2014 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.

Division of Surface Water
Ecological Assessment Section
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Supplement to macroinvertebrate Field Methods - Quantitative Sampling procedures described in Vol. III, Subsection 1, Part A, Pages V-1-2 to V-1-5.

The primary sampling gear used by the Ohio EPA for the quantitative collection of macroinvertebrates in streams and rivers is the modified multiple-plate artificial substrate sampler (Hester and Dendy 1962). The sampler is constructed of 1/8 inch (3 mm) tempered hardboard cut into 3 inch (7.5 cm) square plates and 1 inch (2.5 cm) square spacers. Other items such as plastic washers can also be used as spacers. A total of eight plates and twelve spacers are used for each sampler. The plates and spacers are placed on a 3 inch (7.5 cm) long, 1/4 inch (6 mm) diameter eyebolt so that there are three single spaces, three double spaces, and one triple space between the plates. The total surface area of the sampler, excluding the eyebolt, approximates 1 square foot (roughly 0.1 square meter). A sampling unit consists of a composite cluster of five substrates tied to a construction block that is colonized in-stream for a six week period beginning no earlier than June 15 and ending no later than September 30.

A composited set of five artificial substrate samplers of eight plates each has been used by the Ohio EPA in collecting macroinvertebrate samples since 1973. At this level of effort, it has been found that consistent, reproducible ICI values can be scored despite the collections of often highly variable numbers of individual organisms. Results of analyzing replicate composites of five artificial substrates have shown that variability among calculated ICI values is at an acceptable level. The reliability of the sampling unit not only depends on a standardized colonization surface area, but equally important are the actual physical conditions under which the units are placed in the aquatic environment. It is imperative that the artificial substrates be located in a consistent fashion with particular emphasis on sustained current velocity over the set. With the exception of water quality, the amount of current tends to have the most profound effect on the types and numbers of organisms collected using artificial substrates in Ohio. For an accurate interpretation of the ICI, current speeds should be no less than 0.3 feet/second (10 cm/second) under normal summer-fall flow regimes. The optimal current speed is between 0.7 and 1.5 feet/second (21-46 cm/second). These conditions can usually be adequately met in all sizes of perennial Ohio streams but can be a problem in small headwater streams or those streams so highly modified for drainage that dry weather flows maintain intermittent, pooled habitats only. In these situations, sampling can be conducted, but an alternative interpretation of the ICI value and/or the use of other assessment tools may be necessary.

Detailed supplement to macroinvertebrate Field Methods - Qualitative Sampling procedures described in Vol. III, Subsection 1, Part B, Page V-1-5.

Ohio EPA collects qualitative, natural substrate samples at every macroinvertebrate sampling site, either alone or in conjunction with quantitative (artificial substrate) collections. As a general rule, quantitative sampling is conducted at sites greater than 20 square miles where current velocities and stream depth are almost always adequate for artificial substrate placement. For routine monitoring and assessments, qualitative sampling alone is conducted at the smaller drainage sites. Quantitative samples can be collected in smaller drainage areas where the flow and later depth are sufficient if the data quality objectives indicates the need.

Ohio EPA's primary sampling period is during the summer months of June 15th through September 30th. Since visual inspection is so important, sampling during high water or when the stream is brown and turbid from recent rains is avoided. Ideally, sampling is conducted when the water column is relatively clear, the stream is well within its banks and has experienced an extended period of stable flow. This also assures clear definition among the four types of instream macrohabitats – riffle, run, pool and margin.

A pool is a generally deep and sluggish stream section often with slow or non-detectable current. In contrast, a riffle is typically a short, shallow, high gradient stream section, often with coarse substrates and turbulent flow. A run is the transitional area between riffles and pools that often connects the two habitats. Runs are often moderately shallow with visible current but the water surface is typically smooth and unbroken. Runs are the preferred habitat for artificial substrate placement. For Ohio EPA's sampling purposes, margin habitats are most often the sluggish edges of the wetted channel, usually adjacent to pools, in protected shallows along the edges of bars, or below obstructions and log jams that break the current.

Whenever possible, attempts should be made to include a riffle habitat or at least some semblance of a riffle or constricted flow habitat at each sampling site. Lentic habitats (*i.e.,* lakes, ponds, impoundments) are typically avoided unless they are characteristic of the entire survey sampling area or there are unavoidable water quality issues that must be addressed. While sampling zones are not precisely measured, a sampling reach including a variety of riffle, run, pool and margin habitats rarely extends more than 50-100 yards.

For qualitative evaluations, Ohio EPA's primary sampling tool is a Tri-net "Indestructible"® brand dip net with 500 micron netting used in combination with a white pan. When sampling primary headwater habitat streams (drainage area of 1 sq. mi. or less) it is often helpful to use a dip net with a round rim of about 10 inch diameter in order to get the net rim flush with the stream bottom. Net sampling methodologies vary by habitat but are as follows:

*Note: In addition to using dip nets, macrohabitats are always sampled by visually inspecting individual pieces of coarse substrates and woody debris that may be present.

Riffle Sampling: Stand in the middle of the riffle, place the net firmly on the stream bottom with the opening facing upstream and let the current fill and inflate the netting. Using your foot, kick, grind and agitate the substrates immediately upstream from the opening and let the current carry the dislodged material down and into the net. Large substrates can also be hand rubbed in front of the net to increase efficiency. Coarse substrates can usually be roughly kicked and agitated without releasing excessive sediment and debris. However, when sampling riffles with loose deposits of fine sand or gravel, avoid extremely vigorous kicking as the net will quickly fill with sand and make picking difficult. The same is true of log or "stick" riffles full of muck, peat and detritus. Use a lighter touch to avoid filling the net with excessive debris.

Fall sampling may result in large quantities of leaf litter caught in the net which can also interfere with picking. In these instances, move to a deeper, slower section of stream, hold the dip net in the water with the ring above the surface, pick a handful of leaf litter and vigorously shake it in the suspended net to dislodge any attached organisms. When finished, discard the leaves. In this way, most of the coarse litter can be removed, resulting in a clean sample with minimal loss of organisms. Note: when discarding the leaves from the net, keep an eye out for shoe-shaped blackfly pupal cases and midge tubes that may remain attached.

Run Sampling: The same kick methods used in riffle habitats are also employed in runs. Simply make slight adjustments in technique if current velocities are not sufficient to carry the dislodged organisms into the net. For example, sweep the net back towards the collector after kicking to capture debris suspended in the water column or kick and drag net upstream simultaneously to capture dislodged debris.

Pool Sampling: Because of the increased depth and slow current, a different netting method is used in pools. For maximum species richness, try to locate deposits of loose (*i.e.,* not strongly embedded), coarse substrates, kick and churn the bottom, then work the net back and forth in a "Figure 8" motion, through the plume of disturbed sediment just above the stream bed. In addition to sampling prime pool habitats, at least one kick net sample should be taken from the more typical pool substrate (often sandy or silty and often unproductive aside from red midges) simply to define the typical pool habitat condition. Note: The presence of "red" midges is not an automatic indicator of degraded water quality. Numerous varieties of midges contain hemoglobin and have a bright red appearance but can range in sensitivity from tolerant (*e.g.,* the genus *Chironomus*), to facultative (*e.g.,* the genus *Stictochironomus*), to sensitive (*e.g.,* the species *Microtendipes "caelum"*).

Regarding netting techniques and particularly in pools, never "mine" the stream bottom (*i.e.*, use the dip net as a shovel) as this will only fill the net with sand and sediment. This practice results in unproductive, inefficient sampling and causes difficulty sorting through the massive debris.

Margin Sampling: Stream margin habitats vary considerably but may include:

- Undercut banks
- Tree root wads (dense and fibrous)
- Tree root mats (woody or leathery)
- Grass edges
- Water willow (usually on bars and along gravelly margins)

  http://www.wildflower.org/gallery/result.php?id_image=10499
- Rip-rap
- Shallow, silty edges along bars and pools
- Eroded banks with coarse substrate deposits along the toe
- Bare clay and hardpan

For Ohio EPA's sampling purposes, the trait that distinguishes margin habitats is sluggish or non-detectable current. Riffle and run margins exposed to strong current are usually the same as the riffles and runs themselves. These habitats rarely yield populations appreciably different from those in the main channel and are typically avoided by field personnel, unless aquatic macrophytes are present in which case certain baetid mayflies and case-building caddisflies may be present. Prime margin sampling areas are often found adjacent to pools, in protected or sluggish shallows along the edges of bars and inside bends, or below obstructions and log jams that break the current.

When sampling margins, special attention should be given to locating undercut banks with root mats or root wads, grassy edges and water willow. These areas should always be sampled if present. Use the dip net to reach into undercut banks, then knock and sweep the net in a "piston-like" motion, repeatedly sweeping the net up and over the same spot to capture dislodged organisms and suspended material. Use care when sampling thick woody tree roots or limbs as the net can often snag and rip and the contents may be lost.

Certain streams lack any appreciable or defined margin habitat. These may include extensive bedrock streams, particularly under low-flow conditions, or high-gradient, cascading streams with large boulder substrates. Under extreme low-flow conditions, former margin and undercut bank habitats may be exposed and unavailable for sampling. In these instances, simply record "No Margin" under field sheet observations. The same is true for sites lacking pool, run or riffle habitats.

Picking Organisms: After kick-netting, the net debris is dumped into a white pan and live organisms are picked out with forceps or pipets. Organisms are preserved in a 4-ounce sample collection jar filled with 95% ethyl alcohol (ETOH). Standard lab preservative is 70% alcohol but Ohio EPA uses 95% ETOH for field collections since a fair amount of dilution water and fluids are inadvertently added during sampling. Larger organisms (e.g., crayfish) are often preserved separately in order to avoid damage to other delicate specimens in the sample jar. In addition to the white pan, use forceps and manual picking of individual pieces of cobble, boulder, logs, macrophytes, etc., in order to find case-building, mining, or other attached forms not easily dislodged and captured with the dip net. Examples of taxa to look out for are listed below in the specific habitat sampling section.

All types of aquatic macroinvertebrates at a site are collected but particular emphasis is directed at locating EPT (*i.e.,* Ephemeroptera, Plecoptera, and Trichoptera) taxa since these are especially important in assessing water quality conditions. Ultimately, a narrative water quality evaluation is produced based on the qualitative sampling results using best professional judgment, community composition, field observations, and use of Ohio EPA's historic collection database. Most taxa in the Ohio EPA data base have been assigned a pollution tolerance category (*e.g.,* intolerant, moderately intolerant, facultative, moderately tolerant, tolerant, and very tolerant) and this cumulative information is used in evaluations. Since data are based on Ohio EPA methods and collections from Ohio streams, they may not be directly applicable to other states. As yet, the macroinvertebrate group has not produced a "qualitative Invertebrate Community Index", equivalent to the quantitative ICI, but data and potential metrics based on the natural substrate samples are being evaluated.

Field notes describing the predominant and most common populations from each macrohabitat are recorded on the field sheet (see attachment). Sampling is conducted for a minimum of 30 minutes and continues until, within a reasonable amount of time, no new taxa are found. Under normal circumstances in most typical stream settings, a sampling crew of two usually spends 50 to 90 minutes at a site to ensure thorough coverage. The 30-minute total sampling minimum is rarely employed and usually reserved for the most severely degraded small ditches or streams or in acid mine drainage environments.

Obviously, poorer quality and more polluted or simplified stream segments will often yield fewer taxa and require less sampling time than high quality, natural channels. However, the intensity of the sampling effort and rigor devoted to each site should not vary, regardless of aesthetics or perceived stream quality. It is important to devote the same sampling effort, if not the same sampling time, to each site evaluated.

Before or after sampling, photographs of the sampling area (usually upstream and downstream views) are taken and GPS coordinates are recorded. If GPS is not available, note the location on a 7.5-minute USGS topographic quadrangle map.

When deciding where to sample on-site and, in order to maximize taxa collection richness, special attention should be directed at specific habitats and micro-niches that may be available. The factors and micro-habitats include:

Riffle and Runs. Current velocities facilitate kick-net sampling in these habitats but the sampler should also pick up individual, unembedded coarse substrates for close examination and hand picking. As mentioned previously, hard-to-dislodge taxa such as the caddisflies *Brachycentrus*, *Ceraclea*, *Glossosoma*, *Goera*, *Helicopsyche*, Hydropsychidae, *Hydroptila*, *Leucotrichia*, *Neophylax*, *Nyctiophylax*, *Oecetus*, Philopotamidae, *Polycentropus*, *Protoptila*, *Psilotreta*, and *Pycnopsyche*; the baetid mayfly *Acentrella* on the tops of rocks in fast current; heptageniid mayflies; perlid stoneflies; the lepidopteran *Petrophila*; limpet snails; various midges; bryozoan colonies; and sponge colonies may be found. Look for green or brown, leathery silk covered retreats on the tops and sides of rocks for *Petrophila*. On the sides of rocks, a brown silken retreat stretched across a crevice or ledge may reveal *Nyctiophylax* larvae. The crane fly genus *Antocha* and the caddisfly *Psychomyia flavida* often create visible long tubes of sand and silt on the tops of rocks in fast current. Blackfly larvae and pupal cases are often found on the tops of rocks in exposed current. As a rule, all areas of the rocks from riffles and runs should be closely examined.

When sponges and bryozoans are encountered, scrape off chunks of the colonies and add to the sample jar. However, the colonies should also be examined for associated insect larvae. Inspect sponge colonies for spongilla flies (a neuropteran), case building *Ceraclea* caddisflies (*also in bryozoan colonies*) and the red midge *Xenochironomus xenolabis*.

Bedrock riffles can be sampled with dip nets and should not be entirely ignored as these habitats often contain baetid mayflies. To avoid crushing the organisms, lightly brush the area in front of the net opening by hand, rather than kicking with your foot.

Pools. Pay special attention to pools and pool margins containing loose deposits of gravel and rubble. These substrates may be silty but, if un-embedded, they are often productive. As mentioned previously, an efficient sampling method for these areas is to disturb and churn the substrate with your foot, then work the net back and forth through the plume of lighter, suspended material.

Because of inefficiencies inherent in sampling deeper pool depths, field personnel should also pick up larger pieces of rubble, flagstone or woody debris for close examination and hand-picking. These substrates may include the heptageneiid mayflies *Stenacron* and *Stenonema femoratum*; polycentropid caddisflies; the case-building caddisfly genera *Ceraclea*, *Helicopsyche*, *Lepidostoma*, *Mystacides*, *Neophylax*, *Oecetis* and *Pycnopsyche*; perlid stoneflies; and water penny beetle larvae. Regarding polycentropids, *Nyctiophylax* larvae construct a silken roof over a depression in a piece of wood or a rock while *Polycentropus* inhabits a loose, ill-defined structure of silk and silt on the underside of rocks or woody debris. The large inflated nets of *Neureclipsis* have a cornucopia or French-horn shape easily visible in clear water. Both

Neureclipsis and *Polycentropus* nets deflate when removed from the water. Poke through the soft, silken nets with forceps to find the larvae.

Margin Habitat Sampling. Look for grassy edges and undercut banks with fibrous root wads and root mats that are pliant and will not puncture or snag the net. Also sample patches of aquatic macrophytes and emergent patches of "water willow" growing along bars and shallows. These stream edge habitats often yield large numbers of crustaceans, baetid mayflies, leptocerid caddisflies, beetles and odonates often missing from riffles and runs.

Soft Clay Margins. Pool margins next to exposed clay hard-pan banks or with soft deposits of mucky clay often produce burrowing mayflies. Special attention should be directed at these areas if encountered. If the water is clear, the paired openings of the mayfly burrowing tubes can sometimes be spotted before netting (see photo at right).

Shallows. Shallow margins and edges, particularly along gravel and rubble bars, are often ignored during sampling but may be highly productive. Beetles, corixids, caenid mayflies, baetid mayflies, and midges are often encountered in large numbers. In larger rivers, these are prime locations for discovering the sprawling mayfly genus *Anthopotamus*, particularly if the habitat includes some scattered, coarse substrates. In primary headwater habitat streams search the silty margins of pools for mayflies of the family Ephemerellidae and the caddisfly *Molanna*.

Woody debris. Look for larger, relatively stable and unembedded logs and pieces of woody debris, usually in pools or margins. Clean, stable pieces in slight current are most productive as they are not entirely covered with silt and muck. These substrates often house the case-building caddisflies *Pycnopsyche* and various leptocerid genera; the tube-making caddisfly *Lype diversa*; wood associated riffle beetles like *Macronychus*, *Ancyronyx* and *Helichus*; odonates; and the wood burrowing midges *Orthocladius (Symposiocladius) lignicola*, *Stenochironomus* sp., *Xestochironomus* sp. and *Xylotopus par*. Close examination may uncover the long surface tubes of finely chewed wood associated with the caddisfly genus *Lype*. To extract the larvae, slowly run your forceps through the tube and the larvae will eventually emerge from the opposite end.

Embedded coarse substrates. Avoid whenever possible. These substrates are rarely productive and are often stained black on the undersides as a result of anaerobic oxidation. However, the condition of the substrates is important information in regards to water quality evaluations and should be noted.

Unionids. A passive search for living or fresh-dead mussels should also be conducted during the site sampling process. Mussel research by G. T. Watters (Ohio State Univ., pers. comm.) found the presence of fresh-dead specimens is nearly as predictive of live populations as finding the

live specimens themselves. These are the only non-living organisms that are included in the site inventory. Pay close attention to shallows, gravel bars, and the floodplain immediately adjacent to the wetted channel. A muskrat midden is an ideal source of shells and, if found, should not be overlooked. A review of historical records and fore-knowledge of the potential presence of mussels at a given site is a valuable aid in the search process.

Signs of "fresh-dead" shells include decomposing flesh especially at muscle attachment points or a nacre that is shiny, unweathered and retains its color and luster. Fresher shells usually have an intact hinge but this does not, by itself, define a fresh-dead specimen. Note: Final decisions on whether or not a shell meets the fresh-dead criteria are often made upon return to the lab. Some shells look weathered and old in the field, but once they are cleaned and dried, they exhibit the above characteristics. For this reason, when questionable shells are encountered, they should be collected and returned to the office for final determination.

When live specimens are found, but associated dead specimens are not, digital photos can be substituted for identification to avoid killing the organism. Try to take photos from multiple angles, including umbo (beak) and side views. Include a ruler or other object of known size in the picture for scale. Return all live specimens to the stream in the area they were found.

In summary, a standard qualitative sampling regimen with a two-person crew would be conducted as follows:

1) Drive to and locate the precise stream sampling site using a finalized Study Plan and aided by a road atlas, USGS topographic map, aerial photos (Google, Bing maps, etc), GPS coordinates, etc.

2) Whenever possible, county and township roads and less traveled state routes, parks, or private properties are used for access. Avoid interstates, turnpikes, limited access highways, and narrow, busy highways that lack sufficient berms to pull off safely. Do not park on bridges or between the road and a guardrail. Pulling completely off the road or parking beyond and slightly inside the guardrail is ideal. Turn on blinkers and deploy traffic cones if close to traffic.

3) Label collection jars with a waterproof marking pen such as a magic marker or Sharpie and date the jar on-site prior to stream sampling. This eliminates problems with writing on a damp or alcohol-soaked label. Gear transported to the stream should include dip nets, sampling jar, white sorting pans, forceps, waterproof Ohio EPA Macroinvertebrate Field Sheets and pens, insect spray, GPS, wristwatch or timer, and digital camera.

4) When deciding on a sampling location, the most optimal, natural stream habitats, including riffles, runs and pools and a woody riparian buffer, should be selected. If the entire stream reach is modified or riparian buffers are absent, try to utilize the best habitat available, given the physical limitations. If the field person has a choice, choose

upstream from bridge crossings over downstream to avoid potential flow alteration, runoff and habitat disruption influences immediately downstream from highways. Highway departments often over-widen stream channels in the immediate vicinity of bridges to protect the infrastructure during high flows. Walk upstream (preferred) or well downstream from these areas whenever possible. Sampling under bridges is not prohibited, but these areas are generally avoided, due to the atypical habitat and the permanently shaded channel underneath.

5) Before sampling, carefully inspect dip nets and rinse pans to make certain no organisms have been transported from previous sampling sites.

6) Begin kick net sampling and note the start time. Initially, it is best to divide sampling duties between crew members – one starts with a riffle, while the other samples margins and undercut banks, then each proceeds to cover the run and pool habitats, etc. After each macrohabitat is fully assessed, fill out the population observations on the back of the Macroinvertebrate Field Sheet. Memorizing the observations and waiting until all sampling is completed can be difficult so it is best to keep a running account of the collection information as each habitat is addressed.

7) Since the collections are qualitative, it is not necessary to preserve or account for every organism observed. Avoid over collection of easily identifiable, mono-specific taxa (***e.g.,*** the hellgrammite *Corydalus cornutus*), or other taxa identified to a known, and field identifiable, taxonomic level [***e.g.,*** the mayfly genera *Isonychia*, *Caenis*, and *Tricorythodes*, oligochaetes (Order Oligochaeta), flatworms (Class Turbellaria), etc.]. In contrast, many more specimens of diverse taxonomic groups such as baetid and heptageniid mayflies, hydropsychid caddisflies and midges should be collected.

8) Once all the main habitats have been assessed, crew members can conduct additional kick-net sampling to familiarize themselves with other habitats, concentrate on picking individual pieces of rocks, logs and woody debris, search for mussels, or actively search for rarer taxa suspected of occurring at the site. Sampling is considered complete when all habitats and micro-niches (within a reasonable distance) have been assessed and, by gross visual observation, no new taxa are found. Note the beginning and ending sampling times on the field sheet. An absolute minimum of 30 total sampling minutes is spent at each site.

9) After sampling is completed, one collector should complete the field observations on the Macroinvertebrate Field Sheet while the other takes digital photos, GPS coordinates, and an inventory of sampling equipment to make certain no items are left behind. Be certain to thoroughly clean the dip nets after sampling to make sure no organisms are transferred to the next site.

10) Regarding the field notes under <u>Biological Characteristics</u> for each macrohabitat on the back of the field sheet:

These headings are relatively subjective but self-explanatory. "Predominant Organisms" is usually reserved for the most numerous populations or major taxonomic groups in each macrohabitat (about one to four taxa groups are usually listed). The descriptions used should be as specific as possible, given the limitations of field observations. For example, use the phrase hydropsychid caddisflies instead of "caddisflies", baetid mayflies instead of "mayflies", red midges instead of "midges", if the more detailed descriptors apply. Under the heading "Other Common Organisms", list other groups or taxa that are routinely encountered but not considered predominant. Rarer collections or specimens of particular interest can be noted under the habitat where they are collected or under "Other Notable Collections". It is not necessary to note every different organism found, as this will be accurately determined later, by lab identification. However, any notations that improve or aid in the collector's knowledge and assessment of the site are encouraged.

Density is a relative measure based on field observations and refers to numbers of organisms observed within each macrohabitat (low, moderate, high). Diversity is a similar, subjective observation related to how many different populations or types of different taxa are being found.

The "Comments" section should include a narrative summary of the collector's thoughts and impressions of the site, a description of any potential Causes or Sources of impairment if the site appears impaired, and an initial, narrative evaluation (ranging from *Very Poor* to *Exceptional* and obviously subject to change) based on the collections and the collector's field impressions.

Detailed supplement to macroinvertebrate Laboratory Methods – Quantitative Sampling procedures described in Vol. III, Subsection 1, Part C, Pages V-1-5 and V-1-6.

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 (± 25%), 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 (± 25%) 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 "E" 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:

Mike Bolton
Ohio EPA Groveport Field Office
4675 Homer Ohio Lane
Groveport Ohio, 43125
614-836-8781
Michael.Bolton@epa.ohio.gov

Supplement to macroinvertebrate Laboratory Methods and Data Analysis - Qualitative Sampling procedures described in Vol. III, Subsection 1, Part D, Page V-1-11.

Macroinvertebrate samples which do not have a valid ICI score should be assigned a narrative evaluation based on the qualitative sample. Use these narratives to rate the macroinvertebrate community condition: Exceptional (meets EWH expectations), Very Good (just below EWH expectations), Good (meets WWH/CWH expectations), Marginally Good (just below WWH/CWH expectations), Fair (does not meet WWH/CWH expectations but does meet MWH expectations), Low Fair (does not meet MWH expectations), Poor (meets LRW expectations), and Very Poor (does not meet LRW expectations). Qualitative sample narrative evaluations are assigned based on community attributes such as EPT (Ephemeroptera – mayfly, Plecoptera – stonefly, and Trichoptera – caddisfly) diversity and predominance, sensitive taxa (ST) diversity and predominance, and tolerant taxa predominance. Sensitive taxa are taxa with a tolerance category of intolerant (I) or moderately intolerant (MI) in the Ohio EPA database. The macroinvertebrate tolerance categories can also be found on the most recent edition of the Ohio EPA Macroinvertebrate Taxa List, which can be found on the Ohio EPA website at: http://epa.ohio.gov/dsw/bioassess/BioCriteriaProtAqLife.aspx . The EPT and sensitive taxa diversity expectations in Table 1 and Figures 1 and 2 below are provided as an aid in assigning narrative evaluations.

Table 1. EPT and sensitive taxa expectations for Ohio EPA qualitative samples, from Figures 1 and 2.

Parameter **Stream Size**[a]	**WWH/CWH**	**EWH**
Qualitative EPT		
Headwaters	Range: 9-11 (see Fig. 1)	Range: 13-17 (see Fig. 1)
Wadable	12	18
Small Rivers	12	18
Large Rivers	11	Range: 16-17 (see Fig. 1)
Qualitative Sensitive Taxa		
Headwaters	Range: 10-11 (see Fig. 2)	Range: 15-17 (see Fig. 2)
Wadable	Range: 12-13 (see Fig. 2)	Range: 18-20 (see Fig. 2)
Small Rivers	13	20
Large Rivers	Range: 11-13 (see Fig. 2)	Range: 17-20 (see Fig. 2)

a Stream Size is defined by drainage area (mi^2): Headwaters ~1 - <20, Wadable 20 - <200, Small Rivers 200 - <1000, Large Rivers ≥ 1000.

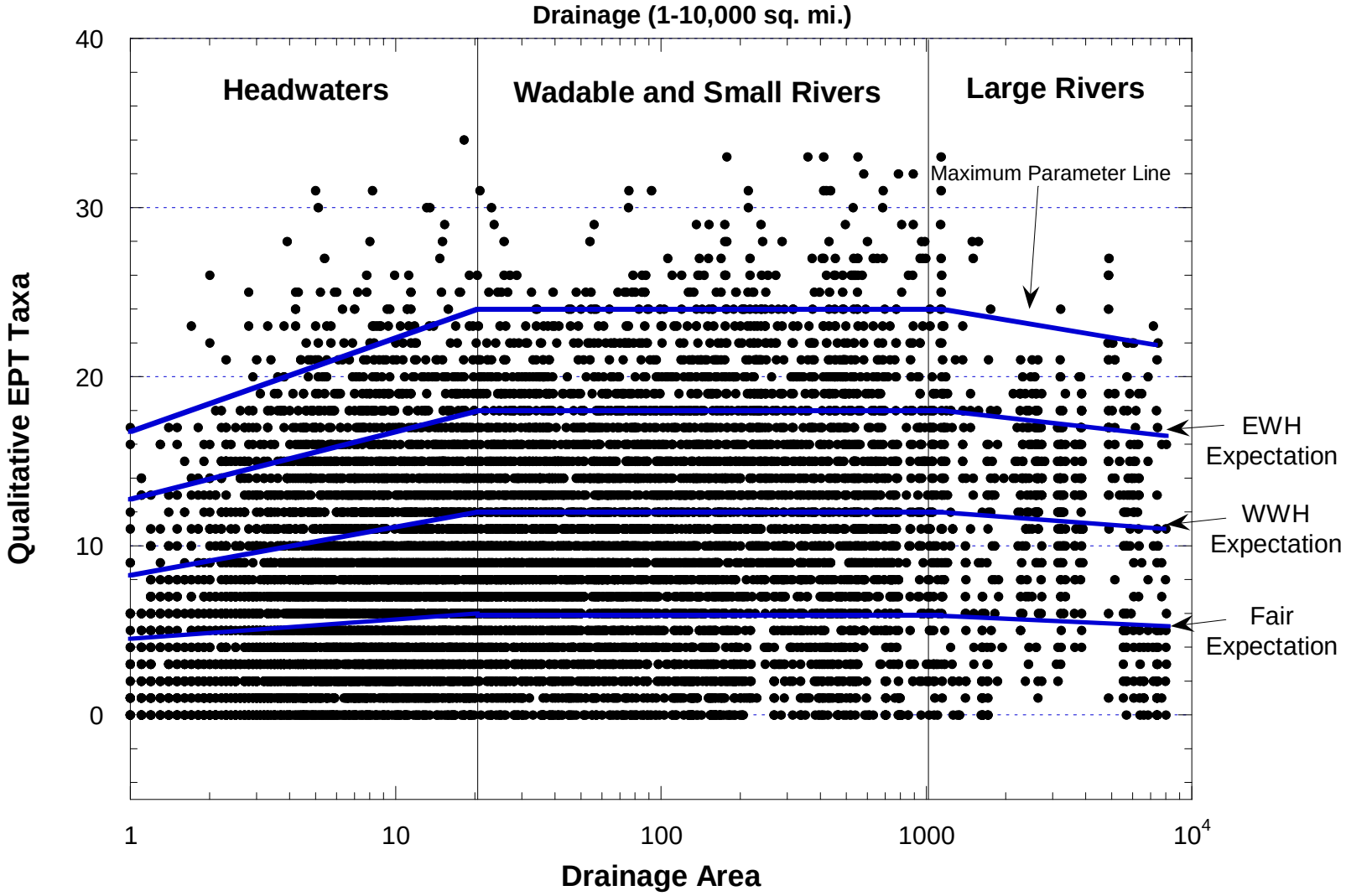

Figure 1. Plot of all Ohio EPA macroinvertebrate qualitative EPT data with the Maximum Parameter Line placed to include the contiguous data which is then quadrasected to estimate the EWH, WWH and Fair expectations (11,152 data points).

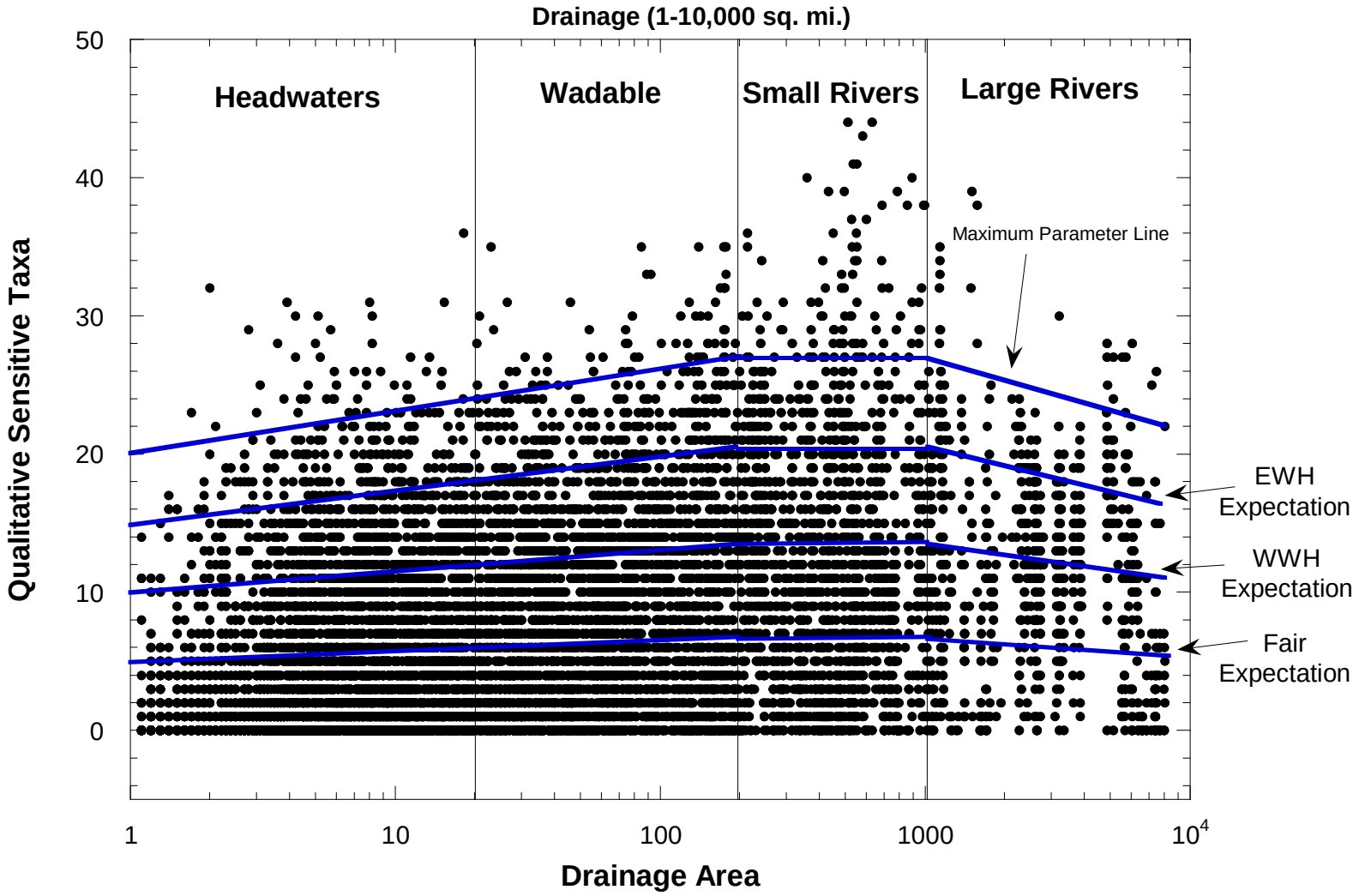

Figure 2. Plot of all Ohio EPA macroinvertebrate qualitative sensitive taxa data with the Maximum Parameter Line placed to include the contiguous data which is then quadrasected to estimate the EWH, WWH and Fair expectations (10,932 data points).

Volume III, pp. V-1-7 to V-1-9. Replaces Tables V-1-1, V-1-2 and all previous versions of this table 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. Genera that are reasonably considered to be monotypic in Ohio are also listed.

Taxon	Subtaxon	Taxonomic Level	Taxonomic Key(ies)
Porifera		Species	Pennak 1989
	If no gemmules are present identify to family (Spongillidae).		
Cnidaria		Genus	Smith 2001
	monotypic genera: *Cordylophora lacustris* and *Craspedacusta sowerbyi*		
Platyhelminthes		Class (Turbellaria)	Thorp & Covich 2010
Nemertea		Phylum (Nemertea)	Smith 2001
Nematomorpha		Phylum (Nematomorpha)	Smith 2001
	Paragordius sp.	Genus	Smith 2001
Ectoprocta		Genus	Thorp & Covich 2010
	monotypic genera: *Cristatella mucedo*, *Hyalinella punctata*, *Lophopodella carteri*, *Paludicella articulata*, *Pectinatella magnifica*, *Pottsiella erecta*		
Entoprocta		Species (*Urnatella gracilis*)	Thorp & Covich 2010
Annelida	Polychaeta	Species (*Manayunkia speciosa*)	Smith 2001
	Oligochaeta	Class (Oligochaeta)	Smith 2001
	Hirudinida	Species	Klemm 1982, Klemm et al. 2014
Crustacea	Anostraca	Species	Pennak 1989
	Conchostraca (Laevicaudata & Spinicaudata)	Species	Pennak 1989
	Isopoda	Genus	Smith 2001, Williams 1972
	Amphipoda	Genus	Thorp & Covich 2010, Smith 2001, Holsinger 1972
	Gammaridae: *Gammarus*	Species	Holsinger 1972
	monotypic genera: *Apocorophium lacustre*, *Echinogammarus ischnus*, *Hyalella azteca*, *Synurella dentata*		

Taxon	Subtaxon	Taxonomic Level	Taxonomic Key(ies)
Crustacea (continued)	Mysidacea	Species (*Tephromysis louisianae*)	Smith 2001
	Cambaridae	Species	Jezerinac & Thoma 1984, Jezerinac 1995, Jezerinac 1993, Taylor 2000, Thoma et al. 2005, Thoma & Stocker 2009, Crocker & Barr 1968
	Palaemonidae	Species	Thorp & Covich 2010
Arachnida	Hydrachnidia	Informal grouping of the water mites	Smith 2001
Ephemeroptera		Genus	Merritt et al. 2008, Mayfly Central 2014
	Baetidae: *Acerpenna*, *Diphetor*, *Baetis*, *Labiobaetis*	Species	Morihara & McCafferty 1979
	Baetidae: *Labiobaetis*	Species	McCafferty & Waltz 1995
	Baetidae: *Acentrella*, *Heterocloeon*, *Iswaeon*, *Plauditus*	Species	Ohio EPA 2013
	Baetidae: *Paracloeodes*	Species	Bolton 2011
	Baetidae: *Centroptilum*, *Procloeon*	Indicate if the taxa have hind wingpads or not.	
	Baetidae: *Procloeon viridoculare*	Species	Lowen & Flannagan 1992
	Heptageniidae: *Heptagenia*	Species	Burks 1953
	Heptageniidae: *Maccaffertium*, *Stenonema*	Species	Bednarik & McCafferty 1979
	Ephemerellidae: *Dannella simplex*	Species	Allen & Edmunds 1962
	Ephemerellidae: *Teloganopsis deficiens*	Species	Allen & Edmunds 1963
	Caenidae: Brachycercinae	Species	Sun & McCafferty 2008
	Baetiscidae: *Baetisca*	Species	Pescador & Berner 1981

Taxon	Subtaxon	Taxonomic Level	Taxonomic Key(ies)
Ephemeroptera (continued)	Ephemeroidea: *Ephemera*, *Hexagenia*, *Litobrancha*, *Ephoron*	Species	McCafferty 1975
	monotypic genera: *Cloeon dipterum*, *Diphetor hageni*, *Iswaeon anoka*, *Stenonema femoratum*, *Choroterpes basalis*, *Habrophlebia vibrans*, *Teloganopsis deficiens*, *Litobrancha recurvata*		
Odonata		Genus	Needham et al. 2000, Merritt et al. 2008
	Coenagrionidae (except *Argia*)	Family	Merritt et al. 2008
	Anisoptera: *Boyeria*, *Lanthus*, *Neurocordulia*	Species	Needham et al. 2000
	monotypic genera: *Archilestes grandis*, *Basiaeschna janata*, *Epiaeschna heros*, *Nasiaeschna pentacantha*, *Hagenius brevistylus*, *Progomphus obscurus*, *Stylogomphus albistylus*, *Didymops transversa*, *Epitheca (Epocordulia) princeps*, *Helocordulia uhleri*, *Erythemis simplicicollis*, *Pachydiplax longipennis*, *Perithemis tenera*, *Plathemis lydia*		
Plecoptera		Genus	Stewart & Stark 2002, Merritt et al. 2008
	Perlidae: *Acroneuria*, *Paragnetina*	Species	Hitchcock 1974
	Perlidae: *Agnetina*, *Perlinella*	Species	Poulton & Stewart 1991
	Perlodidae: *Isoperla*	Species	Hitchcock 1974, Frison 1942, Poulton & Stewart 1991
	Perlodidae: *Diploperla*	Species	Kondratieff et al. 1981
	Perlodidae: *Malirekus*	Species	Stewart & Stark 2002
	monotypic genera: *Nemoura trispinosa*, *Paraleuctra sara, Eccoptura xanthenes*, *Clioperla clio*, *Haploperla brevis*		
Hemiptera	Belostomatidae, Naucoridae, Nepidae, Pleidae, Notonectidae	Genus	Merritt et al. 2008
	Corixidae	Genus	Hilsenhoff 1995, Merritt et al. 2008
	monotypic genus: *Nepa apiculata*		
Megaloptera		Genus	Merritt et al. 2008
	Corydalidae: *Nigronia*	Species	Neunzig 1966
	monotypic genus: *Corydalus cornutus*		
Neuroptera		Genus	Merritt et al. 2008

Taxon	Subtaxon	Taxonomic Level	Taxonomic Key(ies)
Trichoptera		Genus	Wiggins 1996, Merritt et al. 2008
	Philopotamidae	Species	Ross 1944
	Hydropsychidae: *Diplectrona*	Species	Wiggins 1996
	Hydropsychidae: *Ceratopsyche*	Species	Schuster & Etnier 1978, Schefter & Wiggins 1986
	Hydropsychidae: *Hydropsyche*	Species	Schuster & Etnier 1978
	Hydropsychidae: *Macrostemum*	Species	Ross 1944
	Hydropsychidae: *Parapsyche*	Species	Flint 1961
	Rhyacophilidae: *Rhyacophila*	Species	Prather and Morse 2001
	Phryganeidae: *Oligostomis*	Species	Lloyd 1921
	Brachycentridae: *Brachycentrus*	Species	Flint 1984
	Odontoceridae: *Psilotreta*	Species	Parker & Wiggins 1987
	Leptoceridae: *Ceraclea*	Species	Resh 1976
	Leptoceridae: *Mystacides*	Species	Yamamoto & Wiggins 1964, Wiggins 1996
	Leptoceridae: *Nectopsyche*	Species	Glover & Floyd 2004
	Leptoceridae: *Oecetis*	Species	Floyd 1995
	Leptoceridae: *Triaenodes*	Species	Glover 1996
	monotypic genera: *Dolophilodes distinctus*, *Lype diversa*, *Psychomyia flavida*, *Cyrnellus fraternus*, *Potamyia flava*, *Leucotrichia pictipes*, *Mayatrichia ayama*, *Helicopsyche borealis*, *Leptocerus americanus*		
Lepidoptera	Crambidae	Genus	Merritt et al. 2008
Coleoptera	Gyrinidae, Haliplidae, Dytiscidae, Noteridae, Hydrophilidae, Psephenidae, Dryopidae, Elmidae, Ptilodactylidae, Lutrochidae	Genus	Merritt et al. 2008, Hilsenhoff 1995
	Dytiscidae: Hydroporini	Tribe (Hydroporini)	Merritt et al. 2008
	Scirtidae	Family	Merritt et al. 2008

Taxon	Subtaxon	Taxonomic Level	Taxonomic Key(ies)
Coleoptera (continued)	Elmidae: *Dubiraphia* (except *D. vittata* group)	Species (adults only)	Hilsenhoff 1973
	Elmidae: *Optioservus*	Species (adults only)	Brown 1972
	monotypic genera: *Agabetes acuductus*, *Helocombus bifidus*, *Sperchopsis tesselata*, *Dicranopselaphus variegata*, *Psephenus herricki*, *Ancyronyx variegata*, *Macronychus glabratus*, *Microcylloepus pusillus*, *Lutrochus laticeps*, *Anchytarsus bicolor*		
Diptera		Genus	Merritt et al. 2008, McAlpine et al. 1981
	Ceratopogonidae (except *Atrichopogon*, *Forcipomyia*), Dolichopodidae, Syrphidae (except *Eristalis*, *Chrysogaster*), Sciomyzidae, Ephydridae (except *Ephydra*, *Hydrellia*, *Ochthera*, *Setacera*)	Family	Merritt et al. 2008
	Tipulidae: *Tipula abdominalis*	Species	Gelhaus 1986
	Psychodidae: *Pericoma albitarsis*, *Telmatoscopus albipunctatus*	Species	Johannsen 1935
	Ceratopogonidae: *Atrichopogon*	Species	Johannsen 1935
	Chironomidae	Genus/Species[1]	Andersen et al. 2013, Bolton 2012, Epler 2001
	Chironomidae: *Eukiefferiella*, *Tvetenia*	Species group	Bode 1983
	Chironomidae: *Paracladopelma*	Species	Jackson 1977
	Muscidae: *Limnophora*	Species	Johannsen 1935
	monotypic genera: *Protoplasa fitchii*, *Bittacomorpha clavipes*, *Protothaumalea americana*, *Apsectrotanypus johnsoni*, *Brundiniella eumorpha*, *Cantopelopia gesta, Clinotanypus pinguis*, *Hayesomyia senata*, *Nilotanypus fimbriatus*, *Radotanypus florens*, *Telopelopia okoboji*, *Thienemannimyia norena*, *Trissopelopia ogemawi*, *Pagastia orthogonia*, *Prodiamesa olivacea*, *Diplocladius cultriger*, *Doncricotopus bicaudatus*, *Psilometriocnemus triannulatus*, *Xylotopus par*, *Endotribelos hesperium*, *Gillotia alboviridis*, *Hyporhygma quadripunctatum*, *Kribiodorum perpulchrum*, *Lauterborniella agrayloides*, *Paralauterborniella nigrohalteralis*, *Xenochironomus xenolabis*, *Zavreliella marmorata*, *Neostempellina reissi*, *Sublettea coffmani*, *Zavrelia aristata*, *Chlorotabanus crepuscularis*, *Atherix lantha*		

Taxon	Subtaxon	Taxonomic Level	Taxonomic Key(ies)
Mollusca	Gastropoda	Genus/Species[1]	Burch 1982
	Gastropoda: Hydrobiidae	Family	Burch 1982
	Bivalvia: Corbiculidae	Species (*Corbicula fluminea*)	Smith 2001, Burch 1972
	Bivalvia: Dreisseniidae	Species	Benson et al. 2014
	Bivalvia: Pisidiidae	Genus	Smith 2001, Burch 1972
	Bivalvia: Unionidae	Species	Watters et al. 2009

1 After the specimen is identified to genus check the most recent edition of the Ohio EPA Macroinvertebrate Taxa List (located on the Ohio EPA website at: http://epa.ohio.gov/dsw/bioassess/BioCriteriaProtAqLife.aspx) to see if it should be identified further.

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

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