APPENDIX 4

MACROINVERTEBRATE SAMPLING

Appendix 4
Macroinvertebrate Sampling

The following is copied verbatim from the INDR (2001) sampling protocol, pages 6-14: 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 or 45.7 cm) 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 (5-10 cm) 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 3 samples, collect the remaining samples 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 (see appendix). 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 of 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 3 grams of borax to one liter of solution to neutralize the pH of 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 sampling 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.

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 4 multi-plate artificial substrates occurs at each sampling site. The colonization period lasts a minimum of 4 weeks and must not exceed 6 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 8 - $^1/_8$" x 4" x 4" (or 20.6 cm x 10.2 cm x 10.2 cm) wood plates and 12 - $^1/_8$" thick and 1" diameter (or 30.8 cm thick and 2.5 cm diameter) cylindrical PVC spacers. The total surface area of the multi-plate unit is 145.6 in^2 (0.094 m^2) (OEPA 1989). Placement of the spacers between the wood plates on a $\frac{1}{4}$" (0.64 cm) threaded steel rod is as follows: 3 single spacers on top, 3 double spacers in the middle, and 1 triple spacer on the bottom.

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 (15.2 cm to 45.7 cm per second).
2. Deploy the artificial substrates in runs with depths of 1 to 3 feet (30.8 to 91.4 cm). 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 4 – 6 week colonization period if flow levels decline. The distance from the top plate to the surface of the water is normally between 4 and 8 inches (10.2 to 20.3 cm). The bottom plate should be at least 3 inches (7.62 cm) 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 3 feet (0.91 m) from shore. Place the 4 sampling units in a diamond configuration approximately 3 to 5 feet (0.91 to 1.5 m) 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 in critical in the placement of artificial substrates.

Illustrate on a hand-sketched map, the location of the substrates with distances to at least 2 landmarks on the 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.

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 3 '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 3 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 (see appendix):

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 500 μm mesh collection bag over the sampling unit and draw tightly 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 gentle 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 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.

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 of natural substrate sampling. The multi-habitat sampling requires 2 or 3 crew members. 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.

Sampling approach – Subdivide the sampling reach into 3 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 500-600 m. Collect macroinvertebrates from all accessible types of benthic substrates by handpicking or sieving. Common techniques used to collect insects include:

- 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 1 area too long. When 3 crewmembers conduct the sampling, each crewmember should try to collect approximately 40-50 organisms. When 2 crewmembers are sampling, each should try to collect 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 sampling 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.

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., Chronomidae 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. 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.