Differences in plankton biodiversity in two freshwater sources

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Abstract

Plankton in freshwater ponds play an important role in supporting the trophic chain and transferring energy from one trophic level to the next. Previous studies have examined plankton diversity in marine systems, however, there is little research on freshwater plankton diversity. Our study's aims were to investigate plankton biodiversity in two bodies of freshwater at the University of British Columbia Vancouver campus and to determine the drivers of species richness in freshwater ponds. We hypothesized that the two sites would differ in species richness, and that temperature and oxygen levels would influence the number of species. We predicted that temperature and oxygen levels would be correlated with increased species richness. We also predicted that biodiversity would increase with increasing acidity, up to a certain threshold. Our study sampled two bodies of freshwater to determine the species richness of each site, and investigated abiotic factors (oxygen concentration, temperature, and pH) that could be influencing plankton biodiversity. We found that there were no significant differences in the number of species found at the two locations. In addition, our results indicated that pH was negatively correlated with species richness, and that temperature and oxygen concentration did not influence biodiversity. Taken together, these findings highlight the importance of acidity in aquatic ecosystems, and emphasize the need for further research in understanding biodiversity and their drivers in freshwater ecosystems.

Introduction

Plankton are microscopic organisms that are found in both marine and freshwater ecosystems. At the bottom of the food chain, plankton form the foundation of healthy aquatic systems (Shao et al., 2019). Plankton can be classified into two categories: phytoplankton and zooplankton.

Phytoplankton are plant-like microorganisms that are able to photosynthesize and fix carbon. They are shown to drive the global carbon cycle, contribute to nutrient cycling, and are responsible for almost half of the world's primary production (Otero et al., 2020). Phytoplankton carry out photosynthesis by removing carbon dioxide from water, which allows more of it to diffuse from the air, thereby reducing the atmospheric concentrations of this gas (Flakowski, 2012). The carbon dioxide that is consumed by phytoplankton is then gradually recycled back into the atmosphere upon consumption by other marine creatures to obtain energy and nutrients for their own growth (2012). Phytoplankton provide organic matter for the vast majority of the marine ecosystem and limit the growth of crustaceans, fish, sharks, porpoises, and other marine creatures (2012). The productivity of phytoplankton can provide insight into how much carbon dioxide is taken from the atmosphere. In addition, the photosynthetic activity of phytoplankton leads to an increase in dissolved oxygen concentration in their environment.

Zooplankton also play a critical role in marine ecosystems by controlling phytoplankton production and by serving as a food source for organisms in higher trophic levels, such as larval and juvenile fish (Dagg et al., 2008). They function as intermediaries that transfer organic nutrients from phytoplankton to larger marine organisms and are therefore, another major component of the global carbon cycle. Zooplankton consume the carbon dioxide that is fixed by phytoplankton and then move large quantities of carbon from the ocean's surface to deeper layers where it would otherwise not reach (Richardson, 2008).

The composition, abundance, and trophic efficiency of plankton are tightly regulated by the physical and chemical environment in which they are found. For example, cold waters are replete with nutrients which means that primary production would be high and the food web is efficient (Richardson, 2008). In contrast, warm waters are depleted with nutrients and result in an inefficient food web. The increased amounts of carbon dioxide in the atmosphere result in warmer temperatures on the ocean surface. The warming of the ocean is slowing down the plankton-driven carbon pump and reducing the concentration of dissolved oxygen available; this can lead to disastrous consequences for the marine ecosystem with most organisms potentially suffocating (Sekerci & Petrovskii, 2018). Plankton play a crucial role in the carbon cycle and phytoplankton in particular, contribute to increased oxygen concentrations in the marine environment. Therefore, it is important to understand the environmental drivers of plankton biodiversity between different ecosystems.

In this study, we compared the biodiversity of plankton at two freshwater locations on the University of British Columbia Vancouver campus. These locations include the Nitobe Memorial Garden and the UBC Botanical Garden. Additionally, we sought to identify if plankton diversity is associated with oxygen concentration, pH, and water temperature. Oxygen concentration was predicted to positively correlate with phytoplankton biodiversity because of their role in producing oxygen during photosynthesis. The biomass of phytoplankton has also been shown to correlate with the amount of solar radiation and temperature (Vallina et al., 2014). Before a phytoplankton bloom, increased temperature has a positive effect on biomass whereas after and during a bloom, temperature negatively affects the biomass of phytoplankton (Lewandowska et al, 2014). Since we conducted our study in March, we expected temperature to have a positive effect on biodiversity because phytoplankton blooms typically occur during early spring (Lewandowska et al., 2014). Overall, the low water temperatures and lack of sunlight at this time of the year meant that we expected there to be less variety and abundance compared to the warmer months. We also predicted that biodiversity would increase with increasing acidity (decreasing pH), given that microorganisms such as phytoplankton tend to show better tolerance to acidic conditions than they do to alkaline waters (Henson et al., 2021).

Methods and Materials

Field sites and sample collection

We investigated the plankton biodiversity in freshwater ponds in the UBC Botanical Garden (49.253° N, -123.247° W) and Nitobe Memorial Garden (49.266° N, -123.259° W). The pond at the Botanical Garden was surrounded by cattails (*Typha)* while the pond in Nitobe Memorial Garden had relatively little vegetation on the edge of the pond. In the

Nitobe Garden, there were several koi fish inhabiting the pond, while insect larvae were the only notable macroscopic animal in the Botanical Garden pond. In addition, the pond at Botanical Garden was partially sourced by tap water.

We collected samples from the two sites on March 12, 2022. The weather was partly cloudy, and it had not rained for several days. We sampled one pond per garden, and at each pond, four points were indiscriminately selected to collect water from. These four points were at least one meter away from each other. At each point, we collected water samples and measured abiotic factors such as temperature, oxygen concentration, and pH. To collect our samples, we scooped approximately 50 mL of water from the pond using a 100 mL plastic collecting jar. Each sample was collected approximately 30 cm from the edge of the water. We then measured the temperature and oxygen concentration of the pond using an oxygen probe, and the pH using a pH meter. We took measurements at each of the four points in the pond, at the water's surface. After collecting, all water samples were stored uncovered in a 4°C fridge for 96 hours.

Species identification

We identified species in the water from the ponds, four days after collecting samples. To concentrate the water samples, we used a centrifuge to condense the samples to examine samples efficiently. Firstly, we transferred the samples from collection jars into 50 mL Falcon tubes to spin in the centrifuge. We centrifuged each sample for 5 minutes. Then we used a 2-200 μL micropipette to transfer the concentrated sample into smaller tubing for easier access during microscopic examination. To identify the species found in each sample, we micropipetted 30 μL of the concentrated sample onto a slide, and examined it under a compound microscope. We examined each sample three times. During this process, we took photos of microorganisms in order to identify them with respect to size, shape, and prominent features such as the presence of a flagellum. We used a dichotomous key (Stein, 1975) to identify the organisms we found to the most specific level of identification possible. Any unidentifiable organisms were labeled as “mystery plankton”, “mystery zooplankton”, or “mystery phytoplankton”.

Figure 1. An example of an organism (*Euglena)* viewed under a microscope (400 x).

Statistical analysis

To determine if species richness differed between sites, we used a two sample t-test. Residual vs fitted plots, Q-Q plots, and scale-location plots were used to see if data met test assumptions. To normalize species richness data, the number of species found at each site were log-transformed. We created linear models using various combinations of explanatory variables (temperature, oxygen concentration, pH) to characterize their influence on species richness. Using the model with the highest adjusted R^2 value, we tested the effect of oxygen concentration and pH on species richness using ANCOVA tests. R (version 4.1.1) was used for all statistical analyses, and alpha was set to 0.05 to determine if the results were significant.

Results

We found a total of 10 unique organisms in the Botanical Garden and 7 in Nitobe Memorial Garden (Table 1).

Table 1. Names of organisms found in freshwater ponds at the Botanical Garden and Nitobe Memorial Garden. A total of 10 unique organisms were found in the Botanical Garden pond, and 7 were found in the Nitobe Garden pond. “Mystery alga”, “Mystery zooplankton”, and “Mystery plankton” refer to organisms we failed to identify.

	Botanical Garden	**Nitobe Memorial Garden**
Phytoplankton	*Peranema* *Euglena* Mystery alga 1	*Chlamydomonas* *Haematococcus* Cryptophyta Mystery alga 2
Zooplankton	Nematoda *Amoeba proteus* Tardigrada *Paramecium* Mystery zooplankton	*Colpidium* *Amoeba proteus*
Unknown organisms	Mystery plankton 1 Mystery plankton 2	Mystery plankton 3

There was no significant difference in the plankton biodiversity, in terms of species number, between Nitobe Memorial Garden and the Botanical Garden (t-test, $t = 1.46$, $P = 0.19$). On average, 4 (± 0.48) species were found at the Botanical Garden, while 2.75 (± 0.85) species were found at the Nitobe Memorial Garden (Fig. 2).

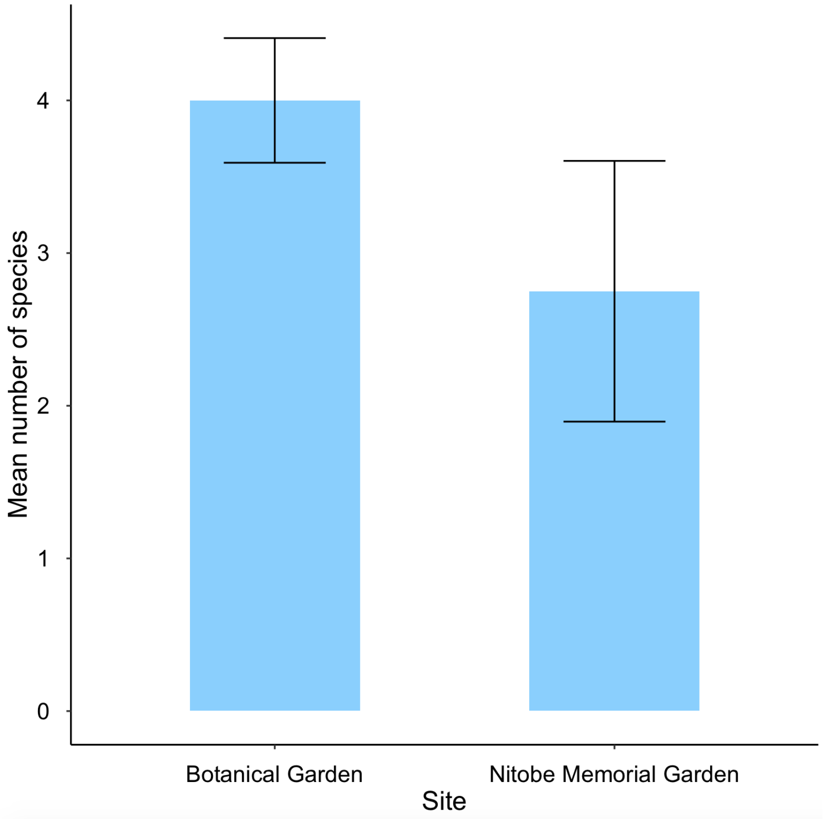

Figure 2. Mean number of freshwater plankton species found at the UBC Botanical Garden and Nitobe Memorial Garden. Four samples were collected from freshwater ponds at each site, and the number of unique species was counted. Error bars indicate standard error. t-test, t = 1.46, *P* = 0.19.

The linear model that best fit the data included dissolved oxygen percentage and pH as additive independent variables (adjusted $R^2 = 0.583$). In this model, species richness decreased with increasing pH (ANCOVA, F = 8.38, *P* = 0.03) (Fig. 3), while the amount of oxygen had no influence on species richness (ANCOVA, F = 3.42, *P* = 0.12).

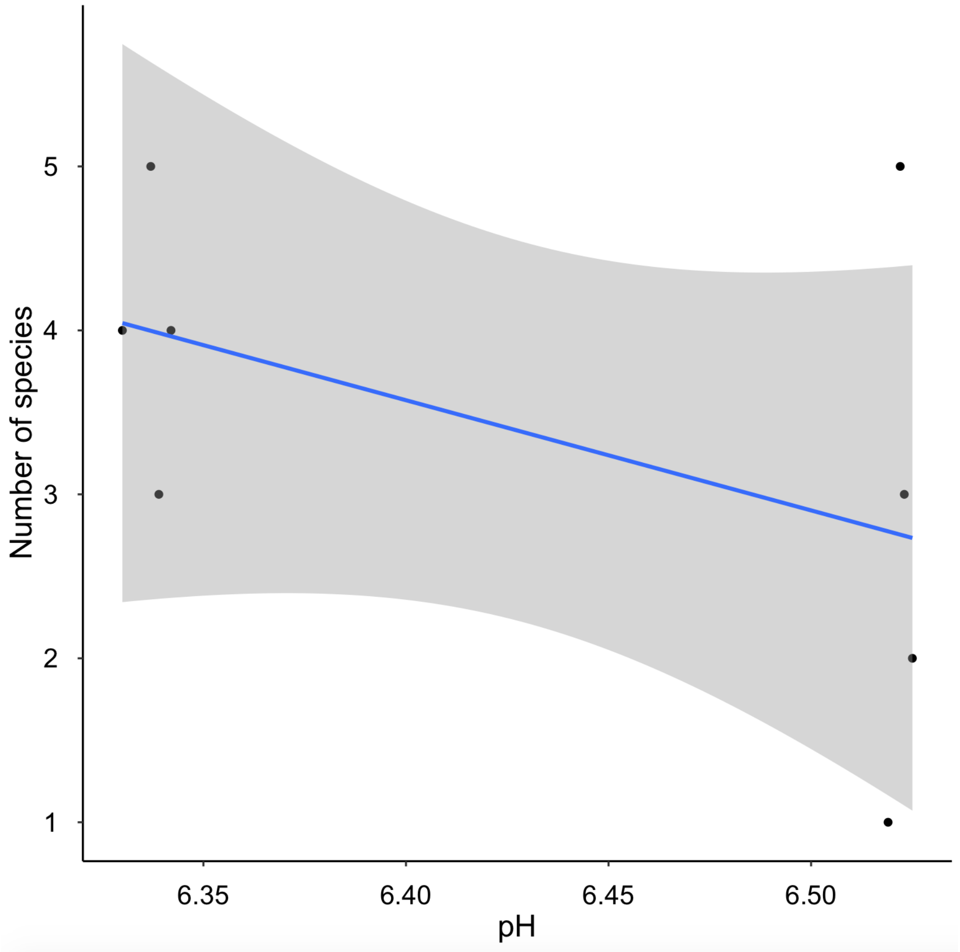

Figure 3. Relationship between pH and the number of plankton species found in freshwater ponds. Water samples (n = 8) were collected and examined from the UBC Botanical Garden and Nitobe Memorial Garden. The blue line indicates the linear model, and the shaded area shows the standard error. ANCOVA, F = 8.38, *P* = 0.03.

Discussion

The aim of our study was to determine how plankton species richness differs between two bodies of freshwater, and to identify factors that may be influencing freshwater plankton biodiversity. Contrary to our prediction, the results failed to reject the null hypothesis as we found that the freshwater microorganism communities do not significantly differ in the number of species between ponds found in the Botanical Garden and Nitobe Garden. However, it was interesting to note that different species were found in each location. We encountered more zooplankton species in the Botanical Garden than in the Nitobe Memorial

Garden. This difference could be due to the higher levels of macroscopic vegetation, qualitatively observed, at the Botanical Garden. On the other hand, the Nitobe site had considerably less vegetation in the pond, but also contained koi fish. The differences in the macro-organisms found in the ponds may be an explanation for why the species identities differed in the two sites, despite having similar species richness.

Previous studies have explored the effects of temperature, salinity, and dissolved oxygen concentration mainly in marine phytoplankton (Karacaoğlu et al., 2011), however the observed impact of increasingly warming and acidifying oceans (Henson et al., 2021) may help us predict the effects of these factors in freshwater systems. The relationship between pH and the number of species was plotted (Fig. 3) and a negative correlation was shown; as pH increased, species richness decreased. It appears that slightly acidic conditions, such as a pH of 6.35 (Fig. 3), are preferred by plankton over more alkaline conditions approaching a neutral pH of 7. Previous experiments discovered that after 2-3 years, later generations of phytoplankton exhibited developing adaptations, or a tolerance to lower pH (Henson et al., 2021). These previous research's findings may explain our observation of finding greater numbers of species in lower pH. As pH has been shown to influence species richness, we can predict that pH levels may be crucial for planktons' survival and play a role in maintaining biodiversity in freshwater habitats.

In addition to pH levels, other abiotic factors such as oxygen levels in the two ponds were considered. Unlike pH, the difference in oxygen concentration levels were not statistically significant and were found to have minimal effects on species richness in both sites. It is important to note that phytoplankton undergo photosynthesis and are responsible for oxygen release (Henson, et al., 2021), while zooplankton rely on oxygen intake, and are sensitive to low dissolved oxygen (DO) levels (Karpowicz et al., 2020). Despite the higher number of zooplankton species than phytoplankton species identified in our Nitobe Samples

(Table 1), the oxygen concentration differences were negligible and did not contribute to the species richness in each pond. This suggests differences may exist in abundances of each type of plankton that overall maintain a balance of gas exchange between the inhabitants of each pond. To ensure all plankton species are included in sampling, future studies could involve the use of a plankton tow net, rather than collecting water directly from a pond.

We predicted that environmental factors such as oxygen levels and pH will impact the biodiversity of the samples, but only pH had a statistically significant effect. The reason for this could be because most microorganisms have mechanisms to counter the need for oxygen whilst the demand for suitable pH is less forgiving. Some phytoplankton, such as green algae and cyanobacteria, are prime examples of facultative anaerobes, which use oxygen when available but are not obligatory (Pearson, 1970). However, if there is a drastic change in pH, their cell walls would break down and result in the death of the organism (Libretexts, 2021). Therefore, despite our prediction that both oxygen and pH would have a significant impact on biodiversity, it was only pH that showed significant effects. Our findings are supported by a previous study, which also found pH to be an effective indicator of phytoplankton biodiversity (Crespo-Mendes et al., 2018). These findings suggest that we can make accurate predictions of biodiversity simply through looking at the pH levels, which prevents the need to mass sample and undergo tedious species identification processes.

Conclusion

Here, we showed that species richness in Botanical Garden and Nitobe Garden ponds are similar, and that pH is negatively related to species richness. These results may point to identifying the key changes in abiotic factors that threaten aquatic species today. In particular, understanding the plankton biodiversity of a system, as well as the abiotic factors influencing species richness in aquatic ecosystems has implications in future ecological studies, especially in light of climate change.

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