Investigating the Variable Abundance of *Arctopsyche grandis* in Colorado Streams

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Abstract

My project explored the spatial distribution of net-spinning caddisflies (*Arctopsyche grandis*) in streams around RMBL. These caddisflies are considered ecosystem engineers, as their silk nets stabilize stream substrates, creating favorable habitats for other benthic organisms, and they are suspension feeders, using their nets to collect drifting organic matter. Previous research by Dr. Demi and Dr. Peckarsky shows their abundance varies substantially in streams in close proximity, with similar watershed characteristics. A survey of individuals from multiple sites in Copper Creek and the East River was conducted, including gut content analysis, water quality measurements (temperature, conductivity), measurements of suspended organic matter, stream velocity measurements, and substrate size analysis. This project aimed to identify whether hydrological differences, temperature, resource availability, and water quality influence the abundance of these important invertebrates. Results showed a significant positive relationship between the percentage of fungi within the diet and historical abundance when combined with temperature, the percentage of animals in the diet, and seston quantity. Additional multivariate results showed a significant relationship with fungi percentage, didymo percentage, and temperature in relation to 2026 abundance. However, no other single variable in linear or multivariate regression analysis showed a significant relationship with abundance. This may be due to a small sample size and a short sampling period. Fungi are related to the decomposition of detritus, and thus the quality of detritus, which was the second most common diet item, consumed may be an important factor for this species' growth and success.

Introduction

Freshwater ecosystems are host to abundant, diverse, and sensitive populations of macroinvertebrate species that are often used as bioindicators of water quality and contaminants (Flores & Zafaralla, 2013; Ferreira et al., 2011). Freshwater ecologists explore the reasons behind, and implications of, the global disruption of these ecosystems by using biotic indices to influence conservation efforts to restore and protect these fragile ecosystems (Strayer & Dudgeon, 2010; Ferreira et al., 2011). These macroinvertebrates and other species are affected by human activity and climate change (Strayer & Dudgeon, 2010). Climate change increases pressures on the biotic communities in freshwater ecosystems, leading to changes in temperature and streamflow due to shifts in precipitation and snowmelt patterns that can lead to water quality

shifts, habitat degradation, flooding, and drought (Nimma et al., 2025). The increasing abiotic and biotic stresses associated with anthropogenic impacts on freshwater systems have the ability to alter community composition, species distributions, and metabolic rates of freshwater organisms (Voelz & Ward, 1996; Spooner & Vaughn, 2008). Further investigation of the spatial distribution of species in freshwater ecosystems offers more information on how habitat filtering, based on traits or combinations of traits, can allow some organisms to thrive in certain regions (Heino et al., 2013; Verberk et al., 2010). Trait-mediated population gradients may be responsible for highly variable abundances of specific species within similar or close-proximity locations, supporting the need for investigation of populations at small scales (Heino et al., 2013; Verberk et al., 2010).

Streams and rivers offer unique variable habitats that shift and require the communities to alter strategies or seek refuge in more suitable microhabitats (Voelz & Ward, 1996; Spooner & Vaughn, 2008). Factors affecting variation in stream communities include temperature shifts, streamflow, substrate particle size and stability, seston (suspended organic matter) size, resource availability, predation, and competition (Peckarsky & Dodson, 1980; Voelz & Ward, 1996; Spooner & Vaughn, 2008). While streams and rivers may be viewed as continuous systems, it is important to acknowledge the microhabitat shifts that can occur, as small as between riffles (Voelz & Ward, 1996). Manipulative studies have found that many variables can influence the shift of community composition and species spatial distribution within a single stream (Peckarsky & Dodson, 1980; Voelz & Ward, 1996; Spooner & Vaughn, 2008). Observations have found that alterations to stream flow and water depth, particularly due to beaver activity, can increase the number of fish in an area, leading to trophic cascades (Hanson & Campbell, 1963). Additional studies have found that substrate types, stability, and possibly size contribute to the species distribution at a microhabitat scale (Cobb et al., 2001; Flecker & Allen, 1984). A large amount of research has contributed to the list of biotic and abiotic factors that influence species distribution in a single stream, and species-specific studies can offer new knowledge for modeling systems and the scientific community.

Macroinvertebrate populations in streams and rivers offer numerous benefits to the ecosystem as a whole. Specifically, *Arctopsyche grandis*, a net-spinning caddisfly, is considered an ecosystem engineer as their silk nets stabilize stream substrates, creating a favorable habitat for other benthic organisms, and their suspension-feeding strategy contributes to water quality by

catching drifting organic matter in its nets (Bertagnolli et al. 2023; Wallace, 1975). Studies on net-spinning caddisflies (family Hydropsychidae) consist of comparisons between species or spatial distribution within a single stream (Cogo et al., 2020; Fairchild & Holomuzki, 2002; Ross & Wallace, 1982). These studies concluded that numerous variables can influence the distribution of net-spinning caddisflies, like temperature, altitude gradients, streamflow, seston size and quantity, or diet (Cogo et al., 2020; Ross & Wallace, 1982; Fairchild & Holomuzki, 2002). Understanding the factors that influence macroinvertebrate abundance in mountain streams can contribute to further understanding of how these aquatic insects are spatially distributed and what traits or variables may influence these patterns.

Previous unpublished research by B. L. Peckarsky and L. M. Demi shows the abundance of *Arctopsyche grandis* varies substantially in streams of close proximity with similar watershed characteristics within the upper East River basin, located near the Rocky Mountain Biological Laboratory (RMBL) in Gunnison County, Colorado, USA. My project explored the spatial distribution of these net-spinning caddisflies (*Arctopsyche grandis*) in East River and Copper Creek around RMBL to understand abundance trends in these streams. My project investigates reasons for variable abundance in multiple sites in two adjacent streams to help provide information about spatial distribution among streams, not just within a single stream or microhabitat. This project aims to identify whether hydrological differences, temperature, resource availability, and water quality influence the abundance of these important invertebrates. Additionally, this work can contribute to current long-term studies investigating macroinvertebrate communities or offer insight into other avenues of research, like predator-prey dynamics, nutrient cycling, and other future RMBL projects.

Questions & Hypothesis

This study aims to answer two questions: 1) Why does the abundance vary so highly across similar streamsheds? 2) What variables contribute to the spatial distribution of this species? I hypothesize that if temperature, flow, substrate size, diet, and resource availability (seston) are responsible for the abundance of *Arctopsyche grandis*, then there will be defined relationships found for both streams.

Materials & Methods

Sites

The East River and Copper Creek share similar watershed characteristics, making them ideal locations to assess the specific variables that may influence species distribution. Using previously collected but unpublished data, three sites in each watershed were chosen, each watershed containing a high, medium, and low abundance site as well as a high, medium, and low elevation site (Table 1). All three sites for each stream are upstream of the confluence of Copper Creek into the East River to ensure that assessment could be done on the individual streams (Figure 1).

Table 1. Site location information. Site information includes latitude and longitude using Universal Transverse Mercator measurements, elevation in meters, average temperature, and average historic abundance.

Site Name	Latitude (UTM X)	Longitude (UTM Y)	Elevation (m)	Average Temp (°C)	Average Historic Abundance (no./m^2)
East River Oregon	0327768	4318925	3096	6.9	3.0
East River Avery	0326970	4315699	2936	10.1	0.5
East River Levi	0327577	4313572	2875	11	16.1
Copper Creek 2nd Crossing	0332372	4316831	3216	7.3	1.4
Copper Creek Above Judd Falls	0329024	4313954	3011	7.6	0.1
Copper Creek Bridge	0327768	4313686	2902	8.2	0.1

Species Abundance

In order to obtain an estimate of species abundance at each site, a Hoffman Box sampler was used to sample a small portion of the stream (0.104 m^2 of stream bottom) and then scaled to an estimate of density per m^2. The Hoffman sampler was used four times across each site, and

the number of *Arctopsyche grandis* individuals was counted, then used to estimate the average abundance for the site.

Gut Contents

Specimen Collection: A total of 20 specimens from each site were collected over 4 sampling dates (5 specimens per collection), unless it was not possible to obtain that many specimens at low-abundance sites. This was to ensure that the content analysis was representative of the population, as the gut may be partially full or empty depending on the specimen. In order to obtain a fully representative sample, the individuals ranged in size during collection. All specimens were placed in preservative (70% ethanol) when collected in the field to prevent the emptying of gut contents between collection and dissection (Rosi-Marshall et al. 2013).

Specimen Dissection: Dissections were conducted under a stereoscopic microscope at 10X magnification. Each specimen was analyzed individually with an assigned ID number containing the site and a number; if there was insufficient gut content from single individuals, then multiple individuals were pooled for analysis. Each specimen had the entire gut tract (fore-gut and hind-gut) removed with forceps, and the contents were expressed into a separate well, leaving only the contents and avoiding contamination and misidentification by including the gut lining and any other organic material (Rosi-Marshall et al. 2013).

Gut Content Identification: Once the gut contents had been completely expressed from the gut lining, the contents were removed from the well plate and placed on a filter for vacuum filtration to remove excess water from the dissection process. Once the filter with the gut contents was dried, it was placed on a microscope slide, and particles were identified using a compound microscope. Particles were measured and identified as animal, diatom, *Didymosphenia geminata* ("Didymo", a particular diatom of interest), detritus, fungi, or unknown. Different components of the diet were ranked by their quality, with insects being the highest quality, next diatoms/algae, then fungi, and followed by detritus. A minimum of 50 particles were identified and measured using imaging software (ImageJ) to determine the relative abundance of different food sources in the diet (Rosi-Marshall et al., 2013).

Seston

Seston analysis was conducted by collecting a minimum of 2L of water from each site during each sampling day and filtering onto ashed, pre-weighed glass fiber filters using vacuum

filtration on site. To ensure enough organic matter was collected, additional known amounts of water were run through the filter. Once a sample was filtered, it was folded and then stored in a labeled envelope to prevent loss and contamination. Each filter was then oven-dried for 48 hours at 60 °C to obtain a dry mass, and then placed in a furnace for 6 hours at 500 °C to obtain an ash-free mass as the inorganic material was burnt off. The dry mass contained the entirety of the sample, but the difference between dry mass and ash-free mass enabled us to obtain the amount of organic matter.

Stream Chemistry

Conductivity was measured as a proxy for productivity using an Oakton CTS1 conductivity meter during the first and the last sampling session at all six sites. Conductivity was measured because it enabled us to assess the amount of dissolved minerals, salts, or inorganic materials in the water, which is a general indicator of productivity.

Temperature

Temperature was measured using HOBO loggers that recorded the temperature every hour for the duration of the study (June 23, 2026 - July 23, 2026).

Stream Flow

Stream flow was measured using a Marsh-McBirney flowmeter once at each site. This measurement enabled us to understand the differences in the velocity of water movement between sites located along the two streams. Three transects across the site were selected, and five measurements of stream flow were recorded and averaged to determine the mean current velocity at each site.

Substrate

Using previously collected, unpublished substrate particle size distribution data (B. L. Peckarsky, unpublished), we assessed the ability of the substrate to provide a suitable habitat at each site by comparing it to *Actopsyche* abundance data collected during the study.

Results

Gut content analysis shows that across all sites and individuals, the highest amount of particle type consumed by *Arctopsyche* is animal, followed by detritus, diatoms, Didymo, fungi, and unknowns (Figure 2). Both linear regressions and multiple regressions reveal that only a single variable and three combinations of variables are significant predictors of historic abundance (Table 2a). The only variable by itself that had a significant impact on *A. grandis* historical abundance is the percentage of fungi in the diet, which was positively related to abundance ($R^2 = 0.71$, $p = 0.03$) (Table 2a). This variable, then combined with the percentage of animal material in the diet, provided another significant predictor of abundance ($R^2 = 0.92$, $p = 0.02$); the combination of temperature and fungi content also showed a significant predictor ($R^2 = 0.90$, $p = 0.03$); and finally, the combination of seston quantity and fungi percentage was a significant predictor of abundance ($R^2 = 0.87$, $p = 0.047$) (Table 2a). Linear regression analysis using only 2026 abundance data collected during this study provided different significant relationships (Table 2b). There is no single variable that provides a significant relationship with 2026 abundance. However, multivariate regressions show that the percent of fungi combined with temperature and percent animals yield the same significant relationship ($R^2=0.90$, $p=0.03$; $R^2=0.89$, $p=0.04$). An additional three-variable predictor regression with the percent of fungi, percent of Didymo, and temperature offers a positive significant relationship with 2026 abundance ($R^2= 0.98$, $p=0.02$)(Table 2b). Multiple regressions were run using temperature and an additional variable, as temperature explained the most variation in abundance as an abiotic factor despite not yielding a significant difference ($R^2= 0.394$, $p=0.182$) (Table 2a; Table 2b). Limited sample size contributed to the decision to limit the multiple regression analyses to a maximum of three variables. Temperature and seston quantity explain the highest amount of variation in *Arctopsyche* abundance when compared to the combination of other variables but remain non-significant ($R^2=0.63$, $p=0.469$).

Discussion

The objective of this study was to determine the variables that explain the spatial distribution and the variable abundance of *Actopsyche grandis* observed in the six study sites. While the limited sample size may explain why more significant relationships between single and multiple drivers of *A. grandis* were not observed, the R^2 values suggest that some abiotic variables, like temperature alone and in combination with seston quantity, stream flow, substrate size, and conductivity, explain between 40-60% of the variation between sites. These observations are consistent with a larger study of 18 alpine and sub-alpine streams in the European Alps across multiple years that shows increasing temperature, particularly warming due to a decrease in glacier presence, shifts community composition (Niedrest & Fureder, 2020). Warming streams were also categorized by an increase in the presence of Trichoptera, the order of caddisflies that includes *Arctospyche grandis* (Niedrest & Fureder, 2020). However, the significance of the relationship with temperature was in relation to the macroinvertebrate community diversity, not density (Niedrest & Fureder, 2020). Trichoptera and *Arctospyche grandis* in particular have been identified to have a lower temperature optimum, growing well at around 4^{o}C, allowing them to live in cooler locations when other variables, like resource availability and water chemistry, are conducive (Neidrest & Fureder, 2020; Cuffney & Minshall, 1981).

Only one particle type within the gut was found to have a significant effect on the abundance of this species between these two rivers. The presence of aquatic hyphomycetes (fungi) is important for the decomposition of leaf detritus material that is often consumed by macroinvertebrates (Gessner & Robinson, 2003). The presence of fungi can increase the nutritional value of undecomposed detritus matter, increasing the reward for the organisms that consume it (Mecom, 1972; Cummins & Klug, 1979). This observation may indicate that the colonization of bacteria and fungi on detritus provides an important food source for *A. grandis* that influences abundance. However, the colonization of detritus by microbes, including fungi, takes time and may be related to the amount of seston in the stream, the temperature of the water, and the time that leaves or needles remain in the channel (Gessner & Robinson, 2003). This notion offers some explanation for why the variables of temperature and seston are significant in combination with fungi percentage in *A. grandis* diet. Additionally, the quality of fungi on the detritus material also impacts nutrition and growth of consumers, as colonized leaves with higher

quality fungi increase growth more than colonized detritus with lower quality fungi (Arsuffi & Suberkropp, 1986; Chung & Suberkropp, 2009). This furthers the need to investigate the relationship between abundance and fungi content within the gut. However, as this study was observational, manipulative studies could further determine whether this relationship is due to the influence of temperature and seston on fungi or another relationship between these variables.

Additionally, diet composition and resource availability may also play a role in determining the spatial distribution of *A. grandis*. A study of 900 caddisfly individuals of various species found that *Arctopsyche grandis* was the most predatory out of all the species sampled, mostly consuming other caddisfly species during the summer months, during periods of physiological change associated with growth and development (Mihuc et al., 1996). This observation suggests that during the summer months, the quantity of animal material consumed is important to ensure successful growth and development of this species, which may influence distribution based on food availability (Mihuc et al., 1996; Mecom, 1972; Cummins & Klug, 1979). Mihuc et al. 1996 also found that outside of this carnivorous period, *A. grandis* consumed mostly algae and diatoms to sustain themselves. This observation suggests that the prominent algae bloom of *D. geminata* may provide a food resource that influences the distribution and abundance of *A. grandis* in the study streams. This algal species provides large biosilica cells and a stalk composed of polysaccharides, proteins, and possibly some uronic acid. The discovery in this study that *A. grandis* consumes Didymo in areas with blooms suggests they are receiving some nutritional benefit (Ejaz et al., 2021).

The fact that *A. grandis* was found to be consuming both the cells and the stalk of Didymo provides support for further research regarding this relationship in these sites, and possible expansion to other sites. The presence of Didymo shifts macroinvertebrate communities, particularly mayflies, stoneflies, and caddisflies, due to the disruption of food resources (Brogan et al., 2024; Anderson et al., 2014). However, the finding that *A. grandis* is consuming Didymo regularly in regions with blooms, and that the relationship between proportional consumption shifts to significant when compared to 2026 abundance data, suggests that they may be more resilient to the ecosystem dynamic shifts that the bloom causes for other species, or evolving to consume this large diatom which may further shift community composition (Brogan et al., 2024; Anderson et al., 2014). The relationship between this species of caddisflies and Didymo needs to

be explored to fully understand the implications for the macroinvertebrate community as a whole.

Additional variables not specifically tested in this study may also explain the abundance and spatial distribution of *A. grandis*, like stream substrate stability (Peckarsky et al., 2014; Cobb et al., 1992). Stream substrate stability can influence macroinvertebrate communities due to the unstable environment and constant change in microhabitat (Peckarsky et al. 2014; Cobb et al. 1992). Similar locations to the sites used for this study have already been assessed and show a large difference in substrate stability based on a composite substrate stability index. For example, Copper Creek has a much more unstable substrate than the East River (Peckarsky et al. 2014), providing a potential, untested explanation of why *A. grandis* abundance is generally lower in Copper Creek. Detailed comparisons of the substrate stability among sites in this study may provide a further explanation for variation in *A. grandis* abundance within a single stream and create stronger links between substrate particle size and flow.

Further study of these particular sites, expansion, and increasing sampling efforts may provide more detailed information on the relationship between abiotic factors and the distribution and abundance of *A. grandis*. Previously cited studies, in conjunction with the results of this study, suggest that with larger sampling efforts, a more definitive answer about the filters that determine spatial distribution for this species. In this study, several environmental variables explained a large proportion of the variation in *A. grandis* abundance, and further exploration of how these relationships impact this species can contribute to a better understanding of the ever-shifting macroinvertebrate community composition due to climate change and anthropogenic impacts in freshwater ecosystems. Alpine mountain streams should be focused on particularly due to the decline in snowfall, which decreases snowmelt and ultimately impacts stream flow and temperature (Stewart, 2009; Barnhart et al., 2016). A large portion of the significant variables correspond with proportional consumption and diet quality, underscoring the need to explore the relationships between food quality and quantity of suspension feeders like *A. grandis*. Exploration of these components can provide more explanation for both habitat filtering in these streams and in streams in other alpine environments.

Figures

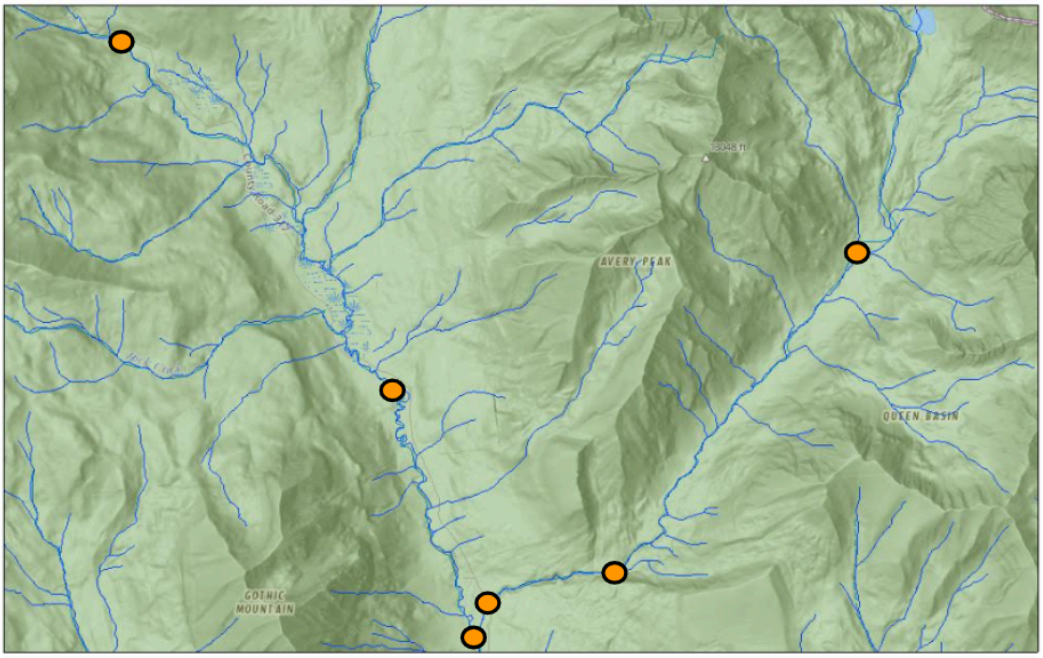

Figure 1. Site location map. Three sites within the East River watershed and three sites within the Copper Creek watershed are all represented with orange dots.

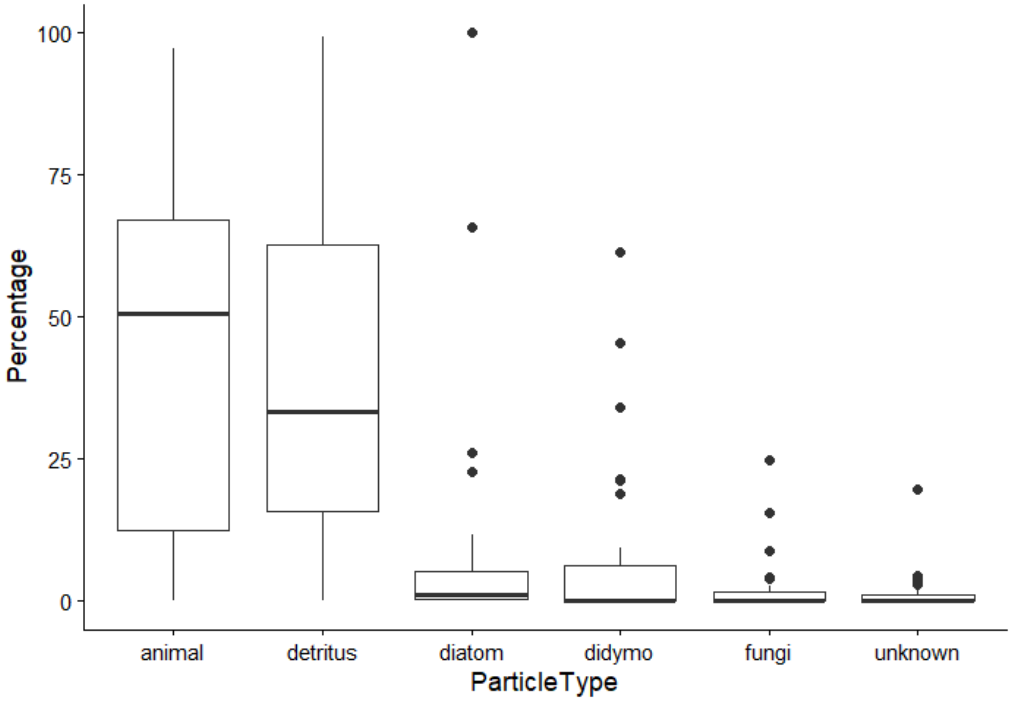

Figure 2. Average proportions of *A. grandis* diet. Percentage proportions of each diet type, including the average percentage of each particle type across all individuals sampled, and error bars.

Table 2a. Linear Regression Model Table. Contains the predictor variables compared to historical abundance data; each variable's slope for single- and multiple-regression, when applicable; an R-squared value; and a p-value.

Predictor Variables	Slope Variable A	Slope Variable B	Slope Variable C	R2	p-value
Seston	-7843.62	–	–	0.024	0.77
Stream Flow	-1.289	–	–	0.005	0.89
Substrate Size	-0.0088	–	–	0.002	0.93
Temperature	1.729	–	–	0.394	0.182
Conductivity	0.03514	–	–	0.087	0.571
Animal Percentage	0.2014	–	–	0.49	0.12
Didymo Percentage	0.3996	–	–	0.52	0.10
Diatom Percentage	-0.0694	–	–	0.08	0.57
Detritus Percentage	-0.08129	–	–	0.12	0.49
Fungi Percentage	3.0909	–	–	0.71	0.03
Fungi + Animal Percentage	9.5147	-0.5207	–	0.92	0.02
Temp + Fungi Percentage	1.2175	2.6811	–	0.90	0.03
Seston + Fungi	24155.73	4.081	–	0.87	0.047
Temperature + Seston	2.388	-2729.88	–	0.63	0.469
Temperature + Substrate	1.729	7.721	–	0.39	0.471
Temperature + Conductivity	1.639	0.012	–	0.40	0.461
Temp + Animal + Fungi	0.6598	-0.3560	7.2616	0.96	0.06
Temp + Fungi + Didymo	0.8917	2.3295	0.1630	0.96	0.07

Table 2b. Linear Regression Model Table. Contains the predictor variables compared to 2026 abundance data; each variable's slope for single- and multiple-regression, when applicable; an R-squared value; and a p-value.

Predictor Variables	Slope Variable A	Slope Variable B	Slope Variable C	R2	p-value
Seston	-9969.42	–	–	0.01	0.84
Stream Flow	0.03048	–	–	<0.0001	0.99
Substrate Size	0.0049	–	–	0.0002	0.98
Temperature	3.589	–	–	0.49	0.12
Conductivity	0.06019	–	–	0.07	0.60
Percent Animal	0.3470	–	–	0.42	0.16
Percent Didymo	0.7943	–	–	0.59	0.07
Percent Diatoms	-0.1234	–	–	0.075	0.59
Percent Detritus	-0.1651	–	–	0.147	0.45
Percent Fungi	5.464	–	–	0.64	0.06
Temperature + Seston	4.767	-48786	–	0.70	0.16
Percent Fungi + Seston	7.450	48443	–	0.82	0.08
Temperature + Flow	3.600	1.433	–	0.49	0.36
Fungi + flow	3.805	6.870	–	0.68	0.18
Temperature + Substrate Size	3.609	0.022	–	0.49	0.36
Percent Fungi + Percent Animal	18.559	-0.0613	–	0.89	0.04
Temperature + Percent Animal	3.308	0.315	–	0.83	0.07
Temperature + Percent Didymo	2.184	0.582	–	0.73	0.13
Temperature + Percent Fungi	2.720	4.548	–	0.90	0.03
Temperature + Percent Fungi + Percent Didymo	2.009	3.781	0.356	0.98	0.02
Temperature + Percent Animal + Percent Fungi	1.7363	-0.6281	12.6294	0.96	0.06

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