The Effect of Freshwater and Saltwater on Plant Species

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Abstract

Saltwater and freshwater plants differ in the way they look, feel and process substances. If you take saltwater plants and place them in a freshwater biome, or freshwater plants and place them in a saltwater biome, the plant would die. Why is this so? To solve this problem, my group ventured into the Sunken Forest on Fire Island, an area with both a saltwater and freshwater biome. The forest contains a salt marsh, bay area, and beach for us to find saltwater plants, and a temperate forest and freshwater bog to obtain our freshwater samples. To recognize their species, we DNA barcoded all samples but only received feedback on five samples and discovered the species name of two samples, one freshwater and one saltwater plant (*Smilax sieboldii* and *Phragmites australis*, respectively). Potential errors in gel electrophoresis and several DNA Barcoding procedures. Next, we compared and contrasted the characteristics of the saltwater plants with the freshwater plants, and made a venn diagram to indicate their differences. We learned that both types of plants originate from the same area, are thin, and receive the same amounts of sunlight. However, saltwater plants tended to be turgid, constantly green, elongated, rough, and skinny, while freshwater plants tended to be rounder, vary in color, shorter, wider, less turgid, and had a waxy texture. The data shows there is a clear difference in appearance between freshwater and saltwater plants. This discovery in plant differences between ecosystems could lead to findings in a specific area's food web, since plants are the basis of all food webs. We have hypothesized that saltwater plants are darker and more slender, while freshwa-

Introduction:

What causes an aquatic plant to be able to take in saltwater instead of freshwater, or freshwater instead of saltwater? Our group is trying to discover our own lead on the situation. We would like to find out what physical traits are characteristic of native saltwater plants and native freshwater plants. Our group aims to solve this

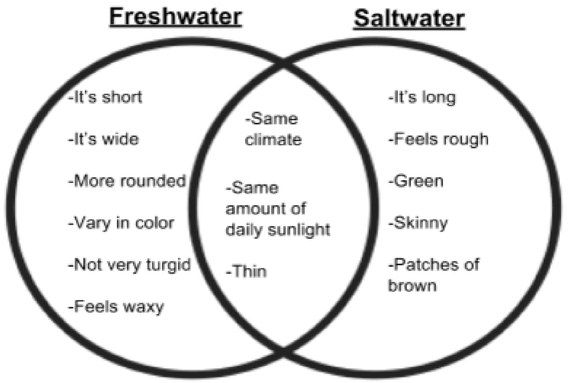
Methods:

The samples were collected on Fire Island, eight of the sixteen were collected from saltwater and the other eight samples were collected from freshwater. We also took pictures of each spot we extracted our samples from and labeled a plastic bag with its sample number. We observed the specimens and compared their physical characteristics using the chart in our section for Data Analysis. We then stored the samples in a freezer for later use. We were then able to use the science lab for a day to complete the DNA barcoding of all the plant subjects used in the project.

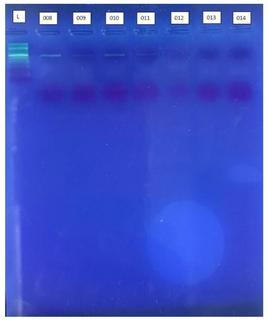
The first step was to take a specimen tissue sample. Next, we added the lysis solution and then grinded to mix all the DNA into the lysis solution. The specimen must be incubated for ten minutes and after, one minute of centrifugation occurs. The supernatant was transferred to a new tube. Next, the silica resin was added to bind the DNA. After mixing the supernatant, it was incubated at a lower temperature, then centrifuged for 30 seconds. Next, the supernatant was removed and wash buffer was added to the remaining pellet and mixed. After, it was centrifuged for 30 seconds and the supernatant was removed and wash buffer was added again and mixed. Following this the sample was centrifuged and the remaining supernatant was removed again. Distilled water is added to remove the DNA from the silica. Following this action it is incubated for 5 minutes, then centrifuged for 30 seconds. The supernatant was then transferred to a fresh tube. Following this procedure is the amplification of the DNA by PCR. The first step in this process is to add PCR reagents, followed by adding two microliters of DNA, which was amplified in a thermal cycler creating a Polymerase Chain Reaction. The final phase in this process is the Electrophoresis, Sequencing and Analysis of the specimens. We poured the gel, loaded the gel with our samples, and ran it for 30 minutes at 130 volts. The samples were sent for sequencing and analyzed using bioinformatics. We matched the barcode sequence of the unknown sample against the barcode library for identification.

Results:

In our experiment, our sixteen plant samples were DNA barcoded because we wanted to research information on why certain species in certain ecosystems thrive. In full demands and hopes out of the sixteen samples only five showed banding in the gel. We were expecting to get bands for the majority of the samples analyzed, but there are many reasons which could have resulted in this. The main issue was probably DNA extraction. During extraction we could have used the brown part of the leaf that contained little amount of chloroplasts or even none. The primer that was used attached to the chloroplast DNA. Another reason is that we did not grind the plant tissue enough to get the DNA out of it. Other sources of error include our pipetting technique. We were using micropipettes for the first time and may have had bubbles in the tips of the micropipettes or not measured correctly. We were contacted by the lab that sequenced our DNA. They think there was an error on their end which caused us to have low quality sequences, although our gel banding was strong. We are following up and currently retesting our DNA.



Plant	Ecosystem	Species in Database
Plant 8	Freshwater	Smilax sieboldii
Plant 9	Saltwater	Phragmites australis
Plant 10	Freshwater	None of the species given have a matching appearance to sample 10
Plant 11	Saltwater	BLAST searches reference a local database created from NCBI sequences that are shorter than 20kbp and match any of the following terms: chloroplast, barcode, mitochondrion, rbcL, ITS1, ITS2, matK, cytochrome oxidase subunit I, COI, CO1, COX1, lef9, 28S ribosomal RNA, 5.8S ribosomal RNA, 18S ribosomal RNA, 23 ribosomal RNA. (No species retrieved because of no sequence)
Plant 16	Freshwater	BLAST searches reference a local database created from NCBI sequences that are shorter than 20kbp and match any of the following terms: chloroplast, barcode, mitochondrion, rbcL, ITS1, ITS2, matK, cytochrome oxidase subunit I, COI, CO1, COX1, lef9, 28S ribosomal RNA, 5.8S ribosomal RNA, 18S ribosomal RNA, 23 ribosomal RNA. (No species retrieved because of no sequence)



Conclusion:

After sequencing all the five samples that displayed banding, only two had the species identified. The other three did not receive a species name because sample 11 and sample 16 had a short sequence and no reverse strand. The images for sample 10 in the database did not match up with the actual leaf of our plant. Only two of the plants, sample 8 and sample 9 were identified with a species name (***Smilax sieboldii*** and ***Phragmites australis***). The whole purpose of our experiment was to determine why and how plants in two different ecosystems thrive and survive. Luckily, the two samples that we received a species name were from two different ecosystems. We had the chance to study both the samples individually and our hypothesis turned out to be correct. Certain plants do have specific adaptations for it to survive in an ecosystem. One major difference between the two plants is texture. The rough texture of these plants allows it to live in saltwater and the smooth texture of a plant allows it to live in freshwater. Ocean plants have adapted to the salinity by breaking down salt into chlorine and sodium ions. Some plants store the salt and later dispose it via their respiratory process. Many plants live close to the seashore and they may have succulent (thick) leaves where they store water. The plants use the water to dilute the saltwater concentration. Reducing the leaf surface is another way of adapting to the condition in a saltwater biome. Marsh grass extracts the salt and you can see white salt crystals on its leaves.[3] Freshwater plants are suitable for light (sandy), medium (loamy) and heavy (clay) soils. Suitable pH is acid, neutral and basic (alkaline) soils. They can grow in semi-shade (light woodland) or no shade and they prefer moist soil.[4] Along with the disappointing factor that not every plant in our experiment was abled to be barcoded, our group was anticipating on making an evolutionary tree, but the database on DNA Subway did not allow us because an alignment that is suitable for creating a phylogenetic tree will have to have an overall high consensus score (represented by the height of the black bars on the lower portion of the alignment window) which our group did not have. After learning the species name of sample 8 was *Similax sieboldii* and sample 9 was *Phragmites australis* we had a chance to compare both ecosystems. Research does not stop here, however. Our findings can be used to try and learn how saltwater plants are able to remove the salts from the ingested water to make the water usable for itself, and identify structures in saltwater plants that freshwater plants do not have. For future work involving this experiment our group can use this information and add a different ecosystem to our project or even use this information for analyzing the two different species separately.

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National Institutes of Health SEPA Science Education Partnership Award Supported by the National Institutes of Health

CSH Cold Spring Harbor Laboratory DNA Learning Center

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