Macro Invertebrates: A Comparison of Macro Invertebrate Diversity at Three Sites and an Investigation of the Presence of the Trematode *Ribeiroia ondatrae* at Neskowin Marsh

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Introduction

Macro invertebrates are animals that have no backbone, but are visible to the naked eye. Macros live in several different places, in and out of water. The macros that live on stream bottoms are called benthic macro invertebrates (BMIs) (Murdoch, 1999).

The most common BMIs are mayflies and stoneflies, aquatic insects that live in the riffles of a stream. These riffles may be caused by an uneven streambed or an aggregation of large rocks. For the riffles to be good homes for BMIs, they must have all different sizes of rocks, not all of one or another size. The moving water provides a continuous flow of food for the macros in the form of plant and animal matter (Murdoch, 1999).

Why worry about macro invertebrates, you say? Because BMIs are good indicators of stream health for several reasons. One is because they represent important links in the food chain as recyclers of nutrients and food for fish. Another is that there are some macros that are not tolerant of pollution, and some that are. Together they can teach us a lot about the health of a stream. Many BMIs have short life cycles. To study the effects of pollution on salmon we would have to wait several years. With macros, we only have to wait one season. Plus, macros are very easy to catch (Murdoch, 1999).

Macro invertebrates have four different feeding habits. There are shredders, collectors, grazers, and predators. Shredders, like stoneflies, feed on larger, mostly dead plant material (called detritus). Collectors, such as caddis and black flies, feed on the small particles left by the shredders. Grazers, like snails and beetles, roam the streambed scraping algae from rocks and plants. Damselfly and dragonfly larvae as well hellgrammites fall into the category of predators, who attack other organisms and feed off of them. It is important to understand this interaction to fully realize how one group depends on the others. The greater diversity of these different groups, the better the water quality in that stream (Murdoch, 1999).

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It's a strange phenomenon that has been taking place more and more often around the world. Frogs with horrible deformities keep being born. No one is positive what is causing these deformities, but many think that small trematode ribeiroia are responsible (Schell, 1970).

Trematodes (or flukes) represent a class of animals in the Phylum Platy helminthes. This phylum contains all so-called 'flatworms'. The phylum contains three classes, the Class Turbellaria (free-living flatworms), Class Trematoda (flukes), and Class Cestoidea (tapeworms). The animals in the two latter groups are parasitic, living as internal or external parasites for vertebrates or invertebrates (Schell, 1970).

The Class Trematoda contains those parasitic flatworms having a non-ciliated body in the adult stage and a well developed digestive tract. The organs for attachment to the host vary greatly in the different species of trematodes (Schell, 1970).

The Class Trematoda contains the sub-classes Monogenen, Aspidogastrea, and Digenea. The Monogenetic and usually the Aspidogastrean groups have only one host in their life cycle. Monogenetic flukes are chiefly ectoparasites that live on the skin and gills of fish. Others have been known to occur in the mouth and bladder of amphibians and reptiles. Holdfast organs are highly developed in this group and are located on a prohaptor at the anterior end and on an opisthohaptor at the posterior end of the body (Schell, 1970).

The majority of known trematodes are assigned to the sub-class Digenea, which are endoparasites of vertebrates. Their exact anatomy varies from group to group, but they can basically be classified as trematodes having one or two muscular suckers, a digestive tract with generally unbranched intestinal ceca, and a complex life cycle involving the production of several larval stages which develop in one or more intermediate hosts. The larval stages can also reproduce (Schell, 1970).

Some trematodes are thought to be harmless, but they all require some nutriment for survival and the host can only provide this. Trematodes can injure the host tissue by the action of muscular suckers, hooks, spines, anchors, or clamps. These tiny wounds can become centers for bacterial invasions. Internal trematodes can even clog small ducts, such as the pancreatic duct, if too many trematodes are present (Schell, 1970).

Neskowin marsh is a small area of marshland located on the Oregon coast, sandwiched between a golf course and the ocean. This marsh has recently been added to the Oregon Coast National Wildlife Refuge Complex. Several different species of plants and animals live in this small, fragmented piece of habitat. It is also a big hot spot for several types of macro invertebrates.

Because of the areas high population of snails and very polluted water, the U.S. Fish and Wildlife Service is interested in studying this area more to see if frog deformities could be a problem there. That’s why they requested that we (the macro invertebrate group) conduct a study on the marsh.

Our study consists of three separate samples of two hundred snails (one in November, one in February, and one in April). After each collection, we separate the snails and observe them one at a time underneath a microscope. Eventually, the trematode worms begin to wriggle out of the snails.

The main reason that trematodes may be present here is because of the highly acidic water, which I mentioned earlier. High pollution means more algae, which leads to a greater population of snails, which means more hosts for trematodes (Schell, 1970). The macro group’s hypothesis is that the marsh is polluted because it is divided by a golf course. Golf courses use fertilizer to get their grass just right, and this may be the reason that the marsh is so polluted. We will take samples on both the uphill and downhill sides of the course to see if there is a change in the amount of trematode worms. If there are more trematodes on the downhill side, then our hypothesis will be correct.

Methods

It is not difficult to collect macro invertebrates. However, there are several steps that must be taken to ensure that all of the specimens are properly sorted, counted, and observed. This is were our work starts.

To ensure that we get a large enough sample size, one must make numerous collections in several different places. We made dips, or collections in the stream, for macros on September 16th, 2002, and February 7th, 2003 in Pringle Creek at Fairview Training Center, as well as on September 19th, 2002 at Hill Creek. We also collected snails on November 19th, 2002, and February 18th, 2003 at Neskowin Marsh.

To collect in Pringle or Hill Creek, we simply park near by and walk down to the stream. Our only tools are D-nets, rock scrubbers, and chest waders. After making three or four dips, we combine the collections into one container. We make two kinds of collections: Department of Environmental Quality level 2 and level 3 collections. For a level two collection, we sort the combined findings by species and count the different types. Then, we send these results to scientists. For a level three collection, we leave all dirt and plant material in the container, and put a medium sized clump of the mixture into separate bottles. We then send these bottles to scientists in Corvallis were they sort them.

Research Question #1: Are trematode worms present in Neskowin Marsh?

When we collect snails in Neskowin Marsh, we take canoes out on the water and search for snails with hands and nets. The snails we find we place into plastic bottles to be sorted back at school. The U.S. Fish and Wildlife Service has asked us to conduct this study to see if trematodes are present, so that is our next step.

Back at Waldo, we give each snail its own temporary plastic cup home. We use magnifying glasses as well as the naked eye to find the trematodes. To confirm each find, we look at the suspected snails under a microscope. We were even fortunate enough to catch a trematode worm and place it on a microscope slide. The moisture on the worm evaporated, leaving a petrified trematode.

We record all of our findings and sort the snails by size and species. We also record the number of infected snails, what species they are, and whether they are large, medium, or small. When we are finished with the snails, we release them either into our school’s courtyard, or back into Neskowin Marsh.

Our last step is sending our results to the U.S. Fish and Wildlife Service. They analyze the data, and decide whether or not frog deformities could be a problem in Neskowin Marsh.

Results

By collecting snails in Neskowin Marsh, I learned that trematode worms are present, and that frog deformities could become a problem there. The results of my study are presented in Table 1.

Table 1

Date	Physa	Helisoma	Total	Cercairea Found
11/20/2002	101	6	107	8
2/18/2003	57	0	57	4
4/30/2003	19	4	23	1

This table shows that we found a total of 13 infected snails in three collections in Neskowin Marsh.

This is a picture of an *Echinostoma revolutum* cercaria from a Physa snail found on our first trip to Neskowin Marsh. This photograph was taken with a FlexCam microscope camera.

By collecting macro invertebrates in Pringle Creek, I found that there are only one species of mayflies in the creek, versus several different species in Hill Creek.

Table 2

Taxon	Abundance	Percent
Hydra	1	1.18
Oligochaete	52	61.18
Juga	1	1.18
Chydoridae	1	1.18
Ostracoda	1	1.18
Crangonyx	1	1.18
TOTAL: Non insects	57	67.06
Baetis tricaudatus	19	22.35
TOTAL: Ephemeroptera	19	22.35
Corynoneura	1	1.18
Eukiefferiella	1	1.18
Orthocladius Complex	1	1.18
Orthocladius	4	4.71
Polypedilum	1	1.18
Thienemannimyia Complex	1	1.18
TOTAL: Chironomidae	9	10.59
GRAND TOTAL	85	100.00

Table 3
(Pringle Creek, 9-16-2002: Water Temp. – 61 F)

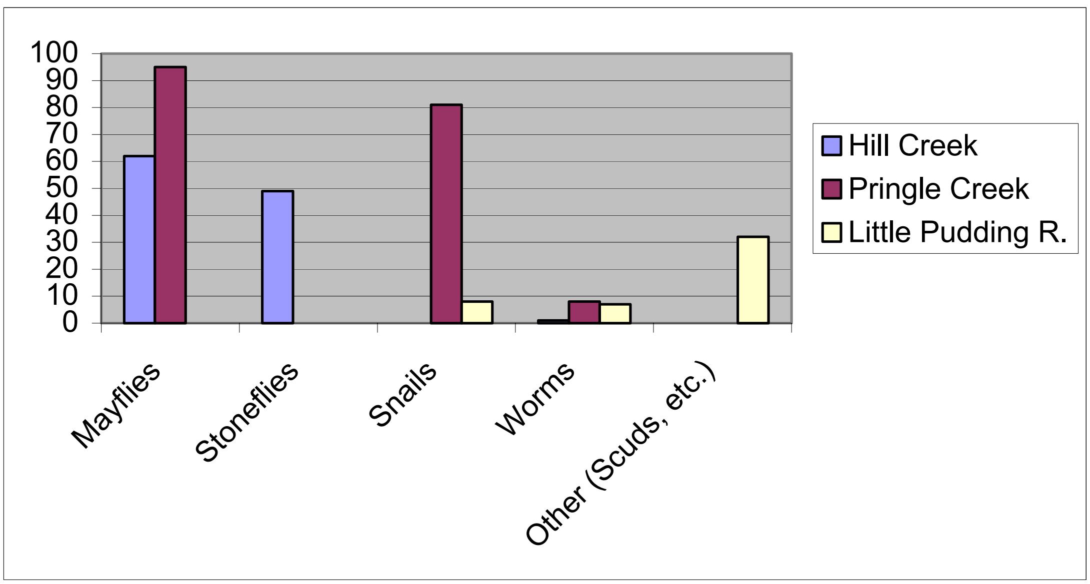
Type	Number
Snails	81
Worms	9
Mayflies	95
Stoneflies	0
Others	0

Table 4
(Hill Creek, 9-19-2002: Water Temp. – 50 F)

Type	Number
Stonefly	49
Mayfly	62
Worm	1
Snail	0
Others	0

Table 5
(Little Pudding River, 2 locations, 4-22-2003: Water Temp. – 58 F)

Type	Forrest Anderson	The Woods
Mayflies	0	0
Stoneflies	0	0
Worms	3	4
Snails	1	7
Other (scuds, etc.)	17	15



This bar graph clearly shows the different numbers of macro species we collected on each of our first collections at our three sites. We found a lot of mayflies at Hill Creek and Pringle Creek, but the main difference was the number of species of mayflies. Pringle Creek had only one (Baetis tricaudatus), and Hill Creek had at least four, many of them much larger than any other macros we've seen before.

Discussion

Conducting research on a topic can lead to a very educated conclusion. Without researching your topic first hand, it is impossible to discuss your topic in an educated way, because research websites found on the web could differ from your own results. That is also the case with my research project on macro invertebrates and trematode parasites found in snails.

The research conducted by my group and I show that trematode worms by the name of *Echinostoma revolutum* are present there. We have no evidence that points to the presence of the trematode worm *Ribeiroia onatrae*.

The question that needs to be answered now, however, is not if the parasites are present, but rather what caused them to be there. To fully answer this question, one must consider all of the variables that played a roll in the study. In this study, the main variables are pollution, season, snail life cycle, and collecting methods.

Pollution. We are sure that it is a problem in the marsh because of the golf course above it, which uses fertilizers that run into the marsh. Further evidence towards this conclusion is the marshes high turbidity and P.H.

Season. We searched for snails in three very different times of the year. Temperature and rainfall could have possibly affected our results, but it is more likely that our research would have shown more dramatic changes if this was true.

The life cycle of a snail lasts roughly one year. It is possible that our collecting started at the beginning of the snail's life cycle, when they were more abundant. If this is true, our last collecting date would have landed near the end of the snail's life cycle, making them much less abundant.

When referring to my study on macro invertebrates, I can draw a few educated conclusions on the data that I found. First of all, our data shows that the streams with less pollution have a higher amount of macro invertebrates, particularly pollution intolerant species of mayflies and stoneflies. Streams with more pollution tend to have a higher abundance of scuds and other pollution tolerant species.

However, if the theory that snails tend to live in more polluted areas were algae grows is true, then our data contradicts this theory. The data from our first trip to Hill Creek shows that we found more snails than anything else. However, in the Little Pudding River, our data shows that snails are second to scuds. It is possible that the weather or our collecting methods affected the outcome of our data, so it is good to review each one.

Our collections occurred in different seasons, were water temperatures change and different species of macros move to different parts of the stream, but it is more likely that our collecting methods may have swayed the outcome of our data.

At each collection, we used D-nets and headed into the stream with chest waders. We then carefully scrubbed all rocks and dirt within a two-foot square around the end of the net. It is possible that, because of our random collecting areas, we could have found a different amount of snails, scuds, stoneflies, mayflies, and worms, despite the weather. Perhaps there are certain places in each of our collection sites that certain species like to hang out, but obviously, in some of these collections, we did not find the spot.

Conclusion

We found several trematode specimens in Neskowin Marsh, but we have no conclusive evidence that points to Ribeiroia onatrae (the trematode worm possibly responsible for frog deformities). We were, however, able to capture a trematode worm by the name of Echinostoma revolutum, and place it on a microscope slide to be studied.

I feel that further research on this topic would be very beneficial. There are a few recommendations I can make to the U.S. Fish and Wildlife Service (USFWS) to make research on this subject much easier:

Just like in any research project, it is always easier to draw conclusions from data when you have a lot of data. To do this, one must travel to their research site for collections as many times as possible. It would also be interesting to see how different collecting methods would change the outcome of the study. That is a variable that could be very easily toyed with and changed for the better. I make these recommendations to the USFWS help any other group attempting this study.

By contemplating the results of my study on macros, I can conclude that the diversity of certain macros changes depending on the condition, cleanliness, and temperature of the stream. Another group could very easily continue our study on macro invertebrates.

There are a few recommendations I can make to any group interested in this study. For one, to get enough data, one must collect numerous times to be able to understand the balance of the stream. It would also be beneficial to have a certain place that you collect each time, so that your data is not effected by were the species are hanging out at a certain time.

Having spent my whole eighth grade year as a field researcher, there are a few things I have learned. For one, enjoy nature while you are collecting. Do not necessarily stay completely fixated on your study while there. It will help you understand the balance of your site more easily. And never, ever forget to bring home the data.

Having spent my last three years in JGEMS, I have come to appreciate nature on a deeper level than I thought I could. It is easier for me to understand the balance of nature and how humans fit into the whole mix of things. Without JGEMS, I would not be the kind of person I am now.

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