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The Effects of Temperature and N:P Ratios on Didymo Algae Growth

Abstract:

Didymosphenia geminata (didymo) is a species of algae that is native to North America, but has been producing unprecedented blooms during the last several decades. Climate change and nitrogen deposition are two hypothesized drivers of this phenomenon. The purpose of this research project is to better understand the conditions that drive didymo algae blooms in the Colorado Rocky Mountains. We hypothesize that didymo blooms will be greatest at high temperatures and under high N:P ratio treatments. In order to test this hypothesis, we used an experimental stream side channel experiment along the East River at the Rocky Mountain Biological Laboratory. There were five different N:P ratio treatments (2, 8, 16, 32, 100) and three temperature treatments combined in a factorial design (5 x 3). This experimental design will help us determine the correlation between temperature and N:P ratio treatments on didymo algae blooms. There were two statistical tests that we used for our data analysis, which were an ANCOVA and ANOVA test. We compared Added N:P Ratios with Didymo Cell Density, and we correlated Temperature Treatment with its Temperature Value. Based on these statistical tests that we ran, we determined that there was no statistical significance among N:P ratio treatments. On

the other hand, there was a statistical difference between temperature treatments, but the temperature values were not different.

Introduction:

Didymosphenia geminata, commonly classified as didymo and “rock snot”, is a freshwater diatom that has been found in the Arctic, Asia, Europe, and North America, based on fossil records from the last glaciation (Taylor and Bothwell 2014, Spaulding et al. 2020). During the early period of the 21st century, blooms have begun to appear over large areas. (Taylor and Bothwell 2014). These algae blooms often occur in cold, high altitude, high flow, and low nutrient conditions (Spaulding and Elwell 2007, Kumar et al. 2009, Bishop and Spaulding 2017), such as in streams of the Rocky Mountains. During bloom formation, excessive extracellular stalk production by individual cells coalesce to form a continuous mat that covers the stream bottom (Taylor and Bothwell 2014).

Didymo negatively impacts the microhabitats that are inhabited by benthic organisms (Marshall 2007, Anderson et al. 2014, Larned and Kilroy 2014), causing changes in the composition of macroinvertebrate communities. Macroinvertebrates play an important role in food webs and are important in both top-down and bottom-up processes that affect food web structure and function (Wallace and Webster 1996, Nery and Schmera 2016). Chironomidae, a family of non-biting midges, experience an increase in their population levels during didymo blooms, due to the high amount of nutrients that are produced. On the other hand, Heptageniidae mayflies experience a decrease in their population size because of habitat degradation (Brogan et al., 2022). Chironomids will seek refuge in low flow didymo algae mats, while Heptageniidae will graze diatoms on smooth, flat rocks (Peckarsky and Cowan 1995). Baetidae, a family of

mayflies, will not be affected by the didymo algae blooms because they forage the diatoms that grow on polysaccharide stalks (e.g., Kilroy et al. 2009, Gillis and Chalifour 2010, James et al. 2010, Bray et al. 2020).

Didymo blooms have become increasingly common over large areas, and scientists have been researching what the possible root cause of this phenomenon might be. Nitrogen deposition and climate change are the two possible drivers, and they must operate over a large spatial scale. Nanus et al. (2009) explains that inorganic nitrogen has been increasing due to nitrogen deposition as a result of nitrogen emissions from cars and fossil fuel combustion. They described that decades of elevated nitrogen deposition has resulted in algal communities shifting from nitrogen to phosphorus limitation in alpine lakes across the Rocky Mountains.

Blooms of didymo have spread across large areas of its native range over recent decades but drivers of this phenomenon have not been identified. Two possible drivers are climate change and atmospheric nitrogen deposition. Based on published literature, we know that blooms occur in rivers that have low phosphorus concentrations. (Kilroy and Bothwell 2012, Bothwell et al. 2014). Spaulding et al. explains that rivers that are downstream of dams will result in more frequent algae blooms (Kirkwood et al. 2007, Spaulding and Elwell 2007), and the dams will increase the amount of phosphorus that will be sequestered, resulting in declines in phosphorus in the river (Powers et al. 2014). The blooms occur as a result of excess stalk production (Taylor and Bothwell 2014). The production of extracellular stalks is thought to allow the algae to extend beyond the benthic boundary layer and into the water column, thus providing better access to potentially limiting nutrients (Taylor and Bothwell 2014). Stalk production is therefore thought to be a response to nutrient limitation conditions, although this hypothesis has been poorly supported (Taylor and Bothwell, 2014).

This research project will explore how temperature and nitrogen-phosphorus ratios influence didymo cell density, which are characteristic of algae blooms. We hypothesize that high temperature and high N:P ratios will result in didymo algae blooms (Belando et al., 2019). In a similar species of stalk-producing diatom, Belando et al. (2019), reported highest cell densities and the highest proportion of stalked cells in combined high temperature and N:P ratio treatments.

The nitrogen-phosphorus ratios that are present within a river will influence the river algae environment. This is because high N:P ratios will result in massive didymo algae blooms, negatively affecting the river's environment. This is because didymo algae blooms will form mats on the surface of the river rock substrates within the water column. Nanus et al. uses spatial geographical mapping data to illustrate the high inorganic nitrogen deposition that has been present in river systems that span all across Colorado. These rivers and streams are typically located at high elevations in the Rocky Mountains (Nanus et al., 2009). Nutrient concentrations in high elevation streams of western Colorado are typically low in phosphorus, and are thereby phosphorus-limited. However, some streams that are either nitrogen-limited or nitrogen-co limited (Moslemi 2010; B. W. Taylor, unpublished data).

Methods:

This research was conducted at the Rocky Mountain Biological Laboratory, along the East River in Gothic, Colorado. The objective for this research project is to better understand how temperature and N:P ratios influence didymo cell density and bloom formation.

We used a streamside channel experiment to test the effects of temperature and N:P ratios on didymo bloom formation. The experiment consisted of 15 channels, subject to either 1 of 3

temperature treatments and 1 of 5 nutrient enrichment treatments. The three temperature treatments were warm, mixed, and ambient. In order to create the three temperature treatments, we used a ½ horsepower irrigation pump (General Electric) to transport ambient river water through a polyvinyl chloride piping system. From the pump, the water was split into two separate lines. The warm temperature treatment consisted of three solar heating coil systems, which would heat up the ambient river water. The ambient and warm water temperature pathways converged to form the mixed temperature treatment, forming three unique temperature treatments.

We collected the temperature data using HOBO data loggers from July 12th to August 1st. These temperature loggers were placed towards the bottom of the trash bins, where there was a transport pipe for the outflow. When inputting these values into a datasheet, we focused our area of study onto four main variables, which were the temperature value, treatment type, date, and time.

These three temperature treatments were collected into three different storage reservoirs (30 gal trash cans), to allow us to maintain constant flow across all experimental channels. We drilled connector piping to a five-way garden hose valve, where we randomized the pathways to different channels using pieces of garden hose to allow the different water treatments to flow into the channels. The experimental channels were 9 ft long vinyl rain gutters fitted with end caps on either end. In order to allow for proper outflow, we cut a v-shape into the downstream ends of the gutter channels. Outflow from each channel was collected in another vinyl rain gutter and piped back to the East River through PVC piping. There were five replicate channels for each temperature treatment.

We tested the effects of elevated levels on N by using five N:P treatments. For the purpose of figuring out our N:P treatments, we examined water quality data from the National Water Quality Monitoring Council in Gunnison from the USGS-CO. Based on the data provided, we determined that we would be using a constant phosphorus concentration, which was 0.004 mg/L. Since our leading prediction for didymo bloom formation is nitrogen deposition we are analyzing a wide range of nitrogen values. The nitrogen concentrations that we gathered from our data collection comprised of 0.0036 mg/L for a 2:1 ratio, 0.014 mg/L for a 8:1 ratio, 0.029 mg/L for a 16:1 ratio, 0.058 mg/L for a 32:1 ratio, and 0.18 mg/L for a 100:1 ratio. The ranges of concentrations and N:P ratios were based on data from the Gunnison water quality database. We decided to keep the phosphorus concentration static across the ratios because nitrogen is hypothesized the main driver behind didymo blooms.

These nitrogen and phosphorus were added into the gutter system through a 20 L plastic bucket drip system, so we can control the flow rate of these additives. Within the drip system, there were three drips in the system per bucket that were randomized to different channel locations, and we made sure that the flows from each drip would be equal and allow the bucket to drain within 1-2 days.

Within the gutter system, we used 3.81cm by 3.81cm tiles to measure didymo stalk counts, and rocks were used to observe didymo concentration levels. The didymo were scrapped off nine out of the thirty tiles randomly every ten days off the surface of the tiles, and they were collected in a tray, which was rinsed with water into a graduated cylinder. The graduated cylinder allowed us to measure the total volume of the sample, and this sample was placed into a 100 mL whirl bag.

The number of didymo cells that are counted will depend on the number of fields of view that we use per sample slide. We used up to 25 fields of view for each slide or when 100 didymo cells are observed, and this calculation was measured by multiplying number of cells of the i th taxon counted in the counting chamber, total volume of the sample, volume of the sample counted (fields counted), and the area in square centimeter of substratum sampled.

We used a Leica Microsystems Compound microscope to estimate the didymo cell density, and we filtered a 1mm subsample of the each algal slurry onto a 25 mm cellulose membrane filter, allowed each filter to dry and then placed a drop of immersion oil onto the filter to turn the filter paper transparent. This concoction was then placed on a slide under the microscope for observation. We would record a total of twenty five transects or count one hundred didymo, depending on which condition was met first.

The first statistical analysis that was used in the experiment was analysis of covariance (ANCOVA) test. ANCOVA is a linear regression model that will use categorical and numerical variables to better understand the response of a phenomenon depending on the amount of exposure to specific treatments. This type of statistical test will determine if there is a statistical difference between significant means, while controlling the effects for covariates. The ANCOVA test was used to test the relationship between the Added N:P Ratios and the Didymo Cell Counts, when referring to the temperature data.

The second statistical analysis that was used in the experiment was conducted through an analysis of variance (ANOVA) test. ANOVA is another linear regression model that will use an interval or ratio dependent continuous variable and a nominal or ordinal categorical variable. This test has been designed to determine the difference among the means in a population depending on the amount of variation within and between the samples. The ANOVA test was

used to test the correlation between the temperature treatments and their corresponding temperature values.

An ANCOVA test was used to better understand how N:P ratios and temperature influence didymo algae growth. We began our data analysis by compiling a google spreadsheet that organized all of our data for the experiment, and we specifically analyzed the channel number, temperature value, N:P ratio, and date. We used rStudio to conduct our data analysis, and we used the “ggplot2” and “plyr” packages in order to make our data visualizations. We made sure that any NA values that were in the dataset or calculation would not effect the final result of our graphs by using a boolean function to prevent this. In addition to this, we wrote a command line to output error bars for every point that was computed onto the figure, so we could illustrate any error or uncertainty in our measurements. The information above allowed us to create a mean variable of the didymo counts from each channel, and these contained three samples, consisting of three subsamples within each sample. For our first data visualization, we examined the statistical difference between N:P ratios (molar) and didymo cell density (n/cm²).

Results:

There was a significant temperature effect on didymo cell density (ANCOVA, $p < 0.05$), but there was no N:P ratio or interaction effect (ANCOVA, $p > 0.05$). We used an analysis of variance to test the efficacy of our solar heating system in creating temperature differences between the three temperature treatments. The analysis of variance test shows that there is no significant difference in water temperature between the three temperature treatments (ANOVA, $p > 0.05$). N:P ratios individually and with temperature, where the p-values that were computed are .7309 and .5998. On the other hand, the analysis of the covariance test displayed statistical

significance when comparing temperature and didymo cell density, where the p-value for that relationship was 0.0104. There was a wide range of cell densities in Figure 1, which spanned from about 0 to 38. The summary statistics from the first figure computed a significant temperature effect, which means that the average cell densities were higher in the warm treatment (12-38). Comparing these values to the other treatments, the mixed temperature treatment didymo cell counts extended from about 4 to 8, and the cold temperature treatment didymo cell counts fluctuated from about 0 to 17.

For the second data visualization, we used an ANOVA test again to determine how the three temperature treatments influence the temperature values. The three temperature treatments were not significantly different in terms of the didymo cell density mean value, and there were a significant number of outliers, which means that there was a high range of variability within the dataset. The analysis of variance test showed that there is no significant statistical relationship between the temperature treatments and the temperature values. The summary statistic that was computed declared that there was no statistical significance within the data, based on the p-value provided of 0.159. This means that there is no correlation between these two variables. The average temperatures in the temperature treatments in the second data visualization were all about the same, which was about 12 degrees celsius. When comparing the ranges in temperature, including outliers, among all three temperature treatments, the warm temperature treatment ranged from about 5 to 30. In addition, the mixed temperature treatment ranged from about 2 to 28, and the cold temperature treatment ranged from about 2 to 26.

Discussion:

The hypothesis of this experiment was that high N:P ratios and high temperatures would result in didymo algae blooms. The results of this experiment were able to partially support the hypothesis. Based on the results, there was no statistical significance among N:P ratio treatments, but there was a statistical difference between the temperature treatments, even though the temperature values were not different. These results are still significant because this means that there is a temperature effect that fuels didymo algae blooms, so we should invest further research into this scientific breakthrough.

Based on the results of the experiment, we are able to verify that half of the hypothesis

High nitrogen deposition has been observed in a recent phenomenon since the 2010s. The streamside channel allowed us to run a randomized and replicable experiment when transporting the river water and nutrient treatments to different channels. In addition to this, our data collection methods enabled us to randomly select tiles to sample for our data analysis techniques.

We determined that N:P ratios and their interaction with temperature had no effect on didymo cell density. On the other hand, the results section concluded that there was a statistical significance between temperature and didymo cell density.

Based on our results, we can interpret this to mean that nitrogen-deposition and phosphorus nutrients do not have an effect on didymo cell density within the East River, which means that didymo have the potential to grow in nutrient poor conditions. Alternatively, the channel experiment experienced a significant sediment issue, preventing the water to flow through the system properly, and this means that the N:P ratios might have had an effect on didymo algae blooms that our data couldn't back up. On the other hand, there was a temperature effect on didymo cell density, but there was not an effect on the river water temperature values. The second data visualization exhibited that temperature has no observable effect on didymo

algae blooms. This means that changes in river temperature will not directly influence didymo development due to these changing conditions based on our data. Because of this information, we can make the inference that didymo algae have been observed outside of historical records because of an external temperature factor that will require more scientific experiments to figure out.

When considering the second data visualization, we learned that there is no statistical significance between temperature type and the temperature values. The rationale behind these outliers in the data may be due to periods of time when the loggers were not exposed to the water due to piping failures, causing the trash bins to heat up significantly. Because of this, the figure computed a high p-value, meaning that the temperature values were not different from each other. Even though this data refutes the first data visualization, there is still significance within the first figure data because it means that there is an external cause behind the temperature anomaly.

Spaulding et al. explained that didymo blooms are able to handle a wide range of river water temperatures, and these events will happen in warm river water temperatures that are between 4°C to 27°C (Spaulding et al., 2007). They also mentioned that didymo algae blooms require a specific pH, which depends on the river acid-base concentrations (Spaulding et al., 2007). Spaulding et al. claims that didymo algae blooms have been observed in both low and high nitrogen and phosphorus concentrations, meaning that N:P ratios may not have an observable effect on these bloom events (Spaulding et al., 2007).

The two main contributions to this contemporary phenomenon may be due to climate change and nitrogen deposition. If these two factors are the leading cause of this phenomenon, then the results of this study are important because there will be an increased presence of didymo

algae bloom developments spreading to more areas around North America. Anthropogenic actions are the leading cause of the didymo species from spreading around North America, specifically through human transfer. Didymo algae bloom developments are harmful for the ecosystems that are located within the rivers, and the extracellular stalks that didymo produce are not able to be broken down by bacteria and fungi.

During the completion of this experiment, there were many limitations that arose, such as construction failures, project maintenance, or data collection and analysis errors. Instead of conducting data collection every ten days, we should have instead increased the duration of the sample period, so the original sample period may have been too short for didymo to colonize and begin to bloom. In addition to this, we had difficulty preventing air and sediment entering the system, preventing proper water flow throughout the channels. This is a problem because water would not be able to flow properly through the system, impacting the didymo formation on the tiles. In addition, the pump would have variable failures preventing the trash bins from filling up to the level that was required for the channels to maintain a proper flow rate and water level. Finally, we needed to make some changes to our piping system, due to water leaks and replace broken parts occasionally. Similar to this, the drip system buckets initially were not outflowing properly for some of the days because the drip rates were not high enough for the water to exit the bucket within our two day refills time period. Because of these construction failures, the streamside channel was sometimes not working correctly the entire time, which could have influenced our results. For the data collection and analysis, there were some implications with the tile sampling and compound microscope work. When collecting the tile samples twice during the research period, there were some instances where sediment would build up on the surface of the tiles, preventing didymo from forming on the surface. When working in the lab for the data

analysis section of the research project, there may have been a limitation of my ability to identify didymo algae under the compound microscope. There were some didymo that I noticed were extremely hard to see because they blended in with the surrounding environment on the slide.

In the future, we could expand our research study to other river systems around areas within North America that are experiencing this same phenomenon, since we have empirical evidence that temperature has an influence on didymo cell density. In addition to this, we could study different external factors that influence didymo algae bloom development, such as how various flow rates affect didymo cell density. This experiment could serve as a pilot experiment that can be replicated to check our observed results from our own experiment, so we can have multiple sources on the cause of didymo algae blooms.

References:

- ❖ Taylor, B. W., & Bothwell, M. L. (2014). The Origin of Invasive Microorganisms Matters for Science, Policy, and Management: The Case of *Didymosphenia geminata*. *Bioscience*, 64(6), 531–538. <https://doi.org/10.1093/biosci/biu060>
- ❖ *Nutrient limitation controls the strength of behavioral trophic cascades in high elevation streams—Peckarsky—2013—Ecosphere—Wiley Online Library*. (n.d.). Retrieve in d June 18, 2024, from <https://esajournals.onlinelibrary.wiley.com/doi/full/10.1890/ES13.00084.1>
- ❖ Brogan, M. S., Peckarsky, B. L., & Resasco, J. (2024). Consequences of nuisance algal blooms of *Didymosphenia geminata* on invertebrate communities in Rocky Mountain streams. *Freshwater Science*, 43(1), 52–64. <https://doi.org/10.1086/729357>

- ❖ Whitton, B. A., Ellwood, N. T. W., & Kawecka, B. (2009). Biology of the freshwater diatom *Didymosphenia*: A review. *Hydrobiologia*, 630(1), 1–37.
<https://doi.org/10.1007/s10750-009-9753-5>
- ❖ Spaulding, S. A., & Elwell, E. (2007). Increase in nuisance blooms and geographic expansion of the freshwater diatom *Didymosphenia geminata*. In *Open-File Report* (2007–1425). U.S. Geological Survey. <https://doi.org/10.3133/ofr20071425>
- ❖ Belando, M. D., Martínez, P. M., & Aboal, M. (2019). Interactive effects of warming and eutrophication on population dynamics and stalk production of epiphytic diatoms in transitional waters. *Estuarine, Coastal and Shelf Science*, 229, 106413.
<https://doi.org/10.1016/j.ecss.2019.106413>
- ❖ Nanus, L., Clow, D. W., Saros, J. E., Stephens, V. C., & Campbell, D. H. (2012). Mapping critical loads of nitrogen deposition for aquatic ecosystems in the Rocky Mountains, USA. *Environmental Pollution*, 166, 125–135.
<https://doi.org/10.1016/j.envpol.2012.03.019>

Figures:

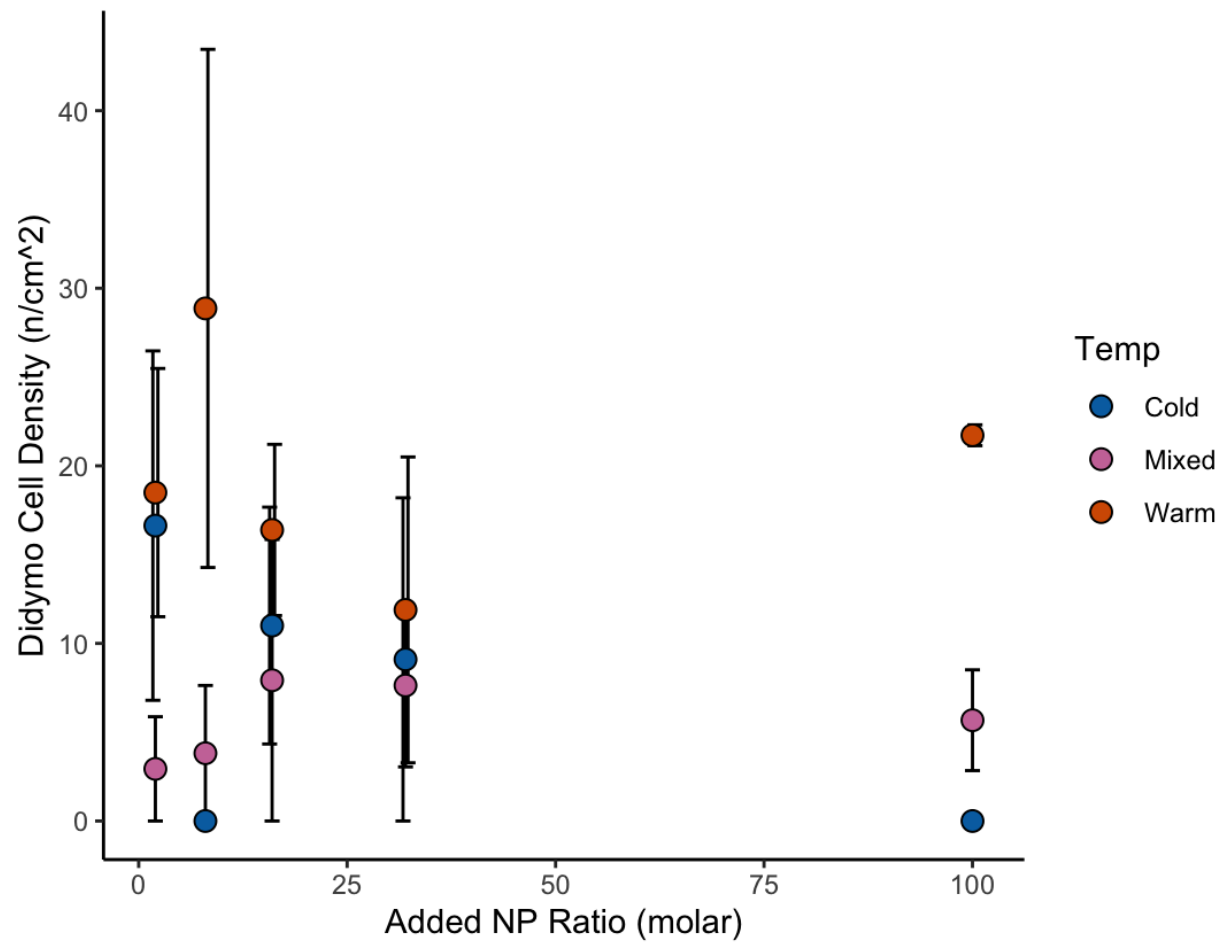


Figure 1: Added NP Ratio (molar) and Didymo Cell Density (n/cm²)

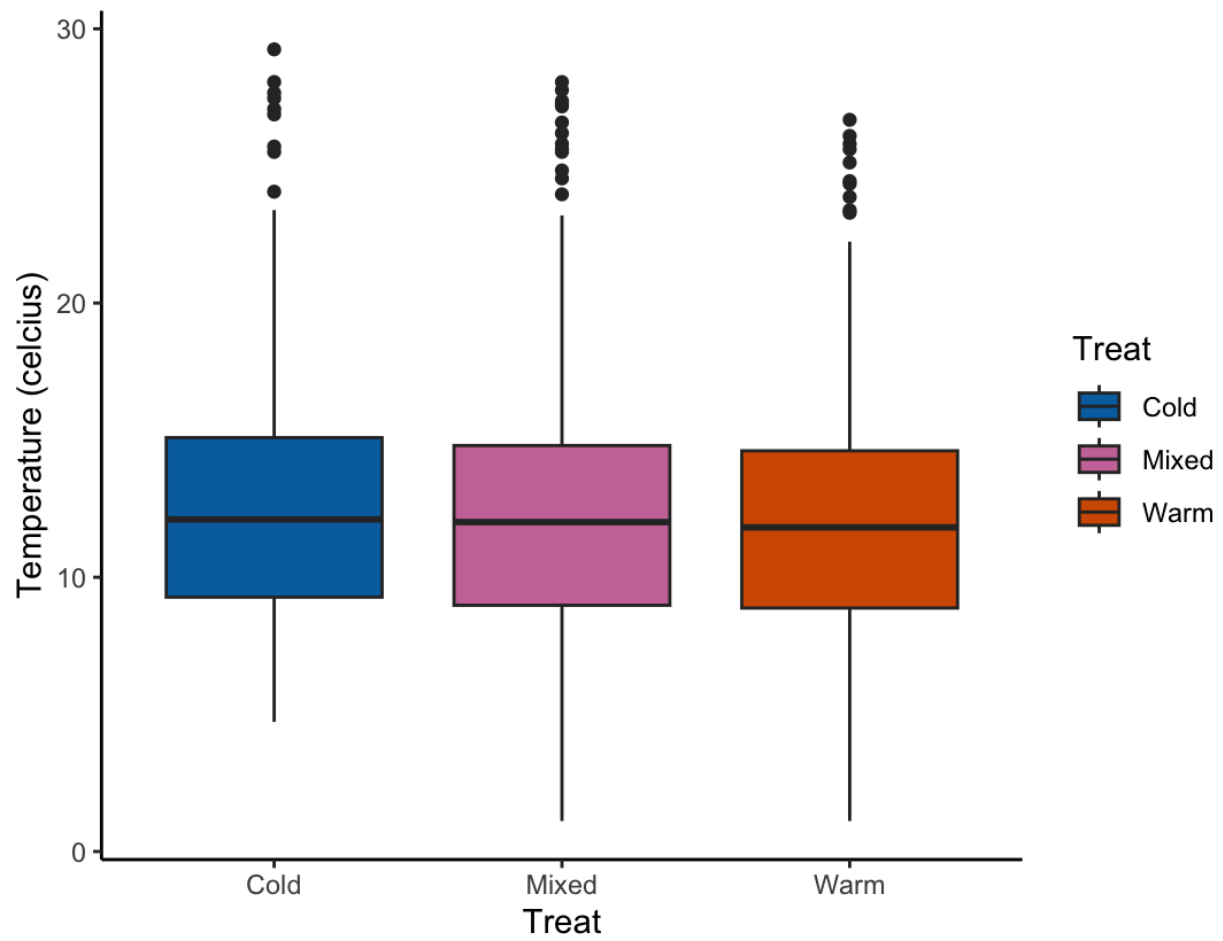
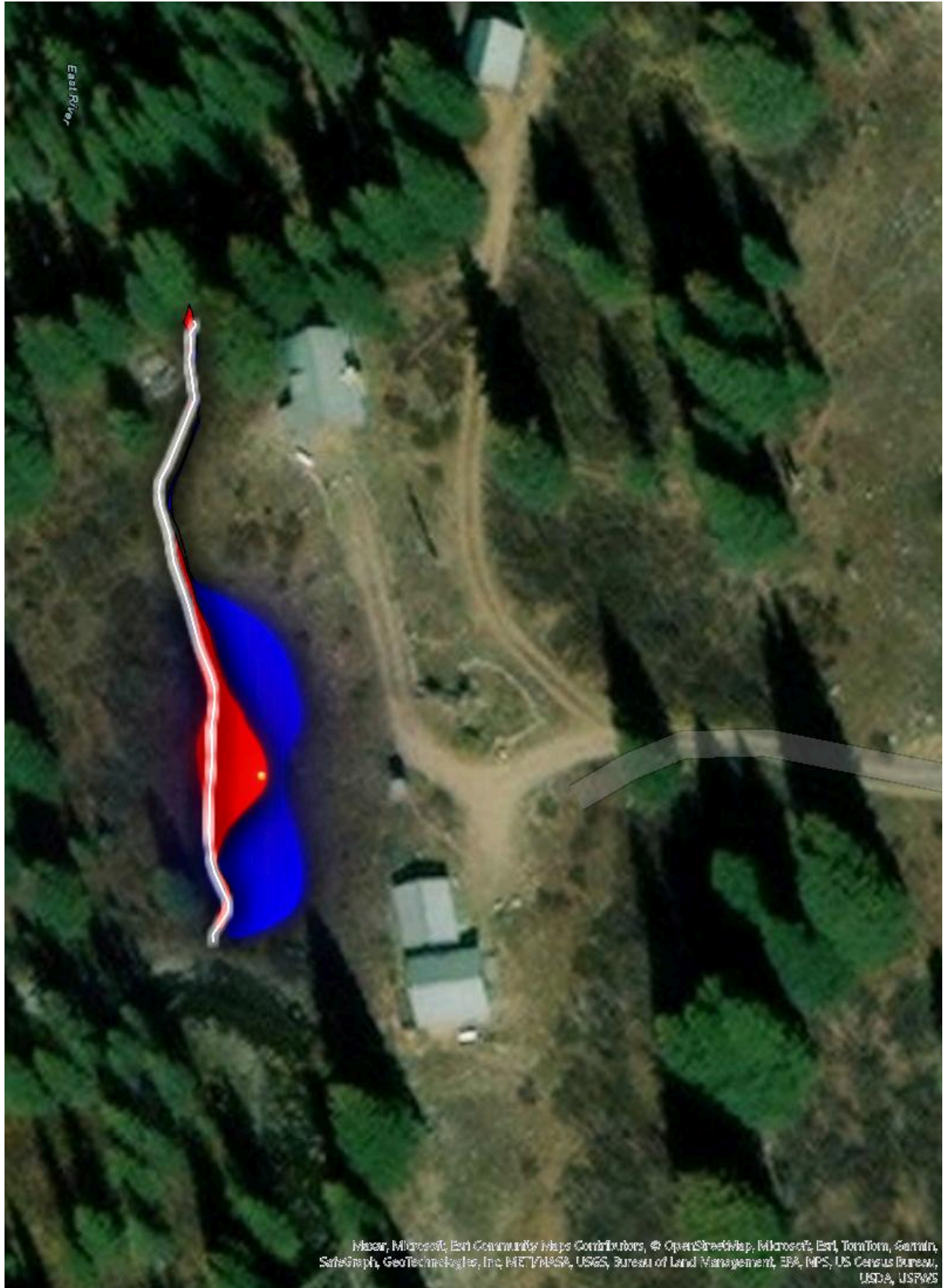


Figure 2: Temperature Treatment and Temperature Values (celcius)



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Figure 3: arcGIS site mapping Didymo Project Site