PROPOSAL NARRATIVE

<u>Increasing water temperatures within *Hexagenia* habitat to see overall survival and growth response</u>

1. Proposal Narrative

A. Abstract

Rivers are under threat by climate change, including global warming, which could limit their value to both humans and wildlife that depend on them. However, it remains unknown how many riverine organisms are being affected by climate change. Mayfly (Order: Epheroptera) are a common aquatic insect in large rivers that contribute greatly to river ecosystem functions, such as nutrient processing, and serve as indicators of overall river overall health. Although recent evidence suggests mayfly (*Hexagenia* spp.) populations in the Upper Mississippi River are currently in significant decline, less is known about what is driving declines in populations. In this study, I will explore how increasing water temperature (15℃ as the control, 20℃ and 25℃) affects mayfly survival and growth rate using a controlled and replicated laboratory mesocosm system that mimics natural river habitat. I predict that mayfly survival and growth rates will be reduced due to the increase in water temperature. The outcomes of my research could be a precaution for how mayflies in the Upper Mississippi River and the La Crosse community might be affected by increasing temperatures associated with climate change. As our region and world continue to warm, the experimental approach could be adapted to test the sensitivity of other insects to increasing temperature and my results could inform how aquatic insects will respond to climate change.

B. Background/Statement of the Problem/Significance of the Project

Rivers play a critical role in nature and wildlife that depend on them for habitat and food resources. Rivers also serve as important purposes for humans, including flood control, recreation, transportation, irrigation, power generation and as a food source (Kammerer, 1987). However, human activities have altered the structure and function of rivers by changing river flow regimes, causing pollution, and introducing non-native species into the waters (Karr and Chu, 1999). More recently, anthropogenic climate change and associated widespread climate warming threatens rivers and sensitive riverine biota through increasing water temperature. Therefore, understanding how changes in water temperature affect riverine biota has become a priority of ecological research and river management.

Rivers are home to diverse communities of aquatic insects and other macroinvertebrates. Aquatic insects are indicators of water quality and ecosystem health because they are sensitive ecological changes within their habitats (Luiza-Andrade, 2020, Jooste 2020). For example, many aquatic insects, such as mayflies, depend on fast water currents that rivers provide and high levels of dissolved oxygen (Klubertanz, 2016). Alterations of river flow regimes and water quality often reduce river flow and lower dissolved oxygen and thus can have a negative effect on mayfly populations (Klubertanz, 2016). Without mayflies and other aquatic insects, rivers may no longer be able to support other plants and animals considering insects serve as an important food resource. Furthermore, natural river ecosystem functioning, such as nutrient processing and improving water quality, may also be negatively affected by the loss of aquatic insects considering these organisms help process organic matter and collect and filter fine particles of nutrients and sediments from the river. Therefore, it is important to understand how global change, notably increasing water temperatures, will affect aquatic insects in order to predict changes in river ecosystem functioning and water quality.

In recent years, temperatures around the Midwest have been unpredictable and varying depending on the season. The National Weather Service has generated future outlooks of temperature and predict that temperatures over the majority of Wisconsin predicting to be 33% higher than average for the months of May through June and a precipitation probability 40% above average these predictors are essential (NWS, 2020). These predictors could mean the increase or decrease in the growth and emergence of aquatic insects. For example, increasing water temperature in accordance to future climate conditions negatively affected the egg survival and reduced adult survival and body size rate of *Enallagma civile,* a predatory species of dragonflies (Starr and McIntyre, 2020). Constrastingly, Jooste, Samways and Deacon (2020) found no adverse effects of increased water temperature of beetles and dragonflies in South

African ponds, although species did show strong habitat preference for cool-water refuges. These past studies on the response of riverine biota help guide future experiments for how to manage our environments properly for the species living within them. However, there is still much to be researched for other important species, such as mayflies (Order Ephemeroptera), that can be conducted including behavior, predator versus prey manipulations, competition rates and size rates.

Mayflies, *Hexagenia,* which are large burrowing flies that can be found in larger bodies of water such as the Mississippi River and Lake Michigan and Superior (Klubertanz, 2016), are detritivores that help prevent the buildup of biomasses like algae and detritus in rivers and lakes (Kammerer, 1987). Despite their short-lived life span, and small body size (4 mm to 3 cm long) they manage to fend off their list of predators and serve as good indicators of water pollution (Salles, 2000). Mayflies are an important food resource in that their eggs are eaten by snails and other insect larvae and the nymphs and adults are preyed on by frogs, fish, birds, water beetles and other predatory insects (Salles, 2000). Mayflies are one small aquatic insect but serve an important role in the food chain and depend on the river for nutrition which is why rivers are such a valuable aspect to our environment.

Recent evidence suggests *Hexagenia* populations have dropped 52% in the Upper Mississippi River area from the years 2012 to 2019, which creates some serious concern for what is to come given global change (Stepanian et al., 2020, Figure 1). The causes to this drastic decline are likely due to rising water temperatures within the Great Lakes from global warming and rising pesticide use around the waterways (Stephanian et al., 2020). However, to our knowledge, there are no laboratory experiments that have explored the effects of increased water temperature on the growth and survival of the *Hexagenia* mayfly. Therefore, there is a gap in our understanding of mayfly presence and emergence in river ecosystems.

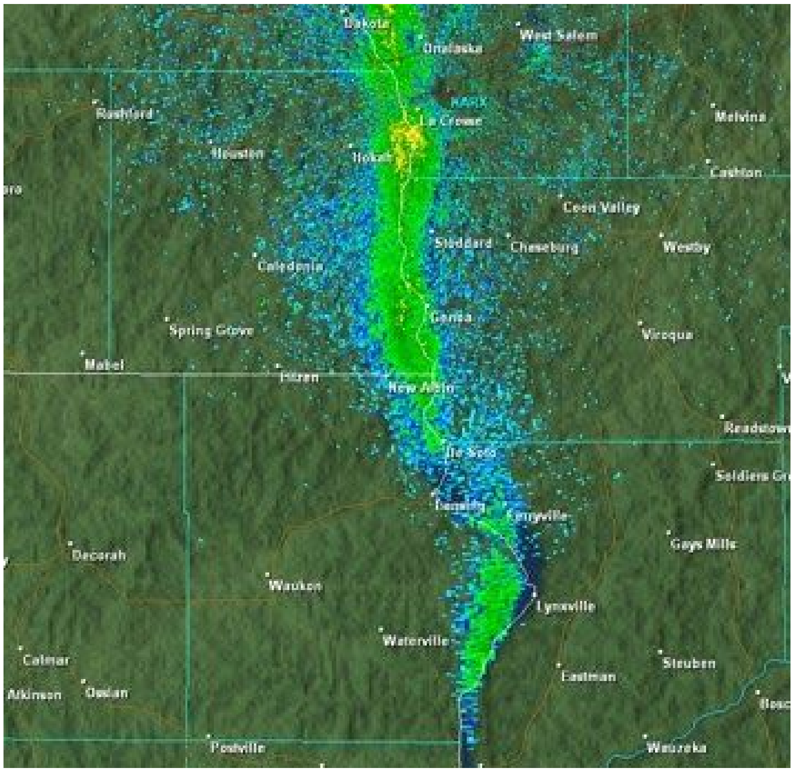

Figure 1. A NexRad radar image showing the density of mayflies covering Northern cities around the Mississippi River in 2018. (Courtesy of La Crosse Fish & Wildlife Conservation Office with credit to: National Weather Service)

C. Objectives / Specific Aims

The aim of this research project is to test the effect of increased water temperature on the growth and survival of *Hexagenia* mayflies by observing the number of mayflies that survive within each manipulated water habitat. This information could help inform the presence and success of future mayfly emergence events that impact the La Crosse community. This can be one step into determining the precautions that may need to go into consideration in regards to water quality of the waterways for La Crosse residents.

To this end, I predict that:

- Increased water temperature will reduce *Hexagenia* mayfly survival
- Increased water temperature will reduce *Hexagenia* mayfly growth rates.

D. Methods

Mesocosm design

I will use pre-fabricated mesocosms (*Living Streams* by Frigid Units, Toledo, Ohio) that mimic natural flowing water habitats. Each rectangular mesocosm (200 cm x 60 cm x 58 cm, 500-liter capacity) will be filled to a height of 20 cm with a representative substrate from the Mississippi River to provide suitable mayfly habitat. Dechlorinated tap water will be used to fill the remainder of the mesocosm, providing a 15 cm water depth. Water will be pumped continuously into mesocosms from a 500-L tank at a velocity of 84 L/s providing a surface water depth of 15 cm. Water temperature will be manipulated (∓ 1℃) using a 1000-W heater and digital thermostat and allow for up to 3 different treatment temperatures. A 12:12-light:dark cycle will be applied using Grolux (35W, 8500 K, Sylvania Inc., Noida, India) aquarium lights above mesocosms.

Experimental design

To measure the effect of different water temperatures on mayflies, I will use three different treatment factors, 15℃ as the control, 20℃ and 25℃. There will be a total of 12 mesocosms total with each treatment having four replicates. The mayflies will be collected from the Mississippi River using a standard dip net and the mayfly density in the mesocosms will be similar to their natural environment. The experiment will take approximately two to four weeks to allow for appropriate temperature manipulation and measure of growth and survival rates. I will be measuring the growth by measuring 100 of the mayflies within each mesocosm using a ruler and then taking an average of each mesocosm. Measuring the survival could be challenging but I plan on at the end of the two to four week observation period separating them into sorting trays, living or dead, and then counting them. I plan on using the alive mayflies for length measurements and growth estimations.

After considering the steps and action I will need to take for this research project, I am estimating the completion of the whole research project to take approximately four months to complete. It will take about a month to prepare the mesocosms for the mayflies habitat, to get the water and substrate all under control and running before adding the mayflies. I think it will take a week to be able to collect a significant amount of mayflies from around the Mississippi River and bring them back to the lab. The actual manipulation of the water flow inside the mesocosms along with observation and data collection will take between two and four weeks and another week for data processing. I am anticipating my data analysis will take two weeks and then to compile all the data and research into writing will take me around a month to complete. As stated previously, the ultimate goal of this research experiment is to see how manipulating the water temperatures within a Mayfly habitat alters the overall survival rate.

E. Final Products and Dissemination

I will incorporate my findings and research into a poster to be presented at UWL's Research & Creativity Symposium. Next, I plan to publish my research in UWL's Journal of Undergraduate Research or a peer-reviewed journal article.

F. Budget justification

Throughout this research project, I will be spending a minimum of 5-10 hours per week inside the lab with the possibility of additional time when needed for extensive parts of the experiment. During this time in the lab I will be maintaining the water temperature for each habitat by adding water if necessary and taking the temperature of each mesocosm 4-5 times a week. I also will need to observe and count the number of mayflies within each mesocosm every three to four days. I am requesting a $1,000 scholarship for all the hard work I will be conducting over the course of the four months I think it will take me to complete the research project.

I am requesting $1,000 to purchase water heaters, flow pumps, and supplies such as thermometers and a type of rock sediment to replicate a waterbed source for the 12 mesocosms used in my experiment. This will allow me to manipulate water temperatures and homogenize flow rates across the experimental treatments. I will be collecting the mayflies with a standard dip net from the Mississippi River to use for my research.

References

Fisheries, Midwest Region. La Crosse Fish & Wildlife Conservation Office. (2018).

Jooste, M.L., Samways, M.J. & Deacon, C. 2020. Fluctuating pond water levels and aquatic insect persistence in a drought-prone Mediterranean-type climate. *Hydrobiologia* 847, 1315–1326. https://doi-org/10.1007/s10750-020-04186-1

Kammerer, J.C. 1987. Rivers, Streams, and Creeks. *USGS.* OFR, 87-242.

Karr, J.R. and Chu, E.W. (1999) Restoring Life in Running Waters: Better Biological Monitoring. Island Press, Washington DC.

Klubertanz, T. H. 2016. Mayfly larvae of Wisconsin. *Board of Regents of the University of Wisconsin System* G4074: 1-44, 2016.

Luiza-Andrade, Ana, Calvao, Batista, L., Dias-Silva, Karina, Faria, Justino, A.P., Brasil, Schlemmer L., et al. (2020) Aquatic Insects and Their Environmental Predictors: A Scientometric Study Focused on Environmental Monitoring in Lotic Environmental. *Environmental Monitoring and Assessment* 192.3 (2020): Environmental Monitoring and Assessment Vol.192(3).

Salles, A. (2000). Ephemeroptera: Mayflies. *University of California Berkeley.*

Starr, S.M., McIntyre, N.E. (2020) Effects of Water Temperature Under Projected Climate Change on the Development and Survival of *Enallagma civile* (Odonata: Coenagrionidae). *Environmental Entomology* Volume 49(1), Pages 230–237. https://doi-org/10.1093/ee/nvz138

Stepanian, P. M., Entrekin, S. A., Wainwright, C. E., Mirkovic, D., Tank, J. L., & Kelly, J. F. (2020). Declines in an abundant aquatic insect, the burrowing mayfly, across major North American waterways. Proceedings of the National Academy of Sciences, 201913598. https://doi.org/10.1073/pnas.1913598117

Three month outlooks official forecast May-Jul 2020. (2020). *National Weather Service.*