

# The effect of salinity on the growth rate of *Nannochloropsis salina*

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## Introduction

Water and land are the largest quantity resources necessary to cultivate algae for biofuel production. It is necessary to find ways to cultivate algae without impinging on fresh water resources, especially in arid regions. It has been shown that certain species of algae such as *N. salina* can be cultivated in non-traditional water resources such as produced water from oil and gas production, which is known to have a high salinity. The purpose of this experiment was to gain insight into the effect that salinity has on the growth rate of *N. salina* algae cells with the intention of exploring alternate water sources such as produced water that can be used for the cultivation of algae. The production of biofuel as a viable source of renewable energy has become a well sought out research topic. Algae as a viable source of biofuel has become of special interest. Algae have been shown to have a high yield of oil per acre of cultivation. The production of biofuel via algae does not compete with food production. Algae consumes carbon dioxide, as well as purify wastewater. With oil and gas production increasing in the United States the issue of the disposal of “produced water” from processes such as fracking has become a large issue. Therefore the possibility that certain algae species could be used to treat this wastewater while being grown for the production of biofuel has become of specific interest.

## Hypothesis

Algae cells grown in hypersaline media will experience a higher rate of growth than algae cells that are grown in hyposaline media because *Nannochloropsis salina* is a marine algae species.

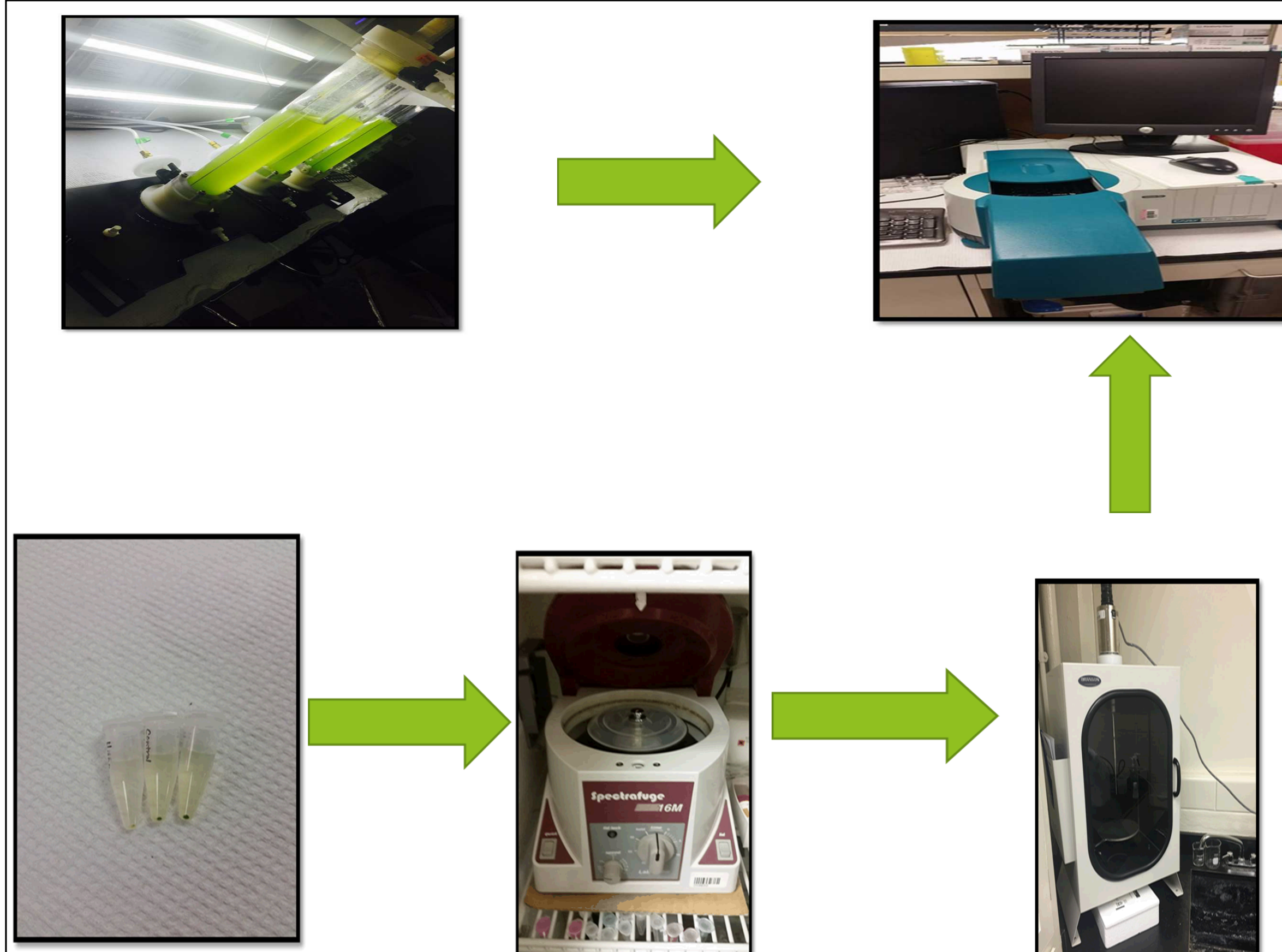
## Conclusion

In conclusion, the algae cells that were grown in the hypersaline media experienced a much higher rate of growth. This observation is demonstrated by the comparison of the quantitative value, rate of change, for each of the different cultures. Data should continue to be collected and replicated in order to strengthen the hypothesis that algae cells grown in hypersaline media do experience a faster rate of growth. The much higher growth rate for the algae cells exposed to hypersaline media suggests that even higher salt contents should be tested. The exponentially higher growth rate for algae cells that were exposed to hypersaline media can help to provide more insight into techniques for mass culturing algae.

## Sandia Recognition

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy’s National Nuclear Security Administration under contract DE-AC04-94AL85000.

## Methods & Materials



The cultures were grown in air-lift bioreactors where each culture was grown in medias that were 50% (hyposaline), 100% (control), and 150% (hypersaline) the salinity of seawater, bubble with ambient air, and provided approximately 200-300 mmol m<sup>-2</sup> s<sup>-1</sup> photons of white light. Optical density(OD) at 750 nm and cell counting via a hemacytometer were used to determine the relationship between OD and cell density, then OD was tracked throughout the experiment to quantify the rate of cell growth. In order to test the chlorophyll content of the cells 1mL samples were taken from each culture, put into the centrifuge, froze in liquid nitrogen, suspended in methanol, sonicated and then centrifuged once again. The supernatant from the top of the sample was then run through the spectrometer to test for the presence of chlorophyll.

## Hyperspectral Imaging

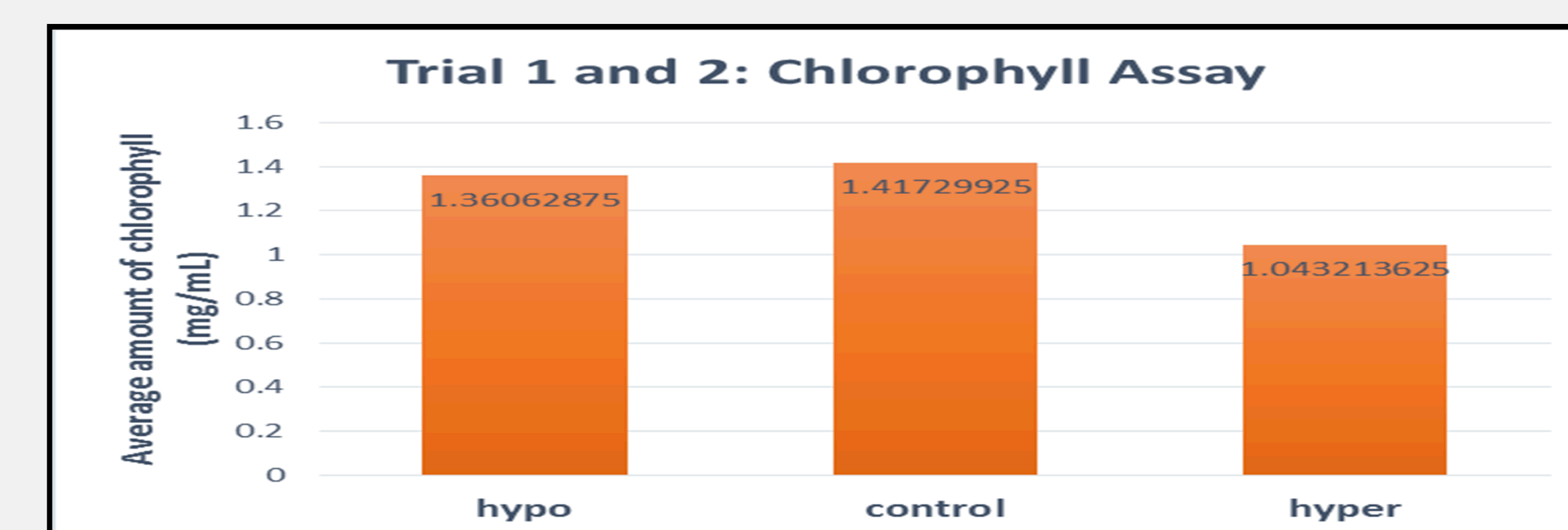
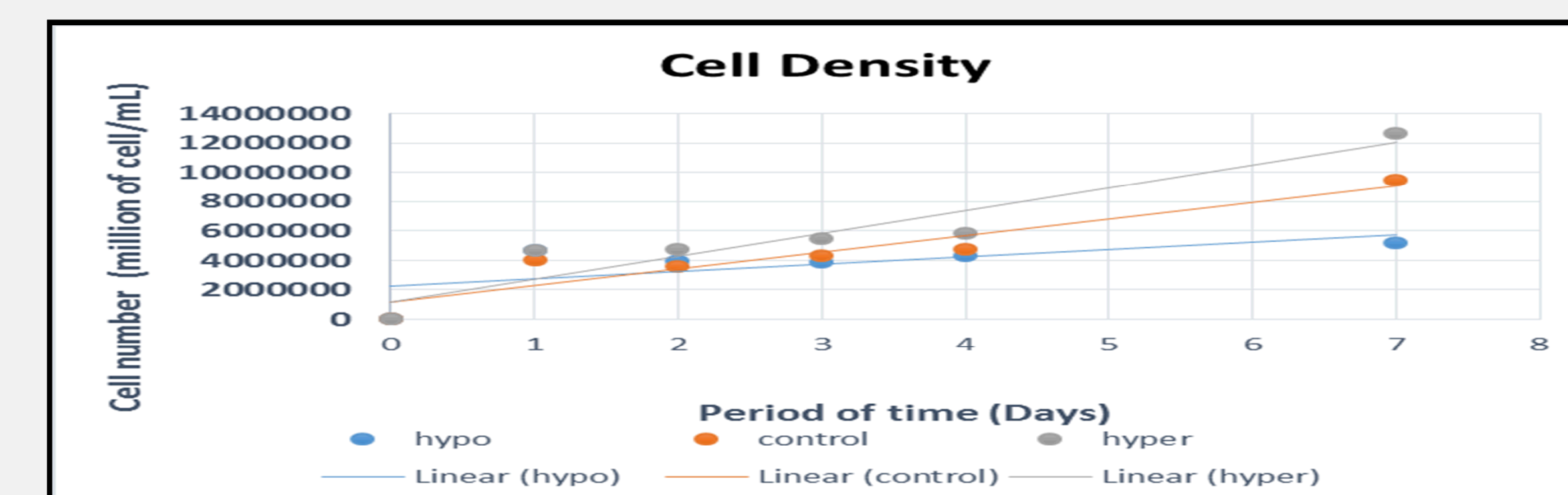
	2 hr 7/7	6 hr 7/7	Day 1 7/8	Day 2 7/9	Day 3 7/10	Day 5 6/29	Day 6 6/30	Day 7 7/1
Hypo saline								
Normal Saline								
Hyper saline								

Image analysis was cell restricted. Individual images from the same experiment were combined into a composite data set. In vivo hyperspectral confocal fluorescence imaging was used in order to determine the pigment localization, as well as the distribution in cyanobacteria cells. Three signals were detected: Chl 685, Chl 670 and auto fluorescence. The Chl 670 signal could potentially be a degraded chlorophyll product. The Chl 685 signal was the detection of chlorophyll A. High Autofluorescence could indicate dead cells. In the future this technique could be used as a method of obtaining descriptive images that could help to obtain insight into the health of the algae cells as well as the prevalence of different biochemical components.

## Results

- First graph Below:** It was observed that the algae cells exposed to the hypersaline media experienced a higher growth rate in comparison to the algae cells that were exposed to the hyposaline growth media, as well as experienced a higher growth rate in comparison to algae cells that were grown in the control media. Calculations of the average rate of change yielded a growth rate of 502,162 cells per day in respect to algae cells grown in hyposaline media, 1,000,000 cells per day in respect to algae cells grown in the control media, and 2,000,000 cells per day in respect to algae cells grown in hypersaline media. Quantitatively this led to the observation that the number of algae cells grown in hypersaline media approximately doubled in respect to the algae cells grown in the control media and approximately quadrupled with respect to the algae cells grown in the hyposaline media.
- Second Graph Below:** It was observed that as the algae cells were exposed to a media with increasing salinity the chlorophyll content decreased.

## Graphical Data



## Future Research

I believe that future work should be done in order to replicate these findings. Replication of this experiment could lead to moving forward with culturing methods that promote large scale algae production. Replication could also lead to further testing to see whether or not *N. salina* could be grown in produced water. Future research should also be done on the effect that salinity has on the lipid production of *N. salina* and whether or not other salts (ionic compounds) would have an effect on the growth rate of the algae.

## Acknowledgements

I would like to acknowledge all of those that made this research possible: John Rosegen, Michael Bennett, Lyndsay Ryan, along with the rest of Dr. Hanson’s and Dr. Lidke’s labs. I would also like to acknowledge Sandia National Labs along with the University of New Mexico for allowing us to use their microscopy equipment.