

Mapping Stream Health Throughout Grayson County, Virginia by Testing Benthic

Macroinvertebrates

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Abstract

Grayson County, Virginia is home to some of the largest Christmas tree productions in the United States. With this production comes the use of a multitude of pesticides, including the insecticide Sniper, Dimethoate, and Safari; the miticide Endivior and the herbicide Roundup. These pesticides have a varying effect on aquatic life, especially on benthic macroinvertebrates. This project focused on collecting benthic macroinvertebrate samples scattered throughout Grayson County and then analyzing the samples to determine what the stream health is at the current time of year. Overall, the stream health is good and has a lot of macroinvertebrates that are sensitive to pollution present. Future research on this project should include testing throughout different times of year (spring and summer) as well as testing upstream and downstream of Christmas tree farms, the following experiment was conducted upstream of Christmas tree farms.

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Introduction

Grayson County is in the southwest portion of Virginia along the North Carolina Border. Its dramatic landscapes include the highest peak in Virginia (Mount Rodgers) as well as the second oldest river in the world (New River) (Grayson County, VA, 2021). The county is quite rural, with a 2021 population of approximately 15,000 people (Census, 2021).

Christmas tree production is one of the largest agricultural products in Grayson County, Virginia; the county is ranked 1st in the state and 12th in the country in terms of production (USDA, 2017). The most used pesticides (at least 5% of acreage treated in 2018) on Christmas trees are: Sniper, Dimethoate, Endivor, Safari, and Roundup (Sidebottom, 2019). While Sniper, Dimethoate, Endivor, and Safari are insecticides/miticides, Roundup is an herbicide. Dimethoate is an organophosphate and an Acetylcholinesterase (AChE) inhibitor (Uhl, P., et al., 2016). Bifenthrin, the active ingredient in Sniper, is a pyrethroid, is a sodium channel modulator (Johnson, et al., 2011). Endivor, which is a miticide, has the active ingredient, Spirodiclofen, which is a tetronic and tetramic acid derivative and inhibits acetyl CoA carboxylase (Cavusoglu, et al., 2021). Dinotefuran, a neonicotinoid, is an active ingredient in Safari, and is a nicotinic acetylcholine receptor (nAChR) (EPA, 2004). Roundup's active ingredient is Glyphosate, which is an amino acid inhibitor. (EPA, 2022).

Bifenthrin is extremely toxic to aquatic life including fish and aquatic invertebrates, according to the company product label that was approved by the EPA, bifenthrin should not be applied near surface water and to not apply when weather conditions favor drift and runoff as these can be hazardous to neighboring aquatic areas (EPA, 2015). Occurrence of pyrethroids, such as Bifenthrin, in the aquatic environment is confirmed to be implicated with population decline of aquatic species like fish; Bifenthrin, in particular, is of public concern owing to its greater contribution to aquatic toxicity among all pyrethroids (Yang, et al, 2018). The World

Health Organization (WHO) has listed Bifenthrin, which is a third-generation type I pyrethroid that has been synthetically made as a class II moderately toxic compound (WHO, 2009). There are multiple elimination routes of pyrethroids in different environmental media, these include hydrolysis, photodegradation, volatilization, and microbial degradation (Yang, et al., 2018). Hydrolysis is a double decomposition reaction with water as one of the reactants (Britannica, 2022). Photodegradation is degradation of a photodegradable molecule that is caused by the absorption of photons, particularly those wavelengths found in sunlight, such as infrared radiation, visible light, and ultraviolet light (Yousif and Haddad, 2013). Volatilization, specifically when it comes to pesticide volatilization, can be defined as the movement of pesticide vapors through the air (EPA, 2022). Microbial degradation is the degradation of organic material in terrestrial, limnological, and marine environment by microorganisms and is based on the growth of microorganisms on organic compounds, which is termed chemoheterotrophic growth style, and depends on the availability of organic nutrients for growth (Kothe, 2011). Pyrethroids, like Bifenthrin, are potent neurotoxins that interfere with nerve cell function by interacting with voltage-dependent sodium channels as well as other ion channels, resulting in repetitive firing of neurons and eventually paralysis (Werner and Moran, 2008). Aquatic organisms, in particular insects, crustaceans, and fish are highly sensitive to pyrethroid insecticides and acute toxicity to these organisms are generally observed at concentrations below 1 ug/L; while sublethal effects have been reported as low as 1 ng/L concentrations (Werner and Moran, 2008).

Dimethoate is a major pesticide in Christmas tree production and is known for its moderate toxicity to animals. The EPA has classified Dimethoate as a possible human carcinogen based on tumor occurrence when it was exposed to mice (EPA, 2008). Dimethoate can be oxidized to form omethoate, which has a higher LC50 than its parent compound dimethoate. The LC50 for Dimethoate is 353 mg/kg for skin contact on a rat (PubChem Dimethoate, 2022), while the LC50

for Omethoate is 700 mg/kg for skin contact on a rat (PubChem Omethoate, 2022) to acetylcholinesterase than dimethoate is, it is also found more in the environment due to different oxidized agents in the water and soil (Anicijevic, V., et al., 2022). According to Vladan J. Anicijevic, et al. 2022, heavy contamination of aquatic ecosystems by dimethoate can lead to mass mortality of fish and other aquatic organisms (Anicijevic, V., et al., 2022). Due to dimethoate's soil persistence being low as well as its increased water solubility, its potential to runoff into surface waters and leach into groundwater is very high (Van Scoy, et al., 2016). Dimethoate can biodegrade in any type of natural waters including river water and has a half-life of 8 days (WFDUK, N.D.). Aquatic invertebrates have a high mortality and are very sensitive to technical grade dimethoate as well as various formulations that contain dimethoate (WFDUK, N.D.P).

Spirodiclofen, the active ingredient in Endivior, is mainly used to protect against mites. It is regarded as hydrolytically unstable at pH of 7 and has a half-life of 52.1 days; it is degraded by ester cleavage with the formation of spirodiclofen-enol (FAO, 2019). The EPA has said that there is limited assessment of risk to aquatic organisms because of definitive toxicity endpoints and dose response not being developed due to solubility issues that they had encountered (EPA, 2005). The solubility of Spirodiclofen in water at pH 4 is 50 ug/l, and at pH 7 is 190 ug/l (PubChem Spirodiclofen, 2022). The EPA also mentions that the expected exposure level of Spirodiclofen in the environment should be below the agency's level of concern by one to two orders of magnitude according to their study (EPA, 2005). The freshwater invertebrates acute OPP aquatic life benchmark from the EPA is > 22.75 ug/L for acute toxicity and 11 ug/L for chronic toxicity (EPA Aquatic Life Benchmarks, 2022). With this information it is possible that though it might be present in the water at times that Spirodiclofen has little to no effect on aquatic organisms. Spirodiclofen rapidly binds to sediment and then rapidly degrades into the BAJ 2740-enol degradant in a sediment layer, the time to reach 50% degradation was less than 12 days

(EPA, 2014). Spirodiclofen has a low solubility in water which also means there should be a low potential for leaching; it is relatively non-volatile from most water, or moist soil surfaces (EPA, 2014). There are indications that because of a Kow of 5.83 there should be a large potential for bioaccumulation, especially in fish (EPA, 2014). A compound's Kow is the octanol-water partition coefficient and is a measure of the compound's hydrophilicity/lipophilicity (Cumming and Rucker, 2017).

Dinotefuran, which is the active ingredient in Safari, has little known toxic effects to aquatic organisms (Zhou, et al., 2022). Dinotefuran has a half-life of 50 to 100 days in soil, and can move into the water body through drift, rain wash and soil runoff, thus causing harm to aquatic organisms (Di, et al., 2020). One way that dinotefuran can be toxic to aquatic organisms, such as zebrafish, is by disturbing metabolic pathways which are related to amino acid, lipid, and energy metabolism (Zhou, et al., 2022). According to Liu, et al., dinotefuran is fairly safe for birds, mammals, and most aquatic organisms (Liu, et al., 2018). Dinotefuran has a moderate molecular weight, a low Kow, with a value of 0.238, and an increased water solubility, which suggests that there is potential for runoff as well as low bioaccumulation (EPA, 2004).

Dinotefuran is stable to hydrolysis in a range of pH from 4-9, and it has been shown to be relatively persistent to metabolism in both anaerobic and aerobic conditions, with a half-life of approximately 50 to 100 days (EPA, N.D.). According to the EPA once dinotefuran is in rivers, ponds, or other bodies of water its fate is somewhat uncertain (EPA, N.D.). The data found through the EPA shows that while dinotefuran is practically non-toxic to both freshwater and estuarine/marine fish, as well as freshwater invertebrates; it is highly toxic to estuarine/marine invertebrates (EPA, N.D.).

Glyphosate is the main ingredient in Roundup, in 2015 the World Health Organization (WHO) classified glyphosate as probably carcinogenic to humans (Van Bruggen, et al., 2018).

Roundup is the best-known product that is glyphosate formulated with polyoxyethylene amine (POEA) (Benbrook, 2016). Glyphosate is very resistant to degradation due to the inert C-P linkage that is present within its chemical structure (Chekan et al., 2016). Though it is resistant to degradation, breakdown is possible and one of the first decomposition products often is aminomethyl phosphonic acid, AMPA (Sun, et al., 2019). Rain and erosion can transport soil particles that contain glyphosate and AMPA into surface water where it can remain in that particular phase or be dissolved. Dissolved glyphosate and AMPA in the surface water can then absorb to the bottom sediment (Van Bruggen, et al., 2018). Ultimately glyphosate will end up in sea water, where it is highly persistent (Mercurio, et al., 2014). Glyphosate and the surfactant POEA can have negative impacts on the health of a variety of aquatic animals including protozoa, mussels, crustaceans, frogs, and fish (Van Bruggen, et al., 2018). Glyphosate formulations with POEA are generally more toxic than those without the surfactant, which is very important due to the fact that aquatic animals seem to be more sensitive to POEA than their terrestrial counterparts (Van Bruggen, et al., 2018).

Project Overview

Materials needed for stream sampling: (VA SOS Equipment List, N.d.)

1. Muddy Bottom Net (D-frame net)
2. Petri Dish
3. Spray Bottle
4. Rubber-soled waders
5. Sanitized mason jars
6. Mason jar lids and bands
7. IR thermometer

- a. Sample 1: Helton Valley Baptist Church
 - i. Helton Creek
- b. Sample 2: Route 749/Highway 58
 - i. Middle Fork Helton Creek
- c. Sample 3: Just before Rout 748 (Cabin Creek Lane) on Highway 58
 - i. Cabin Creek
- d. Sample 4: Rt. 734
 - i. Big Wilson Creek
- e. Sample 5: Just after Rt. 725 on Highway 58
 - i. Mill Creek
- f. Sample 6: Rocky Falls Lane/Wilson Hwy
 - i. New River
- g. Sample 7: Just past Rt. 831 on Highway 21
 - i. Peach Bottom Creek
- h. Sample 8: 36°37'30.2" N 81°26'19.8" W
 - i. Wilson Creek (Across from tree farm)
- i. Sample 9: Ripshin Rd./ Laurel Creek Rd.
 - j. Laurel Creek

- 3. At each sample, the water temperature, average stream width, average stream depth, and flow rate are recorded

Method for sample collection:

- 1. The sample collection will follow the muddy bottom stream sampling procedure from Virginia Save our Streams (VA SOS, 2022):
 - a. Muddy bottom sampling is intended for sampling streams that

primarily don't have rocky bottoms of riffles and are composed of muddy or sandy substrate, overhanging bank vegetation, and submerged woody and organic debris.

- b. An aquatic D-Frame net with 500-micron mesh and an extendable 4 foot pole. Collection will be along a 100 meter section of the stream.
- c. A sample consists of 20 jabs in productive habitats. The following are considered productive habitats for muddy bottom stream sampling:
 - i. Woody Snags
 - ii. Stream Banks
 - iii. Riffles (cobbles)
 - iv. Submerged aquatic vegetation
- d. Each of these habitats should be sampled in proportion to its abundance in the stream sample area
- e. This samples will then be poured into a sanitized quart mason jar and filled with stream water

Method for sample identification:

1. The samples will be scooped into a petri dish, a spoonful at a time, and stream water will be pipetted into the petri dish.
2. Once the petri dish is full, the sample will be looked over under a microscope to start to count macroinvertebrates.
3. Once the investigator finds and identifies the macroinvertebrate, they will remove the macroinvertebrate from the sample so double counting does not occur. Once it is removed it will be written down and recorded.

4. These steps will be repeated until the entire quart mason jar has been sampled.

Method for stream health identification:

1. On the Virginia Save our Stream website there are data forms for muddy bottom samples (Appendix A). The number of macroinvertebrates, and other relevant stream information will be filled out on the first two pages of the data forms
2. The first metric consists of the total number of mayflies, stoneflies, and most caddisflies (not common net-spinning) divided by the total number of macroinvertebrates collected and multiplied by 100. This calculated value is what will be used in the multimetric index as metric one.
3. The second metric consists of the total number of Gomphidae (clubtail) Dragonflies, divided by the total number of macroinvertebrates collected and multiplied by 100. This calculated value is what will be used in the multimetric index as metric two.
4. The third metric consists of the tolerant insects, this includes black flies, clams, dragonflies and damselflies, flatworms, leeches, lunged worms, midges, scuds, sowbugs, and worms. These are macroinvertebrates that are more tolerant to low stream health, such as streams with low dissolved oxygen. These tolerant macroinvertebrates will then be added up and divided by the total number of macroinvertebrates collected and multiplied by 100. This calculated value is what will be used in the multimetric index as metric three.
5. The fourth metric consists of non-insects, this includes clams, crayfish, flatworms, gilled snails, leeches, lunged snails, scuds, sowbugs, and worms.

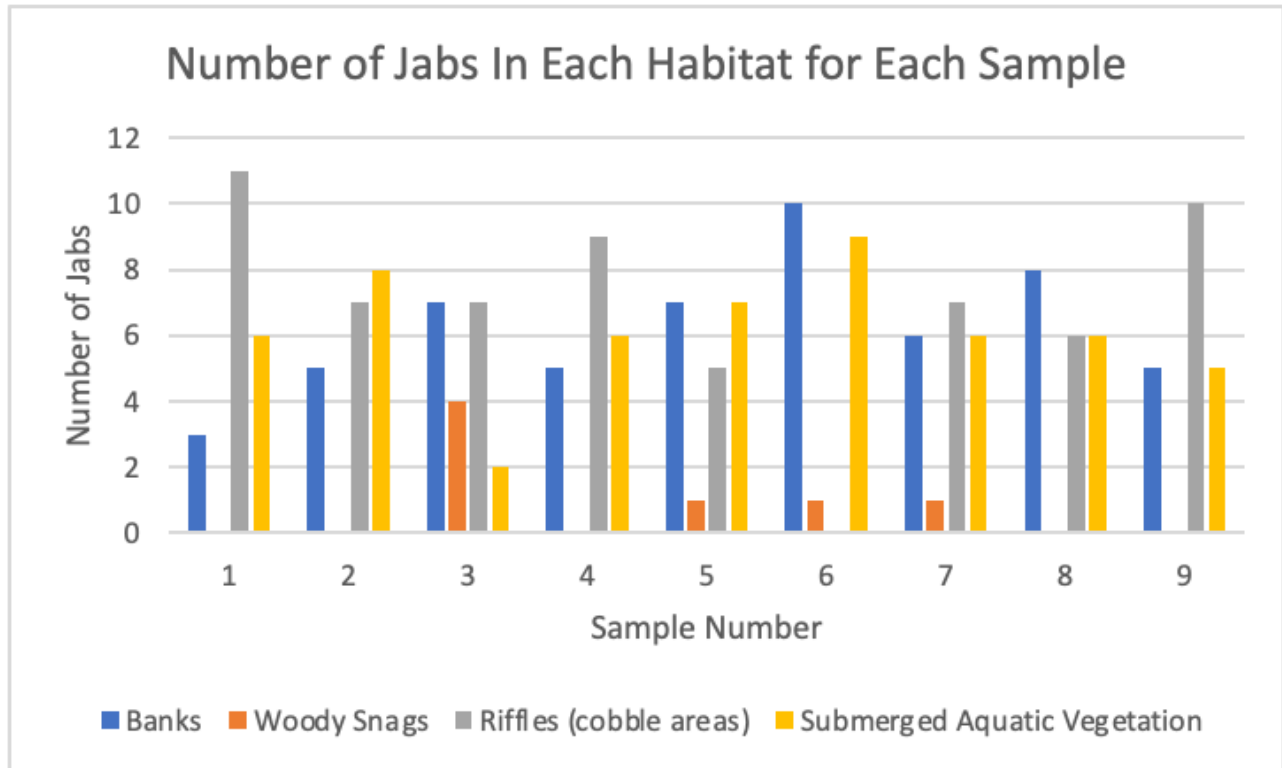
These non-insects will then be added up and divided by the total number of macroinvertebrates collected and multiplied by 100. This calculated value is what will be used in the multimetric index as metric four.

6. The multimetric index, or stream health score is then calculated using the four different metrics and plugging them into the table on page four in appendix A. If metric one is greater than 7.8, it will be given a score of 6, if it is between 0.85-7.8, it will be given a score of 3, if it is less than 0.85 it will be given a score of 0. If metric two is greater than 0.5, it will be given a score of 6; if it is between 0-0.5, it will be given a score of 3; if it is 0, it will be given a score of 0. If metric three is less than 63, it will be given a score of 6; if it is between 63-85, it will be given a score of 3; if it is greater than 85, it will be given a score of 0. If metric four is less than 27, it will be given a score of 6; if it is between 27-70, it will be given a score of 3; if it is greater than 70, it will be given a score of 0.
7. Once the previous step is done, the total number of 6s, 3s, and 0s will be added up and put into the appropriate section of the table. The number of 6s will then be multiplied by 6, the number of 3s will be multiplied by 3, the number of 0, will be multiplied by 0.
8. The total number from the previous step will then be added up, this will then give the stream health score. There are three different types of ecological conditions that can be determined:
 - a. Acceptable Ecological Condition (total score greater than 14)
 - b. Ecological conditions cannot be determined at this time (total score between 8-14)

c. Unacceptable Ecological Condition (total score between 0-7)

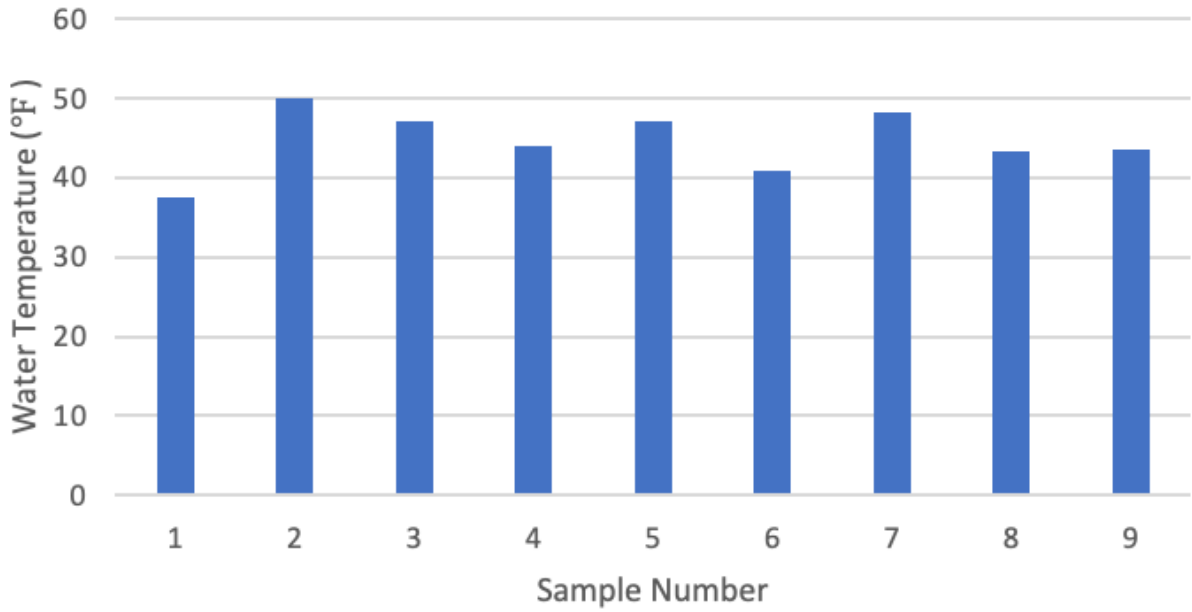
Summary of Outcomes, Discussion, and Recommendations

Results for number of jabs taken from each habitat type (20 jabs total) for each of the 9 samples (Tables for each sample will be in Appendix B):

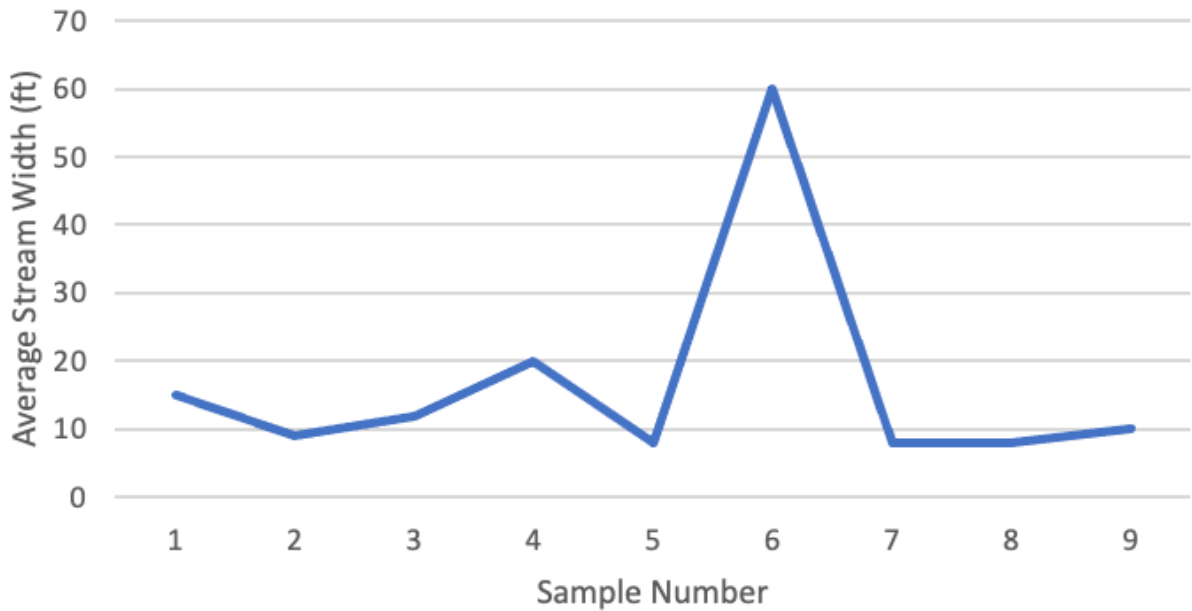


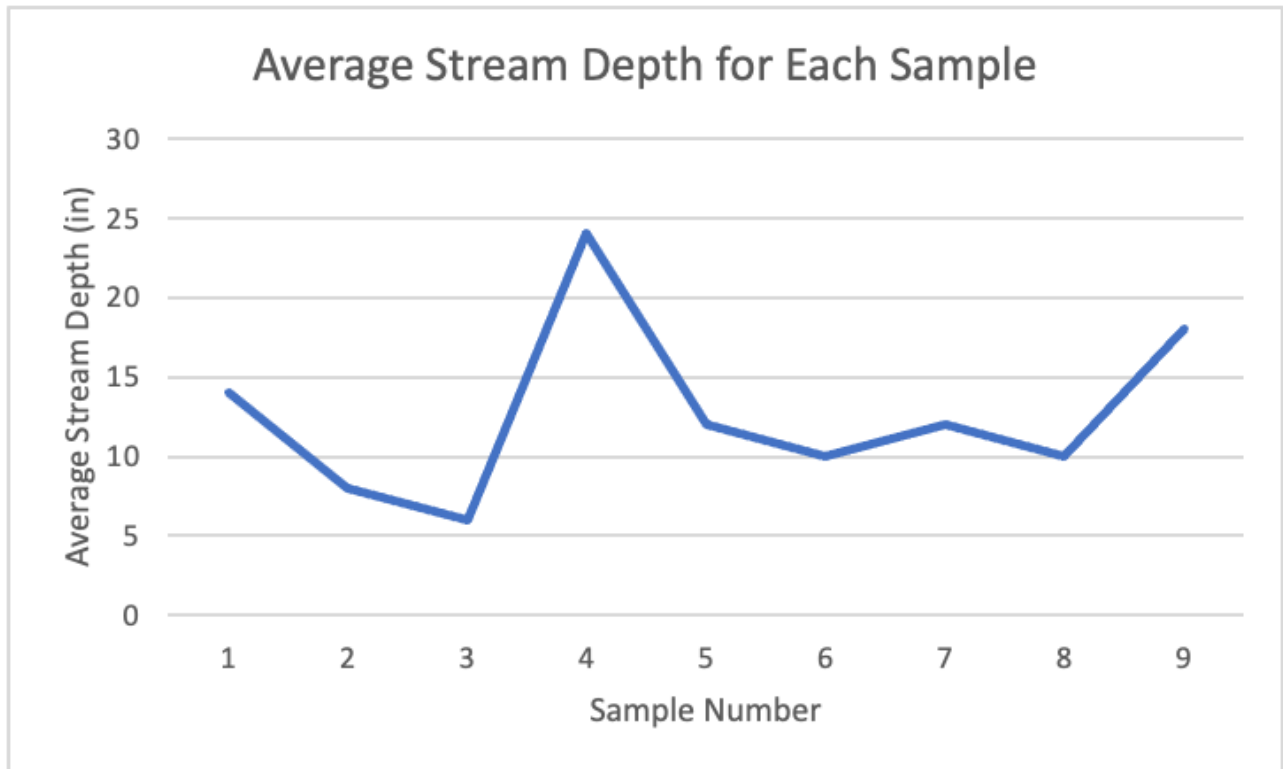
Results for water temperature, average stream width, and average stream depth for each of the 9 samples (Tables for each sample will be in Appendix C):

Water Temperature for Each Sample

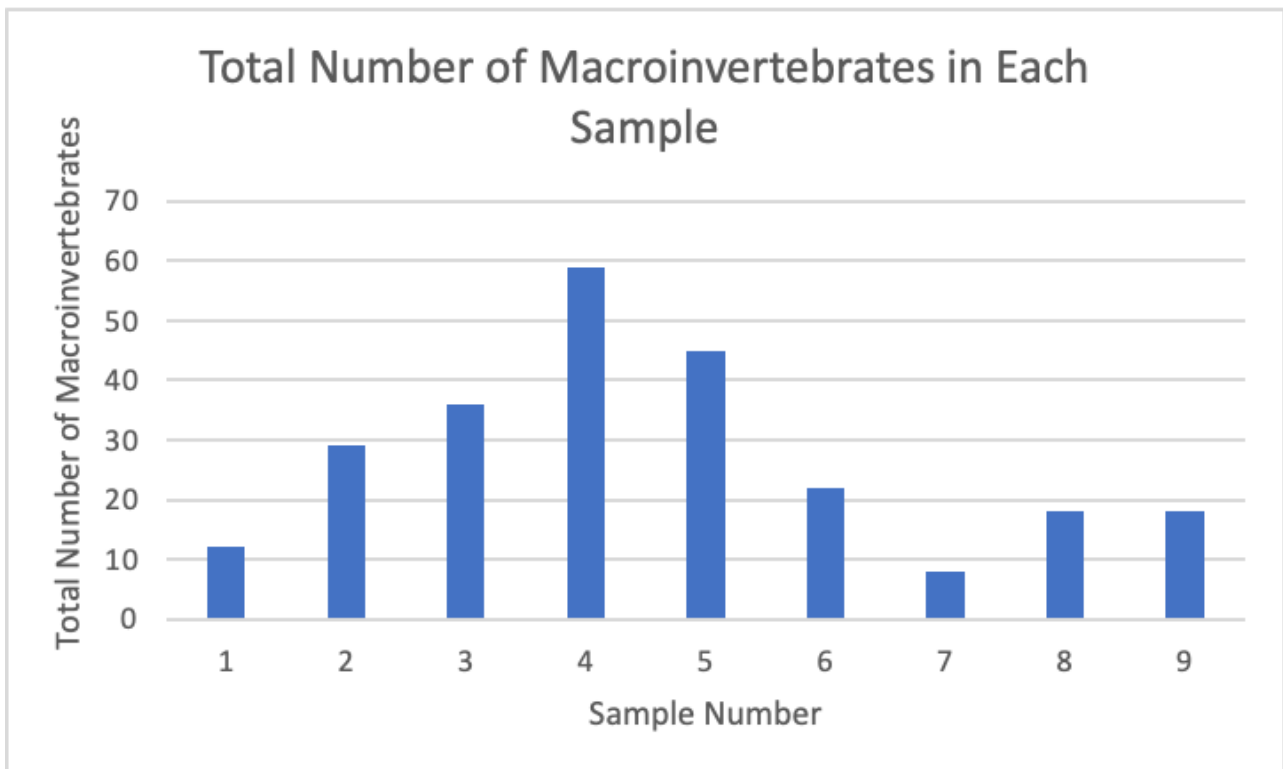


Average Stream Width for Each Sample





Macroinvertebrate Count for each of the 9 samples (Tables for each sample will be in Appendix D):



Diversity of Each Sample:

Sample 1



- Alderflies, Fishflies, and Hellgrammites
- Beetles
- Black Flies
- Caddisflies (not Common Netspinning)
- Clams
- Common Netspinning Caddisflies

Sample 2



- Alderflies, Fishflies, and Hellgrammites
- Beetles
- Black Flies
- Caddisflies (not Common Netspinning)
- Clams
- Common Netspinning Caddisflies

Sample 3



- Alderflies, Fishflies, and Hellgrammites
- Beetles
- Black Flies
- Caddisflies (not Common Netspinning)
- Clams
- Common Netspinning Caddisflies

Sample 4



- Alderflies, Fishflies, and Hellgrammites
- Beetles
- Black Flies
- Caddisflies (not Common Netspinning)
- Clams
- Common Netspinning Caddisflies

Sample 5



- Alderflies, Fishflies, and Hellgrammites
- Beetles
- Black Flies
- Caddisflies (not Common Netspinning)
- Clams
- Common Netspinning Caddisflies

Sample 6



- Alderflies, Fishflies, and Hellgrammites
- Beetles
- Black Flies
- Caddisflies (not Common Netspinning)
- Clams
- Common Netspinning Caddisflies

Sample 7



- Alderflies, Fishflies, and Hellgrammites
- Beetles
- Black Flies
- Caddisflies (not Common Netspinning)
- Clams
- Common Netspinning Caddisflies

Sample 8



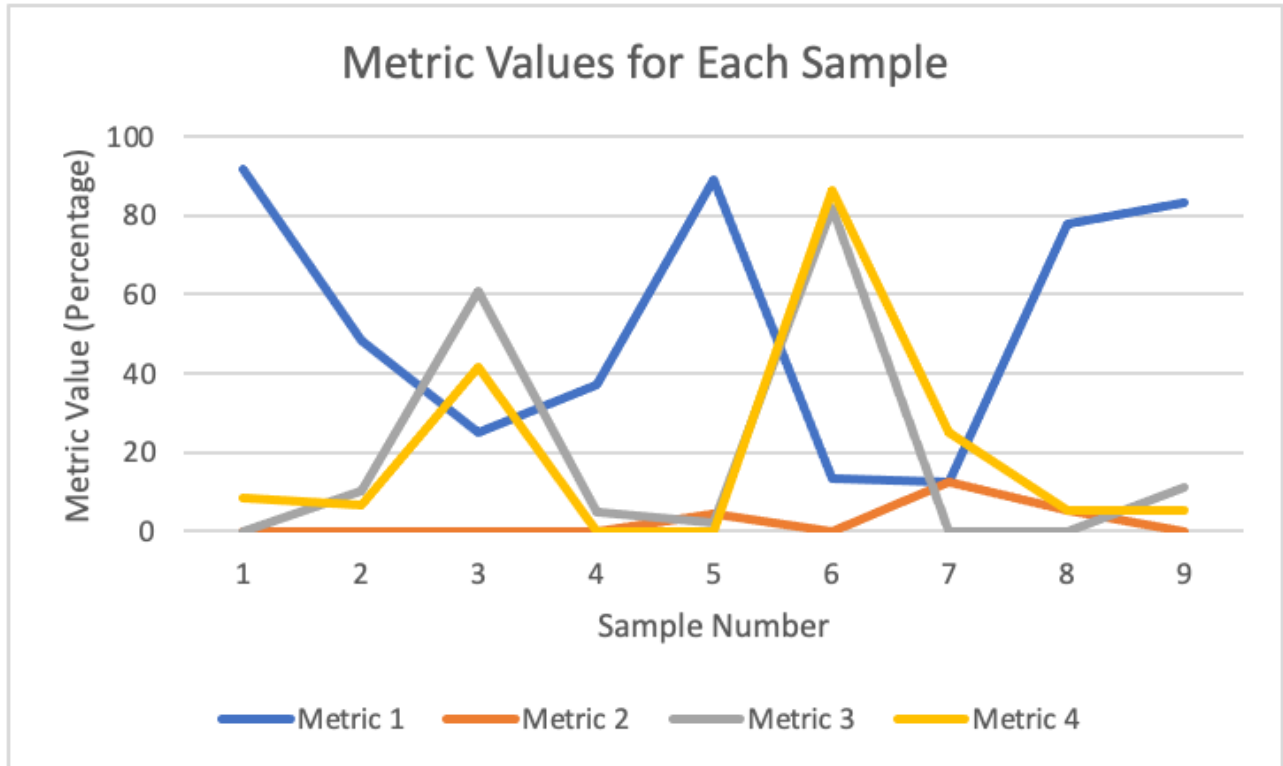
- Alderflies, Fishflies, and Hellgrammites
- Beetles
- Black Flies
- Caddisflies (not Common Netspinning)
- Clams
- Common Netspinning Caddisflies

Sample 9

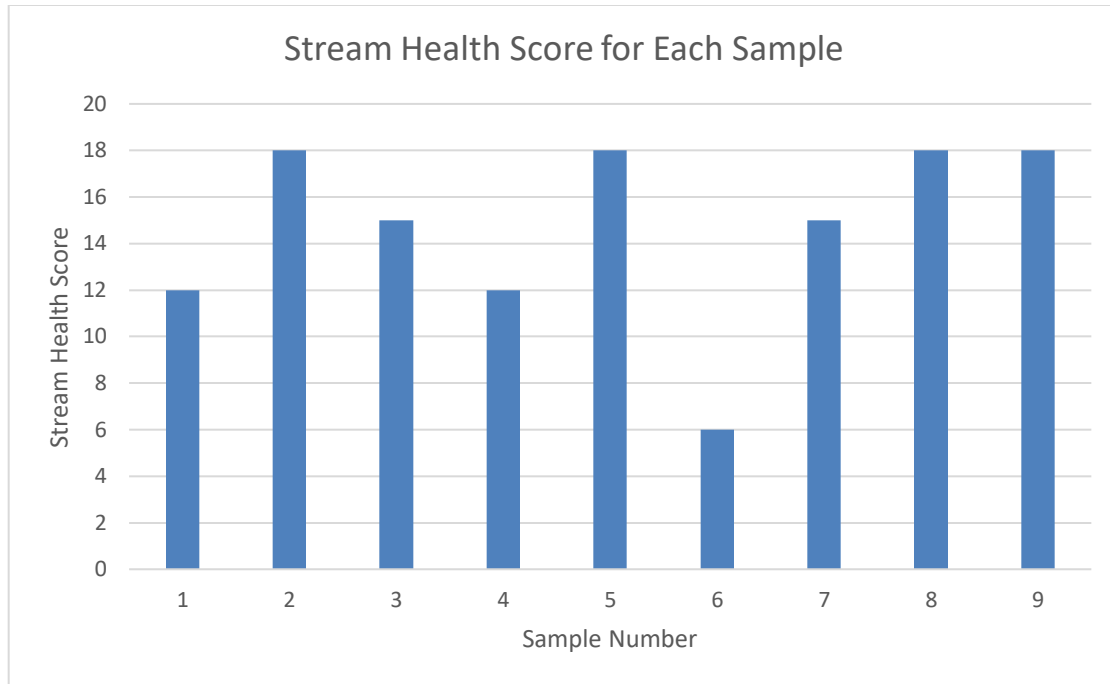


- Alderflies, Fishflies, and Hellgrammites
- Beetles
- Black Flies
- Caddisflies (not Common Netspinning)
- Clams
- Common Netspinning Caddisflies

Results for Individual Metrics for each of the samples (Tables for each sample will be in Appendix E):



Results for the Multimetric Index (Stream Health Score) for each sample (Tables for each sample will be in Appendix F):



Sample	1	2	3	4	5	6	7	8	9
Stream Health	A	E	A	E	A	U	A	A	A

A= Acceptable ecological conditions

E= Ecological conditions cannot be determined at this time

U= Unacceptable Ecological Range

Discussion

Six of the nine samples are within the acceptable ecological condition multimetric index score range, while two samples are within the ecological conditions which cannot be determined at this time multimetric index score range and one sample was in the unacceptable ecological range. This is a bit surprising since samples 8 and 9 were nearby tree farms, though this could be due to the time of collection. The time of collection was after most pests and weeds are dead for the year; the first frost date in this region of VA is the first week in October, while the experiment didn't take place until mid-November, this means that the tree farms may not have been spraying pesticides and insecticides at this time of year. The first

frost date in this region of VA is the first week in October, while the experiment didn't take place until mid-November. The cold weather also might have been a factor in how many macroinvertebrates there were throughout the water systems. The testing date had already been past multiple freezing temperatures that had occurred, as well as being at higher elevation.

Further research into this topic should include the experiment being repeated in the middle of summer when tree farms will be spraying pesticides and insecticides, it should also include more samples where possible. The experiment also could benefit from doing this testing upstream and downstream of a tree farm to determine how much the macroinvertebrate population changes. There could also be an interesting experiment on doing an experiment comparing muddy and rocky bottom sampling.

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Appendices

Appendix A:



IZAACK WALTON LEAGUE OF AMERICA



Biological Monitoring Data Form for Muddy Bottom Streams

Name of Stream: _____ Site ID: _____

Your Name: _____ Name of Certified Monitor(s): _____

Group or Organization Name: _____ Number of Participants: _____

Latitude: _____ Longitude: _____

County/State: _____ Survey Date: _____ Start Time: _____ End Time: _____

Description of Site Location: _____

MUDDY BOTTOM SAMPLING

Record the number of jabs taken from each habitat type (20 jabs total). Total jabs taken from a particular habitat type should be proportionate to the overall percentage of the habitat type in the sample area.

Banks _____ Woody Snags _____

Riffles (Cobble Areas) _____ Submerged Aquatic Vegetation _____

PHYSICAL CONDITIONS (check all that apply)

Today: Sunny Overcast Intermittent Rain Steady Rain Heavy Rain Snow

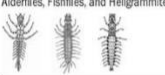











Yesterday: Sunny Overcast Intermittent Rain Steady Rain Heavy Rain Snow











Day Before Yesterday: Sunny Overcast Intermittent Rain Steady Rain Heavy Rain Snow

Water Temperature _____ F° or C° Avg. Stream Width _____ ft. Avg. Stream Depth _____ in. Flow Rate _____
(circle F° or C°) (high, normal, low, negligible)

OTHER COMMENTS _____

MACROINVERTEBRATE COUNT

Macroinvertebrate	Tally	Count
Alderflies, Fishflies, and Hellgrammites 		
Beetles 		
Black Flies 		
Caddisflies (not Common Netspinning) 		
Clams 		
Common Netspinning Caddisflies 		
Crayfish 		
Dragonflies (not Gomphidae) and Damselflies 		
Dragonflies: Gomphidae (clubtail) 		
Flat Worms 		
Gilled Snails 		
Leeches 		

Macroinvertebrate	Tally	Count
Lunged Snails 		
Mayflies 		
Midges 		
Souls 		
Shrimp (freshwater) 		
Sowbugs 		
Stoneflies 		
True Bugs 		
True Flies 		
Worms 		
Other benthic macroinvertebrates		
Total number of organisms in the sample (include "other" category)		

INDIVIDUAL METRICS

	Organism Groups	Number of Organisms		Total Number of Organisms in the Sample		Percent <i>(This is your value for this metric.)</i>
Metric 1	Mayflies + Stoneflies + Most Caddisflies (<i>not</i> Common Netspinning)		÷		Multiply by 100	_____ %
Metric 2	Gomphidae (clubtail) Dragonflies		÷		Multiply by 100	_____ %

Metric 3: Tolerant

Organism Groups	Number of Organisms
Black Flies	_____
Clams	_____
Dragonflies and Damselflies	_____
Flatworms	_____
Leeches	_____
Lunged Snails	_____
Midges	_____
Scuds	_____
Sowbugs	_____
Worms	_____
<hr/>	
Total Tolerant	_____
	÷
Total number of organisms in sample	_____
	Multiply by 100
Percent <i>(This is your value for Metric 3.)</i>	_____ %

Metric 4: Non-Insect

Organism Groups	Number of Organisms
Clams	_____
Crayfish	_____
Flatworms	_____
Gilled Snails	_____
Leeches	_____
Lunged Snails	_____
Scuds	_____
Sowbugs	_____
Worms	_____
<hr/>	
Total Tolerant	_____
	÷
Total number of organisms in sample	_____
	Multiply by 100
Percent <i>(This is your value for Metric 4.)</i>	_____ %

MULTIMETRIC INDEX (STREAM HEALTH SCORE)

Metric Number	Metric Organism	Your Metric Value	6	3	0
1	Mayflies + Stoneflies + Most Caddisflies		Greater than 7.8 _____	0.85 - 7.8 _____	Less than 0.85 _____
2	Gomphidae (clubtail) Dragonflies		Greater than 0.5 _____	Greater than 0 - 0.5 _____	0 _____
3	Tolerant		Less than 63 _____	63 - 85 _____	Greater than 85 _____
4	Non-Insects		Less than 27 _____	27 - 70 _____	Greater than 70 _____
			Total # of 6s: _____	Total # of 3s: _____	Total # of 0s: _____
		SUBTOTALS	Multiply by 6: _____	Multiply by 3: _____	Multiply by 0: _____

Add the three subtotals to get the Save Our Streams Multimetric Index Score: _____

- Acceptable Ecological Condition (Greater than 14)**
- Ecological conditions cannot be determined at this time (8 - 14)**
- Unacceptable Ecological Condition (0 - 7)**

STREAM CONDITIONS

<p>Fish water quality indicators:</p> <input type="checkbox"/> scattered individuals <input type="checkbox"/> scattered schools <input type="checkbox"/> trout (pollution sensitive) <input type="checkbox"/> bass (somewhat sensitive) <input type="checkbox"/> catfish (pollution tolerant) <input type="checkbox"/> carp (pollution tolerant)	<p>Barriers to fish movement:</p> <input type="checkbox"/> beaver dams <input type="checkbox"/> man-made dams <input type="checkbox"/> waterfalls (> 1 ft.) <input type="checkbox"/> none <input type="checkbox"/> other _____	<p>Surface water appearance:</p> <input type="checkbox"/> clear <input type="checkbox"/> clear, but tea-colored <input type="checkbox"/> colored sheen (oily) <input type="checkbox"/> foamy <input type="checkbox"/> milky <input type="checkbox"/> muddy <input type="checkbox"/> black <input type="checkbox"/> grey <input type="checkbox"/> other _____	<p>Streambed deposit (bottom):</p> <input type="checkbox"/> grey <input type="checkbox"/> orange/red <input type="checkbox"/> yellow <input type="checkbox"/> black <input type="checkbox"/> brown <input type="checkbox"/> silt <input type="checkbox"/> sand <input type="checkbox"/> other _____
<p>Odor:</p> <input type="checkbox"/> musky <input type="checkbox"/> oil <input type="checkbox"/> sewage <input type="checkbox"/> other _____ <input type="checkbox"/> none	<p>Stability of streambed (bed sinks beneath your feet in):</p> <input type="checkbox"/> no spots <input type="checkbox"/> a few spots <input type="checkbox"/> many spots	<p>Algae appearance:</p> <input type="checkbox"/> light green <input type="checkbox"/> dark green <input type="checkbox"/> brown coated <input type="checkbox"/> matted on stream bed <input type="checkbox"/> hairy	<p>Algae located:</p> <input type="checkbox"/> everywhere <input type="checkbox"/> in spots _____ % bed covered
<p>Stream channel shade:</p> <input type="checkbox"/> More than 75% full <input type="checkbox"/> 50% - 74% high <input type="checkbox"/> 25% - 49% moderate <input type="checkbox"/> 1% - 24% slight <input type="checkbox"/> none	<p>Streambank composition (=100%):</p> _____% trees _____% shrubs _____% grass _____% bare soil _____% rocks _____% other	<p>Streambank erosion:</p> <input type="checkbox"/> More than 75% severe <input type="checkbox"/> 50% - 75% high <input type="checkbox"/> 25% - 49% moderate <input type="checkbox"/> 1% - 24% slight <input type="checkbox"/> none	<p>Riffle composition (=100%):</p> _____% silt (mud) _____% sand (1/16" - 1/4" grains) _____% gravel (1/4" - 2" stones) _____% cobbles (2" - 10" stones) _____% boulders (> 10" stones) (Not applicable to Muddy Bottom Sampling.)

LAND USES IN THE WATERSHED (UPSTREAM AND SURROUNDING SAMPLING SITE)

Indicate whether the following land uses within a one-mile radius of your sampling site have a high (H), moderate (M), slight (S), or no (N) potential impact to the quality of your stream.

_____ Oil & gas drilling	_____ Urban uses (parking lots, highways, etc.)	_____ Agriculture (type: _____)
_____ Housing developments	_____ Sanitary landfill	_____ Trash dump
_____ Forestry	_____ Active construction	_____ Fields
_____ Logging	_____ Mining (type: _____)	_____ Livestock Pasture
		_____ Other _____

COMMENTS: Describe the amount and type of litter in and around the stream and indicate the current and potential future threats to the stream's health.

Please send your data sheets to your regional coordinator or submit them online at www.vasos.org. If you have any questions about this protocol, please contact the VA SOS Coordinator at vasos@wla.org. Data sheets must be stored for five years after sampling. If you are unable to keep your datasheets, please contact the VA SOS Coordinator.

Appendix B:

Sample Number	1	2	3	4	5	6	7	8	9
Banks	3	5	7	5	7	10	6	8	5
Woody Snags	0	0	4	0	1	1	1	0	0
Riffles (cobble areas)	11	7	7	9	5	0	7	6	10
Submerged Aquatic Vegetation	6	8	2	6	7	9	6	6	5

Appendix C

Sample	1	2	3	4	5	6	7	8	9
Water Temp (F°)	37.6	50.1	47.2	44.0	47.2	40.8	48.2	43.3	43.5
Avg. Stream Width (ft)	15	9	12	20	8	60	8		10
Avg. Stream depth (in)	14	8	6	24	12	10	12		18

Appendix D:

Sample 1:

Macroinvertebrate	Count
Alderflies, Fishflies, and Hellgrammites	0
Beetles	0
Black Flies	0
Caddisflies (not Common Net-spinning)	0
Clams	0
Common Net-spinning Caddisflies	0
Crayfish	0

Dragonflies (not Gomphidae) and Damselflies	0
Dragonflies: Gomphidae (clubtail)	0
Flat Worms	0
Gilled Snails	1
Leeches	0
Lunged Snails	0
Mayflies	9
Midges	0
Scuds	0
Shrimp (freshwater)	0
Sowbugs	0
Stoneflies	2
True Bugs	0
True Flies	1
Worms	0
Other Benthic macroinvertebrates	0
Total number of organisms in the sample	12

Sample 2:

Macroinvertebrate	Count
Alderflies, Fishflies, and Hellgrammites	0
Beetles	2
Black Flies	0
Caddisflies (not Common Netspinning)	2
Clams	0
Common Netspinning Caddisflies	2

Crayfish	0
Dragonflies (not Gomphidae) and Damselflies	0
Dragonflies: Gomphidae (clubtail)	0
Flat Worms	1
Gilled Snails	0
Leeches	0
Lunged Snails	2
Mayflies	5
Midges	0
Scuds	0
Shrimp (freshwater)	0
Sowbugs	0
Stoneflies	12
True Bugs	0
True Flies	3
Worms	0
Other Benthic macroinvertebrates	0
Total number of organisms in the sample	29

Sample 3:

Macroinvertebrate	Count
Alderflies, Fishflies, and Hellgrammites	0
Beetles	1
Black Flies	1
Caddisflies (not Common Net-spinning)	2
Clams	0

Common Netspinning Caddisflies	0
Crayfish	0
Dragonflies (not Gomphidae) and Damselflies	1
Dragonflies: Gomphidae (clubtail)	0
Flat Worms	4
Gilled Snails	4
Leeches	0
Lunged Snails	1
Mayflies	7
Midges	9
Scuds	1
Shrimp (freshwater)	0
Sowbugs	0
Stoneflies	0
True Bugs	0
True Flies	0
Worms	6
Other Benthic macroinvertebrates	0
Total number of organisms in the sample	36

Sample 4:

Macroinvertebrate	Count
Alderflies, Fishflies, and Hellgrammites	0
Beetles	25
Black Flies	0
Caddisflies (not Common Netspinning)	2

Clams	0
Common Netspinning Caddisflies	9
Crayfish	0
Dragonflies (not Gomphidae) and Damselflies	0
Dragonflies: Gomphidae (clubtail)	0
Flat Worms	0
Gilled Snails	0
Leeches	0
Lunged Snails	0
Mayflies	9
Midges	3
Scuds	0
Shrimp (freshwater)	0
Sowbugs	0
Stoneflies	11
True Bugs	0
True Flies	0
Worms	0
Other Benthic macroinvertebrates	0
Total number of organisms in the sample	59

Sample 5:

Macroinvertebrate	Count
Alderflies, Fishflies, and Hellgrammites	0
Beetles	0
Black Flies	0

Caddisflies (not Common Netspinning)	4
Clams	0
Common Netspinning Caddisflies	0
Crayfish	0
Dragonflies (not Gomphidae) and Damselflies	1
Dragonflies: Gomphidae (clubtail)	2
Flat Worms	0
Gilled Snails	0
Leeches	0
Lunged Snails	0
Mayflies	26
Midges	0
Scuds	0
Shrimp (freshwater)	0
Sowbugs	0
Stoneflies	10
True Bugs	0
True Flies	2
Worms	0
Other Benthic macroinvertebrates	0
Total number of organisms in the sample	45

Sample 6:

Macroinvertebrate	Count
Alderflies, Fishflies, and Hellgrammites	0
Beetles	0

Black Flies	0
Caddisflies (not Common Netspinning)	2
Clams	17
Common Netspinning Caddisflies	0
Crayfish	0
Dragonflies (not Gomphidae) and Damselflies	0
Dragonflies: Gomphidae (clubtail)	0
Flat Worms	0
Gilled Snails	1
Leeches	0
Lunged Snails	0
Mayflies	1
Midges	0
Scuds	0
Shrimp (freshwater)	0
Sowbugs	0
Stoneflies	0
True Bugs	0
True Flies	0
Worms	1
Other Benthic macroinvertebrates	0
Total number of organisms in the sample	22

Sample 7:

Macroinvertebrate	Count
Alderflies, Fishflies, and Hellgrammites	0

Beetles	0
Black Flies	0
Caddisflies (not Common Netspinning)	1
Clams	0
Common Netspinning Caddisflies	1
Crayfish	0
Dragonflies (not Gomphidae) and Damselflies	0
Dragonflies: Gomphidae (clubtail)	1
Flat Worms	0
Gilled Snails	2
Leeches	0
Lunged Snails	0
Mayflies	0
Midges	0
Scuds	0
Shrimp (freshwater)	0
Sowbugs	0
Stoneflies	0
True Bugs	0
True Flies	2
Worms	0
Other Benthic macroinvertebrates	0
Total number of organisms in the sample	8

Sample 8:

Macroinvertebrate	Count
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Alderflies, Fishflies, and Hellgrammites	0
Beetles	0
Black Flies	0
Caddisflies (not Common Netspinning)	0
Clams	0
Common Netspinning Caddisflies	1
Crayfish	0
Dragonflies (not Gomphidae) and Damselflies	0
Dragonflies: Gomphidae (clubtail)	1
Flat Worms	0
Gilled Snails	1
Leeches	0
Lunged Snails	0
Mayflies	12
Midges	0
Scuds	0
Shrimp (freshwater)	0
Sowbugs	0
Stoneflies	2
True Bugs	0
True Flies	1
Worms	0
Other Benthic macroinvertebrates	0
Total number of organisms in the sample	18

Sample 9:

Macroinvertebrate	Count
Alderflies, Fishflies, and Hellgrammites	0
Beetles	0
Black Flies	0
Caddisflies (not Common Netspinning)	0
Clams	1
Common Netspinning Caddisflies	0
Crayfish	0
Dragonflies (not Gomphidae) and Damselflies	1
Dragonflies: Gomphidae (clubtail)	0
Flat Worms	0
Gilled Snails	0
Leeches	0
Lunged Snails	0
Mayflies	8
Midges	0
Scuds	0
Shrimp (freshwater)	0
Sowbugs	0
Stoneflies	7
True Bugs	0
True Flies	1
Worms	0
Other Benthic macroinvertebrates	0
Total number of organisms in the sample	18

Appendix E:

Sample 1:

	Organisms Groups	Number of organisms		Total Number of Organisms in the Samples		Percent (This is your value for this metric)
Metric 1	Mayflies + Stoneflies + Most Caddisflies (not Common Netspinning)	11	Divide	12	Multiply by 100	91.67%
Metric 2	Gomphidae (clubtail) Dragonflies	0	Divide	12	Multiply by 100	0

Metric 3: Tolerant

Organism Group	Number of Organisms
Black Flies	0
Clams	0
Dragonflies and Damselflies	0
Flatworms	0
Leeches	0
Lunged Snails	0
Midges	0
Scuds	0
Sowbugs	0
Worms	0

Total		Total number of		Percent (This is your
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Tolerant		organisms in sample		Metric 3 Value)
0	Divide	12	Multiply by 100	0%

Metric 4: Non-insect

Organism Group	Number of Organisms
Clams	0
Crayfish	0
Flatworms	0
Gilled Snails	1
Leeches	0
Lunged Snails	0
Scuds	0
Sowbugs	0
Worms	0

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 4 Value)
1	Divide	12	Multiply by 100	8.33%

Sample 2:

	Organisms Groups	Number of organisms		Total Number of Organisms in the Samples		Percent (This is your value for this metric)
Metric 1	Mayflies + Stoneflies + Most	14	Divide	29	Multiply by 100	48.28%

	Caddisflies (not Common Netspinning)					
Metric 2	Gomphidae (clubtail) Dragonflies	0	Divide	29	Multiply by 100	0

Metric 3: Tolerant

Organism Group	Number of Organisms
Black Flies	0
Clams	0
Dragonflies and Damselflies	0
Flatworms	1
Leeches	0
Lunged Snails	2
Midges	0
Scuds	0
Sowbugs	0
Worms	0

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 3 Value)
3	Divide	29	Multiply by 100	10.34%

Metric 4: Non-insect

Organism Group	Number of Organisms
Clams	0
Crayfish	0

Flatworms	0
Gilled Snails	0
Leeches	0
Lunged Snails	2
Scuds	0
Sowbugs	0
Worms	0

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 4 Value)
2	Divide	29	Multiply by 100	6.90%

Sample 3:

	Organisms Groups	Number of organisms		Total Number of Organisms in the Samples		Percent (This is your value for this metric)
Metric 1	Mayflies + Stoneflies + Most Caddisflies (not Common Netspinning)	9	Divide	36	Multiply by 100	25%
Metric 2	Gomphidae (clubtail) Dragonflies	0	Divide	36	Multiply by 100	0%

Metric 3: Tolerant

Organism Group	Number of Organisms
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Black Flies	1
Clams	0
Dragonflies and Damselflies	1
Flatworms	3
Leeches	0
Lunged Snails	1
Midges	4
Scuds	1
Sowbugs	0
Worms	6

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 3 Value)
22	Divide	36	Multiply by 100	61.11%

Metric 4: Non-insect

Organism Group	Number of Organisms
Clams	0
Crayfish	0
Flatworms	3
Gilled Snails	4
Leeches	0
Lunged Snails	1
Scuds	1
Sowbugs	0
Worms	6

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 4 Value)
15	Divide	36	Multiply by 100	41.67%

Sample 4:

	Organisms Groups	Number of organisms		Total Number of Organisms in the Samples		Percent (This is your value for this metric)
Metric 1	Mayflies + Stoneflies + Most Caddisflies (not Common Netspinning)	22	Divide	59	Multiply by 100	37.29%
Metric 2	Gomphidae (clubtail) Dragonflies	0	Divide	59	Multiply by 100	0%

Metric 3: Tolerant

Organism Group	Number of Organisms
Black Flies	0
Clams	0
Dragonflies and Damselflies	0
Flatworms	0
Leeches	0
Lunged Snails	0
Midges	3
Scuds	0

Sowbugs	0
Worms	0

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 3 Value)
3	Divide	59	Multiply by 100	5.08%

Metric 4: Non-insect

Organism Group	Number of Organisms
Clams	0
Crayfish	0
Flatworms	0
Gilled Snails	0
Leeches	0
Lunged Snails	0
Scuds	0
Sowbugs	0
Worms	0

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 4 Value)
0	Divide	59	Multiply by 100	0%

Sample 5:

	Organisms Groups	Number of organisms		Total Number of		Percent (This is
--	-------------------------	----------------------------	--	------------------------	--	-------------------------

				Organisms in the Samples		your value for this metric)
Metric 1	Mayflies + Stoneflies + Most Caddisflies (not Common Netspinning)	40	Divide	45	Multiply by 100	88.89%
Metric 2	Gomphidae (clubtail) Dragonflies	2	Divide	45	Multiply by 100	4.44

Metric 3: Tolerant

Organism Group	Number of Organisms
Black Flies	0
Clams	0
Dragonflies and Damselflies	1
Flatworms	0
Leeches	0
Lunged Snails	0
Midges	0
Scuds	0
Sowbugs	0
Worms	0

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 3 Value)
1	Divide	45	Multiply by 100	2.22%

Metric 4: Non-insect

Organism Group	Number of Organisms
Clams	0
Crayfish	0
Flatworms	0
Gilled Snails	0
Leeches	0
Lunged Snails	0
Scuds	0
Sowbugs	0
Worms	0

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 4 Value)
0	Divide	45	Multiply by 100	0%

Sample 6:

	Organisms Groups	Number of organisms		Total Number of Organisms in the Samples		Percent (This is your value for this metric)
Metric 1	Mayflies + Stoneflies + Most Caddisflies (not Common Netspinning)	3	Divide	22	Multiply by 100	13.64%
Metric 2	Gomphidae (clubtail) Dragonflies	0	Divide	22	Multiply by 100	0%

Metric 3: Tolerant

Organism Group	Number of Organisms
Black Flies	0
Clams	17
Dragonflies and Damselflies	0
Flatworms	0
Leeches	0
Lunged Snails	0
Midges	0
Scuds	0
Sowbugs	0
Worms	1

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 3 Value)
18	Divide	22	Multiply by 100	81.82%

Metric 4: Non-insect

Organism Group	Number of Organisms
Clams	17
Crayfish	0
Flatworms	0
Gilled Snails	1
Leeches	0
Lunged Snails	0

Scuds	0
Sowbugs	0
Worms	1

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 4 Value)
19	Divide	22	Multiply by 100	86.36%

Sample 7:

	Organisms Groups	Number of organisms		Total Number of Organisms in the Samples		Percent (This is your value for this metric)
Metric 1	Mayflies + Stoneflies + Most Caddisflies (not Common Netspinning)	1	Divide	8	Multiply by 100	12.5%
Metric 2	Gomphidae (clubtail) Dragonflies	1	Divide	8	Multiply by 100	12.5%

Metric 3: Tolerant

Organism Group	Number of Organisms
Black Flies	0
Clams	0
Dragonflies and Damselflies	0
Flatworms	0

Leeches	0
Lunged Snails	0
Midges	0
Scuds	0
Sowbugs	0
Worms	0

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 3 Value)
0	Divide	8	Multiply by 100	0%

Metric 4: Non-insect

Organism Group	Number of Organisms
Clams	0
Crayfish	0
Flatworms	0
Gilled Snails	2
Leeches	0
Lunged Snails	0
Scuds	0
Sowbugs	0
Worms	0

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 4 Value)
2	Divide	8	Multiply by	25%

			100	
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Sample 8:

	Organisms Groups	Number of organisms		Total Number of Organisms in the Samples		Percent (This is your value for this metric)
Metric 1	Mayflies + Stoneflies + Most Caddisflies (not Common Netspinning)	14	Divide	18	Multiply by 100	77.78%
Metric 2	Gomphidae (clubtail) Dragonflies	1	Divide	18	Multiply by 100	5.56%

Metric 3: Tolerant

Organism Group	Number of Organisms
Black Flies	0
Clams	0
Dragonflies and Damselflies	0
Flatworms	0
Leeches	0
Lunged Snails	0
Midges	0
Scuds	0
Sowbugs	0
Worms	0

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 3 Value)
0	Divide	18	Multiply by 100	0%

Metric 4: Non-insect

Organism Group	Number of Organisms
Clams	0
Crayfish	0
Flatworms	0
Gilled Snails	1
Leeches	0
Lunged Snails	0
Scuds	0
Sowbugs	0
Worms	0

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 4 Value)
1	Divide	18	Multiply by 100	5.56%

Sample 9:

	Organisms Groups	Number of organisms		Total Number of Organisms in the Samples		Percent (This is your value for this metric)
Metric	Mayflies +	15	Divide	18	Multiply	83.33%

1	Stoneflies + Most Caddisflies (not Common Netspinning)				by 100	
Metric 2	Gomphidae (clubtail) Dragonflies	0	Divide	18	Multiply by 100	0%

Metric 3: Tolerant

Organism Group	Number of Organisms
Black Flies	0
Clams	1
Dragonflies and Damselflies	1
Flatworms	0
Leeches	0
Lunged Snails	0
Midges	0
Scuds	0
Sowbugs	0
Worms	0

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 3 Value)
2	Divide	18	Multiply by 100	11.11%

Metric 4: Non-insect

Organism Group	Number of Organisms
Clams	1

Crayfish	0
Flatworms	0
Gilled Snails	0
Leeches	0
Lunged Snails	0
Scuds	0
Sowbugs	0
Worms	0

Total Tolerant		Total number of organisms in sample		Percent (This is your Metric 4 Value)
1	Divide	18	Multiply by 100	5.56%

Appendix F:

Sample 1:

Metric Number	Metric Organism	Metric Value	6	3	0
1.	Mayflies + Stoneflies + Most Caddisflies	91.67	Greater than 7.8 <u>X</u>	0.85- 7.8 _____	Less than 0.85 _____
2.	Gomphidae (clubtail) Dragonflies	0	Greater than 0.5 _____	0-0.5 _____	0 <u>X</u>
3.	Tolerant	0	Less than 63 _____	63-85 _____	Greater than 85 _____
4.	Non-insects	8.33	Less than 27 <u>X</u>	27-70 _____	Greater than 70 _____

			Total # of 6s: <u> 2 </u>	Total # of 3s: <u> 0 </u>	Total # of 0s: <u> 1 </u>
		Subtotals	Multiply by 6: <u> 12 </u>	Multiply by 3: <u> 0 </u>	Multiply by 0: <u> 1 </u>

Add the three subtotals to get the Save Our Streams Multimetric Index Score: 12

Acceptable Ecological Condition (Greater than 14)

Ecological conditions cannot be determined at this time (8-14)

Unacceptable Ecological Condition (0-7)

Sample 2:

Metric Number	Metric Organism	Metric Value	6	3	0
1.	Mayflies + Stoneflies + Most Caddisflies	48.28	Greater than 7.8 <u> X </u>	0.85- 7.8 _____	Less than 0.85 _____
2.	Gomphidae (clubtail) Dragonflies	0	Greater than 0.5 _____	0-0.5 _____	0 <u> X </u>
3.	Tolerant	10.34	Less than 63 <u> X </u>	63-85 _____	Greater than 85 _____
4.	Non-insects	6.90	Less than 27 <u> X </u>	27-70 _____	Greater than 70 _____
			Total # of 6s:	Total # of 3s:	Total # of 0s:

			<u>3</u>	<u>0</u>	<u>1</u>
		Subtotals	Multiply by 6: <u>18</u>	Multiply by 3: <u>0</u>	Multiply by 0: <u>0</u>

Add the three subtotals to get the Save Our Streams Multimetric Index Score: 18

X Acceptable Ecological Condition (Greater than 14)

___ Ecological conditions cannot be determined at this time (8-14)

___ Unacceptable Ecological Condition (0-7)

Sample 3:

Metric Number	Metric Organism	Metric Value	6	3	0
1.	Mayflies + Stoneflies + Most Caddisflies	25%	Greater than 7.8 <u>X</u>	0.85- 7.8 ___	Less than 0.85 _____
2.	Gomphidae (clubtail) Dragonflies	0	Greater than 0.5 _____	0-0.5 _____	0 <u>X</u>
3.	Tolerant	61.11	Less than 63 <u>X</u>	63-85 _____	Greater than 85 _____
4.	Non-insects	41.67	Less than 27 _____	27-70 <u>X</u>	Greater than 70 _____
			Total # of 6s: <u>2</u>	Total # of 3s: <u>1</u>	Total # of 0s: <u>1</u>
		Subtotals	Multiply by 6:	Multiply by 3:	Multiply by 0:

			<u>12</u>	<u>3</u>	<u>0</u>
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Add the three subtotals to get the Save Our Streams Multimetric Index Score: **15**

X Acceptable Ecological Condition (Greater than 14)

 Ecological conditions cannot be determined at this time (8-14)

 Unacceptable Ecological Condition (0-7)

Sample 4:

Metric Number	Metric Organism	Metric Value	6	3	0
1.	Mayflies + Stoneflies + Most Caddisflies	37.29	Greater than 7.8 <u>X</u>	0.85- 7.8 _____	Less than 0.85 _____
2.	Gomphidae (clubtail) Dragonflies	0	Greater than 0.5 _____	0-0.5 _____	0 <u>X</u>
3.	Tolerant	5.08	Less than 63 <u>X</u>	63-85 _____	Greater than 85 _____
4.	Non-insects	0	Less than 27 _____	27-70 _____	Greater than 70 _____
			Total # of 6s: <u>2</u>	Total # of 3s: <u>0</u>	Total # of 0s: <u>1</u>
		Subtotals	Multiply by 6: <u>12</u>	Multiply by 3: <u>0</u>	Multiply by 0: <u>0</u>

Add the three subtotals to get the Save Our Streams Multimetric Index Score: 12

Acceptable Ecological Condition (Greater than 14)

Ecological conditions cannot be determined at this time (8-14)

Unacceptable Ecological Condition (0-7)

Sample 5:

Metric Number	Metric Organism	Metric Value	6	3	0
1.	Mayflies + Stoneflies + Most Caddisflies	88.89%	Greater than 7.8 <input checked="" type="checkbox"/>	0.85- 7.8 _____	Less than 0.85 _____
2.	Gomphidae (clubtail) Dragonflies	4.44	Greater than 0.5 <input checked="" type="checkbox"/>	0-0.5 _____	0 _____
3.	Tolerant	2.22	Less than 63 <input checked="" type="checkbox"/>	63-85 _____	Greater than 85 _____
4.	Non-insects	0	Less than 27 _____	27-70 _____	Greater than 70 _____
			Total # of 6s: <input type="checkbox"/> <u>3</u>	Total # of 3s: _____	Total # of 0s: _____
		Subtotals	Multiply by 6: <u>18</u>	Multiply by 3: _____	Multiply by 0: _____

Add the three subtotals to get the Save Our Streams Multimetric Index Score: 18

Acceptable Ecological Condition (Greater than 14)

Ecological conditions cannot be determined at this time (8-14)

Unacceptable Ecological Condition (0-7)

Sample 6:

Metric Number	Metric Organism	Metric Value	6	3	0
1.	Mayflies + Stoneflies + Most Caddisflies	13.64	Greater than 7.8 _____	0.85- 7.8 <u> X </u>	Less than 0.85 _____
2.	Gomphidae (clubtail) Dragonflies	0	Greater than 0.5 _____	0-0.5 _____	0 <u> X </u>
3.	Tolerant	81.82	Less than 63 _____	63-85 <u> X </u>	Greater than 85 _____
4.	Non-insects	86.36	Less than 27 _____	27-70 _____	Greater than 70 <u> X </u>
			Total # of 6s: <u> 0 </u>	Total # of 3s: <u> 2 </u>	Total # of 0s: <u> 2 </u>
		Subtotals	Multiply by 6: <u> 0 </u>	Multiply by 3: <u> 6 </u>	Multiply by 0: <u> 0 </u>

Add the three subtotals to get the Save Our Streams Multimetric Index Score: **6**

 Acceptable Ecological Condition (Greater than 14)

 Ecological conditions cannot be determined at this time (8-14)

 X Unacceptable Ecological Condition (0-7)

Sample 7:

Metric Number	Metric Organism	Metric Value	6	3	0
1.	Mayflies + Stoneflies + Most Caddisflies	12.5	Greater than 7.8 _____	0.85- 7.8 <u> X </u>	Less than 0.85 _____
2.	Gomphidae (clubtail) Dragonflies	12.5%	Greater than 0.5 <u> X </u>	0-0.5 _____	0 _____
3.	Tolerant	0	Less than 63 _____	63-85 _____	Greater than 85 _____
4.	Non-insects	25	Less than 27 <u> X </u>	27-70 _____	Greater than 70 _____
			Total # of 6s: <u> 2 </u>	Total # of 3s: <u> 1 </u>	Total # of 0s: <u> 0 </u>
		Subtotals	Multiply by 6: <u> 12 </u>	Multiply by 3: <u> 3 </u>	Multiply by 0: <u> 0 </u>

Add the three subtotals to get the Save Our Streams Multimetric Index Score: 15

 X Acceptable Ecological Condition (Greater than 14)

_____ Ecological conditions cannot be determined at this time (8-14)

_____ Unacceptable Ecological Condition (0-7)

Sample 8:

Metric Number	Metric Organism	Metric Value	6	3	0
1.	Mayflies +	77.78	Greater than	0.85- 7.8	Less than

	Stoneflies + Most Caddisflies		7.8 <u>X</u>	_____	0.85
2.	Gomphidae (clubtail) Dragonflies	5.56	Greater than 0.5 <u>X</u>	0-0.5 _____	0 _____
3.	Tolerant	0	Less than 63 _____	63-85 _____	Greater than 85 _____
4.	Non-insects	5.56	Less than 27 <u>X</u>	27-70 _____	Greater than 70 _____
			Total # of 6s: <u>3</u>	Total # of 3s: <u>0</u>	Total # of 0s: <u>0</u>
		Subtotals	Multiply by 6: <u>18</u>	Multiply by 3: <u>0</u>	Multiply by 0: <u>0</u>

Add the three subtotals to get the Save Our Streams Multimetric Index Score: 18

X Acceptable Ecological Condition (Greater than 14)

___ Ecological conditions cannot be determined at this time (8-14)

___ Unacceptable Ecological Condition (0-7)

Sample 9:

Metric Number	Metric Organism	Metric Value	6	3	0
1.	Mayflies + Stoneflies + Most Caddisflies	83.33	Greater than 7.8 <u>X</u>	0.85- 7.8 _____	Less than 0.85 _____
2.	Gomphidae	0	Greater than	0-0.5	0

	(clubtail) Dragonflies		0.5 _____	_____	<u> X </u>
3.	Tolerant	11.11	Less than 63 <u> X </u>	63-85 _____	Greater than 85 _____
4.	Non-insects	5.56	Less than 27 <u> X </u>	27-70 _____	Greater than 70 _____
			Total # of 6s: <u> 3 </u>	Total # of 3s: <u> 0 </u>	Total # of 0s: <u> 1 </u>
		Subtotals	Multiply by 6: <u> 18 </u>	Multiply by 3: <u> 0 </u>	Multiply by 0: <u> 0 </u>

Add the three subtotals to get the Save Our Streams Multimetric Index Score: **18**

 X Acceptable Ecological Condition (Greater than 14)

___ Ecological conditions cannot be determined at this time (8-14)

___ Unacceptable Ecological Condition (0-7)