Water Those: Barcoding Water Mites to Evaluate Water Quality

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Abstract

Water mites are excellent bioindicators for water quality, in part due to their sensitivity to changes in their aquatic environment. The discovery of water mites in planaria dishes and other freshwater invertebrate cultures, thus, raises several questions regarding the water used for classroom experimentation, especially after visual comparison to other known water mites species proved to have no clear matches. Genotyping these local water mites allows for both determination of a potentially new species, and evaluation of water quality. To amass data, arthropods were scraped from contaminated dishes and placed into Eppendorf tubes to be processed. Because initial results were inconclusive, other samples were prepared, this time with clusters of arthropods in place of singular specimen. Positive results could indicate either whether the unidentified organisms are a known or new species. Research on both the identified species and closely related species could provide insight on how it can be used as a bioindicator for water quality.

Introduction

Known formally as *Hydrachnidiae*, *Hydracarina,* or *Hydrachnellae,* water mites fall under the phylum *Arthropoda*. Like other arthropods, water mites are invertebrate animals protected by an exoskeleton, and are defined by their segmented body and jointed appendages. There are approximately 5,000 species of water mites worldwide; and at least 1,500 species can be found in North America alone (EcoSpark 2011). Most of them can be classified into the genera *Hydrachna, Hydrodroma, Arrenurus,* and *Unionicola.* (Microscopy UK, No Date) Although water mites differ radically in shape and size, they share similar diets, habitats, and life cycles.

Aquatic mites play an important role in the regulation of populations of other invertebrates in part due to their predatory-parasitic nature. But water mites can serve an additional function as bioindicators of water quality.

Our aim for this project is to identify the water mites that have colonized our planaria dishes. We hypothesize that they are of the genus *Arrenurus*; After classifying the arthropods and figuring out what genus and species they belong to, we can then determine what makes the specific arthropods that we have especially excellent as bioindicators. We can use the data we discover to find better and more accurate ways to test water quality. Water indicators such as water mites are not only more accurate, but also cheaper and safer than chemicals.

Procedure

- Prepared Eppendorf tubes by labelling them into sets of A, B, C, and D.
- Added specimen to Eppendorf tube A of each set. Added 300µl of lysis solution to each tube, and with a plastic pestle ground the tissue until exoskeleton has been degraded. (Later attempts reduced amount of lysis buffer to 150 µl.)
- Incubated overnight at 65°C to further degrade exoskeleton. Centrifuged for 1 min.
- Transferred 150 µL of supernatant to tube B of each set. Added 3 µL of DNA silica resin beads to the tube, then incubated in water bath at 57°C, for 10 minutes.
- Transferred supernatant into tube C. Added 300 µl ice-cold ethanol wash buffer to tube B. Washed the resin pellet, centrifuged for 30 sec, transferred supernatant into tube C. Repeated the wash.
- Micropipetted out remaining wash buffer from pellet. Air dried tube B to remove any excess ethanol.
- Added 100 µL of ddH_20 to tube B. Incubated at 57° C for 10 min. Transferred 50 µL into tube D – this is the DNA.
- Amplified 2 µL of DNA using 12.5 µL Taq Master Mix and 10.5 µL COI invertebrate primer. PCR was performed using a mastercycler and the program DMISHORT: 95C 1 min, 95C 30 sec, 50C 30 sec, 72C 45 sec, 34 cycles, 72C 1 min
- A 2% agarose solution was prepared with 50mL 1X TBE buffer. Wells were loaded with 2µL SYBR Green and 5µL of PCR product. For comparison 5 µL of positive control (provided by UBP), 2 µL of DNA ladder, both stained with SYBR Green, were also loaded.
- Gel was run for 35 minutes at 90V, and viewed using UV transillumination.

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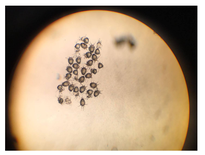
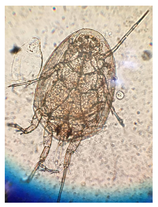
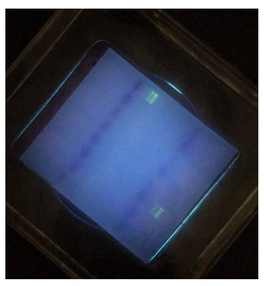
Results

It was hard to gather enough arthropods for DNA extraction and PCR. They were incredibly small, were often stuck to the bottom of the dishes, and difficult to discern. It was difficult to gauge how many, if at all, had been successfully placed into the tubes and lysed. The arthropods likely drifted to the very bottom tip of the tubes, which could not be reached with the plastic pestles. As a result, we had to grind the specimen against the sides of the tubes, which could've resulted in residue along the walls of the tubes and outside of the solution, thus further decreasing potential concentration of DNA. Despite attempting to compensate for these issues with overnight incubation, this was most likely the greatest source of error in our experiment. Our gel results did not contain any visible PCR product.

We suspect that mistakes with DNA extraction were more likely to result in lack of PCR product than the PCR protocol. We are concerned that extraction errors that the silica resin washes and removal of excess ethanol were inadequate. Any of these errors would've interfered with the PCR process.







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Discussion

Upon reflection after the experiment was completed, we realized that there were quite a few factors that led to inconclusive results. First, the nature of the arthropods we were working with made it particularly difficult to collect data. Since they were accidental contaminants of dishes intended to hold planaria, their origins were unknown to us; we had to try our best to breed larger populations in our dishes without any knowledge of their optimal conditions, food, and mating habits. They exhibited periods of heavy reproduction, banding together at the surface of the water in large groups and moving incredibly quickly; we were able to spot orange eggs on the dishes with our microscopes but never managed to observe the hatching of one. The most frustrating part was that the populations would then rapidly drop, most likely due to starvation since we were unable to find a food that allowed them to thrive. In fact, we tried to grow them in four different mediums: the first contained light-colored yeast from our stock container of *Daphnia*, the second contained dark brown food pellets from our stock container of *Planaria*, the third contained miniscule amounts of chicken liver that we had been feeding the *Planaria* from the dish that first saw the rise of these organisms, and the fourth contained distilled water to serve as our control group. We collected small populations of about 10 arthropods for each dish, and left them in the same conditions that the original dishes had been in. All four of the dishes saw minimal growth. A final, small spurt of growth foreshadowed imminent death. We both froze and dried samples for extraction. We intended to extract from fresh samples, but there were nor arthropods still alive at the time of extractions.

If given more time, we would repeat our steps once again adjusting the amount of extraction materials by factors of ¾, ½, and possibly even ¼ if the others produced inconclusive results. By modifying the amount of extraction materials, we would have much more concentrated samples of the DNA and would therefore have a higher chance of success given that the other steps were to be done correctly. We would also try to cluster more arthropods into each tube, to increase DNA yield.