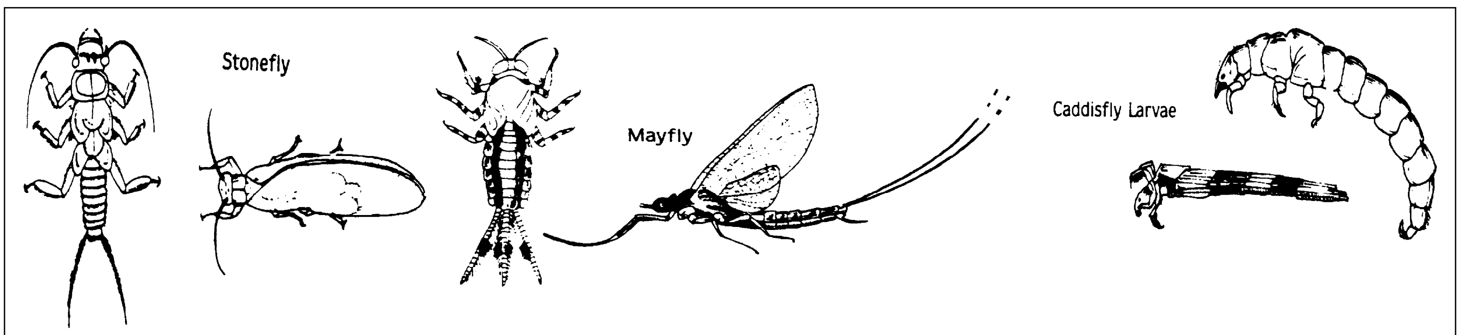
Fun Hands-on Science Education Doing.....
Benthic Macroinvertebrate Studies!!!

Getting to know the local benthic macroinvertebrates in your river is fun and a great way to learn about how healthy your river is. A river is a combination of physical, chemical and biological characteristics. Many chemical characteristics change overtime, sometimes very rapidly to natural and human-caused changes and still have very little cumulative effect on living organisms in rivers. Benthic and riparian area studies help us understand how aquatic communities respond to stress integrated over time whereas to chemical monitoring focuses on the stress and exposure characteristics of pollution. may tell us more about the condition of rivers and streams than conventional chemical monitoring techniques. Studying river insects and riparian habitat tells us about changes that streamflow and chemistry monitoring may miss.

The health and integrity of the ecological community of insects can occur only when chemical, physical and biological **stressors** are negligible or minor. For instance, river insects that have preferred environmental conditions, such as stoneflies (Order: Plecoptera) that like cool, clear water with lots of oxygen, often disappear from the river community when urban development increases rapid water runoff and decreases water quality. For example, watersheds with historic and current overgrazing or urban areas with over 25% of impervious surface tend to have benthic communities that are less diverse and more dominated by pollution-tolerant insects than watersheds that are less disturbed by human communities (May et.al. 2000). The focus on benthic macrointebrates helps evaluate the chemical, physical, and biological characteristics and their cumulative effects in the health of many riparian-dependent organisms that need wet areas to survive.

The word “benthic” means bottom dwelling and refers to organisms that live on the bottom (substrate) of a river, stream or lake. The term “macroinvertebrate” means the organisms without a spine (invertebrate) that can be seen without the aid of a microscope or can be seen by the unaided eye.



Since aquatic insects provide great long-term markers / **indicators** of stream and watershed health you can use these activities to do your own investigations about river health. What kinds of questions do you have about your rivers health and how can studying benthic macroinvertebrates help you read the landscape and tell a story the health of your river?

River characteristics that affect benthic macroinvertebrate communities

This section briefly presents the physical, chemical, and biological characteristics of the river ecosystem and describes how these change upstream to downstream

1. Physical Characteristics

Elevation: The height above sea level impacts how far the river drops from source to mouth. This affects a number of characteristics such as gradient, temperature, and shading.

Gradient: The slope of the river determines the current velocity and bottom composition.

Flow: The amount of water in the river determines the amount of bottom covered by water. In New Mexico, drought and irrigation diversions can severely impact the benthic community and water quality of rivers by constraining the insects to stagnant pools or completely removing their habitat when rivers are dewatered.

Water clarity: The clarity or turbidity of the water affects the depths to which light can penetrate and stimulate biological activity. This is usually the result of road building and sediment being deposited in the stream.

Shading: The shade provided by trees, shrubs, and banks helps moderate stream temperatures in the summer and provides food for stream animals.

Temperature: Some macroinvertebrates and fish are very sensitive to temperature levels and fluctuations. It also affects the amount of oxygen that water can hold (cold water can hold more oxygen than warm water) and that is available to macroinvertebrates and fish.

Percent of area of impervious surface and disturbed soil and plant communities: Studies have shown that as the total impervious area in a watershed exceeds as little as 5%, the benthic and macroinvertebrate communities begin to degrade rapidly (May et.al, 2000). Large fires or extensive overgrazing can severely alter runoff quantity and quality and flow patterns, which can have negative effects on macroinvertebrate communities.

2. Chemical Characteristics

Nutrients (phosphorus and nitrogen): Phosphorus and nitrogen are essential plant and animal nutrients that, in excess amounts, can cause rapid increases in biological activity of plants and bacteria and in high enough amounts may become toxic. Excessive amounts of plants reduce the amount of habitat available to some macroinvertebrates, fish eggs and fish. This may disrupt the stream ecology so that certain biological communities experience severe mortality.

Dissolved oxygen (DO): Water contains oxygen in dissolved form. Oxygen is added to the water through turbulence, gas exchange at the water's surface, and as a by-product of plant photosynthesis. Oxygen gets removed from water by chemical oxidation, respiration of aquatic animals, and decomposition. Some aquatic life require high and stable levels in order to flourish.

3. Biological Characteristics

In-stream (autochthonous) versus Riparian (allocthonous) food production: This characteristic is the amount of living plant material produced in-stream versus that which drops in from the area along the stream. In-stream production depends on the availability of sunlight and the availability of nutrients. Some food types produced in the stream, like algae, are important food and habitat sources for some macroinvertebrates.

Behaviors and adaptations of invertebrates

Habitat
Refers to the place where an organism naturally lives and grows. Effected by the chemical and physical factors of a system, but organic substrates such as plants, logs, or detritus can be important biological components of habitat, running vs standing water, substrate size

Movement
Most freshwater invertebrates have special body shapes and behaviors that enable them to be in a place that meets their essential requirements for acquiring food and oxygen, avoiding competition with other invertebrates and hiding from predators. The major groups are clingers, climbers, crawlers, sprawlers, burrowers, swimmers, skaters.

Feeding invertebrate feeding is usually categorized according to the type of food that is consumed or how food is obtained. Typical foods for freshwater invertebrates are detritus, wood algae, live vascular plants, and other animals. Invertebrates are categorized but their body structure and behavioral habit they use to obtain their food. These categories are called functional feeding groups that include shedders, collectors, scrapers, piercers, engulfer-predators.

Breathing- Most kind of Fresh water invertebrates depend upon dissolved oxygen in the water for their breathing. Oxygen enters the organisms either thought their general body surface or through gills that are specialized for this purpose or both. These are referred to as closed breathing systems. Some invertebrate wiggle their bodies to increase oxygen diffusion.

Life history- Refers to biological events in an organism's life from birth to death. Reproduction usually involves mating by a male and female. There are also numerous examples of asexual reproduction among freshwater invertebrates. Most invertebrates hatch from eggs and are small immature forms that must undergo growth. These smaller growth forms are called larva, juveniles or, just immature. This is important to remember since most invertebrates will be studied in this larval or immature stage of development. They are only considered adults when they have developed structures and organs required for reproduction and laying eggs.

Stress tolerance- This term refers to the ability of organisms to withstand disturbances in their environment. There are many different types of disturbances that can occur in freshwater environments. Of these many are human caused others are forces of nature. These are often referred to as pollution by where substances or energy released into the environment that bring undesirable change or environmental stresses. Environmental stresses is a broader terms that refers to any action that bring about undesirable change. For the purpose of this lab groups will be rated according to NM Watershed Watch Order Key that rates them as pollution sensitive, somewhat pollution tolerant, or pollution tolerant.

Benthic macroinvertebrate collection and analysis methods

The workbook presents methods of collection and analysis of benthic macroinvertebrate samples.

Collection:
Frame dip-net sampling.

This method allows you to sample in several areas of the stream to create a composite sample that is more representative of the diverse **substrate** habitats than the kicknet sample.

Sorting:
1. Identification, sorting and counting to the taxonomic level of Major Groups which include some groups in Genus, Order and Class. This level of analysis provides a general idea of the richness of biodiversity of the benthic macroinvertebrate community. Key indicator insect groups include stoneflies, mayflies and caddisflies. This level of analysis is most appropriate for younger students and schools short on time in the field and class to identify, sort and count the insects.

Sample collection methods

When to Collect

Collect at least one sample per year during the fall after the summer monsoons have ended. Sample every year at about the same time to do a long-term study. This provides much more valuable information than data from one sample. Fall samples are valuable since they reflect the effects of summer high temperatures (and in some rivers, low flows) that place stress on organisms. If you have time, collect a second sample in the spring just before spring runoff occurs from snowmelt.

Where to sample

Where to sample depends on the purpose of your research. Sample in **riffles** if you want to learn the most information about the insects that have low tolerance to pollution. Over the years most schools and professionals in New Mexico have sampled in riffles. However, to learn about the broad diversity of benthic insects in your stream, you want to sample in a variety of habitats such as slow water areas, small logs, undercut banks, leaves, and in riffles using the dip net method in Collection Level 2.

Collection

Frame dip-net sampling.

This method allows you to create a composite sample from several areas of the stream that is more representative of the diverse **substrate** habitats than the kicknet sample. In addition, little sample gets lost around the sides if care is taken while scraping rocks.

<u>Collection Steps</u>

Assemble a team of three people, one to hold the kicknet and two to scrape all rocks in a 18 inch x 18 inch square area just upstream of the kicknet bar in the substrate. Make sure to bury the lower edge of the frame well (up to 2 inches once past the cobbles) into the substrate so that no specimens are lost under the net. Have two students turn over each rock in the sample area and then one student kick (more like do the “twist”) inside the sample area. Carefully lift the frame and net bag towards the current to prevent sample loss.

Take the net and gently gather the sample in a five gallon paint bucket or white tray by pouring water through the net, gently scraping the net with a wide paint brush and finally picking the net with forceps. Pour the sample from the paint bucket through a sieve bucket lined with 500 µm (micron) net. Once the entire sample is in the sieve bucket, gently scrape the sample with fingers and the brush into a 1 quart Mason jar. Check the sides of the buckets for clinging critters during every transfer step. Remove these with forceps and place them into the sample.

Sample sorting methods in the field and in the classroom/lab

Analysis to the Orders and Classes

This level of analysis provides a general idea of the richness of biodiversity of the benthic macroinvertebrate community. Key indicator insect groups include mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Tricoptera). This level of analysis is most appropriate for younger students and schools short on time in the field and class to identify, sort and count the insects.

Order level analysis can be done in the field or in the classroom. Set students in groups of 2-3 people per workstation. Each work station needs to have the same number of forceps as students, a white tray filled with sample, and labelled petri dishes. The number of labelled petri dishes depends on the time you have to sort the sample and level of detail you expect for the research.

Site Instructions for Field Methods
for Macro-invertebrate Collection and Sorting

I. Field Sampling

<u>Choosing a site</u> – This choice depends on the purpose of your study. You will be looking to compare riffle and pool sections of the same reach, to compare riffles and/or pools from different reaches of the same stream, to compare different streams, or to monitor the condition of your stream over time. Fall is the best time to collect samples if collecting on a yearly basis. You and your students should make a plan and consistently follow it.

<u>Macro-invertebrate collection</u> – If you are using a square net try to consistently collect from the same amount of river bottom area each time - 3 samples at a site that in total encompass approximately one square meter. Gently disturb the bottom material with your hands – large rocks with attached organisms should be carefully brushed to dislodge organisms for capture into the net. If performing Field sorting omit Storage handling and labeling instructions, unless sample will be preserved for later lab analysis.

II. Field Sorting

<u>Equipment</u> – magnifying tools, 2 trays, forceps, Petri dishes with names of Major groups, Pipets, 500 micron sieve, identification keys, ethyl and isopropyl alcohol, labels for vials, and much patience. [Vial labels should match sample labels – additionally include organism's identity i.e. mayfly]

<u>First sort</u> – Put entire bolus in a sampling tray. Have students sort through sample tray and pick out as many organisms as they can have them sort into a clean sample tray for easy identification. If the water is too cloudy, re-rinse the material. If there is much debris redistribute the sample into several trays. Retrieving insects is most efficient in a tray with clear water and a relatively white background for good contrast. As you carefully and systematically move bugs and debris from one side of the tray to the other remove each organism. Pay special attention to collect even the tiniest invertebrates, Allow adequate time to perform sorting making sure that at least 100 individuals are sorted. Identify major groups of Invertebrates and sort into corresponding petri dish. Utilize magnifying tool if required to see and identify smaller invertebrates. Record the information collected on recording sheet for analysis.

Common group names to label petri dishes

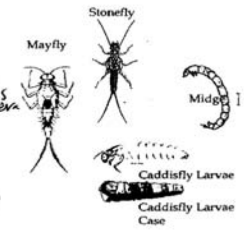
E	P	T	D	C	O
Ephemeroptera	Plecoptera	Trichoptera	Diptera	Chironomidae	Other
Mayfly	*Stonefly*	*Caddisfly*	*True Flies*	*Midge*	*Assorted*

III. Recording field data

Data –After performing identification according to New Mexico Watershed Watch Order key and counting the numbers of individuals in each major group, perform multiple sweeps over the trays to ensure a representative sample has been collected. Have you students determine whether major invertebrates are from Group One Taxa- Pollution sensitive, Group Two Taxa-Somewhat Pollution tolerant, or Group Three Taxa- Pollution tolerant. Compare the numbers of individuals and percentages within each group to determine what is the most dominant group represented at the site. This will give you a rough idea as to the quality of the water. Calculate Water Quality Value According to Watershed Watch group tolerance index score.

I.III Sample Storage

Benthic Macroinvertebrate Sorting & Recording Sheet



Stream name:	Jaramillo creek / valles Caldera
Site name:	site 2
Date of Sampling:	5/1/2012
Date of counting bugs & completing sheet:	5/1/2012
Names of bug sorters:	Los Alamos Middle school

Scientific Name of Group	Common Name	Presence or Absence	Number found	Est. # of taxa/group	% of total (if counted)
Order: EPHEMEROPTERA	Mayflies	X	18		10%
Order: PLECOPTERA	Stoneflies				
Order: TRICHOPTERA	Caddisflies	X	9		5%
Order: DIPTERA, *Family: CHIRONOMIDAE*	Midges	X	21		12.5%
Order: DIPTERA, *Family: SIMULIIDAE*	Blackflies	X	58		34.5%
Order: DIPTERA, *Family: TIPULIDAE*	Craneflies	X	21		12.5%
Order: COLEOPTERA	Beetles	X	14		8%
Order: ODONATA	Dragonflies & Damselflies				
Order: MEGALOPTERA	Dobsonflies				
Order: LEPIDOPTERA	Moths				
Order: AMPHIPODA	Scuds	X	1		0.6%
Order: ISOPODA	Sowbugs				
Order: HEMIPTERA	True Flies	X	18 creeping water Bugs		10%
Class: OLIGOCHAETA	Briste Worms				
Class: GASTROPODA	Snails	X	1		0.6%
Class: PELECYPODA	Clams				
Class:PLANARIAN	Flat Worms	X	2		1.2%
Class: Hirundinea	Leeches				
Other types (not listed)	Aquatic Worm	X	5		3%
	Grand Total:		168		

for NM Dept of Game & Fish Aquatic Education v.1 08

Storage and handling – Carefully sieve the organisms (and probably debris) from each sampling and place in a 1 quart freezer bag. Try not to include much water. Add 90% ethyl alcohol to cover and preserve the organisms. The three sample bags should be stored together in a 1 gallon freezer bag for that site. Make sure to add enough alcohol to cover entire sample. Recheck, mix, and add additional alcohol to the sample to adequately fix the sample.

Labeling – Put a composite sample in one bag or keep riffle samples separate from multi-habitat samples. Each sample must be properly labeled with a tag inside the bag – remember to use a pencil to ensure the labeling survives the alcohol solution. Each label should record:

River
Location id/ sample #
State
County
Collector
Date

Example

Santa Fe River
La Bajada riffle/ samp 2
New Mexico
Santa Fe County
C Herrera
4 Sept 08

IV. Lab Work

Work on each sample bag separately – the data from all three samples can be combined for analysis.

Equipment – magnifying tools (especially dissecting microscopes), trays, forceps, tiny water color brushes, good lighting, Petri dishes, 500 micron sieve, identification keys, 6 stoppered vials, ethyl and isopropyl alcohol, labels for vials, and much patience. [Vial labels should match sample labels – additionally include organism's identity i.e. mayfly]

First sort – Carefully rinse the contents of a sample in the sieve with cold water to remove the alcohol. Put the organisms and debris in a tray and cover with several centimeters of water. If the water is too cloudy, re-rinse the material. If there is much debris redistribute the sample into several trays. Retrieving insects is most efficient in a tray with clear water and a relatively white background for good contrast. As you carefully and systematically move bugs and debris from one side of the tray to the other remove each organism and place into the appropriate vial. Vials should be designated for each of the six major groups including Mayfly, Stonefly, Caddisfly, True flies, Midges, and Other assorted.

Each vial should be half-filled with ethyl alcohol and when finished insert the appropriate label. Collect all the macro-invertebrates in the sample. If the quantity of certain organisms is exceptionally high you may have to do an appropriate subsampling. Sieve the remaining debris and return to the original freezer bag with label. Re-preserve this with 70% isopropyl alcohol and save.

V. Analysis / Interpretation

Preliminary analysis – At this point you have performed a rough separation into 5 taxa – Ephemeroptera (order level), Plecoptera (order), Trichoptera (order), Diptera (order), and Chironomidae (family level). Count and record the organisms at this level. Notice that taxa refer to two different levels of identification – taxon is flexible term that refers to the level of identification made – for example if you were to further identify mayflies to families you would have additional taxa and not count the order Ephemeroptera as a taxon. The "Other" vial's contents can be separated into taxa – i.e. orders and in some cases classes. Record your results on the appropriate data collection sheet.

Advanced sorting and analysis – From this starting point samples may be further identified to family, genus and species levels, thus yielding additional data and allowing more advanced analysis. (see Benthic Macroinvertebrate Metrics guidance).

Water Quality Biotic Index
Use the benthic macroinvertebrate sorting and recording sheet to complete Biotic Index Calculation Sheet. This will give you a score between 0 and 3 with 3 representing good

Name: ______________________ Date: _________________

Benthic Macroinvertebrate Adaptations & Behavior

Select two organisms from the Watershed Watch Order Key, draw each in the boxes including key features. Then research or use your imagination to identify the behaviors and adaptions. Use **Reese Voshell** Jr's book *A Guide to Common Freshwater Invertebrates of North America* as a primary reference, if possible. Check the box to right with your best estimate.

Moving
___ Jumper, ___ Floater, ___ Climber, ___ Skater, ___ Swimmer,
___ Crawler, ___ Burrower, ___ Clinger

Eating Style
___ Scraper, ___ Shredder,
___ Collector (filterer), ___Predator

Place in Food Chain
___ Herbivore (eats plants), ___ Carnivore (eats animals), ___ Omnivore (eats plants and animals), ___ Detrtivore (eats dead and decaying matter)

Tolerance to Pollution
___ Intolerant (suggests good water quality),
___ Somewhat tolerant(wide range of quality
___ Tolerant (suggests poor water quality)

Moving
___ Jumper, ___ Floater, ___ Climber, ___ Skater, ___ Swimmer,
___ Crawler, ___ Burrower, ___ Clinger

Eating Style
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Tolerance to Pollution
___ Intolerant (suggests good water quality),
___ Somewhat tolerant(wide range of quality
___ Tolerant (suggests poor water quality)

Benthic Macroinvertebrate Biotic Index Calculation Sheet

Data Collector's Names____

Date________________________ Begin Time______________ End Time______________

Sensitive Groups		***Somewhat Sensitive Groups***		***Tolerant Groups***	
	Put a "X" if found		Put a "X" if found		Put a "X" if found
Caddisfly larvae	______	Clams larvae	______	Aquatic worms	______
Mayfly larvae	______	Crane fly larvae	______	Blackfly larvae	______
Stonefly larvae	______	Crayfish	______	Leeches	______
Dobsonfly larvae	______	Damselfly larvae	______	Midge Fly Larva (Red)	______
Riffle beetle larvae	______	Dragonfly larvae	______	Pouch/pond snail	______
Gilled snails larvae	______	Scuds	______	Tubificid worm	______
Water penny larvae	______	Sowbugs	______		
		Midge fly larvae (not red)	______	Total # of animals with "X"	______
Total # of animals with "X"	______	Snipe fly larvae	______		
	x 3=______				x 1=______
		Total # of animals with "X"	______		
Group score:			x 2=______	Group score:	
		Group score:			

a) Total # sensitive ____ + Total # somewhat sensitive ____ + Total # tolerant ____ = ____________
Total # of animals (a)

Sensitive Group Score___ + Somewhat Sensitive Group Score____ + Tolerant Group Score______=___________
Total value (b)

Divide total value (b) _______ ÷ total # of animals __________ = __________ for water quality index score

How healthy is the stream?
Excellent ………….. .2.3 – 3.0
Good ………………. 1.5 - 2.2
Fair ………………… 0.8- 1.4
Poor …………………0.0 - 0.7

The calculations can be applied to counts of individual organisms. To do this replace the "X" with the number of individuals, total up the individuals and then multiply the total individuals by Group value (1,2, or 3).

by RiverSource v.3 2015 May