Tenmile Lakes Watershed Quality Assurance Project Plan

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Produced by
TLBP

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5. Goal

It is the goal of the Tenmile Lakes' Basin Partnership to document the baseline ambient water quality conditions of lake and stream water in the basin. We plan to do this through water quality monitoring, continuous temperature monitoring, algae sampling, nutrient sampling, delta building surveys, and project effectiveness monitoring.

6. Problem Definition/Background:

The Tenmile Lakes' Watershed covers approximately 98 square miles (62720 acres). There are ten lakes within the watershed with a combined surface area of about 4.7 square miles (3008 acres) or 5% of the watershed. These lakes and their drainages together can be divided into three sub basins.

The most northern, the Eel Lake subbasin consists of North Clear, Edna, Teal, Schuttpelz, and Hall Lakes which are all drained by Clear Creek into Eel Lake. Eel Lake is drained by Eel Creek, which flows into Tenmile Creek.

The southwestern area, the Saunders Creek subbasin, covers the drainages of Saunders Lake, South Clear Lake, and Saunders Creek. Saunders Creek flows along the eastern edge of the dunes and into Tenmile Creek. These two sub basins, combined, cover approximately 17.5 square miles (11,200 acres).

The Tenmile Subbasin, the easternmost in the watershed, includes Tenmile and North Tenmile Lakes and their respective drainage areas, which combined, cover about 70 square miles (44,800 acres). Tenmile Creek carries the water from this sub basin for about five miles, past the junctions of Eel and Saunders creeks, to the ocean.

A northern portion of the watershed is in Douglas County and the remainder is in Coos County. The city of Lakeside is found within the Tenmile subbasin on the banks of Tenmile Lake and Tenmile Creek. There is also a small dunes sub basin that consists of the dune area and it's aquifers, which is located on the western side of the watershed.

The Tenmile watershed is predominantly forested uplands. Since these forests cover the majority of the drainage, they intercept most of the rain that falls within the watershed, and so act as the catch basin for the entire watershed. Most of the steep upper forested slopes and their forested headwater

streams are found within the Elliott State Forest and are managed by the Oregon Department of Forestry. The Elliott State Forest covers approximately 33.5 square miles (21,440 acres), making the State the largest single landowner within the watershed. Privately owned forestland covers approximately 34.7 square miles (22,208 acres).

The largest landowner within the privately owned section is Menasha Corp. with approximately 11.4 square miles (7,296 acres). Roseburg Lumber manages approximately 4.3 square miles (2,752 acres). Small timber companies and private parties own the remaining forestland.

Most agricultural land found within the watershed is located on the alluvial areas associated with the lower reaches of the nine major headwater tributaries flowing into North and South Tenmile Lakes. There is approximately 2.83 square miles (1,811 acres) of farmland in use today within the watershed. Most agricultural land within the watershed is used for grazing cattle and other livestock. There are an estimated 338 acres of wetlands between the elevations of 6.5' and 12.5' within the watershed.

Over the years, TLBP has conducted studies in the Tenmile basin to identify potential issues within the watershed. The first was a study of Tenmile Lake and its watershed to better understand the role of the watershed and the lake in generating and processing sediment, phosphorus, and nitrogen. Fieldwork was initiated in November 1998 and extended to August 1999 under Phase I of the study. Phase II of the work was funded to collect additional stream nutrient data, extend the sediment analyses, conduct additional phytoplankton sampling, and revise the SWAT watershed model using updated information on land use and stream chemistry. In 2002, a watershed assessment was implemented to characterize conditions within the Tenmile Lakes basin, and to provide a roadmap for restoration activities geared towards improving salmonid habitat and water quality. Existing information was gathered and integrated with data collected by the Tenmile Lakes Basin Partnership (TLBP). As Oregon Watershed Enhancement Board (OWEB) assessments are meant to be large-scale screenings of watershed processes, the wide range of subjects is meant to indicate areas that need more attention. Of primary concern is the explanation of complex watershed processes for the benefit of watershed council members, the general public, and natural resource managers involved in the enhancement and management of the Tenmile Lakes basin. Ultimately, increased understanding of watershed dynamics and land use practices, along with community involvement will lead to a Tenmile Lakes Watershed which will maintain and improve all beneficial uses.

The goals of this project is to build a long-term data set based on these two studies. The participants in this project, including the watershed council, Oregon DEQ, OWEB, State Forestry, City of Lakeside, private agriculture/forestry, and lakefront residents who want to document the baseline ambient water quality conditions of lake and stream water in the basin**.** The data collected within this project will be used by the watershed council and state agency staff to; characterize current water quality conditions, identify specific water quality problem areas, and assist in the development of enhancement and restoration projects. The watershed council will also use the data to educate and inform local residents on the connections between land use and water quality. Results of this monitoring program will be released per written request after it has cleared TLBP's review process. TLBP's review process consists of four steps:

1. Tenmile Lakes Watershed Council
2. Watershed Coordinator
3. Monitoring Coordinator
4. TLBP Monitoring Committee

Once this review process is completed, results will be available for distribution.

6. Project Task/Description:

The Tenmile Watershed Monitoring Project will consist of; stream monitoring, algae and nutrient monitoring, and *project effectiveness monitoring*. This stream monitoring will involve regularly scheduled field sampling events to collect data on water temperature, dissolved oxygen, pH, and turbidity. Seasonal water quality sampling of temperature of all 28 sites, and of these 28,selected sites

will be monitored for dissolved oxygen, pH, and turbidity. This portion of the monitoring project will be conducted from June to October starting in June 2004. Sites located on Big Cr. and Murphy Cr. will be monitored monthly through out the year. Continuous temperature monitoring will also be conducted at all 28 sites from June 1st to October 15th. Sites may change depending on access to private lands.

Nutrient sampling will occur at 4 lake sites and 1site on Tenmile Creek. This sampling will occur in the months of May, July, September, November, and February.

Algae sampling will occur in the lake and the sampling regime will be:

June- 1x
July-2x
August-2x
September-2x
October-1x
November-1x

Both algae and nutrient samples will be sent to a laboratory for analysis.

Delta Building is a new project to monitor the rates of sediment accumulation at the mouths of tributaries that feed into the lake. This project will occur when the lake is at its lowest level.

Project Effectiveness is a biannual survey of our 76 project sites. A survey is conducted with photo points to show the effectiveness of: Fish passage, stream fencing, and riparian plantings, in stream woody debris and road decommissioning.

Other annual projects the watershed monitors are; eagle/osprey nesting, purple martin nesting, aquatic weed inventories, purple loosestrife, and western pond turtle surveys.

The data produced by our various projects will be entered and stored in a project-computerized database established by the watershed council. It will also be shared with all participating state agencies after a written request has been submitted and all data and reports have cleared TLBP's review process. Members of the TLBP technical advisory committee, together with state agency staff, will analyze the data by comparing it to state water quality standards. The Monitoring Coordinator will write and distribute a final, year-end report by January of each succeeding year.

MAJOR TASKS	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
Algae Sampling						X	X	X	X	X	X	
Seasonal Ambient WQ Monitoring	X	X	X	X	X	X	X	X	X	X	X	X
Seasonal Lake Nutrient Sampling		X			X		X		X		X	
Lab Analysis		X			X	X	X	X	X	X	X	
Data Processing, Analysis, Reporting	X								X	X	X	X
Delta Building									X			
Project Effectiveness	X	X				X	X	X				X

7. Measurement Quality Objectives: Objectives

All data will be gathered and handled in accordance with the *Oregon Plan for Salmon and Watersheds* "Water Quality Monitoring Guide Book" chapter 4 page 23. TLBP will use parameters set at data quality level B from DEQ's Data Quality Matrix.

Matrix	**Parameter**	**Precision**	**Accuracy**	**Measurement Range**

Water	Temperature	± 2.0 °C	± 1 °C	-5 to 35 °C
Water	PH	± 0.5 SU	± 0.5 SU	0 to 14 SU
Water	Turbidity	± 30% of Std. Value	± 30% of Std. Value	0 1000 NTU
Water	Dissolved Oxygen	± 1mg/l	± 1mg/l	1 to 20 mg/l
Water	Conductivity			

Data that falls outside these parameters will be rejected, and noted to allow us to refine our techniques. It is TLBP's hopes to get data quality within these parameters in the first year of sampling. It is always difficult to maintain stringent guidelines when working under tight budgetary constraints, and working with borrowed equipment. All nutrient and algal data will rely on the outsourced laboratories QA/AC parameters.

Precision: Duplicate sample results will be used to determine the precision of water quality measurements for each sampling event. If the results of the sample and duplicate sample fall out of the acceptable limits, the discrepancy will be noted, and the data will possibly be discarded. Accuracy for pH, turbidity, and Dissolved Oxygen will be determined by following DEQ's recommendations for equipment calibration. Temperature checks described in sections 15 and 16 will be used to determine the accuracy of temperature measurements. Project monitoring will be conducted within parameters setup by OWEB. All wildlife surveys will follow ODFW guidelines.

Representativeness: For the purpose of this project, representativeness will depend on the parameter being monitored. For the parameters of dissolved oxygen, pH, and turbidity, samples will be collected at or near the center of the stream channel where the water is well mixed and most representative of the ambient conditions. In lake samples, these parameters will be collected with the same procedure used to sample the water column for algae and nutrients.

Comparability: This monitoring program will ensure comparability with similar projects by following the standardized sampling protocols and procedures developed by state agencies, or by the Watershed. These protocols are described in detail in the Oregon Watershed Enhancement Board (OWEB) **Oregon Plan for Salmon and Watersheds Water Quality Monitoring Guidebook.**

Completeness: It is anticipated that samples will be collected from at least 90% of selected sites during all sampling events unless unanticipated weather-related events or safety issues prevent sampling.

8. Training Requirements and Certification:

All data gatherers and processors have received or will receive training from DEQ or other persons trained by DEQ or from Watershed staff. Contact Steve Hanson at the DEQ lab for additional information or training at (503) 229-5449.

9. Documentation and Records:

This QAPP will be submitted to Tenmile Watershed Council members, and to each person on the technical advisory committee. The QAPP will also be posted on the watershed's website, **tlbp.presys.com**, and will also be available at the watershed's office. It is the responsibility of the Monitoring Coordinator to revise the QAPP and to send out the updates to all interested parties. All laboratory results will be stored at TLBP's office and will be included in the yearend report. Separate field data sheets for ambient monitoring will be maintained at the watershed office for each sampling event during the sampling season. See Attachment #2 for examples of monitoring field data sheets. All data will be archived at the watershed office for 5 years and then transferred to data CDs for permanent storage in TLBP's archives. Information recorded on data sheets is to include: Project name, data and time of sampling events, water body name, basin name, general weather conditions,

names of field staff, time of each sample or measurement, results, equipment ID numbers, and precision and accuracy classifications (see section 7) for each piece of equipment. Field staff will also maintain data sheets for each study for all pertinent field observations during the sampling season.

Logbooks will be kept for the pH, and turbidity meters. Detailed records of calibrations and checks against standards will be kept in these logbooks. Logbooks will be stored with the individual meters.

Duplicate samples for D.O., Ph, and turbidity will be done at a rate of one duplicate for every ten samples. Deviations greater than allowable data quality limits will be noted and the problem will be identified, noted, and resolved. This data will be flagged and possibly eliminated from the data set. Duplicate algae samples will be taken and archived for later comparison if necessary.

For continuous temperature monitoring, separate data sheets will be in the office for the duration of the sample season (June 1 to October 15 (±)) for each continuous temperature logger. Logger used in year round temperature monitoring will be replaced and downloaded every four months. For and example of typical data sheet, see attachment #2 at the end of this document. Information to be recorded on these data sheets should include: project name, logger ID number, data filename, site name and location (latitude/longitude, or UTM), logging interval, start and end date of monitoring period, pre- and post-deployment accuracy check results, and field audit results. DEQ data submittal forms will also be used for transfer data to LASAR database.

Project Effectiveness Monitoring data will be entered in TLBP's, OWEB approved data sheets. OWEB methods can be found on it's website **www.oweb.state.or.us**. Examples these data sheets, see attachment #2.

All other project data will be entered in data sheets created in either word or excel. Laboratory results will be entered in site specific spreadsheets, and hard copies will be stored in readily accessible files for 5 years and then transferred to data CDs for permanent storage in TLBP's archives.

A comprehensive database, available through state agencies or developed by the TLBP, will be used to store all data resulting from this project. All data will be reviewed by: 1. Monitoring Coordinator, 2.TLBP Monitoring Committee, 3. Tenmile Lakes Watershed Council, 4. Watershed Coordinator. After this review process, data will be shared among participating staff personnel, agencies, volunteer groups and interested private citizens with written request. A final report will be available in January of the following year. The final report will be available at the TLBP office, as well as the watershed's website, **tlbp.presys.com**.

As the scope of TLBP's monitoring program expands, the QAPP will be revised by the Monitoring Coordinator to include any new monitoring projects.

10. Sampling Process Design:

This Water Quality Monitoring Project consists of seven parts: 1) ambient baseline water quality monitoring, 2) continuous temperature monitoring, 3) algae sampling, 4) nutrient sampling, 5) Delta Building 6) Project Effectiveness, & 7) Winter monitoring. Monitoring Coordinator and the watershed field crew will conduct all sampling.

Baseline Water Quality Monitoring

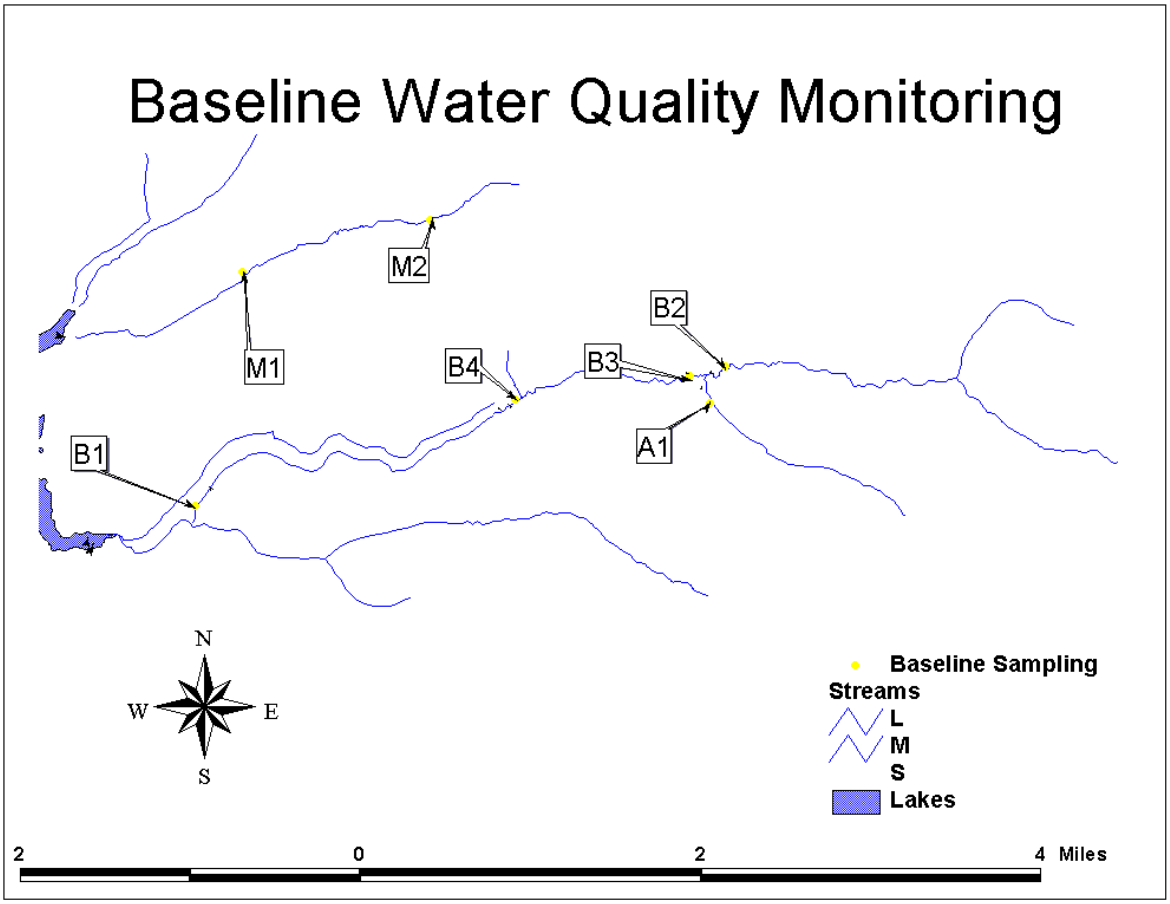
Objective:

1. *To monitor water quality in stream reaches with different land use areas.*

With in Tenmile Lakes Watershed, we have a myriad of land use activities along our tributaries. The goal of this monitoring is to gain a baseline water quality data set between two streams with different land use activities. Murphy Cr. is a tributary that is a freshwater wetland with timber harvest occurring on the adjacent hills. Big Cr. is a tributary with both agricultural and timber harvest operation. Monthly samples or field measurements will be collected at the sites listed in the table below for temperature, dissolved oxygen, pH, specific conductivity, and turbidity. This study is also referred to in the winter monitoring portion of this QAPP. During these monthly sampling visits,

TLBP will try and coordinate the sampling to occur at the same time, at each site, every month. The continuous temperature loggers will be replaced and downloaded every four months. These sites have been selected according the recommendations in Chapter 3 of the OWEB Monitoring Guidebook, with consideration to access to sites and landowner approval. An individual ID number, site description and UTM will identify the sites for this study. See map listed with Temperature Monitoring for site location.

Site ID #	Site Name/Location	Lat/Long
M1	Murphy Lower	43.6/124
M2	Murphy Upper	43.623361/124.07817
B1	Big Cr. Bridge	43.598705/124.09608
B4	Big Cr. Dam Pool	43.608507/124.06136
B3	Big Cr. Riffle	43.611724/124.04059
B2	Big Cr. Upper	43.611724/124.04059
A1	Alder Fork	43.6114/124.039



All field sampling work will be collected according to the protocols and procedures described in the OWEB Monitoring Guidebook. Field data sheets will be completed for each sampling event and field staff will complete data sheets for recording observations and other information pertinent to the project. A consistent monthly sampling will be the normal monitoring schedule, unless weather or other environmental conditions create unsafe conditions for field staff. If conditions do prevent the field staff from conducting a sampling event, they should notify the Monitoring Coordinator as soon

as possible, record the current conditions in the project notebook, and re-schedule the sampling event for the earliest possible date.

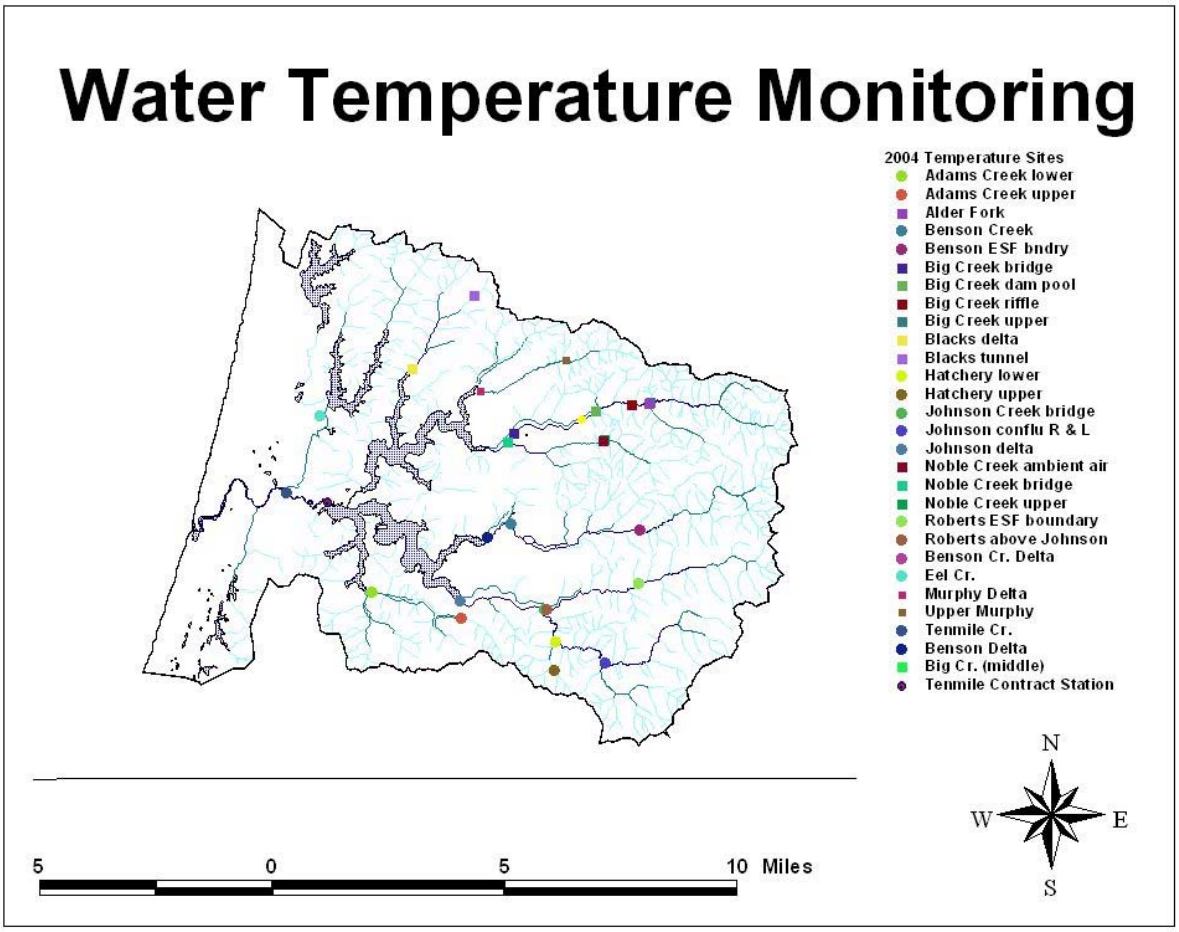
Temperature Monitoring

Objective:

1. *Develop a long-term temperature database for stream reaches.*

The temperature-monitoring network is designed for the purpose of collecting water temperature data from June 1st through Oct. 15th (±). Temperature data will be collected using continuous recorders (Vemco Temps), set at 30-minute intervals, and deployed at the sites shown in the table below. Monitoring sites have been or will be selected according to the recommendations described in Chapter 3 of the OWEB Monitoring Guidebook.

Site ID #	**Site Name/Location**	**Lat/Long**
1	Eel Cr.	43.606212/124.17707
2	Blacks Delta	43.621291/124.13934
3	Upper Blacks Cr.	43.6/124
4	Murphy Lower	43.6/124
5	Murphy Upper	43.623361/124.07817
6	Big Cr. Bridge	43.598705/124.09608
7	Big Cr. Middle	43.608011/124.06434
8	Big Dam Pool	43.608507/124.06136
9	Big Cr. Riffle	43.611724/124.04059
10	Big Upper	43.533971/124.09732
11	Alder Fork	43.6114/124.039
12	Noble Bridge	43.596615/124.09817
13	Noble Upper	43.599466/124.05742
14	Noble Ambient air	43.599466/124.05742
15	Benson Cr. Delta	43.567521/124.10546
16	Benson Cr. Bridge	43.618142/124.09672
17	Benson Cr. ESF boundary	43.572345/124.0405
18	Johnson delta	43.546923/124.11505
19	Johnson Bridge	43.545969/124.07938
20	Johnson Conf R&L	43.530306/124.05267
21	Roberts above Johnson	43.5444/124.078
22	Roberts ESF	43.555673/124.03895
23	Adams Lower	43.549/124.153
24	Adams Upper	43.5/124
25	Hatchery Lower	43.535/124.074
26	Hatchery Upper	43.530413/124.07692
27	Tenmile Cr.	43.5/124
28	Tenmile Contract Station	43.5/124



Continuous temperature loggers will be checked for accuracy before and after field deployment according to the procedure outlined in Chapter 6 of the OWEB Water Quality Monitoring Guidebook. In addition, the field installation procedures also described in Chapter 6 will be followed. Loggers will be set to record a data point every thirty minutes. After temperature loggers have been deployed, field staff will conduct independent field audits after deployment, at least once a month during the monitoring season, and just before removal from the field at the end of the season. The procedure for conducting a field audit on continuous temperature loggers is described in the section "Field Checking Instrument Performance" of Chapter 6 of the OWEB Monitoring Guidebook. A separate field data sheet will be maintained for each logger for recording the results of the accuracy checks and field audits.

<u>Algae Sampling Program</u>

Objective:

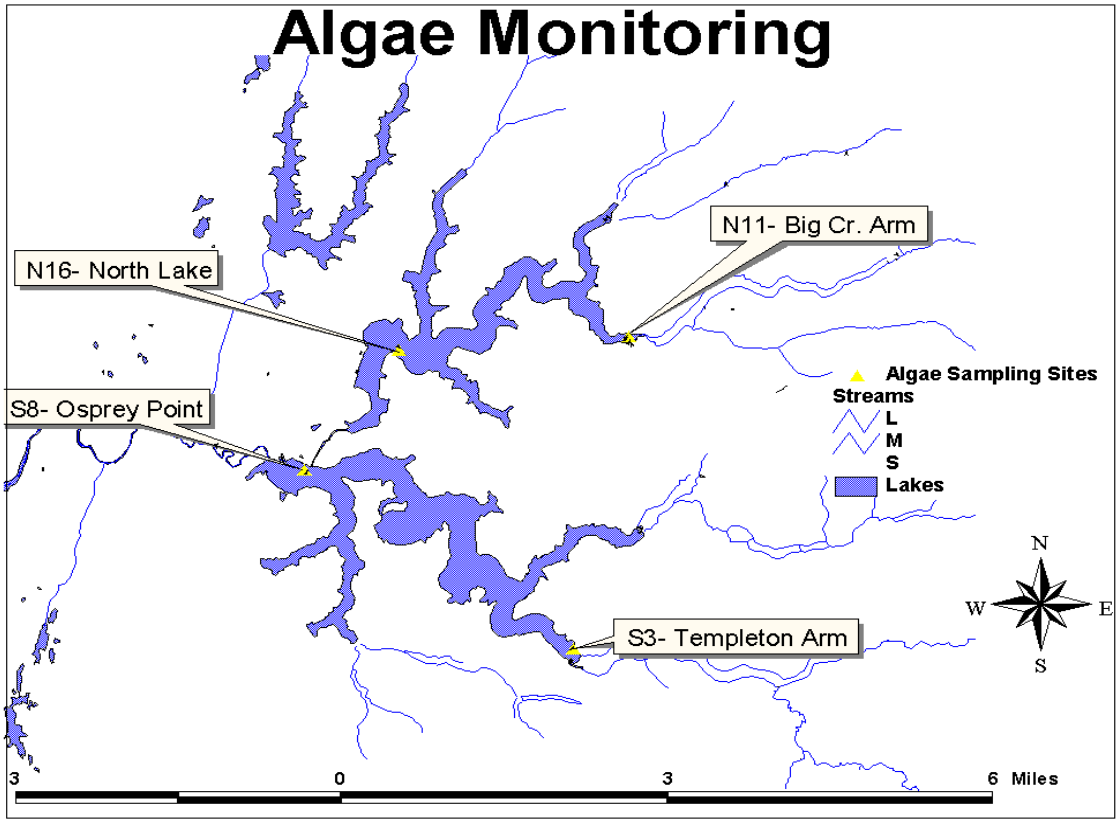
1. *Determine species composition and the lake conditions (i.e. temperature, nutrients present, weather) that influence algal blooms in the Tenmile Lakes system.*

A team of 2-3 field staff will collect water samples at the sites listed in the table below. Nine sampling events will be scheduled:

June- 1x
July-2x
August-2x
September-2x
October-1x
November-1x

Algae sampling will follow TLBP algae sampling protocol (Attachment #1). Water samples will be sent to a laboratory for algal taxonomy and toxin analysis. The algae samples are used to monitor species composition over time. Sites selected are a subset of sites that were created by a consulting firm hired to do a comprehensive nutrient and algae study in 1999 and 2001. Monitoring of algae has continued at the four lake sites to monitor toxic algal blooms during the summer months. These sites give us a representative sample of the lakes. S3 is in a shallow arm of the lake with little lakefront development, and agricultural lands along the tributary that feeds into this site. S8 is located on the south lake, in front of the canal that connects the two lakes. N11 is in a shallow arm with a high density of lakefront development with agricultural land use along the tributary that feeds into this site. N16 is a deep-water site located on the main body of the north lake. Sampling is increased during mid to late summer due to increased algal blooms, with more intensive sampling occurring when toxic algae is present in quantities >2000 cells/ml.

Site ID #	**Site Name/Location**	**Lat/Long**
S3	Templeton Arm/ South Lake	Will be added this summer
S8	South Lake Canal	
N16	Middle of North Lake	
N11	Big Creek Arm/ North Lake	



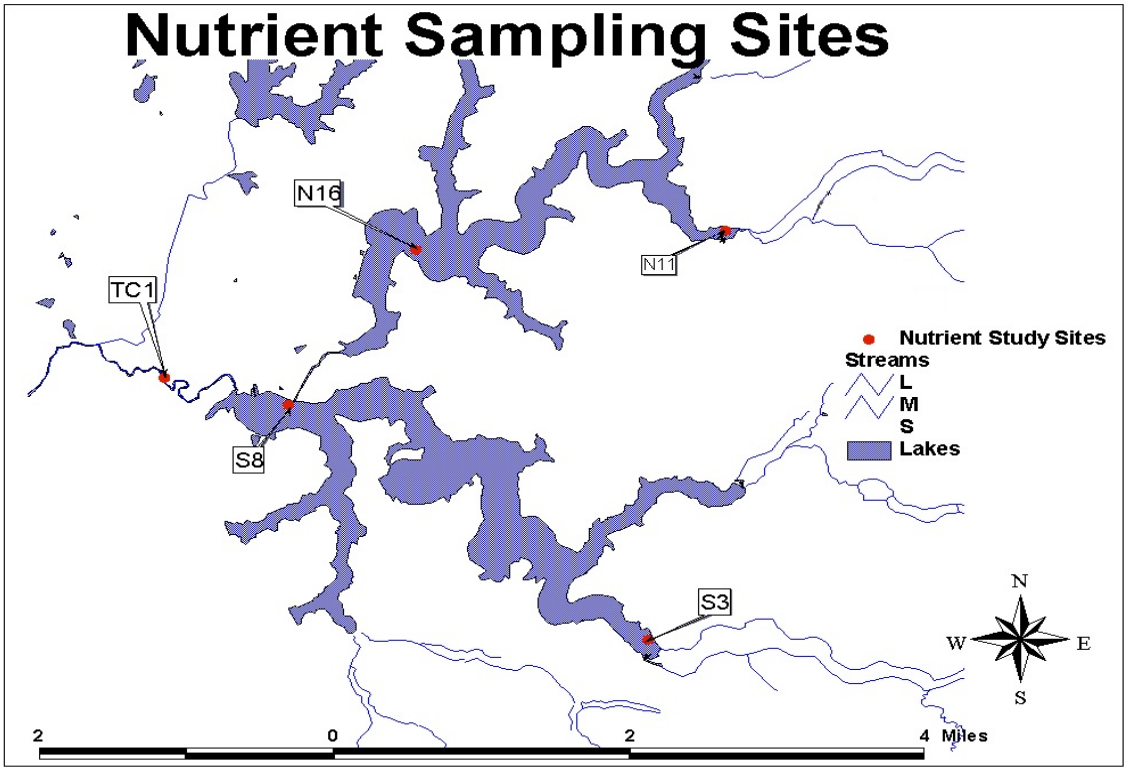
Nutrient Sampling Program

Objective:

1. *To collect and analyze water samples to determine trends and which nutrients algal blooms utilize.*

   A lake nutrient sampling program will be implemented to monitor the seasonal changes in: PO4, NO2, NO3, NH4, Si (OH), Chl a, and Total Phosphorus. Sampling will occur in May, July, September, November, and February. Due to budgetary constraints, the watershed can only afford 5 sampling events. It is understood by the watershed that our data set will have glaring gaps. By sampling in this fashion, it is our goal to show a baseline nutrient trend. This data will then be interpreted with weather and lake conditions obtained by our lake gauge station. When more funding becomes available, the watershed will go to a monthly sampling regime, sampling both top and bottom to get an accurate nutrient analysis. Nutrient sampling will be done concurrently with algae sampling in the months of July and September to monitor any uptake in nutrients from algae growth. Sampling will follow TLBP's nutrient sampling protocol (Attachment #1). The current list of sampling sites are listed below.

Site ID #	Site Name/Location	Lat/Long
S3	Templeton Arm/ South Lake	Will be added this summer
S8	South Lake Canal	
N16	Middle of North Lake	
N11	Big Creek Arm/ North Lake	
TC1	Tenmile Cr.	

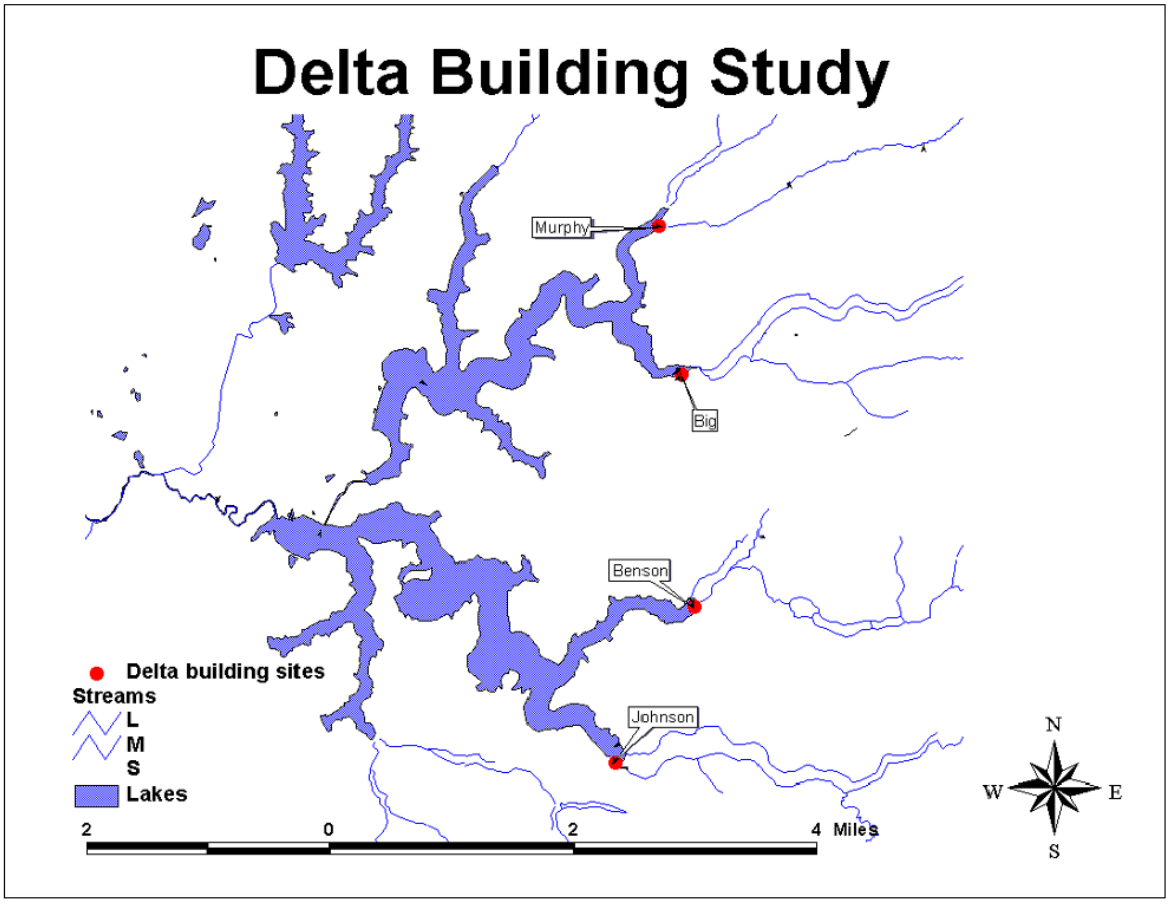


All nutrient samples will be sent to an outside laboratory for nutrient analysis. See attachment #1 for protocols and data sheet.

Delta Building

Objectives:

1. *To track sediment accumulation in Tenmile Lakes.*
   Since the late 1940's, Tenmile Lakes has seen a sharp increase in sediment accumulation at the mouths of the tributaries that feed the lake. To monitor this sediment accumulation, TLBP plans to go out to selected sites when lake levels are at there lowest, and using a laser level, survey the delta along transects on the delta. The delta will also be measured for length, and width at the transect points. We can then get a 3-dimentional map of the delta. Every succeeding year, a survey team will go out at the same lake height, and re-survey the sites. Sites are Benson Cr., Johnson Cr., Tributary A on Big Creek Arm, and Murphy Cr.

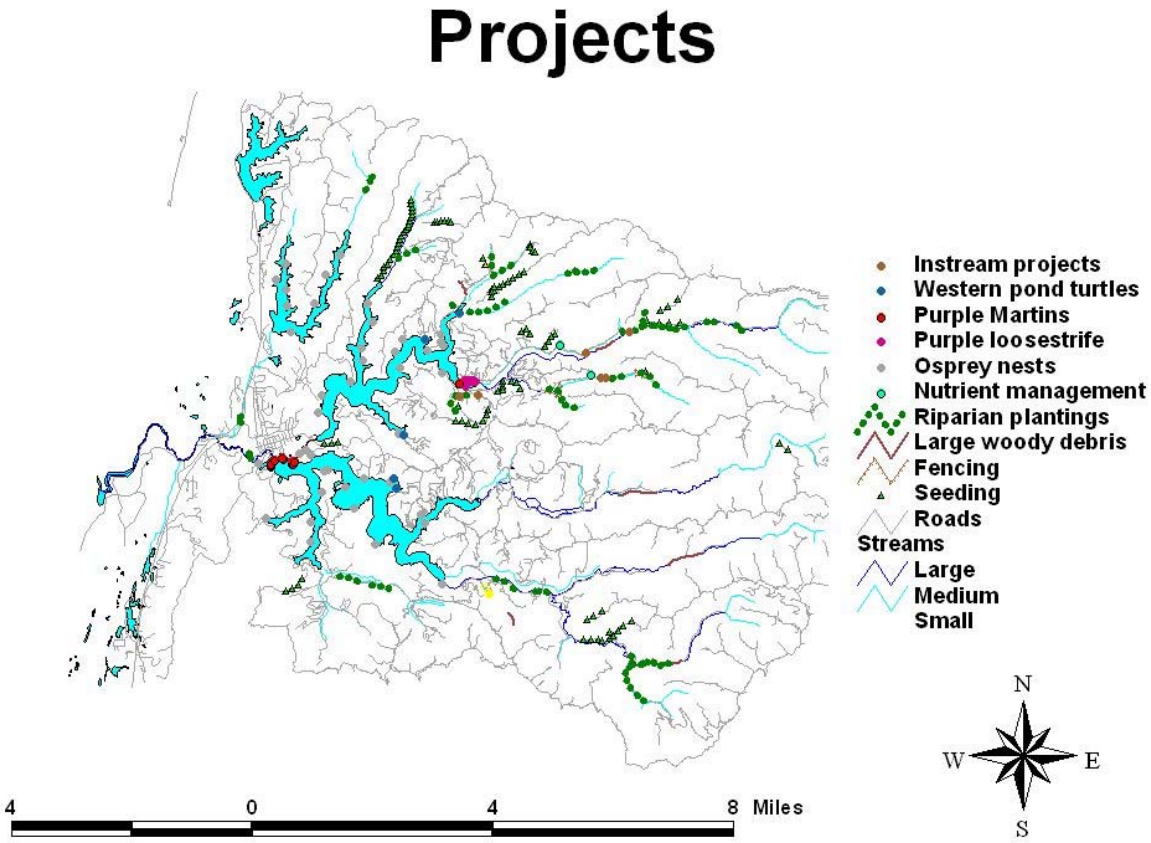


Project Effectiveness

Objectives:

1. *Evaluate and monitor OWEB funded project to fulfill contractual obligations.*

This is a bi-annual survey of our projects; fish passage, riparian fencing, riparian planting, in stream woody debris, and road decommissioning. These surveys involve visiting a photo point to record current status of the project with a camera, and filling out a monitoring data sheet (see attachment #2). Other projects that are on an annual or two year rotation include; purple loose strife, purple martin nesting, eagle/osprey nesting, and western pond turtle surveys. All of these projects follow guidelines set-up by TLBP and ODFW.



Winter Monitoring

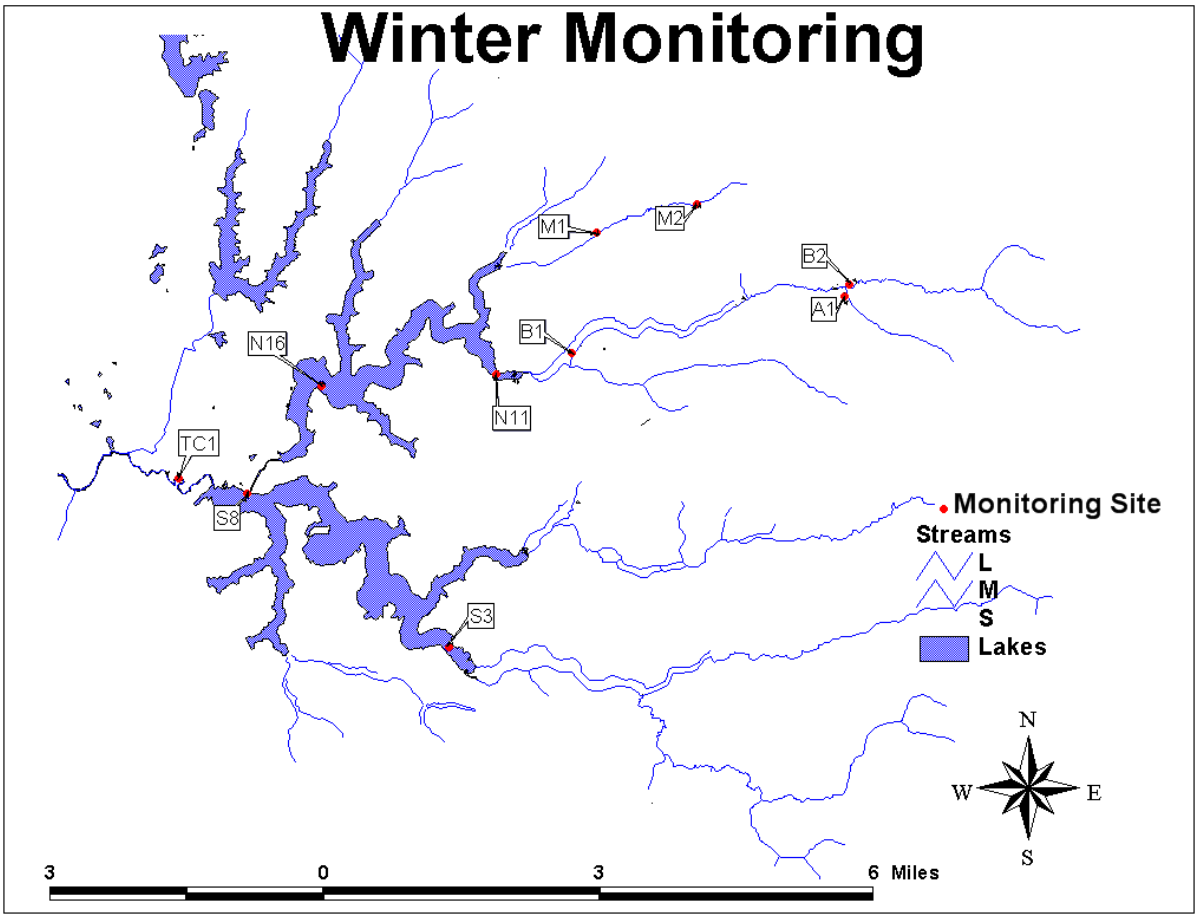
Objectives:

Monitor water conditions on two creek systems, Big Cr. and Murphy Cr., and at predetermined lake sampling sites to determine water quality conditions during the winter months.

During the winter season, vemcos will be placed on Big and Murphy creek. Continuous temperature monitoring devices will be replaced and downloaded every four months. These sites will be visited every two weeks, and samples for D.O., Turbidity, and pH will be taken. The vemcos will be audited according to the protocols established by OWEB. In addition, water samples will also be taken from sites on the lake, as well as at Tenmile Cr. The sample schedule will be every two weeks with each site being sampled with in a specific time of day over the season. Through this sampling regime, TLBP hopes to understand how water conditions are affected by winter storm runoff. These samples will include pH, D.O., specific conductance and Turbidity.

Site ID #	Site Name/Location	Lat/Long
M1	Murphy Lower	
M2	Murphy Upper	43.623361/124.07817

B1	Big Cr. Bridge	43.598705/124.09608
B2	Big Cr. Upper	43.533971/124.09732
A1	Alder Fork	43.6114/124.039
TC1	Tenmile Creek	



11. Sampling Method Requirements:

Sampling will be accomplished using the standard protocols described in the OWEB Water Quality Monitoring Guidebook and TLBP protocols (attachment #1) for lake sampling. DEQ, TLBP, and laboratories that analyze nutrients and algae have provided the monitoring equipment for this project. Field measurements will be recorded immediately after the sample is collected. Continuous temperature loggers will sample at 30 minute intervals with a maximum of four months in the field before being replaced and downloaded. The only laboratory analysis required will be for algae taxonomy/toxicology and nutrient analysis. Any problems discovered with sampling methods will be resolved by the monitoring coordinator and the technical advisory committee and any laboratory that is associated with the parameter being sampled. The table below lists the equipment used for each water quality parameter:

Matrix	Parameter	Equipment	Container	Preservation	Holding Time
Water	Temperature	NIST Traceable Thermometer	Instream	None	Immediately
Water	PH	Orion Model 210A Mtr.	500 ml poly	None	Immediately
Water	Dissolved Oxygen	HACH Digital Titrator	300 ml BOD btl.	Winkler Titr.	8 hr.
Water	Turbidity	HACH 2100P Meter	Screw top bottle	None	Immediately
Water	Conductivity	YSI Model 30 Meter	Instream	None	Immediately
Water	Nutrients	Tube Sampler	250ml bottles/ filter media	Frozen	Overnight to testing lab
Water	Algae	Tube Sampler	250/1000ml bottles	None/1% Lugols	Overnight to testing lab

12. Sample Handling and Custody Procedures:

Ambient water quality measurements will be taken immediately in the field after samples have been collected. Dissolved Oxygen samples, labeled with the site id, will be preserved in the field and will be analyzed when sampling crew returns from the field. Continuous temperature loggers will stay in the field for a maximum of four months before being replaced and downloaded. Algae samples are immediately packaged in a cooler with frozen gel packs and sent next day air to aquatic taxonomist and aquatic toxicologist. Chain of custody forms are filled out and distributed accordingly. All of these steps are clearly outlined in TLBP's Algae Sampling Protocol. Nutrient samples are portioned and filtered accordingly and are frozen overnight to arrest biological activity. Samples are then shipped next day air to a nutrient sampling laboratory for analysis. Again, all of these steps are clearly outlined on TLBP's Nutrient Monitoring Protocol (attachment #1).

13. Analytical Methods Requirements:

All parameters are measured using the protocols previously mentioned in section 11 above. Dissolved Oxygen samples will utilize the Azide Modification of Winkler Method described in the Hach Digital Titrator operations manual. Other data aqusition methods can be found in the sampling protocols (see attachment #1). Nutrient and algal analysis is outsourced to independent laboratories. See attachment #3.

Parameter	Equipment/Method	Container	Preservation	Holding Time
Water Temperature: *single*	NIST Traceable Thermometer	Instream or bucket	none	immediately
Water Temperature: *continuous*	Vemco data logger	Instream	none	N/A
Dissolved Oxygen	HACH OX-DT Kit	300 ml BOD btl	Winkler Titration	8 hr.
Conductivity	YSI Model 30 Meter	Instream or sampling bucket	none	immediately
Turbidity	HACH 2100P Meter	Screw top bottle	none	immediately
Total Phosphorus	EPA 365.3	125 mL plastic bottle	Acidified to pH <2; stored < 4° C	28 days
Nitrate-Nitrite-N	EPA 353.3	125 mL plastic bottle	Acidified to pH <2; stored < 4° C	28 days
pH	Orion 210A Meter	Instream or sample bucket	none	24 hours

14. Quality Control Requirements:

Duplicate quality assurance (QA) samples for all measurements will be taken at a minimum of 10% of the total number of monitoring sites (1 duplicate for every 10 sites) during each sampling period (i.e. monthly for pH, turbidity, nutrient sweep, total phosphorus, chlorophyll a, and D.O.). Duplicate algae samples will be taken and archived at the TLBP office. The field team will check the continuous temperature loggers for accuracy before and after each field deployment and they will conduct independent field audits, using and NIST traceable thermometer or equal, once a month. Any sampling problems that arise for quality control checks will brought to the immediate attention to the Monitoring Coordinator. The monitoring coordinator will meet with the Technical Advisory Committee to resolve the sampling problems.

Precision and accuracy classifications will be assigned as described in section 7. When precision and accuracy classifications differ for a measurement, the lower classification will be chosen to define the overall data quality level (DQL) of the measurement.

15. Instrument/Equipment Testing, Inspection, and Maintenance Requirements:

All field monitoring equipment will be tested for accuracy and /or calibrated in accordance with the procedures outlined in the appropriate chapters of the OWEB Water Quality Monitoring Guidebook and the manufacturer's user's manuals. The NIST Traceable Thermometer will be returned to the DEQ for an annual accuracy check. DEQ will complete the accuracy check and re-certify the thermometer as NIST traceable. Any faulty instruments will be returned to DEQ for replacement, and any faulty equipment will be repaired or replaced by TLBP. All equipment and supplies are stored in TLBP's archive room. Supplies are checked on a regular basis to allow for successful data acquisition. Protocols (attachment #1) begin with field crews making sure all equipment and supplies are in working order before the sampling event occurs. Spare parts stored at TLBP include: batteries, nets, 250 ml bottles, 1000ml bottles, 100 and 1000 ml graduated cylinders, VEMCOs, thermometer, Ph probe, Ph calibration buffers, D.O. titration chemicals, nutrient filters, syringes, and buckets. If equipment failures occur, protocols listed in manufacturers operation manual will be followed. If the equipment cannot be repaired by the watershed staff, it will be sent to the manufacturer for repair, or to DEQ laboratory for replacement.

16. Instrument Calibrations and Frequency:

The pH meter will be calibrated (Two Buffer Calibration) prior to use according to method described in manufacturer users manual. The Hach 2100P Turbidimeter will be re-calibrated with formazin or Stablcal standards quarterly. Daily accuracy checks with field standards will also be done prior to collecting any field measurements. There is no calibration for the Hach DO Digital Titrator. However, split samples will be performed periodically with DEQ staff to check the accuracy of the field kit. The NIST Traceable Digital Thermometer is calibrated at the factory and will be returned to the local DEQ office to check for accuracy. If the thermometer fails, it will be returned to the factory for recalibration. Continuous temperature loggers are factory-calibrated, and they will be checked for accuracy by the using warm and cold water baths as described in OWEB's Water Quality Monitoring Guidebook. The staff at TLBP will perform calibration.

17. Inspection/Acceptance Requirements:

The specific pieces of monitoring equipment that will be used to collect data for this project by the watershed include, but are not limited to: Hach 2100P Turbidimeter, Hach DO Digital Titrator, NIST Traceable Digital Thermometer, Orion Model 210A, VEMCO mini-loggers, Nalgene 25mm .45micron pore size filters, Whatman GF/F 25mm filters, syringes, syringe filter holder, Wisconsin plankton net, tube sampler, Secchi disk, wide and narrow mouth 60 ml bottles, 1000ml bottles, 300ml BOD bottles, 250ml bottles with 1% lugols, 100 and 1000ml graduated cylinders, Garmin76 GPS, laser level, and a Fuji Finepix S3100 digital camera. This equipment has been loaned to, or given to, TLBP by Oregon DEQ, testing laboratories, or was funded through a grant from OWEB. The TLBP

will be responsible for maintaining the equipment and restocking all field supplies when necessary. The names and telephone numbers of vendors and/or manufacturers' representatives are available upon request to the DEQ Laboratory Volunteer Monitoring Coordinator (503) 229-5449. All algae and nutrient sampling equipment will be provided by TLBP and contracted laboratories. If the appropriate supplies are not available for sampling, the corresponding parameter will not be sampled until the sampling equipment is available.

18. Data Acquisition Requirements:

U.S.G.S. 7.5 minute topographic maps will be used to identify site locations, land-use activities, and landscape features. Hand-held GPS units will be used, when available, to collect latitude and longitude readings on site.

Data acquired from outsourcing laboratories will have submitted acceptable QA/QC protocols (see attachment #3). Data from samples sent to these sources will be compared to other data collected during the sampling event to see if any abnormalities influenced the data.

19. Data Management:

Field crews will either take data sheets into the field, or use waterproof notepads to collect information (see attachment #2 for types of data collected). If notepads are used, all data will be transferred to the appropriate data sheet before returning from the field. The Monitoring Coordinator and field crews will check all field data sheets for completeness and accuracy at the end of each field day. Errors will be corrected prior to delivering the data sheets to the project manager and/or data manager. All data, including metadata, will be entered into excel spreadsheets or word documents designed for this project by the watershed council. In the event that statistical analysis is required, Systat or Statistica will be used. This database will be compatible with hardware and software used by state water quality agencies. This data will be backed up on CD-RWs and stored in the TLBP office. As required by the project QA/QC program, all data will be examined and evaluated again by a second review person from the Technical Advisory Committee. The data will then be made available to DEQ, in December of that year, depending on when the data review is completed. Data will be submitted to DEQ through a spreadsheet created by DEQ's Volunteer Monitoring Coordinator.

20. Assessment and Response Actions:

The Monitoring Coordinator and the Watershed Coordinator will be responsible for reviewing the entire Monitoring Project on a regular basis. The Monitoring Coordinator will also receive guidance and advice from state agencies. The Monitoring Coordinator will coordinate the training of all volunteers before any monitoring activities are done, and schedule refresher training sessions as needed.

All field activities may be reviewed by state agency QA staff at the request of the Watershed Coordinator. Data quality audits will be performed by the Monitoring Coordinator once a year, and any/all identified procedural problems will be corrected based on the recommendations by the Monitoring Coordinator.

21. Reports:

Project reports will be developed through a joint effort by the field monitoring team, the Monitoring Coordinator and the Watershed Coordinator. The reporting process will begin after the end of the field monitoring season and final reports will be ready for distribution by January of the succeeding year. Reports will be submitted to Tenmile Lakes Watershed Council, and will be made available to state agencies and the general public whenever such reports are requested, after it has cleared TLBP's review process. Reports will include the data results, data analysis and interpretation, pertinent field observations, QA/QC assessments.

22. Data Review, Validation, and Verification:

The Monitoring Coordinator, and the Watershed Coordinator will review all data resulting from this project and committee members will review all data resulting from this project to determine if it meets the QA Plan objectives. At the discretion of the watershed council, state agency staff may be asked to review and comment on the data. Decisions to accept, qualify or reject data will be made by the Monitoring Coordinator, Watershed Coordinator, and Watershed Counsel.

23. Validation and Verification Methods:

As required by the project QA Program, duplicate samples will be collected at a rate of 1 duplicate per 10 samples collected. This will ensure data that is used falls within the data quality levels. The Monitoring Coordinator will verify the accuracy of the data through regular accuracy checks. Data that continues to be outside the quality levels will be further investigated to determine the cause, using alternate methodology, if available. Duplicates of algae samples will be archived, and retested if any abnormalities occur. During nutrient analysis, a duplicate of each parameter sampled will be taken to monitor field sampling technique and accuracy.

Once the data has been entered in the project database, the Monitoring Coordinator will print a paper copy of the data and proofread it against the original field data sheets. Data will be entered in a computer database on a weekly basis. Errors in data entry will be corrected at that time. Outliers and inconsistencies will be flagged for further review or be discarded. Data quality problems will be discussed as they occur and in the final report to data users.

24. Reconciliation with Data Quality Objectives:

As soon as possible after each sampling event, calculations and determinations for precision, completeness, and accuracy will be made and corrective action implemented if needed. Data may be discarded and re-sampling may occur. The cause of the failure will be evaluated. If the cause is found to be equipment failure, calibration and/or maintenance techniques will be reassessed and improved. If the problem is found to be sampling team error, team members will be retrained. Any limitations on data use will be detailed in both interim and final reports, and other documentation as needed. If failure to meet project specifications is found to be unrelated to equipment, methods, or sample error, specifications may be revised for the next sampling season. Revisions will be submitted to state agencies for review and/or approval.

Attachment #1

TLBP Lake Sampling Protocol

Office Tasks

1. Check sampling date on the program-sampling schedule. Check schedule to see if nutrient sampling need to be done.
2. Check operations of Turbidity, and Ph meters. Make sure the batteries are good and are operating properly. Stock an extra set of batteries
3. Calibrate meters. Consult user manual. Turbidity meter needs to be calibrated every 3 months, Ph everyday.
4. Get a 4-300ml BOD bottles and chemicals need to fix the water sample for D.O. analysis back at the office.
5. Gather and label 4 liter, and 8 250ml bottles for algae sampling. If nutrient sampling is to be done, check nutrient protocols for how many extra bottles will be needed.
6. Check equipment and supply checklist for sampling tasks. Confirm that the sampling equipment and supplies are on board the boat.
7. Check boat protocol, nutrient protocol, and equipment list.

Lake Sampling (algae)

8. Position the boat at the designated sample site. Locate using gps or shoreline landmarks. Anchor the boat, and allow too stabilize. Make sure not to stir up sediment.
9. Complete observations portion of the sampling form. Record the lake and site name, date, and time of sampling and name of samplers.
10. Measure temperature at site and record.
11. Obtain and record Secchi measurement at each site.
12. Calculate 3x the Secchi measurement and record.
13. Lower plankton net to 3x Secchi and gently haul the net to the surface.
14. Empty contents into a well rinsed bucket. Clean net so that the entire haul is in the bucket.
15. Repeat 2 more times and combine contents of all 3 hauls into the bucket. Record the total volume of the sample into the bucket (this number is essential).
16. Place 100 mls in 250 ml opaque sample bottle containing 1% Lugol's preservative. This sample will be shipped to plankton taxonomist Jim Sweet of Aquatic Analysts, who will perform microscopic analysis for *Microcystis* and *Anabaena* density (cell ml –1).
17. Place the rest of the bucket contents in a labeled 1 liter bottle, and record, with no preservative to be shipped overnight to the laboratory of Dr. Wayne Carmichael At Wright State University who will analyze them for microcystin and anatoxin-a*.

It is essential for these samples that the number of hauls (which will be 3 unless otherwise discussed), the length if each haul (depth), and total volume of the sample be recorded on both the sample bottle label and the "Chain of Custody" form.

18. In addition, at each station, 3 1-meter hauls of the "tube sampler" will be deposited in a bucket and a 100 ml sub sample placed in a 250 ml opaque sample bottle. These samples will be archived.
19. Take ph measurement at site and record. Use water from at least .5m below the surface.
20. Take a 100ml sample from the bucket for and place in sample cell for the turbidity meter. Make sure the contents of the bucket have not settled. If so, obtain another sample from the "tube sampler". Follow turbidity meter's directions and then analyze and record on the data sheet.
21. Take a water sample and place in a 250 ml bottle. Fix water sample with the procedures listed in the Hach digital titrator user manual so this sample can be analyzed for D.O. back at the office.
22. Before leaving site, check the data sheet for accuracy.
23. During the months of May, July, September, November, and February, nutrient samples will be taken. Follow nutrient sampling protocol sheet for these months

Getting the algae samples out same day is priority number one.

Nutrient Sampling Protocol

Nutrient Sweep Sample

Materials:
Sample bottles (60 ml HDPE) Narrow mouth
60 ml syringes
Syringe filters (surfactant free cellulose, 25mm, .45micron pore size, Nalgene)

Procedure

24. Rinse sample bottle and cap with deionized water twice.
25. Remove the plunger from the syringe and rinse the syringe with sample water twice.
26. Fill the syringe fully with sample water...insert plunger.
27. Invert syringe and expel the air bubble.
28. Attach a filter to the syringe; filter about 5ml of sample into sample bottle to rinse.
29. Filter about 45-50 ml of sample into the nutrient bottle…the bottle should be no more than 2/3 full. **Do not overfill the bottle!!! Water expands when frozen and if the bottle is too full, the ice will force its way out and take the nutrient ions with it.**
30. Secure cap on bottle and put on ice until sample can be frozen. Allow sample to freeze overnight before shipping.
31. Discard filter

Total Phosphorus Sample

Materials:
60 ml syringes
Wide-Mouth Polypropylene (PP) 60ml bottles
Sharpie Pen

Procedure

32. Take water sample directly into syringe (pre-rinse 2-3x w/sample)
33. Place 20mls into bottle.
34. Label with marker –DO NOT label with tape or paper. Bottles will be autoclaved and tape cannot withstand the temperature and pressure.
35. Secure cap on bottle and put on ice until sample can be frozen. Allow sample to freeze overnight before shipping.

Chlorophyll a Sample

Materials:
Clean bottles
Filtration apparatus
60 ml syringes
GF/F filters
10 ml screw top tubes
Forceps

Procedure

36. Rinse sample bottle and cap with deionized water twice.
37. Remove the plunger from the syringe and rinse the syringe with sample water twice.
38. Fill the syringe fully with sample water...insert plunger.
39. Invert syringe and expel the air bubble.

40. Place 25mm GF/F filter within the supplied filter holder and place on the end of the luer-lock syringe (remove plunger first). If you need to filter more than 60mls---filter 60 mls,

remove the filter holder, then remove the plunger, place the filter holder back on the syringe and continue filtering. **DO NOT REMOVE THE PLUNGER FROM THE SYRINGE WITHOUT FIRST REMOVING THE FILTER HOLDER. IF YOU DO, THE FILTER MAY BREAK.**

41. Filter a known volume of water through the GF/F filter-and record the filtration volume. Filter enough so that there is a small amount of color on the filter. As with any analysis that requires filtration, the more you can filter, the better, but you also do not want to clog the filter.
42. Ideally, water sample should be filtered right away. They can be filtered up to 24hours from the water sample being taken, but sample should be stored in the dark and chilled until filtered.
43. Take filter out for filter holder with forceps and fold in half and place in the supplied 10 ml screw top tubes.
44. Store in a freezer overnight. Tubes with sample filters should be shipped frozen.

Shipping Protocols (algae)

45. Take liter bottles for Dr. Wayne Carmichael and check for correct label information.
46. Fill out chain of custody form and make 3 copies.
47. Correctly label shipping containers to be sent to:

(1000ml) **(250ml)**
Dr. Wayne Carmichael **Jim Sweet Aquatic Analysts**
Wright State University **22 Acme Rd.**
Department of Bio. Sciences **White Salmon, Wa 98672**
3640 Colonel Glenn Hwy
Dayton, Oh 45435-0001

4. Separate cooler samples (1000ml) from box (250ml) Lugols preserved samples.
Place at least 2 frozen gel packs with cooler samples.

48. Place 1 copy of chain custody form (sealed in zip-lock bag) in each shipping container.
49. Fax a copy of chain of custody to Jacob Kahn (541) 552-1024.
50. Place original chain of custody form in file.
51. Make final check of sample containers & shipping labels.
52. Seal sample containers.
53. Get funds for shipping from City Hall (approx $80).
54. Samples are to be shipped **next day air** always.
55. Take samples to N.B. United Parcel Service, and ship **(Next Day Air)**. Samples must reach shipping office by 3pm. **KEEP RECIEPT AND COPY.**
56. Make sure all copies are placed in files for future reference.
57. Confirm with Jake that he received fax (541) 482-1575.
58. Collect UPS receipt, copy and file.

Shipping Protocols (Nutrients)

1. Take sample bottles and place in a freezer overnight.
2. Call Fed/Ex to schedule a pickup-up for the following day.
3. Place sample bottles in cooler with frozen gel packs.
4. Nutrient samples will be **shipped next day air** to:

Kathy Korgslund
Ocean Sciences Bldg, Room 346
University of Washington
1492 NE Boat St.
Seattle, WA 98195

5. Place datasheet containing amount of water filtered or placed into sample bottles in a zip lock bag and add to container
6. Make final check of sample containers & shipping labels.
7. Seal shipping container.

Samples must be shipped next day air and must be shipped out by Wednesday.

Stream Monitoring Protocol

1. These measurements will be done concurrently with the monthly vemco audits. See audit protocols for more information on vemco audits.
2. Review safety protocol before going into the field.

3. Check equipment list
4. Check oxygen, pH, temp, and turbidity meters. Make sure the batteries are good and the instruments are calibrated. Some instruments will have to be calibrated in the field.
5. When arriving at a site, write down date, time, and location on sample form.
6. When collecting a water sample, collect water away from stream bank, in the main current.
7. Try to disturb as little bottom sediment as possible. Try not to collect water that contains bottom sediment.

D.O.

1. Review use of Digital Titrator by reading manual.
2. Collect a clean sample in a 300 ml bottle. Allow the sample to overflow to insure air bubbles are not trapped.
3. Add contents of one Managous Sulfate Powder Pillow and one Alkaline Iodide-Azide Powder Pillow.
4. Immediately insert the stopper so air is not trapped in the bottle. Invert several times to mix.
5. Wait until the floc in the solution has settled.
6. Remove stopper and add the contents of one Sulfamic Acid Powder Pillow
7. Replace stopper and invert the sample several times to mix. The sample can now be stored for 4 hours if titration cannot be conducted in the field
8. Store sample in a dark place with a temp range of 10-20 C.
9. See titrator user manual to finish D.O.
10. Enter the D.O. mg/l on the data sheet.

PH

PH meter should be calibrated before use for that day.

1. Rinse the electrode well with deionized water.
2. Set in sample and wait a few minutes until the meter settle on a number.
3. Record temperature and pH in the appropriate column on the data sheet.

Temperature

1. Review stream sample procedures. Make sure to sample next to vemco site.
2. Place temperature probe at least 4 inches below the surface.
3. Allow the temperature reading to stabilize at a constant temperature reading
4. Record the temperature on the data field sheet.

A water sample can be collected in a liter container and brought back to the lab for turbidity and pH. D.O. must be fixed in the field or titrated in the field. Temperature, for obvious reasons, must be taken in the field.

Data sheet for this data will be separate from audit data sheet.

Attachment 2

Tenmile Lakes Basin Partnership Fax: 541-759-3711
P.O. Box L Ph: 541-759-2414
Lakeside, Or 97449 tlbp@presys.com

Contact: Dr. Jacob Kann: 541-482-1575
Mike Mader: 541-759-2414

Tenmile Lakes Algal Toxin Samples Chain of Custody

Location: Tenmile Lakes, Oregon_____

Sampled by: _____ Date and time: _____

Shipped by: _____ Date and Time: _____

Received by: _____ Date and Time: _____

Please circle below address (es) where samples were sent

Laboratory, please keep this form and return copy to Contact above with final data report(s) laboratories:

Wayne Carmichael, Mary Stukenberg	Jim Sweet
(937) 775-3173 and (937) 775-2714 wayne.carmichael@wright.edu mary.stukenberg@wright.edu	jwsweet@aol.com
Aquatic Biology/Toxicology Department of Biological Sciences Wright State University 3640 Colonel Glenn Hwy, Dayton, OH 45435	Jim Sweet Aquatic Analysts 22 Acme Rd. White Salmon, WA 98672-8201

					Record for Plankton Net Samples		
Date sampled	Time Sampled	Site Label	Anatoxin-a and Mycrocystin to be performed if cells >2000/ml	Sample Type G= Grab P= Plankton Net	Haul Length	Number of hauls	Final Volume of Sample (ml)*

*Note: This is the total volume of the 3 combined net hauls in the bucket –before taking sub-sample for preservation. This is not the total volume of water filtered.

Tenmile Watershed Riparian Monitoring Form.

Monitoring Date:

Monitoring Personnel:

Project Name.	
Project Goals.	
Project location and site number.	
Implementation date.	
Average seedling height at time of implementation.	
Current average seedling height.	
Canopy closure and shade %.	
In-stream temperature at riparian site.	
Predation observations.	
Plant communities surrounding riparian project.	
Maintenance records/<u>dates.</u> **Comments.** **Goal Observations**	

<u>Tenmile Watershed Culvert Monitoring Form.</u>

Monitoring Date:

Monitoring Personnel:

Project Name.	
Project Goals.	
Project location and site number.	
Current Sediment Delivery. High-Med-Low	
Current Culvert Condition.	
Current Outlet Drop. (Inches)	
Current inlet diversion. %	
Erosion Percent at Inlet and Outlet.	
Sediment Depth in Culvert. (Inches)	
Maintenance dates/records. Field observations. Goal Observations	

Tenmile Watershed Bridge Monitoring Form.

Monitoring Date:

Monitoring Personnel:

Project Name.	
Project Goals.	
Bridge location.	
Current sediment delivery. High-Med-Low	
Current bridge condition.	
Current/approach condition.	
Armoring and fill condition.	
Erosion/scouring above project site?	
Maintenance records/<u>dates</u>. Bridge observations. Goal Observations.	

<u>Tenmile Watershed Restoration Project Monitoring Form.</u>
Monitoring Date:
Monitoring personnel:

Project Name.	
Project Goals.	
Project type and location.	
Current vegetation type and growth. %	
Current project condition.	
Current in-stream temperature and flow condition.	
Erosion % at project site.	
Spawning gravel accumulation. %	
Maintenance records/dates. **Field observations.** **Goal Observations.**	

Tenmile Watershed Draw/Gulch/Canyon Monitoring Form.

Monitoring Date:

Monitoring personnel:

Sub-basin	
Site location **UTM=**	
Slide presence.	
Slope%	
Vegetation, **Age@InitiationPoint.**	
Length x Width x Depth	
Volume of sediment.	
Photo #	
Field observations notes. **Slide potential =**	

North & South Tenmile Lakes Microcystis Survey									
Sampled by:									
Date:									
Site#	Time	Temp (F)	Secchi(ft)	Secchi(ft)x 3	Conc. Plank (3 hauls, preserved) ml	Dup Conc. Plank (3 Hauls, preserved) ml	Raw Water Toxicity (not preserved) ml	Sample Length ft	Sample Volume ml
North & South Tenmile Lakes Nutrient Survey									
Site#	pH	Duplicate pH	Turbidity	Duplicate Turbidity	D.O.	Duplicate D.O.	Chl a (mls) filtered	TP (mls)	Nutrient Sample (mls)

Circle one

Audit form for field checking temperature recorders

Hobo or Vemco

Auditor's name: MM JK AE DZ Date:

Site: Basin:

Location: UTM N_____ E_____ or T_____R_____S_____ or Lat_____Lon_____

Picture taken? Y or N Time_____a.m./p.m. File name if digital photo_____

Launch date: Audit temperature at launch:

Launch time: Audit time:

Watch synchronized with temperature recorders? Yes or No Computer time correct? Yes or No

Stream/Channel characteristics at launch

Serial # of data logger	% Substrate Fill out a new line if conditions have changed significantly					Water depth at placement site (m)	Wetted width (m)	Active channel (m)	Canopy closure %
	bedrock	boulder	cobble	gravel	sand/silt				

Audit temperatures/Conditions

Name of downloaded data file (Bin#### for Vemco, xxxx.dtf for Hobos)	Device serial #	Hobos		Cycle end date (Date device removed from stream)	NIST digital temperature DEQ or TLBP?	Device temperature @ audit time (You won't know until unit is downloaded)	Time	Water depth (m)	Wetted width (m)
		Launch date (If different than above)	Launch time (If different than above)						

Comments:

Notes: Try to arrange audit time to coincide with logger cycle, i.e., if the logger is set to sample at ten minutes after the hour, take the audit temperature as near to ten after as you can. If the logger is at the bottom of a pool, attempt to read the temperature at the bottom of the pool. Take a picture of the site. Make sure to name data files with a date component so that data does not inadvertently get erased. Depth to thalweg means depth to deepest part of the study site.

TLBP Site Monitoring Form

Sampler_____

Site_____ UTM_____

Date and Time	Temp	Turbidity	D.O.	pH	Duplicates (list parameter duplicated)	Observations

Attachment 3

University of Washington
Marine Chemistry Laboratory

Detection Limits For:

Chlorophyll a (Chl a), Dissolved Organic Carbon (DOC), Total Suspended Solids (TSS), and Particulate Organic Carbon/Nitrogen (POC/PON).
Chl a (and Phaeopigments):
Fluorometric analysis done on a Turner Model TD700 fluorometer. Published detection limit is 0.02 μg/L.** This is the lowest EXTRACT concentration measurable on the instrument.
Turner Designs (1999) TD-700 Laboratory Fluorometer Operating Manual. p. 49.
DOC
Analysis performed on a Shimadzu TOC-Vcsh Total Organic Carbon Analyzer. Published detection limit is 50 μg C/L.
With a CV of ~1.5%
Shimadzu Corporation (2001) Total Organic Carbon Analyzer TOC-Vcsh/csn User's Manual. p. 249.

TSS
If necessary, we can see differences down to 1 μg. Basically we're restricted by the limits of the balance we use to weigh our filters. **

POC/PON
Analysis performed on a CEC 440-SHA Elemental Analyzer (Leeman Labs, Inc. currently supported by Exeter Analytical, Inc.). Detection limit is ~10μg C/filter and ~1μg Nitrogen/filter (as determined by this lab). **

**The Caveat: because these three analyses require filtration, the minimum sample concentration detectable is variable. The more one is able to filter, the lower the detection limit.

NUTRIENTS

Range	0-3uM PO4	0-50uM Si(OH)4	0-25uM NO3	0-3uM NO2	0-3uM NH3
MDL (uM)	.02	.21	.15	.01	.05
MDL(mg/l)	.0006	.0059	.0021	.0001	.0007

TNP

MDL(uM)	.02	.38
MDL(mg/l)	.0006	.0053

POLICY FOR QUALITY ASSURANCE AND QUALITY CONTROL

The primary objective of the Marine Chemistry Lab is to provide our customers with consistently high quality, accurate data. Our customers provide samples from a wide range of aquatic environments, from pristine freshwater streams to lakes, estuaries, coastal and open ocean water, and anoxic saltwater basins like the Black Sea.

We provide the high quality analytical services our clients require by adhering to the methods described in this manual including proper sampling techniques, sample analysis, data reduction, and quality control procedures. As professional and contributing members of the research oceanographic community we employ methods widely used and accepted by oceanographers worldwide (e.g., UNESCO, 1994).

REFERENCES

UNESCO (1994) Protocols for the Joint Global Ocean Flux Study (JGOFS) core measurements. IOC Manual and Guides 29.

GENERAL LABORATORY QUALITY ASSURANCE

LABORATORY SAFETY

The Dept. of Environmental Health and Safety at the University of Washington requires every laboratory to complete a Laboratory Chemical Hygiene Plan (CHP). The CHP contains a detailed floor plan of the lab showing the locations of safety equipment (eyewash stations, emergency showers, and fire extinguishers) and chemical storage, standard operating procedures (SOPs) for hazardous chemicals, action plans for chemical spills, and a detailed plan to hazardous waste disposal. This CHP, a file containing material safety data sheets (MSDS) for all chemicals used in the laboratory and a Merck Index are kept in the lab and are easily accessible.

Safety inspections of the laboratory are done twice a year by EH&S and the Seattle Fire Department.

All MCL personnel are required to take the Laboratory Safety classes and the Fire Extinguisher classes offered by the UW Staff Training and Development Office.

Labcoats, safety glasses, and protective gloves are readily available in the lab.

REAGENTS

Water: Distilled deionized water (DIW) produced in the building's reverse osmosis system is piped directly into the lab. This water is further deionized by running it through two IWT® ionxchanger (model 2 research) columns. DIW is made fresh before use.

Chemicals: Only reagent grade chemicals are used, and in some cases only specific brands of chemicals are used; for example, Mallinckrodt sodium citrate cannot be used for the ammonium analysis because it produces a huge negative blank in seawater samples relative to DIW.

Each reagent is made up in its own designated volumetric flask to alleviate cross-contamination. Graduated cylinders used in reagent preparation are designated for that use only.

All primary standard chemicals are heated to 105˚C for at least two hours, then cooled and stored in a dessicator.

GLASSWARE AND PIPETTES

General use laboratory glassware is washed with a phosphate-free laboratory detergent and rinsed copiously with DIW.

Class "A" volumetric glassware is cleaned with chromic acid solution, then calibrated gravimetrically to determine its exact volume at 20˚C. All pipettes used in the preparation of standards are also calibrated.

The volumetric glassware is calibrated annually and before any long research cruises. Pipettes are calibrated every six months and before research cruises.

SAMPLE MANAGEMENT

No hazardous samples are accepted for analysis. Hazards include preservation with azides or mercuric chloride or samples that contain any radioactive tracer.

All samples accepted for analysis are logged on the MCL sample inventory form (Appendix A). Customers' logsheets or chain-of-custody forms must accompany the samples and include station and depth information. Bottle labels should not wash off or drip off when the sample is being thawed. MCL can provide appropriate sample bottles and supplies to customers on a rental (non-consumable items) or material issue (consumables) basis to assure that samples are collected and stored properly prior to analysis.

BALANCES

All of the analytical balances in the laboratory are maintained on a service contract with the UW Scientific Instrument Division. The balances are serviced and calibrated yearly. The Cahn electronic balance is recalibrated by the manufacturer every two years.

DATA STORAGE

All raw and final data are archived for at least 10 years. Final data are provided to the customer in hard copy and electronic formats (generally as Microsoft Excel spreadsheets).

STANDARD OPERATING PROCEDURE: NUTRIENT ANALYSES

Five major nutrients (o-phosphate, silicate, nitrate, nitrite, and ammonium) are analyzed in seawater or freshwater with a Technicon AutoAnalyzer II or Alpkem RFA/2 system.

CALIBRATION AND QUALITY CONTROL PROCEDURES FOR AutoAnalyzer II OPERATIONS

SAMPLE BOTTLES

Sample bottles are narrow mouth 60 ml Nalgene® HDPE bottles with a leakproof screw cap. The sample tray of the AutoAnalyzer II turntable has been modified to carry these bottles.

The bottles are cleaned after each use by rinsing with 10% HCl followed by several DIW rinses.

SAMPLE COLLECTION AND PRESERVATION

Samples are collected by rinsing the bottle and cap 3 times with sample and then filling the bottle no more than 2/3 full. Freshwater samples and samples with a high particulate content are filtered through 2.5 cm Whatman® GF/F glass microfibre filters (nominal pore size 0.7 μm) or Nalgene® surfactant-free cellulose acetate membrane filters (pore size 0.45 μm). Filtration is done in the field to avoid changes in the dissolved material that can occur during transportation and storage.

Samples are frozen upright as quickly as possible and stored in a freezer at -20˚C.

SAMPLE CONDITIONS

The first step to quality control is to make note of the sample conditions before analysis; have they been stored correctly, are there problems because of overfilling, do they contain H_2S, or do they contain particulates? Any problems are noted on the raw data sheet and on the data sheet header that goes to the customer.

Samples are thawed overnight in a refrigerated cold room. This method of thawing allows the silicate to depolymerize and the other nutrients to remain stable. Only one day's run of samples is thawed at a time.

REAGENTS

Consistency of methods is a key to good quality analyses. Each reagent is made up in its own designated volumetric flask to alleviate cross-contamination, especially concerning the ammonium reagents. Only reagent grade chemicals are used, and in some cases only specific brands of chemicals are used; for example, Mallinckrodt sodium citrate cannot be used for the ammonium analysis because it produces a huge negative blank in seawater samples relative to the DIW baseline. Every new batch of reagent is recorded in the lab notebook along with any surfactant added or pH adjustment. Graduated cylinders used in reagent preparation are designated for that use only. Fresh DIW is used for reagents and in the analytic stream of the AutoAnalyzer II.

SAMPLE RUNS

The AutoAnalyzer II is ready for a run when a clear non-noisy DIW+reagent baseline is established on each channel. Sample runs are usually 30-50 analyses long, including blanks, calibration standards, check standards (for QC), and samples (see Appendix G).

Working standards for calibration are made to match the expected concentrations and salinity of the samples. The ranges specified in each analytical method are linear (appendix B). At the minimum, a three-point standard curve is run containing a matrix blank plus two concentrations of standard that cover the sample range. If the concentration of the samples is expected to be wide (example: a deep-sea water column profile from 0 to 2000 m), then more standard concentrations are added to the curve.

Two check standards of concentrations different from those used in the standard curve are prepared using the same matrix water as that of the standards. These are the QC samples; their concentrations should reflect the lower and mid-high points in the analytical range. These check standards are monitored on a control chart (example, Appendix C).

Each run begins with a calibration standard curve (matrix blank, concentration 1, concentration 2, each in duplicate) followed by check standard. Samples follow the standards and QC usually grouped according to sampling stations and are arranged by increasing depth in the water column. Each station group is separated by a "lead-in" sample which is a duplicate of the first sample of the next group. This "lead-in" sample eliminates carryover between the typical high concentration sample from depth and the following low concentration surface sample. The second check standard, run in duplicate (the first of this duplicate serves as the "lead-in" sample), follows the samples. The run is finished with another calibration standard and matrix blank and allowed to go to baseline. Timing for each analysis is a 2-minute sample followed by a 40-second DIW wash.

Two sets of duplicate samples are run if enough water is provided by the customer. When at sea with an unlimited water supply and a 24-hour operation, customers are encouraged to take multiple samples at multiple depths to establish analytical precision at different concentration ranges. An example follows; the means and standard deviations for this data set are as follows:

	PO_4		$Si(OH)_4$		NO_3		NO_2		NH_4	
depth	mean	s	mean	s	mean	s	mean	s	mean	s
2 m n=5	1.79	.05	46.54	.01	19.60	.06	.35	.01	.74	.02
10 m n=5	1.97	.01	50.44	.33	21.76	.11	.36	.00	.77	.00
165 m n=10	1.72	.01	38.41	.31	17.79	.04	.25	.01	1.30	.02

All raw data (peak heights) are recorded in ink. Each raw data sheet contains the date, run ID, secondary standard batch ID, and analyst's initials. Beginning and ending factors are calculated and the data are entered onto an Excel spreadsheet which computes the final nutrient concentrations (see calculation equations, page 21).

NUTRIENT STANDARDIZATION PROTOCOLS

PREPARATION OF PRIMARY STANDARDS

Primary standards are prepared using precisely weighed (to 0.1 mg) primary standard chemicals (phosphate, silicate, nitrate, nitrite, ammonium) dissolved in deionized distilled water (DIW) and made up to accurately known volumes. The precise weights and volumes used are listed in the reagent sections of each nutrient chemistry. The weights of the standards are corrected to *in vacuo* using the buoyancy correction of the laboratory conditions. Each primary standard is made up separately and stored in a dark HDPE leakproof bottle in the refrigerator. It is identified by a sequential number and an "A" (i.e., 2A). New primary standards are made up every six months.

Concentrations of the primary standards are: 10.0 mM phosphate, 3.00 mM silicate, 100 mM nitrate, 10.0 mM nitrite, and 10.0 mM ammonium, respectively.

PREPARATION OF SECONDARY STANDARDS

A mixed secondary standard containing phosphate, nitrate, nitrite, and ammonium is made up monthly or as needed. Silicate is not included in the mixed secondary standard because the concentration varies over a wide range, especially in fresh water. Adding the primary silicate standard directly to the working (tertiary) standard gives the analyst more flexibility to choose the correct concentration range for the specific samples being analyzed.

The primary standards are allowed to come to room temperature. Separate aliquots of the phosphate, nitrate, nitrite, and ammonium standards are pipetted into a calibrated class "A" volumetric flask. The solution is brought to volume with DIW. The temperature and flask ID is noted. This secondary standard is dated, then coded using a sequential number and a "B". It is stored in a dark HDPE leakproof bottle in the refrigerator.

The typical concentrations of the secondary standard are 250 µM phosphate, 250 µM nitrite, 250 µM ammonium, and 3000 µM nitrate.

PREPARATION OF TERTIARY OR WORKING STANDARDS

Working standards are made up daily or at least every 4–6 hours when at sea. The standard matrix is made to match that of the samples. If freshwater samples are being run, the working standards are made up in DIW. Otherwise the matrix is matched to within 3 psu (practical salinity units) of the samples. Only filtered low nutrient natural seawater (LNSW) is used to make up standards. This seawater is collected at sea, stored in barrels, and is usually between 35–36 psu salinity. The LNSW is diluted with DIW to obtain other salinity matrices, i.e., standards of about 28 psu are used when running Puget Sound samples.

Aliquots from the silicate primary standard and the mixed secondary standard are combined and diluted with the appropriate matrix water in calibrated 250 ml "A" volumetric flasks. Concentrations of these working standards are adjusted to cover the expected range of the samples and to fall within the linear range of the analytical channel.

Typically the working standards are adjusted to fall within the following ranges: phosphate: 0–3 µM, silicate: 0–100 µM, nitrate: 0–40 µM, nitrite: 0–3 µM, ammonium: 0–3 µM.

CALIBRATION OF THE STANDARDS

Each new batch of primary standards, except for ammonium, is checked against the Marine Nutrients Standards Kit, available from Ocean Scientific International (OSI), containing certified nutrient standards and LNSW. A tertiary (working) standard in LNSW is made up using the new primary and secondary standards. The nutrient concentrations of this standard are 3.00 µM phosphate, 60.0 µM silicate, 39.0 µM nitrate, and 3.00 µM nitrite.

A second working standard in LNSW with the same nutrient concentrations is made up using the Marine Nutrients Standards Kit. The two standards are analyzed in the same run and the peak heights are compared. The peak heights should compare within .02 µM phosphate, .20 µM nitrate, .40 µM silicate, and .05 µM nitrite.

At this time there is no certified seawater standard for ammonium. On recent major research cruises (JGOFS), extensive cross-referencing between ammonium standards from Oregon State University, Texas A&M, Scripps Institution of Oceanography, and the University of Washington was done. All of these standards were in excellent agreement. The chemical lot of the University of Washington standards is still in use. Commercially available ammonium calibration standards in DIW will be used for future comparisons.

New batches of "B" secondary standards are compared with the previous secondary standard. The responses (peak heights) must compare within .5% for phosphate, silicate, and nitrate and within 1% for nitrite and ammonium.

This lab has participated in the U.S. Environmental Protection Agency (EPA) Performance Evaluation program since 1990, which ends with the WP040 samples. We will continue the twice-yearly analysis of PE samples for phosphate, nitrite, nitrate, and ammonium with the ERA

QuikResponse PE program offered by Environmental Resource Associates. Silicate PE samples will be obtained from Ocean Scientific International.

METHODS AND FLOW DIAGRAMS

This section contains detailed procedures for mixing reagents and flow diagrans showing the AutoAnalyzer II reagent stream pertinent to each of the following analyses:

o-phosphate, silicate, nitrate + nitrite, nitrite, ammonium

A description of the 5-channel AutoAnalyzer II system is found in Appendix H.

O-PHOSPHATE ANALYSIS

METHOD OUTLINE

O-Phosphate is analyzed using a modification of the Bernhardt and Wilhelms (1967) method. Ammonium molybdate is added to a water sample to produce phosphomolybdic acid, which is then reduced to phosphomolybdous acid (a blue compound) following the addition of dihydrazine (or hydrazine) sulfate. The sample is passed through a 50 mm flowcell and absorbance is measured at 820 nm.

REAGENTS

Ammonium molybdate

H_2SO_4 solution: Pour 420 ml of DIW into a 2 liter Ehrlenmeyer flask or beaker; place this flask or beaker into an ice bath. SLOWLY add 330 ml of concentrated H_2SO_4. This solution gets VERY HOT!! Cool in the ice bath. Make up as much as necessary in the above proportions.

stock: Dissolve 27 g ammonium molybdate in 250 ml of DIW. Bring to 1 liter volume with the cooled sulfuric acid solution. Add 0.1 ml Wiconate® surfactant. Store in a dark HDPE bottle and refrigerate.

working: Use stock as needed.

Dihydrazine sulfate

stock: Dissolve 6.4 g dihydrazine sulfate in DIW, bring to 1 liter volume with DIW and refrigerate.

working: Use stock as needed.

Note: Hydrazine sulfate may also be used. Dilute 10 g to 1 liter with DIW.

Primary Standard

.6804 g KH_2PO_4 in 500 ml DIW. Concentration = 10.0 mM.

The preparation of primary, secondary, and working standards is discussed on pages 9–11 of this section.

SILICATE ANALYSIS

METHOD OUTLINE

Silicate is analyzed using the basic method of Armstrong et al. (1967). Ammonium molybdate is added to a water sample to produce silicomolybdic acid which is then reduced to silicomolybdous acid (a blue compound) following the addition of stannous chloride. The sample is passed through a 15 mm flowcell and absorbance is measured at 820 nm.

REAGENTS

Tartaric acid

stock: Dissolve 200 g tartaric acid in DIW and bring to 1 liter volume with DIW. Add 0.1 ml of 40% surfynol 465/485 surfactant. Store at room temperature in a dark HDPE bottle.

working: Use stock as needed.

Ammonium molybdate

stock: Dissolve 50 g of ammonium molybdate in DIW and bring to 1 liter volume with DIW. Store at room temperature in a dark HDPE bottle. Discard if a white precipitate forms.

working: Combine 200 ml ammonium molybdate stock with 300 ml 1.2N HCl.

Stannous chloride

stock: Dissolve 40 g of stannous chloride in 100 ml 5N HCl. Refrigerate in a dark HDPE bottle. Discard if a whitish solution forms.

working: Bring 5 ml of stannous chloride stock to 200 ml final volume with 1.2N HCl. Make up every two days; refrigerate between days in a dark HDPE bottle.

Primary Standard

1.1294 g Na_2SiF_6 to 2.0 liter volume with DIW. Concentration = 3.00 mM.

The preparation of primary, secondary, and working standards is discussed on pages 9–11 of this section.

NITRATE/NITRITE ANALYSIS

METHOD OUTLINE

A modification of the Armstrong et al. (1967) procedure is used for the analysis of nitrate and nitrite. For nitrate + nitrite analysis, a water sample is passed through a cadmium column where the nitrate is reduced to nitrite. This nitrite is then diazotized with sulfanilamide and coupled with N-(1-naphthyl)-ethylenediamine to form an azo dye. The sample is then passed through a 15 mm flowcell and absorbance is measured at 540 nm. A 50 mm flowcell is required for nitrite (NO_2). The procedure is the same for the nitrite analysis less the cadmium column. Nitrate concentration equals the (nitrate + nitrite) concentration minus the nitrite concentration.

REAGENTS

Sulfanilamide

stock: Dissolve 10 g sulfanilamide in 1.2N HCl and bring to 1 liter volume with 1.2N HCl. Add 0.1 ml of 40% surfynol 465/485 surfactant. Store at room temperature in a dark HDPE bottle. The solution is stable indefinitely.
working: Use stock as needed.

N-(1-naphthyl)-ethylenediamine dihydrochloride (N-1-N)

stock: Dissolve 1 g N-1-N in DIW, bring to 1 liter volume with DIW. Add 0.1 ml 40% surfynol 465/485 surfactant. Store at room temperature in a dark HDPE bottle. Discard if the solution turns a reddish brown.
working: Use stock as needed.

Imidazole buffer

NOTE: Make the stock the day before use.

stock: Dissolve 13.6 g imidazole in 3.8 liters DIW. Stir for at least 30 minutes to completely dissolve. Add 60 ml of $CuSO_4$ + NH_4Cl mix (see below), bring solution to 4 liters volume with DIW. Using a calibrated pH meter, adjust to pH of 7.85 with 1.2N HCl (about 15 ml). Add 0.1 ml 40% surfynol 465/485 surfactant. Store at room temperature in a HDPE bottle. The solution is stable indefinitely.
working: Use stock as needed.

$CuSO_4$ + NH_4Cl mix

Dissolve 20 g cupric sulfate in DIW, bring to 1 liter volume with DIW (2% solution stock). Dissolve 250 g ammonium chloride in DIW, bring to 1 liter volume with DIW. Add 5 ml of 2% $CuSO_4$ solution to this NH_4Cl stock. Store at room temperature in a dark HDPE bottle. This solution is stable indefinitely.

Primary Standards

NO_3: Dissolve 5.0554 g KNO_3 in DIW, bring to 500 ml final volume. Concentration = 100 mM.

NO_2: Dissolve .3451 g $NaNO_2$ in DIW, bring to 500 ml final volume. Concentration = 10.0 mM.

The preparation of primary, secondary, and working standards is discussed on pages 9–11 of this section.

CADMIUM COLUMNS

Processing the cadmium

Use cadmium granules approximately 2 mm in size. Place the necessary amount of cadmium in an oversized Erhlenmeyer flask to allow sufficient room for mixing and rinsing. Begin with several small rinses of the Cd with 2N HCl, stir with a stirring rod for a few minutes each rinse. Then rinse with DIW a few times, again using a glass stirring rod.

Rinse with 0.3N nitric acid; this pits the surface of the cadmium and increases the surface area. Rinse with DIW a few times.

Rinse a few times with 2N HCl again, then a few more rinses with DIW.

Add enough DIW to the cadmium to cover the granules, then begin adding 2% $CuSO_4$, a little at a time. Stir with a rod in between each addition, but do not decant. Keep adding until the solution remains slightly bluish in tone, and becomes slightly cloudy. This should be a slow, careful process. The cadmium should now be blackish with lots of particulates (broken-off pieces of cadmium). When preparing Cd for 5–7 columns, expect to use more than 400 ml of 2% $CuSO_4$ over the course of about 15 additions (a little at a time). Continue adding until the Cd particles develop the instant the $CuSO_4$ is added. This should be the last addition of $CuSO_4$.

The cadmium is now treated. Decant almost all of the solution from the cadmium, minimizing air exposure. Rinse and decant many times with DIW, use a stir rod during the rinses. Continue rinsing until the rinse water is no longer cloudy, and the cadmium appears dark, spotty, and grayish. During this entire procedure do not stir too vigorously to avoid breaking up the Cd granules.

Use an approximately 10–12 cm long, 6 mm diameter, glass tube with a 1 mm wall and a 4 mm ID. Pack the bottom with a small ball of glass wool and attach a nipple fitting to the bottom end with silicon tubing. Cap this fitting off with a piece of tied-off tygon tubing. Attach a small funnel to the top of the column. Fill the column and funnel with a 50% imidazole buffer solution and load the cadmium into the column. Tap gently to settle the Cd and fill until there is no dead space. Remove the funnel and insert a wetted wad of glass wool. Cap off with the appropriate nipple fitting and silicon tubing. Cap top end with tied-off tygon tubing. Store immersed in 50% imidazole buffer solution until ready to go on the NO_3 channel.

Priming the column

The column needs to be primed whenever it is new or has been topped off with new granules. If the column is not primed, the response may not be stable. For a new column, prime by running about 200 ml of 50 µM NO_3 standard through the system with the column on. Flush the column afterward by running imidazole buffer and DIW through the system for 30–45 minutes. If the

column has just been topped off, run 100 ml of 25–50 µM standard through the system, then flush by running imidazole buffer and DIW through the system for about 30 minutes.

Topping off

It is very important to keep the column full of cadmium to minimize dead space. As samples are run, the cadmium volume will be reduced through use and settling. This dead space *will affect the data*. To top off the column, turn the AutoAnalyzer on and the column off. Tap the column gently to get the maximum settling. Remove the tubing cap and the glass wool from the top of the column. Attach a funnel to the top of the column and fill with buffer solution using a syringe. With a spatula, transfer prepared Cd granules to the column via the funnel, tap with a pencil, and continue filling. Leave enough space for the glass wool. Remove the excess buffer in the funnel with a syringe, remove the funnel, and insert a wetted wad of glass wool in the top of the column. Reconnect the tubing cap, turn on the column to flush the cadmium, and then prime the column.

Cadmium column efficiency

The cadmium column efficiency for reducing nitrate to nitrite is not always 100%; therefore, comparisons need to be made regularly, especially with a new column or one just topped off. While an efficiency of 100% is ideal, an efficiency of less than 100% may not have any significant effect on the accuracy of the nitrate determinations. The check standards that are analyzed during each sample run provide a measure of the accuracy of the analysis.

Prepare a 30 µM nitrite standard and a 30 µM nitrate standard. Run at least four of each, alternating one after the other through the NO_3 channel with the Cd column on. Measure the peak heights and calculate the percentage efficiency; NO_3 peak height ÷ NO_2 peak height = % efficiency.

WOCE and JGOFS protocols call for better than 95% efficiency (UNESCO, 1994).

AMMONIUM ANALYSIS

METHOD OUTLINE

A modification of the Slawyk and MacIsaac (1972) procedure is used for the analysis of ammonium. A water sample is treated with phenol and alkaline hypochlorite in the presence of NH_3 to form indophenol blue (Berthelot reaction). Sodium nitroferricyanide is used as a catalyst in the reaction. Precipitation of Ca and Mg hydroxides is eliminated by the addition of sodium citrate complexing reagent. The sample stream is passed through a 55˚C heating bath, then through a 50 mm flowcell and absorbance is measured at 640 nm.

REAGENTS

Complexing reagent: Sodium citrate

stock: Dissolve 1120 g sodium citrate dihydrate in approximately 3.8 liters of DIW. Bring to 4 liter volume with DIW. Adjust pH to 7.0 using H_2SO_4. Store in a HDPE bottle; it is stable indefinitely.

working: Use stock as needed.

NOTE: J.T. Baker reagent grade sodium citrate dihydrate has a lower absorbance in DIW than other brands.

Alkaline phenol

stock: Add 60 ml of 10N NaOH to 700 ml of DIW. Add 12 ml of liquified phenol, bring to 1 liter volume with DIW. Store in a dark HDPE bottle and refrigerate.

working: Use stock as needed.

10N NaOH: Dissolve 400 g NaOH in DIW *(solution will get very hot!)*. Bring to 1 liter volume with DIW.

Sodium hypochlorite

working: Add 2.5 ml of 5.25% NaOCl (Clorox bleach)® to DIW and bring to 100 ml final volume. Make daily.

Sodium nitroprusside (sodium nitroferricyanide)

stock: Dissolve 0.5 g sodium nitroprusside in DIW; bring to 1 liter final volume with DIW. Store in a dark HDPE bottle at room temperature; the reagent is stable indefinitely.**working:** Use stock as needed.

Primary Standard

Dissolve .2673 g NH_4Cl in DIW; bring to 500 ml volume with DIW. Concentration = 10.0 mM.

or

Dissolve .1321 g $(NH_4)_2SO_4$ in DIW; bring to 1 liter volume with DIW. Concentration = 2.00 mM.

The preparation of primary, secondary, and working standards is discussed on pages 9–11 of this section.

REFRACTIVE INDEX CORRECTIONS

When seawater samples are analyzed relative to a deionized, distilled water baseline, a refractive index correction must be applied [Whitledge et al. (1981) and UNESCO (1994)]. Freshwater samples require no refractive index correction. To determine refractive index corrections, one reagent is left out of each analytical stream and samples of a similar salinity matrix (±3 psu) are run. The reagents omitted are: hydrazine (PO_4), $SnCl_2$ ($Si(OH)_4$), NEEDA (NO_3 and NO_2).

For ammonium, leaving out any of the reagents does not produce a representative refractive index. For our analytical manifold, this refractive index usually ranges between -7.0 and -5.0 and varies between batches of sodium citrate reagent. To obtain a proper correction, we use natural deep sea water (adjusted to the matrix of the standard) which contains no ammonium. The refractive index correction is the total absorbance measured minus the distilled water baseline absorbance.

Refractive index corrections remain relatively constant for samples of similar salinity as long as there is no change in the manifold, flowcell path length or standard calibration control.

CARRYOVER CORRECTIONS

We do not include carryover corrections in the final nutrient calculation because there is insignificant interaction between peaks. "Lead-in" peaks are used to eliminate potential carryover between assumed low to high (or vice versa) peaks, such as a surface seawater sample and a following 1000 m seawater sample.

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Aquatic Analysts
Algae Analytical and Quality Assurance Procedures

May 4, 2004

Sample Handling

Sample Collection and Preservation

Phytoplankton is collected by filling bottles with natural water samples. Samples are collected at either discrete depths, or integrated through the photic zone of lakes. A volume of 250 mL is sufficient for most samples.

These samples are preserved with 1% Lugol's solution immediately after collection. Refrigeration is not necessary, and holding times are a year or more.

Sample Tracking

All samples received in the laboratory are immediately logged into a Sample Receipt Log. All samples are stored in a dedicated area until they are processed. After samples are processed and analyzed and data reports have been submitted to clients, samples are placed in storage for at least one year.

Sample Preparation

Permanent microscope slides are prepared from each sample by filtering an appropriate aliquot of the sample through a 0.45 micrometer membrane filter (APHA Standard Methods, 1992, 10200.D.2; McNabb, 1960). A section is cut out and placed on a glass slide with immersion oil added to make the filter transparent, followed by placing a cover slip on top, with nail polish applied to the periphery for permanency. A benefit to this method is that samples can be archived indefinitely; we have over 18,000 slides archived.

Microscopic Analyses

Algae Identifications

Aquatic Analysts has an extensive library of algae literature, including journal reprints, standard reference books, and internet reference sites. We also maintain files, notes, and photographs of algae we've encountered during the past 29 years of identifying algae. Most algae are identified by cross-referencing several taxonomic sources.

Enumeration

Algal units (defined as discrete particles - either cells, colonies, or filaments) are counted along a measured transect of the microscope slide with a Zeiss standard microscope (1000X, phase

contrast). Only those algae that were believed to be alive at the time of collection (intact chloroplast) are counted. A minimum of 100 algal units are counted. (Standard Methods, 1992, 10200.F.2.c.).

Biovolume Estimates

Average biovolume estimates of each species are obtained from calculations of microscopic measurements of each alga. The number of cells per colony, or the length of a filament, are recorded during sample analysis to arrive at biovolume per unit-alga. Average biovolumes for algae are stored in a computer, and measurements are verified for each sample analyzed.

Data Analyses and Reports

Sample Reports

Results of sample and data analyses are provided to the client in electronic format (email and/or CD disk), and in hard copies. Deliverables include individual sample reports, similarity indices, data summaries, combined species lists, and a brief narrative discussion of the results.

Individual sample reports include sample identification, a trophic state index, total sample density, total sample biovolume, and a list of algae species with their absolute and relative densities and biovolumes. All data are reported in Excel format.

Data summaries include sample identification, total density, total biovolume, the trophic state index, and the top 5 most common algae species (codes) and their relative densities. The summary format (Excel) allows for easy calculations and graphs of algae sample data.

Combined species lists of all species within related groups of samples allow greater sensitivity in comparing different lakes, sites, dates, or depth. Algae species are compiled according to their relative densities.

Trophic State Index

A Trophic State Index based upon phytoplankton biovolume has been developed from a data set of several hundred lakes located throughout the Pacific Northwest (Sweet, 1986, Report to EPA). The index was derived in a similar fashion as Carlson (1977) derived indices for Secchi depth, chlorophyll concentration, and total phosphorus concentration. The biovolume index ranges from 1 for ultraoligotrophic lakes to 100 for hypereutrophic lakes. Values agree well with Carlson's indices.

The index is defined as:

TSI (biovolume) = (Log-base 2 (B+1)) * 5

Where B is the phytoplankton biovolume in cubic micrometers per milliliter divided by 1000.

Similarity Index

A similarity index is useful in comparing phytoplankton communities between two samples. The index compares the relative abundances of each species present in two samples and yields a

value ranging from 0 for totally dissimilar samples, to 100 for identical samples. The formula for the index (modified from Whittaker, 1967) is:

Similarity Index = 100 - (Sum of DIFFERENCE / 2)

Where DIFFERENCE is the absolute value of the difference of the percent density of a given species in two samples.

Quality Assurance

Microscope Calibration

Aquatic Analysts use a Zeiss Standard phase-contrast microscope primarily with a 1000X magnification for identification and enumeration of algal samples. The diameter of the field of view at 1000X magnification is 0.182 mm. The effective area of a filter is 201 millimeters square.

Algae are enumerated along a measured transect, measured accurately to 0.1 mm with a stage micrometer. The algal densities are calculated from the area observed (transect length times diameter of field of view), the effective filter area, and the volume of sample filtered.

The microscope was calibrated using a standard concentration of latex spheres provided by EPA (Cincinnati, OH). The concentration of these spheres was 12,075 per milliliter. Duplicate preparations of the standard spheres were analyzed; the average result was 11,700 spheres per milliliter (96.9 percent). The computer program used to calculate algae densities compensates for this 3.1% error.

Replicates

Replicate algae samples are analyzed at the client's request. We encourage blind replicates for approximately 10% of all samples collected. Replicates are assessed for algae abundance (relative mean difference of densities) and species composition (similarity indices, species lists).

Independent Analyses

Aquatic Analysts has participated in the analyses of split algae samples on several occasions, with general agreement between samples in terms of algae density and algae species compositions.

Internal Data Verification

A custom computer program handles all calculations and data analyses. Final sample reports are compared with laboratory bench sheets before releasing data.

Data summaries, tables of similarity indices, abundance graphs, and combined species lists are searched for inconsistencies, outliers, and interrupted patterns that may indicate possible errors.

Archives

Aquatic Analysts maintains an herbarium of all microscope slides analyzed (over 18,000 to date). These may be reviewed if questions arise after data are reported. In addition, all computer data (sample tracking data, raw count data, final reported data, data analyses, narrative reports) are archived on CD's in permanent storage.