The purpose of this document is to describe the procedures and methods to measure the nutrient variables used to assess streams and large rivers.

2.0 Responsibilities

Bureau personnel who conduct nutrient sampling activities or who supervise those who do are responsible for implementing this procedure.

3.0 Background and Precautions

Nutrient survey and sampling corresponds to two levels of assessment. Level I is a screening level survey that includes qualitative observations and determination of algal biomass and measurements of chemical parameters. Level II involves quantitative measurement of algal biomass, long term Sonde deployment and nutrient sampling.

Nutrient Level I Screening should be conducted once per season (spring, summer, and fall) with at least two screenings by the end of June. This ensures that data are available for Level I assessments to be completed before the biological sampling index period (BSIP). All nutrient survey and sampling field activities will be conducted in accordance with this SOP.

4.0 Definitions

Algae are non-vascular plants with no true roots, stems, or leaves. They are mostly aquatic and range from tall stalks of kelp to fuzzy growths of green filamentous algae to microscopic silica encased diatoms. In the context of this SOP, algae refers to the visible growth of non-rooted aquatic vegetation attached to the stream substrate.
Biological Sampling Index Period (BSIP) includes the time of year when biological samples (periphyton, fish, and macroinvertebrates) are collected for stream biological assessment.

Emergent macrophytes grow on the substrate of rivers and streams in depths of water generally less than a meter and are typically rooted in the sediment with a portion of the plant in the water and part extending into the air. Common emergent macrophytes include plants such as reeds (Phragmites spp.), bulrushes (Scirpus spp.) and cattails (Typha spp.).

Filamentous algae are algae that grow as fibers or strands of cells forming filaments or mats that are attached to substrate. The filaments can either be branched or unbranched.

Macrophyte is a general term that applies to many types of aquatic vegetation including flowering vascular plants, mosses, and ferns and are primarily the large aquatic vegetation rooted in the substrate, although they are also represented by small leaves with roots floating on the surface (duckweed and water fern). Four categories of macrophytes are defined by their relationship to the air, water, and substrate: emergent, floating-leaved, submerged, and freely floating (USEPA 2000).

Periphyton is an assemblage of organisms that grow on underwater surfaces and includes algae, bacteria, fungi, protozoa, and other organisms. In the context of this SOP, periphyton refers to the slime layer or biofilm growing on substrate. It is composed primarily of microscopic organisms while the algae, noted in the percent coverage field, are mainly macroalgae.

Phytoplankton are small (often microscopic) aquatic plants that are suspended in water.

Rapid Bioassessment Protocol (RBP) is the protocol developed by the USEPA for collecting, processing, and assessing fish, benthic macroinvertebrates, and periphyton from wadeable streams. (Barbour et al 1999).

5.0 Equipment and Tools

Refer to the Rivers/Streams MASS Equipment Checklist for the necessary field equipment.

6.0 Process Description

Nutrient surveys will be made in and around a riffle, unless riffles make up less than 25% of the reach or the velocities in the riffles are so great that algal growth is limited (i.e. velocities exceed 1.75 ft/sec) and more algae are found in glides than in riffles. The Rapid Bioassessment Protocol (RBP) states that, “single habitat sampling should be used when biomass of periphyton will be assessed,” and, “the recommended substrate/habitat combination is cobble obtained from riffles and runs with current velocities of 0.33 - 1.6 ft/sec” (Barbour et al 1999). If the velocity appears to be limiting algal growth or riffles make up less than 25% of the reach length, then select a glide (a length of stream with intermediate velocity) in which to make observations. If access to the reach is limited, use a transect across the stream at the sample location. However, effort should be made to find a representative riffle whenever possible. The methods for examining and rating algal and periphyton cover are described in section 6.1 below.

The biomonitoring index period (BIP) was created to provide a consistent window in which biological populations are sampled. This is necessary because seasonal variation in abundance and distribution of biological populations, particularly benthic macroinvertebrates (BMI), can pose challenges when comparing results between sites, over time, or against metrics developed from biological samples taken during a specific time period. The BIP has historically been established as August 15-October 15 for Mountain sites and August 15-November 15 for Foothills and Xeric sites. However, the window between scouring flow events (typically June-August) and the end of the BIP can be restrictive and result in data gaps.
A “scouring flow event” is defined as flow that scours course substrate and impacts aquatic communities. SWQB determines this as follows:

The occurrence of recent scouring of course substrate can be determined with an evidence based-approach. Note recent depositional features, incisions, downcuts, wrack lines at or above assessed bankfull, absence of periphyton growth, folded in-channel vegetation, folded vegetation on the floodplain, and translocation of the current year leaf litter on active floodplain. If nearby gage data are available, note any high flows with gage height 3x the previous week’s average. The occurrence of four or more of the above factors is considered a scour event. Attempt to pin down the timing of the event with gage data or new vegetation emergence. If timing cannot be determined, assume that the event occurred the previous day and schedule resampling accordingly.

If BMIs are scheduled to be collected at a particular site, then Nutrient Level 2, physical habitat and BMI data will be collected simultaneously during the BIP and at least six weeks after a scouring flow event.

If BMIs are not planned or needed at a site, Level 2 Nutrient and sediment surveys may be taken during baseflow conditions, post snowmelt runoff, and prior to the monsoon season (i.e. outside of BIP) at least six weeks after a scouring flow event. Further, periphyton are considered part of a Level 2 Nutrient assessment rather than biological monitoring and be subject to growing season, rather than BIP restrictions.

### Table 1. Established Growing Seasons for Ecoregions of New Mexico.

<table>
<thead>
<tr>
<th>Class</th>
<th>Ecoregion</th>
<th>Biomonitoring Index Period (BIP)</th>
<th>Growing Season Start</th>
<th>Growing Season End</th>
<th>Growing Season Length</th>
<th>1st Chloro-a Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mountain &gt;7,500 ft</td>
<td>22 &amp; 23</td>
<td>August 15-October 15</td>
<td>1-July</td>
<td>15-Oct</td>
<td>3 ½ months</td>
<td>July 15</td>
</tr>
<tr>
<td>Mountains &lt;7,500 ft &amp; Plateau</td>
<td>20, 21, 22 &amp; 23</td>
<td>August 15-November 15</td>
<td>15-Jun</td>
<td>1-Nov</td>
<td>4 ½ months</td>
<td>July 1</td>
</tr>
<tr>
<td>S. Deserts and Plains</td>
<td>24, 25, 26, &amp; 79</td>
<td>August 15-November 15</td>
<td>15-May</td>
<td>15-Nov</td>
<td>6 months</td>
<td>June 1</td>
</tr>
</tbody>
</table>

1. Nutrient Level 1 data may show full support
2. Nutrient Level 2 data may show full support
3. Sonde deployment may be initiated for Nutrient Level 2

The growing season is generally defined as the days between last and first occurrence of 0° Celsius for an ecoregion. SWQB used National Oceanic and Atmospheric Administration (NOAA) data to determine growing seasons for New Mexico based on spring and fall freeze data. NOAA median last and first frost dates were assigned to the different Level 3 Omernick ecoregions and ecoregions with similar dates were grouped together. The average of each grouping was used to determine the growing season for the grouped ecoregions. Ecoregions 22 and 23 were highly variable and thus split at 7,500 feet elevation.

Established periphyton can develop rapidly with adequate nutrients, temperature, and light. SWQB observed rapid growth early in the growing season and determined that two weeks into the growing season was an appropriate buffer to allow sufficient periphyton development before beginning Level 2 sampling.

### 6.1 Level I Nutrient Survey: Field Measurements and Qualitative Observations

The Level I Nutrient Assessment uses a qualitative determination of algal biomass in addition to instantaneous field measurements of dissolved oxygen (D.O.) concentration, percent D.O. saturation at the local elevation, and pH as well as samples for total phosphorus and total nitrogen. Typically, samples
are collected and analytes measured as part of a general water quality survey. Sample collection methods for the parameters listed above are described in the *Sonde Deployment* (6.2) and *Chemical Sampling* (8.2) SOPs. There is no separate form for the Level I Nutrient Survey as the data are recorded on the Stream Field Data Form and Supplemental Field Sheet and compiled in the SWQB Water Quality Database (NMEDAS) and survey binder.

Level 1 surveys may be conducted outside of the growing season and independent of any other data collection activities. They are necessary to determine if a Level 2 Nutrient survey is required for an assessment unit (AU). Level 1 surveys may be performed throughout the survey season; however, they must be conducted after the beginning of the growing season to determine “Full Support” for nutrients. This is because the periphyton may not have had sufficient time to develop prior to the beginning of the growing season. “Non-Support”, however, may be shown at any point during the sampling season. Additionally, a Level 1 survey may not be used to determine “Full Support” if conducted less than six weeks after a scouring event. Table 1 provides established growing seasons for ecoregions of New Mexico.

6.1.1 Periphyton and Algae

A qualitative algal biomass survey is part of the Level I Nutrient Survey and is conducted as part of regular water quality surveys. The information is recorded on the Supplemental Field Sheet which is included as part of the Chemical Sampling SOP (8.2). An estimate of percent algal coverage and a rating of periphyton thickness are qualitative indicators of algal biomass. This survey should be conducted at each sample site and completed once per season, i.e. in spring, summer, and fall. These seasons refer to the character of the hydrograph for a given waterbody. Typically in New Mexico, spring is characterized by higher flows resulting from snow melt, whereas summer and fall are characterized by low flow conditions interrupted by short duration high flow events from seasonal rain storms. During the summer nutrient survey, the study lead should note which, if any, sites might need to be assessed using a reference approach due to high productivity caused by nutrient loading from natural sources such as springs or nutrient rich geologic formations. This will allow for scheduling of appropriate Level II Nutrient Surveys to be conducted at the test site and a suitable reference site.

Step-by-Step Process for Rating Periphyton and Algae:

1) Select a suitable riffle. If the velocity appears to be limiting algal growth or riffles make up less than 25% of the reach length near the water quality sample site, select a suitable glide.

2) Starting at either the top or bottom of the selected reach, visualize a line running diagonally through the reach. Select a landmark on the far bank that corresponds to the end of the diagonal transect. Note the midpoint, ¼, and ¾ distance. Five observations will be made along this diagonal: near the left bank, at ¼, ½, and ¾ the width, and near the right bank. In this manner, locations across the length and cross-section of the reach will be observed.

3) From your starting point (on the bank at either the top or bottom of the diagonal through the reach) and at each of the 5 locations along the diagonal observe as far as you can see along the transect across the stream and estimate the percent algal cover in the wetted width. Look for strands of non-rooted aquatic vegetation attached to the substrate and determine if they cover <25%, 25-50%, 50-75%, or >75% of the stream bed.

4) Take a step into the stream, reach down and pick up the cobble or large gravel nearest the front of your foot. Observe the particle and look and feel for the presence of slime. Rate the periphyton thickness on the substrate using the following scale (Stevenson 1996):

Periphyton Rating:
- 0 indicates substrate is rough with no apparent growth;
- 1 indicates a thin layer of microalgae is visible (tracks can be drawn in the film);
5) Walk along the diagonal, stopping at ¼, ½, and ¾ the width, and near the far bank and make observations of both the algal cover across the wetted width and periphyton thickness on the substrate. If you cannot safely wade across the entire reach, go as far as possible then turn back and continue observations on a diagonal back to the bank at the opposite end of the reach from where you started. For reaches that you cannot wade halfway across, pick up and/or examine the substrate in 5 locations that you can reach and rate the algal and periphyton cover as described above. The observations should be made over an area that is approximately as long as the river is wide.

6) Once you reach the end of the transect or make 5 observations at sites that are non-wadeable, record the average percent algal coverage and periphyton rating on the Stream Field Data Form (Supplemental Field Sheet).

7) Note where in the stream growth is occurring (e.g., in low flow areas, only near seeps, on fine substrate, only on large stable substrate, etc). The physical characteristics of the substrate can influence the amount of algal coverage. As some substrates are too soft or unstable for algae to anchor to, the overall percent algal coverage may reflect the amount of stable substrate in the stream rather than the potential biomass.

6.1.2 Anoxic Layer

As the aquatic vegetation observations are conducted, check under stones and in depositional areas for the presence of an anoxic layer. Anoxic conditions are characterized by a black color and rotten egg smell associated with hydrogen sulfide. Note on the Supplemental Field Sheet the presence or absence of an anoxic layer and the location.

6.1.3 Water Chemistry and Flow

If the Assessment Unit (AU) being surveyed is not part of a current water quality survey, take instantaneous measurements of D.O. concentration, percent D.O. saturation at the local elevation, and pH as described in the Sonde Deployment (6.2) SOP. Additionally, collect water samples for analysis of Total Phosphorus, Total Kjeldahl Nitrogen and Nitrate + Nitrite as described in the Chemical Sampling (8.2) SOP. Fill out a Stream Field Data Form. If there are no gages in the AU, take flow measurements as described in the Flow (7.0) SOP. Fill out a Flow Field Sheet.

6.2 Level II Nutrient Survey: Quantitative Measures

The Level II survey involves quantitative measurement of selected indicators. A Level II Survey will be conducted if the Level I Assessment indicates that nutrient impairment may be occurring in an AU or if the AU was previously listed for nutrient impairment.

Level 2 surveys may be conducted beginning two weeks into the growing season (Table 1) and at least six weeks after a scouring event, including initiation of the >72 hour sonde deployment. Sondes may not be deployed to show “Full Support” less than two weeks into the growing season because periphyton may not have sufficient time to develop and impact physical variables such as dissolved oxygen and pH. However, a particularly eutrophic AU may show “Non-Support” prior to the initiation date due to early periphyton growth.

If it is determined that BMI collection and a Level 2 Nutrient survey are required for a particular AU, this sampling event must be conducted during the appropriate BIP and completed simultaneously. Additionally, a Level 2 survey may not be used to determine “Full Support” if conducted less than six weeks after a scouring event.
Step-by-Step Process to Collecting Nutrient Data:

1) Sonde Deployment
A multi-parameter meter (sonde) should be deployed for at least 72 hours and set to record in 15 minute intervals D.O. (concentration and percent local saturation), pH, specific conductance, temperature, and turbidity values. Fill out a Sonde Deployment-Retrieval Field Sheet. Refer to *Sonde Calibration and Maintenance (6.1)* and *Sonde Deployment (6.2)* SOPs.

2) Water Chemistry and Flow
If the AU being surveyed is not part of a current water quality survey, water samples should be collected for analysis of Total Phosphorus, Total Kjeldahl Nitrogen and Nitrate + Nitrite. Fill out a Stream Field Data Form. Refer to the *Chemical Sampling (8.2)* SOP for sample collection and preservation methods. If there are no gages in the AU, take flow measurements as described in the *Flow (7.0)* SOP. Fill out a Flow Field Sheet.

3) Periphyton Sampling
Collect a sample of benthic algae and fill out the Habitat & Biota Worksheet. It is important to accurately measure and record the area sampled so the final concentration per area of substrate can be calculated. The methods for collecting and handling periphyton samples from both wadable and non-wadable systems are described in the *Biological Sampling – Periphyton (Section 11.2)* SOP. Note the habitat type and dominant substrate at the sampling location. Note the presence and location of watercress in the stream as it can indicate the presence of springs that may be a natural source of nutrients. Watercress is an emergent macrophyte that can be identified by the following (USDA 1988): (1) it has small whitish flowers with four petals, several arranged closely, (2) has roots from stem nodes, and (3) has pinnately divided leaves with very large terminal leaflets on mature leaves. Note the location of springs, wetlands, and upwelling areas. Upwelling areas may be indicated by patches of dense algal growth.

4) Algal Bioassays (OPTIONAL)
If stream observations indicate that algal biomass is a problem in the stream or an NPDES permit is being written for the Assessment Unit, a limiting nutrient analysis and algal growth potential test may be performed (USEPA 1975 and 1978).

Two one-gallon samples of water (no acidification) should be collected and immediately stored on ice. General methods for collecting surface water samples are described in the *Chemical Sampling (8.2)* SOP. Deliver samples to UNM Biology Department within two days of collecting the samples. Please call Dr. Barton @ 505-277-2537 before taking the samples to his lab. Place the results in the survey binder when received.

### 6.3 Quality Control Activities

Quality control activities for water samples and instantaneous field measurements are described in the *Chemical Sampling (8.2)* SOP and the *Sonde and Thermograph (6.2)* SOP, respectively. Quality control activities regarding Level II periphyton sampling are described in the *Biological Sampling – Periphyton (Section 11.2)* SOP.

### 7.0 Related Forms

There are no forms specific to this SOP however the following forms from other SOPs are necessary to complete this SOP:

- Sonde Deployment Instructions (from SOP 6.2)
- Sonde Deployment-Retrieval Field Sheet (from SOP 6.2)
- Habitat & Biota Worksheet (from SOP 5.0)
Stream Field Data Form (Supplemental Field Sheet) (from SOP 8.2)
Equipment Checklist (from SOP 5.0)
Flow Field Sheet (from SOP 7.2)

8.0 Revision History

Revision 1. Updated index period guidelines.
Revision 2. Minor language clarifications.

REFERENCES


