Ecotoxicological Impacts of Dicamba and Sulfur on the Marine Microalga *Tetraselmis* spp.

Emma Archambault[ac,1], Alex Ascher[a,2], Katie Lankowicz[b,2], Isabelle Seé[ac,2]

[a]Quahog Bay Conservancy
[b]Gulf of Maine Research Institute
[c]University of Maine
[1]Author
[2]Project Advisor

ABSTRACT

This study dives into the ecotoxicological impacts of dicamba and sulfur on marine microalgae. Dicamba is a common herbicide and an active ingredient in various at-home lawn care products used in commercial agriculture. Sulfur is an ingredient often found in organic pesticides that claim to be safer for the environment. Both pesticides were tested to gather information on their impacts on the growth and mortality of common microalgae, *Tetraselmis* spp. Experiments were done using concentrations of dicamba at 1 ppb, 10 ppb, 100 ppb, and sulfur at 10 ppb, as well as a control. Concentrations of mobile and immobile cells were quantified multiple times over 18 to 24 hours using a hemocytometer. The ratio of mobile cell concentration to immobile cell concentration was used as a proxy to identify the competing effects of growth and mortality within each treatment. While time influenced the growth-mortality ratio, no trends were consistent across treatments or trials. Findings imply that the tested concentrations have no significant direct impact on *Tetraselmis* spp. Further research would be beneficial in understanding the long-term effects of higher concentrations of these contaminants.

INTRODUCTION

Agricultural pesticide runoff is a pressing issue for the health of our marine ecosystems. With climate change, the frequency of heavy precipitation is expected to increase. This heavy rainfall leads to an increase in agricultural runoff into oceans. Runoff may contain harmful chemicals used as pesticides and herbicides in crops (US EPA, 2022). With an increasing amount of herbicides being released into aquatic environments due to runoff during strong rainfall, a thorough understanding of different chemical contaminants' ecotoxicological impact on marine ecosystems and organisms is essential (Vagi et. al, 2013).

Previous studies have examined the effect of glyphosate, one of the active ingredients in *Roundup®* brand pesticides, on algal mortality and growth. It is well-documented that glyphosate is damaging to algae. It slows photosynthesis and reduces chlorophyll content in a variety of marine algal species (Gil et. al, 2018). These sublethal effects impair algal growth and survival in the environment. With findings that glyphosate damages algae, there is a need to investigate the impacts of other active ingredients commonly found in herbicides and pesticides.

Dicamba is an herbicide popular in residential and commercial applications where some plants have become tolerant to glyphosate herbicides (Craig, 2018). It is present at a concentration of 0.029% by volume in *Roundup for lawns*® herbicide. Dicamba is highly mobile in soil and can easily contaminate ground and surface waters similar to glyphosate. Once introduced to the marine environment, dicamba can have both lethal and sublethal effects across multiple trophic levels (Homa et. al 2024). Dicamba's lethal tendencies in plants are due to its similarity to hormones naturally occurring in plants. Dicamba can inhibit growth by mimicking growth hormones that plants use. Dicamba's impact on aquatic organisms, such as microalgae, is a huge environmental concern.

Other chemical crop sprays marketing themselves as more "eco-friendly" are gaining popularity in residential and commercial use, particularly among organic growers. One active ingredient found in "eco-friendly" crop sprays, such as *Captain Jack's Orchard Spray*®, is sulfur. Orchard Spray contains sulfur as an active ingredient at a concentration of 10.0% volume. Sulfur is highly soluble in water and occurs naturally in seawater as sulfate at concentrations of approximately 2.649 mg/L (Lenntech, 2024). Typically crop sprays containing sulfur and other naturally occurring ingredients are marketed as environmentally safer and healthier. These types of crop sprays are gaining popularity, especially among organic growers. With the increased usage of pesticides with sulfur, sulfur runoff can be expected to increase and seep into oceans over time. Sulfur is an essential component in the growth of microalgae. Some studies have shown that microalgae as well as other plants have limited sulfur storage (Zhang, et. al, 2020). There is little information on the direct effects of sulfur on algae, but there are known environmental effects of sulfur oxidation in the atmosphere causing toxic acid rain, negatively impacting the environment and entering our oceans. This acid rain from increased sulfur use in agriculture ending up in the atmosphere is making oceans more acidic (Carlowicz, 2008). Prolonged exposure to high acidity water will be detrimental to algae and other marine organisms.

Microalgae, such as *Tetraselmis* spp. are primary producers in marine ecosystems and their presence is vital to marine food webs. As coastal oceans become more polluted we must understand how a variety of contaminants affect primary producers such as microalgae, and therefore other organisms at higher trophic levels within marine food webs. With little research on the effects of sulfur and dicamba concentrations on algae, it is important to include these common contaminants in our study. This laboratory experimental study evaluates sulfur and dicamba effects on *Tetraselmis* spp. mortality and growth. Changes in algal growth and mortality rates will be calculated by comparing mobile and immobile cell counts under varying concentrations of contaminants and an uncontaminated control group. It is expected that *Tetraselmis* spp. mortality will increase with increased dicamba concentration as compared to a control group. *Tetraselmis* spp. mortality may increase compared to an uncontaminated control when exposed to 10 ppb sulfur concentration, but the effects may be less severe than dicamba as sulfur is a natural nutrient. Through this research, the potential risks posed by this herbicide to aquatic ecosystems can be identified. Understanding the potential negative impacts of common herbicides on algal growth and mortality is necessary for developing effective pesticide application management strategies.

METHODS

Dilutions

To first dilute dicamba and sulfur, we made sterile seawater at 25‰ salinity using instant ocean salt and deionized water. We used a refractometer to test salinity to ensure it was at 25‰ to match the salinity of our algae culture, which replicates natural seawater conditions. We created our diluted dicamba solutions from a stock solution of *Roundup For Lawns*®, using serial dilutions to achieve 10 ppb, 100 ppb, and 1000 ppb concentrations. Similarly, dilutions of *Captain Jack's Orchard Spray*® were completed to achieve a 100 ppb concentration.

Experimental methods

Using our previously described dilutions, 4 treatments were prepared at the following concentrations: 0 ppb (control), 1 ppb dicamba, 10 ppb dicamba, 100 ppb dicamba, and 10 ppb sulfur. Each treatment consisted of 2 mL of a *Tetraselmis* spp. culture in a sealed cryotube.

To quantify the impact of each of the treatments, 15 µL of the sample was extracted and analyzed from each cryotube multiple times throughout the experiment. *Tetraselmis* spp. is a fast-growing algae, so we needed to separate the potential impacts of contaminants on both growth and mortality. To do this, both the total cells per sample and the number of immobile cells per sample were counted. Immobile cells were assumed to be dead. The number of mobile, or living, cells was calculated as the difference between the total and immobile cells. Cells were counted using a hemocytometer under a stereomicroscope. For each count, the number of cells within four gridded counting chambers was counted and then averaged. Each counting chamber has a known volume of 0.1 µM, so the concentration of cells/mL can be calculated as the average number of cells multiplied by 10,000. After initial observations, two additional observations were done *ad libitum* at roughly the same times over 18-26 hour periods.

After the experiments were completed, all data were organized in Excel. The change in concentration of mobile (living) cells over time was used as a representation of growth rate, while the change in concentration of immobile (dead) cells over time was used as a representation of mortality rate. The ratio of mobile to immobile cells over time is therefore a representation of the relative impact of growth and mortality on the total number of *Tetraselmis* spp. cells in each culture. Graphs of growth and mortality were created to visualize change over time for each trial.

Statistical methods

All statistical analyses were completed in R software (R Core Team, 2024). Initially, a generalized linear model (GLM) was fitted to the data to visualize the potential effects of each treatment on the ratio of growth to mortality across all three trials. However, the generalized linear model does not account for either random errors associated with each trial or the dependence of observations collected over time in a repeated measures design.

An analysis of covariance (ANCOVA) test was chosen as the most appropriate method to make quantitative comparisons of the effects of contaminant treatments while accounting for both time-dependent observations and random errors associated with unique trials. We first ensured that the data fit all assumptions of outliers, linearity, normality, homogeneity of variances, sphericity, independence, and homogeneity of regression slopes. All the assumptions were met.

RESULTS

Time series visualizations

Time series were created to see changes in growth, mortality, and growth-to-mortality ratio across the treatments throughout the experiment (Figure 1). These plots indicated that growth and mortality rates varied over time and across different treatment levels. However, no consistent trend emerged that suggests any clear impact of the treatments compared to the control.

Figure 1: Growth rate over time

Generalized linear models

Throughout the experiment, we observed no significant impact of either sulfur or dicamba at multiple concentrations on algal growth and mortality (Figure 2). The ratio of growth to mortality increased in each treatment over time, independent of contaminant concentration (Figure 2). The rate of change over time was similar among all treatments, as visualized by the slopes in the GLM (Figure 2). The GLM also revealed a slight difference in intercepts between the control and treatments, though this result is likely a sampling artifact rather than a biologically significant result. It does not address the interactive effect of treatment and time on the ratio of growth to mortality, which is why we chose to use a more statistically robust repeated measures ANCOVA for the final interpretation of the results.

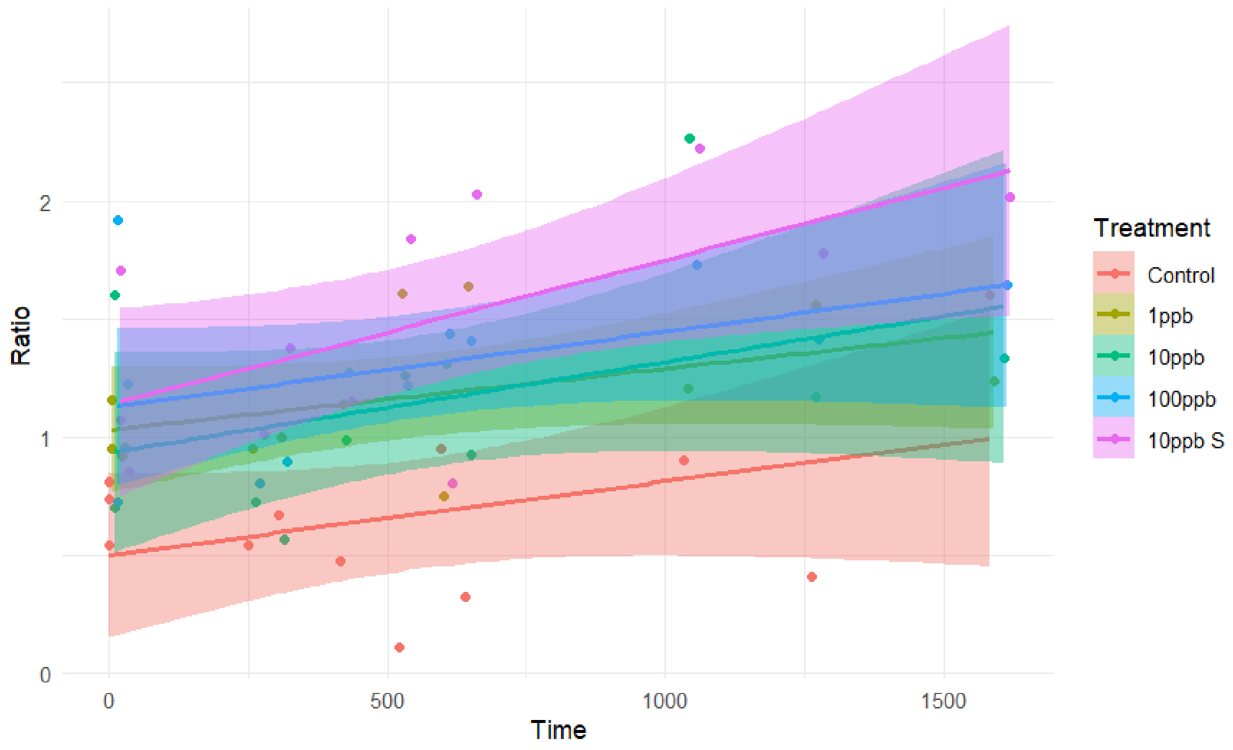

Figure 2: Ratio of mobile to immobile algae over 12 hours

Repeated Measures Analysis of Covariance

A repeated measures ANCOVA test showed that time had a significant effect on the ratio of growth to mortality ($p=0.023$), but this effect was not consistent across treatments or trials. Time and treatment as individual covariates did not significantly impact growth to mortality rate. Overall, our results did not show that dicamba or sulfur contaminant concentrations significantly impacted algal growth and mortality compared to the control.

DISCUSSION

This study aimed to assess the ecotoxicological impacts of dicamba and sulfur on marine microalgae, one of the key primary producers in marine ecosystems. Our findings based on three experimental trials show that dicamba at varying concentrations and sulfur had no significant impact on *Tetraselmis* spp. growth and mortality rates.

Dicamba did not significantly impact algae mortality or growth at 1 ppb, 10 ppb, or 100 ppb throughout the experiment. This result is surprising, based on the documented effects of other pesticides on algal mortality. Glyphosate in other studies has been found to impact photosynthesis and chlorophyll production (Gill et al. 2018). However, the impact of dicamba on algal physiology may differ from that of glyphosate. This could potentially be the reason for the lack of impact observed in this study. Dicamba herbicide is used so widely because of its success in regulating plant growth. Dicamba is easily absorbed by and spread throughout plants where it mimics the hormone auxin, increasing ethylene and abscisic acid in plant tissue causing abnormal cell division and ultimately inhibiting growth. (Minnesota Department of Agriculture, 2024; Rosa, 2024). While dicamba is an inhibitor of growth in terrestrial plants, its mechanisms and effects may differ in marine green microalgae. Dicamba belongs to the benzoic herbicides family which has been found to have low toxicity against green algae whereas other herbicides such as pendimethalin, prosulfocarb, and hiobencarb have higher toxicity to green algae (Li et. al, 2024). The findings from this experiment, as well as other studies, suggest dicamba may not affect hormonal mechanisms in green marine microalgae the same as they do in other microalgae studied at higher auxin concentrations (Wang, et. al, 2021).

Similarly, sulfur, one of the active ingredients in *Captain Jack's Orchard Spray*®, did not affect algae mortality or growth at 10 ppb. Sulfur is known to occur naturally in seawater and is involved in biogeochemical cycles in marine organisms. The impact of sulfur in this study was not significant, this could mean that the low concentrations used are not detrimental, but a higher direct exposure could potentially hurt the algae. It is known that plants and algae have a threshold for how much sulfur they can take in, this threshold may not have been surpassed in the experiment, allowing the algae to continue to grow regularly.

Analysis using repeated measures ANCOVA showed the impact of time on growth to mortality ratio, but revealed no significance between treatments. This shows that even though time affected the ratio of growth to mortality, the effect was not increased in the presence of dicamba or sulfur. Longer exposure periods at higher concentrations might be essential to measuring the sublethal effects of dicamba and sulfur. Standardized sampling at specific times would improve the results as well as incorporate many more sampling points.

Even though this study was inconclusive with the impact of sulfur and dicamba on green microalgae it is very important for the broader implications of pesticide contamination on marine ecosystems. As seen in this experiment minimal exposure did not have a significant impact, but chronic exposure at higher concentrations might be harmful. Additionally, conditions experienced in the real world were not met in the lab. Other factors such as the combined use of many different crop sprays, other contaminants, fluctuating temperatures, and mixing from tides may impact results. There is a need for more research to determine these effects of prolonged exposure, especially with an increase in popularity and usage of agricultural chemicals and increased rainfall and runoff into bodies of water with the progression of climate change. Impacts of contaminants on low trophic level organisms like *Tetraselmis* spp. could have cascading impacts on the health of higher trophic-level organisms and the livelihood of coastal communities. Further understanding of contaminant effects can be used in the development of management strategies for handling these chemicals and minimizing risk to marine ecosystems.

ACKNOWLEDGEMENTS

I would like to thank Pat Scanlan for supporting and funding Quahog Bay Conservancy and making this internship possible. This hands-on learning experience has been amazing, allowing me to see the real-world applications of classroom knowledge. I am thankful to Alex Ascher, Katie Lankowicz, and Isabelle Seé for their guidance and support throughout this project, it would not have been possible without their assistance. A special thank you to Tait Nygaard for providing us with hands-on field experience and the opportunity to explore aspects of marine science that cannot be seen in a classroom or through a computer screen. I am grateful to Dr. Walt Golet for making this experience through the University of Maine a possibility and for introducing us to the science conducted in your lab and at sea. Lastly, I want to thank Reuven Frye, Courtney Swenson, and my wonderful roommate Sydney Ulland for all the laughs and memories. Perchance this has been one of the best summers yet. I learned so much from everyone, encountered incredible marine life, and connected with brilliant individuals in marine science.

+

REFERENCES

Frignoca, I. (2017). Friends of Casco Bay Residential Pesticide Study [Pdf]. *Friends of Casco Bay*. https://www.cascobay.org/friends-of-casco-bay-testimony-in-opposition-to-ld-1505-an-act-to-create-consistency-in-the-regulation-of-pesticides/

Carlowicz, M. (n.d.). The oceans feel impacts from acid rain. *Https://Www.Whoi.Edu/*.

Retrieved August 12, 2024, from

https://www.whoi.edu/oceanus/feature/the-oceans-feel-impacts-from-acid-rain/

Craig, K. (2018). A review of the chemistry, pesticide use, and environmental fate of sulfur dioxide, as used in California. In P. De Voogt (Ed.), *Reviews of Environmental Contamination and Toxicology Volume 246* (Vol. 246, pp. 33–64). Springer International Publishing. https://doi.org/10.1007/398_2018_11

Dicamba—General information | minnesota department of agriculture. (n.d.). Retrieved August 6, 2024, from https://www.mda.state.mn.us/pesticide-fertilizer/dicamba-general-information

Gill, J. P. K., Sethi, N., Mohan, A., Datta, S., & Girdhar, M. (2018). Glyphosate toxicity for animals. *Environmental Chemistry Letters*, *16*(2), 401–426. https://doi.org/10.1007/s10311-017-0689-0

Homa, J., Stachowiak, W., Olejniczak, A., Chrzanowski, Ł., & Niemczak, M. (2024). Ecotoxicity studies reveal that organic cations in dicamba-derived ionic liquids can pose a greater environmental risk than the herbicide itself. *Science of The Total Environment*, *922*, 171062. https://doi.org/10.1016/j.scitotenv.2024.171062

Jørgensen, B. B., Findlay, A. J., & Pellerin, A. (2019). The biogeochemical sulfur cycle of marine sediments. *Frontiers in Microbiology*, *10*, 849. https://doi.org/10.3389/fmicb.2019.00849

Li, S., & Mcintyre, H. (2024). Toxicity assessment of 36 herbicides to green algae: Effects of mode of action and chemical family. *Agrochemicals*, *3*(2), 164–180. https://doi.org/10.3390/agrochemicals3020012

Major ion composition of seawater—Lenntech. (n.d.). Retrieved July 31, 2024, from https://www.lenntech.com/composition-seawater.htm

Mfarrej, M. F. B., & Rara, F. M. (2019). Competitive, sustainable natural pesticides. *Acta Ecologica Sinica*, *39*(2), 145–151. https://doi.org/10.1016/j.chnaes.2018.08.005

Rosa Repetti, M. (n.d.). *Dicamba—An overview | sciencedirect topics*. Retrieved August 6, 2024, from https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/dicamba#:~:text=Dicamba%20is%20a%20chlorinated%20benzoic,Green%20and%20Owen%2C%202011).

Rydh Stenström, J., Kreuger, J., & Goedkoop, W. (2021). Pesticide mixture toxicity to algae in agricultural streams – Field observations and laboratory studies with *in situ* samples and reconstituted water. *Ecotoxicology and Environmental Safety*, *215*, 112153. https://doi.org/10.1016/j.ecoenv.2021.112153

Sylwestrzak, Z., Zgrundo, A., & Pniewski, F. (2021). Ecotoxicological studies on the effect of roundup® (Glyphosate formulation) on marine benthic microalgae. *International Journal of Environmental Research and Public Health*, *18*(3), 884. https://doi.org/10.3390/ijerph18030884

US EPA, O. (2022, October 19). *Climate change impacts on agriculture and food supply* [Overviews and Factsheets]. https://www.epa.gov/climateimpacts/climate-change-impacts-agriculture-and-food-supply

Vagi, M. C., Kostopoulo, M. N., Petsas, A. S., Lalousi, M. E., Rasouli, M. E., & Lekkas , T. D. (2013). Toxicity of Organophoshorous pesticides to the marine alga Tetraselmis Suecica. *Global NEST Journal*, *7*(2), 222–227. https://doi.org/10.30955/gnj.000375

Wang, C., Qi, M., Guo, J., Zhou, C., Yan, X., Ruan, R., & Cheng, P. (2021). The active phytohormone in microalgae: The characteristics, efficient detection, and their adversity resistance applications. *Molecules*, *27*(1), 46. https://doi.org/10.3390/molecules27010046

Zhang, Y., Huang, Z., Zheng, H., Wang, Q., & Li, A. (2020). Growth, biochemical composition and photosynthetic performance of *Scenedesmus acuminatus* under different initial sulfur supplies. *Algal Research*, *45*, 101728. https://doi.org/10.1016/j.algal.2019.101728