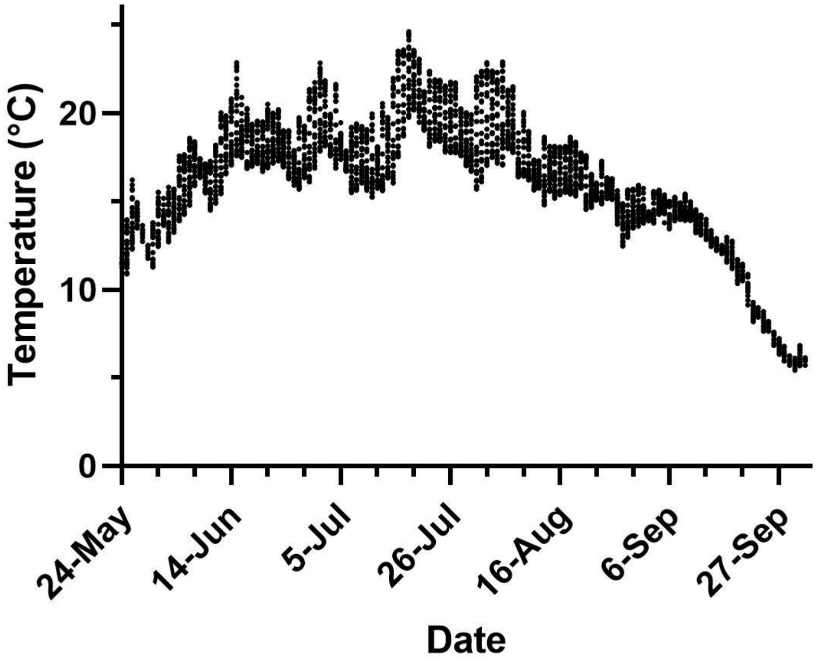

Fig. S1. Water temperature of Nancy Lake, Alaska (61.692°N, 150.020°W) between May 24, 2021 and October 2, 2021. Temperature was recorded every 4 hours using a Pro v2 Onset Hobo water temperature data logger (Onset Computer Corporation, Bourne, MA, USA).

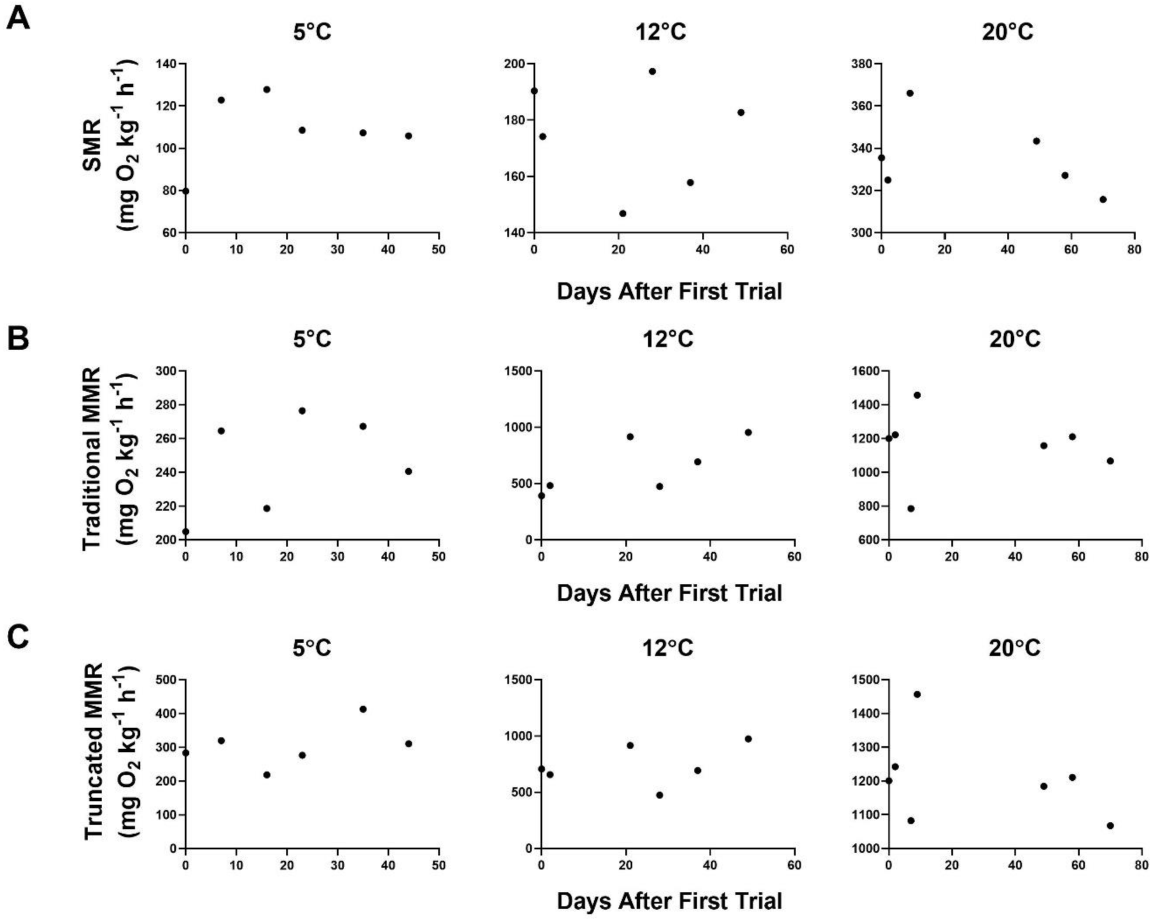

Fig. S2. Metabolic rate of threespine stickleback *Gasterosteus aculeatus* relative to the date of the first trial for each temperature group. (A) Standard metabolic rate (SMR) and maximum metabolic rate (MMR) calculated using the (B) traditional and (C) truncated method. Traditional MMR was calculated with all DO values within each measurement period whereas truncated MMR was calculated for the highest rate of $\dot{M}O_2$ within each measurement period (minimum length = 100 s). Each point represents the metabolic rate estimate for one animal.

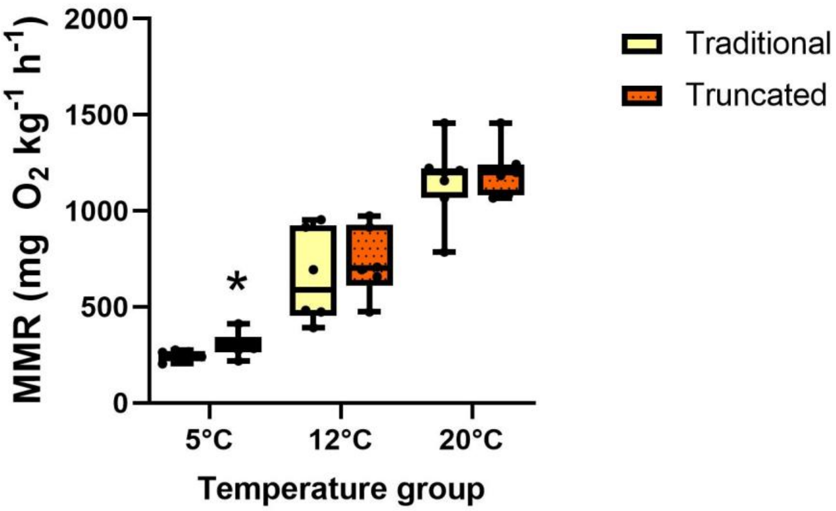

Fig. S3. Comparison of maximum metabolic rate (MMR) estimates for threespine stickleback *Gasterosteus aculeatus* acclimated to three temperatures. The "Truncated" method measured the highest rate of $\dot{M}O_2$ within each measurement period (minimum length = 100 s). The "Traditional" method included all DO measurements within each measurement period. Boxes designate quartiles, line designates median and whiskers designate minimum and maximum values (n = 6 for all temperature groups except MMR at 20°C where n = 7). MMR estimates did not differ by method for any temperature group (Wilcoxon or paired t-test: $P > 0.05$).

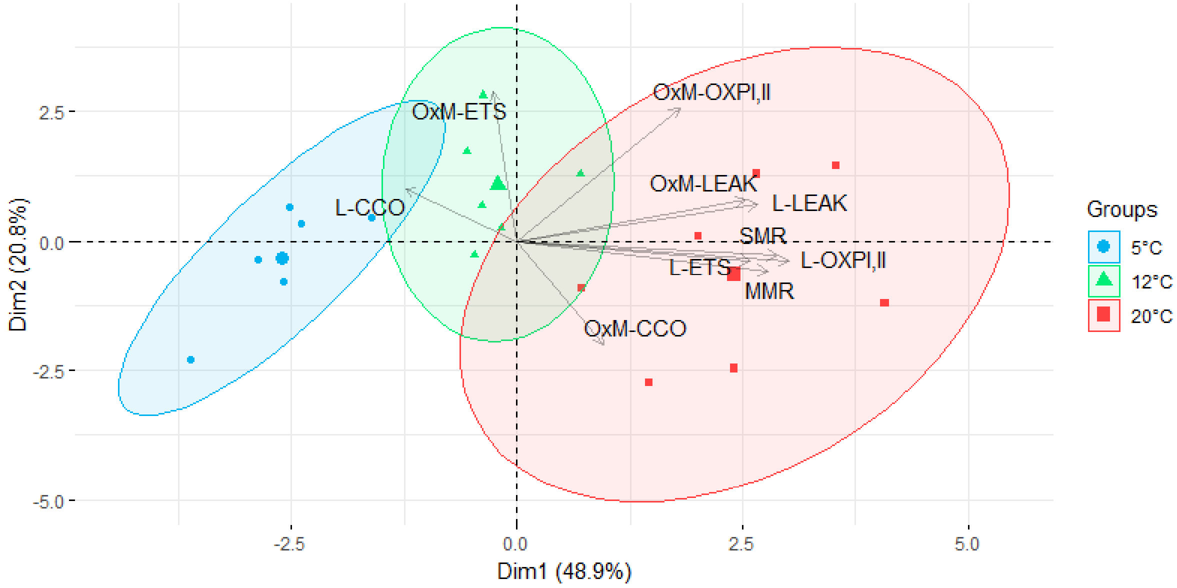

Fig. S4. Principal component analysis of metabolic rate and mitochondrial function in liver and oxidative skeletal muscle from threespine stickleback *Gasterosteus aculeatus* at three acclimation temperatures. Clustering within each temperature group is represented by 0.90 confidence ellipses around the barycenter shown with an enlarged symbol for each temperature group (*n* = 6-7 for each group). L = liver; OxM = oxidative skeletal muscle; SMR = standard metabolic rate; MMR = maximum metabolic rate; OXP I,II = rate of oxidative phosphorylation through complexes I,II; LEAK = rate of leak in the presence of oligomycin; ETS = rate of uncoupled respiration; CCO = uncoupled rate of respiration through cytochrome *c* oxidase (Complex IV); Dim = dimension.

Table S1. Metabolic rate and aerobic scope estimates for threespine stickleback *Gasterosteus aculeatus* acclimated to three temperatures.

Temperature	SMR (mg O_2 kg^{-1} h^{-1})	MMR (mg O_2 kg^{-1} h^{-1})	AAS (mg O_2 kg^{-1} h^{-1})
5°C	108.69 ± 6.87^{A}	303.68 ± 26.24^{A}	194.99 ± 28.31^{A}
12°C	174.87 ± 7.93^{B}	736.86 ± 74.36^{B}	561.98 ± 78.56^{B}
20°C	335.49 ± 7.23^{C}	1206.26 ± 48.60^{C}	891.46 ± 45.89^{C}

Data are mean ± s.e.m. *n* = 6 for all measurements except 20°C MMR where *n* = 7. Superscript letters indicate significant differences among temperature groups within the same method as identified by a one-way ANOVA. SMR = standard metabolic rate, MMR = maximum metabolic rate, AAS = absolute aerobic scope.

Table S2. Correlation coefficients for mass-specific mitochondrial properties in liver and oxidative skeletal muscle of threespine stickleback *Gasterosteus aculeatus* acclimated to three temperatures.

Temperature	Mitochondrial properties	Pearson's r	*P*
5°C	OXP I,II (log)	0.47	0.34
	LEAKOmy	0.43	0.40
	CCO	0.60	0.21
12°C	OXP I,II (log)	0.68	0.14
	LEAKOmy	-0.06	0.90
	CCO	0.30	0.56
20°C	OXP I,II (log)	-0.07	0.88
	LEAKOmy	0.68	0.09
	CCO	-0.32	0.49

OXP I,II = oxidative phosphorylation respiration rate supported by complex I and II; LEAKOmy= leak respiration rate in the presence of oligomycin; CCO = rate of uncoupled respiration through cytochrome *c* oxidase. *n* = 14–15