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Thermal tolerance of fish to heatwaves in agricultural streams: What does not kill you makes you stronger?

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Abstract

1. Heatwaves are increasing in frequency and intensity under climate change. Freshwater ecosystems are among the most thermally impacted systems, within which agricultural streams are experiencing the most extreme heatwaves and deserve prioritised focus. Heatwaves are approaching the upper thermal limits of many fishes but have received little attention to date.
2. To study whether and how fish tolerate heatwaves from a physiological perspective, we simulated single, multiple, and extended heatwaves at 32 and 34°C in the laboratory, based on high-resolution summer temperatures recorded in agricultural versus forested streams in Illinois, U.S.A.
3. By investigating the effects of heatwaves on 25°C acclimated fathead minnow *Pimephales promelas*, an important prey species across North America, we witnessed its high thermal resilience, including a rapid return to metabolic homeostasis after single and multiple heatwaves, measured by oxygen consumption rate. During an extended heatwave, fathead minnow were still able to partially lower oxygen consumption rate after the initial exposure. We also found transient increases in their critical thermal maximum, especially after higher intensity and frequency of heatwaves. However, the thermal resilience of fathead minnow did come with costs, including reduced anaerobic capacity indicated by decreased lactate dehydrogenase activity and impaired antioxidant defence indicated by reduced superoxide dismutase in white muscle.
4. By monitoring metabolic costs and physiological adjustments of fish during and after heatwaves, we showed that fathead minnow were resilient to simulated current and near-future heatwaves, which may allow them to cope with thermal extremes expected in agricultural streams.
5. Overall, the real-time monitoring of fish responses to heatwaves incorporates natural dynamics of thermal patterns. It facilitates mechanistic understandings of how fish react to thermal challenges in the real world and offers opportunities to incorporate high-resolution metabolic costs into future bioenergetic modelling.

KEYWORDS
climate change, critical thermal maximum temperature, enzyme, metabolic rates, respirometry

1 | INTRODUCTION

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

To understand how fish respond to thermal stress, physiological methods have been widely used. Among all physiological aspects, energy metabolism, measured as the rate of oxygen consumption, is arguably one of the most important for fish, as it influences locomotion, growth, and reproduction (Brown et al., 2004). Thermal sensitivity of metabolism has been explained by the oxygen- and capacity-limited thermal tolerance hypothesis (Pörtner, 2010), despite its limitations (Clark et al., 2013; Jutfelt et al., 2018). Oxygen- and capacity-limited thermal tolerance conjectures that during warming, tissue oxygen demand increases exponentially until it reaches a critical temperature, where oxygen demand for maintenance exceeds cardiorespiratory capacity, causing loss of performance (Blasco et al., 2020; Schulte, 2015). To evaluate the impacts of such critical temperatures on the thermal tolerance and metabolism of aquatic organisms, many physiological studies targeting long-term temperature increases have been conducted. However, short-term heatwaves have received considerably less attention (Morash et al., 2018), partially due to a lack of real-time, high-resolution field temperature information. This limits the extrapolation of lab-derived results to real-world conditions, causing us to overlook real-time metabolic costs and adjustments of fish in nature. Examples include increased standard metabolism of Atlantic salmon *Salmo salar* under a more variable environment (Oligny-Hébert et al., 2015), and elevated excess post-exercise oxygen consumption by Nile perch *Lates niloticus* after acute thermal challenges (Nyboer & Chapman, 2017). Thus, bioenergetic models that only include long-term temperature increases, while failing to consider heatwaves, are limited in predictive power.

By monitoring how fish react to extreme heatwaves, we cannot only have a better mechanistic understanding of the real-time adjustments of thermal tolerance, but also examine how prior experience to heatwaves could shape later reversible changes in thermal tolerance (i.e. heat hardening) (Bowler, 2005; Gunderson & Stillman, 2015; Schaefer & Ryan, 2006). Such *carryover effects* (O'Connor et al., 2014) could be important for fish overcoming the increasing frequency and intensity of heatwaves under global warming. However, heat hardening normally comes with costs, including reduced aerobic scope due to elevated metabolism, thus impairing fitness-related performance like prey consumption and predator avoidance (Farrell, 2009; Oligny-Hébert et al., 2015). Also, rapid increases in body temperature during heatwaves can lead to oxidative damage in ectotherms (Guzzo et al., 2019; Heise et al., 2006; Kaur et al., 2005). By monitoring the physiological responses of fishes under different frequencies and intensities of heatwaves, we can better understand their protective mechanisms and costs, then predict potential thermal thresholds for ecosystem stability.

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

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

2 | Methods

2.1 | Monitoring of stream water temperature in summer

Streams for this study were selected among locations where surveys on fish, macroinvertebrates, local habitat, and water quality have been conducted by the Illinois Department of Natural Resources in the Kaskaskia River Basin, Illinois, U.S.A. from 1991 to 2007. For each stream reach (a tributary-to-tributary segment), land-cover proportions in the local catchment (areas directly drained into the reach) were obtained from the Great Lakes Regional Aquatic Gap Analysis Project (Myers et al., 2002; Holtrop et al., 2005; Cao et al., 2015). Candidate sites were grouped by hydrologic unit code (HUC 8) subbasin and stream order, then ranked within each group based on the percentage of *agriculture* and *forest* (control) land cover in the local catchment. Within each sub-basin-stream order group, first and second ranked agricultural and forested sites were grouped to create two pairs of candidate sites. Final paired sites were: (1) Twomile Slough (94% agriculture vs. 0% forest, 39°54′55″N, 88°19′50″W) and Little Creek (26% agriculture vs. 37% forest, 39°17′43″N, 89°01′31″W); (2) Lake Fork (92% agriculture vs. 1% forest, 39°52′34″N, 88°31′07″W) and Jordan Creek (31% agriculture vs. 19% forest, 39°19′51″N, 88°47′24″W).

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

2.2 | Fish collection and husbandry

Laboratory experiments were conducted from May 2019 to November 2020 using fathead minnow acquired from Anderson Minnows Farm (Lonoke, AR, U.S.A.). Fish were housed at the Aquatic Research Facility at the University of Illinois Urbana-Champaign. Fathead minnow were obtained at four different times, with the first three batches tested for CT_{max} and enzyme performance under multiple, single, and extended heatwaves, respectively (see 2.3 below), with each batch having its own 25°C control group to minimise potential batch effects. The last batch was tested for $\dot{M}O_2$ only. Upon arrival at the research facility, fish were distributed randomly into eight 110-L tanks, with maximum density of four fish/10 L. Dissolved oxygen (DO) was maintained at >90% saturation. Water temperature was kept at 25°C using immersion heaters to mimic daily average water temperature in the Kaskaskia River Basin in the summer based on temperature records in 2019 (unpublished) and 2020 (Figure 1), and stream gauge monitoring station (USGS, 2016). Photoperiod was 06:00–20:00 to mimic summer conditions. Fish were fed to satiation daily with fish flakes and brine shrimp. An acclimation period at 25°C lasted at least 2 weeks prior to the start of any experiment. Before any test described below, fathead minnow were fasted at least for 24 hr. All procedures were approved by the University of Illinois Urbana-Champaign Institutional Animal Care and Use Committee (#19088).

2.3 | Short-term heatwave simulations

From temperature loggers, we confirmed that the highest daily temperatures were 33.8°C for agricultural streams in August 2020, and only 29.7°C for forested streams in July 2020. A temperature of 32°C has been common daily high temperature in agricultural streams, with 10 days in total (Figure 1). Thus, to accurately examine the effects of short-term heatwaves on fish during laboratory simulations, 32°C and 34°C were chosen to represent current and future extreme temperatures, respectively. To evaluate whether there were carryover effects derived from previous daily heatwave exposures, we simulated not only single heatwave reaching 32°C and 34°C, but also multiple heatwaves across three consecutive days. Finally, besides single and multiple heatwaves, we added an extended heatwave simulation to further evaluate the effects of heatwave duration on fathead minnow and document real-time adjustments of fish during extended heatwaves.

For single heatwave, we increased the water temperature in holding tanks from 25°C to 32°C or 34°C within 30 min at around 9 am, by pumping 25°C water in holding tanks through stainless steel coils in nearby thermostatic hot water bath for a rapid increase, coupled with heaters/chillers (TK-500; TECO, Ravenna, Italy) for finer adjustments. Once reaching 32°C or 34°C, the temperature was maintained for 1 hr. After this 1-hr heatwave, we cooled the water back to 25°C within 30 min. This rate of temperature increase and decrease is faster than present-day field data (i.e., 30 min vs. 2–4 hr; Figure 1) because we sought to mimic more intense heatwaves to account for future changes. For multiple heatwaves, we repeated the heatwave simulation at the same time (i.e., 9 am) on 3 consecutive days. For extended heatwave, we increased the water temperature from 25°C to 32°C or 34°C within 30 min at 9 am, then held the water at 32°C or 34°C for up to 48 hr. After exposure to single heatwave, the last of multiple heatwaves, and after the first hour during extended heatwave, the short-term physiological responses of fathead minnow were evaluated within 48 hr by quantifying CT_{max}, muscle enzymes, and $\dot{M}O_2$, with procedures described in 2.4, 2.5, and 2.6, respectively.

2.4 | Critical thermal maximum

To quantify the impacts of heatwaves on thermal tolerance, CT_{max} tests were conducted 1, 6, 24, and 48 hr after single heatwave, the last of multiple heatwaves, and after the first hour during extended heatwave. At each time point, six fish in holding tanks were transferred to a 75-L testing tank containing 55 L water. The testing

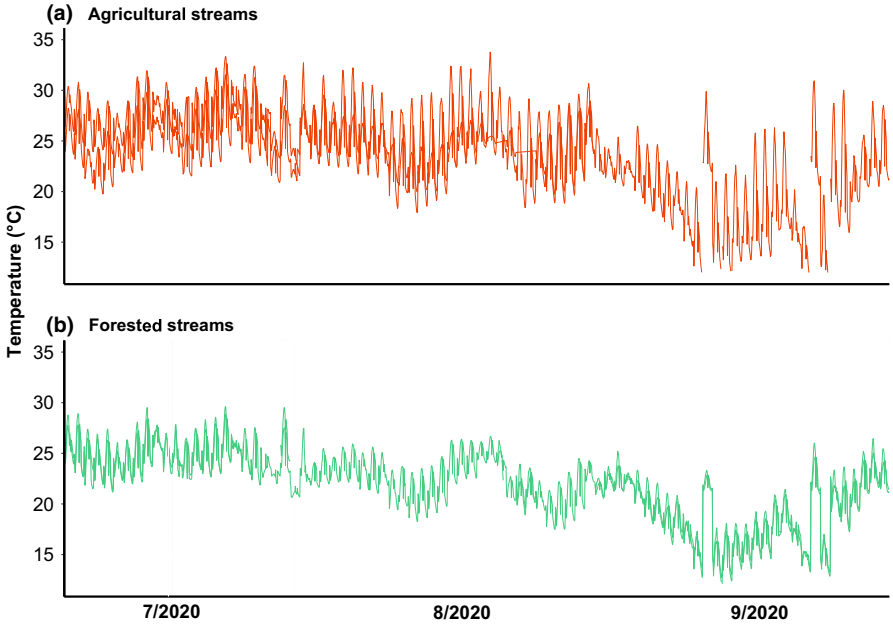

FIGURE 1 Water temperature in two agricultural streams (a) and two reference forested streams (b) in Kaskaskia River basin, Illinois, U.S.A. from 9 July 2020 to 30 September 2020. Temperatures in two agricultural or forested streams were not averaged but highly overlapping in the figure. Mean (±*SD*) temperatures for agricultural versus forested streams: July: 26.5 ± 2.6 versus 25.0 ± 1.8°C; August: 24.8 ± 2.9 versus 22.5 ± 2.0°C; September: 20.0 ± 4.2 versus 19.2 ± 3.1°C. temperature ranges for agricultural versus forested streams: July: 19.8–33.4 versus 21.1°C–29.7°C; August: 17.9–33.8 versus 17.5°C–27.5°C; September: 11.0–30.9 versus 11.9°C–26.6°C. data were missing in August 2020 in agricultural Jordan Creek and September 2020 in forested Lake fork due to loss of loggers.

tank contained a 1,000-W electric immersion heater (SmartOne EasyPlug Axial Bottom Heater; Integrated Aqua Systems, Vista, CA, U.S.A.), two circulating pumps (CompactON 300; Eheim, Deizisau, Germany), and an aerator (Tetra Whisper; Tetra, Blacksburg, VA, U.S.A.). Six plastic compartments (20 × 10 × 10 cm) were attached to the sides of the tank to hold fish. These compartments were perforated with holes for water circulation, but kept fish confined to minimise disturbance from each other, making it easier for monitoring (Dai & Suski, 2019). Six fish were introduced into compartments during each trial and given 30 min acclimation at 25°C (±0.2°C) with nearly 100% DO saturation (>7.5 mg/L).

After 30 min acclimation, the air stone was removed from the tank, and water temperature was increased by 0.3°C/min (Beitinger et al., 2000). The temperature at which fish started to lose body equilibrium, defined by disorganisation of locomotion and failure to maintain dorsoventral orientation for 3 s, was recorded as CT_{max} (Beitinger et al., 2000; Morgan et al., 2018). Fish were quickly removed once they lost equilibrium and measured for total length. During the trial, temperature was recorded every min (Pro Plus Multiparameter Instrument; YSI, Yellow Springs, OH, U.S.A.). DO stayed near 100% saturation (>7.5 mg/L). All fish were only tested once.

2.5 | Muscle enzymes

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

Lactate dehydrogenase (LDH) activity was measured to quantify the anaerobic capacity of white muscle using a Lactate Dehydrogenase Activity Assay Kit (MAK066; Sigma-Aldrich, St. Louis, MO, U.S.A.). For this, 20 mg of muscle was thawed on ice, homogenised (Bullet Blender Blue; Next Advance, Troy, NY, U.S.A.) in 100 µl of cold LDH Assay Buffer, and then centrifuged at 10,000 × gravity (*g*) for 15 min at 4°C. Owing to high activity, the supernatant was further diluted 1:20. A 2-µl aliquot of diluted sample was then measured with 48 µl LDH Assay Buffer and 50 µl Master Reaction Mix per well on 96 well plates at 25°C and λ = 450 nm per the manufacturer's instructions. Total protein concentrations (mg/ml) was determined using 5 µl of undiluted supernatant in each sample, BSA standard (P0834; Sigma-Aldrich, St. Louis, MO, U.S.A.), and Bradford Reagent (B6916; Sigma-Aldrich, St. Louis, MO, U.S.A.) (Bradford, 1976) at 25°C and λ = 595 nm after 10 min incubation. The LDH activity was corrected for total protein concentration (µmol mg^{-1} protein min^{-1}).

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

Superoxide dismutase (SOD), an antioxidant that defends against reactive oxygen species, was quantified using a Superoxide Dismutase Assay Kit (706,002; Cayman Chemical, Ann Arbor, MI, U.S.A.). For this, 10 mg of muscle was thawed on ice, homogenised in 200 µl cold HEPES buffer (1 mM EGTA, 210 mM mannitol, and 70 mM sucrose, pH 7.2), and centrifuged at 10,000 × *g* for 15 min at 4°C. The supernatant was diluted 1:6. The SOD was measured at 25°C and λ = 450 nm per the manufacturer's instructions and corrected for total protein concentration (U/mg protein).

2.6 | Oxygen consumption rate

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

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

Fathead minnow were collected from holding tanks, measured for total mass and total length, then gently introduced into respirometry chambers in the afternoon and left undisturbed until around 10 am the next morning. Resting $\dot{M}O_2$ on day 1 (RMR1) was calculated as the lowest 10th percentile of measurements taken throughout this overnight period (Chabot et al., 2016). Then, for fish in the single heatwave treatment, water temperature in the respirometry tank was increased rapidly from 25°C to 32°C or 34°C within 30 min, maintained for 1 hr, then cooled back to 25°C within 30 min. For fish in the multiple heatwave treatment, the first two heatwaves were simulated in holding tanks before fish were introduced into the respirometry chambers in the afternoon prior to the last heatwave at 10 am the next morning. For fish in the extended heatwave treatment, water temperature in the respirometry tank was increased from 25°C to 32°C or 34°C within 30 min the next morning, then maintained at 32°C or 34°C during the rest of the monitoring period. For the control group, the temperature in the respirometry tank was maintained at 25°C, and control fish received all the same procedures. In all treatments, $\dot{M}O_2$ of fathead minnow was monitored for 24 hr after the start of temperature changes in the respirometry tank, and the lowest 10th percentile of measurements within this 24 hr was calculated as resting $\dot{M}O_2$ on day 2 (RMR2). Eight fish were measured for each treatment. All chambers were deeply cleaned before every trial and the UV steriliser was constantly used during every trial to minimise the influence of background respiration. Final $\dot{M}O_2$ accounted for background bacterial respiration by measuring respiration for 30 min before and after trials and assuming a linear increase in background respiration over time.

2.7 | Data analysis

All data were analysed using R 4.0.2 (R development Core Team, 2020). Following single, multiple, and extended heatwaves, respectively, to quantify the effects of different temperatures on thermal tolerance and enzyme responses, linear mixed models were run using the lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) packages. For thermal tolerance, we used CT_{max} as the dependent variable, with time (1, 6, 24, and 48 hr) and temperature (25°C, 32°C, and 34°C) as fixed factors. For enzyme analyses, we used LDH activity, CS activity, or SOD as dependent variables, with time (1, 6, and 24 hr) and temperature (32°C and 34°C) as fixed factors. The interactions between time and temperature were also included both thermal tolerance and enzyme models, with total length nested within temperature as random factor. Another random factor, compartment nested within temperature, was initially included in thermal tolerance models but later removed by comparing Akaike information criterion values. Model assumptions of linearity, normality, and homogeneity of residuals were confirmed by inspecting plots of model residuals versus fitted values. Tukey's HSD post hoc analyses were performed using the emmeans package (Lenth et al., 2018) to determine differences across factors. Marginal r^2 explaining model variance with fixed factors only and conditional r^2 explaining variance for the entire model were calculated using the MuMIn package (Barton, 2020).

Oxygen consumption rates ($\dot{M}O_2$) per individual were calculated by the average slope of each 9-min measurement period obtained from the linear regression between oxygen consumption over time (mg O_2/hr), then corrected for the volume of the respirometry chamber and total mass of the fish. $\dot{M}O_2$ with $r^2 < 0.9$ were filtered out for quality control (Svendsen et al., 2016). RMR1

and RMR2, with average $\dot{M}O_2$ during the initial 1-hr heatwave exposures, and average 1 hr $\dot{M}O_2$ at 1 and 6 hr after the initial 1-hr heatwave, were calculated for each fish. To evaluate the effects of different temperatures on $\dot{M}O_2$ under single, multiple and extended heatwaves, respectively, linear mixed models were used, with log($\dot{M}O_2$) as the dependent variable, and time (RMR1, RMR2, 1-hr heatwave $\dot{M}O_2$, 1 hr $\dot{M}O_2$ at 1 and 6 hr after initial 1-hr heatwave), temperature (25°C, 32°C, and 34°C), and log(total mass) as fixed factors, The interactions between time and temperature were also included, with fish ID as random factor to account for potential non-independence of data (i.e. different time period of $\dot{M}O_2$ for each fish). Other random factors were initially included in full models but later removed by comparing Akaike information criterion values, including chamber nested within treatment and test date nested within treatment. Assumptions of linearity, normality, and homogeneity of residuals were again confirmed as described above, Tukey's HSD post hoc analyses were performed to separate means. Marginal r^2 and conditional r^2 were also calculated.

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

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

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

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

3 | RESULTS

3.1 | Critical thermal maximum

Critical thermal maximum in fathead minnow elevated more after 34°C heatwaves compared to 32°C heatwaves, with multiple heatwaves transiently eliciting higher CT_{max} than a single heatwave (Figure 2, Table 1). At 32°C, CT_{max} neither increased nor decreased compared to 25°C, except a 0.7°C increase at 1 hr after multiple heatwaves (Figure 2b). However, at 34°C, after single heatwave, CT_{max} increased 1.2°C at 6 hr compared to 25°C (Figure 2a); after multiple heatwaves, CT_{max} increased significantly, with 1.4°C at 1 hr and 0.8°C at 48 hr compared to 25°C (Figure 2b); during extended heatwave at 34°C, CT_{max} first decreased 1.1°C at 1 hr compared to 25°C, but then gradually recovered, until reaching 1.8°C higher by 24 hr (Figure 2c). Fathead minnow under extended heatwave at 34°C experienced >50% mortality after 24 hr, so we stopped CT_{max} tests for the 48-hr group.

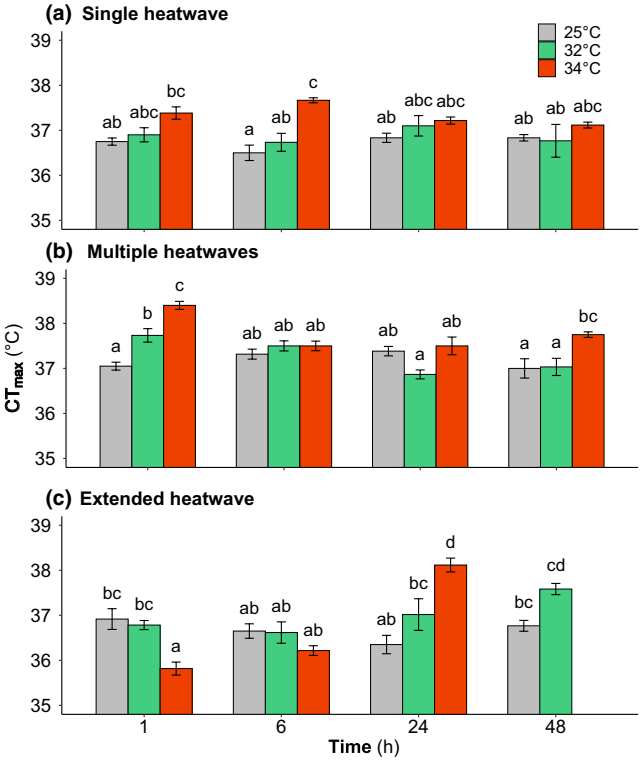

FIGURE 2 Critical thermal maximum (CT_{max}, ±*SE*) of fathead minnow *Pimephales promelas* at 1, 6, 24, and 48 hr after single (a), multiple (b), and extended (c) heatwaves. Groups ($n = 6$ in each group) with different letters are significantly different ($P < 0.05$) from each other.

3.2 | Muscle enzymes

Enzyme activities were generally lower under 34°C heatwaves compared to 32°C (Tables 2–4, Figures S1–S3). LDH activity did not change from 32°C to 34°C after single or during extended heatwave. However, after multiple heatwaves, LDH activity fell by 32% within 24 hr from 32°C to 34°C, with increased activity from 1 to 24 hr detected for both temperatures (Table 2). CS activity did not change from 32°C to 34°C across all types of heatwaves, despite an increase in CS activity from 1 to 24 hr under single heatwave for both temperatures (Table 3). SOD decreased by 21% and 16% within 24 hr from 32°C to 34°C under multiple and extended heatwaves, respectively. An increase in SOD from 1 to 24 hr was detected under single heatwaves for both temperatures (Table 4).

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

Under single and multiple heatwaves, the $\dot{M}O_2$ of fathead minnow increased sharply during the initial 1-hr heatwaves, then decreased quickly to control levels. Fathead minnow did not show higher $\dot{M}O_2$ from 32°C to 34°C during the 1-hr heatwave. Under the extended heatwave, $\dot{M}O_2$ still decreased after the initial 1 hr metabolic spike, but never completely returned to control levels

TABLE 1 Results of linear mixed effect models examining factors affecting critical thermal maximum (CT_{max}) in single, multiple, and extended heatwaves

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

Note: Fixed factors include temperature (25°C, 32°C, and 34°C), time (1, 6, 24, and 48 hr) and their interaction. Model also includes total length nested within temperature as random factor. Marginal r^2 = 0.384, 0.602, and 0.626, while conditional r^2 = 0. 662, 0.627, and 0.689 for single, multiple, and extended heatwaves, respectively. Significant factors are shown in bold.

TABLE 2 Results of linear mixed effect models examining factors affecting lactate dehydrogenase activity in single, multiple, and extended heatwaves

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

Note: Fixed factors include temperature (32°C and 34°C), time (1, 6, and 24 hr) and their interaction. Model also includes total length nested within temperature as random factor. Marginal r^2 = 0.052, 0.336, and 0.105, while conditional r^2 = 0. 052, 0.390, and 0.242 for single, multiple, and extended heatwaves, respectively. Significant factors are shown in bold.

during the entire treatment period, with fish under 34°C showing higher $\dot{M}O_2$ than under 32°C after the initial 1-hr exposure (Figure. 3, Table 5).

For fish in the single heatwave, a 1-hr heatwave at 32°C and 34°C increased $\dot{M}O_2$ by 1.6-fold and 1.8-fold compared to 25°C, with Q_{10} values of 3.8 and 3.2 relative to the control fish, respectively (Figure 3a,d). After this 1-hr heatwave, $\dot{M}O_2$ returned to 25°C control levels for the remaining 24 hr.

For fish in the multiple heatwaves, the third 1-hr heatwave at 32°C and 34°C increased $\dot{M}O_2$ by 1.6-fold and 1.9-fold compared to 25°C, with Q_{10} values of 3.9 and 3.3 relative to the control fish, respectively (Figure 3b,e). After heatwaves, $\dot{M}O_2$ again returned to 25°C control levels for the remaining 24 hr.

For fish in the extended heatwave, the first 1 hr of the heatwave at 32°C and 34°C increased $\dot{M}O_2$ by 1.2-fold and 1.7-fold compared to 25°C, with Q_{10} values of 3.0 and 3.0 relative to the control fish, respectively (Figure 3c,f). After the initial 1-hr heatwave exposure, the $\dot{M}O_2$ of fish declined but never returned to 25°C control levels, which remained at 0.7–1.0 fold and 1.1–1.6 fold higher under 32°C and 34°C, respectively.

Post-heatwave oxygen consumption after 32°C and 34°C single heatwaves were 0.26 and −0.05 mg O_2 for the first hour, respectively. The negative value represents a decrease in post-heatwave oxygen consumption of testing fish compared to the 25°C control group. After 32°C and 34°C multiple heatwaves, post-heatwave oxygen consumption was 0.17 and 0.21 mg O_2, respectively. As a

TABLE 3 Results of linear mixed effect models examining factors affecting citrate synthase activity in single, multiple, and extended heatwaves

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

Note: Fixed factors include temperature (32°C and 34°C), time (1, 6, and 24 hr) and their interaction. Model also includes total length nested within temperature as random factor. Marginal r^2 = 0.318, 0.249, and 0.151, while conditional r^2 = 0. 547, 0.550, and 0.223 for single, multiple, and extended heatwaves, respectively. Significant factors are shown in bold.

TABLE 4 Results of linear mixed effect models examining factors affecting superoxide dismutase in single, multiple, and extended heatwaves

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

Note: Fixed factors include temperature (32°C and 34°C), time (1, 6, and 24 hr) and their interaction. Model also includes total length nested within temperature as random factor. Marginal r^2 = 0.184, 0.183, and 0.236, while conditional r^2 = 0.793, 0. 183, and 0.573 for single, multiple, and extended heatwaves, respectively. Significant factors are shown in bold.

comparison, extended heatwaves resulted in 0.45 and 0.58 mg O_2 in post-heatwave oxygen consumption at 32°C and 34°C during the same period, respectively.

4 | DISCUSSION

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

4.1. Real-time metabolism under extreme heatwaves

When water was cooled to 25°C after 32°C or 34°C heatwaves, fathead minnow recovered metabolic homeostasis rapidly, shown by

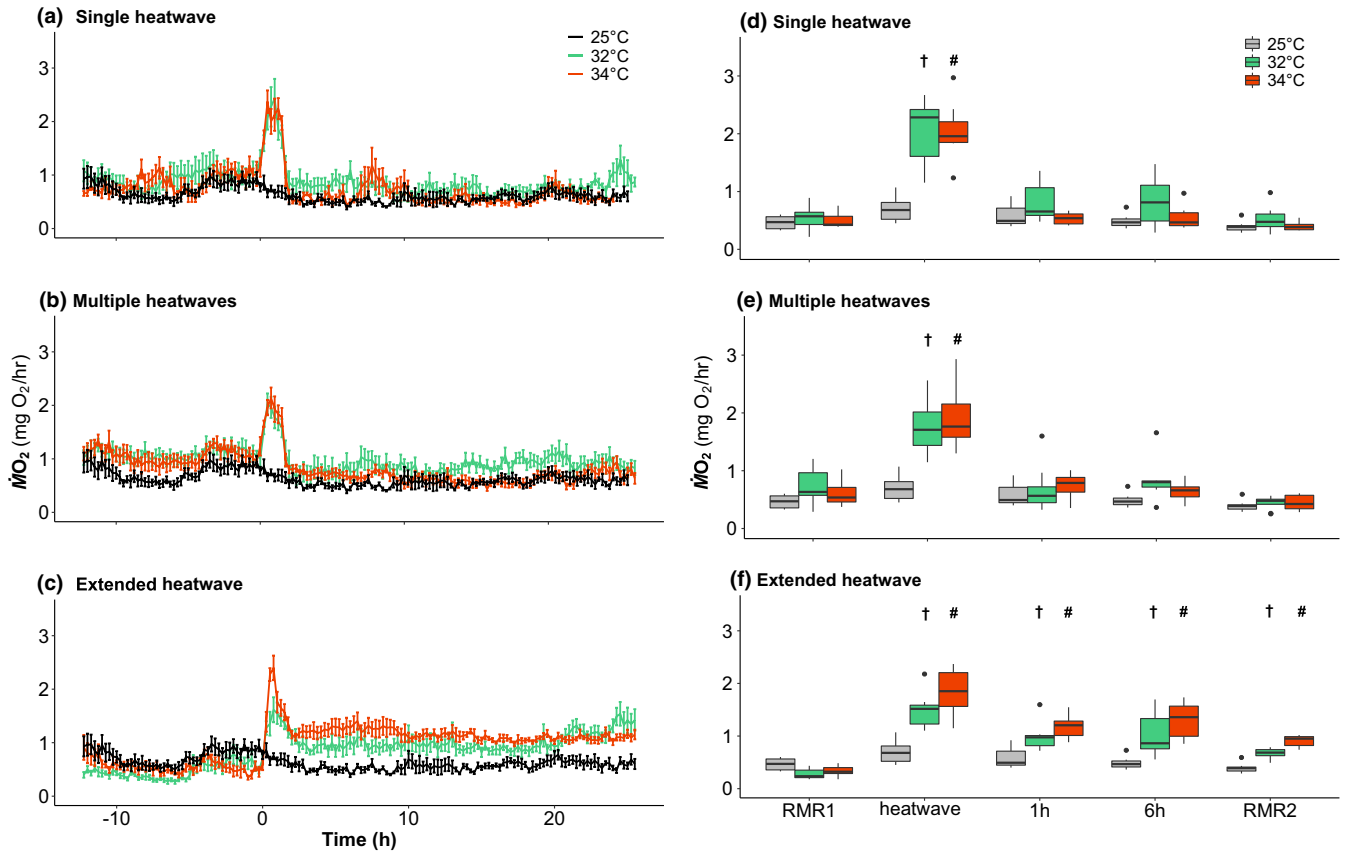

FIGURE 3 Oxygen consumption rates ($\dot{M}O_2$, ±*SE*) of fathead minnow *Pimephales promelas* ($n = 8$ in each temperature × type of heatwaves) during single (a, d), multiple (b, e), and extended (c, f) heatwaves. Time at 0 hr represents the start of temperature changes. In single (a, d) and multiple heatwaves (b, e), temperature changes included 30-min heating, 1-hr heatwaves, and 30-min cooling. In extended heatwaves (c, f), temperature changes included 30-min heating and extended 24-hr heatwaves. † and # represents $\dot{M}O_2$ is higher than reference level (i.e., $\dot{M}O_2$ of 25°C control group at the same time). In (d, e, and f), horizontal line = median; box = first quartile to third quartile; vertical line = 1.5 interquartile range.

minimal increases in oxygen consumption 1 hr post-heatwave compared to the 25°C control. During extended heatwaves, $\dot{M}O_2$ still decreased up to 55% within 24 hr after the initial thermal challenge, despite not returning to resting levels. This implies partial thermal compensation to preserve aerobic scope (Seebacher et al., 2015; Havird et al., 2020). However, under extended exposures to heatwaves, such compensation seems insufficient to help fish restore homeostasis, indicated by costs such as: (1) reduced antioxidant defence at higher temperatures; and (2) fish mortality under 34°C extended heatwave after 24 hr. Overall, the rapid return of $\dot{M}O_2$ during and after heatwaves could help fathead minnow conserve energy and maintain aerobic performance (Fry, 1971). Considering the short duration of heatwaves, this quick decline in oxygen uptake after heatwaves probably helps fathead minnow maintain sufficient aerobic scope for foraging, growth, and reproduction (Pörtner, 2010; Schulte, 2015).

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

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

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

Note: Model includes fish ID as random effect. Marginal $r^2 = 0.816$, 0.711, and 0.820, while conditional $r^2 = 0.901$, 0.847, and 0.889 for single, multiple, and extended heatwaves, respectively. Code in R: Lmer(data = Type_of_Heatwave, logMO2 ~ time*temperature + logMass + [1|fish]). Significant factors are shown in bold.

TABLE 5 Results of linear mixed effect models examining factors affecting metabolic rate ($\dot{M}O_2$) in single, multiple, and extended heatwave at 25°C, 32°C, and 34°C related to Figure 3, time represents different measurement periods, including resting metabolic rate on day 1, the first 1-hr heatwave (heatwave), 1 hr after the first 1-hr heatwave (1 hr), 6 hr after the first 1-hr heatwave (6 hr), and resting metabolic rate on day 2

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

4.2. Thermal tolerance changes and enzyme responses

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

The quick disappearance of transient CT_{max} increases, together with the death of fish during 34°C extended heatwave, implies high costs of heat hardening, especially when the duration

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

4.3. Implications and conclusions

We witnessed high thermal resilience of fathead minnow to extreme heatwaves. This result suggests that some fish species, particularly thermally tolerant ones, can withstand current and near-future heatwaves in disturbed ecosystems such as agricultural streams. This result stimulates several questions for future studies: (1) What will the responses to extreme heatwaves be for more thermally sensitive fish? Distributions of cold-water species have been predicted to be more restricted with global temperature increases (Comte et al., 2013). But this rate of habitat loss could have been underestimated because short-term heatwaves oftentimes have not been included in models. (2) What if during heatwaves, fish must migrate or maintain other essential activities? For Atlantic salmon, it has been verified that they had higher standard metabolism during migration under more variable environments (Oligny-Hébert et al., 2015). This could limit their metabolic scope, thus reducing their energy budget for later reproduction. (3) Can heatwaves impact predator–prey interactions, thus altering nutrient and energy pathways in local freshwater ecosystems? In marine ecosystems, short-term heatwaves in Western Australia during 2011 had long-lasting effects on the range contraction of kelp *Ecklonia radiata* and the range expansion of algal turfs, thus altering local fish community composition (Harvey et al., 2022).

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

In the era of rapid climate change, degraded freshwater ecosystems could experience extreme heatwaves earlier compared to better protected ecosystems, thus requiring a prioritised focus. Here, we show that, under current and near-future heatwaves in agricultural streams, a thermally tolerant fish, fathead minnow, demonstrated high thermal resilience by minimising metabolic costs and transiently enhancing upper thermal tolerance, despite the costs of reduced anaerobic potential and impaired antioxidant defence. When conducting physiological evaluations and bioenergetic modelling on the thermal impacts on fish, we suggest that subsequent studies consider and monitor short-term extreme events that last from hours to days, as their effects are typically overlooked, but may have a disproportionate influence on individuals and populations relative to their short duration.

AUTHOR CONTRIBUTIONS

Q.D., J.K.R., and C.D.S. conceived the ideas and designed methodology for lab part. Q.D., C.D.S., L.E.H., and Y.C. conceived the ideas and designed methodology for field part. Q.D. collected and analysed the data. Q.D. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.zkh18939c.

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REFERENCES

Bartoń, K. (2020). MuMIn: multi-model inference. R package version 1.43.17. CRAN: The Comprehensive R Archive Network.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Beitinger, T. L., Bennett, W. A., & McCauley, R. W. (2000). Temperature tolerances of north American freshwater fishes exposed to dynamic changes in temperature. *Environmental Biology of Fishes*, *58*(3), 237–275. https://doi.org/10.1023/A:1007676325825

Betzelberger, A. M., Gillespie, K. M., McGrath, J. M., Koester, R. P., Nelson, R. L., & Ainsworth, E. A. (2010). Effects of chronic elevated ozone concentration on antioxidant capacity, photosynthesis and seed yield of 10 soybean cultivars. *Plant, Cell & Environment*, *33*(9), 1569–1581. https://doi.org/10.1111/j.1365-3040.2010.02165.x

Blasco, F. R., Esbaugh, A. J., Killen, S. S., Rantin, F. T., Taylor, E. W., & McKenzie, D. J. (2020). Using aerobic exercise to evaluate sub-lethal tolerance of acute warming in fishes. *The Journal of Experimental Biology*, *223*(9), jeb218602. https://doi.org/10.1242/jeb.218602

Bowler, K. (2005). Acclimation, heat shock and hardening. *Journal of Thermal Biology*, *30*(2), 125–130. https://doi.org/10.1016/j.jtherbio.2004.09.001

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, *85*(7), 1771–1789. https://doi.org/10.1890/03-9000

Caissie, D. (2006). The thermal regime of rivers: A review. *Freshwater Biology*, *51*(8), 1389–1406. https://doi.org/10.1111/j.1365-2427.2006.01597.x

Cao, Y., Stodola, A., Douglass, S., Shasteen, D., Cummings, K., & Holtrop, A. (2015). Modelling and mapping the distribution, diversity and abundance of freshwater mussels (family Unionidae) in wadeable streams of Illinois, U.S.A. *Freshwater Biology*, *60*(7), 1379–1397. https://doi.org/10.1111/fwb.12575

Carveth, C. J., Widmer, A. M., & Bonar, S. A. (2006). Comparison of upper thermal tolerances of native and nonnative fish species in Arizona. *Transactions of the American Fisheries Society*, *135*(6), 1433–1440. https://doi.org/10.1577/T05-025.1

Chabot, D., Steffensen, J. F., & Farrell, A. P. (2016). The determination of standard metabolic rate in fishes. *Journal of Fish Biology*, *88*(1), 81–121. https://doi.org/10.1111/jfb.12845

Clark, T. D., Sandblom, E., & Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: Respirometry, relevance and recommendations. *Journal of Experimental Biology*, *216*(15), 2771–2782. https://doi.org/10.1242/jeb.084251

Closs, G. P., Krkosek, M., & Olden, J. D. (2016). *Conservation of freshwater fishes*. Cambridge University Press.

Colvin, N. E., Racey, C. L., & Lochmann, S. E. (2008). Stocking contribution and growth of largemouth bass stocked at 50 and 100 mm into backwaters of the Arkansas River. *North American Journal of Fisheries Management*, *28*(2), 434–441.

Comte, L., Buisson, L., Daufresne, M., & Grenouillet, G. (2013). Climate-induced changes in the distribution of freshwater fish: Observed and predicted trends. *Freshwater Biology*, *58*(4), 625–639. https://doi.org/10.1111/fwb.12081

Corey, E., Linnansaari, T., Cunjak, R. A., & Currie, S. (2017). Physiological effects of environmentally relevant, multi-day thermal stress on wild juvenile Atlantic salmon (Salmo salar). *Conservation Physiology*, *5*, 13.

Dai, Q., & Suski, C. D. (2019). Effects of acclimation temperature on critical thermal limits and swimming performance of the state-endangered bigeye chub Hybopsis amblops. *Aquatic Biology*, *28*, 137–147. https://doi.org/10.3354/ab00715

Dugdale, S. J., Bergeron, N. E., & St-Hilaire, A. (2013). Temporal variability of thermal refuges and water temperature patterns in an Atlantic salmon river. *Remote Sensing of Environment*, *136*, 358–373. https://doi.org/10.1016/j.rse.2013.05.018

Enzor, L. A., Hunter, E. M., & Place, S. P. (2017). The effects of elevated temperature and ocean acidification on the metabolic pathways of notothenioid fish. *Conservation Physiology*, *5*, 15.

Farrell, A. P. (2009). Environment, antecedents and climate change: Lessons from the study of temperature physiology and river migration of salmonids. *Journal of Experimental Biology*, *212*(23), 3771–3780. https://doi.org/10.1242/jeb.023671

Fry, F. E. J. (1971). 1—The effect of environmental factors on the physiology of fish. In W. S. Hoar & D. J. Randall (Eds.), *Fish physiology* (Vol. *6*, pp. 1–98). Academic Press. https://doi.org/10.1016/S1546-5098(08)60146-6

Guasch, H., Armengol, J., Martí, E., & Sabater, S. (1998). Diurnal variation in dissolved oxygen and carbon dioxide in two low-order streams. *Water Research*, *32*(4), 1067–1074. https://doi.org/10.1016/S0043-1354(97)00330-8

Gunderson, A. R., & Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1808), 20150401. https://doi.org/10.1098/rspb.2015.0401

Guzzo, M. M., Mochnacz, N. J., Durhack, T., Kissinger, B. C., Killen, S. S., & Treberg, J. R. (2019). Effects of repeated daily acute heat challenge on the growth and metabolism of a cold water stenothermal fish. *The Journal of Experimental Biology*, *222*(12), jeb198143. https://doi.org/10.1242/jeb.198143

Harvey, B. P., Marshall, K. E., Harley, C. D. G., & Russell, B. D. (2022). Predicting responses to marine heatwaves using functional traits. *Trends in Ecology & Evolution*, *37*(1), 20–29. https://doi.org/10.1016/j.tree.2021.09.003

Havird, J. C., Neuwald, J. L., Shah, A. A., Mauro, A., Marshall, C. A., & Ghalambor, C. K. (2020). Distinguishing between active plasticity due to thermal acclimation and passive plasticity due to Q_{10} effects: Why methodology matters. *Functional Ecology*, *34*(5), 1015–1028. https://doi.org/10.1111/1365-2435.13534

Hebert, C. E., Weseloh, D. V. C., Idrissi, A., Arts, M. T., O'Gorman, R., Gorman, O. T., Locke, B., Madenjian, C. P., & Roseman, E. F. (2008). Restoring piscivorous fish populations in the Laurentian Great Lakes causes seabird dietary change. *Ecology*, *89*(4), 891–897. https://doi.org/10.1890/07-1603.1

Heck, M. P., Schultz, L. D., Hockman-Wert, D., Dinger, E. C., & Dunham, J. B. (2018). Monitoring stream temperatures—A guide for non-specialists. In Monitoring stream temperatures—A guide for non-specialists (USGS Numbered Series No. 3-A25; Techniques and Methods, Vols. 3-A25, p. 84). U.S. Geological Survey. https://doi.org/10.3133/tm3A25

Heise, K., Puntarulo, S., Nikinmaa, M., Abele, D., & Pörtner, H.-O. (2006). Oxidative stress during stressful heat exposure and recovery in the North Sea eelpout *Zoarces viviparus* L. *Journal of Experimental Biology*, *209*(2), 353–363. https://doi.org/10.1242/jeb.01977

Holtrop, A. M., Dolan, C. R., & Epifanio, J. M. (2005). Ecological classification of Rivers for environmental assessment and management: Stream attribution and model preparation. 48.

Jentsch, A., Kreyling, J., & Beierkuhnlein, C. (2007). A new generation of climate-change experiments: Events, not trends. *Frontiers in Ecology and the Environment*, *5*(7), 365–374. https://doi.org/10.1890/1540-9295(2007)5[365:ANGOCE]2.0.CO;2

Johnson, T. B., Bunnell, D. B., & Knight, C. T. (2005). A potential new energy pathway in Central Lake Erie: The round goby connection. *Journal of Great Lakes Research*, *31*, 238–251. https://doi.org/10.1016/S0380-1330(05)70317-8

Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D. J., Lefevre, S., Nilsson, G. E., Metcalfe, N. B., Hickey, A. J. R., Brijs, J., Speers-Roesch, B., Roche, D. G., Gamperl, A. K., Raby, G. D., Morgan, R., Esbaugh, A. J., Gräns, A., Axelsson, M., … Clark, T. D. (2018). Oxygen- and capacity-limited thermal tolerance: Blurring ecology and physiology. *Journal of Experimental Biology*, *221*(1), jeb169615. https://doi.org/10.1242/jeb.169615

Kaur, M., Atif, F., Ali, M., Rehman, H., & Raisuddin, S. (2005). Heat stress-induced alterations of antioxidants in the freshwater fish *Channa punctata* Bloch. *Journal of Fish Biology*, *67*(6), 1653–1665. https://doi.org/10.1111/j.1095-8649.2005.00872.x

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(1), 1–26. https://doi.org/10.18637/jss.v082.i13

Leiva, F. P., Calosi, P., & Verberk, W. C. E. P. (2019). Scaling of thermal tolerance with body mass and genome size in ectotherms: A comparison between water- and air-breathers. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *374*(1778), 20190035. https://doi.org/10.1098/rstb.2019.0035

Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2018). Emmeans: estimated marginal means, aka least-squares means. R package version 1.1.

Little, A. G., Hardison, E., Kraskura, K., Dressler, T., Prystay, T. S., Hendriks, B., Pruitt, J. N., Farrell, A. P., Cooke, S. J., Patterson, D. A., Hinch, S. G., & Eliason, E. J. (2020). Reduced lactate dehydrogenase activity in the heart and suppressed sex hormone levels are associated with female-biased mortality during thermal stress in Pacific salmon. *Journal of Experimental Biology*, *223*(14), jeb214841. https://doi.org/10.1242/jeb.214841

Logan, C. A., & Buckley, B. A. (2015). Transcriptomic responses to environmental temperature in eurythermal and stenothermal fishes. *Journal of Experimental Biology*, *218*(12), 1915–1924. https://doi.org/10.1242/jeb.114397

Loheide, S. P., & Gorelick, S. M. (2006). Quantifying stream–aquifer interactions through the analysis of remotely sensed thermographic profiles and in situ temperature histories. *Environmental Science & Technology*, *40*(10), 3336–3341. https://doi.org/10.1021/es052074

Madeira, D. (2016). Are fish in hot water? Effects of warming on oxidative stress metabolism in the commercial species *Sparus aurata*. *Ecological Indicators*, *8*, 324–331.

Matthews, K. R., & Berg, N. H. (1997). Rainbow trout responses to water temperature and dissolved oxygen stress in two southern California stream pools. *Journal of Fish Biology*, *50*(1), 50–67. https://doi.org/10.1111/j.1095-8649.1997.tb01339.x

Morash, A. J., Neufeld, C., MacCormack, T. J., & Currie, S. (2018). The importance of incorporating natural thermal variation when evaluating physiological performance in wild species. *The Journal of Experimental Biology*, *221*(14), jeb164673. https://doi.org/10.1242/jeb.164673

Morgan, R., Finnøen, M. H., & Jutfelt, F. (2018). CT max is repeatable and doesn't reduce growth in zebrafish. *Scientific Reports*, *8*(1), 7099. https://doi.org/10.1038/s41598-018-25593-4

Myers, D. N., McKenna, J., Passino-Reader, D., & Stewart, J. S. (2002). Great Lakes aquatic GAP project. *Gap Analysis Bulletin*, *11*(2), 59–64.

Nelson, J. A. (2016). Oxygen consumption rate v. rate of energy utilization of fishes: A comparison and brief history of the two measurements. *Journal of Fish Biology*, *88*(1), 10–25. https://doi.org/10.1111/jfb.12824

Nichol, J. E., Choi, S. Y., Wong, M. S., & Abbas, S. (2020). Temperature change and urbanisation in a multi-nucleated megacity: China's Pearl River Delta. *Urban Climate*, *31*, 100592. https://doi.org/10.1016/j.uclim.2020.100592

Nyboer, E. A., & Chapman, L. J. (2017). Elevated temperature and acclimation time affect metabolic performance in the heavily exploited Nile perch of Lake Victoria. *Journal of Experimental Biology*, *220*, 3782–3793. https://doi.org/10.1242/jeb.163022

O'Connor, C. M., Norris, D. R., Crossin, G. T., & Cooke, S. J. (2014). Biological carryover effects: Linking common concepts and mechanisms in ecology and evolution. *Ecosphere*, *5*(3), art28. https://doi.org/10.1890/ES13-00388.1

Oligny-Hébert, H., Senay, C., Enders, E. C., & Boisclair, D. (2015). Effects of diel temperature fluctuation on the standard metabolic rate of juvenile Atlantic salmon (*Salmo salar*): Influence of acclimation temperature and provenience. *Canadian Journal of Fisheries and Aquatic Sciences*, *72*(9), 1306–1315. https://doi.org/10.1139/cjfas-2014-0345

Op de Beeck, L., Verheyen, J., & Stoks, R. (2017). Integrating both interaction pathways between warming and pesticide exposure on upper thermal tolerance in high- and low-latitude populations of an aquatic insect. *Environmental Pollution*, *224*, 714–721. https://doi.org/10.1016/j.envpol.2016.11.014

Page, L. M., & Burr, B. M. (2011). *Peterson field guide to freshwater fishes of North America north of Mexico*. Houghton Mifflin Harcourt.

Plaut, I. (2001). Critical swimming speed: Its ecological relevance. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *131*(1), 41–50. https://doi.org/10.1016/S1095-6433(01)00462-7

Pörtner, H.-O. (2010). Oxygen- and capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology*, *213*(6), 881–893. https://doi.org/10.1242/jeb.037523

Rahmstorf, S., & Coumou, D. (2011). Increase of extreme events in a warming world. *Proceedings of the National Academy of Sciences*, *108*(44), 17905–17909. https://doi.org/10.1073/pnas.1101766108

Raptis, C. E., van Vliet, M. T. H., & Pfister, S. (2016). Global thermal pollution of rivers from thermoelectric power plants. *Environmental Research Letters*, *11*(10), 104011. https://doi.org/10.1088/1748-9326/11/10/104011

R development Core Team (2020). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Rezende, E. L., Castañeda, L. E., & Santos, M. (2014). Tolerance landscapes in thermal ecology. *Functional Ecology*, *28*(4), 799–809. https://doi.org/10.1111/1365-2435.12268

Rohr, J. R., Civitello, D. J., Cohen, J. M., Roznik, E. A., Sinervo, B., & Dell, A. I. (2018). The complex drivers of thermal acclimation and breadth in ectotherms. *Ecology Letters*, *21*(9), 1425–1439. https://doi.org/10.1111/ele.13107

Rubalcaba, J. G., Verberk, W. C. E. P., Hendriks, A. J., Saris, B., & Woods, H. A. (2020). Oxygen limitation may affect the temperature and size dependence of metabolism in aquatic ectotherms. *Proceedings of the National Academy of Sciences*, *117*(50), 31963–31968. https://doi.org/10.1073/pnas.2003292117

Rummer, J. L., Couturier, C. S., Stecyk, J. A. W., Gardiner, N. M., Kinch, J. P., Nilsson, G. E., & Munday, P. L. (2014). Life on the edge: Thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology*, *20*(4), 1055–1066. https://doi.org/10.1111/gcb.12455

Sandblom, E., Clark, T. D., Gräns, A., Ekström, A., Brijs, J., Sundström, L. F., Odelström, A., Adill, A., Aho, T., & Jutfelt, F. (2016). Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. *Nature Communications*, *7*(1), 11447. https://doi.org/10.1038/ncomms11447

Schaefer, J., & Ryan, A. (2006). Developmental plasticity in the thermal tolerance of zebrafish Danio rerio. *Journal of Fish Biology*, *69*(3), 722–734. https://doi.org/10.1111/j.1095-8649.2006.01145.x

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology*, *218*(12), 1856–1866. https://doi.org/10.1242/jeb.118851

Seebacher, F., White, C. R., & Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, *5*(1), 61–66.

Smith, G., Badgley, C., Eiting, T., & Larson, P. (2010). Species diversity gradients in relation to geological history in north American freshwater fishes. *Evolutionary Ecology Research*, *12*(6), 693–726.

Stillman, J. H. (2019). Heat waves, the new Normal: Summertime temperature extremes will impact animals. *Ecosystems, and Human Communities*, *34*, 15–100.

Svendsen, J. C., Tudorache, C., Jordan, A. D., Steffensen, J. F., Aarestrup, K., & Domenici, P. (2010). Partition of aerobic and anaerobic swimming costs related to gait transitions in a labriform swimmer. *Journal of Experimental Biology*, *213*(13), 2177–2183. https://doi.org/10.1242/jeb.041368

Svendsen, M. B. S., Bushnell, P. G., Christensen, E. A. F., & Steffensen, J. F. (2016). Sources of variation in oxygen consumption of aquatic animals demonstrated by simulated constant oxygen consumption and respirometers of different sizes. *Journal of Fish Biology*, *88*(1), 51–64. https://doi.org/10.1111/jfb.12851

USGS. (2016). National Water Information System data available on the World Wide Web (USGS Water Data for the Nation) [WWW Document]. URL http://waterdata.usgs.gov/nwis/ (accessed June 1st, 2019).

Verberk, W. C. E. P., Durance, I., Vaughan, I. P., & Ormerod, S. J. (2016). Field and laboratory studies reveal interacting effects of stream oxygenation and warming on aquatic ectotherms. *Global Change Biology*, *22*(5), 1769–1778. https://doi.org/10.1111/gcb.13240

Wang, H., Hondzo, M., Xu, C., Poole, V., & Spacie, A. (2003). Dissolved oxygen dynamics of streams draining an urbanized and an agricultural catchment. *Ecological Modelling*, *160*(1–2), 145–161. https://doi.org/10.1016/S0304-3800(02)00324-1

Yousefi, M., Jouladeh-Roudbar, A., & Kafash, A. (2020). Using endemic freshwater fishes as proxies of their ecosystems to identify high priority rivers for conservation under climate change. *Ecological Indicators*, *112*, 106137. https://doi.org/10.1016/j.ecolind.2020.106137

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