Benthic Macroinvertebrate Sample Collection and Processing

Wadeable Streams: Rapid Bioassessment Protocols (RBP)
Elements of Rapid Bioassessment Protocols

Integrated assessment
- compares habitat, water quality and biological measures with empirically defined reference conditions.

Emphasizes sampling of a single habitat type
- (riffles or runs)

Standardized collection methods
- (eg. 5-minute kicknet)

Standardized sub-sample protocols
- (+- 175 individuals)
Sample Collection

• What to Collect
• Where to Collect
• How to Collect
Sample Processing

• Sample Preservation
• Sub-Sampling
• Identification and Enumeration
Benthic Macroinvertebrates:

Invertebrate organisms that are large enough to be seen by the unaided eye, can be retained by a US Standard No. 30 sieve (595 µm mesh) and live at least part of their life cycles within or upon available substrates (cobble/gravel, snags, etc.) in a body of water.
<table>
<thead>
<tr>
<th>Major Group</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insecta</td>
<td>Mayflies, Caddisflies, Stoneflies, Beetles, etc.</td>
</tr>
<tr>
<td>Turbellaria</td>
<td>Flatworms</td>
</tr>
<tr>
<td>Annelida</td>
<td>Oligochaeta (aquatic earthworms)</td>
</tr>
<tr>
<td></td>
<td>Hirudinea (leeches)</td>
</tr>
<tr>
<td>Crustacea</td>
<td>Amphipoda (Scuds), Isopoda (aquatic sowbugs), Decapoda (crayfish, shrimp)</td>
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<tr>
<td>Hydracarina</td>
<td>Water mites</td>
</tr>
<tr>
<td>Gastropoda</td>
<td>Snails</td>
</tr>
<tr>
<td>Pelecypoda</td>
<td>Freshwater mussels</td>
</tr>
</tbody>
</table>
Where to Collect the Benthic Macroinvertebrate Sample

Goal: To collect the sample from the optimal benthic macroinvertebrate habitat in the reach.
RBP Benthic Macroinvertebrate Sample Protocols

5 - Minute Kicknet Sample

- Riffles and/or Runs
  - Gravel/Cobble - primary or sole method
  - Sand - supplements snag sample

Snag Sample

- Riffles and/or Runs
- Sand/Silt - Primary method, supplemented by kicknet sample
Preferred Habitat Types for RBP Sample

- Riffles
- Runs / Glides
- Pools
Riffle
Pool
Prioritization of Substrate Characteristics

- Microhabitat heterogeneity
- Dissolved oxygen
- Filter feeders
- Nutrient flow
- Algal community development
I - Cobble/Gravel
II - Debris Jams
III - Emergent Vegetation
IV - Rootwads
Steps in Sample Collection

- Collection
- Processing
- Preservation and Labeling
Field Equipment: Sample collection

- D-frame kick net (mesh ≤ 595 µm)
- Lopping Shears for Snag Samples
- Screen Sieves (mesh ≤ 500 µm)
- Hip and Chest Waders
Field Equipment: Sample Processing

- Sorting Trays
- Subsampling Device (e.g., Mason jar lid)
- Jewelers Forceps
- Sample Jars and Vials
- Preservative - 70% ethanol or 10% Formalin
- Sample Labeling Material
## Riffle/Run with Gravel/Cobble Substrate

<table>
<thead>
<tr>
<th>Step</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Work downstream to upstream</td>
<td>• Start near edge of bank</td>
</tr>
<tr>
<td>Bottom edge of net on substrate</td>
<td>• Opening facing upstream</td>
</tr>
<tr>
<td>Disturb substrate upstream of the net</td>
<td>• Use feet or hands</td>
</tr>
<tr>
<td>Let current carry material into net</td>
<td></td>
</tr>
<tr>
<td>Continue in zig-zag manner</td>
<td>• Cover as much of the riffle as possible</td>
</tr>
<tr>
<td>5 minutes of actual “kick time”</td>
<td></td>
</tr>
</tbody>
</table>
5 – Minute Kicknet Sample Method
<table>
<thead>
<tr>
<th>Snag sample – Silty/Sandy Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Collect woody debris/snags</strong></td>
</tr>
<tr>
<td>- From debris piles/jams in riffle/run areas</td>
</tr>
<tr>
<td>- Prefer 0.5 - 2.5 cm diameter</td>
</tr>
<tr>
<td>- Submerged in the stream ≥ 2 weeks</td>
</tr>
<tr>
<td>- Use lopping shears</td>
</tr>
<tr>
<td><strong>Minimize loss of macroinvertebrates</strong></td>
</tr>
<tr>
<td>- Place kicknet downstream of snag while cutting</td>
</tr>
<tr>
<td>- Place snag in sorting tray, sieve bucket or net</td>
</tr>
<tr>
<td><strong>Collect 5-minute kicknet sample</strong></td>
</tr>
<tr>
<td>- Disturb sand/silt as deeply as possible</td>
</tr>
<tr>
<td>- Kick debris piles</td>
</tr>
<tr>
<td>- Combine kicknet sample with snag sample</td>
</tr>
</tbody>
</table>
Snag Sample Method
RBP Sample Processing
Sample Processing

- Sub-sampling
- Preservation
- Labeling
Sub-sampling RBP 5 - Minute Kicknet Sample

- Wash the sample in the stream to remove excess silt and sand
- Place washed sample in sorting tray
- Visually inspect large debris
- Gently shake or stir sorting tray to evenly distribute contents
Washing the Sample
Isolate 1 - 4 portions of the sample, place in separate sorting tray

Pick ALL Macroinvertebrates from this portion, place in sample vial containing 70% ethanol

Repeat process until 140 - 210 macroinvertebrates are picked (continue to pick last portion until all benthics are removed)
Picking the RBP Sample
<table>
<thead>
<tr>
<th>Sub-sampling Snag + 5 Minute Kick Sample</th>
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</thead>
<tbody>
<tr>
<td>Inspect snags and remove all macroinvertebrates by picking or washing, place in sorting tray.</td>
</tr>
<tr>
<td>Inspect under loose bark and in crevices of snags.</td>
</tr>
<tr>
<td>Combine organisms from snags with supplemental 5-minute kicknet and process as previously described for the 5-minute kicknet sample.</td>
</tr>
</tbody>
</table>
Inspecting Snags
Notes on Sub-sampling

- If the sample contains less than 140 individuals repeat the sample process and combine with the first prior to sorting.

- When picking a subsample continue to pick the last portion until all benthics are removed.

- If the density of organisms in the last subsample is great enough that it will yield many more than 175 individuals subsample the subsample.

- If, after several portions are picked, it is obvious that entire sample contains relatively few benthics pick entire sample without using mason jar lid/EPA square.
RBP Sample Preservation

Field Processed Sample

• 70% Ethanol
• 40% Isopropyl alcohol

Laboratory Sample

• Thoroughly wash sample
• Fill sample containers no more 1/2 full
• 10% Formalin solution to fill jar, leaving at least one inch of head space
• 95% Ethanol only if samples will be picked soon
Preservatives
Sample Labeling

- Station Number and Location Description
- Date and Time of Collection
- Collection method (5-minute kicknet, snag, etc.)
- Preservative Used
- Estimate of number of individuals in sample
- Name of Collector(s)
- Replicate number (if needed)