BIOLOGICAL SAMPLING PROCEDURES FOR WADEABLE STREAMS AND RIVERS IN IOWA

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1.0 Introduction

This document describes the biological sampling procedures currently followed in the stream biocriteria project that began in 1994 with a pilot study (IDNR 1994a). The sampling procedures described in this document represent a sampling methodology framework; some modification of the methods has resulted from the pilot study experience. Revision of this working document may occur periodically in the future to reflect further changes in the methodology.

Benthic macroinvertebrate and fish communities serve as indicators of stream biological integrity in Iowa (IDNR 1993). These biological indicators provide information to evaluate both reference sites and test (impacted) sites in the state. Reference sites represent minimally disturbed stream habitats located in each ecoregion or subecoregion of the state. Test sites represent disturbed, or allegedly disturbed, stream habitats throughout the state. The data obtained from the pilot study and sampling in subsequent years will allow the Water Resources section of Iowa's Department of Natural Resources (IDNR) to develop narrative and numeric biocriteria for wadeable streams and rivers in Iowa.

2.0 General Sampling Considerations

2.1 Sampling season

Biological sampling for biocriteria development purposes occurs during an "index period" that lasts approximately from June 15 through September 30. Sampling conditions are usually favorable and fish populations are relatively sedentary during the summer months. Therefore, sampling can proceed efficiently and the impact of seasonal related fish movements on sampling results is minimal.

2.2 Sampling conditions

Biological sampling commences when stream flow levels are similar to base flow conditions and the water is sufficiently clear for effective sampling. All sampling activities take place during daylight hours, generally between 8:00 A.M. and 5:00 P.M.

To ensure the validity of sampling results, the following sampling restrictions apply:

1) Sampling prohibited during elevated stream flow or turbidity levels, which reduces sampling effectiveness.
2) Sampling prohibited during periods of extremely low flow which are stressful to stream biota (i.e., flows equal or below the estimated 7Q10 flow).
3) Sampling prohibited within one week of a minor runoff event that may result in a minor disruption to the aquatic community (i.e., less than bank-full flow).

4) Sampling prohibited for one year following a major flood event (i.e., out-of bank flow) that significantly disrupts the aquatic community resulting in a lengthy recolonization.
5) Sampling prohibited for one year following a major drought event (i.e., dry stream conditions) that significantly depletes the aquatic community resulting in a lengthy recolonization.

Safety is the primary consideration in the field because of the use of hazardous sampling methods (e.g., electrofishing) and because field personnel work in physically demanding conditions. Crew leaders provide all field staff with instructions on the safe use of sampling equipment before entering the stream. IDNR does not condone sampling during inclement weather (i.e., extreme wind, lightning, or rain).

2.3 Sampling area

All sampling activities take place within a designated stream reach. Generally, a designated sampling reach should exhibit natural habitat qualities representative of other streams in the region and are consistent with the sampling objective (e.g., reference or test site). Described below are guidelines for the designation of a sampling reach. The guidelines can also be found in the document: *"Habitat Evaluation Procedures for Wadeable Streams of Iowa"* (IDNR 1994b).

A sampling reach length of 150 meters (492.13 feet) is the recommended minimum for wadeable streams (OEPA 1989; Meador et. al 1993). A designated sampling reach of 500 feet shall be the minimum length of stream sampled regardless of the frequency of habitat repetition. Apply the following protocol to determine the length of the designated sampling reach:

1) To ensure adequate habitat representation in streams with pool/riffle sequences or channel bends, the designated sampling reach should include:
    a) two distinguishable pool/riffle sequences or
    b) two well defined channel bends (in streams lacking pool/riffle sequences)
2) For those streams lacking pool/riffle sequences or channel bends, the following criteria apply:
    a) Streams $\leq$40 feet in mean width, the designated sampling reach shall be 30X the mean width with a 1200 feet maximum length.
    b) Streams >40 feet in mean width, the designated sampling reach shall be 20X the mean width with 1200 feet maximum length.

The above are guidelines with the realization that some sampling reaches may extend longer than 1200 feet due to an erroneous estimate of stream width or because of the distance between adequate pool/riffle sequences or channel bends.

2.4 Sequence of sampling activities

Prior to sampling, a reconnaissance visit to each prospective site is completed to identify access points to the stream, delineate sampling reach boundaries, deploy benthic macroinvertebrate artificial substrates (if required), and assess fish sampling equipment needs.

Approximately eight hours are required to complete one sampling event. Sampling activities can occur over two consecutive days only if stream conditions remain stable overnight. To minimize the potential impact of one sampling task on all subsequent tasks, the following list is the recommended sequence of sampling activities:

1) Estimate/measure mean stream width, delineate sampling reach, and place block nets (where applicable);
2) Collect water samples for physicochemical water quality parameters;
3) Collect semi-quantitative benthic macroinvertebrate samples;
4) Collect qualitative, multi-habitat benthic macroinvertebrate sample;
5) Conduct fish sampling;
6) Complete physical habitat evaluation

3.0 Benthic Macroinvertebrate Sampling

3.1 Sampling crew

A professional aquatic biologist with expertise in benthic macroinvertebrate sampling and identification supervises the benthic macroinvertebrate sampling. Crewmembers (one or more in addition to the leader) should have a background in aquatic biology and be familiar with benthic macroinvertebrate sampling techniques and common taxa found in Iowa.

3.2 Data collected

Collected from each sampling location are the qualitative and semi-quantitative benthic macroinvertebrate data. The qualitative data gathered ultimately provides a list of the macroinvertebrate taxa sampled from all major types of benthic habitat encountered in the sampling reach. Semi-quantitative data results ultimately provide the relative abundance of each taxon sampled from a standardized sample of productive benthic habitat. The methods employed do not provide quantitative information suitable for calculating density or biomass of benthic macroinvertebrates.

Triplicate samples of either: (a) rock substrates in riffle or shallow run habitat or (b) multi-plate artificial substrates deployed in moderately swift run habitat provide the semi-quantitative data. Combining the list of taxa found in the semi-quantitative samples and the taxa list from the composited multi-habitat sample, generates the qualitative list of taxa.

3.3 Semi-quantitative sampling procedures

To obtain the semi-quantitative samples, use the modified-Hess sampler, the Surber sampler, or the modified Hester-Dendy (multi-plate artificial) substrates, depending on the habitat characteristics of the designated sampling reach. In streams lacking productive riffle or run habitat, use multi-plate artificial substrate sampling devices for collecting macroinvertebrates. A 4-6 week colonization period is required for their use. Artificial substrates are routinely deployed during the reconnaissance visit or in conjunction with a nearby sampling trip. To minimize travel costs, the combination of field trips whenever possible is strongly encouraged.

3.3.1 Modified-Hess sampler

The modified-Hess sampler is an open-ended mesh-enclosed cylinder. The upstream side is a mesh window that allows water to flow through the sampler while keeping all drifting macroinvertebrates out of the sampler. The downstream side of the cylinder has a funnel-shaped mesh collection bag and collection container for capturing macroinvertebrates dislodged as substrates inside the sampler are agitated. The modified-Hess sampler is most effective in shallow riffles and runs (<1.5 feet) with abundant rock substrates. This sampling device performs well in streams where there is a mixture of substrate particle sizes and the sampler can be penetrated 2-4 inches into the stream bottom.

Whenever possible, collect the triplicate samples from the same riffle or run. If the riffle or run is too small to obtain three samples, collect the remaining sample(s) from another suitable riffle or run in the sampling reach. Record observations on the amount and type of periphyton growing on the substrates, the amount of embeddedness of coarse substrates, and the amount of macroinvertebrate colonization on the field data sheet (Appendix 6.1). Apply the following protocol when collecting the modified-Hess samples:

1. Approach the riffle, or sampling area, from downstream to minimize disturbance;
2. Select the area to place the sampler and push the sampler 2-4 inches in to the substrate, with the funnel collection bag downstream;
3. Carefully wash all cobbles and large gravel particles within the cylinder and remove all clinging organisms before discarding;

4. Vigorously agitate the remaining substrate to approximately the same depth as the base of the sampler;
5. Try to rinse as many macroinvertebrates as possible off the sampler and funnel net down into the collection container;
6. Transfer the contents of the collection container and all remaining organisms on the sampler into the sample container.

Process the triplicate modified-Hess samples individually and do not composite them in the field. Add a 10% formalin solution to the sample containers to field preserve them for later analysis. Buffer the sample by adding three grams of borax to one liter of solution to neutralize the pH of the formalin and prevent shrinkage and damage to the tissue of preserved organisms (USGS 1993).

Label the sample containers with indelible ink. The information on the label must include stream name, site identification number, sampling date, collector, and a unique sample identification number. Complete a sample documentation form for each sample according to University of Iowa Hygienic Laboratory (UHL) Limnology field sampling protocols. Record the sample identification numbers on the field observation data sheet.

3.3.2 Artificial substrates

In streams that lack productive riffle or run habitat, use the modified Hester-Dendy artificial substrates to obtain the semi-quantitative samples. Deployment of four multi-plate artificial substrates occurs at each sampling site. The colonization period lasts a minimum of four weeks and must not exceed six weeks. The advantages of artificial substrates, which include habitat standardization and macroinvertebrate productivity, seem to outweigh their disadvantages that include habitat artificiality and taxa selectivity.

Each artificial substrate consists of eight 1/8" x 4" x 4" wood plates and 12 1/8" thick and 1" in diameter cylindrical PVC spacers. The total surface area of the multi-plate unit is 145.6 in^2 (OEPA 1989). Placement of the spacers between the wood plates on a 1/4" threaded steel rod is as follows: three single spacers on top, three double spacers in the middle, and one triple spacer on the bottom.

3.3.3 Artificial substrate placement

Try to deploy the artificial substrates in moderately swift run habitat with firm substrate (sand or sand/gravel, not silt or muck). Apply the following deployment criteria to ensure consistent artificial substrate placement across sampling sites and ecoregions:

1) Deploy the artificial substrates in flowing water having a current velocity of 0.5 to 1.5 feet per second.

2) Deploy the artificial substrates in runs with depths of one to three feet. Consider the anticipated flow stability when determining the appropriate distance from the top plate to the surface of the water. Ideally, deploy the sampling unit in the photic zone of the water column and sufficiently deep to ensure that the top plates remain submersed throughout the four to six week colonization period if flow levels decline. The distance from the top plate to the surface of the water is normally between four and eight inches. The bottom plate should be at least three inches above the bottom to prevent sedimentation of the sampling unit.
3) Deploy the artificial substrate units in the main axis of flow and at least three feet from shore. Place the four sampling units in a diamond configuration approximately three to five feet apart.

Whenever possible, locate the sampling units near the downstream boundary of the sampling reach to enable the benthic macroinvertebrates residing on natural substrates in the sampling reach an opportunity to colonize the artificial substrates via drift. Careful consideration of the susceptibility to vandalism and damage from high flows is critical in the placement of artificial substrates.

Illustrate on a hand-sketched map the location of the substrates with distances to at least two landmarks on shore indicated. Attachment of brightly colored nylon flagging tape to the artificial substrate units may make them easier to find after colonization. Using wooden survey stakes or flagging tape to mark the approximate locations of artificial substrates is also accepted.

3.3.4 Field sample processing

Retrieve the artificial substrates in a downstream to upstream manner. Remove all artificial substrate units present after the colonization period from the stream. Evaluate the status of the substrates and choose the three "best" substrates to process. "Best" is those substrates that are still completely submersed at time of retrieval and free from an extraordinary amount of silt or debris. Samples obtained from heavily damaged or silted units are discarded only after determining that three acceptable samples, containing at least 100 organisms per sample, are available.

Examine each artificial substrate during removal and record the following observations on the field data sheet (Appendix 6.1):

1) amount and type of periphyton growth on the plates;
2) amount of sedimentation and/or other damages to the plates;
3) amount of benthic macroinvertebrate colonization.

Remove the artificial substrates from the streambed with care to minimize the loss of

macroinvertebrates. Carefully remove any extraneous debris, such as leaves or sticks, residing against the sampling unit before removing the unit from the stream bottom. Place a 500μm mesh collection bag over the sampling unit and draw tightly closed at the base to insure that any dislodged organisms are not lost while the artificial substrate is pulled from the stream bed.

Empty the artificial substrate unit and other contents of the collection bag into a white enamel pan containing a small amount of clean water. Remove all clinging organisms from the collection bag with forceps and place in the pan. Disassemble the artificial substrate unit and remove the macroinvertebrates from the plates by gently scraping each plate surface with a single-edge razor blade or pocketknife. Rinse and examine all extraneous debris (e.g., leaves and sticks) for macroinvertebrates and then discard. Transfer the pan contents to a labeled sample jar containing a 10% formalin solution. Use separate labeled containers for the artificial substrate samples and do not composite the samples in the field or laboratory.

Label the sample jars with the following information: stream name, site identification number, sampling date, collector, and the unique sample identification number. Fill out a sample documentation form for each sample according to UHL Limnology field sampling protocols. Record the sample identification numbers on the field observation data sheet.

3.4 Multi-habitat sampling procedures

The purpose of sampling multi-habitat is to increase the number of macroinvertebrate taxa represented on the qualitative list of taxa for the sampling site. Habitat-specific sampling (e.g., riffle-only sampling) is known to result in an underestimate of taxa richness for an entire reach of stream compared to multi-habitat sampling methods (Lenat 1988; Mackey 1984).

Multi-habitat sampling is preferably conducted on the same day but after, or simultaneous to, the retrieval of artificial substrates or natural substrate sampling. The multi-habitat sampling requires two or three crewmembers. Before initiating the sampling, crewmembers must review sampling procedures and divide-up tasks. Time allocation for natural substrate multi-habitat sampling and processing is approximately 1.5 person-hours. In stream reaches that have complex benthic habitat and/or high biological diversity, extend the sampling time to ensure adequate sampling of the reach. Indicate the amount of extra sampling time on the field data sheet.

3.4.1 Benthic habitat inventory

The benthic habitat inventory will aid the crew in organizing sampling activities and characterizes the types of benthic habitat that occur in the sampling reach. The inventory identifies the occurrence of benthic substrates within three types of macrohabitat (i.e., riffle, run, and pool) in the sampling reach. Record each benthic substrate/macrohabitat combination, occurring in significant quantity to be biologically meaningful, on the field observation sheet (Appendix 6.1). Additionally, rank each type of benthic substrate (e.g., woody debris) in terms of relative abundance to the other benthic substrates (i.e., 1 = most abundant, 2 = second most abundant, etc.). Assign these rankings in the left margin of the column listing the types of benthic substrates on the field observation sheet (Appendix 6.1).

3.4.2 Sampling approach

Subdivide the sampling reach into three areas: upper, middle, and lower reach. One crewmember is responsible for each of the areas. Typically, crewmembers use standard No.30 brass sieves to collect and concentrate organisms; however, wash buckets, kick-nets, or other sampling gear are also accepted. The mesh size of all nets, sieves, wash-buckets, or other sampling gear used in multi-habitat sampling ranges from 500-600μm. Collect macroinvertebrates from all accessible types of benthic substrates by handpicking or sieving. Common techniques used to collect insects includes:

- Sieving the gravel, fine substrate, clay hardpan, and overhanging vegetation;
- Disturbing the rocky riffle and run areas by foot and using the sieve as a drift capture tool;
- Handpicking macroinvertebrates from large cobbles and boulders, woody debris, and any other large substrates found in the stream.

It is important to sample as many different substrates as possible by not lingering in one area too long. When three crewmembers conduct the sampling, each crewmember should try to collect approximately 40-50 organisms. When two crewmembers are sampling, each crewmember should try to collect approximately 60-75 organisms. It is important to collect as many different types of organisms as possible. However, if during sampling it appears the taxa richness is minimal, the number of organisms per crewmember mentioned above still applies.

Each crewmember carries a plastic sample container that serves as a temporary receptacle during sampling. At the end of the allotted sampling time (1.5 combined person-hours), combine the sample containers into one labeled sample jar containing a 10% formalin solution. Label the sample container with the following information: stream name, site identification number, sampling date, collector(s), and a unique sample identification number. Complete the sample documentation for the multi-habitat sample according to UHL Limnology field sampling protocol. Record the unique sample identification number on the field observation data sheet.

3.5 Laboratory macroinvertebrate sample processing

Field preserved benthic macroinvertebrate samples are transported to UHL, transferred into 85% ethanol solution, and stored until identification. Obtain (pick) a random subsample of 100 organisms from each triplicate semi-quantitative sample (Modified-Hess or artificial substrate). Sort and identify every organism in the composited qualitative multi-habitat samples (all picks). Initially sort all organisms by order in preparation of the more detailed taxonomic analysis.

Identify the macroinvertebrates in the samples to the "lowest practical taxonomic level." The lowest practical taxonomic level varies between and within invertebrate orders depending on the availability of appropriate taxonomic keys and the amount of time and expertise needed to attain precise determinations. The lowest practical taxonomic level is usually genus or species; however, in certain problematic taxa (e.g., Chironomidae and Oligochaetes) it is family level. If desired, retain several representative individuals of each problematic taxon for a more precise taxonomic analysis later. Follow UHL protocol for taxonomic verification and laboratory QA/QC procedures.

Record the totals of each taxon in the subsample on laboratory bench sheets. Development of an electronic format for data entry is currently underway. The data will eventually reside in the STORET/EDAS database. Following data storage, compare data printouts against laboratory bench sheets similarly to the verification process of the DNR/UHL ambient stream monitoring data in STORET.

4.0 Fish community sampling

4.1 Data collected

The sampling methods described below provide a representative, semi-quantitative sample of the fish community inhabiting the designated sampling

reach. The data collected allow the estimate of the following community parameters of the fish sample:

1) species composition;
2) species relative abundance (i.e., number of fish of each species as a percentage of the total number of captured fish);
3) fish abundance (i.e., catch per unit effort);
4) proportion of fish with external abnormalities.

The methods employed do not provide quantitative information suitable for fish population or biomass estimates.

4.2 Sampling crew

A crew of three to six members using electrofishing techniques samples the fish in the designated sampling reach. A professional biologist with expertise in electrofishing technique and fish identification supervises the crew. The members of the crew must have an educational background in fisheries or aquatic biology. Training in proper electrofishing technique and fish identification is also required. The crew leader is responsible for reviewing sampling duties and safety precautions with the sampling crew before initiating sampling.

4.3 Sampling approach

Fish sampling occurs in the designated stream reach, generally ranging in length from 500-1200 feet. Refer to page four of this document for the explanation of defining a sampling reach. Sketch a map of the sampling reach, including benchmarks and major habitat features, during the physical habitat evaluation.

For this project, fish sampling requires the use of direct current electricity supplied by the electrofishing gear. Sample small headwater streams, with average base-flow widths of less than 15 feet, using a single backpack shocker. Two backpack shockers are used simultaneously in wadeable streams that are too wide (>15 feet) to sample effectively with a single backpack unit. A tow boat electrofishing unit consisting of generator, electrical control box, retractable electrodes, and a live well is used in relatively deep or wide wadeable streams which can not be

effectively sampled with backpack shockers. Record the equipment used during fish sampling on the Fish Data Sheet (Appendices 6.2-6.4).

The crew completes a single pass through the sampling reach proceeding from downstream to upstream to capture fish. It is important to uniformly sample all types of fish habitat. Sample riffles, woody debris snags, and other productive habitats thoroughly by methodically sweeping each area with the shocker electrode(s) in an effort to capture all fish. All stunned fish are collected in 3/16"-mesh landing nets and transferred to plastic buckets or holding tanks until processing. Whenever practical, the upstream and downstream boundaries of the sampling reach are blocked using 3/4" block nets to prevent highly mobile fish (e.g., Catostomid species) from leaving the sampling area.

The amount of time elapsed during the fishing effort is recorded on the Fish Data Sheet (Appendices 6.2-6.4) to allow the catch per unit effort to be determined. This recording can be the elapsed time in hours and minutes or as total seconds used on the backpack shocker(s). Record measurements of stream reach length and average stream width during the habitat evaluation. These measurements allow calculations of the catch per unit effort on a linear or aerial basis.

4.4 Fish identification and examination

Capture stunned fish in the 3/16"-mesh landing nets and transfer the catch to plastic buckets or holding tanks. To prevent unnecessary fish mortality, provide fresh water periodically and keep captivity time to a minimum. At the end of the reach or chosen stopping point, identify and enumerate captured fish before releasing. Fish can be processed and released at several points along the sampling reach or processed at the end of the reach, whichever is more efficient. Exclude fish collected that are less than one inch long from the sample. In addition, examine each for the presence of external abnormalities such as skeletal deformities, eroding fins, lesions, and tumors (DELTs). Record the number of fish per species on the Fish Data Sheet

(Appendices 6.2-6.4). Tally the number of fishes exhibiting one or more abnormality that is external and the types of external abnormalities by species on the Field Data Sheet. Fish examination and DELT coding procedures are adopted from the Ohio EPA fish sampling procedures (OEPA 1989; Appendix 6.5). Fish taxonomy references are available in the field (e.g., IDNR 1987) to assist crewmembers with identification. Preserve all unidentifiable fish in the field with a 10% formalin solution for later laboratory identification by a state fish taxonomist. A voucher/reference collection is currently being established to facilitate accurate future identifications.

5.0 References

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6.0 Appendices

Appendix 6.1 Stream Benthic Macroinvertebrate Community Survey From

Appendix 6.2 Stream Fish Community Survey Form - Mississippi Basin

Appendix 6.3 Stream Fish Community Survey Form - Missouri Basin

Appendix 6.4 Stream Fish Community Survey Form - Coldwater Streams

Appendix 6.5 Methods for Examination of Fish External Abnormalities - Adopted from the Ohio EPA