Zooplankton and Flagellate Abundance in UBC's Nitobe Garden Pond

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Abstract

Zooplankton and flagellates play an important role in freshwater ecosystems, contributing as primary producers and grazers that form a significant part of the base of aquatic food webs. Their sensitivity to environmental changes makes them valuable bioindicators for assessing water quality and ecosystem health. This study examines their abundance in Nitobe Garden Pond, a freshwater system on the University of British Columbia campus. Findings are compared with a previous BIOL 342 study conducted in 2018 to identify potential ecological shifts over time. Abiotic factors, including temperature, dissolved oxygen (O_2), and pH, were measured at three distinct sites to assess environmental uniformity and its influence on plankton distribution. Zooplankton abundance was significantly higher than in 2018 ($p = 0.0001898$), while flagellate counts showed no statistical change ($p = 0.2681$). Elevated dissolved oxygen levels (9.96 mg/L compared to 7.8 mg/L) and a slight increase in water temperature (11°C compared to 10°C) may have contributed to the observed zooplankton growth.

Introduction

Freshwater ecosystems are dynamic environments where microorganisms, such as flagellates and zooplankton, play essential roles in maintaining ecological balance. They are highly contributory to nutrient cycling, energy transfer, and the overall stability of aquatic habitats. Their sensitivity to environmental changes makes them bioindicators for assessing water quality and ecosystem health. Abiotic factors– including temperature, dissolved oxygen, carbon dioxide, light intensity, and nutrient availability– strongly influence the abundance and diversity of flagellates and zooplankton.

Temperature and seasonal light variations, in particular, directly affect the growth and succession of plankton communities. For example, studies in glacial lakes have shown that habitat heterogeneity–including depth differences and nutrient gradients–supports greater diversity among zooplankton species (Spoljar et al., 2017). Similarly, Richardson et al. (2008) demonstrated the strong influence of temperature on phytoplankton growth and seasonal succession. In Lake Baikal, winter diatoms such as *Aulacoseira baicalensis* were most abundant at temperatures below 8°C, while summer cyanobacteria like *Synechocystis limnetica* thrived at temperatures above 8°C°. These findings highlight the role of temperature in shaping community composition and emphasize the importance of localized environmental conditions in determining plankton diversity.

In addition to abiotic factors, biotic interactions significantly impact plankton communities. Grazing pressures from zooplankton can regulate flagellate abundance, particularly in nutrient-poor environments dominated by larger species like *Daphnia*. Conversely, nutrient enrichment can stimulate flagellate growth when grazing pressure is reduced (Pomati et al., 2020). Such interactions reveal a complex interplay of abiotic and biotic factors that control plankton community dynamics.

This research investigates the abundance of zooplankton and flagellates to explore how localized environmental conditions, such as temperature and dissolved oxygen, have changed over time and influenced plankton communities. By comparing current data with previous studies, this work seeks to evaluate ecological shifts and their implications for the system's health.

Methodology

Nitobe Garden Pond, a freshwater system on the University of British Columbia campus, served as the study site due to its accessibility, manageable size, and diverse microhabitats. These characteristics made it an ideal location for examining ecological variability and comparing plankton community data from previous studies.

Three distinct sampling locations were chosen to represent a range of environmental conditions within the pond. The first site, situated along the pond's bank near an outcrop in the middle, featured shallow waters surrounded by vegetation and rocks. The second site, located beneath the bridge spanning the pond, had deeper waters with reduced sunlight due to the shading of the bridge. The third site, near a drainage collection area where water flowed toward a storm drain, was characterized by shallow waters, accumulated debris, and higher nutrient input from runoff.

Abiotic factors were recorded at each site before plankton sampling to capture and document the environmental conditions during collection. Air and water temperatures were measured using respective thermometers or probes. Dissolved oxygen (O_2) concentrations were measured with a dissolved oxygen meter, and carbon dioxide (CO_2) concentrations were measured using a CO_2 testing kit. pH levels were determined using pH strips. Additional factors, such as cloud cover and recent rainfall, were documented to account for potential influences on plankton abundance and distribution.

To ensure systematic and unbiased sampling, each site was divided into a 10x10 grid, with unique numbers assigned to each grid cell. Sampling points were selected using a random number generator to minimize bias. Samples were collected from the center of each chosen grid cell whenever feasible. Physical constraints–such as restricted access to certain areas and the inability to enter the water–occasionally required adjustments. In cases where physical adjustments were needed, the plankton net misaligned with grid boundaries or sank outside the grid, introducing minor variability to the data.

Plankton samples were collected using a 100-micron plankton net. Three replicate samples of approximately 25 mL were collected at each site and stored in mason jars. To preserve the samples for analysis, they were kept at 12°C in a cold storage room provided by the UBC Biology Department.

In the lab, plankton samples were analyzed using an Axiostar Plus dissecting microscope to identify and quantify organisms. Observations were conducted at two magnifications: 10x (with one ocular unit corresponding to 100 micrometers) and 40x (with one ocular unit corresponding to 25 micrometers). Using a micropipette, 15 μL of the sample was transferred onto a glass slide. Organisms were counted and classified using an identification tree (Appendix A) and random sampling methods. To ensure randomness in sampling, 15 snapshots were taken without prior visual selection, and organisms within these snapshots were identified and classified. The identification tree was used to categorize organisms based on observable characteristics such as size, body structure, and the presence of flagella. This approach provided consistent quantification of zooplankton and flagellate abundances and allowed us to try and minimize variability when comparing previous studies with limited data.

Results

Table 1 summarizes the abiotic measurements recorded at the three sampling sites, indicating minimal variability between locations. Air temperature remained consistent at 12°C and water temperature showed no variation., remaining steady at 11°C. Dissolved oxygen (O_2) concentrations ranged narrowly from 9.90 to 9.93 mg/L, while carbon dioxide (CO_2) levels exhibited slight differences, with the highest concentration (8 ppm) recorded at site 3. pH levels were stable across all sites, averaging 7.82. These findings suggest that Nitobe Garden Pond maintains relatively uniform environmental conditions across the sampled locations.

Site	Air temperature (℃)	Water temperature (℃)	O2 Concentration (mg/L)	CO2 Concentration (ppm)	pH
1	12	11	9.93	7	7.83
2	12	11	9.98	7	7.81
3	12	11	9.96	8	7.81
Avg.	12	11	9.96	7.3	7.82

Table 1. Measurement of air temperature, water temperature, O_2 concentration, CO_2 concentration, and pH. Data were collected from three sites at the Nitobe Garden Pond.

Zooplankton consistently outnumbered flagellates across all sampling sites (Table 2). A total of 400 plankton individuals were recorded, with zooplankton contributing 235 and flagellates 165. Zooplankton abundance exceeded flagellate abundance at most sites; only two out of nine samples recorded higher flagellate counts. Overall, Site 2 had the lowest total plankton abundance, followed by Site 1, while Site 3 exhibited the highest abundance (Appendix B). Within individual sites, two of the three replicates often showed similar zooplankton and flagellate counts, while the third sample tended to deviate. This variability may reflect localized environmental conditions or slight differences in sampling technique.

Site/Sample	Zooplankton Count	Flagellate Count	Total
S1 S1	35	29	64
S1 S2	19	22	41
S1 S3	34	21	55
S2 S1	23	17	40
S2 S2	16	8	24
S2 S3	7	15	22
S3 S1	40	15	55
S3 S2	19	17	36
S3 S3	42	21	63
Total	235	165	400

Table 2: Total abundance of zooplankton and flagellate across each site and sample. Samples consisted of 25mL of liquid and 25 µL of the sample was analyzed. Data was collected from the Nitobe Garden Pond.

The previous BIOL 342 report (Table 3) documented a diverse community of plankton species, including *Pediastrum*, *Dinobryaceae*, *Asterionella*, and many others. Adjusted for our classification system, there were no recorded zooplankton and all species were categorized as flagellates. This lack of zooplankton contrasts with the current study's findings, where zooplankton were abundant.

Category	Species	Abundance
Flagellates	Pediastrum	82
	Scenedesmus	13
	Dinobryaceae	63
	Ankistrodesmus	3
	Phacus	1

	Bracteacoccus	2
Other (Diatoms/Phytoplankton)	Asterionella	136
Total		300
Zooplankton	None	0

Table 3: Classification of plankton species sampled from Nitobe Garden Pond into flagellates and zooplankton based on morphological characteristics and identification tree criteria. Data was collected through a previous BIOL342 report on phytoplankton diversity and abundance in UBC freshwater sources.

Compared with the previous BIOL 342 study conducted in 2018 (Table 4), the current study recorded higher total plankton abundance, oxygen concentrations, and water temperature. Total plankton abundance increased from 300 in 2018 to 400 individuals in 2024. Dissolved oxygen levels also rose, averaging 9.96 mg/L compared to 7.8 mg/L. Water temperatures were slightly higher at 11°C compared to 10°C. While differences in water temperature could be attributed to variations in weather or sampling dates, the increase in dissolved oxygen and plankton abundance suggests potential improvements in environmental conditions conducive to plankton growth. Direct CO_2, pH, and air temperature comparisons could not be made as these variables were not measured in the previous study.

Study	Total plankton abundance	Oxygen Concentration (mg/L)	Water Temperature (℃)
Current study	400	9.96	11
Past study	300	7.8	10

Table 3: Comparison between total plankton abundance, oxygen concentration, and water temperature between the samples collected in the study and a previous study. Data was collected through a previous BIOL342 report on phytoplankton and diversity in UBC freshwater sources.

Statistical Analysis

Statistical analysis was conducted to assess the observed trends and determine the significance of differences in plankton abundances and abiotic factors between the current and previous datasets. A two-sample t-test was applied to dissolved oxygen, zooplankton abundance, and flagellate abundance, as these datasets were assumed to follow a normal distribution. The t-test is suitable for evaluating whether the means of two independent groups (current and previous studies) differ significantly, given the sufficient sample sizes and continuous nature of these variables. The Wilcoxon Signed-Rank Test was used for water temperature and total plankton abundance, as these datasets did not meet the normality assumptions required for a t-test. This non-parametric test was deemed more suitable for detecting differences in medians rather than means, particularly given the lack of variability within these datasets due to them having one value.

Water temperature indicated no statistically significant difference between the current study (11°C) and the previous study (10°C), as indicated by the Wilcoxon test (p-value = 0.0719; Appendix G). Dissolved oxygen levels significantly increased, rising from 7.8 mg/L in the previous study to 9.96 mg/L in the current study, with a t-test p-value of 8.02e-13 (Appendix H). Total plankton abundance increased from 300 individuals to 400, though this increase was not statistically significant (p-value 0.0719; Appendix G). Despite the lack of significance, the data hints at an upward trend in overall plankton abundance. Zooplankton demonstrated a statistically significant increase, with a t-test p-value of 0.0001898 (Appendix I). In contrast, flagellate abundance showed no statistically significant change (t-test p-value = 0.264, indicating stability in their populations between 2018 and 2024 (Appendix J).

Discussion

The findings from this study indicate a significant increase in the relative abundance of zooplankton in the Nitobe Garden Pond, along with an increase in plankton abundance compared to previous data. Zooplankton were consistently more abundant than flagellates across all sampling sites, with statistical analysis revealing a highly significant increase in zooplankton counts (t-test p-value = 0.0001898). Conversely, flagellate counts showed no statistically significant change over time (t-test, p-value = 0.268), indicating stability in their population. These results suggest that environmental and ecological variables have preferentially supported zooplankton growth over flagellates and more overall plankton numbers.

Regarding abiotic differences, dissolved oxygen levels significantly increased from 7.8 mg/L to 9.96 mg/L (t-test, p-value = 8.02e-13). This elevated oxygen concentration may have created favourable conditions for zooplankton growth, as higher oxygen levels are preferential and can support increased numbers of zooplankton compared to more anoxic conditions (Weinstock et al. 2022). In contrast, water temperature showed no statistically significant difference between 11°C and 10°C (p-value - 0.0719), suggesting that temperature was less likely a driving factor in the observed differences.

Several limitations were encountered during the study, which may have introduced variability and uncertainty into the results. In terms of sampling constraints, access to certain areas of the pond was restricted, and some sampling points could not be aligned perfectly with the designated grid system. These limitations may have affected consistency and contributed to edge effects. Accurately identifying and classifying plankton under the microscope posed a significant challenge. The limited overlap in species composition between the current and previous datasets required adaptations to the classification tree, forcing some phytoplankton to be categorized as either zooplankton or flagellates based on their closest observable characteristics. While artificial intelligence was used to attempt to assist with identification and quantification, it was unreliable and could not be used for the majority of data. This approach likely introduced biases, as phytoplankton do not neatly fit into either category. This reduced the reliability of direct comparisons and statistical analysis. Additionally, the reliance on older datasets for comparison inherently introduces uncertainties. Differences in sampling techniques, sampling areas, and timing between the current and previous studies could have contributed to the observed differences, making it challenging to isolate ecological changes from differences in data approaches.

Regarding the statistical analysis, limitations arose when attempting to determine the significance of water temperature and total plankton abundance due to the lack of variability within the dataset. Both variables consisted of only one value for each study–10°C or 11°C for water temperature, and 300 or 400 individuals for total plankton abundance. We artificially expanded the dataset to create enough data points to use the Wilcoxon Signed-Rank Test to address this limitation. While this approach enabled the calculation of p-values and allowed for a comparison between the datasets, it was ultimately determined to be an inaccurate and inappropriate method for representing and analyzing the data. This highlighted an important learning outcome: when datasets do not meet the assumptions or requirements necessary for statistical analysis, they should not be performed as they may yield misleading or invalid results.

Future studies should address the limitations of this research by standardizing sampling techniques to ensure comparability over time and minimize variability caused by inconsistent methods. Developing a more detailed and flexible classification system would improve the accuracy of species identification and account for variations in plankton compositions. Expanding the scope of abiotic measurements–such as light availability, nutrient concentrations, and turbidity–could provide deeper insight into the environmental drivers influencing abundance and distribution. Additionally, using more advanced technology like machine learning for organism identification and spatial modelling could enhance data quality and enable a more comprehensive understanding of the ecological drivers and composition of the freshwater system.

Conclusion:

This study examined abiotic factors and plankton abundance across three sites in UBC's Nitobe Garden Pond. Measurements of air temperature, water temperature, O_2 concentration, and CO_2 concentration were relatively consistent across all sites, suggesting a uniform pond environment where plankton abundance is unlikely to be strongly influenced by these variables. Compared to the similar 2018 study, differences included an increase in zooplankton abundance and dissolved oxygen and no significant difference in water temperature or flagellate abundance. The results of this study suggest that the increase in dissolved oxygen concentration between 2018 and 2024 could be a driving factor in the large zooplankton growth. This would align with previous research showing zooplankton can thrive in higher oxygen environments. These findings emphasize the importance of long-term monitoring to detect ecological shifts and understand the drivers of plankton distribution. While this study provides insights into the abundance of plankton in the pond, it also emphasizes the importance of addressing methodological challenges in research.

References:

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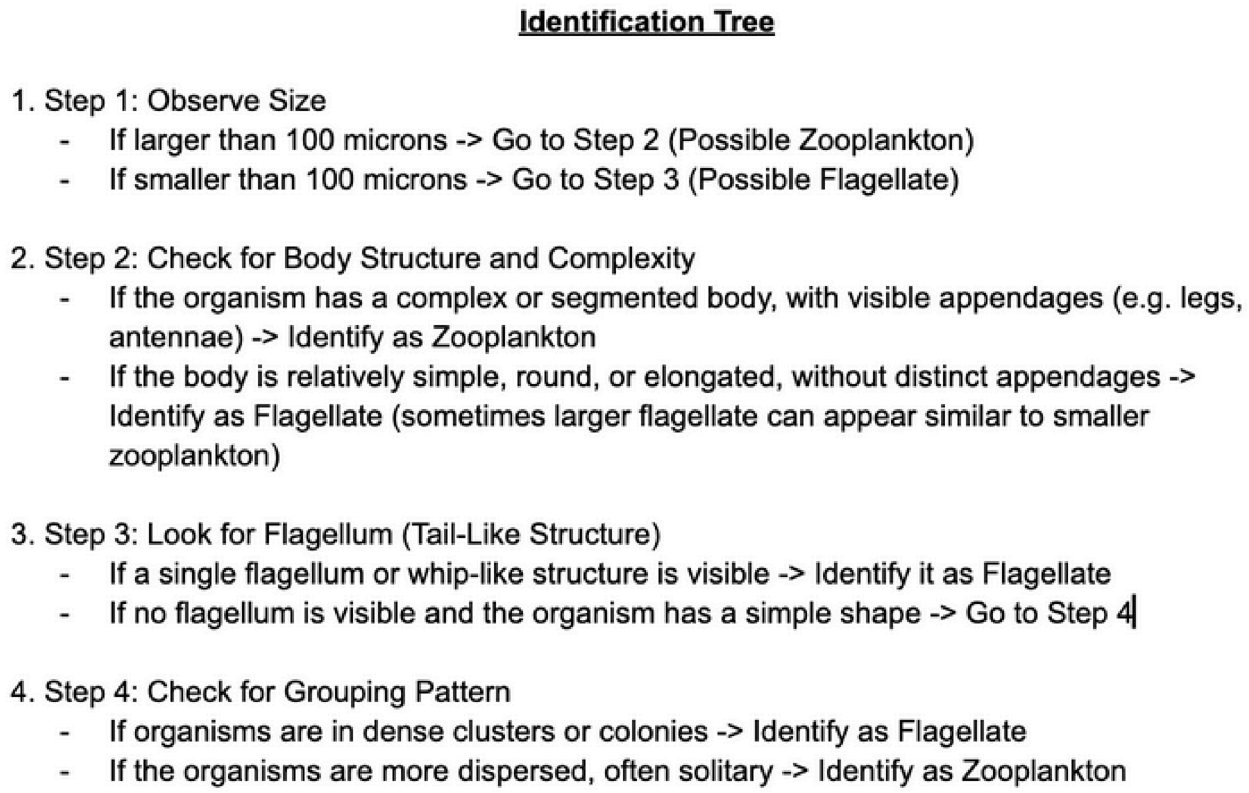
Appendix:

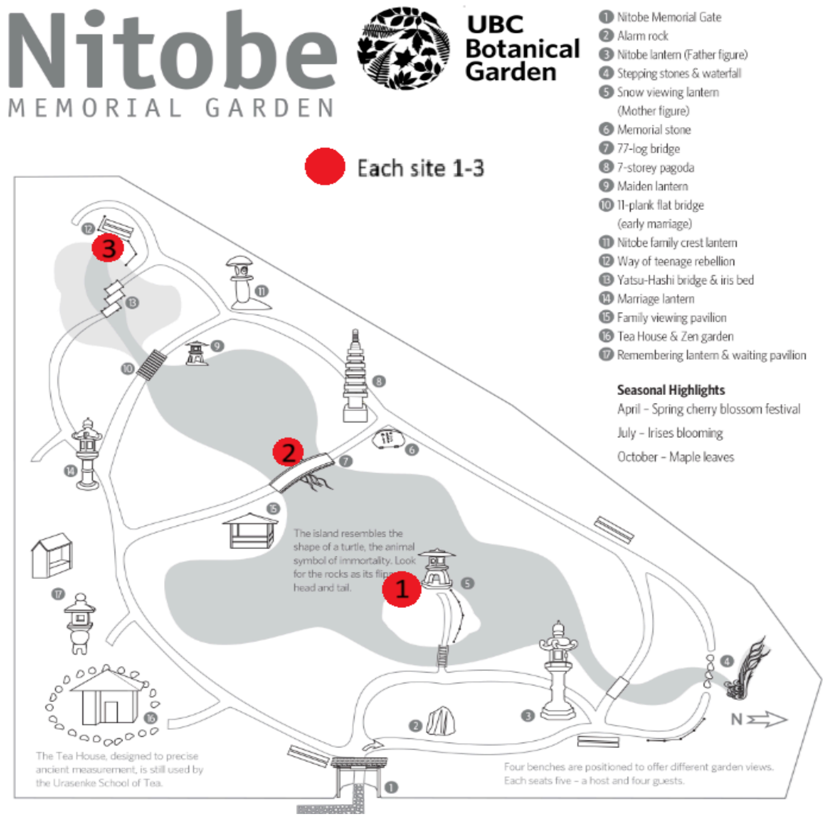
A- Identification tree used to differentiate between zooplankton and flagellates during analysis of the microscopy photos



Identification Tree

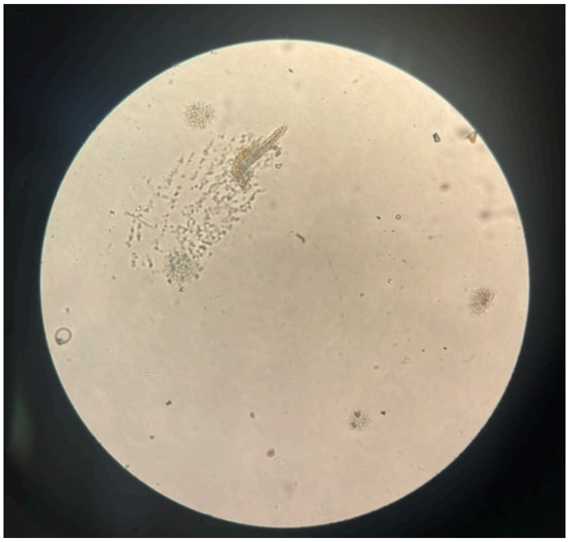
1. Step 1: Observe Size
    - If larger than 100 microns -> Go to Step 2 (Possible Zooplankton)
    - If smaller than 100 microns -> Go to Step 3 (Possible Flagellate)
2. Step 2: Check for Body Structure and Complexity
    - If the organism has a complex or segmented body, with visible appendages (e.g. legs, antennae) -> Identify as Zooplankton
    - If the body is relatively simple, round, or elongated, without distinct appendages -> Identify as Flagellate (sometimes larger flagellate can appear similar to smaller zooplankton)
3. Step 3: Look for Flagellum (Tail-Like Structure)
    - If a single flagellum or whip-like structure is visible -> Identify it as Flagellate
    - If no flagellum is visible and the organism has a simple shape -> Go to Step 4
4. Step 4: Check for Grouping Pattern
    - If organisms are in dense clusters or colonies -> Identify as Flagellate
    - If the organisms are more dispersed, often solitary -> Identify as Zooplankton

B- Map of Nitobe Memorial Garden Pond showing the 3 sampling siteshttps://botanicalgarden2015.sites.olt.ubc.ca/files/2016/01/Nitobe-Map_May-2016.pdf

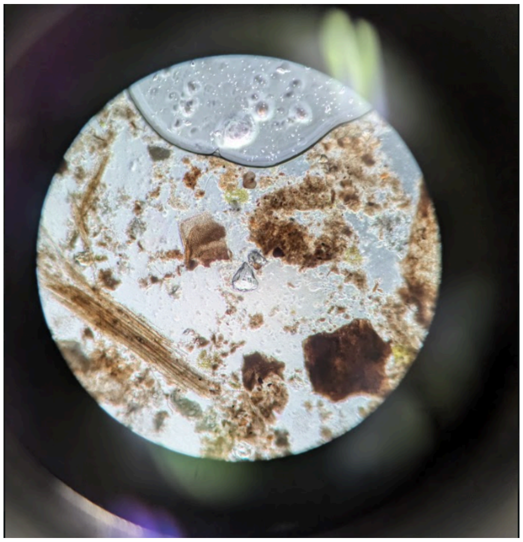


C- Examples of photos from the microscope analysis

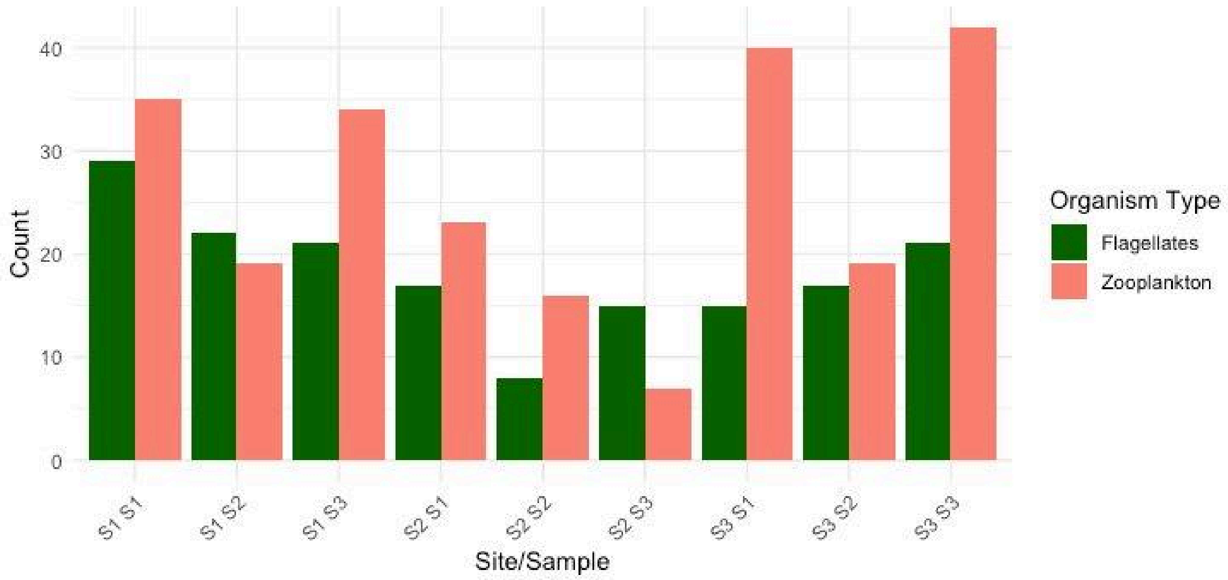
Site 1 Sample 3:



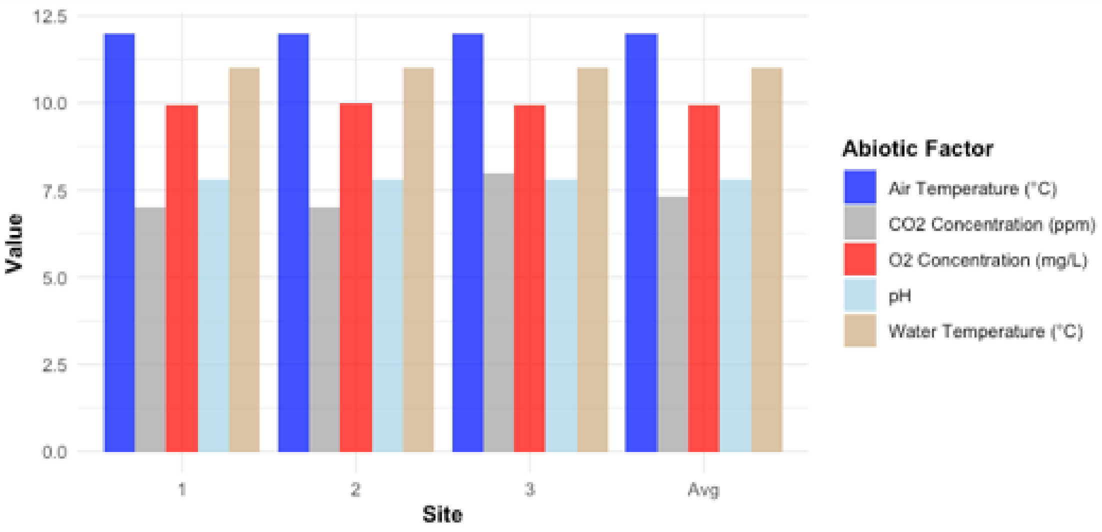
Site 3 Sample 1:



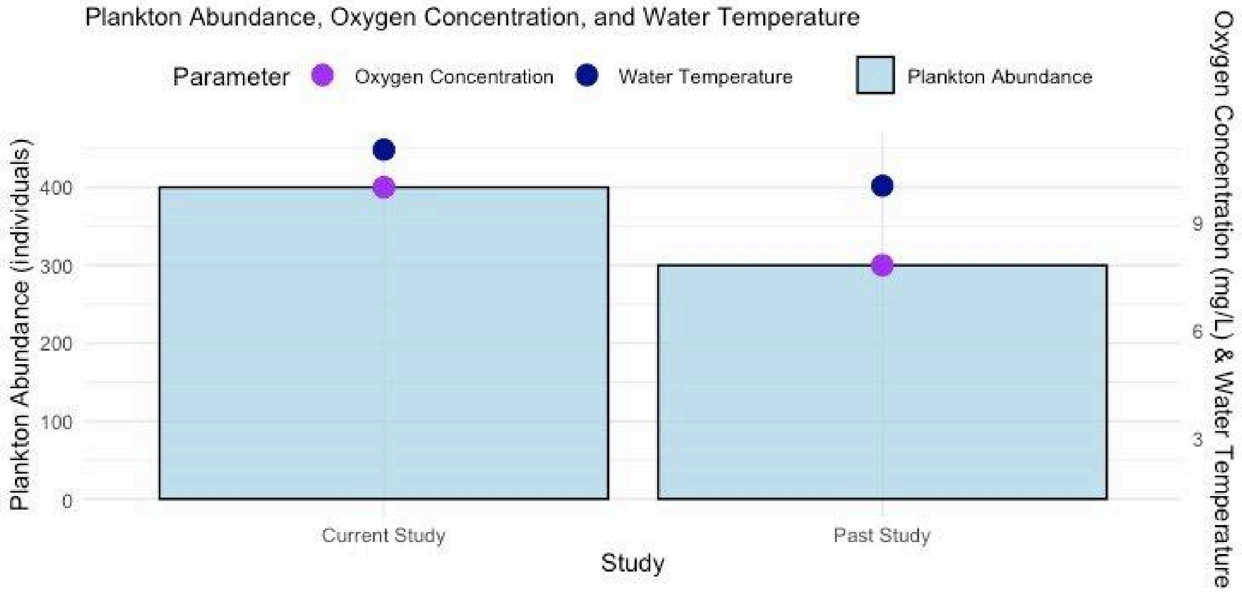
D- Zooplankton and Flagellate Abundance by Site and Sample



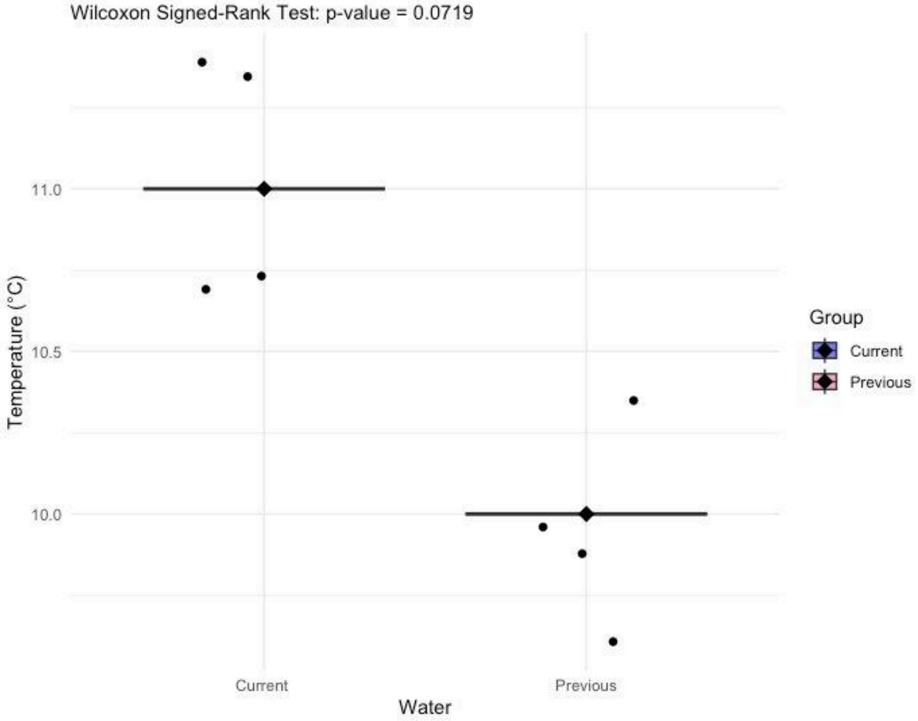
E- Abiotic Measurements across 3 sites and the average



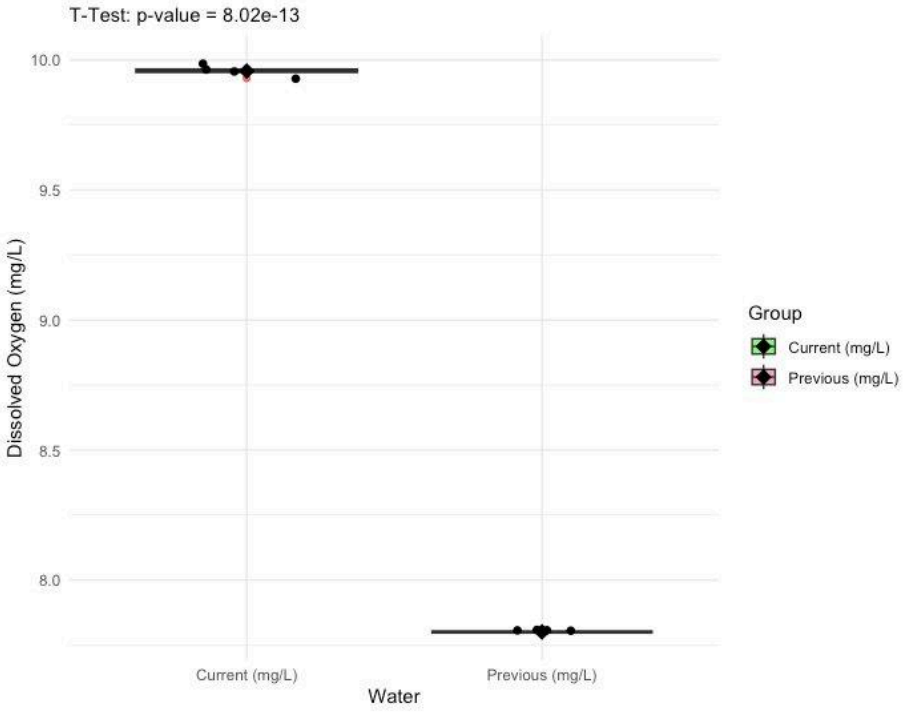
F- Abiotic and Biotic differences between studies (2024 and 2018)



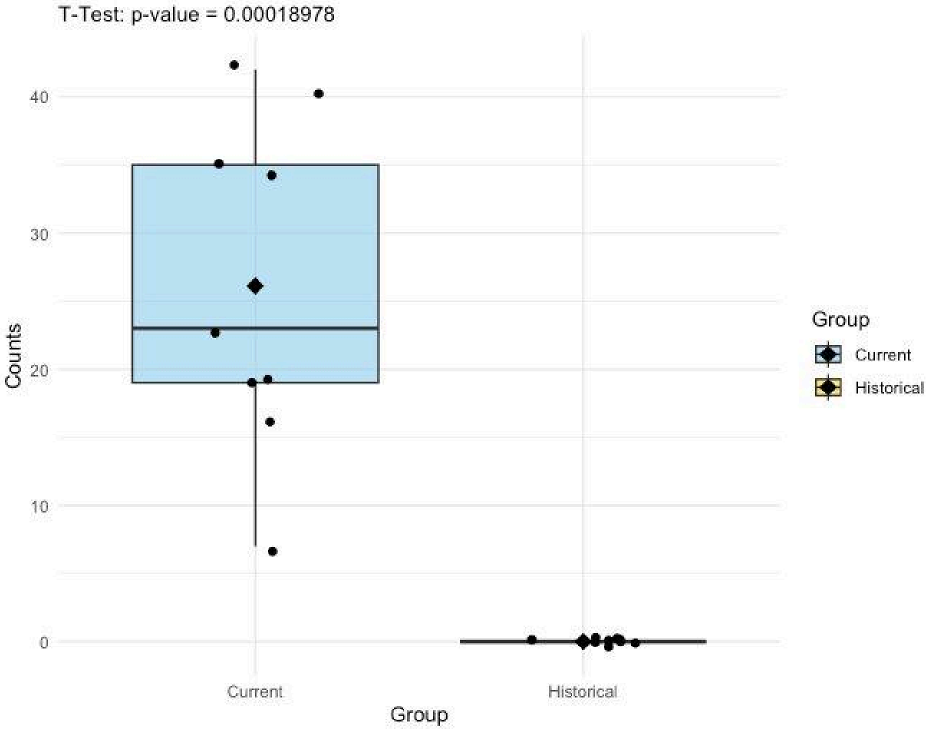
G- Water temperature between sites (℃)



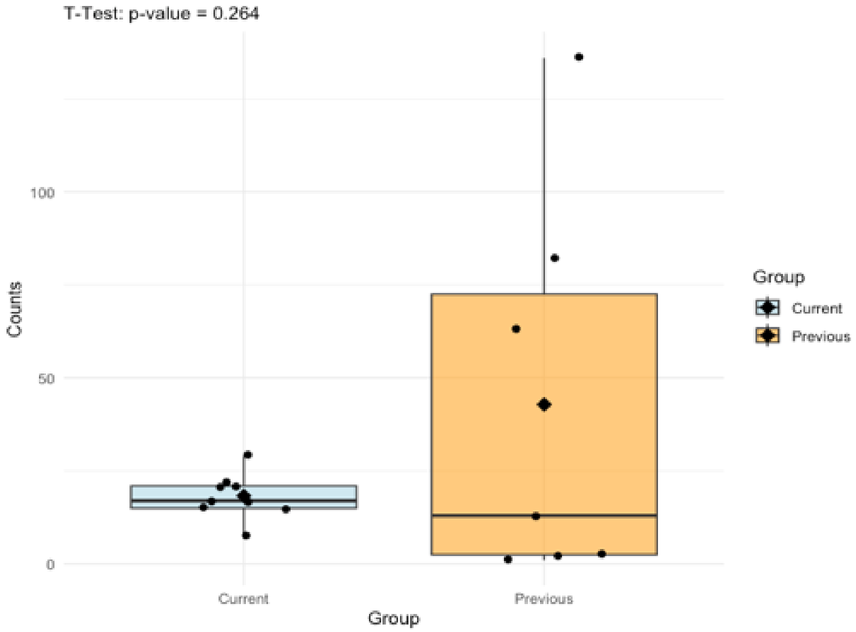
H- Dissolved Oxygen Levels between sites (mg/L)



I- Observed zooplankton abundance between 2024 and 2018



J- Observed flagellate abundance between 2024 and 2018



K- Total observed plankton abundance between 2024 and 2018