Investigating the Effects of L-lysine on *M. aeruginosa* and *D. pulex*

Massimo Grisanti
Advisor: Kevin Crowthers, Ph.D

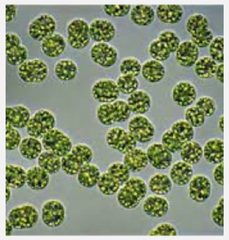
Background

So, what is algae?

Algae are a group of aquatic photosynthetic organisms essential for oxygen production. In fact, algae produce an estimated 70% of all atmospheric oxygen ("Oxygen levels," 2022). Algae can range from being microscopic in size to reaching over 200 feet in length in some kelp species (Lewin et al., 2023).

Harmful Algae Blooms

Harmful algae blooms, also referred to as HABs, occur when high temperatures and nutrient concentrations allow algae to exponentially grow. Once in a bloom state, the algae can then cover the surface of the water, blocking sunlight for aquatic species and decreasing oxygen levels, causing death. Furthermore, some algal species can then produce harmful toxins, called cyanotoxins, which can harm humans and animals. As lakes and ponds are shut down due to harmful algae blooms, towns lose thousands of dollars as their beaches are closed, children in camp are stuck in the blistering heat during the hottest hours of the day, and people who may swim in the infected waters unknowingly could suffer from potentially fatal effects.



Microcystis Aeruginosa

M. aeruginosa is a common species of harmful algae. *Microcystis* can cause rashes, burns, and blisters on the skin after contact, as well as vomiting, nausea, headaches, diarrhea, pneumonia, and fever when ingested ("Microcystis: Toxic blue-green algae," 2009)

My Connection

I was inspired to research this problem after the beach I work at was closed over the summer due to a *Microcystis* bloom. The beach was closed for 3 weeks as we had to wait for the algae to clear. With so much free time from being out of work, I started researching harmful algae blooms only to discover that similar events were happening to beaches and ponds across the nation at unprecedented rates. This ultimately inspired me to research more effective ways to mitigate harmful algae blooms, as it was clear this issue would only get worse with rising temperatures.

Research Question

How will amino acid L-lysine affect the growth of zooplankton with a bloom of *M. aeruginosa*?

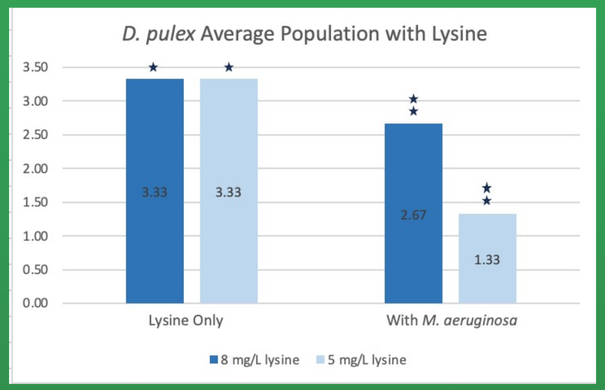
Hypothesis

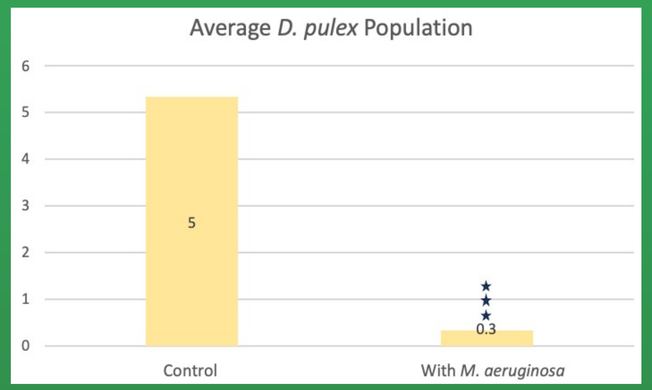
If L-lysine is added to a bloom of *M. aeruginosa*, it will inhibit the growth of algae while promoting the growth of zooplankton.

Key Takeaways

- Harmful algae blooms are a rising problem as temperatures and pollution increase
- Current methods are ineffective, costly, and/or harmful to the environment
- Amino acid L-lysine has been shown to kill *Microcystis* cells, effectively inhibiting its growth
- No testing had been done to determine the potential environmental consequences of using lysine to control *Microcystis* blooms.
- This study used zooplankton species *Daphnia pulex* to show that lysine did not have a significant effect on its population, thus indicating that lysine would not impact the marine food web

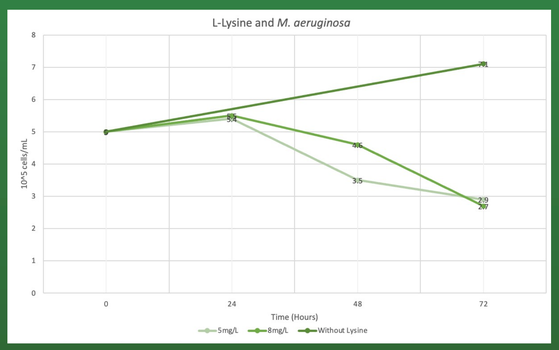
Changes in the *D. pulex* Population

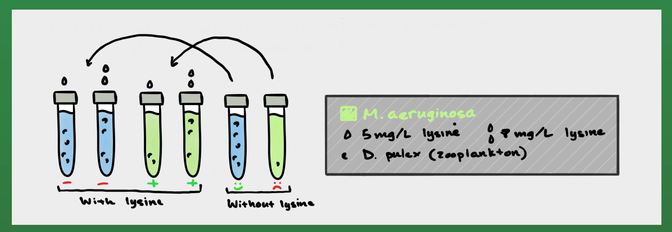




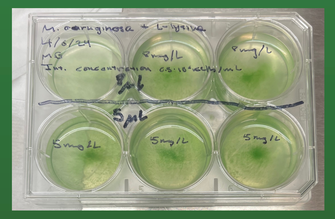
While the zooplankton only had a 6.67% survival rate in the presence of *M. aeruginosa*, the introduction of lysine significantly increased their chance of survival to 53% and 33%, with the 8mg/L lysine having a greater effect than the 5 mg/L lysine concentration. Furthermore, the average zooplankton population in the lysine-only test groups remained above half (2.5) of the original population, indicating the zooplankton would be able to grow in a real-world setting.

M. aeruginosa and Lysine





While *M. aeruginosa* on its own grew from a cell density of 5×10^5 cells/mL to 7.1×10^5 cells/mL, the introduction of 5 mg/L and 8 mg/L lysine killed *Microcystis* cells, causing it to drop to 2.9×10^5 cells/mL and 2.7×10^5 cells/mL, respectively, after 3 days.



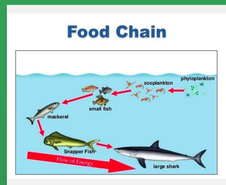
Methods

Procedure

D. pulex was randomly distributed into groups of 5 in 6-well plates. The zooplankton were then separated into 6 test groups: 5 mg/L lysine, 8 mg/L lysine, 5mg/L lysine and *M. aeruginosa*, 8 mg/L lysine and *M. aeruginosa*, just *M. aeruginosa*, and a control with no added substances. For test groups containing *M. aeruginosa,* 1 mL of a preexisting subculture was added to 9mL spring water. Additionally, a control groups containing just *D. pulex* and spring water was included.

For two days, the zooplankton in each test group would be counted and recorded. The number of deaths in each group after 48 hours would indicate the effects of lysine and *M. aeruginosa* on the zooplankton population.

Lastly, a test without *D. pulex* containing *M. aeruginosa* and lysine was included to confirm the effects of lysine documented in previous studies (Tian et al., 2018).



Food Web

D. pulex was chosen as a species to model the aquatic ecosystem because they are primary consumers, so any change in the zooplankton population will subsequently affect larger species.

Significance

The goal of this research project was to determine the sustainability of using L-lysine to control harmful *Microcystis* blooms. To measure these effects, zooplankton species *D. pulex* was used as a model species for the aquatic ecosystem. *M. aeruginosa* was used to better understand the dynamic between lysine and zooplankton in a bloom setting. The effects of lysine were recorded for both species separately, as well as together in order to mimic a harmful algae bloom. It was found that while lysine alone killed 33% of the *D. pulex* population, in the presence of *M. aeruginosa* it promoted the growth of *D. pulex* while killing *M. aeruginosa* cells, supporting the hypothesis. Furthermore, the results suggest that higher concentrations of lysine have a greater effect on promoting the *D. pulex* population, as higher concentrations will kill *M. aeruginosa* cells at a greater rate. It is important to note that this test is only one stride in the process of the determining the sustainability of L-lysine as a method to control *Microcystis* blooms, so further research, such as in-situ tests, should be conducted before implementing it in the real world. With global temperature and pollution rising, lysine may be a step in the right direction for keeping our water safe and usable.

References

Lewin, R. A., and Anderson, R. A. (2023). Algae. *Encyclopedia Britannica.* https://www.britannica.com/science/algae

Oxygen levels. (2022, March 8). Understanding Global Change. https://ugc.berkeley.edu/background-content/oxygenlevels/#:~:text=Photosynthesizing%20algae%20in%20the%20ocean,photosynthesizing%20bacteria%20that%20produce%20oxygen.

Tian, L., Meng, C., Chongyang, R., Yiying W., and Li L. Anticyanobacterial Effect of L-Lysine on Microcystis Aeruginosa. *RSC Advances 8,* no. 38 (n.d.): 21606–12. https://doi.org/10.1039/c8ra00434j.