CE3502. ENVIRONMENTAL MEASUREMENTS, MONITORING AND DATA ANALYSIS

Lab: 8
Environmental Engineering Topic: Stream Ecology
Statistics Topic: ANOVA

Environmental Engineering Background

The effects of pollutants on the environment often are subtle. Pollutants often are present in low concentrations that are not lethal to organisms. Nevertheless, some species of organisms may be more susceptible to the pollutants than others. Because ecosystem health is the real goal of pollution control, it is appropriate for environmental engineers and scientists to assess ecosystem health rather than just to measure the concentrations of pollutants. One of the common methods for assessment of ecosystem health is to perform biological surveys. Such surveys can show (1) whether the total number and variety of species present in one location differs substantially from other similar locations, (2) whether the number of individual organisms in one location differs significantly from the numbers present in other similar locations, and, (3) if the surveys are repeated several times, whether the populations are changing with time.

There is always considerable variability in the numbers of individuals and numbers of species present in natural environments. Consequently, any effort to quantify organisms or species must use statistically rigorous techniques. Large numbers of samples (organisms), large survey areas, and multiple areas for surveying are required. The results of such surveys are multiple groups of data that must be assessed for differences; ANOVA is the standard statistical tool for such an assessment.

Biological indicators often are used to assess stream health. Streams and rivers, because of water flow, are fundamentally different than lakes. The relatively large surface area, the shallow depth, and the constant water movement enhance oxygen transfer from the atmosphere, and, as a result, anoxia in streams and rivers is seldom encountered. However, high nutrient loadings (causing prolific algal growth) or high loadings of organic matter can lead to oxygen drawdown to the point where the oxygen is insufficient for fish. More commonly, excessive algal growth or sewage inputs lead to deposition of fine organic matter on the streambed; this organic matter then decomposes and leads to low oxygen or anoxic conditions in the surface sediments. These low oxygen conditions are inimical to the presence of many macroinvertebrates. The high-oxygen-requiring invertebrates are replaced by other species with greater tolerance for low oxygen conditions. Consequently, one method of assessing the "health" of a stream is to survey the macroinvertebrates that are present. The pages at the end of this handout describe one grouping of organisms that can indicate the stream health with respect to oxygen.

Some of the basic chemical descriptors of a stream environment include the temperature, the dissolved oxygen content, the nutrient content, the turbidity, and various factors related to the composition of the dissolved ions. Water "hardness" distinguishes waters with high Ca^{2+} and Mg^{2+} concentrations (hard water) from waters with low concentrations. Hard water is common in any area with limestone present in the soils.

Salinity refers to the amount of salt present; it is an important indicator of seawater in coastal areas or of relict seawater and evaporite deposits in continental areas. One other generic descriptor of the dissolved ions is termed "total dissolved solids" (TDS). Total dissolved solids is defined as the mass of solids dissolved per unit volume of water. The TDS concentration may be measured be evaporating a pre-filtered sample of water to dryness, and then weighing the residue of solids. A second method is to measure the conductivity of the water. Conductivity is a measure of the ability of the water to conduct electrical current; the conductivity depends on the total number of ions present in the water. Typically, there is a linear relationship between conductivity and TDS.

The TDS concentration in a body of water is affected by many different factors (Table 1). A high concentration of dissolved ions is not, by itself, an indication that a stream is polluted or unhealthy. It is normal for streams to dissolve and to accumulate fairly high concentrations of ions from the minerals in the rocks and soils over which they flow. If easily soluble minerals are present in the rocks and soils (e.g., limestone, gypsum, halite), then high concentrations of TDS may develop naturally.

Table 1. Common sources of total dissolved solids

Source of ions	**Typical ions**
Mineral dissolution	Ca^{2+}, Mg^{2+}, HCO_3^-, SO_4^{2-}, $Si(OH)_2$
Ground water discharge	Ca^{2+}, Mg^{2+}, HCO_3^-, Fe^{2+}, $Si(OH)_2$
Seawater (inflow of marine waters, saltwater intrusion in groundwaters)	Na^+, Cl^-, Mg^{2+}
Evaporation of water – irrigation in arid areas causes large increases in TDS	Ca^{2+}, Mg^{2+}, HCO_3^-, SO_4^{2-}, Na^+, Cl^-
Agricultural runoff	NH_4^+, NO_3^-, PO_4^{2-}
Urban runoff (road salt), disinfection	Na^+, Cl^-
Acid deposition	H^+, NO_3^-, SO_4^{2-}

Total Dissolved Solids is sometimes used as a "watchdog" test. Any change in the ionic composition between testing sites in a stream can be detected easily using a conductivity probe. The TDS concentrations will change in response to any inputs of major ions, but they will not change in response to trace metals nor to organic pollutants. Because many of the dissolved ions are conservative, the TDS often increases from the headwaters of a stream to its mouth as a result of the increasing flowpath of water over soils as well as the cumulative anthropogenic effects. Sudden changes in TDS along the length of a stream seldom occur naturally, and therefore TDS is a useful indicator of pollution.

As indicated in Table 1, there are many possible anthropogenic sources of ions that may contribute to elevated TDS. Fertilizers from farm fields or lawns can add a variety of ions. Runoff from roads that have been salted in winter can increase TDS. Wastewater treatment plants typically discharge waters high in TDS; the high ion content results not so much from the nutrients in the wastewater as from the chlorine that is added in this country as a disinfectant. Irrigation water that is returned to a stream often will have high dissolved solids.

Only in extreme cases is TDS itself harmful to aquatic life. However, irrigation water can often have demonstrable effects on stream biota. In the province of Ontario,

Canada, major changes in fish populations have been occurred as a result of runoff of salt from roads. Onondaga Lake in New York had such high TDS (a result of glass manufacturing) that marine organisms rather than freshwater organisms were found in the lake. High TDS generally is regarded as undesirable by humans. In drinking water, high TDS can act as a laxative as well as create an undesirable taste. Soaps and detergents are less efficient in hard water than in soft water.

TDS values in lakes and streams typically lie in the range of 50 to 250 mg/L. In areas of particularly hard water or high salinity, TDS concentrations may be as high as 500 mg/L. In the U.S., there is a non-binding recommendation that TDS in drinking water not exceed 500 mg/L. Domestic wastewater may have levels up to 1000 mg/L.

Table 2. TDS concentrations in selected rivers

Location	Season	TDS (mg/L)	Season	TDS (mg/L)
Rio Grande River, El Paso, TX	Spring	510	Fall	610
Mississippi River, Memphis, TN	Spring	133	Fall	220
Sacramento River, Keswick, CA	Spring	71	Fall	60
Ohio River, Benwood, WV	Spring	300	Fall	143
Hudson River, Poughkeepsie, NY	Spring	90	Fall	119

Huron Creek Watershed Management Plan

Aquatic ecosystems are frequently impacted heavily by human activities in the watershed. While point-source discharges of pollutants are regulated, the more diffuse effects of human activities in the watershed are often not specifically regulated and can be more difficult to control. For instance, activities as diverse as building a house, spreading sand on roads, farming, and logging all can lead to runoff of fine soil into streams. In the face of multiple sources of "pollutants" and habitat degradation, it often is desirable to plan holistically and to consider all of the factors affecting the water quality. Watershed Management Plans represent an effort (1) to assess all of the threats facing streams and rivers, (2) to involve a wide array of the "stakeholders" who have an interest either in the health of the ecosystem or in the practices threatening the ecosystem, and (3) to develop plans to protect and maintain the health of the ecosystem. Both state and federal regulations now require Watershed Management Plans when state or federal monies are being used, or when stream or river ecosystems are not meeting the legal limits for water quality.

Huron Creek in Houghton is a typical example of a heavily impacted "urban" stream. The stream is relatively short (~ 5 km); it begins in an undeveloped tract of land south of Green Acres Road, flows through a new housing development in Dodgeville,

flows through an impounded wetland (the site of former mining activities) before receiving the discharge from holding ponds that collect runoff from the Copper Country Mall and Walmart parking areas. Below Walmart, the stream flows through a steep gorge with parking lots on both sides, it flows through a former landfill site, it flows past houses with septic systems and it flows adjacent to Highway 26 from which it receives road runoff. The stream empties into the Keweenaw Waterway in the public park west of town. Roughly half of the original stream channel has been altered or moved. Because of the steep terrain and the impervious surfaces in the drainage basin, the stream is very "flashy"; i.e., flow increases rapidly following precipitation events and then decreases rapidly. Potential sources of contaminants include road and parking lot runoff, soil erosion associated with construction and road building, septic leachate, metals from mine tailings, and landfill leachate. Until 2006, Huron Creek was regarded primarily as a conduit to carry water and waste.

A group of stakeholders has developed a Watershed Management Plan for Huron Creek. This process involves (1) identifying and involving stakeholders in the discussion and planning process; (2) identifying concerns, gathering and analyzing information and data, defining challenges/opportunities, developing objectives, and documenting data and decisions; (3) developing a plan for addressing the objectives, selecting among management alternative(s), listing ways (strategies) for implementing the selected alternative(s), and determining how to measure progress; and (4) implementing and evaluating efforts. Michigan Tech faculty and students have been involved in the planning process from the outset, and this class will contribute to the collection of data that will help to measure and to evaluate progress in dealing with current problems.

Statistical Background

A detailed description of the theory behind ANOVA as well as the procedures for conducting ANOVA is provided in the text book (Chapter 9 of Navidi 2006; Chap. 9 in McBean and Rovers 1998; Chap. 24 Berthouex and Brown 2002).

Laboratory Procedures

Materials needed

Rubber gloves
Net (optional)
Zip-loc bags (10/group)
Conductivity probe
500 mg/L TDS standard solution
(Temperature probe)
pH probe (or field pH meter)
Hydrolab or other field DO meter
Field Turbidity meter
Stop watch (or velocimeter)
Boots
Wash bottle with distilled water
Plastic beaker
White wash trays
forceps, eye droppers
ice-cube trays
Kimwipes
pH buffer solutions (4,7,10)
meter stick, tape measure

Procedure:

Each lab section will visit two stream locations (Green Acres Road culvert, Frog Pond, Sharon Ave. culvert, Chutes and Ladders Park). At each sampling location, each group

will collect 10 measurements using one of the field probes (Conductivity, pH, DO, Turbidity), and 5 net "jabs" for enumeration of organisms. Material collected in the nets will be placed in Nalgene bottles and zip-loc bags and returned to the lab for counting of organisms. To maintain consistency and minimize field time, groups will perform the same measurements at each sampling site that is visited. We will drive past each site so that you can see the differences, and you will receive the data from all of the sites.

A. *Conductivity (TDS) and Temperature measurements*
    1. Collect Conductivity measurements using Hach dQ40 meter with conductivity probe that has been calibrated in the lab by the TA.
        i. Turn on the meter, place the probe in the water and determine how long it takes for the reading to stabilize; record the reading of temperature and conductivity.
        ii. Repeat step *i* 9 more times by moving around the river; the goal is to measure the variability of conductivity at each site.

B. *Dissolved Oxygen Measurements (Hach dQ40 or dQ20 with LDO)*
    1. The Hach LDO probe will be calibrated in the lab by the TA prior to class.
    2. When the group is at the side of the stream, turn on the Hach probe by pressing the power button.
    3. Immerse the probe in the stream and determine how long it takes for the probe to reach a stable reading.
    4. Examine the variability in temperature and DO across the width of the stream channel. Based on this survey, choose a sampling strategy that will capture all of the variability that you observed.
    5. Collect a total of 10 paired measurements of temperature and dissolved oxygen; allow the readings to stabilize at each sampling point before recording the values.

C. Measurement of pH (Hach dQ40 with pH probe)
    1. The pH meter will be calibrated in the lab by the TA prior to the class.
    2. When the group is at the side of the stream, turn on the pH meter by pressing the power button; the meter will power up in the pH measure mode.
    3. Immerse the probe in the stream and determine the time required for the reading to stabilize. You will have to allow this amount of time whenever you move to a new location.
    4. Examine the variability in pH across the width of the stream channel. Based on this survey, choose a sampling strategy that will capture all of the variability that you observed.
    5. Collect a total of 10 measurements of pH; allow the readings to stabilize at each sampling point before recording the values.
    6. Once back in the lab, record the pH of three buffer solutions.

D. Turbidity Measurement
    1. You will collect 10 samples at each station by filling the small glass vials that are then inserted into the meter for measurement. To minimize the need for drying the outside of the vial, dip a plastic beaker in the stream to obtain your sample and then pour from the beaker into the vials. Sample across the width of the stream at varying heights above the bed; always sample upstream of where you are standing to avoid sampling artifacts from sediments that you have disturbed. You have 5-10 vials; fill them and then empty and refill them as needed to obtain 10 measurements.
    2. To make the measurements, first turn on the meter by pressing the power button. Wipe the vial with a kimwipe, and then insert the vial into the turbidimeter. Press *Read* button, wait for a reading to appear and record on the attached sheet.
    3. When you return to the lab, measure and record all of the standards in the carrying case.

E. Invertebrate collection
    1. Each group will collect invertebrates by making 5 "jabs" with a net. In a jab, the D-net is pulled upstream for about 0.5 m while scraping the bottom of the stream. Alternatively, one may push the net firmly to the stream bottom (open side facing upstream), and while holding the net stationary use one's foot to stir up the stream bottom for 0.5 m upstream of the net. If there are rocks or logs in the stream, the approach has to be modified; in this case, the rocks in the sampling area are held upstream of the net and the surfaces are scrubbed with a brush so all dislodged organisms float downstream into the net.
    2. After making the five jabs, the contents of the net are emptied into a plastic tray. It may be necessary to wash organisms out of the net with water; be sure that all organisms are retrieved from the net. Any rocks, branches or large plant parts are placed into plastic Ziploc bags. The water and finer materials are poured into 1-L plastic bottles for transport to the lab.
    3. In the lab, dump the rocks and large objects from the plastic bag(s) into a white plastic basin. Place the basin in the sink and, while running tap water slowly, scrub the rock/object gently to dislodge any organisms. When all objects have been scrubbed, pour the contents of the plastic 1-L bottle into the tub also.
    4. Collect and sort the organisms by placing each type of organism into a separate portion of an ice-cube tray. You may use forceps and eye droppers to pick out the organisms. Record the number and types (use key at end of handout) of organisms on each rock.
    5. Leave a record of all measurements with the TA before leaving the lab.

F. Flow Assessment

1. We will measure the dimensions necessary to estimate flow rates in sections of the stream with defined geometries (culverts). Flow will be estimated from Manning's formula:

$$Q = 1.49S^{0.5}A(R^{2/3})/n$$

To determine A,R and n we must record the depth of water (y), the culvert diameter (d), and the construction material. We have no means of measuring S, so it will be estimated. Further relationships that are invoked for a circular culvert:

$$\theta = 2\cos^{-1}\left(1-2\frac{y}{d}\right)$$

$$A = \frac{d^2}{8}\left(\theta - \sin\theta\right)$$

$$P = \frac{d}{2}\theta$$

$$R = \frac{A}{P} = \left(1-\frac{\sin\theta}{\theta}\right)\frac{d}{4}$$

where θ is in radians, and y,d,A,P and R are in consistent units (English or metric).

2. For a circular culvert, measure the water depth and culvert diameter and record the construction material. For square or rectangular culverts, record the water depth, the culvert width and the construction material.

Lab Report

Everyone will receive the data from all groups.

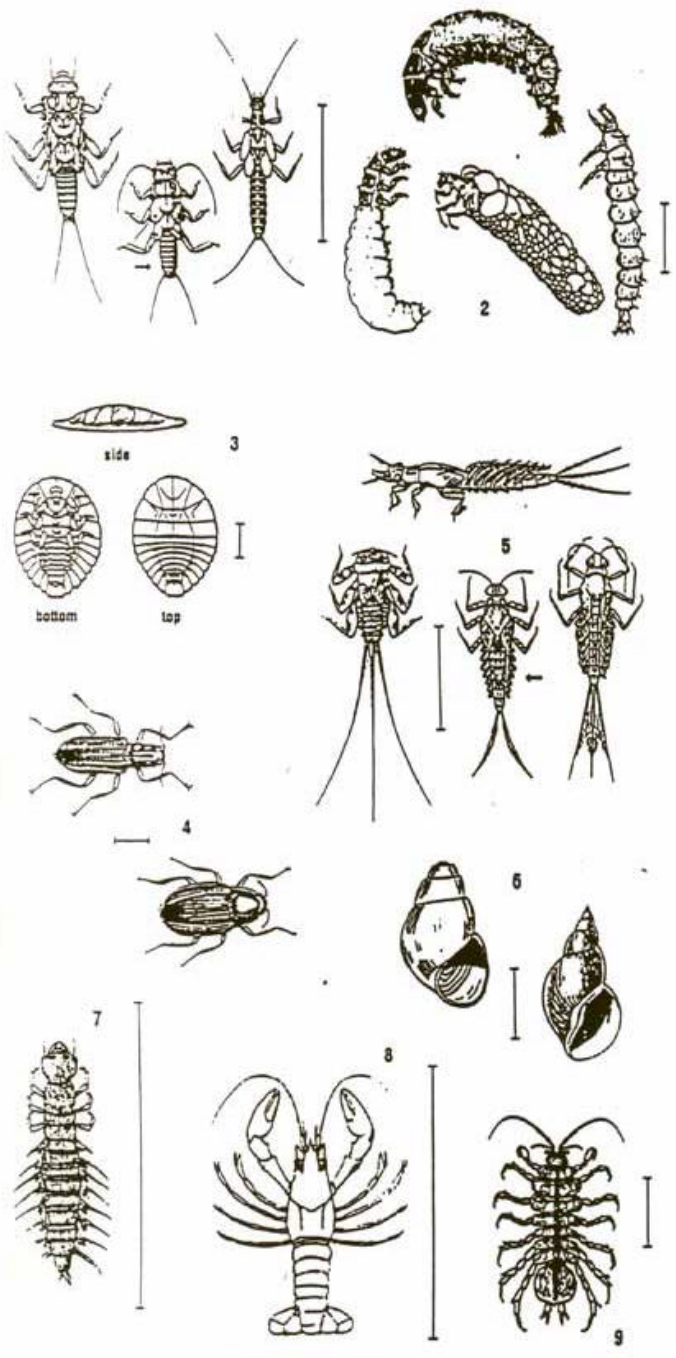
Results:

1. Tabulate the summary statistics (Mean, median, standard deviation, COV) for the temperature, conductivity, turbidity, pH, and DO for each sampling location;
2. Tabulate the Biosurvey results for each station; add the results for all groups so that for each type of organism you have the total number of individuals collected at a given site as well as the five (or four) group totals; you will also want the total number of organism types found by each group at each station;
3. Make a pie chart for each station showing the percentage of each type of organism (this may be presented in the discussion if appropriate);
4. Plot the residuals for conductivity, turbidity, DO, pH, temperature, and number of organism types; in the report make a table in which is tabulated whether each variable passes the test for heteroscedasticity. Include in the report the residuals graph for any variables that do not pass this test.
5. Provide the ANOVA results for the conductivity (or TDS), turbidity, DO, pH, temperature, flow, and number of organism types (assuming that all passed the test for heteroscedasticity);

Discussion

1. Are there qualitative differences in the assemblages of organisms at the different sampling stations?

2. Does the table of temperature, conductivity, DO, pH and turbidity suggest that there are systematic differences in water quality among the sampling locations? Describe and illustrate any spatial trends that you observe.
3. Does the ANOVA indicate that there are any significant differences among the sampling stations?
4. If there are significant differences, which station is different? Explain what factors might cause the sites to differ if there are differences.



Bar lines indicate relative size

Stream Insects & Crustaceans

GROUP ONE TAXA

Pollution sensitive organisms found in good quality water.

1 *Stonefly: Order Plecoptera.* 1/2" - 1 1/2", 6 legs with hooked tips, antennae, 2 hair-like tails. Smooth (no gills) on lower half of body. (See arrow.)

2 *Caddisfly: Order Trichoptera.* Up to 1", 6 hooked legs on upper third of body, 2 hooks at back end. May be in a stick, rock or leaf case with its head sticking out. May have fluffy gill tufts on lower half.

3 *Water Penny: Order Coleoptera.* 1/4", flat saucer-shaped body with a raised bump on one side and 6 tiny legs on the other side. Immature beetle.

4 *Riffle Beetle: Order Coleoptera.* 1/4", oval body covered with tiny hairs, 6 legs, antennae. Walks slowly underwater. Does not swim on surface.

5 *Mayfly: Order Ephemeroptera.* 1/4" - 1", brown, moving, plate-like or feathery gills on sides of lower body (see arrow), 6 large hooked legs, antennae, 2 or 3 long, hair-like tails. Tails may be webbed together.

6 *Gilled Snail: Class Gastropoda.* Shell opening covered by thin plate called operculum. Shell usually opens on right.

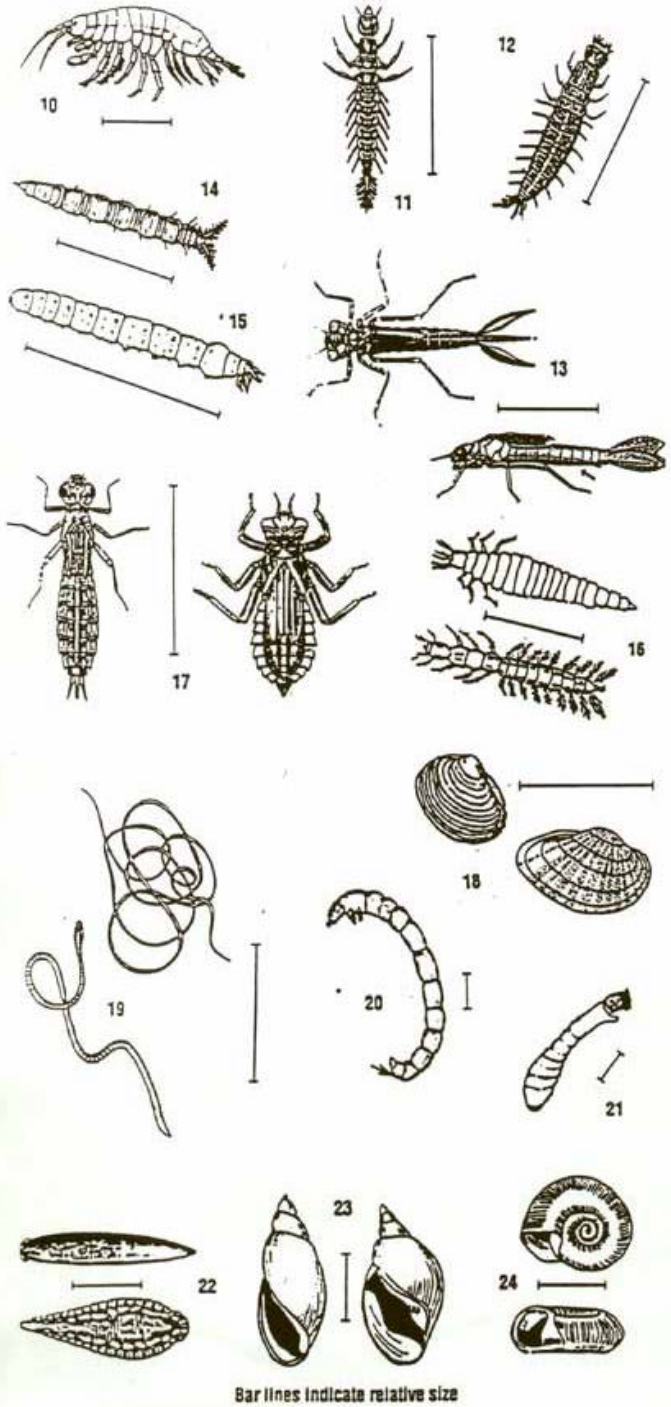
7 *Dobsonfly (Hellgrammite): Family Corydalidae.* 3/4" - 4", dark-colored, 6 legs, large pinching jaws, eight pairs feelers on lower half of body with paired cotton-like gill tufts along underside, short antennae, 2 tails and 2 pairs of hooks at back end.

GROUP TWO TAXA

Somewhat pollution tolerant organisms can be in good or fair quality water.

8 *Crayfish: Order Decapoda.* Up to 6", 2 large claws, 8 legs, resembles small lobster.

9 *Sowbug: Order Isopoda.* 1/4" - 3/4", gray oblong body wider than it is high, more than 6 legs, long antennae.



Bar lines indicate relative size

GROUP TWO TAXA continued

10 *Scud: Order Amphipoda.* 1/4", white to grey, body higher than it is wide, swims sideways, more than 6 legs, resembles small shrimp.

11 *Alderfly larva: Family Sialidae.* 1" long. Looks like small hellgrammite but has 1 long, thin, branched tail at back end (no hooks). No gill tufts underneath.

12 *Fishfly larva: Family Corydalidae.* Up to 1 1/2" long. Looks like small hellgrammite but often a lighter reddish-tan color, or with yellowish streaks. No gill tufts underneath.

13 *Damselfly: Suborder Zygoptera.* 1/2" - 1", large eyes, 6 thin hooked legs, 3 broad oar-shaped tails, positioned like a tripod. Smooth (no gills) on sides of lower half of body. (See arrow.)

14 *Watersnipe Fly Larva: Family Athericidae (Atherix).* 1/4" - 1", pale to green, tapered body, many caterpillar-like legs, conical head, feathery "horns" at back end.

15 *Crane Fly: Suborder Nematocera.* 1/3" -2", milky, green, or light brown, plump caterpillar-like segmented body, 4 finger-like lobes at back end.

16 *Beetle Larva: Order Coleoptera.* 1/4" 1", light-colored, 6 legs on upper half of body, feelers, antennae.

17 *Dragon Fly: Suborder Anisoptera.* 1/2" - 2", large eyes, 6 hooked legs. Wide oval to round abdomen.

18 *Clam: Class Bivalvia.*

GROUP THREE TAXA

Pollution tolerant organisms can be in any quality of water.

19 *Aquatic Worm: Class Oligochaeta.* 1/4" - 2", can be very tiny, thin worm- like body.

20 *Midge Fly Larva: Suborder Nematocera.* Up to 1/4", dark head, worm-like segmented body, 2 tiny legs on each side.

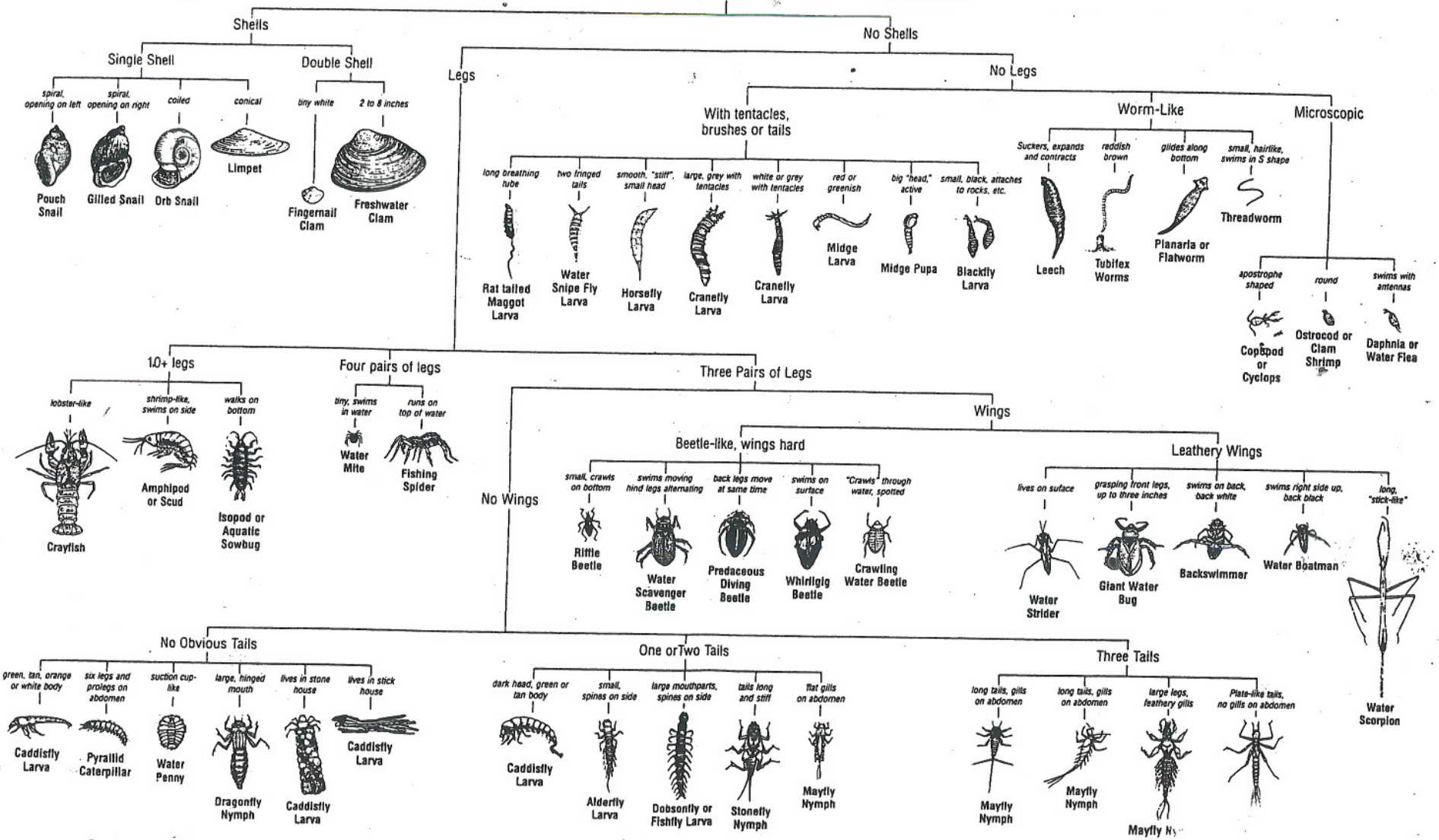
21 *Blackfly Larva: Family Simulidae.* Up to 1/4", one end of body wider. Black head, suction pad on end.

22 *Leech: Order Hirudinea.* 1/4" - 2", brown, slimy body, ends with suction pads.

23 *Pouch Snail and Pond Snails: Class Gastropoda.* No operculum. Breathe air. Shell usually opens on left.

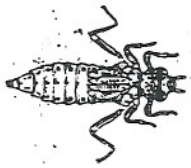
24 *Other snails: Class Gastropoda.* No operculum. Breathe air. Snail shell coils in one plane.

Key to Macroinvertebrate Life in the River

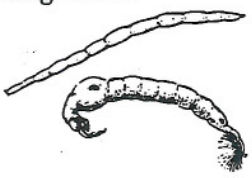


Common Aquatic Invertebrates

Dragonfly nymph P/S

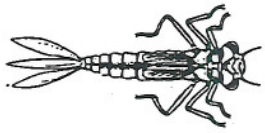


Midge larvae P/S

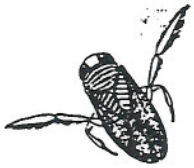


Crane fly larva P/S

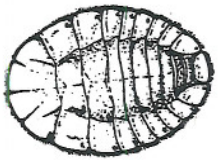
Damselfly nymph P/S



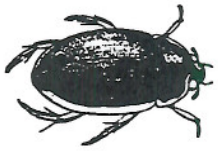
Water boatman (adult) P



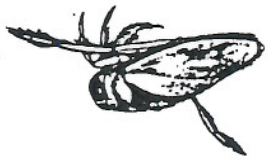
Water penny (beetle larva) S



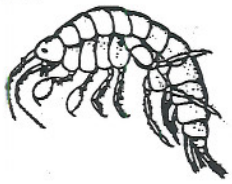
Water scavenger beetle adult P/S



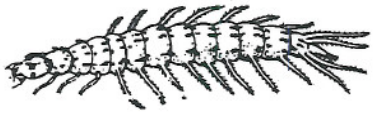
Backswimmer (adult) P



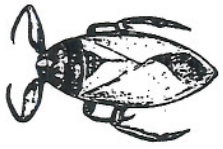
Scud P



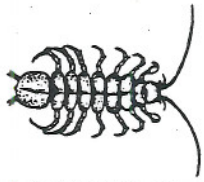
Whirligig beetle larva P/S



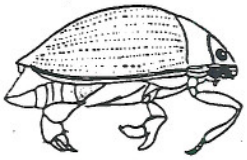
Giant water bug (adult) P/S



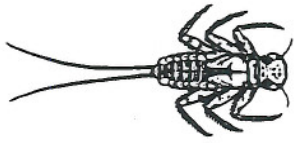
Aquatic sowbug P



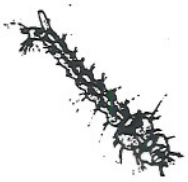
Whirligig beetle adult P



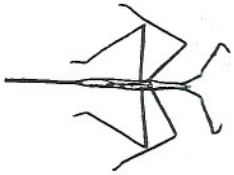
Mayfly nymph S



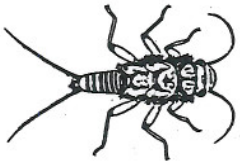
Mosquito larva P



Water scorpion (adult) P

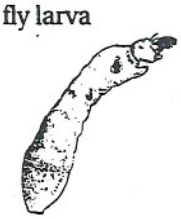


Stonefly nymph S



Water strider (adult) P

Black fly larva S



Caddisfly larva P/S

Dobsonfly larva (hellgrammite) S

Group No: _____

Numbers of organisms for each site:

Station										
Group	1/6	2/7	3/8	4/9	5	1/6	2/7	3/8	4/9	5
Organism type:	Counts									
Blackfly										
Caddisfly										
Mayfly										
Dragonfly										
Stonefly										
Dobsonfly										
Cranefly										
Alderfly										
Fishfly										
Midge larvae (chironomid)										
Midge pupae										
Amphipod (scud)										
Isopod (sowbug)										
Flat worms										
Segmented worms										
Leaches										
Threadworms										
Water mite										
Water spider										
Water strider										
Whirligig beetle										
Water boatman										
Backswimmer										
Giant water bug										
Predaceous diving beetle										
Snail										
Clam										
Fingernail clam										

Station _____ Group Number: _____
Analyte (circle):
Conductivity DO & temperature pH Turbidity

Summary of up to 30 measurements:

1.	2.	3.	4.	5.
6.	7.	8.	9.	10.
11.	12.	13.	14.	15.
16.	17.	18.	19.	20.
21.	22.	23	24.	25.
26.	27.	28.	29.	30.

Station _____ Group Number: _____
Analyte (circle):
Conductivity DO & temperature pH Turbidity

Summary of up to 30 measurements:

1.	2.	3.	4.	5.
6.	7.	8.	9.	10.
11.	12.	13.	14.	15.
16.	17.	18.	19.	20.
21.	22.	23	24.	25.
26.	27.	28.	29.	30.