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BACTERIAL DIVERSITY OF GEORGIA LAKES

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Biotech Research

Background

Lakes are freshwater ecosystems known to be home to various types of bacteria, which play an important role in lake ecosystem processes like nutrient cycling and bioremediation. A lake's level of bacterial diversity can be used to measure its health and stability, with greater diversity indicating greater stability. Each lake included in this study is affected by a different form of pollution: Allatoona by wastewater discharge, Lanier by sewage discharge, and Sinclair by coal pollution. Pollution can have an impact on the bacterial diversity of lake ecosystems. For example, sewage and wastewater discharge have been shown to decrease bacterial diversity. While coal pollution has not been shown to increase bacterial diversity, the large amount of nutrients it releases into the environment may decrease bacterial diversity by favoring bacteria that thrive in extreme conditions. Additionally, lake pollution can pose a public health threat. Sewage discharge has been shown to favor the growth of pathogenic bacteria, which can infect people who choose to swim in the lake. There is an ample gap in the research in studying the bacterial diversity of Georgia's lakes. Only one study has been conducted in Georgia, however this study did not include Lakes Allatoona, Lanier and Sinclair. This project aims to compare the bacterial diversity of these lakes, which could be used as a baseline to determine relative ecosystem stability of them. This could then indicate the possible need for remediation efforts to improve the lake ecosystem's health and stability.

Research Question

How do Georgia Lakes, impacted by different types of pollution, differ in terms of bacterial diversity and richness?

Hypothesis

We hypothesize that the sample lakes will rank in most to least relative biodiversity in the following order: Allatoona, Lanier, Sinclair.

1) Allatoona will have the highest relative biodiversity because wastewater has no significant effect on bacterial diversity of freshwater ecosystems.
2) Lanier will have the second-highest relative biodiversity due to the reduction of bacterial species richness and diversity that is caused by sewage discharge.
3) Sinclair will have lowest relative biodiversity because of the impacts of sewage discharge into the lake not being extreme and bring bacterial diversity towards a negative way.

Methods

Water Collection:

- Two bottles were collected from each lake.
- Using a theremometer, temperature is taken and recorded.
- Afterwards, the sterile, autoclaved, 1.5 L bottles are taken out. The cap is taken off, the foil is peeled off the mouth of the bottle.

Water Filtration:

- Two separate filtration processes were done for Ecoplate and Nanopore. Six samples were collected
- Vaccum apparatus was used with a large flask to collect the water with the filter funnal placed.
- We had a filter funnel where we placed the filter paper on top.2.5um filter was used for Ecoplate and the water was saved. 0.5 um filter was used for Nanopore and left for DNA extraction.

EcoPlate:

- 120 ul of each water sample was pipetted from the filtered lake water into each well of the plate. The plates were labelled and then incubated at 28 C for 120 hours maximum.
- After clear formation of purple color has occurred in all the wells of each plate, they are taken to the microplate reader. Absorbance is measured on the plates at 590 nm 24 to 120 hours after incubation.

DNA Extraction:

- The DNA Extraction process used the ZymoBIOMICS™ DNA Miniprep Kit.

Nanopore:

- The DNA that was extracted underwent sequencing with the MinION gene sequencer from Oxford Nanopore Technologies. We amplified the extracted DNA using polymerase chain reaction (PCR) with 16S primers to target only bacterial DNA to ensure the sequences from the PCR product consisting of solely bacterial genes.
- After PCR, the products were purified and captured the amplified DNA using magnetic AMPure XP beads in conjuction with 70% ethanol. A magnet was employed to separate the eluted DNA from the magnetic beads and was used to be analyzed on the Qubit for DNA concentration.
- The library was then introduced into the MiniION flow cell for sequencing.

Future Research

Future research should be focused on comparing a wider range of Georgia lakes. Samples collected from other lakes affected by the same types of pollution as Lakes Lanier, Allatoona, and Sinclair, should be collected to help draw conclusions about the correlation between pollution type and lake bacterial diversity. Additionally, new samples from the three lakes included in this study should be taken and sequenced again, with a focus on attempting to reduce the number of unknown sequences. This may be able to be done by changing the DNA extraction process to prevent DNA sequence breakdown.

Conclusion

This study was focused on Lake Lanier, Lake Allatoona, and Lake Sinclair - three of the major reservoirs in Georgia that were all impacted by different types of pollutants. While treated wastewater and sewage have varying effects on microbial communities, through our preliminary research it seems that coal by-products appear to have a more severe and lasting impact on bacterial diversity. All these changes don't only influence the stability of the aquatic ecosystems involved but also pose a major risk to public health with the recreational use of the lake water. With the limited research available on these specific lakes, especially within Georgia, this study aims to fill a critical gap by comparing microbial diversity across different pollution contexts. It is essential to make efforts to be able to preserve microbial balance as well as ensure long-term sustainability of freshwater ecosystem.

Acknowledgements

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Results

% of metabolite wells w/ >=2 wells showing growth

Metabolite Type	Lanier	Allatoona	Sinclair
Amines	100%	50%	100%
Amino Acids	50%	83%	100%
Carbohydrates	57%	100%	100%
Carboxylic acids	89%	78%	89%
Miscellaneous	33%	66%	33%
Polymers	50%	100%	100%

Figure 1: The percent of each metabolite group that had a positive result (two or more wells showed a clear color change) on the Eco Plate for each lake after incubating for 120 hours.. A color change indicates that the bacteria in the sample used the metabolite in that well. This data reveals what metabolites the bacteria in each lake sample preferred.

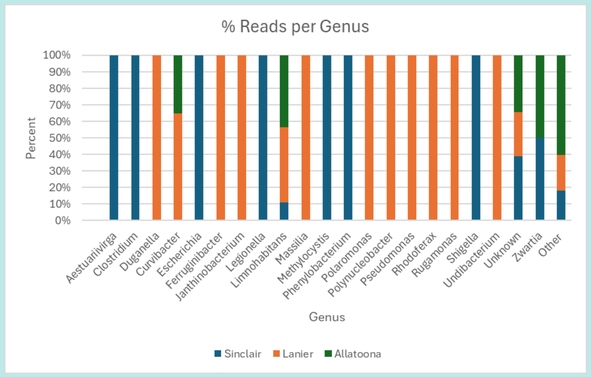

Figure 2: The percent of reads per genus that are from each lake sample. Percent reads is defined as the percentage of all reads for that sample that were a part of that genus. The genus "Other" includes all genera that had a % read of less than 1%.

Discussion

Eco Plate

- The Lake Sinclair Eco Plate showed utilization of 27 of 31 metabolites, and 100% utilization of polymer, carbohydrate, amino acid, and mine metabolites. This was able to show Lake Sinclair's microbial community is capable of breaking down a wide range of organic compounds.
- Allatoona also showed a 100% utilization of both carbohydrate and polymer metabolites, but had lower usage of other metabolites, which presents a likely difference in the microbial community makeup of Allatoona and Sinclair.
- Lake Lanier's lower usage of all metabolites compared to the other two lakes may be an indicator of reduced microbial diversity or lower nutrient ability within the lake's ecosystem.
- The Eco Plates suggest that Lake Sinclair is the most metabolically diverse lake of the three, followed by Allatoona, and finally by Lanier.

Nanopore Gene Sequencing

- Lake Sinclair showed the greatest taxonomic diversity after gene sequencing, which corroborates the results from the Eco Plate which suggested that Lake Sinclair showed the highest bacterial diversity based on metabolic activity.
- Allatoona and Lanier both had fewer unique genera identified than Lake Sinclair, indicating their relative lower diversity.
- Lake Lanier's high Shannon index (2.41) indicates that there is a more even distribution of the genera present in the lake.
- Sinclair's low richness index value (54) suggests that the microbial community may contain fewer but more specialized bacteria, resulting in a lower richness value.
- This supports the idea that coal pollution creates harsher environments by polluting the ecosystem with ions that may be detrimental to some bacteria.

the sequencing and metabolic data form a consistent result - Lake Sinclair is the most microbially diverse, followed by Allatoona and then Lanier does not align with our initial hypothesis of Sinclair having the lowest relative biodiversity as well as Allatoona having the highest relative biodiversity.