Stream Ecosystem Structure: Aquatic Macroinvertebrate Sampling for Restoration Projects

Controls on Macroinvertebrate Community Structure

The river continuum concept (Vannote et al. 1980) describes a predictable change in macroinvertebrate community structure from headwaters to large rivers caused by differences in food resources along the stream/river continuum. Macroinvertebrate assemblages in headwater streams that are dominated by allochthonous inputs possess more species that shred plant material (shredders) than higher order reaches. Middle order reaches are dominated by autochthonous resources (e.g. algae) because they contain open canopies and are relatively shallow. In these reaches, individuals that can remove the algae from the benthic substrates (Scrapers) will be more prevalent than the shredders. In large rivers, autochthonous resources still dominate. However, these resources are floating algae, plant material, and other detritus. In large rivers, species that filter particles from the water column and collect particles from the benthos (filter-gatherers and collector gatherers respectively) dominate the macroinvertebrate assemblage. However, individual species from all functional feeding groups can exist in all streams. As a result, diversity may be determined by the diversity of food resources available to the macroinvertebrate community. Similarly, species can be lost from watersheds when food resources are eliminated (e.g. the removal of the riparian forests and allochthonous inputs). Also, streams in desert and tropical watersheds do not follow the same patterns described by the RCC. Regardless, in these systems macroinvertebrate communities are predictable by the food resources present along the river continuum.

Besides food resources, physical properties of the stream can determine community diversity. While no species is strictly restricted to depositional (e.g. pool) or erosional (e.g. riffle) habitats, many species are more common in one or the other. Benthic substrates differ in each habitat and can support different species. Species adapted to moving between or clinging to cobble substrates in erosional habitats will differ from those adapted to burrowing into sandy substrates of depositional habitats. Discharge, temperature, flow permanence, and other environmental features can also determine what species can exist in a given stream reach. Both availability and type of food and habitat resources will determine what species assemblages exist in a stream reach.

Anthropogenic alterations to the watershed tend to decrease species richness and increase the dominance of a few pollution tolerant taxa (decrease evenness). Also, watersheds heavily influenced by human development are often where invasive species are introduced into lotic ecosystems (e.g. corbicula). Removal of riparian plants, increased impervious surfaces, and increased toxic inputs are important mechanisms for decreased diversity in urbanized watersheds.

The above information is adapted from:

Cummins, K.W. and R.W. Merritt. 1996. Ecology and Distribution of Aquatic Insects. Pages 74-86 *in* **An Introduction to the Aquatic Insects of North America**. R.W. Merritt and K.W. Cummins eds. 3rd edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

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Further References:

Dudley, W.D. and B.W. Feltmate. 1992. **Aquatic Insects**. CAB International, Wallingford, U.K. 358pp.

McCafferty, W.P. 1981. Aquatic Entomology: **The Fishermen's and Ecologists' Illustrated Guide to Insects and Their Relatives**. Science Books International, Boston, MA. 448pp.

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Sampling Benthic Invertebrates

A chapter (Merritt et al. 1996) is provided to explain the various types of sampling devices and the conditions when they are most effective. I will concentrate on the use of Surber Samplers and D-net/Kick-net sampling devices.

Most protocols suggest that sampling riffles only is the best method to obtain data on benthic invertebrate communities for use in IBI and bioassessments. Studies have shown that riffles contain the majority of species found in the stream (though unique species to exist in stream margins), and riffle species are sensitive to disturbance. Also, sampling a single habitat standardizes the samples taken across many sites. However, to assess the diversity of macroinvertebrates in the entire stream, it is important to sample the stream margin and other low flow habitats (Roy et al. 2003). As a result, multi-habitat samples must be taken when species richness is an important part of the bioassessment. Quantitative multi-habitat samples are not easily obtained (see citation above). The easiest method to obtain purely quantitative samples is with a Surber Sampler. This device only works in riffle and run habitats. A D-net is more appropriate for multi-habitat habitat sampling but samples are not quantitative. When designing a study, the goals of the project must taken into consideration when determining the sampling methodology. Specialized equipment may be required if a quantitative, multi-habitat sampling design is required.

Using the Surber Sampler

Benthic collections using a Surber type sampling device must be taken from shallow riffles and/or runs. Because flowing water carries macroinvertebrates into the collecting net as the benthos is disturbed, this sampling device requires specific velocity and depth conditions to work properly. Obviously, samplers of different sizes can accommodate different conditions (e.g. larger samplers can be used in deeper areas, smaller samplers sometimes work better in shallow headwater streams with low velocity).

Figure 1. Sampling using the quantitative Surber Sampler. (Photo courtesy of Emily Duncan)

General Field Collection Protocol

1. **Choose the Sampling Locations:** An appropriate riffle must be chosen in the reach. (Shallow runs can be chosen if riffles are not present or cannot be sampled.) Stream depth must be equal to or lower than the height of the surber sampler but the stream must also be deep enough that water can flow into the sampler. Stream velocity must be adequate for water to pull dislodged organisms into the net. The actual location in the riffle must be chosen at random. A random number table can be used to pick coordinates in the riffle for the exact sampling location. If the location chosen does not meet the standards required for sampling, another site must be chosen. It is more important that the sampling procedure be done appropriately and consistently across all samples.

2. **Set the Surber Sampler:** The base of the sampler (all the way around) (Figure 1) must be firmly against or embedded into the substrate. If space exists below the bottom of the frame, particularly along the back or the sides, organisms dislodged from the benthos may not be captured and diversity and/or abundance may be underestimated.

3. **Initial Benthic Disturbance:** When the sampler is firmly on the bottom, one or two individuals (depending on sampler size) will disturb the streambed in the area inside the samplers frame (Fig.1). Each large rock must be individually cleaned of all invertebrates. This can be done by hand or with a kitchen scrub brush. It is important to make sure that dislodged individuals either flow into the net or are cleaned directly into the net. Often, it is best to clean the rocks while holding them in the net of the sampler. Each large rock should be visually inspected to make sure that all organisms are removed.

4. **Final Benthic Disturbance:** After large rocks on the streambed surface are cleaned and removed, the remaining finer substrates should be disturbed by hand or with some sort of tool (hammer claw, screwdrives, etc.). The benthos is disturbed to a set depth (usually a few inches) depending on the characteristics of the benthos. The overall depth that the streambed is disturbed will vary depending on the site conditions. The sampling depth should remain constant across all sites and throughout the project. Armored streams require greater sampling depths. However, benthic invertebrates will not be carried into the sampling net from deep holes. When working in urban streams, be careful about disturbing the substrate by hand because sharp objects such as glass (and even hypodermic needles) may exist in the substrate.

Other Considerations

This type of sampling provides the advantage of giving a spatially quantitative measure of the benthic invertebrate community. This method should be used when bioassessments require calculating absolute densities. Also, quantitative measures are more appropriate for most statistical analysis.

Water that does not flow (e.g. pools) should not be sampled with this type of device. Also, very shallow, slow moving portions of streams (often encountered in headwaters) also should not be sampled with this device. An alternative quantitative method for sampling pools is with a Hess sampler (Merritt et al. 1996)

This sampler can also not be used when the substrate is very large. The size of the largest benthic substrates must be smaller than the area covered by the base of the surber sampler's frame (Fig 1). Different rules of thumb exist for how to sample substrates half included in the sampling field. In general, I believe it is appropriate to carefully move larger substrates that are partially included in the sampling field and to hand clean only the portion initially included in the sampling area. However, by embedding the frame into the substrate, most particles will sort themselves out and often will be found either entirely inside or outside the frame's base. Areas with larger particles will require larger Surber Samplers or different sampling device.

In urban areas it is important to wear gloves because urban waters may contain disease. Latex rubber gloves should be used when handling samples. Arm length rubber gloves should be considered when sampling with a surber sampler. All equipment should be washed with clean water when field work is finished.

Using the D-net / Kick-net Sampling

D-net and Kick-net sampling devices perform in essentially the same way in riffle and run habitats. Kick-nets consist of a large screen net, usually about a meter wide, between two poles. (Merritt et al. 1996). D-nets are essentially dip-nets with a "D" shaped frame with a fine mesh net (Figure 2). Samples are qualitative or semi-quantitative. Kick-nets usually require 2 people for sampling while a D-net only requires one. However, high velocity streams may require more people for sampling with both devices. Similar to the Surber Sampler, both use flowing water to collect benthic invertebrates when they are dislodged from the stream-bed in riffle and run habitats. However, the D-net offers the advantage of also being able to collect organisms from

still water habitat (pools, stream margins, etc., even wetlands) (Turner and Trexler 1997). As a result, multi-habitat samples can be taken with the D-net. No single mesh size is appropriate for all studies. Smaller mesh sizes will collect more organisms but also finer sediments. Larger mesh sizes will collect less sediment but will allow very small organisms to pass through the net.

Figure 2. Sampling using the D-net. (Photo courtesy of Emily Duncan)

General Protocol Field Collection

Sampling Riffles Using the Kick Method

1. **Choose the Sampling Locations:** An appropriate riffle must be chosen in the reach. Stream depth must be equal to or lower than the height of the D-net. Also, velocity must be adequate for water to pull dislodged organisms into the net. The actual location in the riffle must be chosen at random. A random number table can be used to pick coordinates in the riffle for the exact sampling location. If the location chosen does not meet the standards required for sampling, another site must be chosen. It is more important that the sampling procedure be done appropriately and consistently across all samples. When the reach is determined, physical and chemical parameters (e.g. stream width, flow, conductivity, dissolved oxygen, etc.) should be measured and recorded prior to each sampling event. The parameters measured should be based on the goals of the bioassessment and at a minimum should include the basic physical and chemical properties that are expected to influence invertebrate communities.

2. **Set the D-net Sampler:** The net is placed along the stream bottom (so nothing escapes below the bottom of the net). It is important to make sure that when stream-bed particles are large, the bottom of the D-net frame is between the substrates and the entire width of the net is in contact with the stream bed.

3. **Benthic Disturbance:** The benthos is disturbed by the substrate with your feet while holding the net downstream. Because flow carries benthic organisms into the net (again), kicking should be done primarily towards and away from the net (longitudinally, with stream flow) to ensure that dislodged invertebrates do not flow to the side of the D-net. In order to make the sampling semi-quantitative, kicking is done for a fixed time and in an estimated fixed area. For example, a certain length of stream (one meter is a good length) is sampled at the width of the sampler. Do not sample wider than the net's width. The amount of kicking time will depend on the benthic conditions. For example, armored streams require more sampling time while sandy bottom streams require very little time to thoroughly disturb the benthos.

Sampling Still Water Areas Using the Modified Jab Sampling Method

A jab sample method can be used in still water areas. Studies have shown that sweepnets can be an effective method for assessing invertebrate populations (Turner and Trexler 1997). In lotic ecosystems, low velocity sections of the streams may be shallow (such as backwaters) but often will be much deeper than the height of the D-net. This causes any samples taken with the following methods to be purely qualitative (unlike shallow areas where the entire water column passes through the net).

1. **Choose the Sampling Locations:** An appropriate pool (or other still water area) must be chosen in the reach. No minimal stream depth is required. However, shallower pools will be more effectively sampled. The actual location in the pool must be chosen at random. A random number table can be used to pick coordinates in the riffle for the exact sampling location. If the location chosen does not meet the standards required for sampling, another site must be chosen. It is more important that the sampling procedure be done appropriately and consistently across all samples.

2. **Set the Surber Sampler:** The net is placed along the stream bottom to catch any organisms dislodged initially and carried downstream by low velocities. The net will be moved during sampling so a tight fit with the benthos is less important at this stage.

3. **Benthic Disturbance:** The benthos is disturbed by the substrate with your feet while holding the net downstream. In this habitat, flow will not carry benthic organisms into the net. Kicking should be done slowly so the benthos is disturbed but not disbursed laterally across the pool. Individuals performing the sampling should still try and disturb a fixed area of the stream bed.

4. **Sweep Through the Disturbed Material:** As soon as the benthos is disturbed, the net should be moved through the water column and the suspended benthos materials. As it is moved through this area, the net should be jabbed into the bottom of the stream in order to collect material near the bottom and to further disturb the benthos (this is the traditional jab sample method). This should be repeated several times in order to collect as much material as possible. By moving the net upstream, directly upward, back downstream, and then back to the benthos in a circular manner, the dislodged benthic material will be less dispersed than if the net is simply swept back and forth through the water column.

5. **Sampling Other Structures:** For stream margins, root wads, undercut banks, and large woody debris, a similar qualitative sample can be taken. Instead of disturbing the benthos, an

estimated fixed area is disturbed along the edge of these structures. Again, these samples are purely qualitative. The material is disturbed and the net swept through the water column in a similar way.

Field Processing

The simplest processing method is to take all the benthic material back to the lab. However, this can be impractical for large samples. Also, a larger sample will be more difficult to sort. As a result, it is often a good idea to wash the sample down and remove any large debris (rocks, sticks, leaves) in the field. After the sample is taken with the D-net or Surber Sampler, the benthic material is transferred to a sieve (hole size smaller than or equal to the net on the sampling device). A wash bottle is used to rinse the invertebrates and smaller debris from the larger particles. The sample is then transported from the sieve to a collection container.

During the field sieving process, all vertebrates (and any large invertebrates not of interest) should be released in the field. Small fish and salamanders are often caught in both D/kick-net and Surber Samples. Besides being ecologically responsible, most benthic permits do not allow the incidental take of vertebrates from streams.

Samples should be preserved in at least 70% ethanol. However, the sample will already contain some water and a higher concentration of ethanol should be probably be added to the sample in order to end with at least a 70% concentration. Higher concentrations will not hurt the sample.

Samples must be labeled properly. It is not a good idea to only write on the outside of the container with a marker. Ethanol is a good solvent and can remove many permanent markers. Also, labeling lids can also be problematic. Lids can be taken off and accidentally switched in the lab. The best idea is to use an internal label. Pre-printed labels on waterproof paper that indicate specific site and date information written in pencil work the best (and are inexpensive). A sample label is provided below (Figure 3).

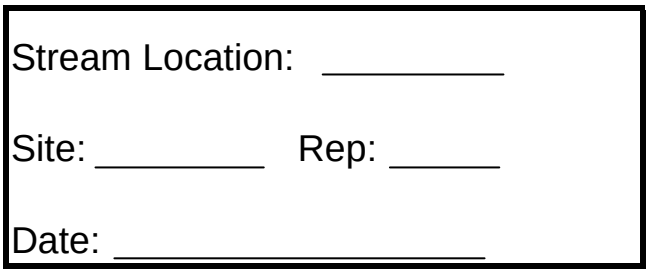

Figure 3. Example of a label used in sample containers.

Lab Processing

Because the Surber Sampler is a quantitative sampler, subsampling should be done by fractioning the entire sample. Fixed count subsamples are less appropriate because the subsamples would not be quantitative. Two different methods can be used for this type of subsampling. A plankton splitter can be used to fractionate samples (into halves) until the desired fraction is separated. Also, samples can be spread over a sieve in the lab and divided into quarters (or halves). To spread the sample out evenly, place the sieve into a small tray with water at a level just above where the sieve's screen will fall when placed in the tray. The water

will help even out the sample. It is important when subsampling not to purposely remove any large organisms in order to include them in the subsample. Each fraction examined should be chosen at random and may exclude larger fauna.

D/kick-net samples can be subsampled and sorted the same was as described above for the samples taken with a Surber Sampler. In fact, some studies suggest that fixed count sub-sampling decreases the ability for macroinvertebrate data to discern between levels of imparity (Doberstein et al. 2000). However, others have shown that fixed count subsamples can be used to detect stream stress for bioassessments. This procedure is most appropriately used with D-net and Kick-net sampling. The general idea is to spread the sample out over a gridded pan and to pick all insects from randomly selected sections until a fixed number of individuals is collected. While 100 individuals has been shown to provide comparable results, 300 individual samples have been proven to most accurately characterize the benthic community in terms of its comparability (Sovell and Vondracek 1999).

Sample Identification

A complete description of the identification of all macroinvertebrates cannot be covered in this course. The best general text and identification key for aquatic insects is Merritt and Cummins (1996 – see list of identification keys). I will present some of the basics in terms of procedures and strategies for identifying aquatic invertebrates. Following this section, I provide a list of identification guides for aquatic macroinvertebrates.

All good keys provide overviews of the morphology of the organisms covered in that key. Understanding the morphological structures of aquatic macroinvertebrates is the most critical aspect of proper identification. Be sure to understand what the key is describing. Always check the referenced figures. If necessary, find other sources that have better diagrams of the morphological structures asked for in the key. The descriptions of organisms in identification keys are usually for late instar insect larvae or adults (depending on the species). Early instar larvae may not be identifiable. All identifications should be performed with a dissecting scope and a high quality light source. This equipment is expensive to buy but is necessary to identify organisms to genus or species. Other useful tools include forceps, dissecting needles, eyedroppers, and watch glasses/petri dishes. A black or white background is best for viewing specimens under the dissecting scope.

A good quality control program should be in place for both sample processing and identification. Sorted subsamples should be resorted to ensure subsampling procedures are done correctly. Also, another taxonomist should recheck all identifications. This could mean sending out a reference collection to a trained professional to provide a consensus on macroinvertebrate identifications.

The Above Information is Adapted From:

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Works Cited:

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Identification Keys:

General Insects, Terrestrial and Aquatic Adults

Borror, D.J., C.A. Triplehorn, and N.F Johnson. 1992. **An introduction to the study of insects**. 6th edition. Saunders College Publishing, Philadelphia.

General Use Keys to Aquatic Larvae

Brigham, A.R., W.U. Brigham, and A. Gnilka (eds.). 1982. Aquatic insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Mahomet, Illinois.

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Midges (Chironomidae)

Epler, J.H. 2001. **Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida**. Special Publication SJ2001-SP13. North Carolina Department of Environment and Natural Resources, Raleigh, NC, and St. Johns River Water Management District, Palatka, FL.

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Wiederholm, T. (Ed.) 1983. Chironomidae of the Holarctic region - Keys and diagnoses, Part 1: Larvae. Entomologica Scandanavica Suppliment 19:1-457.

Other Useful Keys to Species

Adler, P.H., D.C. Currie, and D.M. Wood. 2004. **The Black Flies (Simuliidae) of North America**. Cornell Press. Ithaca, New York.

Brown, H.P. 1976. **Aquatic Dryopid Beetles (Coleoptera) of the United States, Water Pollution Control Research Series 18050 ELDO4/72**. U.S. Environmental Protection Agency, Cincinnati, Ohio.

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