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PRIMARY RESEARCH PAPER

Metabolic rates of a widely distributed crayfish shift along a latitudinal gradient

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Abstract Species are often exposed to novel thermal regimes as a result of anthropogenic activities. Understanding the extent to which populations are locally adapted to the thermal regime may allow us to better predict the response of organisms to novel thermal conditions. We collected virile crayfish, *Faxonius virilis*, from eight populations along a latitudinal gradient and measured their routine metabolic rates (RMR) and thermal tolerance. Countergradient variation suggests that organisms from northern latitudes may spend more energy foraging as an adaptation to the shorter growing season. Thus, we hypothesized that crayfish RMR would be positively related to latitude. We also expected high latitude populations to have a greater sensitivity to acute temperature change and a lower thermal tolerance. In support of our hypothesis, there was a significant positive relationship between latitude and crayfish RMR at night when crayfish are most active, and crayfish from high latitude populations were more thermally sensitive. Thus, changes in the thermal regime are likely to alter the activity level of this species, which could alter its ecological impacts. In addition, virile crayfish across the latitudinal gradient had a high thermal tolerance, which may contribute to the success of this species in novel environments.

Keywords Invasive species · Climate · Respirometry · Ectotherm · Freshwater · Behavior

Introduction

Anthropogenic activities often alter freshwater thermal regimes. Water temperatures have increased in many freshwater ecosystems due to climate change, and warming trends are predicted to continue (Austin & Colman, 2007; Kaushal et al., 2010; Knouft & Ficklin, 2017). Other major drivers of thermal alteration include urban land use in the watershed and the creation of impoundments (Walsh et al., 2005; Kaushal et al., 2010; Olden & Naiman, 2010). Human introductions of species also often result in the establishment of species in novel thermal environments (Atwater et al., 2018). Therefore, understanding how freshwater organisms respond to novel thermal conditions is broadly important for understanding anthropogenic influences on freshwater biota. Scientists often use the climate niche of a species to predict the response of a population to changing thermal conditions (*e.g.*, when predicting likelihood of an invasion or whether a

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species' range will expand or contract due to climate change). However, for species that have large distributions, populations across the range experience different thermal regimes. Understanding the extent to which populations within a species are locally adapted to the thermal regime may allow us to better predict the response of populations to novel thermal conditions.

Temperature is a key factor that can affect the physiology, growth, and behavior of organisms. This is particularly true for aquatic ectotherms, as their body temperature closely follows the temperature of the surrounding water. Temperature is positively related to an organism's metabolic rate (Gillooly et al., 2001). If temperatures and associated metabolic rates are too low, inactivity can limit foraging and growth. Conversely, if temperatures are too high, an organism will require more food and oxygen to meet metabolic demands, so high temperatures can also limit growth and survival (Westhoff & Rosenberger, 2016). Metabolic rates can also be related to behavioral traits (Burton et al., 2011). For example, within several species of fish, the standard metabolic rate (*i.e.*, the minimal, or maintenance, metabolic rate of an ectotherm in an inactive state) is positively correlated with aggression, boldness, and activity level (Metcalfe et al., 2016). Standard metabolic rates can also be negatively correlated with traits such as boldness, likely because individuals with lower maintenance costs have more energy to allocate to physical activity (Careau et al., 2019). Routine metabolic rates (RMR; *i.e.,* the average metabolic rate when an animal is exhibiting routine to minor activity in a respirometer (Chabot et al. 2016)) can also be related to behavioral traits. For example, fast species of stream fish in the Amazon have higher RMRs than more sedentary ones (Campos et al., 2018). In widely distributed species, regional differences in temperature could select for different behavioral traits because of the effects of temperature on metabolism. For example, warm climates may select for behavioral traits that promote food acquisition (high boldness and activity) to offset increased energetic demands (Segev et al., 2017). As the climate warms, the traits in high latitude populations may shift toward those currently found in low latitude populations (Fukami & Wardle, 2005).

Populations across the geographic range of a species may also experience different season lengths, which could select for particular physiological or behavioral traits. A short growing season at high latitudes may select for traits that compensate for season length by promoting food acquisition and rapid growth (Van Petegem et al., 2016). There is some evidence for this pattern in freshwater crayfish. Specifically, populations of rusty crayfish *Faxonius rusticus* (Girard, 1852) and virile crayfish *Faxonius virilis* (Hagen, 1870) from higher latitudes had faster growth rates and higher activity levels in common conditions than populations of the same species from lower latitudes (Sargent & Lodge, 2014; Reisinger et al., 2020). However, these studies did not measure metabolism, so it is unclear whether these differences in behavioral traits are related to differences in metabolic rates.

Previous studies have found among-population variation in the metabolic response of freshwater ectotherms to temperature, suggesting that there is local adaptation to the thermal regime within widely distributed species (Narum et al., 2013; McKenzie et al., 2021). For example, daphnia from subarctic populations had higher oxygen consumption rates in cold water than those from temperate populations, and the opposite pattern occurred in warm water (Chopelet et al., 2008). Thermal tolerance can also shift across latitude, with greater upper thermal limits in populations from warm climates (Stitt et al., 2014; Stoks et al., 2014; Nyboer et al., 2020). Finally, metabolic sensitivity (*i.e.*, the extent to which an organism's metabolic rate changes as temperature changes) can shift across latitude, but the direction of this relationship varies among species (Seebacher et al., 2015; Beachum et al., 2020). While there is substantial evidence that populations can be locally adapted to the thermal regime, this is not always the case (Rangel & Johnson, 2019). Few studies have examined physiological adaptation across the geographic range of freshwater invertebrate species (Stoks et al., 2014).

We tested the extent to which metabolic rates, thermal sensitivity, and thermal tolerance shift over the range of the virile crayfish. This species is ideal for examining local adaptation to the thermal regime because of its broad native range which includes much of the north central United States and south central Canada east of the continental divide. It has also been widely introduced and has established populations across the USA, in the United Kingdom and the Netherlands (Larson et al., 2018). Therefore, it is particularly relevant to understand how populations respond to local thermal conditions. Crayfish are omnivorous and often reach high biomass in freshwater ecosystems, so they can impact many levels of freshwater food webs and alter ecosystem processes such as organic matter processing and energy flow (Whitledge & Rabeni, 1997; Jackson et al., 2014; Alp et al., 2016). Thus, changes in metabolic rates and associated changes in virile crayfish feeding behavior could have large ecological consequences. An earlier study compared the behavior of virile crayfish from a population in northern Wisconsin to one from southern Indiana, USA. In common conditions, crayfish from the higher latitude population were bolder, more active and had higher foraging rates than those from the lower latitude population (Reisinger et al., 2020). We were interested in whether northern and southern populations also differed in their metabolic rates, thermal sensitivity, and thermal tolerance.

To examine metabolic rates and thermal responses, we collected virile crayfish from eight populations across a latitudinal gradient from northern Wisconsin to central Illinois. We measured the RMR of crayfish acclimated to temperatures that represented mean summer temperatures at the low and high end of the latitudinal gradient. RMR was measured both at night, when crayfish are typically active, and during the day, when crayfish are typically inactive (Fero & Moore, 2014). Based on previous research (Reisinger et al., 2020), we hypothesized that crayfish from higher latitudes would have higher activity levels, and thus would have higher RMRs at night when crayfish are out of shelter and actively foraging. We hypothesized that RMRs during the day would be lower in crayfish from high latitude populations, allowing these crayfish to reserve their energy for high activity periods (Careau et al., 2019). We also expected virile crayfish to be adapted to the temperature regime at their home latitude. Thus, we predicted that individuals from low latitude populations would be less thermally sensitive to acute temperature changes than those from high latitude populations (Seebacher et al., 2015). To test for thermal sensitivity, we measured RMR as temperatures increased. Additionally, we predicted that crayfish from lower latitudes would have a higher tolerance of acute thermal stress (*i.e.*, critical thermal maximum), which we assessed by testing crayfish righting response as temperatures increased (Mundahl & Benton, 1990).

Methods

Crayfish collection and husbandry

We hand collected virile crayfish from wadable streams (5.2 m – 7.6 m wetted width) from June to August 2019. Sites were selected to span a latitudinal gradient from Northern Wisconsin to Southern Illinois ($N = 8$; Fig. 1; Table 1). Virile crayfish were the most common crayfish collected at each site, and crayfish were typically found under rocks or in macrophyte beds. We used only male crayfish in experiments to reduce potential variation in metabolic rates due to sex. Following collection, crayfish were shipped overnight in insulated containers to the Fisheries and Aquatic Sciences Laboratory at the University of Florida, Gainesville, FL. Crayfish used for experiments ranged in size from 17 to 36 mm carapace length (mean ± SD = 27 ± 4 mm) and 1.7 – 14.5 g blotted wet weight (5.2 ± 2.7 g). Most crayfish were non-breeding form (form II; 73%). Metabolic rates in crayfish are fairly consistent during the intermolt phase but can increase substantially during late premolt as well as postmolt stages (Huuskonen et al. 2014). Thus, we avoided using crayfish that appeared be about to molt as indicated by a white "waist" between the cephalothorax and abdomen and/or a visible separation between the carapace and first abdominal tergite (Penn, 1943), and only used individuals with hardened exoskeletons.

At the laboratory, crayfish were housed in individual perforated, 0.5-L, deli containers within larger bins containing constantly aerated well water. We labeled perforated containers so that each crayfish had a unique identification number. All crayfish were housed in the laboratory for a minimum of 2 weeks before experiments began. Crayfish were exposed to the natural photoperiod through windows in the laboratory. We fed each crayfish three shrimp pellets (OmegaSea, LLC) twice per week. Water was changed within 24 h after feeding to maintain water quality.

Thermal acclimation

Prior to experiments, we acclimated crayfish to one of two experimental temperatures (23 °C or 28 ℃). Crayfish from each site along the latitudinal gradient were tested at each of these temperatures. We selected

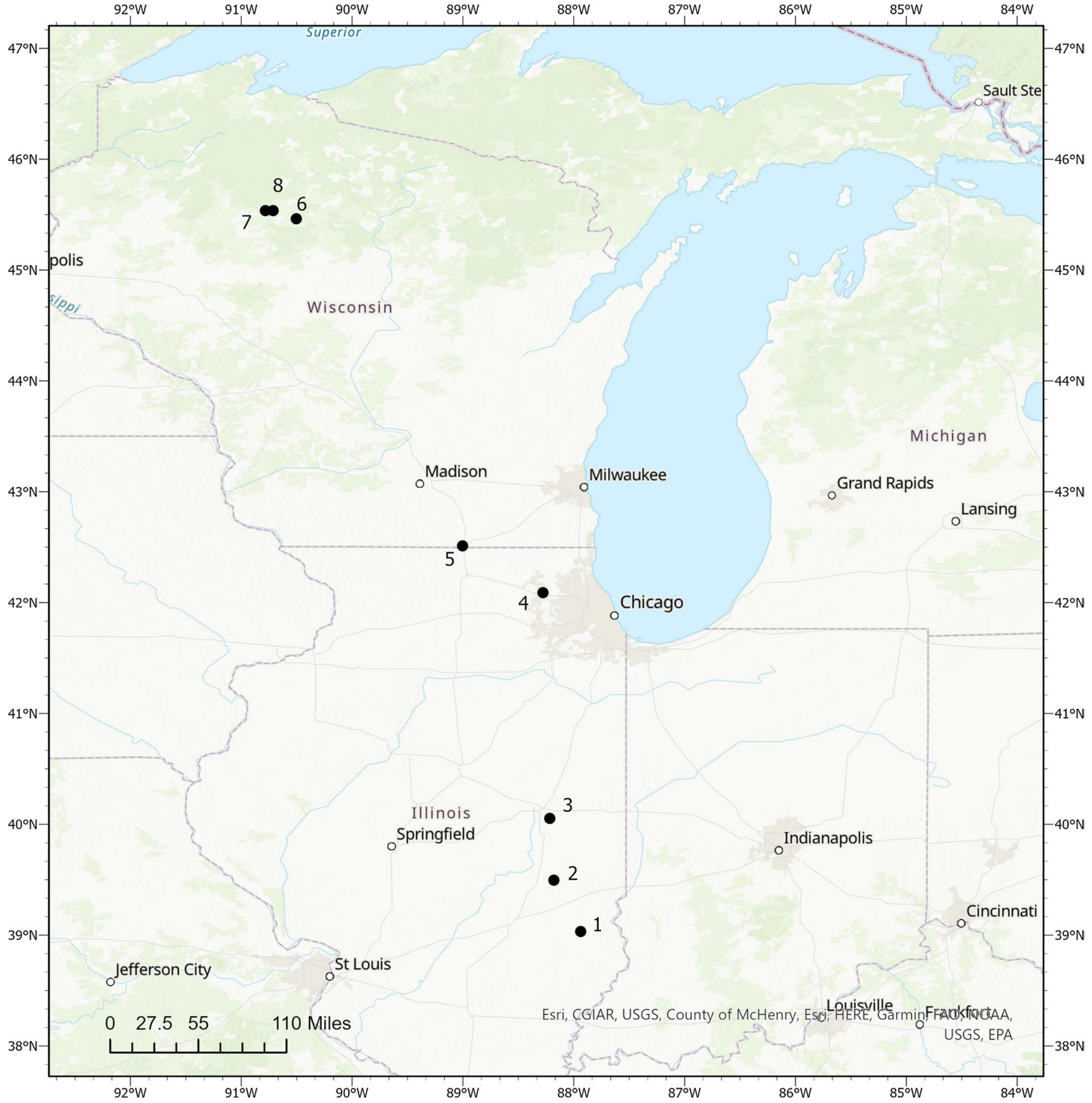

Fig. 1 Virile crayfish collection sites (1–8)

these temperatures to represent mean summer water temperature in streams across the latitudinal gradient. To assess water temperatures across this gradient, we used stream temperature data from USGS gaging stations in northern Wisconsin (4,063,700 Popple River, 4,067,500 Menominee River) and southern Illinois (33,811,495 Little Wabash River, 4,067,500 Embarras River). Mean July stream temperature in 2018 was 21.8 ± 2.6 °C (SD) and 24.5 ± 1.4 °C for Wisconsin stations and 28.4 ± 1.4 °C and 27.9 ± 1.9 °C for Illinois stations. Thus, testing crayfish respiration rates at 23 °C and 28 °C allowed us to assess metabolism at temperatures that were typical across this range. The warmest water temperatures recorded for this period at the northern Wisconsin stations were 27.4 °C and 27.8 °C, which is slightly cooler than the 28 °C experimental temperature, suggesting that this temperature represents the warmest conditions that

Table 1 Coordinates and site numbers for crayfish collection locations along the latitudinal gradient from Northern Wisconsin to Southern Illinois

Site number	Location	Latitude	Longitude
1	Southern Illinois	39.031884	−87.936199
2	Southern Illinois	39.493687	−88.181281
3	Central Illinois	40.054500	−88.215100
4	Northern Illinois	42.088369	−88.279892
5	Southern Wisconsin	42.512860	−89.004200
6	Northern Wisconsin	45.463291	−90.503065
7	Northern Wisconsin	45.537648	−90.782278
8	Northern Wisconsin	45.537117	−90.710476

crayfish from this region would experience. Crayfish were acclimated to experimental temperatures (23 °C or 28 °C) for a minimum of one week prior to experiments. During temperature acclimation, crayfish were housed in the same perforated containers within larger bins described above. Temperatures in bins were maintained within ± 1 °C using 300-W aquarium heaters (Finnex, Inc.) that were connected to digital temperature controllers (Inkbird).

Metabolic rates of acclimated crayfish (23 °C and 28 °C)

We used intermittent respirometry experiments to measure crayfish RMR from July – November 2019 at 23 and 28 °C. Experiments were conducted in four-chamber fiber optic respirometry systems with AutoResp™ 2.3.0 software (Loligo Systems, Inc.). Crayfish were placed in acrylic chambers (405–600 mL) that were submersed in a 190-L tank filled with well water. Water in the large tank was constantly aerated so that it was near 100% saturation with dissolved oxygen (DO), and temperature was maintained with within +/− 1 °C using 300-W aquarium heaters (Finnex, Inc.) that were connected to digital temperature controllers (Inkbird). Each chamber was attached to two water pumps (Eheim 300 L/h), a recirculation pump that circulated water through the chamber and past an optical DO sensor during the closed-circuit cycle while we were measuring respiration rates and a flush pump that brought fresh, oxygenated water from the outer tank into the chamber between cycles (Figs. 2 and 3). Each chamber also contained a known volume of gravel to provide natural substrate for crayfish to hide in or interact with during the experiment. The chambers were large enough to allow crayfish to move around and carry out normal behaviors such as walking around the chamber or digging through the gravel (Figs. 2 and 3). The addition of gravel also reduced the water volume within chambers (final volume: 305—398 ml), which allowed crayfish to draw down oxygen at a greater rate. Because we were interested in measuring RMR rather than standard metabolic rate (SMR; the minimum maintenance cost of a resting organism), we used a respirometer:organism volume ratio of ~ 67, which was larger than the 20–50 range recommended for SMR (Svendsen et al. 2016).

We obtained day and nighttime RMR from 14 – 21 crayfish for each site (7 – 11 replicates per temperature treatment; 149 crayfish total). Each experiment ran for approximately one day. Crayfish were deprived of food for 30–48 h prior to being tested for night experiments to ensure digestive tracts were emptied and energy was not spent on digestion. Crayfish were added to chambers between 15:00 and 18:00 in the afternoon and allowed to acclimate to experimental chambers for 4–7 h. While conducting experiments, sunrise was between 06:37 and 06:57 and sunset was between 17:32 and 20:32. Nighttime respiration was measured from 22:00 to 02:00 the following morning. Daytime respiration was measured from 09:00 to 14:00. Throughout this time, the pumps cycled between the flush phase (approximately 12 min) where oxygenated water was drawn into chambers and the measurement phase where chambers were closed to the outside tank but water recirculated within them (17 – 40 min). The length of the flush phase was adjusted in each experiment to ensure that DO in all chambers was brought up to ~ 100% saturation before each measurement phase. The length of the measurement phase was adjusted so that crayfish did not draw DO below 80% saturation – thus maintaining normoxic conditions. The length of these cycles allowed us to run 3—8 replicate cycles for each crayfish during the day and nighttime measurement periods. During each cycle, RMR was calculated by AutoResp™ 2.3.0 (Loligo System) software as:

$$\mathrm{MO2} = \left([\mathrm{O2}]_{t0} - [\mathrm{O2}]\right)_{t1}) * (\mathrm{V}/\mathrm{t}) * (1/\mathrm{WW})$$

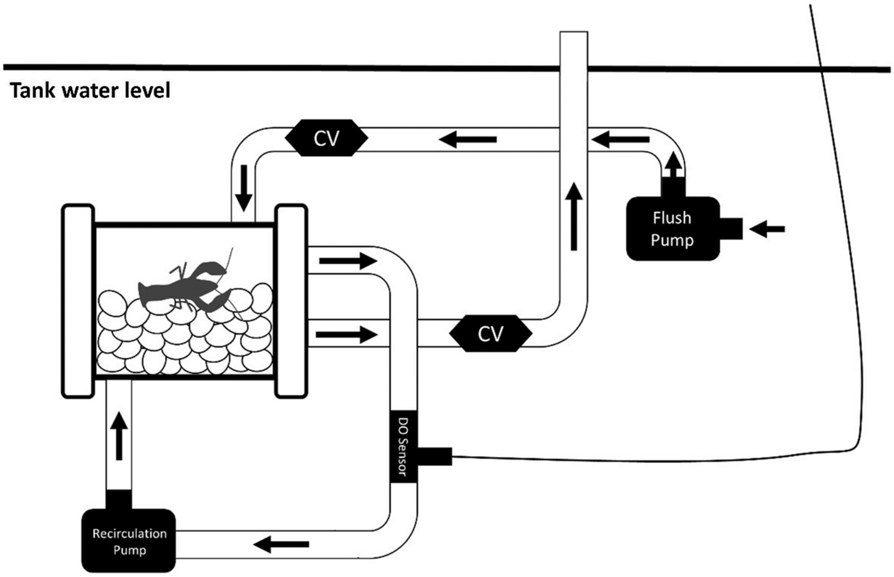

Fig. 2 Diagram showing the respirometry system used. A flush pump was used to pump fresh water into the chamber. The recirculation pump was used to circulate water continuously in the chamber and a dissolved oxygen (DO) sensor was attached to the recirculation tubing to measure DO. Check valves (CV) prevent outside water from flowing into the chambers when the flush pump was not being used. Arrows represent that flow of water throughout the respirometry system

where $[O2]_{t0}$ = oxygen concentration at time t0 (mg O_2/liter), $[O2]_{t1}$ = oxygen concentration at time t1 (mg O_2/liter), t = t1 – t0 (hour), WW = wet mass of the experimental animal (kg).

To correct for background oxygen demand from potential bacterial growth during respiration runs, respiration rate was measured in all chambers before and after each trial for ~ 1 h without crayfish. The mean background respiration rate was calculated as (MO_2 before + MO_2 after)/2 for each chamber and was then subtracted from the crayfish oxygen consumption to yield the corrected RMR. After background respiration was measured at the end of the trial, all materials used (chambers, rocks, tubing, pumps) were soaked in a bleach solution (1:1260 bleach to water ratio), rinsed with tap water, and then dried to reduce bacteria in the system prior to the next trial.

Statistical analysis (Respirometry: acclimated crayfish)

For each individual, RMR_{night} was calculated as the mean of all corrected RMR estimates during the nighttime measurement period (22:00 – 02:00) and RMR_{day} was the mean of all corrected RMR estimates during the daytime measurement period (09:00 – 14:00). We used a linear mixed effects model to test whether the mass-specific RMR of virile crayfish was related to the fixed effects of home latitude, day/night, temperature (23 °C or 28 °C), and all interactions between these variables. We included crayfish reproductive form (I or II) as a covariate in the model. Crayfish ID was included as a random effect in the model because there were two metabolic rate measurements per crayfish (daytime and nighttime). We also included site as a random effect in the model.

Because of the potential impact of molting cycle temporarily increasing metabolic rates, we did not include measurements from crayfish in our analyses when metabolic rates were unusually high (daytime metabolic rates greater than 300 mgO_2/kg/hr) or when we observed crayfish molting within a week of the experiment. In all of these cases except for two, we were able to retest crayfish at a later date (at least 7 days later) and consistently obtained lower metabolic rates during the second experiment ($N = 12$). For retested crayfish, we used only the second measurement in the analysis.

Mass-specific metabolic rates of crayfish are often negatively related to crayfish size (McFeeters et al. 2011). In our dataset, crayfish size was negatively related to mass-specific metabolic rate for the daytime measurements at 23 °C ($P < 0.001$); however, it was not related to metabolic rate within daytime measurements at 28 °C or nighttime measurements at either temperature ($P > 0.2$). Because we were primarily interested in the relationship between metabolic rates and home latitude, we corrected for the influence of crayfish size on mass-specific metabolic rate for the

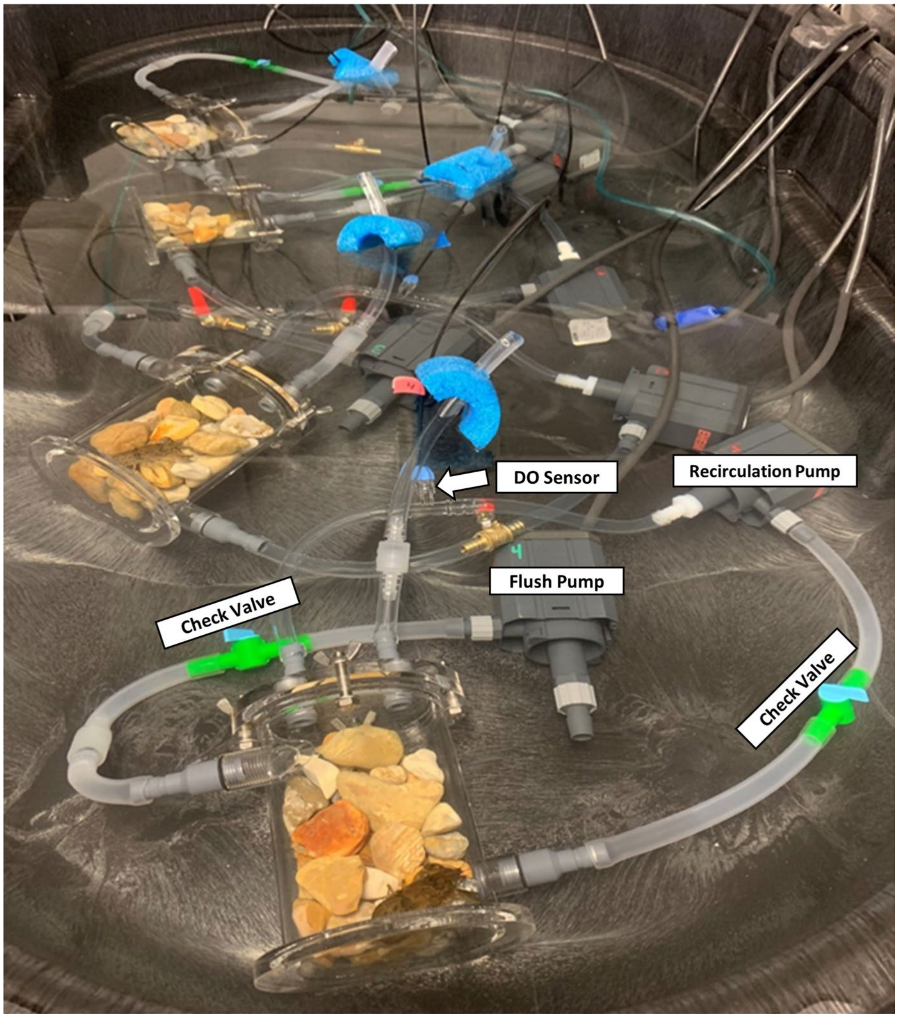

Fig. 3 Photograph depicting respirometry system

daytime measurements at 23 °C prior to conducting the linear mixed effects model. We scaled metabolic rates for these measurements to that of an average size crayfish in our dataset (5 g) using the slope of the relationship between MO_2 and crayfish body mass (− 6.512). Specifically, we used the equation MO_2 (5 g) = $MO_{2(BM)}$ + (BM-5)*6.512, where $MO_{2(BM)}$ is oxygen consumption of animal with body mass BM (Rosewarne et al. 2014). Correcting for size effects in the daytime 23 °C treatment did not affect our main results. The significant three-way interaction between home latitude, day/night, and temperature was present in mixed effects models without scaling to size in any treatment or with metabolic rates in all treatments scaled to that of a 5 g crayfish ($P < 0.02$).

Sensitivity to acute thermal stress

We used the same intermittent respirometry setup described above to assess acute thermal sensitivity of virile crayfish metabolic rates across the latitudinal gradient. Experiments were conducted from January – February 2020. Crayfish were from five sites along the latitudinal gradient (sites 1, 2, 4, 7, 8) and had been collected at the same time as for the acclimated experiments. We tested 5 replicate individuals per site except site 7 where only 3 crayfish were available. Crayfish ranged in size from 18 to 35 mm carapace length (mean ± SD = 27 ± 4 mm) and 2.3 – 13.6 g blotted wet weight (5.9 ± 2.6 g). Most crayfish were non-breeding form (form II; 65%). For this experiment, crayfish were acclimated for at least 1 week to

23 °C as described above. We added crayfish to the respirometry chambers in the afternoon (between 15:00 and 18:00) and left them to acclimate overnight at 23 °C. We began measuring metabolic rates at 9:00 the following morning and raised the temperature by 2 °C every hour until we reached 34 °C. We measured RMR of crayfish every hour. All other methods for this experiment were identical to those for the acclimated exposure respirometry experiment described above.

Statistical analysis (acute thermal sensitivity)

To assess acute thermal sensitivity of crayfish across the latitudinal gradient, we first plotted RMR against temperature and fitted a linear and exponential regression to the data (SigmaPlot 13). We used Akaike information criterion corrected for small sample sizes (AICc) to choose the best fit model (lowest AICc value). We then used the best fit model to estimate the RMR of each crayfish at 23, 28 and 34 °C, and used the following equation to calculate Q_{10}, where T_1 is 23 °C and T_2 is 34 °C (Rangel and Johnson 2019):

$$Q_{10} = \left(\frac{MO_{2T_2}}{MO_{2T_1}}\right)^{\frac{10}{T_2-T_1}}$$

We used linear mixed effects models to test the relationship between the Q_{10} coefficient and the fixed effect home latitude. Carapace length and reproductive form were included as covariates in this model and site was included as a random effect. We also tested whether metabolic rates at 23 °C and 34 °C were related to home latitude in this experiment. We scaled metabolic rates for these measurements to that of an average size crayfish using the same methods as above prior to conducting these analyses. We used linear mixed effects models to test whether the mass-specific RMR of virile crayfish at 23 °C and 34 °C was related to the fixed effect of home latitude and included site in each of these models as a random effect. We also included reproductive form as a covariate in these models. Finally, we compared respiration rates in the acute respirometry experiment at 23 °C and 28 °C using a paired t-test. This analysis allowed us to compare the effects of temperature on respiration rate in the acute thermal stress experiment to those from the acclimated experiment.

Critical thermal maximum (acute)

To assess the thermal tolerance of virile crayfish across the latitudinal gradient, we conducted a critical thermal maximum (CTMax) experiment in November 2019. CTMax was measured as the temperature at which the righting response was lost (*i.e.*, a crayfish placed on its dorsal surface is not able to right itself within 30 s; Mundahl & Benton, 1990). We tested crayfish from the same five sites used in the thermal sensitivity experiment (sites 1, 2, 4, 7, 8), and tested four to five replicate individuals per site. Crayfish ranged in size from 21 to 35 mm carapace length (mean ± SD = 27 ± 3 mm) and 48% were non-breeding form (form II).

The experiment was conducted in 53 L tanks that were constantly aerated and heated using two 300-W aquarium heaters (Finnex, Inc.). Crayfish were acclimated to 23 °C for a minimum of one week prior to the experiment. During the experiment, crayfish were contained within individual, 5-L perforated containers within the larger tanks. We began the experiment at 08:00 at 23 °C and increased the temperature by 2 °C per hour until the temperature was 40 °C. We tested the righting response of each crayfish once per hour by gently removing it from the container and placing it on its dorsal surface in an open area of the tank, while keeping the crayfish submerged. Crayfish were given a maximum of 30 s to right themselves. Crayfish that did not right themselves within 30 s were removed from the tank.

Statistical analysis (CTMax)

We used linear mixed effects models to test the relationship between CTMax and the fixed effect home latitude. Carapace length and reproductive form were included as covariates in the model and site was included as a random effect.

Results

Metabolic rates of acclimated crayfish (23℃ and 28℃)

There were no significant overall effects of home latitude ($F_{1,6} = 1.37$, $P = 0.280$), day/night ($F_{1,145} = 1.18$, $P = 0.278$), or temperature ($F_{1,140} = 2.16$, $P = 0.144$) on the mass-specific RMR

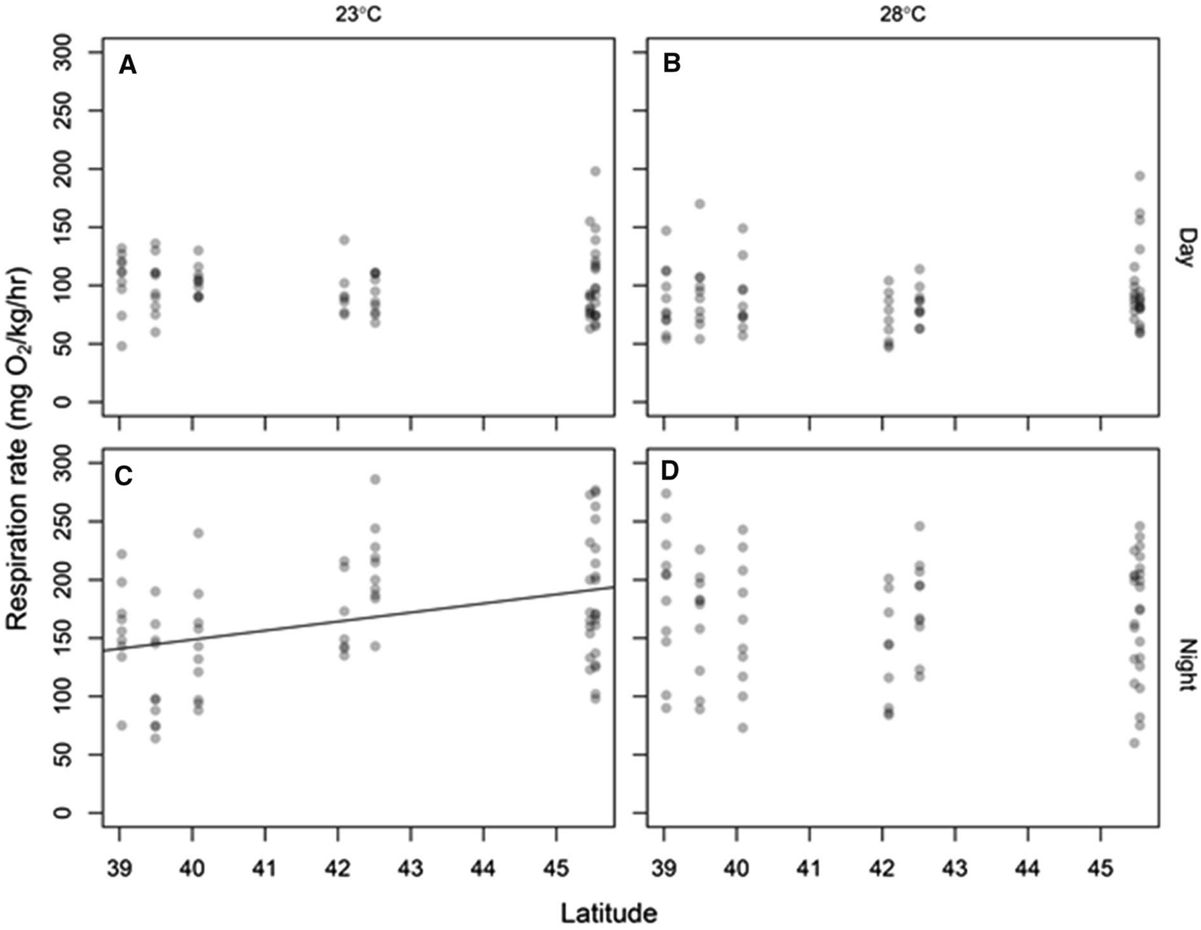

Fig. 4 The relationship between home latitude and mass-specific metabolic rates in virile crayfish. Metabolic rates were measured during the day when crayfish are typically inactive (**A** and **B**) and at night when crayfish are typically active (**C** and **D**). Crayfish were acclimated and tested at either 23 °C (**A** and **C**) or 28 °C (**B** and **D**)

of virile crayfish that had been acclimated to experimental temperatures. However, a significant three-way interaction existed between these variables ($F_{1,145} = 9.46$, $P = 0.003$; Fig. 4). This interaction was the result of a significant positive relationship between home latitude and nighttime RMR in the 23 °C treatment (linear regression: $F_{1,70} = 11.77$, $P = 0.001$, $R^2 = 0.13$; Fig. 4C). There was no effect of home latitude on nighttime RMR in the 28 °C treatment ($P > 0.8$; Fig. 4D). The trend between latitude and RMR at night was not present in the 28 °C treatment because the crayfish from low latitude populations had higher RMR at this temperature. Specifically, for the three lowest latitude populations, the mean RMR at night increased by 25% between 23 and 28 °C (mean ± SD = 137 ± 46 and 171 ± 54 mgO_2/kg/hr, respectively). For the three highest latitude populations, the mean RMR at night decreased by 8% between 23 and 28 °C (184 ± 54 and 169 ± 53 mgO_2/kg/hr, respectively). There was also no effect of home latitude on RMR during the day in either temperature treatment ($P > 0.4$; Fig. 4A and B), indicating that latitude only had an effect on metabolic rate during times when crayfish are typically active.

In addition to the significant three-way interaction, there were also significant two-way interactions in the model. A significant interaction existed between home latitude and day/night ($F_{1,145} = 4.65$, $P = 0.033$), which was driven by the positive effect of home latitude on nighttime respiration rates in the 23 °C treatment. A significant interaction also existed between

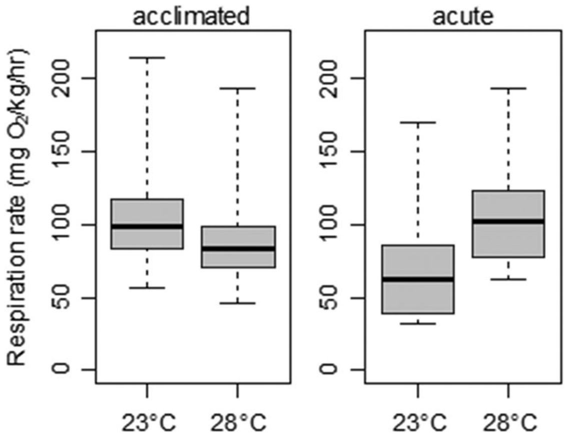

Fig. 5 Box plot depicting the daytime metabolic rate of virile crayfish at 23 and 28 °C for the experiment in which crayfish were acclimated for at least one week at each temperature (acclimated) and the experiment in which crayfish were acclimated at 23 °C and then exposed to an acute increase in temperature (acute). The gray box depicts the interquartile range, the whiskers depict the range, and the solid line depicts the median. The metabolic rate only increased between 23 and 28 °C when crayfish were not acclimated to the warmer temperature

day/night and temperature ($F_{1,145} = 10.16$, $P = 0.002$). Daytime RMRs were higher on average at 23 °C than 28 °C (mean ± SD = 99 ± 25 and 89 ± 29 mgO_2/kg/hr, respectively), but nighttime RMRs were similar across temperatures (167 ± 54 and 167 ± 52 mgO_2/kg/hr). All other interactions between variables were not statistically significant. Finally, breeding form, which was included as a covariate, had a significant effect on metabolic rate. Crayfish that were in non-breeding form (form II) had a higher mass-specific RMR than those that were in breeding form (form I; $F_{1,111} = 6.70$, $P = 0.011$). Non-breeding form crayfish in our experiment were also smaller on average than those in breeding form (26 ± 3 mm and 29 ± 4 mm, respectively).

Sensitivity to acute thermal stress

In contrast to the respirometry experiment in which crayfish were acclimated to either 23 °C or 28 °C, mass-specific RMR increased with temperature in the acute thermal stress experiment (mean ± SD at 23 °C = 69 ± 33, 28 °C = 107 ± 36; $T = 14.56$, $df = 22$, $P < 0.001$; Fig. 5). The relationship between temperature and RMR was best fit by an exponential model for 57% of crayfish and by a linear model for all other individuals. All best fit models were statistically significant ($P < 0.05$). An exponential relationship between metabolic rate and temperature was more common among crayfish from northern Wisconsin (88%) than crayfish from Illinois (40%; Fig. 6). In support of this, there was a marginally significant trend suggesting that home latitude had a positive effect on thermal sensitivity (Q_{10}; $F_{1,21} = 4.24$, $P = 0.052$; Fig. 7). While there may have been an

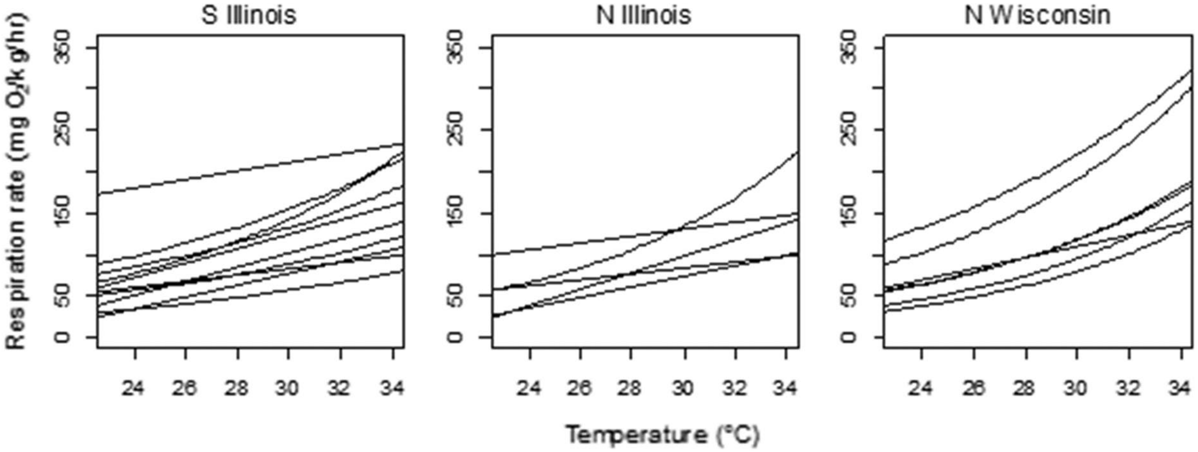

Fig. 6 The relationship between temperature and metabolic rate for virile crayfish from different regions (Southern Illinois, Northern Illinois, and Northern Wisconsin) that were exposed to an acute increase in temperature. Solid lines depict the best fit model (linear or exponential) for each crayfish

effect of home latitude on thermal sensitivity, there was no significant effect of latitude on mass-specific metabolic rate at either 23 °C ($F_{1,20} = 0.73$, $P = 0.402$) or 34 °C ($F_{1,3} = 0.69$, $P = 0.466$; Fig. 7). There was no effect of reproductive form on mass-specific metabolic rates in this experiment ($P > 0.5$).

Critical thermal maximum (acute)

The CTMax for virile crayfish ranged from 31 °C to 40 °C (mean ± SD = 37 ± 2 °C). There was no significant effect of home latitude on the CTMax of virile crayfish ($F_{1,19} = 0.08$, $P = 0.786$; Fig. 8). There were also no effects of either carapace length or reproductive form on the CTMax ($P > 0.1$).

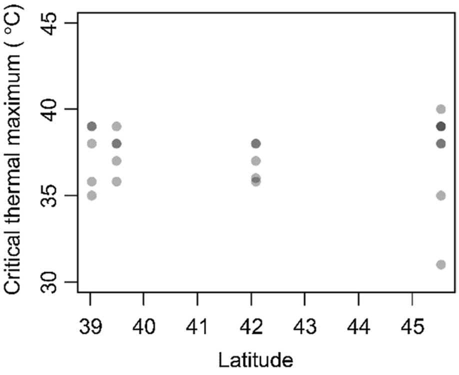

Fig. 8 The relationship between home latitude and critical thermal maximum (the temperature at which a crayfish does not right itself within 30 s) in virile crayfish. Crayfish in this experiment were initially acclimated to 23 °C

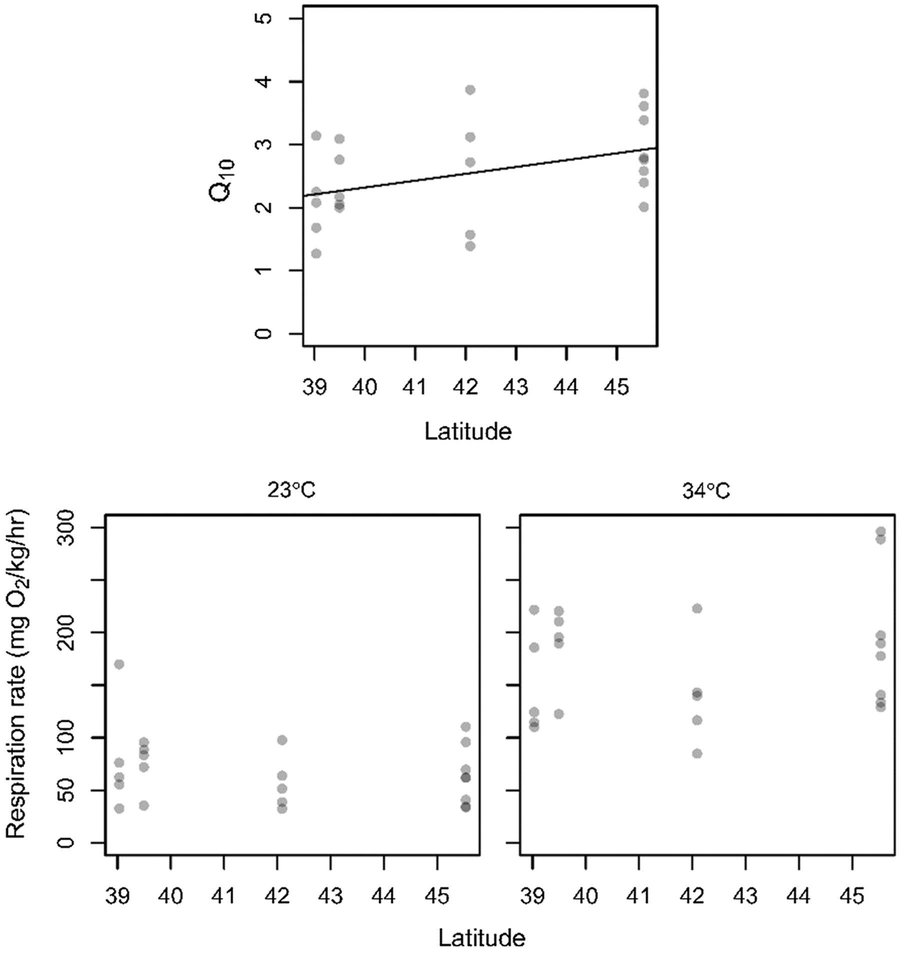

Fig. 7 Results from the thermal sensitivity experiment including the relationship between home latitude and thermal sensitivity (Q_{10}) and the relationship between home latitude and mass-specific metabolic rates at 23 °C and 34 °C in virile crayfish. Crayfish in this experiment were initially acclimated to 23 °C

Discussion

In support of our hypothesis, we found a relationship between latitude and virile crayfish RMR at night. Specifically, crayfish from higher latitudes had a higher RMR than those from low latitudes at night when they were acclimated and tested at the colder temperature (23 °C). It is likely higher metabolic rates at night resulted from higher activity levels because crayfish are typically outside of shelters and active at night (Fero & Moore, 2014), and there was no effect of latitude on metabolic rate during the day when crayfish are typically inactive. In support of this, a previous study recorded crayfish behavior on video and found that RMR increased at night due to increased activity (Taylor & Dunn, 2018). The relationship with latitude we observed is consistent with local adaptation to the growing season. Due to the shorter growing season, individuals from high latitudes must invest more energy in foraging to achieve the same growth rates on an annual basis as those from low latitudes, known as countergradient variation (Conover et al., 2009; Van Petegem et al., 2016). Our results are also consistent with earlier studies showing increased foraging, activity, and growth rates in crayfish populations from colder climates (Sargent & Lodge, 2014; Reisinger et al., 2020).

We did not observe the same positive relationship between latitude and RMR at night when crayfish were acclimated and tested at the warmer temperature (28 °C instead of 23 °C). This was largely because the RMR of low latitude populations increased at 28 °C so that RMR more closely matched that of high latitude populations. These changes are likely to represent changes in activity because they occurred at night. The relationship between activity level and temperature can differ from the relationship between metabolic rate and temperature (Halsey et al., 2015). In addition, activity can affect behaviors such as food acquisition. For example, a previous study focused on virile crayfish from Missouri found that metabolic rates increased between 22 and 30 °C, but feeding rate declined after 26 °C (Whitledge & Rabeni, 2002). Thus, animals may be more or less active at certain temperatures as an adaptation to the environment (*e.g.,* to increase the likelihood that they will be active at times when there is a high probability of obtaining food or a low probability of encountering predators; Halsey et al., 2015). Our data indicate that the temperatures at which virile crayfish were most active differed across the latitudinal gradient, suggesting that populations from lower latitudes are adapted to be less active at cooler temperatures than those from high latitudes. It is likely beneficial for high latitude individuals to be active at colder temperatures because water temperatures may rarely reach 28 °C in northern streams.

In contrast to the relationship with latitude we observed at night, there was no effect of latitude on RMR during the day, suggesting that the energy required for daytime maintenance and/or low activity levels was similar across the range. This occurred at both temperatures in both the acclimated and acute respirometry experiments. We initially hypothesized that crayfish from high latitude populations would have lower RMR during the day, allowing them to reserve their energy for high activity periods (Careau et al., 2019), but this hypothesis was not supported by our data. Thus, the latitudinal patterns in metabolic rates we observed are likely driven by differences in behavior and not the energetic requirements for maintenance.

Virile crayfish across the geographic range were able to acclimate to test temperatures, but temperature still had divergent effects on activity level. When virile crayfish were not acclimated to test temperatures (acute respirometry experiment), increases in temperature resulted in increases in RMR. In contrast, when crayfish were acclimated to test temperatures for at least one week (acclimated respirometry experiment), we did not observe an overall effect of temperature on RMR. This suggests virile crayfish can alter their physiology to compensate for the effects of temperature on their metabolic rates, like many other ectotherms (Seebacher et al., 2015). Previous studies have also found that temperature acclimation can affect the physiology of this species, and that one week is sufficient time for acclimation (Claussen, 1980).

The differences we observed in activity levels across populations could influence the ecological role of crayfish in freshwater ecosystems. A positive relationship between RMR and feeding rate has been observed in other decapod species (Taylor & Dunn, 2018). Crayfish often account for a substantial proportion of the consumer biomass in freshwater ecosystems (Momot, 1995; Whitledge & Rabeni, 2002) and have strong effects on freshwater community structure and ecosystem functions (McManamay et al., 2011; Hansen et al., 2013; Jackson et al., 2014). For example, virile crayfish can affect macrophyte biomass, benthic invertebrate community composition, and leaf litter decomposition rates (Chambers et al., 1990; Jackson et al., 2014). Higher crayfish foraging can also increase nitrogen and phosphorus in the water column due to higher excretion rates (Dodds et al., 2000). Therefore, high latitude virile crayfish populations may have greater foraging rates, which could increase their impacts on freshwater ecosystems.

In addition to temperature influencing activity, our results suggest that virile crayfish from high latitude populations were more thermally sensitive than those from low latitude populations. Specifically, RMR increased to a greater extent with temperature (Q_{10}) in virile crayfish from higher (colder) latitudes than those from lower (warmer) latitudes. This may be an adaptation of high latitude populations that allows them to respond quickly to rising temperatures and to be physiologically active during the short growing season (Nielsen et al., 1999). Some studies focused on ectotherms have found a similar pattern, where thermal sensitivity was positively related to latitude (Nielsen et al., 1999; Seebacher et al., 2015). However, other studies have not detected an effect of latitude on thermal sensitivity (*e.g.*, Rangel & Johnson, 2019).

In contrast to thermal sensitivity, we did not detect an effect of latitude on thermal tolerance (CTM). Virile crayfish across all latitudes exhibited high thermal tolerances. In our study, the mean CTM for virile crayfish was 37.3 ± 1.9 °C, which was similar to that in a previous study that measured thermal sensitivity in Wisconsin virile crayfish (CTM = 38.5 °C; Claussen, 1980) but substantially higher than the mean CTM of all crayfish species included in a recent literature review (33.4 ± 3.5 °C (SD); Westhoff & Rosenberger, 2016). Thus, virile crayfish populations from higher latitudes may be able to successfully become established in warmer climates characteristic of lower latitudes.

In summary, our results suggest that virile crayfish have a broad thermal niche, which likely contributes to the success of this invasive species in novel environments. Virile crayfish are native across a wide geographic range in the midwestern USA and Canada, but they have also invaded many new regions in North America (northeastern USA, southeastern USA, western USA) as well as portions of the UK and the Netherlands (Larson et al., 2018). Our findings indicate that virile crayfish from populations across the latitudinal gradient were able to acclimate to the temperatures in our experiment (23 and 28 °C). Further, even individuals from high latitude populations had a high thermal tolerance. While virile crayfish have a broad thermal niche and can be successful across different climate conditions, our results suggest that their thermal sensitivity (Q_{10}) and behavioral response to temperature (*i.e.*, nighttime activity level) are adapted to the local thermal regime. Thus, populations introduced from different latitudes could have different ecological impacts due to their behavioral response to temperature, and the traits in high latitude populations may shift toward those currently found in low latitude populations as the climate warms. Overall, we found evidence of both local adaptation to the thermal regime and a broad thermal tolerance in this species, suggesting that research focused on among-population variation across the range of a species is relevant for predicting its response to novel thermal conditions.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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