Does the Duckweed Microbiome Change Seasonally?

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INTRODUCTION

Duckweeds are in the family Lemnaceae and includes five genera, *Spirodela*, *Landoltia*, *Lemna*, *Wolffiella*, and *Wolffia*. They tend to grow best in smaller bodies of water such as ponds. Duckweeds are small water plants with a short generation time, that primarily reproduce clonally (Bog, Appenroth, and Sree 2019). Doubling times can range from about a day to 2-3 days (Cheng and Stomp 2009). They can be used for biogas, biofuel, and even animal feed (Bog, Appenroth, and Sree 2019).

Duckweeds have been shown to accumulate Cd, Se, Cu, and Cr (Zayed, Gowthaman, and Terry 1998). Its yields are among the highest for plants grown in nutrient rich wastewater (Xu et al. 2012). This is important because duckweed biomass is considered a viable option as raw material for microbial fermentation in industrial settings (Cheng and Stomp 2009; Xu et al. 2012). This is especially notable in the case of yeast fermentation, which produces ethanol (Cheng and Stomp 2009; Xu et al. 2012). Duckweeds are also a known bioremediator, where plants are used to remove pollutants from the environment.

Duckweeds are considered mitigators of eutrophication because they can recover nutrients and even ammonia in water sources. Historically in the United States, duckweeds haves been used in wastewater treatment due to their ability to pull nutrients (Cheng and Stomp 2009). Duckweeds have specifically been used for swine wastewater treatment in North Carolina. The introduction of duckweeds was associated with a 62–76% reduction in chemical oxygen demand (COD) and 52–73% reduction in total organic carbon (TOC) (Cheng and Stomp 2009).

Observing the trends and patterns of microbe morphology on New Hampshire, duckweeds can give insight on how to increase the efficiency of duckweeds as a phytoremediator locally. There is interest in learning what microbes are associated with duckweeds during different seasons when some ecosystems services are more prevalent than others. Chen et al. (2023) found that surface-flow constructed wetlands, which includes duckweeds, reduced greater amounts of nitrogen in autumn. These findings suggest that understanding how these plant-associated microbes change can help elucidate what ecosystem services are provided when.

It is important to better understand how microbial communities change and come together over time. It has been historically observed that microbes seasonally change in aquatic and terrestrial systems (Lima et al. 2022; Thoms and Gleixner 2013; Zhang et al. 2022). But there are few studies on variation of plant associated microbial communities through the seasons. Different metrics such as dissolved oxygen, pH, plant exudates, phosphate concentration etc. have been known to change seasonally, subsequently altering what microbes are dominant in the environment. Zhang et al. (2022) found that in three different aquatic plants, the highest abundance and diversity of rhizosphere bacteria was seen in the autumn months.

This study will take morphological data, like the color of the colonies and the percent coverage of the

Yeast Mannitol Agar (YMA) plate associated with that color, to view the seasonal changes in microbial communities. The microbes found on these plates are bacterial and fungal communities from duckweed microbiome samples collected in New Hampshire in 2022.

METHODS

Data Collection

This data set was generated by members Alyssa Daigle and Ciana Lazú of the UNH O'Brien Lab. Using the aseptic technique, nutrient agar plates were streaked with water samples from six Durham, NH locations (Mill Pond, Woodman Road, Durham Reservoir, LaRoche Pond, Thompson Farm, and Upper Mill Pond) to achieve microbe growth. Plates were generated from each sampling date, where the sampling season ranged from May to December in 2022. Additionally, at each location, samples were taken from a "left" and a "right" side (approximately 5 feet apart) to assess diversity within each location. Microbe diversity was quantified by assessing the percent coverage of each microbe on the agar plates. Data were recorded in Microsoft Excel to be read into RStudio using read_csv().

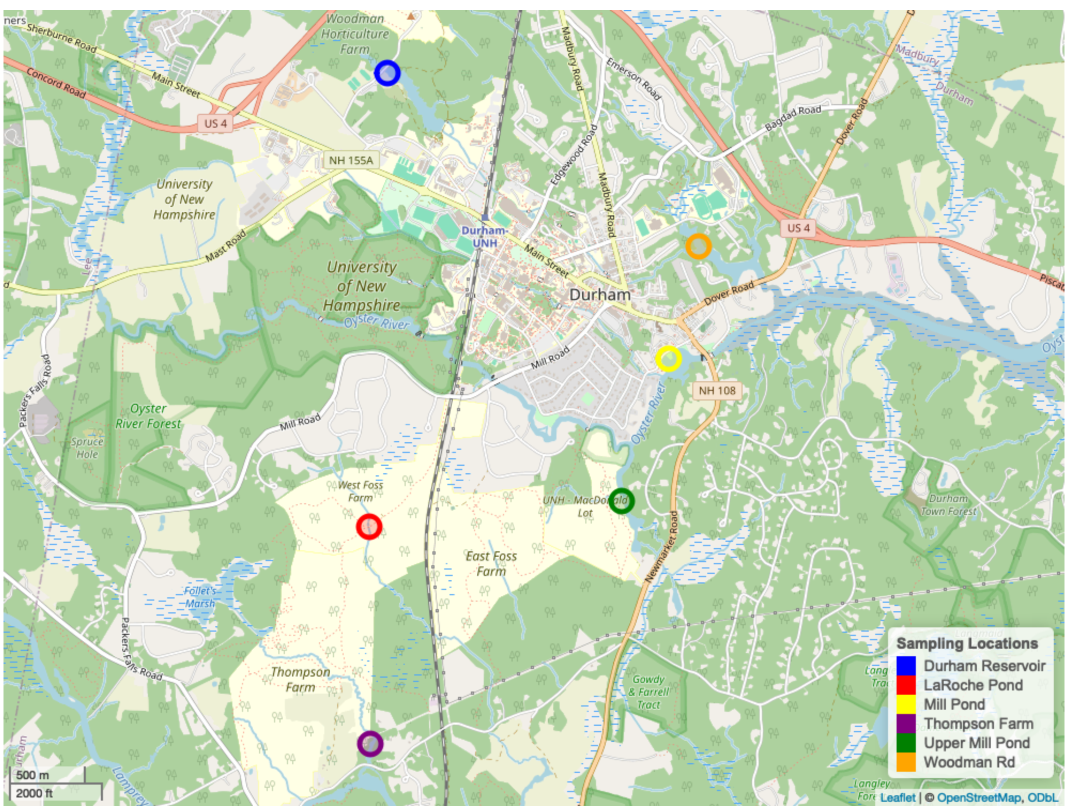

Figure 1: Map of the sampling locations in Durham, New Hampshire.

Data Cleaning + Data Frames

Analyses and data cleaning were conducted in the programming software R version 4.3.1 (R Core Team, 2018). From the original data frame, a matrix was generated where columns represented microbe colors, and each row represented a sampling date. The matrix quantified the percent coverage for each microbe color on every date for every location and side. This matrix was then converted back into a data frame using mutate(), where columns for Location, Side, and Date were pulled from the original data frame. Pivot_longer() was used to reverse the rows and columns to generate the final data frame, "all_colors_df", which was used for generating line plots. Renaming variables and filtering columns occurred using functions in the dplyr package.

Another data frame called "location_microbe" was generated from "all_colors_df" to count the total number of microbe colors reported at each location. Dplyr functions were used to group the data by location and microbe color name, where the total number of microbe colors was summed into a new column. Finally, a data frame called "avg_percent_colors" was generated from the original matrix where the data were grouped by location and the mean percent coverage for each microbe color was calculated using summarise(). Pivot_longer() was used to display the columns as location, microbe color name, and value (average percent coverage).

Plotting and Mapping

All plots were created using the package "ggplot2". The function "geom_line()" was used to create the line plot and quadratic regression plot and "geom_bar()" was used to generate the bar plots. In the package "ggpubr", the function "stat_cor()" was used to amend statistics to the quadratic regression plot.

To generate a map of the sampling site, the packages "leaflet" and "mapview" were installed. A custom data frame was generated by creating three objects: one with a list of location names, one with a list of location longitudes, and one with a list of location latitudes. The "data.frame" function was used to combine these objects into a data frame called "sampling_locations." A new object called "sampling_map" was created by piping the data frame into leaflet, and the resulting map was saved using mapshot().

Statistical Analysis

The Date column in the "all_colors_df" data frame was formatted as Julian dates and then changed into numeric values to prepare for the quadratic regression. From this data frame, the "lm" function from the "stats" package was used to perform a quadratic regression where Julian date was the predictor of the percent coverage of each microbe color.

All code for data cleaning and figure generation can be found in the following repository: https://github.com/alyssa-daigle/BIOL806_Final.git.

RESULTS

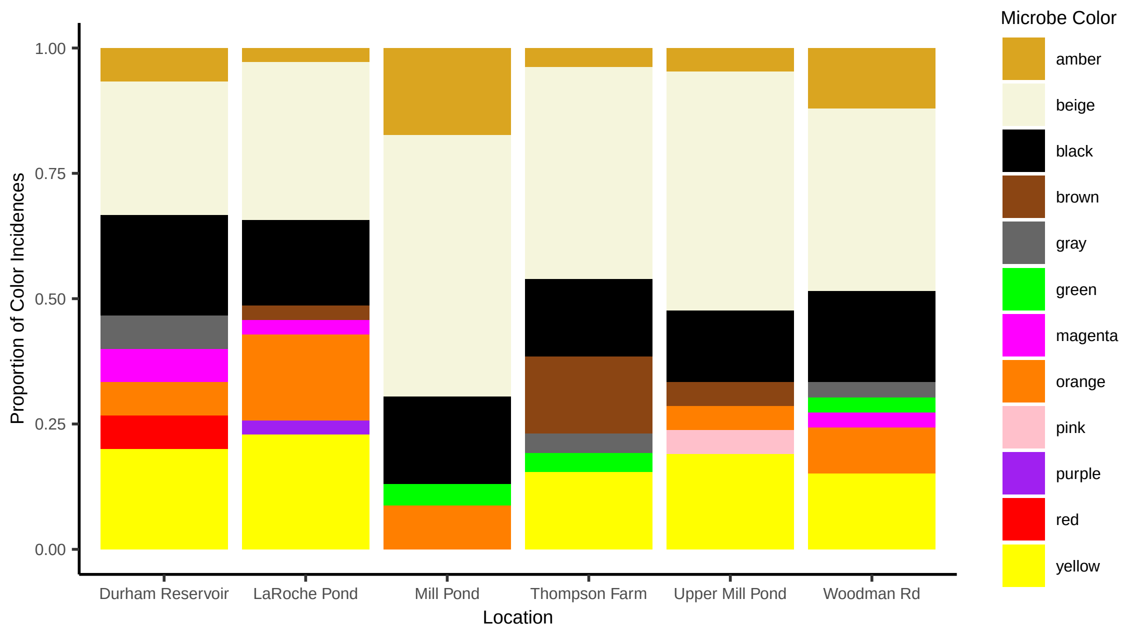

Figure 2: The incidence of each microbe color as a proportion at each location.

Beige, amber, and black are found at each of the locations as seen in Figure 2. Magenta, pink, purple, grey, and green appear to be rarer in general. Plates at each site are mostly composed of beige microbes (Figure 3). The rarer colors mentioned above also do not tend to cover much of the plate. Most sites have 7-8 microbe colors (Figure 4). The exception to this is Mill Pond, which has 5 microbe colors on average on each plate.

There are some interesting trends seen in Figure 5. The Left sampling site of Mill Pond has an increase of beige microbes through July to December, but this same trend is not seen in the Right sampling site. Woodman Rd has some spikes of magenta and black microbes on the Right sampling site around July to September. The Left sampling site also has a spike of black microbes around the same time. Durham Reservoir has a decrease of black microbes on the Left sampling site from August to September. On the Right sampling site, there is a slight increase of black microbes from August to October. LaRoche Pond has a high percent coverage of a rarer colored microbe (magenta) in mid-September on the Left sampling site. Thompson Farm, on the Left sampling site, has a high percent coverage of the beige microbes which is not seen again after November. There is also a spike of black microbes at both the sampling sites. The spike on

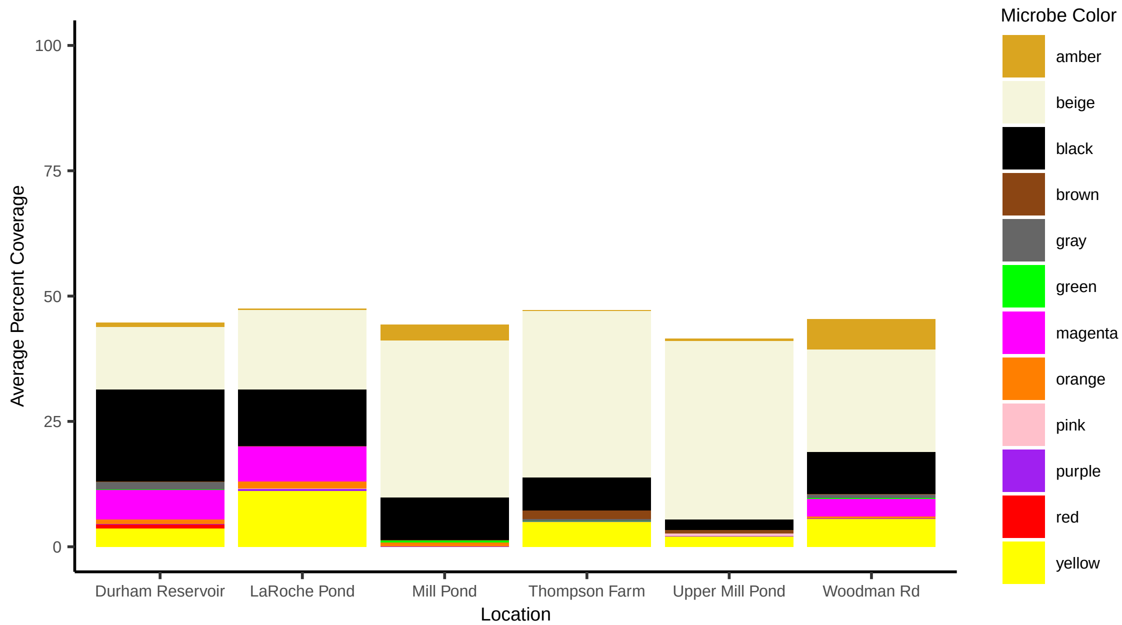

Figure 3: The average percent plate coverage for each microbe color at each location.

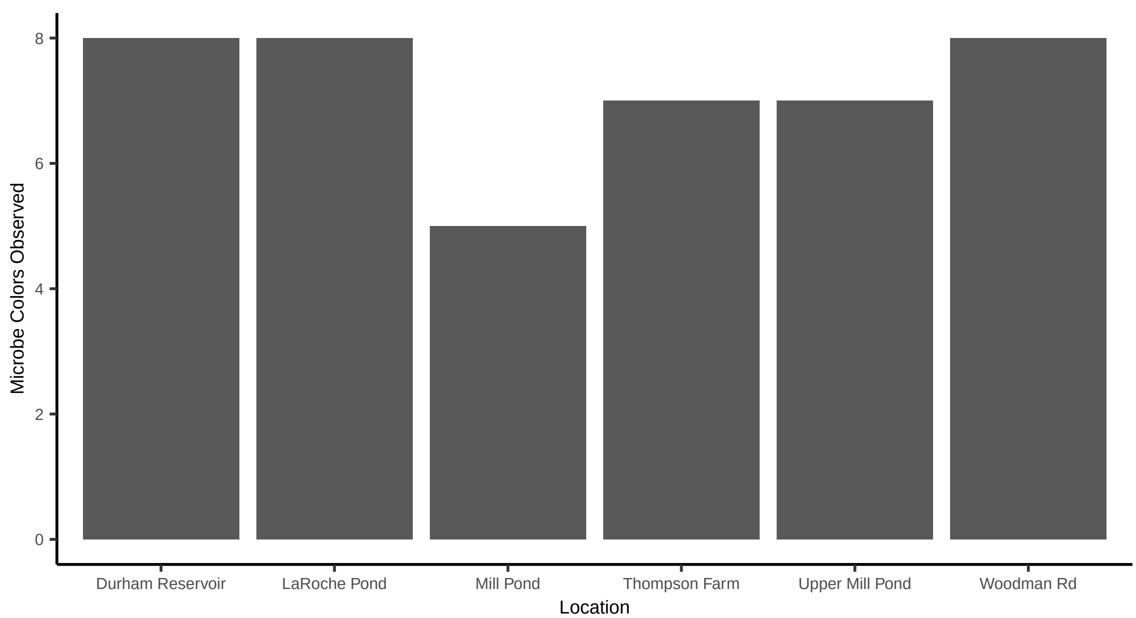

Figure 4: Total number of microbes observed from each plate at each location.

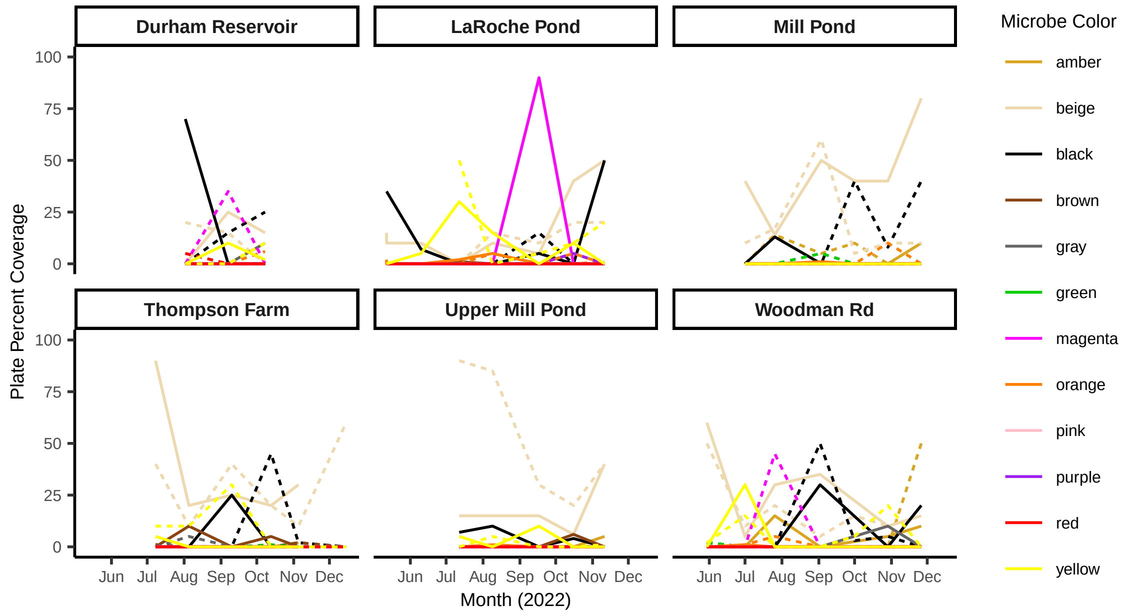

Figure 5: Percent plate coverage of each color over time at each location on the Left vs Right side. Bold line represents Left side, dashed line represents Right side.

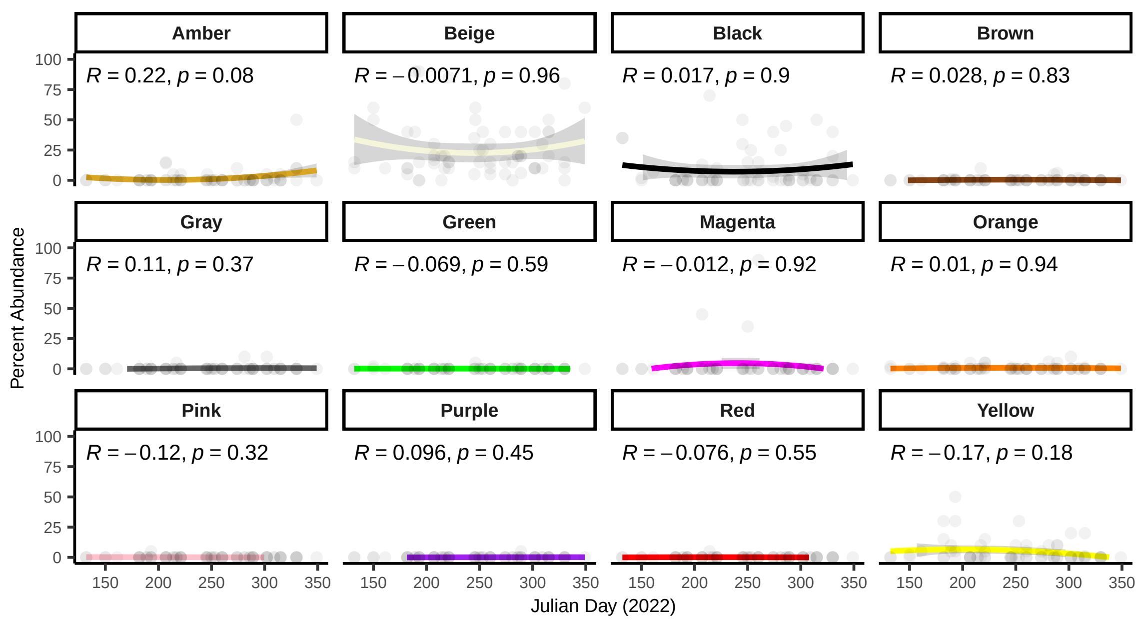

Figure 6: Quadratic regression analyzing the relationship between Julian Day and the percent abundance of each microbe color.

the Left sampling site is around early September while the spike on the Right sampling site is around early October. Upper Mill Pond has a high percent coverage of beige microbes on the Right sampling site. The beige colored microbe started to decline around mid-August. On the Left sampling site, different colored microbes have a low percent coverage, though beige microbes begin to increase around mid-October.

The R2 value is 0.00016, suggesting this model does not explain most of the variation in the response variable, the percent coverage of each microbe (Figure 6). The F-stat is 0.06174 and the p-value is 0.9401, meaning this model is not statistically significant. 0.063 is the change in the percent coverage for a one-unit change in Julian, and 4.10 is the change in the rate of change of the percent coverage concerning Julian.

DISCUSSION

This study aimed to find a connection between time (namely the seasons) and when certain microbe colors appear on the plates. There was no significant correlation between time and color of the microbe (Figure 4). This contradicts previous work that found that microbes significantly change by season (Lima et al. 2022; Thoms and Gleixner 2013; Zhang et al. 2022).

It is a possibility that with more data, there may be more of an association with microbes and different seasons. A lack of long-term data is one of the main limitations of this study. Additionally, there was no identification of any of the microbes, meaning only morphological data was used to correlate microbes with seasonality. If microbes were identified by family or genus, a significant pattern correlated to season may become apparent. Notably, previous research based their findings on identification of microbes rather than on morphological data.

Future research should mediate these limitations by increasing the number of samples and identifying microbes. This data also may benefit by utilizing a program to calculate the percent coverage on each plate to make the data more precise and accurate.

References

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