Proceedings of the National Academy of Sciences

Supplementary Information for the article:

Reduced physiological plasticity in a fish adapted to stable temperatures

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This supplementary information file includes:

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Link to raw data

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References

Supplementary text

Estimation of the number of generations AB fish held in lab

According to the ZFIN website (https://zfin.org/action/genotype/view/ZDB-GENO-960809-7), the AB line was brought into the lab in the 1970s. By 1991, the AB fish were 70 generations removed from the AB-wt fish used to establish the line. From this we can assume that 3.4 generations were produced per year (since 21 years = 70 generations). Since we do not have an exact number of generations from then, as each lab has different protocols, we extrapolated these numbers to give us an estimate of the number of generations the AB-wt (lab) fish had been in a laboratory environment at the start of this experiment. Extrapolating from 1991-2017 (26 years × 3.4 generations) gives 88.4 generations and a total of 158 generations from establishing the line. We therefore used the estimate of over 150 generations in this paper.

Supplementary Data

Link to all raw data: https://figshare.com/s/91f03a69303257e477f1
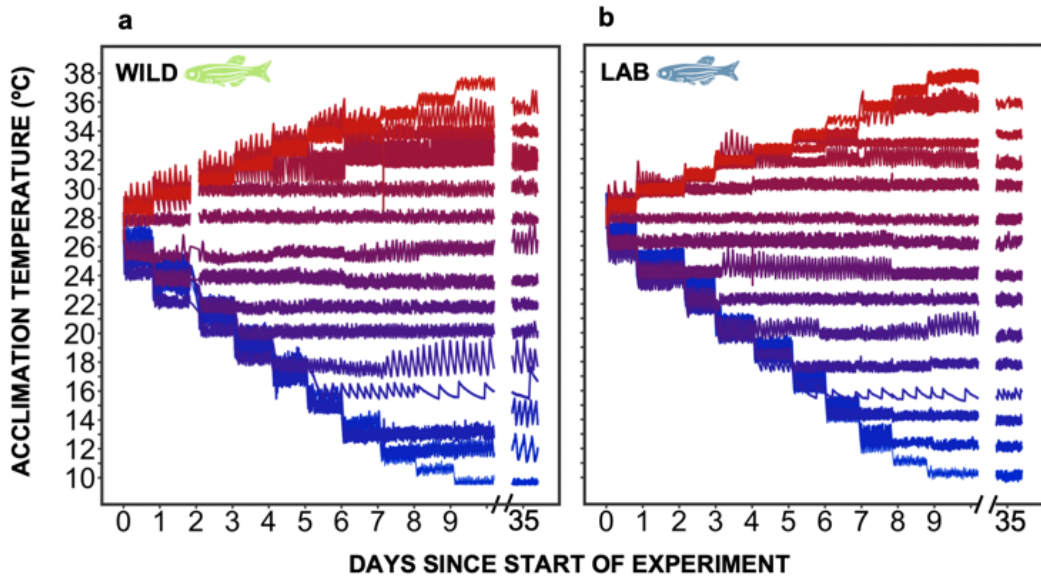
Supplementary figures

Figure S1: Thermal profiles showing the raw traces for how acclimation temperatures (10-38°C; (a) wild fish, 15 aquaria & (b) lab fish, 15 aquaria) were reached and maintained over the entire experimental period (38°C was terminated before the end of the experiment due to high mortality)
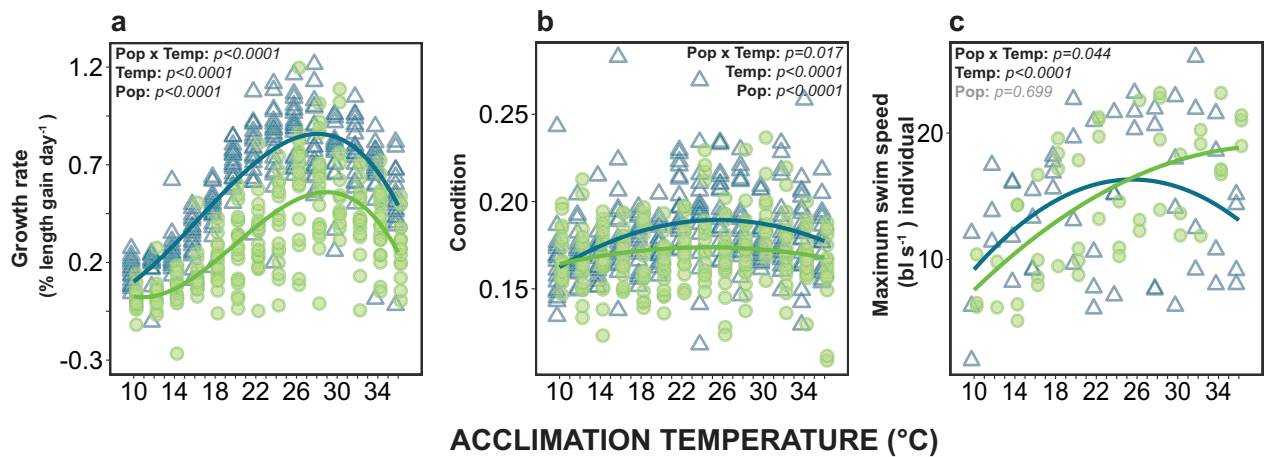

Figure S2: Growth (length), condition and maximum swim speed (individuals) of lab and wild fish acclimated from 10-36(38)°C. b - Specific growth rate for length for each individual fish. c - Condition of fish after 35 days of acclimation (calculated as the Fulton´s condition index). d - Maximum swim speed of individual fish measured in a swim respirometer. (see Table S4 & S6)
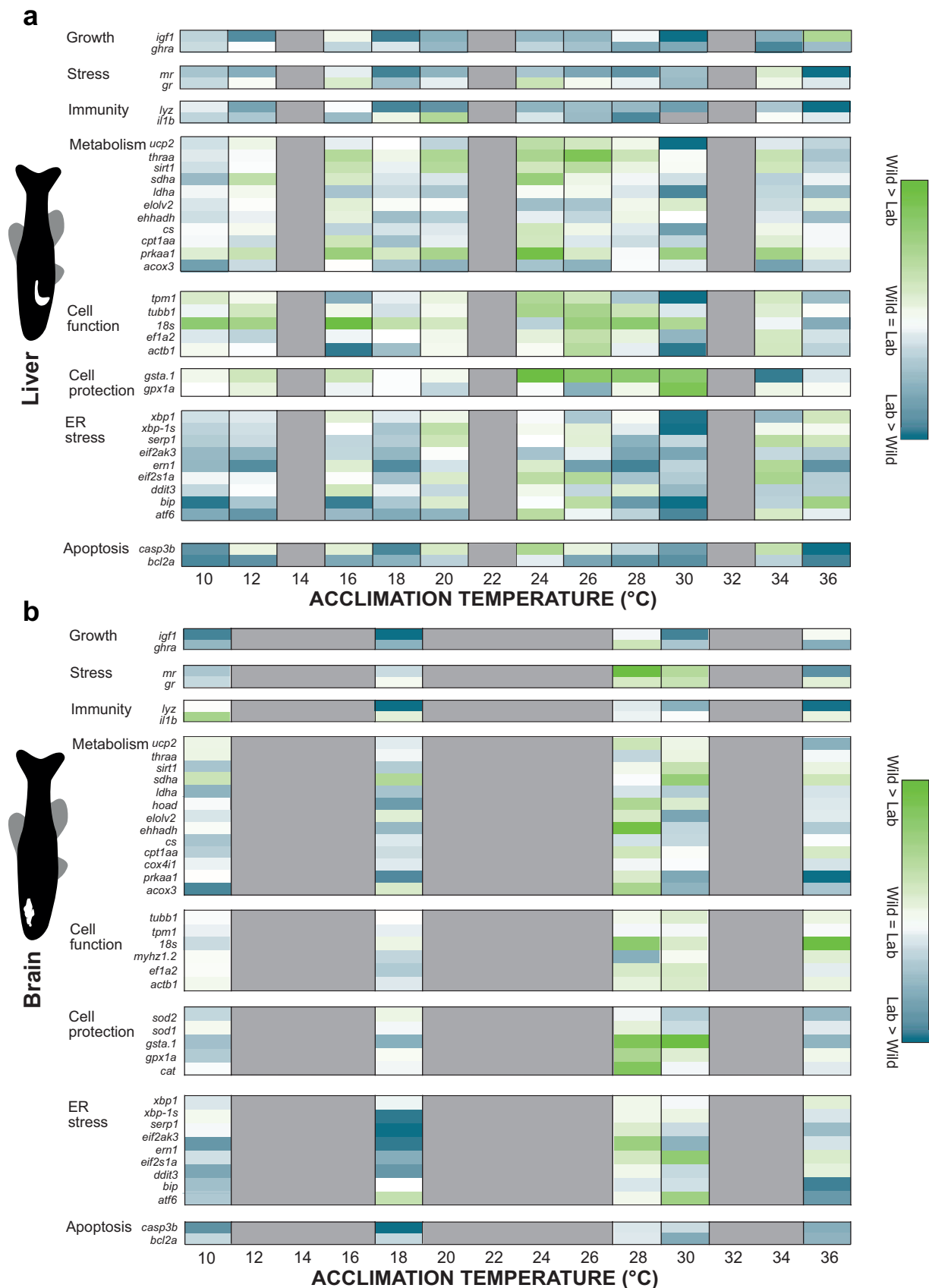
Figure S3: Heat map showing the difference in liver (a) and brain (b) gene expression between wild and lab fish. If there are no differences between wild and lab fish then the colour is white, this then proceeds along a colour gradient with the strongest green showing a higher expression in wild fish and the darkest blue a higher expression in lab fish. Genes are grouped according to their function and colours represent relative expression within these groups. Fish were only sampled at 10, 18, 28, 30 & 36°C in the brain and at 10, 12, 16, 18, 20, 24, 26, 28, 30, 34, and 36°C in the liver.
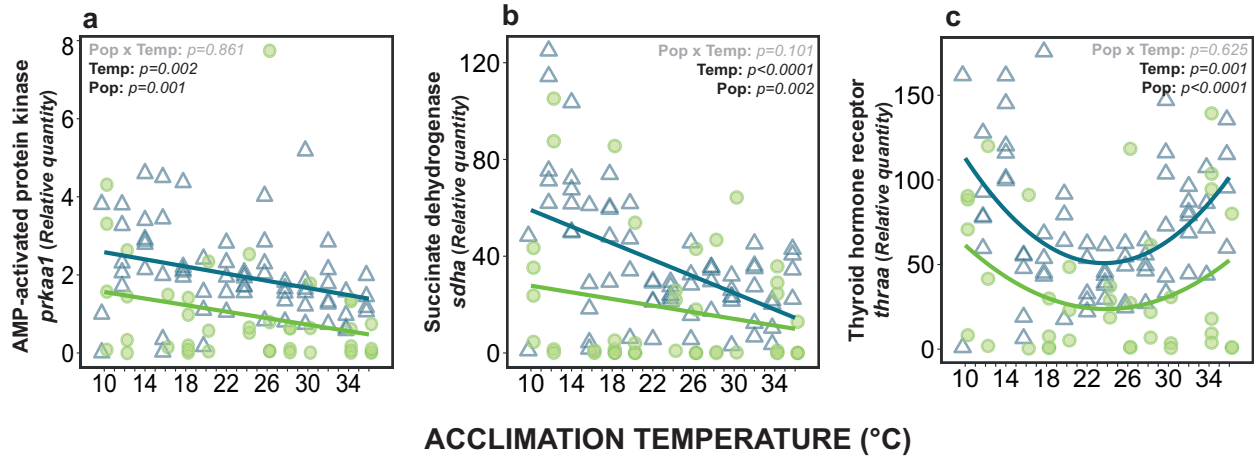
Figure S4: Metabolic gene expression using qPCR in wild and lab zebrafish acclimated from 10-36°C. a - Relative quantity of AMP-activated protein kinase subunit alpha-1 (*prkaa1*); b - Relative quantity of succinate dehydrogenase subunit A (*sdha*); c- Relative quantity of thyroid hormone receptor alpha-A (*thraa*). Wild fish are illustrated by green circles and lab fish by blue triangles.
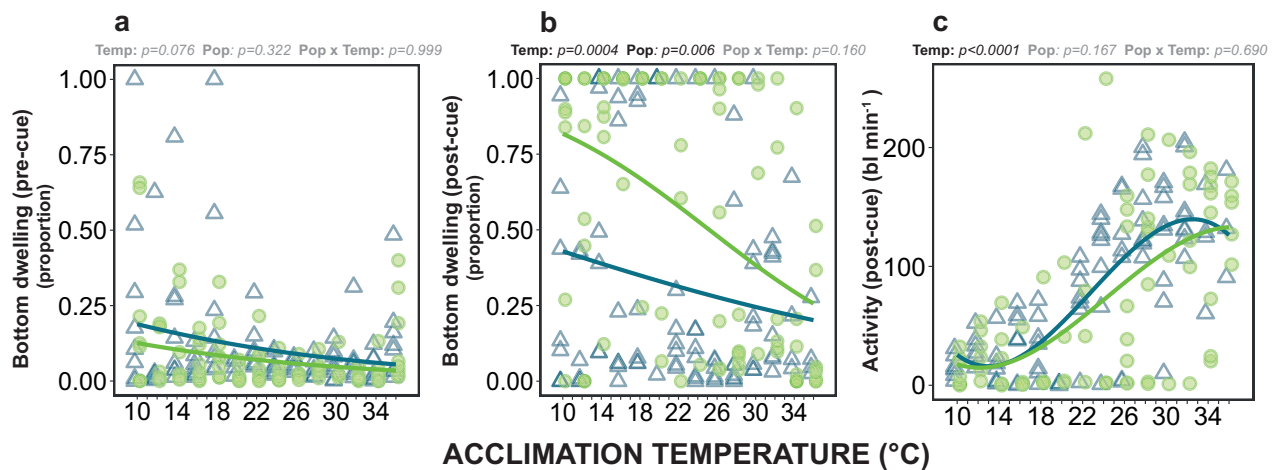
Figure S5: Behaviours corresponding to Fig. 2e & f, pre (a) and post (b) alarm cue time spent bottom dwelling and post-cue activity (c) in lab and wild zebrafish acclimated from 10-36°C. Wild fish: green circles, lab fish: blue circles. Statistically significant differences indicated on each panel: Temp –significant effect of temperature on trait, Pop – significant difference between wild and lab fish (at 23°C), Pop x Temp – significant interaction (see Table S4-S6)
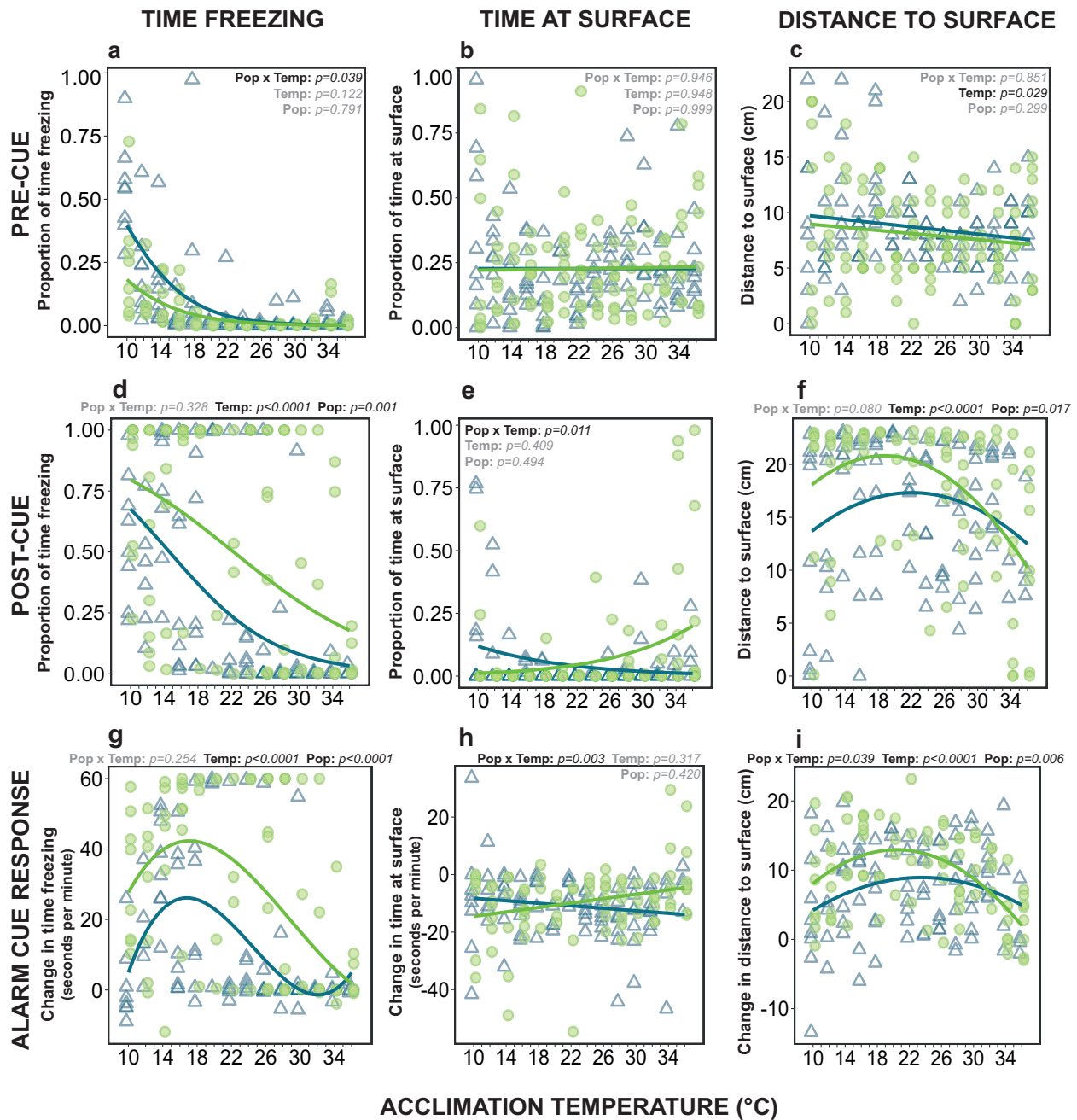
Figure S6: Additional behaviours: time spent freezing, time at the surface, and distance from the surface, in lab and wild zebrafish acclimated from 10-36°C. All behaviours were analysed pre-alarm cue (baseline) and post-alarm cue. The alarm cue response was quantified as the change in behaviour. Wild fish: green circles, lab fish: blue circles. Statistically significant differences indicated on each panel: Temp –significant effect of temperature on trait, Pop – significant difference between wild and lab fish (at 23°C), Pop x Temp – significant interaction (see Table S4-S6)
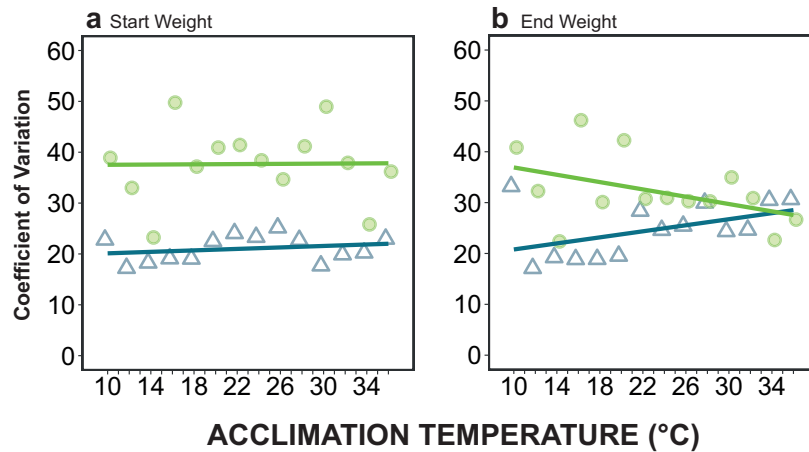
Figure S7: Coefficient of variation for weight of the fish at the (a) start and (b) end of the experiment. During phenotyping not all individuals from each tank were measured but the measured ones were assumed to be representative for that temperature. The coefficient of variation in weight at the start and end of the experiment shows how much variation there was at each temperature and how representative the measured individuals are. At the start of the experiment the two populations (wild: green circles; lab: blue triangles) show consistent variation within population but overall there is less variation in the lab than wild. By the end of the experiment the variation at the colder temperatures was similar as the start but at the warmer temperatures both wild and lab fish had converged and show similar variation.
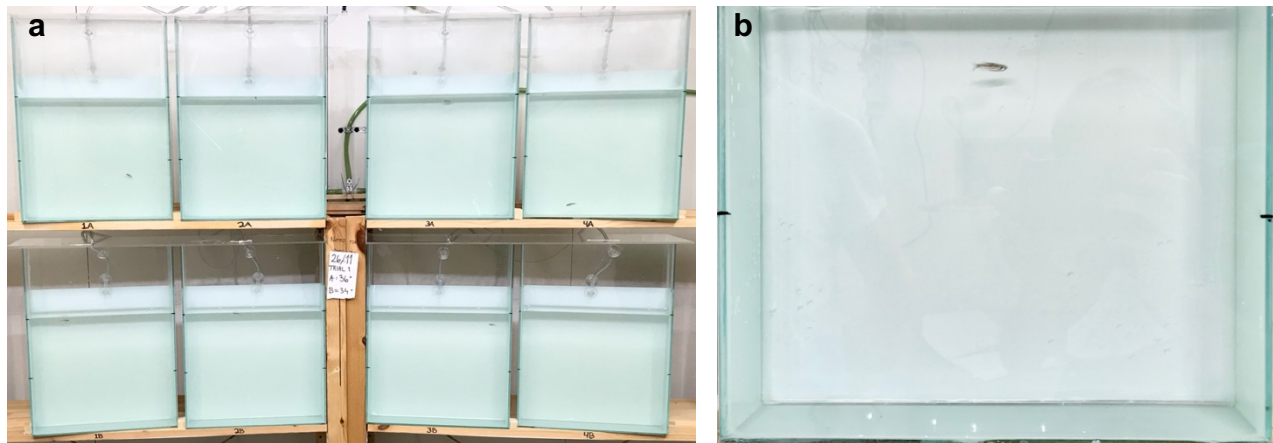
Figure S8: Design of the behavioural arena. The behavioural tests were conducted in eight parallel arenas (a) with one fish in each (b). The tanks had white back and sides, and clear front where the video recording was taken from. A pipette tip introduced chemical cues at the centre top of the arenas.

Supplementary Tables

Table S1: Statistical output from ANOVA´s comparing whether acclimation temperature (T), population (P; wild or lab) and the interaction between them (TxP) differs in a range of traits.

|	Temperature (T)		Population (P)		Interaction (T x P)		Weight (W)	
	F-value^{df}	p-value	F-value^{df}	p-value	F-value^{df}	p-value	F-value^{df}	p-value
Behaviour								
Activity	$74.77^{3,193}$	**<0.0001**	$77.52^{1,193}$	**<0.0001**	$3.75^{3,193}$	**0.012**	-	-
Change in activity	$7.71^{2,163}$	**0.0006**	$42.20^{1,163}$	**<0.0001**	$3.54^{2,163}$	**0.031**	-	-
Change in bottom-dwelling	$13.18^{2,175}$	**<0.0001**	$28.38^{1,175}$	**<0.0001**	$3.43^{2,175}$	**0.034**	-	-
Physiology								
Response time	$42.16^{2,97}$	**<0.0001**	$1.07^{1,97}$	0.303	$3.82^{2,97}$	**0.025**	-	-
Max swim speed	$109.34^{3,74}$	**<0.0001**	$6.21^{1,74}$	**0.015**	$1.64^{3,74}$	0.187	-	-
Thermal tolerance	$3559.9^{2,212}$	**<0.0001**	$34.89^{1,212}$	**<0.0001**	$11.88^{2,212}$	**<0.0001**	$10.82^{1,212}$	**0.0012**
SMR	$57.46^{2,69}$	**<0.0001**	$2.93^{1,69}$	0.091	$3.18^{2,69}$	**0.048**	$6.04^{1,69}$	**0.017**
MMR	$78.61^{3,72}$	**<0.0001**	$20.19^{1,72}$	**<0.0001**	$3.80^{3,72}$	**0.014**	-	-
Aerobic scope	$32.47^{3,17}$	**<0.0001**	$17.64^{1,17}$	**0.0006**	$1.149^{3,17}$	0.358	-	-
Growth rate	$197.69^{3,522}$	**<0.0001**	$277.74^{1,522}$	**<0.0001**	$4.68^{3,522}$	**0.003**	-	-
Cellular								
RBC size	$23.33^{2,108}$	**<0.0001**	$0.005^{1,108}$	0.944	$1.35^{1,108}$	0.264	-	-
RNA:DNA	$0.07^{1,128}$	0.790	$3.55^{1,128}$	0.062	$0.60^{1,128}$	0.440	-	-
Genes								
ghra muscle	$38.51^{2,111}$	**<0.0001**	$28.95^{1,111}$	**<0.0001**	$0.44^{2,111}$	0.646	-	-
igf1 muscle	$9.12^{1,107}$	**0.003**	$5.87^{1,107}$	**0.017**	$5.36^{1,107}$	**0.022**	-	-
hsp70 muscle	$150.69^{1,113}$	**<0.0001**	$13.25^{1,113}$	**0.0004**	$142.29^{1,113}$	**<0.0001**	-	-
hsp90 muscle	$221.64^{1,113}$	**<0.0001**	$1.10^{1,113}$	0.297	$0.57^{1,113}$	0.452	-	-
hsp70 liver	$46.62^{1,106}$	**<0.0001**	$9.51^{1,106}$	**0.003**	$41.43^{1,106}$	**<0.0001**	-	-
hsp90 liver	$379.07^{1,105}$	**<0.0001**	$22.15^{1,105}$	**<0.0001**	$194.74^{1,105}$	**<0.0001**	-	-
hsp70 brain	$22.04^{2,31}$	**<0.0001**	$4.82^{1,31}$	**0.036**	$9.41^{2,31}$	**0.0006**	-	-
hsp90 brain	$70.69^{2,31}$	**<0.0001**	$12.52^{1,31}$	**0.001**	$13.82^{2,31}$	**<0.0001**	-	-

Table S2: Model estimates and standard errors. Population estimates are related to the intercept (lab population at 23°C)

	Population		Temperature β^1		Temperature β^2		Temperature β^3		Weight
	Lab	Wild	Lab	Wild	Lab	Wild	Lab	Wild	
Behaviour									
Activity (pre-cue) bl min⁻¹	109.96 ± 4.19	52.17 ± 6.08	575.42 ± 61.57	35.73 ± 91.17	-159.77 ± 63.13	-278.02 ± 91.14	-44.42 ± 62.05	-127.22 ± 90.85	-
Activity (alarm cue response) bl min⁻¹	-33.55 ± 5.88	-55.48 ± 8.70	86.73 ± 92.08	-3.88 ± 129.84	126.48 ± 94.50	350.248 ± 133.27	-	-	-
Bottom dwelling (alarm cue response) sec min⁻¹	12.41 ± 2.19	16.63 ± 3.18	-38.52 ±33.75	-107.89 ± 46.18	-58.84 ± 34.20	-68.66 ± 47.26	-	-	-
Physiology									
Response time ms	2.22 ± 0.09	0.13 ± 0.13	-7.40 ± 0.92	3.31 ± 1.33	2.33 ± 0.89	-1.63 ± 1.34	-	-	-
Max swim speed bl sec⁻¹	7.82 ± 0.20	0.70 ± 0.28	19.66 ± 1.83	0.77 ± 2.56	-7.07 ± 1.85	-5.50 ± 2.56	-6.34 ± 1.85	1.31 ± 2.57	-
Thermal tolerance °C	38.00 ± 0.18	0.61 ± 0.10	100.04 ± 1.78	-10.27 ± 2.48	-15.10 ± 2.06	3.73 ± 2.63	-	-	3.05 ± 1.14
SMR mg h⁻¹ g⁻¹	0.50 ± 0.07	-0.09 ± 0.03	2.37 ± 0.27	-0.71 ± 0.34	0.39 ± 0.25	-0.38 ± 0.32	-1.15 ± 0.43	1.18 ± 0.61	-1.31 ± 0.49
MMR mg h⁻¹ g⁻¹	1.00 ± 0.04	0.26 ± 0.06	4.82 ± 0.43	-1.07 ± 0.60	-1.15 ± 0.44	-1.05 ± 0.62	-0.69 ± 0.29	0.59 ± 0.43	-
Aerobic scope mg h⁻¹ g⁻¹	0.69 ± 0.05	0.32 ± 0.08	1.80 ± 0.28	-0.45 ± 0.41	-0.99 ± 0.27	-0.30 ± 0.39	-5.38 ± 1.33	-0.93 ± 1.91	-
Growth % day⁻¹	1.93 ± 0.05	-1.03 ± 0.07	10.66 ± 1.29	-3.78 ± 1.87	-17.83 ± 1.34	3.96 ± 1.93	-	-	-
Survival proportion	3.30 ± 0.40	-0.47 ± 0.51	-4.03 ± 1.00	0.04 ± 1.37	-9.63 ± 1.63	1.36 ± 2.12	-	-	-
Cellular									
Red blood cell size FSC-A x 10³	72.47 ± 0.72	-0.11 ± 1.09	78.08 ± 19.71	25.68 ± 29.11	85.74 ± 21.21	-32.23 ± 30.41	-	-	-
RNA:DNA	2.03 ± 0.05	-0.14 ± 0.07	0.002 ± 0.006	-0.008 ± 0.009	-	-	-	-	-
Genes (Relative quantity)									
ghra muscle	0.57 ± 0.02	-0.18 ± 0.03	-1.45 ± 0.24	0.22 ± 0.36	0.88 ± 0.24	-0.25 ± 0.35	-	-	-
igf1 muscle	0.16 ± 0.02	-0.08 ± 0.01	-0.01 ± 0.02	0.01 ± 0.01	-	-	-	-	-
hsp70 muscle (exp)	0.26 ± 0.06	0.28 ± 0.10	0.01 ± 0.01	-0.01 ± 0.01	-	-	-	-	-
hsp90 muscle (exp)	0.85 ± 0.25	-0.30 ± 0.40	0.01 ± 0.01	-0.01 ± 0.01	-	-	-	-	-
hsp70 liver (exp)	0.31 ± 0.07	-0.11 ± 0.12	0.01 ± 0.01	-0.01 ± 0.01	-	-	-	-	-
hsp90 liver (exp)	0.32 ± 0.47	-0.05 ± 0.76	0.01 ± 0.01	-0.01 ± 0.01	-	-	-	-	-
hsp70 brain	0.96 ± 0.13	-0.44 ± 0.20	2.61 ± 0.84	-4.28 ± 1.21	5.02 ± 0.82	-3.10 ± 1.22	-	-	-
hsp90 brain	0.88 ± 0.08	-0.42 ± 0.12	2.58 ± 0.50	-2.04 ± 0.71	5.21 ± 0.48	-3.17 ± 0.72	-	-	-

Table S3: Primers used for gene expression analysis by qPCR

Gene name	Abbreviation	Gene function	Forward Primer Sequence	Reverse Primer Sequence	Accession number
Heat shock proteins					
Heat shock protein 90-alpha 1	hsp90	Chaperone for damaged proteins	tgcgcagggttttcatcatg	accaggttttgcggatcac	BC075757.1
Heat shock protein 70	hsp70	Chaperone for damaged proteins	tcaagcgcaacacaaccatc	atttgcccagcaggttgttg	AF210640
Growth					
Insulin like growth factor 1	igf1	Growth mediator	acacagggggcagaaactat	aagatggggcttaaacgtcc	NM_131825.2 (1)
Growth hormone receptor α	ghra	Growth hormone receptor	aagccagacttctaccacgag	ctaatacaccaacagccccac	EU649774.1 (1)
Stress					
Mineralocorticoid receptor	mr	Mineralocorticoid receptor	cccattgaggaccaaatcac	agtagagcatttgggcgttg	DQ017617.1 (2)
Glucocorticoid receptor	gr	Glucocorticoid receptor	acagcttcttccagcctcag	ccggtgttctcctgtttgat	EF567112.1 (2)
Immunity					
Lysozyme	lyz	Immune response	gatttgagggattctccat	ccgtagtccttccccgtatc	BC162644.1
Interleukin 1 beta	Il1b	Immune response	tggacttcgcagcacaaaa	cacttcacgctcttggatga	NM_212844.2
Muscle function / Cell function					
Tropomyosin 1	tpm1	Muscle structure and function	tggtcgtgcacaaagttagc	ctgccttcacagtagcacca	NM_200934.3
Myosin, heavy polypeptide 1.2	myhz1.2	Muscle structure and function	gctgaggaacaagccaactc	agctgcctcttttccctttc	NM_001161446.1
Metabolism					
Uncoupling protein 2	ucp2	Mitochondrial uncoupling	tggctcaacccactgatgta	caatggtccgatatgcgtc	NM_131176.1 (3)
Thyroid hormone receptor alpha-A	thraa	Thyroid hormone receptor	ctatgaacagcacatccgacaagag	cacaccacacacggctcatc	NM_131396.1
Sirtuin 1	sirt1	Lipid metabolism, protein deacetylation	ctggggtgtctgtttcctgt	actgtccggggtagatttcc	XM_001334404.6
Succinate dehydrogenase subunit A	sdha	Oxidative phosphorylation	tggtatgccgttcagccgta	ggccaagtctttggcattgg	BC164560.1 (4)
Lactate dehydrogenase A4	ldha	Anaerobic metabolism	ttggcagtggcacaaacttg	atgttctccaacgacccaac	NM_131246.1
Hydroxyacyl CoA dehydrogenase +	ehhadh	Lipid metabolism	tgggatgcacttctttttcgc	atcagcatgcggtttccaac	NM_207068.1
Delta-6 fatty acyl desaturase	fadsd6	Lipid metabolism	atacctgaagccgctgctaa	caaatgcagagcgaaaaaca	AF309556.1
Citrate synthase	cs	Carbohydrate metabolism	agtccacgcattgcacaaac	ttcgctgctggacaataacg	NM_199598.1
NADH Dehydrogenase Fe-S protein 3 *+¥	ndufs3	Oxidative phosphorylation	atgtgcagcaagttcaggtg	tgcggaactgagcatttgtg	BC165450.1
Cytochrome C oxidase subunit 4il *+	cox4i1	Oxidative phosphorylation	ttggcaactacggcatttcg	tcagctgctgcacaaacttg	NM_214701.1
Elongation of very-long-chain fatty acids-like 2	elovl2	Lipid metabolism	cactggacgaagttggtgaa	gttgaggacacaccaccaga	NM_001040362.1 (5)
Carnitine palmitoyltransferase 1A	cpt1aa	Lipid metabolism	gcattgaccttcagctcagc	ctgccaacaccagcacgaac	NM_001044854.1 (6)
AMP-activated protein kinase subunit alpha-1	prkaa1	Cellular energy homeostasis	cacatgaatgccaagattgc	tcactccactgctccagatg	NM_001110286.1
Acyl-CoA oxidase 3	acox3	Lipid metabolism	acctttgggatcatggactg	tgttgtttcgtgcctcaaag	NM_213147.1 (7)
Cell function					
Beta 1 tubulin	tubb1	Cytoskeletal structure	cctgctgggaactgtattgt	tcaatgagttccttgccaat	NM_001326422.1 (8)
18s Ribosomal RNA	18s	Ribosomal structure	tcgctagttggcatcgtttatg	cggaggttcgaagacgatca	KY486501.1 (9)
Elongation factor 1α *	ef1a	Protein synthesis	tcacatcgcctgcaagtttg	tcatgtcacgcacagcaaag	AM422110.2
Hypoxia inducible factor 1 subunit alpha b *+¥	hif1ab	Hypoxia response transcription factor	ccaccacccaaaaactccct	ggagtggggggcgataaaaca	NM_001310042.1 (10)
Beta actin 1	actb1	Cytoskeletal structure	ggcatcacaccttctacaatga	tacgaccagaagcgtacagaga	NM_131031.2
Cell protection					
Superoxide dismutase 2, mitochondrial +	sod2	Antioxidant	attgaggattgcagcgtgtg	ttcttggcagcttggaaacg	NM_199976.1
Superoxide dismutase 1, soluble +	sod1	Antioxidant	acaacacaaacggctgcatc	tttgcaacaccactggcatc	NM_131294.1
Glutathione-S-transferase Alpha 1	gsta.1	Detoxifying enzyme and antioxidant	ccccaaaatacaggcgtttc	gcccggctggaggaact	NM_213394.1
Glutathione peroxidase 1a	gpx1a	Antioxidant	agatgtcattcctgcacacg	aaggagaagcttcctcagcc	NM_001007281.2
Catalase +	cat	Antioxidant	aaaatatggccgggcatctg	acagccatcaggttttgcac	AJ007505.1
ER stress					
X-box binding protein 1	xbp1	ER stress mediator	gggttggataccttggaaa	agggccagggctgtgagta	NM_131874.1 (11)
X-box binding protein 1 (spliced form)	xbp-1s	ER stress mediator	tgttgcgagacaagacga	cctgcacctgctgcggact	AF420256 (12)
Stress-associated endoplasmic reticulum protein 1	serp1	ER stress mediator	ccgaaatcacacgtcaactg	catgcgaatcctctgtttgg	NM_200034.1

Eukaryotic translation initiation factor 2-alpha kinase 3 (homolog of PERK)	eif2ak3	ER stress mediator	ttttgctgcagaacggacatg	atcttcaccgggacacttcac	XM_005156585.4 (12)
Endoplasmic to nucleus signalling 1 (homolog of IRE1)	ern1	ER stress mediator	ccagtggctgtgaagaggatt	agttgcacagttctatggcga	NM_001020530 (12)
Eukaryotic translation initiation factor 2 subunit 1 alpha a (homolog of eIF2α)	eif2s1a	ER stress mediator	ccaaagatgagcagctggaga	atccgacacagcctgcttaaa	NM_199569.1(12)
DNA-damage inducible transcript 3 (homolog of CHOP)	ddit3	ER stress mediator	gagttggaggcgtggtatga	ccttggtggcgattggtgaa	NM_001082825 (12)
Heat shock protein 5 (homolog of GRP78)	bip	ER stress mediator	cgaagaagccagatatcgatga	acggctcttttccgttgaag	NM_213058 (13)
Activating transcription factor 6	atf6	ER stress mediator	ctgtggtgaaacctccacct	catggtgaccacaggagatg	NM_001110519 (12)
Apoptosis					
Caspase 3	casp3b	Pro apoptotic mediator	accgctttgtcatcggaatc	tgtttgttgactggcaccac	NM_001048066.2
BCL2 Apoptosis regulator A	bcl2a	Pro apoptotic mediator	ttgactgtgatgctcgtcac	tgcacgtgttttgtgtgtcg	NM_001030253.2

* Used as housekeeping gene in muscle; + used as house keeping gene in liver; ¥ used as housekeeping gene in brain

Table S4: Statistical output from ANOVA´s comparing whether acclimation temperature (T), population (P; wild or lab) and the interaction between them (T×P) differs in a range of traits (supplementary data traits only)

	Temperature (T)		Population (P)		Interaction (T x P)	
	F-value^{df}	p-value	F-value^{df}	p-value	F-value^{df}	p-value
Behaviour						
Time freezing (alarm cue response)	$17.62^{3,173}$	**<0.0001**	$28.36^{1,173}$	**<0.0001**	$1.37^{3,173}$	0.254
Time at surface (alarm cue response)	$1.01^{1,177}$	0.317	$0.65^{1,177}$	0.420	$8.77^{1,177}$	**0.003**
Distance to surface (pre-cue)	$4.86^{1,209}$	**0.029**	$1.09^{1,209}$	0.299	$0.44^{1,209}$	0.851
Distance to surface (post-cue)	$10.70^{2,178}$	**<0.0001**	$5.86^{1,178}$	**0.017**	$2.56^{2,178}$	0.080
Distance to surface (alarm cue response)	$11.22^{2,175}$	**<0.0001**	$7.90^{1,175}$	**0.006**	$3.31^{2,175}$	**0.039**
Activity (post-cue)	$50.40^{3,164}$	**<0.0001**	$1.93^{1,164}$	0.167	$0.49^{3,164}$	0.690
Physiology						
Growth % length day^{-1}	$300.29^{3,520}$	**<0.0001**	$340.52^{1,520}$	**<0.0001**	$7.48^{3,520}$	**<0.0001**
Condition	$17.81^{2,522}$	**<0.0001**	$36.47^{1,522}$	**<0.0001**	$4.11^{2,522}$	**0.017**
Max swim speed (indiv)	$12.75^{2,95}$	**<0.0001**	$0.15^{1,95}$	0.699	$3.24^{2,95}$	**0.044**
Genes						
prkaa1 muscle	$9.90^{1,106}$	**0.002**	$15.68^{1,106}$	**<0.0001**	$0.44^{2,111}$	0.646
sdha muscle	$18.45^{1,101}$	**<0.0001**	$9.95^{1,101}$	**0.002**	$2.73^{1,101}$	0.101
thraa muscle	$7.40^{2,102}$	**0.001**	$25.33^{1,102}$	**<0.0001**	$0.47^{2,102}$	0.625

Table S5: Statistical output from Chi-squared tests for one physiological and six behavioural traits. The test sequentially adds acclimation temperature (T), population (P; wild or lab) and the interaction between them (T×P) into the model to compare whether there is a significant difference from the NULL model.

	NULL	Temperature (T)		Population (P)		Interaction (T x P)	
	Residual Deviance	Residual Deviance^{df}	p-value	Residual Deviance^{df}	p-value	Residual Deviance^{df}	p-value
Behaviour							
Time freezing (pre-cue)	48.192	$24.99^{1,211}$	**<0.0001**	$22.60^{1,210}$	0.122	$22.53^{1,209}$	0.791
Time freezing (post-cue)	173.94	$138.30^{1,182}$	**<0.0001**	$127.79^{1,181}$	**0.001**	$126.84^{1,180}$	0.328
Time at surface (pre-cue)	39.53	$39.52^{1,211}$	0.948	$39.52^{1,210}$	0.999	$39.52^{1,209}$	0.946
Time at surface (post-cue)	50.86	$50.18^{1,182}$	0.409	$49.71^{1,181}$	0.494	$43.24^{1,180}$	**0.011**
Bottom-dwelling (pre-cue)	39.82	$36.68^{1,211}$	0.076	$35.70^{1,210}$	0.322	$35.70^{1,209}$	0.999
Bottom-dwelling (post-cue)	162.86	$150.21^{1,182}$	**0.0004**	$138.32^{1,181}$	**0.0006**	$136.35^{1,180}$	0.160
Physiology							
Survival	247.07	$78.225^{2,27}$	**<0.001**	$77.71^{1,26}$	0.474	$77.30^{2,24}$	0.812

Table S6: Model estimates and standard errors for extended data phenotypes. Population estimates are related to the intercept (lab population at 23°C) Estimates from proportion data are derived from glm models with a binomial error structure and therefore need to be back-transformed to obtain exact proportions.

	Population		Temperature β1		Temperature β2		Temperature β3	
	Lab	Wild	Lab	Wild	Lab	Wild	Lab	Wild
Behaviour								
Time freezing (pre-cue) proportion	-3.38 ± 0.74	-0.64 ± 1.20	-0.23 ± 0.08	0.03 ± 0.13	-	-	-	-
Time freezing (post-cue) proportion	-1.27 ± 0.30	1.22 ± 0.38	-0.16 ± 0.04	0.05 ± 0.05	-	-	-	-
Time freezing (alarm cue response) sec min-1	11.84 ± 2.09	15.39 ± 3.01	-94.38 ± 32.22	-65.96 ± 43.88	-56.76 ± 32.50	-46.27 ± 44.73	89.94 ± 30.53	-49.911 ± 42.90
Time at surface (pre-cue) proportion	-1.23 ± 0.23	-0.01 ± 0.33	-0.01 ± 0.03	0.01 ± 0.04	-	-	-	-
Time at surface (post-cue) proportion	3.27 ± 0.97	0.61 ± 1.40	-23.50 ± 13.87	65.34 ± 19.91	45.05 ± 13.81	1.84 ± 19.02	-	-
Time at surface (alarm cue response) sec min-1	-10.99 ± 1.15	1.33 ± 1.67	-0.22 ± 0.15	0.60 ± 0.20	-	-	-	-
Distance to surface (pre-cue) cm	8.66 ± 0.40	-0.60 ± 0.57	-0.08 ± 0.05	0.01 ± 0.07	-	-	-	-
Distance to surface (post-cue) cm	15.63 ± 0.65	2.24 ± 0.94	-3.35 ± 9.31	-27.63 ± 12.69	-19.40 ± 9.27	-8.23 ± 12.77	-	-
Distance to surface (alarm cue response) cm	7.23 ± 0.62	2.44 ± 0.90	3.22 ± 9.54	-31.22 ± 13.05	-21.92 ± 9.67	-16.38 ± 13.36	-	-
Activity (post-cue) bl min-1	77.55 ± 5.20	-9.95 ± 7.59	630.68 ± 76.60	-40.46 ± 106.06	-16.62 ± 76.56	71.78 ± 106.82	-183.83 ± 74.51	97.09 ± 106.15
Bottom-dwelling (pre-cue) proportion	-2.14 ± 0.32	-0.48 ± 0.51	-0.05 ± 0.04	-0.01 ± 0.06	-	-	-	-
Bottom-dwelling (post-cue) proportion	-0.82 ± 0.23	1.07 ± 0.32	-0.04 ± 0.03	-0.06 ± 0.04	-	-	-	-
Physiology								
Growth % length day-1	0.56 ± 0.01	-0.26 ± 0.02	2.97 ± 0.30	-0.59 ± 0.44	-5.08 ± 0.31	1.47 ± 0.45	-1.70 ± 0.31	-0.51 ± 0.45
Condition	0.18 ± 0.001	-0.01 ± 0.002	0.07 ± 0.03	-0.06 ± 0.05	-0.18 ± 0.04	0.11 ± 0.05	-	-
Max swim speed (indiv) bl sec-1	14.05 ± 0.69	0.83 ± 1.00	19.31 ± 18.19	66.79 ± 26.68	-47.90 ± 19.34	26.37 ± 28.19	-	-
Genes (Relative quantity)								
prkaa1 muscle	1.99 ± 0.15	-0.95 ± 0.24	-0.05 ± 0.02	0.01 ± 0.03	-	-	-	-
sdha muscle	36.81 ± 2.96	-15.84 ± 4.99	-1.71 ± 0.39	0.99 ± 0.60	-	-	-	-
thraa muscle	71.64 ± 4.53	-37.24 ± 7.46	-27.90 ± 48.70	9.27 ± 75.49	193.75 ± 50.50	-72.34 ± 75.21	-	-

Table S7: Optimal temperatures (T_{opt}) and thermal performance breadths (TPB; 80% performance) of lab and wild populations of zebrafish for five phenotypes. The difference in thermal performance breadth is calculated as: $TPB_{Wild} - TPB_{Lab}$. Optimal values are calculated from the best fitted model (second or third order polynomial)

Phenotype	Value$_{opt}$		Value$_{80\%}$		T_{opt} (°C)		Lower $T_{80\%}$ (°C)		Upper $T_{80\%}$ (°C)		TPB (°C)		Difference TPB (°C)
	Lab	Wild	Lab	Wild	Lab	Wild	Lab	Wild	Lab	Wild	Lab	Wild	
Maximum swim speed (U_{crit} group; bl s^{-1})	10.7	11.52	8.56	9.22	30.34	29.13	22.93	21.43	36.20	35.41	13.27	13.98	0.71
Growth (% weight gain day^{-1})	2.98	1.78	2.38	1.42	28.02	28.30	21.34	22.98	33.65	32.78	12.31	9.80	-2.51
Aerobic scope (AS, mg h^{-1} g^{-1})	1.19	1.36	0.95	1.09	29.31	27.08	23.70	19.24	33.98	34.36	10.28	15.12	4.84
Maximum metabolic rate (MMR, mg h^{-1} g^{-1})	1.66	1.68	1.33	1.35	31.34	29.11	24.90	20.01	36.67	38.39	11.77	18.38	6.61
Growth (% length gain day^{-1})	0.86	0.56	0.69	0.45	28.11	28.97	24.48	28.66	33.64	33.38	9.16	4.72	4.44

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