Synthesis 1 Peer Review

I have interpreted the synthesis to be about the cyanobacteria *Microcystis aeruginosa* and its toxic effects on the environment that have been exacerbated by human factors. The synthesis underscores the research that has been conducted on *M. aeruginosa* which demonstrates the evolutionary changes that have occurred in the cyanobacteria due to human pollution. In addition, the synthesis touches on the effects that the environment has had on the cyanobacteria and the subsequent effect the cyanobacteria has had on biotic factors in its environment. Finally, the synthesis emphasizes that understanding this cyanobacteria is an important step in alleviating the overproduction of *M. aeruginosa* toxin that threatens human health.

Overall, I think you have an interesting topic and a lot of good ideas in your paper. You appear to have strong evidence to back up your claims and use a variety of evidence to prove your different points. In general, your synthesis flows well and has a clear message. My main suggestion would be to clarify what "trait" you are studying. A few times, I was confused as your paper seems to talk about toxin production, then growth rate, then toxin production again. I would recommend clarifying what trait is affected by the environment and how it affects the environment. I think that with this clarification, your paper will seem more cohesive and flow even better.

With regards to writing, I believe that your main ideas are in the synthesis, but some areas could benefit from some adjustments. The writing in your introduction (the first two paragraphs) is excellent; the writing really drew my interest and was clear to understand. The flow made sense and made me want to learn more about the cyanobacteria's toxic effect. I think the third paragraph has important points to your argument, however I felt a little bogged down by all of the experimental details. As a reader with little knowledge on the subject, I was confused by the details in the parenthesis, as I did not understand the acronyms ("TN", "TP") and the significance of the different percentages. From my perspective, it may be beneficial to summarize the objective of the research, the variables (ex. "…both temperature and nutrient levels were varied in different conditions"), and the end results. The paragraph would still benefit from strong scientific evidence but would also be easy for the casual reader to understand. Similar to paragraph three, I believe that paragraph four would benefit from removing some of the details and simply summarizing and emphasizing the overall findings of the research. In general, I would say that numbers are not crucial to understanding how these studies back up your argument. Rather, you could reference comparative amounts (ex. "minimal nutrient" vs "medium saturated with nutrient") to emphasize the effect of different nutrient levels on *M. aeruginosa* growth.

With regards to your wording and structure, I found that some portions of your paper flowed well but a few other sections could benefit from stronger transitions or more explanatory topic sentences. For example, I think in paragraphs one, two, four, six, and seven you do a great job in setting up what is going to be talked about in the paragraphs. However, in the other paragraphs I was slightly confused by the topic sentences which, in turn, confused me on the substance of the paragraph. However, I think this would be a minor fix.

Another area to consider is to be more careful about some of your wording. I would prioritize working on this, as while many times throughout the paper you were clear on what you wanted to talk about, sometimes I was still not clear on the "trait" you were referencing. Overall, I think your paper does a good job of talking about the effects of *M. aeruginosa* on the environment. However, when referring back to the synthesis instructions, the guidelines focus on how a certain "trait" affects the environment or is affected by the environment itself. I initially read your paper and believed that the trait was toxin production. However, much of the paper then focused on the growth rate and I was unclear what the connection was between growth rate and toxin production. My interpretation was that as humans are polluting the lake, the pollution increases the growth rate of the cyanobacteria. Due to the sheer increase in numbers of cyanobacetria, more toxin is produced. However, if this is the case, the environment is not necessarily affecting the trait of *toxin production*. Rather, the increase in toxin production is an indirect effect of more cyanobacteria present in the lake. The only way I could see around this is if, perhaps, your trait is the specific *metabolism* of the cyanobacteria. It would then make more sense that pollution would affect the metabolism and cause mutations for metabolism (and subsequently growth) to increase and, as toxin is a production of the metabolism, toxin levels increase as well. The effect of an altered metabolism on the environment would still be that the metabolism products (toxins) are present in greater quantities and causing mutations. By emphasizing one trait, I think your paper will have better cohesion. The reason I say metabolism, and not "growth rate" being affected, is that I am not sure if "growth rate" is a trait. But I may be wrong.

With regards to the scientific engagement of your synthesis, I think you did a really good job picking out sources to back up your claims. Your detailed explanations of the experiments clearly demonstrate that the studies are relevant and have important implications for your argument. As mentioned before, you may find that cutting down on some of the experimental details and conditions will make your paper a little clearer and focused on the main ideas. I think you do a great job in explaining how the abiotic conditions of the environment have affected the evolution of *M. aeruginosa*. I suggest that you mirror this depth when you talk about the effect that *M. aeruginosa* has on the environment. For example, it is very clear that the toxins have detrimental effects on the fish species living in the lake. However, is there any evidence of the toxins causing evolutionary changes in these biotic factors? If not, that may be an interesting question for future pursuit. In addition, have scientists directly proven that it is the pollutants that increase nutrient level which increase cyanobacteria growth? An alternative hypothesis to explain the observed patterns of increased toxicity could be that the pollutants had other nutrients that affected the cyanobacteria's metabolism (and subsequently increased toxin output) and it wasn't necessarily just an increase in nutrients. Your conclusion about larger implications and further questions was clear and backed up by sufficient scientific research.

To reiterate, you have a very good start to your paper. I would just recommend cleaning up some of the details and making it explicit to your reader what "trait" you are focusing on.

Paper:

Microcystis aeruginosa is a single cell cyanobacteria that are able to perform photosynthesis. These bacteria are about 4-6 µm in size and often form colonies that are moist and sticky. This cyanobacteria reproduces asexually and has a genome consisting of 6312 protein-encoding genes. These organisms are found throughout the surface of fresh water environments. However in recent years this cyanobacteria has been causing massive problems in fresh water aquatic environments (*Microcystis Aeruginosa*).

In the last two decades Lake Taihu in Jiangsu Providence, China has suffered a massive cyanobacteria bloom. Although cyanobacteria may provide oxygen through photosynthesis, those that reside in Lake Taihu also produce toxic chemical microcytin that act as a hepatotoxin in vertebrates. Microcystis aeruginosa is the cyanobacteria that is responsible for the contamination of drinking water in Lake Taihu and it has been the center of attention for many researchers in China and around the world (Stone, 2011).

One reason the massive outbreak of M. aeruginosa in Lake Taihu has been attributed to the rising temperature and massive pollution of the lake. However the exact role of these two interaction is limited. Deng et al. have investigated how eutrophication and temperature change affects the growth of M. aeruginosa in laboratory setting. Three nutrient levels, which consisted of KNO_3 and K_2HPO_4 at certain concentration (Low:TN~2mg/L and TP~0.06mg/L, Medium: TN~7mg and TP~0.3mg/L, High: TN~10mg/L and TP~1mg), and six temperature levels (unheated, 12 °C, 14 °C, 16 °C, 18 °C and 20 °C) were used to grow the cyanobacteria. Deng et al. measured the amount of cyanobacteria in each set condition for 12 days and found that high temperature of 20 °C and high nutrition level yielded the highest M. aeruginosa concentration. Temperature shift alone yielded little to no increase in cyanobacteria concentration however the researchers have concluded that the growth of these organism was more sensitive to the nutrient level. These findings have showed that the increase dumping of pollutant such as fertilizers and human waste has caused eutrophication of the lake and thus Deng et al. concludes that rise in both nitrogen and phosphorous in the lake has caused the intensity of cyanobacteria blooms (Deng et al.,2014).

From the above experiment it becomes clear that humans have been causing the shift in ecosystem in which these cyanobacteria resides. Rouco et al. investigated the traits that was ultimately affected by this change in ecosystem (Rouco et al., 2011). Growth rate was one of the trait that Rouco et al. looked at and conducted laboratory experiments in order to understand the effects of adaptation, chance and history on evolutionary change of the growth rate. Culture of three strains of M. aeruginosa was cultivated in BG-11 medium for the duration of one year. The growth rate was measured every four months. Rouco et al. discovered that the growth rate of all three strain increased during the first 8 months. From the data that was collected the researchers have concluded from statistical inference that the mutations that increased growth rate were selected and mutations that decrease growth rate were eliminated. Thus adaptation and to a lesser degree chance contributed to the evolution of growth rate of cyanobacteria in a nutrient rich environment.

Growth rate is a key evolutionary change that was caused in the increased level of nutrient however the effect of these high nutrient in the molecular level of evolution is still unknown. Steffen et al. tackled this question by investigating the global changes in transcriptional pattern of M. aeruginosa in various level of nitrogen and phosphorous concentration response (Steffen et al., 2014). M. aeruginosa were grown in one standard CT medium which consists of 1.67mM of nitrate and 164µM of phosphate. Five different nutrient treatment were also used to culture the organism. RNA samples were collected once these

Comment [1]: Italicize

Comment [2]: confusing wording of this sentence

Comment [3]: Not sure what these stand for, seems a bit superfluous if you don't explain the significance of these letters/numbers

Comment [4]: Wording a bit unclear…

Comment [5]: Tense continuity

Comment [6]: How is it adaptive? Is there some sort of advantage they get from growing faster or are the mutations a consequence of the environment (make clear)

Comment [7]: Not sure if you need to spell this out

organism reached maximum growth rate and was sequenced. From the sequence result urea treated cells resulted in the significant up-regulation of transcription of ureA-B, MAE_14800, nirA, ntcA and glnB which are all involved in nitrogen metabolism. Due to the increased use of urea in fertilizers Steffen et al. stated that these dumping of fertilizers into the water where M. aeruginosa reside may have caused an evolutionary change in M. aeruginosa growth, however further research needs to be conducted in order to fully answer the effect of up-regulation of these genes in organisms evolution.

The evidence above shows that growth rate of M. aeruginosa have been greatly affected by the abiotic change in the ecosystem. However it is clear that this evolutionary change of cyanobacteria have also caused a deadly effect on the ecosystem through biotic interaction. From preliminary data microcystins are a hepatotoxin and also induce liver tumors. Jia et al. investigated the role of microcystins in local fish that reside in Lake Taihu that are commonly consumed by humans (Jia et al., 2014). Four fish species: silver carp, bighead carp, crucian carp and common carp were collected from the lake. Microcystins were extracted from various organs of the fish and was determined that the toxin was throughout each fish's muscles, kidneys, heart, intestinal walls, and liver. It was discovered that smaller fish accumulated higher levels of the toxin in their organs, and potentially led to the decrease in biomass of large predators (Yunkai et al., 2010). All of these fish had microcystin concentration more than one fold higher in their muscles which humans consume. The amount of microcystin concentration is above the tolerable level set by WHO.

M. aeruginosa possess a serious threat to the health of human beings. The massive outbreak in Lake Taihu caused some researchers to look at ways to reduce the population of cyanobacteria other than reducing the pollutant dumped into the lake. Sankar et al. have investigated the effect of copper oxide nanoparticles in M. aeruginosa and found a growth inhibitory effect on these organisms (Sankar et al., 2014). It only took 48 hours to incubate the organism in the nanoparticle for it to take effect. Decreased content of chlorophyll a, b, and carotenoid were observed. Copper oxide nanoparticles may be a good growth repressor however further field testing needs to be conducted. Lirong et al. on the other hand investigated the effect novel wattle extract on cyanobacteria growth in both indoor and outdoor experiments (Lirong et al., 2010). In the outdoor experiment a sample of pond water in Kofu, Shanli, Japan was used. Wattle extract inhibited growth on M. aeruginosa in both experiments and the researchers have concluded that wattle extract is both effective and safer for the environment.

Comment [8]: Unsure what this is

Both of these solutions may well inhibit the growth of M. aeruginosa however the overall effect of copper oxide nanoparticles and wattle extract on the whole ecosystem of the lake needs further analysis. Because of the human pollution and overall warming of the environment, Lake Taihu's M. aeruginosa population sky rocketed. In turn the harmful effect of microcytin greatly affects the species within the lake and ultimately affect humans who consume fish that are high in toxin concentration. The detrimental rate of growth of M. aeruginosa in Lake Taihu surely served as a wake-up call to all those that are depended on their local fresh water supply.

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