Title: Physiological Response of Red Mangrove Seedlings Under Differing Light Regimes

The Mangrove Blues
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Abstract

We studied the physiological differences between sun and shade seedlings of *Rhizophora mangle*, the red mangrove. Using the seedlings and their leaves, five experiments were made to determine whether true adaptations were available to shade seedlings to cope with higher stress. The reason why the shade seedlings were in higher stress is because they have to deal with the salt and intense competition for sunlight to photosynthesize.

We measured seedling density, stomata density, transpiration rates, succulence, and chlorophyll content. A seedling density count was conducted to assess the amount of seedlings per square meter of the sun and shade areas. The count showed there was a significant difference in which there were more seedlings per meter square of sun area than shade area. Stomata density was done to determine how much stomata was on a square cm on the leaf back side. A higher stomata density rate may indicate that there is more gas exchange occurring which in turn would bring in more carbon dioxide to be used in photosynthetic reaction. There was no significant difference in the stomata density of shade versus sun leaves. A potometer was used to measure transpiration rates of sun and shade leaves. A higher level of transpiration would point to more water uptake by the leaf which may indicate a more efficient xylem system. There was no significant difference in the transpiration rates of shade versus sun leaves. Leaf succulence was tested to find out the amount of water per cm square of each leaf. There was significantly higher succulence in shade as compared to sun leaves which may help prevent dehydration in saline water. Our chlorophyll experiment was done to find out the total chlorophyll there was per cm square of leaf. Having more chlorophyll aids in capturing more light for photosynthesis. The chlorophyll in the sun and shade leaves where significantly different; there was more chlorophyll in the shade leaves than in the sun leaves. In conclusion the five experiments pointed to shade seedling adaptations to cope with the extra stress. Out of the five experiments, only one was genetically affected which is the stomata density; the environment affected the other four.
Introduction
General background on mangroves and their significance
Mangroves are one of the most important species of trees in south Florida. Mangroves have a large affect on the fishing economy and also help prevent erosion (Heald & Odum 1969). Mangroves are also an important species of trees is because it is an important source of firewood and timber. Mangroves also provide habitat for many of South Florida’s animals. There are only three true mangroves in South Florida they are the red mangrove (*Rhizophora mangle*), white mangrove (*Languncularia racemosa*), and also the black mangrove (*Avicennia germians*). There is also an associate mangrove called the buttonwood (*Canocarpus erectus*). Mangroves are found through out the tropics and usually live near the water and coastal areas.

Every mangrove species in south Florida has its own unique characteristics. For example the red mangroves is the closest to the shoreline. Then the black mangrove is found finally, the white mangrove is usually further into the shoreline, but they are not in perfect lines and are scattered around the area. The mangroves are one of the few trees that can survive under salt water. After the seeds of the mangrove are germinated when they fall of the tree and are known as viviparous propagules. These viviparous propagules are “born live” in that they are germinated seeds. Propagules can survive on water approximately 6 to 8 months. When the propagule lands on reachable soil it will begin to grow into an adult and grow seedlings, which can form another mangrove forest.

Lack of mangrove understory: the prevailing theories
Mangroves have no understory (Janzen 1985). Janzen (1985) hypothesized that other plants could not handle the salt stress and low light stress at the same time as the mangroves could. He also said that mangroves were evergreens and that is why you don’t find them farther north. Lugo (1986) also said there was no understory because of other stress factors like nutrients, oxygen, and freshwater. Further, Snedaker and Lahmann (1988) believed that there was no understory because these other plants could not evolve in this habitat. Since mangroves were the first tropical plants to evolve saltwater they grew to out compete other species for sunlight. So when other plant tried to evolve there was too much stress and lack of sunlight that they died off.

Considering this lack of understory, we are interested in how mangrove seedlings survive in this environment and what physiological adaptations they may develop to handle the stress. We believe that shade seedlings will be more effective in terms of photosynthesis and water use to compensate for physical stress than the sun seedlings. In our study we will test the following hypotheses:

H₁) Seedling density will be more abundant in sunny areas than in shady areas because there is more sunlight.
H₂) Sun leaves will have a higher stomatal density than the shade leaves because there is a higher rate of photosynthesis.
H₃) Shade leaves will have greater succulence than sun leaves because they are under more stress.
Sun leaves will have a higher rate of transpiration because there is a higher rate of photosynthesis.

There will be a higher amount of chlorophyll in shade leaves then in sun leaves because they are under more stress.

Methods
Site description of Matheson Hammock

We chose Matheson Hammock Park and marina as our studies site because it was a park with a lot of mangroves in different conditions. We sampled mangroves on 7/10 & 7/21. The temperature was really hot and humid. The forest floor was generally sunlight because not much complete canopy after Hurricane Andrew. We chose two study areas sun/shade. We accessed the sun site by a pavement road that we walked down was dominated by red mangroves. The sun field site was very dense and hard to walk through. Nearby our gathering area there was a creek about 20 ft away. In the field site were a lot of crabs moving around. It was high tide during sampling and our study site was partially flooded. The shade study site did not have a fully closed canopy. The site was quite dense with adult trees and prop roots and we had a hard time getting around. There was a creek located about 15 ft away from our study site. Also the ground was much higher and dryer then the sunny site. Another interesting thing is that there were not as many crabs in the shady site than in the sunny site.

Field and lab protocols
Abundance assessment and leaf harvesting

On July 10, 2000 we assessed mangrove seedling abundance in sun and shade plots at Matheson Hammock. A 1m² quadrat was haphazardly tossed to calculate seedling density in each plot. We counted mangrove seedlings inside each square quadrat. We first went to a sunny area where we estimated about 80-85% of full sunlight reaching the seedlings. We observed and studied two adjacent patches with most red mangroves, with Bostrychia sp. (red alga) on their prop roots. We then sampled a shaded area where we calculated about 20% of full sunlight reaching seedlings. The soil in this particular area was covered with unknown algae. Results were analyzed to determine if there was a difference between the number of red mangrove seedlings growing in the shaded area than the sunny area.

Red Mangroves leaves were collected on 7/10 and 7/21 from each study area for use in lab experiments. We collected numerous leaves from different seedlings and placed them in ziploc bags. These leaves were collected in both, shade and sun areas and were stored in a lab refrigerator.

Stomatal density

Stomata density was studied to determine if there was a difference in the stomata between the shade & sun leaves. Twelve sun leaves and twelve shade leaves were divided among four teams of students & teachers. The group put three coats of nail polish on the underside of all the leaves to create a 1x2cm² transparent impression and let them dry for
1 hour. We then used tweezers to peel off the nail polish impression. In continuation we prepared the stomata impression by putting them on microscope slides. We counted the stomata twice on each leaf, first by the teacher and then by a student and if the count difference was less than 10% we would recount. The microscope magnification was 100x for a 0.01cm² field of view.

**Leaf succulence**

Red mangrove leaves were collected from a shaded area and sun area to determine leaf succulence. These leaves were placed in separate ziploc bags and put in a refrigerator for 2 days then taken out to weigh them. The weight of each leaf was recorded and each leaf was numbered. Then they were placed in small boxes made of aluminum foil; one each for 13 sun leaves and 13 shade leaves. Next, each small aluminum foil box containing the leaf was placed in a drying oven for 48 hours at 70°C to desiccate samples. At completion the dried leaves were weighed. To find out the percentage of the water in each leaf, the difference of the wet leaves weight and the dry leaves weight was divided by the wet weight.

**Transpiration**

Transpiration of 5 sun leaf and 5 shade leaves were measured with a potometer (Fig.1). A 10 ml pipette was filled with deionized water and connected them to the red mangrove leaves with a plastic tubing. To simulate nature’s coordinates a fan was set on high and began our experiment at 10:10 a.m. Also one hour after we started our experiment, we set up a lamp in front of every potometer to increase the transpiration rate. Every 1-hour we recorded the water level on the pipette to see how much water had transpired by the leaves. The experiment ended at after 5 hours. On the following class session we used a LI3000 leaf area meter to determine the surface area of the leaves.

**Chlorophyll concentration**

We extracted and measured the concentration of chlorophyll in each red mangrove leaf. Using a hole punch, we created three disks from each red mangrove leaf. We then measured each disk, which averaged 0.3 cm² with a LI3000 leaf area meter. We then placed the three disks in a mortar, added 10-ml of 95% ethanol and ground contents with a pestle. We transferred liquid to a centrifuge tube and placed it in the centrifuge for five minutes. The supernatant (liquid fraction from sample) is then transferred with a Pasteur pipette to a test tube. Absorbency data was recorded for sun and shade leaves with a Sequoia-Turner Model 390 spectrophotometer (fig. 2). We then calculated the total chlorophyll, chlorophyll-a, chlorophyll-b, check of total chlorophyll, and a-b chlorophyll ratio based on the absorbence values at 665, 654, and 649 nm. We used the following equations:

Eq 1) Total Chlorophyll = [6.10*(A665)]+[20.04*(A649)]
Eq 2) Chlorophyll-a = [13.7*(A665)] – [5.76*(A649)]
Eq 3) Chlorophyll-b = [25.8*(A649)] – [7.60*(A665)]
Eq 4) Check of total chlorophyll = [1000*(A654)] /39.8
Results

Abundance assessment

According to our data the red mangroves seedlings are more abundant in the sun area than the shaded area. The sun treated area mean abundance was 57.7 individuals m$^{-2}$ with a standard deviation of 23.4. The shaded area had 1.0 individual m$^{-2}$ with a standard deviation of 0.79 (Fig. 3). There was a significant difference between sun and shade area with a student t-test (p=3.9E $^{-13}$).

Stomatal density

The results show that the leaves in the shade had more stomata than the leaves in the sun. The mean for the sun leaves was 214.96 stomata’s per 0.01cm$^2$ and for shade leaves were 24.71 per 0.01cm$^2$. The standard deviations for the sun leaves were 38.66 and for the shade leaves were 91.97 (Fig. 4). There was no significant difference for stomatal density based on the t-test (p= 0.7382).

Leaf succulence

A t-test indicated the percentage of water contained in red mangrove sun leaves compared to those in the shade were significantly different (p-value=0.0005). The mangrove leaves in the sun contained an average of 65.73% water, with a standard deviation of 2.53 (Fig. 5). Mangrove leaves that were in the shade contained an average of 73.13% with a standard deviation of 6.10.

Transpiration

The result of this experiment is that the sun leaves took up more water than the shade leaves over a 5 hour period of time. The average amount of water taken in by the shade leaves where 2.11 E-07 ml H2O/ per cm$^2$ leaf surface area per second. The sun leaves transpired 2.18 E-07 ml H2O/per cm$^2$ per leaf surface area per second (see Fig. 6). There was not any significant difference with a t-test between the transpiration of the sun/shade leaves. The value was 0.8915 and we could not reject our null hypothesis. There is a significant difference in the size of the leaves. The Leaf size of the sun leaves where much larger than the shade leaves (Fig. 7).

Chlorophyll concentration

The chlorophyll concentration was higher in shade leaves then in sun leaves (Fig. 8). The sun treatment’s total chlorophyll mean was 45.3μg / 1cm$^2$ with a standard deviation of 13.3. The shade treatment’s total chlorophyll mean was 68.4μg / 1cm$^2$ with a standard deviation of 17.2 (fig.8). A t-test showed that there was a significant difference in the amount of total chlorophyll of red mangrove sun and shade leaves (p=0.044).

We also measured the amount of chlorophyll-a and chlorophyll-b in the sun and shade leaves. The shade treatment’s chlorophyll-a mean was 48.2μg / 1cm$^2$ with a standard deviation of 10.8 while the sun treatment’s chlorophyll-a mean was 33.1μg / 1cm$^2$ with a standard deviation of 12.3. Furthermore, the shade treatment’s chlorophyll-b mean was 20.3μg / 1cm$^2$ with a standard deviation of 7.4 and the sun treatment’s
chlorophyll-b mean was 12.3 μg / 1cm² with a standard deviation of 4.7 (fig. 9). The t-test shows that there was not a significant difference between sun and shade leaves for either chlorophyll-a (p=0.076) or chlorophyll-b (p=0.630).

Discussion

Abundance assessment
We found out that the amount of sunlight reaching the forest floor has a strong influence in the mangrove seedling establishment. We reject our Null Hypothesis because there was a significantly higher seedling density in sun exposed areas composed to shaded area according to our t-test results. More seedlings may grow in that area because there is more light available for photosynthesis and the plants would grow faster. We predict that if we had found seedlings growing under a full closed canopy and without any shade (100% of sunlight) the difference may have been more extreme. We also suspect that a cement sidewalk and road located approximately 20 ft away from our sunny site may have created a disturbance. These results are consistent with other researchers who report a lack of understory in Mangrove forests (Janzen 1985, Lugo 1986, Snedaker and Lahmann 1986).

Stomatal density
There was no significant difference expected between the sun and shade leaves in the stomatal density experiment. We suspected more stomata in the sun leaves than in the shade leaves because they likely have higher rates of photosynthesis. The laboratory procedure was most likely successful because we were able to create high quantity transparent impression instead of ripping the plant in half. Also, we drew an “x” on the cover slip with a blue marker, to partition the 0.01cm² area so we could count the stomata in different sections which would reduce the amount of human error while counting.

To improve results emphasis should next time be placed on acquiring leaves that were all the same in size. Also, having more people replicating the count instead of 2 per group. The shade leaves had an insignificant amount more stomata than the sun leaves; possibly to overcome the stress of lower light condition. It would also be interesting to measure the stomata size in future.

Leaf succulence
The results of the succulence experiment indicate that the sun leaves contain less water than those leaves that were in the shade. However, the null hypothesis was not rejected since there was no significant difference. The reason why the mangrove leaves in the sun possibly contain less water than those leaves in the shade is because the sun leaves receive more sunlight. Due to this, the water contained in the sun leaves evaporates more. The leaves that are in a shaded area do not receive direct sunlight unlike those leaves that are in the sun area. For this reason the water in the shade leaves do not evaporate as much. Alternately increased water storage by shade leaves may be a mechanism to cope with stress for reduced sunlight.

A source of possible error that could have affected our results of this experiment is that there was no complete sunny or shade Mangrove habits for seedlings. The leaves that were collected and used as sun leaves were really receiving 75-80% sunlight and the leaves that were used as shade leaves were under 75-80% shade. The results of this experiment could have been
more accurate if the sun leaves were receiving 100% sunlight and if the shade leaves were under 100% shade.

Transpiration

We did not see the expected difference in transpiration between sun and shade leaves. Our results compared to last year’s program’s results in that transpiration rates were about the same. Ball and Critchley (1982) stated that the gas exchanged between the black mangrove had no significant difference between shade and sun leaves. The gas exchange and transpiration are both dependent on stomata openings.

Our project would have been more reliable if we had cut the stems of the leaves inside the water to prevent air bubbles. The air bubbles can block xylem and reduce leaf transpiration. A greater sample size would benefit the experiment. Also we could have run the experiment in full sunlight instead of the low light in the classroom. The fluorescent light in the classroom might not have the photosynthetically active the wavelength needed by the plants thus reducing transpiration rates. In a plant experiment the intensity of the sunlight can have a large affect on the results (Raven et al 1986). One minor concern was that evaporation might have happened during the experiment. The fans might have affected the experiment because the wind of the fan could reach the experiment that was in affect on the other side. This experiment benefits the main project by giving you information to know how much water each kind of leaf.

Another observation made in this experiment is that the sun leaves looked larger than the shade leaves. It was proven that the sun leaves were significantly larger (p=0.1316) when they were weighed. The sun leaves might have been larger because they receive more sunlight, which gives them more energy to go through the process of photosynthesis more rapidly than the shade leaves. The shade leaves receive less sunlight, so they have less energy to photosynthesize and therefore don’t grow as much as the sun leaves.

Chlorophyll concentration

The results of the experiment show that the shade leaves had more total chlorophyll than sun leaves. We rejected our null hypothesis for total chlorophyll because there was a significant difference according to our t-test results. Our chlorophyll b was enriched compared to chlorophyll a but there was no significant difference. Our results are similar to Ball and Critchely (1982) who found that the shade leaves of the grey mangrove, *Avicennia marina*, had more total chlorophyll and chlorophyll b was enriched compared to chlorophyll a. According to Raven et al. (1986) chlorophyll b absorbs light at different wavelengths than chlorophyll a and extends the range of light that could be used for photosynthesis. We believe that our shade leaves had more chlorophyll b because they were under more stress and need to improve photosynthetic efficiency. If leaves from total shade and total sun were available we believe the difference between chlorophyll concentrations would have been greater.

Conclusions

In conclusion our study was based on physiological differences between sun and shade seedlings of the red mangrove, *Rhizophora mangle*. Of our five experiments stomatal density seemed to be genetically controlled by the plant while the other four
seemed to be environmentally regulated. We believe there are two reasons why the red mangrove seedlings are successful in this saline environment. Red mangrove seedlings survive in the understory because they are physiologically regulated by environmental conditions. The other reason is because red mangroves evolved viviparous propagules with a large reserve of food which allow them to survive in the stressful understory.
Literature Cited


Figure 1. The potometer set up.
Figure 2. The spectrophotometer set up.
Figure 3. Red mangrove seedling density in 1m$^2$ quadrats found in sun and shade (n=20)
Figure 4. Stomatal density on red mangrove leaves found in sun and shade (n=5)

![Stomatal Density Graph]

Figure 5. Leaf succulence of red mangrove leaves found in sun and shade (n=5)

![Leaf Succulence Graph]

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![Leaf Succulence Graph]

Figure 5. Leaf succulence of red mangrove leaves found in sun and shade (n=5)

![Leaf Succulence Graph]
Figure 6. Transpiration rates of red mangrove leaves found in sun and shade (n=5)

Figure 7. Red mangrove leaf sizes found in sun and shade (n=20)

Figure 8. Total chlorophyll concentration per unit area for red mangrove leaves found in sun and shade (n=5)
Figure 9. Chlorophyll-a and chlorophyll-b concentrations per unit area for red mangrove leaves found in sun and shade (n=5)