

Effects of Nutrient Runoff on the Photosynthetic Rate and Carbon Concentrating Mechanisms of *Thalassia Testudinum*

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Introduction

Thalassia testudinum is a keystone species in the Caribbean and Gulf Coast marine ecosystems as the most abundant seagrass, creating essential habitats for other organisms. The surrounding marine ecosystem relies greatly on the presence of *T. testudinum*, serving as an important food source for green sea turtles, sea urchins, and manatees, in addition to other fish and invertebrate species (Short, Carruthers, van Tussebroek, Zieman, 2010). As the dominant seagrass in the Florida gulf coast and northwestern Cuban shelf, *T. testudinum* constitutes 97.5% of the seagrasses present in those regions (Dineen, 2001). In the occurrence of high nutrient inputs, *T. testudinum* is outcompeted by other species which are better able to withstand those conditions, in addition to the overgrowth of epiphytic algae which hinder the seagrass' ability to access sunlight (Strong, 1992). As the epiphytes increase from the nitrates and phosphates in the fertilizer, they eventually bacterially decompose, a process which consumes the dissolved oxygen present in the water (Birkholder 2007).

Currently, one of the most imminent threats to *T. testudinum* is the process of cultural eutrophication as a result of nutrient runoff from coastal agricultural sites (Short, et al., 2010). In the NOAA's 2017 annual forecast, generated from a series of models based on nutrient runoff data from the U.S. Geological Survey, the largest ever dead zone in the Gulf of Mexico was

recorded ever since the beginning of dead zone mapping in 1985 (Belva, 2017). This was in consequence of the nutrient pollution from agriculture and developed land runoff from the Mississippi River watershed, which negatively impacted the aquatic ecosystems, and subsequently, the coastal habitats and resources.

As the demand for agricultural yields increases rapidly, many agricultural units have turned to fertilizers to increase the yields of their crops. This anthropogenic nutrient input causes eutrophication in coastal regions, resulting in dead zones. The goal of this research is to find a correlation between the nutrient levels of the sea water and the oxygen production of the *T. testudinum*'s photosynthetic rate and its carbon concentrating mechanisms, which directly impact the chemical makeup of the ocean water and its corresponding internal structures. Previous studies indicated that consequences of the higher nutrient concentrations include physiological responses such as ammonium toxicity and water-column nitrate inhibition through the *T. testudinum*'s external and internal photosynthetic pathways of carbon conversion and limitation. The promotion of the growth of macroalgae and diatoms due to the increase in the water's nutrient concentration stimulates the growth of macroalgae and diatoms, of whose bacterial decomposition consumes even more of the dissolved oxygen present in the water table. Due to the subsequent decreased and limited reactant of sunlight, a decrease in the photosynthetic rate is expected, and presumably a change in the usage of the carbon pathways due to a change in the chemical makeup of the water with the introduction of new algal and bacterial growth.

Methods

Various samples of *Thalassia testudinum* were collected from the Gulf Coast from St. Teresa and taken back to the Florida State University Coastal and Marine Laboratory and contained in the laboratory's mesocosms with varying temperature and nutrient concentration through the manipulation of the primary water source which fed into the individual units. Three shoots of *T. testudinum* were placed within each unit, with six units given the nutrient treatment and six as control. The control was set so that the units contained standard seawater with *T. testudinum* growing inside of it, and with both sets of tanks so that the temperature can be compared to the responses as well. Pulses of the nutrient treatment were done two to three times a week over the course of four weeks, with measurements of the respiration rate, photosynthetic rate, and the carbon concentrating mechanisms done once a week.

In order to measure the seagrass' photosynthetic and respiration rates as a consequence of higher nutrient concentrations, an optical oxygen meter FireStingO2 was used, which utilizes fiber-optical oxygen sensors to measure the quantity of oxygen present in the samples which were taken weekly. When measuring respiration, the collected samples were placed under a dark setup with no access to light, and the oxygen present in the sample was recorded over the course of 300 seconds. To measure photosynthesis, synthetic white, blue, and red light were shone onto the samples with other ambient light being blocked out from the sample; similarly, the oxygen was again recorded over a time span of 300 seconds. In order to measure the net rate of oxygen consumption during respiration and oxygen production from photosynthesis, the individual

samples taken from the units were massed, and the rate of oxygen was simply calculated by taking the slope of the quantitative oxygen against time.

To measure the samples' usage of its carbon concentrating mechanisms, the internal and external photosynthetic pathways of bicarbonate conversion to carbon dioxide in the plant cells were inhibited using the anion-exchange inhibitor 4,4'-diisothiocyanostilbene-2,2'-disulphonic acid (DIDS) and acetazolamide (AZ). The presence of carbon in the ocean largely exists in the form of inorganic bicarbonate, HCO_3^- , whereas the plant cells require carbon dioxide to perform photosynthesis. In marine organisms especially, plant cells have evolved to develop internal and external photosynthetic pathways to diversify methods of carbon acquisition, which uptake bicarbonate in the ocean and convert it into usable carbon for photosynthetic usage (Burkholder, 2007). Since AZ inhibits the enzyme that readily converts bicarbonate which exists in the ocean water into carbon dioxide into the cell, and DIDS blocks the direct intake of bicarbonate into the cell, it leaves only the ambient carbon in the ocean water as the seagrass' source of carbon for photosynthesis. By doing so, the relative use of each pathway during photosynthesis was quantified and tested if the proportion of use of these pathways is affected by higher nutrient concentrations in water.

The inhibitors AZ and DIDS were diluted with sodium hydroxide and put into the samples of *T. testudinum* with an excess concentration, so that there would be sufficient inhibitors regardless of the mass of the sample taken. Magnetic stir bars were placed into the tubes with samples and kept constant throughout the entire data collection. In addition, since the sodium hydroxide was used as a solvent and changes the pH of the seawater, a baseline comparison of only an addition of sodium hydroxide was measured as well. A solution of 0.02

mol AZ and NaOH and 0.03 mol DIDS, and 100 microliters of NaOH and DIDS were dissolved in the seawater, while 200 microliters of AZ was placed into the seawater. This enabled us to compare the *T. testudinum*'s usage of its various external and internal carbon pathways, if it changed as a result of the higher nutrient concentration. Furthermore, by measuring the mass of the sample of *T. testudinum*, the oxygen evolution rate per unit of mass of the seagrass can be compared between the different samples taken from the manipulation of nutrient levels.

Results

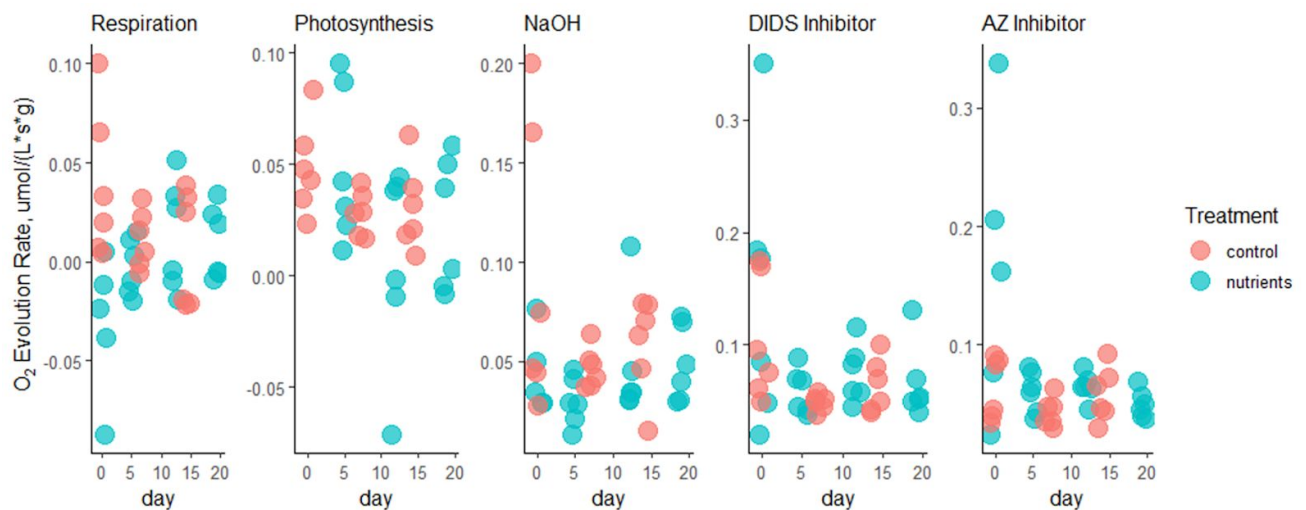
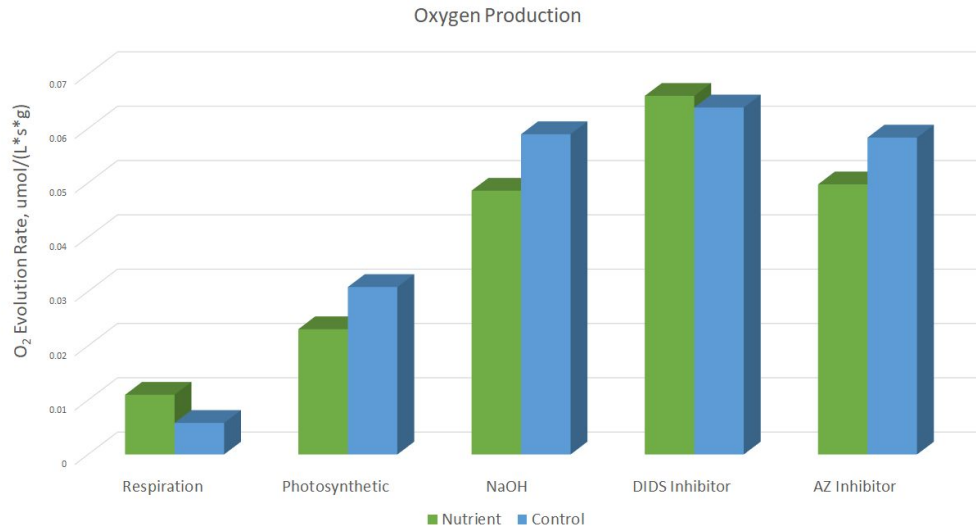


Figure 1. Oxygen evolution rate recorded for photosynthesis, respiration, and with each carbon pathway with inhibitors.

Overall, the respiration rate of the samples treated with nutrients was higher than that of the control, since there was a more negative rate of oxygen depletion; thus, more oxygen was being consumed. It was shown to be statistically significant, as the net respiration rate was directly affected by the higher nutrient concentration (ANOVA, 39, $p=0.0381$). The photosynthetic rate of the nutrient-treated samples was lower, which was expected.



Although the sodium hydroxide enabled the inhibitors to be dissolved, the baseline comparison of the solvent by itself, without the inhibitors, also showed a significant change in the oxygen evolution rate (ANOVA, 39, $p=0.0331$). Presumably, the presence of a strong base such as sodium hydroxide influences the carbon concentrating mechanisms of the cells of *T. testudinum* due to the denaturing of the enzyme which catalyzes the process of bicarbonate to carbon dioxide for photosynthetic usage. Since the enzymes without influence of a change in pH are considered to be at an optimum pH, the strong base of NaOH could have caused the enzyme to no longer be effective in the conversion reaction; the basic trait of the solvent causes the inner bonds which make up the amino acid structure of the enzyme to change, thereby denaturing it. This process could explain the results of the lower oxygen evolution rates with the AZ inhibitor of the samples treated with nutrients in comparison to the control, since the inhibitor DIDS contrastingly displays higher oxygen evolution rates in the treated samples than in the control.

Discussion

Higher nutrient concentration has been shown to contribute to decreased populations of seagrass, in which the experimental units showed the positive feedback loop of excessive diatomic growth and algal competition. The epiphytes outcompeted the *T. testudinum*, even though they were regularly cleaned twice a week. Inevitably, there would still be epiphytic growth which limits the photosynthetic ability of the seagrass. The growth of macroalgae on the surface of the blades of seagrass hindered its ability to access sunlight, thereby decreasing its photosynthetic rate as well. As the epiphytes continued to grow, their decomposition additionally consumed even more of the dissolved oxygen present in the water, which traditionally leads to the die-off of other marine organisms with a limited amount of available oxygen.

The results indicate that the respiration rate of the seagrass given the treatment of higher nutrient concentrations were greater than that of the control, while the photosynthetic rate was lower, indicating that the potential effects of higher nutrient concentrations include the overgrowth of epiphytes which harm the seagrass' ability to photosynthesize, while their increased respiration rates consume even more oxygen, feeding into a positive feedback loop which can lead to large scale dead zones, such as the one present in the Gulf of Mexico. This was simply a smaller scope of a larger scale nutrient pollution issue which occurs globally; the impact of higher nutrient concentration on seagrasses such as *T. testudinum* affects not only the seagrass structure, but also the organisms which depend on it for habitats and food supplies, making it essential in observing and understanding the effect of anthropogenic pollution in the ocean.

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