Reduced physiological plasticity in a fish adapted to stable temperatures

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Significance statement

Plastic individuals can buffer environmental changes, maintaining a stable performance across gradients. Plasticity is therefore thought to be particularly beneficial for the survival of wild populations that experience large environmental fluctuations, such as diel and seasonal temperature changes. Maintaining plasticity is widely assumed to be costly, however empirical evidence demonstrating this cost is scarce. Here we predict that if plasticity is costly, it would be readily lost in a stable environment, such as a laboratory. To test this, we measured a diverse range of phenotypic traits, spanning gene expression, physiology, and behaviour, in wild and lab zebrafish acclimated to 15 temperatures. We show that lab fish have lost plasticity in many traits, demonstrating that maintaining plasticity carries a cost.

Abstract

Plasticity can allow organisms to maintain consistent performance across a wide environmental range. However, it remains largely unknown how costly plasticity is, and whether a trade-off exists between plasticity and performance under optimal conditions. Biological rates generally increase with temperature, and to counter that effect fish use physiological plasticity to adjust their biochemical and physiological functions. Zebrafish in the wild encounter large daily and seasonal temperature fluctuations, suggesting they should display high physiological plasticity. Conversely, laboratory zebrafish have been at optimal temperatures with low thermal fluctuations for over 150 generations. We treated this domestication as an evolution experiment and asked whether this has reduced the physiological plasticity of lab fish compared to their wild counterparts. We measured a diverse range of phenotypic traits, from gene expression through physiology to behaviour, in wild and lab zebrafish acclimated to 15 temperatures from 10°C to 38°C. We show that adaptation to the lab environment has had major effects on all levels of biology. Lab fish show reduced plasticity and are thus less able to counter the direct effects of temperature on key traits like metabolism and thermal tolerance, and this difference is detectable down to gene expression level. Rapid selection for faster growth in stable lab environments appears to have carried with it a trade-off against physiological plasticity in captive zebrafish compared with their wild counterparts.

Main text

In ectotherms, body temperature is affected by the environmental temperature, and higher body temperatures increase biological rates (e.g. enzyme activity, metabolic rates)(1). Thermal performance curves illustrate this relationship, whereby performance under acute temperature exposure typically peaks at an optimal temperature (T_{opt}) and then decreases at non-optimal temperatures(2–4). However, over longer exposure to a temperature, individuals can remodel their physiology to restore homeostasis and counteract direct thermal effects(2, 5). Such thermal acclimation or compensation, can improve performance in varying environments through physiological plasticity(5–7). Physiological plasticity describes the ability of an organism to adjust their physiology in different environments, in this case to counteract the direct effect of temperature. Thermal performance breadth describes the range of temperatures over which performance is above or equal to 80% of the maximum(3), and this breadth can be extended through acclimation. Consequently, physiological plasticity has been hypothesised to be adaptive in heterogeneous environments(8), and would be reflected by broad thermal performance curves for a wide range of traits after acclimation(5). Maintaining high performance over a large thermal range may require large scale alterations in gene expression and therefore a greater capacity to modify transcriptional processes(9).

Having the ability to perform consistently across a wide range of temperatures could come with physiological or biochemical trade-offs. Such trade-offs could include investment in sensory and regulatory mechanisms, production costs (e.g. simultaneous expression of optimal and suboptimal enzyme isoforms, as each is optimised for a specific environmental condition), or could be genetically costly (e.g. if plastic genes are linked to genes for non-beneficial/costly traits)(10, 11). If true, a trade-off could be predicted between physiological plasticity on one hand, and performance at optimal temperatures on the other(12–14). Thermal performance curves with a narrower breadth and a higher peak could therefore be expected in populations that have evolved in the absence of environmental heterogeneity(15). In this case, selection should favour developmental pathways specific to the narrow environmental range experienced and select for

peak performing non-plastic individuals(16). Despite this, little empirical evidence exists linking higher plasticity to decreased performance in a narrow range of stable environments(17, 18).

Here we evaluate whether adaptation to homogenous environments reduces physiological plasticity over time using a novel approach comparing wild-caught versus laboratory (lab) zebrafish. We examine a suite of traits that span all levels of their biological organisation in response to acclimation to temperatures across their entire thermal range. By using a multi- rather than single-trait approach we aim to enhance our understanding of the role of plasticity at the whole organism level (19, 20).

Zebrafish are one of the most commonly used experimental animals(21). In the wild, zebrafish experience large seasonal thermal fluctuations which can range from 17.3 up to 38.6°C(22, 23). Biomedical research laboratories rear zebrafish at optimal temperature for growth and fecundity (28-28.5°C)(24–26). The AB wild-type zebrafish line was originally brought into laboratories in the 1970s and has undergone a domestication process for more than 150 generations (our estimate based on Howe et al.(27); see Supplementary text). Consequently, the line has adapted to life in small aquaria, high population densities, dry food, and to handling by humans(28, 29). One previously overlooked consequence of domestication is the potential adaptation to optimal temperature with low thermal variance and few thermal extremes(30). The domestication of zebrafish can thus be viewed as a long-term evolution experiment to optimal temperature with little thermal variation. We hypothesise that maintaining thermal plasticity comes at a cost, and adaptation to a stable thermal environment has thus reduced the physiological plasticity in domesticated zebrafish compared to wild zebrafish. We therefore predict that wild zebrafish should be able to acclimate and adjust their biochemistry and physiology through physiological plasticity to counteract direct thermal effects on biological rates to a higher degree than lab zebrafish. Wild zebrafish should thus be able to maintain close to normal function over a wider range of non-optimal temperatures, while lab zebrafish should display high performance at a narrower thermal range and faster deterioration of performance at non-optimal temperatures (Fig.

100 1a). Additionally, as lab zebrafish might be adapted to life at optimal temperatures, we predict that
101 the lab zebrafish should show higher performance in the measured traits at optimal temperatures
102 (Fig. 1a). We also predict that wild fish have more consistent performance over different
103 temperatures (i.e. shallower slope in the thermal reaction norm) (Fig. 1b). This would require the
104 wild fish to have an enhanced regulatory capacity compared to the lab fish (Fig. 1c).
105
106 To test these predictions, we exposed 300 juvenile lab zebrafish and 300 juvenile wild-caught F_1
107 generation zebrafish to the full range of temperatures that zebrafish can survive at for 35 days
108 (Fig. S1). After one month of thermal acclimation at 15 different temperatures (10-38°C), both
109 wild and lab fish were subjected to a range of phenotypic testing at their acclimation temperature.
110 The phenotyping included behaviour (swimming activity, alarm cue response), growth rate,
111 metabolism (standard and maximum metabolic rate; SMR and MMR), startle response time,
112 maximum swim speed, thermal tolerance, gene expression, RNA:DNA ratio and red blood cell
113 size (see Methods for further details). The 38°C exposure caused elevated mortality in both the
114 wild and lab zebrafish and was thus terminated (Fig. 4g).
115
116 We found that lab fish had lower physiological plasticity in many of the phenotypic traits
117 compared to wild fish, consistent with the hypothesis of loss of physiological plasticity following
118 adaptation to stable environments (predicted in Fig. 1a, b). SMR was higher in the lab fish at the
119 highest temperatures (Fig. 2a), indicating a lower ability to compensate for the direct effect of
120 temperature. This suggests that lab fish have a higher aerobic requirement to sustain baseline
121 maintenance, which corresponded with the higher expression found in metabolic genes in all
122 studied tissues at 36ºC (Fig. 3g and Fig. S3). In addition, a reduced thermal performance breadth
123 was detected in the MMR of the lab fish, where the breadth was 6.6°C less than the wild fish (Fig.
124 2b & Table S7).
125
126 As aerobic scope is the difference between MMR and SMR, the lower MMR and higher SMR
127 results in a narrower thermal aerobic scope profile in the lab fish (Fig. 2c). This could in turn

explain the differences in maximum swim speed, where the thermal performance breadth of lab fish was within 1°C of the wild fish, but the peak performance was lower (Fig. 2d & Table S1, S2 & S7). Aerobic scope is indicative of the total aerobic capacity of the fish, and a narrower aerobic scope in the lab fish means simultaneous aerobic processes (e.g. growth, digestion, swimming) can only occur at reduced rates at non-optimal temperatures(31, 32).

Reductions in the physiological plasticity of lab fish were also detected in startle response time (Fig. 2e) and thermal tolerance (Fig. 2f). The lab fish had steeper thermal reaction norms than the wild fish and were thus less able to counteract the direct effect of temperature through acclimation. These steeper reaction norms were especially visible at the lower acclimation temperatures where lab fish had a longer response time and a lower thermal tolerance than wild fish. Consequently, lab fish appear less well adapted to respond to stimuli or cope with acute thermal challenges at lower acclimation temperatures. These findings indicate a loss of physiological plasticity in diverse physiological traits in the lab fish and reduced performance at suboptimal temperatures.

The loss of physiological plasticity in lab fish was also detectable at the gene level. This was apparent as a dramatic increase in heat shock protein expression (*hsp70* and *hsp90*) at the highest acclimation temperatures in muscle, liver and brain of lab fish (Fig. 3a-f). Wild fish had a heat shock response at 36°C but to a lesser extent than the lab fish. The higher heat shock expression in the lab fish could be due to failure of function(33–36), for example a lack of heat tolerant isozymes(37) that may have been expressed at a higher degree in the wild fish (as predicted in Fig. 1c). This suggests higher continuous heat stress and heat injury(38) in the lab fish than in the wild fish. Additionally, there may have been a breakdown of regulation, such as a higher production of oxygen radicals due to leakier mitochondria(39), which could have led to more protein damage in the lab fish. The latter explanation is supported by the higher expression of genes relating to the induction of apoptosis and endoplasmic reticulum (ER) stress at the warmest temperatures in the lab fish across all three tissues sampled, suggesting that they experienced greater levels of cellular stress from misfolded proteins (Fig. 3g & Fig S3). The gene expression data therefore strengthen

the evidence of reduced physiological plasticity in the lab fish and supports the suggestion that although the lab fish are coping, they are less able to adjust their biochemistry and physiology to non-optimal temperatures than the wild fish.

If maintaining plasticity is costly, lab fish should show reduced plasticity in combination with higher performance at optimal temperature. This prediction was confirmed, as growth rates of the lab fish were 40% (weight) and 35% (length) higher than wild fish at the optimal temperature (T_{opt}; Fig. 4a; Fig. S2a). These higher growth rates in the lab fish could be due to reduced costs of physiological plasticity, but it is currently unclear what the main components of these costs are. They have been suggested to be genetic(10) or production costs (e.g. continuous production of thermally non-optimal enzyme isoforms(40)), or investment in the sensory and regulatory mechanisms required for maintaining physiological plasticity(10, 41–43). This explanation is consistent with the high physiological plasticity in many traits, and low growth rates observed in the wild fish.

Redundant genes that are not expressed can lose function due to neutral processes such as mutations or genetic drift(44). Physiological traits are often polygenic(45) and due to the likely complex genetic architecture of several of our measured traits, mutations would have to occur at a range of loci to cause functional changes. The slow mutational rate (thousands of generations) relative to the time the lab fish have spent in stable conditions (150 generations) (46, 47) makes it unlikely that this reduction of physiological plasticity in lab fish is due solely to mutation events. Changes in allele frequencies due to genetic drift, as well as directional selection for the stable lab environment, therefore, appear likely to be the mechanisms behind the phenotypic differences we observed. Furthermore, different lab strains of zebrafish that originate from different sources and collected from the wild at different times all have similar levels of heterozygosity and allele frequency patterns (48). This similarity suggests directional selection is the main driving force of adaptation to the lab environment rather than genetic drift. Alternatively, differences in the

183 regulation of gene expression could be the mechanism generating the phenotypes we observed
184 (49).
185
186 We hypothesised that lab fish should show higher growth only at optimal temperatures (Fig. 1a),
187 but interestingly their growth was greater across all temperatures (Fig. 4a). Domestication
188 generally increases growth rate in captivity in a range of organisms(50–52), and the elevated
189 growth rate at all temperatures could be a spill-over effect from strong selection on growth
190 performance at optimal temperature. In fish, higher activity in the growth hormone/IGF-1 axis
191 has been implicated in elevating growth after domestication(53), which match the higher
192 expression of the related genes *ghra* and *igf1* in the muscle of lab fish (Fig. 4b, c) in the present
193 experiment. However, the growth hormone/IGF-1 axis is only thought to be one of several
194 mechanisms that contribute to the elevated growth of domesticated fish(54). Adaptation to the
195 diet, high density, and aquaria are additional components of domestication that may have
196 contributed to the higher growth of lab fish(55).
197
198 Meal digestion, assimilation, and growth are energetically costly and can reduce the aerobic
199 scope available for other processes(32, 56). The lower aerobic scope despite higher growth rate,
200 meaning less available residual aerobic scope, may contribute to the lower spontaneous activity
201 and maximum swim speed seen in the lab fish (Fig. 4d, 2e). Lab fish also responded
202 behaviourally less to an alarm cue (Fig. 4e, f) across all temperatures than wild fish did. In the
203 lab, such alarm behaviours are redundant as conditions are homogenous, food is abundant and
204 there is no predation. A higher activity, maximum swim speed, and alarm cue response should be
205 beneficial in the wild to escape predators, find food and gain information about the
206 environment(57), but the resulting heightened alertness in the wild fish may be energetically
207 demanding(58, 59), and could thus also contribute to the lower growth rate of wild fish.
208 Conversely, by allocating less resources to growth, the wild fish may have been able to increase
209 overall performance across a broader range of temperatures, highlighting a potential trade-off
210 between physiological plasticity and growth.

At the coldest temperatures (10°C to 12°C), our results indicate that physiological plasticity is insufficient for normal function in both populations. Maximum swim speed, MMR, and alarm cue response showed a similar performance decrease in both wild and lab fish (Fig. 2d, b; Fig. 4e, f). Activity, growth and survival (Fig. 4a, d, g) were also reduced at these temperatures. The capacity that physiological plasticity has to restore high performance may be limited at these temperatures due to constraints in the underlying biochemical and physiological mechanisms of the traits(5, 37). These are temperatures that fall outside the range usually encountered in the wild(60), and the fish may therefore lack sufficiently cold-optimised enzyme isoforms(40, 61) or lipid regulation allowing sufficient membrane fluidity(62, 63). We have shown that while wild zebrafish are able to maintain high performance across a wider range of temperatures compared to a lab strain of zebrafish, there are environmental extremes where otherwise adaptive physiological plasticity fails to compensate for direct thermal effects.

Not all traits differed between the wild and lab zebrafish, for example, red blood cell (RBC) size increased similarly at high and low temperatures in both strains (Figure 4h). An increase in RBC size may be due to RBC swelling caused by a physiological heat stress response and β-adrenergic stimulation(64–66). This may provide an explanation for the larger cell size at higher acclimation temperatures and corresponds to the increased expression of heat shock proteins we observed (Fig. 3d-f). The increase at low temperatures may be an adaptive acclimation response, similar to the larger cells generally observed in both cold water adapted and acclimated fishes (67–70). Similarly, there were also differences in RNA:DNA ratio across temperature or between the wild and lab fish (Figure 4i) despite large differences in whole organism growth rate and gene expression (Figure 4a-c). RNA:DNA is a commonly used as a proxy for recent growth rate(71, 72), yet another study in zebrafish(73) also found a change in whole organism growth and gene expression but not RNA:DNA, suggesting that RNA:DNA may be a poor proxy for growth in zebrafish.

In this study, we show that lab zebrafish have lost a certain degree of physiological plasticity in many traits across all levels of biological organisation: behavioural, physiological, and genetic. The lower plasticity and higher growth in the lab population is consistent with the hypothesis of a trade-off between plasticity and performance. A loss of physiological plasticity and improved growth rates in lab zebrafish is likely the result of selection for higher growth and adaption to stable and optimal temperatures in the laboratory in combination with other domestication effects, showing that physiological plasticity can be rapidly reduced in stable environments.

Methods

Experimental animals

The experiment was conducted November-December 2017 at the Norwegian University of Science and Technology (NTNU), Trondheim, Norway. A total of 300 wild zebrafish and 300 lab zebrafish were used in the experiment and acclimated to temperatures from 10-38°C. The wild zebrafish were the offspring of wild-caught zebrafish collected from multiple sites in West Bengal, India and brought into the lab at NTNU in November 2016 (30, 74, 75). The wild fish were produced specifically for this experiment by random mating. The lab zebrafish were from the AB-wt strain, obtained from the Kavli Institute, Trondheim in October 2017. At the Kavli Institute the AB zebrafish line had been reared in an automated and controlled zebrafish rack system which minimised any fluctuations in water parameters, including temperature. Both wild and lab fish were produced using the same reproduction protocol as outlined in detail in (75). All fish were held in similar holding tanks at 28°C until the experiment commenced. The experiments were approved by the Norwegian Food Safety Authority (permit number: 8578).

Thermal acclimation

At the start of the experiment all fish were anaesthetised in buffered MS222 (conc. 110 mg/L); tagged using visible implant elastomers (VIE) (76); weighed (±0.001g); and their standard length (±0.01 mm) was measured. Within each population the fish were then haphazardly distributed between 15 aquaria with 20 fish per aquaria. Each aquarium was assigned to one temperature from 10 to 38°C. The aquaria were 63-L, well aerated and contained a filtration system and a plastic plant. Fish were fed *ad libitum* twice a day with dry fish flakes (Tetra pro) and water was changed regularly. To avoid acute thermal stress, the water temperatures were increased or decreased from 28°C in a daily-stepwise manner using titanium heaters (TH-100, Aqua Medic, Bissendorf, Germany) controlled by thermostats (ITC-306T, Inkbird, Shenzhen, China), until final acclimation temperatures were reached (taking zero to nine days). Acclimation temperatures above 28°C were increased at a rate of 1°C day^{-1}, whereas lower acclimation temperatures were reached by reducing

the temperature by 2°C day^{-1} until 12°C and by 1°C day^{-1} thereafter. Temperatures were monitored in real time and continuously recorded (Picotech TC-08, Cambridgeshire, UK) in each aquarium (Fig. S1). Temperatures were kept constant at the desired temperatures for the duration of the experiment. The aquaria for both wild and lab fish were distributed between three climate-controlled rooms, one for the coldest (10-14°C), one for intermediate (16-26°C), and one for the warmest temperatures (28-38°C). Acclimation temperatures and wild versus lab aquaria within each room were randomly distributed to reduce any within-room effects. The measurements of the fish traits were initiated 23 days after the start of the thermal ramping and continued until the final sampling on day 35.

Due to differing growth rates of wild and lab fish, the wild fish were 4-5 weeks old at start of the experiment while the lab fish were 3-4 weeks old. This enabled us to keep the size and life stage similar at the time of final sampling. In addition, due to the high number of fish and the many traits measured it was not possible to measure the wild and lab fish at the same time. The lab fish were therefore tagged, measured, and acclimated twelve days after the wild fish. Staggering the start time allowed phenotyping on both populations after the same acclimation time. The experimental dates for the wild fish were 02/11/2017 until 07/12/2017 and the lab fish from 14/11/2017 until 19/12/2017.

Quantification of phenotypic traits

All phenotypic tests were performed at the water temperature at which fish were acclimated. Growth and survival were measured for all fish. We measured behaviour, metabolic rates, response time, maximum swim speed and thermal tolerance in a subset of fish at each acclimation temperature. The same individuals had multiple traits quantified whenever possible. As some variables required longer duration measurements than other quicker tests, it was not possible to measure everything on all individuals and sample sizes therefore differ between variables. We minimized any differences that might occur due to sampling time by measuring two different temperatures each day and ensuring the temperature order of the wild and lab fish was the same.

Organs were sampled for further analyses (e.g. biochemical and gene expression) from the fish that did not undergo any phenotyping. Phenotyping was not possible at 38°C due to high mortality in both the wild and lab zebrafish resulting in these tanks being terminated.

Optimal temperature and thermal performance breadth

For performances estimated to have a third order polynomial relationship with temperature, the performance breadth was defined and calculated as the difference between the two temperatures where performance was 80% of the maximum performance (3, 77). Performance breadth was excluded in cases where it went above 39°C (i.e. activity) as this was outside the bounds of the experimental temperatures and exceeded long-term upper thermal tolerance. Optimal temperature was calculated as the peak of the polynomial curve, representing maximum performance.

Growth and survival

Weight (±0.001 g) and standard length (±0.01 mm) were measured at the start of the experiment during the tagging procedure and again at the end of the experiment. Specific growth rate (SGR) was then calculated for each fish as the percentage of weight gained per day (78). Mortality in the aquaria during the experiment was recorded and used to calculate the survival.

Behaviour and alarm cue response

Behaviour was measured individually for eight fish from each acclimation temperature in both wild and lab zebrafish, using a method similar to Vossen et. al (79). All behavioural trials within each strain (lab or wild) were completed within three days. The behavioural assay tanks measured 30 × 7 × 40 cm (length × breadth × height), with the water column at 25 cm, and with backs and sides painted opaque to minimize visual disturbance to the fish. Each tank was closed with a lid to maintain water temperature. The tanks were filmed at a front view using a USB camera (Kurokesu C1 IR, Vilnius, Lithuania). Eight assay tanks were filmed simultaneously spread evenly onto two shelves. The eight fish belonging to the same acclimation temperature were split in two

and assayed on different days, thus each trial measured four fish from one acclimation temperature and four fish from another acclimation temperature.

Video recording was started once all eight fish had been carefully netted and put into their respective tanks. After 15 minutes, a conspecific cue (homogenized whole zebrafish) was added to the water via tubes connected to a timer-activated water pump, triggering an alarm cue response. Each tank's tube ended in a pipette tip positioned above the water line and aimed against the tank's wall to minimize water disturbance. The video was stopped, and the trial ended 10 minutes after the cue was added. After each trial the tanks were thoroughly cleaned to remove any chemical cues and were filled with fresh water.

Behaviour was analysed from videos using the automatic tracking software EthoVision® XT12 (Noldus, Wageningen, Netherlands). Each assay tank was divided into three zones: surface (top 10 % of water column), middle (83%), and bottom (7%; a zone small enough to identify bottom-dwelling behaviour from random swimming) and the video analysed separately pre-cue (15 min) and post-cue (2 min). The behaviours quantified from the videos were: total distance moved (body lengths per min; converted from cm), distance to the surface (cm), duration at the surface (seconds per minute), duration at the bottom (bottom dwelling; seconds per minute) and time spent freezing (total seconds per minute not moving). The alarm-cue response was calculated as the change in behaviour post cue relative to the baseline pre-cue behaviour (by subtracting the pre-cue behaviour from the post-cue behaviour).

Metabolic rates

Standard metabolic rate (SMR)

Standard metabolic rate (SMR) was measured using intermittent-flow respirometry (Loligo® Systems, Viborg, Denmark) following best practices outlined in Clark et al. (31). Rates of oxygen consumption were recorded in four of the eight fish that had undergone the behaviour trials and two SMR systems were run consecutively for two different acclimation temperatures. All SMR

measurements within each strain (wild or lab) were completed within seven days. Each system contained four chambers (0.018 L), which were placed inside a temperature-controlled water bath with well-aerated water. Fish were tested individually with one fish per chamber. Each chamber was connected to a flush pump and a recirculation pump. When the flush pump was turned off the system was closed and the oxygen consumption ($\dot{M}O_2$) of the fish was recorded. The recirculation pump ensured there was constant mixing of the water within the chamber. The duration of the flush and measuring periods were adjusted for each acclimation temperature to prevent the dissolved oxygen falling below 80% air saturation, with warmer temperatures requiring shorter measurement periods. The oxygen concentration within the chamber was measured using fibre-optic leads focused on contactless sensor spots (Firesting O_2, PyroScience, Aachen, Germany) that were fixed on the inside of the chamber. The optodes were calibrated to 100% dissolved oxygen before adding the fish to the chamber and an external temperature sensor was used throughout. All fish had been starved for 24 hours prior to beginning the SMR measurements. Each fish was carefully transferred to their chamber to minimise stress and measurements were started immediately once all fish were in place. Oxygen consumption was then continuously recorded overnight for 18 hours to get resting values by allowing sufficient time for the fish to recover from the handling stress and the novel environment. At the end of the trial the fish were removed from their chambers, anesthetised, identified, and weighed, before entering the next performance test. After the fish had been removed from their chambers, background measurements were recorded for 10 minutes in all the chambers to account for bacterial respiration and algal photosynthesis. The system was then thoroughly bleached and rinsed before starting the next trial. All $\dot{M}O_2$ data were analysed using the respR package in R (80) whereby SMR was calculated as the average of the lowest 10% of rates after outliers were removed, background respiration was subtracted, and the value converted into mg O_2^{-1} h^{-1} g^{-1} by accounting for the volume of the respirometer and mass of the fish (31).

Maximum metabolic rate (MMR)

Groups of five to seven fish were used to measure MMR using a method similar to (81). At the lowest acclimation temperatures, the size of the fish was very small and therefore oxygen consumption rates were very low. This meant measurements were not possible on individual fish as the volume of water in the swim tunnel was too large to accurately detect changes in the oxygen levels. In addition, zebrafish swim better in groups than individually. Three groups of fish were tested from each acclimation temperature. The fish were added to a customised 0.4 L circular glass food container (6 × 14 cm, 365+ IKEA, Älmhult, Sweden). The chamber contained a raised stainless steel mesh platform with a magnetic stirring bar underneath creating a water flow. A piece of 4 cm diameter plastic pipe was added to the middle of the chamber to keep the fish swimming in the outer circumference of the chamber. Two holes were drilled into the lid, one that was connected to a flush pump for water exchange, and one with a chimney for water outlet and to allow an optode (Firesting O_2, PyroScience, Aachen, Germany) to be placed inside the chamber for measurements of oxygen consumption. Once the fish were added, the chamber was placed into a larger tank of water that contained plastic plants to minimise stress and kept the temperature constant. The tank lay on top of a magnetic stir plate and the chamber was sealed ensuring no air bubbles remained. The speed of the water was adjusted using the magnetic stir plate and the fish were first allowed to swim at a comfortable speed (i.e. the slowest speed at which the fish showed steady swimming) for 10 minutes. The speed was then increased for five minutes up until the fish could not maintain swimming. The speed was then adjusted down slightly (10%) so the fish were able to regain swimming and maintain their position in the water for at least one minute. The fish were held at this speed for a maximum of ten minutes during which time the oxygen consumption was measured. The rate of oxygen consumption during this time was used for the MMR calculations. MMR was analysed using the respR package in R similar to the SMR. Since the measurement was taken on a group of fish, their individual masses were summed for use in the calculations, again accounting for the volume of the respirometer. The resulting MMR values were shown in mg O_2^{-1} h^{-1} g^{-1}.

Aerobic scope (AS)

412 The aerobic scope was calculated by subtracting the mean SMR from the mean MMR at each
413 acclimation temperature for the wild and lab zebrafish.
414
415 ## Maximum swim speed (U_{crit})
416 *Group swim performance*
417 Maximum group swim speed (U_{crit}) was also obtained using a group swimming method similar to
418 (82). The maximum swim speed was the speed at which the fish were able to maintain their
419 position in the water column for at least one minute. If two or more fish were not able to keep up
420 with the group, then the speed was reduced. The arbitrary speeds of the stir plate were converted
421 to cm s^{-1} by producing a calibration curve for the inner, middle and outer part of the chambers.
422 The fish mainly swam at the outer edge, and the water speed where the fish were positioned was
423 calculated as the average of the middle and outer speeds. The speeds were then converted to body
424 lengths per second. The mean length of each group of fish (measured at the end of the experiment)
425 was used for calculation of body lengths per second.
426
427 *Individual swim performance*
428 In addition to the group measurement of maximum swim speed, a swim tunnel respirometer (1.5
429 L) (Loligo® Systems, Viborg, Denmark) was used to test the maximum swim speed of individual
430 fish, similar to the methods in (83). Each fish was individually added to the swim chamber and
431 left to swim at a low speed (i.e. the slowest speed at which the fish showed steady swimming) for
432 five minutes to become accustomed to the chamber. The speed was then increased, and the fish
433 were tested at two intermediate speeds for five-minute intervals. After this, the speed was
434 gradually increased until the fish was not able to maintain its position in the water column. The
435 speed was then adjusted down slightly, and the fish was left to swim for 30 seconds. This final
436 speed was used as the maximum swimming speed (U_{crit}). The arbitrary speeds of the motor were
437 converted to cm s^{-1} by producing a calibration curve. Speeds were calibrated at four temperatures
438 to check for a temperature effect on water speed, but there was no difference between them. The
439 speeds were converted to body lengths per second using the length of the fish measured at the end

of the experiment. This method of testing swim capacity was inferior to the group swimming in that many individuals appeared to show low motivation to swim, often letting themselves rest on the back grid at speeds much lower than their maximum. Testing groups of fish in species of shoaling fish can reduce stress and increase performance (84), so the group swimming was deemed the more reliable method. Oxygen consumption measurements from the single fish were too low to measure reliably at the lower temperatures and were therefore not included.

Response time

Response time, sometimes referred to as reaction latency, was measured in the same fish that had undergone the behaviour and SMR tests (n=2-4 per acclimation temperature and population) using methods similar to (85). Each fish was tested individually three times. Similarly to the SMR measurements, all response time trials for each strain were completed within seven days. The fish was placed into a 63-L (60 × 30 × 35cm) aquarium with a reduced water level (10 cm depth; 18-L). The water temperature was controlled using a water-bath with heating and cooling elements to ensure the fish were tested at their acclimation temperature. The tank had a startle weight hanging above it, which was released by pulling a trigger string. The weight fell through a large diameter water PVC pipe without touching the sides. The pipe was placed so that it protruded 1 cm below the surface so that the fish could not see the weight before it hit the water. A mirror was placed under the aquarium at a 45° angle so that a high-speed camera (RX100 MkV, Sony, Kōnan, Japan) could record the fish from below. Once the fish was motionless and within the cameras field of view the weight was released. The event was recorded at 1000 frames sec^{-1}. Each fish was tested three times. However, if the fish did not respond it was tested up to five times. The response time was measured by counting the number of frames (each frame represented 1 ms) from when the weight hit the water until the fish first responded using VLC media player software (VLC, https://www.videolan.org/vlc/). The fish usually responded to the drop with a C-start, a common escape/startle reflex in fish. The response time is presented as the median time for each individual.

Thermal tolerance

Thermal tolerance was the final performance taken for the fish on the termination day of the experiment (day 35). The acute upper thermal tolerance was measured using the critical thermal maxima (CT_{max}) test following the protocol described by (30, 75, 86). At each acclimation temperature, 6-8 fish were tested together. Starting at their respective acclimation temperatures, the temperature was ramped up at a rate of 0.3°C min^{-1} until the fish lost equilibrium and/or showed disorganized swimming (30, 86). At this point they were removed from the tank, their individual CT_{max} was recorded, and the fish were euthanized before the final measurements (length and weight) were made and the termination of the experiment.

Molecular analyses

The fish that did not undergo the performance tests outlined above were euthanised, measured (weight and length), and sampled on the termination day (day 35). Within each population and at each acclimation temperature, 3-6 fish were used for molecular analyses. The brain, liver, and a muscle sample were dissected out of the fish on ice. These samples were stored in RNAlater™ (Invitrogen, Massachusetts, USA) and frozen at -80°C until analysis.

Primers for 44 genes, suspected and previously reported to be related to thermal acclimation, were designed for real-time PCR with Primer3plus (http://primer3plus.com/) using GenBank (https://www.ncbi.nlm.nih.gov/genbank/) or were taken from previous publications, as indicated in Table S3. Molecular analysis was performed at University of Greenwich, UK and MRC London Institute of Medical Sciences, UK. Total RNA from muscle, liver, and brain was extracted using the ReliaPrep™ RNA Cell Miniprep Kit (Promega, Southampton, UK), including a DNA wipe-out step with DNAse I. For muscle samples this method was combined with a Tri-Reagent (Sigma-Aldrich, Missouri, USA) extraction prior to the RNA Kit step. RNA concentration was determined by Nanodrop ND-2000 (Thermo Fisher Scientific, Massachusetts, USA) and diluted to a common concentration of 50 ng μl^{-1} for muscle and liver, and 20 ng μl^{-1} for brain with nuclease free water (Promega, Southampton, UK). The GoScript™ Reverse Transcriptase (Promega, Southampton,

UK) kit was used with Oligo(dT) primers to transcribe 500 ng of muscle RNA, 200 ng of liver RNA and 100 ng of brain RNA into cDNA. cDNA was stored at -20°C until further use.

From all liver and muscle samples, plus a selection of brain samples, the expression of 44 genes (Table S3) was analysed with two technical replicates using the qPCR Biomark^{TM} HD system (Fluidigm, California, USA) based on 96.96 dynamic arrays (GE chips), as previously described in (87). A pre-amplification step was performed with a 500 nM pool of all primers in Preamp Master Mix (Fluidigm, California, USA) and 1.25 µl cDNA per sample for 2 min at 95°C followed by 10 cycles with 15 s at 95°C and 4 min at 60°C. Obtained pre-amp products were diluted 1:10 with low EDTA-TE buffer. The pre-amplified product was loaded onto the chip with SsoFast-EvaGreen Supermix Low Rox (Bio Rad Laboratories, California USA) and DNA-Binding Dye Sample Loading Reagent (Fluidigm, California, USA). Primers were loaded onto the chip at a concentration of 50 µM in Assay Loading Reagent (Fluidigm, California, USA) and low EDTA-TE Buffer. A dilution series was included to calculate primer efficiency and non-template controls were included to ensure samples were contamination free. The chip was run according to the Fluidigm 96.96 PCR protocol with a melting temperature (T_M) of 60°C. qBase+ 3.1 software (Biogazelle, Zwijnaarde, Belgium - www.qbaseplus.com) was used to select housekeeping (HK) genes and to verify stability of HK gene expression throughout analysed samples (88). The following HKs were used for muscle: *hif1ab*, *ef1a*, *cox4i1*, *ndufs3* with a stability of $M < 0.15$; for liver *ehhadh*, *cat*, *sod1*, *sod2*, *hif1ab*, *cox4i1*, *ndufs3* were used with $M < 0.15$; for brain *hif1ab*, *ndufs3* were used with a stability of $M < 0.15$ (89). qBase+ was used to calculate compensated normalised relative quantity (CNRQ) in relation to a reference sample (i.e. lab zebrafish, 10°C, sample 2) (89).

RNA:DNA ratio measurements

RNA:DNA ratio can be used as a snapshot proxy for protein production and growth in fish and can be responsive to thermal acclimation (90). RNA and DNA were quantified according to (91, 92). A pre-cooled stainless-steel ball was added to frozen muscle tissue in a 2 ml round-bottom

Eppendorf tube. The tubes were placed in a pre-cooled (-80°C) homogenization block and homogenized (Tissuelyser, QIAgen, Hilden, Germany) for 2 min (25 Hz). The tubes were moved to a homogenization block at room temperature, 100 µl sarcosil Tris-EDTA (STEB) buffer (0.5% v/v) was added, and homogenization was repeated. 400 µl Tris-EDTA (pH 8.0) was added, and the tubes were centrifuged at 16 000 g, 4°C for 15 min. DNA and RNA was quantified from 10-20 µl of supernatant, using the Qubit (Life Technologies, California, USA) RNA and dsDNA BR (broad range) assays, according to the supplier's instructions. All measurements were carried out in duplicate.

Flow cytometry

Cell size has been connected to the performance in thermal tolerance in ectotherms (93). Here we used flow cytometry as a quick method for red blood cell size estimates. Blood was collected from the tail according to the protocol of (94). Collected blood (0.5-5.0 µl) was transferred to 0.9 × phosphate buffered saline (PBS, 1 ml) and centrifuged at 0.5 g for 10 min. The supernatant was removed, and cells were resuspended in 0.9 × PBS (0.5 ml). Samples were analysed using a Novocyte flow cytometer (ACEA, San Diego, USA). To ensure that only single red blood cells (RBCs) were included in the analysis, two gates were created: one that selected events with low side scatter (SSC), and one that selected the events with both low forward scatter height (FSC)-H and low forward scatter area (FSC-A), which generally included the highest counts in the plot. Poor quality samples were excluded from the analysis. These included samples with low counts (<10,000 events), samples in which < 4% of events were within the selected gate, and samples with unstable FSC-A signal over time. The mean of FSC-A from the gated cell population was used as a measure of RBC size.

Statistical analyses

Each phenotypic trait was analysed separately, whereby the measured trait was the response variable and acclimation temperature, population (wild or lab) and their interaction (acclimation temperature × population) were the predictor variables. Weight was included as a covariate and

was kept when it improved the model. This decision was based on an ANOVA which compared models with and without weight, including it when $p < 0.05$. Temperature was centred on the mean acclimation temperature (23°C). For most phenotypic traits, model selection was used to test whether the effect of temperature was linear, quadratic (2^{nd} order polynomial), or cubic (3^{rd} order polynomial). The quality of the models were compared based on an ANOVA (significance criteria $p < 0.05$) and model parsimony using AIC ($\Delta AIC > 2$). When the ANOVA and the AIC-values showed different results, the simplest model was chosen. Only linear and quadratic temperature effects were tested when there was no biological reason to assume a cubic temperature effect or in those instances when the data was limited. Note that the same model (i.e. linear or quadratic temperature effect) was fitted to both populations for each trait to allow direct comparison. The significance of the predictor variables in the best model were estimated using a two-way ANOVA. Where the response variable was not normally distributed and/or the distribution was bound at the extremes (i.e. phenotypic traits: survival, bottom dwelling, time spent freezing and time spent at surface), the data were converted to proportions and GLM´s were fitted with a binomial error distribution. Model selection was performed using parsimony ($\Delta AIC > 2$) and the significance of the predictor variables in the best model were estimated using a chi-square test ($p < 0.05$). When the response variable rapidly increased or decreased with temperature and was not bound (i.e. phenotypic traits: *hsp70* and *hsp90* in muscle and liver tissue), an exponential model was deemed the most appropriate model fit.

All figures in the main text show the raw data for ease of interpretation. Statistical outputs from ANOVAs are shown in Table S1 and S4 and model estimates in Table S2 and S6. Outputs from chi-squared tests are shown in Table S5. All analyses were carried out in R 3.4.3 (R Core Team, 2017) with effect sizes with p-values less than 0.05 considered statistically significant.

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Data availability

All datasets and code generated from this study are available in the fighare repository (doi:10.6084/m9.figshare.17121464).

Author contributions

F.J. and G.D. designed the experiment. All authors performed the experiment. J.J.M. and A.L. carried out the gene expression analysis. T.R. carried out the RNA:DNA and flow cytometry analysis. A.H.A and R.M analysed the metabolic rate data and E.R.Å and M.H.F the behavioural data. R.M produced the final figures and carried out the statistical analysis on all the final datasets. R.M wrote the manuscript with significant input from all co-authors.

Competing interests

The authors declare no competing interest.

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References

1. F. J. Fry, Responses of vertebrate poikilotherms to temperature. *Thermobiology*, 375–409 (1967).

2. M. J. Angilletta, P. H. Niewiarowski, C. A. Navas, The evolution of thermal physiology in ectotherms. *J. Therm. Biol.* **27**, 249–268 (2002).

3. R. B. Huey, R. D. Stevenson, Integrating Thermal Physiology and Ecology of Ectotherms: A Discussion of Approaches. *Integr. Comp. Biol.* **19**, 357–366 (1979).

4. P. M. Schulte, T. M. Healy, N. A. Fangue, Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr. Comp. Biol.* **51**, 691–702 (2011).

5. F. Seebacher, C. R. White, C. E. Franklin, Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Change* **5**, 61–66 (2015).

6. A. R. Gunderson, J. H. Stillman, Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc. R. Soc. B Biol. Sci.* **282**, 20150401 (2015).

7. J. G. Kingsolver, R. B. Huey, Evolutionary Analyses of Morphological and Physiological Plasticity in Thermally Variable Environments. *Integr. Comp. Biol.* **38**, 545–560 (1998).

8. T. Garland, S. A. Kelly, Phenotypic plasticity and experimental evolution. *J. Exp. Biol.* **209**, 2344–2361 (2006).

9. G. N. Somero, The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers.' *J. Exp. Biol.* **213**, 912–920 (2010).

10. T. J. DeWitt, A. Sih, D. S. Wilson, Costs and limits of phenotypic plasticity. *Trends Ecol. Evol.* **13**, 77–81 (1998).

11. S. M. Scheiner, M. Barfield, R. D. Holt, The genetics of phenotypic plasticity. XI. Joint evolution of plasticity and dispersal rate. *Ecol. Evol.* **2**, 2027–2039 (2012).

12. L.-M. Chevin, R. Lande, G. M. Mace, Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory. *PLOS Biol.* **8**, e1000357 (2010).

13. R. B. Huey, J. G. Kingsolver, Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.* **4**, 131–135 (1989).

14. C. L. Richards, O. Bossdorf, N. Z. Muth, J. Gurevitch, M. Pigliucci, Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol. Lett.* **9**, 981–993 (2006).

15. M. Liefting, A. A. Hoffmann, J. Ellers, Plasticity Versus Environmental Canalization: Population Differences in Thermal Responses Along a Latitudinal Gradient in Drosophila Serrata. *Evolution* **63**, 1954–1963 (2009).

16. E. C. Snell-Rood, J. D. V. Dyken, T. Cruickshank, M. J. Wade, A. P. Moczek, Toward a population genetic framework of developmental evolution: the costs, limits, and consequences of phenotypic plasticity. *BioEssays* **32**, 71–81 (2010).

17. C. J. Murren, *et al.*, Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. *Heredity* **115**, 293–301 (2015).

18. R. A. Relyea, Costs of Phenotypic Plasticity. *Am. Nat.* **159**, 272–282 (2002).

19. A. Forsman, Rethinking phenotypic plasticity and its consequences for individuals, populations and species. *Heredity* **115**, 276–284 (2015).

20. M. E. Nielsen, D. R. Papaj, Why study plasticity in multiple traits? New hypotheses for how phenotypically plastic traits interact during development and selection. *Evolution* **n/a** (2022).

21. C. Nusslein-Volhard, R. Dahm, *Zebrafish* (OUP Oxford, 2002).

22. R. Mondal, A. Bhat, Temporal and environmental drivers of fish-community structure in tropical streams from two contrasting regions in India. *PLOS ONE* **15**, e0227354 (2020).

23. R. E. Engeszer, L. B. Patterson, A. A. Rao, D. M. Parichy, Zebrafish in The Wild: A Review of Natural History And New Notes from The Field. *Zebrafish* **4**, 21–40 (2007).

24. J. Schaefer, A. Ryan, Developmental plasticity in the thermal tolerance of zebrafish Danio rerio. *J. Fish Biol.* **69**, 722–734 (2006).

25. M. Westerfield, *The zebrafish book: a guide for the laboratory use of zebrafish (Brachydanio rerio)* (University of Oregon press, 1995).

26. C. Lawrence, The husbandry of zebrafish (Danio rerio): A review. *Aquaculture* **269**, 1–20 (2007).

27. D. G. Howe, *et al.*, ZFIN, the Zebrafish Model Organism Database: increased support for mutants and transgenics. *Nucleic Acids Res.* **41**, D854-860 (2013).

28. J. M. Ramsay, *et al.*, Whole-body cortisol response of zebrafish to acute net handling stress. *Aquac. Amst. Neth.* **297**, 157–162 (2009).

29. D. Wright, A. j. w. Ward, D. p. Croft, J. Krause, Social Organization, Grouping, and Domestication in Fish. *Zebrafish* **3**, 141–155 (2006).

30. R. Morgan, *et al.*, Are model organisms representative for climate change research? Testing thermal tolerance in wild and laboratory zebrafish populations. *Conserv. Physiol.* **7** (2019).

31. T. D. Clark, E. Sandblom, F. Jutfelt, Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *J. Exp. Biol.* **216**, 2771–2782 (2013).

32. F. Jutfelt, *et al.*, 'Aerobic scope protection' reduces ectotherm growth under warming. *Funct. Ecol.* **n/a** (2021).

33. C. Queitsch, T. A. Sangster, S. Lindquist, Hsp90 as a capacitor of phenotypic variation. *Nature* **417**, 618–624 (2002).

34. S. L. Rutherford, Between genotype and phenotype: protein chaperones and evolvability. *Nat. Rev. Genet.* **4**, 263–274 (2003).

35. S. L. Rutherford, From genotype to phenotype: buffering mechanisms and the storage of genetic information. *BioEssays* **22**, 1095–1105 (2000).

36. S. L. Rutherford, S. Lindquist, Hsp90 as a capacitor for morphological evolution. *Nature* **396**, 336–342 (1998).

37. G. N. Somero, Temperature Adaptation of Enzymes: Biological Optimization Through Structure-Function Compromises. *Annu. Rev. Ecol. Syst.* **9**, 1–29 (1978).

38. L. B. Jørgensen, H. Malte, M. Ørsted, N. A. Klahn, J. Overgaard, A unifying model to estimate thermal tolerance limits in ectotherms across static, dynamic and fluctuating exposures to thermal stress. *Sci. Rep.* **11**, 12840 (2021).

39. E. Cadenas, K. J. A. Davies, Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic. Biol. Med.* **29**, 222–230 (2000).

40. G. N. Somero, Proteins and Temperature. *Annu. Rev. Physiol.* **57**, 43–68 (1995).

41. E. Kussell, S. Leibler, Phenotypic Diversity, Population Growth, and Information in Fluctuating Environments. *Science* **309**, 2075–2078 (2005).

42. F. Seebacher, *et al.*, Plasticity of Oxidative Metabolism in Variable Climates: Molecular Mechanisms. *Physiol. Biochem. Zool.* **83**, 721–732 (2010).

43. A. Patapoutian, A. M. Peier, G. M. Story, V. Viswanath, ThermoTRP channels and beyond: mechanisms of temperature sensation. *Nat. Rev. Neurosci.* **4**, 529–539 (2003).

44. R. Albalat, C. Cañestro, Evolution by gene loss. *Nat. Rev. Genet.* **17**, 379–391 (2016).

45. D. L. Crawford, P. M. Schulte, A. Whitehead, M. F. Oleksiak, "Evolutionary Physiology and Genomics in the Highly Adaptable Killifish (Fundulus heteroclitus)" in *Comprehensive Physiology*, (John Wiley & Sons, Ltd, 2020), pp. 637–671.

46. R. C. Lacy, Loss of Genetic Diversity from Managed Populations: Interacting Effects of Drift, Mutation, Immigration, Selection, and Population Subdivision. *Conserv. Biol.* **1**, 143–158 (1987).

47. J. Masel, O. D. King, H. Maughan, The Loss of Adaptive Plasticity during Long Periods of Environmental Stasis. *Am. Nat.* **169**, 38–46 (2007).

48. J. Suurväli, *et al.*, The Laboratory Domestication of Zebrafish: From Diverse Populations to Inbred Substrains. *Mol. Biol. Evol.* **37**, 1056–1069 (2020).

49. M. Kærn, T. C. Elston, W. J. Blake, J. J. Collins, Stochasticity in gene expression: from theories to phenotypes. *Nat. Rev. Genet.* **6**, 451–464 (2005).

50. T. Kimura, Y. Hamada, Growth of wild and laboratory born chimpanzees. *Primates* **37**, 237–251 (1996).

51. T. Gjedrem, Genetic improvement of cold-water fish species. *Aquac. Res.* **31**, 25–33 (2000).

52. M. J. Zuidhof, B. L. Schneider, V. L. Carney, D. R. Korver, F. E. Robinson, Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. *Poult. Sci.* **93**, 2970–2982 (2014).

53. I. A. Fleming, T. Agustsson, B. Finstad, J. I. Johnsson, B. T. Björnsson, Effects of domestication on growth physiology and endocrinology of Atlantic salmon (Salmo salar). *Can. J. Fish. Aquat. Sci.* **59**, 1323–1330 (2002).

54. D. R. Causey, *et al.*, Proteomic comparison of selective breeding and growth hormone transgenesis in fish: Unique pathways to enhanced growth. *J. Proteomics* **192**, 114–124 (2019).

55. B. D. Robison, W. Rowland, A potential model system for studying the genetics of domestication: behavioral variation among wild and domesticated strains of zebra danio (Danio rerio). *Can. J. Fish. Aquat. Sci.* **62**, 2046–2054 (2005).

56. S. M. Secor, Specific dynamic action: a review of the postprandial metabolic response. *J. Comp. Physiol. [B]* **179**, 1–56 (2009).

57. I. Plaut, Critical swimming speed: its ecological relevance. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **131**, 41–50 (2001).

58. K. J. Millidine, J. D. Armstrong, N. B. Metcalfe, Presence of shelter reduces maintenance metabolism of juvenile salmon. *Funct. Ecol.* **20**, 839–845 (2006).

59. S. S. Killen, D. Reid, S. Marras, P. Domenici, The interplay between aerobic metabolism and antipredator performance: vigilance is related to recovery rate after exercise. *Front. Physiol.* **6**, 111 (2015).

60. R. Spence, *et al.*, The distribution and habitat preferences of the zebrafish in Bangladesh. *J. Fish Biol.* **69**, 1435–1448 (2006).

61. P. W. Hochachka, G. N. Somero, *Biochemical Adaptation: Mechanism and Process in Physiological Evolution* (Oxford University Press, 2002).

62. J. R. Hazel, Thermal Adaptation in Biological Membranes: Is Homeoviscous Adaptation the Explanation? *Annu. Rev. Physiol.* **57**, 19–42 (1995).

63. J. R. Hazel, E. Eugene Williams, The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Prog. Lipid Res.* **29**, 167–227 (1990).

64. N. M. Templeman, S. LeBlanc, S. F. Perry, S. Currie, Linking physiological and cellular responses to thermal stress: β-adrenergic blockade reduces the heat shock response in fish. *J. Comp. Physiol. B* **184**, 719–728 (2014).

65. M. Nikinmaa, Effects of adrenaline on red cell volume and concentration gradient of protons across the red cell membrane in the rainbow trout, Salmo gairdneri. *Mol.Physiol.* **2**, 287–297 (1982).

66. M. E. Forster, *et al.*, Catecholamine release in heat-stressed Antarctic fish causes proton extrusion by the red cells. *J. Comp. Physiol. B* **168**, 345–352 (1998).

67. W. C. E. P. Verberk, *et al.*, Shrinking body sizes in response to warming: explanations for the temperature–size rule with special emphasis on the role of oxygen. *Biol. Rev.* **96**, 247–268 (2021).

68. I. L. E. van de Pol, A. Hermaniuk, W. C. E. P. Verberk, Interacting Effects of Cell Size and Temperature on Gene Expression, Growth, Development and Swimming Performance in Larval Zebrafish. *Front. Physiol.* **12**, 738804 (2021).

69. A. Hermaniuk, I. L. E. van de Pol, W. C. E. P. Verberk, Are acute and acclimated thermal effects on metabolic rate modulated by cell size? A comparison between diploid and triploid zebrafish larvae. *J. Exp. Biol.* **224**, jeb227124 (2021).

70. W. A. Van Voorhies, Bergmann Size Clines: A Simple Explanation for Their Occurrence in Ectotherms. *Evolution* **50**, 1259–1264 (1996).

71. F. J. Bulow, RNA–DNA Ratios as Indicators of Recent Growth Rates of a Fish. *J. Fish. Res. Board Can.* **27**, 2343–2349 (1970).

72. L. Buckley, E. Caldarone, T.-L. Ong, RNA–DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. *Hydrobiologia* **401**, 265–277 (1999).

73. Y. Masuda, H. Oku, T. Okumura, K. Nomura, T. Kurokawa, Feeding restriction alters expression of some ATP related genes more sensitively than the RNA/DNA ratio in zebrafish, Danio rerio. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **152**, 287–291 (2009).

74. J. Sundin, *et al.*, On the Observation of Wild Zebrafish (Danio rerio) in India. *Zebrafish* (2019) https:/doi.org/10.1089/zeb.2019.1778 (November 24, 2019).

75. R. Morgan, M. H. Finnøen, H. Jensen, C. Pélabon, F. Jutfelt, Low potential for evolutionary rescue from climate change in a tropical fish. *Proc. Natl. Acad. Sci.* **117**, 33365–33372 (2020).

76. C. Hohn, L. Petrie-Hanson, Evaluation of visible implant elastomer tags in zebrafish (Danio rerio). *Biol. Open* **2**, 1397–1401 (2013).

77. B. L. Phillips, J. Llewelyn, A. Hatcher, S. Macdonald, C. Moritz, Do evolutionary constraints on thermal performance manifest at different organizational scales? *J. Evol. Biol.* **27**, 2687–2694 (2014).

78. K. D. Hopkins, Reporting Fish Growth: A Review of the Basics1. *J. World Aquac. Soc.* **23**, 173–179 (1992).

781 79. L. E. Vossen, *et al.*, Low concentrations of the benzodiazepine drug oxazepam induce
782 anxiolytic effects in wild-caught but not in laboratory zebrafish. *Sci. Total Environ.* **703**,
783 134701 (2020).

784 80. J. Harianto, *respR: Analyse, convert, and automate respirometry-related and time-series*
785 *data - januarharianto/respR* (2019) (September 3, 2019).

786 81. G. E. Nilsson, S. Östlund-Nilsson, R. Penfold, A. S. Grutter, From record performance to
787 hypoxia tolerance: respiratory transition in damselfish larvae settling on a coral reef.
788 *Proc. R. Soc. B Biol. Sci.* **274**, 79–85 (2007).

789 82. T. Usui, *et al.*, The French press: a repeatable and high-throughput approach to exercising
790 zebrafish (Danio rerio). *PeerJ* **6**, e4292 (2018).

791 83. S. S. Killen, D. P. Croft, K. Salin, S. K. Darden, Male sexually coercive behaviour drives
792 increased swimming efficiency in female guppies. *Funct. Ecol.* **30**, 576–583 (2016).

793 84. B. M. Culbert, K. M. Gilmour, S. Balshine, Social buffering of stress in a group-living fish.
794 *Proc. R. Soc. B* (2019) (December 8, 2019).

795 85. S. Gingins, D. G. Roche, R. Bshary, Mutualistic cleaner fish maintains high escape
796 performance despite privileged relationship with predators. *Proc. R. Soc. B Biol. Sci.* **284**,
797 20162469 (2017).

798 86. R. Morgan, M. H. Finnøen, F. Jutfelt, CT max is repeatable and doesn't reduce growth in
799 zebrafish. *Sci. Rep.* **8**, 1–8 (2018).

800 87. J. J. Miest, C. Arndt, M. Adamek, D. Steinhagen, T. B. H. Reusch, Dietary β-glucan
801 (MacroGard®) enhances survival of first feeding turbot (Scophthalmus maximus) larvae
802 by altering immunity, metabolism and microbiota. *Fish Shellfish Immunol.* **48**, 94–104
803 (2016).

804 88. J. Vandesompele, *et al.*, Accurate normalization of real-time quantitative RT-PCR data by
805 geometric averaging of multiple internal control genes. *Genome Biol.* **3**, research0034.1
806 (2002).

807 89. J. Hellemans, G. Mortier, A. De Paepe, F. Speleman, J. Vandesompele, qBase relative
808 quantification framework and software for management and automated analysis of real-
809 time quantitative PCR data. *Genome Biol.* **8**, R19 (2007).

810 90. A. M. Malzahn, C. Clemmesen, H. Rosenthal, Temperature effects on growth and nucleic
811 acids in laboratory-reared larval coregonid fish. *Mar. Ecol. Prog. Ser.* **259**, 285–293
812 (2003).

813 91. E. Caldarone, M. Wagner, J. St Onge-Burns, L. J. Buckley, Protocol and guide for
814 estimating nucleic acids in larval fish using a fluorescence microplate reader (2001).

815 92. C. Izzo, Z. A. Doubleday, A. G. Schultz, S. H. Woodcock, B. M. Gillanders, Contribution
816 of water chemistry and fish condition to otolith chemistry: comparisons across salinity
817 environments. *J. Fish Biol.* **86**, 1680–1698 (2015).

93. F. P. Leiva, P. Calosi, W. C. E. P. Verberk, Scaling of thermal tolerance with body mass and genome size in ectotherms: a comparison between water- and air-breathers. *Philos. Trans. R. Soc. B Biol. Sci.* **374**, 20190035 (2019).

94. G. L. Pedroso, *et al.*, Blood collection for biochemical analysis in adult zebrafish. *J. Vis. Exp. JoVE*, e3865–e3865 (2012).

Figure legends

Figure 1: Predictions of how reduced physiological plasticity can be detected: a - We predict that lab zebrafish (blue) will show higher performance in the measured traits at the optimal temperature (T_{opt}) but have a narrower thermal performance breadth (≥ 80% performance) than wild zebrafish (light green). b - We predict that wild zebrafish will show a higher capacity for adjusting their biochemistry and physiology (through acclimation or physiological plasticity) to counteract direct thermal effects on biological rates. This will be evident in wild zebrafish maintaining close to normal biological rates (i.e. metabolism) across different temperatures, post acclimation, while lab zebrafish will display more extreme rates as temperatures increase or decrease. c – To allow a consistent performance after acclimation, we predict greater regulation in the underlying mechanisms in the wild zebrafish compared to the lab zebrafish.

Figure 2: Thermal performance curves of wild and lab zebrafish for metabolic rates and other physiological traits after acclimation to temperatures from 10°C to 36°C. a - Standard metabolic rate (SMR), each point represents an individual fish; b - Maximum metabolic rate (MMR), each point represents a group of 6 fish (measured together); c - Aerobic scope, the difference between mean MMR and mean SMR. d - Maximum swim speed (BL sec^{-1}) where each point represents a group of 6 fish; e - Response time (latency to respond to a stimulus), each point represents the median response time of an individual fish; f - Thermal tolerance (using the critical thermal maxima method: CT_{max}) of individual fish. Optimal temperatures for performance, and thermal performance breadths (80% performance), are illustrated beneath panel b-d and exact

values as shown in Table S7. The p-values for the statistical models are indicated on each panel: Temp - effect of temperature on trait, Pop - difference between wild (light green circles) and lab (blue triangles) fish (intercept at 23°C), Pop x Temp - interaction (see Table S1-S2).

Figure 3: Comparison of gene expression in wild and lab zebrafish acclimated to temperatures from 10°C to 36°C. Heat shock protein (*hsp*) expression (a-f): *hsp70* and *hsp90* in the muscle (a, d), liver (b, e) and brain (c, f). Statistical model results indicated on each panel: Temp - effect of temperature on expression, Pop - difference between wild (light green circles) and lab (blue triangles) fish (at 23°C), Pop x Temp - interaction. (g) Heatmap showing the difference in muscle gene expression (log transformed) between wild and lab zebrafish. Genes are grouped by functional groups and colours represent relative expression within these groups where green shows a higher expression in wild fish and blue a higher expression in lab fish. White illustrates no difference. Full gene names and functions can be found in Table S3.

Figure 4: Growth, behaviour, survival and cell size in wild (green circles) and lab (blue triangles) zebrafish acclimated from 10°C to 36°C. Growth rate (a-c): a - Specific growth rate for weight for each individual fish; b - Relative quantity of growth hormone receptor α (*ghra*) in muscle tissue from qPCR. c - Relative quantity of insulin-like growth factor 1 (*igf*) in muscle tissue from qPCR. Behavioural (d-f): d - Swimming activity (distance moved) for individual fish pre-alarm cue; e - Change in activity in response to an alarm cue; f - Change in time bottom-dwelling (log sec^{-1}) in response to alarm cue, compared with pre-alarm cue. Survival (panel g) - Percentage survival of wild and lab fish after 35 days of acclimation to temperatures from 10-38°C. Red blood cell size (panel h). RNA:DNA ratio (proxy of current growth) (panel i). Maximum performance and upper and lower thermal performance breadths (80% performance) are illustrated beneath panel a. Statistically significant differences indicated on each panel: Temp

870 - significant effect of temperature on trait, Pop - significant difference between wild and lab fish
871 (at 23°C), Pop x Temp - significant interaction (see Table S1-S2 & S5).