

HOW CLIMATE CHANGE AND AGRICULTURE COULD RESHAPE THE MIDWESTERN
STREAM FISH COMMUNITIES

BY

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DISSERTATION

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ABSTRACT

Global climate change is a major driver of freshwater fish community changes. Agriculture further intensifies the impacts of climate change in the Midwestern USA. To quantify such impacts on fish communities, physiological experiments can provide a mechanistic understanding, but have scaling issues, while ecological modeling can handle large-scale projections but lack resolution locally. It is with optimizing and integrating these approaches that we can offer better guidance in conservation planning. In this dissertation, I sought to demonstrate that physiological experiments can effectively quantify the impacts of short-term, small-scale extreme events, heatwaves, on individual species and species interactions. When it comes to longer-term and broader-scale understanding, ecological modeling coupled with physiological information can handle watershed-scale projections of different climate and agriculture scenarios. Thus, combining these two approaches together could help generate more effective and accurate conservation decisions.

In this dissertation, Chapter 2 used physiological experiments to show that widespread, warmwater prey, fathead minnow (*Pimephales promelas*), had high thermal resilience under extreme heatwaves in agricultural landscapes, including rapid returns to metabolic homeostasis and transient increases in critical thermal maximum (CT_{max}). However, even for fathead minnow, experiencing heatwaves came with costs, including reduced anaerobic capacity and impaired antioxidant defense. Chapter 3 was built upon this finding and took these conclusions one step further to the fish community, by quantifying the influence of heatwaves and summer temperatures on both predator and prey fishes, as well as cool and warmwater fishes. Compared to the warmwater predator largemouth bass (*Micropterus salmoides*) and prey fathead minnow, the

coolwater predator walleye (*Sander vitreus*) had many disadvantages in the local community under summer temperature with heatwaves, including higher standard metabolic rate, limited aerobic scope, low metabolic activity when encountering prey, and reduced swimming performance after experiencing a heatwave. Finally, Chapter 4 further broadened the scope to the watershed level by using ecological models coupled with physiological information for each species. In Chapter 4, I used contemporary fish and environmental records to build base models. These models showed that flow, temperature, nitrate, and watershed area were among the top predictors of functional dispersion, functional evenness, and species richness in local communities. Functional dispersion, the metric including physiological information, had > 50 % better performance than traditional species richness based on R^2 . Then, based on different climate and agricultural conservation scenarios, I projected possible fish community changes in the Kaskaskia River Watershed. Species richness and functional dispersion were predicted to decrease under climate change in most sites across the watershed, up to 20% and 4% by 2099, respectively. When potential agricultural conservation practices were simulated under climate change, the decreasing trends of species richness and functional dispersion remained, suggesting climate change outweighed potential agriculture conservation efforts.

When considered together, the chapters included in this dissertation demonstrate the importance of 1) considering different scales (i.e., individual species, communities, and the whole watershed) and 2) considering both short-term extreme events and long-term overall trends when designing conservation plans. This dissertation also shows the value and the potential of combining experimental physiology and ecological modeling on freshwater conservation.

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CHAPTER 1: INTRODUCTION

In the era of rapid climate change, ecosystems have irreversibly been thermally affected (Buisson et al., 2008). Freshwater ecosystems are among the most threatened (Closs et al., 2016), with the highest extinction rates among vertebrates (Grooten & Almond, 2018; Tickner et al., 2020). This is mainly because over 1/3 of global freshwater discharge has been altered by agricultural, industrial, or urban development (Albert et al., 2021). Within agricultural regions in particular, thermal impacts are further aggravated by reduced riparian cover, disappearing thermal refuges, altered flow patterns, and decreased groundwater recharge (Caissie, 2006; Loheide & Gorelick, 2006; Dugdale et al., 2013). Where global climate change and regional agricultural disturbances act together, such as in Midwestern agroecosystems, understanding 1) how different species react; 2) how different trophic levels interact; 3) how the entire fish community responds, will be key to designing effective conservation plans. To achieve these goals, physiological experiments and ecological modeling are two ideal approaches, with physiological experiments offering mechanistical understandings of fine-scale responses of fishes under climate change, while ecological modeling is capable of projecting broader-scale longer-term community changes. Combining these two methods together provides the potential to maximize both advantages.

Under climate change, thermal stressors can be viewed through both short-term and long-term perspectives. In the short term, heatwaves, referred to as the extreme weather from hours to days, are increasing in both frequency and intensity (Jentsch et al., 2007). Thus, heatwaves could disproportionally shape species performance and distribution despite their short duration (Jentsch et al., 2007; Sandblom et al., 2016). To confer resilience under heatwaves, species have to rely on phenotypic plasticity, or acclimation (Seebacher et al., 2015). Long-term warming requires aquatic

species to develop evolutionary adaptations to elevate physiological performance. Otherwise, species distributions will change, or extinction will be inevitable (Jutfelt, 2020). Responses among species under climate change are not independent. For species coexisting in a local community, interactions, such as competition and predator-prey interactions, should also be considered.

At a fine scale, we can use physiology to mechanistically understand how aquatic species respond and adapt to thermal stressors, with metabolism and locomotion of particular interest. Metabolism, measured as the rate of oxygen consumption, is arguably one of the most important (Brown et al., 2004). Standard metabolic rate, the minimal metabolism for the subsistence of merely essential homeostasis, is highly sensitive to temperatures (Chabot et al., 2016). The capacity of maintaining low standard metabolic rate during warming, or being energy efficient, means competitive advantages for certain species under climate change. Aerobic scope, defined as the difference between maximum and standard metabolic rates, is also important as it directly determines species fitness (Clark et al., 2013). Aerobic scope offers metabolic potential for essential activities, including growth, reproduction, and locomotion. Among these activities, locomotion, also called swimming capacity, is a physiological trait extensively studied due to its importance in prey consumption and predator avoidance in stream fishes (Farrell, 2009; McKenzie, 2011). To date, studies on metabolism and locomotion have largely overlooked the effects of short-term heatwaves, and have only viewed temperature as a factor remaining stable at different levels (Morash et al., 2018). Also, metabolism and locomotion have been largely examined using individual species, overlooking species interactions that can be critical for ecosystem functions as altered species interactions could impact nutrient and energy pathways, further changing community composition (Rall et al., 2010; Domenici et al., 2020). Thus, only if we consider thermal stress in the context of both short-term and long-term perspectives, can we

obtain a more comprehensive, mechanistic understanding of how fish species react and interact under climate change.

When it comes to considerations of thermal stressors at a broader scale, ecological modeling is another approach to investigate changes in freshwater ecosystems, and can be used in combination with mechanistic physiological methods. With increasing sampling effort and environmental information, modeling approaches offer ecological insights into thermal impacts on species distributions at a larger scale compared to physiological approaches (Elith & Leathwick, 2009; Buckley et al., 2010). Instead of separating mechanistic physiology methods from correlative modeling methods, it is feasible to incorporate physiological understandings into ecological modeling, thus better utilizing physiological mechanisms to optimize species distribution predictions (Kearney & Porter, 2009). By simulating fish community changes under different global climate change and regional agriculture practice scenarios, we can evaluate the effectiveness of different conservation efforts, thus offering realistic and efficient management recommendations.

Watersheds in Illinois have been deeply reshaped by agriculture, while still having a productive and speciose nature (Smith et al., 2010), with the Kaskaskia River Watershed in the southwest of Illinois, USA being an illustrative example. Such freshwater systems provide a perfect opportunity to investigate how global climate change and regional agricultural impacts act together on freshwater fish communities. Thus, the overall objective of this dissertation is to integrate physiological experiments with ecological modeling to quantify how Midwestern fish communities could be altered by climate and agricultural changes, and project how fish

communities may change in the future. More specifically, I sought to answer the following questions ranging from small to large time and spatial scale in my three chapters:

- **Chapter 2:** Over short-time scales, from the perspective of individual species, how do and how will individual species in highly degraded agricultural streams respond physiologically to summer heatwaves? For this, I:
 1. Monitored and recorded high-resolution summer stream temperatures in both agricultural and forested sites in the Kaskaskia River Watershed
 2. Simulated current and next-future heatwaves in the laboratory on one widespread warmwater prey species, fathead minnow (*Pimephales promelas*)
 3. Examined critical thermal tolerance, enzyme performance, and metabolism during heatwaves to quantify the physiological performance of fathead minnow
- **Chapter 3:** Over short-time scales, from the perspective of an aquatic community, how do and how will species with different thermal preferences respond to climate change with heatwaves, which could lead to changes in community composition in the Kaskaskia River Watershed? For this, I:
 1. Simulated stream temperatures in the laboratory with heatwaves expected with future climate change, using warmwater prey, fathead minnow, warmwater predator, largemouth bass (*Micropterus salmoides*), and coolwater predator, walleye (*Sander vitreus*)
 2. Quantified the metabolism and swimming performance individually during heatwaves
 3. Examined the metabolism of both predators and prey during predator-prey interactions.

- **Chapter 4:** Over longer-time scales, from the watershed perspective, how will climate and agricultural changes affect riverine biodiversity, when incorporating both ecological and physiological information from fish species? For this, I:
 1. Quantified the contemporary spatial distribution of fish biodiversity based on functional diversity and species richness
 2. Projected potential changes in biodiversity under future climate change scenarios
 3. Defined the effectiveness of different agricultural conservation practices on biodiversity under climate change

CHAPTER 2: THERMAL TOLERANCE OF PREY FISH TO HEATWAVES IN AGRICULTURAL STREAMS: WHAT DOES NOT KILL YOU MAKES YOU STRONGER?

2.1 Introduction

In the era of rapid climate change, heatwaves, referred to as short-term extreme weather from hours to days, could disproportionally shape species performance and distribution (Jentsch et al., 2007; Sandblom et al., 2016). Globally, within all ecosystems, freshwater ecosystems are among the most thermally threatened (Closs et al., 2016; Yousefi et al., 2020). Regionally, the intensity of heatwaves in freshwater ecosystems could be further elevated, such as by thermal pollution from power plants (Raptis et al., 2016) and high surface energy flux from urbanization (Nichol et al., 2020). Besides these, agriculture is another major factor that intensifies heatwaves in freshwater ecosystems, due to 1) increased solar radiation after the reduction of riparian cover (Caissie, 2006); 2) reduced thermal refuges following channelization (Dugdale et al., 2013); and 3) decreased cool groundwater recharge after agricultural water use (Loheide & Gorelick, 2006). Additionally, the impacts of heatwaves on aquatic organisms in agricultural landscapes could further be exacerbated by low dissolved oxygen from fertilizer use and toxicants from pesticide application (Wang et al., 2003; Verberk et al., 2016; Op de Beeck et al., 2017).

To understand how fish respond to thermal stress, physiological approaches have been widely used. Among all physiological aspects, energy metabolism, measured as the rate of oxygen consumption, is arguably one of the most important for fish, as it influences locomotion, growth, and reproduction (Brown et al., 2004). Thermal sensitivity of metabolism has been explained by the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis (Pörtner, 2010), despite

with limitations (Clark et al., 2013; Jutfelt et al., 2018). OCLTT conjectures that during warming, tissue oxygen demand increases exponentially until reaching a critical temperature, where oxygen demand for maintenance exceeds cardiorespiratory capacity, causing loss of performance (Schulte, 2015; Blasco et al., 2020). To evaluate the impacts of such critical temperatures on the thermal tolerance and metabolism of aquatic organisms, many physiological studies targeting long-term temperature increases have been conducted. However, short-term heatwaves have obtained considerably less attention (Morash et al., 2018), partially due to a lack of real-time, high-resolution field temperature information. This limits the extrapolation of lab-derived results to real-world conditions, thus causing us to overlook real-time metabolic costs and adjustments of fish in nature, such as increased standard metabolism of Atlantic salmon (*Salmo salar*) under more variable environment (Oligny-Hébert et al., 2015), as well as elevated excess post-exercise oxygen consumption (EPOC) of Nile perch (*Lates niloticus*) after acute thermal challenges (Nyboer & Chapman, 2017). Thus, bioenergetic models only including long-term temperature increases, while not considering heatwaves, are limited in predictive power.

By monitoring how fish react to extreme heatwaves, we cannot only have a better mechanistic understanding of the real-time adjustments of thermal tolerance, but also examine how prior experience to heatwaves could shape later reversible changes in thermal tolerance (i.e. heat hardening) (Bowler, 2005; Schaefer & Ryan, 2006; Gunderson & Stillman, 2015). Such “carryover effects” (O’Connor et al., 2014) could be important for fish overcoming the increasing frequency and intensity of heatwaves under global warming. However, heat hardening normally comes with costs, including reduced aerobic scope due to elevated metabolism, thus impairing fitness-related performance like prey consumption and predator avoidance (Farrell, 2009; Oligny-Hébert et al., 2015). Also, rapid increases in body temperature during heatwaves can lead to oxidative damage

in ectotherms (Kaur et al., 2005; Heise et al., 2006; Guzzo et al., 2019). By monitoring the physiological responses of fishes under different frequencies and intensities of heatwaves, we can better understand their protective mechanisms and costs, then predict potential thermal thresholds for ecosystem stability.

Streams and rivers in the Midwestern USA are known for their productive and speciose nature (Smith et al., 2010). With such diversity of prey and predators, a key question is: what species should be prioritized for consideration under climate change? A common prey fish in North America, fathead minnow (*Pimephales promelas*), became my focus here for the following reasons. First, the performance of prey fish under heatwaves is critical for ecosystem stability, as they act as important nutrient and energy pathways (Johnson et al., 2005; Hebert et al., 2008). Dominant prey species, like fathead minnow, are critical from a food web perspective, considering both their widespread natural distributions across North America and the fact of being widely stocked to support fisheries (Colvin et al., 2008; Page & Burr, 2011). Second, despite the upper thermal tolerance of smaller species being less impaired during warming relative to larger ones, the acclimation potential of smaller species is lower (Rohr et al., 2018; Leiva et al., 2019). Fathead minnow, as a eurythermal prey species, has around a 2 °C lower critical thermal maximum (CT_{max}) compared to its eurythermal predators largemouth bass (*Micropterus salmoides*) and channel catfish (*Ictalurus punctatus*) when acclimated at 25 °C (CT_{max} : 36.1 °C vs. 37.8 – 38.7 °C) (Beitinger et al., 2000; Carveth et al., 2006). Thus, heatwaves could have different consequences for small prey relative to large predators, influencing the balance in nutrient and energy pathways. The potential impacts of heatwaves on prey fish still lack enough attention, deserving a more comprehensive understanding.

To further understand the physiological mechanisms by which stream fishes respond to short-term heatwaves, I exposed fathead minnow to different frequencies and intensities of heatwaves and then quantified 1) short-term CT_{max} ; 2) enzyme responses related to antioxidant defense, as well as aerobic and anaerobic capacity; and 3) whole-organism oxygen consumption rate ($\dot{M}O_2$). The frequency and magnitude of heatwaves were based on *in situ* monitoring of agricultural streams in Illinois, USA. I predicted that fathead minnow undergoing higher frequency and magnitude of heatwaves would show higher CT_{max} , but with more costs in terms of prolonged increases in whole-organism metabolism, reduced metabolism-related enzyme performance, and reduced antioxidant defense. Quantifying the physiological responses of fathead minnow to heatwaves facilitates a better mechanistic understanding of thermal impacts on fish. It also motivates future bioenergetic modeling to consider both short-term and long-term thermal impacts.

2.2 Methods

2.2.1 Monitoring of stream water temperature in summer

Streams for this study were selected among locations where surveys on fish, macroinvertebrates, local habitat, and water-quality have been conducted by the Illinois Department of Natural Resources in the Kaskaskia River Watershed, Illinois, USA from 1991-2007. For each stream reach (a tributary-to-tributary segment), land-cover proportions in the local catchment (areas directly drained into the reach) were obtained from the Great Lakes Regional Aquatic Gap Analysis Project (Holtrop et al., 2005; Cao et al., 2015). Candidate sites were grouped by hydrologic unit code (HUC 8) subbasin and stream order, then ranked within each group based on the percentage of “agriculture” and “forest” (control) land cover in the local catchment. Within each subbasin-stream order group, first and second ranked agricultural and forested sites were

grouped to create two pairs of candidate sites. Final paired sites were 1) Twomile Slough (94% agriculture vs. 0% forest, 39°54'55"N, 88°19'50"W) and Little Creek (26% agriculture vs. 37% forest, 39°17'43"N, 89°01'31"W); 2) Lake Fork (92% agriculture vs. 1% forest, 39°52'34"N, 88°31'07"W) and Jordan Creek (31% agriculture vs 19% forest, 39°19'51"N, 88°47'24"W)

On July 8th, 2020, one temperature logger (U26-001; Onset HOBO, Bourne, MA, USA) was covered by a PVC solar shield, connected to rebar with a carabiner and zip ties, and deployed 0.5 m above the substrate and 15 m upstream from a bridge in each stream mentioned above (Heck et al., 2018). Loggers were programmed to record water temperature every 10 min. Data were downloaded monthly until November 2020, and missing loggers were replaced.

2.2.2 Fish collection and husbandry

Laboratory experiments were conducted from May 2019 to November 2020 using fathead minnow acquired from Anderson Minnows Farm (Lonoke, AR, USA). Fish were housed at the Aquatic Research Facility at the University of Illinois Urbana-Champaign. Fathead minnow were obtained at four different times, with the first three batches tested for critical thermal maximum (CT_{max}) and enzyme performance under multiple, single, and extended heatwaves, respectively (see 2.2.3 below), with each batch having its own 25 °C control group to minimize potential batch effects. The last batch was tested for oxygen consumption rate ($\dot{M}O_2$) only. Upon arrival at the research facility, fish were distributed randomly into eight 110 L tanks, with maximum density of four fish 10 L⁻¹. Dissolved oxygen (DO) was maintained at > 90 % saturation. Water temperature was kept at 25 °C using immersion heaters to mimic daily average water temperature in the Kaskaskia River Watershed in the summer based on temperature records in 2019 (unpublished) and 2020 (Figure 2.1), and stream gauge monitoring stations (USGS, 2016). Photoperiod was

06:00 – 20:00 to mimic summer conditions. Fish were fed to satiation daily with fish flakes and brine shrimp. An acclimation period at 25 °C lasted at least two weeks prior to the start of any experiment. Before any test described below, fathead minnow were fasted at least for 24 h. All procedures were approved by the University of Illinois Urbana-Champaign Institutional Animal Care and Use Committee (#19088).

2.2.3 Short-term heatwave simulations

From temperature loggers, I confirmed that the highest daily temperatures were 33.8 °C for agricultural streams in August 2020, and only 29.7 °C for forested streams in July 2020. A temperature of 32 °C has been common daily high temperature in agricultural streams, with 10 days in total (Figure 2.1). Thus, to accurately examine the effects of short-term heatwaves on fish during laboratory simulations, 32 and 34 °C were chosen to represent current and future extreme temperatures, respectively. To evaluate whether there were carryover effects derived from previous daily heatwave exposures, I simulated not only single heatwave reaching 32 and 34 °C, but also multiple heatwaves across three consecutive days. Finally, besides single and multiple heatwaves, I added an extended heatwave simulation to further evaluate the effects of heatwave duration on fathead minnow and document real-time adjustments of fish during extended heatwaves.

For single heatwave, I increased the water temperature in holding tanks from 25 to 32 or 34 °C within 30 min at around 9 am, by pumping 25 °C water in holding tanks through stainless steel coils in nearby thermostatic hot water bath for a rapid increase, coupled with heaters/chillers (TK-500; TECO, Ravenna, Italy) for finer adjustments. Once reaching 32 or 34 °C, the temperature was maintained for 1 h. After this 1 h heatwave, I cooled the water back to 25 °C within 30 min.

This rate of temperature increase and decrease is faster than present-day field data (i.e., 30 min vs 2-4 h) (Figure 2.1) because I sought to mimic more intense heatwaves to account for future changes. For multiple heatwaves, I repeated the heatwave simulation at the same time (i.e., 9 am) on three consecutive days. For extended heatwave, I increased the water temperature from 25 to 32 or 34 °C within 30 min at 9 am, then held the water at 32 or 34 °C for up to 48 h. After exposure to single heatwave, the last of multiple heatwaves, and after the first hour during extended heatwave, the short-term physiological responses of fathead minnow were evaluated within 48 h by quantifying CT_{max} , muscle enzymes, and $\dot{M}O_2$, with procedures described in 2.2.4, 2.2.5, and 2.2.6, respectively.

2.2.4 Critical Thermal Maximum (CT_{max})

To quantify the impacts of heatwaves on thermal tolerance, CT_{max} tests were conducted at 1, 6, 24, and 48 h after single heatwave, the last of multiple heatwaves, and after the first hour during extended heatwave. At each time point, six fish in holding tanks were transferred to a 75 L testing tank containing 55 L water. The testing tank contained a 1000 w electric immersion heater (SmartOne EasyPlug Axial Bottom Heater; Integrated Aqua Systems, Vista, CA, USA), two circulating pumps (CompactON 300; Eheim, Deizisau, Germany), and an aerator (Tetra Whisper; Tetra, Blacksburg, VA, USA). Six plastic compartments (20 × 10 × 10 cm) were attached to the sides of the tank to hold fish. These compartments were perforated with holes for water circulation, but kept fish confined to minimize disturbance from each other, making it easier for monitoring (Dai & Suski, 2019). Six fish were introduced into compartments during each trial and given 30 min acclimation at 25 °C (± 0.2 °C) with nearly 100% DO saturation (> 7.5 mg L⁻¹).

After 30 min acclimation, the air stone was removed from the tank, and water temperature was increased by $0.3\text{ }^{\circ}\text{C min}^{-1}$ (Beitinger et al., 2000). The temperature at which fish started to lose body equilibrium, defined by disorganization of locomotion and failure to maintain dorsoventral orientation for three seconds, was recorded as CT_{max} (Beitinger et al., 2000; Morgan et al., 2018). Fish were quickly removed once they lost equilibrium and measured for total length. During the trial, temperature was recorded every min (Pro Plus Multiparameter Instrument; YSI, Yellow Springs, OH, USA). DO stayed near 100% saturation ($> 7.5\text{ mg L}^{-1}$). All fish were only tested once.

2.2.5 Muscle enzymes

To quantify the activity of metabolism-related and antioxidant defense-related enzymes at 1, 6, and 24 h after same heatwave simulations as CT_{max} , another six fish were collected from holding tanks, euthanized (cerebral percussion), and measured for total length. White muscle was excised below the dorsal fin, rinsed with phosphate-buffered saline (PBS) solution at pH 7.4 to remove blood cells and clots, then immediately frozen in liquid nitrogen before being stored at $-80\text{ }^{\circ}\text{C}$ prior to analyses. All enzyme analyses and protein concentration measurements described below were performed in duplicate using the spectrophotometric method. Samples collected in the $25\text{ }^{\circ}\text{C}$ control group could not be analyzed due to the pandemic, limiting my ability to detect enzyme performance changes from baseline levels.

Lactate dehydrogenase (LDH) activity was measured to quantify the anaerobic capacity of white muscle using a Lactate Dehydrogenase Activity Assay Kit (MAK066; Sigma-Aldrich, St. Louis, MO, USA). For this, 20 mg of muscle was thawed on ice, homogenized (Bullet Blender Blue; Next Advance, Troy, NY, USA) in 100 μL of cold LDH Assay Buffer, and then centrifuged

at $10,000 \times$ gravity (g) for 15 min at 4 °C. Owing to high activity, the supernatant was further diluted by 1:20. Two μL of diluted sample was then measured with 48 μL LDH Assay Buffer and 50 μL Master Reaction Mix per well on 96 well plates at 25 °C and $\lambda = 450$ nm per the manufacturer's instructions. Total protein concentrations (mg ml^{-1}) was determined using 5 μL of undiluted supernatant in each sample, BSA standard (P0834; Sigma-Aldrich, St. Louis, MO, USA), and Bradford Reagent (B6916; Sigma-Aldrich, St. Louis, MO, USA) (Bradford, 1976) at 25 °C and $\lambda = 595$ nm after 10 min incubation. The LDH activity was corrected for total protein concentration ($\mu\text{mol mg}^{-1} \text{ protein min}^{-1}$).

Citrate synthase (CS) activity was measured to quantify the aerobic capacity of white muscle using a MitoCheck Citrate Synthase Activity Assay Kit (701040; Cayman Chemical, Ann Arbor, MI, USA). For this, 20 mg of muscle was thawed on ice, homogenized in 200 μL cold buffer (250 mM sucrose, 10 mM Tris-base, and 1 mM EGTA, pH 7.4) and centrifuged at $10,000 \times$ g for 15 min at 4 °C. The supernatant was used in the assay without further dilution. The CS activity was measured at 25 °C and $\lambda = 412$ nm per the manufacturer's instructions. Same as LDH activity, CS activity was corrected for total protein concentration ($\mu\text{mol mg}^{-1} \text{ protein min}^{-1}$).

Superoxide dismutase (SOD), an antioxidant that defends against reactive oxygen species, was quantified using a Superoxide Dismutase Assay Kit (706002; Cayman Chemical, Ann Arbor, MI, USA). For this, 10 mg of muscle was thawed on ice, homogenized in 200 μL cold HEPES buffer (1mM EGTA, 210 mM mannitol, and 70 mM sucrose, pH 7.2), and centrifuged at $10,000 \times$ g for 15 min at 4 °C. The supernatant was diluted by 1:6. The SOD was measured at 25 °C and $\lambda = 450$ nm per the manufacturer's instructions and corrected for total protein concentration ($\text{U mg}^{-1} \text{ protein}$).

2.2.6 Oxygen consumption rate ($\dot{M}O_2$)

To quantify how different heatwaves impacted whole-organism oxygen consumption rates ($\dot{M}O_2$, mg O₂ h⁻¹), intermittent-flow respirometry was used (Loligo Systems, Viborg, Denmark) (Clark et al, 2013). In the system, four glass cylindrical chambers (inner diameter: 45 mm; length: three were 145 mm, one was 114 mm) were submerged in a 98 L experimental tank (radius: 570 mm; height: 150 mm) in a dark room, with aerated water temperature controlled at 25 ± 0.2 °C. Heatwave simulations were identical to those mentioned above. A UV sterilizer (9 Watt, Pond Boss; West Palm Beach, FL, USA) minimized bacterial respiration during trials.

The measurement cycle was determined following pilot trials and went as follows: a 5 min flush, 1 min wait, and 9 min measure period, resulting in 15 min cycle. A mixing pump (Universal 300; Eheim, Deizisau, Germany) ran to move water through the chamber and around an external circuit of gas-tight tubing to avoid any oxygen gradient. A flush pump that was connected to each chamber (Universal 300; Eheim, Deizisau, Germany) and controlled by AutoResp 2.2.2 (Loligo Systems, Viborg, Denmark) allowed for the exchange of fresh aerated water from the tank during every 5 min flush period. During the 9 min measurement period, DO in the chambers was monitored every sec using a 4-channel Minisensor Oxygen Meter with associated sensors (PreSens; Regensburg, Germany) and remained above 80% saturation.

Fathead minnow were collected from holding tanks, measured for total mass and total length, then gently introduced into respirometry chambers in the afternoon and left undisturbed until around 10 am the next morning. Resting $\dot{M}O_2$ on day one (RMR1) was calculated as the lowest 10th percentile of measurements taken throughout this overnight period (Chabot et al., 2016). Then, for fish in the single heatwave treatment, water temperature in the respirometry tank

was increased rapidly from 25 to 32 or 34 °C within 30 min, maintained for 1 h, then cooled back to 25 °C within 30 min. For fish in the multiple heatwave treatment, the first two heatwaves were simulated in holding tanks before fish were introduced into the respirometry chambers in the afternoon prior to the last heatwave at 10 am the next morning. For fish in the extended heatwave treatment, water temperature in the respirometry tank was increased from 25 to 32 or 34 °C within 30 min the next morning, then maintained at 32 or 34 °C during the rest of the monitoring period. For the control group, the temperature in the respirometry tank was maintained at 25 °C, and control fish received all the same procedures. In all treatments, $\dot{M}O_2$ of fathead minnow was monitored for 24 h after the start of temperature changes in the respirometry tank, and the lowest 10th percentile of measurements within this 24 h was calculated as resting $\dot{M}O_2$ on day two (RMR2). Eight fish were measured for each treatment. All chambers were deeply cleaned before every trial and the UV sterilizer was constantly used during every trial to minimize the influence of background respiration. Final $\dot{M}O_2$ accounted for background bacterial respiration by measuring respiration for 30 min before and after trials and assuming a linear increase in background respiration over time.

2.2.7 Data analysis

All data were analyzed using R 4.0.2 (R development Core Team, 2020) with $\alpha = 0.05$. Following single, multiple, and extended heatwaves, respectively, to quantify the effects of different temperatures on thermal tolerance and enzyme responses, linear mixed models were run using the lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) packages. For thermal tolerance, I used CT_{max} as the dependent variable, with time (1, 6, 24, and 48 h) and temperature (25, 32, and 34 °C) as fixed factors. For enzyme analyses, I used LDH activity, CS activity, or

SOD as dependent variables, with time (1, 6, and 24 h) and temperature (32 and 34 °C) as fixed factors. The interactions between time and temperature were also included in both thermal tolerance and enzyme models, with total length nested within temperature as a random factor. Another random factor, compartment nested within temperature, was initially included in thermal tolerance models but later removed by comparing Akaike information criterion (AIC) values. Model assumptions of linearity, normality, and homogeneity of residuals were confirmed by inspecting plots of model residuals versus fitted values. Tukey's HSD post hoc analyses were performed using the emmeans package (Lenth et al., 2018) to determine differences across factors. Marginal r^2 explaining model variance with fixed factors only and conditional r^2 explaining variance for the entire model were calculated using the MuMIn package (Bartoń, 2020).

Oxygen consumption rates ($\dot{M}O_2$) per individual were calculated by the average slope of each 9 min measurement period obtained from the linear regression between oxygen consumption over time ($\text{mg O}_2 \text{ h}^{-1}$), then corrected for the volume of the respirometry chamber and total mass of the fish. $\dot{M}O_2$ with $r^2 < 0.9$ were filtered out for quality control (Svendsen et al., 2016). RMR1 and RMR2, with average $\dot{M}O_2$ during the initial 1 h heatwave exposures, and average 1 h $\dot{M}O_2$ at 1 h and 6 h after the initial 1 h heatwave, were calculated for each fish. To evaluate the effects of different temperatures on $\dot{M}O_2$ under single, multiple and extended heatwaves, respectively, linear mixed models were used, with $\log(\dot{M}O_2)$ as the dependent variable, and time (RMR1, RMR2, 1 h heatwave $\dot{M}O_2$, 1 h $\dot{M}O_2$ at 1 h and 6 h after initial 1 h heatwave), temperature (25, 32, and 34 °C), and $\log(\text{total mass})$ as fixed factors. The interactions between time and temperature were also included, with fish ID as a random factor to account for potential non-independence of data (i.e. different time period of $\dot{M}O_2$ for each fish). Other random factors were initially included in full models but later removed by comparing AIC values, including chamber nested within treatment

and test date nested within treatment. Assumptions of linearity, normality, and homogeneity of residuals were again confirmed as described above, Tukey's HSD post hoc analyses were performed to separate means. Marginal r^2 and conditional r^2 were also calculated.

To measure thermal sensitivity under heatwaves, Q_{10} was calculated by:

$$Q_{10} = \frac{R_2 \frac{10^\circ\text{C}}{T_2 - 25^\circ\text{C}}}{R_1}$$

where R_2 and R_1 represent $\dot{M}O_2$ during the 1 h heatwave across different heatwave treatments and the 25 °C control group, respectively; T_2 represents the heatwave temperature.

To quantify the post-heatwave oxygen consumption, within 1 h after water being cooled back to 25 °C in single and multiple heatwave groups, the area (i.e., mg O_2) above $\dot{M}O_2$ of the 25 °C control group was calculated. The area during the same period in the extended heatwave group was also calculated for comparisons.

2.3 Results

2.3.1 $CT_{\max}$

$CT_{\max}$ in fathead minnow elevated more after 34 °C heatwaves compared to 32 °C heatwaves, with multiple heatwaves transiently eliciting higher $CT_{\max}$ than a single heatwave (Figure 2.2, Table 2.1). At 32 °C, $CT_{\max}$ neither increased nor decreased compared to 25 °C, except a 0.7 °C increase at 1 h after multiple heatwaves (Figure 2.2b). However, at 34 °C, after single heatwave, $CT_{\max}$ increased 1.2 °C at 6 h compared to 25 °C (Figure 2.2a); after multiple heatwaves, $CT_{\max}$ increased significantly, with 1.4 °C at 1 h and 0.8 °C at 48 h compared to 25 °C (Figure

2.2b); during extended heatwave at 34 °C, CT_{max} first decreased 1.1 °C at 1 h compared to 25 °C, but then gradually recovered, until reaching 1.8 °C higher by 24 h (Figure 2.2c). Fathead minnow under extended heatwave at 34 °C experienced > 50% mortality after 24 h, thus I stopped CT_{max} tests for the 48-h group.

2.3.2 Muscle enzymes

Enzyme activities were generally lower under 34 °C heatwaves compared to 32 °C (Tables 2.2-2.4, Figures A.1-A.3). LDH activity did not change from 32 to 34 °C after single or during extended heatwave. However, after multiple heatwaves, LDH activity fell by 32% within 24 h from 32 to 34 °C, with increased activity from 1 to 24 h detected for both temperatures (Table 2.2). CS activity did not change from 32 to 34 °C across all types of heatwaves, despite an increase in CS activity from 1 to 24 h under single heatwave for both temperatures (Table 2.3). SOD decreased by 21% and 16% within 24 h from 32 to 34 °C under multiple and extended heatwaves, respectively. An increase in SOD from 1 to 24 h was detected under single heatwaves for both temperatures (Table 2.4).

2.3.3 Oxygen consumption rates ($\dot{M}O_2$)

Under single and multiple heatwaves, the $\dot{M}O_2$ of fathead minnow increased sharply during the initial 1 h heatwaves, then decreased quickly to control levels. Fathead minnow did not show higher $\dot{M}O_2$ from 32 to 34 °C during the 1 h heatwave. Under the extended heatwave, $\dot{M}O_2$ still decreased after the initial 1 h metabolic spike, but never completely returned to control levels during the entire treatment period, with fish under 34 °C showing higher $\dot{M}O_2$ than under 32 °C after the initial 1 h exposure (Figure 2.3, Table 2.5).

For fish in the single heatwave, a 1 h heatwave at 32 and 34 °C increased $\dot{M}O_2$ by 1.6-fold and 1.8-fold compared to 25 °C, with Q_{10} values of 3.8 and 3.2 relative to the control fish, respectively (Figures 2.3a and 2.3d). After this 1 h heatwave, $\dot{M}O_2$ returned to 25 °C control levels for the remaining 24 h.

For fish in the multiple heatwaves, the third 1 h heatwave at 32 and 34 °C increased $\dot{M}O_2$ by 1.6-fold and 1.9-fold compared to 25 °C, with Q_{10} values of 3.9 and 3.3 relative to the control fish, respectively (Figures 2.3b and 2.3e). After heatwaves, $\dot{M}O_2$ again returned to 25 °C control levels for the remaining 24 h.

For fish in the extended heatwave, the first 1 h of the heatwave at 32 and 34 °C increased $\dot{M}O_2$ by 1.2-fold and 1.7-fold compared to 25 °C, with Q_{10} values of 3.0 and 3.0 relative to the control fish, respectively (Figures 2.3c and 2.3f). After the initial 1 h heatwave exposure, the $\dot{M}O_2$ of fish declined but never returned to 25 °C control levels, which remained at 0.7-1.0 fold and 1.1-1.6 fold higher under 32 and 34 °C, respectively.

Post-heatwave oxygen consumption after 32 and 34 °C single heatwaves were 0.26 and -0.05 mg O₂ for the first hour, respectively. The negative value represents a decrease in post-heatwave oxygen consumption of testing fish compared to the 25 °C control group. After 32 and 34 °C multiple heatwaves, post-heatwave oxygen consumption was 0.17 and 0.21 mg O₂, respectively. As a comparison, extended heatwaves resulted in 0.45 and 0.58 mg O₂ in post-heatwave oxygen consumption at 32 and 34 °C during the same period, respectively.

2.4 Discussion

By simulating heatwaves in the laboratory using current and near-future daily extreme temperatures in agricultural streams, I found that fathead minnow did not exhibit pronounced adverse short-term physiological responses. Instead of expected prolonged increases in metabolism after exposures to heatwaves, fathead minnow rapidly recovered metabolic homeostasis by returning oxygen consumption rates ($\dot{M}O_2$) to resting levels within one hour after single and multiple heatwaves at both 32 and 34 °C. When fish were held at 32 or 34 °C during extended heatwaves, $\dot{M}O_2$ still decreased after an initial metabolic spike. Fathead minnow also increased their upper thermal tolerance transiently, with an increase in $CT_{max} > 1$ °C after three different types of heatwaves at 34 °C. However, such resilience to heatwaves did come with costs, identified by decreased anaerobic capacity and impaired antioxidant defense from 32 to 34 °C, as well as the mortality during 34 °C extended heatwave after 24 h.

2.4.1 Real-time metabolism under extreme heatwaves

When water was cooled to 25 °C after 32 or 34 °C heatwaves, fathead minnow recovered metabolic homeostasis rapidly, shown by minimal increases in oxygen consumption 1 h post-heatwave compared to the 25 °C control. During extended heatwaves, $\dot{M}O_2$ still decreased up to 55% within 24 h after the initial thermal challenge, despite not returning to resting levels. This implies partial thermal compensation to preserve aerobic scope (Seebacher et al., 2015; Havird et al., 2020). However, under extended exposures to heatwaves, such compensation seems insufficient to help fish restore homeostasis, indicated by costs such as: 1) reduced antioxidant defense at higher temperatures; and 2) fish mortality at 24 h after experiencing a 34 °C extended heatwave. Overall, the rapid return of $\dot{M}O_2$ during and after heatwaves could help fathead minnow

conserve energy and maintain aerobic performance (Fry, 1971). Considering the short duration of heatwaves, this quick decline in oxygen uptake after heatwaves probably helps fathead minnow maintain sufficient aerobic scope for foraging, growth, and reproduction (Pörtner, 2010; Schulte, 2015).

Besides rapid metabolic adjustments following initial heatwave exposures, previous heatwave experience did not help fish gain more efficient $\dot{M}O_2$ during later heat exposures, as the increase in $\dot{M}O_2$ was 1.6-fold vs. 1.6-fold at 32 °C, and 1.8-fold vs. 1.9-fold at 34 °C after single vs. multiple heatwaves. Besides aerobic performance, the limited excess post-heatwave oxygen consumption after single and multiple heatwaves suggests a limited oxygen debt from anaerobic metabolism. Thus, with such duration and intensity of heatwaves in the future, oxygen compensation to clear excess lactic acid build-up after anaerobic metabolism seems manageable for fathead minnow (Svendsen et al., 2010; Nelson, 2016). However, extended heatwaves did cause higher aerobic and anaerobic costs for fish, indicated by elevated $\dot{M}O_2$ and higher LDH activity during extended heatwaves compared to 1 h heatwaves. Overall, the duration and frequency of heatwaves can play an important role in the build-up of energetic costs over the summer period. Under current thermal conditions in agricultural streams, fathead minnow should still be able to minimize both aerobic and anaerobic costs to conserve energy for other activities after short-duration heatwaves.

I did not use classical aerobic scope measurements with $\dot{M}O_{2min}$ and $\dot{M}O_{2max}$ under different acclimation temperatures to evaluate the effects of heatwaves on fish (Clark et al., 2013). Rather, I chose to quantify the real-time changes in $\dot{M}O_2$ during and after heatwaves. The classical method of defining aerobic scope is ideal for evaluating optimal temperature ranges for physiological

performance during long-term temperature increases (Rummer et al., 2014). However, in addition to long-term increases in average temperatures, climate change and agricultural disturbances can also threaten the thermal safety of fish through short-term extreme heatwaves, which are becoming increasingly common (Rahmstorf & Coumou, 2011; Stillman, 2019). We should consider such short-term thermal threats from both 1) the magnitude, which could exceed the thermal safety range of fish, causing death directly, and 2) the duration, which could narrow the thermal safety margins, possibly impairing fish performance (Rezende et al., 2014). To overcome such heatwaves, fish require highly flexible and tolerant physiological performance. Real-time $\dot{M}O_2$ offers the chance to observe such physiological responses on a fine scale, as shown by the rapid adjustments of $\dot{M}O_2$ during and after heatwave exposures. This use of fine-scale $\dot{M}O_2$ measurements also provides better estimations of metabolic costs, thus offering a chance to incorporate dynamic metabolism into bioenergetic models to improve model performance. As shown during extended heatwave at both 32 and 34 °C, fish could still rapidly adjust $\dot{M}O_2$ after an initial metabolic spike. If classical aerobic scope at 25, 32, and 34 °C were measured separately and compared with each other, such quick adjustments to $\dot{M}O_2$ likely would have been overlooked, causing incorrect estimation of aerobic scope reduction and metabolic costs during short-term heatwaves.

2.4.2 Thermal tolerance changes and enzyme responses

Short-term heatwaves increased the upper thermal tolerance of fathead minnow. One hour after multiple heatwaves, fathead minnow showed their highest CT_{max} after all 34 °C heatwaves, and showed the only CT_{max} increase after all 32 °C heatwaves. This indicates that previous heatwave experience did have carryover effects and helped fathead minnow better prepare for

subsequent heatwaves. This result is different from the effects of repeated daily heatwaves on the stenothermal Atlantic salmon (*Salmo salar*), which did not show increased CT_{max} with more heatwaves (Corey et al., 2017). Such different results in heat hardening can likely be attributed to different capacities of the inducible heat shock response between stenothermal and eurythermal fishes (Logan & Buckley, 2015), as well as a diminished oxygen limitation from gill surface area in smaller fishes (Rubalcaba et al., 2020). Besides the CT_{max} increase 1 h after multiple heatwaves at 32 °C, fathead minnows did not change CT_{max} within 48 h under all types of 32 °C heatwaves. This suggests that 32 °C heatwaves in Midwestern agricultural streams were still within the upper thermal tolerance of fathead minnow, and thus did not cause excessive thermal stress that would stimulate significant heat hardening. Considering the results after all heatwaves, fathead minnow showed the highest CT_{max} after 34 °C heatwaves, up 1.4 °C to 38.4 °C, but this endpoint is still lower than the CT_{max} after long-term acclimation at extreme temperatures. For example, after weeks of acclimation at 32 °C, the CT_{max} of fathead minnow was shown to be over 40 °C using the same 0.3 °C min⁻¹ ramping speed and the same end point indicated by loss of equilibrium (Beitinger et al., 2000). Such differences in CT_{max} indicates the limited effects of short-term heatwaves on increasing thermal tolerance. Higher thermal tolerance cannot be established overnight. Instead, it appears after fish undergo gradually physiological acclimations.

The quick disappearance of transient CT_{max} increases, together with the death of fish during 34 °C extended heatwave, implies high costs of heat hardening, especially when the duration of heatwaves is taken into consideration, with longer heatwaves potentially narrowing thermal safety margins. The performance of both metabolism-related and antioxidant defense-related enzymes confirms this. More specifically, superoxide dismutase (SOD), citrate synthase (CS) activity, and lactate dehydrogenase (LDH) activity all increased temporally, either after single or multiple

heatwaves, implying post-heatwave compensation costs. Besides temporal changes, the lower SOD in white muscle from 32 to 34 °C represents a reduced energy investment in antioxidant defense (Little et al., 2020). However, with higher respiratory requirements during extreme heatwaves, excessive oxidative stress from the production of reactive oxygen species should be buffered with more antioxidant agents (Heise et al., 2006). This contradictory result shows that fathead minnow either passively lost antioxidant defense due to the denaturation of enzymes or inability of synthesis (Madeira, 2016), or allocated energy towards activities with higher priority (Betzberger et al., 2010). Apart from more oxidative damage, anaerobic capacity, indicated by LDH activity, was downregulated within 24 h from 32 to 34 °C after multiple heatwaves. This downregulation means fish relied less on glycolysis for ATP (Enzor et al., 2017), signaling energy conservation after experiencing high energetic costs from previous heatwave exposures. Unfortunately, this energy conservation also implies lower anaerobic potential that could translate to reduced burst swimming performance for predator avoidance and forage (Plaut, 2001).

2.4.3 Implications and conclusions

I witnessed high thermal resilience of fathead minnow to extreme heatwaves. This result suggests that some fish species, particularly thermally tolerant ones, can withstand current and near-future heatwaves in disturbed ecosystems such as agricultural streams, where experienced > 4 °C higher heatwaves than nearby forested streams. This result stimulates several questions for future studies: 1) What will the responses to extreme heatwaves be for more thermally sensitive fish? Distributions of cold-water species have been predicted to be more restricted with global temperature increases (Comte et al., 2013). But this rate of habitat loss could have been underestimated because short-term heatwaves oftentimes have not been included in models. 2)

What if during heatwaves, fish must migrate or maintain other essential activities? For Atlantic salmon, it has been verified that they had higher standard metabolism during migration under more variable environments (Oligny-Hébert et al., 2015). This could limit their aerobic scope, thus reducing their energy budget for later reproduction. 3) Can heatwaves impact predator-prey interactions, thus altering nutrient and energy pathways in local freshwater ecosystems? In marine ecosystems, short-term heatwaves in Western Australia during 2011 had long-lasting effects on the range contraction of kelp (*Ecklonia radiata*) and the range expansion of algal turfs, thus altering local fish community composition (Harvey et al., 2022).

In nature, the impacts of heatwaves should never be analyzed alone. Rather, other factors that could have additive or synergistic effects on biota should be considered in concert with thermal changes, such as low dissolved oxygen from nutrient enrichment or toxicant exposure from pesticides in agricultural regions (Wang et al., 2003; Verberk et al., 2016; Op de Beeck et al., 2017). Also, the impacts of heatwaves should be analyzed dynamically. With diel fluctuations of temperatures in streams, fish could recover at night when water cools, even during prolonged heatwaves that last days. However, low dissolved oxygen at night (Matthews & Berg, 1997; Guasch et al., 1998) could cause respiration pressure, disturbing the recovery process. Thus, impacts of heatwaves on fish in real streams are more complicated than I simulated and tested in this study.

In the era of rapid climate change, degraded freshwater ecosystems could experience extreme heatwaves earlier compared to better protected ecosystems, thus requiring a prioritized focus. Here, I show that, under current and near-future heatwaves in agricultural streams, a thermally tolerant fish, fathead minnow, demonstrated high thermal resilience by minimizing

metabolic costs and transiently enhancing upper thermal tolerance, despite the costs of reduced anaerobic potential and impaired antioxidant defense. When conducting physiological evaluations and bioenergetic modeling on the thermal impacts on fish, I suggest that subsequent studies consider and monitor short-term extreme events that last from hours to days, as their effects are typically overlooked, but may have a disproportionate influence on individuals and populations relative to their short duration.

2.5 Figures and tables

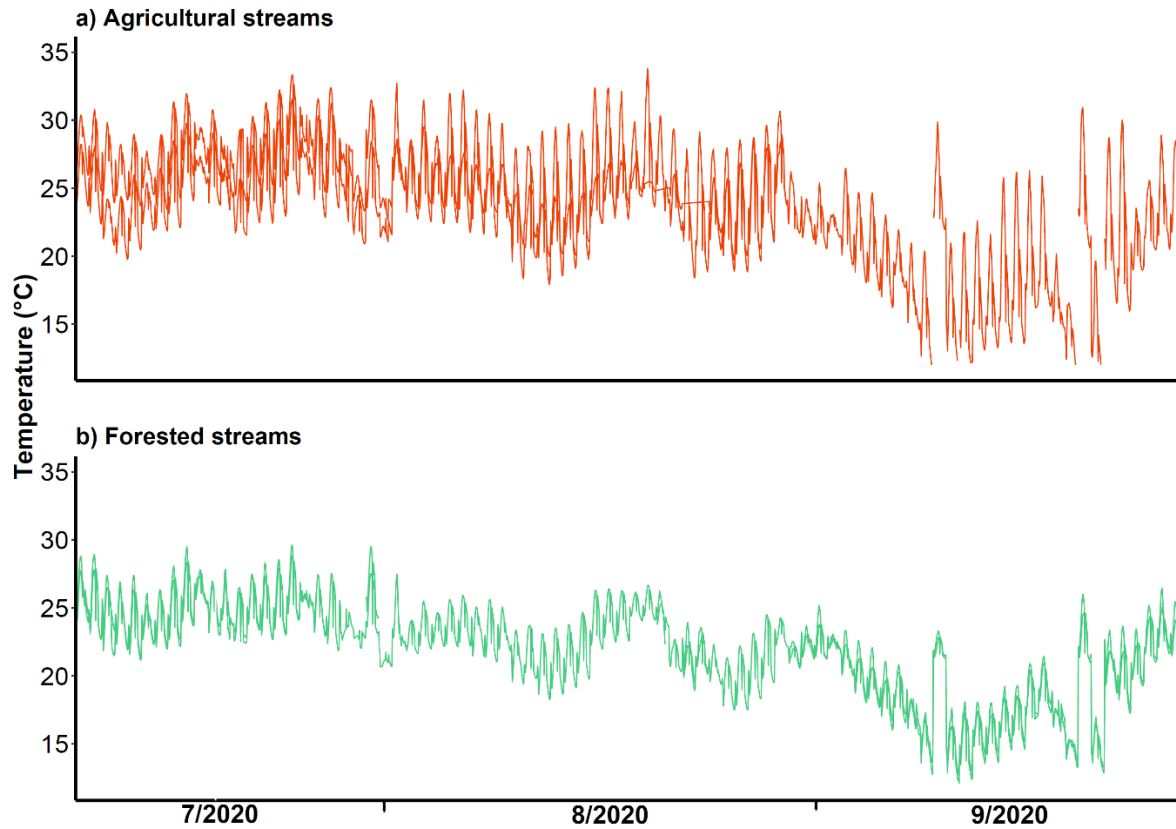


Figure 2.1 Water temperature in two agricultural streams (a) and two reference forested streams (b) in Kaskaskia River Watershed, Illinois, USA from July 9th, 2020 to September 30th, 2020. Temperatures in two agricultural or forested streams were not averaged but highly overlapping in the figure. Mean ($\pm$ sd) temperatures for agricultural vs. forested streams: July: 26.5 ± 2.6 vs. 25.0 ± 1.8 °C; August: 24.8 ± 2.9 vs. 22.5 ± 2.0 °C; September: 20.0 ± 4.2 vs. 19.2 ± 3.1 °C. Temperature ranges for agricultural vs. forested streams: July: $19.8 - 33.4$ vs. $21.1 - 29.7$ °C; August: $17.9 - 33.8$ vs. $17.5 - 27.5$ °C; September: $11.0 - 30.9$ vs. $11.9 - 26.6$ °C. Data were missing in August, 2020 in agricultural Jordan Creek and September, 2020 in forested Lake Fork due to loss of loggers.

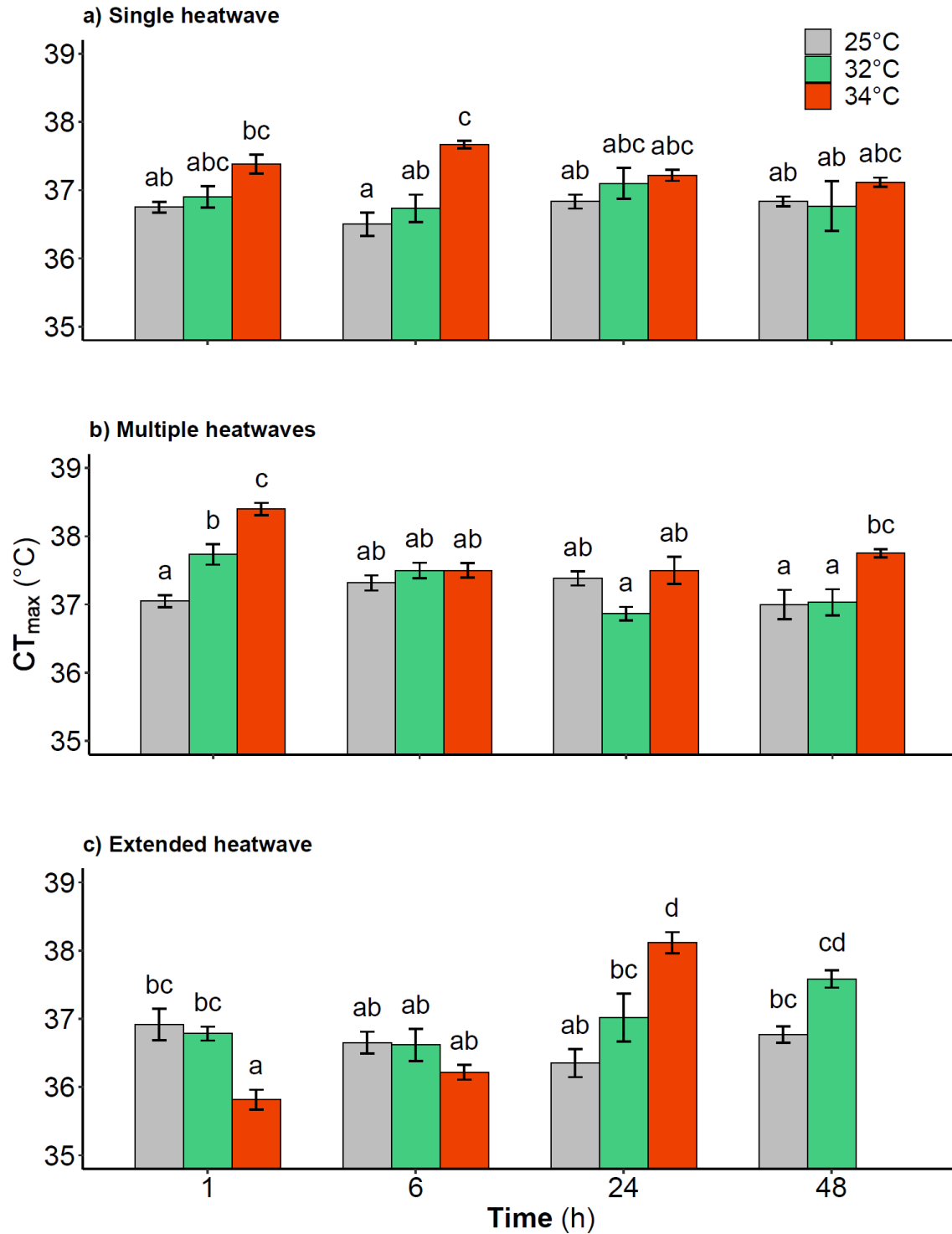


Figure 2.2 Critical Thermal Maximum (CT_{max} , $\pm$ se) of fathead minnow (*Pimephales promelas*) at 1, 6, 24, and 48 h after single (a), multiple (b), and extended (c) heatwaves. Groups ($n = 6$ in each group) with different letters are significantly different ($P < 0.05$) from each other.

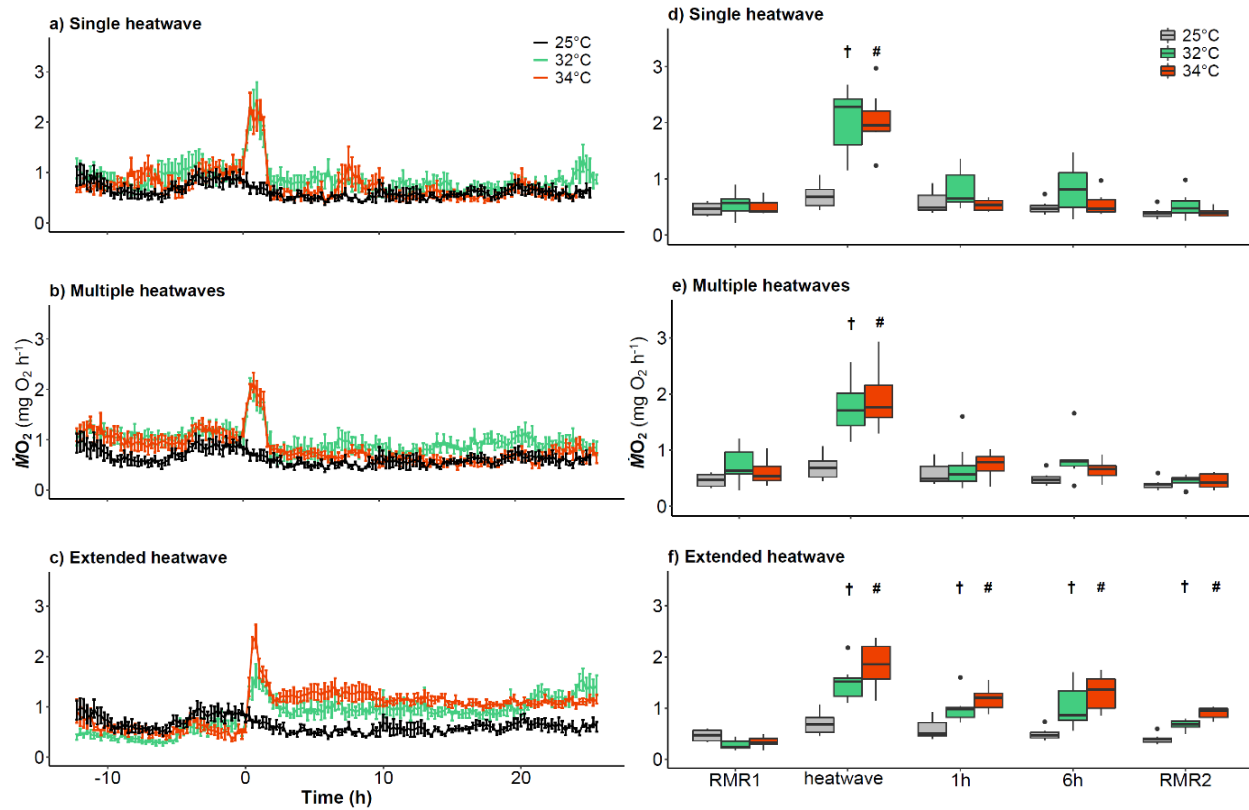


Figure 2.3 Oxygen consumption rates ($\dot{M}O_2$, $\pm$ se) of fathead minnow (*Pimephales promelas*) (n = 8 in each temperature $\times$ type of heatwaves) during single (a, d), multiple (b, e), and extended (c, f) heatwaves. Time at 0 h represents the start of temperature changes. In single (a, d) and multiple heatwaves (b, e), temperature changes included 30 min heating, 1 h heatwaves, and 30 min cooling. In extended heatwaves (c, f), temperature changes included 30 min heating and extended 24 h heatwaves. † and # represents $\dot{M}O_2$ is higher than reference level (i.e., $\dot{M}O_2$ of 25 °C control group at the same time). In d), e), and f), horizontal line = median; box = first quartile to third quartile; vertical line = 1.5 interquartile range

Table 2.1 Results of linear mixed effect models examining factors affecting **critical thermal maximum (CT_{max})** of fathead minnow (*Pimephales promelas*) in single, multiple, and extended heatwaves. Fixed factors include temperature (25, 32, and 34 °C), time (1, 6, 24, and 48 h) and their interaction. Model also includes total length nested within temperature as a random factor. Marginal $r^2 = 0.384$, 0.602, and 0.626, while conditional $r^2 = 0.662$, 0.627, and 0.689 for single, multiple, and extended heatwaves, respectively. Significant factors are shown in **bold**.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	<i>P</i>
Single temperature	1.952	0.976	2	14.950	9.893	0.002
Time	0.178	0.059	3	28.219	0.602	0.619
Single temperature*Time	1.630	0.272	6	27.858	2.754	0.031
Multiple temperature	4.383	2.191	2	23.692	21.124	<0.001
Time	2.654	0.885	3	59.386	8.528	<0.001
Multiple temperature*Time	3.736	0.623	6	58.641	6.003	<0.001
Extended temperature	0.916	0.458	2	31.597	2.533	0.095
Time	5.943	1.981	3	48.807	10.955	<0.001
Extended temperature*Time	12.527	2.505	5	50.165	13.856	<0.001

Table 2.2 Results of linear mixed effect models examining factors affecting **lactate dehydrogenase (LDH) activity** of fathead minnow (*Pimephales promelas*) in single, multiple, and extended heatwaves. Fixed factors include temperature (32 and 34 °C), time (1, 6, and 24 h) and their interaction. Model also includes total length nested within temperature as a random factor. Marginal $r^2 = 0.052, 0.336$, and 0.105 , while conditional $r^2 = 0.052, 0.390$, and 0.242 for single, multiple, and extended heatwaves, respectively. Significant factors are shown in **bold**.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	<i>P</i>
Single temperature	1.894	1.894	1	30	0.252	0.619
Time	8.739	4.370	2	30	0.582	0.565
Single temperature*Time	3.782	1.891	2	30	0.252	0.779
Multiple temperature	22.379	22.379	1	18.771	7.493	0.013
Time	28.662	13.331	2	29.113	4.799	0.016
Multiple temperature*Time	1.034	0.517	2	29.113	0.173	0.842
Extended temperature	10.477	10.477	1	27.034	0.495	0.488
Time	45.405	22.703	2	28.631	1.072	0.356
Extended temperature*Time	33.013	16.507	2	28.631	0.779	0.468

Table 2.3 Results of linear mixed effect models examining factors affecting **citrate synthase (CS) activity** of fathead minnow (*Pimephales promelas*) in single, multiple, and extended heatwaves. Fixed factors include temperature (32 and 34 °C), time (1, 6, and 24 h) and their interaction. Model also includes total length nested within temperature as a random factor. Marginal $r^2 = 0.318, 0.249$, and 0.151 , while conditional $r^2 = 0.547, 0.550$, and 0.223 for single, multiple, and extended heatwaves, respectively. Significant factors are shown in **bold**.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	<i>P</i>
Single temperature	0.00010	0.00010	1	17.448	0.622	0.441
Time	0.00213	0.00106	2	25.731	6.743	0.005
Single temperature*Time	0.00070	0.00035	2	25.731	2.229	0.128
Multiple temperature	0.00003	0.00003	1	15.094	0.200	0.661
Time	0.00024	0.00012	2	24.315	0.813	0.455
Multiple temperature*Time	0.00163	0.00082	2	24.315	5.457	0.011
Extended temperature	0.00001	0.00001	1	22.807	0.060	0.809
Time	0.00066	0.00033	2	27.073	2.582	0.094
Extended temperature*Time	0.00014	0.00007	2	27.073	0.549	0.584

Table 2.4 Results of linear mixed effect models examining factors affecting **superoxide dismutase (SOD)** of fathead minnow (*Pimephales promelas*) in single, multiple, and extended heatwaves. Fixed factors include temperature (32 and 34 °C), time (1, 6, and 24 h) and their interaction. Model also includes total length nested within temperature as a random factor. Marginal $r^2 = 0.184, 0.183,$ and 0.236 , while conditional $r^2 = 0.793, 0.183,$ and 0.573 for single, multiple, and extended heatwaves, respectively. Significant factors are shown in **bold**.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	<i>P</i>
Single temperature	1.015	1.015	1	17.396	2.178	0.158
Time	3.962	1.981	2	14.982	4.252	0.034
Single temperature*Time	0.183	0.091	2	14.982	0.196	0.824
Multiple temperature	8.885	8.885	1	30.000	6.379	0.017
Time	1.370	0.685	2	30.000	0.492	0.616
Multiple temperature*Time	0.694	0.347	2	30.000	0.249	0.781
Extended temperature	3.978	3.978	1	25.509	6.590	0.016
Time	0.214	0.107	2	24.899	0.177	0.839
Extended temperature*Time	2.189	1.094	2	24.899	1.813	0.184

Table 2.5 Results of linear mixed effect models examining factors affecting metabolic rate ($\dot{M}O_2$) of fathead minnow (*Pimephales promelas*) in single, multiple, and extended heatwave at 25, 32, and 34 °C. Related to Figure 2.3, time represents different measurement periods, including resting metabolic rate on day one (RMR1), the first 1 h heatwave (heatwave), 1 h after the first 1 h heatwave (1 h), 6 h after the first 1 h heatwave (6 h), and resting metabolic rate on day two (RMR2). Model includes fish ID as a random effect. Marginal $r^2 = 0.816, 0.711$, and 0.820 , while conditional $r^2 = 0.901, 0.847$, and 0.889 for single, multiple, and extended heatwaves, respectively. Code in R: `lmer(data = Type_of_Heatwave, logMO2 ~ Time*Temperature + logMass + (1|Fish))`. Significant factors are shown in **bold**.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	P
Single temperature	0.056	0.028	2	17.986	4.371	0.03
Time	3.491	0.873	4	73.248	136.224	<0.01
Single temperature*Time	0.753	0.094	8	73.232	14.680	<0.01
Log(Mass)	0.175	0.175	1	17.917	27.318	<0.01
Multiple temperature	0.148	0.148	2	19.915	8.477	<0.01
Time	0.240	0.060	4	82.059	93.830	<0.01
Multiple temperature*Time	0.500	0.125	8	82.049	8.636	<0.01
Log(Mass)	0.107	0.107	1	19.978	11.492	<0.01
Extended temperature	0.345	0.172	2	17.865	22.137	<0.01
Time	3.737	0.932	4	74.973	119.738	<0.01
Extended temperature*Time	1.121	0.140	8	74.972	18.015	<0.01
Log(Mass)	0.038	0.038	1	17.827	4.882	0.04

CHAPTER 3: COOL VS. WARMWATER FISHES AND PREDATOR-PREY INTERACTIONS UNDER CLIMATE CHANGE WITH HEATWAVES

3.1 Introduction

Under climate change, heatwaves are damaging ecosystems globally, from terrestrial forest fires to marine coral bleaching (Spinks et al., 2019; Canadell & Jackson, 2021). In Chapter 2, I witnessed that the intensity and duration of heatwaves in streams and rivers could be further exacerbated across Midwestern agricultural landscapes. Such heatwaves under climate change inevitably elevate metabolic demands of fish species (Deutsch et al., 2015). Compared to warmwater species (e.g., fathead minnow (*Pimephales promelas*) in Chapter 2), coolwater or coldwater species could have lower resilience and higher metabolic costs to heatwaves, thus, influenced more by climate change in terms of behavior, growth, and possibly survival (Somero, 2010; Scott & Johnston, 2012; Nagelkerken & Munday, 2016).

The differing responses of cold, cool, and warmwater species to climate change could fundamentally change the structure of local fish communities (Comte et al., 2013). Under climate change, habitats may no longer provide preferred thermal ranges of many native species, giving opportunities to other species with competitive advantages (Milazzo et al., 2013). For example, shifts from the dominance of coolwater predator walleye (*Sander vitreus*) to warmwater largemouth bass (*Micropterus salmoides*) have already been projected in 91 % of Wisconsin lakes by 2089 (Hansen et al., 2017). In Ontario, Canada, coolwater walleye and cisco were predicted to decline by 22 and 26 %, respectively, with up to 422 % increase in warmwater smallmouth bass (*Micropterus dolomieu*) populations at the same locations (Van Zuiden et al., 2016). Besides long-term projections, localized short-term heatwaves could temporarily pass the “good growth” range

for coolwater species, giving warmwater species competitive advantages (Nelson & Palmer, 2007). Short duration of such heatwaves will not allow acclimation or adaptation of species, thus the performance of fishes heavily relies on transitory physiological and behavioral responses (Jutfelt, 2020). For fish communities where cool and warmwater species coexist now, the influence of climate change, especially heatwaves, could be the deterministic factor that ultimately reshapes the composition of local fish communities (Rogers et al., 2019).

Predator-prey relationships in local fish communities can also be altered under climate change in addition to interspecific competition. Increasing productivity during warming will not necessarily benefit large teleost predators that have narrow diet ranges. Instead, generalists may outcompete these large specialists (van Denderen et al., 2018). Also, temperature dependence of predation success could be the key factor determining the relative performance of predators and prey. Higher temperatures could impact the attack speed of coolwater predators, but not the escape speed of warmwater prey (Grigaltchik et al., 2012; Öhlund et al., 2015), causing certain thermal thresholds to become the tipping point of predator-prey interactions. Besides behavior, temperature also affects energy gain (ingestion) and loss (metabolism). The temperature-dependent performance of ingestion and metabolism may differ among different trophic levels, further perturbing the stability of local communities (Rall et al., 2010).

Large-scale ecological modeling can identify potential trajectories for fish community changes, while physiological experiments help verify or reject such ecological projections by identifying the mechanisms by which fishes respond to climate change, especially heatwaves. On the organism level, thermal sensitivity of metabolism has been partially explained by the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis (Pörtner & Peck, 2010), as explained

in Introduction of Chapter 2. Compared to warmwater species, coolwater fishes may face steeper increases in tissue oxygen demand during warming, thus reaching the critical temperatures that cause impairment or loss of performance earlier (Schulte, 2015). On the individual level, behavioral changes, especially temperature-regulated swimming, are key factors in determining prey capture, predator avoidance, and migration of different species (Killen et al., 2010). During warming, coolwater species may exceed their thermal optimum for swimming while warmwater species may be less impacted. Difference in changes of swimming performance alters the balance of local fish communities by influencing survival and fitness of different species (Lee et al., 2003). With the understanding of physiological mechanisms, we can better predict and explain the trajectories of many fish community changes.

Under climate change with heatwaves, the potentially different responses between coolwater and warmwater species could drive the changes of fish communities. To offer the potential mechanisms to changes in Midwestern fish communities in the future, I focused on three species that are both ecologically and recreationally important, and have long-term sampling records in the Kaskaskia River Watershed: 1) largemouth bass, one of the most abundant warmwater predator species; 2) walleye, a coolwater predator species that coexist with largemouth bass that are becoming less abundant but very popular in Midwestern USA; 3) fathead minnow, a widespread and ecologically important prey species in local food webs (Stein et al., 2021; Metzke et al., 2022). I quantified differences in physiological performance of 1) walleye vs. largemouth bass predators and 2) fathead minnow vs. both predators at summer temperature with heatwaves in the laboratory. These physiological performance measures included whole-organism maximum and standard metabolic rates, and aerobic scope (MMR, SMR, and AS); whole-organism oxygen consumption rate ($\dot{M}O_2$) during heatwaves and predator-prey interactions; critical swimming speed

(U_{crit}) for each species. The magnitude of heatwaves was based on *in situ* monitoring of forested streams in the Kaskaskia River Watershed, Illinois, USA. Compared to warmwater largemouth bass and fathead minnow, I predicted that coolwater walleye would respond poorly to summer conditions, no matter with heatwave or not, in terms of the narrowest AS, limited metabolic response during predator-prey interaction, and impaired U_{crit} . Quantifying and comparing the physiological responses of cool vs warmwater species under climate change with heatwaves could offer mechanistic evidence how and why coolwater fishes are going to be replaced by warmwater competitors in Midwestern USA, thus better guiding the future management practices of the freshwater ecosystems under climate change.

3.2 Methods

3.2.1 Fish collection and husbandry

Laboratory experiments were conducted from May 2021 to December 2022, using fathead minnow acquired from Anderson Minnows Farm (Lonoke, AR, USA), largemouth bass acquired from Jake Wolf Memorial Fish Hatchery (Topeka, IL, USA), and walleye acquired from Keystone Hatcheries (Richmond, IL, USA). Fish were housed at the Aquatic Research Facility at the University of Illinois Urbana-Champaign. Before fish arrival, all holding tanks were cultured with nitrifying bacteria using Fritz Turbo Start 700 (Fritz Aquatics, Mesquite, TX, USA), coupled with ammonium chloride, for up to three weeks. Dissolved oxygen (DO) in all tanks was maintained at > 90 % saturation.

Upon arrival at the research facility, fathead minnow were distributed randomly into five 110 L tanks, with a maximum density of four fish 10 L^{-1} . Fathead minnow used as food for predator

species (i.e., largemouth bass and walleye) were distributed randomly into another five 110 L tanks, with maximum density of six fish 10 L⁻¹. Juvenile largemouth bass (n = 50) were evenly distributed in four 567 L tanks. Juvenile walleye (n = 50) were evenly distributed in three 567 L tanks (Tables 3.1 & 3.3). Fathead minnow were fed to satiation daily with fish flakes and brine shrimp. To ensure predator species became familiar with fathead minnow as prey, both largemouth bass and walleye were fed with at least three fathead minnow per individual every two days. Before any test described below, fathead minnow were fasted at least for 24 h while largemouth bass and walleye were fasted at least for 48 h. Tank water temperatures were initially set to match shipping temperature, and then were increased 1 °C per day until reaching 25 °C (TK-500; TECO, Ravenna, Italy) to mimic average summer water temperature in the Kaskaskia River Watershed based on temperature records in 2020 (Dai et al. In Press) and Illinois stream gauge monitoring stations (USGS, 2016). The acclimation period at 25 °C lasted at least two weeks prior to the start of any experiment (Beitinger & Lutterschmidt, 2011). All procedures were approved by the University of Illinois Urbana-Champaign Institutional Animal Care and Use Committee (#20098).

3.2.2 Maximum and standard metabolic rates (MMR and SMR)

To quantify maximum and standard whole-organism oxygen consumption rate ($\dot{M}O_2$, mg O₂ kg⁻¹ h⁻¹) of both prey (i.e., fathead minnow) and predators (i.e., largemouth bass and walleye) at summer temperature (25 °C), intermittent-flow respirometry was used (Loligo Systems, Viborg, Denmark) (Clark et al, 2013). In the system, four small glass cylindrical chambers (inner diameter: 45 mm; length: 145 mm) were used for small prey species, and four large glass cylindrical chambers (inner diameter: 140 mm; length: 500 mm) were used for large predator species. Chambers were submerged in a 440 L holding tank (length: 1100 mm; width: 1000 mm; height:

400 mm) in a quiet room, with aerated water temperature controlled at 25 ± 0.2 °C. A UV sterilizer (9 Watt, Pond Boss; West Palm Beach, FL, USA) minimized bacterial respiration during trials.

After pilot trials, the measurement cycle for all three species was the same: a 5 min flush, 1 min wait, and 14 min measure period, resulting in 20 min cycle. A mixing pump (Universal 300; Eheim, Deizisau, Germany) ran to move water through the chamber and around an external circuit of gas-tight tubing to avoid any oxygen gradient. A flush pump connected to each chamber (Universal 300; Eheim, Deizisau, Germany) and controlled by AutoResp 2.2.2 (Loligo Systems, Viborg, Denmark) allowed for the exchange of fresh aerated water from the tank during every 5 min flush period. During the 14 min measurement period, DO in the chambers was monitored every sec using a 4-channel Minisensor Oxygen Meter with associated sensors (PreSens; Regensburg, Germany) and remained above 80% saturation.

To quantify the maximum metabolic rate (MMR) for fathead minnow, largemouth bass, and walleye, I first gently introduced individuals into a 88 L circular arena (radius: 280 mm; height: 360 mm) at around 2 pm. Fish were then manually chased by a dip net for 3 min or until exhaustion, quantified as being unable to show burst swimming over 30 s (Norin & Clark, 2016). After this chasing period, I immediately measured fish for total mass and total length, then introduced the individual into the cylindrical chamber (during the flush cycle). Once the chamber was sealed, I manually switched from the flush period to the 1 min wait period to start the automatic measurement cycles. The $\dot{M}O_2$ generated during the first cycle was considered to be MMR. Fish were then left undisturbed overnight until around 12 pm the next day. I calculated the average over the lowest 10% of $\dot{M}O_2$ measurements among all cycles for each individual as SMR (Chabot et al.,

2016). The difference between MMR and SMR for each species was calculated as aerobic scope (AS).

3.2.3 Heatwave exposure

From temperature loggers in the Kaskaskia River Watershed (Dai et al. In Press), I confirmed that the highest daily temperature in July 2020 was 29.7 °C for forested streams. Walleye are considered a coolwater species (relative to warmwater species such as largemouth bass and fathead minnow) (Bozek et al., 2011), and the chronic and acute thermal tolerances of walleye in the summer have previously been reported as 25 and 30 °C, respectively (Nevada Division of Environmental Protection, 2018). Thus, the heatwave intensity in this study was set at 30 °C to avoid immediate mortality of walleye, but to mimic maximum summer temperatures in forested streams in the Kaskaskia River Watershed as the critical thermal challenge. To accurately examine the effects of short-term heatwaves on fish during laboratory simulations, the duration of 30 °C heatwave was maintained for 1 h, coupled with a 1 h ramping period from 25 °C and a 1 h cooling period back to 25 °C. Temperature changes were achieved by pumping water in the respirometry tank or swim tunnels through stainless steel coils in nearby thermostatic hot/cold water baths, coupled with heaters/chillers (TK-500; TECO, Ravenna, Italy) for finer adjustments. This rate of temperature changes is faster than present-day field data (i.e., 1 h vs 2-4 h) as I sought to mimic more intense heatwaves predicted in the near future.

3.2.4 Oxygen consumption rate ($\dot{M}O_2$) with the heatwave and predator-prey interactions

Similar to the MMR/SMR procedure described above, fathead minnow, largemouth bass, or walleye were collected from holding tanks, measured for total mass and total length, gently

introduced into respirometry chambers at around 2 pm in the afternoon, and then left undisturbed until around 9 am the next morning. Resting $\dot{M}O_2$ (RMR) was calculated as the lowest 10% of measurements taken throughout this overnight period. Then, for fish experiencing the heatwave treatment, water temperature in the respirometry tank was increased rapidly from 25 to 30 °C within 1 h, maintained for 1 h, then cooled back to 25 °C within 1 h at around 12 pm. For the control group, the temperature in the respirometry tank was held at 25 °C, and control fish received all the same procedures.

One hour after 12 pm (i.e., 1 pm), I introduced either prey or predators to measure the metabolic response during predator-prey interaction for 2 h until around 3 pm. For fathead minnow, this means I either introduced four largemouth bass or four walleye and let them swim freely around four respirometry chambers in the holding tank. This ensured fathead minnow, as prey, could receive both visual and olfactory cues from predators during the interaction period. For walleye or largemouth bass, I introduced 20 fathead minnow into four mesh cages (five in each cage) right above four large respirometry chambers, plus another 20 fathead minnow in the respirometry tanks for free swimming. This again ensured walleye or largemouth, as predators, could receive both visual and olfactory cues from prey during the interaction period.

Eight fish were measured for each treatment. All chambers were deeply cleaned before every trial and the UV sterilizer was used constantly to minimize background respiration. Final $\dot{M}O_2$ accounted for background bacterial respiration by measuring respiration for 20 min cycle before and after trials and assuming a linear increase in background respiration over time.

3.2.5 Swimming performance (U_{crit}) with the heatwaves

Tests of critical swimming speed (U_{crit}) were performed in a 5 L ($30 \times 7.5 \times 7.5$ cm) flow-controlled swim tunnel for fathead minnow, and a 30 L ($46 \times 15 \times 15$ cm) flow-controlled swim tunnel for largemouth bass and walleye (Loligo; www.loligosystems.com). Swim tunnels were calibrated using a flow meter (HFA; Höntzsch) to convert motor speed to water velocity (cm s^{-1}). For these tests, individual fish were gently introduced into the swim tunnel at 25 ± 0.2 °C, with water velocity at either 1 body length (BL) s^{-1} for fathead minnow, or 0.5 BL s^{-1} for largemouth bass or walleye. Right after sealing the swim tunnel, the heatwave simulation commenced using procedures identical to those described above (i.e., 1 h ramping, 1 h at 30 °C, and 1 h cooling). Water temperature in the control group was maintained at 25 °C for the 3 h period. Following the heatwave, water velocity was increased by 0.5 BL s^{-1} every 5 min (Dai & Suski, 2019) for fathead minnow or every 20 min for largemouth bass and walleye (Hasler et al., 2009), until fish became exhausted, determined when the fish failed to move off the rear screen of the chamber for > 5 s. Once showing exhaustion, the fish was gently removed from the swim tunnel, measured for total mass and total length. Eight fish were measured for each treatment.

Critical swimming speed (U_{crit}) was calculated as:

$$U_{crit} = U + (t/T) \times \Delta U$$

where U (cm s^{-1} or BL s^{-1}) is the highest sustained water velocity fish achieved for the full 5 min for fathead minnow or 20 min for largemouth bass or walleye, ΔU is the velocity increment (i.e. 0.5 BL s^{-1}), t (min) is the time fish swam during the final increment, and T is the time increment (i.e. 5 or 20 min) (Brett, 1964). Solid blocking correction was performed for largemouth bass but

not for fathead minnow or walleye because the fish square area of fathead minnow or walleye relative to the cross-sectional area of swim tunnels was $< 5\%$ (Bell & Terhune, 1970).

3.2.6 Data analysis

All data were analyzed using R 4.0.2 (R development Core Team, 2020) with $\alpha = 0.05$. Oxygen consumption rates ($\dot{M}O_2$) per individual were calculated by the average slope of each 14 min measurement period obtained from the linear regression between oxygen consumption over time ($\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$), then corrected for the volume of the respirometry chamber and total mass of the fish. $\dot{M}O_2$ with $r^2 < 0.9$ were filtered out for quality control (Svendsen et al., 2016).

To compare MMR and SMR of each species, linear mixed models were run using the lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) packages, with $\dot{M}O_2$ as the dependent variable, and the type of $\dot{M}O_2$ (MMR or SMR) as the fixed factor. Fish ID was included as a random factor because multiple $\dot{M}O_2$ were collected from each fish. Other random factors including test date and total length were initially included in full models but were later removed based on higher AIC values. Model assumptions of linearity, normality, and homogeneity of residuals were confirmed by inspecting plots of model residuals versus fitted values and quantile-quantile plots. Marginal r^2 explaining model variance with fixed factors only and conditional r^2 explaining variance for the entire model were calculated using the MuMIn package (Bartoń, 2020).

To quantify the effects of heatwaves and predator-prey interactions on $\dot{M}O_2$ for each species, linear mixed models were again run, with $\dot{M}O_2$ as the dependent variable, treatment (heatwave or no-heatwave) and time (RMR, 1 h heatwave, 1 h after heatwave, and first hour during predator-prey interaction) as fixed factors. The interactions between treatment and time were also

included, with fish ID as a random factor to account for potential non-independence of data (i.e. different time period of $\dot{M}O_2$ for each fish). Other random factors were initially included in full models including chamber nested within treatment and test date nested within treatment but were later removed based on larger AIC values. Assumptions of linearity, normality, and homogeneity of residuals were again confirmed as described above. Tukey's HSD post hoc analyses were performed to separate means using the emmeans package (Lenth et al., 2018). Marginal r^2 and conditional r^2 were also calculated.

To quantify the effects of heatwaves on swimming performance for each species, linear mixed models were again used, with U_{crit} ($cm\ s^{-1}$ or $BL\ s^{-1}$) as the dependent variable and treatment (heatwave or no-heatwave) as the fixed factor. Total length nested within treatment was included as a random factor. Other random factors were initially included in full models but later removed based on larger AIC values, including test date nested within treatment and total mass nested within treatment. Assumptions of linearity, normality, and homogeneity of residuals were again confirmed as described above.

3.3 Results

3.3.1 MMR and SMR

After $\geq$ two weeks acclimation at 25 °C, the mass specific MMR for fathead minnow was 190 % higher than SMR ($F_{1,7} = 36.13$, $P < 0.01$, $R^2_m = 0.61$, $R^2_c = 0.75$), leaving the aerobic scope as $441 \pm 193\ mg\ O_2\ kg^{-1}\ h^{-1}$ (mean $\pm$ sd) (Table 3.1, Figure 3.1a). For largemouth bass, the mass specific MMR was 151 % higher than SMR ($F_{1,7} = 89.30$, $P < 0.01$, $R^2_m = 0.82$, $R^2_c = 0.86$), leaving the aerobic scope as $123 \pm 37\ mg\ O_2\ kg^{-1}\ h^{-1}$ (Table 3.1, Figure 3.1b). For walleye, the mass specific

MMR was only 96 % higher than SMR ($F_{1,6} = 109.76$, $P < 0.01$, $R^2_m = 0.85$, $R^2_c = 0.90$), leaving the aerobic scope as $214 \pm 54 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ (Table 3.1, Figure 3.1c).

3.3.2 $\dot{M}O_2$ with the heatwave and predator-prey interactions

The warmwater prey species, fathead minnow, rapidly increased $\dot{M}O_2$ during the 1 h heatwave, and showed elevated $\dot{M}O_2$ when encountering largemouth bass, but not walleye (Table 3.2, Figures 3.2-3.3). During the heatwave, fathead minnow increased $\dot{M}O_2$ by 1.3-fold compared to 25 °C during 10-11 am. This $\dot{M}O_2$ during the heatwave was higher than RMR by 1.9-fold. After the heatwave, the $\dot{M}O_2$ of fathead minnow returned to RMR level. However, once largemouth bass were introduced into the tank, fathead minnow showed an additional 1.6-fold increase in $\dot{M}O_2$ compared to the post-heatwave period. This $\dot{M}O_2$ was 1.3-fold of the response during the heatwave and was not affected by whether or not fish experienced the heatwave previously. In contrast, the introduction of another predator species, walleye, resulted in no significant $\dot{M}O_2$ response for fathead minnow relative to the post-heatwave period.

The warmwater predator species, largemouth bass, also showed a rapid increase in $\dot{M}O_2$ during the 1 h heatwave, but an even higher $\dot{M}O_2$ upon encountering fathead minnow, particularly if they experienced a heatwave (Table 3.2, Figures 3.4-3.5). During the heatwave, largemouth bass increased $\dot{M}O_2$ by 0.5-fold relative to 25 °C during 10-11 am. This $\dot{M}O_2$ during the heatwave was higher than RMR by 0.6-fold. During the post-heatwave period, the $\dot{M}O_2$ of largemouth bass returned to RMR level. Once fathead minnow were introduced into the holding tank, largemouth bass again showed a significant $\dot{M}O_2$ increase, with 0.6-fold increase in $\dot{M}O_2$ compared to the “post-heatwave” period for fish not experiencing heatwave before or 1.0-fold increase experiencing heatwave before. Thus, largemouth bass that experienced the heatwave had higher

$\dot{M}O_2$ by 0.4-fold compared to fish that did not experience the heatwave, and this $\dot{M}O_2$ level was even 0.5-fold higher than $\dot{M}O_2$ during the heatwave.

The coolwater predator species, walleye, also showed a rapid increase in $\dot{M}O_2$ during the 1 h heatwave, but did not respond metabolically to prey exposure. During the heatwave, walleye increased $\dot{M}O_2$ by 0.6-fold compared to 25 °C during 10-11 am. This $\dot{M}O_2$ during the heatwave was higher than RMR by 0.6-fold. During the post-heatwave period, the $\dot{M}O_2$ of walleye returned to RMR level. Once fathead minnow were introduced into the holding tank, walleye did not show significant response in terms of $\dot{M}O_2$ values relative to post-heatwave. The 48-h mortality rate of walleye used in the analysis was zero.

3.3.3 Swimming performance (U_{crit}) with the heatwave

After experiencing 1 h 30 °C heatwave, fathead minnow did not show a statistically significant decrease in swimming performance, despite with $P < 0.1$ (U_{crit} in BL s⁻¹: $F_{1, 6.9} = 5.09$, $P = 0.06$; U_{crit} in cm s⁻¹: $F_{1, 7.2} = 4.26$, $P = 0.08$) (Table 3.2, Figure 3.6a-3.6b). Largemouth bass showed no change in swimming performance after experiencing the heatwave (U_{crit} in BL s⁻¹: $F_{1, 13.0} = 0.45$, $P = 0.51$; U_{crit} in cm s⁻¹: $F_{1, 13.0} = 0.74$, $P = 0.41$) (Table 3.2, Figure 3.6c-3.6d). However, with 1 h 30 °C heatwave exposure, walleye, the only coolwater species in the study, showed 25% lower U_{crit} (BL s⁻¹) ($F_{1, 11.3} = 13.40$, $P < 0.01$) and 28% lower U_{crit} (cm s⁻¹) ($F_{1, 11.3} = 9.17$, $P = 0.01$) (Table 3.2, Figure 3.6e-3.6f).

3.4 Discussion

By simulating the heatwaves in the laboratory using the current summer average and extreme temperatures in forested streams in Midwestern USA, where recent sampling records of

fish communities include both coolwater and warmwater species (Stein et al., 2021), I found that, compared to warmwater predator largemouth bass and prey fathead minnow, the coolwater predator walleye had limited relative AS above SMR at 25 °C, low metabolic activity when encountering prey, and reduced swimming performance after experiencing 1 h 30 °C heatwave. All of these are clear evidence of thermal disadvantages for walleye relative to warmwater species who share a similar trophic position in local fish communities. The warmwater predator largemouth bass had relatively high aerobic potential based on AS, evidenced by greatly increased $\dot{M}O_2$ when encountering prey. The swimming performance of largemouth bass was not affected by 1 h 30 °C heatwave. The warmwater prey fathead minnow, again as in Chapter 2, showed thermal resilience, evidenced by AS being two times SMR and quick $\dot{M}O_2$ response to heatwave and predator existence. The swimming performance of fathead minnow was minimally affected by the heatwave.

3.4.1 SMR, MMR, and AS without heatwaves

Walleye at 25 °C showed lower aerobic potential compared to largemouth bass and fathead minnow, implying competitive disadvantages against warmwater fishes in summer. To my knowledge, this is the first study on MMR, SMR, and AS of walleye, using standardized intermittent-flow respirometry. Galarowicz and Wahl (2003) previously used closed-vessel respirometry to measure SMR of walleye. Results were 210-377 mg O₂ kg⁻¹ h⁻¹ when acclimated at 25 °C. The lower end of their results is close to my SMR of juvenile walleye (Table 3.1). This is reasonable as the method of closed-vessel respirometry could overestimate SMR because measurements by Galarowicz and Wahl (2003) were made right after fish were introduced into the chamber due to the nature of closed-vessel respirometry, rather than overnight resting values in

this study. The SMR of the warmwater predator, largemouth bass, was only 37 % of walleye. To rule out the possibility that such difference is only due to body size, I referred to Redpath et al. (2010) and found smaller largemouth bass (79 – 95 g) still showed lower SMR (i.e., 104 – 115 O₂ kg⁻¹ h⁻¹) at similar temperatures. Besides predators, prey fathead minnow, which body weight is < 4 % of walleye, showed similar SMR (216 vs. 224 kg⁻¹ h⁻¹) to walleye. This is additional evidence that coolwater walleye require more energy for homeostasis, thus potentially less energy efficient (Rummer et al., 2014; Lefevre et al., 2021). This is a competitive disadvantage of walleye against warmwater competitors and prey.

The FAS of walleye was shown to be narrower than both largemouth bass and fathead minnow (ie., 1.0 vs. 1.5 and 1.9). With narrower aerobic scope, walleye have less capacity for digesting prey, even after successful predation, due to limited room for the specific dynamic action of food, possibly affecting growth (Clark et al., 2013). Coupled with being less energy efficient due to higher homeostatic maintenance, walleye could perform poorly compared to other co-existing warmwater predator species in the local community (Schulte, 2015). This is either because narrow FAS leave no buffer for extreme heatwave events that could cause direct death, extirpating the walleye population locally, or because the limited FAS constrains the ability of fish to “multitask” physiologically, including growth, forage, escape, and later reproduction (Killen et al., 2007; Allan et al., 2015), reducing competitive ability. With current summer temperatures in the Kaskaskia River Watershed, the competitive disadvantages of coolwater walleye compared to warmwater largemouth bass is evident physiologically.

3.4.2 Metabolic rates with the heatwave and predator-prey interactions

During the 1 h heatwave at 30 °C, walleye had almost same mean $\dot{M}O_2$ as MMR at 25 °C (434 vs. 438 O₂ kg⁻¹ h⁻¹), while mean $\dot{M}O_2$ of largemouth bass was only 65 % of MMR (135 vs. 206 O₂ kg⁻¹ h⁻¹). This indicates that a 1 h 30 °C heatwave, which is already occurring in the Kaskaskia River Watershed (Dai et al. In Press), could already push walleye to their upper aerobic limits, leaving walleye little aerobic potential for activities such as predation and digestion. Otherwise, the distribution of walleye could be more constrained in deeper water column of larger water bodies. Besides that, such thermal stressors could cause elevated oxidative damage, as well as increased bacterial and parasitic infections, that could lead to reduced fitness (Stillman, 2019). If persisting for extended hours, such heatwaves may eventually cause equilibrium loss or even delayed mortality of walleye locally (Lefevre et al., 2021), or range shifts (Comte et al., 2013). The same intensity and duration of heatwaves seem to put little stress on largemouth bass. With the frequency and severity of heatwaves predicted to increase in the near future (Stillman, 2019), there could still be thermal safety margin (Madeira et al., 2017) for largemouth bass, but not for coolwater walleye in the Kaskaskia River Watershed. This difference in physiological performance could be larger if the heatwaves happen in later spring or early autumn, when the “acclimation” effect is weaker (Beitinger & Bennett, 2000). Besides comparisons between predators, small prey fathead minnow had up to over 150 % $\dot{M}O_2$ relative to mean MMR at 25 °C (941 vs. 627 O₂ kg⁻¹ h⁻¹), compared to large predator largemouth bass. The difference in the response from different sized warmwater species could represent varying energetic costs during heatwaves, favoring predator species. All of these, including the different performance of coolwater vs. warmwater predators and predators vs. prey species, could influence the local community structure in the long term (Cohen et al., 2018).

Beyond testing heatwave effects on the metabolism of individuals independently, I also incorporated predator-prey interactions using visual and chemosensory stimuli. Hall and Clark (2016) witnessed an elevated metabolic cost for prey when noticing the presence of predators, especially when being “attacked” (visual cue) outside of respirometry chambers. Both warmwater species in this study, fathead minnow and largemouth bass, showed a significant metabolic reaction when exposed to their prey or predators. Surprisingly, largemouth bass showed an even higher $\dot{M}O_2$ during the prey exposure period compared to when experiencing 1 h 30 °C heatwave. Thus, such heatwaves show limited impacts on their predation capacity. During the testing period, despite not quantifying behavioral responses, anecdotally, fathead minnow showed burst swimming activities within their chambers when exposed to largemouth bass but not when exposed to walleye. Also, when largemouth bass were exposed to their prey, I witnessed a number of predation attempts. In contrast, despite active daily consumption of live fathead minnow during acclimation period, walleye did not respond metabolically to the introduction of fathead minnow, no matter previously experiencing heatwave or not. Also, when being introduced as predators, fathead minnow did not respond actively to the presence of walleye. These results emphasize the inactivity of walleye at or near their upper thermal limit. Thus, the limited aerobic potential of walleye following exposure to summer extreme temperatures could inhibit their ability to perform burst swimming or other predation-related activities (Schulte, 2015). Also, both chronic thermal stress at 25 °C and acute thermal stress during the 30 °C heatwave may interrupt endocrine balance, causing loss of appetite for walleye (Lefevre et al., 2021). By quantifying the metabolism changes of predators and prey during heatwaves and later interactions, I identified that coolwater walleye were unable to have enough aerobic potential to safely overcome heatwaves and generate effective predation responses.

3.4.3 Swimming performance under summer conditions following the heatwave

Largemouth bass showed no reduced U_{crit} after the 1 h 30 °C heatwave, being around 2 BL s^{-1} at 25 °C. This is similar to previous tests of 2.17 ± 0.12 BL s^{-1} at 25 °C (Hasler et al., 2009). Fathead minnow showed a noticeable, but non-significant, reduction in swimming performance, suggesting a reduced predator-avoidance capacity after heatwaves, especially facing thermal-tolerant predators like largemouth bass. The U_{crit} of fathead minnow at 25 °C is close to previous reports (i.e., 45.68 ± 4.53 vs. ~ 38 cm s^{-1}) (Kolok et al., 2004). Unlike warmwater predator and prey, the swimming performance of coolwater walleye was significantly impaired following a 1 h 30 °C heatwave, with ≥ 25 % decrease in U_{crit} (both in BL and cm). Such impairment of swimming further confirms the risk of narrow AS, as swimming is largely fueled by aerobic metabolism (Rubio-Gracia et al., 2020). With similar total length to largemouth bass, walleye would be expected to have larger U_{crit} due to its higher swimming potential in streams and rivers (Peake et al., 2000). However, the advantage of swimming performance of walleye could largely vanish under the heatwave and elevated summer temperatures, leaving largemouth bass, or other warmwater predators, to be a better competitor in riverine environments (Domenici et al., 2020), where swimming is important for maintaining position, movement, attacking, and escape (Hasler et al., 2009; Rubio-Gracia et al., 2020).

This study is the first to test the swimming performance of walleye at 25 °C, close to its chronic upper thermal tolerance (Nevada Division of Environmental Protection, 2018). It is reported that under lower temperature, 20.5 °C, U_{crit} of walleye (Fork length = 180 mm) were projected to be 0.35 and 0.64 cm s^{-1} with water velocity increased by 10 cm s^{-1} for every 60-min and 10-min period, respectively (Peake et al., 2000). My results offer higher-resolution guidance

for future stream flow management on walleye after considering not only static but fluctuating summer temperatures. Also, for other coolwater or coldwater species, swimming performance not only at constant temperatures, but also under heatwaves, should be considered when designing migration paths or corridors.

3.4.4 Conclusion

Recent modeling work using recruitment and abundance data, coupled with abiotic projections under climate change, has projected shifts from coolwater walleye to warmwater largemouth bass in Midwestern lakes (Hansen et al., 2017). I sought to use ecophysiology to define possible mechanisms related to these community shifts, focusing on metabolic and swimming parameters. My results, including metabolic rates and swimming performance, show that walleye, or other similar coolwater species, will have competitive disadvantages against warmwater species like largemouth bass, which could be replacing native coolwater fishes (Hansen et al., 2017; Missaghi et al., 2017). What is more, other stresses, such as low dissolved oxygen and high ammonia, often coexist with heatwaves and could further impact the more sensitive coolwater species (Cai & Summerfelt, 1992). By incorporating physiology into modeling on biodiversity (Bruggeman & Kooijman, 2007; Thuiller et al., 2013), we could more efficiently predict the impacts of climate change, then offer conservation guidance for coolwater species (e.g., walleye and Salmonidae species) that are economically and spiritually important (Nesper, 2002; Powers, 2000).

In the era of rapid climate change, in addition to changes in average temperature, heatwaves could also shape or change species performance. Here, I show that, under current summer heatwaves in forested streams, the performance of coolwater and warmwater species could vary

greatly, thus potentially altering predator-prey relationships and competition outcomes. This chapter partially answers the question I raised in the discussion part of Chapter 2 as I extend the ecophysiological experiments from individual prey species to key predators and prey in local fish communities of the Midwest. However, to scale our predictions up to regional level, I need to combine both lab-derived results and ecological modeling for better management guidance. Thus, in the next chapter, I will illustrate this part.

3.5 Figures and tables

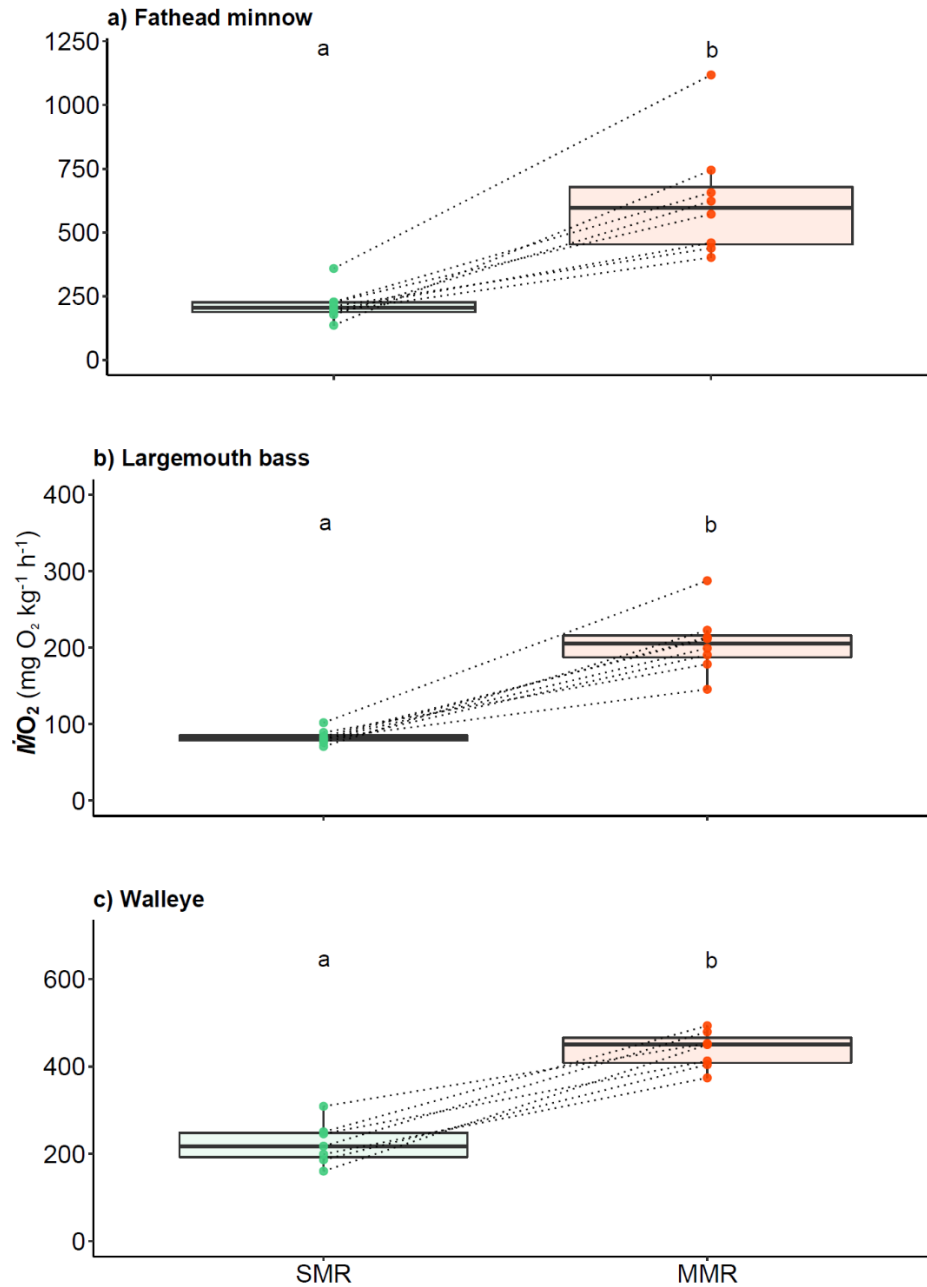


Figure 3.1 Standard and maximum metabolic rate (SMR and MMR, $\pm$ se) of fathead minnow (*Pimephales promelas*), largemouth bass (*Micropterus salmoides*), and walleye (*Sander vitreus*) at 25 °C. Groups ($n = 8$ per group) with different letters are significantly different ($P < 0.05$) between SMR and MMR.

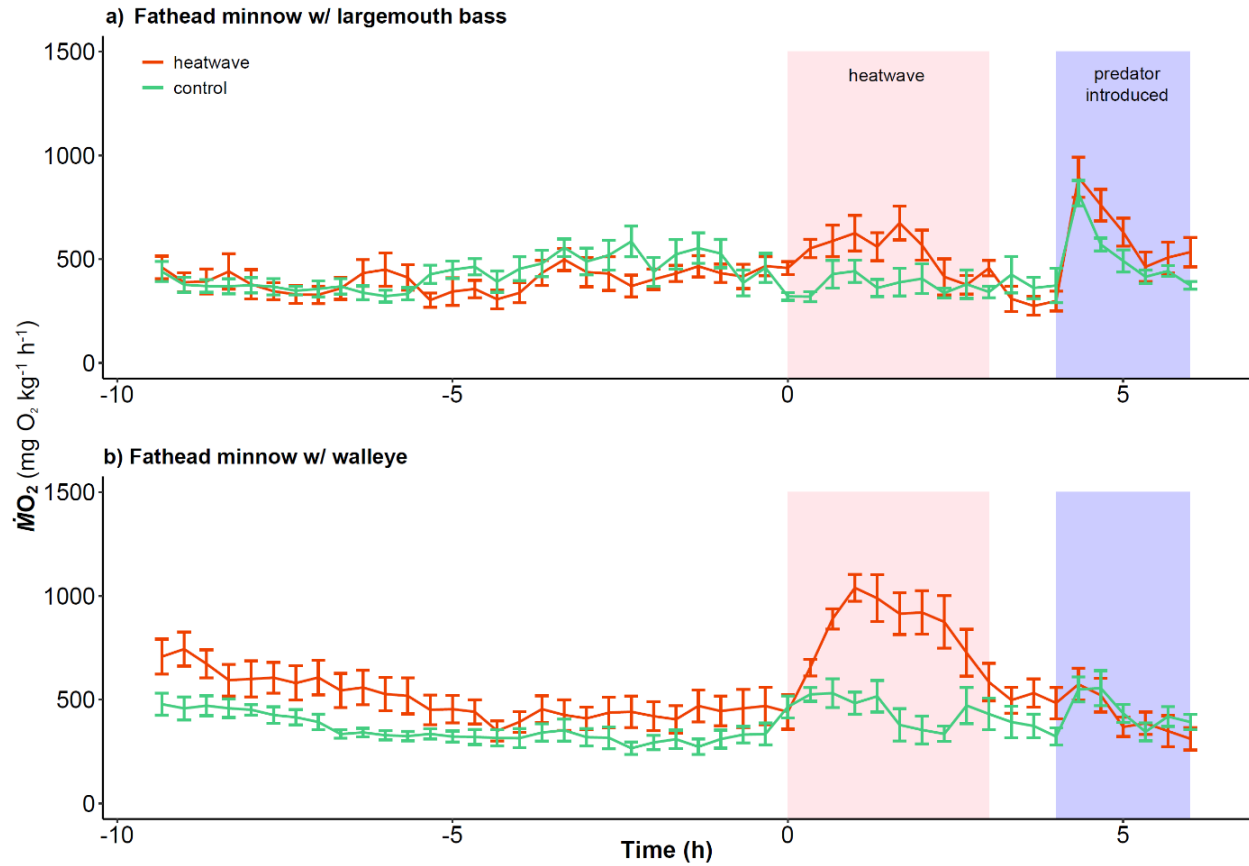


Figure 3.2 Oxygen consumption rates ($\dot{M}O_2$, $\pm$ se) of fathead minnow (*Pimephales promelas*) ($n = 8$ per group) during the heatwave (pink background) and predator-prey interactions (purple background). Time at 0 h represents the start of temperature changes. Heatwave included 1 h heating, 1 h heatwaves at 30 °C, and 1 h cooling. Predators were either largemouth bass (*Micropterus salmoides*) (a) or walleye (*Sander vitreus*) (b).

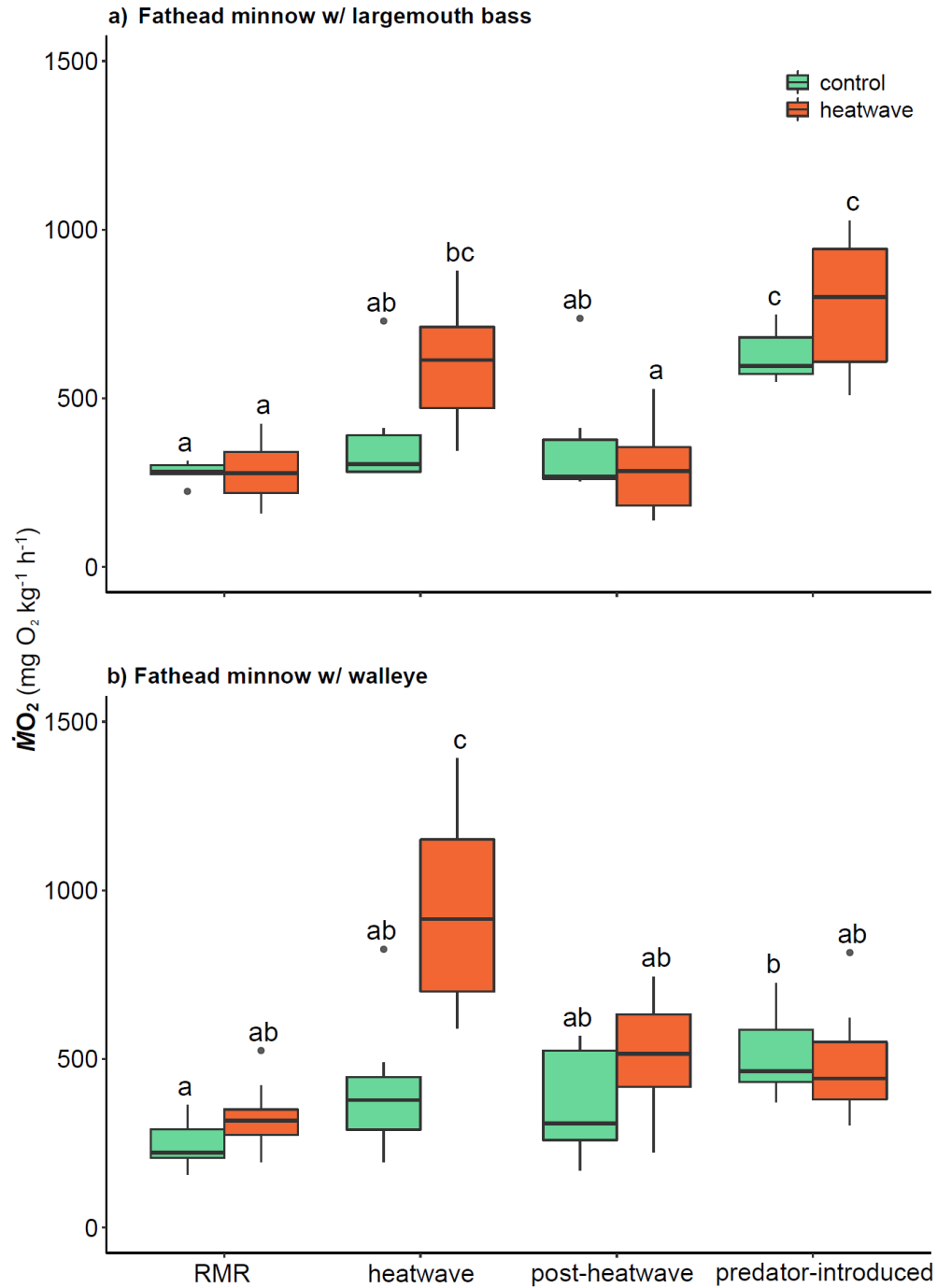


Figure 3.3 Oxygen consumption rates ($\dot{M}O_2$, $\pm$ se) of fathead minnow (*Pimephales promelas*) ($n = 8$ in per group) during the heatwave and predator-prey interactions. Predators were either largemouth bass (*Micropterus salmoides*) (a) or walleye (*Sander vitreus*) (b). Horizontal line = median; box = first quartile to third quartile; vertical line = 1.5 interquartile range. Different letters represent statistical differences ($P < 0.05$).

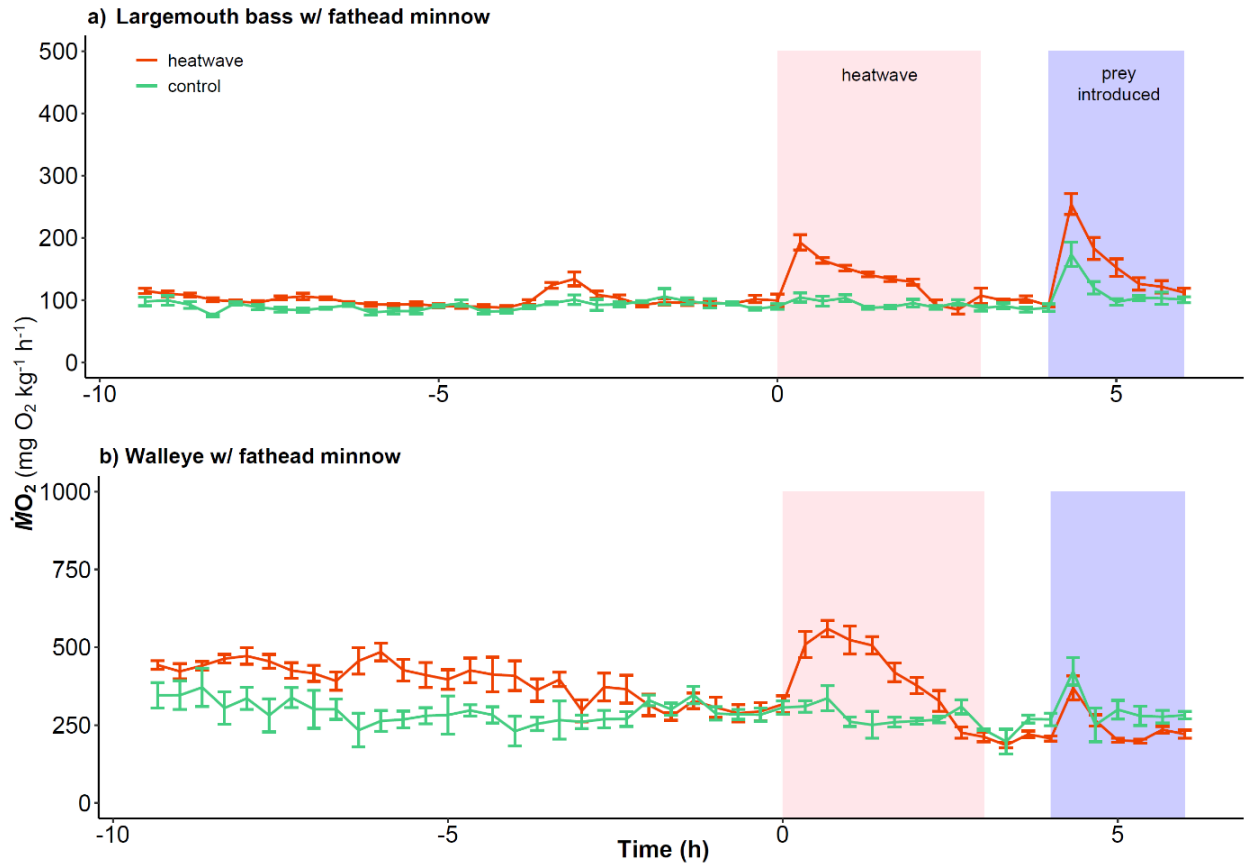


Figure 3.4 Oxygen consumption rates ($\dot{M}O_2$, $\pm$ se) of largemouth bass (*Micropterus salmoides*) (a) or walleye (*Sander vitreus*) (b) ($n = 8$ in each predator group) during the heatwave (pink background) and predator-prey interactions (purple background). Time at 0 h represents the start of temperature changes. Heatwave included 1 h heating, 1 h heatwaves at 30 °C, and 1 h cooling. Prey were fathead minnow (*Pimephales promelas*).

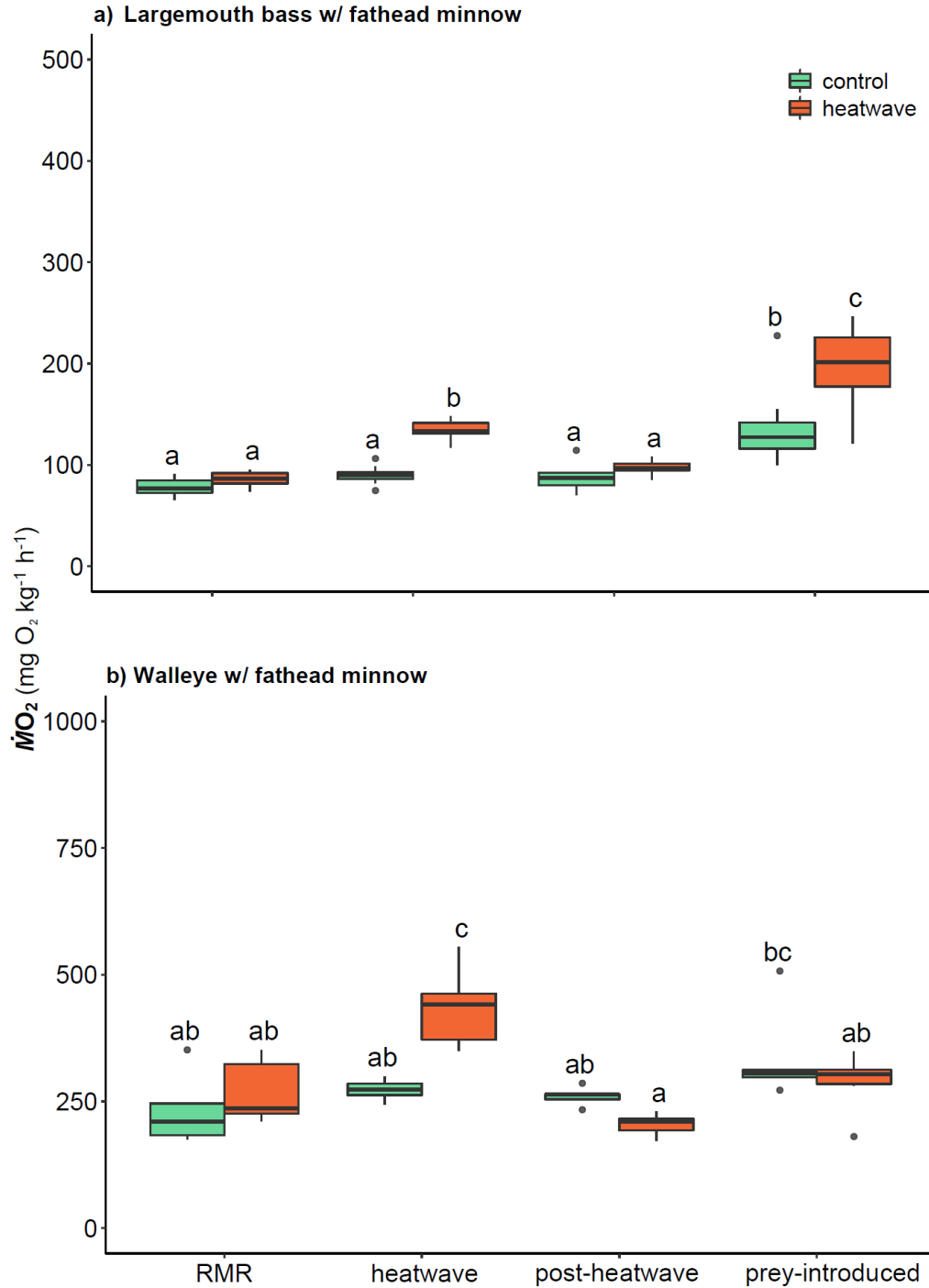


Figure 3.5 Oxygen consumption rates ($\dot{M}O_2$, $\pm$ se) of largemouth bass (*Micropterus salmoides*) (a) or walleye (*Sander vitreus*) (b) ($n = 8$ in per group) during the heatwave and predator-prey interactions. Horizontal line = median; box = first quartile to third quartile; vertical line = 1.5 interquartile range. Different letters represent statistical differences ($P < 0.05$).

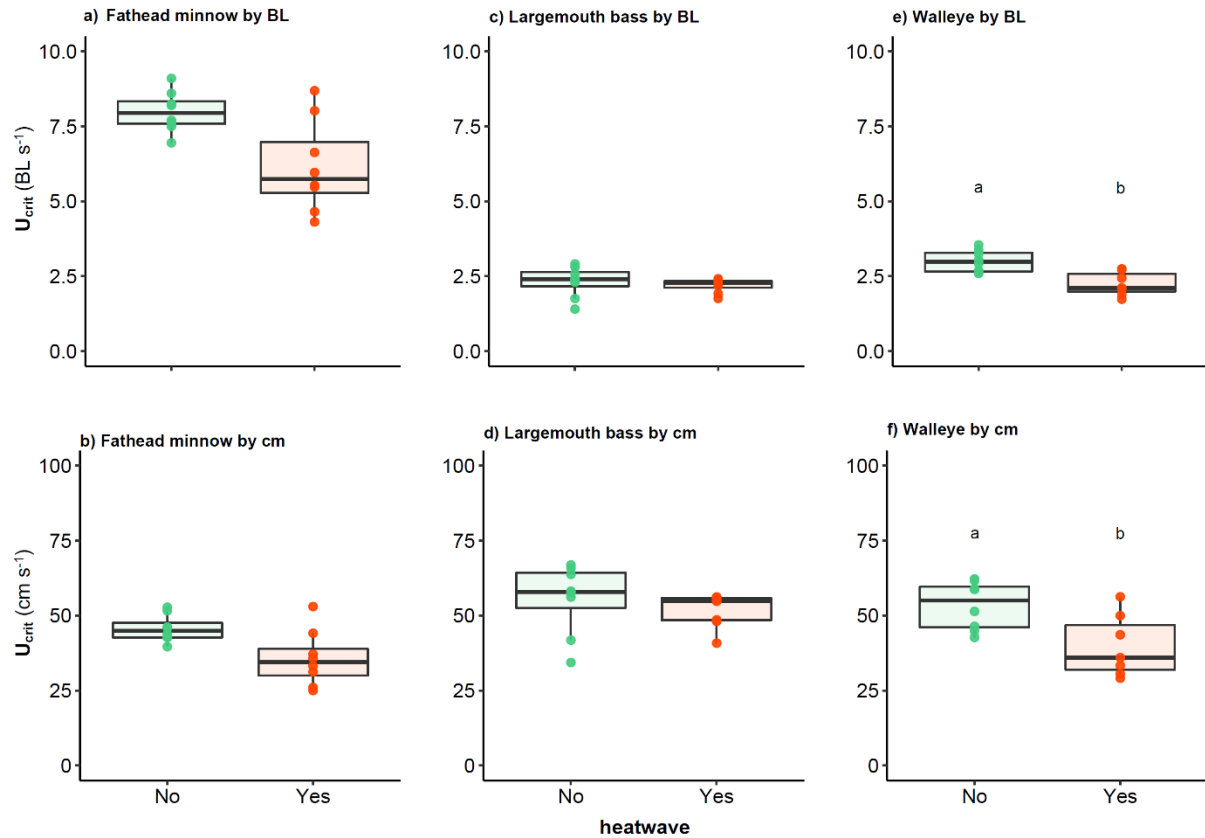


Figure 3.6 Critical swimming speed (U_{crit} , $\pm$ se) by body length (BL) or cm of fathead minnow (*Pimephales promelas*) (a, b), largemouth bass (*Micropterus salmoides*) (c, d), and walleye (*Sander vitreus*) (e, f) ($n = 8$ in per group) after heatwave or not at 25 °C. Horizontal line = median; box = first quartile to third quartile; vertical line = 1.5 interquartile range. Different letters represent statistical differences ($P < 0.05$) between heatwave group and control group.

Table 3.1 Mean ($\pm$ sd) whole-animal and mass-specific metabolic rates ($\dot{M}O_2$) and body size during respirometry for fathead minnow (*Pimephales promelas*), largemouth bass (*Micropterus salmoides*), and walleye (*Sander vitreus*).

Species	Temperature ($^{\circ}C$)	n	Total length (mm)	Total mass (g)	Whole animal (mg O_2 h $^{-1}$)			Mass specific (mg O_2 kg $^{-1}$ h $^{-1}$)		
					SMR	MMR	AS	SMR	MMR	AS
Fathead minnow	25	8	59 $\pm$ 6	1.7 $\pm$ 0.5	0.38 $\pm$ 0.15	1.07 $\pm$ 0.43	0.69 $\pm$ 0.32	216 $\pm$ 65	627 $\pm$ 230	441 $\pm$ 193
Largemouth bass	25	8	225 $\pm$ 11	188.3 $\pm$ 28.8	15.47 $\pm$ 1.91	38.40 $\pm$ 7.29	22.92 $\pm$ 8.83	82 $\pm$ 9	206 $\pm$ 41	123 $\pm$ 37
Walleye	25	7	171 $\pm$ 19	49.1 $\pm$ 17.2	10.87 $\pm$ 3.67	21.03 $\pm$ 5.33	10.16 $\pm$ 2.87	224 $\pm$ 49	438 $\pm$ 43	214 $\pm$ 54

SMR, standard metabolic rate; MMR, maximum metabolic rate; AS, aerobic scope.

Table 3.2 Results of linear mixed effect models examining factors affecting mass specific metabolic rate ($\dot{M}O_2$) under heatwave and predator-prey interactions for fathead minnow (*Pimephales promelas*), largemouth bass (*Micropterus salmoides*), and walleye (*Sander vitreus*). Related to Figures 3.2-3.5, time represents different measurement periods, including resting metabolic rate (RMR), 1 h heatwave at 30 °C (heatwave), 1 h post heatwave (post-heatwave), and first 1h of predator-prey interaction (interaction). Model includes fish ID as a random factor. Marginal $r^2 = 0.62, 0.58, 0.75$, and 0.57 , while conditional $r^2 = 0.74, 0.74, 0.77$, and $0.0.65$ for fathead minnow with largemouth bass predator, fathead minnow with walleye predator, largemouth bass with fathead minnow prey, and walleye with fathead minnow prey, respectively. Significant factors are shown in **bold**.

Species		Sum Sq	Mean Sq	NumDF	DenDF	F value	P
fathead minnow with largemouth bass predator	Heatwave or not	32233	32233	1	13.35	2.35	0.15
	Time	1543626	514542	3	36.83	37.54	<0.01
	Heatwave or not *Time	178811	59604	3	36.83	4.35	0.01
fathead minnow with walleye predator	Heatwave or not	156466	156466	1	14	8.68	0.01
	Time	1224529	408176	3	42	22.64	<0.01
	Heatwave or not *Time	709031	236344	3	42	13.11	<0.01
largemouth bass with fathead minnow prey	Heatwave or not	11215	11215	1	14	25.52	<0.01
	Time	68427	22809	3	42	51.90	<0.01
	Heatwave or not *Time	7926	2642	3	42	6.01	<0.01
walleye with fathead minnow prey	Heatwave or not	2863	2863	1	9	0.98	0.35
	Time	102772	34257	3	27	11.78	<0.01
	Heatwave or not *Time	84203	28068	3	27	9.65	<0.01

Table 3.3 Mean ($\pm$ sd) critical swimming speed (U_{crit}) by body length (BL) and cm and body size for fathead minnow (*Pimephales promelas*), largemouth bass (*Micropterus salmoides*), and walleye (*Sander vitreus*).

Species	Treatment	Temperature (°C)	Total length (mm)	Total mass (g)	U_{crit} (BL s ⁻¹)	U_{crit} (cm s ⁻¹)
Fathead minnow	30 °C heatwave	25	58 $\pm$ 2	1.74 $\pm$ 0.24	6.16 $\pm$ 1.54	35.68 $\pm$ 9.31
	Control	25	57 $\pm$ 1	1.73 $\pm$ 0.24	7.99 $\pm$ 0.68	45.68 $\pm$ 4.53
Largemouth bass	30 °C heatwave	25	237 $\pm$ 10	201.38 $\pm$ 32.20	1.98 $\pm$ 0.21	46.77 $\pm$ 4.76
	Control	25	240 $\pm$ 10	222.88 $\pm$ 35.11	2.08 $\pm$ 0.47	49.75 $\pm$ 10.43
Walleye	30 °C heatwave	25	176 $\pm$ 21	47.14 $\pm$ 19.40	2.25 $\pm$ 0.40	39.83 $\pm$ 10.38
	Control	25	179 $\pm$ 24	55.63 $\pm$ 20.32	3.00 $\pm$ 0.36	53.37 $\pm$ 7.97

CHAPTER 4: WATERSHED-SCALE PROJECTIONS OF BIODIVERSITY UNDER AGRICULTURAL CONSERVATION PRACTICES AND CLIMATE CHANGE

4.1 Introduction

Global climate change is driving ecosystem reorganization (Antão et al., 2020; Comte et al., 2021). Freshwater ecosystems are among the most threatened in the world, with the highest extinction rates among vertebrates (Grooten & Almond, 2018). In rivers, thermal and flow regimes are two top factors altered by climate change (Webb, 1996; Jones, 2011) that are driving ecosystem changes through displacing fishes sensitive to temperature and flow, with more tolerant ones (Comte et al., 2021).

Regional land-use is another factor interacting with climate change to influence biodiversity. This interaction can exert additive, synergistic, or antagonistic impacts on riverine ecosystems (Radinger et al., 2016). Agriculture is the greatest driver among all landscape changes, encompassing > 40 % world's land surface (Raven & Wagner, 2021). In agricultural landscapes, the impacts of climate change on temperature and flow can be intensified due to 1) increased solar radiation following the reduction of riparian cover (Caissie, 2006), 2) altered flow patterns from subsurface drain tiles and surface ditches (Blann et al., 2009), and 3) decreased groundwater recharge after water extraction for irrigation (Loheide & Gorelick, 2006). Moreover, nutrient input from fertilizers, toxicants from pesticides, and fine sediment from soil erosion can further stress already fragile riverine communities (Wantzen & Mol, 2013; Op de Beeck et al., 2017; Wang et al., 2020). To improve water quality in agricultural watersheds, best management practices (BMPs) have been promoted across the world, which mainly aim to reduce non-point source pollution, including sediment and nutrient loading, to ensure long-term economic sustainability and

environmental resilience (Bekele et al., 2012). However, the effects of such conservation efforts on local biodiversity have yet to be rigorously evaluated (Allan, 2004; South et al., 2019). In theory, agricultural conservation using BMPs, by the name itself, should maintain a balance between agricultural production and ecosystem stability.

Diversity indices based on presence and abundance data have been important tools used to evaluate the effectiveness of conservation efforts on riverine ecosystems under climate change (Oberdorff et al., 2011). Unfortunately, most biodiversity measures cannot identify the mechanism(s) responsible for changes to the community behind the numbers, such as the replacement of sensitive species by tolerant ones, or the invasion of nuisance species (Murdoch et al., 2020). In the last two decades, trait-based ecosystem vulnerability analysis has gained greater attention when modeling biodiversity responses under climate change, as it can incorporate both taxonomic diversity, and the physiological, morphological, or life-history characteristics of each species (Poff, 1997; McGill et al., 2006; Zakharova et al., 2019). Such an approach offers the potential to discover mechanistic explanations for community changes, which can be applied beyond the species level across different ecosystems (Olden et al., 2006; Frimpong & Angermeier, 2010). Functional diversity indices, developed from the quantification of functional traits (Petchey & Gaston, 2006), can elucidate species' roles in ecosystem functioning, thus offering better explanatory power of how climate and land-use drive ecosystem changes (Cadotte et al., 2011). When it comes to conservation and restoration, functional diversity could be a better choice for decision-making processes.

To illustrate how climate and agriculture can affect riverine biodiversity using trait-level and community-level modeling, I studied the Kaskaskia River Watershed in Illinois, USA, due to

1) its > 50 years' standardized sampling program of productive and speciose fish community throughout the watershed, 2) extensive monitoring on water quality, 3) heavily altered riverine environment by agriculture, and 4) previous species-level projections (Cao et al. 2016; Acero Triana et al., 2021). As such, I sought to quantify 1) the contemporary spatial distribution of functional diversity and species richness for fish communities, 2) the key environmental drivers on biodiversity, 3) the potential changes in biodiversity under future climate, and most importantly, 4) whether agricultural conservation practices, including crop rotation, cover crops, reduced tillage, and reduced or split fertilizer application, could help with biodiversity conservation.

4.2 Materials and methods

4.2.1. Study area

The Kaskaskia River Watershed is a major tributary of the Mississippi River, located in central and southwestern Illinois, USA (Figure 4.1). It drains 14,880 km², 10.2% of Illinois (USACE, 2017). Prior to European settlement in the 1700s, the watershed was mainly covered by prairies and forests. Today, the land cover has been altered to predominately croplands (63%), mostly corn and soybeans. Forest and grasslands have decreased to 16% and 9%, respectively, while urban or impervious surface has increased to 9% (USDA, 2016). Besides land cover changes, many meandering streams and rivers have also been channelized and dredged, with drain tiles installed in most farmland (Blann et al., 2009). Two large reservoirs, Lake Shelbyville and Carlyle Lake, were constructed on the main channel during 1960-1970s (Shasteen et al., 2013). Changes in land cover and flow regime accelerated the fragmentation and degradation of aquatic ecosystems in the Kaskaskia River Watershed (Larimore & Bayley, 1996; Acero Triana et al., 2021) .

4.2.2 Data source

4.2.2.1 Fish sampling data

Fish data were combined from two datasets: 1) the Intensive Basin Survey monitored by Illinois Department and Natural Resource (IDNR) and Illinois Environmental Protection Agency (IEPA); 2) Monitoring and Assessment of Aquatic Life program from Illinois Natural History Survey (INHS). Sampling sites were determined by fisheries biologists, based on geology, biodiversity, pollution, and historical sampling records (Cao et al., 2016). Fish sampling followed standard IDNR protocols (IDNR, 2010) using an electric seine or backpack electrofishing, as well as boat electrofishing, during May-October from 1952 to 2015 (Cao et al., 2016). To avoid sampling inconsistency and represent the latest fish community composition across the Kaskaskia River Watershed, I only included the latest year per sampling site after 1995. Due to the productive and speciose nature of Midwestern streams and rivers (Smith et al., 2010), I further excluded sites with < 20 individuals and < 5 species to avoid any bias from improper field operations (Cao et al., 2015). These criteria resulted in 91 fish sites in 1st-4th order streams and rivers distributed across the entire watershed (Figure 4.2).

4.2.2.2 Environmental data

I grouped environmental data into two categories: 1) variables affected by climate and agricultural practices, including those describing water quality, water temperature, and flow, referred to as climate-related variables hereafter, and 2) non-climate variables that were not expected to change over the period from 1995-2100.

For climate-related data, I utilized a hydrologic model previously generated for the entire Kaskaskia River Watershed (Acero Triana et al., 2021). This hydrologic model was based on the Soil and Water Assessment Tool (SWAT), using topography, soil, land cover, meteorology, and agricultural conservation practices as inputs. The output, climate-related variables, included streamflow, suspended sediment, nitrate, and dissolved oxygen. Besides these, water temperature was estimated by regression between air and water temperature records at two gauges in the watershed where water temperature measurements are available. All five variables were calculated with mean, minimum, maximum, and standard deviation annually based on daily values from 1990 to 2015 for the baseline model (contemporary) and from 2015 to 2100 for the projection. The spatial resolution of these variables was at the subbasin level ($n = 175$), where a subbasin was defined as the drainage area of one stream or river segment. Future environmental projections were built upon the baseline model, using future climate conditions and scenario-based agricultural conservation practices.

Future climate projections were based on 32 different temperature and precipitation projections from the Atmosphere-Ocean Global Climate Models (AOGCM) under the highest representative concentration pathway (RCP8.5), referred as “business as usual”. As part of these projections, minimum, mean, and maximum annual air temperature were projected to increase by 0.92, 0.61, and 0.79 °C per decade, with a total increase of 8.82, 5.62, and 7.56 °C by 2100, respectively. Mean annual precipitation was projected to increase slightly, with minimum, mean, and maximum precipitation close to 750, 1000, and 1500 mm, by 2100, respectively (Acero Triana, 2020).

The scenario-based agricultural conservation practices considered in the model were, 1) crop rotation, 2) cover crops, 3) reduced tillage, and 4) reduced or split fertilizer application. These four practices were prioritized by landowners in the Kaskaskia River Watershed, and believed to help improve the sustainability and resilience of the watershed, based on a previous survey (Shipley et al., 2020), coupled with state conservation reports (IDNR, 2017; IEPA, 2019). Twelve agricultural conservation scenarios were generated that incorporated these four conservation practices, with different magnitudes (Acero Triana et al., 2021). These agricultural conservation practices were predicted to be very effective for nutrient and sediment input reduction into streams, with nitrate predicted to decrease up to 40 and 30 % during 2020-2029 and 2030-2039, and suspended sediment predicted to decrease up to 40% and 40% during the same period. For future projections, I first averaged 32 climate scenarios for changes in air temperature and precipitation, then applied the 12 conservation scenarios above climate change.

For non-climate data, I referred to previous species distribution models across streams in Illinois, USA (Cao et al., 2016). Based on the rankings of how non-climate environmental variables influenced fish species abundance, I selected 15 variables: soil permeability, land cover (% forest cover and % agricultural cover), channel gradient, watershed slope (total-watershed and local-watershed slope), bedrock depth (< 15 m and < 30 m below), dam (upstream dam, distance to upstream dam, downstream dam, and distance to downstream dam), stream order, sinuosity, and watershed area. These 15 variables were selected among > 300 candidate environmental variables compiled from the Great Lakes Regional Aquatic Gap Analysis Project (Brenden et al., 2008; Steen et al., 2008; McKenna et al., 2013; Cao et al., 2016). The resolution was at the reach level, where the reach was defined as a stream section between the confluences of an upstream tributary and a downstream tributary.

4.2.3 Data analysis

4.2.3.1 Functional traits

Compared to taxonomy-based models, trait-based models have the potential to simplify data and provide a mechanistic understanding about fish distribution and community changes (Frimpong & Angermeier, 2010). Among all traits, ecological traits involving life history, trophic level, and habitat preference are well studied, widely available, and have been proven to effectively predict species distributions under environmental change (Olden et al., 2006; Blanck et al., 2007; Parker et al., 2018; Mao et al., 2021). Besides these, physiological tolerance is directly related to habitat selection for fish, making physiological traits ideal to model future species distribution when evolutionary potential is excluded (Giacomini et al., 2013; Lange et al., 2016). I thus used both ecological and physiological trait groups (Table 4.1) for each species.

4.2.3.2 Functional diversity indices

Out of many functional diversity indices, I focused on two: functional dispersion (FDis) and functional evenness (FEve) as 1) they represent different aspects of functional structure; 2) they are not sensitive to species richness, and 3) they can be used to test unlimited numbers of traits (Laliberté & Legendre, 2010; Parker et al., 2018; Mao et al., 2021). I did not test functional richness (FRic) because 1) it cannot account for the relative abundance of species (Laliberté & Legendre, 2010; Pla et al., 2011) and 2) it had low quality of reduced-space representation in my models. I then used traditional species richness as an alternative.

FDis measures the variability of species traits in a local community by quantifying the spread of traits from the centroid in high-dimensional space for each species. It accounts for species

abundance by shifting the centroid towards more abundant species. FDis has lower boundary at zero, and no upper boundary. The fewer traits that abundant species share, the higher the FDis value will be (Laliberté & Legendre, 2010; Pla et al., 2011).

FEve measures the regularity of species traits. It is calculated by minimum spanning tree method, which connects all trait points in a high-dimensional space, using the lowest total tree branch length. FEve quantifies how evenly individuals are distributed in the trait space. FEve has a boundary between zero and one. The fewer common traits that species share, or the less evenly distributed species abundance is, the higher FEve value will be (Gaëlle & Jean-Claude, 2018).

To evaluate how different trait types were associated with all environmental variables described earlier, I calculated FDis and FEve based on 1) ecological traits (FDis_E and FEve_E), 2) physiological traits (FDis_P and FEve_P), and 3) both ecological and physiological traits (FDis and FEve) for all 91 sampling sites. These indices were weighted with abundance, which was square-root transformed to reduce sampling bias from the weight of common species (e.g., American gizzard shad (*Dorosoma cepedianum*)). I also calculated species richness (SR) at each site for comparison. All functional indices were generated using the package “FD” (Laliberté et al., 2014) in R 4.0.2 (R Core Team, 2020). The cailliez correction was implemented for the species-by-species distance matrix because traits involve both quantitative and qualitative variables (Laliberté et al., 2014). As a result, seven diversity indices (FDis_E, FEve_E, FDis_P, FEve_P, FDis, FEve, and SR) were calculated for models.

4.2.3.3 Random forest modeling

I used random forest (RF) regression to assess how climate-related and non-climate environmental variables influence biodiversity. I selected RF to model current and future biodiversity changes because 1) it does not require assumptions of data distributions; 2) it can include environmental variables with complex interactions; and 3) it allows more predictors than observations in models (Cutler et al., 2007). To define the current relationship between biodiversity measures and environmental data for FDis, FEve, and SR at each sampling site, I used 1) five years' averaged climate-related variables or 2) five years' averaged climate-related variables with additional non-climate variables as predictors in RF after comparing model R^2 from single year to up to five-year's average. In all models, I also included year as an extra variable to check for temporal influences as samples were collected over 20 years. I trained all models with 500 trees to stabilize estimates of variable importance, then excluded all variables with negative importance from the output. All RF models were built using package "ranger" (Wright & Probst, 2020), with relative importance per variable calculated using the "importance" function. The positive, negative, or multimodal relationships between environmental variables and biodiversity indices were evaluated using partial-dependence in the package "randomForest" (Liaw & Wiener, 2002). Based on model performance measured from pseudo- R^2 values, I selected the best fitting models for FDis, FEve, and SR, respectively, then, using the best models to predict future biodiversity changes every 10 years, either under only climate change, or climate change with the 12 agriculture conservation scenarios described above.

4.3 Results

4.3.1 Contemporary spatial patterns of the diversity measures

Random Forest (RF) models were generated based on 86 fish species recorded at 91 sites using environmental variables as independent variables and FDis, FEve, or SR as dependent variables (Figures. 4.2-4.4), with all 7×2 combinations (columns $\times$ rows in Table 4.2). Among FDis, FEve, and SR, FDis models had the best fits ($R^2 = 0.26 - 0.44$), followed by SR models ($R^2 = 0.25 - 0.29$), with FEve models having the lowest performance ($R^2 = 0.02 - 0.10$) (Table. 4.2). Models had better performance overall when considering both climate-related and non-climate explanatory variables than climate-related variables only, with R^2 increasing up by 0.12. For functional indices, when including both ecological and physiological traits, models had better performance than either ecological traits or physiological traits alone.

Within all models with $R^2 \geq 0.10$, partial-dependence plots helped explain how FDis, FEve, and SR can be driven by environmental variables (Table 4.3). When only climate-related variables were included in models, flow and temperature had a dominating importance: Ecology-based FDis_E was positively related to flow, which explained up to 69% of the variance explained by all environmental variables, ecology-based FEve_E was positively related to temperature, explaining up to 39% of the variance explained by all environmental variables, while physiology-based FDis_P and SR were negatively related to temperature, explaining up to 54%. When both climate-related and non-climate variables were included in models, flow and temperature still showed high importance, up to 34% and 44% of the variance explained by all environmental variables, respectively. However, non-climate variables, including watershed area, bedrock depth, and % agriculture land cover, also played an important role in these models. Among non-climate variables,

watershed area was ranked high, up to 52% and 40% of the variance explained by all environmental variables for FDis and FDis_E, respectively.

4.3.2 Climate and agricultural conservation projections

Based on model performance, I selected FDis and SR to predict the influence of future climate and agricultural conservation practices on biodiversity in the Kaskaskia River Watershed (Figures 4.5-4.6, Tables 4.4-4.5). Assuming agriculture practices stayed the same from 2019 to 2100, when including both climate-related and non-climate variables, SR was generally expected to decrease, up to 6% on average from 2019 to 2049. From 2049 to 2099, SR was expected to increase, from - 6 % back to - 4 % relative to 2019 levels. For site-specific predictions, the largest decrease was shown to be - 20 % in 2049 compared to 2019. When climate-related variables were considered alone, the trend of SR changes stayed the same, but average SR was predicted to be lower than SR in models with both climate-related and non-climate variables. Compared to predictions of SR changes, FDis had similar trends, with up to - 1.1 % from 2019 to 2049. After 2049, FDis showed a gradual increase, eventually back to - 0.5 % in 2099 relative to 2019. The site-specific predictions of FDis had up to - 4.0 % in 2049 compared to 2019, then a gradual increase, with - 1.6 % in 2099 compared to 2019 in the worst site. Similar to SR, when climate-related variables were considered alone, the trends of FDis stayed the same but were predicted to be lower on average.

All 12 agricultural conservation scenarios were not able to reverse the decreasing trends of SR and FDis (Figures 4.7-4.8, Tables 4.4-4.5). No agricultural conservation scenarios would enhance average SR over 1 %. Site-specific predictions (Figures 4.9-4.10) showed larger differences across streams when implementing agricultural conservation compared to no

conservation, with the largest decrease in SR changing from – 20 % to – 22% by 2049. Similar to SR, the overall decrease of FDis was not reversed with agricultural conservation, with the enhancement of overall FDis less than 0.2 %.

4.4 Discussion

I examined environmental drivers of stream fish biodiversity measures in the Kaskaskia River Watershed, Illinois, USA: SR, FDis, and FEve. I then extrapolated the potential changes in biodiversity to all sampling sites across the watershed to 2099. When global climate change and regional agricultural conservation practices acted together in this productive and speciose watershed, my mapping and projection concluded that agricultural conservation practices including crop rotation, cover crops, reduced tillage, and reduced or split fertilizer application, which mainly focus on reducing nutrient and sediment loss, did little to help reverse declines in biodiversity expected under climate change.

4.4.1 Environmental drivers of functional diversity

Higher functional diversity represents better efficiency of resource use by the local community, oftentimes appearing in more heterogeneous environments (Díaz & Cabido, 2001). Thus, compared to changes in species richness, functional diversity helps better define the causation of community changes due to environmental disturbances (McGill et al., 2006). Because functional diversity is calculated through trait-based approaches, whether selected traits are highly relevant to local environmental conditions will determine the performance of functional diversity in predicting environmental impacts on the local community.

In this study, I showed that combining ecology with physiology for functional indices improves model performance, evidenced by the higher R^2 in RF models. FDis measures the variability of species traits in a local community, and physiological traits are an ideal choice to quantify such variability. The physiological traits here quantify the tolerance of fishes to water quality, temperature, and flow. Thus, habitats containing fish with different tolerances manifest environmental heterogeneity locally, such as different flow regimes and thermal refuges, offering more niches for different species. Compared to FDis which reflects the dissimilarity among individuals in the community, neither ecological nor physiological traits worked well for FEve. FEve reflects the evenness among individuals in the community. Itself alone may not be useful for detecting the effects of climate changes and agriculture, although further investigation is needed (Parker et al., 2018).

Among all environmental factors, flow and temperature were the two most important drivers of FDis and FEve. In FEve models, within the explanation power, temperature factors contributed up to ~ 40 % among all independent variables and positively related FEve. The Kaskaskia River Watershed already has high summer temperatures, thus may not provide ideal habitats for coolwater species. This was confirmed by the fact that only eight of the 86 fish species sampled classified as coolwater fish (Buisson et al., 2008; Hansen et al., 2017). Thus, warmer water in the Kaskaskia River Watershed under future climate scenarios may not significantly change fish composition, but, in fact, may increase productivity to better support local warmwater fish species after the disappearance of coolwater species (Rathert et al., 1999; Wehrly et al., 2003), benefiting FEve. Minimum flow is another key factor positively correlated to FEve, likely because it influences both habitat size and connectivity (Jaeger et al., 2014). In FDis models containing ecological traits, flow was the top predictor, accounting for up to 70% in relative importance

among all variables, positively related to FDis. This is understandable as flow enables fish migration to support local diversity, even for sinking populations (Falke & Fausch, 2010). Also, more flow means more habitats, thus offering more niche space for more species, especially larger predators (Xenopoulos & Lodge, 2006; Petermann et al., 2015).

When physiological traits were considered alone, temperature became the top predictor, and was negatively correlated with FDis. This could be because few coolwater fishes may drive the variability of traits in FDis, even when only few sites show their presence. Besides temperature and flow, a medium level of nitrate was also positively related to FDis. Except all climate-related variables, non-climate variables also explained a significant portion of functional diversity distributions. Among all non-climate variables, watershed area accounted for up to 52% of variable importance for FDis, likely because bigger watershed area yields more discharge, thus providing more habitat space (Xenopoulos & Lodge, 2006). Dams were never a top predictor in any FDis or FEve models, suggesting limited impacts on fish species in the Kaskaskia River Watershed. This is likely due to 1) a low percentage of migrating fish species in the Midwestern USA and 2) such limited migrating fish species already distributed across the whole Kaskaskia basin (Smith, 2010), making the impact of damming invisible at the community level. SR models also showed similar results with FDis, but with lower R^2 , suggesting that trait-based functional dispersion could be more informative for predicting climate-change impacts, and potentially a better solution to explore mechanisms between the biodiversity and the environment (Parker et al. 2018).

4.4.2 Climate change and agricultural conservation on future biodiversity

When climate change was considered alone, I found a downward trend of FDis and SR by 2099, with the most severe decrease expected during 2029 to 2049; after 2049, such a decrease

was expected to generally ease or stop. Under RCP8.5 global warming scenarios, I expected air temperature to increase 0.61 °C per decade in the Kaskaskia River Watershed, together with slight increases in precipitation (Acero Triana et al., 2021). With the fact that within 86 fish species present, only 15 were sensitive to water quality and eight preferring cool water, such climate trend in the Kaskaskia River Watershed may only threaten a small percentage of the local fish community. This is different from many other watersheds worldwide, such as in Europe (Buisson et al., 2013; Markovic et al., 2014) and the Verde River Basin of North America (Jaeger et al., 2014) where the majority of fish communities were threatened by climate change. But the climate change projections here lack enough consideration of short-term extreme events, such as heatwaves and drought. Thus, the impacts of climate change could be underestimated in the Midwestern fish communities. For the majority of fish species in such Midwestern agricultural watersheds, climate change in the 21st century may benefit their fitness (Rountrey et al., 2014). In Illinois, climate changes could increase their growing season by two weeks (Wuebbles et al., 2021). However, caution should be made here as I do not have lab-derived data or long-term field monitoring information on how increased temperatures could alter physiological function, like metabolism (e.g., standard metabolic rate and aerobic scope), for warmwater species, thus altering their growth and reproduction, and eventually their fitness and distributions (Rummer et al., 2014; Stillman, 2019). Under elevated temperature, the oxygen demand of fish tissue can increase exponentially until a critical temperature, where oxygen demand for maintenance exceeds cardiorespiratory capacity, causing a loss of performance (Pörtner, 2010). Studies on metabolism during warming have gained attention, but mostly on cold-water or coolwater species, not warmwater ones (Oligny-Hébert et al., 2015; Lennox et al., 2018).

We cannot avoid the impacts of climate change locally, but we do have the potential to reduce climate impacts through appropriate regional land-use management (Lawrence et al., 2014; Oliver & Morecroft, 2014). Through simulating 12 agricultural conservation scenarios targeted at the economic sustainability and resilience of the watershed (Shipley et al., 2020), I expected positive responses in biodiversity. However, none of these conservation scenarios elicited any measurable biodiversity changes. This indicates that management for ecosystem services (i.e., minimizing nutrient and sediment input into rivers) will not have co-benefits of biodiversity (Smith et al., 2015; Xiao et al., 2018). This is understandable because the conservation practices were prioritized to reduce nutrient and sediment input, not improve the variability of temperature or flow under climate change, which were the strongest drivers of biodiversity in my models. I therefore would expect biodiversity increases when conservation practices focus on mitigating temperature and flow impacts, such as building wooded riparian zones or recovering river sinuosity (Bowler et al., 2012; Beechie et al., 2015). However, the economic benefits of such conservation efforts, such as Conservation Reserve Program by USDA, may not elicit the interests of a majority of farmers. Only if we find a way to make conservation practices benefit both biodiversity and agriculture production, and both of such economic benefits can be quantified (Chan et al., 2011; Hermoso et al., 2018), would we anticipate long-term collaboration between farming and conservation.

Biodiversity, no matter species richness or functional diversity, may not be prioritized when landowners consider wildlife conservation in the Kaskaskia River Watershed (Shipley et al., 2020). Instead, the focus of landowner interests may involve enhancing the abundance of sportfish species, such as walleye (*Sander vitreus*), muskellunge (*Esox masquinongy*), and smallmouth bass (*Micropterus dolomieu*) due to their importance in recreation or food. Because these species are

also sensitive to temperature and nutrients, we can motivate the conservation interests of landowners through conserving sports fishes, which could further benefit the whole biodiversity by acting as ‘umbrella species’ (Roberge & Angelstam, 2004). Also, how to control nuisance or invasive species, especially Silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Hypophthalmichthys nobilis*), by restoring natural flow and temperature regimes that promote the competitiveness of native species against invasive species, is another practical way to enhance native biodiversity when designing agricultural conservation plans (Rahel et al., 2008; Lawrence et al., 2014). Overall, it may be difficult to stimulate public interest in conserving biodiversity alone, but if we can combine it with other benefits, like agriculture production and recreation, we could gain more support from landowners for win-wins of ecosystem service actions for biodiversity. To design such conservation plans, how to mitigate temperature and flow impacts on “important” species is a realistic direction to begin with.

4.4.3 Future conservation planning

In this study, I modeled the relationships between fish diversity and a range of environmental variables, then predicted biodiversity changes under climate change and agriculture conservation projections. The watershed-wide average of both species richness and functional diversity were predicted to decrease moderately. However, the magnitude of the decrease varied greatly across sites. I therefore recommend that future efforts prioritize the monitoring of sites predicted to have the highest and lowest biodiversity when designing basin-wide conservation practices. With such prioritization, we can establish long-term monitoring programs on how key drivers (e.g., flow, temperature, and nitrate) shape local fish communities. Also, local riverine

biodiversity relies on dispersal (Falke & Fausch, 2010). How to improve connectivity among sites is another key factor when designing conservation programs.

We should better collaborate with local residents when designing conservation practices (Allen, 2005; Stubbs, 2014). To achieve this, while targeting the restoration of biodiversity, I recommend incorporating both key species enhancement and invasive species control into planning. With such an approach, residents can better appreciate the importance of freshwater diversity besides economic growth and environmental stability under climate change (Ban et al., 2013).

4.5 Figures and tables

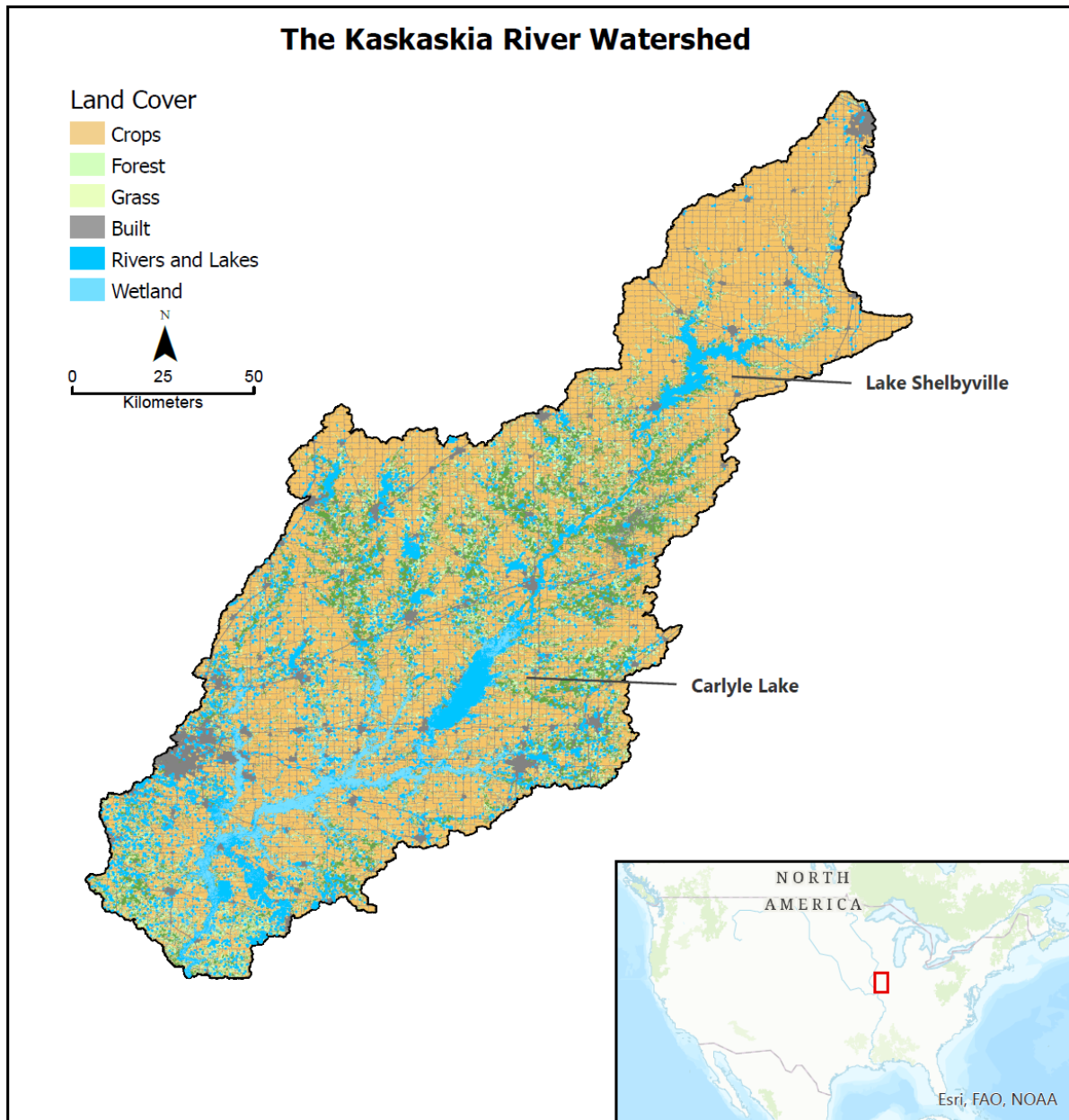


Figure 4.1 Land cover of the Kaskaskia River Watershed.

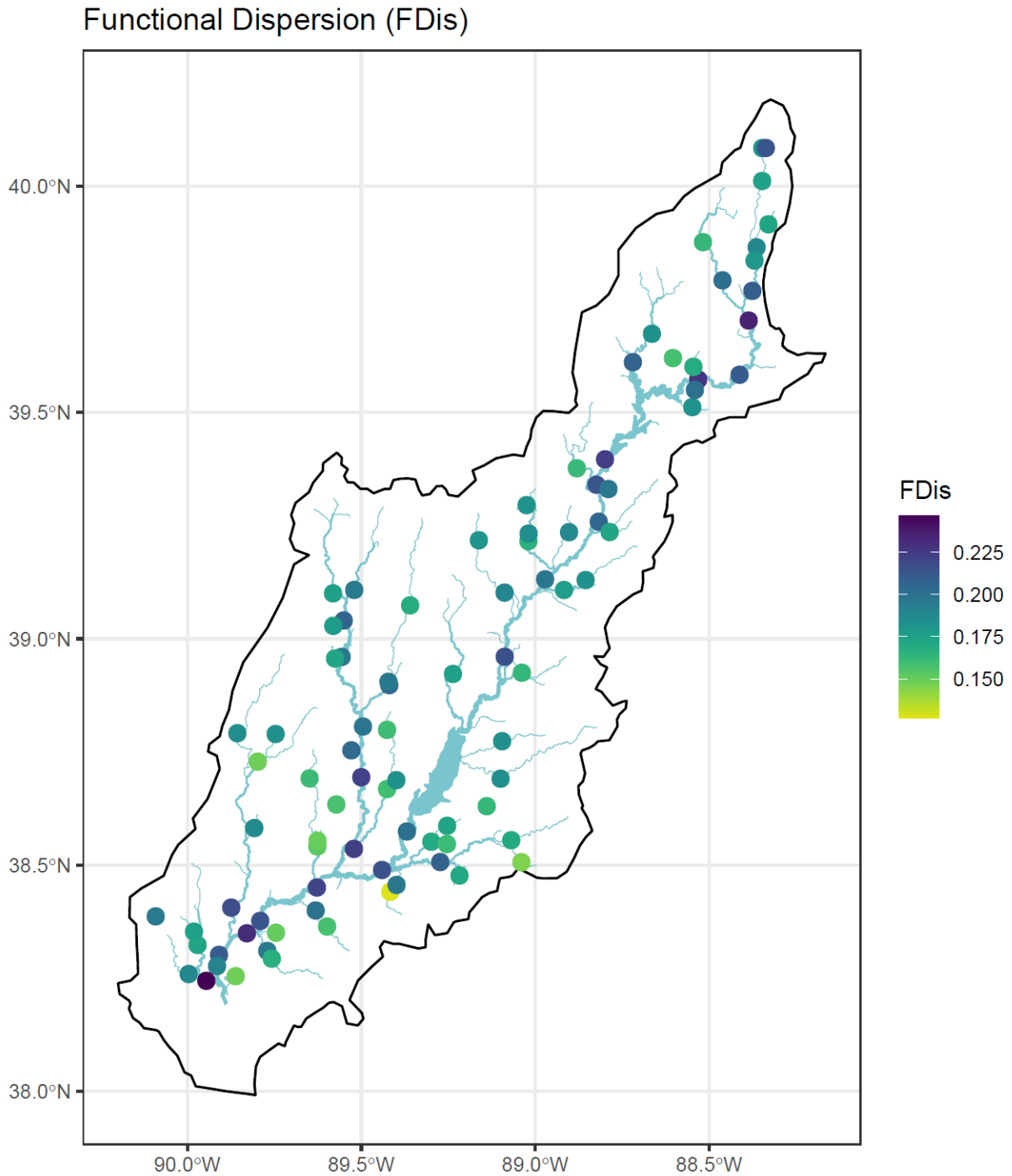


Figure 4.2 Functional dispersion (FDis) at 91 sites at over 1995-2015 in the Kaskaskia River Watershed, estimated based on six ecological and five physiological traits of 86 species.

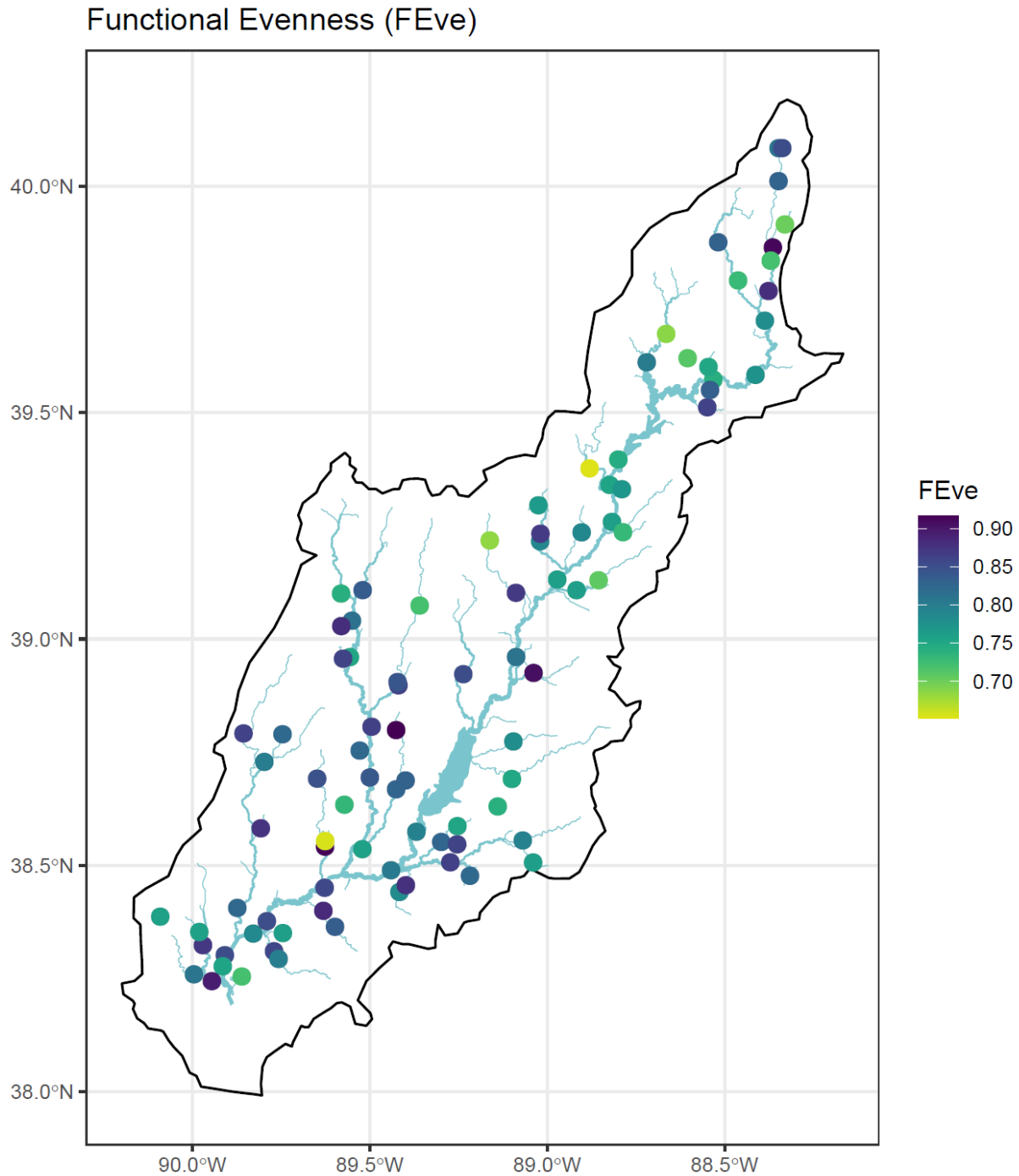


Figure 4.3 Functional evenness (FEve) at 91 sites at over 1995-2015 in the Kaskaskia River Watershed, estimated based on six ecological and five physiological traits of 86 species.

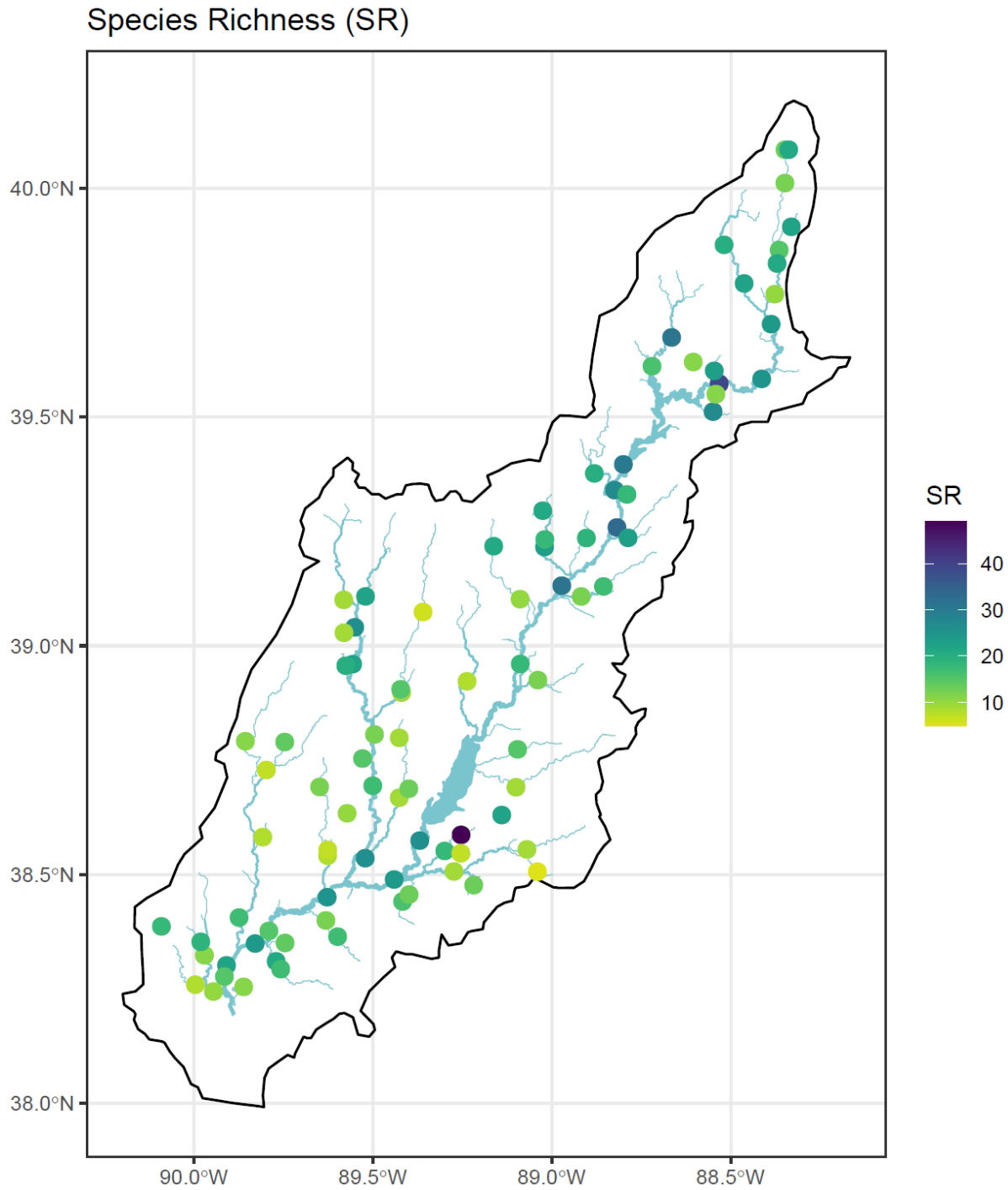


Figure 4.4 The number of species collected or species richness (SR) at 91 sites over 1995-2015 in the Kaskaskia River Watershed.

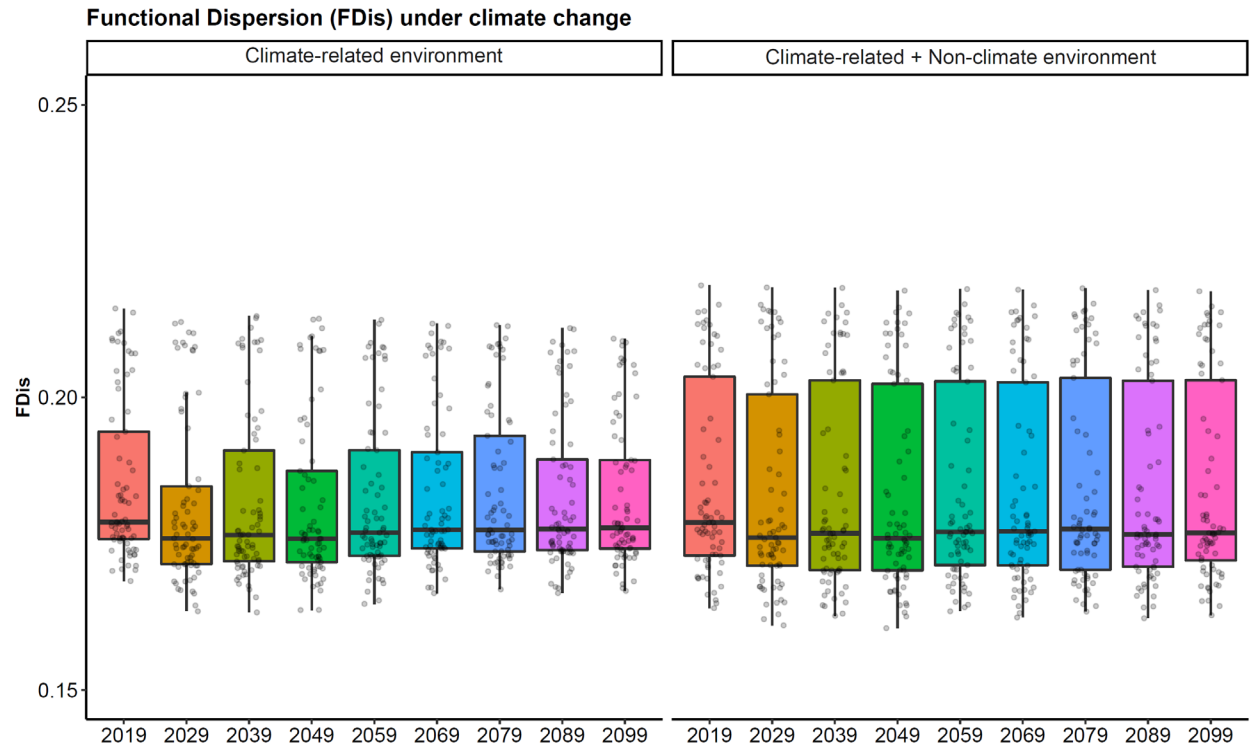


Figure 4.5 Predicted changes of functional dispersion (FDis) in the Kaskaskia River Watershed under averaged RCP8.5 climate change scenarios, using six ecological and five physiological traits of 86 species for calculation. Climate-related variables includes water temperature, flow, suspended sediment, nitrate, and dissolved oxygen.

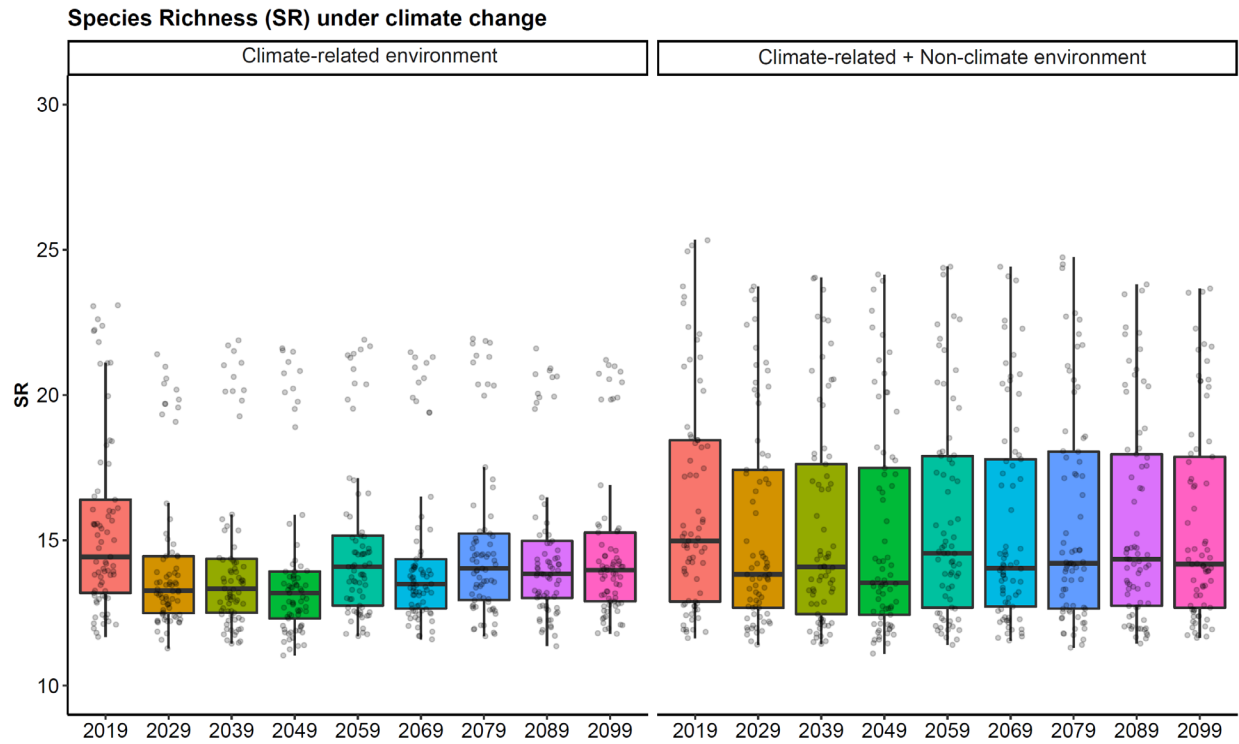


Figure 4.6 Predicted changes of species richness (SR) in the Kaskaskia River Watershed under averaged RCP8.5 climate change scenarios. Climate-related variables includes water temperature, flow, suspended sediment, nitrate, and dissolved oxygen.

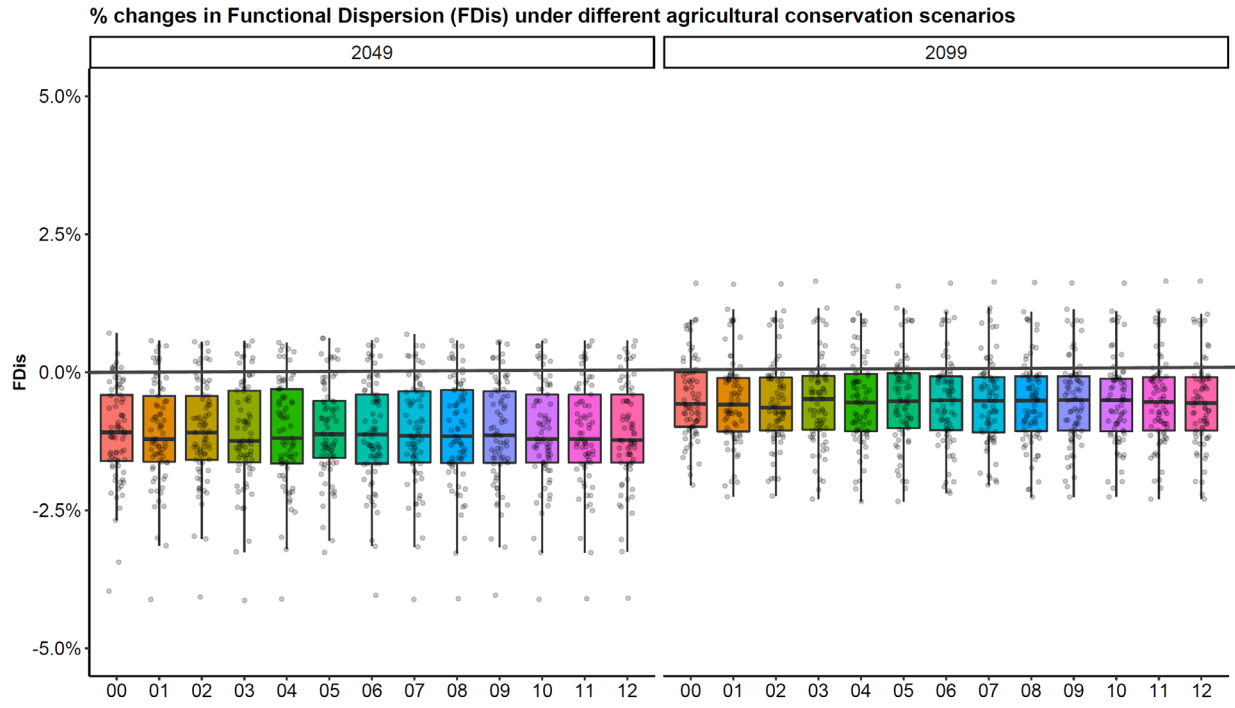


Figure 4.7 Predicted changes of functional dispersion (FDis) under different agricultural conservation scenarios with climate change in the Kaskaskia River Watershed. The baseline is model projection in 2019. 00 represents no agricultural conservation, 01 and 02 represent 10% and 20% reduction of nitrate application in corn field, 03 and 04 represent the different splits of nitrate application. 05 – 07 represent different crop rotations in corn and soybean fields. 08 and 09 represent the use of winter wheat as cover crops in corn and soybean fields. 10 – 12 represent the reduced till or no-till in corn and soybean fields. Models use both ecological and physiological traits of each species for FDis measurement, and both climate-related and non-climate variables for environmental information.

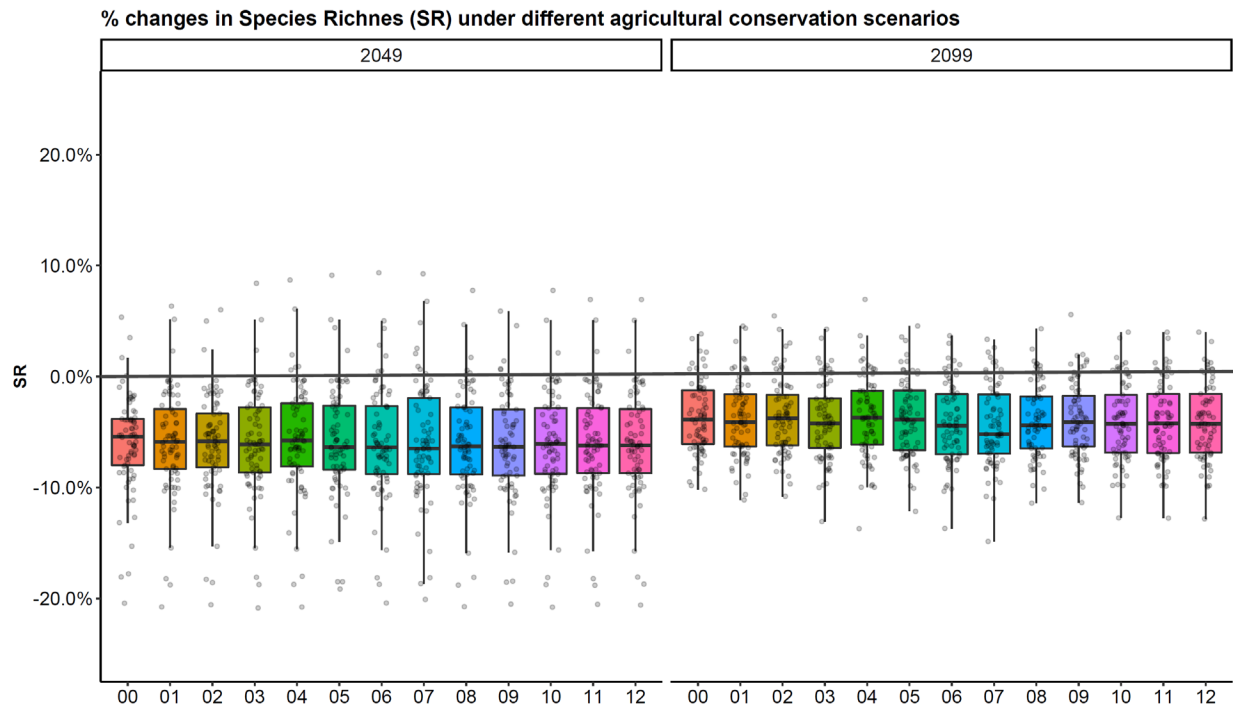


Figure 4.8 Predicted changes of species richness (SR) under different agricultural conservation scenarios with climate change in the Kaskaskia River Watershed. The baseline is no agriculture conservation in 2019. 00 represents no agricultural conservation, 01 and 02 represent 10% and 20% reduction of nitrate application in cornfield, 03 and 04 represent the different splits of nitrate application. 05 – 07 represent different crop rotations in corn and soybean fields. 08 and 09 represent the use of winter wheat as cover crops in corn and soybean fields. 10 – 12 represent the reduced till or no-till in corn and soybean fields. Models use both climate-related and non-climate variables for environmental information.

Predicted Functional Dispersion (FDis) changes under agricultural conservation scenarios 04 compared to no conservation

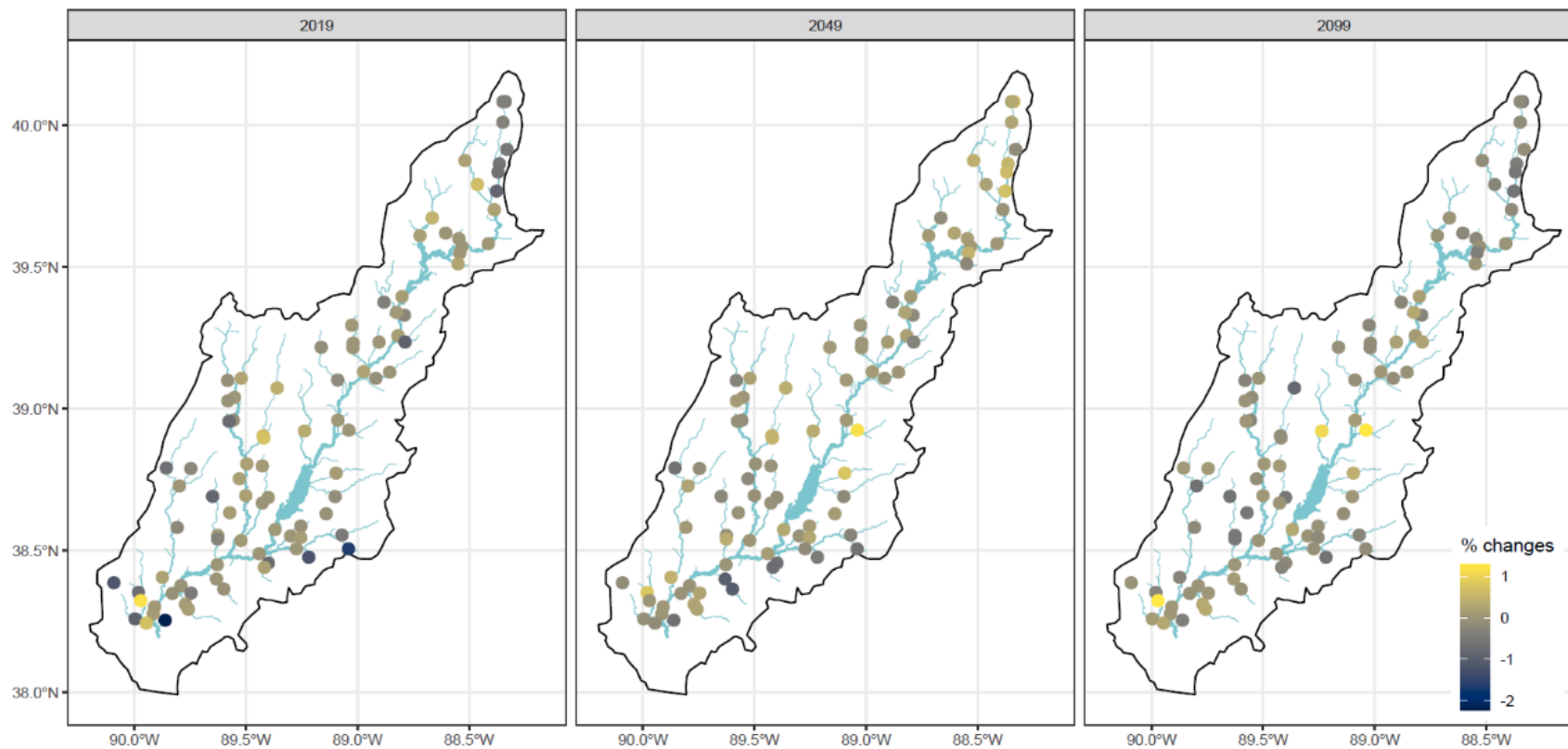


Figure 4.9 Predicted functional dispersion (FDis) changes from agricultural conservation scenarios 04 (splitting nitrate application in 50% non-tiled cornfield) to no conservation under climate change.

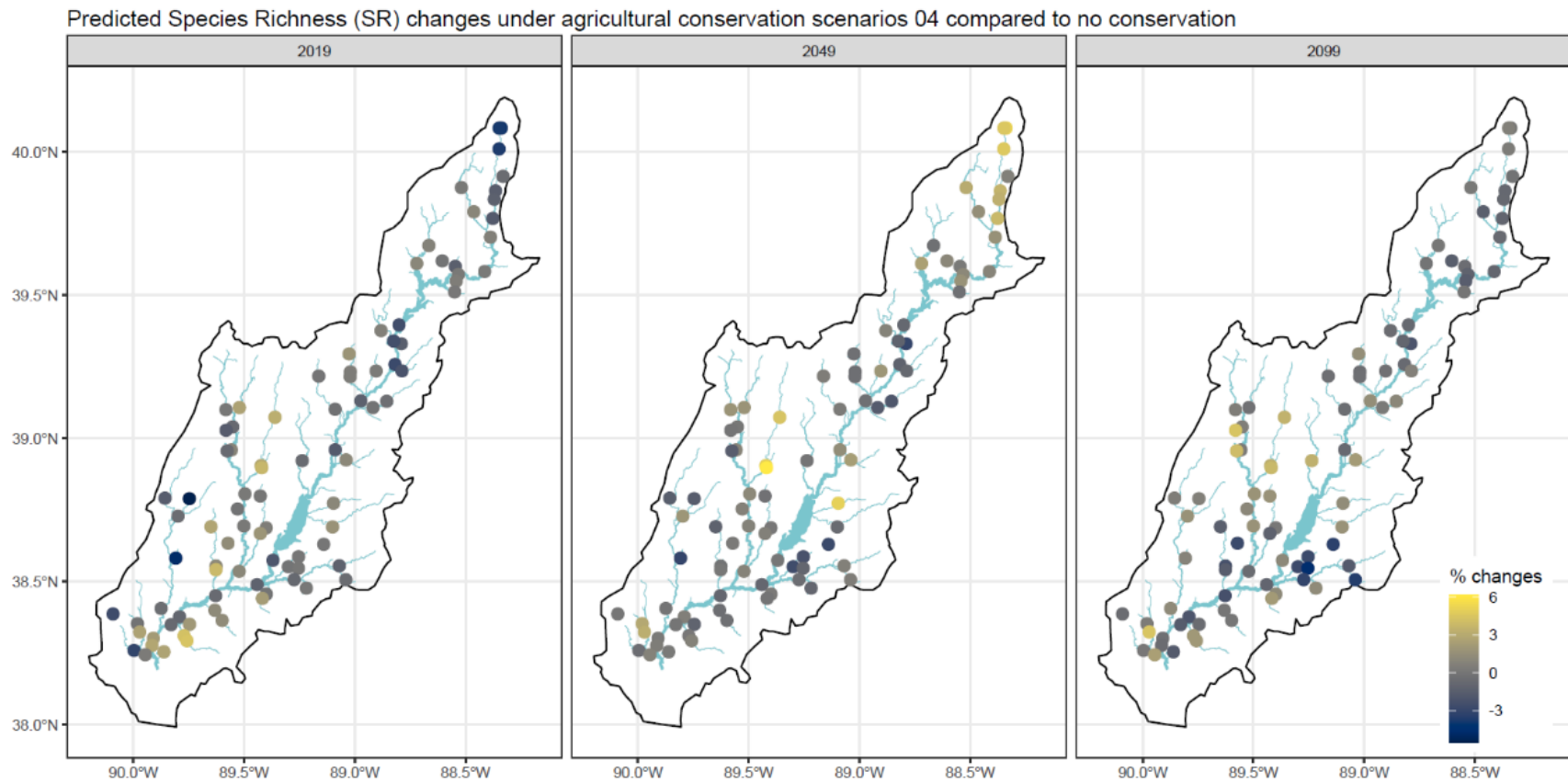


Figure 4.10 Predicted species richness (SR) differences from agricultural conservation scenarios (splitting nitrate application in 50% non-tiled cornfield) to no conservation under climate change.

Table 4.1 Functional traits of fish used to calculate functional diversity indices in the Kaskaskia River Watershed, extracted from FishBase (<http://www.fishbase.org/>), Fishtraits Database (<http://www.fishtraits.info/>), BioNet (<https://programs.iowadnr.gov/bionet/Fish/Species/List>), Environment Protection Agency (https://archive.epa.gov/water/archive/web/html/app_c-1.html), and previous study (Whittier et al., 2007).

Category	Trait	Type	Description and (or) units
Ecology	Habitat preference	Categorical	1) small substrate, 2) large substrate, 3) vegetation or debris, and 4) pelagic
	Trophic level	Numeric	2-4.5 Based on food items
	Lifespan	Numeric	Maximum age (years)
	Mature age	Numeric	Mean age at maturation (years)
	Fecundity	Numeric	No. of eggs or offspring per breeding season (log transformation)
	Parental care	Factor	With or without parental care
Physiology	Water quality tolerance	Factor with levels	1) tolerant, 2) intermediate, and 3) intolerant
	Thermal tolerance	Factor with levels	Summer maximum temperature: 1) cool: 18-25 °C and 2) warm ≥ 25 °C
	Swimming factor	Numeric	Ratio of minimum depth of the caudal peduncle to the maximum caudal fin depth
	Flow preference	Factor with levels	1) slow, 2) moderate, and 3) fast
	Maximum total length	Numeric	Maximum total body length (mm)

Table 4.2 R^2 values in random forest models for functional dispersion and evenness based on 1) ecological traits (FDis_E and FEve_E), 2) physiological traits (FDis_P, FEve_P), and 3) both ecological and physiological traits (FDis, FEve). Species richness (SR) is also calculated for comparisons.

Environmental variables	Ecology		Physiology		Ecology & Physiology		SR
	FDis_E	FEve_E	FDis_P	FEve_P	FDis_LP	FEve_LP	
SWAT	0.26	0.10	0.37	0.02	0.32	0.10	0.25
SWAT & Non-climate environment	0.36	0.10	0.39	0.09	0.44	0.10	0.29

SWAT, Sol & Water Assessment Tool

Table 4.3 Responses of biodiversity measures to the top-5 environmental predictors in random forest (RF) models. The order of numbers represents variable importance ranking in each model (measured with “importance” function in “ranger”). The symbol in parenthesis denotes the relationship between the variable and the response (+ positive, – negative, ~ multimodal) inferred through partial-dependence plots (measured with “partialPlot” function in “randomForest”). See Table 4.2 for definitions of the abbreviations of functional diversity and species richness. Climate-related variables include water temperature, flow, suspended sediment, nitrate, and dissolved oxygen. Mtry in Random Forest represents the number of variables randomly sampled as candidates at each split.

Environmental Variables		Climate-related						Climate-related & Non-climate					
Responses		FDis_LP	FEve_LP	FDis_L	FEve_L	FDis_P	SR	FDis_LP	FEve_LP	FDis_L	FEve_L	FDis_P	SR
Temperature	Mean		3 (+)		1 (+)	3 (-)	5 (-)		3 (+)		4 (+)	3 (-)	
	Min		4 (+)		5 (+)	5 (-)			5 (-)				
	Max		2 (+)		3 (+)	1 (-)	1 (-)		2 (+)		3 (+)	1 (-)	3 (-)
	Std				4 (+)	2 (-)	3 (-)					2 (-)	
Flow	Mean	1 (+)		1 (+)			2 (+)	2 (+)		2 (+)			
	Min	5 (+)	1 (+)	5 (+)	2 (+)		4 (+)		1 (+)	4 (+)			
	Max	2 (+)		3 (+)				3 (+)					
	Std	3 (+)		2 (+)				4 (+)		3 (+)			
Suspended Sediment	Mean												
	Min												
	Max		5 (-)										
	Std												
Nitrate	Mean												
	Min	4 (-)		5 (+)				5 (-)					4 (-)
	Max					4 (-)							
	Std												
Dissolved Oxygen	Mean												
	Min												
	Max												
	Std												
Soil permeability													1 (-)
Land cover	% Forest												
	% Agriculture								4 (-)		2 (-)		5 (+)
Channel gradient													
Watershed slope	Total watershed												
	Local watershed											4 (-)	
Bedrock depth	< 15 m										5 (+)		
	< 30 m										1 (-)	5 (+)	
Dam	Upstream												
	Distance to upstream												
	Downstream												
	Distance to downstream												
Stream order													
Sinuosity										5 (-)			
Watershed area								1 (+)		1 (+)			2 (+)
mtry in RF		5	10	2	3	6	5	16	14	16	5	12	10

Table 4.4 Percentage of predicted functional dispersion (FDis) changes using no conservation practices in 2019 (CP00) as baseline, which was mean = 0.185, min = 0.164 and max = 0.219. Both ecological and physiological traits were included in functional diversity. Both climate-related and non-climate environmental variables were included in random forest models. In each cell, three numbers represent percent changes of mean, min, and max FDis. CP00 represents no agricultural conservation, CP01 and CP02 represent 10% and 20% reduction of nitrate application in corn field, CP03 and CP04 represent the different splits of nitrate application. CP05 – 07 represent different crop rotations in corn and soybean fields. CP08 and CP09 represent the use of winter wheat as cover crops in corn and soybean fields. CP10 – 12 represent the reduced till or no-till in corn and soybean fields.

	2019	2029	2039	2049	2059	2069	2079	2089	2099
CP00	0.0, 0.0, 0.0	-1.0,-1.8,-0.2	-0.8,-0.8,-0.2	-1.1,-2.1,-0.4	-0.6,-0.3,-0.3	-0.5,-1.0,-0.3	-0.3,-0.4,-0.2	-0.5,-1.1,-0.4	-0.4,-0.8,-0.5
CP01	-0.1, 0.2,-0.1	-1.1,-1.0,-0.2	-0.9,-1.0,-0.1	-1.1,-1.8,-0.6	-0.6,-0.6,-0.3	-0.6,-0.7,-0.4	-0.4,-0.2,-0.3	-0.6,-0.4,-0.4	-0.5,-0.2,-0.5
CP02	-0.1, 0.2,-0.1	-1.1,-1.1,-0.2	-0.9,-0.9,-0.1	-1.0,-1.7,-0.6	-0.5,-0.5,-0.3	-0.6,-0.6,-0.4	-0.4, 0.0,-0.2	-0.6,-0.1,-0.4	-0.5,-0.2,-0.5
CP03	-0.1, 0.2,-0.1	-1.1,-1.2,-0.2	-0.9,-1.1,-0.2	-1.1,-1.9,-0.5	-0.6,-0.6,-0.3	-0.6,-1.0,-0.4	-0.4,-0.5,-0.3	-0.6,-0.6,-0.5	-0.5,-0.4,-0.5
CP04	-0.1, 0.2,-0.1	-1.1,-1.7,-0.2	-0.9,-1.3,-0.1	-1.1,-1.9,-0.5	-0.6,-1.0,-0.3	-0.6,-1.0,-0.4	-0.4,-0.7,-0.3	-0.6,-0.5,-0.4	-0.5,-1.1,-0.5
CP05	-0.1, 0.2,-0.1	-1.0,-1.1,-0.2	-0.9,-0.9,-0.1	-1.0,-1.5,-0.5	-0.5,-0.5,-0.3	-0.5,-0.8,-0.4	-0.3,-0.7,-0.3	-0.5,-0.7,-0.4	-0.5, 0.1,-0.5
CP06	-0.1, 0.2,-0.1	-1.1,-1.1,-0.2	-0.9,-1.0,-0.2	-1.0,-1.8,-0.5	-0.6,-0.8,-0.4	-0.6,-1.1,-0.4	-0.4,-0.6,-0.3	-0.6,-0.6,-0.5	-0.5,-0.3,-0.5
CP07	-0.1, 0.2,-0.1	-1.1,-1.1,-0.2	-0.9,-1.0,-0.2	-1.0,-1.8,-0.6	-0.6,-0.8,-0.4	-0.6,-0.7,-0.4	-0.4,-0.7,-0.3	-0.6,-0.6,-0.4	-0.5,-0.3,-0.5
CP08	-0.1, 0.2,-0.1	-1.1,-1.1,-0.2	-0.9,-0.9,-0.1	-1.0,-1.9,-0.5	-0.6,-0.7,-0.3	-0.6,-0.6,-0.4	-0.4,-0.3,-0.3	-0.6,-0.5,-0.5	-0.5,-0.3,-0.5
CP09	-0.1, 0.2,-0.1	-1.1,-1.1,-0.2	-0.9,-0.7,-0.1	-1.0,-1.8,-0.5	-0.6,-0.8,-0.3	-0.6,-0.6,-0.4	-0.4,-0.6,-0.3	-0.6,-0.3,-0.4	-0.5,-0.2,-0.5
CP10	-0.1, 0.2,-0.1	-1.1,-1.1,-0.2	-0.9,-1.0,-0.1	-1.1,-1.9,-0.6	-0.6,-0.5,-0.3	-0.6,-0.9,-0.4	-0.4,-0.4,-0.3	-0.6,-0.5,-0.4	-0.5,-0.3,-0.5
CP11	-0.1, 0.2,-0.1	-1.1,-1.2,-0.2	-0.9,-1.0,-0.1	-1.1,-1.9,-0.6	-0.6,-0.5,-0.3	-0.6,-0.8,-0.4	-0.4,-0.4,-0.3	-0.6,-0.6,-0.4	-0.5,-0.2,-0.5
CP12	-0.1, 0.2,-0.1	-1.1,-1.1,-0.2	-0.9,-1.0,-0.1	-1.1,-1.9,-0.5	-0.6,-0.6,-0.3	-0.6,-0.8,-0.4	-0.4,-0.5,-0.3	-0.6,-0.5,-0.4	-0.5,-0.3,-0.5

Table 4.5 Percentage of predicted species richness (SR) changes using no conservation practices in 2019 (CP00) as baseline, which was mean = 16.2, min = 11.6 and max = 25.3. Both ecological and physiological traits were included in functional diversity. Both climate-related and non-climate environmental variables were included in random forest models. In each cell, three numbers represent percentage changes of mean, min, and max SR. CP00 represents no agricultural conservation, CP01 and CP02 represent 10% and 20% reduction of nitrate application in corn field, CP03 and CP04 represent the different splits of nitrate application. CP05 – 07 represent different crop rotations in corn and soybean fields. CP08 and CP09 represent the use of winter wheat as cover crops in corn and soybean fields. CP10 – 12 represent the reduced till or no-till in corn and soybean fields.

	2019	2029	2039	2049	2059	2069	2079	2089	2099
CP00	0.0, 0.0, 0.0	-5.1,-1.9,-6.3	-4.7,-1.7,-5.1	-6.0,-4.6,-4.7	-2.7,-2.0,-3.6	-4.1,-0.7,-3.6	-2.9,-2.9,-2.3	-3.7,-1.6,-6.0	-4.0, 0.1,-6.6
CP01	-0.2, 0.5,-2.8	-5.2,-2.4,-8.2	-4.9,-2.4,-6.3	-6.0,-4.0,-5.8	-2.5,-2.0,-3.8	-4.5,-1.9,-5.2	-3.3,-2.5,-3.1	-3.8,-1.5,-6.5	-4.2,-1.3,-7.0
CP02	-0.2, 0.5,-2.8	-5.2,-2.7,-8.2	-4.9,-1.9,-6.2	-6.1,-3.8,-5.8	-2.5,-1.5,-3.7	-4.4,-1.7,-5.0	-3.2,-2.1,-2.9	-3.7,-1.4,-6.3	-4.0,-1.4,-5.9
CP03	-0.2, 0.5,-2.8	-5.3,-2.4,-8.3	-4.9,-2.5,-6.4	-6.1,-5.0,-6.1	-2.7,-1.4,-3.9	-4.5,-2.0,-5.2	-3.4,-2.7,-3.2	-4.0,-2.1,-5.7	-4.3,-1.6,-7.1
CP04	-0.2, 0.5,-2.8	-5.1,-2.3,-8.3	-4.4,-2.6,-6.4	-5.7,-4.1,-5.8	-2.2,-2.0,-3.9	-4.2,-2.0,-5.1	-3.0,-1.5,-3.2	-3.6,-2.2,-5.9	-4.1,-1.5,-7.3
CP05	-0.2, 0.5,-2.8	-5.0,-2.7,-8.3	-4.7,-2.4,-6.4	-5.9,-4.9,-5.9	-2.5,-1.3,-3.9	-4.2,-1.9,-5.1	-3.2,-2.6,-3.2	-3.7,-2.1,-6.6	-4.1,-1.5,-7.2
CP06	-0.2, 0.5,-2.8	-5.2,-2.6,-8.2	-4.8,-3.1,-6.5	-6.0,-4.4,-6.0	-2.7,-2.2,-3.9	-4.5,-2.1,-5.2	-3.4,-4.0,-3.1	-4.0,-2.3,-5.9	-4.6,-1.6,-7.4
CP07	-0.2, 0.5,-2.8	-5.2,-2.6,-8.2	-4.8,-2.8,-6.6	-6.1,-4.0,-6.1	-2.9,-2.0,-4.0	-4.6,-2.4,-5.4	-3.5,-4.6,-3.3	-4.1,-2.4,-6.0	-4.7,-1.7,-7.8
CP08	-0.2, 0.5,-2.8	-5.2,-2.4,-8.2	-4.8,-2.3,-6.5	-6.1,-5.1,-5.9	-2.7,-1.2,-3.9	-4.6,-2.2,-5.3	-3.3,-2.2,-3.2	-3.8,-2.1,-6.5	-4.3,-1.8,-7.1
CP09	-0.2, 0.5,-2.8	-5.2,-2.5,-8.2	-4.8,-2.6,-6.5	-6.2,-4.7,-5.8	-2.7,-1.2,-3.7	-4.6,-1.9,-5.2	-3.3,-1.6,-3.2	-3.7,-2.1,-5.9	-4.2,-1.6,-7.1
CP10	-0.2, 0.5,-2.8	-5.2,-2.2,-8.3	-4.9,-2.6,-6.5	-6.1,-4.0,-5.9	-2.6,-2.0,-3.9	-4.5,-2.0,-5.1	-3.4,-2.8,-3.2	-3.8,-2.0,-6.5	-4.4,-1.6,-7.2
CP11	-0.2, 0.5,-2.8	-5.3,-2.2,-8.3	-4.9,-2.5,-6.5	-6.1,-4.0,-5.9	-2.6,-2.0,-3.9	-4.5,-2.0,-5.3	-3.4,-2.9,-3.2	-3.8,-2.0,-6.5	-4.4,-1.5,-7.2
CP12	-0.2, 0.5,-2.8	-5.2,-2.5,-8.3	-4.9,-2.7,-6.5	-6.1,-4.3,-5.9	-2.6,-2.0,-3.9	-4.5,-2.0,-5.3	-3.4,-2.9,-3.2	-3.9,-2.0,-6.5	-4.4,-1.5,-7.2

CHAPTER 5: SUMMARY

How will global climate change and regional agricultural impacts act together to influence freshwater fish communities in the future? For all agricultural landscapes around the world, being able to quantitatively “answer” this question helps design effective conservation plans in advance. There are different approaches that can be used to answer this question, ranging from short-term, fine-scale physiological experiments to long-term, broad-scale ecological modeling. Each approach has both advantages and limitations. In this work, I demonstrated the importance of using physiological experiments to quantify the impacts of short-term extreme events on either individual species or species interactions. Such impacts could have been largely overlooked in broad-scale conservation planning but set the threshold for the stability of local fish communities. I also showed the value of watershed-scale modeling using the rich ecological and environmental records in Illinois, as well as future climate and agricultural projections. Taking this one step further, I incorporated physiological metrics into ecological modeling to improve model performance. Such models enable us to effectively focus on the most important environmental factors, and the most urgent areas for conservation.

In **Chapter 2**, I recorded high-resolution water temperature data in various forested and agricultural streams across the Kaskaskia River Watershed. Such records demonstrated how agricultural landscapes could intensify summer heatwaves in local streams. I then simulated the heatwaves happening in agricultural streams in the lab to quantify their impacts on fathead minnow (*Pimephales promelas*), an important prey species across North America. Fathead minnow showed thermal resilience to heatwaves, including rapid returns to metabolic homeostasis and transient increases in critical thermal maximum (CT_{max}). However, even for fathead minnow, a thermal-

tolerant warmwater species, undergoing heatwaves still came with costs, including reduced anaerobic capacity and impaired antioxidant defense. In this chapter, I showed the value of using real-time monitoring on fish responses to heatwaves, the natural thermal dynamics oftentimes overlooked in previous conservation planning. Such physiological experiments facilitate the mechanistic understanding of how fish react to thermal challenges in the real world. This chapter is unique because it offers the potential for more accurate projections of climate change impacts on fish communities when incorporating such high-resolution metabolic costs into models.

In **Chapter 3**, I took these concepts one step further. Instead of only focusing on a widespread warmwater prey fish, fathead minnow, I sought to compare the influence of heatwaves and summer temperatures on both predator and prey fishes, as well as cool and warmwater fishes. By doing this, I sought to use physiology to define possible mechanisms related to community shifts under climate change. The physiological metrics I focused on were metabolic and swimming parameters. By simulating summer average and extreme temperatures appearing in forested streams, where both coolwater and warmwater species existed, I demonstrated that, compared to warmwater predator largemouth bass (*Micropterus salmoides*) and prey fathead minnow, the coolwater predator walleye (*Sander vitreus*) had higher standard metabolic rate, limited aerobic scope, low metabolic activity when encountering prey, and reduced swimming performance after experiencing a heatwave. All of these demonstrated the thermal disadvantage for walleye relative to warmwater species in local fish communities. This chapter is unique because instead of using data on recruitment and abundance as is typically done in modeling, it used a physiological approach to define potential mechanisms related to community shifts between cool and warmwater species, and interaction changes between predators and prey.

In **Chapter 4**, I further broadened scope, moving from the shifts of communities to a whole watershed under climate and agriculture changes. Instead of pure physiological experiments, I used ecological modeling and incorporated physiological understandings for different species into models. I used contemporary fish and environmental records to build base models. Models showed water flow, water temperature, nitrate, and watershed area were among the top predictors for three biodiversity measures. Functional dispersion, the metric that included physiological information, had > 50 % better performance than species richness, in terms of R^2 . Then, based on different climate and agricultural conservation scenarios, I projected potential fish community changes in the Kaskaskia River Watershed. Species richness and functional dispersion were predicted to generally decrease in most sites across the watershed, up to 20% and 4% by 2099, respectively, in spite of some sites predicted to increase in biodiversity. When potential agricultural conservation practices prioritized by local landowners were simulated under climate change, the decreasing trends of species richness and functional dispersion remained, suggesting climate change outpowered potential agriculture conservation benefits. Considering the fact that all agricultural conservation plans included in models were target at nutrient and sediment reduction into rivers, to design better agricultural conservation practices that also conserve freshwater biodiversity, I recommend evaluating whether conservation practices, either within the watershed or within the river itself, could also improve natural temperature and flow regimes based on my models. Overall, this chapter is unique because it used both physiological and ecological information for different species in the fish community to model the ecosystem-level responses under various agricultural conservation practices with climate change.

Overall, **Chapter 2** illustrated the importance of considering fine-scale thermal dynamics on physiological performance of individual species; **Chapter 3** extended such considerations of

thermal dynamics from individual species to local fish community; **Chapter 4** further broadened the scope to a watershed level, combining the ecological modeling with physiological understandings to project the ecosystem-level responses under future long-term climate and agricultural changes. All chapters in this dissertation demonstrate the importance of considering different scales (i.e., individual species, communities, and the whole watershed) when designing conservation plans. This dissertation also shows the value and the potential of combining experimental physiology and ecological modeling on freshwater conservation.

REFERENCES

- Acero Triana, J. S. (2020). *Assessing the impacts of environmental stressors on watershed ecosystem services integrating hydro-ecologic models and socio-cultural perspective* (Doctoral dissertation, University of Illinois at Urbana-Champaign).
- Acero Triana, J. S., Chu, M. L., & Stein, J. A. (2021). Assessing the impacts of agricultural conservation practices on freshwater biodiversity under changing climate. *Ecological Modelling*, 453, 109604. <https://doi.org/10.1016/j.ecolmodel.2021.109604>
- Albert, J. S., Destouni, G., Duke-Sylvester, S. M., Magurran, A. E., Oberdorff, T., Reis, R. E., Winemiller, K. O., & Ripple, W. J. (2021). Scientists' warning to humanity on the freshwater biodiversity crisis. *Ambio*, 50(1), 85–94. <https://doi.org/10.1007/s13280-020-01318-8>
- Allan, B. J. M., Domenici, P., Munday, P. L., & McCormick, M. I. (2015). Feeling the heat: The effect of acute temperature changes on predator–prey interactions in coral reef fish. *Conservation Physiology*, 3(1). <https://doi.org/10.1093/conphys/cov011>
- Allan, J. D. (2004). Landscapes and Riverscapes: The Influence of Land Use on Stream Ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, 35(1), 257–284. <https://doi.org/10.1146/annurev.ecolsys.35.120202.110122>
- Allen, A. W. (2005). The Conservation Reserve Enhancement Program. 19.
- Antão, L. H., Bates, A. E., Blowes, S. A., Waldock, C., Supp, S. R., Magurran, A. E., Dornelas, M., & Schipper, A. M. (2020). Temperature-related biodiversity change across temperate marine and terrestrial systems. *Nature Ecology & Evolution*, 4(7), 927–933. <https://doi.org/10.1038/s41559-020-1185-7>
- Ban, N. C., Mills, M., Tam, J., Hicks, C. C., Klain, S., Stoeckl, N., Bottrill, M. C., Levine, J., Pressey, R. L., Satterfield, T., & Chan, K. M. (2013). A social–ecological approach to conservation planning: Embedding social considerations. *Frontiers in Ecology and the Environment*, 11(4), 194–202. <https://doi.org/10.1890/110205>
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Bartoń, K. (2020). MuMIn: multi-model inference. R package ver. 1.43. 17. CRAN: The Comprehensive R Archive Network.
- Beechie, T. J., Pess, G. R., Imaki, H., Martin, A., Alvarez, J., & Goodman, D. H. (2015). Comparison of potential increases in juvenile salmonid rearing habitat capacity among alternative restoration scenarios, Trinity River, California. *Restoration Ecology*, 23(1), 75–84. <https://doi.org/10.1111/rec.12131>
- Beitinger, T. L., & Bennett, W. A. (2000). Quantification of the Role of Acclimation Temperature in Temperature Tolerance of Fishes. *Environmental Biology of Fishes*, 58(3), 277–288. <https://doi.org/10.1023/A:1007618927527>
- Beitinger, T. L., Bennett, W. A., & McCauley, R. W. (2000). Temperature Tolerances of North American Freshwater Fishes Exposed to Dynamic Changes in Temperature. *Environmental Biology of Fishes*, 58(3), 237–275. <https://doi.org/10.1023/A:1007676325825>
- Beitinger, T. L., & Lutterschmidt, W. I. (2011). TEMPERATURE | Measures of Thermal Tolerance. In *Encyclopedia of Fish Physiology* (pp. 1695–1702). Elsevier. <https://doi.org/10.1016/B978-0-12-374553-8.00200-8>

- Bekele, E. G., Demissie, M., & Lian, Y. (2012). *Optimizing the Placement of Best Management Practices (BMPs) in Agriculturally-Dominated Watersheds in Illinois*. 2890–2900. [https://doi.org/10.1061/41173\(414\)302](https://doi.org/10.1061/41173(414)302)
- Bell, W. H., & Terhune, L. D. B. (1970). Water tunnel design for fisheries research. Fisheries Research Board of Canada, Biological Station.
- Betzelberger, A. M., Gillespie, K. M., Mcgrath, J. M., Koester, R. P., Nelson, R. L., & Ainsworth, E. A. (2010). Effects of chronic elevated ozone concentration on antioxidant capacity, photosynthesis and seed yield of 10 soybean cultivars. *Plant, Cell & Environment*, 33(9), 1569–1581. <https://doi.org/10.1111/j.1365-3040.2010.02165.x>
- Blanck, A., Tedesco, P. A., & Lamouroux, N. (2007). Relationships between life-history strategies of European freshwater fish species and their habitat preferences. *Freshwater Biology*, 52(5), 843–859. <https://doi.org/10.1111/j.1365-2427.2007.01736.x>
- Blann, K. L., Anderson, J. L., Sands, G. R., & Vondracek, B. (2009). Effects of Agricultural Drainage on Aquatic Ecosystems: A Review. *Critical Reviews in Environmental Science and Technology*, 39(11), 909–1001. <https://doi.org/10.1080/10643380801977966>
- Blasco, F. R., Esbaugh, A. J., Killen, S. S., Rantin, F. T., Taylor, E. W., & McKenzie, D. J. (2020). Using aerobic exercise to evaluate sub-lethal tolerance of acute warming in fishes. *The Journal of Experimental Biology*, 223(9), jeb218602. <https://doi.org/10.1242/jeb.218602>
- Bowler, D. E., Mant, R., Orr, H., Hannah, D. M., & Pullin, A. S. (2012). What are the effects of wooded riparian zones on stream temperature? *Environmental Evidence*, 1(1), 3. <https://doi.org/10.1186/2047-2382-1-3>
- Bowler, K. (2005). Acclimation, heat shock and hardening. *Journal of Thermal Biology*, 30(2), 125–130. <https://doi.org/10.1016/j.jtherbio.2004.09.001>
- Bozek, M. A., Baccante, D. A., & Lester, N. P. (2011). *Walleye and Sauger Life History*. 70.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1), 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Brenden, T. O., Wang, L., Seelbach, P. W., Clark, R. D., Wiley, M. J., & Sparks-Jackson, B. L. (2008). A spatially constrained clustering program for river valley segment delineation from GIS digital river networks. *Environmental Modelling & Software*, 23(5), 638–649. <https://doi.org/10.1016/j.envsoft.2007.09.004>
- Brett, J. R. (1964). The Respiratory Metabolism and Swimming Performance of Young Sockeye Salmon. *Journal of the Fisheries Research Board of Canada*, 21(5), 1183–1226. <https://doi.org/10.1139/f64-103>
- Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a Metabolic Theory of Ecology. *Ecology*, 85(7), 1771–1789. <https://doi.org/10.1890/03-9000>
- Bruggeman, J., & Kooijman, S. A. L. M. (2007). A biodiversity-inspired approach to aquatic ecosystem modeling. *Limnology and Oceanography*, 52(4), 1533–1544. <https://doi.org/10.4319/lo.2007.52.4.1533>
- Buckley, L. B., Urban, M. C., Angilletta, M. J., Crozier, L. G., Rissler, L. J., & Sears, M. W. (2010). Can mechanism inform species' distribution models? *Ecology Letters*, 13(8), 1041–1054. <https://doi.org/10.1111/j.1461-0248.2010.01479.x>
- Buisson, L., Grenouillet, G., Villéger, S., Canal, J., & Laffaille, P. (2013). Toward a loss of functional diversity in stream fish assemblages under climate change. *Global Change Biology*, 19(2), 387–400. <https://doi.org/10.1111/gcb.12056>

- Buisson, L., Thuiller, W., Lek, S., Lim, P., & Grenouillet, G. (2008). Climate change hastens the turnover of stream fish assemblages. *Global Change Biology*, 14(10), 2232–2248. <https://doi.org/10.1111/j.1365-2486.2008.01657.x>
- Cadotte, M. W., Carscadden, K., & Mirotchnick, N. (2011). Beyond species: Functional diversity and the maintenance of ecological processes and services. *Journal of Applied Ecology*, 48(5), 1079–1087. <https://doi.org/10.1111/j.1365-2664.2011.02048.x>
- Cai, Y., & Summerfelt, R. C. (1992). Effects of temperature and size on oxygen consumption and ammonia excretion by walleye. *Aquaculture*, 104(1), 127–138. [https://doi.org/10.1016/0044-8486\(92\)90143-9](https://doi.org/10.1016/0044-8486(92)90143-9)
- Caissie, D. (2006). The thermal regime of rivers: A review. *Freshwater Biology*, 51(8), 1389–1406. <https://doi.org/10.1111/j.1365-2427.2006.01597.x>
- Canadell, J. G., & Jackson, R. B. (Eds.). (2021). *Ecosystem Collapse and Climate Change* (Vol. 241). Springer International Publishing. <https://doi.org/10.1007/978-3-030-71330-0>
- Cao, Y., Hinz, L., Metzke, B., Stein, J., & Holtrop, A. (2016). Modeling and mapping fish abundance across Wadeable streams of Illinois, USA, based on landscape-level environmental variables. *Canadian Journal of Fisheries and Aquatic Sciences*, 73(7), 1031–1046. <https://doi.org/10.1139/cjfas-2015-0343>
- Cao, Y., Stodola, A., Douglass, S., Shasteen, D., Cummings, K., & Holtrop, A. (2015). Modelling and mapping the distribution, diversity and abundance of freshwater mussels (Family Unionidae) in Wadeable streams of Illinois, U.S.A. *Freshwater Biology*, 60(7), 1379–1397. <https://doi.org/10.1111/fwb.12575>
- Carveth, C. J., Widmer, A. M., & Bonar, S. A. (2006). Comparison of Upper Thermal Tolerances of Native and Nonnative Fish Species in Arizona. *Transactions of the American Fisheries Society*, 135(6), 1433–1440. <https://doi.org/10.1577/T05-025.1>
- Chabot, D., Steffensen, J. F., & Farrell, A. P. (2016). The determination of standard metabolic rate in fishes. *Journal of Fish Biology*, 88(1), 81–121. <https://doi.org/10.1111/jfb.12845>
- Chan, K. M. A., Hoshizaki, L., & Klinkenberg, B. (2011). Ecosystem Services in Conservation Planning: Targeted Benefits vs. Co-Benefits or Costs? *PLoS ONE*, 6(9), e24378. <https://doi.org/10.1371/journal.pone.0024378>
- Clark, T. D., Sandblom, E., & Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: Respirometry, relevance and recommendations. *Journal of Experimental Biology*, 216(15), 2771–2782. <https://doi.org/10.1242/jeb.084251>
- Closs, G. P., Krkosek, M., & Olden, J. D. (2016). *Conservation of Freshwater Fishes*. Cambridge University Press.
- Cohen, J. M., Lajeunesse, M. J., & Rohr, J. R. (2018). A global synthesis of animal phenological responses to climate change. *Nature Climate Change*, 8(3), 224–228. <https://doi.org/10.1038/s41558-018-0067-3>
- Colvin, N. E., Racey, C. L., & Lochmann, S. E. (2008). Stocking contribution and growth of largemouth bass stocked at 50 and 100 mm into backwaters of the Arkansas River. *North American Journal of Fisheries Management*, 28(2), 434–441.
- Comte, L., Buisson, L., Daufresne, M., & Grenouillet, G. (2013). Climate-induced changes in the distribution of freshwater fish: Observed and predicted trends. *Freshwater Biology*, 58(4), 625–639. <https://doi.org/10.1111/fwb.12081>

- Comte, L., Olden, J. D., Tedesco, P. A., Ruhi, A., & Giam, X. (2021). Climate and land-use changes interact to drive long-term reorganization of riverine fish communities globally. *Proceedings of the National Academy of Sciences*, 118(27), e2011639118. <https://doi.org/10.1073/pnas.2011639118>
- Corey, E., Linnansaari, T., Cunjak, R. A., & Currie, S. (2017). Physiological effects of environmentally relevant, multi-day thermal stress on wild juvenile Atlantic salmon (*Salmo salar*). *Conservation Physiology*, 5, 13.
- Cutler, D. R., Edwards Jr., T. C., Beard, K. H., Cutler, A., Hess, K. T., Gibson, J., & Lawler, J. J. (2007). Random Forests for Classification in Ecology. *Ecology*, 88(11), 2783–2792. <https://doi.org/10.1890/07-0539.1>
- Dai, Q., & Suski, C. D. (2019). Effects of acclimation temperature on critical thermal limits and swimming performance of the state-endangered bigeye chub *Hybopsis amblops*. *Aquatic Biology*, 28, 137–147. <https://doi.org/10.3354/ab00715>
- Deutsch, C., Ferrel, A., Seibel, B., Pörtner, H.-O., & Huey, R. B. (2015). Climate change tightens a metabolic constraint on marine habitats. *Science*, 348(6239), 1132–1135. Scopus. <https://doi.org/10.1126/science.aaa1605>
- Díaz, S., & Cabido, M. (2001). Vive la différence: Plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution*, 16(11), 646–655. [https://doi.org/10.1016/S0169-5347\(01\)02283-2](https://doi.org/10.1016/S0169-5347(01)02283-2)
- Domenici, P., Allan, B. J. M., Lefrançois, C., & McCormick, M. I. (2020). The effect of climate change on the escape kinematics and performance of fishes: Implications for future predator–prey interactions. *Conservation Physiology*, 7(coz078). <https://doi.org/10.1093/conphys/coz078>
- Dugdale, S. J., Bergeron, N. E., & St-Hilaire, A. (2013). Temporal variability of thermal refuges and water temperature patterns in an Atlantic salmon river. *Remote Sensing of Environment*, 136, 358–373. <https://doi.org/10.1016/j.rse.2013.05.018>
- Elith, J., & Leathwick, J. R. (2009). Species Distribution Models: Ecological Explanation and Prediction Across Space and Time. *Annual Review of Ecology, Evolution, and Systematics*, 40(1), 677–697. <https://doi.org/10.1146/annurev.ecolsys.110308.120159>
- Enzor, L. A., Hunter, E. M., & Place, S. P. (2017). The effects of elevated temperature and ocean acidification on the metabolic pathways of notothenioid fish. *Conservation Physiology*, 5, 15.
- Falke, J. A., & Fausch, K. D. (2010). *From Metapopulations to Metacommunities: Linking Theory with Empirical Observations of the Spatial Population Dynamics of Stream Fishes*. 28.
- Farrell, A. P. (2009). Environment, antecedents and climate change: Lessons from the study of temperature physiology and river migration of salmonids. *Journal of Experimental Biology*, 212(23), 3771–3780. <https://doi.org/10.1242/jeb.023671>
- Frimpong, E. A., & Angermeier, P. L. (2010). Trait-based approaches in the analysis of stream fish communities. *American Fisheries Society Symposium*, 109–136.
- Fry, F. E. J. (1971). 1—The Effect of Environmental Factors on the Physiology of Fish. In W. S. Hoar & D. J. Randall (Eds.), *Fish Physiology* (Vol. 6, pp. 1–98). Academic Press. [https://doi.org/10.1016/S1546-5098\(08\)60146-6](https://doi.org/10.1016/S1546-5098(08)60146-6)
- Gaëlle, L., & Jean-Claude, G. (2018). Assessing functional evenness with the FEve index: A word of warning. *Ecological Indicators*, 90, 257–260. <https://doi.org/10.1016/j.ecolind.2018.03.020>

- Galarowicz, T. L., & Wahl, D. H. (2003). Differences in growth, consumption, and metabolism among walleyes from different latitudes. *Transactions of the American Fisheries Society*, 132(3), 425–437.
- Giacomini, H. C., DeAngelis, D. L., Trexler, J. C., & Petrere, M. (2013). Trait contributions to fish community assembly emerge from trophic interactions in an individual-based model. *Ecological Modelling*, 251, 32–43. <https://doi.org/10.1016/j.ecolmodel.2012.12.003>
- Grigaltchik, V. S., Ward, A. J. W., & Seebacher, F. (2012). Thermal acclimation of interactions: Differential responses to temperature change alter predator–prey relationship. *Proceedings of the Royal Society B: Biological Sciences*, 279(1744), 4058–4064. <https://doi.org/10.1098/rspb.2012.1277>
- Grooten, M., & Almond, R. E. A. (2018). Living planet report - 2018: Aiming higher. *Living Planet Report - 2018: Aiming Higher*. <https://www.cabdirect.org/cabdirect/abstract/20183346851>
- Guasch, H., Armengol, J., Martí, E., & Sabater, S. (1998). Diurnal variation in dissolved oxygen and carbon dioxide in two low-order streams. *Water Research*, 32(4), 1067–1074. [https://doi.org/10.1016/S0043-1354\(97\)00330-8](https://doi.org/10.1016/S0043-1354(97)00330-8)
- Gunderson, A. R., & Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences*, 282(1808), 20150401. <https://doi.org/10.1098/rspb.2015.0401>
- Guzzo, M. M., Mochnacz, N. J., Durhack, T., Kissinger, B. C., Killen, S. S., & Treberg, J. R. (2019). Effects of repeated daily acute heat challenge on the growth and metabolism of a cold water stenothermal fish. *The Journal of Experimental Biology*, 222(12), jeb198143. <https://doi.org/10.1242/jeb.198143>
- Hall, A. E., & Clark, T. D. (2016). Seeing is believing: Metabolism provides insight into threat perception for a prey species of coral reef fish. *Animal Behaviour*, 115, 117–126. <https://doi.org/10.1016/j.anbehav.2016.03.008>
- Hansen, G. J. A., Read, J. S., Hansen, J. F., & Winslow, L. A. (2017). Projected shifts in fish species dominance in Wisconsin lakes under climate change. *Global Change Biology*, 23(4), 1463–1476. <https://doi.org/10.1111/gcb.13462>
- Harvey, B. P., Marshall, K. E., Harley, C. D. G., & Russell, B. D. (2022). Predicting responses to marine heatwaves using functional traits. *Trends in Ecology & Evolution*, 37(1), 20–29. <https://doi.org/10.1016/j.tree.2021.09.003>
- Hasler, C. T., Suski, C. D., Hanson, K. C., Cooke, S. J., Philipp, D. P., & Tufts, B. L. (2009). Effect of water temperature on laboratory swimming performance and natural activity levels of adult largemouth bass. *Canadian Journal of Zoology*, 87(7), 589–596. <https://doi.org/10.1139/Z09-044>
- Havird, J. C., Neuwald, J. L., Shah, A. A., Mauro, A., Marshall, C. A., & Ghalambor, C. K. (2020). Distinguishing between active plasticity due to thermal acclimation and passive plasticity due to Q_{10} effects: Why methodology matters. *Functional Ecology*, 34(5), 1015–1028. <https://doi.org/10.1111/1365-2435.13534>
- Hebert, C. E., Weseloh, D. V. C., Idrissi, A., Arts, M. T., O’Gorman, R., Gorman, O. T., Locke, B., Madenjian, C. P., & Roseman, E. F. (2008). Restoring Piscivorous Fish Populations in the Laurentian Great Lakes Causes Seabird Dietary Change. *Ecology*, 89(4), 891–897. <https://doi.org/10.1890/07-1603.1>

- Heck, M. P., Schultz, L. D., Hockman-Wert, D., Dinger, E. C., & Dunham, J. B. (2018). Monitoring stream temperatures—A guide for non-specialists. In *Monitoring stream temperatures—A guide for non-specialists* (USGS Numbered Series No. 3-A25; Techniques and Methods, Vols. 3-A25, p. 84). U.S. Geological Survey. <https://doi.org/10.3133/tm3A25>
- Heise, K., Puntarulo, S., Nikinmaa, M., Abele, D., & Pörtner, H.-O. (2006). Oxidative stress during stressful heat exposure and recovery in the North Sea eelpout *Zoarces viviparus* L. *Journal of Experimental Biology*, 209(2), 353–363. <https://doi.org/10.1242/jeb.01977>
- Hermoso, V., Cattarino, L., Linke, S., & Kennard, M. J. (2018). Catchment zoning to enhance co-benefits and minimize trade-offs between ecosystem services and freshwater biodiversity conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 28(4), 1004–1014. <https://doi.org/10.1002/aqc.2891>
- Holtrop, A. M., Dolan, C. R., & Epifanio, J. M. (2005). *Ecological Classification of Rivers for Environmental Assessment and Management: Stream attribution and Model preparation*. 48.
- IDNR. 2010. Fisheries manual of operation: filed sampling protocols for rivers and streams. IDNR, Division of Fisheries/Illinois Natural History Survey, 1816 S Oak Street, Champaign, IL 61821.
- IDNR, 2017. 2017 CREP Annual Report.
- IEPA, IDOA, University of Illinois Extension, 2019. Illinois Nutrient Loss Reduction Strategy Biennial Report 2018-2019. Springfield.
- Jaeger, K. L., Olden, J. D., & Pelland, N. A. (2014). Climate change poised to threaten hydrologic connectivity and endemic fishes in dryland streams. *Proceedings of the National Academy of Sciences*, 111(38), 13894–13899. <https://doi.org/10.1073/pnas.1320890111>
- Jentsch, A., Kreyling, J., & Beierkuhnlein, C. (2007). A new generation of climate-change experiments: Events, not trends. *Frontiers in Ecology and the Environment*, 5(7), 365–374. [https://doi.org/10.1890/1540-9295\(2007\)5\[365:ANGOCE\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2007)5[365:ANGOCE]2.0.CO;2)
- Johnson, T. B., Bunnell, D. B., & Knight, C. T. (2005). A Potential New Energy Pathway in Central Lake Erie: The Round Goby Connection. *Journal of Great Lakes Research*, 31, 238–251. [https://doi.org/10.1016/S0380-1330\(05\)70317-8](https://doi.org/10.1016/S0380-1330(05)70317-8)
- Jones, J. A. (2011). Hydrologic responses to climate change: Considering geographic context and alternative hypotheses. *Hydrological Processes*, 25(12), 1996–2000. <https://doi.org/10.1002/hyp.8004>
- Jutfelt, F. (2020). Metabolic adaptation to warm water in fish. *Functional Ecology*, 34, 1138–1141. <https://doi.org/10.1111/1365-2435.13558>
- Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D. J., Lefevre, S., Nilsson, G. E., Metcalfe, N. B., Hickey, A. J. R., Brijs, J., Speers-Roesch, B., Roche, D. G., Gamperl, A. K., Raby, G. D., Morgan, R., Esbaugh, A. J., Gräns, A., Axelsson, M., ... Clark, T. D. (2018). Oxygen- and capacity-limited thermal tolerance: Blurring ecology and physiology. *Journal of Experimental Biology*, 221(1). <https://doi.org/10.1242/jeb.169615>
- Kaur, M., Atif, F., Ali, M., Rehman, H., & Raisuddin, S. (2005). Heat stress-induced alterations of antioxidants in the freshwater fish *Channa punctata* Bloch. *Journal of Fish Biology*, 67(6), 1653–1665. <https://doi.org/10.1111/j.1095-8649.2005.00872.x>
- Kearney, M., & Porter, W. (2009). Mechanistic niche modelling: Combining physiological and spatial data to predict species' ranges. *Ecology Letters*, 12(4), 334–350. <https://doi.org/10.1111/j.1461-0248.2008.01277.x>

- Killen, S. S., Atkinson, D., & Glazier, D. S. (2010). The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecology Letters*, 13(2), 184–193. <https://doi.org/10.1111/j.1461-0248.2009.01415.x>
- Killen, S. S., Costa, I., Brown, J. A., & Gamperl, A. K. (2007). Little left in the tank: Metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings of the Royal Society B: Biological Sciences*, 274(1608), 431–438. <https://doi.org/10.1098/rspb.2006.3741>
- Kolok, A. S., Peake, E. B., Tierney, L. L., Roark, S. A., Noble, R. B., See, K., & Guttman, S. I. (2004). Copper tolerance in fathead minnows: I. The role of genetic and nongenetic factors. *Environmental Toxicology and Chemistry*, 23(1), 200–207. <https://doi.org/10.1897/02-609>
- Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, 82(1), 1–26. <https://doi.org/10.18637/jss.v082.i13>
- Laliberté, E., & Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, 91(1), 299–305. <https://doi.org/10.1890/08-2244.1>
- Laliberté, E., Legendre, P., Shipley, B., & Laliberté, M. E. (2014). Package ‘FD’. Version, 1, 12.
- Lange, K., Townsend, C. R., & Matthaei, C. D. (2016). A trait-based framework for stream algal communities. *Ecology and Evolution*, 6(1), 23–36. <https://doi.org/10.1002/ece3.1822>
- Larimore, R. W., & Bayley, P. B. (1996). The fishes of Champaign County, Illinois, during a century of alterations of a prairie ecosystem. *Illinois Natural History Survey Bulletin*; v. 035, no. 02.
- Lawrence, D. J., Stewart-Koster, B., Olden, J. D., Ruesch, A. S., Torgersen, C. E., Lawler, J. J., Butcher, D. P., & Crown, J. K. (2014). The interactive effects of climate change, riparian management, and a nonnative predator on stream-rearing salmon. *Ecological Applications*, 24(4), 895–912. <https://doi.org/10.1890/13-0753.1>
- Lee, C. G., Farrell, A. P., Lotto, A., MacNutt, M. J., Hinch, S. G., & Healey, M. C. (2003). The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon stocks. *Journal of Experimental Biology*, 206(18), 3239–3251. <https://doi.org/10.1242/jeb.00547>
- Lefevre, S., Wang, T., & McKenzie, D. J. (2021). The role of mechanistic physiology in investigating impacts of global warming on fishes. *Journal of Experimental Biology*, 224(Suppl_1), jeb238840. <https://doi.org/10.1242/jeb.238840>
- Leiva, F. P., Calosi, P., & Verberk, W. C. E. P. (2019). Scaling of thermal tolerance with body mass and genome size in ectotherms: A comparison between water- and air-breathers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 374(1778), 20190035. <https://doi.org/10.1098/rstb.2019.0035>
- Lennox, R. J., Eliason, E. J., Havn, T. B., Johansen, M. R., Thorstad, E. B., Cooke, S. J., Diserud, O. H., Whoriskey, F. G., Farrell, A. P., & Uglem, I. (2018). Bioenergetic consequences of warming rivers to adult Atlantic salmon *Salmo salar* during their spawning migration. *Freshwater Biology*, 63(11), 1381–1393. <https://doi.org/10.1111/fwb.13166>
- Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2018). Emmeans: Estimated marginal means, aka least-squares means. R package version, 1(1), 3.
- Liaw, A., & Wiener, M. (2002). Classification and regression by randomForest. *R news*, 2(3), 18–22.

- Little, A. G., Hardison, E., Kraskura, K., Dressler, T., Prystay, T. S., Hendriks, B., Pruitt, J. N., Farrell, A. P., Cooke, S. J., Patterson, D. A., Hinch, S. G., & Eliason, E. J. (2020). Reduced lactate dehydrogenase activity in the heart and suppressed sex hormone levels are associated with female-biased mortality during thermal stress in Pacific salmon. *Journal of Experimental Biology*, 223(14). <https://doi.org/10.1242/jeb.214841>
- Logan, C. A., & Buckley, B. A. (2015). Transcriptomic responses to environmental temperature in eurythermal and stenothermal fishes. *Journal of Experimental Biology*, 218(12), 1915–1924. <https://doi.org/10.1242/jeb.114397>
- Loheide, S. P., & Gorelick, S. M. (2006). Quantifying Stream–Aquifer Interactions through the Analysis of Remotely Sensed Thermographic Profiles and In Situ Temperature Histories. *Environmental Science & Technology*, 40(10), 3336–3341. <https://doi.org/10.1021/es0522074>
- Madeira, C., Mendonça, V., Leal, M. C., Flores, A. A. V., Cabral, H. N., Diniz, M. S., & Vinagre, C. (2017). Thermal stress, thermal safety margins and acclimation capacity in tropical shallow waters—An experimental approach testing multiple end-points in two common fish. *Ecological Indicators*, 81, 146–158. <https://doi.org/10.1016/j.ecolind.2017.05.050>
- Madeira, D. (2016). Are fish in hot water? Effects of warming on oxidative stress metabolism in the commercial species *Sparus aurata*. *Ecological Indicators*, 8.
- Mao, Z., Gu, X., Cao, Y., Luo, J., Zeng, Q., Chen, H., & Jeppesen, E. (2021). How does fish functional diversity respond to environmental changes in two large shallow lakes? *Science of The Total Environment*, 753, 142158. <https://doi.org/10.1016/j.scitotenv.2020.142158>
- Markovic, D., Carrizo, S., Freyhof, J., Cid, N., Lengyel, S., Scholz, M., Kasperdius, H., & Darwall, W. (2014). Europe’s freshwater biodiversity under climate change: Distribution shifts and conservation needs. *Diversity and Distributions*, 20(9), 1097–1107. <https://doi.org/10.1111/ddi.12232>
- Matthews, K. R., & Berg, N. H. (1997). Rainbow trout responses to water temperature and dissolved oxygen stress in two southern California stream pools. *Journal of Fish Biology*, 50(1), 50–67. <https://doi.org/10.1111/j.1095-8649.1997.tb01339.x>
- Mcgill, B., Enquist, B., Weiher, E., & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, 21(4), 178–185. <https://doi.org/10.1016/j.tree.2006.02.002>
- McKenna Jr, J. E., Carlson, D. M., & Payne-Wynne, M. L. (2013). Predicting locations of rare aquatic species’ habitat with a combination of species-specific and assemblage-based models. *Diversity and Distributions*, 19(5–6), 503–517. <https://doi.org/10.1111/ddi.12059>
- McKenzie, D. (2011). *The energetics of Fish Swimming* (pp. 1075–1080).
- Metzke, B. A., Burr, B. M., Hinz Jr, L. C., Page, L. M., & Taylor, C. A. (2022). *An Atlas of Illinois Fishes: 150 Years of Change*. University of Illinois Press.
- Milazzo, M., Mirto, S., Domenici, P., & Gristina, M. (2013). Climate change exacerbates interspecific interactions in sympatric coastal fishes. *Journal of Animal Ecology*, 82(2), 468–477. <https://doi.org/10.1111/j.1365-2656.2012.02034.x>
- Missaghi, S., Hondzo, M., & Herb, W. (2017). Prediction of lake water temperature, dissolved oxygen, and fish habitat under changing climate. *Climatic Change*, 141(4), 747–757. <https://doi.org/10.1007/s10584-017-1916-1>

- Morash, A. J., Neufeld, C., MacCormack, T. J., & Currie, S. (2018). The importance of incorporating natural thermal variation when evaluating physiological performance in wild species. *The Journal of Experimental Biology*, 221(14), jeb164673. <https://doi.org/10.1242/jeb.164673>
- Morgan, R., Finnøen, M. H., & Jutfelt, F. (2018). CT max is repeatable and doesn't reduce growth in zebrafish. *Scientific Reports*, 8(1), 7099. <https://doi.org/10.1038/s41598-018-25593-4>
- Murdoch, A., Mantyka-Pringle, C., & Sharma, S. (2020). The interactive effects of climate change and land use on boreal stream fish communities. *Science of the Total Environment*, 700, 134518.
- Nagelkerken, I., & Munday, P. L. (2016). Animal behaviour shapes the ecological effects of ocean acidification and warming: Moving from individual to community-level responses. *Global Change Biology*, 22(3), 974–989. <https://doi.org/10.1111/gcb.13167>
- Nelson, J. A. (2016). Oxygen consumption rate v. rate of energy utilization of fishes: A comparison and brief history of the two measurements. *Journal of Fish Biology*, 88(1), 10–25. <https://doi.org/10.1111/jfb.12824>
- Nelson, K. C., & Palmer, M. A. (2007). Stream Temperature Surges Under Urbanization and Climate Change: Data, Models, and Responses1. *JAWRA Journal of the American Water Resources Association*, 43(2), 440–452. <https://doi.org/10.1111/j.1752-1688.2007.00034.x>
- Nesper, L. (2002). *The Walleye War: The Struggle for Ojibwe Spearfishing and Treaty Rights*. U of Nebraska Press.
- Nevada Division of Environmental Protection. (2018). FINAL_Guidance_for_Developing_Temperature_Criteria_for_Nevada_Waters.pdf. URL https://ndep.nv.gov/uploads/water-wqs-docs/FINAL_Guidance_for_Developing_Temperature_Criteria_for_Nevada_Waters.pdf (accessed 6.1.22)
- Nichol, J. E., Choi, S. Y., Wong, M. S., & Abbas, S. (2020). Temperature change and urbanisation in a multi-nucleated megacity: China's Pearl River Delta. *Urban Climate*, 31, 100592. <https://doi.org/10.1016/j.uclim.2020.100592>
- Norin, T., & Clark, T. D. (2016). Measurement and relevance of maximum metabolic rate in fishes. *Journal of Fish Biology*, 88(1), 122–151. <https://doi.org/10.1111/jfb.12796>
- Nyboer, E. A., & Chapman, L. J. (2017). Elevated temperature and acclimation time affect metabolic performance in the heavily exploited Nile perch of Lake Victoria. *Journal of Experimental Biology*, jeb.163022. <https://doi.org/10.1242/jeb.163022>
- Oberdorff, T., Tedesco, P. A., Hugueny, B., Leprieur, F., Beauchard, O., Brosse, S., & Dürr, H. H. (2011). Global and Regional Patterns in Riverine Fish Species Richness: A Review. *International Journal of Ecology*, 2011, e967631. <https://doi.org/10.1155/2011/967631>
- O'Connor, C. M., Norris, D. R., Crossin, G. T., & Cooke, S. J. (2014). Biological carryover effects: Linking common concepts and mechanisms in ecology and evolution. *Ecosphere*, 5(3), art28. <https://doi.org/10.1890/ES13-00388.1>
- Öhlund, G., Hedström, P., Norman, S., Hein, C. L., & Englund, G. (2015). Temperature dependence of predation depends on the relative performance of predators and prey. *Proceedings of the Royal Society B: Biological Sciences*, 282(1799), 20142254. <https://doi.org/10.1098/rspb.2014.2254>
- Olden, J. D., Poff, N. L., & Bestgen, K. R. (2006). Life-History Strategies Predict Fish Invasions and Extirpations in the Colorado River Basin. *Ecological Monographs*, 76(1), 25–40. <https://doi.org/10.1890/05-0330>

- Oligny-Hébert, H., Senay, C., Enders, E. C., & Boisclair, D. (2015). Effects of diel temperature fluctuation on the standard metabolic rate of juvenile Atlantic salmon (*Salmo salar*): Influence of acclimation temperature and provenience. *Canadian Journal of Fisheries and Aquatic Sciences*, 72(9), 1306–1315. <https://doi.org/10.1139/cjfas-2014-0345>
- Oliver, T. H., & Morecroft, M. D. (2014). Interactions between climate change and land use change on biodiversity: attribution problems, risks, and opportunities. *Wiley Interdisciplinary Reviews: Climate Change*, 5(3), 317–335.
- Op de Beeck, L., Verheyen, J., & Stoks, R. (2017). Integrating both interaction pathways between warming and pesticide exposure on upper thermal tolerance in high- and low-latitude populations of an aquatic insect. *Environmental Pollution*, 224, 714–721. <https://doi.org/10.1016/j.envpol.2016.11.014>
- Page, L. M., & Burr, B. M. (2011). *Peterson Field Guide to Freshwater Fishes of North America North of Mexico*. Houghton Mifflin Harcourt.
- Parker, J., Cao, Y., Sass, G. G., & Epifanio, J. (2018). Large river fish functional diversity responses to improved water quality over a 28 year period. *Ecological Indicators*, 88, 322–331. <https://doi.org/10.1016/j.ecolind.2018.01.035>
- Peake, S., McKinley, R. S., & Scruton, D. A. (2000). Swimming performance of walleye (*Stizostedion vitreum*). *Canadian Journal of Zoology*, 78(9), 1686–1690. <https://doi.org/10.1139/z00-097>
- Petchey, O. L., & Gaston, K. J. (2006). Functional diversity: Back to basics and looking forward. *Ecology Letters*, 9(6), 741–758. <https://doi.org/10.1111/j.1461-0248.2006.00924.x>
- Petermann, J. S., Farjalla, V. F., Jocque, M., Kratina, P., MacDonald, A. A. M., Marino, N. A. C., de Omena, P. M., Piccoli, G. C. O., Richardson, B. A., Richardson, M. J., Romero, G. Q., Videla, M., & Srivastava, D. S. (2015). Dominant predators mediate the impact of habitat size on trophic structure in bromeliad invertebrate communities. *Ecology*, 96(2), 428–439. <https://doi.org/10.1890/14-0304.1>
- Pla, L., Casanoves, F., & Rienzo, J. D. (2011). *Quantifying Functional Biodiversity*. Springer Science & Business Media.
- Plaut, I. (2001). Critical swimming speed: Its ecological relevance. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 131(1), 41–50. [https://doi.org/10.1016/S1095-6433\(01\)00462-7](https://doi.org/10.1016/S1095-6433(01)00462-7)
- Poff, N. L. (1997). Landscape Filters and Species Traits: Towards Mechanistic Understanding and Prediction in Stream Ecology. *Journal of the North American Benthological Society*, 16(2), 391–409. <https://doi.org/10.2307/1468026>
- Pörtner, H. O., & Peck, M. A. (2010). Climate change effects on fishes and fisheries: Towards a cause-and-effect understanding. *Journal of Fish Biology*, 77(8), 1745–1779. <https://doi.org/10.1111/j.1095-8649.2010.02783.x>
- Pörtner, H.-O. (2010). Oxygen- and capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology*, 213(6), 881–893. <https://doi.org/10.1242/jeb.037523>
- Powers, M. (2000). The Spirit of the Salmon: How the Tribal Restoration Plan Could Restore Columbia Basin Salmon. *Environmental Law*, 30, 867.
- R Core Team (2020). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. URL <https://www.R-project.org/>.

- Radinger, J., Hölker, F., Horký, P., Slavík, O., Dendoncker, N., & Wolter, C. (2016). Synergistic and antagonistic interactions of future land use and climate change on river fish assemblages. *Global Change Biology*, 22(4), 1505–1522. <https://doi.org/10.1111/gcb.13183>
- Rahel, F. J., Bierwagen, B., & Taniguchi, Y. (2008). Managing Aquatic Species of Conservation Concern in the Face of Climate Change and Invasive Species. *Conservation Biology*, 22(3), 551–561. <https://doi.org/10.1111/j.1523-1739.2008.00953.x>
- Rahmstorf, S., & Coumou, D. (2011). Increase of extreme events in a warming world. *Proceedings of the National Academy of Sciences*, 108(44), 17905–17909. <https://doi.org/10.1073/pnas.1101766108>
- Rall, B. C., Vucic-Pestic, O., Ehnes, R. B., Emmerson, M., & Brose, U. (2010). Temperature, predator–prey interaction strength and population stability. *Global Change Biology*, 16(8), 2145–2157. <https://doi.org/10.1111/j.1365-2486.2009.02124.x>
- Raptis, C. E., van Vliet, M. T. H., & Pfister, S. (2016). Global thermal pollution of rivers from thermoelectric power plants. *Environmental Research Letters*, 11(10), 104011. <https://doi.org/10.1088/1748-9326/11/10/104011>
- Rathert, D., White, D., Sifneos, J. C., & Hughes, R. M. (1999). Environmental correlates of species richness for native freshwater fish in Oregon, U.S.A. *Journal of Biogeography*, 26(2), 257–273. <https://doi.org/10.1046/j.1365-2699.1999.00274.x>
- Raven, P. H., & Wagner, D. L. (2021). Agricultural intensification and climate change are rapidly decreasing insect biodiversity. *Proceedings of the National Academy of Sciences*, 118(2), e2002548117. <https://doi.org/10.1073/pnas.2002548117>
- Redpath, T. D., Cooke, S. J., Suski, C. D., Arlinghaus, R., Couture, P., Wahl, D. H., & Philipp, D. P. (2010). The metabolic and biochemical basis of vulnerability to recreational angling after three generations of angling-induced selection in a teleost fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 67(12), 1983–1992.
- Rezende, E. L., Castañeda, L. E., & Santos, M. (2014). Tolerance landscapes in thermal ecology. *Functional Ecology*, 28(4), 799–809. <https://doi.org/10.1111/1365-2435.12268>
- Roberge, J.-M., & Angelstam, P. (2004). Usefulness of the Umbrella Species Concept as a Conservation Tool. *Conservation Biology*, 18(1), 76–85. <https://doi.org/10.1111/j.1523-1739.2004.00450.x>
- Rogers, L. A., Griffin, R., Young, T., Fuller, E., St. Martin, K., & Pinsky, M. L. (2019). Shifting habitats expose fishing communities to risk under climate change. *Nature Climate Change*, 9(7), 512–516. <https://doi.org/10.1038/s41558-019-0503-z>
- Rohr, J. R., Civitello, D. J., Cohen, J. M., Roznik, E. A., Sinervo, B., & Dell, A. I. (2018). The complex drivers of thermal acclimation and breadth in ectotherms. *Ecology Letters*, 21(9), 1425–1439. <https://doi.org/10.1111/ele.13107>
- Rountrey, A. N., Coulson, P. G., Meeuwig, J. J., & Meekan, M. (2014). Water temperature and fish growth: Otoliths predict growth patterns of a marine fish in a changing climate. *Global Change Biology*, 20(8), 2450–2458. <https://doi.org/10.1111/gcb.12617>
- Rubalcaba, J. G., Verberk, W. C. E. P., Hendriks, A. J., Saris, B., & Woods, H. A. (2020). Oxygen limitation may affect the temperature and size dependence of metabolism in aquatic ectotherms. *Proceedings of the National Academy of Sciences*, 117(50), 31963–31968. <https://doi.org/10.1073/pnas.2003292117>

- Rubio-Gracia, F., García-Berthou, E., Guasch, H., Zamora, L., & Vila-Gispert, A. (2020). Size-related effects and the influence of metabolic traits and morphology on swimming performance in fish. *Current Zoology*, 66(5), 493–503. <https://doi.org/10.1093/cz/zoaa013>
- Rummer, J. L., Couturier, C. S., Stecyk, J. A. W., Gardiner, N. M., Kinch, J. P., Nilsson, G. E., & Munday, P. L. (2014). Life on the edge: Thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology*, 20(4), 1055–1066. <https://doi.org/10.1111/gcb.12455>
- Sandblom, E., Clark, T. D., Gräns, A., Ekström, A., Brijs, J., Sundström, L. F., Odelström, A., Adill, A., Aho, T., & Jutfelt, F. (2016). Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. *Nature Communications*, 7(1), 11447. <https://doi.org/10.1038/ncomms11447>
- Schaefer, J., & Ryan, A. (2006). Developmental plasticity in the thermal tolerance of zebrafish *Danio rerio*. *Journal of Fish Biology*, 69(3), 722–734. <https://doi.org/10.1111/j.1095-8649.2006.01145.x>
- Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology*, 218(12), 1856–1866. <https://doi.org/10.1242/jeb.118851>
- Scott, G. R., & Johnston, I. A. (2012). Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. *Proceedings of the National Academy of Sciences*, 109(35), 14247–14252. <https://doi.org/10.1073/pnas.1205012109>
- Seebacher, F., White, C. R., & Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, 5(1), 61–66. <https://doi.org/10.1038/nclimate2457>
- Shasteen, D. K., Bales, S. A., & Stodola, A. P. (2013). Freshwater mussels of the Kaskaskia River basin. Illinois Natural History Survey.
- Shipley, N. J., Johnson, D. N., van Riper, C. J., Stewart, W. P., Chu, M. L., Suski, C. D., Stein, J. A., & Shew, J. J. (2020). A deliberative research approach to valuing agro-ecosystem services in a worked landscape. *Ecosystem Services*, 42, 101083. <https://doi.org/10.1016/j.ecoser.2020.101083>
- Smith, G., Badgley, C., Eiting, T., & Larson, P. (2010). Species diversity gradients in relation to geological history in North American freshwater fishes. *Evolutionary Ecology Research*, 12.
- Smith, P., Cotrufo, M. F., Rumpel, C., Paustian, K., Kuikman, P. J., Elliott, J. A., McDowell, R., Griffiths, R. I., Asakawa, S., Bustamante, M., House, J. I., Sobocká, J., Harper, R., Pan, G., West, P. C., Gerber, J. S., Clark, J. M., Adhya, T., Scholes, R. J., & Scholes, M. C. (2015). Biogeochemical cycles and biodiversity as key drivers of ecosystem services provided by soils. *SOIL*, 1(2), 665–685. <https://doi.org/10.5194/soil-1-665-2015>
- Somero, G. N. (2010). The physiology of climate change: How potentials for acclimatization and genetic adaptation will determine ‘winners’ and ‘losers.’ *Journal of Experimental Biology*, 213(6), 912–920. <https://doi.org/10.1242/jeb.037473>
- South, E. J., Edward DeWalt, R., & Cao, Y. (2019). Relative importance of Conservation Reserve Programs to aquatic insect biodiversity in an agricultural watershed in the Midwest, USA. *Hydrobiologia*, 829(1), 323–340. <https://doi.org/10.1007/s10750-018-3842-2>
- Spinks, R. K., Munday, P. L., & Donelson, J. M. (2019). Developmental effects of heatwave conditions on the early life stages of a coral reef fish. *The Journal of Experimental Biology*, 222(16), jeb202713. <https://doi.org/10.1242/jeb.202713>

- Steen, P. J., Zorn, T. G., Seelbach, P. W., & Schaeffer, J. S. (2008). Classification Tree Models for Predicting Distributions of Michigan Stream Fish from Landscape Variables. *Transactions of the American Fisheries Society*, 137(4), 976–996. <https://doi.org/10.1577/T07-119.1>
- Stein, J. A., King, S. M., Balto, G., Stanhope, K. A., McNamara, T., Fiolek, C., & Johnson, C. (2021). *Research and Analysis of Fisheries in Illinois: FINAL PERFORMANCE REPORT 1 July 2020–30 June 2021*. Illinois Natural History Survey.
- Stillman, J. H. (2019). *Heat Waves, the New Normal: Summertime Temperature Extremes Will Impact Animals, Ecosystems, and Human Communities*. 34, 15.
- Stubbs, M. (2014). Conservation Reserve Program (CRP): Status and Issues. 24.
- Svendsen, J. C., Tudorache, C., Jordan, A. D., Steffensen, J. F., Aarestrup, K., & Domenici, P. (2010). Partition of aerobic and anaerobic swimming costs related to gait transitions in a labriform swimmer. *Journal of Experimental Biology*, 213(13), 2177–2183. <https://doi.org/10.1242/jeb.041368>
- Svendsen, M. B. S., Bushnell, P. G., Christensen, E. a. F., & Steffensen, J. F. (2016). Sources of variation in oxygen consumption of aquatic animals demonstrated by simulated constant oxygen consumption and respirometers of different sizes. *Journal of Fish Biology*, 88(1), 51–64. <https://doi.org/10.1111/jfb.12851>
- Thuiller, W., Münkemüller, T., Lavergne, S., Mouillot, D., Mouquet, N., Schiffrers, K., & Gravel, D. (2013). A road map for integrating eco-evolutionary processes into biodiversity models. *Ecology Letters*, 16(s1), 94–105. <https://doi.org/10.1111/ele.12104>
- Tickner, D., Opperman, J. J., Abell, R., Acreman, M., Arthington, A. H., Bunn, S. E., Cooke, S. J., Dalton, J., Darwall, W., Edwards, G., Harrison, I., Hughes, K., Jones, T., Leclère, D., Lynch, A. J., Leonard, P., McClain, M. E., Muruven, D., Olden, J. D., ... Young, L. (2020). Bending the Curve of Global Freshwater Biodiversity Loss: An Emergency Recovery Plan. *BioScience*, 70(4), 330–342. <https://doi.org/10.1093/biosci/biaa002>
- USACE. (2017). Kaskaskia River Project Master Plan 2017. [WWW Document]. URL https://www.mvs.usace.army.mil/Portals/54/docs/recreation/rend/Kasky%20Master%20Plan%202018%20DRAFT_16OCT2018.pdf?ver=2018-10-16-162924-713 (accessed 5/21/2019)
- USDA. (2016). National Agricultural Statistics Service Cropland Data Layer [WWW Document]. Public Cropland Data Layer. URL <https://nassgeodata.gmu.edu/CropScape/> (accessed 5/21/2019).
- USGS. (2016). National Water Information System data available on the World Wide Web (USGS Water Data for the Nation) [WWW Document]. URL <http://waterdata.usgs.gov/nwis/> (accessed 6/1/2019).
- van Denderen, P. D., Lindegren, M., MacKenzie, B. R., Watson, R. A., & Andersen, K. H. (2018). Global patterns in marine predatory fish. *Nature Ecology & Evolution*, 2(1), 65–70. <https://doi.org/10.1038/s41559-017-0388-z>
- Van Zuiden, T. M., Chen, M. M., Stefanoff, S., Lopez, L., & Sharma, S. (2016). Projected impacts of climate change on three freshwater fishes and potential novel competitive interactions. *Diversity and Distributions*, 22(5), 603–614. <https://doi.org/10.1111/ddi.12422>
- Verberk, W. C. E. P., Durance, I., Vaughan, I. P., & Ormerod, S. J. (2016). Field and laboratory studies reveal interacting effects of stream oxygenation and warming on aquatic ectotherms. *Global Change Biology*, 22(5), 1769–1778. <https://doi.org/10.1111/gcb.13240>

- Wang, H., Hondzo, M., Xu, C., Poole, V., & Spacie, A. (2003). Dissolved oxygen dynamics of streams draining an urbanized and an agricultural catchment. *Ecological Modelling*, 160(1–2), 145–161. [https://doi.org/10.1016/S0304-3800\(02\)00324-1](https://doi.org/10.1016/S0304-3800(02)00324-1)
- Wang, H.-Y., Shen, S.-F., Chen, Y.-S., Kiang, Y.-K., & Heino, M. (2020). Life histories determine divergent population trends for fishes under climate warming. *Nature Communications*, 11(1), 4088. <https://doi.org/10.1038/s41467-020-17937-4>
- Wantzen, K. M., & Mol, J. H. (2013). Soil erosion from agriculture and mining: a threat to tropical stream ecosystems. *Agriculture*, 3(4), 660–683.
- Webb, B. W. (1996). Trends in stream and river temperature. *Hydrological Processes*, 10(2), 205–226. [https://doi.org/10.1002/\(SICI\)1099-1085\(199602\)10:2<205::AID-HYP358>3.0.CO;2-1](https://doi.org/10.1002/(SICI)1099-1085(199602)10:2<205::AID-HYP358>3.0.CO;2-1)
- Wehrly, K. E., Wiley, M. J., & Seelbach, P. W. (2003). Classifying Regional Variation in Thermal Regime Based on Stream Fish Community Patterns. *Transactions of the American Fisheries Society*, 132(1), 18–38. [https://doi.org/10.1577/1548-8659\(2003\)132<0018:CRVITR>2.0.CO;2](https://doi.org/10.1577/1548-8659(2003)132<0018:CRVITR>2.0.CO;2)
- Wright, M. N., Wager, S., & Probst, P. (2020). Ranger: A fast implementation of random forests. R package version 0.12, 1.
- Wuebbles, D. J. (2021). Impacts, Risks, and Adaptation in the United States: 4th US National Climate Assessment, Volume II. In *World Scientific Encyclopedia of Climate Change: Case Studies of Climate Risk, Action, and Opportunity Volume 3* (pp. 85–98).
- Xenopoulos, M. A., & Lodge, D. M. (2006). Going with the Flow: Using Species–Discharge Relationships to Forecast Losses in Fish Biodiversity. *Ecology*, 87(8), 1907–1914. [https://doi.org/10.1890/0012-9658\(2006\)87\[1907:GWTFUS\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[1907:GWTFUS]2.0.CO;2)
- Xiao, H., Dee, L. E., Chadès, I., Peyrard, N., Sabbadin, R., Stringer, M., & McDonald-Madden, E. (2018). Win-wins for biodiversity and ecosystem service conservation depend on the trophic levels of the species providing services. *Journal of Applied Ecology*, 55(5), 2160–2170. <https://doi.org/10.1111/1365-2664.13192>
- Yousefi, M., Jouladeh-Roudbar, A., & Kafash, A. (2020). Using endemic freshwater fishes as proxies of their ecosystems to identify high priority rivers for conservation under climate change. *Ecological Indicators*, 112, 106137. <https://doi.org/10.1016/j.ecolind.2020.106137>
- Zakharova, L., Meyer, K. M., & Seifan, M. (2019). Trait-based modelling in ecology: A review of two decades of research. *Ecological Modelling*, 407, 108703. <https://doi.org/10.1016/j.ecolmodel.2019.05.008>

APPENDIX A: SUPPLEMENTAL FIGURES FOR CHAPTER 2

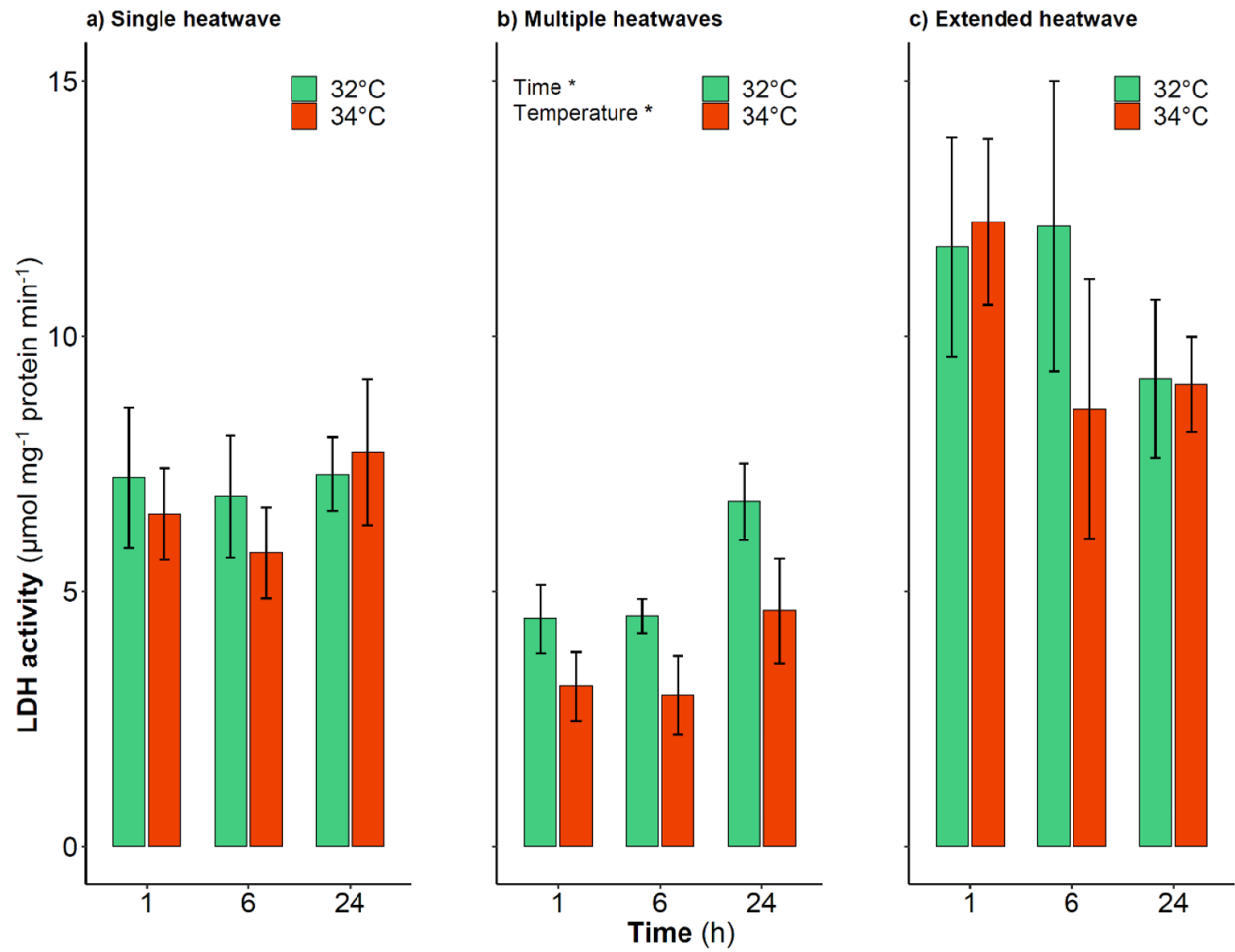


Figure A.1 Lactate dehydrogenase (LDH, $\pm$ se) activity of fathead minnow (*Pimephales promelas*) at 1, 6, and 24 h after single (a), multiple (b), and extended (c) heatwaves ($n = 6$ in each time $\times$ temperature group). Asterisk near legends represents significant difference ($P < 0.05$) between 32 and 34 °C or/and among 1, 6, 24 h.

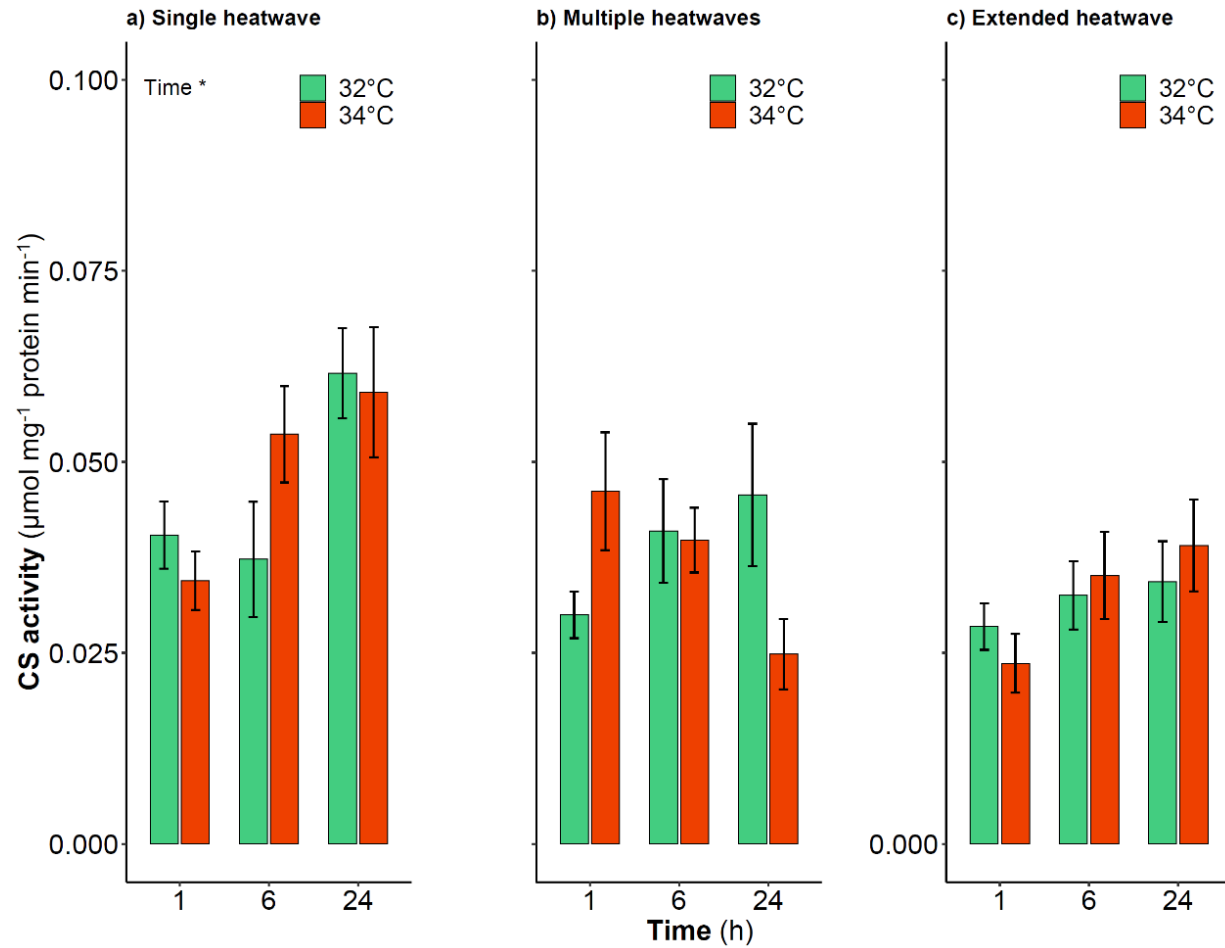


Figure A.2 Citrate synthase (CS, $\pm$ se) activity of fathead minnow (*Pimephales promelas*) at 1, 6, and 24 h after single (a), multiple (b), and extended (c) heatwaves ($n = 6$ in each time $\times$ temperature group). Asterisk near legends represents significant difference ($P < 0.05$) between 32 and 34 °C or/and among 1, 6, 24 h.

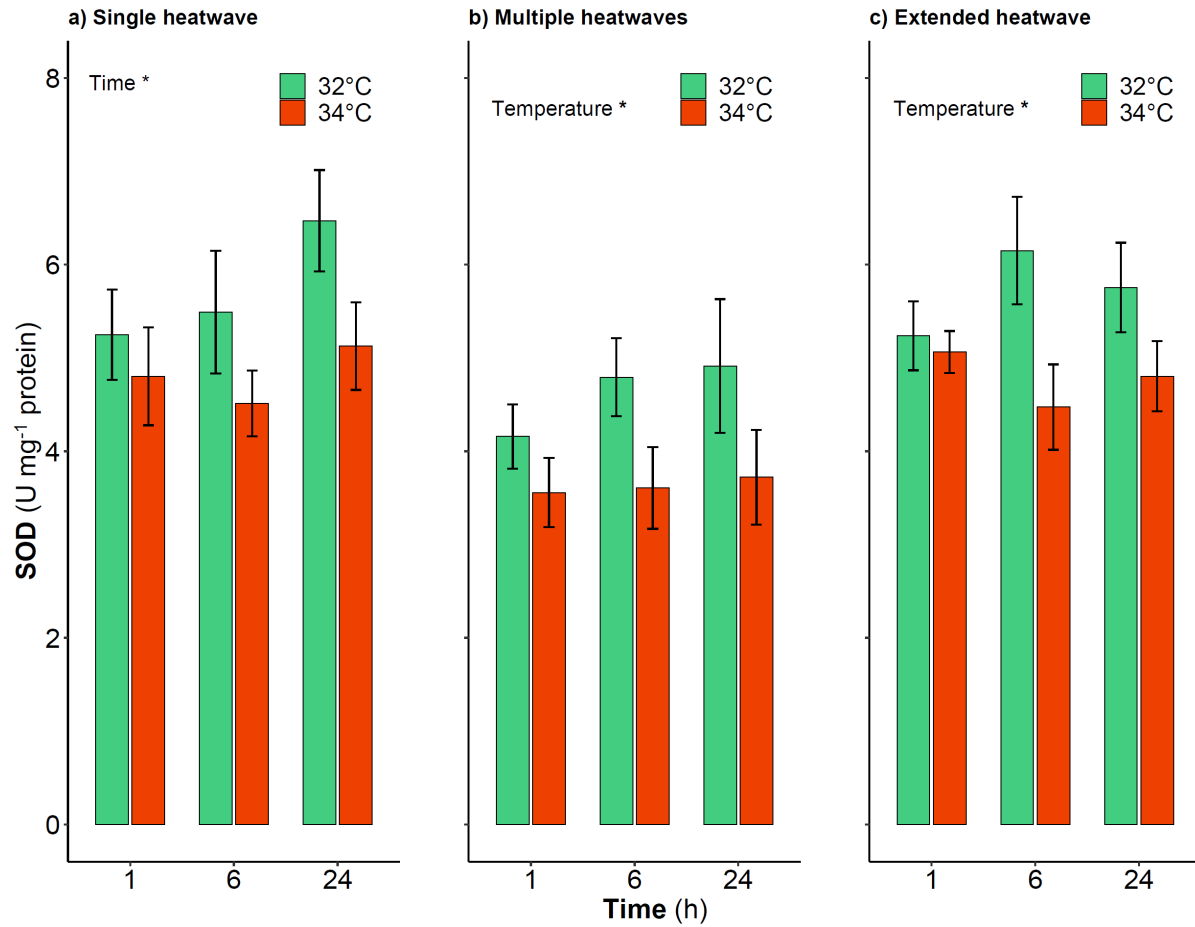


Figure A.3 Superoxide dismutase (SOD, $\pm$ se) of fathead minnow (*Pimephales promelas*) at 1, 6, and 24 h after single (a), multiple (b), and extended (c) heatwaves ($n = 6$ in each time $\times$ temperature group). Asterisk near legends represents significant difference ($P < 0.05$) between 32 and 34 °C or/and among 1, 6, 24 h.