



**Fig. S1.** Comparison of glucose values from the hexokinase kinetic assay and the glucose meter using a linear regression ( $y = 1.054x + 1.416$ ,  $P < 0.001$ ,  $r^2 = 0.54$ ) for juvenile brook trout (*Salvelinus fontinalis*) held at temperatures spanning their thermal distribution ( $n = 10$  per temperature treatment).

**Table S1.** Transcript information for the thirteen target and three reference genes retrieved from a previously published brook trout transcriptome in Sutherland et al., (2019).

Subject	Length (bp)	Species	Database	Accession no.	Score	E. value	% Identity
QSF_HSP70.6.8	1932	<i>Oncorhynchus mykiss</i>	Swissprot	HSP70_O NCMY	2732	0	86.5
QSF_SERPH.3.3	1233	<i>Salmo salar</i>	Refseq_rna	NM_0011 39968.1	840	0	92.6
QSF_ATP1A3.7.8	972	<i>Oncorhynchus mykiss</i>	Refseq_rna	NM_0011 24630.1	458	0	84.8
QSF_G6PC.1.2	1059	<i>Felis catus</i>	Swissprot	G6PC_FE LCA	1059	1e <sup>-25</sup>	49.9
QSF_CIRBP.4.24	468	<i>Salmo salar</i>	Refseq_rna	NM_0011 39676.1	152	7e <sup>-79</sup>	88
QSF_GPX1.1.4	255	<i>Oncorhynchus mykiss</i>	Refseq_rna	NM_0011 24525.1	418	0	91.2
QSF_HSP90B.2.6	1161	<i>Salmo salar</i>	Refseq_rna	NM_0011 23532.1	818	0	95.5
QSF_LOC101171 85.1.1	228	<i>Oryzias latipes</i>	Refseq_pro tein	XP_00408 6462.1	312	1e <sup>-31</sup>	76.4
QSF_RL7.4.13	942	<i>Salmo salar</i>	Refseq_rna	NM_0011 40480.1	523	0	93.6
QSF_RS9.1.4	267	<i>Rattus norvegicus</i>	Swissprot	RS9_RAT	200	1e <sup>-16</sup>	97.4
QSF_RL8.1.6	774	<i>Danio rerio</i>	Swissprot	RL8_DA NRE	1267	1e <sup>-177</sup>	93.8
QSF_IRK8.1.1	1266	<i>Salmo salar</i>	Refseq_rna	NM_0011 40360.1	1828	0	97.2
QSF_NKCC1A.1. 2	3453	<i>Salmo salar</i>	Refseq_rna	NM_0011 23683.1	479	0	94.1
QSF_LOC100136 366.1.1	4557	<i>Salmo salar</i>	Refseq_rna	NM_0011 23534.1	4750	0	96.9
QSF_LOC100136 607.1.1	561	<i>Oncorhynchus mykiss</i>	Refseq_rna	NM_0011 24597.1	633	0	97.6
QSF_LO C101477634.1.1	681	<i>Maylandia zebra</i>	Refseq_rna	XM_0045 48350.1	259	1e <sup>-148</sup>	84.8

**Table S2.** Results of one-way analysis of variance (ANOVA) and Kruskal-Wallis test for the mRNA abundance of genes measured in gill and liver tissue of juvenile brook trout (*Salvelinus fontinalis*) acclimated to 5, 10, 15, 20, 23, and 25°C.

Tissue	Gene	Df	Sum of Squares	Mean of Squares	F values/ Chi-squared	P-value
Gill	<i>atp1a3</i>	5, 59	24.39	4.878	3.674	<b>0.006</b>
	<i>irk8</i>	5, 59	14.60	2.920	1.739	0.142
	<i>nkcc1a</i>	5, 59	35.33	7.066	5.069	<b>&lt; 0.001</b>
	<i>vatb</i>	5, 59	-	-	2.268	0.811
	<i>vate1</i>	5, 59	10.28	2.056	1.475	0.213
	<i>cftr</i>	5, 59	2.96	0.591	0.4	0.846
	<i>cirbp</i>	5, 59	14.83	2.966	1.469	0.216
	<i>gpx1</i>	5, 59	6007	1201.4	5.735	<b>&lt; 0.001</b>
	<i>serpinh1</i>	5, 59	10.55	2.111	1.933	0.104
	<i>hspa8</i>	5, 59	11.94	2.387	2.252	0.063
	<i>hsp90ab1</i>	5, 59	25.33	5.066	4.321	<b>0.002</b>
Liver	<i>g6pc</i>	5, 59	67.56	13.51	3.123	<b>0.016</b>
	<i>gpx1</i>	5, 59	36.22	7.245	4.924	<b>0.001</b>
	<i>hspa8</i>	5, 59	36.59	7.318	8.828	<b>&lt; 0.001</b>
	<i>hsp90ab1</i>	5, 59	54.31	10.86	15.14	<b>&lt; 0.001</b>
	<i>nupr1</i>	5, 59	4444	888.8	4.918	<b>0.001</b>
	<i>serpinh1</i>	5, 59	4.63	0.9258	0.573	0.720

*atp1a3*, Na<sup>+</sup>/K<sup>+</sup>-transporting ATPase subunit alpha-3; *irk8*, ATP-sensitive inward rectifier K<sup>+</sup> channel 8; *nkcc1a*, Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> co-transporter-1-a; *vatb*, B subunit of V-type H-ATPase; *vate1*, E 1 subunits of V-type H-ATPase; *cftr*, cystic fibrosis transmembrane conductance regulator; *cirbp*, cold-inducible RNA-binding protein; *gpx1*, glutathione peroxidase-like peroxiredoxin;

*serpinh1*, *serpinh1*; *hspa8*, heat shock cognate 71 kDA protein; *hsp90ab1*, heat shock protein 90-beta; *g6pc*, glucose-6-phosphatase; *nupr1*, nuclear protein-1.

Data were analyzed using a one-way ANOVA except if data were not equally variable or normally distributed, in which case a Kruskal-Wallis test was used; significant *P*-values are represented as bolded text

**Table S3.** Results of the two-way analysis of variance (ANOVA) for muscle lactate, as well as plasma cortisol and glucose of juvenile brook trout (*Salvelinus fontinalis*) acclimated to five temperatures (5, 10, 15, 20, and 23°C) and exposed to one of three treatments (unhandled, acute stress, and acute-stress recovery).

Variable	Group	Df	Sum of Squares	Mean of Squares	F value	P-value
Muscle lactate	Treatment	2	13.135	6.568	125.415	< <b>0.001</b>
	Temperature	4	4.107	1.027	19.606	< <b>0.001</b>
	Treatment × Temperature	8	4.106	0.513	9.801	< <b>0.001</b>
Plasma cortisol	Treatment	2	37274	18637	46.15	< <b>0.001</b>
	Temperature	4	6593	1648	4.08	<b>0.004</b>
	Treatment × Temperature	8	13919	1740	4.31	< <b>0.001</b>
Plasma glucose	Treatment	2	2.9573	1.4787	64.803	< <b>0.001</b>
	Temperature	4	0.7413	0.1853	8.122	< <b>0.001</b>
	Treatment × Temperature	8	1.2648	0.1581	6.929	< <b>0.001</b>

Significant *P*-values are represented as bolded text

**Table S4.** Results of the one-way ANOVA and Kruskal-Wallis test for standard metabolic rate (SMR) and maximum MR (MMR), recovery time, aerobic scope (AS), and factorial aerobic scope (FAS) of juvenile brook trout (*Salvelinus fontinalis*) acclimated to 5, 10, 15, 20, and 23°C.

<b>Variable</b>	<b>Df</b>	<b>Sum of Squares</b>	<b>Mean of Squares</b>	<b>F value/ Chi-Squared</b>	<b>P-value</b>
<b>SMR</b>	4, 40	-	-	30.43	<b>&lt; 0.001</b>
<b>MMR</b>	4, 40	1.272	0.318	8.84	<b>&lt; 0.001</b>
<b>Recovery Time</b>	4, 40	506.7	126.7	27.69	<b>&lt; 0.001</b>
<b>AS</b>	4, 40	0.598	0.149	1.29	0.292
<b>FAS</b>	4,40	23.91	5.978	7.57	<b>&lt;0.001</b>

Significant *P*-values are represented as bolded text