Sexual dimorphism of metabolism in vampire crabs (*Geosesarma hagen*) is temperature and size-dependent

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Running Title: Sexual dimorphism of metabolism in crabs

Key Words: Brachyura, respirometry, ectotherm, longitudinal, climate, brooding

Word Count: 2,109

Summary Statement: We quantify how temperature, body mass, and sex affect metabolic rate in vampire crabs. We discuss the relevance of this study to ectotherms, ecology, and evolution.

Resumen

El metabolismo es un vínculo clave entre la fisiología de un organismo y el medio ambiente. La plasticidad en las tasas metabólicas permite a los organismos responder y adaptarse al cambio ambiental. Comprender cómo cambia el metabolismo en respuesta a los aumentos de la temperatura ambiente y cómo estos cambios tendrán efectos diferenciales entre los individuos es un objetivo fundamental. Exploramos la relación entre la tasa metabólica, la temperatura, el tamaño corporal y el sexo en cangrejos vampiros terrestres (*Geosesarma hagen*) a dos temperaturas ecológicamente relevantes. Encontramos dimorfismo sexual de las tasas metabólicas a temperaturas más cálidas, pero solo en cangrejos más pequeños, sugiriendo que las hembras pequeñas son más susceptibles a las condiciones de calentamiento. El coeficiente de temperatura (Q_{10}) para *G. hagen* fue de 1,52, enseñando un cambio esperado del 52% en la tasa metabólica por cada aumento de 10℃. Este trabajo demuestra cómo contabilizando los efectos correlacionados de las variables abióticas y bióticas al cuantificar el metabolismo en ectotérmicos es importante para aprender cómo el cambio climático puede afectar a las poblaciones naturales.

Abstract

Metabolism is a key link between an organism's physiology and environment. Plasticity in metabolic rates allows organisms to respond and adapt to environmental change. Understanding how metabolism shifts in response to increases in ambient temperature and how these changes will have differential effects across individuals is a critical goal. We explored the relationship between metabolic rate, temperature, body size, and sex in terrestrial red devil vampire crabs (*Geosesarma hagen*) at two ecologically-relevant temperatures. We found sexual dimorphism of metabolic rates at warmer temperatures, but only in smaller crabs, suggesting small females are most susceptible to warming conditions. The temperature coefficient (Q_{10}) for *G. hagen* was 1.52, showing an expected 52% change in metabolic rate for every 10℃ increase. This work demonstrates how accounting for the correlated effects of abiotic and biotic variables when quantifying metabolism in ectotherms is important for learning how climate change may affect natural populations.

Introduction

Understanding how biological processes affect metabolism and how metabolic rates shift in response to environmental change is an important goal in biology. Metabolism is the sum of processes by which energy and materials are transferred and modified throughout the body (Brown et al., 2004). In heterotrophs, which oxidize *ex-vivo* organic carbon to produce energy, the metabolic rate is the rate of respiration (Brown et al., 2004; Martin and Fuhrman, 1955; Norin and Metcalfe, 2019). Organisms with relatively higher metabolic rates must gather enough food to meet higher energetic demands and ambient conditions may change an organism's energy requirements. Metabolic rates are reliable indicators of energy expenditure and plasticity in metabolic rates allows organisms to meet varying energetic demands across space and time (Speakman et al., 2004). Within the context of climate change and conservation, understanding the ecology and evolution of metabolic rates is crucial. Metabolic rates are directly tied to ecology and evolution through growth, maintenance, reproduction and heritability (Kleiber, 1947; Norin and Metcalfe, 2019; Pettersen et al., 2018; West et al., 1997). Knowing the abiotic and biotic factors that affect energetic demands (metabolism) is an important milestone in predicting how animal communities will cope with accelerating changes in climate.

While there exists a vast literature on metabolic rates, few studies have quantified the combined effects of temperature, body size, and sex on metabolism. The classical explanation for how temperature affects metabolic rates states that higher temperatures increase the rate of biochemical reactions (Gillooly et al., 2001; Robinson et al., 1983). In ectotherms, metabolic rates are usually quantified by estimating the standard metabolic rate (SMR), which is the rate of metabolism of adult individuals at a particular temperature (McNab, 2002). In endotherms, metabolic rates have historically been quantified as the basal metabolic rate (BMR), or the minimum energy expenditure for living (Hulbert and Else, 2004), but standard metabolic rates are also used to compare rates among endotherms exposed to different temperatures (Frappell and Butler, 2004). Typically, metabolic Q_{10} values, or the expected change in metabolic rate given a change of 10°C (Gillooly et al., 2001), are used as indicators of how sensitive

metabolic rates are to changes in temperature. Increases in body mass are also associated with higher metabolic rates since body mass is a reliable indicator of the amount of metabolically active tissue and the respective energetic demands of organisms of different body sizes (Gillooly et al., 2001; Kleiber, 1947; Robinson et al., 1983). Finally, on the premise that reproduction is energetically costly, we know that egg production results in higher metabolic rates in both endotherms and ectotherms (Gilbert and Manica, 2010; Glazier, 1991; Nilsson and Råberg, 2001). A broader understanding of how temperature, body size, and sex determine metabolic rate is necessary for predicting how individuals and species will respond to changing environments.

Brachyuran crabs (true crabs) are an ideal system for understanding how metabolic rates vary with body size, temperature, and sex. True crabs have a near-circumglobal distribution and inhabit most environments on Earth including marine, freshwater and terrestrial habitats (Bliss, 2015; Boudreau and Worm, 2012; Green, 1997; Kawai and Cumberlidge, 2016; Watson-Zink, 2021). True crabs also cover a wide range of body sizes from a few millimeters, as in the parasitic male pea crab with a carapace width of 5.3 mm (Kane and Farley, 2006), up to several meters, as in Japanese spider crabs with a leg span of up to 4 m (McClain et al., 2015). Brachyurans also deploy a variety of developmental strategies, from extended pelagic development with free-swimming larvae to direct terrestrial development with extensive maternal care (Anger et al., 2015; Vogt, 2013). Many true crabs, particularly ones with markedly amphibious or terrestrial lifestyles, also possess novel secondary organs for respiration in addition to their gills, and they flexibly shunt between these organs depending on their respiratory media (Morris, 2002).

The goal of this study is to determine how temperature, body size, and sex impact metabolic rates (gill respiration) in the red devil vampire crab, *Geosesarma hagen*. This terrestrial species is endemic to Java, Indonesia where surface temperatures average 31°C and range from 16 to 41°C (Jaelani and Handayani, 2022; Sholihah and Shibata, 2019). Vampire crabs are commonly found kilometers inland in muddy, wet burrows near freshwater streams and can grow to be about 11–14 cm across. Since vampire crabs possess direct development and juvenile crabs hatch directly from eggs held in the mother’s abdominal flap, with no preceding pelagic zoeal

stage (as is present in all marine crab species), these crabs are completely independent of seawater. Additionally, this species tends to form few (~50) relatively large (1–1.5 mm) eggs, implying that there is a higher degree of maternal investment in the offspring due to greater yolk provisioning, which may reflect a greater maternal budget for reproduction than species with smaller and more numerous eggs (Hohle and Singheiser, 2016). This species allows us to test how sex and body size influence terrestrial crabs with high parental investments.

In this study, we test the hypothesis that warmer water, larger body sizes, and females (with relatively greater reproductive investment) are all related to higher metabolic rates (gill respiration) in *G. hagen*. Specifically, we predict larger mothers in warmer water will have the highest metabolic rates while smaller males in cooler water will have the lowest metabolic rates. Overall, this study is important in understanding how metabolic rates in ectothermic animals might be impacted by climate change and changing abiotic and biotic conditions.

Materials and methods

Data Collection

We obtained N = 15 adult intermolt red devil vampire crabs, Geosesarma hagen Ng, Schubart, and Lukhaup (2015) from Aqua Imports (Boulder, CO, USA). Of the 15 crabs, seven were females, five of which were brooding. These animals were housed individually in transparent plastic containers (18.09 cm L x 11.13 cm W x 13.34 cm H) within opaque plastic containers with mesh-covered holes for air circulation and light penetration. We added cardboard barriers between each crab to minimize aggression. Each tank contained about five centimeters of moist soil mixed with sphagnum moss, a small cup for shelter, and another small cup for water with a shallow layer of mineral clay. We kept the animals in a humidity-controlled (80% relative humidity) room kept at an average of 26°C with a 12 hour light-dark cycle (night from 3pm-3am). Crabs were fed ¼ teaspoon of *Drosophila hydei* that were dusted with Repashy Calcium plus (Repashy Ventures, Inc.; Oceanside, CA, USA) three times per week. Each crab was also given three pinhead crickets twice a week. All water for their care and experimentation was distilled water mixed with SaltyShrimp (Garnelenhaus, Germany)

Mineral GH/KH + Powder. Prior to experimentation, we weighed the animals and determined their sex.

We used the Q-Box AQUA Aquatic Respirometry Package from Qubit Systems (Kingston, Ontario, Canada) to measure oxygen use and water temperature through time (Fig. S1). We randomly assigned crabs to each treatment and placed them in aerated water at either 21 or 28°C. These temperatures were chosen as a compromise between minimizing discomfort in the crabs and covering a wide range of temperatures experienced by the species in nature (Sholihah and Shibata, 2019). The experiment was run inside a Fisherbrand Isotemp incubator (Santa Clara, California, United States) to stabilize water temperatures. We recorded data for each crab for at least 16 minutes and we terminated trials at either 35 minutes or when the water reached 90% of the initial dissolved oxygen to minimize respiratory stress. For each trial, the crab was placed in the experimental chamber for 10 minutes with flowing fresh water to acclimate.

Data Analysis

Prior to data analysis, we downsampled all measurements to a frequency of 12 min^{-1}. We selected this sampling frequency as a trade-off between sampling often and reducing noise in the data. Since the sampling frequency was inadvertently changed during one trial, we interpolated the data to match our target frequency. We also removed the first 25% of the data to reduce noise associated with starting each trial, leaving 12 min (=144 samples) per individual. Removing the first 25% of the data is the standard procedure recommended by the respirometer manufacturer (Qubit Systems Inc., 2022). Next, we estimated the amount of oxygen in the respirometer using Eqn 1.

$$DO = DO_{cor}(V_R - V_A), \quad (1)$$

where DO is dissolved oxygen (mg O_2), DO_{cor} is the per L amount of oxygen in the respirometer after correcting for salinity (mg O_2 L^{-1}), V_R is the respirometer volume (L), and V_a is the animal volume.

We analyzed these data using a longitudinal generalized linear mixed effects (GLMM) model. We tested for interactions between time, temperature, body mass, and sex to determine how metabolic rates change relative to temperature, body mass, and

sex. We used crab ID as a random effect and accounted for time autocorrelation using an autoregressive and moving average (ARMA) correlation structure for each ID. This procedure requires co-optimization of two values: the autoregressive (*p*) and moving average (*q*) parameters. We accomplished the latter by varying each parameter between 0 and 5, then comparing model fits using maximum likelihood and AIC. For interpretation, we selected the best model that accounted for higher degrees (*p*) of temporal autocorrelation.

We performed post hoc analyses by comparing metabolic rates across temperature (21 and 28°C), body mass (0.82–1.08 g), and sex. The average values for temperature and body mass were 21.35°C and 27.35°C, and 0.95 g and 1.04 g, respectively. The selected body masses (0.82–1.08 g) are the range of body masses shared by males and brooding females. Instead of testing for differences among all pairwise combinations of selected variable levels, we created contrasts to compare marginal effects of one variable while holding the other two constant. For example, we tested for an effect of temperature on metabolic rate, while holding mass and sex constant. We adjusted for multiple comparisons using the Bonferroni correction. Finally, we regressed mass-specific metabolic rate against temperature using ID as a random effect and used the model coefficients to estimate the metabolic Q_{10} for *Geosesarma hagen*. We implemented these analyses using nlme version 3.1-158 (Pinheiro et al., 2022; Pinheiro and Bates, 2000) and emmeans version 1.8.8 (Lenth, 2023) in R version 4.2.1 (R Core Team, 2022).

Results and Discussion

We tested whether ambient temperature, body size, sex and their interactions influence metabolic rates in *G. hagen*. We expected that larger females at warmer temperatures and smaller males at lower temperatures would have the highest and lowest metabolic rates, respectively. We found that temperature, body mass, and sex had non-additive effects on metabolic rates (Table 1; Table S1). While metabolic rates seemed to increase with greater temperatures, larger body sizes, and in females relative to males, significant interactions indicated that interpreting the combined effects of each variable is necessary. Thus, we reported effects and confidence intervals for

each combination of traits (Table S2), but we only interpreted a subset of all possible comparisons (Fig. 1, Table 2). Overall, smaller females at 28°C had the greatest average metabolic rate while smaller crabs at 21°C had the lowest metabolic rates (Fig. 1). We also found that, on average, higher temperatures only increased metabolic rates in small females, but not in large females or in males. Smaller females at 28°C had greater metabolic rates (0.0020 mg O_2 min^{-1}) relative to smaller females at 21°C (0.0009 mg O_2 min^{-1}; t-ratio = 4.293, $p_{adj} < 0.001$). We did not find sexual dimorphism of metabolic rates in larger individuals or at lower temperatures, but at 28°C, we found greater metabolic rates in smaller females relative to smaller males (0.0014 mg O_2 min^{-1}; t-ratio = -3.088, $p_{adj} < 0.025$). Finally, we report a Q_{10} for *Geosesarma hagen* of 1.520 indicating the metabolic rate of this species increases by 52% for every 10°C increase.

To our knowledge, this is the first report of metabolic rates in *Geosesarma hagen*. Our results show how the sexual dimorphism of metabolic rates is both temperature- and size-specific in this species. The drivers of sexual dimorphism of metabolic rates across animals were discussed by Somjee et al. (2022), but no empirical examples were given, presumably due to the scarcity of relevant literature. Regardless, our results align with their prediction that sexual dimorphism of metabolic rate could arise from sexual differences in rates of energy use (Smith et al., 2022). The latter interpretation is consistent with our prediction that reproductively mature females should have higher metabolic rates because reproduction is energetically costly. If only a small baseline difference in metabolic rates exists between the sexes, then this would explain why (1) warmer temperatures did not substantially increase metabolic rates in small males, and (2) why sexual dimorphism was apparent at 28°C but not 21°C. It is also possible we lacked statistical power to detect an increase in metabolic rate with temperature in males due to small sample size. If so, the latter would emphasize the greater effect of temperature on females than in males. One of the only other tests of sexual dimorphism of metabolic rates in crabs was done using fiddler crabs (*Uca pugnax*) but they did not exhibit sexual dimorphism when measured at 20–21°C (Valiela et al., 1974). Interestingly, small females, not larger females, had the highest metabolic rates at 28°C and this may be due to smaller crabs having greater rates of heat transfer which would increase metabolic rates (Vandervoort, 2020). Additionally, the metabolic Q_{10} of *G.*

hagen (1.52) is greater than the average crab species which has a Q_{10} of 1.26 (Griffen and Sipos, 2018). This indicates that while *G. hagen* is a primarily terrestrial species, its metabolism is still quite sensitive to temperature.

Our findings indicate higher temperatures on Java could present challenges for *G. hagen*, particularly for small females. Metabolism is an energetically costly process that can account for up to 50% of individual energy budgets in ectotherms (Congdon et al., 1982). If climate change causes rising temperatures in the areas inhabited by *G. hagen*, our findings indicate small females would face greater energy requirements in relation to reproduction. However, it is possible that smaller individuals will have resistance to rising energy requirements associated with warmer temperatures in solely terrestrial settings (Griffen and Sipos, 2018). This is because the rate of aerial respiration in crabs does not change with temperature like aquatic respiration which is possibly due to organ-level adaptation, greater diffusivity of oxygen in air, or the higher oxygen concentration of air relative to water (Fusi et al., 2016). Future studies should seek to determine how respiratory media and sexual differences in energy use might relate to metabolic rates and ambient temperatures in decapods.

This study has several important implications for understanding the ecological and evolutionary processes tied to energy budgets. In this study, we have shown how metabolic rates can differ across levels of temperature, body size, and sex. Broadly speaking, these findings suggest sexual dimorphism and life history play an important role in determining energy consumption. Thus, future studies should aim to quantify the effects of sexual dimorphism and life history on metabolic rates in conjunction with the effects of temperature. Other traits involving behavior (Somjee et al., 2022) and maternal transfer of hormones (Burton et al., 2011) or other resources to offspring also deserve attention. While defining metabolic differences across temperature gradients is an important goal, this study highlights the limitations of interpreting the marginal effects of single variables. Instead, determining the correlated effects of environmental factors on metabolic rates in a spatially-explicit manner should be one of the primary goals of future ecophysiological research. Inspecting spatial patterns will help us understand combined effects of abiotic factors such as temperature, humidity, or climatic refugia on metabolism while also considering the contributions of many other relevant biotic traits.

List of symbols and abbreviations

DO = dissolved oxygen (mg O_2)
DO_{cor} = salinity-corrected oxygen density (mg O_2 L^{-1}),
V_R = respirometer volume (L)
V_A = animal volume (L).
GLMM = generalized linear mixed model
ARMA = autoregressive and moving average correlation structure
AIC = Akaike Information Criterion

Acknowledgements

We thank the O'Connell lab at Stanford University for helping us care for the animals and providing valuable feedback on previous versions of this manuscript. We also thank Monika Kuzma and Billie Kearns from Qubit Systems Inc. for aiding us with the methodology.

Competing interests

The authors declare no competing interests.

Author contributions

BHJ: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing (original draft), Writing (review and editing), Visualization, Supervision, Project administration, Funding acquisition. GAB: Conceptualization, Methodology, Investigation, Data curation, Writing (original draft), Writing (review and editing). MPL: Investigation, Writing (review and editing). LAO: Conceptualization, Methodology, Resources, Writing (review and editing), Supervision, Project administration, Funding acquisition. VMWZ: Conceptualization, Writing (review and editing), Supervision, Funding acquisition.

Funding

This material is based upon work supported by the National Science Foundation Postdoctoral Research Fellowships in Biology Program under Grant No. 2109850 (to BHJ) and Grant 2109869 (to VMWZ). GAB was supported by the Biology Summer Undergraduate Research Program (BSURP). BHJ and LAO are supported by the New York Stem Cell Foundation. LAO is a New York Stem Cell – Robertson Investigator. VWZ is supported by a Stanford Science Fellowship.

Data availability

Data and code may be found on DRYAD (DOI: TO BE UPDATED UPON ACCEPTANCE).

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Figure legends

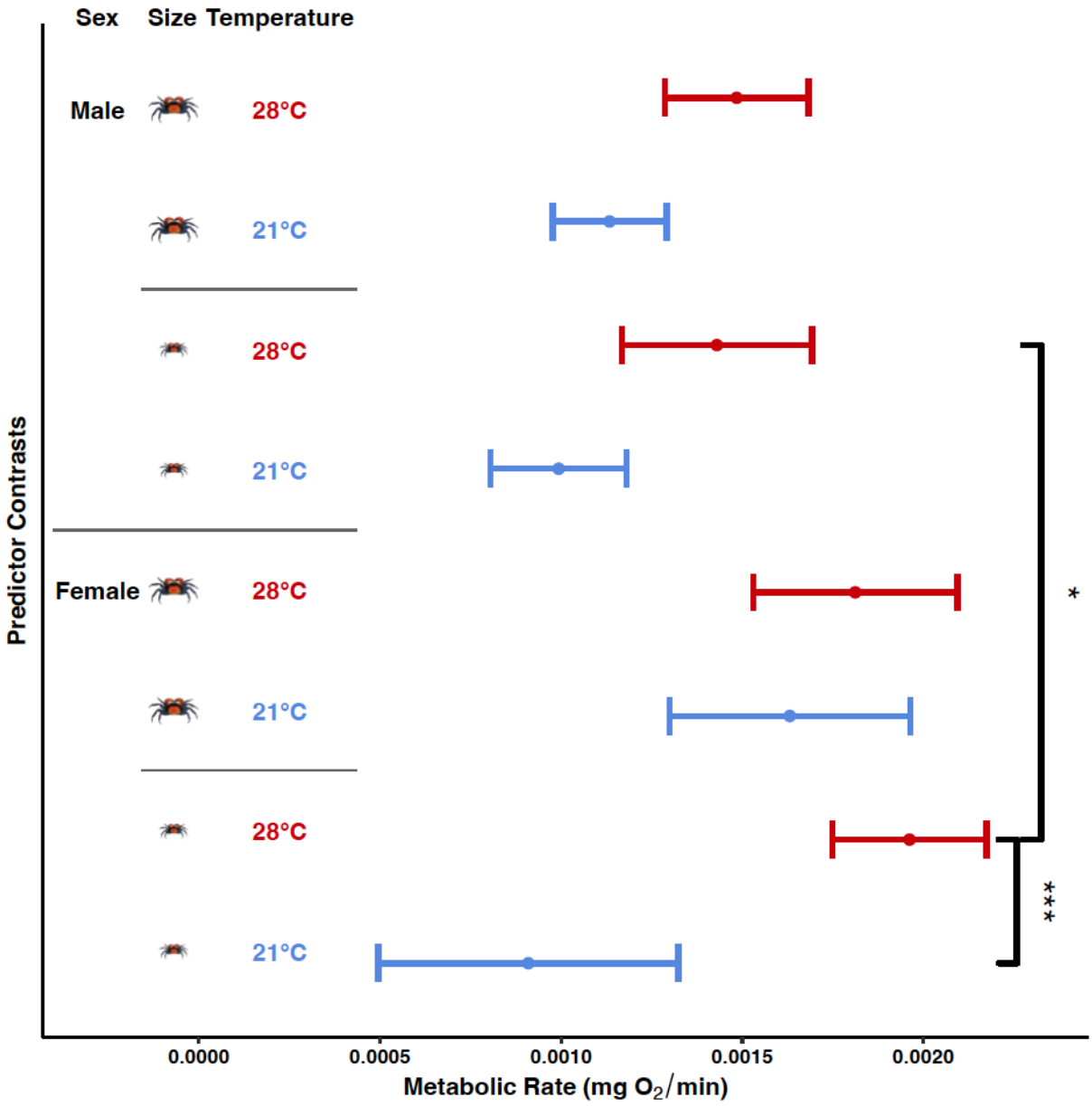

Figure 1. Pairwise slope tests of metabolic rate across combinations of temperature, body mass, and sex. Slope comparisons are based on the best fit model. Combinations of variables were selected based on significant interactions. The y-axis indicates the levels at which we held constant the effects of temperature, body mass, and sex to visualize statistical interactions and perform pairwise comparisons of metabolic rate. The subset of pairwise slope comparisons tested and their associated degrees of freedom are described in the main text and found in Table 2. Small and large

body sizes correspond to 0.82g and 1.08g, respectively. The selected body masses are the range of body masses shared by both sexes. Error bars indicate 95% confidence intervals. Significance levels (*** = 0.0001, * = 0.05) correspond to significant metabolic rate differences.

Tables

Table 1. Generalized linear mixed model fit of the effects of time, temperature, body mass, and sex on Dissolved Oxygen (DO). Temp = temperature, Mass = body mass, M = male, β = regression coefficient, SE = standard error, DF = degrees of freedom. Coefficient units are in brackets. Rows with significant p-values are in bold.

Model Term	β	SE	DF	t-value	p-value
Intercept (mg O_2)	-0.4163	0.2635	2133	-1.5800	0.114
Time (mg $O_2\,min^{-1}$)	**-0.0128**	**0.0053**	**2133**	**-2.3962**	**0.017**
Temp (mg $O_2\,°C^{-1}$)	-0.0001	0.0099	2133	0.0087	0.993
Mass (mg $O_2\,g^{-1}$)	0.0484	0.2796	11	0.1731	0.866
M (mg O_2)	0.1323	0.2777	11	0.4762	0.643
Time:Temp (mg $O_2\,min^{-1}°C^{-1}$)	**0.0005**	**0.0002**	**2133**	**2.7130**	**0.007**
Time:Mass (mg $O_2\,min^{-1}g^{-1}$)	**0.0128**	**0.0056**	**2133**	**2.3031**	**0.021**
Temp:Mass (mg $O_2\,°C^{-1}g^{-1}$)	0.0033	0.0106	2133	0.3084	0.758
Time:M (mg $O_2\,min^{-1}$)	**0.0112**	**0.0056**	**2133**	**2.0147**	**0.044**
Temp:M (mg $O_2\,°C^{-1}$)	-0.0016	0.0105	2133	-0.1486	0.882
Mass:M (mg $O_2\,g^{-1}$)	-0.0998	0.2898	11	-0.3444	0.737
Time:Temp:Mass (mg $O_2\,min^{-1}°C^{-1}g^{-1}$)	**-0.0005**	**0.0002**	**2133**	**-2.2751**	**0.023**
Time:Temp:M (mg $O_2\,min^{-1}°C^{-1}$)	**-0.0004**	**0.0002**	**2133**	**-2.0946**	**0.036**
Time:Mass:M (mg $O_2\,min^{-1}g^{-1}$)	**-0.0113**	**0.0058**	**2133**	**-1.9667**	**0.049**
Temp:Mass:M (mg $O_2\,°C^{-1}g^{-1}$)	-0.0005	0.0110	2133	-0.0451	0.964
Time:Temp:Mass:M (mg $O_2\,min^{-1}°C^{-1}g^{-1}$)	**0.0004**	**0.0002**	**2133**	**1.9744**	**0.048**

Table 2. Pairwise comparisons of metabolic rate (mg O_2 min^{-1}) across temperature, body mass, and sex. Each contrast holds 2 of 3 variables constant; “at” indicates a margin over which comparisons are made. SF = small female, LF = large female, SM = small male, LM = large male, SE = standard error, DF = degrees of freedom, p-adj = Bonferroni adjusted p-value. The selected body masses, 0.82–1.08 g, are the range of body masses shared by both sexes; S = 0.82 g, L = 1.08 g. Rows with significant differences in metabolic rate are in bold.

Contrast	Estimate	SE	DF	t-ratio	p-adj
28°C v. 21°C at SF	**0.0011**	**0.0002**	**2133**	**4.2930**	**<0.001**
28°C v. 21°C at LF	0.0002	0.0003	2133	0.7001	1
LF v. SF at 28°C	-0.0001	0.0001	2133	-1.0457	1
LF v. SF at 21°C	0.0007	0.0003	2133	2.2847	0.269
28°C v. 21°C at SM	0.0004	0.0002	2133	2.5611	0.126
28°C v. 21°C at LM	0.0004	0.0001	2133	2.6664	0.093
LM v. SM at 28°C	0.0001	0.0001	2133	0.6304	1
LM v. SM at 21°C	0.0001	0.0001	2133	2.1646	0.366
LM v. LF at 28°C	-0.0003	0.0002	2133	-1.8653	0.747
LM v. LF at 21°C	-0.0005	0.0002	2133	-2.6529	0.097
SM v. SF at 28°C	**-0.0005**	**0.0002**	**2133**	**-3.0881**	**0.025**
SM v. SF at 21°C	0.0001	0.0002	2133	0.3612	1

Supplementary Information

Table S1. Model comparisons of ARMA correlation structure optimization. P = autoregressive parameter, q = moving average parameter, LogLik = log-likelihood, AIC = Akaike Information Criterion, ΔAIC = AIC difference from the best fitting model. Models within 2 AIC units of the best model are in bold. We interpret the model with p=4 to account for higher levels of temporal autocorrelation. Models for missing parameter combinations (p = 0–5; q = 0–5) did not converge.

p	**q**	**LogLik**	**AIC**	**ΔAIC**
0	1	14530.68	-29023.35	2003.13
0	2	14748.74	-29457.48	1569
0	3	14894.63	-29747.27	1279.22
0	4	15003.43	-29962.87	1063.61
2	**0**	**15533.24**	**-31026.48**	**0**
2	4	15534.45	-31020.91	5.57
2	5	15534.43	-31018.86	7.63
4	**0**	**15534.75**	**-31025.5**	**0.98**
4	2	15534.75	-31021.5	4.99
4	3	15535.16	-31020.31	6.17
5	1	15534.87	-31021.75	4.74

Table S2. Marginal effects of time on dissolved oxygen (DO) across temperature, body mass, and sex. Temp = temperature, Mass = body mass, SE = standard error, DF = degrees of freedom, CL = confidence level.

Temp	Mass	Sex	Time Effect	SE	DF	Lower CL	Upper CL
21	0.82	F	0.0009	0.0002	2133	0.0005	0.0013
28	0.82	F	0.0020	0.0001	2133	0.0017	0.0022
21	1.08	F	0.0016	0.0002	2133	0.0013	0.0020
28	1.08	F	0.0018	0.0001	2133	0.0015	0.0021
21	0.82	M	0.0010	0.0001	2133	0.0008	0.0012
28	0.82	M	0.0014	0.0001	2133	0.0012	0.0017
21	1.08	M	0.0011	0.0001	2133	0.0010	0.0013
28	1.08	M	0.0015	0.0001	2133	0.0013	0.0017

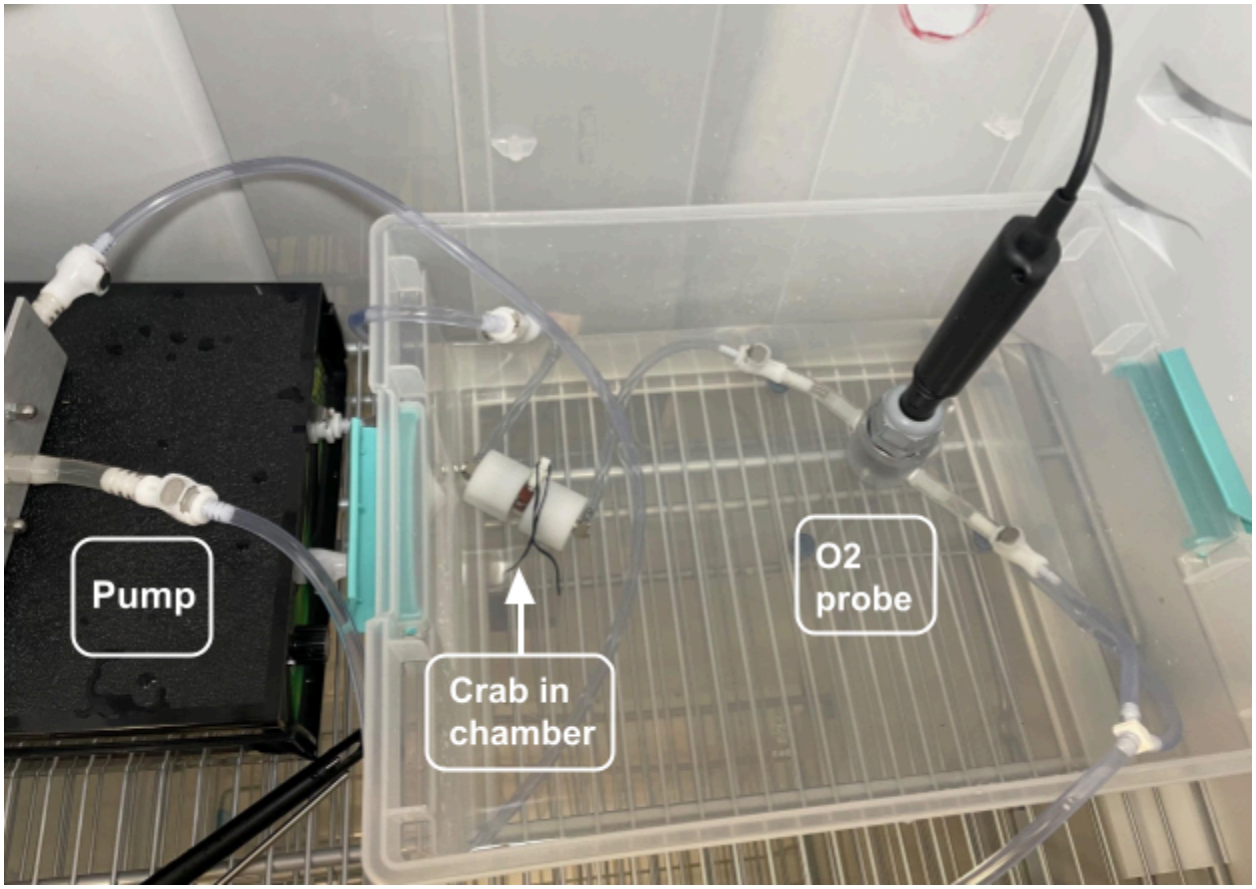

Figure S1. Photograph of respirometer setup. A crab is inside the animal chamber (with a black twist tie). The animal chamber is submerged in water. Respirometer is inside an incubator.