Biological Criteria for the Protection of Aquatic Life:

Volume III. Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Macroinvertebrate Communities

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Division of Surface Water
Ecological Assessment Section

NOTICE TO USERS

The Ohio EPA incorporated biological criteria into the Ohio Water Quality Standards (WQS; Ohio Administrative Code 3745-1) regulations in February 1990 (effective May 1990). These criteria consist of numeric values for the Index of Biotic Integrity (IBI) and Modified Index of Well-Being (MIwb), both of which are based on fish assemblage data, and the Invertebrate Community Index (ICI), which is based on macroinvertebrate assemblage data. Criteria for each index are specified for each of Ohio's five ecoregions (as described by Omernik 1987), and are further organized by organism group, index, site type, and aquatic life use designation. These criteria, along with the existing chemical and whole effluent toxicity evaluation methods and criteria, figure prominently in the monitoring and assessment of Ohio's surface water resources.

Besides this document, the following documents support the use of biological criteria by outlining the rationale for using biological information, the methods by which the biocriteria were derived and calculated, and the process for evaluating results.

Ohio Environmental Protection Agency. 1987a. Biological criteria for the protection of aquatic life: Volume I. The role of biological data in water quality assessment. Div. Water Qual. Monit. & Assess., Surface Water Section, Columbus, Ohio.

Ohio Environmental Protection Agency. 1987b. Biological criteria for the protection of aquatic life: Volume II. Users manual for biological field assessment of Ohio surface waters. Div. Water Qual. Monit. & Assess., Surface Water Section, Columbus, Ohio.

Ohio Environmental Protection Agency. 1989. Addendum to Biological criteria for the protection of aquatic life: Volume II. User's manual for biological field assessment of Ohio surface waters. Division of Water Qual. Plan. & Assess., Ecol. Assess. Sect., Columbus, Ohio.

Ohio Environmental Protection Agency. 1990. The use of biological criteria in the Ohio EPA surface water monitoring and assessment program. Div. Water Qual. Plan. & Assess., Ecol. Assess. Sect., Columbus, Ohio.

Ohio Environmental Protection Agency. 2006. Methods for assessing habitat in flowing waters: Using the Qualitative Habitat Evaluation Index (QHEI). Ohio EPA Tech. Bull. EAS/2006-06-1. Div. of Surface Water, Ecol. Assess. Sect., Columbus, Ohio.

Ohio Environmental Protection Agency. 2014. 2014 updates to Biological Criteria for the Protection of Aquatic Life: Volume II and Volume II Addendum. Users manual for biological field assessment of Ohio surface waters. Div. of Surface Water, Ecol. Assess. Sect., Columbus, Ohio.

Omernik, J.M. 1987. Ecoregions of the conterminous United States. Ann. Assoc. Amer. Geogr. 77(1): 118-125.

Rankin, E.T. 1989. The qualitative habitat evaluation index (QHEI): rationale, methods, and application. Div. Water Qual. Plan. & Assess., Ecol. Assess. Sect., Columbus, Ohio.

In addition to the preceding guidance documents, the following publications by the Ohio EPA should also be consulted as they present supplemental information and analyses used by the Ohio EPA to implement the biological criteria.

DeShon, J.E. 1995. Development and application of the invertebrate community index (ICI), pp. 217-243. in W.S. Davis and T. Simon (eds.). Biological Assessment and Criteria: Tools for Risk-based Planning and Decision Making. Lewis Publishers, Boca Raton, FL.

Rankin, E. T. 1995. The use of habitat assessments in water resource management programs, pp. 181-208. in W. Davis and T. Simon (eds.). Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making. Lewis Publishers, Boca Raton, FL.

Yoder, C.O. and E.T. Rankin. 1995. Biological criteria program development and implementation in Ohio, pp. 109-144. in W. Davis and T. Simon (eds.). Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making. Lewis Publishers, Boca Raton, FL.

Yoder, C.O. and E.T. Rankin. 1995. Biological response signatures and the area of degradation value: new tools for interpreting multimetric data, pp. 263-286. in W. Davis and T. Simon (eds.). Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making. Lewis Publishers, Boca Raton, FL.

Yoder, C.O. 1995. Policy issues and management applications for biological criteria, pp. 327-344. in W. Davis and T. Simon (eds.). Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making. Lewis Publishers, Boca Raton, FL.

Yoder, C.O. and E.T. Rankin. 1995. The role of biological criteria in water quality monitoring, assessment, and regulation. Environmental Regulation in Ohio: How to Cope With the Regulatory Jungle. Inst. of Business Law, Santa Monica, CA. 54 pp.

Yoder, C.O. and M.A. Smith. 1999. Using fish assemblages in a State biological assessment and criteria program: essential concepts and considerations, pp. 17-63. in T. Simon (ed.). Assessing the Sustainability and Biological Integrity of Water Resources Using Fish Communities. CRC Press, Boca Raton, FL.

These documents are available from the following web site link or by contacting:

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http://epa.ohio.gov/dsw/bioassess/BioCriteriaProtAqLife.aspx

CONTENT ACKNOWLEDGEMENTS

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Subsection 1. Macroinvertebrates

Part A) Internal Field and Laboratory Training

Purpose: To ensure continuity of effort and technical proficiency among all Ohio EPA staff conducting macroinvertebrate surveys including the field collection and laboratory processing and identification of macroinvertebrate samples. Training emphasizes:

- taxonomic competency,
- proper placement and retrieval of Hester-Dendy artificial substrate samplers,
- proper technique and effort for qualitative sampling, and
- proper laboratory quantitative sample processing.

1) Full-Time Macroinvertebrate Biologists

- New macroinvertebrate biologists shall be mentored by a full-time staff member for at least their first field season in order to be trained in field techniques by an experienced employee.
- New macroinvertebrate biologists shall have their laboratory sample processing techniques and taxonomy monitored by experienced employees until they are deemed to be competent.
- All macroinvertebrate biologists shall be familiar with the field and laboratory methods found in this document and those for primary headwaters found at: http://epa.ohio.gov/portals/35/wqs/headwaters/PHWHManual_2012.pdf and http://epa.ohio.gov/portals/35/wqs/headwaters/TechRep_Macroinvert_2002.pdf .
- All macroinvertebrate biologists shall be evaluated for field and laboratory (including taxonomic) competency by the collection and processing of a quantitative and qualitative sample from the same site at the same time at least once every three years. All macroinvertebrate biologists shall be evaluated for taxonomic competency by identifying a taxonomic test sample every year that the quantitative sample is not collected. The cycle will reset with the full quantitative evaluation after a new employee has completed one year of training.

2) Part-Time Macroinvertebrate Collectors

- Any Ohio EPA employee who will be collecting macroinvertebrate samples is responsible for acquiring the necessary training and expertise to collect an adequate sample. One way to accomplish this would be to contact the full-time macroinvertebrate biologists in EAS to request field and laboratory training.
- All macroinvertebrate collectors should be familiar with the field methods found in this document and those for primary headwaters found at: http://epa.ohio.gov/portals/35/wqs/headwaters/PHWHManual_2012.pdf and http://epa.ohio.gov/portals/35/wqs/headwaters/TechRep_Macroinvert_2002.pdf .
- All Ohio EPA macroinvertebrate collectors who would like their samples to be considered as valid collections shall be required to pass a field technique and taxonomic competency test by adequately collecting a field sample using Ohio

EPA's standard methods and passing a family level taxonomy test. Procedures to assess the employee's competencies would be similar to those utilized to assess external practitioners in the Credible Data Program for the Level 3 macroinvertebrate collection and assessment only discipline.

Part B) Field Methods – Quantitative Sampling

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 substituted 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 (Figure 1). 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 that is colonized in-stream for at least a six-week period with the retrieval no earlier than June 15 and no later than September 30. A delay in the retrieval of the artificial substrates for a week or two is acceptable if weather or flow conditions preclude a timely pick-up. Samplers placed in streams and rivers are tied to a depth appropriate concrete construction block (4" height for shallow streams or 8" height for deeper rivers) which anchors them in place and prevents the multiple-plates from coming into contact with the natural substrates (Figure 2). In water deeper than four feet, floatation and anchoring devices are often used to establish the samplers just off the bottom and suspended above the anchor. Whenever possible, the samplers are placed in runs rather than pools or riffles and, in all cases, an attempt is made to establish stations in as similar an ecological situation as possible. At the initial placement of the artificial substrate samplers, detailed drawings and field notes are made of the steam or river reach and the exact location of the samplers within the reach. This facilitates the finding of the artificial substrates at retrieval after six weeks. Measurements of water depth and current velocity at the sampler location are made as well as an observation of the degree of canopy cover.

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

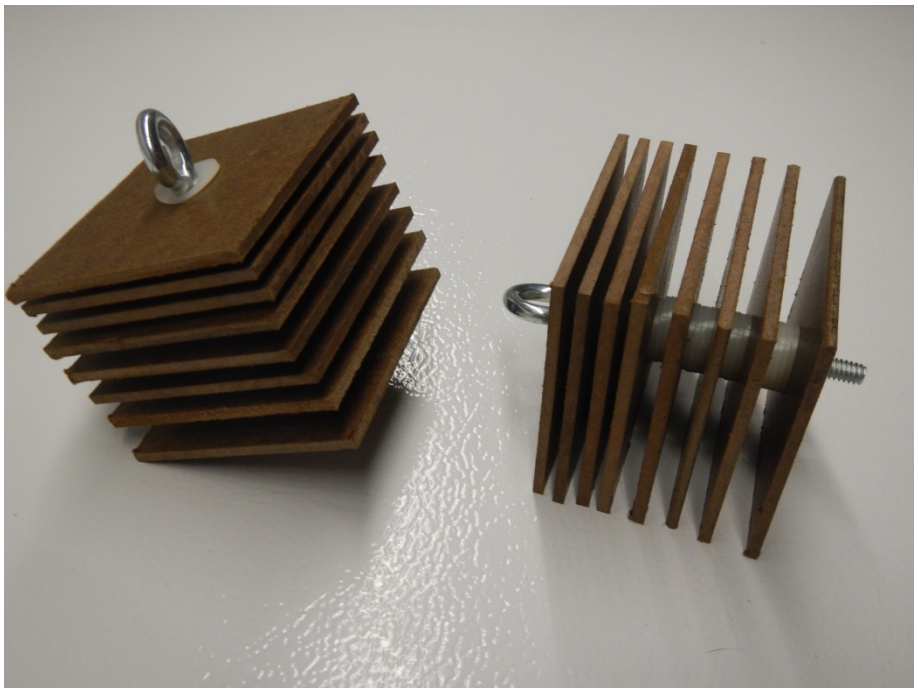

Figure 1 Modified Hester-Dendy multiple-plate artificial substrate sampler used by the Ohio EPA for the collection of a quantitative macroinvertebrate sample.

Figure 2 Configuration of artificial substrate samplers on cement block anchors used by the Ohio EPA to collect quantitative macroinvertebrate collections from small streams (left) and large rivers (right).

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. As a general rule, quantitative sampling is conducted at sites with greater than 20 mi^2 drainage areas. At these locations, current velocities and stream depth are almost always adequate for artificial substrate placement. Quantitative samples can be collected in smaller drainage areas where the flow and water depth are sufficient if the data quality objectives indicate the need.

After the six-week colonization period, retrieval of the samplers is accomplished by cutting them from the block and placing them in one quart plastic containers while still submersed. Care is taken to avoid disturbing the samplers and thereby dislodging any organisms. Enough 37% formaldehyde is added to each container to equal an approximate 10% formalin solution. The plastic containers are stored in coolers and transported back to the home laboratory for subsequent processing (see Part C). In conjunction with the qualitative sampling that will be completed (see next section), a detailed site description sheet is begun for the site (Figures 3a and 3b) and information related to the artificial substrate set and retrieval is noted.

Part C) Field Methods – Qualitative Sampling

The Ohio EPA collects qualitative, natural substrate samples at every macroinvertebrate sampling site, either alone or in conjunction with quantitative (artificial substrate) collections. For routine monitoring and assessments, qualitative sampling alone is conducted at most smaller drainage sites (i.e., sites with drainage areas <20 mi^2).

Ohio EPA's primary sampling period for conducting qualitative sampling is during the summer months from June 15th through September 30th. Since visual inspection of macrohabitats and bottom substrates is so important when collecting the qualitative sample, avoid sampling during high water or when the stream is brown and turbid from recent rains. Ideally, sampling is conducted when the water column is relatively clear, and 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

Stream _____ Station ID _____ RM _____ Date Collected _____

Location _____ Date Set _____

Lat./Long. _____ Wpt# _____ Photo # _____ Collected By _____

Sampling Method: HD(No._____) - DN/HP - Surber - Grab (Type_____) - Other_____

HD Sampler Site: Depth (Set) _____ Depth (Ret) _____ Canopy _____ Current (Set) _____ Current(Ret)_____

HD Condition: Disturbed Yes/No Comment:_____

Debris Yes/No Comment:_____

Silt/Solids None - Slight - Moderate - Heavy

DN/HP Sampling: Start _____ End _____ Habitats: Pool - Riffle - Run - Margin - Backwater

Physical Characteristics

Flow Condition:	Flood - Above Normal - Normal - Low - Interstitial - Intermittent - Dry					
Current Velocity:	Fast	-	Moderate	-	Slow -	ND
Channel Morphology:	Natural - Channelized - Channelized (Recovered) - Impounded					
Bank Erosion:	Extensive	-	Moderate	-	Slight -	None
Riffle Development:	Extensive	-	Moderate	-	Sparse -	Absent
Riffle Quality:	Good	-	Fair -	Poor	Embedded:	Yes/No
Clarity:	Clear	-	Murky -	Turbid	Temp:_____	
Color:	None	-	Green -	Brown -	Grey -	Other (_____)
Canopy:	Open	-	75% -	50% -	25% -	Closed

Substrate Characteristics

	Pool	Riffle	Run
Bedrock( )			
Boulder( )			
Rubble( )			
Coarse Gravel			
Fine Gravel			
Sand			
Silt			
Clay/Hardpan			
Detritus			
Peat			
Muck			
Other( )			
Macrophytes			
Algae( )			
Artifacts( )			
Compaction(F,M,S)			
Depth(Average)			
Width(Average)			

Predominant Land Use (L,R,B)

Forest	Open Pasture	Wetland
Shrub	Closed Pasture	Other
Old Field	Urban	( )
Rowcrop	Residential/Park	
Industrial	Mining/Construction	

Predominant Riparian Vegetation Width

Left	Right	Type
		Large Trees
		Small Trees
		Shrubs
		Grass/Weeds
		None

Margin Habitat

Undercut Banks	Root Mats
Grass	Water Willow
Shallows	Clay/Hardpan
Rip Rap	Bulkhead
Other( )	

Margin Quality: Good - Fair - Poor

Figure 3a Ohio EPA/DSW Ecological Assessment Section macroinvertebrate site description sheet (front).

Biological Characteristics

% Riffle _____

Predominant Organisms: _____

Other Common Organisms: _____

Density: High - Moderate - Low

Diversity: High - Moderate - Low

% Run _____

Predominant Organisms: _____

Other Common Organisms: _____

Density: High - Moderate - Low

Diversity: High - Moderate - Low

% Pool _____

Predominant Organisms: _____

Other Common Organisms: _____

Density: High - Moderate - Low

Diversity: High - Moderate - Low

% Margin _____

Predominant Organisms: _____

Other Common Organisms: _____

Density: High - Moderate - Low

Diversity: High - Moderate - Low

Other Notable Collections: _____

Comments: _____

_____

_____

_____

_____

Evidence of Pollution: _____

Potential Pollution Sources: _____

Predominant Taxa	**Overall Density**	**Field Evaluation**

Figure 3b Ohio EPA/DSW Ecological Assessment Section macroinvertebrate site description sheet (back).

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 (*e.g.,* 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 mi^2 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. In addition to using dip nets, macrohabitats are also always sampled by visually inspecting individual pieces of coarse substrates and woody debris that may be present. Net sampling techniques vary by macrohabitat but are as follows.

1) Net Sampling Techniques by Macrohabitat Type

Riffles

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.

Runs

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.

Pools

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.

Margins

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),
- rip-rap,
- shallow, silty edges along bars and pools,
- eroded banks with coarse substrate deposits along the toe, and
- 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.

2) 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 three insect orders are especially important in assessing water quality conditions. Ultimately, in the absence of an ICI score, 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 data base. 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 tolerance assignments are based on Ohio EPA methods and collections from Ohio streams, a particular taxon's tolerance assignment may not be directly applicable to other states, although assignments do generally concur with those found in recent literature.

Field notes describing the predominant and most common populations from each macrohabitat are recorded on the field sheet (Figure 3b). Sampling is conducted for a minimum of 30 minutes and continues until, within a reasonable amount of time, no new

taxa are being observed or collected. 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 diversity, special attention should be directed at specific micro-niches that may be available. Some specific pointers for sampling these unique areas in the major macrohabitats are as follows.

Riffles 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 may be discovered such as the caddisfly taxa *Brachycentrus*, *Ceraclea*, *Glossosoma*, *Goera*, *Helicopsyche*, Hydropsychidae, *Hydroptila*, *Leucotrichia*, *Neophylax*, *Nyctiophylax*, *Oecetus*, Philopotamidae, *Polycentropus*, *Protoptila*, *Psilotreta*, and *Pycnopsyche*; the baetid mayfly genus *Acentrella*; heptageniid mayflies; perlid stoneflies; the lepidopteran *Petrophila*; limpet snails; various midges; bryozoan colonies; and sponge colonies. Look for green or brown, leathery silk covered retreats on the tops and sides of rocks for *Petrophila*. The genus *Acentrella* can often be found on the tops of rocks in fast current. 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 caddisflies of the genus *Ceraclea* (*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 not 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 mayfly taxa *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 genera of the caddisfly family Polycentropodidae, genus *Nyctiophylax* larvae construct a silken roof over a depression in a piece of wood or a rock while genus *Polycentropus larvae* inhabit loose, ill-defined structures of silk and silt on the underside of rocks or woody debris. The large inflated nets of the genus *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.

Margins

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, damselflies, and dragonflies often missing from riffles and runs.

Other Habitat Types

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 genus *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*; damselflies and dragonflies; 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.

3) Special Note on Freshwater Mussel Sampling (Family Unionidae)

To collect and possess freshwater mussel shells in Ohio, collectors are required to apply for and receive valid Scientific Collector's Permits from the Ohio Department of Natural Resources and the U.S. Fish and Wildlife Service. At no time are living mussels permitted to be harvested or collected, and, if found, should be disturbed as little as possible.

A passive search for living or "fresh-dead" mussels should also be conducted during the qualitative 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 prior 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 laboratory. 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 laboratory for final determination.

When live specimens are found, but associated dead specimens are not, digital photos can be substituted for identification and documentation. Photos should be taken from multiple angles, including umbo (beak) and side views. Include a ruler or other object of known size in the picture for scale. Handle the live mussels carefully and return specimens to the stream in the area and habitat where they were found.

Part D) Laboratory Methods – Artificial Substrate Sample

Sampling information for each site is immediately entered into the Ecological Analysis and Assessment Application (EA^3) when samples are returned to the laboratory. A macroinvertebrate sheet number is generated and that number is marked on sample containers and jars and is carried forward in all subsequent steps in the processing and laboratory analysis of the sample. Metadata entries needed to generate the EA3 macroinvertebrate sheet number include the sample type (*i.e.*, qualitative sample, qualitative/quantitative sample, or qualitative/quantitative w/replicate sample), station ID number, site river mile, collector, and collection date.

1) Initial Processing

Individual multiple-plate sampler containers are initially flushed with clean water while being held over stacked U.S. Standard Testing Sieves number 30 (0.589 mm openings) and number 40 (0.425 mm openings) in order to remove as much of the formalin preservative as possible. Care is taken to hold the artificial substrate sampler within the container while it is being flushed and drained over the set of sieves. After flushing, the individual artificial substrate is removed from the container and placed in a full bucket of clean water; the container undergoes a final rinse and any organisms and debris are drained into the sieves. This process is repeated for all five substrates. The five substrates are then disassembled in the bucket of water, cleaned of organisms and debris, and discarded. During this process, visual inspections of each plate are made and, if observed, sections of bryozoan colonies are removed from the plates and saved for identification; only colonies, not individuals, are counted. Once all the plates have been cleaned and discarded, the organism/debris mixture is poured through the set of sieves. The collected organism/debris material is then thoroughly rinsed with clean water to remove as much silt and other fines as possible. As a final step, the organisms and material collected from each sieve are flushed with 70% alcohol preservative into two jars; each jar is topped off with 70% alcohol preservative and capped tightly. The

Ohio EPA uses a larger four-ounce glass jar for the coarser #30 screen material and a smaller eight-dram glass jar for the #40 screen material. Along with the qualitative sample jar from the site, the three jars should be bound together with a rubber band. The #30 and #40 screen jars should be labeled with site location information including the macroinvertebrate sheet number so they can be matched together if separated.

2) Sorting, Counting, and Organism Identifications

#30 Screen Sample – Sorting and Counting

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.

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.

- Midges — Approximately 100 larvae (+ 25%), cleared, mounted and identified. (Note: no midges are removed during the pre-pick).
- Mayflies — Approximately 75 (within diverse families such as Heptageniidae or Baetidae).
- 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. 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 is 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.

Midges are cleared in a 10% KOH solution and "wet-mounted" 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 drawbacks 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 – Sorting and Counting

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 for the #30 screen sample. 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:

- *Corynoneura* spp. (antenna as long or longer than head capsule),
- *Thienemanniella* spp. (antenna about ½ head capsule length, A2 may be dark),
- *Nilotanypus fimbriatus* (elongate head capsule),
- *Labrundinia* spp. (elongate head capsule, body preserves in a sigma [∑] shape),
- *Stempellina* spp. (curved transportable sand case), and
- *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. 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).

Organism Identifications

Any taxonomic key in the laboratory may be used as an aid in the identification of an organism. However, the final identification and name used are taken from the references in Table 1. Also indicated in this table is the level of taxonomy attainable with the keys listed. Some specific details regarding organism identifications are as follows.

- Species level identifications are made where possible and practical. Generic or higher level classifications are made if specimens are damaged beyond identification, in those cases where taxonomy is incomplete or laborious and time-consuming, or where the specimen is an unidentifiable early instar.
- 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.
- Nematodes; microcrustaceans of the order Cladocera (water fleas), class Ostracoda (seed shrimps), and class Copepoda (copepods); the order Collembola (springtails); and certain semi-aquatic insect families (i.e., Gerridae [water striders], Hydrometridae [marsh treaders], Gelastocoridae [toad bugs], Lampyridae [lightning bugs], and adult Psephenidae (water pennies]) are not included in counts and identifications.
- Organisms determined to be dead before the time of collection are discarded.
- When only one sex or life stage can be identified, it is assumed that the other sex or stage is the same taxon.
- Early instars that cannot be identified are extrapolated where possible.

Table 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(s)
Porifera		Species	Thorp & Covich 2010
	If no gemmules are present, identify to family (Spongillidae).		
Cnidaria		Genus	Smith 2001
	monotypic genera: *Cordylophora caspia* 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

Taxon	Subtaxon	Taxonomic Level	Taxonomic Key(s)
Annelida	Polychaeta	Species (*Manayunkia speciosa*)	Smith 2001
	Oligochaeta	Class (Oligochaeta)	Smith 2001
	Hirudinida	Species	Klemm 1982, Klemm et al. 2015
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*		
	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 2015
	Baetidae: *Acerpenna*, *Diphetor*, *Baetis*	Species	Morihara & McCafferty 1979
	Baetidae: *Acentrella*, *Heterocloeon*, *Iswaeon*, *Plauditus*	Subgenus/Species	Ohio EPA 2015
	Baetidae: *Anafroptilum*, *Neocloeon*	Genus	Jacobus & Wiersema 2014

Taxon	Subtaxon	Taxonomic Level	Taxonomic Key(s)
Ephemeroptera (continued)	Baetidae: *Labiobaetis*	Species	Morihara & McCafferty 1979, McCafferty & Waltz 1995
	Baetidae: *Paracloeodes*	Species	Bolton 2011
	Baetidae: *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
	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. 2014, Merritt et al. 2008
	Coenagrionidae (except *Argia*)	Family	Merritt et al. 2008
	Anisoptera: *Boyeria*, *Lanthus*, *Neurocordulia*	Species	Needham et al. 2014
	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

Taxon	Subtaxon	Taxonomic Level	Taxonomic Key(s)
Plecoptera (continued)	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, Rasmussen & Pescador 2002
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

Taxon	Subtaxon	Taxonomic Level	Taxonomic Key(s)
Trichoptera (continued)	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
	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

Taxon	Subtaxon	Taxonomic Level	Taxonomic Key(s)
Diptera (continued)	Psychodidae: *Pericoma albitarsis*, *Telmatoscopus albipunctatus*	Species	Johannsen 1934-1937
	Ceratopogonidae: *Atrichopogon*	Species	Johannsen 1934-1937
	Chironomidae	Genus/Species[1]	Andersen et al. 2013, Bolton 2012, Epler 2001, Epler 2014
	Chironomidae: *Eukiefferiella*, *Tvetenia*	Species group	Bode 1983
	Chironomidae: *Paracladopelma*	Species	Jackson 1977
	Muscidae: *Limnophora*	Species	Johannsen 1934-1937
	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*		
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 web site at: http://epa.ohio.gov/dsw/bioassess/BioCriteriaProtAqLife.aspx) to see if it should be identified further.

Part E) Macroinvertebrate Data Analysis

1) Quantitative Sampling: Invertebrate Community Index (ICI) Assessment

The principle measure of overall macroinvertebrate community condition used by the Ohio EPA is the Invertebrate Community Index (ICI), a measurement derived in-house using data collected over several decades. The ICI is a modification of the Index of Biotic Integrity (IBI) for fish developed by Karr (1981). The ICI consists of ten structural community metrics, each with four scoring categories of 6, 4, 2 and 0 points (Table 2). The point system evaluates a sample against a data base of relatively undisturbed ecoregional reference sites throughout Ohio which are used to delineate the scoring ranges. Six points are scored if a given metric has a value comparable to those of exceptional stream communities, 4 points for those metric values characteristic of more typical good communities, 2 points for metric values slightly deviating from the expected range of good values, and 0 points for metric values strongly deviating from the expected range of good values. Metrics 1-9 are all generated from the artificial substrate sample data while Metric 10 is based solely on the qualitative sample data. The summation of the individual metric scores (determined by the relevant attributes of an invertebrate sample with consideration given to sampling site drainage area) results in the ICI score which ranges from 0 (very poor community condition) to 60 (exceptional community condition). Narrative quality ranges of the ICI scaled to Level 3 ecoregion in Ohio are provided in Table 3. More discussion of the derivation and use of the ICI including descriptions of each metric, the data plots used to score each metric, and other information used to assess the ICI score and sampling site can be found in Ohio EPA (1987b and 1989) and DeShon (1995).

Table 2 Invertebrate Community Index (ICI) metrics and scoring criteria derived from macroinvertebrate community data collected from ecoregional reference sites in Ohio.

Metric	Scoring Criteria 0 2 4 6
1. Total Number of Taxa 2. Total Number of Mayfly Taxa 3. Total Number of Caddisfly Taxa 4. Total Number of Dipteran Taxa 5. Percent Mayflies 6. Percent Caddisflies 7. Percent Tribe Tanytarsini Midges 8. Percent Other Dipterans and Non-Insects 9. Percent Tolerant Organisms 10. Total Number of Qualitative Ephemeroptera, Plecoptera, And Trichoptera (EPT) Taxa	Scoring of each metric varies with site drainage area; see Ohio EPA (1989) or DeShon (1995) for scoring plots.

Table 3 Narrative quality ranges of the Invertebrate Community Index (ICI) scaled to Level 3 ecoregions[2] in Ohio.

Narrative	Invertebrate Community Index (ICI) Range				
	Huron/Erie Lake Plains HELP (1)	Interior Plateau IP (2)	Erie/Ontario Lake Plains EOLP (3)	Western Allegheny Plateau WAP (4)	Eastern Corn Belt Plains ECBP (5)
Exceptional	46 - 60				
Very good	42 - 44				
Good	34 - 40	30 - 40	34 - 40	36 - 40	36 - 40
Marginally Good	30 - 32	26 - 28	30 - 32	32 - 34	32 - 34
Fair	22 - 28	22 - 24	22 - 28	22 - 30	22 - 30
Low Fair	14 - 20				
Poor	8 - 12				
Very Poor	0 - 6				

2) Qualitative Sampling: Narrative Assessment

Macroinvertebrate samples which were collected only with qualitative procedures or for which a valid ICI score is not available are assigned a narrative evaluation based on the qualitative sample. The following narratives are used to rate the macroinvertebrate community condition in relation to the various designated aquatic life beneficial uses codified in the Ohio Water Quality Standards:

- Exceptional (meets Exceptional Warmwater Habitat [EWH] expectations),
- Very Good (just below EWH expectations),
- Good (meets Warmwater Habitat [WWH] or Coldwater Habitat [CWH] expectations),
- Marginally Good (just below WWH or CWH but still meets expectations),
- Fair (does not meet WWH or CWH expectations but does meet Modified Warmwater Habitat [MWH] expectations),
- Low Fair (does not meet MWH expectations),
- Poor (meets Limited Resource Water [LRW] expectations), and
- Very Poor (does not meet LRW expectations).

Qualitative sample narrative evaluations are assigned based on community attributes including, but not limited to, EPT (Ephemeroptera-mayfly, Plecoptera-stonefly, and Trichoptera-caddisfly) diversity and predominance, sensitive taxa (ST) diversity and predominance, tolerant taxa predominance, and observations of taxa diversity and quality in the various macrohabitats present at the sampling site. Sensitive taxa are taxa with a tolerance category of intolerant (I) or moderately intolerant (MI) in the Ohio EPA data base while tolerant taxa are designated as moderately tolerant (MT), tolerant (T), or very tolerant (VT). The macroinvertebrate tolerance designation for most taxa is

[2] from Omernik (1987)

included with the most recent version of the Ohio EPA macroinvertebrate taxa list which can be found on the Ohio EPA web site at:
http://epa.ohio.gov/dsw/bioassess/BioCriteriaProtAqLife.aspx.
The EPT and sensitive taxa diversity expectations in Table 4 and Figures 4 and 5 are provided as an aid in assigning narrative evaluations.

Table 4 EPT and sensitive taxa expectations for qualitative samples collected using Ohio EPA sampling procedures (also see Figures 4 and 5).

Parameter Stream Size[3]	WWH/CWH	EWH
Qualitative EPT		
Headwater	Range: 9-11 (see Fig. 4)	Range: 13-17 (see Fig. 4)
Wading	12	18
Small Rivers	12	18
Large Rivers	11	Range: 16-17 (see Fig. 4)
Qualitative Sensitive Taxa		
Headwater	Range: 10-11 (see Fig. 5)	Range: 15-17 (see Fig. 5)
Wading	Range: 12-13 (see Fig. 5)	Range: 18-20 (see Fig. 5)
Small Rivers	13	20
Large Rivers	Range: 11-13 (see Fig. 5)	Range: 17-20 (see Fig. 5)

[3] Stream size is defined by drainage area (mi^2): Headwaters ~1 - <20, Wading 20 - <200, Small Rivers 200 - <1000, and Large Rivers ≥ 1000.

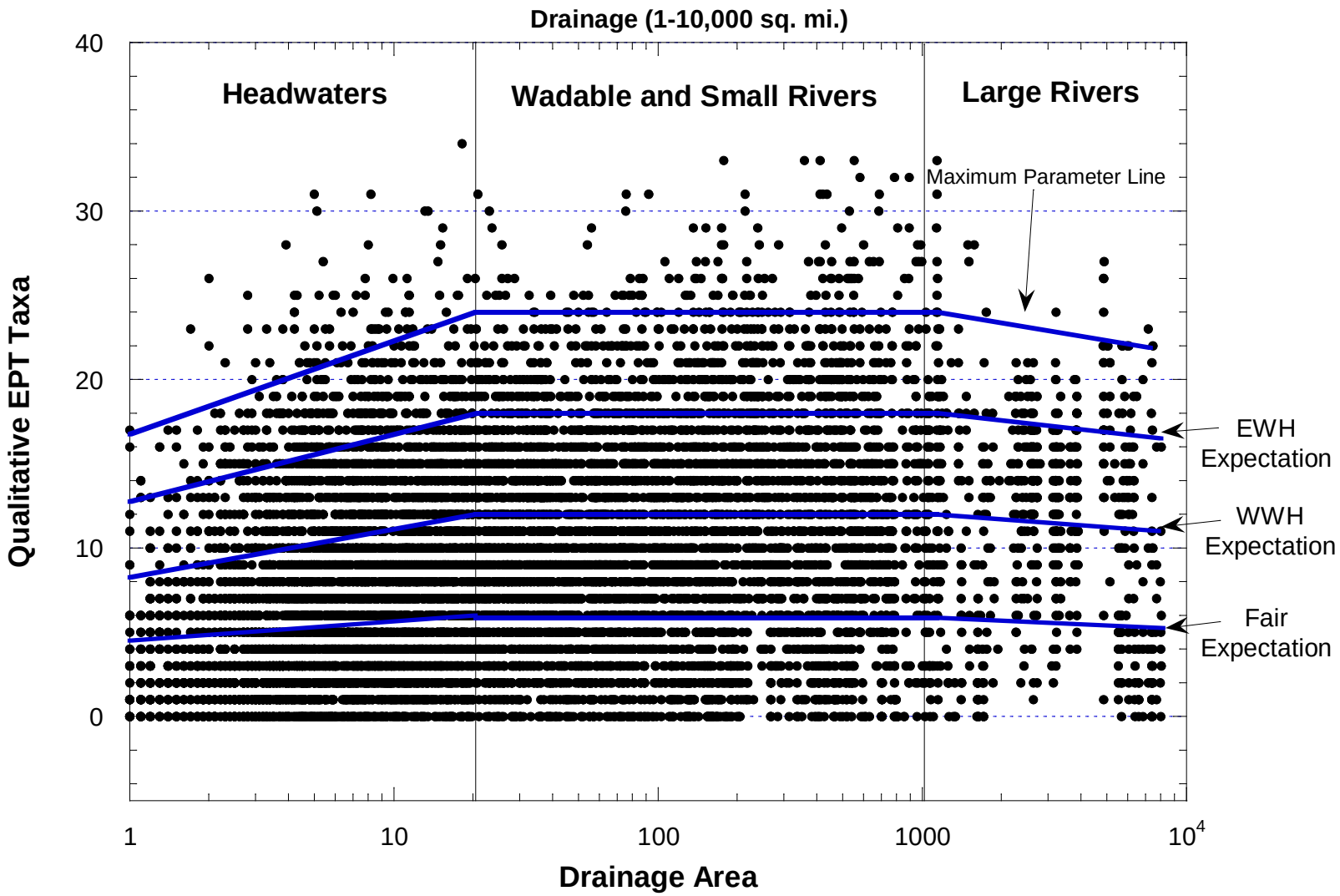

Figure 4 Plot of all Ohio EPA macroinvertebrate qualitative EPT data with the Maximum Parameter Line placed to include the contiguous data which is then quadrisected to estimate the EWH, WWH, and Fair expectations (11,152 data points)

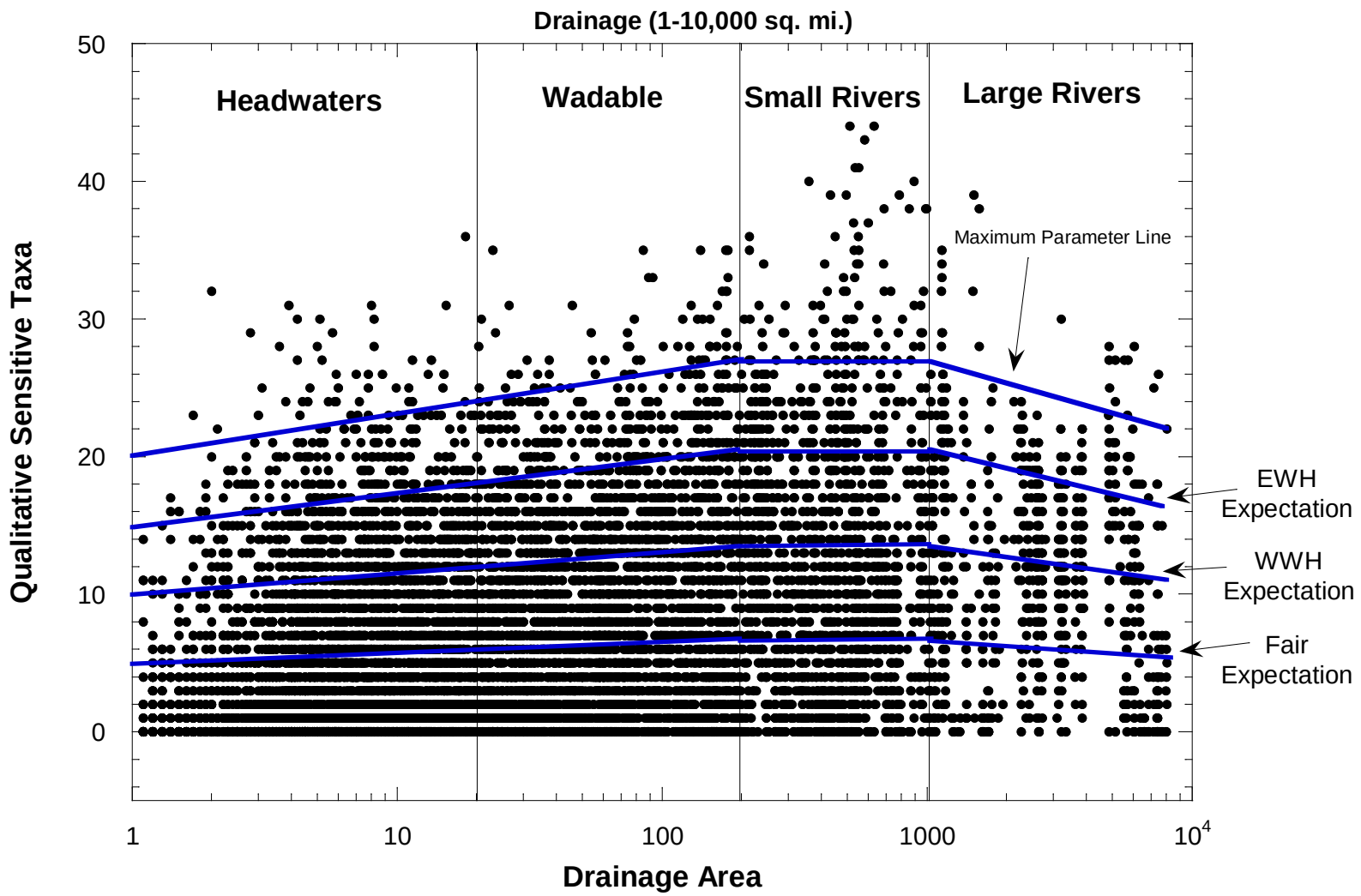

Figure 5 Plot of all Ohio EPA macroinvertebrate qualitative sensitive taxa data with the Maximum Parameter Line placed to include the contiguous data which is then quadrisected to estimate the EWH, EEH, and Fair expectations(10,932 data points).

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Subsection 2. Fish

Part A) Training

To ensure continuity of effort and technical proficiency among all staff performing electrofishing surveys, the following items are minimal skills expected of all fish crew leaders:

- Field appraisal of stream conditions (flow and turbidity)
- Determination of a sampling location characteristic of a broader reach
- Qualitative Habitat Evaluation Index (QHEI)
- Selection of appropriate gear for site
- Use of personal protective equipment (PPE) and communication/clarification of electrofishing hazards. Knowledge of and compliance with EAS field safety protocols.
- Taxonomic competency
- Processing the catch (data sheets, standardized terms, weights, counts, and subsampling)
- Effective sampling techniques and adherence to protocols for various gear types
- Critical importance of field observations

Prior to Leading a Fish Crew

1. It is expected that anyone who will lead a fish crew have a full understanding of biocriteria, principals of electrofishing, Ohio EPA sampling techniques and associated safety protocols.
2. Attendance at biocriteria training is strongly encouraged. At a minimum, prospective district fish crew leaders will review the PowerPoint presentations incorporated into the biocriteria training available from EAS.
3. Attendance at QHEI training is required.
4. Fish ID Test – District and new EAS fish crew leaders will arrange with EAS management to take the fish test prior to field season. The laboratory practical will include the following:
    a. Twenty-five fish are to be identified to species level with the aid of a relevant taxonomic key(s), an academic treatment(s) of fishes of the mid-west [e.g., Fishes of Ohio, (Trautman 1981)], and appropriate laboratory facilities (stereoscope, light source, dividers, fine forceps, fine probe, etc.) over a 1.5 hour period.
    b. Misidentification of more than 25% will necessitate reevaluation at a later date.

Initial Training for new EAS Fish Crew Leaders

Field sampling

a. All new full-time field personnel in the Fish Evaluation Group of EAS receive in-house training in electrofishing, proper PPE and safety protocols, and biocriteria prior to the start of the field season. A senior staff member also accompanies the new field crew leader for at least the first two weeks of the field sampling season, though often for the entire first field season, providing instruction in all aspects of fish field work. After such time that the prospective crew leader has demonstrated basic proficiency, they are then permitted to proceed with field work; unsupervised, with periodic conferences with the Fish Evaluation Group supervisor to assure the sampling effort is being conducted in accordance with the procedures described herein.

b. New temporary summer field personnel are directed to review this document, and are given pre-field season training on the procedures involved in the fish sampling program including an electrofishing orientation and associated safety and PPE protocols.

c. During the initial week of electrofishing sampling, all new field personnel are evaluated by sorting a collection of different Ohio fish to species and counting the abundance to determine their familiarity with Ohio fish taxonomy and their ability to accurately count large numbers of fish. Full-time field crew leaders perform or supervise all of the actual field identifications and counts with the summer personnel assisting.

Initial Calibration for District Fish Crew Leaders

Field sampling

a. For the first field season, each District fish crew leader will complete a minimum of 10 fish sites with an EAS fish crew leader.
b. Sites will be within a planned survey area of the District boundaries.
c. Sites will be drawn from a mix of drainage areas so as to include both longline and wading work. Boat work (if District fish crew leader will be collecting such data) is a separate 10 sites.
d. Only three people will be on a sampling crew; EAS fish crew leader, District fish crew leader, and one intern or full-time staff person.

Fieldwork

e. Before sampling occurs, the EAS fish crew leader will review the role of each crew member involved in each sampling technique (shocker, assist netter, longline or wading gear handler).

f. Crew members should assume each role for at least one full site. For the majority of sites, the District fish crew leader will serve as either the primary sampler (shocker) or assist netter and will receive detailed instructions on technique from the EAS fish crew leader while actively sampling. The District fish crew leader will fine tune their skills in each role through mentoring by the EAS fish crew leader.

Fish Identification

g. EAS fish crew leader will review key taxonomic characteristics of collected species with District fish crew leader as samples are processed.
h. District fish crew leaders must demonstrate the ability to accurately identify most fish collected, and likewise fully comprehend knowledge gaps so as to confidently recognize fish taxa of which they are unfamiliar.
i. District fish crew leaders will be able to accurately sort and identify 30 fish within 15 minutes by the final site.
j. Species that are difficult to identify in the field or are otherwise unfamiliar to the District fish crew leader must be added to the site voucher so as to allow for species diagnosis in the laboratory at a later date.

Vouchers

k. For the initial field season, District fish crew leaders will voucher two individuals of each species collected per site. Exceptions to this include species represented in the voucher by a diagnostic photograph(s)[4] or where only one individual of a given species was collected as part of the sampling effort. Photographic vouchers should be limited to material not easily preserved, larger specimens (>8" in length).
l. Districts will complete voucher processing with EAS assistance at the EAS office before January.

Habitat evaluations

m. QHEIs will be completed individually by the EAS fish crew leader and District fish crew leader for each site. Upon completion, discussion of habitat attributes and related field observations should occur.
n. Both QHEI forms for each site should be submitted to EAS for comparison purposes.

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[4] Photographic vouchers must clearly depict the specimen so that a trained observer may positively identify the fish depicted. Clear depiction includes distinct morphological, meristic, or other relevant anatomical features that will allow for a definitive identification of that particular species, and may necessitate multiple photographs. The District fish crew leader must label or otherwise clearly annotate photographs and preserved specimens. At a minimum, supporting documentation must include the water body, specific location, date of sampling.

Post Field Season

1. Vouchers – As specified in 4b above, District fish crew leaders will arrange to complete voucher processing at EAS so that vouchers are completed before January.
2. QHEI scores – QHEI results will be summarized and sent to respective EAS and District personnel. A follow-up phone conference will occur between EAS and District staff regarding the QHEI results to ensure consistency among practitioners.

Future Field Seasons (Post Initial Calibration)

Field sampling

a. For subsequent field seasons, District fish crew leaders and EAS fish crew leaders will select, in conjunction with study plan coordinators, three fish sites to be sampled independently by both the District and EAS.
b. Sites will be within a planned survey area of the District boundaries.
c. Sites will be a mixture of drainage areas that cover both longline and wading work. Boat work (if District fish crew leader will be collecting such data) is a separate three sites.
d. District fish crew leaders will complete the first fish pass, marking the beginning and end of the zone with tree marking paint (or other identifier), and completing a QHEI.
e. District fish crew leader will email EAS fish crew leader upon completion of first pass, so that EAS fish crew leader may plan accordingly for second pass. EAS fish crew leader will complete second pass and a QHEI at each site after adequate recovery time for fish community has occurred.
f. QHEIs should be completed by each crew leader at each site.

Vouchers

g. District fish crew leaders will voucher two individuals of each species collected per site. When only large individuals of a species are collected (>8" length), photographs[5] may be considered an adequate form of voucher.
h. Districts will complete voucher processing at EAS before the end of the calendar year.

[5] Photographic vouchers must clearly depict the specimen so as to enable a trained observer to positively identify the fish depicted. Clear depiction includes distinct morphological, meristic, or other relevant anatomical features that will allow for a definitive identification of that particular species, and may necessitate multiple photographs. The District fish crew leader must label or otherwise clearly annotate photographs and preserved specimens. At a minimum, supporting documentation must include the water body, specific location, date of sampling.

Post Field Season Meeting

i. District fish crew leader and EAS fish crew leader will meet and discuss QHEI and fish results prior to the end of the year.
j. Discrepancies between scores will be addressed accordingly.

Expectations of Fish Crew Leaders

To keep both taxonomic and sampling skills sharp, it is expected that each fish crew leader, whether at a District or within EAS, will actively lead a fish crew and sample a minimum of three sites every other year. In the event that a fish crew leader does not complete this requirement, they will be required to repeat the Initial Calibration and Fish ID test before resuming fish crew leader activities.

Part B) Field Methods

1) Sampling Site Selection

The selection of fish sampling sites is based upon several factors including, but not limited to, the following:

- Pollution sources (point and non-point),
- Stream beneficial use designation evaluations (attainment status, verified, unverified, undesignated, not listed in WQS),
- Historical sampling for trends assessment,
- Physical habitat features, natural or anthropogenic, that may include geology, physiography, impoundment, stream order/size, macrohabitat, drainage improvements, etc.

Sampling resources are allocated based on the size of a given study area and related aspects including drainage area, number of tributaries, 12-digit hydrologic unit codes (HUCs), linear stream miles and the number and complexity of the priority issues requiring field evaluation. Optimum placement of sampling sites is determined recognizing practical access and resource constraints. The principal objectives of each survey determine where sampling sites will be located. Generally, sites are located upstream from major pollution sources to determine the background condition for the study area. Should the upstream portion of the stream or watershed be impacted, an alternate site may be chosen on an adjacent stream with similar watershed characteristics. Reference sites within the same ecoregion may also be used in this role; these are listed in Ohio EPA (1989). The role of upstream sites is not necessarily to provide a biological performance level, against which downstream sites are compared, since the ecoregion biocriteria fill this niche for the respective aquatic life use designations. Upstream sites are, however, important in defining any site or watershed specific background conditions that might temporarily or permanently influence eventual aquatic life use attainment in the downstream reaches. Selection of sampling sites within a segment is accomplished by selecting the most typical habitat available in an effort to represent the current potential of that segment. An attempt should be made to sample typically similar macrohabitats at all sampling sites established within the study area.

A general approach for evaluating point source discharges, is to have at least one site situated upstream from the primary process wastewater outfall(s), one within the mixing zone (as needed, e.g., effluent toxicity), and additional sites are located at intervals downstream from the mixing zone (i.e. dependent on stream size and mixing characteristics) to determine the near and far field impacts, the longitudinal extent and severity of any impact, and to determine if and where recovery occurs. Spacing of the downstream sampling sites is based on physical macrohabitat characteristics, access to the segment, other adjacent point and nonpoint sources, stream size, and other factors. An attempt is made to place sampling sites between point sources where sufficient distance between each exists. Sampling sites may also be situated in the mouths of major adjoining tributaries to determine any potential effects on the receiving waters.

Localized areas of macrohabitat modification such as instream impoundments or channelized sections alter macrohabitat available for fish and can affect community structure and function. Generally, these areas are not typical of the macrohabitat in a free-flowing river or stream. However, these areas are often times impacted by the principal sources targeted for evaluation in certain study areas (particularly in urban areas); therefore, sampling sites are located within these modified areas as needed. These areas should be sampled to understand the underlying influence that they exert on biological performance and aquatic life use attainment.

When possible, fish sampling zones should include all representative macrohabitat present, including riffles, runs, pools, and glides. Ideally, the fish zone is measured prior to sampling with the end of the zone at a riffle. Situating the zone so that it ends at a riffle facilitates capture of fish.

2) Fish Sampling Procedures

Introduction

The principal method used by Ohio EPA to obtain fish relative abundance and distribution data is pulsed direct current (D.C.) electrofishing. As with any single method there exists inherent sampling selectivity and sampling bias. Pulsed D.C. electrofishing is, however, widely viewed as the single most effective method for sampling fish communities in lotic habitats. Thirteen different fish sampling techniques, methods or gear types have been assigned sampler type codes. Eight codes are currently recognized as valid for generating fish relative abundance data for the purpose of calculating Index of Biotic Integrity (IBI) and Modified Index of well-being (Mlwb) scores from which aquatic life use attainment is partially judged (Table 5). The remaining codes are assigned to methods that were evaluated over the course of several years before the existing sampling protocol was established. This system of letter codes supersedes a system of numerical codes used prior to 1984. The use of any one of these sampling methods is dependent on the type of information required and the type of aquatic habitat being sampled.

The boat mounted and wading electrofishing methods are the most commonly used fish sampling techniques by Ohio EPA in lotic habitats. The boat electrofishing methods

(sampler type A) are used to sample the larger streams and rivers (Table 5). Wading methods (sampler types D and E) are used in wading streams. Sampler type B (18' boat, circular electrode array) is used in the deeper rivers (*e.g.*, Ohio River) and embayments (*e.g.*, Lake Erie tributary river mouths). Sampler type C (boat longline) is used in free-flowing rivers to sample riffle habitats and is used only in conjunction with standardized boat protocols (type A), where a more thorough and intensive species inventory of riffle habitat is needed. The resulting data are recorded separately from the boat catch and are not used to calculate the IBI or Mlwb. Sampler types G and H are backpack electrofishing-seining combination and seining methods, respectively, and are no longer in routine use. The fyke net and hoop net methods (types I and J) may be necessary in lentic, wetland, or large river habitats. The experimental gill net method (type K) may be necessary to sample for mid-channel and pelagic species.

Fish sampling is preferably conducted between mid-June and early October, when stream and river flows are generally low, pollution stresses are potentially the greatest, and the fish community is most vulnerable to electrofishing. Sampling may be conducted outside of this time period, but the resulting data may not be applicable for index calculation and aquatic life use assessment or evaluation. The use and applicability of these data are evaluated on a case-by-case basis. Special studies are conducted by the Fish Evaluation Group on an as needed basis to examine the effectiveness, selectivity and efficiency of each sampling method.

Pulsed D.C. Electrofishing Methods and Equipment

Selection of the appropriate sampler type is dependent upon depth, size and macrohabitat of the water body being sampled. This is a critical part of the sampling process since gear type determines data applicability for the purpose of evaluating attainment of aquatic life uses. Thus, it is important that the appropriate sampler type be used.

Boat electrofishing methods (sampler type A) are typically used in medium to large sized streams and rivers where wading methods would be impractical, inefficient, ineffective and unsafe. Applicable waters include streams and rivers, and lakes with sufficient depth to accommodate a fully loaded 12', 14', 16' or 18' boat. The typical drainage area range requiring the use of boat methods is between 150 and 500 mi^2, but obviously may include significantly larger waters. However, site conditions, regardless of drainage area, ultimately determine applicability, as boat methods have been used for sites as small as 75mi^2 where pool depths will permit a fully loaded boat. The appropriate boat size is determined by the size and depth of the waterbody in question, with 12' and 14' boats typically employed to sample small and medium sized rivers, with 16' and 18' boats used in the largest and deepest rivers, impoundments, and embayments. For day time electrofishing, 18' boat methods are designated sampler type A, if a straight electrode array is deployed or sampler type B if a circular array is employed. Night electrofishing may be appropriate for the largest rivers (*e.g.*, Ohio River, impounded sections of the Muskingum River) where the drainage area exceeds 6,000 – 7,000 mi^2. Depending on the electrode array used, this method is termed sampler type N (straight array) or sampler type M (circular array).

Table 5 Designation of sampler types and description of fish sampling methods evaluated by Ohio EPA in developing established fish sampling protocol (revised March 30, 2015). Methods in gray font have been discontinued.

Sampling Method	Sampler Type	Relative Abundance	Data Collected	
			#	Wt[a]
Boat-mounted electrofishing – straight electrode array	**A**	Per 1.0 km	X	X
Boat-mounted electrofishing – circular electrode array	**B**	Per 1.0 km	X	X
Boat longline – riffle method[b]	**C**	Per 0.3 km	X	X
Tote barge electrofishing	**D**	Per 0.3 km	X	X
Longline electrofishing	**E**	Per 0.3 km	X	X
Backpack electrofishing	**F**	Per 0.3 km	X	X
Backpack electrofishing-seine combination[c]	**G**	Per 0.3 km	X	
Seines[d]	**H**	Per 0.3 km	X	
Fyke net[d]	**I**	Per 24 hours	X	X
Trap/modified hoop nets[d]	**J**	Per 24 hours	X	X
Gill net[d]	**K**	Per 24 hours	X	X
Boat-mounted electrofishing – straight electrode array NIGHT[f]	**N**	Per 1.0 km	X	X
Boat mounted electrofishing – circular electrode array NIGHT[f]	**M**	Per 1.0 km	X	X
Reserved	**L, O-Z[e]**			

a - Weight data is taken if Mlwb is needed.
b - Used in conjunction with sampler Type A, as needed.
c - Discontinued method.
d - Discontinued method and is not suitable for calculating IBI or Mlwb scores.
e - These codes are available for methods developed in the future.
f - Reserved for large rivers, lacustuaries and Lake Erie

Wading methods are used in smaller, wading size streams that cannot accommodate the boat methods due to the physical limitations of the stream channel. These are referred to as wading sites and range from the smallest headwater areas (<20 mi^2 drainage area) to sites of 400 - 500 mi^2. The Sportyak/rollerbeast electrofishing method (sampler type D) is used in streams that range in size from 5-20 meters in width and 0.5 – 1.0 meter in depth (average). There is a great deal of overlap in terms of drainage area between the sites where either the wading or boat sampler types may be most appropriate. The key factors in making the choice between these two methods is pool width and depth and access for the sampling equipment.

The longline electrofishing method (sampler type E) is used in areas where the pools are separated by shallow riffles which make the use of the Sportyak/rollerbeast electrofishing method impractical. Both methods will sample the same site with equal efficiency. The backpack electrofishing method (sampler type F) is used in very shallow, small headwaters streams where the longline method is not necessary to secure an adequate sample. Streams that are more than five times the width of the

anode net ring and more than twice the depth of the same should not be sampled with the backpack electrofishing method. The seining methods (sampler types G and H) were used in the past, but have been discontinued by Ohio EPA. These sampler types are retained to store in Ohio EPA's database data generated by non-Ohio EPA entities and to make possible the use of historical data. Results generated by these latter methods (sampler types G and H) may not be suitable for determining aquatic life use attainment using the IBI and Mlwb.

Selection of any of the previously described methods is based on the best professional judgment of the field crew leader and information gathered through communication with district office field staff that have visited the site and in a reconnaissance of the stream. Reconnaissance should take place during low flow conditions if at all possible. Drainage area, stream length, and stream order are good physical indicators which aid in the selection of the appropriate sampling gear. Information to be collected during the reconnaissance includes the general width and depth of the stream, presence of riffles, dams, log jams and other impediments to navigation, stream access, and location of pollution sources and tributaries. All of these factors are used in choosing the appropriate sampler type(s).

Electrofishing should be conducted only under "normal" summer flow and clarity. What constitutes "normal" can vary from stream to stream. Generally, "normal" water conditions in Ohio occur during below annual average river discharge levels. Under these conditions, the surface of the water generally will have a "placid" appearance. Abnormally turbid conditions are to be avoided as are elevated flow and current. All of these adversely affect sampling efficiency and may render data ineligible for Mlwb and IBI calculation. Most Ohio surface waters have some background turbidity due to planktonic algae and suspended sediment and very few, if any, are entirely clear. Rainfall and subsequent runoff can cause increased turbidity due to the increased presence of suspended sediment (clays and silt). In most areas, this imparts a light to medium brown coloration in the water. Floating debris such as sticks and other trash are usually obvious on the surface. Visibility under such conditions is seldom more than a few inches. Such conditions should be avoided and sampling should be delayed until the water returns to its "normal" clarity. High flow should be avoided for the obvious safety reasons, but this also reduces sampling efficiency. The boat methods are particularly affected as it becomes more difficult for the driver to maneuver the boat into areas of cover and current heterogeneity. These cautions apply to all of the electrofishing methods.

Netters are required to wear polarized sunglasses to minimize surface glare, thus increasing the visibility of stunned fish. An exception to this is with night sampling where sunglasses are not worn.

Boat Electrofishing Methods and Equipment

Equipment type, electrode design, and sampling methods follow the rationale and procedures outlined in Gammon (1973, 1976) and Novotny and Priegel (1974). Figure 6 provides a diagrammatic description of the boat apparatus. A Smith-Root 5.0 GPP,

Type VI-A[6], 3.5 GPP, and 2.5 GPP electrofishing units[7] are used in the 12', 14', 16' and 18' boats. The Type VI-A unit rectifies 60HZ 240 VAC (which is supplied by a 3500 or 4500 watt gasoline powered alternator to pulsed DC) and the associated pulse configuration consists of a triangular wave that can be adjusted to 60 or 120 pulses/second. Six voltage settings from 166 to 996 VDC in 166 volt increments are available. The voltage setting used in a particular situation is determined on a trial and error basis by increasing the voltage setting until a pulse width of 4-5 milliseconds produces an amperage reading of 8 amperes. In Ohio waters during June through October, relative conductivity values normally range from 300-600 umhos/cm. This generally results in a voltage selection of 336, 504, or 672 VDC. Conductivity values below this range may require higher voltage settings, whereas higher conductivity values may require lower voltage settings. The Smith-Root Model 3.5 GPP, 5.0 GPP, and 2.5 GPP gas powered alternator and pulsator also delivers pulsed DC current. The pulse configuration consists of a fast rise, slow decay pulse which can be interrupted into 30, 60 or 120 pulses/second. The duty cycle is a low range of 0-500 volts that typically starts at about 60% (300 volts). The percent range is adjusted to reach 4-12 amp output. At times, a combination of 120 pps and range adjusted below 60% are needed to attain the proper output as measured in amps. Other comparable pulsed D.C. electrofishing units are acceptable for use as long as their performance is comparable to the aforementioned designs.

Pulsed DC current is transmitted through the water by an arrangement of anodes and cathodes suspended in the water from the boat. On the 12', 14' and 16' boats, four 32" long ¼" diameter stainless steel aircraft cable anodes are hung from a retractable aluminum boom which extends in front of the boat. Boom length varies according to boat size and is approximately 3.05m on the 18' boat, 2.75m on the 16' boat, 2.15m on the 14' boat, and 2.0m on the 12' boat. Boom width varies from approximately 1.55 to 1.65m, being wider on the larger boats. Four anodes are positioned on the front of the

[6] Use of product or company name does not signify endorsement.
[7] Smith-Root, Inc. 14014 N.E. Salmon Creek Ave., Vancouver, Washington 98686[1].

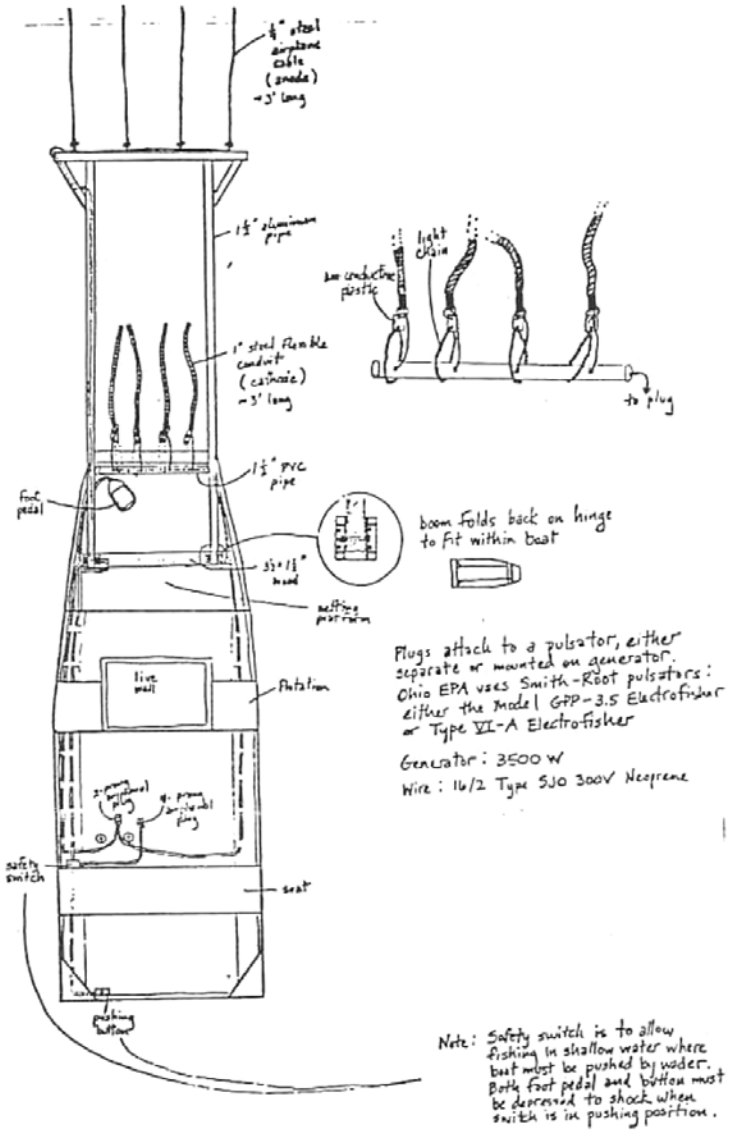

Figure 6 Diagram of the boat electrofishing apparatus used by Ohio EPA to sample large river and stream fish communities.

boom in a line perpendicular to the length of the boat. Four 64" lengths of 1" O.D. flexible galvanized steel conduit serve as cathodes, and are suspended directly from the bow in a line perpendicular to the length of the boat. The width of this array ranges from 0.75m on the 12' boat to 0.90m on the larger boats. Anodes and cathodes are replaced when damaged or worn. Safety equipment includes a positive pressure cut-off foot-pedal switch located on the bow deck, and an emergency cut-off switch on the pulse box/rectifier. One additional positive pressure (hand activated) emergency cut-off switch is located along the inside of the transom. This switch is used when water depth will not permit the use of an outboard motor and thus the driver must wade in the stream and hand maneuver the boat while actively sampling. Adjacent to the stern seat, a three-way toggle switch activates the positive pressure switch on the transom so that both it and the foot-pedal on the bow must be depressed to close the circuit. The three-way toggle

switch acts as an additional emergency cut-off; the boat has multiple and redundant "kill" switches. There is a magnetic-hydraulic circuit breaker on the Type VI-A electrofishing units, which is an additional emergency cut-off.

The 18' electrofishing boat can be used with either a standard straight electrode array (sampler type A) or with a circular electrode array (sampler type B). The circular array is outfitted according to the specifications listed in Novotny and Priegel (1974). Anode configuration is circular and can be altered by adding or removing electrodes or changing the surface area exposure of each electrode depending on the conductivity of the water. Anodes are added in very low conductivity water (<100-150 umhos) or removed in extremely high conductivity water (>900 umhos). For deep water sampling, additional length of cathodes may be attached to the bow. These sampling methods are used in rivers where average sampling zone depth is consistently deeper than 1.5-2.0 meters (*e.g.*, Lake Erie river mouths, lower Muskingum River, Ohio River, etc.) and in lakes, reservoirs, and impoundments, but may be employed on an as needed basis elsewhere.

For the deep water sampling described above, sampling is conducted at night. For night electrofishing, the equipment typically includes four 75 watt flood lamps attached to a deck mounted guard rail, the lighting system powered by a separate motorized generator. Other lamp arrays and power sources can and have been employed, provided they safely and adequately illuminate the shock field and working areas of the boat deck.

A field crew consists of a minimum of three persons (whenever possible), a boat driver, a netter, and a support vehicle driver. Limited access to most rivers and streams requires the electrofishing boat to be launched at an upstream point with a two person crew; a primary netter and a boat driver. The third crew member is responsible for maintaining contact with the electrofishing boat and meeting the boat at points downstream. Smaller rivers that are not continuously navigable are sampled by locating put-in and take-out access points at each sampling location.

The netter's primary responsibility is to capture all fish sighted; the driver's responsibility is to maneuver the boat as effectively as possible giving the netter the best opportunity to capture stunned fish (the driver may assist in netting stunned fish that appear at the rear or behind the boat). A boat net with a 2.5 meter long handle and ¼ inch square heavy Delta knotless mesh netting is used to capture fish as they are attracted to the anode array and/or stunned. An effort is made to capture every fish sighted by both the netter and driver. Both tasks are skill dependent with the boat maneuvering task requiring the most experience to achieve proficiency.

Each sampling zone is fished in a downstream direction by slowly and steadily maneuvering the electrofishing boat as close to shore and submerged objects as possible by rowing or motoring. This may require frequent turning, backing, shifting (forward, reverse), changing speed, etc. in areas of moderate to extensive cover. The electrofishing boat is pushed on the transom by the driver when the water is too shallow

to motor or row. A hand actuated positive pressure cut-off switch located on the inside of the transom is used during this procedure in addition to the bow foot-pedal switch for safety. Both the netter and driver are clad in chest waders, rubber gloves, and a jacket type personal flotation device.

Boat Sampling Site Selection

Sampling sites are selected along the shoreline with the most diverse macrohabitat features. The site ideally begins at a riffle facing upstream, and is generally along the gradual outside bends of the larger rivers but is not invariable. Wherever practical or possible, each zone should include a riffle-run type of habitat. This, of course, is determined by the availability of such areas. Boat electrofishing zones generally measure 0.5 kilometers (km) in length, although shorter distances may be necessary. Distance can be measured any number of ways, the most common methods by Ohio EPA being optical laser range finder or a forester's hip chain. When using a laser rangefinder, each zone is measured in increments until an accumulated distance of 0.5 km is reached. It is critically important that lines are shot in such a way so as to capture the curvature of the wetted channel. Regarding the forester's hip chain, sites are measured by periodically securing the hip chain thread to a stationary object while wading or motoring the boat through the length of the sampling zone, again making sure to follow the thalweg so as to capture the full, sinuous, length of the reach. Laser range finders are verified to measure accurately prior to being used in the field on a marked course. Max depth is estimated to the nearest 0.1m by regularly probing the zone with a graduated dip net, either over the course of the sample event or while marking the zone. The maximum depth is then recorded on the QHEI sheet. Notations may also be included on the QHEI regarding the range of depths encountered. The boundaries of each electrofishing zone are typically marked on stationary objects (*e.g.*, trees, bridge piers, etc.) with fluorescent foresters' tree paint. The starting point is marked with a capital "S" and the ending point is marked with a visible capital "E". This enables accurate duplication of the site on subsequent sampling dates. On rare occasions, if the sampling zone is disjunct, additional marks are necessary. An X marks where sampling stops and an arrow indicates where sampling resumes. The location of each sampling zone is indexed by river mile (using the river mile index from the Ohio EPA PEMSO RMI system of River Mile maps: (http://www.arcgis.com/home/webmap/viewer.html?webmap=992b6fe112e14623bf3cfcc3a048f7e5&extent=-86.7944,38.2065,-78.6564,42.1167).

Boat Electrofishing Techniques

Each boat site is sampled two or three times during the seasonal index period. Three to four weeks should elapse between sampling events per station. Individual sampling zones are largely fished with the current in a downstream direction, generally following the thalweg. For highly sinuous waters, this may necessitate crossing the channel to continue working the outside bend. The boat driver must pay close attention to channel features as they relate to fish habitat (structure/cover, pools, runs, glides, riffle, etc.) and work diligently to fish all major habitat types represented within the given sampling reach. Generally, boat electrofishing proceeds close to the shore and submerged

objects. It is absolutely critical to sample carefully, particularly at physically complex sites where abundant and varied structure/cover, mixed current velocities and a diversity of channel forms and associated substrate types are present. Figure 7 provides a diagrammatic portrayal of how two different boat electrofishing zones should be sampled. In zones with extensive woody debris and slow current, it is necessary to maneuver the boat in and out of the "pockets" of habitat formed by the debris. Under these conditions, if the water depth approaches 1-2 m, it is usually necessary to "wait" for the fish to appear at or near the surface. In moderately fast or swift current, it is necessary to conduct fast turns and other variable maneuvers in order to put the netter in a good position to capture stunned fish. The efficiency is enhanced if the boat can be kept moving downstream at a pace just slightly greater than the current velocity. Fish are usually oriented into the current and must either swim into the approaching electrical field or turn sideways to escape downstream. This latter movement presents an increased voltage gradient making the fish more susceptible to the electrical current. It is often necessary to sample through fast water sections two to three times. Portions of zones with continuous swift current can be effectively sampled by either "backing" the boat downstream and occasionally pausing to allow the netter to capture stunned fish or maneuvering the boat downstream, perpendicular to the current, so that the anode array (the electric field) is able to draw fish from shoreline cover attendant to run, pausing periodically, as described above. The driver may need to assist with netting when large numbers of fish are stunned. Attempting to electrofish such fast water areas (runs and swift glides) in an upstream direction only will greatly diminish sampling efficiency and the resulting catch. The exception to this would include only riffles and upper margins of point bars immediately downstream from riffle areas. For these areas, the boat is positioned opposite the current at the face of the riffle. When all is set, the circuit is closed, the shock field energized and the fish are captured as they are stunned and drift downstream. A similar technique is employed to fish associated point bars.

Although sampling effort and resulting abundance estimates (relative number and relative weight) are based upon zone length or distance fished, the amount of time spent electrofishing each zone is an important consideration. Time fished can legitimately vary depending on any number of factors (e.g., physical complexity, current velocity, fish abundance).

However, there is a general minimum amount of time that should be spent sampling each boat zone. Based on an analysis of 1,187 electrofishing samples where time fished was compared to various catch results (*e.g.*, numbers, weight, species), it was determined that these parameters are sensitive to the relative level of minimum effort expended. Inspection of the results show that at least 1300 to 1600 seconds should be spent sampling any 0.5 km boat electrofishing zone. Typical boat sample time is between 2300 and 2600 seconds and may be longer depending upon the aforementioned factors.

Captured fish are immediately placed in an on-board live well for later processing. Water is replaced regularly in warm weather to maintain adequate dissolved oxygen levels in the water and to minimize mortality. This is achieved by the combined means

of bucketing water into the live well and a siphon to remove water from the live well. In this way the entire volume of the live well is replaced multiple times over the course of a given sampling event.

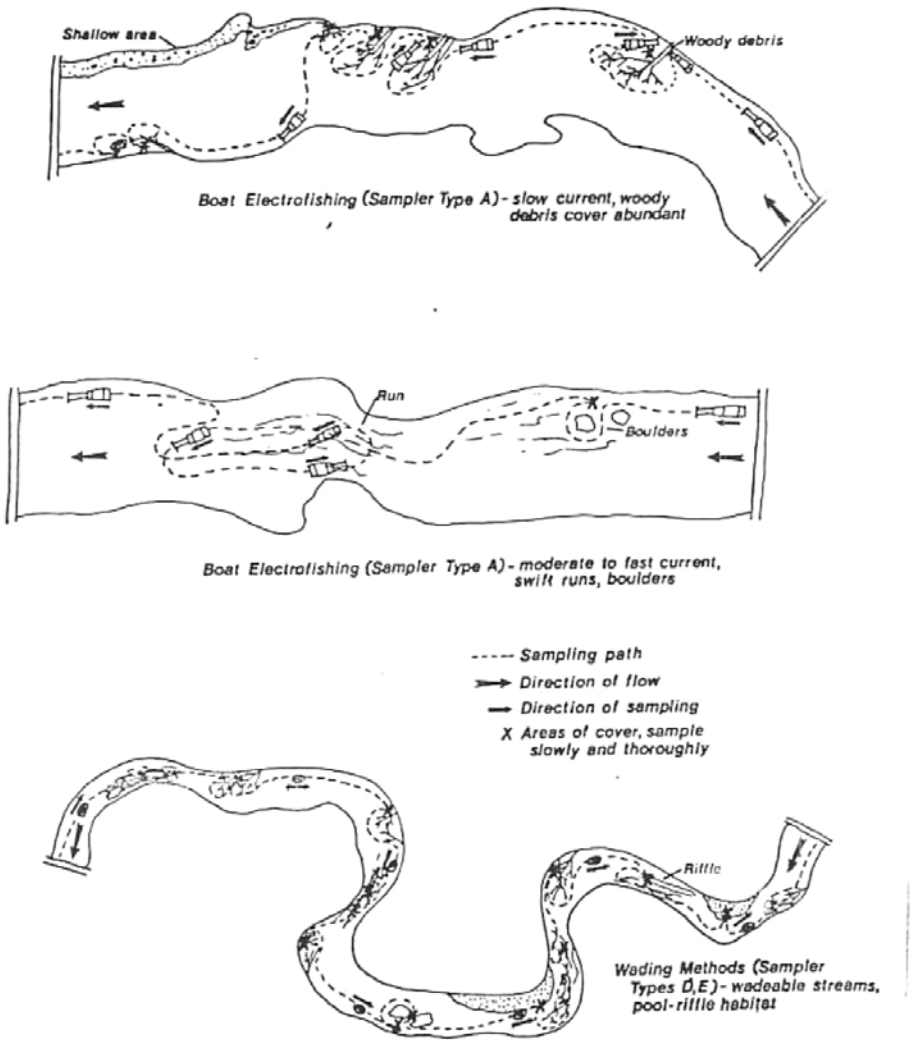

Figure 7 Diagrammatic portrayals of proper boat electrofishing technique at two different river sampling locations and wading method technique in a typical pool-run-riffle stream habitat.

Wading Electrofishing Methods and Equipment and Sampling Techniques

Ohio EPA wading methods consist of three electrofishing gear types: Tote barge (sampler type D), longline (sample type E), and backpack (sampler type F). Type D and E systems are almost exclusively powered by a model No.1736 DCV Baldor (formerly T&J) 1750 watt, three-phase, bridge rectified, 250 volt pulsed DC electrofishing unit. For intermediate sites, not amenable to either standard wading or boat methods, a 2500 watt Smith-Root 2.5 GPP electrofisher may be employed as a power source for sampler types D and E, in place of the 1750 watt Baldor unit. Gear types D and E account for the vast majority of monitoring work performed by Ohio EPA over the past 30 years. At the time of the first publication of this document (Ohio EPA 1989) two backpack electrofishers (sampler type F) were employed by Ohio EPA: Michigan DNR Model and the Coeffelt BP-2. Both are powered by 12 volt DC motorcycle batteries, and yield an output of 100-200 volts, pulse DC. Since that time other units have been adopted including the Smith-Root 15-C, power supplied by a small gasoline motor driven 60 Hz, 120 volt alternator. Recently, different, improved or otherwise new designs have been brought to market, some employing high output, light weight, lithium ion batteries. However, Ohio EPA does not routinely employ backpack electrofishing units in bioassessment surveys.

Tote barges are used to sample smaller, wading size streams where depth and access preclude boat methods. Any small, lightweight watercraft will suffice, provided it is fitted with a proper cathode array and will safely accommodate a gasoline powered electrofishing unit, live well and sundry equipment. Presently, Ohio EPA employs two barge types: 1) Sportyak, the trade name of a 2.1m, polyethylene plastic boat, to which can be affixed a rear axle and wheels to facilitate mobility (Figure 8), and 2) Roller Pram (commonly referred to as a Rollerbeast), a wholly unique conveyance developed by Ohio EPA, comprised of two large polyethylene lawn rollers or barrels, affixed to a welded aluminum frame, with a watertight compartment attached to the frame between the barrels sufficient to carry all necessary equipment (Figures 9 and 10). The great advantage of the Rollerbeast is that the lawn rollers or barrels not only provide excellent buoyancy but also significantly reduce the physical effort required to launch, retrieve and maneuver through a given stream reach, as the craft may be rolled, rather than dragged over the varied obstacles common to riverine environments (riffles, gravel bar, logs, etc.). Additional features are easily added to the Rollerbeast to increase crew efficiency, and may include a rear push-bar handle, and fixed, light weight shelving.

Either singularly or in combination, the cathode array on the tote barge may include a dedicated stainless steel plate, stainless steel air craft cable, or the frame of the roller pram itself, provided a surface area of at least 1000 cm^2 is achieved. The anode takes the form of a standardized, custom manufactured, teardrop stainless steel netted ring, attached to a ~1.8 m long fiberglass (Extran) tube. The electrofisher is activated by a waterproof, positive pressure magnetic or positive pressure mechanical switch mounted on the anode net pole (see Figure 11 for a diagrammatic description). A combination of a retractile cord and water resistant cable carries the switch circuit and current to the anode from the power source (e.g., Baldor unit or Smith Root 2.5 GPP).

The longline electrofishing (sampler type E) method is used in streams that are too shallow to efficiently sample with the tote barge method. The backpack electrofishing method (sampler type F) may be used in lieu of the longline electrofishing method in only the smallest headwaters streams following the restrictions that were previously stated.

Procedures for sampling require a three person crew, all wearing chest waders and rubber gloves. Polarized sunglasses are worn to diminish glare and facilitate seeing fish. The primary netter operates the anode net ring while one crew member guides the tote barge and the third crew member assists in capturing fish. This method is also diagrammed in Figure 7. All habitat types are thoroughly sampled in an upstream direction for a distance of 150-200 meters. The primary netter works the net ring beneath undercut banks, in and around brush piles, log jams, large boulders and other submerged structures. An effective technique for capturing fish under such objects is to thrust the anode ring into and under the structure with the current on and then quickly withdraw the anode ring in one swift motion. This has the effect of drawing fish out from under such structure making their capture possible. Sampling effort is usually concentrated on one side of the stream and some switching from one stream bank to the other may be necessary to sample all habitat types. In riffle and run areas, the primary netter rakes the anode ring from upstream to downstream, allowing it to drift with the current. At the same time, the assist netter blocks an area downstream from the anode ring. This minimizes escape and avoidance of the electrical field by riffle species. When the holding tank is full of fish or sampling is completed, the fish are processed (see Fish Counting and Weighing Procedures).

Sampling methods G-K have not been employed by Ohio EPA since the early 1980's. Detailed descriptions of these methods are presented in Appendix 1.

Figure 8 Sportyak with attached rear axle and wheels.

Figure 9 Side view of Rollerbeast with shelves and push-bar. Live well is adjacent to generator in the middle.

Figure 10 Rollerbeast without shelves.

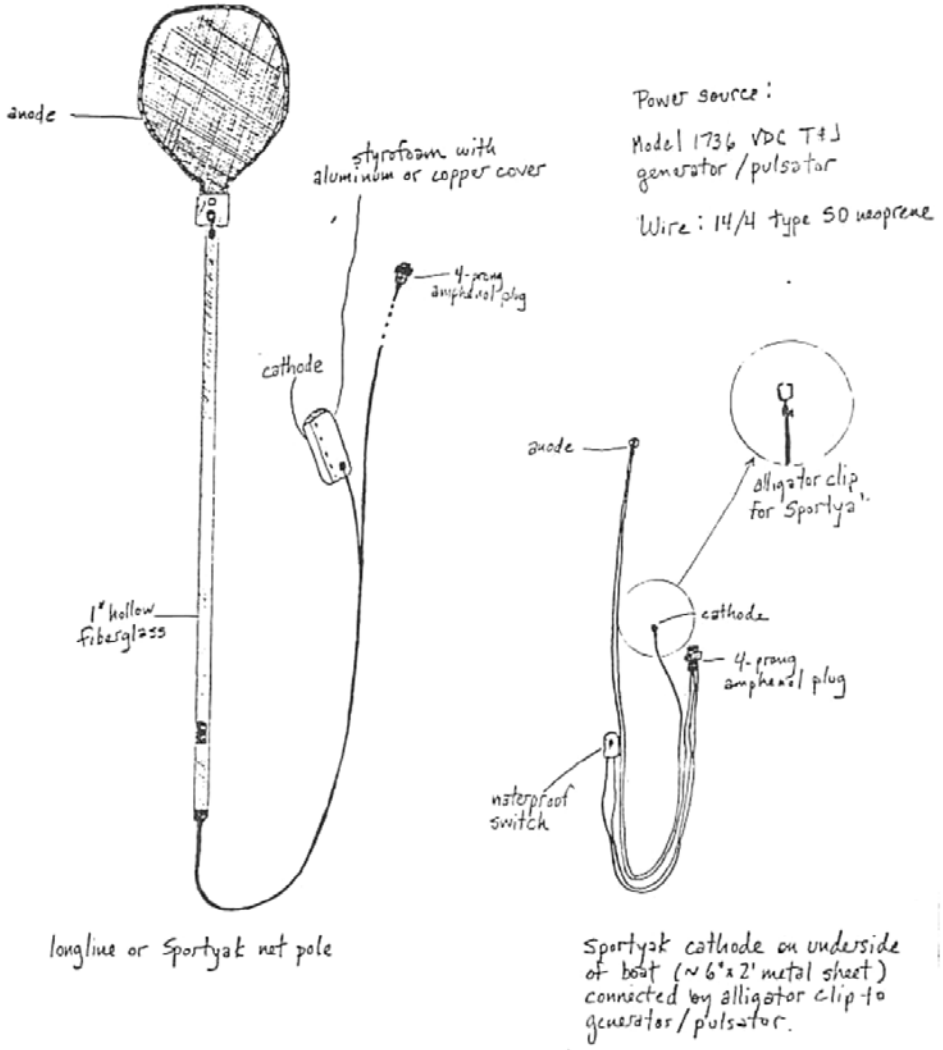

Figure 11 Diagram of the net pole/electrode apparatus with the Sportyak-generator and long-line electrofishing methods by Ohio EPA to stream fish communities.

3) Field Counting and Weighing Procedures

Handling Live Specimens

Captured fish are placed in a livewell, and the catch is processed immediately following the end of sampling. Water in the livewell is refreshed by the combined means of bucketing water into the live well by the tote barge tender and a siphon to remove water from the livewell. In this way the entire volume of the live well is replaced multiple times over the course of a given sampling event. Fish are then sorted by species, examined for gross external anomalies, counted and weighed, the latter at sites greater than 20 mi^2, then released.

Field Identification

The majority of captured fish are identified to species in the field; however, any uncertainty about the field identification of individual fish requires their preservation for later laboratory identification (see Part C). Fish are preserved for future identification in formalin and labeled by date, river or stream, river mile, and crew member initials. Identification is required to the species level at a minimum and may be necessary to the subspecies level in certain instances (*e.g.*, banded killifish). The collection techniques used may not be consistently effective for fish less than 15-20 millimeters in length, thus inclusion in the catch is not recommended. Also, Angermier and Karr (1986) and Angermier and Schlosser (1988) recommended that fish of this size, which may include young-of-year, not be included in IBI calculations as they may unduly bias through over representation.

Weighing Procedures

For samples of species which are comprised entirely of one size class (*e.g.*, adults, juveniles, young-of-year), two methods may be used. For larger species (*e.g.*, carp, redhorse, most sunfish), where the adult fish are of a similar size, the catch may be weighed as separate individuals or in aggregate as a species. All results are recorded on the fish data sheet (Figure 12). For catches with more than 15 individuals per species, representative subsamples by size class should be taken for each species, so as to have a reasonable estimate to extrapolate to the remaining individuals within the size class. Note that the first 15 individuals encountered is not likely to be representative of the entire sample. If extremely high numbers of a particular species are collected and the fish are of a relatively uniform size, the number of individuals may be determined by mass weighing all fish collected and extrapolating the numbers from a counted and weighed subsample. Young of year are noted on data sheets, but not used to calculate IBI or MIwb scores.

Ohio EPA **FISH DATA SHEET** Sheet ID For Office Use Only _____ New Station (requires lat/long & county) ☐ Mix Zone ☐ Page____of ____

Station ID_____ River Code_____RM_____Date_____Time_____

Stream_____ Location _____

Comments _____

Lat _____Long _____ County _____ ALP _____ Time Fished _____

Crew _____Netter _____Others _____ Sampler Type _____

Distance _____Flow _____Temp. C _____ Secchi _____Source _____ Project _____

	Fins Code	Number Weighed	Total Counted	Total Weight	Weights	Counts	DELT ANOMALIES (Deformities, Erosions, Lesions, Tumors; Multiple DELTs on one fish)					
1	V 10x						D	E	L	T	M	*
2	V 10x						D	E	L	T	M	*
3	V 10x						D	E	L	T	M	*
4	V 10x						D	E	L	T	M	*
5	V 10x						D	E	L	T	M	*
6	V 10x						D	E	L	T	M	*
7	V 10x						D	E	L	T	M	*
8	V 10x						D	E	L	T	M	*
9	V 10x						D	E	L	T	M	*

* A-anchor worm; B-black spot; C-leeches; F-fungus; N-blind; P-parasites; S-emaciated, W-swirled scales Y-popeye; Z-other

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Figure 12 Fish Data Sheet.

Individual fish weighing less than 1000 grams are weighed to the nearest 1gram on a spring dial scale (1000 gram capacity x 2 gram intervals). Fish weighing more than 1000 grams are weighed to the nearest 25 grams on a spring dial scale (10,000 – 12,000 gram capacity in 50 gram increments.) All scales are checked once with National Bureau of Standards Class F check weights (2000 grams in 1 gram increments) and adjusted as necessary.

3) Assessment of External Anomalies

At sites >20 mi^2 drainage area, all fish that are weighed and counted, whether done individually, in aggregate, or as a subsample, are examined for the presence of gross external anomalies, and their occurrence is recorded on the fish data sheet (Figure 12) and subsequently entered into EA^3. For sites <20mi^2 drainage area, all counted fish and subsampled fish are examined for the presence of gross external anomalies and recorded on the fish data sheet and subsequently entered into EA^3.

In order to standardize the procedure for counting and identifying anomalies, the following criteria should be followed.

Gross external anomalies are visible to the naked eye when the fish are captured, identified, sorted, weighed, and counted. Table 6 lists the types of anomalies that are recorded on the fish data sheet and subsequently entered into EA^3. External anomalies are expressed as percent (weighted) of affected fish among all fish weighed for boat and wading sites, and as a percent of all fish counted for headwater sites. This is computed for each type of anomaly for each species in each sample. For wading and boat sites, it is computed as a weighted number (*i.e.*, based on percent incidence among weighed fish times the total number of that fish species in the sample). Then the total percent anomalies for a specific type of anomaly or group of anomalies can be calculated for one or more sites.

Table 6 Codes utilized to record external anomalies on fish.

Code	Description
D	Deformities of the head, skeleton, fins and other body parts.
E	Eroded fins.
L	Lesions, ulcers.
T	Tumors.
M	Multiple DELT anomalies (e.g. lesions and tumors, etc.) on the same individual fish.
A	Anchor worm.
B	Black Spot.
C	Leeches.
F	Fungus.
I	*Icthyophthirus multifilis* (Ich).

N	Blind – one or both eyes; includes missing and grown over eyes (does not include eyes missing due to popeye disease).
S	Emaciated (poor condition, thin, lacking form).
P	External parasites (other than those already specified).
Y	Popeye disease.
W	Swirled scales.
Z	Other, not included above.

The following is a review of some anomalies commonly encountered in freshwater fishes. These characteristics should be used in determining the types of external anomalies present and in coding the fish data sheet (Figure 12).

- **Deformities:** Can include malformation of the head, spinal vertebrae, fins, barbels and abdomen and have a variety of causes including but not limited to toxic chemicals, heavy metals, viral and bacterial (*e.g.*, *Mycobacterium*) infections, and parasites (*e.g.*, *Myxosoma cerebalis*; Post 1983). Fish with extruded eyes (popeye disease) or obvious injuries should not be included or otherwise identified as having or being deformed. Detailed examples of selected deformities are provided below:
  - Spinal deformities may include *lordosis* (concave curvature of the caudal region of the spine), *scoliosis* (lateral curvature of the spine), *kyphosis* (convex curvature of the thoracic region of the spine resulting in a "humpback" condition), and *perosomus* (truncated, compressed, or otherwise shortened body) (Lemly 1997).
  - *Craniofacial deformities* may include all readily discernable anomalies of head and jaw, including but not limited to deformation of skull (e.g., pugheadedness), malformed, misaligned or asymmetrical jaw, such as retrognathia, maxillary and mandibular (Lemly1997 and Smith et al. 2002).
  - *Anomalous barbels* are defined as being stubbed, irregular, clubbed, bifurcated or otherwise significantly malformed. In practical terms this subcategory is limited to ictalurids, more commonly bullheads and channel catfish. Fish presenting shortened or missing barbels due to active tissue necrosis are excluded as these are presently characterized as being eroded (E) within the DELT metric.
  - *Distended abdomen* **may not be** teratological in nature, but this condition is presently counted as a deformity within the DELT metric since the adoption of biocriteria in 1989. Numerous etiologies may result in grossly distended abdomen [edema, dystocia (egg binding), internal neoplasm, etc.], but the main objective is to capture abdominal edema or what is commonly called dropsy. Affected fish present severe abdominal swelling due to the accumulation of fluid in tissue and body cavity. The associated internal pressure can cause the fish's scales to protrude, giving it a bristly or pinecone-like appearance.

- **Eroded fins:** These are the result of a chronic disease principally caused by flexibacteria invading the fins causing a necrosis of the tissue (Post 1983). Necrosis of the fins may also be caused by gryodactylids, a small trematode parasite. When necrosis occurs in the tissue at the base of the caudal fin, it is referred to as peduncle disease. Erosions also occur on the preopercle and operculum and these should be included. In Ohio streams and rivers, this anomaly is generally absent in least impacted fish communities, but can have a high incidence in polluted areas. It occurs most frequently in areas with multiple stresses, particularly low or marginal dissolved oxygen or high temperatures in combination with chronic toxicity (Pippy and Hare 1969; Sniezko 1962).

- **Lesions (Ulcers):** These appear as open sores or exposed tissue and can be caused by viral (*e.g.*, *Lymphocystis*) and bacterial (*e.g.*, *Flexibacter columnaris*, *Aeromonas*, *Vibrio*) infections. Prominent bloody areas on fish should also be included. Small, characteristic sores left by anchor worms and leeches should not be included unless they are enlarged by this infection. Obvious injuries, however, should not be included unless they, too, are likewise infected. As with eroded fins, lesions often times appear in areas impacted by multiple stresses, particularly marginal dissolved oxygen in combination with sublethal levels of toxics. Note: Although the term lesion has been commonly used in fisheries literature to describe the above conditions, a better and more accurate term to describe a festering sore would be ulcer, as lesion is in fact a very general term referring to an injured or disease area of the body. This clarification is purely sematic and does not in any way affect the manner in which DELT anomalies are accounted for, or defined by Ohio EPA.

- **Tumors:** These result from the loss of carefully regulated cellular proliferative growth in tissue and are generally referred to as neoplasia (Post 1983). In wild fish populations, tumors can be the result of exposure to toxic chemicals. Baumann et al. (1987) identified polynuclear aromatic hydrocarbons (PAHs) as the cause of hepatic tumors in brown bullheads in the Black River (Ohio). Viral infections (*e.g.*, *Lymphocystis*) can also cause tumors. Parasites (*e.g.*, *Glugea anomala* and *Ceratomyxa shasta*; Post 1983) may cause tumor like masses, but these should not be considered as tumors. Parasite masses can be squeezed and broken between the thumb and forefinger, whereas true tumors are firm and not easily broken (P. Baumann, pers. comm.).

The following may be noted on data sheets, but are not considered DELTs:

- *Anchor worm (Lernaea cyprinacea):* This is a common parasitic copepod and can be identified by the presence of an adult female which appears as a slender, worm-like body with the head attached (buried) in the flesh of the fish. A small, characteristic sore is left after the anchor worm detaches. Attachment sites are included in the determination of light and heavy infestations. If the former attachment site becomes infected and enlarged as the result of an infection, it should be recorded as a lesion.

- *Black spot:* This disease is common on fish in Ohio streams and is caused by the larval stage of a trematode parasite (*e.g.*, *Uvulifer ambloplitis* and *Crassiphiala bullboglossa*). They are easily identified as small black cysts (approximately the size of a pin head) on the skin and fins. Black spot has been reported as being most prevalent on fish inhabiting relatively shallow stream and lake habitats which have an abundance of aquatic vegetation with snails and fish eating birds, two of its intermediate animal hosts. It may also increase in frequency in mildly polluted streams or where fish are crowded due to intermittent pooling.

- *Leeches:* These parasites belong to the family Piscicolidae and are usually greenish brown in color and 5-25 millimeters long (Allison et al. 1977). Leeches can be identified by the presence of two suckers (one on each end) and the ability to contract or elongate their body. They may occur almost anywhere on the external surface of fish, but are most frequently seen on the anterioventral surface of bullheads (*Ictalurus*). Field investigators should become familiar with the small sores or scars left by leeches as these are included in the determination of light and heavy infestations. If these sores become enlarged and infected, they are also regarded as lesions. Leeches are seldom harmful to fish unless the infestation is heavy.

- *Fungus:* This is a growth that can appear on a fish's body as a white cottony growth and is most frequently caused by the fungus *Saprolegnia parasitica*. The fungus usually attacks an injured or open area of the fish and can eventually cause further disease or death.

- *Icthyophthirus multifilis (Ich):* This is a protozoan that manifests itself on a fish's skin and fins as a white spotting. This disease rarely occurs in wild fish populations.

- *Popeye:* This disease is generally identified by bulging eyes and can be caused by gas accumulation in areas where the water is gas supersaturated. It occurs most frequently in Ohio as the result of fluid accumulation from viral infection, nematodes (*Philometra*), or certain trematode larvae (Rogers and Plumb 1977).

Information on external anomalies is recorded because many are either caused or exacerbated by environmental factors and often times indicate the presence of multiple, sublethal stresses. Komanda (1980) found that morphological abnormalities are uncommon in unimpacted natural fish populations. The effects of temperature, salinity, dissolved oxygen, diet, chemicals, organic wastes, etc., especially during the ontogeny and larval stages of fishes can be the cause of many types of anomalies (Berra and Au, 1981) and thereby the presence of anomalies may act as an indicator of pollution stress. A high frequency of DELT anomalies (deformities, eroded fins, lesions, and tumors) is a good indication of a stress caused by sublethal stresses, intermittent stresses, and chemically contaminated substrates. The percent DELT anomalies is a metric of the IBI (Ohio EPA 1987). Field investigators are urged to refer to texts on fish

health for further information and pictures of specific anomalies. If necessary, affected fish should be preserved for laboratory examination.

4) The Qualitative Habitat Evaluation Index (QHEI)

The Qualitative Habitat Evaluation Index (QHEI) is a physical habitat index designed to provide an empirical, quantified evaluation of the general lotic macrohabitat characteristics that are important to fish communities. A detailed analysis of the development and use of the QHEI is available in Rankin (1989) and Ohio EPA (2006) which are both available on the Ohio EPA website (http://epa.ohio.gov/dsw/bioassess/BioCriteriaProtAqLife.aspx). The QHEI assessment should be completed at the time the fish sampling is completed; only one QHEI score is required at a two-pass or three-pass site.

Part C) Laboratory Methods

1) Handling Preserved Materials

Preservation Techniques

Fish that are preserved for subsequent identification or for vouchers are immersed in a fixative solution as soon as possible after capture. This helps retain chromatophore patterns which aid in identification. The recommended fixative is a solution of one part commercially prepared formalin and nine parts water. Large fish or containers with closely packed fish require stronger concentrations of formalin. Strong solutions of formalin can cause gaping or distortion of the mouth and gills, thus care should be taken to obtain correct concentrations when making the solutions. Specimens more than a few inches long should be slit along the right side of the abdomen prior to preservation; fish heavier than one or two pounds should also be injected in the muscles on each side of the backbone. Fish normally remain in the formalin solution for at least two to three weeks to fix the tissues. Fish are then rinsed in clean water to wash off any excess formalin. The specimens are then soaked in clean water for one week to allow leaching of formalin. The specimens are then rinsed in clean water and placed in a 35% alcohol solution for two to three weeks, then switched to a 50% alcohol solution for two to three weeks, and lastly placed in a 70% aqueous solution of ethyl alcohol for permanent storage.

Preserving containers are labelled with a permanent marker as soon as the specimens are collected and detail essential aspects of the sample as completely as possible. Minimum information to be recorded includes the stream or river name, location, date, river mile, and crew member initials. This information may be written on the initial preserving container with a permanent marker. If instead a paper label inserted inside the voucher container is used, it should be 100% waterproof and labeled with India ink or a soft lead pencil.

Laboratory Identification and Verification

As discussed previously, fish are field identified by the field crew leader when the field identification is certain. However, if there is any uncertainty, the fish are preserved and brought back to the laboratory for verification. In the Ohio EPA EAS laboratory, keys available to identify preserved fish include, but are not limited to, Becker (1983), Clay (1975), Pflieger (1975), Scott and Crossman (1973), and Trautman (1957, 1981). Scientific nomenclature follows the recommendations of the American Fisheries Society (http://fisheries.org).

Identifications are verified in-house by at least one trained, full-time Ohio EPA staff. Once taxonomic verification is made, the information is transferred to the fish data sheet for the respective location and either entered into or corrected in EA[3]. If there remains any question as to the identity of a specimen, it is taken to the Ohio State Museum of Zoology (OSUMZ) for identification by the Curator of Fishes.

Disposition

Ohio EPA maintains an up-to-date reference collection of Ohio and midwestern U.S. region fishes at the Ohio EPA Groveport Field Office. New species or unique specimens are added to the collection as they are encountered. Duplicate specimens are deposited in the OSUMZ where they are permanently catalogued.

2) Data Handling and Analysis

Data Sheets

Fish data sheets (see example, Figure 12) are completed in the following manner.

(1)Header

- Station ID – Internal Ohio EPA use only.
- **Netter**: Last name of primary sampler (shocker) for site.
- **Crew Leader**: Last name of fish crew leader responsible for day's work.
- **Other**: Last names of remaining crew members not identified under the preceding categories.
- Date – Month/day/year.
- Time – Time of day.
- Stream – River or stream being sampled.
- Location – Location described as adjacent to, upstream or downstream from a notable landmark.
- River Code – Internal Ohio EPA use only.
- River Mile – River mile to the nearest 0.1 mile determined by inspection of Ohio EPA's River Mile maps: (http://www.arcgis.com/home/webmap/viewer.html?webmap=992b6fe112e14623bf3cfcc3a048f7e5&extent=-86.7944,38.2065,-78.6564,42.1167).
- Distance – Electrofishing distance in meters to the nearest 1 m.

- Sampler Type – Sampler type letter code should be noted here (letter codes can be found in Table 5).
- Data Source – Internal use by Ohio EPA only.
- Time Fished – Actual time devoted to sampling fish in seconds or minutes and seconds.
- Secchi – Secchi reading, if taken. Secchi discs and/or transparency tubes may be used to determine if sufficient clarity is present for sampling.
- County – The county where sampling is occurring.
- ALP – Internal Ohio EPA use only.
- Project – Internal Ohio EPA use only.
- Comments – Provided for any unique observations regarding the site (e.g., paucity of fish, sewage odor noted, cattle in stream above sampling zone, stream much lower than first pass,…) or any field parameters such as D.O. or pH that may be collected while sampling.

(2) Sampling Results

- FINS Code – Each species and any hybrids are recorded by a family species code following the system presented in Tables 7, 8, or 9 of Volume II. Gross external anomalies if any, are recorded for each species according to guidance stated previously.

Additional information that can be entered into EA^3 includes purpose of the data, latitude and longitude, site drainage area (mi^2), local gradient, sample designation, flow, temperature (^oC), dissolved oxygen (mg/l), and any notations in the comments field.

Data Storage and Compilation

All completed fish data sheets are logged by the field crew leaders to prevent loss and assure that all sites are sampled according to the plan of study. Upon returning to the office, the fish crew leader logs the data sheets into electronic master tracking sheets kept at the Ohio EPA EAS Office. Data is then entered into EA^3 which was developed by Ohio EPA for the purpose of storing and analyzing fish relative abundance data. The data sheets are then assembled in a notebook along with QHEI sheets. This is then filed for future reference at the Ohio EPA EAS Office. Any subsequent changes that are made to the fish data sheets are initialed and dated. After all data for a survey have been entered into EA^3, the entered data are proofread by the field crew leader for accuracy. All corrections or updates are then entered into EA^3. Occasionally data from a sampling run may be considered invalid for calculating IBI and Mlwb scores (e.g. due to elevated water levels during sampling, etc.). Although these data are entered into FINS they are designated as invalid samples for calculating community evaluation indices.

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Appendix 1

Retired Fish Sampling Methods

Backpack Electrofishing/Seine Sampling Methods and Equipment

The procedures and equipment used with the backpack electrofishing/seine methods (sampler type G) are generally the same as the backpack electrofishing method (sampler type F), except that seines are used in conjunction with the backpack electrofishing unit. This method was used to generate relative abundance data suitable for calculating the IBI in the years 1977-1981. The use of seines was discontinued in 1982 due to the relatively high degree of variability in the data caused by differing levels of skill between field crews. A detailed description of the methods can be found in earlier versions of this manual. While this method and seines alone may be used by non-Ohio entities to generate fish relative abundance data, it may not be acceptable to generate IBI or Mlwb scores for aquatic life use attainment purposes. This will be evaluated on a case-by-case basis.

Passive Gear Methods and Equipment

Passive gear methods are those in which the sampling device is stationary and the capture of fish is dependent on their movements onto the sampling device. These methods are not used on a routine basis by Ohio EPA and are considered experimental. Four types of passive gear (fyke nets, trap nets, modified hoop nets, and gill nets) may be used to supplement boat electrofishing data in large rivers, estuaries, marshes, wetlands, lakes or impoundments. Fyke nets and trap nets are used in shallow water while modified nets and gill nets are used in deep or open water.

Fyke nets (Sampler type I) are used in areas where a side channel can be completely blocked off by the two side leads which “funnel” fish into the net. Locations such as tributaries, marsh channels, or other channels off of the main channel are potential sampling sites. Fyke nets are set by anchoring the cod end just upstream from the channel confluence with the river, with the open end facing the main channel. The two side wings are angled toward the shoreline which blocks as much of the channel as possible. A center lead extends into the main channel helping to guide fish into the net. The Maine fyke net consists of a 4.5 meter body (11.4 millimeter stretched mesh) supported by five square spring steel frames with three internal throats on the first three frames. Two 9 meter x 1.2 meter wings and one 22.5 meter center lead are attached to the open end of the net. The cod end and all leads are anchored and floats attached to each anchor.

Trap nets (Sampler type J) are used to sample impoundments and wide river channels with slow velocity conditions. Trap nets are set in structurally complex areas where fish movement and density are anticipated to be highest in order to maximize net catches. One center lead is fastened to shore and the net is set perpendicular to the shore with the cod end anchored and marked with a float. Net dimensions are similar to those of the fyke net except a shorter 15 meter center lead is used. Modified hoop nets (Sampler type J) are used when sampling the deeper mid-channel areas. Modified hoop nets have been used to successfully capture fish moving upstream and downstream. By connecting two hoop nets together facing in opposite directions and placing them parallel to the flow, it is possible to discern fish movement in both the upstream and downstream directions. Modified hoop nets are set in mid-channel parallel to the flow and anchored and marked with floats at both ends.

Gill nets (Sampler type K) are set in open water areas to sample fishes in large rivers, lakes, and impoundments where portions of the fish community are not accessible to shoreline electrofishing. Gill nets can be set at the surface, mid-depth, or on the bottom, depending on the objectives of the sampling and intended target species within the fish community. Gill nets are anchored in open water areas and marked with floats on both ends. Monofilament experimental gill nets are 37.5 meters long with 7.5 meter panels of 15.2 millimeter, 22.9 millimeter, 25.4 millimeter, 40.6 millimeter, and 50.8 millimeter bar mesh.

All passive gear is checked and emptied 12 to 24 hours after setting. Standard procedures are used to process fish captured by passive gear. Data collected by passive gear can used to determine relative abundance which is expressed as numbers/24 hours and weight (kg)/24 hours. These results have not been used by Ohio EPA to calculate IBI and Mlwb scores for aquatic life use attainment purposes.