

# Thermal tolerance depends on season, age and body condition in imperilled redbside dace *Clinostomus elongatus*

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Urbanization tends to increase water temperatures in streams and rivers and is hypothesized to be contributing to declines of many freshwater fishes. However, factors that influence individual variation in thermal tolerance, and how these may change seasonally, are not well understood. To address this knowledge gap, we studied redbside dace *Clinostomus elongatus*, an imperilled stream fish native to rapidly urbanizing areas of eastern North America. In wild redbside dace from rural Ohio, USA, acute upper thermal tolerance (i.e. critical thermal maximum, CT<sub>max</sub>) ranged between ~34°C in summer (stream temperature ~22°C) and 27°C in winter (stream temperature ~2°C). Juveniles had higher CT<sub>max</sub> than adults in spring and summer, but in winter, CT<sub>max</sub> was higher in adults. Thermal safety margins (CT<sub>max</sub> – ambient water temperature; ~11°C) were less than the increases in peak water temperature predicted for many redbside dace streams due to the combined effects of climate change and urbanization. Furthermore, behavioural agitation occurred 5–6°C below CT<sub>max</sub>. Safety margins were larger (>20°C) in autumn and winter. In addition, redbside dace were more sensitive (2.5°C lower CT<sub>max</sub>) than southern redbelly dace *Chrosomus erythrogaster*, a non-imperilled sympatric cyprinid. Body condition (Fulton's *K*) of adult redbside dace was positively correlated with CT<sub>max</sub>, but in juveniles, this relationship was significant only in one of two summers of experiments. Next, we measured CT<sub>max</sub> of captive redbside dace fed experimentally manipulated diets. In adults, but not juveniles, CT<sub>max</sub> was higher in fish fed a high- vs. low-ration diet, indicating a causal link between nutrition and thermal tolerance. We conclude that redbside dace will be challenged by predicted future summer temperatures, especially in urbanized habitats. Thus, habitat restoration that mitigates temperature increases is likely to benefit redbside dace. We also suggest habitat restoration that improves food availability may increase thermal tolerance, and thus population resilience.

**Key words:** Critical thermal maximum, endangered, energy stores, nutrition, species at risk

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## Introduction

Environmental temperature is one of the main factors that determines the habitat suitability and thus geographic distribution of fishes and other ectotherms (Fry, 1971; Bozinovic *et al.*, 2011). Climate change is therefore expected to affect the distribution and persistence of many species (Heino *et al.*, 2009; Bellard *et al.*, 2012; Sunday *et al.*, 2012). Temperature changes are predicted to have an especially pronounced effect on species with limited ability to shift distribution. For example, headwater stream fishes cannot move upstream to cooler water (Buisson *et al.*, 2008), and both natural and anthropogenic fragmentations of stream habitats can restrict shifts in species distribution and may limit recolonization after local extinction events (Zwick, 1992; Gido *et al.*, 2016). Stream fish communities are therefore thought to be structured in large part by thermal conditions (Kowalski *et al.*, 1978; Rahel and Hubert, 1991; Beauchene *et al.*, 2014) and may be especially sensitive to environmental warming (Dudgeon *et al.*, 2006).

In addition to the effects of climate change on stream temperatures, urbanized streams often experience additional thermal disruption as a result of land-use changes (e.g. high heat capacity of paved surfaces, reduced tree canopy cover) that can increase mean summer water temperatures by 5–8°C (Paul and Meyer, 2001; Mohajerani *et al.*, 2017). In the absence of adequate storm water infrastructure, rain falling on pavement can be heated appreciably and then run off into streams and rivers, which can cause their water temperatures to increase acutely by as much as 4–8°C (Poole and Berman, 2001; Nelson and Palmer, 2007; Hester and Bauman, 2013; Somers *et al.*, 2013). Increases in maximum environmental temperatures may be a key factor causing the local extinction of many species (Román-Palacios and Wiens, 2020). However, the thermal tolerances of many stream fishes are unknown, which limits our ability to evaluate habitat suitability or predict impacts of anthropogenic changes and thus population persistence of these fishes (Schulte *et al.*, 2011).

Thermal tolerance of fishes typically depends on thermal acclimation history, although the magnitude of thermal acclimation response varies amongst species (Beitinger *et al.*, 2000; Somero, 2005). Understanding seasonal changes in thermal tolerance is a major goal of conservation physiology, both to identify when animals are most susceptible to thermal stress, and because species-specific differences in the scope for thermal plasticity may influence susceptibility to habitat alteration and climate change (and Olden, 2017; Farrell and Franklin, 2016).

Thermal tolerance of fishes can also depend on intrinsic factors. For example, juvenile and subadult fishes are often more tolerant of warm conditions compared to larvae (e.g. Drost *et al.*, 2016; Moyano *et al.*, 2017) and sexually mature adults (Pörtner and Farrell, 2008). Sex differences in thermal tolerance are rarely reported (Hanson *et al.*, 2008), which could indicate a bias against publication of null results, but in

the few species for which sex differences have been explicitly studied, female fishes tend to be more thermally tolerant than males (Baker *et al.*, 1970; Benfey *et al.*, 1997; Nakajima *et al.*, 2009). Understanding how thermal tolerance varies amongst ages and sexes has important implications for predicting demographic effects of thermal stress in fish populations.

The amount of stored energy or food availability has long been thought to influence thermal tolerance of fishes and other ectotherms by increasing the ability of an animal to support the energy demands of mounting a physiological response (Hutchison, 1976; Pörtner and Peck, 2010; Nguyen *et al.*, 2017). However, few studies have explicitly tested this hypothesis, and thus far, the results from this handful of studies are mixed. Under aquaculture conditions, thermal tolerance of cutthroat trout *Oncorhynchus clarki henshawi* was correlated with body condition (Robinson *et al.*, 2008), and dietary enrichment can also increase thermal tolerance (Tejpal *et al.*, 2014; Kumar *et al.*, 2016). Similarly, food-restricted fishes often suffer decreased thermal tolerance (Woiwode and Adelman, 1992; Lee *et al.*, 2016). However, thermal tolerance of white sturgeon *Acipenser transmontanus* was unaffected by feeding ration (Lee *et al.*, 2016), and in *A. medirostris* thermal tolerance was lower at high feeding rations (Verhille *et al.*, 2015). Dietary enrichment also decreased thermal tolerance of barramundi *Lates calcarifer* (Gomez Isaza *et al.*, 2019). One possibility for the mixed results is that the effects of nutrition depend on fish size or thermal conditions (Robinson *et al.*, 2008; Verhille *et al.*, 2015), though this possibility has not been well investigated. Furthermore, these studies have all been conducted under highly artificial aquaculture conditions, and the relationship between energy reserves and thermal tolerance in wild fish is unknown. If such a relationship does exist in wild fish, it could have important implications for the management of imperilled species.

Redside dace *Clinostomus elongatus* are small minnows that were historically abundant in cool-water streams across much of northeastern North America, but populations have declined dramatically, and reddsides are now considered endangered in Canada (COSEWIC, 2007; Redside Dace Recovery Team, 2010) and are imperilled across much of their range in the United States (reviewed in Serrao *et al.*, 2018). Through most of the year, adult and juvenile reddsides primarily occupy pools, where they are found in mixed-species shoals (McKee and Parker, 1982). In the spring, however, reddsides move into shallow riffles where they spawn in gravel nests constructed by larger bodied fishes (Koster, 1939). Some other minnows, such as the southern redbelly dace *Chrosomus erythrogaster* also spawn in these nests, leading to occasional hybridization with reddsides (Koster, 1939; Trautman, 1957).

One hypothesis for the widespread decline of reddsides populations is thermal stress resulting from the impact of urbanization on adjacent waterbodies and exacerbated by climate change (Parker *et al.*, 1988; COSEWIC, 2007; Redside

Dace Recovery Team, 2010). Habitat degradation could also reduce food availability and/or increase energy expenditure of fishes (Du *et al.*, 2019), and therefore reduce body condition (Maceda-Veiga *et al.*, 2014) and indirectly affect thermal tolerance. However, our knowledge of reddsides dace thermal tolerance is limited to a single study of adult fish acclimated to varying temperatures under laboratory conditions (Novinger and Coon, 2000), and there is no published data about sex-specific or life-stage differences in thermal tolerance, or how these may change seasonally. Furthermore, very few studies have investigated how thermal tolerance is affected by body condition in any wild fish species. Thus, our first objective was to understand how thermal tolerance (measured as the critical thermal maximum, CT<sub>max</sub>) and body condition of wild reddsides dace changes seasonally, and whether this depends on age or sex. Next, to examine whether reddsides dace are uniquely susceptible to warming temperatures compared to similar species that are not imperilled, we compared the CT<sub>max</sub> of reddsides dace, redbelly dace and hybrids between these species. Finally, using both natural variation in body condition of wild fish (Fulton's *K*) and a captive feeding experiment, we investigated the hypothesis that thermal tolerance is dependent on body condition and energy reserves. We also asked whether the size of the namesake red spot on the side of reddsides dace is an indicator of body condition or thermal tolerance.

## Materials and Methods

### Animal collection and housing

Redsides dace were captured via seining from the north branch of the Kokosing River, Morrow County, Ohio, USA (40.545909, -82.654234) on five occasions between 2018 and 2019 (August 27–29, November 4–8, February 21–25, May 1–4, August 6–9). Although reddsides dace are threatened or endangered across most of their range, populations in Ohio are abundant and stable (Zimmerman, 2017). Captured fish ( $n = 30$ –50 per season, total  $n = 211$ , Table S1) were quickly (<20 min) transported in 20 L of buckets to a stream side laboratory for CT<sub>max</sub> measurements (see below for details). Juveniles and adults were differentiated in the field using body size and the presence (adults) vs. absence (juveniles) of red body pigmentation; sex of adults was confirmed after each trial via dissection. In June 2019, we also compared thermal tolerance of reddsides dace ( $n = 12$ ), southern redbelly dace *C. erythrogaster* ( $n = 14$ ) and hybrids between these two species ( $n = 9$ ). Hybrids of these species are known to occur in central Ohio and were identified by an overall body shape resembling that of reddsides dace, but with a smaller, terminal mouth and two black lateral stripes (Trautman, 1957; personal communication from Brian Zimmerman, University of Ohio).

During the first sampling period (August 2018), three temperature loggers (HOBO Water Temp Pro, Onset, Cape Cod, MA, USA) were deployed at the deepest points of

separate natural pools along the same ~500-m section of the Kokosing River where all experimental fish were captured. These temperature measurements were used to estimate ambient conditions experienced by reddsides dace and to calculate thermal safety margins (see below). Presence of reddsides dace in each of these pools was confirmed, via seining, when loggers were deployed.

To experimentally investigate the influence of body condition on thermal tolerance, an additional group of fish ( $n = 50$ ) was transported to the Freshwater Restoration Ecology Centre, University of Windsor, LaSalle, Ontario, Canada, in November 2018. These fish were held in round fibreglass tanks (850 L, 10–14°C, pH = 7.1–7.8) and fed frozen bloodworms and commercial fish flakes 3–4 times weekly for 8 months prior to experimentation. All experiments were approved by the University of Windsor Animal Care Committee, and collection permits were issued by the Ohio Department of Natural Resources Division of Wildlife.

### Thermal tolerance measurements

Thermal tolerance (CT<sub>max</sub>) was measured by increasing water temperature at a controlled rate (0.33°C/min) until fish could no longer remain upright in the water column (Becker and Genoway, 1979). This CT<sub>max</sub> approach has been widely used to assess thermal tolerance of fishes, as loss of equilibrium is considered to reflect the 'ecological death' of animals under natural conditions (Lutterschmidt and Hutchison, 1997). Furthermore, while the rate of heating in these trials exceeds what fishes are likely to experience under natural conditions, CT<sub>max</sub> measured using this protocol is typically a good predictor of ecological thermal limits (Pörtner and Peck, 2010). During each CT<sub>max</sub> trial, individual reddsides dace were confined within a mesh-sided plastic container (12 × 8 × 8 cm) that floated within a well-aerated and well-mixed water bath (VWR model 1203). Five fish were tested at a time and were able to see each other through the sides of the individual housing containers. Temperature and dissolved oxygen (always >80%) were continually monitored (YSI Pro Plus multi-parameter instrument, Yellow Springs, OH, USA) during each trial. In each trial, fish were first acclimated to the holding container for 15 min at ambient stream temperature before heating began. We selected a 15-min acclimation period because in preliminary trials (conducted in August 2018) we found no difference in CT<sub>max</sub> between adult reddsides dace acclimated for 15 vs. 60 min ( $33.7 \pm 0.1$  vs.  $33.5 \pm 0.2^\circ\text{C}$ ,  $n = 17$ ,  $t$ -test  $P = 0.51$ ). At CT<sub>max</sub>, fish were removed from the water bath, immediately euthanized via cerebral percussion, photographed, weighed, fixed in 10% buffered formalin for 48 h and stored in 70% ethanol for subsequent dissection. Thermal safety margins (Sandblom *et al.*, 2016) were calculated as the difference between CT<sub>max</sub> and the highest stream temperature we recorded in the 2 weeks preceding or following the CT<sub>max</sub> measurement.

In laboratory CT<sub>max</sub> trials (related to experimental manipulation of body condition, see below), fish were video recorded, and the temperature at which they first showed signs of behavioural agitation were noted (McDonnell and Chapman, 2015). Logistical limitations prevented video recording and measurement of agitation temperature in the field. In reddsides, thermal agitation was reflected by a characteristic burst of activity in which the fish began to vigorously swim into a corner of the holding container. The agitation window of each fish was calculated as the difference between CT<sub>max</sub> and agitation temperature. The size of the agitation window is inversely related to thermal tolerance, as a small window reflects that a fish experiencing acute warming can continue to engage in routine behaviours for a longer period before reaching CT<sub>max</sub> (Wells *et al.*, 2016).

### Manipulation of body condition

To directly test the link between body condition and thermal tolerance, we provided captive reddsides with low- or high-ration diets ( $n = 12$  adults and 12 juveniles per diet treatment) for 10 weeks (July 3–Sept 18, 2018) at 14°C before measuring CT<sub>max</sub>. To ensure identical temperature and environmental regimes between groups, fish were housed in two tanks with each subdivided in half, such that each tank contained low- and high-ration fish. The tanks were divided by fine mesh that prevented food from passing through, and both received water from a central filtration system that further ensured equal temperatures between tanks. The low-ration group was fed frozen bloodworms (~5–6% of total body mass) twice per week, while the high-ration group was fed the same amount of bloodworms 6–7 times per week. We chose to vary feeding frequency rather than quantity of food during a single feeding to minimize the effects of possible dominance hierarchies on access to food and ensure that all fish in each treatment group were able to acquire food at each feeding. Fish were held in groups as reddsides are a social species, and we wanted to minimize any effects of isolation stress on thermal tolerance (LeBlanc *et al.*, 2011; Yusishen *et al.*, 2020).

### Red spot size and organ investment

The area of the red spot (present in adults only) was measured using ImageJ (<https://imagej.nih.gov/ij/>) from photographs of the left side of each fish that were taken immediately after measurement of CT<sub>max</sub> (Beausoleil *et al.*, 2012). The same observer (C.N.) measured the area of each red spot twice, and the mean of these measurements was used.

To understand how overall body condition is influenced by investment in different organs, and how this pattern changes across seasons, each fish was carefully dissected, and the liver (an indicator of glycogen reserves), digestive tract (investment in feeding; gut contents were not removed) and gonads (reproductive investment) were removed and weighed. The remaining carcass was also weighed as an indicator of muscle mass. Formalin fixation and ethanol storage inevitably reduce tissue mass, but loss of mass tends to stabilize after

3 weeks (Rombough, 1994). Thus, we conservatively waited 6 weeks before taking final organ mass measurements, which underestimate fresh mass. Careful dissection and weighing of fresh tissues in the field were not logistically possible. Fresh (measured in the field) and fixed (immediately before dissection) whole-body masses were highly correlated ( $r = 0.997$ ) and decreased by an average of  $6.6 \pm 1.2\%$  (mean  $\pm$  SEM) after fixation.

### Calculations and statistical analysis

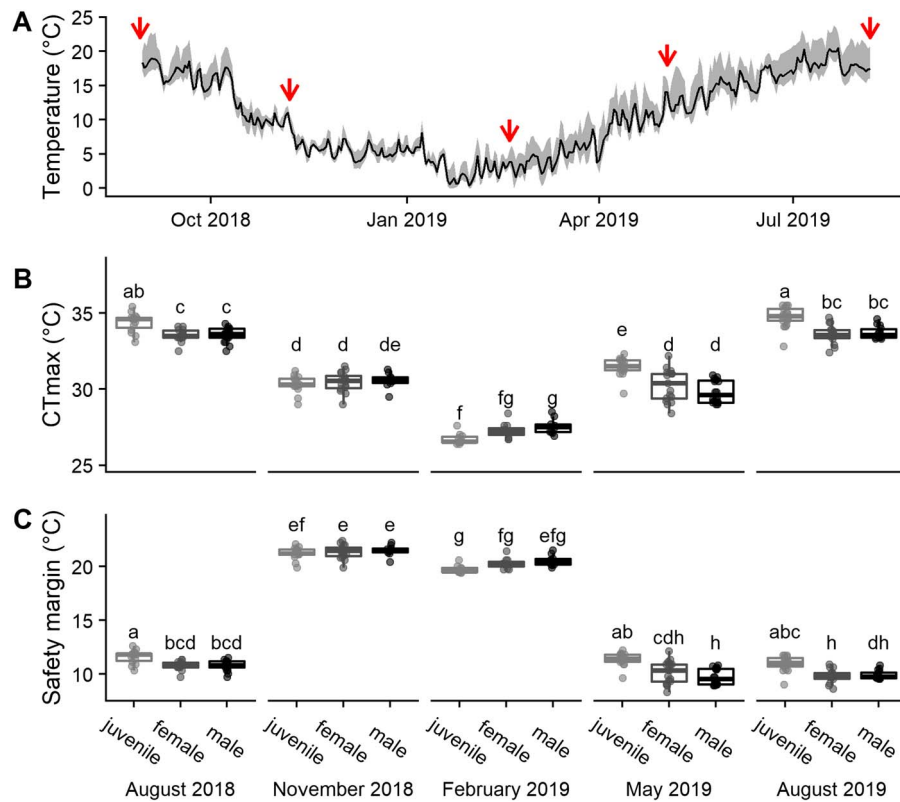
Body condition was calculated from fresh mass ( $M$ ) and standard length ( $L$ ) using two approaches: the traditional Fulton's condition factor ( $K = M/L^3 \times 100$ ) and relative condition factor ( $K_{rel} = M/aL^b$ ; where  $\log(a) = \text{intercept}$  and  $b = \text{slope}$  calculated from the  $\log(L)$  vs.  $\log(M)$  relationship) as recommended by Froese (2006).  $K_{rel}$  was calculated independently for both adults and juveniles. The reddsides dace scaling exponents we calculated were very close to the value of  $b = 3$  assumed by Fulton's  $K$  (adult  $b = 2.93$ , 95% CI = 2.70–3.16; juvenile  $b = 3.15$ , 95% CI = 2.94–3.36; Fig. S1), and therefore  $K$  and  $K_{rel}$  were highly correlated (adult  $R^2 = 0.997$ , juvenile  $R^2 = 0.967$ ). For simplicity and ease of comparison with previous studies (e.g. Beausoleil *et al.*, 2012), we therefore used Fulton's  $K$  for all subsequent statistical analyses. The results of our analyses were qualitatively similar if  $K_{rel}$  was used instead of Fulton's  $K$  (data not shown).

Differences in CT<sub>max</sub>, thermal safety margins and body condition between sexes, ages and seasons (and all interactions) were evaluated using linear models in R (package lme4; Bates *et al.*, 2015) followed by *post hoc* Tukey's tests (package lsmeans; Lenth, 2016). Each of our five field visits (i.e. August 2018, November 2018, February 2019, May 2019, August 2019) were treated as independent samples. To account for possible variation amongst trials (because five fish were tested per trial), trial number was included as a random effect for all analyses; no other covariates were included in these analyses.

To evaluate the relationship between body condition and CT<sub>max</sub>, adult and juvenile reddsides dace were analyzed separately due to a significant three-way interaction between body condition, age and season on CT<sub>max</sub> ( $F_{4,187} = 2.8$ ,  $P = 0.029$ ). Sex and season (and all interactions) were included as covariates for the analysis of adults; only season was included in the juvenile model. As before, trial number (because five fish were tested per trial) was included as a random effect for all analyses.

To assess seasonal changes in absolute red spot size and organ investment, body length was included as a covariate to account for differences in fish size. Adults and juveniles were analyzed separately due to statistical interactions between age and season, and the non-overlapping size ranges of these age classes. To assess the relative contributions of carcass, digestive tract, liver and gonad mass to overall body condition, we performed Pearson correlations using organ masses adjusted





**Figure 1:** Seasonal variation in water temperature, reddsides thermal tolerance and thermal safety margins. **(A)** Water temperature at our study site. The black line indicates daily mean temperature averaged from three pools within a ~500-m stretch of river where dace were captured. The shaded grey region represents the full range of daily temperatures. Red arrows show time points when thermal tolerance experiments were conducted. **(B)** Critical thermal maximum (CT<sub>max</sub>) of juvenile (light grey) and adult reddsides dace (female in dark grey and males in black). **(C)** Thermal safety margins (CT<sub>max</sub> – ambient stream temperature) of reddsides dace. Different letters represent significant differences between groups ( $P < 0.05$ ). For each boxplot, dots show individual data points, the bold horizontal line in the middle of the box represents the median, the top and bottom of the box represent the first and third quartiles, and the whiskers show the highest and lowest values within  $1.5 \times$  the interquartile range.

to the common standard length of each age and sex (i.e. juvenile = 4.55, female = 7.68, male = 7.39 cm).

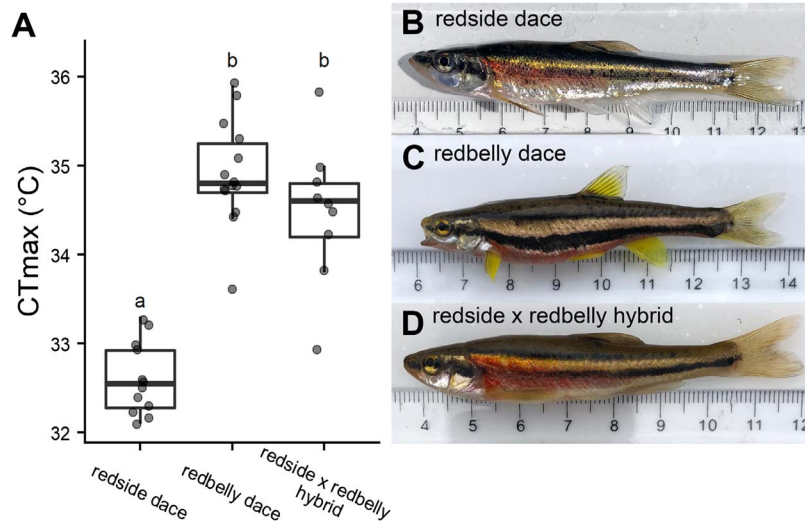
Differences in CT<sub>max</sub> and body condition between fish fed low- and high-ration diets were evaluated with linear models as described above. Two acclimation tanks were used per treatment; these along with trial number (nested within tank) were included as random effects in our statistical models.

## Results

### Thermal tolerance and thermal safety margins

Water temperature at our field site varied between a July high of 23.8°C to a January low of  $-0.06^\circ\text{C}$  (Fig. 1A). Mean daily temperature fluctuations were  $3.5 \pm 0.1^\circ\text{C}$ , and ranged between 0.1 and 7.5°C (Fig. 1A). Temperatures were highly similar amongst the three pools at our field site in which we deployed temperature loggers (Fig. S2).

Seasonal changes in CT<sub>max</sub> generally followed changes in water temperature, but the magnitude of seasonal variation differed between juveniles and adults (season  $\times$  age  $F_{8,157} = 6.8$ ,  $P < 0.0001$ ; Fig. 1B). In May and August, CT<sub>max</sub> was  $\sim 1^\circ\text{C}$  higher in juveniles than in adults (August 2018 juveniles vs. males  $t = 3.5$ ,  $P = 0.002$ , juveniles vs. females  $t = 3.5$ ,  $P = 0.002$ ; May 2019 juveniles vs. males  $t = 6.4$ ,  $P < 0.0001$ , juveniles vs. females  $t = 4.6$ ,  $P < 0.0001$ ; August 2019 juveniles vs. males  $t = 4.3$ ,  $P = 0.0001$ , juveniles vs. females  $t = 4.6$ ,  $P < 0.0001$ ). In February, however, CT<sub>max</sub> tended to be higher (by  $\sim 0.7^\circ\text{C}$ ) in adults (juveniles vs. males  $t = 2.5$ ,  $P = 0.035$ , juveniles vs. females  $t = 1.7$ ,  $P = 0.20$ ; Fig. 1B). There was no difference in CT<sub>max</sub> between adult males and adult females ( $F_{1,126} = 0.01$ ,  $P = 0.92$ , season  $\times$  sex  $F_{4,125} = 1.4$ ,  $P = 0.23$ ; Fig. 1B). Thermal safety margins in May and August ( $\sim 11^\circ\text{C}$ ) were only half of what they were in the winter ( $\sim 22^\circ\text{C}$ ; Fig. 1C). Due to their higher CT<sub>max</sub> in spring and summer, juveniles had  $\sim 1^\circ\text{C}$  higher safety margins than adults in May (juveniles vs. males  $t = 6.4$ ,  $P < 0.0001$ , juveniles vs. females  $t = 4.6$ ,  $P < 0.0001$ ) and



**Figure 2:** Thermal tolerance of reddsides dace *Clinostomus elongatus*, southern redbelly dace *Chrosomus erythrogaster* and their hybrids. (A) Critical thermal maximum (CT<sub>max</sub>) of fish captured from the wild in June 2019. Different letters represent significant differences between groups ( $P < 0.05$ ). For each boxplot, dots show individual data points, the bold horizontal line in the middle of the box represents the median, the top and bottom of the box represent the first and third quartiles, and the whiskers show the highest and lowest values within  $1.5 \times$  the interquartile range. (B–D) Representative photographs of each species and a hybrid. Ruler shows scale in centimetres.

August (2018 juveniles vs. males  $t = 3.5$ ,  $P = 0.002$ , juveniles vs. females  $t = 3.5$ ,  $P = 0.002$ ; 2019 juveniles vs. males  $t = 4.3$ ,  $P = 0.0001$ , juveniles vs. females  $t = 4.6$ ,  $P < 0.0001$ ), but adult safety margins tended to be larger in February (juveniles vs. males  $t = 2.5$ ,  $P = 0.035$ , juveniles vs. females  $t = 1.7$ ,  $P = 0.20$ ; Fig. 1C).

CT<sub>max</sub> of reddsides dace was  $\sim 2^\circ\text{C}$  lower than that of redbelly dace (overall  $F_{2,29} = 59.4$ ,  $P < 0.0001$ ; *post hoc*  $t = 10.5$ ,  $P < 0.001$ ; Fig. 2), and that of reddsides  $\times$  redbelly hybrids ( $t = 7.6$ ,  $P < 0.0001$ ). CT<sub>max</sub> of hybrids was not different from that of redbelly dace ( $t = 1.8$ ,  $P = 0.17$ ; Fig. 2).

### Body condition affects thermal tolerance

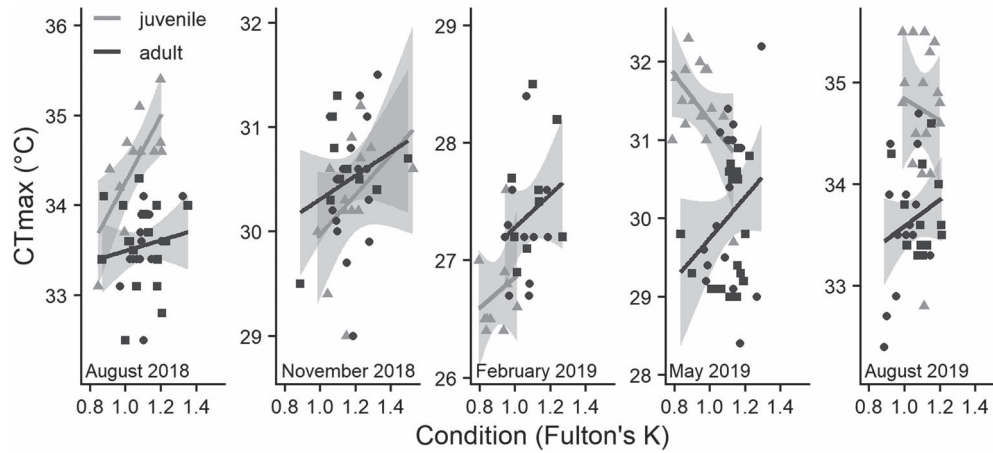
Body condition of wild adult reddsides dace was positively correlated with CT<sub>max</sub> (overall slope =  $1.54 \pm 0.62$ ,  $F_{1,120} = 6.37$ ,  $P = 0.013$ ), and this relationship did not depend on season or sex (condition  $\times$  sex  $F_{1,120} = 1.64$ ,  $P = 0.20$ ; condition  $\times$  season  $F_{4,118} = 0.24$ ,  $P = 0.91$ ; condition  $\times$  season  $\times$  sex  $F_{4,120} = 0.34$ ,  $P = 0.85$ ; Fig. 3). In juvenile fish, the relationship between body condition and CT<sub>max</sub> depended on season (condition  $\times$  season  $F_{4,56} = 3.5$ ,  $P = 0.013$ )—there was a positive correlation in August 2018 (slope =  $3.34 \pm 1.39$ ,  $t = 2.4$ ,  $P = 0.020$ ), a negative correlation in May 2019 (slope =  $-3.46 \pm 1.69$ ;  $t = 2.0$ ,  $P = 0.046$ ), and no statistically significant relationship at any other time (all  $P > 0.10$ ). Body size was not related to CT<sub>max</sub> in either juveniles (length  $F_{1,60} = 0.5$ ,  $P = 0.46$ , mass  $F_{1,55} = 0.16$ ,  $P = 0.78$ ) or adults (length  $F_{1,116} = 0.2$ ,  $P = 0.69$ , length  $\times$  sex  $F_{1,121} = 0.8$ ,  $P = 0.37$ , mass  $F_{1,119} = 0.1$ ,  $P = 0.73$ , sex  $\times$  mass  $F_{1,121} = 0.09$ ,  $P = 0.76$ ; Fig. S3). Area of the lateral red spot was not a

significant predictor of CT<sub>max</sub> ( $F_{1,114} = 1.2$ ,  $P = 0.28$ , spot size  $\times$  season  $F_{4,111} = 1.3$ ,  $P = 0.26$ ; Fig. S4A). Red spot area was positively correlated with body condition only in May 2019 (spot size  $\times$  season  $F_{4,104} = 3.4$ ,  $P = 0.022$ , May *post hoc* slope =  $0.14 \pm 0.05$ ,  $t = 3.1$ ,  $P = 0.003$ ; Fig. S4B).

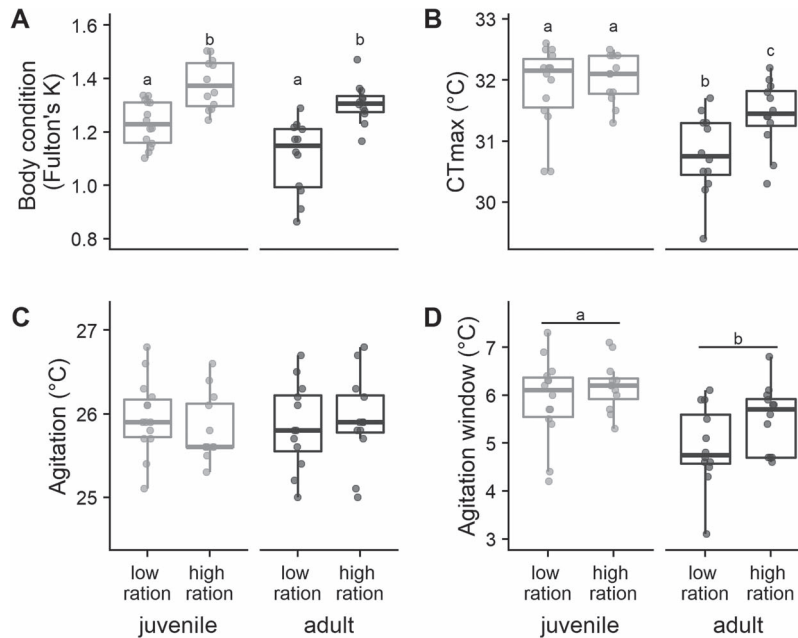
Supplemental feeding of captive reddsides dace caused a significant increase ( $\sim 15\%$ ) in the body condition of both juveniles ( $F_{1,23} = 23.1$ ,  $P = 0.0002$ ) and adults ( $F_{1,17} = 28.6$ ,  $P < 0.0001$ ; Fig. 4A). CT<sub>max</sub> of high-ration, high-condition adults was  $\sim 0.6^\circ\text{C}$  higher than CT<sub>max</sub> of low-ration adults ( $F_{1,19} = 7.3$ ,  $P = 0.014$ ; Fig. 4b), but there was no difference in CT<sub>max</sub> between juveniles fed different rations ( $F_{1,17} = 2.2$ ,  $P = 0.16$ ). There was no effect of supplemental feeding on agitation temperature of juveniles ( $F_{1,18} = 0.6$ ,  $P = 0.44$ ) or adults ( $F_{1,18} = 0.2$ ,  $P = 0.63$ ; Fig. 4c) or on the agitation window (juvenile  $F_{1,16} = 2.1$ ,  $P = 0.17$ , adult  $F_{1,20} = 3.3$ ,  $P = 0.09$ ; Fig. 4D). However, the agitation window was  $\sim 0.8^\circ\text{C}$  larger in juveniles than adults ( $F_{1,40} = 20.2$ ,  $P < 0.0001$ ; Fig. 4D).

### Seasonal variation in body condition and organ size

Seasonal changes in body condition (Fulton's  $K$ ) depended on the age and sex of reddsides dace (interaction  $F_{8,193} = 6.0$ ,  $P < 0.0001$ ; Fig. 5A). Males maintained body condition throughout the year (all  $t < 1.3$ ,  $P > 0.67$ ), while females tended to be in the best condition in November and lowest in August (Fig. 5A). Juvenile condition was significantly lower ( $\sim 15\%$ ) in February and May than in August or November (all  $t > 3.0$ ,  $P < 0.01$ ; Fig. 5A). Relative carcass mass (body mass – organ masses), which presumably reflects



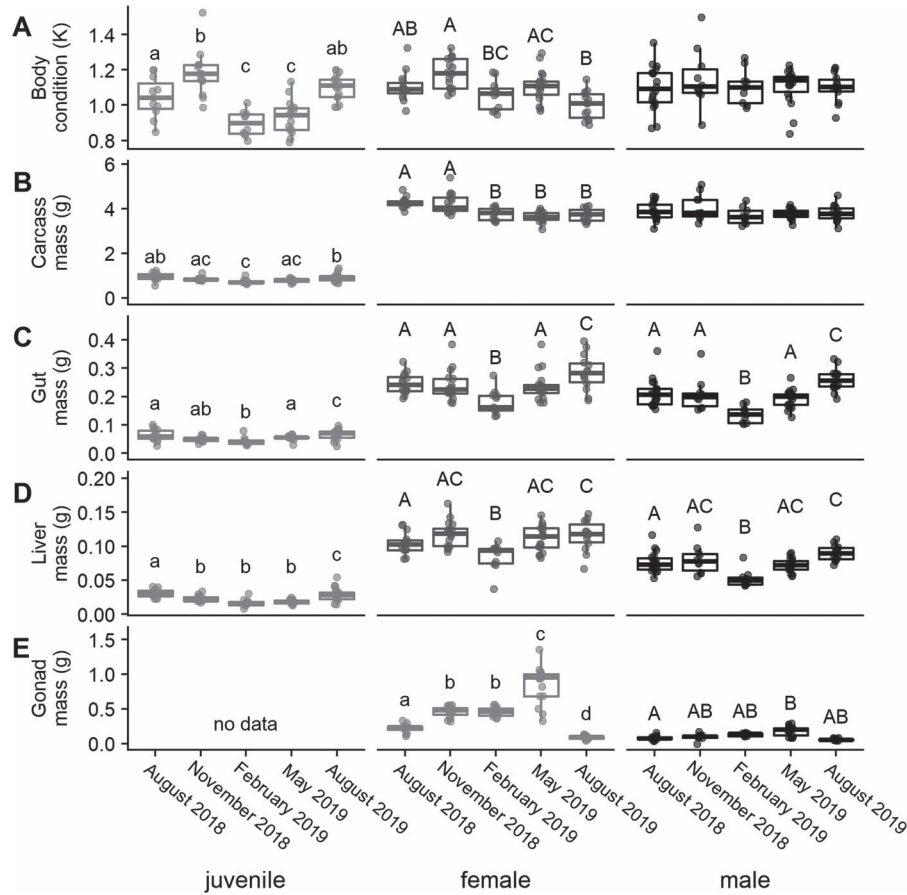
**Figure 3:** Thermal tolerance of adult redside dace is correlated with body condition (Fulton's  $K$ ;  $M/L^3$ , where  $M$  is body mass and  $L$  is standard length) across seasons. Grey triangles indicate juveniles, black circles indicate adult females and black squares indicate adult males. Grey bands indicate 95% confidence intervals. There was a positive relationship between body condition and critical thermal maximum (CTmax) in adults ( $P = 0.013$ ). In juveniles, there was a significant positive relationship in August 2018 ( $P = 0.020$ ), a negative relationship in May 2019 ( $P = 0.046$ ), and no significant relationship at any other time (all  $P > 0.05$ ).



**Figure 4:** Supplemental feeding increases body condition and thermal tolerance of adult redside dace. **(A)** Body condition ( $M/L^3$ , where  $M$  is body mass and  $L$  is standard length) of juvenile and adult redside dace fed twice per week (low ration) or daily (high ration). **(B)** Critical thermal maximum (CTmax) of redside dace. **(C)** Agitation temperature of redside dace. **(D)** Agitation window (i.e. difference between agitation temperature and CTmax) of redside dace. Different letters represent significant differences between groups ( $P < 0.05$ ). For each boxplot, dots show individual data points, the bold horizontal line in the middle of the box represents the median, the top and bottom of the box represent the first and third quartiles, and the whiskers show the highest and lowest values within  $1.5 \times$  the interquartile range.

variation in the amount of swimming muscle, was the lowest in juveniles in February, but there was no consistent pattern in adults (age  $\times$  season  $F_{8,194} = 5.8$ ,  $P < 0.0001$ ; Fig. 5B). After controlling for variation in body size, both gut (age  $\times$  season  $F_{8,194} = 4.4$ ,  $P < 0.0001$ ) and liver (age  $\times$  season  $F_{8,194} = 4.3$ ,  $P < 0.0001$ ) masses were generally the smallest

in February in both juveniles and adults (Fig 5C and D). Gonad size changed amongst seasons in a sex-dependant pattern (sex  $\times$  season  $F_{4,130} = 43.5$ ,  $P < 0.0001$ ; Fig. 5E). In females, ovary mass approximately doubled between August and November ( $t = 6.2$ ,  $P < 0.0001$ ), followed by a second doubling between February and May ( $t = 9.7$ ,  $P < 0.0001$ ;



**Figure 5:** Seasonal changes in reddsides dace body condition and organ size. Organ size data in panels B–E are size-corrected to the mean length of each group (juvenile = 4.55, female = 7.68, male = 7.39 cm). Different lowercase letters represent significant differences amongst seasons within juveniles, and different capital letters represent significant differences within adults ( $P < 0.05$ ). A lack of letters indicates no seasonal differences ( $P > 0.05$ ). Juveniles and adults were not statistically compared. For each boxplot, dots show individual data points, the bold horizontal line in the middle of the box represents the median, the top and bottom of the box represent the first and third quartiles, and the whiskers show the highest and lowest values within  $1.5 \times$  the interquartile range.

Fig. 5E). In males, gonad size was constant from August to February (all  $t < 2.3$ ,  $P > 0.15$ ), but approximately doubled during spawning season in May ( $t = 3.1$ ,  $P = 0.019$ ; Fig. 5E). Gonads in juveniles were too small to be reliably dissected or weighed.

In general, body condition of reddsides dace was positively correlated with the mass of the carcass, digestive tract, liver and gonad (in adults), though the strength of these correlations depended on age, sex and season (Table 1).

## Discussion

Understanding the thermal tolerance of fishes is an important first step for predicting population level responses to warming caused by habitat alteration and climate change, especially for species at risk (e.g. Schulte *et al.*, 2011). We found that reddsides dace show considerable seasonal plasticity in thermal toler-

ance. Seasonal changes in CT<sub>max</sub> were largest in juvenile fish, which had higher CT<sub>max</sub> than adults in summer, but lower CT<sub>max</sub> than adults over winter. Summer CT<sub>max</sub> was also lower than predicted peak water temperatures for urbanized reddsides dace streams due to the combined effects of climate change, land-use changes and storm water run-off. Our study also provides the first evidence that body condition plays an important role in determining the thermal tolerance of wild fish, though the mechanism linking these traits remains unknown. Thus, habitat degradation that decreases food availability or otherwise reduces fish body condition might exacerbate the effects of warming.

## Thermal tolerance varies across seasons and ages

Seasonal changes in thermal tolerance of reddsides dace generally followed ambient water temperature. Although there are



**Table 1:** Summary of correlations between body condition and carcass or organ masses in redbside dace across an annual cycle.

		Carcass	Digestive tract	Liver	Gonad
Juveniles	August 2018	0.15 (0.66)	−0.13 (0.71)	−0.20 (0.56)	NA
	November 2018	<b>0.88 (&lt;0.001)</b>	0.31 (0.30)	0.53 (0.06)	NA
	February 2019	0.62 (0.06)	0.42 (0.23)	0.53 (0.12)	NA
	May 2019	0.58 (0.02)*	0.47 (0.08)	0.24 (0.39)	NA
	August 2019	<b>0.68 (0.003)</b>	0.24 (0.36)	0.12 (0.64)	NA
Females	August 2018	<b>0.91 (&lt;0.001)</b>	0.45 (0.11)	0.00 (0.99)	0.71 (0.004)*
	November 2018	<b>0.84 (&lt;0.001)</b>	0.44 (0.08)	0.30 (0.25)	0.10 (0.71)
	February 2019	<b>0.86 (&lt;0.001)</b>	0.48 (0.14)	0.64 (0.03)*	<b>0.84 (0.001)</b>
	May 2019	0.38 (0.13)	0.34 (0.18)	0.29 (0.25)	<b>0.70 (0.002)</b>
	August 2019	<b>0.93 (&lt;0.001)</b>	<b>0.74 (0.002)</b>	<b>0.76 (0.002)</b>	0.58 (0.03)*
Males	August 2018	<b>0.89 (&lt;0.001)</b>	0.49 (0.04)*	<b>0.68 (0.002)</b>	<b>0.81 (&lt;0.001)</b>
	November 2018	0.77 (0.009)*	0.54 (0.11)	0.48 (0.16)	0.35 (0.32)
	February 2019	<b>0.96 (&lt;0.001)</b>	0.79 (0.01)*	0.57 (0.11)	0.72 (0.03)*
	May 2019	<b>0.75 (&lt;0.001)</b>	0.22 (0.38)	0.44 (0.06)	0.48 (0.04)*
	August 2019	<b>0.93 (&lt;0.001)</b>	−0.05 (0.86)	0.19 (0.52)	0.02 (0.93)

Values are Pearson correlation coefficients, with *P* values given in parentheses. Significant relationships before Bonferroni correction for multiple comparisons (*P* < 0.05) are noted with asterisks, and significant relationships after Bonferroni correction (juveniles *P* < 0.0033; adults *P* < 0.0025) are shown in bold.

few comparative data for other cyprinids, seasonal patterns of thermal tolerance in sympatric redbelly dace (Scott, 1987) and the shiners *Notropis stramineus* and *N. cornutus* (Kowalski et al., 1978) follow a similar pattern to what we measured in redbside dace. Furthermore, our measurements of CTmax in Ohio redbside dace were comparable to previously reported values CTmax of adult redbside dace captured in New York State and laboratory-acclimated to similar temperatures (6, 12, 20°C) as we measured in the winter, spring/fall and summer in Ohio (Novinger and Coon, 2000). These populations (Ohio and New York) are genetically distinct and probably diverged around 500,000 years ago (Serrao et al., 2018).

Age only affected thermal tolerance at temperature extremes in the peak of summer and winter. The acclimation response ratio ( $\Delta\text{CTmax}/\Delta\text{water temperature}$ ), a metric of the ability of fishes to adjust thermal tolerance, was ~30% higher in juveniles (~0.53) compared to adults (~0.41). These values are similar to the average response ratios of 0.37 recently reported for 82 freshwater fishes (Comte and Olden, 2017) and 0.34 for 111 freshwater and marine species (Morley et al., 2019). These response ratios also generally predict the thermal tolerance of the fish we used in the laboratory feeding study, which were acclimated at 14°C and had CTmax values ~1–2°C higher than wild fish in November and May when mean water temperature was similar at 10–11°C. While few studies have investigated the thermal acclimation capacity of different life stages, our

results are opposite to the pattern reported in imperilled longfin dace *Agosia chrysogaster*, in which juveniles had lower CTmax and acclimation response ratios than adults (Troia et al., 2015). The physiological and ecological significance of these intra- and interspecific differences is uncertain but may reflect a trade-off between maximum and minimum temperature tolerances (Gutiérrez-Pesquera et al., 2016). In this view, decreased CTmax of juvenile redbside dace in winter may represent adaptive plasticity that enhances tolerance of cold conditions. Regardless, these results highlight the need to evaluate thermal tolerances of different life stages independently for different fish species, as generalizations may not be possible.

Thermal safety margins, representing the maximum survivable acute increase in temperature, were relatively small (~10–11°C) for both juvenile and adult redbside dace when considering that storm water inputs in urban areas can acutely raise water temperatures by up to 8°C (Hester and Bauman, 2013), and climate change is projected to further increase summer air temperatures in redbside dace habitat by 2–7°C by the end of the century (Zhang et al., 2019). These air temperature increases are expected to raise stream water temperatures by 2–5°C, depending on the specific local characteristics of each stream (e.g. hydrology, hydrogeology, surrounding landscape; Stoneman and Jones, 1996; Morrill et al., 2005; Chu et al., 2009). The thermal safety margins we calculated for redbside dace are similar to those reported for other mid-latitude cyprinids (average

~9°C) and freshwater fishes in general (~8°C), calculated from the dataset aggregated by Morley *et al.* (2019). However, thermal safety margins may overestimate the ability of animals to cope with warming environments, as we noticed behavioural agitation at temperatures 5–6°C below CT<sub>max</sub>. This suggests that routine behaviours of redbreasted sunfish may be altered at temperatures only ~5°C above the maximum stream temperatures we recorded at our rural field site. This agitation window is similar to what has been reported for other fishes (e.g. McDonnell and Chapman, 2015; Wells *et al.*, 2016; McDonnell *et al.*, 2019), and transcriptomic responses to thermal stress can also be induced 4–6°C below CT<sub>max</sub> (Komoroske *et al.*, 2015), indicating sublethal effects that may impact future survival and reproduction. While agitation windows are not as commonly reported in the literature as thermal safety margins, behavioural agitation likely impact population-level responses to thermal stress and is an important area for future research. Finally, our heating rate of 0.33°C/min, while commonly used in thermal tolerance studies, may overestimate tolerance to chronically elevated temperatures when damage has more time to accrue (Beitinger *et al.*, 2000; Bates and Morley, 2020). Thus, our results indicate that the combined thermal consequences of climate change, urbanization and storm water run-off poses a serious threat to the persistence of redbreasted sunfish.

### Body condition influences thermal tolerance

We found a clear relationship between body condition and thermal tolerance of adult redbreasted sunfish. In wild fish, there was a robust positive relationship between body condition and CT<sub>max</sub> of adults that was consistent throughout the year. In the laboratory, CT<sub>max</sub> of fish from the same source population could be manipulated by varying feeding ration and thus body condition, suggesting that thermal tolerance is determined in part by nutritional status. These results are consistent with previous laboratory studies that show decreased thermal tolerance of fishes after food restriction (Woiwode and Adelman, 1992; Lee *et al.*, 2016). Thermal tolerance of redbreasted sunfish was independent of body mass and length, consistent with most previous studies of fishes (e.g. Recsetar *et al.*, 2012; Chrétien and Chapman, 2016). Notably, supplemental feeding did not affect the agitation temperature or agitation window of redbreasted sunfish, suggesting that the mechanisms that determine behaviourally stressful temperatures are at least somewhat decoupled from ultimate thermal limits. Therefore, while our results highlight that nutritional status is an important consideration when assessing thermal limits of fishes, temperatures that cause disruption of routine fish behaviour may be independent of food availability.

Contrary to the pattern in adults, body condition did not generally influence thermal tolerance of juvenile redbreasted sunfish. This contrast may reflect age-related differences in energy allocation and thus the utility of body condition for

approximating energy reserves. Body condition can be a good predictor of fat and protein content (e.g. Pangle and Sutton, 2005), but it is not always reliable (Sutton *et al.*, 2000; Trudel *et al.*, 2005). Adults body condition was always positively correlated with carcass mass (i.e. body without viscera) and was generally correlated with organ size (Table 1), and thus probably indicates overall energy reserves. However, juvenile body condition was only related to carcass mass. Redbreasted sunfish grow rapidly and attain 50–70% of their final adult size in the first year of life (Dieterman *et al.*, 2018). In juveniles, available energy may therefore be allocated primarily to somatic growth to increase overall body length, rather than to growth or fuel storage in other tissues and organs. Body condition may therefore be a poor indicator of energetic status in juveniles.

There is no single physiological explanation for the upper thermal limits of ectotherms (Gangloff and Telemeco, 2018), and therefore, the mechanism linking body condition and thermal tolerance in adult redbreasted sunfish is unclear. The oxygen and capacity limitation of thermal tolerance hypothesis suggests that thermal tolerance is determined by the ability of ectotherms to maintain tissue oxygen supply in the face of increasing demand at high temperatures (Pörtner *et al.*, 2017). Assuming body condition reflects available energy reserves, one possibility is that high-condition fish have a greater capacity to increase gill ventilation and cardiac output to enhance oxygen supply. Large energy reserves could also enhance anaerobic capacity and prolong survival at temperatures that exceed the limits of aerobic metabolism. An alternative hypothesis is that maximum thermal limits are reached when animals can no longer maintain cell integrity or membrane potential of the nervous system (Gangloff and Telemeco, 2018; Jutfelt *et al.*, 2019). In this view, faster and/or larger mobilization of energy stores in relatively high-condition redbreasted sunfish may help defend cellular integrity in the face of thermal stress, thus increasing CT<sub>max</sub>. Finally, dietary supplementation of the serotonin-precursor tryptophan has been shown to dampen the stress response to acute warming and increase CT<sub>max</sub> (Tejpal *et al.*, 2014). Dietary supplementation of antioxidants can also increase thermal tolerance (Mahanty *et al.*, 2017; Dong *et al.*, 2020). Relatively high-condition redbreasted sunfish may similarly benefit from an increased availability of key nutrients.

Seasonal changes in body condition and relative organ sizes generally reflected patterns common in temperate fishes (e.g. Hunt, 1969; Foltz and Norden, 1977; Schwalm and Chouinard, 1999; Zaldúa and Naya, 2014). These changes were especially pronounced in juvenile redbreasted sunfish, presumably because of smaller overall energy reserves. Similarly, body condition of juvenile blacknose sunfish *Rhinichthys atratulus* decreased over winter to a much greater extent than in adults (Cunjak and Power, 1986). Given the apparent link between condition and thermal tolerance, perhaps the relatively low body condition of juvenile redbreasted sunfish over winter may be partly responsible for the low CT<sub>max</sub> of juve-

niles vs. adults at this time point. Furthermore, overwintering survival of juvenile redbelly dace may be particularly sensitive to stressors that affect metabolism and energy stores, given that body condition is a strong predictor of overwintering survival in other fishes (Pangle *et al.*, 2004).

Body colouration is thought to be an indicator of overall quality and nutritional status of fishes (Barber *et al.*, 2000), as pigments are typically acquired from food and are important for stress responsiveness (Kittilsen *et al.*, 2009; Parolini *et al.*, 2018). For example, in bluegill sunfish *Lepomis macrochirus*, thermal tolerance was higher in fish with darker body colouration (Emery, 1974). Furthermore, a previous study found that the area of the lateral red spot in redbelly dace was positively related to sperm motility and competitive fertilization success (Beausoleil *et al.*, 2012). We found that the size of the red spot was positively correlated with body condition only during the breeding season (May 2019) and was never related to CT<sub>max</sub>. One possibility for the weakness of these relationships is that hue, saturation and/or brightness of the red spot are better indicators of physiological status than simple size (e.g. Beausoleil *et al.*, 2012); logistical limitations prevented measurement of these characteristics in the current study.

### Thermal tolerance of redbelly dace

Redside dace are endangered across much of their range, but most sympatric cyprinids are not currently of conservation concern (Holm *et al.*, 2009; Zimmerman, 2017). While there is probably no single reason why redbelly dace populations are particularly sensitive to habitat alteration, relatively low tolerance of thermal stress may be an important factor. Consistent with this view, CT<sub>max</sub> of redbelly dace was much lower than that of the redbelly dace, a species that has maintained healthy populations but itself has relatively low thermal tolerance compared to other co-occurring fishes (Hopper *et al.*, 2020). Furthermore, the maximum summer CT<sub>max</sub> of redbelly dace we report here ranks in the bottom 30% of North American cyprinid fishes that have been studied (reviewed by Beitinger *et al.*, 2000), consistent with the general view that these cool-water specialists may be particularly susceptible to warming temperatures (McKee and Parker, 1982; Parker *et al.*, 1988).

At our field site, we occasionally encountered hybrids of redbelly and redbelly dace and questioned how these hybrids might impact redbelly dace populations if they had enhanced thermal tolerance (i.e. hybrid vigour) as seen in some other fishes (Bettoli *et al.*, 1985; Liu *et al.*, 2017). For example, hybrids of *Poeciliopsis monacha* and *P. lucida* have higher thermal tolerance than either parental species and can inhabit desert streams uninhabitable to non-hybrids (Bulger and Schultz, 1979). Similarly, cytoplasmic hybrids between northern redbelly dace *Chrosomus eos* and finescale dace *Chrosomus neogaeus* have replaced pure *C. eos* in the northern part of their range, suggesting higher fitness at cold temperatures (Mee and Taylor, 2012). In our study, hybrid

redbelly × redbelly dace shared the high thermal tolerance of redbelly dace. While the reproductive potential of these hybrid dace is currently unknown, the higher thermal tolerance of these hybrids may make them superior competitors to redbelly dace in warming habitats with unknown consequences for population dynamics and persistence.

### Perspectives

Habitat warming exponentially increases the food requirements of fishes and other ectotherms because of the combined effects of elevated metabolic rate and reduced energy conversion efficiency at high temperatures (Brett *et al.*, 1969; Mehner and Wieser, 1994). Small increases in water temperature, as low as 2°C, can also decrease appetite and thus exacerbate the energetic consequences of warming (Brett and Higgs, 1970; Morgan *et al.*, 2001). This confluence of factors, recently coined ‘metabolic meltdown’ (Huey and Kingsolver, 2019), suggests that warming will make it increasingly difficult for fishes to attain enough energy to meet metabolic needs. Our results indicate another, previously unappreciated, consequence to metabolic meltdown—decreases in body condition can also decrease thermal tolerance and further threaten survival in warming habitats. Food limitation appears to be an important cause of local extinctions (Cahill *et al.*, 2013), and redbelly dace may be particularly prone to nutrient limitation, as these fish primarily rely on flying insects as prey (Daniels and Wisniewski, 1994) and insect abundances are in decline globally (Sánchez-Bayo and Wyckhuys, 2019; Wagner, 2020).

Human impacts on aquatic habitats can cause declines in the body condition of fishes (Maceda-Veiga *et al.*, 2014). We found that decreases in condition lower thermal tolerance, and therefore, care should be taken to not disturb habitats containing threatened species, especially when temperatures are high. Our results also suggest that restoration of degraded and urbanized habitat, a high-priority strategy in the effort to conserve redbelly dace (e.g. COSEWIC, 2007; Redside Dace Recovery Team, 2010), is likely to benefit redbelly dace given their narrow thermal safety margins during the spring and summer. We especially suggest actions that limit peak water temperature, such as riparian plantings to increase stream shading and suitable storm water management infrastructure to capture run-off (Booth *et al.*, 2013; Vietz *et al.*, 2016). Restoration to increase habitat and abundance of prey insects may also benefit redbelly dace populations, as well-fed individuals are more resilient to thermal stress.

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