

# **<sup>1</sup>MODULE 4: COLLECTION AND IDENTIFICATION OF MACROINVERTEBRATES FROM DIFFERENT CHANNEL UNITS**

## **Introduction**

Stream macroinvertebrates and their assemblages are influenced by physicochemical features of the water body and landscape-level features of the catchment basin. This occurs because organisms generally live and reproduce under a restricted set of abiotic characteristics (also biotic characteristics, but we won't discuss those yet). Thus, the number of species and abundance of different taxonomic groups often varies among different reaches and channel unit types because reaches and channel units vary in abiotic conditions. For example, chironomids, many of which eat FPOM, are often found in higher abundance in pools, which typically have much more fine sediment, than in riffles. In our initial studies abiotic conditions in our study channel units (Rocky Creek pool and riffle and Bear Creek plane-bed channel unit) varied substantially. It is the purpose of this laboratory to collect macroinvertebrates in the different channel units in order to test the hypothesis that macroinvertebrate assemblages differ among the channel units.

## **Exercise 1: Macroinvertebrate sampling**

In this exercise we will sample macroinvertebrates in our channel units. Macroinvertebrates can be sampled in many different ways using many different types of samplers. I will not provide a full discussion of sampling methods and sampler types in this handout, but have provided (on reserve in the library) a handout called "Design of Aquatic Insect Studies: Collecting, Sampling and Rearing Procedures" and we will view a video on the subject before we go out to collect. I have also provided in the library a handout called "Benthic Stream Algae: Distribution and Structure" which describes sampling methods for algae and an algal key. If you desire further information please see these handouts.

Macroinvertebrate sampling can be either quantitative or qualitative. Quantitative samples typically estimate the density of macroinvertebrates as numbers per unit of stream bottom (usually in square meters). Qualitative sampling methods do not estimate density, but typically give a general assessment of the taxa present, possibly with some observations on their relative abundance. In this laboratory, we will use qualitative sampling to determine the types of aquatic organisms present and their relative abundance.

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In each channel unit we will sample macroinvertebrates using timed, kick net samples. We will use a net called a D-net to take our samples. To take one sample, a student will place the net in the stream downstream of where they are standing. Then the student will disturb the sediment in front of the net and move across the channel unit kicking up the sediment and catching the macroinvertebrates released in the downstream net. One to two minutes should provide many organisms. Students should try to disturb sediments to about a depth of 10 cm because most of the organisms that we will be concerned with live in the top 10 cm.

The mesh size of the net is very important because the larger the mesh, the more types of organisms that can be washed through and, therefore, not be retained on the net. This can cause bias in sampling, since taxa type is often related to size (e.g., chironomids are often very small and stoneflies are very large). Thus, the correct mesh size for a project must be determined by the project's goals. We will use nets with mesh size of 500 $\mu$ m which will capture most organisms that we will be able to identify in the laboratory.

Each sample should be brought back to the stream side and dumped into a wash bucket or sieve. The wash bucket and sieves have mesh size of about 580  $\mu$ m. Large debris, like stones and sticks, should be removed and organisms cleaned off their surfaces by hand. Organisms from the debris should be placed in the wash bucket or sieve. The wash bucket or sieve should be dipped into the stream and fine sediments can be washed out. The material remaining in the wash bucket should be concentrated by washing into another sieve. The material from that sieve should then be washed into a plastic container with 70% ethanol, and covered with 70% ethanol as a preservative. Each sample should be labeled with the channel unit, the date, the collector, and the sample number (1-12) both on the inside (with labeling material provided) and on tape on the outside of the container.

We will collect 4 samples from each channel unit (total of 12 for the class). How we divide up the sampling will depend on the number of groups in each laboratory section. The class data will be combined for the report. Taking 4 samples in each channel unit will allow students to do statistical analyses on their results (using techniques presented in the analysis laboratory).

## **Exercise 2: Sample processing**

Samples will be returned to the laboratory and the samples will be processed. We will be working with these samples for a few weeks, so you must take care to label all your containers clearly. Also, remember that we will share data, so following the plan will ensure that all of the data are comparable. **The general plan for processing the samples appears below:**

1. First, we need to remove a **random sample** of 200 bugs from each sample because we will never be able to identify all the bugs in each sample. We want the sample to be random because if we don't remove the macroinvertebrates randomly we tend to pick out the

largest, most conspicuous “bugs” and the smaller bugs are under represented in our subsample. There are many ways to ensure that we remove a random sample. We could dump the contents of each sample into a gridded sorting pan with some alcohol, stir or slosh the contents of the pan to distribute the bugs, and then use a random number table to choose one grid and remove all the material from that grid. A simpler way that works quite well is to stir the sample in the plastic container with a spoon and then close your eyes and pick two or three spoonfuls from the sample. Either way that you do it, the subsample (from the grid or from the spoon) should be placed in a clean container with 70% alcohol. Be sure to label the new container with all the information from the original, plus indicate that it is a subsample (using the labeling material provided).

Take small amount of your random subsample and place in a watch glass with some alcohol. Under a stereo microscope use the forceps and pipettes provided to **pick out all bugs, worms etc.** and place in another clean plastic container with some 70% alcohol. **Be sure to label this new container with all the information from the original, plus indicate that it is the “picked portion.” Thus, each sample will have 3 plastic containers—the original container, the subsample and the picked portion, all of which should be clearly labeled!** All the organisms should be picked from the subsample. We need a total of around 200 organisms. If you pick out 200 from your first subsample, then that sample is done. However, this is unlikely to occur. After you are finished with the first subsample, throw the debris out in the sieve in the sink. Continue to take subsamples as above until about 200 organisms have been removed. If two people work on one sample, please work together & pick into the same container. When all samples in the class have been processed this way, move on to step two. **The picking process will probably take more than one class period, so be sure that all your samples are in the plastic containers and labeled ready for the next period.**

2. Sort the organisms from each of the “picked samples” based on their general appearance. While you are sorting you can put those organisms that are similar into the well-plates provided and cover them with more 70% ethanol. **Place similar organisms into separate wells in 12- or 24-well plates. This process will probably take more than one class period, so at the end of the period, the organisms from the wells should be transferred to vials with screw lids, covered with 70% ethanol and labeled. Never leave organisms from one week to the next in the well plates as the alcohol will evaporate and the sample will be unuseable.**

3. Use the keys to begin to identify the organisms. We will discuss how to use the keys in class. Begin with the “Key to Major Taxonomic Groups of Freshwater Invertebrates” which is found in this laboratory manual. If the organism is an insect proceed to the insect key which you will find on reserve in the library. Organisms should be identified to the lowest taxonomic unit as given in Table 1 (only those taxonomic units in bold are likely to be found). Insects will be identified to Order. Organisms in each taxonomic unit (e.g., molluscs, gastropods; mayflies) should be placed in a separate vial and labeled.

**You must be organized through out this process. Keep similar organisms together in well plates and vials. Make sure everything is adequately labeled. We will begin to analyze these organisms in the next laboratory.**

### **Literature Cited**

Cummins, R.W. and M.A. Wilzbach. 1985. Field procedures for analysis of functional feeding groups of stream macroinvertebrates. Pymatuning Laboratory of Ecology, University of Pittsburgh.

Merritt, R.W. and K.W. Cummins 1996. Trophic relations of macroinvertebrates. Pp. 453 - 474, in "Methods in Stream Ecology" , Hauer, F.R. and Lamberti, G.A. (Eds), Academic Press, N.Y.

### **Assignment:**

There is no assignment for this laboratory; however, we will make extensive use of these data in the next Module.

## **Table 1. Common Freshwater Macroinvertebrate Taxa**

- I. Kingdom Animalia--**
  - A. Phylum Porifera--**freshwater sponges
  - B. Phylum Cnidaria (Coelenterata)--**freshwater jellyfish & hydra
  - C. Phylum Platyhelminthes--**flatworms (i.e., planarians)
  - D. Phylum Nematoda--**nematodes
  - E. Phylum Mollusca**
    - 1. **Class Gastropoda--**snails & limpets
    - 2. **Class Bivalvia--**clams and mussels
  - F. Phylum Annelida-**
    - 1. **Class Oligochaeta--**segmented worms
    - 2. **Class Hirudinea--**leeches
  - G. Phyla Ectoprocta (Bryozoa) & Entoprocta--**Lophophorate animals
  - H. Phylum Arthropoda--**animals with an exoskeleton
    - 1. **Subphylum Chelicerata--**water spiders and mites
    - 2. **Subphylum Crustacea--**crayfish, freshwater shrimp, skuds (amphipods) and isopods and microscopic copepods, cladocerans and others
    - 3. **Subphylum Uniramia--**insects & relatives
      - a. **Class Insecta--**and insects and springtails
        - a. **Order Collembola--**springtails
        - b. **Order Ephemeroptera--**mayflies
        - c. **Order Plecoptera--**stoneflies
        - d. **Order Trichoptera--**caddisflies
        - e. **Order Odonata--**damsel flies and dragonflies
        - f. **Order Hemiptera--**true bugs (e.g., water striders)
        - g. **Order Coleoptera--**beetles
        - h. **Order Lepidoptera--**moths
        - i. **Order Megaloptera--**Alderflies
        - j. **Order Diptera--**true flies

## <sup>12</sup>Key to the Major Taxonomic Groups of Freshwater Invertebrate

1a. Unicellular organisms; microscopic (most 10 to 200 μm and too small to be seen by the naked eye); present as individuals or colonies; heterotrophic and/or autotrophic; typically not captured because mesh sizes of sampling gears are too large; protists. . . . . Kingdom Protista



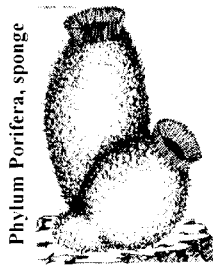
Kingdom Protista, ciliate



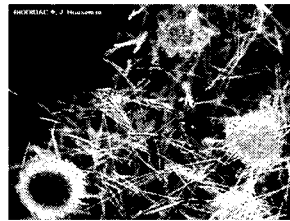
Kingdom Protista, ciliate

1b. Multicellular, heterotrophic organisms (sometimes with symbiotic autotrophs) . . . . . 2

2a (1b). Multicellular animals without discrete organs and with a tissue-level construction: body form and size variable, in flowing water may be crusts on rocks or twigs; typically not found in with sampling gears, but can be found by examining rock and twigs; sponges . . . . . **Phylum Porifera**



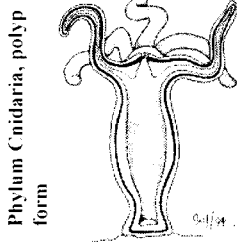
Phylum Porifera, sponge



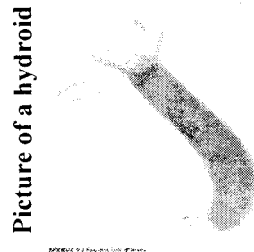
Gemmules of a freshwater sponge

2b. Multicellular animals with organ or organ-system construction and 2-3 embryonic cell layers . . . . . 3

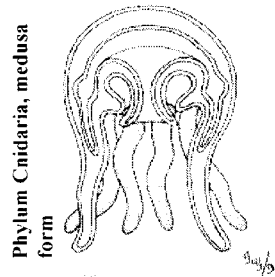
3a (2b). Two embryonic cell layers; adults with a central body cavity opening to the exterior; radially symmetric; typical lifecycle alternates between polyps (hydroid form) and medusae (jellyfish form, e.g., *Craspedacusta*); typically not found in with sampling gear as specimens often destroyed; polyps found by examining rocks and twigs; medusa often ephemeral . . . . . **Phylum Cnidaria**



Phylum Cnidaria, polyp form



Picture of a hydroid



Phylum Cnidaria, medusa form



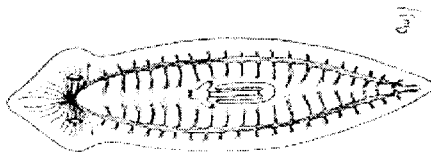
*Craspedacusta*

3b. Three embryonic cell layers; adults with cellular ectoderm, mesoderm, and endoderm; bilateral symmetry typical (few asymmetric and/or colonial) . . . . . 4

<sup>1</sup>Most figures were used with permission of the BIODIDAC project funded by the "Programme de perfectionnement linguistique" from Heritage Canada and the Government of Quebec through RUFHQ and the University of Ottawa. A few figures are public domain or are the authors.

<sup>2</sup>Last modified 8/22/2001; Keys modified from Thorp and Covich (1991)

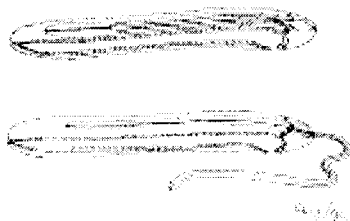
4a (3b). Flattened or cylindrical, bilateral, acoelomate worms with one opening to the digestive tract (incomplete); in fresh water streams typically < 5mm in length; bodies may be deformed by preservatives; not often captured with typical sampling methods; flatworms (some groups occasionally called planarians ) ..... Phylum Platyhelminthes, Class Turbellaria



**Phylum Platyhelminthes, planarian**

4b. Pseudocoelomate or coelomate eumetazoans with a complete digestive tract (mouth to anus) ..... 5

5a (4b). Flattened, unsegmented worms with an eversible proboscis; not typically taken in most sampling regimes; ribbon worm ..... Phylum Nemertea



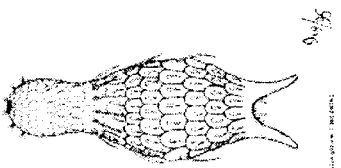
**Phylum Nemertea, ribbon worms**

5b. Not with above characteristics ..... 6

6a (5b). Pseudocoelomates; legs, lophophorate tentacles, and, segmentation absent; gastrotrichs, rotifers, roundworms or hairworms; small; only roundworms typically taken with most sampling gears ..... 7

6b. Coelomates; legs, lophophorate tentacles, and/or segments present; snails and mussels, segmented worms, bryozoans, water bears and arthropods (mites, insects and crustaceans) ..... 10

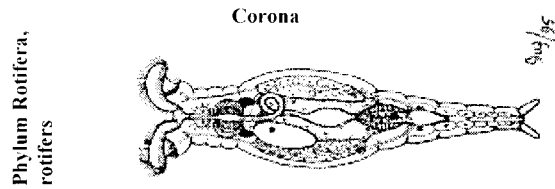
7a (6a). Small, microscopic (50 - 800µm), spindle shaped, ventrally flattened; cuticle usually ornamented with spines or scales ..... Phylum Gastrotricha



**Phylum Gastrotricha, gastrotrich**

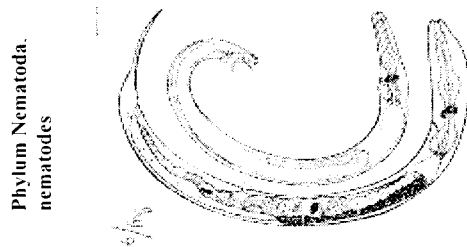
7b. Not with the above characteristics ..... 8

8a (7b). Not worm-like, microscopic (typically 100 - 1000  $\mu\text{m}$  in length); pseudocoelomates with apical corona, mastax and jaws; typically found in the plankton and not found with most benthic sampling gears; wheel animals, rotifers . . . . . **Phylum Rotifera**



8b. Not with the above characteristics; nonsegmented worms . . . . . 9

9a (8b). Complete digestive tract present; cylindrical body usually tapering at both ends; most under 1 cm long (except family Mermithidae); roundworms . . . . . **Phylum Nematoda**

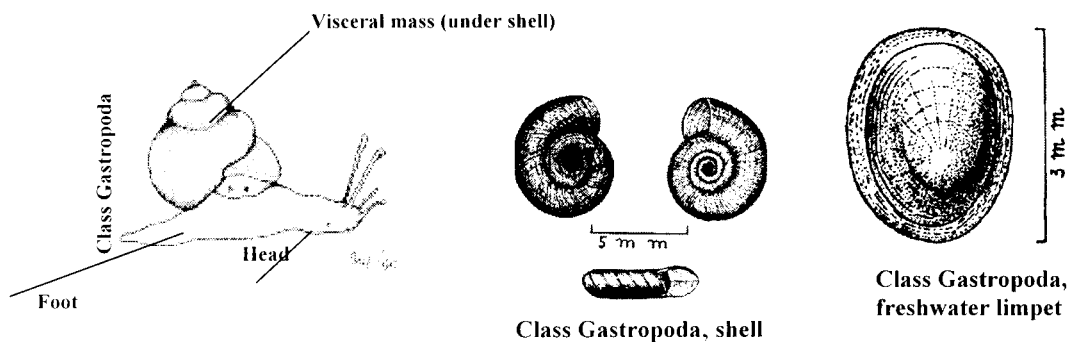


9b. Anterior and posterior tips of the body rounded; length several cm to 1 m, width 0.25-3mm; only adults with free-living stage; not typical but may be encountered; hairworms or horsehair worms . . . . . **Phylum Nematomorpha**

10a (6b). Soft-bodied coelomates, usually with a hard calcareous shell; most with ciliated gills, a ventral muscular foot, and a fleshy mantle covering the internal organs; snails and mussels . . . . . **Phylum Mollusca**.....11

10 b. Body not enclosed in a single, spiraled shell or in a hinged, bivalved shell, or if a bivalved shell is present, then the animal has jointed legs . . . . . 12

11a (10a). Body enclosed in a single shell, which usually has obvious spiral coils (limpets are the exception); body basically partitioned into head, foot, and visceral mass; snails and limpets . . . . . **Phylum Mollusca, Class Gastropoda**



11b. Body enclosed in two, hinged shells; head absent: mussels and clams . . . . . **Phylum Mollusca, Class Bivalvia**



**Freshwater mussel shell**



**Life cycle of freshwater mussel**

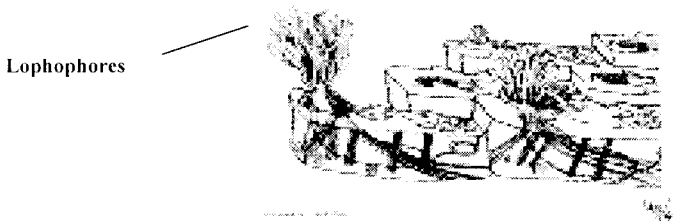


**The zebra mussel**

12a (10b). Legs absent in all life stages . . . . . **13**

12b. Adults and most larval stages with legs; if larvae without legs or prolegs (small nonjointed appendages in some insects), then head region with paired mandibles (jaws); chitinous exoskeleton covers all or part of body . . . . . **Phylum Arthropoda.....15**

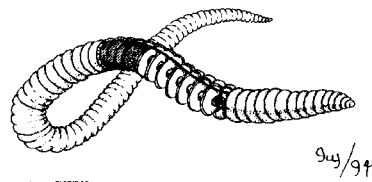
13a (12a). Feeding tentacles (lophophores) present; many colonial and asymmetric; not often found with typical benthic samples; can be found by examining rocks and twigs: bryozoans . . . . . **Phyla Ectoprocta & Entoprocta**



**Bryozoan closeup with lophophore**

13b. Lophophorate tentacles absent; segmented worms . . . . . **14**

14a (13b). No suckers and usually four bundles of chaetae on each segment except the first (**Classes Oligochaeta** and Aphanoneura), or posterior, disc-shaped sucker on segment 11 and head composed of peristomium and three segments (Class Branchiobdellida); Oligochaeta, common in typical benthic samples, others typically uncommon, Aphanoneura small (1-2mm), even microscopic, Branchiobdellida, parasites of crustaceans . . . **Phylum Annelida, Class Oligochaeta**



**Class Oligochaeta, tubificids and others**



**Freshwater tubificids**

