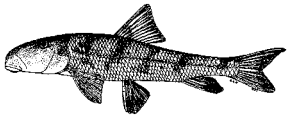
*State of Ohio*
*Environmental Protection Agency*

**Division of Surface Water**

# 2008 Updates to Biological Criteria for the Protection of Aquatic Life: Volume III. Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Macroinvertebrate Communities.



**August 26, 2008**

Ted Strickland, Governor
Chris Korleski, Director

**Detailed supplement to macroinvertebrate sample processing procedures described in Vol. III, Subsection 1, Part C, pp. V-1-5 and V-1-6.**

## Quantitative Sample

**Summary**: Processing the artificial substrate sample is relatively simple and straight forward. First, conduct a thorough pre-pick of the coarse, #30 screen sample in a white enamel pan or under low magnification to remove obvious rare taxa and to make an initial inventory of the taxa present. After the pre-pick, sub-sample to obtain the required number of midges and manageable numbers of other large populations (*e.g.*, hydropsychid caddisflies, heptageniid mayflies, etc.). After counting and identifying the #30 material, the finer, #40 screen sample is sub-sampled into manageable cuts, scanned and counted to the lowest recognizable group, then extrapolated into the list of taxa already identified and enumerated in the #30 screen. The numbers from the pre-pick, #30 screen and #40 screen are then combined to arrive at the total number for each taxon.

### Recommended Equipment

Dissecting Microscope
Compound Microscope with at least 40X, 100X, and 400X magnification
Folsom Sample Splitter or other sample splitting device
70% Ethyl Alcohol (ETOH as preservative)
Squeeze Bottles
10% Potassium Hydroxide (KOH) solution (for clearing midge larvae)
Small, 10 ml beakers (for clearing midge larvae)
Hot plate (for clearing midge larvae)
Microscope slides and cover slips
Euparal (slide mounting medium for making permanent reference or voucher slides)
Glacial Acetic Acid (for Euparal permanent mounts)
100% Ethyl Alcohol (for Euparal permanent mounts)
Fine forceps
White enamel pan
Petri dishes with lids (some scored with equally spaced rows to facilitate scanning under the microscope)
Watch glasses (hold multiple sub-sample fractions)

### Pre-picking

After cleaning and sieving, the artificial substrate sample should be placed in two containers. The Ohio EPA uses a larger four-ounce glass jar for the coarser #30 screen material and a smaller eight-dram vial for the #40 screen material. Along with the qualitative sample jar from the site, the containers should be bound together with a rubber band. The #30 and #40 screen containers should be labeled with site location information including a common log or site number so they can be matched together if separated.

**Note**: If the cleaned sample still contains a lot of silt that would cloud the sample during processing, the analyst may want to re-screen the #30 sample through a fine sieve (# 40 or # 60) prior to processing.

The first step in lab identification is a thorough pre-pick of the #30 screen material to initially remove as many different taxa for identification as possible. This can be done by eye in a white enamel pan, with the aid of a magnifying lens, or by using low magnification under the dissecting scope. [Note: midges (Chironomidae) are excluded from pre-picking unless the total number in the sample is extremely low]. Besides picking out obvious rare and different taxa (different orders, families, and genera), the user should try to select enough specimens from large or diverse taxonomic groups (*e.g.*, hydropsychid caddisflies, baetid mayflies, heptageniid mayflies) so that most, if not all, available species are removed. After picking, the remaining sample is sub-sampled for midges (about 100 larvae) and to identify a manageable number of the other large organism groups (*e.g.*, 75 mayflies, 75 caddisflies, minimum). These cuts are primarily for abundance information since, excluding midges, the majority of taxa should be accounted for in the pre-pick.

**Sub-sampling**

Nearly all artificial substrate samples will require some degree of sub-sampling in order to count and identify a manageable number of organisms. Ohio EPA uses a clear plastic Folsom sampler splitter (alcohol resistant) to split the sample material into equal halves. The sample is poured into the splitter drum, rocked back and forth to evenly distribute the material, then turned over to split the sample in half and pour the material into the tubs positioned underneath. If additional cuts are needed, one of the fractions is poured back into the drum and the process is repeated, over and over, until the desired number of cuts is made.

As a general rule, when processing a typical sample with large numbers of mayflies, caddisflies and midges, the user should reach the following, minimum numeric targets between the pre-pick and the sub-sample:

| | |
|---|---|
| 1) Midges | Approximately 100 larvae ($\pm$ 20%), cleared, mounted and identified. (Note: no midges are removed during the pre-pick). |
| 2) Mayflies | Approximately 75 (within diverse families such as Heptageniidae or Baetidae). |
| 3) Caddisflies | Approximately 75 (within diverse families such as Hydropsychidae). |

Except for the Midge targets, these are general guidelines to ensure adequate sample analysis. It is acceptable to identify more than 75 mayflies or caddisflies but, if large numbers are present and require sub-sampling, the user should at least meet the minimum targets. On the other hand, if the sample contains very few mayflies or caddisflies, it may be impractical or impossible to reach the75 count guidelines. In these instances, the user should account for the available taxa during the pre-pick or during sub-sampling. [**Note**: While the pre-pick may be done by eye in a white enamel pan,

sub-samples are always processed under 10X magnification using the dissecting scope. The back of the bench sheet can be used to keep track of the cuts and counts, and to make calculations.]

Midges (Chironomidae) are treated differently than other taxa in the sample and the number identified should always remain near the 100 (± 20%) count target, regardless of population density. The user can reach the target range by sub-sampling enough times to reach the target (preferred) or by over-picking (*i.e*, exceeding the target), then sub-sampling the midges down to the target number (not as efficient and wastes time because more midges than needed are picked).

Since population densities on the artificial substrates vary, a different sample fraction is often needed to quantify the different populations. For example, the user may cut a sample four times (to 1/16th) to pick out 100 midges but may need to work through an additional 1/8th or 1/4th cut to find an adequate number of mayflies or caddisflies. To process these populations, Ohio EPA recommends making all the cuts needed first, then working backwards (beginning with the smallest fraction) until an adequate number of each taxa group is picked for identification. In the event of an over-cut (*i.e.*, not enough specimens in the fraction to meet the target number), simply work backwards through the next fraction (or the next, or the next) until enough specimens are picked out (or counted). It is important to remember that once the user begins picking a taxa group from a cut, every specimen from that group must be counted in that fraction. For example, if the cut contains 200+ midges, picking doesn't stop at the 100 specimen target but must continue until all specimens are removed. In this example, it would probably be more efficient to return the fraction to the sample splitter and perform additional cuts.

As a rule, it is better to over-cut than to not make enough cuts and spend excessive time picking and counting more organisms than needed. Specimens that are too small to identify with confidence (such as early instar heptageniid mayflies or hydropsychid caddisflies) are extrapolated into the counts of the larger specimens, already identified in that group.

Once adequate numbers of midges, mayflies, caddisflies, etc., have been picked/counted, the user can stop processing through additional cuts. However, Ohio EPA methods require that at least 1/8th of the sample is viewed under magnification in order to ensure sample processing consistency between users. This last step is especially important when processing samples with extremely high densities so rarer taxa are not overlooked.

**Midge Identification**

Midges are cleared in a 10% KOH solution and "wet-mount" on slides for identification with a compound microscope. Specimens are typically cleared in a 10 ml beaker on a hot plate, set slightly below boiling, for about 30 minutes or until the midges are sufficiently cleared. Voucher specimens are slide mounted in Euparal. Specimens cleared in KOH that are going to be mounted in Euparal need to go through the dehydration series: minimum of 5 minutes in glacial acetic acid, 15 min. in 70 % ETOH, and 15 min. in 100% ETOH. Another option would be to mount all the specimens directly into CMC 10 that will both clear and mount the specimen. The draw backs to this option are that some characters are not easily seen using this method and this mounting medium is only semi-permanent. Slides usually develop air fingers over time.

**# 40 Screen Sample**

As a general rule, the finer, #40 screen sample is sub-sampled into smaller, manageable fractions then scanned and counted by major taxonomic group (e.g., early instar Hydropsychidae, early instar Hepatageneiidae, Chironomidae, etc.). The user should try to look at about a minimum of 100 organisms in the #40 screen fraction to ensure adequate sample coverage. As a general rule, the number of sub-samples is often similar to the number used during the #30 screen Ids. Like the #30 screen sub-sampling procedures, if population densities in the scanned cut are too high, return the material to the sample splitter and make additional cuts.

The material in the #40 screen is identified to the lowest practical level and counted. Many specimens will be early instars and may not be identifiable past the genus or family level. For this reason, these counts are extrapolated into the taxa already identified and enumerated in the #30 screen. Midges are also counted and extrapolated into the #30 screen material with a few exceptions. These include certain easily recognized midge taxa that are so small the mature larvae often pass through the #30 screen and are caught in the #40. These taxa include:

1) *Corynoneura spp*. (antenna as long or longer than head capsule)
2) *Thienemanniella spp*. (antenna about ½ head capsule length, $A_2$ may be dark)
3) *Nilotanypus fimbriatus* (elongate head capsule)
4) *Labrundinia spp*. (elongate head capsule, body preserves in a sigma "Σ" shape)
5) *Stempellina spp*. (curved transportable sand case)
6) *Stempellinella spp*. (straight transportable sand case)

Since these taxa don't accurately represent populations throughout the sample, they are removed, identified, and counted separately from the other midges in the sample. **Note:** If the user happens to pick out and identify #40 screen midges that are not among the six taxa listed above, ignore the identifications and treat them as unidentified Chironomidae (to be extrapolated into the already identified, # 30 screen midges).

**Oligochaete Identification**

For normal sampling purposes in Ohio streams and rivers, Ohio EPA does not identify aquatic segmented worms beyond the Class level (Oligochaeta). Since specimens are fragile and often broken, the simplest counting method is to count the number of end pieces and divide by two.

**Totaling the Sample**

For each taxa add the pre-pick, # 30, and # 40 portions of the sample. Midge totals are arrived at by first calculating the ratio of the individuals identified for each taxon by the total number of individuals identified and then multiplying this ratio for each taxon by the total number of midges calculated for the entire sample. Numbers for the special # 40 screen midges are removed from this tabulation and calculated separately. This combined list represents the total number of taxa and their density found on the artificial substrates. **Note**: The analyst should only report distinct taxa on the

final taxa list. Do not report immature, damaged or pupal specimens unless the analyst is certain those individuals are distinctly different from taxa already identified. Specimens that were dead prior to sample collection (*e.g.*, empty snail or clam shells, exuviae, rotten specimens) are not counted.

**Reference Specimens/QA**

One or more specimens of each new taxon identified should be retained in a permanent reference collection and verified by a taxonomic expert. The Ohio EPA is willing to verify reference specimens if they are brought to the Ohio EPA Groveport Field Office laboratory. Contact:

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Ohio EPA Groveport Field Office
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Groveport Ohio, 43125
614-836-8781
mike.bolton@epa.state.oh.us

**Volume III, pp. V-1-7 to V-1-9. Replaces Tables V-1-1 and V-1-2 with Table V-1.**

Table V-1. Current taxonomic keys and the level of taxonomy routinely used by the Ohio EPA in streams and rivers for various macroinvertebrate taxonomic classifications.

- Porifera: Species (Pennak 1989)
- Cnidaria: Genus (Smith 2001)
- Platyhelminthes: Class (Thorp and Covich 2001)
- Nemertea: Phylum (Smith 2001)
- Nematomorpha: Phylum/genus (Smith 2001)
- Ectoprocta: Genus/species (Thorp and Covich 2001)
- Entoprocta: Species (Thorp and Covich 2001)
- Annelida
  - Oligochaeta: Class (Smith 2001)
  - Hirudinea: Species (Klemm 1982)
- Arthropoda
  - Crustacea
    - Isopoda: Genus (Smith 2001)
    - Amphipoda: Genus (Smith 2001)
      - Gammarus: Species (Holsinger 1972)
    - Decapoda
      - Cambarus and Fallicambarus: Species (Jezerinac and Thoma 1984, Jezerinac 1993)
      - Orconectes and Procambarus: Species (Jezerinac 1978)
      - Palaemonetes: Species (Pennak 1989)
  - Arachnida: Hydrachnidia (Smith 2001)
  - Insecta
    - Ephemeroptera: Genus (Merritt et al. 2008)
      - Baetidae: Genus/species (Morihara and McCafferty 1979, McCafferty and Waltz 1990, Lugo-Ortiz and McCafferty 1998, Ohio EPA 2007a)
      - Pseudocloeon: Species (McCafferty and Waltz 1995)
      - Heptageniidae
        - Maccaffertium and Stenonema: Species (Bednarik and McCafferty 1979)
      - Ephemerellidae
        - Dannella: Species (Allen and Edmunds 1962)
        - Serratella: Species (Allen and Edmunds 1963b)
      - Brachycercinae: Species (Sun and McCafferty 2008)
      - Baetiscidae
        - Baetisca: Species (Pescador and Berner 1981)
      - Ephemeroidea: Species (McCafferty 1975)
    - Odonata: Family/genus (Merritt et al. 2008)
      - Anisoptera: Genus/species (Needham *et al.* 2000)
    - Plecoptera: Genus (Stewart and Stark 2002)
      - Perlidae
        - Acroneuria: Species (Hitchcock 1974)
        - Paragnetina: Species (Hitchcock 1974)
      - Perlodidae: Species (Hitchcock 1974)
    - Hemiptera: Genus (Hilsenhoff 1995, Merritt et al. 2008)
    - Megaloptera: Genus (Merritt et al. 2008)
      - Nigronia: Species (Neunzig 1966)
    - Neuroptera: Genus (Merritt et al. 2008)
    - Trichoptera: Genus (Wiggins 1996, Merritt et al. 2008)
      - Philopotamidae: Species (Ross 1944)
      - Hydropsychidae
        - Hydropsyche and Ceratopsyche: Species (Schuster and Etnier 1978, Schefter and Wiggins 1986)
      - Rhyacophilidae
        - Rhyacophila: Species (Prather and Morse 2001)
      - Odontoceridae
        - Psilotreta: Species (Parker and Wiggins 1987)
      - Leptoceridae
        - Ceraclea: Species (Resh 1976)
        - Mystacides: Species (Yamamoto and Wiggins 1964)
        - Nectopsyche: Species (Glover and Floyd 2004)
        - Oecetis: Species (Floyd 1995)
        - Triaenodes/Ylodes: Species (Glover 1996)
    - Lepidoptera: Genus (Merritt et al. 2008)
    - Coleoptera: Genus (Merritt et al. 2008)
      - Dryopoidea: Genus/species (Brown 1972)
    - Diptera: Family/genus (Merritt et al. 2008)
      - Ceratopogonidae
        - Atrichopogon: Species (Johannsen 1935)
      - Chironomidae: Genus/species groups (Wiederholm 1983, Epler 2001, Ohio EPA 2007b)
        - Ablabesmyia: Species (Roback 1985, Epler 2001)
        - Labrundinia: Species (Roback 1987, Epler 2001)
        - Tanypus: Species (Roback 1977, Epler 2001)
        - Brillia: Species/species group (Epler 2001)
        - Corynoneura: Species (Ohio EPA 2007b)
        - Eukiefferiella and Tvetenia: Species groups (Bode 1983)
        - Nanocladius: Species (Epler 2001)
        - Parakiefferiella: Species (Ohio EPA 2007b)
        - Rheocricotopus: Species (Saether 1985, Epler 2001)
        - Thienemanniella: Species (Hestenes and Saether 2000, Epler 2001)
        - Chironomus: Species groups (Oliver and Roussel 1983)
        - Dicrotendipes: Species (Epler 2001)
        - Endochironomus and Tribelos: Species (Grodhaus 1987, Epler 2001)
        - Parachironomus: Species (Epler 2001, Ohio EPA 2007b)
        - Paracladopelma: Species (Jackson 1977)
        - Polypedilum: Species groups/species (Maschwitz 2000, Ohio EPA 2007b)
        - Tanytarsini: Genus/species groups/species (Epler 2001, Ohio EPA 2007b)
      - Muscidae: Species (Johannsen 1935)
- Mollusca
  - Gastropoda: Genus/species (Burch 1982)
  - Bivalvia
    - Sphaeriidae: Genus (Burch 1972)
    - Unionidae: Species (Waters 1995)

**Volume III, pp. V-1-11 to V-1-15.** The following is a complete list of macroinvertebrate taxonomic references.

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