September 28, 2009

Jeff Reynolds
Division of Surface Water - OEPA
Credible Data Program
50 W. Town St, Suite 700, P.O. Box 1049
Columbus, OH 43216-1049

Re: Study Plan for 2009 Biological Survey of Sycamore Creek

Mr. Reynolds,

Enclosed, please find the Final Study Plan for 2009 Biological Survey of Sycamore Creek. The document has been revised slightly since Revision 1 of the Draft Study Plan (dated August 25, 2009), to reflect the use of tote barge electrofishing as opposed to longline electrofishing.

If you should have any questions, please give me a call at the number below or contact me by e-mail at jpapineau@enviroscienceinc.com.

Respectfully,

Joe Papineau
GIS Analyst / Biologist

Enclosure:

OCT -1 AM 11:06
EPA - DSW

3781 DARROW ROAD, STOW, OHIO 44224
330-688-0111 / FAX: 330-688-3858 / TOLL FREE: 800-940-4025

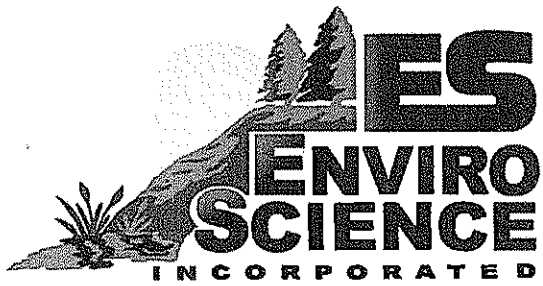
ENVIRO SCIENCE INCORPORATED

Final Study Plan For
2009 Biological Survey of Sycamore Creek, Pickerington, Fairfield County, Ohio

Prepared for:

Ohio Environmental Protection Agency
50 West Town Street, Suite 700
Columbus, Ohio 43215

Prepared by:



EnviroScience, Inc.
3781 Darrow Road Stow, OH 44224
(800) 940-4025
Fax (330) 688-3858

September 25, 2009

2009 OCT -1 AM 11:06
OHIO EPA - DSW

INTRODUCTION

EnviroScience, Inc. proposes to conduct a biocriteria use attainment study of a section of Sycamore Creek in Pickerington, Fairfield County, Ohio during the 2009 sampling season. The study will be conducted on behalf of Schottenstein Zox and Dunn Co., LPA for the City of Pickerington Wastewater Treatment Plant (WWTP). In total, four sampling sites (Attachment A, Figure 1.0) have been established within the study area for evaluation of the following:

- Fish Community Evaluation (Index of Biotic Integrity) – except at RM 4.33
- Benthic Macroinvertebrate Evaluation (Invertebrate Community Index or Qualitative Community Tolerance Value)
- Habitat Characterization (Qualitative Habitat Evaluation Index)

The purpose of this study is to determine if sites downstream of the Pickerington WWTP outfall are in attainment of Warm Water Habitat (WWH) criteria. Site locations have been chosen to capture potential effects of elevated total dissolved solids (TDS) that may be introduced to Sycamore Creek via the Pickerington WWTP outfall.

EnviroScience proposes to conduct this evaluation under the supervision of personnel who have fulfilled the requirements Ohio's Credible Data Program and have received their Level 3 Qualified Data Collector (QDC) status (Appendix B, C and D):

Name:	Joel Bingham (effective December 28, 2006; May 14, 2008)
Address:	3781 Darrow Rd., Stow, OH 44224
Phone:	330.688.0111
Email:	jbingham@enviroscienceinc.com
QDC Level:	3
QDC Specialty:	Fish Community Biology and Habitat (QHEI)
QDC Number:	046

Name:	Rhonda Mendel (effective March 6, 2008)
Address:	3781 Darrow Rd., Stow, OH 44224
Phone:	330.688.0111
Email:	rmendel@enviroscienceinc.com
QDC Level:	3
QDC Specialty:	Benthic Macroinvertebrate Biology
QDC Number:	160

Name:	Joe Papineau (effective September 10, 2008)
Address:	3781 Darrow Rd., Stow, OH 44224
Phone:	330.688.0111
Email:	jpapineau@enviroscienceinc.com
QDC Level:	3
QDC Specialty:	Chemical Water Quality Assessment
QDC Number:	240

Additional staff that will be assisting with sample collection, analysis and data entry may include:

Name:	Jamie Krejsa
Title:	Vice President / Director of Ecological Services
Project Role(s):	Agency Coordination, Sample Collection, Data Review
QDC Status:	None

Name:	Dave Czayka
Title:	Biologist
Project Role(s):	Sample Collection, Data Entry
QDC Status:	Training completed for Level 2 Habitat (QHEI, HHEI), training in progress for Level 3 Fish

Name:	Nicole Jordan
Title:	Biologist
Project Role(s):	Sample Collection, Data Entry
QDC Status:	Level 2 Habitat (QHEI), Level 2 Benthic Macroinvertebrates
QDC Number:	063

Name:	Jeff Niehaus
Title:	Biologist
Project Role(s):	Sample Collection, Data Entry
QDC Status:	None

Name:	Andrew Farber
Title:	Biologist
Project Role(s):	Sample Collection, Data Entry
QDC Status:	None

All work conducted will be done so under the supervision of Level 3 QDC staff. Level 3 QDC staff will be present during all field activities and will also review all data and results for completeness, accuracy and strict compliance with all Level 3 protocols.

BIOCRITERIA STUDY

EnviroScience will evaluate fish and benthic macroinvertebrate communities, stream and riparian habitat, and several in-field chemistry parameters to assess the ecological conditions of study area. Biological sampling will follow the methods within *Biological Criteria for the Protection of Aquatic Life Volume I-III, Addenda and Updates* (Ohio EPA, 1987a, 1987b, 1989a, 1989b, 2006b, 2008). Four sites will be sampled within the study area:

- RM 4.7 – Historical Ohio EPA sampling site upstream of the Pickerington WWTP outfall selected to provide upstream reference data on fish and macroinvertebrate communities, and to allow for comparison to historical data
- RM 4.33 – Macroinvertebrate-only sampling site within the Pickerington WWTP effluent mixing zone selected to provide data on potential impacts to Sycamore Creek's macroinvertebrate community within the mixing zone
- RM 4.2 - Historical Ohio EPA sampling site downstream of the Pickerington WWTP outfall selected to provide data on potential impacts to Sycamore Creek's fish and macroinvertebrate communities, and to allow for comparison to historical data
- RM 3.8 – Additional downstream sampling site selected to provide data on potential impacts to Sycamore Creek's fish and macroinvertebrate communities

Sampling site locations will be documented using a dGPS with a horizontal accuracy of less than one meter.

EnviroScience proposes to conduct one round of fish sampling considering the drainage area (<20 sqmi) of Sycamore Creek in the study area.

Field chemistry data will be collected with a YSI 556® water quality monitoring system during all field activities. Field chemistry parameters will include temperature (to 0.01° C), pH (to 0.01 SU), conductivity (to 1 µS/cm), and dissolved oxygen (to 0.01 mg/L). Date, time and general weather conditions will also be recorded alongside each set of field chemistry parameters. The unit will be calibrated according to the manufacturer's specifications prior to each visit to the field. Collection of field chemistry data will be performed by (or under the direct supervision of) Joseph Papineau, EnviroScience's QDC Level 3 for chemical water quality assessment.

During sampling, Joel Bingham, acting as the QDC Level 3 for fishery evaluations, will preserve two voucher specimens of each taxon at each sampling site per request of the Ohio EPA. Macroinvertebrate vouchers will be preserved by Rhonda Mendel, the QDC Level 3 for macroinvertebrate evaluations. If a specimen is too large or protected then the specimen will be photo documented with a digital camera in the field and released. Voucher specimens will be retained for 10 years in the EnviroScience voucher collection. In addition to the voucher specimens, each sampling site will be photo

documented to show the existing condition of the stream channel and riparian areas. Voucher specimens of fish and/or macroinvertebrates collected from each sampling location will be available upon request.

Specific tasks involved will be as follows:

Fish Community Evaluation

Fish community data will be collected from three of the four sites (excluding RM 4.33 to avoid overlap with RM 4.2), noted in Figure 1.0 (Appendix A) and Table 1, on Sycamore Creek area using one pass wading methods developed by Ohio EPA (1989b, Volume III Updates 2006). At all fish sampling locations, a Smith-Root® 2.5 GPP portable pulsed DC electrofisher will be used to collect fish specimens in all available habitats. Tote barge electrofishing will be used for all fish sites, and sampling will proceed in an upstream direction for 200 meters. The electrofishing crew will consist of two netters: one individual controlling the current output and one individual identifying and maintaining fish in livewells until they are released to the stream.

Immediately after collection, stunned fish will be identified, counted and weighed. During processing, all fish are examined for deformities, erosions, lesions and tumors (DELT anomalies). Photographs will be taken to document representative DELTs. Fish are released immediately after this process, except for those retained for a voucher specimen. Every effort is made to minimize handling and holding times. All collected data will be recorded in the field on data sheets modeled closely after Ohio EPA field data sheets (Appendix E).

Data analyses will include the Index of Biotic Integrity (IBI) for all fish sites (Ohio EPA 1987b, 1989a; Volume II Updates 2006, 2008).

Table 1. Fish and Macroinvertebrate Community Sampling Sites

Site Name	Latitude	Longitude	USGS Quad	Description	Drainage Area*	Sample Types	Distance	No. Passes (Fish)
RM 4.7	-82.7597	39.8781	Reynoldsburg	Downstream side of upper Hill Rd bridge	17.4 sqmi	Tote barge, HD/Kick	200 m	One
RM 4.33	-82.7611	39.8726	Canal Winchester	Approx. 25m downstream of outfall, just upstream of WWTP road bridge	19.2 sqmi	HD/Kick	-	-
RM 4.2	-82.7603	39.8708	Canal Winchester	Upstream side of lower Hill Rd bridge	19.3 sqmi	Tote barge, HD/Kick	200 m	One
RM 3.8	-82.7583	39.8661	Canal Winchester	Approx. 600m downstream of outfall	19.5 sqmi	Tote barge, HD/Kick	200 m	One

*Drainage areas provided by USGS Stream Stats at http://water.usgs.gov/osw/streamstats/ohio.html

Macroinvertebrate Community Evaluation

Macroinvertebrates will be collected at each of the four sampling sites, noted in Figure 1.0 (Appendix A) and Table 1, within the general locations of fish sampling, and in accordance with Ohio EPA protocols (Ohio EPA, 1989b; Volume III Updates 2006, 2008). Deployment of Hester-Dendy (HD) multiple-plate artificial substrate macroinvertebrate samplers will occur during fish sampling. Collection of qualitative kick samples will occur during Hester-Dendy collection, six weeks after deployment.

At each site, Hester-Dendy samplers will be positioned in the euphotic zone (one to two feet below the water surface) and in adequate flow to maximize the abundance and diversity of macroinvertebrates. Ideally, samplers will be placed at the head of a recovery pool immediately below a riffle. A velocity measurement will be taken immediately upstream of the sampler and recorded on the Hester-Dendy deployment sheet (Appendix E). Two sets of five samplers are placed on cement blocks and anchored to the bottom of the stream or stream bank. Care will be taken to place the samplers at an adequate depth so they remain submerged during low flow periods. Samplers will be retrieved at the end of a six-week colonization period. Although both sampler sets are collected, only one will be processed.

Because of the potential for reduced flows and water depths at the sampling sites, the Qualitative Community Tolerance Value (QCTV) narrative method may be used to evaluate these sites. Ohio EPA has developed this method for areas which lack adequate flow and depth. The QCTV score is based on the median pollution tolerance

values of all organisms from a sample. Lower QCTV scores indicate the presence of organisms that can tolerate degraded conditions.

At each of the four sites, qualitative "kick" samples will be collected from all available natural substrates. For each kick sample, a D-frame net will be placed on the substrate while the area immediately upstream is disturbed to allow macroinvertebrates to flow into the net. In areas of low velocity, it is necessary to "jab" or scrape the net across the substrate to collect the sample. Qualitative sampling will continue for at least 30 minutes or until no new taxa are evident in gross examination. Hester-Dendy samplers and qualitative samples will be preserved in 95% ethanol, labeled, and returned to the laboratory for processing.

In the laboratory, Hester-Dendy samplers will be dismantled and scrubbed to remove organisms and debris. The mixture of debris and organisms from the samplers will be poured through a #30 sieve, which has been placed over a #40 sieve. Qualitative samples will be poured from their container into a shallow white-bottomed pan, and invertebrates will be picked from the remaining debris under a dissecting microscope. All sample processing and subsampling of the material from the #30 sieve and #40 sieves will follow the methods detailed by Ohio EPA (Volume III Updates 2006). Macroinvertebrate identifications will be carried out to the taxonomic level required by Ohio EPA (typically genus/species). Midges (Diptera, Chironomidae) will be cleared in KOH and mounted on microscope slides in CMC-10 (Masters Company, Inc.) prior to identification. Oligochaete worms (Oligochaeta) will be identified to class. All identifications and abundance values will be recorded on EnviroScience's macroinvertebrate bench sheets. EnviroScience biologists will adhere to the QA procedures specified by Ohio EPA. Suspect identifications will be verified by a second taxonomist. Voucher specimens of fish and/or macroinvertebrates collected from the different sampling locations will be available upon request.

Macroinvertebrate data collected from Hester-Dendy samplers will be analyzed using Ohio EPA's methods for calculating Invertebrate Community Index (ICI) values (Ohio EPA 1987b, 1989a; Volume II Updates 2006, 2008). Macroinvertebrate data collected from qualitative kick samples will be analyzed using the QCTV according to the following procedure, as outlined by Ohio EPA:

a. Compile the taxa list (genus/species as indicated)
b. Assign weighted tolerance values for qualifying taxa (Ohio EPA, based on abundance of weighted ICI scores)
c. Rank the order
d. Take the median score
e. Compare to the Ecoregion Scoring Criteria
f. If above the 25th percentile for the ecoregion, the site receives a "Good" or "Excellent" rating.

g. If below the 75th percentile for the ecoregion, the site receives a "Fair" or "Poor" rating.
h. Good and Excellent ratings would meet criteria for WWH ICI attainment
i. Fair and Poor would not meet criteria for WWH ICI attainment.

Habitat Characterization

EnviroScience will evaluate in-stream habitat and riparian zone characteristics at each site using the Qualitative Habitat Evaluation Index (QHEI) during electrofishing activities. The QHEI, as developed by the Ohio EPA, is a physical habitat index that provides a quantified evaluation of the lotic macrohabitat characteristics important to the biological community (Ohio EPA, 1989c; QHEI Updates 2006).

SCHEDULE

Benthic macroinvertebrate samplers (Hester-Dendys) will be deployed the week of August 24, 2009. These samplers will be collected along with qualitative kick samples six weeks later, during the week of October 5, 2009. Fish sampling will be conducted the week of August 31, 2009. Field events will be scheduled during approximate base flow conditions for Sycamore Creek. Unfavorable field conditions (i.e. elevated flow and turbidity) may necessitate slight adjustments to the fish sampling schedule.

DELIVERABLES

Collected data will be analyzed and the scores from the indices described above will be used to establish the aquatic life use attainment status of each sampled reach of Sycamore Creek (Ohio EPA 1987b, 1989a; Volume II Updates 2006, 2008). Reported findings will include all values from the fish and macroinvertebrate surveys, habitat results, and physiochemical parameters observed in the study area. Summaries for each sampling site, as well copies of all field data sheets, will be provided to Ohio EPA. Additionally, chemical water quality and habitat assessment data will be submitted to Ohio EPA electronically via Ohio EPA's Credible Data online database, and Level 3 fish and macroinvertebrate data hardcopy submittals will be accompanied by a signed certification that the data were collected and processed according to applicable Credible Data guidelines.

Literature Cited

Fausch, D.O., J.R. Karr, and P.R. Yant. 1984. Regional Application of an Index of Biotic Integrity Based on Stream Fish Communities. Transactions of the American Fisheries Society. 113: 39-55.

Karr, J.R. 1981. Assessment of Biotic Integrity Using Fish Communities. Fisheries. 6(6): 21-27.

Ohio Environmental Protection Agency. 1987a. Biological criteria for the protection of Aquatic life: Vol I. The role of biological data in water quality assessment. Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus Ohio.

Ohio Environmental Protection Agency. 1987b. Biological criteria for the protection of Aquatic life: Vol II. Users manual for biological field assessment of Ohio's surface waters. Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus Ohio.

Ohio Environmental Protection Agency. 1989a. Addendum to Biological criteria for the protection of Aquatic life: Vol II. User's manual for biological field assessment of Ohio's surface waters. Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus Ohio.

Ohio Environmental Protection Agency. 1989b. Biological criteria for the protection of Aquatic life: Vol III. Standardized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities. Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus Ohio.

Ohio Environmental Protection Agency. 1989c. The Qualitative Habitat Evaluation Index (QHEI): Rationale, Methods, and Application. Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus, Ohio.

Ohio Environmental Protection Agency. 2006a. Methods for Assessing Habitat in Flowing Waters: Using the Qualitative Habitat Ecology Index (QHEI). Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus, Ohio.

Ohio Environmental Protection Agency. 2006b. Updates to Biological criteria for the protection of aquatic life: Volumes II & III. Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus Ohio.

Ohio Environmental Protection Agency. 2008. Updates to Biological criteria for the protection of aquatic life: Volumes II & III. Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus Ohio.

Appendix A, Figure 1.0
(Sampling Site Locations)

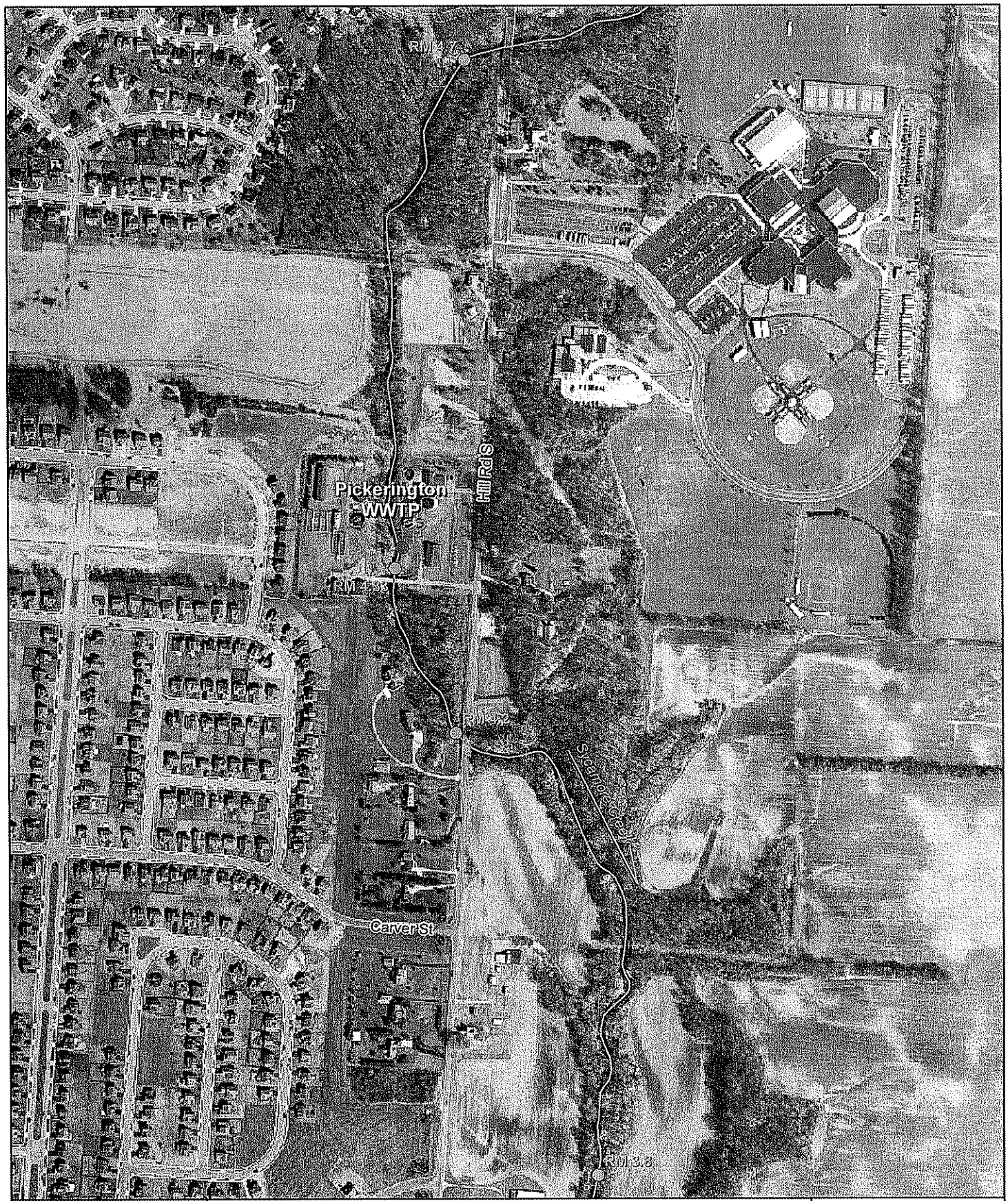


Figure 1.0. Study Plan for Sycamore Creek, Pickerington, OH.

- Biocriteria Sampling Site
- WWTP Outfall
- Stream

300 150 0 300 Feet

100 50 0 100 Meters

N

0.5ft aerial imagery courtesy of OSIP.

ES ENVIRO SCIENCE INCORPORATED

Appendix B, QDC Level 3 Documentation

OhioEPA

State of Ohio Environmental Protection Agency

STREET ADDRESS:
Lazarus Government Center
50 W. Town St., Suite 700
Columbus, Ohio 43215

TELE: (614) 644-3020 FAX: (614) 644-3184
www.epa.state.oh.us

MAILING ADDRESS:
P.O. Box 1049
Columbus, OH 43216-1049

OHIO E.P.A.

MAY 14 2008

ENTERED DIRECTOR'S JOURNAL

CERTIFIED MAIL

Effective Date: May 14, 2008
Expiration Date: May 13, 2010

I certify this to be a true and accurate copy of the official documents as filed in the records of the Ohio Environmental Protection Agency.

By: _____ Date: 5-14-08

Joel Bingham
Oxbow River & Stream Restoration
5511 Young Road
Stow, Ohio 44224

Re: Qualified Data Collector Approval, Surface Water Volunteer Monitoring Program

Dear Joel:

The Division of Surface Water Volunteer Monitoring (Credible Data) Program has reviewed your Qualified Data Collector (QDC) application. Pursuant to Ohio Revised Code (ORC) 6111.53 and Ohio Administrative Code (OAC) 3745-4-03, you are approved as a QDC for the following level and specialty:

QDC Level: 3
QDC Specialty: Fish Community Biology
QDC number: **046**

Please use this QDC number on all correspondence, study plans, etc. submitted to Ohio EPA.

As noted at the top of this letter, this status is effective as of the date of this letter and expires two years from that date. You may now submit study plans to the Volunteer Monitoring Program.

A renewal application must be submitted in accordance with OAC 3745-4-03(C). As provided in this rule, renewal of status is contingent upon active participation in the Volunteer Monitoring Program at the designated level and specialty. Lack of such participation will prevent you from renewing your status, but you may re-apply for initial QDC status.

As a reminder, your status is contingent upon the absence of any trespassing violation (within the previous five years) by you or any person sampling under your supervision. Always obtain land owner permission prior to sampling.

Additionally, collection (and retention) of aquatic biological samples (this includes fish, macroinvertebrates, mollusks, and shells) requires a collector's permit from the Ohio Department of Natural Resources/Division of Wildlife. Obtain this permit prior to collection of any biological samples.

You are hereby notified that this action of the Director is final and may be appealed to the Environmental Review Appeals Commission pursuant to Section 3745.04 of the Ohio Revised Code. The appeal must be in writing and set forth the action complained of and the grounds upon which the appeal is based. The appeal must be filed with the Commission within thirty (30) days after notice of the Director's action. The appeal must be accompanied by a filing fee of seventy dollars ($70.00) which the Commission, in its discretion, may reduce if by affidavit you demonstrate that payment of the full amount of the fee would cause extreme hardship. Notice of the filing of the appeal must be filed with the Director within three (3) days of filing with the Commission. Ohio EPA requests that a copy of the appeal be served upon the Ohio Attorney General's Office, Environmental Enforcement Section. An appeal may be filed with the Commission at the following address: 309 South Fourth Street, Room 222, Columbus, Ohio 43215.

Sincerely,

Chris Korleski
Director

OhioEPA
State of Ohio Environmental Protection Agency

OHIO E.P.A.

DEC 27 2006

ENTERED DIRECTOR'S JOURNAL

STREET ADDRESS:
Lazarus Government Center
122 S. Front Street
Columbus, Ohio 43215

TELE: (614) 644-3020 FAX: (614) 644-3184
www.epa.state.oh.us

MAILING ADDRESS:
P.O. Box 1049
Columbus, OH 43216-1049

Effective Date: December 28, 2006
Expiration Date: December 27, 2008

CERTIFIED MAIL

Joel Bingham
Oxbow River & Stream Restoration
5511 Young Road
Stow, Ohio 44224

Re: Qualified Data Collector Approval, Surface Water Volunteer Monitoring Program

Dear Mr. Bingham:

The Division of Surface Water Volunteer Monitoring (Credible Data) Program has reviewed your Qualified Data Collector (QDC) application. Pursuant to Ohio Revised Code (ORC) 6111.53 and Ohio Administrative Code (OAC) 3745-4-03, you are approved as a QDC for the following level and specialty:

QDC Level: 3
QDC Specialty: Stream Habitat Assessment (QHEI)
QDC number: 046

Please use this QDC number on all correspondence, study plans, etc. submitted to Ohio EPA.

As noted at the top of this letter, this status is effective as of the date of this letter and expires two years from that date. You may now submit study plans to the Volunteer Monitoring Program.

A renewal application must be submitted in accordance with OAC 3745-4-03(C). As provided in this rule, renewal of status is contingent upon active participation in the Volunteer Monitoring Program at the designated level and specialty. Lack of such participation will prevent you from renewing your status, but you may re-apply for initial QDC status.

I certify this to be a true and accurate copy of the official documents as filed in the records of the Ohio Environmental Protection Agency.

By: Date: 12-27-06

Bob Taft, Governor
Bruce Johnson, Lieutenant Governor
Joseph P. Koncelik, Director

Printed on Recycled Paper

Ohio EPA is an Equal Opportunity Employer

As a reminder, your status is contingent upon the absence of any trespassing violation (within the previous five years) by you or any person sampling under your supervision. Always obtain land owner permission prior to sampling.

Additionally, collection (and retention) of aquatic biological samples (this includes fish, macroinvertebrates, mollusks, and shells) requires a collector's permit from the Ohio Department of Natural Resources/Division of Wildlife. Obtain this permit prior to collection of any biological samples.

The approval of this certification is a final action of the director of Ohio EPA and may be appealed to the Environmental Review Appeals Commission pursuant to ORC 3745.04. The appeal must be in writing and set forth the action complained of and the grounds upon which the appeal is based. It must be filed with the Commission within thirty days after notice of the director's action. Notice of the filing of the appeal must be filed with the director within three days after the appeal is filed with the commission. An appeal may be filed with the Commission at the following address: 309 South Fourth Street, Room 222, Columbus, Ohio 43215.

Sincerely,

Joseph P. Koncelik
Director

OhioEPA
State of Ohio Environmental Protection Agency

STREET ADDRESS:
Lazarus Government Center
50 W. Town St., Suite 700
Columbus, Ohio 43215

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www.epa.state.oh.us

MAILING ADDRESS:
P.O. Box 1049
Columbus, OH 43216-1049

OHIO E.P.A.
MAR - 6 2008
DIRECTOR'S OFFICE

CERTIFIED MAIL

Effective Date: March 6, 2008
Expiration Date: March 5, 2010

I certify this to be a true and accurate copy of the official documents as filed in the records of the Ohio Environmental Protection Agency.

By: _____ Date: 3-6-08

Rhonda Mendel
EnviroScience, Inc.
470 Grand Valley Drive
Howard, Ohio 43028

Re: Qualified Data Collector Approval, Surface Water Volunteer Monitoring Program

Dear Rhonda:

The Division of Surface Water Volunteer Monitoring (Credible Data) Program has reviewed your Qualified Data Collector (QDC) application. Pursuant to Ohio Revised Code (ORC) 6111.53 and Ohio Administrative Code (OAC) 3745-4-03, you are approved as a QDC for the following level and specialty:

QDC Level: 3
QDC Specialty: Benthic Macroinvertebrate Biology
QDC number: **160**

Please use this QDC number on all correspondence, study plans, etc. submitted to Ohio EPA.

As noted at the top of this letter, this status is effective as of the date of this letter and expires two years from that date. You may now submit study plans to the Volunteer Monitoring Program.

A renewal application must be submitted in accordance with OAC 3745-4-03(C). As provided in this rule, renewal of status is contingent upon active participation in the Volunteer Monitoring Program at the designated level and specialty. Lack of such participation will prevent you from renewing your status, but you may re-apply for initial QDC status.

As a reminder, your status is contingent upon the absence of any trespassing violation (within the previous five years) by you or any person sampling under your supervision. Always obtain land owner permission prior to sampling.

Additionally, collection (and retention) of aquatic biological samples (this includes fish, macroinvertebrates, mollusks, and shells) requires a collector's permit from the Ohio Department of Natural Resources/Division of Wildlife. Obtain this permit prior to collection of any biological samples.

You are hereby notified that this action of the Director is final and may be appealed to the Environmental Review Appeals Commission pursuant to Section 3745.04 of the Ohio Revised Code. The appeal must be in writing and set forth the action complained of and the grounds upon which the appeal is based. The appeal must be filed with the Commission within thirty (30) days after notice of the Director's action. The appeal must be accompanied by a filing fee of seventy dollars ($70.00) which the Commission, in its discretion, may reduce if by affidavit you demonstrate that payment of the full amount of the fee would cause extreme hardship. Notice of the filing of the appeal must be filed with the Director within three (3) days of filing with the Commission. Ohio EPA requests that a copy of the appeal be served upon the Ohio Attorney General's Office, Environmental Enforcement Section. An appeal may be filed with the Commission at the following address: 309 South Fourth Street, Room 222, Columbus, Ohio 43215.

Sincerely,

Chris Korleski
Director

State of Ohio Environmental Protection Agency

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MAILING ADDRESS:
P.O. Box 1049
Columbus, OH 43216-1049

I certify this to be a true and accurate copy of the official documents as filed in the records of the Ohio Environmental Protection Agency.

Effective Date: September 10, 2008
Expiration Date: September 9, 2010

CERTIFIED MAIL

By: _____ Date: 9-10-08

RECEIVED DIRECTOR'S JOURNAL
SEP 10 2008
OHIO E.P.A.

Joseph Papineau
EnviroScience, Inc.
3781 Darrow Road
Stow, Ohio 44224

Re: Qualified Data Collector Approval, Surface Water Volunteer Monitoring Program

Dear Joseph:

The Division of Surface Water Volunteer Monitoring (Credible Data) Program has reviewed your Qualified Data Collector (QDC) application. Pursuant to Ohio Revised Code (ORC) 6111.53 and Ohio Administrative Code (OAC) 3745-4-03, you are approved as a QDC for the following level and specialty:

QDC Level: 3
QDC Specialty: Chemical Water Quality Assessment
QDC number: **240**

Please use this QDC number on all correspondence, study plans, etc. submitted to Ohio EPA.

As noted at the top of this letter, this status is effective as of the date of this letter and expires two years from that date. You may now submit study plans to the Volunteer Monitoring Program.

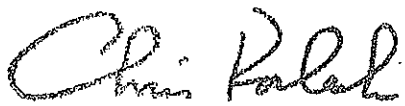
A renewal application must be submitted in accordance with OAC 3745-4-03(C). As provided in this rule, renewal of status is contingent upon active participation in the Volunteer Monitoring Program at the designated level and specialty. Lack of such participation will prevent you from renewing your status, but you may re-apply for initial QDC status.

As a reminder, your status is contingent upon the absence of any trespassing violation (within the previous five years) by you or any person sampling under your supervision. Always obtain land owner permission prior to sampling.

Additionally, collection (and retention) of aquatic biological samples (this includes fish, macroinvertebrates, mollusks, and shells) requires a collector's permit from the Ohio Department of Natural Resources/Division of Wildlife. Obtain this permit prior to collection of any biological samples.

You are hereby notified that this action of the Director is final and may be appealed to the Environmental Review Appeals Commission pursuant to Section 3745.04 of the Ohio Revised Code. The appeal must be in writing and set forth the action complained of and the grounds upon which the appeal is based. The appeal must be filed with the Commission within thirty (30) days after notice of the Director's action. The appeal must be accompanied by a filing fee of seventy dollars ($70.00) which the Commission, in its discretion, may reduce if by affidavit you demonstrate that payment of the full amount of the fee would cause extreme hardship. Notice of the filing of the appeal must be filed with the Director within three (3) days of filing with the Commission. Ohio EPA requests that a copy of the appeal be served upon the Ohio Attorney General's Office, Environmental Enforcement Section. An appeal may be filed with the Commission at the following address: 309 South Fourth Street, Room 222, Columbus, Ohio 43215.

Sincerely,



Chris Korleski
Director

Appendix C, Ohio DNR Scientific Collection Permits

DIVISION OF WILDLIFE
Ohio Department of Natural Resources

Division of Wildlife Headquarters
2045 Morse Road, Bldg. G
Columbus, Ohio 43229-6693
1-800-WILDLIFE

Chief, Division of Wildlife **David M Graham**

WILD ANIMAL PERMIT: 10-207

SCIENTIFIC COLLECTION

DATE ISSUED
5/26/2009

JOEL D. BINGHAM
3781 DARROW RD.
STOW, OH 44224

Others authorized on permit
NO

is hereby granted permission to take, possess, and transport at any time and in any manner specimens of wild animals, subject to the conditions and restrictions listed below or any documents accompanying this permit. This permit, unless revoked earlier by the Chief, Division of Wildlife, is effective from:

3/16/2009 **to:** **3/15/2010**

This permit must be carried while collecting wild animals and be exhibited to any person on demand.

THIS PERMIT IS RESTRICTED AS FOLLOWS:

1. Permittee may collect specimens for survey and inventory purposes. No endangered species may be retained. 1-2 Unidentified fish specimens may be retained for identification.
2. Twenty-four hours prior to sampling, permittee must contact the local wildlife officer or nearest wildlife district office to advise location(s) and duration of sampling activities. Contact the Division of Wildlife if undocumented aquatic invasive species are discovered.
3. All voucher specimens are to be deposited at Hiram College, The Ohio State University, Museum of Biological Diversity.
4. Collection is prohibited in the Killbuck, Chagrin River, mainstem Darby Creek and Fish Creek (Williams County) without explicit written permission from the Division of Wildlife. Permission is granted to survey the Darby watershed in the vicinity of Middleburg, for a stream restoration project.
5. Permittee must provide an annual report of collecting activities to the Division of Wildlife. Report shall provide species, quanity and locations of collection.

Locations of Collecting:

STATEWIDE

Equipment and method used in collection:

ELECTROSHOCKER, HESTER DANDY SAMPLER, SEINE, AMPHIBIAN FUNNEL TRAPS, KICK NET

Name and number of each species to be collected:

BENTHIC MACROINVERTABRATES, INCLUDING MUSSELS, AND FISH FOR SURVEY PURPOSES. SPORT FISH OVER 6" MAY NOT BE RETAINED. THREATENED OR ENDANGERED WILD ANIMALS ARE TO BE PHOTO-DOCUMENTED AND RETURNED.

This permit is not valid for collecting migratory birds, their nests, or eggs unless a current permit from the U.S. Fish and Wildlife Service has been obtained.

RESTRICTIVE DOCUMENTS ACCOMPANYING THIS PERMIT? NO

NO ENDANGERED SPECIES MAY BE TAKEN WITHOUT WRITTEN PERMISSION FROM THE CHIEF

DIVISION OF WILDLIFE
Ohio Department of Natural Resources

Division of Wildlife Headquarters
2045 Morse Road, Bldg. G
Columbus, Ohio 43229-6693
1-800-WILDLIFE

Chief, Division of Wildlife **David M Graham**

WILD ANIMAL PERMIT: 10-211

SCIENTIFIC COLLECTION

DATE ISSUED
5/26/2009

DAVID CZAYKA
3781 DARROW RD.
STOW, OH 44224

Others authorized on permit

NO

is hereby granted permission to take, possess, and transport at any time and in any manner specimens of wild animals, subject to the conditions and restrictions listed below or any documents accompanying this permit. This permit, unless revoked earlier by the Chief, Division of Wildlife, is effective from:

3/16/2009 to: 3/15/2010

This permit must be carried while collecting wild animals and be exhibited to any person on demand.

THIS PERMIT IS RESTRICTED AS FOLLOWS:

1. Permittee may collect specimens for survey and inventory purposes. No live endangered species may be retained. Unidentified fish specimens may be retained for identification.
2. Twenty-four hours prior to sampling, permittee must contact the local wildlife officer or nearest wildlife district office to advise location(s) and duration of sampling activities. Contact the Division of Wildlife if undocumented aquatic invasive species are discovered.
3. All voucher specimens are to be deposited at The Ohio State University, Museum of Biological Diversity, Cleveland Museum of Natural History or common species may be held at Enviroscience Laboratory.
4. Collection is prohibited in the Killbuck, Big Darby, Little Darby, Chagrin River and Fish Creek (Williams County) without explicit written permission from the Division of Wildlife.
5. Permittee must provide an annual report of collecting activities to the Division of Wildlife. Report shall provide species, quanity and locations of collection.

Locations of Collecting:

STATEWIDE WITH EXCEPTIONS

Equipment and method used in collection:

ELECTROFISHING, HESTER-DENDY, SEINE AND BONGO NETS

Name and number of each species to be collected:

FISH AND AQUATIC MACRO-INVERTEBRATES FOR SURVEY AND INVENTORY PURPOSES. SPORT FISH OVER 6" MAY NOT BE RETAINED.

This permit is not valid for collecting migratory birds, their nests, or eggs unless a current permit from the U.S. Fish and Wildlife Service has been obtained.

RESTRICTIVE DOCUMENTS ACCOMPANYING THIS PERMIT? NO

NO ENDANGERED SPECIES MAY BE TAKEN WITHOUT WRITTEN PERMISSION FROM THE CHIEF

DIVISION OF WILDLIFE
Ohio Department of Natural Resources

Division of Wildlife Headquarters
2045 Morse Road, Bldg. G
Columbus, Ohio 43229-6693
1-800-WILDLIFE

Chief, Division of Wildlife **David M Graham**

WILD ANIMAL PERMIT: 10-213

SCIENTIFIC COLLECTION

DATE ISSUED
5/26/2009

JAMIE R. KREJSA
3781 DARROW RD.
STOW, OH 44224

Others authorized on permit

NO

is hereby granted permission to take, possess, and transport at any time and in any manner specimens of wild animals, subject to the conditions and restrictions listed below or any documents accompanying this permit. This permit, unless revoked earlier by the Chief, Division of Wildlife, is effective from:

3/16/2009 **to:** **3/15/2010**

This permit must be carried while collecting wild animals and be exhibited to any person on demand.

THIS PERMIT IS RESTRICTED AS FOLLOWS:

1.MAY COLLECT FISH AND AQUATIC INVERTABRATES FOR SURVEY AND INVENTORY. ALL ENDANGERED SPECIMENS ARE TO BE RELEASED AT CAPTURE SITE. NO ENDANGERED SPECIES MAY BE PERMANENTLY COLLECTED.
2.TWENTY-FOUR HOURS PRIOR TO COLLECTING ACTIVITIES, PERMITTEE MUST CONTACT THE LOCAL WILDLIFE OFFICER OR THE NEAREST WILDLIFE DISTRICT OFFICE TO ADVISE LOCATION AND DURATION OF SAMPLING.
3.WHEN COLLECTING ON LAKE ERIE, CONTACT LAKE ERIE ENFORCEMENT UNIT AT (419)625-8062 FORTY-EIGHT (48) HOURS PRIOR TO COLLECTING. COLLECTION IS PROHIBITED IN THE BIG DARBY, LITTLE DARBY, FISH CREEK (WILLIAMS COUNTY), AND KILLBUCK CREEK WITHOUT THE WRITTEN PERMISSION OF THE DIVISION OF WILDLIFE.
4.ACCURATE RECORDS MUST BE MAINTAINED OF SPECIES, QUANITY AND LOCATIONS SPECIMANS WERE COLLECTED.
5.AN ANNUAL REPORT OF COLLECTING AND EDUCATIONAL ACTIVITIES MUST BE SUBMITTED TO THE OHIO DIVISION OF WILDLIFE.

Locations of Collecting:
STATEWIDE, WITH NOTED EXCEPTIONS

Equipment and method used in collection:
SEINE, ELECTROSHOCKER, POMAR DREDGE, HESTER DENDY, DIP NETS AND KICK NETS.

Name and number of each species to be collected:
FISH AND BENTHIC MACROINVERTEBRATES. SPORT FISH OVER SIX INCHES MAY NOT BE RETAINED. LIVE MUSSELS MUST BE IMMEDIATELY RELEASED AT CAPTURE SITE..

This permit is not valid for collecting migratory birds, their nests, or eggs unless a current permit from the U.S. Fish and Wildlife Service has been obtained.

RESTRICTIVE DOCUMENTS ACCOMPANYING THIS PERMIT? NO

NO ENDANGERED SPECIES MAY BE TAKEN WITHOUT WRITTEN PERMISSION FROM THE CHIEF

DIVISION OF WILDLIFE
Ohio Department of Natural Resources

Division of Wildlife Headquarters
2045 Morse Road, Bldg. G
Columbus, Ohio 43229-6693
1-800-WILDLIFE

Chief, Division of Wildlife **David M Graham**

WILD ANIMAL PERMIT: 10-210

SCIENTIFIC COLLECTION

DATE ISSUED
5/26/2009

NICOLE JORDAN
3781 DARROW RD.
STOW, OH 44224

Others authorized on permit
NO

is hereby granted permission to take, possess, and transport at any time and in any manner specimens of wild animals, subject to the conditions and restrictions listed below or any documents accompanying this permit. This permit, unless revoked earlier by the Chief, Division of Wildlife, is effective from:

3/16/2009 **to:** **3/15/2010**

This permit must be carried while collecting wild animals and be exhibited to any person on demand.

THIS PERMIT IS RESTRICTED AS FOLLOWS:

1. Permittee may collect specimens for survey and inventory purposes. No live endangered species may be retained. Unidentified fish specimens may be retained for identification.
2. Twenty-four hours prior to sampling, permittee must contact the local wildlife officer or nearest wildlife district office to advise location(s) and duration of sampling activities. Contact the Division of Wildlife if undocumented aquatic invasive species are discovered.
3. All voucher specimens are to be deposited at The Ohio State University, Museum of Biological Diversity, Cleveland Museum of Natural History or common species may be held at Environscience Laboratory.
4. Collection is prohibited in the Killbuck, Big Darby, Little Darby, Chagrin River and Fish Creek (Williams County) without explicit written permission from the Division of Wildlife.
5. Permittee must provide an annual report of collecting activities to the Division of Wildlife. Report shall provide species, quanity and locations of collection.

Locations of Collecting:
STATEWIDE WITH EXCEPTIONS

Equipment and method used in collection:
ELECTROFISHING, HESTER-DENDY, SEINE AND BONGO NETS

Name and number of each species to be collected:
FISH AND AQUATIC MACRO-INVERTEBRATES FOR SURVEY AND INVENTORY PURPOSES. SPORT FISH OVER 6" MAY NOT BE RETAINED.

This permit is not valid for collecting migratory birds, their nests, or eggs unless a current permit from the U.S. Fish and Wildlife Service has been obtained.

RESTRICTIVE DOCUMENTS ACCOMPANYING THIS PERMIT? NO

NO ENDANGERED SPECIES MAY BE TAKEN WITHOUT WRITTEN PERMISSION FROM THE CHIEF

DIVISION OF WILDLIFE
Ohio Department of Natural Resources

Division of Wildlife Headquarters
2045 Morse Road, Bldg. G
Columbus, Ohio 43229-6693
1-800-WILDLIFE

Chief, Division of Wildlife **David M Graham**

WILD ANIMAL PERMIT: 10-209

SCIENTIFIC COLLECTION

DATE ISSUED
5/26/2009

JOSEPH PAPINEAU
3781 DARROW RD
STOW, OH 44224

Others authorized on permit
NO

is hereby granted permission to take, possess, and transport at any time and in any manner specimens of wild animals, subject to the conditions and restrictions listed below or any documents accompanying this permit. This permit, unless revoked earlier by the Chief, Division of Wildlife, is effective from:

3/16/2009 to: 3/15/2010

This permit must be carried while collecting wild animals and be exhibited to any person on demand.

THIS PERMIT IS RESTRICTED AS FOLLOWS:

1. Permittee may collect specimens for survey and inventory purposes. No endangered species may be removed from the wild. Unidentified fish specimens may be retained for identification.
2. Twenty-four hours prior to collection activities, permittee must contact the local wildlife officer or nearest wildlife district office to advise location(s) and duration of sampling activities. Report all undocumented aquatic invasive species to the Division of Wildlife within twenty-four hours of discovery.
3. All voucher specimens are to be deposited at The Ohio State University, Museum of Biological Diversity.
4. Collection is prohibited in the Killbuck, Big Darby, Little Darby, Chagrin River and Fish Creek (Williams County) without explicit written permission from the Division of Wildlife.
5. Permittee must provide an annual report of collecting activities to the Division of Wildlife. Report shall provide species, quanity and locations of collection.

Locations of Collecting:
STATEWIDE, WITH NOTED EXCEPTIONS

Equipment and method used in collection:
ELECTROSHOCKER, SEINE, HESTER DANDY SAMPLER, KICK NET, HAND

Name and number of each species to be collected:
AQUATIC MACROINVERTABRATES, AMPHIBIANS AND FISH FOR SURVEY AND INVENTORY. SPORT FISH OVER 6" MAY NOT BE RETAINED.

This permit is not valid for collecting migratory birds, their nests, or eggs unless a current permit from the U.S. Fish and Wildlife Service has been obtained.

RESTRICTIVE DOCUMENTS ACCOMPANYING THIS PERMIT? NO

NO ENDANGERED SPECIES MAY BE TAKEN WITHOUT WRITTEN PERMISSION FROM THE CHIEF

DIVISION OF WILDLIFE
Ohio Department of Natural Resources

Division of Wildlife Headquarters
2045 Morse Road, Bldg. G
Columbus, Ohio 43229-6693
1-800-WILDLIFE

Chief, Division of Wildlife **David M Graham**

WILD ANIMAL PERMIT: 10-212

SCIENTIFIC COLLECTION

DATE ISSUED
5/26/2009

JEFFERY R. NIEHAUS
3781 DARROW RD
STOW, OH 44224

Others authorized on permit

NO

is hereby granted permission to take, possess, and transport at any time and in any manner specimens of wild animals, subject to the conditions and restrictions listed below or any documents accompanying this permit. This permit, unless revoked earlier by the Chief, Division of Wildlife, is effective from:

3/16/2009 **to:** **3/15/2010**

This permit must be carried while collecting wild animals and be exhibited to any person on demand.

THIS PERMIT IS RESTRICTED AS FOLLOWS:

1. Permittee may collect specimens for survey and inventory purposes. No live endangered species may be retained. Unidentified fish specimens may be retained for identification.
2. Twenty-four hours prior to sampling, permittee must contact the local wildlife officer or nearest wildlife district office to advise location(s) and duration of sampling activities. Contact the Division of Wildlife if undocumented aquatic invasive species are discovered.
3. All voucher specimens are to be deposited at The Ohio State University, Museum of Biological Diversity, Cleveland Museum of Natural History or common species may be held at Enviroscience Laboratory.
4. Collection is prohibited in the Killbuck, Big Darby, Little Darby, Chagrin River and Fish Creek (Williams County) without explicit written permission from the Division of Wildlife.
5. Permittee must provide an annual report of collecting activities to the Division of Wildlife. Report shall provide species, quanity and locations of collection.

Locations of Collecting:

STATEWIDE WITH EXCEPTIONS

Equipment and method used in collection:

ELECTROFISHING, HESTER-DENDY, SEINE AND BONGO NETS

Name and number of each species to be collected:

FISH AND AQUATIC MACRO-INVERTEBRATES FOR SURVEY AND INVENTORY PURPOSES. SPORT FISH OVER 6" MAY NOT BE RETAINED.

This permit is not valid for collecting migratory birds, their nests, or eggs unless a current permit from the U.S. Fish and Wildlife Service has been obtained.

RESTRICTIVE DOCUMENTS ACCOMPANYING THIS PERMIT? NO

NO ENDANGERED SPECIES MAY BE TAKEN WITHOUT WRITTEN PERMISSION FROM THE CHIEF

Appendix D, Non-trespass/Photo/Voucher Affirmations

I, the undersigned, do hereby affirm that I have not been convicted of or pleaded guilty to a violation of section 2911.21 of the Revised Code (criminal trespass) or a substantially similar municipal ordinance within the previous five years.

Joel Bingham
Printed Name

Restoration Biologist
Title

Signature

08/03/09
Date

3781 DARROW ROAD, STOW, OHIO 44224
330-688-0111 / FAX: 330-688-3858 / TOLL FREE: 800-940-4025

ES ENVIRO SCIENCE INCORPORATED

I, the undersigned, do hereby affirm that I have not been convicted of or pleaded guilty to a violation of section 2911.21 of the Revised Code (criminal trespass) or a substantially similar municipal ordinance within the previous five years.

David Czayka
Printed Name

Biologist
Title

Signature

8-3-09
Date

3781 DARROW ROAD, STOW, OHIO 44224
330-688-0111 / FAX: 330-688-3858 / TOLL FREE: 800-940-4025

ES ENVIRO SCIENCE INCORPORATED

I, the undersigned, do hereby affirm that I have not been convicted of or pleaded guilty to a violation of section 2911.21 of the Revised Code (criminal trespass) or a substantially similar municipal ordinance within the previous five years.

Andrew Farber
Printed Name

Environmental Inspector / Biologist
Title

Signature

8/6/09
Date

3781 DARROW ROAD, STOW, OHIO 44224
330-688-0111 / FAX: 330-688-3858 / TOLL FREE: 800-940-4025

ES ENVIRO SCIENCE INCORPORATED

I, the undersigned, do hereby affirm that I have not been convicted of or pleaded guilty to a violation of section 2911.21 of the Revised Code (criminal trespass) or a substantially similar municipal ordinance within the previous five years.

Nicole Jordan
Printed Name

Biologist
Title

Signature

8/5/09
Date

3781 DARROW ROAD, STOW, OHIO 44224
330-688-0111 / FAX: 330-688-3858 / TOLL FREE: 800-940-4025

ES ENVIRO SCIENCE INCORPORATED

I, the undersigned, do hereby affirm that I have not been convicted of or pleaded guilty to a violation of section 2911.21 of the Revised Code (criminal trespass) or a substantially similar municipal ordinance within the previous five years.

James Kretsa
Printed Name

VP
Title

Signature

8-3-09
Date

3781 DARROW ROAD, STOW, OHIO 44224
330-688-0111 / FAX: 330-688-3858 / TOLL FREE: 800-940-4025

ES ENVIRO SCIENCE INCORPORATED

I, the undersigned, do hereby affirm that I have not been convicted of or pleaded guilty to a violation of section 2911.21 of the Revised Code (criminal trespass) or a substantially similar municipal ordinance within the previous five years.

Rhonda J. Mendel
Printed Name

Senior Macroinvertebrate Taxonomist
Title

Signature

8/3/09
Date

3781 DARROW ROAD, STOW, OHIO 44224
330-688-0111 / FAX: 330-688-3858 / TOLL FREE: 800-940-4025

ES ENVIRO SCIENCE INCORPORATED

I, the undersigned, do hereby affirm that I have not been convicted of or pleaded guilty to a violation of section 2911.21 of the Revised Code (criminal trespass) or a substantially similar municipal ordinance within the previous five years.

Jeffrey R Nicholas — Printed Name

Biologist — Title

Signature

8-3-2009 — Date

3781 DARROW ROAD, STOW, OHIO 44224
330-688-0111 / FAX: 330-688-3858 / TOLL FREE: 800-940-4025

ES ENVIRO SCIENCE INCORPORATED

I, the undersigned, do hereby affirm that I have not been convicted of or pleaded guilty to a violation of section 2911.21 of the Revised Code (criminal trespass) or a substantially similar municipal ordinance within the previous five years.

JOSEPH L. PAPINEAU
Printed Name

CeIS ANALYST/BIOLOGIST
Title

Signature

8/3/09
Date

3781 DARROW ROAD, STOW, OHIO 44224
330-688-0111 / FAX: 330-688-3858 / TOLL FREE: 800-940-4025

ES ENVIRO SCIENCE INCORPORATED

During sampling, I, Joel Bingham, acting as the QDC Level 3 project manager for fishery evaluations, will preserve two voucher specimens of each taxon at each sampling site. If a specimen is too large or protected then the specimen will be photo documented with a digital camera in the field and released. Voucher specimens will be retained for 10 years in the EnviroScience voucher collection. In addition to the voucher specimens, each sampling site will be photo documented to show the existing condition of the stream channel and riparian areas.

Joel Bingham	Restoration Biologist
Printed Name	Title
Joel B	8/03/09
Signature	Date

3781 DARROW ROAD, STOW, OHIO 44224
330-688-0111 / FAX: 330-688-3858 / TOLL FREE: 800-940-4025

ES ENVIRO SCIENCE INCORPORATED

During sample identification, I, Rhonda Mendel, QDC Level 3 project manager for macroinvertebrate evaluations, will preserve two voucher specimens of each taxon at each sampling site. If a specimen is too large or protected then the specimen will be photo documented with a digital camera in the field and released. Voucher specimens will be retained for 10 years in the EnviroScience voucher collection. In addition to the voucher specimens, each sampling site will be photo documented to show the existing condition of the stream channel and riparian areas.

Rhonda J. Mendel
Printed Name

Senior Macroinvertebrate Taxonomist
Title

Rhonda J. Mendel
Signature

8/3/09
Date

3781 DARROW ROAD, STOW, OHIO 44224
330-688-0111 / FAX: 330-688-3858 / TOLL FREE: 800-940-4025

ES ENVIRO SCIENCE INCORPORATED

Appendix E, Field Data Sheets

ENVIRO SCIENCE

ation ID _____ Date _____ Time _____

eam/River _____ Location _____

mments _____ County _____

itude _____ Longitude _____

amplers _____ Sample Type _____

Distance _____ Flow _____ Temp. _____ D.O. _____ pH _____

ecies	Number Weighed	Total Counted	Total Wieght	Weights/Counts					Delt Anomalies Deformities,Erosions Lesions,Tumors,Other
									D E L T O
									D E L T O
									D E L T O
									D E L T O
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ecies	Number Weighed	Total Counted	Total Wieght	Weights/Counts					Delt Anomalies Deformities, Erosions Lesions, Tumors, Other
									D E L T O
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									D E L T O
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									D E L T O
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									D E L T O
									D E L T O
									D E L T O
									D E L T O

Pg ___ of ___

pecies	Number Weighed	Total Counted	Total Wieght	Weights/Counts					Delt Anomalies Deformities, Erosions Lesions, Tumors, Other
									D E L T O
									D E L T O
									D E L T O
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									D E L T O
									D E L T O
									D E L T O
									D E L T O

Ohio EPA Volunteer Monitoring Program
Macroinvertebrate Field Sheet

Station ID _____ Sheet ID _____ Date Collected _____

Project _____ Sampler Type _____ # HDs _____ Collected By _____

ALP _____ RM _____ Data Comments _____ Date Analyzed _____

Lat./Long. _____ Analyzed By _____

Stream _____ Location _____

Flow _____ Temp. C) _____ HDs - Current Set (fps) _____ Ret. (fps) _____ Ql. Time Sampled (min.) _____

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

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

HD Condition: Disturbed Yes/No Comment:_____

Debris Yes/No Comment:_____

Silt/Solids None - Slight - Moderate - Heavy

DN/HP Sampling: Total Time _____ Habitats: Pool - Riffle - Run - Margin - Backwater

---

Physical Characteristics

Flow Condition: Flood - Above Normal - Normal - Low - Interstitial - Intermittent - Dry

Current Velocity: Fast - Moderate - Slow - ND

Channel Morphology: Natural - Channelized - Channelized (Recovered) - Impounded

Bank Erosion: Extensive - Moderate - Slight - None

Riffle Development: Extensive - Moderate - Sparse - Absent

Riffle Quality: Good - Fair - Poor Embedded: Yes/No

Clarity: Clear - Murky - Turbid Temp:_____

Color: None - Green - Brown - Grey - Other (_____)

Canopy: Open - 75% - 50% - 25% - Closed

Substrate Characteristics

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

Predominant Land Use (L,R,B)

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

Predominant Riparian Vegetation Width

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

Margin Habitat

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

Margin Quality: Good - Fair - Poor

Biological Characteristics

Riffle:

Predominant Organisms: _____

Other Common Organisms: _____

Density:High - Moderate - Low

Diversity: High - Moderate - Low

Run:

Predominant Organisms: _____

Other Common Organisms: _____

Density:High - Moderate - Low

Diversity: High - Moderate - Low

Pool:

Predominant Organisms: _____

Other Common Organisms: _____

Density:High - Moderate - Low

Diversity: High - Moderate - Low

Margin:

Predominant Organisms: _____

Other Common Organisms: _____

Density:High - Moderate - Low

Diversity: High - Moderate - Low

Other Notable Collections: _____

Potential Pollution Sources: _____

Evidence of Pollution: _____

Photo Numbers: _____

Comments: _____

_____

_____

_____

_____

_____

Appendix F, EnviroScience QA/QC Plan

EnviroScience, Inc.

QUALITY ASSURANCE PROGRAM PLAN

for Aquatic Survey

Prepared by:

EnviroScience, Inc.
3781 Darrow Road, Stow , OH 44224
(330) 688-0111, 1-800-940-4025
Fax: (330) 688-3858

October 2, 2008

Table of Contents

1.0 INTRODUCTION 1
2.0 PROJECT ORGANIZATION 1
2.1 Responsible Corporate Official 1
2.2 Quality Assurance Manager- 1
2.3 Field Operations Manager 1
2.4 Taxonomist 2
2.5 Data Recorder 2
2.6 Geographic Information System Technician 3
2.7 Additional Biologists and/or Technicians 3
3.0 METHODS AND SAMPLING PROCEDURES 3
3.1 Site Selection 4
3.2 Water Chemistry 4
3.3 Habitat Evaluation 5
3.4 Benthic Macroinvertebrates 5
3.4.1 Hester-Dendy Sampling 5
3.4.2 Surber Sampling 6
3.4.3 Ponar Sampling 6
3.4.4 Kick Sampling 6
3.4.5 Benthic Macroinvertebrate Sample Processing and Identification 6
3.5 Fish 7
3.5.1 Electrofishing 7
3.5.2 Seining 8
3.5.3 Hoopnetting 9
3.5.4 Trawling 9
3.5.5 Fish Sample Processing 9
3.5.6 Fish Identification/Verification 10
4.0 SAMPLE AND DOCUMENT CHAIN OF CUSTODY 10
4.1 Sample Collection and Transport 10
4.2 Voucher Specimens 11
5.0 FIELD EQUIPMENT CALIBRATION PROCEDURES 11
5.1 Fish Weights 11
5.2 Multiple Parameters 12
6.0 DATA REDUCTION, VALIDATION AND REPORTING 12
6.1 Data Reduction for Field and Technical Data 12
6.1.1 Chemistry 12
6.1.2 Habitat Evaluation 12
6.1.3 Macroinvertebrates 12
6.1.4 Fish 13
6.2 Validation 14
6.3 Data Reporting 15
6.3.1 Data Summary 15
6.3.2 Report Generation 15
7.0 CORRECTIVE ACTION AND QUALITY ASSURANCE REPORTS TO MANAGEMENT 18
7.1 Corrective Action 18
7.2 Quality Assurance Reports to Management 18
REFERENCES 19

List of Appendices

Appendix A Standard Operating Procedures for Chemistry
Appendix B Standard Operating Procedures for Habitat Evaluation
Appendix C Standard Operating Procedures for Macroinvertebrate Sampling and Data Analysis
Appendix D Standard Operating Procedures for Fish Sampling and Data Analysis
Appendix E Standard Operating Procedures for Mussel Projects

List of Acronyms

APHA	American Public Health Association
DELT	Deformities, Erosion, Lesions, Tumors
OHEPA	Ohio Environmental Protection Agency
FOM	Field Operations Manager
GPS	Global Positioning System
IBI	Index of Biotic Integrity
MIWB	Modified Index of Well-Being
OEPA	Ohio Environmental Protection Agency
QAM	Quality Assurance Manager
QHEI	Qualitative Habitat Evaluation Index
QUAPP	Quality Assurance Program Plan
RBP	Rapid Bioassessment Program
RCO	Responsible Corporate Official
SOP	Standard Operating Procedures
USEPA	United States Environmental Protection Agency

1.0 INTRODUCTION

The purpose of this document is to provide a Quality Assurance Program Plan (QUAPP) for aquatic surveys that is an orderly assemblage of project organization, general procedures, data reduction, validation, reporting, corrective action and quality assurance reports to management. The appendices which follow the text are EnviroScience's Standard Operating Procedures (SOPs) for analysis of chemistry, habitat, macroinvertebrates, mussels and fish.

2.0 PROJECT ORGANIZATION

In any given aquatic survey project, the organization of personnel remains constant. All field work is performed by a staff of three to five experienced biologists and/or technicians, one of which is the Field Operations Manager (FOM). The FOM reports to both the Quality Assurance Manager (QAM) and Responsible Corporate Official (RCO). The RCO ultimately oversees the progress of each project and coordinates with the FOM and QAM on all aspects of the project. In the laboratory, responsibilities are as stated below. Additional biologists are used for benthic macroinvertebrate sample processing in the laboratory, if needed.

2.1 Responsible Corporate Official

Responsibilities:

Oversee the design, initiation and progress of each project
Maintain communication with the QAM and FOM
Cost management

2.2 Quality Assurance Manager

Responsibilities:

Create and update quality assurance practices
Maintain communication with the RCO and FOM
Provide general oversight of field operations to maintain adherence to SOP's

2.3 Field Operations Manager

Responsibilities in the field:

Serve as chief client liaison
Maintain communication with the RCO and QAM

Coordinate and monitor the performance of field activities
Maintain SOP's in performance of field operations
Maintenance of all sampling equipment
Assuring all safety requirements are met prior to and during field operations

Responsibilities in the laboratory:

Serve as chief client liaison
Oversee processing of field samples
Data analysis
Coordinate generation of the final report
Maintain communication with the RCO and QAM

2.4 Taxonomist

Responsibilities in field:

Obtain voucher specimens for EnviroScience reference collection
Assist in field responsibilities
Maintain communication with the FOM and field crew

Responsibilities in the laboratory:

Serve as chief taxonomist
Maintain voucher specimens for EnviroScience's reference collection
Maintain up to date reference libraries
Assist in data analysis
Assist in laboratory responsibilities
Assist in report generation
Maintain communication with the FOM and laboratory crew

2.5 Data Recorder

Responsibilities in the field:

Serve as primary data recorder
Maintain, organize, and preserve all data obtained in the field
Assist in field responsibilities
Maintain communication with the FOM and field crew

Responsibilities in the laboratory:

Reduce data collected in the field
Assist in data analysis
Assist in laboratory responsibilities
Assist in report generation
Maintain communication with the FOM

2.6 Geographic Information System Technician

Responsibilities in the field:

Collect reference and data points
Maintain, organize, and preserve all data obtained in the field
Assist in field responsibilities
Maintain communication with the FOM and field crew

Responsibilities in the laboratory:

Reduce data collected in the field for use in map development
Assist in watershed and land use data analysis
Assist in laboratory responsibilities
Develop maps of study area and assist in report generation
Maintain communication with the FOM

2.7 Additional Biologists and/or Technicians

Responsibilities in the field:

Assist field operations
Maintain communication with the FOM and field crew

Responsibilities in the laboratory:

Assist in processing of field samples
Maintain communication with the FOM and laboratory crew

3.0 METHODS AND SAMPLING PROCEDURES

All methods and sampling procedures used during an aquatic survey project strictly adhere to EnviroScience's SOP's. These SOP's are based on the U. S. Environmental Protection Agency's (USEPA) Rapid Bioassessment Protocols (RBP; Plafkin et al.

1989), protocols used by the Ohio EPA for water quality monitoring (OEPA 1987), and the Qualitative Habitat Evaluation Index (QHEI) as developed by the Ohio EPA (Rankin, 1989). Strict adherence to these SOP's forms the basis of EnviroScience's Quality Assurance Program.

EnviroScience may use one or a select combination of the procedures outlined in this document to fulfill all requirements for any given project. The following sections provide a brief overview of each procedure. Detailed procedures are contained in the SOP's attached as appendices to this document.

3.1 Site Selection

The sampling sites chosen for each project vary according to a number of factors including size of the waterbody, watershed size, tributary location(s), waterbody topography, potential source(s) of pollution, the type of sampling required, and local recreational uses. Sites are typically chosen with coordination between the client, EnviroScience personnel, and staff of the appropriate regulating agency(s). These sites are confirmed after field reconnaissance, including the establishment of at least one reference site.

3.2 Water Chemistry

Field chemistry is performed for those parameters having a minimal recommended holding time. These parameters include dissolved oxygen, pH, conductivity, turbidity, flow, water temperature and air temperature. These parameters, except turbidity, are measured using a Hydrolab Reporter™ Water Quality Multiprobe and the Hydrolab Scout® 2 Display Unit or the YSI™ 556, which is calibrated at the start of each workday and the results recorded in a bound notebook. The Hydrolab or YSI unit is placed directly into the water being tested and lowered to the appropriate depth. After the readings are allowed to stabilize, all field chemistry data is recorded on a chemistry data sheet. Field chemistry testing is performed in accordance with *Standard Methods for the Examination of Water and Wastewater* (APHA, 1992). EnviroScience's SOP's for chemistry can be found in Appendix A: Chemistry, Collection Methods and Field Testing Procedures.

When needed, analytical chemistry is performed by an outside accredited laboratory for parameters with greater than twenty-four hour holding times and for those requiring preservatives.

Ambient water samples for analytical chemistry analysis are collected with a pre-cleaned and rinsed stainless steel bucket. Sample containers are filled carefully to avoid loss of preservatives. The bucket is rinsed between each sampling site. In

waterbodies of deeper depths (approximately three feet or greater), analytical chemistry water samples are collected with a two-liter Kemmerer bottle at the appropriate depth. These water samples are poured into a pre-cleaned and rinsed stainless steel bucket before being transferred to the appropriate sample containers.

When EnviroScience obtains ambient water samples for laboratory analysis, the samples are placed in coolers containing ice that maintain the sample at approximately 4 ^{0}C during transport. An EnviroScience courier transports the samples from the field to the laboratory, or the samples are shipped to ensure next-day delivery.

3.3 Habitat Evaluation

A habitat evaluation is performed once during each project, and typically coincides with sampling of the biological communities. This evaluation may be repeated if sites are altered between sampling events by man-induced activities or natural causes. In-stream habitat and riparian zone characteristics important to the aquatic ecosystem are evaluated using the Qualitative Habitat Evaluation Index (QHEI; Rankin, 1989). The metrics used in the QHEI evaluate stream substrates, in-stream cover, channel morphology, pool and riffles, and the riparian zone, and provide a quantified evaluation of habitat quality and the ability to support biological communities. For a detailed description of the QHEI, see Appendix B: Standard Operating Procedures for Habitat Evaluation.

3.4 Benthic Macroinvertebrates

Four different sampling techniques are used to sample macroinvertebrates in an aquatic system. These include modified Hester-Dendy multiplate artificial substrate samplers, ponar sampling, kick sampling and surber sampling. Modified Hester-Dendy samplers can be used in shallow and deep waterways. Ponar sampling is most often used in deep waterways. Kick sampling and surber sampling are limited to depths of water shallow enough to maintain personnel safety. In order from most quantitative to most qualitative is the Hester-Dendy sampling, surber sampling, ponar sampling, and kick sampling. For detailed information on macroinvertebrate sampling, see Appendix C: Standard Operating Procedures for Macroinvertebrate Sampling.

3.4.1 Hester-Dendy Sampling

Hester-Dendy samplers are constructed of 1/8 inch tempered hardboard cut into eight, three inch x three inch (7.62 cm) square plates and twelve one inch x one inch (2.54 cm) square spacers for each sampler. The plates and spacers are placed on a 1/4 inch eyebolt so that there are three single spaces, three double spaces, and one triple space between each plate. This sequence provides various sized niches for

colonization by a representative community of benthic macroinvertebrates typical of a lentic/lotic system.

3.4.2 Surber Sampling

Surber samples are typically collected in areas with gravel and cobble substrates and good flow. A 30.5 x 30.5 cm surber sampler with a U.S. #30 mesh net is used to obtain macroinvertebrate samples in waterways of less than 32 cm in depth. The sampler is positioned into the stream with the net pointing downstream. Samples are collected manually by one or two field biologists only in the area encompassed by the sampler.

3.4.3 Ponar Sampling

A predetermined number of ponar samples are taken at each site. The standard ponar is most often used for deeper waterways and is attached to a winch and cable that is mounted to EnviroScience's sampling boat.

3.4.4 Kick Sampling

When taking a kick sample, a Biologist positions a D-framed net with U.S. #30 mesh in the water and disturbs the substrate by foot, so that the dislodged organisms are carried downstream into the net. Sampling is conducted for a specified amount of time, according to the protocol being followed. The net contents are emptied into a pan of stream water, examined, sieved with a U.S. no. 30 standard sieve, transferred to container, labeled and preserved with 80% ethyl alcohol. The net will be thoroughly cleaned before further sampling by vigorous rinsing in the stream.

3.4.5 Benthic Macroinvertebrate Sample Processing and Identification

Organisms removed from the samplers are placed in containers with 70-80% ethanol. All field samples are labeled with the sample site, date, project name, and samplers initials, and transported to EnviroScience's macroinvertebrate laboratory for processing. In the laboratory, each sample is labeled with an identification number and logged in EnviroScience's Sample Log Book. All specimens maintain the original sample identification number throughout the identification process. If necessary the samples are re-preserved in 70- 80% ethanol at this time.

The project manager monitors the progress of macroinvertebrate identification throughout the laboratory portion of the aquatic survey project. The project manager divides the samples from a project among available taxonomists. Specific taxa may be distributed to certain taxonomists where those individuals exhibit particular expertise.

Each sample is sorted and separated according to taxonomic group. If a large number of organisms are collected, it may be sub-sampled. The subsampling method is pre-determined based on the objective of the project. Macroinvertebrates are identified to the lowest feasible taxonomic level for data analysis (i.e., genus/species). For organisms of the family Chironomidae (Diptera), preserved larvae are mounted in a clearing agent on slides prior to identification. As organisms are identified, the individuals in each taxonomic group are counted and the numbers recorded on EnviroScience's Macroinvertebrate Bench Sheets.

The project manager provides a proficiency test for less experienced taxonomists by first giving them a set of referenced "unknowns" composed of commonly occurring taxa. When the less experienced taxonomist is finished with their unknowns, they are verified by a more experienced taxonomist. Where differences occur, the less experienced taxonomist is directed to the questionable key couplet and the organism is jointly re-identified by both taxonomists if needed. Less experienced taxonomists decisions are verified on a sample to sample basis and re-identifications are made where needed. EnviroScience understands that macroinvertebrate identification may become more subjective from generic to specific level. Questionable or unusual taxa are verified by an outside taxonomist. A final check for correct identification is made against EnviroScience voucher specimens which were verified by an outside authority in benthic macroinvertebrate identification. Original vouchers will be verified by Dr. Benjamin A. Foote of Kent State University, Eric Chapman of Kent State University, Dr. John H. Olive of the University of Akron, or other known authorities in benthic macroinvertebrate identifications. Specimens encountered in a study that are not in EnviroScience's permanent reference collection are immediately verified and transferred to EnviroScience's permanent collection.

3.5 Fish

Four different sampling techniques are used to sample juvenile and adult fish. These methods include electrofishing, seining, hoopnetting, and trawling. Electrofishing is a quantitative method used to sample general fish populations in all habitat types. Seining is employed to sample fish populations in near shore habitats, and hoopnets collect bottom fish species. The combination of these methods often provides a representative picture of the fish population in a given area. See Appendix D: Standard Operating Procedures for Fish Sampling.

3.5.1 Electrofishing

A Smith Root ® 5.0 GPP Portable DC Pulsed Electrofisher is used for wading and boat electrofishing. Wading methods use a long line extension, whereas boating methods

use anodes mounted to a boom in front of a boat. For headwater sites, a Smith Root ® Model 60B Backpack Electrofisher is used. The voltage of the electrofishing unit is adequately adjusted to representatively sample the smaller individuals while minimizing adverse effects to larger individuals. The degree to which fish are affected by electric current is a function of their surface area. Generally, larger fish are more sensitive to the electric currents. Immediately after collection, the fish are transported in live-wells to the field station for processing.

3.5.2 Seining

A 30 foot (9.1 meters) by 6 foot (1.8 meters) seine with 1/8 inch (.317 centimeters) mesh is used to collect fish representative of near-shore communities. Sampling consists of two biologists pulling the seine in an upstream direction and then drawing it into shore. If no fish are obtained in a seining attempt, the haul is repeated in an undisturbed area within the site. Seining is performed at most three times within a study area. If no fish are obtained with the third haul, the site is recorded as having zero fish. On occasion, the seine may snag on the substrate and fish escape. If this occurs, the seine is repeated within an undisturbed area. When an area within a study site can not be seined, a different area within a larger sampling zone is sampled, if available. Personnel safety is often a factor in determining a site to be unseinable. Fish captured by seining are immediately placed into a livewell for processing at the field station.

3.5.3 Hoopnetting

In deeper waterways, hoopnetting is performed by using 3.0 foot (0.9 meters) diameter hoopnets with 1 inch (2.54 centimeters) bar mesh. Hoopnets are either baited or not baited and set parallel to shore with the mouth opening at the downstream end. After a 24 hour period, the nets are checked for fish and subsequently reset for another 24 hour period. If fish are present, they are collected and processed. The nets are removed after 48 hours. Fish captured in the nets are immediately placed into a livewell for processing at the field station.

3.5.4 Trawling

In deeper waterways, trawling is performed using a 16 foot (4.91 meters) semi-balloon otter trawl with a 1/8 inch (0.317 cm) mesh cod liner. Trawling is conducted by pulling the trawl with the boat in an upstream direction for approximately five minutes in each sampling zone. A trailing buoy is attached to the cod end to allow for easy retrieval in the event of snags. Snags often occur in study areas that contain significant quantities of large debris. Any trawl attempt that is interrupted by a snag or obstruction is restarted. Damaged nets are repaired before the next trawl is initiated. While trawling, the length of the tow rope is maintained at two to three times the depth of the sampling zone. A trawling run is considered successful when a mid-depth sample is taken without getting snagged. Fish captured from trawling are immediately placed into a livewell for processing at the field station.

3.5.5 Fish Sample Processing

Fish identifications are made primarily by the Fish Taxonomist. The Fish Taxonomist is responsible for choosing those biologists that can identify fish correctly and efficiently. Any doubtful identifications are directed to the Fish Taxonomist who makes the final identification. Fish with questionable identification are transported to EnviroScience's laboratory for further examination and identification. The FOM supervises the progress of fish identification throughout the aquatic survey project.

After identification, all fish are weighed to the nearest 1/10 (0.1) of a gram. A calibrated portable top loader scale (300 g capacity x 0.1 g intervals) is used for smaller specimens. Fish weighing 300 grams or more are weighed on a calibrated Hobart® meat scale (30 lb capacity x 0.01 lb intervals). Weights obtained in pounds are converted to kilograms (1 lb = approximately 0.454 kg). Standard lengths or total lengths are recorded to the nearest centimeter. Standard lengths are measured from the anterior-most tip of the head in a straight line to the base of the caudal fin, whereas total lengths are measured from the anterior-most tip of the head in a straight line to the tip of the caudal fin. Length, weight and anomaly data are recorded on EnviroScience's

Fish Data Sheets. Anomalies include deformities, erosions, lesions and tumors (DELT anomalies; Ohio EPA, 1989). With the exception of those retained for vouchers, all collected fish are released upon total recovery from the initial shock.

3.5.6 Fish Identification/Verification

Fish identification predominantly occurs in the field while performing an aquatic survey. Representative fish having uncertain identity or those being used for vouchers are preserved in 10% buffered formalin and returned to the EnviroScience laboratory for further examination. For each aquatic survey project, a reference collection is produced that includes at least one specimen of all species encountered in the study area. Reference specimens are verified to EnviroScience's permanent reference collection. Original vouchers are verified by Dr. Lowell P. Orr of Kent State University. Specimens encountered in a study that are not represented in EnviroScience's permanent reference collection will be immediately verified and subsequently transferred to EnviroScience's voucher collection.

4.0 SAMPLE AND DOCUMENT CHAIN OF CUSTODY

4.1 Sample Collection and Transport

Any samples collected as part of an aquatic survey project require the use of a chain of custody or sample submission form. These are used to document the integrity of all samples. To maintain a record of sample collection, transfer between personnel, shipment, and receipt by the laboratory, a chain of custody or sample submission form is filled out for each sample within a given project. The chain of custody or sample submission form contains the following information:

Client information (name, address, primary contact, phone)
Type of Sample Submission / Chain of Custody form (Fish, Macroinvertebrate, Chemistry)
Date of sample collection
Time of sample collection
Type of sample (Fish, Macroinvertebrate, Chemistry)
Station or site at which the sample was collected
Sample ID #
Number of Samples
Type of sample method
Collector's name and signature
Flow rate
Weather Conditions

Spaces to be signed as custody is transferred between individuals
Shipping information
Condition of the sample when received in the laboratory (For EnviroScience use only)

EnviroScience uses separate sample submission / chain of custody forms for fish, macroinvertebrates, in-field chemistry and for each laboratory performing chemical analyses. Each form is modified to accommodate additional information involved in that type of sampling. For example, fish sample submission / chain of custody forms have blanks to fill in the sampling method used to collect the fish (electrofishing, seining, etc.). This information is in addition to that listed above.

The FOM is responsible for ensuring that the sample submission / chain of custody forms are properly completed. The FOM will also place the sample submission / chain of custody form in a resealable plastic bag and tape it to the inside of the shipping vessel containing the respective samples. An example of EnviroScience's Sample Submission / Chain of Custody Forms for In-field Chemistry can be found in Appendix A attached to this document. Sample submission / chain of custody forms for samples collected by EnviroScience and analyzed by an outside company are supplied by the performing laboratory.

4.2 Voucher Specimens

A sample submission / chain of custody form is also used when fish and macroinvertebrate specimens are in possession of authorities used for outside verification. One form accompanies each specimen or group of specimens en route to the laboratories.

5.0 FIELD EQUIPMENT CALIBRATION PROCEDURES

The following is a brief description of calibration procedures used on field equipment. Detailed descriptions of calibration procedures and sampling techniques are provided in Appendix A: Standard Operating Procedures for Chemistry and Appendix D: Standard Operating Procedures for Fish.

5.1 Fish Weights

Fish are weighed on a portable top loader scale (300 g capacity x 0.1 g intervals) and a Hobart® meat scale (30 lb capacity x .01 lb intervals). Both scales are checked daily with National Bureau of Standards Class F check weights (up to 2000 g in 1 g increments). The top loader scale can be adjusted when necessary. The Hobart® meat scale is not adjustable but the difference, when present, is noted and recorded as such.

5.2 Multiple Parameters

A Hydrolab Reporter™ connected to a Hydrolab Scout® 2 display unit is also used when multiple parameters are required during sampling. The Hydrolab is calibrated prior to each workday. Calibrations are recorded in EnviroScience's calibration log book. Sample parameters include dissolved oxygen, pH, conductivity, and stream temperature. In-field chemistry is acquired by collecting a water sample using a stainless steel bucket and then lowering the Hydrolab into the sample. Readings are recorded once the display unit values stabilize. Prior to collection, the stainless steel bucket is carefully rinsed with water from the sample site. See Appendix A: Standard Operating Procedures for Chemistry.

6.0 DATA REDUCTION, VALIDATION AND REPORTING

6.1 Data Reduction for Field and Technical Data

6.1.1 Chemistry

All in-field chemistry data are transcribed into a computer database spreadsheet. As with fish and macroinvertebrates, a printout of the database spreadsheet is manually compared to the original chemistry data sheets to detect transcription errors. Once the spreadsheets are checked, graphs are generated to display general trends in chemical parameters throughout the study area. These graphs are double checked for transcription errors.

6.1.2 Habitat Evaluation

While in the field conducting various sampling procedures, one biologist performs the habitat evaluation and fills out the form thoroughly. It is the responsibility of the FOM to oversee completion of the habitat evaluation and address any questions that may arise. If a score is questioned by any of the biologists, the field team discusses that attribute of the particular site and a score is determined.

6.1.3 Macroinvertebrates

As stated in Section 3.4.5, all macroinvertebrate samples receive an identification number and retain that number throughout the identification process. Once the identification of all macroinvertebrates for a site is complete, data from the macroinvertebrate bench sheets is entered into a computerized database. To detect

transcription errors, a printout of the database spreadsheet is manually compared to the original macroinvertebrate bench sheets.

If data is evaluated using USEPA methods, then one of three methods are used (RBP I, II, or III). RBP I is a rapid screening method used to identify areas as impacted, non-impacted, or potentially affected that require further evaluation. Organisms are identified to Order/Family and their relative abundances determined. Based on an evaluation of the taxa present and habitat, the site's level of impairment is described and sources of potential pollutants are identified. RBP II is also used as a screening tool, or as a protocol to prioritize sites for further evaluation. Organisms are identified to the family level and then applied to eight metrics that evaluate different components of the community. It is more intensive than RBP I and therefore, more sensitive to detecting impairment. RBP III is the most rigorous technique because of the level of identification (genus/species), and provides the most detailed analysis of the macroinvertebrate community and degree of impairment. Data from RBP II and III are evaluated using the same metrics to determine Biological Condition, but the criteria for RBP III have been modified to accommodate the more detailed taxonomy. Biological Condition scores are calculated following methods described in *Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish* (Plafkin et al., 1989). Once scores have been determined, they are double checked by a second biologist. Sample sites are then evaluated as to their degree of impairment.

If data are evaluated using OEPA methods, then a biologist prepares the Invertebrate Community Index (ICI) worksheets. The worksheets are completed manually and are double checked by a second biologist. If any discrepancies are noted, the ICI is triple checked by a third biologist. After a minimum of double checking, the ICI scores are entered into a computer spreadsheet and graphs are generated. This worksheet is modeled after an Ohio EPA computer program (OEPA 1981). The ICI consists of ten structural community metrics, each with four scoring categories. The point system evaluates a sample against a database of 247 relatively undisturbed reference sites throughout Ohio. The ICI scores are calculated following methods described in *Biological Criteria for the Protection of Aquatic Life,* Volume II. *Users Manual for Biological Field Assessment of Ohio Surface Waters* (Ohio EPA, 1987).

6.1.4 Fish

Fish data is generally recorded in the field with indelible ink pens on an EnviroScience Fish Data Sheet. Information recorded includes species name, weight (g), standard length (cm) and observed anomalies. Once the Fish Data Sheets are completed for a given site, the Data Recorder places the sheets into resealable plastic bags in a portable filing box. It is the responsibility of the Data Recorder to maintain the Fish Data Sheets throughout the duration of the aquatic survey project.

All fish data is entered into a computerized database. To minimize transcription errors, a printout of the database is manually compared to original worksheets. Once the spreadsheets are checked, a biologist prepares the Index of Biotic Integrity (IBI) worksheets. The worksheets are completed manually and are double checked by a second biologist. If any disagreement exists, the IBI is triple checked by a third biologist. After the IBI scores are determined, the score for each site is placed into the computer database and graphs are generated. This worksheet is modeled after the Ohio EPA computer program (OEPA, 1987). Graphs generated from IBI scores are also double checked for transcription errors.

Modified Index of Well Being (MIwb) scores are calculated at sites with more than a twenty mile^2 drainage area and are interpreted in conjunction with the IBI scores. The MIwb is initially calculated by a spreadsheet. The MIwb is also hand calculated by a second biologist for every other site to verify no errors were made in data entry or in set-up of the spreadsheet. Once calculated, the MIwb scores are used to generate graphs. The graphs are also double checked for errors. Relative abundance and relative weights are adjusted to provide abundance and weight on a per kilometer basis.

IBI and MIwb scores are calculated following methods described in *Biological Criteria for the Protection of Aquatic Life,* Volume II. *Users Manual for Biological Field Assessment of Ohio Surface Waters* (Ohio EPA, 1987). It should be noted that the IBI and MIwb indices are usually used to evaluate streams up to 6,500 mi^2 and are not calibrated for lakes. Nonetheless, these indices are the most useful tools available for quantifying fish community data.

6.2 Validation

Data generated and reported by EnviroScience is validated using a number of distinct processes, many of which have been described in previous sections of this document. The following list summarizes the major techniques used to validate field and laboratory data:

- Field activities, and in particular sample collection and chain of custody procedures, are reviewed daily by the FOM to ensure that all activities were completed in strict accordance with EnviroScience SOP's. Chain of custody forms are compiled and reviewed again as part of report generation activities.

- Periodically during the course of field activities, log books and data sheets are reviewed by the FOM to ensure completeness. These reviews

and a final check performed prior to report generation also verify that field instruments have been properly calibrated.

- Macroinvertebrate identifications are validated through a series of internal quality control checks involving peer review, periodic proficiency testing and verification of vouchers by an outside authority.

- Fish identifications are supervised and principally performed by an experienced taxonomist. Any questionable fish and one of each species encountered are verified by an outside authority.

- For both macroinvertebrate and fish identifications, EnviroScience maintains permanent reference collections of organisms verified by outside authorities.

- All computerized spreadsheets and databases are at least double checked against original log books, data sheets and bench sheets.

6.3 Data Reporting

6.3.1 Data Summary

The water quality data is summarized to explain the relationships between chemistry, habitat and the biological communities. All data is presented in English units with metric units in parenthesis (i.e., 5 ft (1.5 m)). Where practical, data is presented as graphs and tables. All potential limiting factors are identified, the data is compared with water quality from similar studies if applicable, and reasonable goals are established for continued improvement in water quality factors.

6.3.2 Report Generation

The report format for each aquatic survey project generally includes the following:

1. Executive Summary,
2. Statement of project purpose,
3. General overall project description,
4. Heading and summary for each project task with accompanying appendices,
5. All pertinent data, including field sheets,
6. Water quality and index calculations,
7. All pertinent project correspondence,
8. Necessary maps, charts, graphs, computations and computational breakdowns,
9. Project conclusion.

After all data pertaining to a project is generated and graphs compiled, the report is drafted. A detailed description of the type and format of the report to be produced is prepared prior to the initiation of field work. The following is a sample Table of Contents:

TABLE OF CONTENTS (*SAMPLE*)

1.0 Introduction

2.0 Methods
- 2.1 Habitat Evaluation
- 2.2 Macroinvertebrates
  - 2.2.1 In-field Sampling Procedures
  - 2.2.2 Labeling and Record Keeping
  - 2.2.3 Sample Processing
  - 2.2.4 Macroinvertebrate Identification
  - 2.2.5 Macroinvertebrate Data Analysis
- 2.3 Fish Populations
  - 2.4.1 Electrofishing Procedures
  - 2.4.2 Sample Processing
  - 2.4.3 Identification/Enumeration
  - 2.4.4 Fish Data Analysis
- 2.5 Field Chemistry

3.0 Results
- 3.1 Habitat
- 3.2 Macroinvertebrate Community Assessment
- 3.3 Fish Community Assessment
- 3.4 Field Chemistry

4.0 Discussion

5.0 Conclusion

6.0 References

7.0 CORRECTIVE ACTION AND QUALITY ASSURANCE REPORTS TO MANAGEMENT

7.1 Corrective Action

When a significant condition adverse to the quality of an aquatic survey project occurs, corrective action is initiated. Condition identification, cause, and proposed corrective action is documented and reported to the FOM and QAM. Implementation of corrective action is verified and documented by the FOM. All project personnel have the responsibility, as part of their normal work duties, to promptly identify and report conditions adverse to the quality of an aquatic survey project. The FOM will oversee the corrective actions and will report to the RCO as part of routine reports to management (See Section 7.2).

Corrective actions may be taken due to a number of circumstances, including, but not limited to:

- When quality assurance in this document or field SOP's have not been followed,
- When data are later determined to be incorrect (for example, incorrect identification of a fish species or macroinvertebrate species),
- When equipment or instrumentation is found to be incorrectly operated, calibrated or maintained,
- When a strict chain of custody for samples collected during an aquatic survey project is not maintained,
- As a result of routine audits performed by the QAM and other project management.

7.2 Quality Assurance Reports to Management

Throughout an aquatic survey project, quality assurance reports are provided by the QAM on a monthly basis to the RCO. These reports will describe in detail the progress of the aquatic survey project from a quality assurance perspective. These reports describe problems noted during the period, describe corrective measures implemented, and detail any resulting financial or schedule impacts. These reports are placed in the permanent project file and are available upon the request of the client.

REFERENCES

American Public Health Association, American Water Works Association, and Water Environment Federation. Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WEF.

Plafkin, J. L., M. T. Barbour, K. D. Porter, S. K. Gross, and R. M. Hughes. 1989. Rapid Bioassessment Protocols for use in Streams and Rivers: Benthic Macroinvertebrates and fish. U.S. Environmental Protection Agency, Assessment and Watershed Protection Division. Washington, D. C.

Rankin, E. T. 1989. The Qualitative Habitat Evaluation Index (QHEI): Rationale, Methods, and Application. Ohio Environmental Protection Agency, Division of Surface Water, Ecological Assessment Section. Columbus, Ohio.

Wetzel, R. G. and G. E. Likens. 1979. Limnological Analysis. W. B. Saunders Co., Philadelphia, PA. pp. 146-148.

APPENDIX A

STANDARD OPERATING PROCEDURES FOR IN-FIELD CHEMISTRY

ENVIROSCIENCE, INC.

Standard Operating Procedures Document:

Chemistry: Collection Methods and Field Testing

1.0 PURPOSE

To provide methods for collection, preservation, and processing of chemistry samples used to evaluate surface water quality.

2.0 SCOPE

This Standard Operating Procedure (SOP) includes procedures for sample site location, collection techniques, instrument calibration, in-field data collection, and chain of custody. All procedures are modified from and strictly adhere to those stated in the Manual of Ohio EPA Surveillance Methods and Quality Assurance Practices (Ohio Environmental Protection Agency, 2006).

3.0 SAMPLE SITE LOCATION

In most cases, chemistry samples are collected at sites specified in the clients National Pollution Discharge Elimination System (NPDES) permit. Field chemistry samples are also taken at each sampling site during biological sampling events. When site specific complications or limitations prevent collection of an adequate sample, written authorization for a change in location will be obtained from the permitting agency.

4.0 PROCEDURES FOR SAMPLE COLLECTION

Chemistry samples are usually collected as "grab" samples, which represent conditions that exist at the time of collection. Grab samples obtained from the water surface may be collected directly into a sample container. More commonly, these samples are collected using a stainless steel bucket with a length of quality rope or a high density polyethylene dipper. All are pre-cleaned in the laboratory with a non-phosphate detergent before use and rinsed three times with ambient water before a sample is obtained. The bucket or dipper is filled and chemical parameters with brief holding times (i.e. dissolved oxygen, temperature, pH and conductivity) are measured directly from the bucket. Water samples that require laboratory analysis are allocated to individual sample bottles using a polyethylene funnel.

Subsurface grab samples are collected at a specific depth in the water column with a Kemmerer water sampler. Subsurface samples are allocated to individual sample bottles directly from the sampler through a polyethylene funnel.

Stormwater samples may be collected any number of ways depending on the flow from the outfall. During a heavy storm surge, a stainless steel bucket is used to collect sample water, which is then partitioned to sample containers through a high density polyethylene funnel. Lighter flows may be collected directly into the sample container or by funneling the water into the appropriate container. EnviroScience personnel are required to wear latex gloves and protective eye wear when sampling stormwater outfalls.

The type of collection bottle, preservative, and holding time used in any sampling event is dependent on the chemical parameter being tested. The requirements for containers, preservatives, and holding times are listed in the Manual of Ohio EPA Surveillance Methods and Quality Assurance Practices (Ohio EPA, 2006). Once collected, the sample containers are labeled using indelible ink with the site location, date, and time of the sampling event and then placed in a cooler with ice to ensure a temperature of about 4 ^{0}C during shipping.

5.0 CHAIN OF CUSTODY PROCEDURES

Chemistry samples for parameters that are not suitable for field analysis (i.e. time limitations and equipment logistics problems) must be transported to an accredited laboratory for further examination. An EnviroScience Sample Submission and Chain of Custody Form (Figure 5.1) is filled out for every four samples taken. The form and sample are released with a signature and time of delivery to the receiving party, and a photocopy is made for EnviroScience records. The sample submission and chain of custody form contains the following information:

Client information (name, address, primary contact, phone)
Date and time of sample collection
Station or site at which the sample was collected
Sample ID #
Number of Samples
Type of sample method
Collector's name and signature
Flow rate
Weather Conditions
Spaces to be signed as custody is transferred between individuals
Shipping information

Spaces to fill in for the condition of the sample when received in the laboratory (For EnviroScience Use Only)

Table 5.1 EnviroScience Chain of Custody Form

EnviroScience uses separate sample submission and chain of custody forms of the laboratory contracted to perform chemical analyses.

6.0 INSTRUMENT CALIBRATION AND TESTING METHODS

This standard procedure applies to the calibration, operation and maintenance of the Hydrolab Reporter™ Water Quality Multiprobe, the Hydrolab Scout® 2 Display Unit and the YSI 556.

6.1 Summary of Method

6.1.1 The Hydrolab Reporter™ Water Quality Multiprobe or the YSI 556 is lowered into a stainless steel sampling bucket filled with water to be tested. The probe is gently waved through the water in order to ensure a constant flow of water over the membrane. Once the reading has stabilized, the value is read directly from the display unit and is recorded on an in-field chemistry data sheet (Figure 6.1). If readings are required at a specific depth within the water column, the probe is lowered by rope and weight to that depth and allowed to equilibrate.

6.2 Sample Handling and Preservation

6.2.1 Not Applicable.

6.3 Interferences

6.3.1 Not Applicable.

6.4 Apparatus

6.4.1 Hydrolab Reporter™ Water Quality Multiprobe
6.4.2 Hydrolab Scout® 2 Display Unit.
6.4.3 External Battery Source, Panasonic® Sealed Rechargeable Battery
6.4.4 YSI 556

6.5 Reagents

6.5.1 Not Applicable.

Figure 6.1 In-field Chemistry Data Sheet

Station	Date	Time	Water Temp	pH	Dissolved Oxygen	Conductivity
Duplicate						

Comments and Calibrations: _____

Field Manager Signature: _____

6.6 Procedure

6.6.1 Calibration:

6.6.1.1 Calibrate the multiprobe by pouring one of the following calibration fluids into the Hydrolab® calibration cup:

Temperature-	none
Dissolved Oxygen-	none
Conductivity-	1.413 mS/cm KCl solution
pH-	7 and 10 standard solutions

6.6.1.2 Immerse the probe into the cup, and allow the reading to stabilize.

6.6.1.3 After the reading has stabilized, hit the calibrate button and select the appropriate probe from the list: 'p' for pH, 'C' for conductivity, 'O' for dissolved oxygen (in units of mg/L).

6.6.2 Temperature Calibration

6.6.2.1 Probe accuracy will be checked monthly by comparison to the results from a NBS calibrated thermometer.

6.6.3 Dissolved Oxygen Calibration

6.6.3.1 Remove the probe from the moist calibration chamber and inspect the membrane for debris or air bubbles. If necessary, the membrane must be changed.

6.6.3.2 Point the multiprobe toward the ceiling and fill the calibration cup with tap water until the water just reaches the o-ring securing the membrane.

6.6.3.3 Remove any water from the surface of the membrane

6.6.3.4 Cover the calibration cup with the calibration cover

6.6.3.5 Allow the reading to stabilize.

6.6.3.6 Hit Calibrate on the display unit and select the DO meter (mg/L)

6.6.3.7 The display will read 'Std'. Type in the local barometric pressure in mmHg (760 is sea level), always within 608-912 mmHg.

6.6.3.8 To determine the local bp, call the local weather bureau for a barometric reading

6.6.3.9 Hit Enter

6.6.3.10 Enter DO reading that has been determined from Winker's titration.

6.6.3.11 Hit Enter

6.6.3.12 Record calibration in EnviroScience's In-field Chemistry Calibration Log.

6.6.3a Winkler Titration:

6.6.3a.1 Carefully fill four 300 ml BOD bottles (no air bubbles) from a common source.

6.6.3a.2 The contents of one bottle is gently poured into a beaker and the oxygen concentration is determined

6.6.3a.3 To each of three 300 ml bottles add

a. 2 ml manganous sulfate solution

b. 2 ml alkaline iodide-azide solution
Insert the stopper and invert several times to mix, allow to settle and mix again, allow to settle again.

c. 2 ml conc. sulfuric acid using a serological pipette - (USE CAUTION WITH CONCENTRATED ACIDS) stopper and invert to mix.

6.6.3a.4 Pour contents of bottle into a 600 ml beaker, add a stir bar, place on magnetic stirrer, and titrate with 0.0375 N sodium thiosulfate solution until a straw yellow color is produced.

6.6.3a.5 Add starch (a quick spray commercial starch) in small increments, the solution will turn blue.

6.6.3a.6 Continue titration with 0.0375 N sodium thiosulfate until the blue color disappears. Be careful to continue titration in small increments (i.e., one drop at a time).

6.6.3a.7 Record the number of milliliters used, each ml 0.0375 N sodium thiosulfate titrant is equivalent to 1 mg dissolved oxygen when all 300 ml of sample are titrated.

6.6.4 Conductivity Calibration

6.6.4.1 EnviroScience's multiprobe uses a fresh water cell block. If a salt water cell block is required, please see the Hydrolab Operating Manual.

6.6.4.2 Place the sensor in a 0.01 M KCl solution (1413 S/cm)

6.6.4.3 Thoroughly rinse the sensors several times with deionized water.

6.6.4.4 Rinse the sensors twice with the KCl solution

6.6.4.5 With the sensors pointed toward the ceiling, fill the calibration cup with the KCl standard solution. Make sure there are no bubbles interfering with the sensors.

6.6.4.6 Allow the multiprobe readings to stabilize

6.6.4.7 Hit the Calibrate button and go to Conductivity, 'C'.

6.6.4.8 Type in the calibration standard value (1413)

6.6.4.9 Hit enter

6.6.4.10 Record calibration in EnviroScience's In-field Chemistry Calibration Log.

6.6.5 pH Calibration

6.6.5.1 Make sure the glass pH probe is not coated with any contaminant. If so, clean with a soft, non-scratching cloth (i.e., a cotton ball) and rubbing alcohol. Fill the calibration cup with the 7 pH buffer solution.

6.6.5.2 Hit Calibrate and go to the pH sensor, 'p'. Enter the buffer value at the Std.' cue.

6.6.5.3 Hit Enter

6.6.5.4 Rinse the electrode and calibration cup with deionized water

6.6.5.5 Fill the calibration cup with a buffer solution that is near the anticipated value of the sample.

6.6.5.6 Hit Calibrate and go to the pH sensor, 'p'. Enter the buffer value at the 'Std.' cue.

6.6.5.7 Hit Enter

6.6.5.8 Record calibration in EnviroScience's In-field Chemistry Calibration Log.

6.6.6 Sample:

6.6.6.1 Place the multiprobe into the sample water and provide constant, gentle mixing.

6.6.6.2 Allow 1 minute for the reading to stabilize. A longer period may be needed if the readings do not stabilize within 1 minute. If there are problems with the readings stabilizing, the sensors need to be cleaned and/or calibrated.

6.6.6.3 Record values on the EnviroScience's In-field data sheet (Figure 1).

6.6.7 Maintenance:

6.6.7.1 Upon completion of field work, the multiprobe sensors should be cleaned with soap and water, and subsequently rinsed with deionized water.

6.6.8 Temperature Maintenance

6.6.8.1 No maintenance required.

6.6.9 Dissolved Oxygen Maintenance

6.6.9.1 DO sensor maintenance is required if calibration procedures cannot be performed, if the membrane covering the sensor is damaged, or if a bubble exists underneath the membrane.

6.6.9a **Membrane Replacement**:

6.6.9a.1 Prepare 2M electrolyte solution by dissolving KCL crystals in a dropper bottle with distilled water. Fill the bottle to the top.

6.6.9a.2 Unscrew the sensor guard from the probe and remove the o-ring and membrane. Thoroughly rinse the sensor with deionized water.

6.6.9a.3 Fill the probe with electrolyte as follows:

a. Grasp the probe in your left hand. Fill the sensor body with electrolyte

b. Secure a membrane under your left thumb. Add more electrolyte to the probe until a large meniscus completely covers the gold cathode.

c. With the thumb and forefinger of your other hand, grasp the free end of the membrane.

d. Using a continuous motion stretch the membrane up, over, and down the other side of the sensor. Stretching forms the membrane to the contour of the probe.

e. Secure the end of the membrane under the forefinger of the hand holding the probe.

f. Roll the o-ring over the end of the probe. There should be no wrinkles in the membrane or trapped air bubbles. Some wrinkles may be removed by lightly tugging on the edges of the membrane beyond the o-ring.

g. Trim off excess membrane with scissors or sharp knife. Check that stainless steel temperature sensor is not covered by excess membrane.

6.6.9.2 It is recommended that the DO probe soak in water overnight to allow the membrane to relax to its final shape

6.6.10 Conductivity Maintenance

6.6.10.1 Remove the white cell block covering the six pin-shaped electrodes

6.6.10.2 Remove the six o-rings

6.6.10.3 Polish each electrode with an emery cloth, or with #400 wet/dry sandpaper

6.6.10.4 DO NOT touch the pH glass probe with the chosen abrasive!!

6.6.10.5 Clean the electrodes and the cell block with a swab soaked in alcohol

6.6.10.6 Re-install the o-rings (replace if necessary, if they are flat)

6.6.10.7 Re-install the white cell block (DO NOT over tighten the screws!)

6.6.10.8 Rinse the sensor with deionized water, and if possible let newly polished sensor soak overnight in tap water

6.6.11 pH Maintenance

6.6.11.1 Clean the glass pH electrode only when obviously dirty. If the readings are slow or non-reproducible, then the electrodes most likely need cleaned.

6.6.11.2 Clean with a non-scratching cloth using rubbing alcohol (cotton-ball is recommended). Rinsing with methyl alcohol will also help.

6.6.11.3 If readings continue to be slow, soak the electrode in 0.1 M HCl for five minutes. Rinse with water, followed by rinsing with pH buffer for 10 minutes

6.6.11.4 The reference electrode should also be maintained by replacing the electrolyte.

a. Remove the cover sleeve gently and empty the old electrolyte.
b. Fill the sleeve with a 3-4 N standard KCl solution saturated with silver chloride.

c. Replace the cover sleeve by pointing the multiprobe to the floor and push the cover sleeve back on until the sleeve has just covered the o-ring.

d. Point the multiprobe up and push the sleeve the rest of the way down.

e. Excess air and electrolyte should come out the porous junction at the tip of the sleeve. If the junction is not clean and does not pass electrolyte regularly, then it needs to be replaced.

6.6.11.5 It is recommended to allow the sensor to soak overnight in tap water for re-equilibration of the electrodes.

7.0 Data Treatment

7.1 Values for temperature, dissolved oxygen, conductivity, and pH are read directly from the Hydrolab Scout® 2 Display Unit

8.0 Quality Control

8.1 The Hydrolab Reporter™ Water Quality Multiprobe is calibrated in the

laboratory monthly using the Winkler Titration method mentioned above. Calibrations are recorded in EnviroScience's bound infield lab/calibration log.

8.2 Duplicates are analyzed at a frequency of one of every ten sampling stations and the results are recorded on the chemistry data sheet. These duplicates are then transferred into EnviroScience's bound infield lab/calibration log.

9.0 Preventative Maintenance

9.1 Batteries for the Hydrolab Reporter™ Water Quality Multiprobe are checked before each field excursion. Spare batteries always accompany field personnel during sampling procedures.

9.2 The Hydrolab Reporter™ Water Quality Multiprobe is checked for corrosion and other associated problems at the beginning of each month. This inspection takes place at the same time that the meter is calibrated against the Winkler Titration Method.

9.3 The Hydrolab Reporter™ Water Quality Multiprobe is serviced yearly in EnviroScience's laboratory. A reconditioning kit is used from Hydrolab and the date of service recorded in EnviroScience's bound infield lab/calibration log.

REFERENCES

American Public Health Association, American Water Works Association, and Water Environment Federation. 1992. Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WEF.

Ohio Environmental Protection Agency. 2006. Manual of Ohio EPA Surveillance Methods and Quality Assurance Practices. Division of Environmental Services. Columbus, Ohio.

APPENDIX B

STANDARD OPERATING PROCEDURES FOR HABITAT EVALUATION

ENVIROSCIENCE, INC.

Standard Operating Procedures Document:

Habitat Evaluation / Methods

1.0 PURPOSE

To provide field methods for the evaluation of habitat important to in-stream biological communities.

2.0 SCOPE

This Standard Operating Procedure (SOP) includes general information on the USEPA and OEPA methodologies for habitat evaluation, procedures for sample site description, procedures for completing the site description sheets, and procedures for determining overall scores. The USEPA follows RBP methodology, and the OEPA uses the Qualitative Habitat Evaluation Index (QHEI).

3.0 GENERAL INFORMATION

The RBP habitat evaluation and the QHEI provide a quantified assessment of the in-stream and riparian zone characteristics important to biological communities. The RBP evaluation examines three components of habitat; substrate and in-stream cover, channel morphology, and riparian and bank structure. Nine metrics are used to evaluate these three characteristics by assigning scores to each and obtaining a total. The highest possible score is 135. Each site is then compared with a reference site to determine an overall habitat rating.

The QHEI index is calculated by assigning scores to six individual metrics and then adding these for a total QHEI score. The highest possible score is 100. The QHEI is calculated at each sampling station once each sampling year. If major disturbances occur within the sample site, either man-induced or natural, the QHEI is recalculated prior to re-sampling. For a complete description of the development and use of the QHEI, see Rankin (1989).

In order to determine physical habitat quality accurately, standardized definitions for pool, run, and riffle habitats, are essential. Below are definitions of different habitats taken from Platts et al. (1983).

Riffle and Run Habitats:

Riffle - areas of the stream with fast current velocity and shallow depth; the water surface is visibly broken.

Run - areas of the stream that have a rapid, non-turbulent flow; runs are deeper than riffles with a faster current velocity than pools and are generally located downstream from riffles where the stream narrows; the stream bed is often flat beneath a run and the water surface is not visibly broken.

Pool and Glide Habitats:

Pool - an area of the stream with slow current velocity and a depth greater than riffle and run areas; the stream bed is often concave and stream width frequently is the greatest; the water slope is nearly zero.

Glide - this is an area common to most modified stream channels that do not have distinguishable pool, run, and riffle habitats; the current and flow is similar to that of a canal, the water surface gradient is nearly zero.

4.0 PROCEDURES FOR HABITAT EVALUATION

4.1 RBP HABITAT EVALUATION

Below is a description of the three principal characteristics evaluated using RBP methods and their associated metrics, their components, and guidelines on scoring each metric. When each metric score is determined, the corresponding number on the field data sheet is circled (Figure 4-1). When habitat is between two classifications, both boxes are checked and scores averaged.

Substrate and In-stream Cover
- Bottom substrate and available cover, maximum 20 points
- Embeddedness, maximum 20 points
- Flow/Velocity, maximum 20 points

Channel Morphology
- Channel Alteration, maximum 15 points
- Bottom scouring and deposition, maximum 15 points
- Pool/riffle, run/bend ration, maximum 15 points

Riparian and Bank Structure
- Bank Stability, maximum 10 points
- Bank vegetation, maximum 10 points
- Streamside cover, maximum 10 points

Figure 4-1. RBP Habitat Evaluation Score Sheet

Figure 4-1. RBP Habitat Evaluation Score Sheet (continued)

4.1.1 RBP HABITAT EVALUATION METRICS

Bottom substrate and available cover- This metric evaluates the proportion of substrate types on
the stream bottom, and the proportion of in-stream habitat. Substrate and habitat types include gravel, cobble, woody debris, undercut banks, and other stable habitats. Higher proportions result in higher scores.

Embeddedness- This metric measures the degree stream bottom substrates and the interstitial
spaces are covered by find sediment. Lower percentages of embeddedness result in higher scores.

Flow/Velocity- If flow is less than 0.15 cms (5 cfs; i.e. "Low flow"), this metric evaluates the
ambient discharge of the stream where higher flows receive higher scores. Evaluating the flow also requires determining if the stream is considered as "cold-water" or "warm-hater". Higher flows result in higher scores. If flow is greater than 0.15 cms (5 cfs), this metric accounts for water depth as well as discharge.

Channel Alteration- This metric evaluates the degree to which the deposition of sediment
particles have altered stream flow. Excess sediment can aggrade the stream bottom in some areas, scour the stream bottom in others, cause channelization, and increase embeddedness. Channels with little alteration result in higher scores for his metric.

Bottom scouring and deposition- This metric evaluates the proportion of stream bottom that has
been affected by scour or and deposition. Excess sediment in the water column can increase these characteristics. Higher proportions of each result in lower scores.

Pool/riffle, run/bend ratio- This metric evaluates the ratio of pools to riffles and runs to bends.
Both of which ultimately affect habitat diversity. It calculated as the distance between riffles divided by stream width. Lower numbers result in higher scores.

Bank Stability- This metric evaluates the stability of stream banks by determining the degree of
erosion and bank sloping. Banks with reduced erosion and lower slopes receive higher scores.

Bank vegetation- The metric also evaluates the stability of the stream bank. The proportion of

stream bank covered by vegetation and/or boulders and cobble determines the score, where higher proportions result in higher scores.

Streamside cover- This metric evaluates the vegetation types adjacent to the stream. Riparian
zones with greater vegetation receive higher scores, where shrubs and trees are considered best.

4.1.2 RBP SCORING CRITERIA

Each parameter is rated as being in one of four categories (excellent, good, fair, or poor), and each category has a range of values with which the parameter was scored (Table 4-1). The sum of the scores from these metrics yields a total score that numerically rates the habitat of each stream reach. The total maximum score possible is 135 points. Habitat is considered excellent if it falls within the range of 111-135, good between 77-110, fair between 39-76, and poor below 38. The downstream sites are compared with upstream reference conditions by calculating a percent comparability, which is the ratio of downstream to the reference site scores. Determining percent comparability assesses how well the study site compares to the "best-attainable", or reference, condition (Plafkin et al., 1989), and is an indicator of the study site's potential to support a biological community similar to that of the "best-attainable" condition. Based on its similarity to the expected conditions, the site is classified as *Comparable to Reference*, *Supporting*, *Partially Supporting*, or *Non-Supporting*.

Table 4-1. Rapid Bioassessment Habitat Evaluation Scores

	Condition
Condition / Parameter	
Excellent	
Good	
Fair	
Poor	
Substrate and In-stream Cover	

1. Bottom substrate and available cover
16-20
11-15
6-10
0-5

2. Embeddedness
16-20
11-15
6-10
0-5

3. Flow/Velocity
16-20
11-15
6-10
0-5

Channel Morphology

4. Channel Alteration
12-15
8-11
4-7
0-3

5. Bottom scouring and deposition
12-15
8-11
4-7
0-3

6. Pool/riffle, run/bend ratio
12-15
8-11
4-7
0-3

Riparian and Bank Structure

7. Bank stability
9-10
6-8
3-5
0-2

8. Bank vegetation
9-10
6-8
3-5
0-2

9. Streamside cover
9-10
6-8
3-5
0-2

4.2 QUALITATIVE HABITAT EVALUATION INDEX

4.2.1 QHEI SITE DESCRIPTION

The following sample site information is recorded at the top of the QHEI site description sheet (Fig. 4.2).

1. Stream, River Mile (RM) - Official stream names will be obtained from the Gazetteer of Ohio Streams (Ohio DNR, 1960) for Ohio streams. For streams in other states, stream names will be obtained from USGS 7.5 minute topographic maps. River miles will also be obtained from USGS 7.5 minute topographic maps.

2. Specific Location, Station - When describing the sampling station, proximity of local landmarks such as bridges, roads, discharge outfalls, railroad crossings, parks, tributaries, dams, etc. will be noted. Each sampling site will also be given a station number. This number will sometimes correlate with the river mile at which it is located.

3. Field Sampling Crew, Date - The crew leader will complete the QHEI form and list the field crew on the form, the crew leader will be listed first. The date will also be recorded on the QHEI site description sheet.

4.2.2 PROCEDURES FOR COMPLETING THE QHEI SITE DESCRIPTION SHEET

Below is a description of the six QHEI metrics, their components, and guidelines on scoring each metric. When each metric classification is determined, the corresponding box on the QHEI form is checked. In cases where the habitat is intermediate between two classifications, both boxes are checked and their scores averaged.

4.2.2.1 METRIC 1: SUBSTRATE

Substrate type - Two substrate boxes will be checked on the QHEI form. If one type of substrate comprises at least 75-80% of the stream bottom, it will be checked twice, otherwise the two most common substrate types will be checked. Boxes for artificial substrates are included on the sheets. No more than two boxes will be checked. Although only two boxes will be checked, all substrate types which comprise at least 5% of the site, or occur in sufficient quantity to support species associated with that substrate, will be noted in the spaces provided. If time allows, the percentage of each substrate will be determined. If there are more than four substrate types, each comprising at least 5% of the site, the appropriate box will be checked.

Figure 4.2 EnviroScience QHEI Site Description Sheet

Stream_____ Location_____ RM_____ Date_____
Station #_____ Crew:_____ **QHEI SCORE:**

1] **SUBSTRATE** (Check ONLY two substrate TYPE BOXES; but mark all types present in SPACES) **SUBSTRATE SCORE:**

Type	pool	riffle		pool	riffle		Substrate Quality			
Bldr/Slab(10)	____	____	Bedrock(5)	____	____	*Substrate Origin (Check all)*		*Silt Cover (Check one)*	
Boulder (9)	____	____	Gravel (7)	____	____	Limestone (1)	Hardpan (0)	Silt Heavy (-2)	Silt Moderate(-1)
Cobble (8)	____	____	Sand (6)	____	____	Tills (1)	Rip/Rap (0)	Silt Normal(0)	Silt Free (1)
Hardpan (4)	____	____	Detritus(3)	____	____	Sandstone (0)	Shale (-1)	*Extent of Embeddedness(Check one)*	
Muck (2)	____	____	Artificial(1)	____	____	Coal Fines (-2)		Extensive (-2)	Moderate (-1)

Total number of substrate types: >4 (2) ≤4 (0) Low (0) None (1)
NOTE: (Ignore sludge that originates from point-sources; score is based on natural substrates)
COMMENTS_____

2] **INSTREAM COVER** **COVER SCORE:**

Type (Check all that apply)			Amount (Check ONLY 1 OR check 2 and average)	
Undercut Banks (1)	Deep Pools (2)	Oxbows (1)	Extensive > 75% (11)	Moderate 25-75% (7)
Overhanging Vegetation (1)	Root Wads (1)	Aquatic Macrophytes (1)	Sparse 5-25% (3)	Nearly Absent < 5% (1)
Shallows (in slow water) (1)	Boulders (1)	Logs or Woody Debris (1)		

COMMENTS_____

3] **CHANNEL MORPHOLOGY** (Check ONLY one per category OR check 2 and average) **CHANNEL SCORE:**

Sinuosity	Development	Channelization	Stability	Modifications/Other	
High (4)	Excellent (7)	None (6)	High (3)	Snagging	Impounded
Moderate (3)	Good (5)	Recovered (4)	Moderate (2)	Relocation	Islands
Low (2)	Fair (3)	Recovering (3)	Low (1)	Canopy Removal	Leveed
None (1)	Poor (1)	Recent or no Recovery (1)		Dredging	Bank Shaping
				One Side Channel Modifications	

COMMENTS_____

4] **RIPARIAN ZONE AND BANK EROSION** (check ONLY one per category OR check 2 and average **RIPARIAN SCORE:**

Riparian Width L/R (per bank)	Erosion/Runoff-Floodplain Quality L/R (most predominant per bank)		Bank Erosion L/R (per bank)	
Wide >50m (4)	Forest, Swamp (3)	Urban or Industrial (0)	None or Little (3)	
Moderate 10-50m (3)	Open Pasture/Rowcrop (0)	Shrub or Old Field (2)	Moderate (2)	
Narrow 5-10m (2)	Residential, Park, New Field(1)	Conserv. Tillage (1)	Heavy or Severe (1)	
Very Narrow 1-5m (1)	Fenced Pasture (1)	Mining /Construction (0)	None (0)	

COMMENTS_____

5] **POOL/GLIDE AND RIFFLE/RUN QUALITY** **POOL SCORE:**

Pool/Glide Quality

Maximum Depth (Check 1)	Morphology (Check 1)	Pool/Run.Riffle Current Velocity (Check all that apply)	
> 1m (6)	Pool Width > Riffle Width (2)	Torrential (-1)	Eddies (1)
0.7 - 1m (4)	Pool Width = Riffle Width (1)	Fast (1)	Interstitial (-1)
0.4 - 0.7m (2)	Pool Width < Riffle Width (0)	Moderate (1)	Intermittent (-2)
< 0.4m (1)		Slow (1)	NO POOL (0)
< 0.2m (pool = 0)			

COMMENTS_____

Riffle/Run Quality **RIFFLE SCORE:**

Riffle/Run Depth	Riffle/Run Substrate	Riffle/Run Embeddedness		
Generally > 10 cm, Maximum > 50 cm (4)	Stable (e.g., Cobble, Boulder) (2)	Extensive (-1)	Moderate (0)	
Generally > 10 cm, Maximum < 50 cm (3)	Moderately Stable (e.g., Pea Gravel) (1)	Low (1)	None (2)	
Generally 5-10 cm (1)	Unstable (Gravel, Sand) (0)	NO RIFFLE (0)		
Generally < 5 cm (Riffle = 0)				

COMMENTS_____

6] **GRADIENT** **GRADIENT SCORE:**
Feet/Mile:_____ %Pool:_____ %Riffle_____ %Run_____

Figure 4.2 QHEI SHEET (continued)

Is reach representative of stream? (Y/N)_____.

If not, why?_____

_____

Additional comments/Pollution impacts:_____

_____

_____

	Gear	Distance	Water Clarity	Water Stage		
First Pass	_____	_____	_____	_____	Subjective Rating (1 - 10)	Aesthetic Rating (1 - 10)
Second Pass	_____	_____	_____	_____		
Third Pass	_____	_____	_____	_____		

Canopy (% open):_____ Gradient: Low Moderate High

Photos:_____

Stream measurements: Average Width:_____ Average Depth:_____ Maximum Depth:_____

Length	Width	Depths>>>				Pool/Glide/ Riffle/Run					

Cross Sections of Stream

Drawing of Stream

Substrate quality - The origin of the substrate refers to the "parent" material the substrate is derived from. All substrate origins present will be noted. If >50% of the substrate surface is surrounded, impacted in, or covered by sand and/or silt, the substrate is considered embedded. While naturally sandy streams are not considered embedded, streams in which the original substrate has been buried by sand due to anthropogenic activities are considered embedded. If the substrate is considered embedded, the appropriate box will be marked. The extensiveness of embedded substrates within the sampling zone will also be noted and are characterized as follows: Extensive (75% of site area), Moderate (50-75%), Sparse (25-50%), Low (< 25%).

The extent of silt cover will be recorded. In areas where a 1 inch layer of silt covers nearly the entire stream bottom, the silt cover is considered Heavy. If there is an extensive covering of silt with a few areas of cleaner substrates, such as in riffles, the silt cover is considered Moderate. When small amounts of silt are present the silt cover is considered Normal. The Silt Free box will be checked if the substrates are exceptionally clean.

Substrate types:

1. Bedrock - solid rock forming a continuous surface.
2. Boulder - rounded stones over 256 mm in diameter (10 in.) or large "slabs" more than 256 mm in length (boulder slabs).
3. Cobble - stones from 64-256 mm (2 ½ - 10 in.) in diameter.
4. Gravel - mixture of rounded course material from 2-64 mm (1/12 - 2 ½ in.) in diameter.
5. Sand - materials 0.06 - 2.0 mm in diameter; gritty texture when rubbed between fingers.
6. Silt - 0.004 - 0.06 mm in diameter, generally this is fine material which feels "greasy" when rubbed between fingers.
7. Hardpan - particles less than 0.004 mm in diameter, usually clay, which forms dense, gummy surface that is difficult to penetrate.
8. Marl - calcium carbonate ; usually grayish-white; often contains fragments of mollusk shells.
9. Detritus - dead, unconsolidated organic material covering the bottom; this may include sticks, wood and other partially decayed course plant material.
10. Muck - black, fine, flocculent, completely decomposed organic matter (does not include sewage sludge).
11. Artificial - substrates such as rock baskets, gabions, bricks, trash, concrete etc.; placed in the stream for reasons OTHER than habitat mitigation.

The substrate score is based on the underlying material, therefore, sludge which originates from point sources is not included. Sludge is defined as a thick layer of organic matter of human or animal origin.

Substrate metric score: The theoretical maximum score is > 20 points; however, the maximum score allowed for the QHEI metric score is 20 points

4.2.2.2 METRIC 2: IN-STREAM COVER

The in-stream cover metric includes both the cover type and cover amount. Each cover type representing at least 5% of the sampling area, or present in sufficient amount to support species commonly found in that habitat, should be marked. Cover which is located in shallow areas of the stream (< 20 cm) is generally not included due to its limited utility. Some examples of cover providing little use in shallow areas are log-jams, boulders, undercut banks, overhanging vegetation, and rootwads.

Stream cover is considered Extensive if it is present throughout the sampling area (> 75% of site). If cover is found in 25-75% of the area it is considered Moderate. When less than 25% of the sampling area has cover, it is considered Sparse. Cover is considered nearly absent when it is present in less than 5%, this usually occurs in recently channelized or other modified streams. If the cover amount is intermediate between two of the above categories, two boxes are checked and the average is taken. The cover types are:

1. undercut banks
2. overhanging vegetation
3. shallows (in slow water)
4. logs or woody debris
5. deep pools (> 70 cm)
6. oxbows
7. boulders
8. aquatic macrophytes
9. rootwads (tree roots that extend into stream)

If rootwads are prominent as a major component, they should be checked along with undercut banks. However, if they are only a small component of undercut banks, only undercut banks will be checked.

Cover Metric Score: The theoretical maximum score is > 20 points; however, the maximum in-stream cover metric score is limited to 20 points.

4.2.2.3 METRIC 3: CHANNEL MORPHOLOGY

The channel morphology metric represents the quality of the stream channel and therefore, relates to the stability and diversity of the macrohabitat. Channelization, channel development, channel stability, and channel sinuosity (the degree to which the stream meanders) are all considered in this metric.

1. Sinuosity - A straight channel is considered to have no sinuosity. A channel with only one or two slight, outside bends within the sampling area is considered low sinuosity. A sampling reach with more than two outside bends, one of which is well defined, has a moderate sinuosity. If the sampling area has more than two or three well defined bends with deep areas on the outside bend and shallow areas on the inside bend, the sinuosity is high. Sinuosity can be conceptually described as the ratio of the stream distance between two points on the channel of a stream and the straight-line distance between these same two points, taken from a topographic map. Only one box will be checked.

2. Development - This metric evaluates the amount of riffle/pool complexes. If no riffles or pools are present or if the riffles and pools present are shallow with the riffle bottoms composed of sand or fine gravel, the development is Poor. The development is also Poor if glide habitats are prominent. If riffles are absent or poorly developed, but pools are well developed with variation of depth, the development is Fair. If there is a distinct transition between riffles and pools, pools having variation of depth, and riffles with larger substrates such as gravel, rubble or boulders present, the development is Good. When the development of the sampling area is similar to the Good category and the area contains deep riffles and runs (> 0.5 m) and pools with maximum depths >1 m, the development is considered Excellent. In streams sampled with wading methods, a sequence of riffles, runs, and pools must occur more than once in a sampling zone in order for the development to be considered Excellent. Only one box will be checked.

3. Channelization - This metric refers to anthropogenic channel modifications. If an area has been channelized in the past, but has recovered the majority of its natural channel characteristics, it is classified as Recovered. If the area is presently regaining some of its natural characteristics, it is considered Recovering. Examples of recovering habitats are those found in the Huron/Erie Lake Plain ecoregion (NW Ohio), where channelization occurred long ago, and mature trees make up the riparian border, but poor channel characteristics are still present. In areas where recent channelization has occurred or no recovery is apparent, the box for Recent or No Recovery is checked. Examples of areas with no recovery are drainage ditches and grass lined or rip-rapped banks. The specific type of habitat modification will be noted on the QHEI form, although no score is given.

4. Stability - Channels where the banks and substrate are stable and there is little or no erosion or bedload are given a High score. Stream channels which are artificially stable, such as concrete, are also given a High score. Although these streams

generally have a negative influence on fish, it is for reasons other than stability. Channels which have stable riffle/pool characteristics, but sometimes exhibit effects of instability such as high bedloads, eroding or false banks, or wide fluctuations in water level, are considered Moderate in stability. Channels which have severely eroding banks, high bedloads which creep downstream, or fine substrates in riffles which change location all have a Low stability.

5. Modifications/Other - Modifications and sources of such modifications are marked on the QHEI form but are not scored.
   Channel Morphology Metric Score: Generally only one box is checked for each category, however, with some sampling areas, conditions may be intermediate between two categories. In these cases both boxes are checked and the average is taken. The maximum score for the channel morphology metric is 20 points.

4.2.2.4 METRIC 4: RIPARIAN ZONE AND BANK EROSION

With this metric, the quality of both the riparian buffer zone and the floodplain vegetation is considered. Both the left and right banks (looking downstream) are observed and given scores for width of the floodplain, floodplain quality, and bank erosion.

1. Width of the Floodplain - Floodplain width refers to the width of the riparian (stream side)
   vegetation. This width is estimated for forest, shrub, swamp, and old field vegetation. Old field is a fairly mature successional field with stable, woody plant growth. Weedy urban and industrial lots are not included because of their high runoff potential. Width estimates are made for both the right and left banks and average of these two scores is then calculated.

2. Floodplain Quality - This refers to the area immediately outside of the riparian zone or greater than 100 feet from the stream, whichever is wider. The floodplains are categorized as urban, residential, pasture, construction, etc. and floodplain quality types are determined for both the left and right banks. The two predominant floodplain types (one for each bank) should be checked and then averaged. The floodplain area is not limited to the riparian zone but is much less encompassing than the stream basin. The floodplain can have direct erosional and runoff effects during normal wet weather.

3. Bank Erosion - Below is a list of Streambank Soil Alteration Ratings from Platts et al. (1983). In this scoring, false banks are considered those that are no longer adjacent to the normal flow of the channel but have been moved back into the floodplain most commonly due to livestock. One box will be checked for each stream-side and an average calculated.

a. None - This rating includes stable streambanks which are not being altered by water flows or animals (e.g. livestock) (Score 3).

b. Little - This rating includes stable streambanks which are slightly altered along the transect line. Streams are considered to have little bank erosion if less than 25% of the streambank is receiving any stress and any stress being received is light. If less than 25% of the streambank is false, broken down or eroded the bank erosion is also considered to be little (Score 3).

c. Moderate - Bank erosion is considered moderate when at least 50% of the streambank is in stable condition and 25- 50% of the streambank is false, broken down or eroded (Score 2).

d. Heavy - Bank erosion is considered heavy if less than 50% of the streambank is in a stable condition and over 50% of the streambank is false, broken down or eroded (Score 1).

e. Severe - Bank erosion is considered severe when less than 25% of the streambank is in stable condition and over 75% of the streambank is false, broken down, or eroded (Score 1).

Riparian Zone and Bank Erosion Score: Generally, one box for each bank should be checked, however, if conditions are considered intermediate between two categories, two boxes can be checked and an average taken. Scores should be given for each of the three categories for each bank. Scores for the left and right banks are averaged for one score per category per sampling area. The maximum score for this metric is 10 points.

4.2.2.5 METRIC 5: POOL-GLIDE AND RIFFLE-RUN QUALITY

This metric measures the quality of pool, glide and/or riffle-run habitats. Pool depth and morphology, overall diversity of current velocities (in pools and riffles), riffle-run depth, and substrate type and quality are all included in this metric.

4.2.2.5.1 Pool-Glide Quality

1. Maximum depth of pool or glide- Only one box is checked (Score 0 to 6). If the maximum depth of a pool or glide is less than 20 cm, it is considered to have lost its function and a score of 0 is given. In this case, no other characteristics need to be scored.

2. Morphology - If pools are wider than riffles, the "wide" box is checked. If pools and riffles are the same width the "equal" box is checked. If riffles are wider than pools, the "narrow" box is checked (Score 0 to 2). If the entire stream (including areas outside the sampling zone) is pool or riffle, check riffle = pool.

3. Current Types - Each current type present in the stream (including riffles and runs) should be checked (score -2 to 4). Definitions of current types are found below:

a. Torrential - These currents are very turbulent and fast flowing with large standing waves and a very broken, unconnected water surface. They are usually limited to gorges and dam spillway tailwaters.

b. Fast - Fast currents are generally non-turbulent with small standing waves in riffle/run areas. The water surface may be partially broken but the surface is visibly connected.

c. Moderate - These currents are non-turbulent and water surface is connected. The flow is detectable and visible so that floating objects are readily transported downstream.

d. Slow - With slow currents, water flow is sluggish but still perceptible.

e. Eddies - These are small areas with a circular current motion. They are usually formed in pools immediately downstream from riffle / run areas.

f. Interstitial - Interstitial currents are perceptible only in the interstitial spaces between substrate particles in riffle / run areas.

g. Intermittent - Intermittent areas have no evident flow. These areas are comprised of pools separated by dry areas.

Pool-Glide quality score: The theoretical maximum score for pool quality is > 12; however, the maximum score assigned for this metric is limited to 12 points.

4.2.2.5.2 Riffle / Run Quality

If no riffles are present within the site, score 0 for this metric.

1. Riffle / Run Depth - Select the box which most closely describes the depth of the riffle (score 0 to 4). If the depth is less than 5 cm, the riffle is considered to have lost its function and the entire riffle metric scores a 0.

2. Riffle / Run Substrate Stability - Select the box that best describes the substrate type and stability of the riffle habitats (Score 0 to 2).

3. Riffle /Run Embeddedness - This category represents the degree to which cobble, gravel, and boulder substrates are surrounded or covered by sand or silt. Substrates are considered embedded if > 50% of the substrate surface is embedded (these substrates can not be easily dislodged). Substrates which are concreted are also included. If > 75% of the riffle area of the sampling zone has embedded substrates, the Extensive box should be checked. Moderate should be checked if 50-75% of the area has embedded substrates, Sparse should be checked if 25-50% has embedded substrates, and Low should be checked if < 25% of the area contains embedded substrates.

Riffle-Run quality score: The maximum score for riffle run quality is 8 points.

4.2.2.6 METRIC 6: MAP GRADIENT

The map gradient is calculated from USGS 7.5 minute topographic maps by measuring the elevation drop through the sampling area. This is done by measuring the stream length between the first contour line upstream and the first contour line downstream of the sampling site and dividing the distance by the contour interval. If the contour lines are closely " packed", a minimum distance of at least one mile should be used. Some judgement may need to be exercised in certain anomalous areas (e.g. in the vicinity of waterfalls, impounded areas, etc.) and this can be compared to an in-field, visual estimate which is recorded on the back of the habitat sheet.

Scoring for ranges of stream gradient takes into account the varying influence of gradient with stream size, preferably measured as drainage area in square miles or stream width. Gradient classifications (Table 4-2) were modified from Trautman (p. 139, 1981) and scores were assigned, by stream size category, after examining scatter plots of IBI vs. natural log of gradient in feet/mile.

The maximum QHEI metric score for this metric is 10 points.

See Table 4-3 to compute the total QHEI score.

4.2.2.7 PROCEDURES FOR RECORDING ADDITIONAL INFORMATION

Additional information is recorded on the reverse side of the QHEI Site Description Sheet (Fig. 4-2) and is described as follows:

1. Additional Comments / Pollution Impacts - Different types of pollution sources (e.g. wastewater treatment plant, feedlot, industrial discharge, non-point source inputs) are noted with their proximity (in 0.1 mile increments) to the sampling site. Any evidence of litter either in-stream or on the stream bank is also noted.

2. Sampling Gear / Distance Sampled - The type of fish sampling gear used during each pass is specified and any variation in sampling procedures is noted (e.g., sampler type A specifies sampling along one shoreline of 0.5 km, but due to local restrictions, sampling may be performed on both shorelines to accumulate 0.5 km). The total sampling distance in kilometers for each sampling site for each pass is recorded.

3. Water Clarity - the following descriptions can be used as a guide:

    a. Clear - bottom is clearly visible (if shallow enough) and the water contains no apparent color or staining.

Table 4-2. Classification of Stream Gradients for Ohio, corrected for stream size. Modified from Trautman (p. 139, 1981).

Average Stream Width (m)	Drainage Area (sq mi)	Gradient (ft/mile) Very Low	Low	Low-Moderate	Moderate	Moderate-High	High	Very High	
0.3-4.7	0-9.2	0-1.0	1.1-5.0	5.1-10.0	10.1-15.0	15.1-20	20.1-30	30.1-40	
		2	4	6	8	10	10	10	
4.8-9.2	9.2-41.6	0-1.0	1.1 - 3.0	3.1-6.0	6.1-12.0	12.1-18.0	18.0-30	30.1-40	
		2	4	6	10	10	8	6	
9.2-13.8	41.6-103.7	0-1.0	1.1 - 2.5	2.6-5.0	5.1-7.5	7.6-12.0	12-20	20.1-30	
		2	4	6	8	10	8	6	
13.9 30.6	103.7-622.9	0-1.0	1.1 - 2.0	2.1-4.0	4.1-6.0	6.1-10.0	10.1-15	15.1-20	
		4	6	8	10	10	8	6	

> 30.6 > 622.9	- 6	0 - 0.5 8	10	0.6-1.0 10	10	1.1-2.5 8	2.6-4.0	4.1-9.0	> 9.0

* Any site with a gradient > than the upper bound of the "very high" gradient classification is assigned a score of 4.

**Taken from OEPA (1987)

Table 4-3. Computing the Total QHEI Score: The components for each metric are summed to obtain the individual metric scores. The QHEI metric scores can not exceed the metric maximum score. These metric scores are then summed to obtain the total QHEI score.

QHEI Metric	Metric Component	Component Scoring range	Metric Max. Score
1) Substrate	a) Type	0 to 21	20
	b) Quality	-5 to 3	
2) In-stream Cover	a) Type	0 to 10	20
	b) Amount	1 to 11	
3) Channel Morphology	a) Sinuosity	1 to 4	20
	b) Development	1 to 7	
	c) Channelization	1 to 6	
	d) Stability	1 to 3	
4) Riparian Zone	a) Width	0 to 4	10
	b) Quality	0 to 3	
	c) Bank Erosion	1 to 3	
5a) Pool Quality	a) Max. Depth	0 to 6	12
	b) Current	2 to 4	
	c) Morphology	0 to 2	
5b) Riffle Quality	a) Depth	0 to 4	8
	b) Substrate Stability	0 to 2	
	c) Substrate Embdnss	-1 to 2	
6) Gradient		2 to 15	10
Total Maximum Score			100

b. Stained - usually a brownish (or other) color to the water; the bottom may be visible in shallow areas.

c. Turbid - bottom seldom visible at more than a few inches; caused by suspended sediment particles.

The apparent source of staining (e.g. tannic acid, leaf decay, etc.) and turbidity (e.g. runoff (clay / silt), algae / diatoms, sewage, etc.) may be specified under additional comments.

4. Water Stage - This is the general water level of the stream during each pass; suggested descriptors are:

a. flood
b. high
c. elevated
d. normal
e. low
f. interstitial

(Note: sampling should not be conducted during flood or high flows).

5. Canopy - This is the percentage of the sampling site that is not covered or shaded by woody bank vegetation. In wide streams and rivers this determination should be made along both sides of the river or stream (i.e., the percent of the sampling path that is open).

6. Gradient - Check the box that best describes the gradient at the site. This will be used to check the accuracy of gradients taken from topographic maps.

7. Field Crew - The names of all individuals involved with the sampling / site description at each site are included.

8. Photographs - The number of each photograph taken is recorded and the subject of the photograph is briefly described.

9. Stream measurements (optional) - When measuring the individual sampling sites, length, width, and average and maximum depth information will be recorded. Each measurement will be recorded as either a riffle, run, pool, or glide by placing an X in the correct box to the right of the measurements recorded; see the introduction for definitions of riffles, runs, etc.

The number of width measurements is left to the discretion of the field crew leader. Short riffles may require only one or two width measurements while long pools will probably require more depending on the degree of variation that exists in the stream's width. Depth measurements should be made in association with individual width measurements. Depths should be taken at the steam margins and various points across the stream. Up to nine depth measurements may

be taken depending on the variability in the stream bottom. Maximum depth is the deepest spot in the stream section sampled. One purpose of this information is to calculate pool volume.

10. Stream Diagram - Cross-sections: Two or three cross-sections of the stream are drawn to provide information on features of the stream bank, steam bottom, stream channel, and floodplain. Definitions of these terms follow Platts et al. (1983):

a. Channel - The cross-section containing the stream that is distinct from the surrounding area due to breaks in the general slope of the land, lack of terrestrial vegetation, and changes in the composition of the substrate materials. The channel is made up of streambanks and stream bottoms.

b. Banks - The portion of the channel cross-section that tends to restrict lateral movement of water. The banks often have a slope steeper than 45° and exhibit a distinct break in slope from the stream bottom. Also, an obvious change in substrate materials is a reliable delineation of the bank.

c. Stream bottom -The portion of the channel cross-section not classified as bank. The bottom is usually composed of stream sediments or water transported debris and may be covered by rooted or clinging aquatic vegetation. In some geologic formations, the stream bottom may consist of bedrock rather than sediments.

d. Flood plain - The area adjacent to the channel that is seasonally submerged under water. Usually the floodplain is a low area covered by various types of riparian vegetation.

e. Stream Map - The entire sampling zone should be drawn. Physical features, sampling path description, and any other pertinent information should be included on this map.

REFERENCES

Ohio Environmental Protection Agency. 1987. Users Manual for Biological Field Assessment of Ohio Rivers and Streams. Div. of Water Quality Monitoring and Assessment, Columbus.

Ohio Environmental Protection Agency. 2006. Manual of Ohio EPA Surveillance Methods and Quality Assurance Practices Volume I. Div. of Environmental Services, Columbus.

Ohio Department of Natural Resources. 1960. Gazetteer of Ohio Streams. Ohio DNR, Div. of Water, Columbus, Ohio. Ohio Water Plan Inventory Rept. No. 12. 179 pp.

Platts, W.S.; Megahan, W.F.; and G.W. Minshall. 1983. Methods for Evaluating Stream, Riparian, and Biotic Conditions. General Technical Report INT-138. Odgen, Utah. U.S. Dept. of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 70 pp.

Rankin, E.T. 1989. The Qualitative Habitat Evaluation Index (QHEI): Rationale, Methods, and Application. Ohio EPA, Div. Water Qual. Planning and Assessment, Columbus, Ohio.

Trautman, M.B. 1981. The Fishes of Ohio. (2nd edition). Ohio State Univ. Press, Columbus. 782 pp.

APPENDIX C

STANDARD OPERATING PROCEDURES FOR MACROINVERTEBRATE SAMPLING AND DATA ANALYSIS

ENVIROSCIENCE, INC.

Standard Operating Procedures Document:

Benthic Macroinvertebrate Collection Methods
Benthic Macroinvertebrate Sample and Laboratory Processing
Benthic Macroinvertebrate Data Analysis

1.0 PURPOSE

To provide standard operating procedures (SOP's) for the collection and laboratory practices used in an aquatic survey of benthic macroinvertebrates.

2.0 SCOPE

These SOP's apply to using benthic macroinvertebrates for evaluating the biological integrity of surface waters. Information and sampling procedures for the modified Hester-Dendy Artificial Substrate Sampler, the Surber Sampler, the Ponar Sampler and the Kick net are described in the text. Included are general information on each of the sampling methods and procedures for field data collection, labeling and record keeping, sample preservation, sample collection, processing and sample sorting, subsampling, organism identification, and data analysis. Procedures in this text are modified from and strictly adhere to those of the Ohio and United States Environmental Protection Agencies (OEPA 1987, Barbour et al. 1999).

3.0 GENERAL INFORMATION

3.1 Modified Hester Dendy Sampler

Habitats and Substrates Sampled: All types of habitats in rivers, streams, lakes and reservoirs; not efficient in wetlands; uses hardboard substrate.

Effectiveness of the Device: Colonization depends on substrate; selective for certain types of organisms; three to five replicates

Advantages: Provides a quantitative estimate of the community; excellent for water quality monitoring; uniform substrate type; high level of precision; samples contain negligible amount of debris; provides habitats of known area for a known time at a known depth.

Limitations: Requires three site visits for installation, check, and collection; subject to vandalism; biased for aquatic insects which have the ability to attach; may require additional weight for stability; six week wait for colonization results.

3.2 Surber Sampler

Habitats and Substrates Sampled:	Shallow, flowing streams less than 32 cm. in depth with good current; cobble substrate, mud, sand and gravel.
Effectiveness of Device:	Relatively quantitative when used by an experienced biologist; performance depends on current and substrate.
Advantages:	Encloses area sampled; easily transported or constructed; samples a unit area.
Limitations:	Difficult to set in some substrate types (i.e. large streams; cannot be used in deep waterways.

3.3 Ponar Sampler

Habitats and Substrates Sampled:	Freshwater lakes, rivers, estuaries and reservoirs with hard and soft sediments such as clay, hard pan, sand, gravel and muck; best for use in deep waters
Effectiveness of Device:	Not entirely adequate for hard, bedrock substrates; very efficient for deep burrowing organisms in soft sediments; collects both qualitative and quantitative samples.
Advantages:	Better penetration than other grabs; side plates and screens reduce washout, shock waves and disturbances; best quantitative grab sampler for deep freshwater use. substrate
Limitations:	A very heavy grab may require use of a boat with a winch and cable; stones, pebbles and other debris can lodge jaws open causing loss of sample.

3.4 Kick Net Sampling

Habitats and Substrates Sampled: Shallow, flowing streams of approximately 1 m in depth with good current; cobble substrate, mud, sand and gravel.

Effectiveness of Device: Qualitative; performance depends on current, substrate and sampler.

Advantages: Easily transported; samples each habitat representative in the study area.

Limitations: Difficult to set in some substrate types (i.e. large moving streams; cannot be used in deep waterways.

4.0 PROCEDURES FOR FIELD DATA COLLECTION

A description of the physical habitats within the stream reach being studied provides essential interpretive information. A written site description and schematic drawing of the stream describe the aquatic habitats and help locate specific stream features. If necessary, black and white photographs are taken to readily illustrate the stream and its riparian corridor. These photographs are taken from several perspectives in order to accurately portray the entire project area. The following features of the stream and its riparian corridor are recorded during an aquatic survey.

1. Site Specific Information Stream Name, Project Identification, Date, Weather Conditions, Locality, Drainage, Field Personnel, and Reference Map

2. Stream Flow Characteristics Flow, Flow Regime, Stream Gradient, Current Velocity

3. In-stream Habitat Characteristics Stream Morphology (emphasizing widths, lengths, and depths of all habitat types), Substrate Types, In-stream Cover, Visible Pollution, In-stream Vegetation

4. Riparian Habitat Characteristics Land Use Immediately Adjacent To The Stream, Livestock Access To The Stream

This information is recorded on EnviroScience Stream Data Sheets (Figure 4.1).

Figure 4.1 Stream Data Sheets (Front)

Project:

Deployment Date:_____ **Scheduled Collection Date:**____
Stream /River: _____ **Locality:**_____
Picture #:_____ **G.P.S. Reading:**_____
Station #: _____ **Biologist:**_____

A) Substrate

Substrate	Riffle	Run	Pool
% Detritus			
% Clay			
% Silt			
% Sand			
% Gravel			
% Cobble			
% Boulders			
% Bedrock			

B) Pollution: 1) Type:
2) Source:

C) Water Chemistry

Chemistry Parameter	Deployment	Retrieval
Clarity		
Water Temp.		
Air Temp.		
Conductivity		
pH		
Dissolved Oxygen		
Flow		

Figure 4.1 Stream Data Sheets (Back)

D) Habitat Analysis:

1) Riparian Zone

2) Land Use (Circle All that apply):

a) Industrial b) Urban c) Residential d) Pasture
e) Forest g) Oil-Field h) Other

3) Livestock (Circle One):

a) Present b) Absent

4) Stream Channel (Circle All that apply):

a) Natural b) Channelized c) Recovering
d) Leveed e) Impounded f) Backwater

5) Stream Morphology (% Stream Area):

% Riffle	
% Run	
% Pool	

6) Instream Cover (% Stream Area):

% Undercut Banks	
% Boulders	
% Logs	
% Vegetation	
% Overhanging Brush	

7) Qualitative Sampling (Circle One): Yes No

a) If Yes, which type: Ponar Kick

8) Date of Collection (__/__/__)

9) Site Description (Remarks and Notes):

5.0 PROCEDURES FOR SAMPLE COLLECTION

5.1 Procedures for Sampling with a modified Hester Dendy Sampler

1. For maximum abundance and diversity of macroinvertebrates, artificial substrate samplers are positioned in the euphotic zone (one to two feet below the water surface), or on the substrate at shallow water stations. Where present, samplers are ideally placed at the head of a recovery pool immediately below riffle-type habitat. Samplers are placed on eight inch cement blocks and anchored to the substrate to avoid loss during floods. Each sampler is constructed of 1/8 inch tempered hardboard cut into eight three-inch square plates, separated by twelve one inch square spacers. The plates and spacers are placed on a 1/4 inch eyebolt with three single spaces, three double spaces, and one triple space between the plates. The total surface area of the sampler, excluding the eyebolt, is 145.6 square inches. Care was taken to place the samplers at an adequate depth so they would remain submerged during periods of low flow. Also, samplers are placed to not allow a Hester-Dendy to touch the stream bottom, which will permit siltation and subsequent loss of sample. At least three to five samplers are installed at each collecting site. Two sets of Hester-Dendy samplers are installed at each site to provide a backup set in case one set is lost (i.e. flood, vandalism, excess siltation, or emerged due to low flow); however, only one set from each site is processed. The samplers are left in place for at least six weeks to allow for organism colonization. The exposure time is consistent among sites during the study. If study time limitations reduce this period, the data is evaluated with caution and the appropriate notation.

2. The samples are made as representative as possible by placing the samplers in similar habitats at each sampling station (e.g. riffle/run areas). In impounded and low flow areas the samplers are deployed where the highest flow reading can be recorded. In streams up to a few meters in width, the samplers are installed at approximately midstream. In larger streams, the samplers are installed at approximately one-quarter of the total width from the nearest bank.

3. Loss of macroinvertebrates is minimized when retrieving multi-plate samplers by approaching from downstream, cutting them from the block and placing them in polyethylene containers while still submersed. Care is taken to avoid disturbing the samplers and attached organisms. Formalin is added to the containers until there is approximately 10-15% in solution.

5.2 Procedures for Sampling with a Surber Sampler

1. The sampler is positioned securely on the substrate, parallel to the flow of the water, with the net pointing downstream. The sampler is put in place quickly to reduce the escape of rapidly moving organisms.

2. Precautions are made to make sure there are no gaps under the edges of the frame that would allow for washing of water under the net and loss of benthic organisms. Gaps that may occur along the edge of the sampler are eliminated by careful shifting of substrate along the outside edge of the sampler. Care is taken not to disturb the substrate upstream from the sample site.

3. Once the sampler is in position on the stream bottom, it is held there during sampling so the area delineated remains constant. All rocks in the sampling area are carefully turned over and rubbed or brushed in front of the net with the hands or a soft brush to dislodge the organisms that are clinging to them. At times it is necessary to hand pick some of the heavier mussels and snails that are not carried into the net by the current.

4. Remaining gravel and sand are stirred vigorously with the hands to a depth of 10 cm (4.0 inches) to dislodge bottom-dwelling organisms.

5. The sample is removed by inverting the net into a previously rinsed bucket containing ambient stream water. The bucket is poured and rinsed into a No. 30 sieve which is then partially submerged in water and agitated until all fine materials have passed through. This is done to reduce sample volume and expedite sample processing in the laboratory. All observed organisms and debris are removed with forceps and placed into a polyethylene container containing 70-80% ethanol. The net is carefully examined for any organisms that are clinging to it. These organisms are also removed with forceps for inclusion with the sample. The net and bucket are rinsed between samples.

5.3 Procedures for Sampling with a Ponar Sampler

1. The standard version of the Ponar grab sampler is used to obtain qualitative samples. It is attached to a winch and cable that is mounted to the stern of EnviroScience's sampling boat. The Ponar is lowered to the substrate of the waterway and immediately retrieved upon closure of the mechanism. The lowering of the sampler occurs by slowing releasing the winch at a constant rate to avoid premature closure of the mechanism. Retrieval occurs by cranking the winch back up until the Ponar grab sampler is visible just below the surface of the water.

2. A large plastic tub is placed beneath the Ponar grab sampler as it is raised above the surface of the water. This is performed in order to retrieve any macroinvertebrates which may exit the sampler when it is raised above the water.

3. Together, the tub and the Ponar grab sampler are maneuvered onto the boat and lowered to the floor. Once secure, the sampler is opened and all contents are rinsed into the tub. The tub is poured and rinsed into a floatable No. 30 sieve which is then partially submerged in water and agitated until all fine materials have passed through. All organisms and debris are rinsed or removed with forceps and placed into a polyethylene container containing 70-80% ethanol.

5.4 Procedures for Sampling with use of the Kick Net

1. The D-frame kick net fitted with U.S. No. 30 mesh is positioned securely on the substrate, with the frame perpendicular to the flow of water and the net pointing downstream. It is put in place quickly to reduce the escape of rapidly moving organisms.

2. Once the kick net is in position on the stream bottom, an area directly upstream of the net opening is vigorously kicked to loosen the natural substrates. Any rocks are rubbed or brushed in front of the net to dislodge organisms and pupal cases clinging to them. Stream current carries the loosened substrate and debris into the mouth of the net. Some of the heavier mussels and snails that are not carried into the net must be hand picked. Collections are made for a minimum of thirty (30) minutes and continued until no new taxa are evident in gross examination. All available substrate types are sampled within a sample area.

3. The sample is removed by inverting the net into a previously rinsed bucket containing ambient stream water. The bucket is poured and rinsed into a sieve which is then partially submerged in water and agitated until all fine materials have passed through. This is done to reduce sample volume and expedite sample processing in the laboratory. All observed organisms and debris are rinsed or removed with forceps and placed into a polyethylene container containing 70-80% ethanol formalin. The net is carefully examined for any organisms that are clinging to it. These organisms are also removed with forceps for inclusion with the sample. The net and bucket are rinsed between samples.

6.0 PROCEDURES FOR LABELING AND RECORD KEEPING

1. All samples are labeled in the field at the time of collection with the location, type of sample, date, and sampler's initials. Labels are made on water-resistant paper and placed inside the sample container. A lead based soft pencil or indelible ink is used to protect against bleeding or discoloration of the sample preservative. The outside of each container and lid are also labeled with the same information as the paper labels and the field notebook. The field notebook should include the sampling method, weather, substrate characteristics, depth, name of client, and any other physical or environmental conditions noted.

2. After returning to the laboratory, each sample is assigned a unique sequential identification (ID) number. This number identifies the sample in a permanent ledger where the information from the field notebook and chain of custody forms is recorded. The chain of custody, sample ID number, and the ledger document the transfer of the sample from the field to the laboratory. The sample ID number is also placed prominently on the sample container before storing, and ultimately on all specimen vials, microscope slides and other items connected with the sample.

The preceding procedures for labeling and record keeping are used with all sampling methods.

7.0 PROCEDURES FOR SAMPLE PRESERVATION

1. In the field, soft bodied organisms, which include specimens of leeches, aquatic oligochaetes and other macroinvertebrates, are placed in polyethylene jugs containing 70-80% ethanol. This aids in preserving and sustaining body parts that are important in identification and prepares the sample for sorting.

2. If sorting does not occur within two weeks upon arrival to the laboratory, the original 70-80% ethanol is carefully poured off and replaced with new 70-80% ethanol.

3. All specimens which are permanently preserved are done so by placing into glass vials containing 70-80% ethanol and 5% glycerin. These vials are tightly fitted with rubber stoppers or screw caps. Teflon tape is used to create a better seal on the screw-cap vials.

The preceding procedures for sample preservation are performed with all sampling methods.

8.0 PROCEDURES FOR IN-HOUSE PROCESSING AND SAMPLE SORTING

8.1 Hester Dendy Samplers

1. Multiplate samplers are taken to the laboratory where the plates are composited, placed into a sample tray of water and dismantled. The individual tiles are scrubbed with a soft brush and carefully rinsed into the tray. The tiles are visually inspected for clinging organisms, and the water in the tray is washed through a No. 30 sieve placed on top of a No. 40 sieve. The sieves may be held under gently running water to wash debris into one end of the sieve for transfer into a sample jar containing 70% ethanol, or directly into sorting tray. After each sample is sorted, notations are made in the sample log book, including the date and the initials of the sorter. Samples are checked by the lab supervisor to confirm that organisms are not overlooked.

8.1.2 Surber, Ponar and Kick net Samples

1. Organisms and debris obtained from Surber, Ponar and Kick net samples are removed from the respective polyethylene container and rinsed into a No. 30 sieve. The material may then be gently washed under running water and placed into a sorting tray.

2. After each sample is sorted, notations are made in the sample log book, including the date and the initials of the sorter. Samples are checked by the lab supervisor to confirm that organisms are not overlooked.

8.2 Procedures for Subsampling and Sorting Samples

1. Once a sample has been rinsed it can be transferred to a sorting tray and distributed homogenously with just enough liquid remaining to keep the sample moist. The pan is divided into 2in X 2in grids that are numbered to aid in carrying out subsampling when necessary.

2. The material is then portioned out into a Petri dish and carefully inspected under a dissection microscope for organisms. When this material has been fully examined it is placed into a separate container and labeled as "sorted portion." The entire sample is examined in a similar fashion until it is completely sorted.

3. Subsampling techniques are used when the number of individuals from a specific group (Order) is expected to exceed a minimum standard recommended by the Ohio EPA. At least 70 mayfly, 70 caddisfly, and 100 chironomid larvae must be collected before subsampling techniques are initiated.

4. Subsampling is completed by random extraction of organisms from the sample until adequate numbers are counted. Remaining organisms are extrapolated and recorded to obtain a relative number for the sample using the following equation:

Number of organisms in sorted portion x Number of grids in the subsampler = approx. number of organisms in sample

5. If the calculated number of organisms in the whole sample is greater than 100 organisms over the target count (e.g., 350 organisms for a 250 target count), then material can be subsampled to save sorting effort. Subsampling should continue by random selection of grids to be sorted until the target number of organisms is met. If subsampling is performed, a brief (~5 min) search for large or rare taxa should be completed of the unsorted portion. These organisms should be placed in a separate vial and clearly labeld "Large/Rare taxa."

9.0 PROCEDURES FOR ORGANISM IDENTIFICATION

1. Members of the Dipteran family Chironomidae (midges) are cleared by mounting on a microscope slide in CMC-9 (Masters Company, Inc.) and allowed to dry. After drying, each slide is preserved by sealing the cover slip with nail polish. Each slide is then ready for identification.

2. Larger organisms are identified in petri dishes under a dissecting microscope.

3 Identifications are taken to the lowest practical taxonomic level (generally genus/species) or that required by the state agency where the particular study takes place. Experienced taxonomists identify each organism and place them into vials with an identification label and a sticker showing the sample identification number on the lid. The identification labels display the sample identification number, location, site, date of collection, and initials of the taxonomist written in indelible ink. The identified taxa and number of individuals for each taxa are recorded on EnviroScience's Aquatic Invertebrate Bench Sheets (Table 10.1). A separate set of bench sheets is used for every sample.

4. Questionable or unusual taxa are verified on a consensus basis between at least three experienced taxonomists. A final check for correct identification is made against EnviroScience's permanent reference collection for confirmation. The reference collection consists of organisms that have been verified by an outside authority in macroinvertebrate identification. At the client's request, a reference collection with one member of each taxa found may be produced and verified against the EnviroScience voucher collection.

Table 9.1 Aquatic Invertebrate Bench Sheets

Collected From:	Station:	
Locality:	Sample Number:	
Type of Sample:	River Mile:	
Date Collected:	Date Identified:	
Collector:	Biologist/Taxonomist:	
Date Picked:		
Technician:		
TAXA	NUMBER OF INDIVIDUALS	TOTAL
PORIFERA		
TURBELLARIA, PLANARIIDAE		
ANNELIDA		
OLIGOCHAETA		
HIRUDINEA (LEECH)		
Helobdella stagnalis		
CEOLENTERATA		
ARTHROPODA		
ARACHNIDA		
CRUSTACEA		
CLADOCERA		
COPOPODA		
ISOPODA, Asellidae		
Caecidotea sp.		
Lirceus sp.		
AMPHIPODA		
CRANGONYCTIDAE		
Crangonyx sp.		
GAMMARIDAE		
TALTRIDAE		
Hyalella azteca		
DECAPODA		

Table 9.1 Aquatic Invertebrate Bench Sheets

Sample I.D. # :	Station #:	
TAXA	NUMBER OF INDIVIDUALS	
INSECTA		
EPHEMEROPTERA		
BAETIDAE		
Baetis sp.		
CAENIDAE		
Caenis sp.		
EPHEMERIDAE		
Ephemera		
Hexagenia		
EPHEMERELLIDAE		
Ephemerella		
HEPTAGENIIDAE		
Stenacron sp.		
Stenonema sp.		
ISONICHIIDAE		
Isonychia sp.		
POTOMANTHIDAE		
Anthopotamus sp.		
LEPTOHYPHIDAE		
Tricorythodes sp.		
ODONOTA		
ANISOPTERA (DRAGONFLY)		
CORDULIIDAE		
AESHNIDAE		
GOMPHIDAE		
ZYGOPTERA (DAMSELFLY)		
CALOPTERYGIDAE		
Calopteryx sp.		
COENAGRIONIDAE		
Argia sp.		
Enallagma sp.		
LESTIDAE		
Lestes sp.		
PLECOPTERA		
PERLIDAE		
Perlesta sp.		

Table 9.1 Aquatic Invertebrate Bench Sheets

Sample I.D. # :	Station #:	
TAXA	NUMBER OF INDIVIDUALS	
HEMIPTERA		
CORIXIDAE		
Trichorixa sp.		
GERRIDAE		
MESOVELIIDAE		
Mesovelia sp.		
MEGALOPTERA		
CORYDALIDAE		
Corydalus conutus		
Nigronia sp.		
SIALIDAE		
Sialis sp.		
TRICHOPTERA		
HYDROPSYCHIDAE		
Cheumatopsyche sp.		
Hydropsyche depravata grp.		
Hydropsyche/Ceratopsyche		
PSYCHOMYIIDAE		
HYDROPTILIDAE		
Hydroptila sp.		
LEPTOCERIDAE		
Nectopsyche sp.		
POLYCENTROPODIDAE		
Cyrnellus sp.		
PHILOPOTAMIDAE		
Chimarra sp.		
COLEOPTERA		
DRYOPIDAE		
DYTISCIDAE		
ELMIDAE		
Ancyronyx sp.		
Dubiraphia sp.		
Machronychus sp.		
Stenelmis sp.		
GYRINIDAE		
HYDROPHILIDAE		
Berosus sp.		
PSEPHENIDAE		
Psephenus herricki		

Table 9.1 Aquatic Invertebrate Bench Sheets

Sample I.D. # :	Station #:	
TAXA	NUMBER OF INDIVIDUALS	
DIPTERA		
ATHERICIDAE		
Atherix lantha		
CERATOPOGONIDAE		
CULICIDAE		
EMPIDIDAE		
SIMULIIDAE		
Simulium sp.		
TABANIDAE		
TIPULIDAE		
Hexatoma sp.		
Tipula sp.		
CHIRONOMIDAE residual + pupae		
CHIRONOMINAE		
CHIRONOMINI		
TANYTARSINI		
ORTHOCLADIINAE		
TANYPODINAE		
MOLLUSCA		
GASTROPODA		
BIVALVA		
TOTAL NUMBER OF INDIVIDUALS =		
TOTAL NUMBER OF TAXA =		
EPT TAXA:		
NOTES:		

10.0 Data Analysis

1.) Invertebrate Community Index

The principle measure of overall macroinvertebrate community condition used by EnviroScience is the Invertebrate Community Index (ICI), a measurement derived by the Ohio EPA from information collected over many years. The ICI is a modification of the Index of Biotic Integrity (IBI) for fish developed by Karr

(1981). The ICI consists of ten structural community metrics, each with four scoring categories of 6, 4, 2, and 0 points (Table 11.1). The point system evaluates a sample against a database of 247 relatively undisturbed reference sites throughout Ohio. Six points will be scored if a given metric has a value comparable to those of exceptional stream communities, 4 points for those metric values characteristic of more typical good communities, 2 points for metric values slightly deviating from the expected range of good values, and 0 points for metric values strongly deviating from the expected range of good values. The summation of the individual metric scores (determined by the relevant attributes of an invertebrate sample with some consideration given to stream drainage area) results in the ICI value. When the ICI is used for stream evaluation, both Hester-Dendy artificial substrate samplers and qualitative kick samplers are collected at each site. Metrics 1-9 are all generated from the artificial substrate sampler data while Metric 10 is based solely on qualitative sample data. More discussion of the derivation of the ICI including descriptions of each metric and the data plots and other information used to score each metric can be found in Ohio EPA (1987).

2.) When the ICI is not appropriate for data analysis, or if supplementary data is requested, benthic metrics following that of Plafkin et al. (1989) and Barbour et al. (1999) are calculated.

A.) The Rapid Bioassessment Protocols (RBP) developed by Plafkin et al. (1989) was designed primarily for qualitative data sets, and incorporates the following eight metrics into one index:

Taxa richness
Hilsenhoff Biotic Index
Ratio of Scrapers and Filtering Collector Abundances
Ratio of EPT and Chironomidae Abundances
% Contribution of Dominant Taxon
EPT Index
Ratio of Shredders and Total Abundances
Jaccard Coefficient of Community

Table 10.1. Invertebrate Community Index (ICI) Metrics and Scoring Criteria Based on Macroinvertebrate Community Data From 247 Reference Sites

Metric	Scoring Criteria 0 2 4 6
1. Total number of taxa 2. Total number of Mayfly Taxa 3. Total number of Caddisfly Taxa 4. Total number of Dipteran Taxa 5. Percent Mayflies 6. Percent Caddisflies 7. Percent Tribe Tanytarsini Midges 8. Percent Other Dipterans and Non-Insects 9. Percent Tolerant Organisms 10. Total Number of Qualitative Ephemeroptera, Plecoptera, and Trichoptera (EPT) Taxa	Scoring of each metric varies with drainage area; see Ohio EPA (1987).

Throughout Ohio.

Reference sites are chosen, and the final evaluation of Biological Condition at comparison sites is determined as follows. Each metric is assigned a Biological Condition Score (Figure 11.1, Plafkin et al., 1989) according to the macroinvertebrate data. This score is determined after comparing data from the comparison site to data from the reference site to calculate a percent similarity. Scores are assigned according to the percent similarity, and are either 0, 2, 4, or 6, with a higher number indicating healthier communities. Scores for each metric are totaled at each site and compared by percent comparability to the reference site. The percent comparability is used to assign one of the following Biological Condition Categories to each comparison sites: Nonimpaired (>83% comparable), Slightly Impaired (54-79%), Moderately Impaired (21-50%), or Severely Impaired (<17%).

B.) The RBP's were finalized in 1999 (Barbour et al.) to synthesize existing methods currently in use by various state agencies, and to provide a tool applicable to a wider range of planning and management purposes. The revised RBP manual synthesizes the many benthic metrics in use today, and are broadly categorized under four classes to describe different aspects of the invertebrate assemblage: taxa richness, measures of composition, measures of tolerance or intolerance, and measures of feeding group. Each metric listed has been tested for practicality, however each must be tested on a regional basis. Metrics are chosen in combination to survey more than one aspect of the community, thus giving a

complete assessment of the biological condition. Metrics are calibrated and formed into a multimetric index, and sites are assessed for biological condition and judgement of impairment. Barbour et al. (1999) gives a complete description of methods used to formulate and calibrate a multimetric index, and for establishing biological condition.

Figure 10.1 Flowchart for evaluation of Biological Condition following Plafkin et al. (1989).

a = Score is a ratio of study site to reference site x 100
b = Score is a ratio of reference site to study site x 100
c = Score is determined from actual value

	Biological Condition Scoring Criteria			
Metric	6	4	2	0
1. Taxa richness[a]	> 80%	60-80%	40-60%	< 40%
2. Hilsenhoff Biotic Index[b]	>85%	70-85%	50-70%	< 50%
3. % Contribution of Dominant Taxon[c]	< 20%	20-30%	30-40%	>40%
4. EPT Index[a]	> 90%	80-90%	70-80%	< 70%
5. Percent Number of Mayflies[a]	—	--	—	—
6. Number of Intolerant Taxa[a]	> 80%	60-80%	40-60%	< 40%
7. Percent Abundance of Intolerant Taxa[a]	> 80%	60-80%	40-60%	< 40%
8. Community Loss Index[c]	< 0.5	0.5-1.5	1.5-4.0	> 4.0

Bioassessment	
% Comparable to Reference Score	Biological Condition Category
> 83%	Nonimpaired
54-79%	Slightly impaired
21-50%	Moderately impaired
< 17%	Severely impaired

REFERENCES

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Revision to Rapid Bioassessment Protocols for use in Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish. EPA 841-D-97-002. U. S. Environmental Protection Agency.

Ohio EPA. 2006. Manual of Ohio EPA Surveillance Methods and Quality Assurance Practices. Division of Water Quality Monitoring and Assessment, Surface Water Monitoring Section. Columbus, Ohio.

Ohio EPA. 1987. Biological criteria for the protection of aquatic life: Vol. II. Users manual for biological field assessment of Ohio surface waters. Division of Water Quality Monitoring and Assessment, Surface Water Monitoring Section. Columbus, Ohio. 119 p.

Ohio EPA. 1989. Biological criteria for the protection of aquatic life: Vol. III. Standerdized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities. Division of Water Quality Monitoring and Assessment, Surface Water Monitoring Section. Columbus, Ohio. 58 p.

Plafkin, J. L., M. T. Barbour, K. D. Porter, S. K. Gross, and R. M. Hughes. 1989. Rapid Bioassessment Protocols for use in Streams and Rivers: Benthic macroinvertebrates and Fish. EPA/440/4-89-001. U.S. Environmental Protection Agency, Assessment and Watershed Protection Division, Washington, DC. 20460.

United States EPA. 1990. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/4-90/030.

APPENDIX D

STANDARD OPERATING PROCEDURES FOR FISH SAMPLING AND DATA ANALYSIS

ENVIROSCIENCE, INC.

Standard Operating Procedures Document:

Fish Survey/Collection Methods
Fish Survey/Laboratory Processing

1.0 PURPOSE

To provide field and laboratory methods of water quality studies using fish to evaluate the biological integrity of surface waters.

2.0 SCOPE

This Standard Operating Procedure (SOP) includes procedures for sample site selection, field-data collection, fish sample collection, the handling of live specimens, in-field organism identification, in-field fish processing, examination of external anomalies, labeling and record keeping, and preserved material handling. Procedures in this text are modified from and strictly adhere to those of the Ohio and United States Environmental Protection Agencies (OEPA 1987; Barbour et al. 1999).

3.0 PROCEDURES FOR SAMPLE SITE SELECTION

Fish sampling sites are chosen by considering several factors, they are:

1. point source discharge location
2. stream use designation issues
3. physical habitat variations (e.g. dams, changes in geology, changes in stream order,
   presence of a stream confluence, etc,)
4. non-point source pollution location
5. macrohabitat variations
6. ecoregion boundary proximity

A set number of biological sampling sites are allotted for each project. This allotment is based on the issues being evaluated. In selecting sampling sites within the study area, the most typical habitats available are chosen in an effort to represent the area accurately. Every attempt to sample similar macrohabitats within the study area is made. Resource availability and access to sites are also considered in determining ideal sampling locations, however, sampling site locations are ultimately determined by the objectives of each individual survey.

Reference sites are generally located upstream from all pollution sources in order to determine the background condition of the study area. These upstream sites are important in specifying any background conditions that might temporarily or permanently influence aquatic life use attainment in the downstream reaches.

In studies involving point source discharges, at least one sampling site is situated upstream from the discharge, one is situated within the mixing zone, and other sites are located at intervals downstream from the mixing zone (i.e. dependent on stream size and mixing characteristics). This arrangement allows for determination of near and far field impacts, the extent and severity of longitudinal effects, and the location of recovery if such recovery occurs within the study area.

4.0 PROCEDURES FOR FIELD DATA COLLECTION

A description of the physical habitat within the sample stream reach provides essential interpretive information. A Stream Data Sheet (Figure 4.1) is completed for each sampling site and includes physical and geographical information, a written site description, and a schematic drawing of the stream. If necessary, photographs are taken to readily illustrate the stream and its riparian corridor. These photographs may be taken from several perspectives to accurately portray the entire project area.

In addition to the stream data sheet, a Qualitative Habitat Evaluation Index (QHEI) will be completed upon request. The QHEI is a physical habitat index which provides a quantified evaluation of the lotic macrohabitat characteristics important to fish communities. A more detailed description of the QHEI can be found in EnviroScience's QHEI SOP.

5.0 PROCEDURES FOR FISH SAMPLE COLLECTION

Pulsed direct current (D.C.) electrofishing is the principal method used by EnviroScience to obtain relative fish abundance and distribution data. Although sampling selectivity and bias is inherent in all collection methods, pulsed D.C. electrofishing is widely viewed as the single most effective method for sampling fish communities. Other sampling methods may be used to supplement electrofishing. Fish sampling is conducted between mid-June and early October, when the fish community is most stable and sedentary, stream and river flows are generally low, and pollution stresses are potentially the greatest.

5.1 Procedures for Selection of the Appropriate Sampler Type

Selection of sampler type is based on macrohabitat, and the size and depth of the sample water body. Sampler type selection is a critical part of the survey since data

quality essentially determines the applicability of the survey results in evaluating attainment of aquatic life uses.

Fig 4.1 Stream Data Sheet

STREAM DATA FOR ENVIROSCIENCE

Project: Date:

Project Type: Permit #:

Locality:

Drainage:

Collector / Biologist: Map:

Type of Sampler Used: Collection Depth:

Sample# Station#

Flow Stability: Intermittent Continuous Gradient:

Current: None___ Slow___ Fast___ Rapid____ Flow:

Substrate	Riffle	Run	Pool
Detritus	%	%	%
Clay	%	%	%
Silt	%	%	%
Sand	%	%	%
Gravel	%	%	%
Cobble	%	%	%
Boulders	%	%	%
Bedrock	%	%	%

Land Use: Industrial___ Urban___ Residential___ Pasture__ Oil-Field
Row-Crop___ Forest ___ Other ___

Livestock: Present___ Absent___ Access to stream: Yes___ No __

Fig 4.1 Stream Data Sheet (continued)

SAMPLE #_____ **STATION #** _____

Pollution: Type:

Source:

Stream Morphology: Riffle _____% Run_____% Pool_____%
Stream Channel: Natural Channelized Recovering Leveed
Impounded Backwater

Instream Cover (% stream area): Undercut Banks____% Boulders____%
Logs____% Vegetation____%
Overhanging Brush____%_____

Weather Conditions: Sunny_____ Partly Cloudy _____ Mostly Cloudy__
Rain: Today____ Yesterday_____ 2-days Prior__

Air Temperature:_____ Water Temperature:_____

Dissolved Oxygen:_____ Conductivity:_____

Site Description (Remarks and Notes):

5.2 Procedures for Boat Electrofishing

Boat electrofishing methods are used in moderate to large sized streams and rivers where wading methods are impractical and inefficient. Streams and rivers with pools deep enough to accommodate EnviroScience's electroshocking boat and equipment will be sampled with boat electrofishing methods. The electrofisher uses pulsed direct current (DC) from anodes mounted to a boom on the front of the boat. The available peak current from the electrofishing unit is 1,000 volts and 5,000 watts. The output of the unit is adjusted according to the conductivity of the water body being sampled. The lower the conductivity of the water, the higher the voltage needed to effectively sample the area. The current flowing through the water is directly related to the voltage applied; the higher the voltage, the greater the current.

Fish are usually oriented into the current and must either swim into the electric field or turn sideways to escape downstream. This sideways movement creates an increased voltage gradient making the fish more susceptible to the electric current. The degree to which fish are affected by electric current is a function of their surface area. Generally, larger fish are more sensitive to the electric currents. The power output is therefore adjusted to representatively sample smaller individuals while minimizing adverse effects on larger individuals.

In the largest rivers where the drainage area exceeds 6,000-7,000 square miles, night electrofishing will be conducted. At night, fish tend to come within four to six feet of the surface to feed where they are more susceptible to the electric current. Four (4) 75 watt flood lamps mounted to the boom of the boat are further used to attract fish closer to the surface. When exposed to the electric current, the fish become temporarily stunned and float to the surface where they are netted and placed in a livewell for recovery.

5.2.1. Procedures for Boat Sampling Site Selection

Sampling sites are selected along the shoreline containing the most diverse macrohabitat features. In larger rivers this is usually the outside river banks. Mid-stream areas will also be electrofished to sample those fish not typically found along the shoreline and, if present, to sample mid-stream habitat structures. When possible, run-type habitats will also be included within the sampling zone. Boat electrofishing zones are normally 0.5 kilometers in length, but shorter distances may be necessary in some situations. The length of the sampling reach will be measured and marked on shore using fluorescent orange paint, making future relocation of the sampling reach easier. The sampling reach boundaries will include a starting point marked with an arrow pointing in a downstream direction, and an ending point marked with a capital "E". If the sampling zone is not continuous, additional marks will be necessary; a capital "X" will mark the spot where the zone stops and an arrow will mark where it

resumes. To characterize the sampling area and to identify areas too deep to electrofish, water depth is determined at ten locations within each sampling zone using a Humminbird® wide view depth finder. The location of each sampling zone is indexed by river mile and marked on 7 ½ minutes USGS topographical maps for permanent reference.
Each boat sampling zone will be electrofished two or three times during the sampling season. If only two passes are to be made, there will be a period of at least five to six weeks between passes. If three passes are planned, a minimum of three or four weeks will elapse between passes.

5.2.2. Procedures for Boat Sampling Techniques

A field crew consists of four biologists, a boat driver, one or two netters, and one or two field station biologists. The field station biologists will maintain contact with the electrofishing boat and are responsible for meeting the boat at the designated biological field station after the completion of fish sampling at each site. The field station biologists also process fish while the driver and netters go to the next site.

Sampling zones are electrofished from upstream to downstream with sampling beginning at the upstream end and proceeding downstream. A minimum of 1,300 to 1,600 seconds is normally spent sampling for each 0.5 km sampling zone; however, time may vary depending on the number of fish being collected, the water current, the extent of cover within the zone, or specific requirements of the survey.

All members of the boat crew wear rubber boots, rubber gloves, and a jacket type personal flotation device. Shocking is controlled by the primary netter at the bow of the boat using a foot pedal equipped with a "dead man" switch. Positive pressure cut-off switches located inside the electroshocking unit allow any member of the crew to turn off the electroshocker if necessary. The netters are responsible for capturing all fish sighted and the driver is responsible for maneuvering the boat so that the netter has the best opportunity to capture stunned fish (the driver may also capture stunned fish within reach).

The boat is carefully maneuvered by directing the boat's bow as close as possible to the shore and/or submerged objects while shocking the near shore area. Sampling should be performed carefully and adjusted for different habitats, particularly at sites where there is extensive woody debris or a moderately fast to swift current. In zones with extensive woody debris it is necessary to maneuver the boat in and out of the macrohabitats formed by the debris. As the water current increases it becomes necessary to maneuver the boat quickly so that the netter has time to capture the stunned fish. However, as the water depth increases to greater than 100-200 cm, it may become necessary to wait for the fish to surface. The driver may help in netting the fish when large numbers are stunned. For optimum results both the boat and

electric field should be kept moving downstream slightly faster than the current. It may be necessary to pass over fast water sections twice or back troll the boat downstream, pausing occasionally to allow the netter to capture the stunned fish. Electrofishing fast waters in an upstream direction will only reduce sampling efficiency.

As fish are attracted to the anode and/ or stunned, they are collected with a boat net. The boat nets have 2.5 m long handles and 7.62 mm Atlas mesh knotless netting. Efforts are made to capture all fish which are spotted. Captured fish are immediately placed in one of three on-board livewells. When sampling is complete the fish are transported to the field station where they are processed and released unharmed.

When sampling is performed during the day, netters are required to wear polarized sunglasses to facilitate spotting stunned fish in the water during each electrofishing run. Polarized sunglasses are not necessary with night sampling.

5.3 Procedures for Wading Electrofishing

In smaller streams, where access by boat is not possible, sport-yak or long-line wading methods are used for electrofishing purposes. The sport-yak method employs a 10' tote barge in which the generator, electrofishing unit, and livewells are placed. This assembly is towed behind the electrofishing crew. Where the majority of the sampling reach is too shallow for the sport-yak barge, the long-line method is employed. In this assembly the generator, electrofishing unit, and livewells remain on shore while the anode is maneuvered through the sampling reach on a 90 m cable.

A two or three person crew, all wearing chest waders and rubber gloves, is required for sampling wadeable sites. The primary netter will operate the anode net ring while the other crew members aid in capturing fish. All habitat types are thoroughly sampled in an upstream direction for a distance of 150-200 meters. The primary netter works the net ring beneath undercut banks and in and around submerged structures such as brush piles, log jams, and boulders. In order to capture fish located in these areas, it may be necessary to thrust the anode ring into and under the structure with the current on and then quickly withdraw the anode ring in one swift motion. This will draw fish out from under such structures enabling one of the netters to capture the stunned fish. In order to sample all habitat types, sampling should occur along both stream banks. In riffle and run areas the primary netter takes the anode ring from upstream to downstream, allowing it to drift with the current. In order to prevent riffle species from escaping or avoiding the electrical field, a second netter blocks off an area downstream and a seine is placed downstream to catch any additional fish. Captured fish are placed in livewells and processed when the livewell is full or sampling is completed.

While one sampling pass may be sufficient in small or simple streams, two or three individual passes are recommended with the wading methods. The number of passes

should be recorded and will be used in calculating biological indices and making environmental evaluations (Ohio EPA, 1987). Relative abundance data is expressed in terms of both numbers and weight (kg) / 0.3 km.

5.4 Procedures for Hoopnetting

In larger rivers hoopnets with a 3.0 foot (0.97-meter) diameter mouth and 1 inch (2.5-cm) bar mesh are often used in conjunction with electroshocking. The strategy of the hoopnet is to sample fish that are on or near the bottom that can not be sampled by other methods. The nets are placed parallel to the shore with the mouth opening downstream. After a 24 hour period, the nets will be checked and reset. If any fish are present at this time they are removed and processed. The nets are again checked at 48 hours and removed. All fish sampled in the nets will be placed in livewells and processed on shore.

5.5 Procedures for Seining

A 30 foot (9.1 meters) by 6 inch (1.8 meters) seine with 1/8 inch mesh is often used in conjunction with electroshocking. The strategy of seining is to sample near shore species in larger rivers and lakes, and to sample all species in smaller streams. Sampling consists of two field biologists pulling the seine in an upstream direction and then drawing it into shore. If no fish are found in a seining attempt, the haul is repeated in an undisturbed area within the site. Seining is performed at most three times at each site. If no fish are found on the third seine haul, the site is recorded as having zero fish obtained for that seining site. The seine haul will be repeated if it becomes snagged on the substrate. Seining is not practical in all situations. If an area near a specific site can not be seined, the field manager will choose a stream run in the same area as the site if one is available. Field notes will be taken on the start and end points of all seining runs. All captured fish are placed in live wells and transported to the field station to be processed.

5.6 Procedures for Trawling

A 16 foot (4.91 meters) semi-balloon otter trawl with 1/8 inch (0.32 meter) mesh cod liner is often used in conjunction with electroshocking. The trawling method is used in larger rivers to sample mid-depth fish species. Trawling is conducted in an upstream direction with a typical duration of 5 minutes per trawl sample. A trailing buoy is attached to the cod end of the trawl to facilitate retrieval when the trawl becomes entangled on snags, boulders, or other objects. The length of the tow rope will be maintained at two to three times the depth of the sampling zone. After each successful trawl, the net will be brought up and the catch emptied into a live well. When the trawl becomes entangled on debris or other structure, the trawl will be brought to the surface

and, if undamaged, the catch emptied and the trawl returned to the water to complete the trawl run. If the trawl has been damaged, it is mended and the trawl run restarted.

6.0 PROCEDURES FOR IN-FIELD SAMPLE PROCESSING

6.1 Procedures for Handling Live Specimens

In all collecting methods, captured fish are placed in a livewell until processed. In order to maintain dissolved oxygen levels and minimize mortality, aerators are placed in the livewell and water is replaced as needed. When the livewell is full or sampling is completed, fish are identified to species, examined for external anomalies and weighed. Fish are released immediately after this process and every effort is made to minimize handling and holding times.

6.2 Procedures for Fish Identification

The majority of captured fish are identified in the field by the sampling crew with the aid of Trautman (1981). Questionable species are verified by the FOM, however some species may require laboratory identification. When laboratory identification is necessary, fish are preserved in borax buffered 10% formalin in the field, labeled by date, river or stream, and river mile or
site designation. Fish are identified by an expert in fish taxonomy to the species level, and may be identified to the sub-specific level in some cases (e.g. banded killifish). These specimens, are kept in EnviroScience's permanent library as reference organisms.

Collection techniques may not be consistently effective with fish less than 15 mm in length. These fish (young of the year) are therefore not included in catch totals or IBI calculations, as they may produce bias in the aquatic ecosystem health measure (Angermier and Karr, 1986; Angermier and Schlosser, 1988).

6.3 Procedures for Weighing and Measuring Organisms

In the case of samples comprised entirely of one size class of the same species (e.g. adults, juveniles, young-of-the-year), weighing may be performed either on individuals or in aggregate as a species. For catches with more than 15 individuals per species, a subsample of 15 fish is weighed, either as individuals or in aggregate. If there is a noticeable variation in sizes between individual fish of a species, individual weights are taken using the subsampling technique.

With smaller species (e.g. most minnows and darters) mass weighing in aggregate is recommended. If more than 50 individuals of one species are collected in the catch a subsample of at least 50 fish is weighed and the remainder are counted. If extremely high numbers are collected and the fish are of a relatively uniform size, the number of individuals may be determined by mass weighing all fish collected and extrapolating the numbers from a counted and weighed subsample. All results are recorded on the fish data sheet (Figure 6.1).

Samples that are comprised of two distinct size classes of fish (e.g. adults and juveniles) of a species are processed as two separate size groups. Adults and juveniles are recorded separately on the fish data sheet by adding an "A" to the species code for adults and a "B" for juveniles. For example, if both adult and juvenile white suckers occur in the same sample the adult numbers and weights are recorded as *Catastomus commersoni commersoni*-A with juvenile numbers and weights recorded as *Catastomus commersoni commersoni*-B. Although each is listed separately on the fish data sheet they are treated as a subsample of the same species in any subsequent data analyses..

Individual fish weighing less than 1000 grams (g) are weighed to the nearest 1 g on a portable top loader scale (300 g capacity x 0.1 g intervals). Fish weighing more than 1000 g are weighed on a Hobart 7 meat scale (30 lb capacity x .01 lb intervals) and these weights are then converted to grams. All scales are checked once each week with National Bureau of Standards Class F check weights (up to 2000 g in 1 g increments) and adjusted as necessary.

6.4 Procedures for Examination of External Anomalies

All fish that are weighed whether individually, in aggregate, or subsampled (only the fish that are actually weighed) are examined for the presence of gross external anomalies. The occurrence of anomalies is recorded on the fish data sheet. Gross external anomalies are defined as externally visible skin or subcutaneous disorders. These anomalies should be noted when the fish are captured, identified, sorted, weighed, and counted. Exact counts of anomalies present (i.e. the number of tumors, lesions, etc. per fish) are not made; although, light and heavy infestations are noted for certain types of anomalies. Anomalies are expressed as percent (weighted) of affected fish among all fish weighed times the number of fish in the sample. This is computed for each type of anomaly for each species and in each sample. The total percent for a specific type of anomaly or group of anomalies can be calculated for one or more sites. The types of anomalies which are recorded on the fish data sheet are presented in Table 6.1.

Figure 6.1: EnviroScience Fish Data Sheet

Field Crew:_____ Page ____ of

Date:_____ Time of Day:_____ Clients:_____

River/Stream:_____ River Mile:_____ Location: _____
Sample Type:_____ Distance Fished:_____ Time Fished:__' __"

Total Seconds Fished: _____ Depth of Sample:_____ Data Source:_____

Observed Flow:_____ Number of Species: _____

Station Number: _____ Sample ID Number:_____

Species	Total Weight	Total Counted	Weight (Grams)					Anomalies				

Table 6.1 Anomaly Abbreviations (OEPA, 1987)

(AL) = Anchor Worm, Light Infestation : parasitic worm hanging from fish; characteristic sore is left after detachment; five or fewer worms and/or sores is considered a Light Infestation.

(AH) = Anchor Worm, Heavy Infestation : six or more attached worms and/or attachment sites.

(BL)= Black Spot Light Infestation : small black cysts; light infestations, spots do not cover most of the body and the average distance between spots > the diameter of the eye.

(BH)= Black Spot Heavy Infestation : black spots cover most of the body and fins

(CL)= Leeches Light Infestation : five or fewer attached leeches and/or attachment sites

(CH)= Leeches Heavy Infestation : six or more attached leeches and/or attachment sites

(D) = Deformities : misshaped stomach, head, spinal vertebrae or other body parts

(E) = Eroded Fins

(F)= Fungus : white cottony growth

(I) = Ich : protozoan identified by white spotting on skin and fins of fish (rare in wild)

(L) = Lesions : open sores, bloody areas

(M)= Multiple DELT Anomalies

(N) = Blind : cloudy eyes

(P) = Parasites

(Y) = Popeye : bulging eyes

(S) = Emaciated : underweight

(W)= Swirled Scales

(T) = Tumors : tumor like masses caused by parasites are NOT included here

(Z) = Other

External anomalies can indicate the presence of multiple, sublethal stresses, intermittent stresses, and chemically contaminated substrates. They are believed to be caused and/or aggravated by environmental factors. The effects of temperature, salinity, dissolved oxygen, diet, chemicals, organic wastes, etc., especially during the ontogeny and larval stages of fishes, may be the cause of many types of anomalies (Berra and Au, 1981). Morphological abnormalities are uncommon in unimpacted, natural fish populations (Komanda, 1980). For these reasons, the presence of anomalies on fish may act as an index of pollution stress and are used in the Index of Biotic Integrity (IBI) (OEPA, 1987). Field investigators should refer to texts on fish health and pictures of specific anomalies. If necessary, affected fish should be preserved for laboratory examination.

The following is a review of some anomalies commonly encountered in freshwater fishes. These characteristics are used in determining the types of external anomalies present. All definitions of fish anomalies given are taken from Manual of Ohio EPA Surveillance Methods and Quality Assurance Practices, Volume I (OEPA 2006).

1) Deformities (D): Deformities may result from the presence of toxic chemicals, viruses, bacteria, infections, and protozoan parasites. They may affect the head, spinal vertebrae, fins, and stomach shape. Fish with obvious injuries or extruded eyes (see popeye disease) are not included.

2) Eroded Fins (E): These generally result from flexibacteria invading the fins and causing necrosis of the fish tissue (Post, 1983). Necrosis of the fins can also result from the presence of the small trematode parasite, gryodactylid. When necrosis occurs in the tissue at the base of the caudal fin it is referred to as peduncle disease. Other erosions included occur on the preopercle and operculum.

3) Lesions and Ulcers (L): These appear as open sores, exposed tissue, or prominent bloody areas and are caused by viral and bacterial infections. Small, characteristic sores left by anchor worms and leeches, and obvious injuries are not included unless they are enlarged due to this infection. Lesions often times appear in areas impacted by multiple stresses, such as marginal D.O. in combination with sublethal levels of toxins.

4) Tumors (T): Tumors, also known as neoplasia, result from the loss of carefully regulated cellular growth in the tissue (Post, 1983). Abnormally high amounts of cellular growth in the tissue can be caused by the presence of toxic chemicals. Tumor like masses caused by parasites are not considered as tumors.

5) Anchor worm (*Lernaea cyprinacea*) (A): The anchor worm is a parasitic copepod which resembles a slender worm and hangs from the fish. The adult female buries its head into the flesh of the fish and leaves a small, characteristic sore when it

detaches. The number of attachment sites is included in the determination of light and heavy infestations. A former attachment site which has become infected and enlarged is recorded as a lesion.

6) Black spot (B): This disease is easily identified as small black cysts on the skin and fins and is caused by the larval stage of a trematode parasite. Black spot is prevalent on fish located in habitats comprised of an abundance of aquatic vegetation with snails and fish eating birds, two intermediate belonging to the family *Piscicolidae* and are usually greenish brown in color and 5-25 mm long (Allison et al., 1977). Leeches can be identified by the presence of two suckers (one on each end) and the ability to contract or elongate their body. They may occur almost anywhere on the external surface of bullheads (*Ictalurus* sp.). Small sores or scars left by leeches are included in the determination of light and heavy infestations. If these sores become enlarged and infected they are also regarded as lesions. Leeches are seldom harmful to fish unless the infestation is very heavy.

7) Fungus (F): Fungus appears as a white cottony growth on the skin of the fish. It is generally caused by *Saprolegnia parastitica* and attacks injured and open areas of the fish. Fungus may eventually cause additional disease or death.

8) Ich or *Icthyophthirus multifilis* (I): Ich is a protozoan identified by white spotting on the skin and fins of the fish; however, it rarely occurs in wild fish populations.

9) Popeye (Y): This disease is identified by bulging eyes and can be caused by gas accumulation in areas where the water is gas supersaturated.

6.5 Procedures for Labeling and Record Keeping

Fish data sheets (Figure 6.1) are completed at the time of fish processing. They include the following information:

1) Field Crew - All members of the field crew are listed here with the FOM listed first.

2) Date, Time - military time is used, sampling start and completion times are recorded.

3) River / Stream - major river or stream being sampled.

4) Location - described as adjacent to, upstream, or downstream from a notable landmark.

5) Date - month / date / year

6) River Mile - river mile (from the middle of the electrofishing zone) to the nearest 0.1 mile
determined by inspection of USGS river mile index maps.

7) Distance- electrofishing distance in kilometers to the nearest 0.01 km (electrofishing only).

8) Sampler Type - sampler type should be noted here

9) Depth - average depth for the sampling zone, determined using a Humminbird7 wide view
depth finder.

10) Time Fished - actual time devoted to sampling fish in seconds as recorded by the electroshocking unit (electrofishing only).

11) Number of Species - number of species of fish captured during each sampling (hybrids and
exotics are not included and should be indicated separately).

12) Species Codes - each species and any hybrids are recorded by a family-species code similar
to that of OEPA (1987).

Each species is recorded on the fish data sheet along with fish weight, standard length, and anomalies. In order, fish data sheets are filed according to survey, river mile, and date at the EnviroScience laboratory.

Any fish which are retained for further identification or as voucher specimens are labeled by date, river or stream, river mile or site description, and method collected. When these fish are identified they are then labeled with a species code.

7.0 PROCEDURES FOR HANDLING PRESERVED MATERIALS

7.1 Procedures for Sample Preservation

Fish which are preserved as voucher specimens, or needed for subsequent identification should be immersed in a fixative solution as soon as possible. Immediate fixing is necessary in order to maintain chromatophore patterns useful in identification. The recommended fixative is a solution of one part commercially prepared formalin and nine parts water. One teaspoon of borax is added per two gallons of fixative. The borax acts as a buffer to neutralize the acidic effect of the formalin, retarding shrinkage, preventing the hardening of soft body parts, and preventing decalcification of the

tissues (Ladler et al. 1962). If fish are kept at temperatures greater than 80 F (26.7 C), a stronger solution of one part formalin to seven or eight parts water should be used. Stronger solutions are also needed for large or closely packed fish. Because strong solutions of formalin can cause distortion or gaping of the mouth and gills, care should be taken to obtain correct concentrations of formalin solutions. Fish longer than a few inches should be slit along the right side of the abdomen before preserving. Fish heavier than one to two pounds should be injected in the muscles on each side of the backbone. Specimens are kept in the formalin solution for two to three weeks, rinsed in water to wash off excess formalin, and allowed to drain for two hours. They are then placed in a 35% alcohol wash for two to three weeks, switched to a 50% alcohol wash for two to three weeks, and placed in a 70% aqueous solution of ethyl alcohol for permanent storage.

Labels are made using 100% waterproof rag paper, labeled with indelible ink and placed in preserving container. Labels include genus and species, stream or river name, location, date, river mile, and principal collector.

7.2 Procedures for Laboratory Identification and Verification

Fish are identified in the field by trained field biologists. If accurate identification is not possible, fish are preserved and brought back to the laboratory for verification. In the EnviroScience laboratory several keys are used for identification (Trautman 1957, 1981; Eddy and Underhill, 1978; Hubbs and Lagler, 1980).

Identifications are verified by at least two trained EnviroScience staff. When verification is complete, the information is recorded. Any specimens which can not be identified are taken to the Ohio State University Museum of Biological Diversity (OSUMBD) for identification by the curator of fishes.

7.3 Procedures for Disposition of Samples

EnviroScience maintains an up-to-date reference collection of Ohio and Midwestern U.S. fishes. New or unique species are continually added and duplicates are donated to OSUMBD.

8.0 ENTRAINMENT SORTING PROCEDURES

8.1 Procedures for Laboratory Identification and Verification

Collected fishes are fixed in 10% buffered formalin, and brought back to the laboratory for verification in a labeled container. Samples are rinsed through a No.35 (500µm) sieve, and discarded formalin solution is later neutralized with Formalex®. Sample contents are carefully rinsed into sorting trays and sorted using mounted Wild M5 dissecting microscopes. Several larval fish keys (Auer, 1982; Nelson and Cole, 1975) as well as numerous species, family, and region-specific keys, notes, and articles published in various fisheries journals are used for identification. Quality assurance measures are conducted on all entrainment sample replicates to ensure sorting efficiency.

Identifications are verified by at least two trained EnviroScience staff. When verification is complete, the information including species common name, scientific name, quantity, and life-stage is recorded on bench sheets. Any specimens which can not be identified are taken to The Ohio State University Museum of Biological Diversity (OSUMBD) for identification by the curator of fishes.

8.2 Procedures for Disposition of Samples

EnviroScience maintains an up-to-date reference collection of Ohio and Midwestern U.S. fishes. New or unique species are continually added and duplicates are donated to OSUMBD. One voucher specimen per species collected is preserved in 70% ethyl

alcohol, after fixation in 10% buffered formalin for a minimum of three days. A label containing Sample number, Scientific name, Sample Locality (Site/County, State), Sample Date, EnviroScience log number, Taxonomist, and Client, written with indelible ink, is included in the voucher container. Parafilm® should be applied to the voucher container before capping.

9.0 ANALYTICAL PROCEDURES

Relative abundance data are analyzed for community (all species included) and population (single species) parameters. Relative abundance is expressed in terms of numbers/unit distance (or time for passive gear) and weight (kg)/unit distance (or time for passive gear). Community analyses include the number of species per sample, cumulative species per sampling location, Shannon indices (H) based on numbers and weight, the modified Index of Well-Being (MIWB), and the IBI. The specific details of how these indices and evaluations are derived is described in the OEPA Users Manual for Biological Field Evaluation of Ohio Surface Waters (Ohio EPA, 1987).

REFERENCES

Allison, L.N., J.F. Hnath and W.G. Yoder. 1977. Manual of Common Diseases, Parasites, and Anomalies of Michigan Fishes. Michigan Dept. Nat. Res., Lansing. Fish Mgmt. Rept. No. 8, 132pp.

American Public Health Association, American Water Works Association, and Water Environment Federation. 1992. *Standard Methods for the Examination of Water and Wastewater.* APHA, AWWA, WEF.

Angermier, P.L. and J.R. Karr. 1986. Applying an index of Biotic Integrity Based on Stream Fish Communities: Considerations in Sampling and Interpretation. N. Am. J. Fish. Mgnt. 6: 418-429.

Angermier, P.L. and I.J. Schlosser. 1988. Assessing Biotic Integrity in the Fish Community of a Small Illinois Stream. N. Am. J. Fish. Mgmt. 7:331-338.

Auer, N. A. 1982. Identification of Larval Fishes of the Great Lakes Basin with Emphasis on the Lake Michigan Drainage. Great Lakes Fishery Commission, Ann Arbor, MI 48105. Special Pub. 82-3:744 pp.

Barbour, M. T., J. Gerritsen, B. D. Snyder, J. B. Stribling. 1999. Revision to Rapid Bioassessment Protocols for Use in Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish. EPA 841-D-97-002.

Berra, T.M. and R. Au. 1981. Incidences of Teratological Fished from Dedar Fork Creek, Ohio. Ohio J. Sci. 81(5): 225.

Eddy, S.E. and J.C. Underhill. 1978. Freshwater Fishes, third edition. Wm. C. Brown Company Publishers, Dubuque, Iowa.

Hubbs, C. L., and K.F. Lagler. 1980. Fishes of the Great Lakes Region. The University of Michigan Press, Ann Arbor.

Komanda, N. 1980. Incidence of Gross Malformations and Vertebral Anomalies of Natural and Hatchery *Plecoglossus altivelis*. Copeia 1980: 29-35.

Lagler, K.F., J.E. Bardach, and R.R. Miller. 1962. Ichthyology. John Wiley and Sons, Inc. 545pp.

Nelson, D. D. and R. A. Cole. 1975. The Distribution and Abundance of Larval Fishes Along the Western Shore of Lake Erie at Monroe, Michigan. Institute of Water Research, Michigan State University. Techical Report No. 32.4:14 pp.

Ohio Environmental Protection Agency. 1987. Users manual for Biological Field Assessment of Ohio Rivers and Streams. Div. of Water Quality Monitoring and Assessment, Columbus.

Ohio Environmental Protection Agency. 2006. Manual of Ohio EPA Surveillance Methods and Quality Assurance Practices. Div. of Environmental Services, Columbus, Ohio.

Post, G. 1983. Textbook of Fish Health. TFH Publications, Inc., Neptune City. 256 pp.

Trautman, M.B. 1957. The Fishes of Ohio. Ohio State Univ. Press, Columbus. 683 pp.

Trautman, M.B. 1981. The Fishes of Ohio. (2nd edition) Ohio State Univ. Press, Columbus. 782 pp.