Guidance for Assessing and Characterizing Potentially Toxic Cyanobacteria Blooms

The purpose of this document is to provide guidance for water quality managers who intend to monitor water bodies prone to cyanobacteria blooms. The objectives of the guidance are to:

- Help water quality managers identify monitoring objectives.
- Suggest analytical parameters and information relevant to a cyanobacteria monitoring program.
- Provide an adaptable framework for a monitoring plan.
- Provide sampling information specific to cyanobacteria and algae monitoring.
- Provide resources to more detailed information.

This guidance is not a complete surface water sampling, step-by-step protocol. The Oregon Department of Environmental Quality (DEQ) recommends that water quality managers refer to DEQ's Field Sampling Reference Guide (1998) and Methods of Operations Manual (2004) for detailed guidance to surface water sampling and analysis.

1.0 Background

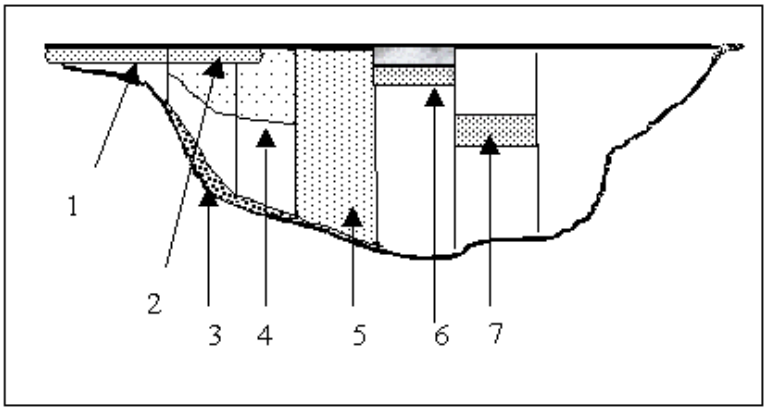
The geologic record indicates that cyanobacteria were among the planet's first life forms. Their metabolic activity has contributed oxygen to the Earth's atmosphere for over a billion years. Today, they get little credit for their crucial contribution to our existence. Rather, water quality managers view cyanobacteria, commonly but incorrectly termed blue-green algae, as unsightly and potentially hazardous to humans and their pets.

The metabolic processes of algae (green algae and cyanobacteria) can result in dissolved oxygen and pH fluctuations that stress aquatic communities. A decaying bloom can also consume all the available dissolved oxygen in a small or shallow water body. Cyanobacterial decay particularly concerns water quality managers because as the cells lyse (break apart) they may release toxic substances. Because the mechanism of toxic release is poorly understood, water quality managers will often act on the assumption that visible blooms are potentially toxic.

Cyanobacteria have a primitive cell structure (prokaryotic) that lacks a membrane bound nucleus, but several adaptive characteristics allow them to out-compete more complex algal species. The majority of cyanobacteria are photoautotrophs and, like green algae, need only water, CO_2, inorganic nutrients, and light to survive. Photosynthesis is their principle mode of energy metabolism.

Because some cyanobacteria respond similarly to environmental factors, they can be grouped as "ecostrategists", typically inhabiting different niches of aquatic ecosystems. Figure 1 illustrates the diverse potential locations of phytoplankton blooms in a thermally-stratified, shallow lake. The World Health Organization (WHO, 1999) publishes a comprehensive reference to cyanobacteria and their adaptations. For example, some cyanobacteria can utilize the longer wavelengths of visible light allowing them to proliferate deeper in the water column, below surface algae. To increase their survival at the lake's surface, some cyanobacteria have UV absorbing sheath pigments. Still others are able to survive long periods in complete darkness. Cyanobacteria use gas vacuoles to regulate their buoyancy and take advantage of optimal light and nutrient conditions. The cyanobacteria also tend to have a higher affinity for nutrients than green

algae species. Cyanobacteria are also more likely to dominate the algal community in a water bodies with long retention times and temperatures exceeding 25ºC.



Typical locations of phytoplankton blooms in thermally-stratified, shallow lakes or reservoirs:
1 - shoreline scums; 2 - planktonic scums on open water; 3 - phytoplankton scums in or on the lake sediments; 4 - dispersed populations of phytoplankton within the epilimnion; 5 - homogeneous populations of phytoplankton dispersed throughout the water column during well-mixed, non-stratified conditions; 6 - scums under ice; 7 - sub-surface or metalimnetic phytoplankton maxima (modified from Lindholm and Meriluoto 1989)

Figure 1: Potential locations of phytoplankton blooms in a water body, from Lindholm and Meriluoto, 1989, as cited in WHO, 1999, Chap. 10.

Cyanobacteria also have the ability to release toxins. The following paragraphs are excerpted from WHO (1999).

> In spite of their aquatic origin, most of the cyanobacterial toxins that have been identified to date appear to be more hazardous to terrestrial organisms than to aquatic biota. Cyanobacteria produce a variety of unusual metabolites, the natural function of which is unclear, although some elicit effects on other biota. Research has focused on compounds that affect humans and livestock either as toxins or as pharmaceutically useful substances.
>
> The best understood mechanisms of cyanobacterial toxicity range from hepatotoxic, neurotoxic and dermatoxic effects to general inhibition of protein synthesis. Cyanotoxins fall into broad groups of chemical structure: cyclic peptides, alkaloids and lipopolysaccharides (LPS), as presented in Table 1. To assess the specific hazard of cyanobacterial toxins one must understand their chemical and physical properties, their occurrence in waters used by people, the regulation of their production, and their fate in the environment.
>
> The majority of studies on the occurrence, distribution and frequency of toxic cyanobacteria were conducted in the 1980's using mouse bioassays. Analytical methods suitable for quantitative toxin determination only became available in the late 1980's, but studies of specific cyanotoxins have been increasing since then. The results of both approaches indicate that neurotoxins are generally less common, and the cyclic peptide toxins (nodularians and microcystins), which primarily cause liver injury, are more widespread and are very likely to occur if certain cyanobacteria taxa are present.

Table 1: General features of the cyanotoxins, from WHO 1999.

General features of Cyanotoxins			
	Toxin Group[1]	**Primary Target organ in mammals**	**Cyanobacterial genera[2]**
Cyclic Peptides	Microcystins	Liver	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis*
	Nodularian	Liver	*Nodularia*
Alkaloids	Anatoxin-a	Nerve Synapse	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*
	Anatoxin-a (S)	Nerve Synapse	*Anabaena*
	Aplysiatoxins	Skin	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria)*
	Cylindrospermopsins	Liver[3]	*Cylindrospermopsis, Aphanizomenon, Umezakla*
	Lyngbyatoxin-a	Skin, G.I. Tract	*Lyngbya*
	Saxitoxins	Nerve Axons	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
Lipopolysaccharides	(LPS)	Potential irritant; affects any exposed tissue	ALL

2.0 Initial Assessment and Preparation for Developing a Monitoring Plan

Before undertaking a monitoring project, water quality managers should thoroughly review the history of the water body and surrounding land use. Considering the water body's history may help the water quality manager decide when, where and what to sample. This review may also help identify any current management strategies that might be changed to lessen the likelihood of an algal or cyanobacteria bloom occurring.

The left column of Figure 2 lists some common alterations to a water body's natural state. The middle column suggests how each of these alterations may influence the frequency and magnitude of cyanobacteria blooms, even if blooms naturally occurred before human-caused development.

[1] Many structural variants may be known for each toxin group.

[2] Not produced by all species of a particular genus

[3] Whole cells of toxic species elicit widespread tissue damage to kidney and lymphoid tissue

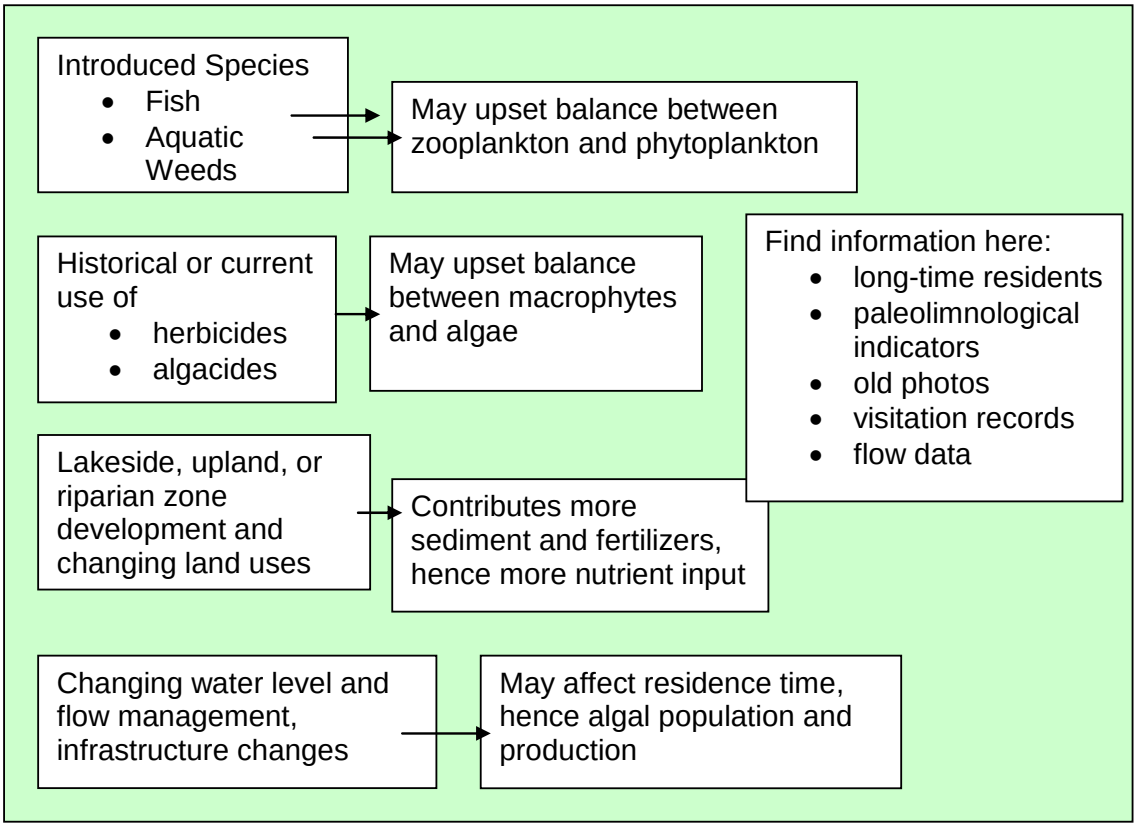


Figure 2: Aspects of historical and current lake management to consider before designing a monitoring program.

The right column in Figure 2 suggests information sources that water quality managers may find helpful as they make decisions about how to design their monitoring program. The following sources may provide clues about how water body management has changed:

- Review aerial photos (Find historical aerial photos at the University of Oregon Map Library: http://libweb.uoregon.edu/map/orephoto/consult.htm).
- Interview long-time residents about development and lake management.
- Review visitation records if a public park adjoins the water body.
- Review reports and public records from hydroelectric power providers.
- Review county tax and development records.
- Review irrigation and drainage district records.
- If budget allows, a paleolimnological analysis may provide signals of natural and human-caused alterations as well as an approximation of the natural state of the water body.

After a historical and landscape assessment of the lake and watershed, make direct observations of lake conditions to assess whether or not an algal bloom is occurring or imminent. The stage of bloom formation will dictate the most appropriate monitoring and management options, as presented in Table 2 (WHO, 1999).

Table 2: Information to assess whether a cyanobacterial problem exists or is likely, from WHO 1999.

Observation	Sources of Information	Management Options
1. Potential for bloom formation	Water quality monitoring data (nutrients, temperature, etc)	Basis for proactive management
2. History of bloom formation	Cyanobacterial blooms may follow marked seasonal and annual patterns	Can inform proactive management
3. Monitoring for Cyanobacteria and/or cyanotoxins	Turbidity, discoloration, cell microscopic identification, cell counts and toxin analysis provide increasingly reliable information	Possible only during event and enables only reactive management
4. "Scum Scouting"	In areas of high public interest the general public and untrained agency staff may play a role in identifying and reporting obvious hazards such as scums	Possible only during event and enables only reactive management
5. Reporting of animal deaths and human illness	Requires both volition and a mechanism for data collection which may not exist	Possible only during event and informs only reactive management
6. Epidemiological detection of disease patterns in the human population	Requires both effective reporting and large scale effects before detection is likely	Normally well after an event; can inform future management strategies

3.0 Developing a Monitoring Plan

The "Volunteer Monitor's Guide to Quality Assurance Project Plans" (EPA 1996) is a good reference for developing a monitoring plan. The first step in developing a monitoring plan should be to define project objectives. The questions you intend to answer with the monitoring data will follow from project objectives. Water quality managers should base their objectives and monitoring questions, at least partially, on the information gathered in the historical assessment and observation of the current bloom condition.

Public health concerns, as well as the desire for water quality information, will probably drive project objectives when monitoring cyanobacteria blooms. Before data is collected water quality managers should develop a plan to communicate that data to an appropriate audience. Example objectives that may apply to your project are to:

- assess and respond to public health concerns
- assess potential causes of the bloom and identify patterns in bloom development
- track compliance with established alert levels
- trace the effects of management changes.

The monitoring questions that arise from your project objectives may depend on whether a cyanobacteria bloom is present or just expected.

3.1 Before the Cyanobacterial Bloom

If a lake is not yet experiencing a cyanobacteria bloom, a water quality manager may have time to collect data that contributes to understanding the conditions that lead to cyanobacteria dominance. A monitoring design to document long term, or seasonal variability would be more appropriate than one focusing on short term spatial variation. The monitoring plan should emphasize collecting depth integrated samples that represent average conditions as well as those from discrete depths. Compiling climatic information over several years will also help water quality managers understand how the current year's conditions compare with long term averages. Public meteorological and hydrologic data are available from sources such as Oregon State Climate Service (http://www.ocs.oregonstate.edu/index.html) and US Geological Survey (http://or.water.usgs.gov/).

Some relevant questions one might attempt to answer by collecting and analyzing monitoring data are:

1. What are dominant algae species at different times of year?
2. What are dominant algae species at different depths in the lake?
3. How do total nutrient concentrations in the water column change throughout the year?
4. What are the most significant nutrient sources to the water body?
5. Does the lake stratify in the summer?
6. What are current hydrologic and weather patterns (precipitation, flow, temperature) and how do they compare with long-term averages?

3.2 During the Cyanobacteria Bloom

If a bloom occurs, then a lake manager will likely shift the monitoring focus to public health protection. Under these circumstances, appropriate monitoring should include samples that represent worst-case conditions in areas people are most likely to contact the water. Analyses from samples that represent areas of the lake without a visible cyanobacteria bloom will be helpful for risk communication to the public. A reactive monitoring design would collect data that could answer questions such as:

1. Where is the bloom most concentrated? Does that change based on wind direction?
2. What are the dominant species in the bloom?
3. Are the cyanobacteria cells producing toxins? At what concentration?
4. Are toxin concentrations changing with bloom growth and die-off?
5. Are there other associated concerns or patterns – pH, DO, taste and odor problems?

3.3 Representative Sampling Locations and Frequency

In either situation, before or during a cyanobacteria bloom, a water quality manager will need to choose appropriate locations that will provide sufficient and representative data to answer the monitoring questions. Depending on project objectives and budget, sampling locations may include surface scums, integrated and discrete subsurface samples, and sediment samples. A preliminary sampling design should consider physical lake characteristics such as:

- Lake shape. Lake shape may influence the movement of water and distribution of cyanobacteria and algae populations. A lake with few embayments and sheltered areas may require fewer sampling points than a lake with a more complex shoreline. One sample from a lake whose lakebed slopes regularly from shore to center may represent open-water conditions, while characterizing a waterbody with more complex lakebed topography (e.g. a reservoir) would probably require more samples. While a project budget may require depth integrated samples for a deep lake, discrete sampling may prove more informative in shallow lakes or at depths of particular interest.

- Tendency to stratify. The size, orientation, and depth of a lake will influence its tendency to temperature stratify. If seasonal lake profile information is not available, a lake manager should consider collecting this information before or during cyanobacteria bloom monitoring. A stratified lake provides several micro-environments that algae and cyanobacteria species may exploit. When the thermocline occurs within the euphotic zone (approximately two times the Secchi depth), this area may be particularly favorable for algae or cyanobacteria growth because of optimal nutrient, temperature and light conditions.

- Retention time. How long water remains in the lake will influence cyanobacteria growth. Shorter retention times, less than five to 10 days, are less likely to correlate with cyanobacteria blooms (WHO, 1999, Chap. 6). In general, the less time the water spends in the lake, the less frequent the need for sampling to characterize the situation.

Sampling frequency will also depend on visible changes, public health concerns, the desired schedule for risk communication, project budget, and likely analytical turn-around times. Table 3 outlines an example monitoring strategy that may be appropriate for a recreational water body with an active cyanobacterial bloom.

Table 3 Example Monitoring Strategy: Simplified from WHO guidelines (WHO, 1999, Chap. 13) and consultation with the Oregon Dept. of Human Services, Health Division.

Quantity of Cyanobacterial Species in Sample	Sampling for Cyanobacteria					Public Info/Outreach
	Location(s)	Considerations	Type	Frequency	Considerations	
500 - 1,999 cells/ml	Areas of likely use or contact	Thermal stratification Embayments	Cyanobac-terial Population	1/week	Confirm expected laboratory turn-around time Review results before increasing frequency	Internal communication of laboratory results. External communication with local and state health departments and management agencies.
2,000 – 20,000 cells/ml	Areas of likely use or contact Worst case area with scum formation Reference, open water sample	Thermal stratification Depth of euphotic zone Wind direction Embayments Potential bloom decay	Cyanobac-terial Population	1/week or as conditions visually change	Confirm expected laboratory turn-around time and day of week results will be available. Review results before increasing frequency	Internal communication of laboratory results. External communication with local and state health departments, managing entities, and other appropriate agencies. Post information sheets on and around waterbody.
			Cyanobac-terial Toxin	Collect and save up to 1 week	Laboratory turn-around time may be several weeks. Analyze for likely toxins based cyanobacterial population results.	
20,000 – 100,000+ cells/ml	Areas of likely use or contact Worst case area with scum formation Reference, open water sample	Thermal stratification Depth of euphotic zone Wind direction Embayments Potential bloom decay	Cyanobac-terial Population	As conditions visually change	Confirm expected laboratory turn-around time and day of week results will be available. Review results before increasing frequency	Internal communication of laboratory results. External communication with local and state health departments, managing entities, and other appropriate agencies. At 100,000 cells/mL, post advisory on and around waterbody. Consider posting early in the week during recreational season. Results may warrant closure or partial closure.
			Cyanobac-terial Toxin	Collect and save up to 1 week	Laboratory turn-around time may be several weeks. Analyze for likely toxins based cyanobacterial population results.	

4.0 Sample Collection, Processing and Shipping

Data collection may involve a range of techniques depending on the stage of the bloom formation and the project budget. Visual monitoring and simple field tests may be effective for triggering increased sampling frequency. Much of the analytical work, such as nutrient analysis and algal identification, is relatively inexpensive, though the analysis does require specialized skills and equipment. Toxin analysis will likely be less common in a monitoring program because of expense and longer turn-around times.

4.1 Visual and Remote Observations

A visual assessment of the water body, looking for areas of discoloration or surface scum collection, can provide valuable preliminary information. These observations could involve a homeowner or other volunteer-based program, as well as lake managers. Tracking Secchi depth or turbidity measurements regularly may indicate an imminent bloom. With a relatively small investment of a microscope, water quality managers can be trained in on-site identification of the most common cyanobacteria species.

With a larger project budget, remote sensing technologies may be used for the early detection of cyanobacterial blooms. The current sensor technologies not only allow for the detection of chlorophyll *a*, but also the detection of the cyanobacterial specific pigments, phycocyanin and phycoerythrine. Phycocyanin is typically indicative of freshwater cyanobacteria and phycoerythrine is typically indicative of marine cyanobacteria. Ground truthing samples are still required to make full use of remotely sensed imagery due to the differences in the physical and chemical properties of each water body. The resource list at the end of this protocol provides sites and contacts where one can find more information about remote sensing for cyanobacteria blooms.

4.2 Biological Analysis

After preliminary observations and possibly the confirmed presence of cyanobacteria species, water quality managers should consider submitting samples for quantitative algal/cyanobacterial identification. If taxonomic identification indicates that the cyanobacteria dominate the plankton community, water quality managers may use chlorophyll *a* analysis as a surrogate to track bloom growth.

The contracted laboratory will likely provide the water quality manager with appropriate dark bottles already containing sufficient Lugol's solution to preserve the samples. If that is not the case, Figure 3 provides the recipe.

Figure 3: Lugol's Solution.

Lugol's solution is commonly used for short-term (e.g. a few months, but possibly a year or more) storage of cyanobacteria. Dissolve one gram of iodine crystals and two grams of potassium iodide in 300 ml of water. Use three drops of this solution in a 100 ml sample (it should look like very weak tea).

Hazards: Harmful if swallowed. May cause irritation. Avoid breathing vapors, or dusts. Use with adequate ventilation. Avoid contact with eyes, skin, and clothes. Wash thoroughly after handling. Keep container closed. Conditions aggravated/target organs: Persons with pre-existing skin, eye, thyroid, and respiratory disorders, as well as persons with iodine allergies, will be more susceptible. May cause skin and eye irritation. Conditions aggravated: Thyroid disorders,

After a sample is capped in dark bottle with Lugol's solution, the sample can be stored at room temperature until identification. The resource list at the end of the protocol provides contact information for field/laboratory supply companies from which water quality managers can purchase appropriate bottles.

If the monitoring strategy calls for actions based on quantified levels of cyanobacteria, the water quality manager should confirm with the taxonomic laboratory that they will report toxin-producing cyanobacteria in "cells/mL." Laboratories may also present results as density (number colonies/mL or natural units/mL) or biovolume (μm^3/mL). With an approximate cell volume measurement, one can estimate "cells/mL" from biovolume units (Jim Sweet, Aquatic Analysts, personal communication, April 2005). The taxonomic laboratory may be able to provide average cell volume measurements or calculate an approximate cell count from data reported as biovolume.

4.3 Chemical Analysis

Chemical analysis can complement the biological information by providing information about potential causes and consequences of the cyanobacteria blooms. A monitoring program should include analysis of nutrient concentrations, particularly nitrogen and phosphorus. If nutrient information has already been collected, and the water quality manager has a good understanding of seasonal fluctuation and rate of change, the bloom monitoring program may require little additional nutrient sampling. Sufficient nutrient data to calculate the ratio of nitrogen (N) to phosphorus (P) before and during the bloom may be useful for evaluating whether or not a low N:P ratio (in general, lower than 10:1 molecules) may be one of the causes of the bloom (WHO 1999, Chap. 2).

Water quality managers may wish to incorporate toxin analysis into their monitoring plan when observations or biological analyses indicate rapid bloom growth and potential die-off. Consult first with the contract laboratory and confirm the required sample volume. Collect samples for toxin analysis in 1-liter dark polyethylene bottles and transport them on ice (4ºC). Do not chemically preserve or freeze the sample as this would cause cells to lyse. Lysing cells makes quantification of the dissolved toxin and intracellular toxin indiscernible. Send samples on ice overnight to the contract laboratory. Sample holding time is approximately one week, as long as samples are kept in the dark and at 4ºC.

4.4 Field Procedures

Most cyanobacteria monitoring programs will follow general surface water or lake sampling procedures. Good references for field procedures are DEQ's Methods of Operations Manual (MOMs, DEQ, 2004), Chapters 3 (Surface Water) and 5 (Lakes) and DEQ's Field Sampling Reference Guide (DEQ, 1998). MOMs Chapter 3 also describes the procedures for additional field analysis (pH, DO, alkalinity, etc.) and preparation of samples for nutrient analysis.

Necessary equipment will depend on the surface water body being sampled and the parameters being tested. Appendix A lists potentially applicable equipment and supplies for a cyanobacteria monitoring program. Sampling devices may include stainless steel buckets, pumps, or discrete depth samplers, depending on site accessibility and water body characteristics. Any devices should first be cleaned and rinsed with deionized water. The sampling plan should include analysis of blank samples drawn from blank water poured into the sampling devices.

Metadata recorded at the time of sampling will be important when interpreting the analytical results. Record the date and time of sampling in a field notebook and the precise sample location (latitude and longitude by GPS). Also record the weather, air temperature, and wind characteristics.

Please direct questions and comments regarding this guidance to:

Karen Font Williams
Oregon Department of Environmental Quality
Northwest Region, Water Quality
(503) 229 – 6254
williams.karen@deq.state.or.us

And

Dave Gilbey
Oregon Department of Environmental Quality
Water Quality
(503) 229 – 5358
gilbey.david@deq.state.or.us

References

U.S. Environmental Protection Agency, 1996. Volunteer Monitor's Guide to Writing Quality Assurance Project Plans, EPA 841-B-96-003.

World Health Organization, 1999. Toxic Cyanobacteria in Water; A Guide to their Public Health Consequences, Monitoring and Management.
http://www.who.int/docstore/water_sanitation_health/toxicyanobact/begin.htm

Oregon DEQ, 2004. Methods of Operations Manual
Oregon DEQ, 1998. Field Sampling Reference Guide.
http://www.deq.state.or.us/lab/qa/techdocs.htm

Resources

Academia
www.clr.pdx.edu
http://water.umn.edu/

Laboratory Analysis
http://www.wright.edu/biology/faculty/carmichael/labhome/labhome.htm Wayne Carmichael's Lab
http://www.wright.edu/biology/faculty/carmichael/labhome/CyanoHAB%20Services.htm
http://www.deq.state.or.us/lab/lab.htm - Contact Gene Foster (503) 229 – 5983 x273

Monitoring Equipment and Supplies
www.turnerdesigns.com
www.HACH.com
Other supply vendors: Wildco, VWR, Fisher Scientific, Ben Meadows, Forestry Supply

Oregon Department of Human Services - Health Division
http://www.dhs.state.or.us/publichealth/esc/docs/maadvisories.cfm

CDC Center for Disease Control
http://www.cdc.gov/hab/default.htm

Oregon Department of Environmental Quality
www.deq.state.or.us

Internet Resources

Oregon Cyanobacterial List Serve Group
Contact Dr. Mark Systma (systmam@pdx.edu) at Portland State University to be added to Cyanobacterial listserv

World Health Organization
http://www.who.int/docstore/water_sanitation_health/toxicyanobact/begin.htm

Oregon Lakes Association
www.oregonlakes.org

Cyanocite
http://www-cyanosite.bio.purdue.edu/

Appendix A – Equipment Checklist

Sampling Equipment

- Stainless steel buckets and rope to lower
- Secchi disk
- Van Dorn bottle with messenger (or peristaltic pump)
- Phytoplankton net
- Hauling system to raise and lower probes and samplers – calibrated rope and pulley, hydraulic or electric winch.
- Water quality probes and meters – single parameter (e.g.,conductivity/temperature, light) or multiparameter (e.g., YSI, Hydrolab).

Sample bottles

- Dark polyethylene 1L bottles
- Dark polyethylene 250 mL bottles with Lugol's sol'n preservative
- DO, BOD bottles and BOD bottle caps
- Basic polyethylene l liter
- Nutrient polyethylene 500 mL
- Dissolved phosphate polyethylene 250 L

Reagents and supplies*

- Small dropper bottle of concentrated H_2SO_4 (for preservation)
- Dissolved Oxygen (By Winkler Titration)
- Alkalinity and pH
- Conductivity/Salinity

Filtering

- Glass filter jar
- Filter base & top and rubber stopper
- Hand vacuum pump (With vacuum gage for chlorophyll *a*)
- 250 mL graduated cylinder
- Filters:
    - membrane for dissolved metals, boron, silicon, chlorides, or sulfates
    - glass fiber for dissolved orthophosphate only and chlorophyll
- Chlorophyll *a* supplies:
    - Numbered plastic petri dishes
    - Aluminum containers for petri dishes shipping
    - Dry ice and dry ice container

Miscellaneous

- Stainless steel stirring spoon
- Paper to wrap glass bottles in for shipping
- Distilled water and container
- Blank water and container
- Waste water container
- Towels
- Ice Chests
- Wet ice
- DO saturation tables
- Calculator
- Clip board
- Field notebook
- Shipping/chain of custody forms
- Bathymetric map
- Rain gear
- Hip boots
- Cell phone
- GPS unit

*See MOMs methods for procedures and supplies