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The effects of warming on red blood cell carbonic anhydrase activity and respiratory performance in a marine fish

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ABSTRACT

Measures of fitness are valuable tools to predict species' responses to environmental changes, like increased water temperature. Aerobic scope (AS) is a measure of an individual's capacity for aerobic processes, and frequently used as a proxy for fitness. However, AS is complicated by individual variation found not only within a species, but within similar body sizes as well. Maximum metabolic rate (MMR), one of the factors determining AS, is constrained by an individual's ability to deliver and extract oxygen (O_2) at the tissues. Recently, data has shown that red blood cell carbonic anhydrase (RBC CA) is rate-limiting for O_2 delivery in red drum (*Sciaenops ocellatus*). We hypothesized increased temperature impacts MMR and RBC CA activity in a similar manner, and that an individual's RBC CA activity drives individual variation in AS. Red drum were acutely exposed to increased temperature (+6 °C; 22 °C to 28 °C) for 24 h prior to exhaustive exercise and intermittent-flow respirometry at 28 °C. RBC CA activity was measured before temperature exposure and after aerobic performance. Due to enzymatic thermal sensitivity, acute warming increased individual RBC CA activity by 36%, while there was no significant change in the control (22 °C) treatment. Interestingly, average MMR of the acute warming treatment was 36% greater than that of control drum. However, we found no relationships between individual RBC CA activity and their respective MMR and AS at either temperature. While warming similarly affects RBC CA activity and MMR, RBC CA activity is not a predictor of individual MMR.

1. Introduction

Global climate change is invariably altering the environmental conditions marine and coastal species are experiencing, even across small spatiotemporal scales. The trio of ocean deoxygenation, acidification, and warming are of particular concern for ectothermic organisms that are most susceptible to external changes. In the past few decades, extensive research has focused on the impacts of environmental change on overall fitness, and Fry's basic concepts of whole organism metabolic rate (Fry, 1947, 1971; Fry and Hart, 1948) have emerged with renewed interest (reviewed by Farrell, 2016). Absolute aerobic scope (here abbreviated as AS) is calculated as the difference between standard and maximum metabolic rate, where standard metabolic rate (SMR) is the estimate of the basal energetic requirements of an organism to sustain life, and maximum metabolic rate (MMR) is an estimate of an organism's peak aerobic capacity. Recent work has also highlighted the importance of using different metrics of aerobic scope, such as factorial aerobic scope (FAS; Clark et al., 2005; Esbaugh et al., 2021; Halsey et al., 2018; Seibel and Deutsch, 2020). FAS, the ratio of MMR to SMR (i.e., maximum O_2 supply to minimum O_2 demand) has been utilized in the context of the metabolic index, and has been hypothesized to define marine biogeographical distribution on the basis of thermal- and hypoxia-based constraints (Deutsch et al., 2015; Deutsch et al., 2020). In short, aerobic scope is frequently used as a snapshot of an individual's fitness – the available energy that can be used for all life's non-vital processes (e.g., growth, feeding, digestion, reproduction, etc.; reviewed by Clark et al., 2013). Understanding environmental impacts on aerobic scope, and therefore individual fitness, is critical when attempting to predict species' responses to climate change.

Increasing water temperature raises the cost of basal energetic requirements and predictably increases SMR (reviewed by Schulte, 2015). However, the relationship between MMR and increased water temperature is more complex. In general, MMR also rises with increasing temperature; however, many species exhibit a breakpoint after which MMR declines, leading to a warming-induced collapse of AS. For example, red drum (*Sciaenops ocellatus*) demonstrate a linear increase in

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MMR from 18 °C to 25 °C and then plateau at 28 °C (Ackerly and Esbaugh, 2021). The oxygen- and capacity-limited thermal tolerance hypothesis (OCLTT) suggests such collapses are the cause for upper thermal limitations in aquatic ectotherms (Pörtner and Knust, 2007; Pörtner and Lannig, 2009), which remains a subject of debate (Jutfelt et al., 2018). In contrast, many other studies have shown a right-shifted thermal performance curve where MMR and other O_2 transport measures (e.g., heart rate, cardiac output) increase until mortality (e.g., Clark et al., 2005; Norin et al., 2014). This interspecific variation in response to increased temperature is further complicated by individual variation in aerobic performance within a species and when standardized for body size (Norin and Clark, 2016).

Cardiac output and morphology have been implicated in driving individual variation of aerobic performance (Claireaux et al., 2005); however, MMR is constrained by an individual's ability to both deliver O_2 (e.g., cardiac performance) and extract O_2 at the tissues (Clark et al., 2011; Eliason et al., 2011; Farrell et al., 2009; Fry and Hart, 1948; Norin and Clark, 2016). While August Krogh described O_2 transport as passive movement (Krogh, 1910, 1919a, 1919b), in recent years researchers have found that fish with pH-sensitive hemoglobins (Hbs) may invest energy to increase O_2 extraction (Alderman et al., 2016; Damsgaard et al., 2020; Harter et al., 2019; Harter and Brauner, 2017; Randall et al., 2014; Rummer and Brauner, 2011; Rummer and Brauner, 2015; Rummer et al., 2013). Red blood cell carbonic anhydrase (RBC CA) has since emerged as a potentially critical rate-limiting step for O_2 transfer in this process. Until recently, RBC CA had been thought to primarily function in carbon dioxide (CO_2) excretion, catalyzing the movement of metabolically-produced CO_2 from aerobically-demanding tissues into the RBC and then excreting CO_2 at the gills. However, the enzyme had also been considered well in excess of what was necessary for CO_2 excretion (Maren and Swenson, 1980; Perry, 1986). Our recent work found that RBC CA activity dictates the rate of intracellular acidification, and thus the Hb-O_2 offloading rate, in red drum (Dichiera and Esbaugh, 2020). If MMR is constrained by O_2 extraction at the tissues in this species, the relationship between RBC CA activity and Hb-O_2 offloading rate may drive aerobic performance. In addition, red drum display remarkable variation in RBC CA activity even when standardized for body size (Fig. S1). Thus, intraspecies variation in RBC CA activity may play a key role in defining intraspecies variation of MMR (and therefore AS) and contribute to the thermal responsiveness of MMR.

On this background, the current study explored the relationship between RBC CA and MMR in red drum using an acute thermal stress experiment. We hypothesized that: 1) a warming-induced elevation of MMR would be coincident with a similar magnitude of elevated RBC CA activity; and 2) RBC CA activity would predict individual variation in MMR in red drum, with a robust relationship across different temperatures. Red drum were chosen for this work as they are native to the shallow estuaries found along the coast of the Gulf of Mexico and are commonly exposed to periods of rapid warming in early summer. Furthermore, there exist previously determined relationships between RBC CA and Hb-O_2 extraction (Dichiera and Esbaugh, 2020), as well as established data pertaining to the effects of warming on SMR, MMR, and AS (Ackerly and Esbaugh, 2021; Ern et al., 2016) for red drum.

2. Materials and methods

All experimental protocols were approved by the University of Texas at Austin Institutional Animal Care and Use Committee (AUP-2018-00231).

2.1. Experimental animals

Red drum ($N = 72$, 24.3 ± 1.4 g, mass ± S.E.M.) were maintained for several months at the University of Texas Marine Science Institute Fisheries and Mariculture Laboratory in Port Aransas, TX. Most fish ($N = 56$) were purchased from Ekstrom Aquaculture, LLC in Palacios, TX and a small number ($N = 16$) were reared in-house from embryos. All fish were maintained in control conditions (22.15 ± 0.02 °C, 34.8 ± 0.1 ppt) in 600 L recirculating tanks for at least two weeks before experimental trials began and were fed ad libitum daily.

2.2. Acute warming exposure

Fish were fasted for 24 h prior to acute temperature exposure. Individuals were randomly assigned to either control (22.25 ± 0.02 °C; $N = 17$) or acute warming (28.12 ± 0.04 °C; $N = 15$) treatments. For all exposures fish were placed in 4 L glass tanks placed within a water bath maintained at 22 °C. For the acute warming treatment, the water bath temperature was increased 2 °C per hour until tank temperatures reached 28 °C, while the control treatment was maintained at 22 °C. Fish were then left overnight for an additional 21 h prior to respirometry.

2.3. Blood sampling and measurements

Fish were elastomer tagged and blood sampled one week prior to acute temperature exposure (initial) and immediately after testing aerobic performance, as detailed below (final; approximately 45 h at either 22 °C or 28 °C). Fish were sampled for whole blood under anesthesia via immersion in tricaine methanesulfonate (MS-222100 mg L^{-1} buffered with 500 mg L^{-1} $NaHCO_3$). Blood samples were collected via caudal puncture into a heparinized syringe, transferred to a microcentrifuge tube, and immediately placed on ice. Hematocrit (% RBCs in whole blood) was determined using microcapillary tubes, centrifuged in a hematocrit centrifuge for 2 min at 10,000*g*. Whole blood samples were also centrifuged for 2 min at 10,000*g* to separate RBCs from plasma. RBCs were washed three times with cold isotonic saline (300 mOsm L^{-1} NaCl) to remove any remaining plasma or extracellular components. Mean corpuscular hemoglobin concentration (MCHC) was determined using standard Drabkin's reagent (Fisher Scientific) and packed RBCs (forgoing the need to standardize to Hct). Absorbance was read at 540λ and concentration calculated using an extinction coefficient of 11 mmol L^{-1} cm^{-1} according to Van Assendelft and Zijlstra (1975).

RBC CA activity was measured using a modified ΔpH method (Henry, 1991). RBC samples were lysed using a 500-times dilution in deionized water and kept at −80 °C until activity assays. A 10 μL aliquot of each sample was added to 7 mL buffer medium (225 mM D-mannitol, 13 mM Tris Base, 75 mM sucrose, pH 7.4) and 200 μL of CO_2-saturated distilled water was injected into the medium using a Hamilton syringe. All reactions were performed in triplicate, and at 22 °C if initial timepoint and/or control treatment, or 28 °C if final acute warming treatment. The uncatalyzed reaction rate (the rate of pH change for CO_2-saturated distilled water only) was subtracted from the observed rate to obtain the true catalyzed reaction rate. The buffer capacity (μmol H^+ pH $unit^{-1}$) was determined prior to measurements via addition of 50 μL 0.1 N HCl to the buffer medium, then calculating the differences in initial and final pH, standardized to volume. This value was used to convert the catalyzed and uncatalyzed reaction rates from pH units min^{-1} to mol H^+ min^{-1}. RBC CA activity was standardized to protein content (μmol H^+ min^{-1} μg $protein^{-1}$) measured using Pierce™ Coomassie Plus (Bradford) Assay Reagent (Thermo Scientific™). To account for the documented relationship between body mass and RBC CA activity in red drum, all data underwent a second normalization for fish body mass using the relationship described by Dichiera and Esbaugh (2020).

2.4. Aerobic performance

A standard chase protocol was used for MMR (Roche et al., 2013), in which each individual was chased to exhaustion for 3 min in their respective treatment temperature (22.30 ± 0.03 °C; 28.11 ± 0.04 °C), and then exposed to air for 1 min. Individuals were immediately placed into intermittent flow respirometry chambers (ranging from 694 to 1200 mL depending on individual mass, Loligo® Systems, Denmark)

with a 3 min flush period, 1 min wait period, and 4 min closed measurement cycle (comprising 1 loop). Respirometry chambers were kept in a water bath at the respective treatment temperatures (22.09 ± 0.05 °C; 27.97 ± 0.04 °C). Oxygen concentrations (MO_2) were measured using fiber optic probes (Presens) connected to Autoresp (Loligo® Systems, Denmark). MO_2 was calculated as the negative slope of the oxygen partial pressure in each period. To account for the contribution of bacterial respiration to MO_2 measurements, the average of two 30-min loops (3 min flush period, 1 min wait period, and 26 min closed measurement cycle) before and after the fish entered the chamber were used to determine the contribution of bacterial respiration over time. These values were deducted from the raw MO_2 values, assuming a linear increase in bacterial respiration over time. MMR was determined as the highest MO_2 recorded within the first five measurements. SMR was determined as the lowest 10th percentile of recorded MO_2 over 23–24 h. Both were mass-corrected using species-specific scaling exponents from Pan and Esbaugh et al. (2016)(SMR) and Ackerly and Esbaugh (2020) (MMR). Absolute AS (AS) was calculated as the difference between MMR and SMR, and factorial aerobic scope (FAS) was calculated as the ratio of MMR to SMR. Excess post-exercise oxygen consumption (EPOC) was measured as the time (min) required to return to routine metabolic rate (RMR) following exhaustive exercise. RMR was calculated as the average MO_2 over the entire 23–24 h respirometry period.

2.5. Statistical analyses

All data are reported as mean ± S.E.M. Two-way repeated measures ANOVAs were used to determine the effects of temperature and time (pre-exposure vs. post-respirometry) on hematological parameters. Two-tailed student *t*-tests were used to determine the effect of temperature on aerobic performance using $\alpha < 0.05$ to determine significance. In circumstances where normality failed, a non-parametric Mann-Whitney rank sum test was used. Linear regressions were used to examine potential relationships between RBC CA activity and aerobic performance. All statistical analyses and graphs were performed and created in SigmaPlot (13.0).

3. Results

3.1. The effects of acute temperature change on RBC CA activity

Two-way repeated measures ANOVA demonstrated that time had a significant effect on RBC CA activity ($F_{1,45} = 4.686$, $P = 0.042$) but there were no interactions between time and temperature, nor an effect of temperature. To better address the effects of the temperature treatment on RBC CA activity we ran paired student's t-tests (using Bonferroni-corrected $\alpha < 0.025$). RBC CA activity did not change from initial to final sampling timepoints within the control treatment ($t = -0.339$, $P = 0.742$; Fig. 1A); however, RBC CA activity significantly increased in the warming treatment with a 36% increase from the initial sampling at 22 °C to the final sampling at 28 °C ($t = -2.768$, $P = 0.018$; Fig. 1B). To assess whether this increase in enzyme activity was due to a kinetic effect of temperature or an increase in CA content after ~45 h acute warming treatment, we ran a subset of samples ($N = 5$) at both 22 °C and 28 °C and found a similar increase in enzyme activity of 37% (Fig. S2). There were no statistically significant effects of time and temperature on body mass or MCHC (Table 1). Time had a significant effect on hematocrit ($F_{1,30} = 24.211$, $P < 0.001$) but there were no interactions between time and temperature.

3.2. The effects of acute temperature change on aerobic performance

When comparing aerobic performance across treatments, there were significant increases in MMR (Mann-Whitney, $U = 19$, $P < 0.001$), SMR (Mann-Whitney, $U = 17$, $P < 0.001$) and AS (Mann-Whitney, $U = 62$, $P = 0.014$) with increased temperature (Fig. 2). Conversely, FAS significantly decreased with increased temperature (Mann-Whitney, $U = 57$, $P = 0.008$). EPOC also significantly decreased with increased temperature ($t = -3.575$, $P = 0.001$). Simply put, recovery was quicker for the acute warming treatment than the control treatment (Fig. 3A). RMR was significantly higher for the acute warming fish than the control fish (Mann-Whitney, $U = 32$, $P < 0.001$; Fig. 3B). However, temperature had a similar effect on RMR and MMR, as evidenced by the similar RMR: MMR for control and acute warming treatments ($t = 1.687$, $P = 0.102$; Fig. 3C).

3.3. The relationship between RBC CA activity and individual aerobic performance

RBC CA activity did not have any relationships with MMR, AS, FAS, or EPOC when temperature treatments were combined (Fig. 4). Scaling of MMR and SMR did not impact these relationships, as RBC CA activity did not have any relationships with non-scaled aerobic performance metrics ($P > 0.05$; data not shown). Regressions were run for each temperature as well, but RBC CA activity did not have any relationships with MMR, AS, or FAS at either temperature, or EPOC for the acute

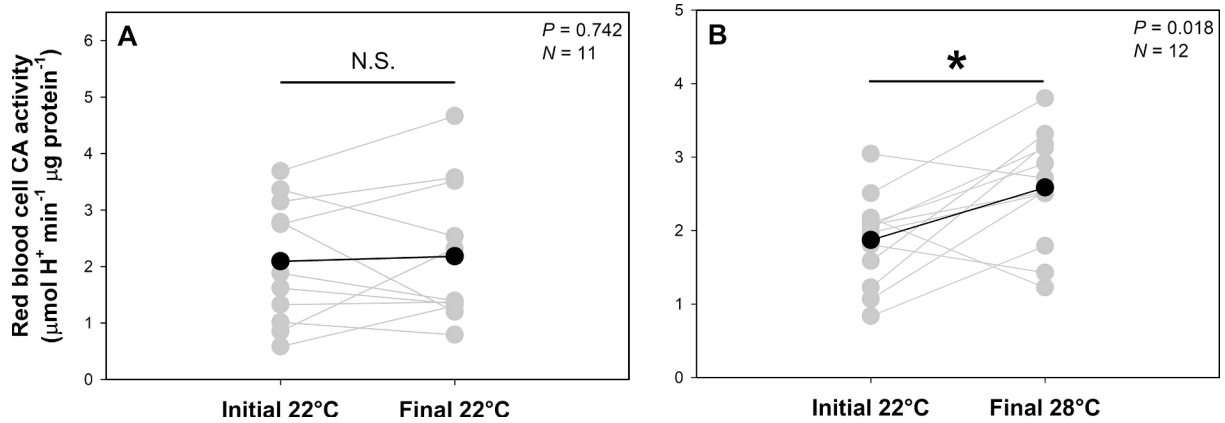


Fig. 1. Individual pre-warming and post-exercise RBC CA activity for red drum, *Sciaenops ocellatus*. (A) RBC CA activity for individuals acclimated and exercised at 22 °C. Individuals did not show a significant difference between their initial RBC CA activity at 22 °C and their final RBC CA activity at 22 °C ($P = 0.742$). (B) RBC CA activity for individuals acclimated initially at 22 °C, then acutely exposed, exercised, and sampled at 28 °C. Individuals showed a significant increase from their initial RBC CA activity at 22 °C and their final RBC CA activity at 28 °C ($P = 0.018$). RBC CA activity was measured at control temperature (22 °C) for the initial measurement and measured at the respective treatment temperatures (22 °C or 28 °C) for the final measurement. Activity was normalized to protein content.

Table 1
Hematological parameters for red drum, *Sciaenops ocellatus*, for both control (22 °C) and acute warming (28 °C) treatments pre-warming and post-exercise. All data is presented as mean ± S.E.M. Sample sizes are indicated in parentheses. There was a significant effect of time (two-way repeated measures ANOVA, $\alpha > 0.05$) on hematocrit ($F_{1,30} = 24.211$, $P < 0.001$), and RBC CA activity ($F_{1,45} = 4.686$, $P = 0.042$). However, there was no interaction between time and treatment. * denotes a significant effect of time from initial sampling to final sampling.

	Treatment			
	Control (22 °C)		Acute warming (28 °C)	
	Initial	*Final*	*Initial*	*Final*
Weight (g)	20.8 ± 2.5 (17)	21.1 ± 2.8 (17)	23.1 ± 3.6 (15)	24.3 ± 3.7 (15)
Hematocrit (%)*	30.9 ± 1.4 (7)	23.6 ± 2.5 (7)	35.1 ± 2.1 (8)	23.6 ± 21.8 (8)
Mean corpuscular hemoglobin concentration (mM)	9.95 ± 1.37 (11)	10.37 ± 2.01 (11)	12.64 ± 1.79(11)	11.63 ± 1.38(11)
RBC CA activity (μmol H^+ min^{-1} μg $protein^{-1}$)*	2.09 ± 0.33 (11)	2.18 ± 0.38 (11)	1.87 ± 0.18 (12)	2.59 ± 0.22 (12)

warming treatment. However, RBC CA activity did have a positive linear relationship with EPOC for the control treatment only ($r^2 = 0.335$, $P = 0.030$; Fig. S3).

4. Discussion

Using an acute thermal stress, we explored the relationship between RBC CA activity and respiratory performance, testing two hypotheses: 1) acute warming increases MMR and RBC CA activity in a similar fashion; and 2) individual variation in RBC CA activity predicts variation in MMR, and therefore AS. While acute warming impacted the average measures of aerobic performance and RBC CA activity similarly, we did not find evidence individual variation in RBC CA activity was determinative of individual variation in MMR or AS.

As predicted, all absolute measures of metabolic rate increased with temperature. MMR was significantly elevated after ~24 h exposure to +6 °C. SMR and RMR were also significantly higher at 28 °C than that of the control 22 °C treatment. Our data broadly agree with the prior findings of Ackerly and Esbaugh (2021), where the authors found both SMR and MMR increased when acclimated to increased temperature for 3 weeks. However, a comparison of the relative sensitivities to temperature between the two studies reveals that the acute exposure described here yielded a steeper thermal response slope over the same temperature range for both SMR and MMR. The SMR observations may indicate that red drum exhibit a degree of metabolic thermal acclimation that actively lowers SMR when acclimated to higher temperatures, which has previously been described in shorthorn sculpin (*Myoxocephalus scorpius*; Sandblom et al., 2014). In contrast, the MMR observations would indicate deterioration of MMR when chronically exposed to elevated temperatures, which again coincides with observations from shorthorn sculpin (Sandblom et al., 2014). Further studies are clearly warranted to directly test the role of acclimation on the thermal responsiveness of metabolic traits in red drum.

MMR and SMR represent measures of maximum O_2 supply and minimum O_2 demand, respectively; however, the ecological significance of warming is generally ascribed based on calculations of AS, which describes the energy available to a fish beyond what is required to sustain baseline physiological function. AS can be described in absolute (i.e., MMR-SMR) or factorial (i.e., MMR/SMR) terms, and the chosen calculation can have important consequences for how research findings are interpreted in an ecological context (Halsey et al., 2018). Here we report our findings using both absolute AS and FAS, with contrasting conclusions. Because the absolute increase of MMR following warming exceeded that of SMR, the acute temperature change resulted in a significant increase in absolute AS. However, the relative thermal sensitivity of SMR greatly exceeded that of MMR and thus FAS significantly decreased at the higher experimental temperature. Absolute AS is best viewed as a proxy for the absolute amount of O_2 beyond what is required for vital physiological function. In red drum, this measure has been positively correlated to competitive performance whereby fish with higher absolute AS values were more likely to be dominant in paired competitions (Khursigara et al., 2018), which suggests that absolute AS is linked to individual performance. Conversely, FAS is a ratio of maximum O_2 supply to minimum O_2 demand, and has been linked to latitudinal habitat limitations in red drum and other species (Deutsch et al., 2020). Note that 28 °C is well below the CT_{max} reported for red drum (36.5C ± 0.2 °C; Ern et al., 2016), which is a trait that has also been linked to latitudinal range boundaries in aquatic ectotherms (Sunday et al., 2011, 2012).

The conflicting conclusions based on absolute AS and FAS make it difficult to assess whether the metabolic shifts observed with warming described above are beneficial or detrimental to red drum – a dilemma that has been discussed previously for other species (Halsey et al., 2018). We would hypothesize that the greater access to ATP for non-vital functions coincident with elevated absolute AS would have a more direct impact on performance with respect to activity and feeding (i.e., specific dynamic action; Sandblom et al., 2014). This hypothesis is corroborated when looking at the effect of warming on EPOC, which was calculated as the amount of time required for MO_2 to return to RMR. Note that RMR is the average oxygen consumption observed over the course of the SMR trial, and thus a good representation of average metabolic rate. When red drum were exposed to acute warming, their EPOC returned to RMR at a faster rate (Fig. 3A). While it is tempting to suggest that the faster recovery time was due to the elevated RMR exhibited by warmed fish (Fig. 3B), the ratio of RMR to MMR was similar in the two temperature treatments (Fig. 3C). As such, it is apparent that the acutely warmed fish recover to routine metabolic performance more quickly than the control fish. The best available data for red drum suggest that absolute AS peaks between 25 °C and 28 °C, while FAS is highest at low temperatures and continually declines with warming (Ackerly and Esbaugh, 2021; Esbaugh et al., 2021). As such, the available evidence suggests EPOC performance is guided by the absolute AS thermal performance curve, and not that of FAS.

With respect to RBC CA, we hypothesized that warming would induce an increase in RBC CA activity that matched the warming induced increase in MMR. Using a paired experimental design, we observed that RBC CA activity increased by 36% from the pre-warming 22 °C time point to the 28 °C sampling time point. Interestingly, this perfectly matches the 36% increase in MMR at 28 °C compared to 22 °C. While it is important to note that the post-warming sample also incurred an exercise stress as part of the MMR measurement protocol, we observed no increase in RBC CA activity in the control 22 °C experiment. Therefore, the change in RBC CA activity was not due to the short period of exercise but was a product of warming. A remaining question was whether the observed increase in CA activity was due to increased protein abundance (i.e., acclimation), or whether acute warming simply improved the enzyme reaction rate. To address this, we tested a subset of samples at both 22 and 28 °C and observed a 37% increase in CA activity (Fig. S2). This experiment clearly demonstrated that elevated RBC CA activity observed in our warming experiment was due to the thermal

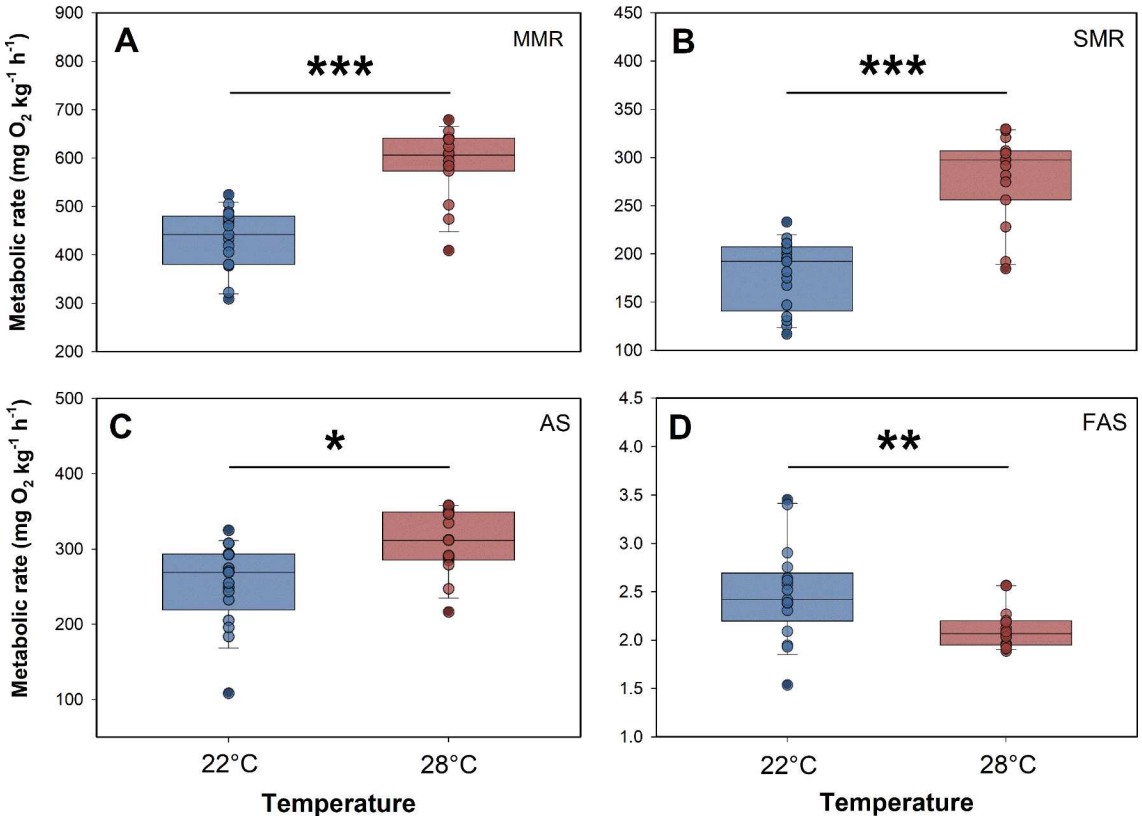


Fig. 2. Natural and temperature-induced variation in metabolic rate for red drum, *Sciaenops ocellatus*. Blue denotes control treatment (22 °C) and red denotes acute warming treatment (28 °C). There were significant increases in (A) maximum metabolic rate ($P < 0.001$), (B) standard metabolic rate ($P < 0.001$), (C) absolute aerobic scope ($P = 0.014$) with increased temperature. (D) Factorial aerobic scope significantly decreased with increased temperature ($P = 0.008$). All data are represented as box plots, where the minimum, first quartile, median, third quartile and maximum are presented for each treatment, and individual data are overlayed (22 °C: $N = 17$; 28 °C: $N = 15$). Note that the y-axis for factorial aerobic scope is unitless. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

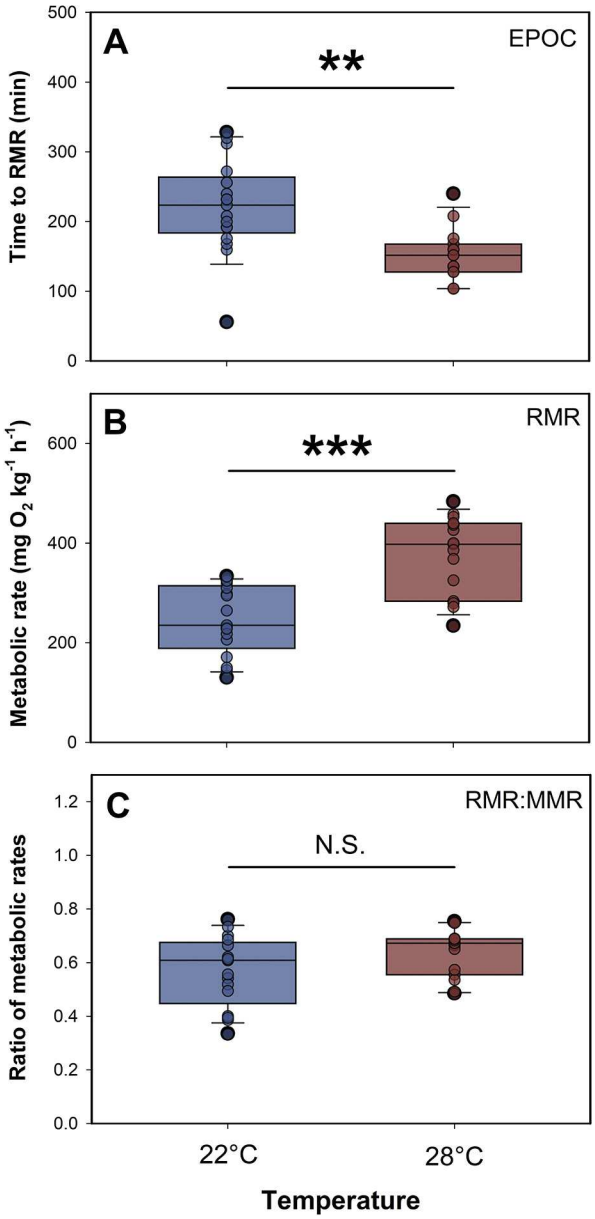
sensitivity of the RBC CA reaction rate, as opposed to rapid acclimation during the 24 h SMR measurement period.

Despite the similar warming effects on RBC CA activity and MMR described above, our data did not show a significant relationship between individual variation in RBC CA activity and individual variation in MMR. We did observe that RBC CA activity explained ~34% of the variation in EPOC in the control treatment (22 °C); however, this trend was not observed at 28 °C nor when the data sets were combined. As such, the relationship between RBC CA and EPOC at 22 °C appears spurious. Thus, our second hypothesis was not supported; however, this should not be used to invalidate RBC CA's role in O_2 extraction. Instead, it seems that other O_2 extraction processes meaningfully contribute to individual variation in aerobic performance. There exist a plethora of characteristics (e.g., Hb-O_2 binding affinity and carrying capacity, beneficial localization and abundance of plasma-accessible CA, β-adrenergic sodium proton exchange proteins, etc.) that have been suggested to improve O_2 extraction at the tissues (Alderman et al., 2016; Berenbrink et al., 2005; Brauner and Harter, 2017; Damsgaard et al., 2020; Harter et al., 2019; Harter and Brauner, 2017; Harter et al., 2018; Nikinmaa et al., 2019; Randall et al., 2014; Rummer and Brauner, 2011; Rummer and Brauner, 2015; Rummer et al., 2013). Similarly, the variation in aerobic performance we measured is also likely to be closely tied to variation in cardiac performance. Indeed, many studies exist that detail how MMR and its variation are limited by the cardiovascular system (Claireaux et al., 2005; Hillman et al., 2013; Norin and Clark, 2016). Incidentally, cardiac output has been seen to double with a 10 °C increase in water temperature (Clark et al., 2008; Gollock et al., 2006), which could greatly impact MO_2 in the context of the Fick equation. As we continue to characterize red drum aerobic performance under various environmental conditions (Ackerly and Esbaugh, 2020, 2021; Ern et al., 2016; Esbaugh et al., 2016; Johansen and Esbaugh, 2017; Khursigara et al., 2018; Pan et al., 2017), there exists a gap in our knowledge specifically regarding the contribution of cardiac performance.

In conclusion, our work here demonstrated that acute warming stress exerted similar effects on RBC CA activity and MMR, which subsequently improved absolute AS and EPOC recovery. However, contrary to our hypothesis, individual variation in RBC CA activity was not significantly related to individual variation in MMR. This was not necessarily surprising as the cardiorespiratory traits that combine to define MMR are intertwined and complex. For example, respiratory O_2 extraction in the heart feeds cardiac performance and improves further O_2 transport. Failure in any one of these systems will impact the performance of all components and reduce MMR. It is therefore important to consider the cardiorespiratory system holistically, particularly when trying to understand the relationships between respiratory performance and temperature.

Contributions

A.M.D. and A.J.K. designed and conducted experiments. A.M.D. ran statistical analyses, created figures, and wrote first draft of manuscript. A.J.E. provided constructive feedback on experimental design and statistical analyses, and funds to conduct experiments. All authors contributed to manuscript revisions.



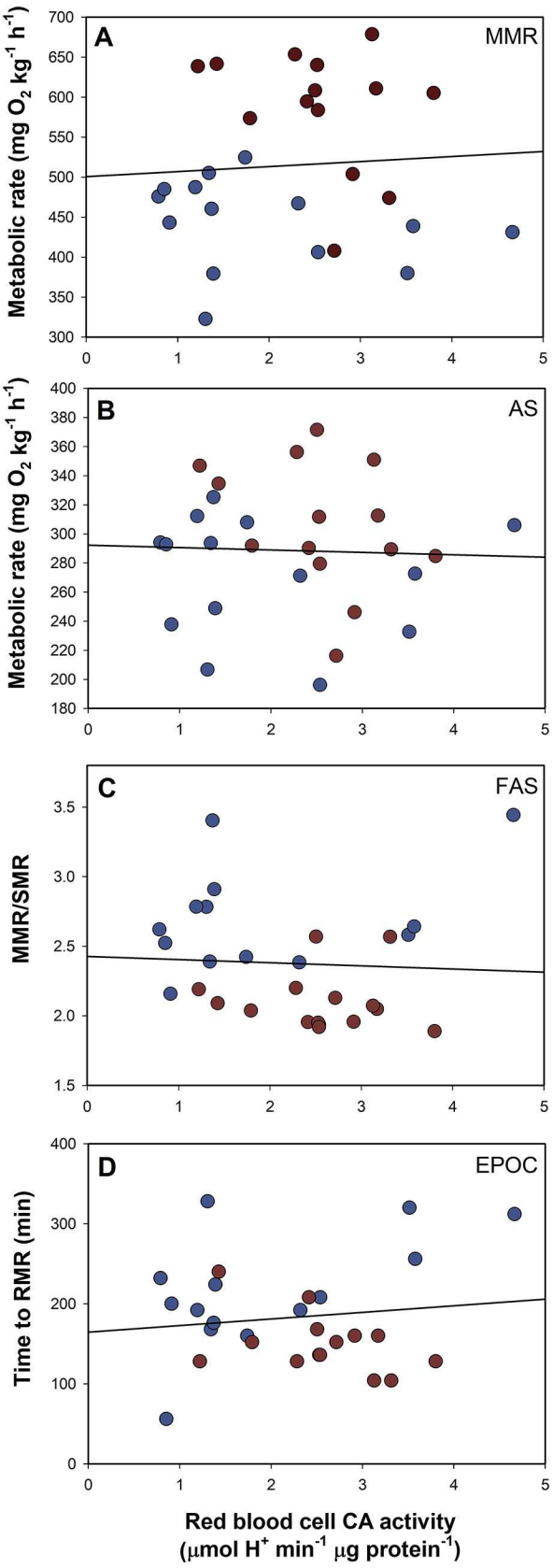


Fig. 3. Natural and temperature-induced variation in excess post-exercise oxygen consumption in red drum, *Sciaenops ocellatus*. Blue denotes control treatment (22 °C) and red denotes acute warming treatment (28 °C). (A) There was a significant decrease in time to resting metabolic rate (RMR) with increased temperature ($P = 0.001$). Recovery was quicker for the acute warming treatment (152.0 ± 9.4 min) than the control treatment (221.7 ± 16.3 min). (B) RMR was significantly higher for the acute warming treatment ($P < 0.001$). (C) RMR:MMR was not significantly different ($P = 0.102$) between treatments, indicating RMR increased with a similar magnitude to MMR with increased temperature. All data are represented as box plots, where the minimum, first quartile, median, third quartile and maximum are presented for each treatment, and individual data are overlayed (22 °C: $N = 17$; 28 °C: $N = 15$). Note that RMR:MMR is a unitless ratio. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Funding information

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Fig. 4. Relationships between red blood cell carbonic anhydrase and aerobic performance for red drum, *Sciaenops ocellatus*. Blue denotes control treatment (22 °C) and red denotes acute warming treatment (28 °C). RBC CA activity did not have any statistically significant relationships with (A) maximum metabolic rate, (B) absolute aerobic scope, (C) factorial aerobic scope, nor (D) excess post-exercise oxygen consumption (22 °C: $N = 14$; 28 °C: $N = 14$). Note that the y-axis for factorial aerobic scope is unitless. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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