Soft Bodied Algae and Diatom

Identification, Enumeration, and Processing

Standard Operating Procedure

EA-SOP-2016
Revision 1

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Table of Revisions

Revision Number	Revision Date	Revisor	Reason for Revision
1	20190304	M. Payne	Including comprehensive language for the NEON project

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1.0 Sample Preparation

1.1 Sample receipt, importation, and data entry

Before a sample is processed, a Sample Receiver confirms the following information with the packing slip or chain of custody form against each sample container received: sample identification number, pertinent location and replicate information, sample date, habitat type, area sampled, and sample volume, if available. Notes on sample condition are also noted. Chain of Custodies/Receipt Logs are then signed and returned to the client via data portal. Samples placed in 4° storage.

The sample information is then imported into EcoAnalysts' Laboratory Information System, AEGIS, which allows the generation of labels containing unique sample identification numbers. Sample receipt and location is also tracked in a software system, Ajera. Samples are then labeled and will remain in 4° storage until analysis has begun.

Technicians and Taxonomists record sample specific information into a software data system, SIMs, which carries over the sample identifiers generated from AEGIS.

When new bottles of chemicals are opened, the open date must be written on the bottle. Do not use chemicals that are past their expiration date. Use proper personal protective equipment when handling chemicals, including gloves, lab coats, and fume hoods. Ensure chemicals are disposed of properly.

1.2 Measuring Initial Sample Volume

Sample volume provided by the client is used unless the client requests that the sample volume be re-verified.

1) Carefully measure sample volume using a graduated cylinder of appropriate size and recorded on the data sheet.
2) Return sample to its original container using distilled water, reseal, and properly store for further analyses.
3) Wash glassware and rinse with deionized water before taking volume measurements from additional samples.

1.3 Subsampling for Soft-Bodied Algae and Diatom Splits

1) Shake sample vigorously to evenly disperse the algae.
2) Remove subsample from the middle of the dispersed sample and place into a vial labeled with the sample ID number and the appropriate suffix, "-soft" or "diatom".
    a) If the sample is silty, remove a 10 mL subsample.
    b) If the sample is clear, remove a 20 mL subsample.
    c) EcoAnalysts samples a minimum of 10 mL for soft algae and a minimum of 10 mL for diatoms.
3) Record subsample volume on the vial and data sheet for both soft body algae and the diatom subsamples.
4) Return remaining sample to the original container and set aside to be shipped back to the client upon the completion of the project, if requested.

2.0 Identification and Enumeration of Soft Algae

This section describes EcoAnalysts' process for counting and identifying soft algae to the lowest practical taxon, and identification and enumeration of live diatoms to genus.

2.1 Palmer Maloney Counting Chamber (Primary Method)

2.1.1 Achieving Desired Cell Density

1) Gently invert sample a minimum of 30 times to homogenize sample for even distribution of cells.
2) Place exactly 0.1 mL of homogenized sample in a Palmer Maloney counting chamber using a micropipette. Microalgae may sometimes contain clumps composed of filamentous and colonial algae, which interferes with random distribution, as well as accurate identification and enumeration. Multiple species comprised in a clump must be partitioned on the cover slip using a pair of fine tipped forceps prior to identification.
3) Examine at 400X magnification using a compound light microscope to assess if periphyton is too dense or dilute for identification and enumeration. Dilute or concentrate sample as necessary to achieve desirable cell density (approximately 15-20 counting units per field of view). If dilution or concentration is needed, record the new volume and concentration ratio on the data sheet. Dilution is achieved by incorporating more DI water into your aliquot. Concentrate a sample by adding more sample volume to the aliquot, record this initial volume, centrifuge for 40 minutes, siphon off the supernatant, and record the final volume.

2.1.2 Counting Soft Algae

1) Count and identify soft algae and diatoms to the lowest practical taxon using the transect method until at least 300 natural units are encountered. For colonial algae, count each colony as one natural unit and report the total number of cells in each colony (except in cases where cells/colony are exceedingly high, then estimate based on the number of cells in each 10 by 10 micron area of the colony), or in the case of filaments, record each filament as one natural unit and report the number of cells per filament in each transect (where cells are not clearly delineated, cells will be defined as 10µ lengths and noted in data). Diatoms will be identified to genus where possible. Dead diatoms will be recorded.
2) Counting and identification is made at 400X magnification using a Leica compound light microscope with DIC and the latest taxonomic references. Record all identifications and counts in counting software. Please differentiate between "live" diatoms that still have the presence of protoplast, or mark as "dead" if the frustule is empty. Diatoms marked as "dead" will not be included in the 300 count.
3) Create a digital image reference collection. Images should include a scale bar, species name, and reference used. Image File should include the Client sample ID and Species name.

3.0 Oxidation (Cleaning) Methods for Diatoms

This section describes the necessary preparation to count and accurately identifying diatoms to the lowest practical taxon. The primary method of cleaning diatoms with the use of hydrogen peroxide is the preferred method and is effective on most samples received. Diatoms that are heavily sedimented may not come clean with the use of hydrogen peroxide and the secondary cleaning method may be implemented. If samples contain lots of flocculant material burn mounting of diatoms may implemented. Chosen method should be noted against the samples.

Diatom cleaning -Wear goggles, gloves, lab coat, perform under hood

3.1 Primary Cleaning Method: Hydrogen Peroxide/Potassium Dichromate Oxidation

Note preservative type (typically either formaldehyde or gluteraldehyde based) for proper treatment and disposal of material accumulated in the aspirator flask (as a result of siphoning supernatant material from the individual centrifuge tubes) as needed. Gluteraldehyde based preservative waste is disposed of in the labeled yellow container; formaldehyde based preservative waste is disposed of in the labeled 2-gallon container under the hood and neutralized with form-zero.

1) Separation and Disposal of Preservative Material.
    a. If your original total sample volume is 30 ml or greater: Agitate the sample by inverting and up-righting the sample container vigorously 5 times, then pour a 15 ml volume of well mixed sample into a 40 ml graduated plastic centrifuge tube with appropriate label (e.g., Sample ID number). If your original total sample volume is less than 30 ml, mix as above, and pour no more than ½volume into the centrifuge tube as above making note of volume used on the diatom slide map, and in the project file.
    b. Bring volume to 35 ml line with DI H2O. Centrifuge at 1500 rpm for 20 minutes. Diatoms are present in the plug at the bottom of the centrifuge tube.
    c. Carefully siphon off the supernatant material using the aspirator and vacuum flask apparatus setup under the hood. Note: monitor fluid level in the vacuum flask to allow for proper vacuum and avoidance of waste material uptake in hosing. When turning on or off water at the tap, make sure that the hosing is unclamped and that the pipette is not in liquid. Take care not to disturb the concentrated diatom material – it is advised not to siphon below the point of narrowing (approx. 2.5 ml line) of the centrifuge tube.
2) Oxidation of Organic Matter with H2O2 and Heat. Note: H2O2 is a strong oxidizing agent. Wear safety goggles and gloves. Handle H2O2 and heat under the fume hood.
    a. Add sufficient DI H2O (deionized water) to the centrifuge tube containing sample material to bring the volume to 15 ml (or less depending on the sample volume cleaned in Step 1.a.)
    b. Seal the tube with cap, shake to mix and pour sample material from the centrifuge tube into a respectively labeled beaker (approx. 200 ml size).
    c. Add 15 mls of 30% Hydrogen peroxide to the centrifuge tube as a rinse and pour into the beaker containing the sample. Place beaker onto the hotplate and allow to heat at 90-110 C (set the hot plate remote dial to ‘2’) for 2 hours until all organic matter is removed. Wait to begin timing until after bubbling and steaming is observed. Use

glass petri plates to cover the beakers and protect samples from contamination during the heating and cooling process.

3) Cooling of Cleaned Diatom Material and Inspection.
    a. Turn off the hotplate and allow the samples to cool overnight.
    b. Note: samples with heavy organic content may require a longer heating period and additional H2O2. In order to tell if organic material is removed, use a clean fine tip glass pipette to take a minute bit of settled material from the beaker bottom, prepare a wet mount using glass slide and cover slip, and examine under the scope to ensure diatom frustules are clear.
    c. If clear, proceed to Step 4.
    d. If not clear, centrifuge, siphon to approximately 5 ml taking care not to disturb sediment plug and repeat Step 2.
4) Sample Rinse.
    a. Mix and pour oxidized sample material from a beaker into a respectively labeled cleaned graduated 40 ml centrifuge tube. Rinse the beaker with a small amount of DI H2O and add to the centrifuge tube with sample taking care not to exceed the 35 ml volume line. If less than 35 ml, add DI H2O to bring up to the 35 ml line.
    b. Mix and centrifuge for 20 minutes at 1500 rpm.
    c. Carefully siphon off supernatant from centrifuge tube (Note: take care not to disturb the sediment plug). Refill to the 35 ml line with DI H2O, mix and centrifuge again for 20 minutes at 1500 rpm. This is the first full rinse.
    d. Repeat Step 4.c. This is the second full rinse.
    e. After the second full rinse, check pH for neutrality (pH about 6-7), siphon off the supernatant as before, i.e., down to 3 ml (the bend 'line' of the centrifuge tube).
5) Check for Diatom Density.
    a. Mix sample well. Take 100 µl of sample material, place on slide with cover slip and examine under the scope for diatom cell density. This is your opportunity to determine if the sample should be left at this concentrated volume relative to your start volume or brought back up with DI H2O to your start volume (typically 15 ml).
    b. NOTE: If diatoms are so scarce so as to be difficult to find, leave at this volume and determine a concentration factor for calculation of actual sample volume represented. Remember that if you start out with a sample volume in Step 1 of 15 ml, and stop here with a cleaned sample volume of 3 ml, your concentration factor would be 5, i.e., 1000ul of this cleaned concentrated sample represents 5000 µl of the original sample material.
    c. Otherwise, add enough DI H2O to fill the centrifuge tube to the sample volume you started with in Step 1.a. (Note: your sample concentration is the same now as it was at start – only clean!)

3.2 Secondary Cleaning Method: Concentrated Acid Oxidation

1) Add a 10 mL subsample of preserved algal sample to a 1000 mL beaker and place on a hot plate set at 100 degrees Celsius under a fume hood.
2) Carefully add 20 mL of concentrated nitric or sulfuric.

3) Add a micro-spatula of potassium dichromate to catalyze the reaction and continue to heat the mixture at 100 degrees Celsius for 1 to 2 hours.
4) Remove mixture from heat and allow to cool for a few hours.
5) Fill the beaker with deionized water.
6) Wait 1 hour for each centimeter of water depth in the beaker and then siphon off the supernatant. Siphon from the center of the water column to avoid siphoning light algae that have adsorbed onto the sides and surface of the water column.
7) Refill the beaker with deionized water.
8) Repeat steps 6 and 7 until a neutral pH is achieved and all color is remove and the sample becomes clear (usually 6-8 rinses).
9) After the sample becomes clear, siphon down to 1 dram (3.7 mL) and transfer to a 1 dram vial labeled with the sample ID number for storage.

3.3 Burn Mount Cleaning Method

1) Place coverslips onto an aluminum coverslip plate.
2) Take a known portion of untreated original sample that has been vigorously shaken and drip it onto a coverslip and allowed to dry at room temperature.
3) After drying, the cover slips are examined at 400X magnification.
   a) A density of 15-30 diatom valves per field of view is best for counting purposes. If the desired density is not achieved, the cover slip is remade after the proper dilution or concentration of the sample has been made and recorded on the data sheet.
4) After the appropriate density is achieved, place the aluminum plate with the coverslips onto a hot plate set at 570°C for 30 minutes or until the organic material is incinerated.
5) Using NAPHRAX™, permanently mount onto a slide labeled with the corresponding sample ID number scribed on it.
6) Place the finished slides in an appropriately labeled slide box with the project name and identify as burn mounts.

4.0 Preparation of Diatom Slides Using Naphrax™ Mounting Medium

This section describes the procedure for depositing cleaned sample material onto a cover slip and permanently mounting this coverslip to a slide using a high resolution mounting medium (Naphrax™).

<u>Slide Making - Wear goggles, lab coat, gloves</u>

THE FOLLOWING STEPS SHOULD BE CONDUCTED UNDER A FUME HOOD. NAPHRAX™ IS TREATED WITH A TOLUENE SOLVENT TO MAKE FLUID. AS TOLUENE IS HEATED AND BOILS OFF AS A GAS, THE NAPHRAX™ HARDENS. PREVENT INHALATION OF TOLUENE.

NOTE: After slides have been made, remaining sample will be prepared for long term storage (see steps for Sample Handling below) – i.e., do not discard any remaining cleaned sample material.

4.1 Deposit Cleaned Material on Coverslip and Microscope Slide Mounting

1) Pipette 100 µl of sample material onto slide and apply cover slip. Examine under the microscope to determine the best concentration range for permanent slide preparation. Diatom cells should be dense enough for ease of count but not overlapping with each other or non-diatom material. For example, should diatom abundance and density of material look good based on examination of your 100 µl volume, you determine 2 more concentrations to be applied in attempt to improve prep quality. Each sample therefore has associated with it 3 possible slides for diatom cell counts. You may go higher and lower, 2 lower, 2 higher – it is a judgment call. You need to maintain record of actual volumes used. NOTE: If even 1000 µl of straight sample provides too little material for counting diatoms, you may have to concentrate the sample if sufficient sample material is available. Centrifuge the sample and remove supernatant volume to allow for a known concentration factor. For example, if you want to concentrate a sample by 2, and the volume is 30 mls, centrifuge, remove 15 mls of supernatant, mix – the sample is now 2x the original concentration. Remember to apply this factor to your indicated sample volume on slide labels (e.g., 100 µl of applied sample material now represents 200 µl of actual sample) and make note of the change in concentration on the storage vial.
2) Prepare 3 cover slips per sample. Coverslips must be cleaned with 95% ethanol and Kim Wipe to remove oils, etc. Place cover slips on a metal block keeping track of which samples and concentrations (to be determined) are associated with each square. Note: placement of coverslips on pennies positioned on the block squares may help to prevent 'spillage'.
3) For each sample you will need 4 clean small beakers (40 ml or smaller). Beakers can be reused if all are cleaned well and rinsed with DI H2O between samples. One beaker is to be used for DI H2O. The remaining 3 beakers are to be used for the 3 best concentrations for slide cover prep as described below.
4) Note: a slide cover holds and disperses well a 1 ml (1000 µl) liquid volume. Your objective is to place an approximate 1 ml volumetric mixture of your predetermined sample amount + DI H2O onto a cover slip for drying. Given that you know how much sample material you want to apply to the cover slip, pipette sufficient DI H2O into a clean beaker such that the total volume of liquid upon addition of the sample material equals 1 ml. Add your sample material.
5) Mix well and apply All Beaker Content (the 1ml or so volume) over the entire surface of the coverslip. Note: Knowledge of sample volume is critical; knowledge of DI H2O less so. The DI H2O will evaporate off during the drying process and is important only for application and dispersal of diatoms contained in a known sample volume.
6) Repeat for remaining 2 sample volumes of interest.
7) Allow coverslips to dry under a heat lamp during the day when monitoring is possible (fire safety hazard prevention). Samples can be left to air dry overnight without the use of a heat lamp if needed.
8) When dried, place a cover slip diatom side face up on a glass slide (to prevent disturbance of cell material) and examine under the light microscope to gauge quality. Take care to replace the cover slip diatom side face up when returning to the metal block.

Note: Microscope slides may have a 'rough' end and 'smooth end'. Keep 'rough' side up if present. Label a microscope slide using a diamond etcher on a smooth end with sample ID and volume (e.g., 6949-1-1 100 µl).

4.2 Preserve and Store Cleaned Material

1) After slides are analyzed according to the appropriate protocol, and no additional slides need to be made, process the vials containing the remaining cleaned material for long-term storage by adding two - four drops of 100% buffered formalin to each vial and tightly cap the vials.
2) Transfer the vials to an appropriate storage cabinet for long-term storage or return to client.

5.0 Diatoms Identification and Enumeration of Microscope Slides

This section describes the identification and enumeration process of diatom taxa mounted on microscope slides.

5.1 Diatom Counts

1) Count and identify diatoms, at 1000X magnification using a Nikon compound light microscope and the latest taxonomical references, to the lowest practical taxon using the transect method until at least 300 cells (600 valves) are encountered, unless a different count is requested by the client.
2) Record all identifications and counts on an approved data sheet.
3) Create a digital image reference collection. Images should include a scale bar, species name, and reference used. Image File should include the Client sample ID and Species name.

5.2 Special Handling for Low Density of Diatoms

In some cases, the number of diatoms in a sample is very sparse. This is usually because diatoms were rare in the habitats sampled, or the sample bottles contain a small amount of material. In these cases, additional procedures are required to either make a satisfactory slide for analysis or to determine that analysis of a sample is not practical. We may also ask the client if this situation has occurred in the past and if diatoms slides are necessary to create.

1) If a satisfactory slide can be made by increasing the concentration of cleaned diatom material by two to five times, use a micro-pipette to remove the required amount of water from the vial of material after it has been allowed to settle for at least eight hours. Record the concentration factor.
2) If a concentration of cleaned material greater than two to five times is required, then the original sample may be subsampled again. Digest the subsample and prepare a new vial of cleaned material. If the sample is still too dilute, combine two vials of cleaned subsample material. Records all steps and volumes along the way, including the final concentration factor.
3) If, after following the steps above to concentrate the cleaned material, the density of diatoms on a cover slip does not meet the criteria of 30 to 40 cells per field at 400-450X magnification, proceed to make the densest slide possible.

a. First, make a determination of whether it is practical to analyze the sample by quickly scanning the slide in its entirety under 100X magnification. Estimate the total number of individuals on the slide.
b. To make a determination of whether or not the slide is countable, take into account the density of diatoms, evidence of dissolution, and amount of debris (silt, clay, broken diatom remains, and other siliceous organisms) that would make it difficult to identify specimens accurately.
    i. As a general guideline, if accurate identifications are possible, and at least 100 specimens could be counted within four hours, a determination will be made to analyze the slide.
    ii. The diatom taxonomist may be asked to take the extraordinary measure of counting a slide for beyond the four hours in extreme cases.

4) When evaluating a slide with few diatoms, a taxonomist may occasionally see evidence suggesting that a sample contains lightly silicified diatoms that may not have survived the digestion process. In these rare instances, a burn mount may be made to determine whether diatoms did exist in the original sample.
    a. Although this method does not rid the sample material entirely of organic debris, the diatoms on the slide can be identified.
    b. After the burn mount slide is prepared, examine the slide to determine if diatoms are present and whether analysis of the slide is warranted.
        i. Slides prepared using the burn mount method cannot be counted if too much organic material remains on the slide.
        ii. Generally, burn mounts are used only as a last resort, and to confirm that weakly silicified diatoms are not present in the sample.
5) Create a digital image reference collection. Images should include a scale bar, species name, and reference used. Image File should include the Client sample ID and Species name.

6.0 Identification and Enumeration of Macroalgae

Procedures to describe the qualitative method for the identification and enumeration of large filamentous or plant-like macroalgae. Algal taxa are identified to the lowest possible level and abundance ranking is assigned. Macroalgae will be preserved in glutaraldehyde, so ensure proper PPE (gloves, lab coat, hood) and waste disposal are utilized.

1) Gather the sample group of macroalgae and open the corresponding import file and SIMs to record sample information.
2) Scan the sample in the container for the presence of macroalgae, and if none is present record this against the sample in the notes field.
3) For those samples containing macroalgae: remove using forceps and place the macroalgae in a petri dish for separation between differing algal forms
4) Place small portions of differing algal organisms onto separate slides. Create a wet mount for microscopy.
    a. For those samples containing no macroalgae use a pipette to create the slide.

5) Place the slide under a compound microscope and look for characteristics that will allow identification to the lowest possible level. Record the taxon on the bench sheet or directly into SIMs.
6) Notes from the taxonomists will include shape and size of colonies, and distinctive characteristics.
7) After taxa have been identified, place all the macroalgae under a dissecting microscope in order to assign an abundance rating to each taxon.

   The table below will be used as guidance (Aycock & Acker, 2016)

Abundance Rating	Abundance Rating Descriptor	Definition per sample
1	Rare	Species only observed once or twice
2	Frequent	Species observed as a few small clumps or clusters but not seen in the majority
3	Common	Species observed in many small clumps or cluster and seen in the majority
4	Very Common	Species observed in large clumps or clusters and seen in the majority
5	Abundant	Species is dominate however overall macroalgae community isn't extremely large
6	Very Abundant	Species dominates a very large macroalgae community

8) Pull small portions of each taxa and place in a small scintillation vial with the sample ID, client ID, and taxon name.
9) Create a digital image reference collection. Images should include a scale bar, species name, and reference used. Image File should include the Client sample ID and Species name.
10) Place the reminder of the identified macroalgae in a vial with the sample ID, client ID, and name it as ID'd portion of the sample.

7.0 Calculating Cell Density

To estimate algal cell density, keep track of the volume of the sample counted in the following method:

1) Record the strip length and width and then multiply the area by the depth.
2) If applicable, multiply the counted volume by the dilution or concentration factor.
3) The total algal cell density is calculated as follow:

$$=[N / (V_{sc} * CR)] * V_T$$

Where: N = the total number of cells counted
V_{sc} = the volume of sample counted
CR = the concentration or dilution factor (if applicable, or CR=1)
V_T = total sample volume

4) Calculate the density of each taxon using the same formula, except N will represent the number of counts for the individual taxa.

8.0 Quality Assurance / Quality Control

8.1 Quality Assurance of Taxonomic Identifications

1) High quality digital images are taken of each taxon encountered in the project. This is one of the best voucher systems for permanently archiving soft algae specimens. These images include taxa names, photographer/taxonomist name, date, and project ID number.
2) A minimum of 10% of all samples may be analyzed by an independent phycologist to ensure taxonomic accuracy and reproducibility of the processing and analysis methods.

8.2 Quality Control Calculations and remediation

3) Percent Difference in Enumeration will be calculated as follows:

$$PDE = (|n_1 - n_2| / (n_1 + n_2)) * 100$$

Where:
n₁ is the number of individuals counted by Taxonomist 1
n2 *is the number of individuals counted by Taxonomist 2*
PDE must be ≤5%

4) Percent Community Similarity will be calculated as follows:

$$PS_C = 100 - 0.5\Sigma(|ai - bi|) \; s \; i{=}1$$

Where:
a_i is the percentage of species *i* in Count A (Taxonomist 1)
b_i is the percentage of species *i* in Count B (Taxonomist 2)
PS_C must be ≥85%

5) In addition, Soft-bodied algae proportional counts will be subjected to the following criteria:
   a. The common algae identified by both taxonomists should match.
   b. Taxa accounting for more than 10% relative abundance should be identified similarly by both taxonomists (synonyms are acceptable).

6) If either or both QA values, steps 3 & 4, fall below the acceptance level:
   a. Taxonomists discuss any discrepancies. In some cases, it is necessary to re-examine the digital images and/or specimens to resolve discrepancies.
   b. The final data are adjusted according to the recommendation of both taxonomists.

7) A quality control report describing results and corrective steps taken, if necessary, will be provided concurrently with data delivery, if requested.

8.3 External QA/QC

1) At the request of the client sample can be shipped to an external lab for QA/QC procedures. Reconciliation will be completed in the same manner.

2) External Labs may also be contacted at our request for taxon verification.

9.0 Archiving and Shipments to the Biorepository

9.1 Archiving

1) Counted portions for Soft Algae samples will be stored at room temperature after identification. Because of the nature of soft-algae samples, degradation is expected and the samples lose their taxonomic value over time. Synoptic Reference Collection photos are the best vouchering system of soft-algae samples. Because of the siliceous cell walls, diatoms remain viable for years with proper storage. Each microscope sample will be labeled with a unique identifier which will link it to all information necessary for the identification of the sample, including water body name, site identification number, and sampling date. Cleaned portions of Diatom samples will also be stored at room temperature until the material is freeze-dried.
2) Samples will be placed into appropriate containers in an orderly fashion. Adequately preserved soft algae, freeze dried diatom sample, diatom slides, and macroalgae samples will be stored until notified to ship to the biorepository.

9.2 Shipping to the Biorepository

1) Once shipping date has been triggered, ship sample components by Domain and Date received.
2) Complete a NEON manifest for each shipment as well as and EcoA Chain of Custody to track all components being shipped. Ensure proper labeling exists on all sample components: original client ID and contents. Ensure samples are tracked with proper NEON sample classes.
3) Check which analysis was required for each sample group. For each group ship the following:
   A) Macroalgae-Individual taxon scintillation vials and the vial containing the ID'd portion.
   B) Periphyton & Phytoplankton- Soft Body fraction that has been identified, diatom slides, and freeze-dried remainder of the diatom sample.
4) Once paperwork is complete and samples are accounted for, hand the samples off the to the Hazardous Materials Shipper for proper packaging.
5) The Haz-mat shipper will add tracking numbers to the manifest and notify the project coordinator that the shipment has been set for pick-up.
6) Project coordinator will notify those who are needed that a shipment is coming.

10.0 Data Management and Reporting

1) Data are entered into a custom-built taxonomy counting program which creates an electronic file for each sample.
2) Since the counting program automatically tallies the number of cells for each taxon, no handwritten bench sheets are required – this entirely eliminates the potential for transcription error. Ecoanalysts can print electronic bench sheets, per client request, at any point during the contract period.
3) Sample identifier information is entered, followed by the taxa and counts and notes.
4) After all samples in the project are identified, the data are formatted in the output specified by the client.
5) A CD of taxa photographs will be compiled upon request.
6) Any remaining sample materials are retained for 30 days after project completion, or until final disposition is determined by the client.

11.0 Hazardous Waste Disposal

1) Lugol's solution at these concentrations are not considered hazardous

2) Glutaraldehyde waste and materials used to prepare the samples must be disposed of in the yellow 20-gallon chemical pack to be collected Emerald Hazardous Waste disposal

References

ANSP Protocols for Analysis of NAWQA Algae Samples

Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, Second Edition

National Science Foundation, National Ecological Observatory Network. 2016. AOS Protocol and Procedure: Periphyton, Seston and Phytoplankton Sampling. NEON Document #: NEON.DOC.003045, Revision A.

Aycock, L. and F. Acker. Identification and Enumeration of Macroalgae. Academy of Natural Sciences of Drexel University Patrick Center for Environmental Research. Procedure No. P-13-76, Revision 0 (10/16).

Training

Please sign and date below to indicate your understanding of the procedures and protocols within this document:

Name	Date	Position