



# ORSANCO BIOLOGICAL INDICES AND ASSESSMENT METHODOLOGY

The Ohio River Valley Water Sanitation Commission is an inter-state agency tasked with monitoring the Ohio River and ensuring it supports defined beneficial public uses and maintains a healthy aquatic community. ORSANCO biologists, through assistance with state and federal partners, have developed a means to assess the relative condition of the Ohio River aquatic community. This document details how these assessments are completed.

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# ORSANCO Biological Indices and Assessment Methodology

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## **1.0 Assessment Overview**

ORSANCO biologists conduct surveys to assess the unique assemblages present within the larger biotic community of the Ohio River, of which there are more than 160 fish species and over 500 macroinvertebrate taxa. This diverse biological community is supported by the large drainage area of the Ohio River basin and the heterogeneity of habitats present in the 981 miles of the Ohio River. Biologists collect instream habitat, water quality, and aquatic vegetation data to categorize this heterogeneity into habitat classes. These paired biotic and abiotic datasets are collected annually at probabilistic sites within a subset of the 19 navigational pools (i.e. impoundments created by navigational dams) on the Ohio River. The biotic data are assessed by two independent multi-metric biological indices, the modified Ohio River Fish Index (*mORFI*n) and the Ohio River Macroinvertebrate Index (ORMI)n), and different statistical thresholds are applied to each habitat class. The score of each site is aggregated and a central tendency measure is used to arrive at two condition ratings: one each for the fish and macroinvertebrate assemblages. These results are reviewed by ORSANCO biologists and the Biological Water Quality Subcommittee (BWQSC), which comprises representatives from ORSANCO member states and federal partners. ORSANCO biologists and the BWQSC follow standardized methods for the evaluation of the *mORFI*n and ORMI)n results by comparing them to established biocriteria to arrive at a final assessment score and condition rating for each navigational pool.

## **2.0 Data Collection**

### **2.1 Sampling Design**

ORSANCO biologists conduct biological surveys in two to four pools per year, depending on resource availability. This schedule allows for an assessment of each pool to be completed every six to seven years. Each assessment consists of the probabilistic sampling of 15 randomly generated sites, which has been established by Blocksom et al. (2005) to be an appropriate number of sites to achieve an accurate assessment of the fish assemblage within a pool.

To maintain consistency across sampling years, surveys are conducted during an index period which runs between July 1<sup>st</sup> and October 31<sup>st</sup>. This restriction maximizes the opportunity to sample assemblages at normal flow conditions. The target areas for fish surveys are 500 m littoral reaches, incorporating all available habitat types and using non-lethal electrofishing methods approved by federal and state agencies. Fishes are collected at night when fish activity is at its diurnal peak, maximizing the number of individuals and species captured (Simon and Sanders 1999). This method attempts to provide most accurate representation of the fish assemblage. Collected individuals are identified to species, measured to total length using 3 cm size classes, enumerated, and evaluated for any DELTs (deformity, erosion, lesion, tumors) before being returned to the water. A subset of smaller individuals may be preserved (10% formalin) in order to confirm identification in the laboratory.

### **2.2 Macroinvertebrate Collection**

Two sampling methods are used to collect macroinvertebrates: Hester-Dendy (HD) samplers and multi-habitat kicks (MH). HDs are the primary sample used for pool assessment purposes as they best reflect the Ohio River macroinvertebrate community. MHs are used for trend assessments and a method of scoring single visit sites. HDs are constructed (Appendix C), placed on the river bottom in 3 m of water at the downstream end of each 500 m sampling site, and secured to the shore. Similar to fishes,

macroinvertebrate sampling is restricted to a defined time period within each year. HDs are deployed for six weeks, beginning September 1<sup>st</sup>, allowing adequate time for macroinvertebrate colonization. After the six week colonization period, HDs are retrieved and MHs are conducted (Appendix C). At each transect, all available habitats (e.g., sand flats, woody debris, boulders, etc.) are sampled relative to the proportion of their availability. The MHs from each transect are then combined to represent the assemblage at each site. The resulting samples are identified to the finest taxonomic resolution and enumerated separately for HDs and MHs at each site.

### 2.3 Instream Habitat Assessment

Instream substrates and depth are sampled using a copper sounding rod (Appendix C). The substrate is probed and the occurrence of each type (e.g., boulder, cobble, gravel, sand, fine, and/or hardpan) is recorded at 3 m intervals from shore, resulting in up to 11 measurements per transect. This is repeated at each transect within the site. Quantitative surveys of submerged aquatic vegetation (SAV) began in 2016 in response to an observed increase of non-native vegetation (e.g., *Hydrilla verticillata*). SAV surveys are conducted with a double-sided rake (Appendix C) at the same transect intervals as instream substrate.

## 3.0 Biological Indices

### 3.1 Habitat Class Designation

The resulting substrate composition and depth measurements are used to classify each site into one of five habitat classes (Figure 1). Class 'A' and 'B' sites are dominated by coarse substrates in areas less than 3 m in depth (>80.9% and >50.4%, respectively). Class 'C' sites are moderate in their substrate composition, containing less than 50.4% coarse and 76.9% fine substrates. Class 'D' and 'E' sites have predominantly fine substrates (>76.9%), only differing in depth; 'D' sites are shallower than 'E' sites. This distinction was made based upon the relative percentage of the site that is less than 6m in depth; a site greater than 65.4% is classified as 'D'; those with smaller percentages are classified as 'E' sites.

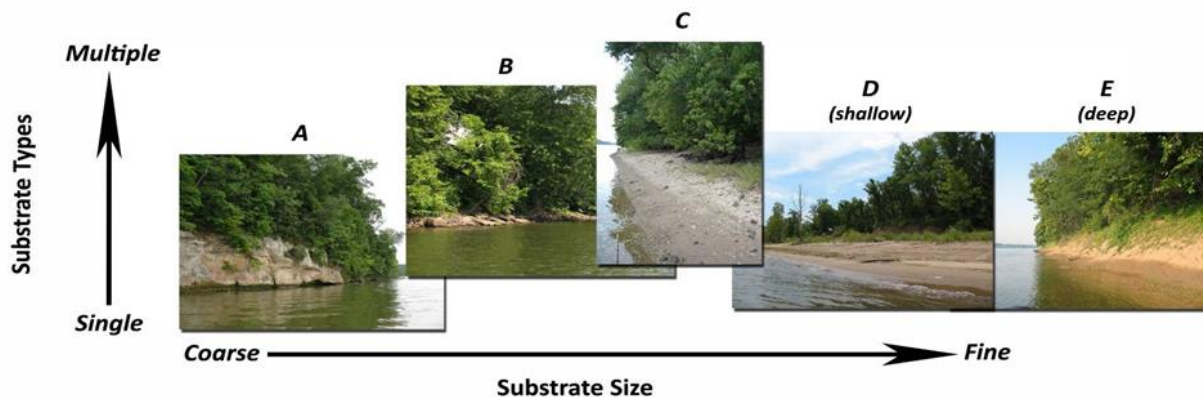


Figure 1. Habitat classes of the Ohio River exhibit a gradient from highly coarse substrates to the sand/fine dominated substrates differing by water depth.

Other abiotic data collected at probabilistic sites includes water temperature, pH, conductivity, and Secchi depth, and water quality surface grab samples. These data are collected at the time of electrofishing to inform best electrofishing practices and continue building datasets used in the

derivation of condition gradients that were integral to the development of the ORMIn. Additionally, water quality and SAV data are available for review by the BWQSC.

### 3.2 Metric Selection

Prior to metric analysis, all taxa are classified based on their life histories (Appendix A). These classifications were compiled from pertinent literature, reviewed by regional experts on fishes and macroinvertebrates, and ultimately approved by the BWQSC. These classifications are used to generate metrics associated with raw and/or proportional counts of fish and macroinvertebrates (Table 1).

**Table 1. The metrics that comprise the modified Ohio River Fish Index (*mORFIn*) and Ohio River Macroinvertebrate Index (*ORMIn*) scores; macroinvertebrate metrics shown relative to those specific to 3 m depth contour settings of Hester-Dendy (HD) samplers and multi-habitat D-net samples with a minimum of 200 individuals collected (MH 200). The metric selection process generally followed Hughes et al. (1998) and McCormick et al. (2001) by examining candidate metrics for scoring range, variability, responsiveness, and redundancy (Emery et al. 2003). Irruptive species are not included in the calculation of metrics denoted by an “\*”.**

<b>Fish Metric</b>	<b>Definition</b>
Native Species	Number (No.) of species native to the Ohio River, excluding non-natives and hybrids
Intolerant Species	No. of species intolerant to pollution and habitat degradation, excluding non-natives and hybrids
Sucker Species	No. of sucker species (e.g. redhorse and buffalo), excluding non-natives and hybrids
Centrarchid Species	No. of black bass, sunfish, crappie species, excluding non-natives and hybrids
Great River Species	No. of species primarily found in large rivers, excluding non-natives and hybrids
% Piscivores*	% of individuals that consume other fish
% Invertivores*	% of individuals that consume invertebrates
% Detritivores*	% of individuals that consume detritus (dead plant material)
% Tolerants*	% of individuals tolerant to pollution and habitat degradation
% Simple Lithophils*	% of individuals belonging to breeding groups that require clean substrates for spawning
% Non-natives*	% of individuals not native to the Ohio River, including both non-natives and hybrids
No. DELT anomalies	No. of individuals with Deformities, Erosions, Lesions, Tumors present
Catch per unit effort (CPUE)	Total abundance of individuals, excluding non-natives, hybrids, and tolerants
<b>Macroinvertebrate Metric - HD</b>	<b>Definition</b>
No. Taxa	No. of unique taxa
EPT Taxa	No. of taxa that belong to are either the Ephemeroptera, Plecoptera, or Trichoptera orders
Predator Taxa	No. of taxa that are predators
% Collector-Gatherer Taxa	% of taxa that feed on fine particulate organic matter
% Caenids*	% of individuals (ind.) that belong to the pollution tolerant <i>Caenidae</i> family of Ephemeropterans
% Odonates*	% of ind. that belong to the Odonata order
% Intolerants*	% of ind. intolerant to pollution and habitat degradation
% Clingers*	% of ind. that cling to instream habitat
<b>Macroinvertebrate Metric – MH 200</b>	<b>Definition</b>
No. Sprawlers	No. of individuals from species possessing the sprawler habitat designation
% Odonates excluding Gomphidae*	% of ind. that belong to the Odonata order, excluding those from the tolerant Gomphidae family
% EPT Taxa	% of taxa that belong to are either the Ephemeroptera, Plecoptera, or Trichoptera orders
% Collector-Gatherer Taxa	% of taxa that feed on fine particulate organic matter

Metrics represent various aspects of the community: diversity, abundance, feeding and reproductive guilds, pollution tolerance, and physical health of individuals. Given the highly modified nature of the Ohio River, metrics were not evaluated relative to a pre-impoundment reference condition. Instead, the metrics of each index were selected for their ability to distinguish between levels of abiotic degradation. The sensitivity of the original ORFIn metrics were evaluated by comparing performance between sites with no observed impact to those in proximity to known point sources (Emery et al. 2003). The original ORFIn was modified (*mORFIn*) in 2010 to include continuous scoring methods (Section 3.2).

Paired sediment toxicity, sestonic nutrients, water quality, and macroinvertebrate samples were used to derive the ORMIn index. The addition of these abiotic variables was supported by a cooperative USEPA

EMAP-GRE grant (2007-2011) and therefore not available during the generation of the ORFIn. Sites within the ORMIn calibration dataset were categorized into least and most impacted based upon their position along a condition gradient comprising these abiotic variables (e.g., Angradi et al. 2009). Macroinvertebrate metrics were generated for each sample type (HD and MH) and separately evaluated for their ability to delineate between sites that fell on the extremes of the abiotic condition gradient (i.e. least impacted versus most impacted sites).

### 3.3 Metric Calculation and Longitudinal Adjustment

In order to maximize responsiveness and ecological importance of individual metrics, some alterations to metric calculations were necessary. Irruptive species (e.g., Gizzard Shad, *Dorosoma cepedianum* and Zebra Mussels, *Dreissena polymorpha*) are excluded from all proportional metrics as their abundance in a sample may be more reflective of their behavior than site condition (Fausch et al. 1990; Simon and Emery 1995; Simon and Sanders 1999). Additionally, when irruptive species abundances are extreme they can cloud the effect of other species in the sample. For a similar reason, all hybrid and non-native taxa are excluded from count metrics (Yoder and Rankin 1995). Lastly, when calculating all fish or macroinvertebrate taxa metrics, only the most taxonomically resolute records are counted per sample. In some cases the condition of an individual may prevent species identification (e.g., early life stage, damaged specimen, etc.). This can result in a sample containing phylogenetically similar individuals identified to different taxonomic levels. For example, a sample may contain two specimens identified as *Dicrotendipes* sp. and *Dicrotendipes lucifer*. For the purposes of generating the %Collector-Gatherer Taxa metric, only the *Dicrotendipes lucifer* would be included in the calculation to avoid the potential for over-inflation. The *Dicrotendipes* sp. specimen would only be counted as a taxa in the event that a more taxonomically resolute specimen was not identified from the sample. However, both specimens would count towards metrics pertaining to total numbers of individuals.

The *m*ORFIn and ORMIn were specifically generated for application on the Ohio River, which has a longitudinal distance of 981 miles. To account for variation in taxa distribution, each metric was evaluated for correlation with river mile (Emery et al. 2003). Longitudinal variation was accounted for using the equation:

$$[m(x) + b] + \text{residual constant}$$

Linear regressions were used to determine significant effects, and regression residuals were calculated for each metric that demonstrated a trend (Table B1, Appendix B). The lowest residual observed in the calibration dataset was added, as a constant, to remove any negative values. The sum of the constant and residual value was used in the remaining metric calculations as it represents the relative value of the raw metric with longitudinal variation removed. For any metric that did not display a longitudinal trend, the raw observed value was simply used to calculate a metric score.

All metrics are scored on a hundred point scale. The '100' score threshold was set at the 95<sup>th</sup> percentile value, and the '0' score threshold was set to the 5<sup>th</sup> percentile value, according to the CALU method (Blocksom 2003). This method employs continuous scoring (e.g., 0-100) in which all sites are used to set thresholds rather than relying on reference sites, and the lower and upper thresholds are set based on the statistical distribution of the data. Positive metrics (i.e. metrics whose increased values reflects a healthy community) were scored as:

$$\frac{\text{observed value} - 5\text{th percentile}}{95\text{th percentile} - 5\text{th percentile}} \times 100$$

Negative metrics (i.e. metrics whose increased values reflect an unhealthy community) were scored as:

$$\frac{95\text{th percentile} - \text{observed value}}{95\text{th percentile} - 5\text{th percentile}} \times 100$$

It is mathematically possible for a metric to be scored beyond the 0-100 range. In these scenarios a metric scoring below zero is given a 0 score and a site scoring above 100 is lowered to 100. For each index, metric scores (Table 1) are averaged by site and then all sites are averaged for final *mORFI*n and *ORMI*n scores.

### 3.4 Index Scores and Assessment

With either the *mORFI*n or *ORMI*n, species-specific habitat preferences precludes aggregation of scores across sites. To remove bias attributable to varying habitat preferences, metric scores are calculated relative to statistical thresholds of each habitat class. Using calibration datasets, the statistical distribution (5<sup>th</sup>, 25<sup>th</sup>, 50<sup>th</sup>, 75<sup>th</sup>, and 95<sup>th</sup> percentiles) of average metric scores was calculated for each of the five habitat classes based on the presence or absence of SAV (Table B2 and Table B3, Appendix B). For the *ORMI*n, sites classified with habitats A or B are treated equally. Likewise, habitats D or E are treated equally. This was necessary to have a sufficient dataset from which to calculate the statistical thresholds (Figure 2). A discrete score (0, 10, 20, 30, 40, 50, 60) was assigned to the minimum and maximum observed average metric scores and each of the statistical thresholds (Figure 2). Final *mORFI*n and *ORMI*n scores are calculated based on how a given site scores relative to the thresholds of calibration sites within the same habitat class:

$$\left[ \left( \frac{\text{observed average metric score} - \text{lower threshold}}{\text{upper threshold} - \text{lower threshold}} \right) \times 10 \right] + \text{discrete score of lower threshold}$$

As with the individual metric scores, it is mathematically possible for a metric to be scored beyond the 0-60 range. In these scenarios a metric scoring below zero is given a 0 score; a site scoring above 60 is lowered to 60. To aid in the communication of our assessment results, six condition ratings (“Very Poor”, “Poor”, “Fair”, “Good”, “Very Good”, and “Excellent”) were applied to the ranges between each statistical threshold (Figure 2).

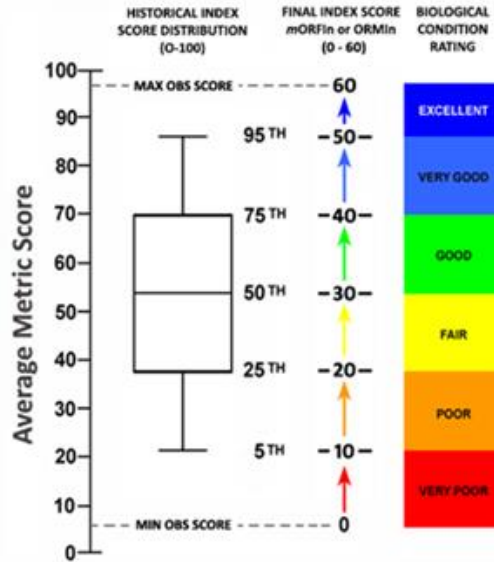


Figure 2. Multi-step approach to convert average metric score (0-100) of each individual site into final index scores (0-60) based on the varying expectations of the different habitat classes. Each Habitat Class (A-E) has different thresholds used to convert average metric score to final *mORFIn* and *ORMIn* score (Appendix B).

ORSANCO uses the 25<sup>th</sup> percentile as the statistical threshold with which to define biocriteria and determine aquatic life use (ALU) attainment (i.e. possessing intact biological communities). The biocriteria (i.e. 25<sup>th</sup> percentile) for both the *mORFIn* and *ORMIn* is an index score of 20.

Final index scores for the probabilistic sites are averaged within a given pool and compared to this biocriteria. Following similar methods to Blocksom et al. (2005), it was determined that the macroinvertebrate assemblage can be accurately represented with data from a minimum of 10 probabilistic sites; the fish index requires a minimum of 15 probabilistic sites. A pool is assessed to be in *full support* of its ALU designation if both the *mORFIn* and *ORMIn* scores are greater than or equal to 20 (i.e. a condition rating “Fair”, “Good”, “Very Good”, or “Excellent”). A pool is in *partial support* of its ALU designation if only one of the indices’ scores greater than or equal to 20 while the other is less than 20 but greater than 10 (i.e. a “Poor” rating). Any pool in which both indices score below a 20, or in which at least one index scores below 10 (i.e. a “Very Poor” rating), would be considered in *non-support* of its ALU designation. An inability to collect requisite assemblage data, data qualifications, and resource availability can contribute to scenarios that preclude assessment determination for either index within an assessment cycle. The *Evaluation of Biological Pool Survey Results* section of the ORSANCO Biological SOP further details how ORSANCO and the BWQSC proceed in these cases (Appendix C).

#### 4.0 Assessment Reporting

ORSANCO summarizes the results of the biological surveys and pool assessments in annual reports made available to the public via our website: [www.orsanco.org/publications/pool-assessments](http://www.orsanco.org/publications/pool-assessments). These biological assessments are part of a broader water quality monitoring effort that ORSANCO conducts on behalf of Ohio River main stem states (Illinois, Indiana, Kentucky, Ohio, Pennsylvania, and West Virginia). Every two years, a combined assessment of Ohio River designated uses is completed in cooperation with state 305(b) coordinators ([www.orsanco.org/publications/biennial-assessment-305b-report](http://www.orsanco.org/publications/biennial-assessment-305b-report)). This



biennial assessment reports the conditions of Ohio River water quality and the ability to which the river supports each of its four designated uses: aquatic life, public water supply, contact recreation, and fish consumption.

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## Fish Taxa Designations

**Table A1. Common and Latin names for fish taxa collected on the Ohio River in rows. Columns pertain to twelve of the thirteen fish metrics comprising the modified Ohio River Fish Index (Native = Native Species, Int = Intolerant Species, Sucker = Sucker Species, Cent = Centrarchidae Species, GR = Great River Species, Pisc = %Piscivorous Individuals, Inv = %Invertivorous Individuals, Detr = %Detritivorous Individuals, Tol = %Tolerant Individuals, SL = %Simple Lithophilic Individuals, NN = %Non-native Individuals, CPUE = Catch Per Unit Effort). The DELT anomaly metric is not displayed as the presence of Deformities, Erosions, Lesions, and or Tumors observed on collected fish is recorded by the sampler and not influenced by any designation. Taxa included in the calculation of a particular metric are denoted with an “X”. Taxa with an “\*” are considered irruptive species and are excluded from some metric calculation (See Section 3.2).**

Common Name	Family	Latin Name	Native	Int	Sucker	Cent	GR	Pisc	Invert	Detr	Tol	SL	NN	CPUE
Petromyzontidae sp	Petromyzontidae	Petromyzontidae sp	X											X
Silver Lamprey	Petromyzontidae	Ichthyomyzon unicuspis	X											X
Ohio Lamprey	Petromyzontidae	Ichthyomyzon bdellium	X	X										X
Chestnut Lamprey	Petromyzontidae	Ichthyomyzon castaneus	X											X
Lampetra sp	Petromyzontidae	Lampetra sp	X											X
American Brook Lamprey	Petromyzontidae	Lethenteron appendix	X											X
Paddlefish	Polyodontidae	Polyodon spathula	X	X			X					X		X
Longnose Gar	Lepisosteidae	Lepisosteus osseus	X					X						X
Spotted Gar	Lepisosteidae	Lepisosteus oculatus	X					X						X
Shortnose Gar	Lepisosteidae	Lepisosteus platostomus	X				X	X						X
Bowfin	Amiidae	Amia calva	X					X						X
American Eel	Anguillidae	Anguilla rostrata	X				X							X
Alewife	Clupeidae	Alosa pseudoharengus											X	
Skipjack Herring	Clupeidae	Alosa chrysochloris	X				X							X
Gizzard Shad*	Clupeidae	Dorosoma cepedianum	X											X
Threadfin Shad	Clupeidae	Dorosoma petenense	X											X
Goldeye	Hiodontidae	Hiodon alosoides	X	X			X					X		X
Mooneye	Hiodontidae	Hiodon tergisus	X	X			X					X		X
Northern Pike	Esocidae	Esox lucius	X					X						X
Muskellunge	Esocidae	Esox masquinongy	X					X						X
Muskellunge X Northern Pike	Esocidae	Esox lucius x masquinongy						X					X	
Leuciscidae sp	Leuciscidae	Leuciscidae sp	X											X
Common Carp X Goldfish	Cyprinidae	C. carpio x C. auratus									X		X	
Common Carp	Cyprinidae	Cyprinus carpio								X	X		X	
Grass Carp	Cyprinidae	Ctenopharyngodon idella									X		X	
Silver Carp	Cyprinidae	Hypophthalmichthys molitrix									X		X	
Bighead Carp	Cyprinidae	Hypophthalmichthys nobilis									X		X	
Goldfish	Cyprinidae	Carassius auratus								X	X		X	
Golden Shiner	Leuciscidae	Notemigonus crysoleucas	X								X			
Red Shiner	Leuciscidae	Cyprinella lutrensis							X		X		X	

ORSANCO Biological Indices and Assessment Overview – Appendix A: Taxa Designations

Common Name	Family	Latin Name	Native	Int	Sucker	Cent	GR	Pisc	Invert	Detr	Tol	SL	NN	CPUE
Cypress Minnow	Leuciscidae	Hybognathus hayi	X											X
Mississippi Silvery Minnow	Leuciscidae	Hybognathus nuchalis	X				X							X
Notropis sp	Leuciscidae	Notropis sp	X											X
Miss. Silv. Minn. X Silver Chub	Leuciscidae	H. nuchalis x M. storeriana											X	
Striped Shiner	Leuciscidae	Luxilus chrysocephalus	X											X
Common Shiner	Leuciscidae	Luxilus cornutus	X						X					X
Channel Shiner	Leuciscidae	Notropis wickliffi	X	X					X					X
Redfin Shiner	Leuciscidae	Lythrurus umbratilis	X											X
Spottail Shiner	Leuciscidae	Notropis hudsonius	X											X
Rosyface Shiner	Leuciscidae	Notropis rubellus	X	X					X					X
Spotfin Shiner	Leuciscidae	Cyprinella spiloptera	X											X
Emerald Shiner*	Leuciscidae	Notropis atherinoides	X											X
Silver Shiner	Leuciscidae	Notropis photogenis	X											X
Ghost Shiner	Leuciscidae	Notropis buchmanii	X				X		X					X
Silverband Shiner	Leuciscidae	Notropis shumardi	X											X
Sand Shiner	Leuciscidae	Notropis stramineus	X						X					X
River Shiner	Leuciscidae	Notropis blennioides	X				X		X			X		X
Bigeye Shiner	Leuciscidae	Notropis boops	X											X
Steelcolor Shiner	Leuciscidae	Cyprinella whipplei	X						X					X
Pugnose Minnow	Leuciscidae	Opsopoeodus emiliae	X											X
Shoal Chub	Leuciscidae	Macrhybopsis hystoma	X				X		X					X
Bigeye Chub	Leuciscidae	Hybopsis amblops	X	X					X			X		X
Silver Chub	Leuciscidae	Macrhybopsis storeriana	X				X		X			X		X
River Chub	Leuciscidae	Nocomis micropogon	X											X
Central Stoneroller	Leuciscidae	Camptostoma anomalum	X											X
Suckermouth Minnow	Leuciscidae	Phenacobius mirabilis	X						X					X
Bluntnose Minnow	Leuciscidae	Pimephales notatus	X							X	X			
Fathead Minnow	Leuciscidae	Pimephales promelas	X							X	X			
Bullhead Minnow	Leuciscidae	Pimephales vigilax	X											X
Silverjaw Minnow	Leuciscidae	Notropis buccatus	X											X
Western Blacknose Dace	Leuciscidae	Rhinichthys obtusus	X									X		X
Gravel Chub	Leuciscidae	Erimystax x-punctatus	X											X
Streamline Chub	Leuciscidae	Erimystax dissimilis	X											X
Creek Chub	Leuciscidae	Semotilus atromaculatus	X											X
White Sucker	Catostomidae	Catostomus commersonii	X							X	X	X		
Carpoides sp	Catostomidae	Carpoides sp	X		X					X				X

ORSANCO Biological Indices and Assessment Overview – Appendix A: Taxa Designations

Common Name	Family	Latin Name	Native	Int	Sucker	Cent	GR	Pisc	Invert	Detr	Tol	SL	NN	CPUE
Ictiobinae sp	Catostomidae	Ictiobinae sp	X		X					X				X
Quillback	Catostomidae	Carpiodes cyprinus	X		X					X				X
River Carpsucker	Catostomidae	Carpiodes carpio	X		X					X				X
Highfin Carpsucker	Catostomidae	Carpiodes velifer	X		X					X				X
Shorthead Redhorse	Catostomidae	Moxostoma macrolepidotum	X	X	X				X			X		X
Smallmouth Redhorse	Catostomidae	Moxostoma breviceps	X	X	X				X			X		X
Moxostoma sp	Catostomidae	Moxostoma sp	X		X							X		X
Silver Redhorse	Catostomidae	Moxostoma anisurum	X		X				X			X		X
River Redhorse	Catostomidae	Moxostoma carinatum	X	X	X				X			X		X
Black Redhorse	Catostomidae	Moxostoma duquesnii	X	X	X				X			X		X
Golden Redhorse	Catostomidae	Moxostoma erythrurum	X		X				X			X		X
Northern Hogsucker	Catostomidae	Hypentelium nigricans	X	X	X				X			X		X
Blue Sucker	Catostomidae	Cycleptus elongatus	X	X	X		X		X			X		X
Ictiobus sp	Catostomidae	Ictiobus sp	X		X					X				X
Smallmouth Buffalo	Catostomidae	Ictiobus bubalus	X		X					X				X
Bigmouth Buffalo	Catostomidae	Ictiobus cyprinellus	X		X					X				X
Black Buffalo	Catostomidae	Ictiobus niger	X		X					X				X
Spotted Sucker	Catostomidae	Minytrema melanops	X		X							X		X
White Catfish	Ictaluridae	Ameiurus catus											X	
Blue Catfish	Ictaluridae	Ictalurus furcatus	X				X							X
Yellow Bullhead	Ictaluridae	Ameiurus natalis	X								X			
Ictalurus sp	Ictaluridae	Ictalurus sp	X											X
Brown Bullhead	Ictaluridae	Ameiurus nebulosus	X								X			
Channel Catfish	Ictaluridae	Ictalurus punctatus	X											X
Black Bullhead	Ictaluridae	Ameiurus melas	X								X			
Noturus sp	Ictaluridae	Noturus sp	X						X					X
Mountain Madtom	Ictaluridae	Noturus eleutherus	X						X					X
Slender Madtom	Ictaluridae	Noturus exilis	X						X					X
Tadpole Madtom	Ictaluridae	Noturus gyrinus	X						X					X
Brindled Madtom	Ictaluridae	Noturus miurus	X						X					X
Freckled Madtom	Ictaluridae	Noturus nocturnus	X						X					X
Northern Madtom	Ictaluridae	Noturus stigmosus	X											X
Stonecat	Ictaluridae	Noturus flavus	X	X					X					X
Flathead Catfish	Ictaluridae	Pylodictis olivaris	X					X						X
Rainbow Trout	Salmonidae	Oncorhynchus mykiss											X	
Trout-Perch	Percopsidae	Percopsis omiscomaycus	X						X					X

ORSANCO Biological Indices and Assessment Overview – Appendix A: Taxa Designations

Common Name	Family	Latin Name	Native	Int	Sucker	Cent	GR	Pisc	Invert	Detr	Tol	SL	NN	CPUE
Pirate Perch	Aphredoderidae	Aphredoderus sayanus	X						X					X
Banded Killifish	Fundulidae	Fundulus diaphanus							X				X	
Western Mosquitofish	Poeciliidae	Gambusia affinis	X											X
Blackstripe Topminnow	Fundulidae	Fundulus notatus	X						X					X
Striped Mullet	Mugilidae	Mugil cephalus	X											X
Mississippi Silverside	Atherinopsidae	Menidia audens	X											X
Brook Silverside	Atherinopsidae	Labidesthes sicculus	X	X										X
Inland Silverside	Atherinopsidae	Menidia beryllina	X											X
Atlantic Needlefish	Belonidae	Strongylura marina	X											X
Banded Sculpin	Cottidae	Cottus carolinae	X											X
Morone Sp	Moronidae	Morone sp	X					X						X
Striped Bass	Moronidae	Morone saxatilis						X					X	
Hybrid Striped Bass	Moronidae	M. saxatilis x chrysops						X					X	
White Perch	Moronidae	Morone americana											X	
White Bass	Moronidae	Morone chrysops	X					X						X
Yellow Bass	Moronidae	Morone mississippiensis	X											X
Centrarchidae sp	Centrarchidae	Centrarchidae sp	X			X								X
Rock Bass	Centrarchidae	Ambloplites rupestris	X			X		X						X
Lepomis Hybrid	Centrarchidae	Lepomis hybrid											X	
Lepomis sp	Centrarchidae	Lepomis sp	X			X								X
Green Sunfish	Centrarchidae	Lepomis cyanellus	X			X					X			
Warmouth	Centrarchidae	Chaenobryttus gulosus	X			X								X
Bluegill	Centrarchidae	Lepomis macrochirus	X			X			X					X
Pumpkinseed	Centrarchidae	Lepomis gibbosus	X			X			X					X
Orangespotted Sunfish	Centrarchidae	Lepomis humilis	X			X			X					X
Longear Sunfish	Centrarchidae	Lepomis megalotis	X			X			X					X
Redear Sunfish	Centrarchidae	Lepomis microlophus							X				X	
Micropterus sp	Centrarchidae	Micropterus sp	X			X		X						X
Smallmouth Bass	Centrarchidae	Micropterus dolomieu	X	X		X		X						X
Largemouth Bass	Centrarchidae	Micropterus salmoides	X			X		X						X
Spotted Bass	Centrarchidae	Micropterus punctulatus	X			X		X						X
Largemouth Bass X Spotted Bass	Centrarchidae	M. salmoides x punctulatus						X					X	
Largemouth Bass X Smallmouth Bass	Centrarchidae	M. salmoides x dolomieu						X					X	
Pomoxis sp	Centrarchidae	Pomoxis sp	X			X								X
White Crappie	Centrarchidae	Pomoxis annularis	X			X		X						X
Black Crappie	Centrarchidae	Pomoxis nigromaculatus	X			X								X

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Common Name	Family	Latin Name	Native	Int	Sucker	Cent	GR	Pisc	Invert	Detr	Tol	SL	NN	CPUE
Eastern Sand Darter	Percidae	Ammocrypta pellucida	X						X			X		X
Etheostoma sp	Percidae	Etheostoma sp	X											X
Mud Darter	Percidae	Etheostoma asprigene	X											X
Johnny Darter	Percidae	Etheostoma nigrum	X						X					X
Greenside Darter	Percidae	Etheostoma blennioides	X	X					X					X
Variagate Darter	Percidae	Etheostoma variatum	X											X
Rainbow Darter	Percidae	Etheostoma caeruleum	X						X			X		X
Orangethroat Darter	Percidae	Etheostoma spectabile	X						X			X		X
Bluntnose Darter	Percidae	Etheostoma chlorosomum	X											X
Tippecanoe Darter	Percidae	Etheostoma tippecanoe	X											X
Tippecanoe Darter	Percidae	Etheostoma tippecanoe	X											X
Fantail Darter	Percidae	Etheostoma flabellare	X						X					X
Stripetail Darter	Percidae	Etheostoma kennicotti	X											X
Bluebreast Darter	Percidae	Etheostoma camurum	X											X
Banded Darter	Percidae	Etheostoma zonale	X	X					X					X
Yellow Perch	Percidae	Perca flavescens	X											X
Percina sp	Percidae	Percina sp	X											X
Logperch	Percidae	Percina caprodes	X	X					X			X		X
Dusky Darter	Percidae	Percina sciera	X	X					X			X		X
Channel Darter	Percidae	Percina copelandi	X	X			X		X			X		X
Gilt Darter	Percidae	Percina evides	X											X
Longhead Darter	Percidae	Percina macrocephala	X											X
Blackside Darter	Percidae	Percina maculata	X						X			X		X
Slenderhead Darter	Percidae	Percina phoxocephala	X	X					X			X		X
River Darter	Percidae	Percina shumardi	X				X		X			X		X
Sander sp	Percidae	Sander sp	X					X				X		X
Walleye	Percidae	Sander vitreus	X					X				X		X
Saugeye	Percidae	S. vitreus x canadensis						X				X	X	
Sauger	Percidae	Sander canadensis	X					X				X		X
Freshwater Drum	Sciaenidae	Aplodinotus grunniens	X											X

## Macroinvertebrate Taxa Designations

**Table A2. Taxonomic serial numbers (TSN) and Latin names for macroinvertebrate taxa collected on the Ohio River in rows. Columns pertain to designations used in the calculation of Ohio River Macroinvertebrate Index scores for samples collected by either Hester-Dendy or multi-habitat kicks samples (Appendix C). EPT = Ephemeroptera, Plecoptera, or Trichoptera Taxa; Pred = Predaceous Taxa; CG = Collector-Gatherer Taxa; CL = Clinger Taxa; SP = Sprawler Taxa; Intol = Intolerant Taxa). Taxa denoted with an “X” are pertinent to the calculation of metrics associated with each column designation. Taxa with an “\*” are considered irruptive species and are excluded from some metric calculations (See Section 3.2).**

TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
48893	<i>Cordylophora caspia</i>			X	X					
50844	Hydridae sp			X	X					
50845	Hydra sp			X	X					
50846	Hydra americana			X	X					
	Xenacoelomorpha sp			X						
54468	Tricladida sp			X						
54502	Planariidae sp									
1038742	<i>Girardia tigrina</i>									
54554	<i>Cura foremanii</i>									
57411	Nemertea sp		X							
59490	Nematoda sp									
63274	Mermithidae sp		X							
64183	Nematomorpha sp									
64358	Polychaeta sp									
68422	Oligochaeta sp			X						
68854	Naididae sp			X						
69290	Hirudinea sp									
	<i>Erpobdella microstoma</i>									
69296	Piscicolidae sp									
69316	<i>Myzobdella lugubris</i>									
69357	Glossiphoniidae sp									
69363	Placobdella sp									
69364	<i>Placobdella papillifera</i>									
69365	<i>Placobdella parasitica</i>									
69366	<i>Placobdella ornata</i>									
69368	<i>Placobdella montifera</i>									
69384	<i>Actinobdella</i> sp									
69386	<i>Actinobdella inequiannulata</i>									
69396	<i>Helobdella</i> sp									
69398	<i>Helobdella stagnalis</i>									
69399	<i>Helobdella triserialis</i>									
69401	<i>Helobdella fusca</i>									
69404	<i>Helobdella transversa</i>									
69407	Hirudinidae sp									
69438	Erpobdellidae sp			X						
69445	<i>Erpobdella punctata</i>			X						
69459	Gastropoda sp									
	Littorinimorpha sp									
70307	<i>Viviparus georgianus</i>									
70311	<i>Campeloma</i> sp									

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TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
70312	Campeloma decisum									
70328	Cipangopaludina sp									
70332	Cipangopaludina japonica									
70493	Hydrobiidae sp									
70508	Probythinella lacustris									
70510	Cincinnatia cincinnatiensis									
70548	Somatogyrus sp									
70667	Birgella subglobosus									
70747	Amnicola sp									
71541	Pleurocera/Elimia sp						X			
71541	Pleuroceridae sp									
71542	Goniabasis sp									
71549	Pleurocera sp						X			
71550	Pleurocera acuta						X			
71554	Pleurocera canaliculata						X			
71601	Leptoxis sp						X			
71647	Leptoxis trilineata						X			
71654	Elimia sp						X			
71879	Lithasia sp						X			
71885	Lithasia obovata						X			
71886	Lithasia verrucosa						X			
71889	Lithasia armigera						X			
76483	Lymnaeidae sp									
76484	Lymnaea sp									
76497	Fossaria sp									
76529	Pseudosuccinea columella									
76568	Ancylidae sp									
76569	Ferrissia sp									
76572	Ferrissia rivularis									
76577	Laevapex fuscus									
76591	Planorbidae sp									
76592	Gyraulus sp									
76595	Gyraulus parvus									
76599	Helisoma sp									
76600	Helisoma anceps									
76626	Menetus sp									
76643	Micromenetus sp									
76654	Planorbella sp									
76671	Planorbella trivolvis									
76676	Physidae sp									
76677	Physa sp									
76698	Physella sp									
79118	Bivalvia sp									
79913	Unionidae sp									
80060	Quadrula quadrula									
80166	Truncilla donaciformis									
80182	Leptodea fragilis									



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TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
80196	Ligumia recta									
80282	Potamilus alatus									
81339	Dreissena polymorpha*				X					
81381	Corbiculidae sp									
81385	Corbicula sp									
81387	Corbicula fluminea									
81388	Pisidiidae sp									
81391	Sphaerium sp									
81400	Pisidium sp									
81427	Musculium sp									
83103	Neumania sp		X							
83832	Cladocera sp									
83863	Sida crystallina									
83972	Leptodora kindtii									
83973	Chydoridae sp									
83992	Chydorus sp									
84195	Ostracoda sp									
85131	Candonidae sp			X						
85132	Candona sp			X						
85257	Copepoda sp									
85258	Calanoida sp									
86110	Harpacticoida sp									
88530	Cyclopoida sp									
89407	Argulus sp									
89856	Mysidae sp									
90045	Mysis relicta									
90276	Taphromysis louisianae									
90979	Almyracuma bacescui									
92658	Asellus sp									
92666	Lirceus sp			X						
92668	Lirceus fontinalis			X						
92686	Caecidotea sp			X						
92692	Caecidotea racovitzai			X						
93294	Amphipoda sp									
93589	Corophium sp									
93745	Gammaridae sp									
93773	Gammarus sp									
93780	Gammarus fasciatus									
93861	Stygobromus sp			X						
94025	Hyalella sp			X						
94026	Hyalella azteca			X						
95081	Crangonyx sp			X						
95599	Decapoda sp									
96396	Palaemonetes kadiakensis			X						
97336	Cambaridae sp			X						
97343	Cambarus bartonii			X						
97421	Faxonius sp			X						

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TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
97424	Faxonius rusticus			X						
97466	Faxonius obscurus			X						
97473	Faxonius propinquus			X						
99237	Collembola sp			X						
99245	Isotomidae sp			X		X				
100504	Heptageniidae sp	X			X		X			
100507	Stenonema sp	X			X					
100516	Stenonema femoratum	X			X					
100516	Stenonema femoratum	X			X					
100602	Heptagenia sp	X			X		X			
100676	Leucrocuta sp	X			X		X			
100713	Stenacron sp	X		X	X					
100714	Stenacron interpunctatum	X		X	X					
100740	Stenacron gildersleevei	X		X	X		X			
100755	Baetidae sp	X		X						
100771	Labiobaetis sp	X		X						
100800	Baetis sp	X		X						
100808	Baetis intercalaris	X		X						
100817	Baetis tricaudatus	X		X			X			
100873	Centroptilum sp	X		X						
100903	Callibaetis sp	X		X						
101041	Isonychia sp	X								
101095	Leptophlebiidae sp	X		X			X			
101148	Leptophlebia sp	X		X						
101232	Ephemerellidae sp	X		X	X		X			
101405	Tricorythodes sp	X		X						
101468	Brachycercus sp	X		X		X	X	X		
101478	Caenis diminuta group	X		X		X		X		
101478	Caenis sp	X		X		X		X		
101480	Caenis amica	X		X		X		X		
101483	Caenis diminuta	X		X		X		X		
101486	Caenis hilaris	X		X		X		X		
101488	Caenis latipennis	X		X		X		X		
101489	Caenis punctata	X		X		X		X		
101494	Baetisca sp	X		X		X	X			
101525	Ephemeridae sp	X		X						
101537	Hexagenia sp	X		X						
101552	Hexagenia limbata	X		X						
101589	Pentagenia vittigera	X		X						
101593	Odonata sp		X						X	
101596	Aeshnidae sp		X						X	
101647	Boyeria vinosa		X						X	
101649	Basiaeschna janata		X						X	
101654	Nasiaeschna pentacantha		X						X	
101664	Gomphidae sp		X						X	X
101665	Gomphus sp		X						X	X
101666	Stylurus sp		X						X	X

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TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
101680	Gomphus externus		X						X	X
101681	Gomphus hybridus		X						X	X
101685	Gomphus lividus		X						X	X
101692	Gomphus rogersi		X						X	X
101697	Gomphus vastus		X						X	X
101705	Gomphus descriptus		X						X	X
101730	Dromogomphus sp		X						X	X
101732	Dromogomphus spinosus		X						X	X
101735	Hagenius brevistylus		X			X			X	X
101761	Stylogomphus sp		X						X	X
101770	Arigomphus sp		X						X	X
101797	Libellulidae sp		X			X			X	
101799	Pachydiplax longipennis		X			X			X	
101803	Perithemis sp		X			X			X	
101804	Perithemis tenera		X			X			X	
101851	Didymops sp		X			X			X	
101852	Didymops transversa		X			X			X	
101865	Erythemis sp		X			X			X	
101866	Erythemis simplicicollis		X			X			X	
101893	Libellula sp		X			X			X	
101918	Macromia sp		X			X			X	
101922	Macromia taeniolata		X			X			X	
101934	Neurocordulia sp		X						X	
101936	Neurocordulia molesta		X				X		X	
101937	Neurocordulia yamaskanensis		X						X	
101939	Neurocordulia obsoleta		X						X	
101947	Somatochlora sp		X			X			X	
102020	Corduliidae sp		X			X			X	
102035	Epitheca sp		X						X	
102052	Calopteryx sp		X						X	
102077	Coenagrionidae sp		X						X	
102078	Ischnura sp		X						X	
102102	Enallagma sp		X						X	
102108	Enallagma divagans		X						X	
102108	Enallagma divagans		X						X	
102125	Enallagma basidens		X						X	
102135	Nehalennia sp		X						X	
102139	Argia sp		X		X				X	
102140	Argia apicalis		X		X				X	
102140	Argia apicalis/tibialis		X		X				X	
102143	Argia fumipennis		X		X				X	
102146	Argia moesta		X		X				X	
102147	Argia sedula		X		X				X	
102148	Argia tibialis		X		X				X	
102489	Peltoperla sp	X			X		X			
102517	Nemouridae sp	X				X				
102540	Amphinemura sp	X				X				

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TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
102643	Capniidae sp	X				X	X			
102789	Taeniopteryx sp	X				X				
102798	Taeniopteryx nivalis	X				X				
102844	Leuctra sp	X				X	X			
102914	Perlidae sp	X					X			
102917	Acroneuria sp	X	X		X		X			
102918	Acroneuria lycorias	X	X		X		X			
102921	Acroneuria arida	X	X		X		X			
102923	Acroneuria evoluta	X	X		X		X			
102942	Neoperla sp	X	X		X					
102995	Isoperla sp	X	X		X		X			
103012	Isoperla bilineata	X	X		X					
103202	Chloroperlidae sp	X	X		X		X			
103244	Perlinella sp	X	X		X		X			
103246	Perlinella drymo	X	X		X		X			
103248	Perlinella ephyre	X	X		X		X			
103251	Perlesta sp	X	X		X					
103364	Corixidae sp									
103369	Sigara sp									
103423	Trichocorixa sp		X							
103444	Hesperocorixa sp									
103484	Corisella sp									
103491	Palmacorixa sp									
103603	Neoplea sp		X							
103684	Belostoma sp		X							
103754	Ranatra nigra		X							
103769	Gelastocoris sp					X				
109216	Coleoptera sp									
111858	Haliplus sp		X							
111923	Peltodytes sp									
111926	Peltodytes duodecimpunctatus									
111928	Peltodytes lengi									
111932	Peltodytes sexmaculatus									
111942	Peltodytes dietrichi									
111963	Dytiscidae sp		X							
112374	Neoporus sp		X							
112374	Coptotomus venustus		X							
112654	Gyrinus sp		X							
112711	Dineutus sp		X							
112713	Dineutus discolor		X							
112714	Dineutus assimilis		X							
112811	Hydrophilidae sp									
112812	Berosus sp									
112973	Enochrus sp									
113265	Staphylinidae sp		X		X					
113929	Scirtes sp				X					
113948	Cyphon sp				X					

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TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
114006	Helichus sp				X					
114009	Helichus lithophilus				X					
114011	Helichus basalis				X					
114072	Psephenus herricki				X		X			
114087	Ectopria sp				X					
114093	Elmidae sp				X					
114095	Stenelmis sp				X					
114102	Stenelmis crenata				X					
114105	Stenelmis humerosa-sinuata gr				X					
114126	Dubiraphia sp				X					
114131	Dubiraphia vittata				X					
114177	Optioservus sp				X		X			
114193	Ancyronyx sp			X	X					
114194	Ancyronyx variegatus			X	X					
114212	Macronychus sp			X	X					
114213	Macronychus glabratus			X	X					
114667	Anchytarsus sp				X					
115002	Sialis sp		X							
115031	Nigronia serricornis		X		X					
115033	Corydalus sp		X		X					
115086	Climacia sp		X							
115087	Climacia areolaris		X							
115090	Sisyra sp				X					
115095	Trichoptera sp	X								
115276	Chimarra obscura	X			X		X			
115278	Chimarra aterrima	X			X		X			
115334	Psychomyiidae sp	X		X	X					
115335	Psychomyia sp	X		X	X		X			
115398	Hydropsychidae sp	X			X					
115402	Diplectrona modesta	X			X		X			
115408	Cheumatopsyche sp	X			X					
115453	Hydropsyche sp	X			X					
115454	Hydropsyche betteni	X			X					
115458	Hydropsyche bidens	X			X					
115468	Hydropsyche frisoni	X			X					
115471	Hydropsyche incommoda	X			X					
115477	Hydropsyche phalerata	X			X					
115481	Hydropsyche simulans	X			X					
115485	Hydropsyche orris	X			X					
115551	Potamyia sp	X			X					
115552	Potamyia flava	X			X					
115570	Ceratopsyche sp	X			X		X			
115577	Ceratopsyche bronta	X			X					
115580	Ceratopsyche morosa	X			X					
115580	Ceratopsyche morosa group	X			X					
115603	<del>Macros</del> Macroinvertebratesstemum sp	X			X					
115629	Hydroptilidae sp	X					X			

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TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
115641	Hydroptila sp	X			X					
115657	Hydroptila waubesiana	X			X					
115714	Ochrotrichia sp	X		X	X					
115722	Ochrotrichia tarsalis	X		X	X					
115779	Oxyethira sp	X		X			X			
115828	Orthotrichia sp	X		X	X					
115833	Neotrichia sp	X			X		X			
115892	Phryganea sp	X								
115933	Limnephilidae sp	X								
116474	Molanna sp	X					X			
116547	Leptoceridae sp	X	X							
116565	Trienodes sp	X	X							
116572	Trienodes injustus	X	X				X			
116575	Trienodes marginatus	X	X							
116598	Mystacides sp	X		X			X			
116607	Oecetis sp	X	X		X					
116607	Oecetis furva group	X	X		X					
116609	Oecetis cinerascens	X	X		X					
116613	Oecetis inconspicua	X	X		X		X			
116636	Oecetis persimilis	X	X		X					
116651	Nectopsyche sp	X	X				X			
116659	Nectopsyche exquisita	X	X							
116660	Nectopsyche pavida	X	X							
116661	Nectopsyche candida	X	X							
116684	Ceraclea sp	X					X			
116705	Ceraclea flava	X		X			X			
116722	Ceraclea tarsipunctata	X					X			
116958	Micrasema sp	X					X			
117043	Polycentropodidae sp	X			X					
117044	Polycentropus sp	X			X					
117091	Cyrnellus sp	X			X					
117092	Cyrnellus fraternus	X			X					
117095	Neureclipsis sp	X			X					
117098	Neureclipsis crepuscularis	X			X					
117104	Nyctiophylax sp	X			X					
117232	Lepidoptera sp									
117641	Pyralidae sp						X			
118831	Diptera sp									
118840	Tipulidae sp									
119037	Tipula sp									
119656	Antocha sp			X	X					
119704	Limonia sp									
120094	Hexatoma sp		X							
120830	Ormosia sp			X						
121027	Dicranota sp		X				X			
121227	Blephariceridae sp					X	X			
125351	Psychodidae sp			X						

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TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
125468	Psychoda sp			X						
125514	Pericoma sp			X						
125904	Chaoborus sp		X			X				
125923	Chaoborus punctipennis		X			X				
125930	Culicidae sp									
125956	Anopheles sp									
126640	Simuliidae sp				X					
126774	Simulium sp				X					
127076	Ceratopogoninae sp		X							
127076	Ceratopogonidae sp		X			X				
127150	Atrichopogon websteri				X					
127278	Dasyhelea sp			X		X				
127338	Bezzia/Palpomyia sp		X							
127729	Probezzia Kieffer		X							
127761	Sphaeromyia sp		X							
127917	Chironomidae sp			X						
127994	Tanypodinae sp		X			X				
127996	Clinotanypus sp		X							
127998	Clinotanypus pinguis		X							
128010	Coelotanypus sp		X							
128018	Coelotanypus tricolor		X							
128070	Natarsia sp		X			X				
128078	Thienemannimyia group		X			X				
128078	Labrundinia/Nilotanypus sp		X			X				
128079	Ablabesmyia sp		X			X				
128079	Ablabesmyia rhamphe group		X			X				
128081	Ablabesmyia annulata		X			X	X			
128093	Ablabesmyia janta		X			X				
128097	Ablabesmyia karelia group		X			X				
128097	Ablabesmyia mallochi		X			X				
128112	Ablabesmyia parajanta		X			X				
128113	Ablabesmyia peleensis		X			X				
128121	Ablabesmyia rhamphe		X			X				
128130	Conchapelopia sp		X			X				
128161	Guttipelopia sp		X			X				
128171	Krenopelopia hudsoni		X			X				
128173	Labrundinia sp		X			X				
128174	Labrundinia becki		X			X				
128178	Labrundinia pilosella		X			X				
128183	Larsia sp		X			X				
128202	Nilotanypus sp		X			X				
128207	Paramerina sp		X			X				
128215	Pentaneura sp		X			X				
128236	Thienemannimyia sp		X			X				
128245	Thienemannimyia senata					X				
128259	Zavreliomyia sp		X			X				
128277	Procladius sp		X			X				

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TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
128285	Procladius bellus		X			X				
128324	Tanypus sp		X			X				
128457	Orthoclaadiinae sp			X						
128457	Cricotopus/Orthoclaadius sp				X					
128478	Brillia flavifrons									
128511	Cardiocladius sp		X							
128515	Cardiocladius obscurus		X							
128520	Chaetocladius sp			X		X				
128563	Corynoneura sp			X		X				
128567	Corynoneura lobata			X		X				
	Corynoneura floridaensis				X					
	Corynoneura sensu			X		X				
128575	Cricotopus (Isocladius) intersectus group				X					
128575	Cricotopus sp				X					
128575	Cricotopus (Isocladius) sylvestris group				X					
128578	Cricotopus (Isocladius) sp nr. absurdus			X	X					
128583	Cricotopus (Cricotopus) bicinctus				X					
128600	Cricotopus (Cricotopus) fugax				X					
128645	Cricotopus (Isocladius) sylvestris				X					
128651	Cricotopus (Cricotopus) tremulus				X					
128653	Cricotopus (Cricotopus) triannulatus				X					
128659	Cricotopus (Cricotopus) trifascia				X		X			
128666	Cricotopus (Cricotopus) vierriensis				X					
128670	Diplocladius sp			X		X				
128682	Epoicocladius sp			X			X			
128683	Epoicocladius flavens			X						
128689	Eukiefferiella sp			X		X				
128693	Eukiefferiella clairpennis			X		X				
128699	Eukiefferiella discoloripes			X		X				
128703	Eukiefferiella brevicar			X		X				
128704	Eukiefferiella brehmi gr			X		X	X			
	Eukiefferiella tirolensis			X		X				
128712	Georthoclaadius sp									
128718	Gymnometriocnemus sp					X				
128737	Heterotrissocladius sp			X		X				
128750	Hydrobaenus sp					X				
	Hydrosmittia sp					X				
128776	Limnophyes sp			X		X				
128811	Lopescladius sp			X		X	X			
128818	Mesosmittia sp			X						
128844	Nanocladius sp			X		X				
128852	Nanocladius crassicornus			X		X				
128853	Nanocladius distinctus			X		X				
128859	Nanocladius minimus			X		X				
128874	Orthoclaadius sp			X		X				
128913	Orthoclaadius lignicola			X		X	X			



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TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
128962	Paracricotopus sp			X		X				
128968	Parakiefferiella sp			X		X				
128975	Parakiefferiella bathyphila			X		X				
128978	Parametriocnemus sp			X		X				
128982	Parametriocnemus lundbecki			X		X				
128989	Paraphaenocladus sp			X		X				
129018	Psectrocladius sp			X		X				
129027	Psectrocladius elatus			X		X				
129041	Psectrocladius simulans			X		X				
129052	Pseudorthocladus sp			X		X	X			
129071	Pseudosmittia sp									
129086	Rheocricotopus sp			X		X				
129102	Rheocricotopus robacki			X		X				
129110	Smittia sp			X						
129152	Stilocladius sp			X		X				
129162	Synorthocladus semivirens			X						
129182	Thienemanniella sp			X		X				
129189	Thienemanniella similis			X		X				
129190	Thienemanniella xena			X						
	Thienemanniella lobapodema			X		X				
129201	Tvetenia paucunca			X		X				
129228	Chironominae sp			X						
129236	Axarus sp			X		X				
129254	Chironomus (C.) sp			X						
129254	Chironomus sp			X						
129254	Chironomus (C.) decorus group			X						
129313	Chironomus riparius			X						
129350	Cladopelma sp			X						
129368	Cryptochironomus sp		X			X				
129376	Cryptochironomus fulvus		X			X				
129394	Cryptotendipes sp			X		X				
129428	Dicrotendipes sp			X						
129448	Dicrotendipes modestus			X						
129448	Dicrotendipes modestus/tritonus			X						
129450	Dicrotendipes neomodestus			X						
129452	Dicrotendipes nervosus			X						
129458	Demicryptochironomus sp			X			X			
129458	Dicrotendipes lucifer			X						
129458	Dicrotendipes lucifer/simpsoni			X						
129459	Einfeldia sp			X						
129470	Endochironomus sp				X					
129474	Endochironomus subtendens				X					
129483	Glyptotendipes sp									
129484	Glyptotendipes amplus									
129488	Glyptotendipes lobiferus									
129506	Goeldichironomus sp			X						
129512	Goeldichironomus holoprasinus			X						

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TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
129516	Harnischia sp			X						
	Harnischia complex			X						
129522	Kiefferulus sp			X			X			
129526	Lauterborniella agrayloides			X						
129531	Lipinella sp			X						
129533	Microchironomus nigrovittatus			X						
129535	Microtendipes sp				X					
129535	Microtendipes rydalensis group				X					
129541	Microtendipes pedellus				X					
	Microtendipes pedellus group				X					
129547	Microtendipes rydalensis				X					
129548	Nilothauma sp			X						
129561	Pagastiella sp									
129564	Parachironomus sp		X			X				
129565	Parachironomus abortivus		X			X				
129579	Parachironomus frequens		X			X				
129583	Parachironomus pectinatellae		X			X				
129590	Parachironomus tenuicaudata group		X			X				
129595	Parachironomus hirtalatus		X			X				
129597	Paracladopelma sp			X		X				
129619	Paralauterborniella nigrohalteralis			X	X					
129623	Paratendipes sp			X						
129624	Paratendipes albimanus			X						
129637	Phaenopsectra sp				X					
129647	Phaenopsectra obediens				X					
129652	Phaenopsectra punctipes				X					
	Polypedilum flavum									
129657	Polypedilum fallax group									
129657	Polypedilum sp									
129657	Polypedilum scalaenum group									
129657	Polypedilum illinoense group									
129657	Polypedilum trigonus									
129676	Polypedilum fallax									
129684	Polypedilum halterale									
129684	Polypedilum halterale group									
129686	Polypedilum illinoense									
129708	Polypedilum scalaenum									
129719	Polypedilum tritum									
129733	Robackia demeijerei			X						
129744	Stelechomyia perpulchra									
129746	Stenochironomus sp			X						
129785	Stictochironomus cafrarius group			X						
129785	Stictochironomus sp			X						
129820	Tribelos sp			X						
129823	Tribelos fuscicorne			X						
129827	Tribelos jucundum			X						
129837	Xenochironomus sp		X							

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TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
129838	Xenochironomus xenolabis		X							
129851	Pseudochironomus sp			X						
129872	Tanytarsini sp									
129873	Cladotanytarsus sp			X						
129881	Cladotanytarsus mancus			X						
129884	Constempellina sp			X						
129890	Micropsectra sp			X			X			
129935	Paratanytarsus sp			X		X				
129952	Rheotanytarsus exiguus group				X					
129952	Rheotanytarsus sp				X					
129962	Stempellina sp			X			X			
129969	Stempellinella sp			X		X				
129975	Sublettea sp									
129978	Tanytarsus sp									
129997	Tanytarsus guerlus group									
	Tanytarsus glabrescens group				X					
130150	Stratiomyidae sp			X		X				
130160	Allognosta sp			X		X				
130627	Stratiomys sp			X		X				
130694	Nemotelus sp			X						
130934	Tabanidae sp		X			X				
131078	Chrysops sp		X			X				
135830	Empididae sp		X			X				
135893	Roederiodes sp				X					
136327	Hemerodromia sp		X			X				
136824	Dolichopodidae sp		X			X				
144653	Sciomyzidae sp									
146893	Ephydriidae sp			X						
150730	Limnophora sp		X							
155469	Bryozoa sp				X					
156691	Plumatella sp				X					
156754	Urnatella gracilis									
183774	Dicrotendipes tritonus			X						
189328	Zavreliella marmorata			X						
193743	Dicrotendipes simpsoni			X						
204822	Gloiobdella elongata									
205210	Menetus dilatatus									
206622	Procloeon sp	X		X						
206630	Epitheca princeps princeps		X						X	
206642	Trienodes abus	X	X							
555638	Desserobdella phalera									
568515	Cricotopus "Santa Fe"			X	X					
568515	Cricotopus (Isocladius) sp				X					
568515	Cricotopus "Ozarks"				X					
568537	Polypedilum nubifer									
568545	Leptohyphidae sp	X		X						
568546	Acerpenna sp	X		X						

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TSN	Taxa Name	EPT	Pred	CG	CL	SP	Intol	Caenidae	Odonata	Gomphidae
568559	Anthopotamus sp	X								
591727	Macromiinae sp		X			X			X	
591728	Corduliinae sp		X			X			X	
591869	Gomphus (Gomphurus) sp		X						X	
592764	Aphylla angustifolia		X						X	
593022	Stylurus notatus		X						X	
598191	Hydropsychinae sp	X			X					
598221	Hydroptilinae sp	X					X			
609583	Pseudocentropiloides usa	X		X						
650401	Faxonius cristavarius									
656501	Echinogammarus sp									
656749	Apocorophium lacustre									
656834	Echinogammarus ischnus									
666581	Nigronia fasciata		X		X					
693963	Crambidae sp									
697957	Stenonema sp	X			X					
698241	Stenonema pulchellum	X			X					
698255	Stenonema vicarium	X			X		X			
698469	Stenonema mediopunctatum	X			X					
698471	Stenonema terminatum	X			X					
698515	Stenonema integrum	X			X					
733321	Acari sp		X							

## Longitudinal Adjustments and Metric Scoring Thresholds

**Table B1.** Various values necessary to adjust and score each fish and macroinvertebrate metric. The linear equation components are shown for those metrics that exhibited a longitudinal trend in regards to river-mile; metrics not requiring longitudinally adjustment are labeled “n/a”. In order to adjust any observed value, the relative components are inserted into equation A, where ‘x’ equals the Ohio river-mile (0-981) at which the fish or macroinvertebrate sample was obtained. Equations B and C use constants shown in the “Scoring Threshold” columns. These equations are used calculate metrics depending on whether the metric reflects a positive (+) or negative (-) component of the biological assemblage (denoted in the “Metric Direction” column). The observed value used in equations B and C is either the river-mile adjusted or raw value depending on whether the specific metric required longitudinal adjustment or not, respectively. See Section 3.2 for more details.

Index	Metric	Metric Direction	Longitudinal Adjustment			Scoring Threshold	
			Slope (m)	Intercept (b)	Residual Constant	95 <sup>th</sup> Percentile	5 <sup>th</sup> Percentile
<b>mORFin</b>	Native Taxa	+	-0.0048	18.34	13.84	20.76	7.01
	Intolerant Taxa	+	-0.0030	4.73	4.11	6.82	1.65
	Sucker Taxa	+	n/a	n/a	n/a	6.00	0.00
	Centrarchid Taxa	+	n/a	n/a	n/a	3.00	0.00
	Great River Taxa	+	-0.0037	3.93	3.78	6.96	1.58
	% Piscivores	-	n/a	n/a	n/a	9.43	0.00
	% Invertivores	+	-0.0095	27.76	27.04	60.71	5.11
	% Detritivores	-	n/a	n/a	n/a	10.80	0.00
	% Tolerants	-	n/a	n/a	n/a	28.85	0.00
	% Simple Lithophils	+	-0.0321	43.04	37.65	77.57	11.16
	% Non-natives	+	0.0044	28.15	31.12	65.78	7.96
	No. DELT anomalies	-	n/a	n/a	n/a	4.00	0.00
Catch per unit effort (CPUE)	+	-0.1457	304.30	272.27	666.99	51.17	
<b>ORMin - HD</b>	No. Taxa	+	-0.0030	23.43	18.41	25.51	8.56
	EPT Taxa	+	-0.0018	5.99	5.27	8.60	2.29
	Predator Taxa	+	0.0007	4.46	5.14	8.51	2.02
	% Collector-Gatherer Taxa	-	-0.0069	36.31	19.58	30.84	7.77
	% Caenids	-	-0.0017	1.43	1.41	3.66	0.15
	% Odonates	+	0.0003	1.41	1.65	6.84	0.11
	% Intolerants	+	0.0003	1.34	1.63	6.46	0.02
	% Clingers	+	0.0047	25.98	24.80	57.19	4.88
<b>ORMin MH 200</b>	No. Sprawlers	-	-0.1604	117.44	111.33	233.63	4.47
	% Odonates excluding Gomphidae	+	-0.0012	2.21	2.21	11.47	0.00
	% EPT Taxa	+	-0.0139	22.11	15.52	27.08	1.89
	% Collector-Gatherer Taxa	-	-0.0091	35.04	20.12	32.93	5.24

## Habitat Class Thresholds and Final Index Score Calculation

**Table B2.** The statistical thresholds for each habitat class used to generate final *mORFI*n scores from average metric scores. The average metric values are scored relative to the historical performance of samples obtained from sites belonging to the same habitat class using the equation below. See Section 3.3 for more details.

$$\text{Index Score} = \text{Discrete Score of Lower Threshold} + \left[ \frac{(\text{observed average metric score} - \text{lower threshold})}{(\text{upper threshold} - \text{lower threshold})} \right]$$

Statistical Thresholds	Discrete Score	<i>mORFI</i> n – SAV Absent					<i>mORFI</i> n – SAV Present				
		Habitat Classes					Habitat Classes				
		A	B	C	D	E	A	B	C	D	E
min observed	0	24.64	14.52	12.53	18.13	32.57	34.70	31.43	28.85	18.13	32.57
5 <sup>th</sup> Percentile	10	36.62	37.89	30.13	31.55	32.57	36.62	38.91	35.67	31.55	32.57
25 <sup>th</sup> Percentile	20	50.03	46.71	44.55	41.80	39.59	50.03	46.71	47.95	42.50	42.14
50 <sup>th</sup> Percentile	30	55.97	55.05	52.23	49.72	50.53	55.97	55.05	54.73	49.72	50.53
75 <sup>th</sup> Percentile	40	62.59	63.77	61.14	57.90	59.18	62.59	63.77	61.14	57.90	59.18
95 <sup>th</sup> Percentile	50	72.54	75.71	73.19	68.58	67.26	72.54	75.71	73.19	68.58	67.26
max observed	60	75.88	81.46	81.50	76.99	67.26	75.88	81.46	81.50	76.99	67.26

**Table B3.** The statistical thresholds for each habitat class used to generate final *ORMI*n scores from average metric scores. The average metric values are scored relative to the historical performance of samples obtained from sites belonging to the same habitat class using the equation below. See Section 3.3 for more details.

$$\text{Index Score} = \text{Discrete Score of Lower Threshold} + \left[ \frac{(\text{observed average metric score} - \text{lower threshold})}{(\text{upper threshold} - \text{lower threshold})} \right]$$

Statistical Thresholds	Discrete Score	<i>ORMI</i> n – HD SAV Absent			<i>ORMI</i> n - HD SAV Present			<i>ORMI</i> n - MH >200 Individuals		
		Habitat Classes			Habitat Classes			Habitat Classes		
		A-B	C	D-E	A-B	C	D-E	A-B	C	D-E
min observed	0	24.61	4.81	7.59	13.00	18.12	23.24	22.16	19.27	21.18
5 <sup>th</sup> Percentile	10	26.69	17.99	19.05	26.70	22.93	28.94	27.02	22.90	21.55
25 <sup>th</sup> Percentile	20	39.12	33.25	33.35	38.48	40.92	36.27	41.61	32.93	34.08
50 <sup>th</sup> Percentile	30	47.06	45.31	44.20	46.05	50.36	45.65	47.82	41.97	40.62
75 <sup>th</sup> Percentile	40	59.75	53.51	49.98	56.40	55.37	54.51	53.58	50.98	59.44
95 <sup>th</sup> Percentile	50	65.44	59.67	60.54	68.73	62.10	66.88	59.34	61.73	76.21
max observed	60	66.33	62.49	62.97	79.17	64.13	71.72	73.08	75.93	76.94

**STANDARD OPERATING PROCEDURES**  
**FOR THE**  
**BOAT ELECTROFISHING POPULATION SURVEY**



**Ohio River Valley Water Sanitation Commission**  
**5735 Kellogg Avenue**  
**Cincinnati, Ohio 45230**  
**(513) 231-7719**  
**March 2023**

## **INTRODUCTION**

This document describes the procedures for ORSANCO's Boat Electrofishing Population Surveys and provides guidelines for the proper collection and processing of Ohio River Basin Fish harvested during electrofishing activities. This SOP has been developed to maintain continuity and ensure data quality.

### **1.0 Field Equipment**

The following equipment is used to conduct electrofishing surveys:

#### **1.1 Electrofishing Watercraft**

- 1.1.1 19-ft. aluminum johnboat w/ 115hp *and* 25-30hp outboard motors
- 1.1.2 MLES® Infinity/ HC80 Electrofishing System w/ 5000-7500W gasoline generator
- 1.1.3 Dual, Retractable Anode Array
- 1.1.4 Minimum (6) LED DC flood and spot lighting array and marine navigation lighting
- 1.1.5 Positive pressure cut-off foot pedal switch
- 1.1.6 50 – 75-gallon plastic or aluminum live well with 12V agitator-type aerator
- 1.1.7 Marine radio
- 1.1.8 Personal LED headlamps and handheld lights
- 1.1.9 Fire extinguisher, first aid kit, portable AED, marine safety and emergency kit

#### **1.2 Fish Collection**

- 1.2.1 Fiberglass handled nets with 0.25-in mesh
- 1.2.2 Rubber-soled footwear for each crew member
- 1.2.3 Personal Floatation Device for each crew member
- 1.2.4 Gloves for each crew member
- 1.2.5 Ear and eye protection for each crew member

#### **1.3 Fish Processing**

- 1.3.1 Fish identification reference keys
- 1.3.2 Fish measuring board
- 1.3.3 Weighing scales
- 1.3.4 Voucher collection containers and preservative
- 1.3.5 Sorting buckets and trays

#### **1.4 Water Chemistry Measurements**

- 1.4.1 In-Situ AquaTROLL 500
- 1.4.2 Secchi disk
- 1.4.3 Laser rangefinder



## 2.0 Electrofishing Survey Procedures

### 2.1 Training

- 2.1.1 Each ORSANCO staff member involved in electrofishing activities will be trained in electrofishing procedures by a permitted ORSANCO field crew leader.
- 2.1.2 Field crew leader qualification requires a biologist with supervised boat electrofishing experience, demonstrated competency with modern electrofishing equipment and techniques, demonstrated competency with fish identification and marked proficiency with SOP execution. Field crew leaders are appointed based on eligibility, experience and professional ability as determined by and at the sole discretion of the Technical Programs Manager.
- 2.1.3 Staff shall perform at least two training sessions to the satisfaction of the Technical Programs Manager before performing any program sampling.
- 2.1.4 Each staff member involved in electrofishing activities will be certified in cardiopulmonary resuscitation (CPR) and basic first aid procedures.

### 2.2 Field Methods

#### 2.2.1 Site Selection

Probabilistic sites are selected and sample frames are generated using RF3 river double lines for the Ohio River and river mile coverage provided by ORSANCO. A generalized random tessellation stratified survey design for a linear network with reverse hierarchical randomization is used to select all sampling locations. This survey design provides coordinates for 15 primary sampling sites and up to 25 overdraw sampling sites in each of the selected pools. Probabilistic sites are evaluated for safety by the Field Crew Leader. If a sampling location is deemed unsafe (proximity to dam, active loading zone, etc.) the site location may be shifted to ensure safe sampling. Site locations will be shifted first up to 500m upstream of the original coordinates on the same bank. If moving upstream does not provide a safe sampling environment, then the top of the sampling zone may be shifted up to 500m downstream of the original coordinates. If that also fails to provide a safe sampling location, the top of the sampling zone may be moved to the opposite bank, directly across from the original coordinates. If this does not yield a safe sampling environment, the site is thrown out and an overdraw site is selected by draw order and used in its place.

#### 2.2.2 Zone Measurement

Standard electrofishing zones are 0.5-km length. Distances are measured with handheld GPS systems in conjunction with boat mounted chart plotters and laser rangefinders.

### 2.2.3 Zone Delineation

The boundaries of each electrofishing zone may be clearly marked on a stationary object (e.g. trees, rocks, etc.) with biodegradable orange or red surveyor's flagging. This enables accurate location of the site on subsequent sampling dates. Care must be taken not to mark objects on private property.

### 2.2.4 Site Indexing

Each sampling zone location is indexed to the nearest tenth of a river mile using Geographic Information Systems (GIS) software. GPS is used to ground-truth and obtain coordinates (latitude and longitude in decimal degrees) on site at the upstream boundary of each zone.

## 2.3 Water Chemistry Parameters

2.3.1 Dissolved oxygen, conductivity, temperature, pH, and Secchi disk transparency depth are recorded at the upstream end of each electrofishing zone prior to sampling. General weather and ambient condition observations are recorded at this time. This information is recorded at the appropriate locations on the data sheet (Attachment A).

### 2.3.2 Secchi Disk Transparency

A measurement of water column transparency is obtained by observing a specially marked, circular disk (Secchi disk) which is lowered through the water column until it is not visible. The Secchi disk rope is accurately graduated in meters, 0.15 meter (6") graduations. Observing 1 meter above the water surface, the disk is lowered into the water, under illumination provided by electrofishing lights, to the depth at which the disk disappears. The disk is raised and the depth at which the gradation between black and white just reappears is recorded.

## 2.4 Fish Sampling Procedures

### 2.4.1 Electrofishing Boat Design

A description of the electrofishing boat is given in Section 1.1

### 2.4.2 MLES® Infinity / HC80 System Settings

The Ohio River's relative conductivity values normally range from 300 to 500 mmhos/cm. This generally results in a voltage selection of 180-250 volts DC at 60-120 pulses/ second with a duty cycle approaching 25%. These settings will generally produce the target power approaching 6000-7000W. The operator may adjust settings to produce the target power as necessary to ensure desired effect on fish. The operator may use higher voltage settings at lower conductivity

readings and lower voltage settings at higher conductivity readings to obtain the desired power output.

#### 2.4.3 Pulsed DC Electrofishing

Pulsed DC electricity is transmitted through the water by the electrode array. Safety features include a positive pressure cut-off switch located on the bow of the boat controlled by a netter and an emergency shut-down switch operated by the driver.

- 2.4.4 Surveys will be conducted at night beginning just after dusk. Night electrofishing is conducted to take advantage of increased foraging activity and diurnal movements of fishes that occur along the shoreline in the evening hours.
- 2.4.5 Individual sampling zones are electrofished from upstream to downstream by slowly and steadily maneuvering the boat close to the shore and instream structure in a “zigzag” pattern.
- 2.4.6 Time electrofished (seconds) is recorded from the MLES® control box immediately after electrofishing. A minimum of 1800 seconds is required to provide a sufficient sample. More time may be necessary for zones that incorporate more complex habitats such as those with extensive woody cover such as logs or stumps. Less time may be acceptable for zones that exhibit fast flow and/or minimal structure or instream cover.
- 2.4.7 A sampling crew consists of two netters and a driver. All personnel are clad in rubber-soled footwear, clear protective eyewear, and an orange Type I USCGA personal floatation device with reflective material. PFDs will be worn by all personnel when the watercraft is underway. The netters may also wear protective gloves. Personnel will wear helmets when operating in areas where Silver Carp are present.
- 2.4.8 As the driver maneuvers the boat through the electrofishing zone, netters remove affected fish from the water. The fish are then placed into a well-aerated live well to be processed immediately after electrofishing.
- 2.4.9 It is recommended that sampling take place under stable, low-flow conditions at a stage level within 1m of “normal, flat pool”, and when Secchi disk transparency depths are at least 0.3m (13”).

### 3.0 Fish Processing Procedures

- 3.1 Fish may be sorted into sorting containers by family or species
- 3.2 Processing priority is as follows:
  - 1. stressed individuals
  - 2. threatened and/or endangered species

3. large individuals
4. general population

Total length of each fish is measured to the nearest 3cm size class using a 1-meter measuring board. Total weight (when taken) of each fish is recorded to the nearest gram using either a 1.0-kg scale or a 4.0-kg scale, depending on fish weight. Small individuals of a given species may be sorted into 3-cm size classes and a total number recorded for all individuals. Large fish (>30-cm) should be measured individually, even if in large numbers. All areas of the data sheet (Figure 1) are filled out completely and legibly for each individual or size class.

#### **4.0 Fish Disposal Procedures**

- 4.1 All living specimens, except voucher and questionable specimens are returned to the water. Voucher specimens are preserved in 10% formalin solution. When handling formalin, eye protection will be worn at all times. All handling of formalin or formaldehyde will take place in a well-ventilated area. Waste formalin will be recycled for later use.
  
- 4.2 Fish not surviving will be buried on shore or returned to deep water for nutrient recycling. If many fish are not surviving, the project leader must investigate probable causes and implement immediate corrective action. Probable causes to be examined (but not limited to) are:
  1. lack of sufficient aeration
  2. slow / inefficient fish processing; improper fish handling
  3. electrofishing settings incorrect (Section 2.4.2).

#### **5.0 Data Handling and Analysis**

- 5.1 Field data sheets are physically checked, digitally photographed after each site and collected at the conclusion of each study by the Principal Investigator. All data is entered electronically as soon as possible after any field operation. Upon return, all electronic data and photo backups are moved to ORSANCO servers for QAQC and storage. Details can be found in the Data Entry and Database Usage SOP.
  
- 5.2 ORSANCO staff compiles and reviews all data prior to entry into ORSANCO databases. Data reduction procedures are documented in the Biological Monitoring and Assessment Quality Assurance Program Plan, Section 9.
  
- 5.3 For specific routine data assessment procedures, see Section 12 of the Biological Monitoring and Assessment Quality Assurance Program Plan.

#### **6.0 Reference and Voucher Collections**

Any species contained in the voucher collection but not in the reference collection will be properly labeled and added to the reference collection. Reference collections will be stored at ORSANCO.

**7.0 Corrective Action**

Immediate Corrective Actions (ICAs) and Long Term Corrective Actions (LTCAs) will be determined by crew leaders and documented as per the procedure outlined in the Biological Monitoring and Assessment Quality Assurance Program Plan, Section 13.

**8.0 References**

Kolz, A.L., J.B. Reynolds, and J. Boardman. 1991. Principles and Techniques of Electrofishing. U. S. Fish & Wildlife Service Instructional Course Packet #2101

Ohio Environmental Protection Agency. 1989. Biological Criteria for the protection of aquatic life: Volume III. Standardized Biological Field Sampling and Laboratory Methods for assessing Fish and Macroinvertebrate Communities. Division of Water Quality Monitoring and Assessment. Columbus, Ohio.

Pflieger, W.L. 1975. The Fishes of Missouri. Western Publishing Co. 343 pp.

Tennessee Valley Authority. 1987. Field Operations Biological Resources Procedures Manual. Tennessee Valley Authority, Division of Natural Resource Operations. Knoxville, Tennessee.

Trautman, M.B. 1981. The Fishes of Ohio. Revised Edition. Ohio State University Press. Columbus, Ohio. 782 pp.

Date:	Crew:	SiteID:	River:	Rmi:	LDB / RDB	Site Pic: Y/N	Entered by:								
Lat:	Long:	Secchi	Temp	Cond	DO	ph									
Start Time:	Volts:	Amps:	End Time:	Seconds:	Minnow Jar: Y/N	Count:	QA:								
Notes															
<b>Species</b>	<b>SC</b>	<b>Count</b>	<b>SC</b>	<b>Count</b>	<b>SC</b>	<b>Count</b>	<b>SC</b>	<b>Count</b>	<b>SC</b>	<b>Count</b>	<b>SC</b>	<b>Count</b>	<b>SC</b>	<b>Count</b>	<b>Flag</b>
Gizzard Shad	60.5														
Freshwater Drum	60.1														
Longnose Gar	30.1														
Channel Catfish	13.1														
Flathead Catfish	15.1														
Common Carp	90.1														
Smallmouth Buffalo	107.1														
River Carpsucker	102.1														
Highfin Carpsucker	102.5														
Quillback	1015														
Smallmouth Redhorse	103.1														
Golden Redhorse	105.1														
Silver Redhorse	103.5														
Hybrid Striper	150.3														
White Bass	150.5														
Morone Sp	150														
Largemouth Bass	166.1														
Smallmouth Bass	165.5														
Spotted Bass	166.5														
Bluegill	162.1														
Longear Sunfish	164.5														
Green Sunfish	161.1														
White Crappie	167.1														
Black Crappie	167.5														
Walleye	177.1														
Sauger	177.5														
Saugeye	177.3														

Figure 1. Electrofishing Datasheet

**STANDARD OPERATING PROCEDURES**

**FOR**

**HABITAT DATA COLLECTION FOR**

**FISH POPULATION SURVEYS**



**Ohio River Valley Water Sanitation Commission  
5735 Kellogg Avenue  
Cincinnati, Ohio 45230  
(513) 231-7719  
March 2023**

## **INTRODUCTION**

This document describes the procedures for ORSANCO's Habitat Data Collection. This SOP has been developed to maintain continuity and ensure data quality.

### **1.0 Field Equipment**

The following equipment is used to conduct the habitat surveys:

#### **1.1 Watercraft**

- 1.1.1 19-ft. aluminum johnboat
- 1.1.2 Two 10-ft, ¾ in. copper poles capped at one end, and wrapped with marking tape at each one foot interval along the length of the pole. Poles are fitted with male and female adapters for assembly and breakdown.
- 1.1.3 Laser rangefinder
- 1.1.4 Marine radio

### **2.0 Habitat Data Collection Procedures**

#### **2.1 Training**

- 2.1.1 Each ORSANCO staff member involved in the habitat data collection program will be trained in collection procedures by a staff member having at least one year of habitat data collection experience on the Ohio River.
- 2.1.2 *Field crew leader qualification* requires a biologist with supervised boat experience, demonstrated competency with modern equipment and techniques, and marked proficiency with SOP protocol execution. Field crew leaders are appointed based on eligibility, experience, and professional ability as determined by and at the sole discretion of the Technical Programs Manager.
- 2.1.3 Staff shall perform at least two training sessions to the satisfaction of the Crew Leader and Technical Programs Manager before performing any program sampling.

#### **2.2 Field Methods**

- 2.2.1 Site Selection

Sites are selected and sample frames are generated using RF3 river double lines for the Ohio River and river mile coverage provided by ORSANCO. A generalized random tessellation stratified survey design for a linear network with reverse hierarchical randomization was used to select all sampling locations. This survey design provided coordinates for 15 primary sampling sites and up to 25 overdraw sampling sites in each of the selected pools. Probabilistic sites are evaluated for safety by the Field Crew Leader. If a sampling location is deemed unsafe (proximity to dam, active loading zone, etc.) the site location may be shifted to ensure safe sampling. Site locations will be shifted first up to 500m upstream of the original coordinates on the same bank. If moving upstream does not provide a safe sampling environment, then the top of the sampling zone may be shifted up to 500m downstream of the original coordinates. If that also fails to provide a safe sampling location, the top of the sampling zone may be moved to the opposite bank, directly across from the original coordinates. If this does not yield a safe sampling environment, the site is thrown out and an overdraw site is selected by draw order and used in its place.

### 2.2.2 Zone Measurement

Electrofishing zones are 0.5-km length. Distance is measured with a handheld GPS unit and a laser rangefinder. Sampling zones are measured by navigating to the upstream end of the electrofishing zone, marking the start point in the GPS unit while simultaneously measuring distance from shore with the laser rangefinder. These two pieces of equipment are used to measure zone length and distance from shore while maneuvering the boat to the end of the zone.

### 2.2.3 Zone Delineation

The boundaries of each electrofishing zone are clearly marked on stationary object (e.g. trees, rocks, etc.) with florescent orange paint and/or orange surveyor's flagging. This enables accurate location of the site on subsequent sampling dates. Care must be taken not to mark objects on private property.

### 2.2.4 Site Indexing

Each sampling zone location is indexed to the nearest tenth of a river mile using Geographic Information Systems (GIS) software. GPS is used to ground-truth and obtain coordinates (decimal degrees) on site at the upstream boundary of each zone.

## 2.3 Habitat Data Collection Procedure

- 2.3.1 Beginning at shoreline at the upstream end of the zone, one crew member takes/ records a GPS mark at the water's edge. Sediment at the shoreline is recorded. The driver then slowly backs the boat away from shore in a straight line perpendicular to the shoreline, as a crewmember maintains a fix on the target point with the rangefinder and calls off distance to shore. At each 10' interval sediment and depth are recorded by lowering one end of the copper pole to the substrate. Sediment and depth are recorded every 10' (3m) to 100' (30m) out from shore, using boat-mounted depthfinders to determine depths greater than 20'. This procedure is repeated at each of the six 100 m zone marks, starting at the upper end and finishing at the lower.



### **3.0 Data Handling and Analysis**

Field data sheets are checked and initialed by at least two crewmembers, digitally photographed and are collected at the conclusion of each study by the Principal Investigator. Details can be found in the Date Entry and Database Usage SOP. ORSANCO staff compiles and reviews all data prior to entry into the ORSANCO data base.

### **4.0 Corrective Action**

Immediate Corrective Actions (ICAs) and Long Term Corrective Actions (LTCAs) will be determined by crew leaders and documented as per the procedure outlined in the Biological Monitoring and Assessment Quality Assurance Program Plan, Section 13.

Date:		Crew:									
River/Site # :		Site Description:									
Rmi:											
RDB / LDB (circle one)		Straight Stretch / Inside Bend / Outside Bend / Backchannel (circle one)									
Latitude		N									
Longitude		W									

Transect	DFS (ft)	Depth	B	C	G	S	F	H	X	Transect	DFS (ft)	Depth	B	C	G	S	F	H	X
0	0	0								300	0	0							
0	10									300	10								
0	20									300	20								
0	30									300	30								
0	40									300	40								
0	50									300	50								
0	60									300	60								
0	70									300	70								
0	80									300	80								
0	90									300	90								
0	100									300	100								

Outfalls/ Discharges (Type & #)										Outfalls/ Discharges (Type & #)									
Bank Grade		Flat / Gradual / Sloped / Steep / Cliff								Bank Grade		Flat / Gradual / Sloped / Steep / Cliff							
AMD		None / Minor / Major / Holy Rustoleum!								AMD		None / Minor / Major / Holy Rustoleum!							
Road/ RR/ Bridge (dist from water in m)										Road/ RR/ Bridge (dist from water in m)									

Transect	DFS (ft)	Depth	B	C	G	S	F	H	X	Transect	DFS (ft)	Depth	B	C	G	S	F	H	X
100	0	0								400	0	0							
100	10									400	10								
100	20									400	20								
100	30									400	30								
100	40									400	40								
100	50									400	50								
100	60									400	60								
100	70									400	70								
100	80									400	80								
100	90									400	90								
100	100									400	100								

Outfalls/ Discharges (Type & #)										Outfalls/ Discharges (Type & #)									
Bank Grade		Flat / Gradual / Sloped / Steep / Cliff								Bank Grade		Flat / Gradual / Sloped / Steep / Cliff							
AMD		None / Minor / Major / Holy Rustoleum!								AMD		None / Minor / Major / Holy Rustoleum!							
Road/ RR/ Bridge (dist from water in m)										Road/ RR/ Bridge (dist from water in m)									

Transect	DFS (ft)	Depth	B	C	G	S	F	H	X	Transect	DFS (ft)	Depth	B	C	G	S	F	H	X
200	0	0								500	0	0							
200	10									500	10								
200	20									500	20								
200	30									500	30								
200	40									500	40								
200	50									500	50								
200	60									500	60								
200	70									500	70								
200	80									500	80								
200	90									500	90								
200	100									500	100								

Outfalls/ Discharges (Type & #)										Outfalls/ Discharges (Type & #)									
Bank Grade		Flat / Gradual / Sloped / Steep / Cliff								Bank Grade		Flat / Gradual / Sloped / Steep / Cliff							
AMD		None / Minor / Major / Holy Rustoleum!								AMD		None / Minor / Major / Holy Rustoleum!							
Road/ RR/ Bridge (dist from water in m)										Road/ RR/ Bridge (dist from water in m)									

ADDITIONAL SITE ON REVERSE SIDE

Figure 1. ORSANCO Habitat Field Sheet.

**STANDARD OPERATING PROCEDURES**

**FOR**

**SUBMERGED AQUATIC VEGETATION COLLECTION**



**Ohio River Valley Water Sanitation Commission  
5735 Kellogg Avenue  
Cincinnati, Ohio 45230  
(513) 231-7719  
March 2023**

## **INTRODUCTION**

This document describes the procedures for ORSANCO's Aquatic Vegetation Collection. This SOP has been developed to maintain continuity and ensure data quality.

### **1.0 Field Equipment**

The following equipment is used to conduct aquatic vegetation surveys:

#### **1.1 Watercraft**

- 1.1.1 19-ft. aluminum boat
- 1.1.2 Doubled-headed vegetation rake
- 1.1.3 Laser Rangefinder
- 1.1.4 Marine radio

### **2.0 Aquatic Vegetation Collection Procedures**

#### **2.1 Training**

- 2.1.1 Each ORSANCO staff member involved in the aquatic vegetation data collection program will be trained in collection procedures by a staff member having at least one year of habitat data collection experience on the Ohio River.
- 2.1.2 *Field crew leader qualification* requires a biologist with supervised boat experience, demonstrated competency with modern equipment and techniques, and marked proficiency with SOP protocol execution. Field crew leaders are appointed based on eligibility, experience, and professional ability as determined by and at the sole discretion of the Technical Programs Manager.
- 2.1.3 Staff shall perform at least two training sessions to the satisfaction of the Crew Leader and Technical Programs Manager before performing any program sampling.

#### **2.2 Field Methods**

##### **2.2.1 Site Selection**

Sites are selected and sample frames are generated using RF3 river double lines for the Ohio River and river mile coverage provided by ORSANCO. A generalized random tessellation stratified survey design for a linear network with reverse hierarchical randomization is used to

select all sampling locations. This survey design provides coordinates for 15 primary sampling sites and up to 25 overdraw sampling sites in each of the selected pools. Probabilistic sites are evaluated for safety by the Field Crew Leader. If a sampling location is deemed unsafe (proximity to dam, active loading zone, etc.) the site location may be shifted to ensure safe sampling. Site locations will be shifted first up to 500m upstream of the original coordinates on the same bank. If moving upstream does not provide a safe sampling environment, then the top of the sampling zone may be shifted up to 500m downstream of the original coordinates. If that also fails to provide a safe sampling location, the top of the sampling zone may be moved to the opposite bank, directly across from the original coordinates. If this does not yield a safe sampling environment, the site is thrown out and an overdraw site is selected by draw order and used in its place.

#### 2.2.2 Zone Measurement

Electrofishing zones are 0.5-km length. Distance is measured with a handheld GPS unit and a laser rangefinder. Sampling zones are measured by navigating to the upstream end of the electrofishing zone, marking the start point in the GPS unit while simultaneously measuring distance from shore with the laser rangefinder. These two pieces of equipment are used to measure zone length and distance from shore while maneuvering the boat to the end of the zone.

#### 2.2.3 Zone Delineation

The boundaries of each sampling zone are clearly marked on stationary object (e.g. trees, rocks, etc.) with florescent orange/ red surveyor's flagging. This enables accurate location of the site on subsequent sampling dates. Care must be taken not to mark objects on private property.

#### 2.2.4 Site Indexing

Each sampling zone location is indexed to the nearest tenth of a river mile using Geographic Information Systems (GIS) software. GPS is used to ground-truth and obtain coordinates (decimal degrees) on site at the upstream boundary of each zone.

### 2.3 Aquatic Vegetation Data Collection Procedure

2.3.1 Beginning at the shoreline at the upstream end of the zone, one crew member takes/ records a GPS mark at the water's edge, marking the top of the zone (0m). Five transects are marked in 100m intervals downstream of 0m. Between each of these six transects visual methods are used to provide a qualitative estimate of woody cover, submerged, and emergent vegetation occurrence between each transect. The driver then slowly backs the boat away from shore in a straight line perpendicular to the shoreline, as a crewmember maintains a fix on the target point with the rangefinder and calls off distance to shore. Aquatic vegetation is gathered at each 10' interval using a double sided rake. In water shallower than 15' deep (boat-mounted depthfinders are used to determine depths greater than 15'), a rake attached to a pole is lowered to the substrate. The rake is then twisted around twice and pulled straight out of the water. This procedure is repeated at each of the six transects throughout the zone (0m, 100m, 200m, 300m,

400m, 500m). The plants collected at each transect will be documented and vouchered at completion of the zone.

2.3.2 Data recorded at each transect point include:

2.3.2.1 **Sampling device** – ‘P’ – pole

2.3.2.2 **Rake fullness** – ‘0’ – No plants present, ‘1’ – Only a few plants present, not enough to cover entire length of rake, tines still visible, ‘2’ – There are enough plants to cover entire length of rake in a single layer, tines not fully covered, ‘3’ – the rake is completely covered in plants, tines not visible. These measures are obtained for each species observed on the rake. Plants that are dislodged via the rake but either fall off or float to surface are included in fullness measures. When a species is observed in the vicinity of a sample point, within 5 feet, but not sampled by the rake, the species is recorded as visually observed and included in total number of species observed.

2.3.2.3 **Species name** – Taxa codes for each species will be recorded.

2.3.2.4 **Voucher type** – ‘P’ – photo, ‘S’ – specimen

**3.0 Data Handling and Analysis**

- 3.1 Field data sheets are checked and initialed by at least two crewmembers, digitally photographed, and are collected at the conclusion of each study by the Principal Investigator. Details can be found in the Date Entry and Database Usage SOP.
- 3.2 ORSANCO staff compiles and reviews all data prior to entry into ORSANCO databases.

**4.0 Corrective Action**

- 4.1 Immediate Corrective Actions (ICAs) and Long Term Corrective Actions (LTCAs) will be determined by crew leaders and documented as per the procedure outlined in the Biological Monitoring and Assessment Quality Assurance Program Plan, Section 13.

Date:		Crew:		River/Site # :		RDB / LDB	(circle one)		
Notes									
<b>Species Name (abbreviate &amp; circle rake fullness observed)</b>									
<b>Transect</b>	<b>DFS (ft)</b>	<b>Device</b>							
0	0	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
0	10	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
0	20	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
0	30	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
0	40	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
0	50	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
0	60	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
0	70	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
0	80	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
0	90	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
0	100	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
<b>Voucher Type</b>			Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample
<b>Visual Veg. Observation:</b>									
<b>Transect</b>	0 - 100	Emergent %:		Emergent Type:		Submergent %:		Woody Cover %:	
<b>Species Name (abbreviate &amp; circle rake fullness observed)</b>									
<b>Transect</b>	<b>DFS (ft)</b>	<b>Device</b>							
100	0	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
100	10	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
100	20	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
100	30	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
100	40	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
100	50	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
100	60	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
100	70	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
100	80	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
100	90	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
100	100	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
<b>Voucher Type</b>			Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample
<b>Visual Veg. Observation:</b>									
<b>Transect</b>	100 - 200	Emergent %:		Emergent Type:		Submergent %:		Woody Cover %:	
<b>Species Name (abbreviate &amp; circle rake fullness observed)</b>									
<b>Transect</b>	<b>DFS (ft)</b>	<b>Device</b>							
200	0	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
200	10	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
200	20	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
200	30	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
200	40	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
200	50	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
200	60	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
200	70	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
200	80	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
200	90	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
200	100	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
<b>Voucher Type</b>			Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample
<b>Visual Veg. Observation:</b>									
<b>Transect</b>	200 - 300	Emergent %:		Emergent Type:		Submergent %:		Woody Cover %:	

Figure 1. ORSANCO Aquatic Vegetation Field Sheet – Front

Date:		Crew:		River/Site # :		RDB / LDB	(circle one)		
Notes									
<b>Species Name (abbreviate &amp; circle rake fullness observed)</b>									
<b>Transect</b>	<b>DFS (ft)</b>	<b>Device</b>							
300	0	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
300	10	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
300	20	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
300	30	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
300	40	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
300	50	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
300	60	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
300	70	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
300	80	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
300	90	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
300	100	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
<b>Voucher Type</b>			Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample
<b>Visual Veg. Observation:</b>									
<b>Transect</b>	300 - 400	Emergent %:		Emergent Type:		Submergent %:		Woody Cover %:	
<b>Species Name (abbreviate &amp; circle rake fullness observed)</b>									
<b>Transect</b>	<b>DFS (ft)</b>	<b>Device</b>							
400	0	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
400	10	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
400	20	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
400	30	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
400	40	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
400	50	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
400	60	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
400	70	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
400	80	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
400	90	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
400	100	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
<b>Voucher Type</b>			Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample
<b>Visual Veg. Observation:</b>									
<b>Transect</b>	400 - 500	Emergent %:		Emergent Type:		Submergent %:		Woody Cover %:	
<b>Species Name (abbreviate &amp; circle rake fullness observed)</b>									
<b>Transect</b>	<b>DFS (ft)</b>	<b>Device</b>							
500	0	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
500	10	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
500	20	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
500	30	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
500	40	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
500	50	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
500	60	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
500	70	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
500	80	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
500	90	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
500	100	P R	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
<b>Voucher Type</b>			Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample	Photo Sample
<b>Visual Veg. Observation:</b>									
<b>Species Name (abbreviate &amp; record total biomass)</b>									
<b>Species Biomass:</b>			g	g	g	g	g	g	g

Figure 2. ORSANCO Aquatic Vegetation Field Sheet – Back.







Fullness Rating	Coverage	Description
0		No plants present.
1		Only few plants. There are not enough plants to entirely cover the length of the rake head in a single layer.
2		There are enough plants to cover the length of the rake head in a single layer, but not enough to fully cover the tines.
3		The rake is completely covered and tines are not visible.

Figure 3. Illustration of rake fullness ratings modified from Hauxwell et al.. 2010.

**STANDARD OPERATING PROCEDURES**  
**FOR**  
**MACROINVERTEBRATE SAMPLING**  
**USING MODIFIED HESTER-DENDY SAMPLERS**



**Ohio River Valley Water Sanitation Commission**  
**5735 Kellogg Avenue**  
**Cincinnati, Ohio 45230**  
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**March 2023**

## **INTRODUCTION**

This document describes the procedures for ORSANCO's aquatic macroinvertebrate population surveys using the modified Hester-Dendy (H-D) multi-plate sampling method.

### **1.0 Sampling Schedule**

Sampling schedules should be established which take advantages of low flow conditions in summer and early fall. The samplers are set out in late August to mid-September, and are collected six weeks after placement. (Attachment 1, 2017 Intensive Survey Sampling Workplan)

#### **1.1 Personnel Qualifications**

*Field crew leader qualification* requires a biologist with supervised boat experience, demonstrated competency with modern equipment and techniques, and marked proficiency with SOP protocol execution. Field crew leaders are appointed based on eligibility, experience, and professional ability as determined by and at the sole discretion of the Technical Programs Manager.

### **2.0 Sampling Procedures**

#### **2.1 Hester-Dendy (H-D) Specifications**

Samplers are constructed of 1/8 inch tempered masonite cardboard cut into three inch square plates and one inch square or circular spacers. A 3/8 inch hole is drilled in the center of each plate and spacer. Eight plates and twelve spacers are placed on a 1/4 inch X 4 inch eye bolt such that there are three single spaces (1/8"), three double spaces (1/4"), and one triple space (3/8") between the plates. Plates and spacers are secured to the eye bolt with two 1/4 inch washers and one standard 1/4 inch nut. See Hester and Dendy (1962).

#### **2.2 Sampling Unit Assembly**

A sampling unit is a series of five H-D samplers bound together with plastic ties and secured to a cement paver. The five samplers are tied together, eyebolt to eyebolt in a circular pattern. The group is then secured to the top of the paver with a plastic tie once paracord has been tied to the paver's eyebolt.

#### **2.3 Placement**

Each sampling unit should be placed in an area safe from disturbance in substrate representative of the 500m zone. In the event that the zone is not suitable for deployment, the field crew leader may then choose to set in a nearby area that would best represent the zone, ideally selecting a location within the site. Once a location is chosen, a boat driver backs out slowly from shore, until 10' depth is achieved. A knot is tied in the paracord attached to the paver to indicate 10', to aid in placing the sampling unit at the proper depth. The sampling unit is lowered into the water by the paracord, allowed to settle on the bottom, and GPS coordinates are recorded. The boat then returns to shore, letting line out so that it may be tied off securely. The line may be tied to anything deemed secure by the collector. GPS coordinates

are recorded from the tie-off location and notes are taken by the field crew leader. Efforts will made to disguise the line to prevent tampering.

#### 2.4 **Colonization period**

The sampling unit must remain undisturbed for a period of at least six weeks, but should not exceed eight weeks.

#### 2.5 **Sampling Unit Retrieval**

During retrieval, the sampling unit is approached from downstream to ensure minimal disturbance. Upon location of the retrieval line, the collector will cut the line, being sure to keep the line taught to minimize disturbance. The collector then backs out slowly until the boat is directly over the sampling unit, at which time the unit is slowly pulled to the surface. A tub or collection basin is submerged and positioned next to the unit to catch any escaping sample. The five H-D samplers are then carefully cut from the paver and transferred to a numbered 5-gallon bucket once securely on board. All paracord is collected and removed from the sampling location. Plates are disassembled on site or after returning to shore.

#### 2.6 **Plate Disassembly and Sample Preservation**

The five H-D samplers are disassembled in the bucket with special care taken not to spill or lose any of the sample material. The plates are brushed or scraped using another plate or gloved fingers while submerged, and all sampler plates and non-plastic spacers are discarded. Metal hardware is retained for reuse. After all parts have been rinsed and removed from the bucket, the water is then poured through a standard #30 sieve, the bucket is rinsed through the sieve until clean, and all residue placed in a sample container. The sieve is rinsed repeatedly to ensure that all organisms have been removed from the sieve. Once all organisms and residue are in the sample container, 70% ethanol is added to cover the sample with at least one inch of preservative.

#### 2.7 **Sample Packaging and Labeling**

Each sample is properly preserved in a plastic sample container. The lid is then sealed with electrical tape after the internal label is added. Each container is labeled inside and out with collection site, date of collection, sample number, and sample type (MH or HD). All samples are recorded on a standard chain of custody form (Figure 1).

#### 2.8 **Sample Storage**

Preserved samples are held at ORSANCO at room temperature until they can be shipped to the contract laboratory for speciation and enumeration.

**2.9 Documentation**

Habitat and environmental conditions, such as water quality parameters at each sampling location are noted and recorded. A standard macroinvertebrate sampling sheet is used to record the locations of sampler placement and retrieval.

**3.0 Corrective Action**

Immediate Corrective Actions (ICAs) and Long Term Corrective Actions (LTCAs) will be determined by crew leaders and documented as per the procedure outlined in the Biological Monitoring and Assessment Quality Assurance Program Plan, Section 13.

**4.0 Materials List**

PLACEMENT:

- 1. Waders
- 2. Assembled H-D samplers
- 3. Cement paver
- 4. Plastic zip ties
- 5. Rope / paracord

RETRIEVAL:

- 1. Waders
- 2. Knives / grappling hooks
- 3. 5-gallon buckets
- 4. Socket wrenches
- 5. Common screwdriver
- 6. Squirt bottle
- 7. Distilled water
- 8. Sorting pans or buckets
- 9. #30 sieves
- 10. Plastic sample jars and lids
  
- 11. Electrical tape
- 12. Permanent markers
- 13. Waterproof paper and ink for labels in sample jars
- 14. Any instruments needed for measuring WQ parameters



**STANDARD OPERATING PROCEDURES**

**FOR**

**MACROINVERTEBRATE SAMPLING**

**USING A QUALITATIVE MULTIPLE HABITAT APPROACH**



**Ohio River Valley Water Sanitation Commission  
5735 Kellogg Avenue  
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(513) 231-7719  
March 2023**

## **INTRODUCTION**

This document describes the procedures for ORSANCO's aquatic macroinvertebrate population surveys using the qualitative multiple habitat sampling method.

### **1.0 Sampling Schedule**

The qualitative sampling methods are to be performed upon retrieval of the Hester-Dendy sampling units.

### **2.0 Sampling Procedures**

#### **2.1 Net Specifications**

Samples are collected with standard D-frame 500µm mesh dip nets.

#### **2.2 Collecting Technique**

Multi-habitat samples are collected at 6 transects, every 100m, throughout each 500m zone. At each transect, 10 of any combination of jabs, sweeps, kicks, etc. are taken within 10m of the transect point. Efforts should be made to sample all available habitats within the 10m radius. The net is rinsed of debris and organisms into a 500µm mesh sieve bucket at each transect. All transect samples are combined to make one composite sample.

#### **2.3 Sample Processing and Preservation**

After all 6 transects have been collected, the remaining slurry is poured through a standard #30 sieve, the sieve bucket is rinsed through the #30 sieve until clean and all residue placed in a sample container. The #30 sieve is rinsed repeatedly into a white sorting pan or bucket to ensure that all organisms have been removed. Once all organisms and residue are in the sample container, 70% is added to cover the sample with at least one inch of preservative.

#### **2.4 Sample Packaging and Labeling**

Each sample is properly preserved in a plastic sample container. The lid is then sealed with electrical tape after the internal label is added. Each container is labeled inside and out with collection site, date of collection, sample number, and sample type (MH or HD). All samples are recorded on a standard chain of custody form (Figure 1).



## 2.5 **Sample Storage**

Preserved samples are held at ORSANCO at room temperature until they can be shipped to the contract laboratory.

## 2.6 **Documentation**

Habitat and environmental conditions are noted and recorded in a log.

## 3.0 **Corrective Action**

Immediate Corrective Actions (ICAs) and Long Term Corrective Actions (LTCAs) will be determined by crew leaders and documented as per the procedure outlined in the Biological Monitoring and Assessment Quality Assurance Program Plan, Section 13.

## 4.0 **Materials List**

1. Waders
2. D-frame net
3. Buckets
4. Squirt bottles
5. Distilled water
6. Sorting pans or buckets
7. #30 sieves and 500µm mesh sieve buckets
8. Plastic sample jars and lids
9. Electrical tape
10. Permanent markers
11. Waterproof paper and ink for labels in sample jars
12. Any instruments needed for measuring WQ parameters



**STANDARD LABORATORY PROCEDURES**

**FOR THE**

**MACROINVERTEBRATE SAMPLE ANALYSIS**



**Ohio River Valley Water Sanitation Commission  
5735 Kellogg Avenue  
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March 2023**

## **INTRODUCTION**

ORSANCO has adopted a version of Ohio EPA's laboratory methods for the analysis of its macroinvertebrate samples. Our methods are based Ohio EPA document:

Biological Criteria for the Protections of Aquatic Life: Volume III. "Standardized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities". Ohio Environmental Protection Agency. 1989. Division of Water Quality Monitoring and Assessment, Columbus, Ohio.

### **1.0 Laboratory Procedures**

Macroinvertebrate samples are immediately given an acceptable identification code and recorded into a log book upon arrival at the laboratory. Other information in the logbook includes the sample location, retrieval date, the person who conducts the analysis, and any other comments considered pertinent to the collections and sample analysis.

Composite samples are passed through U.S. Standard Testing Sieves #30 (0.589mm openings) with all remaining material preserved and properly labeled and coded in containers of 95% ethanol.

The following procedures are used during sample analysis:

- A) Sorting of the sample is done in a white enamel pan followed by scanning under the dissecting microscope (10x magnification). Subsamples are produced using the following guidelines:
  - 1. The Folsom sample splitter is used for all subsampling.
  - 2. After an entire sample has been sorted, subsampling within families containing unmanageable numbers is acceptable.
  - 3. Very large samples may be subsampled prior to sorting – but only after examination in a white enamel pan to remove obvious rare taxa, e.g. crayfish, hellgrammites, non-hydropsychid caddisflies.
  - 4. A minimum of 250 organisms is identified, with at least 50-100 midges, 70 caddisflies, 70 mayflies.
- B) Dipterans of the family Chironomidae are prepared for identification by clearing the larvae in hot 10% KOH for 30 minutes and then mounting in water on a microscope slides. Permanent slides for the voucher collection are mounted in Euparal mounting medium.
- C) Organisms determined to be dead at the time of collection are discarded.
- D) When only one sex of life stage can be identified it is assumed that the other sex or stage is the same species.
- E) Sections of bryozoan colonies are removed from the plates and saved for identification. Only colonies, not individuals, are counted.

- F) Early instars that cannot be identified are extrapolated where possible.
- G) Species level identifications are made wherever possible and practical.
  - 1. A minimum of 30% of taxa identified to species level per sample is desired unless precluded by the delivered state of the specimens. Generic or higher level classifications are made if specimens are damaged beyond identification, specimen is an unidentifiable early instar, or where established taxonomy is incomplete.
  - 2. The subclass Oligochaeta is exempt from this requirement. These specimens need not be speciated, only enumerated.
- H) Organisms are listed in a standard laboratory table format (“TRU CODE” included as a column when possible).
- I) Two end fragments of an oligochaete are counted as an individual. Fragments without ends are not counted.
- J) Any taxonomic key in the laboratory can be used as an aid in the identification of an organism. However, the final identification and name used are taken from the asterisked references in the attached tables. Also indicated is the level of taxonomy attainable with the keys listed.
- K) Data should be received by ORSANCO by the turn-around timeframe of 90-110 days. There will be a late fee of 5% per sample per week for analyses returned after the 110th day of the laboratory's receipt of sample.

## **2.0 Corrective Action**

Issues or potential improvements pertaining to the analysis of macroinvertebrate samples may arise during the execution of this protocol. Corrective actions may therefore be implemented at the discretion of ORSANCO Biological Staff. Immediate Corrective Actions (ICAs) and Long Term Corrective Actions (LTCAs) will be determined by the Project Leader and documented as per the procedure outlined in the Biological Monitoring and Assessment Quality Assurance Program Plan, Section 13.

**STANDARD OPERATING PROCEDURES**

**FOR**

**DATA ENTRY AND DATABASE USAGE**



**Ohio River Valley Water Sanitation Commission  
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March 2023**

## **INTRODUCTION**

This document describes the procedures for ORSANCO's biological data entry and database use. This document provides guidelines for the proper entry, archiving, and processing of Ohio River Basin location, fish, macroinvertebrate, habitat, water quality, and background data collected during monitoring and assessment activities. This SOP has been developed to maintain continuity and ensure the quality of the data collected and subsequent products derived from these data.

### **1.0 Data Entry Schedule**

Data entry occurs throughout the field season once data have been collected and all data sheets are returned to ORSANCO. Database architecture, appending, and verification occur at the cessation of field activities.

### **2.0 Data Entry Procedures**

Raw data sheets are assembled in a file along with site description sheets, maps of the sampling sites, and the final study plan. Raw data are entered by biological staff under the supervision of the Project Leader into Microsoft Excel© spreadsheets and imported into a Microsoft Access© relational database under the supervision of the Project Leader. Data are to be manually entered sequentially row by row from the raw datasheet specific to each location. Data entry will continue for all data types until each datasheet is initialed as complete. Any changes made to data sheets are initialed, dated, and verified by the Project Leader. Digital photograph backups of all datasheets will be compiled, organized by date and location, and stored electronically on ORSANCO servers.

After all data for a survey have been entered into the database, the entries are proofread by the Field Sampling Leader for accuracy; a final call on whether the data is acceptable will be made by the Project Leader. All corrections or updates are then applied to the database.

### **3.0 Database Usage**

The biological Microsoft Access© database(s) will reside and be maintained on ORSANCO servers and their security and maintenance overseen by ORSANCO IT staff.

Access to these data servers is possible only by ORSANCO staff using a valid login and password. All data on all servers are backed up incrementally on a daily basis as changes / modifications occur. Additional full-system backups occur weekly and are overseen by ORSANCO IT staff. Additionally an emergency backup of all biological database information is maintained on an external hard drive. This emergency backup is updated incrementally as changes are made to the database. The emergency backup is overseen and maintained by ORSANCO biological staff under the supervision of the Technical Programs Manager.

All databases are comprised of tables containing both text and numeric information. Queries can be performed by any member of ORSANCO biological staff with a valid login and password or any staff member authorized by and under supervision of ORSANCO staff.

Data queried from databases will be used in numerous statistical analyses tailored to provide insight as they relate to specific projects at the discretion of ORSANCO biological staff.

#### **4.0 Database Verification**

Database(s) is (are) populated / appended and verified for accuracy by the Project Leader. Data tables are sorted to identify and correct clerical errors in taxonomy, location information, location naming convention, river mile, and collection information. A minimum of 10% of all data in each database is hand verified by comparison to raw datasheets. If more than 10% of the verifications are incorrect, 100% of digitally entered data are verified against original raw datasheets to ensure correct entry.

#### **5.0 Documentation**

Any and all data, documents / products, or reports derived from analyses of data within ORSANCO databases are available to the public via written request only after QAQC and review. The use of Microsoft Office© tools (Access, Excel etc.) will be documented upon request. QAQC responsibility for data entry and database usage/ verification as related to the Biological Monitoring and Assessment Program effectively terminates at the annual compilation of a final, verifiably accurate and up to date database for use by ORSANCO biological staff.

#### **6.0 WQX Data Submission**

As per Clean Water Act Section 106 funding requirements, ORSANCO uploads all applicable monitoring data to WQX. All applicable data must first pass QAQC requirements before they are formatted per established guidelines, verified, and submitted electronically via the WQX portal under the supervision of the Technical Manager.

#### **7.0 Corrective Action**

Issues or potential improvements pertaining to data entry and database usage may arise during the execution of this protocol. Corrective actions may therefore be implemented at the discretion of the Project Leader. Immediate Corrective Actions (ICAs) and Long Term Corrective Actions (LTCAs) will be determined by staff and documented as per the procedure outlined in the Biological Monitoring and Assessment Quality Assurance Program Plan, Section 13.



**STANDARD OPERATING PROCEDURES**

**FOR**

**MULTI-PARAMETER WATER QUALITY SENSOR**

**DEPLOYMENT AND APPLICATION**



**Ohio River Valley Water Sanitation Commission  
5735 Kellogg Avenue  
Cincinnati, Ohio 45230  
(513) 231-7719  
March 2023**

## **INTRODUCTION**

This document describes ORSANCO's Multi-parameter Water Quality Sensor (i.e., continuous data loggers) deployment, application, and data handling procedures.

### **1.0 Field Equipment**

The following equipment is used to deploy Multi-parameter Water Quality Sensors:

#### **1.1 Multi-parameter Water Quality Sensor Unit Types**

- 1.1.1 Onset HOBO® Dissolved Oxygen/Temperature Data Logger
- 1.1.2 YSI 6600V2
- 1.1.3 In-Situ AquaTROLL 500
- 1.1.4 YSI EXO2

#### **1.2 Watercraft (where applicable)**

- 1.2.1 Approved workboat equipped with dual engines and all appropriate safety gear
- 1.2.2 Handheld GPS and boat-mounted chartplotter
- 1.2.3 Laser rangefinder / measuring tape
- 1.2.4 Marine radio
- 1.2.5 Tool kit
- 1.2.6 Data sheets / field notebook

#### **1.3 Sensor Mount / Sampling Platform and Security**

- 1.3.1 PVC protective sheath
- 1.3.2 Parachute (550) cord (in stream) or steel cable (infrastructure-attached sites)
- 1.3.3 Concrete cinder block, heavy grade zip-tie fasteners, 2' rebar sections

### **2.0 Training**

- 2.1 All crew members will receive training by an ORSANCO staff member with at least one year of large river field sampling experience as a Field Crew Leader. All crew members will be responsible for familiarizing themselves with all applicable ORSANCO SOPs related to their workload. Crew training will take place in accordance with procedures outlined in the Biological Monitoring and Assessment Quality Assurance Program Plan, Section 4. All field sampling will occur under the direct supervision of an ORSANCO Field Crew Leader.

- 2.2 *Field crew leader qualification* requires a biologist with supervised boat experience demonstrated competency with modern equipment and techniques, and marked proficiency with SOP protocol execution. Field crew leaders are appointed based on eligibility, experience, and professional ability as determined by and at the sole discretion of the Technical Programs Manager.
- 2.3 Staff shall perform at least one training session to the satisfaction of the Field Crew Leader and Technical Programs Manager and be familiar with all ORSANCO Field Safety protocols before performing any program sampling.

### **3.0 Field Methods for Multi-parameter Water Quality Sensor Deployment**

#### **3.1 Sensor Initialization, Calibration and Sampling Platform Construction**

Each Multi-parameter Water Quality Sensor will be initialized and indexed prior to deployment using instrument-specific software packages. Once initialized, each sensor will be prepared as per manufacturer guidelines and carefully packed for transport to the deployment site(s). Sensors that are factory sealed require calibration by the manufacturer, and are shipped back for inspection and calibration within the manufacturer-specified timeframes. All other water quality sensors will be calibrated weekly. This calibration will be conducted in accordance with all manufacturer's instructions and guidelines. Regardless of type, all sensors are returned to the manufacturer for routine maintenance within the manufacturer-specified timeframes.

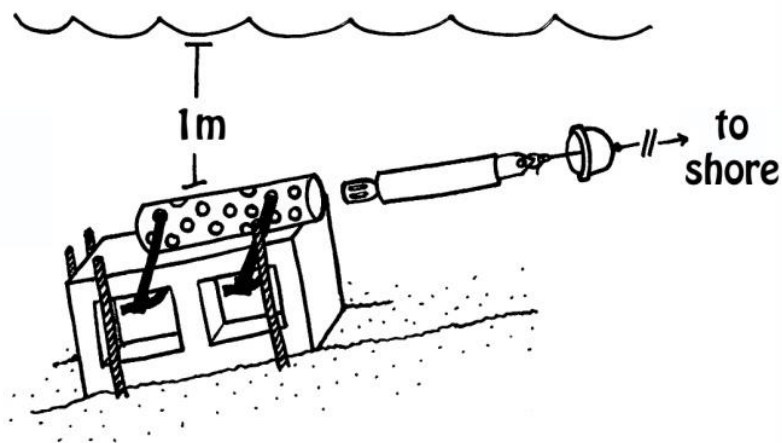
Upon deployment, sensors are unattended and deployment sites are of two types:

- in-situ in stream - or*
- in-situ infrastructure-attached.*

Each in-situ in stream deployment for Onset HOBO® data loggers consists of:

- 3.1.1 1 (one) full size foundation-grade concrete cinder block and 2' rebar sections
- 3.1.2 1 (one) 2" PVC sheath and cap; each sheath drilled with a sufficient number of 0.5" holes to allow adequate water flow around the sensor
- 3.1.3 1 (one) water quality sensor
- 3.1.4 adequate parachute cord to secure sensor to sheath cap (cap is attached to PVC sheath via friction) and to onshore tie-off location (e.g., tree or other fixed object)

*In-situ in-stream deployment illustration*

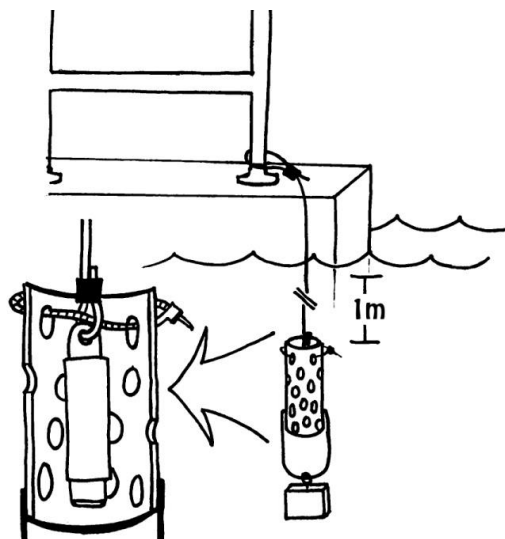


Each in-situ infrastructure-attached deployment for Onset HOBO® data loggers consists of:

- 3.1.5 1 (one) 2" PVC sheath drilled with a sufficient number of 0.5" holes to allow adequate water flow around the sensor
- 3.1.6 1 (one) water quality sensor
- 3.1.7 heavy duty cable tie (for conductivity sensors) or bolt with wing nut to secure sensor to PVC sheath
- 3.1.8 adequate steel cable to secure sheath to attached infrastructure for security and retrieval
- 3.1.9 lead or concrete weight attached to bottom of sheath via steel cable

Each PVC protective sheath must be sufficiently robust to handle the impact of large, fast-moving debris flowing with the water.

*In-situ infrastructure deployment illustration*



### 3.2 Site Selection and Equipment Location Considerations

Exact sampling sites will change periodically and will be documented in annual work plans.

- 3.2.1 *In-situ in stream* water quality sensor deployment site locations that are coupled with existing monitoring / assessment site locations should be placed within the sampling reach, preferably near the downstream end. Care should be taken in choosing exact deployment sites. Areas of potentially high human activity (fishing areas, boat docks, etc) should be avoided to minimize tampering with the sensors. Areas with evidence of beaver (*Castor canadensis*) activity should also be avoided to minimize the threat of chewing through the parachute cord. Bends, sandbars, and eddies are sources of non-uniform flow that can result in areas of erosion and /or aggradation and are not ideal deployment locations. Confluences are typically areas of high turbulence and non-uniform flow due to dynamic mixing of water bodies. Due to active mixing, monitoring in confluences is not recommended. Monitor a fair distance downstream to obtain more valid data. Distance downstream will depend on site conditions. Avoid areas of streambank erosion for any stable, robust long-term monitoring stations. Sediment transport can introduce problems related to streambed aggradation and sediment build-up on sensors (Miles 2008; Wagner 2006). Each exact deployment site is GPS marked and indexed as inconspicuously as possible.
- 3.2.2 *In-situ infrastructure-attached* sensor deployment site locations on bridges, aprons, dams / locks and other structures should be deployed at sturdy attachment points. Parameters measured and monitoring objectives should be considered to determine appropriate depths for sensor deployment. For example, conductivity sensors should be deployed as near to the bottom of the river as possible if the goal is to record maximum conductivity readings, whereas sensors should be deployed approximately 1 (one) foot below the surface to record surface temperature. Turbulence and flow disturbance at in stream infrastructure (bridges, etc.) can affect sensor performance and increases maintenance needs. Localized heavy erosion can occur downstream of these structures and aggradation can occur upstream. Avoid deploying sensors at or immediately downstream of outfalls, discharge points and spill-prone areas unless specifically targeting their effects (Miles 2008; Wilde 2006). Each deployment site is GPS marked and indexed as inconspicuously as possible.

### 3.3 Sensor Maintenance and Deployment Site Visit Intervals

#### 3.3.1 *Onset HOBO data loggers*

During each deployment site visit the sensor will be retrieved from its PVC sheath and wiped clean using a terry cloth (or similar) rag. At this point data will also be downloaded to a data shuttle and any other required maintenance will be performed (e.g., removal of zebra mussels or algae from sheath, replacement of cable ties, bolts or nuts as needed).

##### 3.3.1.1 *In-situ in stream water quality sensors*

Deployment site visits will occur no less than every 6 (six) weeks that the sensors are deployed.

3.3.1.2 *In-situ infrastructure-attached water quality sensors*

Deployment site visits will occur no less than every 60 (sixty) days that the sensors are deployed.

3.3.2 *YSI EXO2 Datasonde / In-Situ AquaTROLL 500*

During each visit the sensor will be retrieved from its PVC sheath and wiped clean using a terry cloth (or similar) rag. Data will be downloaded to a data shuttle and any other required maintenance will be performed (e.g., removal of zebra mussels or algae from sheath).

3.3.2.1 Deployment site visits will occur no less than once every three (3) weeks that the sensors are deployed.

3.4 **Documentation**

3.4.1 *Installation Documentation:* Written documentation of all deployment locations records of installation including receipts, owners' manuals, and modifications made, etc. are kept in site files.

3.4.2 *Photo documentation:* Complete photographic documentation of each deployment site and installation are recommended. Photos of the site

including upstream and downstream and cross-section photos at each sampling site, and photos of installations, etc. should be taken at a variety of site conditions wherever appropriate.

3.4.3 *Ongoing Site Visit Documentation:* Records of site visits (in addition to deployment and retrieval information), maintenance performed, problems encountered and their solutions, etc. will be maintained and kept in site files.

4.0 **Data Collection, Handling, and Management**

4.1 **Data Collection and Handling**

All raw data will be entered into site files by hand or by using sensor specific software packages. Data entry is carried out by the Field Crew Leader.

4.1.1 *Sensor Data Retrieval*

Data from each sensor will be downloaded to appropriate data shuttles or laptop computers upon sensor retrieval, deployment site visits, or sensor return to ORSANCO offices.

#### 4.1.2 *Discrete Data Collection*

At the time of sensor deployment and each subsequent deployment site visit (including retrieval) appropriate water quality data will be recorded using a recently calibrated secondary unit (YSI, Hydrolab, In-Situ, etc.) for side by side comparisons with the sensor data to determine accuracy.

### 4.2 **Data Management**

All data is housed and backed up on servers located at ORSANCO offices and available for use in relational databases and spreadsheet programs. Findings, data analyses, and assessment outcomes (where appropriate) derived from water quality sensor data will be detailed in program-specific reports and made available upon completion and program approval.

## 5.0 **Corrective Action**

Immediate Corrective Actions (ICAs) are implemented and resolved expeditiously by leaders in the field. Documentation of ICAs takes place on site and is archived with raw data sheets and on Corrective Action forms when appropriate. In the case of Long Term Corrective Actions (LTCAs), the following steps should be taken:

1. Define the problem and discuss with Technical Programs Manager
2. Technical Programs Manager assigns responsibility for investigating the problem
3. Responsible person(s) determines corrective action to eliminate the problem
4. Corrective action plan must be approved by Technical Programs Manager
5. Implement corrective action
6. Establish effectiveness of corrective action (follow-up)
7. Verify that corrective action has eliminated the problem
8. Issue report (documentation, CA Form(s)) to Technical Programs Manager

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**STANDARD OPERATING PROCEDURES**

**FOR**

**WATER CHEMISTRY SAMPLE COLLECTION**



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March 2023**



## **INTRODUCTION**

This document describes the field operations and quality control activities that ORSANCO will use for collecting water chemistry data. It is a step-by-step guide for proper collection, preservation, and shipment of river water samples, developed to insure the quality of data collected by field personnel.

### **1.0 Sampling Locations**

ORSANCO will sample at any of 15 randomly selected sites in each of three to four pools as well as fixed sampling locations annually. Sites used in this study are selected following a probability design frame developed for ORSANCO by USEPA.

### **2.0 Sampling Schedule**

Water chemistry will consist of single point samples taken separately from each designated location during the July-October sampling period. Samples are collected so as to arrive at the laboratory on a weekday (Monday through Friday) and within 5 days of collection unless prohibited by laboratory-specified hold times.

### **4.0 Sample Kit and Field Equipment**

The basic field kit required for water chemistry sampling is described below. Replacement supplies are provided by ORSANCO as needed.

#### **TEST EQUIPMENT**

In-Situ AquaTROLL 500 handheld sonde or YSI Professional Plus and Pro ODO or comparable multi-meter kit

#### **CONTAINERS**

2-liter Plastic / Acrylic Kemmerer / Van Dorn sampler with spigot

Sample collection containers (provided by laboratory)

#### **MISC SUPPLIES**

2 - 50 foot Nylon Ropes

Ice

Waterproof-Marking Pen

Vinyl Tape

Safety Glasses

Disposable Latex Gloves

Strapping Tape

Shipping Labels

Zip-Lock Bags

Insulated Coolers

Chain of Custody Forms

USCG Approved PFD

Water Quality Report Forms

Rubber bands

## 5.0 River Water Sample Collection

5.1 *Kemmerer / Van Dorn*: the acrylic sampler will be filled approximately 100 feet from shore and will come from a depth approximately halfway between the surface and bottom. The collection point will be located at the downstream end of the 500m shoreline reach delineating the bounds of the biological collection area.

*Surface grab*: surface grab sample containers will be filled approximately 100 feet from shore and will come from a depth approximately 1 foot below the surface at the downstream end of the 500m shoreline reach.

5.2 The contract laboratory will provide sample containers labeled with the analytical parameter, station number, and preservative inside a cooler for shipping. Additionally, each field sampler will mark the bottles with the collection date, time, and his/her initials using a waterproof-marking pen.

<u>BOTTLE SIZE</u>	<u>LABEL</u>	<u>PRESERVATIVE</u>
500 mL amber glass	Phenols	H <sub>2</sub> SO <sub>4</sub>
250 mL plastic	Cyanide (CN <sup>-</sup> )	NaOH
1 Liter plastic	Direct Ammonia, TOC TP, Nitrate-Nitrite	H <sub>2</sub> SO <sub>4</sub>
1 Liter plastic	TSS	None
1 Liter plastic	Cl, SO <sub>4</sub>	None
250 mL plastic	Hardness	HNO <sub>3</sub>

5.3 The appropriate amount of river water will be distributed to the sample bottles, capped, and inverted several times to thoroughly mix the solution.

5.4 Sample bottles will be placed in an insulated cooler with crushed ice to maintain at 4° C.

5.5 All pertinent information and field observations will be recorded on Chain of Custody Water Quality Report forms, using one form per station. One copy will accompany the sample to the laboratory and a second copy will be submitted to ORSANCO. Chain of Custody Water Quality Report forms will be sealed in a plastic sealable bag and placed inside the shipping cooler.

## 6.0 Field Measurements

6.1 Temperature, pH, conductivity, and dissolved oxygen are measured in the field using portable sondes. Proper calibration and maintenance of these instruments are required to obtain valid data. Instruments will be examined frequently for signs of corrosion or instability and any malfunctions will be reported.

- 6.2 All field measurements, temperature, pH, dissolved oxygen, and conductivity will be made simultaneously with a Multi Probe System/ Data Logger. Units will be calibrated each week of use or more frequently if required following appropriate SOPs.

## 7.0 Sample Shipment / Delivery

Sample bottles will be shipped or hand delivered to the laboratory in insulated coolers containing sufficient ice to maintain a 4°C temperature during shipment by each sampler. Shipped samples will be sent so that they arrive at the laboratory within 24 hours of collection.

- 7.1 Crushed ice will be obtained locally before beginning sample collections.
- 7.2 As sample bottles are filled, they will be stored in coolers with ice until all locations are sampled. Information will be recorded on the Water Quality Report / Chain of Custody forms.
- 7.3 Place address label and shipping carriers label will be affixed to the cooler lid and secured with clear tape.
- 7.4 Coolers will be delivered to the nearest shipping office for express overnight service to the laboratory.
- 7.5 A copy of the Water Quality Report form / Chain of Custody and a copy of the shipping label will be retained by ORSANCO.

## 8.0 Quality Control

- 8.1 The interior of all plastic / acrylic samplers (Kemmerer / Van Dorn / surface container) will be kept scrupulously clean. After each sample the container will be emptied, and visually inspected for residual debris or oil films that may contaminate the next sample. If contamination is observed, the sampler will be cleaned with a non-phosphate detergent and thoroughly rinsed with deionized water. Procedures for decontamination will be to clean sampling equipment with a non-phosphate detergent and thoroughly rinse with water. The decontamination procedure will be performed prior to and immediately after sampling and as stated above if contamination is present in the sampling container.
- 8.2 During summer months, additional ice and coolers may be needed to keep samples chilled during shipment or delivery to the laboratory. Fewer bottles will be placed in each cooler and more ice added just before delivery to the carrier. Placing the bottles and ice within a large plastic garbage bag inside the cooler and sealing the bag will help minimize water leakage during shipment.
- 8.3 Containers with chemical preservatives will be handled carefully to prevent spillage on hands and clothing. Acid spills will be neutralized with sodium bicarbonate (baking soda) and the area will be washed with water. Sampling equipment will be cleaned and wiped dry before storage.

- 8.4 Sample blanks and duplicates will be prepared in the field and submitted to the laboratory on a periodic basis. These quality control samples serve as a check on the sampling method, equipment contamination, and laboratory performance in meeting analytical precision and accuracy.
- 8.4.1 Sample blanks will be prepared with distilled water following the exact steps used for the routine river samples. Sample bottles will be labeled as "Field Blank" along with the date and time of collection.
- 8.4.2 Duplicates will be prepared by collecting another river water sample and filling a second bottle according to steps outlined under Part 5 above. Sample bottles will be labeled as "Duplicate" along with the date and time of collection.

**Table 4.** Analytical Parameters, Methods and Reporting Levels

<b>Parameters</b>	<b>Analytical Method</b>	<b>Detection Limit</b>
Ammonia Nitrogen	350.3	0.03 mg/L
Chloride	325.3	1.0 mg/L
Hardness	SM 2340C	1.0 mg/L
Nitrate + Nitrite	353.3	0.02 mg/L
Phenolics	420.1	0.005 mg/L
Total Kjeldahl Nitrogen	4500-N	0.20 mg/L
Sulfate	HACH 8051	1.0 mg/L
Total Suspended Solids	160.2	1.0 mg/L
Total Phosphorus	365.3	0.01 mg/L
Total Organic Carbon	415.1	0.5 mg/L

**STANDARD OPERATING PROCEDURES**

**FOR**

**BOTTOM SEDIMENT SAMPLE COLLECTION**

ORSANCO follows EMAP-GRE sediment collecting procedures found in the EMAP-GRE Field Operation Manual, Section 11, p207-214, excerpted in the following document.



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March 2023**

## **INTRODUCTION**

At or near each sampling location, a fine-sediment sample is collected using either a hand scoop or a “petite Ponar” grab sampler. The objective is to collect a 4-L composite sample that is representative of MCS depositional areas at the site. The composite sample will be subsampled in the lab for multiple analyses. Section 2.0 describes the sediment sample collection procedures in detail.

### **1.0 Sample Collection Procedure**

- 1.1 Sediment samples are collected at each 100 meter transect within the sampling zone.
- 1.2 Locate sediment samples in areas or patches of fine substrate (silt / sand, silt, clay, muck) in a zone bounded on the shore side by the apparent low-water mark from daily flow fluctuations and bounded on the river side by the 0.3-m (usually about mid-biceps) depth contour (recommended maximum sample depth; deeper sampling may be possible). The low-water mark at a site can often be detected by the presence of periphyton or attached filamentous algae just below the low-water mark. If samples cannot be safely collected by wading at a station due to vertical banks or other reason go to step 2.5.
- 1.3 Be sure to avoid the area that has just been kick sampled. Sampling up-river from kick sample locations is recommended. If fine substrates are not present within 5 m up- or downriver from the station, do not collect sediment at that station and flag the station on the form.
- 1.4 If fine substrate is present, use a small scoop to collect a sample of about 225 cm<sup>2</sup> (15 x 15 cm [6 x 6 inches]) of the top 2 cm of substrate (this volume is approximately equal to six scoops). Place the sample in a clean bucket. Use gloves for handling sediment. Do not assume rip rapped shorelines lack fine sediment. Look for fines between the large rocks
- 1.5 If wading is not possible, use a petite Ponar sampler or similar device deployed from the boat to collect a sediment sample adjacent to the station. Release the petite Ponar sample onto a tub and use the scoop to collect about 225 cm<sup>2</sup> (15 x 15 cm [6 x 6 inches]) of the top 2 cm of the sample. Estimate sample area visually. Place the subsample in the sediment composite bucket and discard the rest of the Ponar sample.
- 1.6 Repeat steps 1.2-1.5 at each of the 6 littoral stations. Record the total number of replicates (stations) included in the composite. Note in a comment the stations at which sediment was collected using a non-wading method.
- 1.7 It is important that a sufficient sediment (not less than 4 L) sample for analysis be collected. If multiple stations have no fine sediment, it is permissible to collect extra sample at stations that do have fine sediment or between stations. Be sure to note this in a comment.
- 1.8 Using a large stainless steel spoon, thoroughly mix the composite sample in the bucket and transfer 4 L of the composite in a 30 x 50-cm 3-mil thick polyethylene bag. Try to limit the amount of sediment adhering to the inside of the bag near the top. Grasp the bag just above the sediment to express the air.

Twist and knot the bag to seal it. Write the site number and date directly on the bag with a permanent marker and place it in a cooler with ice.

1.9 Go to section 3 for sample labeling and preservation procedures.

## 2.0 Sample Labeling and Preservation

2.1 To avoid clutter in the boat, sediment samples may be transported to the ramp or base location (if it is close to the ramp) in a cooler with ice for final labeling and preservation.

2.2 Place the sediment sample inside a second 3-mil polyethylene bag, twist the top, and knot to seal. Prepare a label (Figure 1.) for outside the outer bag. Using a fine-point permanent marker, fill in the site number and sample date. Place the label on the outer bag and cover it with clear tape. Place the sample on ice or in a refrigerator. Do not freeze sediment samples.

<p><b>SEDIMENT GRAB (SG)</b></p> <p>GRW04449-____</p> <p>____/____/200__</p> <p>Composite volume _____ L</p> <p>Site visit number 1 2</p> <p>300255</p>
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Figure 1. Example sediment sample label.

## 3.0 QA Considerations

- It is permissible to collect sediment between stations to insure a composite volume of at least 4L. Note deviations from standard procedure in a comment.
- Do not assume rip rapped shorelines lack fine sediment. Look for fines between the large rocks.
- Mix the composite sediment sample thoroughly before extracting the final 4L composite.
- When sampling debris pulled from the river, be sure to sample the upper surface.
- Monitor sediment samples in your possession to insure they do not warm up or freeze.

## 4.0 Safety Considerations.

- Use extreme care walking on riprap. Rocks can shift unexpectedly and serious falls are possible.
- Use caution when sampling in swift or deep water. Wear a suitable PFD and consider using a

safety tether held by an assistant. For most people, conditions are rarely suitable for collecting a periphyton or sediment sample in water deeper than 0.6 m.

- Do not attempt to collect periphyton or sediment from vertical or near vertical banks.
- Professional-quality breathable waders with a belt are recommended for littoral sampling. Neoprene boots are an alternative, but should have sturdy, puncture-resistant soles.
- Use caution using the Ponar-type samplers. The jaws are sharp and may close unexpectedly. Replace frayed lines and worn parts.
- Raise the Ponar sampler from and into a plastic tub rather than from the boat deck. This prevents feet from getting under the sampler.
- Don't try to remove large pieces of debris from the river by yourself.
- Use safety glasses and gloves when handling formalin.
- Avoid contact with sediment samples. Use gloves if necessary.

## 5.0 References

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**GUIDELINES**

**FOR**

**THE EVALUATION OF BIOLOGICAL POOL SURVEY RESULTS**



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## INTRODUCTION

This SOP has been developed to maintain continuity and ensure physical habitat and biological data collected per ORSANCO SOPs are used to appropriately qualify and assess an Ohio River navigational pool for aquatic life use. Each individual navigational pool will serve as a separate and distinct Assessment Unit (AU). ORSANCO's Biological Water Quality Subcommittee (BWQSC) believes that a subset of 15 randomly selected sites within each navigational pool can accurately represent the condition of fish and macroinvertebrate assemblages within each AU. This document describes the procedure the BWQSC will use to determine biological assessment endpoints as defined by *aquatic life use support* following these six steps:

## EVALUATION GUIDELINES

**Step 1:** Do biological data require explanation or *qualification*? *Qualification*: data passes QAQC yet is not representative of results achieved through standardized collection protocol as influenced by non-pollutant factors (e.g. elevated discharge or velocity and subsequent hydrologic events, equipment malfunction, loss or sampling device tampering, instream habitat or other factors that would contribute to either Type 1 or Type 2 error).

- If **yes**, proceed to **Step 2**.
- If **no**, proceed to **Step 3**.

**Step 2:** If data require qualification based on input from the biological staff, can qualified data be described in such a way or statistically adjusted (account for variation due to above listed stressors) that they are comparable to other assessment data and therefore useable in indicator results?

- If **yes**, include sample data and proceed to **Step 3**.
- If **no**, exclude sample data from further analyses, and proceed to **Step 3**.

**Step 3:** Were the minimum number of samples collected for each indicator? 15 fish samples, and a minimum of 10 macroinvertebrate (macroinvertebrate) samples need to be collected. The 10 macroinvertebrate samples must be comprised of deep Hester-Dendy's. Minimum sample number criteria (15 fish and 10 macroinvertebrate respectively) are standardized and necessary to ensure comparability between assessments.

- If neither indicator from a single pool meets the required minimum, **additional samples will be necessary** to obtain an assessment. Budget and staffing resources must be sufficient to do so. The pool may remain unassessed for that cycle **only** if resources for additional samples are insufficient and/or a pool assessment was made in the previous cycle.
- Proceed to **Step 4** with an indicator failing to meet the required minimum.
- Proceed to **Step 5** with an indicator meeting the required minimum.

**Step 4:** Was an assessment made in the previous cycle using the indicator?

- If **yes**, pool remains unassessed for that indicator.
- If **no**, **additional samples will be necessary** to avoid two successive assessment cycles where the same indicator was not used. Budget and staffing resources must be sufficient to do so. If resources for additional samples are insufficient, the BWQSC can use the other indicator results for assigning support status.

**Step 5:** Calculate the arithmetic mean (average) of *mORFIn* (fish) score and / or average *ORMIn* (macroinvertebrate) score for the pool, as well as the 90% confidence interval. Does the 90% confidence interval overlap the established biocriterion (i.e. 20-point threshold – 25<sup>th</sup> percentile of observed index score)?

- If **yes**, continue to **Step 6**.
- If **no**, the BWQSC accepts the indicator results. Results can then be used in press releases to stakeholders and the public as well as reported to the 305(b) workgroup for use in assessment and proceed.

**Step 6:** The BWQSC will review all paired abiotic and biological data (comparability) in order to determine whether to:

- **Accept** the average indicator result for use in press releases to stakeholders and the public as well as reported to the 305(b) workgroup with the caveat “non-significant departure from the criterion”. This caveat would not preclude the assessment from attaining its aquatic life use designation

OR

**Decline** the average indicator result for use in assessment and proceed. Pool remains unassessed for the excluded indicator.