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The Effect of Changes in pH on the Production and Respiration Rates of Native Tape Grass, Vallisneria Americana

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Abstract

In the past century, the world has seen an increase in the amount of carbon dioxide (CO_2) in the atmosphere. The rise in CO_2 can put stress on aquatic ecosystems due to ocean acidification, an overall decrease in the pH of the ocean's waters. Freshwater ecosystems, already stressed by pollution and recent increases in the number of invasive species are also showing signs of acidification due to the increase in CO_2. The effect of the rise in acidity is known to be harmful to calcifying organisms, but the effect on freshwater submerged aquatic vegetation (SAV) is not well studied. The invasive SAV *Hydrilla verticallata* (*Hydrilla*) and native SAV *Vallisneria americana* (tape grass) often compete for similar environments in the freshwater portions of the Chesapeake Bay. Previous studies on the effects of pH changes on *Hydrilla* found that the SAV may be experiencing phenotypic plasticity allowing it to continue to produce and respire even at the most acidic treatments. This study looked at the respiration and production rates of *Vallisneria americana* under differing pH's. Samples of tape grass were incubated in water with a pH ranging from 8.2 units to 5.7 units in a light gradient box for the production treatments and a dark box for the respiration treatments. It was found that at the pH's closest to the control 7.2, the 6.7 and 8.2 treatments, tape grass experienced no production. In the 5.7 and 6.2 treatments, tape grass experienced significantly higher production rates. Data was collected and analyzed using a One-Way ANOVA and a Tukey's HSD test. There was a significant difference found in both the production and respiration rates at the varying pH levels. With the stress of an increase in acidity and invasive species, the results of this study suggest that tape grass will continue to produce and respire as a crucial part of freshwater ecosystems.

Introduction

For many years, alien/invasive species have been introduced to ecosystems that are foreign to them. Most of the alien species fail to root themselves in the new environments; however, the few species that do, can become very harmful to the environment that they inhabit. Invasive species can be harmful to the environment due to the fact that they have very few predators and a high output of propagules, cause less biodiversity, disrupt food webs and nutrient cycles, and cause changes in biogeochemical and hydrological species (Chadwell and Englehardt, 2008; McChesney, 2010). Invasive species are also more likely to root themselves in areas that have a low number of competitors (Chadwell and Engelhardt, 2008). The success of invasive species is also affected by the environmental conditions of the area they are invading. Invasive species may or may not be affected in the same way as native species are to environmental changes such as pollution, eutrophication, and rises in CO_2 and acidity.

As more and more fossil fuel burning cars and factories are being built around the world, the amount of CO_2 being released into the atmosphere is increasing. In the last 250 years, the amount of CO_2 in the atmosphere has increased from approximately 280 parts per million volume to nearly 384 parts per million volume in 2007 (Doney et. al., 2009). The increasing amount of CO_2 not only causes a problem for people and animals but also aquatic ecosystems around the world. Carbon dioxide rapidly diffuses into the water causing the pH to become more acidic (Arnold et. al., 2012). In the marine environment, the CO_2 reacts with seawater, forming carbonic acid and can ultimately result in ocean acidification. In the last 150 years, increases in atmospheric CO_2 concentrations have led to an increased rate of CO_2 diffusion into the oceans, causing the pH of the oceans to drop from 8.21 to 8.10 and it is predicted that in the future the ocean pH is expected to decrease an additional 0.3 to 0.4 pH units. (Arnold et. al., 2012).

Ultimately, this would lead to the oceans becoming more and more acidic. If the pH of oceans rises at a rate quicker than the vegetation can adapt, submerged aquatic vegetation species could be endangered.

While marine ecosystems seem to be the main concern, it is plausible that freshwater ecosystems will also experience increasing pH as CO_2 diffuses from the atmosphere into lakes and rivers (Strumm and Morgan, 1996). In a study conducted in 2009, it was found that marine SAV's are less vulnerable to acidic water and can continue to respire and produce as the pH rises (Doney et. al., 2009). Christopher and Haller found that at higher and lower pH, aquatic plants were not able to grow as much when a foreign pesticide was introduced (2010). Their experiment found that dry weights of the plants decreased when the pH of the water was lower or higher (Christopher and Haller, 2010). The results of the previously mentioned study indicate that as the pH of freshwater ecosystems becomes more acidic, vital submerged aquatic vegetation (SAV) could suffer.

Tape Grass (*Vallisneria americana*) and *Hydrilla* (*Hydrilla verticillata*) are two common Chesapeake Bay SAV's typically found in the freshwater portions of bay tributaries. These two plants provide services that are vital to Bay health such as being habitat for fish and aquatic invertebrates, food for waterfowl, and their roots hold the sediment, reducing erosion (Moore et. al., 2010). Tape grass and *Hydrilla* often competefor similar environments. *Hydrilla,*an invasive plant, spreadsby using tubers that sprout new growth. As *Hydrilla* grows, it forms a matt across the top of the water that often stretches for long distances (McChesney, 2010). *Hydrilla* can also grow up to an inch in a single day and can start growing earlier in the day as it requires less sunlight to grow, making it ideal for holding sediment in an ecosystem (Langland, 1996). Its native rival, tape grass, grows by a root system and is a semi-permanent plant that forms

meadows across the bottom (McChesney, 2010). Chadwell and Engelhardt examined the competition between tape grass and *Hydrilla* and found that pre-existing tape grass beds did not slow the colonization of the area by *Hydrilla* (2008). These results indicate that *Hydrilla* may be a superior competitor in the absence of additional environmental stressors.

If freshwater habitats continue to become increasingly acidic, both of these plants could suffer causing a significant gap in both habitat and food web structure in freshwater ecosystems. It is important to know how each of these species will be affected by pH changes in the water. While *Hydrilla* is an invasive species, tape grass may not be able to initially colonize an area if it experiences stress from lower pH. Alternately, *Hydrilla* may not be as successful as an invader if it experiences stress from lower pH.

In a study conducted in 2014, the effect of different pH treatments was tested on *Hydrilla verticillata*. The results of this study indicate that pH differences did have an effect on the production rates of *Hydrilla*. The study found that *Hydrilla* production rate was highest in the control 7.2 treatment, followed by the experiment's most acidic treatment, 5.7, and the most basic treatment, 8.2. Intermediate pH's tested 6.2 and 6.7 showed the lowest production rates. The results suggest that phenotypic plasticity, the ability of plants to make small changes to their structures and functions to better survive in an environment, may be occurring in *Hydrilla* (Radcliffe; Schlichting, 1986).

As a compliment to the *Hydrilla* study, this current study aims to determine the effect of pH change on the production rates of native tape grass (*Vallisneria americana*). The results of these complementary studies may help scientists better understand the relationship between pH change and production rates of tape grass as well as understand more about the competitive relationship between tape grass and *Hydrilla* as the overall pH of freshwater ecosystems

becomes more acidic. In this experiment, sections of tape grass were incubated during an approximately 1-2 hour period. The amount of dissolved oxygen before and after the incubation period was measured as a reading of the amount of respiration or production occurring. The respiration rates and production rates were calculated by amount of dissolved oxygen per milligram of tape grass per hour.

Hypotheses

With respect to the respiration rate (R) of *Vallisneria americana*, as measured by the amount of dissolved oxygen consumed during short incubations under differing pH levels, the Null Hypothesis states that the respiration rates would be equal. It was hypothesized that, as the pH increased the respiration rates would also increase, and as pH decreased the respiration rates would also decrease.

H_o: $R_{5.7}=R_{6.2}=R_{6.7}=R_{7.2}=R_{8.2}$ Where: 5.7=pH 5.7; 6.2=pH 6.2; 6.7=pH 6.7;

H_a: $R_{5.7}<R_{6.2}<R_{6.7}<R_{7.2}<R_{8.2}$ 7.2=pH 7.2; 8.2= pH 8.2

With respect to the production rate (P) of *Vallisneria americana*, as measured by the amount of dissolved oxygen produced during short incubations under differing pH levels, the Null Hypothesis states that the production rates would be equal. It was hypothesized that as the pH increased the production rates would increase, and as pH decreased the respiration rates would decrease.

H_o: $P_{5.7}=P_{6.2}=P_{6.7}=P_{7.2}=P_{8.2}$

s

Methods and Materials

This study was conducted at the Virginia Institute of Marine Science (VIMS) in Gloucester Point, Virginia. The *Vallisneria americana* for this study was purchased from

American Native Plants in Perry Hall, Maryland. Water used for the experiment was collected from cultivation tanks at VIMS on August 11th, 2014. Five darkened two liter treatment bottles of water filtered through a 0.5 μm filter were adjusted to a pH of 5.7, 6.2, 6.7, 7.2, and 8.2.   The pH was adjusted using Top Fin pH increase, sodium carbonate, and Top Fin pH decrease, sodium bisulfate. The pH's, the independent variable, were chosen based on Radcliffe's study, which were found using data collected from the Chesapeake Bay Program in the Chickahominy River (Station RET 5.1A). Station RET 5.1A contains well studied *Hydrilla* and tape grass beds. The pH's were determined by using the mean summer value (7.2) and the extremes of pH (6.7-8.2) reached during a period from January 2008 to December 2012. The treatments of 5.7 and 6.2 were chosen to test more acidic extremes (Radcliffe, 2014). The tape grass was acclimated overnight in each treatment in five gallon buckets of unfiltered water with stirrers to circulate gases and nutrients.

The respiration and production rates of *Vallisneria americana,* the dependent variables, were measured by the amount of dissolved oxygen (D.O.) that was found before and after light and dark incubations. To determine production rates, the initial D.O. of each two liter bottle was measured using a HACH 40d luminescent DO sensor that had been calibrated to account for drift due to atmospheric conditions (VIMS, 2014). To measure the respiration rates, a 30 mL piece of tape grass was incubated in a 60 mL darkened Biochemical Oxygen Demand (BOD) bottles in a temperature controlled dark box with temperature kept constant. Each pH treatment contained four dark samples. To measure production rates, a 30 mL piece of tape grass was incubated in 60 mL BOD bottles in a flow through, temperature controlled light gradient box under varying photosynthetically active radiation (PAR, ~60 – 1300 μE m^{-2} s^{-1}) to imitate the varying light that occurs throughout a day. Each pH treatment(5.7, 6.2, 6.7, 7.2, 8.2) contained twelve samples

spread across the light gradient to mimic the differences in light intensity found throughout the day. Final D.O. measurements were taken after 1 to 2.5 hour incubations using the D.O. probe specified above. After the shoots were removed, they were dried for approximately 48 hours at 65 degrees Celsius and massed to determine the dry weight to determine the mass, making it possible to equate for the size of the plant when looking at the production and respiration rate. The tape grass was massed using a Mettler Toledo AB-S/FACT analytical balance. Production rates were graphed using the Jassby and Plat PI curve equation (Appendix Equation 1). The data was analyzed using a One-Way ANOVA for the production rates as well as a Tukey's HSD test to determine where the significance lies between tests. A One-Way ANOVA was used to determine if there was any significance in the respiration rates and a Tukey's HSD test was used to determine which treatments were significantly different.

Results

In this study, respiration and production rates were measured at varying pH's of 5.7, 6.2, 6.7, 7.2, 8.2. Production rates were measured by the amount of D.O. produced during an incubation period in a light gradient box. Respiration rates, measured by the amount of D.O. used during an incubation period in a dark box, showed some variation (Figure 1-below). The highest respiration rate occurred in the slightly acidic treatment of pH 6.7 followed by the treatments of pH 6.7 and pH 8.2 (Figure 1). The control group pH 7.2 and the treatment of pH 5.7 showed lower respiration rates. The p-value (p-value=8.383 x 10-3) found using a One-Way ANOVA was used to analyze the results and found there to be a significant difference between the treatments (Appendix Table 1). A Tukey HSD test was used to determine the significance of each test. It was found that the pH 6.7 treatment was significantly different from the pH 7.2 and the pH 5.7. The pH 6.2 treatment and the pH 8.2 treatment were not significantly different from

the pH 5.7, pH 6.7, or pH 7.2 treatments, and the pH 5.7 is not significantly different from the
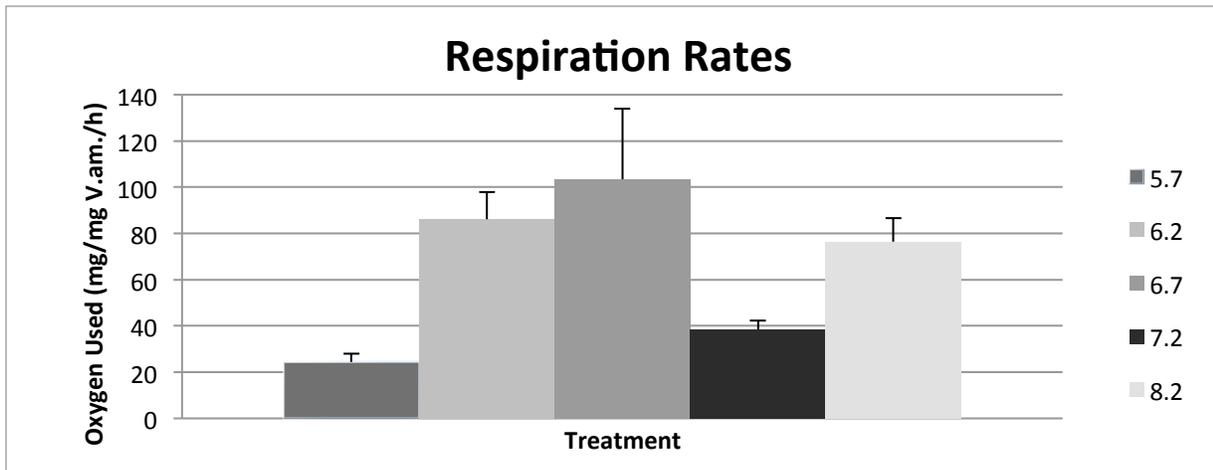
pH 7.2 treatment (Appendix Table 2).

Figure 1 shows the difference between the respiration rates at each pH with error bars in standard error.

Production rates were measured by the amount of dissolved oxygen produced during an

incubation period in a light gradient box. Figure 2 shows a significant difference between the

production rates at differing pHs.

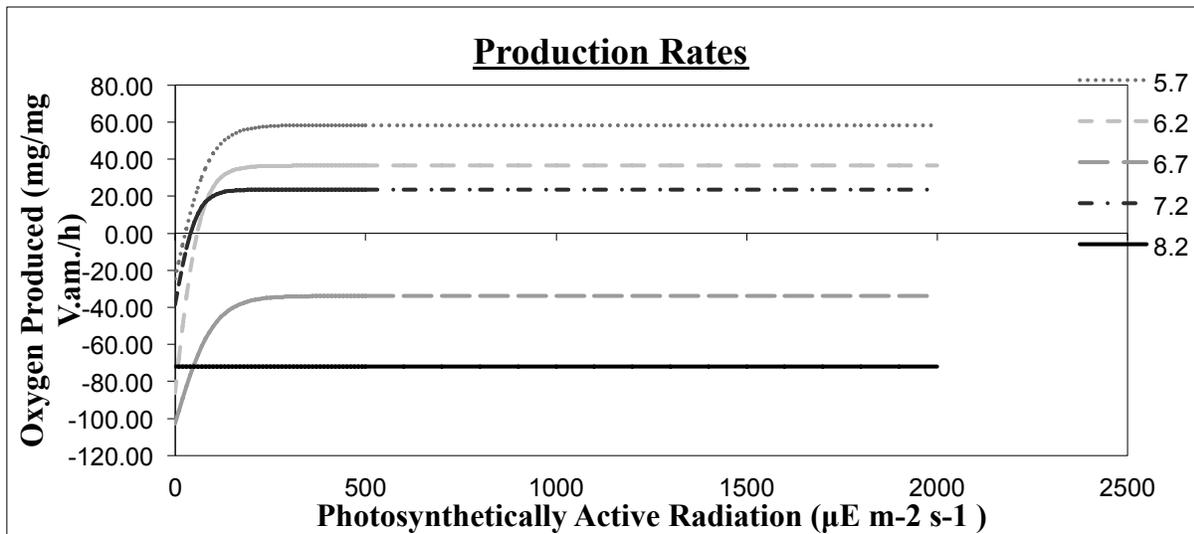Figure 2 shows the difference between production rates.

Production rates had similar variance across the test as the respiration rates showed. The

treatment of pH 5.7 had the greatest production rates followed by pH 6.2 and the control

treatment pH 7.2 (Figure 2). The treatments of pH 6.7 and pH 8.2 experienced no positive

production only respiration as oxygen was used rather than released.

A One-Way ANOVA showed a significant difference between the treatments and the pH (p-value=8.877x10^{-12}) (Appendix Table 3). A Tukey HSD test was done to determine if any test were significantly different. It was found that the pH 6.7 and the pH 8.2 treatments were not significantly different from each other; however, they were significantly different from the pH 5.7, 6.2, and 7.2 treatments. There was no significant difference between the pH 5.7, pH 6.2, and pH 7.2 treatments (Appendix Table 4).

Discussion and Conclusion

The respiration rates (Figure 1) show that the 6.7 treatment had the highest respiration rate. The 7.2 treatment and 5.7 treatment were the two lowest respiration rates with the 5.7 treatment being slightly lower. The 6.2 and 8.2 treatments experienced a moderate amount of respiration. The null hypothesis can be rejected as a One-Way Anova showed a significant difference between the different pH treatments (Appendix Table 1). It was found that the 6.7 treatment was significantly different from the 5.7 and 7.2 treatment but no other treatments were significantly different. The alternate hypothesis cannot be supported since there is not an increasing trend in rates as the pH becomes more basic.

The production rates (Figure 2) shows that the 5.7 treatment had the highest rate of production. The 6.2 and 7.2 treatments had the next highest production rates with the 6.2 treatment being slightly higher than the 7.2 treatment. The 6.7 and 8.2 treatment showed no positive production. It was found that the 6.7 and 8.2 treatments were significantly different than the 5.7, 6.2, and 7.2 treatments. However, the 6.7 and 8.2 treatments were not significantly different from each other and the 5.7, 6.2, and 7.2 treatments were not significantly different from each other. The null hypothesis can be rejected as a One-Way ANOVA showed a significant difference between the different pH treatments (Appendix Table 2). The alternate

hypothesis cannot be supported as the production rate does not increase as the pH becomes more basic.

The data shows that as the world's waters become more acidic, tape grass may struggle to survive. As stated above, tape grass at the control pH of 7.2 functions at a median level, where respiration and production occurs but not a high level. When the plant was pushed slightly out of its "comfort zone" to pH's of 6.7 and 8.2, the plant did not experience production. This could mean that the plant was dying and releasing oxygen. However, when the tape grass was exposed to a pH of 5.7 and a pH of 6.2, it had significantly higher production rates. This may be a sign that the tape grass is going into overdrive to try to survive. During this period of overdrive, the tape grass could be experiencing phenotypic plasticity. Phenotypic plasticity is the ability of a plant to alter is functions due to environmental stressors such as rising acidity (Schlichting, 1986). It is believed that *Hydrilla* also experienced phenotypic plasticity during a study that looked at the effect of pH on the production and respiration rates (Radcliffe, 2014).

Hydrilla is a known competitor of tape grass. In a similar study, *Hydrilla* experienced similar results when exposed to the same pH range. At a pH of 7.2 the plant performed with high production rates. At the pH's of 6.7 and 8.2 the *Hydrilla* experienced very little production but still produced oxygen. At the pH's of 6.2 and 5.7 the plant produced more oxygen but still less than the control treatment of 7.2.

Tape grass didn't experience any production at the pH of 6.7 when the *Hydrilla* experience a low amount of production. Since the pH of the world's water is changing at a slow rate, this could mean that *Hydrilla* could out compete tape grass. As the pH becomes more acidic, tape grass may not be able to handle the stress of slightly acidic waters. If *Hydrilla* is able to survive long enough to adapt, it could possibly out compete tape grass reducing the

number of tape grass beds. In 2006, *Hydrilla* was out competing tape grass and was found in Otter Point Creek (Maryland) covering 100 percent of 19 out of 64 sample sights (McChesney, 2010). Because *Hydrilla* was more productive at the pH of 6.7 than the tape grass, as tape grass is stressed by the rising acidity, *Hydrilla* could possibly take over beds. Further studies could look at the effect of this on the freshwater ecosystem and water fowl.

Though *Hydrilla* may out compete tape grass in a short time scale, tape grass may be able to, due to phenotypic plasticity, alter its production and respiration functions to survive until the pH reaches past a critical point. In the current study, the tape grass had very low production rates at the pH of 6.7. However, at some point between the pH of 6.7 and the pH of 6.2, the tape grass reached a critical point where it began to produce at a higher level than the control treatment, pH of 7.2. *Hydrilla* saw a similar affect, however, the critical point for *Hydrilla* is between a pH of 6.2 and a pH of 5.7 (Radcliffe, 2014) (Appendix Graph 1). Tape grass may perform better because its critical point is slightly less acidic allowing it to start producing at a higher level early than *Hydrilla*. However, in the short term, while the pH is slightly acidic, the change is acidity could be detrimental to tape grass as all of its energy will be put into surviving rather than reproducing. Studies that look at the long term effect of pH on tape grass are needed to understand how they will behave after long term exposure to acidic waters.

Waterfowl and fish rely on tape grass beds as a source of food and as protection. If the waters in freshwater ecosystems become more acidic at a faster rate than tape grass can adapt to, food webs in freshwater ecosystems could become disturbed. Further studies could also work to determine the exact critical point at which both hydrilla and tape grass began producing at a higher rate.

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Appendix

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	15181.29	4	3795.322	5.962856	0.008383	3.35669
Within Groups	7001.434	11	636.494			
Total	22182.72	15				

Table 1 shows the p-value of Tape Grass Respiration is significant

| Treatment	5.7	6.2	6.7	7.2	8.2
5.7		Not Significant	**Significant**	Not Significant	Not Significant
6.2	Not Significant		Not Significant	Not Significant	Not Significant
6.7	**Significant**	Not Significant		**Significant**	Not Significant
7.2	Not Significant	Not Significant	**Significant**		Not Significant
8.2	Not Significant	Not Significant	Not Significant	Not Significant	

Table 2 shows the Tukey HSD test significance for the respiration treatments.

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	126976.2701	4	31744.06751	24.10478367	8.87738E-12	2.530694205
Within Groups	76381.34991	58	1316.919826			
Total	203357.62	62				

Table 3 shows the p-value of Tape Grass Production is significant

Treatment	5.7	6.2	6.7	7.2	8.2
5.7		Not Significant	**Significant**	Not Significant	**Significant**
6.2	Not Significant		**Significant**	Not Significant	**Significant**
6.7	**Significant**	**Significant**		**Significant**	Not Significant
7.2	Not Significant	Not Significant	**Significant**		**Significant**
8.2	**Significant**	**Significant**	Not Significant	**Significant**	

Table 4 show the Tukey HSD significance for the production treatments.
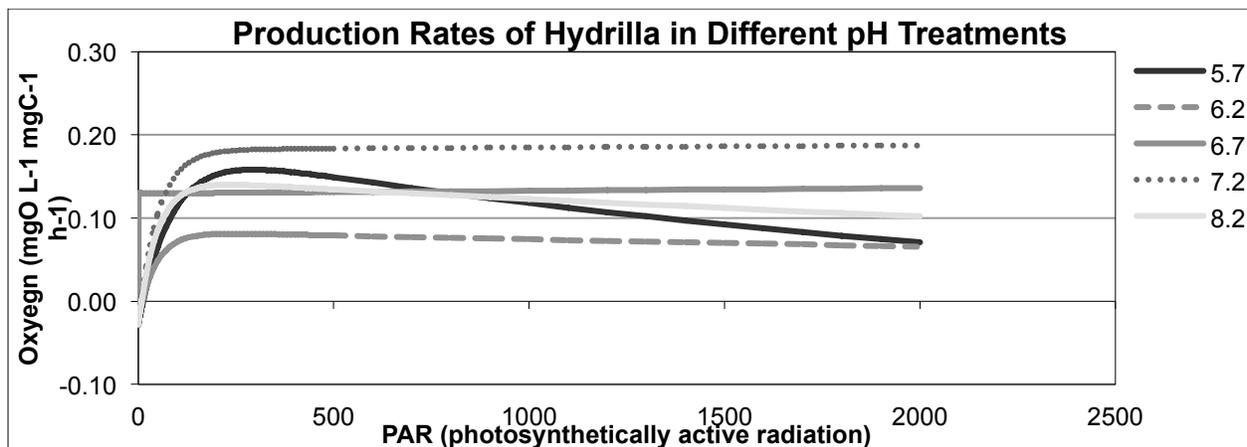
Graph 1 shows the Production Rates of Hydrilla in a study done by Radcliffe in 2014.

Equation 1
Jasby and Platt 1976 PI Curve Equation:

$$P = P_m \tanh\left(\frac{\alpha I}{P_m}\right) + R_d$$

Where:
P= rate of photosynthesis
P_m= light saturated, maximum photosynthetic rate
α = initial slope of photosynthesis-irradiance curve
I= irradiance
R_d= rate of dark respiration