The Potential for *Microcystis* Growth and Microcystin Exposure in the San Francisco Bay Estuary

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ABSTRACT

Microcystis is a genus of freshwater cyanobacteria responsible for causing harmful algal blooms (HABs). HAB's are typically caused by eutrophication from sewage contamination and agricultural runoff into bodies of water. *Microcystis* and some other species of cyanobacteria produce microcystin, a potent liver toxin, which reaches dangerously high concentrations in bodies of water during HAB's. Microcystins can upset diatom diversity leading to upsets in trophic dynamics, and can harm/kill several species of fish, shellfish, and mammals to include humans. It is common to see signs warning of toxic algae in the water at East Bay Regional Parks and many lakes close their swimming beaches annually as a result of HABs. Despite *Microcystis* being a primarily freshwater genus, researchers in the past decade have detected microcystins in the brackwater areas of the San Francisco Bay estuary and have determined microcystins to be the cause of death of some marine fish and mammals. Several salt tolerance experiments conducted on *Microcystis* in different areas of the world have had varying results based on region, and therefore there was no distinct salinity tolerance of the local strain of *Microcystis* to the East Bay. To address this gap I conducted a salt shock laboratory experiment with *Microcystis* collected from East Bay Regional Parks, and found the upper salt tolerance for survival and colony formation to be between 16 & 18 PSU. I then used salinity and flow data from the EPA, USGS, and my own measurements to map the salinity gradient of the San Francisco Bay area. By identifying regions of low salinity in the bay, I was able to predict where *Microcystis* blooms are likely to occur.

KEYWORDS

Cyanobacteria, Harmful Algae Blooms, Estuary, San Francisco Bay, Runoff, Microcystins

INTRODUCTION

Harmful algal blooms are a recurring environmental health issue in warm, slow moving bodies of water, often caused by eutrophication from sewer contamination and fertilizer runoff in agricultural areas (Carpenter 2005). The majority of HAB's that occur in freshwater are made up of the blue-green algae, Cyanobacteria, which produce biotoxins called microcystins that are harmful to fish, animals, and humans (Preece et al. 2017, Lehman et al. 2010). HAB's can occur rapidly and with little warning which makes them a deadly threat that park rangers and environmentalists must actively monitor. They are a significant issue in the San Francisco bay area particularly because of the high level of pollution, which triggers blooms in freshwater bodies and estuaries (Preece et al. 2017). East Bay Regional Parks has posted signs at many nearby lakes warning visitors not to enter the water because toxic algae may be present.

In the past two decades scientists have found evidence that microcystins can be present in the ocean near sources of freshwater runoff. These toxins, produced by the cyanobacteria genus *Microcystis*, can bioaccumulate in shellfish, and cause harm to marine fish and mammals. It has even been the proven cause of fish and sea otter deaths (Miller et al. 2010, Preece et al. 2017). Many scientists find this evidence intriguing because *Microcystis* is primarily known as a freshwater species (Tonk et al. 2007). Some hypothesize that the presence of microcystins in seawater may be due to *Microcystis* surviving and growing in the brackwater near runoff sources in the bay ("Identifying the Source and Taxa That are Producing Microcystins Detected in San Francisco Bay | U.S. Geological Survey" n.d., Miller et al. 2010, Preece et al. 2017).

Salt tolerance experiments have previously been conducted with *Microcystis* aeruginosa but for unknown reasons they have posed different results showing growth tolerance at a wide range of practical salinity units (PSU = grams NaCl per liter water). Results vary from 1 PSU slowing growth to it being unaffected up to 10 PSU (Georges des aulnois et al. 2020, Sellner et al. 1988, Tonk et al. 2007). This is an issue because it makes it difficult to predict where *Microcystis* might survive in the bay without a definitive gauge of what salinity it can tolerate. Therefore it is imperative that a locally derived sample of *Microcystis* be tested for salinity tolerance in order determine how the growth curve of *Microcystis* might vary in different areas of the bay. Using this data will help us predict which areas are potentially at high risk based on their lower salinity. Some possible areas are Suisun and San Pablo bay as they are fed directly from the Sacramento and San Joaquin rivers which are the main sources of freshwater runoff into the

bay.

METHODS

Study Area

I conducted this study in the upper San Francisco Bay estuary. Cyanobacteria samples used were taken from Lake Del Valle and Lake Chabot with permission of East Bay Regional Parks.

Salt-Shock of *Microcystis*

To determine the survivable salinity range of local *Microcystis*, I conducted a laboratory salt-shock experiment. According to varying previous studies growth will cease between 8-14 PSU for a freshwater strain (Tonk et al. 2007, Georges del Aulnois 2020, Reed and Walsby 1985). However, these studies were conducted using variable techniques and with taxa from different sources which lead to a wide range of results that are not precise enough for this study. For my salt shock experiment, I isolated *Microcystis* colonies from water samples taken from Lake Chabot and Lake Del Valle, both local freshwater sources in the east bay. These isolated colonies were grown in two separate erlenmeyer flasks containing BG11 broth, capped with tin foil and placed in sunlight at 20C for optimal growth (Rippka et al. 1978) and grown for a month to maintain a stock culture.

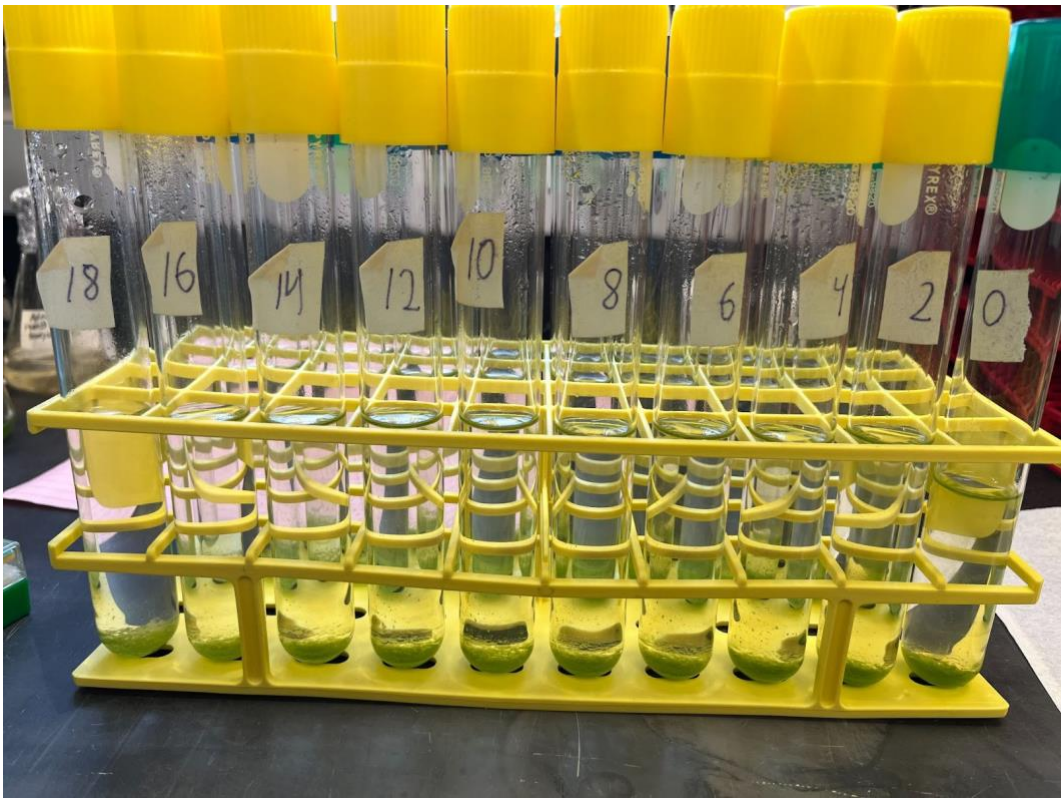

Figure 1. Experimental Setup. Triplicate test tubes containing 33mL BG-11 medium, 2mL *Microcystis* culture grown on BG-ll, and NaCl as required to make concentrations of 0-18 g/L.

For the salt shock, I prepared ten triplicate test tubes with 33 mL BG-11 broth each and added NaCl to create a brackwater medium in the tubes which would simulate nutrient rich water in the bay. The salt concentration in the triplicates ranged 0-18 PSU in increments of two (fig 1). Using sterile technique I added 2mL of the *Microcystis* culture to each test tube. Over a 7 day period I observed growth visually and vortexed the tubes on days 3 and 5 to simulate a displacement event such as a flood washing the bacteria into the bay. On day 7 took photographs of each of the bacteria in salinity level at under 400x magnification and took observations of cell size, colony formation, mucus presence, and cell replication.

Mapping Salinity

In order to map the salinity gradient of the northern area of the San Francisco bay estuary I synthesized water flow and salinity data collected by the EPA and USGS and tested current conditions myself. The most recent available salinity and flow monitoring report from the EPA and USGS provides baseline data for approximating salinity in different regions of the bay and

the steepness of the gradient, but this data is now over five years old. Based on this data I predicted a low of 0 PSU near the mouth of the San Joaquin river, and a high of >20 PSU off of Albany Beach (Hencks and Burau 2017). To gather more recent data for mapping, I measured salinity using a hydrometer in ten notable areas from the San Joaquin River into the central bay. The sites were Antioch Bridge, Bay point regional shoreline, the Martinez marina, the California Maritime Academy, Point Pinole regional shoreline, Miller/Knox regional shoreline, Albany Beach, the Berkeley marina, Alameda Beach, and Alameda Creek Vista Point. All data points were plotted on a map of the San Francisco bay estuary and different regions were shaded in by color code to show their salinity range.

Predicting Future Blooms

In order to determine which areas of the San Francisco bay estuary are most susceptible to *Microcystis* blooms and contamination I used my salinity maps and salt shock results to create a caution zone map of the bay. Based on observed salinity tolerance of *Microcystis* I used the shaded zones of the salinity map to estimate which areas *Microcystis* could survive in stagnant water based on the local salinity. The regions were coded by risk level, for instance if the samples thrived at 2 PSU and an area was shaded as 1-2 PSU, that area was identified as high risk. This map provides a comprehensive predictor of susceptible areas in the upper bay where fish, animals, and humans could be at risk for exposure to microcystins in the Summer season. Docks, boat ramps, and slow moving pools in high risk areas are important areas to monitor for blooms in the near future.

RESULTS

Cell Behavior and Growth

After 7 days of growth I took slide images at 400x magnification of the control and all the salt water cultures and compared cell size, colony formation, mucus presence, and active cell divisions (Figure 2). The control culture grew rapidly, forming a large colony adhering to the bottom and sides of the tube and forming a thick surface layer. Under magnification it had tightly packed colonies with high cohesion, rapid cell divisions, no observable dead cells, and little

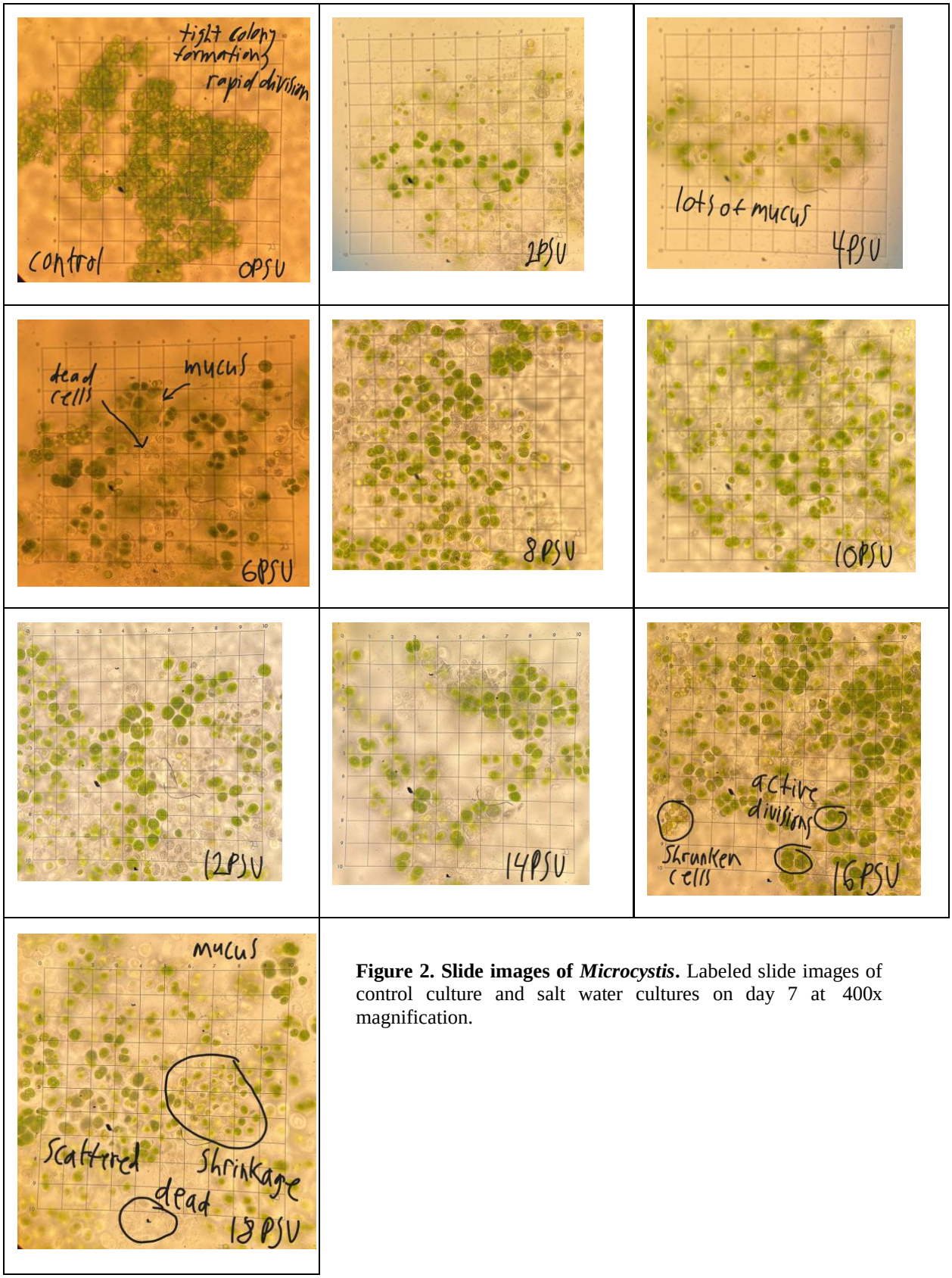

Figure 2. Slide images of *Microcystis*. Labeled slide images of control culture and salt water cultures on day 7 at 400x magnification.

mucus. The cultures in 2-14 PSU generally showed similar characteristics with colonies dispersed throughout the test tube and thin surface layers. Under magnification cells were less tightly packed, enveloped in mucus, and had active cell divisions. Notably, there were shriveled and dead cells along the edges of the colonies. At 16 PSU few colonies were suspended in the medium, a small surface layer was visible, and the colonies on the bottom had turned a lighter shade of green. Under magnification there were several clumps of shriveled cells on the edges of the mucus and the only living cells were those still tightly packed and surrounded by mucus. Amongst the living cells there were multiple active divisions. At 18 PSU all colonies were settled in the bottom of the test tube, there was no surface layer, and the colonies were a very light green. Under magnification cells were sparsely scattered with about 50% dead or shrunken, no mucus, and very few active cell divisions.

Mapping Salinity

Salinity readings in PSU were as follows: Antioch Bridge- 0.10, Bay point regional shoreline- 0.20, Martinez marina- 0.37, the California Maritime Academy- 2.0, Point Pinole regional shoreline- 8.1, Miller/Knox regional shoreline- 12.0, Albany Beach- 13.0, the Berkeley marina-13.0, Alameda Beach- 20.5, and Alameda Creek Vista Point- 14.0.

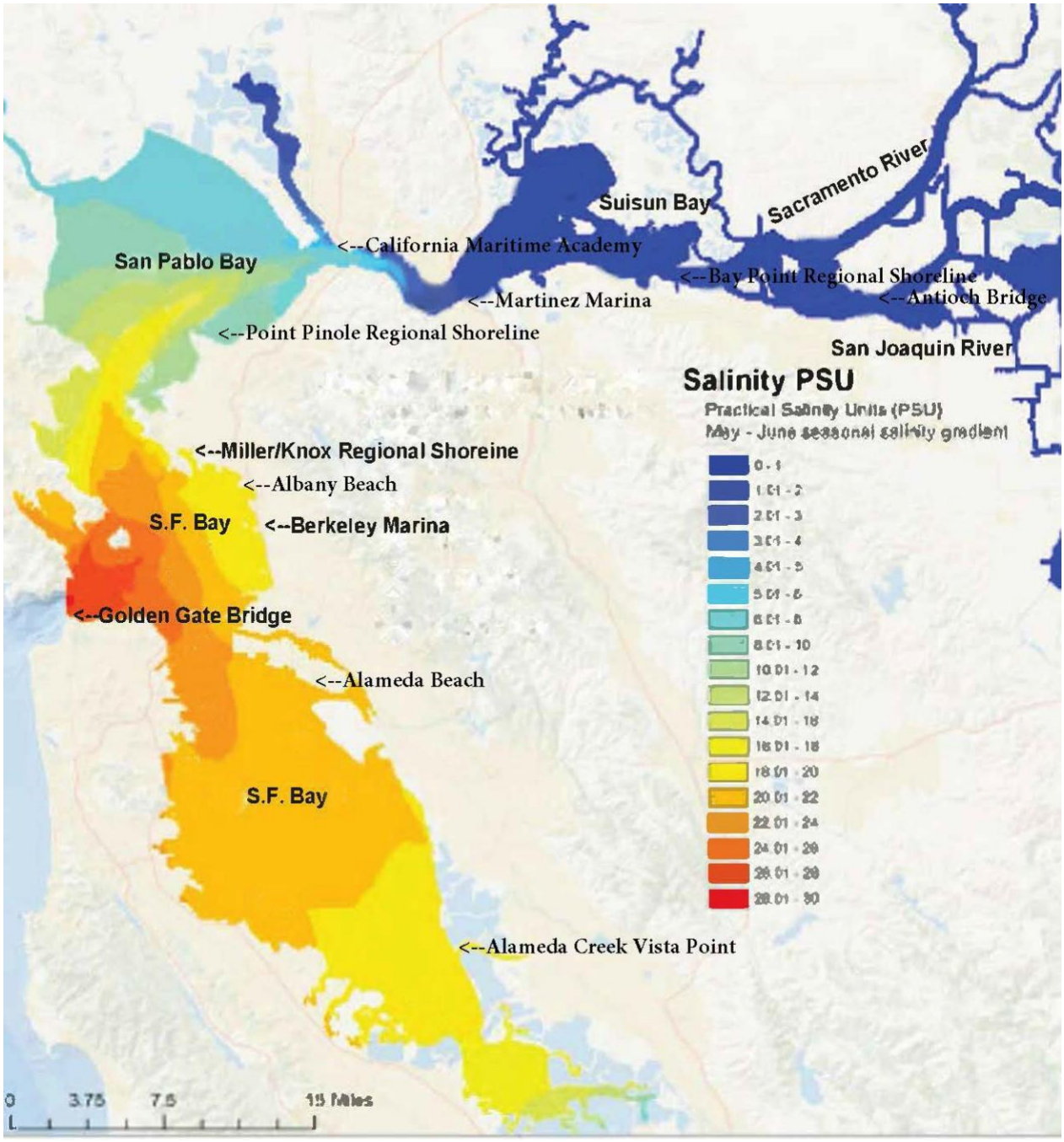

Figure 2. Map of bay area salinity gradient. Salinity data compiled by USGS during the early Summer season from previous years. The ten points where I measured salinity in April 2023 are labeled.

DISCUSSION

I determined the upper salt tolerance of this local strain of *Microcystis* is between 16 & 18 PSU based on the differences in colony growth and cell behavior between tubes 14, 16, and 18. This means that *Microcystis* deposited into the bay through freshwater runoff, given the correct nutrient conditions, could form blooms and release microcystins in stagnant areas of water in marinas, near boat docks and ramps, along shorelines/coves, and in tidal marshes where the brackwater salt concentration is below 16 PSU. Factors which could increase risk of blooms are events such as heavy rains and heavy snow melt which would increase the rate of freshwater outflow into the bay and cause eutrophication events from sewage spillover and fertilizer runoff.

Microcystis Response to Salt Shock

Immediately with the addition of NaCl the colonies began forming differently in the test tubes and decreased in growth rate overall, however the only samples observed with no growth or surface colonies at all were the 18PSU triplicate. In the slide images (Figure 1) we can see that the cells in all the saltwater media are dispersed in a type of extracellular mucus that envelops each colony. Based on the cells being smaller and shriveled on the edges, this mucus appears to have been formed by the cells as a protective response to the salt, and likely contains dead cells and extracellular microcystin toxins (Tonk et al. 2007). The shrunken cells indicate an inability to maintain turgor pressure at high salinities which explains the clumps of shrunken cells in samples 16 and 18 (Reed and Walsby 1985). In sample 18 over half the cells were shrunken, there were little to no cell divisions, and no colonies grew on the surface or sides of the test tube; meaning that *Microcystis* has an upper salinity tolerance for survival between 16 & 18 PSU.

2023 Spring-Summer Salinity Gradient in the Bay

Given the abnormally heavy rainfall and snowfall in Northern California this year (“Precipitation Piles on in California” 2023), which increased freshwater runoff into the bay, I predicted there would be a lower salinity for the 2023 Summer season than in previous years.

This prediction was correct because when comparing my readings to previous data I found that the salinity at my 10 shoreline locations this April were on average ~2g/L below their historical salinity (Anchor QEA, LLC et al. 2016, Hencks and Burau 2017).

Expanded High Risk Areas

Given that the salt shock experiment I conducted suggests the upper salt tolerance of *Microcystis* to be between 16-18 PSU, and the lower salinity in the bay, I predict that several areas of the bay will be susceptible to *Microcystis* blooms this year where previously they were not. Notable areas of concern are shorelines, marinas, docks, boat ramps, and tidal marshes in the East Bay from Point Pinole Regional shoreline to the Berkeley Marina and essentially all brackwater areas where the salinity falls below 16 PSU. The areas around freshwater deposits into the bay such as Alameda Creek vista point, are at particularly high risk (Miller et al. 2010).

Another risk factor accompanying the heavy precipitation this year is eutrophication due to an excess nutrient runoff from ground soils. This nutrient runoff comes in the form of pollutants, fecal matter, fertilizers, and eroded soil minerals, which are high in nitrogen and phosphorus. Over enrichment of nutrients is the primary cause of harmful algal blooms in aquatic ecosystems (Aguilera et al. 2019, Carpenter 2005). A eutrophication event in addition to lower than normal salinity in the bay could lead to harmful *Microcystis* blooms throughout the East bay resulting in a dangerous accumulation of microcystin toxins in the water.

Limitations and Future Directions

This study primarily addressed the relationship between *Microcystis* and salt concentration in the San Francisco Bay estuary. My research with this local strain of *Microcystis* is only applicable to the bay because *Microcystis* strains in other regions of the world have been shown to have varying salt tolerance (Tonk et al. 2007). There are also several other species of cyanobacteria in the East Bay that produce microcystins and could potentially cause blooms in and around the bay if they exhibit some level of salt tolerance. *Microcystis* competition with other cyanobacteria is a factor I did not consider. Finally, more specific growth data could be

taken with samples grown at multiple salinities between 16 & 18 to determine a more specific upper tolerance.

Important future research that could help us understand future blooms is a study on *Microcystis* growth in different nutrient environments. Understanding what types of pollutants cause blooms would help us better understand what specific events might trigger blooms in certain areas of the bay. I would also research the impact of cyanobacteria blooms on trophic cascades and the food web. Some useful research has already been done on the impact of Microcystins at the diatomic level (Lehman et al. 2010). Looking into broader trophic dynamics and bioaccumulation of microcystins in the food chain is important to understanding the broader ecological repercussions of the estuarine blooms we may face in the near future.

Conclusions

Microcystis growth and microcystin accumulation are very real imminent threats to the people and wildlife of the bay and the Summer to Fall of 2023 will likely see blooms in new locations throughout the East bay. If an excess of nutrients is or has been deposited into an area of stagnant water and the salinity is below 16 PSU, *Microcystis* could very well thrive. These results offer a strong explanation for the previous detection of microcystins in the bay where researchers previously were unsure of the primary source ("Identifying the Source and Taxa That are Producing Microcystins Detected in San Francisco Bay | U.S. Geological Survey" n.d., Miller et al. 2010, Preece et al. 2017). Areas of stagnant water along the East bay shoreline should be monitored for *Microcystis* growth and if colonies are observed, then the water should be tested for microcystins. Fish deaths and shifts in diatom diversity in these areas would also be prominent bioindicators of microcystins in the water (Lehman et al. 2010).

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