Appendix E: KTWQP Nutrient, Phytoplankton, Periphyton and Algal Toxin SAP

Aquatic Analysts

Algae Analytical and Quality Assurance Procedures

August 24, 2007

Sample Handling

Sample Collection and Preservation

Phytoplankton are 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 21,000 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 30 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 upon request. 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:

$$TSI\ (biovolume) = (\text{Log-base } 2\ \ (B+1)) * 5$$

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. On occasion, we also contract independent algae analysts for second opinions on some difficult to identify algae species.

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 21,000 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.