The Effects of Dissolved and Suspended Solids on Freshwater Meiofauna

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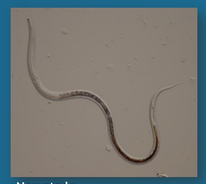

Nematoda


Rotifera, *Lecane* sp.


Arthropoda, Cladocera


Arthropoda, Copepoda


Tardigrada


Rotifera, *Brachionus* sp.

Abstract

Meiofauna include small-sized animals (< 1mm) distributed in all aquatic ecosystems on Earth, where they play fundamental trophic and ecological roles. The biodiversity of marine meiofauna and its links with anthropogenic activities is routinely investigated, however, freshwater communities are less known. This is particularly true for the Southeastern United States, which is greatly investigated and elected a hotspot of biodiversity for larger species but very little is known about the meiofauna. The purpose of this research is to reveal the biodiversity of meiofauna from the Tennessee River and test for potential correlations with anthropogenic activities. As a proxy for pollution, dissolved and suspended solids were considered in this study. The research hypothesis is that meiofaunal biodiversity would be affected by possible changes of dissolved and suspended solids in the water column. Possible mechanisms causing biodiversity shifts could be ascribed to osmotic stresses of animals to cope with variation in dissolved solids or, more indirectly, because different sunlight penetration caused by suspended solids would affect primary production.

To test the hypothesis, water samples were collected from nine stations located along the Tennessee River in Hamilton County. Each station was visited three times, and, during each visit, environmental parameters (including dissolved and suspended solids) were measured. Meiofauna biodiversity (estimated as richness, community composition, and phylogenetic diversity) was revealed using a metagenomic approach. Statistical analyses were applied to test for possible correlations between the biodiversity estimates and the measured environmental parameters.

Results show a high biodiversity of meiofauna with more than 200 amplicon-sequence variants distributed across 10 metazoan phyla. Environmental conditions are highly variable among stations and statistical analyses show that while both dissolved solids (TDS) and turbidity (suspended solids, NTU) did not significantly affect meiofauna biodiversity in the collected samples, various other water and sediment metrics were found to be significant predictors of meiofauna biodiversity.

In conclusion, the results of this project not only reveal for the first time the meiofauna biodiversity from the Tennessee River, but also suggest that meiofauna could be used as a bioindicator for several anthropogenic activities in freshwater ecosystems.

Introduction

Research Question: Do differing amounts of dissolved and suspended solids alter the meiofaunal biodiversity observed at sites along the Tennessee River?

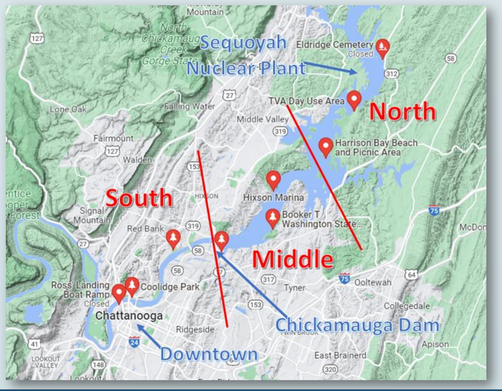
Hypothesis: As levels of dissolved and suspended solids change in the water column from sedimentation, the biodiversity of meiofaunal communities will change as well.

Reasoning for Hypothesis:

1. Variation of dissolved and suspended solids in the water may cause osmotic stress for meiofaunal animals
2. More indirectly, different sunlight penetration could be caused by suspended solids and would affect primary production, limiting a vital food source for many meiofaunal species

Project Design:

- Hamilton County, Tennessee was divided into 3 samplings areas (Bottom, Middle, and Top) and 3 sampling stations were selected in each area for a total of 9 stations.
- Each of these 9 stations was visited and sampled a total of 3 times from February to April of 2021.



Methodology

Sampling Activity

1. Collection of water quality data (Horiba U500 water quality monitor)
2. Collection of station images using a cell phone
3. Collection of water samples (via plankton net and filtering apparatus, pictured)
4. Collection of sediment samples (via benthic coring tool).

eDNA Metabarcoding

1. DNA was extracted from water filter samples using a Qiagen DNeasy PowerSoil ® kit with an added overnight digestion step with ProteinaseK enzymes.
2. Extracted eDNA was amplified by two rounds of PCR (the first to amplify the 18s gene in question, the second to attach the Illumia adapter IDs to the amplified strands).
3. PCR products were pooled and sent to the sequencer
4. Sequencing results were analyzed using Geneious Prime, Qiime2, and BASH coding to classify the DNA of the organisms present in each sample.

Granulometry

1. Sediment samples were dried
2. Dry sediment samples were added to the sieving apparatus, which allowed us to sort the sediment grains by size
3. The breakdown of grain size allowed us to use GRADISTAT software to statistically analyze each sample and label each with a sample type (i.e., poorly sorted, muddy sandy gravel)
4. Each of the sample components was then recombined and subjected to muriatic acid and washing steps. The muriatic acid was used to remove the inorganic carbon (carbonate) from the samples. After washing, samples were re-dried in an oven at 60-100 °C.
5. Sediment samples were then placed in a muffle oven at 475°C to burn off the remaining carbon. This allows us to determine what percentage of the entire sample was composed of organic materials.

Computational Analysis

1. Genetic sequences were obtained by a sequencing core facility after adapter removal and preliminary checks for quality control
2. Raw genetic reads were imported into the QIIME2 platform for analysis and further processing
3. The taxonomy of each sequence variant was determined by comparing the top five hits identified with BLAST against the SILVA 128 database and assigning them with the best consensus taxonomy (BLAST hits were only considered if the percent identity of the match fell within 0.5% identity of the top hit and if the alignment of the hit spans was >120 bp)
4. Unassigned sequences, (and chordates, protists, plants, fungi, larger invertebrates) were removed and excluded from subsequent analyses
5. Final dataset consisted of 203 sequence variants (ASVs) and a total of 10,790,074 genetic reads.

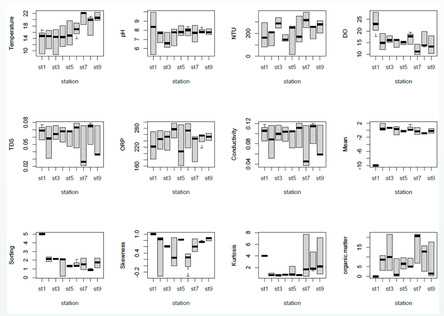
Statistical analysis

1. Tested if environmental parameters were different among stations, among each of the three visits, the combination of the two factors (station * visit), sampling area (Bottom, Middle, Top) using the analysis of variance (ANOVA)
2. Biodiversity was assessed as richness (# of observed unique amplicon sequence variants in each sample), community composition (distribution of ASVs among samples), and (iii) phylogenetic diversity (=ommunity composition considering the phylogenetic distance among features)
3. To test whether explanatory variables (environmental parameters) were significant predictors of community composition as well as UniFrac (response variables), we used a permutational multivariate analysis of variance (PERMANOVA) applied on distance matrices
4. We performed a Non-metric Multidimensional Scaling analysis (NMDS) to visualize dissimilarities among communities

Results

Environmental Parameters

- Most of the measured parameters showed **differences among stations and among the three areas.**
- Differences among the three visits and the interaction between station/area and visit did not show significant differences, corroborating the hypothesis that **stations and areas (bottom, middle, top) are characterized by unique abiotic conditions**, regardless of weather events.
- Abiotic parameters with the highest variability were **temperature, dissolved solids, and turbidity, followed by conductivity and oxidation potential**.
- ANOVA tests showed that differences in temperature are positively correlated to differences in NTU ($p < 0.01$) and oxidation potential ($p < 0.01$) and negatively correlated to variation in DO ($p<0.01$).

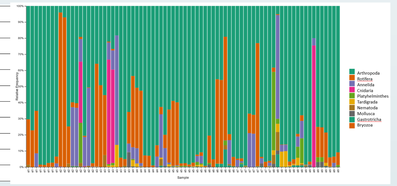


Biodiversity Estimates

Richness

- **10 phyla and 203 different meiofaunal ASVs** were recorded.
- Most common phylum found was **Arthopoda** in which 83 unique ASVs were documented.
- After Arthropods, organisms from phylum **Rotifera** (39 ASVs) and **Nematoda** (25 ASVs) were the second and third most abundant phyla.
- The rarest taxa documented was the **Bryozoan** phylum with only 1 representative ASV identified.
- Phyla were **well distributed across stations**, except for Cnidaria and Bryozoa which were less represented overall.

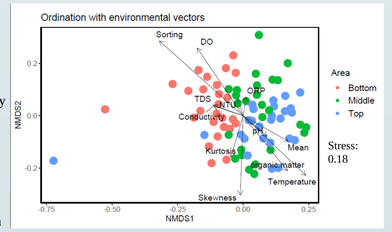
Phyla Detected	Number of ASVs within each phylum
Arthropoda	83
Rotifera	39
Nematoda	25
Platyhelminthes	16
Annelida	14
Gastrotricha	13
Tardigrada	7
Cnidaria	3
Mollusca	2
Bryozoa	1



- **Station 7** had the highest biodiversity and species richness as 120 of the 203 total ASVs were found there over the course of the sampling period.
- **Station 1** exhibited the lowest diversity values where only 76 out of 203 ASVs were documented.
- ANOVA statistical analysis revealed that variation in **temperature and oxidation potential** (ORP) are the two abiotic parameters that better explain variation in richness ($p=0.01$ and 0.02 respectively).

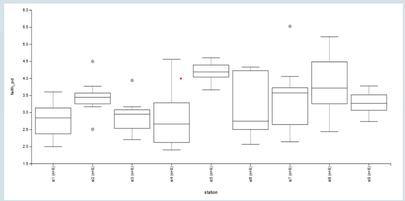
Community Composition

- PERMANOVA analyses revealed that community composition is significantly different among stations ($p<0.001$) and the three areas ($p<0.001$).
- The abiotic parameters that significantly explain variation in community composition were **temperature** ($p<0.001$) and **dissolved oxygen** ($p<0.001$) as well as **oxidation potential** ($p<0.05$), and **various sedimentological parameters**.
- The NMDS plot shows that variations in the **temperature and dissolved oxygen** have the greatest influence on community composition.
- **Total dissolved solids and conductivity** affect the community composition almost identically and have an **opposite effect of turbidity**



Phylogenetic Diversity

- Phylogenetic diversity is significantly different among some stations and between the bottom and both middle and top areas ($p<0.05$).
- Variation in **temperature** was the only abiotic parameter that significantly explained variation in the phylogenetic diversity ($p<0.05$).
- Phylogenetic diversity **did not seem to significantly change in relation to when stations were visited.**



Main Conclusions

1. This study was the **first assessment of meiofaunal biodiversity** from the Tennessee River

2. The biodiversity of freshwater meiofauna can be explained by variation in abiotic parameters, therefore, freshwater meiofauna can be used as a **valuable bioindicator for environmental changes** (especially temperature)

3. The **203 unique ASVs** found were certainly unanticipated given (i) the small sampling area (just over a 20 river-mile length), (ii) the high connectivity of the stations on the river, (iii) the seemingly homogenous habitat of this area of the river, and (iiii) the poor water quality and sedimentation conditions of the Tennessee River

4. This higher biodiversity supports the idea that the Southeastern United States is not only a **hotspot for freshwater biodiversity** for bigger species, but also for small-sized animals.

5. Environmental parameters differ across stations and areas (bottom, middle, top), but each station or area's **parameters remain fairly constant** regardless of when the sampling activity was performed; this result held true even for sampling trips that occurred soon after weather events and causing visible changes in the water level. However, biodiversity measured as **richness and community composition changes across stations and areas also depending on when the sampling activity was performed**.

6. We hypothesize that some of the **variation in the environmental parameters observed may be due to anthropogenic effects** in each area. Additionally, we find that the meiofaunal diversity is different among these three study sections with the Bottom portion containing some of the least diverse stations and the Top area holding the most diverse stations, suggesting that either the **level of urbanization, the thermal influence of the Sequoyah Nuclear Plant, or the habitat isolation created by the Chickamauga Dam may also influence meiofaunal biodiversity**. [More samples from other urbanized, riverine areas would be needed to more definitively support this hypothesis.]

7. We do not see any strong evidence to suggest that variation in the levels of dissolved (total dissolved solids) and suspended (turbidity) solids have any effect on the meiofaunal biodiversity. The final results of this study lead us to **reject this hypothesis as neither turbidity nor total dissolved solids statistically affected the meiofaunal biodiversity** measured as richness, community composition, and phylogenetic diversity, at least in our samples.

Going Forward

Future researchers should consider using a larger sample size, such as more sampling stations and site visits to ensure accurate results. It would also be beneficial to examine the planktic and benthic meiofaunal species together to get a clearer picture of their community as a whole. A study examining the effects of urbanization and specific land use on local meiofauna populations would further serve to utilize these animals as effective bioindicators of anthropogenic, environmental changes

Acknowledgements, Contact, and References

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References: Scan QR Code