

Ecological Effects of Higher Water Temperature in Experimental Montane Streams

by

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## **Abstract**

Montane rivers are some of the most pristine water sources on earth, providing a wide variety of ecosystem services to downstream regions. However, these cold-water ecosystems are at risk due to climatic warming and other anthropogenic impacts. Importantly, they are potentially being impacted by disproportionately higher rates of warming when compared to those at lower elevations. Thus, I experimentally tested for the effects of elevated water temperatures on benthic communities by constructing and heating mountain-fed stream mesocosms. The experimental design consisted of 12 three-meter-long high density polyethylene troughs, which were each modified to simulate flowing headwaters. Attached algal biofilms and macroinvertebrate communities were each sampled three times during the 28-day experiment. High performance liquid chromatography of concentrated algal pigment collections from the experiment was used to quantify the effects of heating on primary producers. My results revealed no significant effects of warming on chlorophyll-inferred algal biomass. However, there was some evidence that algal community composition in the heated mesocosms had diverged from controls by the end of the experiment. Macroinvertebrate samples were taxonomically analysed using the morphological species concept and light microscopy. The effects of environmental warming on the abundance, taxonomic richness and community composition of benthic macroinvertebrates were all non-significant. My findings support other evidence of species composition rather than more robust functional aggregate properties (e.g. primary and secondary production) of aquatic algal communities being among the most sensitive and earliest responders to ecosystem stress. This cost-effective experimental stream facility provides an opportunity for testing future hypotheses regarding the direct and interactive effects of warming and other stressor (e.g., exotic sportfish introductions) on mountain stream ecosystems.

## **Preface**

This thesis is an original work by Benjamin Mercer. I designed and developed the experimental setup. Dr. Rolf Vinebrooke conceived the original research question and focus. Blake Stuparyk, Morgan Walz and Tamika Nagao assisted in the construction of the experimental mesocosms and sampling. The original version of the research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name “Cumulative impacts of invasive sportfish and rising temperatures on mountain stream ecosystems”, AUP00004029, April 26<sup>th</sup>, 2022. No parts of this thesis have been published.

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## **Chapter 1: Introduction**

One of the main stressors impacting aquatic ecosystems worldwide is global warming, leading to increased water temperatures, and altered rates of flow within the riverine network (Woodward et al. 2010; Jones et al. 2017). Higher temperatures are problematic as many stream ecosystems contain sensitive taxa that often reside within a narrow thermal niche. Therefore, exceeding the thermal tolerance for species results in habitat loss as well as stress induced mortality, ultimately impacting regional biodiversity and ecosystem services (Birrell et al. 2020; Elser et al. 2020). This becomes especially important when considering fish species used for human sustenance (Bonsal et al. 2020). Further increased temperatures will also result in glacier loss in headwater regions (Elser et al. 2020) which in turn leads to less runoff during the ice-free season and shifts streams towards being reliant on precipitation (Hannah et al. 2007; Khamis et al. 2014a; Brighenti et al. 2019).

Empirical evidence of the effects of higher temperatures on lotic ecosystems is lacking when compared to other environmental stressors (Table 1). My literature review of 23 lotic mesocosm experiments showed that the impacts of nutrient and sediment loading are the most commonly examined stressors. In comparison, climatic factors such as warming, and drought related impacts have remained relatively understudied (Table 1). Initially, the stressors of interest in experimental streams were metals including copper, iron, or other effluent originating from mining operations (Clements et al. 2019). More recently the focus has shifted towards testing the impacts of drought on stream ecosystems. This includes a wide variety of measured response variables, such as temperature, flow rates of droughts and arguably the most common being sediment deposition. Various studies have also examined the impacts of nutrient additions. While studies on pollutants such as herbicides and pesticides have been relatively consistent

throughout. Instead, most studies that examine the impacts of climate related variables have involved manipulations of water flow. Mencelez et al. (2020) recently also highlighted that the manipulation of water temperature occurred in less than 10% of the mesocosm experiments they examined.

**Table 1.** Frequency of stressor use in experimental stream mesocosm based on 23 papers included in the literature review.

Stressor Type	Number of Experiments
Drought	1
Flow	4
Metals	1
Nutrients	9
Pesticides	3
Pollutants	2
Predation	2
Salinity	3
Sediment	9
Warming	1

Aquatic macroinvertebrates are often the primary focal taxa of stream mesocosm experiments (Mencelez et al. 2020). Similarly, macroinvertebrates are frequently studied in survey-based approaches (Brighenti et al. 2019). Previous work has indicated that macroinvertebrate communities tend to shift in composition and abundance based on individual

species' thermal tolerances as water temperatures increased (Niedrist and Fureder 2021). Although there is some evidence that certain species, including stoneflies may be able to withstand certain amounts of warming (Hotaling et al. 2020). Thus, highlighting the need for a better understanding of how benthic communities respond to increased rates of warming, especially over longer more intensive heating regimes. One of the primary concerns with increased warming is the loss of biodiversity of sensitive high elevation taxa (Piano et al. 2020). This results from many of these species having very narrow thermal tolerances in which they can survive (Giersch et al. 2017).

A study on natural or whole stream warming experiments showed little effects of an increase in water temperatures on benthic invertebrate diversity (Hogg and Williams 1996). This is further reinforced by lake mesocosm experiments focused on zooplankton species, where temperature effects if detected were often marginally significant (MacLennan et al. 2015; Loewen and Vinebrooke 2016). The lack of detectable effects of warming is likely impart due to limited exposure time (Loewen and Vinebrooke 2016) coupled with only moderate rates of temperature increases that are held constant rather than varied to stimulate extreme fluctuations.

While warming is often observed to have negative impacts on macroinvertebrates and other larger species, algal taxa may in fact benefit from moderate rates of warming because of their smaller size (Daufresne et al. 2009). This is in part due to higher temperatures tending to favour and select for smaller bodied individuals as there is often a higher metabolic cost associated with increased temperature (Daufresne et al. 2009; Birrell et al. 2020; Elser et al. 2020). Also, there is potential for increased rates of photosynthesis associated with higher water temperatures (Bondar-Kunz et al. 2021). As such the impacts of warming have been shown to

exhibit positive impacts on certain members of the benthic community. Specifically with respect to increased algal biomass (Piggott et al. 2012).

Fewer stream mesocosm experiments have examined the impacts of warming on periphytic algal communities when compared to macroinvertebrates (Menczelesz et al. 2020). Given that algae comprise the base of the lotic food web more research into environmental impacts is greatly needed. Some evidence from previous experimental warming studies indicates that higher temperatures may in fact be of little concern for benthic algal communities where biomass will increase while community composition varies little (Costello et al. 2018). While other studies indicate that the impacts of warming are context dependent, and the effects may be altered due to nutrient loading or sediment type (Piggott et al. 2015b). As such further determining how increased algal biomass will influence higher trophic orders will also be important as climatic changes and warming continues.

This is further mirrored by many survey-based studies that tend to also neglect to include periphyton in stream research, especially in higher elevation headwater streams (Hieber et al. 2001; Rott et al. 2006; Brighenti et al. 2019). Thus, full community studies are quite rare in the literature. As such there should be a push to examine stressor impacts in a more holistic fashion to obtain a better understanding of impacts on stream community. A remedy for this is increased use of stream mesocosms as these studies often observe the stressor impact on multiple levels of biota (Menczelesz et al. 2020).

### *Stream Mesocosms*

There is a variety of different stream mesocosm designs present within the literature. The most common being the straight trough design, which simulates a low-order stream reach (Menczelesz et al. 2020). These designs can be flow through where water enters in the upstream

portion of the trough then flows out in the downstream portion, often returning to the stream of which the water originated. However, this design has also been adapted into recirculating systems, which have the benefit of more control in terms of water chemistry and temperature (Tzilkowski et al. 2010). It also allows for the mesocosms to be constructed and subsequently used experimentally in a more controlled environment such as a lab setting. The biggest drawback being that this approach would likely oversimplify stream processes as it lacks the ecological realism of a natural system (Tzilkowski et al. 2010). Another method of accounting for natural processes would be to construct in-situ mesocosms, often present directly in the stream channel itself. This would capture the natural variation in the stream but runs the risk of logistic issues in terms of flow rates and debris potentially harming the experimental setup. Which it is likely for these reasons that this approach is not common.

In more recent years a newer stream mesocosm design has gained traction, this being the ExStream system designed by Jeremy Piggott and company (Liess et al. 2009; Piggott et al. 2015a; Piggott et al. 2015b). The original stream mesocosm system was constructed in New Zealand and in the years since several more have been established globally. The ExStream design is an array of small circular mesocosms each containing a small motor and paddle to create flow. Despite the stream flowing in a circular direction the authors claim that this design closely mimics a natural stream reach. An incredible benefit of this design is that it allows for many replicates (total of 128) (Piggott et al. 2015a; Piggott et al. 2015b). It also allows for multiple different stressors and combinations thereof to be tested simultaneously. Current research using this design has primarily examined combinations of nutrient addition, sedimentation, and reduction in flow.

There is a need however for a greater number of experiments focused on the construction and test of mid sized stream mesocosms. Currently most of the experimental streams can be classified as large or small systems, while intermediate size stream troughs are rare (Tzilkowski et al. 2010). Including more medium sized experimental mesocosms would likely better approximate the impacts and changes in smaller headwater streams. Another benefit of this approach is that the costs associated with construction and experimental procedures would likely be reduced. Thus, allowing for greater accessibility to experimental stream research.

The majority of stream mesocosm research occurs at low elevations throughout Europe and North America, with much fewer experimental set ups and even less long-term facilities being constructed elsewhere (Menczelesz et al. 2020). Thus, there exists a bias towards those specific stream communities, which may not accurately represent the impacts of stressors across the globe. As such there is a greater need for mesocosm experiments to occur on a broader geographic scale at higher elevations to better encapsulate how headwater systems will respond to further changes, and how in turn these shifts will influence and alter downstream ecosystems.

### *Montane Streams*

Lotic montane aquatic ecosystems provide essential services to communities ranging from potable water and food production (Brighenti et al. 2019; Bonsal et al. 2020). To values that are much more difficult to quantify such as recreational opportunities and simply the value of their existence for emotional to spiritual wellbeing. However, these fragile ecosystems are experiencing drastic alterations due to a wide variety of stressors such as forestry, oil and gas exploration, introduction of non-native and invasive species and warming (Schindler and Donahue 2006).

Impacts in higher elevation ecosystems are often amplified when compared to lower altitude systems (Pepin et al. 2015). This is especially true for warming, where it has been observed that mountain habitats are experiencing rates of warming as much as three times that of lower elevations (Jones et al. 2017) in North America, whereas the average yearly temperature in European regions has increased by two degrees Celsius in the past century (Brighenti et al. 2019). Historically, scientific research has predominantly focused on impacts of environmental and anthropogenic impacts within lake ecosystems, whereas stream ecosystems have been overlooked (Hotelling et al. 2017). Thus, there exists a gap in knowledge as to how montane stream communities will respond to higher water temperatures.

## **Conclusion**

As headwater streams are being impacted by a wide variety of stressors, we can see that there is an increasing need for research into lotic ecosystems to better determine stressor impacts on benthic communities. Whole stream experiments would likely accurately capture impacts of environmental stressors; however, they are logistically challenging and often introduce variance that cannot be accounted for. Stream mesocosms however offer a controlled environment to test for stressor induced changes on various processes occurring in lotic ecosystems (Stewart et al 2013). Although, some caution should be taken as these experimental systems can over simplify natural processes therefore lack ecological realism (Stewart et al. 2013; Reiber et al. 2022). Despite this, experimental mesocosms still serve an important role in better understanding and potentially mitigating increased stressor loads on these fragile systems.

A major gap in the stressor literature is how warming and subsequent increases in water temperature influence benthic communities present in lotic systems. As the climate crisis inevitably worsens there is an increasing need and urgency to better understand how freshwater

ecosystems will respond to ever increasing rates of warming. Since there is a lack of experimental stream systems in Canada and more specifically in the Rocky Mountains research in this area is crucial, not only to determine impacts on benthic communities within this region but to also further expand the use, development, and replicability of stream mesocosms globally. The gap in knowledge surrounding both increased water temperatures in combination with the lack of experimental stream research in the Canadian Rocky Mountains, provides the core foundation and motivation for our study.

### *Thesis Research Objectives*

- 1) Design and construct experimental stream mesocosms to experimentally test impacts of environmental change on mountain lotic ecosystems.
- 2) Determine the impacts of higher water temperatures on macroinvertebrate and algal communities from montane streams.

To achieve the first objective of this study a series of stream mesocosms were created at the University of Calgary's Biogeoscience Institute located in the Kananaskis region of Alberta, Canada (<https://ucalgary.ca/research/research-groups/biogeoscience-institute>). Twelve troughs consisting of high-density black polyethylene were each supported on an aluminum frame and fed water using electrical water pumps set at a flow rate that simulated low order stream conditions. Water was circulated through each mesocosm via a holding tank positioned below the end of each trough. Each trough was then inoculated with algal communities, which had colonized artificial substrates conditioned for one month into nearby streams. Similarly, macroinvertebrate communities that had colonized cobble baskets deployed in each stream were also introduced into each of the mesocosms.



To achieve my second objective, electrical heaters were placed in half of the holding tanks to increase water temperatures and elicit responses by benthic communities in six of the stream mesocosms. The responses of the algal communities were quantified via chlorophyll and other pigment analyses using high performance liquid chromatography. The morphological species concept and dissecting light microscopy were used to measure the effects of higher temperatures on the community structure of macroinvertebrates.

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## **Chapter 2: Ecological Effects of Higher Water Temperature in Experimental Montane Streams**

### **Introduction**

Do rising temperatures affect mountain stream ecosystems? Here, global warming is of particular concern to ecologists and natural resource managers working in mountainous regions as evidence shows that the rate of warming increases with elevation (Lepori et al. 2015; Pepin et al. 2015; Fell et al. 2017; Giersch et al. 2017). As such, warming rates have increased upwards of three times over the North American Rocky Mountains compared to that of the global average (Jones et al. 2017). A better understanding of the impacts of warming on montane stream communities becomes increasingly pertinent when considering the potential subsequent loss of ecosystem services and biodiversity because of higher temperatures (Brown et al. 2007; Bregghenti et al. 2019). Knowledge gaps exist in the ecological impacts of environmental warming on mountain streams because they remain relatively understudied in this context compared to lakes and streams in other areas (Hotaling et al. 2017).

Mountain streams provide key habitats for a wide range of biodiversity, often including rare species not found elsewhere (Giersch et al. 2017; Piano et al. 2020). Headwater streams also provide a wide range of ecosystem services ranging from recreational and spiritual aspects to potable water and food sources for many human populated regions throughout the world (Schindler and Donahue 2006). Often characterized by varied flow regimes and cold temperatures (Ward 1994; Hieber et al. 2005), headwater streams are highly heterogeneous systems due to the water source of origin, ranging from glacial and snow melt runoff, to ground water and pluvial inputs (Brown et al. 2007; Hotaling et al. 2017). Due to the heterogeneous nature of these systems, there is often high regional biodiversity (Finn and Poff 2005, Hotaling et

al. 2017). However, regional biodiversity is under threat as community homogenization is likely to occur as water temperatures, and other stressor impacts, alter stream habitats and allow for lower elevation species to migrate upwards (Brown et al. 2007; Hotaling et al. 2017). As these fragile, yet important, riverine networks are being subject to increasing amounts of natural and anthropogenic stressors including increased industrial activity, glacier recessions and increased temperatures (Khamis et al. 2014b), a better understanding of how montane streams will respond to further environmental change is crucial in the face of an increasingly changing world.

I set out to design and construct a series of experimental stream mesocosms to test for differential effects of a moderate increase in water temperature on algal and macroinvertebrate communities. I hypothesized that a moderate increase in water temperature stimulates algal growth while suppressing more sensitive grazing macroinvertebrates, resulting in further positive indirect effects on primary producers (i.e., a trophic cascade). My rationale for this hypothesis involved the relative greater biological diversity of lower trophic levels (e.g., primary producers) providing them a better buffer against the impacts of environmental warming (Petchey et al. 1999; Hooper et al. 2005; Reusch et al. 2005). The use of artificial stream troughs allowed me to better control environmental variables such as setting the exact increase in water temperature while consistently maintaining flow rates and depths across all mesocosms. This also allowed me to reduce most of the natural variation that may confound results often present in surveys and space for time substitution studies. The experimental streams also allowed me to test for stressor impacts without influencing the natural environment and thus is a much more sustainable way to perform ecological research.



## **Methods**

### *Study Area*

My study was conducted at the University of Calgary's Biogeoscience Institute (51.028251, -115.035720) near Barrier Lake, Alberta . Streams sampled for benthic macroinvertebrate inoculum were Barrier and Lusk Creek (51.027652, -115.031746 and 51.032809, -115.014817 respectively). Barrier Creek is a 2<sup>nd</sup> order montane stream with sections of cobble and sandy substrate flowing through a predominantly forested area. Lusk Creek is a 4<sup>th</sup> order montane headwater stream with faster flowing water and medium cobble size. Lusk Creek had fewer trees surrounding the creek bed and the dominant vegetation was a combination of both trees and grasses. Lusk creek has also been previously stocked with sportfish for recreational angling. Land use surrounding both streams was predominantly natural area with only some areas of human influence (i.e., adjacent roads and the field stations).

### *Experimental Design and Setup*

Macroinvertebrates and attached algal communities, or “periphyton” were collected for the experiment using cobble baskets, tiles, and kick sweeps. Cobble baskets (30cm x 30cm x 15cm) were constructed of 1.27 cm mesh size galvanized steel rolled fencing. Panels were linked together using zip ties as a more cost-effective solution. All baskets were filled with 40mm Montana Rainbow Rock™ to provide substrate. Square unglazed clay tiles (100 cm<sup>2</sup>) were placed in the baskets on top of the substrate to provide a standardized surface for colonization by periphytic algae. A total of 108 cobble baskets were deployed in Barrier Creek on May 17<sup>th</sup>, 2022, and were retrieved on August 8<sup>th</sup>, 2022. All the cobble baskets were recovered, however a one of the tiles was lost during the colonization period. After retrieval all baskets were rinsed to ensure removal of invertebrates and placed in troughs with no flow to further ensure the removal

of invertebrates. A total of nine baskets were placed in each of the experimental stream channels. Prior to the experiment, stream mesocosms were additionally inoculated with macroinvertebrates from two montane streams. The first stream was Barrier creek which was used for the fishless treatments and then Lusk creek which was intended for use in the treatments that mimicked sportfish presence. This was initially done to maintain some aspect of the original study design and test for stressor interactions; however, this blocked design based on stream of origin was dropped later in the experiment. Macroinvertebrate samples for the inoculum were obtained using a three-minute kick net following the Canadian Aquatic Biomonitoring Network (CABIN) standard. The samples were transferred from the net to a cooler with stream water added then transported back to the experimental streams. Each trough received one full kick net sample.



**Figure 1.** An overview of mesocosm construction and experimental design. A) The cobble baskets deployed in Barrier Creek. B) Completed stream mesocosms. The initial test to determine the location of any leaks or other issues. C) Experiment start up. The nine cobble baskets each containing cobble for macroinvertebrates and a tile for attached algal, or “periphyton” appear in each mesocosm. Water flowed in the mesocosms from the left to the holding tank positioned on the right.

The stream troughs were constructed from 3-meter-long high-density polyethylene feed bunks (Behlen Country®). These feed bunks were reinforced with a steel frame, increasing the stability, and elevating the bottom of the stream trough off the ground, potentially mitigating some heat loss to the environment. Inflows and outflows were cut into either end of the trough. The inflow diameter measured 3.81cm while the outflow was larger at 5.08cm and was drilled lower to aid passive flow out of the stream. ABS plastic pipes were then inserted into the holes to provide some support and allow for easy connection to the recirculating system. ABS plastic was used primarily due to the cost, it also had the distinct advantage of being more resistant to shock and cooler temperatures when compared to PVC pipes. However, ABS is not as resistant to solar ultraviolet radiation (UV) as PVC plastic. Pipes were attached using 1.27cm steel strapping and bolted into the tops of the troughs. This was done to minimize the amount of movement of the inflow and outflow pipes. Inflows and outflows were then sealed with a combination of silicone and gorilla glue to ensure no leaks occurred. Threaded pipe fittings used to connect 2.54cm hose to the larger inflow/outflow combination of both nylon and ABS plastics. ABS fittings were sealed to pipes using ABS specific pipe cement, then all threaded joints were sealed with plumbers’ tape to ensure a watertight seal and further mitigate the risk of water loss.

The tubing used in the mesocosm system was G911-100 2.54cm vinylite tubing. The benefit of this product is that it is reinforced as well as “Food grade” potable water/other beverage, thus limiting the amount of chemical leeching into the system that may harm sensitive taxa. Another benefit of the clear tubing is that it allows for very rapid location and subsequent removal of any debris that may impede flow. Conversely, there is the potential for increases rates of algal growth due to UV penetration, although this was not observed in this experiment. The tubing was cut into sections of 86.36cm in length to connect the inflows together. An identical length of tubing was used at the outflow to direct water back into the holding tank. The pump was then connected to the inflow sections of the stream using a 6.096-meter-long section of tubing. This length was chosen to minimize the amount of tension and stress placed on the system. All the tubing was connected using a combination of nylon hose barbs and hose “T’s”, which were then fastened in place using hose clamps to insure a tight seal between then tubing and associated fitting.

Ball valves were installed at both the inflow and outflow to better control the rate at which water entered and exited the stream troughs. The valves were connected to the trough and the remainder of the inflow tubing using two small 7.62cm sections of hose. The ball valves were primarily used to regulate inflow and outflow to ensure all troughs had approximately the same discharge rate and to maintain a consistent depth. This allowed for rapid calibration of an entire block of streams and the associated holding tank.

Flow and water recirculation powered by an Aquascape® Ultra 2000 water pump. This specific pump is rated for a max flow rate is upwards of 7571 liters/hour. The pump was then submerged in the holding tank at the outflow end of the streams and served to recirculate and pump the water up to the inflow. Approximately 300 liters were kept in a holding tank at the

base of each stream block. This allowed the pump to remain submerged and disperse water equally to all troughs. The flow path for each of the stream blocks was as follows; water pumped from the holding tank to the tops of the stream troughs, the flow was then predominantly driven by gravity/passive flow back into the holding tank for reheating/recirculation.

The water used in the experimental streams was treated potable spring water originating from Barrier Creek. Water treatment processes include membrane filtration with UV radiation followed by disinfection using sodium hypochlorite (Epcor communications). Once all troughs and holding tanks were filled the water then sat for several days to not only test stream trough integrity but allow for any additives to evaporate. Water was then treated with aquarium water treatment solution to remove any potentially harmful trace chemicals because of the initial purification. The treatment solution was added to each of the troughs and the holding tanks to insure adequate amounts in all sections of the mesocosm set up. Water treatment was added in accordance with the recommended concentration per liter dosage outline by the product. Following this all troughs and tanks were periodically refilled with water and treatments solution was added to compensate for evaporative water loss.

Holding tanks were covered with a layer of shade cloth to limit debris input which could have resulted in fouling of the pumps. An added benefit is that the shade cloth limited light penetration into the holding tanks thus reducing potential algal growth. There was also likely a slight insulative property allowing for more consistent temperatures within each tank.

To increase the water temperature in the warming treatments three Fluviat<sup>®</sup> E300 aquarium heaters were placed in the holding tanks. Each heater is rated for warming of 357 liters. Thus, using three heaters was sufficient to warm and then maintain increased temperatures within the entire stream system. To monitor temperature a single HOBO<sup>®</sup> logger was placed at

the mid point of each stream. The loggers then recorded water temperature every hour throughout the duration of the experiment.

Experimental day 0 was August 11<sup>th</sup>, 2022, this corresponds with the day that the warming treatment was started. From here the troughs were then monitored, and communities allowed to establish for 11 days prior to sampling. The first sample day was August 22<sup>nd</sup>, 2022. Subsequent samples occurred every 9 days. (August 31<sup>st</sup> and September 9<sup>th</sup>, 2022). In total the full experiment ran 28 days. Upon completion of sampling the troughs were then drained and disassembled

### *Sampling Methods*

Water chemistry samples were taken on the first and final sampling day. Two 50ml water samples were taken from each of the holding tanks. The water samples were then analysed by the Biogeochemical Analytical Services Laboratory (<http://www.biology.ualberta.ca/basl/>) at the University of Alberta to determine the concentrations of total phosphorus and nitrogen present within the experimental streams. Chemical analysis was performed using flow injection analysis with a Lachat QuickChem 8500 FIA automated ion analyzer.

To sample periphyton, three tiles were removed at random from each trough. This resulted in a total of three subsamples per replicate trough. The tiles selected also corresponded with the cobble baskets sampled for macroinvertebrates. After removal the tiles were scrubbed for 30 seconds using an Oral B 500 electric toothbrush into an enamel pan. The tile, toothbrush and pan were then rinsed with 50ml of deionized (DI) water. The rinse water was then filtered onto a 47 mm diameter Whatman GF/C filter paper using a filter tower to capture algal pigments. The filter papers were then stored in a freezer until analysis at the University of Alberta. Pigment analysis was performed using High Performance Liquid Chromatography (HPLC). The complete

protocol used for the HPLC analysis followed that developed by Vinebrooke and Leavitt (1999). Briefly, filter papers containing the periphytic algae concentrated from scrapings of individual tiles were freeze dried to extract pigments using an 80% acetone and 20% methanol standard solution. Extracts were then dried with N<sub>2</sub> gas and reconstituted in a precise volume of injection solution which was analysed for pigment concentrations using an Agilent 1100 series HPLC. The chromatographs were then interpreted using the Agilent ChemStation software to detect and quantify the concentrations of chlorophyll and taxonomically diagnostic algal pigments (Table 2) obtained from each sample.

**Table 2.** Taxonomically diagnostic algal pigments and their associated taxa (Vinebrooke & Leavitt 1999; Cook et al, 2023)

Diagnostic Pigment	Algal Group
Chlorophyll <i>a</i>	All algae
Chlorophyll <i>b</i>	Chlorophytes, euglenophytes
Alloxanthin	Cryptophytes
Canthaxanthin	Filamentous cyanobacteria
Diadinoxanthin	Chromophytes
Diatoxanthin	Diatoms
Fucoxanthin	Chromophytes, Diatoms
Lutein	Chlorophytes
Zeaxanthin	Cyanobacteria
Myxoxanthophyll	Colonial cyanobacteria
Violaxanthin	Chlorophytes

To sample benthic macroinvertebrates a one min bucket swirl and sieve was performed a total of three times to ensure all macroinvertebrates were dislodged from the substrate. A total of three cobble baskets were randomly selected per trough and then mixed to form a composite

sample. The cobble was then rinsed into a bucket and the stones were then mixed and scrubbed by hand to dislodge any clinging invertebrates. The water was then passed through a filter to capture individuals. The contents in the filter were transferred to a 500ml Nalgene bottle and immediately preserved in 95% ethanol until enumeration occurred. In the lab the preserved samples were sieved again to further remove larger pieces of sediment. The remaining material was divided into petri dishes. From here the samples were sorted and inverts were enumerated and identified to order for a 2-hour maximum sorting time per sample. Taxonomic identification was taken to the lowest feasible resolution for the project. All invertebrate enumeration and identification were performed using an Olympus SZ61 dissecting scope.

### *Statistical Analysis*

To test for a difference in the mean water temperature between the warming and control treatment levels, a standard two tailed t-test was performed and a line graph indicating mean daily temperatures for both treatments was generated. To test for difference in the total nitrogen and total phosphorous a Repeated Measures Analysis of Variance (RM-ANOVA) was used to account for differences between treatments and over time.

A preliminary RM-ANOVA with blocking was used to test for any significant time-dependent effects of experimental warming and blocks (i.e., stream of origin) on macroinvertebrate richness. Taxonomic richness was calculated using the sum of each identifiable Order detected within a sample. To test for differences in taxonomic composition between macroinvertebrate communities in control versus warmed mesocosms, I used Non-metric Multi-Dimensional Scaling (NMDS) and the resulting ordination plots. Here, Hellinger transformation of the taxonomic data was performed prior to ordination analysis. Total chlorophyll was used as a proxy for estimating periphytic algal biomass in each sample.

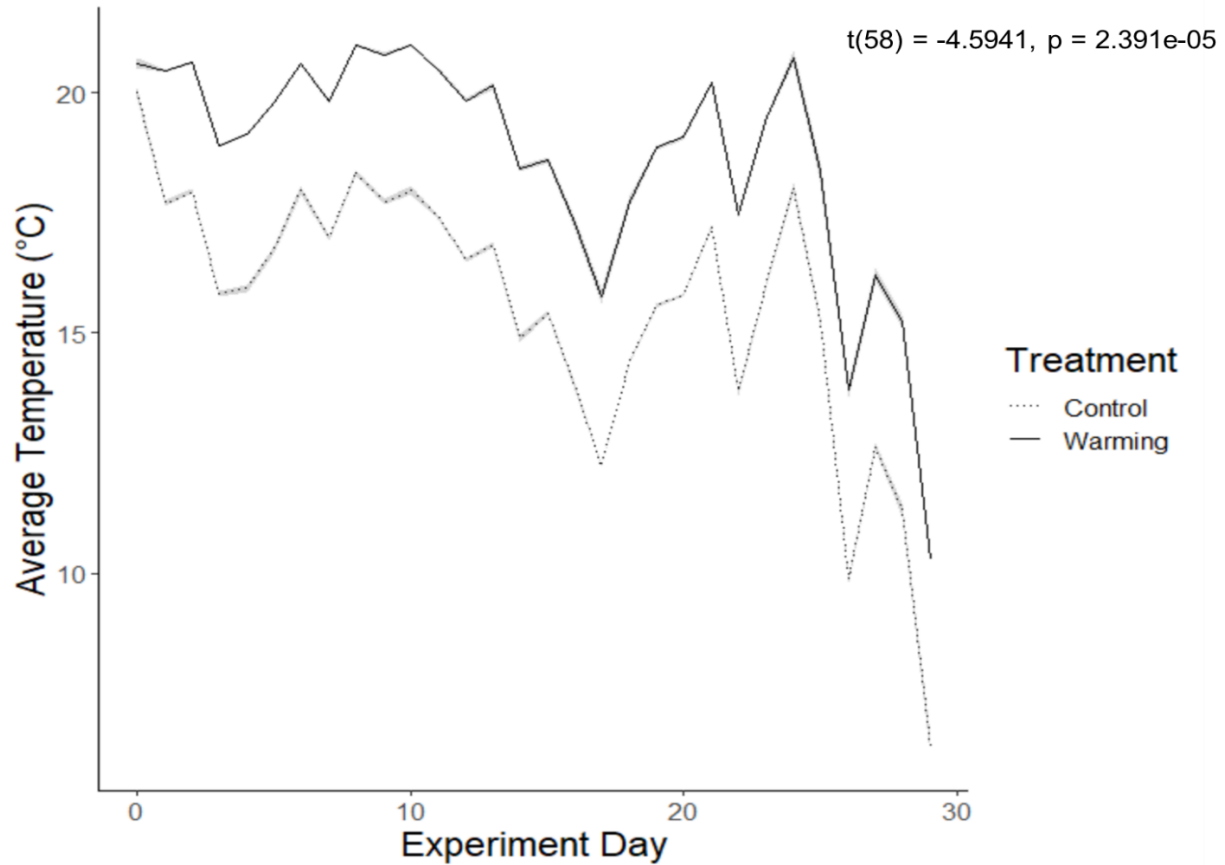


Chlorophyll and accessory pigment concentrations were transformed using  $\log_{10}(x+1)$  prior to statistical analysis. NMDS was also performed using detected accessory algal pigments to determine the taxonomic response of periphyton communities to the experimental factors. All statistical analyses were performed in R Studio (R Studio Team 2020).

## **Results**

### *Temperature*

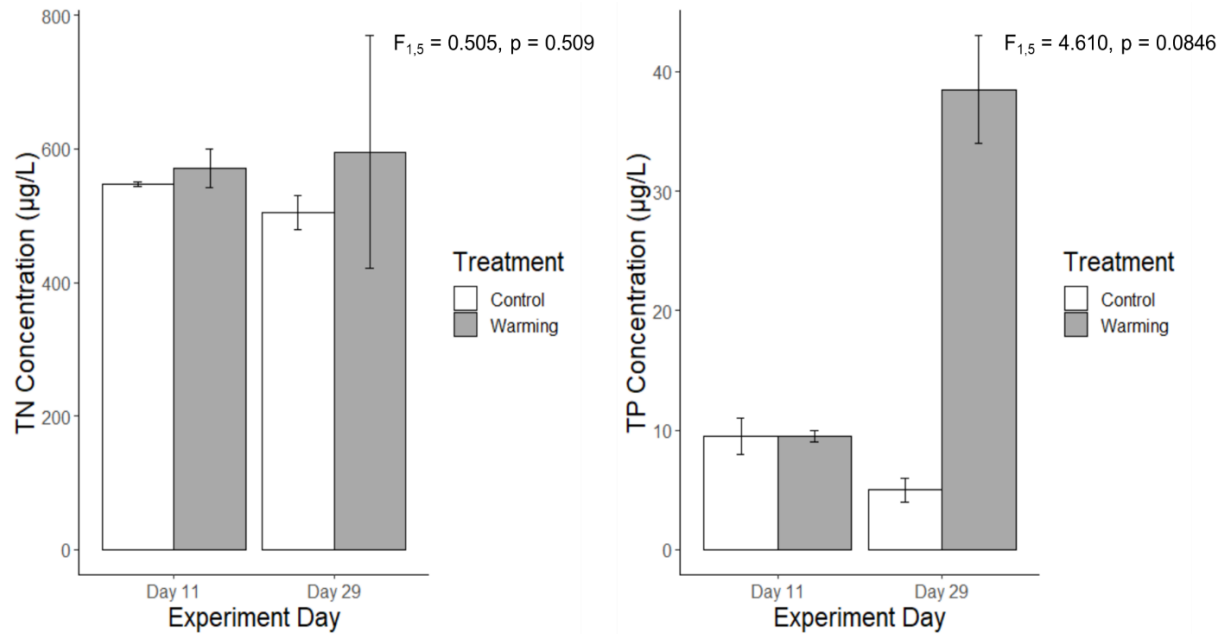
The increase in temperature was successful as the daily mean temperature of the warming treatment consistently exceeded the temperature of the controls (Fig. 2). A two-tailed t-test also indicated that the temperature difference was statistically significant ( $p < 0.05$ ). On average the warming treatment had a temperature increase of 3.14 degrees Celsius when compared to the control troughs. It should be noted that despite large amounts of variation between days there was little variation between all troughs in a treatment group (Fig. 2).



**Figure 2.** Daily mean water temperatures in the control and warming treatments. Shaded area around the lines represents the standard area of the mean.

### *Water Chemistry*

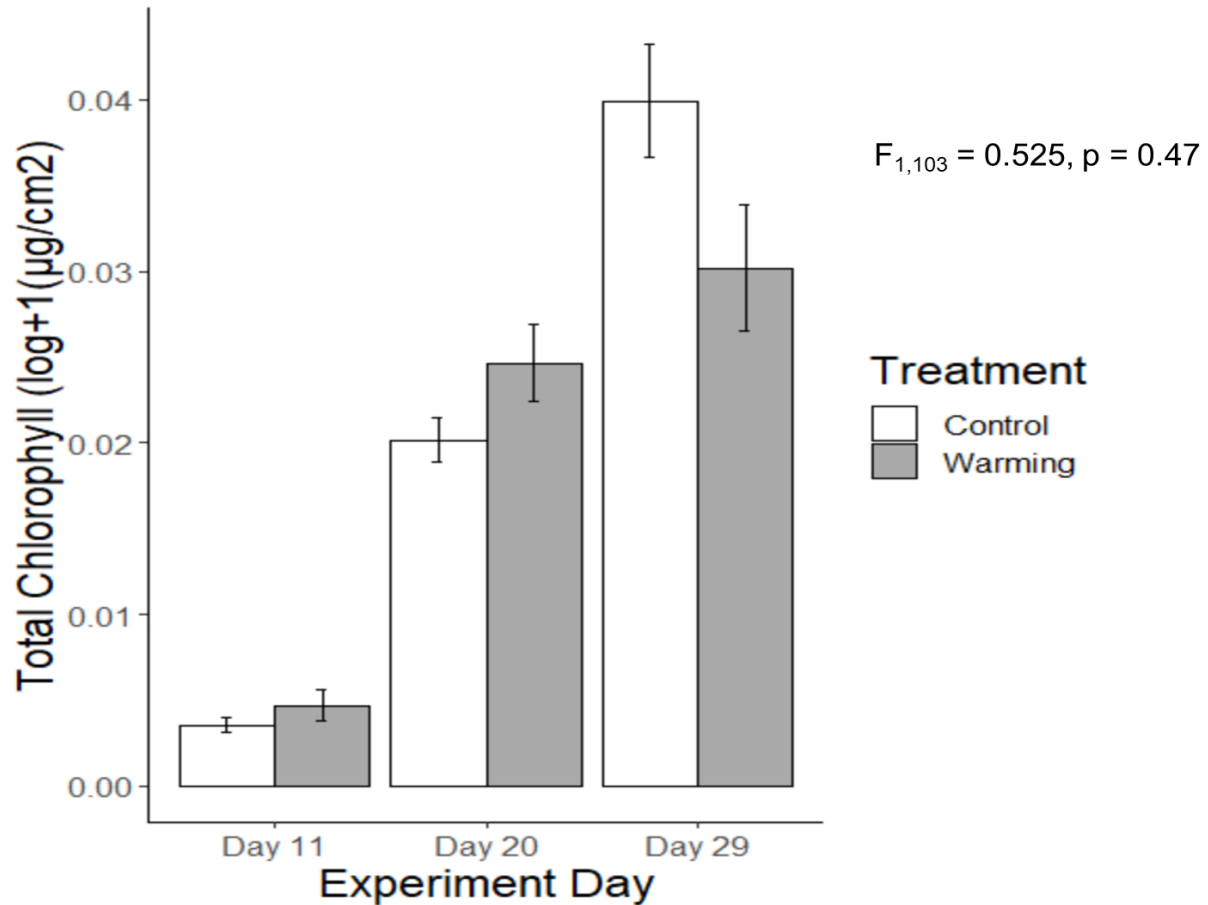
No significant differences in total nitrogen or phosphorus were detected between the heated mesocosm and controls on either sampling date (Fig. 3). Nevertheless, total phosphorus showed a striking increase in final concentration whereas nitrogen concentrations remained relatively stable throughout the experiment. Nitrogen concentrations were orders of magnitude larger than that of phosphorus.



**Figure 3.** Total nitrogen and phosphorus concentrations on day 11 and day 29 of the sampling period. Error bars represent the standard error of the mean (N = 4).

### *Periphyton*

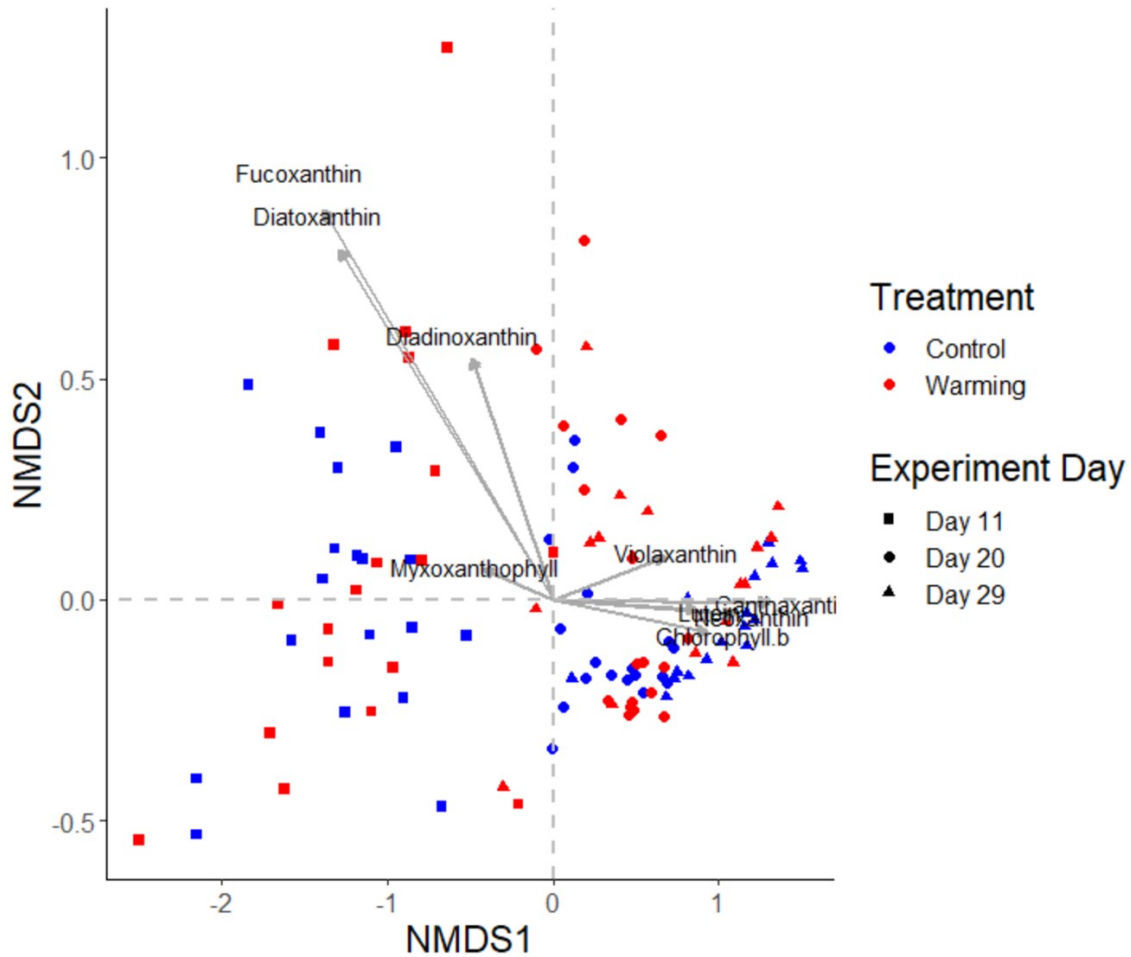
RM ANOVA revealed that there was no significant difference in chlorophyll concentration between heated and control mesocosms throughout the experiment ( $p = 0.47$ ) (fig. 4). Nevertheless, chlorophyll concentrations did significantly increase during the experiment ( $p < 0.05$ ). Interestingly, there was a notable decline and less chlorophyll in the warmed communities than the controls by the end of the experiment.



**Figure 4.** Total chlorophyll concentrations in the control and warming periphytic algal communities during the stream mesocosm experiment. Error bars represent standard error of the mean.

NMDS of taxonomically diagnostic algal pigments did not reveal distinct differences in composition between the heating treatment and controls (Fig. 5). Instead, duration of the experiment best defined the primary x-axis gradient. NMDS axis 1 captured the succession away from diatom-produced pigments (fucoxanthin, diatoxanthin) towards green algae (chlorophyll *b*, lutein, violaxanthin) over the course of the experiment. However, the secondary y-axis did capture a subtle divergence of heated communities from the controls during the experiment as controls tended to reside more in the lower right quadrant while warmed mesocosms were more

prevalent in the upper right quadrant (Fig. 5). In general, green algal pigments were more detected in the controls than in the warmed communities by the end of the experiment.

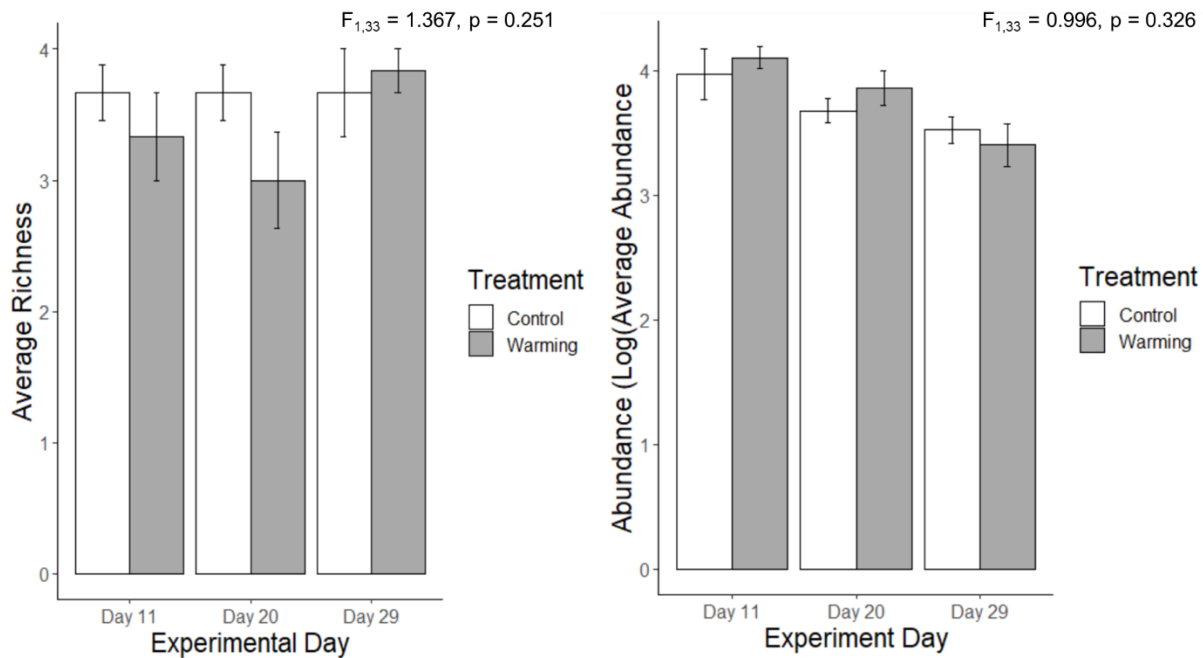


**Figure 5.** NMDS ordination of taxonomically diagnostic algal pigments in responses to duration of the stream mesocosm experiment and the heated treatment.

### *Macroinvertebrates*

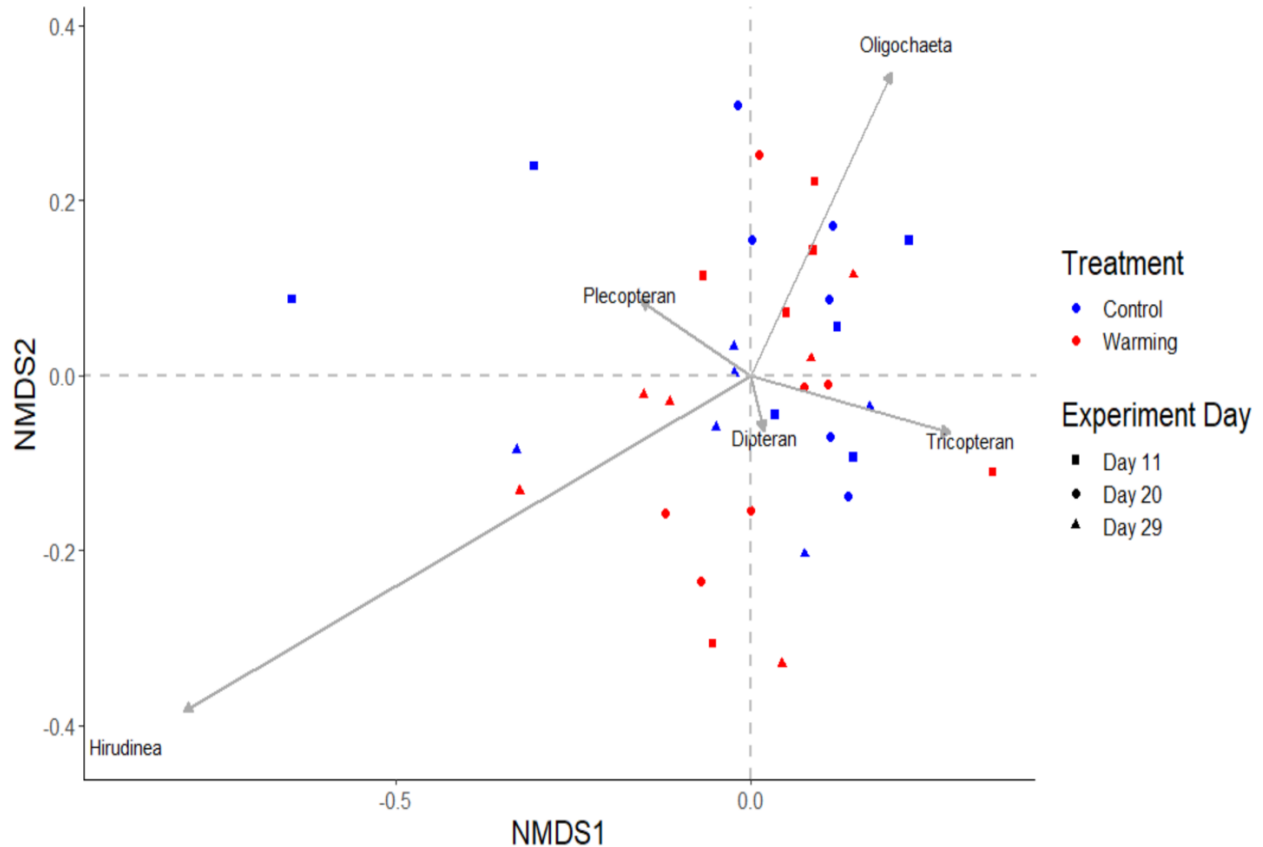
RM-ANOVA revealed no significant effects of heating on the taxonomic richness or total abundance of the macroinvertebrate communities (Fig. 6). Here, RM-ANOVA with blocking had detected that there were no significant blocking effects on these response variables, and so blocking was removed from the analysis. Taxonomic richness remained relatively consistent

throughout the entirety of the experiment with the control treatment having a higher richness in both the first and second sampling events. However, in the final sample the controls had a slightly lower richness when compared to the warming treatment, although the variation within samples was quite large. Total abundance showed a marginal, NS decrease throughout the duration of the experiment. However, there was little difference between the control and warming treatment at each sampling date.



**Figure 6.** Macroinvertebrate taxonomic richness and total abundance in heated and control stream mesocosms during the experiment. Error bars shown are the standard error of the mean (N = 6).

NMDS of macroinvertebrate taxa showed no clear patterns in date of sampling or treatment effects (Fig. 7). Most of the points fall between the main taxa observed within the samples. Very few samples contained Hirudinea thus leading to several of points appearing as outliers and ultimately contributing to the much longer vector observed for this specific order.



**Figure 7.** NMDS ordination of macroinvertebrate Orders in response to duration of the stream mesocosm experiment and the heating treatment.

## Discussion

The results did not support my hypothesis of a warming-induced trophic cascade exerting net positive effects on periphytic algae by suppressing sensitive macroinvertebrates in mountain streams. Instead, my results revealed no statistically significant effects of warming on chlorophyll-inferred algal biomass or the taxonomic richness and abundance of macroinvertebrates. However, some evidence suggested that an increase in total phosphorus and a taxonomic shift in the algal communities were occurring during the experiment in response to the warming treatment. In particular trends were showing that total phosphorus concentration and algal composition shifted on the final sampling date, however there was also an observed

increase in total variance. Thus, my experiment may have lacked the warming effect size and/or temporal scale to detect statistically significant responses by the algae or macroinvertebrates.

Below, I provide greater detail regarding potential explanations for my key findings.

The realized environmental warming treatment of 3.14 degrees Celsius was perhaps not large enough to generate detectable direct effects on benthic communities. Despite this, the temperature increase observed is consistent with previous research where water temperatures are often increased in a range from less than 1 degree to 6 degrees Celsius (Piggott et al. 2012; MacLennan et al. 2015; Piggott et al. 2015; Lowen & Vinebrooke 2016). Similar freshwater mesocosm experiments have also not detected direct effects of moderate increases in temperature on planktonic communities (MacLennan et al. 2015; Lowen & Vinebrooke 2016). Other studies reporting significant direct effects of experimental warming on freshwater biota often involved larger differences in temperature between treatment groups contained in smaller mesocosms, which likely provide less physical refugia (Petchey et al. 1999; Knillman et al. 2013). Instead, freshwater experiments involving elevated temperature when paired with other environmental treatments often did detect significant but muted indirect effects of warming (Piggott et al. 2012; Jackson et al. 2016; Morris et al. 2022). Empirical investigations of the ecological impacts of increased thermal variation rather than simply an increase in mean temperature would likely be more ecologically relevant given the increasingly extreme climatic events resulting from global warming (Vasseur et al. 2014; Pansch et al. 2018).

The lack of significant warming effects on nitrogen and phosphorus concentration in water samples collected from the mesocosms (Fig. 3) might be explained by cobble substrates lacking in nutrient recycling potential. In comparison, other research has shown higher water temperatures stimulate the release of phosphorus from relatively more nutrient rich sediment



layers (Jensen and Andersen 1992; Friberg et al. 2009; Chen et al. 2020). Nevertheless, phosphorus concentrations in my warmed mesocosms were higher than in the controls by the end of the experiment, suggesting that a lag in the chemical response could have perhaps been overcome had the experiment been longer in duration. In comparison, total nitrogen levels remained relatively unaffected by the warming treatment and time. Notably, there was a large increase in the variance observed within the warming treatment on the final sample day. This could potentially be attributed to an increase in nitrogen because of higher water temperatures. However, there is still some debate as to how temperature influences nitrogen use and turn over in aquatic systems (Friberg et al. 2009).

There was some evidence based on chlorophyll-inferred algal biomass that suggested warming had marginally accelerated aging of the periphytic algal communities relative to the controls during the experiment. Similarly, temperatures accelerated algal growth rates (Gudmundsdottir et al. 2011; Piggott et al. 2012; Tison-Rosebery et al. 2022). Further prolonged exposure to higher temperatures can also eventually cause periphytic algae to senesce more quickly (Stevenson et al. 1996). However, variable water temperatures can also result in differences in cellular chlorophyll content between periphytic algae, thereby confounding the interpretation of algal biomass responses to higher temperatures (Westberry et al. 2008; Baulch et al. 2009).

NMDS of the periphytic algal pigments revealed a marginal influence of higher temperatures on periphyton succession during the experiment (Fig. 5). Specifically, warming appeared to have suppressed the development of green algae relative to that in the controls by the end of the experiment. Otherwise, periphyton succession typically involves smaller diatoms being superseded by a diversity of larger filamentous cyanobacteria and green algae. (Hieber et

al. 2001; Piggott et al. 2015b; Brighenti et al. 2019) as was evident in the controls. Therefore, a longer experiment or more pronounced heating treatment would probably have increased in divergence of the warmed versus control periphyton community compositions and possibly also total biomass in my experiment.

The lack of significant effects of environmental warming on the total abundance and taxonomic richness of macroinvertebrate communities (Fig. 6) can also be partly attributed to the sparseness of their populations. This reflects how smaller 1<sup>st</sup> and 2<sup>nd</sup> order mountain streams typically contain low abundances of specialist taxa and fewer generalists, which would otherwise be more abundant in more productive downstream ecosystems (Giersch et al. 2017; Piano et al. 2020). Low surface water flow rates may have also limited adequate oxygenation as dipterans capable of tolerating anoxic events were the most abundant macroinvertebrates, particularly in the warmed mesocosms. The complete absence of ephemeropterans further attests to the potential anoxia in the mesocosms as these organisms generally require well-oxygenated conditions (Elbrecht et al. 2016; Beerman et al. 2018; Blocher et al. 2020; Reiber et al. 2022). However, it should be noted that despite issues with the amount of flow within the streams both trichopterans and plecopterans were found in each of the three sampling dates that occurred.

There are two potential explanations for the presence of EPT taxa in both the warming and control treatments throughout the experiment. The first is that the moderate temperature increase was insufficient to elicit any effects, alternatively there is increasing evidence that some stonefly taxa specifically can adjust their temperature tolerances in response to higher temperatures (Hotaling et al. 2020; Niedrist and Fureder 2021). As stated above the most common taxa found within the samples were dipterans, this was then followed by plecopterans. Trichopterans and oligochaetes were found in many of the samples with Hirudinea being the

rarest of the taxa observed. The prevalence of dipteran species, particularly chironomids is quite common in higher altitude stream systems (Hieber et al. 2005), thus lending support to our findings.

During the first two weeks of the experiment, I observed that there was a greater number of macroinvertebrates present within the warming treatment than in the control troughs. However, this trend is then reversed in the final sampling week where I observed greater abundances in the control treatment. One possible explanation of this is that the moderate amounts of warming within the experiment may have led to some individuals maturing at a faster rate and therefore exit the troughs earlier in the season (Birrell et al. 2020). Another possibility is that the higher water temperature induced thermal related mortality within the warming treatments, ultimately reducing the overall abundance (Piggott et al. 2012). Taxonomic richness in both the control and warming treatments remained almost constant throughout the entirety of the experimental duration (Fig. 6). Therefore, no taxonomic group was lost during the full 28-day experiment despite low rates of surface flow and increased temperatures within half of the stream troughs.

The invertebrate taxonomic ordination showed no clear patterns based on the experimental treatments (Fig 7). Whereas periphyton was clearly influenced by experimental date no similar pattern was observed in the macroinvertebrates. Ultimately this is a direct result of the low abundances per sample as well as the limited taxonomic pool present within the stream mesocosms. Hirudinea were found in very few samples which explains why that taxonomic grouping appears as an outlier in ordination space. All other taxa were found consistently within each of the samples, with some variation that can be seen in the mean taxonomic richness of each treatment. Similar effects of warming have been shown in previous

experimental stream studies. Piggott et al. (2012) showed that increased temperatures only impacted 20% of measured biological variables compared to sedimentation and nutrient additions which impacted 78% and 44% respectively. Thus, reinforcing that impacts of warming are often minimal and difficult to detect in experimental mesocosms.

To better determine the impacts of increased water temperature on macroinvertebrates individual body size could be considered in future studies. This would allow for a functional approach to measuring stressor impacts in experimental streams (Daufresne et al. 2009). I opted to not include body size in this study due to the low abundance found in many samples, as such a consistent number of measurements per sample would not have been achieved. Some larger individuals were found but they were often of very low abundance and infrequent within samples. Reiber et al. (2022) found that mesocosms do not accurately represent benthic macroinvertebrate assemblages, specifically in terms of the abundances of invertebrates found in stream troughs. This likely explained why we observed low abundances throughout the experiment.

## **Conclusion**

Despite a lack of statistically significant results, I successfully completed objective one by effectively designing and constructing a stream mesocosm system to test ecological impacts on benthic communities. As such this research provides an important step in the development and use of feasible mid-sized stream mesocosm. It also provides a replicable design that can be altered and improved upon to examine a variety of multiple stressor scenarios and how they may influence benthic communities. Ultimately increasing the accessibility of stream mesocosm research and the questions that can be examined. However, during this experiment I was unable

to effectively determine the impacts of higher water temperatures on benthic macroinvertebrate and algal communities thus not completing objective two.

For future research permanent stream facilities much like the systems observed in Europe or New Zealand should be constructed. This would allow researchers to address a greater variation of stressor combinations over a longer time frame and examine the subsequent impacts on multiple levels of biological complexity. While simultaneously allowing for increased consistency and replicability in stream mesocosm research. Greater consistency and accessibility of stream research will be vital for science to better understand how imperiled stream ecosystems will respond and change in the face of a continually shifting climate.

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### **Third Chapter: The trials and tribulations of stream mesocosms**

The first issue in a series of misfortunes with this project involved the acquisition of permits. Initially the goal of the experiment was to include live rainbow trout to test for the cumulative impacts of both non-native sportfish and warming. Because of this all-necessary animal care and ethics training was undertaken at the university. I also submitted a proposal for animal use in research, which was approved in late April of 2022. During this approval process no further permit requirements were addressed by the approval committee. However, come late July, only several weeks away from the experiment start day, the examining committee identified several other permits that would be required to perform the research. This included permits to safely transport the fish. Fish holding permits, which should have been established with the host institute however no contact was made, despite it being the responsibility of the animal care unit to notify host institutes of this type of research. As a result, I reached out to the appropriate government agencies as recommended by the provincial aquaculture specialist and the animal care unit. The initial meeting and contact for these new permits occurred on July 29<sup>th</sup>, 2022. No response was received from government offices until September 2<sup>nd</sup>, 2022.

Stemming from the permit issues the entire design of the experiment had to be altered at the last minute, shifting the focus from a multiple stressor and cumulative effects study to a single stressor experiment focused solely on warming. The main issue here being that warming effects have been largely discussed in the literature, so there would likely be few novel findings.

However, the experiment itself remains novel due to the development of the mesocosm design, alongside that previous research in the region has primarily focused on lake impacts, whereas streams have largely been overlooked.

One of the major construction hurdles was that the original stream designs were not durable. The troughs were constructed primarily of plywood with an ABS liner on the interior of the box. However, after transport from the University of Alberta to the Field station, the liner had already begun to develop cracks. The field team attempted to reseal the troughs by using a thick poly liner, however this did not fully seal the troughs. Upon further inspection it was revealed that the plywood had begun to warp in a relatively short time frame, which resulted in further cracking and separation of the ABS liner. Another major issue with the initial trough design was delays in construction. On the date that had been agreed upon for completion of construction, only two of the four sets had been completed. The third and fourth set had yet to be started. It was at this point we began examining other options for contingency plans, and settled on the poly feed bunks and began prototyping from the ground up in late June/early July. Fortunately, I was able to source all of the troughs and other construction materials in a short time span, in part due to United Farm and Auto (UFA) and Home Depot. The completed stream troughs were the finalized in August approximately a week prior to the experiment day 0.

The overall timeline for the project was quite rushed because of these issues. The experiment itself was delayed over a month because of the logistical issues with construction and permit acquisition. Sampling of the streams did not occur until after the original proposed end date of the experiment. This likely lead to seasonality issues with the experiment as this neared the end of the growing season for many of the species studied. Furthermore, this greatly reduced timeline did not allow for the experimental set up and mesocosm design to be as optimized and polished as it likely should have been.

One of the primary changes that should be made to the design of the mesocosms would be to increase the rate of flow, specifically the surface flow to better oxygenate the water

column. This would allow for the mesocosm to better approximate a first order stream/riffle zone. The lack of surface flow is like what resulted in the loss of more sensitive taxa such as ephemeropterans and gave rise to the dipteran dominant communities we observed during enumeration.

The first step in this process would be to increase the size of the pumps used. One significant draw back however is that as pump size increases the cost per unit increases at a much greater rate. To better accommodate the increase in water movement from a larger pump, the tubing used should also be increased in size to a 2-inch diameter. Further increasing the size of the inflows and outflows would also be recommend if increasing the output of the pump. Alongside this the addition of a DO probe would allow researchers to determine if the oxygenation levels within the stream trough are sufficient to ensure the survival of rarer taxa.

The current mesocosm design would also benefit from greater consistency in the rate of flow throughout the experiment. Addition of the ball valves allowed for rapid stream calibration and for the 3 weeks of the experiment the system was relatively stable. However, something occurred within the last week that resulted in the first block of streams having reduced rates of flow. The likely explanation is that the pump becoming fouled with debris, despite the outflows being cleared regularly. Another potential explanation is that there was a significant heat wave then immediately followed by a drastic cooling which may have impacted the system. Although the other three blocks did not appear as impacted.

The construction of the streams themselves would also benefit from several design changes. This predominantly regarding the robustness and longevity of the system. For example, better reinforcement of both inflow and outflow through the use of bulkheads. Although this is a more expensive option, the current design allowed for a fair amount of movement of the inflow

and outflow pipes ultimately increasing the risk of leaks. For greater longevity brass hose barbs and connections could be used as opposed to the nylon fittings, which can degrade over time when exposed to sun. However higher cost per part should be considered.

In an initial attempt to salvage the multiple stressor design and test for fish effects we colonized the streams with macroinvertebrates from two separate streams. However, due to the fact that this was a last-minute pivot all periphyton tiles originated from the same stream, thus not following the same design as invertebrates. Ultimately no block effect based on stream of origin was detected for macroinvertebrates and as such was not including in the final analysis of the data, and we simply focused on a single factor warming experiment. However, this likely introduced some unknown variance into the experiment and should be avoided in future studies to better compare treatment effects.

To better examine the impacts of increased water temperatures on benthic communities, greater variation in temperature should be used as this will likely represent a more realistic scenario of environmental impacts. Despite a statistically significant temperature difference between the control and warming treatment, there was no real effect observed in either the macroinvertebrate or periphytic communities present based on simply increasing mean water temperatures. Another potential explanation for the lack of significant effects was that the sample sizes were simply too small to accurately detect a response to the higher water temperatures. A post-hoc power analysis indicated that in order to detect a temperature effect with a large effect size, a total sample size of 79 would be required. If a medium effect size is assumed, then required sample size increases to a total of 152. Indicating that even if the current experimental design elicited a large effect size the impacts on macroinvertebrate communities would not have been detectable.

Another logistical challenge for the experimental set up was the power supply available at the field station. During the initial set up and colonization period the streams experienced a full power outage. This outage occurred several times throughout the day, with the first outage having an unknown duration. These outages resulted in the loss of flow and heating to the streams, alongside a large degree of water loss. Ultimately the power was then split between two separate buildings at the field station to better serve the streams and limit the strain on the power grid at the station.

Finally, a way of ensuring a better method of preventing debris buildup in the pumps would be beneficial. This could include a more precisely sized netting or cage surrounding the streams and holding tanks to mitigate any terrestrial inputs such as leaves. Conversely a scheduled maintenance regime would likely solve this problem but given the relatively stability of the system and the short experimental time frame this was never fully established.

One of the major issues with this design is that the shared holding tank among three troughs result in a large degree of pseudo replication within the treatment groups. Each stream was in blocks of three thus there is a lack of independence, realistically reducing the number of replicates to two control and two warmed. The reason behind this design choice was largely governed by limited design and construction time, as well as a lack of resources (space, materials and financial). It is also an artifact of the original design where there were four distinct treatments to test for individual and cumulative impacts.

Thus, in future studies more separation between troughs is highly recommend. This could be done by having single troughs as the recirculating system which a distinct pump and holding tank. Another method would be to increase the total number of replicates. However, this creates logistical issues for the total cost of the experimental set up as well as the space requirements for



the stream mesocosms. Although it would ultimately result in a more accurate and statistically sound experimental design. Despite the need for improvements, this stream mesocosm design provides a good template for future research and can serve as a basis for more feasible and economically viable stream experiments.

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## **Appendix**

### *Literature Review Methods:*

For the literature review a search was performed in Web of Science using key words such as “Stream”, “Mesocosm”, “Experimental streams”. From here papers were read and the results themselves were analysed to determine if the research fit the selection criteria. For the paper to be included results had to be presented as a mean with some measure of variance, either standard deviation or standard error. This was needed to calculate the relative response ratio of each study. The reasoning behind the use of a relative response ratio is that it is a more accurate method to compare results while taking into account differences in methodology or sample sizes. If mean values or variances were not included in the results or supplemental information the paper was excluded from the review. Other parameters for inclusion were that the experiment had to be focused solely on lotic systems. Any pond or lake mesocosm study was not included. There was no limit on levels of biological organization or on stressor type being researched. If a study examined multiple types of stressors, then each of these were included as a separate data point in the analysis.

From these criteria a total of 23 distinct papers were used in the final literature review resulting in 46 separate data points accounting for a variety of stressor types and level of biological organization. These stressors included factors such as drought, warming, sedimentation and various types of pollutants or nutrient additions. All studies included focused on either benthic macroinvertebrates or benthic algal communities.