Text S1

Determining P_{cSMR}: The critical oxygen partial pressure (Pc, specifically P_{CSMR}) for standard metabolic rate (SMR; determined as described in methods) (Figure S1), we calculated P_{cSMR} using a breakpoint method (*calo2crit* package; Claireaux & Chabot 2016). A regression line was then fit to all MO_2 measures below SMR in the hypoxia trial (i.e., the oxygen conforming section of the curve; $MO_2 = mPO_2 + b$). P_{cSMR} was then assigned to the PO_2 (partial pressure of oxygen) at which the "oxygen conforming" regression line intersected the SMR (Claireaux & Chabot 2016). Using SMR and the P_{cSMR} derived from this breakpoint method we calculated an oxygen supply capacity (α; $\alpha = SMR/P_{cSMR}$) that was independent of MMR values in this study. This oxygen supply capacity derived from the breakpoint method was then used in comparison to the observed oxygen supply capacity (detailed in methods section).

For comparison, we then calculated P_{cSMR} values from the α-method of Pc determination (Seibel et al. 2021; see methods section) and compared with the breakpoint method. The α-method is inherently more conservative than other methods. Accordingly, in 83% of trials, P_{cSMR} was lower using the α-method than the breakpoint method, however, estimates of P_{cSMR} were still considered comparable between the two methods at all temperatures except 23°C (Welch's t-tests, Bonferroni correction: 23°C, $t(5) = -5.8349$, *p-value* = 0.002).

A line of limiting oxygen was then generated for each individual (Figure 4) to describe the metabolic rate dependence of the P_c or the oxygen dependency of MMR up to the P_{cMAX} beyond which additional oxygen conveys no improvement in performance. The P_{cmax} is an evolved trait that approximates the highest PO_2 to which a species is persistently exposed (Seibel & Deutsch 2020), which for many coastal species is near air-saturation (21 kPa). Oxygen supply capacity was equivalent at the highest metabolic rate achieved, whether during exercise or exposure to hypoxia, thus the mean α describes a line of limiting oxygen (α-lines; Seibel et al. 2021) that dictates critical oxygen limits from rest to maximum metabolism at each temperature (Figure 5) (Seibel & Deutsch 2020, Seibel et al. 2021).

The effect of temporal pseudo replication: In this study, it was necessary to reuse animals in good health after the appropriate recovery period (see methods section), due to limited access to further *S. acanthias* collection, to minimize the number of individuals obtained for captive experiments, and due to some subject attrition in the lab. Individuals were only tested ("run") once per temperature bin, with no "repeat" measures within a target temperature (within-bin independence). No individual was run at more than 3 temperatures across the study. For individuals in year 2 that completed both trial types, the exercise and hypoxia trial at a single temperature was counted as a single "run" as trials were run consecutively and the animal was not removed from the chamber between trials. Of the total 104 runs across temperature, 59.62% (n = 62) trials were first runs, 37.5% (n = 39) were second runs, and 2.88% (n = 3) were tertiary runs. We acknowledge that some samples lack temporal independence across temperature bins. To identify if random error due to individual variation in metabolism, and using recovered animals (temporal non-independence), had a significant impact on the trends of metabolic traits across temperature, we ran several tests.

When modeling SMR and RMR, mixed effects models using the lme4 package (Bates et al. 2012) were easily overparameterized and often resulted in singular fits due to uneven sampling and small effect sizes of "individual" and "run". Similarly non-linear mixed effects model using nlme (Pinheiro et al. 2023) for MMR and alpha also produced singular fits when random effects for individuals and run were included. To explicitly determine if there was an effect of "run" on SMR or RMR, ANCOVA models were run with temperature and "run"(factor), which resulted in non-significant effects of "run" (RMR: $F_{2,100} = 0.10$, $p = 0.90$; SMR: $F_{2,31} = 1.01$, $p = 0.37$). "Run" also had an insignificant effect on alpha, MMR (ANOVA, alpha: $F_{3,11} = 0.85$, $p = 0.46$, MMR: $F_{2,97} = 0.0787$ $p = 0.92$).

To affirm that pseudo replication due to repeated runs did not affect our results, we subset the data for each metabolic metric to only include the first chronological trial for each individual. We then modeled metabolic relationships (each metric according to the "Methods" section), using only the first-run data, which we refer to as the "first-run" model. However, this model was not appropriate for comparison alone, since most secondary and tertiary runs occurred at the higher temperatures as a result of 23°C being tested

last (by necessity) in both years, and 21°C being tested second to last in year 1 (Figure S5) (10°C: 100% run 1; 13°C: 82.35% run 1, 17.65% run 2; 17°C: 76.19% run 1, 23.81% run 2; 21°C: 34.78% run 1, 65.22% run 2; 23°C: 9.52% run 1, 76.19% run 2, 14.29% run 3), skewing the "first run" data to lower temperatures, and decreasing sample sizes for the highest temperature.

To explicitly remove pseudo replication due to multiple runs within an individual, we subsampled the data so that each individual only contained a single measurement. For individuals with multiple runs, we randomly selected only a single run (run#: 1, 2, or 3) for inclusion and discarded its other runs. From this subsampled data we ran the same nonlinear regressions to generate a model output. This process was repeated 10,000 times allowing us to produce a 95% confidence interval if each individual only contained a single run. The "first run" model and the complete model, inclusive of all runs across all individuals and temperatures, were then graphically compared to this generated CI (Figure S3). The complete model (including measurements from all individuals) fell within the simulated CI. However, the "run 1" model (only individuals first run) deviated outside the confidence intervals at higher temperatures, likely due to decreased sample size in the "first run" data at high temperatures. Except for 23°C, the two models generated output values within 5% of each other. Thus, including individual subsequent runs increased sample size and thus precision especially at higher temperatures. We determined the amount of variation reused individuals introduced to the data was negligible, and proceeded with further analysis of metabolic traits, modeling without random effects.

Table S1. T-test results. For all comparisons significance was determined by p value <0.01 for a Bonferroni corrected threshold of alpha = 0.01, with the exception of mass between the sexes, which did not require Bonferonni correction and significance was determined by p value <0.05.

Comparison	Test type	Temp	t-value	df	p-value
RMR vs SMR	Welch's T test	10	3.9584	25.458	**0.00054**
		13	6.7168	11.522	**2.625e-05**
		17	4.5089	11.534	**0.00079**
		21	8.8968	18.503	**4.162e-08**
		23	11.84	20.481	**1.28e-10**
MMR vs predicted MMR•	Welch's T test	10	-1.3852	39.746	0.17
		13	-1.386	30.113	0.18
		17	-1.386	30.113	0.18
		21	-2.3689	46.82	0.022
		23	-2.5873	37.066	0.014
MMR vs predicted MMR$^{\Delta}$	Welch's T test	10	-1.4818	10.503	0.17
		13	1.44	10.564	0.18
		17	0.026871	6.398	0.98
		21	-0.15602	15.331	0.88
		23	1.1856	21.808	0.25
α (overall) vs α (SMR/P_{cSMR})	Welch's T test	10	-0.27778	12.713	0.7856
		13	2.6003	16.039	0.0193
		17	1.1587	7.7801	0.2809
		21	2.2286	22.275	0.03624
		23	4.0288	24.935	**0.00046**
α (overall) vs α (SMR/P_{cSMR})*†	Paired T test	10	2.1457	7	0.06905
		13	2.4893	5	0.05521
		17	3.4732	5	0.01779
		21	2.8788	3	0.06359
		23	-5.8349	5	**0.002091**
P_{cSMR} (α -method) vs P_{cSMR} (bp method)*	Paired T test	10	-2.6096	7	0.03493
		13	-2.5333	5	0.05232
		17	-3.4195	5	0.01885
		21	-3.11	3	0.05288
		23	-5.8349	5	**0.002091**
Female vs Male mass	Welch's t-test	All	14.33	101.31	**2.2e-16**
Female vs Male MMR	Welch's t-test	10	-1.2981	8.6956	0.2276
		13	-0.60597	14.331	0.554
		17	-0.0594	12.123	0.9536
		21	-1.7143	10.478	0.1159
		23	-0.88277	4.0954	0.4261
Female vs Male α	Welch's t-test	10	-2.0739	8.2239	0.07085
		13	-0.60361	12.34	0.557
		17	-0.41305	6.7897	0.6923
		21	-1.3577	9.2178	0.2069
		23	-0.4469	4.5202	0.6755
Female vs Male RMR	Welch's t-test	10	-0.78159	10.656	0.4515
		13	0.19058	13.62	0.8517
		17	-0.91362	5.5441	0.3989
		21	0.59668	10.17	0.5638
		23	-0.22615	3.9981	0.8322

* = Only individuals that successfully completed both exercise and hypoxia trials
† = Direct comparison made prior to mass correction
Δ = MMR predicted using α=SMR/P_{cSMR}
• = MMR predicted using overall observed α

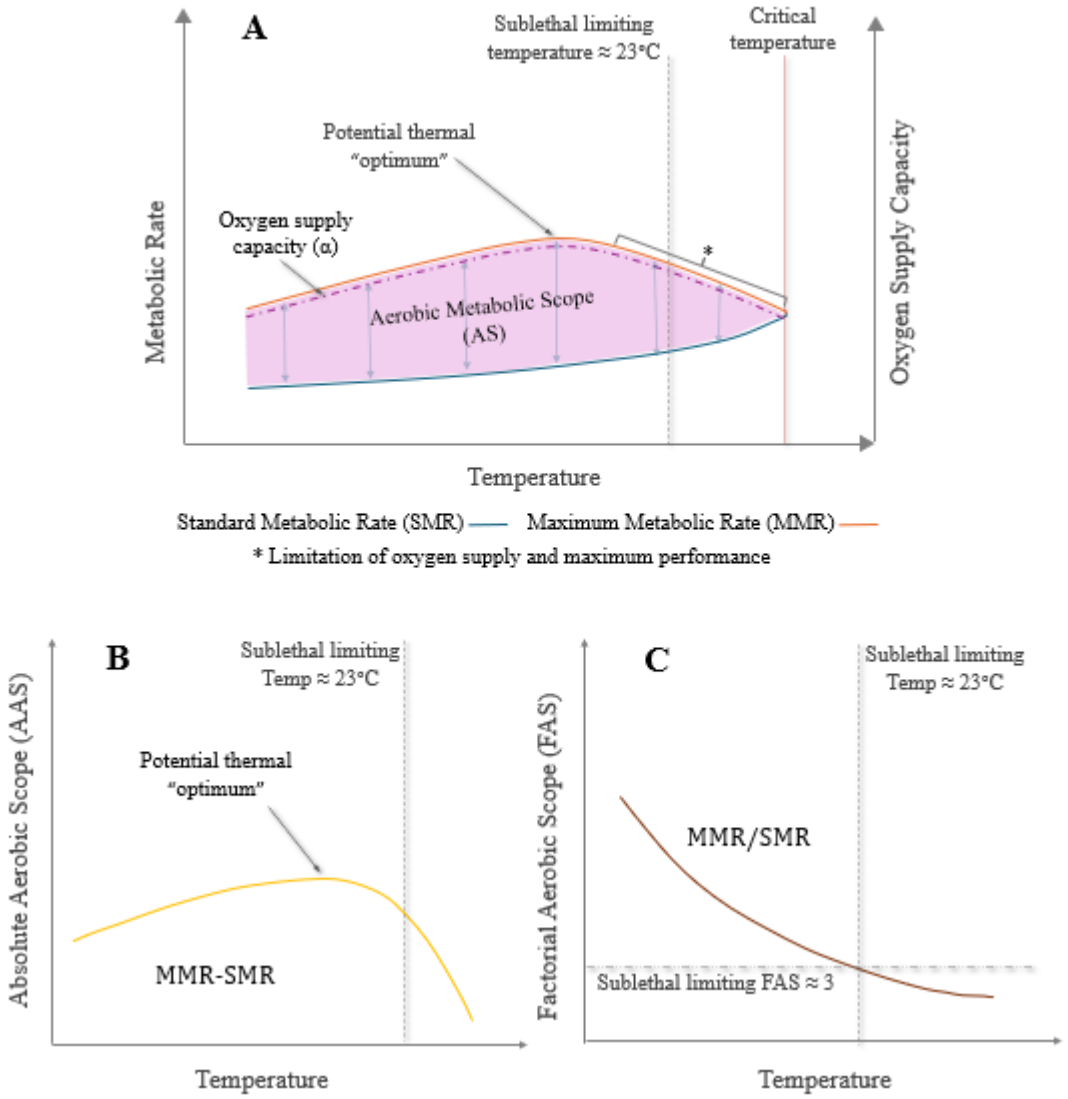

Figure S1. Graphical Hypotheses. **(A)** Standard and maximum metabolic rates were hypothesized to increase with temperature for *Squalus acanthias*, demonstrating differing thermal sensitivities and leading to changes in aerobic metabolic scope across temperature. Maximum performance (solid red curve) was hypothesized to decline significantly at a sublethal limiting high temperature, due a breakdown in physiological capacity to supply oxygen to tissues (oxygen supply capacity; purple dashed curve), leading to limitation of the aerobic metabolic scope (purple shaded region) at high temperature. **(B)** Absolute aerobic scope, (AAS; yellow curve), was hypothesized to resemble a right shifted bell shape curve, increasing with temperature to a peak at an "optimal" temperature (hypothesized to correspond to preferred thermal habitat of wild *S. acanthias,* where fitness is optimized), followed by a significant decline beyond its peak, that would coincide with a sublethal warm temperature limit approaching 23˚C. **(C)** Factorial aerobic scope (FAS; brown curve) was hypothesized to demonstrate an inverse relationship with temperature for *S. acanthias*, and would decline to a minimum limiting threshold near an FAS of 3 at 23˚C for this population.

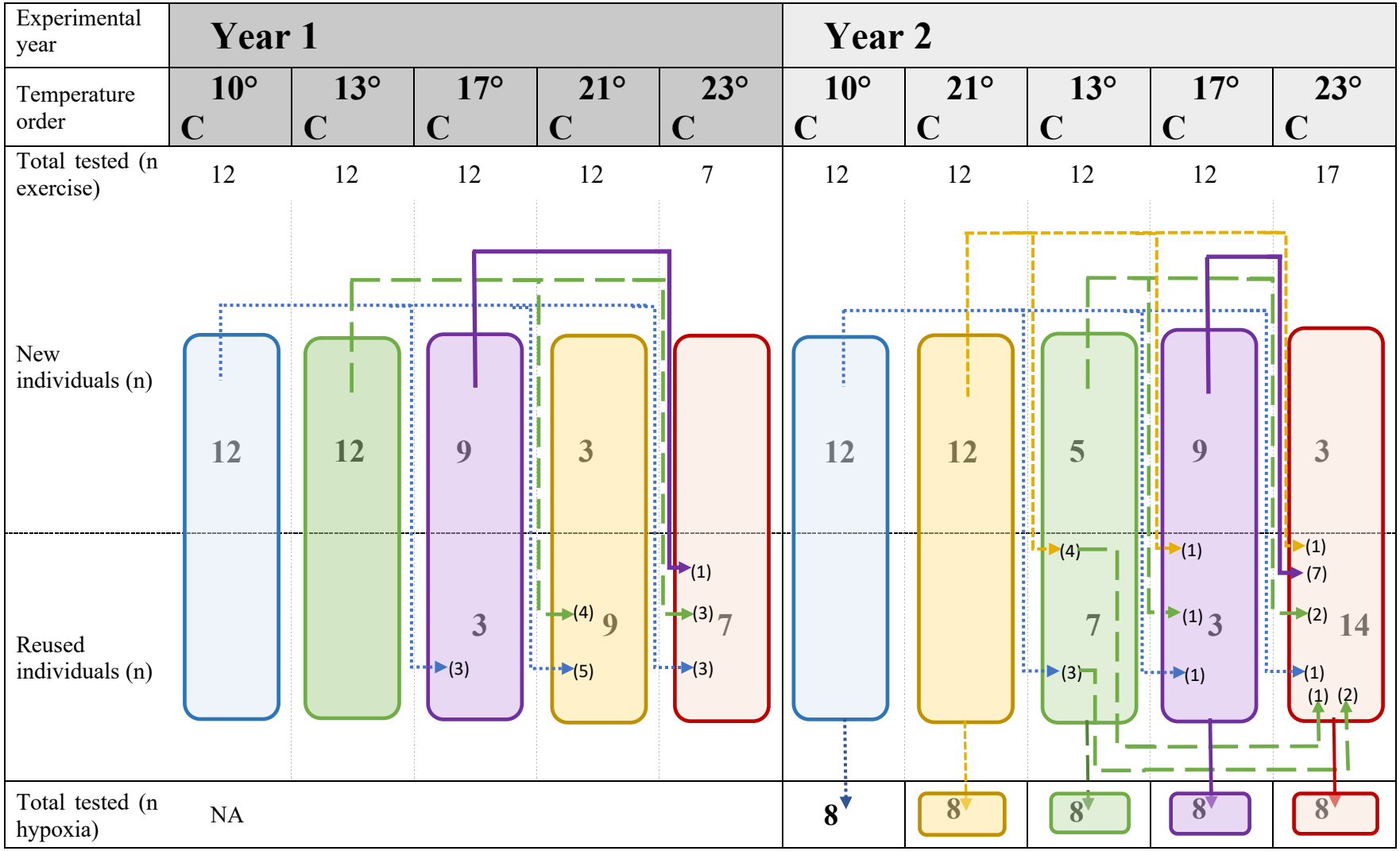

Figure S2. Experimental flow diagram. The diagram depicts experimental groups in year 1 (left) and year 2 (right) grouped by temperature and displayed in the chronological order of experimental temperature testing (left to right). Exercise trials were conducted first to determine maximum metabolic rate (MMR), oxygen supply capacity (α; determined at MMR) and a measure of resting metabolic rate (RMR). The number of new as well as reused individuals are specified for exercise trials in each temperature group. Dashed arrows with temperature specific patterning, denote the previous temperature from which reused individuals were sourced, accompanied by the number of individuals that were sourced from that temperature, in parentheses. In year 2, a subsection of individuals underwent hypoxia testing immediately following exercise trials (without removal from chambers) to determine a measure of resting metabolic rate (SMR), a critical limiting oxygen level for SMR (P_{cSMR}), and a measure of oxygen supply capacity (α; independent of MMR). This figure depicts the flow of experimental temperature groups for the total number of animals that underwent exercise and hypoxia trials. However, the number of usable trials from these totals are detailed in Table 1.

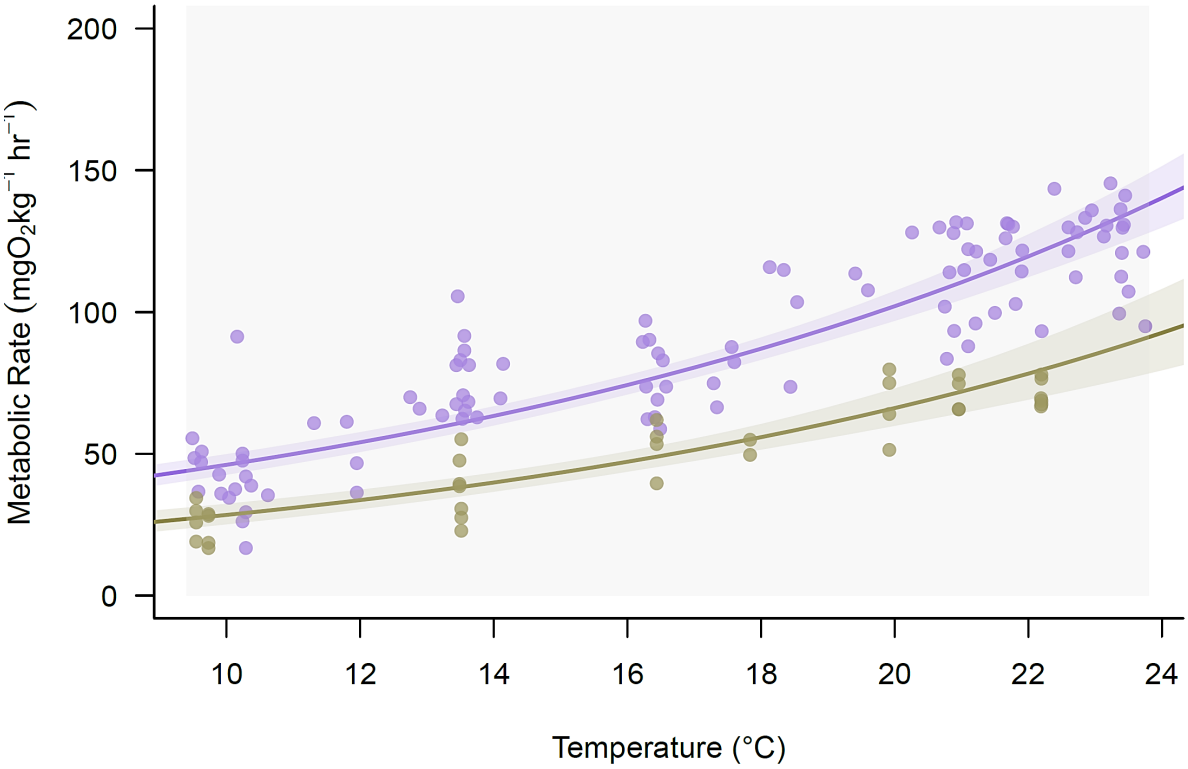

Figure S3. Comparison of resting metabolic rates from exercise (RMR) and hypoxia trials (SMR). Filled circles represent individual metabolic measures. Solid lines represent exponential models of metabolic metrics, and colored shaded regions surrounding models represent 95% confidence intervals. RMR, resting metabolic rate from exercise trial depicted in purple represents the $q_{0.2}$ of resting metabolic measures within the 22-hour exercise trial (RMR; <22hours). SMR, standard metabolic rate, olive-green in color, represents the $q_{0.2}$ of resting metabolic measures prior to oxygen limitation in hypoxia trials (SMR; >22hours). SMR was between 53.6-66.8% of RMR across temperature.

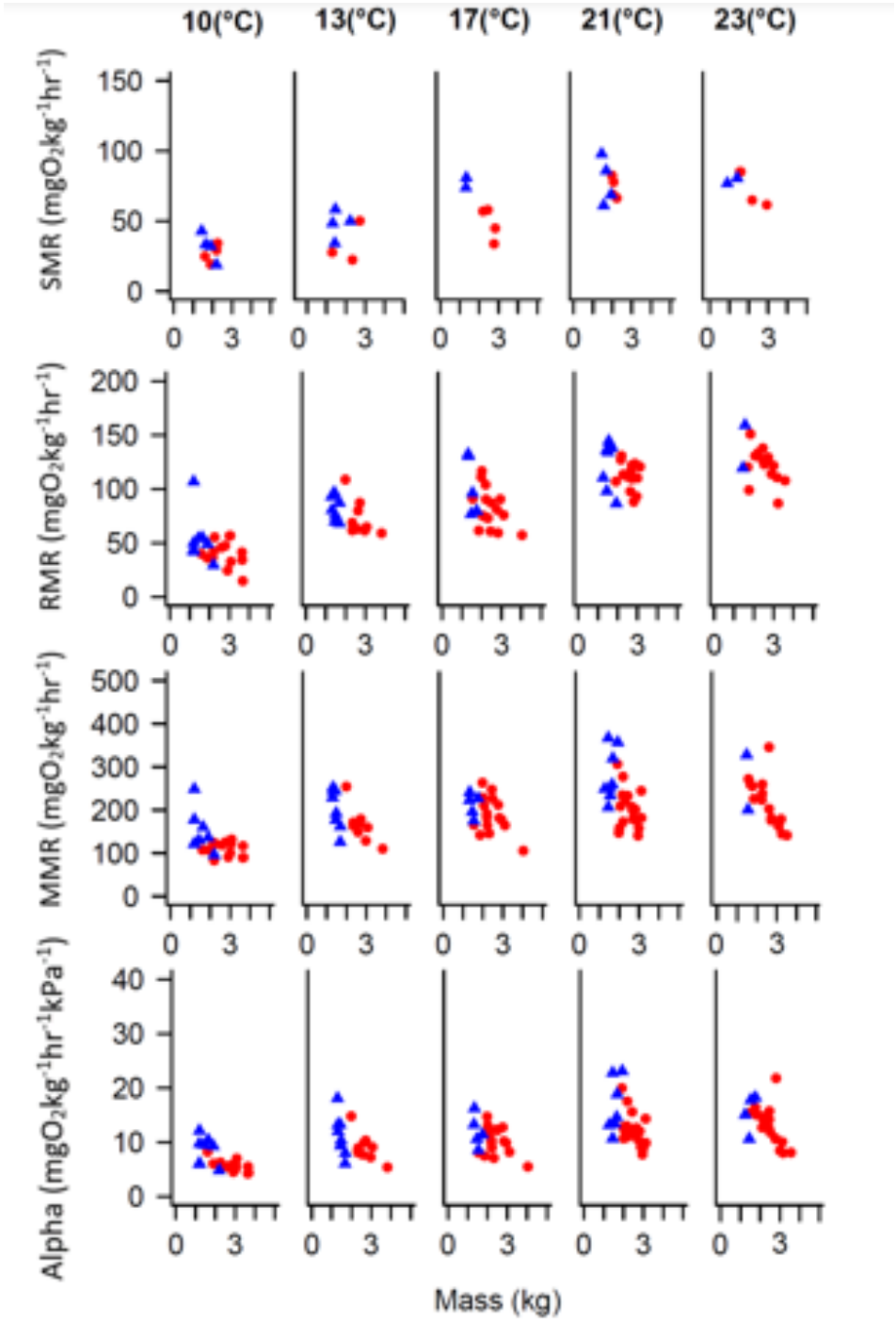

Figure S4. Measured metabolic metrics by sex and mass. Blue filled triangles represent individual males, and red filled circles represent individual females within each target temperature bin.

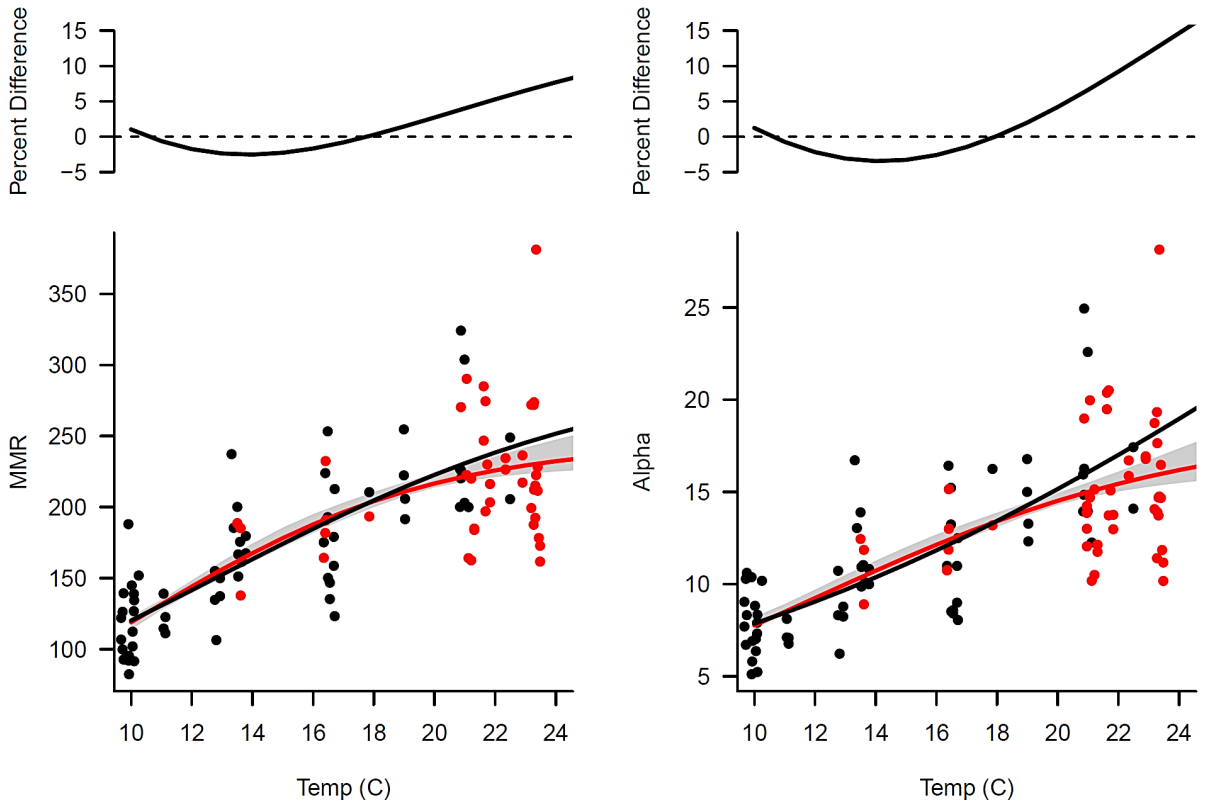

Figure 5. Representative effects of temporal pseudo replication for maximum metabolic rate (MMR) and oxygen supply capacity (α). Of the total trials across temperature, 59.62% (n = 62) trials were primary runs (trials) (individual points in black), 37.5% (n = 39) were secondary runs at a temperature unique to the first, and 2.88% (n = 3) were tertiary runs (second and tertiary runs represented by red points) at a temperature unique to the first and second. The solid black lines represents the "first-run" model, a nonlinear least squares regression (logistic model) for MMR and alpha for each individual's first chronological trial. The solid red lines represents the "null" model, inclusive of all trials regardless of run number. The 95% confidence interval (gray band) was calculated by completing 10,000 repetitions of, 1) randomly selecting only a single run (run#: 1, 2, or 3) from each individual, and 2) running the same nonlinear regressions to generate a model output. The "first-run" model only deviated outside the confidence intervals at higher temperatures, likely due to decreased sample size of first runs at high temperatures (10C: 100% run 1; 13C: 82.35% run 1, 17.65% run 2; 17C: 76.19% run 1, 23.81% run 2; 21C: 34.78% run 1, 65.22% run 2; 23C: 9.52% run 1, 76.19% run 2, 14.29% run 3). Percent difference between the first run model and the null model are represented above each modeled figure for both MMR (left) and α (right).

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