An Analysis of Respirometer/Manometer Methods:

The effects of thallus weight, carbon dioxide buffers, and light levels on the oxygen production of macroalgae from the Biosphere 2 ocean

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Abstract:

Prior to estimating the effect on gas levels from marine algae within the Biosphere 2, a method for measuring photosynthesis and respiration rates by the major algae taxa which occur their must be determined. A Gilson respirometer/manometer was used to make these measurements. Higher rates of photosynthesis were observed with smaller amounts of material in the respirometer bottles. Comparing the effects of two carbon dioxide buffers, pottasium hydroxide and the Pardee buffer yielded inconclusive results. Testing for the effects of different light levels on photosynthesis revealed a direct correlation between light levels and photosynthesis rates.

Introduction:

Determining the effect on gas content in the Biosphere II by the algae in its ocean is a critical insight to the project's ecology. To make an estimate of this factor, two related studies must be performed. One of these must estimate the biomass of different species of algae in the biosphere. The other study must estimate the respiratory and photosynthesis rates of these algae species in the Biosphere II ocean. Estimating the biomass in the Biosphere 2 ocean involves sampling the algae by transects, weighing the samples, and then extrapolating the findings to account for the area of the entire ocean bottom. Determining the photosynthetic an respiratory rates of the algae in the ocean requires a sampling of all the major taxa, followed by analysis of these samples using a respirometer/ manometer or an O_2 meter in the laboratory. Prior to accurately making this assessment of photosynthetic and respiratory rates, a protocol for the experiment that gives the most likely accurate results must be determined by trial and error.

One important factor that needs to be looked into before running the experiment is the amount of material to be measured in each jar of the respirometer. It has been demonstrated that the photosynthesis of macroalgae is less productive when the same mass of thalli are measured in a smaller bottle (Littler 1979). This is assumed to be mainly due to lack of nutrients, but self-shading of the thalli may also be a factor.

A comparison of two types of carbon dioxide buffers is also made by this experiment. These two buffers are potassium hydroxide and diethanolamine from a solution prescribed by the Pardee

buffer method (Umbriet et al., 1972). This buffer is prepared by mixing (in order): 12 ml of diethanolamine, 30 mg of thiorea, 6g of potassium bicarbonate, 4.4 ml of 6M hydrochloric acid, and 13.6 ml of distilled water. After stirring and letting this solution sit in a stoppered flask overnight, it is used to produce a one- percent solution with seawater. (Dawes 1981) This will maintain a constant gas pressure of carbon dioxide in the solution, which is necessary when measuring oxygen uptake in order to avoid inaccurate results. (Umbriet et al., 1972)

The effect of light levels on the thalli will also be compared by this experiment. Since light levels at different depths in the Biosphere 2 ocean had not been measured at the start of this experiment, this test was necessary.

Materials and Methods:

This experiment was performed by Jeff Cohn.

Sample Collection:

Samples were collected from Columbia University's Biosphere 2 ocean. Collections of a single species were performed as early in the morning as possible. For all samples, the depth and its approximate location were noted. Samples were transported from the Biosphere 2 to the University of Arizona's Environmental Research Laboratory as quickly as possible in an aerated cooler at a temperature close to 76° F.

General Respirometer methods:

A Gilson respirometer was used for measuring photosynthesis and respiration rates with Meriam 827 red oil for the manometer fluid. The respirometer was started by: turning on the stirring motor; setting the temperature of the heater to 76° C; turn on the main and auxiliary heaters (while turning off auxiliary heater when the correct temperature is read); set coolant at 77° C; and turn on the lights. The 20-ml flasks were agitated at 65-beats min^{-1}. KY lubricant was used to lubricate the stopcock.

All of the algae samples were run the same day as they were collected. In order to minimize the effects that circadian rhythms could have on the data between different runs, the runs were also all conducted between 10 AM and 3 PM. Also, samples taken from depths differing by more than

0.25 meters should be maintained and run separately. Unless noted, vessels containing the samples were filled with seawater. Wet weights were measured for the thalli placed in each vessel before-hand.

The glassware was cleaned after each run by first them with warm water and soap. They would then be soaked in a 5% HNO_3 solution for 24 hours, followed by rinsing them at least 3 times with de-ionized water. The glassware may be rinsed with an HNO_3 solution and then de-ionized water if there is not sufficient time to soak them for 24 hours.

Light Level Determination:

A Licor Model 189 quantum meter was used to determine the light level originating from below at 392 $\mu Einm^{-2} sec^{-1}$. The sensor was held level with the bottom of the flasks.

Dry Weight Preparation:

The algae from each vessel were dried at 60° C for 48 hours before being weighed.

Methods for Separate Trials:

For the first trial that was run on 2/4/99, the thalli were run for 30 minutes over full strength light. Four flasks contained samples of *Amphiroa,* three contained *Enteromorpha,* another three contained *Hypnea,* and four were blanks (empty). 5 ml of seawater and filter paper saturated with 10% KOH were added to each flask. Wet weights ranged from 192 to 860 mg.

The second trial was run on 2/5/99. First, the samples were run for 40 minutes over full strength light, and then immediately for 30 minutes in darkness. This was done with three flasks containing branched *Amphiroa,* two with coraline green, three containing *Dasya,* three with red sheet algae, and three blanks. Wet weights ranged from 20.2 to 420.8 mg.

On the morning of 2/12/99, the third trial was run. Either 0.2 ml of KOH or 0.6 ml of Pardee buffer was added in addition to seawater to each flask. The flasks were also shaken at 80 beats per minute. The shaker was turned off after five minutes, and then on again at ten minutes. The *Chaetomorpha* thalli were placed in three flasks for each treatment. The *Hypnea thalli* were placed in three flasks with the Pardee treatment and two with the KOH treatment. There were also two Pardee and one KOH blank run. The wet weights ranged from 371.2 mg to 596.1 mg.

The fourth trial was run on the afternoon of 2/12/99 with *Chaetomorpha* thalli. The samples

were run for 15 minutes in the dark, followed by 15 minutes of light, then another 15 minutes of dark, with the final 25 minutes over light. Four flasks were treated with KOH, while seven were treated with Pardee buffer. There were also one KOH and two Pardee buffer blanks. Certain flasks were also covered with different numbers of screens to create lower light levels within the flask. One flask for each treatment was left uncovered. Three Pardee buffer flasks were each covered with one, two, or three screens, reducing the light level inside the flask to 318 $\mu Einm^{-2}\,sec^{-1}$, 277 $\mu Einm^{-2}\,sec^{-1}$, and 241 $\mu Einm^{-2}\,sec^{-1}$, respectively. Another three flasks from each treatment were covered with four, eight, or twelve screens, reducing the light levels to 191 $\mu Einm^{-2}\,sec^{-1}$, 104 $\mu Einm^{-2}\,sec^{-1}$, and 55.5 $\mu Einm^{-2}\,sec^{-1}$, respectively. Additionally, one KOH and two Pardee buffer blanks were run. The wet weights ranged from 391 mg to 507.4 mg.

On 2/16/99, the fifth trial was run with a freshwater species of *Cladophora.* The samples were run for the first 20 minutes in the dark, the next 15 minutes over light, followed by 10 minutes again in the dark. Four flasks had KOH buffer treatments while seven flasks were Pardee buffer treatments. Three of the Pardee buffer flasks were covered with one, two or four screens. There were also one KOH and two Pardee buffer blank flasks. The wet weights ranged from 217 mg to 372.5 mg.

The sixth trial on 2/20/99 was done with all blank flasks. Seven flasks contained unfiltered Biosphere II water, while the other seven contained filtered water. For each of the water types, there were four flasks with a Pardee buffer treatment and three with KOH added. The first 20 minutes were run in the dark, followed by 65 minutes over light. There were 10 minutes before the start time and between the transition from dark to light to allow for equilibration.

The seventh trial was run with *Hypnea* on 2/25/99. The samples were collected near the shoreline at Biosphere II at less than six inches of depth. Two, four, or six screens covered three of the flasks, while three pairs of flasks remained uncovered. An additional flask was covered with eight screens. Three of the remaining flasks were blank, and one flask was leaking and therefore voided. The samples were first run in the dark for twenty minutes, followed by 15 minutes with light. These times do not include the thirty minutes for equilibrium before the start and between the light changes. The wet wets of the thalli in each flask ranged from 263.7 mg to 309.1 mg.

Results:

To convert the manometer readings to find the oxygen produced for each datum, the blanks were averaged at each time where they were recorded, and this average was subtracted from each other datum from that time. Each datum could then be divided by the mass of the thalli in that vessel, and by the time interval between each recording.

For the results obtained on 2/4/99, one *Hypnea* vessel was omitted due to insufficient data, as well as two *Amphiroa* vessels because they were outliers. The mean microliters of oxygen produced for each interval are shown on figure 1. The *Enteromorpha* vessels produced and average of 3.4 microliters of oxygen per gram per minute. The *Amphiroa* and *Hypnea* vessels consumed an average of 1.7 and 1.5 microliters of oxygen per gram per minute, respectively.

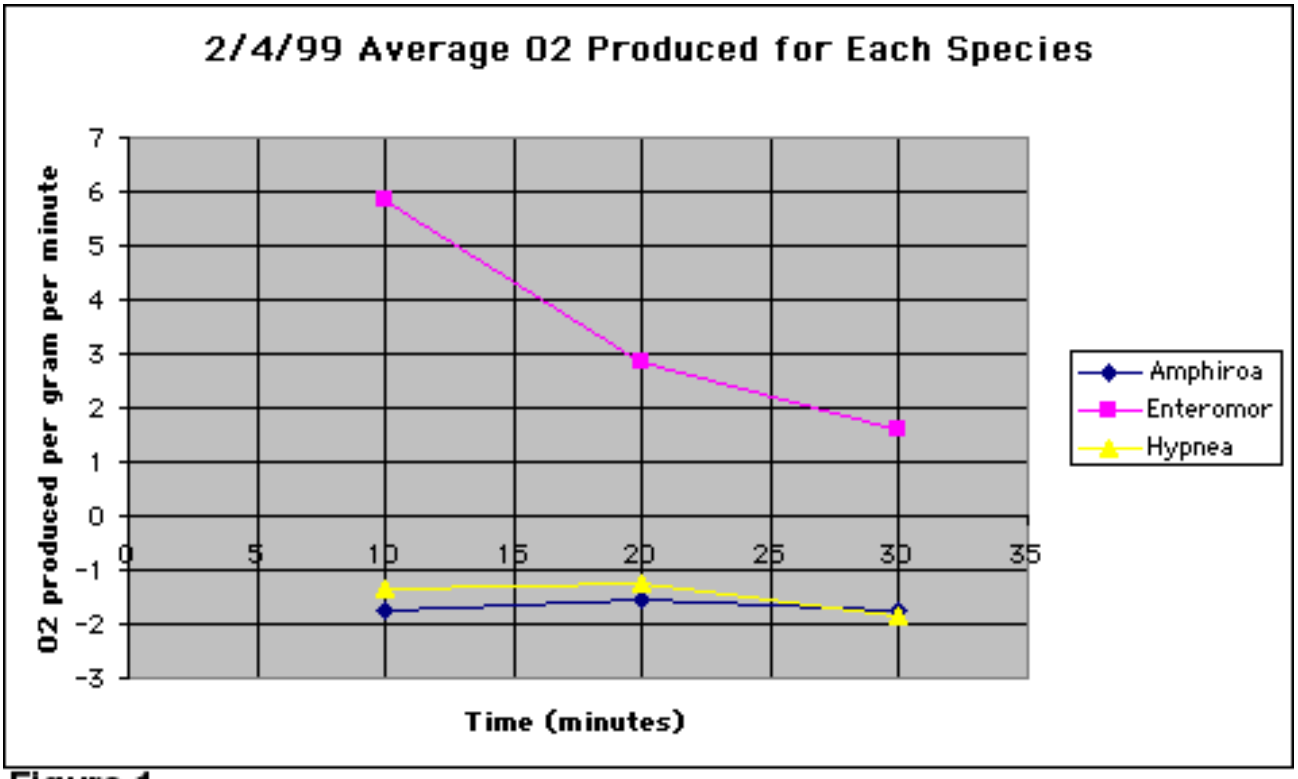

Figure 1

The findings from the trial on 2/5/99 are shown in figure 2 with a separate graph for each species. The low measurement taken at twenty minutes for vessel twelve (coraline green algae)

should probably be considered incorrect due to human error and ignored.

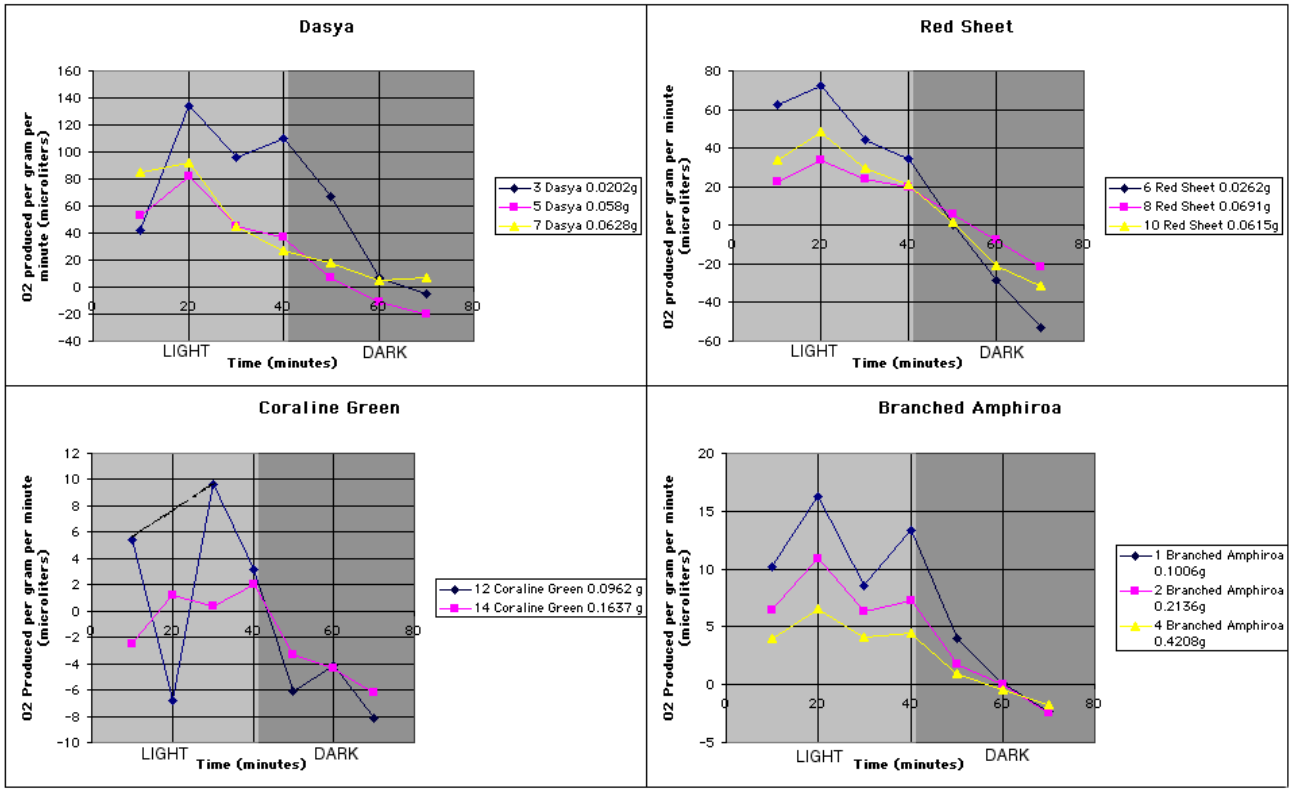

Figure 2 - Results from 2/5/99

Figure 3 shows the mean oxygen production through time for the buffer treatment and species on the morning of 2/12/99. The average of these results over time is shown in the bar chart in figure 4.

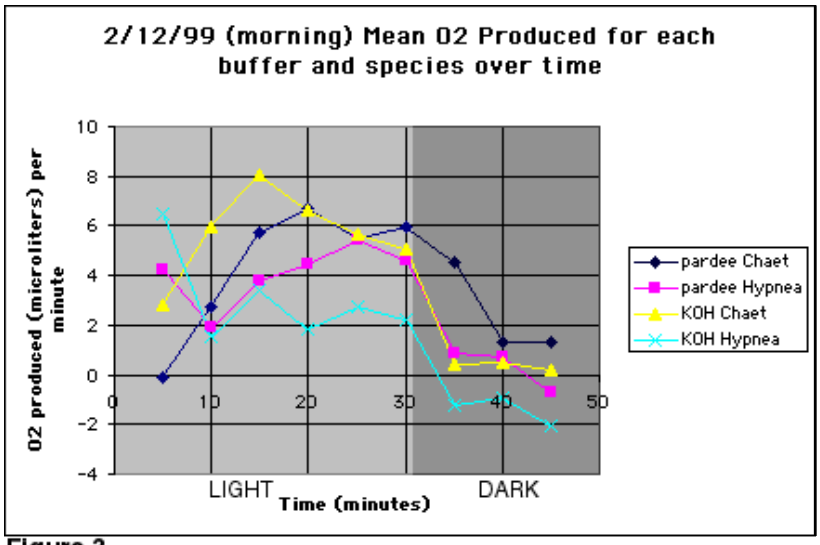

Figure 3

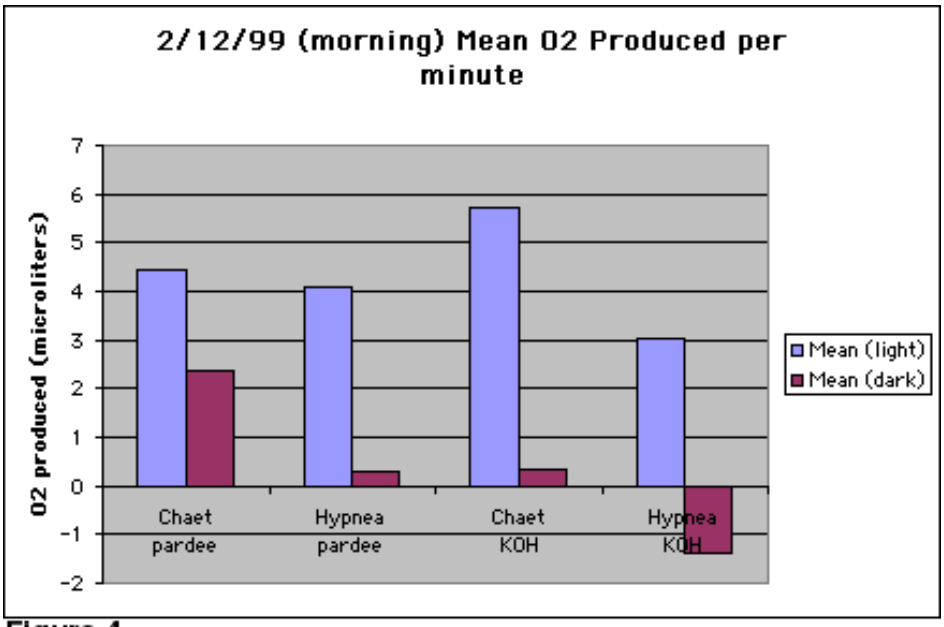

Figure 4

The results obtained on 2/12/99 in the afternoon are shown on figure 5. To create this graph, the results from each vessel were divided by the level of light they were subjected to in microeinsteins. Vessels six and three were then omitted as outliers, and the mean at each point in time was calculated for each buffer treatment.

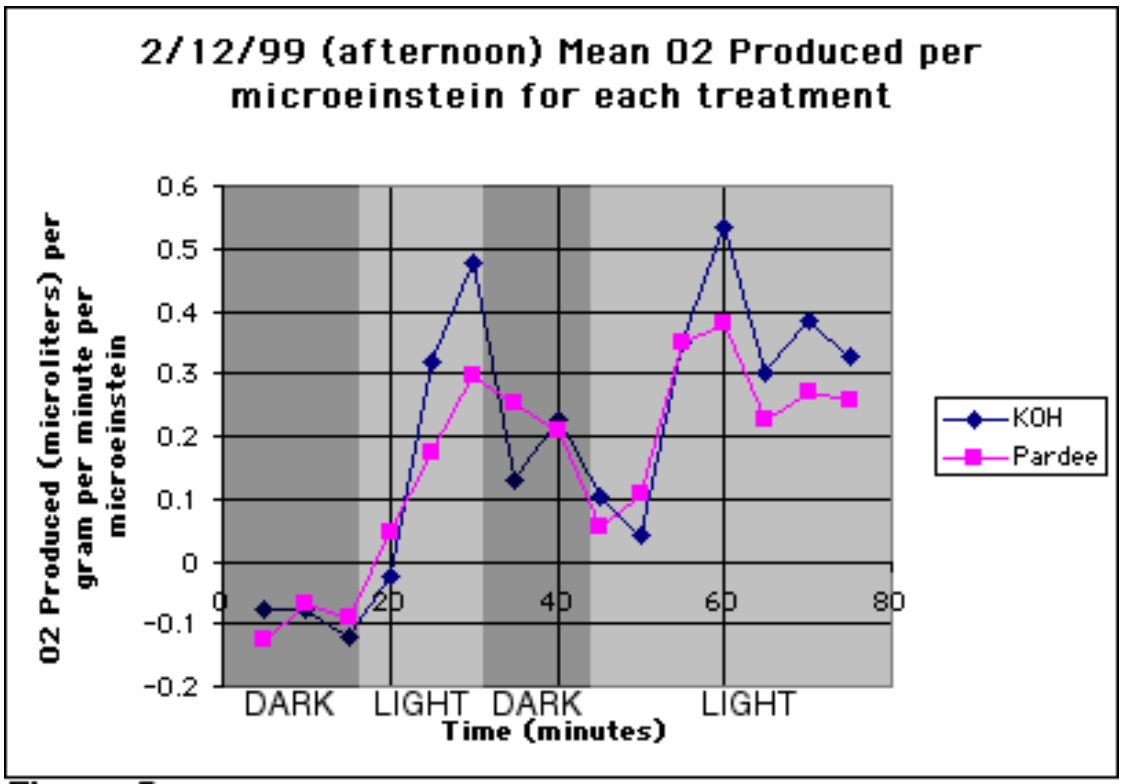

Figure 5

The results for the freshwater species of *Cladophora* on 2/16/99 are shown on the graph in figure 6. This graph shows the mean amount of oxygen produced for each buffer with the light levels other than 392 microeinsteins omitted.

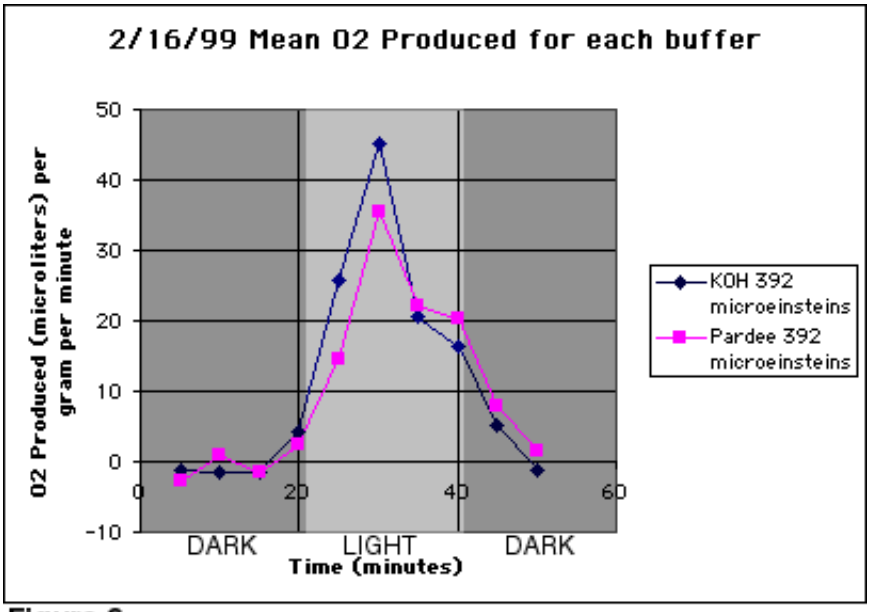

Figure 6

The graph for the mean O2 produced per minute for each treatment of Biosphere 2 water is shown in figure 7.

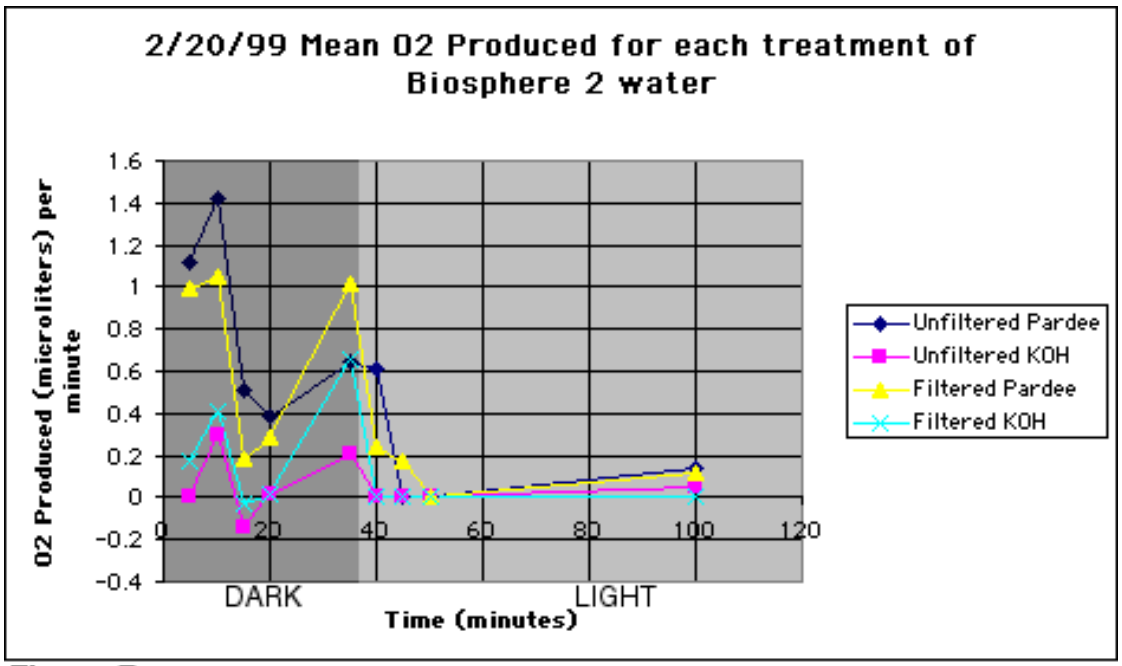

Figure 7

Figure 8 shows the graph for the mean O2 Produced for each light level on 2/25/99. The mean O2 produced for each light level correlates with the intensity for each level examined using linear regression with an r value of 0.94. The mean O2 produced for each light level per minute over light is: 5.66 microliters per gram per minute for 135 microeinsteins, 8.14 microliters per gram per minute for 191 microeinsteins, 8.97 microliters per gram per minute for 277 microeinsteins, and 10.44 microliters per gram per minute for 392 microeinsteins. Figure 9 plots these mean values against their respective light level.

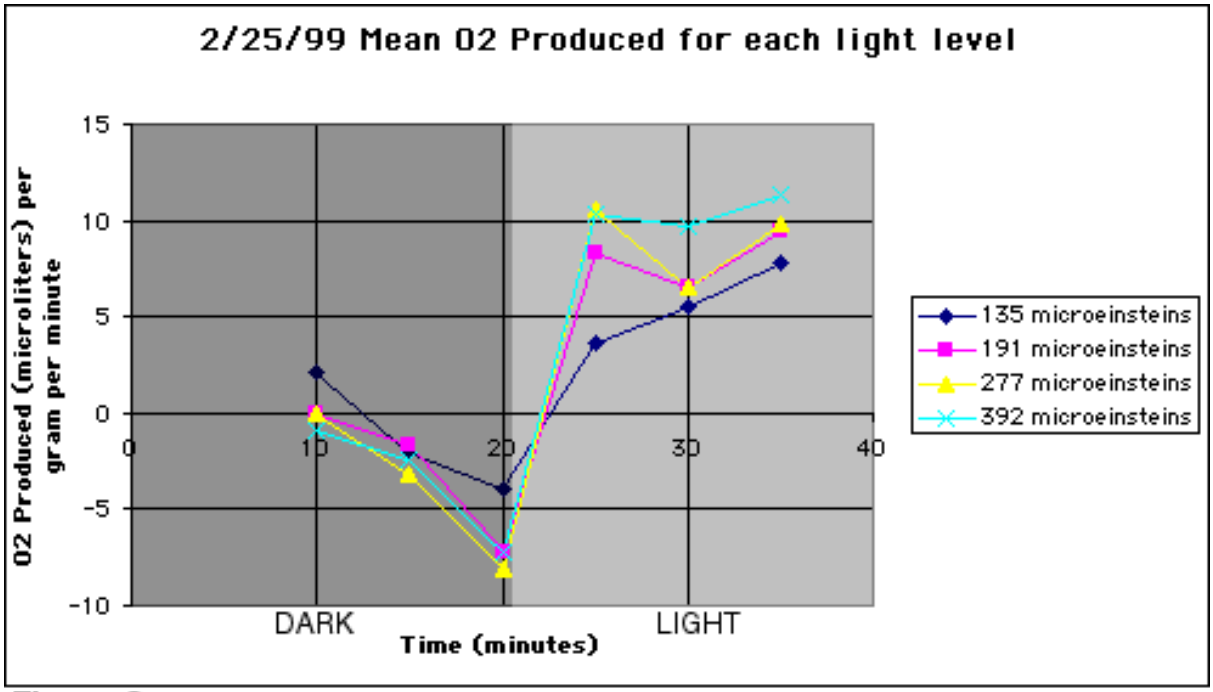

Figure 8

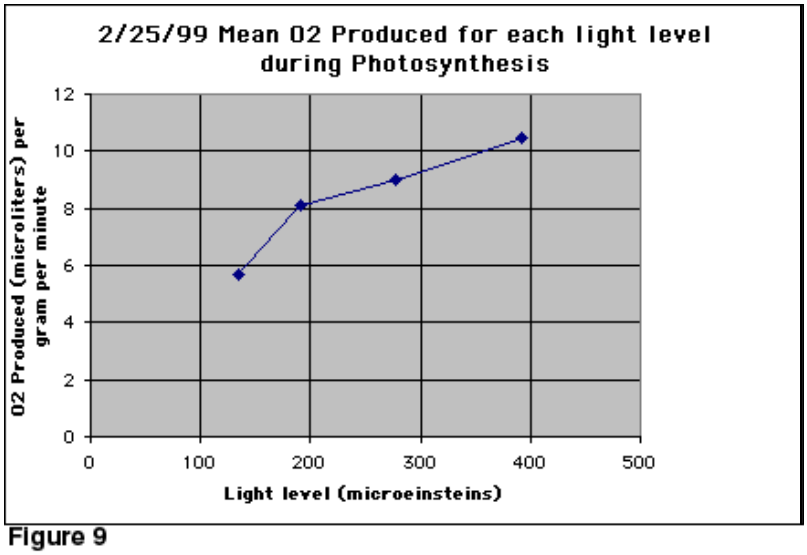

Figure 9

Discussion:

Optimal mass of thalli:

Insights based on the effect that the amount of material in each flask has on respiration readings is best seen in figure 2. For all four species measured in this trial on 2/5/99, a greater rate of photosynthesis per gram per minute was measured in flasks with the smallest mass. This trend is likely caused by depletion of nutrients or shading. (Littler 1979) A threshold was never reached in which a smaller amount of material was too small to be effectively measured. In order to simulate the respiration and photosynthesis if algae in the Biosphere 2 ocean, however, some effect of shading would be desired if it is a significant factor. Especially with the larger thalli, shading would decrease the photosynthesis to mass ratio in naturally occurring algae. This effect would likely vary with different species due to their differences in three-dimensional shape. Such an idea suggests that using complete thalli in their natural orientation to light while in larger bottles would yield the most accurate results.

Type of Buffer used:

The results from this experiment are inconclusive as to the effect of the type of buffer used. The results from the afternoon of 2/12/99 and 2/16/99 shows that the *Chaetomorpha sp.* and freshwater *Cladophora sp.* tested have a slightly higher reading of oxygen production with potassium hydroxide rather than the Pardee buffer. The results on the morning of 2/12/99 (figure 4), however, show a different response between *Chaetomorpha sp.* and *Hypnea sp.* with the two buffers. The rates of photosynthesis are fairly similar for the two species under the Pardee buffer. Yet for the potassium hydroxide buffer, the *Chaetomorpha sp.* yield a much higher photosynthesis rate than *Hypnea sp.* Unlike the other species measured, *Hypnea sp.* produce less oxygen during photosynthesis with potassium hydroxide than with the Pardee buffer. The measurements taken with filtered and unfiltered Biosphere 2 water on 2/20/99 (figure 7) also gave interesting results. During the last 50 minutes of the run under light, both the unfiltered and filtered water treated with Pardee buffer had higher oxygen production than unfiltered water with potassium hydroxide buffer. These results from

the biosphere water suggest that the Pardee buffer may cause water to expel oxygen even if there is not a source of oxygen production within it. Since different buffers have a different effect with *Hypnea sp.* than with other species of algae, it is possible that the buffer may chemically influence the photosynthetic processes in certain algae. It is important that this effect is understood before adding buffers when attempting to accurately measure photosynthesis and respiration rates.

Light level used during photosynthesis measurements:

The correlation of light level to photosynthesis rates that are found in the results from 2/5/99 (figure 9) demonstrate the dramatic effect that the amount of light has on an alga's oxygen production during photosynthesis. This effect shows that replicating the light levels of the Biosphere 2 ocean in the laboratory are critical to determining the actual oxygen production occurring there. The thalli should be run in the respirometer at light levels appropriate to the depths that they were sampled from.

References:

Dawes, Clinton J. Marine Botany. 1981. New York: John Wiley & Sons.

Littler, M.M., 1979. “The effects of bottle volume, thallus weight, oxygen saturation levels, and water movement on apparent photosynthetic rates in marine algae.” Aquatic Botany. **7**: 21-34.

Umbriet, W.W., R.H. Burris, and J.F. Stauffer. Manometric Techniques for the Study of Tissue Metabolism. 1972. Minneapolis: Barges.