Periphyton Field Collection Procedures

B-004-OWQ-WAP-XXX-24-T-R2
Technical Standard Operating Procedure
Office: Water Quality
Branch: Watershed Assessment and Planning
Sections: Probabilistic and Targeted Monitoring

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Purpose

This technical standard operating procedure (TSOP) describes the methods for collecting periphyton in the field. Specifically, this TSOP covers the processes of:

- sample collection preparation
- field collection of:
    - periphyton
- sampling:
    - epilithic
    - epidendric
- sample preservation and paperwork

Periphyton samples are collected during low flow waterway conditions, from September through October. Diatoms are extracted from periphyton samples and processed and identified in the Indiana Department of Environmental Management (IDEM) laboratory located at the Shadeland office (refer to “Processing and Identification of Diatom Samples” TSOP). Diatoms are bioindicators, facilitating the “weight-of-evidence” approach to interpretation of biomonitoring results as recommended by the U.S. Environmental Protection Agency (U.S. EPA). Diatom data will be used in the diatom index of biological integrity (IBI) to learn more about diatom communities at sites and will potentially be used in assessments for aquatic life use or nutrient impairment.

Scope

This TSOP applies to agency staff in the Office of Water Quality (OWQ), Probabilistic and Targeted Monitoring Sections, who are responsible for collecting periphyton samples.

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Authorizing Signatures

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

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This TSOP is consistent with agency requirements.

Quality Assurance Staff
Office of Program Support
Date: 8/30/2024

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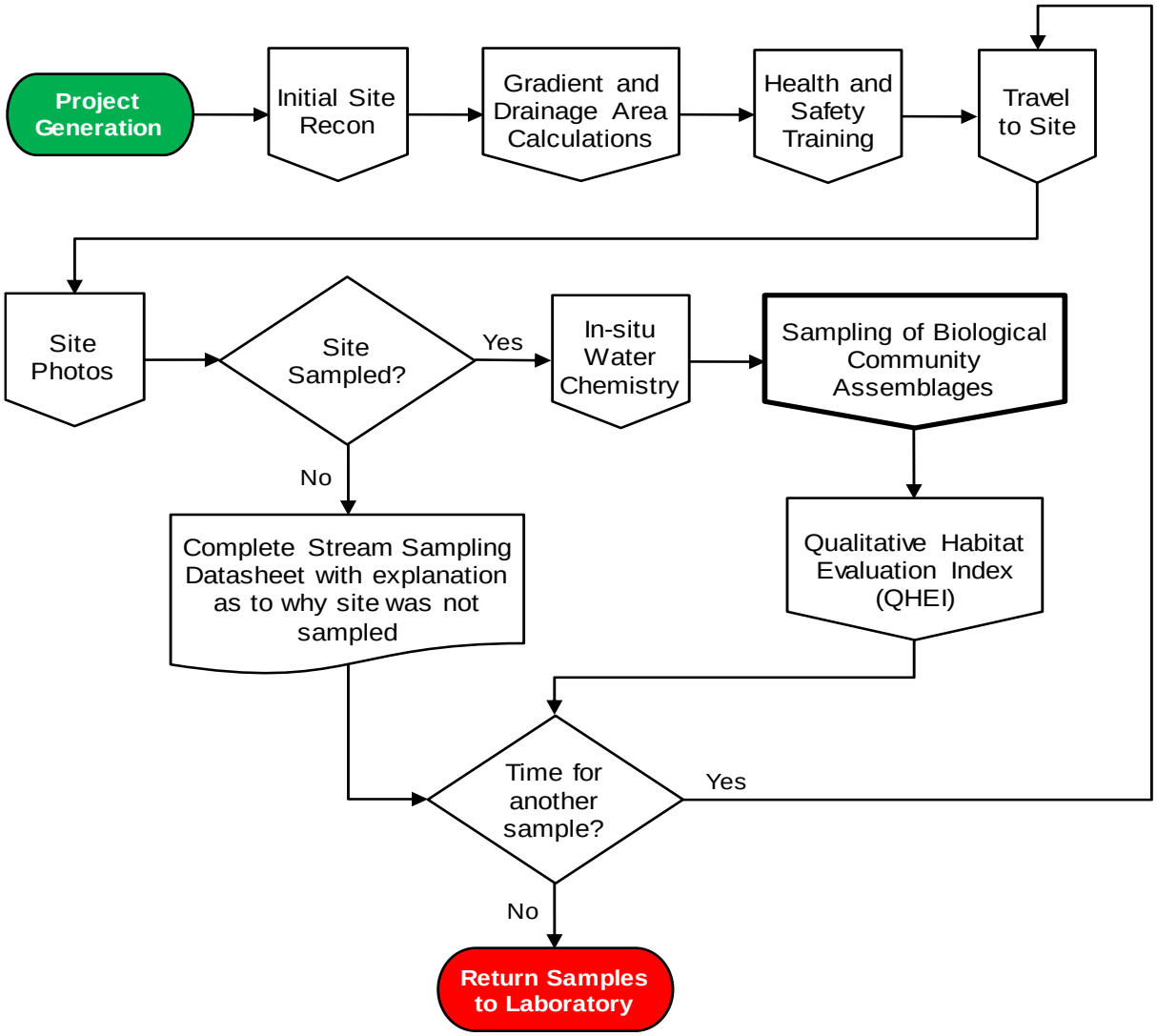
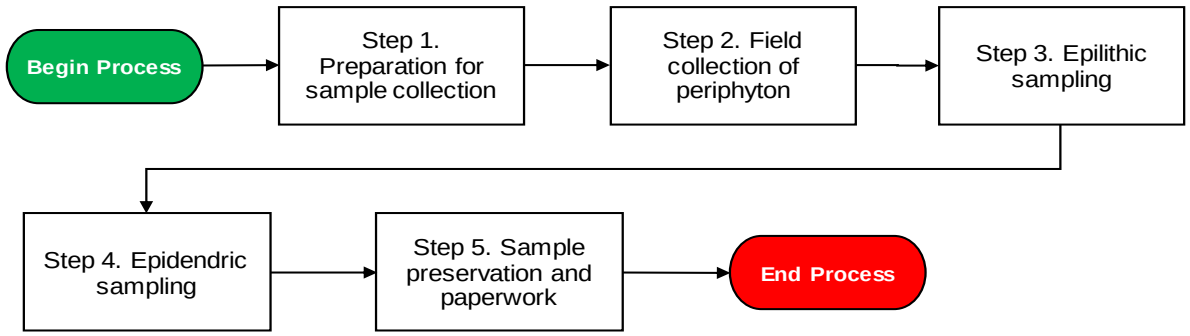


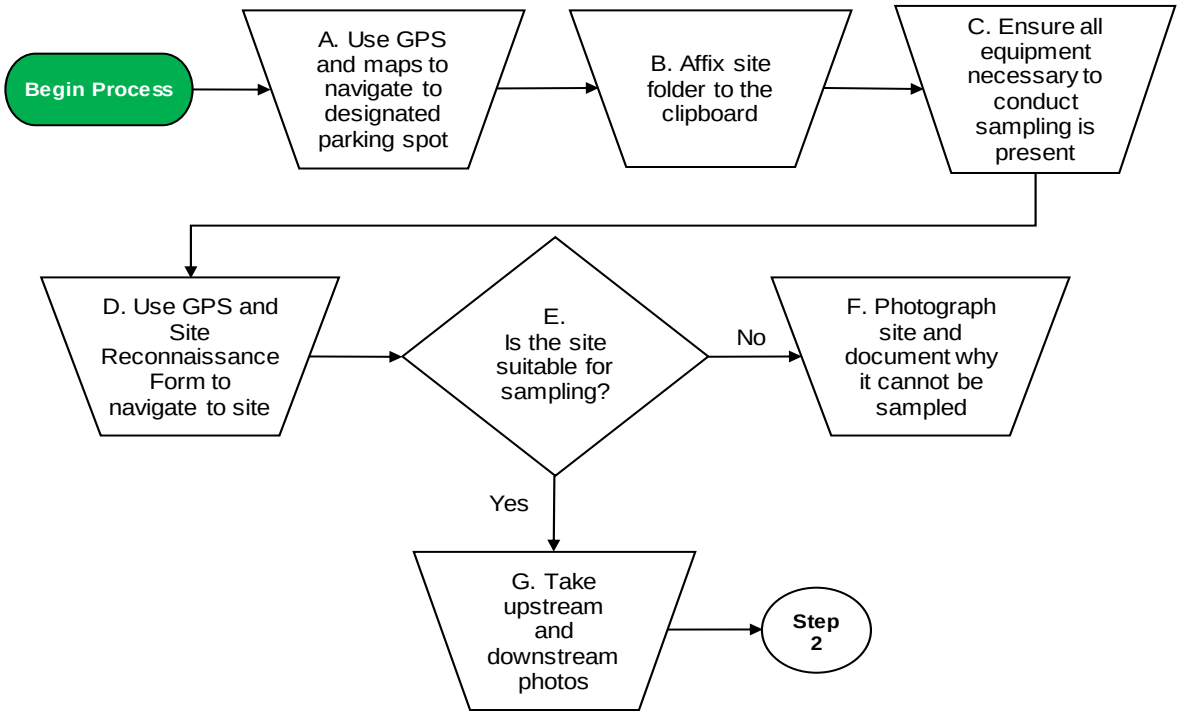
2.0. Procedure

2.1. Procedural Flowchart



2.2. Procedural Steps

Step 1. Preparation for sample collection



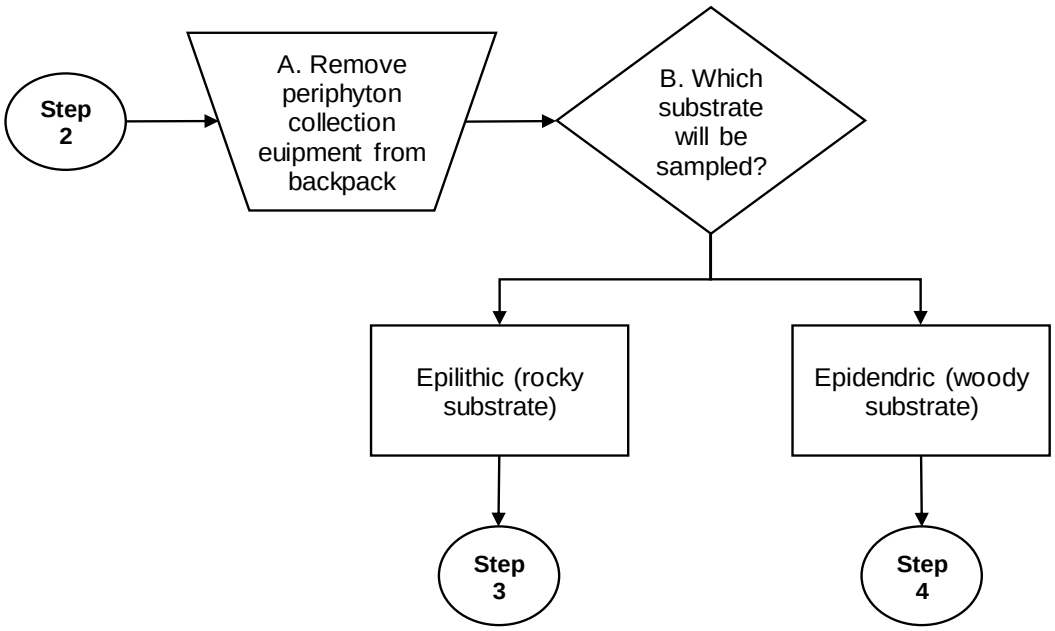
A. The field crew uses a combination of the Indiana Atlas & Gazetteer, aerial maps (Appendix 9.1), topographic maps (Appendix 9.2), and handheld Global Positioning System (GPS) unit to drive to the designated parking spot as described on the Site Reconnaissance Form (Appendix 9.3).
B. The crew chief affixes the site folder, which contains the field sheet, the Algal Biomass Lab Datasheet (Appendix 9.4), the Site Reconnaissance Form, site maps, and field sheets from previous sampling visits to the clipboard.
C. The crew chief, using the Water Chemistry Sampling Checklist (Appendix 9.5), will ensure that all equipment necessary to conduct periphyton sampling is present and stored in backpacks for transport to the sampling site.
D. Using the handheld GPS unit and the Site Reconnaissance Form, the crew chief and field crew proceed to the sampling site with the clipboard, backpacks, and any other equipment that is required to complete sampling.
E. Upon arrival at the x-point, the crew chief determines if the site can be sampled. To be sampled, water must be present in 50% of the stream reach. If the site cannot be sampled,

proceed to Step 1.F. If the site can be sampled, proceed to Step 1.G.

F. If the site cannot be sampled, the field crew will take a photo of the site. The photograph should illustrate why the site was rejected. The crew chief completes the Algal Biomass Lab Datasheet and appropriate forms for the collection of water chemistry (IDEM 2024). The crew chief and field crew return to the vehicle and repeat the process, starting with Step 1.A, if time allows for sampling another site.

G. If the site can be sampled, the field crew will take upstream and downstream photos. Any abnormalities at the site that may be affecting water quality, such as algal blooms, dead fish, or modifications to the bank or stream, are photographed as well. Then, proceed to Step 2 to sample periphyton.

Step 2. Field collection of periphyton



A. The field crew removes the proper equipment for collecting periphyton from the backpack. The crew chief and field crew put on gloves and wear them during sample collection.

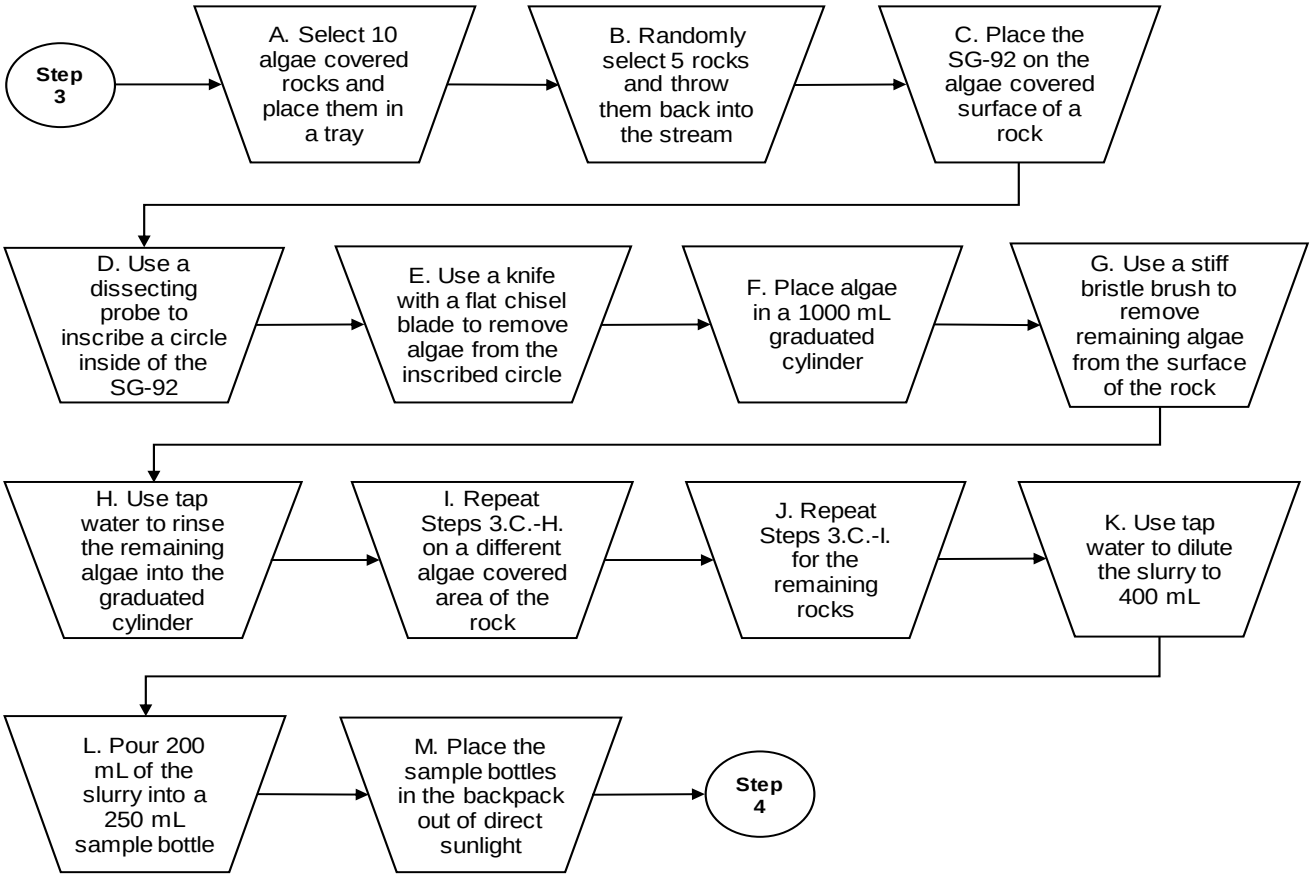
B. The crew chief decides which substrate periphyton will be collected from. Periphyton samples are collected from one of two substrate types: epilithic (rocks) or epidendric (sticks). Selection of the habitat where benthic algal samples are collected should be based on the following hierarchy:

1. Riffles in shallow streams with coarse-grained substrates (epilithic habitat)

2. Woody snags in streams with fine-grained substrates (epidendric habitat)

Rocks represent the most stable substrate, which more accurately reflects stream conditions at a specific site. This is why they are given precedence over sticks. Periphyton samples will be collected according to a modification of one of the epilithic sampling protocols described in Moulton et al. (2002). Proceed to Step 3 for epilithic sampling and Step 4 for epidendric sampling.

Step 3. Epilithic Sampling



A. The field crew collects ten algae covered rocks from a transect across the stream that includes the x-point, and places them into a tray. Rocks may be collected 20-25 meters upstream and downstream from x. Be sure to select rocks that have not been disturbed and try to choose flat rocks to make scraping the algae easier (see Section 2.3.C.).

B. The crew chief or field crew randomly selects five of the ten rocks to be thrown back into the stream.

C. The crew chief places the SG-92 on the algae covered surface of a rock. The SG-92 is a modified syringe sampling device that performs best on smooth cobble surfaces with moderate to dense coverage of microalgal periphyton. The SG-92 barrel should be placed on a smooth part of the cobble. Be sure to choose a spot on the rock that will allow enough room for a second SG-92 scrape to be made on the same rock.

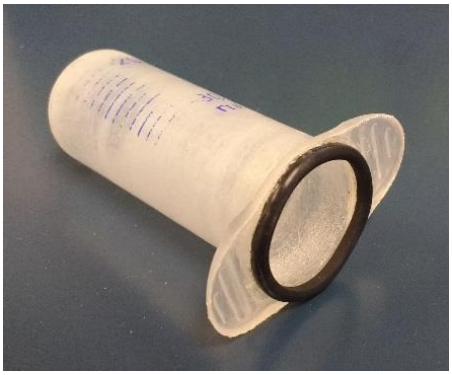
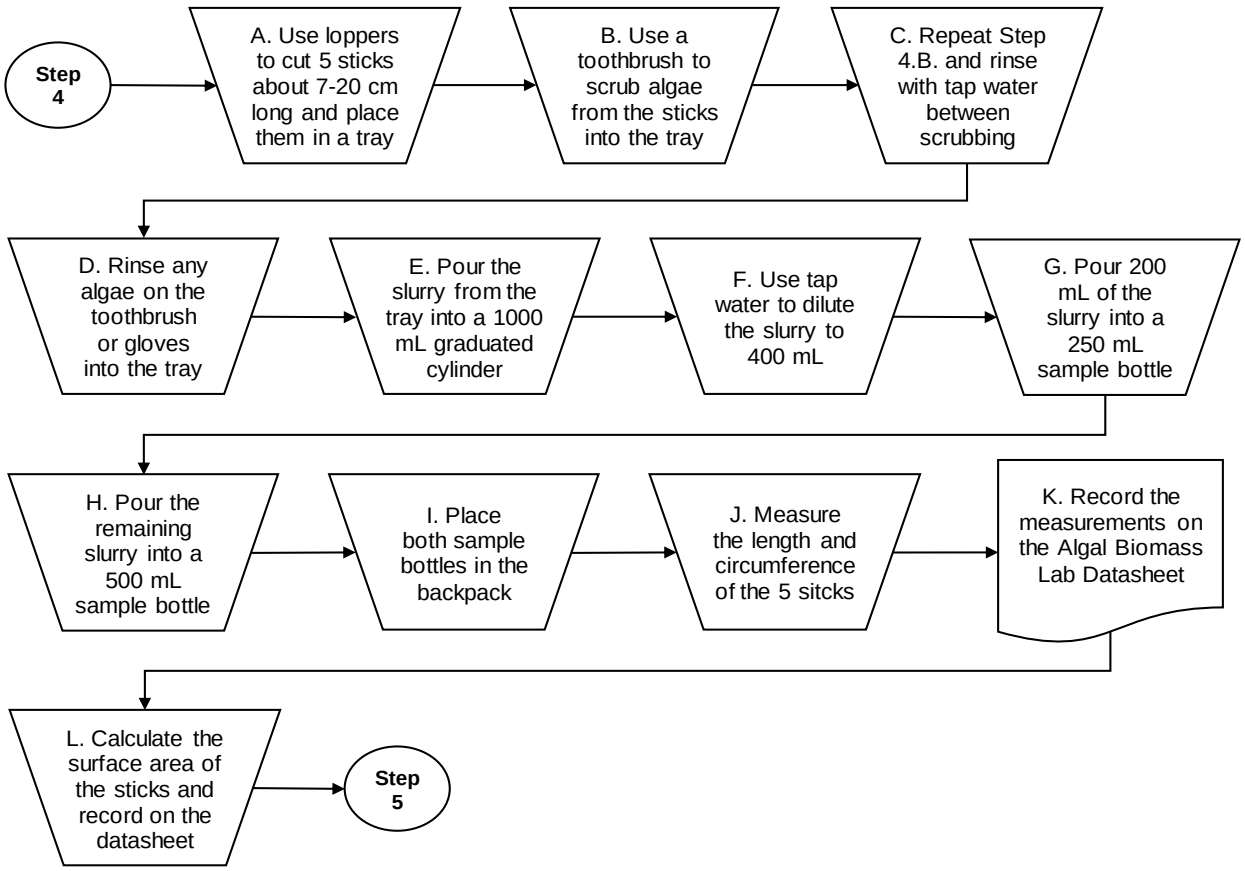


Figure 1. The SG-92 is used for epilithic sample collection. See Appendix 9.7 for assembly instructions.

D. The crew chief uses a dissecting probe to inscribe the inside circumference of the SG-92 onto the rock surface. The SG-92 is removed from the rock once the circle has been inscribed.
E. The crew chief uses a knife with a flat chisel blade to scrape periphyton from the inscribed circle on the rock. Make sure to only scrape algae from within the inscribed circle, since the circle is a known area.
F. The crew chief places the removed periphyton directly into a clean 1000 milliliter (mL) graduated cylinder.
G. The crew chief uses a stiff bristle brush to remove any remaining periphyton from the inscribed circle on the rock.
H. The crew chief uses tap water to rinse the remaining periphyton from the inscribed circle, knife, and brush into the graduated cylinder.
I. The crew chief repeats Steps 3.C. – 3.H. to scrape a different periphyton covered area of the rock.
J. The crew chief repeats Steps 3.C. – 3.I. until two areas are scraped from each of the five rocks, yielding one composite sample of 10 scrapings.
K. The crew chief uses tap water to dilute the slurry to 400 mL.

L. The crew chief homogenizes the sample and pours 200 mL of the slurry into a 250-mL sample bottle. This sample will be used for the diatom identification and enumeration project.
M. The crew chief places the sample bottles in the backpack and out of direct sunlight to prevent photodegradation of the sample.

Step 4. Epidendric Sampling



A. The field crew uses loppers to cut five submerged sticks ranging from 7–20 cm in length and located as close to the x-point as possible. The cut sticks are placed into a tray. Only undisturbed sticks with visible algae and devoid of irregularities are selected for periphyton sampling.
B. The field crew uses a toothbrush to scrub periphyton from the sticks into the tray.
C. The field crew repeats Step 4.B. until all periphyton has been removed and rinses the stick with tap water between scrubbings. Sticks should be rinsed and scrubbed over the tray so that all material lands in the tray. The sticks are scrubbed hard enough to remove periphyton, but not too hard

that the bark would be removed and end up in the tray (see Section 2.3.C.).

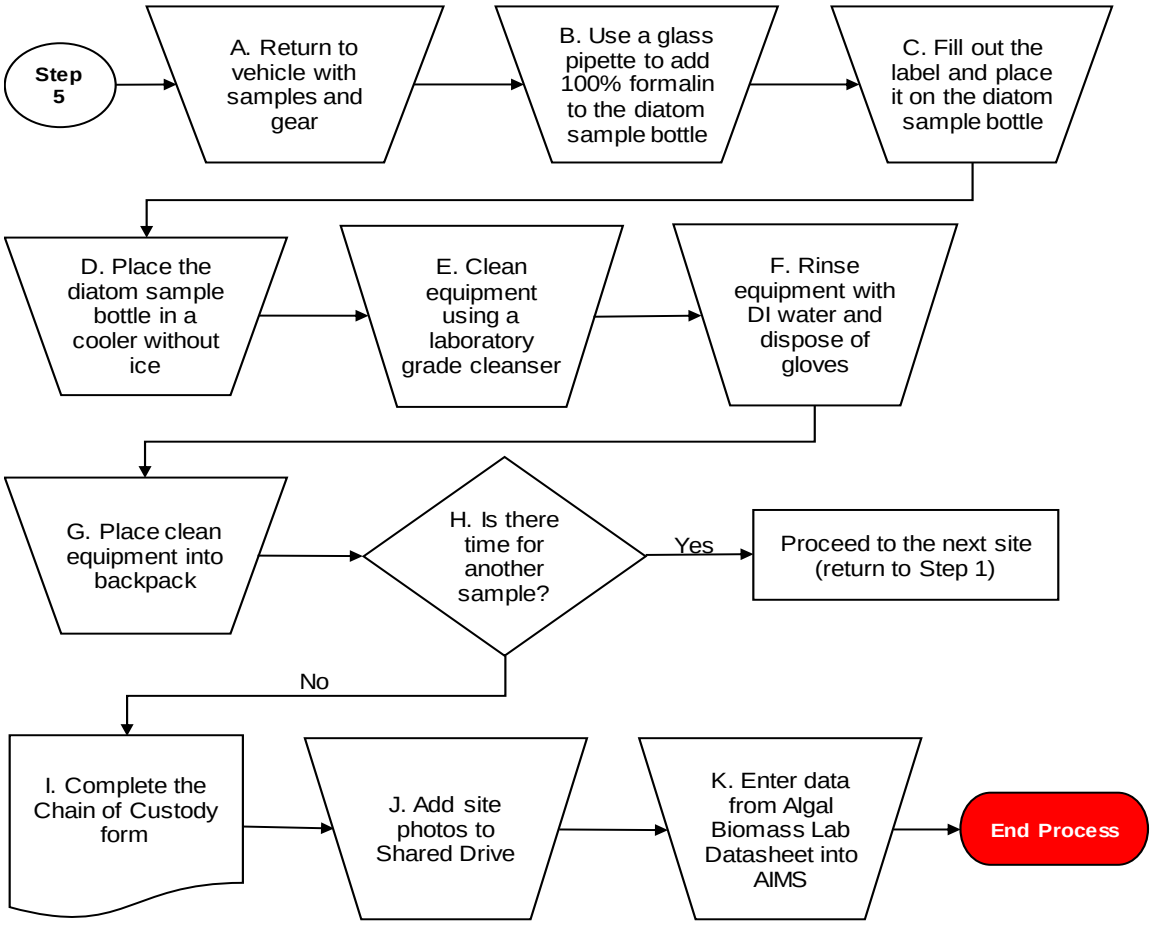
D. The field crew uses tap water to rinse any periphyton remaining on the toothbrush and gloves into the tray.
E. The field crew pours the slurry from the tray into a clean 1000 mL graduated cylinder.
F. The field crew uses tap water to dilute the slurry in the graduated cylinder to 400 mL.
G. The field crew homogenizes the sample and pours 200 mL of the slurry into a plastic 250-mL sample bottle to be used for diatom identification and enumeration.
H. The field crew places both sample bottles in the backpack out of direct sunlight.
I. The field crew uses fabric measuring tape to measure the length and circumference of the five sticks (the circumference should be measured at each end and the middle of the stick).
J. The field crew records the measurements on the Algal Biomass Lab Datasheet (Appendix 9.4).
K. The field crew calculates the lateral surface area of each of the sticks using a formula that assumes the shape is a cylinder:

$$\text{Area} = \sum_{i=1}^{n} \mathrm{U}_i \mathrm{L}_i \text{ where,}$$

$\mathrm{U}_i =$ the average of the three circumferences for the segment

$\mathrm{L}_i =$ the length of the segment

Step 5. Sample Preservation and Paperwork



A. After the periphyton sample has been collected and placed into the backpack, the crew chief and field crew proceed to the vehicle with samples and gear. A new pair of gloves must be worn during preservation.

B. To preserve the diatom sample (the 200 mL sample collected in Step 3.L. or Step 4.G.), the crew chief uses a 10 mL glass pipette to add 100% formalin to the diatom sample bottle. The crew chief uses 2 mL of 100% formalin for every 50 mL of sample (e.g., a 200 mL sample would be preserved with 8 mL of formalin). Formalin is stored inside of a 250-mL plastic bottle placed in a sealable bag and kept in a plastic container. The glass pipette is kept in the sealable bag and used only for the purpose of adding formalin to the diatom sample. (See Section 2.3.A. for important health and safety information, and 2.3.C. for information on why it is important to preserve the diatom sample.)

C. The crew chief fills out the weatherproof diatom label with the date and time of sample collection, formalin volume, sample

volume, and type of substrate sampled. The label is then placed on the diatom sample bottle.

D. The crew chief places the diatom sample in a separate cooler without ice.
E. The field crew uses laboratory grade cleaner to clean all equipment that came in contact with samples (see sections 2.3.A. and 2.3.C.). A wash basin with tap water and cleaner is used to wash the equipment.
F. After washing equipment, the field crew rinses equipment with deionized water. Once all equipment has been washed and rinsed, the field crew may remove and dispose of their gloves.
G. The field crew places clean equipment back into the appropriate totes and backpacks.
H. Is there time to sample another site? If yes, proceed to Step 1.A. If no, proceed to Step 5.I.
I. If there is not enough time to sample another site, the crew chief completes a Chain of Custody form (Appendix 9.6) for diatom samples.
J. When staff return to the office, upstream and downstream photos as well as photos of any site abnormalities that may affect water quality (e.g., algal blooms, fish kills, or modifications to the bank or stream) are downloaded from the GPS unit or work phone and saved to the appropriate folder on the Shared Drive. The filenames should be changed to include a three-digit U.S. EPA site identification number, the direction of flow (i.e. US for upstream or DS for downstream), initials of the photographer, initials of other crew members, and date as MMDDYY (example: 001 US KAK TAF 061511).
K. The crew chief and field crew enter algal biomass data (type of substrate sampled, volume of sample filtered, etc.) from the Algal Biomass Lab Datasheet (Appendix 9.4) into the Assessment Information Management System (AIMS) database. The information is entered in the “Biomass” tab under “Algae”. Ancillary and in-stream observations are entered into AIMS in the days or weeks following sample collection depending on time constraints of the field staff.

2.3. Related Technical Issues

A. Health and Safety Warnings

1. Safety issues are the responsibility of all crew members; however, any questions in the field should be directed to the crew chief. The crew chief is responsible for the completion of all work listed in the TSOP, the health and safety aspects of the sampling event, and successful interactions with landowners and members of the public.
2. All field staff are required to complete basic first aid and cardio-pulmonary resuscitation (CPR) training.
3. According to the memorandum “Change in status of Water Assessment Branch staff in accordance with the Agency training policy”, dated November 29, 2010, OWQ Watershed Assessment and Planning Branch staff are exempt from initial and annual training requirements set forth in Section 6.0 of the IDEM Health and Safety Training Policy (IDEM 2010). The memorandum also states, “as an alternative to the training requirements of the policy, the branch will conduct in-service training at a minimum of four hours per year on topics directly related to duties performed by staff.” New hires or those changing job responsibilities without the minimum four-hour training must be accompanied in the field by a staff member who has met the requirements of the branch health and safety training.
4. Sampling on surface waters requires safety consciousness of staff members and the use of specialized equipment; thus, staff will comply with the IDEM Personal Protective Equipment (PPE) Policy (IDEM 2024a). If an injury or illness arises in the field, staff will follow the IDEM Injury and/or Illness Resulting from Occupational Exposure Policy (IDEM 2024b).
5. To prevent drowning during sample collection, personnel will wear appropriate clothing and PPE when operating boats or sampling in deep water or swift currents. According to the memorandum “Use of Personal Flotation Devices (PFDs) by Branch Personnel”, dated February 29, 2000, staff must wear U.S. Coast Guard approved Type I, II, or III PFDs whenever:
    a. The planned work requires them to enter the water and the maximum water depth at any place at the work site is over the knee (Note: this depth depends on the employee, but it will usually be between 12 and 20 inches or 300-500 mm).
    b. The employee is in a watercraft of any kind that is being launched, is in the water, or is being retrieved from the water.
    c. The employee must work from structures that do not possess guard rails and are over or alongside water where the water depth is, or could reasonably be expected to be, three feet or more.

6. In addition, when work is being done in boats on boundary waters as defined by Indiana Code (IC) 14-8-2-27, or between sunset and sunrise on any waters of the state, all personnel in the watercraft must wear a high intensity whistle and Safety of Life at Sea (SOLAS) certified strobe light.
7. Field personnel collecting and preserving algal community samples will follow policies and procedures established in the Water Chemistry Field Sampling Procedures (IDEM 2024d), the Hazardous Communication Plan (IDEM 2019), the Office of Water Quality Watershed Assessment and Planning Branch Laboratory Safety Plan (IDEM 2021), and Transportation, Use, the Handling, and Storage of Laboratory Chemicals Used to Preserve Biological Samples (IDEM 2024c).
8. To prevent inhaling formalin fumes, staff should position themselves upwind of the sample to which formalin is being added.
9. Before using a chemical listed in this procedure, staff must review the safety data sheets (SDS), which are stored in a binder in the laboratory in Building 41.
10. When handling chemicals, staff must also wear appropriate PPE. For the purposes of this TSOP, required PPE will consist of:
    a. Chemical resistant nitrile gloves
    b. Protective eyewear (safety glasses or goggles)
    c. A face shield

B. Cautions
1. To prevent degradation of algae samples, prevent contact with light and heat sources.
    a. To preserve the representativeness of an algae sample, place sample bottles in the backpack immediately after sample collection (Steps 3.M. and 4.H.). Algae grow relatively quickly when exposed to light.
    b. The diatom samples are preserved with formalin and kept in a dark cooler without ice to prevent degradation (Steps 5.A. – 5.D.).
2. Be mindful that samples are collected from a representative location. For example, if a tributary enters upstream of the site, rocks collected from above the tributary may not be representative of the community at the site, due to different inputs and water quality.
3. It is important to avoid cross-contamination of the samples. All equipment that comes in contact with the samples must be cleaned thoroughly in the field. For this project, all necessary equipment is

cleaned between sampling sites with laboratory grade cleaner (Step 5.E.).

C. Interferences
   1. To prevent loss or corruption of data, always record the correct sample number and site information legibly during field collection and processing procedures.
   2. When collecting periphyton from an epilithic habitat, be sure to choose undisturbed, flat rocks to make scraping algae easier (Step 3.A.). Disturbed rocks do not provide a representative sample of the habitat and irregular rocks make it difficult to collect a complete sample. Choose rocks large enough to allow for two SG-92 scrapes. When selecting rocks, try to walk along the stream edge and/or start downstream before heading upstream. This prevents the disturbance of any potential rocks that may end up in the epilithic sample and helps with visibility as disturbed sediment will flow downstream.
   3. When collecting periphyton from an epidendric habitat, try not to scrape bark off the stick when scrubbing the algae (Step 4.C.).
   4. Be sure to properly preserve the diatom identification and enumeration sample (Step 5.B.). Algal cells may continue to grow and/or decompose in the sample bottle if the samples are not preserved properly.
   5. To avoid cross-contamination of samples, ensure that equipment is thoroughly cleaned between sites (Step 5.E.).

D. Calibration
   1. Not applicable.

E. Troubleshooting
   1. Not applicable.

3.0. Roles

3.1. Responsibilities

A. Crew Chief
   1. Preparation for sample collection
   2. Field collection of periphyton
   3. Epilithic sampling
   4. Epidendric sampling
   5. Sample preservation
   6. Cleanup and paperwork

B. Field Crew

1. Preparation of sample collection
2. Field collection of periphyton
3. Epilithic sampling
4. Epidendric sampling
5. Sample preservation
6. Cleanup and paperwork

3.2. Training requirements

A. Preparation for sample collection
   1. Crew chief
   2. Field crew

B. Field collection of periphyton
   1. Crew chief
   2. Field crew

C. Epilithic sampling
   1. Crew chief
   2. Field crew

D. Epidendric sampling
   1. Crew chief
   2. Field crew

E. Sample preservation
   1. Crew chief
   2. Field crew

F. Cleanup and paperwork
   1. Crew chief
   2. Field crew

4.0. Required Forms, Equipment, or Software List

4.1. Forms

A. Site Reconnaissance Form (Appendix 9.3)

B. Algal Biomass Lab Datasheet (Appendix 9.4)

C. Water Chemistry Sampling Checklist (Appendix 9.5)

D. Chain of Custody (Appendix 9.6)

4.2. Equipment

A. Toothbrush

B. Cloth measuring tape

C. Stencil brush

D. Small hobby knife with chisel blade

E. Dissection probe

F. Modified syringe with O-ring attached (SG-92)

G. Nalgene© HDPE plastic 250 mL sample bottles (for diatom samples)

H. Plastic bins

I. 500 mL Unitary wash bottle

J. 1000 mL graduated cylinder

K. 500 mL plastic sample bottle

L. Loppers

M. Tray

N. Chemical resistant nitrile gloves

O. 100% Formalin (stored in 250 mL bottle)

P. 10 mL glass pipettes

Q. Weatherproof labels (preprinted in the office with AC number, U.S. EPA site identification number, L-site, and site description for each site)

R. Multiple large coolers (1 cooler for diatom samples, multiple coolers for water chemistry samples if they are collected at the same time)

S. Equipment totes

T. Tap water carboy (5-gallon capacity)

U. Deionized water carboy (5-gallon capacity)

V. Permanent marker

W. Trimble Juno 3D and/or SB GPS unit

X. Safety glasses

Y. Eyewash station

Z. First Aid Kit

AA. Hip boots

BB. Chest waders

CC. Life jacket (U.S. Coast Guard approved Type I, II, or III)

DD. Laboratory grade cleaner

EE. Tape measure for stream width

FF. Wading rod

GG. Aluminum foil

HH. Pipette pumps

II. Scrub brush

JJ. Face shield

KK. High intensity whistle

LL. Safety of Life at Sea (SOLAS) certified strobe light

4.3. Software

A. AIMS database

5.0. Records Management

5.1. Site Reconnaissance Form

A. The original hard copy is kept in the site folder and stored in a file cabinet in the Watershed Assessment and Planning Branch library at the IDEM Shadeland office.

B. Information recorded on the Site Reconnaissance Form is entered into the AIMS database.

C. The original hard copy is retained until it is scanned and uploaded to AIMS or the Virtual File Cabinet (VFC).

5.2. Algal Biomass Datasheet

A. The original hard copy is kept in the site folder and stored in a file cabinet in the Watershed Assessment and Planning Branch library at the IDEM Shadeland office.

B. Information recorded on the Algal Biomass Lab Datasheet is entered into the AIMS database.

C. The original hard copy is retained until it is scanned and uploaded to AIMS or VFC.

5.3. Chain of Custody Forms

A. Original hard copies are kept in the site folder and stored in a file cabinet in the Watershed Assessment and Planning Branch library at the IDEM Shadeland office.

B. Field datasheets and chain of custody forms are scanned and stored as attachments in AIMS.

C. Original hard copies are retained until scanned and uploaded to VFC.

5.4. AIMS Database

A. http://aims.idem.in.gov/AIMS/Pages/Login/

6.0. Definitions

6.1. “AA/AB/AC number” – 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 data base).

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. “Algae” – Chlorophyll-bearing, nonvascular aquatic plants. Examples of algae include diatoms, green and red algae, and primitive photosynthetic bacteria such as Cyanobacteria (also called blue-green algae).

6.4. “Algal Biomass Lab Datasheet” – Form used to record information for algal biomass and diatom samples (Appendix 9.4). Form contains information about sampling time, canopy closure, and algae substrate collected.

6.5. “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.

6.6. “Benthic habitat” – Habitat associated with or occurring on the bottom of a body of water.

6.7. “Carboy” – A large container, generally of five-gallon capacity, used to store water.

6.8. “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 (Appendix 9.6).

6.9. “Deionized water” – Water that has had its mineral ions removed through a process called ion exchange.

6.10. “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.

6.11. “Environmental Protection Agency (EPA) site ID” – The identification number given to the 100 random probabilistic sites drawn yearly. Example: INRB16-001.

6.12. “Epidendric” – Benthic habitat consisting of woody substrates on which organisms are attached or loosely associated.

6.13. “Epilithic” – Benthic habitat consisting of natural coarse-grained substrates (gravel, cobble, boulders) or bedrock.

6.14. “Gazetteer” – A geographical dictionary or directory (i.e., listing cities, towns, rivers, mountains, and other geographic features along with the exact location of these features).

6.15. “Periphyton” – A complex mixture of algae, cyanobacteria, heterotrophic microbes and detritus that is attached to submerged surfaces in most aquatic ecosystems.

6.16. “Photodegradation” – The alteration of materials by light.

6.17. “Safety Data Sheet (SDS)” – A sheet containing data regarding the properties of a particular substance or product. It is intended to provide workers and emergency personnel with procedures for handling or working with that substance or product in a safe manner.

6.18. “SG-92” – Modified syringe-sampling device that creates a seal when pressed onto smooth cobble surfaces with moderate-to-dense coverage of microalgal periphyton. This device is used to collect periphyton samples off of rocks or other submerged objects where algae are attached.

6.19. “Site Folder” – A folder for a specific site that contains all pertinent paperwork to do with the site. Site reconnaissance forms, all field data sheets including those for water chemistry, algal biomass, fish community,

macroinvertebrate community, chain of custody forms, etc. are all stored in this folder which is located in a file cabinet in the Watershed Assessment and Planning Branch library at the IDEM Shadeland office.

6.20. “Site Reconnaissance Form” – Form used to gather information such as landowner, equipment needed to complete sampling, and the access route to take to the site (Appendix 9.3).

6.21. “Stream reach” – A segment of a stream equal in length to 15 times the average wetted width of the stream, with a minimum length of 50 meters and a maximum length of 500 meters.

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

6.23. “Transect” – An imaginary line drawn through an area in order to help scientists sample and monitor organisms or conditions along the line. The results obtained from samples along the line give an indication of the organisms or conditions in the entire area.

6.24. “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.

6.25. “Wading rod” – A rod, graduated in feet and tenths of feet, used for stream gaging in shallow water.

6.26. “X-point” – The exact location where sampling should take place in the stream at the probabilistic site. The x-point should be included in transects and/or reaches that are sampled at the site (some biological parameters require sampling an area and not just one point of the waterbody).

7.0. Quality Assurance and Quality Control

7.1. Quality control of periphyton sampling and the enumeration and identification of diatom samples are documented by quality control checks of both field and laboratory data.

	Quality Control Samples	
Sample Type	Duplicate	Blank

Diatom	1 site per week **or** 10% of sites sampled (approximately 5 per basin)	

Table 1 The number of quality control (duplicate and blank) samples for each sample type.

7.2. All sample labels must be accurately and thoroughly completed, including AIMS sample numbers, date, stream name, and sampling location. After sampling has been completed at a given site, all equipment that was in contact with the sample is cleaned with laboratory grade cleaner and rinsed with American Society for Testing and Materials (ASTM) D1193-06 Type III water (ASTM 2011). Chain of Custody forms are filled out in the field to document the collection and transfer to the laboratories. Upon arrival at the laboratories, samples are checked in by the laboratory manager. Once the diatom samples are in storage, there is another Chain of Custody form to document when the sample is removed from storage to be processed and made into a permanent mount.

8.0. References

8.1. USEPA Methodologies:

A. Suter II, G.W. 2016. Weight of Evidence in Ecological Assessment. U.S. Environmental Protection Agency Office of Research and Development, EPA100R16001.

8.2. USGS Methodologies:

A. 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.

B. Wilde, F.D., Radtke, D.B., Gibs, J., and Iwatsubo, R.T. 1999. National Field Manual for the Collection of Water-Quality Data Chapter A4. Collection of Water Samples. U.S. Geological Survey Techniques of Water-Resources Investigations, Book 9, Chapter A4.

8.3. Indiana Codes (IC) or Indiana Administrative Codes (IAC):

A. IC 14-8-2-27: Boundary Waters

8.4. Agency Policies and Standard Operating Procedures:

A. IDEM 2010. IDEM Health and Safety Training Policy. A-030-OEA-10-P-R2. IDEM, Indianapolis, Indiana.

B. IDEM 2019. Hazard Communication (HazCom) Plan. IDEM, Indianapolis, Indiana.

C. IDEM 2021. Office of Water Quality Watershed Assessment and Planning Branch Laboratory Safety Plan. IDEM, Office of Program Support, Indianapolis, Indiana.

D. IDEM 2023. Processing and Identification of Diatom Samples. B-002-OWQ-WAP-TGM-23-T-R1. IDEM, Indianapolis, Indiana.

E. IDEM 2024a. IDEM Personal Protective Equipment Policy. A-059-AW-24-P-R1. IDEM, Indianapolis, Indiana.

F. IDEM 2024b. Injury and/or Illness Resulting from Occupational Exposure. A-034-AW-24-P-R4. IDEM, Indianapolis, Indiana.

G. IDEM 2024c. Transportation, Use, Handling, and Storage of Laboratory Chemicals Used to Preserve Biological Samples. B-063-OWQ-WAP-XXX-24-S-R0. IDEM, Indianapolis, Indiana.

H. IDEM 2024d. Water Chemistry Field Sampling Procedures. B-015-OWQ-WAP-XXX-24-T-R1. IDEM, OWQ, Watershed Assessment and Planning Branch. Indianapolis, Indiana.

8.5. American Society for Testing and Materials Methodology:

A. ASTM D1193-06 (2018), Standard Specification for Reagent Water.

9.0. Appendices

9.1. Example of an aerial map used to locate the site

9.2. Example of a topographic map used to locate the site

9.3. Example of the Site Reconnaissance Form

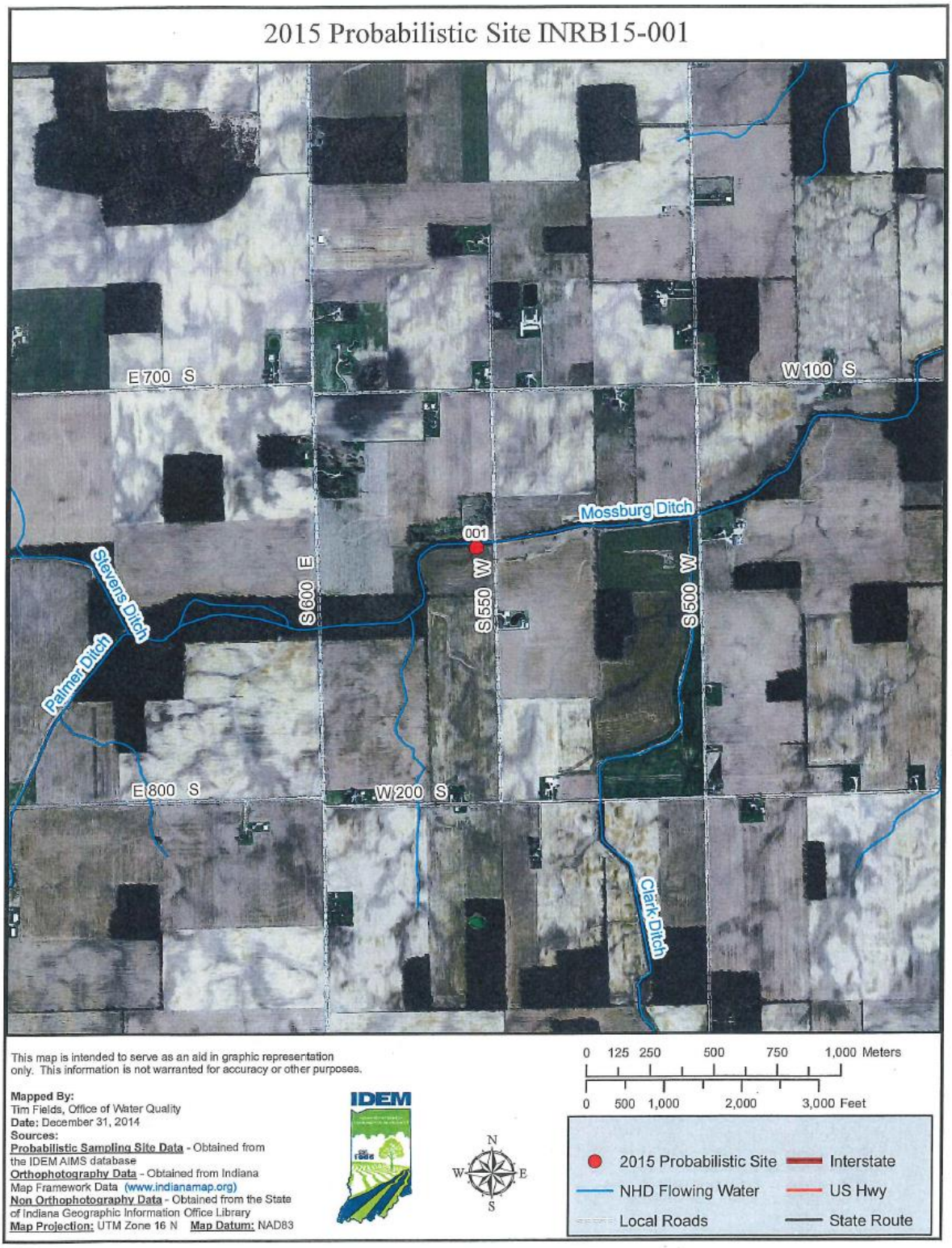
9.4. Algal Biomass Lab Datasheet

9.5. Water Chemistry Sampling Checklist

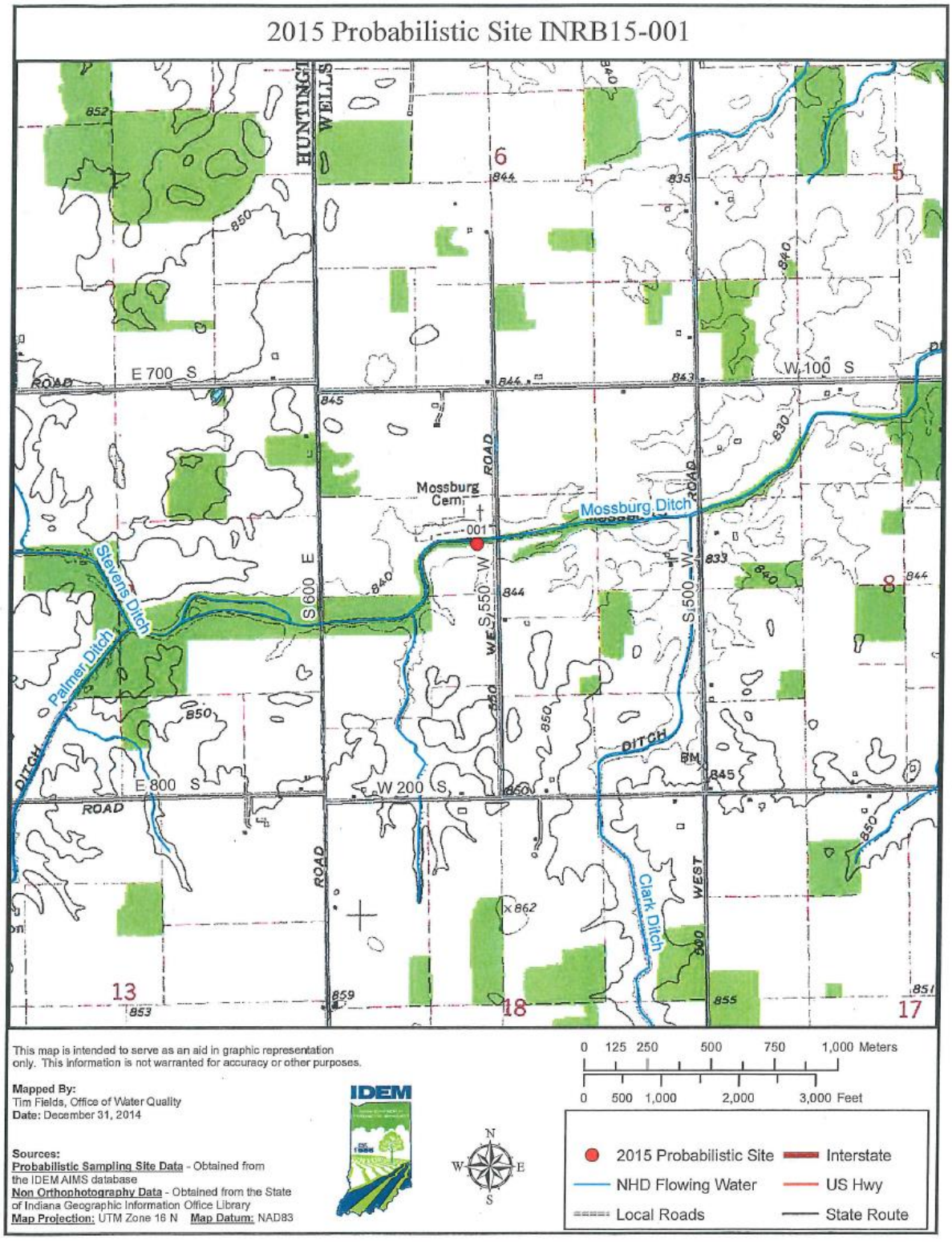
9.6. Chain of Custody Form

9.7. Instructions for making SG-92s for epilithic sampling

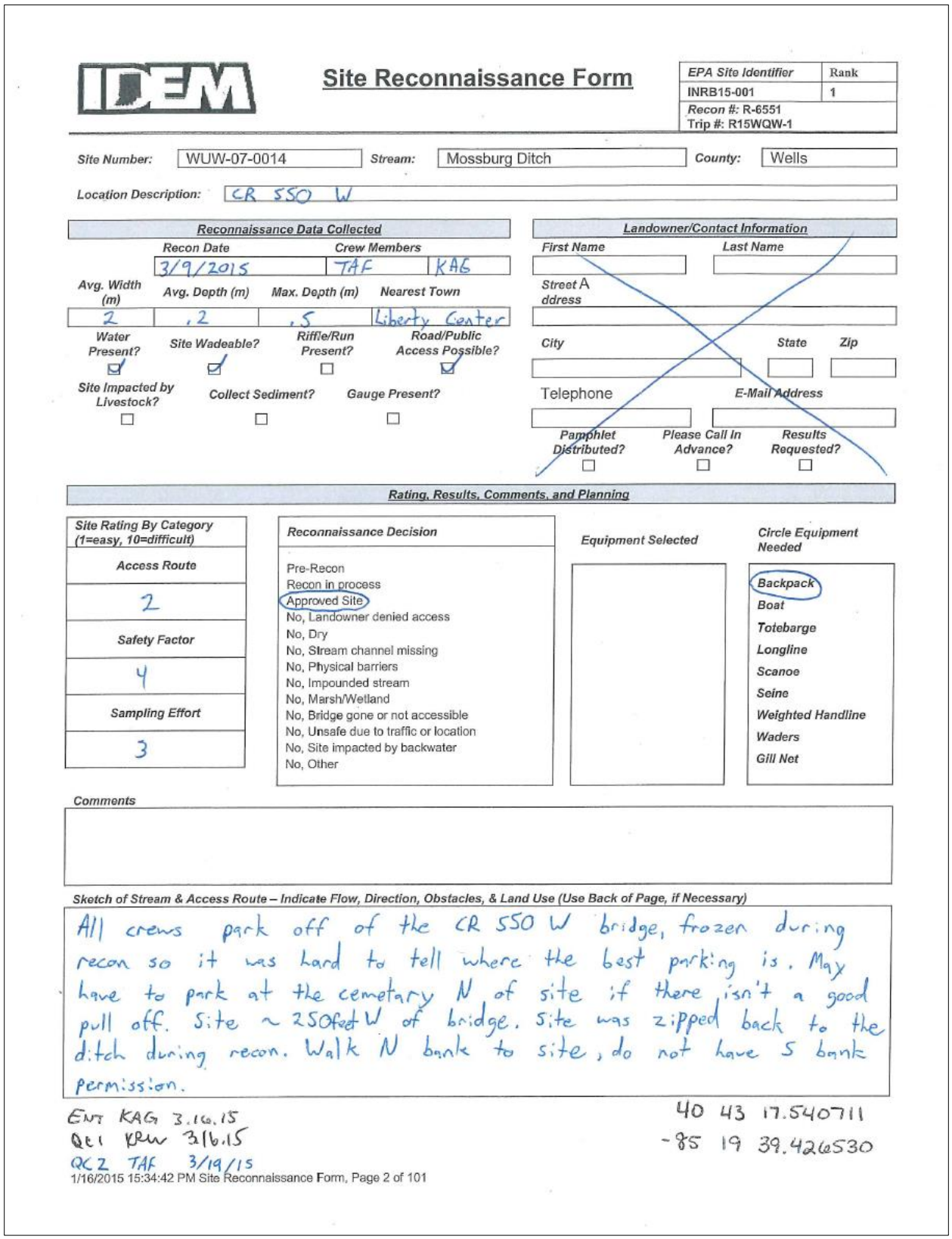
9.1. Example of an aerial map used to locate the site



9.2. Example of a topographic map used to locate the site



9.3. Example of the Site Reconnaissance Form



IDEM

Site Reconnaissance Form

EPA Site Identifier	Rank
INRB15-001	1
Recon #: R-6551 Trip #: R15WQW-1	

Site Number: WUW-07-0014 Stream: Mossburg Ditch County: Wells

Location Description: CR 550 W

Reconnaissance Data Collected

Recon Date	Crew Members	
3/9/2015	TAF	KAG

Avg. Width (m)	Avg. Depth (m)	Max. Depth (m)	Nearest Town
2	.2	.5	Liberty Center

Water Present?	Site Wadeable?	Riffle/Run Present?	Road/Public Access Possible?
☑	☑	☐	☑

Site Impacted by Livestock? ☐ Collect Sediment? ☐ Gauge Present? ☐

Landowner/Contact Information

First Name: Last Name:

Street Address:

City: State: Zip:

Telephone: E-Mail Address:

Pamphlet Distributed? ☐ Please Call In Advance? ☐ Results Requested? ☐

Rating, Results, Comments, and Planning

Site Rating By Category (1=easy, 10=difficult)
Access Route
2
Safety Factor
4
Sampling Effort
3

Reconnaissance Decision

Pre-Recon
Recon in process
Approved Site
No, Landowner denied access
No, Dry
No, Stream channel missing
No, Physical barriers
No, Impounded stream
No, Marsh/Wetland
No, Bridge gone or not accessible
No, Unsafe due to traffic or location
No, Site impacted by backwater
No, Other

Equipment Selected

Circle Equipment Needed

Backpack
Boat
Totebarge
Longline
Scanoe
Seine
Weighted Handline
Waders
Gill Net

Comments

Sketch of Stream & Access Route – Indicate Flow, Direction, Obstacles, & Land Use (Use Back of Page, if Necessary)

All crews park off of the CR 550 W bridge, frozen during recon so it was hard to tell where the best parking is. May have to park at the cemetary N of site if there isn't a good pull off. Site ~ 250 feet W of bridge. Site was zipped back to the ditch during recon. Walk N bank to site, do not have S bank permission.

ENT KAG 3.16.15
QC1 KRW 3/16/15
QC2 TAF 3/19/15

40 43 17.540711
-85 19 39.426530

1/16/2015 15:34:42 PM Site Reconnaissance Form, Page 2 of 101

9.4. Algal Biomass Lab Datasheet

Algal Biomass Lab Datasheet

Sample #	Site	Stream

Supporting Site Information

Traditional Forestry % Closed Canopy: ☐ <=10m ☐ >10m (Measure center only if width <=10m, record to nearest whole percent)

	North	East	South	West	Average x 1.04 =
Left Bank					
Center					
Right Bank					
Total %CC (Average from above, or Center only = %CC)				100 - %CC	

Phytoplankton Information

Sampling Method: ☐ Grab Sample (Dip) ☐ Multiple Verticles Number of Verticles:

Chlorphyll A	Blank	Filter 1	Filter 2	Filter 3	Filter 4
Sample Time					
Sample Volume (mL)					

Periphyton Information

Periphyton Habitat: ☐ Epilithic (Area-Scape) ☐ Epidendric (Cylinder Scrape) ☐ Epipsammic (Petri Dish)

Diatom Sample Collected: ☐ Yes ☐ No Diatom Volume: mL Formalin Volume: mL Slurry Volume mL

Chlorphyll A	Blank	Filter 1	Filter 2	Filter 3	Filter 4
Sample Time					
Sample Volume (mL)					

Periphyton Area Calculation

Cylinder Scrape

Snag #	Length (cm)(L)	Circumference U_1	U_2	U_3	U	Area (L * U)
1						
2						
3						
4						
5						
				Total Area (cm^2)		

Area Scrape (Using SG-92)

Rock#	1	2	3	4	5
Area (cm^2)	7.38	7.38	7.38	7.38	7.38
Total (cm^2)	36.9				

Petri Dish

Number of Discrete Samples (n):	
Total Area of One Sampler (a):	19.01 cm^2
Total Sample Area (n * a):	

Stream Discharge / Rainfall Information

Nearest USGS Gage Site: ☐ Upstream ☐ Downstream ☐ No USGS Gage Near

River miles from site:	Discharge CFS at sampling: CFS
Gage location:	Discharge days since 50% flow exceeded: days
Rainfall data source: ☐ NOAA ☐ CoCoRaHS ☐ Indiana State Climate Office ☐ USGS gage rain gauge ☐ Other:	
Total precipitation at sampling: in. on date:	Cumulative rain 7 days previous to sampling: in.
Rain station location, county:	Inches since last rainfall previous to sampling: in.
	Days since last rainfall previous to sampling: days

Identifier	Date	Reviewer 1	Date	Reviewer 2	Date	Notes:
		☐ Review 1 Completed		☐ Review 2 Completed		

9.5. Water Chemistry Sampling Checklist

Water Chemistry Sampling Checklist

Front Seat

Vehicle Binder
Gazetteer
Electronics
- Phone & Charger
- Rangefinder

Field Box
- Extra Pens
- Extra Labels
- Chain of Custody (+2)
- Analysis Request Form
- Site Folders
- Data Sheets

Clipboard
- Pens
- Fine Tip Sharpies
- Glass Bottle Labels
- Pamphlets
- Business Cards

Back Seat

Carboy – Millipore Water
Personal Box
- Extra Gloves
- Extra Safety Glasses
- Sunscreen
- Hand Sanitizer
- Safety Vest

Peristaltic Pump
Grey Pump Tote
- Pump Tubing
- 0.45 µm Filters
- Gloves

Luggage (if needed)
Rain Gear

Under Back Seat

Winch Bag
First-Aid Kit
Jumper Cables

Back of Truck

Drinking Water Cooler
Life Jackets
Chest/Hip Waders
Ice Coolers (adequate #)
Plastic and Glass Bottles
Grey Chemical Tote
- Safety Glasses
- Eyewash Solution
- Gloves
- Preservatives (HNO_3, H_2SO_4, and NaOH)
- Sharpies
- Bug Spray
- Hand Sanitizer
- Machete
- Liquinox
- Scrub Brushes
- Extra Toothbrushes
- Aluminum Foil

Hydrolab Backpack
- Hydrolab (Calibrated)
- DO Meter

QA Equipment
- Turbidity Meter
- pH/Temp Meter

Pole Sampler
Stainless Steel Bucket
Rope + Carabiner
Orange Safety Cones
Dry Ice Cooler
Tap Water Carboy
DI Water Carboy
Loppers
Formalin
WD40
Diatom Cooler
- 250 mL bottles (15)

Back of Truck Cont.

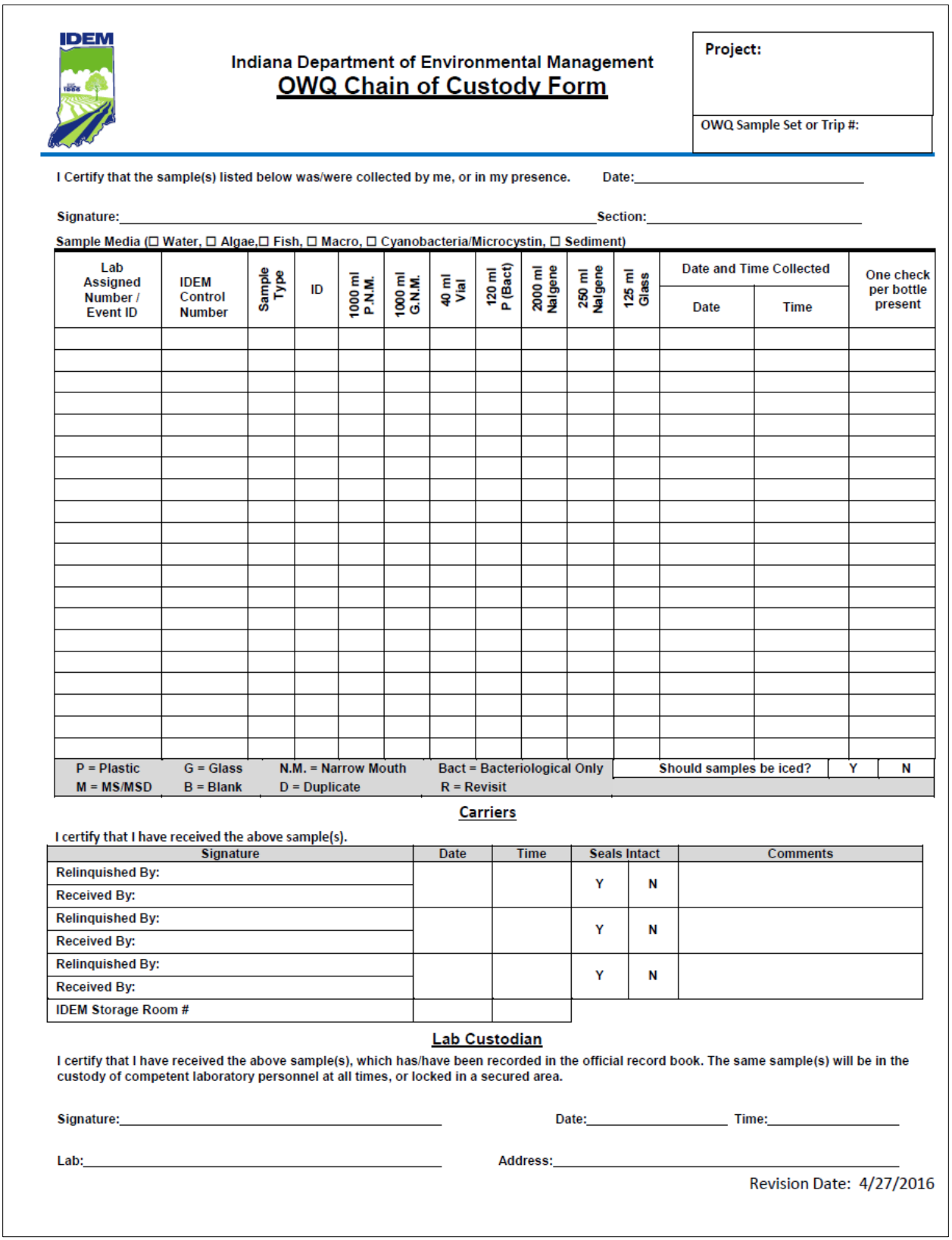
Water Backpack
- Densiometer
- Toothbrushes (2)

Loose Algae Equipment
- Small Plastic Tub (2)
- 1L Graduated Cyl. (2)
- Spray Bottle (2)
- 500 mL Wide Mouth (2)
  - X-Acto Knife
  - Pick
  - Stencil Brushes
  - Stencils

Key
General Water Chemistry
Diatom Sampling
DRP Sampling

(Updated 6/3/2024 by ARS)

9.6. Chain of Custody Form



Indiana Department of Environmental Management

OWQ Chain of Custody Form

Project:

OWQ Sample Set or Trip #:

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

Signature:_____ Section:_____

Sample Media (☐ Water, ☐ Algae,☐ Fish, ☐ Macro, ☐ Cyanobacteria/Microcystin, ☐ Sediment)

Lab Assigned Number / Event ID	IDEM Control Number	Sample Type	ID	1000 ml P.N.M.	1000 ml G.N.M.	40 ml Vial	120 ml P (Bact)	2000 ml Nalgene	250 ml Nalgene	125 ml Glass	Date and Time Collected		One check per bottle present
											Date	Time	

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

M = MS/MSD B = Blank D = Duplicate R = Revisit

Carriers

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

Signature	Date	Time	Seals Intact		Comments
Relinquished By:			Y	N	
Received By:					
Relinquished By:			Y	N	
Received By:					
Relinquished By:			Y	N	
Received By:					
IDEM Storage Room #					

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:_____ Date:_____ Time:_____

Lab:_____ Address:_____

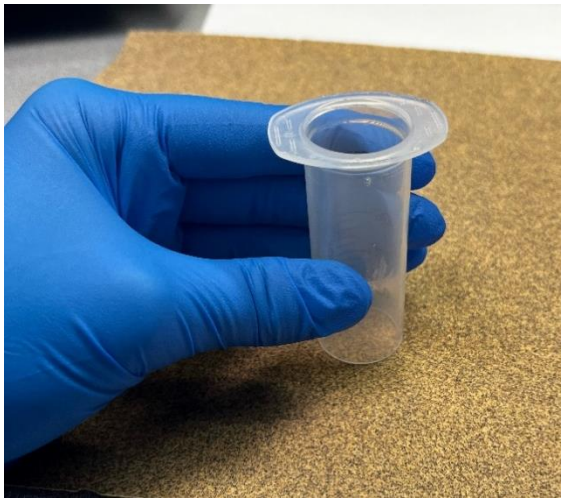
Revision Date: 4/27/2016

9.7. Instructions for making SG-92s for epilithic sampling

The SG-92 is used for sampling periphyton on rocks. SG-92s are constructed prior to sample collection and are washed and re-used after each sampling event. The following steps are modified from Moulton et al. 2002:

Step 1. Use a die-grinder to remove the pointed end of a 30-mL syringe. Once removed, the pointed end can be discarded. Note: Anything that will cut hardened plastic may be used here, we found that the die-grinder works most efficiently without cracking the syringe.

Step 2. Using fine-grit sandpaper, sand the cut-end of the syringe until it is smooth to ensure that there are no rugged or sharp edges.



Step 3. Using fine-grit sandpaper, sand down one side of a rubber O-ring until it is flat. The O-ring should have an inside diameter of 2.06 cm and an outside diameter of 2.70 cm.

Step 4. Attach the flattened end of the O-ring to the flanged end of the syringe using super glue. Sanding down the O-ring prior to attaching it to the syringe will ensure a tighter seal.

Step 5. Once assembled and the super glue is fully dry, the SG-92 may be used for periphyton sample collection. The SG-92 allows collecting samples from a known area. The O-ring helps to elevate the syringe and creates a seal on the rock when scribing the circle. This ensures that the SG-92 does not move around, and the syringe flanges are not disturbing periphyton on the rest of the rock, which could impact the placement of the second circle and/or interfere with a representative sample.