

# 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  $\mu\text{m}$  mesh) and live at least part of their life cycles within or upon available substrates (cobble/gravel, snags, etc.) in a body of water.

# Major Groups of Benthic Macroinvertebrates

## Insecta

- Mayflies, Caddisflies, Stoneflies, Beetles, etc.

## Turbellaria

- Flatworms

## Annelida

- Oligochaeta (aquatic earthworms)
- Hirudinea (leeches)

## Crustacea

- Amphipoda (Scuds), Isopoda (aquatic sowbugs),
- Decapoda (crayfish, shrimp)

## Hydracarina

- Water mites

## Gastropoda

- Snails

## Pelecypoda

- Freshwater mussels

# 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



# Run



# Glide



# 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



# V - Sand



# VI - Bedrock



# Steps in Sample Collection

Collection

Processing

Preservation and Labeling

# Field Equipment: Sample collection

**D-frame kick net (mesh  $\leq$  595  $\mu\text{m}$ )**

**Lopping Shears for Snag Samples**

**Screen Sieves (mesh  $\leq$  500  $\mu\text{m}$ )**

**Hip and Chest Waders**

# Field Equipment: Sample Processing

**Sorting Trays**

**Subsampling Device (eg. Mason jar lid)**

**Jewelers Forceps**

**Sample Jars and Vials**

**Preservative - 70% ethanol or 10% Formalin**

**Sample Labeling Material**

# Riffle/Run with Gravel/Cobble Substrate

Work downstream to upstream

- Start near edge of bank

Bottom edge of net on substrate

- Opening facing upstream

Disturb substrate upstream of the net

- Use feet or hands

Let current carry material into net

Continue in zig-zag manner

- Cover as much of the riffle as possible

5 minutes of actual “kick time”



# 5 – Minute Kicknet Sample Method



# Snag sample – Silty/Sandy Substrate

Collect woody debris/snags

- From debris piles/jams in riffle/run areas
- Prefer 0.5 - 2.5 cm diameter
- Submerged in the stream  $\geq$  2 weeks
- Use lopping shears

Minimize loss of macroinvertebrates

- Place kicknet downstream of snag while cutting
- Place snag in sorting tray, sieve bucket or net

Collect 5-minute kicknet sample

- Disturb sand/silt as deeply as possible
- Kick debris piles
- Combine kicknet sample with snag sample

# 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



## Subsampling 5 - Minute Kicknet 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



## Sub-sampling Snag + 5 Minute Kick Sample

Inspect snags and remove all macroinvertebrates by picking or washing, place in sorting tray.

Inspect under loose bark and in crevices of snags.

Combine organisms from snags with supplemental 5- minute kicknet and process as previously described for the 5-minute kicknet sample.

# 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 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)

# Safety

