Fertilizer Effects on Schistosome Infection Rate Through Nutrient Addition and Nitrogen Fixation Experiments

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Abstract:

Schistosomiasis is the second most devastating socio-economic parasitic disease a person can contract in parts of the world, such as, Africa and China. Although there are no cases of the schistosomiasis in the United States, another schistosome strain infects birds which are present in Colorado. Part of the schistosome's lifecycle lives in freshwater snails maturing before the cercariae infects a larger host. During the duration of 3 weeks, John Mischler and I preformed two experiments: the nutrient addition experiment and the nitrogen fixation experiment. Both examined how fertilizers ultimately affect snails' infection rate. The first experiment questioned which fertilizer element creates the most algae growth. After taking water samples from 17 fertilizer rich ponds in Brush, CO we added nitrogen, phosphorus and nitrogen and phosphorus into individual bottles of pond water with light for 6 days measuring chlorophyll a growth. The results from this experiment have not fully been analyzed. The second experiment questioned how much nitrogen fixation occurred in the sample ponds by measuring a nitrogen fixation by-product, ethylene. The results showed low levels of nitrogen fixation possibly explained by the non-nitrogen fixing phytoplankton using the available nitrogen in the water column to out-compete the nitrogen fixing organisms.

Introduction:

The overall idea of the nutrient addition experiment was to determine which element increases algae growth by measuring the chlorophyll a in water samples from Brush, CO. By discovering this answer, more could be told about how a parasitic schistosome finds a snail as host. If the snail is eating algae growth with more nitrogen or phosphorus in a highly fertilized area, is there a higher infection rate among snails with the parasite because the host snail looks more appealing with the additional nutrients? If the snail has higher/lower levels of nitrogen or phosphorus will there be a higher infection rate of snails in a more fertilized area? On the other hand do fertilizers make a more nutritious snail food (algae) allowing the snail to have a higher resistance to parasitic infection? The nutrient addition experiment examined the effects fertilizers components, nitrogen and phosphorus; have on algae growth in water samples.

Briefly, the conducted experiment used bottles of pond water to signify a micro-environment which could be easily manipulated by the addition of elements used in fertilizers. The individual bottles also allowed for multiple trials on the same pond to be treated in different ways. After 6 days of intense light, the pond water was judged by the amount of chlorophyll a produced.

Next, the nitrogen fixation experiment determined the amount of nitrogen fixation occurring in the sample ponds in comparison with the amount of nitrogen being added by the fertilizers. Typically organisms need about 16 times more nitrogen than phosphorus in order to stay healthy. When there is an uneven ratio of phosphorus and nitrogen being added into the ponds by the fertilizers, an outside source of additional nitrogen needs to come from somewhere. Nitrogen has two pathways to get into the pond, through fertilizers and nitrogen fixation. Nitrogen fixation is the process in which certain phytoplankton convert stable diatomic atmospheric nitrogen into an useable form. This process is extremely energy costly for the phytoplankton but is necessary when there are low levels of nitrogen available. The purpose of the experiment was to determine the amount of nitrogen the nitrogen fixers are producing on their own.

It was predicted the nitrogen from the fertilizers would be the limiting agents in the ponds because the nitrogen fixers would outcompete the fertilizer and convert the available nitrogen into ammonia a usable compound for algae. Within the experiment, acetylene was added into syringes filled with pond water then the amount of ethylene produced by nitrogen fixation was measured.

Methods:

For the nutrient addition experiment, 100ml glass bottles were used to signify a micro pond environment. In preparation for collecting data samples there were 320 glass bottles (100ml) to be sterilized. Each bottle was acid washed then rinsed in purified water. This step was important because although the bottles were new from the manufacture, precautions were taken to ensure there were no foreign particles that would interfere with the experiment. After the bottles were dry each received a label indicating which treatment would be used. For each of the 20 ponds, there were 3 treatments, 1 control along with 4 replications for a total of 16 bottles per pond. The treatments included addition of nitrogen, phosphorus, and a combination of nitrogen and phosphorus. After the labeling was completed, data collection followed.

On Monday July 16th, John and I collected water samples from various ponds at 3 different sites in Brush, CO. The sites were under the control of The Division of Wildlife and during bird season were used for duck hunting. At each pond we would walk into the water and collect from the area with the least amount of visible loose sediment trying to get the clearest pond water. We would collect a gallon bucket along with a 32 oz Nalgene bottle. I would next use a dissolved oxygen meter and record the readings along with the pH and temperature. Unfortunately after a few pond samples the pH and thermometer stopped working and we had an incomplete data set. Another issue that was happened was some of the pond sites were dried up due to the previous dry winter. This meant that our sample size decreased from 20 sample ponds to 17 ponds.

The next day at the lab, there was a set up on the counter with aluminum foil, on the top, the sides of the counter and the back wall with foil. Propped up on two surrounding boxes were florescence lights. Determined by a light meter, there were equal amounts of light hitting each spot on the counter. Next, each bottle was filled up with the sample pond water and a foam cork allowing for the passage of air. Next, the bottle was placed under the light source in random order and the appropriate nutrients were added. Twice a day each individual bottle was shaken remixing the added nutrients along with randomization.

After 6 days of consistent light, we used a filtering system to capture the amount of algae produced in each bottle. There was a circular paper filter over a Buchner funnel leading into a Buchner Flask also attached was a vacuum speeding up the filtering process. After the pond water was completely filtered, the paper filter was wrapped in aluminum foil and labeled. The individual filters were later stored in a freezer currently awaiting analysis, marking the conclusion to the nutrient addition experiment.

After collecting data samples from Brush, back at the lab, the nitrogen fixation experiment was conducted. Using plastic syringes, 30ml of pond were tested on. The tests were repeated using 4 syringes. To measure the amount of nitrogen fixation occurring in the pond water, we added acetylene to the syringes next measuring the converted ethylene determining the amount of nitrogen fixation.

Results:

The results of the nutrient addition experiment are awaiting further analysis.

The nitrogen fixation experiment has determined that there are low levels of ethylene produced in the sample ponds from Brush. This means that there are low levels of nitrogen fixation occurring.

Discussion:

The data analysis has not been performed on the nutrient addition experiment yet.

A possible explanation for the low levels of nitrogen fixation could be related to a competitive advantage between the non-nitrogen fixers and the nitrogen fixers. The nitrogen fixers are being out-competed by the non-nitrogen fixing phytoplankton. The non-nitrogen fixing phytoplankton is able to pull the available existing nitrogen from the water column. There are low levels of nitrogen in the ponds but there is just enough to get by on for the non- nitrogen fixers. On the other hand, the nitrogen fixing phytoplankton is having to pull the nitrogen from the atmosphere and fix the element to a usable form. The fixing of nitrogen is very high in

energy cost which is in this case detrimental to the nitrogen fixers' survival. The nitrogen fixers have to spend a lot of energy to survive by converting nitrogen which the non-fixers are avoiding.

Previous studies have said that in fertilizer rich ponds with such an imbalance of nitrogen and phosphorus that the nitrogen fixers will bring up the nitrogen levels to become more balanced however our research has suggested otherwise. Although we do not have all the data yet, from what we have seen, the nitrogen levels are not being brought up and are staying low. This can be explained by the nitrogen fixers being out-competed by the non-nitrogen fixers.

The future of this research project entails completing the data analysis of the of the nutrient addition experiment in order to determine which element produces the most algae growth. This will further explain how fertilizers affect the infection rate snails with schistosomes.