A. Scope

This method pertains to the collection of diatom samples from wadeable streams for the calculation of the Diatom Nutrient Index (DNI). The DNI is currently being proposed as a new response indicator for total phosphorus (TP) impairments. The DNI would complement the macroinvertebrate community metrics that are currently being assessed as bioconfirmation of a TP impairment. Unlike Wisconsin's macroinvertebrate or fish IBI's the DNI does not assess overall health of the diatom community. Instead it is designed to assess for the impacts of eutrophication. The DNI may also be used to assess the general response of biota in streams to nutrients, compare biological condition before and after restoration activities, or any other nutrient related investigation.

B. Summary of method

Diatoms may be present on the "sunny-side" surface of any substrate in streams. The preferred method for sampling diatoms is to scrape the biofilms off of any hard surface found on the stream bottom. Biofilms will consist of diatoms, other soft algae, bacteria and fungi. Generally, any hard surface that has visible green or golden-brown algae, or a slimy surface when touched will have enough diatoms for collection, identification and enumeration for the calculation of the DNI. The index period for diatom collection in order to calculate the DNI is 01 July – 15 September, taking care to avoid sampling directly after large rainfalls.

In general, cobble sized rocks, or other hard substrates, will be moved from the stream bed and placed into a plastic pan on a stable location on the stream bank. Care should be taken not to remove rocks that have been recently disturbed, such as those overturned by staff wading in the stream. Also, avoid collecting rocks that are in nearly in complete shade, such as under an overpass or near a bank with unusually high canopy shading for that stream system. If the stream system has very high canopy shading throughout select rocks from areas that receive partial sunlight as best as possible. The rocks should be scrubbed with a stiff bristle brush until they no longer feel "slimy". Rocks and brushes will be rinsed with tap water and the algae and rinsate will be collected in a 60 mL bottle and preserved with 3.0 mL of 25% glutaraldehyde. Specific sampling requirements for variety of substrates are listed below in Section E.

1. Standard QA/QC practices

Standard QA/QC procedures include field duplicates and laboratory duplicates by sample splitting. In general, field duplicates will be samples from similar substrates but collected from different specimens (i.e rock or gravel patches). For example, if collecting diatoms from cobble sized rocks make collections from similar sized rocks in similar locations but keep the individual rocks, sample containers and samples separated for the two samples. QA/QC procedures and frequency will be developed on a project specific basis.

C. Safety

Safety precautions of a general nature should be recognized. Collecting samples in extremely hot and humid weather carries the risk of dehydration and heat stroke. Collection of diatoms requires contact with stream water and stream sediment. Use precaution when reaching in streams to remove substrates to sample as glass or other sharp objects may be present and hard to see. Use gloves when collecting substrate if you are unsure if stream substrates can be handled safely.

Glutaraldehyde

Preserving DNI samples requires the use of small amounts of 25% glutaraldehyde. Caution should be used to avoid contact with skin or eyes when preserving the sample, so using gloves and wearing splash proof safety glasses are required. Immediately rinse skin with water in case of contact. Glutaraldehyde is known to be a low risk throat and lung irritant but poses little threat when handled properly and used in well ventilated areas or outdoors. When opening the glutaraldehyde container outdoors do facing upwind to minimize inhalation of vapors. A first aid kit should always be carried with the field crew for general safety considerations. If shipping samples preserved with glutaraldehyde staff will need to have Hazardous Materials Shipping training, valid for three (3) years http://intranet.dnr.state.wi.us/int/es/science/ls/Shipping/Training/.

Glutaraldehyde should be stored in an approved flammable storage container at or below room temperature. At room temperature glutaraldehyde shows no change in concentration or deterioration after one year. It is recommended that an unopened glutaraldehyde container should be stored for no more than two (2) years. Once opened glutaraldehyde should be stored for no longer than one (1) year. Any small spills can be cleaned up with a liquid absorbing material and discarded. Any remaining solution can be washed or rinsed with water ensuring the rinsate is discarded into a municipal sewer and not a natural waterway as glutaraldehyde is especially dangerous to aquatic organisms. See MSDS for more information https://www.sciencelab.com/msds.php?msdsId=9924162.

D. Equipment

- Stiff bristle brushes
  - mini wire brush, toothbrush, or similar
- Small plastic tray can it be glass like a glass cake pan?
  - painters tray, food tray, dish tub, or something with minimal grooves
- Spray bottle with tap water or laboratory squirt bottle
  - hand pump sprayer works better than trigger spray bottles
- Plastic funnel
- 60 mL sample container (glass or HDPE)
- 25% glutaraldehyde
  - ~3 mL per sample
- Disposable eye dropper
- Splash proof safety goggles
- Nitrile gloves
- Petri dish or wide-mouth bottle and spatula without holes/slits (sand gravel samples)
- Disposable plastic syringe or turkey baster (silt samples)
- Quart bottle or larger
  - Nalgene, bug jar, etc.
- Field sheets and pencils

See Section H for links to recommended equipment.

E. General Collection procedures

The following procedures outline a stepwise approach for collecting periphyton samples from each of the three (3) unique riffle units established for the reach. Based on the type of substrate available, select one sampling method for the stream reach. The method should be selected based on the following priority: rock, gravel, sand, silt.

Rocks – cobble sized

1. Collect 2 pieces of substrate (5 – 25 cm diameter) from each of the 3 fast-water habitat units (i.e. riffles) for a total of 6 samples. Make sure to keep track of the upper surface of each piece of substratum (don't sample the underside of a rock).
    a. This may be modified to a minimum of 5 rocks from one riffle if rocks and/or riffles are rare in the reach.

2. Where possible, all of these samples should be taken from a depth of approximately 15 - 20 cm (if the stream is <15cm deep sample at the deepest points).
    a. If substrate within the 5 - 25 cm diameter size-classes is unavailable, refer to the procedures for sampling loose sediments below.

3. On the periphyton data sheet, rate macro-algal cover (including long filaments), moss cover, and periphyton thickness on the rocks you chose for scraping.

4. Place selected rocks into plastic tray on a stable portion of stream bank. Scrape loose algae from the area on the rock with a brush or knife. Use a putty knife if thick algae is hard to remove (brushing dense periphyton clogs up the brush). Rinse the material into the small tray with a minimal amount of water. Repeat for each piece of substratum rinsing the material into the tray to create one composite sample.

5. Rinse the material from the plastic tray into the 1 quart bottle. Carefully examine all of the tools used for dislodging the periphyton and rinse any remaining material into the composite sample using a minimal amount of tap water.

6. Close the 1L bottle containing the composite sample, shake vigorously until all material is fully suspended and homogenized (clumps of algae broken up). Use the turkey baster to continue mixing and extract ~10 mL and place in the 60 mL periphyton sample bottle. Extract additional aliquots until 40 mL has been transferred to the sample container (as indicated by the 40 mL mark on the side of the container).

7. Wearing gloves use pipette to extract 3.0 mL of 25% glutaraldehyde and add to the 60mL sample bottle. Hold pipette in gloved hand and remove glove turning inside out around pipette for disposal.

8. Label these samples as "Rock". Make sure that the date, stream name, and sample identification number are identical to what was recorded on the data sheets.

9. To avoid contamination of subsequent samples, thoroughly rinse all of the sampling tools before leaving the field with stream water and then tap water.

Gravel and Sand

1. Invert the petri dish (or wide-mouth bottle) over a portion of sediments submerged at a depth of approximately 15-20cm (if the stream is <15cm deep sample at the deepest point) and trap the sediments by inserting the spatula under the dish.
    a. For large gravel (3-5 cm) collections can be made by hand by picking up individual rocks and transferred to 1 quart container.

2. Transfer the sample to the 1 quart sample homogenization container. Repeat this step for 5 more samples, three from each riffle in the reach.
    a. This may be modified to a minimum of 5 samples from one riffle if sand/gravel and/or riffles are rare in the reach.

3. Pour a small amount (~20 mL) of tap water over the gravel and sand in the 1 quart bottle, cover, and shake and swirl vigorously to remove algae from gravel and sands. Allow 10 seconds for sands and gravels to settle and pour algal-water suspension into 60 mL sample container. Dump sand/gravel sample back into stream.
    a. Repeat sand/gravel sample collection 4-5 times for each site and combine each sample until one 60 mL sample container is full of algal-water suspension.

4. Continue with steps 6-10 under **Rocks** section but label the sample as “Sand/Gravel”.

Silt and Fine Sediments

1. Invert the petri dish (or wide-mouth bottle) over a portion of sediments submerged at a depth of approximately 15-20cm (if the stream is <15cm deep sample at the deepest point) and trap the sediments by inserting the spatula under the dish. Do not press the petri dish into the sediments – you want to trap only the top few mm of sediments.
    a. Otherwise, use a turkey baster or modified syringe to sample the top 2-3 mm of sediment from an 8-10 cm diameter area of stream bottom.

2. Holding the spatula with the fines trapped under the dish, rinse all the sediments within the dish into the 1 quart sample homogenization container. Repeat above steps to collect 9 total sediment samples within the reach.

3. Continue with steps 6-10 under **Rocks** section but label the sample as “Silt”.

F. Documentation

Record all pertinent data on the Diatom Sampling Sheet (See Section I) and be sure to record site ID (SWIMS ID and Date) on sample bottle. Make a photocopy of the field sheet, one for your personal records and one to be delivered with the sample. Samples and field sheets need to be delivered to Gina LaLiberte at Science Services. All samples must be delivered to Gina LaLiberte by Dec 15th of the sampling year. Earlier delivery is highly encouraged if possible.

G. SOP updates tracking

Version Number	Date	Sections	Name	Approval
1.0	03/17/2015	All	LaLiberte/ Garrison/Shupryt	Mike Shupryt 2015
2.3	11/08/2016	E. Gravel/Sand method -3.a – Clarified language	Shupryt	

H. Recommended Equipment Purchases

Equipment for this project can be purchased from a combination of local hardware stores and on-line laboratory suppliers. Examples of recommended equipment and internet links can be found below. This list is not exhaustive of all the supplies needed, consult section D for full list. Inclusion of a particular brand or dealer below does not constitute an endorsement of that brand or dealer.

- Stiff bristle brushes
  - http://www.homedepot.com/p/Lincoln-Electric-3-Piece-Miniature-Brush-Set-KH590/202939869
- Small plastic tray
  - http://www.homedepot.com/p/Linzer-9-in-Deep-Well-Plastic-Tray-RM-405-SP/100142784
- Spray bottle with tap water
  - http://www.homedepot.com/p/Viagrow-2-l-2-000-ml-Handheld-Garden-Pump-Sprayer-V20819/203548965?keyword=2+l+%282%2C000+ml%29+Handheld+Garden+Pump+Sprayer
  - OR Squirt bottle with tap water http://www.grainger.com/product/GRAINGER-APPROVED-Wash-Bottle-6FAV8?functionCode=P2IDP2PCP
- 25% glutaraldehyde, eye dropper, gloves (3 mL per sample)
  - Product 16210 http://www.emsdiasum.com/microscopy/products/chemicals/glutaraldehyde.aspx
  - Product 2263-1
  - http://www.tedpella.com/Embedding_html/Disposable_Syringes.htm
- Disposable plastic syringe or turkey baster (silt samples)
  - Product 115-55 (60 mL) with tip cut off for larger opening
  - http://www.tedpella.com/Embedding_html/Disposable_Syringes.htm

This document is available electronically on the WDNR's website.

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WDNR PUB-WY- 079-2015

Diatom Sampling Sheet

Date: _____ Collected by: _____

Stream name: _____

Site ID: _____

Riffle coordinates: _____

Substrate sampled (circle): Rock Gravel/Sand Silt/Sediments

Substrate	Macro-algae Cover (0 to 3)	Moss Cover (0 to 3)	Periphyton Thickness (0 to 5)	Dimensions of Area Scraped (if measured)	Petri (check if used)
1					
2					
3					
4					
5					
6					
7					
8					
9					

Moss cover and macro-algal cover:
0: no moss or macro-algae present;
1: some moss or macroalgae, but <5% coverage;
2: 5-25% cover of substratum by moss or macro-algae;
3: > 25% cover of substratum by moss or macro-algae

Periphyton (microalgae) thickness:
0: substrate is rough with no apparent growth;
0.5: substrate is slimy, but biofilm is not visible (tracks cannot be drawn in the film with the back of your fingernail; endolithic algae can appear green but will not scratch easily from the substratum);
1: a thin layer of microalgae is visible (tracks can be drawn in the film with the back of your fingernail);
2: accumulation of microalgae to a thickness of 0.5-1 mm;
3: accumulation of microalgae from 1 mm to 5 mm thick;
4: accumulation of microalgae from 5 mm to 20 mm;
5: layer of microalgae is greater than 2 cm.

Site notes: _____