

**Protocol for Monitoring Springs at Ozark National Scenic Riverways, Missouri.
Heartland I&M Network**

SOP 6: Aquatic Invertebrate Sampling

Version 1.10 (06/06/2018)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #
1.0	6/6/2018	D.E. Bowles	Formalin is no longer used; ethyl alcohol listed the as acceptable preservative	Personnel safety	1.1

This SOP describes field procedures for collecting and processing invertebrate samples in the field. Appropriate QA/QC requirements are highlighted within the procedure descriptions.

I. Collecting Benthic Samples and Associated Habitat Data

Procedure:

- A. Prior to collecting benthic samples and taking habitat measurements, always complete data sheet information for spring name, date and time of survey, and initials of personnel who collect the samples.
- B. Collect one random benthic sample on transects 2, 4, 6, 8, and 10 for a total of five samples per spring. Only three such samples will be collected at Welch Spring where only three transects can be established. Benthic invertebrate samples will always be taken in an upstream direction. Individual benthic samples from the spring-run will be collected using the following *a priori* randomization procedure:
 1. Collect individual benthic samples centered on transects starting at river left, then alternating to the middle, and finally the right. If the crew is working/collecting data in an upstream direction, river right is on the workers' left, and river left is on the right. This sequence is repeated for the remaining transects. Note: the invertebrate sampling frame is centered within the area delineated by the 1m² sampling frame used for assessing physical habitat (see SOP #9).
 2. The starting sequence (left, middle, right) will be altered in each sampling year to the extent practical so that the substrate on each transect will be sampled in a specific area only once ever third year. Note: Sample sequence in any given year

should be altered only if the original starting point presents danger to the collector or if it is not accessible.

3. Samples will be collected with a Slack Surber sampler (analogous to the EPA-style rectangular benthic net) with a $\sim 500\ \mu\text{m}$ mesh net fitted with a sampling area guide (0.5 x 0.5 m rectangle of PVC pipe placed immediately in front of the net) to delineate the approximate sampling area of $0.25\ \text{m}^2$ (Fig. 1). Place the collection net and sample frame firmly against the substrate so that the opening is oriented directly into the spring current.



Figure 1. Invertebrate sampling net with sampling frame attached.

C. Benthic sample collection

1. While one team member holds the net firmly against the stream bottom facing the current, a second team member uses a garden cultivation tool to agitate the entire area within the net sample frame for 2 minutes (Fig. 2). At the end of 2 minutes, the net can be lifted from the stream bottom, but with sufficient caution so as not to spill the sample.
2. Remove any large debris or rocks from the net and inspect for attached organisms. Scrub individual substrate pieces into the collecting net with a soft-bristled brush as needed to remove moss and attached organisms. These pieces can be discarded after any invertebrates have been removed (Note: ensure that any substrate removed from the sample area is returned to its approximate original location immediately following sample collection). Pour and rinse the contents of the net into a wash bucket with a $500\mu\text{m}$ mesh sieve and rinse the sample by swirling the bucket in water using care not to submerge the bucket (Fig. 2). Continue this process until all the fine silt and other sediments have been washed from the sample. Remove the sample contents from the wash bucket and place them in the

sample container (Fig. 2). Inspect the net, bucket, and sieve for any remaining organisms and carefully place them in the sample container. Excess water in the sample container can be drained into the sieve and inspected for organisms so that the preservative is not overly diluted.



Figure 2. Placement of sampling net on stream bottom and agitation of substrate with garden tool (top left), washing the fine sediments from the sample using the wash bucket (top right), and transferring sample from wash bucket to sample container (bottom). Note that sample container is always held over wash bucket to ensure that sample is not accidentally spilled.

3. Once all organisms have been removed from the net, and contents placed in the sample container, add preservative (95% ethyl alcohol) until the sample contents are completely covered. Ensure that the container is properly labeled, insert a completed sample label, and tightly close the lid. **Always complete sample labels in permanent, waterproof ink written on 100% ragbond paper.** Sample debris must be completely covered by preservative. Example sample labels are provided at the end of this SOP.
 4. Repeat this procedure for each discrete sample. Prior to leaving the site, check the samples to ensure they have been properly labeled and tightly closed, and ensure data sheets are properly completed.
- D. Collect associated habitat data for each sample. Refer to SOP #9 (Physical Habitat Measurements) for descriptions and procedures for collecting habitat data.

Sample Labels

Ozark National Scenic Riverways Spring Invertebrate Sample

Spring Name: _____

Transect Number (2, 4, 6, 8 or 10): _____

Sample Location (L, M, R): _____

Date Collected: _____

Collectors: _____

Ozark National Scenic Riverways Spring Invertebrate Sample

Spring Name: _____

Transect Number (2, 4, 6, 8 or 10): _____

Sample Location (L, M, R): _____

Date Collected: _____

Collectors: _____

Ozark National Scenic Riverways Spring Invertebrate Sample

Spring Name: _____

Transect Number (2, 4, 6, 8 or 10): _____

Sample Location (L, M, R): _____

Date Collected: _____

Collectors: _____

Ozark National Scenic Riverways Spring Invertebrate Sample

Spring Name: _____

Transect Number (2, 4, 6, 8 or 10): _____

Sample Location (L, M, R): _____

Date Collected: _____

Collectors: _____

Ozark National Scenic Riverways Spring Invertebrate Sample

Spring Name: _____

Transect Number (2, 4, 6, 8 or 10): _____

Sample Location (L, M, R): _____

Date Collected: _____

Collectors: _____

Ozark National Scenic Riverways Spring Invertebrate Sample

Spring Name: _____

Transect Number (2, 4, 6, 8 or 10): _____

Sample Location (L, M, R): _____

Date Collected: _____

Collectors: _____