The Effects of Hydrogen Sulfide on Kelp Growth using *Nereocystis leutkeana*

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Abstract

Nereocystis leutkeana, or Bull kelp is a fundamental part of near shore marine ecosystems and as carbon dioxide levels in the atmosphere increase, due to climate change, algae blooms grow larger resulting in higher concentrations of hydrogen sulfide being added into the ocean. This experiment shows a correlation between micromolar hydrogen sulfide concentration and a potential decline in growth in brown algae. It may also sow a potential resilience to mm concentrations of HS. this paper could further our understanding of kelp growth in association with rising HS levels in the ocean. Additionally, this study could inspire further research into the evolutionary connections between brown algae and terrestrial plants by comparing processes of photosynthesis in the presence of hydrogen sulfide.

Introduction

What is *Nereocystis leutkena?*

Bull Kelp, *Nereocystis leutkeana,* is a canopy-forming kelp found in the near-shore, subtidal habitats of the Pacific Northwest (Druehl, L. D. 1970, Foreman, n.d.). *Nereocystis,* and kelp in general, are key to forming habitats and are associated with increased levels of biodiversity in areas where they are present (Teagle et al., 2017). Kelps also play an important role as primary producers in the formation of detritus, particulates of kelp that fell off the main blade due to erosion. This organic matter is a main food source for many filter and suspension-feeding animals (Duggins et al., 1989). The significance of *Nereocystis* is that it supports ecosystems by providing habitat and contributing significant amounts of biomass to various invertebrate species (Kelly & Scheibling, 2012). It is important to conserve and protect kelp forests, and with global climate change significantly impacting ocean ecosystems by decreasing productivity, ecological interactions and reducing the number of habitat-forming species, such as kelp, it is important to try and understand the causes and effects of climate change in our oceans (Hoegh-Guldberg & Bruno, 2010).

Why focus on Hydrogen Sulfide?

Increased concentrations of greenhouse gases in the atmosphere, in association with climate change, are causing an increase in the size and frequency of harmful algal blooms (Moore et al., 2008). When algal blooms are decomposed by bacteria through anaerobic respiration they produce high concentrations of hydrogen sulfide in the ocean (Zhao et al., 2019). Not only is hydrogen sulfide increasing in the oceans in association to climate but also due to underwater volcanic vents, and according to speculation hydrogen sulfide is going to become more and more prevalent in our world making it an important thing to understand.

Hydrogen Sulfide and Photosynthesis

Hydrogen sulfide, also know as H_2S, is a common and lethal gas (Guidotti, 2010), has Recently it has been shown that in micromolar amounts hydrogen sulfide can increase the rates of photosynthesis in plants and in turn increase the overall growth of plants (Chen et al., 2011). This phenomenon is also found in the marine plant species: *Zostera Marina* and *Phyllospadix scouleri* (Dooley et al., 2013, Dooley et al., 2015). Doing so by affecting photosystem I of the plant's plastids by supplementing the reaction of photosynthesis alongside H_2O in photosystem II (Dooley et al., 2013).

Brown algae evolved separately from land plants and evolved quite recently compared to land plants (Bringloe et al., 2020), with further research describing the numerous changes brown algae underwent in terms of the composition of its plastids, evolving photosynthesis separately from that of land plants (K. Raval et al., 2024). Red algae, an ancestor of brown algae is separated from green algae by having a different instance of endosymbiosis involving different plastids (Maréchal, 2024), and brown algae evolves with a different and extensive array of different plastids and photosynthetic systems (Terpis et al., n.d.). Whether H_2S affects the photosystems of Brown algae similarly to land plants is a question yet to be answered.

Why do this experiment?

The effects of hydrogen sulfide in ocean ecosystems are not a well-studied phenomenon. With Kelp being such an important part of nearshore subtidal ecosystems it would be important to research the effects hydrogen sulfide would have on the growth of *Nereocystis leukemia*, which is: a fast-growing, abundant, and an important kelp species for the formation of kelp forests (Druehl, L. D. 1970); making it a prime choice for a model organism.

Alongside that, this experiment could help further the understanding of brown algae from an evolutionary standpoint. If the plastids and photosystems of the brown algae, which are so far removed from land plants (Choi et al., 2024), behave in a way similar to land plants when in the presence of hydrogen sulfide a better understanding of the endosymbiosis of plastids could be made if H_2S affects on kelp photosynthesis is similar to that of plants.

This experiment aims to test if hydrogen sulfide, in micromolar concentrations, affects the growth of kelp. Two answers may come from this experiment: 1) The presence of hydrogen sulfide does not affect the growth of kelp: 2) The presence of hydrogen sulfide does affect the growth of kelp. Either answer would lead to a better understanding of how climate change may affect our oceans' ecosystems and ecology.

Materials and Methods

Growing *Nereocystis*

16 *Nereocystis* were collected on San Juan Island in Washington off Beaverton Cove from the boat line (ranging from 0.5 to 1.5 meters of depth) of the abandoned sailboat "Pura Vida", which was abundant in young bull kelp and easily collected. Beaverton Cove is located in an inlet west of Friday Habor Laboratories (48.5542, -123.01757) with medium to low wave action but still very affected by the tide.

4 kelp were placed into 4 large tanks with lengths ranging from 117.5cm-118cm and widths ranging from 56cm-57.1cm. The height of the water was standardized to 5cm for each tank and the volume of each tank was 33L (+/- 0.7L) (fig.1). Flow rates of each tank were standardized to 4.5 ml/sec (+/- 0.1ml). Plastic sheets were fitted to the tops of each tank as well as a screened mesh: the plastic sheets reduced the evaporation rate of administered H_2S and the black screened mesh reduced the amount of light entering into the tanks preventing dangerous amounts of sun exposure as bleaching was an issue in past testing. The black screened mesh was

not added until day 2 of collection 5/24/2024 due to high light levels on day 1.

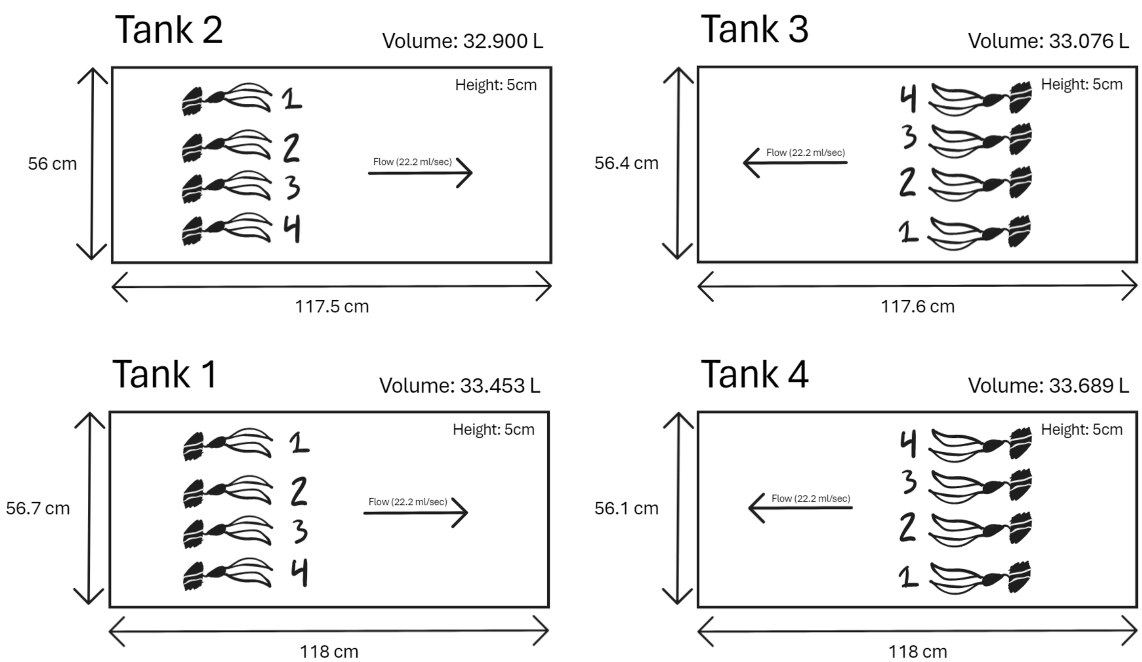

Fig. 1. Diagram of tank setup. Tank 1 acting as the controlled containing 0µM, Tank 2 testing 25µM, Tank 3 testing 50µM, and tank 4 testing 250µM.

Bull Kelp individuals were placed on similar-sized stones and fastened using rubberbands around the stipe. The stipes were cut around 7 cm below the pneumatocyst (bulb) removing the holdfast. Only 2 blades were kept and measured from each *Nereocystis* individual, and if individuals contained more than 2 blades, the two healthiest blades were kept and all others were removed. The pneumatocysts were ensured to be submerged below the water to prevent desiccation. They were placed left to right in a 1-4 position respectively (fig. 1).

Measuring Growth

To measure the growth of the blade a mark was placed 2cm above the pneumatocyst of the *Nereocystis* individual, that mark was measured with a ruler, a technique discussed in *Field*

Studies of the Giant Kelp Nereocystis (Nicholson, 1970). *Nereocystis* was marked using a needled syringe and Black India ink, gently pricking the blade with the syringe twice at the same length to be more easily found when measuring (fig. 2). As the blade grows the mark will grow further from the meristem, this is a more accurate reading of growth than measuring the entire blade because the formation of detritus can cause blade measurements to be unreliable.

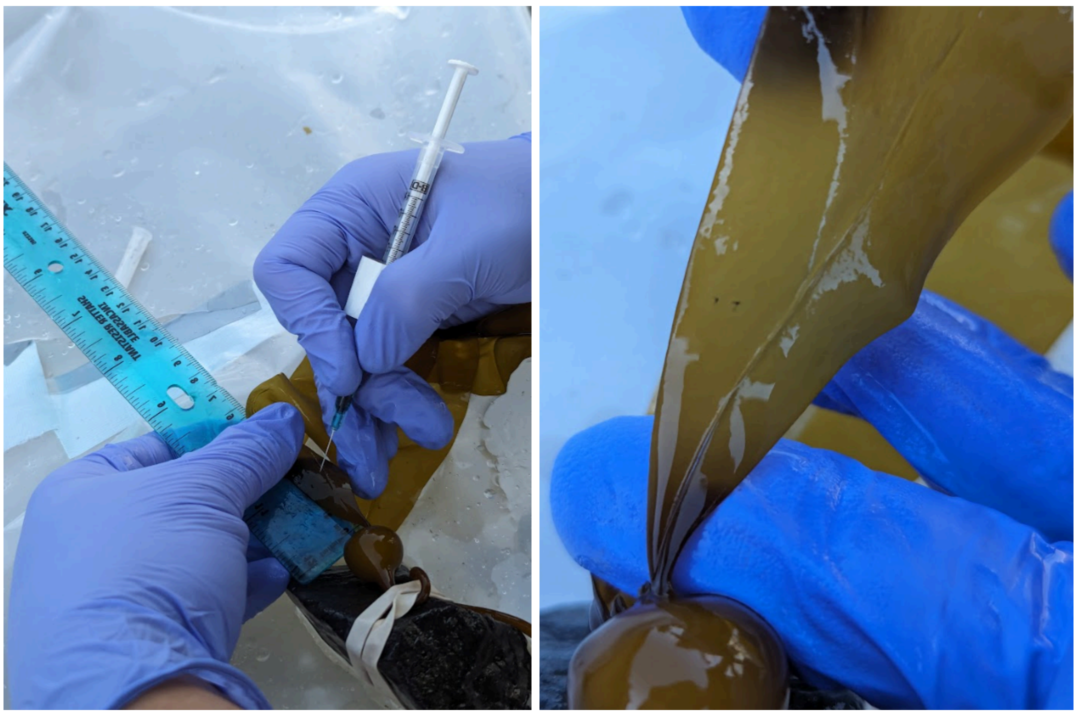

Fig. 2. Photos displaying the ink marking process on *Nereocystis* blade

As each *Nereocystis* had two blades, the length of both blades was collected and then averaged for the length of growth for that individual. This was done in case one of the two blade meristems would die, and then the nutrients and photosynthates collected from both tissues would be translocated to the living meristem adding to its growth (Nicholson, 1970).

Administering Hydrogen Sulfide

3 of the tanks were filled with differing concentrations of H_2S: 25µM, 50µM, and 250µM. So Tank 1 had kelp with 0µM of H_2S, Tank 2 with 25µM, Tank 3 with 50µM and Tank 4

with 250µM. These concentrations of hydrogen sulfide for each tank were decided based on the paper *Increased Growth and Germination Success in Plants Following Hydrogen Sulfide Administration* (Dooley et al., 2013) as well as factoring in a limited budget and resources .

Hydrogen sulfide was administered by titration of 0.4% H_2S water into a 1L bottle of saltwater, diluting it, and then placing those bottles into their respective tanks overnight to allow the hydrogen sulfide to diffuse via a concentration gradient. Flow was turned off to reduce the evaporation of H_2S, as it is an incredibly volatile chemical. After 10-14 hours the flow of the tanks was turned back on for 10-14 hours. Data was collected in the mornings after flow was turned on and H2S was tested, and in the afternoons before administering H_2S. This cycle was repeated 4 times for a total of 4 administered doses of hydrogen sulfide with a final data collection period on the 4th afternoon.

Tank Consistency

To keep consistency between tanks the following things were measured and compared: temperature of the tanks, light readings into the tanks (measured using a Licor meter), and the flow rates of the tanks measured (40ml/sec +/- 0.1ml).

In the mornings after hydrogen sulfide administration before turning the flow of tanks back on the levels of hydrogen sulfide were tested in each of the 3 tested tanks using a CHEMets Visual Test kit, which gave a reading between 0-8ppm of hydrogen sulfide in water using a comparative methylene blue color gradient.

Results

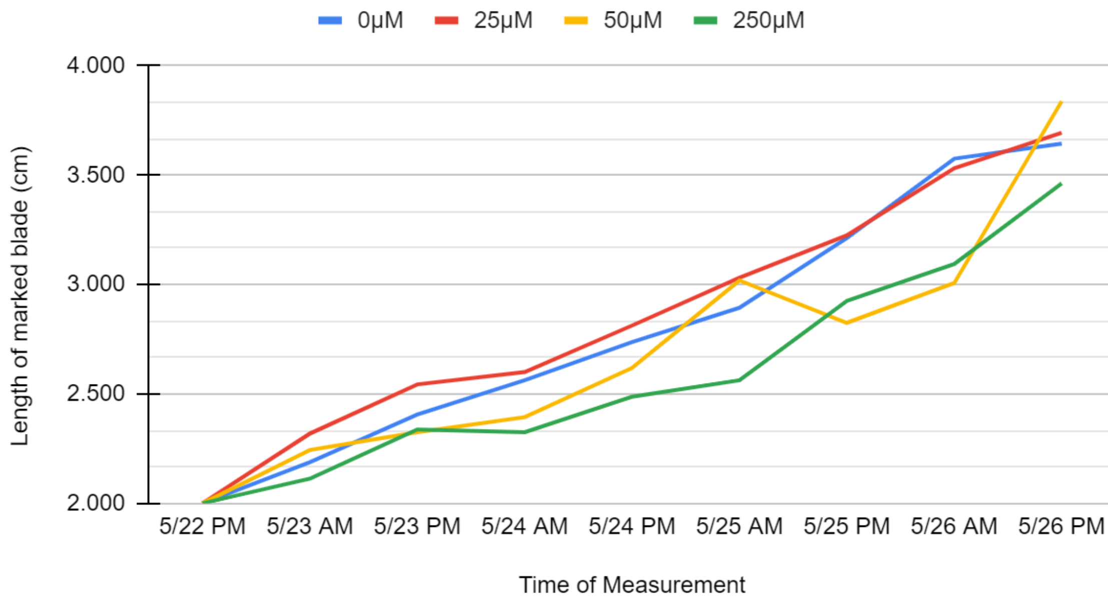

Figure 3. The average length of kelp blades in varying concentrations of H_2S (0µM, 25µM, 50µM, and 250µM). In H_2S concentrations greater than 25µM, lengths are more irregular and for most of the experiment are shorter than the controlled concentration of 0µM. Key above graph showing 0µM (blue), 25µM (red), 50µM (yellow), and 250µM (green) correlation to the graph.

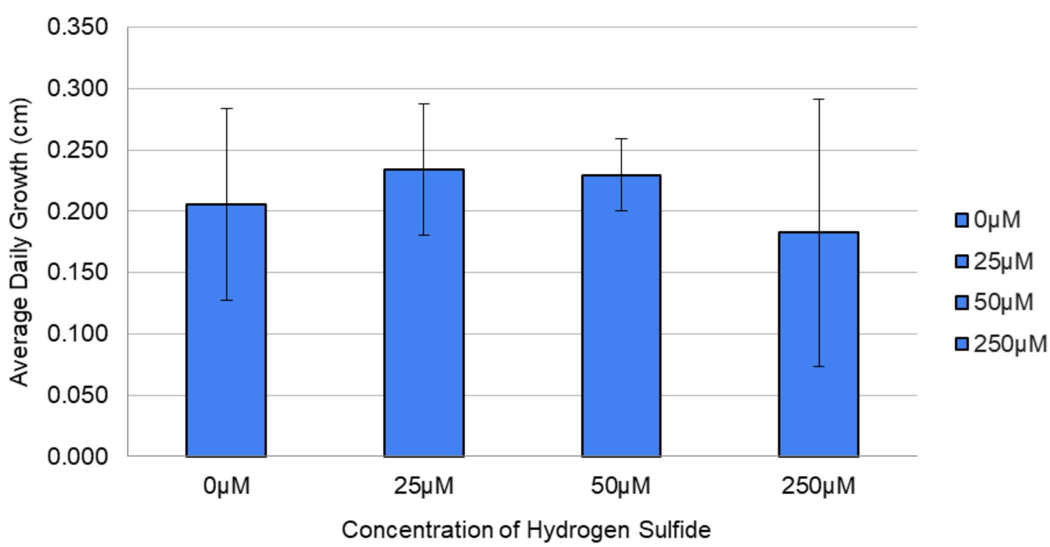

Figure 4. The average daily growth of kelp in varying concentrations of H_2S. Although growth trends varied throughout each day, the overall daily rate of growth for kelp in all the different concentrations was roughly the same. Stand deviation bars are shown. P-value of growth data is 0.835

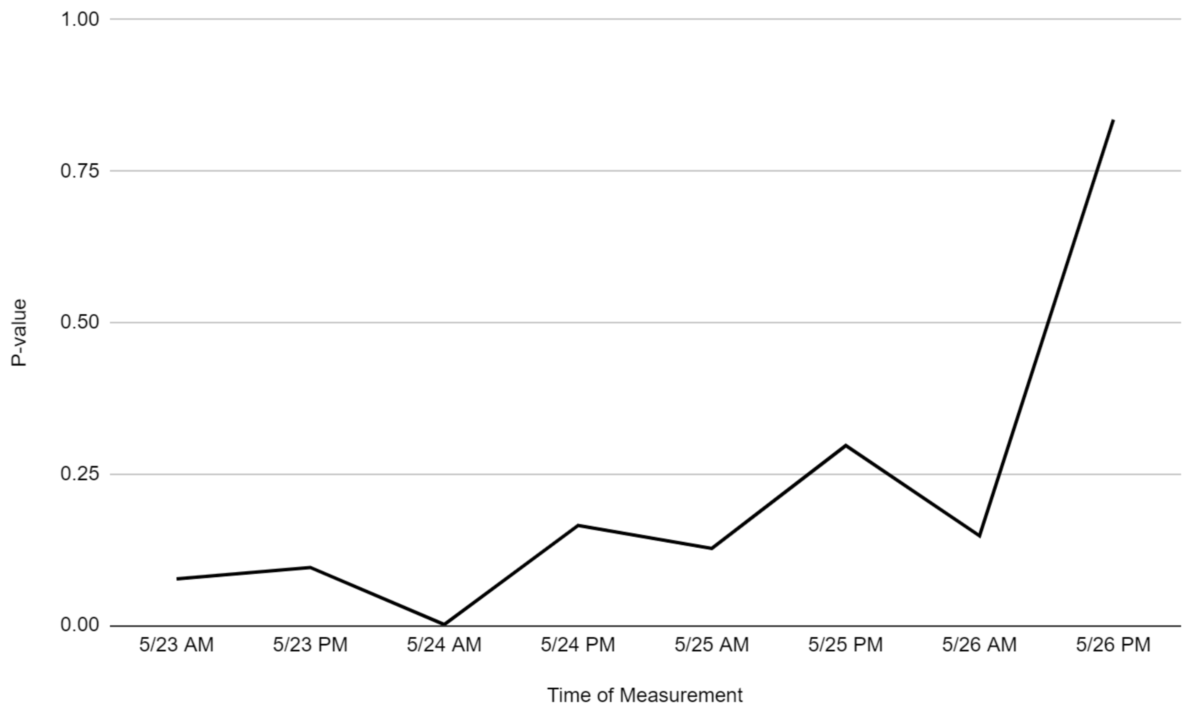

Figure 5. A graph depicting the varying P-values for each period of measurement, calculated by ANOVA. The only measurement of significance was the done at 5/24 AM after 2 administrations of the hydrogen sulfide, with a P-value of 0.001.

External testing was done on the tanks including temperature, light levels, and H_2S concentrations the morning after the administration of H_2S. Temperatures between the tanks ranged from 10.3C° to 12.5C° depending on the day, with all tanks staying within 0.35C° of each other. Light levels in the tanks were high during day 1 reaching 1705.05µmol s^{-1} m^{-2} with variance between tanks with +/- 184.75µmol s^{-1} m^{-2}. Following days (2 and 4, missed data collection on day 3) light levels reached 251.8µmol s^{-1} m^{-2} and 222.1µmol s^{-1} m^{-2} with variance of +/-36.8µmol s^{-1} m^{-2} and +/-34.1µmol s^{-1} m^{-2}. Concentrations of H_2S were collected in the mornings for days 2-4. H_2S presence was 0 except for tank 4 (250µM) on days 2 and 3 with a presence of 1ppm.

Discussion

The effects of hydrogen sulfide on terrestrial plants are a recently discovered and tested phenomenon (Dooley et al., 2013), with a complete lack of research on its effects on algae. With this experiment, it is shown through an ANOVA analysis that the addition of hydrogen sulfide (when administered in intervaled doses) does not affect the final length of kelp after 4 days of trials ($F = 0.286$, df=3, $P = 0.835$)(fig.3). The average daily growth rate for each concentration is also left unaffected ($F = 0.285$, df=3, $P = 0.835$) (fig. 4). However, H_2S does affect the growth trends of *Nereocystis*. Doing an ANOVA analysis of the growth rate on each day the average you get an average p-value of 0.21, with all other days ranging averaging with a P-value of 0.130. The fourth measurement of lengths (5/24 AM) had a significance 0.001, showing that on that second day, there was a very significant difference in the growth of the kelp at those 4 different concentrations of hydrogen sulfide.

The measurements occurring on 5/24 AM show kelp grown in 25µM length was slightly better than kelp grown in normal conditions (0µM). Note, an error occurred in the administration of H_2S in the tank containing 25µM and it is unknown if the administration of H_2S on that day occurred, also in the morning of collection the entire tank was drained of water, the timing of this draining is unknown adding another variable to not knowing if H_2S administration occurred. The growth data of this instance is hence omitted and not to be considered in this analysis, however the length still holds significance. At 50µM kelp was 10.2% shorter than the controlled kelp, and growth rates were 56% smaller than that of the controlled kelp. At 250µM kelp was 9.3% shorter than the controlled with a negative growth rate of -0.0125.

The growth trends of the kelp are rather varying, sometimes showing significant differences and sometimes showing insignificant differences. Comparing the growth rates of kelp grown at 0µM and 25 µM, the kelp grown at a 25µM concentration of hydrogen sulfide had a slightly higher growth rate and less variability in lengths of the 4 kelps. One potential reason for this increase in growth could be correlated to the hypothesis of Dr. Dooley, that hydrogen sulfide can increase plant growth rates by increasing the photosynthesis rates due to an increase in the amount of available reactants (Dooley et al., 2013). However, further testing will need to be done to determine if this hypothesis applies to kelp species, specifically testing photosynthesis by either measuring with fluorometry or by measuring the dissolved oxygen production of the kelp (Siebers et al., 2021) and additional long-term experiments with larger sample size may show that the increase in growth for kelp grown at 25µM is significant.

For kelp grown at 50µM on 4/24 AM a possible reason for the decrease growth could be a shutting down of photosynthesis in photosystem II similarly to that of plants (Dooley et al., 2013) but disimilarly then increasing in growth rate at a rate similar to that controlled at 0µM. If this experiment were to be repeated the use of a flourocam as a way of measuring the photosynthesis in the kelp and seeing if photosystem II does infact shutdown reducing the amount of photsynthates from being produced and thusly growth.

A trend of interest is with *Nereocystis* at 250µM on 5/24 AM. There was decrease in the rate growth in the kelp of this tank. A possible reason for a decrease in length occurring is hormonal interaction with the morphology of the blade, affecting size of the cells themselves rather than affecting the rate of cellular division (Coleman & Martone, 2020), where H_2S triggered shortening of the morphological length of the blade. Further testing will need to be done on the photosynthetic rates as well as changes in the entire length of the blade, by adding multiple tattoos along the blades to see if the entire blade undergoes shrinking or just the meristem end.

There is an increased variability with *Nereocystis* grown at 250µM that can be seen compared to other kelp grown at different concentrations with a standard deviation of 0.1cm for the average daily growth rate. However, the sample size of the experiment is significant enough to be able to come to any definite conclusions and a greater sample size will be needed in order to truly conclude increased variability in growth at increase concentrations.

If this experiment were to be redone I would firstly expand the duration of the experiment. It seemed as though kelp growing at 50µM and 250µM were starting to grow exponentially at the end of the experiment and it would be interesting to see how exponential that growth would be. Also with the addition of more funding and more time a specialized tank could be created with a circulating flow system, to recycle hydrogen sulfide and nutrients, and an enclosed system without air in order to reduce the volatility of the H_2S as much as possible. Growth lights in order to keep growth as consistent as possible. My largest complaint with this experiment is my lack of H_2S monitoring. I am only able to monitor when I am physically at the tanks and can only do so using a color spectrum ppm test which is widely inaccurate due to the nature of the test, relying on different shades of blue for ppms of 1-8. Having a constant H_2S monitoring system that gives exact numerical data would allow for the volatility of Hydrogen sulfide to be easily managed by adding H_2S when concentration would drop, also knowing how long H_2S remained in each tank would help with the accuracy of the experiment.

If further research was made into the effects of hydrogen sulfide on *Nereocysistis* and algae in general a greater understanding of the effects of climate change could be made. As climate change continues, the rate at which hydrogen sulfide is put into the oceans also increases, and that increase in hydrogen sulfide could reduce the growth of kelp and potentially being another factor to the decline of kelp forests (Hollarsmith et al., 2022). A greater understanding for the ways in which kelp and algae photosynthesises could also be made. In future experimentation with the addition of photosynthetic data, a comparison between the photosystems in brown algae and terrestrial plants could be made to further understand brown algae's evolutionary formation of plastids and how they differ from land plants on a mechanistic level.

Conclusion

In summary, this experiment seeked to investigate the effects hydrogen sulfide may have on kelp, by exposing tanks containing *Nereocystis leutkeana* with 3 differing concentrations of H_2S, and exposing them intermittently. It was found that *Nereocystis* at a specific point of exposure to H_2S does have it's growth affected. With concentrations 50µM or greater decreasing its rate of growth. Potentially there may be a sort of resilience threshold of the *Nereocystis* at those higher concentrations (50µM & 250µM) where growth is impeded but an eventual acclimation occurs resuming growth rates, but the data from this experiment is not significant enough to support that claim and further experimentation would be required. This study shows that hydrogen sulfide can affect the growth rates of *Nereocystis* and potentially other kelps.

Acknowledgments

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References

Bringloe, T. T., Starko, S., Wade, R. M., Vieira, C., Kawai, H., De Clerck, O., Cock, J. M., Coelho, S. M., Destombe, C., Valero, M., Neiva, J., Pearson, G. A., Faugeron, S., Serrão, E. A., & Verbruggen, H. (2020). Phylogeny and Evolution of the Brown Algae. *Critical Reviews in Plant Sciences*, *39*(4), 281–321. https://doi.org/10.1080/07352689.2020.1787679

Chen, J., Wu, F.-H., Wang, W.-H., Zheng, C.-J., Lin, G.-H., Dong, X.-J., He, J.-X., Pei, Z.-M., & Zheng, H.-L. (2011). Hydrogen sulphide enhances photosynthesis through promoting chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification in Spinacia oleracea seedlings. *Journal of Experimental Botany*, *62*(13), 4481–4493. https://doi.org/10.1093/jxb/err145

Choi, S.-W., Graf, L., Choi, J. W., Jo, J., Boo, G. H., Kawai, H., Choi, C. G., Xiao, S., Knoll, A. H., Andersen, R. A., & Yoon, H. S. (2024). Ordovician origin and subsequent diversification of the brown algae. *Current Biology*, S0960982223017694. https://doi.org/10.1016/j.cub.2023.12.069

Coleman, L. J. M., & Martone, P. T. (2020). Morphological plasticity in the kelp *Nereocystis luetkeana* (Phaeophyceae) is sensitive to the magnitude, direction, and location of mechanical loading. *Journal of Phycology*, *56*(6), 1414–1427. https://doi.org/10.1111/jpy.13043

Dooley, F. D., Nair, S. P., & Ward, P. D. (2013). Increased Growth and Germination Success in Plants following Hydrogen Sulfide Administration. *PLoS ONE*, *8*(4), e62048. https://doi.org/10.1371/journal.pone.0062048

Dooley, F. D., Wyllie-Echeverria, S., Gupta, E., & Ward, P. D. (2015). Tolerance of Phyllospadix scouleri seedlings to hydrogen sulfide. *Aquatic Botany*, *123*, 72–75. https://doi.org/10.1016/j.aquabot.2015.02.004

Dooley, F. D., Wyllie-Echeverria, S., Roth, M. B., & Ward, P. D. (2013). Tolerance and response of Zostera marina seedlings to hydrogen sulfide. *Aquatic Botany*, *105*, 7–10. https://doi.org/10.1016/j.aquabot.2012.10.007

Druehl, L. D. (1970). The pattern of Laminariales distribution in the northeast Pacific. *Phycologia, 9(3–4), 237–247.* Https://doi.org/10.2216/i0031-8884-9-3-237.1. (n.d.).

Duggins, D. O., Simenstad, C. A., & Estes, J. A. (1989). Magnification of Secondary Production by Kelp Detritus in Coastal Marine Ecosystems. *Science*, *245*(4914), 170–173. https://doi.org/10.1126/science.245.4914.170

Foreman, R. E. (n.d.). *Studies on Nereocystis growth in British Columbia, Canada*.

Guidotti, T. L. (2010). Hydrogen Sulfide: Advances in Understanding Human Toxicity. *International Journal of Toxicology*, *29*(6), 569–581. https://doi.org/10.1177/1091581810384882

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The Impact of Climate Change on the World's Marine Ecosystems. *Science*, *328*(5985), 1523–1528. https://doi.org/10.1126/science.1189930

Hollarsmith, J. A., Andrews, K., Naar, N., Starko, S., Calloway, M., Obaza, A., Buckner, E., Tonnes, D., Selleck, J., & Therriault, T. W. (2022). Toward a conceptual framework for managing and conserving marine habitats: A case study of kelp forests in the Salish Sea. *Ecology and Evolution*, *12*(1), e8510. https://doi.org/10.1002/ece3.8510

K. Raval, P., MacLeod, A. I., & Gould, S. B. (2024). A molecular atlas of plastid and mitochondrial proteins reveals organellar remodeling during plant evolutionary transitions from algae to angiosperms. *PLOS Biology*, *22*(5), e3002608. https://doi.org/10.1371/journal.pbio.3002608

Kelly, J., & Scheibling, R. (2012). Fatty acids as dietary tracers in benthic food webs. *Marine Ecology Progress Series*, *446*, 1–22. https://doi.org/10.3354/meps09559

Maréchal, E. (Ed.). (2024). *Plastids: Methods and Protocols* (Vol. 2776). Springer US. https://doi.org/10.1007/978-1-0716-3726-5

Moore, S. K., Trainer, V. L., Mantua, N. J., Parker, M. S., Laws, E. A., Backer, L. C., & Fleming, L. E. (2008). Impacts of climate variability and future climate change on harmful algal blooms and human health. *Environmental Health*, *7*(Suppl 2), S4. https://doi.org/10.1186/1476-069X-7-S2-S4

Nicholson, N. (1970). Field Studies on the Giant Kelp Nerocystis. *Journal of Phycology*, *6*, 177–182. https://doi.org/10.1111/j.1529-8817.1970.tb02378.x

Popovic, R., Colbow, K., Vidaver, W., & Bruce, D. (1983). *Evolution of 02 in Brown Algal Chloroplasts*. *73*. https://doi.org/10.1104%2Fpp.73.4.889

Siebers, M. H., Gomez-Casanovas, N., Fu, P., Meacham-Hensold, K., Moore, C. E., & Bernacchi, C. J. (2021). Emerging approaches to measure photosynthesis from the leaf to the ecosystem. *Emerging Topics in Life Sciences*, *5*(2), 261–274. https://doi.org/10.1042/ETLS20200292

Teagle, H., Hawkins, S. J., Moore, P. J., & Smale, D. A. (2017). The role of kelp species as biogenic habitat formers in coastal marine ecosystems. *Journal of Experimental Marine Biology and Ecology*, *492*, 81–98. https://doi.org/10.1016/j.jembe.2017.01.017

Terpis, K. X., Salomaki, E. D., Barcytė, D., Pánek, T., Verbruggen, H., Kolisko, M., Bailey, J. C., Eliáš, M., & Lane, C. E. (n.d.). *Multiple plastid losses within photosynthetic stramenopiles revealed by comprehensive phylogenomics*. https://doi.org/10.1101/2024.02.03.578753

Zhao, Y., Zhang, Z., Wang, G., Li, X., Ma, J., Chen, S., Deng, H., & Annalisa, O.-H. (2019). High sulfide production induced by algae decomposition and its potential stimulation to phosphorus mobility in sediment. *Science of The Total Environment*, *650*, 163–172. https://doi.org/10.1016/j.scitotenv.2018.09.010

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