The thermal ecology of prairie stream fishes

by

Bryan Daniel Frenette

B.S., University of Oklahoma, 2011
M.S., University of Oklahoma, 2014

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
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Abstract

Earth's atmosphere has warmed by approximately 1°C over the past century and continues to warm at an increasing rate. The effects of atmospheric warming are already visible in most major ecosystems and are evident across all levels of biological organization. Understanding how organisms respond to spatial and temporal variation in temperature, as well as linking their functional responses to temperature, are critical steps toward predicting the responses of populations and communities to global climate change. The southern redbelly dace (*Chrosomus erythrogaster*) and the central stoneroller (*Campostoma anomalum*) are two minnows (Cyprinidae) that occur in the Flint Hills region of the United States. These species fill similar ecological roles in streams where they co-occur but differ in their overall pattern of occurrence, with dace largely occupying cooler headwater reaches and stonerollers persisting in both headwaters and warmer intermediate-sized streams. Differences in the fundamental thermal niche of these species could underlie the observed differences in their realized thermal niches along a stream-size gradient of temperature. To better understand how temperature drives patterns of occurrence in functionally similar species of fish, I evaluated the thermal ecology of these two minnow species. First, I tested for interspecific differences in physiological functional traits along an ecologically realistic temperature gradient. The critical thermal maximum of the stoneroller was higher than dace at warm acclimation temperatures, indicating a greater capacity to buffer thermal stress. Additionally, temperature drove differences in activity levels between species; dace were more active when temperatures were warm, though behavioral differences between the benthic stoneroller and column-dwelling dace could also influence activity. Second, I tested whether acclimation to a diurnal temperature cycle affected the energy metabolism of dace and stoneroller compared to constant acclimation conditions. Dace acclimated to a diurnal thermal regime exhibited higher maximum metabolic rates, and subsequently higher aerobic scope, when exposed to temperatures above mean conditions. This indicates that diurnal variation in temperature is an important contributor to this species' ability to maintain energy metabolism when exposed to above-average temperature. Third, I leveraged long-term fish community and environmental data to examine responses in body size, abundance, and growth to inter-annual variation in temperature and flow in two cohorts of dace and stoneroller. I found that the average body size of dace in November decreased during years when stream flows were reduced during the spring and summer,

while the average body size of stonerollers increased during years with lower spring flows and stable flow persisting through the summer. The abundances of both species in November was not influenced by inter-annual variation in flow or temperature. Finally, while growth of dace between August and November was not influenced by inter-annual variation in flow or temperature, stonerollers grew less during years were flows were reduced during the spring and summer. Collectively, these studies demonstrate both the importance of using long-term data to infer patterns along environmental gradients and highlight how functional responses to temperature can inform patterns of occurrence along thermal gradients.

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Abstract

Earth's atmosphere has warmed by approximately 1°C over the past century and continues to warm at an increasing rate. The effects of atmospheric warming are already visible in most major ecosystems and are evident across all levels of biological organization. Understanding how organisms respond to spatial and temporal variation in temperature, as well as linking their functional responses to temperature, are critical steps toward predicting the responses of populations and communities to global climate change. The southern redbelly dace (*Chrosomus erythrogaster*) and the central stoneroller (*Campostoma anomalum*) are two minnows (Cyprinidae) that occur in the Flint Hills region of the United States. These species fill similar ecological roles in streams where they co-occur but differ in their overall pattern of occurrence, with dace largely occupying cooler headwater reaches and stonerollers persisting in both headwaters and warmer intermediate-sized streams. Differences in the fundamental thermal niche of these species could underlie the observed differences in their realized thermal niches along a stream-size gradient of temperature. To better understand how temperature drives patterns of occurrence in functionally similar species of fish, I evaluated the thermal ecology of these two minnow species. First, I tested for interspecific differences in physiological functional traits along an ecologically realistic temperature gradient. The critical thermal maximum of the stoneroller was higher than dace at warm acclimation temperatures, indicating a greater capacity to buffer thermal stress. Additionally, temperature drove differences in activity levels between species; dace were more active when temperatures were warm, though behavioral differences between the benthic stoneroller and column-dwelling dace could also influence activity. Second, I tested whether acclimation to a diurnal temperature cycle affected the energy metabolism of dace and stoneroller compared to constant acclimation conditions. Dace acclimated to a diurnal thermal regime exhibited higher maximum metabolic rates, and subsequently higher aerobic scope, when exposed to temperatures above mean conditions. This indicates that diurnal variation in temperature is an important contributor to this species' ability to maintain energy metabolism when exposed to above-average temperature. Third, I leveraged long-term fish community and environmental data to examine responses in body size, abundance, and growth to inter-annual variation in temperature and flow in two cohorts of dace and stoneroller. I found that the average body size of dace in November decreased during years when stream flows were reduced during the spring and summer,

while the average body size of stonerollers increased during years with lower spring flows and stable flow persisting through the summer. The abundances of both species in November was not influenced by inter-annual variation in flow or temperature. Finally, while growth of dace between August and November was not influenced by inter-annual variation in flow or temperature, stonerollers grew less during years were flows were reduced during the spring and summer. Collectively, these studies demonstrate both the importance of using long-term data to infer patterns along environmental gradients and highlight how functional responses to temperature can inform patterns of occurrence along thermal gradients.

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Acknowledgements

Dedication

This dissertation is dedicated to Adrianne Noelle Frenette, for her unwavering love and support during the four years spent laboring over this project, and to Lucas and Phoebe Frenette for being my sources of joy and inspiration.

Preface

The three chapters of this dissertation have been prepared in submission format and include co-authors. Chapter 2 is formatted for *Conservation Physiology* and is under review with this journal. For consistency, Chapters 3 and 4 follow the same formatting style.

Chapter 1 - Temperature, stream fish, and the fundamental thermal niche

Understanding how species respond to their environment is a fundamental goal of ecology. Temperature is a critical abiotic component of all environments on earth and influences processes across levels of biological organization (Magnuson et al. 1979). The body temperatures of ectothermic organisms are dependent on the temperature of the environment, and thus can strongly influence physiological processes (Angilletta 2009). The range of body temperatures allowing for positive population growth define the fundamental thermal niche of ectotherms, and both physiological processes and thermoregulatory behaviors contribute to the fundamental thermal niche (Gvoždík 2018). The fundamental thermal niche defines the entire range of temperature over which a species can potentially occur, but, since other ecological factors also influence a species' niche, the realized thermal niche may not perfectly mirror the fundamental thermal niche. Understanding the mechanisms that underlie an organism's thermal physiology and contribute to the fundamental thermal niche is important if one's goal is to predict how populations will respond under scenarios of global change (Clark et al. 2013). Global climate change is expected to shift the distributions of fish species as temperatures are pushed toward their upper thermal limits (Caissie 2006). However, because fish are confined by the boundaries of their aquatic environments, their ability to disperse to habitats within their thermal niche may be limited, contributing to the loss of populations or species (Woodward et al. 2010; Alahuhta et al. 2019).

The biodiversity of stream fishes has declined over the last several decades in the Great Plains of North American due to anthropogenic impacts (Perkin et al. 2017). Intermittent streams, like those that occur throughout the Great Plains, exhibit cycles of drought and rewetting and are considered harsh environments (Datry et al. 2014). During periods of drought, fish are likely to experience thermally stressful conditions (Matthews 1988; Matthews and Marsh Matthews 2003; Dodds et al. 2004). Fish that live in these environments are adapted to harsh physicochemical conditions, but populations may fluctuate across years as a consequence of drying events, and short-lived fish species may be particularly sensitive to the frequency and severity of drying events (Propst et al. 2008; Jaeger et al. 2014; Hopper et al. 2019). The southern redbelly dace (*Chrosomus erythrogaster*) and the central stoneroller (*Campostoma anomalum*) are two ecologically similar species of small-bodied minnow (Cyprinidae) that occur throughout the Flint Hills region of the Great Plains. Both species occur in small intermittent headwater streams, but populations of dace

do not persist into downstream, intermediate reaches. Headwater streams in these systems are typically cooler, on average, compared to more downstream reaches (Troia and Gido 2014; Troia et al. 2016). Differences in the fundamental thermal niche of dace and stonerollers could be one potential explanation of the observed differences in occurrence along a stream-size gradient of temperature.

The overarching goals of my dissertation were to determine if physiological mechanisms related to the fundamental thermal niche of these two species differed in their response along ecologically relevant gradients of temperature, and to assess how inter-annual variation in environmental conditions (temperature and flow) affected populations of these species at a long-term sampling site. For the second Chapter of this dissertation, I assayed the response of critical thermal maximum, energy metabolism, swimming performance, and spontaneous activity in fish acclimated along a gradient of temperature. This study provides a mechanistic understanding of factors contributing directly to the fundamental thermal niche of these two species and emphasizes the importance of a mechanistic approach to understanding organismal thermal ecology. In Chapter 3, I expanded on the mechanistic approach to understanding contributing factors to an organism's thermal niche by testing the effect of acclimation to a diurnally variable thermal regime on the energy metabolism of dace and stoneroller. Circadian fluctuations in temperature are an often-overlook component of a species' thermal niche (Beauregard et al. 2013; Callaghan et al. 2016; Morash et al. 2018). This study builds on an emerging body of literature that suggests that diurnal variation in temperature can drive differences in metabolic rates compared to acclimation to constant thermal conditions and challenges the common practice of using constant thermal environments in laboratory studies of organismal thermal biology (Schulte et al. 2011; Corey et al. 2017; Guzzo et al. 2019).

Inter-annual variation in environmental conditions can drive variation in population vital rates (Scheurer et al. 2003; Dexter et al. 2014; Hedden and Gido 2019). Furthermore, age classes might differ in their environmental requirements, resulting in intraspecific differences in vital rates. For the fourth Chapter of this dissertation, I used long-term fish community and environmental data to assess how inter-annual variation in temperature and stream flow affected the average body size, abundance, and growth of the dace and stoneroller across years. This study highlights the value of leveraging long-term ecological datasets to infer changes in population

dynamics over spatial and temporal scales and the importance of considering the influence of multiple, often complimentary, environmental factors when assessing population dynamics.

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Chapter 2 - Temperature effects the on performance and physiology of two prairie stream minnows

Abstract

Earth's atmosphere has warmed by approximately 1°C over the past century and continues to warm at an increasing rate. Effects of atmospheric warming are already visible in most major ecosystems and are evident across all levels of biological organization. Linking functional responses of individuals to temperature is critical for predicting responses of populations and communities to global climate change. The southern redbelly dace *Chrosomus erythrogaster* and the central stoneroller *Campostoma anomalum* are two minnows (Cyprinidae) that commonly occur in the Flint Hills region of the United States but show different patterns of occurrence, with dace largely occupying headwater reaches and stonerollers persisting in both headwater and intermediate-sized streams. We tested for differences between species in critical thermal maximum, energy metabolism, sustained swimming, and activity over an ecologically relevant temperature gradient of acclimation temperatures. Typically, metrics increased with acclimation temperature for both species, although stoneroller activity decreased with temperature. We observed a significant interaction between species and temperature for critical thermal maxima, where stonerollers only had higher critical thermal maxima at the coldest temperature and at warm temperatures compared to the dace. We did not find evidence suggesting differences in the energy metabolism of dace and stonerollers. We detected interspecific differences in sustained swimming performance, with dace having higher swimming speed than stonerollers regardless of acclimation temperature. Finally, there was a significant interaction between temperature and species for activity; dace activity was higher at intermediate and warm temperatures compared to stonerollers. We observed subtle interspecific differences in how performance metrics responded to temperature that did not always align with observed patterns of distribution for these species.

Background

Temperature is a critical physical component of the environment that influences organisms across levels of biological organization (Addo-Bedaiko et al. 2000; Angilletta 2009; Culumber et al. 2012; Jensen et al. 2017). Over the past century the Earth's atmosphere has warmed by approximately 1°C and continues to warm at an increasing rate, affecting ecosystems at global,

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regional, and local scales (Walther et al. 2002; Angilletta 2009; Eliason et al. 2011; Pachauri et al. 2014). Effects of climate change are already visible in most ecosystems, leading to shifts in the phenology of organisms, species ranges, and community composition (Eaton and Scheller, 1996; Walther et al. 2002; Angilletta 2009; Barceló et al. 2016). Understanding the role of temperature in shaping the performance of organisms will help researchers predict the impacts of climate warming on species' distributions (Pörtner and Knust 2007; Primack et al. 2009; Killen 2014). A mechanistic understanding of the physiological processes that underly an organism's thermal biology will aid conservation efforts in the face of global change by bolstering the predictive ability of models of species distribution beyond the range of currently available environmental data (Teal et al. 2018).

The fundamental thermal niche of ectotherms, whose body temperatures are dependent on the environment, can be defined broadly as the range of body temperatures allowing for positive population growth (Magnuson et al. 1979; Gvoždík 2018). Physiological mechanisms – including preferred body temperatures, thermal tolerances, and energy metabolism – contribute to the fundamental thermal niche of an organism, as does the role of thermally-related behavior in influencing these mechanisms (Magnuson et al. 1979; Kearney and Porter 2004 & 2009; Gvoždík 2018). The fundamental thermal niche is important in determining the entire range of temperatures over which populations of an organism can persist and is often included in species distribution models (Helaouët and Beaugrand 2009). However, temperature is only one dimension of a species' ecological niche and other components of the niche can also play a role in determining the temperature range where a species occurs. A species' realized thermal niche may, therefore, represent a narrower range of temperatures after considering the influence of other biotic and abiotic factors (and their interactions with temperature) on the fundamental thermal niche (Gvoždík 2018). Understanding the underlying mechanism driving thermal physiology is an important consideration when examining species' distributions along temperature gradients, as well as their expected response to climate change (Grossman et al. 2010; Clark et al. 2013; Stoffels et al. 2016).

The distributions of fish species are expected to shift as climate change pushes water temperatures toward their upper thermal limits (Caissie 2006). Grassland streams exist in one of the world's most threatened biomes and understanding the thermal biology of grassland fishes will aid in predicting how populations of these species will respond to a changing climate (Dodds et al.

2004; Troia et al. 2016). In the Great Plains of North America, the biodiversity of stream fishes has declined over the last several decades, largely due to anthropogenic impacts (Perkin et al. 2017). These systems naturally experience severe fluctuations in environmental conditions due to cyclical patterns of drying and wetting, and fishes may experience thermal stress during periods of drought (Matthews 1988; Dodds et al. 2004). Thermal stress might be exacerbated by fragmentation that inhibits dispersal into habitats with cooler water temperatures (Matthews and Zimmerman 1990; Perkin et al. 2015). Under climate change scenarios, grassland streams are expected to be pushed towards more arid conditions and subjected to increases in the frequency and severity of droughts (Dodds et al. 2015). Physicochemical limitations associated with temperature are likely strong drivers of the distribution of fish species in prairie streams, and interspecific differences in thermal physiology or performance could facilitate replacement of species along a temperature gradient (Troia et al. 2016).

In prairie streams of the Flint Hills region in the central United States, cooler temperatures typically occur in spring-fed headwaters while downstream reaches are warmer (Troia and Gido 2014; Troia et al. 2016). The southern redbelly dace (*Chrosomus erythrogaster*) and the central stoneroller (*Campostoma anomalum*) are two species of minnow (Cyprinidae) that occur in this region. Both species function in similar trophic roles, feeding primarily on algae (Bertrand and Gido 2007). The critical thermal maximum (CTM) of the dace varies seasonally between approximately 17 to 32°C, while the stoneroller exhibits CTM as high as 38°C (Scott 1987; Mundahl 1990). Stoneroller and dace often co-occur in very small streams, but the occurrence of dace rapidly diminishes moving downstream, while stoneroller populations persist (Kansas Fishes Committee 2014). Given the general pattern of increasing summer maximum temperature in downstream reaches (Troia and Gido 2014), it is possible that dace are less tolerant of higher stream temperatures than stonerollers. Considering the apparent restriction of dace to headwater streams, the compounding effects of climate change and other anthropogenic disturbances may lead to the extirpation of dace populations from this region (Perkin et al. 2015; 2017). Understanding the mechanisms that contribute to the thermal niche of these species will aid in forecasting how they will respond to potential increases in temperature and thermal stress associated with drought.

Here we examined differences in physiological and performance responses over an ecologically relevant temperature gradient in the two species. Our general study aim was to test if

dace and stoneroller differ in their physiological response to temperature, and therefore, differ in their fundamental thermal niches. We hypothesized that because stonerollers occur in larger, warmer streams, the response of physiological and performance metrics would be higher for stonerollers than for dace at warmer temperatures. We measured a suite of physiological and performance traits that allowed us to compare thermal responses in these species. These traits include CTM (the maximum temperature at which motor function ceases), aerobic metabolic scope, maximum sustained swimming speed (U_{crit}; highest swimming velocity before fatigue), and activity (units of distance traveled per unit time).

Materials and Methods

Fish distribution sampling

We collected dace and stonerollers from 188 sites in the Neosho and Kansas River basins in the Flint Hills ecoregion of eastern Kansas as part of another stream fish community survey. Sites were selected using a random-stratified design with the goal of capturing gradients of stream hierarchy (drainage basin and stream order) and catchment land use (percentage of cultivated lands and density of road crossings and impoundments). The watershed area of sites sampled ranged between <10 km^2 and >200 km^2. We sampled stream fish communities in the summer of 2017 in several pools and riffles (mesohabitats) at each site. Fish were sampled using single pass backpack electroshocking followed by multiple seine hauls (3 m by 1.2 m, 3.2 mm mesh seine) in all habitat types (large woody debris, vegetation, root wads, etc.) within each mesohabitat. We categorized streams into bins based on watershed area and visualized the abundances of both dace and stoneroller over this stream-size gradient.

Characterizing streams temperatures

Submersible temperature loggers (Onset Corporation, Bourne, MA, USA) were placed in fixed locations at three stream sites – Kings Creek, McDowell Creek, and Deep Creek – near Manhattan, KS. These three streams are tributaries to the Kansas River basin. Kings Creek is a low-order prairie stream found on the Konza Prairie Biological Station (KPBS). McDowell Creek and Deep Creek are more intermediate-sized streams. Hourly temperature was recorded for each stream between 2013 and 2014 and were used to determine the mean daily temperature (°C ± standard deviation) and the coefficient of variation (%) of daily temperature for each stream.

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Experimental animals

For all experiments, dace and stonerollers were collected from the same ~30 m pool in Kings Creek near Manhattan, KS. Fishes were collected through a combination of seining (4.6 m by 1.8 m, 3.2 mm mesh seine) and DC-pulsed backpack electrofishing (Smith-Root model 20B) and then transported to an aquarium facility at Kansas State University in large, aerated coolers (see Table 2.1 for the number and size range of individuals collected). We maintained a photoperiod of 12 h light: 12 h dark and a room temperature of 20°C in the aquarium facility. Fish were housed in either 38 or 76 L aquaria at a density of no more than 1 fish/6 L of water. Collections for critical thermal maximum experiments occurred during the summer of 2015. For aerobic scope and critical swimming velocity experiments, fish were collected between March and August of 2017, and different fish were used in each experiment. Collections for activity experiments occurred in August of 2017.

Fish were held in the laboratory for two days at room temperature before being randomly assigned to an acclimation treatment appropriate to the experiment being conducted (treatments described in following sections). Either aquarium heaters or chillers were used to adjust treatment temperatures at a rate of 2°C/day until they reached the assigned treatment (Fangue et al. 2014). After reaching the treatment temperature, fish were acclimated for 14 days before beginning experiments – similar acclimation lengths have been used in other studies of fish thermal biology (McDonnell and Chapman 2016; Malekar et al. 2018; Zhou et al. 2019). Unless otherwise noted, fish were fed a daily ration of commercial flake food and dried bloodworms. Fish were fasted for 48 hr prior to their use in experiments to ensure a post-absorptive state (Allen-Ankins and Stoffels 2017).

Critical thermal maximum

We exposed dace and stonerollers to acute thermal stress to determine the critical thermal maximum (CTM) after acclimating them to different temperatures in the laboratory. For this experiment, we established five temperature acclimation treatments using separate 76-L tanks (7, 12, 17, 22, and 27°C) and randomly assigned 10 individuals of both species to each tank. These temperatures were chosen to represent possible mean ambient temperatures that occur across seasons in Kings Creek and other nearby grassland streams (see Fig. 2.2). Subsequent experiments following this CTM experiment used a slightly different thermal gradient (10, 15, 20, 25, and

30°C) to more effectively utilize available heaters, chillers, and aquarium space. Following the acclimation procedure described above, test fish were taken from their acclimation tanks and held in an aerated cooler that was kept at a constant temperature matching the acclimation temperature. Experimental trails were conducted on individual fish. Dechlorinated water was added to a 4 L testing chamber. A mesh cylinder was placed in the center of the chamber, and an air stone was placed inside the cylinder to oxygenate and mix the water, preventing a thermal gradient from developing. A mesh screen was placed over the chamber to prevent fish from escaping during a trial. Initial water temperature in the chamber was matched to the acclimation temperature being tested. The chamber was then placed on a heating plate, and one fish was added to the chamber. A HOBO logger (Onset Corporation, Bourne, MA, USA) configured to record temperature at 30 s intervals was immediately added to the chamber with the fish. The water in the chamber was heated at a rate of 0.3°C/min (Culumber et al. 2012; Chen et al. 2018). The temperature at which the fish experienced a loss of equilibrium was used to determine an individual's CTM (Culumber et al. 2012; Culumber et al. 2014). Once a fish reached its CTM, the fish was removed and measured in millimeters total length (mm TL) and weighed in to the nearest 0.01 grams (g) (Table 2.1).

Oxygen uptake and aerobic metabolic scope

We used intermittent flow respirometry to measure mass-adjusted rates of oxygen consumption (MO_2; mg O_2 g^{-1} h^{-1}) for both focal species along an acclimation gradient of temperature like the one described above (10, 15, 20, 25, and 30°C). We used as many as six dace and six stonerollers in each temperature treatment to measure standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic metabolic scope (AMS; total n = 52). We used a four-chamber intermittent flow respirometer (Loligo Systems, Tjele, Denmark) to measure metabolic rates following well-established methods (Crans et al. 2015; Svendsen et al. 2016). The volume of the respirometer chambers was 0.0987 L, and the relative mass of each fish to respirometer volume averaged approximately 0.01 g mL^{-1}.

A submersible pump continuously circulated oxygenated water (≥95% O_2 saturation) from a reservoir tank to the respirometer's water bath. At the beginning of each week, we calibrated the respirometer's optic oxygen probes following the manufacturer's protocol. Dipping probe oxygen mini sensors were inserted into a probe vessel above a thin planar oxygen mini sensor that was housed inside the respirometry chambers (Loligo Systems, Tjele, Denmark). Either submersible

heaters or a recirculating chiller was used to achieve a water temperature in the respirometer that matched the corresponding acclimation treatment. For each trial three fish were randomly drawn from an acclimation tank, and fish were randomly assigned to one of the four respirometry chambers. The remaining chamber was kept empty to serve as a control to measure background respiration during the trials (Jones et al. 2007; Svendsen et al. 2014; Rosewarne et al. 2016). Background respiration (mg O_2 h^{-1}) was calculated following equation (2) from Clark et al. (2013):

$$Mo_2 = \frac{\left[(V_t - V_f) \times \Delta C_{wO_2}\right]}{(\Delta t \times M_f)}$$

where V_t is the respirometer volume, V_f is the fish volume and is assumed to be the same as fish mass (M_f), ΔC_{wO2} is the change in oxygen concentration in the respirometer water, and Δt is the change in time during which the change in oxygen concentration is measured. Since the respirometer chamber used to measure background respiration is empty of fish, fish mass and fish volume are not included in the calculation (Rodgers et al. 2016). In our experiments, background respiration remained near zero (and <0.001% of fish respiration) across the 18h experimental period.

Prior to introduction to the respirometry chamber, fish were measured in mm TL and weighed to the nearest 0.01 g (Table 2.1). The respirometer was programmed to operate with a 240 s flush phase (when oxygenated water is flushed into the chambers), a 60 s wait phase, and a 240 s measure phase (modified from Stoffels 2015). The respirometry phase cycles were controlled using AutoResp^{TM} version 2 automated intermittent respirometry software (Loligo Systems, Tjele, Denmark). Fish were introduced to the respirometer chambers at 1400 h, and oxygen consumption rates were measured over the course of 18 h to allow fish to reach a constant resting state. The rate of oxygen consumption (MO_2) was calculated from the slope of the regression of oxygen content (kPa) over time (h). We excluded the first 2 hours of data to eliminate confounding effects of handling stress and used the average of the 10 lowest MO_2 values collected over the remaining 16 h period to determine an individual's SMR; MO_2 values with a r^2 value < 0.9 were excluded.

Following SMR measurement, we employed a chase protocol to elicit MMR in individual fish (Brennan et al. 2016; Rosewarne et al. 2016). One fish was moved from the respirometry chamber to a circular arena containing aerated water with temperature matching the treatment temperature being tested. Using the handle of an aquarium dip net, the fish was chased in the arena until the fish exhibited burst-glide swimming and was considered exhausted when it would no

longer respond to physical stimuli (in all trials, minimum chase time was six minutes). Following the chase protocol, fish were immediately reintroduced to the respirometer for the quantification of MMR (Rosewarne et al. 2016). We used the highest value of three cycles of the measure phase (240 s) to estimate MMR. This procedure was then repeated for the fish remaining in the respirometer, and all fish were tested between 0800-0900 h.

Critical swimming velocity

We used a 5-L swim tunnel respirometer (Loligo Systems, Tjele, Denmark) to carry out swimming performance trials (Svendsen et al. 2015). We included eight dace and eight stonerollers in each of five temperature treatments (10, 15, 20, 25, and 30°C; total n = 80). We used either submersible heaters or ice packs to maintain water temperature in the swim tunnel within 0.2°C of the temperature treatment being tested. Trials of critical swimming velocity (U_{crit}) were conducted using a velocity increment based on the total length (TL) of the fish (Brett 1964; Morozov et al. 2018). Before being introduced to the swim chamber, individual fish were measured in mm TL and weighed to the nearest 0.01 g (Table 2.1). To discourage the fish from attempting to rest against the back screen of the swim chamber, we placed a partitioning shade over the front half of the chamber and shined light at the back of the chamber. Following methods for other fish species, fish were first acclimated to the chamber with no flow velocity for 30 min (Lehman et al. 2017), and then for 10 min at a water velocity of 1 TL s^{-1} before beginning the experiment. Swimming trials began at a water velocity of 2 TL s^{-1}, with velocity being increased by 1 increment (1 TL) every 5 min and ending once the fish was fatigued (Nelson et al. 2003). Fatigue was determined as the velocity at which the fish became pinned to the back of the swim tunnel and would not return to swimming after visual or acoustic stimuli. The end of a dip net was used to prod the back of the swim chamber to stimulate the fish. U_{crit} was calculated in cm s^{-1} as:

$$Ucrit = Ui + Uii \left(\frac{Ti}{Tii}\right)$$

where U_i is the highest velocity maintained for an entire 5 min interval, U_{ii} is the velocity increment, T_i is the time elapsed at fatigue velocity, and T_{ii} is the time between velocity increments (Brett, 1964; Sfakianakus et al. 2011). We calculated relative U_{crit} (RU_{crit}) by dividing U_{crit} by body

size (TL) to compare the swimming velocity corrected for body size (Brett 1964; Gregory and Wood 1998).

Activity

For activity trials, we used six dace and six stonerollers for each temperature treatment (10, 15, 20, 25, and 30°C). Individual fish were placed into 27.5 by 17.5 cm plastic aquaria (hereafter, "arenas") housed in a recirculating water bath after they were measured in mm TL and weighed to the nearest 0.01 g. (Table 2.1). To prevent fish from seeing other individuals during the trail, the sides of the arenas where painted black. A recirculating chiller or submersible heater was used to maintain the water temperature in the water bath appropriate for the temperature treatment being tested. Each arena was kept aerated while housing fish. Arenas where placed over a 2 cm × 2 cm grid on a white background. Fish were habituated to the arenas for seven days and fed a ration of frozen bloodworms each day during this period. Fish were fasted for 48 hr before we stationed a GoPro® camera above each individual arena, capturing the entire arena in the field of view. The aerator was removed from the arena during video recording to prevent agitation of the water's surface. Fish were recorded continuously for 15 min (Gautrais et al. 2009; Troia et al. 2016). We discarded the first 5 min of video to account for fish potentially being disturbed when the camera was turned on. Activity was quantified from the subsequent 10 min of video by randomly selecting a subsample of ten non-overlapping 30-s intervals during the filmed trail and calculating the average number of 2 cm × 2 cm grids the fish traversed per unit time. All 12 fish belonging to a single acclimation temperature were filmed on the same day, beginning at 0900 h and finishing within 2 h. During filming trials, we recorded two fish concurrently using different cameras. Filming for all five acclimation treatments was completed in five days.

Statistical analyses

We used two-way analysis of covariance (ANCOVA) to test for differences in CTM. We included acclimation temperature (treatment) and species as fixed factors, and body size (mm TL) as a covariate (Angilletta 2006; Allen-Ankins and Stoffels 2017). Post-hoc independent samples *t*-tests with a Bonferroni corrected significance threshold of $\alpha = 0.01$ were conducted to compare the CTM of dace and stonerollers in each temperature treatment. We calculated AMS for an individual as the difference between its MMR and SMR. Data for SMR, MMR, and AMS were analyzed using two-way analysis of variance (ANOVA) and included acclimation temperature (treatment)

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and species as fixed factors. Metabolism data were ln-transformed prior to analysis to meet assumptions of normality. Since we collected and housed fish in the laboratory over different time blocks in some experiments, we first used a linear mixed-effects model and included sampling block as a random factor to account for any confounding effects. All species and acclimation treatments were represented in each sampling block. However, we found no significant effect of sampling block and were able to eliminate this parameter. We did not detect a significant temperature × species interaction for SMR ($F_{4,42} = 1.679$, $P < 0.173$), MMR ($F_{4,42} = 1.135$, $P < 0.353$), or AMS ($F_{4,42} = 1.523$, $P < 0.213$), and subsequently removed this interaction term. Tukey post-hoc tests were used to determine differences between temperature treatments. Data for RU_{crit} was analyzed using two-way ANOVA as described above and was ln-transformed prior to analysis to meet assumptions of normality. We did not detect a significant temperature × species interaction for RU_{crit} ($F_{4,70} = 0.349$, $P < 0.844$), and subsequently removed this interaction term. Post-hoc independent samples t-tests with a Bonferroni corrected significance threshold of $\alpha = 0.01$ were conducted to compare the RU_{crit} of dace and stonerollers in each temperature treatment. Tukey post-hoc tests were used to determine differences between temperature treatments. We used two-way ANCOVA to test for differences in activity. We included acclimation temperature (treatment) and species as fixed factors, and body size (mm TL) as a covariate. Data for activity was square-root-transformed prior to analysis to meet assumptions of normality. Body size did not have a significant effect on activity ($F_{1,46} = 2.432$, $P = 0.126$), and was removed from the analysis.

Results

Fish abundances along a stream-size gradient

Both dace and stonerollers were present and abundant in small streams (watershed areas < 10 to 50 km^2). Stoneroller populations remained abundant as stream size increased toward > 200 km^2, while populations of dace did not persist or were scarcely abundant (Fig. 2.1).

Stream temperatures

We calculated the mean daily temperatures (± 1 SD) and the coefficient of variation (%) for daily temperature for three streams in the Kansas River basin. Kings Creek was approximately 5°C

cooler, on average, during summer months compared to the more intermediate-sized McDowell and Deep Creeks (Fig. 2.2A). Additionally, temperatures in Kings Creek were typically more variable (Fig. 2.2B). Dace and stoneroller co-occur in Kings Creek. Whereas stoneroller occur in both McDowell Creek and Deep Creek, dace are absent or very rare in these streams.

Critical thermal maximum

There was a significant temperature × species interaction in the CTM of dace and stonerollers acclimated along a temperature gradient, after controlling for the effect of body size (Fig. 2.3 $F_{4,83}$ = 6.725, $P < 0.001$). The covariate, body size, was significantly related to CTM ($F_{1,83} = 6.973$, P = 0.01) and regardless of species, larger fish had typically had slightly higher CTM (0.5 to 1°C) than did smaller fish. Not surprisingly, acclimation temperature had the strongest effect on CTM for both species, with dace CTM increasing by approximately 7°C and stoneroller CTM increasing by approximately 6°C between the coldest and warmest acclimation temperatures. Post-hoc independent t-tests indicated that interspecific differences were less pronounced within each temperature treatment. While CTM was similar at intermediate temperatures (12°C, 17°C), stonerollers CTM was approximately 0.5 to 1.5°C higher than dace at low (7°C) and high (22°C, 27°C) acclimation temperatures (Fig. 2.3).

Oxygen uptake and aerobic metabolic scope

SMR, MMR, and AMS did not differ between dace and stonerollers (SMR: $F_{1,46} = 0.87$, $P = 0.356$; MMR: $F_{1,46} = 1.225$, $P = 0.274$; AMS: $F_{1,46} = 3.67$, $P = 0.062$), but all metrics were significantly affected by acclimation temperature (SMR: $F_{4,46} = 7.465$, $P < 0.001$; MMR: $F_{4,46} = 15.342$, $P < 0.001$; AMS: $F_{4,46} = 10.467$, $P < 0.001$). Tukey post-hoc tests revealed that mean values for SMR were significantly higher at 30°C than at 10, 15, and 20°C (Fig. 2.4A), while mean values for MMR were significantly higher at 25 and 30°C than other acclimation temperatures (Fig. 2.4B). Mean values for AMS were significantly higher at 25°C compared to the 10, 15, and 20°C treatments, while AMS at 30°C was significantly higher than the 10 and 15°C treatments (Fig. 2.4C).

Critical swimming velocity

We detected significant effects of temperature ($F_{1,74} = 21.658$, $P < 0.001$) and species ($F_{1,74} = 4.434$, $P = 0.039$) on RU_{crit}. Tukey post-hoc tests revealed that mean values for RU_{crit} were significantly lower at 10°C than other acclimation temperatures (Fig. 2.5A). Dace typically had higher mean RU_{crit} than stoneroller, regardless of acclimation temperature treatment (Fig. 2.5A).

Activity

Two-way ANOVA indicated a significant temperature × species interaction for activity ($F_{4,47} = 3.991$, $P = 0.007$). Post-hoc independent *t*-tests indicated that interspecific differences were less pronounced within each temperature treatment. Dace activity increased toward intermediate temperatures before declining at high temperatures, while stoneroller activity was high at the lowest temperature and declined in warmer treatments (Fig. 2.5B).

Discussion

While stonerollers persist and are abundant across a larger gradient of stream sizes throughout the Flint Hills of Kansas, dace appear to be more restricted to smaller sized streams (Fig. 2.1). Local stream temperature data indicates that smaller headwater streams in this region are typically cooler on average, particularly during the summer (Fig. 2.2) – and studies modelling stream temperature in the Flint Hills support this (Troia and Gido 2014; Troia et al. 2016). We performed a comparative analysis of a series of traits associated with the thermal physiology of stonerollers and dace and found some subtle differences, but the direction and magnitude of these interspecific differences did not always align with our prediction that the measured responses of stonerollers for these traits would be higher than dace at warmer water temperatures. For example, stonerollers exhibited significantly higher CTMs than dace when acclimated to a warm (22°C) temperature, but there was no difference between species at the warmest (27°C) temperature – although stonerollers trended toward higher CTM than dace at this temperature (see Fig. 2.3) This provides some evidence that stonerollers might buffer thermal stress better than dace, and if our stream temperature data is indeed representative (see Fig. 2.2) then stonerollers appear to have higher CTMs than dace under summer temperature conditions that are more typical of intermediate-sized streams. The range of CTM values we observed across acclimation treatments align well with what

is already reported for both species under natural conditions (Scott 1987; Mundahl 1990). However, the lack of difference in CTMs between species at some acclimation temperatures suggests thermal tolerance alone is likely not a strong predictor of species distribution.

The greatest between-species contrast was the interaction between species and temperature for activity, where dace activity increased with temperature while the activity of stonerollers declined – a pattern that was counter to our predictions. The interaction in activity we observed between temperature and species indicates that temperature might affect the behavior of these species differently, or that spontaneous activity may trade off with other physiological functions not measured herein. Alternatively, the lower spontaneous activity of the stoneroller could be the result of the benthic-dwelling stoneroller maintaining a less active lifestyle compared to the dace, which typically inhabits the water column. Additionally, dace typically exhibited higher U_{crit} values compared to stonerollers, regardless of acclimation temperature, indicating that they are stronger swimmers overall. Hydrology can be an important driver of interspecific differences in swimming performance along a stream-size gradient in other systems. Higher sustained swimming performance has been observed in headwater salmonids in Japanese streams (Morita et al. 2016) and in Australian blackfishes (Allen-Ankins and Stoffels 2017) when compared to a downstream species. Since both temperature and hydrology vary along gradients of stream size, this emphasizes the importance of considering the influence of both variables on species distributions due to the covarying nature of these habitat conditions along the river continuum.

Why do dace not occur in larger streams where stonerollers persist? Aerobic metabolism and swimming performances of dace and stonerollers did not differ at high temperatures indicating that these species are similar in their fundamental thermal niche along these axes, yet dace are typically absent from streams that regularly experience these high temperatures. While stonerollers could have a higher capacity to buffer thermal stress than dace in intermediate streams under summer-like thermal conditions, other ecological factors may also contribute to differences in the observed distributions of dace and stonerollers. For example, in grassland streams, more predatory species of fish occur downstream compared to headwaters, and the interaction between predation and competition may contribute to the exclusion of dace from larger, downstream reaches (Schlosser 1982; Chase et al. 2002; Chesson and Kuang 2008). Furthermore, relatively little is known regarding the role of temperature in mediating the outcomes of ecological interactions like competition and predation (Öhlund et al. 2015; Rogers et al. 2018). While an organism's

fundamental thermal niche is an important driver of distributional patterns, the outcome of ecological interactions (both independent of and influenced by temperature) may drive distributions through shaping of the realized thermal niche (Öhlund et al. 2015; Allen-Ankins and Stoffels 2017).

Emerging evidence suggests that subtle, interacting characteristics among closely related and ecologically similar species may drive differences in ecological success (Dupoué et al. 2017; Porreca et al. 2017). We found some evidence that dace and stonerollers differ in components of their thermal biology but that the fundamental thermal niche of both species is very similar, at least for the metrics we examined. Low sample sizes in some of our experiments could have consequences on these results. High individual variability in physiological metrics may have contributed to the lack of differences we observed between species (e.g. Fig. 2.4), and a larger sample of fish tested may have better teased out differences if any do exist. Other ecological factors, such as hydrology, habitat heterogeneity, and biotic interactions could be more important drivers of the distributions of these species than temperature or may interact with temperature, and ultimately affect the realized thermal niche of these species. We addressed several mechanisms relating to the thermal niche of these species, which could potentially influence the distributional patterns we observe. However, other aspects of the life history of these organisms, such as growth or reproduction, could also be important components of their thermal niches, and may differ between species. Our experiments also were conducted using adult fish and the influence of temperature on the early life history of these species is not well understood yet could be an important contributor to differences in growth and survival. Furthermore, the results of ecological interactions between these species could change as a function of temperature. Future research should continue to integrate the interactive effects of temperature and other environmental conditions on organismal, population, and community level processes.

Finally, many laboratory studies are limited in that they use chronic exposures to constant temperatures to assess responses in physiological traits, which may not reflect how organisms respond to temperature change and variation in natural environments (Schulte et al. 2011). Recent work on thermal tolerance in zebrafish (Schaefer and Ryan 2006) and Atlantic salmon (Corey et al. 2017) suggests that exposure to variable thermal regimes increases the thermal limits of these species compared to exposure to chronic temperatures. In grassland streams, intermediate reaches are typically warmer and more stable compared to smaller headwater reaches (see Fig. 2.2).

Incorporating thermal variability into tests of organismal responses to temperature might help elucidate how organisms respond to temperature in variable environments (Schulte et al. 2011; Drake et al. 2017). The increased frequency and severity of drought in prairie streams will likely exacerbated stream drying and potentially eliminate spring refugia (Dodds et al. 2004). For the dace, a species typical of small headwaters but absent downstream, the loss of these important thermal refugia may lead to local extirpation in some systems and contribute to conservation challenges. Understanding how organisms like dace, that may rely on conditions specific to these headwaters, respond along environmental gradients will be an important step in forecasting their response to anthropogenic change.

Figures
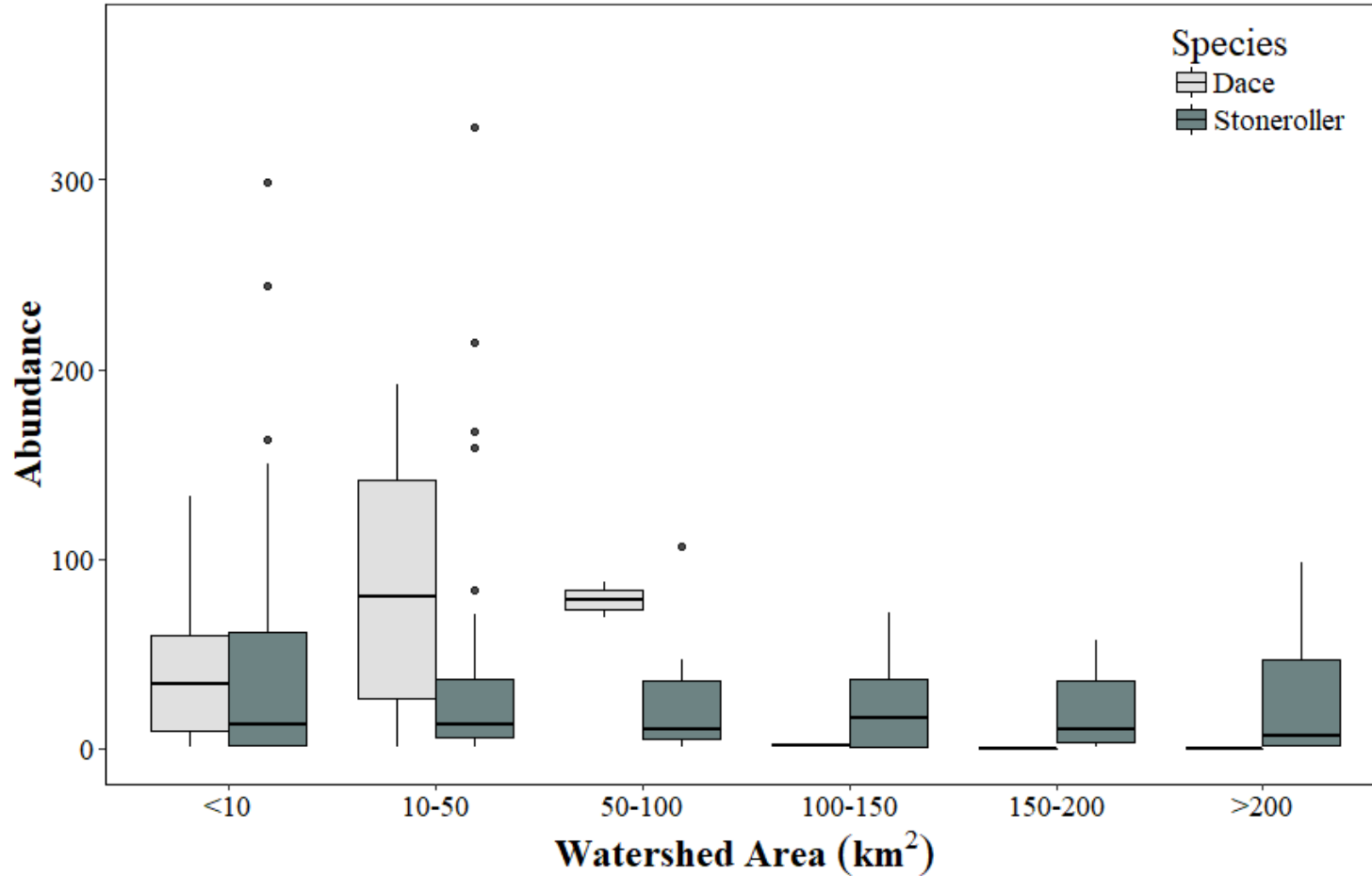
Figure 2.1: Boxplots representing abundances (count of individuals) of southern redbelly dace (light gray) and central stoneroller (dark gray) as a function of watershed area (km^2). Boxes represent the median and the 1st and 3rd quartile, and filled circles represent outliers. Dace populations occur more frequently in smaller watersheds, and stonerollers persist into larger watersheds.
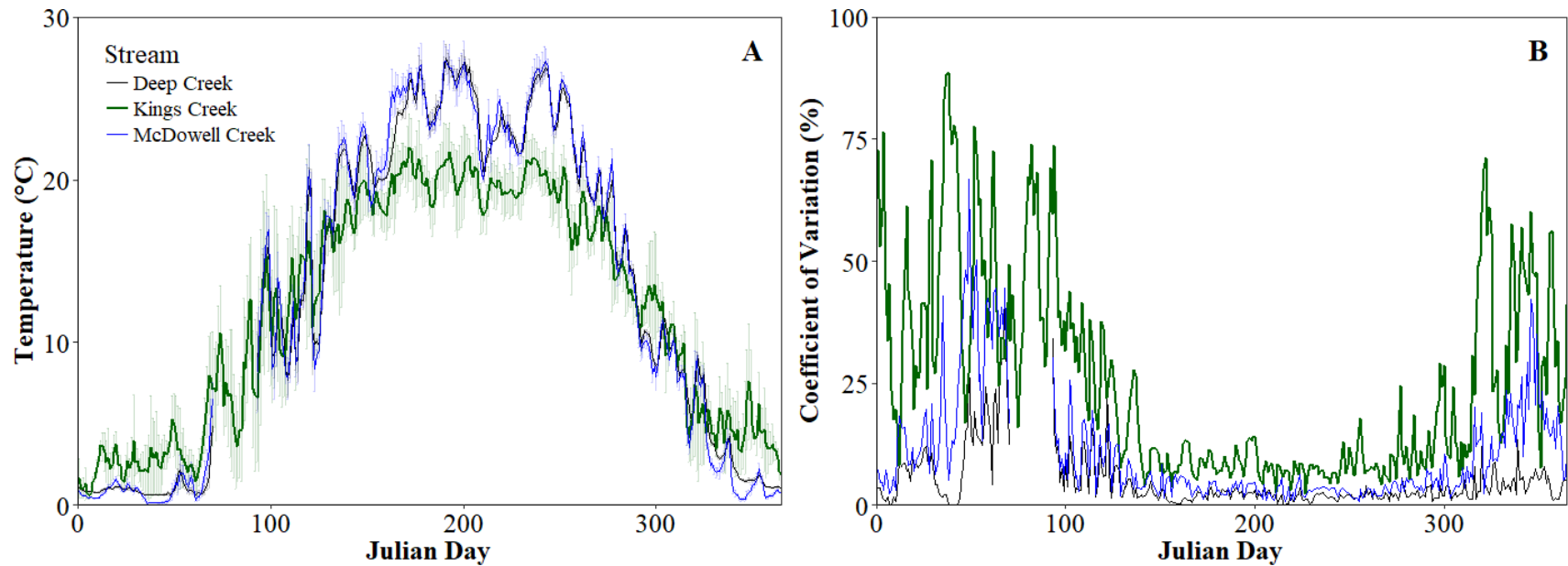
Figure 2.2: Mean (± 1 SD) daily temperature (°C; A) and coefficient of variation (%; B) plotted for Julian day for three prairie streams in Kansas River drainage of the Flint Hills region (USA). Kings Creek (green) is a headwater reach that contains dace and stoneroller. Kings Creek feeds McDowell Creek (blue), which contains stonerollers but not dace. Deep Creek (black) is in the same river basin, is similar in size to McDowell Creek, and contains stoneroller but not dace. Temperature data are from 2013/14.
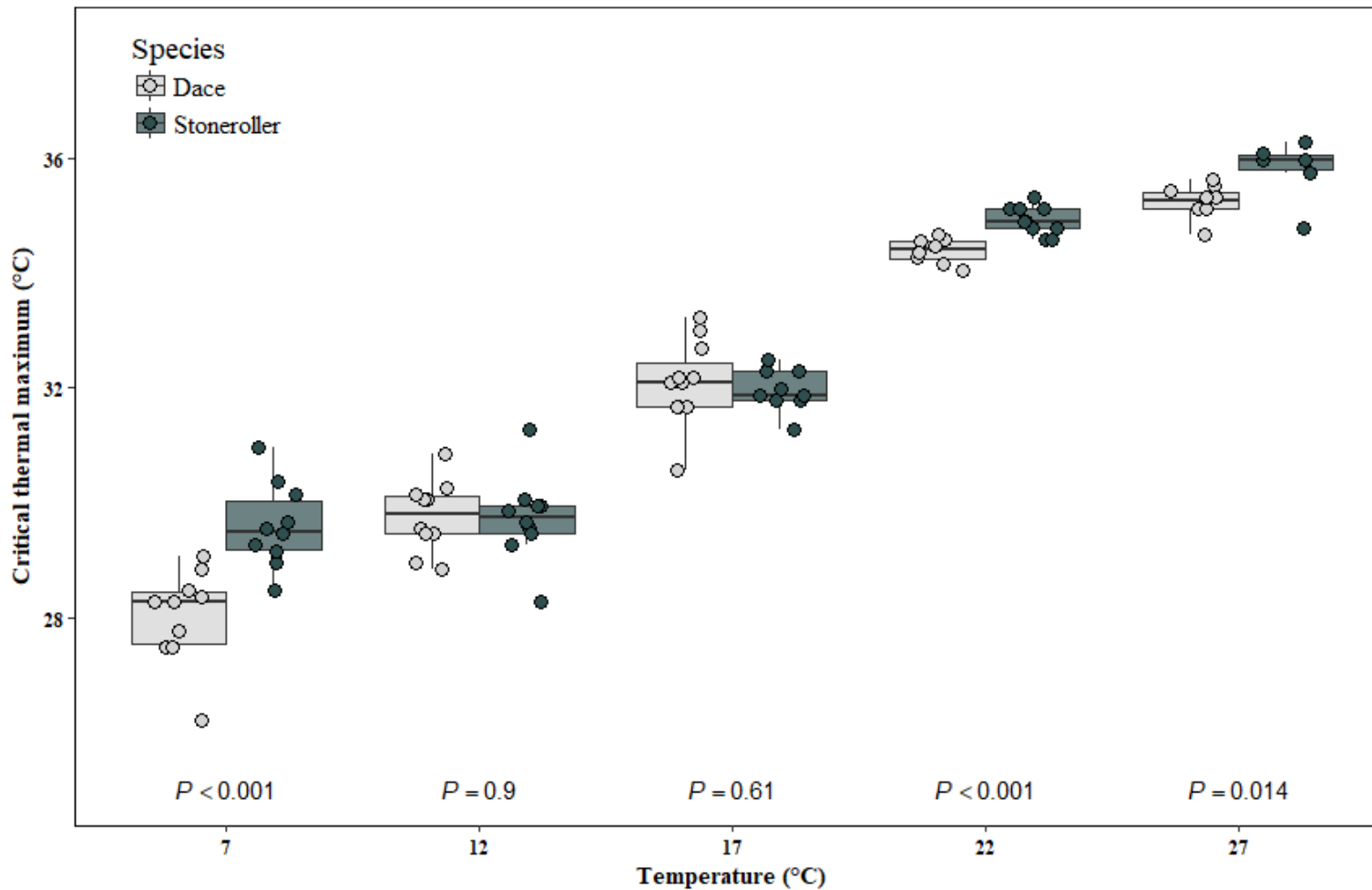
Figure 2.3: Critical thermal maximum (CTMs; °C) of southern redbelly dace (light gray) and central stoneroller (dark gray) acclimated to constant temperatures (°C) along a gradient in the laboratory. Boxes represent the median and the 1st and 3rd quartile. *P*-values represent between-species post-hoc comparisons (independent samples t-tests) and are considered significant at $P \leq 0.01$.
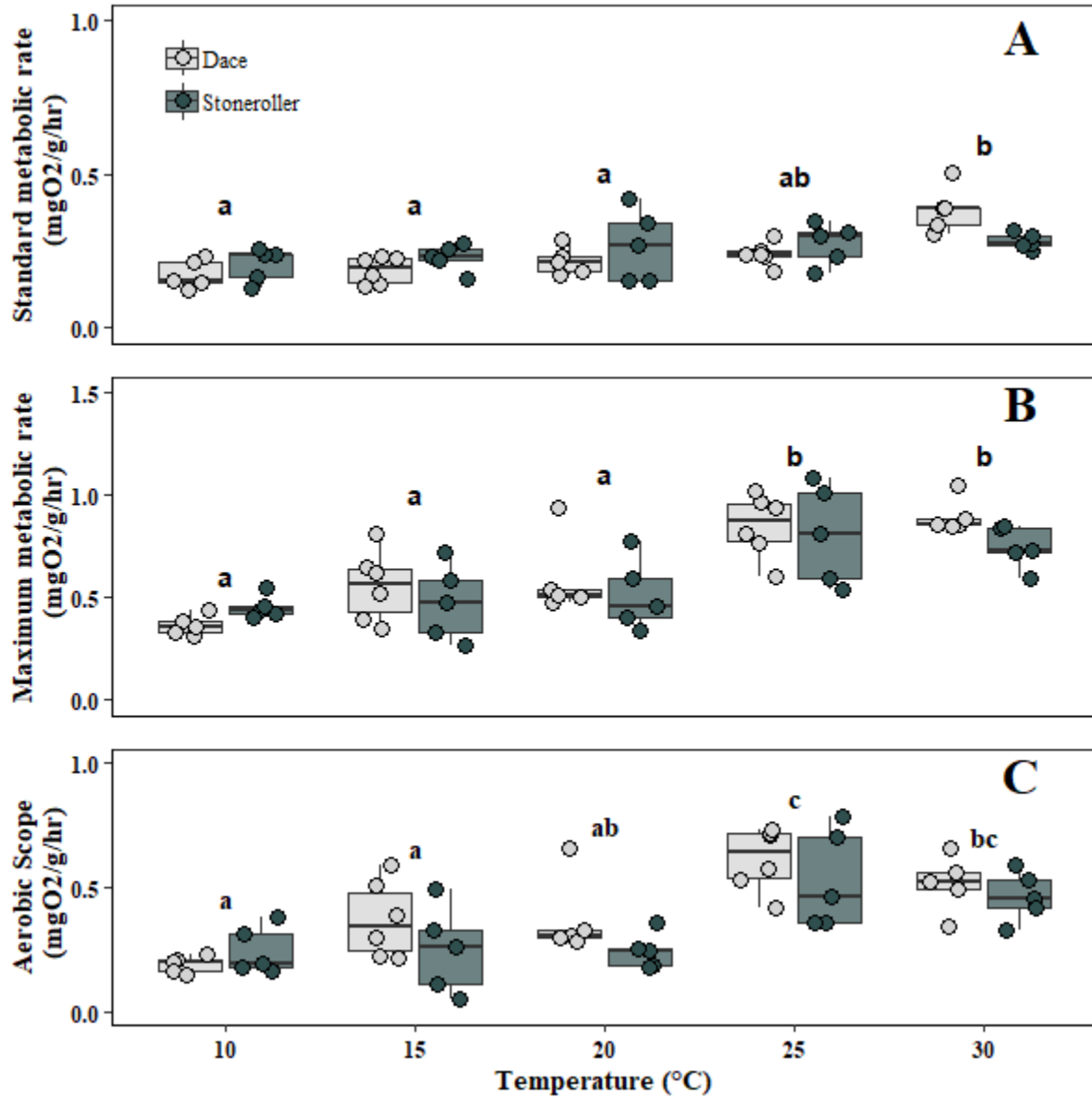
Figure 2.4: Mass-adjusted (**A**) standard metabolic rate (SMR) (mg O_2 g^{-1} h^{-1}), (**B**) maximum metabolic rate (MMR), and (**C**) aerobic metabolic scope (AMS) of southern redbelly dace (light gray) and central stoneroller (dark gray) acclimated to constant temperature (°C) along a gradient in the laboratory. Filled circles represent individual data points. Boxes represent the median and the 1st and 3rd quartile. Lowercase letters (a-c) represent significant differences between temperature treatments.
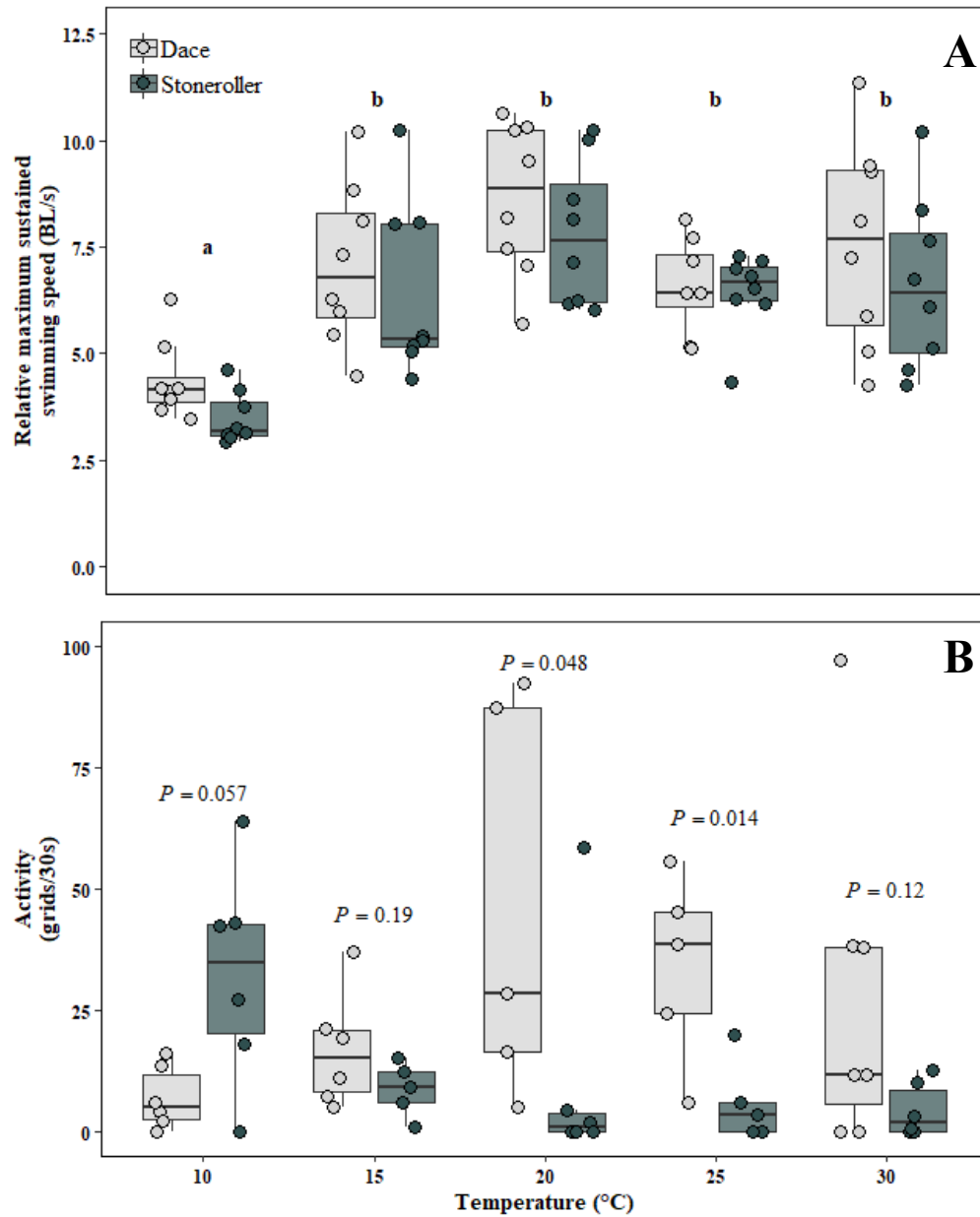
Figure 2.5: (**A**) Relative maximum sustained swimming speed (RU_{crit}; BL/s) and (**B**) activity (the average number of 2 cm x 2 cm grids crossed in 30s; grids/30s), of southern redbelly dace (light gray) and central stoneroller (dark gray) acclimated to constant temperature (°C) along a gradient in the laboratory. Filled circles represent individual data points. Boxes represent the median and the 1st and 3rd quartile. *P*-values represent between-species post-hoc comparisons (independent samples t-tests) and are considered significant at $P \leq 0.01$.

Tables

Table 2.1: Mean (± 1 SD) total length (mm), mean weight (g), and sample size (N = number of individuals) of dace and stonerollers in each temperature treatment used in critical thermal maximum, metabolism, critical swimming velocity, and activity experiments. Rows highlighted in gray represent the mean values across all treatments in the experiment for length and weight, and the total sample size for each species in the experiment.

Experiment	Species					
	Dace			Stoneroller		
	Total length (mm)	Weight (g)	N	Total length (mm)	Weight (g)	N
Critical thermal maximum						
7°C	35 (± 2.1)	0.32 (± 0.06)	10	53 (± 6.2)	1.23 (± 0.43)	10
12°C	36 (± 3.0)	0.38 (± 0.10)	10	53 (± 5.2)	1.16 (± 0.36)	10
17°C	37 (± 6.1)	0.42 (± 0.25)	11	53 (± 6.9)	1.13 (± 0.39)	9
22°C	35 (± 2.3)	0.34 (± 0.05)	8	52 (± 5.1)	1.06 (± 0.30)	10
27°C	36 (± 3.3)	0.36 (± 0.10)	10	62 (± 6.7)	1.66 (± 0.32)	6
Overall	36 (± 3.7)	0.37 (± 0.14)	49	54 (± 6.6)	1.22 (± 0.40)	45
Metabolism						
10°C	52 (± 5.6)	1.28 (± 0.43)	5	54 (± 5.2)	1.33 (± 0.26)	5
15°C	52 (± 6.3)	1.29 (± 0.52)	6	51 (± 5.0)	1.19 (± 0.37)	5
20°C	47 (± 2.6)	0.88 (± 0.14)	5	48 (± 7.1)	1.03 (± 0.40)	5
25°C	53 (± 4.6)	1.33 (± 0.32)	6	52 (± 9.5)	1.31 (± 0.64)	5
30°C	52 (± 4.7)	1.31 (± 0.33)	5	48 (± 5.1)	0.83 (± 0.08)	5
Overall	51 (± 5.0)	1.22 (± 0.38)	27	51 (± 6.6)	1.14 (± 0.41)	25
Critical swimming velocity						
10°C	54 (± 7.7)	1.42 (± 0.61)	8	55 (± 8.6)	1.51 (± 0.55)	8
15°C	55 (± 6.0)	1.50 (± 0.47)	8	58 (± 10.0)	1.84 (± 1.02)	8
20°C	53 (± 9.5)	1.27 (± 0.66)	8	59 (± 7.9)	1.91 (± 0.94)	8
25°C	55 (± 6.4)	1.50 (± 0.63)	8	51 (± 9.5)	1.41 (± 0.60)	8
30°C	55 (± 9.1)	1.39 (± 0.65)	8	55 (± 6.4)	1.42 (± 0.43)	8
Overall	54 (± 7.5)	1.41 (± 0.58)	40	56 (± 8.6)	1.62 (± 0.74)	40
Activity						
10°C	52 (± 4.2)	1.03 (± 0.24)	6	62 (± 7.9)	1.89 (± 0.70)	6
15°C	54 (± 4.3)	1.29 (± 0.46)	6	57 (± 6.9)	1.43 (± 0.34)	5
20°C	53 (± 8.8)	1.16 (± 0.52)	5	53 (± 2.3)	1.22 (± 0.15)	6
25°C	50 (± 9.0)	0.95 (± 0.61)	5	52 (± 3.1)	1.09 (± 0.18)	5
30°C	54 (± 8.6)	1.11 (± 0.55)	7	58 (± 5.3)	1.37 (± 0.35)	6
Overall	53 (± 6.9)	1.11 (± 0.47)	29	57 (± 6.2)	1.41 (± 0.47)	28

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Chapter 3 - Metabolic physiology of minnows exposed to stable and variable thermal environments

Abstract

Laboratory studies of organismal thermal biology often rely on acclimation to constant temperatures to assess physiological responses, yet organisms in their natural environment are exposed temperature variation across spatial and temporal scales. Incorporating thermal variability into tests of organismal responses to temperature might help elucidate how organisms respond to temperature in variable environments. Additionally, metabolic responses under variable thermal conditions may be an important and often overlooked component influencing an organism's fundamental thermal niche. The southern redbelly dace (*Chrosomus erythrogaster*) and the central stoneroller (*Campostoma anomalum*) are two species of functionally similar minnows that display differences in their realized thermal niches along a stream-size gradient of temperature. To test how exposure to a variable thermal regime affects metabolic physiology, we measured the metabolic rates of dace and stonerollers acclimated to either constant (20°C) or diurnally variable (mean = 20°C; range = 17 - 23°C) temperature conditions. We then used intermittent flow respirometry to compare standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS) of dace and stonerollers at 15, 20, and 25°C. Using a model selection approach, we compared responses between dace and stonerollers to determine if temperature variation contributes to differences in the realized thermal niches of these species. We found evidence that acclimation to a diurnally variable thermal environment influences the energy metabolism of these species compared to acclimation to constant thermal conditions, with some evidence for interspecific differences in the response to acclimation treatment. The SMR of stonerollers acclimated to variable conditions was higher at a test temperature of 25°C compared to constant conditions. Dace had lower SMR when acclimated to variable conditions at test temperatures of 15 and 20°C, but higher SMR at a test temperature of 25°C compared to constant conditions. Stoneroller had higher overall MMR and AS compared to dace. Fish acclimated to diurnally variable thermal conditions maintained higher MMR and AS compared to those held at constant conditions. MMR increased with the test temperature of the respirometer, while AS increased between 20 and 25°C, but was similar between 15 and 20°C. Dace that were acclimated to diurnally variable thermal conditions maintained higher MMR and AS compared to dace

acclimated to constant conditions, while the MMR and AS of stonerollers was similar regardless of acclimation treatment. Our results suggest that acclimation to a diurnally variable thermal environment influences the metabolic rates of these species and is an important contributor to their thermal ecology.

Background

Environmental temperature has the capacity to influence organisms across levels of biological organization, which can impact fundamental and realized thermal niches and ultimately observed patterns of distribution and abundance of species (Culumber and Tobler 2018; Gvoždík 2018). The body temperature of ectothermic organisms largely reflects the temperatures present in their environment, and the range of body temperatures at which positive population growth is possible delimits the organism's fundamental thermal niche (Spotila et al. 1992; Gvoždík 2018). Key physiological processes related to an organism's capacity to tolerate thermal stress, their preferred or optimal body temperature, and the effect of temperature on metabolic processes define the fundamental thermal niche, as do thermoregulatory behaviors (Magnuson et al. 1979; Kearney and Porter 2004; 2009; Gvoždík 2018). An organism's realized thermal niche, in contrast, describes the range of environmental temperatures where a species is actually observed to occur after accounting for the influence of other ecological factors (Gvoždík 2018), and may be narrower than, mirror, or exceed the fundamental thermal niche. Variation in environmental temperatures and in solar radiation drive thermal clines along both altitudinal and latitudinal gradients, and how this variation affects the thermal niche of organisms at these spatial scales has received a great deal of focus (Janzen 1967; Ghalambor et al. 2006; Culumber and Tobler 2018). While temperature's role in shaping the thermal niche along these large spatial gradients is well understood, we know comparatively little about how variability in the thermal environment affects the physiology and the thermal niche of organisms at smaller spatial and temporal scales. Current models of climate change predict that, along with increases to average global temperature, both temperature variability and the frequency and severity of extreme temperature events will increase (Xenopoulos et al. 2005). Thus, understanding how organisms respond to variable thermal conditions will be crucial to responding to emerging conservation challenges (Morrongiello et al. 2011; Comte et al. 2013; Corey et al. 2017).

Metabolic responses under variable thermal conditions may be an important – and often overlooked – factor influencing an organism's thermal niche. Scientists studying the thermal physiology of organisms typically conduct studies under conditions where organisms are acclimated to constant temperatures (Morash et al. 2018). These constant conditions typically reflect average conditions and allow researchers to simplify experimental designs to compare thermal response under mean conditions representative of different thermal habitats or at different times of the year. These constant conditions, however, may not accurately reflect organismal responses under ecologically realistic conditions, as organisms experience temperature variation under natural conditions (Callaghan et al. 2016; Morash et al. 2018). In streams and rivers, thermal regimes are influenced by atmospheric conditions, topography, stream discharge, and interaction with groundwater; these factors influence stream temperatures along a spatial gradient with larger streams typically being warmer, on average (Caissie 2006). Water temperatures in streams also vary temporally on both diurnal and annual cycles, and the magnitude of this variability is driven by factors like latitude, stream order, and the type of stream (Mosley 1983; Caissie 2006). Organisms inhabiting streams and rivers, like fish, must contend with the influence of small-scale spatial and temporal variation in temperature on their body temperatures and, in turn, their physiological processes. Current empirical work in zebrafish and Atlantic salmon indicates that thermal variation can increase critical thermal maxima compared to constant acclimation conditions (Schaefer and Ryan 2006; Corey et al. 2017), and that other metrics, such as growth rate and metabolism, can also be influenced by exposure to variable thermal regimes (Flodmark et al. 2004; Meeuwig et al. 2004; Imholt et al. 2011; Beauregard et al. 2013; Morash et al. 2018). Fish communities vary along gradients of mean temperature and the magnitude of temperature variation, and these metrics provide a framework for describing the realized thermal niches of stream fishes based on thermal regime (Wehrly et al. 2003).

Streams in temperate grasslands are characterized by high daily and seasonal extremes in temperature that may be exacerbated by lengthy periods of intermittent flow (Dodds et al. 2015). Fish inhabiting these streams are exposed to natural variation in environmental conditions along an upstream-downstream gradient, and fishes that inhabit upstream reaches experience greater temporal variability in temperature – driving differences in community structure and life history strategy along this gradient (Schlosser 1990). The central stoneroller (*Campostoma anomalum*) and the southern redbelly dace (*Chrosomus erythrogaster*) are two minnow (Cyprinidae) species

that are often abundant in grassland streams throughout the Flint Hills Region of the United States (Frenette et al. 2019); these species fill similar ecological roles as consumers by grazing algae (Bertrand and Gido 2007). However, the distributions of dace are limited to small, cool headwaters while stonerollers persist in warmer, intermediate-sized streams (Frenette et al. 2019). Stonerollers exhibits higher critical thermal maxima (CTM) compared to dace (Scott 1987; Mundahl 1990; Frenette et al. 2019), but otherwise physiological responses to temperature that affect the fundamental thermal niche of these species are similar (Frenette et al. 2019). Yet, how thermal variation at fine temporal scales (such as diurnal changes in temperature) influences the fundamental thermal niche, and subsequently the realized thermal niche, of these organisms is not well understood.

We measured the metabolic rates of dace and stonerollers acclimated to either constant or variable temperature conditions using intermittent flow respirometry to test how exposure to a variable thermal regime affects the metabolic physiology of our focal species. We tested each individual fish at respirometer temperatures of 15, 20, and 25°C to determine how acute exposure to temperatures above and below the normal diurnal range influence metabolic physiology of these species. We also compared metabolic rates between dace and stonerollers to determine if temperature variation contributes to differences in the thermal niches of these species. We hypothesized that fish acclimated to a variable thermal environment would exhibit increased standard metabolic rate (SMR) and decreased maximum metabolic rate (MMR), leading to a decrease in aerobic scope (AS; Morash et al. 2018). While we expect metabolic rates of both species to be similar when tested using conditions matching mean acclimation temperature (Frenette et al. 2019), exposure to temperatures above or below this mean value may affect species differently. We predict that exposure to cooler than average temperature will be less energetically costly for the dace resulting in lower SMR and higher AS compared to the stoneroller, since dace are typically associated with cool headwater streams – and predict that the reverse will be true when exposed to warmer than average temperature – resulting in higher SMR, lower MMR, and lower AS compared to the stoneroller.

Materials and Methods

Fish acquisition and acclimation

Dace and stonerollers were collected from several large (~30 m) pools in a downstream reach of Kings Creek near Manhattan, KS on June 8, 2018. Kings Creek is a typical headwater grassland stream located on the Konza Prairie Biological Station (KPBS), and is a tributary to McDowell Creek, which drains into the Kansas River (Mast and Turk 1999). Channels within Kings Creek range in order from 1-5 and include both intermittent and perennial, spring-fed reaches (Gray and Johnson 1988). Temperatures in Kings Creek are cooler, on average, than intermediate-size streams like McDowell Creek, but are more temporally variable (Frenette et al. 2019). Data collected using submersible temperature loggers (Onset Corporation, Bourne, MA, USA) from 1998 – 2015 at a fixed location in Kings Creek indicate that, generally, summer conditions within the downstream perennial reach of Kings Creek where fish were collected exhibit a mean temperature of approximately 20°C with temperatures typically varying diurnally from between 17°C and 23°C (Fig. 3.1). Fish were collected using a seine (4.6 m by 1.8 m, 3.2 mm mesh) and upon capture were immediately transferred to a large, aerated cooler filled with stream water. After capture, fish were transported to and housed in an aquarium facility at Kansas State University, where they were held under a photoperiod of 12 h light: 12 h dark. Room temperature and ambient water temperature in the aquarium facility was maintained at 20°C. Fish were distributed evenly between four 76-L (72.2 cm x 30.5 cm x 30.5 cm) aquaria at a density of no more than 1 fish/12 L of water. Fish were fed a ration of commercial flake food and dried bloodworms daily. All tanks were maintained under ambient room conditions for two days prior to assigning them to temperature acclimation treatments.

Tanks were assigned to either of two treatments: a constant thermal environment or a thermal environment with diurnal variation in temperature. Fish held in tanks under the constant thermal environment experienced a temperature of 20°C, which represents the mean summer thermal conditions in downstream reaches of Kings Creek. Fish held in tanks under the variable thermal environment experienced a mean temperature of 20°C, but temperatures varied diurnally between 17°C and 23°C, reflecting the typical diurnal temperature swing during summer conditions in Kings Creek. To achieve the variable temperature regime, tanks were equipped with 100 W submersible glass heaters (Aqueon Products, Franklin, WI, USA) and submersible chillers

(Aqua Logic, Inc., San Diego, CA, USA) that were controlled by programmable timers. Tank temperature was either increased or decreased by 1°C every 2 h, reaching a low of 17°C at 0000 h and a high of 23°C at 1200 h. Fish were held under their treatment conditions for 22 days prior to beginning experiments. To ensure that fish were in a post-absorptive state they were fasted for 48 h before the onset of an experimental trial (Allen-Ankins and Stoffels 2017).

Intermittent flow respirometry

We used intermittent flow respirometry to measure mass-adjusted rates of oxygen consumption (MO_2; mg O_2 g^{-1} h^{-1}) for both dace and stonerollers. We used a four-chamber intermittent flow respirometer (Loligo Systems, Tjele, Denmark) with optic oxygen probes to measure SMR and MMR (Crans et al. 2015; Frenette et al. 2019). Oxygen probes were calibrated according to the manufacturer's protocol at the beginning of each week. The volume of the respirometer chambers was 0.0987 L. Oxygenated water (≥95% O_2 saturation) was circulated from a reservoir tank to the respirometer using a submersible pump. A 150 W submersible glass heater (Aqueon Products, Franklin, WI, USA) and a 1/13 HP recirculating chiller (AquaEuro Systems, Gardena, CA, USA) were used to regulate the water temperature in the respirometer and were both housed in the reservoir tank. Prior to beginning a trial, the temperature of the water in the respirometer was brought to either 15, 20, or 25°C. Daily minimum and maximum temperatures can exceed the typical range of 17 - 23°C (Fig. 3.1); we chose these respirometer test temperatures to assess if, in addition to acclimation conditions, exposure to temperatures beyond typical conditions yielded different metabolic responses in dace and stonerollers. At approximately 1400 h on the day of a trial, three fish were then drawn from their acclimation tank and were randomly assigned to a respirometer chamber. One chamber remained empty to measure background respiration (mg O_2 h^{-1}) during the trials, which remained near zero (<0.001 % of fish respiration) across the experimental period (Clark et al. 2013; Rodgers et al. 2016).

The total length (mm TL) and weight (to the nearest 0.01 g) of each fish was measured prior to the fish being placed in the respirometer chamber. Following Frenette et al. (2019), the phase settings of the respirometer were set to a 240 s flush phase, a 60 s wait phase, and a 240 s measurement phase. The respirometry phase cycles were controlled using AutoResp^{TM} version 2 automated intermittent respirometry software (Loligo Systems, Tjele, Denmark). To allow fish to reach a resting state and achieve SMR, rates of oxygen consumption (MO_2) were measured over

the course of 18 h. The rate of MO_2 was calculated from the slope of the regression of oxygen content (kPa) over time (h). We used the average of the 10 lowest MO_2 values collected over the entire 18 h period to determine an individual's SMR, and MO_2 values with a r^2 value <0.9 were excluded.

We subjected fish to a chase trial to determine MMR (Brennan et al. 2016; Rosewarne et al. 2016). Following the 18 h SMR measurement period, one fish was removed from its chamber in the respirometer and placed in a circular arena filled with aerated ($\geq$95% O_2 saturation) water held at a temperature that matched the respirometer temperature. Fish were encouraged to swim by being chased with the handle of an aquarium dip net, were chased until the fish exhibited burst-glide swimming and was considered exhausted when they would no longer respond to physical stimuli (in all trials, minimum chase time was six minutes). Fish were then immediately returned to their respirometer chamber and MMR was measured. We used the highest value of three cycles of the measure phase (240 s) to estimate MMR. All three fish being tested were subjected to the same procedure independently, and all fish were tested between 0800-0900 h. Once all fish had been tested, they were returned to their acclimation tank. Metabolic rates of all individuals were assayed each at respirometer temperatures of 15, 20, and 25°C and fish were given seven days to recover in their acclimation tank between respirometry trials.

Statistical approach

For each experimental trial, AS was calculated as the difference between MMR and SMR. Data were ln-transformed prior to analysis to meet assumptions of normality. To determine which factors best explained variation in observed metabolic rate, we employed a model selection approach using Akaike information criteria adjusted for small sample sizes (AIC_c) with the nlme and MuMIn packages in the R statistical environment (R Core Team 2018; Bartoń 2019; Pinheiro et al. 2019). Linear mixed-effects models including all combinations of the main effects of species (dace and stoneroller), acclimation conditions (constant vs. variable acclimation), and respirometer temperature (15, 20, and 25 °C) were compared to a null model. Since individual fish were tested at each respirometer temperature, individual was included as a random factor. The null model included only the random effect of individuals. We considered models with $\Delta AIC_c < 2$ compared to the top model and with a model weight (w_i) ≥ 0.1 sufficient to explain

the variation in the response variable under evaluation (Burnham and Anderson 2002; Wagenmakers and Farrell 2004).

Results

The top weighted model for SMR included the main effects of acclimation condition, species, and respirometer temperature, and the interaction between acclimation condition and respirometer temperature (Table 1). Stonerollers acclimated to variable conditions had very similar SMR values compared to constant acclimation conditions at 15°C (mean ± 1 SE; 0.16 ± 0.01 and 0.15 ± 0.02 mg O_2 g^{-1} h^{-1}, respectively), while dace acclimated to variable conditions had lower SMR (0.11 ± 0.02 mg O_2 g^{-1} h^{-1}) compared to dace acclimated to constant conditions (0.14 ± 0.01 mg O_2 g^{-1} h^{-1}) and to stonerollers, overall. SMR for stonerollers acclimated to variable conditions was similar compared to constant acclimation conditions at 20°C (0.23 ± 0.02 and 0.25 ± 0.06 mg O_2 g^{-1} h^{-1}), while dace acclimated to variable conditions had slightly lower SMR (0.18 ± 0.2 mg O_2 g^{-1} h^{-1}) compared to dace acclimated to constant conditions (0.22 ± 0.07 mg O_2 g^{-1} h^{-1}) and to stonerollers, overall. Finally, at 25°C, stonerollers acclimated to variable conditions had slightly higher SMR compared to constant acclimation conditions (0.34 ± 0.05 and 0.30 ± 0.05 mg O_2 g^{-1} h^{-1}), while dace acclimated to variable conditions had notably higher SMR compared constant acclimation conditions (0.26 ± 0.02 and 0.33 ± 0.07 mg O_2 g^{-1} h^{-1}) (Fig. 3.2).

The top weighted models for MMR and AS included the main effects of acclimation condition, species, and respirometer temperature (Table 3.1). For both metrics, a second model within 2 ΔAIC_c units was also weighted highly and included these three main effects and the interaction between acclimation condition and species. The MMR of stonerollers (0.83 ± 0.03 mg O_2 g^{-1} h^{-1}) was higher than dace (0.71 ± 0.03 mg O_2 g^{-1} h^{-1}; Fig. 3a). Fish acclimated to variable conditions had higher MMR (0.80 ± 0.04 mg O_2 g^{-1} h^{-1}; Fig. 3b) than fish acclimated to constant conditions (0.73 ± 0.03 mg O_2 g^{-1} h^{-1}). MMR of fish increased (from 0.61 ± 0.02 to 0.99 ± 0.03 mg O_2 g^{-1} h^{-1}; Fig. 3c) with increasing test temperature of the respirometer. Dace acclimated to variable thermal conditions had had higher MMR values (0.76 ± 0.06 mg O_2 g^{-1} h^{-1}) compared to dace acclimated to constant conditions (0.66 ± 0.04 mg O_2 g^{-1} h^{-1}), while stoneroller MMR was similar whether they were acclimated to variable (0.84 ± 0.04 mg O_2 g^{-1} h^{-1}) or constant (0.81 ± 0.05 mg O_2 g^{-1} h^{-1}) thermal conditions (Fig. 3d). The AS of stonerollers (0.59 ± 0.03 mg O_2 g^{-1} h^{-1}) was higher than dace (0.50 ± 0.02 mg O_2 g^{-1} h^{-1}; Fig. 4a). Fish acclimated to variable conditions

had higher AS (0.58 ± 0.02 mg O_2 g^{-1} h^{-1}) than fish acclimated to constant conditions (0.51 ± 0.03 mg O_2 g^{-1} h^{-1}; Fig. 4b). AS of fish was similar at test temperatures of 15 and 20°C (0.46 ± 0.02 and 0.48 ± 0.03 mg O_2 g^{-1} h^{-1}, respectively), but increased at 25°C (0.69 ± 0.02 mg O_2 g^{-1} h^{-1}; Fig. 4c). Finally, dace acclimated to variable thermal conditions had had higher AS values (0.55 ± 0.04 mg O_2 g^{-1} h^{-1}) compared to dace acclimated to constant conditions (0.45 ± 0.03 mg O_2 g^{-1} h^{-1}), while the AS of stonerollers was similar whether they were acclimated to variable (0.60 ± 0.03 mg O_2 g^{-1} h^{-1}) or constant (0.57 ± 0.04 mg O_2 g^{-1} h^{-1}) thermal conditions (Fig. 4d).

Discussion

We examined whether energy metabolism in two minnow species differed when they were acclimated to either a constant thermal environment or a diurnally variable thermal environment that reflected natural summer conditions in their natal stream. We found evidence that acclimation to a diurnally variable thermal environment influences the energy metabolism of these species compared to acclimation to constant thermal conditions, with some evidence for interspecific differences in the response to acclimation treatment. The SMR of fish acclimated to variable conditions was lower at test temperatures of 15 and 20°C but was higher at a test temperature of 25°C. Stoneroller had higher overall MMR and AS compared to dace. Fish acclimated to diurnally variable thermal conditions maintained higher MMR and AS compared to those held at constant conditions. MMR increased with the test temperature of the respirometer, while AS increased between 20 and 25°C, but was similar between 15 and 20°C. Dace that were acclimated to diurnally variable thermal conditions maintained higher MMR and AS compared to dace acclimated to constant conditions, while the MMR and AS of stonerollers was similar regardless of acclimation treatment.

Our results suggest that acclimation to a diurnally variable thermal environment can influence the metabolic rates of dace and stonerollers, with evidence suggesting a stronger effect of diurnal variation on dace metabolic rates – as indicated by higher MMR and AS in dace acclimated to diurnally variable conditions. Exposure to thermal conditions outside of the typical diurnal range influenced the SMR of dace and stonerollers acclimated to variable diurnal thermal conditions differently than those fish acclimated to constant conditions, particularly when exposed to high temperatures. When fish were exposed to test temperatures representative of mean conditions or to temperatures below typical diurnal conditions, those fish that were acclimated to

a diurnal cycle exhibited similar or slightly lower SMR, indicating overall consistency in baseline metabolism at these temperatures. However, when fish experienced temperatures above the normal range of the diurnal cycle, those fish acclimated to variable conditions exhibited higher SMR values, indicative of an increase to baseline energy expenditure when acclimated to variable conditions and experiencing potentially stressful thermal environments. Circadian temperature fluctuations have been documented to increase the SMR of Atlantic salmon (*Salmo salar*) parr and this increase in baseline metabolic cost may have negative consequences for fish growth (Beauregard et al. 2013). However, plastic responses in MMR after acclimation or repeat exposure to variable thermal conditions could compensate for an increase in baseline energy expenditure, allowing fish to maintain or increase their overall aerobic capacity (Guzzo et al. 2019). A study conducted on juvenile lake trout (*Salvenlinus namaycush*) found evidence for plasticity in MMR following simulated forays into warmer environments, allowing fish to maintain a larger window of energy availability in cold water (Guzzo et al. 2019). Acclimation to diurnal variation in temperature that exposes fish to temperatures above mean values may elicit a similar plastic response in the MMR of dace, and likely contributes to their increase in AS when exposed to variable thermal conditions.

Other studies suggest exposure to thermal variation on diurnal scales has the capacity to drive differences in fish physiology when compared to responses under acclimation to constant thermal environments (Morash et al. 2018). For example, the routine metabolic rate (RMR) and MMR of Atlantic salmon parr both decreased when fish are acclimated to a diurnal thermal cycle with a 6°C fluctuation when compared to a mean constant temperature (Morash et al. 2018). The critical thermal maxima (CTM) of wild juvenile Atlantic salmon also increased under similar acclimation conditions compared to stable conditions (Corey et al. 2017). Additionally, growth rates in salmonid fishes like Atlantic salmon and cutthroat trout (*Oncorhynchus clarki henshawi*) decreased with increasing diurnal thermal variability (Imholt et al. 2011; Meeuwig et al. 2004; Morash et al. 2018). Much of the current research on the effects of small-scale thermal variation on fishes has been conducted in salmonids and other cold-water species. Species of warm-water fishes may be less sensitive or exhibit different responses to diurnal thermal variations when compared to cold-water fishes, like salmonids (Eldridge et al. 2015). For dace, increased MMR, and subsequently AS, when experiencing diurnal thermal variation may be an important adaptation to life in streams that vary spatially and temporally in temperature.

Our top models for MMR and AS provide evidence that these metabolic rates in stonerollers are higher, overall, compared to the dace. The standard metabolic rate of ectotherms increases as a function of temperature as the rate of biochemical reactions increase, and other aspects of energy metabolism (MMR, AS) increase with temperature and may plateau or decline as temperatures near the critical thermal maximum (Angilletta 2009). When we compared fish acclimated to and tested at 20°C in another study (Frenette et al. 2019), dace had similar SMR (0.22 ± 0.03 vs 0.22 ± 0.02 mg O_2 g^{-1} h^{-1}; values from the present study presented first), MMR (0.61 ± 0.07 vs 0.59 ± 0.09 mg O_2 g^{-1} h^{-1}), and AS (0.39 ± 0.05 vs 0.37 ± 0.07 mg O_2 g^{-1} h^{-1}). Interestingly, while SMR of stonerollers was similar between these studies (0.25 ± 0.02 vs 0.27 ± 0.05 mg O_2 g^{-1} h^{-1}), MMR (0.76 ± 0.06 vs 0.51 ± 0.08 mg O_2 g^{-1} h^{-1}) and AS (0.51 ± 0.05 vs 0.25 ± 0.03 mg O_2 g^{-1} h^{-1}) were higher in this experiment. So, why is there a disparity in the metabolic rates of stonerollers at 20°C between these experiments? Life history differences could provide one potential explanation, as determining known ages of small-bodied minnows *in vivo* is challenging, and physiological responses to temperature may vary across life history stages (Morash et al. 2018). Additionally, dace are typically associated with cool headwater streams, and as such may be adapted to maintaining lower overall metabolic rates compared to the stoneroller, that occupies warmer habitats (Brown et al. 2004).

Fishes in temperate waters experience thermally variable environments and while the application of constant temperatures in studies of thermal biology have been useful, this approach does not accurately reflect ecological conditions experienced by the animals even at small spatial and temporal scales (Schulte et al. 2011; Drake et al. 2017). Since ectotherms thermoregulate through behavioral modifications, studies of organismal thermal biology should focus on not only how thermal variation affects physiology but behavioral responses as well (Martin and Huey 2008). Ignoring thermal variability in studies focused on organismal thermal biology may be shortsighted considering the variation in temperature that organisms experience even over the course of a single day, and especially considering that current thermal regimes may be altered in a changing climate. Current global change expectations forecast that, along with increases to mean temperatures, temperature variation and the preponderance of severe weather events will increase and understanding how organismal processes are influenced by temperature variation will aid in predicting how these organisms will respond to changes in thermal regimes in a changing climate.

Figures
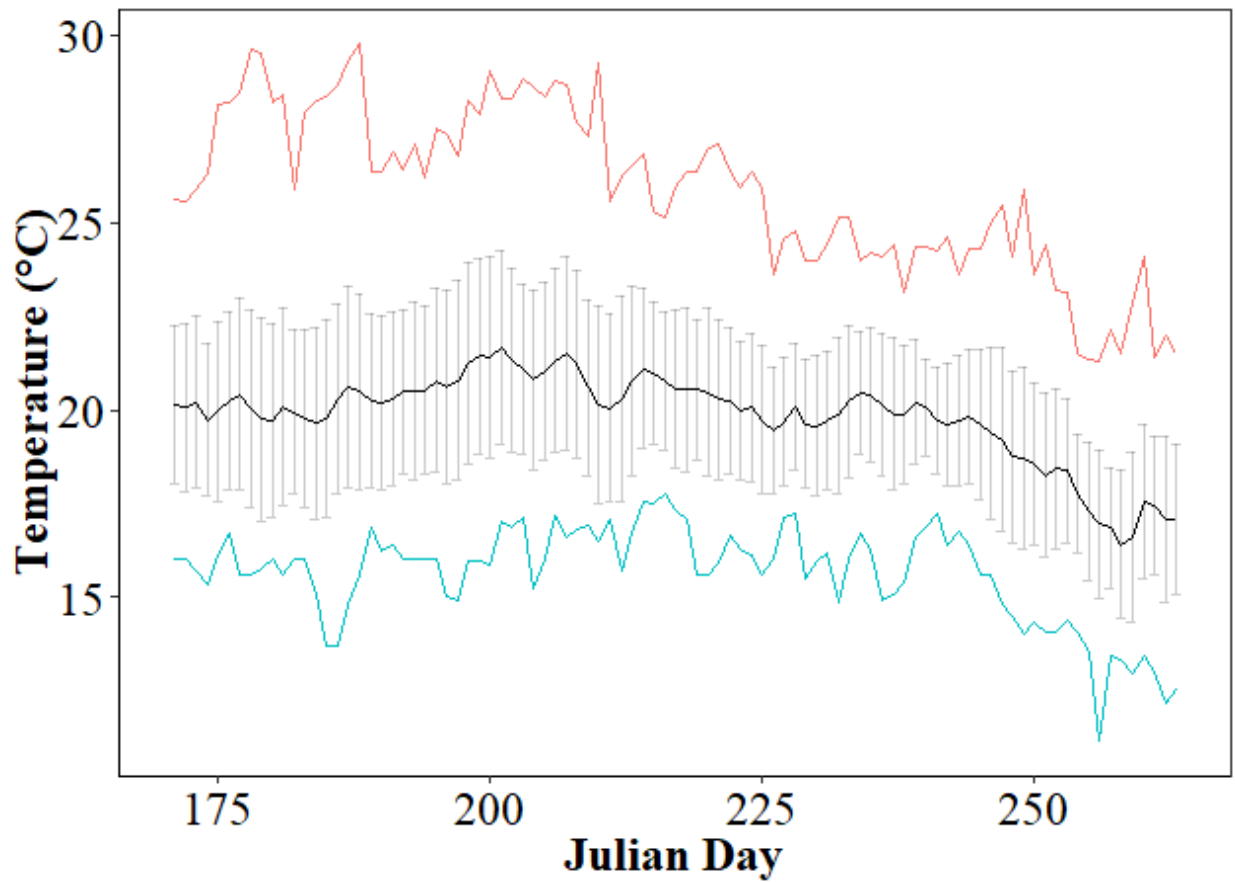
Figure 3.1: Mean (± 1 SD) daily summer temperature (°C; black line) bounded by daily maximum (red line) and daily minimum (blue line) temperatures in a downstream reach of Kings Creek. Kings Creek is a headwater grassland stream in the Flint Hills region of the USA that contains both southern redbelly dace and central stoneroller in high abundance. Temperature data are from 1998-2015.
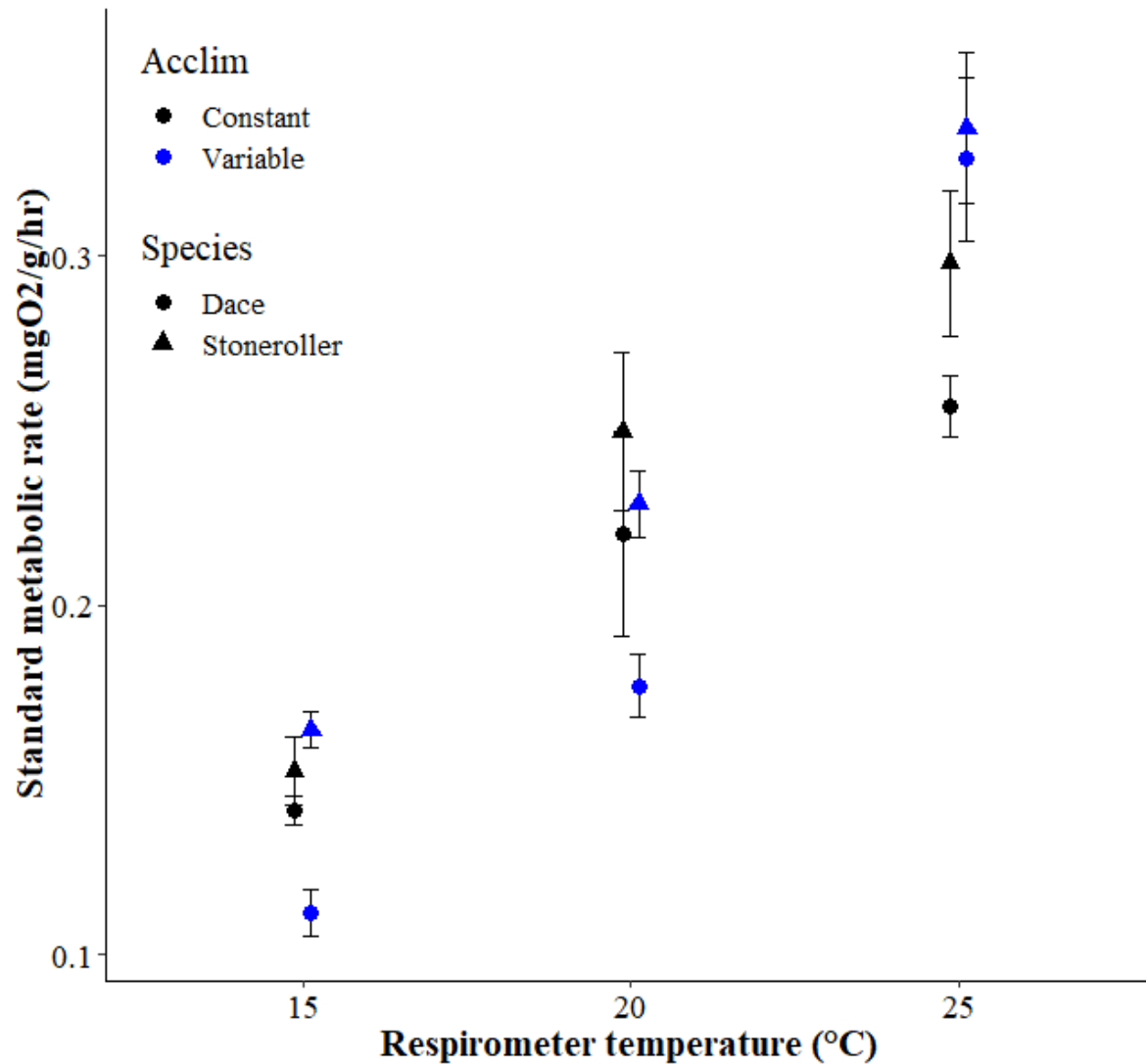
Figure 3.2: Mean (± SE) mass-adjusted standard metabolic rate (SMR; mg O_2 g^{-1} hr^{-1}) for southern redbelly dace (circles) and central stoneroller (triangles) acclimated to either constant (black) or variable (blue) thermal conditions, and tested at 15, 20, and 25°C.
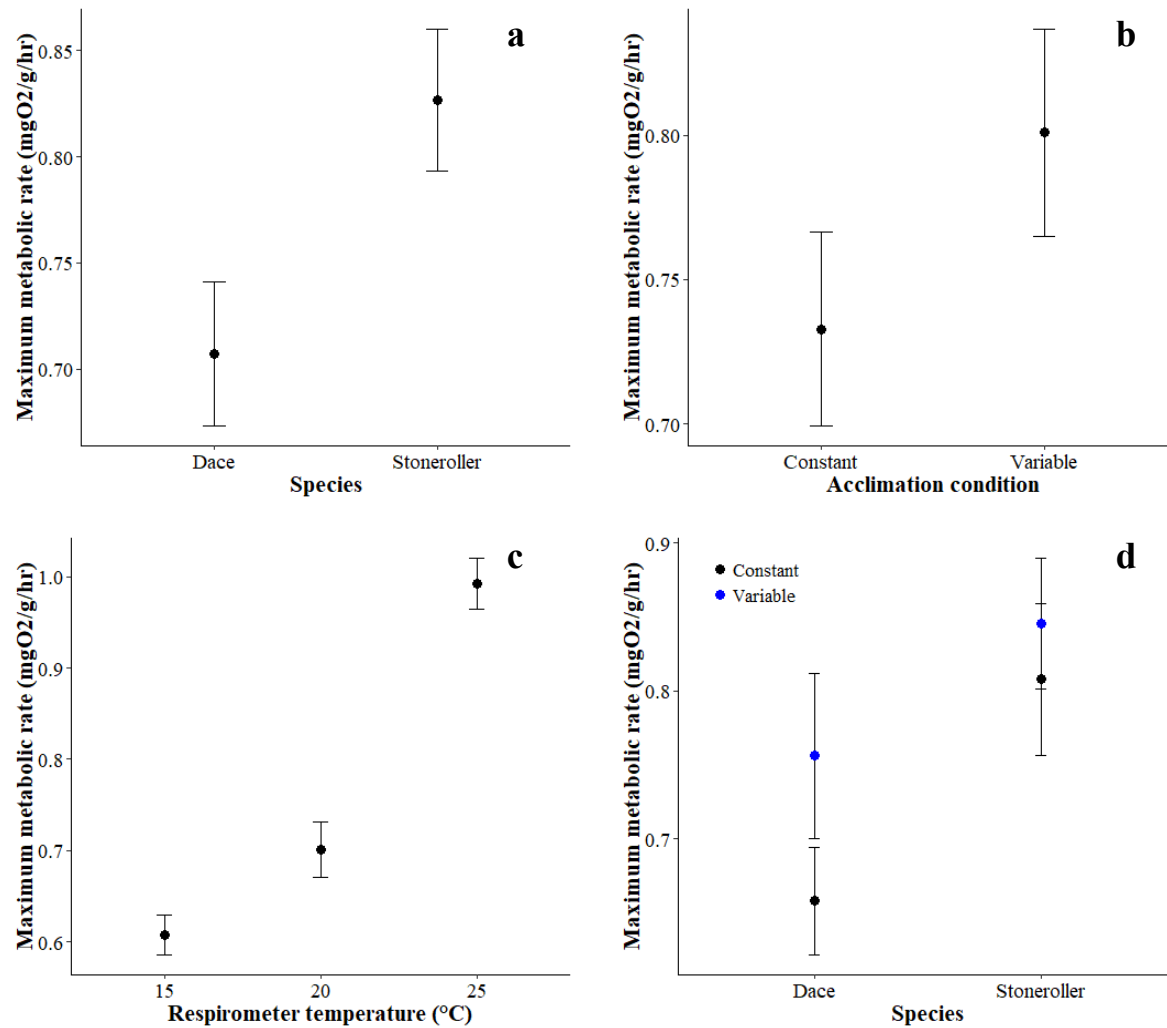
Figure 3.3: Comparisons of mean (± SE) mass-adjusted maximum metabolic rate (MMR; mg O$_2$ g^{-1} hr^{-1}) between species (dace and stoneroller; **a**), acclimation conditions (constant and variable; **b**), respirometer test temperatures (15, 20, and 25°C; **c**), and for the interaction between species and acclimation condition (constant = black, variable = blue; **d**).
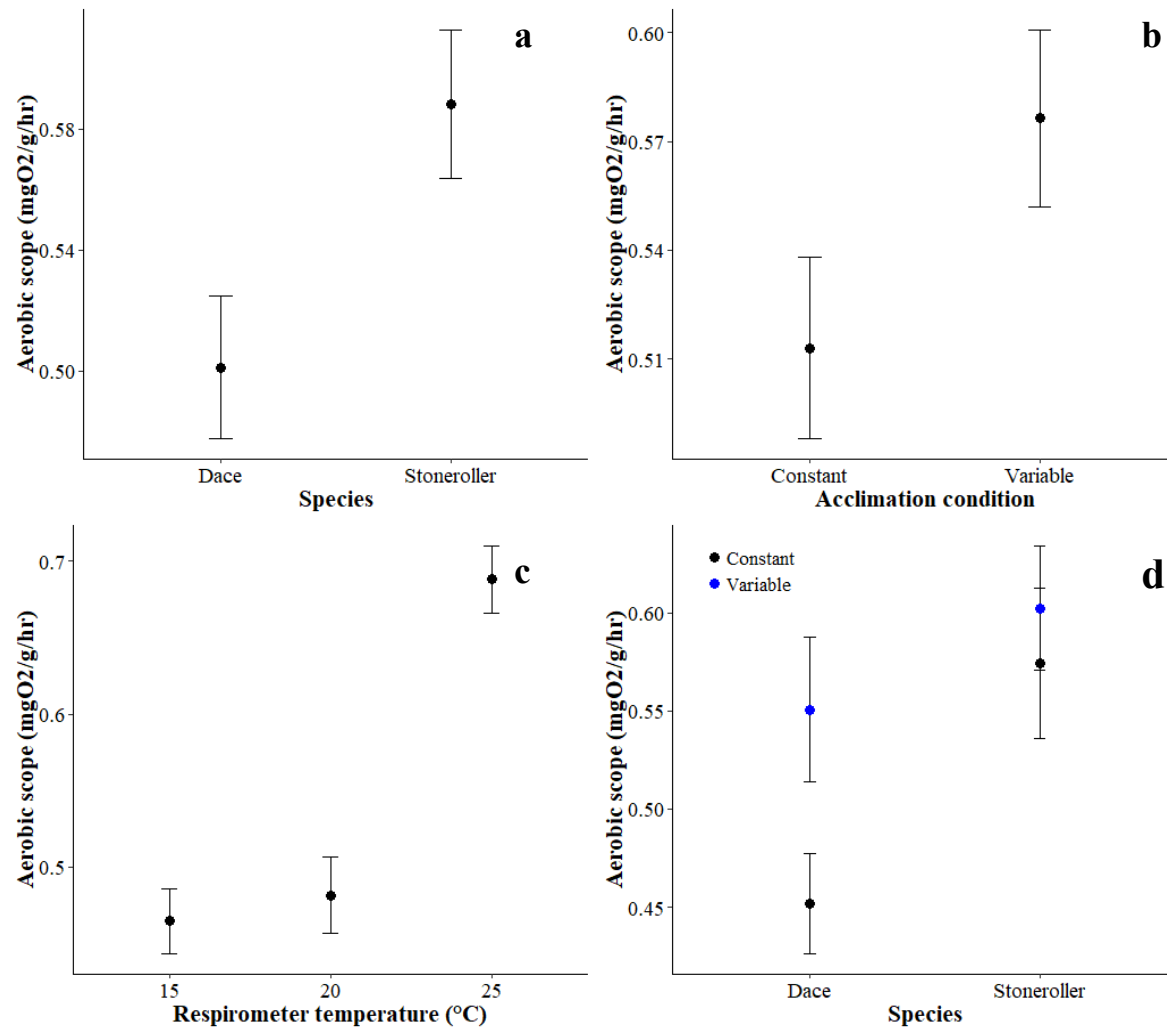
Figure 3.4: Comparisons of mean (± SE) mass-adjusted aerobic scope (AS; mg O_2 g^{-1} hr^{-1}) between species (dace and stoneroller; **a**), acclimation conditions (constant and variable; **b**), respirometer test temperatures (15, 20, and 25°C; **c**), and for the interaction between species and acclimation condition (constant = black, variable = blue; **d**).

Tables

Table 3.1: Models explaining variation in the standard and maximum metabolic rates and aerobic scope of dace and stoneroller acclimated to either variable or constant thermal conditions. Models within 2 ΔAICc units of the top model are also included in the table, as are model weights and the number of parameters (k) included in each model. Abbreviations are defined as: smr = standard metabolic rate; mmr = maximum metabolic rate; ams = aerobic scope (aerobic metabolic scope); acclim = the acclimation treatment (constant or variable); species = the species (dace or stoneroller); treatment = the respirometer test temperature (15, 20, or 25°C).

	Model	ΔAIC$_c$	k	Model weight
Standard	smr ~ acclim + species + treatment + acclim*treatment	0.00	6	0.47
metabolic rate	smr ~ acclim + species + treatment + acclim*species + acclim*treatment	0.76	7	0.32
Maximum	mmr ~ acclim + species + treatment	0.00	5	0.54
metabolic rate	mmr ~ acclim +species + treatment + acclim*species	1.67	6	0.23
Aerobic	ams ~ acclim + species + treatment	0.00	5	0.45
scope	ams ~ acclim +species + treatment + acclim*species	0.38	6	0.37

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Chapter 4 - Responses of two prairie stream fishes to temperature and flow across life history stages

Abstract

Intermittent prairie streams, like those found in the Great Plains of the United States exhibit predictable cycles of drying and wetting, and often experience drought conditions. Fishes inhabiting these systems are adapted to cope with these harsh physicochemical conditions. However, year-to-year variation in environmental factors driven by cycles of drought and rewetting can affect fish growth and abundance and requires investigation. Furthermore, juvenile fish might respond differently to environmental variation than adults due in part to differences in environmental requirements across life history stages. To evaluate how environmental factors associated with temperature and flow affect species and cohort structure across years, we used long-term fish sampling and environmental datasets to examine responses in southern redbelly dace (*Chrosomus erythrogaster*) and central stoneroller (*Campostoma anomalum*) across 13 sampling years. We did not find evidence that cohorts of these species exhibit intraspecific differences in their response to inter-annual variation in temperature or flow. The average body size of dace in November decreased during years where stream flows were reduced during the spring and summer, while the average body size of stonerollers increased during years with lower spring flows and stable flow persisting through the summer. The abundances of both species in November was not influenced by inter-annual variation in flow or temperature. Finally, while growth of dace between August and November was not influenced by inter-annual variation in flow or temperature, stonerollers grew less during years were flows were reduced during the spring and summer. Our results indicate that flows can influence population vital rates, with drought conditions appearing to have a negative effect on populations of dace and stoneroller. Understanding how species are affected, across stages of their life history, by environmental variation will be important in forecasting their expected response to climate change, which is expected to drive more frequent and severe disturbance events in intermittent prairie streams – potentially disrupting the cyclical pattern of drying and wetting.

Background

The use of long-term ecological data provides an invaluable resource to address problems relating to environmental change, the management of natural resources, and the conservation of biodiversity (Lindenmayer et al. 2012). Long-term data is particularly useful if it allows us to predict responses along gradients that may be altered under scenarios of global change (Tilman et al. 2001; Willis et al. 2007; Matthews and Marsh-Matthews 2016). As human populations increase, so too does our use of freshwaters, resulting in increased fragmentation and alterations to natural flow regimes (Woodward et al. 2010; Perkin et al. 2015; 2017). Furthermore, water temperature in freshwater systems is largely driven by climate. Over the past century the Earth's climate has warmed by approximately 1°C and continues to warm, driving potential shifts in the thermal regimes of freshwaters (Woodward et al., 2010; Pachauri et al., 2014). Since aquatic species living in freshwaters are confined by the boundaries of their habitats, their ability to disperse in response to environmental change is limited (Dudgeon et al. 2006; Woodward et al. 2010). To best conserve biodiversity in freshwaters, it is critical to understand how populations and communities respond to environmental factors to best predict their trajectory under scenarios of global change (Dudgeon et al. 2006; Alahuhta et al. 2019).

Intermittent streams, like those found throughout the Great Plains of the United States, are characterized by predictable cycles of drying and wetting and are often considered to be harsh environments (Datry et al. 2014). These streams typically comprise the headwaters in river networks and are important contributors to biodiversity (Meyer et al. 2007). Intermittent streams flow during wet seasons but large reaches are dry at other times of year. Organisms, like fish, that live in intermittent streams are presented with notable environmental challenges (Matthews 1988; Matthews and Marsh Matthews 2003; Dodds et al. 2004). During periods of drying, reductions of flow can isolate stream reaches into pools, limiting the movement of organisms to thermal refugia (Bond et al. 2008). Additionally, reductions in pool surface area during already warm drying events can exacerbate increases in water temperatures. Organisms living in these environments are adapted to cope with harsh physicochemical conditions (Dodds et al. 2004). However, populations may fluctuate between years as a result of the frequency and severity of drying events, and multiple consecutive years of drought can have negative consequences for short-lived species (Propst et al. 2008; Jaeger et al. 2014; Hopper et al. 2019). Recolonization dynamics are often important contributors to the long-term persistence of stream organisms (Whitney et al. 2016; Bogan et al.

2017). Understanding how organisms in intermittent streams respond to variable thermal conditions and flow regimes is of interest to ecologists, especially considering that the compounding effects of anthropogenic disturbance and climate change have contributed to declines in biodiversity in these systems (Perkin et al. 2015; 2017).

While many long-term studies aim to explain how abundances of different populations of organisms change over time, fewer address how proxies to organismal fitness, like growth, might vary through time and along environmental gradients (Scheurer et al. 2003; Dexter et al. 2014; Hedden and Gido 2019). In intermittent streams, cyclical patterns of flooding and drying drive among-year variation in abiotic conditions, like temperature and stream flow, and this variability might have important implication for the growth and abundance of stream organisms. The occurrence of wet years allows populations of fish to recover from drought conditions, and the frequent occurrence of wet years may be necessary to maintain the populations of some species (Bernardo et al. 2003; Propst et al. 2008; Gido and Propst 2012; Mims and Olden 2013; Pool and Olden 2014). Other species with adaptations for low flow conditions, like western mosquitofish (*Gambusia affinis*), may benefit from dry years and increase in population size (Pool and Olden 2014; Hopper et al. 2019; Rogosch et al. 2019). Intermittent streams in grasslands are expected to experience an increase in the frequency and severity of drought under current scenarios of climate change (Dodds et al. 2015). If the frequency of drought years increases and alters the dynamic that allows the recovery of species that rely on wet years, shifts in the fish communities may occur (Ruhí et al. 2010). Existing and ongoing long-term datasets from these systems will be useful in explaining how organisms respond to variable environmental conditions and predict how they will respond to shifts in environmental conditions attributed to global change.

The central stoneroller (*Campostoma anomalum*) and the southern redbelly dace (*Chrosomus erythrogaster*) are two ecologically similar minnow species (Cyprinidae) that occur in the Flint Hills region of the central United States. Both species co-occur in small intermittent headwater streams, but dace occurrence decreases as stream size increases (Frenette et al. 2019a). Dace and stoneroller are both primarily algivorous and occupy similar trophic positions when they co-occur (Bertrand and Gido 2007). These species are both fractional spawners and, when conditions are favorable, may produce multiple cohorts of offspring during spring and summer months (Falke et al. 2010). Recent work suggests they differ in aspects of their fundamental thermal niche, with stonerollers being better at tolerating warm thermal conditions that are

physiologically stressful. Physiological differences likely contribute to the observed difference in occurrence between species along a stream-size gradient (Frenette et al. 2019a; 2019b; Hopper et al. 2019). However, we know relatively little about how populations of these species respond to inter-annual variation in temperature and flow, and whether this variation might affect age classes differently.

Our objective was to test if size, abundance, and growth of dace and stoneroller differ in response to inter-annual variation in discharge and temperature in an intermittent prairie stream, and whether cohorts (age-0 and age-1 fish) respond differently. To achieve this, we used long-term data from Kings Creek on the Konza Prairie Biological Station (KPBS), where fishes were sampled annually in May, August and November. For each sampling year, we used modal progression analysis to classify fish into age classes based on the long-term length-frequency data, and to estimate each cohort's abundance and mean body size in November. We calculated growth for a cohort as the difference in mean body size between August and November samples. Since drought conditions – characterized by reduced flow and increased temperature – can negatively impact fish populations, we hypothesized that the abundance of both species regardless of age class would be negatively impacted by drought-like conditions. However, because dace do not tolerate thermal stress as well as stonerollers, populations of dace are expected to be more severely impacted by drought. We also hypothesized that growth will be negatively influenced by drought-like conditions, especially for age-0 fish that rely on habitats at the periphery of pools or in riffles (Martin et al. 2013) and may be rare or unavailable when stream volumes are reduced due to drying.

Materials and Methods

Sampling occurred between 1998 and 2017 in a downstream perennial reach of the Kings Creek watershed on the Konza Prairie Biological Station (KPBS) near Manhattan, KS. Fish communities were sampled in August and November with single pass DC-pulsed backpack electrofishing. The average area of the stream reach sampled across years measured approximately 110 m^2. Species of fish were identified, measured to total length (mm TL), and released back into the sampled habitat. Stream temperature was recorded using a submerged HOBO temperature logger (Onset Corporation) that continuously recorded hourly temperature. Hourly air temperature at 2 m above ground level for the area was recorded using a CR-10 data logger (Campbell Scientific). Mean

daily discharge data were recovered from the USGS stream gauge for Kings Creek (Station # 06879650).

To prepare our environmental data, we first looked at the strength of the linear relationship between air and water temperatures at our sampling site across eight years where temperature data for the entire year was present. In all cases we found a significant linear relationship between air and water temperatures, with R^2 values ranging between 0.89 and 0.93 (Fig. A1). Our water temperature dataset was incomplete across all years of fish sampling due to the tendency of the data logger to be lost during extreme flow events, and we chose to proceed with the more complete air temperature dataset for our analyses. This left us with 13 complete years of air temperature data ranging from the years 2001 to 2017.

Statistical approach

Modal progression analysis using the software Food and Agriculture Organization-International Center for Living Aquatic Resources Management Stock Assessment Tools (FiSAT) was used to classify individuals into age classes based on length-frequencies (Hedden and Gido 2019). This analysis can separate early age-classes of fish, allowing us to estimate the number and mean size of age-0 and age-1 individuals of both species captured during each sampling period (Isely and Grabowski 2007). For each year of sampling, total lengths of dace and stoneroller sampled in both August and November we categorized into 5 mm bins to characterize cohorts. Bhattacharya's method was used to separate cohorts by identifying slopes using the equation:

$$\ln(N_{i+1}) - \ln(N_i)$$

where N_i and N_{i+1} are the successive frequencies of size bins in a sample (Bhattacharya 1967). Using the estimates from the Bhattacharya's method, a mean of the normal distribution and its standard deviation were calculated, and maximum likelihood was used to separate the normally distributed components of the size-frequency sample, effectively separating the sample into distinct cohorts with estimates of mean total length and population size for each age class present in a sample. Growth (in mm TL) was calculated for each year where a cohort was detectable in both the August and November sample as the difference in mean size between the November and August sample for each cohort (Table 4.1).

Principle component analysis (PCA) was used to summarize inter-annual variation in temperature and discharge. Since both the spring and summer seasons are critical for growth and

reproduction in these species (Settles and Hoyt 1976; Sabaj et al. 2000), we partitioned each year into spring temperature and summer temperature based on the usual equinox dates. For each season in each year, we determined the mean, maximum, and minimum temperature. For each year where we had temperature data, we also calculated the mean and maximum spring and summer discharge. Since days of no flow occurred in spring and summer of nearly every year for which we had data, minimum discharge values were almost universally zero. To provide a more meaningful measure of low discharge, we instead calculated the number of recorded days of zero flow in spring and summer of all years. This left us with 12 environmental variables related to temperature and flow to be included in the PCA (Fig. A2). We determined, based on initial assessment of the PCA results, that maximum and minimum temperature and maximum flow were largely redundant with other variables included in the PCA. We removed these variables from the analysis, reducing the number of assessed environmental variables to 6. We generated a correlation matrix between the variables of interest to evaluate the relationships between associated variables (Table A1).

To test the effects of species and environmental factors on the size, abundance, and growth of dace and stoneroller, we employed a model selection approach using Akaike information criteria adjusted for small sample sizes (AIC_c) using the glmulti package in the R statistical environment (Mazerolle 2019; R Core Team 2017). We built linear models to test the effects age class and of the environmental variables (PC scores) on size, growth, and abundance of dace and stoneroller in the November sample. We chose the November sample because reproduction was assumed to be complete at this point in the year for these species and growth should slow as temperatures decline. To facilitate a comparison between age classes, which differ in overall body and population sizes, we first z-transformed each of the dependent variables. We partitioned the data to look at species separately, and models additively included the main effects of age and the first three principal component axes. To assess whether density-dependent factors influenced growth, we also included z-transformed abundance in the growth models. Models with $\Delta AIC_c < 2$ compared to the top model and with a model weight (w_i) ≥ 0.1 were considered sufficient to explain the variation in the response variable under evaluation (Burnham and Anderson 2002; Wagenmakers and Farrell 2004).

Results

Axes 1 and 2 of the PCA explained 67% of the variability in the environmental data and including axis 3 resulted in 86.1% of the variability being explained (Table 4.2; Fig. 4.1). Years that fall on the positive direction of the PC1 axis are characterized by dry conditions with reduced stream flow and many days of no flow occurring in spring and summer, whereas years falling on the negative direction are characterized by wetter conditions and persistent flow during spring and summer (Fig. A3). Years that fall on the positive direction of the PC2 axis are characterized by cooler average spring and summer temperatures. Finally, years that fall on the positive direction of the PC3 axis are characterized by lower mean flows during spring, higher mean flow during summer and summer temperatures slightly above mean conditions.

The top candidate model explaining the size of dace in November included PC1 (Table 4.3). Regardless of age, the average size of dace was smaller, overall, when conditions during spring and summer were dry (Fig. 4.2). The top candidate model explaining the abundance of dace in November included PC2. However, the null model was within 2 ΔAIC_c units of the top model, indicating that variation in abundance cannot be ruled out from random chance. The top candidate model explaining growth of dace between August and November included PC3. Again, the null model was within 2 ΔAIC_c units of the top model, indicating that variation in growth cannot be ruled out from random chance.

The top candidate model explaining the size of stonerollers in November included PC3. Regardless of age, the average size of stonerollers was larger, overall, when average spring flows were lower and summer flows were higher (Fig. 4.3). The null model was the top candidate model explaining the abundance of stonerollers in November, and no additional models ranked within 2 ΔAIC_c units of the top model. The top candidate model explaining growth of stonerollers between August and November included PC1. Regardless of age, stonerollers grew less when conditions during spring and summer were dry (Fig. 4.4).

Discussion

We employed a model selection approach to explore how body size, abundance, and growth of populations of two prairie stream minnows are influenced by inter-annual variation in temperature and discharge. Our results suggest that when conditions were dry – indicated by low mean flow

and a high number of days without flow during spring and summer – the mean body size of dace in both the age-0 and age-1 cohorts surveyed in November was smaller. This indicates a potential negative influence of drought on the body size of this species. However, abundance and growth rates of dace were not strongly influenced by inter-annual variation in temperature or discharge. When springs were not characterized by high flow and stream flow was maintained during summer, the mean body size of both cohorts of stonerollers surveyed in November were relatively larger, indicating a potential association with stable, flowing habitats. The abundance of stonerollers was not strongly influenced by inter-annual variation in temperature or discharge. Finally, the growth of stonerollers between August and November was lower during dry conditions that were indicative of a drought year, regardless of age class.

Our data suggested that the average relative body size of dace in November, regardless of what cohort they belonged to, was smaller when conditions in Kings Creek were dry during spring and summer. When conditions were indicative of a drought year, dace in the age-0 cohort were approximately 5 mm smaller, and age-1 fish were approximately 9 mm smaller, compared to years when streams were flowing. The fact that drought-like conditions negatively impacted the average body size of dace is consistent with their reported sensitivity to drought. Hopper et al. (2019) showed that, while severe drought decreases populations of both dace and stonerollers (sometimes completely extirpating dace from isolated pools), stonerollers maintain higher critical thermal maximum (CTMax) than dace, indicative of a greater capacity to buffer thermal stress. Frenette et al. (2019a) also found appreciable differences in the CTMax of these species, with stonerollers maintaining higher CTMax when acclimated to warm temperatures. However, temperature was not linked to PC axis associated with dace body size, indicating that other factors driven by drought may contribute to a decrease in the overall body size of dace.

The average body size of stonerollers in November was influenced by a different combination of environmental variables than dace but was still influenced largely by inter-annual variation in spring and summer flow. Stonerollers in both cohorts were larger on average when mean spring flows were lower, summer mean flows were higher, and mean summer temperatures were slightly warmer. During years characterized by these conditions age-0 stonerollers were 6 mm larger and age-1 stonerollers were 3 mm larger than when springs had high flow and summer flows were reduced. Stonerollers are typically associated with riffle habitats (Martin et al. 2013;

Hedden and Gido 2019), and years where streams are slow flowing during spring and maintain flow during summers may be advantageous to the persistence of larger stonerollers.

Inter-annual variation in temperature and flow did not strongly predict the abundances of dace or stonerollers across years. Dace and stonerollers, like other fish species native to streams in the Great Plains, are fractional spawners and can produce multiple broods spread across the spring and early summer breeding season (Heins and Rabito 1986; Falke et al. 2010). If conditions for reproduction are favorable during either the spring or summer, fish may have multiple opportunities to successfully produce at least one successful cohort and maintain abundances across years with variable temperature or flow. Drought conditions (high temperature and lack of stream flow) can impose notable physiological stress on fishes, and individuals will often use early cues to migrate downstream to refugia (Magoulick and Kobza 2003). Additionally, physiological stress from temperature and density-dependent factors during drought can impose high rates of mortality on fishes remaining in isolated pools (Hopper et al. 2019). Our long-term sampling site at Kings Creek, however, is a perennial reach and typically persists during dry years, although reductions of stream flow do occur. The variability in temperature and stream flow during the sampling years included in our study likely did not include conditions severe enough to negatively impact the abundances of populations of dace and stonerollers. It is important to note, however, that a severe drought occurred in summer 2018, resulting in extreme mortality of these species associated with almost complete drying of the study reach evaluated here (Hopper et al. 2019).

Inter-annual variation in temperature and flow did not strongly predict the growth of dace (measured as the change in a cohort's mean body size between August and November) across years but did influence the growth of stonerollers. When conditions in Kings Creek were dry during spring and summer, growth of age-0 stonerollers was reduced by 3.3 mm and growth of age-1 stonerollers was reduced by 4 mm, compared to years when streams were flowing. It is interesting that, regardless of age class, stoneroller growth was influenced by drought conditions while dace growth was not. During drought, flows diminish and restrict fish to isolated pools with limited access to riffles. As pool volume decreases due to evaporation, access to habitats at the pool's edge under normal conditions are lost (Bond et al., 2008). These riffle and edge habitats are typically used by juvenile fishes and may be critical for juvenile recruitment into adult habitats (Lobb III and Orth 1991; Matthews 1998, Pusey and Arthington 2003). Though these fish (which are well adapted to life in the harsh conditions of intermittent streams) can survive when summers are dry

and temperatures are more variable, we would anticipate a reduction in growth rates for juveniles resulting from the loss of access to critical habitat at the pool's edge. Martin et al. (2013) showed that in Kings Creek juvenile fish were more abundant in shallow pools, indicating that these habitats may be more important to juvenile fish in this system. This helps explain, at least, why age-0 dace did not display reduced growth in response to dry or warm conditions. Since stonerollers growth, regardless of age class, is reduced during dry conditions, differences in habitat requirements that are influenced by drought could influence this species differently compared to the dace. Previous work has shown that occurrence of these two common species in our study system are consistent across multiple years of sampling, but that habitat requirements (pool versus riffle) differ between species (Martin et al. 2013; Hedden and Gido 2019). While dace are typically associated with pool habitats, which persist at this perennial site even in dry conditions, stonerollers associate with riffles – and access to riffle habitats may be limited during drought-like conditions, with potentially negative consequences for stoneroller growth (Martin et al. 2013; Hedden and Gido 2019). Additionally, while stonerollers may be more tolerant of environmental stressors associated with warm temperature during drought, conditions during these events may not be conducive to supporting growth. Stonerollers growth has been shown to be density-dependent in an experimental stream study (Pennock and Gido 2016). Thus, during drought, when fish may be densely crowded in pools, growth of stonerollers is likely to be reduced. The influence of density-dependence on growth of dace remains to be evaluated.

We found evidence that body size in populations of dace and stoneroller, and growth in populations of the stoneroller, occurring in an intermittent headwater prairie stream responded to variation in stream flow across multiple years of long-term sampling. Since occurrence of these species varies along a stream-size gradient of temperature, and these species differ in some aspects of their fundamental thermal niche, we were surprised to find little evidence that inter-annual variation in temperature influenced the body size, abundance, or growth of these species (Frenette et al. 2019a; 2019b). However, variability in air temperature across sampling years included in this study was low (Fig. A3) and may not have included thermal conditions warm enough to negatively influence the cool water associated dace. Intermittent streams, like Kings Creek, are highly stochastic in nature due to shifts in temperature and flow conditions between wet and dry years, and the occurrence of fish species at small spatial scales is hard to predict (Jackson et al. 2001; Wuellner et al. 2013). Our results show that variation in flow across years can influence

populations of dace and stoneroller and highlights the inherent value of leveraging long-term ecological data to make inferences about the responses of organisms across spatial and temporal gradients. Intermittent headwater streams are important contributors to freshwater biodiversity and are critical to the maintenance of fish populations (Freeman et al. 2007; Meyer et al. 2007). Fishes in these systems are resilient and are adapted to cycles of drying and flooding, however, climate change is expected to increase the frequency and magnitude of extreme events (Dodds et al. 2004). Understanding how different species respond, across their life history, to abiotic factors directly relevant to this cycle will serve to inform conservation efforts in stream ecosystems.
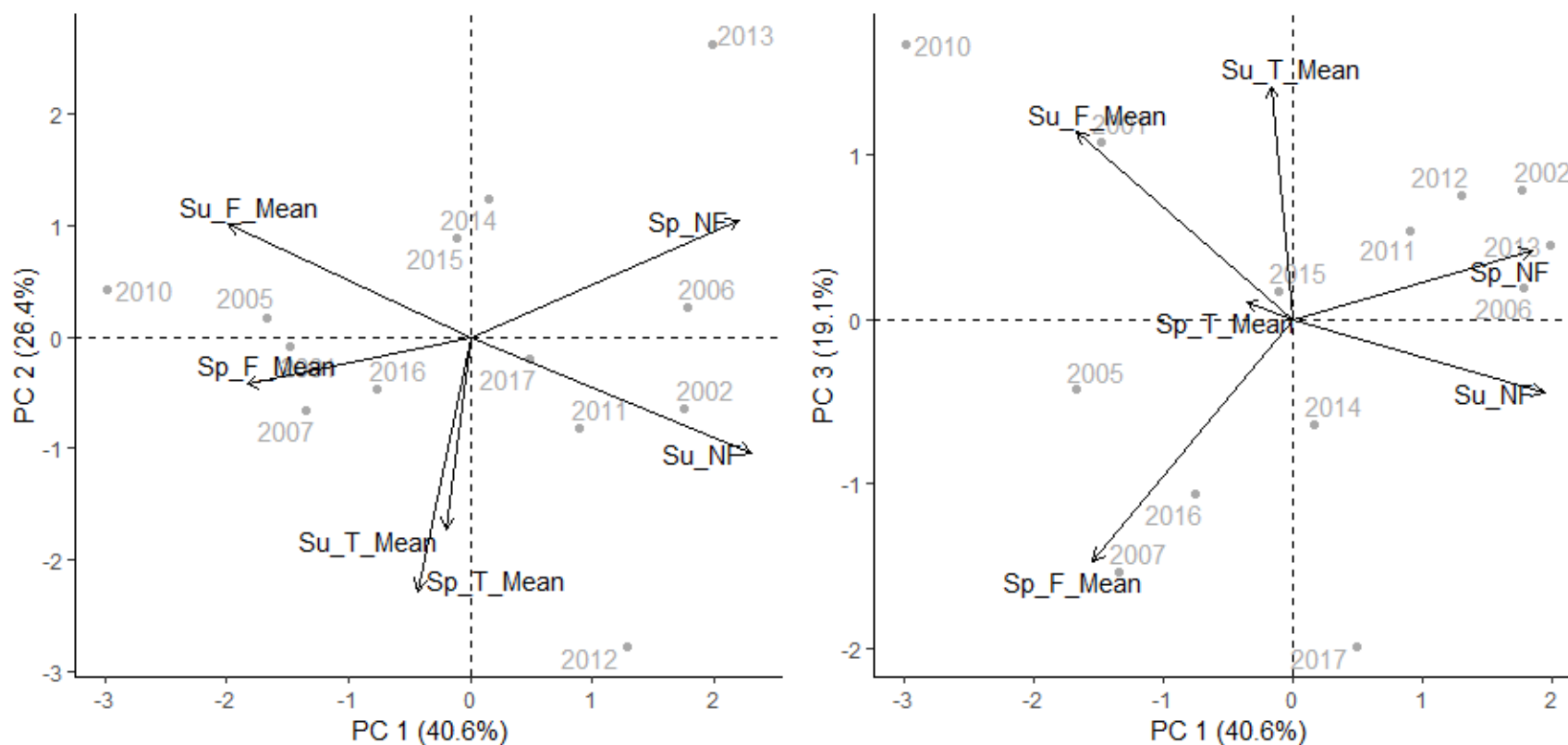
Figure 4.1: Principal components analysis of environmental variables related to temperature and flow in Kings Creek, USA sampled across 13 years. Directionality of the associations between PC axes and environmental factors are presented black lines with arrows. Labels for environmental vectors are: Sp; spring, Su; summer; T; temperature (°C), F; flow (discharge; m³/s), NF; the number of days without flow, Mean; the mean value. The left panel compares the first and second PC axes, and the right panel compares the first and third PC axes. The labeled gray data points represent the year.

Figure 4.2: Association of PC1 on z-score transformed body size (mm TL) of dace (age-0 = black; age-1 = gray). PC axis 1 explains variation in stream flow, with positive loadings representing low flow and numerous days of no flow in both spring and summer.
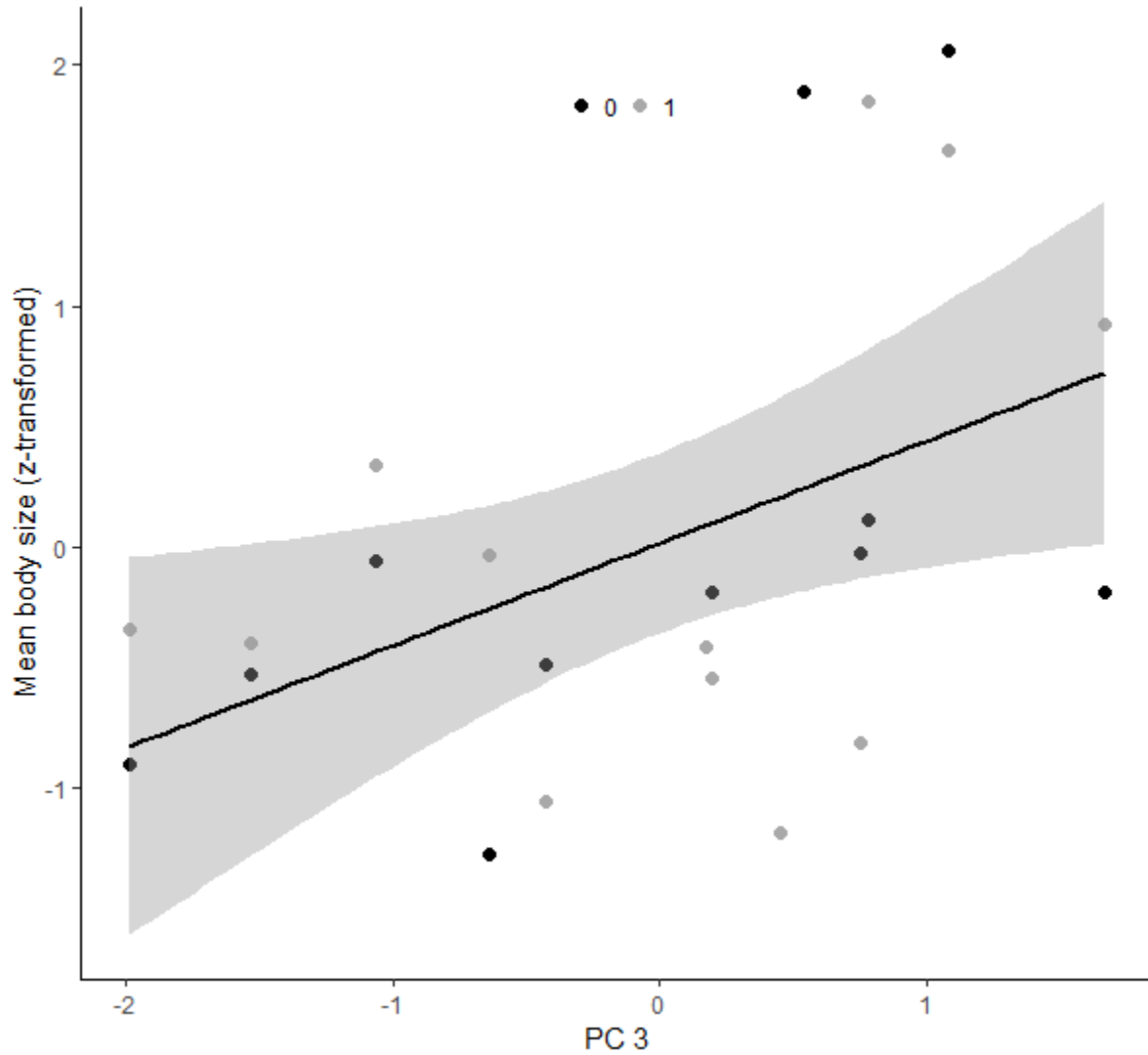
Figure 4.3: Association of PC3 on z-score transformed body size (mm TL) of stoneroller (age-0 = black; age-1 = gray). PC axis 3 explains variation in spring and summer flow and summer temperature, with positive loadings representing lower mean flows during spring, higher mean flow during summer, and summer temperatures near mean conditions.

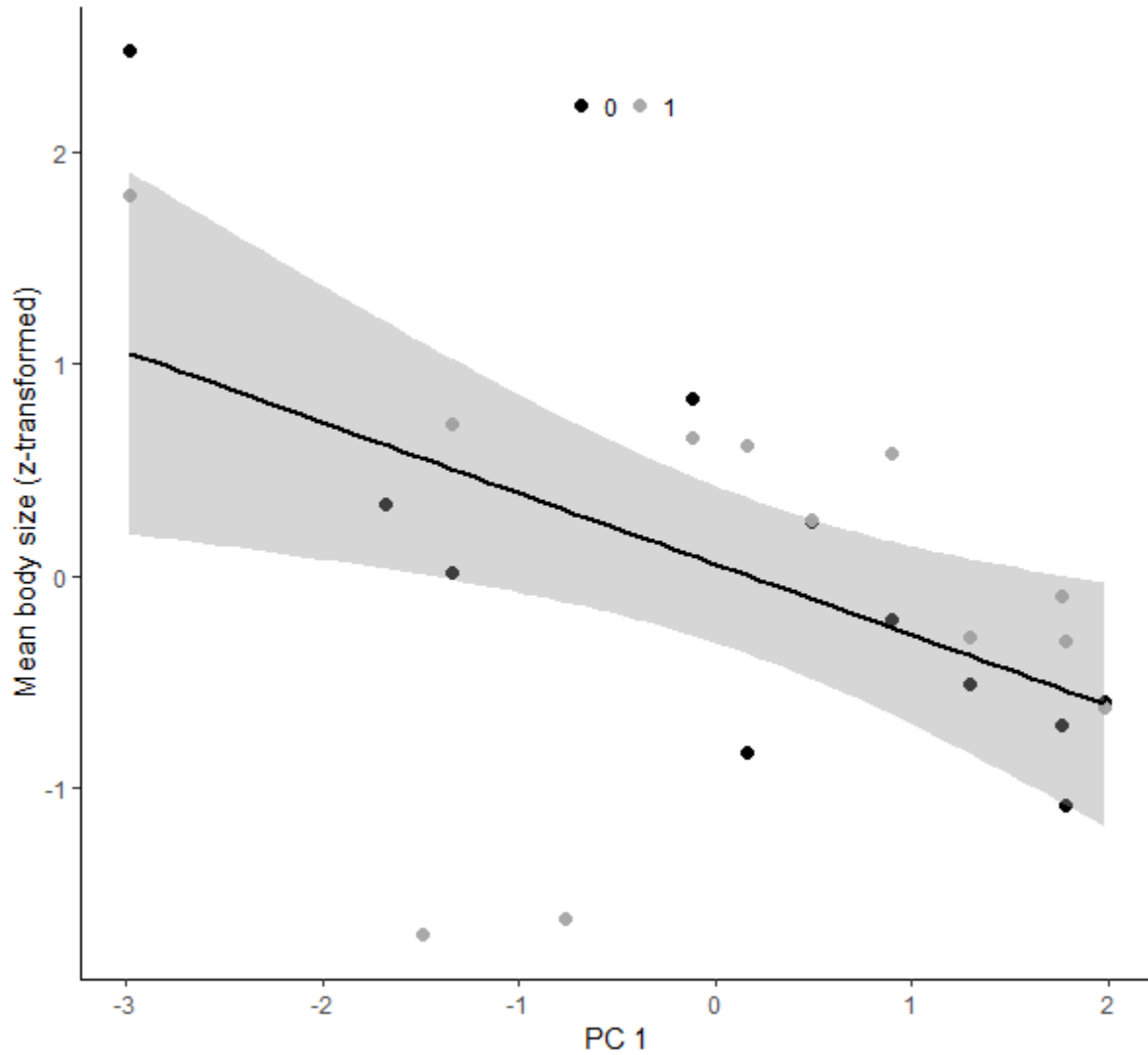Figure 4.4: Association of PC1 on z-score transformed growth (mm TL) of stoneroller (age-0 = black; age-1 = gray). PC axis 1 explains variation in stream flow, with positive loadings representing low flow and numerous days of no flow in both spring and summer.
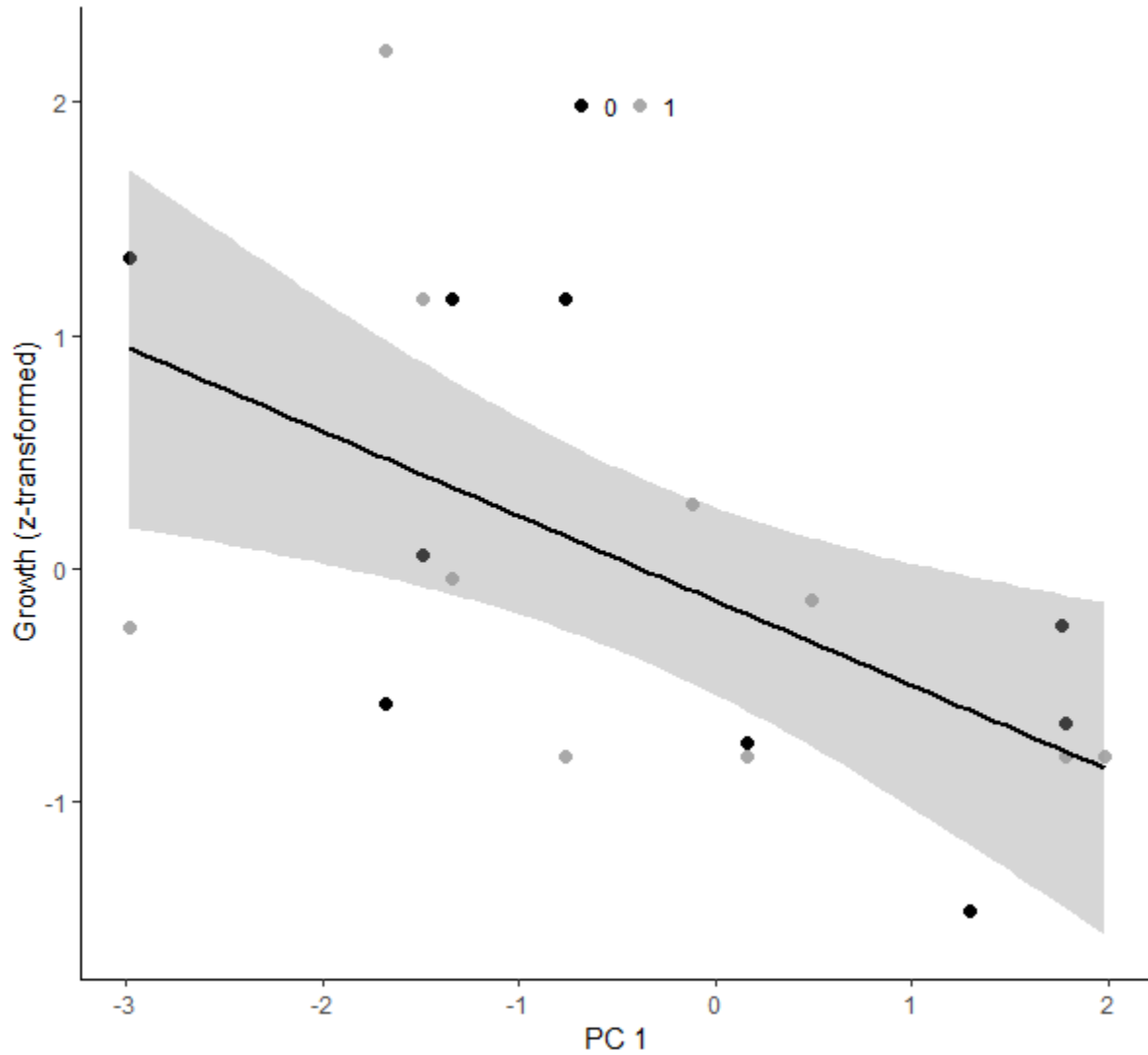
Table 4.1: Predicted mean size (mm TL), abundance (number of individuals/110 m^2), and growth (mm TL) in November for two age classes of dace and stoneroller across multiple sampling years. Years where a cohort of a species was not detected are represented as dashed lines. Mean growth was calculated only when a cohort was present in both August and November of the same year. Values averaged across sampling years are presented in the bottom row of the table.

| | **Dace** | | | | | | **Stoneroller** | | | | | |
| | *Age-0* | | | *Age-1* | | | *Age-0* | | | *Age-1* | | |
	Size	Abundance	Growth	Size	Abundance	Growth	Size	Abundance	Growth	Size	Abundance	Growth
2001	---	---	---	37.5	75	1.9	59.6	25	5.7	86.4	62	8.5
2002	29.4	25	6.9	52.1	19	6.3	49.2	90	5	87.5	20	---
2005	33.2	273	---	---	---	---	46	74	4.2	72.1	52	13.1
2006	28	94	0.3	50.2	231	3.4	47.6	148	4	74.8	174	0
2007	32	172	4.5	59.5	126	3.1	45.8	227	8.3	75.6	128	3.3
2010	41	312	11.9	69.4	340	---	47.6	104	8.7	82.6	433	2.4
2011	31.2	88	7.4	58.3	111	7.7	58.7	63	---	---	---	---
2012	30.1	415	3.2	50.3	580	4.4	48.5	96	2.1	73.4	105	---
2013	29.8	106	6.9	47.3	115	1.9		---	---	71.4	101	0
2014	28.9	162	2.2	58.6	315	0	41.8	184	3.8	77.5	47	0
2015	35	147	6.4	58.9	420	0.4	46.4	123	---	75.5	149	4.7
2016	---	---	---	38.2	261	---	48.3	86	8.3	79.5	128	0
2017	32.9	429	2.3	55.4	239	3.3	43.8	70	---	75.9	100	2.9
Avg	31.1	191	4.5	50.6	260	3.2	48.6	108	5.6	77.7	125	3.5

Table 4.2: The % of explained variability for PC axes 1, 2, and 3 (in parentheses), and the loading values and % contribution of each of 6 environmental parameters to these PC axes. Loadings with an associated % contribution ≥ 10% are highlighted in bold.

	PC1 (40.6%)		PC2 (26.4%)		PC3 (19.1%)	
	Loading	*% contribution*	*Loading*	*% contribution*	*Loading*	*% contribution*
Spring mean temperature	-0.158	1.1	**-0.844**	**45.0**	0.047	0.2
Summer mean temperature	-0.075	0.3	**-0.637**	**25.7**	**0.627**	**34.3**
Spring mean flow	**-0.681**	**19.1**	-0.157	1.6	**-0.650**	**36.9**
No flow days (spring)	**0.815**	**27.3**	0.389	9.5	0.186	3.1
Summer mean flow	**-0.736**	**22.3**	0.375	8.9	**0.503**	**22.1**
No flow days (summer)	**0.856**	**30.1**	-0.385	9.3	-0.198	3.4

Table 4.3: Models explaining variation across years for mean body size (mm TL), abundance (number of individuals) and growth (mm TL) of dace and stoneroller. Models within 2 ΔAIC_c units of the top model are also included in the table, as are model weights and the number of parameters (k) included in each model.

	Model	ΔAIC_c	k	Model weight
Dace size	SizeZ ~ PC1	0.00	3	0.65
Dace abundance	AbunZ ~ PC2	0.00	3	0.28
	AbunZ ~ 1	0.08	2	0.27
	AbunZ ~ PC1	1.52	3	0.13
Dace growth	GrowthZ ~ PC3	0.00	3	0.37
	GrowthZ ~ 1	1.39	2	0.19
Stoneroller size	SizeZ ~ PC3	0.00	3	0.54
Stoneroller abundance	AbunZ ~ 1	0.00	2	0.36
Stoneroller growth	GrowthZ ~ PC1	0.00	3	0.68

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Chapter 5 - Conclusions

Understanding how species respond to their environment is a fundamental goal of ecology, especially considering that environmental requirements can act to limit a species' distribution over ecological gradients. Temperature is an important abiotic variable that influences the ecology of fish, and changes to global mean temperatures resulting from climate change threatens to alter the distributions of freshwater fish species (Caissie 2006; Angilletta 2009). Grasslands are currently one of the world's most threatened biomes and understanding the thermal biology of fishes that inhabit grassland streams will aid in predicting how populations will respond to changes to thermal regimes (Dodds et al. 2004; Troia et al. 2016). In this dissertation, I used experimental methods to examine how the physiological mechanisms of two common species of minnows that occur in prairie streams differ along ecologically relevant gradients of temperature and in response to circadian fluctuations in temperature. Furthermore, I leveraged long-term ecological data to examine how vital rates important to populations of these fish responded to inter-annual variation in temperature and flow.

Differences in the realized thermal niches of ecologically similar species can potentially be explained by underlying differences to their fundamental thermal niches (Gvoždík 2018). In Chapter 2, I measured a suite of functional traits that contribute to the fundamental thermal niche along a thermal gradient in the southern redbelly dace (*Chrosomus erythrogaster*) and the central stoneroller (*Campostoma anomalum*). These species fill similar ecological roles where they co-occur but differ in their pattern of occurrence along a stream-size gradient of temperature, with stonerollers persisting into warmer downstream reaches while dace are more restricted to cool headwaters. Stonerollers exhibited higher critical thermal maxima than dace when acclimated to warm temperatures, indicative of a greater capacity to buffer thermal stress. Dace were more active than stonerollers at warmer temperatures. This pattern was counter to what we predicted but could be attributed to a less active lifestyle in the benthic stoneroller. Dace were better swimmers regardless of temperature, and energy metabolism did not differ between the species, indicating that these axes of the fundamental thermal niche may not be important contributors to observed interspecific differences in the realized thermal niches of dace and stonerollers. However, these experiments were performed on fish acclimated to constant thermal environments. Differences in the response of functional traits to acclimation gradients that include diurnal thermal variation could contribute to interspecific differences in thermal biology (Schulte et al. 2011).

In Chapter 3, I measured how metabolic rates of dace and stonerollers differed when acclimated to either constant thermal conditions or a diurnal thermal cycle representative of what fish would experience, on average, in the wild. I found evidence that metabolic rates in these species differ when fish are acclimated to diurnal fluctuations in temperature, and that dace and stonerollers respond differently to diurnal thermal variation. When acclimated to a diurnally variable thermal environment, both species exhibited higher standard metabolic rates when exposed to temperatures above the experienced diurnal range. Dace acclimated to a diurnal thermal cycle exhibited higher maximum metabolic rates and aerobic scope compared to when acclimated to constant thermal conditions. This result aligns well with other studies that indicate a plastic response of maximum metabolic rate after experiencing variable thermal environments that may allow species to maintain a larger window of energy availability under normal conditions (Guzzo et al. 2019). Additionally, these results emphasize the importance of considering thermal variation at small temporal scales and challenge the conventional practice of relying on constant thermal environments to make inferences about the thermal ecology and physiology of fishes.

Finally, in Chapter 4, I used multiple years of fish community and habitat data to understand how factors important to the population dynamics of dace and stoneroller differed in response to inter-annual variation in temperature and flow. Flow, but not temperature, drove differences in the average body size of dace and stonerollers and in the growth rates of stonerollers in a perennial reach of Kings Creek near Manhattan, KS. The average relative size of dace was smaller following a dry spring and summer. Stoneroller size decreased when access to riffle habitats, which this species associates with, was likely diminished (Martin et al. 2013). Stoneroller growth was lower following drought conditions, driven potentially by lack of access to riffles or by density-dependent factors (Martin et al. 2013; Pennock and Gido 2016.). These results emphasize the importance of considering multiple ecological factors when making predictions about what drives fluctuations in population dynamics over time. However, temperatures during the study period considered herein did not vary drastically from mean conditions.

These studies contribute to the conservation of aquatic biodiversity by highlighting how the response of functional traits along environmental gradients can help explain patterns of distribution and highlight how long-term data can provide insight into how species respond to multiple variable environmental factors over time.

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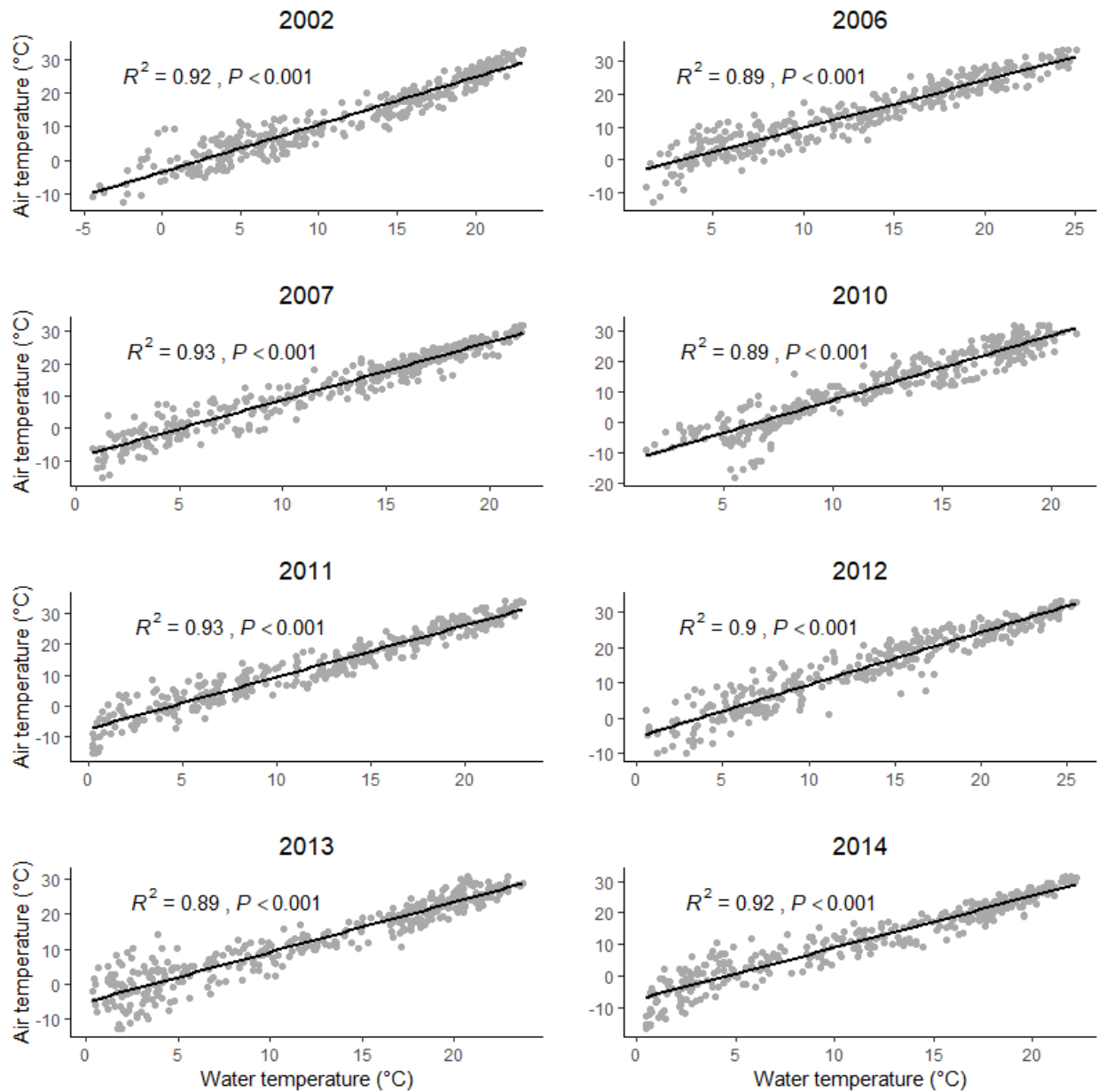# Appendix A - Chapter 4 supplemental tables and figures

Figure A.1: Scatter plots depicting the linear relationship between water and air temperatures (°C) for Kings Creek, USA for eight years between 2002 and 2014.
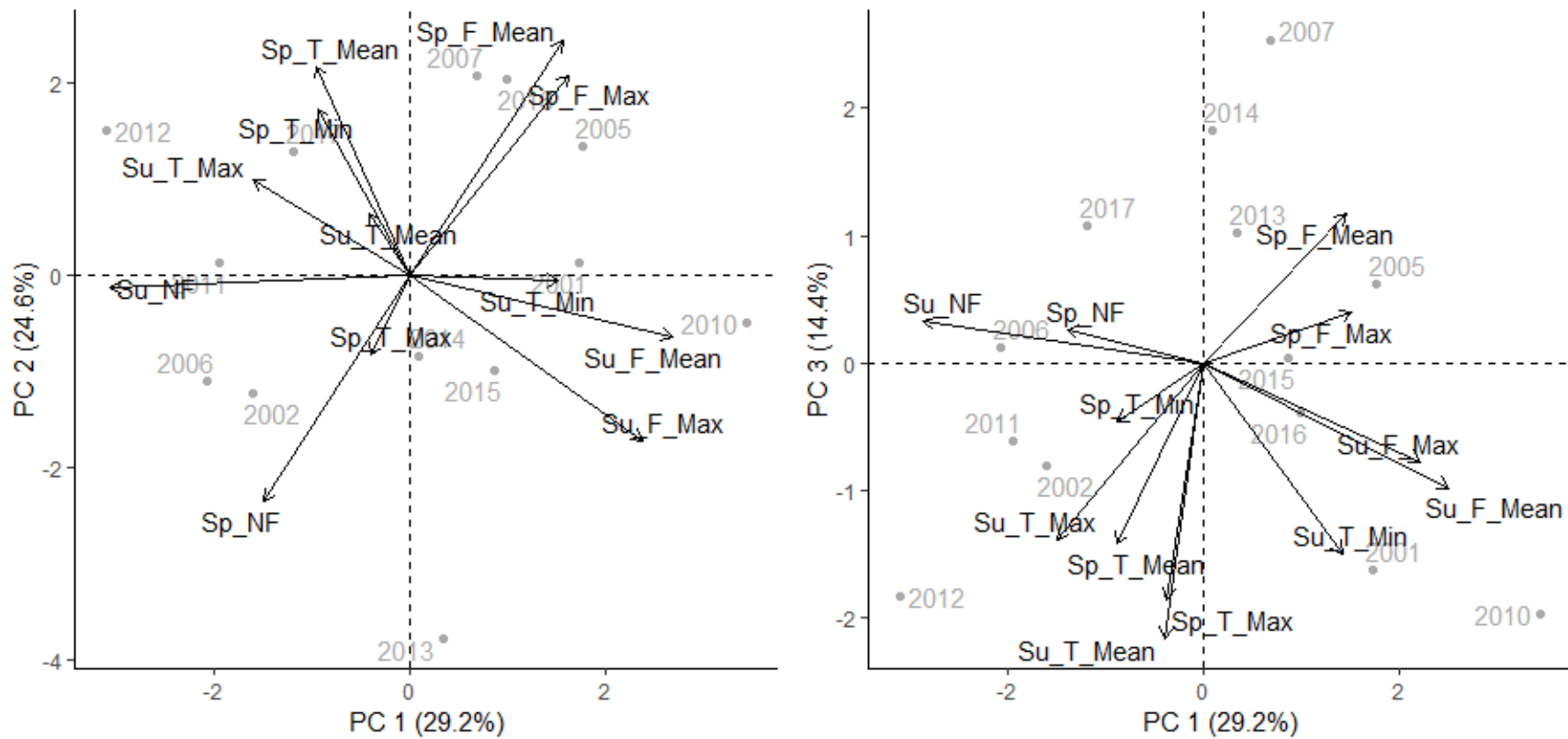
Figure A.2: Principal components analysis of environmental variables related to temperature and flow in Kings Creek, USA sampled across 13 years. Labels for environmental vectors are: Sp; spring, Su; summer; T; temperature (°C), F; flow (discharge; m^3/s), NF; the number of days without flow, Mean; the mean value, Max; the maximum recorded value, Min; the minimum recorded value. The left panel compares the first and second PC axes, and the right panel compares the first and third PC axes. The labeled gray data points represent the year.
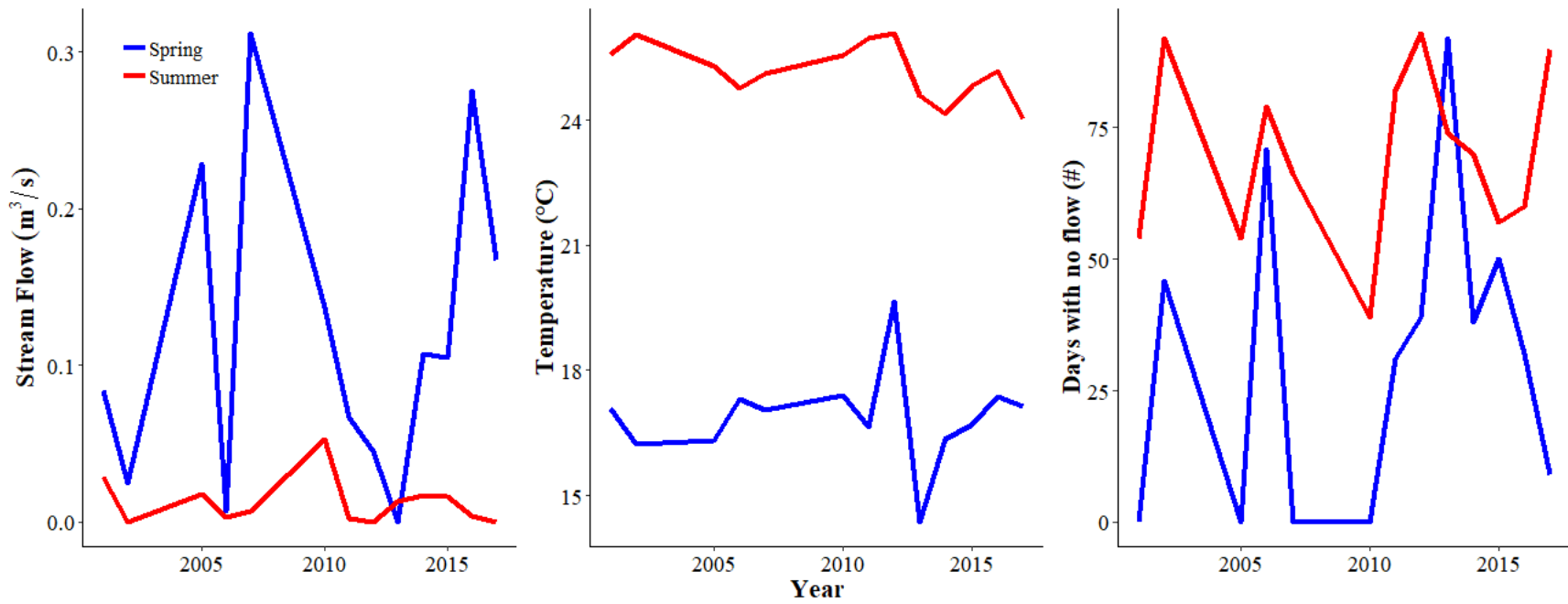
Figure A.3: Inter-annual variation in mean stream flow (m^3/s; left panel), mean temperature (°C; center panel), and the number of days with no flow (right panel) during spring (blue) and summer (red) in a perennial reach of Kings Creek between 2001 and 2017 included in the study.

Table A.1: Correlation matrix (Pearson's r) of the 12 environmental parameters included in the principle component analysis. Values for r are presented above the cross-diagonal. Labels for environmental parameters are: Sp; spring, Su; summer; T; temperature (°C), F; flow (discharge; m^3/s), NF; the number of days without flow, Mean; the mean value.

	Sp T_Mean	Su T_Mean	Sp F_Mean	Sp NF	Su F_Mean	Su NF
Sp_T_Mean	1.00	0.38	0.14	-0.36	-0.10	0.14
Su_T_Mean		1.00	-0.15	-0.19	0.04	0.04
Sp_F_Mean			1.00	-0.67	0.06	-0.43
Sp_NF				1.00	-0.38	0.42
Su_F_Mean					1.00	-0.84
Su_NF						1.00