Processing and Identification of Diatom Samples

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Technical Standard Operating Procedure
Office of Water Quality
Watershed Assessment and Planning Branch
Targeted Monitoring Section

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Purpose

The purpose of this technical standard operating procedure (TSOP) is to describe the method for preparing periphyton (attached algae) samples for taxonomic identification of diatoms. Procedures are performed by Targeted Monitoring Section (TMS) staff of the Watershed Assessment and Planning Branch (WAPB) in the Office of Water Quality (OWQ). Diatom samples are collected from one of three substrate types (in order of preference): epilithic (rocks), epidendric (sticks), or epipsammic (sand). Algae samples are collected according to a modification of one of the epilithic sampling protocols described in Moulton et al. 2002. The primary reference for sample preparation and processing is Barbour et al. 1999.

Scope

This TSOP applies to agency staff in OWQ, Targeted Monitoring Section, who process and identify diatom community samples collected from rivers and streams as part of the Indiana Department of Environmental Management's (IDEM) ambient water quality monitoring program. This TSOP covers the processes of acid digestion, or "cleaning", preparation of permanent diatom mounts, archiving samples, and the enumeration and identification of diatoms in periphyton samples.

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Authorizing Signatures

I approve and authorize this technical standard operating procedure (TSOP):

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Michelle Ruan, Senior Environmental Manager — Date
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This TSOP is consistent with agency requirements.

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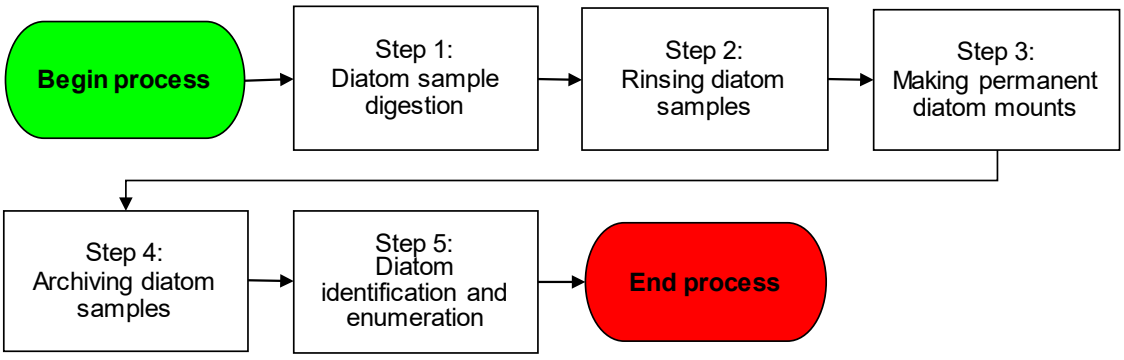
1.0. Overview Flowchart

Not applicable.

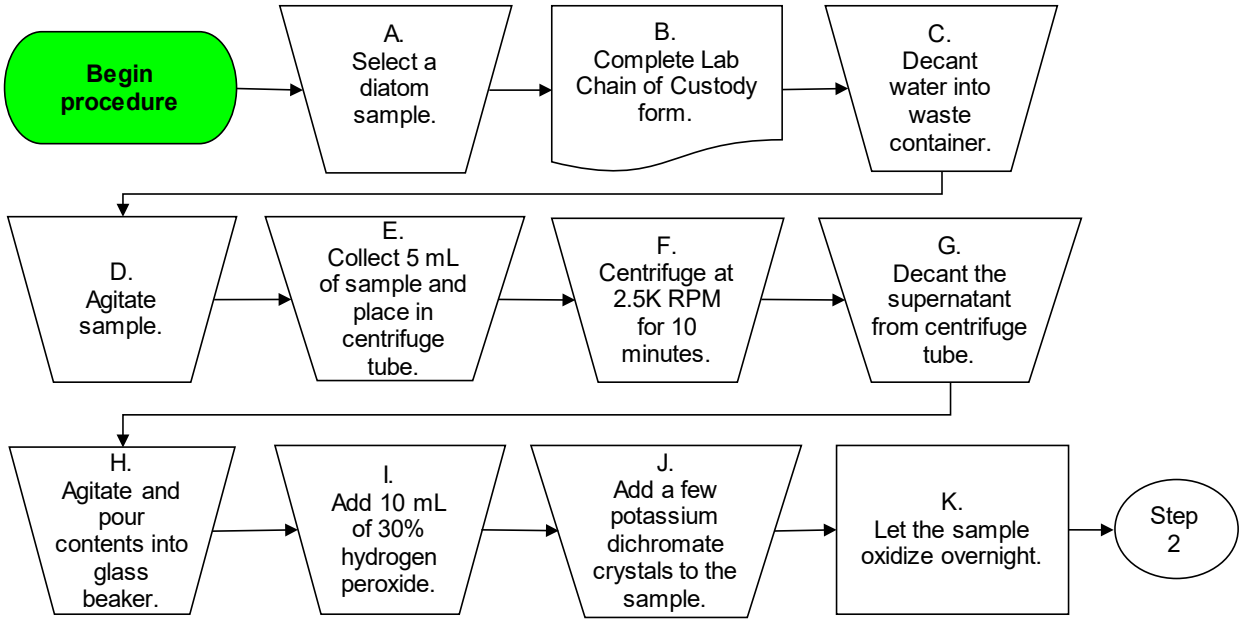
2.0. Procedure

2.1. Procedural Flowchart



2.2. Procedural Steps

Step 1. Diatom Sample Digestion



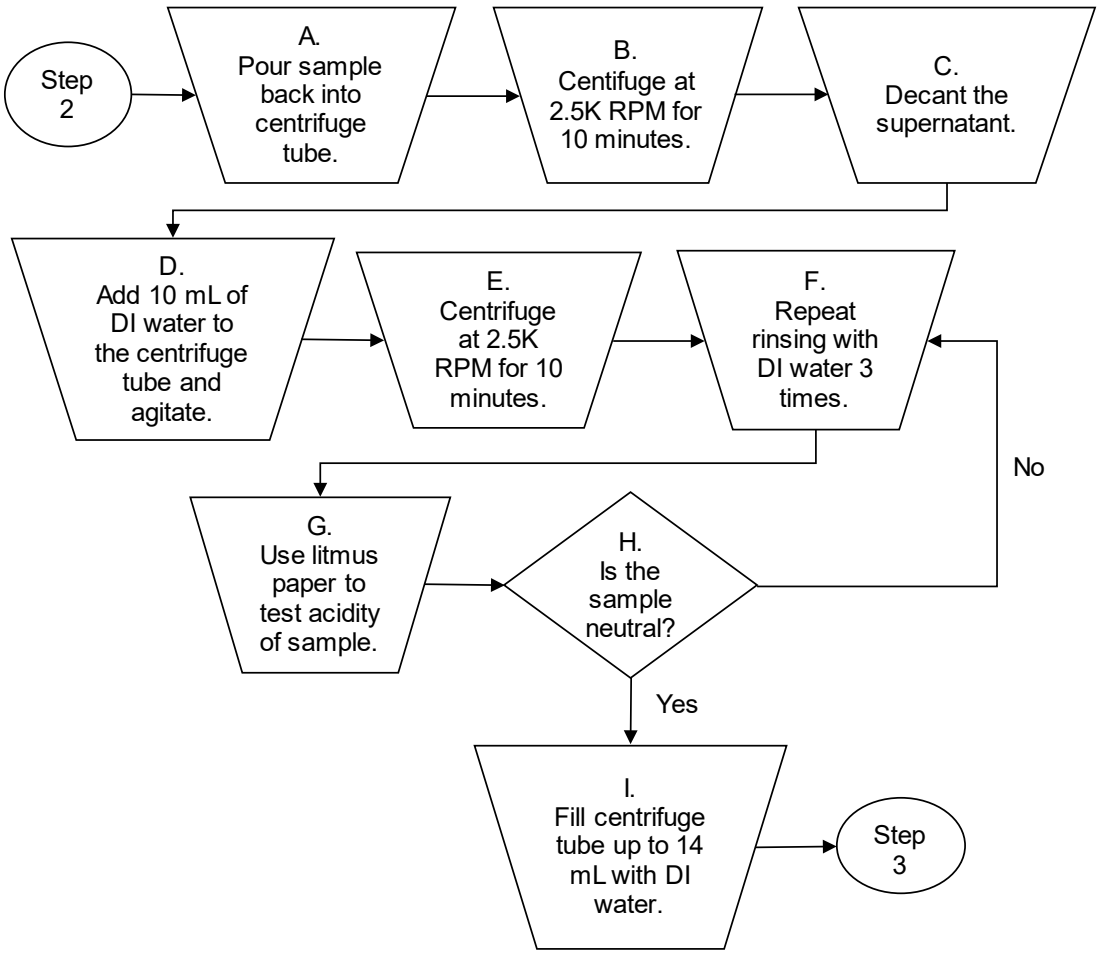
Note: All of Step 1 is to be performed under the fume hood. LS should wear appropriate personal protective equipment (PPE),

such as a lab coat, safety glasses, and chemical resistant nitrile gloves.

A. Laboratory scientist (LS) selects an unprocessed diatom sample. Samples can be processed in any order; however, it is a good idea to process them in chronological order to keep things organized. The samples are preserved with formalin in the field, so there is not a holding time constraint for processing samples. LS transfers the sample to the fume hood while taking care not to shake or disturb the sample contents during the transfer.
B. LS fills out the “Sample Type,” “Event ID,” “IDEM Sample #” (AIMS sample number), number of 250 ml sample containers removed, date and time sample was removed (under “Removed from Storage for Processing”), “Processing Room #”, and “Initials” portions of the Laboratory Chain of Custody form.
C. LS selects an empty 1000 mL glass beaker to serve as a temporary waste container. Using a 10 mL pipettor with reusable pipet tips, LS slowly decants as much water as possible from the sample container and expels it into the waste container (Biggs and Kilroy 2000). LS should stop decanting once it becomes difficult to collect water without disturbing the periphyton that has settled at the bottom of the sample bottle. Make sure to use a different pipet tip for each sample to avoid cross-contamination. The used pipet tips should be washed with a high-quality laboratory grade cleaner and hot water.
D. LS agitates the sample for about 15 seconds making sure it is thoroughly mixed before moving on to the next step.
E. Using the 10 mL pipettor, LS collects 5 mL of the sample and expels it into a plastic 15 mL centrifuge tube. The centrifuge tubes should be labeled with site information, such as the AIMS sample number or the three-digit U.S. EPA site identification number. Make sure to use a plastic centrifuge tube with a screw-top lid. Put the lid on the centrifuge tube.
F. LS places six centrifuge tubes in the holders inside of the centrifuge, leaving a space between each one. The screw-top lids are too big to fit side by side in the centrifuge. Centrifuge six samples simultaneously for 10 minutes at 2,500 revolutions per minute (2.5K RPM). Centrifuging six samples at once makes the most efficient use of time.

G. LS removes the centrifuge tubes from the centrifuge and places them in a test tube rack. Still working under the fume hood, LS uses a clean Pasteur pipet to decant the supernatant from the centrifuge tube and expels it into the waste container. Make sure to use a clean Pasteur pipet for each sample to avoid cross-contamination. Be sure to avoid disturbing the pellet that forms in the tip of the centrifuge tube. Leave the pellet and a little bit of liquid in the tube.
H. LS agitates the contents of the centrifuge tube and pours it into a clean 250 mL glass beaker. The beakers should be labeled with site information using white label tape; each sample should have a centrifuge tube and beaker with matching labels. Add a little bit of deionized (DI) water to the centrifuge tube to rinse out and collect as much of the periphyton material as possible. The DI water and periphyton material is also poured into the glass beaker. The used centrifuge tubes should be washed with a high-quality laboratory grade cleaner and hot water; these will be used again in Step 2.
I. LS is careful to space the beakers at least a few inches apart to avoid cross-contamination in the event of an overflow. LS adds 10 mL of 30% hydrogen peroxide to each of the beakers.
J. LS dons additional PPE, such as a face shield and outer rubber gloves, before working with potassium dichromate. The addition of potassium dichromate will cause a violent exothermic reaction. LS adds a few potassium dichromate crystals to each beaker using a microspatula. If there is no reaction, add a few more crystals until the reaction takes place (Barbour et al. 1999).
K. The reaction is over once the sample color changes from dark purple to yellow and boiling stops (Barbour et al. 1999). LS should cover the beakers tightly with Parafilm M® to ensure that the sample does not evaporate but leave a small opening for the sample to vent. LS lets the sample oxidize overnight with the fume hood running overnight.

Step 2. Rinsing diatom samples



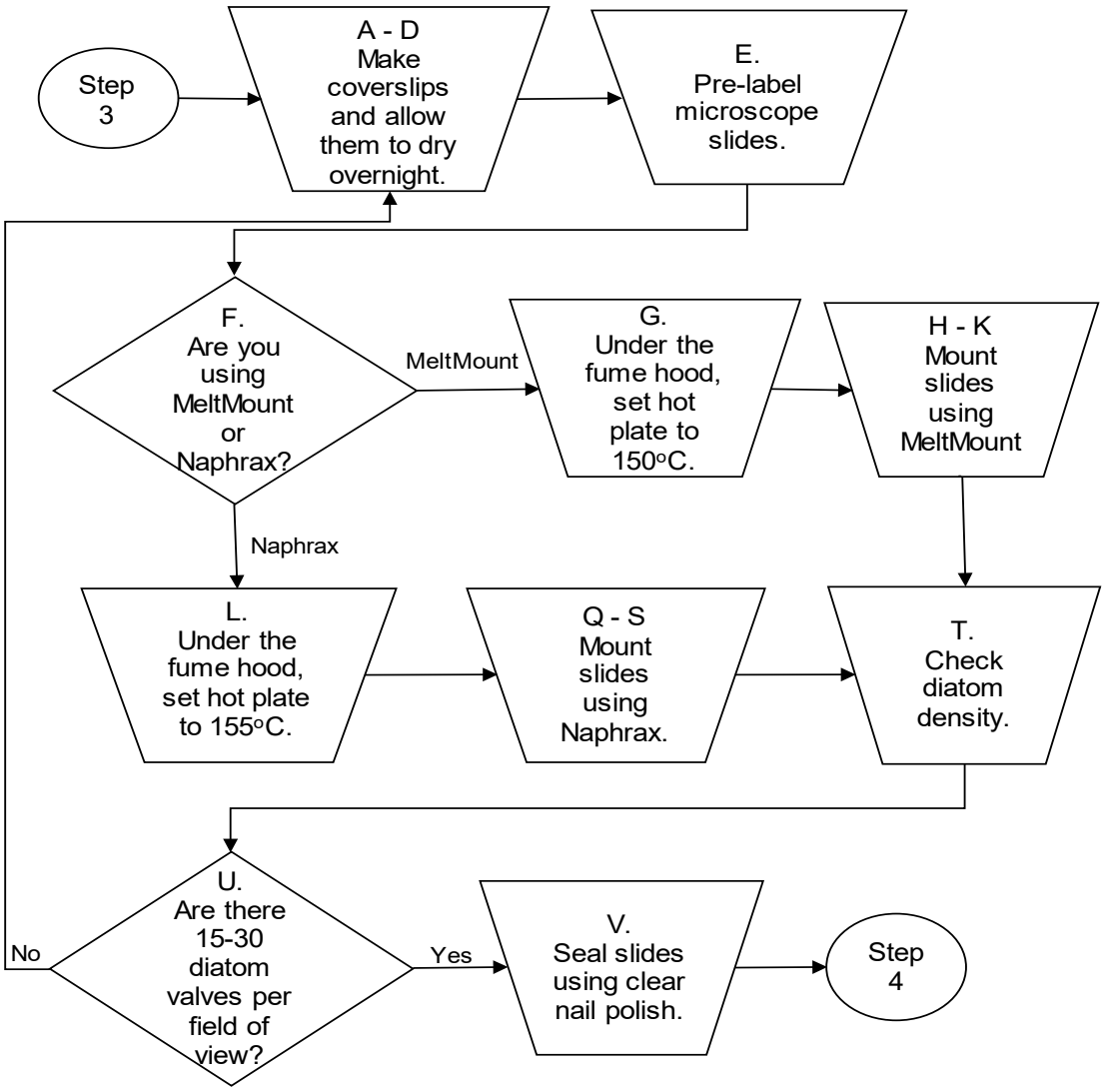
Note: All of Step 2 is to be performed under the fume hood. LS should wear appropriate PPE, such as a lab coat, safety glasses, and chemical resistant nitrile gloves.

A. LS pours the sample back into a labeled 15 mL centrifuge tube (the same one that was used the previous day). LS uses DI water to rinse the sides of the beaker and any remaining material into the centrifuge tube.
B. LS centrifuges the sample for 10 minutes at 2.5K RPM. In the same way as before, LS only puts 6 tubes into the centrifuge at one time and leaves a space in between each tube. Make sure each centrifuge tube has the same volume so that the centrifuge is balanced when it is in use. Otherwise, the centrifuge will rattle and shift around.
C. Using a clean Pasteur pipet, LS decants the supernatant into a waste container and leaves the pellet undisturbed in the bottom

of the centrifuge tube (Barbour et al. 1999). The waste is poured down the drain at the end of this process.

D. LS adds 10 mL of DI water to the centrifuge tube and agitates the sample. LS agitates the sample vigorously enough to break up the pellet so that all the material is “rinsed” with the DI water.
E. LS centrifuges the sample for 10 minutes on 2.5K RPM as before.
F. LS repeats Step 2.C through Step 2.E until the sample has been “rinsed” at least three times and visual inspection of the sample shows that the color fades from yellow to clear.
G. LS uses litmus paper to check the neutrality of the sample. The litmus paper should be blue after being dipped into the sample, which indicates that it has a neutral pH.
H. If the solution tests neutral, LS proceeds to the next step. If the sample is still acidic, LS returns to Step 2.F and repeats the rinsing process until the sample becomes neutral.
I. LS fills the centrifuge tube to 14 mL with DI water and puts the screw-cap lid on. If the pellet is small and LS determines that the diatom sample may be sparse, LS can make the decision to leave the volume at 10 mL.

Step 3. Making permanent diatom mounts.



Note: Steps 3.A through 3.E are to be performed at the benchtop.

A. LS places a large ceramic tile on the benchtop and cleans the tile with isopropyl alcohol. LS pre-soaks square 22 x 22 mm^2 glass coverslips in isopropyl alcohol or ethanol to clean them, disperses them onto the tile, and allows them to dry. The tile helps to minimize disturbance from vibrations which could lead to breaking the surface tension on the coverslips. These steps are performed at the benchtop because airflow from the hood could also break the surface tension of the sample on the coverslips.

B. Using a 100–1000 µL pipettor, LS places 900 µL of DI water in a plastic 1 mL microfuge tube.
C. LS agitates the sample in the centrifuge tube for 15 seconds and then uses the pipettor to quickly collect 100 µL of the sample while it is still mixed. LS adds the 100 µL of sample to the microfuge tube that already contains 900 µL of DI water. There should be a total of 1 mL in the microfuge tube which includes the sample and DI water. Record the sample volume used in the Diatom Laboratory Book.
D. Using the pipettor, LS draws up the contents of the microfuge tube, expels the sample back into the microfuge tube, and draws it up again. LS does this three times to mix the sample, and then expels it slowly and carefully onto a coverslip. There should be enough sample volume to cover the coverslip but not so much that the surface tension is broken (Biggs and Kilroy 2000). Using the pipet tip, LS drags the sample out to all corners of the coverslip, being careful not to break the surface tension. LS leaves the coverslips on the ceramic tile to evaporate overnight.
E. LS uses a thin-tipped permanent marker to label frosted microscope slides with pertinent site information, as shown in Figure 1. The top line should contain the AIMS sample number. The middle line should contain the three-digit U.S. EPA site number and an “A”, “B”, or “C” in parentheses to distinguish the slides from one another. Slides will be made in triplicate for each sample as a quality assurance/quality control measure. The last line should contain the waterbody name and the last two digits of the year that the sample was collected.

Figure 1. A schematic illustrating the correct labelling of a diatom microscope slide.

Note: Steps 3.F through 3.S are to be performed in the fume hood, and LS should wear appropriate PPE, including a lab coat, safety glasses, and chemical resistant nitrile gloves.

F. After allowing coverslips to dry overnight, LS turns on the fume hood and cleans the work surface thoroughly. LS then places a hot plate under the fume hood. If using MeltMount as the mounting medium, proceed to Step 3.G. If using Naphrax as the mounting medium, proceed to Step 3.L.

If using MeltMount as the mounting medium:

G. LS sets the temperature of the hot plate to 150ºC.
H. LS places a pre-labelled microscope slide on the hot plate.
I. LS inverts the MeltMount™ Quick-Stick™ and rests it on the microscope slide and lets the mounting media melt onto the slide (a drop smaller than a dime is the best amount of media to use).
J. Using bent-tipped forceps, LS picks up a coverslip with dried material on it and places it material side down onto the drop of MeltMount™ on the microscope slide. LS is careful to never touch the coverslips with their fingers; coverslips should only be handled using bent-tipped forceps.
K. LS gently taps and maneuvers the coverslip to remove air bubbles that form between the coverslip and the microscope slide. Once all the air bubbles have been removed, remove the slide from the hot plate and let it sit overnight before proceeding to Step 3.T.

If using Naphrax as the mounting medium:

L. LS sets the temperature of the hot plate to 155ºC.
M. LS places pre-labelled microscope slides on to the work surface of the fume hood. For efficiency, LS can work with as many slides at one time that will comfortably fit on the hot plate that will be used in Step 3. P.
N. Using a 1-200μL pipettor, LS draws up 100μL of Naphrax and places it on top of each microscope slide.
O. Using bent-tipped forceps, LS picks up the coverslips with dried samples one at a time and places them material side down onto the drop of Naphrax on each slide. LS is careful never to touch the coverslips with their fingers; coverslips should only be handled using bent-tipped forceps.

P. LS then places each microscope slide onto the hot plate, arranging them around the perimeter of the hot plate and leaving a space between them so they are not touching. The Naphrax will begin to bubble, as shown in Figure 2A. LS leaves the slides on the hot plate for 15 minutes, at which point the Naphrax will have stopped bubbling or slowed significantly (Acker 2002), as shown in Figure 2B. The bubbling occurs when Naphrax is heated because the solvent, toluene, begins to vaporize, allowing the Naphrax to harden (Rhuland et al. 1999).
Q. LS removes the slides from the hot plate one at a time. As each slide is removed, LS will gently maneuver the coverslip using bent-tipped forceps to remove air bubbles between the coverslip and the microscope slide. Naphrax will begin to harden when the slide is taken off the hot plate. If Naphrax hardens before finishing this step and the coverslip can no longer be maneuvered, place the slide back on the hot plate for a few seconds which will allow the Naphrax to become pliable again. After air bubbles have been removed, LS will gently press down on the center of the coverslip to remove excess Naphrax underneath, as shown in Figure 2C. When finished with this step, LS should ensure that the coverslip is parallel to the microscope slide.
R. Once all slides have been removed from the hot plate and cooled, LS uses a glass scraper or forceps to determine if the Naphrax has fully hardened. If the Naphrax is brittle and can be easily chipped when pressed, proceed to Step 3.S. If the Naphrax is still malleable or gummy when pressed, place the slide back on the hot plate for 5 minutes and start over from Step 3.Q.

S. LS uses a glass scraper to carefully scrape off any excess mountant from the sides of each coverslip, as shown in Figure 2D. LS is careful not to lift the coverslip off the microscope slide or crack the coverslip while scraping around it. Allow slides to sit in the fume hood overnight before proceeding to Step 3. T.
T. LS looks at the microscope slide using a microscope with

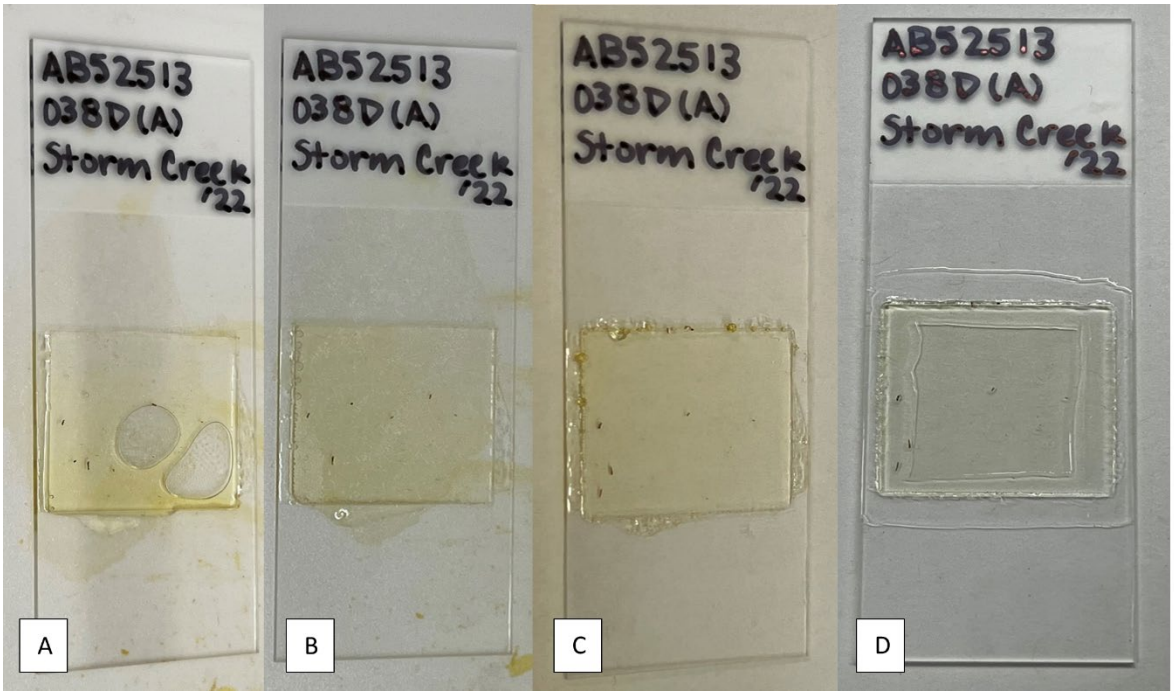


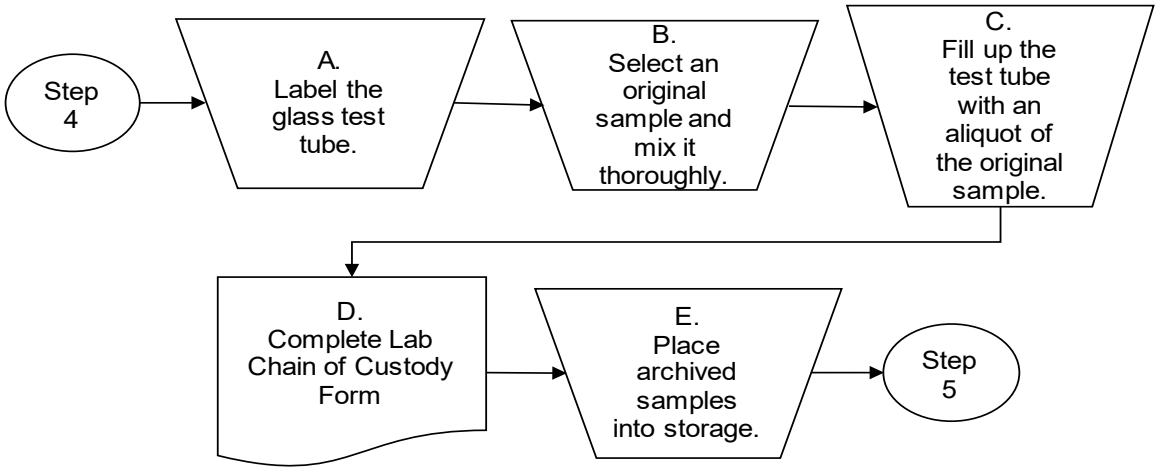
Figure 2. Photos depicting the different stages of the mounting process using Naphrax. A) When a microscope slide is placed on the hot plate, large bubbles form as a result of the solvent, toluene, vaporizing. B) After the slide has been left on the hot plate for 15 minutes, large bubbles cease to form, indicating that the toluene has fully vaporized. C) The slide after LS pressed down on the coverslip to push excess Naphrax out to the sides. D) The finished slide after excess Naphrax has been scraped and the coverslip has been sealed to the slide using clear nail polish.

Nomarski differential interference contrast (DIC) using the 40x objective. An ideal dispersal of diatoms would be 15-30 valves visible at 40x in a field of view. LS should count 5 random fields of view to determine if the volume of sample used is ideal. LS also checks for clumping of diatom valves or excessive sediment in the slide which could make identifications difficult.
U. LS determines if the volume on the coverslip produces an ideal quantity and dispersal of diatom valves. If the volume is sparse (less than an average of 15 valves visible in a field of view),

then LS goes back to Step 3.A and starts the process over by adjusting the volume of sample and DI water. If the volume is dense (greater than an average of 30 valves visible in a field of view) or excessive sediment or clumping of diatom valves impedes counting, then LS goes back to Step 3.A and starts the entire process over by adjusting the volume of sample and DI water. There should be a total of 1 mL on the coverslip after new volumes are used. LS records the new sample volume in the Diatom Laboratory Book. If the diatom dispersal is ideal, LS proceeds to the next step.

V. LS uses clear fingernail polish to seal the edges of the coverslip. Once the fingernail polish is dry, LS puts the slides in a microscope slide box.

Step 4. Archiving samples

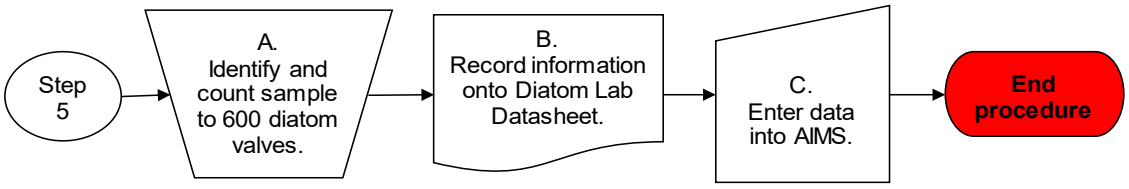


Note: All of Step 4 is to be performed under the fume hood. LS must wear appropriate PPE, including a lab coat, safety glasses, and chemical resistant nitrile gloves.

A. LS labels glass test tubes with the appropriate site information which includes all the information on the sample labels.
B. LS collects a 10-15 mL aliquot of the original sample using a 10 mL pipettor and reusable pipet tip. LS makes sure that the sample is thoroughly mixed before pipetting the sample. The pipet tips need to be washed with laboratory grade cleaner and hot water after they are used.
C. LS expels the aliquot into a 15 mL glass test tube with a screw-top lid, filling the tube up to the neck.

D. LS fills out the Laboratory Chain of Custody form with information regarding the number of slides made and archived samples put into storage. This information completes the Laboratory Chain of Custody.
E. LS puts the test tubes in a storage container, labels the container with the basin and year sampled, and places it into a cabinet in the laboratory.

Step 5. Diatom identification and enumeration



A. IDEM biologist identifies diatom taxa using a microscope with 100x oil immersion objective lens. The ocular micrometer is used to measure diatom valves and should be calibrated before use. A digital camera system with software capable of doing measurements (see Section 4.3) can be used to capture images and measurements of diatom valves. IDEM biologist counts and identifies six hundred diatom valves for each sample.
B. IDEM biologist records site location information along with taxonomic identifications and enumeration information onto the Diatom Benchsheet.
C. IDEM biologist enters the data from the Diatom Benchsheet into the Assessment Information Management System (AIMS) database. Most diatom taxa entered into the database will have a North American Diatom Ecological Database (NADED) code assigned to them. The NADED ID is the taxon code used within the North American Ecological Database, where algal counts for all Phycology Section projects are stored.

2.3. Related Technical Issues

A. Health and Safety Warnings
1. These procedures involve hazardous chemicals, glassware, and equipment. Before using any chemicals mentioned in this procedure, staff must review the relevant Safety Data Sheets (SDS), which are stored in a binder in the lab. An eye wash station, first aid kit, and overhead safety shower are located in the laboratory where these procedures are performed.

2. Handle glassware such as beakers, coverslips, and microscope slides with care. Broken glassware should be disposed of in the “broken glass” container and not the regular trash.
3. For the purposes of this TSOP, required personal protective equipment (PPE) will consist of:
    a. Chemical resistant nitrile gloves
    b. A laboratory coat
    c. Protective eyewear, such as safety glasses or goggles
    d. A face shield
    e. Rubber gloves

B. Cautions
1. Be sure to always record the correct sample number and site information during each step of the processing procedures. Centrifuge tubes, beakers, microfuge tubes, microscope slides, and test tubes should always be labelled with the corresponding sample information.
2. Ensure that accurate measurements of aliquots and subsamples are obtained for quantitative samples.
3. Adding potassium dichromate to the diatom sample during the digestion process will cause a violent exothermic reaction. Be careful to add only a few potassium dichromate crystals at a time to avoid the mixture from boiling over the top of the beaker. This should be done under a fume hood and appropriate PPE (section 2.3) should be worn. The beakers should be placed at least a few inches apart to prevent cross-contamination in the event of overflow.
4. Do not let the sample dry out overnight during the acid digestion process. When the liquid evaporates, it is impossible to get the dried material off the sides of the beaker and the sample will need to be redigested. To avoid this, cover the beaker with Parafilm M®. The Parafilm M® should be pulled tight over the top of the beaker, but a little space should be left open to allow the sample to vent.
5. Be sure to always clean the immersion oil from the microscope objective and microscope slide after use. Use only lens paper to wipe off the objective.
6. When transferring the unprocessed sample to the fume hood to be decanted before processing, avoid shaking or disturbing the contents of the sample bottle. If the sample is mixed during the transfer, it will have to be left to settle for 8+ hours until the solids settle on the bottom of the sample bottle.

7. Make sure to use a different pipet tip for each sample to avoid cross-contamination. This is very important since the data from this project is site specific. The integrity of the project depends on the attention to detail in the laboratory.
8. The tile helps to minimize disturbance from vibrations which could lead to breaking the surface tension on the coverslips. If the surface tension is broken on the coverslip, a new one will need to be made.
9. LS is careful to never touch the coverslips with their fingers; coverslips should only be handled using bent-tipped forceps. The dried material (diatoms) on the coverslip could adhere to the surface of a glove or bare finger. Forceps offer minimal disturbance to the material on the coverslip.
10. When mounting slides using Naphrax, it is important to use caution when scraping slides to not get too close to the edge of the coverslip. If the coverslip cracks while scraping, a new coverslip with the appropriate density will need to be remade, left to dry overnight, and mounted onto a new microscope slide.

C. Interferences
1. When dealing with microscopic organisms, the potential for contamination is great. To avoid cross-contamination of the samples, glassware and tools need to be cleaned thoroughly with a laboratory grade cleaner and hot water. Glassware should be stored in cabinets to avoid airborne contamination. There is also a risk of cross-contamination when the potassium dichromate is added to the sample. Adding too much potassium dichromate or adding it too quickly can cause a violent reaction and the sample could boil over the top of the beaker and contaminate the sample next to it. Therefore, be sure to always space the beakers far apart to avoid contamination in the case of a spill or spatter.
2. When making coverslips, it is important to minimize the risk of uneven dispersal of diatom valves on the coverslip. Do this by drying the coverslips on a ceramic tile which will absorb vibrations from the benchtop and decrease disturbance to the coverslip. Also, try to minimize air currents in the room. Check the first slide of the triplicate set under the microscope to gauge distribution and density of the valves. Clumping of diatom valves reduces the accuracy of the quantitative enumeration and makes identifications difficult. If the diatom density on the slides is too dense, enumeration and identification will be difficult. If the diatom density on the slides is too sparse, then it will likely result in decreased counting efficiency.

If any of these problems occur, adjust the volume of the sample that is placed on the coverslip and start the process over. Make sure to record any new volumes in the Diatom Laboratory Notebook. Diatom density and dispersal should be checked each time a permanent mount is made with a new volume.

3. To avoid making taxonomic errors, refer to taxonomic literature and keys and archived photographic images to resolve uncertainty regarding taxa present in the sample.

D. Calibration
   1. Automatic pipettors are factory calibrated and come with a certificate of proof.
   2. The ocular micrometer on the Nikon Eclipse 80i microscope was calibrated with a stage micrometer to ensure that measurements are accurate. The calibration was done before the ocular reticle was used for measurements. It is unknown whether the stage micrometer is traceable to a NIST standard.

E. Troubleshooting
   1. During the identification and enumeration process, the IDEM biologist might come across broken diatom valves. The valve should be counted if there is more than half of the valve present and the central area is intact. Valves that extend beyond the transect boundary should be counted if more than half of the valve is inside of the transect. For unidentifiable specimens, use the lowest possible classification level. If the genus can be determined, but the species is unknown put "'Genus' sp. 1." For example, if the diatom is identified as the genus Achnanthidium, but the exact species is unknown, use "Achnanthidium sp. 1." If no classification level can be determined, use "unknown pennate sp. 1" or "unknown centric sp. 1" depending on the diatom's shape.
   2. If a sample is still reacting after having been left to oxidize overnight following the digestion process, LS can add 5mL of 30% hydrogen peroxide to the sample to speed up the reaction. The sample should then be covered with Parafilm M® and left to sit overnight or until the reaction stops completely. Once the sample has finished reacting, LS can begin the rinsing process. Rinsing the sample before it has finished reacting can make rinsing difficult and can result in excess organic material in the slide mounts, which would make the identification and enumeration process difficult.

3. If the coverslip lifts off the slide when scraping in Step 3.S, then put the slide back on the hot plate for a few seconds and reseal the coverslip to the slide using forceps.
4. During the slide mounting process using Naphrax, if LS finds that the Naphrax is not bubbling properly or is taking longer than usual to set up, LS can push the slide towards the center of the hot plate. The center of the hot plate is the hottest area as compared to the edges. If the slide is on the edge, sometimes this can result in the reaction taking longer to complete. Pushing the slide to the center could speed up the reaction. Remember to keep an eye on the slide when using the center of the hot plate to avoid overcooking the slide.
5. If a sample is very sparse in diatom valves even after increasing the volume of sample on the coverslip, LS can add 1200 µL of sample (no DI water) to a coverslip. This is the maximum volume that could be placed onto a coverslip before the surface tension breaks.
6. For trouble with the microscope or Nikon Elements software, contact a customer service representative from Nikon.

3.0. Roles

3.1. Responsibilities

A. Laboratory scientist
   1. Exercises proper laboratory safety – reads the SDS and wears appropriate PPE.
   2. Knows proper chemical disposal methods.
   3. Processes diatom samples to be made into permanent diatom mounts.
   4. Makes permanent archive of periphyton samples.

B. IDEM biologist
   1. Identifies and enumerates diatom samples.
   2. Records taxonomic information in AIMS.

3.2. Training requirements

A. Training in the process of diatom acid digestion and creating diatom mounts.
   1. Laboratory scientist
   2. IDEM biologist

B. Training in handling and disposal procedures of laboratory chemicals.
   1. Laboratory scientist
   2. IDEM biologist

C. Training in sample preservation and archiving subsamples.
   1. Laboratory scientist
   2. IDEM biologist

D. Training in identification of freshwater diatoms.
   1. IDEM biologist

E. Training in AIMS database management.
   1. Laboratory scientist
   2. IDEM biologist

4.0. Required Forms, Equipment, or Software List

4.1. Forms

A. Laboratory Chain of Custody Form

B. Field Chain of Custody Form

C. Diatom Benchsheet

4.2. Equipment

A. 250 mL glass beakers

B. 1000 mL beaker

C. 500 mL deionized water squeeze bottles

D. Laboratory grade cleaner

E. Disposable glass Pasteur pipets

F. 10 mL pipettor

G. 10 mL reusable pipet tips

H. 10–100 µL pipettor

I. 10–100 µL pipet tips

J. 100–1200 µL automatic pipettor

K. 100–1000 µL pipettor

L. 100–1000 µL pipettor tips

M. Centrifuge

N. 15 mL centrifuge tubes (plastic)

O. Fume hood

P. 30% hydrogen peroxide

Q. Isopropyl alcohol

R. Potassium dichromate crystals

S. Microspatula

T. Parafilm M®

U. Litmus paper

V. Ceramic tile

W. White labelling tape

X. 1 mL microfuge tubes (plastic)

Y. Bent-tipped forceps

Z. Square glass coverslips measuring 22 x 22 mm^2

AA. Rite-in-the-Rain universal laboratory notebook

BB. Fine tip permanent marker

CC. Glass microscope slides with a frosted edge for writing

DD. Hot plate

EE. MeltMount™ Quick Stick™ mounting media

FF. Naphrax mounting media with toluene

GG. Glass scraper

HH. Kim-Wipes cleaning tissues

II. Microscope slide boxes

JJ. Nikon Eclipse 80i microscope with Normarski differential interference contrast (DIC)

KK. Type A immersion oil for microscopy

LL. Lens paper

MM. Taxonomic identification keys

NN. Clear fingernail polish (any brand)

4.3. Software

A. Nikon Elements D imaging software

5.0. Records Management

5.1. Original copies of Field and Laboratory Chain of Custody forms, and Diatom Benchsheets are stored in a filing cabinet in the Algae Lab at the Shadeland Office. A summary of the analysis and comments are reported in the Rite-in-the-Rain universal laboratory notebook and are stored in the IDEM biologist's cubicle. Identification and enumeration information from the Diatom Benchsheets are entered and stored in AIMS. Chain of Custody forms and Diatom Benchsheets have been scanned and uploaded to AIMS as attachments under the project. Starting with the 2023 season, these documents will be scanned and stored in the Virtual File Cabinet (VFC).

6.0. Definitions

6.1. "AIMS sample number (AA/AB/AC)" – A number assigned to each individual watershed sampling event conducted by Indiana Department of Environmental Management (IDEM) field crews. This number is used to identify the sampling event in the Assessment Information Management System database (AIMS database).

6.2. "Agency staff" – Any employee or representative of the Indiana Department of Environmental Management including regular employees, temporary employees, contractors, and interns.

6.3. "Assessment Information Management System database (AIMS database)" – IDEM database containing information related to water chemistry, aquatic habitat, macroinvertebrate, fish, and algae communities, fish tissue analyses, sediments, and *E. coli* bacteria data collected by agency staff from watershed sampling events.

6.4. "Benthic surface" – Area at the lowest level of a body of water such as a stream or lake, including the sediment surface and some sub-surface layers.

6.5. "Chain of custody COC" –The records documenting the possession of the samples from the time they are obtained until they are disposed of or shipped off-site.

6.6. "Deionized (DI) water" – Water that has had its mineral ions removed. Water with no polar ions in it (such as sodium, chloride, magnesium, calcium, sulfate, nitrate, etc.,) that are normally found in water. Do not confuse de-ionized water with distilled water, because deionized water

may have nonpolar compounds in it such as benzene, toluene, octane, and hexane.

6.7. “Diatom” – Algae with distinctive, transparent cell walls made of silicon dioxide hydrated with a small amount of water. The cell wall is called a frustule and consists of two halves called valves.

6.8. “Diatom sample number” – An identification number used to identify a diatom sample.

6.9. “Diatom valve” – Diatom cell walls are made up of two distinct halves, or valves. The older, larger valve is called the epivalve and the smaller, younger valve is the hypotheca.

6.10. “Epidendric” – The growth habit of living organisms attached to trees or sticks.

6.11. “Epilithic” – The growth habit of living organisms attached to stone or stonelike material.

6.12. “Epipsammic” – The growth habit of living organisms attached to sand grains or moving through sand.

6.13. “IDEM biologist” – Indiana Department of Environmental Management scientist educated and trained to understand the life of living organisms, including their structure, function, growth, origin, evolution, and distribution. In this case, the IDEM biologist needs to have specific knowledge of algae and the taxonomy of diatoms.

6.14. “Laboratory scientist” – The agency staff person responsible for preparing and analyzing water samples following a strict preparation and analysis protocol.

6.15. “Nomarski differential interference contrast (DIC)” – An optical microscopy illumination technique used to enhance the contrast in unstained, transparent samples.

6.16. “North American Diatom Ecological Database (NADED) code – A number assigned to a diatom species that is included in the database at the Academy of Natural Sciences of Drexel University in the Phycology Section.

6.17. “Periphyton” – Material that is attached to submerged benthic surfaces; here, it refers to the algal component.

6.18. “Safety Data Sheet (SDS)” – A document that lists information relating to occupational safety and health for the use of various substances and products.

6.19. “Taxonomist” – A biologist that groups organisms into categories.

6.20. “Technical standard operating procedure (TSOP)” – A standard operating procedure that involves environmental data generation, manipulation, or compilation of an analytical process.

6.21. “Virtual File Cabinet (VFC)” – The agency’s electronic digital image document repository system, that stores, files, indexes, redacts, reassembles, and securely accesses electronic documents of all types both received and created by the various program areas within the agency.

7.0. Quality Assurance and Quality Control

7.1. Quality control (QC) of the diatom project will be documented by QC checks of both field and laboratory data. Replicate diatom field samples will be collected at every tenth site. This will result in a precision evaluation based on a 10% replicate of samples collected. In the laboratory, permanent diatom mounts will be made in triplicate in case one or more of them break or excessive air bubbles develop after the slide has been sealed. Diatom taxa will be counted in duplicate at ten percent of the sites.
Currently, all diatom samples are identified and enumerated by a contracted algal taxonomist from the Department of Biological and Environmental Sciences at Georgia College.

Dr. Kalina Manoylov
BIOL/ENSC Dept
Georgia College
320 N. Wayne St.
Milledgeville, GA 31061

7.2. Information entered on the Field Chain of Custody is duplicated in a laboratory notebook with specific notes.

7.3. While performing processing procedures, it is important to label centrifuge tubes, microfuge tubes, and microscope slides correctly.

8.0. References

8.1. Acker, F., Russell, B., Morales, E. 2002. Protocol P-13-49: Preparation of Diatom Slides Using Naphrax™ Mounting Medium. USGS Report 02-06.

8.2. Barbour, M.T., J. Gerritsen, B.D. Snyder and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition.

EPA/841/B-99/002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

8.3. Biggs, B.J.F and Kilroy, C. 2000. Stream Periphyton Monitoring Manual. NIWA, Christchurch, New Zealand.

8.4. Moulton II, S.R., Kennan, J.G., Goldstein, R.M., and Hambrook, J.A. 2002. Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program. USGS Open-File Report 02-150.

8.5. Ruhland, K., Karst, T., Paterson, A., Gregory-Eaves, R., Smol, J.P., Cumming, B.F. 1999. Standard Sediment Sample Preparation Methods for Siliceous Microfossils (Diatoms and Chrysophyte Scales and Cysts). Paleoecological Environmental Assessment and Research Laboratory; Department of Biology Queen's University.

9.0. Appendices

9.1. Example of a completed Field Chain of Custody form

9.2. Biological Samples Laboratory Chain of Custody form

9.3. Diatom Benchsheet

9.4. Example of a completed page of the Diatom Laboratory Notebook

9.5. List of Taxonomic References

9.1 – Example of a completed Field Chain of Custody form

Indiana Department of Environmental Management

OWQ Chain of Custody Form

Project: *2014 Corvallis Water Chem R3W4, Oct 6, 2014*

OWQ Analysis Set: *14WQW030*

I Certify that the sample(s) listed below was/were collected by me, or in my presence. Date: 10/8/2014

Signature: [signature] Section: PMS

Lab Assigned Number	IDEM Control Number	Sample Type	ID	1000 ml P, N.M.	1000 ml G, N.M.	40 ml VIAL	~~120 ml~~ G (Beet) Slurry Volume (mL)	~~500 ml~~ P, N.M. Sample Volume (mL)	~~250 ml~~ P, N.M. Substrate	Date and Time Collected: Date	Time
	AB18835		5				400	200	Rocks	10/7	11:00
	AB18821		9							10/6	10:20
	AB18832		13							10/7	09:00
	AB18822		21							10/6	12:26
	AB18836		25							10/6	14:30
	AB18833		29							10/7	14:50
	AB18837		33							10/7	12:40
	AB18834		41							10/7	16:30
	AB19355	D	41							10/7	16:30
	~~AB19356~~	~~B~~	~~42~~								
	AB18829	M	43							10/8	09:00
	AB18840		58				↓	↓	↓	10/8	11:10

P = Plastic G = Glass N.M. = Narrow Mouth Bact = Bacteriological Only | Should samples be iced? Y N

Carriers

I certify that I have received the above sample(s).

Signature	Date	Time	Seals Intact		Comments
Relinquished By: [signature]	10/14/14	07:25	Y	N	
Received By: Kristen Arnold					
Relinquished By:			Y	N	
Received By:					
Relinquished By:			Y	N	
Received By:					

Lab Custodian

I certify that I have received the above sample(s), which has/have been recorded in the official record book. The same sample(s) will be in the custody of competent laboratory personnel at all times, or locked in a secured area.

Signature: Kristen Arnold Date: 10/14/14 Time: 07:25

Lab: Address:

10/2/2014 8:48:04 AM Chain of Custody Datasheet, Page 1 of 1

9.2 – Biological Samples Laboratory Chain of Custody form

INDIANA DEPARTMENT OF
ENVIRONMENTAL MANAGEMENT
OFFICE OF WATER QUALITY BIOLOGICAL SAMPLES
LABORATORY CHAIN OF CUSTODY

ROOM # ______

By placing your initials below, you are certifying that the sample(s) listed below was/were processed by you or in your presence in the processing room noted below and returned to the noted storage room.

Sample Type AD = Algae Diatom AS = Algae, Soft F = fish M = macro	**Event ID or Macro #**	**IDEM Sample #**	# of 2000 mL Nalgene Jar	# of 250 mL Nalgene Jar	# of 125 mL Glass Jar	**Removed from Storage for Processing**		Processing Room #	Initials	**Placed in Storage after Processing**		Storage Room #	Initials	# of Olive Voucher Jars	# of Slides	# of Close Top Test Tubes	**Sample Split** P = Permanent T = Temporary
	(YY____) or (__________)	(AB_____)				Date (mm/dd/yyyy)	Time (24hr)			Date (mm/dd/yyyy)	Time (24hr)						
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Lab: Indiana Department of Environmental Management

Address: 2525 N. Shadeland Ave., Laboratory Room 121, 124, 125, Indianapolis, IN 46219

9.3 – Diatom Benchsheet

Indiana Department of Environmental Management
Diatom Benchsheet

Slide #	AB#	L-site #	Stream Name	Location	Identifier	ID Date

Transect #1	Transect #2	Transect #3	Transect #4	Transect #5	Transect #6
X:	X:	X:	X:	X:	X:
Y:	Y:	Y:	Y:	Y:	Y:

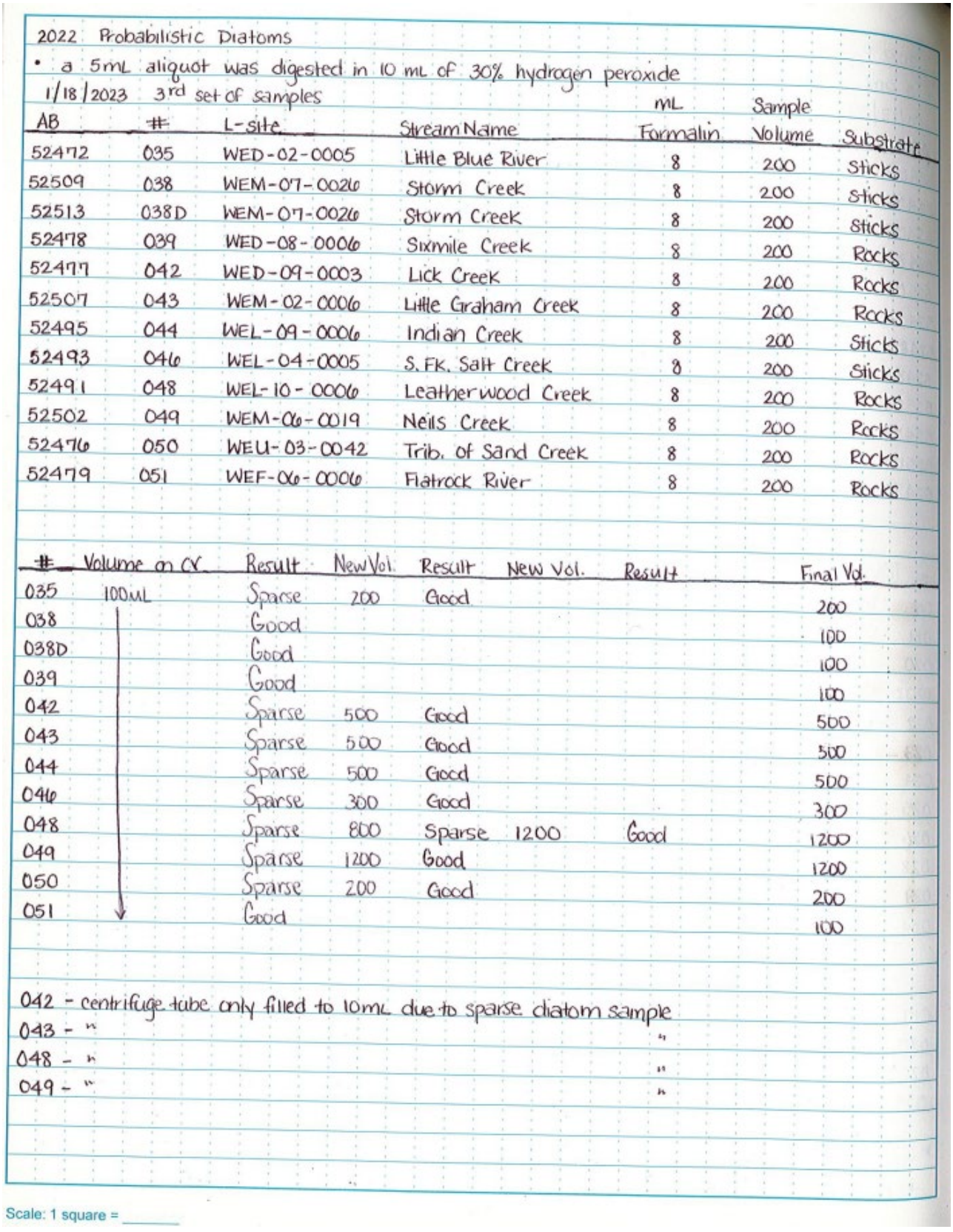
Taxon	Count	Notes

Taxon	Count	Notes

Notes:

Total:

9.4 – Example of a completed page in the Diatom Laboratory Notebook



2022 Probabilistic Diatoms

• a 5mL aliquot was digested in 10 mL of 30% hydrogen peroxide

1/18/2023 3rd set of samples

AB	#	L-site	Stream Name	mL Formalin	Sample Volume	Substrate
52472	035	WED-02-0005	Little Blue River	8	200	Sticks
52509	038	WEM-07-0026	Storm Creek	8	200	Sticks
52513	038D	WEM-07-0026	Storm Creek	8	200	Sticks
52478	039	WED-08-0006	Sixmile Creek	8	200	Rocks
52477	042	WED-09-0003	Lick Creek	8	200	Rocks
52507	043	WEM-02-0006	Little Graham Creek	8	200	Rocks
52495	044	WEL-09-0006	Indian Creek	8	200	Sticks
52493	046	WEL-04-0005	S. Fk. Salt Creek	8	200	Sticks
52491	048	WEL-10-0006	Leatherwood Creek	8	200	Rocks
52502	049	WEM-06-0019	Neils Creek	8	200	Rocks
52476	050	WEU-03-0042	Trib. of Sand Creek	8	200	Rocks
52479	051	WEF-06-0006	Flatrock River	8	200	Rocks

#	Volume on CY	Result	New Vol.	Result	New Vol.	Result	Final Vd.
035	100uL	Sparse	200	Good			200
038		Good					100
038D		Good					100
039		Good					100
042		Sparse	500	Good			500
043		Sparse	500	Good			500
044		Sparse	500	Good			500
046		Sparse	300	Good			300
048		Sparse	800	Sparse	1200	Good	1200
049		Sparse	1200	Good			1200
050		Sparse	200	Good			200
051		Good					100

042 - centrifuge tube only filled to 10mL due to sparse diatom sample
043 - " "
048 - " "
049 - " "

Scale: 1 square = _____

9.5 – List of diatom taxonomic references

Located in the Laboratory

1. “Freshwater flora of Central Europe” (“Suesswasserflora von mitteleuropa”) series published by Gustav Fischer Verlag, Germany, editors: H. Ettl, J. Gerloff, H. Heynig, D. Mollenhauer.

    - Band 2/1: Bacillariophyceae: Naviculaceae 1986.
    - Band 2/2: Bacillariophyceae: Bacillariaceae, Epithemiaceae, Surirellaceae 1988.
    - Band 2/3: Bacillariophyceae: Centrales, Fragillariaceae, Eunotia 1991. Orders Centrales: Melosira, Orthoseira, Ellerbeckia, Aulacoseira, Cyclotella, Cyclostephanos, Stephanodiscus, Thalassiosira, Stephanocostis, Skeletonema, Acanthoceras, Chaetoceros, Rhizosolenia, Pleurosira, Actinocyclus. In der Familie Fragilariaceae: Tetracyclus, Diatoma, Meridion, Asterionella, Tabellaria, Synedra, Fragilaria, Opephora, Hannaea, Centronella. Eunotiaceae: Eunotia, Actinella, Peronia.
    - Band 2/4: Bacillariophyceae: Achnanthaceae und Gomphonema 1-4 1991.

2. “Freshwater Algae of North America: Ecology and Classification.” Wehr and Sheath eds. 2003.

3. “How To Know the Freshwater Algae.” Prescott 3rd ed. 1970.

4. “The Freshwater Algal Flora of the British Isles.” John, Whitton, and Brookes 2002.

5. “Diatoms of North America.” William Vinyard 1979.

Online resource:

1. Spaulding et al. 2021. Diatoms.org: supporting taxonomists, connecting communities. Diatom Research 36(4): 291-304. doi:10.1080/0269249X.2021.2006790