DEPARTMENT OF ECOLOGY
State of Washington

Standard Operating Procedure EAP073, Version 2.5

Minimum Requirements for the Collection of Freshwater Benthic Macroinvertebrates in Streams and Rivers

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Purpose of this Document

The Washington State Department of Ecology develops Standard Operating Procedures (SOPs) to document agency practices related to sampling, field and laboratory analysis, and other aspects of the agency's technical operations.

Publication Information

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The Washington State Department of Ecology's (Ecology's) Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical and administrative experts. Their primary purpose is for internal Ecology use, although sampling and administrative SOPs may have a wider utility. Our SOPs do not supplant official published methods. Distribution of these SOPs does not constitute an endorsement of a particular procedure or method.

Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by Ecology.

Although Ecology follows the SOP in most instances, there may be instances in which Ecology uses an alternative methodology, procedure, or process.

SOP Revision History

Revision Date	Revision History	Summary of Changes	Sections	Reviser(s)
4/11/2022	2.5	Transferred to new template	All	Meghan Rosewood-Thurman

1.0 Purpose and Scope

1.1 This document is the Environmental Assessment Program (EAP) Standard Operating Procedure (SOP) for the collection of freshwater benthic macroinvertebrate (BMI) data in rivers and streams for the Watershed Health Monitoring program (WHM) or related studies during a Data Collection Event (DCE).

1.2 This SOP includes procedures for sites sampled with the Narrow and Wide protocols. See SOP EAP106 (Merritt, 2022), which describes the site verification and layout procedures for the WHM Narrow Protocol and SOP EAP105 (Hartman, 2020a) which describes site layout for the Wide Protocol.

1.3 This document provides minimum requirements for the standardized methods of collecting and preserving aquatic insects, as well as for the taxonomic identification and reporting of the contents of BMI samples.

2.0 Applicability

2.1 The methods described here are compatible with those used by other federal and state agencies in the Pacific Northwest Region (Hayslip, 2007). Data collected using these methods allows us to share data with other agencies, thereby allowing for more efficient use of time in the field and potentially more extensive sampling of the streams and rivers in Washington.

2.2 This SOP is used in combination with other SOPs to complete a DCE for the WHM program. This method explains how to collect macroinvertebrates across the main channel at eight of 11 major transects. Follow the method outlined in this SOP only after the site verification and layout procedures have been completed (Merritt, 2022, and Hartman, 2020).

2.3 To allow for comparable results, any data submitted for analysis using Ecology's bioassessment models by outside entities should be conducted in this manner.

2.4 These methods also pertain to biological assessment conducted for potential regulatory purposes, i.e., directed studies (e.g., TMDL studies) or outside entities assessing sites for potential listing on the state's 303(d) list for "biological impairment" (see Ecology's Water Quality Program Policy 1-11: Bioassessment).

3.0 Definitions

3.1 DCE: The Data Collection Event is the sampling event for the given protocol. Data for a DCE are indexed using a code which includes the site ID followed by the year, month, day, and the time (military) for the start time of the sampling event. For example: WHM07620-000222-DCE-YYYY-MMDD-HH:MM. One DCE should be completed within one working day, lasting four to six hours, on average.

3.2 D-frame kicknet (Fig. 1): A lightweight, packable net used for the collection of aquatic macroinvertebrates. The kicknet is composed of a three to four foot pole with a D-shaped frame attached to the bottom. The flat side of the frame goes against the substrate. The frame is one foot wide and one foot tall. There is a 500-micron mesh net attached to the frame. The kicknet functions across most substrate types, this is the required sampling device for status and trends monitoring.

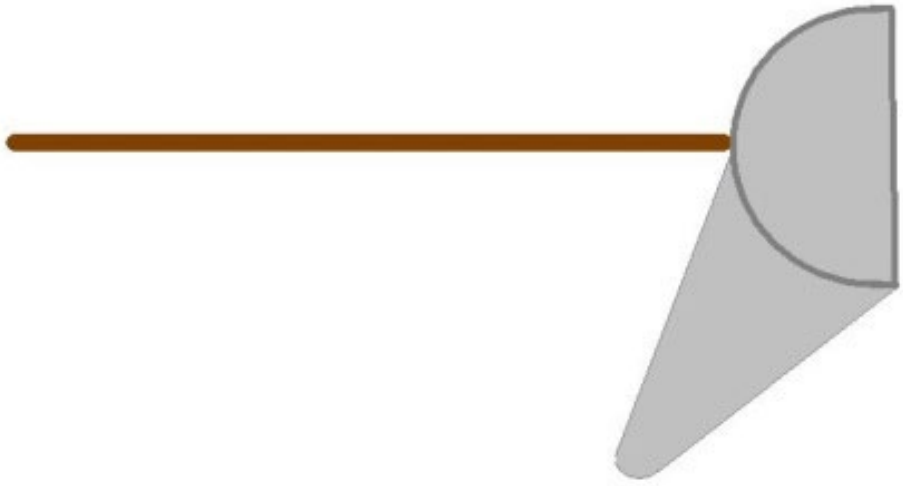

Figure 1. Diagram of a D-frame kicknet**.**

3.3 EAP: Environmental Assessment Program

3.4 Ecology: Washington State Department of Ecology

3.5 EIM: The Environmental Information Management System (EIM)[1] is the Department of Ecology's main database for environmental monitoring data. EIM contains records on physical, chemical, and biological analyses and measurements. Supplementary information about the data (metadata) is also stored, including information about environmental studies, monitoring locations, and data quality. The "Search by map" feature enables plotting coordinates over orthophotographic imagery. EIM also includes a searchable component for Watershed Health Monitoring Data[2]

[1] http://www.ecy.wa.gov/eim/

[2]https://fortress.wa.gov/ecy/eimreporting/Stream/STREAMSearch.aspx?SearchType=Stream&State=newsearch&Section=all

3.6 Hess sampler: A cylindrical mesh frame that is open on either end to allow access to bottom substrates through the top of the cylinder (Figure 2). This cylinder has a 500-micron mesh net attached to part of the wall for sample collection. This sampler prevents escape of sample organisms and prevents outside materials and organisms from drifting into the net.

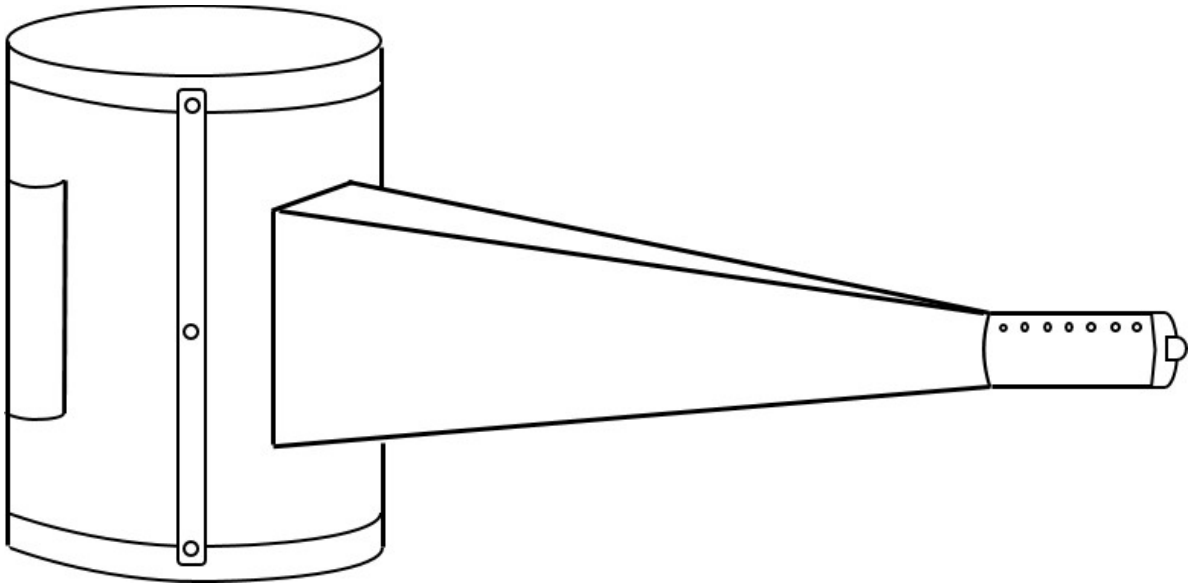

Figure 2. Diagram of a Hess sampler

3.7 Narrow Protocol: The set of Watershed Health Monitoring SOPs that describe data collection at wadeable sites with an average bankfull width of less than 25 m at the index station.

3.8 Narrow Protocol sampling stations: Sampling occurs in a zigzag sequence (Table 1) when moving upstream.

Table 1. Pre-determined station locations on each transect of a standard stream site.

Station	% Transect Distance Left to Right
1	25
2	50
3	75
4	50
5	25
6	50
7	75
8	50

3.9 Reach-wide composite sample: The reach-wide sample is composited from eight predefined stations (Table 1). Each station is located on a separate transect and selected regardless of habitat type. Sampling from multiple dispersed locations provides a representative sample.

3.10 SDS: Safety Data Sheets (previously Material Safety Data Sheets or MSDS) provide both workers and emergency personnel with the proper procedures for handling or working with a particular substance. An SDS includes information such as physical data (melting point, boiling point, flash point, etc.), toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment, and spill/leak procedures.

3.11 Station: Any location within the site where an observation is made or part of a sample is collected. For SOP EAP073 and SOP EAP111 (Larson 2022), eight out of the 11 transects are randomly selected for periphyton and macroinvertebrate sampling. Table 1 defines the sampling path within the stream or river.

3.12 Substrate: The material that rests on the bottom of the stream.

3.13 Surber sampler – A net used for sampling aquatic insects, composed of a 12 × 12 inch square frame with a 500-micron mesh net attached. It has another 12 × 12 inch square frame that sits on the substrate to border the sampling area (Figure 3).

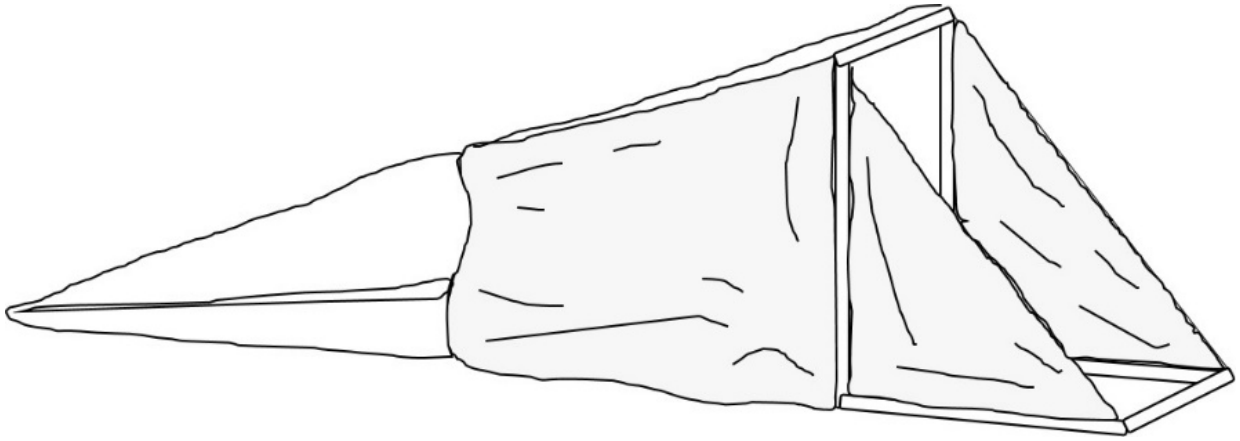

Figure 3. Surber sampler.

3.14 Targeted riffle sampling: A targeted sample represents sampling a single habitat type from a stream reach that extends at least twice its bankfull width. A targeted sample is composed of 8 feet of surface area sampled across multiple riffles or pools. Targeted sampling from a single habitat type can help to reduce the variation in the data and to provide a clear response signal. Individual directed studies may decide on the utility of using targeted riffle sampling; however, projects involved in status and trends monitoring employ only reach-wide composite sampling.

3.15 Transect: A straight line along which observations are made or measurements are taken. This line spans the stream channel and is perpendicular to the direction of flow.

3.16 Wide Protocol: The set of WHM SOPs that describes the sample and data collection at sites that are non-wadeable or greater than a 25 m bankful width. Wide Protocol abbreviates the Narrow Protocol and is typically done by rafts.

3.17 Wide Protocol sampling stations: Eight transects of the stream or river coinciding with the bank of the habitat survey. At each of the selected transects, a sample is collected from a representative portion (as much as practical) of a littoral zone extending 10 m into the stream/river from the wetted bank and 10 m upstream and downstream, respectively from the transect. The sample should also be collected in an area shallow enough to deploy the kicknet and in an area away from backwaters, eddies, or other edge habitat.

4.0 Personnel Qualifications/Responsibilities

4.1 This SOP pertains to all Ecology staff in EAP and any other technicians collecting and entering data for the WHM program.

4.2 For collection of the sample, personnel should at a minimum review the Quality Assurance Monitoring Plans for the status and trends monitoring programs (e.g., Ambient Biological Monitoring (Adams, 2010), WHM) and the training tutorial Sampling Macroinvertebrates in Wadeable Streams in Washington State (EAP 2010). All field staff should be briefed by the field crew leader or project manager on the sampling goals and objectives prior to arriving to the site.

4.3 All field staff must have completed the annual WHM program field training and be familiar with the WHM protocol used for the given DCE. All field staff must be familiar with the electronic tablet and web-based field forms that one uses to record and submit data for the WHM program. Contact the WHM Data Coordinator for further information.

4.4 All field staff must comply with the requirements of the EA Safety Manual (Ecology, 2019). A full working knowledge of the procedures in Chapter 1 “General Field Work,” especially the sections “Working in Rivers and Streams,” and “Fall Protection,” is expected. Sampling from a boat requires one person onboard to be a qualified boat operator as defined by the EAP Boating Plan in Chapter 3 of the EA Safety Manual. All persons onboard must be familiar with Chapter 3 of the EA Safety Manual, “Boating.”

4.5 For taxonomic analysis of the sample, the personnel should be certified for identification of Western United States taxa to the genus or species level by the Society for Freshwater Science (2017). Sample identification and enumeration should be to the lowest practical level as outlined in Quality Assurance Monitoring Plan: Ambient Biological Monitoring in Rivers and Streams: Benthic Macroinvertebrates and Periphyton (Adams 2010).

4.6 All staff must be familiar and comply with the requirements of Ecology's Chemical Hygiene Plan and Hazardous Materials Management Plan (EAP 2018).

4.7 Read the Safety Data Sheets (SDS) for ethanol before beginning the sorting/taxonomic procedures. The SDSs are available in the Ecology Headquarters benthic laboratory and on the Ecology's internal QA website. Use proper protective clothing and equipment as indicated.

4.8 Immediately report to your supervisor any symptoms or reactions that might be related to ethanol exposure.

4.9 Field staff must be trained annually to minimize the spread of invasive species. See SOP EAP070 (Parsons et al. 2021).

5.0 Equipment, Reagents, and Supplies

5.1 Field tablet (charged), electronic field forms

5.2 Clip board with blank paper data forms and pencils (contingency)

5.3 Wide-mouth HPDE jars (128 oz. wide mouth square is a recommended size)

5.4 D-Frame kicknet (pre-cleaned of organisms) with these characteristics:

- Frame mouth that is one ft. (30.5 cm) wide and tall
- 500-µm mesh net

5.5 95% ethanol (3:1 ratio by volume for each part sample)

5.6 Label (waterproof) for jar exterior

5.7 Label (waterproof) for jar interior

5.8 Soft-lead pencil

5.9 Clear tape

5.10 Electrical tape

5.11 Pocket knife

5.12 Wading/rafting gear (pre-cleaned of organisms)

5.13 Disinfection solutions, brushes, or other equipment necessary to minimize the spread of invasive species from site to site.

6.0 Summary of Procedure

6.1 Details of the procedures determined by the purpose for monitoring (Table 2).

Table 2. Details of benthic sampling based on monitoring purpose.

Monitoring purpose	Status & Trends (narrow protocols)	Status & Trends (wide protocols)	Regulatory
Device	D-frame kicknet	D-frame kicknet	D-frame kicknet, or Surber, or Hess
Mesh	500 µm	500 µm	500 µm
Site length	20 bankfull widths (150–500 m)	20 bankfull widths (150–2000 m)	2 bankfull widths (or more)
Sample area	8 ft^2	8 ft^2	8 ft^2
Station distribution	8 transects, 4 margins + 4 central	8 transects, littoral zone on side of stream where habitat is surveyed	Multiple riffles or 8 transects
Time to suspend	30 seconds	30 seconds	30–120 seconds
Sample	Reach-wide composite	Reach-wide composite	Reach-wide or targeted-riffle composite
Season	July 1–Oct 15	July 1–Oct 15	July 1–Oct 15
Subsample goal	500+ organisms	500+ organisms	500+ organisms
Taxonomic resolution	lowest practical	lowest practical	lowest practical

6.2 Field Sampling

6.2.1 For status and trends monitoring purposes (e.g., WHM), the sampling season extends from July 1 to October 15. For regulatory monitoring purposes, sampling should be the same period.

6.2.2 Samples should be collected with a device with 500-micron mesh, including D-frame kicknets, Surber samplers, or Hess samplers. Samples collected for status and trends monitoring (i.e., WHM, Ambient Stream Biological Monitoring, and Sentinel programs) should use a D-frame kicknet.

6.2.3 Samples should be collected from eight square feet of stream bottom and composited in the same jar. These samples should come from multiple locations across the study site.

6.2.4 Samples taken for monitoring status and trends of stream health (e.g., WHM) should be composited (regardless of habitat) from eight randomly selected transects dispersed across a site at least 150 m long.

6.2.5 Samples taken for the purpose of regulatory assessment should be composited from eight feet of surface area taken from multiple fast-water habitats in the study reach. Aliquots may be from either turbulent (e.g., riffles) or non-turbulent habitat (e.g., glides), as long as flow is sufficient to carry organisms into the net.

6.3 Sampling Fast-water Aliquots

6.3.1 Place the sampling device firmly against the stream bottom, facing the flow of water. Eliminate gaps under the frame with the opening of the collection kicknet.

6.3.2 Identify the sample area. The sample area is the 12-inch-by-12-inch area upstream from the base of the kicknet. Gently scrub large substrate particles with your hand (larger than five cm in diameter) in front of the sampling device to remove any organisms that cling to the substrates; allow the flow to carry them into the mesh.

6.3.3 After each particle in the sample area is scrubbed, inspect it for any remaining organisms, and then set it outside of the sample area.

6.3.4 Kick or use a trowel, for a minimum of 30 seconds, to stir up and suspend the substrate in front of the kicknet. Allow the flow of the water to carry the BMI into the mesh

6.4 Sampling Slack-water Aliquots

6.4.1 If flow is unable to carry the BMIs into the mesh, visually inspect the stream bottom for any heavy or large organisms, such as mussels and snails, and place them in the sample jar.

6.4.2 Pick up any loose rocks or large substrate particles and scrub them over the kicknet, allowing the organisms to fall into the mesh, and then set aside.

6.4.3 After scrubbing, vigorously kick the remaining finer substrate within your sample area and drag the kicknet repeatedly (for 30–120 seconds) in a figure eight fashion through the disturbed area just above the bottom.

6.4.4 Move the kicknet continuously so the organisms remain trapped in the kicknet and do not escape; continue kicking.

6.4.5 On completion of sampling, remove the kicknet from the water with a quick upward/upstream motion to wash the organisms to the bottom of the kicknet.

6.4.6 Wash the contents of the kicknet down to the bottom for ease of placing the sample aliquot into a jar. Remove relatively large debris, i.e., pieces of wood or rocks, from the kicknet following inspection for attached invertebrates.

6.5 Place the aliquots in the jar.

6.5.1 Carefully inspect the mesh itself and remove any remaining organisms that may be stuck to the kicknet. Adding a small amount of ethanol to the jar prior to sample collection helps to reduce the number of organisms sticking to the kicknet and minimizes sample degradation during the sampling event.

6.6 Add 95% non-denatured ethanol to equal 2/3 of the volume of the total sample. Sufficient ethanol is necessary to preserve the contents of the jar until taxonomic enumeration.

6.6.1 Existing water in the jar should not dilute the concentration of ethanol below 70%, so if, for example, approximately 100 mL of water is in the jar, add 300 mL of ethanol (ratio is 3:1).

6.7 Add a label printed on waterproof paper to the contents of the jar (Figure 4). Write the jar number, DCE, skipped transects, sampler name, and any additional comments.

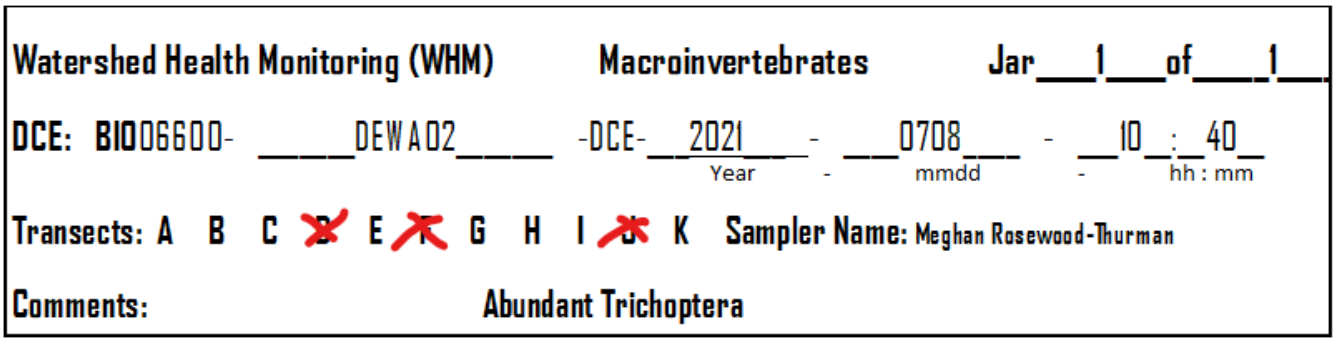

Figure 4. A completed macroinvertebrate label

6.7.1 Seal the jar securely, wrap the lid with electrical tape at the junction with the bottle, and affix a second label printed on waterproof paper to the outside of the jar. Deliver the contents to the taxonomist for identification and enumeration.

6.8 In the eforms on the samples page, select Benthos in the Click Jars Collected for Lab Shipment box (Figure 5). Make any notes about the stream in the notes box, then click save

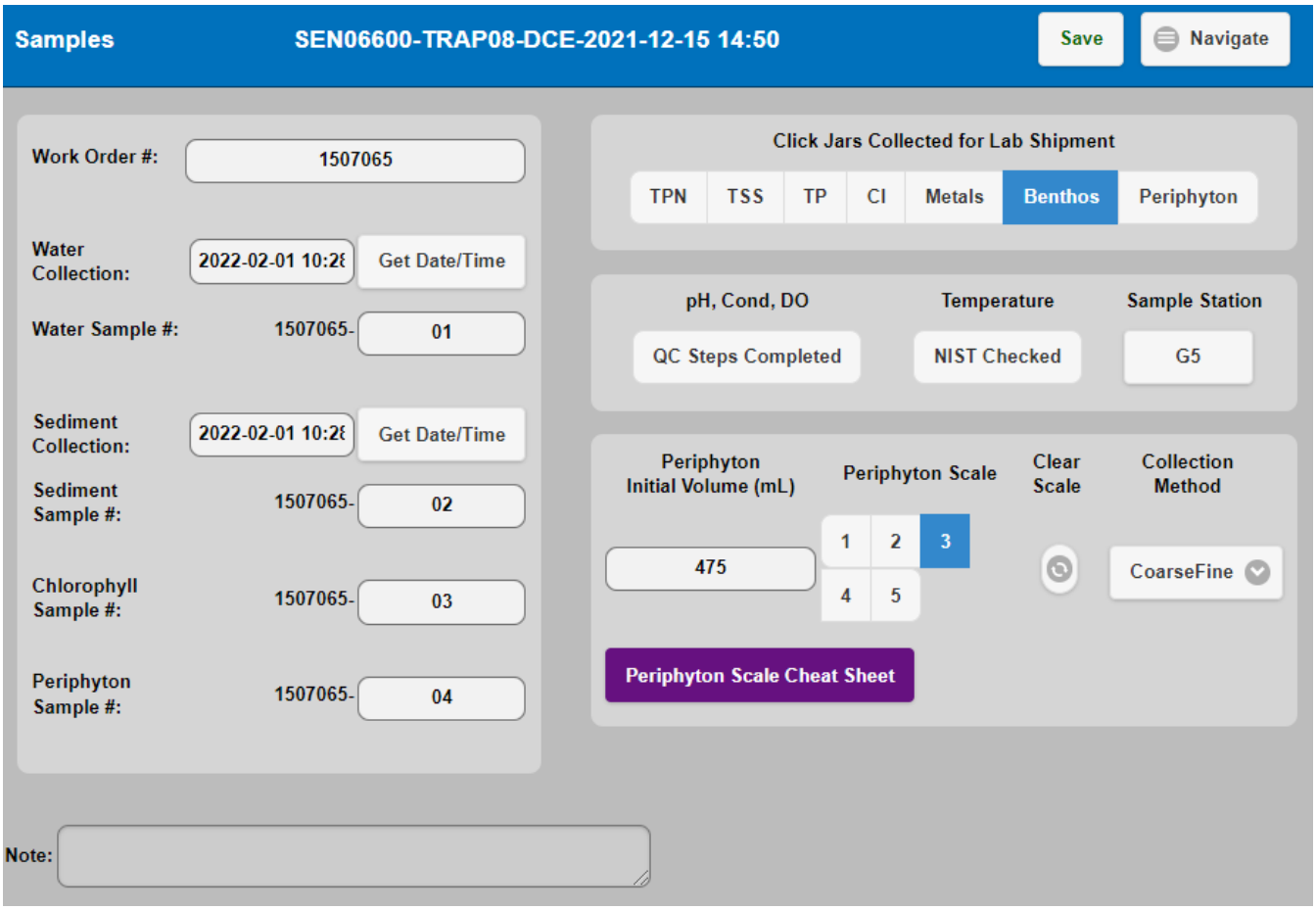

Figure 5. On the sample page, select Benthos and add any additional notes.

7.0 Data Reporting

7.1 At a minimum, identify a target of 500 organisms by the lab for each sample. There are occasional situations that lead to fewer than 500 organisms per sample and do not meet this target. In these cases, the lab should identify the entire sample. Acceptance of smaller count (<500 organisms identified) data into our database for assessment purposes will be allowed at Ecology's discretion.

7.2 Identify each organism to the "lowest practical level." Lowest practical level is generally to genus or species, unless the specimen is underdeveloped or damaged, preventing identification to this level. Adams (2010) outlined the standard taxonomic effort employed by EAP's status and trends monitoring projects (see appendices G & H in Adams [2010]).

7.3 Lab data reported should include at a minimum:

- 7.3.1 Lab name/taxonomist
- 7.3.2 Integrated Taxonomic Information System (ITIS) taxa number
- 7.3.3 Scientific name of taxa
- 7.3.4 Collection date
- 7.3.5 Sampling device
- 7.3.6 Habitat sampling scheme (reach wide or targeted)
- 7.3.7 Protocol used (narrow or wide)
- 7.3.8 Number of organisms identified
- 7.3.9 Density of taxa per meter square
- 7.3.10 Number of taxa by life stage
- 7.3.11 Report number of damaged taxa and indicate if unable to identify to lowest level
- 7.3.12 Report taxa uniqueness for nonspecific identifications (to estimate diversity)

8.0 Records Management

8.1 List every sample on a chain of custody form submitted to the taxonomist. This form should include location, date, and sampling information.

8.2 The taxonomist will submit data to Ecology's EIM database[3] or to Puget Sound Stream Benthos[4]. Arrange with King County DNR to give permissions for the taxonomist to submit data to the Puget Sound Stream Benthos website.

9.0 Quality Control and Quality Assurance

9.1 Field Quality Assurance

9.1.1 PROJECT QA/QC procedures are discussed in the Quality Assurance Monitoring Plan (Cusimano et al., 2006); a new version will be available 2022.

9.1.2 For additional information, see the Quality Assurance Monitoring Plan for Ambient Biological Monitoring in Rivers and Streams: Benthic Macroinvertebrates and Periphyton, Appendix C (Adams 2010).

9.2 Macroinvertebrate Sorting Efficiency

9.2.1 Quality control procedures for initial sample processing and subsampling involves checking *sorting efficiency*. Conduct these checks on 10% of the samples by independent observers who microscopically re-examine the sorted substrate from each sample.

9.2.2 All organisms that were missed are counted. Sorting efficiency is evaluated by applying the following calculation:

$$SE = n_1 / n_2 \times 100$$

Where SE is the sorting efficiency expressed as a percentage, n_1 is the total number of specimens in the first sort, and n_2 is the total number of specimens in the first and second sorts combined.

9.2.3 The person or lab enumerating the sample records sorting efficiency on each bench sheet. If 95% sorting efficiency is not achieved for a given sample, a failure is recorded on the bench sheet and in the database.

9.2.4 The sorted portion of that sample is then completely resorted before the sorting efficiency test is repeated for that sample.

9.2.5 Sorting efficiency statistics for each technician and for the entire laboratory are reviewed monthly.

9.2.6 Sorting efficiency for each sample in a project is reported to the client in the technical summary document. Technicians who do not maintain the target sorting efficiency are given remedial training, and larger portions of the samples they process are examined for the sorting efficiency test until they are able to maintain the target sorting efficiency.

9.2.7 Apply a second evaluation of the subsampling process to a small proportion of samples processed in each month; typically, one sample per week is subjected to the following test of *precision of the subsampling process*.

9.2.8 The procedure is only applied to samples where the target number of organisms was achieved in less than half of the Caton grids. A sample is randomly selected, and a second subsample is resorted from the unprocessed sample remnant.

9.2.9 A second technician performs this sort. The resulting subsample is identified, and Bray-Curtis similarity index is calculated for the results of both subsamples.

9.2.10 Results that are less than 90% similar would indicate the need for more thorough distribution of sample materials in the subsampling tray or more special attention given to easily missed taxa when sorting (i.e., increased magnification).

9.3 Taxonomic Accuracy and Precision

9.3.1 Taxonomic misidentification results in inadequate biological characterization of a stream. Errors in identification should be less than 5% of the total taxa in the sample. Conduct a re-identification of samples for 10% of the total number of samples in each year.

9.3.2 Experienced taxonomists conduct secondary identification in order to maintain confidence in the data set. Send difficult taxa to museum curators whose specialty includes members of the order in question.

9.3.3 Orma J. Smith Museum of Natural History in Caldwell, Idaho, maintains the voucher collections. Prepare voucher collections from the set of samples yearly and ship them to the address below:

The Orma J. Smith Museum of Natural History

College of Idaho

2112 Cleveland Blvd.

Caldwell, ID 83605-4432

[3] https://ecology.wa.gov/Research-Data/Data-resources/Environmental-Information-Management-database
[4] https://pugetsoundstreambenthos.org/Default.aspx

10.0 Safety

10.1 Field Safety

10.1.1 All field staff must comply with the requirements of the EAP Safety Manual (Ecology, 2019).

10.2 Chemical Safety

10.2.1 Keep Ethanol in small quantities tightly sealed containers out of direct sunlight.

10.2.2 Read all relevant Safety Data Sheets (SDS) before beginning this procedure. The SDS are available in the Ecology benthic laboratory located at the EAP Operations Center and on Ecology's internal Quality Assurance website.

10.2.3 Report to supervisor immediately any symptoms or reactions that might be related to ethanol exposure.

11.0 References

11.1 Adams, K. 2010. Quality Assurance Monitoring Plan: Ambient Biological Monitoring in Rivers and Streams: Benthic Macroinvertebrates and Periphyton[5]. Publication 10-03-109. Washington State Department of Ecology, Olympia.

11.2 Cusimano, R., G. Merritt, R. Plotnikoff, C. Wiseman, C. Smith, and WDFW. 2006. Status and Trends Monitoring for Watershed Health and Salmon Recovery: Quality Assurance Monitoring Plan[6].

11.3 EAP [Environmental Assessment Program]. 2010. Sampling Macroinvertebrates in Wadeable Streams in Washington State[7]. Video, 13 min. 26 sec. Washington State Department of Ecology, Olympia. Posted September 22, 2010.

11.4 Ecology [Washington State Department of Ecology]. 2019. Environmental Assessment Program Safety Plan. Washington State Department of Ecology, Olympia.

11.5 Ecology, 2019. Environmental Assessment Program Chemical Hygiene Plan and Hazardous Materials Management Plan. Washington State Department of Ecology, Olympia.

11.6 Ecology [Washington State Department of Ecology]. 2019. Quality Assurance at Ecology. Environmental Assessment Program, Washington State Department of Ecology, Olympia. https://ecology.wa.gov/About-us/How-we-operate/Scientific-services/Quality-assurance.

11.7 Hartman, C. 2020a. Standard Operating Procedure for GIS-Based Verification, Layout, and Data Collection (Wide Protocol).[8] EAP105, Version 1.1Publication 19-03-220. Washington State Department of Ecology, Olympia.

11.8 Hayslip, Gretchen, editor. 2007. Methods for the collection and analysis of benthic macroinvertebrate assemblages in wadeable streams of the Pacific Northwest[9]. Pacific Northwest Aquatic Monitoring Partnership, Cook, Washington.

11.9 Larson, C. and S. Collyard 2022. Standard Operating Procedure EAP111, Version 1.14 Periphyton Sampling, Processing and Identification in Streams and Rivers.[10] Washington State Department of Ecology, Olympia,

11.10 Merritt, G. 2022. Standard Operating Procedures for Verification and Layout of Sites (Narrow Protocol)[11] SOP EAP106. Washington State Department of Ecology, Environmental Assessment Program, Olympia.

11.11 Parsons, J., D. Hallock, K. Seiders, B. Ward, C. Coffin, E. Newell, C. Deligeannis, and K. Welch. 2021. Standard Operating Procedure EAP070, Version 2.3: Minimize the Spread of Invasive Species.[12] Publication 18-03-201. Washington State Department of Ecology, Olympia.

11.12 "Taxonomic Certification Program[13]." Society for Freshwater Science, 2017. Accessed 2/22/2022.

[5] https://fortress.wa.gov/ecy/publications/summarypages/1003109.html
[6] https://fortress.wa.gov/ecy/publications/SummaryPages/0603203.html
[7] https://www.youtube.com/watch?v=b2KOliKIGic&t=142s
[8] https://apps.ecology.wa.gov/publications/documents/1903220.pdf
[9] https://www.pnamp.org/document/1359
[10] https://apps.ecology.wa.gov/publications/documents/1903207.pdf
[11] https://apps.ecology.wa.gov/publications/documents/1803226.pdf
[12] https://fortress.wa.gov/ecy/publications/SummaryPages/1803201.html
[13] https://freshwater-science.org/about/taxonomic-certification