

**The Priming Effect: Discovering the future of lake carbon cycling**

**BIOS 569: Practicum in Field Biology**

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**Abstract:** Inland lakes are key players of the global carbon cycle. It is found that labile organic matter; glucose, cellulose, or root exudates will change the mineralization rate of recalcitrant organic matter in the soil, which alters the carbon cycle. The labile carbon increases the rate that bacteria breaks down recalcitrant carbon. This process is known as the priming effect and is important because it could change our view of how inland lakes fit into the global carbon cycle and influence predictions of projected lake carbon cycling. The priming effect was tested by collecting lake water from five different lakes along UNDERC property in the Upper Peninsula Michigan (East Long, West Long, Hummingbird, Crampton, and Morris). Glucose was used as the source of labile carbon and was placed in the bottles in four treatment groups of 0.25, 1.0, 2.25, and 4.0 mg with three replicates of each. To test how much CO<sub>2</sub> and CH<sub>4</sub> was released headspace gas was extracted five times every five days and put in a gas chromatography machine. It was found that there was more of a rate of recalcitrant carbon being broke down and releasing more CO<sub>2</sub> over all of the five lakes. There was a positive priming effect for the labile carbon increased the rate of recalcitrant carbon being broke down.

**Introduction:**

Lakes have recently been recognized as important components of the global carbon cycle (Tranvik et al. 2009). Leaves can fall into lakes and dissolve into the water column, which contain carbon and can alter the internal carbon cycle of the recipient aquatic ecosystem (Attermeyer et al., 2014). In the study done by Tranvik et al. 2009, they state that the emissions of carbon dioxide from inland waters into the atmosphere are similar in magnitude to the carbon dioxide uptake by the oceans. This meaning that the CO<sub>2</sub> emission from inland lakes is very similar to the CO<sub>2</sub> uptake in the ocean and therefore has the same effect on the atmospheric CO<sub>2</sub>. Furthermore, Tranvik et al. states that the global burial of organic carbon in inland water sediments exceeds organic carbon sequestration on the ocean floor.

In terrestrial ecosystems, the inputs to soils of labile organic matter (LOM) such as glucose cellulose, or root exudates, are known to induce modifications in the mineralization rate of the more recalcitrant organic matter (ROM) initially present, altering soil carbon (C) and nutrient content (Guenet et al., 2010). This process, known as the priming effect, can enhance the recalcitrant organic matter mineralization rate anywhere from 10% to 500% (Guenet et al., 2010). The priming effect is commonly observed in terrestrial environments however not in aquatic environments. This meaning that labile carbon (carbon that is easily broken down from phytoplankton in aquatic systems) increases the rate that bacteria breaks down recalcitrant carbon (carbon that is hard to break down and comes from terrestrial sources such as trees). The priming effect could have significant implications for understanding current and predicting future lake carbon cycling. As of now, no study has tried to find out the general importance of

priming effect, even though recalcitrant and labile organic matter consistently shows up together in aquatic ecosystems (Guenet et al., 2010).

The priming effect was first discovered and described by Lohnis in 1926 and its importance in soil sciences (Guenet et al., 2010). The priming effect could have negative and positive effects on mineralization of ROM. A positive effect results from labile organic matter (LOM) increasing recalcitrant organic matter (ROM) mineralization rate and a negative effect would be the LOM decreasing ROM mineralization rate (Guenet et al., 2010). Since the priming effect in aquatic systems has not been well studied it is difficult to understand the mechanisms of this process. It is theorized that the priming effect could be controlled by the nutrient availability (Guenet et al., 2010). The nutrients present could affect the rate of the mineralization and therefore the amount of carbon released in the atmosphere.

Some theories also suggest that the intensity of the priming effect could be controlled by interactions between LOM decomposers and ROM decomposers (Guenet et al. 2010). Guenet et al states that a positive priming effect could be due to a co-metabolism effect, in which the breakdown of one compound depends on the presence of another. In this case the presence of LOM may supply energy to LOM decomposers for the production of extracellular enzymes, which may in turn degrade ROM into simpler catabolites (Guenet et al. 2010).

The priming effect is important to understand because if the occurrence of phytoplankton blooms increase in inland lakes, then then this increased LOM source will potentially increase the rate of mineralization, thus releasing more CO<sub>2</sub> into the

atmosphere and changing our view of how lakes fit into the global carbon cycle. In this experiment I hypothesize that higher amounts of LOM (glucose) will increase the rate of recalcitrant carbon broke down, increasing the amount of CO<sub>2</sub> into the atmosphere.

### **Methods:**

Lake water was collected from five lakes on UNDERC property in the Upper Peninsula Michigan (East Long, West Long, Hummingbird, Crampton, and Morris). Lake water was filtered the lake water 0.2 µm filters to remove any bacteria from the water while leaving dissolved organic carbon behind with 100 mL of lake water used in each incubation bottle. To test the priming effect hypothesis, there were four treatment groups per lake, one control treatment for each lake, and one glucose control treatment with three replicates for each treatment. The control group for each lake had 100 mL of lake water with no added glucose. The control for glucose (our source of labile carbon) had amounts of 0.25, 1.0, 2.25, and 4.0 mg of glucose with three replicates of each dissolved in 100 mL of tap water. The four treatment groups had 100 mL of lake water with 0.25, 1.0, 2.25, and 4.0 mg of glucose dissolved respectively. All treatments had 1 mL of unfiltered lake water added as the source of natural lake bacteria. The vials were incubated at room temperature (20-22 degrees C) for three weeks.

Eight milliliters of headspace gas from each sample was extracted five times during the incubation after every 5 days and analyzed on a gas chromatograph. Another round of glucose was added in the second week to simulate a pulse of fresh labile carbon. This reflects what would happen in a lake with a phytoplankton bloom. Gas chromatograph was used to estimate the amount of carbon respired and released as CO<sub>2</sub> and CH<sub>4</sub>.

To determine if there was a significant priming effect in each of the lakes, an ANOVA on the slopes of the linear regression of amount of carbon respired as a function of time along with an ANOVA on the total amount of carbon respired after accounting for carbon added was run in the statistical software SYSTAT and R.

**Results:**

It was found that in Morris Lake the control treatment had the lowest rate of carbon respiration. The 0.25 mg of glucose had the second lowest rate, which was expected. The treatment group of 1.0, 2.25, and 4.0 stayed close together over the weeks until week three. That was where the 4.0 mg of glucose started resulting in the highest respiration rate. In the ANOVA test, Morris Lake overall had a p-value of 0.0246 which is significant at the significance level of 0.05. Within its treatments only the control and the 4.0 mg of glucose were statically different in their slopes of carbon respired. It had a p-value of 0.019. In the ANOVA test for the respiration rates, Morris Lake overall had a p-value significant at 0.016. The only respiration rate that was statically different was between the treatment groups of the control and 4.0 mg.

East Long resulted in having the control treatment group as the lowest respiration of carbon (Figure 2). The highest amount of carbon respiration was the treatment groups of 2.25 mg and 1.0 mg of glucose with total carbon respired 2825 and 2494  $\mu\text{mol L}^{-1}$  of  $\text{CO}_2$  respectively. This was higher than the expected treatment group of 4.0 mg of glucose ending with a rate of 1380  $\mu\text{mol L}^{-1}$  of  $\text{CO}_2$ . In the ANOVA test, East Long slopes overall showed were significant at a p-value of 0.00555. The only treatments that were statically different form one another was 2.25 and 0.25 with a p-value of 0.045. In

the ANOVA test for the respiration rates, East Long was significant at 0.0125 and its respiration value was significant between control and 1.0 mg.

For West Long, the control group and the 0.25 mg of glucose stayed around the same values of respiration until the final week in which the 0.25 mg treatment had a high rate (Figure 3). The other three treatment groups of 1.0, 2.25, and 4.0 mg stayed close together in rates until the last week as well. The 4.0 mg treatment group resulted in the highest respiration as expected and 1.0 and 2.25 stayed close to one another. In the ANOVA test, West Long Lake had a significant p-value at 0.0051. Within in West Long, the treatment slopes that were statistically different from each other were 4.0 mg and 0.25 at a p-value of 0.0588, the control and 2.25 with a p-value at 0.024, and the control and 4.0 mg at a p-value of 0.0037. In the ANOVA test for the respiration rates, West Long was significant overall at 0.0014. The respiration rate that was statically significant was between the control and 0.25 mg and the control and 4.0 mg at a p-value of 0.0035 and 0.0011.

In Figure 4, Hummingbird Lake linear relationship was shown. These results were expected as the higher amount of glucose added the higher respiration rate there would be. The control group was at the lowest rate, followed by 0.25, 1.0, 2.25, and 4.0 mg of glucose added. In the ANOVA test, overall the slopes of Hummingbird were not statistically significant at a p-value of 0.162 and 0.134. None of the treatment groups in that lake were statistically different from one another. In the ANOVA test for the respiration rates, Hummingbird respiration values overall were not statically significant and had p-value of 0.262 and 0.147. The treatment groups compared to one another showed no significance as well.

Figure 5 shows the results of the cumulative linear relationship of Crampton Lake. The control group had the lowest rate as expected. 0.25 had the highest rate in the first week and then as time went on it slowed down. The group 4.0 mg of glucose had the highest rate of recalcitrant carbon respired over the five weeks. The treatment group of 1.0 peaked right away until the fourth week and then dropped in week five. As seen in the figure there is a drop in the rate of carbon respiration in every treatment for week five. In the ANOVA test, overall the slopes of Crampton were not statistically significant at a p-value of 0.162 and 0.134. In both lakes none of the treatment groups in that lake were statistically different from one another. In the ANOVA test for the respiration rates, Crampton and respiration values overall were not statically significant and had p-value of 0.262 and 0.147. The treatment groups compared to one another showed no significance as well.

**Discussion:**

The hypothesis stated that the higher amount of labile carbon (glucose), the higher the rate of recalcitrant carbon being broke down there would be, increasing the amount of CO<sub>2</sub> into the atmosphere. The results from this experiment support the hypothesis.

A possible reason would be that East Long could have a limit of the amount of carbon it can hold before it no longer respire at a high rate. East Long could be exposed to different conditions and nutrients preventing it from accepting the glucose added. A difference could be whether East Long is oligotrophic eutrophic. Guenet, 2010 suggests that the priming effect could switch from a positive PE (overmineralization of ROM) in oligotrophic systems to a negative PE (undermineralization of ROM) in eutrophic



conditions. This could be further researched by testing what makes East Long different from the other lakes.

Crampton Lake had a dropped respiration rate in every treatment in week five. This was not expected and drew up many questions. although it is odd that all the treatments resulted in a drop besides 2.25 mg. Another reason why this happened could be that the bacteria that enhances the rate of recalcitrant carbon could of died and therefore resulting in the rate of carbon.

These results show that the priming effect does exist in aquatic environments, specially showing a positive effect. A positive effect is when labile organic matter (LOM) can increase recalcitrant organic matter (ROM) mineralization rate. Currently, the priming effect idea is almost completely ignored by the scientific communities studying marine and continental aquatic ecosystems. However, Guenet et al showed many experimental results and field observations that support their theory that the priming effect occurs in both terrestrial and aquatic ecosystems (2010). A reason why they think that scientists reject is because the priming effect is undetectable without a way of distinguishing organic matter (OM) sources. (Guenet et al. 2010).

The ANOVA and Tukey tests for both the Slopes of the linear regression and the total amount of carbon respired after accounting for carbon added for each lake did not show many significance between each treatment. Also Hummingbird and Crampton were not significant at all in both ANOVA tests. This could be due to the fact there were only three replicates for each treatment. The more replicates there are the better the and closer the data would show the true results of each of the five lakes.

The priming effect is something that needs to be further looked into for it could change how we view the global carbon cycle. It could be further tested whether the contents of the lakes had an effect on how much CO<sub>2</sub> and CH<sub>4</sub> was released such as the nutrient present.

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**Works Cited**

- Attermeyer, K., Hornick, T., Kayler, Z. E., Bahr, A., Zwirnmann, E., Grossart, H.-P., and Premke, K. 2014. Enhanced bacterial decomposition with increasing addition of autochthonous to allochthonous carbon without any effect on bacterial community composition, *Biogeosciences*, 11, 1479-1489, doi:10.5194/bg-11-1479-2014.
- Guenet B, Michael Danger, Luc Abbadie, and Gérard Lacroix 2010. Priming effect: bridging the gap between terrestrial and aquatic ecology. *Ecology* 91:2850–2861. doi.org/10.1890/09-1968.1
- Tranviket Lars J. et.al. 2009. Lakes and reservoirs as regulators of carbon cycling and climate. *Limnology and Oceanography*, 54 part 2, 2298-2314. 10.4319/lo.2009.54.6\_part\_2.2298

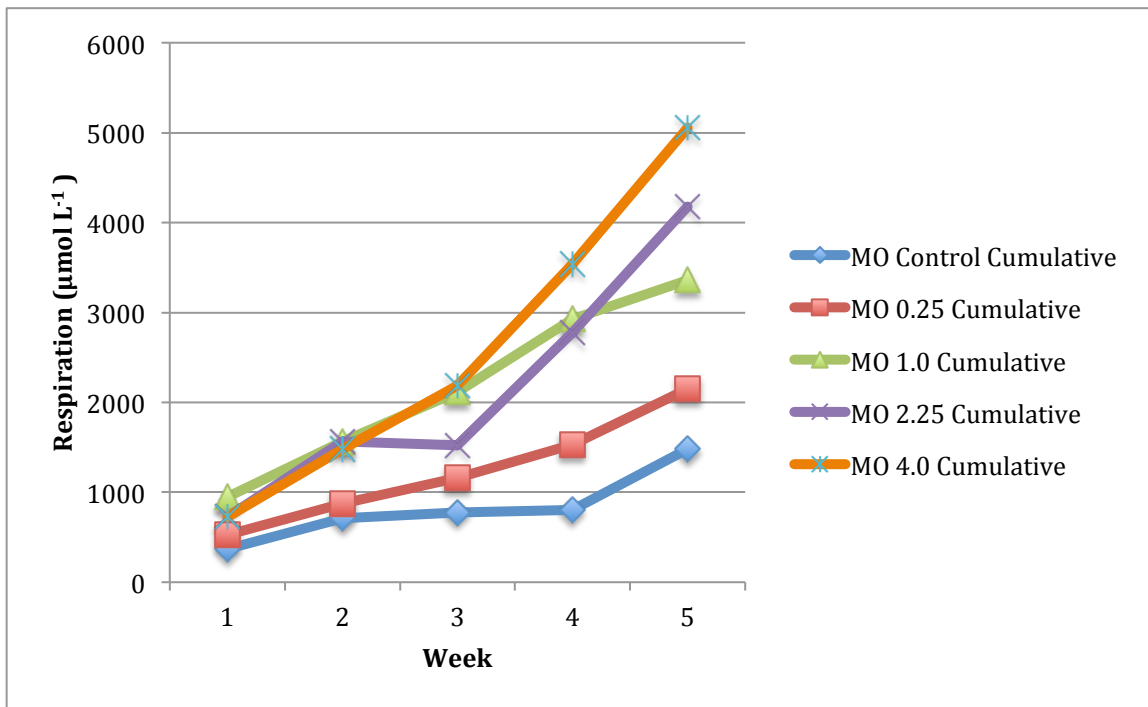
**Figures:**

Figure 1: This figure shows the cumulative linear relationship between Morris Lake and the four treatment groups over five week. The control treatment had the lowest respiration for all five weeks. At the end of the five weeks the 4.0 mg of glucose treatment resulted in having the highest respiration of recalcitrant carbon.

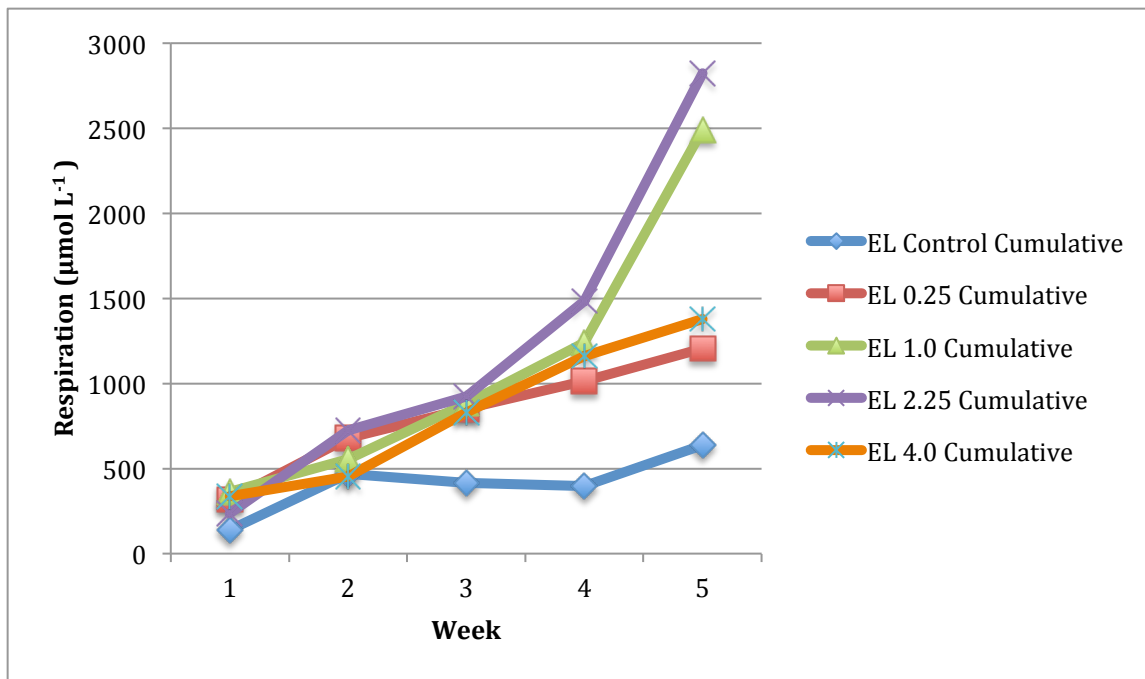


Figure 2: This figure shows the cumulative linear relationship of East Long lake of the four glucose treatment groups over a time period of five weeks. The control group with no glucose added resulted in the lowest carbon respiration of recalcitrant carbon. The highest amount of carbon respiration was the treatment group of 2.25 mg and 1.0 mg of glucose. This was not expected and could be further explained by there could've been lab error. Another possible explanation was East Long Lake could have a limit of the amount of carbon it can hold before it no longer respire.

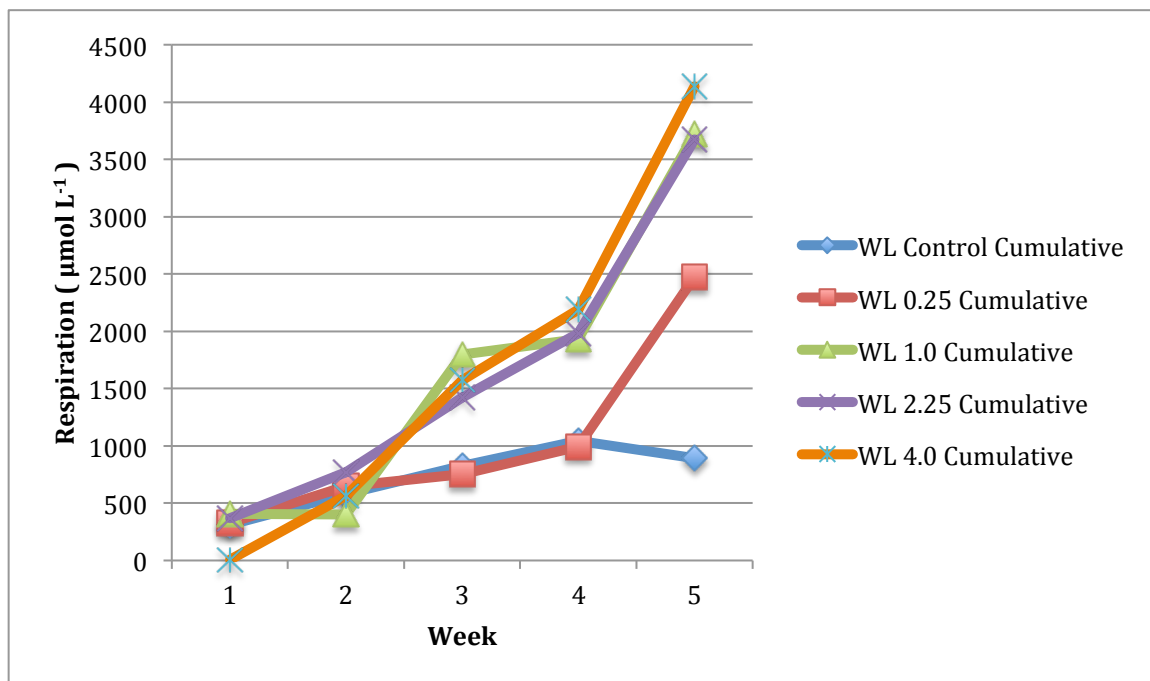


Figure 3: This figure shows the cumulative linear relationship of West Long lake of the four treatment groups of glucose over a course of five weeks. The control group

and the 0.25 mg of glucose stayed around the same value of respiration up until the final week. The last three treatment groups of 1.0, 2.25, and 4.0 mg stayed the same until the last week as well. The 4.0 mg treatment group resulted in the highest respiration as expected.

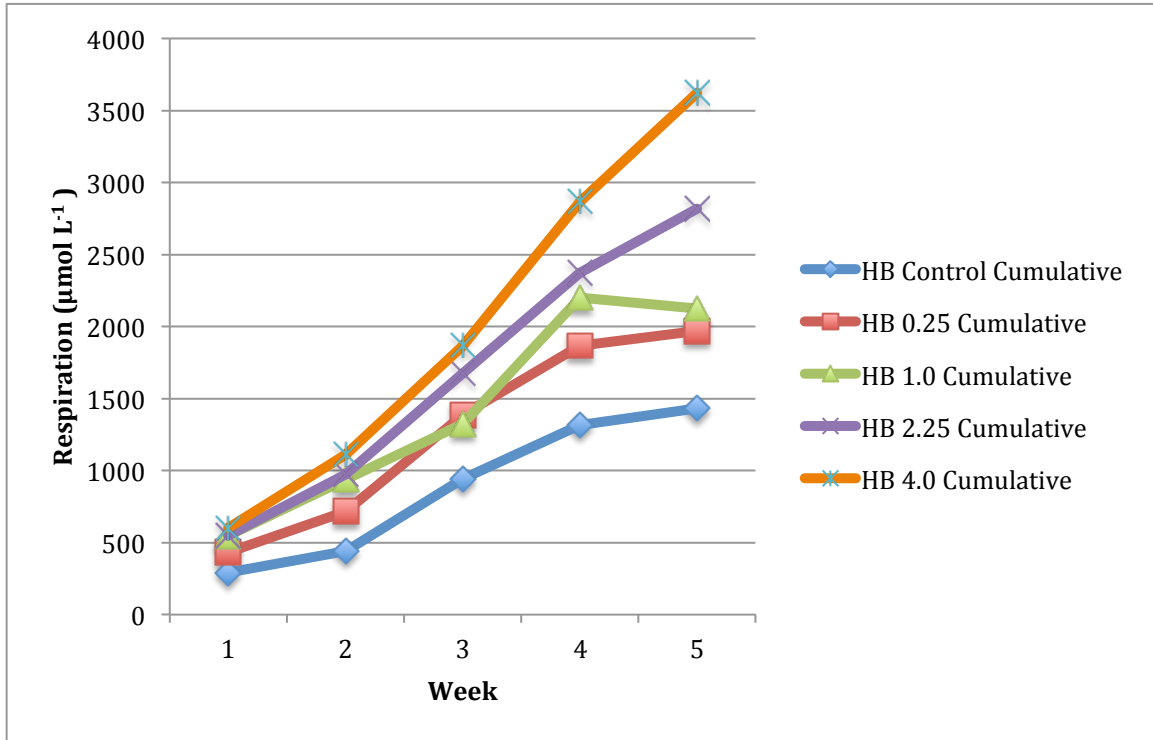


Figure 4: This figure shows the cumulative linear relationship of Hummingbird Lake of the four glucose treatment groups over a time length of five weeks. These results were expected as the higher amount of glucose added the higher respiration rate there would be.

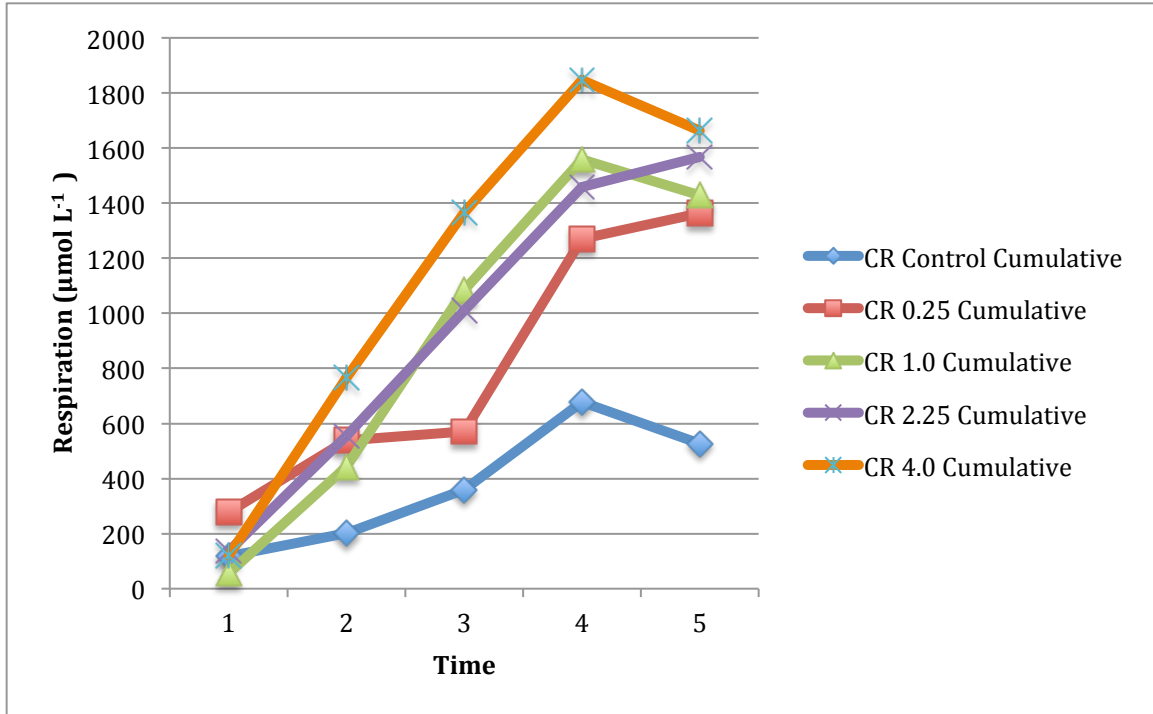


Figure 5: This figure shows the cumulative linear relationship of Crampton Lake of the four treatment groups of glucose over a time period of five weeks. These results were expected as the more glucose was added the higher respiration rate of carbon there would be. This happened up until week five. There is a drop in the rate of carbon respiration in every treatment expect for 2.25 mg. This could be due to a lab error or that Crampton Lake has a limit on the amount of carbon it can handle before it slows down.