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On the Composistion of *Cymodocea nodosa* Root Exudate Under Artificial Blue, Green and Natural Light Conditions

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ABSTRACT Seagrasses are identified as a sentinel species: a good indicator of overall marine ecosystem health and function. At the rhizome, they are known to interact with marine bacteria by exchanging energy in the form of glucose and free amino acids secreted through root exudate in exchange for microbe-fixated nitrogen that can be utilized for plant growth. To analyze potential outcomes of possible future changes in light availability, an experiment was designed to collect and analyze the root exudate of *Cymodocea nodosa* under three light conditions (standard fluorescent light, blue LED, and green LED light). After 72 hours of treatment, the root exudate was examined for glucose, nitrite, nitrate, and ammonia concentrations via spectrophotometry, while respiration was measured utilizing oxygen respirometry. No differences were observed for glucose, free amino acid content, nitrite, or ammonia. The standard fluorescent lightning yielded a significant increase in respiration of *C.nodosa*. Nitrate displayed a significant increase in both blue and green LED lightning. Due to the shortened experimental time frame it is concluded that a more significant effect could be observed if exudate is studied longitudinally.

INTRODUCTION

Around the world, there are approximately 60 species of known seagrasses that grow in large meadows (Orth et al., 2006). As producers in the marine environment, they provide a major source of energy for multiple trophic levels. One way they provide energy is by releasing organic carbon from their roots into nearby sediment, which are known to be locations of high microbial activity that aid in decomposition (Duarte, Holmer, & Marbà, 2013). These microbes are essential to various nutrient cycles within the marine ecosystem as they interact with the environment's detritus (Wasmund, 2017). At higher trophic levels, seagrasses have a direct role

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in sustaining various species reliant on the nutrients seagrasses produce from sunlight. Among them are many species of fish, whose habitat has been encroached on in recent years by commercial fishing activity. Seagrasses provide fish a shelter from predators, an area to feed, a temporary nursery, and a permanent habitat for other invertebrates (Jackson, Rowden, Attrill, S.J, & M. B, 2001). As seagrass meadows shrink worldwide, many of the species' populations that rely on their sustenance will be increasingly threatened.

Given that seagrass meadows produce so much energy for coastal ecosystems, they tend to grow in shallow coastal waters with plenty of sunlight. They have such a high necessity for light that some seagrass species need to obtain up to 25% surface irradiance, while other angiosperms may only need approximately 1% surface irradiance (Drew, 1978). *Cymodocea nodosa* is a warm water pioneer species of seagrass that can be located along the North African coast, coastal Mediterranean and in the benthic zone surrounding the Canary Islands (Cebrián, Duarte, & Marbà, 1996). In fact, *Cymodocea nodosa* has been observed to populate both shallow subtidal zones as well as water as deep as 50-60 meters (Borum, Duarte, Krause-Jensen, & Greve, 2004). It is considered a model species for studying the effects of light-stress due to to its ability to withstand a wider range of surface irradiance.

The Rhizobiome

The site of nutrient transfer between seagrasses and marine bacteria is the rhizome, which grows just below the soil surface in close conjunction with the plant's roots. Some rhizobacteria (bacteria growing within the rhizome of a seagrass) form a symbiotic relationship with plants. Such is the behavior of diazotrophs, which help to fixate nitrogen onto the plant's roots in times when nitrogen availability is limited (Bürgmann, Meier, Bunge, Widmer, & Zeyer, 2005). In exchange for fixated nitrogen, marine bacteria obtain energy resources secreted by seagrasses into the rhizome (Nielsen & Pedersen, 2000). Carbohydrates from photosynthetic processes (10-40% of all photosynthetically produced carbon) and free amino acids that help enrich the surrounding soil with sources of carbon and nitrogen, are the main components of root exudate (Lee, 2007). It is believed that the secretions of these macromolecules provide rhizobacteria with the sustenance needed to fixate nitrogen and perform other life processes (Herbert, 1999). Carbohydrates and amino acids are good energy sources for rhizobacteria, because they have a large amount of accessible carbon.

Factors Affecting the Rhizobiome

Nitrogen is a key component of the bodies of all living organisms, it plays a fundamental role in the formation of all proteins and nucleic acids. While nitrogen is extremely abundant (78% of the earth's atmosphere by volume) it tends to exist as a gas, which is unusable to most species, including *C.nodosa* (Fields, 2004). In order for atmospheric nitrogen to be usable it must be converted to and used in different forms through nitrogen cycling. This is done by bacteria that convert nitrogenous material from the atmosphere and detritus into usable nitrogen sources (such as nitrate, nitrite and ammonia) for living organisms. Rhizobacteria with a symbiotic relationship are often referred to as Plant Growth Promoting Rhizobacteria (PGPR), having been observed to aid in dynamic growth factors of marine angiosperms. *C.nodosa* is able to acquire a usable form of nitrogen (nitrate and nitrite) through its relationship with nitrogen-fixing bacteria, which convert ammonia to nitrate and nitrite in the rhizosphere (Fields, 2004). By measuring the concentrations of ammonia, nitrate, and nitrite in the root exudate of *C.nodosa,* the crucial role that nitrogen plays in the life cycle of *C.nodosa* can be examined.

Amino acids play a part in development and growth, pH control, and stress regulation in plants (Hildebrandt, Nunes Nesi, Araújo, & Braun, 2015). While they play similar roles in cellular regulation and intracellular processes in the rhizosphere as in other organs, it has been found that amino acids play a crucial part in osmoregulation. To regulate the water flowing into or out of the rhizosphere, plants will synthesize different solutes from amino acids and deploy them where necessary to increase cellular

flux for microbes (Moe, 2013). This mechanism allows for plants to direct the flow of their nutrients needed to sustain the microbes living in their rhizosphere. Due to the crucial roles amino acids play in the rhizosphere of plants and beyond, amino acid concentration was selected as a variable of study.

In a recent study, it was observed that alterations in light availability for seagrass had an impact on its released exudates, suggesting that root exudate could be a strong measure for light-stress on plantlife (Martin, Statton, et al., 2018). The light wavelength range of 400-500 nm is ideal for promoting photosynthesis (Reece et al., 2013). A concern for seagrasses is the amount of light attenuation that occurs before the light reaches their leaves. With predicted changes in global climate paired with the continuation of human generated pollution increasing in marine environments, it is expected that many seagrasses will struggle to photosynthesize as efficiently (Gosling et al., 2011). Being a benthic plant, seagrasses have a high amount of chlorophyll-a (absorbance peaks at approximately 665 nm and 440 nm), which is also present in phytoplankton that produce algal blooms (Torres, Ritchie, Lilley, Grillet, & Larkum, 2014).

As human activity in the marine environment becomes increasingly more detrimental, it is predicted that seagrasses will have to compete more for optimal sunlight (Toro-Farmer et al., 2016). One detrimental example of light-stress is observed in the overexposure of leaf tissue to UV light correlating with leaf reddening, which is a sign of anthocyanin poisoning (Novak & Short, 2011). Damage to a plant's leaves can be especially toxic to a plant's health as it greatly inhibits its ability to respire and photosynthesize. With alterations in photosynthetic capacities, it is also predicted that the symbiotic relationship between seagrasses and rhizobacteria will be affected. Considering that light could be scant in some regions or too intense in others with changing climate, it is worth considering if exposure to a consistent single wavelength of light is harmful or beneficial by measuring its photosynthetic repiratory capacity. In order to measure photosynthetic activity, dark respiration tests would be suitable for measuring the plant's respiration, which in turn would allow for a direct measure of the oxygen saturation that the plant is expending (Drew, 1978).

To better understand the relationship between changes in differences of wavelengths of light and its effect on the root exudate generated in seagrasses, an in-situ experiment was designed to study *C. nodosa*. Three light conditions (blue, green, and natural light) all based on a corresponding wavelength (455 nm, 515 nm, and a range of simulated sunlight, respectively) artificially created by light emitting diodes were chosen as the basis of the experimental conditions to analyze the variables of plant respirometry, carbohydrate, free amino acid, nitrate, nitrite, and ammonium concentrations within root exudate. The study aimed to test two hypotheses. Based on the literary research it is hypothesized that there will be a significant difference in free amino acid, carbohydrate, and nitrogenous concentrations observed between groups exposed to different light conditions. It is further hypothesized that a significant difference in photosynthetic plant respiration will be observed between treatment groups.

METHODS

C.nodosa was collected from the bay of Cádiz during low tide at the coordinates 36.490213, -6.264767. This particular seagrass bed contained a mixture of *C.nodosa* and *Zostera noltii*. *C.nodosa* was distinguished from *Z.noltii* by examining the roots of extracted seagrass. *C.nodosa* has a single branched root coming from each node, while *Z.noltii* has multiple branched roots coming from the rhizome. In curating an optimal method to isolate and collect root exudate during light treatment, an initial hydroponic setup was designed to house the specimen in situ. The hydroponic setup utilized a PVC tube with a foam core encased in silicone to provide a barrier of separation between the root and the rest of the plant. Drain ports were made at the bottom of the tubes in order to aid in extraction of root exudate samples. An LED light strip was then wrapped around the inner top of the tube. The LEDs used were Aura LED by Tzumi. However, the design was discarded when it was noted that placement of the plant in the foam core could potentially

damage the specimen, and that a design flaw prevented the formation of a strong seal around the drain port, thus greatly increasing the risk of loss of sample.

Instead, a simpler design was employed, involving a soil medium in a seawater aquarium. The goal was to encase the root portion of each sample plant in a 1 mL Eppendorf tube that could be sealed watertight with Parafilm and then buried in the sand (originating from the banks of Rio San Pedro) of an aquarium, such that each specimen's roots remained anchored to the bottom. Lights corresponding to each treatment condition were wrapped around the aquarium, which was then wrapped up with aluminum foil to prevent light leaking and cross contamination. An approximate surface irradiance was calculated by dividing the watts of the lights by the surface area of the tank. The watts of all the lights used was 100 watts and the surface area of the tank was 900 cm^2. There fore the estimated surface irradiance is 0.111 W/cm^2. The experiment was carried out over a period of three days, approximately 72 hours.

Exudate Collection

In order to collect root exudates, the design required each *C.nodosa* sample be prepared to have a 2 cm rhizome by cutting its original rhizome 1 cm away from the leaf blade on either side, and placed into a 1.5 mL Eppendorf tube with its top removed entirely. To ensure collected exudate samples were not tainted with salt water from the aquarium, each Eppendorf tube was filled with 1 mL of saltwater and wrapped up with parafilm above the rhizome to create a waterproof barrier between the leaf blade and rhizome. Once parafilm was wrapped around each tube, it was further secured with tape. The newly prepared specimens were then placed in the sand of their respective treatment aquaria.

Glucose HK Assay

To determine the amount of glucose in each exudate sample, a Glucose HK kit from Spinreact was utilized. The procedure from the kit was scaled down and modified to work with the root exudate samples, because the kit's original use was for blood and urine samples. The reaction was conducted at room temperature inside of a 96-well microtiter plate. To prepare the reagent, a vial of enzymes containing (2 mmol/L of NAD+, 1000 U/L of Hexokinase, and 1000 U/L of Glucose-6-phosphate) was mixed in a buffer containing (4 mmol/L of TRIS pH 7.5, 2.1 mmol/L of ATP, and 0.8 mmol/L of Mg2+). Each sample was prepared in duplicate on the 96-well microtiter plate. Each sample well contained 50 µL of the exudate sample and 250 µL of the reagent. Blanks were prepared in triplicate using 50 µL of Milli Q water and 250 µL of reagent in each well. Standards were also prepared in triplicate using 50 µL of glucose with a concentration of 100mg/dl and 250 µL of reagent. Lastly, 50 µL saltwater samples from the specimens in each aquarium treatment group and 250 µL of reagent were prepared in triplicate in the 96 vial plate wells. After placement in the wells, samples were allowed to react for 10 minutes. Spectrophotometry of the samples was then performed with a Powerwave 340-Bio-Tek spectrophotometer. Average absorbance readings were recorded for each sample in each treatment group at 340 nm and used to calculate the glucose concentrations released by each specimen.

Measurement of Free Amino Acids

The procedure began with the creation of seven L-alanine standards of differing concentrations (0.25 mM, 0.125 mM, 0.0625 mM, 0.03125 mM, 0.01563 mM, 0.00781 mM, and distilled water) from a stock solution of 0.0445 g of L-alanine mixed with 50 mL of distilled water. Upon creating the solutions, a 96-well microtiter plate was organized for the placement of specimen sample and standard. Once done, 140 µL of sample or standard were placed accordingly in each designated well. Then 80 µL of ninhydrin followed by 80 µL cyanide acetate were added to each well plate containing sample or standard. The well plate was baked at 100°C for 15 minutes before it was placed in a Powerwave 340-Bio-Tek spectrophotometer and analyzed for absorbance maxima at 590 nm.

Measurement of Respiration and Set-up of Lights

Aquaria of the blue and green light conditions were covered with aluminum foil to prevent external light from entering the tank. After 72

hours, the plants were taken out for respiration tests. The respiration procedure was adapted from a previous study done on fish respiration.[20] Homemade cylinders (600mL), made by Dr. Jason Bystriansky, were placed inside a 10 gallon tub. A water pump was connected to each of the cylinders to have a stream of oxygen-saturated and oxygen-unsaturated water flow through the optic fiber of the Loligo Witrox 4 oxygen instrument. Four plants were inserted into each cylinder to test all 8 plants from each light condition in one series of data collection. Oxygen saturation data was collected from the optic fibers of Loligo Witrox 4 in time intervals starting with 30 minutes of exposure to the respective light condition. Afterwards, the lights were shut off and the tub was covered with a black tarp to measure oxygen saturation in the dark for another 30-minute time interval.

Measurements of Nitrite, Nitrate, and Ammonia

To determine the concentrations of nitrite, nitrate, and ammonia for each sample, Salifert assay kits were used. The procedures from the kits were scaled down from their original aquarium application to work with root exudate samples. After calculating absorbances, a standard curve was fitted, and the concentrations of nitrite, nitrate and ammonia were calculated for each sample.

The stock solution for nitrite was prepared by diluting 100 mg of $NaNO_2$ in 0.1 L of H_2O. The stock was then diluted to create the 2.0 mg/L, 1.0 mg/L, and 0.5 mg/L standards. Into a 96-well microtiter plate 100 µL of each standard solution was added with 5 µL of reagent 1 and 5 µL of reagent 2 from the Salifert nitrite kit. Absorbance was measured for each sample and standard using a Bio-Tek Powerwave HT Microplate Spectrophotometer set to 530 nm.

The stock solution for nitrate was created by diluting 100 mg of $NaNO_3$ in 0.1 L of H_2O. Each standard was created by diluting the stock solution to reusable amounts of 100 mg/L, 50 mg/L, and 25 mg/L standard solutions. After placing standards and samples in a 96-well microtiter plate, 12 µL of reagent 2 was added into each well, followed by 100 µL of reagent 1. Absorbance was measured for each sample and standard using a Bio-Tek Powerwave HT Microplate Spectrophotometer set to 540 nm.

The stock solution for ammonia was created by diluting 10 mg of NH_4Cl in 0.1 L of H_2O. The stock was then diluted to create standard solutions of concentrations: 2.0 mg/L, 1.0 mg/L, and 0.5 mg/L. Following the creation of each standard, 80 µL of each standard and sample solution was added to a 96-well microtiter plate with 40 µL of reagent from the ammonia assay kit. Absorbance was measured for each sample and standard using a Bio-Tek Powerwave HT Microplate Spectrophotometer set to 600 nm.

Spectrophotometry

After measuring absorbance in a spectrophotometer for each sample in any of the assays conducted, the Beer-Lambert Law (A=ebc) can be utilized to calculate the concentrations. In the Beer-Lambert law "A" represents the absorbance, "e" represents the molar absorptivity in units of L mol^{-1} cm^{-1}, "b" represents the path length of the cuvette in units of cm, and "c" represents the concentration of the compound in solution, expressed in mol L^{-1}. To determine the unknown concentration of the molecule of focus within each sample a standard curve is fitted with a linear regression using the absorbances measured from the standard solutions. The slope of the linear regression defining the curve is equivalent to the product of path length (b) and molar absorptivity (e). Thus, with the standard curve's slope and the measured absorbance concentration can be calculated.

Statistical Testing

A one-way ANOVA performed in Microsoft Excel was utilized for the statistical analysis of the Glucose HK Assay, measurement of free amino acids, respiration, and nitrogenous content assays.

RESULTS

Glucose HK Assay

The average absorbance readings were converted to µmol and divided by each sample's dry root weight (measured in grams) shown in Figure 1.

No significant difference in the amount of glucose in the root exudates of the three treatment groups was found ($p = 0.610$).

Measurement of Free Amino Acids

The absorbance readings were converted to mM concentration. No significant difference was observed in the amount of free amino acids in the root exudats of the three treatment groups ($p = 0.27301$) as shown in Figure 2.

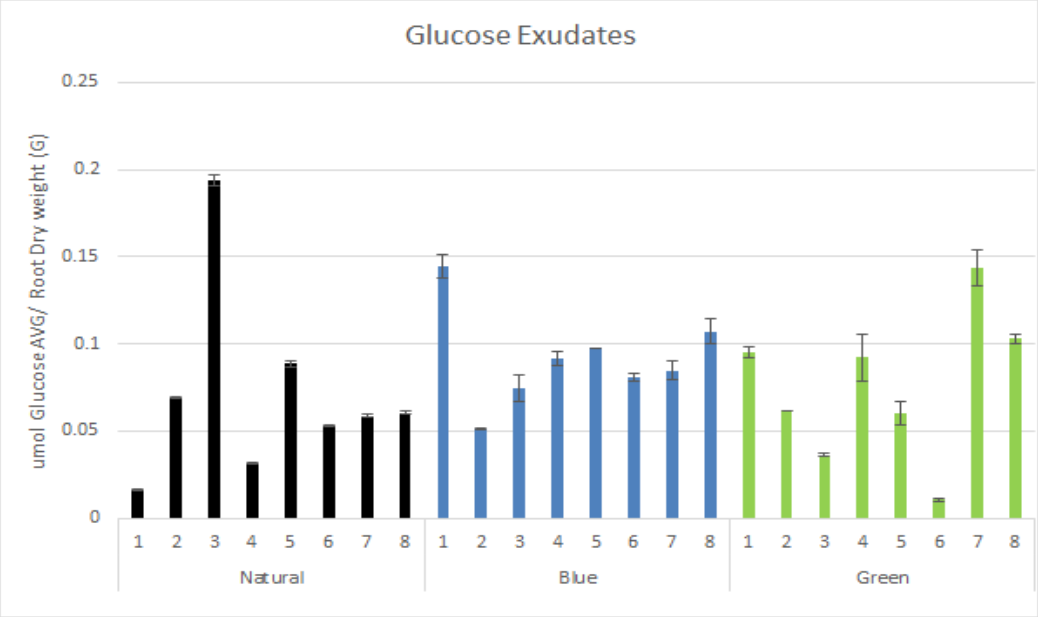

Figure 1. The averages of µmol of glucose g^{-1} root dry weight of all root exudate samples. The error bars indicate the standard error of each sample. The total average of the "natural" light treatment group was 0.07 ± 0.05 µmol of glucose g^{-1} root dry weight, blue light treatment group was 0.09 ± 0.03 µmol of glucose g^{-1} root dry weight, and the green light treatment group was 0.07 ± 0.04 µmol of glucose g^{-1} root dry weight. All listed averages are shown with ± standard error.

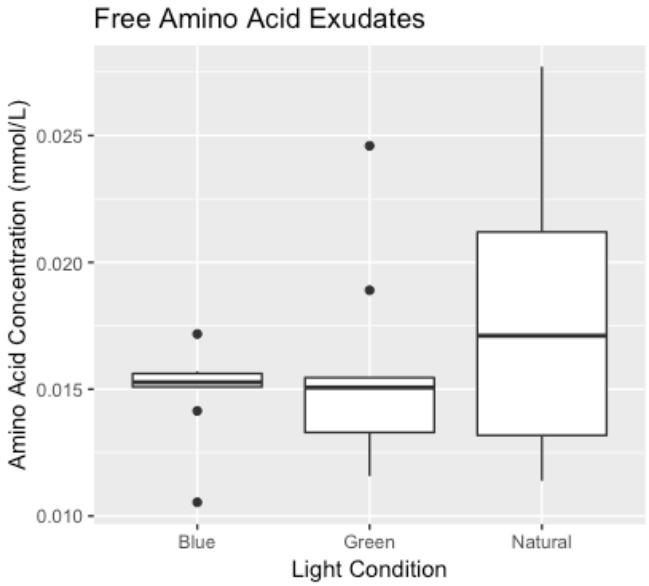

Figure 2. Boxplot of amino acid concentrations per replicate by treatment group. Outliers are represented as dots.

Respiration

The oxygen saturation of each group was plotted against time to show the overall change during the 60-minute period (Figures 3, 4, and 5). The change in percent oxygen per minute by mass was calculated for each light condition. It was found that natural light had the lowest rate of oxygen production at -0.13 % min^{-1} g^{-1}, while blue light had a calculated rate of -0.33% min^{-1} g^{-1} and green light had a calculated rate of -0.31% min^{-1} g^{-1}. There was a significant effect of the wavelength of light on the oxygen saturation ($p=0.05$).

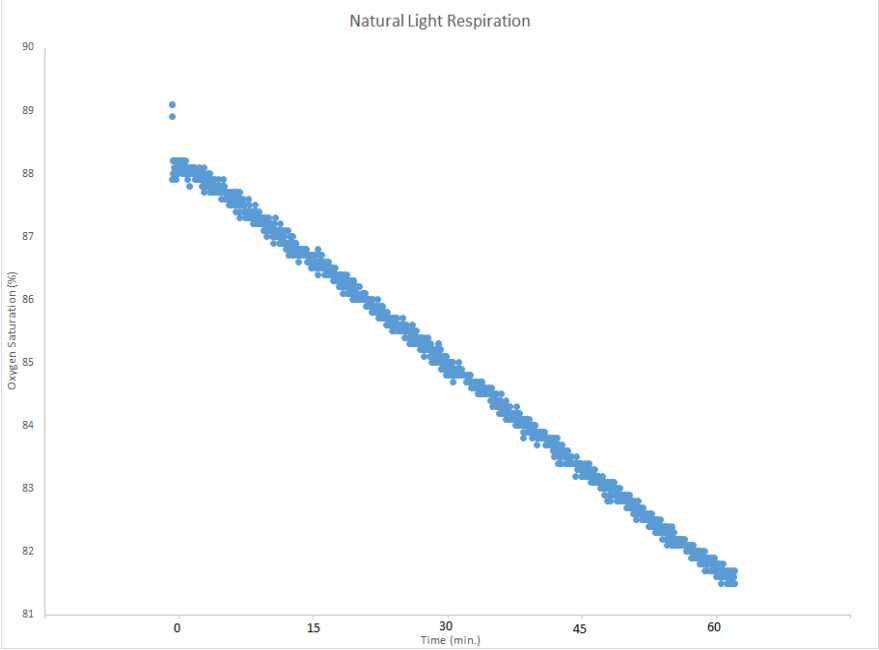

Figure 3. The average change in oxygen saturation for the natural light condition.

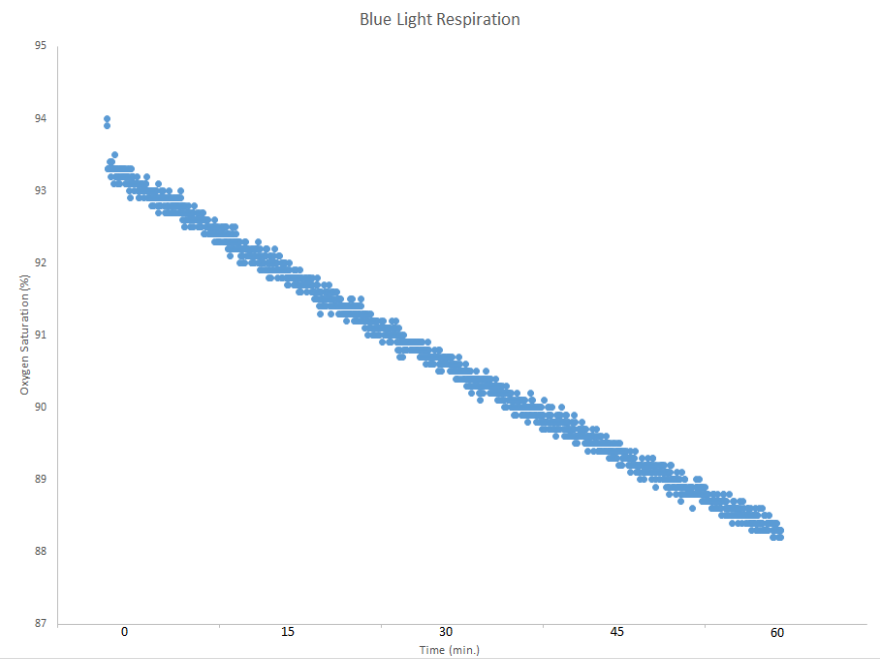

Figure 4. The average change in oxygen saturation for the blue light condition.

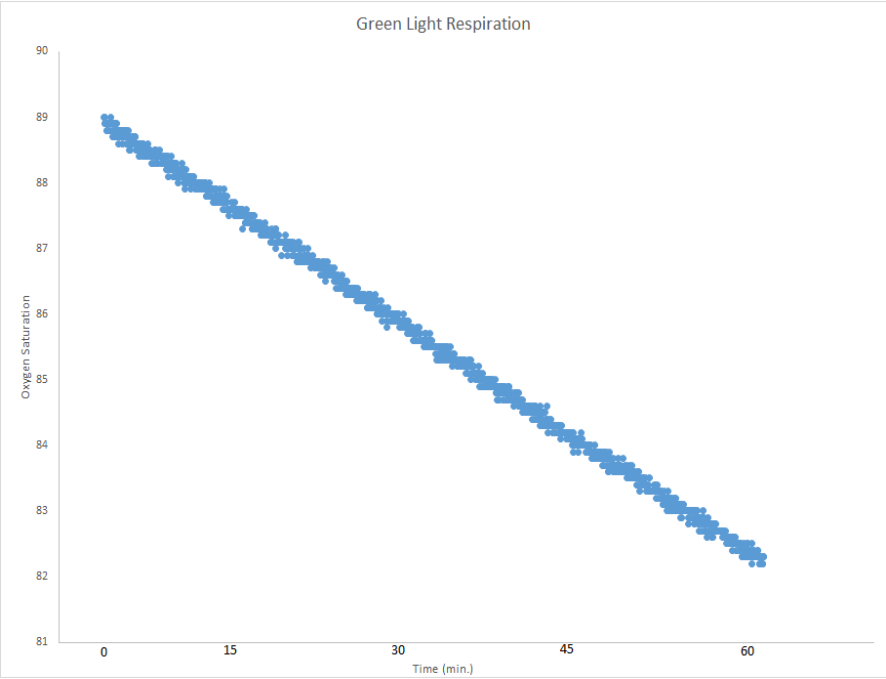

Figure 5. The average change in oxygen saturation for the green light condition.

Nitrate, Nitrite, and Ammonia Assay

The average absorbance and concentration (in µg/mL) for nitrate, nitrite, and ammonia of all 8 samples from each of the three treatment groups can be found in Table 1. Figures 6-8 display the standard curves of nitrate, nitrite, and ammonia used to determine the concentrations. The nitrate assay yielded statistically significant results ($p = 0.015$), and the nitrite results did not yield statistically significant results ($p = 0.865$).

Sample		**Light Wavelength**		
		Natural	**Blue**	**Green**
Nitrate	**Avg Concentration (µg/ml)**	10.10(±1.9)	42.00(±0.7)	48.1(±0.6)
Nitrite	**Avg Concentration (µg/ml)**	0.4(±2.4)	0.7(±1.1)	0.8(±1.0)
Ammonia	**Avg Concentration (µg/ml)**	22.55(±1.15)	55.37(±0.75)	41.40(±0.62)

Table 1. Average concentrations calculated for each treatment group in µg/ml and with ± 1 standard error.

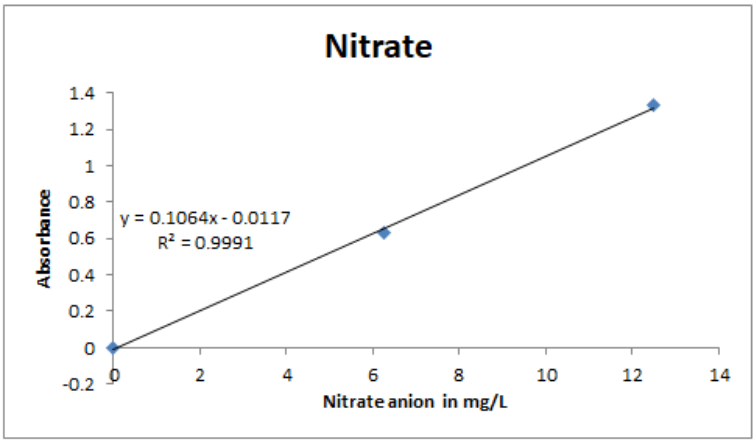

Figure 6. The figure above presents the standard curve of the nitrate stock solutions. The figure plots of Absorbance value for a given concentration. From the figure, a linear relationship was discovered, which can be represented by the equation y=0.1064x-0.0117 and R^2=0.9991.

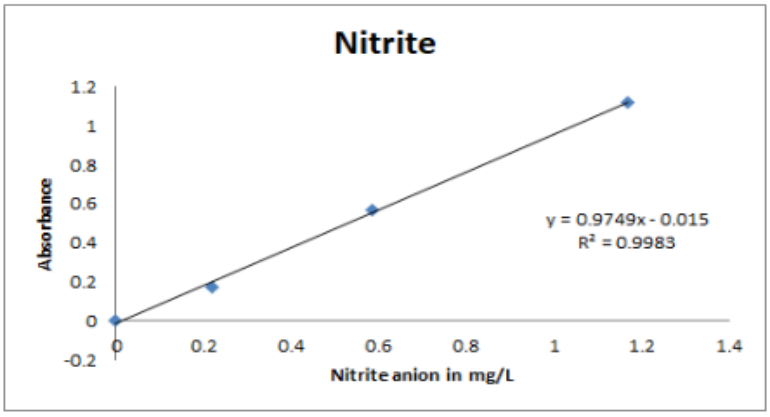

Figure 7. The figure above presents the standard curve of the nitrite stock solutions. The figure plots of Absorbance value for a given concentration. From the figure, a linear relationship was discovered, which can be represented by the equation y=0.9749x-0.015 and R^2=0.9983.

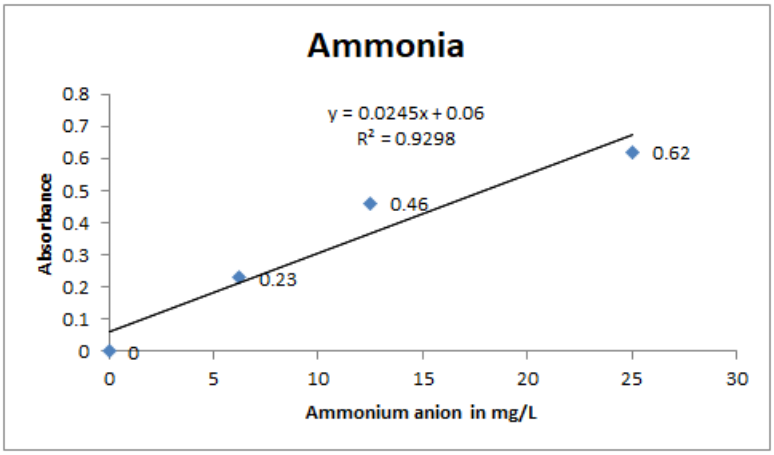

Figure 8: The figure above presents the standard curve of the ammonia stock solutions. The figure plots of Absorbance value for a given concentration. From the figure, a linear relationship was discovered, which can be represented by the equation y=0.0245x-0.06 and R^2=0.9298.

DISCUSSION

ANOVA results from respirometry data suggested that there was a significant effect of light wavelength on oxygen saturation in *C. nodosa*'s root exudate ($p=0.05$). This suggests what nature confirms, that natural light underpins the best environment for the plant's respiration and photosynthetic processes in general. Despite this, the results from this study can be extrapolated to claim that artificial light meant to simulate sunlight may help with the growth and restoration of *C. nodosa*'s and similar species' populations in the future if a large percentage of seagrass meadowbeds are adversely affected by climate change and human activity.

Glucose is one of many carbohydrates that are exuded by plant roots, although due to the ease with which it is processed by microorganisms in the rhizosphere, it is considered one of the more important carbohydrate exudates for microbe populations (Strickland, Wickings, & Bradford, 2012). Glucose has been shown to be an influential exudate component in rhizoremediation, a process where plant exudates support microbial populations responsible for degrading pollutants. In one study observing root exudate composition and its effects on the microbial community, it was found that glucose had the most significant positive effect in increasing mycobacterium populations. Mycobacterium like diazotrophs also help to support a plant, however, rather than fixating nitrogen they help degrade pyrene in contaminated soils. The glucose concentrations observed in this experiment were far below the 150 mM level that has been observed to help with desorption of contaminants such as pyrene (Lu, Sun, & Zhu, 2017). One of the factors that likely contributed to the lower glucose concentration is the shortened experimental timeframe of three days. This shortened span of time along with stress produced from the initial design could explain why root exudate samples yielded no statistically significant glucose results as energy was diverted to homeostatic processes.

Previous literature suggests that amino acid concentration in marine angiosperms can be altered by stress and light availability, while in terrestrial angiosperms amino acids have been linked to communication between host plant and rhizobia. Research on the physiological processes underlying repairs to structural damage to plant tissues suggests that plants could be diverting more energy towards the building of polypeptides and the synthesis of free amino acids than to the degradation of proteins to amino acids for secretion at the root (Nielsen & Pedersen, 2000). This can have a substantial effect on microbial populations as it was found that in low light conditions, seagrasses will invest less energy output to their root exudate, drastically altering the bacterial makeup of the microbiome (Martin, Gleeson, et al., 2018). When conditions are ideal, the relationship between rhizobia and amino acids is best exemplified on land in the molecular mutualism of legume-rhizobia symbiosis, where the legume host provides rhizobacteroids with specific amino acids that allow the cessation of ammonia assimilation and the initiation of biochemical processes that provide the plant with amino acids required to synthesize asparagine (Lodwig et al., 2003). The results of the ANOVA for amino acid concentrations suggested that light condition is significant in affecting concentration values. This can be attributed to the natural cycling of free amino acids occuring between seagrass and rhizobia, as either would explain no significant change between treatment groups. As such, future investigations should aim to collect data from different tissues of the same plant to gain insight into the movement of amino acids.

The link between host plant and rhizobia is based on the nitrogenous needs of both species. The results yielded from the nitrate assay suggest that the average concentration of nitrates in the blue and green light treatment groups was increased relative to the natural light condition. The results for the nitrite assay displayed no statistically significant difference between experimental groups, and the results of the ammonia assay should be considered inconclusive, as a good calibration curve was unable to be fit to it. These results are consistent with the potential for nitrogen fixation to have taken place via diazotrophs; a review on nitrogen fixation in seagrass meadows calculated an observed rate of 0.03 - 150 mg N m^{-2} d^{-1} present in different seagrass beds (Welsh, 2000). Traditionally, it has

been understood that nitrogen-fixating bacteria convert atmospheric N_2 into ammonia, which is used in various processes by the host plant. Research into ammonia toxicity in *Z. marina* suggested that nitrate, unlike ammonia, does not produce cytotoxicity at any concentration (van Katwijk et al., 1997). Taking this into account with the aforementioned observation that rhizobia in terrestrial angiosperms terminate ammonia assimilation when provided with a supply of amino acids demonstrates the potential for mutualism between marine plant and rhizobia species. This suggests that ammmonia is an undesirable source of nitrogen relative to amino acids and nitrates as detailed by the activity of glutamine synthetase, which changes ammonia into glutamine in plant cytosol. Glutamine plays a range of roles as a nitrogenous source for amino acids, nucleotides, and is linked to limiting the uptake of nitrates by downregulating the activity of nitrate reductase through transcription factors, which increases nitrate concentration within the cell (Miller et al., 2008; Kan et al. 2015). Similarly, when analyzing the output and type of amino acids in *Z. marina*'s root exudate, researchers found that alanine and butyric acid (GABA) concentrations substantially increased in eelgrass when it was exposed to anoxic conditions, similar to ones seen in algal blooms (Pregnall, Smith, Kursar, & Alberte, 1984). Both GABA and alanine have been observed to positively correlate with increases in nitrate influx, suggesting that glutamine, GABA, alanine and potentially many other amino acids form the basis of a signaling system regulating the flow of nitrogenous material between rhizobia and host plant (Miller et al., 2008). Still, increases in nitrate concentration under exposure to blue and green light conditions may explain why there was no statistical difference observed in glucose concentrations, as it is implicated as an easily oxidized source of carbon for rhizobacteria that are carbon limited (McGlathery et al., 1998). In order to fully determine whether a communication mechanism based on nitrogen exists in marine angiosperms, or if the results simply reflect an energy producing strategy of microbes, will require further investigation into rhizobia classification and what concentrations and types of amino acids are present in the various tissues of host plants.

The possibilities of these results cannot become clearer without further experimentation. In future studies of *C. nodosa*'s root exudate, researchers should focus on reducing the amount of stress exposed to the plant by developing an in-situ design allowing for the measurement and management of experimental variables before and after each trial (Phillips et al., 2008). Furthermore, identifying rhizobacteria populations through light microscopy and molecular characterization of 16S rRNA that are observed to be living symbiotically in the rhizome would allow insight into the metabolic activity and characteristics of essential microbes, as well as the community dynamics that govern the rhizosphere (Islam et al., 2016). An avenue that future studies could utilize to gain a clearer picture would be the use of an in-situ measurement of exudate compounds. Such an approach would not only allow for measurements to be taken before the possibility for assimilation of carbon containing exudates, but also the verification of a presence of rhizobacteria through light microscopy and molecular characterization of 16S rRNA (Phillips et al., 2008). Finally, extending the length and depth of this study may have resulted in more statistically significant results among select variables and increased the number of variables to strengthen our analysis and findings. By extending the length and depth of future studies, this could enable the development of novel solutions to impending climate changes and expanding human activity in the marine environment.

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