

These parameters include field and laboratory measurements. In general, water chemistry parameters are indicative of the ecosystem quality that the aquatic communities live in and hence can have a profound effect on these organisms. By themselves, they can equate to the generalized notion of “water quality,” indicative of water pollution not meeting water quality standards or indicate a stressor and effect (for example, high nutrient load leading to eutrophication). Analysis of the chemical characteristics of water is fundamental to the effective monitoring of the water quality of aquatic resources. Water chemistry parameters are indicators of the productivity or “trophic” state (e.g., oligotrophic, mesotrophic, or eutrophic) of aquatic ecosystems, the natural variability of conditions within and among trophic states, the capacity of ecosystems to support biotic communities, and potential changes in ecosystem status and trends.

Acid Neutralizing Capacity (ANC), similar to Alkalinity, is the resistance of the water body to acidification. It is measured in the field using unfiltered water (note: when done on filtered water, it is termed Alkalinity; when on unfiltered water it is ANC). Here, we perform the tests on unfiltered water to obtain the actual ANC value for a site, but it is measured using an “alkalinity” test kit. As a measure of the buffering capacity of a lake, it is indicative of the lake resistance to declines in pH, either through natural processes or anthropogenic stressors.

Anions/Cations being monitored include the two predominant anions (negatively charged ions – SO_4^{2-} and Cl^-) and four cations (positively charged ions – Ca^{2+} , Na^+ , K^+ , and Mg^{2+}). These six ions, along with carbonates (estimated with the ANC measurement), make up most of the ions in lake water. These ions are important indicators of the edaphic context of the lake or pond, with different ion concentrations reflecting variation in watershed geology, vegetation, and weathering processes. However, SO_4^{2-} is also common as an indicator of pollution (e.g., from mining waste or fertilizers). It is important to note that SO_4^{2-} is common in volcanic regions such as CRLA and LAVO.

Dissolved organic carbon (DOC) is a measure of detritus in the water column. Sources of DOC can be from autochthonous (within the lake) processes through extracellular release by algae or senescing organisms or bacterial degradation or allochthonous (terrestrial) processes (e.g., leaf litter breakdown carried into the lake by wind or water). Utilization and uptake of DOC by bacteria and phytoplankton is enhanced by higher temperatures and light; hence decreasing trends in DOC may indicate climate change, although acidification is also a potential cause in decreasing DOC (Schindler et al. 1992, Wetzel 2001). Dissolved organic carbon is also important as a cofactor for mercury availability (Barkay et al. 1997).

Nutrients include the dominant forms of nitrogen and phosphorous. Both elements may be limiting nutrients to lake ecosystems, controlling ecosystem productivity, as well as being indicators of eutrophication caused by external stressors (e.g., atmospheric deposition or visitor use activities). Nitrogen will be measured as total nitrogen (TN). Ammonia was also considered, but was below quantifiable limits in most cases (Appendix A) and analytical requirements of quick processing would be logistically difficult for backcountry lakes.

Similar to nitrogen, phosphorous is an important limiting nutrient and can be the most limiting. We will be monitoring total phosphorous.

For both nitrogen and phosphorous, a derived metric, Trophic State Index (TSI), will be calculated to give estimates of the trophic status of the ecosystem (e.g., eutrophic or oligotrophic) (Carlson 1977, 1992).

Oxidation/reduction potential, or redox potential (hereafter), is a measure (or expression) of the oxidizing or reducing intensity of the water column (Wetzel 2001). This refers to the oxidized (loss of electrons) or reduced (gaining electrons) state of chemicals, especially iron and manganese. There is a tendency for reduction to be associated with anoxic conditions, so that stratified lakes will have sharp changes in redox potential. This is because oxygen is an oxidizing agent. Redox potential dictates the ionic charge state of nutrients, some being biologically available and others being unavailable for metabolic purposes. Generally, if a lake is most oxidizing (has a positive redox potential), inorganic waste products from biological processes will be oxidized, changing ammonia into nitrates. Likewise, the reducing zones of a lake are important in denitrification processes. Changes in redox potential are also associated with the trophic condition of lakes, with oligotrophic lakes having higher oxidation, and hypereutrophic lakes being reduced.

Turbidity is a measure of the clarity of the water column. Water with high turbidity (e.g., low clarity) indicates either high amounts of suspended solids (i.e., siltation) or high productivity. Oligotrophic, montane lakes should have low turbidity. Trends in turbidity will indicate shifting changes in land use activities that may impact water quality, or increasing nutrients that may increase productivity and lead to changes in lake trophic status.

2.5.3 Lake Environmental Parameters

Environmental measurements serve as co-variables to help us understand patterns in the aquatic communities and also as monitoring parameters themselves. As co-variables, environmental variables are useful in predicting presence/absence of organisms based on habitat heterogeneity or on total habitat availability and may help explain important spatial variation in other parameters of interest across the sampling frame. They are important components of aquatic resource monitoring because these characteristics help describe the context or template for ecosystem function and condition (Southwood 1977, Warren 1979, Frissell et al. 1986, Larson et al. 1994, 1999). Additionally, as monitoring parameters of their own, trends in specific parameters (e.g., increases in the percent of fine sediments) can indicate a stressor such as land use or visitor impacts. Changes in lake area can also be indicative of reduced water availability (a potential effect of climate change).

Percent substrate type is a measure of the habitat heterogeneity of a lake or pond. The substrate also denotes a “quality” of the ecosystem that influences the aquatic communities (Minshall 1984). Substrate type will be measured using a hand-held GPS unit, to produce a fine-scale map of substrate types that can also be used to determine total amount of substrates (e.g., X# of meters of cobble or silt). The crew will walk the perimeter, recording the estimated percent of each substrate type (cobble, gravel, bedrock, woody debris, etc.) of lake sections. Taken together, the substrate types will convey information about total habitat heterogeneity for all lake organisms.

Area of the site (measured around the circumference at the wetted perimeter) is a basic measure of total habitat availability. The larger the wetted area, the more species that the lake may be

capable of supporting (MacArthur and Wilson 1967), whereas a smaller wetted area may contribute to the lake being more susceptible to changes in the water cycle, i.e., becoming ephemeral with reduced snowpack and precipitation. Area will be measured using a hand-held GPS unit, to produce a fine-scale areal outline. When entered into a Geographical Information System software (i.e., ESRI Arc products), other ecosystem measures will be generated, such as the longest length and width.

Shoreline length is the circumference of the lake wetted perimeter, also to be determined in the field with a GPS unit.

Water clarity is a basic measure of water quality and trophic status of a lake or pond. Generally, the less clear the water is, the higher the productivity is. Water clarity can be extremely useful as an additional measure (along with nutrients and algal biomass) to detect eutrophication of lentic habitats. Similar to nutrients, water clarity measurements can be used to derive a Trophic State Index (Carlson 1977, 1992). Water clarity is measured using a Secchi Disk in the field. Lower Secchi depth readings will correlate with higher turbidity, and although turbidity will be a more sensitive reading, the collection of Secchi depths will allow broader comparisons to patterns nation-wide (many volunteer organizations use Secchi depths for citizen monitoring). Secchi Disk readings, however, will not work at shallow sites where the disk is visible to the bottom of the lake, so they will only be taken and recorded at lakes deep enough for the Secchi Disk to disappear.

2.5.4 Aquatic Communities

The aquatic community of a lake is made up of different taxonomic components, spanning the spectrum of functional roles: primary production, consumption, predation, and decay. Here, we take the view that any one taxonomic component (e.g., zooplankton) is not a “community,” but rather an assemblage that makes up an important part of the entire aquatic community. By sampling all aspects of the aquatic community, we will effectively be sampling the food chain (or trophic web) of the lake, allowing the determination of status and trends in the functional ecology of the lake or pond. By examining the whole aquatic ecosystem, interactions between organisms can be better understood so that predictive models of how the park ecosystems will respond to specific stressors or extirpations can be evaluated (Agrawal et al. 2007).

Aquatic communities are important components of healthy aquatic ecosystems that are determined by and sensitive to the conditions of the habitats within which they reside (Loeb and Spacie 1994). Part of their utility as monitoring indicators is that each assemblage can react to different stressors individually. For example, an increase in sediment inputs can create negative responses in fish due to clogging of gills, whereas certain insects (e.g., certain Chironomidae midges) will respond positively to an increase in fine sediments. An additional advantage is that aquatic communities can integrate responses to stressors over time, with some components responding rapidly to changes, and others responding gradually to longer-term stressors. For instance, benthic macroinvertebrates act as continuous monitors of water quality issues, so that even a point-in-time measurement can provide information about seasonal or annual trends without the need for continuous sampling (Hawkes 1979).

Integrated biological sampling provides cost-effective monitoring of aquatic resources, when compared to other types of monitoring. A review of cost/benefits comparing biological

monitoring to physical, chemistry, and toxicity monitoring showed the greatest gain and understanding from using biomonitoring alone, and that when combined with physical and chemical monitoring, provided the best overall assessment of the ecosystem (Brinkhurst 1996).

Chlorophyll a is a measure of algal productivity and can also be used in Trophic State Index model (Carlson 1977, 1992). Chlorophyll *a* concentration can be affected by an increase or decrease of nutrient input into a system, with increases in Chlorophyll *a* being a consistent response to increased nutrients. In terms of examining the trophic web of the lake, Chlorophyll *a* is a useful proxy for lake primary production. Direct measurements of the pelagic algal community, the phytoplankton, are not measured due to budgetary constraints.

Zooplankton are important members of lake communities. As an individual monitoring component, techniques for analysis and interpretation are still being developed, such as ecological measures of integrity and classification based on zooplankton assemblage (Boix et al. 2005). Zooplankton indices have also been successful in detecting trends and ecological condition of wetlands (Lougheed and Chow-Fraser 2002). By combining zooplankton assemblage data with the other components of the aquatic communities, a robust image of the ecological integrity can be developed (Knapp et al. 2005).

Benthic macroinvertebrates have a rich scientific history as biomonitoring tools (Rosenberg and Resh 1993). Change in this diverse group of aquatic organisms has been demonstrated successfully as indication of ecological impairment (e.g., Lenat et al. 1981, Rosenberg et al. 1986). Benthic macroinvertebrates have formed the basis of predictive models of impairment, using O/E (observed to expected ratios) to compare existing with expected conditions. Such predictive models include the River Invertebrate Prediction and Classification System (RIVPACS, Wright et al. 1989) models, but also integrated multi-metric IBIs and IELs (indices of biological and ecological integrity, respectively) (Karr and Chu 1999). Our intention is to develop similar models for lakes.

Amphibians are perhaps the premiere “early-warning detection system,” in the sense that they are exceptionally sensitive to changes in the water chemistry, chemical pollution, and introduced pathogens. Amphibians world-wide are experiencing population declines due to a large number of distinct and interacting stressors (e.g., exotic species, impaired habitat, pollutants, climate change, etc.). For this reason, many populations are currently imperiled and necessitate monitoring for inherent conservation reasons. Integrated into the lake/pond monitoring, amphibians represent signals of introduced exotic species (Stead et al. 2005, Fellers et al. 2008), emerging wildlife diseases (Collins and Storfer 2003), and declining ecological integrity (Knapp et al. 2005).

Fish are also useful for documenting ecosystem response to stressors. Moreover, as the top predator in the lentic ecosystem, trends in fish populations can indicate large, cascading effects throughout the ecosystem (Power 1990). In the case of LAVO, fish are an interesting conundrum, in that they are introduced and are hence a stressor themselves. Monitoring fish populations can then both serve as indication of ecosystem response to far field stressors and as a direct measure of a specific local stressor itself.

2.5.5 Derived and Integrated Metrics/Indices

From the various classes of monitoring parameters, we will derive a number of useful metrics and indices for assessments of status and trend, as well as for exploratory data analyses (EDA) (Table 5). Some metrics are used as simple correlative parameters (e.g., approximate volume) for classification or data exploration; other metrics serve as explanatory variables (e.g., shoreline development), which should equate to habitat complexity (Wetzel 2001). The actual calculation of these metrics is detailed in SOP #19: Data Reporting and Analysis.

Table 5. Derived metrics and indices to be used in further data analysis, co-variables, or status and trend analyses. For ecological indices, letters in parentheses indicate taxonomic groups that will be used for that index (F = Fish, MI = Macroinvertebrates, Z = Zooplankton, A = Amphibians, All = all groups to be used simultaneously).

Derived calculation	Type	References
Approximate volume	Morphometric	Wetzel 2001
Shoreline development	Morphometric	Wetzel 2001
Physical Habitat Complexity Index	Morphometric	USEPA 2009
Trophic State Index	Ecological	Carlson 1977, 1992
Taxonomic diversity (F, MI, Z)	Ecological	Magurran 2004
Hilsenhoff Biotic Index (MI)	Ecological	Hilsenhoff 1987, 1988
Fish Condition Index (F)	Ecological	Anderson and Gutreuter 1983
Observed/expected ratio (MI, Z, A, All)	Ecological	Knapp et al. 2005, USEPA 2009
Index of Ecological Integrity	Ecological	Karr and Chu 1999

Approximate volume is a calculated value based on the assumption that the lake has a cone-like bottom profile (or hypsographic curve). We use this approximation as opposed to the more costly and logistically difficult procedure of detailed bathymetric maps.

Shoreline development is the ratio of the actual shoreline to that of a circumference of a perfect circle of the same surface area. A perfectly round lake will approach 1, whereas a lake with a complex shoreline will have higher numbers of shoreline development. This metric is a reflection of the possibility of increased development of littoral communities, proportional to the lake size.

Trophic State Index is a concept developed by Dr. Robert Carlson, where algal biomass (measured as Chlorophyll *a*), clarity, and nutrients are used to either directly assess the state of oligotrophy, mesotrophy, or eutrophy of a water body directly (using algal biomass) or indirectly using proxies (Secchi depth and nutrient chemistry).

Physical Habitat Complexity Index is one of a number of indices recently developed by the USEPA as part of the National Lake Assessment program (USEPA 2009). It combines measures of littoral habitat complexity and riparian habitat complexity, and can be used to score the condition of the physical habitat.

Taxonomic diversity measures include Shannon index, Evenness, and simple counts of species richness. Shannon diversity and Evenness are classical measures of diversity that incorporate

dominance, or lack of dominance, of taxonomic groups. We use these in addition to normal measures of diversity, such as basic species richness, because macroinvertebrate responses to stressors are often manifested as dominance changes, with one or two species dominating the assemblage. In these cases, Shannon index and Evenness may provide more power to evaluate stressor response of the aquatic community.

Hilsenhoff Biotic Index is a weighted average of tolerance values derived from empirical observations of macroinvertebrate responses to pollution (Hilsenhoff 1987, 1988). Because these responses have been extended to a variety of impacts, the Hilsenhoff Biotic Index is a useful way of examining macroinvertebrate changes to stressors. Recent work has also tried to adapt zooplankton to tolerance values (Lougheed and Chow-Fraser 2002). As a body of literature is built, we will expand the use of the Hilsenhoff Biotic Index to zooplankton.

Fish Condition Index is based on the ratio of fish weight to length (LeCren 1951), with the basis that fish that have higher weight to length ratios are “healthier” than fish with low ratios.

2.5.5.1. Multi-metric Indices of Ecological Integrity (IEI): The first Annual Report will develop park-specific Indices of Biological Integrity and an Index of Ecological Integrity. Integrated measures that collate information from different parameters are a useful and powerful tool in biological monitoring (Karr and Chu 1999). Multimetric models are widely used to monitor aquatic ecosystems, and are used by county, state, and national monitoring agencies (EVS-Environmental Consultants 2005, Rehn et al. 2008, USEPA 2009). Although less frequently applied to lakes, these tools have proven their worth to management agencies. These multimetric methods employ a variety of parameters to develop a single index of environmental quality. For example, some of the metrics used in macroinvertebrate-based Index of Biological Integrity (IBI) are based on the following categories: (1) taxa richness and composition; (2) tolerant vs. intolerant organism; (3) feeding and habitat requirements; and (4) population attributes. The USEPA has already developed an IBI (called a multi-metric index by the USEPA) for lake diatoms, which has been vetted and developed for our nation’s lakes (USEPA 2009). They are currently developing further IBIs based on macroinvertebrates, but these are still under development. However, these existing and as of yet unreleased tools are geared to either taxa groups not sampled in our protocol, or built and calibrated for lakes of a differing target population (USEPA models are based on lake >4 hectares in size) and hence may not apply to this monitoring protocol (most lakes in LAVO are <4 hectares).

In this protocol, we will develop IBIs (indices based on strictly biological data) and an IEI for LAVO, where we incorporate physical and chemical metrics into the overall model, if appropriate. This will be done using data collected prior to protocol implementation from sampling lakes in and around the parks from a disturbance gradient. Models for CRLA are untenable due to the lack of sites from an adequate disturbance gradient. More detail on the process of developing IBIs and IEIs are given in SOP #19: Data Analysis and Reporting.

2.5.5.2. Observed/Expected Ratio: The RIVPACS (River Invertebrate Prediction and Classification System) is a metric that compares the Observed value (of species richness) to an Expected value. It is calculated as:

The intuitive, easily explained nature of the score and its interpretation is one of the main advantages for using RIVPACS. For example, a score of 0.85 for a site can be interpreted as a site that had 85% of the expected species (i.e., depauperate, perhaps has experienced local extirpations of biodiversity). Likewise, a score of 1.15 indicates that a site had 15% more taxa than expected (i.e., site is of exceptional quality and is supporting a diverse community).

The RIVPACS bioassessment procedure has been extensively developed and refined over the years. The procedure has been extensively utilized by many agencies and predictive models already exist for streams throughout the western United States (USEPA 2006). But as the name implies, it has been focused on river bioassessment programs. However, recent work has been accomplished applying the methods successfully to lake communities (Johnson 2003, Knapp et al. 2005). Similar to the IBI and IEI models, existing and upcoming national work may not be applicable to the monitoring done under this protocol since the sampling frames differ.

More details on the methods needed for developing O/E scores are presented in SOP #19: Data Analysis and Reporting.

2.6 Power Analysis and Level of Change that can be Detected

Power analysis (statistical power of a hypothesis test) in a long-term monitoring program is an important issue to consider. It is extremely necessary to ensure that monitoring can actually assess environmental changes at a level that is relevant to managers. However, evaluation of a power analysis must recognize several aspects: (1) power is affected by three quantities: α , the probability of a Type I error (rejecting the null hypothesis, when the null hypothesis is true); the practically significant difference (degree of change [effect size] of interest for management); n , the sample size; and σ , the amount of natural variability in the population; and (2) types of tests can affect power (e.g., a t -test versus regression, and a one-tailed t -test versus a two-tailed t -test).

Power is defined as $1 - \beta$, and β is dependent upon the actual effect size (or actual change in the measured parameter), which the investigator does not know prior to experimentation or sampling. Hence, a power analysis investigates a range of effect sizes and determines what the power is under a hypothetical effect size, so that a “power curve” is developed. Alternatively, the investigator can specify the desired effect size to detect and evaluate power at that level of change; this is the tactic employed in this analysis. For a specific test of interest, one quantity can be solved for by holding all others fixed. For example, in this protocol we are interested in solving for the power for a given sample size, magnitude of change, α -level, and estimated variability such that we can evaluate which parameters may be more sensitive for trend detection. Such pro-active (or *a priori*) power analysis allows us to evaluate the likelihood of successful trend or change detection for our sampling design. To do this, the model of holding sampling size constant, making estimates of our variance from our pilot project data, and then calculating hypothetical power is the most appropriate for judging the merits of our protocol plan.

Our power analyses are based on the following: (1) data from the pilot project (Appendix A) used to estimate population standard deviation; (2) α level of 0.10 (increased from the standard level of 0.05), using the justification that the “conserver’s risk” (e.g., the Type I error) should be less restrictive (Irwin 2008) given the mandate of the National Park Service to manage for future generations; and (3) the given desired effect size is a 20% change in any parameter. Both the α level and 20% effect size are somewhat arbitrary, although once chosen, α should remain unchanged. An effect size of 20% has been chosen as a reasonable goal because if detected, this level of change is probably a “biologically” significant increase or decrease that may signal need for management actions.

2.6.1 Power Analyses Caveats

The goal of this protocol that is most relevant to power analyses is to “Characterize the habitat, water quality, and biotic communities in a probabilistic sample of mountain lakes and determine status and trends in key univariate and multivariate variables.” However, in determining trends using a linear regression model, the sample size that determines power for trend significance is the number of sampling periods. Hence, low power is a fundamental aspect for the *start* of a monitoring program (Morrison 2007). It is only after many sampling periods that power to detect trends is accomplished. On account of this, our power analyses do not address long-term trend detection, but rather a shift in a parameter between only two sampling occasions (a two sample *t*-test). This is a rather simplistic assessment of power; evaluation of the strength of a monitoring program should recognize that a regression analysis (when a gradient exists) is *more* “powerful” (Somerfield et al. 2002). Over the long term, the “power of the protocol” is going to be greater than presented here.

Second, many of our protocol objectives focus on a multivariate approach because we have chosen to analyze community change using species assemblages. Primary methods for the analysis of community data are non-parametric methods, for which there is no theoretical basis for power analyses (Somerfield et al. 2002). In other words, it is impossible with the current body of statistical literature to run power analyses on our primary method of data analysis. The utility of using univariate measures to assess a sampling program based on multivariate analyses is summarized by Somerfield et al. (2002):

“Multivariate techniques have been shown repeatedly to be more “sensitive” (i.e. powerful) than univariate techniques (Warwick and Clarke 1991, Somerfield and Clarke 1997, Clarke and Warwick 2001) and although there is no general framework for determining power in the multivariate context the repeated demonstration that multivariate technique produce significant results when univariate techniques do not may be taken as evidence that a survey designed to have adequate power in a univariate context (e.g. for diversity indices) should have adequate power in the multivariate context (of changes in whole community composition).”

Third, many of our variables are measured to provide context for other parameters. For example, anions and cations are measured to understand lake chemistry in relation to the zooplankton and Chlorophyll *a* biomass but not as parameters for trend detection by themselves. So, although some parameters may have extremely low power due to high variability, this does not limit their usefulness for the monitoring program.

2.6.2 Water Chemistry Power Analyses

Measurements made during the pilot project at LAVO were used to estimate the power to detect a hypothetical 20% shift (increase or decrease) in the value between two sampling periods (Table 7). Power was based on one sample, 2-tailed *t*-tests using pilot project data. Values were split between shallow samples (taken at 0.5 m below the water surface) and deep samples (0.5 m above the lake bottom). In cases where the lake was less than 2 m deep, a single middle sample was taken.

Power was relatively low in both shallow and deep samples (average of 41% and 43%, respectively). Some parameters uniformly had high power (e.g., ammonia [sampled in the pilot project, but is not part of the final protocol]), whereas some had low power (e.g., sodium). A higher power for parameters taken in the middle of the water column demonstrates the importance of measuring secondary habitat variables. Variability within these lakes was lower, possibly because the habitat was more similar (i.e., they were all shallow lakes with max depth under 2 m). Contrasted to lakes where a deep and shallow sample was collected, the range of lake depths were from 2 m to 25 m, with a correspondingly lower power.

Although some of these powers are low, their main purpose is as explanatory and co-variables to other measurements (e.g., biological parameters). In the synoptic sampling design of this protocol, we do not expect to have the power to detect minor shifts in these parameters measured every 3 years.

Table 6. Results of pilot project measurements in the summer of 2008 from Lassen Volcanic National Park, and power analyses calculated from averages and standard deviations. Power was based on 1-sample, 2-tailed *t*-tests, with the measured pilot project mean, and standard deviation, run against a hypothetical mean representing a 20% change. Hypothetical sample size was 30. Analyses were run in Systat 12. All values (except power) are in mg/L. n in table refers to number of samples from pilot project that average and standard deviation were based on. -- indicates not enough samples (did not meet minimal quantification levels, or rejected for QC purposes).

Measurement	Shallow Sample (n = 16)			Middle Sample (n = 4)			Deep Sample (n = 16)		
	Average	Standard Deviation	Power	Average	Standard Deviation	Power	Average	Standard Deviation	Power
Total Dissolved Nitrogen ¹	0.318	0.189	57%	0.513	0.094	100%	0.323	0.181	60%
Total Dissolved Phosphorous ¹	0.015	0.012	40%	0.029	0.011	90%	0.016	0.014	31%
Ammonia ²	0.016	0.004	99%	--	--	--	0.016	0.005	97%
Sodium	1.229	1.768	19%	0.848	1.073	22%	1.297	1.838	20%
Potassium	0.492	0.453	32%	0.208	0.042	100%	0.474	0.451	30%
Calcium	4.427	12.730	12%	1.470	2.119	19%	4.669	13.025	13%
Magnesium	1.013	1.354	21%	0.535	0.602	25%	1.047	1.387	20%
Sulfate	4.548	16.256	12%	0.023	0.023	29%	4.506	16.118	12%
Chloride	0.239	0.079	94%	0.190	0.032	100%	0.275	0.158	59%
Chlorophyll a	0.568	0.512	33%	--	--	--	0.506	0.269	65%
Dissolved Organic Carbon	4.806	3.410	45%	5.100	1.044	100%	3.259	1.860	59%
		Average	41%		Average	68%		Average	43%

¹Nitrogen and Phosphorous were sampled as dissolved components in the pilot project, but upon review we will implement Total portions (unfiltered) for protocol implementation. ²Ammonia was sampled in the pilot project, but is not a part of the final protocol.

2.6.3 Biological Power Analyses

Summary indices derived from zooplankton and macroinvertebrate samples were used to conduct prospective power analyses on the biological community (Table 8). For both zooplankton and macroinvertebrates, species richness (S) and Shannon index (H') were used to assess power to detect a 20% change between sampling periods (SOP #19: Data Analysis and Reporting details how S and H' are calculated). Analyses were done as above for water chemistry, with the exception that there was no need to separate out depths (all samples were taken in the water column [zooplankton] or littoral zone [macroinvertebrate]). Power was high for macroinvertebrates (99% - 99.5%) and reasonable for zooplankton (74.6% - 82.7%). Both species richness and Shannon index are relatively coarse measures for detecting trends, but the ability to confidently assess change in even these rudimentary characteristics suggest that more robust analyses (e.g., ecological ordination, Analysis of Similarity, etc.) will be able to detect trends.

During the pilot project, fish were only collected in two of the 21 lakes sampled. In Summit Lake, we collected five brook trout (*Salvelinus fontinalis*), and in Reflection Lake, we collected 23 golden shiner (*Notemigonus crysoleucas*). Although these samples do not lend themselves to a park-based trend, we analyzed K, the condition factor of each species to determine our ability to detect trends in K at individual lakes (SOP #19: Data Analysis and Reporting details K calculation) (Table 9). We assumed that a repeat sampling would result in the same sample size (in number of individual fish collected). Power was high for both species.

Table 7. Results of power analyses on macroinvertebrates and zooplankton using 1-sample, 2-tailed t-tests to detect a 20% change in Species richness and Shannon index. Averages and standard deviations were calculated using pilot project data. Analyses were run in Systat 12.

Index	Macroinvertebrates			Zooplankton		
	Average	Standard Deviation	Power	Average	Standard Deviation	Power
Species richness	28.60	7.20	99.5%	13.9	5.75	82.7%
Shannon index	2.16	0.59	99.0%	1.17	0.57	74.6%

Table 8. Results of power analyses for site assessment of 20% change in fish condition factor, K. Analyses were run in Systat 12 using a 1-sample, 2-tailed t-test, with the assumption of follow-up sampling returning the same sample size (n) for each lake. Note that this is for site assessment, not park assessment; only 2 of the sampled lakes had fish, limiting our inferences to the specific lake, and not the entire park unit.

	Average	Standard Deviation	Power
K, Fish condition factor			
Brook Trout (Summit Lake, n = 5)	1.78	0.218	91%
Golden Shiner (Reflection Lake, n = 23)	1.71	0.318	100%

2.6.4 Power Analyses Summary

In sum, power analyses to assess our minimal detectable difference for trend analysis are complicated by (1) sample size for trend is time periods sampled, not number of sites; (2) linear regression integrating split-panel designs are problematic (Starcevich 2009); and (3) our main

statistical technique will be using non-parametric multivariate analyses, which are not amenable to power analyses at this time. Finally, the main parameters from the biological sampling will be observed/expected ratios (O/E) and indices of biological and ecological integrity (IBIs and IEIs), which will be developed later in the sampling program.

Regardless, power analyses to detect a 20% change between two time periods show mostly low power for chemical parameters but relatively high power for the biological community metrics.

3.0 Field and Laboratory Methods

3.1 Data and Sample Collection

The attached Standard Operating Procedures (SOPs) describe field collection methods in detail, including pre-season preparation, water sampling and handling, physical habitat sampling, aquatic community sampling, shipping of samples, and end of season procedures (Table 9).

Table 9. Standard Operating Procedures covering the preparation, collection, and recording of field data for the integrated aquatic community and water quality sampling of lakes.

SOP	Title and Description
SOP #1	Preparations, Equipment, and Safety This SOP is a general overview of the steps necessary for the initiation of a field season. It covers tasks that the Project Lead will have to start early on in the planning process; hiring of field crews, equipment preparation, scheduling of crews, and basic safety are discussed.
SOP #2	Field Crew Training This SOP covers the requirements for getting crews trained for the upcoming season, including field sampling procedures, ethical considerations, and data entry training.
SOP #3	Site Selection This SOP is provided to give an overview of the site selection process and to inform the field crews of how the sites were initially selected, but this protocol will only have to be performed once at the initial implementation of the program.
SOP #4	Order of Work This SOP covers the sequential order of the tasks that the field crew will perform, so that sampling is not compromised by previous tasks (e.g., trying to measure water clarity after stirring up sediments from macroinvertebrate collection).
SOP #5	Site Arrival Tasks This SOP describes the initial on-site arrival tasks of the field crew (e.g., equipment set-up and associated tasks).
SOP #6	Weather, Physical Habitat, and Site Photography This SOP describes the steps for recording weather, immediate physical habitat descriptions, and digital documentation of a site.
SOP #7	Fish Collection and Processing This SOP details the procedures for setting gill nets, collecting and processing fish, guidelines on for the proper handling of fish, and data recording of the sampled fish assemblage.
SOP #8	Water Sample Collection, Zooplankton, and Secchi Disk This SOP describes the procedures necessary for collecting water samples at the deepest point in the lake, collecting zooplankton, and how to measure the water clarity with a Secchi Disk.
SOP #9	Water Sample Filtration and Handling This SOP covers the steps necessary for field handling of water chemistry samples, including labeling, filtering, and storage. It also covers the process of Chlorophyll a sampling and field alkalinity measurements.
SOP #10	Multiprobe Calibration and Use This SOP covers how to calibrate and use the water quality multi-probe sonde for the collection of turbidity, temperature, pH, conductivity, redox potential, and depth using the Eureka Environmental Manta probe and Amphibian data recording unit.

Table 9. Standard Operating Procedures covering the preparation, collection, and recording of field data for the integrated aquatic community and water quality sampling of lakes (continued).

SOP	Title and Description
SOP #11	Amphibian, Invertebrates, and Lake Substrate Walk-around This SOP describes the integrated procedures for a lake walk-around for sampling macroinvertebrates, amphibians, and recording habitat type into a Trimble GPS unit. Embedded within these methods are the measurement of other physical parameters: habitat area, shoreline circumference, longest length, etc.
SOP #12	Post-Site Tasks This SOP details the necessary steps for the field crew required prior to leaving the site, including data quality control checks and disinfection procedures for wildlife diseases (e.g., Chytrid fungus) and exotic organisms (e.g., New Zealand mudsnails, <i>Potamopyrgus antipodarum</i>).
SOP #13	Data Entry This SOP covers the steps associated with entering the data collected during the field season and how to enter it accurately into the Klamath Network Lakes database.
SOP #14	Photo Management This SOP details the steps necessary to manage the digital images collected during the field season.
SOP #15	Post-Field Season This SOP describes tasks to be undertaken by the field crew at the end of the season, including equipment clean-up, inventorying, storage, and post-season de-briefing.
SOP #16	Quality Assurance Project Plan This SOP provides and details the steps necessary to ensure quality control and quality assurance of the data collected under this protocol.
SOP #17	Data Transfer, Storage, and Archive This SOP describes how to transfer data from field sheets and electronic field equipment (Amphibian, GPS, and Camera) into the Klamath Network lakes database along with storage of seasonal data and data archiving processes.

3.1.1 Field Season Preparation

Following the Operation Checklist (Appendix P), preparations should begin as early as October of the prior year, by communicating with the Network Coordinator the needs (personnel, budgetary, and contract laboratory costs). Steps should be taken so that hiring of the field crew can begin in January, so it may be necessary to have Human Resources start the procedure in the previous December. Other preparations that should be arranged prior to the start of the season include arranging for permits and housing for field staff.

Permit requirements may change from sampling period to sampling period, depending on locations of lakes to be sampled and the requirements of the current park staff. Some parks require permits while some will allow research and collections by National Park Service employees without a permit. Minimum Requirement Analysis for sampling in wilderness areas will need to be conducted. The Project Lead will need to coordinate with the Chief of Natural Resources at CRLA, LAVO, and REDW well in advance of the beginning of the field season to ensure that all permits are secured.

Sampling permits for fish are also necessary from the California Department of Fish and Game for the sampling of Freshwater Lagoon because half of the lagoon is located on state land. The permits can be an extensive process to receive, and they must be in the name of the Field Crew

Lead, not the Project Lead, however the application should start before the Field Crew Lead is hired by applying under the Project Lead's identification. A minimum of 6 months should be allotted for the application period. The permit procedure is available at: <http://www.dfg.ca.gov/licensing/pdf/fg1379.pdf> or by contacting:

DFG
License and Revenue Branch
1740 North Market Boulevard
Sacramento, CA 95834

All reporting requirements for park or state permits are the responsibility of the Project Lead.

3.1.2 Field Work

Crews hike or drive to collection sites. Crews work along the shoreline, wading into the shallow zones of the lake either in waders or neoprene booties as conditions warrant. The deeper portion of the lake is accessed using a lightweight, inflatable raft. Crews will be carrying heavy loads into the backcountry; lightweight personal and sampling gear is encouraged. This is especially important because the sampling frame has placed an emphasis on lakes that are accessible and can be sampled in a single day of travel. No overnight camping in the wilderness should be necessary, but will be encouraged if it facilitates sampling multiple sites efficiently.

Crews will collect water samples, physical habitat data, filter samples (e.g., Chlorophyll *a*), invertebrates, and fish samples. Invertebrate samples will be sent to an aquatic entomology laboratory but fish samples will be processed and disposed of in the field. Amphibians will be sampled using Visual Encounter Surveys. Amphibians will be handled only occasionally, as necessary to confirm species identifications.

Crews will perform alkalinity analyses in the field, using a portable test kit with minimal chemical requirements (mild sulfuric acid). All generated waste will be carried out by the crew, and disposed of properly, meeting requirements of the Chemistry Department of Southern Oregon University.

3.1.3 Sample Handling and Shipping

All staff handling samples are required to adhere to quality control procedures to ensure sample integrity. All procedures detailed in the SOPs must be performed. No "short-cuts" by the field crew will be allowed. Water samples must be placed in a designated freezer or refrigerator as soon as possible by the field crew upon return from the field. It is the responsibility of the Project Lead to secure access to such facilities by the field crew. Water samples are shipped overnight to the lab, using the Southern Oregon University administrative agreement to cover charges. Samples should be shipped early in the week, to avoid the potential for samples to show up at the end-of-week workday, at a time when no one is available to receive them. The primary contact for each laboratory should be contacted in advance to inform them that the samples are "in the mail."

Macroinvertebrate samples are stored in 95% Ethanol to ensure adequate preservation. It is the responsibility of the field crew to ensure that enough room in the sample vials exists to achieve this. All macroinvertebrate samples are retained by the Project Lead or field crew until the end of

the season, when they will be shipped to an aquatic entomology laboratory. It is the responsibility of the Project Lead to ensure that samples are shipped legally (note: it is illegal to ship Ethanol and other flammable liquids without special certification and training). The Project Lead should work with the aquatic entomology laboratory to meet these requirements. One possible solution to shipping Ethanol is the temporary replacement of Ethanol with water and overnight shipping. The aquatic entomology laboratory can then replace the water with Ethanol, so that minimal degradation to the samples has been incurred.

3.1.4 Quality Assurance and Quality Control

Quality assurance and quality control procedures are embedded in individual SOPs, so that if they are followed as written, the requirements of the Quality Assurance Project Plan (QAPP) will be met. Overall needs for the QAPP are reiterated in SOP #16: Quality Assurance Project Plan. The QAPP for Klamath Network lakes sampling have been written to meet the requirements of the National Park Service, Water Resources Division (Irwin 2008), the State of California Surface Water Ambient Monitoring Program Quality Assurance Program, and the Klamath Network Data Management Plan. The QAPP addresses the needs of measurement quality objectives, sample contamination, field measurements, sample handling, instrumentation testing and calibration, and audits. The QAPP also includes information on the preferred method to document cumulative bias, which arises when personnel, equipment, or contract laboratories are changed. The cumulative bias procedures will allow the crosswalk of data before and after changes are made to maintain the integrity of the data.

3.1.5 End of Season Procedures

Once sampling is complete at all sites, gear is decontaminated a final time, cleaned, repaired as necessary, and stored. Crews will make a list of gear needing to be replaced or repaired.

The Project Lead will conduct a post-season debriefing with the field crew to discuss the season and make sure that all necessary sampling has been done. Any departures from the protocol will be discussed and analyzed. Necessary revisions and improvements to the protocols will be discussed.

3.2 Field and Laboratory Analyses

Laboratory methodologies and instrumentation have been chosen that match national standards, that are identical to methods used at CRLA, and that match the methods used by the North Coast and Cascades Network. With the exception of the measurements that will be made in the field (acid neutralizing capacity, temperature, dissolved oxygen, pH, conductivity, redox potential, and turbidity [Table 10]), all chemical analyses will be performed by contract laboratories (Table 12).

Field analyses and methodological details are presented in Table 11. A depth profile, measured at the surface and down every 0.5 m interval to just above the bottom substrate will be accomplished with a multi-parameter electronic recording unit (sonde), specifically the *Eureka Environmental Manta* multi-parameter sonde and *Amphibian* data recording unit. In keeping with the nature of a long-term monitoring program, the probe used may change as equipment wears out, technological improvements are made, and companies go in and out of business. Any change of equipment will follow the SOP #16: Quality Assurance Project Plan guidelines for

cumulative bias, to ensure continuity of reliable data and documented using an equipment log book.

Table 10. In situ measurements, methods, and quality standards for depth profiles to be performed at the deepest location of the lake. Specifications from Eureka Environmental, www.eurekaenvironmental.com. NTU = Nephelometric Turbidity Units.

Measurement	Method	Range	Accuracy	Resolution
Depth	Pressure transducer	0 - 25 m	± 0.2%	0.01 m
Dissolved oxygen	Optical luminescence	0 - 25 mg/L	± 1% or 0.2 mg/L, whichever is higher	0.01 mg/L
pH	Reference electrode	2 - 12 units	± 0.2 units	0.01 units
Redox potential	Reference electrode	-999 - 999 mV	± 20 mV	1 mV
Specific Conductance	4-Electrode Graphite Conductivity Sensor	0 - 5 mS/cm	± 1%	0.001 mS/cm
Temperature	30k ohm thermistor	- 5° C - 50° C	± 0.1° C	0.01° C
Turbidity	McVan NEP9500 type	0 - 3000 NTU	<1% when under 400 NTU	0.1 NTU

The sole chemical analysis to be done in the field will be the determination of acid neutralizing capacity from the epilimnion (0.5 m below the surface of the lake) and hypolimnion (0.5 m above the lake bottom substrate). Acid neutralizing capacity measurements will be accomplished using a Hach Digital Titrator Model 16900, following Hach procedure 8203. The range of this test kit is 10 – 4000 mg/L as CaCO₃; accuracy of the Digital Titrator is ± 1% for samples within the range of the test; resolution is one digit (1 mg/L for most circumstances), titrating to a pH endpoint of 4.8. Although laboratory testing could improve the range and resolution, the logistical constraint of short holding times (<14 days) necessitate field analyses. A further consideration is the use of acid neutralizing capacity as a co-variable to examine the ecological integrity of the aquatic ecosystem and not as a single factor for trends alone. We maintain that it is more important to identify ecosystems with high versus low acid neutralizing capacities as a covariable and the accuracy and resolution of the Hach field kit will meet these needs.

Klamath Network and network park units do not have facilities, equipment, or personnel to conduct laboratory analyses in-house, necessitating the contracting to a specialized laboratory. In general, the procedures will follow those recommended by the American Public Health Association (Eaton et al. 2005) and approved by the US Environmental Protection Agency.

Table 11. Laboratory analyses to be conducted by a contract laboratory; minimum MDL, ML, and precision requirements. APHA = American Public Health Association (Eaton et al. 2005); MDL = Method Detection Limit; ML = Minimum level of quantification.

Parameter	Method	Instrumentation ¹	MDL (mg/L)	ML (mg/L)	Precision (± mg/L)
Calcium	APHA 3111 D	Varian SpectrAA220	0.06	0.19	0.06
Chloride	APHA 4110 B	Dionex 1500 Ion Chromatograph	0.01	0.03	0.01
Dissolved Organic Carbon	APHA 5310 B	Shimadzu TOC-VCSH Combustion Analyzer	0.05	0.16	0.05
Magnesium	APHA 3111 B	Varian SpectrAA220	0.02	0.06	0.02
Nitrate	APHA 4500-NO3 F	Technicon Auto-Analyzer II	0.001	0.003	0.001
Potassium	APHA 3111 B	Varian SpectrAA220	0.03	0.1	0.03
Sodium	APHA 3111 B	Varian SpectrAA220	0.01	0.03	0.01
Sulfate	APHA 4110 B	Dionex1500 Ion Chromatograph	0.02	0.06	0.02
Total Nitrogen	APHA 4500-NO3 F; APHA 4500-P J. Persulfate digestion	Total Technicon Auto-Analyzer II	0.01	0.032	0.01
Total Phosphorous	APHA 4500-P B; APHA 4500-P E	Total Technicon Auto-Analyzer II	0.002	0.003	0.002

¹= example instrumentation used by contract laboratory (Oregon State University CCAL) for pilot project.

4.0 Data Management, Analysis, and Reporting

The clear, concise, and consistent recording, analysis, and reporting of data is essential to the success of the long-term monitoring of Klamath Network lakes program and will be a top priority for all personnel involved in the monitoring program. During each phase of the protocol, checks will be used to ensure the accuracy and completeness of monitoring program activities. Responsibilities for each person involved in the project are outlined in SOP #16: Quality Assurance Project Plan. Each person should be familiar with their roles prior to implementing field work. Guidance for data management and analysis (in general, and in the field and laboratory) are available from the Klamath Network Vital Signs Monitoring Plan and its attached Appendix J (the Klamath Network Data Management Plan), available at: http://science.nature.nps.gov/im/units/klmn/Monitoring/MON_Phase_III.cfm. Additional guidance is available on the NPS Data Management web site at: <http://science.nature.nps.gov/im/datamgmt/index.cfm> and from EPA Guidance for Quality Assurance Project Plans (USEPA 1998) available at: <http://www.epa.gov/quality/qs-docs/r5-final.pdf>. Specific SOPs covering the data management portions of these protocols are summarized in Table 12.

Table 12. Summary of SOPs covering aspects of data management, analysis, and reporting.

SOP	Title and Description
SOP #13	Data Entry This SOP provides the details on how to utilize the Lakes database to enter data, store data, QA/QC data, and export data for analysis and integration into NPSTORET.
SOP #14	Photograph Management This SOP provides information on how to manage photographs taken as part of this project, including the required metadata.
SOP #15	Post Season Tasks This SOP describes the data management tasks that need to occur once the field season is over, such as post processing GIS data.
SOP #16	Quality Assurance Project Plan This SOP is a detailed description of the methods to ensure quality data, including QA/QC. Topics covered include chemical blanks, outlier analyses, data completion goals, roles and responsibilities, and ensuring data comparability over time.
SOP #17	Data Transfer, Storage, and Archiving This SOP provides the details on how the PI goes about organizing and submitting the season deliverables so they can be integrated into the master databases and archived in the appropriate locations
SOP #18	Metadata Guidelines This SOP provides details for the type of metadata to be collected and how it is stored and accessed.
SOP #19	Data Reporting and Analysis This SOP describes the methods for basic calculations of metrics and indices used in the protocol. It also details the reporting schedule for summary reports and Analysis and Synthesis reports.
SOP #20	Revising the Protocol This SOP details the steps necessary to revise the protocol.

4.1 Database Design

The water quality component of the Natural Resource Challenge (NRC) requires that all NPS networks archive any physical, chemical, and biological water quality data collected with NRC water quality funds in the NPS STORET (STorage and RETrieval) database. To assist in this

process, networks have the opportunity to make use of a relational database patterned after the Natural Resource Database Template (NRDT) and developed by the Water Resources Division (WRD) called NPSTORET, or they can utilize any of the numerous databases already available as long as they can export that data into a format that meets the STORET Electronic Data Deliverable (NPSEDD) specifications. After analyzing the potential available databases and examining the utility of the NPSTORET database, the Klamath Network has opted to develop a NRDT compliant, network-specific database that meets the NPSEDD specification for all their aquatic and water quality monitoring projects. It was determined that NPSTORET did not have all the functionality needed to account for all the data being collected as part of this integrated protocol.

4.1.1 Metadata Procedures

Creation of metadata files and information is an integral part of any project that collects samples that generate data and information. Metadata consists of information that documents the information contained within data files and information products. The overall goals of metadata creation are to develop a comprehensive document that (1) explains enough about the project data to ensure they are useable by future personnel and the scientific community, and (2) complies with Federal Geographic Data Committee and NPS mandates for federal projects. Metadata development begins at the start of every project; as the project develops, so does the metadata. Within the sideboards set by the program and federal requirements, the process of metadata creation will vary depending on goals and objectives, funding, and scope of the project. It is the responsibility of the Data Manager to set forth the metadata requirements and the process used to create the metadata. These requirements are outlined in SOP #18: Metadata Guidelines.

The metadata for a project should be created prior to implementing the field season and will need to be updated at the end of each field season. The Klamath Network utilizes a Metadata Interview form that describes the various attributes of a dataset. The interview form includes information about the time frame, description, sensitivity, collection location, and purpose of the data, plus various other pieces of information needed to develop the metadata for the dataset. It is the Project Lead's responsibility to complete a new Metadata Interview form before the start of the first field season and at the end of each additional field season.

In addition to metadata associated with each spatial product, database, spreadsheet, and document, the Klamath Network requires metadata to be provided for each photograph used to capture some aspect of a monitoring project (e.g., field crew, sites, sampling method). Photographs are a valuable tool used for a multitude of objectives including conducting outreach, identifying specimens, displaying habitat conditions, documenting field work, and analyzing data. It is the responsibility of the field crew and the Project Lead to follow the photograph procedures provided in SOP #14: Photograph Management. The Project Lead will submit project-related photographs and photograph metadata to the Data Manager at the end of each project.

4.1.2 Storage

The Klamath Network utilizes a standardized folder structure for each of our monitoring and inventory projects. All data deliverables including finalized and draft copies should be organized utilizing this standardized structure, as described in SOP #17: Data Transfer, Storage, and Archiving.

When collecting data electronically in the field, a backup of the database will be made prior to leaving a field site and stored in the appropriate locations. Once out of the field, data from the electronic devices should be stored in a desktop or laptop computer. Details for backing up the database are provided in SOP #13: Data Entry.

When returning to the Klamath Network office, data should be reviewed by the Project Lead. Once the data have undergone all validation and verification processes, they should be transferred to the Data Manager, along with a Data Certification form. Once submitted, the data will be uploaded to a master database that can be used for data analysis. In addition, the data will follow the backup process implemented by Southern Oregon University that includes nightly, weekly, and quarterly backups stored for 2 months (nightly and weekly backups) or 1 year (quarterly backups) and archived utilizing Reference Applications.

4.2 Data Collection and Data Sheet Archival

The Klamath Network, when possible, will make use of tablet PCs, Trimble Pocket PCs, and/or PDAs to collect data associated with this protocol. It is the responsibility of the Field Crew Lead and Project Lead to adequately train field crews in data collection and management methodologies outlined in this protocol. Since this protocol is a long-term commitment and crew turnover is expected, a training session, based on the Data Entry SOP (#14) is necessary each season. A log should be kept outlining the training sessions each crew member attends and logs should be transferred to the Data Manager at the end of each field season.

While the Network will make every attempt to enter data electronically in the field, we recognize there are instances when this will not be possible. Field crews should always have hardcopy field forms available when going to monitoring sites. If data are entered onto hardcopy forms, they should be entered into the database as soon as possible after data collection. Data entry should occur each week unless longer time frames are approved by the Project Lead and Data Manager. At no point should field notebooks be substituted for datasheets. Datasheets should be designed following the specifications outlined in the Klamath Network Data Management Plan (Mohren 2007). All datasheets will be bound and stored in a waterproof storage container at the end of a sampling day. At the end of a sampling period, upon returning to the Klamath Network office, datasheets will be scanned into a PDF format with a naming convention outlined in SOP #15: Post-Field Season. PDF documents will be stored in the project folder located on the Klamath Network server. Original datasheets will be stored in a dry, water-proof container at the Klamath Network office.

4.3 Data Verification and Validation

Data verification is the process of ensuring that data entered into a database accurately duplicate data recorded in the field. Field crew members should implement the following process to verify data:

1. *Visual review at data entry*— This method should always be used when entering data. In this method, the crew member entering the data verifies each record *after input*, prior to the next input. Records are checked to ensure all parameters have been entered and that the values make sense. If hardcopy datasheets are being used, records entered into the databases are compared to the data on the hardcopy datasheets. Errors or missing values are corrected immediately.

2. *Visual review after data entry*— After the data have been entered, and prior to leaving the site, records should be double-checked to ensure they are complete and accurate. When possible, this should be completed by someone other than the person who entered the data.

3. *Final Review*— After following the processes outlined in number 1 and 2 above, it is the Project Lead's responsibility to review a predetermined subset of records that have been entered into the database and compare them to the original hardcopy forms, if available. A timeline should be developed during the project's planning phase to outline the number of records that will be checked and a time frame as to when they will be examined.

While data verification can be completed by someone with little to no knowledge of the data, data validation requires a reviewer to have extensive knowledge on what the data mean and how they were collected. Data validation is the process of reviewing the finalized data to make sure the information presented is logical and accurate. The accuracy of the validation process can vary greatly and is dependent on the reviewer's knowledge, time, and attention to detail. General data validation procedures include:

Data entry application programming— Filters for illegal data will be used to prevent data being entered that exceed their logical value (e.g., 2 m vs. 200 m lake depth). It is important to note that not all fields have appropriate domains and it will be the responsibility of the Project Lead to examine these fields for erroneous data.

Outlier detection and review— An outlier is an unusually extreme value for a variable, given the statistical model being used to analyze the data. It is important to note that not all outliers are a result of data contamination; they may be indicators of important thresholds or extremes in variation of the parameter of interest. Statistical tests such as Grubbs' test, regression mapping, and graphical displays such as scatter plots will be used to examine the data for outliers (Michener et al. 2000). Depending on the analysis methodology, outliers may not need to be removed. A determination will need to be made to define what is considered an "unusually extreme" value indicating data contamination or an environmental aberration that clouds the interpretation of the field measurement. Generally, non-error-associated outliers should be flagged and retained, allowing those conducting data analysis to make determinations about inclusion or rejection.

Review of what makes sense— The Field Lead and Project Lead should be intimately familiar with the types of data being collected, including expected data ranges. The individuals in these roles should review the tabular data to make sure they appear logical. GIS data should be plotted and examined to determine the accuracy of the spatial locations (Sanders 2005).

4.3.1 Data Certification

After data validation and verification, the Project Lead will turn in a Data Certification form(s) (from the Klamath Network Data Management Plan, Mohren 2007) to the Data Manager. This form is used to ensure:

- The data are complete for the period of time indicated on the form.
- The data have undergone the quality assurance checks indicated in the protocol.

- Metadata for all data has been provided.
- Project timelines are being followed and all products from the field season have been submitted.
- The level of sensitivity associated with the deliverable is appropriate.

A new certification form should be submitted each time a product is submitted. If multiple products are submitted at the same time, only one form is necessary.

4.4 Data Analysis and Reporting

Data analysis and reporting guidelines are covered in SOP #19: Data Analysis and Reporting. This SOP covers a comprehensive approach by the Klamath Network of the reporting of data for the next 12 years. There will be two elements of our reporting strategy: (1) Annual Reports describing field sites visited, interesting findings, and status of the measured parameters completed every sampling period and (2) Analysis and Synthesis reports completed every 3 years that focus on trends and the development of indices. These reports and their contents are covered in more detail in SOP #19: Data Analysis and Reporting, but are briefly summarized here.

4.4.1 Annual Reports

Annual reports for this protocol are identical in format to annual reports for vital signs monitored every year. An example of an annual report is provided in Appendix A, from the data collected during the pilot project. These reports will focus on providing managers a current status assessment, defined using measures of central tendency (means or medians) of the park habitats. Reporting tools will focus on mean conditions, along with user-friendly graphical presentations. Unusual or significant findings will also be highlighted. Annual reports serve to update the park units where sampling occurred for their use in management and reporting goals.

Due to necessary turn-around times for contract laboratories, summary reports will be due June 1st of the year following lake/pond sampling. This will provide approximately 180 days for the contract laboratories to process invertebrate and zooplankton samples and an additional 3 months for the Project Lead to complete the report. As appropriate, the report will be formatted to the Natural Resources Technical Report (NRTR) or as Natural Resource Data Series (NRDS).

4.4.2 Analysis and Synthesis Reports

Analysis and Synthesis reports form the basis of trend analysis for the integrated water quality and aquatic communities vital signs. In the spirit of long-term sampling, the protocol will run through several sampling periods before meaningful analyses can be completed. The first Analysis and Synthesis report will occur after the second sampling period. As in the Annual Reports, they will occur every 3 years thereafter. Reporting format will follow the NRTR format.

The initial three Analysis and Synthesis reports will focus on describing the fundamental aspects and gradients of the lakes: (1) Physical, (2) Biological, and (3) Chemical. An individual report will be devoted to each aspect of the lake, starting with the least variable of the three: the physical environment. The second and third will focus on either the biological or chemical.

The fourth Analysis and Synthesis report will be the first major analysis of trends. This will be after five sampling periods and will be due on the 1st of November, 2023. Although this lag between implementation and the first trend analysis seems unduly long (9 years), this is close to the minimum number of sampling periods needed to achieve significant trends with the Mann-Kendall test at the α level of 0.05 level (Rohlf and Sokal 1995) (so trend analyses prior to this would be of limited usefulness). This report will be a comprehensive study on the techniques to detect trends and will outline the methods to be used in future trend analyses, recognizing that the field of ecological statistics and trend analysis will always be an innovative and evolving one.

Future Analysis and Synthesis reports will always include a trend component. The Project Lead is encouraged to explore other aspects of monitoring as well. Possible topics include: (1) Bayesian statistics for lake management questions; (2) Status and trends in a regional context (i.e., integrating data from other regional programs); (3) Various lake/pond biology and ecosystem topics; and (4) Reanalysis of sampling frame (e.g., have new lakes formed or have perennial habitats become ephemeral). In determining the topics to be covered by Analysis and Synthesis reports, park staff at the respective park units should be consulted to explore specific management or research needs that may be answerable using the data from this protocol, and if available integrate data from the park as well.

4.4.3 Data and Product Distribution

The Klamath Network utilizes the Network's Internet and Intranet web sites, Southern Oregon University, and the National I&M databases to disseminate information to the parks and the general community. Prior to dissemination, all information must be associated with appropriate metadata. It is the responsibility of the Data Manager to work with the Project Lead and park staff to determine the sensitivity of the data prior to posting. Constraints will be placed on sensitive data to prevent or limit distribution to the public.

The Klamath Network will send NPSEDD from NPSTORET to the WRD on an annual basis for quality assurance and for upload into the WRD's copy of STORET and the Environmental Protection Agency's (EPA) STORET National Data Warehouse (Figure 6).

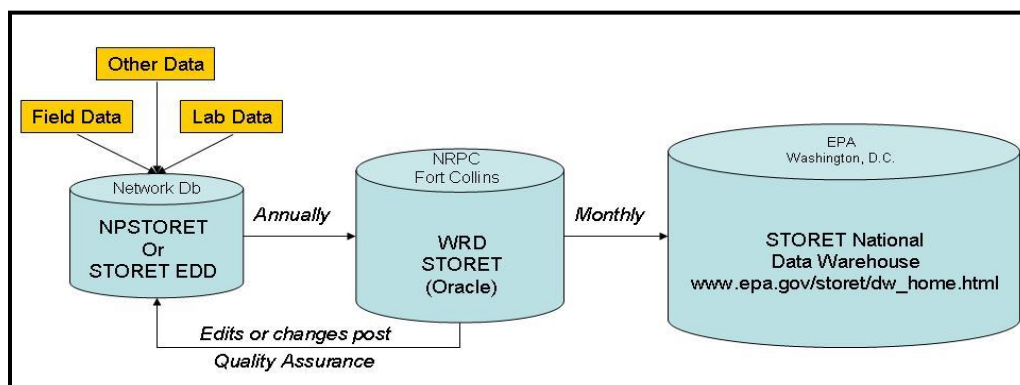


Figure 6. Simplified conceptual model of the Natural Resource Challenge vital signs water quality data flow from collection to distribution.

5.0 Personnel Requirements and Training

5.1 Roles and Responsibilities

The Integrated Aquatic Community and Water Quality Monitoring of Mountain Pond and Lakes in the Klamath Network program is the primary responsibility of the Network Aquatic Ecologist, also referred to as the Project Lead. The Project Lead is a GS-9/11/12 level scientist who is trained and experienced in aquatic ecology, with hands-on experience in lentic and lotic habitat ecology, either through postgraduate education or work experience. The Project Lead is responsible for managing the day-to-day activities of the lakes project; supervises seasonal crew members and provides them with tactical and logistical support during the field season; verifies, validates, and analyzes data; and writes and completes Annual (or Summary) and Analysis and Synthesis Reports.

Assisting the Project Lead are the Network Coordinator, who has overall responsibility for implementing and supervising the Klamath Network Mountain Pond and Lake Long-term Monitoring project; is responsible for the successful completion of all aspects of the program; and directly supervises the Network Project Lead and Data Manager. The Data Manager is responsible for creating and maintaining the seasonal and master database; providing data management guidance and training to project staff; and ensuring the data are accurate, properly documented, stored, archived in a secure manner, and made available to a diverse audience.

The field crew will consist of two members: a senior Field Lead and a junior Field Crew Member. With the number of sites to be visited in this protocol at 42 or less (depending on the number of ephemeral ponds in CRLA), a single crew will be ample to sample all sites in the summer field season.

The Field Lead is supervised by the Project Lead, is accountable for supervising the crew member and any volunteers in the field, and is responsible for the successful completion and verification of monitoring program tasks. This includes but is not limited to the collection, storage, and shipment of field samples and the collection and entry of data into the monitoring program database. The Field Lead also is responsible for the calibration, use, and/or maintenance of monitoring program instruments, equipment, and gear. The Field Lead will have experience in conducting aquatic field work in relatively remote isolated locations, at least some minimum experience in supervising peers, and the ability to live and work cooperatively with others under often stressful and challenging conditions for extended periods.

The Field Crew Member is supervised by the Field Lead and will be responsible for successfully completing all monitoring program tasks, including but not limited to the collection, storage, and shipment of field samples and collection, verification, and storage of field data. Field crew members will have at a minimum some experience in conducting aquatic field work in relatively remote, isolated locations and demonstrate the ability to live and work cooperatively with others under often stressful and challenging conditions for extended periods.

5.2 Training Procedures

A standardized, comprehensive training program for all personnel is necessary to ensure that data collection is consistent and meets the data quality objectives of the Quality Assurance Project

Plan (SOP #16: Quality Assurance Project Plan) and the data standards defined in the Klamath Network Data Management Plan. Consistency is the key to the successful accomplishment of the goals and objectives of this protocol. The training program should last 2 weeks, although actual data collection under the supervision of the Network Project Lead can be accomplished during this period.

The training program should start with classroom sessions, with the Project Lead developing a syllabus and instructional materials that adequately covers the following topics (the list can be expanded):

1. Background on I&M program objectives, sampling design, and data analysis.
2. Field sampling methods and QA/QC concerns.
3. Equipment operations and maintenance.
4. Field and laboratory sample processing and handling.
5. Fish and amphibian species identification, handling, and a primer on wildlife diseases.
6. Recording and storing data, both manually and digitally.
7. Safety in the backcountry.
8. Orienteering.
9. Backcountry rules and ethics.
10. Computer data entry.

This educational period is supplemented with this narrative, protocol, and appendices, but these materials (to be supplied before the Entrance on Date [SOP #1: Preparations, Equipment, and Safety]) are not to be used as a substitute for a training period.

Classroom training material will be developed by the Project Lead and stored in electronic form on the Klamath Network server, following the protocols of the data management plan. Over the course of this protocol implementation, these materials will be refined and improved by the Project Lead.

After the classroom sessions have been completed, additional training will focus on hands-on collection of data in the field. This can take place at index sites within the appropriate park unit and be used as actual data for the program, with the qualification that the Project Lead is on hand to supervise and train the crew in proper techniques, to ensure QA/QC. For example, the Project Lead can take a profile reading with the water quality sonde as a demonstration, which will be actual data for the “record,” which then will be repeated by the field crew members to learn the sampling techniques.

Each crew member will be certified in each SOP, with date certified, individual responsible for certification, and specific SOP certified recorded using the forms provided in Appendix F.

6.0 Operational Requirements

6.1 Annual Workload and Field Schedule

Necessary tasks for the implementation of this protocol are presented in Table 13. Preparation for the upcoming field season starts the year before, ideally in December or earlier. By January, the Project Lead should re-inventory and re-check the condition of the field gear and order replacements or send them to the manufacturer for servicing as necessary. (Checks will be done at the close of the last season, but with 2 years between sampling, gear must be rechecked.) In April, the Project Lead should obtain bids (or verify as sole source) for specimen processing (water chemistry, Chlorophyll *a*, zooplankton, and macroinvertebrates) and initiate contracting to the chosen laboratory. Water chemistry bottles should be acid washed (or confirm that pre-acid washed bottles are ready in suitable numbers) and filters prepared for water sample collection (SOP #1: Preparations, Equipment, and Safety) in April, with all associated tasks completed by the middle of June. Training of the field crew should begin in July, at the start of the field season. Training is an on-going activity; periodic checks will be made to ensure that QA/QC procedures are followed. Although data entry will occur throughout the field season, a final QA/QC will occur with the presence of the field crew, so that any remaining questions may be answered. Upon data certification and receipt of the data deliverables of the specimen contractors, the Project Lead will formulate and write the Annual Report and/or Analysis and Synthesis report, as appropriate. The first stages of this could occur in October. However, initiation of the report writing may be delayed relative to the availability and delivery of the required data. Report(s) should be finalized by June of the following year.

Table 13. Summary of annual tasks and workload for implementation of protocol. N/A indicates not applicable, either an ongoing task, or open ended. See Appendix P, Operation Checklist for more details.

Task	Timeframe to initiate	Deadline
Hiring of field crew	December - January	End of January
Inventory and maintain field gear	January - February	End of February
Purchase required field gear	March	March
Acquire bids for specimen processing, arrange contracting	April	End of May
Prepare water chemistry bottles and filters	April	Middle of June
Training and orientation	July	N/A - Ongoing
Field work	July - September	N/A
Final data entry and QA/QC	September	September
Annual Report and Analysis and Synthesis Reports	October	June of following year

6.2 Facility and Equipment Needs

Facilities necessary for the completion of this protocol include office space with access to computers for the Project Lead, as well as computers for data input from the seasonal field crew. Minimal laboratory facilities are necessary for the steps of acid washing bottles and filter prep, all available through Southern Oregon University Chemistry Department. Seasonal housing for the field crews is also necessary, along with access to refrigeration/freezer usage for storing water samples.

A large amount of equipment is necessary for the completion of this protocol. A complete equipment list is provided in Appendix L, along with quantity needed per site and per sampling season.

6.3 Budget Considerations

Total annual operating budget of the protocol is budgeted for \$80,000. This budgetary figure does not include the costs of the core Network staff (see below). The annual cost is split between WRD budgetary funds and Klamath Network funding. The first year of implementation budget and protocol has been developed to be under this amount so that inflationary cost increases over the long-term will not jeopardize program viability. Our goal has been to ensure that the program stays financially sound for a minimum of 7 years, under an assumption of no programmatic budget increases. We have assumed a typical inflationary increase in all costs (salary, benefits, sample processing, and equipment) of 3% per year. Hence, to come just under the budget of \$80,000, our budget for 2010 (the first year of implementation) is \$64,865.50 (Table 14).

Additional budget considerations and costs come from the core Network staff, consisting of:

- Project Lead (assuming GS-11 level): approximately 20 pay periods at \$2,600 per = \$52,000 (the Project Lead time in a lakes year will also include preparation work for stream monitoring in upcoming year)
- Network Coordinator (assuming GS-12 level): approximately 1 pay period at \$3,200 per = \$3,200.
- Network Administrative Assistant (assuming GS-07 level): approximately 1.5 pay periods at \$1,406 per = \$2,107.
- Data Manager (assuming GS-11 level): approximately 1.5 pay periods at \$2,600 per = \$3,900.
- Total costs of core Network staff = \$61,207.

Table 14. Budget for implementation of the Integrated Aquatic Community and Water Quality of Mountain Ponds and Lakes in the Klamath Network Protocol. Numbers in parentheses and red indicate a programmatic deficit, assuming no budgetary increases.

		2010	2013	2016	2019	2022
		Year 0	Year 3	Year 6	Year 9	Year 12
Program Item (@ 2009 costs)						
Salary						
	GS-7 Field Crew Lead 1 FTE @ 8PP; \$1460.80 per PP	\$ 11,686.40	\$ 12,770.04	\$ 13,954.17	\$ 15,248.10	\$ 16,662.01
	GS-5 Crew Member 1 FTE @ 7PP; \$1179.20 per PP	\$ 8,254.40	\$ 9,019.81	\$ 9,856.19	\$ 10,770.12	\$ 11,768.80
Vehicle						
	Field transport/fuel	\$ 3,000.00	\$ 3,278.18	\$ 3,582.16	\$ 3,914.32	\$ 4,277.28
Travel						
	Lodging and per diem	\$ 2,000.00	\$ 2,185.45	\$ 2,388.10	\$ 2,609.55	\$ 2,851.52
Equipment						
	Consumables, GPS units, Calibration solutions, etc.	\$ 5,000.00	\$ 5,463.64	\$ 5,970.26	\$ 6,523.87	\$ 7,128.80
Specimen Processing						
	Zooplankton; 42 samples @ \$87.5	\$ 3,675.00	\$ 4,015.77	\$ 4,388.14	\$ 4,795.04	\$ 5,239.67
	Macroinvertebrates; 42 samples @ \$235	\$ 9,870.00	\$ 10,785.22	\$ 11,785.30	\$ 12,878.11	\$ 14,072.26
	Chlorophyll a; 84 samples @ \$35	\$ 2,940.00	\$ 3,212.62	\$ 3,510.51	\$ 3,836.03	\$ 4,191.74
	Water chemistry; 84 samples @\$160	\$ 13,440.00	\$ 14,686.25	\$ 16,048.06	\$ 17,536.15	\$ 19,162.23
QAPP						
	10% extra samples; verification; probe maintenance; etc.	\$ 5,000.00	\$ 5,463.64	\$ 5,970.26	\$ 6,523.87	\$ 7,128.80
	Total	\$ 64,865.80	\$ 70,880.61	\$ 77,453.16	\$ 84,635.16	\$ 92,483.12
	Surplus/Deficit	\$ 15,134.20	\$ 9,119.39	\$ 2,546.84	\$ (4,635.16)	\$ (12,483.12)

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Standard Operating Procedure (SOP) #1: Preparations, Equipment, and Safety

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP explains what procedures will be completed prior to implementing the field season, including reviewing the budget, hiring the field crew, reviewing equipment needs, preparing site description forms, creating maps, preparing data forms, setting up the database, setting up equipment, meeting park requirements, planning the training, and scheduling field crews.

The timeline for preparing for the upcoming field season is shown in Table 1 to assist in planning preparations. Appendix P, Operational Checklist is also provided to assist in planning and assuring that timelines are met.

Reviewing the Budget

The Project Lead will work with the Network Coordinator each year to review the budget and ensure it meets salary, equipment, travel costs, and projected costs for sample analyses.

Hiring the Field Crew

The Project Lead will be responsible for hiring the field crew leader and field crew member(s). If possible, priority consideration should be given to previous years' crew members to maximize year-to-year consistency in field operations and methods. However, since the interval between implementing the Lakes and Ponds is every 3 years, these seasonal employees may not be available. Thought should be given to assigning preference to crew members from the Streams protocol owing to familiarity with the Klamath Network park landscapes, procedures, and personnel.

The hiring of the seasonal employees should be initiated in the December of the preceding year to allow adequate time for flying the position, advertising the position, and giving adequate lead time for proper consideration of candidate qualifications. The hiring should be complete no later than March.

Table 15. Approximate timeline for starting preparatory tasks for implementing the Lakes protocol, with a field start date of 15 July. * indicates prior calendar year. EOD = Entrance on Duty date.

Task	Anticipated task start date	Must be completed on date
Reviewing the budget	1 October*	31 October*
Hiring the field crew		
Position information to Human Resources	1 November*	14 November*
Post position for 4 weeks on USAJOBS.gov	1 December*	31 January
EOD date for Field Crew Leader	7 July	
EOD date for Field Crew	14 July	
Equipment needs/reviewed/new gear ordered	1 November*	30 November*
Prepare site folders	1 March	30 April
Prepare data forms	1 March	30 June
Coordinate park requirements	1 January	28 February
Prepare and schedule training	1 March	31 May
Schedule field work	1 May	31 May
Prepare database with sites & contacts	1 May	31 May
Prepare water quality sampling equipment	7 July	14 July
Prepare field sheets	7 July	14 July
Prepare Electronic Units	1 July	14 July

The target qualifications of the Crew Leader are:

1. Prior experience in implementing aquatic monitoring protocols.
2. Limnological experience and training (preferably graduate level education).
3. Water quality analysis experience (e.g., water sample collection and processing).
4. Substantial backcountry orienteering and backpacking experience.
5. Experience and aptitude using Global Positioning Systems.
6. High level of physical fitness (ability to carry an average pack weight of 60 pounds over 3+ miles of rough terrain, spend 4 hours sampling a lake, and to return the 3+ miles in a single 12 hour period).
7. Familiarity with the geography and natural communities of either Lassen Volcanic National Park or Crater Lake National Park.
8. Familiarity with Klamath Network Water Quality protocols (including Wadeable Streams protocol).

9. Leadership experience.
10. Strong organizational skills.
11. Ability to get along well with others in a field crew setting.
12. Certification in wilderness first aid (Wilderness First Responder preferred).
13. Ability to manage field crews for long periods of time.
14. Data management skills.

Note that all qualifications may not be possible in any one individual and it is the task of the Project Lead to select for the best combination of the above qualifications. Once selected, the Crew Leader should review the protocol and discuss any questions with the Project Lead. The Crew Leader's Enter on Date should also begin a week prior to the rest of the crew to assist the Project Lead in this SOP.

The target qualifications of the field crew member are:

1. Knowledge and training in aquatic ecology (a demonstrated high interest in ecology can substitute)
2. High level of physical fitness (ability to carry an average pack weight of 60 pounds over 3+ miles of rough terrain, spend 4 hours sampling a lake, and to return the 3+ miles in a single 12 hour period).
3. Ability to get along well with others in a field crew setting.
4. Backcountry orienteering and backpacking experience.
5. Familiarity with either Lassen Volcanic National Park or Crater Lake National Park.
6. Knowledge of (or preferably, certification in) wilderness first aid.

As for the Field Crew Leader, the best combination of the above qualifications should be selected for by the Project Lead.

Soon after being hired, technicians should be mailed the following:

1. A written description of expectations, duties, and responsibilities, to be signed and returned (Appendix B. Expectation of Field Crew).
2. A copy of the Integrated Water Quality and Aquatic Communities – Lakes and Ponds Protocol, including all appendixes.
3. A list of required and recommended personal equipment.

Equipment Needs

Equipment will be organized and inventoried for the field season by the Project Lead several months (should be done in January) in advance of the training session. This allows time to make needed repairs and order equipment and supplies (Appendix L. Equipment List).

Appendix L presents the complete list of equipment needed to implement this protocol. Although the quantities listed are for the minimum number of lakes or ponds needed to accomplish the sampling objectives, extra consumable supplies should be a part of each field kit (e.g., extra 250 mL vials, extra 50 mL zooplankton vials, extra filters). Not all gear is required to be taken into the field and should instead be kept in the field vehicle or housing (e.g., laptop and computer cables).

Gear must be assured to be in excellent working order, and routine maintenance should be done on gear, as specified in later SOPs (e.g., care and cleaning of water quality multiprobes).

Prepare Site Folders

A folder for each lake and pond site to be sampled is developed and includes a site description and map (see example in Appendix G. Example of Site Folder). This site folder should collate and summarize information relevant to planning and field work from previous site visits to assist the new field crew. An additional laminated sheet of field sites (to be prepared by the Project Lead) will be included with the folders. Field site descriptions are completed by the field crew the first year a field site is sampled. Site descriptions include the trailhead or parking location, time taken to hike to the site in previous field visits, latitude and longitude coordinates, and a written description (if necessary) to help navigate to the site. The Project Lead, along with the Crew Leader, will mark and highlight all sites to be sampled in the field season on a set of USGS 7.5 min topographic maps. Coordinates will also be pre-loaded into the Garmin 60 CSx /76CSx or equivalent GPS unit for navigation purposes prior to the start of the field season. It is the responsibility of the Project Lead to make sure project folders are complete prior to the start of the field season and at the end of the field season.

Prepare Data Forms

An adequate number of data forms to fulfill training needs and provide for the entire field season should be printed on waterproof paper using a laser-jet printer (at least 50 sheets). The sheets will be individually numbered and meet the requirements of the Klamath Network Data Management Plan (Mohren 2007). The data forms will include the data sheets for lake habitat measurements and fish collection, as well as the event, incidental photo, calibration/equipment, and training logs. Example data sheets and forms are provided in Appendix F. Field Data Sheets and Logs.

Preparing Database

Prior to beginning the field work, the project database needs to be set up for field work following the methods described in SOP #13: Data Entry. In order to use the database, a GIS shapefile of

lakes to be surveyed and a contact list of all members of the project need to be developed by the Project Lead working with the GIS Specialist. These two files automatically upload into the Access database and it is imperative that field names are kept the same from year to year. The GIS shapefile should be a polygon file projected as NAD83 Zone 10. The shapefile should be stored on the KLMN server at:

S:\Monitoring\Water_Quality_Monitoring\Lakes\Lakes_GIS\PARK\YYYY and the name of the file is PARK_YYYY.dbf. In both the file pathway and the file name, the “PARK” is the 4 letter park code and YYYY is the year of the survey. The shapefile should contain the following fields:

- a. Network
- b. Park
- c. Site_Name
- d. Site_Type
- e. Site Code
- f. X-Coordinate (utm and lat/long)
- g. Y-Coordinate (utm and lat/long)
- h. Coordinate System
- i. PLSS (Public Land Survey System)
- j. Watershed
- k. Subwatershed
- l. County
- m. USGS Map
- n. GIS ID Number
- o. GIS Shapefile Name

Site name in the above shapefile should correspond with the naming convention chosen for the park in question. For lakes in LAVO, we are following the naming convention used by the state of California. They have assigned a unique number to each water body within the state. Using these numbers, we can track each site, be it named or not (the majority of lakes/ponds in LAVO are unnamed). In the rare case that a site is sampled that does not have a number assigned, the nearest named site is used, with the addition of a 0.1 to the site name. For example, a new site near location 11555 that does not have a code is entered into the database as 11555.1. The codes have been obtained from California Department of Fish and Game and are located in the shapefile on the network server at: S:\Monitoring\Lakes Monitoring\Lakes_GIS\CA_Fish_Game. For CRLA, there is no existing naming convention, so the crews will assign a code to each site as

it is visited. This code will be provided to the CRLA resource staff for cross-referencing in future work.

The contact lists should be in an Excel format and include any individual that may be using the database or collecting data as part of this protocol. The files should include the following fields.

- a. Last Name
- b. First Name
- c. Middle Initial (if available)
- d. Organization
- e. Position Title
- f. Address
- g. Email
- h. Phone Number

Park Requirements

In January, the Project Lead should communicate with Lassen Volcanic, Crater Lake, and Redwood National and State Parks to determine the contact person for each park. The Project Lead should contact each park to inform them of the survey schedule and to arrange for:

1. Crew housing and refrigerator facilities.
2. Necessary permits for sampling/backcountry access.
3. Keys necessary for crews to access facilities.
4. Park-specific radio training for field crews.
5. Park-specific safety issues and protocols.

Prepare for and Schedule Training

The training sessions should be scheduled and materials should be prepared as detailed in SOP #2: Field Crew Training.

Scheduling Field Work

The target field season for the monitoring of mountain lakes and ponds is the middle of July to the middle of September. However, the logistics of accessing high mountain lakes and ponds dictate that the initiation of the field season is flexible. Sampling should begin reasonably soon

after snow melts in Lassen Volcanic and Crater Lake National Parks, allowing for safe access to remote sites (but will not begin prior to July 15th). In years with late-lying snow, the Project Lead and Crew Leader will jointly decide when it is safe enough to commence sampling. For hiring purposes, however, it should be assumed that the start of the field season will be on or around July 15th each year. In the event that a field season is delayed, the preferred option is to delay the crew start date as needed, but another option is to spend extra time training or assisting other monitoring efforts as needed. This may be a reality in heavy snow years, but it is not anticipated that delaying the field season will be a regular occurrence. Effects of heavy snow years will also be mitigated by scheduling the lower elevation lakes first, followed by the higher elevation lakes later in the year.

A further priority in determining field schedule is to match the previous sample dates from prior years to the current years. For example, if Lake Helen in Lassen Volcanic National Park was first sampled on July 16th, then the crews should aim for July 16th plus or minus 5 days the following sampling cycle. This will help the comparability of samples across years.

Personnel Safety

During field operations, the motto of the USGS should be adopted: “Safety first.” All members of the field crew should be thoroughly familiar with the USGS safety manual: *USGS National Field Manual for the Collection of Water Quality Data: Chapter A9. Safety in Field Activities* (Appendix C), which was provided to them prior to the beginning of the field season. This manual should be considered the standard reference for safety questions and safety protocols, and in case any clarification is needed, the Project Lead should be consulted. Although this manual is comprehensive and not all elements may apply to the field crew, it is mandatory reading. These points are reiterated in Appendix O. Job Hazard Analyses. It is the responsibility of the Project Lead to go over and certify each crew member in the Job Hazard Analyses.

The following points also bear reinforcing:

- There are two main dangers of aquatic work: drowning and hypothermia.
- Automatic inflating suspender personal flotation devices (SPFD) must be worn while in boats or wearing waders.
- These must be worn on the outside of all clothing layers to ensure proper functioning. Crews are responsible to check that the CO₂ cartridge is full.
- Crews are highly encouraged to carry a spare set of dry clothes in case of accidental submersion.
- Basic first aid and CPR should be known by at least one member of each crew.
- Crews should pay particular attention to lightning storms; the mixture of water, high elevation, and electricity does not make for safe working conditions.

Preparing Water Quality Sampling Equipment

Preparing water quality equipment is a time-consuming process. Ideally, the field crew leader can be brought on a week before crew training and the commencement of sampling to help the Project Lead with these tasks.

Acid Washing Bottles

Clean, uncontaminated bottles and lids are essential for ensuring accurate results from field and laboratory analyses. Even slight amounts of contamination can contribute to large inaccuracies in measurements. Hence, the following protocols must be adhered to in the strictest sense.

This portion explains the process for preparing bottles for water chemistry sampling. These bottles need to be free from contaminants; washing them in a mild (0.5 Normal Hydrochloric Acid [HCl]) will accomplish this. Hydrochloric Acid, even when diluted to a relatively weak concentration, is still a hazardous substance and all precautions for personal safety should be followed. Employees conducting this SOP should frequently consult with the Project Lead or the SOU Chemistry Stockroom manager concerning facilities and safety requirements. At a minimum, the employee should have available:

- A fume hood with ample work room
- Protective eyewear
- Protective gloves (latex type okay)
- Protective labcoat
- Eyewash/shower station nearby (in the same room is preferable; if not located in the same room, then know location of nearest station)
- Ample amounts of a neutralizing agent (e.g., baking soda; sodium bicarbonate).

The bottles that need to be washed are: 250 mL Amber HDPE Nalgene collection vials, two 2 L Amber HDPE Nalgene collection bottles, and 60 mL Amber Boston Rounds collection vials. Consult SOP #9: Water Sample Filtration and Handling for further details on bottle use.

The numbers of bottles needing to be washed for a field season are 130 each of the 250 mL Amber HDPE Nalgene bottles and 60 mL Amber Boston Rounds. This calculation is based on 59 sites, with the possibility of two samples at each site, plus 10% extra for potential loss or breakage. The 2 L collection bottles should be washed when they are brand new and at the beginning of a field season; however, they will be reused throughout the field season without acid washing in between sites (SOP #9: Water Sampling Filtration and Handling).

Protocol for Acid Washing Bottles:

1. Needed materials:

- a. Safety gloves (latex okay)
 - b. Safety glasses/goggles (prescription eyewear is *not* sufficient)
 - c. Lab jacket/coat
 - d. Concentrated HCl
 - e. Distilled Water
 - f. 1000mL Graduated Cylinder
 - g. Glass jugs for storing and pouring HCl (2)
 - h. Funnel, large
 - i. Shallow trays (2 or more)
 - j. Baking Soda (2+ pounds)
 - k. pH meter
 - l. Fume hood/Chemistry Lab Facilities
 - m. Glass stirring rod
2. Start by diluting concentrated HCl (which is 12 “Normal” [N]) down to 0.5N. Do all pouring and measuring in a fume hood. Use appropriate safety gear. At a minimum, wear a lab coat, gloves, and safety goggles or glasses.

- a. Mix 1 liter at a time (or less, if only small quantities are required)
- b. Use the $C_1V_1=C_2V_2$ formula, where C = concentration and V = volume. So, to make 1 L (or 1000 mL) of 0.5N HCl from 12N HCl, use dimensional analysis to solve for the unknown quantity of the 12N acid needed (here denoted as V_1):

$$(12\text{N})(\text{Unknown } [V_1]) = (0.5\text{N})(1000 \text{ mL})$$

$$12 V_1 = 500$$

$$V_1 = 500/12$$

$$V_1 = 41.667 \text{ mL of 12N HCl necessary}$$

(Note that N [Normality] cancels out and you can round up to 42 mL)

- c. Measure 958 mL Distilled water in large graduated cylinder (note that 958 mL is the amount added to 42 mL HCl for a final volume of 1000 mL).
 - d. Pour (using funnel) into 1 L glass jug.
 - e. Measure 42 mL of HCl in a large graduated cylinder (note that it does not have to be exact - ± 2 mL is probably fine (although err on side of too strong)).
 - f. Using a funnel, add the 42 mL of 12N HCl to the 958 mL distilled water in the glass jug.
 - g. Acidic fumes may be given off in this step, so be sure to perform this in a fume hood with hood turned on.
3. After mixing 0.5N HCl, transfer a small aliquot to another glass jug. This is to facilitate easier pouring of the acid, which should minimize spillage.

4. Lay out the bottles to be washed in a shallow tray. Place caps in a separate, shallow tray.
5. Slowly pour enough 0.5N HCl into each bottle, making sure the acid reaches the top. This must also be done in a fume hood. Pour the acid into the bottle so that spillage is contained in the shallow tray. Some spillage is unavoidable, but careful pouring should minimize the spills. Use an additional funnel if necessary.
6. When all bottles have been filled, let sit for up to 8 hours, as time allows. After the 8 hours, carefully dump the used acid into a 25 liter bucket, again in a fume hood, wearing appropriate protective gear.
7. Refill the bottles with 0.5N HCl for an additional wash period. Repeat step 6 after bottles have sat for 8 hours.
8. For the caps, fill a shallow tray with 0.5 HCl and add the loose caps into the acid. While wearing gloves, ensure that each cap is submerged, so that the cap threads are adequately soaked.
9. Let sit for 8 hours, as time allows. Repeat with fresh acid, as you do for the bottles.
10. After the second acid soak, fill the bottles with distilled water and again let soak for 8 hours, or as time allows. After this, rinse all bottles and caps a minimum of three times under flowing distilled water.
11. Allow to completely dry, using a drying oven at 60° C, if available.
12. Cap the bottles and store in a large Tupperware type container.
13. Label the container with the following: type of bottle, acid washed with 0.5N HCl, date washed, and by whom.
14. Dispose of the waste acid that has been poured into the 25 L bucket by neutralizing the acid. When pouring waste acid into the bucket, *do not fill the bucket over half way*.
 - a. Place the bucket with ~12.5 L of 0.5N HCl in a fume hood. SLOWLY add powdered baking soda into the acid.

- b. The sodium bicarbonate in the baking soda will react with the acid to neutralize the pH. This is an exothermic reaction; the acid will bubble and foam as heat is produced.
 - c. Slowly continue to add baking soda to the acid. When the amount of bubbling and foaming begins to lessen, carefully use the pH meter to monitor the pH of the waste acid. A glass stirring rod can expedite the mixing process to accelerate the neutralizing process. The baking soda addition will gradually increase the pH to 6 or 7. When the pH is above 6.5, the waste can safely be poured down the drain.
15. In case of spills, pour a sufficient amount of baking soda onto the spilled acid. After foaming subsides, the neutralized acid can be cleaned up with paper towels.

Preparing Filters for Dissolved Organic Carbon

Samples for dissolved organic carbon (DOC) must be filtered (0.7 μm glass-fiber filters only – Whatman GF/F; Whatman product number: 1825-047) in the field for proper analyses. The filtering process removes suspended particles that may result in erroneous values for analyses of dissolved constituents. Similar to the bottles, the filters must be prepared by cleaning the contaminants and carbon off of the filters. For this, the filters should be precombusted prior to use. The methodology for this is simple: Place filters in folded rectangles of aluminum foil large enough to cover filters (approximately 110 mm X 55 mm) with the dull side in (touching the foil). The foil and filters should then be combusted in a muffle furnace at 500°C for 4 hours. After cooling, the foil and filters should be placed in a plastic Ziploc type bag for field use. For specific instructions on muffle furnace operations, the manual supplied with the muffle furnace should be consulted. The muffle furnace is obtained through the Southern Oregon University stockroom manager.

Preparing Filters for Water Samples

The standard filter for water chemistry (anions, cations, nutrients) is Millipore Nylon Membrane 0.45 μm (Millipore Catalog number HNWP04700). No special preparation is necessary.

Preparing Bottles for Dissolved Organic Carbon

Amber glass vials for Dissolved Organic Carbon analyses must pre-treated similar to the filters. Vials (without the lids) should be placed into a muffle furnace and heated to 475° C for 8 hours. After 8 hours, the furnace should be shut off and the bottles allowed to cool to air temperature overnight. They should then be capped and stored for us. Note that the lids should not be combusted, only acid washed.

Preparing the Electronic Equipment

GPS Units

Navigation Units

Programming of GPS units needed for navigation (e.g., Garmin 76CSx units) to field locations should be undertaken with the help of the Network Data Manager or GIS Specialist. Entries should be set up to allow the easy use of the GPS unit, e.g., habitats are identified in the unit both

with lake name. The datum NAD 83, Zone 10N is the Klamath Network standard that will be used for this protocol.

Trimble

The following settings should be set by the GIS Specialist or Data Manager prior to field work. Open ArcPad on the GPS unit and select GPS Preferences and enter the following:

GPS	Protocol: <i>Trimble GPSCorrect</i>
Capture	Ensure the box is checked to <i>Enable Averaging</i> Number of Positions to Average: Points: at least 60 Vertices: at least 30 Streaming: Position Interval: 1 Distance Interval: 3 meters
GPS Height	Input antenna height (1.0m if no antenna)
Datum	GPS Datum: <i>D_WGS_1984</i>

GPSCorrect

- In ArcPad, from the GPS menu, select Trimble GPSCorrect. Tap the section list button and then select *Setup*. In **Logging Settings**, ensure that Log GPS to SSF is set to *On*. Set Log H-Star Data to *No*. In **GPS Settings**, most often the slider is set all the way to productivity. Adjust **Real-Time Settings** as desired. If available, activate WAAS satellites to improve GPS accuracy. WAAS satellite are positioned over the equator, so for WAAS to work you will need a view of the southern sky.

GPS Analyst

These settings allow you to keep all GIS data in NAD83 UTM Zone 10N but collect GPS positions in WGS84. Upon upload to GPS Analyst, they will automatically reproject to NAD83 UTM Zone10N.

- Geographic Transformation: *NAD_1983_To_WGS_1984_5*
- Spatial Reference for GPS-enabled feature classes: *NAD_1983_UTM_Zone_10N / NAVD_1988*

Preparing GIS Data to Transfer to GPS Units

The following procedures should be done by the GIS Specialist or Network Data Manager and require that Trimble GPS Analyst extension for ESRI ArcGIS Desktop is enabled.

GPS-Enable Geodatabase and Feature Class

1. Open ArcCatalog.
2. Right-click the geodatabase and select *Properties*. Select the Trimble GPS Analyst tab.

3. Check the *GPS-enable geodatabase* check box as well as check boxes next to the relevant feature classes. Click OK.
4. The Select Geographic Transformation dialog appears. In the dropdown menu, select *NAD_1983_To_WGS_1984_5* and then click OK. At this point, a GPS Sessions feature dataset has been added to the geodatabase.
5. Now each feature class must be individually modified to your particular accuracy requirements. Right-click a feature class you want to GPS-enable and select *Properties*.
6. Select the Fields tab. You are going to add two new fields. Click the next blank row in the Field Name Column and type “Average_Est_Accuracy.” In the same row, click the Data Type column and select *Double* from the dropdown menu. Create one more field, name it “Worst_Est_Accuracy,” and define its data type as *Double*. When finished, click *Apply*.
7. In the Properties window, select the Trimble GPS Analyst tab. Store average estimated accuracy in the field you just created, *Average_Est_Accuracy*. Store worst estimated accuracy in the field *Worst_Est_Accuracy*.
8. Repeat Steps 5 through 7 for each layer you will be using in the field.

Prepare Feature Class Structure for Field Data Collection

Once a feature class is GPS-enabled, it is ready for GPS data but it may not be ready for attribute information you want to input in the field. The GIS Specialist and Data Manger will make sure the appropriate attribute fields are in the feature class.

Check Out Data for ArcPad

Besides checking out GPS-enabled feature classes, you can also check out background layers to view for context while in the field.

1. Open ArcMap.
2. Add layers you will need while working on ArcPad in the field. These include GPS-enabled feature classes, which are editable, as well as background layers. Symbolize the layers and set the zoom to the way you want them to be viewed in ArcPad, then save the ArcMap Document.
3. If the Trimble GPS Analyst toolbar is not currently open in the ArcMap interface, go to the View menu and select it from the Toolbars list. Or, right click any toolbar and select *Trimble GPS Analyst* from the menu.
4. From the Trimble GPS Analyst toolbar, click Get Data for ArcPad.
5. On the first page of the Get Data for ArcPad wizard, check all the layers you want to have while working on ArcPad in the field, then click *Next*.

6. On the next page, check all the feature classes you want to be able to edit, then click *Next*.
7. On the following page, choose a spatial extent option. Keep in mind that the full extent of the selected layer(s) will in some cases create large file sizes. If you choose the current display extent, be sure it covers the geographic area you would like to GPS.
8. On the same page, create a name for the folder that will be created to store the data (the format *project_YYYYMMDD* is recommended). Click and go to the location where you want to store the field data folder.
9. Click *Finish*.
10. **It is very important that at this point that you save the ArcMap Document!** If you neglect to do this, GPS Analyst will not “remember” checking out the data. The ArcMap document should be saved to the following location:

S:\Monitoring\Lakes Monitoring\Lakes_GIS\Seasonal Data\xxxx

Where xxxx is a folder you created that list the year the survey will be started.

Transfer ArcPad Shapefiles to a Handheld

1. Connect the Trimble GPS unit to the computer with a USB cable. Microsoft ActiveSync will open. (If it does not, open it manually. If it did not open because the device was not recognized, you will need to troubleshoot the connection before continuing.)
2. Cancel or exit the ActiveSync partnership wizard if it opens. It is not necessary to establish a partnership.
3. In ActiveSync, click *Explore*. Copy or drag the field data folder (created in section 2.3.3, step #8) onto the mobile device. (Alternately, you can copy or drag the folder to the mobile device icon in Windows Explorer.)

When finished, disconnect the USB connection between the mobile device and computer and verify that the folder and its contents are on the field computer and can be opened in ArcPad.

Preparing the Amphibian Unit

Similar to the GPS units, the Amphibian (the trade name of the Eureka Environmental computer for running the water probe, ***not*** a unit for monitoring “amphibian” species) unit (or similar water quality data logger) should be loaded with pre-made files prior to the start of the field season. See SOP #10: Multiprobe Usage and Calibration for more details.

Preparing the Laptop

A laptop computer, for the use of the field crew, is to be prepared for the upcoming season. The use of the computer is for backing up data (from the Trimble, Amphibian, and digital cameras). File structure will be set up by the Project Lead for this purpose (SOP #12: Post-site Tasks).

Additional files included on the computer will be backups of protocol-associated programming (e.g., ArcPad files for Trimble, site list for loading onto the Navigation GPS unit) as well as electronic copies of the complete protocol, appendixes (i.e., this current document), previous field season images, reports, and any incidental materials of use to the field crew.

The folders that will be included are:

Project_Database. This is the Access database into which data will be entered each night.

Documentation. This folder will contain any documentation that might be needed while in the field (e.g., Lakes Protocol, Equipment User Guides, etc.).

GIS_Data. This folder contains a copy of all the GIS data that were loaded on the Trimble unit and Garmin unit prior to starting the field season. These data are available as a backup in case something goes wrong with the layers on the handheld units.

Identification. This folder will contain any information needed to help with the identification of animals (e.g., Fish Identification Guides, ID Cards).

Other. An addition folder that can be used for any data files that do not “fit” into one of the above categories.

Literature Cited

Mohren, S. R. 2007. Data management plan, Klamath Inventory and Monitoring Network. Natural Resource Report NPS/KLMN/NRR—2007/012. National Park Service, Fort Collins, CO.

Standard Operating Procedure (SOP) #2: Field Crew Training

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP explains what procedures will be completed to ensure that the field crew is adequately trained. Training should include procedures on sample collection and processing, data management methods, identification of fish and amphibians, emergency procedures and safety, as well as National Park Service rules and ethics.

Sample Collection and Processing Training

Ensuring that field crews are adequately trained begins with the selection of qualified individuals who have prior experience in aquatic ecology. However, since it will not always be possible to hire an “idealized” individual, an extensive training period should be implemented so that crews are familiar with all techniques used in the field. These training protocols should always be adhered to, even if highly experienced individuals are hired. The collection and processing techniques presented within these protocols are susceptible to error from improper collection techniques (e.g., water chemistry contamination) or by modifying techniques (e.g., invertebrate collection). Hence, even highly experience crews should be trained and retrained each field season in a regimented and documented format. Quality training is the first step of this protocol’s QAPP (SOP #16: Quality Assurance Project Plan).

Training begins with the delivery of a copy of these full protocols so that crew members can familiarize themselves with all SOPs, rationales, and goals of the project. Pre-reading of the protocols by the crew will ensure that training sessions are as efficient as possible.

Prior to the field season, the Project Lead and Field Crew Leader should consult on the procedures for training and the standards that the crews will be held to. The training will start with classroom sessions, where the Project Lead instructs the two crew members in the following:

1. Background on I&M program objectives, sampling design, and data analyses.
2. Field sampling methods and QA/QC concerns.
3. Equipment operations and maintenance.

4. Field and laboratory sample processing and handling.
5. Fish and amphibian species identification and a primer on wildlife diseases.
6. Recording, managing, and organizing data, both manually and digitally.
7. Safety in the backcountry, including vehicle usage rules.
8. Orienteering.
9. Backcountry rules and ethics.
10. Computer data entry.
11. NPS administrative tasks (e.g., timesheets, sick leave).

Many of the training elements may be best demonstrated in the field; hands-on training is crucial. The preferred format for training is for the Project Lead to accompany the entire field crew to sites during the first week of the field season. The Project Lead conducts a workshop at the first site to be sampled, instructing the crew on proper techniques. In this process, extra time is taken to perform each technique, with the Project Lead also providing rationale for each method to give the crew members the proper context for the methodology.

After the initial demonstration site, the Project Lead should observe the field crew at a minimum of two additional sites, giving corrective comments to ensure proper collection techniques of each protocol. If additional training sites are necessary, the Project Lead and Field Crew Leader should adjust the site schedule as necessary.

The training should extend to tasks and SOPs relating to activities occurring at the field lodging (e.g., SOP #12: Post-site Tasks, on electronic data backup, data storage, probe maintenance, etc.)

As each SOP is discussed, demonstrated in a workshop, and mastered by crews in the field, the training should be documented using Training Logs (Appendix F).

Specific Concerns

In addition to the specific protocols, the field crew should read, be familiar with, and follow the practices of:

- 1) “*Leave No Trace*,” from the Center for Outdoor Ethics (as provided in Appendix D); and
- 2) “*Guidelines for use of live amphibians and reptiles in field and laboratory research*” from the American Society of Ichthyologists and Herpetologists (relevant sections, as provided in Appendix K)

First Aid, Safety, and Emergency Procedures

All project personnel will be working in remote areas at some point during the field season; it is therefore essential that everyone, to the extent possible, be prepared for emergency situations.

Although providing full-scale training in wilderness first aid is beyond the scope of this training program, we recommend the crew read and discuss in detail a simple wilderness first aid booklet prior to beginning field work. Crews should also discuss the Job Hazard Analyses (Appendix O) with the Project Lead and potential responses to theoretical emergency situations, having a clear understanding of what to do if they or someone else on the crew becomes seriously injured or goes missing.

In addition, all crew members should be trained in the use of handheld 2-way radios that will be provided to each team (Appendix E. Icom Radio Use Handbook).

Backcountry Rules and Ethics

In addition to the standard guidance of “*Leave No Trace*” (Appendix D), project personnel should receive instruction on backcountry regulations for the parks, including permit requirements and procedures, campsite restrictions, food storage, fire season restrictions, etc. Note that some of these rules differ among the parks. If possible, arrangements should be made for a backcountry ranger or other qualified Park Service employee to meet with the crew to discuss these topics. Regardless, it is the Project Lead's responsibility to make sure that all crew members understand the rules they must follow.

Data Entry, Management, and Organization

Project Leads, technicians, and interns will be trained in data entry and quality control procedures according to SOP #13: Data Entry and SOP #16: Quality Assurance Project Plan at the onset of the field season. As a group, the data entry protocol will be reviewed. Each pertinent database will be demonstrated on the computer by entering several example records. Time will be allotted for the field crew to practice the data entry system and for questions to be answered.

Standard Operating Procedure (SOP) #3: Site Selection and GRTS

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

The SOP explains the procedure that is undertaken to populate the list of sites that the field crews are to sample for a particular field season. Rationale for choosing these methods are detailed in the protocol narrative and the reader is referred to the relevant section for more details.

Site Selection for CRLA Ponds and Lakes

Crater Lake National Park (CRLA) presents an unusual challenge for selecting perennial habitats. Although there are 37 lakes/ponds identified using USGS topographic and GIS shapefiles, many of these are not perennial. Preliminary site visits during the pilot project indicated that of 23 habitats observed, all but four of these are dry by the end of the water year (31 October 2008). Based on this ratio, we only expect a maximum of seven perennial habitats in CRLA. See the narrative for more discussion on this topic.

Hence, the site selection for CRLA is to sample *all* perennial, lentic habitats. Using the data from the pilot project, the first year of implementation will only visit the sites identified as having water at the end of the water year. As future visits determine water presence/absence, the sampling list will be adjusted accordingly (if repeat visits show a habitat to be perennial).

Site Selection for Lassen Volcanic National Park Ponds and Lakes

The procedure for developing a site list will only be necessary once, at the beginning of the project.

1. Available GIS shapefiles from the National Wetland Inventory project are obtained from their web site (<http://wetlandsfws.er.usgs.gov/>) or obtained from the park GIS Specialist.
2. A list of suitable perennial ponds and lakes is created based on the following criteria:
 - a. The water body is <1000 m from an established trail or road.

- b. The water body is <25 m deep.
 - c. The slope of the surrounding terrain is <30%.
3. The judgment site is removed from the available list (Lake Helen).
4. The remaining list is then used in GRTS (Generalized Random Tessellation Stratified) software, a plug-in for the statistical program “R,” (below text and Figure 1). This application compiles a list of 30 random samples of water bodies in a spatial stratified structure. The number of oversample sites, “over,” should be set to “30” to provide adequate numbers of sites in the case of unsuitable samples sites in the original list.

Running GRTS in “R” requires knowledge of the program and programming that is beyond the scope of this protocol. However, background information and how to information is included here: http://www.epa.gov/nheerl/arm/designing/design_intro.htm

The code used in “R,” once the library spsurvey is added, should look like this:

```
## Choosing Lakes: GRTS Example
path<-"\\Water_Quality_Monitoring\\Pilot_Study\\Lakes_GIS\\GRTS\\working\\"
library(spsurvey)

design <- list(None=list(panel=c(Index=18, Time1=18, Time2=18, Time3=18,
Time4=18,Time5=18, Time6=18, Time7=18, Time8=18, Time9=18,
Time10=18),seltype="Equal",over=100))

grts(design, DesignID="Site", SiteBegin=1, type.frame="area",
      src.frame="shapefile", in.shape=paste(path,"lakes_select",sep=""),
      sp.object=NULL, att.frame=NULL,
      id=NULL, xcoord=NULL, ycoord=NULL, stratum=NULL, mdcaty=NULL,
      startlev=NULL,
      maxlev=11, maxtry=1000, shift.grid=TRUE, do.sample=TRUE,shapefile=TRUE,
      prjfilename=NULL, out.shape=paste(path,"LAVO_WQ_Sites",sep=""))
```

Specific purposes of the programming are detailed in Figure 1.

This is a necessary library to load for GRTS to run successfully

This is the path to the shapefiles that will define the suitable monitoring location

This is a test section of code that is commented out

```
## GRTS Example: Experiment(s) using the library(spsurvey)
path <- "C:\\1_Veg_Monitoring\\Results\\CRLA\\"
library(spsurvey)

##mz.dbf <- read.dbf(paste(path,"Matrix_Zone",sep=""))
##mz.shape <- read.shape(paste(path,"Matrix_Zone",sep=""))

design <- list (None=list (panel=c (Panel1=25,Panel2=50),seltype="Equal",over=10))

grts(design, DesignID="Site", SiteBegin=1, type.frame="area",
     src.frame="shapefile", in.shape=paste(path,"Matrix_Zone",sep=""), sp.object=NULL, att.frame=NULL,
     id=NULL, xcoord=NULL, ycoord=NULL, stratum=NULL, mdcaty=NULL, startlev=NULL,
     maxlev=11, maxtry=1000, shift.grid=TRUE, do.sample=TRUE, shapefile=TRUE,
     prjfilename=NULL, out.shape=paste(path,"sample",sep=""))

tmp <- read.shape(paste(path,"sample",sep=""))
plot(tmp,type="n",xlab="X coord",ylab="Y coord")
points(tmp[tmp$panel=="Panel1",],pch=16,col="red")
points(tmp[tmp$panel=="Panel2",],pch=16,col="green")
points(tmp[tmp$panel=="OverSamp",],pch=16,col="blue")
```

This is the name of the output GRTS points shapefile

This is the name of the input suitable area shapefile

This the crucial section of code in designing the GRTS sampling design. If there are to be multiple "panels" or sets of points each group is designated by the Panel1, Panel2, etc... The number of points for each panel the value after each Panel name. The selection type is always set to since there is equal weighting. The over value is the number of overrun points to be available for replacement points

Figure 7. Layout of GRTS code to be run in the Software "R."

Standard Operating Procedure (SOP) #4: Order of Work

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP lays out an example flow of work for the field crew. The goal is for a two person crew to be able to sample a small pond or lake in 2-3 hours and a larger lake in 3-4 hours. This will then allow adequate access time to sample the largest of lakes in a 10-12 hour workday. Additionally, it is hoped that multiple smaller lakes could also be accomplished in a 10-12 hour workday.

Unlike other SOPs, this SOP should be flexible for different crews. As long as certain principles are held and individual SOPs are adhered to, crews should be free to modify the order of tasks as they see efficient. For example, if a local park specialist accompanies the crew for a day, there may be a more efficient way for the crews to accomplish the workload with a third person.

This SOP explains how the following SOPs are integrated into an overall work flow so that procedures do not interfere with each other (Figure 1). Hence, the following descriptions do not detail the steps, but they refer to the other SOPs. In some cases, a SOP is started early and allowed to continue while the crew focuses their attention on other matters.

The order of work presented represents a schematic of what worked well during the pilot project used in developing these SOPs. The primary considerations in modifying this order of work are:

1. Doing the zooplankton, secchi depth, and the water probe prior to water sample collection may stir up bottom sediments which will give spurious results for water chemistry. Hence, water sample collection should be a priority before other collections. SOP #8: Water Sample Collection has more details on water collection.
2. Deployment of the gill net for fish collection (SOP #7: Fish Collection and Processing) should be started as soon as possible. The net must be deployed for a minimum of 2 hours. At smaller lakes, the other SOPs may be completed within this time window, allowing the crew to sample additional nearby sites that day.

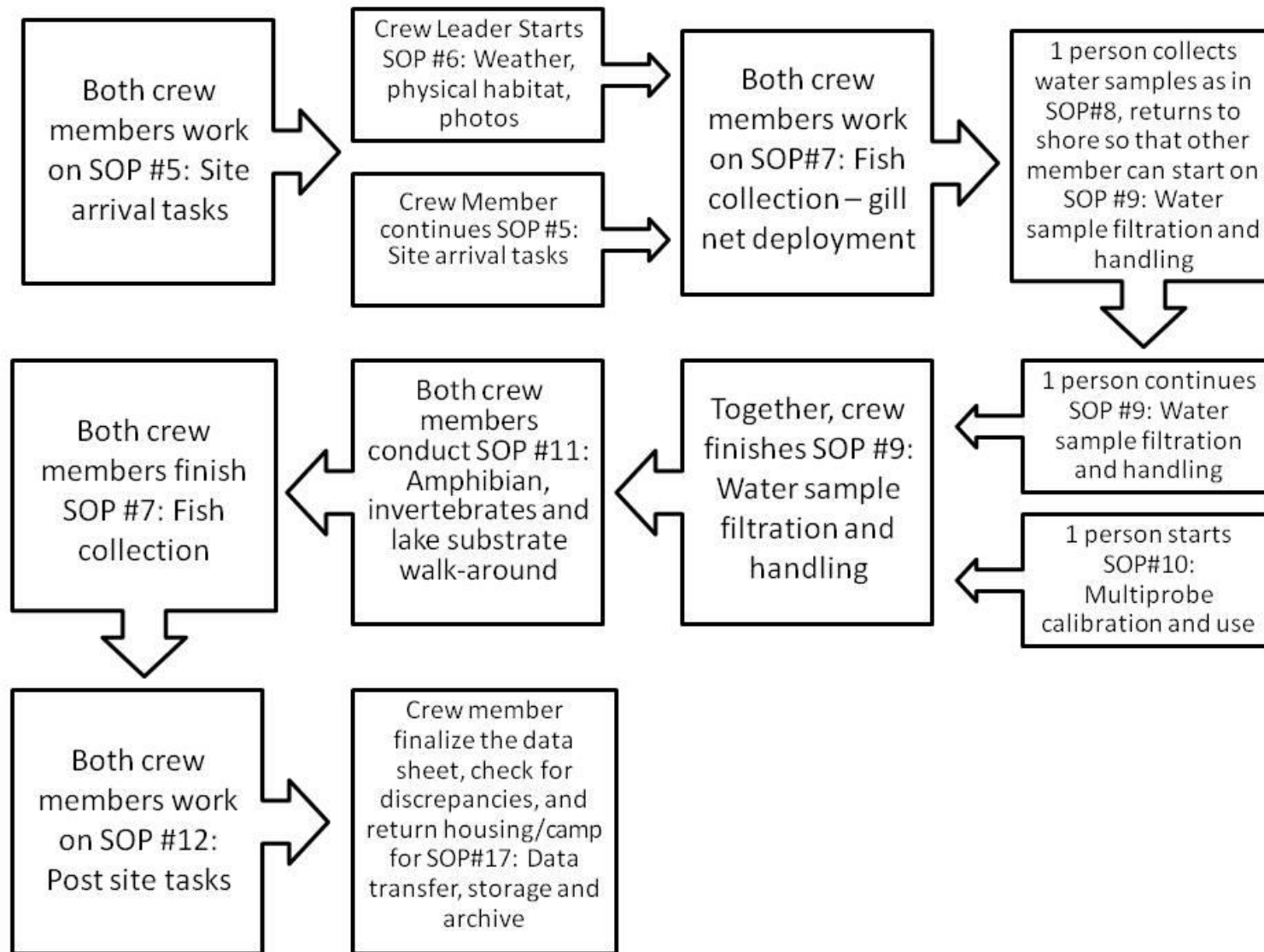


Figure 8. Suggested order of work for efficient sampling of lakes and ponds.

Standard Operating Procedure (SOP) #5: Site Arrival

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP explains the immediate tasks that need to be completed upon site arrival and includes the miscellaneous tasks associated with sampling that may not be included in other SOPs.

To reduce the possibility of transporting disease and exotic organisms to other sites, crews should keep their aquatic footwear and trail footwear separate. Upon reaching the proximity of the site, the crew should remove their hiking footwear, and don their aquatic footwear. Their hiking footwear should be left away from the aquatic habitat, to be used when all aquatic sampling is complete.

Establish a Working Area

1. Scout the lake, either by walking around or a visual scan, to select the best location to set up.
2. Choose this location based on the following criteria:
 - a. Weather conditions – shady for hot days, sunny for cool days, and sheltered for windy days.
 - b. Isolation from trails or campsites
 - i. Although interaction between monitoring staff and the public is generally encouraged, park visitors may ask many questions that will distract the field crew, resulting in delays or processing errors.
 - c. Always ensure that there is shade available for storage of processed water chemistry samples nearby. If this is not possible, be prepared to store the insulated cooler of samples within the lake itself, tethered to the shoreline.
 - d. Lack of woody debris (i.e., large logs, roots, etc.) – this condition is necessary for the setting of the gill net (for fish sampling), as well as access for the inflatable raft. Slippery logs with sharp above or below water branch stubs (sharp stubs of

broken off branches) can be very dangerous; they are best avoided. Moreover, they can snag and damage the gill nets, as well as puncture the raft.

- e. Flat working area – working with vials, jars, and electronic equipment is hampered if the field crew must work on a steep slope. Note that a large flat rock on a slope may be adequate, provided it is stable.
- f. Open vegetation – dense vegetation may facilitate the loss of small pieces of equipment (e.g., forceps).

Preparing Gear and Equipment

1. Inflate the two-person raft. Follow the manufacturer's recommended process for this. Inflate in an area that will minimize the risk of puncture.
 - a. During the inflation process, double check that there are no leaks in the boat. If there are leaks, repair them immediately. The vulcanizing agent in the repair kit requires time to work (up to 30 minutes). For smaller punctures, duct tape may be an alternative solution. For large leaks, overnight repair using Seam Grip® or similar product along with patch material may be necessary.
2. Set up a water chemistry area in the shade. Using a pack towel or small tarp, arrange an area that will be debris/dirt free to minimize/eliminate contamination during water chemistry processing.
3. Unload remaining gear, minimizing “gear scatter.” Keep things organized for specific tasks, and keep electronic gear out of the sun as much as possible. If shade is limited, place gear underneath field packs. Assess and anticipate the solar and shade paths to ensure that sensitive gear will stay in the shade through the sampling period, if possible.

Prepare and Pre-label Sample Vials

This is a procedure that can be done prior to arriving at the site (e.g., the member who is not driving can do this during transit or it can be completed the night before). If it has not been done prior, it should be done upon site arrival. Because the needs of the labels depend on the SOP (e.g., water samples versus invertebrates), the needs are detailed in later SOPs.

Preliminary Data Recording

1. Begin to fill in General Habitat data sheet (Appendix F).
 - a. **Record** the crew names and any additional observers (e.g., park staff).
 - i. Do not record “Bob” or “Nancy.” Give full first and last names. Do not abbreviate the first name (e.g., no S. Smith; rather Sean Smith).
 - b. **Record** the site name and the unique lake code.

- c. **Record** the date (use yyyyymmdd format [e.g., 20081121 for the 21st November 2008]) and arrival time (use 24 hour time). Note this specific format is essential for data management and must be strictly adhered to.
 - d. **Record** the trails and roads used to access the site and the time taken for each.
- 2. Use the Garmin 60/76CSx (or similar) GPS unit to determine and record the Latitude/Longitude of the set-up location. Ensure that the datum is in NAD 83 Zone 10.
- 3. Determine and record the county the lake is in using USGS topographic maps.
- 4. Record qualitative water level:
 - a. High – **Record** as high if the water level appears to overflow established shorelines. For example, if terrestrial grasses and vegetation is inundated and a distinct shoreline is difficult to discern.
 - b. Normal – **Record** as normal if water level appears to reach or nearly reach (within 1.0 meters on average) an established shoreline, and no or little littoral zone is exposed.
 - c. Low – **Record** as low if water level is substantially drawn down from an established shoreline (> 1.0 meters on average), leaving large portions of the littoral zone substrate exposed.
 - d. Dry – **Record** as dry when no standing water is apparent. If this is the case, halt sampling due to ephemeral nature of water body.

Standard Operating Procedure (SOP) #6: Weather, Physical Conditions, and Site Photography

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP explains the process for measuring weather and habitat parameters, along with digital documentation of the site using digital cameras.

Weather

Weather observations are of a mostly subjective basis and are intended to convey a sense of the current conditions experienced by both the field crew and the biota of the lake being sampled. Weather conditions should be based on the conditions present upon site arrival and not upon conditions during or after sampling. Large shifts in weather (e.g., change of two or three units on wind scale, change of sunny conditions to cloudy and rain) can be noted in the general field notes section of the data sheet. However, small changes in weather need not be denoted.

1. Categorize the observed weather conditions upon arrival:
 - a. Cloud cover
 - i. Sunny – visible sky has less than 25% cloud cover.
 - ii. Partly Cloudy – visible sky has between 25 and 50% cloud cover.
 - iii. Mostly Cloudy – visible sky has between 51 and 75% cloud cover.
 - iv. Cloudy – visible sky has over 75% cloud cover.
 - b. Precipitation
 - i. No precipitation.
 - ii. Light rain – use this category for precipitation that is “drizzling” or “sprinkling.” A useful distinction is that a light rain would only require intermittent use of windshield wipers when driving a car.

- iii. Hard rain – use this category for precipitation that is falling rapidly and would quickly result in soaking crew members or gear. This would be a rain that would require frequent use of windshield wipers while driving a car.
 - iv. Light snow – use this category for snow fall that is composed of small flakes that is not actively accumulating snow on the ground. This category is similar to light rainfall, albeit snow.
 - v. Heavy snow – use this category for dense snowfall that is actively accumulating on the ground.
- c. Using a compass, record the direction that the wind is coming from. This can be accomplished in several ways:
 - i. Tie a short piece of flagging to the tip of a walking stick, and note the direction the flag is blown towards (and record the opposite direction – this is the direction the wind is coming from).
 - ii. Using a handful of fine dirt/sediment/vegetation, slowly pour the material out and note which direction the material is blown towards (and record the opposite direction – this is the direction the wind is coming from).
- d. If white caps are present on the lake, note as such. If ripples are present, note as such.
- e. Air temperature
 - i. Measure and record using an armored thermometer. Place in the shade for at least 15 minutes, so that the thermometer has equilibrated adequately.
- f. Wind scale
 - i. Use a Beaufort wind scale to estimate wind speed (Table 1, Figure 1).

Physical Conditions

The measurements covered here are brief. Although other, in-depth measurements are made, they are collected in the process of other SOPs, and as such are covered in later protocols.

1. Inlets/Outlets
 - a. If there are flowing inlets/outlets apparent from the approach hike or initial walk around, record the numbers of each. This will also be recorded in the Trimble GPS survey (SOP #11: Amphibian, Invertebrates, and Lake Substrate Walk-around).
2. General terrain (terrestrial)

- a. Circle the appropriate descriptors to describe the surrounding landscape. If more than one descriptor applies, multiple entries can be marked.
 - i. Forested: If surrounding terrain is composed of obvious, dominant trees.
 - ii. Meadow: If surrounding terrain is composed mostly of grasses, although some shrubs and trees may be apparent.
 - iii. Alpine: If the lake basin and landscape appears to be above the tree line.
 - iv. Sub-Alpine: If the lake basin is below the tree line.

Digital Photographs

Digital photographs should be taken at all sites, including instances when a crew decides that a site is unsuitable for sampling (e.g., too shallow, dry, access time too long, etc.). The minimum number of photographs to be taken is four, looking in the cardinal directions (North, South, East, and West). Direction should be determined using a compass. If time allows, the photos should be taken across the waterscape. If the lake is large, some photos may be taken during the amphibian survey and walk-around (SOP # 11: Amphibian, Invertebrates, and Lake Substrate Walk-around). Metadata for digital documentation must be entered into the datasheet (Appendix F). Although four images is the minimum, crews are encouraged to take additional photos, especially of sampling activities, unusual habitat features, or of scenic interest. However, the metadata standards must be maintained (SOP #14: Photo Management).

Table 16. Wind scale guide. Use your best judgement and try to use multiple descriptors in doing so (e.g., land and lake conditions).

Beaufort number	Wind speed		Description	Wave height		Lake conditions	Land conditions
	mph	m/s		m	ft		
0	<1	<0.3	Calm	0	0	Flat.	Calm. Smoke rises vertically.
1	1-3	0.3-1.5	Light air	0.1	0.3	Ripples without crests.	Wind motion visible in smoke.
2	4-7	1.5-3.3	Light breeze	0.2	0.7	Small wavelets. Crests of glassy appearance, not breaking	Wind felt on exposed skin. Leaves rustle.
3	8-12	3.3-5.5	Gentle breeze	0.6	2	Large wavelets. Crests begin to break; scattered whitecaps	Leaves and smaller twigs in constant motion.
4	13-17	5.5-8.0	Moderate breeze	1	3.3	Small waves.	Dust and loose paper raised. Small branches begin to move.
5	18-24	8.0-10.8	Fresh breeze	2	6.6	Moderate (1.2 m) longer waves. Some foam and spray.	Branches of a moderate size move. Small trees begin to sway.
6	25-30	10.8-13.9	Strong breeze	3	9.9	Large waves with foam crests and some spray.	Large branches in motion. Whistling heard in overhead wires. Umbrella use becomes difficult.
7	31-38	13.9-17.2	High wind, Moderate Gale, Near Gale	4	13	Sea heaps up and foam begins to streak.	Whole trees in motion. Effort needed to walk against the wind.
8	39-46	17.2-20.7	Fresh Gale	5.5	18	Moderately high waves with breaking crests forming spindrift. Streaks of foam.	Twigs broken from trees. Cars veer on road.

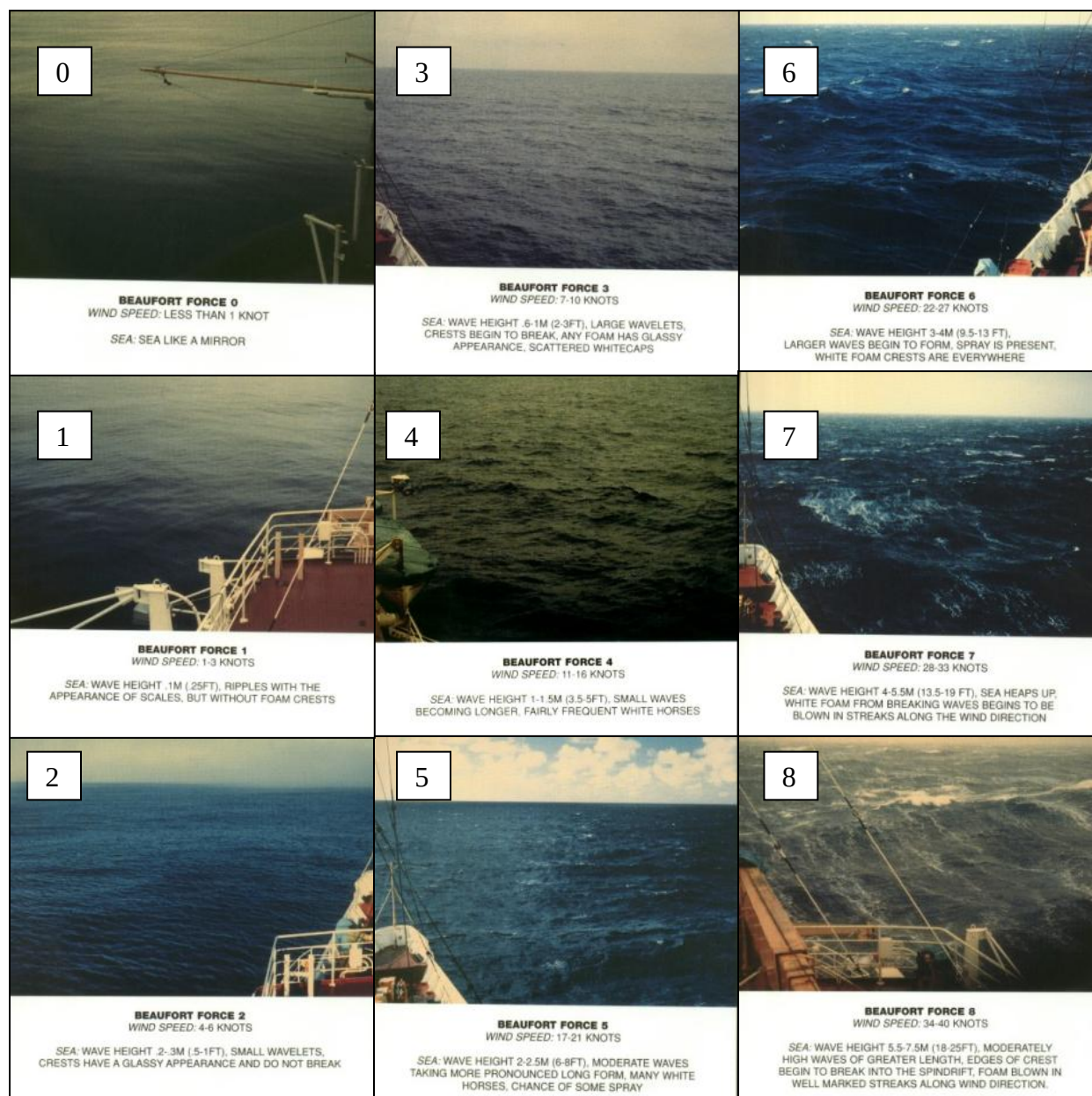


Figure 1. Pictorial guide to Beaufort Wind Scale, to be used in conjunction with Table 1.

Standard Operating Procedure (SOP) #7: Fish Collection and Processing

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP explains the process for collecting fish and how to process collected specimens. The deployment of a gill net is a necessary step in determining the presence/absence and composition of the fish assemblage.

Without sampling a habitat, determination of fish absence (as opposed to presence) is impossible; therefore, in all but the shallowest of habitats, the crews must deploy the gill net and it should be deployed for a minimum of 2 hours (**note: in the cases of many fish [>20], the Crew Leader can make the determination to pull the net in sooner**). If there are no fish collected in the 2 hour period, fish are recorded as “not detected.” The minimum depth required for deploying the gill net is approximately 1 m, but if the habitat bottom is comprised of abundant felled trees or vegetation that would otherwise tangle the net, the Crew Leader can make a judgment call to not deploy the net. If the gill net is not deployed, the crew must be constantly aware for fish signs during the course of the sampling. If no fish or fish signs are observed in this time period, a general note should be made that no fish or fish signs were observed. However, this should not be taken to mean that fish are absent in this habitat. Crews should also be on the lookout for signs fish (e.g., feeding fish, carcasses, fish in shallows, etc.). If observed, **record** observations in the general site descriptions.

Deployment of gill nets, associated with time deployed, will be used to determine “catch per unit effort,” which is a measure of relative abundance. Because catch rate is dependent upon the mesh size (smaller mesh entraps smaller fish; larger mesh entraps larger fish), monitoring for trends requires that gill net specifications be kept constant throughout monitoring. We have elected to use a monofilament, variable mesh size gill net configuration used by the USGS (R. Hoffman, personal communication): Total length of 42 m, 1 m deep, 3 panels: 1) 25mm mesh X 6 m length; 2) 35 mm X 12 m length; and 3) 50 mm X 24 m length. This net (with the smallest mesh closest to the shore) will be deployed out from the lake shore along the bottom of the lake (see below for exceptions).

Note of Caution

The public perception of gill netting for fish is a delicate situation. Crew members should strive to maintain a low profile during gill netting procedures but should be quick to stress the scientific importance of monitoring fish populations in our national parks when there are public inquiries. Likewise, the crew members should be quick to share their findings with the public while gill netting. For instance, anglers might be interested to know what species and size are present. Crew members should also note that they are monitoring fish populations and not trying to eliminate the fish from the lake.

Gill Net Deployment

To prepare for deployment, the crew needs to ready the inflatable raft, inflatable life jacket, and the gill net. To ready the gill net, a 250 mL HDPE Nalgene bottle should be partially filled with lake water. The end line at the top of the gill net should be wrapped within the threads of the bottle, so that when the lid is tightened, the end line is secured to the partially filled bottle. This serves as a rudimentary float for the gill net. A mesh bag with several rocks (weighing approximately 1 kg) should be secured with flagging or similar cord to the bottom leadline at the end of the gill net. The rocks serve as a rudimentary anchor, so that the net rests suspended from the bottom. With a suitable site chosen, the crew is ready to deploy the net.

1. To deploy the gill net, the onshore crew member holds the majority of the net, while the other crew member (holding the float/anchor end) rows the inflatable boat toward the center of the lake.
2. When the entire length of the gill net is pulled from shore to the center of the site, the float and anchor end of the net is released and the net is allowed to sink to the bottom of the lake. While feeding the net out, care and time should be taken to ensure that there are no tangles that may interfere with the capture of fish. The net should be deployed in as straight a line as possible, perpendicular to shoreline. If the size of the lake prevents full deployment of the gill net, the actual deployed length should be noted and recorded. In lakes with large amounts of coarse woody debris on the bottom, the gill net can be allowed to float rather than sink (i.e., do not use the anchor bag). This will decrease the likelihood of net snagging.
3. The crew member on shore secures the shore end of the gill net to vegetation or a rock cairn at the immediate water-land interface. The shore end of the net is positioned at the water-land interface to reduce incidental capture of birds, mammals or other wildlife.
4. The time that the net was first fully deployed should be recorded on the data sheet.

Gill Net Removal and Fish Processing

1. After a minimum of 2 hours of deployment (the crews should have completed SOPs #6, 8, 9, 10, 11, and 12 in this time), the net can be retrieved for fish processing. Note that deployment can be longer than 2 hours.

2. The time that net retrieval is begun should be recorded on the data sheet.
3. The crew member in the inflatable boat paddles out to the end of the gill net. Because the net should be sunk, the crew member must grab the net in a shallow portion of the lake and maintain a hold on the net while moving to the end of the net. The easiest way to accomplish this is to use the net to pull the raft out to the end (but the oars should still be in the boat in case the net gets away or if the wind is heavy).
4. Once the crew member is at the end of the net, he or she should lift it out of the water so that it does not snag on the bottom as the net is taken in. The crew member **on the shore** should pull the net in, weaving the retaining spine in and out of the float line bobs. This will result in a manageable, orderly net ready for disinfection and redeployment at the next lake.
5. While the net is being retrieved, the crew member on the shore is responsible for removing large particles of detritus or woody debris from the net. Removal of woody debris can be a time consuming process; rushing the removal of a twig may cause damage to the gill net. Crews should minimize the amount of damage done to the gill net during the retrieval and cleaning process.
6. As the net is retrieved, fish should be removed. Care is taken to prevent damage or undue pain to fish upon removal. In general, there are two methods to remove the fish from the nets: 1) Push the fish forward through the mesh, assisting the movement of the monofilament around the gills and abdomen. This will be the easiest if the fish is small relative to the size of the mesh. 2) Back the fish out of the mesh. This is more time consuming, but maybe the only option if the fish is large relative to the mesh size. Take care to minimize damage to the fish as the monofilament lines will become entrapped beneath the gill opercula unless great care is taken during fish removal.
7. Collected fish should be placed in a collapsible bucket filled with water. If many fish are caught, multiple buckets may be necessary.
8. After all fish are removed and the gill net is completely retrieved with float bottle and anchor bag removed, the net should be laid (still bundled up) someplace in ample sunlight (if available) so that it can dry thoroughly, and be disinfected according to SOP # 12: Post-Site Tasks.

Fish Processing

Processing of fish should be completed as quickly as possible after fish capture. Four key measurements are made: 1) Species ID; 2) Fork Length and Total Length; 3) Weight; and 4) Indication of Disease. Species determination should be made using fish guides (an example is provided in Appendix H). Specimens that are unidentifiable or questionable (e.g., a fish species that does not match the descriptions in the guide) should be retained as voucher specimens for further study. Vouchered specimens should be placed in doubled Ziploc bags containing 70% ethanol. A paper label (in pencil on weather resistant paper) indicating location, date, and collector should be inserted into the doubled Ziploc bags. Vouchers will be transferred to a large 2 L Nalgene vial at the housing facilities.

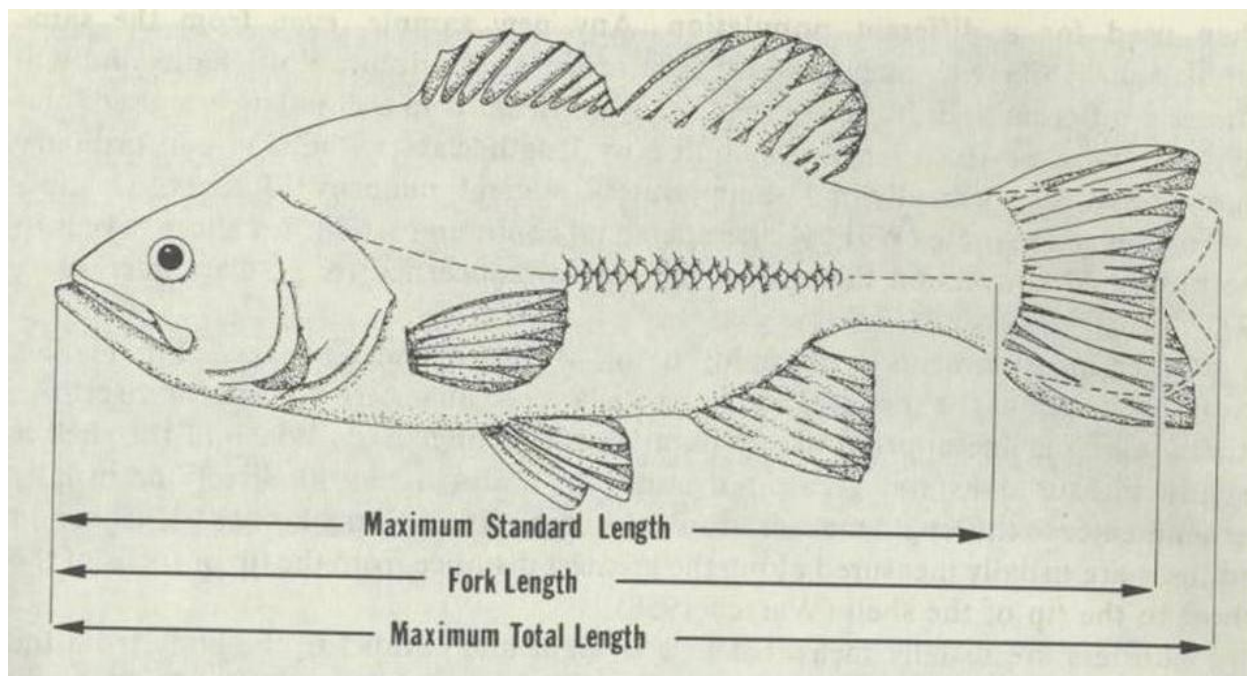


Figure 9. Common length measurements of fish. Maximum Standard Length is measured from the snout to the base of the caudal peduncle. From Gutreuter and Anderson, 1983.

Fork length (FL) is the length measured from the tip of the snout to the middle of the caudal fin (Figure 1). A fish board should be used to determine FL by placing the snout against the backboard and measuring the point where the caudal fin is shortest (i.e., the middle). Total Length (TL) is measured from the snout to the longest measurement, when the tail is condensed (e.g., not splayed out).

Weight is determined using a pesola scale. To weigh a fish, place the fish in an appropriate sized Ziploc bag, and clip the scale to the bag. Record the weight (note that the Ziploc bag weight can be considered negligible for fish weight). There are two scales available for weighing; crew members should use the one most suitable for the particular fish.

Fish should also be inspected for external anomalies or disease (Figure 2). The type of anomaly, location, and severity should be scored on the data sheet following the guidelines in Table 1, adapted from Ohio EPA (1989). Other signs of diseases should be noted as observed (see Figure 3 and Table 2).

All data should be entered and recorded into the appropriate field data sheet (Appendix F).

At the discretion of the crew leader, viable fish should be returned to the lake from which they were captured. Fish that are identified as nuisance exotic species should be euthanized, while exotic sport fish should be returned. Native fish should be returned if viable and with strong signs of life (e.g., responsive to stimuli, active swimming). If an individual is lethargic and unresponsive, the fish should be euthanized. The requirements of the permit issued by the parks should also be consulted as to how nuisance exotic fish should be disposed of.

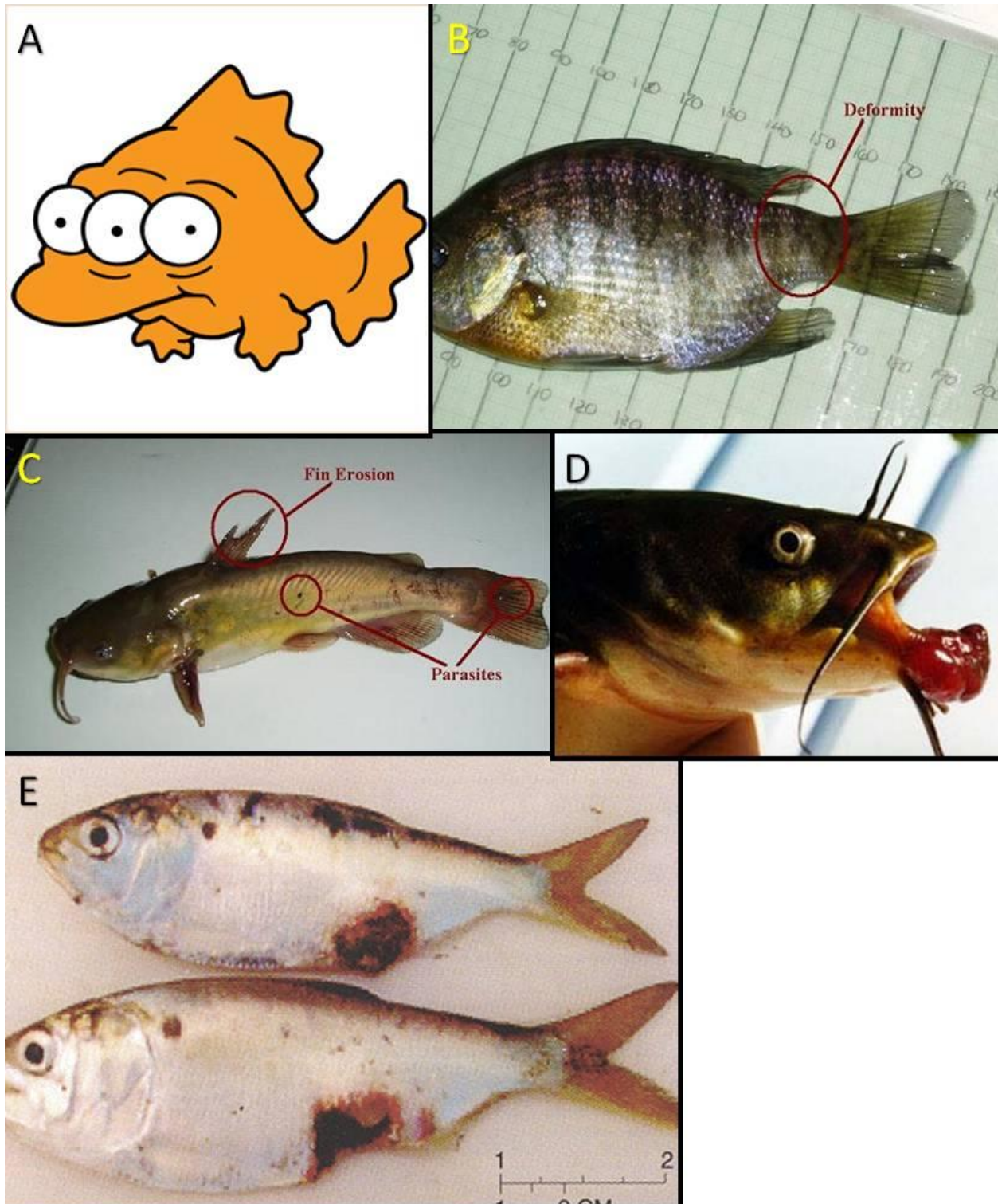


Figure 10. Examples of fish deformities, eroded fins, lesions, and tumors: A. Goldfish from Springfield Lake, Tri-state area (fish caught by H. Simpson); B. Bluegill showing tail deformity; C. Bullhead showing fin erosion and parasites (Images B and C from US Fish and Wildlife Service; D. Unidentified catfish showing tumor (from National Oceanic Atmospheric Administration); E. Menhaden showing lesions (from United States Geological Survey).

Table 17. Deformities, eroded body parts, lesion, and tumor (referred to as “DELT”) scoring guidance; adapted from Ohio EPA (1989).

Type of Anomaly	Severity Grade	
	Mild	Severe
Deformity (fin, head, vertebrate, other body part)	1 deformity	>1 deformity
Eroded body part (fin, gill opercula, etc.)	<2 eroded body parts, or fins not eroded past ray fork	≥3 eroded body parts, or fin eroded to base
Lesion (open sore, exposed tissue, ulcer)	<2 lesions < the size of the largest scale	≥3 lesions, or a lesion > the size of the largest scale, or raw tissue
Tumor	<2 tumors < the diameter of the eye	≥3 tumors, or 1 tumor larger than the diameter of the eye

Table 18. Other diseases that should be noted on field forms as identified (adapted from Ohio EPA 1998).

Other diseases	Description
Anchor worm	A parasitic copepod, appearing as a slender, worm like body with head attached in fish flesh.
Black spot	A trematode parasite that appears as small, black cysts on skin and fins.
Leeches	Hirudinea, generally identifiable by the presence of two suckers, ability to contract or elongate.
Fungus	Appears as white cottony growth.
<i>Ichthyophthirius multifiliis</i>	A protozoan that appears as a white spotting.
Popeye	Bulging eyes; this can be caused by one of several factors.
Whirling disease	Caused by <i>Myxobolus cerebralis</i> , causing spinal, head, and jaw deformities and black tails.

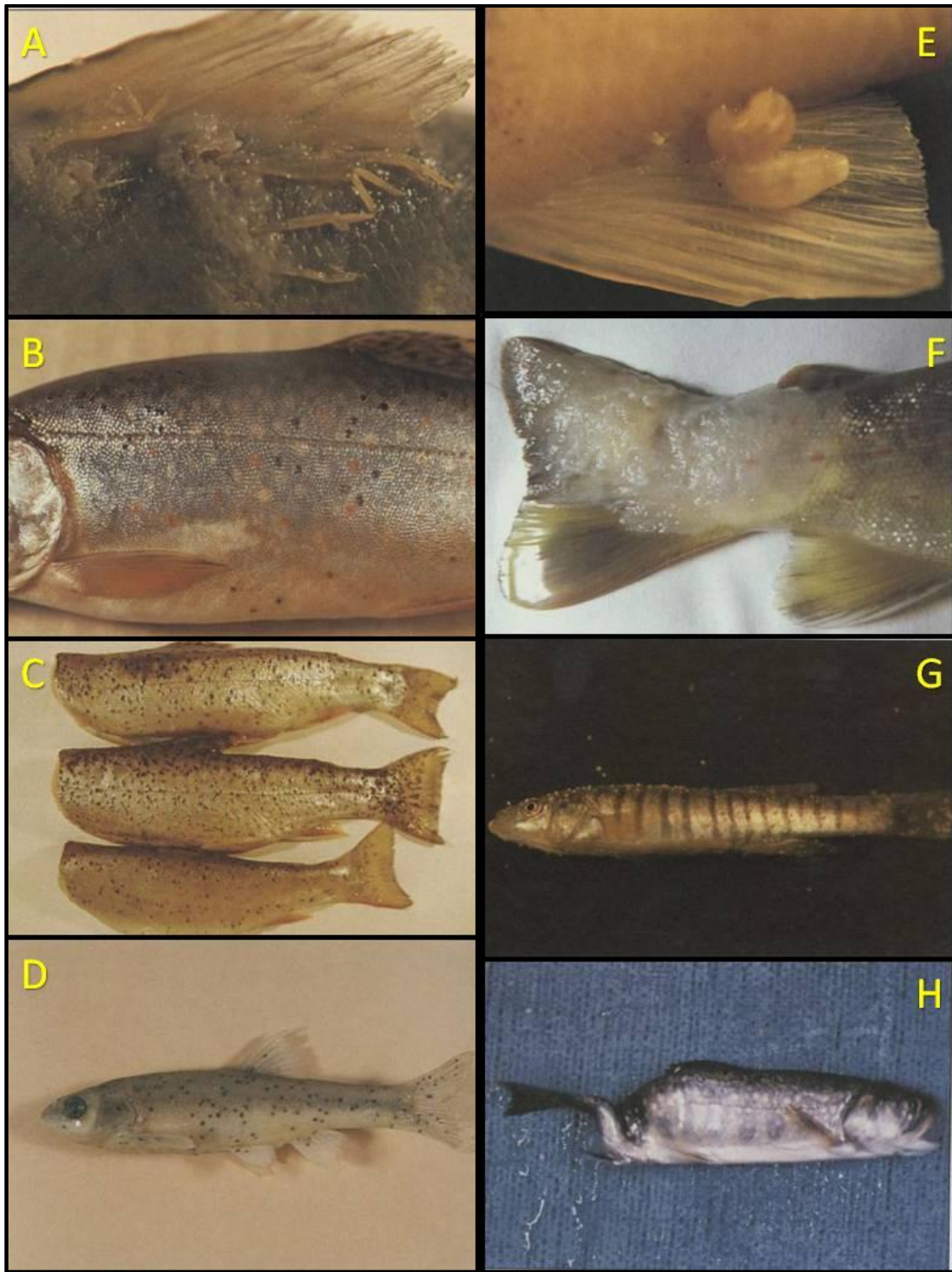


Figure 11. Examples of external fish disease: A. Anchor worms infecting rainbow trout; B. Blackspot disease on brook trout; C. Severe blackspot disease on brook trout; D. Blackspot disease on flathead chub; E. *Myxobolus* leech on fin of yellow perch; F. Fungus growing on fin and peduncle of brown trout; G. *Ichthyophthirius multifiliis* infection on plains killifish; H. Whirling disease (*Myxobolus*) in brook trout. All images from Mitchum (1995).

During euthanasia procedures, public attitudes should be considered. The techniques presented here should be carried out as humanely as possible. The American Society of Ichthyology and Herpetology (ASIH, 2003; Appendix K for amphibians) lists the techniques of pithing, spinal cord dislocation, or decapitation as acceptable forms of euthanasia. Pithing is the insertion of a sharp object (probe or knife blade) into the brain cavity of the animal, followed by a rapid twisting and scrambling. Spinal cord dislocation, or breaking the neck of an organism is accomplishable by a sharp impact to the animal's head. Swift decapitation with a sharp blade has also shown to be humane and quickly result in mortality. Because pithing involves the insertion of a sharp object into an organism that is difficult to hold onto, and because spinal cord dislocation may be unaesthetic to the public, the preferred method for euthanasia is decapitation. However, because fish species can be tolerant to low oxygen levels, decapitation should also be followed by pithing the brain cavity. The Project Lead will insure that all crew members are trained in euthanasia techniques.

Several methods are acceptable for fish carcass disposal: 1) Burying carcasses in a hole a minimum of 200 m from a water body as deep as possible; 2) Sinking carcasses in the deepest part of the lake by puncturing the swim bladder (likely done during decapitation); and 3) Transporting carcasses secured in plastic bags and disposing in a landfill.

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Standard Operating Procedure (SOP) #8: Water Sample Collection, Zooplankton, and Secchi Disk

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP describes the tasks associated with collecting the water sample for laboratory (e.g., nutrients, anions, Chlorophyll *a*, etc.) as well as field analyses (alkalinity; although the methodology for this is in SOP #9: Water Sample Filtration and Handling). It also describes the methodology for associated tasks that should be done during the similar time period (e.g., Secchi Disk depth, max depth, and Zooplankton sampling).

Order of Tasks

Certain tasks presented here pose a risk of stirring up bottom sediments, which can contaminate a water sample. (**The protocol is for sampling the water, not the water and sediments!**) Because of this, it is necessary for the crews to perform the collections in the following order:

1. Find the deepest portion of the lake.
2. Collect the water sample at 0.5 m above the lake bottom.
3. Collect the water sample at 0.5 m below the lake surface.
4. Collect the Zooplankton sample.
5. Take a Secchi Disk measurement.
6. Return to shore for filtering and processing samples.

Finding the Deepest Portion of the Lake

Water collection should occur in the deepest portion of the lake. In lakes or ponds that have a uniform depth, collection should occur near or in the middle of the lake (i.e., maximize the distance from any shoreline). The crew member assigned to this task should paddle the boat to potential deep spots, where the water clarity indicates depth or the lake shoreline suggests the deepest spot may be. As the crew member visits these spots, he or she should test the depth with the hand-held sonar, following the manufacturer's instructions. This generally entails a simple process of holding the sonar vertically, with the tip just below the water surface. The crew member depresses a button or switch, and the depth reading is displayed. Care must be taken to ensure that the sonar is held upright and not at an angle.

Upon finding the deepest portion of the lake, the crew member should make visible references to the lake shores to help triangulate his or her position. The depth should be recorded on the dive slate and the crew member should prepare to collect the deep water sample.

Collecting Water Samples

Water samples should be collected using a Beta Van-Dorn Style water sampler. Crew members should be familiar with the sampler's operation before venturing out into the lake. Prior to collection, the crew should rinse the sampler and the 2 L collection bottles (labeled "shallow" and "deep") with water from the lake.

1. At the deepest portion of the lake, the ends of the bottle should be locked "open" by securing the cable to the appropriate pegs (Figure 1).
2. Once locked open, the bottle should be slowly lowered by the line (marked in 0.5 m intervals) until the bottle is 0.5 m above the lake bottom.
3. Once at the appropriate depth, the weighted messenger attached to the line is released down the line so that the trigger mechanism is activated, releasing the bottle endcaps.
4. The water sampler is then brought back to the surface and drained into the 2 L amber Nalgene collection bottle.
5. The procedure is repeated in the same location for the shallow sample, 0.5 m below the lake surface.

Important note: If upon retrieval of the deep sample, the crew member notices copious sediments, the sample should be retaken in a similarly deep section. Likely, the technician stirred up the bottom sediments while deploying the sampler, resulting in sample contamination. For this reason, the hand-held sonar should be used immediately prior to the water sample collection to confirm that the crew member is still in the deepest portion of the lake, and that they are deploying it at the correct depth.

In windy conditions, it may be difficult for the crew member to maintain their position. Deployment of a simple anchor, made up of a small bag of rocks tied to a long line can help; the crew member needs to be aware that anchor deployment can similarly stir up sediments, contaminating the sample. For this reason, anchors should not be deployed until after the collection of the deep water sample.

If the lake is less than 2 m deep, follow the above protocol, but only take a single sample at the midpoint.

Zooplankton

Zooplankton are collected with a 64 μm Wisconsin style net with a 12.7 cm diameter opening (Wildlife Supply Company product no. 40-A45 or similar), with a "dolphin style" collection cup (Figure 1). A mini-carabiner or similar link should be used to attach the leading ring to a 25 meter rope, marked off in 0.5 meter increments with a permanent marker. Samples are collected

at the same spot in the lake as the water sample collection (deepest part of the lake). Five replicate tows are made.



Figure 12. Wisconsin style plankton net.

Prior to commencing all tows, check that:

- A. The white clamp on the surgical tubing spout is securely closed.
- B. The dolphin collecting cup is securely screwed to the plankton net.
- C. There are no holes or tears in either the net or the collecting cup.

If the lake is shallow (less than 2 meters deep), perform a series of **horizontal tows**:

- 1. While retaining the attached rope at the 5 meter mark, throw the net in a haphazard direction from the inflatable boat.
- 2. When the net lands in the water, start pulling the net towards yourself at a rate of approximately 0.5 m/s. This rate should be fast enough so that the net does not sink or that the collection cup does not drag on the lake substrate.
- 3. While pulling, the net opening should be within 0.5 meters of the water surface.
- 4. When the net is at the boat, lift the net quickly out of the water and into the boat.
- 5. Insert the surgical tubing drain into a 250 mL collection bottle labeled, “ZOOPS,” and release the white clamp. Rinse the interior of the dolphin cup with lake water using a squirt bottle. This can be done either with the collection cup attached to the net or removed. Rinsed adequately so that no debris or organisms remain in the cup – this should be done with less than 50 ml of water.
- 6. Cap the “ZOOPS” bottle, re-secure the dolphin cup, and snap the white clip closed.

7. Repeat steps 1 – 6 until five replicate tows have been made.
 - a. Ensure that tow is made in a different direction.
 - b. Continue adding the collected sample to the single “ZOOPS” collection bottle, so that the samples are composited into a single sample.
8. Take the composite sample back to shore for further processing:
 - a. At the shoreline, remove the dolphin cup from the net to filter out the excess water.
 - b. With the white snap securely fastened, pour the composite sample into the dolphin cup. This will retain the sample while the excess water is filtered through the side mesh panels, tilting the dolphin cup from side to side.
 - c. If the sample has copious sediments/organisms, it may be necessary to “massage” the side panels to help the water filter out.
 - d. When most of the water has been filtered out, place the surgical tubing spout into a 50 ml plastic centrifuge vial. (It may be convenient to drive the conical end into the lake shore sediments to keep the vial stable and upright).
 - e. Unclasp the white snap, allowing what little water is there to flow into the vial with the organisms. Sparingly use the squirt bottle to wash remaining organisms into the vial. The target amount of water in the vial is 15 ml.
 - f. Add a small piece of Alka-Seltzer[®] (or similar magnesium bicarbonate) to the vial. This will produce carbon dioxide gas, which will “relax” the organism for preservation. Allow several minutes for the relaxing process to work.
 - g. Fill the vial with 95% ethanol (the existing water in the vial will dilute it down to an approximate 70% level).
 - h. Label the vial according to SOP #9: Water Sample Filtration and Handling, using an external and internal label.
9. **Record** the tow length and type (horizontal or vertical) on the datasheet or on the field computer.

If the lake is deep (greater than 2 m), perform **vertical tows**.

1. Retaining the rope, lower the net to within in 0.5 to 1 meter of the bottom (using the 0.5 m marks on the rope).
2. Pull the rope vertically at a rate of approximately 0.5 m/s.
3. Inspect the sample to insure that no silt or bottom sediment accidentally entered the net. If there is, repeat the tow.
4. Process the sample as above in steps 4-9, again achieving 5 tows.

Water Clarity

Water clarity should be measured with a 20 cm black and white Secchi disk at the deepest portion of the lake (where the water samples were collected). Sunlight intensity can affect the readings; if possible, the measurements should be made as close as possible to noon. However, the timing of site arrival and weather conditions (e.g., clouds) are subject to variability but measurements can still be made. **Likewise, the use of polarized sunglasses can cause variability between users – ALL SUNGLASSES should be removed prior to measurement.**

1. At the deepest part of the lake, slowly lower the Secchi disk over the sunny side of the boat.
2. Record the depth at which the disk disappears from sight, start pulling it up, and then record the depth that it reappears on the dive slate. Repeat and record for a total of three times. Record time of day and surface conditions.
3. If the disk remains visible all the way to the bottom, check the box for “water clarity – 100%.” Do not write the depth of the lake, as this may be misinterpreted as the depth where the disk disappeared or reappeared when in reality it was still visible.
4. Record notes about the basic clarity or coloration of the water (e.g., brown or reddish tinged water on the dive slate).
5. Return to shore and transfer Secchi depth data to master field sheet.

Standard Operating Procedure (SOP) #9: Water Sample Filtration and Handling

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP describes the process for the labeling, prepping, filtering, and handling of water samples (for filtered water and Dissolved Organic Carbon [DOC]). Storing and shipping are covered in separate SOPs. It also includes steps for processing Chlorophyll *a* samples and field analysis of alkalinity.

All of the tasks associated with this protocol are highly susceptible to contamination or bias from mishandling. As with the other protocols, it is very important that the crew completely follow these protocols to avoid the introduction of contamination and error into the analyses. Special attention should be given to the use of gloves, filter handling, and general cleanliness. Field conditions, especially wind and gusts, can make this difficult. If there are issues caused by contamination or accidents, the samples should be repeated (including the repetition of SOP #8: Water Sample Collection, if necessary).

The tasks are listed in the order they should occur: 1) Labeling, 2) Filtration preparation, 3) Filtering (metals and ions, DOC, and Chlorophyll *a*). After the completion, the process must be repeated for the remaining sample, if the lake was more than 2 m deep.

Labeling

Labeling is an important task and is a common source of mishandling specimens. All labeling (water chemistry [filtered and DOC], Chlorophyll *a*, zooplankton, and macroinvertebrates) should be followed in the following formats for consistency and accuracy. Ideally, vial and bottles are pre-labeled the night before or during transit using an electronic label machine (e.g., Brother Model PT-1400 or similar). Labels produced by these label makers are resilient, are always legible, weather resistant, and do not fade or smear when exposed to solvents (especially Ethanol). The only caution is that they must be placed on a dry and clean surface (hence, they should be done first, before sample processing). Labels should always be placed on the vial or bottle, and not on the lids.

The following information should be included: Site Name; Site Code; date (yyyymmdd format); sample type; and county and state. Site Names and Site Codes are provided in a summary sheet given to the field crews prior to the start of the field season. Sample type is one of the following: Unfiltered water, Filtered water, Dissolved Organic Carbon, Chlorophyll *a*, Zooplankton, or

Invertebrate Sweep. For Unfiltered water, Filtered Water, Dissolved Organic Carbon, and Chlorophyll *a*, there should also be notation of “deep” or “shallow.” Examples of how labels should be laid out are presented in Figure 1.

CLUSTER LAKE 3 CODE310 20080918 FILTERED WATER DEEP SHASTA CO, CA	CLUSTER LAKE 3 CODE310 20080918 INVERTEBRATE SWEEP SHASTA CO, CA
CLUSTER LAKE 3 CODE 310 20080918 DOC SHALLOW SHASTA CO, CA	CLUSTER LAKE 3 CODE310 20080918 CHLOROPHYLL A DEEP SHASTA CO, CA

Figure 13. Preferred layout for labeling sample vials. This format should be used for all samples: Invertebrate sweeps, Dissolved Organic Carbon (DOC) - shallow and deep, Filtered Water – shallow and deep, Chlorophyll *a* – shallow and deep, and zooplankton.

If necessary (e.g., the batteries die or the label-maker breaks), labeling can be accomplished using colored vinyl tape (preferably white) and a permanent marker (e.g., Sharpie). The same information should be recorded as above in a legible manner. Because Ethanol erases permanent markers, samples preserved in Ethanol (Invertebrate Sweeps and Zooplankton) should be labeled using a paper label written in pencil on weather proof paper (e.g., “Rite in the Rain”) and inserted inside the sample bottle.

Because Invertebrate Sweep samples may consist of multiple bottles, multiples should be labeled identically, with an additional label denoting the sequence and total number of bottles (e.g., 1 of 3; 2 of 3; 3 of 3).

Nutrients

Water samples for nutrients are not filtered, so that total nutrients measured.

1. Start by “pre-conditioning” the sample vials. Approximately 20 to 30 ml of sample water from the deep sample should be poured into the labeled, deep unfiltered water vial.
2. Recap, shake, and invert sample vial so that entire interior of bottle is washed.
3. Unscrew the vial lid partway, and shake vigorously so that the vial and cap threads are also washed.
4. Empty and shake the vial and cap to drain excess water.
5. Fill with sample water up to the top, so that a meniscus is actually above the lid of the vial. Carefully cap, minimizing any potential “headspace.”

6. Place the bottle in as cool and insulated a place as possible. This will generally be within the lake itself. The preferred method is to use a cooler pouch (within a mesh bag secured to the lake shore via a cord). If snow banks are nearby, the crew can use the cooler pouch and snow.

Prepping for Filtration

Samples should be returned to the shore as soon as possible after their collection (usually after Secchi disk readings) and processed immediately after they are returned to shore. The crew member should not work on other SOPs until water sample processing is completed. Note that the other crew member can proceed to SOP #10: Multiprobe Calibration and Use.

Set up for processing should be done in the shade, on level ground, and in a place with minimal loose debris. Shelter from wind is also ideal. A field towel should be laid out to provide a work surface relatively free from contaminants. The equipment for filtration should be clean and set up nearby.

Water Filtration

Water for chemical analyses must be filtered and frozen to retard biological and chemical processes that can affect the chemical constituents in the time period between collection and analysis. This time period should not be more than 28 days, but the realities of field work, shipping samples, and the sample backlog of a chemical analytical lab dictate that this period may extend beyond 28 days. Hence, it is very important that samples be adequately filtered and preserved to maintain sample integrity until analyzed. Holding times and methods for dealing with holding time exceedances are detailed in SOP #16: Quality Assurance Project Plan.

Process a single sample in its entirety. In other words, filter the “shallow” water for metal and nutrients, filter the “shallow” water for DOC, filter for Chlorophyll *a*, and then do the alkalinity analysis. Only then should the crew member start processing the “deep” sample.

Because three different filter types are used (one for cations/anions, one for DOC, and one for Chlorophyll *a*), plastic storage bags containing the filters should be clearly labeled to avoid using an inappropriate filter for any specific sample processing.

Procedure for Cations/Anions

Water samples for cations/anions are filtered through a 0.45 µm nylon membrane filter (Millipore, product number: HNWP4700) into an acid washed (SOP# 1: Preparations, Equipment, and Safety), amber high density polyethylene (HDPE) bottle and frozen as soon as possible. Any changes in filtering mechanism or filters should follow the procedures outlined in the QAPP (SOP #16: Quality Assurance Project Plan).

1. Using latex gloves and forceps, insert a clean, unused Millipore HNWP filter into the inline filter holder as shown in Figure 2. Assemble following the diagram and tighten the inflow fitting to the outflow fitting. Tighten until the body o-ring is compressed, and do not over-tighten. Make certain that the inflow fitting is a Luer fitting for attaching a Luer-lok syringe, and not a tube fitting.

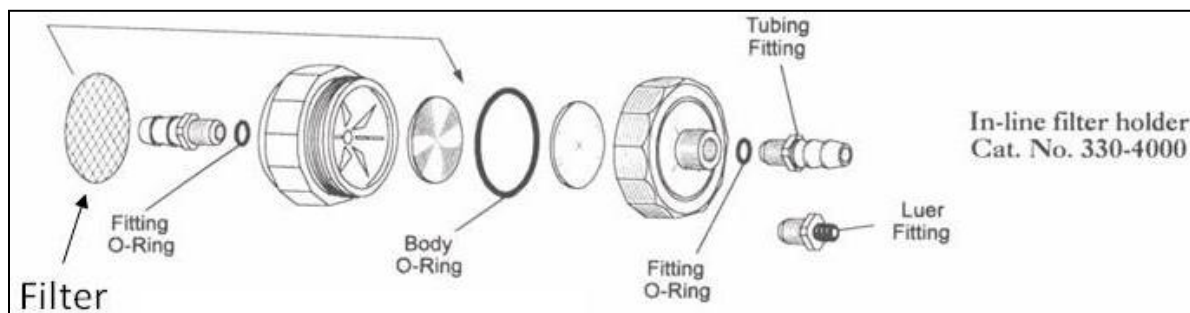


Figure 14. Diagram of inline filter holder (NALGENE®) showing assembly. In this configuration, the input is on the right (use the Luer fitting) and the outflow is on the left.

2. Using a 50 mL nylon syringe, draw 10 mL of sample water into the syringe. With the syringe inverted (i.e., plunger down, nozzle down), pull the plunger down until nearly removed from syringe body. Do not remove the plunger from the body of the syringe. Invert the syringe several times to pre-condition the syringe with sample water. Depress the plunger to expel the water onto the ground or into a waste container.
3. Draw up a full syringe of water from the sample being processed, and attach the syringe to the filter holder using the Luer-Lok fitting. Precondition the filter and filter holder by depressing the plunger and expelling 10 mL of water onto the ground or into a waste container.
4. Expel another 10 to 20 mL of the water into a properly labeled (see above) 250 mL sample collection bottle. Loosely attach the cap, and gently shake the bottle. Dump the water onto the ground or into a waste container.
5. If any water remains in the current syringe, filter into the bottle. Refill the syringe with the sample water and continue to filter water until the bottle is filled. **Do not withdraw the syringe plunger while the syringe is still attached to the filter holder; the filter may shred inside.** Any headspace (air at the top of the bottle) in the bottle should be minimal (and ideally absent). Cap tightly.
6. The filter holder should now be readied for filtering water for DOC analyses.

Procedure for Dissolved Organic Carbon

Dissolved organic carbon (DOC) analyses require that the sample be filtered through 0.7 μ m glass-fiber filters and that the sample be contained in a 60 ml amber Boston Round glass vial. Glass vials and the filters should be prepared as in SOP#1: Preparations, Equipment, and Safety.

1. Sample water should be filtered following the above protocol for cations/anions and nutrients. Differences in the methods are simply the type of filter used (pre-ashed 0.7 μ m glass fiber filter – Whatman product number: 1825-047), the vial used (acid-washed and pre-combusted amber glass), and that the syringe does not need to be preconditioned (having been preconditioned in the above filtration).

2. Avoiding contamination is also crucial for DOC analyses. Skin oils, small soil or litter particles could easily contaminate the sample. Likewise, any headspace in this vial can affect the analyses. The crew member should slowly top off the vial so that a convex meniscus is formed. Upon capping, this should eliminate any headspace.
3. The vial should then be stored in a cool and insulated place as described above.

Procedure for Chlorophyll *a*

In contrast to the above two procedures, for Chlorophyll *a* the filter is collected as the sample and not the filtered water. A total of 500 mL of water should be filtered through a Millipore mixed cellulose ester membrane (0.45 μm , 47 mm diameter, plain white surface, HAWP 047-00 manufacturer number). **Care must be taken not to confuse this filter with the filter for anions/cations.** After filtration, the filter is placed in a square of aluminum foil and stored in a clean plastic scintillation vial. This filter has been chosen to match the filters historically used by the Crater Lake National Park long-term water quality monitoring programs. It dissolves completely in an acetone mixture used by contract laboratories to extract the chlorophyll from the filter for use in a fluorometer.

Because Chlorophyll *a* concentrations are based on the amount of water filtered through the filter, the crew member must be meticulous about the amount filtered. Although the filter can hold more than 50 mL at a time, it is not recommended to uptake more than this amount of water. This will allow for easier tracking of the amount filtered; a total of ten 50 mL syringes to equal 500 mL.

1. The Millipore membrane 0.45 μm filter should be inserted into the filter holder as before using latex gloves and forceps.
2. An initial 50 mL of water is uptaken using the syringe and filtered through the filter. This is repeated until a total of 500 mL has been filtered.
3. Once 500 mL has been filtered, the syringe should be filled with 20 mL of air. This air should be pushed through the syringe. This will help push out residual water that may remain inside the filter holder.
4. The filter should be removed and inserted into a small square of clean aluminum foil, slid into a labeled scintillation vial, and tightly capped. It should then be stored as above.
5. If it becomes difficult to filter, the crew member can use a second filter. In this instance, the two filters will be placed into a single vial. The use of multiple filters for a single sample should be noted on the field sheet in the section for comments.
6. There should be ample water left for both the 500 mL of water to be filtered for the chlorophyll analyses and the alkalinity. **Note: the 500 mL requirement is not a minimum; it is an absolute amount.** If for some reason, excess water is filtered (for example, if a total of 550 mL is filtered), the total amount filtered should be noted on the data sheet. If extenuating circumstances dictate that less was filtered, this should be noted as well.

Procedure for Acid Neutralizing Capacity Field Analysis

Acid Neutralizing Capacity needs to be analyzed as soon as possible (recommendation is less than 24 hours). Acid Neutralizing Capacity is measured in the field using a portable analytical kit (for alkalinity). The general procedure is to add an acid to a sample and track the resulting change in pH. The amount of acid needed to achieve a certain pH is converted to the amount of alkalinity. Field analysis is identical in methodology to lab methodologies, except that a colorimetric endpoint (i.e., target color) is used to signal the target pH level.

The procedures below are for use with the Hach® Digital Titrator. If the Acid Neutralizing Capacity kit brand or style is changed or updated, data comparability procedures should be carried out following SOP #16: Quality Assurance Project Plan.

Special Consideration: This test uses a titration cartridge of concentrated sulfuric acid (H_2SO_4). As a potentially dangerous substance, the crew will review the necessary Material Safety Data Sheet (MSDS) with the Project Lead and be familiar with all safety procedures before handling the kit. The minimum recommended safety gear to be worn during analyses are: safety glasses and latex gloves. It is the responsibility of the Project Lead to ensure that crews have access to this gear.

1. Prior to analysis, an Erlenmeyer flask and graduated cylinder should be preconditioned with sample water.
2. Wear safety gear: gloves and eyewear.
3. Follow Hach protocol method 8203 for specifics on how to perform analysis (Appendix I). Use the below guidelines.
 - a. Titrate to a pH endpoint of 4.8 (light violet-gray). **When assessing color, do not wear sunglasses.**
 - b. For most tests done in lakes of Lassen Volcanic National Park and Crater Lake National Park, Acid Neutralizing Capacities are expected in the range of 10 – 40 mg/L as CaCO_3 .
 - c. Initial analyses should be performed using 100 mL of sample water and a Titrant Cartridge of 0.16 N H_2SO_4 (see Table 1 of Method 8203 in Appendix I).
4. Record amount of sample, strength of acid, and total digits (units required to reach endpoint).
5. Dispose of the waste into a 1 L plastic waste vial, clearly labeled “ANC waste.”
6. Repeat steps 1 – 5. If the calculated value of Acid Neutralizing Capacity falls outside the 10 – 40 mg/L of CaCO_3 range, the sample volume and titrant strength should be adjusted accordingly. If the initial acid strength used was wrong, or if the calculated value is more than 10% off from the first measurement, or if the crew member thinks they missed the endpoint, repeat a third time.

Standard Operating Procedure (SOP) #10: Multiprobe Usage and Calibration

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP describes the usage of a multi-parameter probe (hereafter, multiprobe) for the measurement of the four “Core” parameters required by the Water Resources Division for lentic sites: Temperature, Specific Conductance, pH, and Dissolved Oxygen. Additional parameters that the KLMN has included: ORP (Oxidation/Reduction Potential), turbidity, and depth.

Over the course of the monitoring project, multiprobes will wear out, be lost, damaged, or otherwise need replacing. Although a well maintained probe could easily last a decade or longer, the Program Lead should anticipate the need to either upgrade or replace worn-out components on a biennial basis. When probes are upgraded, repaired, or replaced, steps provided in SOP# 16: Quality Assurance Project Plan should be undertaken to ensure data comparability. It is also the responsibility of the Program Lead to ensure that the probes and display units are in proper functioning order a minimum of 3 months prior to the initiation of field work.

The current multiprobes employed by Klamath Network are the *Manta* multiprobe with the *Amphibian* Display, both manufactured by Eureka Environmental Engineering. Although this SOP should assist in many issues that may arise with multiprobe use, occasional assistance or technical support may be necessary. Their web site and support staff should be regularly contacted for upgrades to software and firmware. Their contact information is:

Eureka Environmental Engineering
2113 Wells Branch Parkway Suite 4400
Austin, TX 78728
Tel: (512) 302-4333
Fax: (512) 251-6842
www.eurekaenvironmental.com
Sales@eurekaenvironmental.com
support@eurekaenvironmental.com

The user’s guides for both the *Manta* and *Amphibian* Display are included in Appendix J. Also contained in Appendix J is a copy of the help file for the program used to manage data collected by the *Manta* and Eureka PC, along with a help file for iPAQ software version of Eureka Pocket

PC interface. The Program Lead and crew should familiarize themselves with all four of these documents prior to multiprobe use. After reading the documents, the Program Lead and crew should begin trials with the multiprobe to insure that all are comfortable using the equipment **prior to field work**.

The following step-by-step guide to use is not a surrogate for reading the manuals. However, it will fill in the gaps of usage that may not be detailed in the manuals. It also details aspects that are specific to ensuring that data are measured, collected, stored, and managed in identical manner through the life of the project.

Multiprobe Data Collection Step by Step

- A. Prior to use, the *Amphibian* and *Manta* components should be checked for proper condition:
 1. The *Amphibian* is a Hewlett-Packard iPAQ model hx2490b pocket PC (hereafter, iPAQ) contained in a waterproof carry case (www.otterbox.com). The container should be inspected for the following (Figure 1).
 - a. Intact and clean body O-ring.
 - b. Functioning side-clasps.
 - c. Intact and clean window O-ring.
 - d. Secured and centered iPAQ.
 - e. Clean serial port, free of dirt.
 - f. Clean and covered round USB/Power Charger port.
 2. The *Manta* Probe should also be inspected (Figure 2).
 - a. Check for cracks in acrylic body.
 - b. Inspect the integral cable connection (Figure 2). These may crack and need replacing with time.
 - c. Check that the serial port is clean and free of dirt.
 - d. Check that the O-rings within the acrylic body are making contact (a thin, dark line is visible).
- B. Remove the red cap covering the serial port on the *Amphibian* and connect the *Manta* serial port to the *Amphibian*. **Always use the thumb screws to secure the probe to the *Amphibian*.** Failure to do so may result in the probe coming off during readings.
- C. Once connected, turn on the *Amphibian* by depressing the power button using the stylus attached to the unit (Figure 3).
- D. The iPAQ uses Windows Mobile 5.0 operating system for functioning. Crew members should become familiar with this operating system by exploring the functions and settings prior to the field season. It is an intuitive operating system and is interfaced using the stylus to point and depress on-screen items, similar to a point and click interface on a desktop PC. Start the Eureka Software by touching the stylus to the Start menu (Program start button). This should result in a drop-down list of program options (Figure 4). Depress “**eureka**” to start the *Manta* software.



Figure 15. *Amphibian* overview – top down (Left); open (Right). *Amphibian* parts: A) Neoprene handle; B) Stylus; C) iPAQ pocket PC; D) Top serial port; E) Case clasps; F) External battery; G) Velcro straps to secure iPAQ; H) Circuit board; I) Bottom USB/battery charger port.



Figure 16. *Manta* overview. *Manta* parts: A) *Manta* body; B) Storage cup; C) 25 m cable; D) Waterproof, integral cable connection; E) Marine-grade serial port.



Figure 17. Hewlett-Packard iPAQ overview, showing relevant buttons to *Manta* multiprobe function.

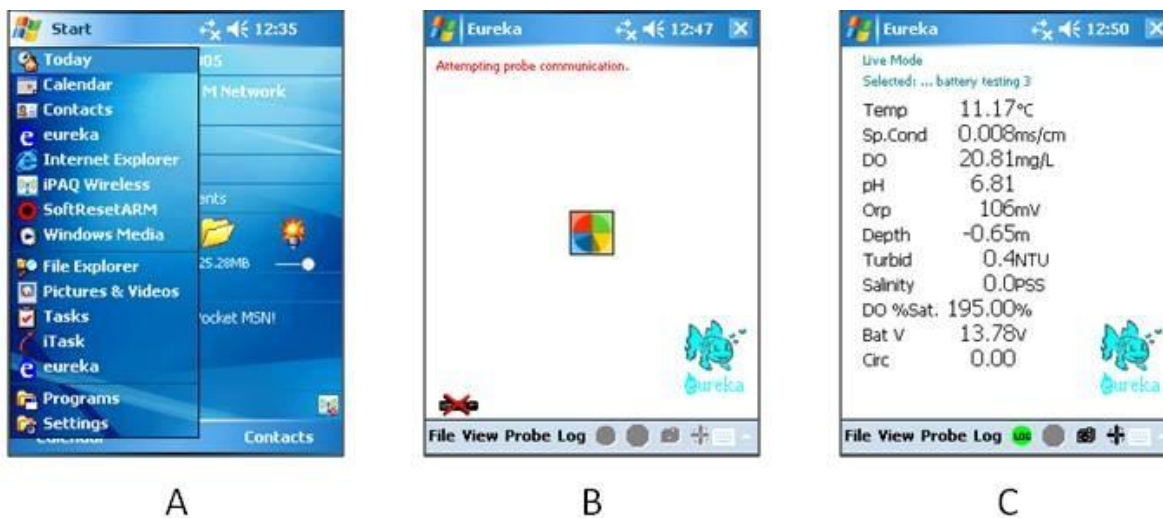


Figure 18. (A) iPAQ start up screen after depressing start; (B) Eureka Start-up; (C) Eureka after probe connection.

- E. Alternatively, the Eureka software can be started by depressing the “hotstart” button on the bottom left of the iPAQ (Figure 3).

- F. Once started, the software will attempt to communicate with the *Manta* Probe. If a multi-color symbol appears, communication is being attempted (Figure 4b). Upon connection, data readouts will appear (Figure 4c).
- G. It is now possible to proceed with the calibration steps (see below section on Calibration).
- H. Before data collection, a proper file must be created in Eureka to store the data in (Figure 5a-c).
1. Under the “**Log**” menu, depress “**Locations.**” Do not use the “” button (Figure 5a).
 2. Depress the “**New**” button, so that there is an active cursor (Figure 5b).
 3. Depress the keyboard icon at the bottom of the screen to create a new “**Location**” (Figure 5c).
 - a. Using the shift key so that capitals and underlines can be used in the file name structure, proceed to tap the keyboard to create the name.
 - b. **The file name structure should be: Park_Lake_Number_Year.**
Example: **LAVO_11555_2008**. In this example, the California Lake ID number is 11555, and it was sampled in 2008, and it is in Lassen Volcanic National Park.
 - c. **DO NOT ABBREVIATE** due to time constraints. “**Lake_1_08**” is not an acceptable file name. Note: Files may (and should) be created ahead of time, either by the crew or the Program Lead. Note that the list will indicate if a file has been “**used**” or is “**empty.**”
 - d. Once the file is created, highlight it by selecting it from the list and depress “**Select.**”
- I. Once the proper location file is selected, logging a data file can commence.
1. Ensure that the proper location file is being used; it is indicated at the top of the data readout screen, in small blue text. For example, in Figure 5a, the active location file is “**battery testing 3.**” If this is not the proper file, return to the log screen and select the proper file.

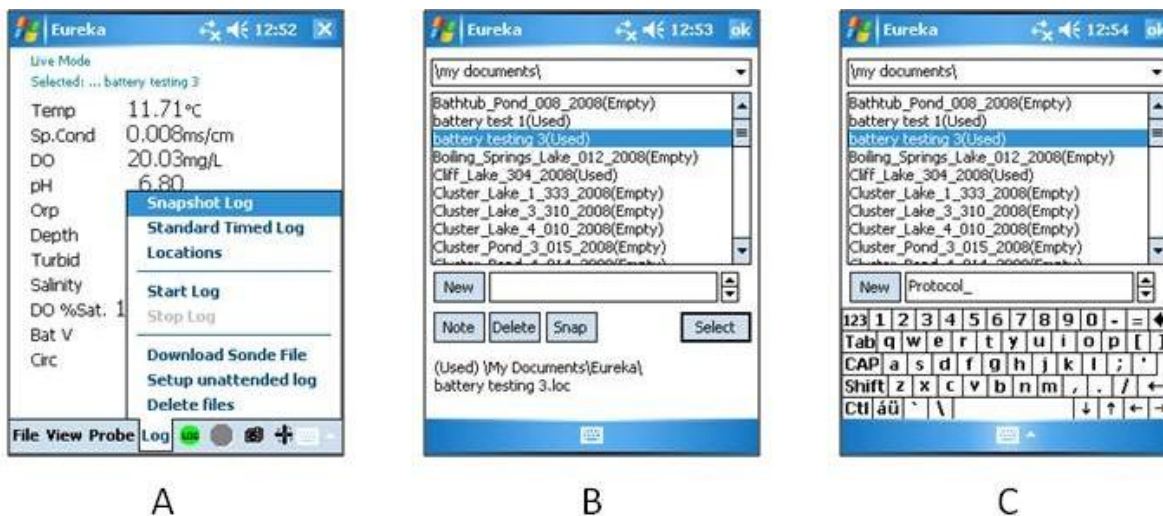


Figure 19. (A) Eureka screen captures for creating “Location” files, under “Log” command; (B) Selecting an existing “Location” file or creating new “Location” files; (C) Using keyboard function to name “Location” file.

- J. At this point, depress the “Log” menu option, NOT the green log logo “

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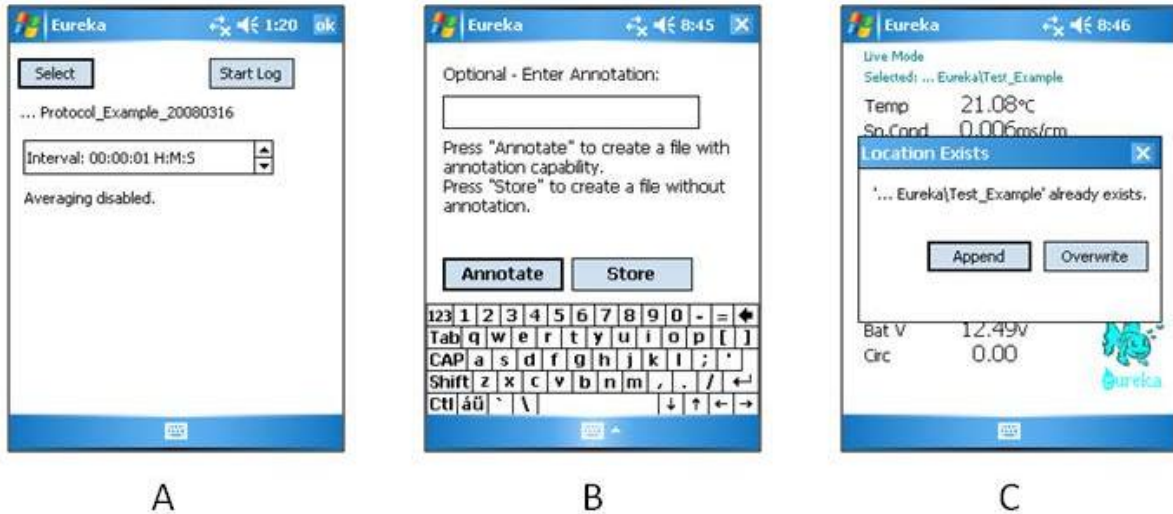


Figure 20. Eureka screen captures for initiation of data logging: (A) Setting the logging interval; (B) Annotation option; and (C) Appending or overwriting an existing file.

Lake/pond Profile Step by Step

With the basic instructions on how to collect data, the following steps should be followed in collecting a lake profile.

- A. While on shore, calibrate Dissolved oxygen (% saturation), and pH, following the steps outlined in “Calibration Step by Step.” Calibrate the depth.
- B. Connect the *Amphibian* to the *Manta* while on shore; replace the storage cup with the weight (Figure 7).



Figure 21. After removal of storage cup, weighted cup on left is attached by screwing into the *Manta* body. Note that the weight is heavy and exerts leverage. Use a firm grasp so that the weighted cup does not interfere or break any of the probes.

- C. Using the inflatable boat, return to the deepest portion of the lake, where the water sample and secchi depth were collected. Use a hand held sonar to confirm depth. Deploy anchor bag (mesh bag with long cord tied to it) if necessary. If deploying anchor bag, use slack so that anchor is **not** directly beneath the boat.
- D. Turn on the unit and allow it to warm up while in the water on the side of the raft.
- E. Confirm that the depth reading is 0.05 m or less, when probe end is just submerged. **If it is not, recalibrate.**
- F. Turn on the circulator by depressing the “” button. Alternatively, the circulator option can be accessed by depressing “**Probe**” menu option, followed by “**Circulator**.” When the circulator is active, the data readout screen (Figure 4c) will display “**Circ 1.00**.” **Note that if the circulator is not turned on, the dissolved oxygen readings will be inaccurate.**
- G. With the probe tip at the surface, wait until all readings appear stable, especially the dissolved oxygen. This may take 1 minute or more. Look for a point where the dissolved oxygen in mg/l fluctuates back and forth (e.g., 9.89 to 9.88 to 9.89, etc.).
- H. When the numbers have stabilized, lower the probe a half meter (e.g., 0.00 m to 0.50 m). Repeat the stabilization process in G. above.
- I. Continue lowering the probe until stabilized readings have been taken every 0.5 m to the bottom of the lake.
- J. Bring the probe back to the surface and shut it down.
- K. **When shutting it down, be sure to exit and close the program. Turning the unit off without shutting down will cause the program to continue to draw power from the batteries.**
- L. Remove the weighted cup and replace it with the storage cup. The storage cup should be approximately 1/3 full. It is not necessary to fill it completely.
- M. Download the data file to the computer and archive according to instructions in SOP #12: Post-Site Tasks.

Calibration Step by Step

Regular calibration is an important component of maintaining quality control on data collected. The calibration, calibration check, and acceptable range schedule for each parameter should be followed as in Table 1.

Table 19. Calibration guidelines for *Manta* multiprobe. The probe should be calibrated at the beginning of the work week. When calibration checks are outside the acceptable range (compared to reference solutions), the probe should be recalibrated in the field. (NIST - *National Institute of Standards and Technology*).

Parameter	Calibration Interval	Calibration Check	Acceptable Range	Notes
Conductance	1/week	When recalibrating	$\pm 5 \mu\text{S/cm}$	
Dissolved Oxygen (% Saturation)	Every site	NA	$\pm 5\%$	
pH	1/week	per sampling site	± 0.3	
Redox (ORP)	1/week	NA	$\pm 40 \text{ mV}$	
Temperature	NA	1/month	$\pm 0.3 \text{ }^{\circ}\text{C}$	Temperature is factory calibrated, however checks against a NIST thermometer should be done 1/month.
Turbidity	1/week	NA	$\pm 3\%$	

In general, the probe should be calibrated the day or evening before a work week commences. The probe should be calibrated in the five main parameters, regardless of whether or not it is in the “acceptable range.” When in the field, prior to measurement, a quick check against a known solution or another reliable probe should be done. If the parameter measurement is outside the acceptable range, the technician should recalibrate prior to making measurements. Note: it is recommended that the check be against a calibration solution, ensuring that the technician has calibration solution on hand to recalibrate if necessary. **Record results of calibrations and calibration checks on the appropriate logsheet (Appendix F).** Additionally, although the calibration may require a multi-point calibration, the calibration check can be against a single value, as close as possible to the anticipated measurement value.

Generic Calibration Step by Step

- A. Attach *Manta* to *Amphibian* as above for multiprobe data collection step by step.
- B. Once operating and probe is reading (as in Figure 4c), remove the storage cup and replace with the calibration cup (Figure 8).



Figure 22. Calibration cup with black covering used for calibration procedure.

- C. At this point, the probe must be maintained “upside-down” or in an inverted state until calibration is complete.
- D. Tap on “**Probe**” menu option, followed by “**Calibration...**” (Figure 9a). From the drop down list, select the parameter to be calibrated (Figure 9b).
- E. Once selected, tap the appropriate number of calibration points:

Parameter	# of calibration points	Calibration points
Depth	1	@0.00 m for 1 second
Dissolved oxygen (%saturation)	1	@100.00% for 60 seconds
pH	3	@7.00, 4.00, 10.00 for 60 seconds
Redox (ORP)	1	@0.00mV for 1 second
Specific conductivity	1	@1413µS/cm for 60 seconds
Turbidity	2	@0.00 and 100.00 NTU for 60 seconds

- F. Choose the appropriate options (e.g., for pH: “**@7.00 for 60 seconds**”).
- G. Prior to initiating (by tapping “**Calibrate**”), rinse the probes with a wash bottle of deionized water, shake dry, and fill with the appropriate calibration solutions, so that the probe is covered.
 - a. If doing dissolved oxygen, do not fill the cup. Rather, fill until just below the dissolved oxygen probe. Place the black rubber cap on top of calibration cup and let the probe equilibrate for 3 or 4 minutes. This creates a 100% saturated air pocket with in the atmosphere.
 - b. If doing depth, there is no “solution.” Simply run the calibration while holding the probe in the air along the shoreline, ensuring that there is water on the probe (gently shake or use corner of cloth to dry, if necessary).
- H. When the appropriate solution is in the cup, and the probe has equilibrated, tap “**Calibrate.**” (Figure 9a). Follow instructions on the *Amphibian* (Figure 9b).
- I. When doing dissolved oxygen, be sure to maintain the probe vertical, so that no water gets on the oxygen membrane.
- J. Once all calibrations are done, click “**ok**” in the upper right corner. Click “**Yes**” to save the calibrations (Figure 9c).
- K. Record results and date and time of calibration on calibration tracking form (Appendix F).
- L. Remove calibration cup and replace with storage cup or with weighted cap for data collection.

Dissolved Oxygen Membrane

When calibrating dissolved oxygen, the calibration may occasionally fail, or while using the probe, the operator may notice that an inordinate amount of time may be required for the probe to equilibrate. Both of these are signs that the membrane may be wrinkled, have an air bubble, damaged, or otherwise “gone bad.” Such a membrane will need to be replaced, along with electrolyte solution. For this reason, the field crew should always carry spare membranes and electrolyte solution with them to the field site.

Excellent instruction on how to replace the membrane is provided in Appendix J, Eureka Environmental *Manta* manual. Both crew members should be trained in membrane replacement. Recalibration will have to be performed after replacement, ideally after a 24 hour period to allow the membrane to relax and stretch. Calibrations after the 24 hour period will be more stable and last longer. If the membrane replacement is done on-site, the calibration is still valid. However, the calibration will not “last,” so that if measurements are retaken later in the day (3+ hours, for example), the probe will need to be recalibrated. In other words, the calibration will not “hold” unless it is calibrated 24 hours later. However, accurate readings can still be taken immediately after replacement.

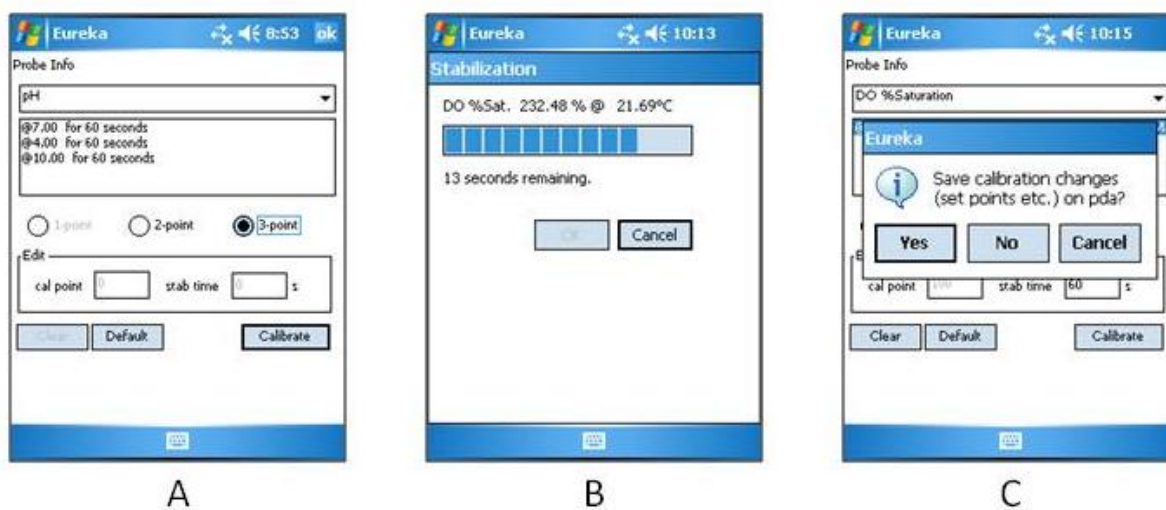


Figure 23. Eureka screen captures for calibrating probe: (A) Selecting parameter; (B) Waiting for calibration stabilization time; and (C) Saving calibration set points.

Standard Operating Procedure (SOP) #11: Amphibian, Invertebrates, and Lake Substrate Walk-around

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

The purpose of this SOP is to describe the procedures to survey for amphibians, collect invertebrates, and characterize the lake environment. Although these are three disparate activities, all data collection is performed in a single step. Working as a pair, the crew walks around the lake or pond. While one person is actively searching for amphibians and collecting invertebrates, the other member is using a Trimble GeoExplorer Handheld Units (hereafter, “Trimble”) to record substrate types, location, species of amphibians observed, number of amphibians encountered, and location of invertebrate sweeps.

This protocol is presented in five sections: 1) use of Trimble, 2) substrate characterization, 3) amphibian survey methods, 4) invertebrate collection techniques, and 5) riparian habitat characterization.

Responsibilities

It is the responsibility of the Project Lead to make certain field crews have all necessary equipment and field forms to help when collecting data. The Project Lead should work closely with the Data Manager to develop a half-day training session on how to use the data collection equipment. It is the Project Lead and field crew member’s responsibility to follow all validation and verification processes when collecting data.

Use of Trimble GPS

The Trimble GPS unit forms the basis of data collection for four components of this protocol; 1) Lake/pond area; 2) type and percentage of near shore lake substrate; 3) numbers, species, and life stages of amphibians; and 4) invertebrate collection locations. Hence, we will be co-collecting habitat type at all locations of amphibians and invertebrate collections. This will allow the calculations of ancillary data, such as the correlation between amphibian abundance and substrates, as well as an accurate assessment of the proportion of substrate types sampled with the invertebrate collections. **On account of this, crew competence with the Trimble is of critical importance.** Crews must be intimately familiar with the use and trouble shooting of the unit to ensure accurate data collection. Crews must ensure that the Trimble always has an

adequate battery charge and that care is taken in the handling of the unit. Note that the collection of lake area is an automatic step and is calculated using GIS applications from the collection of substrate type.

Data Collection

Once the crew is ready to begin collecting data on the perimeter of the lake, amphibians, and invertebrate collection locations, follow the steps listed below. **Start by recording the type of unit used on the datasheet.**

Opening the Project File in ArcPad

- A. A few minutes prior to collecting data, turn on the Trimble by clicking the **Green** button on the bottom of the unit.
- B. Using a stylus, Tap the word [**GPS**] at the bottom, right side of the screen. A drop down menu should appear.
- C. Be patient while ArcPad opens.
- D. Although the default on the Trimble will be to automatically have a list of project open, you may need to open the Lakes_Study_XXXX.apm file manually, where XXXX is the current year (e.g., Lakes_Study_2010.apm for the first year of implementation). This file will be set up by the Data Manager prior to the initiation of the field season (SOP #1: Preparations, Equipment, and Safety).
 1. With ArcPad open, in the upper left corner there is a picture of a file. Use the dropdown arrow next to the file to select the “Open Map” option.
 2. Select the “Lakes_Study_XXXX” project.
- E. You now need to make certain you have captured enough satellites to provide an accurate location.
 1. Tap the [**GPS Position** ] button. Make certain the button looks pressed in.
 2. A small screen will appear showing the satellites. If you see the coordinates in the small screen, you have enough satellites to begin capturing data. If you do not have enough satellites, the Trimble unit will beep repeatedly.
 3. NOTE: If you tap the coordinate, you can change it from lat/long to UTM.
- F. When you open the ArcPad project, it is usually zoomed very far out. In the bottom right corner of the screen look at the scale and see what it is set to (e.g., 1:210102).
 1. Double tap the scale and set it to 1:1000.
 2. You should now be able to see the line. Keep in mind you will need to restart the process to collect the perimeter data.

- G. Once you have satellite coverage, you can begin to collect data.
- H. As you collect data, you may hear a beep and the GPS symbol will change to . This means you are no longer collecting data. Move to a more open area if possible and wait till you get satellite coverage.

Recording the Location of the Lake, Invertebrate Sites, and Amphibians

- A. Make certain you have selected the “Waterbody_Boundary” shapefile by using the edit tool on the drawing toolbar.
- B. Once you have enough satellites, using the drop down arrow next to the small dot on the tool bar, select “Polyline” from the list of options.
- C. If you are ready to start collecting the perimeter of the lake, click the satellite streaming button and start walking around the lake. REMEMBER to keep the Trimble unit pointed as straight up and down as possible.
- D. Walk along the perimeter until you come to: 1) a change in habitat (section 2), 2) an amphibian collection location, or 3) an invertebrate location. Depending on which one of the three options you come to, skip to the appropriate section below.

If You Have Come to a Change in the Habitat

As stated above, you are walking around the lake collecting perimeter (steps A-D above) data and you have come to a change in habitat. At this point, you will record the habitat type you have **just covered, not the new habitat.**

- A. When you are at the end of the habitat you are currently walking through, tap the green arrow  at the bottom left side of the screen.
- B. This will open the Water Boundary form (Figure 1).
 - 1. Using the pick lists, complete the form. If the value you need is not in the pick list, you can use the keyboard to enter the data.
 - a. Site Name: This is a MANDATORY field.
 - b. Primary Habitat: This is a MANDATORY field and should be populated with the most abundant habitat value you **just previously walked through.**
 - c. Secondary Habitat: This is a MANDATORY IF APPLICABLE field and should be populated with the second most abundant habitat value you **just previously walked through.** As a rule of thumb, only include a second habitat if there is more than 20% of this habitat type present.
- C. Once you have completed the form, tap the green “OK” button at the bottom left side of the screen.

- D. On the tool bar, next to the arrow and dot, use the dropdown list to select “Segment Polyline” .
- E. Click the satellite streaming button  and continue walking around the lake.
- F. Repeat steps A-H for each habitat type you walk through.

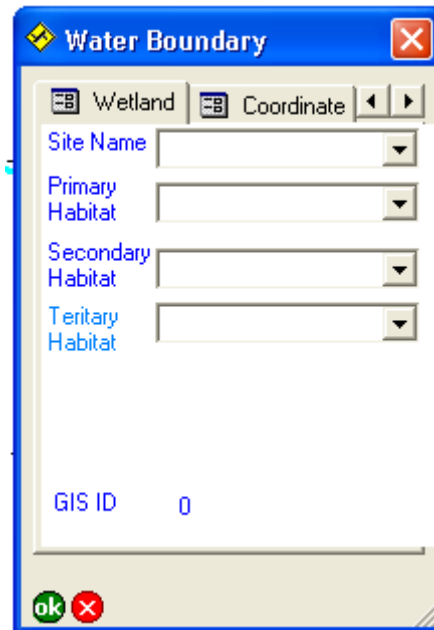


Figure 24. Water Perimeter Data Entry Form on the Trimble GPS Unit.

If You Have Come to a Amphibian or Invertebrate Collection Location

As stated above, you are walking around the lake collecting perimeter data (steps A-C above) and you have come to a location where you see/hear/capture an amphibian or are going to collect invertebrates, or have set the gill net.

- A. Select the “Amphibian” or shapefile using the editing tool under the drawing toolbar.
- B. Using the dropdown arrow next to the small dot or polyline (depending on what data was collected previously), select “Point” from the list of options.
 1. Tap the [**Capture GPS Point** ] button .
 2. This will open the Amphibian form (Figure 2). At the top of the form you will see a count. The GPS will collect 30 point locations and average them for a more accurate location. While the Trimble is collecting the locations, complete the fields in the form.
 3. Enter the following fields.
 - a. **SITE TYPE:** This is a MANDATORY field. Select “Amphibian” from the picklist. NOTE: If this is an invertebrate collection site, select "Invertebrate" or if

it is the point on the shore where the gill net was set, select “Gill Net” and skip to step C below.

- b. NAME: This is a MANDATORY field. Select the scientific name of the species collected. Make your best efforts to determine the genus and the species. If you cannot determine the species just select the genus (e.g., *Rana* sp.). If you cannot determine the genus, select “Unknown Amphibian.”
 - c. LIFE STAGE: This is a MANDATORY field
 - (a) Adult
 - (b) Subadult
 - (c) Metamorph
 - (d) Larvae
 - (e) Egg Sack
 - d. COLLECTION: This is a MANDATORY field. Select the method the species was observed
 - (a) Already Dead
 - (b) Audio
 - (c) D-Net
 - (d) Gill Net
 - (e) Visual
 - e. COUNT: This is a MANDATORY field. Enter the total number of individuals, estimating as needed.
 - f. SIZE: Enter the size of the individual in cm, if applicable (e.g., if in hand).
- C. Once you have completed this form, tap the green “OK” button in the bottom left corner of the form.
- 1. Repeat steps 1-4 for each amphibian that is: 1) a different species or 2) is a different size.
- D. Click the satellite streaming button and continue walking around the lake collecting perimeter data.

Figure 25. Amphibian and invertebrate data entry form.

Ending the Project

Once you have completed collecting the perimeter and sampling at amphibian and invertebrate locations, you are done using the Trimble and can close out the project and shut off the unit. To do this, complete the following steps.

- A. At the top left side of the screen on the toolbar, there is a folder. Using the dropdown arrow, select “Exit.” **Note:** When you exit the project, all the data will be saved so you do not have to worry about clicking a save option.
- B. This will close ArcPad. NOTE: If you click the [X] in the upper right corner of the application, it will only minimize the program and not close it. Be sure to close the project when you are done.
- C. Tap the large button at the bottom of the screen (just once). This shuts off the unit.

Troubleshooting

Every effort will have been made to set up the Trimble unit and map project before the crew goes into the field. However, situations will occur that cannot be planned for and this section lists some potential issues and the methods on how to correct them.

- A. **“NO MATTER WHAT I DO I CANNOT GET SATELLITE COVERAGE.”**
 1. The Trimble units are set to a “Productive” setting versus a “Precision” setting to help ensure field crews can quickly gather data. Since this study is sampling lake areas, it is assumed most field sites will be in fairly open habitats with adequate satellite coverage. However if you cannot, complete the following steps.

- a. Compare the settings on the Trimble unit to the settings described in the Settings sections of this SOP. Make sure they are the same. If you still cannot get enough satellite, then go to step b.
 - b. Move to an open area, wait for satellite coverage, and then move back to the area you are trying to sample. Continue collecting data but if you lose satellite coverage go to step c.
 - c. If you think you cannot get satellite coverage because of the habitat you are sampling in, use the Trimble for those areas of the lake where you can get coverage.
- B. “I HAVE ARCPAD OPEN AND I TAP THE SCREEN BUT ALL IT DOES IT BEEP AT ME.”**
1. Users have the ability to “Lock” ArcPad and occasionally this accidentally occurs. In the upper right corner of the unit is a picture of a lock. Tap it and a message should come up saying “Unlock?” Tap “YES.”
- C. “THE POINT AND/OR POLYLINE OPTIONS ARE GREYED OUT SO I CAN NOT COLLECT DATA.”**
1. Each shapefile needs to be in edit mode. Click the pencil on the tool bar. You should see the Amphibians and Waterbodies_Boundary shapefiles. If you do not, complete the following steps.
 - a. On the top toolbar, click the image that looks like three sheets stacked on top of each other . You should see both shapefiles.
 - b. In the column with the pencil as the heading, make sure each box is checked.
 - c. Click the green “OK” button at the bottom of the screen.
- D. “I KNOW I HAVE DONE EVERYTHING CORRECT BUT AS I WALK THE PERIMETER IT DOES NOT DRAW MY LINE.”**
1. You may be zoomed too far out. In the bottom right corner of the screen look at the scale and see what it is set to (e.g., 1:210102).
 2. Double tap the scale and set it to 1:4000.
 3. You should now be able to see the line. Keep in mind you will need to restart the process to collect the perimeter data.
- E. “THE PROJECT HAS FROZEN, NOTHING WORKS.”**
1. Stop pushing buttons and wait 5 minutes. There may be several things going on and it just needs some time to complete those processes.

2. After waiting 5 minutes, if it has still not worked, you will need to do a hard reset. This should be a LAST RESORT option.
3. Hold the large button on the Trimble Unit down for 30 seconds and then release it. The unit should turn off.
4. Push the large button again to restart the unit.
5. It will ask you if you want to use the backup copy; say “YES.”
6. Reopen your project.

Backing up the Data

Data collected using the Trimble GPS units should be backed up as often as possible. At the end of the day, the GPS data should be backed up onto the laptop computer. This information is repeated in SOP # 12: Post-Site Tasks.

Backing Up Data on the Trimble Units

Make certain you have ActiveSync on the computer where you plan on backing up the data and follow the steps below to back up the data.

1. Connect the Trimble cradle to the computer and place the Trimble unit in the cradle.
2. ActiveSync should start automatically and will let you know when the computer and the Trimble unit are connected.
3. On the computer, open Windows Explorer and look for the Trimble icon that is labeled “Mobile Device” (Figure 3). Double click on the mobile device.
4. Go to the following pathway:

My Windows Mobile-Based Device\Lakes_Study_XXXX, where XXXX is the year of the study.
5. Right click on the file called “Shapefiles” and select “copy.”
6. Go to the location you plan on storing the backup file (on the computer).
7. Right click on the appropriate folder and select “Paste.”
8. You have now made a backup of the GPS data.

An alternative, using a “Secure Digital” card (better known as SD cards) can be used by the crew. If the Trimble is equipped with an SD card, transfer the “Shapefiles” folder to the card using the PocketPC mobile Windows Explorer, and then eject the card. Using an external card reader or internal drive (if laptop has one), copy the “Shapefiles” folder to the dated folder (e.g., 20100725) in the GPS_Backup folder. Replace the SD card back in the Trimble.

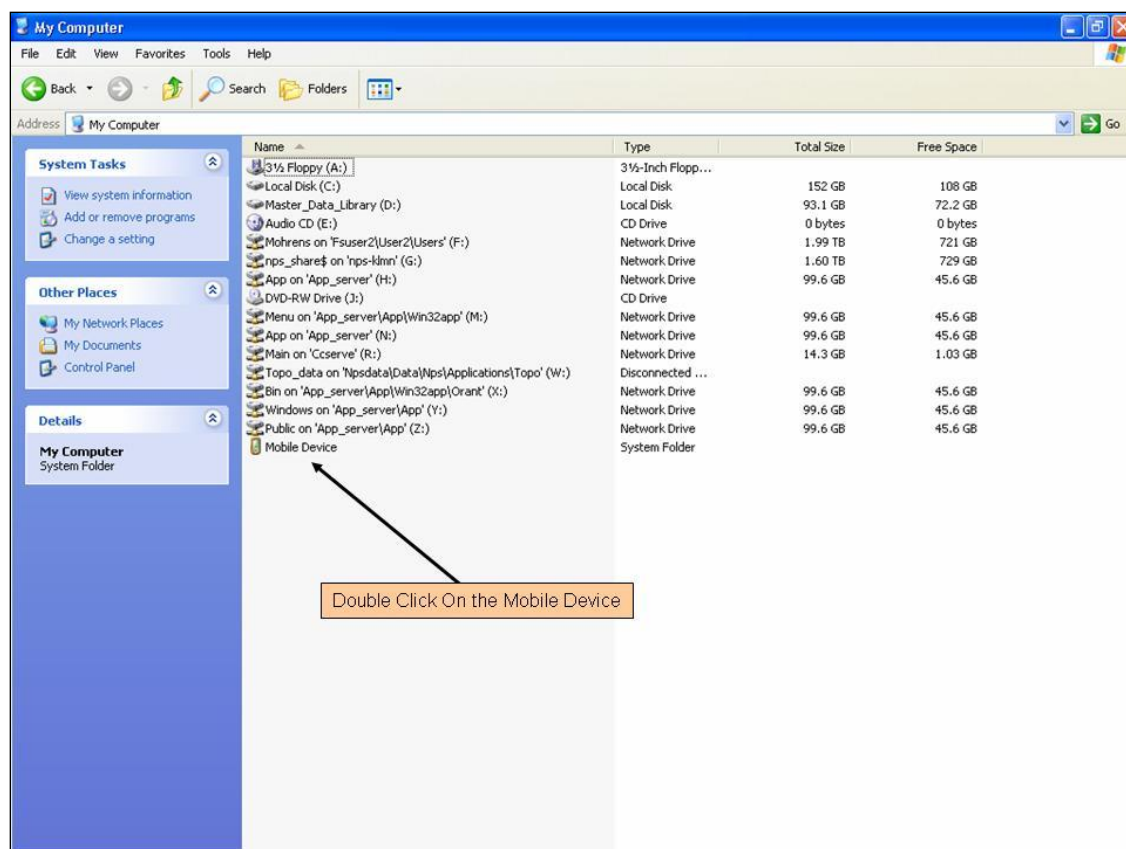


Figure 26. In the Windows Explorer window, users should double click on the mobile device icon to access the Trimble file structure so they can make copies of the GPS data.

Substrate Characterization

Substrate characterization is done by visual observation of the dominant substrate making up the near-shore littoral zone of the lake. This zone is defined by the wetted perimeter to a distance of 3 meters out into the lake. If the slope of the shore is such that substrate 3 meters away is not visible, characterize the habitat based on the most dominant substrate that you can observe. No step by step instructions are included here; this is covered in the basic operation in “Use of Trimble GPS.” The following are guidelines in determining how to implement the step by step.

Only characterize the primary dominant substrates. For example, if you are on a beach area with roughly 45% sand, 30% gravel, 15% woody debris, and 10% detritus, enter sand as the primary substrate. The characterization should be based on the superficial areal coverage and not volume or mass. Hence, if emergent vegetation (like water lilies, *Nymphae* sp.) is covering the majority of the habitat, characterize the emergent vegetation as the primary substrate, even though silt or sand maybe more dominant if based on volume.

If the primary and secondary substrate types appear to be in equal proportions, type it as whichever makes the most immediate impression. Alternatively, ask for a second opinion (there will always be two crew members doing this).

Only start recording a new habitat type in the Trimble when an obvious shift in either primary substrate occurs. An obvious shift is defined by a new substrate that extends a minimum of 2

meters; if the previous substrate type resumes in less than 2 meters, do not type the minor shift in substrates.

Substrate Types

Bedrock

Bedrock is any rock substrate that forms the basal substrate of the lake bottom. Bedrock is generally considered to be greater than 4 m in diameter.

Boulder

Boulders are rock substrates greater than 300 mm (approximately 1 foot) in their longest dimension. Note that they may be submerged or emergent.

Cobble

Cobbles are rock substrates greater than 75 mm (approximately 3 inches) but less than 300 mm in their longest dimension.

Detritus

Detritus is the term used to describe organic debris that is smaller than 25 mm (approximately 1 inch) in their longest dimension. Components will generally be identifiable as small pieces of wood, leaves, or other organic matter that is in the process of decomposition.

Emergent Macrophytes

Emergent macrophytes are plants (or macroalgae) that emerge from the water. This category is also used for macrophytes that are on the water surface (e.g., water lilies).

Gravel

Gravel is composed of rock substrate particles greater than 2 mm but less than 75 mm in their longest dimension.

Sand

Sand is composed of rock substrate particles less than 2 mm in their longest dimension.

Fines

“Fines” is a mix of inorganic and organic particles of a very fine particles size (generally less than 0.5 mm). Unlike sand, which will feel gritty between one’s fingers, fines will feel soft and fine, like baking flour. Fines may be characterized by dark organic particles. Fines without any organic debris would be a sediment like clay (or “muck”). The intricacies of determining the amount of organic component to silt necessitate the lumping of clay into the “fine” category.

Submergent Macrophytes

Submergent macrophytes are plants (or macroalgae) that do not emerge through the water surface.

Woody Debris

Woody debris is organic substrates from terrestrial plants (e.g., wood branches, fallen logs, rotting rootwads, etc.) that make up the lake bottom. They will generally be large logs and branches. If the longest dimension is over 25 mm, characterize as woody debris. If under, then categorize as detritus. Note that woody debris may often be floating.

Amphibian Survey

The purpose of the amphibian surveys is to develop Presence/Not Detected lists for each lake or pond. Qualitative estimates of abundance are a secondary purpose. However, the survey techniques are oriented towards maximizing detection and not strict abundance estimates.

Amphibian surveys are accomplished using Visual Encounter Surveys (VES) and targeted searches. As the crew member recording habitat data walks the lake wetted perimeter, the other walks within the lake or pond body and continuously searches for visual or aural signs of amphibians. As amphibians are observed, the species or genus encountered, life stage, and the approximate number seen are recorded as in Section 1 on Trimble use.

In addition to the continuous search, a minimum of 15 intensive searches throughout the lake perimeter should be conducted. The intensive search is composed of turning over rocks and logs and searching through macrophytes. Amphibians encountered should be recorded in the Trimble as described above.

Intensive searches should be conducted at equal spacing around the lake shore. An initial estimate of the circumference of the lake should be taken prior to the initiation of the walk around. Divide this estimate by 15 to estimate the approximate distance between intensive searches. Although an estimate of distance is used, the actual searches should be done on the most likely habitat for amphibians, even if the most likely substrate is not immediately in this location. The crew member in the water should spend several minutes turning over substrate, actively searching for amphibians. When found, the amphibian species, life stage, and numbers are recorded in the Trimble as described above.

Amphibian Walk-Around Procedure step by step

- A. Start by making a visual estimate of the approximate perimeter, in meters. This estimate does not have to be precise; it is only an estimate. Divide your estimate by 15 to estimate 15 equally spaced sampling location. This distance will form your basis for intensive search locations. Note: if this lake is an index lake and has been sampled previously, the recorded actual perimeter should be available to guide the spacing of sampling locations.
- B. Proceed to walk around the lake. One crew member walks the wetted perimeter and records substrate type (as described above) while the other crew member wades in the water.
- C. As amphibians are encountered in the visual encounter survey, the crew member walking the wetted perimeter will **record the species, life stage, and approximate number in the Trimble** (as described above). It is the responsibility of the crew member in the water to visually search for amphibians, as the other crew member is focusing on perimeter and substrate typing and data entry.
- D. While walking, pace off the approximate distance calculated in A. above. When arriving at this spot, look for the nearest (within 10 m) likely habitat for amphibians. The most likely habitat will be areas with structure (e.g., logs, rocks, macrophytes). At this location, conduct an intensive search.

1. Turn over likely structures (logs and such) and search for amphibians. The intensive search should be done over a minimum of 4 m² for at least 3 minutes.
 2. If the intensive search site is not near any likely habitat, the crew should ensure that there are no amphibians in this area and move on. Note that if the substrate is a sandy beach, the time required may be much less than 3 minutes. Use the 3 minute rule only for complex habitats.
 3. Do not stop searching if you find a single amphibian species. Continue searching for additional species until the entire square area is searched.
 4. The crew member walking the perimeter should **record the data** in the Trimble (as described above).
 5. Note that data from visual surveys and intensive searches are entered identically and that no distinction is made between occurrences.
- E. If necessary, individuals may be captured for species verification. The following handling procedures should be followed (See also: American Society of Ichthyologists and Herpetologists, 2004. Guidelines for use of live amphibians and reptiles in field and laboratory research, Second edition. Available at <http://www.asih.org/files/hacc-final.pdf>.)
1. Capture techniques should be as minimally invasive as possible.
 2. Minimize handling time.
 3. Always wear a protective layer (e.g., neoprene or latex gloves). This will prevent introducing toxins (e.g., insect repellent or sunscreen) through the amphibian skin. It will also prevent tetrodotoxin in *Taricha granulosa*, the Roughskin Newt, from affecting the handler.
 4. Always handle the amphibian with wet hands. If hands dry out during handling, rewet your hands.
 5. Release the specimen in the exact place where the organism was captured.
- F. As the crew maps the wetted perimeter, the crew should reassess the distance between the intensive search areas so that the crew does not return to the starting point before finishing 15 intensive searches.

Amphibian Disease Protocol

Crews should be on the lookout for signs of amphibian diseases. There are two primary diseases of concern for the amphibians of Lassen Volcanic, Crater Lake, and Redwood National Parks: (1) *Batrachochytrium dendrobatidis* (*Bd* for short, also known as “Chytrid Fungus”), and (2) Ranavirus.

For either disease, the main indications that will be observable to field crews will be:

- Massive die-offs – Large numbers of dead adults, larvae, or metamorphs may indicate presence of either disease.

- Lethargic individuals – Amphibians of any life stage or species that exhibit slow responses to capture attempts (or no response) may indicate disease. Do not handle these individuals without gloves!
- Abnormal morphology – The presence of additional limbs, discolored limbs, or peeling of skin may indicate disease. Swelling and redspots in the ventral region can also indicate internal bleeding consistent with diseases.

The ability to routinely test individuals for either disease is beyond the logistical and financial ability of this protocol. For example, the cost to routinely test all frog species for *Bd* and the time for crews to immediately ship specimens off to analytical labs is prohibitive.

Instead, upon identification of a possible disease outbreak, the Field Crew Leader should inform the Project Lead as soon as possible, preferably the same day as the identification. The Project Lead should consult with the following entities:

USGS National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711-6223.
Phone: (608) 270-2400
Online at: <http://www.nwhc.usgs.gov/>

And

National Park Service
Biological Resource Management Division – Wildlife Health
1201 Oakridge Drive, Suite 200
Fort Collins, CO 80525
Phone: (970) 267-2162
Online at: <http://www.nature.nps.gov/biology/>

In providing diagnoses, the USGS National Wildlife Health Center will be able to instruct the Project Lead on specimen collection and shipping procedures to ensure quality specimens for diagnoses. These methods may change from year to year and from likely diseases. Live specimens may be preferred but will require same-day shipping using overnight services.

The National Park Service – Biological Resource Management Division similarly maintains wildlife health veterinarians who may assist the Project Lead in obtaining technical help with the possible disease.

Disinfection after every site is a necessary step to be taken after every habitat (SOP# 12: Post-Site Tasks). **HENCE, DISINFECTION TO PREVENT THE ADDITIONAL SPREAD IS DONE REGARDLESS OF WHETHER OR NOT THE CREW OBSERVES ANY SIGNS OF DISEASE.** The disease may be dormant or asymptomatic in the amphibian populations; failure to disinfect may spread the disease further.

Invertebrate Collection Procedures

Invertebrate collections are done by net sweeps in the littoral zone of each lake. The nature of a lentic habitat necessitates a semi-quantitative collection procedure, described herein and adapted from Knapp et al. (2005). Strictly quantitative collections are prohibited due to the logistics of heavy corers and motile nektonic invertebrates. Plus, the wide dispersion of lentic invertebrates dictates a large total area being sampled to adequately sample the assemblage.

Collections are comprised of 15 “standard sweeps.” A single standard sweep is done by rapidly dragging a D-net (304 mm wide, 0.5 mm mesh size) over a 1 m stretch of substrate. The net should follow the bottom contours and only sample the water column, epibenthos, and surficial sediments. The sweep is followed immediately by a sweep in the opposite direction so that the habitat sampled in the first sweep is resampled. This method is semi-quantitative because the 1 m stretch is visualized and not measured. A 1 m increment marked on the net handle is used to guide the crew member in this visualization so that the sweep length closely approximates 1 m.

After the sweep, the collected material is deposited in a bucket for processing and preserving in 95% Ethanol. If too much material is collected over the 15 sweeps to fit in a volume of 2 L, the collected material can be split in the field. This is done by mixing the material in the bucket so that it is homogenous, pouring the debris on a 500 µm sieve, and dividing the material into two sections. A coin flip determines which half to retain, and which to dispose of. The material can be further split, if necessary. The material is then transferred to 500 ml vials (up to four if necessary), labeled with a weather-proof paper with pencil added to each vial, and preserved with 95% Ethanol. Note that the water content is high in the debris, so that the final concentration will be closer to 75-80% Ethanol.

Invertebrate Collection Step by Step

- A. Prior to beginning the collection, label the collection vials with the pre-made labels SOP #9: Water Sample Filtration and Handling). Attempting to attach the labels to the vials after processing will result in difficulty in securely attaching the labels.
- B. As with the amphibian intensive search sites (Step A), make a visual estimate of the lake perimeter. This will indicate the spacing between invertebrate collection sites. Note that unlike the amphibian intensive search sites, the invertebrate collection occurs at the exact site (i.e., there is NO moving the location to a “better” habitat). The invertebrate collection area should be offset from the amphibian intensive search sites.
- C. Upon arriving at an invertebrate collection site, visualize the area to be swept. Using the 1 m mark on the net handle, make a mental note of the approximate length of 1 m. Sweep the net, by lightly dragging the net on through the surficial sediments in a 1 m length of habitat parallel to the lake shore. The net should be swept with a rapid movement; this will increase the likelihood of capturing fast moving, swimming invertebrates that may exhibit avoidance behavior.
- D. **Immediately**, at the end of the 1 m sweep, twist the net 180 degrees, so that the net is turned around (but without flushing the previously collected debris). Again, **immediately** sweep the net back over the 1 m sweep length, so that any material disturbed into the water column is collected in the net.
- E. Deposit the collected debris (with invertebrates) into a collapsible bucket.
- F. While the crew member is in the water, **the crew member who is walking the wetted perimeter enters the location of the invertebrate collection into the Trimble** (as above in Section 1).

- G. Continue with the walk-around, recording the substrates and amphibian visual search as in the above sections.
- H. Repeat steps A – E at the remaining 14 invertebrate collection sites, adding debris to the collapsible bucket. If required, two or more buckets can be used to collect the debris.
- I. After collecting in 15 sites, process the collected debris by transferring to containers.
 1. While the debris is in the bucket, remove large particles of debris (pinecones, sticks, etc.). Before removal, rinse with water to ensure that no invertebrates are hanging on. Dump removed particles on the shore or back into the lake. Do not spend a lot of time on this; a maximum of 15 minutes is recommended. If the other crew member has other tasks remaining, more than 15 minutes can be spent on this at the discretion of the Crew Leader.
 2. If the remaining collected material is greater than 2 L, it is necessary to do a “field” split.
 - a. Pour the debris onto the 500 μ m sieve. It is best to work in moderate portions of debris; not all the debris must be split at once.
 - b. Stir or agitate the debris so that it the debris is homogenous in the sieve.
 - c. Use the splitter (a length of lightweight steel plate that matches the interior diameter of the sieve) to split the debris into two equal portions.
 - d. Using a coin flip or other random number generator, discard one half of the debris back into the lake. Note that this portion may contain hundreds of invertebrates, so it is best to return it to the lake or pond.
 - e. Repeat a-d until the entire original collection has been split.
 - f. If the sample is still greater than 2 L, repeat a-d again (effectively so that a 25% portion of the original sample is retained).
 3. Once the sample has been reduced to fit within a 2 L volume, transfer the material to the collection vials.
 4. The vials may be filled to the top, but do not compact the material; Ethanol must be able to penetrate the material for preservation.
 5. Fill the vials with 95% Ethanol, so that Ethanol covers the debris.
 6. Fill out a paper label (on Rite-in-the-Rain paper) with a pencil. **Be sure to indicate the total number of vials and the amount of field split.**
 - a. Write “1 of 4,” “2 of 4,” etc. Do not use short hand, as fractions could be confused with percentages (e.g., “1/4” is a fraction [25%], and could be confused with the split count). Use % to indicate the amount of field split (one complete split = 50%; two splits = 25%; three splits = 12.5%; four splits = 6.25%). If there were no splits, indicate with a 100%.
 - b. Record the number of vials and the split percentage on the field data sheet.

Riparian Habitat Characterization

Lake riparian habitat is adapted from the KLMN Wadeable Streams protocol. Riparian habitat is assessed in a 15 by 15 meter plot alongside the invertebrate and amphibian collection stations, with the point of invertebrate defining the middle of the plot. In this zone, the crew characterizes the vegetation type and areal coverage, as well as the dominant tree in this plot and any indications of human influence.

Riparian “Dominant” Tree Characterization

This procedure contributes to the assessment of “old growth” (or simply the largest local tree) characteristics of riparian vegetation and aids the determination of possible historic conditions and the potential for riparian tree growth. Record the type of tree, and, if possible, the taxonomic group. Record this information, along with the laser-measured height, approximate diameter at breast height (dbh), and distance from the wetted margin of the stream on the data form. Visual estimation of these parameters from the stream bank may be difficult to ascertain due to understory and ground cover vegetation blocking line-of-site. If necessary, travel into the riparian zone towards (or possibly away from) the dominant tree until an adequate estimation can be made from an unobstructed position.

1. Select the largest tree in the 15 m × 15 m plot (if any; it is possible that a plot will be barren).
2. Classify this tree as deciduous, coniferous, or broadleaf evergreen (classify western larch as coniferous). Identify, if possible, the species or the taxonomic group of this tree from the following list:
 - a. Acacia/Mesquite
 - b. Alder/Birch
 - c. Ash
 - d. Maple/Boxelder
 - e. Oak
 - f. Poplar/Cottonwood
 - g. Sycamore
 - h. Willow
 - i. Unknown or Other Deciduous
 - j. Cedar/Cypress/Sequoia
 - k. Fir (including Douglas Fir, Hemlock)
 - l. Juniper
 - m. Pine
 - n. Spruce
 - o. Unknown or Other Conifer
 - p. Unknown or Other Broadleaf Evergreen
 - q. Snag (Dead Tree of Any Species)
 - i. If the largest tree is visibly determined to be dead, enter “Snag” as the taxonomic group, regardless of whether or not the species of the dead tree can be identified.
3. Estimate the height of the dominant tree using the laser rangefinder (TruPulse 200B).

- a. Turn the rangefinder on, by pushing the “Fire” button (Figure 4).

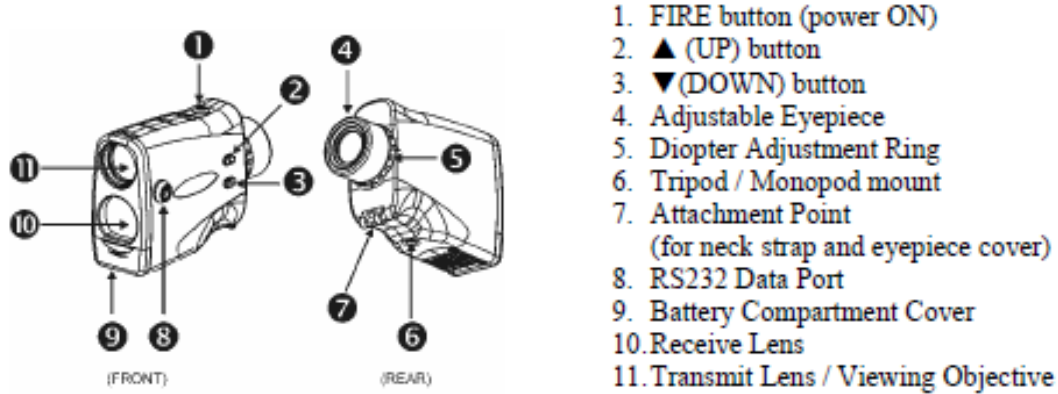


Figure 4. TruPulse 200B laser rangefinder functions.

- b. Ensure that the unit is in Height Measurement Mode (flashing “HD” and solid HT displayed in viewfinder [Figure 5]).

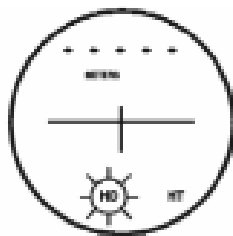


Figure 5. Height measurement mode.

- c. If not, scroll through the settings with either button 2 or 3 (Figure 4).
- d. Start by measuring the horizontal distance to the largest tree in the inter-transect area. Do this by pressing and holding the “Fire” button.
- e. The horizontal distance will briefly flash on the top of the viewer. In most cases, this will be the distance to the tree. **Record this in the proper place.**
- f. The viewfinder will then request for the first angle (this can be the top or the bottom of the tree) (Figure 6).

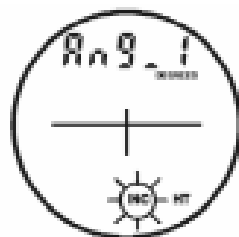


Figure 6. Viewfinder requesting the first angle.

- g. Aiming at the top or bottom, press and hold the “Fire” button. The current angle will be display. When you are as close to the top or bottom as you can be, release the “Fire” Button; this locks in the measured angle.
- h. The unit will now be ready for the second angle (the one you didn’t measure above) (Figure 7).

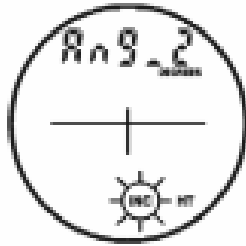


Figure 7. Viewfinder requesting the second angle.

- i. Repeat step g, by pressing and holding the “Fire” button while aiming at the base or top.
- j. Upon release after measuring the second angle, the calculated height will be displayed (Figure 8). **Record this as the height.**

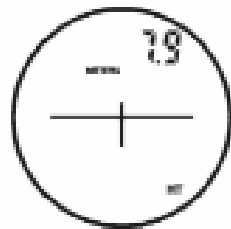


Figure 8. Viewfinder displaying calculated height.

- k. If you are unable to get a clear line of sight to either the top or bottom of the dominant tree in a reasonable amount of time (1-2 minutes of scrambling), shoot to the highest point possible and add a > sign to indicate that this is a minimum measurement of tree height. Although an absolute height is the preferred measure, logistical constraints prevent more than a couple minutes spent trying to see a clear view of the top and bottom.
4. Estimate and record the diameter of the dominant tree at breast height (dbh).
 5. Estimate and record the distance of the dominant tree from the wetted margin of the lake. (This may be done with the laser rangefinder, if the user is standing at the lake margin.)

Visual Riparian Estimates

1. Standing at the invertebrate collection point estimate 7.5 meters on either side and 15 meters deep to approximate the 15m × 15 m plot.
2. Within this 15 m × 15 m area, conceptually divide the riparian vegetation into three layers: a Canopy Layer (>5 m high), an Understory (0.5 to 5 m high), and a Ground Cover layer (<0.5 m high).
3. Within this 15 m × 15 m area, determine the dominant vegetation type for the Canopy layer (vegetation >5 m high) as either Deciduous, Coniferous, broadleaf Evergreen, Mixed, or None. Consider the layer "Mixed" if more than 10% of the areal coverage is made up of the alternate vegetation type. If Mixed is chosen, indicate which other categories are present to constitute "Mixed."
4. Determine separately the areal cover class of large trees (>0.3 m [1 ft] diameter at breast height [DBH]) and small trees (<0.3 m DBH) within the canopy layer. Estimate areal cover as the amount of shadow that would be cast by a particular layer alone if the sun were directly overhead. Record the appropriate cover class on the field data form ("0"=absent: zero cover; "1"=sparse: <10%; "2"=moderate: 10-40%; "3"=heavy: 40-75%; or "4"=very heavy: >75%).
5. Look at the Understory layer (vegetation between 0.5 and 5 m high). Determine the dominant vegetation type for the understory layer as described in step 4 for the canopy layer.
6. Determine the areal cover class for woody shrubs and saplings separately from non-woody vegetation (Herbs, grasses, and forbs) within the understory, as described in step 4 for the canopy layer.
7. Look at the Ground Cover layer (vegetation <0.5 m high). Determine the areal cover class for woody shrubs and seedlings, non-woody vegetation (Herbs, grasses, and forbs), inundated (standing water), and the amount of bare ground present as described in step 4 for large canopy trees.

.Human Influence Estimates

1. Looking from the same plot as the riparian vegetation, examine for the following human influences: (1) walls, dikes, revetments, riprap, and dams; (2) buildings; (3) pavement/cleared lot (e.g., paved, graveled, dirt parking lot, foundation); (4) roads or railroads; (5) inlet or outlet pipes; (6) landfills or trash (e.g., cans, bottles, trash heaps); (7) parks, maintained lawns, campsites, or firepits; (8) pastures, rangeland, hay fields, or evidence of livestock; (9) logging; (10) recent wildfires, and (11) mining (including gravel mining).
2. For each type of influence, determine if it is present and what its proximity is to the stream and riparian plot area.
3. For each type of influence, record the appropriate proximity. Proximity classes are: B ("Bank") Present within the defined 10 m stream segment and located in the stream or on the stream bank; C ("Close") Present within the 15 × 15 m riparian plot area, but away

from the bank; P ("Present") Present, but outside the riparian plot area; and O ("Absent") not present within or adjacent to the riparian plot area.

Standard Operating Procedure (SOP) #12: Post-Site Tasks

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP details the necessary tasks for the field crew to do in between sampling of sites. This includes: (1) Disinfection of field gear; (2) Storage and shipping of water samples; (3) Data backup (photos, multi-probe data, GPS data); and (4) Tasks to prepare for the next day.

Prior to leaving the site, the crew must review each data sheet and electronic form for completion and accuracy and the crew leader must sign each page to verify that the data are complete.

In the event that a certain protocol was not doable, due to equipment failure, safety reasons, etc., an event log (Appendix F: Field Data Sheets, Training Logs) should be filled out prior to leaving the site.

Disinfection

It is the responsibility of the field crew to ensure that they do not participate in the transfer or spread of wildlife diseases or invasives species. To minimize the chance of disease spread, crews should switch out of hiking footwear before they approach the waterbody and switch to water wear (sandals, neoprene booties, or waders). This water footwear is then disinfected before going to another waterbody.

Gear is packed up at the water's edge prior to departure and is isolated from the environment in industrial strength trash bags. **(Gear should be scrubbed with a stiff brush prior to packing to remove excess debris.)** Disinfection is then carried out back at either the park housing or campground.

1. Start disinfection by preparing a treatment barrel and rinse barrel (13+ gallon trashcan).
 - a. Prepare a 3% solution of bleach (Sodium hypochlorite). Standard household bleach (e.g., Clorox®) is 6.15%. This may vary depending on brand.
 - b. Use the $c_1V_1 = c_2V_2$ formula to calculate the amount of bleach needed in the trash can to be diluted. In the case of commercial Clorox, with a final volume of 10 gallons, this is:

$$(6.15\%) \times (\text{initial volume}) = (3\%) \times (10 \text{ gallons})$$

Initial volume $\approx$ 4.9 gallons

- c. Under the above scenario, 4.9 gallons should be poured in one of the trash cans, and 5.1 gallons of water should be used to dilute it to a final volume of 10 gallons.
2. Fill a second trash can with 10 gallons of tap water as rinse water.
3. Place gear in bleach water for 1 minutes. This must be timed and not estimated.
4. Rinse gear in tap water (in second trash can).
5. Allow to dry as thoroughly as possible before packing up again.
6. Used bleach solution must be brought back to a municipal sewage system, where it can be added to standard waste water and safely decontaminated using dilution of tap water. Two 5 gallon jugs are provided to return the bleach water from the park housing units to municipalities.

When the above protocol is not doable (e.g., the crew is camping at isolated areas) or the crew needs to disinfect expensive electronic gear (e.g., the multiprobe), an alternative procedure should be followed:

1. Using a household spray bottle, spray 70% Ethanol over the entire surface of the equipment.
2. Allow Ethanol to permeate the equipment for at least 1 minute.
3. Rinse with tap water and allow to dry (except for the multiprobe).

Gear to be disinfected includes all gear that came into contact with water when sampling, including but not limited to: waders, boots, sandals, nets, boats, paddles, water sampling gear, Secchi Disks, etc.

Sample Storage and Shipping

Storage

Upon return to the crew housing, the samples should be stored as follows:

Dissolved Organic Carbon: placed in a refrigerator at 4° C, in a dark container.

Filtered Water sample: placed in a freezer at -18° C in the dark.

Unfiltered Water sample: placed in a freezer at -18° C in the dark

Chlorophyll a filters: placed in a freezer at -18° C in the dark.

Zooplankton and Macroinvertebrate samples: No special storage necessary, but should be organized and stored in plastic storage bins.

Although the crew should have ensured that labels were adequately attached, accurate, and followed the protocols, the crew should double check labeling at this time too.

Shipping

It is the responsibility of the Project Lead to arrange for the shipping of samples to contract laboratories. Depending on the requirements of the contract laboratories, zooplankton and

macroinvertebrate samples may be held until the field season has ended. Shipping of samples sooner may be desired if the laboratory can improve upon sample turn-around time.

Water samples must be shipped throughout the project so that sampling holding times are minimized. Most water samples can only be held for a maximum of 28 days before the quality control issues arise and EPA regulatory holding periods are exceeded (Eaton et al. 2005).

Shipping preferences will vary depending on the contract laboratory. The basic protocol below is for the contract laboratory used for the pilot project, the Cooperative Chemical Analytical Laboratory, based at Oregon State University.

1. When shipping samples, time is of the essence (e.g., do not start sample preparations on a Friday. Instead, do it on a Monday morning, so you can ship it that afternoon and the receiving lab can get it on Tuesday morning.). Also, confirm with the lab that someone will be there to receive the samples and that they are ready for them to arrive.
2. Start by ensuring that all samples to be shipped out are present and properly labeled.
3. Prepare a sample inventory sheet to provide to the laboratory, both included in the package and for electronic delivery:

NPS LAVO Lake Pilot Project 2008					
Sample no.	Lake Code	Date (YYYYMMDD)	Type	County	State
1	11555	20080909	filtered water deep	Shasta	California
2	11555	20080909	filtered water shallow	Shasta	California
3	10623	20080910	filtered water deep	Shasta	California
4	10623	20080910	filtered water shallow	Shasta	California
5	10624	20080911	filtered water deep	Shasta	California
6	10624	20080911	filtered water shallow	Shasta	California
7	10655	20080917	filtered water	Shasta	California
8	11322	20080917	filtered water deep	Shasta	California
9	11322	20080917	filtered water shallow	Shasta	California

Samples were collected with a horizontal Van Dorn bottle. Samples labeled shallow were taken 0.5 m below the surface of the lake, and samples labeled deep were taken 0.5 m above the bottom of the lake. If there is no "deep" or "shallow", then there was only a single midpoint sample taken. All samples were filtered through a 0.45 micrometer nylon membrane filter.

Contact Information: Dr. Eric Dinger, (541) 552-8574, Eric_Dinger@nps.gov
 1250 Siskiyou Blvd
 Klamath I&M Network
 Southern Oregon University
 Ashland, OR 97520

Account Code: SCD-08G

4. This inventory includes contact information, lake code, dates, type of sample, and sampling information. A sample number is assigned and added (1 – 9, above), to allow the lab and Network to ensure that there is no confusion about what sample is what.

5. Secure a label to each bottle with the sample number, ensuring that the label is on the right bottle.
6. Wrap the bottle or vial lid with parafilm or electrical tape to ensure a tight seal. Place these within a large zip seal type plastic bag.
7. Using blue ice packs to keep the samples cold, place the samples in a medium sized ice chest (e.g., a 48 quart). Place the frozen filtered water samples on the bottom, and then layer some newspaper or cardboard in between the frozen samples and the refrigerator (i.e., non-frozen) dissolved organic carbon samples.
8. When packing the dissolved organic carbon samples (in glass vials), add some padding around the vials to prevent breakage.
9. Print out a copy of the sample inventory sheet, place it in a zip seal type plastic bag, and place on top of the sample (so that it is the first thing the receiving lab will see). **Include a chain-of-custody form, detailed below (included in Appendix F: Field Datasheets, Logs).**
10. Seal the ice chest with packing tape for a secure seal.
11. Use an express carrier (e.g., UPS or Fed-Ex) to send the package **overnight**. If shipping from a university, ensure that the package is delivered to the mail room prior to the carrier pickup. The drop-off point should be planned out in advance, so that the crew or Project Lead is not searching for one at the last minute.
12. If packing and shipping is done by the crew leader, it is the responsibility of the Project Lead to provide the crew leader with either pre-paid shipping labels or an account code to charge the shipping to.
13. After shipping, the Project Lead must follow-up with the recipient to ensure that the samples were safely delivered and received with no loss of sample integrity. Shipping and tracking numbers should be retained to facilitate any follow-up.

The shipping of zooplankton and macroinvertebrate samples are similar to the shipping of water samples. **However, the shipping of Ethanol (a flammable material) is regulated by the Department of Transportation; shipping of undeclared Ethanol is a federal offense.** The regulated shipping of Ethanol is permissible, if the shipper (the Project Lead) is certified through a Department of Transportation training program and special procedures are followed.

A safe, legal work-around to shipping Ethanol is as follows:

1. Prior to shipping, pour off the majority of the Ethanol in the shipping vial (generally 80 to 90 % of the Ethanol). Use a sieve to ensure that no specimens are lost.
2. Replace with tap water.
3. Include a letter to the laboratory, clearly stating “**stored in water – replace with Ethanol upon receiving.**”
4. Warn the lab staff that the incoming samples will need Ethanol replacement and ensure that the samples are sent **overnight**.
5. As with the water samples, send so that they arrive on a working day, when staff will be present to replace the water with Ethanol.

When shipping invertebrate samples (legally with Ethanol, if a certified shipper is known, or by replacing Ethanol with water), use the following guidelines:

- Ship in a hard plastic container (e.g., ice chests).
- Wrap vials with Parafilm or vinyl electrical tape.
- After wrapping with Parafilm, put vials in a zip seal bag.
- Include absorbent material in the vial and packaging in case of breakage.
- Fill out and include a chain-of-custody form (in a waterproof bag, Appendix F.)
- Redundancy is good.

Chain-of-Custody Form (adapted from the Greater Yellowstone Network [O’Ney 2005])

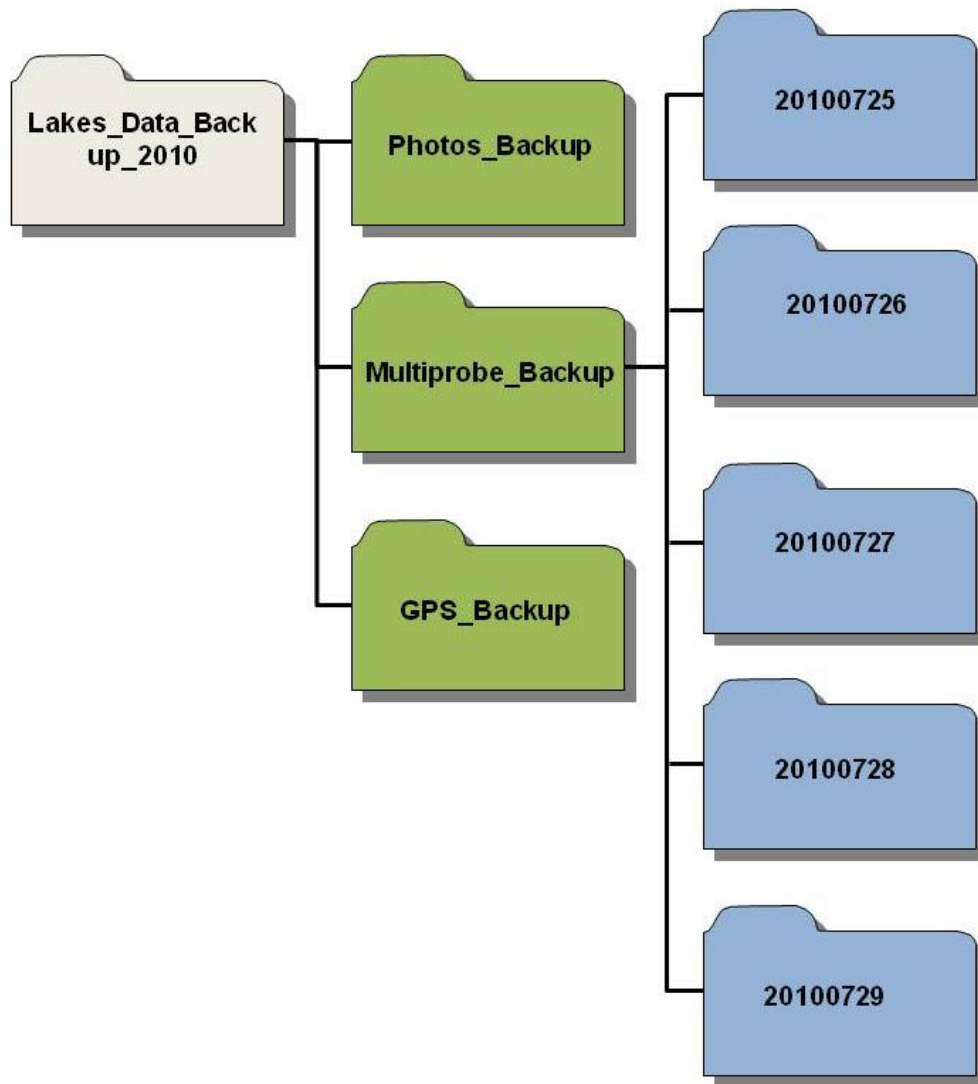
A chain-of-custody form will document the collection and transfer of all samples originating from this protocol. The end purpose is to assure that an accurate written record is created by the field crew that will be accepted as valid evidence to trace a sample or samples from the moment of collection through laboratory testing and reporting of test results.

When shipping samples, the form must be completed and attached to the sample inventory sheet (as above, in a waterproof bag). The Project Lead, shipping the samples, retains a shipping receipt as proof of transfer of custody. Laboratory personnel receiving the samples indicate date and time received upon sample arrival. The original forms are scanned and retained, along with all other logs.

Some aspects of the chain-of-custody form replicate the sample inventory form but should be included on both sheets for regulatory purposes.

Data Back-up

Upon returning to the crew housing, all electronic data should be backed up onto a Network laptop. This includes digital photos, multiprobe data, electronic databases, and Trimble GPS data. Prior to field crew deployment, the Project Lead should preload the laptop with the following hierarchical file structure. This file structure should start within the folder: C:\Documents and Settings\My Documents\Lakes_Protocol.



Et Cetera...

The file of the first hierarchical folder (Lakes_Survey_2010) should be adjusted from field season to field season, with the current year forming the last four digits. The folders for the third level (e.g., 20100725, 20100726) follow the date format of `yyyymmdd`. Every day that has a field sampling activity should have a representative folder within each type of data.

Note that backing up the files *does not include any renaming of files!*

Photos

The digital photos taken for the day should be downloaded after every field visit. Using Windows Explorer, the photos from the camera (connected to the computer using the camera's download cable) should be copied (using shift-left click function to highlight the photos, followed by a right click and selecting "copy") to copy the entire set of the day's photos.

The destination folder should then be opened up in the file structure, ensuring that it is within the Lakes_Survey_yyyy and Photos_Backup with the correct date folder. The photos should then be pasted within the date folder (e.g., 20100725). Keep in mind, photographs from previous days will be on the camera and care should be taken to only back up photographs from that day's surveys. **A double check that the correct files were copied into the correct destination should follow.** The camera can then be disconnected using the "Safely Remove Hardware" function of Microsoft Windows.

Multiprobe Data

The Sonde multiprobe data should be backed up in a similar fashion to the photos.

The *Amphibian* should be connected to the laptop computer via a USB download cable supplied to the field crew. Upon connecting, the iPaq PDA should be auto-detected using Microsoft ActiveSync. For the purposes of downloading the multiprobe data, the ActiveSync functions can be ignored and canceled out of.

Using Windows Explorer, click on the device labeled "Mobile Device" under "My Computer." This will access the files on the PDA. Under the file folder, "My Documents\Eureka," copy the .loc file (this is known as a "location" file for the amphibian unit) and copy and paste into the "Multiprobe_Backup" folder of the appropriate date. At this point, the crew member should ensure that there are data within this file, done by checking that the file size is greater than 0 KB.

After downloading to the laptop, the multiprobe data should be processed to remove readings between every 0.5 meters that were not equilibrated.

1. Open the program "Eureka," software provided by Eureka Environmental
2. Click the "**Open**" button.
3. Navigate to the downloaded .loc file, and open.
4. The data should appear in the window.
5. Click the "**Export**" button, and save as an Excel CSV file in a sub-folder.
6. Using Windows Explorer, navigate to the CSV file, and double click to open in Excel. (Although not an .xlsx file, it will open automatically).
7. Click on the leftmost row numbers to highlight the entire row. Hold shift and click on the last row to be deleted. This will highlight all the rows in between the first row of the series to be deleted and the last.
 - a. Delete all rows until the last, fully equilibrated measurement at 0.5 meter intervals.
 - b. When done, all that should remain are single readings for 0.5 m, 1.0 m, 1.5 m, etc.
 - c. Note that because of slight movements up and down during field measurement, the data retained may not be exactly at 0.5 m intervals (e.g., readings at 0.54, 1.01, 1.49, 2.00, 2.55) – this is okay.
8. Check for potential errors (e.g., circulator not turned on – recorded in the file as "0.").
9. Save as an .xlsx file, retaining the original file naming convention.

GPS Data

The details on backing up GPS data are also provided in SOP #11: Amphibian, Invertebrates, and Lake Substrate Walk-around, but are also summarized here.

Make certain you have ActiveSync on the computer where you plan on backing up the data and follow the steps below to back up the data.

1. Connect the Trimble cradle to the computer and place the Trimble unit in the cradle.
2. ActiveSync should start automatically and will let you know when the computer and the Trimble unit are connected.
3. On the computer, open Windows Explorer and look for the Trimble icon that is labeled “Mobile Device” (Figure 3). Double click on the mobile device.
4. Go to the following pathway:
My Windows Mobile-Based Device\Lakes_Study_2010
5. Right click on the folder called “Shapefiles” and select “copy.”
6. Go to the location you plan on storing the backup file (the dated folder 20100725, for example, within the GPS_Backup folder).
7. Right click on the appropriate folder and select “Paste.”

An alternative, using a “Secure Digital” card (better known as SD cards) can be used by the crew. If the Trimble is equipped with an SD card, transfer the “Shapefiles” folder to the card using the PocketPC mobile Windows Explorer, then eject the card and, using an external card reader or internal drive (if laptop has one), copy the “Shapefiles” folder to the dated folder (e.g., 20100725) in the GPS_Backup folder. Replace the SD card back in the Trimble.

Tasks for the Next Field Day

The field crew, collectively, should do the following:

1. Check consumables and replace field kits/backpacks with necessary supplies, such as filters, latex gloves, water bottles, foil for Chlorophyll *a*, data sheets, etc.
2. Batteries should be recharged, including but not limited to: Icom Radio, Amphibian data logger, GPS units (Trimble and Garmin, if applicable), camera, headlamps, and tablet PCs.
3. Review the field folder for the next day's site. If a repeat Index site, they should look over the photos, field conditions, and access time required by previous crews. If a new site, they should review maps and access routes to plan for the following day.

Literature Cited

Eaton, A. D., L. S. Clesceri, E. W. Rice, and A. E. Greenberg, editors. 2005. Standard methods for the examination of water and wastewater, 21st edition. American Public Health Association, Washington, D.C.

O’Ney, S. E. 2005. Regulatory water quality monitoring protocol, Standard Operating Procedure #7: Quality assurance/quality control procedures. National Park Service, Greater Yellowstone Network, Bozeman, MT.

Project: Klamath Inventory and Monitoring Lakes

Project Lead:

Phone:

Address:

Fax:

Email:

Park(s):

Shipped via: Fed-Ex UPS USPS

Tracking Number:

Field Crew:

Sample (may refer to an attached inventory sheet)	Date Sampled (yyyymmdd)	Time sampled (24 hour format)	Site Name	GRTS Code	Type (Filtered water, Dissolved Organic Carbon, Invertebrates, Zooplankton, Chlorophyll a)

Name and Position	Date/time received	Location received (i.e. Lab name)	Date/time relinquished	Location delivered/sent to:	Signature

Standard Operating Procedure (SOP) #13: Data Entry

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

Introduction

Data entry is a critical component of field studies that must be addressed to ensure the data collected are of good quality. This SOP provides details of how to complete data entry in the project databases, which include site characterization and fish. The database was developed using the NPS Natural Resource Database Template (NRDT) in Access 2007 and utilizes lookup tables and electronic forms that mimic the hardcopy datasheets to reduce transcription errors and allow for easy data entry. Data dictionaries for the database are provided in Appendix M. Once data entry is completed, data are assessed for Quality Assurance/Quality Control processes, covered in SOP #16: Quality Assurance Project Plan. Once quality control processes have been implemented, data are uploaded into a master database that is used to conduct analysis and develop summaries for reports. The master database also has the ability to export the data into the NPSEDD (NPS Electronic Data Deliverables) format for integration into EPA STORET (STORage and RETrival).

This data entry SOP describes a single process for one of two possible methods: (1) entering data into the project database, transcribing the data from paper field sheets; or (2) directly entering the data into a field Tablet PC during data collection. The latter is the preferred method; however, the methodology is identical. The same database is used since the Tablet PC operates a full version of Microsoft Windows operating system with the full functionality of a desktop computer.

Timelines and Responsibilities

Field crew members are responsible for completing the first two rounds of data validation and data entry. Data should be entered as quickly as possible following data collection. While it is the goal of the Network to collect data electronically wherever possible, data will occasionally be recorded using datasheets. Hardcopy datasheets should be entered in the same week the data are collected. It is the responsibility of the Project Lead to ensure data are entered on a weekly basis

and should schedule the field crew's time accordingly. The Project Lead should review the data after the first sampling period where field crews have been working on their own to ensure data are being documented properly. Periodically, the Project Lead should also review a subset of the data to ensure they are being recorded properly throughout the field season. In addition, prior to field crews starting a new park, the Project Lead should review the data to ensure they are accurate and complete.

Preparing the Database for Field Work

In order to prepare the database to be used in the field, the Project Lead will need to provide the Data Manager with a GIS layer of all the sites that will be visited during the upcoming season, a list of field crew members and contact information, and a list of all species expected to be encountered and identifiable in the field that will be recorded as part of this protocol (SOP #2: Field Crew Training). Note: the field species list need not include all potential species that might be subsequently identified in samples by contract laboratories. Once the Data Manager has the lists, he/she can begin to prepare the project database that will be used by the field crews that year.

Entering New Sites

To prepare the project database so it can be used in the field, there are a variety of steps (listed below) that need to be followed.

1. The first thing the Data Manager must do is load a list of sites that will be surveyed that year into the database.
 - a. It is the responsibility of the Project Lead to work with the GIS Specialist to develop this list of sites.
 - b. The final list of sites to be surveyed should be located at (to be done by the Project Lead):
G:\Monitoring\Water_Quality_Monitoring\Lakes\Lakes_GIS\PARK\YYYY
and the name of the file is PARK_YYYY.dbf. In both the file pathway and the file name, the "PARK" is the four letter park code and YYYY is the year of the survey.
 - c. A copy of the site list file should be made and placed in:
G:\Monitoring\Water_Quality_Monitoring\Lakes\Lakes_Data\Survey
Areas\YYYY\PARK.
2. Open the front-end of the database that you have placed in your working directory.
3. Click on the [Administrative Tools] button.
4. Click the [Upload Sites] button.
5. Browse to the location of the file you created in step 1c above.
6. Click the [Upload Sites] button.

7. The sites table should now be populated with the following:

- a. Location_ID
- b. Network
- c. Park
- d. Site_Name
- e. Site_Type
- f. GRTS Code
- g. X-Coordinate
- h. Y-Coordinate
- i. Coordinate System
- j. PLSS
- k. Watershed
- l. Subwatershed
- m. County
- n. USGS Map
- o. GIS ID Number
- p. GIS Shapefile Name

Entering Contact Information

Next, the Data Manager will need to enter the contact information for each of the individuals that could be working on this project, supplied by the Project Lead. To complete this task, follow the steps below.

1. Open the front-end of the database and click the [Lookup Tables] button.
2. Using the pick list, select `tlc_contacts`.
3. Delete any of the contacts that are not going to be involved in the project that year.

Add any new contacts (such as new crew members) that will be involved in the project by May 31st of that year. This information should be provided by the Project Lead as described in SOP #1: Preparations, Equipment, and Safety.

Updating the Pick List

Since this is a standardized protocol, the pick list values should not be changed, with the exception of a few rare occasions. It is the responsibility of the Project Lead to follow all change procedure processes associated with this protocol prior to having the Data Manager

change one of these values. Once proper procedures have been followed, the Data Manager should follow these steps to update a pick list.

1. First, determine which fields need to be updated.
2. Click on the [Lookup tables] button on the main form of the database.
3. Using the pick list, select `tlu_Enumerations`.
4. Find the `Enum_Group` associated with the list of data you want to edit.
5. Delete any values you no longer want to use.
6. Add any new values that are needed. Be sure to complete all fields, including:
 - a. `Enum_Group` exactly as it is in the database.
 - b. Sort Order, which is the order you want the data to appear in the pick list.
 - c. `Enum_Code`, the value that is stored in the database.
 - d. `Enum_Description`, a description of the value that is stored in the database.
7. Once you are done, click the [Close] button in the upper, right corner of the form.
8. It will ask you if you want to save your changes, click [YES].
9. You are done.

Data Entry

It is the responsibility of the Project Lead to ensure that the laptop provided to the crew has the current version of the Klamath Network Mountain Lakes and Pond database.

To start, navigate to `C:\Lakes\Lakes_Data`. Within this folder, double click on the icon:  `KLMN_FE_v1.00_20081124.mdb`. This will open up a “gateway” to enter the data (Figure 1).

Procedures

1. On the left side of the database window that opens up is a tab named “Enter / edit data.”
2. After clicking on “Enter / edit data,” you will be prompted for setting some default values for this entry session (Figure 2).
3. Assure that the information is presented correctly. If you are a new user, follow the menu-driven process to add yourself. Note that name is last name_first name, with proper punctuation (capitalize first and last name, separated by an “underscore”). Set the correct park, datum, zone and protocol (NAD83, Zone 10N is default). Protocol will be Lakes Monitoring for all sites.
4. After clicking “OK,” a list populated with existing data will appear (Figure 3). At the first data entry session, this list should be blank. You should be supplied with a blank database, the previous field season being stored and archived according to the Klamath Network Data Plan (Mohren 2007).

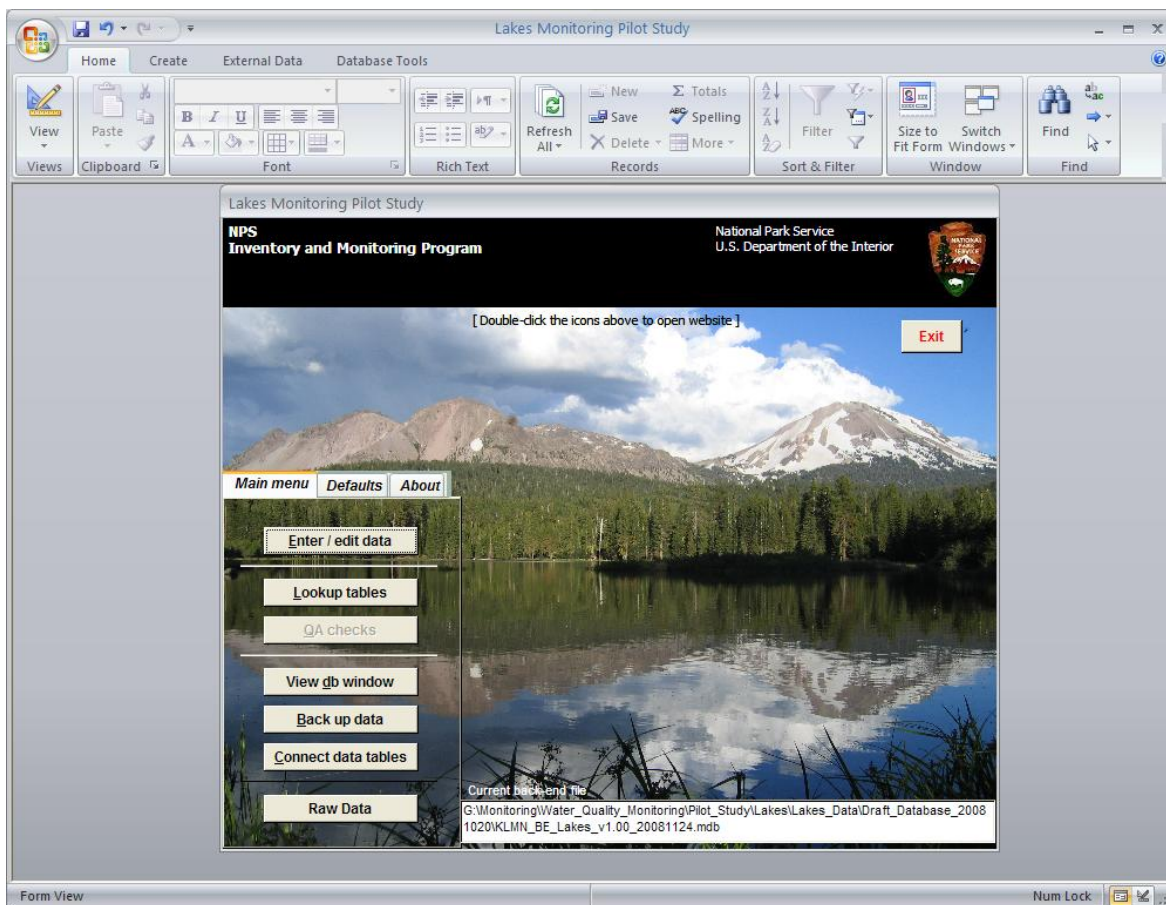


Figure 27. Klamath Network database gateway for entering Mountain Lakes and Ponds data.

Figure 28. Default screen for beginning the data entry process.

5. Click on “Add a new record” in the upper right hand corner.
6. Fill out all fields on the “Data Entry Form – New Record” screen that originates (Figure 4).
 - a. First, in the location field, select the name of the lake that you are sampling. This should automatically populate the X/Y coordinates and the unit code fields.
 - b. Next, enter the following fields:
 - i. Start date – date you started the survey.
 - ii. Arrival time – time you arrived at the lake.
 - iii. Drive time – time taken from staging area/housing till parking for the start of hiking.
 - iv. Hike time – time it took you to hike from your starting location (parking spot or camping site) to the lake.
 - v. Descriptor of location – General (Forested, Alpine, sub-alpine).
 - vi. Trails used to access – Provide a description of the trails you used to access the lake, beginning at the parking location or camp site.
7. Next, complete all the fields in the eight tabs that do not pertain to data collected on the Trimble unit or Manta data logger. Be sure to fill in all areas. Even if there appears to be a field that does not pertain to this particular site, be sure to note it with either N/A for not applicable or with a note in the “Visit notes” section. If necessary, “add a person” (e.g., a park specialist joined the crew for that day).
8. As data are being entered, the person entering the data should visually review each data form to make sure that the data on screen match the field forms.
9. Cycle through the tabs (highlighted in red in Figure 4) to ensure that all data are entered. At the conclusion of the data entry for this site, there should be **NO DATA** on the field form that is not entered into the database.
10. Because this database is designed to contain all data originating from this protocol, there will be fields not entered by the crew: Invertebrates, Water chemistry, Manta data, and Amphibians (embedded in the GIS shapefiles). **Note that there are some fill-able fields (e.g., the time and field split of invertebrates, and amphibian walk-around times). The crew must still enter these data!** These fields will be filled in by data import steps after the contract laboratories provide their data to the Project Lead. During data entry, tabs and fields to be imported later will be “hidden” from the database interface.

Sample Data Gateway - List of data that have been entered

* Double-click on the field label to change sort order. Double-click on a Location Name to open the Locations form for that record or a Visit Date to open the Data Entry form for that record.

Filters
 Park: LAVO Location Name: Year: Visit Date: Filter Is On

Unit*	Site Name*	Location Name*	Entered/updated*	Year*	Visit date*	Protocol*
LAVO		Site-014	10/31/2008 11:16:55 AM	2208	10 Sep 2208	Lakes Pilot Study
LAVO		Site-000	10/31/2008 1:16:46 PM	2008	22 Sep 2008	Lakes Pilot Study
LAVO		Site-001	10/31/2008 3:26:31 PM	2008	03 Oct 2008	Lakes Pilot Study
LAVO		Site-003	10/31/2008 11:34:15 AM	2008	12 Sep 2008	Lakes Pilot Study
LAVO		Site-004	10/31/2008 3:44:56 PM	2008	05 Oct 2008	Lakes Pilot Study
LAVO		Site-006	10/31/2008 12:34:42 PM	2008	17 Sep 2008	Lakes Pilot Study
LAVO		Site-008	10/31/2008 4:17:42 PM	2008	14 Oct 2008	Lakes Pilot Study
LAVO		Site-009	10/31/2008 2:46:05 PM	2008	01 Oct 2008	Lakes Pilot Study
LAVO		Site-010	10/31/2008 12:34:09 PM	2008	16 Sep 2008	Lakes Pilot Study
LAVO		Site-013	10/31/2008 2:35:14 PM	2008	30 Sep 2008	Lakes Pilot Study
LAVO		Site-016	10/31/2008 2:24:05 PM	2008	25 Sep 2008	Lakes Pilot Study
LAVO		Site-018	10/31/2008 4:07:19 PM	2008	06 Oct 2008	Lakes Pilot Study
LAVO		Site-300	10/31/2008 10:05:16 AM	2008	09 Sep 2008	Lakes Pilot Study
LAVO		Site-301	11/4/2008 11:16:42 AM	2008	02 Oct 2008	Lakes Pilot Study
LAVO		Site-303	10/31/2008 2:02:22 PM	2008	24 Sep 2008	Lakes Pilot Study
LAVO		Site-304	10/31/2008 1:34:48 PM	2008	23 Sep 2008	Lakes Pilot Study

Figure 29. Date screen gateway for new record additions.

Data Entry Form - New record

Location: X/Y: Protocol Name: Lakes Pilot Study

Start Date: Arrive Time: Depart Time: Drive Time: Hike Time: # Inlets: # Outlets: Descriptor of Location: Trails used to access: Visit Notes:

Weather Photographs Water Physio-Chemistry Invertebrates Fish Amphibians Water Chemistry Manta Data

Cloud Cover: Precipitation: Air Temperature: Wind Scale: Wind Direction: White Caps / Ruffles on Lake?:

Record: 1 of 1

Note tabs – all must be filled out

Figure 30. "Data Entry Form, new record" for entering Lake data. Note the tabs: the crew must cycle through each in turn to fill out all data.

11. Before concluding entering these site data, the person entering the data should **again** visually review each data form to make sure that the data on screen match those on the field forms.
12. If a new site (“add new”) is necessary, the crew should fill out all applicable fields.
13. When done with the site, the person entering the data should click on “New record” to enter the next site.
14. At the conclusion of the data entry session, click on “Close” to exit the data entry screen.
15. “Exit” out of the main window; you are done.

Basic Guidelines

1. Enter all data exactly as they appear on the field form. If notes were taken short hand during the field visit, and it was your note, it is acceptable to write it as long-hand (i.e., grammatically correct). An example: On field form – “2 hikers seen.” In database entry – “Two hikers were observed hiking by the site.”
2. When entering data, no fields should be left blank. When a field is left blank by the observer that actually required data, enter -9 in that entry field. This value is used because -9 would not be an acceptable data value for any field. An example of when to enter -9 is if the crew ran out of alkalinity reagents and was unable to take a second (or first) reading. This should be noted until “visit notes.”
3. When entering data, try to leave enough time to complete a block of data (e.g., the entire field visit). Do not partially enter a field visit.
4. After entering the data, write your initials and the date on the bottom of the field form in the applicable space.
5. If you have any questions about how to enter data, ask the Project Lead for clarification immediately; do not assume anything.

Data Verification and Validation

These are important steps from SOP #16: Quality Assurance Project Plan, but are repeated here to provide a comprehensive SOP for completing the data entry, including ensuring that the data are entered correctly.

Data Verification

Data verification of the data involves evaluating the correctness, conformance, compliance, and completeness of the entire dataset against the methods or procedures of the protocol (SWAMP 2008). Verification should be done on both field data (including field chemical analyses) and laboratory data (chemical, invertebrate, and zooplankton). It is the responsibility of both the Field Crew and Project Lead.

- Data Verification includes:

- Visual Review at data entry – the technician verifies each value during input. Errors are corrected immediately.
- Visual Review after data entry – after entry, data are printed out and compared to original hardcopy sheets.
- Duplicate data entry – Randomly selected site data are entered as normal, but are duplicate records. Although time consuming in that it repeats data entry efforts, this gives an estimate of the data entry accuracy.
- Review – It is the Project Lead’s responsibility to review a subset of records to ensure that they are identical to the hardcopy datasheets.
- For the duplicate data and review, the minimum number is 20% of the sites (approximately eight total sites).

Data Validation

After verification, the Project Lead reviews it against all criteria in the protocol, especially the QAPP criteria (e.g., holding times, laboratory duplicates, completeness goals, reporting limits). After successful validation, the Data Manager can send the data on to WRD for incorporation into WRD STORET.

- Data Validation includes:
 - Data entry programming steps – The Project Lead, along with the Network Data Manager, will program steps design to prevent errors. For example, maximum depth of a lake entry will not allow the recording of a lake 100 m deep (since there are no lakes in Lassen Volcanic National Park that deep). This is an example of a mistake that might occur if the technician accidentally enters “100” instead of “10.0.”
 - Outlier detection and review – Statistical review and graphical display will be used to detect outliers, which are unusually extreme values of a variable outside the range of normal values. In outlier review, it is important to realize that not all extreme values represent errors, but can reflect the real variation of the data in nature. Generally, outliers that cannot be ascribed to error will be flagged and retained.
 - Review of “what makes sense” – The Field Crew Leader and Project Lead will compare and review the tabular data to confirm that everything “makes sense.” Both should be intimately familiar with the types of data being collected, and as such should be able to detect mistakes. GIS data will be plotted and confirmed to match the spatial locations.

Database Backup

Data backups should be completed every day that new data are entered. Backups are created to

save time in case of mistakes or database file corruption.

Backing up the database is a simple procedure.

1. From the main database gateway (Figure 1), click on “Back up data.” You will then be prompted to confirm the backup. Alternatively, the user will be prompted to back up the database when exiting the program.
2. You will be prompted for a file location for the backup. Place it in: C:\Data\Lakes_Data\Backups. This location should be pre-created by the Project Lead or Data Manager on the laptop.
3. Save the file in this location with the default name given by MS Access. It will include a date and time stamp (e.g., 20090721_1133, for a backup made on the 21st of July, 2009 at 11:33AM).
4. Transfer the complete database and all backup files (including photos, multiprobe data, and GIS data [See SOP #12: Post-Site Tasks]) to the Project Lead, using a CD or flash drive. Deliver or mail to: Aquatic Ecologist (or Project Lead), Klamath Network, 1250 Siskiyou Blvd, Ashland, OR 97520, on a weekly basis.

Maintaining the Data Entry Log

A data entry log must be maintained during data entry (next page, also included in Appendix F). Each “data entry log” line item should be specific to a single data entry session. At the top of the data entry log, record the name of the data entry file and the names of the individuals entering data. During each data entry session, record the date, your initials, the field season year, the number of sites entered, the names of the sites, the number of hours spent entering data, and the name of the exported backup file). In addition, record any comments. Comments should include the reason that “-9” was entered for data that did not conform to the database or unresolved edit needs.

Literature Cited

- Mohren S. R. 2007. Data management plan, Klamath Inventory and Monitoring Network. Natural Resource Report NPS/KLMN/NRR—2007/012. National Park Service, Fort Collins, CO.
- Surface Water Ambient Monitoring Program. 2008. Quality assurance project plan. California State Water Resources Control Board. Available online. (http://www.swrcb.ca.gov/water_issues/programs/swamp/docs/qapp/swamp_qapp_master090108a.pdf.) Accessed 3 December 2009.

Klamath Network Mountain Lakes and Ponds Data Entry Log

Data File:_____ Data Enterer Name:_____

Field Season Year_____ NOTE: Continue site names and comments on following lines as necessary.

Initial s	Date	# of sites entered	Site names	# of hours to enter	Comments	Backup file name

Standard Operating Procedure (SOP) #14: Photo Management

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP includes instructions for managing photos taken for the lakes and pond monitoring protocol. This SOP is adopted from the Klamath Network Landbird Monitoring Protocol.

Introduction

This document covers photographic images collected by the Project Lead and field crew during the course of conducting project-related activities.

Care should be taken to distinguish data photos from incidental or opportunistic photos. Data photos are those taken for at least one of the following reasons:

1. To document a particular feature or perspective for the purpose of site relocation.
2. To capture site habitat characteristics and to indicate gross structural changes over time.
3. To document species detection.
4. To document field crew activities during surveys and site set up (human interest, methods, and aesthetic photos are encouraged).

It is the responsibility of the Project Lead to ensure images are properly named and stored in the correct location along with the image metadata as described below.

Photo Metadata

The Klamath Network Data Plan (Mohren 2007) specifies the required metadata for photo management. As part of the field datasheet (Appendix F), the required metadata is recorded for all site photos. The required metadata that must be recorded by the crew taking the photos are:

- **Photo name**
- **Date photo taken**
- **Name of photographer**

- **Description of photo**
- **Site name**
- **Coordinates (if available)**

Other required metadata (Table 1) (e.g., Category Folder, Rights, Publisher, etc.) are automatically populated by the Data Manager.

Photo name is the file name assigned by the camera (e.g., RIMG0001). Do not include the extension in the file name and be sure to include the letters.

Date photo taken is self explanatory, but should be in the format mm/dd/yyyy. Note that this is different from other protocol procedures (yyyymmdd), but is in this format to comply with photo metadata standards.

Name of photographer is also self explanatory, but full name should be given (i.e., not just initials).

Description of photo should be as detailed, clear, and concise as possible. When entering into the database, caption descriptions are acceptable but keep grammar correct.

Acceptable examples:

- Lake Helen, looking west.
- Aaron Maxwell holding unknown tadpole at Reflection Lake.
- Black bear seen on trail from Cliff Lake, during field crew hike out.

UNACCEPTABLE examples

- L. Helen, west
- Tadpole?
- Bear on trail

Downloading and Processing Procedures

Downloading and processing procedures are detailed in SOP #12: Post-Site Tasks. Metadata for the images is entered during the data entry phase (SOP #13: Data Entry).

Deliver Image Files for Final Storage

It is the Project Lead's responsibility to compile all images into a common folder and to transfer processed images to the Data Manager (SOP #17: Data Transfer, Storage, and Archive).

To transfer images from computers and to transfer the compiled set of images to the Klamath Network Data Manager, copy the folder for the appropriate year images onto a CD, DVD, or flash drive for delivery. These files will be stored in the Lakes_Images folder, a sub folder of the Water Quality Monitoring folder located on the Klamath Network server. Copies of the images will be placed in the Klamath Network Image Library. Metadata for the images will be loaded into the Klamath Network Image Database, which is linked to the photographs in the Klamath Network Image Library. Images and metadata will be backed up and archived following the

methodologies outlined in the Klamath Network Data Management Plan (Mohren 2007). Mohren (2007) should be consulted for additional information on photo management.

Literature Cited

Mohren S. R. 2007. Data management plan, Klamath Inventory and Monitoring Network. Natural Resource Report NPS/KLMN/NRR—2007/012. National Park Service, Fort Collins, CO.

Table 1. Complete list of fields required for photo metadata. Some of these fields are autopopulated by the Data Manager (e.g., Rights, Category Folder, and Publisher).

*Park Code	*Network Code	Project	*Photo Name	*Date	*Photographer	*Description	UTM East	UTM North	Datum	*Category Folder	*Ext.	*Rights	Collection	Publisher	Resource Type
	KLMN	Lakes							NAD 83 Zone 10		.jpg		KLMN	NPS	Image
	KLMN	Lakes							NAD 83 Zone 10		.jpg		KLMN	NPS	Image
	KLMN	Lakes							NAD 83 Zone 10		.jpg		KLMN	NPS	Image
	KLMN	Lakes							NAD 83 Zone 10		.jpg		KLMN	NPS	Image

1) * Required fields.

2) Populated fields are populated with their default values as shown above.

3) Fields include:

Park Code – CRLA, LABE, LAVO, ORCA, REDW, WHIS.

Network Code – KLMN.

Project – Name of the project you are working on.

Photo Name – Name of the photograph, do NOT include the extension.

Date – Date the photograph was taken in the format MM/DD/YYYY.

Description – A DETAILED description of the photograph, including the name of the site, if applicable.

UTM East and North – The UTM coordinates where the picture was taken, if applicable.

Datum – The datum and zone for the UTM coordinates. The default is NAD 83 Zone 10.

Category Folder – The name of the folder where the picture is being stored.

Ext. – The extension; the KLMN requires photographs to be in .jpeg format.

Rights – Right for use or dissemination. Generally, rights are “Public,” except for sensitive species, children, etc.

Publisher – Owner of the photograph, usually NPS.

Resource Type – What is it? Image, PPT, Graphic; usually Image.

Standard Operating Procedure (SOP) #15: Post-Field Season

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP explains procedures that will be completed after the field season, which include handling equipment, data forms, communication with NPS personnel, and reporting. Technicians and interns will assist the Project Lead in completing post season field tasks. This SOP is based on a similar SOP for the Klamath Network Landbird Community Monitoring Protocol (Stephens et al. 2009).

Clean, Inventory, and Store Field Equipment

1. All equipment should be inventoried.
2. All equipment should be cleaned, determined to be in working order, and stored in the proper storage location. Equipment should be prepared for long-term storage (2 years in some cases), including the removal of batteries.
3. Record broken or missing equipment on the inventory sheet. Label the equipment with sufficient information so that someone else will understand the specific problem.
4. Report missing or faulty equipment and/or equipment needing repairs to the Project Lead immediately so that equipment can be repaired or replaced before the following field season. The Project Lead should schedule a time to inform the Network Coordinator about needed repairs, purchases, and projected costs early in the fiscal year planning process (i.e., no later than November 1 following the field season).
5. Vehicles should be filled up with fuel and other fluids (oil, coolant, wiper fluid) and the inside and outside should be thoroughly cleaned. Mileage reports and vehicle maintenance forms should be submitted to the Project Lead.
6. After all data have been backed up (in accordance with previous SOPs), electronic equipment should be checked in to the Data Manager.

GIS Post-Processing and Organization

The GIS Specialist is responsible for:

1. Check in field data to the GPS-enabled Geodatabase and uses GPS Analyst and the field-recorded SSF files to further correct the positioning of the data.

2. Corrected GPS data then will be given FGDC-compliant metadata using ArcCatalog metadata tools and will include notes about processing techniques and final GPS accuracy. These files then will be saved to the network server at \\Water_Quality_Monitoring\Lakes\Lakes_GIS.
3. Using GIS tools, calculate lake circumference, lake area, numbers of invertebrate sweeps per lake and per habitat type, numbers of amphibian searches per lake and per habitat type, and the percentage of lakeshore composed of each habitat type. Transfer the results to the server Water_Quality_Monitoring\Lakes\Lakes_Analysis.
4. The GIS Specialist will also perform quality control checks of the GIS data for correct projection definitions, logical consistency with other GIS data layers, and complete metadata viewable in ArcCatalog.
5. Once the GIS Specialist has completed his/her tasks, the Project Lead should review the shapefiles and work with the GIS Specialist to cleanup any additional issues.
6. Once quality control checks are done, GIS data from each survey year will be imported into one master Geodatabase for Water Quality Monitoring that includes all of the Network parks. This is located on the KLMN server at [\\Data_Management\GIS\database\klmn\data\monitoring\Water_Quality](\\Data_Management\GIS\database\klmn\data\monitoring\Water_Quality).

Data Forms

Data forms should be submitted to the Project Lead at the end of each sampling event. At the end of the field season, it is the Project Lead's responsibility to:

1. Ensure that all surveys have been completed.
2. Ensure that all data have been entered into the databases.
3. File extra field forms in the proper file cabinet for the following year.
4. Organize the datasheets into the proper format. One PDF document will be made for each lake and the file will be named using the project name, lake name, and year surveyed. Datasheets should be organized by:
 - Lake Name Folder
 - Main field sheet
 - Fish field sheet (if any)
5. The file should be named with the following conventions:
 - Parkcode_LakeName_GRTS_Year.pdf for the main field sheet (e.g., LAVO_Lake_Helen_000_2008.pdf)
 - Parkcode_LakeName_Fish_GRTS_Year.pdf for the associated fish sheet (e.g., LAVO_Reflection_Lake_Fish_300_2008.pdf)

Close-out

The Project Lead should communicate with the Park Contact to determine whether keys and/or other equipment need to be returned. Once keys and equipment have been returned, the Network Contact should be notified.

Field Season Reporting

The Field Crew Leader should prepare a brief report (generally not more than three pages) that includes the following:

1. Clear enumeration of which lakes/ponds were completed during the season.
2. Description of any logistic difficulties that arose and explanation of how they were addressed.
3. Clear documentation and explanation of any diversions from established protocols.
4. Discussion of any interesting or potentially important observations that may have been noted during the field season
5. Suggestions for improving the training session or field season logistics in the future.

After this brief report, there should be a debriefing session in which the entire field crew and the Project Lead discuss the field season and any issues in the report. The Network Coordinator and Network Data Manager are encouraged to attend this meeting.

Electronic Equipment

The Field Crew Leader should make certain all electronic equipment is cleaned and in working order. Electronic equipment includes the tablet PCs, GPS units, Trimble units, and cameras. Upon submitting the equipment for check in, all project related materials (images, databases, documents, and shapefiles) should be removed for these units.

Standard Operating Procedure (SOP) #16: Quality Assurance Project Plan

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

Consider the following scenario: A driver is pulled over by an officer of the law for speeding. This officer used a radar gun to determine that the driver (maybe you, the reader) was traveling 12 miles per hour over the speed limit. In your defense, you believe that you were not traveling that fast, and that maybe his or her radar gun was not functioning properly. Maybe the radar gun was reading wrong and that the margin of error on the radar was plus or minus 12 miles per hour? All valid concerns, except that the officer can confidently tell you that the radar gun was calibrated the previous day against a known speed, and that the margin of error is documented at plus or minus 2 miles per hour. Not only that, but they have written documentable proof of it, and in sum, you're *busted*. How was the officer able to know all of this? Because of a Quality Assurance Project Plan!

Just as the officer was confident of the radar gun reading, we have to be confident of our own lake measurements. Our path to confidence is also in a Quality Assurance Project Plan.

This SOP details the Quality Assurance Project Plan (QAPP) for this protocol.

The purpose of the QAPP is to ensure that data produced through this protocol is of a known, documentable, and defensible quality. A clearer way of defining the QAPP purpose is to pose example questions that it is supposed to answer:

- If we measure calcium to be 0.8 mg/L (at noon, in July, from the deepest part of the lake), how do we know that it is actually 0.8 mg/L, and not 0.9 or 0.7 mg/L?
- If we measure calcium at 0.8 mg/L and in the future it is measured at 1.2 mg/L, how do we know that there has been an actual increase in the calcium and not just measurement error?
- If our multiprobe breaks and is replaced, how do we know that probe B (new) is giving comparable results to probe A (old)?
- If we found insect species A in year 1, but not in year 10, is this because the species was locally extirpated, or because of a taxonomic error, or because of change in taxonomy?
- If we are making a probabilistic survey of the Network lakes, how do we know our sample *really* represents the lakes of the Network? If it represents it now, how do we know that it will represent the lakes 30 years from now?

- If our data are compared or shared with other data sources, how do we know that our numbers are representative of other programs?
- If we produce data that do not meet certain standards, how are they handled?
- If we are not able to sample all of the sites or all parameters, do we still make inferences to the park units?
- If a field in the database is left blank how do we know if that data was not collected, did not exist, or was accidentally not entered?
- If measurements made by the Network are used in a court of law, how do we prove that we followed the SOPs to the letter and that the data represents the best measure of "truth" possible?

The answers to these questions are dealt with in two primary methods: documentation and methodology. The documentation includes the most basic form: the current protocol, from the narrative to the last appendix, and of course, the strict adherence to the methods described in the SOPs. The delivery of data must also include documentation. In this form, much of the documentation is metadata provided with the data. It is also in the strict documentation of all events related to this protocol, even though not directly related to the data about lakes or ponds (e.g., the documentation of field crew training and calibration events).

To help ensure quality data, methods are implemented to deal with the inevitable change (e.g., new instrumentation, changing analytical chemistry laboratories). Other methods affecting quality data are data validation and verification steps. In addition, the development of a data collection system that incorporates domain values, pick list, and logic checks is important.

Some aspects of this QAPP will come in the form of guidance. When possible, the following steps and methodology should be carried out. **It is through the documentation of errors, variance, etc. (whatever the source) that the quality of the data is known.** Where possible, this should be included with the metadata. For example, with the cumulative bias procedures (described below), it is recommended that a shift in personnel be accompanied by seven overlapping measurements. For a field crew change, with 3 years in between sampling periods, this is untenable. For the Project Lead, who is responsible for training the field crews, it would be wise for the outgoing Project Lead to sample with the incoming Project Lead. However, personal situations can easily prevent this from happening and the cost of processing seven extra macroinvertebrate samples (estimated at \$1,635, not to mention zooplankton and water chemistry samples) can easily be out of the budget.

Sampling Frame, Sample Size and Minimum Detectable Differences

Sampling Frame

The sampling frame for this protocol is all perennial lakes and ponds in Lassen Volcanic National Park (the only component of our design with a probabilistic sampling design), with the following criteria:

- Perennial – This selection criterion is applied to remove habitats that are influenced by seasonal desiccation which could mask other stressors of interest. It

also ensures that data collection can always occur at the sites, assisting in data completion goals.

- Less than 25 m maximum depth – This selection criterion removes large lakes that are characterized by different physical and environmental processes. While monitoring these lakes is valuable, they would require separate methodology to adequately assess and they are a numerical minority of sites within the Klamath Network.
- Less than 1000 m from a travelable road or trail – This selection criterion reduces logistical constraints to field crews, such as travel time, to ensure that each site can be sampled in the allotted time frame for achieving sampling objectives.
- In mild topographies with lake slopes < 30 degrees – This selection criterion ensures crew safety and that access to lakes is doable.

For Crater Lake National Park, it is all perennial lakes and ponds (outside of the Crater Lake caldera) that fit the above criteria.

As Irwin (2008) points out, long-term monitoring plans must deal with the possibility of the population of interest "drifting" in and out of the sampling frame. An example of a realistic event causing this is the park making new roads or trails (or decommissioning roads and trails). This would change the list of habitats within our sampling frame. An example of a population shift would be a natural disaster (e.g., earthquake or lava flow), causing the creation or loss of lake habitat.

As part of this protocol, the adequacy of the sampling frame will be revisited on a regular interval of 15 years (every four sampling periods). If the sampling frame or population of interest is found to have changed within this period, corrective action, as necessary to accomplish the goals of this protocol, may be undertaken.

Sample Size and Minimum Detectable Differences

This protocol focuses on park-level inferences, using measures of central tendency to track changes in status and trends. To achieve this, with ample statistical power in select parameters (see narrative), a minimum sample of 25 lakes is required. Based on data completeness goals (see below), we have increased the sample size to 30. Where possible, efforts will be focused on ensuring complete sampling of index sites.

Minimum detectable differences are a question of power and are detailed in the power analysis, covered in the protocol narrative.

Data Comparability

Comparability is the measure of confidence that one dataset, element, or method can be considered as similar or identical to another (SWAMP 2008). The goal of this document is to ensure that if the programmatic requirements are fulfilled, then the data collected by this protocol is considered to be a similar quality level.

It is outside the scope of this protocol and Network budget to do comparability studies to other methodologies, be it Forest Service, academic, or state sponsored monitoring. As research or external funding is available to address these comparability studies (and as need dictates), Network staff may work towards these goals. During the development of this protocol, methods and parameters being implemented by other agencies were considered and in some cases applied to this protocol. However, the fundamental goal of this QAPP and overall protocol is to ensure comparability *within* our program. The major source of potential variation that could affect data comparability is protocol, personnel, laboratory, or equipment changes.

Data Completeness

The Klamath Inventory and Monitoring Network recognize that a certain percentage of samples of all types (zooplankton, invertebrates, water chemistry, etc.) will fail. Reasons for failing may include lost samples, dehydrated samples, samples that exceed holding times, or sample contamination.

Data completeness goals are based on a multi-step process detailing the minimum sample size needed to make statistical inferences about the population of interest (Irwin 2008). Once this minimal sampling size is calculated (see Sampling Frame and Sample Size and Minimum Detectable Differences, above), the number of samples that will fail is estimated and the sample size is increased by the same percentage. This procedure is complicated for multi-parameter protocols, such as the one here. Water chemistry samples (with multiple types), invertebrate samples, water probes, and GPS files may all fall short of precision targets, but at different failure rates. However, since we are making inferences to a population of lakes, the ultimate sample size for this protocol is *the number of sites*.

During the initial scoping period, it was determined that a minimum of 25 samples are needed to characterize the population of lakes with desired levels of precision (A. Merton, statistician, personal communication). Following the guidance of Irwin (2008), we increased our sample size to 30 total lakes to accommodate unforeseen problems.

Cumulative Bias

The term bias has many definitions, even within the realm of statistics. Here, bias is taken to be a systematic error in measurements. Over the length of a monitoring program, bias may cumulate from many sources: collector bias, instrument bias, protocol bias, etc. With the obvious expectation that personnel and gear will change and protocols will be revised over the years (SOP# 20: Revising the Protocol), QAPP methodology for dealing with change and minimizing and documenting the cumulative bias are laid out below. Following these procedures will allow for Data Comparability in trend analyses.

Any change in the following categories will be documented in the metadata produced during the project. Just as field crew personnel from year to year are documented, the different laboratories (although changing labs should be a severe option) will also be documented. See Irwin (2008) for more guidance on Cumulative Bias.

Change in Personnel

When possible, it is recommended that personnel changes be accompanied by an overlap of seven measurements. For the lakes protocol, this extrapolates to essentially duplicating the sampling effort at seven lakes, in all measured parameters. This is prohibitively costly to do and hence an alternative method of documenting variance in personnel bias is presented.

At 10% of the lakes (four or five lakes total), efforts of the two crew members should be duplicated (e.g., the field crew leader does a walk-around recording lake habitat and then later in the day the crew member repeats the walk-around). Another example is in repeating Acid Neutralizing Capacity measurements by both crew members. Over the life of the protocol, this should result in a gradually building body of knowledge about the degree of variation caused by different personnel. It is recommended that the annual reports (SOP #19: Data Reporting and Analysis) include the results of this duplication of efforts as an evaluation of protocol success. If large variation between personnel is detected, this should trigger an evaluation of the parameter that the variation is large in. What thresholds of variation and technique of measuring variation might precipitate a re-evaluation will be dependent upon the parameter. Using alkalinity as an example, repeat measurements by different personnel that yield values of 20 mg/L and 23 mg/L are relatively very close and would generally be recognized as being in the neighborhood of low alkalinity, but the percent relative difference is actually somewhat high: $(20 - 23)/20 = 15\%$. Percent relative difference is a commonly used measure for assessing QA/QC goals, but in this case, other techniques such as coefficients of variation may be more applicable. Hence, it is recommended that each parameter be evaluated by the Project Lead.

When repeating measurements at 10% of the lakes, the repetition does not have to occur at the same lakes. So alkalinity could be repeated at lakes numbered 1, 3, 5, and 7, whereas the habitat could be repeated at lakes numbered 2, 5, 9, and 12. The determination of what parameters to be repeated at which lake should be a random determination by the Project Lead prior to the field season, although on the ground changes may be made so that sites are still samplable in a single day. Information for the field crews should be included in the site information folder (for example, see Appendix G). This will spread the repeat effort out across the sampling frame, so that the increased time for any one lake is not beyond the logistical ability of the field crew.

It should also be stressed that control of personnel bias is done through strict, exact SOP adherence and thorough training. Furthermore, it is valuable to limit interpretation to measures that are less likely to be affected by personnel differences. For example, macroinvertebrate abundances can be highly affected by personnel experience; however, relative abundance or Presence/Absence measures are less affected by personnel. Hence, inferences to site or temporal impacts should be evaluated at the more robust measures first.

Change in Equipment

When possible, replacement equipment should match the original equipment. Specifications of replacement gear should also match the original equipment. If being replaced because of planned obsolescence, the new equipment should be tested against the old equipment to establish comparability using seven comparable measurements, *over the entire range of environmental variability*. If the seven (minimum) measurements are only done in a small subset of conditions, the correction provided by duplicating measurements may not apply to the entire sample set.

Change in Contract Laboratories

Bias from different laboratories is a source of error that is under the control of the Project Lead. Generally, a change in labs used should have solid rationales, which include (but are not limited to), the following reasons:

- (1) Laboratory shuts down or otherwise closes.
- (2) Dramatic price increase so that the Network cannot meet sample completeness goals.
- (3) Laboratory consistently fails to meet agreed upon deadlines for data delivery.
- (4) Laboratory internal QA/QC procedures change or their methodology changes.

It is the responsibility of the Project Lead to stay in communication with contracting officers and laboratory managers to ensure that laboratories meet the standards and that lab continuity is maintained where and when appropriate.

The purpose of seven measurements

Using a minimum of seven measurements (taken with old/new equipment, samples analyzed by old/new lab, etc.) is used to assess comparability. If the relative percent difference in the seven samples were low (less than 10%), then the change can be considered comparable. If the bias is greater, the new samples can be standardized to the old samples.

Standardization should only be done for trend analyses, and not for annual reports/status/data delivery to WRD STORET.

Programmatic Elements of the QAPP

The following covers aspects of the day to day data acquisition and data generation that must be followed to meet the requirements of this QAPP.

1. Sampling Methods

All data generated or acquired through this protocol must adhere to all SOPs. Crews should be trained and training documented to demonstrate that this aspect has been met.

2. Sample Handling and Custody

Certain basic requirements concerning filter choice, holding container, storage method, and storage time (Table 1). Biotic samples (invertebrates and zooplankton require no special handling or holding time, except to be preserved in 95% Ethanol. Chains of custody (Appendix F) must be maintained and logged to record the transfer and shipment of samples.

3. Measurement Quality Objectives and Reporting Limits

Measurement quality objectives (MQOs) are a set of attributes (e.g., precision, bias, sensitivity, detection limits, etc.) that determine whether or not a test result is accepted (Table 2). Reporting limits determine at what level an analyte test can be reported at a quantifiable level, or whether or not the proper determination is "detected, below quantifiable level" or "non-detect." Note that many labs are able to provide a "quantified" number for analytes below these levels. For analytical and exploratory purpose, the Project Lead may choose to use these numbers for calculations. However, for reporting and regulatory purposes, the reporting limits *must be adhered to*. Here, any value under "Method Detection Limit" must be reported as "Non-Detect" and values between "Minimum Level of Quantification" and "Method Detection Limit" must be

reported as "Detected, below quantification limits," when the data is transferred to WRD STORET. Internal database will retain the raw value as reported by the lab, even if the values are below the Minimum level of quantification.

Table 20. Required sampling specifics to meet necessary goals of data quality for water chemistry analytes.

Analytes	Units	Required container (see SOP#1)	Required filter	Required sample volume	Required preservation	Maximum holding time
Filtered water sample:						
Anions (Cl, SO ₄)	mg/L	Acid washed, HDPE	Whatman GF/C (1822-047)	50 ml	Frozen (-18°C)	28 days
Cations (Na, Ca, K, Mg)	mg/L	Acid washed, HDPE	Whatman GF/C (1822-047)	50 ml	Frozen (-18°C)	28 days
Dissolved total nitrogen	mg/L	Acid washed, HDPE	Whatman GF/C (1822-047)	100 ml	Frozen (-18°C)	28 days
Dissolved total phosphorous	mg/L	Acid washed, HDPE	Whatman GF/C (1822-047)	100ml	Frozen (-18°C)	28 days
NO ₃ + NO ₂ -N	mg/L	Acid washed, HDPE	Whatman GF/C (1822-047)	60 ml	Frozen (-18°C)	28 days
Total:				360 ml		
Other:						
Chlorophyll	mg/L	HDPE (storage for filter)	Millipore mixed Cellulose ester membrane (HAWP 047-00)	500 ml filtered	Frozen (-18°C)	28 days
Dissolved organic carbon	mg/L	Acid washed, furnace fired Amber Glass container	Whatman GF/F (1825-047)	60 ml	Refrigerated at 4°C	28 days

Table 21. Minimal Measurement Quality Objectives and reporting limits for chemical parameters necessary to be met by contract laboratories.

Parameter	Method Detection Limit (mg/L)	Minimum Level of Quantification (mg/L)	Precision (± mg/L)
Calcium	0.06	0.19	0.06
Chloride	0.01	0.03	0.01
Dissolved Organic Carbon	0.05	0.16	0.05
Dissolved total nitrogen	0.01	0.032	0.01
Dissolved total phosphorous	0.002	0.003	0.002
Magnesium	0.02	0.06	0.02
NO ₃ - NO ₂ -N	0.001	0.003	0.001
Potassium	0.03	0.1	0.03
Sodium	0.01	0.03	0.01
Sulfate	0.02	0.06	0.02

4. Chemical Laboratory Quality Controls

Chemical contract laboratories are required to meet certain responsibilities for assuring quality control. For this protocol, the required controls are:

- a) Instrument calibration prior to initiating analysis run (three to six NIST traceable standards).
- b) Standards analyzed every 10 samples.
- c) Detection limit standard run at least once in analysis run.
- d) A minimum of 10% of the samples must be duplicated (lab duplicate, not field duplicate).
- e) Field duplicates should be run (Responsibility of Project Lead to provide).
- f) Periodic blanks should be run.

Filter blanks and bottle blanks should be included in the analysis. It is the responsibility of the Project Lead to have the field crew collect a minimum of four field duplicates during the field season. These are samples handled identical in every way to the original sample, but they are used to generate measures of precision for the analyses. The Project Lead must also provide bottle blanks (Acid-washed bottles filled with deionized water). Likewise, deionized water should be filtered using the protocol and then treated as samples. The purpose of these bottle and filter blanks is to ensure that there are no contamination sources in the bottle or filter prep that may jeopardize results. Blanks, either filter or bottle, will be tracked in the database.

When duplicates or standards are greater than 10% deviant, the instruments should be recalibrated and the analyses repeated back to the last successful standard check.

Laboratories not conforming to these criteria should be rejected by the program during the initial contracting period.

5. Taxonomic Laboratory Quality Controls

Taxonomic laboratories should employ and use only taxonomists certified by the North American Benthological Society (NABS, www.benthos.org; an international scientific organization of aquatic ecologists) as trained taxonomists. There is currently no certification program for zooplankton taxonomists. For zooplankton taxonomists, a taxonomist C.V. or resume should be obtained and kept on record by the Project Lead as proof of taxonomic ability. This will standardize the knowledge so that invertebrate identifications can be assured as being correct. Taxonomic laboratories must also either: (1) complete a voucher collection of each taxon identified, or (2) retain and curate the portion of sample sorted and identified. Either of these will allow for double checking taxonomic accuracy by later project leaders or subject area experts. As the budget allows, periodic consideration should be made to sending taxonomic specimens to secondary labs to confirm taxonomic identifications.

6. Data Entry and Data Management

Data entry and data management are covered in SOP# 13: Data Entry, but QA/QC procedures are described here.

Although the Klamath Network has opted to develop and maintain a Network-specific database, the database meets the requirement of the WRD to be consistent with NPSEDD (electronic data deliverables), so that all data will interface with both WRD STORET and EPA STORET. This

standardization and standard metadata requirements improve comparability of long-term datasets.

Prior to leaving the site, hard copy data sheets, electronic data entry forms, specimen labels, and data logger data will be reviewed by the field crew. If possible, both field crew members should review all of the data. Field crew members should utilize built-in database utilities and their personal knowledge of the data to ensure all fields on the forms are complete and the entered data is logical and appropriate.

Data collected during the project will be entered no less frequently than once a week. This will ensure (a) that details about the sampling event are still recent for the data recorders and (b) that the Project Lead can check on the progress of the data entry and associated tasks earlier rather than later. Hence, mistakes either in data collection or entry can be caught in time to rectify the problem.

Checks done by the Project Lead, both during the field season and at the end of the season include the following:

- Completeness checks - Are all forms in the database entered?
- Double checks - Are the entered values the same as on the datasheet? (A minimum of 20% should be checked; if there are errors, 100% of the values should be checked.)
- Outlier checks - Are there any values that are outside the normal range of variability (such as measurement or natural variability, etc), that might be suspect? Full details on how to deal with outliers is presented in Irwin (2008).
- Data flagging - Are there any values that have been flagged by the crew? For example, the GPS was not working on a particular day, so the crew is not sure about the specific habitat that was sampled or the wind was so high that the crew was unable to maintain a stable position over the deepest part of the lake for multiprobe profile readings. The Project Lead must determine if to accept, reject, or redo a flagged value, including having the field crew return to the habitat for re-measurement.
- Formal data verification - Verification of the data involves evaluating the correctness, conformance, compliance, and completeness of the entire dataset against the methods or procedures of the protocol (SWAMP 2008). Verification should be done on both field data (including field chemical analyses) and laboratory data (chemical, invertebrate, and zooplankton).
 - Data verification includes:
 - Visual review at data entry – the technician verifies each value during input. Errors are corrected immediately.
 - Visual review after data entry – after entry, data are printed out and compared to original hardcopy sheets.
 - Duplicate data entry – Randomly selected site data are entered as normal, but are duplicate records. Although time consuming in that it repeats data entry efforts, this gives an estimate of the data entry accuracy.
 - Review – It is the Project Lead’s responsibility to review a subset of records to insure that they are identical to the hardcopy datasheets.
 - For the duplicate data and review, the minimum number is 20% of the sites (approximately eight total sites).

- Formal data validation - After verification, the Project Lead reviews it against all criteria in the protocol, especially the QAPP criteria (e.g., holding times, laboratory duplicates, completeness goals, reporting limits). After successful validation, the Data Manager can send the data onto WRD for incorporation into WRD STORET.
 - Data Validation includes:
 - Data entry programming steps – The Project Lead, along with the Network Data Manager, will program steps designed to prevent errors. For example, maximum depth of a lake entry will not allow the recording of a lake 100 m deep (since there are no lakes in Lassen Volcanic National Park that deep). This is an example of a mistake that might occur if the technician accidentally enters “100” instead of “10.0.”
 - Outlier detection and review (see Irwin 2008) – Statistical review and graphical display will be used to detect outliers (unusually extreme values of a variable outside the range of normal values). In outlier review, it is important to realize that not all extreme values represent errors, but can reflect the real variation of the data in nature. Generally, outliers that cannot be ascribed to error will be flagged and retained.
 - Review of “what makes sense” – The Field Crew Leader and Project Lead will compare and review the tabular data to confirm that everything “makes sense.” Both should be intimately familiar with the types of data being collected and as such should be able to detect mistakes. GIS data will be plotted and confirmed to match the spatial locations.

What Happens Now?

The QAPP is designed to identify, reduce, and, if possible, correct the data collected by the project. It is also designed to quantify the quality of the data, so that the precision of measurements of this protocol are to a known amount.

Data errors can never be entirely eliminated. Variation in the data due to collector, measurement, or equipment error can never be reduced to zero. If errors are so large so that data completeness goals are not met to the quality objectives of this protocol, corrective action should occur. The corrective steps should be commensurate with the severity of the errors. Possible steps include:

- Editing and documenting the error.
- “Re-dos,” when possible (e.g., re-entering all the data).
- Revising specific SOPs.
- Increasing the training period.
- Eliminating SOPs with large, uncontrollable variation.
- Changing contract laboratories.
- Programmatic review.

Literature Cited

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Standard Operating Procedure (SOP) #17: Data Transfer, Storage, and Archive

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP explains the procedures for data transfer to the Network Data Manager. In addition, data certification, storage, and archiving and a timeline for project deliverables are addressed.

Data Transfer

All project deliverables, including but not limited to raw data, processed data, Metadata Interview forms, updated data dictionary (if necessary), images with metadata, training logs, datasheets and logs, data entry logs, equipment logs, special event logs, spatial files, and Certification forms will be transferred to the Klamath Network Data Manager following the timeline listed in Table 1. It is the responsibility of the Project Lead to ensure all products and associated documentation are delivered following the outlined timeline. At no point in time should a new field season start if project deliverables have not been completed unless prior permission has been granted by the Network Coordinator and Data Manager.

Certification Form

The Klamath Network will utilize a Certification form submitted by the Project Lead to ensure:

1. The data are complete for the period of time indicated on the form.
2. The data have undergone all quality assurance checks.
3. Metadata for all data has been provided (when applicable).
4. Project timelines are being followed and all products from the field season have been submitted.
5. The correct level of sensitivity is associated with the deliverables.

A new Certification form should be submitted each time a product is submitted. If multiple products are submitted at the same time, only one Certification form is needed for those products. Certification Forms can be obtained from the Network [Internet](#) and [Intranet](#) web sites

or by contacting the [Network Data Manager](#). An example of the Certification form is included at the end of this SOP.

Field Forms

Hardcopies of the datasheets will be provided to the Network Data Manager following the timeline below. It is the responsibility of the Data Manager to scan the datasheets into PDF documents within 1 month of receiving the hardcopies. The datasheets will be organized in the order in which they are to be scanned when they are transferred to the Data Manager (SOP #15: Post-Field Season). For the lakes, there will be three separate sets:

1. Site Field Forms (standard datasheet)
2. Fish Collection Forms
3. Protocol Associated Log Sheets

The details of scanning and naming structure of the files are covered in SOP #15: Post-Field Season.

The scanned log sheets will include:

- Calibration logs
- Training logs
- Crew Expectation logs (Appendix B)
- Chain of Custody Forms
- Any QA/QC documents arising from the QAPP (SOP# 16)

Electronic files will be stored in the documentation folder located at S:\Monitoring\Lakes_Monitoring\Lakes_Data\Datasheets\Seasonal_Data\YYYY on the Network server. Additional details on storage methods are described below.

Databases

At the end of the field season, the project database will be provided to the Network Data Manager along with the Certification form, Metadata Interview form, and, if necessary, an updated data dictionary (SOP#18: Metadata Guidelines). It is the responsibility of the Project Lead to examine the project database for accuracy and completeness prior to transferring the database to the Data Manager. Once the database(s) have been transferred to the Data Manager, he or she will run the data through one more round of validation/verification checks and then load the data into a master database that contains all the data from previous years.

Photos

Images and associated metadata will be transferred to the Data Manager in the format explained in SOP #14: Photo Management.

GIS Shapefiles

A GIS shapefile is created and data stored therein using ArcPad 8, as described in SOP #11: Amphibian, Invertebrates, and Lake Substrate Walk-around. Throughout the field season, this file will be backed up on a daily basis, even though the additional data collected each day is always inputted into the same file. Once validated through QA/QC procedures (SOP#16: Quality Assurance Project Plan), backups will be destroyed via deletion. As described in SOP #15: Post-Field Season, it is the responsibility of the GIS Specialist to complete the post-processing task of all GIS files created as part of this project. Once shapefiles have been processed, quality controlled by the GIS Specialist and Project Lead, posted to the project GIS folder, and uploaded to the GIS Server, the Project Lead should submit the Certification form to the Data Manager.

Training and Contact Information

Prior to implementing field work, a list of contact information for each person involved in the Lakes project will need to be submitted to the Network Data Manager. Contact information will include:

First Name

Last Name

Middle Initial

Organization

Position Title

Mailing Address

Email Address

Work Phone Number

Each person conducting field work as part of this protocol will need to follow the training procedures outlined in SOP #2: Field Crew Training. Log books developed in a standardized Excel format will need to be delivered to the Network Data Manager following the timeline listed above.

Reports

There is the potential for a variety of reports to be developed utilizing data collected as part of this lake monitoring project, including Annual Reports, Analysis and Synthesis reports, scientific publications, one page summary reports, and comprehensive reports.

Annual Reports and Analysis and Synthesis reports will be the responsibility of the Project Lead and should be submitted in the NPS Technical Report Series or Data Series format as appropriate, unless utilizing another series format for publication.

The reports described in SOP #19: Data Reporting and Analysis and any scientific publications should be submitted to the Network Data Manager upon completion.

Summary reports are one page reports completed by the Network staff and used to sum up information in the annual and analyses reports. These reports should be completed within 1 month of receiving the annual or analyses reports. These reports will be sent to all park employees and will provide links to the larger reports.

Lastly, the reports will be entered into NatureBib, the National Park Service natural resource bibliography application to catalog, search, and manage natural resource-related information sources pertaining to national parks. Additional information on NatureBib is available at: <http://science.nature.nps.gov/im/apps/nrbib/>. Reports and species information will be linked to NPSpecies, a master NPS database for documenting the occurrence and status of species in more than 270 national park units that contain significant natural resources. More information is available at: <http://science.nature.nps.gov/im/apps/npspp/>.

Data Storage

Project folders have been created for each monitoring protocol the KLMN plans to implement (Figure 1). Project folders contain five standard folders using a naming convention that includes the project title and one of the following: Documents, GIS, Data, Images, or Analysis. These five folders will contain all the data and information for a project as follows:

Lakes_Documents. This folder contains the reports, budgets, work plans, emails, protocols, contracts, datasheets, and agreements associated with a specific project.

- a) **Lakes_GIS.** This folder contains shapefiles, coverages, layer files, geodatabases, GPS files, GIS/GPS associated metadata, and spatial imagery associated with a project.
- b) **Lakes_Data.** This folder contains the KLMN Lakes database, data delivered by contract laboratories, raw data from the multiprobe and .dbf files from the six field databases.
- c) **Lakes_Images.** This folder contains any photographs related to the project and associated image metadata. In addition, copies of all photographs and metadata will be transferred into the KLMN Image database. Details on the KLMN Image database can be found in the KLMN Data Management Plan.
- d) **Lakes_Analysis.** This folder will contain derived data and associated metadata created during analysis.

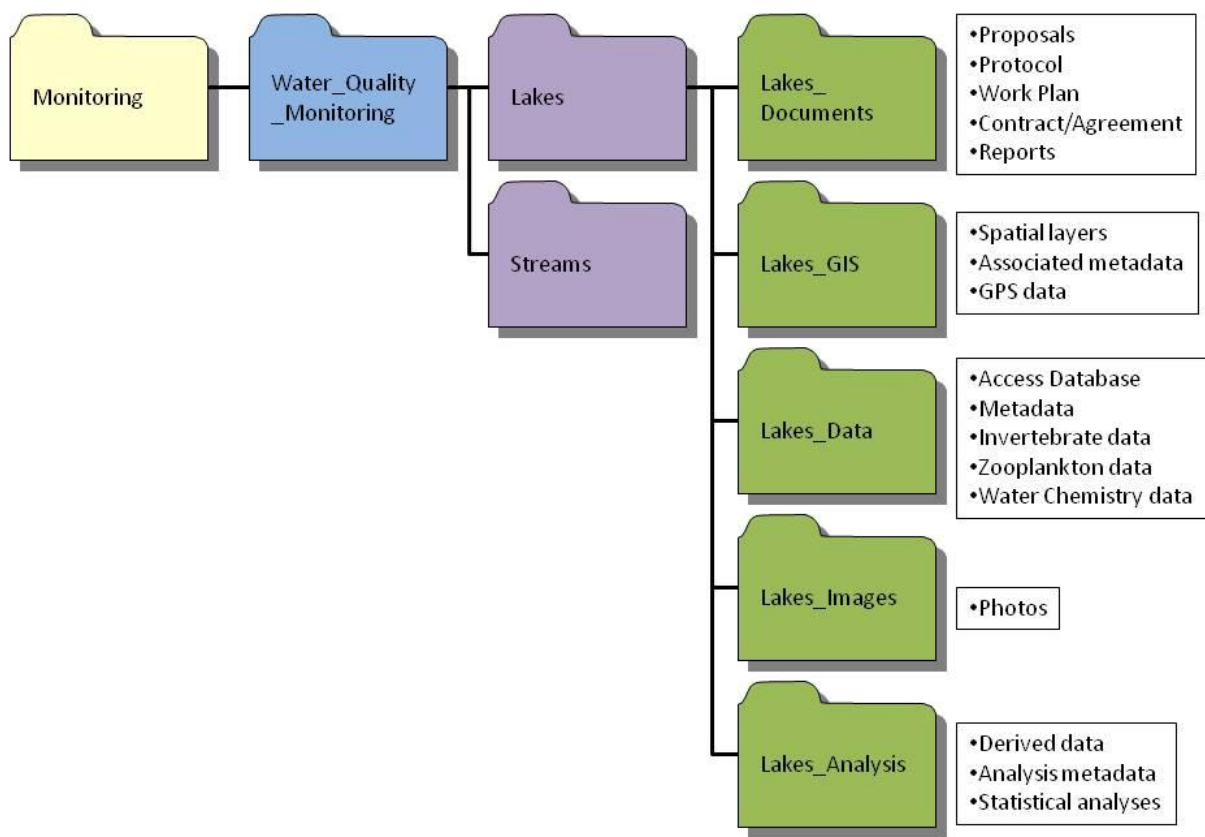


Figure 31. The Klamath Network Mountain Lakes and Ponds file structure to store all data and protocol information.

Network staff will have read-only access to all products to prevent changes to the information. If write access is needed, you will need to contact the Network Data Manager. It is the responsibility of all Network staff to inform the Data Manager when they have added new material to the project folder.

Storage, Backup, and Archiving

A copy of the project folder will be stored in the Network Archive drive whenever any new information is added to the folder. The Network Archive and Network drives are subject to all backup and archiving processes described in the Network Data Management Plan. The Network relies on Southern Oregon University (SOU) for the backup and long-term storage requirements. Nightly backups are done by SOU to store information that has been edited. This is not a full backup but is intended to protect products that have been manipulated. This information is stored for a 1 week period before it is recycled. SOU begins a weekly full backup of their servers on every Friday and stores the files on tape drives. Backups are stored for 60 days before the tapes are reused. SOU will run quarterly backups on March 31st, June 30th, October 31st, and December 31st of each year. Files stored on a quarterly basis are maintained for 1 year before being recycled (Mohren 2007).

Despite the QA/QC measures in place, finding errors in datasets in the future is inevitable. In such instances, archived data will not be corrected; however, an updated product will be placed into the archive drive along with the digital error and data entry log detailing the correction.

Table 22. Deliverable products, responsible individual, due date, and store location for all products developed while implementing the *Klamath Network Mountain Lakes and Ponds Monitoring Protocol*.

Deliverable Product	Primary Responsibility	Target Date	Instructions for Network
Contact Information	Project Lead	Two weeks prior to the start of the field season	Lakes Database Stored in the Lakes_Data Folder.
Metadata Interview Form	Project Lead	Prior to beginning the first field season and by Feb 1 st of the following year when updates occur	Store in Lakes_Data ⁵ , Use to create and revise full metadata.
Updated Data Dictionary	Project Lead	Prior to beginning the first field season and by Feb 1 st of the following year when updates occur	Store in Lakes_Data ⁵ , Use to create and revise full metadata.
Full Metadata (Parsed XML)	Network Data Manager	Prior to beginning the first field season and by March 1 st of the following year	Store in Lakes_Data ⁵ , Upload the Parsed XML Record to the NPS Data Store ²
Protocol Changes (if made)	Project Lead	2 weeks prior to implementing the change	Store in Lakes_Document ⁵ , Update Protocol on Websites and NPS Data Store, Send Copy to Parks
Data Certification Report	Project Lead	every time a product(s) is submitted	Store in Lakes_Document ⁵
Field Data Forms, Fish Forms	Project Lead	Feb 1 st of the following year	Scan Original, Marked-up Field Forms as PDF Files and Store in Lakes_Document ⁵
Equipment, datasheet, event, and training logs	Project Lead	Feb 1 st of the following year	Store in Lakes_Documents ⁵
QA/QC Field Database	Project Lead	Prior to field crew being released	Store in Lakes_Data ⁵ ,
QA/QC Invert Database Upload	Project Lead	Feb 1 st of the following year and prior to conducting analysis or summaries of reports	Store in Lakes_Data ⁵ ,
QA/QC Manta Database Upload	Project Lead	Feb 1 st of the following year and prior to conducting analysis or summaries of reports	Store in Lakes_Data ⁵ ,

Table 1. Deliverable products, responsible individual, due date, and store location for all products developed while implementing the *Klamath Network Mountain Lakes and Ponds Monitoring Protocol* (continued).

Deliverable Product	Primary Responsibility	Target Date	Instructions for Network
Digital Photographs and Metadata	Project Lead	Feb 1 st of the following year	Store in Lakes_Image ⁵ , Copies of Photographs in Network Image Library, Copies of Image Metadata into Network Image Database linked to Photographs
Annual Report	Project Lead	June 1 st of the following year and prior to starting any new fieldwork	Store in Lakes_Document ⁵ , Upload to NPS Data Store ² , Send Copy to Parks, Post on the Network Internet and Intranet Websites
Analyses and Synthesis Report	Project Lead	Every three years on November 1 st	
Other Publications	NPS Staff, Project Lead	As completed	
Other Records	Network Contact	Review for retention every Oct 1 st	Digital Files that are Slated for Permanent Retention Should be Uploaded to the Network Lakes Project Folder. Retain or Dispose of Records Following NPS Director's Order #19 ⁴ .
WRD Data Uploads	Project Lead/Data Manager	After all final QA/QC, annual report finalized	Transfer to WRD in NPSEDD format

¹ The Network Image Library is a hierarchical digital filing system stored on the Network file servers. The image library is linked to an image database that stores metadata on each image.

² NPS Data Store is a clearinghouse for natural resource data and metadata (<http://science.nature.nps.gov/nrdata>). Only non-sensitive information is posted to NPS Data Store.

³ NatureBib is the NPS bibliographic database (<http://www.nature.nps.gov/nrbib/index.htm>). This application has the capability of storing and providing public access to image data (e.g., PDF files) associated with each record.

⁴ NPS Director's Order 19 provides a schedule indicating the amount of time that the various kinds of records should be retained. Available at: <http://data2.itc.nps.gov/npspolicy/DOrders.cfm>.

⁵ The Network Lakes project folder located on the shared file server at the Network office. The project folder contains five folders including: Lakes_Documents, Lakes_Data, Lakes_Analysis, Lakes_GIS, and Lakes_Image used to separate and store data and information collected as part of the Klamath Network Mountain Lakes and Ponds monitoring.

Literature Cited

Mohren, S. R. 2007. Data management plan, Klamath Inventory and Monitoring Network. Natural Resource Report NPS/KLMN/NRR—2007/012. National Park Service, Fort Collins, Colorado.

KLMN Certification Form (also included in Appendix F)

1) Certification date: _____

2) Certified by: _____

Title: _____

Affiliation: _____

3) Agreement code: _____

Project title: _____

4) Range of dates for certified data: _____

5) Description of data being certified: _____

6) List the parks covered in the certified data set, and provide any park-specific details about this certification.

Park	Details

7) This certification refers to data in accompanying files. Check all that apply and indicate file names (folder name for images) to the right:

_____ Hardcopy Datasheet(s): _____

_____ PDF Datasheet(s): _____

_____ Database(s): _____

_____ Spreadsheet(s): _____

_____ Spatial data theme(s): _____

_____ GPS file(s): _____

_____ Geodatabase file(s): _____

_____ Photograph(s): _____

_____ Data Logger(s) files: _____

_____ Other (specify): _____

_____ Certified data are already in the master version of a park, KLMN or NPS database.

Please indicate the database system(s): _____

8) Is there any sensitive information in the certified data which may put resources at greater risk if released to the public (e.g., spotted owl nest sites, cave locations, rare plant locations)?

_____ No _____ Yes Details: _____

9) Were all data processing and quality assurance measures outlined in the protocol followed?

Yes / No

If No, Explain _____

10) Who reviewed the products?

11) Results and summary of quality assurance reviews, including details on steps taken to rectify problems encountered during data processing and quality reviews.

Standard Operating Procedure (SOP) #18: Metadata Guidelines

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP explains the procedures for completing metadata for products developed using this protocol. This includes, but is not limited to, databases, documents, GPS data, and GIS data. Details on metadata for photographs can be found in SOP #14: Photo Management. This SOP is based on metadata recommendations developed by the KLMN (Mohren 2007), the Natural Resource GIS Program (NR-GIS Data Store 2005a-i), and by the NPS North Coast Cascade Network (NCCN 2006a, NCCN 2006b, NCCN 2006c).

Introduction

Documentation is a critical step towards ensuring products collected as part of this monitoring effort are usable for their intended purposes now and well into the future. This involves the development of metadata, which is defined as structured information about the content, quality, condition, and other characteristics of a product. In addition to spatial information, metadata includes information about data format, collection and analysis methods, time of collection, originator, access/use constraints, and distribution. Metadata provides the means to catalog products, within Intranet and Internet systems, making them available to a broad range of potential users. While most frequently developed for geospatial data, metadata describing non-geospatial datasets is also important (NCCN 2006a).

Timelines

It is the responsibility of the Project Lead to submit metadata or metadata products (e.g., Metadata Interview form, data dictionary) to the Klamath Network Data Manager, in the proper format, when he or she submits the product in which the metadata is associated. SOP #17: Data Transfer, Storage, and Archive provides details on the products to be delivered to the Data Manager, the due dates for those products, and the person responsible for those products.

Responsibilities and Standards

Metadata is one of the most important pieces of documentation to help guarantee the long-term usability of data. The degree of documentation will vary depending on the product, but a few standards will always hold true.

1. Metadata for spatial data collected through I&M funded projects will meet the Federal Geographic Data Committee (FGDC), National Biological Information Infrastructure (NBII), and NPS standards.
2. Project Leads will be expected to submit a data dictionary (for tabular and spatial data) and Metadata Interview form (for spatial data) prior to the start of the first field season.
3. Project Leads will be expected to review and revise all data dictionaries and Metadata Interview forms at the end of each field season and report changes following the timeline listed SOP #17: Data Transfer, Storage, and Archive.
4. It is the responsibility of the Data Manager to develop the official metadata based on the data dictionary and Metadata Interview form provided by the Project Lead.
5. It is the Data Manager's responsibility to parse and transfer metadata to the NPS Data Store, if applicable.
6. The Data Manager will work with the Project Lead and park staff to determine the sensitivity level of any data.

Reports

Three main types of reports are expected to be developed during this monitoring effort, including Annual Reports, Analysis and Synthesis reports, and scientific publications (SOP #19: Data Analysis and Reporting). It is the responsibility of the individual creating the reports to ensure the following guidelines are met.

1. First and last name of all authors are included on the reports.
2. Affiliation of the authors is included on the report.
3. Version numbers are used on all drafts of the report.
4. Date the report was completed is included on the report.
5. The date representing the information presented in the report is included in the report.
6. Series number is included in the report when applicable.
7. The NatureBib accession number has been added to the keywords field in the properties of the document in the format: NatureBib #123456. This will be created by the Network Data Manager.

Spatial Data, Databases, and Spreadsheets

The Data Manager is responsible for creating and maintaining the official metadata for all GIS and GPS products, relational databases, and spreadsheets. It is the responsibility of the Project Lead to provide Metadata Interview forms and data dictionaries to the Data Manager prior to

implementing the field work as described in SOP #17: Data Transfer, Storage, and Archive. The Data Manager will use the Metadata Interview form, data dictionary, and protocol to develop complete metadata for each product. Each year field work occurs, the Project Lead must submit a data dictionary (if changes to the product have occurred) and Metadata Interview form following the timeline in SOP #17: Data Transfer, Storage, and Archive.

Steps for Metadata Creation

Step 1: Metadata Interview Form and Information Gathering (Figure 1)

- A. The Project Lead should obtain and complete the KLMN Metadata Interview form at project onset to facilitate compiling the information required to create compliant metadata.
 - 1. The KLMN Metadata Interview form is posted on the KLMN Internet and Intranet web pages. In addition, the form can be obtained by contacting the Network Data Manager.
 - 2. Best attempts should be made to populate the Metadata Interview form as completely as possible prior to starting field work. However, it is recognized that changes to the form will occur throughout the project.
- B. A data dictionary must be created to provide information to help the Data Manager create or update the official metadata and in some cases, the project database. The data dictionary should include:
 - 1. The name and purpose of each table, shapefile, coverage, or feature class.
 - 2. A list of attribute names, type, size, and description by table. An example of a data dictionary in the proper format is provided at the end of this SOP.
 - 3. If the database was not designed by the Klamath Network, it is the responsibility of the Project Lead to provide the Data Manager with the data dictionary.
- C. If a taxa list other than a current ITIS certified taxa list was used, the Project Lead will need to provide the list that was used at the end of each field season. Taxa lists should include:
 - 1. Taxon group (Bird, Mammal, Reptile, Invertebrate, etc.)
 - 2. Scientific name
 - 3. Common name
 - 4. Any special code that defines a species
- D. The Project Lead should send a copy of any additional information that might be valuable for the development of metadata.

Step 2: Sensitivity Review. Sensitive data (species locations, site locations, etc.) may not be subject for release to the public.

- A. The current version of NPS Data Store does not screen for sensitive information. Therefore, any data with a sensitive status will not be posted on the Data Store.

- B. The Network Data Manager will be responsible for posting data as sensitive. Status of the data will be based on comments provided by the Project Lead under the “Sensitivity” question in the Metadata Interview form and Certification form. In addition, the Klamath Network will consult with park staff if the sensitivity status of any data is questionable.

Step 3: Metadata Software Selection

- A. The Klamath Network will utilize ArcCatalog, NPS Metadata Tools and Editor, and the Database Metadata Extractor to create metadata for all projects.
 - 1. ArcCatalog automatically harvests spatial organization and reference information, as well as entity and attribute information for GIS datasets.
 - 2. The NPS Metadata Tools and Editor is provided as a standalone program or as an extension for ArcCatalog and is available at:
<http://science.nature.nps.gov/nrdata/tools/>
 - i. It can be used for metadata creation and editing.
 - ii. It can import, export, and parse metadata.
 - iii. It cannot harvest entity and attribute information; however, this is an anticipated feature for the next version.
 - 3. The NPS Database Metadata Extractor (MTE) is a custom software application for authoring, editing, and managing NPS metadata. The MTE operates either as an extension to ArcCatalog versions 8.3/9.x or as a stand-alone desktop application. Eventually, this tool will be incorporated into the NPS Metadata Tools and Editor. Features of this tool include:
 - i. Automatically harvests entity (table) and attribute (field) metadata from Access databases, including domains.
 - ii. Allows the user to edit and review the harvested metadata and make batch edits.
 - iii. Allows the user to export metadata to a FGDC-compliant XML file.
 - iv. Allows exported XML to be used in the Metadata Tools and Editor either by opening it to start a new metadata record or by updating it with a template to fill in Section 5 of an existing metadata record.

Step 4: Additional Requirements

- A. Along with the required metadata, the Klamath Network requires the following information be included in the metadata document.
 - 1. The name and agreement code for the project. These references can be entered in the Related Key element in the Program Information section (NPS Section 0) on the NPS Profile.
 - 2. References to all products (GIS, GPS, Databases, Reports) generated by the projects. These references can be entered in the repeatable Cross Reference element of the Identification Information section.

3. Standard language for NPS liability should be inserted into the Distribution Liability metadata element of the Distribution Information (FGDC Section 6).
 - i. This can be found at: <http://www.nps.gov/gis/liability.htm>.

Step 5: Biological Data Profile

If a dataset includes biological information, the Biological Data Profile provides a set of extended metadata elements to document the species observed, taxonomic information, methods, and analytical tools.

- A. The most direct, and KLMN preferred, means to populating the Biological Data Profile metadata elements are outlined in [Biological Profile \(National Biological Information Infrastructure - NBII\) Metadata Guide](#) (NR-GIS Data Store 2005a).
 - i. This approach primarily utilizes the NPS Metadata Tools and Editor and may also require the entity and attribute harvesting capability of NPS Database Metadata Extractor for Access datasets.
- B. The following two documents describe alternative approaches to completing the Biological Data profile for a metadata record. Note that the first requires the use of additional metadata creation software (Spatial Metadata Management System, or SMMS):
 - i. [Metadata Tools Used in the Creation of the FGDC Biological Data Profile](#) (Callahan and Devine, 2004).
 - ii. [National Biological Information Infrastructure \(NBII\) Metadata Steps](#) (McGuire 2004).

Step 6: Metadata Review

The Data Manager should review metadata for quality control (QC) prior to posting to NPS Data Store. A useful QC Checklist is available for download on the NPS Intermountain Region GIS web site at: http://imgis.nps.gov/tips_templates.html.

Step 7: Metadata Parsing and Exporting to XML Format

The NPS Data Store requires that metadata records be parsed into FGDC-structured metadata and then exported to XML format.

- A. If using ArcCatalog, these steps can both be done directly with the NPS Metadata Tools and Editor. See [Parsing Metadata with the NPS Metadata Tools and Editor](#) (NR-GIS Data Store 2005i) for more information.
- B. If using other applications, export the metadata first to ASCII text format and then parse with Metadata Parser (MP). MP can simultaneously output an XML format metadata file as well.
 - i. MP must be customized to handle NPS, Biological Data, or ESRI Profile metadata elements. For specifics, refer to:
 - a. The README.txt file included in the zipped NPS Metadata Profile configuration files, available from the NPS Data Store web site at: <http://science.nature.nps.gov/nrdata/docs/metahelp/metahelp.cfm>.

- b. [Parsing Metadata with the NPS Metadata Tools and Editor](#) (NR-GIS Data Store 2005i).

Step 8: Metadata Posting

Post the metadata to the NPS Data Store.

- A. Authorized NPS staff may request upload and edit access to the NPS Data Store through the NPS Natural Resource Universal Web Login (UWL), available at: <https://science1.nature.nps.gov/nrdata/>. This is also the portal for uploading data.
- B. More information about metadata upload format requirements is available at: <http://science.nature.nps.gov/nrdata/docs/metahelp/metainfo.cfm> and in [Metadata and Data Uploading Guidance](#) (NR-GIS Data Store 2005g).

Step 9: Editing/Updating Metadata Already Posted to NPS Data Store

As of Version 1, the NPS Data Store application allows online editing of NPS Theme Category and ISO Theme Keyword information and the deletion of single metadata records and/or datasets only (see help documentation at:

<http://science.nature.nps.gov/nrdata/docs/metahelp/edithelp.cfm>).

- A. For metadata records simply needing edits to NPS Theme Category or ISO Theme Keyword elements, refer to [Editing Category Information](#) (NR-GIS Data Store 2005f).
- B. If a metadata record posted to the NPS Data Store contains errors or requires edits to other elements, it will need to be deleted from the NPS Data Store, edited, and then reposted. Refer to [Deleting Single Records](#) (NR-GIS Data Store 2005e).
 - i. The user should first download the metadata record (save in XML format) to the local system, then edit as needed in a text editor or metadata software program.
 - ii. The edited metadata record can then be resubmitted to the NPS Data Store.
 - iii. If the dataset documented by the metadata record requires no edits, it will not need to be reposted. Simply ascertain that the metadata file still specifies the correct pathway to the dataset on the NR-GIS Data Server before resubmitting the metadata file.

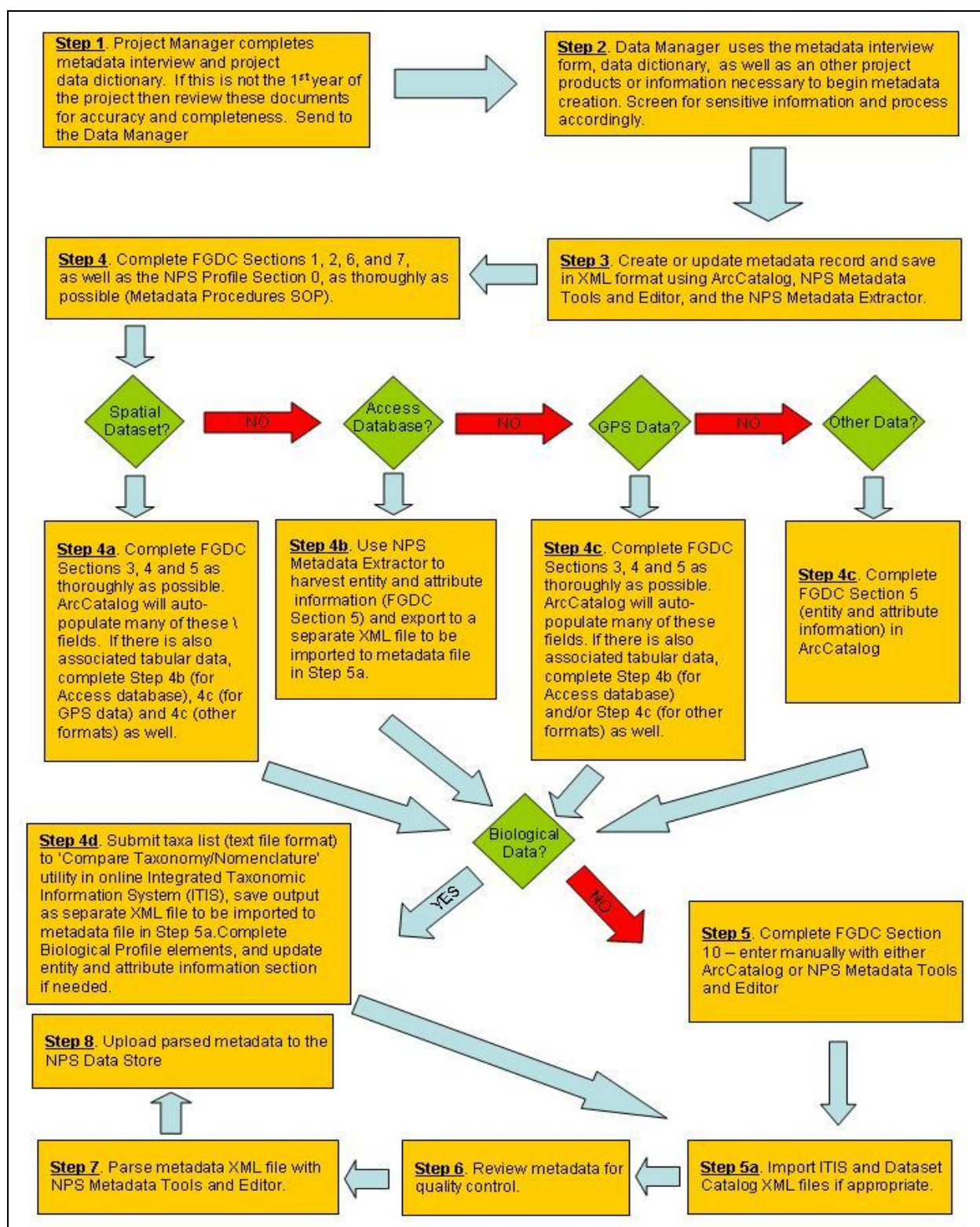


Figure 32. The following diagram shows the general workflow for metadata creation for spatial and tabular data.

Literature Cited

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Electronic Metadata Interview

[Note: Please make your responses directly within this word document in "Red" type.]

1. Have you already prepared metadata for this dataset?
 - a. If yes, please send a copy of the document or reference to where it can be found and skip to item **18**.
2. What is the title of the dataset?
3. Who is the originator(s)/owner of the dataset? (Include address and telephone number)?
 - a. If someone else should answer question about the data, please list the name, address, and telephone number.
 - b. Are there other organizations or individuals who should get credit for support, funding, or data collection and analysis?
4. Does the dataset contain any sensitive information that should not be released to the public? NPS?
 - a. Explain why the data should not be released to the public.
 - b. Explain why the data should not be released to non-park NPS staff.
5. Is the dataset published or part of a larger publication?
 - a. If so, what is the reference?
6. Include a brief (no more than a few sentences) description of the dataset.
7. Why were the data collected in the first place?
8. What is the time period represented by the dataset?
9. Were the data developed primarily through:
 - a. Field visits
 - b. Remote instrumentation (i.e., Temperature recorders, etc.)
 - c. Existing data sources
10. What is the status of the data you are documenting? – *complete, in progress, planned*
 - a. Will the dataset be updated? If so, how frequently?
11. Where were the data collected? Include description and coordinates, if known.
12. List some keywords to help search for this dataset.
 - a. Thematic, Place, Temporal, Strata, Taxonomy
 - b. If a controlled vocabulary was used, what is the reference?
13. List any related datasets that could be documented for cross-reference.
14. The FGDC Biological Profile includes the means to document tabular datasets, taxonomy, field methods, and the use of analytical tools or models.

- a. Was your dataset developed using a model or other analytical tool?
 - i. If so, what is the reference?
 - ii. If the model or tool is available, include a contact and/or URL.
 - b. Does the dataset contain biological information? If no, skip to item 15.
 - i. What species or communities were examined?
 - ii. Did you use a taxonomic authority or field guide for identification? If so, what is the reference?
 - iii. Briefly summarize your field methods (cut and paste from other documents!).
If you used existing protocols or methods, list the references.
 - iv. If you use a different taxonomic hierarchy than what is available in ITIS, then you need to supply the taxonomic hierarchy for all species within the dataset.
15. Is your dataset archived in a databank or data catalog? If yes, please include a reference to the documentation and skip to item 16. If No:
- a. What measures did you take to make certain that your dataset was as nearly correct as possible?
 - b. Were there any things that you excluded from your data collection (e.g., stems less than a certain diameter or streams without surface flow)?
 - c. What is the form of your dataset? - *spreadsheet, ASCII file, GIS layer, database, other*
 - d. What is the filename for your dataset?
 - i. For each file or table, list the fields in the dataset and for each field list:
 - ii. The definition of the field.
 - iii. If the data are coded (Enumerated Domain), list the codes and the definitions.
 - iv. If the codes come from a published code set (Codeset Domain), list the reference.
 - v. If the data are measured (Range Domain), list the units and the minimum and maximum allowable values (“no limit” is acceptable).
 - vi. Otherwise, the domain is unrepresentable. Include a brief description of what is in the field.
16. Is this a GIS dataset? If no, skip to item 17.
- a. Include a path to where the data can be accessed over the network or send a copy of the ArcInfo export file, an ArcView shapefile, or an ArcCatalog exported metadata file (txt or xml).
 - i. Include projection parameters, if necessary.
 - b. List any source datasets you used. For each source, list:
 - i. Source name, originator, and publication date.

- ii. Source time period and scale.
 - iii. Source presentation form and media type.
 - iv. Contribution of source to your analysis.
 - c. List the processing steps you used to create your dataset, including the approximate date of processing.
17. Is the dataset available for distribution? If no, go to 18.
- a. Are there legal restrictions on who may use the data?
 - b. Do you have any advice for potential users of the dataset?
 - c. What are your distribution instructions?
18. You are done. Send this completed document with the relevant responses to this interview to your metadata coordinator (Klamath Network Data Manager – Currently: Sean Mohren. Sean.Mohren@nps.gov, 541-552-8576)

Example Data Dictionary

Dataset: ASXXYY

File Type: mdb

Relationship: Area Search Header (one) to Area Search Data (many)

Table: Area Search Header

Field Name	Field	Required (Y/N)	Type	Length	Decimal	Definition	Enumerated Domain	Range Domain
RecNum	1	Y	Numeric	Integer	0	Auto Number based on order of entry; Key Field		Integer starting at 1, no limit
Project	2	Y	Character	20		Project or Region Code		
Site	3	Y	Character	20		Site name (often 4-letter code)	Each 4-letter code represents itself	
Point	4	Y	Character	2		Point (Search area)	Typically designated A or B	
Month	5	Y	Character	2		Month		01 to 12
Day	6	Y	Character	2		Day		01 to 31
Year	7	Y	Character	4		Year		
PrimObs	8	Y	Character	4		Primary observer's initials		
SecObs	9	N	Character	20		Secondary observer's initials, if multiple observers, initials separated by " , "		
Temp	10	Y	Character	3		Temperature		Degrees Celsius, range=-10 to 40
CldCvr	11	Y	Character	3		Cloud Cover		Percentage, range=0 to 100%

Definitions and Acronyms

ArcCatalog

Module in ESRI's ArcGIS software within which metadata for spatial datasets (coverages, shapefiles) can be created.

Biological Data Profile

Set of definitions for the documentation of biological data through the creation of extended elements to the FGDC Content Standard for Digital Geospatial Metadata (CSDGM).

<i>CSDGM</i>	Content Standard for Digital Geospatial Metadata. The FGDC-promulgated metadata standard established to provide a common set of terminology and definitions for documenting digital geospatial data.
<i>Dataset Catalog</i>	NPS Inventory and Monitoring Program tool for metadata creation, ideal for abbreviated dataset documentation but not for fully FGDC-compliant metadata creation.
<i>ESRI®</i>	Environmental Systems Research Institute. A GIS software company.
<i>FGDC</i>	Federal Geographic Data Committee. The interagency committee that promotes the coordinated development, use, sharing, and dissemination of geographic data.
<i>GIS</i>	Geographic Information System. A computer system for capturing, manipulating, analyzing, and displaying data related to positions on the Earth's surface.
<i>ISO</i>	International Organization for Standardization. A network of national standards institutes of 150 countries, responsible for the “ISO 19115” international metadata standard.
<i>Metadata</i>	Data about the content, quality, condition, and other characteristics of a dataset, documented in a standardized format.
<i>MP</i>	Metadata Parser. A command-line program developed by the USGS to locate syntax errors in metadata files, verify FGDC-compliance, and convert between file formats.
<i>NBII</i>	National Biological Information Infrastructure. Collaborative program instrumental in developing the Biological Data Profile of the FGDC’s CSDGM.
<i>NPS Profile</i>	The NPS Natural Resource and GIS Metadata Profile extends the FGDC CSDGM to incorporate NPS-specific elements such as park and project details. The NPS Profile includes the Biological Data Profile and the ESRI Profile.
<i>NPS Data Store</i>	The NPS Natural Resource, GIS, and I&M Programs’ web-based system (incorporating a database, data server, and secure web interface) to integrate data dissemination and metadata maintenance for Natural Resource, GIS, and other program data sets, digital documents, and digital photos.
<i>SGML</i>	Standard Generalized Markup Language. An ISO standard flexible markup language (predecessor to XML) used in many applications, including electronic publishing on the Web.
<i>XML</i>	Extensible Markup Language. A simple and flexible text format (a profile, or subset, of SGML) that facilitates large-scale electronic publishing and exchange of data on the Web.

Standard Operating Procedure (SOP) #19: Data Analysis and Reporting

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This SOP describes the general scheme for the data analysis and reporting of the Klamath Network Lakes protocol. It is separated into two sections: (1) General information on reporting and analyses for Annual Reports and Analysis and Synthesis reports; and (2) Specific guidelines for water quality and aquatic community analyses. The purpose of section one is to dictate the reporting schedule and content of the reports so that they meet protocol objectives. The purpose of section two is to ensure continuity of methods among personnel and reports to assist in standardization.

Cautionary note on target and statistical population:

Recognition and adherence to the differences between the definitions of “populations” is an important component of data analysis and reporting. This protocol, using a probabilistic design, makes inferences to lakes that were not sampled; but these inferences are limited based on the both the target and statistical population.

In simple terms, the target population refers to the lakes that fit the protocol’s sampling frame, i.e., all lakes within Lassen Volcanic National Park that are within 1 km of trail or road, are in > 30% slope, and are less than 25 meters deep. From this target population, the sites to be sampled are drawn. When we make inferences (such as status), we extrapolate our sample size to this entire target population. For example, our sampling protocol cannot assess status for lakes deeper than 25 meters. Additionally, these inferences are further limited by the statistical population.

The statistical population includes all possible values that we could measure from the lake, bounded both by space and time. “Space and time” is a surrogate for the application of this protocol; so that our statistical population for something as simple as shoreline temperature is as follows:

“The larger conceptual universe of temperature values that could theoretically be sampled and measured using a hand-held thermometer, between the hours of 10 a.m. and 3 p.m. between 15 July and 15 October in lakes that fit the sampling frame.”

It is these numbers that we can make extrapolations and status for; for example, we cannot make inferences to shoreline temperatures in January or June.

However, instead of defining the precise statistical population for every response variable measured under this protocol we offer a general definition:

“The larger conceptual universe of response variable values, bounded by the limits (spatially, temporally, and methodologically) of this protocol.”

Reporting

The target audience of all reports (Annual and Analysis and Synthesis) is a broad group of interested parties, including park superintendents, resource managers, Inventory and Monitoring staff, external scientists, partners, and the public. The timelines and specific purposes of each report are detailed in Table 1.

Annual Reports

Annual reports serve as the main conduit for informing the audience of the current years' monitoring activities. An example of an annual report is given in Appendix A of this protocol and should serve as a template for future reports, although we will revise it with input from managers. In all annual reports, an emphasis will be put on using summary statistics (measures of central tendency and dispersion) for the key parameters of the protocol (Table 2). Findings of special interest to resource managers or the public will also be highlighted. Examples of this are instances of wildlife diseases or new records of non-native species. In general, the annual reports will not lend themselves to hypothesis testing; rather, hypothesis testing (on trends) will be covered in later Analysis and Synthesis reports. However, special interests or patterns observed may lend themselves to hypothesis testing. For example, it may be appropriate to test for differences in species' distributions from the west side of a park to the east side of the park. Recommendations for protocol revisions will also be suggested as necessary; however, actual protocol revisions will follow the steps outlined in SOP #20: Revising the Protocol. In years where there are both Annual Reports and Analysis and Synthesis Reports, these are separate publications.

Annual reports should include results of cumulative bias studies, if necessary (see SOP #16: Quality Assurance Project Plan).

Table 23. Overview of data reporting for Klamath Network Lakes Protocol. Year refers to the year initiated (reports will be due the following year).
 *Analysis and Synthesis reports in 2024 and beyond do not have a “scheduled” topic. Rather, the Network staff at that time is encouraged to explore new and emerging avenues of summaries and analyses (with emphasis on park relevant material), but will always include a trend component.

Report type	Year(s)	Purpose	Method and References (if applicable)
Annual Report	Every sampling year	Summarize monitoring activities	
		Describe current status	Means/Variance/Horvitz-Thompson estimators (Manly 2009)
		Document changes/recommendations to monitoring protocols	
		Increase communication between I&M program and all parties	
Analysis and Synthesis	2016	Description of lake physical habitat gradients and patterns	Wetzel 2001
	2019	Description of lake chemistry gradients and patterns	Wetzel 2001
	2021	Description of lake biological gradients and patterns	Wetzel 2001
	2023 and every 3 years after	Trend Analyses (Select univariate & multivariate - IEI, O/E, species composition)	Time series (e.g., Mann-Kendall; progressive change) (Chatfield 2004; Phillipi et al. 1998)

Table 24. Recommended variables for inclusion Annual Reports, along with basic status and recommendations on reporting per lake. ANC = Acid Neutralizing Capacity, TSI = Trophic State Index, O/E = Observed/Expected, IEI = Index of Ecological Integrity, CPUE = Catch Per Unit Effort,

Response variable	Mean	95% CI	Median	Range	Per lake
Water Chemistry					
Total nitrogen	X	X	X	X	X
Total phosphorous	X	X	X	X	X
Dissolved organic carbon	X	X	X	X	X
Calcium	X	X	X	X	X
Sulfate	X	X	X	X	X
Sodium	X	X	X	X	X
Chloride	X	X	X	X	X
ANC	X	X	X	X	X
TSI	X	X	X	X	X
Profile graphs					X
Physical					
Shoreline development	X	X	X	X	X
Substrate compositon					X
Macroinvertebrates					
Taxa richness	X	X	X	X	X
Shannon diversity	X	X	X	X	X
O/E ratio	X	X	X	X	X
IEI	X	X	X	X	X
Species list					X
Zooplankton					
Taxa richness	X	X	X	X	X
Shannon diversity	X	X	X	X	X
O/E ratio	X	X	X	X	X
IEI	X	X	X	X	X
Species list					X
Amphibians					
Species presence/Not detected					X
Fish					
Species presence/Not detected					X
CPUE					X
<i>Kn</i>					X

Analysis and Synthesis Reports

Reports 1-3

The first Analysis and Synthesis report will be prepared after the second sampling period (2013 and 2016). This initial report has been delayed so that some measurements of temporal variation in parameters can be included. The first, second, and third Analysis and Synthesis reports are focused on describing various gradients, parameters and patterns of three components of the lake ecosystems: physical, chemical, and biological. The fourth will be the first analysis of trends for the lakes program. Follow-up Analysis and Synthesis reports will be conducted every 3 years (although an Annual Report will also be produced every 3 years, these will be separate products), and other topics will be decided in the future. Likely topics include items of interest to park managers or emerging new analytical techniques and tools as yet unknown.

The Project Lead is responsible for the accomplishment of the Analysis and Synthesis reports. The Project Lead should be knowledgeable in park resources and statistical analyses to ensure that meaningful reports are produced. If a Project Lead is being hired and their expected tenure will include the writing of these reports, a background in statistics and preferably these specific areas should be a prerequisite of the job. If the Project Lead does not have the required skills (for example, an interim Network staff member is overseeing the collection of data or non-aquatic ecologist has filled a general network position), it is the responsibility of the Network Coordinator to supplement the skill set of the Project Lead, either with personal assistance or the contracting of an outside resource (academic or USGS personnel).

Analysis and Synthesis Report 4: Trend Analyses

The fourth Analysis and Synthesis report will be the first analysis of trends in selected parameters. This will be performed after a total of five sampling periods, so that the sample size for a temporal effect will still be limited. Doing trends analyses before this point, although a major goal of this protocol, would be premature.

The trend report will be analyzed with a variety of parametric and non-parametric techniques, on both univariate and multi-variate parameters (Table 3). In general, in assessing change, a "weight of evidence approach" will be undertaken. For instance, if several tests (Mann-Kendall, regression, *and* multivariate) all agree that a significant change has occurred, this will be taken as strong evidence of biologically significant change, whereas a single test showing significant change (e.g., only the Mann-Kendall) will be taken as weaker evidence of biologically significant change.

This report will also explore the standardization of the trends analyses, allowing future Analysis and Synthesis reports to include repeatable trend analyses through preparation of standardized "R" scripts, and other analyses incorporating new annual data.

We also expect that new techniques will emerge for studying trends that allow complex dynamics of species composition changes to be more clearly demonstrated. Emerging techniques will also be considered, and if applicable, applied to the trends Analysis and Synthesis report.

Table 25. Proposed analyses for trend detection in Analysis and Synthesis Report 6. TSIs = Trophic State Indices; DOC = Dissolved Organic Carbon; ANC - Acid Neutralizing Capacity; IEI = Index of Ecological Integrity, respectively. * = note that although these parameters are "univariate," they are derived from a broader suite of multivariate information, and being tested with univariate techniques, provide a robust assessment of trend.

Univariate parameters	Analytical tests	References	Proposed Software
Lake Trophic Status -			
TSIs (total nitrogen, total phosphorous & Secchi depth)			
Lake morphometry parameters -			
Volume, area, shoreline development			
Chemistry -			
Anions, cations, DOC, nutrients, ANC	Parametric and non-parametric time series analysis (Regression models and Mann-Kendall rank correlation tests).	Quinn and Keough (2002), Chatfield (2006), Zar (2009)	Systat, "R", or similar
Biological* -			
Taxa richness, Shannon Index, Hilsenhoff Biotic Index, O/E scores, IEI, Fish condition index, Chlorophyll biomass			
Multivariate parameters			
Macroinvertebrates assemblages, Zooplankton assemblages, Lake communities	Indices of multi-variate seriation	Warwick and Clarke (1991), Philippi et al. (1998), Clarke and Warwick (2001)	Primer-E, PC-ORD, or similar

When doing trend analyses, the Project Lead should adjust data for any cumulative bias from protocol/equipment/laboratory changes that were noted in previous Annual Reports, if necessary.

The essential “statistical toolboxes” for these analyses are listed in Table 2. Time series analysis (i.e., trends) is a topic spanning several textbooks filled with multiple techniques and approaches, and even an elementary introduction is beyond the scope of this SOP. However, a good starting point for these analyses will be two of the most elementary forms of time series, and these should be the backbone of the trends reports. To assist in the implementation, some guidelines are presented below. It is also valuable to examine basic biplots of the data with time as the dependent variable to explore possible non-linear patterns or trends in the data.

Linear Regression – Although multiple models of linear regression exist (see below), reporting and interpretation of trend will be based on (1) slope estimate and standard error of slope; and (2) significance of slope via analysis of variance (ANOVA) F tests. The slope estimate provides the effect size of the trend (if any) and the direction, positive or negative. The standard error of the slope is an estimate of the precision of the slope. The actual effect size of the slope should be evaluated by the Project Lead for biological significance. The statistical significance is provided by the ANOVA F test (Quinn and Keough 2002). The Project Lead should also investigate using analysis of covariance (ANCOVA) to examine the categorical factor (site) differences.

Mann-Kendall Trends Analysis – This is a non-parametric test for trends based on the Kendall’s Tau (τ), a rank-order correlation coefficient of concordance. For example, if in five time periods (1 – 5), the response value increases with each period, there will be 100% concordance. If only four of the five are in concordance, there would be closer to 80% concordance. Significance is tested by randomizing the time elements and developing a distribution of tau values based on random patterns (i.e., no effect of time). If the observed value is higher than 95% of the randomized values, the trend is statistically significant.

Indices of multivariate seriation – This is a multivariate correlational test similar to the Mann-Kendall Trends Analyses; however, the correlation is tested between the elements of two symmetrical matrices: one based on the ecological similarity (measured with a similarity index, such as Bray-Curtis) and one based on temporal distances between samples. A correlation coefficient is calculated by ranking the order of the elements and calculating the Kendall’s Tau for concordance. Similar to the Mann-Kendall test, significance is tested by randomizing one matrix element and comparing the observed correlation coefficient to the resulting randomized distribution.

Guidelines for Water Quality and Aquatic Community Analyses

The purpose of this section is to ensure standardization so that analyses of data from this program are comparable across years.

pH: Because pH is a logarithmic value, pH must be converted to the antilog (i.e., raw hydrogen ion concentration), averaged, then reconverted to pH. This should be done for averaging profile readings or if multiple locations were measured within a lake. See example in Table 4. However,

when averaging pH among lakes, for example to calculate an average pH for all of the Lassen Volcanic National Park lakes, a standard average should be used.

Table 26. Example depth and pH readings taken in a hypothetical lake and how to average pH readings. Note that a straight average of the pH readings = 7.75; not 7.85, the correct value.

Depth	pH	Raw value ¹	Average raw value	Average pH ²
0.5m	7.3	19952623.15	71413970.35	7.85
1.0m	7.6	39810717.06		
1.5m	8.0	100000000.0		
2.0m	8.1	125892541.2		

¹can be calculated in MS Excel using " $=\text{POWER}(10, \text{value})$ ", where 10 is the logbase, and "value" is the measured pH. ²Average value reconverted using the " $=\text{LOG}(\text{value}, 10)$ " function in MS Excel where value is the averaged raw value and 10 is the baselog.

Approximate volume ($\sim V$): Because we only measure the maximum depth (z_m), the volume of a lake can only be *approximated* on the assumption that the lake has a cone-like bottom profile. Although this is not necessarily a valid assumption, a working measure for using volume as a co-variable can be calculated.

—

Shoreline development (D_L): This is a ratio of the length of the shoreline (L) to the perimeter of a circle with an equal area. Hence, a calculated D_L near unity (1.0) would be a very circular habitat. With increasing D_L , the shoreline (and hence habitat) complexity increases. A lake with a large D_L would be a lake with many bays and coves and associated variation in shoreline habitats.

=====

Trophic status indicators (TSI): Trophic status indicators (TSIs) developed by Carlson (1977) serve as an index of lake enrichment or trophic state. They are calculated as below:

Where SD = Secchi depth in meters. TSI for Secchi depth should only be calculated for lakes where a recorded Secchi depth exists (i.e., lakes where a secchi disk was visible to the lake bottom should not be recorded, and TSI should not be calculated).

Where TP = Total phosphorous in $\mu\text{g/L}$.

Where TN = Total nitrogen in $\mu\text{g/L}$.

Where *Chl a* = Chlorophyll *a* in µg/L.

Taxonomic Resolution

Taxonomic resolution may vary from site to site and year to year. One reason is that mature invertebrates (i.e., later instars of insect larvae) are more likely to have developed the diagnostic features necessary for identification. Another reason is that some taxa have only genus level keys (e.g., Ephemeroptera) and others better developed species keys (e.g., Coleoptera: Dytiscidae). Damaged individuals may also limit taxonomic resolution. Lastly, taxonomic expertise of the individual identifying the specimen may cause differences in resolution.

Standardization of taxonomic resolution is accomplished by requiring contract laboratories to only employ taxonomists certified by the North American Benthological Society (www.benthos.org), and by timing the collection of samples to similar times of the year. However, the varying amounts of taxonomic resolution present a problem in determining the total number of unique taxa in which to base taxa richness and Shannon index calculations. To this end, the contract laboratory provides the determination of which taxa not identified to the lowest practical level are “unique.” This allows the taxonomist to identify a species to genus/species level for one specimen, and only identify a specimen of the same family to the family level. If he or she determines that the specimen keyed to family level is “unique,” this indicates that the specimen is probably not represented by the individuals identified to the genus/species level and should be treated as a separate new taxon, despite the reduced resolution.

Abundances

Abundances should be calculated for a) Zooplankton (per cubic meter); b) Macroinvertebrates (per square meter); and c) Fish (catch per unit effort). Both zooplankton and macroinvertebrates, for logistical reasons, are sub-sampled. Although the sub-sampling is quantitative in nature, additional potentially compounding error is added to the sample. Hence, data interpretation and reporting for zooplankton and macroinvertebrates should focus on relative abundances. Although abundances for individual taxa can be ecologically relevant, the presentation of abundances for 100+ taxa over a long-term time series does not lend itself to easily interpretable summaries. Hence, presentation of abundance data should be at the gross level for these groups (e.g., all macroinvertebrates per square meter). Abundance of individual taxa should only be included if there are special considerations justifying it (e.g., endangered or invasive species).

Shannon Index (H')

This information index incorporates both relative abundance and taxa richness (Shannon 1949, Magurran 2004). It is calculated as:

Where p_i = the proportion of the i th species (e.g., abundance of taxa i divided by the total abundance of the sample).

The calculation is straightforward and can easily be done in MS Excel or another spreadsheet. However, two important considerations must be made: 1) taxonomic resolution and 2) which

logarithmic base to use. Taxonomy should be based on unique taxa (see above). Although examples of using different logarithmic base for the transformation exist in the literature, there is growing momentum to standardize on the natural log (ln) (Magurran 2004). **All Shannon Indices calculated for this monitoring program should use the natural log.**

Hilsenhoff Biotic Index (HBI)

This index is specific to macroinvertebrates. It is a weighted average of tolerance values derived from empirical observations of macroinvertebrate responses to pollution (Hilsenhoff 1987, 1988). It is calculated as:

Where n_i = the number of individuals for taxa i , T_i = the assigned tolerance value of taxa i , and N = the total number of individuals for a sample.

For consistency, a single source for tolerance values should be utilized. The source for this protocol is tolerance values developed by Mr. Robert Wisseman of Aquatic Biology Associates and is available at: <http://www.cbr.washington.edu/salmonweb/taxon/>. This source has been chosen because: 1) it was developed specifically for Pacific Northwest taxa, and 2) it includes non-insect tolerance values.

One advantage of the HBI is that tolerance values have been developed for Order, Family, and lower taxonomic levels. Hence, individuals that were only identified to Family can still be incorporated in the index, without making assumptions or collapsing taxonomic information.

Additional work has been done on adapting this method to zooplankton. However, the development of tolerance values for zooplankton is still relatively limited; either in geographic location (e.g., the Iberian Peninsula; Boix et al. 2005), or in habitat type (e.g., wetlands; Loughheed and Chow-Fraser 2002). When tolerance values for lake zooplankton in the Pacific Northwest become available, they should be integrated into the data analysis, with a corresponding revision to this SOP.

Fish Condition Index (Kn)

The Fish Condition Index (Kn) is based on a ratio of fish weight to length (LeCren 1951). It can be used to track the relative condition of different fish populations as they are encountered in the monitoring program. It is calculated as:

Where W = weight of the fish in grams and L = the length of the fish in millimeters.

After calculating the Kn for each individual fish, an average Kn can be reported for each species in each habitat. Note that the “Fallacy of Averages” must be avoided (Welsh et al. 1988). The fallacy in this case is that an investigator might average the weights of fish, average the length of the fish, and then calculate an “average” Kn . Because W and L are not independent, the use of average W and L to calculate an index is mathematically improper. The example below

demonstrates how averaging weight and length, and then calculating *Kn* on average weight and average length results in an erroneous value (Table 5).

Table 27. Example of hypothetical fish measurements that can result in erroneous averaged values.

Fish number	Weight (g)	Length (mm)	Condition index (<i>Kn</i>)	Actual average <i>Kn</i> : 42.2
1	200	80	39.1	
2	300	90	41.2	
3	100	60	46.3	
Average:	200	76.7		

"average *Kn*" = 44.4

Since the fish collection techniques used in these protocols may result in low numbers collected, any reporting of *Kn* should include the reporting of the sample size it is based on. See Anderson and Gutreuter (1983) for more information on *Kn*.

Note that comparisons of *Kn* between species is not appropriate, nor is comparing the same species at multiple sites. Rather, it should be compared for a single population (defined as the fish of one species at one site) across years.

Water Quality Exceedances

Although this protocol is not designed to monitor for standards exceedances, reporting should include any instances of exceedances where encountered. Because the protocol sampling is a single point in time, any reports of exceedances should not constitute a call for management action. Instead it is a signal that there may be impairment and the parameter exceeded should be investigated using state standards (e.g., 4 day average of parameter X) to determine actual exceedance.

Both the state of California and the state of Oregon have promulgated water quality standards. However, many of the standards are for toxic substances (e.g., Polyaromatic Hydrocarbons) and do not overlap with monitored parameters under these protocols. Of the California standards, they have yet to develop standards for the monitored parameters. For Oregon, most standards are centered on allowable increases or decreases from natural conditions. Table 6 presents the Oregon standards, along with National Park Service and Environmental Protection Agency standards.

These standards may be updated, expanded, and revised by the respective agencies. The Project Lead should periodically (once per sampling event) check for updates. The sources used in Table 6 are:

Oregon - <http://www.deq.state.or.us/WQ/standards/standards.htm> (accessed on 21st January 2009).

California - <http://www.epa.gov/region09/water/ctr/> (no overlapping parameters with current protocol; accessed on 21st January 2009).

Table 28. Summary of water quality standards available for testing exceedances. California currently does not have any overlapping standards with monitored parameters. All standards presented are for instantaneous readings. Drinking Water standards for the EPA are provided as comparison only, not for regulatory compliance.

	Oregon Standards	NPS Potential Criteria	EPA Freshwater	EPA Drinking Water
Acid Neutralizing Capacity ¹	> 20 mg/L	> 25 mg/L		
Chloride	< 230 mg/L		< 860 mg/L	< 250 mg/L
Chlorophyll a ²	0.015 mg/L			
Dissolved Oxygen	> 6.5 mg/L	> 4 mg/L		
pH		> 6.5	< 9.0	< 8.5
Turbidity		< 50 NTU		
Total N as (NO ₂ + NO ₃)				< 10 mg/L

¹Measured in CaCO₃; ²standard for lakes that do not stratify. Standard for stratifying lakes is 0.01 mg/L, Oregon Chlorophyll a standards based on average of 3 samples over 3 consecutive months.

EPA Standards - <http://www.epa.gov/waterscience/criteria/wqctable/> (accessed on 21st January 2009)

NPS Potential Criteria – Embedded in NPS Storet, v. 1.71, these are suggested values to screen against, but are not regulatory standards.

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Standard Operating Procedure (SOP) #20: Revising the Protocol

Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

This document explains how to make and track changes to the Lakes Protocol, including its accompanying SOPs. While this monitoring protocol has been developed using current standardized methodology, all long-term monitoring programs need to be flexible to adapt to changes. As new technologies, methods, and equipment become available, this protocol will be updated as appropriate. Current best practices will be weighed against the continuity of protocol information in determining revisions. Project staff should refer to this SOP whenever edits are necessary and should be familiar with the protocol versioning system in order to identify and use the most current versions of the protocol documents. All changes will be made in a timely manner with the appropriate level of review.

All edits require review for clarity and technical soundness. Small changes to existing documents (e.g., formatting, simple clarification of existing content, small changes in the task schedule or project budget, or general updates to information management handling SOPs) may be reviewed in-house by project cooperators and Klamath Network staff. However, major changes to data collection or analysis techniques, sampling design, or response design will trigger an outside review. The Project Lead should coordinate with the Klamath Network Coordinator to determine if outside review is needed.

Revision Procedures

The following procedures will ensure that both minor and major revisions to this document will align with the monitoring plan.

1. Discuss proposed changes with other project staff prior to making modifications. It is imperative to consult with the Data Manager prior to making changes because certain types of changes may jeopardize dataset integrity unless they are planned and executed to avoid this. Also, because certain changes may require altering the database structure or functionality, advance notice of changes is necessary to help minimize disruptions to project operations. Consensus should be reached regarding who will be making the changes and in what timeframe.

2. Make the agreed-upon changes in the appropriate protocol document. Note that the protocol is split into separate documents for each appendix and SOP. Also note that a change in one document may necessitate other changes elsewhere in the protocol. For example, a change in the narrative may require changes to several SOPs. Similarly, renumbering an SOP may mean changing document references in several other documents. Also, the project task list and other appendices may need to be updated to reflect changes in timing or responsibilities for the various project tasks.
3. Document all edits in the Revision History Log embedded in the protocol narrative and each SOP. Log changes only in the document being edited (e.g., if there is a change to an SOP, log those changes only in that document). Record the date of the changes (i.e., the date when all changes were finalized), author of the revision, the change and the paragraph(s) and page(s) where changes are made, the (brief) reason for making the changes, and the new version number. Version numbers increase incrementally by hundredths (e.g., version 1.01, 1.02) for minor changes. Major revisions should be designated with the next whole number (e.g., version 2.0, 3.0). Record the previous version number, date of revision, and author of revision; identify paragraphs and pages where changes are made, rationale for revisions, and the new version number.
4. Circulate the changed document for internal review among project staff and cooperators. Minor changes and clarifications will be reviewed in-house. When significant changes in methodology are suggested, revisions will first undergo internal review by the project staff. Additional external review including, but not limited to, National Park Service staff with appropriate water quality, aquatic communities, and statistical expertise will be required.
5. Upon ratifying and finalizing changes:
 - a. Ensure that the version date (last saved date field code in the document header) and file name (field code in the document footer) are updated properly throughout the document.
 - b. Make a copy of each changed file to the protocol archive folder (i.e., a subfolder under the Protocol folder in the project workspace).
 - c. The copied files should be renamed by appending the revision date in YYYYMMDD format. In this manner, the revision date becomes the version number and this copy becomes the “versioned” copy to be archived and distributed.
 - d. The current, primary version of the document (i.e., not the versioned document just copied and renamed) does not have a date stamp associated with it.
 - e. To avoid unplanned edits to the document, reset the document to read-only by right-clicking on the document in Windows Explorer and checking the appropriate box in the Properties popup.
 - f. Inform the Data Manager so the new version number(s) can be incorporated into the project metadata.

6. As appropriate, create PDF files of the versioned documents to post to the Internet and share with others. These PDF files should have the same name and be made from the versioned copy of the file.
7. Send a digital copy of the revised monitoring plan to the Network Coordinator and Network Data Manager. The revised monitoring plan will be forwarded to project and park staff who had been using a previous version of the affected document. Ensure that field staff has a hardcopy of the new version.
8. The Network Data Manager will place a copy of the revised protocol in the proper folder on the Klamath Network shared drive. In addition, the Network Data Manager will archive the previous version in the Klamath Network archive drive.
9. The Network Data Manager will post the revised version and update the associated records in the proper I&M databases, including but not limited to NatureBib, NPS Data Store, KLMN Intranet and Internet web sites, and the Protocol database.
10. Update the following table to inform of the most up-to-date current version of all protocol sections.

Table 29. Protocol version tracking table showing latest version and effective date. To be updated with any revisions.

	Latest Version	Effective Date
Protocol Narrative	1.0	01/10/11
SOP#1 :Preparations, Equipment and Safety	1.0	01/10/11
SOP#2 :Field Crew Training	1.0	01/10/11
SOP#3 :Site Selection	1.0	01/10/11
SOP#4 :Order of Work	1.0	01/10/11
SOP#5 :Site Arrival Tasks	1.0	01/10/11
SOP#6 :Weather, Physical Habitat, and Site Photography	1.0	01/10/11
SOP#7 :Fish Collection and Processing	1.0	01/10/11
SOP#8 :Water Sample Collection, Zooplankton, and Secchi Disk	1.0	01/10/11
SOP#9 :Water Sample Filtration and Handling	1.0	01/10/11
SOP#10 :Multiprobe Calibration and Use	1.0	01/10/11
SOP#11 :Amphibian, Invertebrates, and Lake Substrate Walk-around	1.0	01/10/11
SOP#12 :Post-Site Tasks	1.0	01/10/11
SOP#13 :Data Entry	1.0	01/10/11
SOP#14 :Photo Management	1.0	01/10/11
SOP#15 :Post-Field Season	1.0	01/10/11
SOP#16 :Quality Assurance Project Plan	1.0	01/10/11
SOP#17 :Data Transfer, Storage, and Archive	1.0	01/10/11
SOP#18 :Metadata Guidelines	1.0	01/10/11
SOP#19 :Data Reporting and Analysis	1.0	01/10/11
SOP#20 :Revising the Protocol	1.0	01/10/11
Appendix A: Annual Report from Pilot Project	1.0	01/10/11
Appendix B: Expectation of Field Crew	1.0	01/10/11
Appendix C: USGS Safety Manual, Chapter A9	1.0	01/10/11
Appendix D: Leave No Trace Handbook	1.0	01/10/11
Appendix E: Icom Radio Use Handbook	1.0	01/10/11
Appendix F: Field Data Sheets, Training Logs	1.0	01/10/11
Appendix G: Example of Site Folder	1.0	01/10/11
Appendix H: Example of Fish Identification and Disease Guide	1.0	01/10/11
Appendix I: Hach Digital Titrator Manual	1.0	01/10/11
Appendix J: Eureka Environmental Manta and Amphibian Water Quality Probe Manuals	1.0	01/10/11
Appendix K: American Society of Ichthyology and Herpetology Amphibian Handling Manual	1.0	01/10/11
Appendix L: Equipment List	1.0	01/10/11
Appendix M: Database Structure	1.0	01/10/11
Appendix N: Macroinvertebrate Tolerance Values	1.0	01/10/11
Appendix O: Job Hazard Analyses	1.0	01/10/11
Appendix P: Operational Checklist	1.0	01/10/11

Appendixes

All appendixes can be found online at the Klamath Network website:

http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/VS_Lakes_Protocol.cfm

Appendix A. Annual Report from Pilot Project

http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendixes/Appendix_A_Annual_Report_Final_20091015.pdf

Appendix B. Expectation of Field Crew

http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendixes/Appendix_B_Responsibilities_Final_20091015.pdf

Appendix C. USGS Safety Manual, Chapter A9

http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendixes/Appendix_C_Safety_Manual_Final_20090719.pdf

Appendix D. Leave No Trace Handbook

http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendixes/Appendix_D_Leave_No_Trace_Final_20090719.pdf

Appendix E. Icom Radio Use Handbook

http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendixes/Appendix_E_Radio_SOP_Final_20090719.pdf

Appendix F. Field Data Sheets, Training Logs

http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendixes/Appendix_F_Data_Sheets_Logs_Final_20091019.pdf

Appendix G. Example of Site Folder

http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendixes/Appendix_G_Site_Folder_Final_20091019.pdf

Appendix H. Example of Fish Identification and Disease Guide

http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendixes/Appendix_H_Fish_Amphibian_Guide_Final_20090719.pdf

Appendix I. Hach Digital Titrator Manual

http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendixes/Appendix_I_Hach_Titrator_Final_20090719.pdf

Appendix J. Eureka Environmental Manta and Amphibian Water Quality Probe Manuals

http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendixes/Appendix_J_Eureka_Multiprobe_Final_20090719.pdf

Appendix K. American Society of Ichthyology and Herpetology Amphibian Handling Manual
http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendices/Appendix_K_ASIH_Amphibian_Handling_Final_20090719.pdf

Appendix L. Equipment List
http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendices/Appendix_L_Equipment_Final_20091021.pdf

Appendix M. Database Structure
http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendices/Appendix_M_Database_Final_20091209.pdf

Appendix N. Macroinvertebrate Tolerance Values
http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendices/Appendix_N_Tolerance_Values_Final_20110311.pdf

Appendix O. Job Hazard Analyses
http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendices/Appendix_O_Job_Hazard_Analysis_Final_20110311.pdf

Appendix P. Operational Checklist
http://science.nature.nps.gov/im/units/klmn/Monitoring/vs/Lakes/Documents/Protocol/Appendices/Appendix_P_Checklists_20110220.docx.pdf

The Department of the Interior protects and manages the nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its special responsibilities to American Indians, Alaska Natives, and affiliated Island Communities.

NPS 963/112291, January 2012

National Park Service
U.S. Department of the Interior



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